The Fluorescence of Indoles and its Application in Drug Analysis

by

KAIS KHALIL ALI

A thesis presented for the degree of

MASTER OF PHILOSOPHY

in the

University of Aston in Birmingham

August 1977
Department of Pharmacy
University of Aston in Birmingham
Gosta Green
Birmingham B4 7ET
U.K.

SUMMARY

The Fluorescence of Indoles and its Application in Drug Analysis. Kais Khalil Ali, M.Phil. Thesis 1977.

The literature on fluorescence of indoles and its application is briefly reviewed. The effect of polar solvents such as n-butanol, methanol, cyclohexanol, borneol, tert-butanol, 3-ethyl-3-pentanol, acetonitrile, tetrahydrofuran, pyrrolidine, cyclohexylamine, triethylamine, quinuclidine, triethylenediamine and acetic acid on the fluorescence of some indole derivatives such as unsubstituted indole; 2,3-cyclodecamethylene-indole; 2,3-dimethylindole; 2-Butyl-3-propylindole; 2-methylindole; 2-tert-butylindole; 3-methylindole; tryptophol; 7-methylindole; 5-methylindole; 5-methoxyindole; 5-fluoroindole; 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 6-methoxy-1,2,3,4-tetrahydrocarbazole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole; 1,2,3,-trimethylindole; 1,2-dimethylindole and 1,3-dimethylindole in cyclohexane is reported.

It was noted that the formation of an exciplex is dependent upon electronic and steric effects of both solvent and indole structures. The nature of the exciplex is discussed and a dipole-dipole with some charge-transfer interaction is proposed for the mechanism of exciplex formation.

The synthesis of some of the above indoles is described. The analysis of drugs such as 5-Hydroxyindole-3-acetic acid, 5-Hydroxytryptophan, indomethacin and oxypertine by native fluorescence, O-phthalaldehyde reaction and gas chromatography methods is described.

Key words: indole, fluorescence, exciplex, drug, analysis.

ACKNOWLEDGEMENT

The author would like to express his gratitude and thanks to Dr A.Z. Britten for his invaluable help and encouragement during the course of this work, also staff and research colleagues in the Department of Pharmacy, especially Professor D.G. Wibberley and Dr. W.J. Irwin for many useful discussions. Thanks are also due to the state Company for Drug Industries (S.D.I.) of Iraq for their financial support and study leave throughout the course of this work.

Grateful thanks are also extended to Miss K. Ensor for her reading part of the manuscript.

C O N T E N T S

1. INTRODUCTION

Luminescence attracted the attention of scientists a long time ago, but in the last years it has found applications in industrial practice. During the initial period of application of luminescence to practical work, its methods lacked a theoretical basis and the observed facts were not well understood. Moreover, the development of fluorometry was also hampered by the lack of suitable apparatus, e.g. light sources, light filters, and photometers. These difficulties have been overcome and the necessary light filters, monochromators, quartz mercury-vapor and xenon lamps, and other apparatus required for a spectrophotofluorometer are now readily available.

Luminescence was first discovered [1] as fluorescence in aqueous solution of wood extract (lignum nephriticum). Later the synthetic dyes were for a long time the most important compounds of the investigations, which were naturally restricted to the visible region of the spectrum. It was plausible, therefore, that the first attempts to find relations between the fluorescence of compounds and their constitution were based on the semiempirical observations on these compounds.

Photoluminescence is defined as the radiation emitted by a molecule, or an atom, after it has absorbed radiant energy and been raised to an excited state [2]. Photoluminescence consists of fluorescence and phosphorescence. Both processes have been used by the analytical chemist for trace analysis. The kinds of analysis which have been performed to date, using fluorescence, have been very varied, including trace metal determination, analysis for organic material, and particularly for determining trace constuents of biological systems.

In order to understand the luminescence processes it is necessary to become familiar with the nature of the electronic and excited state processes.

1.1. Electronic States and the Absorption and Emission of Electromagnetic Radiation.

Electronic states are concerned with properties of all of the electrons in all of the orbitals, so that, when an electron moves from one orbital to another, the state of the molecule is changed and it is important to consider the states of the molecule involved rather than considering only the orbitals involved in such an electron promotion. Thus, there is the ground state, the normal state of the molecule, or the state of lowest energy. There is only one ground state for any molecule. But, there are many different possible excited states for even very simple molecules, the exact nature of which depend upon the particular types of orbitals involved in the excitation. However, electronic states of organic molecules can be grouped into two broad categories; singlet and triplet states. A singlet state is one in which all of the electrons in the molecule have their spins paired. The resulting spin is zero. For instance, the ground state of most organic molecules will be a singlet state. Triplet states are those in which one set of electron spins have become unpaired, that is all electrons in the molecule except two have paired spins. In other words, the distinctions between singlet and triplet states, is the multiplicity of that state which is a term used to express the orbital angular momentum of given state of an atom or molecule [3]. Thus, if the multiplicity is 1, the state is called singlet, if it is three the state is called triplet. Singlet and triplet states differ significantly in their properties as well as in their energies. Triplet states always lie lower in energy than their corresponding singlet states. The singlet states may be said to be stacked in one vertical column, while the triplet states are stacked in another vertical column. Each of the electronic states has a number

of vibrational levels superimposed on it and these may be assigned as O, 1, 2, 3, 4, 5 etc. The vibrational levels arise because a molecule in a given electronic state may absorb small increments of energy corresponding to changes in vibrational modes. At room temperature a combination of thermal agitation and attractive and repulsive forces keeps the atoms vibrating in the lowest vibrational level of the ground state. Therefore absorption occurs from the Zeroth vibrational level of the ground state.

Light is a form of electromagnetic radiation i.e. energy, and is characterized by a frequency, a wavelength, and in a vacuum, a constant velocity. However, when light enters matter, two things may happen to it. It may pass through the matter with little absorption taking place. In this case there is little loss of energy, or, on its passage through the matter, the light may be absorbed, entirely or in part. In either case absorption involves a transfer of energy to the medium. Thus, the absorption itself is a highly specific phenomenon and radiation of a particular energy can be absorbed only by characteristic molecular structures. However, luminescence processes can be interpreted only in terms of the excited state from the luminescence emission and its relationship to the ground state of the molecule.

1.2. Fluorescence and Phosphorescence Processes

The process of fluorescence may be represented in schematic diagram, Figure 1.

When the quantum of light strikes a molecule, it is absorbed in about 10^{-15} second or within the period of frequency of the light, which is short in relation to the time required for all other electronic processes and for nuclear motion. The internuclear distances therefore remain constant during the absorption of light which will then raise the energy of the molecule by promoting an electron to a higher state, known as the excited state.

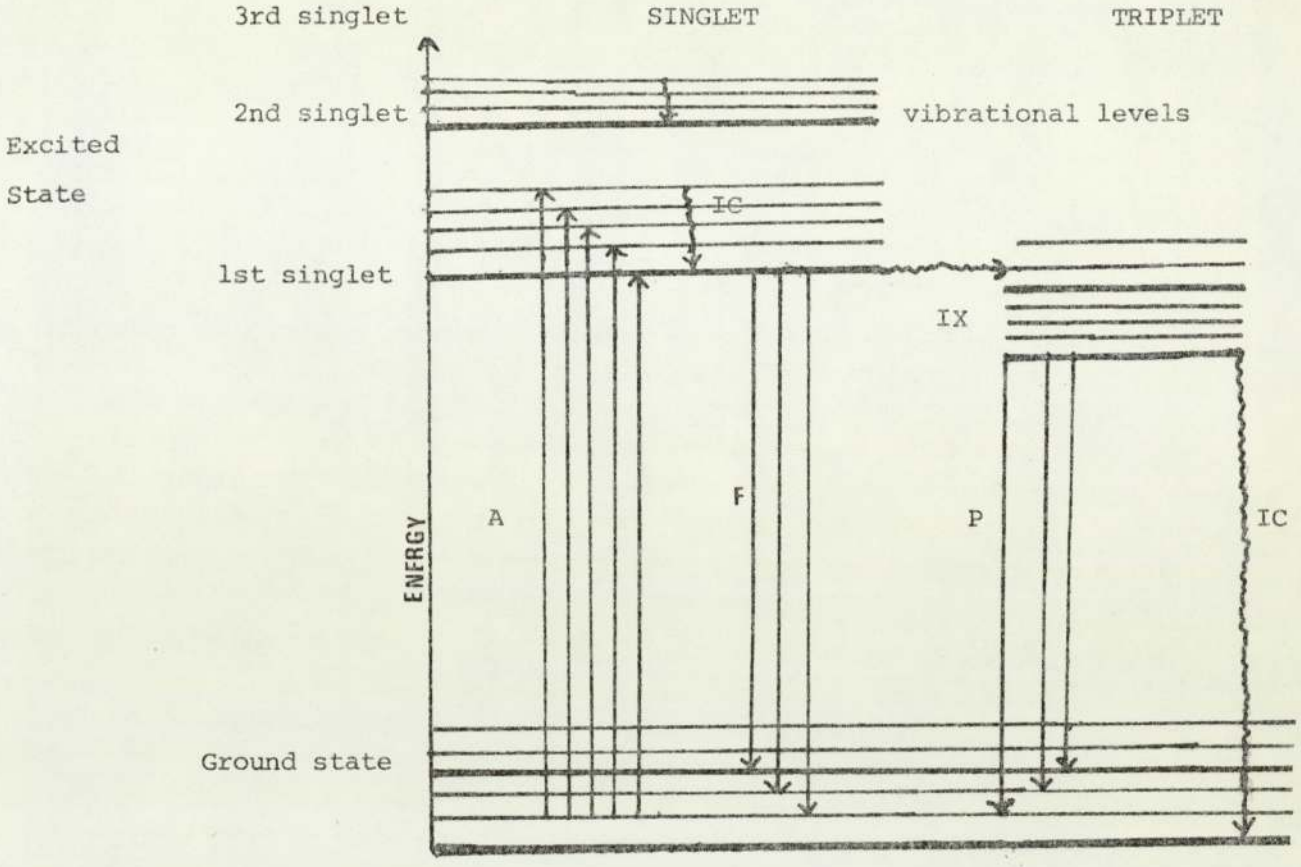


Figure 1

The excited state persists for a finite time which is about 10^{-8} second [3], because the life of an excited singlet state is approximately 10^{-9} second to 10^{-7} second [2]. After excitation there occurs deactivation by radiationless processes and return to the lowest vibrational level of the excited state. This process occurs within 10^{-13} to 10^{-11} second .This is a very important point because it means that before an excited molecule in solution can emit a photon, it will undergo vibrational relaxation, and therefore, photon emission will always occur from the lowest vibrational level of an excited state. In this case, the probability for return to the ground state is very high by photon emission (F arrows), this process is called Fluorescence (2).

In some gases at extremely lowe pressures the reverse transition occurs before vibrational deactivation can occur. This has the same energy as the absorption transition, thus the emitted light has the same wavelength as the absorbed light. However, one tends to see photon emission from higher vibrational levels of the excited states in gas phase spectra at low pressure. This process is called resonance radiation [3], which does not occur at higher vapour pressures or in solutions. The fraction of excited molecules that will fluoresce is called quantum efficiency of fluorescence. So that for highly fluorescent molecules the quantum effiency of fluorescence approaches unity, while for molecules which have fluorescence so weak that it is difficult to observe itthe quantum effiency of fluorescence is very low, and approaches zero.

Light of a frequency which is incapable of producing in a molecule a transition to the excited electronic state, is nevertheless capable of being absorbed by the molecule. The absorbed photon excites electrons in the ground state to higher vibrational levels, and so there is no electronic transition. The energy is entirely conserved and a photon of the same energy is emitted within 10^{-15} second , while the electrons return then to their original state. Since the absorbed and emitted photon are of the same energy the emitted light has the same wavelength as the exciting light. Light emitted in this case is referred to as Rayleigh scattering. However, in the case of electronic transitions the change in photon energy causes a shift of the fluorescence spectrum to a long wavelength relative to the absorption spectrum. This is called the Stokes shift.

From the preceding discussion it would appear that all molecules which absorb light energy should fluoresce, but some molecules in an excited singlet state or triplet state are found to return to the ground state without the emission of a photon, converting all the excitation

energy into heat. This radiationless process is called internal conversion (↓c wavy arrows in Fig. 1). On the other hand, most absorbing molecules have two excited electronic states [4] , Singlet and Triplet, which are related to each other as shown in the schematic diagram, Fig. 1. Transition from the ground singlet state to the excited singlet state constitutes normal absorption (A); the reverse is fluorescence (F). The transition from the ground state to the triplet states by direct absorption is very weak, but may be an efficient process for the excitation of triplet states from the lowest singlet state. This process is called intersystem crossing (↓x wavy arrow in Fig. 1.) and is a spin-dependent internal conversion process. Molecules in the excited triplet state, can return to the ground state via the singlet state only by taking energy from the environment, so that, the emitted light is the same as that produced by normal fluorescence, but the lifetime in this case of the excited state is longer than 10^{-8} second Intersystem crossing [5] is enhanced. (i) if the energy difference between the lowest singlet state and the triplet state just below it is small, (ii) by longer lifetime of an excited singlet state as this should increase the fraction of excited molecules which undergo intersystem crossing, compared with those that fluoresce. Once intersystem crossing has occurred, the molecule undergoes the usual internal conversion process and falls to the zeroth vibrational level of the triplet state. At low temperature thermal energy is not available and the return to the ground state can proceed only via the forbidden transition from the triplet state to the ground state, because the energy difference between the triplet state and the ground state is smaller than the difference between the lowest singlet state and the ground state. Since the probability of this is quite low, the excited state persists for a long time. i.e. the lifetime of a triplet state is much longer than that of a singlet state, i.e. several seconds at ordinary temperature, so

that the emitted light will be of a lower frequency and longer wavelength than the fluorescent light. In other words the excited triplet state represents a tautomeric form of the ground state in which a pair of electron spins are uncoupled. The emission produced by the transition from a triplet state to a ground state (P arrows), is called Phosphorescence.

It is seen that phosphorescence differs from fluorescence mainly in so far as the persistence of the luminescence following the excitation by the light source and by the marked enhancement and increase of the emission time with the lowering of temperature. Because of these reasons, phosphorescence is almost never observed in solution at room temperature, although it has been detected for some molecules by using a very sensitive detector[6] .

Coming back to the fluorescence, the process takes place when a molecule absorbs a photon light and an electron is raised to a higher level of energy. The molecule is, therefore, left in an excited state. The electronic change yields a band spectrum of absorption which covers the whole series of transitions, electronic, vibrational and rotational. If the molecule does not decompose as a result of the increase in energy and if all the energy is not dissipated by subsequent collision with other molecules, then after a short time (10^{-8}, 10^{-7} second), the electron returns to the lower energy level, emitting a photon in this process. The difference between the energy of the initial state (ground state) and the final state (excited singlet state) determines the energy of the emitted radiation (fluorescence). However the emitted fluorescence has a greater wavelength or lower energy than the light which is absorbed.

1.3. Fluorescence Quenching Processes

Fluorescence may cause a problem when measuring the absorption, so absorption raises certain problems during fluorometric assay. Obviously, light must be absorbed before fluorescence does occur. However, it is also apparent that when absorption is so great as to make the solution opaque, no light will pass through to cause excitation. . At intermediate concentration, even though light does penetrate through the solution, it is not evenly distributed along the light path, so that the portions nearest the light source absorb much of it and gradually less and less is available for the remainder of the solution, i.e. molecules near the light source absorb some of incident light, so that the transmitted light has less energy than the original incident light. However, this transmitted light will be the incident light for the remainder of molecules in the solution. The result is that most of the excitation occurs at the entrance, with less and less through the remainder of the cell, thus, the non-uniform distribution of the fluorescence in a strongly absorbing solution presents a problem for detection. A general rule is that a linear response will be obtained until the concentration of the fluorescent substance is sufficiently great so as to absorb a significant amount of the exciting light. A solution having an absorbancy of say 0.5 in the spectrophotometer would be expected to lower the apparent fluorescence, and to obtain a linear response the solution must absorb less than 5% of the exciting radiation [7].

Again all molecules which absorb light energy should fluoresce, but even under ordinary conditions a large number of strongly absorbing substances are non-fluorescent. This means that the fluorescence efficiency of such molecules is very low so that fluorescence is not as widespread as light absorption, because of competing quenching processes

tending to decrease the quantum yield of fluorescence. This may be caused not only by a high concentration of the molecules of the fluorescent substance but also by the presence of other organic and inorganic materials or by radiationless transitions which occur when the excited singlet electronic state leads to an electronic state in which a chemical bond is broken. These are termed Predissociative transitions, that is the molecules contain linkages with a bond strength lower than the energy required for electronic excitation. There are two further types of processes that compete with fluorescence; radiationless transfer of excitation energy to an appropriate acceptor, and reversible and irreversible chemical reactions. Often, excited state reactions are different from reactions of the same molecule in the ground state.

A collisional quenching process is a bimolecular process depending upon contact between the excited state and the quencher. This process requires that the lifetime of the excited state involved should be greater than 10^{-9} second . There are two mechanisms by which collisional loss of excitation energy can occur; enhancement of intersystem crossing by the quencher, or electron transfer. The effect of the quencher on intersystem crossing and enhancement of phosphorescence is well known, for example; (i) heavy atoms effect both the radiative and nonradiative triplet singlet transitions. McGlynn and Smith (8) have studied the external heavy atom effect and have suggested it might be a useful means of enhancing the phosphorescence, intensity of some compounds.

(ii) Alkyl halides and halide ions are effective quenchers either by an external or internal effect. Hood and Winfordner [9] have studied the influence of ethyliodide-ethanol as a solvent. They found that a 5:1 v:v ethanol-ethyl iodide enhanced the phosphorescence signal.

(iii)Paramagnetic metal chelates [10] are also effective quenchers.

(iv) The ability of oxygen to quench excited the singlet state of many

molecules[11] especially aromatic hydrocarbons in solution. Nitric oxide also quenches the fluorescence of aromatic hydrocarbons with about the same efficiency as oxygen. In both instances the quenching mechanism is due to the small energy gap between singlet and triplet states because of the presence of n-electrons of oxygen.

However this concept does not include cases, where the decrease in fluorescence yield is caused by the partial interception of excitation energy or of the fluorescence emission for instance, by an absorbing impurity. The same considerations apply if the solvent absorbs in the spectral region of the excitation of the emission.

Another possibility for collisional quenching is by an electron transfer mechanism, which occurs when the fluorescent molecule reacts with the quencher and abstracts an electron from it to form the ion pair. This ion pair can dissociate to give either a triplet state and quencher or simply a ground state and quencher [2].

A non-collisional energy transfer can also occur over distances larger than the contact distances of molecular collision. These processes are non-radiative processes of energy transfer from one molecule to another, that is non-collisional transfer of the energy between donor and acceptor[13].

Delayed fluorescence is another energy transfer process,which may occur either when two triplets collide with one another producing one molecule in the excited singlet state and the other in a ground state. The fluorescence emission of the excited singlet state may then be observed. Or it may occur, if the lowest singlet and triplet are so close in energy that thermal activation will occasionally promote triplets up to excited singlet state, from which they fluoresce. In both cases an emission has the spectral characteristics of fluorescence

but a lifetime just shorter than the lifetime of phosphorescence [14]. However, delayed fluorescence should not be confused with the excimer fluorescence which has a spectrum different from normal fluorescence.

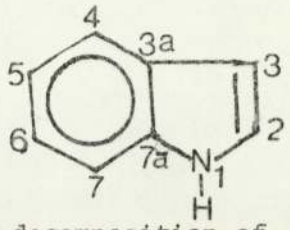
Recently [15] it has been found that the emission of solution of certain organic molecules changes at increasing concentration, and a new broad emission band appears to the red side of the fluorescence spectrum. This is known to be due to the formation of a short-lived dimer between an excited molecule and an unexcited molecule which breaks up shortly after formation with emission of the characteristic spectrum. When two identical molecules are involved, the excited dimer is called an excimer. On the other hand such interaction between unlike molecules is called an exciplex. It should be emphasised that this type of interaction is only formed with the excited state and cannot be detected by absorption spectrophotometry[16]. Weak acids and bases usually show solvent dependent fluorescence emission. The influence of pH upon the fluorescence of organic molecules was reported by Williams [17], who discussed the relationship between molecular dissociation and fluorescence. Thus, phenol fluoresces maximally at pH 1 and its fluorescence diminishes as the pH is raised and becomes essentially zero at pH 13. On the other hand, monohydroxyl and dihydroxybenzoic acids fluoresce in alkaline solution, and hydroxyphenyl acetic acid and related compounds show the maximal fluorescence at a neutral pH. Therefore a change in hydrogen ion concentration can cause a marked change in both the intensity and the position of fluorescence emission with little effect on the absorption spectrum. Such findings have been reported for several indole compounds [18], where changes in pH produced a large change in fluorescence intensity without producing a comparable change in absorption. On the other hand, indole has been shown to have highly solvent dependent fluorescence

properties [16, 19, 20, 21, 22]. Since solvent effects are important in the quantitative study of protein fluorescence, a reappraisal in terms of their probable significance for the fluorescence of tryptophanyl and tyrosyl residues in proteins [23, 24] is required.

2. THE FLUORESCENCE OF INDOLE

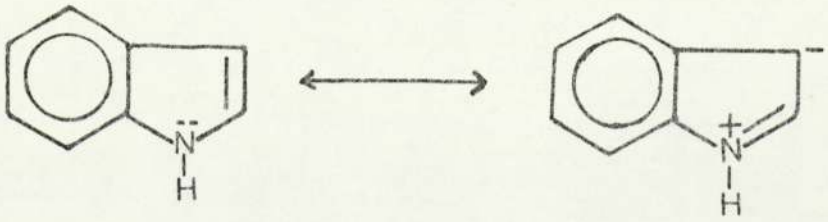
2.1 GENERAL CONSIDERATIONS

Indole is the commonly used name for benzopyrrole in which the benzene ring is fused to the 2 and 3 position of the pyrrole ring [25]. The indole structure occurs widely in natural compounds. It occurs in some plant materials and may be formed by the decomposition of tryptophan in putrefaction of proteins. The simpler naturally occuring indole derivatives include 3-methylindole, skatole, as well as indigo which is a common purple dye. Indole was discovered in 1866 during an investigation of indigo. The discovery of many alkaloids containing the indole nucleus led to further interest in indole chemistry. During this period the finding that the essential amino acid, tryptophan and the plant hormone heteroauxin were indole derivatives added stimulus to research in indole chemistry.



Recently indole derivatives have achieved increased significance in medicinal chemistry. There are a number of indole derivatives of physiological and pharmacological interest, and some of them are in use as drugs [12].

Indole [26] has a planar heteroaromatic structure with a ten pi-electron system formed by a pair of electrons from the nitrogen atom and eight electrons from the eight carbon atoms. The indole ring is reactive towards electrophilic substitution with the 3-position being the most susceptible to attack. This is in agreement with an explanation based on a simple resonance approach which makes the 3-position electron rich.



Early work on the fluorescence of indole began in the 1950's (27) when it was proposed that the protein fluorescence in the ultraviolet region was the result of the emission from tyrosine and tryptophan residues. Later it was found that pyrrole in aqueous solution is non-fluorescent, but indole was highly fluorescent[28] . This property of indole has been valuable in the detection and identification of indole compounds, especially in biological systems. Fluorescence is an extremely sensitive technique with a very low limit of detection. It has therefore been applied in studying the chemical composition and the physico-chemical properties of proteins, as well as other macromolecules and interaction of proteins with one another and smaller molecules, and in the quantitative estimation of tryptophan and tyrosine in protein hydrolysates [3]. Also the fluorescence behaviour of the amino acids, tryptophan, and tyrosine have been used in the study of protein structures [29]. It has been found that the ultraviolet fluorescence of proteins containing tryptophan, is predominantly due to the fluorescence of this amino acid [23]. A knowledge of the fluorescence properties of tryptophan is therefore of importance in the understanding of protein fluorescence. The measurements of the quantum yields and the wavelength distributions are useful in studying the spectral properties of tryptophanyl side chains buried within protein structures [30].

The estimation of the fluorescence of indole (31, 32, 18, 33, 28) provides a basis for the analysis of protein emission due to tryptophan fluorescence. However, the changes in the fluorescence of indole produced by solvents, with little effect on the ultraviolet absorption spectra imply that the excited state is highly polarized [16]. This makes indole in the excited state a strong acid bearing the positive charge on the nitrogen. Studies of solvent, pH, concentration and temperature effects on the fluorescence spectra lend insight into the influence of substituent groups in molecules on acid base equilibria, hydrogen bonding and the nature of the excited state[24,34].

The following salient features in the fluorescence of indole have been reported:

(1) There is a large Stokes shift and loss of vibrational structure in the fluorescence spectra of indole in a polar solvent as compared with a non-polar solvent without a corresponding shift in the ultraviolet absorption spectra[35,36].

(2) The absorption of indole between 250 and 300 nm is due to two overlapping transitions, designated $^1L_a \leftarrow {}^1A$ and $^1L_b \leftarrow {}^1A$. The two excited states are differentially shifted by solvents. Thus ^1L_a lies lower in polar solvents, but ^1L_b lies lower in non-polar solvents. However indole in polar solvents exhibits emission from both the ^1L_a and the ^1L_b state[37,36].

(3) The red shifts in the fluorescence spectrum of indole and related compounds, rather than being due to generalised solvent effects, have their origin in exciplex formation, i.e. formation of an excited state complex of indole and a polar molecule[16,38]. Thus the position of the emission maximum for a given tryptophanyl residue in a protein would depend upon its ability to form such an excited state complex.

It is clear that an understanding of the fluorescence behaviour of indole is necessary for the better use of fluorescence analysis. Of particular value would be the knowledge of the effect of the molecular environment on the position of emission maximum of indole.

Accordingly, it was decided to examine the effect of solvent on the fluorescence of indole and its derivatives as well as the structural and electronic requirements for indole exciplex formation.

2.2. SOLVENT EFFECT

Solvent can affect the structure of spectra absorption curves as well as the intensities and wavelengths of maxima[7], and this effect on the fluorescence and absorption maxima of indole has been well documented[35].

Solvents produce shifts to longer or shorter wavelengths, usually, of the order of 5 to 10 nm and seldom more than 17 nm[39].

McRae[40] studied the solvent effect on electronic spectra and derived a general expression for the solvent induced frequency shift by means of second order perturbation. He considered a solute molecule to be reducible to a point of dipole in an environment of isotropic dielectric solvent. In media of low viscosity, Brownian motion, which causes the molecules to become mobile will be a hindrance to charge transfer between molecules.

Polar solvents produce a red shift of the fluorescence of indole and substituted indole. This was attributed by Van Duuren[35] to the dielectric properties of the solvent, i.e. there is a shift to a longer wavelength with increased dielectric constant of the solvent. Furthermore, he suggested that there is an interaction between the solvent and the activated indole molecule but not between the solvent and the ground state of the indole molecule. Thus polar contributing mesomeric states of indole make a larger contribution in the activated indole molecule than in the ground state. Such polar structures would be more sensitive to the dielectric constant of the surrounding solvent, accounting for pronounced shifts in the fluorescence spectra.

Mataga et al[41] reported that hydrogen bonding is mainly responsible for the red shift mechanism in polar solvents. This was supported by Hardin et al[30], who pointed out that the red shift in polar solvents resulted from a hydrogen bonding mechanism, while the shift caused in non-polar solvents was mainly from the altered polarizability of the medium. Thus they applied this to predicting the wavelength position of the tryptophan absorption bands of proteins. In contrast indole can behave as proton donor or proton acceptor with a hydrogen bonding solvent[24], and this factor was taken into consideration by Van Duuren.

Hydrogen bonding is not necessary for the red shift to occur as was

found by examination of the fluorescence emission of 1,2-dimethylindole and 1-methyl-2-phenylindole in various solvents. These substances cannot undergo hydrogen bonding with dioxane, nevertheless their fluorescence emission maxima follows the same shift to longer wavelengths with higher dielectric constants. Mataga and co-workers[42], interpreted the red shift in terms of a generalized dipole-dipole interaction between solvent and solute in the excited state. However, they reported that the large shift of the fluorescence band in the polar solvents indicated a large increase of the dipole moment in the excited state, and greater stabilization was achieved by solute-solvent interaction in the fluorescent state than in the ground state, and Frank-Condon excited state[43]. Thus the fluorescence spectra of indole in n-hexane/ethanol mixtures indicated the relative intensity of the fluorescence band(^{1}Lb) decreases, and a broad band shifts further to the red as the concentration of ethanol is increased. In view of this, it may be feasible, that the ^{1}La state in which indole seems to have a large dipole moment (in contrast to the ^{1}Lb state), is strongly stabilized by the interaction of the solute with surrounding polar solvent molecules. This may become the lowest excited state during the radiationless process from the Frank-Condon to the equilibrium excited state. Therefore the difference of solvent shifts in fluorescence spectra are due to the dipole-moment produced in the excited state molecules[44]. In contrast, it has been reported[45] that the absorption spectra of indole in mixed solvents show a definite change. These changes are small at low polar solvent concentration but nevertheless are significant relative to the spectra in non-polar solvents, and indicate the possible presence of a ground state complex of indole in polar solvents.

Walker et al[16,38] have shown that these shifts, rather than being due to generalised solvent effects, have their origin in exciplex formation which is responsible for a red shift and loss of vibrational

structure in the fluorescence spectra of indole and its derivatives in polar solvents. However, they found that addition of only small amounts of ethanol or butanol to a cyclohexane solution of indole caused a large shift where a change in bulk dielectric properties was insignificant and because of this they postulated the formation of a complex between solute and solvent in the excited state. On the other hand it was reported[46] that the difference in solvent shifts of fluorescence spectra of molecules was due to the interaction energies between solute molecules and the solvent molecules surrounding them due to orientation polarization. Furthermore it was suggested[33] that the large red shift on the fluorescence of indoles depends on the possibility of solvent reorientation of the solvent molecules during the lifetime of the excited state rather than from the formation of stoicheiometric exciplex, explaining their results in terms of a solvent relaxation process.However, application of the formula for orientation - polarisation interaction with an excited dipole, developed by Mataga[42], yielded a poor correlation between the dipole moment and the red shift observed.

2.3 EXCIPLEXES

Exciplex is a term derived from excited complex[47] which means stable only in the excited state[48]. The fluorescence of indole and its derivatives in polar solvents shows a loss of vibrational structure relative to fluorescence spectra in pure solvent, and a pronounced red shift[35]. These characteristics are attributable to the formation of excited state complex between indole and one or two polar solvent molecules. Lumry et al[49] on varying the proportion of a cyclohexane-ethanol solution of indole observed a maximum shift at alcohol concentration, so low as to have a negligible effect on the solvent dielectric constant. As a result they suggested that a specific solvent solute excited state complex termed exciplex was responsible for the red shift emission. It was reported[50,51] that photochemical

reactions are now thought to proceed via weak exciplex or intermediate strength partial charge-transfer mechanisms. New compounds made by photochemical addition of electron rich olefins to naphthalene and anthracene, were postulated to have formed through the initial formation of exciplexes.

Weak exciplexes[52] are often the precursors of a strong exciplex. The weak exciplex is the result of dipole-dipole interactions between the excited molecule and one or more polar molecules, i.e., the exciplex formation is based on the basis of stoicheiometric complex formation between the exciplex, having a large dipole moment, and small dipolar molecules. This suggestion supports Chandross and Thomas[53] who first realised that all exciplexes were not charge transfer complexes, their conclusion being based on the studies of N,N-dimethyl-3-(1-naphthyl)propylamine, which forms an intramolecular charge transfer exciplex at room temperature. The emission from this compound is due entirely to the intramolecular exciplex in benzene. On the other hand, upon addition of a small amount of acetonitrile, they observed the appearance of a second emission band, shifted to the red from the original exciplex emission. These observations were ascribed to weak exciplexes defined as stoichiometric dipole-dipole stabilized complexes, which are formed between an excited species and one or more polar molecules[54], the dipole moment increasing upon excitation. However, in solvents of high dielectric constant the exciplex is not usually stable because the direct formation of radical ions becomes the most favoured reaction of the donor-acceptor pair. Birks[55] suggested that exciplex formation is caused by a charge-transfer interaction to which an excitation (dipole-dipole) interaction may contribute.

In recent work by Taylor[56] on the geometry of aromatic hydrocarbon-N,N-dimethylaniline exciplexes, it is shown that charge transfer and steric effect are the important stabilizing force in exciplexes. Further

studies on the intramolecular exciplex formation have been carried out by Chandross et al[57] where naphthalene and alkylamines were shown to readily form an exciplex when electronically excited. The exciplex did not seem to have strong geometrical preferences. In the anthracene-$(CH_2)_n$-dimethylaniline system. Okada and Co-workers[58] found that, for n=3, a charge transfer complex formed easily in all solvents. The excited complex could not form for n=1 or 2 in solvent of low polarity. They nevertheless, concluded that a parallel sandwich geometrical structure might be favourable, but not necessarily for the exciplex formation.

Walker et al[16,38] have explained the red shift and the loss of vibrational structure in polar and mixed solvents by assuming the presence of an excited state solute-solvent complex termed exciplex. A general kinetic scheme of exciplexes given by Walker is as follows:-

Excitation of uncomplexed solute	$A+h\nu \longrightarrow A^*$
Fluorescence of uncomplexed solute	$A^* \longrightarrow A+h\nu$
Internal quenching of uncomplexed solute	$A^* \longrightarrow A$
Exciplex Formation	$A^*+ns \longrightarrow Asn^*$
Exciplex dissociation	$Asn^* \longrightarrow A^*+ns$
Exciplex internal quenching	$Asn^* \longrightarrow A+ns$
Exciplex fluorescence	$Asn^* \longrightarrow A+ns+h\nu'$

Where A and S represent the solute and polar solvent molecules respectively, Ans is the ground state complex and Asn* is the excited state polar solvent complex. They showed that the 1:2 solute-polar solvent exciplex is formed with indole and 1-methylindole in an n-pentane and n-butanol mixture at room temperature and also with other associating solvents. No evidence for a 1:1 complex was found for these solvents, only a 1:1 exciplex was found with non-associating solvents. They suggested that the stability of the exciplex state is a result of configurational interaction of singlet states which are of both neutral and charge-transfer type; i.e. charge-transfer interaction between excited indole and solvent is explained due to dipole-dipole and polarization

interactions[53]. On the other hand, an excited state pyrene-aromatic amine complex was interpreted by Mataga and co-workers[59] to be a result of interaction of excitation and charge resonance states, though they stress the importance of the charge-transfer state in the stabilization of the complex. Furthermore, it was suggested[19] that exciplex formation does not depend on hydrogen bonding as shown by the fact that it occurs with 1-methyl substituted indole compounds and with non-hydrogen bonding solvents . Lockwood[60] proposed that the formation of the exciplex of N-methylindole with a hydroxylic solvent precludes the possibility of hydrogen bonding. However, in some environments, such as in the interior of protein molecules indole fluorescence may produce an emission spectrum without vibrational structure or red shift, due to the environmental stabilization of ^1La relative to the ^1Lb state through hydrogen bonding rather than exciplex formation. Longworth [61] has confirmed exciplex formation between 1,2-dimethylindole and isopropanol in 3-methylpentane at room temperature. The progressive red shift found to take place implied an equilibrium. The stoeichiometry of the reaction was found to be 1:1. He was unable to confirm the 1:2 exciplexes which were reported by Walker[16]. Moreover he explained that increasing the viscosity and cooling the system would inhibit exciplex formation. It was reported [45] that exciplex formation of indole in the methanol-cyclohexane mixture depends on the association rate during the lifetime of the excited state, furthermore Selinger et al[32] suggested that exciplex formation depends on the dielectric constant of the solvent, as well as to some extent its viscosity.

The study by McDonald and Selinger[62] of the excitation spectrum of the naphthalene-diethylaniline exciplex, provides evidence that exciplexes are formed by exciting either the donor or acceptor. However, at this stage they eliminated the possibility that energy

transfer within the collision complex immediately proceded electron transfer. On the other hand it was reported[58] in the fluorescence of the aromatic hydrocarbon amine exciplex that the separation between donor and acceptor is large. The stabilisation energy of a charge-transfer state may be smaller than in the case of ordinary exciplexes, however since the dipole moments in the charge-transfer states are much larger than those of the ordinary exciplexes, the solvation in a polar solvent may be very effective for the stabilization of the charge-transfer state.

Numerous experimental results and theoretical consideration have led to the conclusion that the fluorescent state of the exciplex may be identical with that of the corresponding electron donor-acceptor complex, while their Frank-Condon excited states should be different from each other. However, few cases of exciplexes and electron donor-acceptor fluorescence in the electron donor-acceptor systems have been reported[63]. Therefore, the excited state is indeed the only state of the exciplex which has been explained in a very similar manner to the Mulliken[64] theory of charge-transfer complexes.

2.4 CHARGE-TRANSFER COMPLEX

The term electron donor and electron acceptor are relative terms and there is no reason why any molecule should not be a charge donor and acceptor under the appropriate conditions. The term charge-transfer complex was first put forward by Mulliken[64], who developed the theory to explain the phenomenon. The donor-acceptor complex[65] is formed upon mixing a solution of molecules having a low ionization potential (electron donor) with a solution of high electron affinity (electron acceptor) in the ground state. Rose[66] proposed that two conditions have to be satisfied for charge-transfer absorption to occur, (i) the donor shall have a high energy filled orbital and the acceptor low energy

vacant orbital, (ii) the above orbitals shall overlap. This overlap may be sterically determined or derived either from chance collisions between components or from complex formation, i.e. before a charge interaction can take place, the molecules must be in sufficient proximity, so that the difference in electropotential can be recognised. With large molecules, steric hindrance may well prevent such close approach. The energy of a complex[67] is found to be correlated with the electron affinity of the acceptor and with the ionization potential of the donor, indicating that the interaction involves transfer from D to A. Itoh et al[68] proposed that the electron donor-acceptor complex is the result of the electronic interaction and geometrical arrangement between the excited electron acceptor and the donor in the ground state. The primary process of the exciplex formation seems to be different from those of both ground state donor and acceptor in the electron donor-acceptor complex formation. Charge-transfer complexes have well defined simple stoicheiometries. There are various methods of determining the stoicheiometries[69]. One of the best methods is that of plotting some property of complex formation against the concentration of the partners. The structure of the complex will be determined by the intermolecular forces. The structure giving the minimum potential energy, where all intermolecular forces are considered being the most probable. Slifkin[69] classified the complexes into three broad classes according to the type of donor. The first class where the donor electron is a π electron includes conjugated systems and polycyclic aromatic hydrocarbons as typical members of this group. The second class of donor is that in which the donated electron is an n-electron or lone pair electron. This is most frequently located on the nitrogen in the amino group, and the third class is that in which charge-transfer can take place between localized regions of positive charge and negative charge in adjacent molecules. The charge-transfer band can be affected by

solvents. Weak charge complexes which have little dative character in the ground state, show slight wavelength shifts of the charge-transfer band maximum, which correlate approximately with the polarity dielectric constant[70]. Moreover Foster and Thomson[71] suggested that the stronger charge-transfer complex, with predominantly dative character in the ground state, can dissociate into the component ions in solvents of high dielectric constant, so that increasing the polarity of the solvent causes the charge-transfer band to be replaced by the spectra of the ions. Weller[72] suggested that two effects of increasing dielectric constant on the charge-transfer emission are noticed; a red shift and a considerable decrease in the intensity. Furthermore it was reported[73] that an increase of the solvent polarity leads to a significant displacement of the maximum of the complex fluorescence to a longer wavelength, the displacement of the fluorescent maximum being dependent on the dielectric constant of the solvent. The solvent effect dependent red shift can easily be understood on account of the dipolar nature of the excited complex whose energy should decrease as the solvating property of the solvent increases. The strong decrease in intensity of the complex emission has been discussed[74] in connection with the results of the lifetime measurements on the same complex in different solvents. Beens and co-workers[75] have shown that the emission spectra of the complex are solvent dependent, insofar as their maxima are red shifted with increasing solvent polarity. They chose anthracene-diethylaniline as the system to estimate the dipole moment effect, and found that increasing the dipole moment causes a red shift of the emission maximum.

Indole forms intermolecular charge-transfer complex with various electron acceptor molecules. The formation of a 1:1 intermolecular charge-transfer complex between indole and tetracyanoethylene in dichloromethane at room temperature has been reported[76]. There are

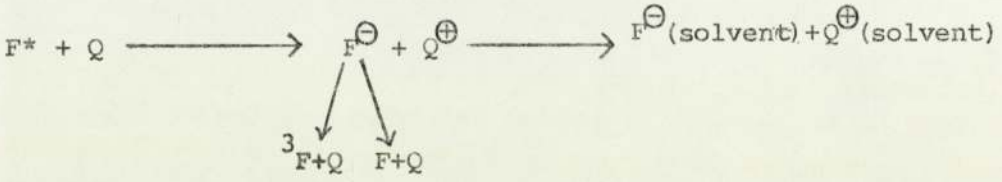
many organic electron acceptors which form charge-transfer complexes with indole; such as anthraquinone, naphthoquinone, maleic anhydride and trinitrobenzene[77].

Several authors have reported on the charge-transfer complexes of indole with other electron-acceptors[78]. However, it was suggested[69] that the apparent strong charge donation ability of the indoles arises from the presence of strongly negatively charged carbon atoms, rather than arising from the donation of a π-electron from the conjugated electron system in the indole ring. Green and Malrieu[80] have calculated the super-delocalizability, a measure of reactivity, at C3 in the indole nucleus and found that this correlates better with the maximum position of the charge transfer band than it does with energy of the highest occupied molecular orbital of the indole. Furthermore, Foster et al[81] who studied the complex of the indole and its derivatives with organic acceptors, (trinitrobenzene, and dinitrobenzene) by NMR found that the strength of interaction correlates to some extent with the electron donating ability of indole. Methylation especially at the 2 or 3 position increases the association constant, while methylation in benzene ring at 7-position does not produce such a marked increase. It may be that substituents on the benzene ring decrease the association constant through hindrance. Thus suggesting the importance of a specific steric configuration for the complex.

2.5 FLUORESCENCE QUENCHING

True quenching is not related to absorption or light scattering, but is due to an interaction of the fluorescent molecule with solvent or with other solute in such a manner as to lower the efficiency and/or lifetime of the fluorescence process[3]. This means that the compound will exhibit less fluorescence when it is in the presence of the quenching substance.

Hopkin and Lumry[22] have shown that there are at least two pathways of decay. One appears to be outright electron ejection and the other is collisional scavenging of electrons. Some quenching reactions, however, are the result of a specific interaction between the fluorescent species and the quencher. Hercules[2] has explained the quenching effect by a charge-transfer mechanism as shown in the following:



The excited state fluorescent molecule F* reacts with the quencher (Q) abstracting an electron from it to form the ion pair $F^{\ominus}$, $Q^{\oplus}$. This ion pair can dissociate to give either a triplet state ^3F and quencher Q, or simply a ground state and quencher. In the presence of a polar solvent, both $F^{\ominus}$ and $Q^{\oplus}$ can be solvated and can then carry out characteristic radical ion reactions in solution. However, it was reported[83] that the charge-transfer interaction appeared to be the predominant quenching mechanism for the singlet states of aromatic compounds with amines. For example, this was found for the case of naphthalene and bicyclic azo compounds as a model for the theory. Moreover Goldschmidt et al[84] postulated that aromatic molecules quenched via charge-transfer interaction and they explained this by a detailed mechanism leading to the triplet state of excited aromatic molecules. However, Labianca and co-workers[85] suggested that the charge-transfer interaction and quenching activity could be divided in two types:-

(i) either within a group of quenchees and quencher there may be large variations in binding energies of the exciplexes and relatively small variations in the rate constant for internal conversion, or

(ii) the ion pair may lie lower in energy than the fluorescent molecule, so that complete transfer of an electron is an irreversible decay process.

There is convincing evidence that charge and electron transfer processes are important in the formation and decay of exciplex species. Ander et al[86] reported that rate of quenching depends on the donor-acceptor properties of the quenchee and quencher.

In the study of the quenching of the fluorescence of naphthalene and other aromatic hydrocarbons by conjugated dienes it was suggested[87] that exciplexes were intermediates in the quenching process. It was proposed[74] that by increasing the solvent polarity the exciplex fluorescence intensity and its lifetime decreases. It was explained that the fluorescence quenching in polar solvents arose by electron transfer between excited and quencher molecules which formed a sandwich type charge-transfer complex. Recently [88] it was pointed out that quenching of one exciplex can lead to the formation of another exciplex. It has also been reported[72] that the effect of amines as fluorescence quenchers can be ascribed to the formation of charged products with excited fluorescers. However, the principle path[84] of intersystem crossing in the excited aromatic hydrocarbon-amine complex in both polar and non-polar solvents, is a fast process competing with the vibrational and solvent relaxation, following the electron transfer in the encounter complex. It has been reported[89] that, for aliphatic amines quenching ketone fluorescence, the quenching rate constant decreases on going from tertiary to primary amines.

The fluorescence of many electron rich aromatics is quenched efficiently by methylchloro-acetate and chloroacetamide. This is explained[90] by the charge-transfer from the aromatic to the quencher to form exciplex binding. However, the fluorescence properties of many indole derivatives[91] have been found to be dependent upon the nature of their structure as well as upon the temperature and solvent composition. Steiner and Kirby[92] reported on the quenching of the fluorescence of indole derivatives and concluded that the quenching could take place

through (i) transfer of an electron by collisional contact of the quencher with an excited indole.

(ii) preliminary ejection of an electron from an excited indole to vicinal solvent molecules.

(iii) the formation of a transient complex of the charge-transfer type between excited indole and the quencher.

They have also proposed[92] that molecules which are known to be efficient electron scavengers might lead to deactivation of the excited state of indole derivatives by an electron transfer from the ring system to the quencher molecule. Water and amines[38] quench indole fluorescence due to electron ejection which is the major quenching process in water and the indole exciplex. It is also reported that iodide[93], hydrogen and hydroxide ions[94], appear to quench indole fluorescence, but the mechanism, in most cases has not been proven. Recci and Kilichowska[95] have reported that lanthanide quenches indole fluorescence by a ground-state complex formation, in which the quenching occurs by virtue of the formation of a non-fluorescent complex between indole and lanthanide. Most charge-transfer complexes[76] appear to be non-fluorescent. Thus the addition of a donor to a fluorescent acceptor in solution or vice-versa cause a quenching of the fluorescence which is proportional to the association constant (Kc) of the complex, and can thus be utilized for measuring it. A Stern-volmer type of equation may be used to obtain the constant Kc.

$$\frac{Fo}{F} = 1+Kc[D]$$

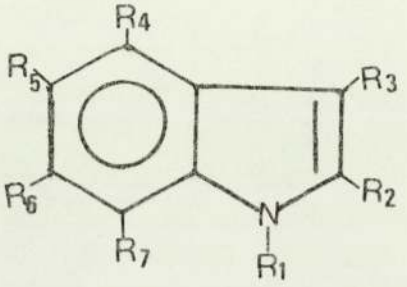
Where Fo and F are the fluorescence intensities in the absence and presence of quencher respectively and $[D]$ is the concentration of the quencher plot of relative fluorescence yield vs. quencher concentration gives the association constant.

2.6 Scope of investigation

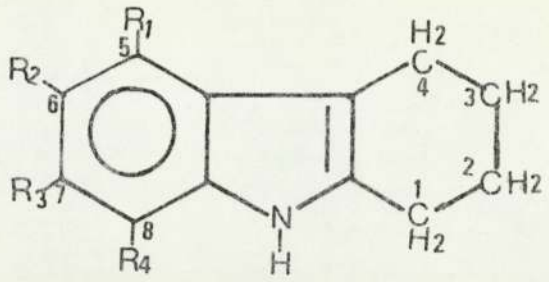
This was to examine the structural and electronic requirements for indole exciplex formation. The polar solvents used were, methanol, n-butanol, cyclohexanol, tert-butanol, borneol, acetonitrile, pyrrolidine, triethylamine, cyclohexylamine, quinuclidine [1-azobicyclo(2,2,2)octane] ABCO, triethylenediamine [1,4-diazobicyclo(2,2,2)-octane] DABCO, 3-ethyl-3-pentanol, tetrahydrofuran, and acetic acid. For comparison, fluorescence spectra of several indole derivatives with substituents on the pyrrole ring and on the benzene ring have been investigated.

The indoles studied were

(1)



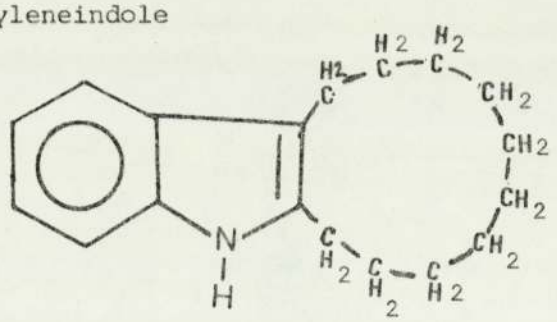
	1	R_1	R_2	R_3	R_4	R_5	R_6	R_7
i)	Indole	H	H	H	H	H	H	H
ii)	1,2-Dimethylindole	CH_3	CH_3	H	H	H	H	H
iii)	1,3-Dimethylindole	CH_3	H	CH_3	H	H	H	H
iv)	1,2,3-Trimethylindole	CH_3	CH_3	CH_3	H	H	H	H
v)	3-Methylindole	H	H	CH_3	H	H	H	H
vi)	2-Methylindole	H	CH_3	H	H	H	H	H
vii)	2-Tertbutylindole	H	$-C(CH_3)_3$	H	H	H	H	H
viii)	2,3-Dimethylindole	H	CH_3	CH_3	H	H	H	H
ix)	5-Methylindole	H	H	H	H	CH_3	H	H
x)	5-Fluoroindole	H	H	H	H	F	H	H
xi)	5-Methoxyindole	H	H	H	H	OCH_3	H	H
xii)	7-Methylindole	H	H	H	H	H	H	CH_3
xiii)	Tryptophol	H	H	CH_2CH_2OH	H	H	H	H
xiv)	2-Butyl-3-propylindole	H	$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_3$	H	H	H	H



(2)

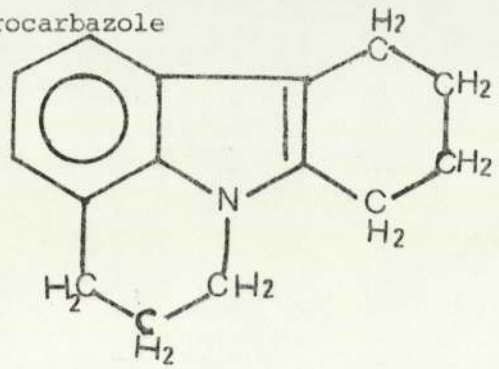
		R_1	R_2	R_3	R_4
xv)	6-Methyl-1,2,3,4-tetrahydrocarbazole	H	CH_3	H	H
xvi)	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	H	CH_3	CH_3	H
xvii)	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	H	CH_3	H	CH_3
xviii)	6-n-butyl-1,2,3,4-tetrahydrocarbazole	H	$CH_2CH_2CH_2CH_3$	H	H
xix)	6-Methoxy-1,2,3,4-tetrahydrocarbazole	H	OCH_3	H	H

xx) 2,3-Cyclodecamethyleneindole



(3)

xxi) 8,9-Cyclotrimethylene-
1,2,3,4-tetrahydrocarbazole



(4)

2.7 RESULTS

The shifts in emission maximum (nm) caused by polar solvents with different indole derivatives are summarized in tables 1-14. Some of the recorded emission spectra are included in the appendix.

Consideration of the indole derivatives show that the magnitude of a red shift produced by n-butanol is of the order; tryptophol; 2,3-dimethylindole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 3-methylindole; 2,3-cyclodecamethyleneindole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 2-butyl-3-propylindole; 2-methylindole; 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; 2-tert-butylindole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 1,2,3-trimethylindole;indole; 6-methoxy-1,2,3,4-tetrahydrocarbazole; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole; 1,2-dimethylindole; 1,3-dimethylindole. It seems that there is an obvious relationship between a red shift and increasing the concentrations of n-butanol by 0.04-0.2M, i.e. the higher concentration such as 0.2M causes a greater red shift than 0.16 M. n-Butanol does not produce any red shift in 5-methylindole, 5-methoxyindole; 5-fluoroindole and 7-methylindole, (table 1.) Methanol showed similar behaviour, producing a red shift as produced by n-butanol (table 2.)

On the other hand cyclohexanol appears to have less effect than methanol, (table 3). Borneol (table 4) is shown to be more effective in producing a red shift than tert-butanol (table 5) and 3-ethyl-3-pentanol (table 6), but the three solvents still appeared to have a lesser effect in producing a red shift than cyclohexanol. The red shift produced in the indole derivatives tested by acetonitrile is in order of; tryptophol; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 2,3-dimethylindole; 2-butyl-3-propylindole; 2,3-cyclodecamethyleneindole; i,2,3-trimethylindole; 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; 3-methylindole; 1,2-dimethylindole; 2-methylindole; 1,3-dimethylindole; 2-tert-butylindol; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 6-methoxy-1,2,3,4-tetrahydro-

carbazole. Indole does not show a clear red shift nor is there a red shift produced in 5-methylindole; 5-fluoroindole; 5-methoxyindole and 7-methylindole, however higher concentrations of 0.16 M acetonitrile produce a shift more to the red than lower concentrations (table 7). There is in general a small quenching effect shown by the alcohols. The extent of quenching varies with the structure of the alcohol as shown for 2-butyl-3-propylindole in n-butanol and methanol, spectra 1 and 2. The quenching also varies with the indole structure as seen for 2-methylindole; 1,2,3-trimethylindole and 6-methoxy-1,2,3,4-tetrahydrocarbazole in butanol spectra 3, 4 and 5.

Tetrahydrofuran, produces smaller red shifts than the alcohols (table 8), and it was found that tetrahydrofuran is not an effective fluorescence quencher for the most of the indoles, as shown for tryptophol and 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole, spectra 6 and 7.

Pyrrolidine produces less red shift than n-butanol and acetonitrile in the compounds shown previously (table 9), but it appears to be a most effective quencher for all compounds tested, as shown for example, 6-methyl-1,2,3,4-tetrahydrocarbazole; n-butyl-1,2,3,4-tetrahydrocarbazole; 2-methylindole and 5-methylindole, spectra 8-11. The quenching effect is proportional to the concentrations of the amine and even the lowest concentration of pyrrolidine, such as 0.04 M, is effective as a quencher. Although cyclohexylamine produces similar shifts to pyrrolidine (table 10), its behaviour as a quencher is weaker than pyrrolidine see spectra 8 and 11-13. Triethylamine seems to be less able to produce a red shift than the above amines. A red shift produced in the following compounds is in the following order, 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; tryptophol; 6-methyl-1,2,3,4-tetrahydrocarbazole; 3-methylindole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 2,3-cyclodecomethyleneindole; 2,3-dimethylindole; 2-methylindole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 6-methoxy-1,2,3,4-tetrahydrocarbazole (table 11). Triethylamine also appears to be less of a quencher than cyclohexylamine, although a low concentration of

triethylamine such as 0.04 M produces a small reduction in the fluorescence intensity as seen from spectra 14 and 15.

Quinuclidine produced greater shifts than triethylamine with indole, 5-methylindole and 2,3-dimethylindole. Like triethylamine it had no effect on the emission of 1,2,3-trimethylindole. Triethylenediamine, showed an almost identical effect to quinuclidine (tables 12 and 13) and spectra 16 and 17.

Acetic acid was found in general to produce no red shift, with the exceptions of 6,7-dimethyl- and 6-methyl-1,2,3,4-tetrahydrocarbazole. It did however act as an efficient quencher. (table 14 and spectra 18-22). Shifts (nm) and fluorescence emission maximum (nm) listed in tables 1-14, are correct to within $\pm$ 2 nm.

Table 1

Red shift (nm) caused by n-butanol

n-butanol M	Indole	2,3-cyclodecamethyleneindole	2-Butyl-3-propylindole	2,3-Dimethylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydro-carbazole	6-Methyl-1,2,3,4-tetrahydro-carbazole	6-n-butyl-1,2,3,4-tetrahydro-carbazole	6-Methoxy-1,2,3,4-tetrahydro-carbazole	6,8-Dimethyl-1,2,3,4-tetrahydro-carbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	1,2-Dimethylindole	1,3-Dimethylindole	3-Methylindole
maximum emission (nm) at OM	303	317	321	319	305	307	311	305	312	327	312	321	316	317	328	316	325	329	313	322	311
0.04	1	8	10	10	4	3	11	0	0	0	0	3	5	3	2	2	2	2	1	1	9
0.08	2	15	13	15	10	7	23	0	0	0	0	7	13	13	3	4	3	4	1	1	15
0.12	4	18	18	19	14	10	27	0	0	0	0	11	18	16	5	7	5	7	1	4	19
0.16	6	20	19	22	17	14	30	0	0	0	0	13	22	19	5	11	6	11	4	2	21
0.20	8	21	20	24	20	16	31	0	0	0	0	19	24	21	6	14	6	13	6	3	22

Table 2

Red shift (nm) caused by methanol

Methanol M	Indole	2,3-Cyclodeca-methyleneindole	2,3-Dimethylindole	2-Butyl-3-propylindole	7-Methylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm)	303	317	318	321	305	314	322
at OM							
0.04	1	6	10	3	0	0	2
0.08	3	12	15	5	0	1	2
0.12	5	17	21	7	0	2	2
0.16	7	19	23	9	0	5	2
0.20	8	24	25	11	0	6	6

Table 3

Red shift (nm) caused by cyclohexanol

Cyclohexanol M	Indole	2,3-Cyclodeca-methyleneindole	2,3-Dimethylindole	7-Methylindole	2-Butyl-3-propylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm)	303	317	319	305	321	313	322
at OM							
0.04	1	7	9	0	8	0	2
0.08	4	13	13	0	14	0	2
0.12	5	15	19	0	16	2	2
0.16	6	17	21	0	18	2	2
0.20	7	19	22	0	19	4	3

Table 4

Red shift (nm) caused by borneol

Borneol M	Indole	2,3-Cyclodecamethyleneindole	7-Methylindole	1,2-Dimethylindole	1,3-Dimethylindole	1,2,3-Trimethylindole
Maximum emission (nm) at OM	302	317	305	314	323	329
0.04	2	7	0	1	1	1
0.08	4	14	0	2	1	4
0.12	5	15	0	2	2	5
0.16	5	17	0	3	2	7
0.20	6	18	0	3	3	9

Table 5

Red shift (nm) caused by tert-butanol

Tert-butanol M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Butyl-3-propylindole	7-Methylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm)	302	317	319	321	305	313	322
at OM							
0.04	2	7	9	8	0	1	0
0.08	3	12	13	11	0	2	1
0.12	5	13	16	14	0	2	2
0.16	5	16	18	16	0	3	2
0.20	7	18	21	17	0	3	3

Table 6

Red shift (nm) caused by 3-ethyl-3-pentanol

3-ethyl-3-pentanol M	Indole	2,3-Cyclodecamethyleneindole	2-Butyl-3-propylindole	7-Methylindole	1,2,3-Trimethylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm)	303	317	321	303	329	313	322
at OM							
0.04	1	7	6	0	2	1	1
0.08	2	11	10	0	3	2	1
0.12	3	15	14	0	4	3	2
0.16	3	17	15	0	4	3	2
0.20	4	18	-	0	6	3	3

Table 7

Red shift (nm) caused by acetonitrile

Acetonitrile M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Butyl-3-propylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene -1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	1,2-Dimethylindole	1,3-Dimethylindole	3-Methylindole
Maximum emission (nm) at OM	302	317	319	321	305	307	311	305	313	327	312	320	316	317	327	316	327	329	312	323	311
0.04	1	8	9	8	2	2	8	0	0	0	0	3	6	5	2	1	2	5	4	2	5
0.08	2	10	12	10	5	3	13	0	0	0	0	7	10	11	3	2	6	10	8	4	10
0.12	3	13	14	12	7	7	16	0	0	0	0	11	14	14	3	4	7	13	12	7	11
0.16	3	15	17	15	9	7	19	0	0	0	0	15	17	18	4	5	7	15	14	8	14

Table 8

Red shift (nm) caused by tetrahydrofuran

Tetrahydrofuran M	Indole	2,3-Cyclodecamethyleneindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
Maximum emission (nm) at OM	302	317	306	311	305	312	327	312	321	317	316	327	316	325	329	311
0.04	0	6	2	6	0	0	0	0	3	5	5	2	0	0	0	6
0.08	0	8	2	9	0	0	0	0	5	7	9	4	0	0	0	7
0.12	0	9	4	11	0	0	0	0	7	8	10	4	0	0	0	9
0.16	0	10	4	11	0	0	0	0	8	10	12	4	0	0	0	9
0.20	0	11	4	12	0	0	0	0	9	11	12	4	0	0	0	9

Table 9

Red shift (nm) caused by Pyrrolidine

Pyrrolidine M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-Butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
Maximum emission (nm) at OM	303	318	319	305	307	311	304	312	327	313	320	317	316	329	317	325	329	311
0.04	3	5	5	4	3	7	3	2	1	3	2	5	4	0	1	0	0	5
0.08	3	8	8	7	5	13	3	2	1	4	4	10	12	2	2	0	0	9
0.12	4	10	11	7	7	14	4	3	2	5	7	13	14	2	5	0	0	11
0.16	5	12	13	9	8	19	4	3	2	5	9	15	14	6	6	0	0	14
0.20	5	12	15	11	9	19	4	3	2	5	9	15	14	6	7	0	0	14

Table 10

Red shift (nm) caused by Cyclohexylamine

Cyclohexylamine M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
Maximum emission (nm) at OM	303	317	318	306	311	303	312	327	312	320	317	316	327	317	325	329	312
0.04	3	9	9	3	10	2	2	0	3	4	8	7	3	1	0	0	10
0.08	4	12	12	5	16	3	2	0	3	7	10	11	4	2	0	0	11
0.12	5	13	14	7	17	3	2	0	4	9	14	13	4	3	0	0	12
0.16	5	14	14	8	19	6	4	0	4	12	14	13	4	5	0	0	14
0.20	6	14	14	9	19	6	4	0	5	13	15	15	4	6	0	0	14

Table 11

Red shift (nm) caused by triethylamine

Triethylamine M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyll,2,3,4-tetrahydrocarbazole	6-n-Butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole
Maximum emission (nm)	303	317	319	305	306	311	305	312	327	312	320	317	316	327	325	329	311	316
at OM																		
0.04	3	2	5	1	0	7	0	0	0	2	3	5	4	2	0	0	11	1
0.08	3	6	7	2	0	12	0	0	0	2	7	8	6	2	0	0	12	2
0.12	3	7	8	4	0	14	0	0	0	3	11	11	12	3	0	0	12	2
0.16	3	9	9	6	0	14	0	0	0	3	13	13	12	3	0	0	13	2
0.20	3	10	10	7	0	14	0	0	0	3	16	13	12	3	0	0	13	2

Table 12

Red shift (nm) caused by quinuclidine (ABCO)

quinuclidine M	Indole	2,3-Dimethyl-indole	1,2,3-Trimethyl-indole	5-Methyl-indole
Maximum emission (nm) at OM	304	320	329	312
0.04	5	10	0	2
0.08	6	13	0	2
0.12	6	14	0	3
0.16	6	14	0	3
0.20	6	14	0	3

Table 13

Red shift (nm) caused by Triethylenediamine (DABCO)

Triethylene-diamine M	Indole	2,3-Dimethyl-indole	1,2,3-Trimethyl-indole	5-Methyl-indole
Maximum emission (nm) at OM	304	318	329	312
0.04	3	10	0	2
0.08	4	12	0	2
0.12	5	14	0	3
0.16	5	14	0	3
0.20	5	14	0	3

Table 14

Red shift (nm) caused by Acetic Acid

Acetic acid	Maximum emission at OM	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	8,9-Cyclomethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
		303	317	319	311	305	312	327	312	320	318	317	327	325	329	311
0.04		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
0.08		0	0	0	0	0	0	0	0	2	2	0	0	0	0	0
0.12		0	0	0	0	0	0	0	0	2	2	0	0	0	0	0
0.16		0	0	0	0	0	0	0	0	2	2	0	0	0	0	0
0.20		0	0	0	0	0	0	0	0	2	2	0	0	0	0	0

2.8 General Discussion

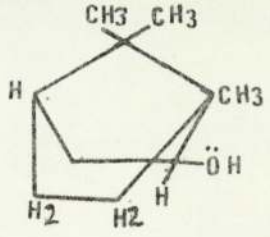
From the previous work on exciplexes, alcohols[16] have been used in the studies of the exciplexes of indole and indole derivatives. In this work methanol, n-butanol and sterically hindered alcohols such as, cyclohexanol, tert-butanol, borneol and 3-ethyl-3-pentanol were used to study structural and electronic requirements for indole exciplex formation. Alcohols may donate a proton as well as lone pair electrons. In contrast acetonitrile, of high dielectric constant[79] should be able to donate the lone pair electrons particularly readily due to the absence of steric hindrance effect. The red shift produced by hydroxylated solvents may arise by several mechanisms. One could be the result of proton transfer. Therefore, acetic acid an efficient proton donor was used to check this idea. Or the red shift may be due to interaction with oxygen in the excited state without transfer of proton. Tetrahydrofuran was used to examine this effect. The amines were chosen as stronger electron donors to show their effects on the exciplex formation. Cyclohexylamine (primary amine), Pyrrolidine (secondary amine), and triethyl amine (tertiary amine) were selected to distinguish between those amines which can act as proton donors and those which can only act as electron donors. Steric effects were examined using quinuclidine and triethylenediamine.

A consideration of the electronic and steric structure of some solvents tested is necessary to partly explain their effectiveness or otherwise, in interacting with the indole derivatives.

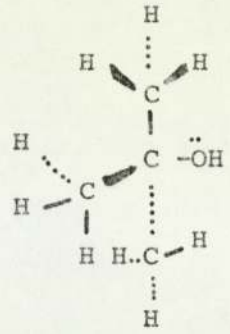
$CH_3-CH_2-CH_2-CH_2-\ddot{O}H$

n-butanol

XXII

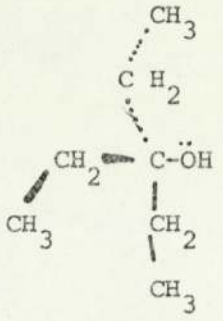


XXIII borneol



tert-butanol

(XXIV)



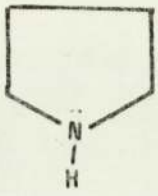
3-ethyl-3-pentanol

(XXV)

$CH_3-C\equiv N:$

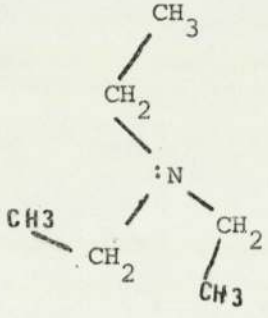
acetonitrile

(XXVI)



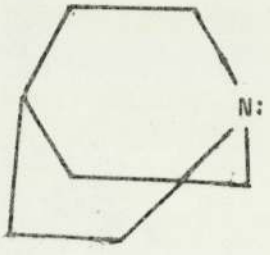
pyrrolidine

(XXVII)



triethylamine

(XXVIII)



quinuclidine

(XXIX)

It can be expected that in the alcohol series structure such as (XXIII) and (XXV) will have greater hindrance for access to their lone pairs than tert-butanol (XXIV) and n-butanol (XXII). Similarly in the amine group, it can be expected that triethylamine (XXVIII)will have hindrance for access to its lone pair in comparison with say quinuclidine (XXIX), cyclohexylamine or pyrrolidine (XXVII).

Triethylamine is known not to form a complex with BF_3 which contrasts with other amines such as quinuclidine and triethylenediamine. This has been attributed due to the effect of the spatial requirement of the "floppy" ethyl groups [82].

On the other hand acetonitrile (XXVI) should be free of steric hindrance effects about its lone pair and may be expected to show exciplex formation which is less dependent upon the indole structure.

The indole derivatives used in this work have been mostly alkylated derivatives. The methyl substituted indoles were chosen to examine electronic effects of the position of substitution on exciplex formation. The indoles, such as 2,3-cyclodecamethyleneindole, 2-tert-butylindole and 2-butyl-3-propylindole, were used to determine steric requirements of exciplex formation. Finally indoles such as 5-methoxy, 5-fluoroindole and tryptophol were studied to examine the effect of hetero atoms and their inductive effects in exciplex formation. The results of this work showed that the substitution in the pyrrole ring was effective in producing a red shift while substitution in benzene ring did not produce any red shift. Therefore it was decided to use derivatives containing substituents in both the pyrrole and benzene rings to evaluate their effects on exciplex formation.

2.8.1. Effect of nature and position of indole substituents upon exciplex formation

Examination of the results obtained from the different indoles with any one polar solvent show the following effects. Most of the 2- or 3-alkyl or 2,3-dialkyl indoles show 10-24 nm red shifts with all the alcohols,

acetonitrile,pyrrolidine, cyclohexylamine, triethylamine,quinuclidine, triethylenediamine and tetrahydrofuran. In general the red shift is greater for these indoles than for the unsubstituted indole itself. The notable exception being 2-tert-butylindole which in general shows much smaller to smaller red shifts with the polar solvents.

This is particularly noticeable in its interaction with triethylamine where it does not appear to form an exciplex (spectra 23). Likewise its interactions with tetrahydrofuran and acetonitrile show reduced red shifts in comparison to the other 2- and, or 3- alkylated indoles (spectra 24 and 25). Its reduced interaction with acetonitrile (table 7) is to be noted in that the latter appears to be less sensitive to indole structure in its ability to form exciplexes.

It appears therefore that the presence of 2- or 3- or both 2- and 3- alkyl substituents enhances exciplex formation. However the reduced red shifts in the emission of 2-tert-butylindole and of 2-methylindole strongly suggest that a steric requirement exists for exciplex formation. Thus the 2,3-positions of the indole play a major role in the exciplex formation. This is further strengthened by observation that in general, 2,3-dimethylindole shows greater red shifts in comparision with 2,3-cyclodecamethyleneindole with the alcohols.

It is also possible that these results are compatible with the operation of a hyperconjugative effect in the excited state indole exciplex structure. In the case of 2-tert-butylindole no hyperconjugation is possible. If this were the only effect it may be expected that it would exciplex to a similar extent as indole itself. This is not the case as can be seen from its interactions with n-butanol and tetrahydrofuran, which demonstrate that the bulky tert-butyl groups does have an enhancing effect on exciplex production.

In marked contrast can be seen the effect of N-alkylation of the indole system upon exciplex formatation. Comparision of 2-methyl, 3-methyl and 2,3-dimethylindole interactions, with the alcohols, the amines and tetrahydrofuran with the interactions of the same N-alkylated derivatives and indole itself shows in many instances a change from a moderate to strong exciplex for the former indoles to very weak or no exciplex formation for the latter compounds. It could be concluded from these observations that this result is due to an electronic effect and/or the absence of hydrogen bonding due to the lack of an N-H group in the N-methyl derivatives. However it is seen that 1,2,3-trimethylindole shows stronger exciplex formation with n-butanol, borneol, 3-ethyl-3-pentanol and acetonitrile than indole itself. It is therefore unlikely that hydrogen bonding due to the presence of an indolic NH group plays any part in exciplex association. This has been previously reported[60]. That the result is not entirely due to an electronic effect may be concluded from the fact that acetonitrile forms exciplex with the 2,3-alkylindoles almost to the same extent as it does with N-alkylated compounds. Likewise all the amines and tetrahydrofuran appear not to give exciplexes with the N-alkylindoles whereas they do with the corresponding NH indoles.

It would appear that a steric effect is again of major importance in the production of the exciplex. This is demonstrated by the interaction of 3-ethyl-3-pentanol with 2,3-cyclodecamethyleneindole and 1,2,3-trimethylindole (table 6 and spectra 26, 27). The latter compound shows a red shift of 4-6 nm in comparison to the 17-18 nm obtained for the former. Only the structurally more compact acetonitrile is able to form exciplexes with the N-alkyl indoles almost as readily as with N-unsubstituted derivatives.

Substitution of the 5- or 7- positions of the indole by methyl groups is found to prevent exciplex formation with all alcohols, acetonitrile, tetrahydrofuran and triethylamine. Further, consideration suggests that

of the two possible factors which produce this the determining one in these instances is an electronic effect. This is concluded from the fact that whereas 5-methylindole shows no exciplex formation with the alcohols, acetonitrile, triethylamine and tetrahydrofuran the same compound when alkylated in the 2,3-positions forms relatively strong exciplexes with these solvents. This is clearly demonstrated by the emission data of 6-methyl-1,2,3,4-tetrahydrocarbazole (tables 1, 7, 8 and 11 and spectra 28-31 and 32-35 respectively). That a steric effect is also present is seen from the behaviour of 6-n-butyl-1,2,3,4-tetrahydrocarbazole with the same solvents. This compound shows similar red shifts, though of slightly reduced magnitude, than the corresponding 6-methyl derivative. Hence the blocking effect of alkyl groups in the benzene ring is completely overcome by the presence of 2,3-dialkyl substitution. It is difficult to see how any effect other than an electronic inductive type can explain these observations. To further ascertain this hypothesis the interaction of 5-methoxy and 5-fluoroindole with polar solvents was studied. With the exception of pyrrolidine and cyclohexylamine all the other polar solvents did not form exciplexes with these two indoles. However when 5-methoxyindole was alkyl substituted in the 2,3-positions, as in 6-methoxy-1,2,3,4-tetrahydrocarbazole then exciplex formation took place. The shifts observed were however only small to moderate with the largest occurring with n-butanol which showed a 6 nm red shift. These red shifts observed for 6-methoxy-1,2,3,4-tetrahydrocarbazole were considerably smaller than those for the 6-methyl-1,2,3,4-tetrahydrocarbazole. This result would appear to be in keeping with the presence of only an electron pushing or donating effect due to the 6-methyl groups and that of the presence of an electron pushing mesomeric effect and an opposing electron withdrawing effect due to the methoxy group.

It is also found in general that dialkyl substitution in the 6,7- and 6,8-positions of tetrahydrocarbazoles shows smaller red shifts than found for 6-alkyltetrahydrocarbazole with polar solvents. This is demonstrated by 6,7-, 6,8-dimethyl and 6-methyl- and 6-n-butyl-1,2,3,4-tetrahydrocarbazoles respectively. The former pair of compounds show 0-19 nm shifts while the latter molecules show 11-24 nm shifts (tables 1,7, 8 and 9-11 and spectra 7-9, 32-38). This may be due to the greater electron pushing effect of the dialkyl groups which reduces the interaction leading to the exciplex formation.

The results are consistent with the production of the largest shifts with tryptophol with alcohols, acetonitriles, tetrahydrofuran and amines (tables 1,7-11). This may be due to presence of the hydroxyl group which may facilitate the exciplex formation between the polar solvents and the indole system of tryptophol.

2.8.2. Effect of nature and structure of solvent upon exciplex formation

n-Butanol appears to be very effective in exciplex formation, however it produces greater red shifts in the 2-,3- alkyl -and 2,3-dialkylindoles as compared with other indoles (table 1). It does not produce red shifts with 5- and 7- substituted indoles. Methanol showed similar behaviour to n-butanol (table 2), but consideration of the result of 2-butyl-3-propylindole with methanol shows a 11 nm shifts as compared with 20 nm with n-butanol; this is thought to be due to the reduced solubility of methanol in cyclohexane in the presence of this indole. The red shifts produced by methyl and n-butyl alcohol are seen to be generally slightly greater than those obtained with cyclohexanol and the alcohols XXIII-XXV, (tables 1-6 and spectra 39-42 and 26).

Comparison of the shifts produced by methanol and cyclohexanol and the alcohols XXIII-XXV show a consistently smaller shift due to the latter compounds. These alcohols are more bulky than methanol and approach to their oxygen atoms is likely to be subject to some steric hindrance due to the presence of ring structures of "floppy" alkyl groups. This

effect is again seen in the emission spectra of 2,3-cyclodecamethyleneindole which shows smaller shifts with cyclohexanol and tert-butanol than does 2,3-dimethylindole (table 3-6 and spectra 40, 42-44). This may be due to some steric hindrance from the bulky 2,3-cyclodecamethylene ring system which further reduces such interaction. This strongly suggests that a steric requirement exists for exciplex formation. Tetrahydrofuran in general shows smaller shifts than alcohols (table 8). This could be due to its smaller dielectric constant[79], which reduces the magnitude of such interaction. Acetonitrile (XXVI) which is a compact polar molecule, might be expected to show less dependence upon indole structure for exciplex formation and the results obtained indicate that this is so (table 7). The only exceptions are 2-tert-butylindole and N-alkylated compounds because interaction with the former showed reduced red shifts in comparison with other 2-3-alkylindoles and the interaction with the latter compounds produced greater shifts than produced by alcohols.

The red shifts produced by pyrrolidine (XXVII) appear to be smaller in 2- or 3- alkyl, 2,3-dialkyl, N-alkylindoles and in indole itself in comparison with those produced by alcohols and acetonitrile. But pyrrolidine appears more effective in producing shifts in 5- or 7-alkylindoles than the alcohols. The shifts showed by 5-methyl, 5-methoxy, 5-fluoro and 7-methylindole clearly demonstrate this effect (table 9 and spectra 11). Cyclohexylamine shows similar shifts to pyrrolidine with the indoles tested (table 10). Pyrrolidine is also found to quench the fluorescence intensity of most of the indoles (spectra 8-11 and 45-47). In comparison with other polar solvents pyrrolidine is the most effective quencher. Triethylamine (XXVIII) produces smaller red shifts than cyclohexylamine and pyrrolidine in the indoles tested (tables 9-11), and it does not show red shifts with 5- or 7-alkylindoles (table 11). Likewise, triethylamine appears to be much poorer quencher than pyrrolidine (spectra 14,15, 48 and 49). Although the lowest concentration of triethylamine,

such as 0.04M produces a small quenching effect, higher concentrations do not cause any further significant quenching. The smaller shifts produced by triethylamine than other amines could be the result of the presence of "floppy" ethyl groups which reduce the interaction with indoles. This is clearly demonstrated by quinuclidine (XXIX) and triethylenediamine which produce shifts in indole, 2,3-dimethylindole and 5-methylindole greater than triethylamine (tables 11-13 and spectra 16,17, and 49). In general interactions of amines with indoles either produced marked red shifts or a marked quenching effect on the fluorescence intensity. The shifts are greater with the 2- or 3- alkyl or 2,3-dialkyl substituted indoles (tables 8-11 and spectra 8-11). The behaviour of triethylamine, quinuclidine and triethylenediamine with indoles suggests that a steric requirement exists for the exciplex formation between indoles and amines. Acetic acid did not show any marked shifts with indole derivatives with the exception of a small shift of 2 nm for 6-methyl- and 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole (table 14). It did however appear to be an efficient quencher for all the indoles tested (spectra 18-22). This effect may be due to formation of non-fluorescent protonated indolenine[96] in the excited state.

2.8.3. The Nature of Indole Exciplexes

The red shifts in the fluorescence of indole and some of its derivatives which occur in nonpolar-polar solvent mixtures at low polar solvent concentrations, where bulk solvent properties are only slightly changed, have been interpreted in several ways. The most generally accepted[16,19,21,22] view is that the large red shifts arise from the formation of a complex between the solvent and the excited state indole system. The exact mechanism of formation, the geometrical requirements, factors influencing exciplex stability and chemical nature of the indole exciplexes is not understood. To date only a small number of indole/exciplexes have been observed and only limited studies have been done. It was the purpose of the present

study to examine the dependence of exciplex formation or lack of it on the type of substituents present on the indole system and the type and structure of the polar solvent. In this way it was hoped a better understanding could be obtained about the nature of the exciplex in terms of the factors mentioned above.

Examination of the complexation of methyl substituted indoles with primary alcohols such as n-butanol and methanol in cyclohexane solution shows there is marked dependence of exciplex formation upon position of the methyl group. Thus it is seen that 2- and 3-methylindole show large red shifts, and when both 2 and 3 positions are occupied then even larger red shifts result. On the other hand the presence of a methyl group on either the 5,7, or 1 positions prevents any red shift being observed. This is in contrast to the reports [16,22] that 1-methylindole and 5-methylindole form exciplexes with polar molecules. It is to be noted that in these reports there is no data given showing the extent of the red shift observed and in the case of 1-methylindole the concentration of alcohol in pentane solution was taken up to 1.1M commencing with 0.33M as the first concentration used. In the present study alcohol or other polar solvent concentration never exceeded 0.20M and commenced at 0.04M. This was done to ensure that the bulk solvent properties of the cyclohexane were little changed by the addition of the polar solvents. Under these conditions no red shift is observable for 5- and 7-methylindoles. It is highly likely that in the published work the presence of approximately 1.0M polar solvent in the nonpolar solvent can no longer be assumed to have no effect on the bulk properties. There is no doubt that the presence of a 5-alkyl group on the indole nucleus has a marked blocking effect on exciplex formation as concluded from the complete absence of any red shift in the fluorescence of such compounds. This result could arise from either an electronic or a steric effect or a combination of both exerted by the presence of a 5- or 7-methyl group. In order to gain an insight as to which of these two effects was operative a number of di- and tri-alkylated indoles were examined. It was found that the presence of 2,3 -

disubstitution in some cases, completely cancelled the effect of the 5- or 7-methyl group and red shifts were observed equal in magnitude to those for indoles with only 2,3-diakylsubstitution. Clearly the blocking effect of a 5-methyl group does not arise from steric effects inhibiting exciplex formation. This can be taken as quite certain for even in the case of a 5-n-butyl-2,3-dialkylindole a large red shift was obtained.

It appeared therefore that alkyl groups affect exciplex formation by virtue of their electronic effect which is that of positive electron induction into the indole nucleus. To test this hypothesis complexation of 5-methoxy and 5-fluoroindole was examined. Both compounds were found to give no red shift with most of the polar solvents tested. The result obtained for 5-methoxyindole was confirmed in a recent publication[54]. It was also found that if the 5-methoxyindole was also 2,3-dialkyl substituted then exciplex formation was restored though the red shift obtained was less than for the 5-methyl-2,3-dialkylindole. At first sight there appears to be a difficulty in understanding the blocking of complexation by two groups as unlike as methyl and fluoro or methoxyl. It is however known that the methoxyl group has an electron donating effect which results from the combination of an electron withdrawing inductive effect and an electron donating mesomeric effect. Consequently if only an overall electronic effect determines whether an exciplex forms or not then clearly both the methyl and methoxyl groups as well as the fluoro group possess the same property. The magnitude of this may differ between the groups and so may account for the smaller red shift observed for the 2,3-dialkyl-5-methoxyindole in which the strong electron withdrawing effect inductive of the oxygen attenuates its mesomeric electron donating effect.

It appeared therefore that an electron donating effect from the direction of the 5,6 and 7-positions of indole, that is from the benzene ring side, towards the pyrrole ring resulted in inhibition of complexation. The same result however was found to hold if the 1-position was alkylated.

This observation was at first surprising since it would be expected that the electron inductive effect of 1-methyl group would be in approximately the opposite direction to that of a 5-methyl group and so might have been expected to result in a greater red shift. It was therefore decided to study whether a steric effect was also operative in exciplex formation. Examination of the red shifts produced by 2- and 3-methylindoles and also those of 1,2-and 1,3-dimethylindoles shows that the 3-substituted compounds give a significantly greater red shift. This taken with the effect of 1-alkyl substitution mentioned earlier suggested that a steric requirement based on the 1 and 2 positions of the indole may be operative in exciplex formation. Consequently 2-tert-butylindole was prepared as a model compound in which steric hindrance to approach to the 2-position would be expected to be present. Indeed it was found that the red shifts obtained with polar solvents for this compound were significantly smaller than the shifts observed for 2-methylindole with the same solvents. There appears therefore to be a steric requirement for exciplex formation for indoles. It cannot be concluded definitely that this is the case because substitution by a 2,3-decamethylene ring or 2-butyl-3-propyl groups did not show any marked reduction in the red shift when compared to 2,3-dimethylindole. Therefore if there is a geometrical requirement it is unlikely to be very strongly defined. The presence of a geometrical requirement was further tested by using alcohols in which the hydroxyl group was placed in a slightly sterically hindered environment. Therefore the alcohols cyclohexanol, tert-butanol, borneol and 3-ethyl-3-pentanol were used in combination with some indole derivatives.

Examination of tables 1 to 6 show that there is a small but consistent reduction in the red shift obtained with the above alcohols and 2,3-cyclodecamethyleneindole.

Since it had been suggested[55] that the exciplex may arise from a charge-transfer interaction it was decided to see whether amines would

produce exciplexes and whether their interaction differed in any way from the alcohols. It was considered that if the exciplex formation involved the use of a "lone pair" of electrons then the amines could be expected to be more reactive than alcohols in this capacity. A primary, secondary and tertiary amine was chosen in the form of cyclohexylamine, pyrrolidine and triethylamine. This choice was based on the considerations that in general tertiary amines have lower oxidation potentials than primary and secondary amines[97] and therefore may act as more efficient donors in charge-transfer complexes and also there may be an involvement of the -NH group in the exciplex formation.

Examination of the results obtained with these three amines show that both the primary and the secondary amines give red shifts with most of the indoles that also interact with the alcohols. Triethylamine, the tertiary amine, however is seen not to give exciplexes as readily as the other two amines. This is seen by examination of table 11 and comparison with tables 9 and 10. As mentioned earlier, triethylamine is known to be a sterically hindered amine in reactions involving its lone pair. It was therefore considered that its complexation was reduced as a result of the spatial requirements of its ethyl groups. To test this hypothesis the complexation of quinuclidine (azobicyclooctane), and triethylenediamine (diazobicyclooctane) with indoles was studied. These two tertiary amines were found to complex with indole, 2,3-dimethyl- and 5-methylindole more efficiently than did triethylamine. A further observation on the behaviour of the azobicyclooctane is that it apparently gives the maximum red shift at lower concentrations with no further shift in emission at higher concentrations. This is in contrast to the other amines and polar solvents.

A further feature found in the interaction of amines with the indoles is the marked quenching effect of cyclohexylamine and pyrrolidine and its absence in the case of triethylamine and quinuclidine and triethylenediamine. The latter three amines behaved analogously to the alcohols in that generally

only a small quenching effect was observed and in many cases either no quenching or enhancement of fluorescence intensity took place. Clearly the primary and secondary amines provide some energy conversion mechanism not present in the tertiary amine or alcohols. It is possible that this involves the NH group which is present in the former but not the latter. If this is so then there is some difference between the NH group of amines and the OH group of alcohols which accounts for the non-quenching of exciplex emission by alcohols. The other differences between alcohol and amine exciplexes of indoles was the observation of a small red shift for 5- and 7-methyl for 5-fluoroindoles with cyclohexylamine and for 5- and 7-methyl)-, 5-fluoro- and 5-methoxyindole with pyrrolidine. With triethylamine only the 5-fluoroindole shows a small red shift. Also the presence of 1-methyl or alkyl group on the indole has a blocking effect to exciplex formation with the amines studied. For example, 1,2,3-trimethylindole and 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole with n-butanol give a 13 and 6 nm red shift respectively. The same two compounds give no red shift at all with any of the amines.

It is concluded that amines interact with indoles to form exciplexes which are generally of slightly lower stability than the exciplexes with alcohols. This conclusion is drawn from the fact that the red shifts with amines are less than those found with alcohols. Like the alcohols the amines appear to show a steric requirement for the complexation. Furthermore with primary and secondary amines the complexation generally results in quenching which implies the possibility of an effect either not present or present to a reduced extent with the alcohols. And lastly it is found that whereas substitution in the 5- and 7-positions of the indole nucleus by overall electron pushing groups is effective in inhibiting exciplex formation with alcohols it is seen that in the case of amine-indole exciplexes it is the presence of alkyl groups on the 1-position that is most effective in preventing exciplex formation. With a view to

help resolve the mechanism and nature of exciplex formation three other polar solvents were tested. Acetic acid was chosen to test the function of protons in such complexation. From the spectra 18-22, it can be seen that acetic acid is an effective quencher of the emission of all the indoles examined and from table 14 it is clear that the quenching is not accompanied by an red shift. This can be taken to demonstrate that the exciplexs do not arise from a proton transfer between the polar solvent and the indole. Indeed it may be that proton transfer takes place from primary and secondary amines and accounts for their quenching effect on the emission of indoles.

Tetrahydrofuran was tested to see what function if any the hydroxyl group of alcohols plays in exciplex formation. Comparison of tables 8 and 1 shows that tetrahydrofuran always shows red shifts much reduced from those with butanol. Also it does not show any shift with those indoles which give only an 8-9 nm shift with 0.20M n-butanol. Clearly the presence of a hydroxyl group is important in exciplex formation.

Finally examination of table 7 and comparison with the results of n-butanol in table 1, demonstrates that acetonitrile complexes with all the same indoles as does the alcohol. The red shifts obtained at 0.16M acetonitrile, owing to its reduced solubility in cyclohexane, are generally less than those given with n-butanol.

The exceptions are the indoles with 1-methyl or 1-alkyl substituents which show slightly greater red shifts with acetonitrile. This is in contrast to the results obtained with the amines where no complexation is observed.

What is the nature of the indole exciplex and its mechanism of formation?. The original suggestion[53] that the indole exciplexes were charge-transfer complexes between excited state indole and the polar solvent is difficult to visualise. It is not obvious which should be

the donor and the acceptor molecule in an indole-alcohol interaction. If the indole acts as the donor, as may be expected from other observations[77,76,78] then it is not clear how the alcohol can act as the acceptor. By analogy with amine-aromatic charge-transfer complexes[83] it could be argued that just as the amines generally act as donor molecules then the alcohols would also act as the donor partner of an indole-complexation. If this is the case then again it is not at all clear why and how the excited state indole acts as an acceptor complex. It may well be that in the excited state indole there is a charge-transfer from say the pyrrole ring system into the benzene ring which enables the pyrrole ring to play the part of an acceptor molecule in a charge-transfer interaction. Furthermore, if the exciplex was entirely or even largely the result of charge-transfer interaction it may be expected that amines would form stronger complexes than alcohols with indoles. This would be reflected in the larger red shifts produced. From the experimental work it is seen that this is not the case, since the amines do not give larger red shifts than the alcohols and indeed do not show any marked variation in themselves as donor molecules. For it might be expected that the oxidation potential of say quinuclidine, a tertiary amine, is less than that of cyclohexylamine, a primary amine, and so might produce a greater shift in the spectrum as has been found for some exciplexes[54]. Comparison of the shifts produced by those two amines with indole and 2,3-dimethylindole tables 10, 12 and 13, show identical red shifts in both cases. If on the other hand the indole residue acts as donor it is not clear why 2-methylindole or 3-methylindole forms an exciplex with a greater red shift than indole itself with polar solvents and yet 5- or 7-methylindole does not form any exciplex at the same conditions. Similarly how does 2,3-disubstitution of a 5-methylindole restore complexation, or why does 5-methylindole not form exciplexes with alcohols but it does form weak exciplexes with amines?

An improved understanding may be obtained if it is accepted that there are at least two different classes of exciplexes[52], and that not all exciplexes are the result of charge-transfer interaction. It had been found[53] that N,N-dimethyl-3-(1-naphthyl)propylamine formed an intramolecular exciplex of a charge-transfer type. At room temperature the emission is entirely due to this exciplex with λmax about 430 nm in benzene. On addition of a small amount of acetonitrile, dioxane, or ethanol to this solution it was found that a second emission band appeared, red shifted from the original exciplex emission. This was ascribed due to the formation of a weak exciplex resulting from a stoichiometric dipole-dipole stabilised complex between an excited species and one or more polar molecules. Thus there is a distinction between weak and strong exciplexes. The latter are charge-transfer complexes with their characteristic properties such as a strong dependence of λmax of fluorescence on the difference between the oxidation and reduction potentials of the donor and acceptor,respectively, in non-polar solvents. Similarly, a linear correlation[98] has been found between the donor ionisation potential and the fluorescence quenching of the acceptor and because of the polarity of the strong exciplex it is very sensitive to the polarity of the solvent[54].

Weak exciplexes[52,53] are seen as resulting from the dipole-dipole interaction between the excited molecule and one or more polar molecules. If the excited state molecule has a large dipole moment which is well localised then it may form a weak exciplex with one or more molecules having a polar group. The presence of the polar molecule may attenuate the field of the localised charge and possibly in some cases undergo further change to form a partial charge-transfer complex or a strong exciplex with complete electron transfer. Although there may be little requirement for a single preferred geometry in a weak exciplex, the dipole-dipole interactions may produce an induction of charge in the polar partner

by the excited state molecule and result in some stereochemical direction. Evidence has been found for geometrical requirements in the case of intramolecular exciplexes[99]. The dipolar nature of the weak exciplex will invalidate the use of the Lippert-Mataga[42] equation in calculating the dipole moment of the excited state. This problem was encountered by Lumry et al[49] who could not calculate a dipole moment sufficiently large to account for the red shift of indole emission at low alcohol concentrations[16]. Likewise the emission λmax will not depend strongly upon the ionisation potentials and electron affinities for weak exciplexes. However substituents on the complexing partners may well exert strong effects upon the stability of the exciplex. The properties of weak exciplexes are therefore likely to differ from those of the strong, charge-transfer exciplexes. It is possible that weak exciplexes in some cases are the precursor of strong exciplexes.

It is on the basis of weak exciplexes formed by the dipole-dipole interaction between indoles and polar molecules that the complexation observed in this work can be best interpreted. It follows that the direction and the magnitude of the excited state dipole of indole and its derivatives will be the most important factor which will decide whether an exciplex forms or not.

Studies of the direction of the excited state indole and methylindoles have been made although only a small number of literature reports are available. Thus Tyutyulkov and Dietz[112] have calculated that excited state dipole direction of indole is as shown in the figure 2.

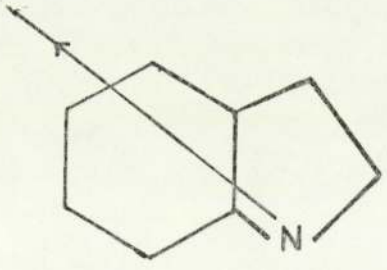


Figure 2

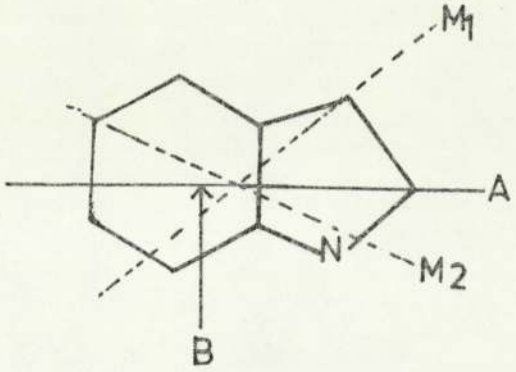


Figure 3

More recently Lombardi et al[113] using the optical Stark Effect determined that the dipole moment of the indole in the lowest singlet $\pi \rightarrow \pi^*$ state lies at 26° from the A molecular axis as shown in fig. 3. They were unable to say which direction the dipole took and therefore presented the two possible orientations for it as M_1 or M_2. The magnitudes of the dipole moments for the excited state indoles have not been investigated to any large extent and so only a few reports are given. For example Mataga et al[42] estimated that the change in the dipole moment for indole from the ground state to the excited state was 5D.

Gladchenko et al[114] estimated that the excited states indole dipole moment was 5.6D and its direction was along the short axis of the molecule. Kawski[115] has calculated dipole moments for indole, 1-methyl-,2-methyl-, 1,2- and 2,3-dimethylindole in the ground state and excited state. He gives the changes in the dipole between the ground and excited states to be of the order of 2-3D with only a small difference in this between indole and methylindoles. Finally Lumry et al[54] in a discussion on the lack of exciplex formation by 5-methoxyindole estimates that the dipole change for this molecule on excitation is only of the order of 1.1D.

The magnitude of the dipole moment of the molecule can be expected to be attenuated by the presence of substituents. The direction of the attenuation and its degree will be dependent upon the position of the substituent with respect to the direction of the dipole moment and the specific electronic effect exerted by the substituent. The resultant direction and magnitude of the interaction of two dipole moments can be obtained by vector addition. For example the resultant magnitude and direction of the interaction of two vectors $\bar{X}$ and $\bar{Y}$ is given by $\bar{xy}$ as shown in fig. 4.

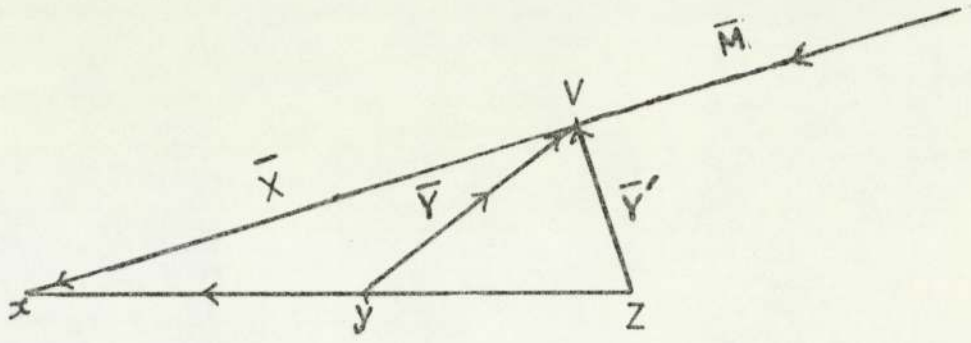


Figure 4

It is readily seen that provided the angle between $\bar{X}$ and $\bar{Y}$ is less than 60° the result is to give a dipole moment of lower magnitude than $\bar{X}$. At all angles greater than 60° the result will be a greater dipole moment given the directions of the interacting moments are as in $\bar{X}$ and $\bar{Y}'$ in the fig.4. In this instance the magnitude of the moment becomes $\overline{xz}$. If the interacting moments are exactly parallel as given by $\bar{X}$ and $\bar{M}$ then the result and moment is the sum of the two moments $(\bar{X} + \bar{M})$, and the direction is unchanged. This analysis can be applied in the case of the effect of position of substitution by alkyl groups (or positive electron inductive groups) on the magnitude of the dipole moment of the excited state indole.

Drawing fig. 5 on a larger scale it is possible to see the effect of a methyl group substituted at different positions on the magnitude of the excited state dipole moment. The direction of the excited state dipole moment which gives the greatest agreement with the observed data is that given by Ml. The assumption is made here that reduction in the magnitude of the excited state dipole moment will reduce the tendency for the formation of a weak exciplex and may in some instances prevent complexation altogether.

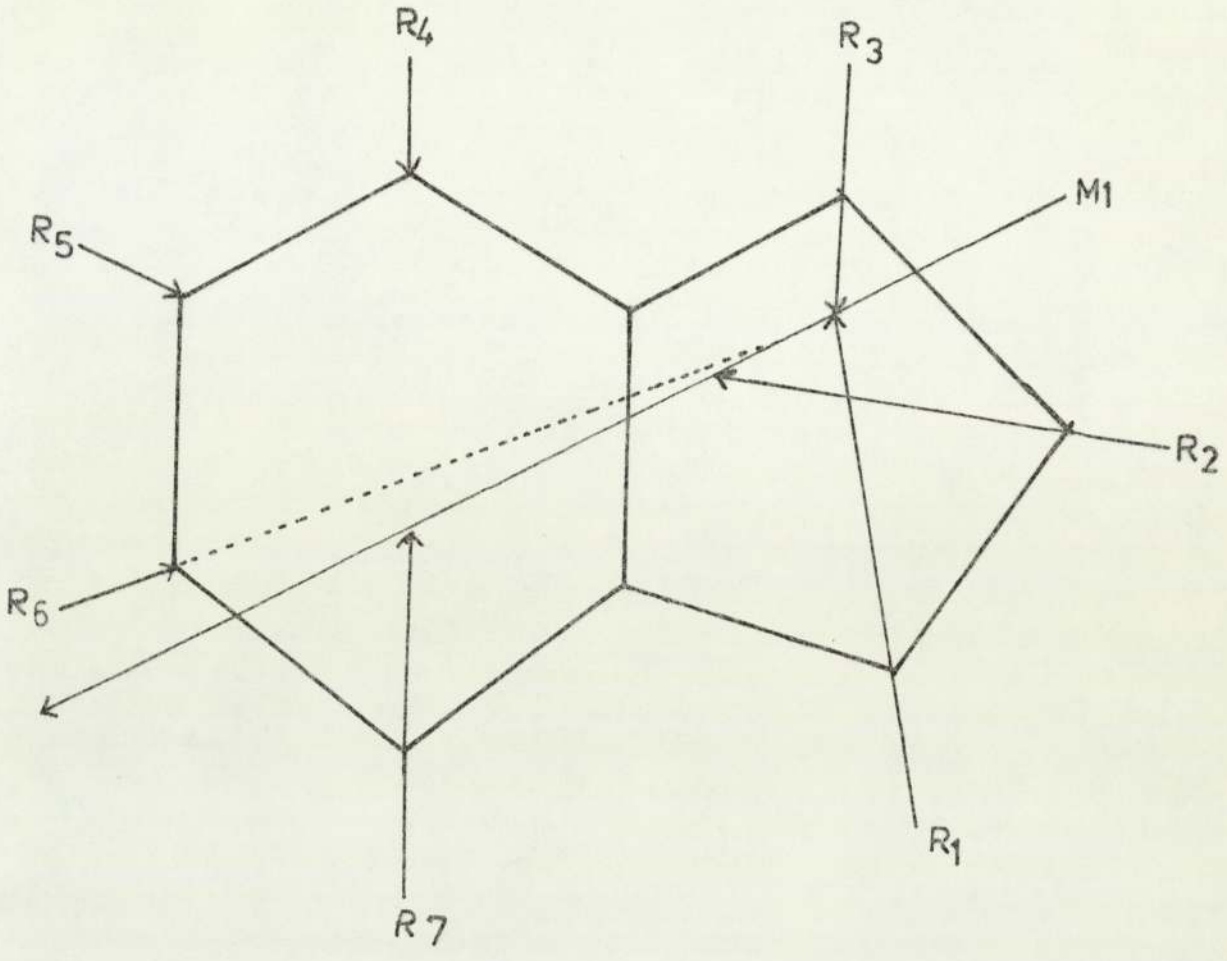


Figure 5

It can now be seen that depending on the exact angles of direction of the interacting moments alkyl groups at position 1,5,6 and 7 can easily reduce the magnitude of the excited state dipole. Methyl groups at positions 5 and 6 appear to be particularly favourably directed for this purpose. But likewise positions 1 and 7 exert an opposing dipole and so reduce the excited state dipole. On the other hand it is seen that positions 2 and 3 and probably 4 will all reinforce the excited state dipole and thus produce a greater overall polarity in the excited molecule.

This analysis appears to fit broadly all the experiments observed in

this work on the exciplex formation between polar solvents and position of substitution of the indole system. Some of the more subtle differences which have been observed between the behaviour of specific solvents and particular type of indole substituents may arise as a result of one or two other factors. For example, the direction of orientation of the polar solvent molecule in the exciplex may differ according to the structure of the polar solvent molecule. It is possible that in the case of alcohols the direction is dictated by the polarity of the $(R)-\overset{-}{O}-\overset{+}{H}$ bond. If this is the case then the group R will lie in the exciplex in the general direction of the 2,3-positions of the indole if the exciplex geometry is taken to be one where the two interacting molecules take up positions close to one another and parallel to the directions of their dipole moments. In the case of acetonitrile if the polarisation of the $(R)-\overset{+}{C}\equiv\overset{-}{N}$ is taken as the important factor then the R group will tend to lie towards the benzene ring of the indole molecule. The same will apply for tertiary amines. The primary and secondary amines would be expected to behave as the alcohols but may show some tendency to adopt positions in between those of the alcohols and acetonitrile. The effect of these orientations may be one which induces a change in the direction of the dipole moment of the excited state indole and hence may result in a change in magnitude of the dipole moment so that differences such as the weak complexation of 5-methyl- and 5-fluoroindole may take place with cyclohexylamine but not with n-butanol. Similarly the 1-alkyl substituted indoles may not form an exciplex with the amines but will complex with alcohols. An alternative explanation may rest in the fact that the weak exciplex is not one due to a pure dipole-dipole interaction and that there is indeed a contribution from a charge-transfer interaction. The degree of charge transfer will depend upon the usual factors and will vary with the structure of the indole

and the polar solvent molecule. Such differences are known to exist for complexation between different partners. If the generalised wavefunction ψ of a bimolecular, excited state complex is given by Mataga et al[59].

$$\psi = \Sigma_i aiAi^*s + \Sigma_i biASi^* + CA^+ S^- + dA^- S^+$$

where ai, bi, c, d are coefficients and Ai*, Si* refer to various neutral and excited singlet states of the partners A and S_i then it is found that for excimer formation a = b and c = d. However for aromatic hydrocarbon-amine complexes a > b and d > c.

It may be that in the case of the amine-indole exciplexes there is a greater contribution of the charge-transfer interaction with a resultant quenching being observed with the primary and secondary amines. This however would leave unexplained the lack of quenching of fluorescence by the tertiary amines. This particular result may be better accommodated by the presence of another mechanism in the case of primary and secondary amines which involves the presence of the H atom of the NH group. As a result of some degree of charge-transfer interaction between the indoles and the primary and secondary amines the N-H group may acquire a strong acid character with the effect of providing a proton source. The exciplex would then lose proton which would be picked by the excess of amine molecules and thus provide a route for deactivation of the excited state.

That other structural features in the indoles may play a part in complexation can be deduced from the interactions of tryptophol with polar solvents. This indole was selected to see whether the presence of an hydroxyl group in the molecule would result in an intramolecular exciplex which would show no further red shifts on the addition of polar solvent molecules. It was found that tryptophol in fact shows the largest red shifts of all indoles with polar solvents as seen from tables 1,7,8,9 and 10. This may arise from the presence of the hydroxyl group in the tryptophol which enhances the interaction between this indole and the polar molecules due to its own dipole moment and its hydrogen bonding properties. It may also

be that a weak intramolecular exciplex is formed which due to its greater polarity shows a stronger interaction with polar solvents and so produces the larger red shifts.

This work has further illustrated the variety of complexation reaction available to indole derivatives. It has produced a possible explanation for some of the observed interactions of indoles and polar solvents in non-polar solutions and the dependence of this interaction upon the structures of the partners. At the same time it has revealed the further need for a study of the complexation of indoles with polar molecules if the fluorescence properties of proteins are to be more clearly understood. For presumably the tryptophan residue which often is largely the source of protein fluorescence is subject to a variety of environmental interactions within the protein structures.

2.9 EXPERIMENTAL

2.9.1. Materials and Methods

All indoles and solvents were checked for purity prior to use by ultraviolet spectroscopy. Indole and 5-fluoroindole (Koch-Light Lab.); 2,3-dimethylindole; 3-methylindole; 2-methylindole and 5-methoxyindole (Aldrich Chemical Co. Ltd.); 5-methylindole (I.C.N. pharmaceutical) and 7-methylindole (Fluka A.G.-chemischefabrik), were pure industrial preparations. 2,3-Cyclodecamethyleneindole; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole and 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole (gifts from Dr. Britten) were recrystallized from methanol. 1,3-Dimethylindole and tryptophol (gifts from Dr. Britten) were very pure. 1,2-Dimethylindole (gift from Dr. Britten) was purified by vacuum sublimation, and 1,2,3-trimethylindole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 2-tert-butylindole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 2-butyl-3-propylindole; 6-methoxy-1,2,3,4-tetrahydrocarbazole, were made and their purity checked in the laboratory.

Cyclohexane was purified by shaking with conc. H_2SO4 and distilled over activated charcoal. Methanol, n-butanol, cyclohexanol and tert-butanol, were purified by distillation with potassium hydroxide. Acetonitrile was purified by distillation and pyrrolidine by distillation over zinc powder. Tetrahydrofuran was purified by distillation over lithium aluminium hydride.

The free base of quinuclidine Hcl(ABCO) was liberated[100] by combining very concentrated aqueous solutions of the hydrochloride with potassium hydroxide, the solid amine was removed by filtration, dried in a vacuum desiccator, dissolved in ether, filtered and the ether removed by vacuum distillation. Borneol, triethylenediamine, acetic acid, triethylamine and 3-ethyl-3-pentanol were pure industrial preparations.

The ultraviolet absorption for most of the above solvents gave a 100% transmission at 200-400 nm. A concentration of 25×10^{-5} M indole in cyclohexane was used to check the ultra-violet absorption spectra.

This was too high for fluorescence work and was therefore diluted to 5×10^{-5}M.

The ultra-violet spectra were recorded on a Pye Unicam 8000 Spectrophotometer and an Aminco-Bowman spectrofluorometer and a Hewlett Packard 7035B X-Y recorder was used to obtain the fluorescence emission spectra at room temperature.

The slit settings on the instrument were:

Excitation wavelength	285 nm
Excitation inner slit	3 mm
Excitation outer slit	3 mm
Emission inner slit	2 mm
Emission outer slit	0.5 mm
Photomultiplier slit	1 mm
Amplifier setting at	0.3
Amplifier sensitivity	36

The above settings were used for all the experiments except in the case of 1,2-dimethylindole when the photomultiplier slit was changed to 0.5 mm.

All indoles were at 5×10^{-5} M concentration in cyclohexane solutions containing 0.04, 0.08, 0.12, 0.16 and 0.2M of the alcohols, amines, acetonitrile, tetrahydrofuran and acetic acid as the polar solvents. Emission spectra were recorded in the above solvents and the shift of maximum emission in each case is listed in the results. Some of the emission spectra are included in the appendix.

2.9.2. Synthesis of Indole Derivatives

The starting materials used were pure industrial preparations. Ultra-violet (UV) and Infra-red (IR) spectra were recorded on Pye Unicam SP 8000 and SP 200 spectrophotometers respectively. Gas liquid chromatography was used to check the purity of 1,2,3-trimethylindole using a Perkin Elmer F11 with the following conditions.

column	3% OV1
oven temperature	150°C
injection temperature	2
N_2 pressure	21 lb/in^2
H_2 pressure	20 lb/in^2
air pressure	19 lb/in^2
chart speed of recorder	5 mm/min.

1) 1,2,3-Trimethylindole

Hedney and Ley[101] described N-alkylation of indole and pyrroles in dimethylsulphoxide, and made 1-benzylindole from indole and benzylbromide. The same procedure has been used for making 1,2,3-trimethylindole from 2,3,-dimethylindole and methyliodide.

Procedure

Dry dimethyl sulphoxide (200 ml) was added to pot. hydroxide (22.4 gm) pellets and the mixture stirred for 15-20 mins. 2,3-Dimethylindole (14.52 gm "0.1 mol") was then added and the mixture was stirred for 3/4 hour. Methyliodide (28.382 gm "12.5 ml," 0.2 mol) was added and the mixture was cooled briefly and stirred for a further 3/4 hour. Water (200 ml) was added, the mixture was extracted with ether (3 x 100 ml), and each extract was washed with water (3 x 50 ml). The combined ether layer was dried using $Cacl_2$ and a small quantity of anhydrous sod. sulphate, the ether was removed under reduced pressure. The red liquid was purified by vacuum distillation to yield a pale yellow, clear, oily liquid of 1,2,3-trimethylindole 14 gm (88%). It has a melting point 18°C[102].

2) 2-Tert-butylindole

The Fischer indole synthesis[103] was used for making 2-tert-butylindole from methyl-tert-butyl-Ketone with polyphosphoric acid.

Procedure

To a mixture of (5 gm) methyl-tert-butyl-Ketone and (5 ml) phenylhydrazine, which was first heated on water bath for 10 minutes,

about (20 gm) of polyphosphoric acid was added. The mixture was stirred and warmed gently on oil bath until 155°C (at which point there is a sudden rise in temperature). The mixture was maintained at about 155°C until the temperature began to drop spontaneously and then the mixture was cooled with water and 100 ml of water was added. This was then extracted with ether (4 x 60 ml) and each extract was filtered through a layer of the same quantity of anhydrous sod. sulphate. The combined dried ether extracts yield the indole. The ether was evaporated to yield an oily brown product 5 gm (58%). The product was purified by vacuum distillation. Yellow waxy crystals were produced which when recrystallized from methanol gave colourless crystals which become darker with time and gave a melting point of 60-63°C. Reference melting point was 65-69°C(104).

3) 2-Butyl-3-propylindole

This compound has not previously been made, it was synthesised by the same method as 2-tert-butylindole, using (6.3 gm) dibutyl-ketone, (5 ml) phenylhydrazine and about (20 gm) polyphosphoric acid. The sudden rise in temperature in this reaction was at 145°C. A brown thick liquid was produced after evaporation of the ether, which was purified by vacuum distillation to yield a pale yellow, oily, clear liquid with a characteristic odour, 8.7 gm (79.8%). The compound was checked by IR (thin film), which shows peaks at 3400 cm^{-1} and 740 cm^{-1} due to the indole N-H and CH(1,2-disubstituted) benzene ring respectively. The picrate of this compound gave chocolate brown needles with a melting point of 90-94°C.

4) 2-Decyl-3-nonylindole

This compound has also not previously been made. It was prepared by the method of preparation of 2-tert-butylindole, using (5 gm) didecyl-ketone, (1.6 ml) phenylhydrazine and about (6.6 gm) of polyphosphoric acid. The beginning of the reaction was a sudden rise in temperature at 155°C. A gummy orange product was produced after evaporation of ether, with 6.2 gm (69.9%) yield. Two recrystallizations from methanol yielded orange

amorphous crystals, with a melting point of 50-55°C. The compound is unstable when left in ordinary conditions and should be kept under nitrogen.

5) 6-Methyl-1,2,3,4-tetrahydrocarbazole

This compound was synthesised in two stages. The first stage being the synthesis of p-tolylhdrazine HCl and then cyclization to get the carbazole.

(i) p-tolylhydrazine HCl was made by the Fischer method[105], which was modified to avoid the precipitation of sod. chloride. This method gives after recrystallization the hydrochloride of the hydrazine in the pure state.

Procedure

P-toluidine (7.1 gm) was dissolved in 150 ml water, and 12 ml conc. HCl was added. The solution was cooled in iced water with mechanical stirring and a solution of sod. nitrite (4 gm $NaNo_2$ in 15 ml H_2O) was added. When the addition was complete the diazotised solution was poured into the sod. sulphite solution (37.5 gm $Na_2SO3. H_2O$).

The mixture has a red/orange colour which after some time (one hour at least) became more yellowish. Then 4.5 ml glacial acetic acid and a little zinc dust was added and the solution was warmed on a water bath for 3/4 hour - 1 hour. This was filtered and 225 ml conc. HCl was added to the filtrate and the solution cooled in iced water. White crystals deposited which were filtered with suction and washed with dil. Hcl and dried in a vacuum disiccator. The yield was 4 gm (38%) of p-tolylhydrazine HCl with a melting point of 234-237°C.

(ii) Cyclization of p-tolylhydrazine Hcl to produce the tetrahydrocarbazole

The preparation of 1,2,3,4-tetrahydrocarbazole was reported by Rogers and Croson[106], when they cyclised phenylhydrazine Hcl with cyclohexanone.

Procedure

A mixture of (1.69 gm) cyclohexanone and (7.2 gm) of acetic acid,

contained in 250 ml three necked round bottomed flask, equipped with reflux condenser, and a slip-sealed stirrer, was heated under reflux and stirred while (3.16gm) p-tolylhydrazine Hcl was added in one hour. The mixture was heated under reflux for an additional hour and poured into a beaker and stirred by hand until it solidified. It was then cooled to about 5°C and filtered with suction. The filter cake was washed with 2 ml water and with 2 ml of 75% ethanol and the crude solid was vacuum dried. The yield was 2.8 gm (75.45%); when recrystallized from methanol white crystals were formed with a melting point of 138-142°C. Reference melting point 142-3°C.[107]

6) 6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole

2,4-Dimethylaniline was used as a starting material to prepare 2,4-dimethylphenylhydrazine Hcl which after cyclization yielded the 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole.

2,4-Dimethylphenylhydrazine Hcl was prepared by the same method (i) as for 6-methyl-tetrahydrocarbazole using 2,4-dimethylaniline as a starting material. The product of yellow crystals had a melting point of 149-152°C. Cyclization of the above product was carried out as method (ii) with some changes.

Procedure

A mixture of (9.8 gm) of cyclohexanone and (36 gm) acetic acid contained in 250 ml three necked round bottom flask equipped with reflux condenser, and a slip-sealed stirrer,was heated under reflux and stirred while (17.1 gm) of 2,4-dimethylphenylhydrazine Hcl was added during 1 hour. The mixture was heated under reflux for an additional hour, cooled to about 5°C to give a thick brown liquid, which was purified by vacuum distillation to give a pale yellow oil. This when recrystallized from methanol-petroleum ether (boiling point 80-100°C), gave white crystals with a melting point of 92-95°C. Reference melting point was 92-94°C.[108].

7) 6-n-Butyl-1,2,3,4-tetrahydrocarbazole

p-n-Butylaniline (9.94 gm) was used as a starting material to prepare p-n-butylphenylhydrazine Hcl by the same method (i) as for 6-methyl-tetrahydrocarbazole; the product of p-n-butylphenylhydrazine HCl gave yellow crystals with a melting point of 80-85°C and a yield of 11.2 gm (83.79%). Cyclization of this product was carried out as in method (ii) in 6-methyl-tetrahydrocarbazole, using (10.02 gm) of p-n-butylphenylhydrazine HCl, (5 gm) cyclohexanone and (18 gm) acetic acid. The crude solid yielded 8.2 gm (75.3%) of violet crystals. When recrystallized from methanol, pale violet crystals were formed which were dissolved in ether and shaken with conc. HCl. The ethereal layer was washed with 20% HCl, and filtered through anhydrous sod. sulphate. The ether was removed under reduced pressure and grey crystals were formed. It had a melting point of 100-104°C. Reference melting point of 102-104°C.(109)

8) 6-Methoxy-1,2,3,4-tetrahydrocarbazole

This compound was prepared as in mthod (ii) in 6-methyl-1,2,3,4-tetrahydrocarbazole using p-methoxy-phenylhydrazine HCl as a starting material with the following changes.

Procedure

A mixture of (2.5 gm) of cyclohexanone and (9 gm) acetic acid was heated until boiling. The mixture was transferred to a 250 ml three necked, round bottom flask equipped with reflux condenser and a slip-sealed stirrer. This was heated under reflux and stirred while (4.4 gm) p-methoxy-phenylhydrazine HCl was added during 1 hour. The mixture was heated under reflux for an additional hour, cooled to about 5°C and filtered with suction. The filter cake was washed with (2.5 ml) water and then with (2.5 ml) of 75% ethanol. The product was light brown, with a yield of 4.6 gm (81.3%). This was twice recrystallized from methanol which gave light tan needles with a melting point of 94-98°C, and a reference melting point of 93-107°C.(110)

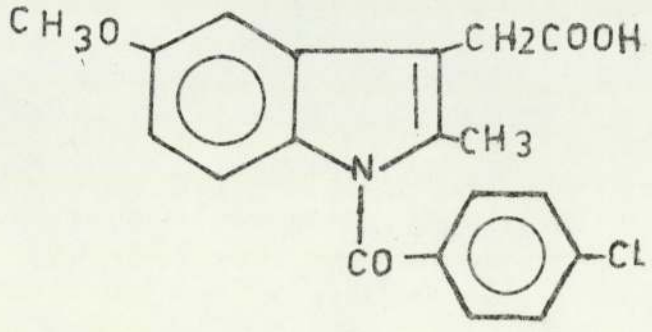
9) Many attempts to prepare 5,8-dimethyl-1,2,3,4-tetrahydrocarbazole and 7,8-dimethyl-1,2,3,4-tetrahydrocarbazole from 2,5-dimethylaniline and 2,3-dimethylaniline respectively, were unsuccessful due to the diazo-coupling effect.[111]

3. FLUOROMETRIC ANALYSIS OF 5-METHOXYINDOLES

3.1. Introduction

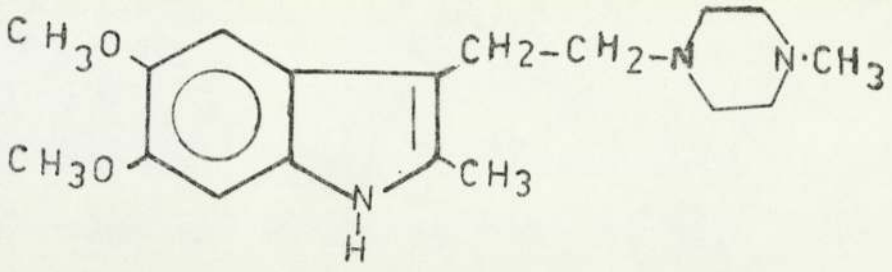
Fluorimetry is used widely in the analysis of drug compounds.[116]. The native fluorescence of 5-methoxyindoles is sufficiently intense and specific to permit its use for quantitative assay purposes. The procedures for analysis have been based on their native fluorescence in acidic, neutral or alkaline solutions. The fluorescence intensities and emission wavelengths vary with the pH of the solutions[18]. The compounds chosen for the purpose of analysis were, Indomethacin (XXX) [1-(P-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid] an anti inflammatory drug[12], oxypertine (XXXI) (1-[2-(5,6-dimethoxy-2-methyl-3-indolyl)ethyl]-phenylpiperazine), which is a psychosedative indole derivative with a tranquilizing effect[117], and the biologically important[118] compounds 5-Hydroxyindole-3-acetic acid (XXXII) and 5-Hydroxytryptophan (XXXIII).

Indomethacin



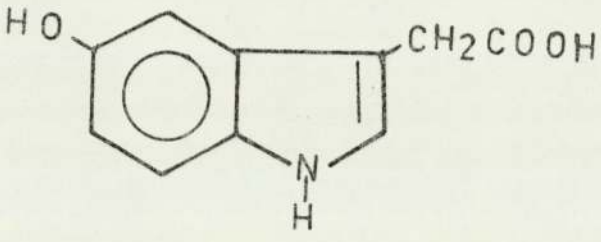
(XXX)

Oxypertine



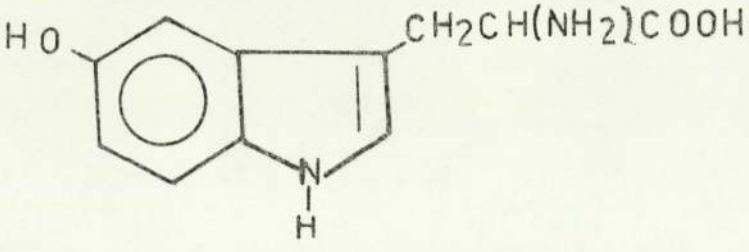
(XXX1)

5-Hydroxyindole-3-acetic acid



(XXX11)

5-Hydroxytryptophan



(XXX111)

Quantitative determinations of these compounds were carried out to investigate the possibility of using fluorimetry as a general method for the analysis of all 5-methoxyindoles. Udenfriend and co-workers[18] reported that in a neutral or slightly acid solution, 5-Hydroxyindoles fluoresce at 330 nm when excited at 295 nm, but when the acidity is increased by the addition of Hcl, the 330 nm fluorescence decreases and a new fluorescence peak appears at 550 nm. In concentrated acidic solutions the 550 nm fluorescence is maximal and the 330 nm fluorescence is extremely low. This shift of fluorescence from the ultraviolet to the visible, with increasing acidity is completely reversible and is not accompanied by a noticeable change in the absorption spectrum. Chemically induced fluorescence may also be used for analysis of compounds which do not show native fluorescence under the above conditions.

Shore and co-workers[119] reported a fluorescence assay procedure for histamine, based on the reaction of the amine with o-phthalaldehyde (OPT) in alkaline solution. Under the same conditions negative results were obtained with a number of compounds, including serotonin[120]. Later Curzon and Giltrow[121] studied the reactivity of various aromatic compounds with amino acids under acidic conditions, and found that aromatic 1,2-dialdehydes reacted with amino acids such as tryptophan to yield coloured products. However, the use of o-phthalaldehyde in the reaction may yield a fluorescent product, and therefore the sensitivity of the determination may be increased.

A comparison of these determinations with gas chromatography was carried out, as it was believed that the latter might be more sensitive.

Quantitive analysis was carried out to determine detection limits by native fluorescence, the o-phthalaldehyde reaction and gas chromatography.

The detection limit[122] is defined as the concentration which gives a reading equal to 2 x standard deviation of a series of (at least) ten determinations at or near the blank level. The following equations were used to determine the standard deviation.

mean (average) $\bar{x} = \frac{\Sigma x}{n}$

where x is the recorded value and n is the number of recorded values.

standard deviation (σ) $= \sqrt{\frac{\Sigma x^2 - n\bar{x}^2}{n-1}}$

3.2 Native Fluorescence

Native fluorescence of 5-Hydroxyindoles has been used for their quantitative analysis for several years[120]. Holet and Hawkins[123] estimated indomethacin in serum by its native fluorescence in alkaline solution (phosphate buffer pH8), and this estimation was used to study indomethacin absorption. It was reported[116] that indomethacin produces a strong fluorescence in 0.1 N NaoH at 408 nm when excited at 295 nm. Aurther et al[124] have determined indomethacin in ethanol by its native fluorescence at 410 nm when excited at 328 nm.

Native fluorescence had been used to determine 5-Hydroxyindole-3-acetic acid and 5-Hydroxytryptophan in brain tissue[118,125].

Table 15 shows the results obtained for the determination of these compounds by their native fluorescence. The acidic solution of 5-Hydroxyindole-3-acetic acid shows two emissions when activated at 295 nm, one at 340 nm and the other at 545 nm. The lower limit determined at the former emission was 0.1 ug/ml and at the latter emission 0.01 ug/ml. Linearity over the range of 0.1-0.6 ug/ml and 0.01-0.05 ug/ml respectively was achieved (Fig. 6 and 7).

Table 15

Detection limit by native fluorescence

Compound	solvent	detection limit ug/ml	excitation wavelength nm	emission wavelength nm
	8 NHcl	0.1	295	340
5-Hydroxyindole	8 NHcl	0.01	295	545
-3-acetic acid	0.1N NaoH	0.01	360	460
	ethanol	0.002	295	340
	8NHcl	0.01	295	340
5-Hydroxytryptophan	8 NH cl	0.004	295	540
	ethanol	0.001	295	340
oxypertine	ethanol	0.002	295	350
Indomethacin	0.1N NaoH	0.01	310	375

The alkaline solution of 5-Hydroxyindole-3-acetic acid was found to be weakly fluorescent when excited at 295 nm, but showed strong fluorescence at 460 nm when excited at 360 nm. The minimum limit determined was 0.01 ug/ml. A linear relation between concentration over the range of 0.01-0.1 ug/ml and fluorescence intensity was obtained (fig. 8). In contrast the lower limit determined of 5-Hydroxyindole-3-acetic acid in a neutral solution of ethanol was 0.002 ug/ml at 340 nm when activated at 295 nm, and linearity over the range of 0.002-0.005 ug/ml was achieved (fig. 9).

5-Hydroxytryptophan also shows two emissions in an acid solution (8N Hcl) at 340 nm and 540 nm when excited at 295 nm. The lower limits determined at 340 nm was 0.01 ug/ml ahd at 540 nm was 0.004 ug/ml. Linearity over the ranges of 0.01-0.08 ug/ml and 0.004-0.016 ug/ml

respectively was obtained (fig. 10 and 11). The alkaline solution of 5-Hydroxytryptophan appears to be a very weakly fluorescent even at high concentrations such as 25 ug/ml when scanning between the range 200-600 nm for excitation and emission. In neutral solution of ethanol, in comparison, it seems highly fluorescent at 340 nm when excited at 295, with a detection limit of 0.001 ug/ml, and linearity over the range 0.001-0.01 ug/ml. (fig. 12). Aurther et al[124] reported that indomethacin in ethanol emits at 410 nm when activated at 328 nm. On carrying this out it was found that indomethacin does not show emission at these wavelengths, or for that matter at any excitation and emission wavelengths. Several attempts were carried out to determine the fluorescence of indomethacin in 3NHcl, 5NHcl, 8NHcl and perchloric acid. It has been proposed[123] that indomethacin fluorescence in a phosphate buffer at pH8, but again it was found that such a solution did not fluoresce. However indomethacin appears highly fluorescent in an alkaline solution such as 0.1N NaoH. This is due to the decomposition of indomethacin by strong alkali[126]. The lower limit detected in this case at 375 nm, when excited at 310 nm, was 0.01 ug/ml and linearity over the range of 0.01-0.05 ug/ml was achieved (fig. 13). Oxypertine is insoluble in both acidic and alkaline aqueous solutions, thus the detection limit of oxypertine was determined only in neutral ethanol at 350 nm when activated at 295 nm and was found to be 0.002 ug/ml. A linear relation between fluorescence intensity and concentration over the range of 0.002-0.01 ug/ml was obtained (fig. 14).

From the above investigation it can be concluded that a change in the solvent and pH plays a major role in the native fluorescence determination of the compounds examined. This is because such changes may produce alteration in the emission and excitation wavelengths as well as in the sensitivity. Therefore by careful selection of the solvent media and the excitation and emission wavelengths it may be possible to determine these

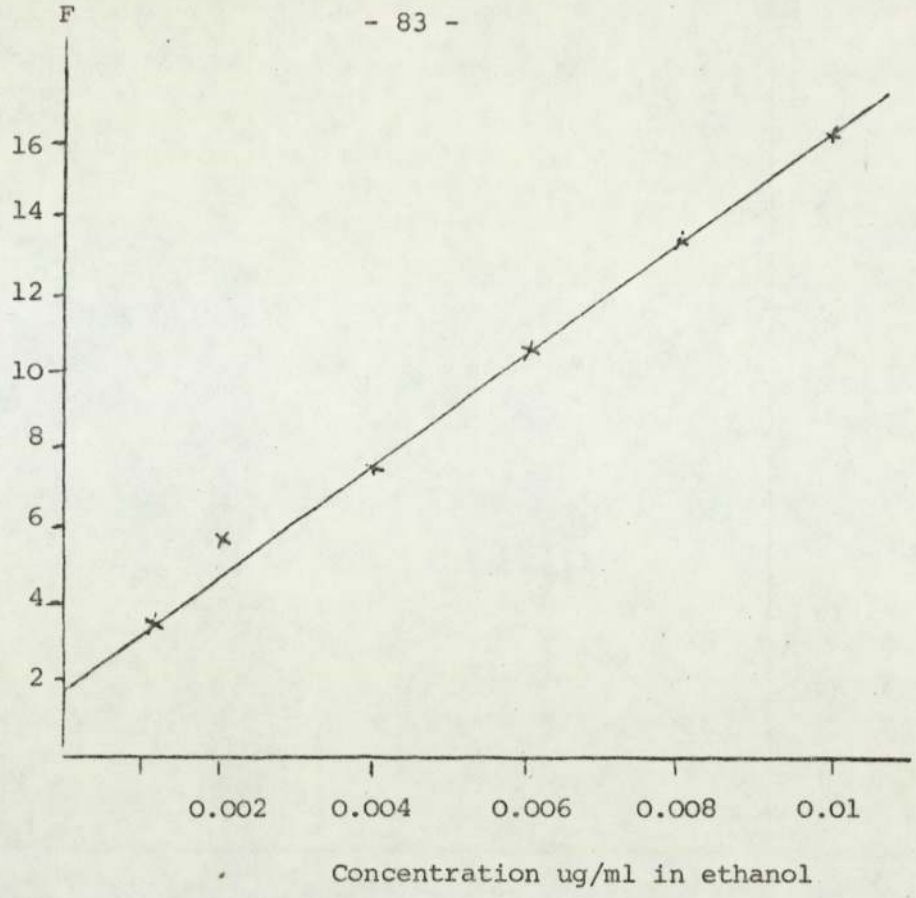


Figure 12. 5-Hydroxytryptophan by native fluorescence

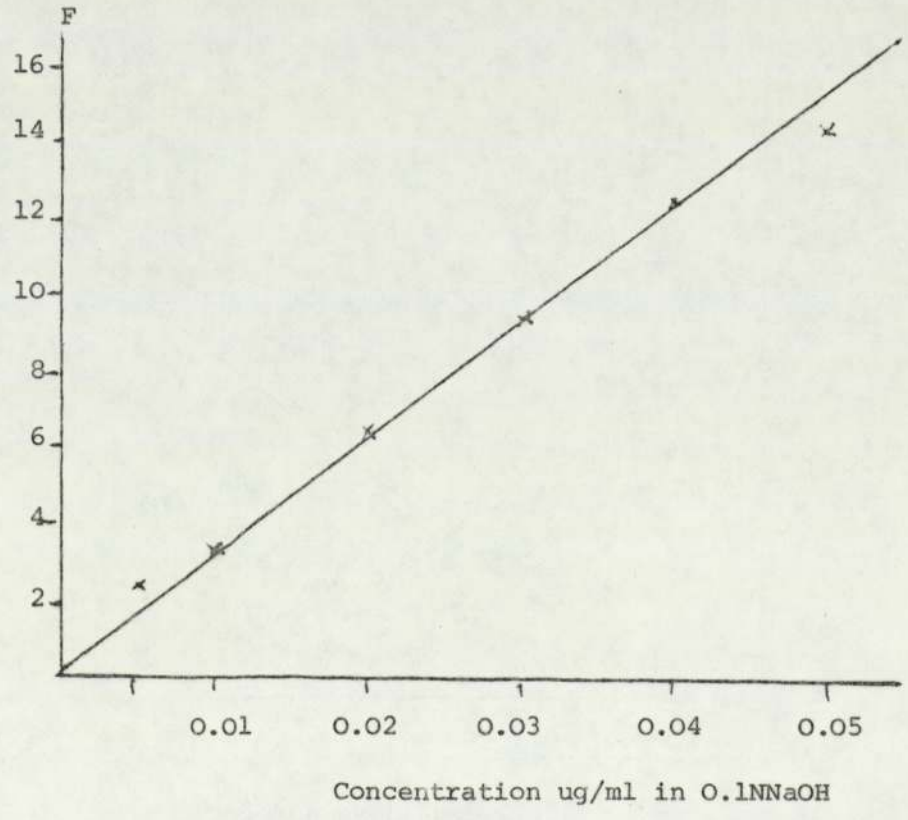


Figure 13. Indomethacin by native fluorescence

substances without interfering from other substances frequently found to be present in the same solutions. This is particularly a problem with biological fluids.

3.3 -O-Phthalaldehyde Reaction Method (OPT)

The compounds shown previously were reacted with o-phthalaldehyde because it was reported[127] that 3- and 5-substituted indoles form highly fluorescent complex with o-phthalaldehyde. Quantitative determinations were carried out by the above reaction, and the results are summarized in table 16.

Table 16

Detection limits by o-phthalaldehyde method

Compound	detection limit ug/ml	excitation wavelength nm	emission wavelength nm
5-Hydroxyindole-3-acetic acid	0.3	360	470
5-Hydroxytryptophan	0.1	360	470
Indomethacin	0.05	360	470
Oxypertine	0.01	380	420

Examination of the detection limits obtained shows the following; 5-Hydroxyindole-3-acetic acid when reacted with o-phthalaldehyde shows a weaker fluorescence in comparison with native fluorescence. The detection limit at 470 nm when excited at 360 nm was 0.3 ug/ml and linearity over the range of 0.3-1ug/ml was achieved (fig. 15), while the maximum detection limit in acid at 340 nm was 0.1 ug/ml. 5-Hydroxytryptophan determination by the OPT method appears to be less sensitive in comparison to the native fluorescence method. The detection limit of 5-Hydroxytryptophan in the o-phthalaldehyde reaction method was 0.1 ug/ml at 470 nm when excited at 360 nm, and linearity relation between fluorescence intensity and

concentration over the range 0.1-0.8 ug/ml was achieved. (fig. 16). The maximum detection limit of 5-Hydroxytryptophan by native fluorescence at 340 nm was 0.01 ug/ml in the acid solution. Similarly indomethacin shows a stronger fluorescence by native fluorescence in comparison with the o-phthalaldehyde reaction method. The detection limit of the former was 0.01 ug/ml at 375 nm while for the latter was 0.05 ug/ml at 470 nm when excited at 360 nm. Finally oxypertine determination also seemed less sensitive with the o-phthalaldehyde reaction than by native fluorescence. The detection limit was 0.01 ug/ml at 520 nm when excited at 380 nm, with a linear relation between fluorescence intensity and concentratoon over the range of 0.01-0.06 ug/ml. (fig. 17). The detection limit by native fluorescence at 350 nm was 0.002 ug/ml.

In general, the native fluorescence method appears more sensitive in comparison with the o-phthalaldehyde reaction. The sensitivity of the former was 3, 10, 5 and 50 times more sensitive than the latter for the compounds 5-Hydroxyindole-3-acetic acid, 5-Hydroxytryptophan, indomethacin and oxypertine respectively. However the o-phthalaldehyde method although less sensitive is useful to determine the above compounds in solution which contain substances that emit at the wavelengths of native fluorescence. The o-phthalaldehyde reaction could be used to shift the wavelength of detection of the emission so that there was no interference from the fluorescence of the other constituents present in the same solution.

3.4 Gas Chromatography

Quantitative analysis of the compounds shown previously, with the exception of indomethacin, was carried out by gas chromatography. Many attempts were made to chromatograph these compounds using stationary phases 3% OV1[128], 4% SE30 and OV17[129]. All were unsuccessful. The compounds are very polar and involatile and require conversion into volatile derivatives. Donike[130] reported that the N-trimethylsilylation rate of the indoles by N-Methyl-N-TMS-trifluoruoacetomide is increased by the addition of a

basic derivative and he suggested using TMS imidazole for this purpose. This was carried out on the compounds and seemed to be unsuitable because it did not give a satisfactory chromatogram. N,O-Bis(trimethylsilyl)-acetamide was used to make the derivatives as it produced sharp non-tailing peaks in the gromatogram. The lower limit of detection for 5-Hydroxyindole-3-acetic acid was 0.266 ug, and linear relation between peak height and sample amount over the range of 0.266 -1.06 ug was achieved (fig. 18). The lower limit for 5-Hydroxytryptophan determination was 2 ug, and linearity over the range 2-10 ug was obtained (fig. 19). Helleberg[131] has described a method in which electron-capture gas liquid chromatography permits the specific determination of serum and urine levels of indomethacin from therapuetic doses. However he was able to detect down to a limit of sensitivity of 50 ng/ml of indomethacin. Quantitative analysis of oxypertine by gas chromatography was carried out by silylating the column with silyl-8. The lower limit determined was10 ug and linear relation between peak height and sample volume over the range of 10-16 ug was achieved (fig. 20).

The above results show that the sensitivity of the gas chromatographic method was poorer than the native fluorescence and o-phthalaldehyde reaction methods. This may be due to the detector used, because the type of detector plays a major part in the sensitivity. Electron capture detection for several indole derivatives is approximately 1000 times more sensitive than a flame ionization detector under the same conditions[132].

Conclusion

It has been found that the sensitivity of quantitative analysis by native fluorescence depends upon the pH of the solvent media. Native fluorescence seems to be more sensitive than the o-phthalaldehyde reaction method which in turn is more sensitive than the gas chromatigraphy method The sensitivity of the latter depends upon the stationary phase of column and the type of detector.

3.5 Experimental

An Aminco-Bowman Spectrofluorometer was used for determining the fluorescence. The compounds analysed were pure industrial preparations; 5-Hydroxyindole-3-acetic acid and 5-Hydroxytryptophan (Aldrich Chemical Co. Inc.,) Indomethacin was from Merk Sharp and Dohme and Oxypertine was obtained from Winthrop Lab. Analar reagents were used where appropriate.

Native Fluorescence

A stock solution of each substance was diluted to known concentrations. Ten measurements of blanks were taken in each determination which were, 8N Hcl in the case of the acidic solution, ethanol in the case of the neutral solution and O.1N NaoH in the case of the alkaline solution.

Excitation and emission wavelengths are listed in table 15. The slit setting on the instrument for all compounds were

Excitation inner slit	3 mm
Excitation outer slit	3 mm
Emission inner slit	2 mm
Emission inner slit	2 mm
Photomultiplier slit	4 mm

O-Phthalaldehyde reaction

O-phthalaldehyde solution was prepared by dissolving 40 mg O-phthalaldehyde in 100 ml absalute methanol (freshly prepared). Ten measurements of the blank were taken in each determination. Excitation, and emission wavelengths are listed in table 16. The reactions were carried out as follows:-

To 1 ml of O.05 N Hcl containing 1 ug, 1.5 ug, 2.5 ug, 3 ug, 4 ug or 5 ug of 5-Hydroxyindole-3-acetic acid in 6 test tubes respectively and 10 test tubes of blanks, 0.05 ml of the O-phthalaldehyde solution was added. After mixing, 4 ml of 10 N Hcl was added to each tube and after mixing again, the tubes were heated in a boiling water bath for 1 hour. After cooling to room temperature the fluorescence was measured with the following settings.

Excitation inner slit	3 mm
Excitation outer slit	3 mm
Emission inner slit	2 mm
Emission outer slit	2 mm
Photomultiplier slit	4 mm

The same experiment was carried out with 5-Hydroxytryptophan. In the case of oxypertine and indomethacin the settings of the instrument and the solvent were changed as follows:-

To 1 ml of ethanol containing 0.025 ug, 0.05 ug, 0.1 ug, 0.15 ug, 0.2 ug, 0.25 ug or 0.3 ug of oxypertine in 7 test tubes respectively and 10 test tubes of blanks, 0.05 ml of theO-phthalaldehyde solution was added. After mixing, 4 ml of 10N Hcl was added to each tube and after mixing again, the tubes were heated in a boiling water bath for 1 hour. After cooling to room temperature the fluorescence was measured with the following settings.

Excitation inner slit	5 mm
Excitation outer slit	5 mm
Emission inner slit	3 mm
Emission outer slit	5 mm
Photomultiplier slit	4 mm

The same experiment was carried out with indomethacin so that each ml of solution contained either 0.15 ug, 0.25 ug, 0.4 ug, 0.5 ug, or 0.6 ug with the same conditions and setting of the instrument.

Gas Chromatography

All separations were carried out on a F11 Perkin-Elmer Gas Chromatography equipped with flame ionization detector. A Perkin-Elmer 56 Recorder was used to record the separation by the 3% SE30 (100-120 mesh) chrom WAWHMDS column. Derivatization was carried out in reactive vials, and the following methods were used to make the derivatives:-

(i) For 5-Hydroxyindole-3-acetic acid; solutions of 2 mg, 4 mg, 6 mg, 8 mg and 10 mg 5-Hydroxyindole-3-acetic acid in 5 ml acetonitrile were prepared. To 0.2 ml of each above solution in vials and a blank of 0.2 ml acetonitrile in another vial, 0.1 ml N,O-Bis(trimethylsilyl)-acetamide was added. The vials were then heated in a boiling water bath for 2 hours then cooled. The determination was carried out under the following operating conditions:-

Oven temperature	220°C
Injection temperature	310°C
N_2 pressure	25lb/in^2
H_2 pressure	20lb/in^2
Air pressure	20lb/in^2
Sensitivity range	2 x 10^2
Chart speed of recorder	5 mm/min.

(ii) For 5-Hydroxytryptophan; to 0.2 ml of acentonitrile containing either 0.6 mg, 1.2 mg, 1.8 mg, 2.4 mg or 3 mg 5-Hydroxytryptophan in vials with a blank vial containing 0.2 ml of acetonitrile, 0.1 ml N,O-Bis(trimethyl-slilyl)-acetomide was added. The vials were then heated in a boiling water bath for 2 hours and then cooled, and the separation was determined under the following operating conditions:

Oven temperature	235°C
Injection temperature	340°C
N_2 pressure	25lb/in^2
H_2pressure	20lb/in^2
Air pressure	20lb/in^2
Sensitivity range	10 x 10^2
Chart speed of recorder	5 mm/min.

(iii) In the case of oxypertine the column was silylated by injecting 35 ul of silyl-8 with an oven temperature of 200°C. The temperature was kept at 200°C for 10 minutes and then reduced to room temperature. Operating conditions in this case were:

Oven temperature	300°C
Injection temperature	405°C
N_2 pressure	25lb/in^2
H_2 pressure	20lb/in^2
Air pressure	20lb/in^2
Sensitivity range	5 x 10^2
Chart speed of recorder	5mm/min.

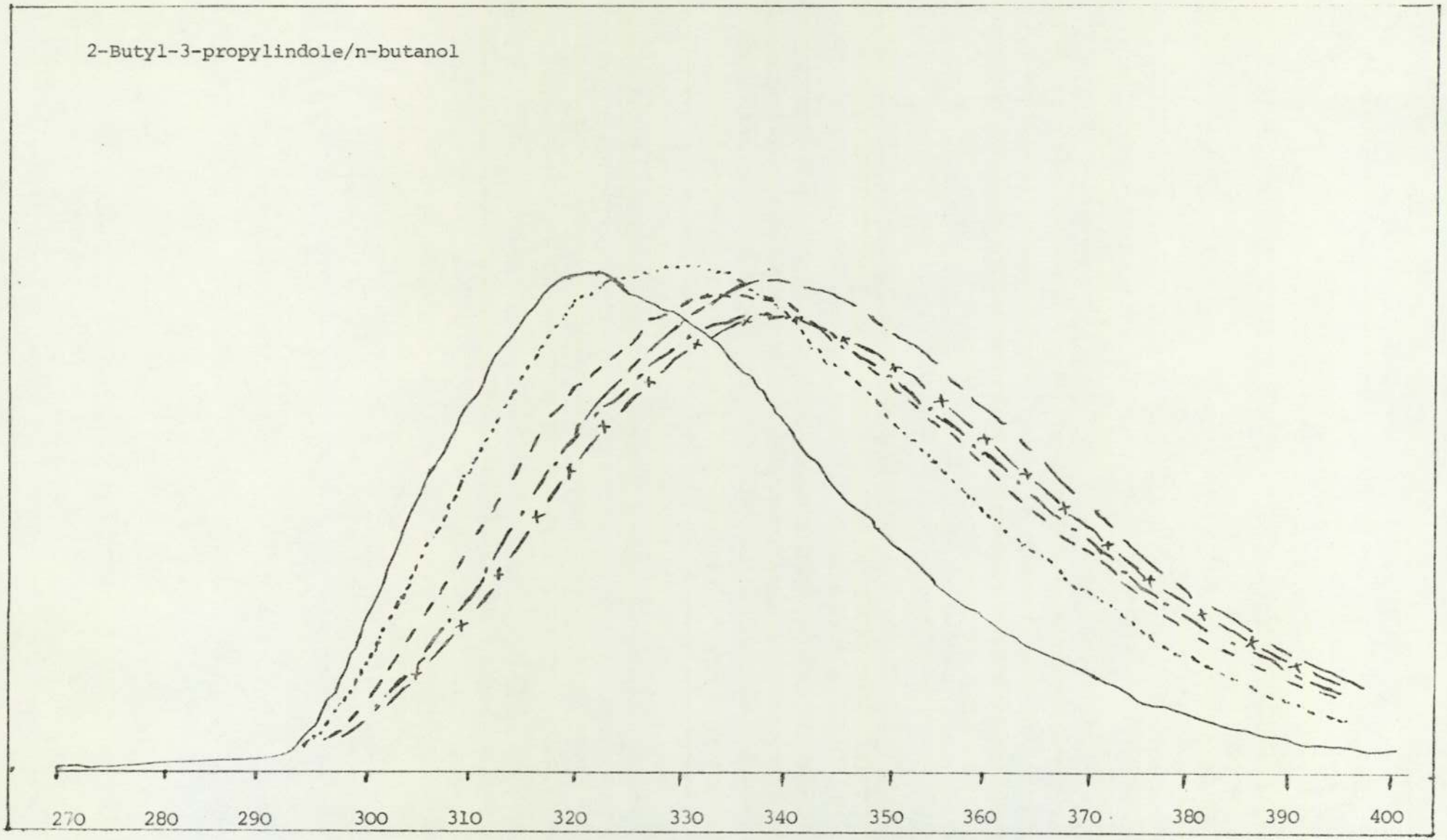
Under the above conditions the following amounts; 8 ug, 9 ug, 10 ug, 12 ug, 14 ug, and 16 ug of oxypertine was determined.

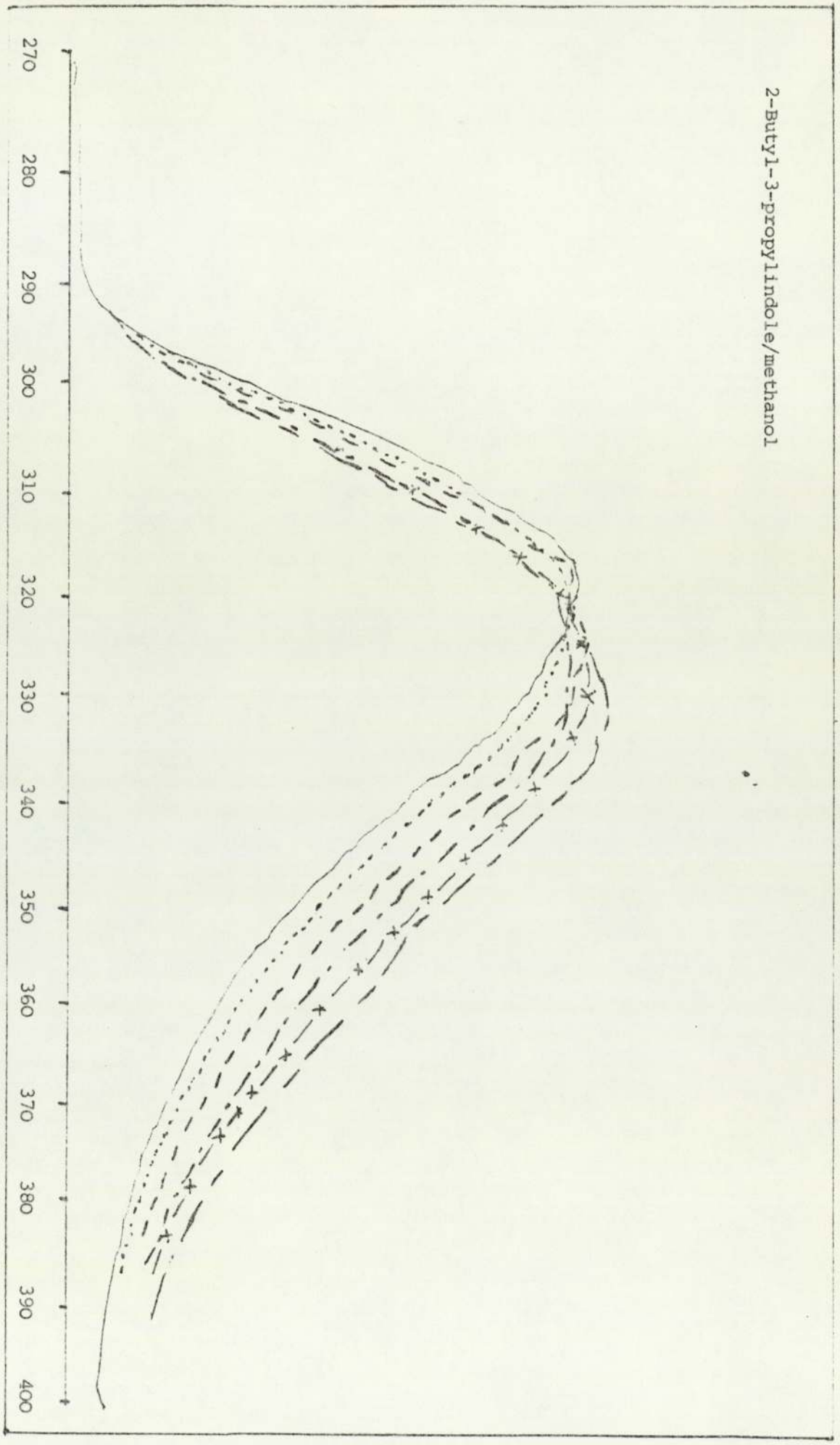
Appendix

Spectra 1 to 49 are presented of the indoles with the given polar solvent in cyclohexane solution at the polar solvent concentrations of:

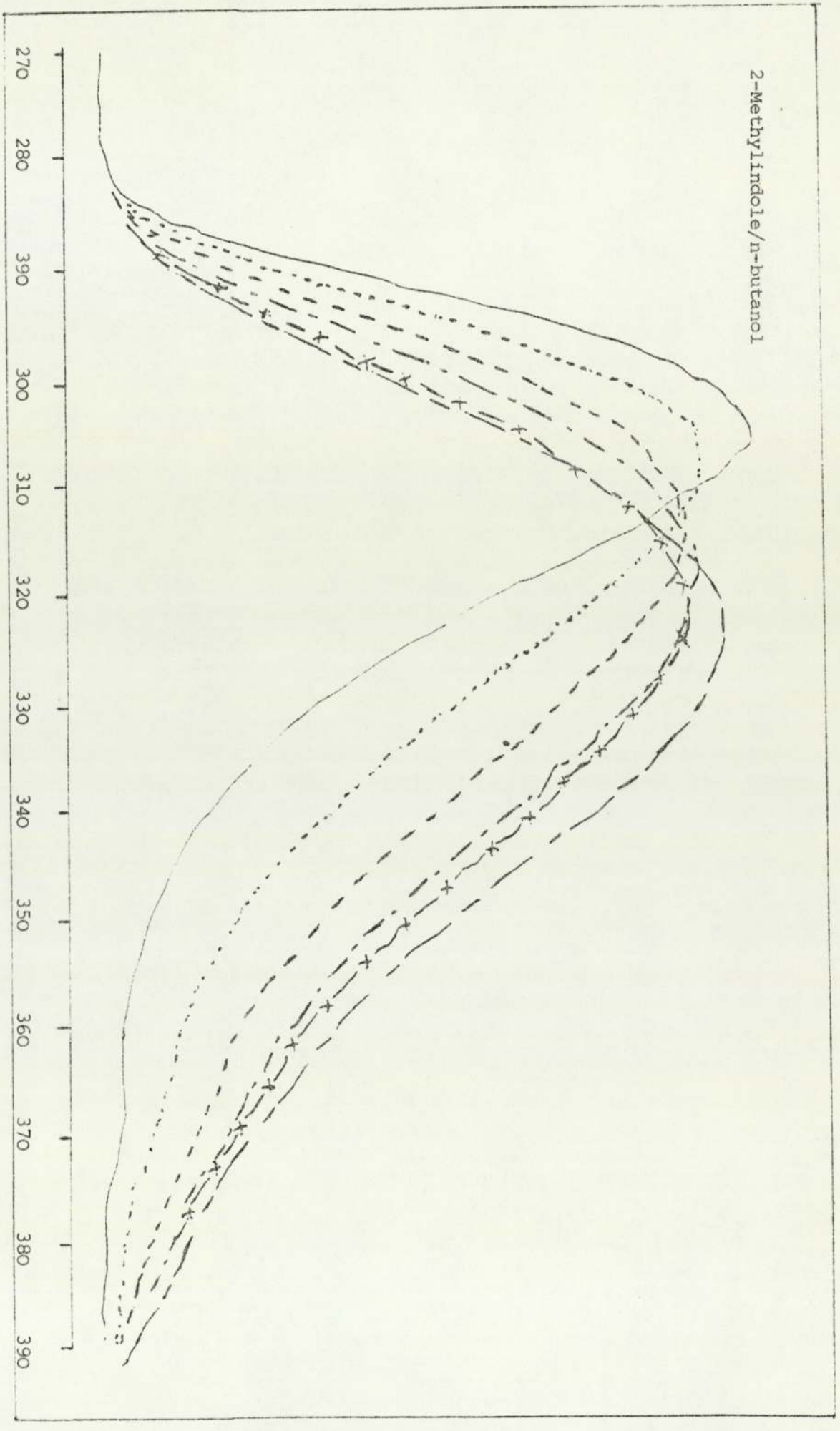
(1)	————————————————	0.00M
(2)	....	0.04M
(3)	------------------------------------	0.08M
(4)	.—.—.—.—.—.—.—.—.—.—.—.—.	0.12M
(5)	—x—x—x—x—x—x—x—x—x—x—x—x	0.16M
(6)	——— - ——— - ——— - ——— -	0.20M

The wavelengths and shifts of wavelength are given in nanometers (nm).

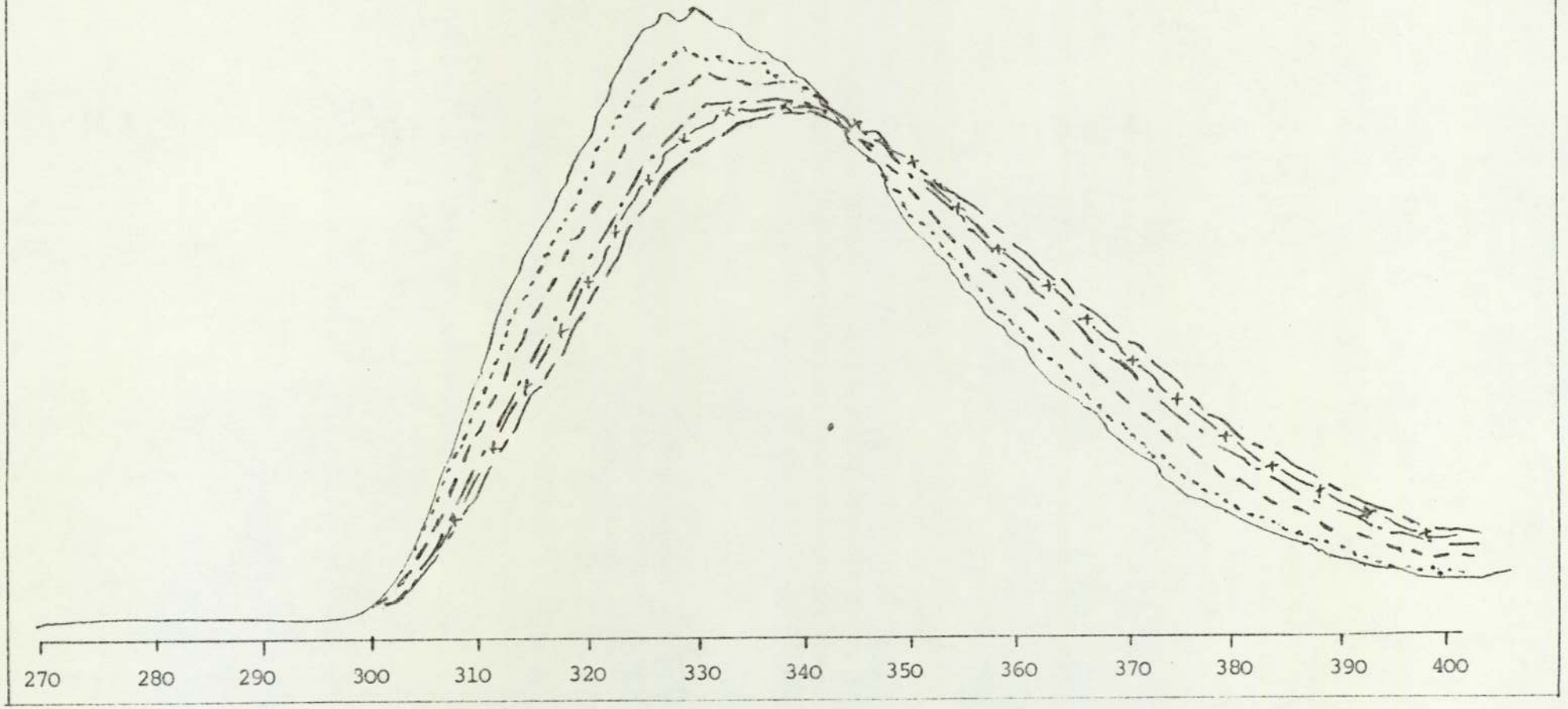

2-Butyl-3-propylindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

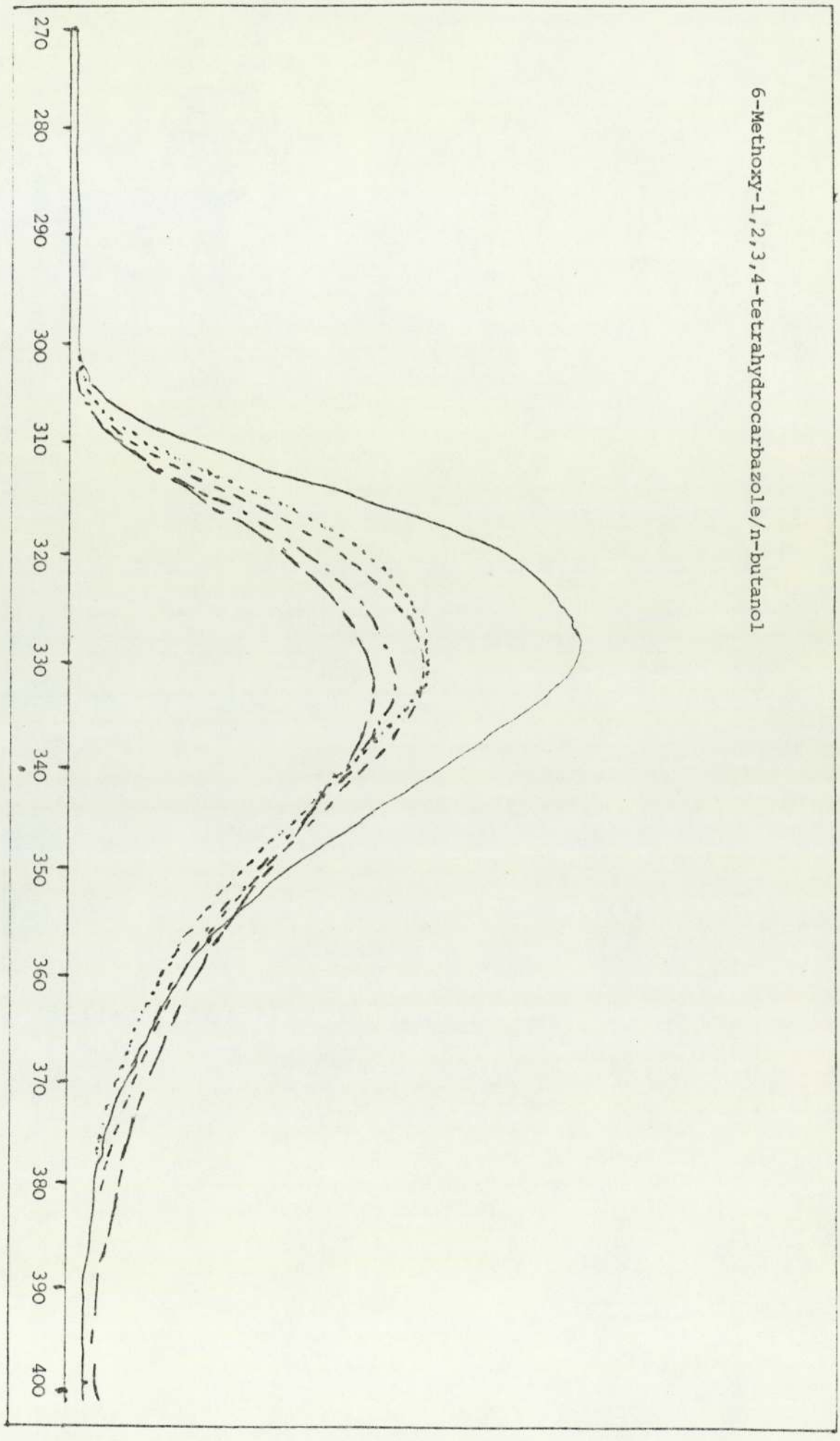

2-Butyl-3-propylindole/methanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

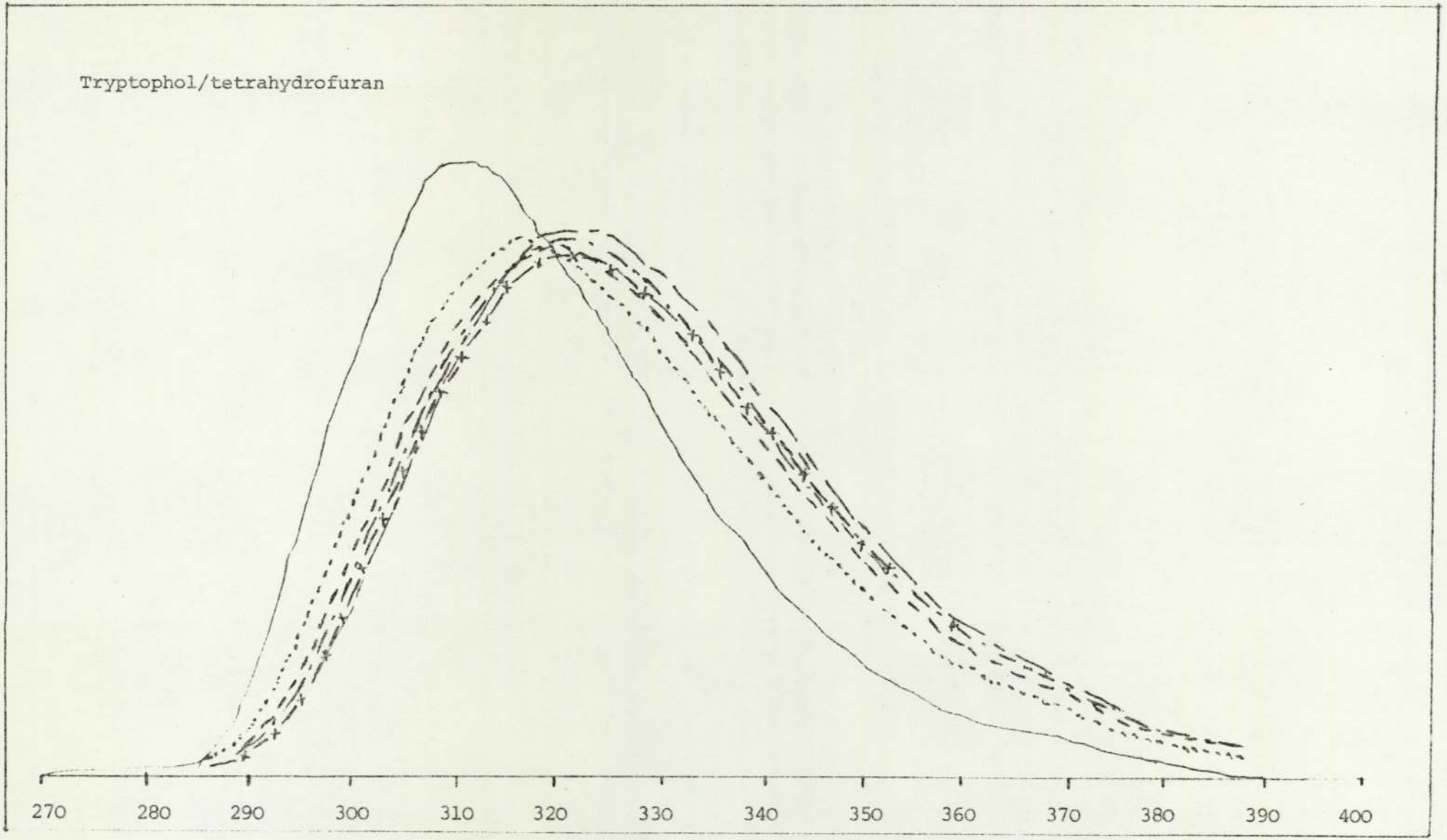
2


2-Methylindole/n-butanol
270
280
390
300
310
320
330
340
350
360
370
380
390

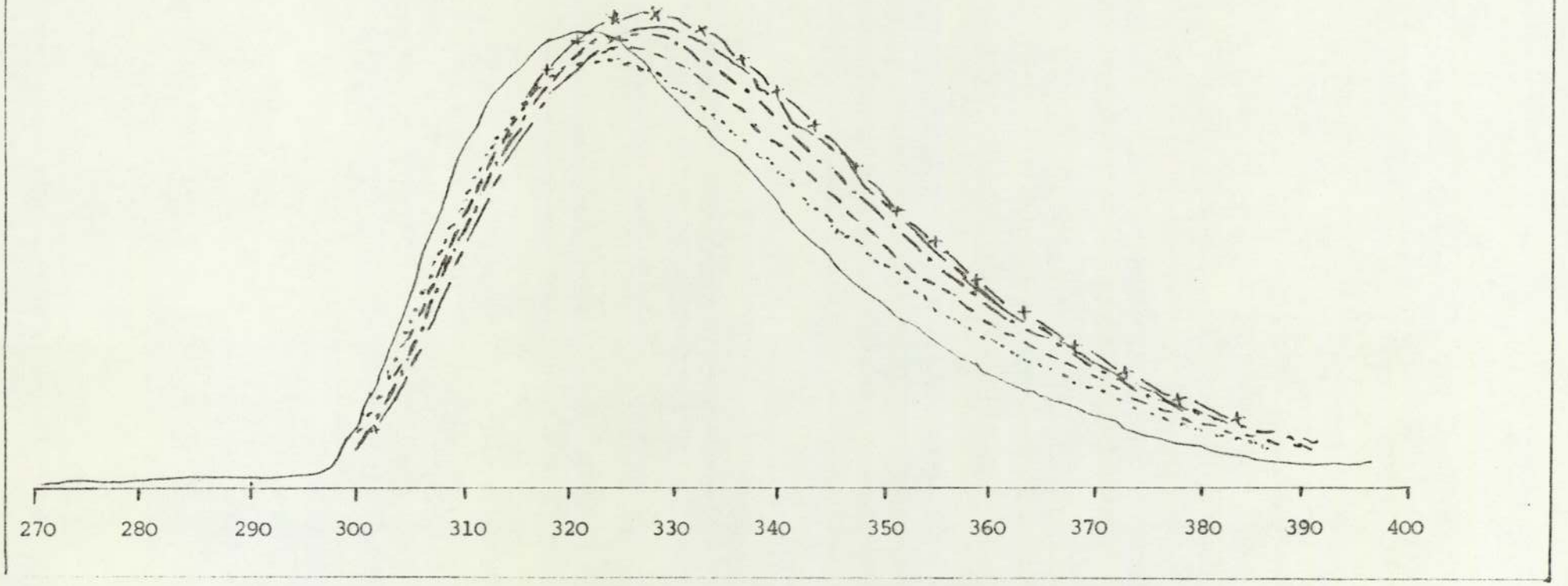
1,2,3-Trimethylindole/n-butanol



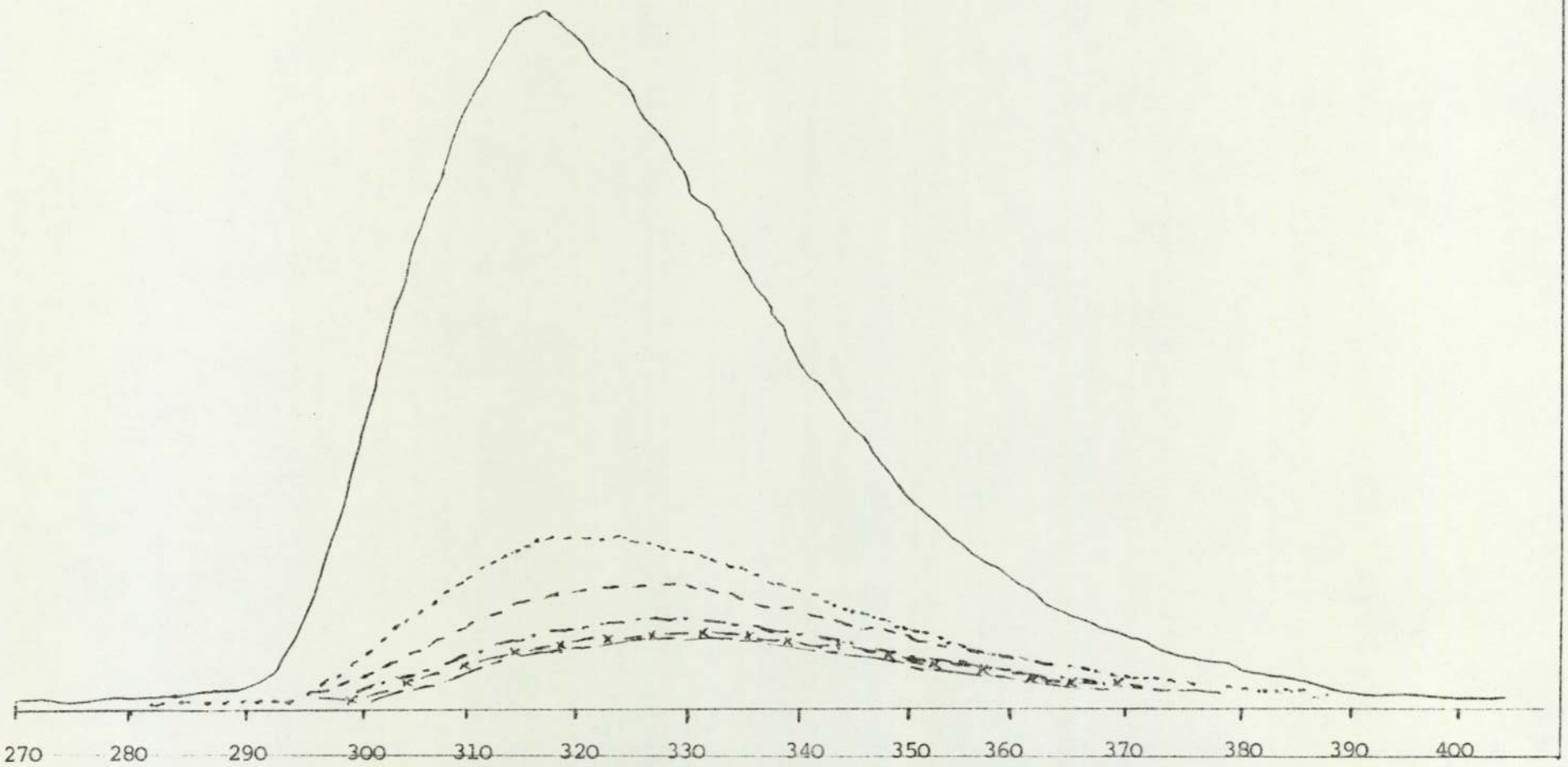

6-Methoxy-1,2,3,4-tetrahydrocarbazole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

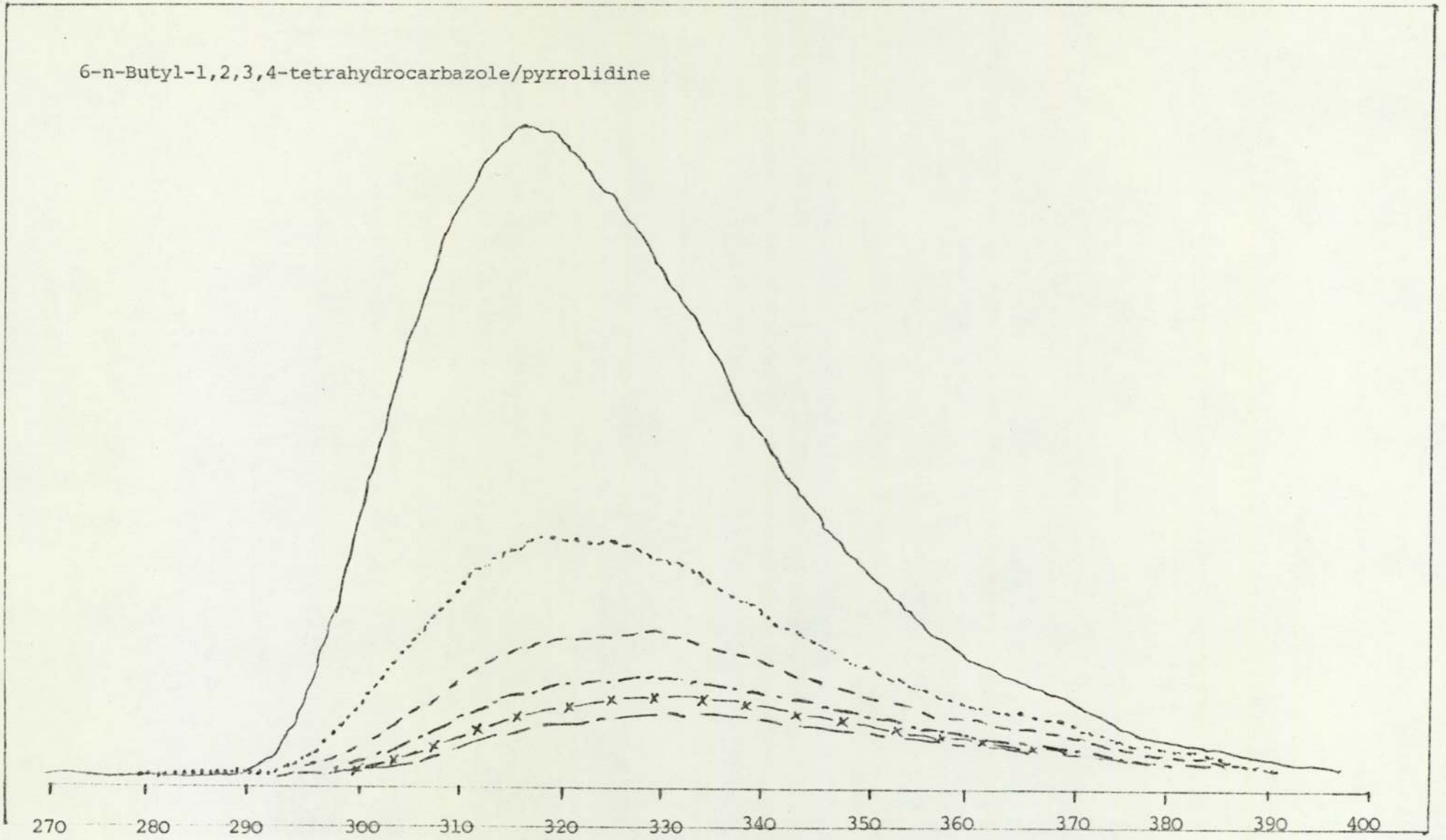

Tryptophol/tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390
400

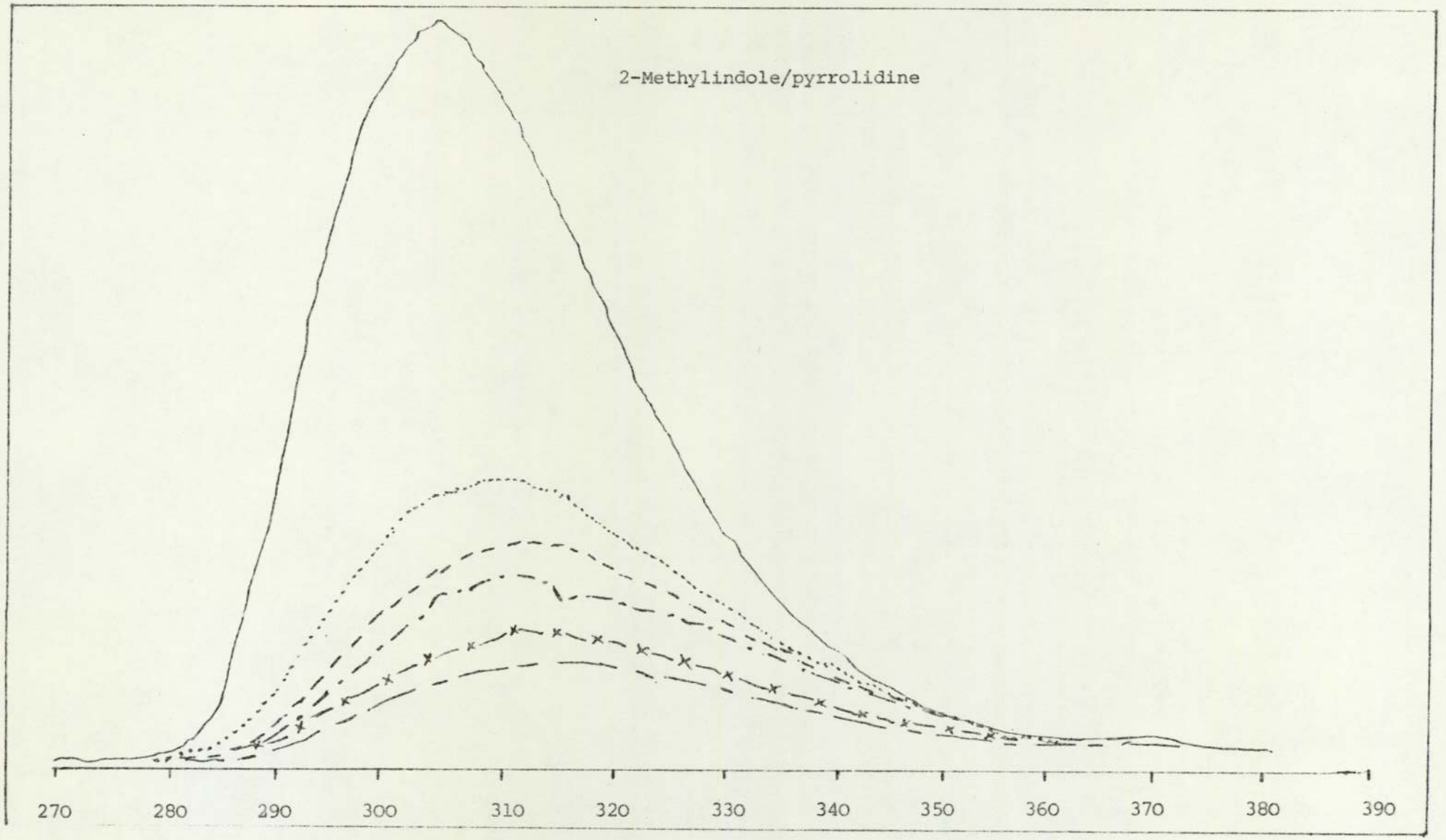
6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole/tetrahydrofuran

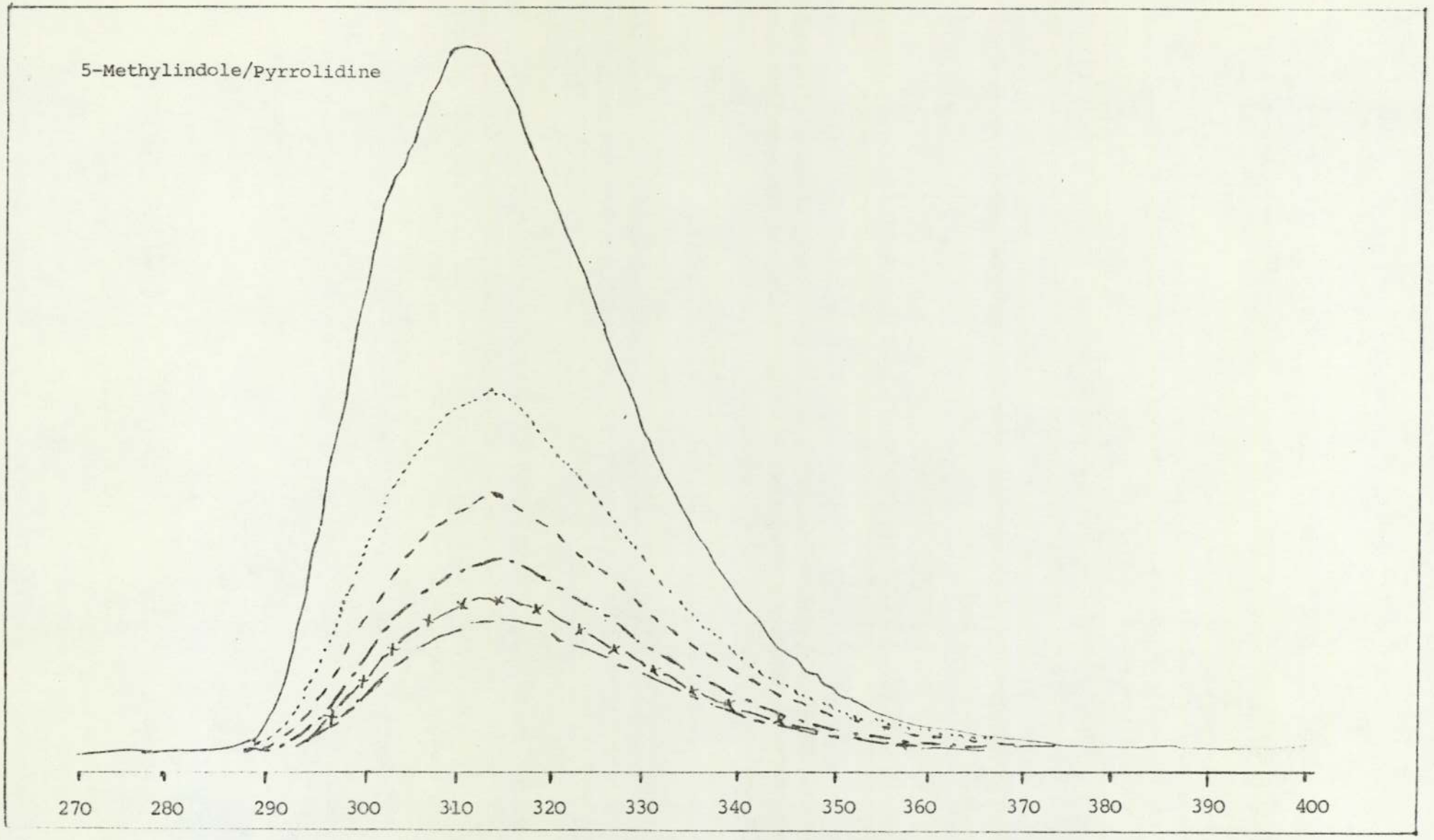


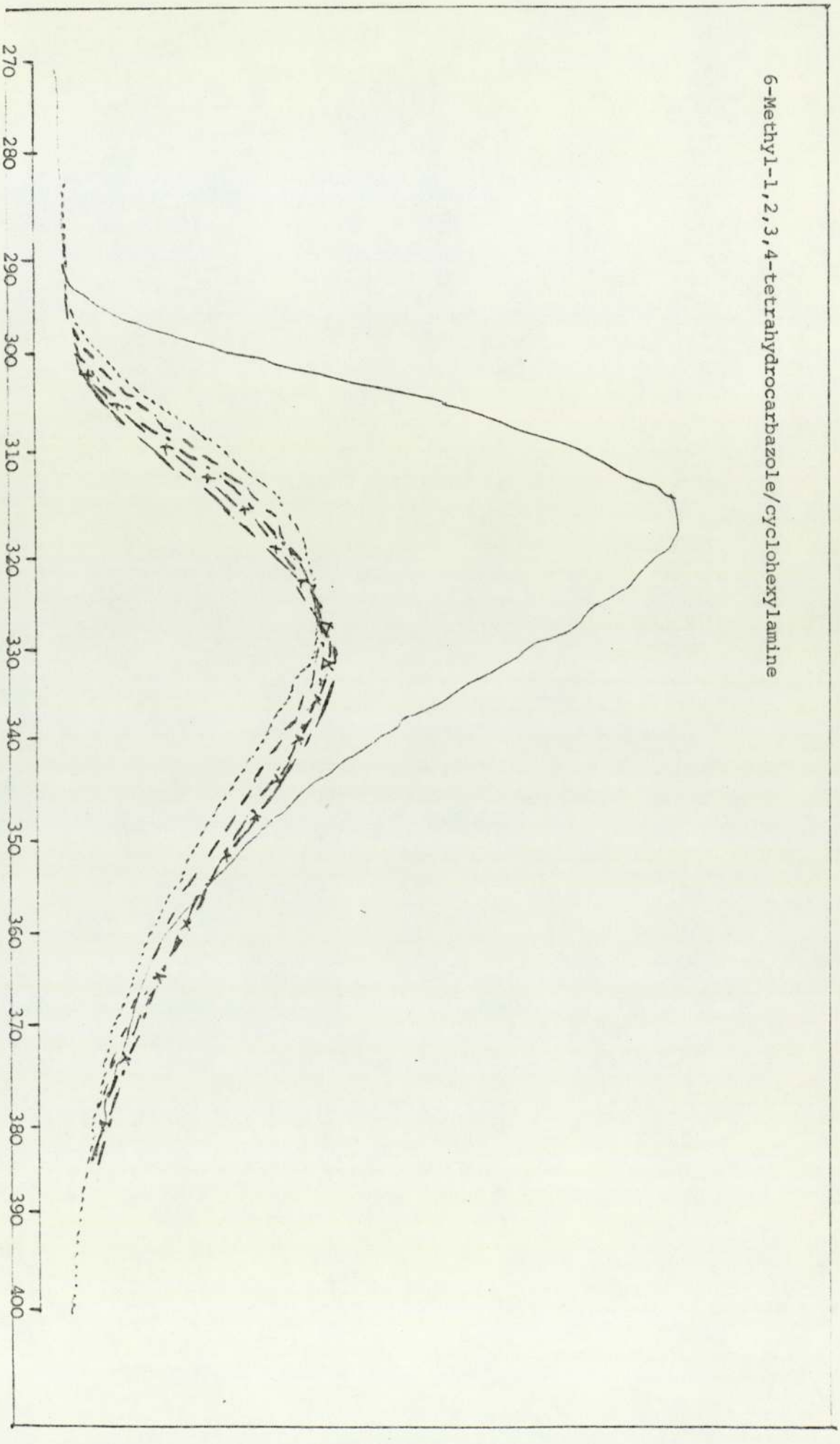
6-Methyl-1,2,3,4-tetrahydrocarbazole/pyrrolidine

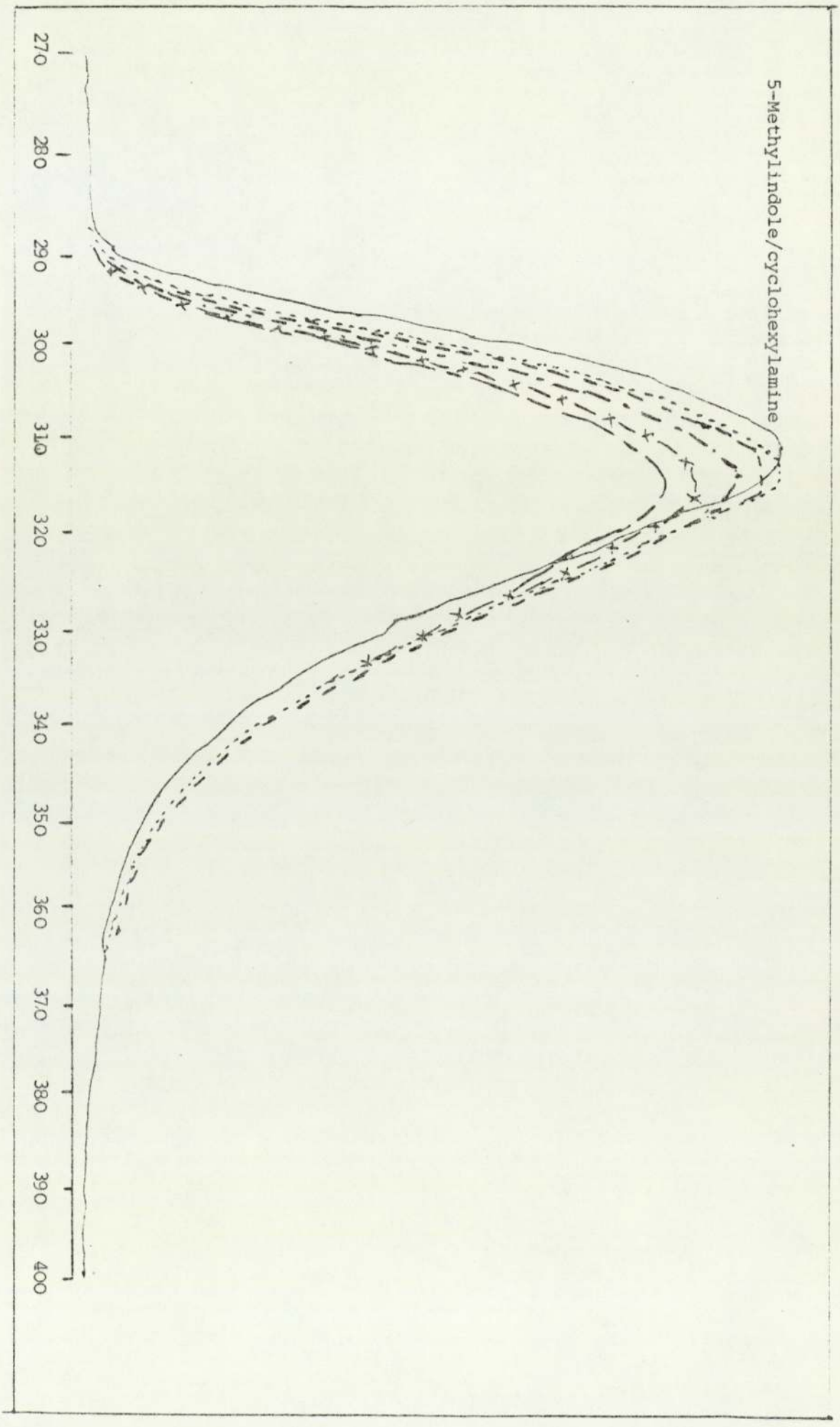


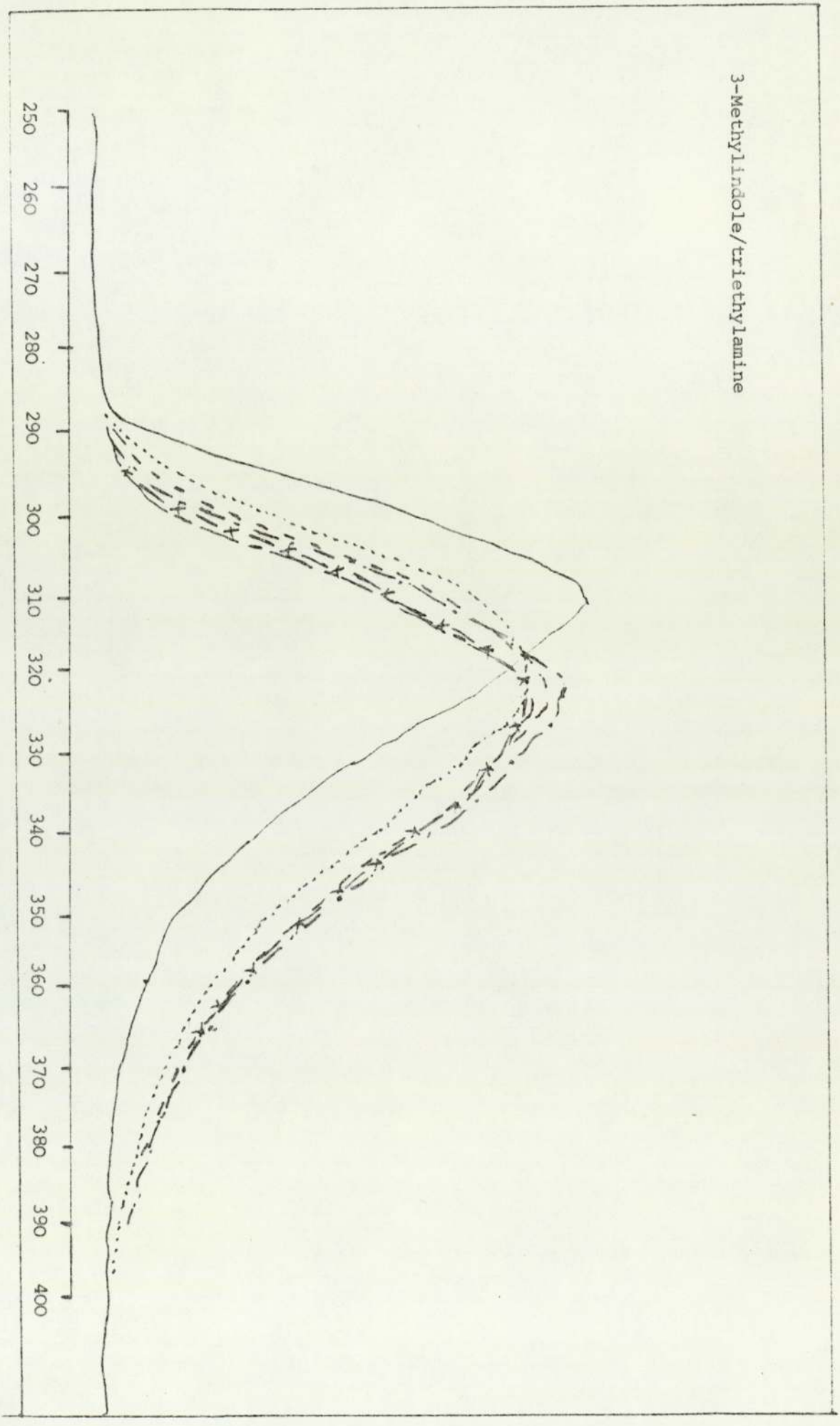

6-n-Butyl-1,2,3,4-tetrahydrocarbazole/pyrrolidine
- 100 -
270
280
290
300
310
320
330
340
350
360
370
380
390
400

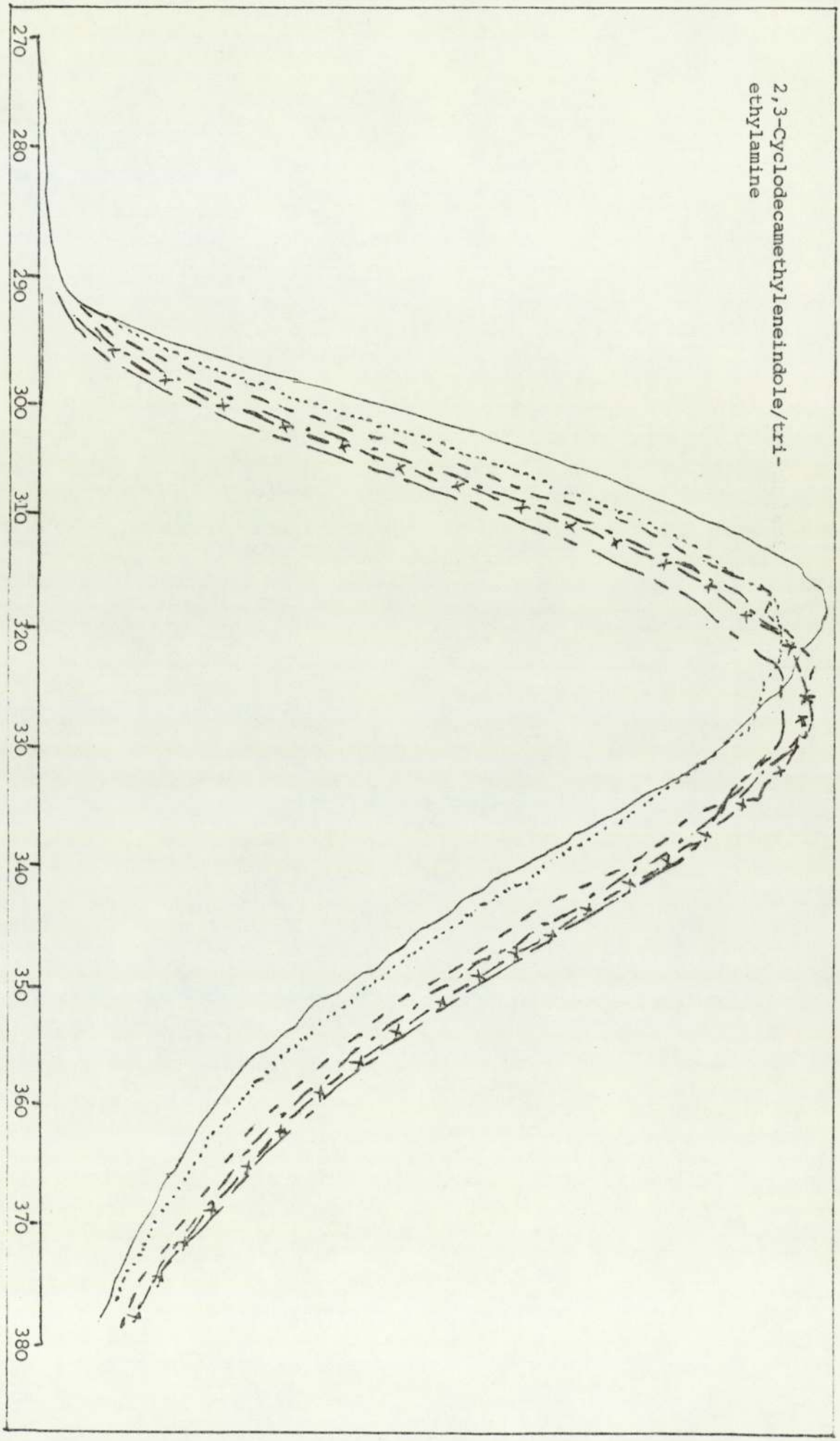

2-Methylindole/pyrrolidine
270
280
290
300
310
320
330
340
350
360
370
380
390

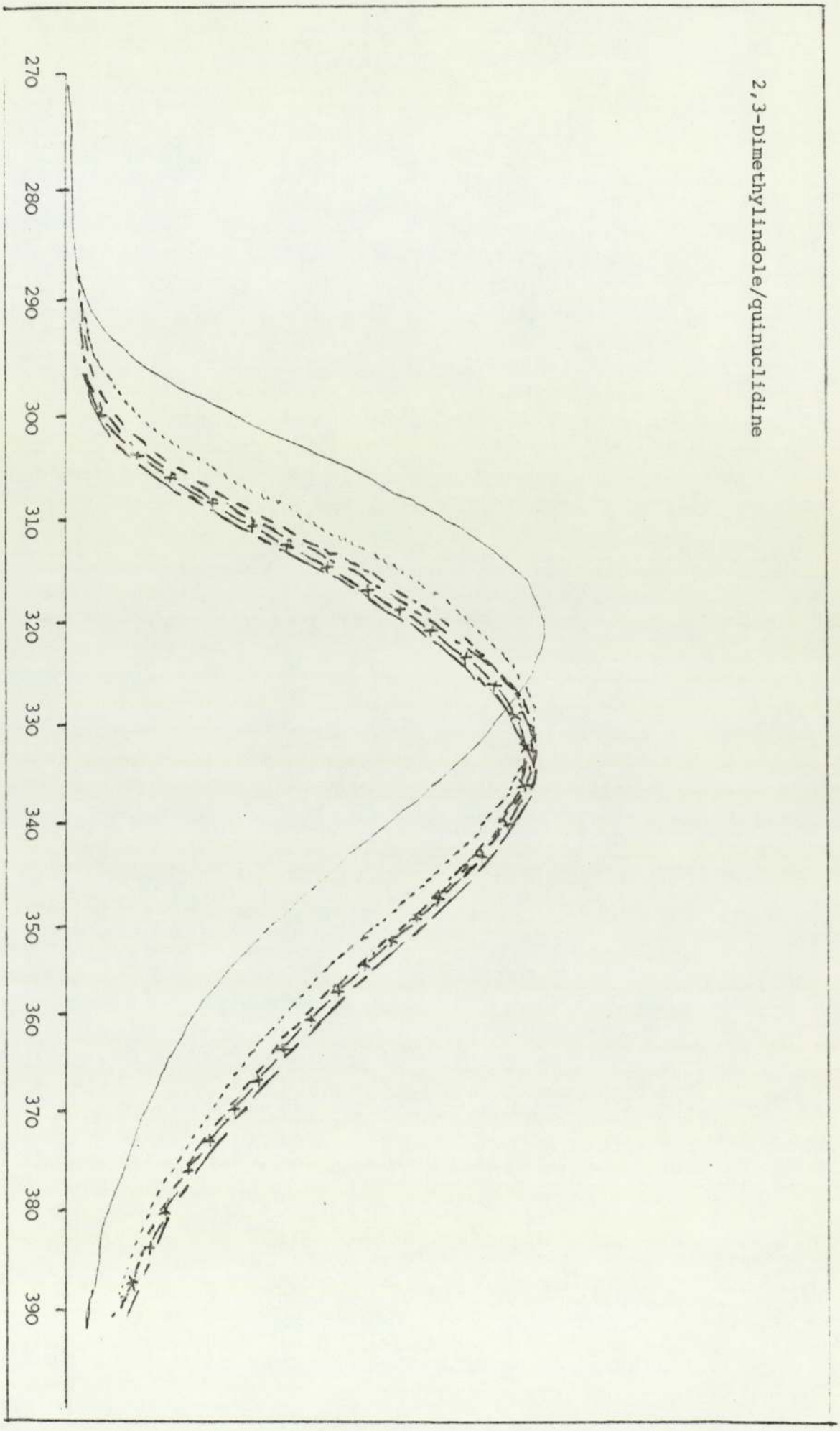

5-Methylindole/Pyrrolidine
270
280
290
300
310
320
330
340
350
360
370
380
390
400


6-Methyl-1,2,3,4-tetrahydrocarbazole/cyclohexylamine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

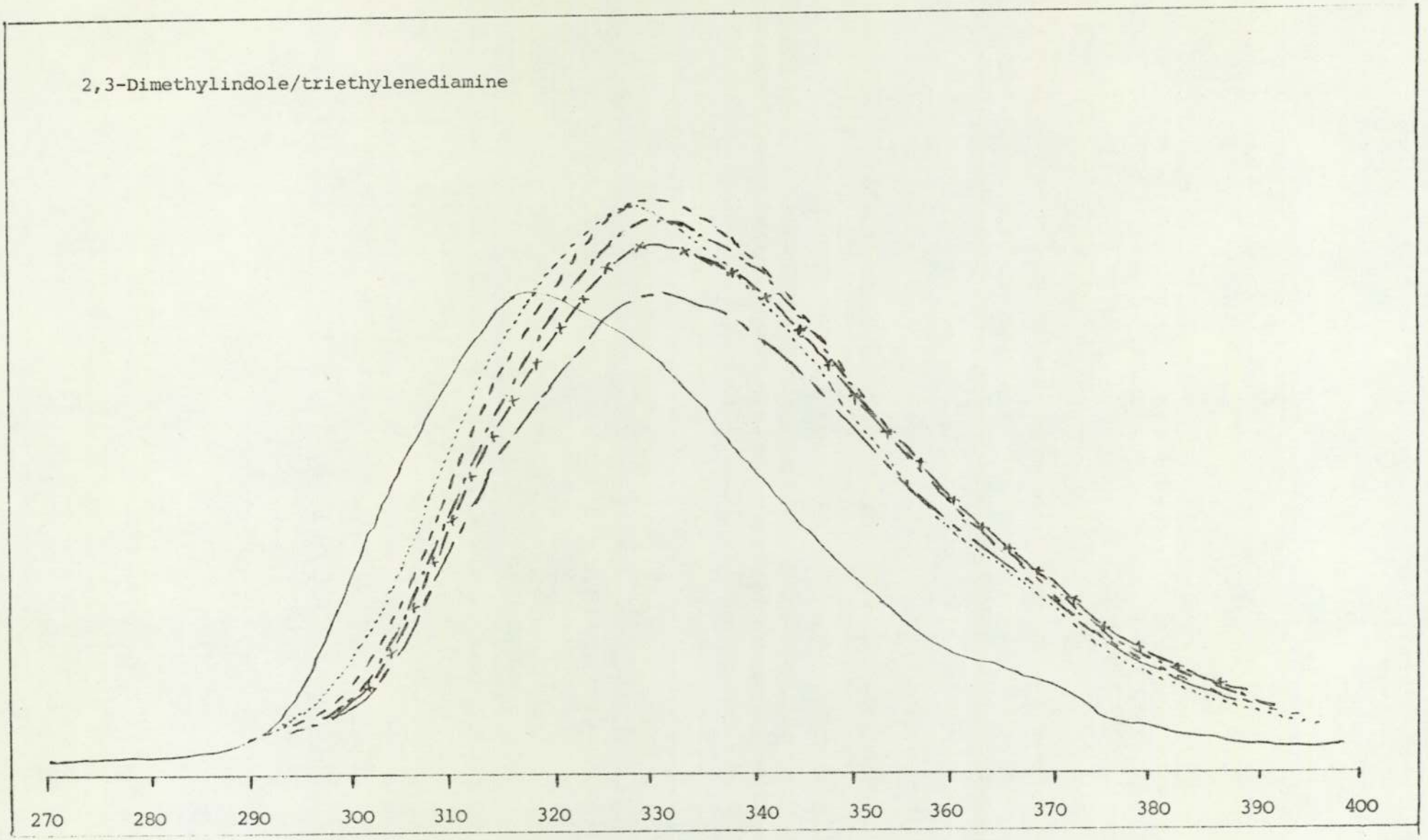

5-Methylindole/cyclohexylamine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

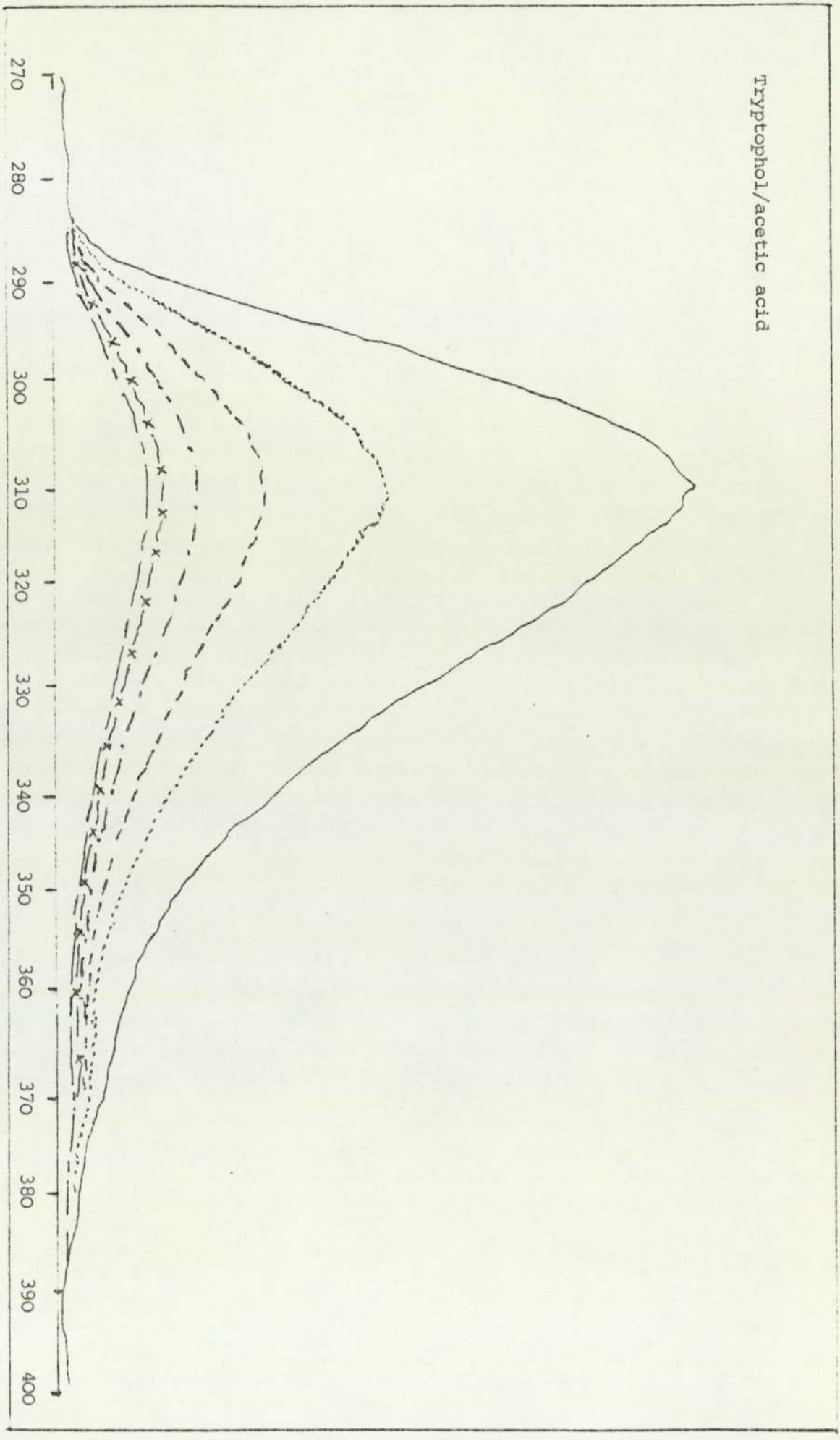

3-Methylindole/triethylamine
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400


2,3-Cyclodecamethyleneindole/tri-
ethylamine
270
280
290
300
310
320
330
340
350
360
370
380

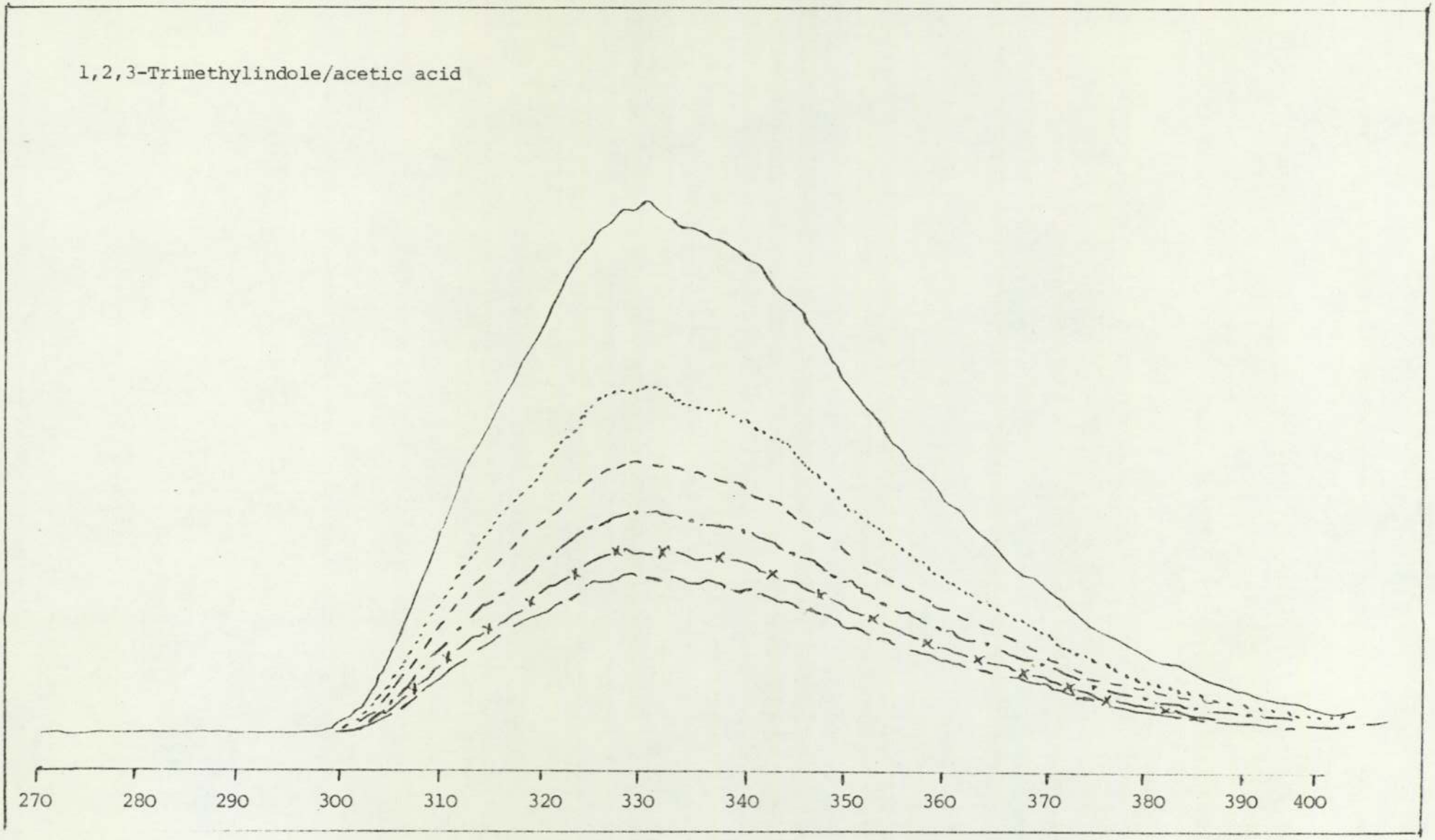

2,3-Dimethylindole/quinuclidine
270
280
290
300
310
320
330
340
350
360
370
380
390

2,3-Dimethylindole/triethylenediamine

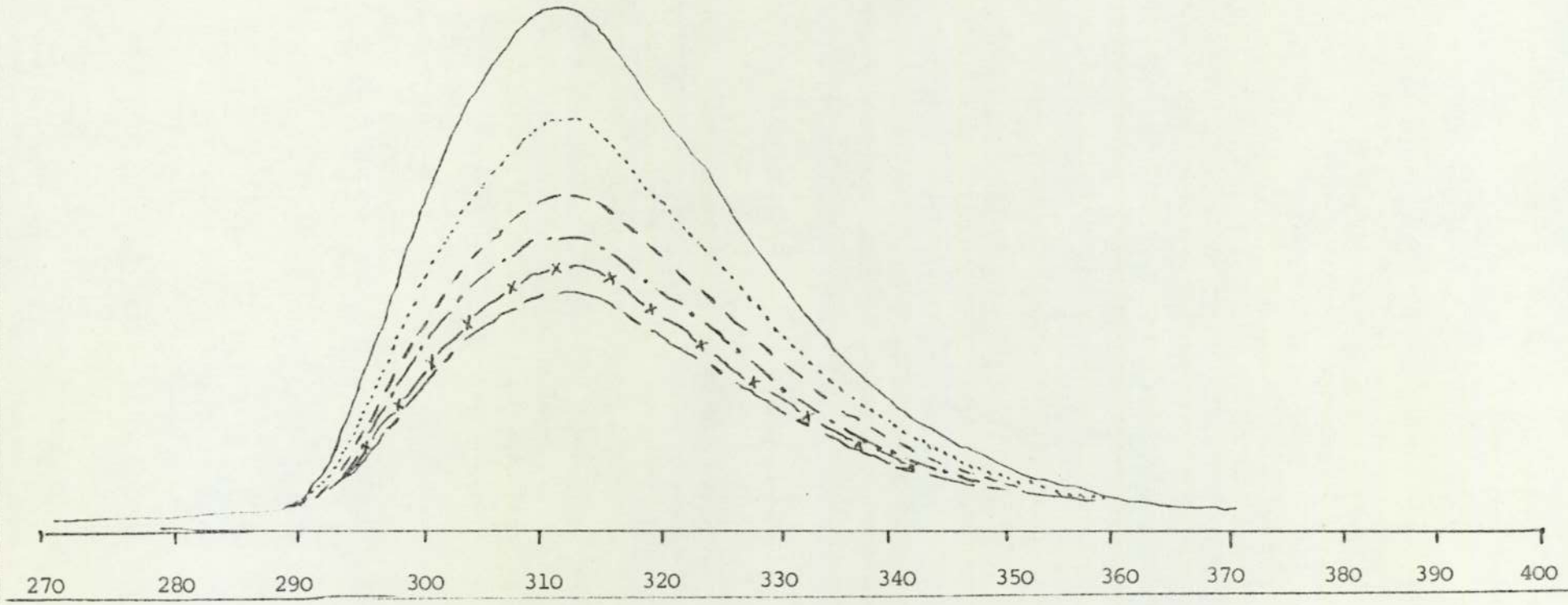


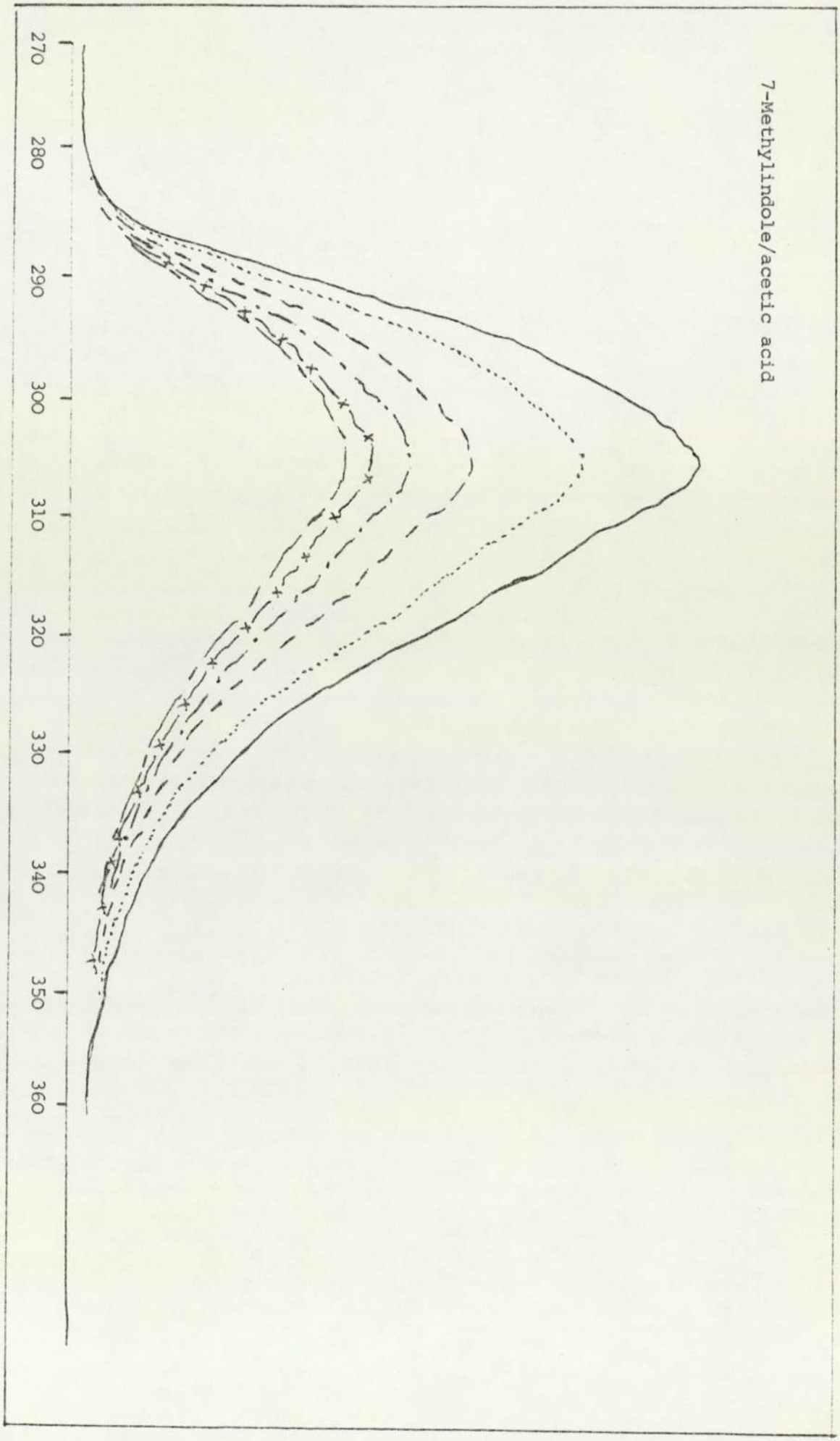

Tryptophol/acetic acid
270
280
290
300
310
320
330
340
350
360
370
380
390
400

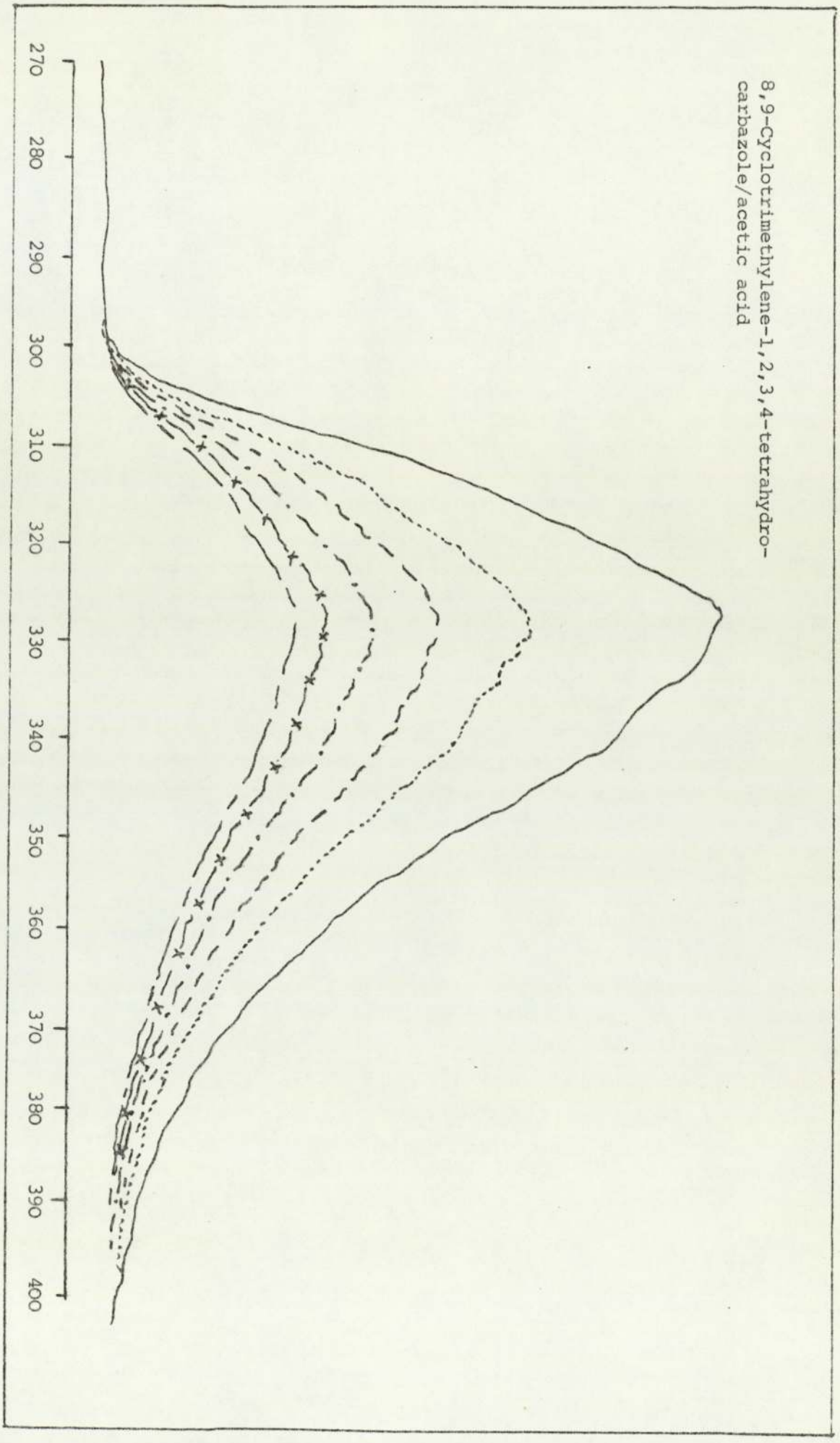
1,2,3-Trimethylindole/acetic acid

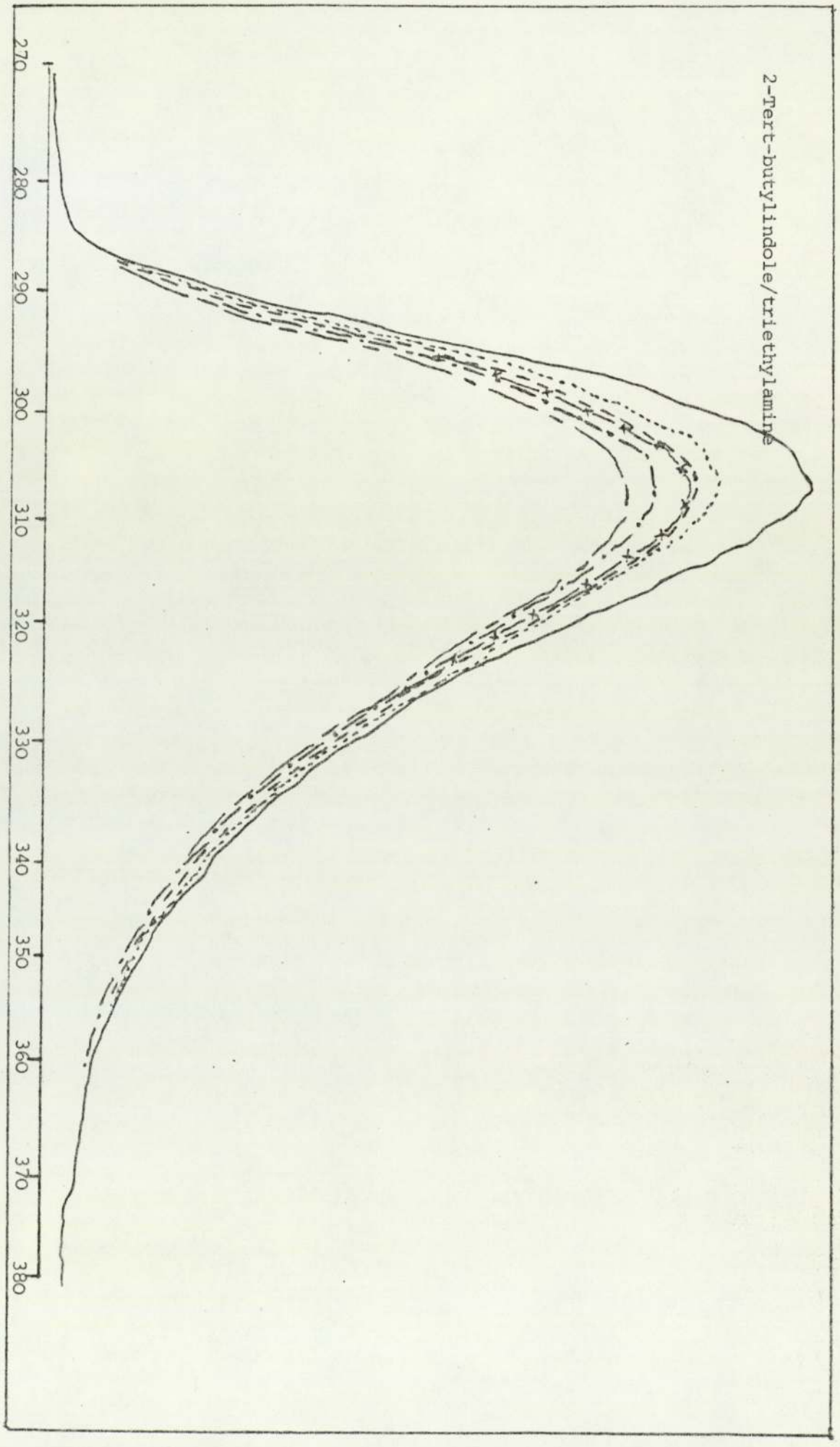


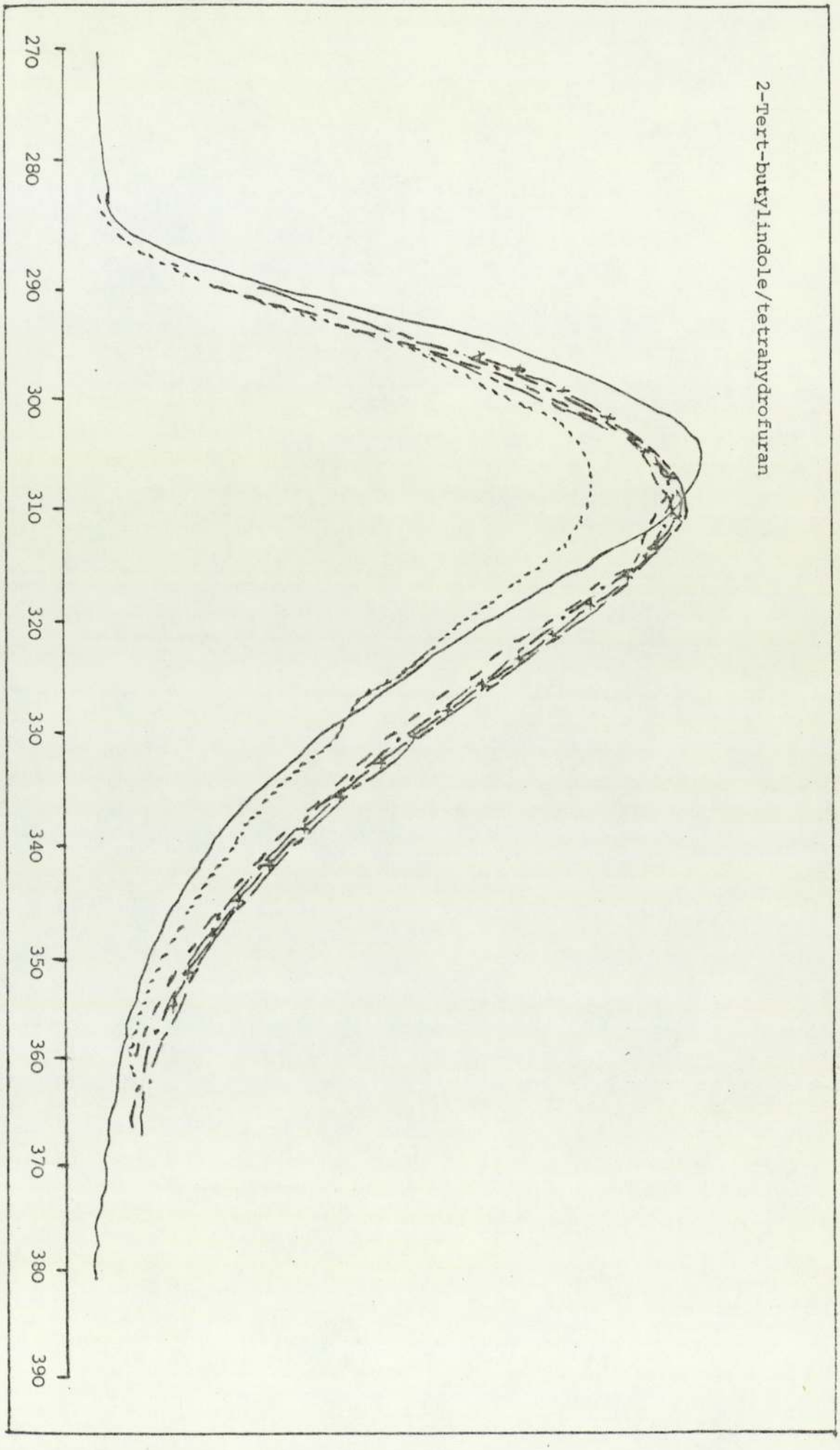
5-Fluoroindole/acetic acid

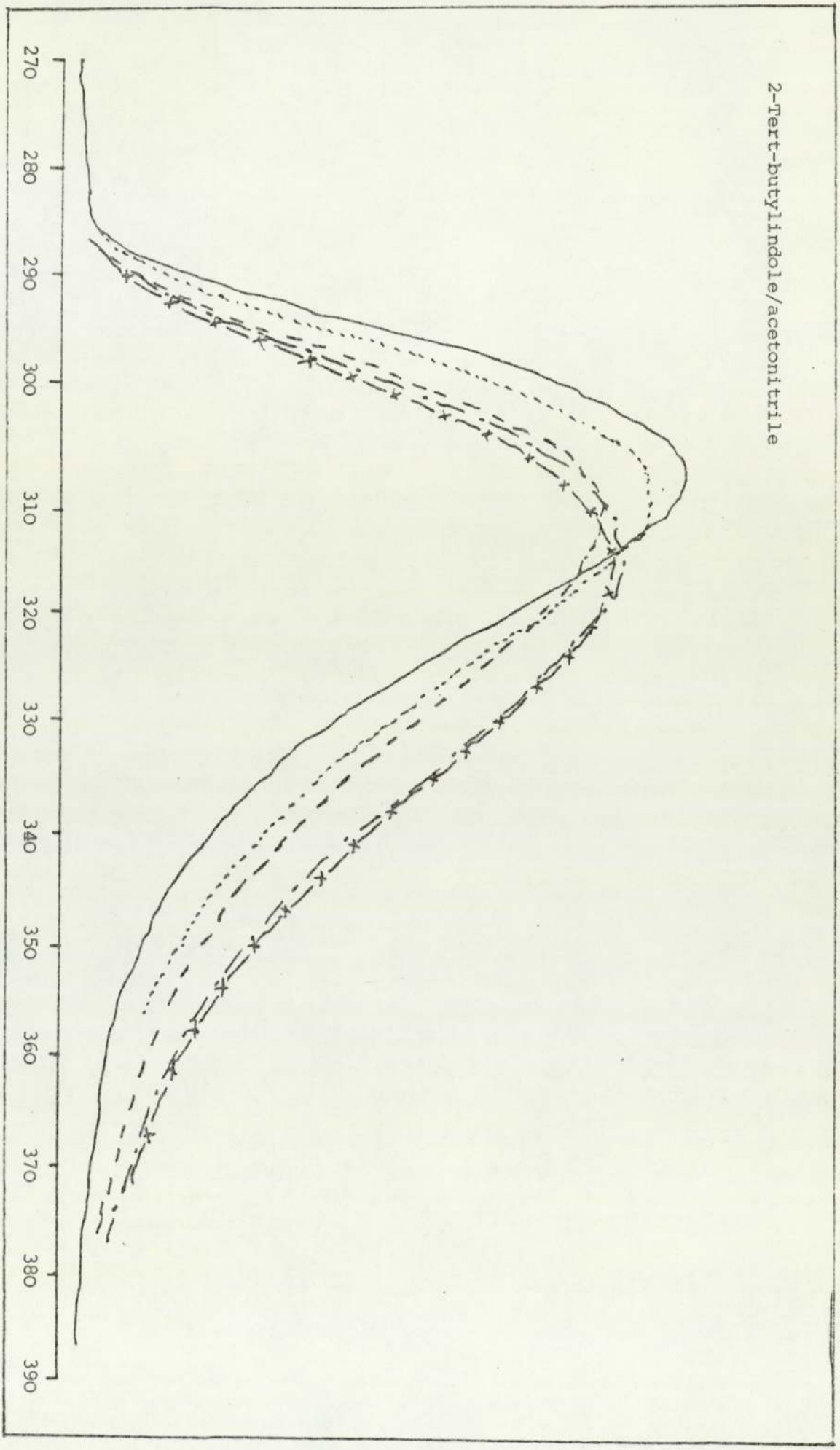


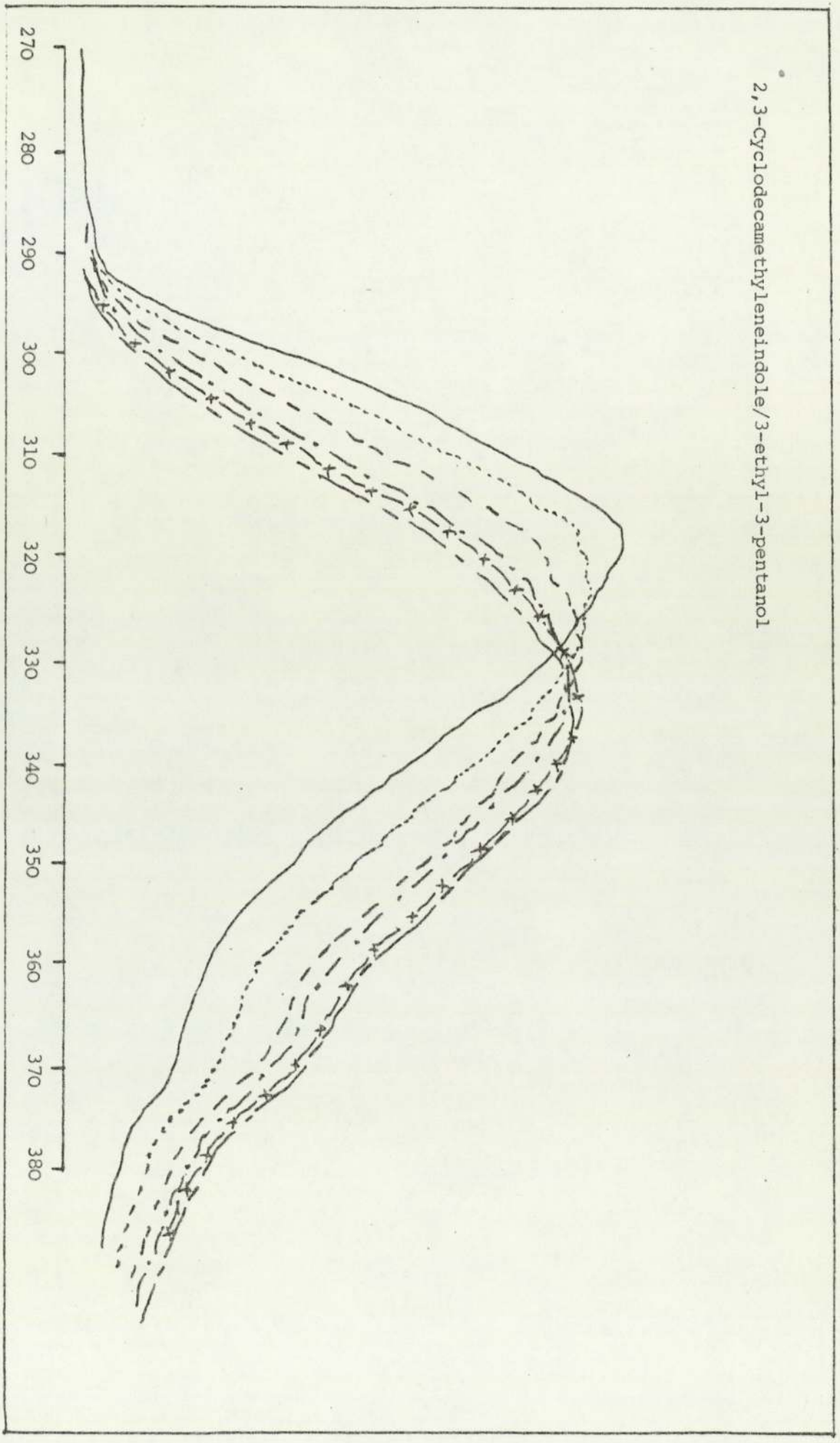

7-Methylindole/acetic acid
270
280
290
300
310
320
330
340
350
360


8,9-Cyclotrimethylene-1,2,3,4-tetrahydro-
carbazole/acetic acid
270
280
290
300
310
320
330
340
350
360
370
380
390
400

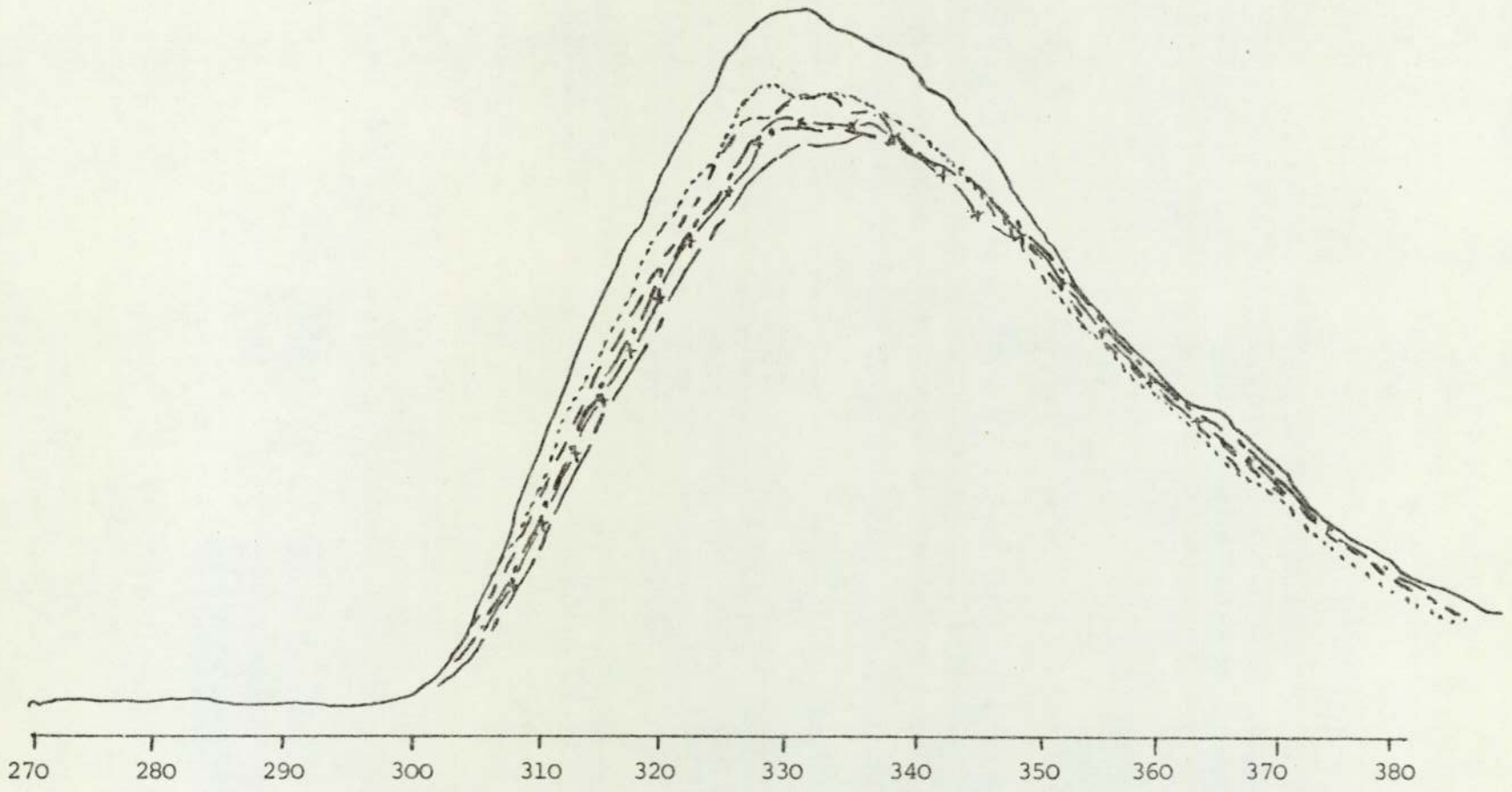

2-Tert-butylindole/triethylamine
270
280
290
300
310
320
330
340
350
360
370
380

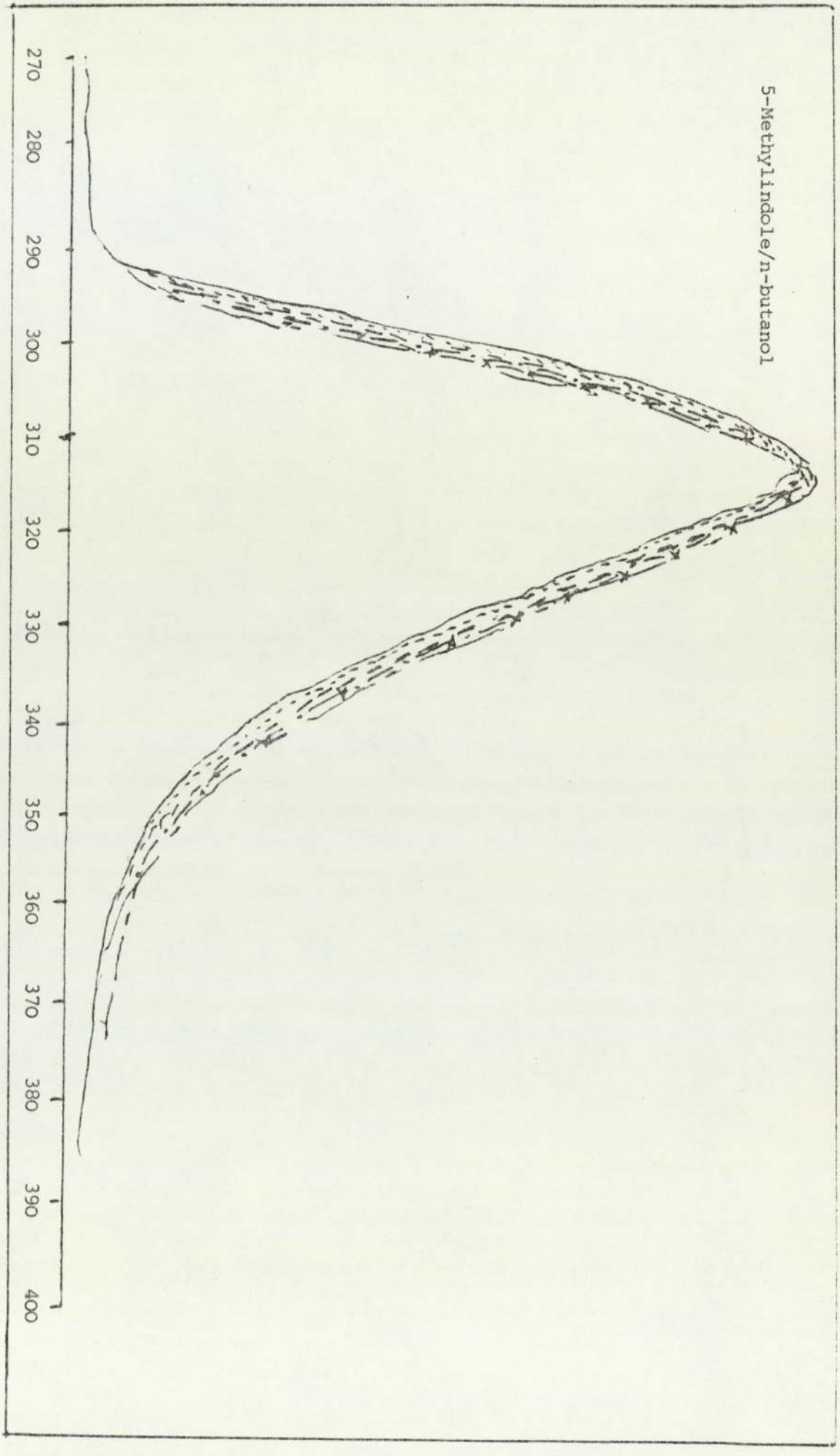

2-Tert-butylindole/tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390

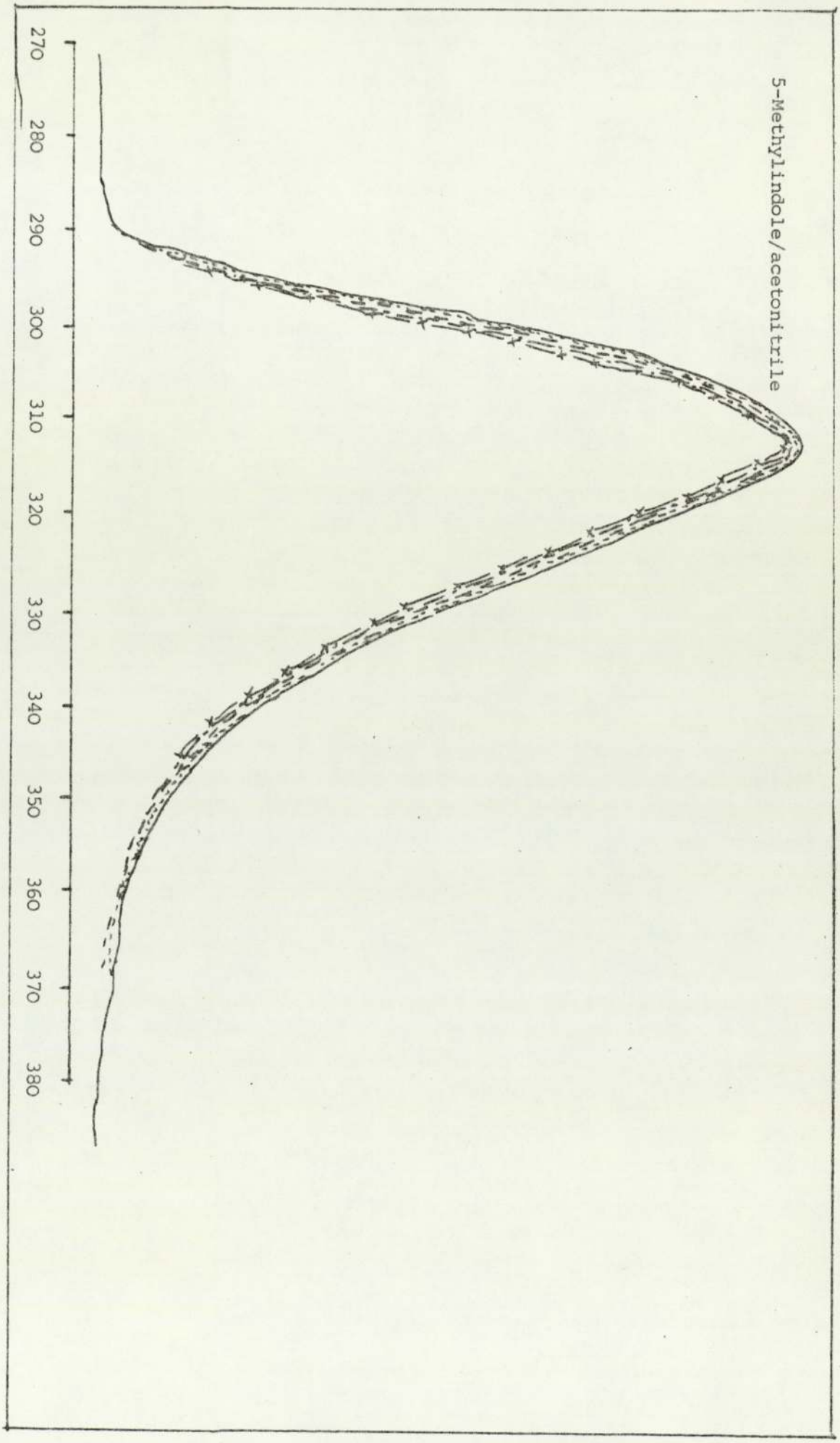

2-Tert-butylindole/acetonitrile
270
280
290
300
310
320
330
340
350
360
370
380
390

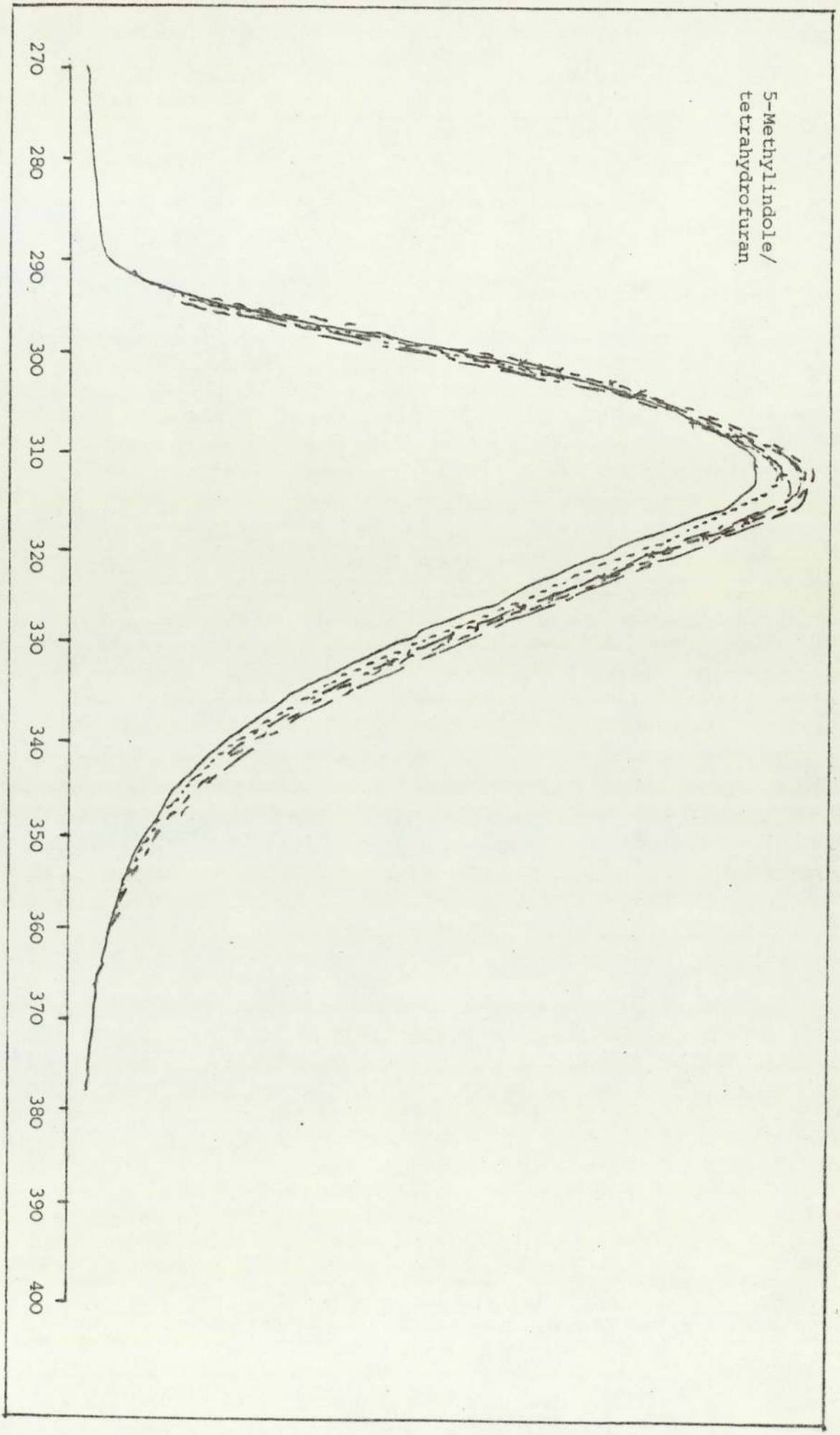

2,3-Cyclodecamethyleneindole/3-ethyl-3-pentanol
270
280
290
300
310
320
330
340
350
360
370
380

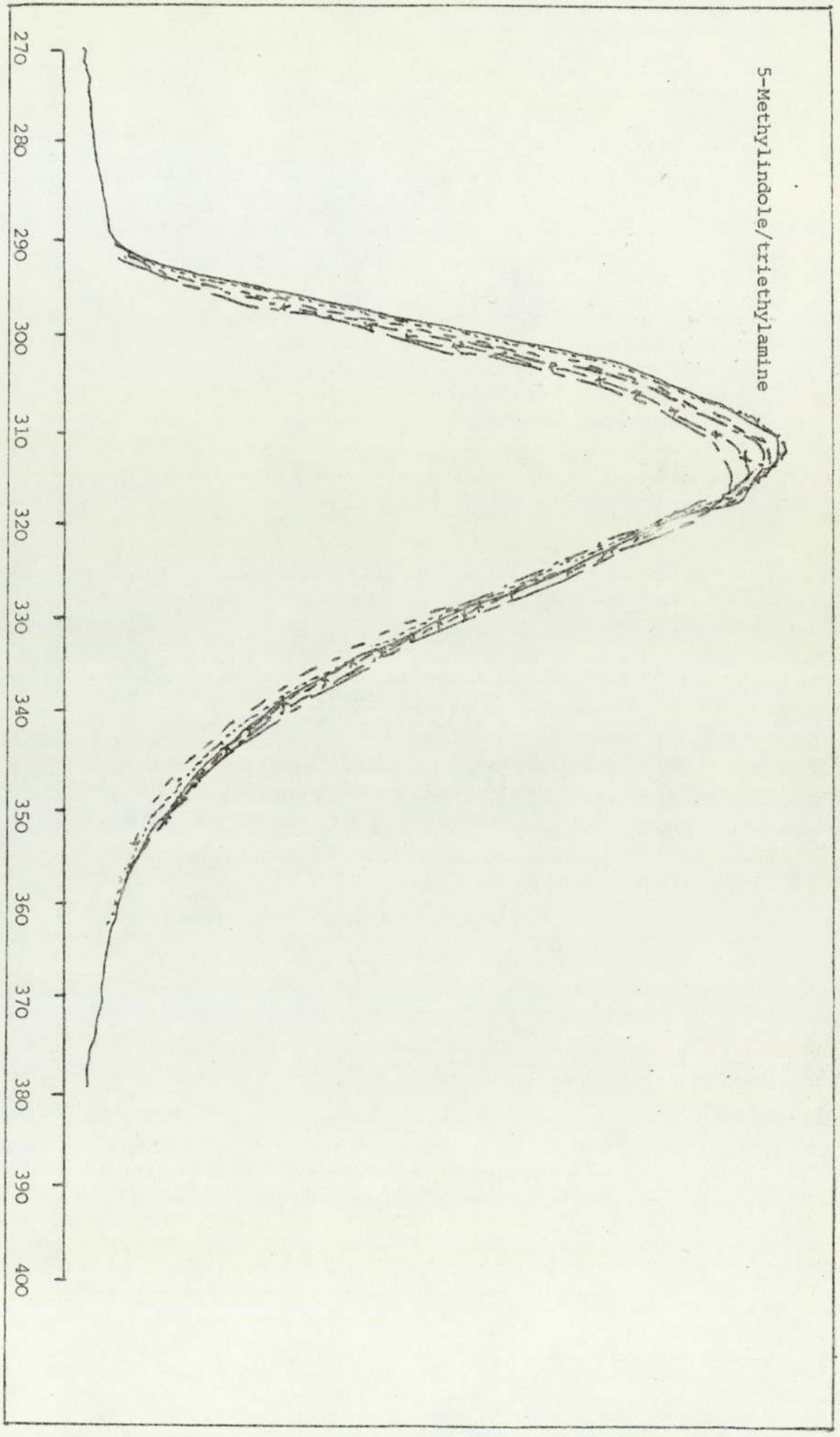
1,2,3-Trimethylindole/3-ethyl-3-pentanol

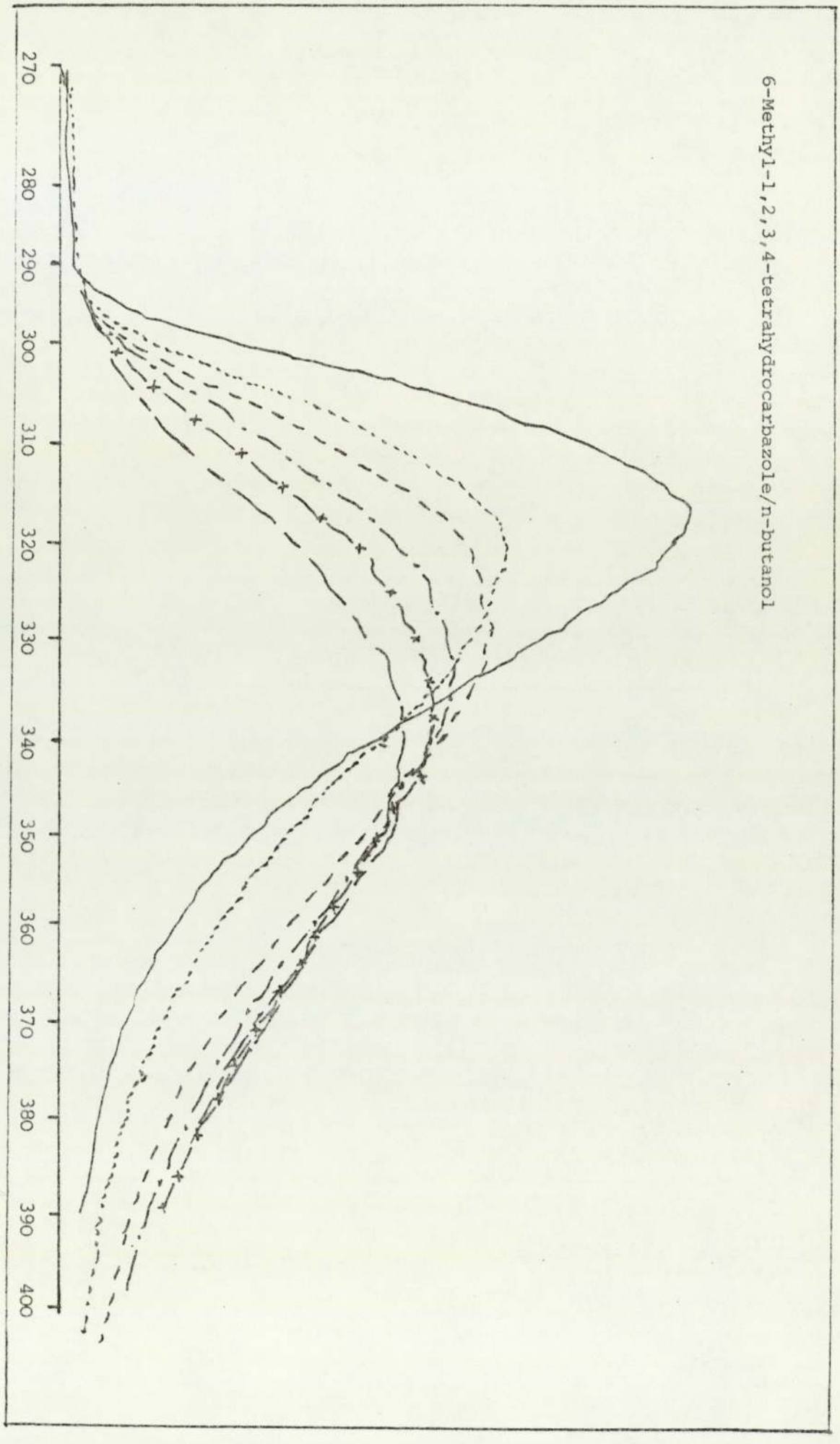


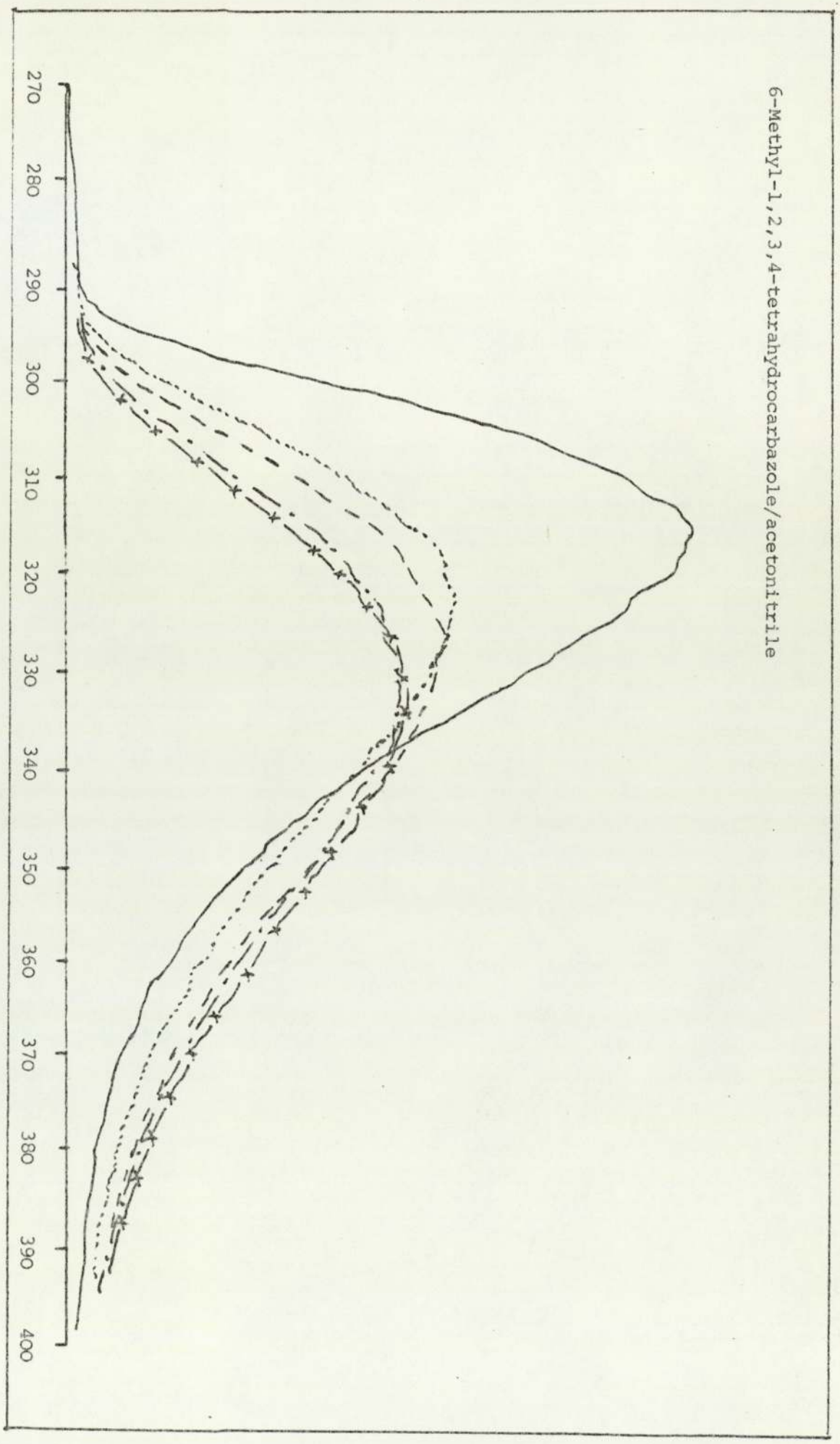

5-Methylindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400


5-Methylindole/acetonitrile
270
280
290
300
310
320
330
340
350
360
370
380

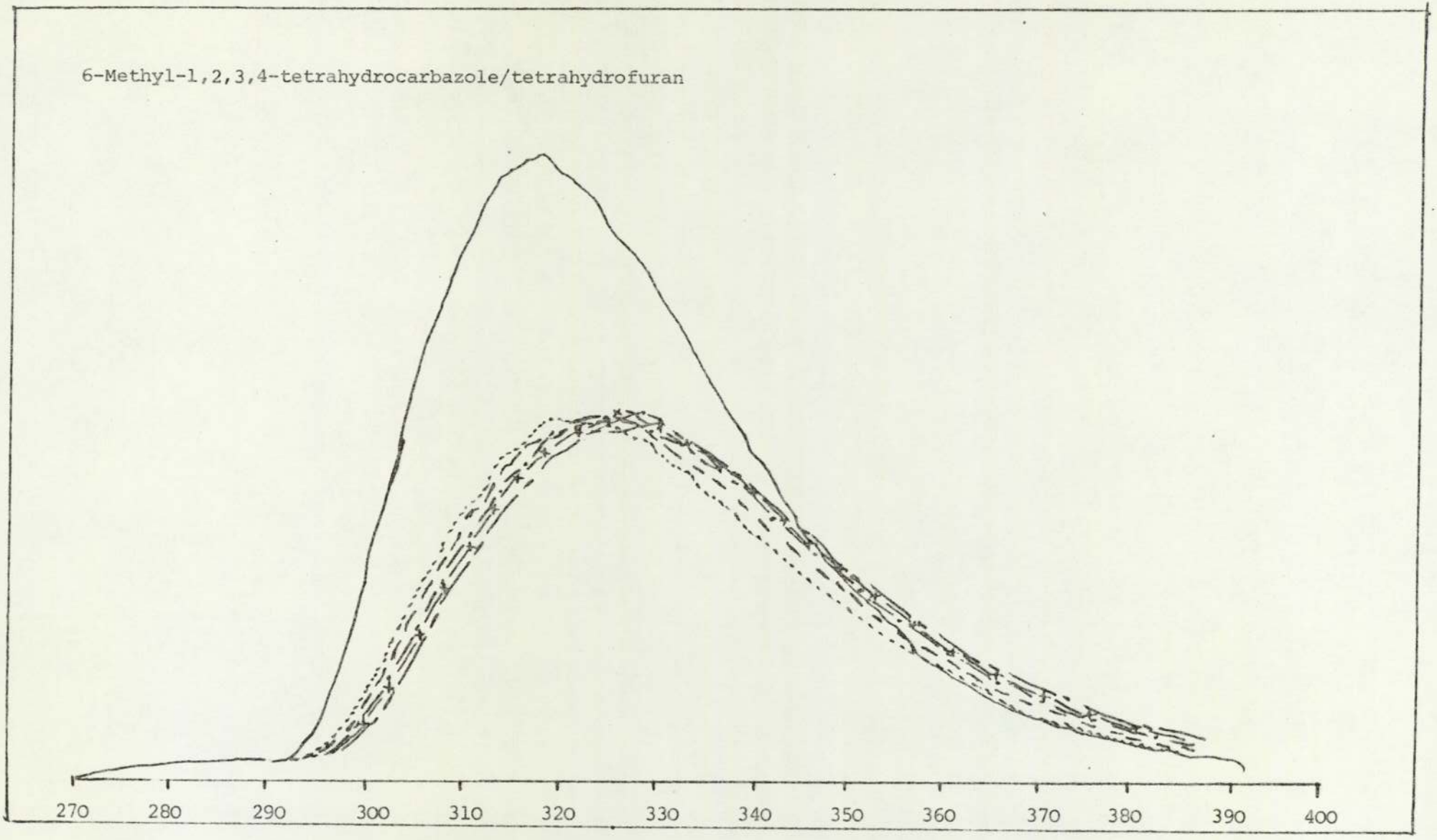

5-Methylindole/
tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390
400


5-Methylindole/triethylamine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

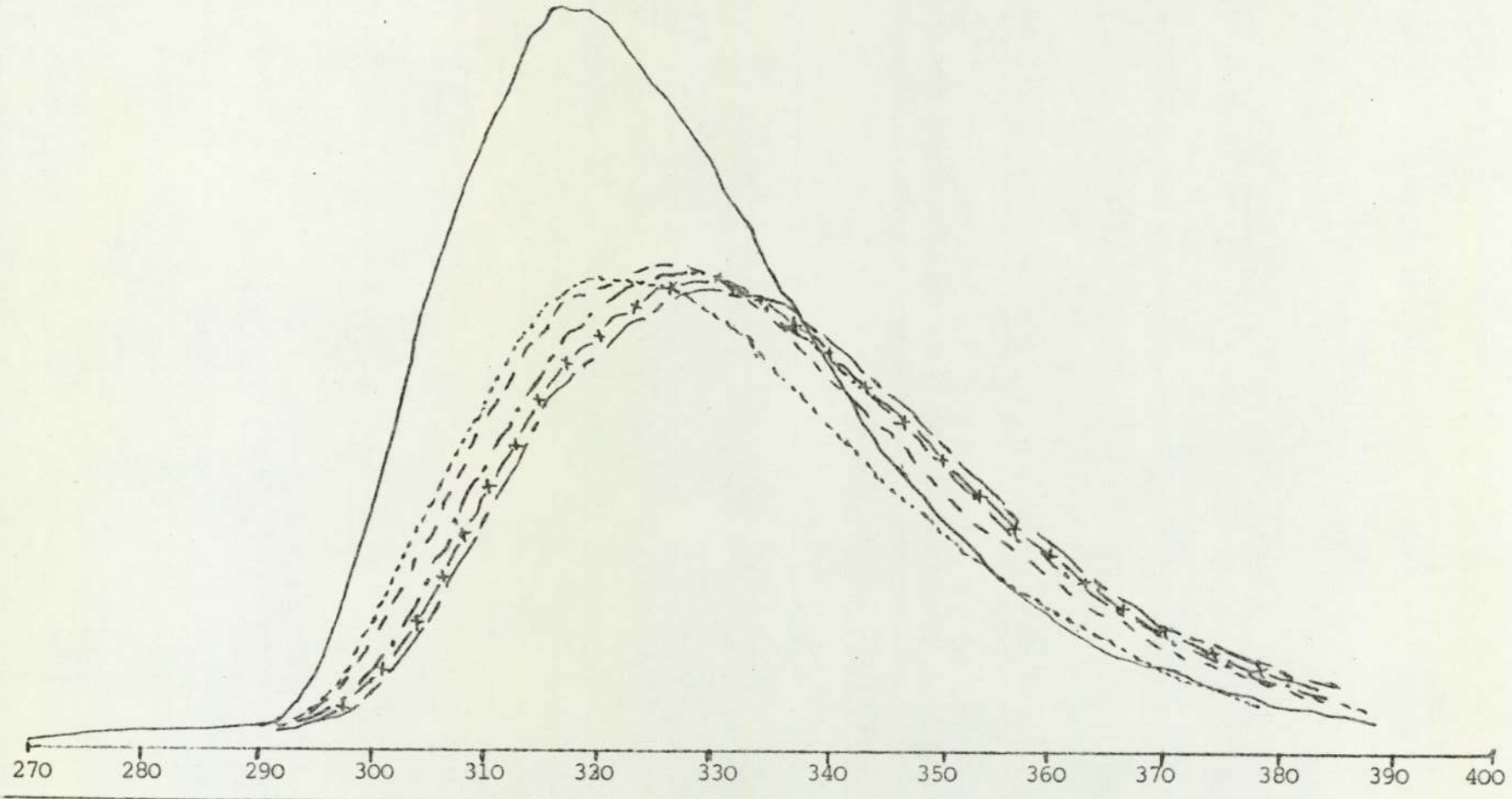

6-Methyl-1,2,3,4-tetrahydrocarbazole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

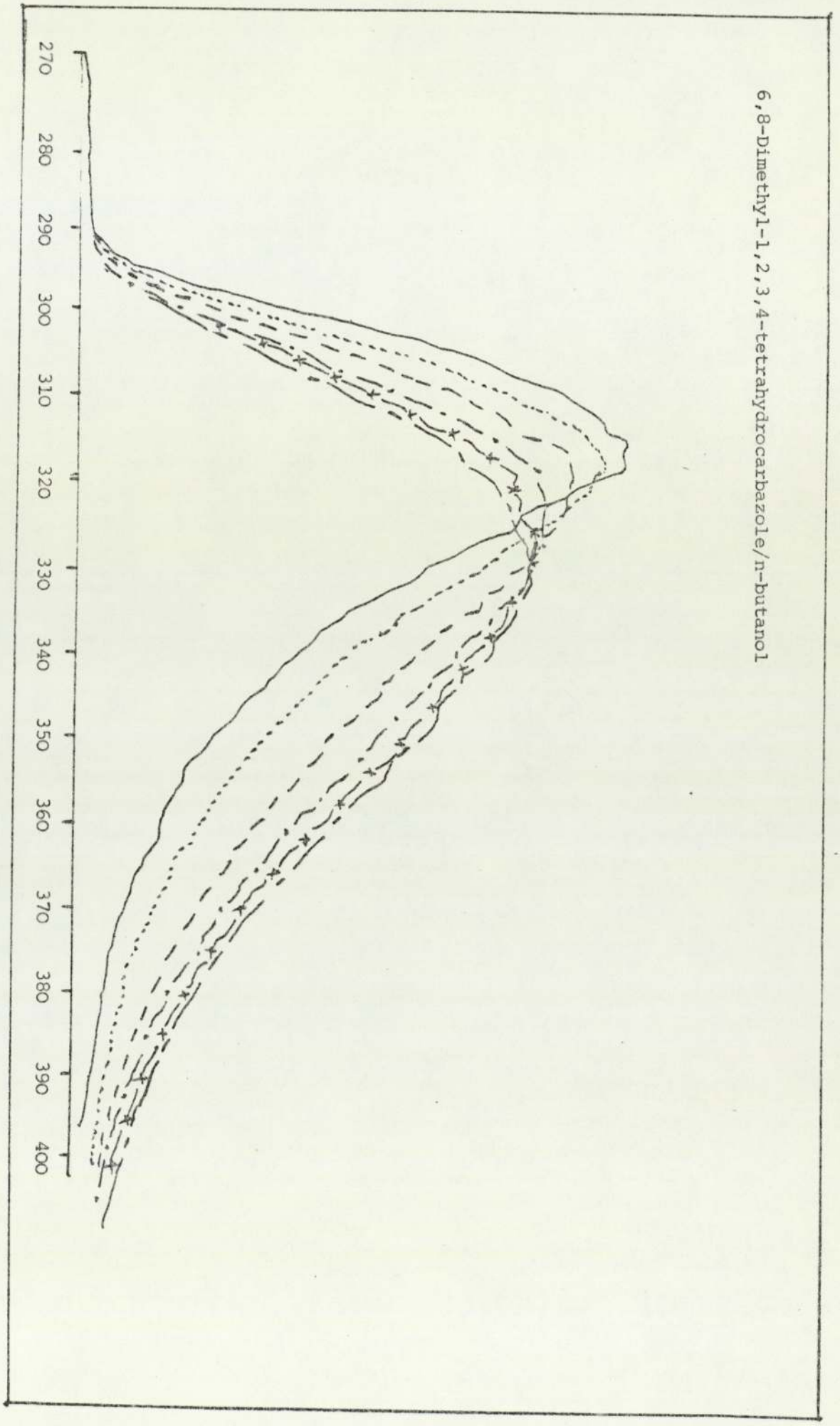

6-Methyl-1,2,3,4-tetrahydrocarbazole/acetonitrile
270
280
290
300
310
320
330
340
350
360
370
380
390
400

6-Methyl-1,2,3,4-tetrahydrocarbazole/tetrahydrofuran



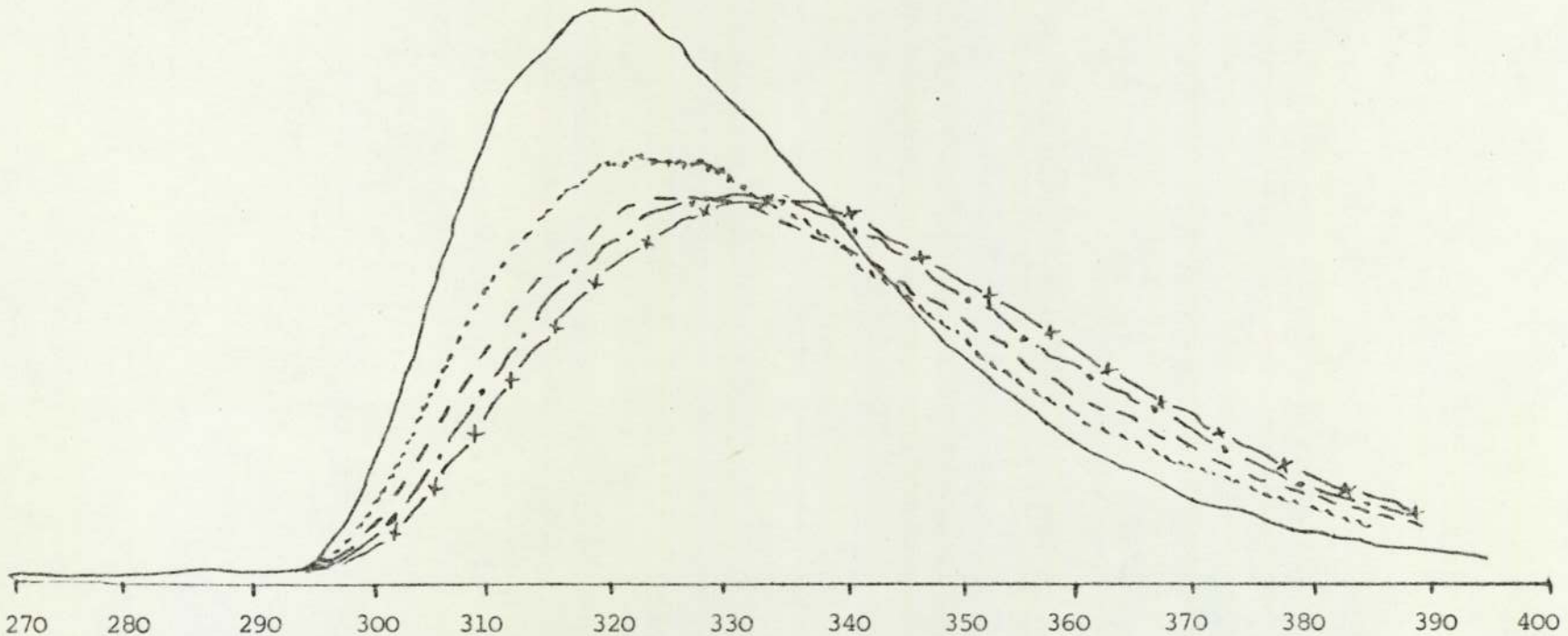
6-Methyl-1,2,3,4-tetrahydrocarbazole/triethylamine



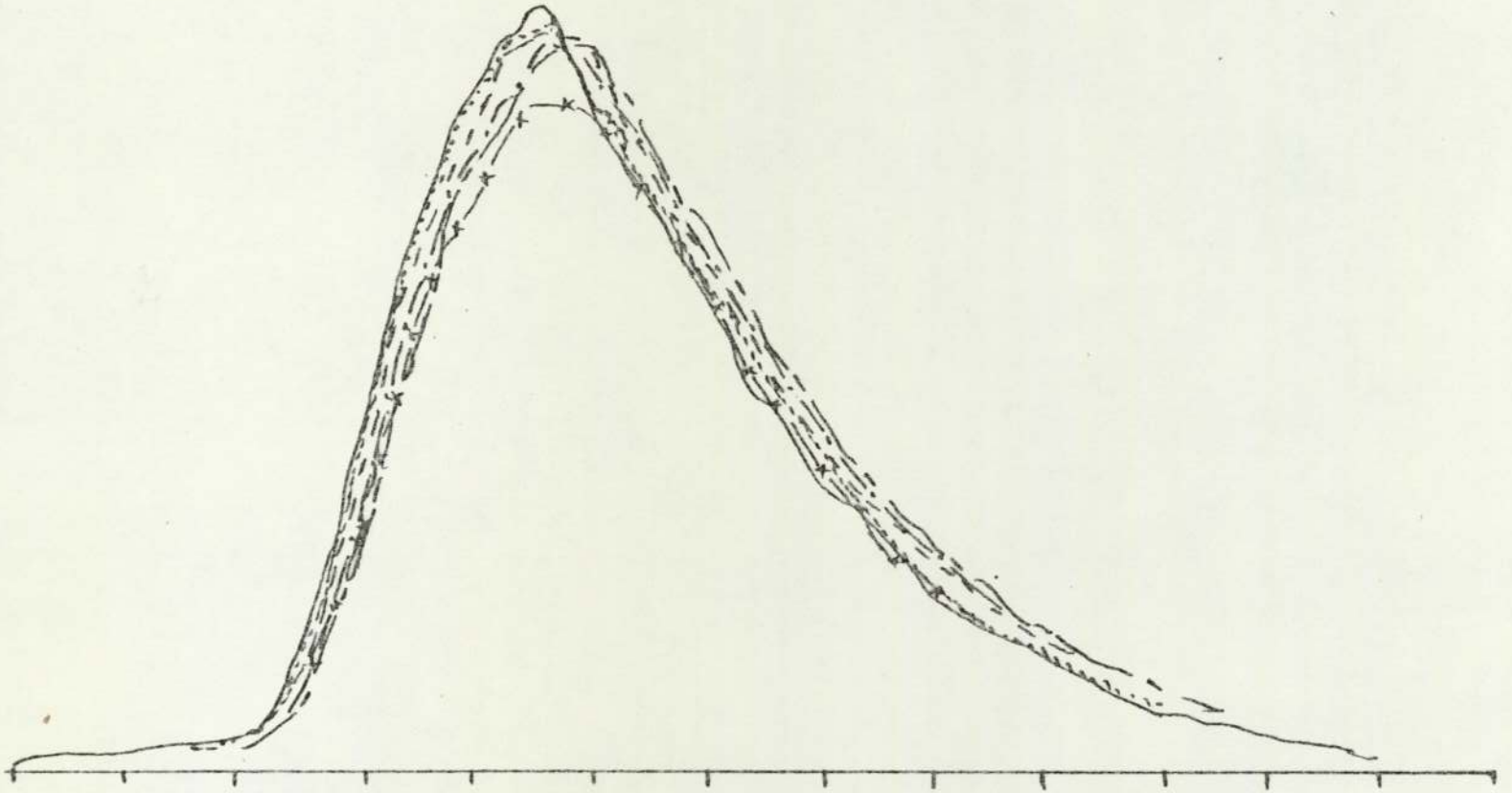


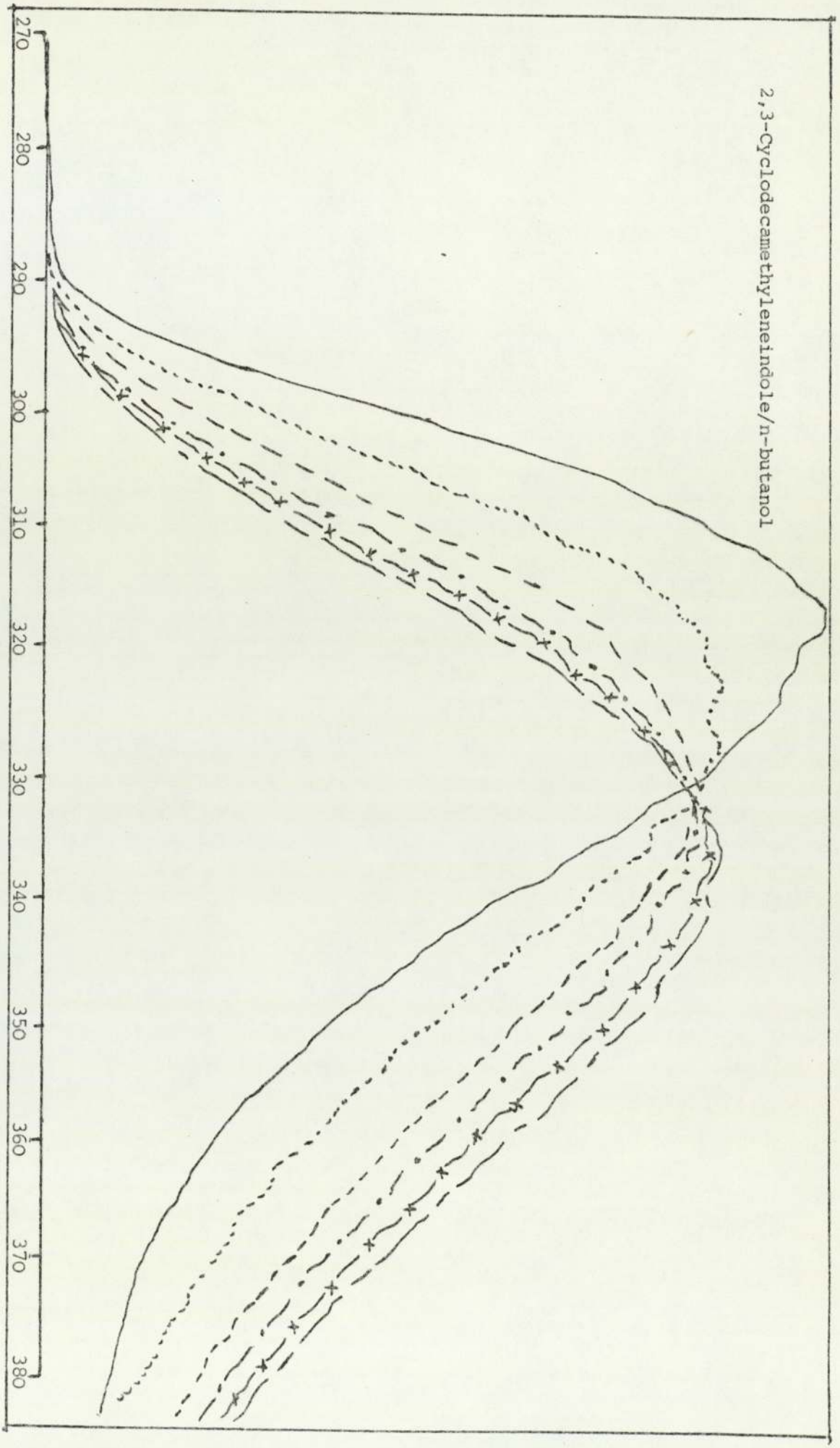
6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole/n-butanol

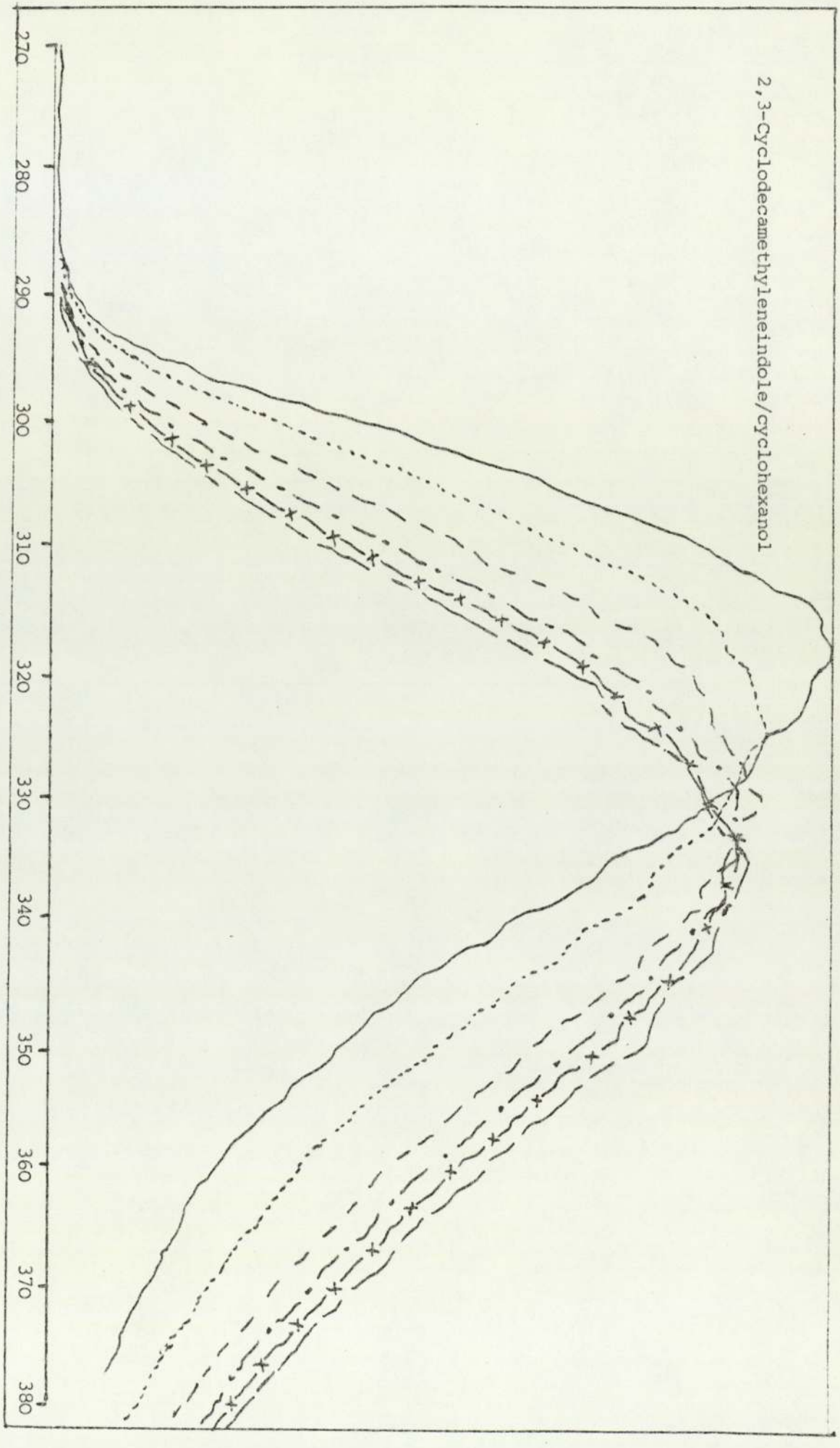
6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole/acetonitrile

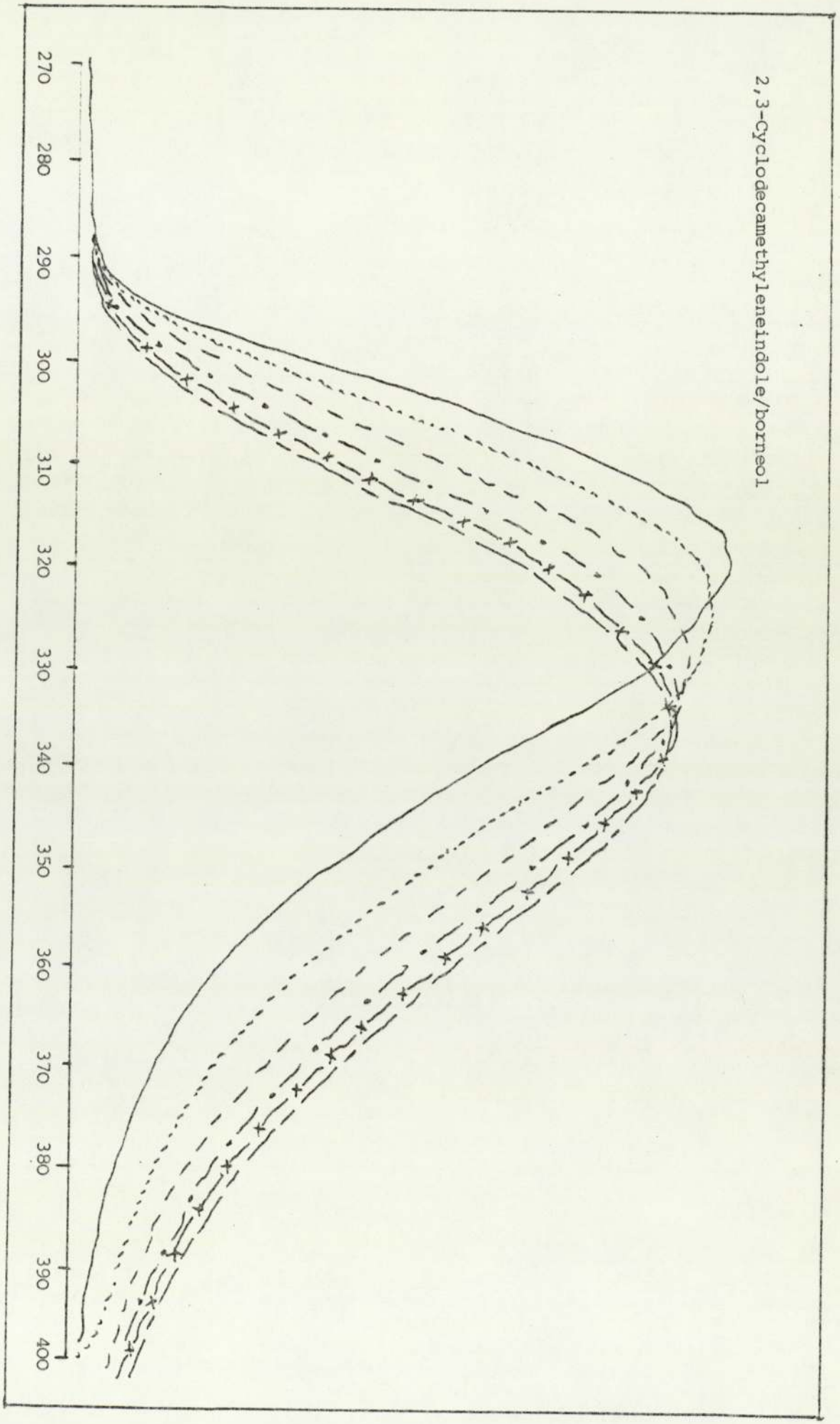


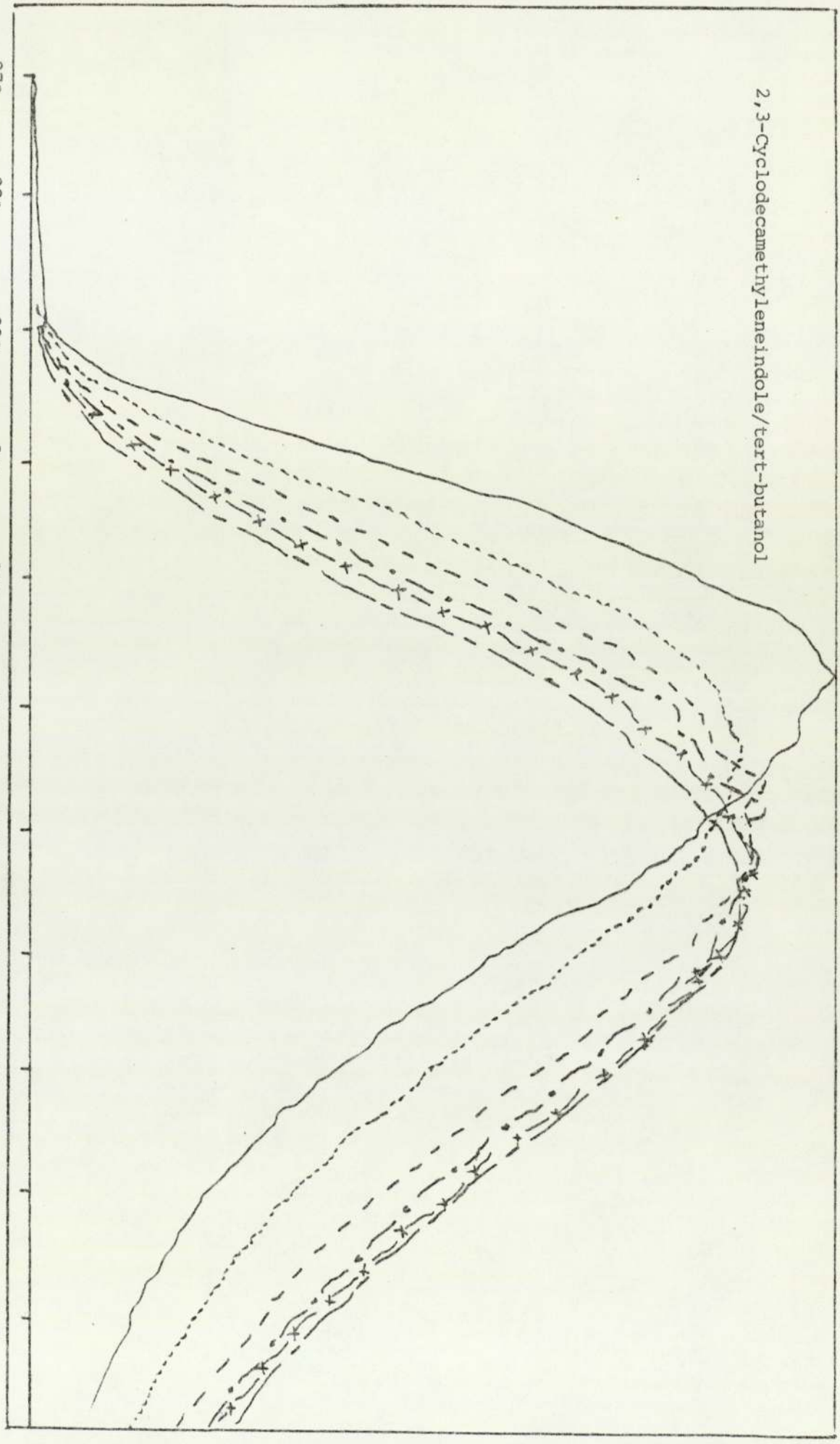
6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole/triethylamine

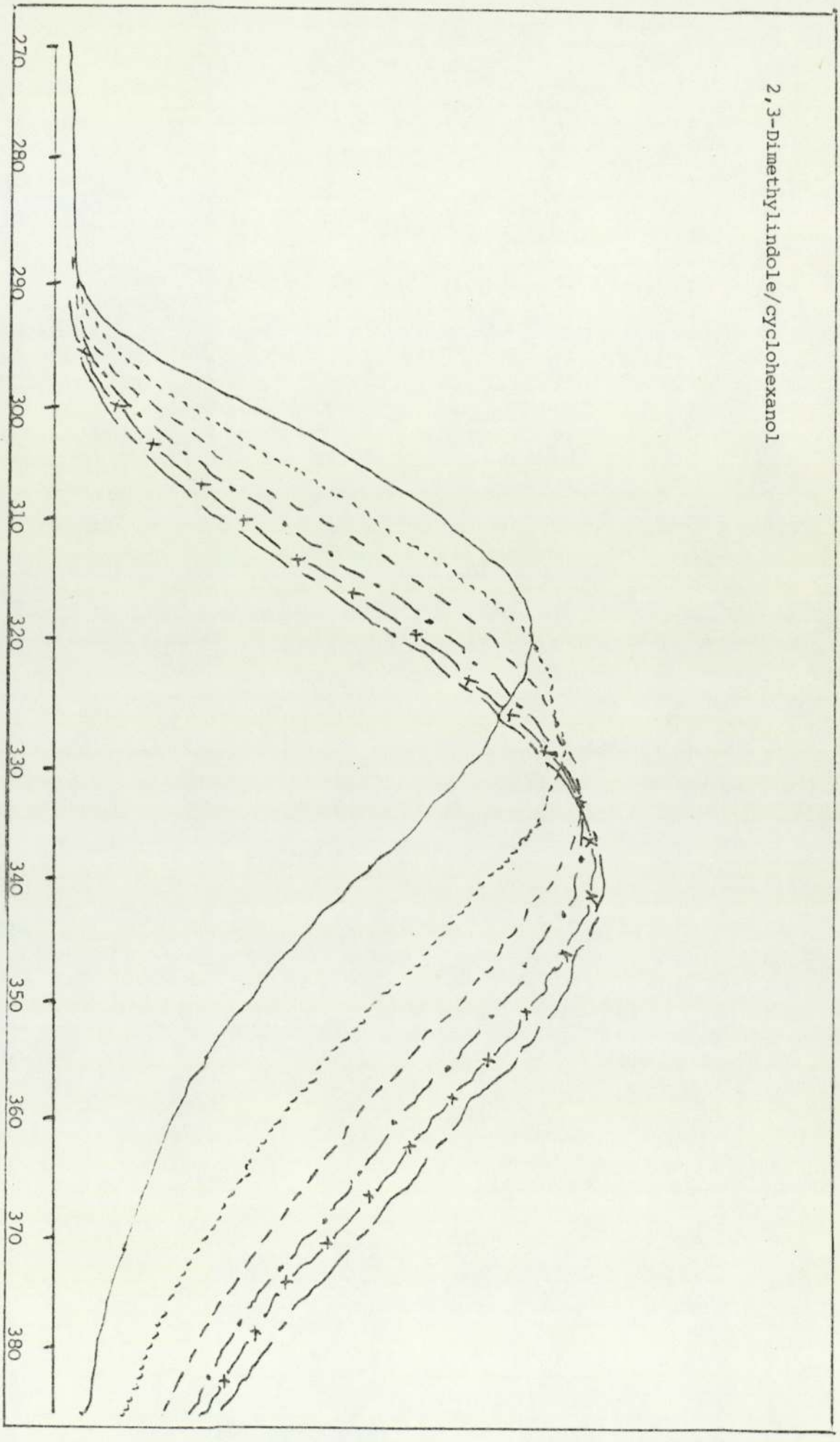


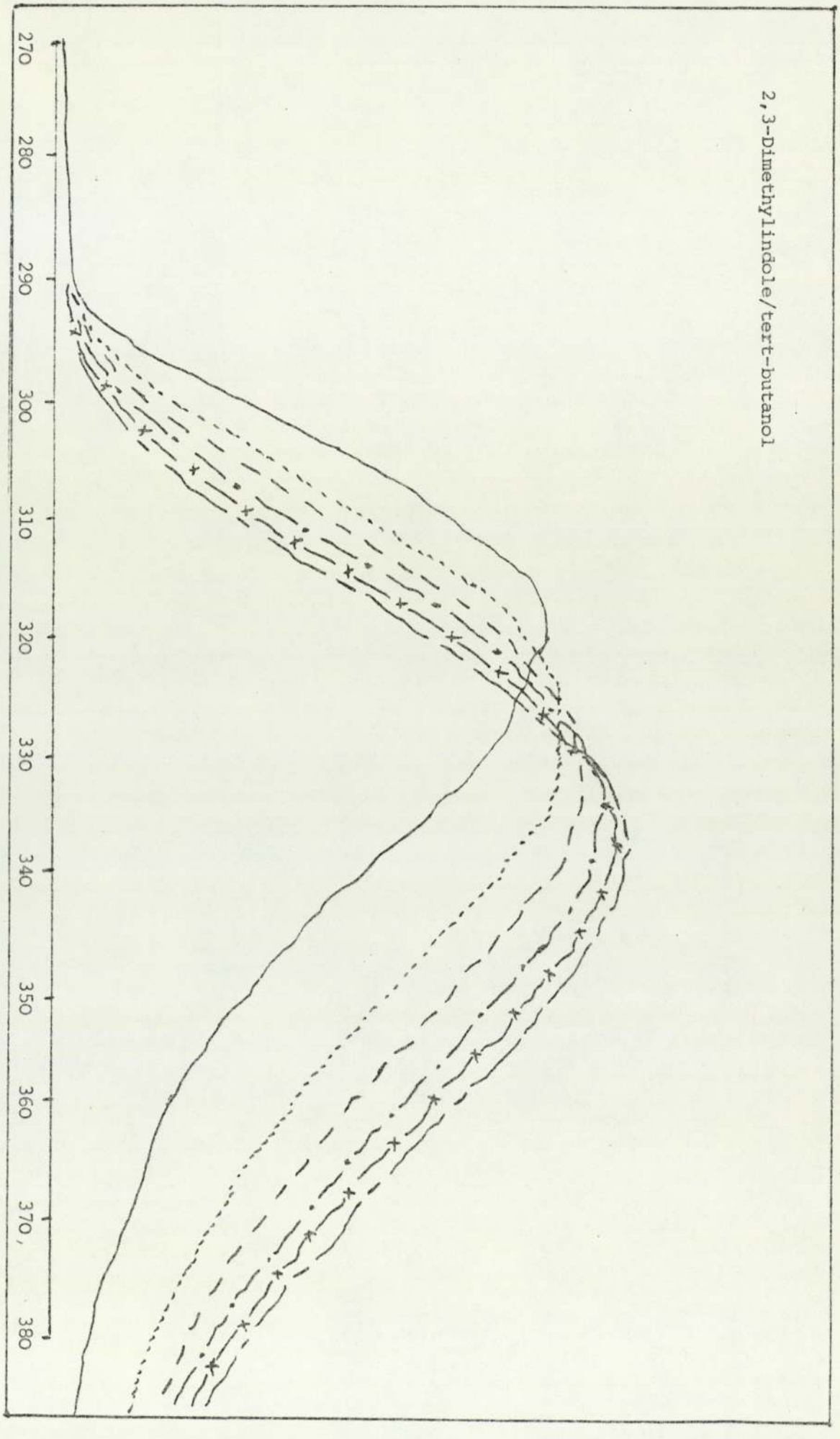

2,3-Cyclodecamethyleneindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380

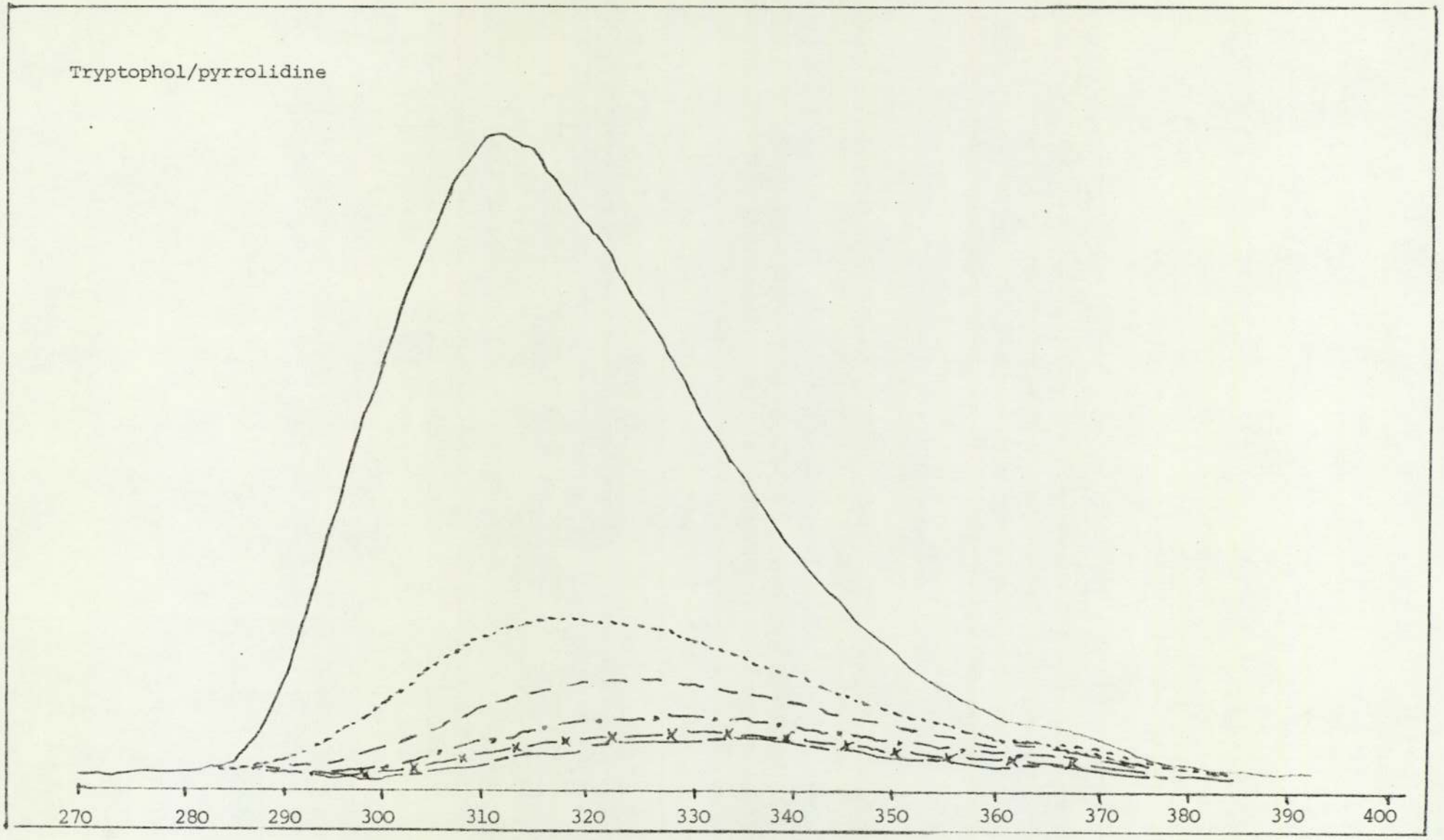

2,3-Cyclodecamethyleneindole/cyclohexanol
270
280
290
300
310
320
330
340
350
360
370
380


2,3-Cyclodecamethyleneindole/borneol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

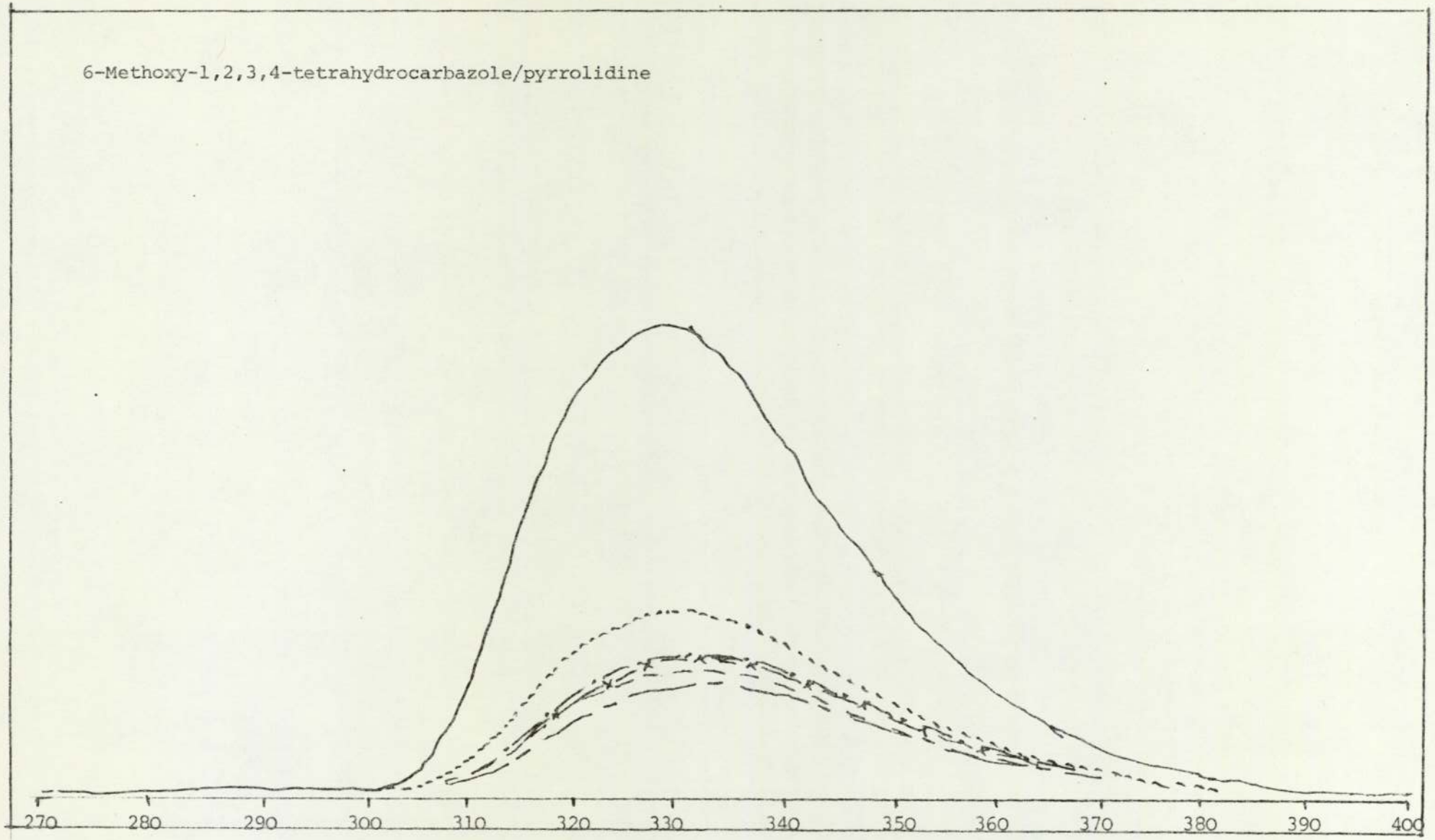

2,3-Cyclodecamethyleneindole/tert-butanol
270
280
290
300
310
320
330
340
350
360
370


2,3-Dimethylindole/cyclohexanol
270
280
290
300
310
320
330
340
350
360
370
380

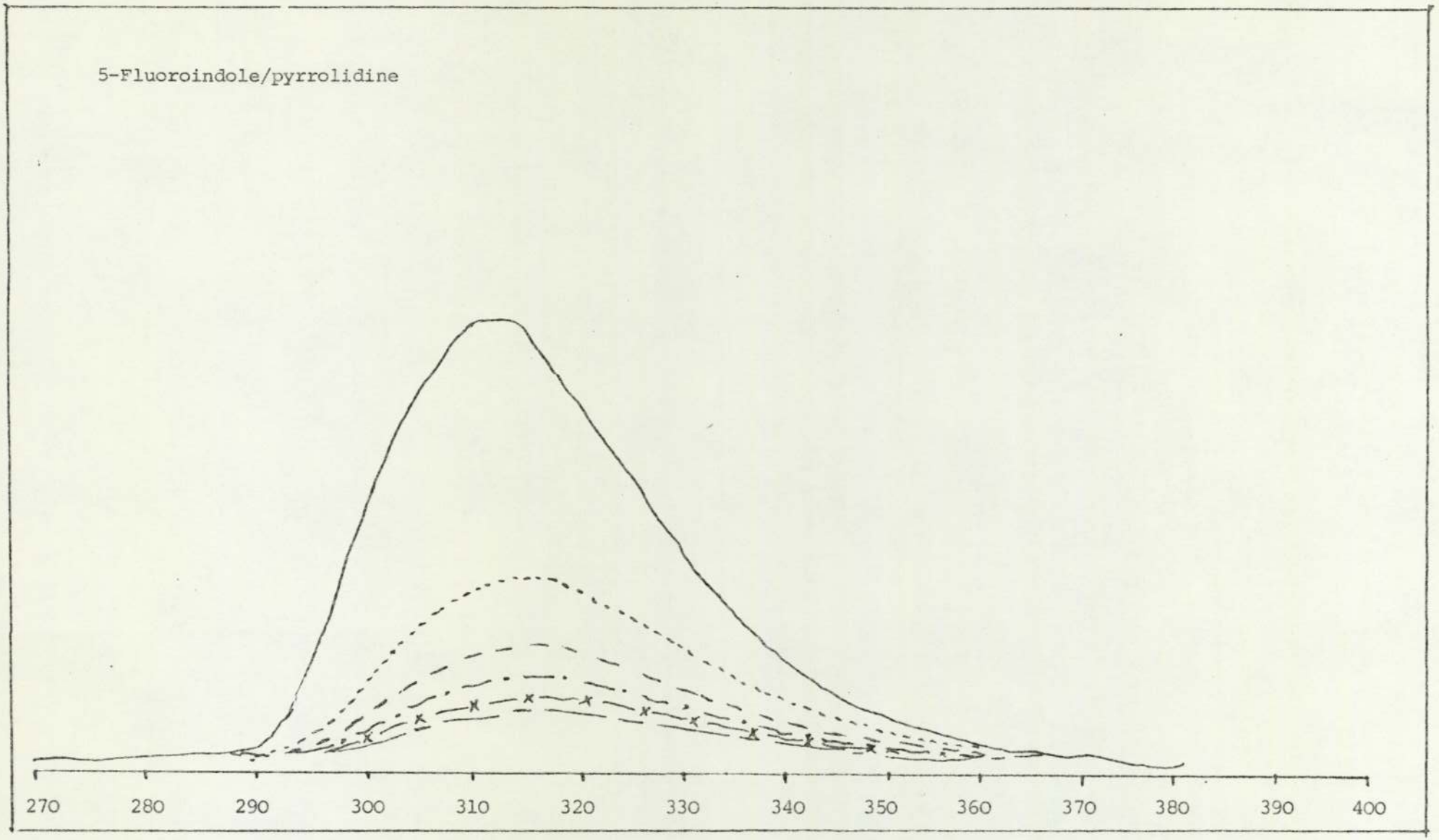

2,3-Dimethylindole/tert-butanol
270
280
290
300
310
320
330
340
350
360
370
380

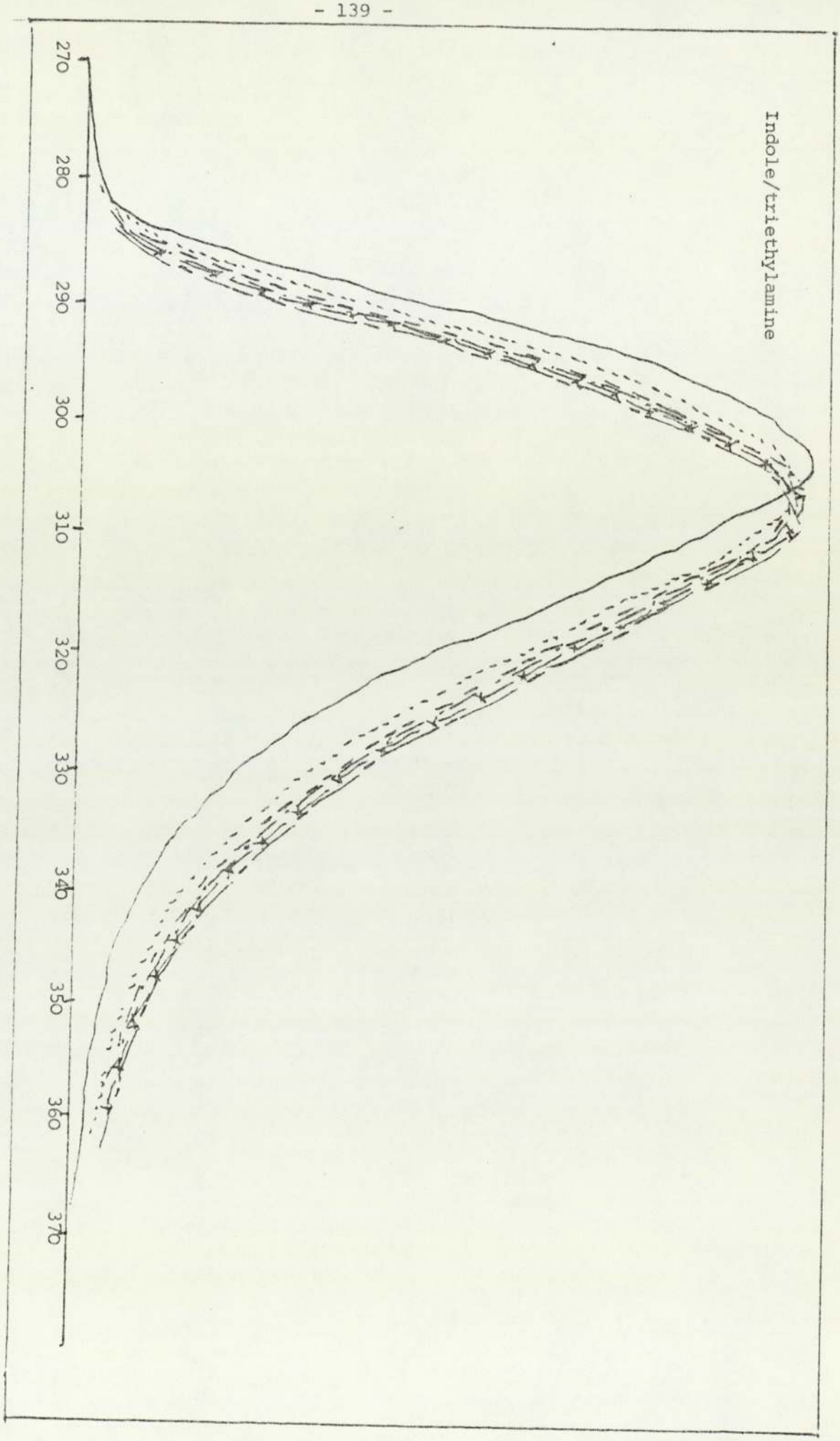

Tryptophol/pyrrolidine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

6-Methoxy-1,2,3,4-tetrahydrocarbazole/pyrrolidine

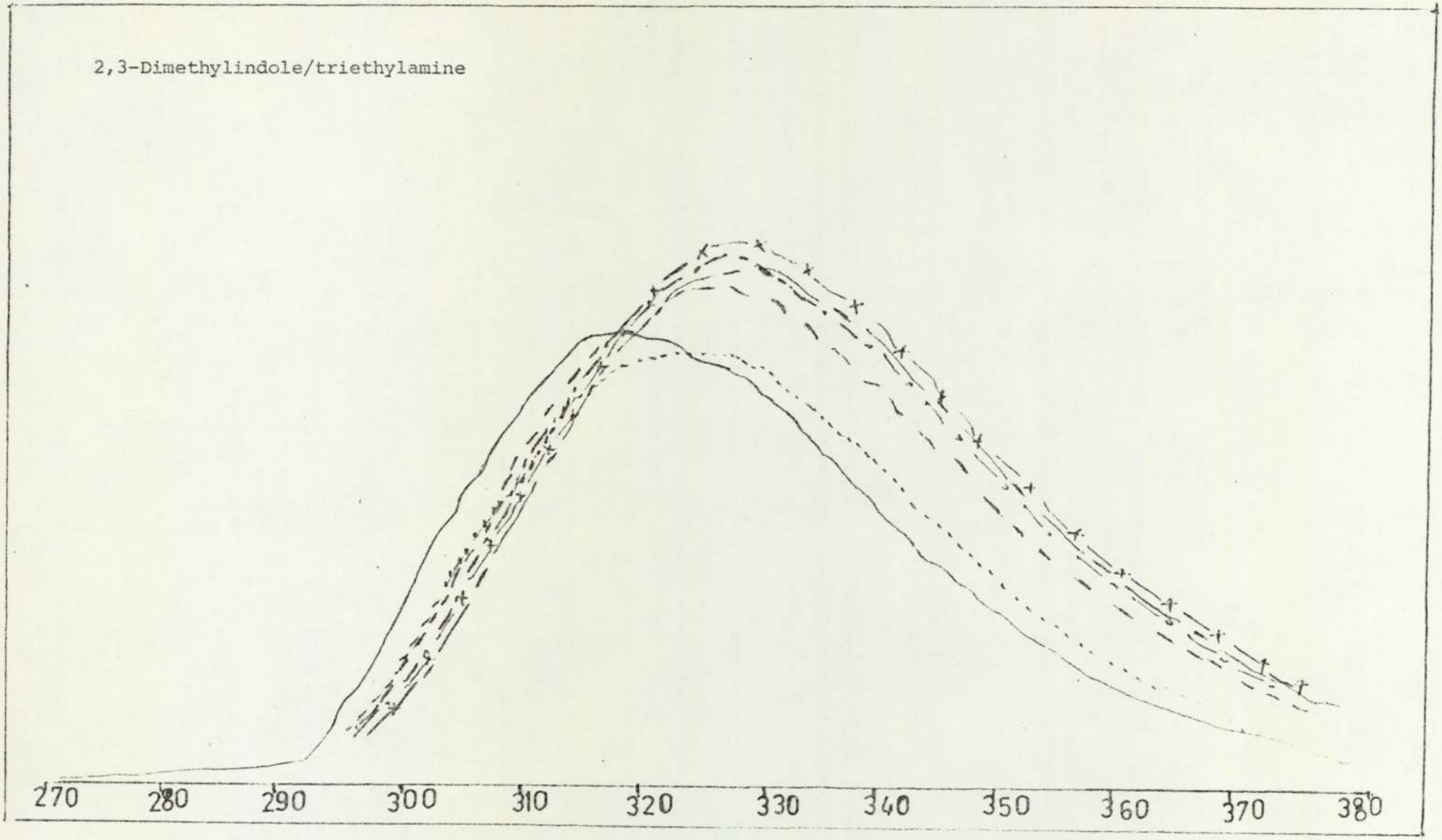


5-Fluoroindole/pyrrolidine




Indole/triethylamine
270
280
290
300
310
320
330
340
350
360
370

2,3-Dimethylindole/triethylamine



BIBLIOGRAPHY

1. P. Pringsheim, "Fluorescence and Phosphorescence", Interscience, London (1965).

2. D.M. Hercules, "Fluorescence and Phosphorescence Analysis", Interscience, London (1966).

3. S. Udenfriend, "Fluorescence Assay in Biology and Medicin", vol.I, Academic Press, London (1962).

4. G.N. Lewis and M. Kasha, J. Am. Chem. Soc., 66, 2100 (1944).

5. M. Kasha, Radiation Res. Suppl., 2, 243 (1960).

6. C.A. Parker and C.G. Hatchard, J. Phys. Chem., 66, 2506, (1962).

7. E.J. Bowen and F. Wokes, "Fluorescence of Solutions", Longmans, Green, London, 1953.

8. S.P. McGlynn, J. Daigre and F.J. Smith, J. Chem. Phys., 39, 675 (1963).

9. L.V.S. Hood and J.D. Wineforder, Anal. Chem., 38, 1922 (1966).

10. J. Nag-Chandhuri, L. Stoessell, and S.P. McGlynn, J. Chem. Phys., 38, 2027 (1963).

11. E.L. Wehry, "Fluorescence", edited by G.G. Guilbault, Edward Arnold Ltd., (London) (1967).

12. C.A. Winter, E.A. Risley and G.W. Nuss, J. Pharmacol. exp. Ther., 141, 369 (1963).

13. A.N. Terenin and V.L. Ermolaev, Trans. Faraday Soc., 52, 1042 (1956).

14. N.J. Turro, "Molecular Photochemistry", W.A. Benjamin, New York (1967).

15. J.B. Birks, "Organic Molecular Photophysics", Wiley, London (1970).

16. M.S. Walker, T.W. Bednar and R. Lumry, J. Chem. Phys., 47, 1020 (1967).

17. R.T. Williams, J. Roy. Inst. Chem., 83, 611 (1959).

18. S. Udenfriend, D.F. Bogdanski and H. Weissback, Science, 122, 972 (1955).

19. M.S. Walker, T.W. Bednar and R. Lumry, Photochem. Photobiol., 14, 147 (1971).

20. R.W. Ricci, Photochem. Photobiol., 12, 67 (1970).

21. M.S. Walker, T.W. Bednar and R. Lumry, "Molecular Luminescence",edited

by E.C. Lim, W.A. Benjamin, Nww York (1969).

22. T.R. Hopkins and R. Lumry, Photochem. Photobiol., 15, 555 (1972).

23. F.W.J. Teale, Biochem. J., 76, 381 (1960).

24. B.L. Van Duuren, Chem. Rev., 63, 325 (1963).

25. W.J. Houlihan, "Indoles" part 1, Wiley London (1972).

26. R.J. Sundberg, "The Chemistry of Indoles", vol. 18, Academic Press., London (1970).

27. J.W. Longworth, "Excited states of proteins and nucleic acids" edited by I. Weinrub and R.F. Steiner, MacMillan, London (1971).

28. J.W. Bridges and R.T. Williams, Biochem. J., 107, 225 (1968).

29. R.W. Cowgill, Biochem. Biophys. Acta, 200, 18 (1970).

30. E.H. Strickland, C. Billups and E. Kay, Bioch.,11, 3657 (1972).

31. L.J. Andrews and L.S. Forster, Photochem. Photobiol., 19, 353 (1974).

32. B.K. Selinger and R.J. McDonald, Aust. J. Chem., 25, 897 (1972).

33. J. Eisnger and G. Navon, J. Chem. Phys. 50, 2069 (1969).

34. R.W. Ricci, J. Chem. Education, 51, 692 (1974).

35. B.L. Van Duuren, J. Org. Chem., 26, 2954 (1961).

36. S.V. Konev, "Fluorescence and Phosphorescence of Proteins and Nucleic acids", Plenum Press, New York (1967).

37. P.S. Song and W.E. Kurtin, J. Amer. Chem. Soc., 91, 4892 (1969).

38. M.S. Walker, T.W. Bednar and R. Lumry, J. Chem. Phys. 45, 3455, (1966).

39. A. Gillam and E.S. Stern "Electronic Absorption Spectroscopy", E.Arnold Ltd., London (1950).

40. E.G. McRae, J. Phys. Chem., 61, 562 (1957).

41. N. Mataga and S. Tsuno, Bull. Chem. Soc. Japan, 30, 368 (1957).

42. N. Mataga, Y. Torihashi and K. Ezumi, Theoret. Chem. Acta. (Berl.) 2, 158 (1964).

43. N. Mataga, Y. Kaifu and M. Koizumi, Bull. Chem. Soc. Japan, 28, 690 (1955).

44. S. Sambursky and G. Wolfsohn. Trans. Far. Soc. 36, 427 (1940).

45. W.B. DeLauder and P H. Wahl, Biochem. Biophys. Acta, 243, 153 (1971).

46. N. Mataga, Y. Kaifu and M. Koizumi, B ull. Chem. Soc. Japan, 29, 465 (1956).

47. J.B. Birks, "Photophysics of Aromatic Molecules", Wiley, London (1970).

48. M. Ottolenghi, Accounts Chem. Res., 6, 153 (1973).

49. R. Lumry, S. Yanari, and F.A. Bovey, Amer. Chem. Soc. Div. Org. Coating Plastics Chem. Preprints 142, 18R (1962).

50. D. Creed and R.A. Caldwell, J. Amer. Chem. Soc., 96, 7369 (1974).

51. N.C. Yang and J. Libman, J. Amer. Chem. Soc., 94, 1405 (1972).

52. E.A. Chandross, "The Exciplex", edited by M. Gordon and W.R. Ware, Academic Press. London (1975).

53. E.A. Chandross and H.T. Thomas, Chem. Phys. Letters, 9, 397 (1971).

54. M.V. Hershberger and R.W. Lumry, Photochem. Photobiol., 23, 391 (1976).

55. J.B. Birks, Nature, 214, 1187 (1967).

56. G.N. Taylor, E.A. Chandross and A.H. Schiebel, J. Amer. Chem. Soc., 96, 2693 (1974).

57. E.A. Chandross and C.J. Dempster, J. Amer. Chem. Soc., 92, 3586 (1970).

58. T. Okada, T. Fujita, M. Kubota, S. Maski, N. Mataga, R. Ide, Y. Sakata and S. Misumi, Chem. Phys. Letters, 14, 563 (1972).

59. N. Mataga, K. Ezumi and T. Okada, Mol. Phys., 10, 201 & 203 (1966).

60. G. Lockwood, A. Thesis of Ph.D University of Aston, Gosta Green, Birmingham (1974).

61. J.W. Longworth, Photochem. Photobiol., 7, 587 (1968).

62. R.J. McDonald and B.K. Selinger, Aust. J. Chem. 24, 1797 (1971).

63. M. Irie, S. Tomimoto and K. Hayashi, J. Phys. Chem., 76, 1419 (1972).

64. R.S. Mulliken, J. Amer. Chem. Soc., 74, 811 (1952), J. Phys. Chem., 56, 801 (1952).

65. R.S. Mulliken and W.B. Person, Annu. Rev. Phys. Chem., 13, 107 (1962).

66. J. Rose, "Molecular Complex", Pergamon, London (1967).

67. C.H. Linschitz and L. Pekkarinen, J. Amer. Chem. Soc., 82, 2411 (1960).

68. M. Itoh, T. Mimura and T. Okamoto, Bull. Chem. Soc. Japan, 47, 1078 (1974).

69. M.A. Slifkin, "Charge Transfer Interactions of Biomolecules," Academic Press. London (1971).

70. L.J. Andrews and R.M. Keefer, "Molecular Complexes in Organic Chemistry", Holden-Day, London (1964).

71. R. Forster and T.J. Thomson, Trans. Farad. Soc., 58, 860 (1962)

72. A. Weller, Pure Appl. Chem., 16, 115 (1968).

73. M.G. Kuzmin and L.N. Guseva, Chem. Phys. Letters, 3, 71, (1969).

74. H. Knibbe, K. Roellig, F.P. Schaefer and A. Weller, J. Chem. Phys., 47, 1184 (1967).

75. H. Beens, H. Knibbe and A. Weller, J. Chem. Phys., 47, 1183 (1967).

76. R. Foster and H. Hanson, Tetrahad., 21, 255 (1965).

77. A. Szent-Gyorgi, I. Isenberg and J. McLaughlin, Proc. Natn. Acad. Sci., 47, 1089 (1961).

78. E. Fujimori, Pro. Natn. Acad. Sci., 45, (1959).

79. R.L. Schneider, Eastman Organic Chemical Bulletin, 47, (1975).

80. J.P. Green and J.P. Malrieu, Pro. Natn. Acad. Sci., 54, 659 (1965).

81. R. Foster and C.A. Fyfe, J. Chem. Soc. (B) 926 (1966).

82. D.J. Gram and G.S. Hammond, "Organic Chemistry", M. Grow Hill Book Comp. Inc., London (1974).

83. T.R. Evans, J. Amer. Chem. Soc., 93, 2081 (1971).

84. C.R. Goldschmidt, R. Potashnik and M. Ottolenghi, J. Phys. Chem., 75, 1025 (1971).

85. D.A. Labianca, G.N. Taylor and G.S. Hammond, J. Amer. Chem. Soc., 94, 3679 (1972).

86. S. Ander, H. Blume, G. Heinrch and D.S. Frohlinde, Chem.Commun.,745 (1968).

87. L.M. Stephenson and G.S. Hammond, Pure Appl. Chem., 16, 125 (1968).

88. H. Ohta, D. Creed, P.H. Wine, R.A. Caldwell and L.A. Melton, J. Amer. Chem. Soc., 98, 2002 (1976).

89. N.J. Turro and R. Engel, J. Amer. Chem. Soc., 91, 7113 (1969).

90. M.T. McCall, G.S. Hammond, O. Yonemitsu and B. Witkop, J. Amer. Chem. Soc., 92, 6991 (1970).

91. I. Weinryb and R.F. Steiner, Biochemistry, 7, 2488 (1968).

92. R.F. Steiner and E.P. Kirby, J. Phys. Chem.,73, 4130 (1969).

93. S.S. Lehrer, Biochemistry, 10, 3254 (1971).

94. A. White, Biochem. J., 71, 217 (1959).

95. R.W. Ricci and K.B. Kilichowske, J. Phys. Chem., 78, 1953 (1974).

96. A.Z. Britten (private communication).

97. R.O. Loutfy and R.O. Loutfy, Can. J. Chem. 50, 4052 (1972).

98. D. Rehm and A. Weller, Ber. Bunsenges Phys. Chem., 73, 834 (1969).

99. R.S. Davidson and K.R. Trethewey, J. Chem. Soc. Comm, 827 (1976); J.H. Borkent, J.W. Verhoeven and Th. J. D E. Boer, 42, 50 (1976).

100. A.M. Halpern, J.L. Roebber and K. Weiss, J. Chem. Phys., 49, 1348 (1968).

101. H. Heaney and S.V. Ley J. Chem. Sci. Perkin, 1, 499 (1973).

102. Dictionary of Organic Compounds, vol. 5, 3178, E. and F.N. Spon Ltd., London (1965).

103. H.M. Kissman, D.W. Farnsworth and B. Witkop, J.Amer. Chem. Soc., 74, 3948 (1952).

104. Bell, Malcolm Rie [1-(carboxyalkyl)indole], Chem. Abst., 72, 66807 m (1970).

105. S. Veibel et N. Hauge, Bull. Soc. Chem., 5, 1506 (1938).

106. C.U. Rogers and B.B. Corson, Organic Synthesis (Collective), vol. 4, 884 (1963).

107. N.K. Kochetkov, N.F. Kucherova and V.P. Evdakov (Derivatives of indole), Chem. Abst., 51, 12883i (1957).

108. G. Anderson and N. Campbell, J. Chem. Soc., 2855 (1950).

109. A. Smith and J.H.P. Utley, J. Chem. Soc., C, 1-5 (1970).

110. A.H. Milne and M.L. Tomlisnoson, J.Chem. Soc., 2789 (1952); E. Campaigne and R.D. Lake, J. of Org. Chem., 24, 478 (1959); A. Smith and J.H. Utley, J.Chem. Soc., C, 1-5 (1970).

111. J.D. Robert and M.C. Caserio, "Basic Principles of Org. Chem.," W.A. Benjamin, California (1964).

112. N. Tyutyulkov, F. Dietz, Bulgarian Academy of Sciences, Comm. Depart. Chem., 2, No. 1, 55 (1969).

113. Chiang-Tung Chang, Chung-Uung Wu, A.R. Muirhead and J.R. Lombardi, Photochem. Photobiol., 19, 347 (1974).

114. L.F. Gladechenko and L.G. Pikulik, Chem. Abst., 67, 432 gge (1967).

115. A. Kawski and G. Jacyno, Bull. Acad. Pol. Sci, 22, 339 (1974); A. Kawski and J. Sepiol, ibid, 20, 707 (1972).

116. N. Strojny and J. Arthur F. de Silva. J. of Chromatographic Science 13, 583 (1975).

117. M.M. Airaksinen, P.J. Neuvonen and I. Jaakonmaki, Biochem. Pharmacol., 19, 2181 (1970).

118. E. Giacalone and L. Valzelli, Pharmacology, 2, 171 (1969).

119. P.A. Shore, A. Burkhalter and V.H. Coh. J. Pharmacol. Exp. Therap., 127, 182, (1959).

120. R.P. Maickel, "Methods of Neurochemistry," vol. 2, edited by Ranir Fried, Marcel Dekker, New York (1972).

121. G. Curzon and J. Giltrow, Nature, 173, 314 (1954).

122. R.L. Pecsok, L.D. Shields, T. Cairns and I.G. McWilliam , "Modern Methods of Chemical Analysis," (2nd ed.) Wiley, London (1968).

123. L.P.J. Holt and C.F. Hawkins, Brit. Med. J., 1, 1354 (1965).

124. J. Arthur F. de Silva, N. Strojny and K. Stika, Analytical Chemistry, 48, 144 (1976).

125. R.G. Wiegand and E. Scherfling, J. Neurochem., 9, 113 (1962).

126. "The Merk Index", 8th ed., Merk and Co., Rahway N.J., 1968.

127. R.P. Maickel and F.P. Miller, Analytical Chemistry, 38, 1937 (1966).

128. D.G. Ferry, D.M. Ferry, P.W. Maller and E.G. McQueen, J. Chromatography, 89, 110 (1974).

129. E. Gelpi, E. Peralta and J. Segura, J. of Chromatographic Science, 12, 701 (1974).

130. M. Donike, Chromatographia, 9, 440 (1976).

131. L. Helleberg, J. Chromatography, 117, 167 (1976).

132. J.L. Brook, R.H. Biggs, P.A. St. John and D.S. Anthony, Analytical Biochemistry, 18, 453 (1967).

The Fluorescence of Indoles and its Application in Drug Analysis

by

KAIS KHALIL ALI

A thesis presented for the degree of

MASTER OF PHILOSOPHY

in the

University of Aston in Birmingham

August 1977
Department of Pharmacy
University of Aston in Birmingham
Gosta Green
Birmingham B4 7ET
U.K.

SUMMARY

The Fluorescence of Indoles and its Application in Drug Analysis. Kais Khalil Ali, M.Phil. Thesis 1977.

The literature on fluorescence of indoles and its application is briefly reviewed. The effect of polar solvents such as n-butanol, methanol, cyclohexanol, borneol, tert-butanol, 3-ethyl-3-pentanol, acetonitrile, tetrahydrofuran, pyrrolidine, cyclohexylamine, triethylamine, quinuclidine, triethylenediamine and acetic acid on the fluorescence of some indole derivatives such as unsubstituted indole; 2,3-cyclodecamethylene-indole; 2,3-dimethylindole; 2-Butyl-3-propylindole; 2-methylindole; 2-tert-butylindole; 3-methylindole; tryptophol; 7-methylindole; 5-methylindole; 5-methoxyindole; 5-fluoroindole; 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 6-methoxy-1,2,3,4-tetrahydrocarbazole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole; 1,2,3,-trimethylindole; 1,2-dimethylindole and 1,3-dimethylindole in cyclohexane is reported.

It was noted that the formation of an exciplex is dependent upon electronic and steric effects of both solvent and indole structures. The nature of the exciplex is discussed and a dipole-dipole with some charge-transfer interaction is proposed for the mechanism of exciplex formation.

The synthesis of some of the above indoles is described. The analysis of drugs such as 5-Hydroxyindole-3-acetic acid, 5-Hydroxytryptophan, indomethacin and oxypertine by native fluorescence, O-phthalaldehyde reaction and gas chromatography methods is described.

Key words: indole, fluorescence, exciplex, drug, analysis.

ACKNOWLEDGEMENT

The author would like to express his gratitude and thanks to Dr A.Z. Britten for his invaluable help and encouragement during the course of this work, also staff and research colleagues in the Department of Pharmacy, especially Professor D.G. Wibberley and Dr. W.J. Irwin for many useful discussions. Thanks are also due to the state Company for Drug Industries (S.D.I.) of Iraq for their financial support and study leave throughout the course of this work.

Grateful thanks are also extended to Miss K. Ensor for her reading part of the manuscript.

C O N T E N T S

1. INTRODUCTION

Luminescence attracted the attention of scientists a long time ago, but in the last years it has found applications in industrial practice. During the initial period of application of luminescence to practical work, its methods lacked a theoretical basis and the observed facts were not well understood. Moreover, the development of fluorometry was also hampered by the lack of suitable apparatus, e.g. light sources, light filters, and photometers. These difficulties have been overcome and the necessary light filters, monochromators, quartz mercury-vapor and xenon lamps, and other apparatus required for a spectrophotofluorometer are now readily available.

Luminescence was first discovered [1] as fluorescence in aqueous solution of wood extract (lignum nephriticum). Later the synthetic dyes were for a long time the most important compounds of the investigations, which were naturally restricted to the visible region of the spectrum. It was plausible, therefore, that the first attempts to find relations between the fluorescence of compounds and their constitution were based on the semiempirical observations on these compounds.

Photoluminescence is defined as the radiation emitted by a molecule, or an atom, after it has absorbed radiant energy and been raised to an excited state [2]. Photoluminescence consists of fluorescence and phosphorescence. Both processes have been used by the analytical chemist for trace analysis. The kinds of analysis which have been performed to date, using fluorescence, have been very varied, including trace metal determination, analysis for organic material, and particularly for determining trace constuents of biological systems.

In order to understand the luminescence processes it is necessary to become familiar with the nature of the electronic and excited state processes.

1.1. Electronic States and the Absorption and Emission of Electromagnetic Radiation.

Electronic states are concerned with properties of all of the electrons in all of the orbitals, so that, when an electron moves from one orbital to another, the state of the molecule is changed and it is important to consider the states of the molecule involved rather than considering only the orbitals involved in such an electron promotion. Thus, there is the ground state, the normal state of the molecule, or the state of lowest energy. There is only one ground state for any molecule. But, there are many different possible excited states for even very simple molecules, the exact nature of which depend upon the particular types of orbitals involved in the excitation. However, electronic states of organic molecules can be grouped into two broad categories; singlet and triplet states. A singlet state is one in which all of the electrons in the molecule have their spins paired. The resulting spin is zero. For instance, the ground state of most organic molecules will be a singlet state. Triplet states are those in which one set of electron spins have become unpaired, that is all electrons in the molecule except two have paired spins. In other words, the distinctions between singlet and triplet states, is the multiplicity of that state which is a term used to express the orbital angular momentum of given state of an atom or molecule [3]. Thus, if the multiplicity is 1, the state is called singlet, if it is three the state is called triplet. Singlet and triplet states differ significantly in their properties as well as in their energies. Triplet states always lie lower in energy than their corresponding singlet states. The singlet states may be said to be stacked in one vertical column, while the triplet states are stacked in another vertical column. Each of the electronic states has a number

of vibrational levels superimposed on it and these may be assigned as O, 1, 2, 3, 4, 5 etc. The vibrational levels arise because a molecule in a given electronic state may absorb small increments of energy corresponding to changes in vibrational modes. At room temperature a combination of thermal agitation and attractive and repulsive forces keeps the atoms vibrating in the lowest vibrational level of the ground state. Therefore absorption occurs from the Zeroth vibrational level of the ground state.

Light is a form of electromagnetic radiation i.e. energy, and is characterized by a frequency, a wavelength, and in a vacuum, a constant velocity. However, when light enters matter, two things may happen to it. It may pass through the matter with little absorption taking place. In this case there is little loss of energy, or, on its passage through the matter, the light may be absorbed, entirely or in part. In either case absorption involves a transfer of energy to the medium. Thus, the absorption itself is a highly specific phenomenon and radiation of a particular energy can be absorbed only by characteristic molecular structures. However, luminescence processes can be interpreted only in terms of the excited state from the luminescence emission and its relationship to the ground state of the molecule.

1.2. Fluorescence and Phosphorescence Processes

The process of fluorescence may be represented in schematic diagram, Figure 1.

When the quantum of light strikes a molecule, it is absorbed in about 10^{-15} second or within the period of frequency of the light, which is short in relation to the time required for all other electronic processes and for nuclear motion. The internuclear distances therefore remain constant during the absorption of light which will then raise the energy of the molecule by promoting an electron to a higher state, known as the excited state.

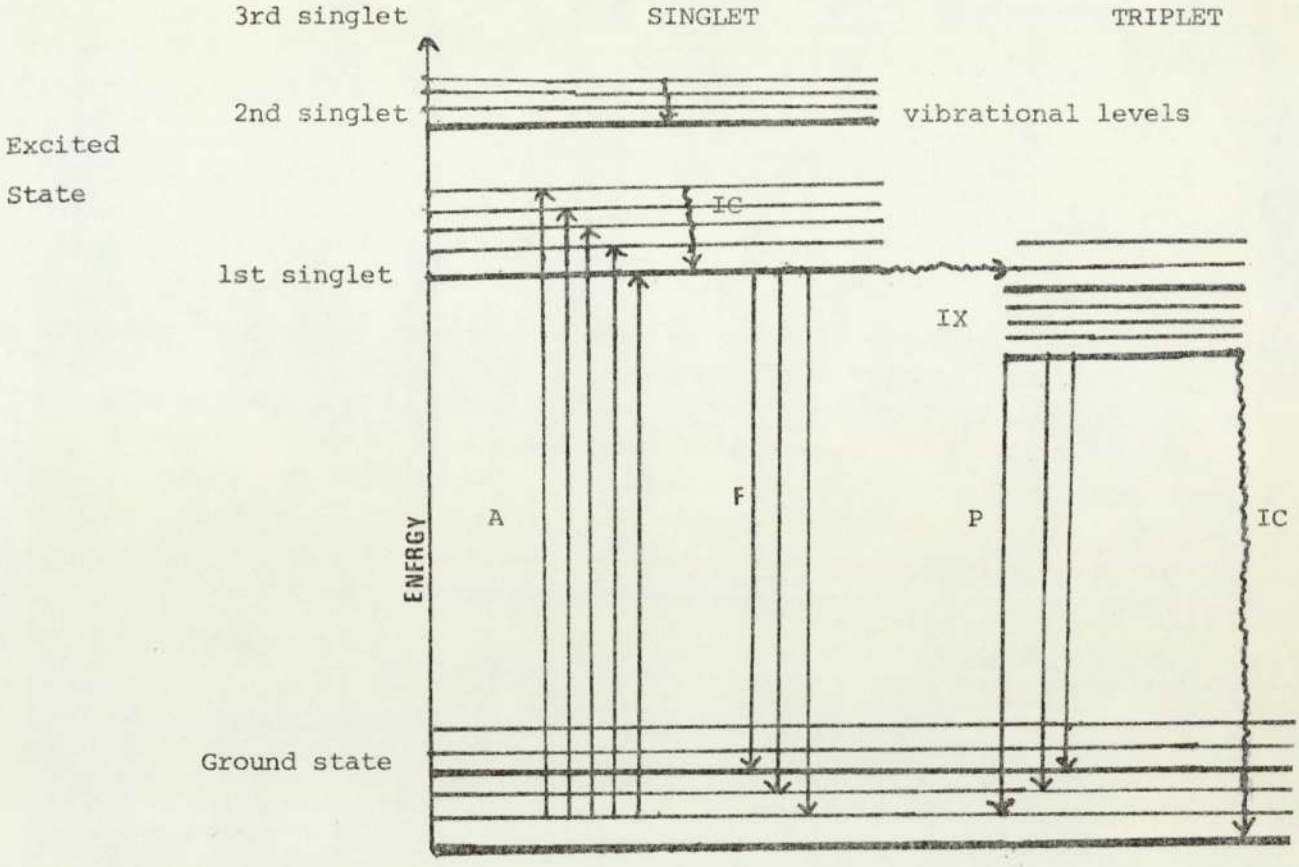


Figure 1

The excited state persists for a finite time which is about 10^{-8} second [3], because the life of an excited singlet state is approximately 10^{-9} second to 10^{-7} second [2]. After excitation there occurs deactivation by radiationless processes and return to the lowest vibrational level of the excited state. This process occurs within 10^{-13} to 10^{-11} second .This is a very important point because it means that before an excited molecule in solution can emit a photon, it will undergo vibrational relaxation, and therefore, photon emission will always occur from the lowest vibrational level of an excited state. In this case, the probability for return to the ground state is very high by photon emission (F arrows), this process is called Fluorescence (2).

In some gases at extremely lowe pressures the reverse transition occurs before vibrational deactivation can occur. This has the same energy as the absorption transition, thus the emitted light has the same wavelength as the absorbed light. However, one tends to see photon emission from higher vibrational levels of the excited states in gas phase spectra at low pressure. This process is called resonance radiation [3], which does not occur at higher vapour pressures or in solutions. The fraction of excited molecules that will fluoresce is called quantum efficiency of fluorescence. So that for highly fluorescent molecules the quantum effiency of fluorescence approaches unity, while for molecules which have fluorescence so weak that it is difficult to observe itthe quantum effiency of fluorescence is very low, and approaches zero.

Light of a frequency which is incapable of producing in a molecule a transition to the excited electronic state, is nevertheless capable of being absorbed by the molecule. The absorbed photon excites electrons in the ground state to higher vibrational levels, and so there is no electronic transition. The energy is entirely conserved and a photon of the same energy is emitted within 10^{-15} second , while the electrons return then to their original state. Since the absorbed and emitted photon are of the same energy the emitted light has the same wavelength as the exciting light. Light emitted in this case is referred to as Rayleigh scattering. However, in the case of electronic transitions the change in photon energy causes a shift of the fluorescence spectrum to a long wavelength relative to the absorption spectrum. This is called the Stokes shift.

From the preceding discussion it would appear that all molecules which absorb light energy should fluoresce, but some molecules in an excited singlet state or triplet state are found to return to the ground state without the emission of a photon, converting all the excitation

energy into heat. This radiationless process is called internal conversion (↓c wavy arrows in Fig. 1). On the other hand, most absorbing molecules have two excited electronic states [4] , Singlet and Triplet, which are related to each other as shown in the schematic diagram, Fig. 1. Transition from the ground singlet state to the excited singlet state constitutes normal absorption (A); the reverse is fluorescence (F). The transition from the ground state to the triplet states by direct absorption is very weak, but may be an efficient process for the excitation of triplet states from the lowest singlet state. This process is called intersystem crossing (↓x wavy arrow in Fig. 1.) and is a spin-dependent internal conversion process. Molecules in the excited triplet state, can return to the ground state via the singlet state only by taking energy from the environment, so that, the emitted light is the same as that produced by normal fluorescence, but the lifetime in this case of the excited state is longer than 10^{-8} second Intersystem crossing [5] is enhanced. (i) if the energy difference between the lowest singlet state and the triplet state just below it is small, (ii) by longer lifetime of an excited singlet state as this should increase the fraction of excited molecules which undergo intersystem crossing, compared with those that fluoresce. Once intersystem crossing has occurred, the molecule undergoes the usual internal conversion process and falls to the zeroth vibrational level of the triplet state. At low temperature thermal energy is not available and the return to the ground state can proceed only via the forbidden transition from the triplet state to the ground state, because the energy difference between the triplet state and the ground state is smaller than the difference between the lowest singlet state and the ground state. Since the probability of this is quite low, the excited state persists for a long time. i.e. the lifetime of a triplet state is much longer than that of a singlet state, i.e. several seconds at ordinary temperature, so

that the emitted light will be of a lower frequency and longer wavelength than the fluorescent light. In other words the excited triplet state represents a tautomeric form of the ground state in which a pair of electron spins are uncoupled. The emission produced by the transition from a triplet state to a ground state (P arrows), is called Phosphorescence.

It is seen that phosphorescence differs from fluorescence mainly in so far as the persistence of the luminescence following the excitation by the light source and by the marked enhancement and increase of the emission time with the lowering of temperature. Because of these reasons, phosphorescence is almost never observed in solution at room temperature, although it has been detected for some molecules by using a very sensitive detector[6] .

Coming back to the fluorescence, the process takes place when a molecule absorbs a photon light and an electron is raised to a higher level of energy. The molecule is, therefore, left in an excited state. The electronic change yields a band spectrum of absorption which covers the whole series of transitions, electronic, vibrational and rotational. If the molecule does not decompose as a result of the increase in energy and if all the energy is not dissipated by subsequent collision with other molecules, then after a short time (10^{-8}, 10^{-7} second), the electron returns to the lower energy level, emitting a photon in this process. The difference between the energy of the initial state (ground state) and the final state (excited singlet state) determines the energy of the emitted radiation (fluorescence). However the emitted fluorescence has a greater wavelength or lower energy than the light which is absorbed.

1.3. Fluorescence Quenching Processes

Fluorescence may cause a problem when measuring the absorption, so absorption raises certain problems during fluorometric assay. Obviously, light must be absorbed before fluorescence does occur. However, it is also apparent that when absorption is so great as to make the solution opaque, no light will pass through to cause excitation. . At intermediate concentration, even though light does penetrate through the solution, it is not evenly distributed along the light path, so that the portions nearest the light source absorb much of it and gradually less and less is available for the remainder of the solution, i.e. molecules near the light source absorb some of incident light, so that the transmitted light has less energy than the original incident light. However, this transmitted light will be the incident light for the remainder of molecules in the solution. The result is that most of the excitation occurs at the entrance, with less and less through the remainder of the cell, thus, the non-uniform distribution of the fluorescence in a strongly absorbing solution presents a problem for detection. A general rule is that a linear response will be obtained until the concentration of the fluorescent substance is sufficiently great so as to absorb a significant amount of the exciting light. A solution having an absorbancy of say 0.5 in the spectrophotometer would be expected to lower the apparent fluorescence, and to obtain a linear response the solution must absorb less than 5% of the exciting radiation [7].

Again all molecules which absorb light energy should fluoresce, but even under ordinary conditions a large number of strongly absorbing substances are non-fluorescent. This means that the fluorescence efficiency of such molecules is very low so that fluorescence is not as widespread as light absorption, because of competing quenching processes

tending to decrease the quantum yield of fluorescence. This may be caused not only by a high concentration of the molecules of the fluorescent substance but also by the presence of other organic and inorganic materials or by radiationless transitions which occur when the excited singlet electronic state leads to an electronic state in which a chemical bond is broken. These are termed Predissociative transitions, that is the molecules contain linkages with a bond strength lower than the energy required for electronic excitation. There are two further types of processes that compete with fluorescence; radiationless transfer of excitation energy to an appropriate acceptor, and reversible and irreversible chemical reactions. Often, excited state reactions are different from reactions of the same molecule in the ground state.

A collisional quenching process is a bimolecular process depending upon contact between the excited state and the quencher. This process requires that the lifetime of the excited state involved should be greater than 10^{-9} second . There are two mechanisms by which collisional loss of excitation energy can occur; enhancement of intersystem crossing by the quencher, or electron transfer. The effect of the quencher on intersystem crossing and enhancement of phosphorescence is well known, for example; (i) heavy atoms effect both the radiative and nonradiative triplet singlet transitions. McGlynn and Smith (8) have studied the external heavy atom effect and have suggested it might be a useful means of enhancing the phosphorescence, intensity of some compounds.

(ii) Alkyl halides and halide ions are effective quenchers either by an external or internal effect. Hood and Winfordner [9] have studied the influence of ethyliodide-ethanol as a solvent. They found that a 5:1 v:v ethanol-ethyl iodide enhanced the phosphorescence signal.

(iii)Paramagnetic metal chelates [10] are also effective quenchers.

(iv) The ability of oxygen to quench excited the singlet state of many

molecules[11] especially aromatic hydrocarbons in solution. Nitric oxide also quenches the fluorescence of aromatic hydrocarbons with about the same efficiency as oxygen. In both instances the quenching mechanism is due to the small energy gap between singlet and triplet states because of the presence of n-electrons of oxygen.

However this concept does not include cases, where the decrease in fluorescence yield is caused by the partial interception of excitation energy or of the fluorescence emission for instance, by an absorbing impurity. The same considerations apply if the solvent absorbs in the spectral region of the excitation of the emission.

Another possibility for collisional quenching is by an electron transfer mechanism, which occurs when the fluorescent molecule reacts with the quencher and abstracts an electron from it to form the ion pair. This ion pair can dissociate to give either a triplet state and quencher or simply a ground state and quencher [2].

A non-collisional energy transfer can also occur over distances larger than the contact distances of molecular collision. These processes are non-radiative processes of energy transfer from one molecule to another, that is non-collisional transfer of the energy between donor and acceptor[13].

Delayed fluorescence is another energy transfer process,which may occur either when two triplets collide with one another producing one molecule in the excited singlet state and the other in a ground state. The fluorescence emission of the excited singlet state may then be observed. Or it may occur, if the lowest singlet and triplet are so close in energy that thermal activation will occasionally promote triplets up to excited singlet state, from which they fluoresce. In both cases an emission has the spectral characteristics of fluorescence

but a lifetime just shorter than the lifetime of phosphorescence [14]. However, delayed fluorescence should not be confused with the excimer fluorescence which has a spectrum different from normal fluorescence.

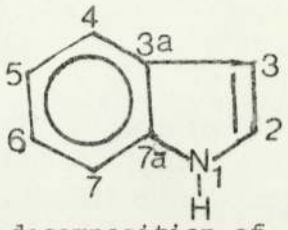
Recently [15] it has been found that the emission of solution of certain organic molecules changes at increasing concentration, and a new broad emission band appears to the red side of the fluorescence spectrum. This is known to be due to the formation of a short-lived dimer between an excited molecule and an unexcited molecule which breaks up shortly after formation with emission of the characteristic spectrum. When two identical molecules are involved, the excited dimer is called an excimer. On the other hand such interaction between unlike molecules is called an exciplex. It should be emphasised that this type of interaction is only formed with the excited state and cannot be detected by absorption spectrophotometry[16]. Weak acids and bases usually show solvent dependent fluorescence emission. The influence of pH upon the fluorescence of organic molecules was reported by Williams [17], who discussed the relationship between molecular dissociation and fluorescence. Thus, phenol fluoresces maximally at pH 1 and its fluorescence diminishes as the pH is raised and becomes essentially zero at pH 13. On the other hand, monohydroxyl and dihydroxybenzoic acids fluoresce in alkaline solution, and hydroxyphenyl acetic acid and related compounds show the maximal fluorescence at a neutral pH. Therefore a change in hydrogen ion concentration can cause a marked change in both the intensity and the position of fluorescence emission with little effect on the absorption spectrum. Such findings have been reported for several indole compounds [18], where changes in pH produced a large change in fluorescence intensity without producing a comparable change in absorption. On the other hand, indole has been shown to have highly solvent dependent fluorescence

properties [16, 19, 20, 21, 22]. Since solvent effects are important in the quantitative study of protein fluorescence, a reappraisal in terms of their probable significance for the fluorescence of tryptophanyl and tyrosyl residues in proteins [23, 24] is required.

2. THE FLUORESCENCE OF INDOLE

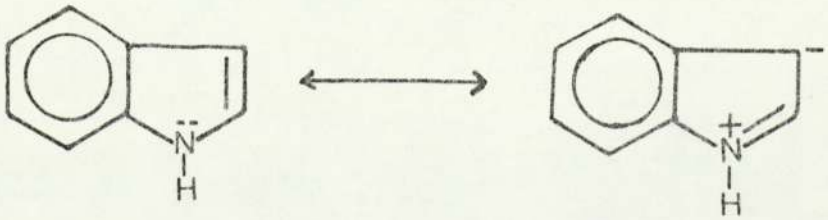
2.1 GENERAL CONSIDERATIONS

Indole is the commonly used name for benzopyrrole in which the benzene ring is fused to the 2 and 3 position of the pyrrole ring [25]. The indole structure occurs widely in natural compounds. It occurs in some plant materials and may be formed by the decomposition of tryptophan in putrefaction of proteins. The simpler naturally occuring indole derivatives include 3-methylindole, skatole, as well as indigo which is a common purple dye. Indole was discovered in 1866 during an investigation of indigo. The discovery of many alkaloids containing the indole nucleus led to further interest in indole chemistry. During this period the finding that the essential amino acid, tryptophan and the plant hormone heteroauxin were indole derivatives added stimulus to research in indole chemistry.



Recently indole derivatives have achieved increased significance in medicinal chemistry. There are a number of indole derivatives of physiological and pharmacological interest, and some of them are in use as drugs [12].

Indole [26] has a planar heteroaromatic structure with a ten pi-electron system formed by a pair of electrons from the nitrogen atom and eight electrons from the eight carbon atoms. The indole ring is reactive towards electrophilic substitution with the 3-position being the most susceptible to attack. This is in agreement with an explanation based on a simple resonance approach which makes the 3-position electron rich.



Early work on the fluorescence of indole began in the 1950's (27) when it was proposed that the protein fluorescence in the ultraviolet region was the result of the emission from tyrosine and tryptophan residues. Later it was found that pyrrole in aqueous solution is non-fluorescent, but indole was highly fluorescent[28]. This property of indole has been valuable in the detection and identification of indole compounds, especially in biological systems. Fluorescence is an extremely sensitive technique with a very low limit of detection. It has therefore been applied in studying the chemical composition and the physico-chemical properties of proteins, as well as other macromolecules and interaction of proteins with one another and smaller molecules, and in the quantitative estimation of tryptophan and tyrosine in protein hydrolysates [3]. Also the fluorescence behaviour of the amino acids, tryptophan, and tyrosine have been used in the study of protein structures [29]. It has been found that the ultraviolet fluorescence of proteins containing tryptophan, is predominantly due to the fluorescence of this amino acid [23]. A knowledge of the fluorescence properties of tryptophan is therefore of importance in the understanding of protein fluorescence. The measurements of the quantum yields and the wavelength distributions are useful in studying the spectral properties of tryptophanyl side chains buried within protein structures [30].

The estimation of the fluorescence of indole (31, 32, 18, 33, 28) provides a basis for the analysis of protein emission due to tryptophan fluorescence. However, the changes in the fluorescence of indole produced by solvents, with little effect on the ultraviolet absorption spectra imply that the excited state is highly polarized [16]. This makes indole in the excited state a strong acid bearing the positive charge on the nitrogen. Studies of solvent, pH, concentration and temperature effects on the fluorescence spectra lend insight into the influence of substituent groups in molecules on acid base equilibria, hydrogen bonding and the nature of the excited state[24,34].

The following salient features in the fluorescence of indole have been reported:

(1) There is a large Stokes shift and loss of vibrational structure in the fluorescence spectra of indole in a polar solvent as compared with a non-polar solvent without a corresponding shift in the ultraviolet absorption spectra[35,36].

(2) The absorption of indole between 250 and 300 nm is due to two overlapping transitions, designated $^1L_a \leftarrow {}^1A$ and $^1L_b \leftarrow {}^1A$. The two excited states are differentially shifted by solvents. Thus ^1L_a lies lower in polar solvents, but ^1L_b lies lower in non-polar solvents. However indole in polar solvents exhibits emission from both the ^1L_a and the ^1L_b state[37,36].

(3) The red shifts in the fluorescence spectrum of indole and related compounds, rather than being due to generalised solvent effects, have their origin in exciplex formation, i.e. formation of an excited state complex of indole and a polar molecule[16,38]. Thus the position of the emission maximum for a given tryptophanyl residue in a protein would depend upon its ability to form such an excited state complex.

It is clear that an understanding of the fluorescence behaviour of indole is necessary for the better use of fluorescence analysis. Of particular value would be the knowledge of the effect of the molecular environment on the position of emission maximum of indole.

Accordingly, it was decided to examine the effect of solvent on the fluorescence of indole and its derivatives as well as the structural and electronic requirements for indole exciplex formation.

2.2. SOLVENT EFFECT

Solvent can affect the structure of spectra absorption curves as well as the intensities and wavelengths of maxima[7], and this effect on the fluorescence and absorption maxima of indole has been well documented[35].

Solvents produce shifts to longer or shorter wavelengths, usually, of the order of 5 to 10 nm and seldom more than 17 nm[39].

McRae[40] studied the solvent effect on electronic spectra and derived a general expression for the solvent induced frequency shift by means of second order perturbation. He considered a solute molecule to be reducible to a point of dipole in an environment of isotropic dielectric solvent. In media of low viscosity, Brownian motion, which causes the molecules to become mobile will be a hindrance to charge transfer between molecules.

Polar solvents produce a red shift of the fluorescence of indole and substituted indole. This was attributed by Van Duuren[35] to the dielectric properties of the solvent, i.e. there is a shift to a longer wavelength with increased dielectric constant of the solvent. Furthermore, he suggested that there is an interaction between the solvent and the activated indole molecule but not between the solvent and the ground state of the indole molecule. Thus polar contributing mesomeric states of indole make a larger contribution in the activated indole molecule than in the ground state. Such polar structures would be more sensitive to the dielectric constant of the surrounding solvent, accounting for pronounced shifts in the fluorescence spectra.

Mataga et al[41] reported that hydrogen bonding is mainly responsible for the red shift mechanism in polar solvents. This was supported by Hardin et al[30], who pointed out that the red shift in polar solvents resulted from a hydrogen bonding mechanism, while the shift caused in non-polar solvents was mainly from the altered polarizability of the medium. Thus they applied this to predicting the wavelength position of the tryptophan absorption bands of proteins. In contrast indole can behave as proton donor or proton acceptor with a hydrogen bonding solvent[24], and this factor was taken into consideration by Van Duuren.

Hydrogen bonding is not necessary for the red shift to occur as was

found by examination of the fluorescence emission of 1,2-dimethylindole and 1-methyl-2-phenylindole in various solvents. These substances cannot undergo hydrogen bonding with dioxane, nevertheless their fluorescence emission maxima follows the same shift to longer wavelengths with higher dielectric constants. Mataga and co-workers[42], interpreted the red shift in terms of a generalized dipole-dipole interaction between solvent and solute in the excited state. However, they reported that the large shift of the fluorescence band in the polar solvents indicated a large increase of the dipole moment in the excited state, and greater stabilization was achieved by solute-solvent interaction in the fluorescent state than in the ground state, and Frank-Condon excited state[43]. Thus the fluorescence spectra of indole in n-hexane/ethanol mixtures indicated the relative intensity of the fluorescence band(^{1}Lb) decreases, and a broad band shifts further to the red as the concentration of ethanol is increased. In view of this, it may be feasible, that the ^{1}La state in which indole seems to have a large dipole moment (in contrast to the ^{1}Lb state), is strongly stabilized by the interaction of the solute with surrounding polar solvent molecules. This may become the lowest excited state during the radiationless process from the Frank-Condon to the equilibrium excited state. Therefore the difference of solvent shifts in fluorescence spectra are due to the dipole-moment produced in the excited state molecules[44]. In contrast, it has been reported[45] that the absorption spectra of indole in mixed solvents show a definite change. These changes are small at low polar solvent concentration but nevertheless are significant relative to the spectra in non-polar solvents, and indicate the possible presence of a ground state complex of indole in polar solvents.

Walker et al[16,38] have shown that these shifts, rather than being due to generalised solvent effects, have their origin in exciplex formation which is responsible for a red shift and loss of vibrational

structure in the fluorescence spectra of indole and its derivatives in polar solvents. However, they found that addition of only small amounts of ethanol or butanol to a cyclohexane solution of indole caused a large shift where a change in bulk dielectric properties was insignificant and because of this they postulated the formation of a complex between solute and solvent in the excited state. On the other hand it was reported[46] that the difference in solvent shifts of fluorescence spectra of molecules was due to the interaction energies between solute molecules and the solvent molecules surrounding them due to orientation polarization. Furthermore it was suggested[33] that the large red shift on the fluorescence of indoles depends on the possibility of solvent reorientation of the solvent molecules during the lifetime of the excited state rather than from the formation of stoicheiometric exciplex, explaining their results in terms of a solvent relaxation process.However, application of the formula for orientation - polarisation interaction with an excited dipole, developed by Mataga[42], yielded a poor correlation between the dipole moment and the red shift observed.

2.3 EXCIPLEXES

Exciplex is a term derived from excited complex[47] which means stable only in the excited state[48]. The fluorescence of indole and its derivatives in polar solvents shows a loss of vibrational structure relative to fluorescence spectra in pure solvent, and a pronounced red shift[35]. These characteristics are attributable to the formation of excited state complex between indole and one or two polar solvent molecules. Lumry et al[49] on varying the proportion of a cyclohexane-ethanol solution of indole observed a maximum shift at alcohol concentration, so low as to have a negligible effect on the solvent dielectric constant. As a result they suggested that a specific solvent solute excited state complex termed exciplex was responsible for the red shift emission. It was reported[50,51] that photochemical

reactions are now thought to proceed via weak exciplex or intermediate strength partial charge-transfer mechanisms. New compounds made by photochemical addition of electron rich olefins to naphthalene and anthracene, were postulated to have formed through the initial formation of exciplexes.

Weak exciplexes[52] are often the precursors of a strong exciplex. The weak exciplex is the result of dipole-dipole interactions between the excited molecule and one or more polar molecules, i.e., the exciplex formation is based on the basis of stoicheiometric complex formation between the exciplex, having a large dipole moment, and small dipolar molecules. This suggestion supports Chandross and Thomas[53] who first realised that all exciplexes were not charge transfer complexes, their conclusion being based on the studies of N,N-dimethyl-3-(1-naphthyl)propylamine, which forms an intramolecular charge transfer exciplex at room temperature. The emission from this compound is due entirely to the intramolecular exciplex in benzene. On the other hand, upon addition of a small amount of acetonitrile, they observed the appearance of a second emission band, shifted to the red from the original exciplex emission. These observations were ascribed to weak exciplexes defined as stoichiometric dipole-dipole stabilized complexes, which are formed between an excited species and one or more polar molecules[54], the dipole moment increasing upon excitation. However, in solvents of high dielectric constant the exciplex is not usually stable because the direct formation of radical ions becomes the most favoured reaction of the donor-acceptor pair. Birks[55] suggested that exciplex formation is caused by a charge-transfer interaction to which an excitation (dipole-dipole) interaction may contribute.

In recent work by Taylor[56] on the geometry of aromatic hydrocarbon-N,N-dimethylaniline exciplexes, it is shown that charge transfer and steric effect are the important stabilizing force in exciplexes. Further

studies on the intramolecular exciplex formation have been carried out by Chandross et al[57] where naphthalene and alkylamines were shown to readily form an exciplex when electronically excited. The exciplex did not seem to have strong geometrical preferences. In the anthracene-$(CH_2)_n$-dimethylaniline system. Okada and Co-workers[58] found that, for n=3, a charge transfer complex formed easily in all solvents. The excited complex could not form for n=1 or 2 in solvent of low polarity. They nevertheless, concluded that a parallel sandwich geometrical structure might be favourable, but not necessarily for the exciplex formation.

Walker et al[16,38] have explained the red shift and the loss of vibrational structure in polar and mixed solvents by assuming the presence of an excited state solute-solvent complex termed exciplex. A general kinetic scheme of exciplexes given by Walker is as follows:-

Excitation of uncomplexed solute	$A+h\nu \longrightarrow A^*$
Fluorescence of uncomplexed solute	$A^* \longrightarrow A+h\nu$
Internal quenching of uncomplexed solute	$A^* \longrightarrow A$
Exciplex Formation	$A^*+ns \longrightarrow Asn^*$
Exciplex dissociation	$Asn^* \longrightarrow A^*+ns$
Exciplex internal quenching	$Asn^* \longrightarrow A+ns$
Exciplex fluorescence	$Asn^* \longrightarrow A+ns+h\nu'$

Where A and S represent the solute and polar solvent molecules respectively, Ans is the ground state complex and Asn* is the excited state polar solvent complex. They showed that the 1:2 solute-polar solvent exciplex is formed with indole and 1-methylindole in an n-pentane and n-butanol mixture at room temperature and also with other associating solvents. No evidence for a 1:1 complex was found for these solvents, only a 1:1 exciplex was found with non-associating solvents. They suggested that the stability of the exciplex state is a result of configurational interaction of singlet states which are of both neutral and charge-transfer type; i.e. charge-transfer interaction between excited indole and solvent is explained due to dipole-dipole and polarization

interactions[53]. On the other hand, an excited state pyrene-aromatic amine complex was interpreted by Mataga and co-workers[59] to be a result of interaction of excitation and charge resonance states, though they stress the importance of the charge-transfer state in the stabilization of the complex. Furthermore, it was suggested[19] that exciplex formation does not depend on hydrogen bonding as shown by the fact that it occurs with 1-methyl substituted indole compounds and with non-hydrogen bonding solvents . Lockwood[60] proposed that the formation of the exciplex of N-methylindole with a hydroxylic solvent precludes the possibility of hydrogen bonding. However, in some environments, such as in the interior of protein molecules indole fluorescence may produce an emission spectrum without vibrational structure or red shift, due to the environmental stabilization of ^1La relative to the ^1Lb state through hydrogen bonding rather than exciplex formation. Longworth [61] has confirmed exciplex formation between 1,2-dimethylindole and isopropanol in 3-methylpentane at room temperature. The progressive red shift found to take place implied an equilibrium. The stoeichiometry of the reaction was found to be 1:1. He was unable to confirm the 1:2 exciplexes which were reported by Walker[16]. Moreover he explained that increasing the viscosity and cooling the system would inhibit exciplex formation. It was reported [45] that exciplex formation of indole in the methanol-cyclohexane mixture depends on the association rate during the lifetime of the excited state, furthermore Selinger et al[32] suggested that exciplex formation depends on the dielectric constant of the solvent, as well as to some extent its viscosity.

The study by McDonald and Selinger[62] of the excitation spectrum of the naphthalene-diethylaniline exciplex, provides evidence that exciplexes are formed by exciting either the donor or acceptor. However, at this stage they eliminated the possibility that energy

transfer within the collision complex immediately proceded electron transfer. On the other hand it was reported[58] in the fluorescence of the aromatic hydrocarbon amine exciplex that the separation between donor and acceptor is large. The stabilisation energy of a charge-transfer state may be smaller than in the case of ordinary exciplexes, however since the dipole moments in the charge-transfer states are much larger than those of the ordinary exciplexes, the solvation in a polar solvent may be very effective for the stabilization of the charge-transfer state.

Numerous experimental results and theoretical consideration have led to the conclusion that the fluorescent state of the exciplex may be identical with that of the corresponding electron donor-acceptor complex, while their Frank-Condon excited states should be different from each other. However, few cases of exciplexes and electron donor-acceptor fluorescence in the electron donor-acceptor systems have been reported[63]. Therefore, the excited state is indeed the only state of the exciplex which has been explained in a very similar manner to the Mulliken[64] theory of charge-transfer complexes.

2.4 CHARGE-TRANSFER COMPLEX

The term electron donor and electron acceptor are relative terms and there is no reason why any molecule should not be a charge donor and acceptor under the appropriate conditions. The term charge-transfer complex was first put forward by Mulliken[64], who developed the theory to explain the phenomenon. The donor-acceptor complex[65] is formed upon mixing a solution of molecules having a low ionization potential (electron donor) with a solution of high electron affinity (electron acceptor) in the ground state. Rose[66] proposed that two conditions have to be satisfied for charge-transfer absorption to occur, (i) the donor shall have a high energy filled orbital and the acceptor low energy

vacant orbital, (ii) the above orbitals shall overlap. This overlap may be sterically determined or derived either from chance collisions between components or from complex formation, i.e. before a charge interaction can take place, the molecules must be in sufficient proximity, so that the difference in electropotential can be recognised. With large molecules, steric hindrance may well prevent such close approach. The energy of a complex[67] is found to be correlated with the electron affinity of the acceptor and with the ionization potential of the donor, indicating that the interaction involves transfer from D to A. Itoh et al[68] proposed that the electron donor-acceptor complex is the result of the electronic interaction and geometrical arrangement between the excited electron acceptor and the donor in the ground state. The primary process of the exciplex formation seems to be different from those of both ground state donor and acceptor in the electron donor-acceptor complex formation. Charge-transfer complexes have well defined simple stoicheiometries. There are various methods of determining the stoicheiometries[69]. One of the best methods is that of plotting some property of complex formation against the concentration of the partners. The structure of the complex will be determined by the intermolecular forces. The structure giving the minimum potential energy, where all intermolecular forces are considered being the most probable. Slifkin[69] classified the complexes into three broad classes according to the type of donor. The first class where the donor electron is a π electron includes conjugated systems and polycyclic aromatic hydrocarbons as typical members of this group. The second class of donor is that in which the donated electron is an n-electron or lone pair electron. This is most frequently located on the nitrogen in the amino group, and the third class is that in which charge-transfer can take place between localized regions of positive charge and negative charge in adjacent molecules. The charge-transfer band can be affected by

solvents. Weak charge complexes which have little dative character in the ground state, show slight wavelength shifts of the charge-transfer band maximum, which correlate approximately with the polarity dielectric constant[70]. Moreover Foster and Thomson[71] suggested that the stronger charge-transfer complex, with predominantly dative character in the ground state, can dissociate into the component ions in solvents of high dielectric constant, so that increasing the polarity of the solvent causes the charge-transfer band to be replaced by the spectra of the ions. Weller[72] suggested that two effects of increasing dielectric constant on the charge-transfer emission are noticed; a red shift and a considerable decrease in the intensity. Furthermore it was reported[73] that an increase of the solvent polarity leads to a significant displacement of the maximum of the complex fluorescence to a longer wavelength, the displacement of the fluorescent maximum being dependent on the dielectric constant of the solvent. The solvent effect dependent red shift can easily be understood on account of the dipolar nature of the excited complex whose energy should decrease as the solvating property of the solvent increases. The strong decrease in intensity of the complex emission has been discussed[74] in connection with the results of the lifetime measurements on the same complex in different solvents. Beens and co-workers[75] have shown that the emission spectra of the complex are solvent dependent, insofar as their maxima are red shifted with increasing solvent polarity. They chose anthracene-diethylaniline as the system to estimate the dipole moment effect, and found that increasing the dipole moment causes a red shift of the emission maximum.

Indole forms intermolecular charge-transfer complex with various electron acceptor molecules. The formation of a 1:1 intermolecular charge-transfer complex between indole and tetracyanoethylene in dichloromethane at room temperature has been reported[76]. There are

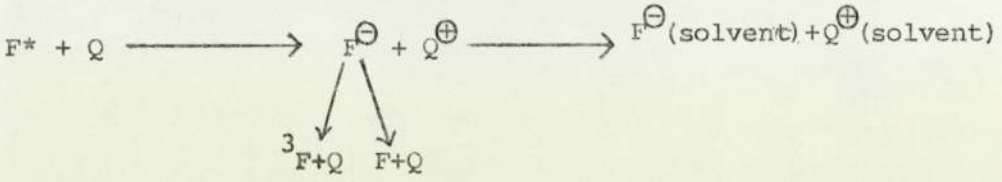
many organic electron acceptors which form charge-transfer complexes with indole; such as anthraquinone, naphthoquinone, maleic anhydride and trinitrobenzene[77].

Several authors have reported on the charge-transfer complexes of indole with other electron-acceptors[78]. However, it was suggested[69] that the apparent strong charge donation ability of the indoles arises from the presence of strongly negatively charged carbon atoms, rather than arising from the donation of a π-electron from the conjugated electron system in the indole ring. Green and Malrieu[80] have calculated the super-delocalizability, a measure of reactivity, at C3 in the indole nucleus and found that this correlates better with the maximum position of the charge transfer band than it does with energy of the highest occupied molecular orbital of the indole. Furthermore, Foster et al[81] who studied the complex of the indole and its derivatives with organic acceptors, (trinitrobenzene, and dinitrobenzene) by NMR found that the strength of interaction correlates to some extent with the electron donating ability of indole. Methylation especially at the 2 or 3 position increases the association constant, while methylation in benzene ring at 7-position does not produce such a marked increase. It may be that substituents on the benzene ring decrease the association constant through hindrance. Thus suggesting the importance of a specific steric configuration for the complex.

2.5 FLUORESCENCE QUENCHING

True quenching is not related to absorption or light scattering, but is due to an interaction of the fluorescent molecule with solvent or with other solute in such a manner as to lower the efficiency and/or lifetime of the fluorescence process[3]. This means that the compound will exhibit less fluorescence when it is in the presence of the quenching substance.

Hopkin and Lumry[22] have shown that there are at least two pathways of decay. One appears to be outright electron ejection and the other is collisional scavenging of electrons. Some quenching reactions, however, are the result of a specific interaction between the fluorescent species and the quencher. Hercules[2] has explained the quenching effect by a charge-transfer mechanism as shown in the following:



The excited state fluorescent molecule F* reacts with the quencher (Q) abstracting an electron from it to form the ion pair $F^{\ominus}$, $Q^{\oplus}$. This ion pair can dissociate to give either a triplet state ^3F and quencher Q, or simply a ground state and quencher. In the presence of a polar solvent, both $F^{\ominus}$ and $Q^{\oplus}$ can be solvated and can then carry out characteristic radical ion reactions in solution. However, it was reported[83] that the charge-transfer interaction appeared to be the predominant quenching mechanism for the singlet states of aromatic compounds with amines. For example, this was found for the case of naphthalene and bicyclic azo compounds as a model for the theory. Moreover Goldschmidt et al[84] postulated that aromatic molecules quenched via charge-transfer interaction and they explained this by a detailed mechanism leading to the triplet state of excited aromatic molecules. However, Labianca and co-workers[85] suggested that the charge-transfer interaction and quenching activity could be divided in two types:-

(i) either within a group of quenchees and quencher there may be large variations in binding energies of the exciplexes and relatively small variations in the rate constant for internal conversion, or

(ii) the ion pair may lie lower in energy than the fluorescent molecule, so that complete transfer of an electron is an irreversible decay process.

There is convincing evidence that charge and electron transfer processes are important in the formation and decay of exciplex species. Ander et al[86] reported that rate of quenching depends on the donor-acceptor properties of the quenchee and quencher.

In the study of the quenching of the fluorescence of naphthalene and other aromatic hydrocarbons by conjugated dienes it was suggested[87] that exciplexes were intermediates in the quenching process. It was proposed[74] that by increasing the solvent polarity the exciplex fluorescence intensity and its lifetime decreases. It was explained that the fluorescence quenching in polar solvents arose by electron transfer between excited and quencher molecules which formed a sandwich type charge-transfer complex. Recently [88] it was pointed out that quenching of one exciplex can lead to the formation of another exciplex. It has also been reported[72] that the effect of amines as fluorescence quenchers can be ascribed to the formation of charged products with excited fluorescers. However, the principle path[84] of intersystem crossing in the excited aromatic hydrocarbon-amine complex in both polar and non-polar solvents, is a fast process competing with the vibrational and solvent relaxation, following the electron transfer in the encounter complex. It has been reported[89] that, for aliphatic amines quenching ketone fluorescence, the quenching rate constant decreases on going from tertiary to primary amines.

The fluorescence of many electron rich aromatics is quenched efficiently by methylchloro-acetate and chloroacetamide. This is explained[90] by the charge-transfer from the aromatic to the quencher to form exciplex binding. However, the fluorescence properties of many indole derivatives[91] have been found to be dependent upon the nature of their structure as well as upon the temperature and solvent composition. Steiner and Kirby[92] reported on the quenching of the fluorescence of indole derivatives and concluded that the quenching could take place

through (i) transfer of an electron by collisional contact of the quencher with an excited indole.

(ii) preliminary ejection of an electron from an excited indole to vicinal solvent molecules.

(iii) the formation of a transient complex of the charge-transfer type between excited indole and the quencher.

They have also proposed[92] that molecules which are known to be efficient electron scavengers might lead to deactivation of the excited state of indole derivatives by an electron transfer from the ring system to the quencher molecule. Water and amines[38] quench indole fluorescence due to electron ejection which is the major quenching process in water and the indole exciplex. It is also reported that iodide[93], hydrogen and hydroxide ions[94], appear to quench indole fluorescence, but the mechanism, in most cases has not been proven. Recci and Kilichowska[95] have reported that lanthanide quenches indole fluorescence by a ground-state complex formation, in which the quenching occurs by virtue of the formation of a non-fluorescent complex between indole and lanthanide. Most charge-transfer complexes[76] appear to be non-fluorescent. Thus the addition of a donor to a fluorescent acceptor in solution or vice-versa cause a quenching of the fluorescence which is proportional to the association constant (Kc) of the complex, and can thus be utilized for measuring it. A Stern-volmer type of equation may be used to obtain the constant Kc.

$$\frac{Fo}{F} = 1+Kc[D]$$

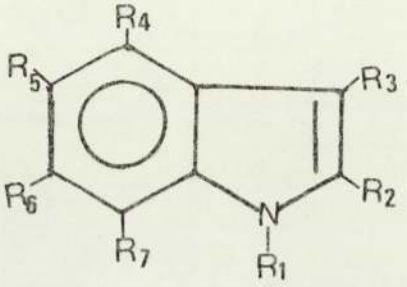
Where Fo and F are the fluorescence intensities in the absence and presence of quencher respectively and $[D]$ is the concentration of the quencher plot of relative fluorescence yield vs. quencher concentration gives the association constant.

2.6 Scope of investigation

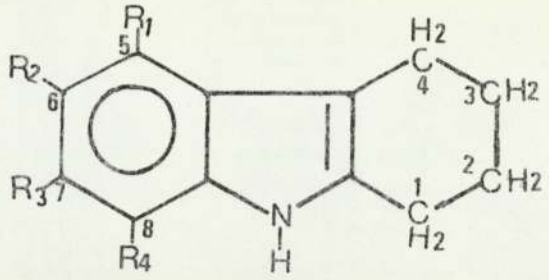
This was to examine the structural and electronic requirements for indole exciplex formation. The polar solvents used were, methanol, n-butanol, cyclohexanol, tert-butanol, borneol, acetonitrile, pyrrolidine, triethylamine, cyclohexylamine, quinuclidine [1-azobicyclo(2,2,2)octane] ABCO, triethylenediamine [1,4-diazobicyclo(2,2,2)-octane] DABCO, 3-ethyl-3-pentanol, tetrahydrofuran, and acetic acid. For comparison, fluorescence spectra of several indole derivatives with substituents on the pyrrole ring and on the benzene ring have been investigated.

The indoles studied were

(1)



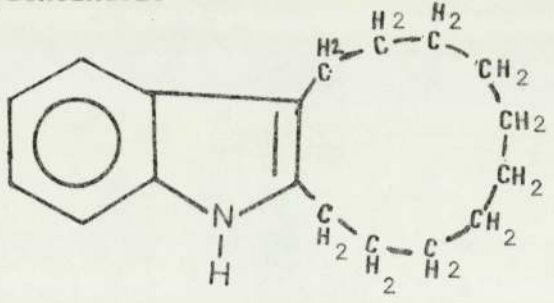
	1	R_1	R_2	R_3	R_4	R_5	R_6	R_7
i)	Indole	H	H	H	H	H	H	H
ii)	1,2-Dimethylindole	CH_3	CH_3	H	H	H	H	H
iii)	1,3-Dimethylindole	CH_3	H	CH_3	H	H	H	H
iv)	1,2,3-Trimethylindole	CH_3	CH_3	CH_3	H	H	H	H
v)	3-Methylindole	H	H	CH_3	H	H	H	H
vi)	2-Methylindole	H	CH_3	H	H	H	H	H
vii)	2-Tertbutylindole	H	$-C(CH_3)_3$	H	H	H	H	H
viii)	2,3-Dimethylindole	H	CH_3	CH_3	H	H	H	H
ix)	5-Methylindole	H	H	H	H	CH_3	H	H
x)	5-Fluoroindole	H	H	H	H	F	H	H
xi)	5-Methoxyindole	H	H	H	H	OCH_3	H	H
xii)	7-Methylindole	H	H	H	H	H	H	CH_3
xiii)	Tryptophol	H	H	CH_2CH_2OH	H	H	H	H
xiv)	2-Butyl-3-propylindole	H	$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_3$	H	H	H	H



(2)

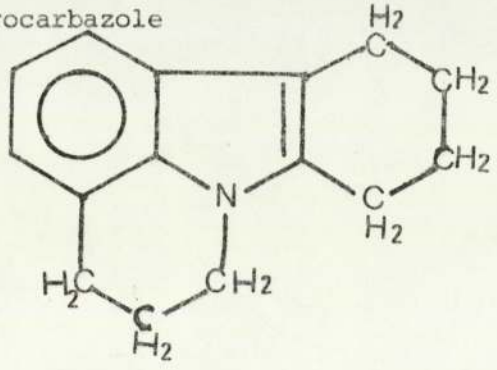
		R_1	R_2	R_3	R_4
xv)	6-Methyl-1,2,3,4-tetrahydrocarbazole	H	CH_3	H	H
xvi)	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	H	CH_3	CH_3	H
xvii)	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	H	CH_3	H	CH_3
xviii)	6-n-butyl-1,2,3,4-tetrahydrocarbazole	H	$CH_2CH_2CH_2CH_3$	H	H
xix)	6-Methoxy-1,2,3,4-tetrahydrocarbazole	H	OCH_3	H	H

xx) 2,3-Cyclodecamethyleneindole



(3)

xxi) 8,9-Cyclotrimethylene-
1,2,3,4-tetrahydrocarbazole



(4)

2.7 RESULTS

The shifts in emission maximum (nm) caused by polar solvents with different indole derivatives are summarized in tables 1-14. Some of the recorded emission spectra are included in the appendix.

Consideration of the indole derivatives show that the magnitude of a red shift produced by n-butanol is of the order; tryptophol; 2,3-dimethylindole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 3-methylindole; 2,3-cyclodecamethyleneindole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 2-butyl-3-propylindole; 2-methylindole; 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; 2-tert-butylindole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 1,2,3-trimethylindole;indole; 6-methoxy-1,2,3,4-tetrahydrocarbazole; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole; 1,2-dimethylindole; 1,3-dimethylindole. It seems that there is an obvious relationship between a red shift and increasing the concentrations of n-butanol by 0.04-0.2M, i.e. the higher concentration such as 0.2M causes a greater red shift than 0.16 M. n-Butanol does not produce any red shift in 5-methylindole, 5-methoxyindole; 5-fluoroindole and 7-methylindole, (table 1.) Methanol showed similar behaviour, producing a red shift as produced by n-butanol (table 2.)

On the other hand cyclohexanol appears to have less effect than methanol, (table 3). Borneol (table 4) is shown to be more effective in producing a red shift than tert-butanol (table 5) and 3-ethyl-3-pentanol (table 6), but the three solvents still appeared to have a lesser effect in producing a red shift than cyclohexanol. The red shift produced in the indole derivatives tested by acetonitrile is in order of; tryptophol; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 2,3-dimethylindole; 2-butyl-3-propylindole; 2,3-cyclodecamethyleneindole; i,2,3-trimethylindole; 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; 3-methylindole; 1,2-dimethylindole; 2-methylindole; 1,3-dimethylindole; 2-tert-butylindol; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 6-methoxy-1,2,3,4-tetrahydro-

carbazole. Indole does not show a clear red shift nor is there a red shift produced in 5-methylindole; 5-fluoroindole; 5-methoxyindole and 7-methylindole, however higher concentrations of 0.16 M acetonitrile produce a shift more to the red than lower concentrations (table 7). There is in general a small quenching effect shown by the alcohols. The extent of quenching varies with the structure of the alcohol as shown for 2-butyl-3-propylindole in n-butanol and methanol, spectra 1 and 2. The quenching also varies with the indole structure as seen for 2-methylindole; 1,2,3-trimethylindole and 6-methoxy-1,2,3,4-tetrahydrocarbazole in butanol spectra 3, 4 and 5.

Tetrahydrofuran, produces smaller red shifts than the alcohols (table 8), and it was found that tetrahydrofuran is not an effective fluorescence quencher for the most of the indoles, as shown for tryptophol and 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole, spectra 6 and 7.

Pyrrolidine produces less red shift than n-butanol and acetonitrile in the compounds shown previously (table 9), but it appears to be a most effective quencher for all compounds tested, as shown for example, 6-methyl-1,2,3,4-tetrahydrocarbazole; n-butyl-1,2,3,4-tetrahydrocarbazole; 2-methylindole and 5-methylindole, spectra 8-11. The quenching effect is proportional to the concentrations of the amine and even the lowest concentration of pyrrolidine, such as 0.04 M, is effective as a quencher. Although cyclohexylamine produces similar shifts to pyrrolidine (table 10), its behaviour as a quencher is weaker than pyrrolidine see spectra 8 and 11-13. Triethylamine seems to be less able to produce a red shift than the above amines. A red shift produced in the following compounds is in the following order, 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole; tryptophol; 6-methyl-1,2,3,4-tetrahydrocarbazole; 3-methylindole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 2,3-cyclodecomethyleneindole; 2,3-dimethylindole; 2-methylindole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 6-methoxy-1,2,3,4-tetrahydrocarbazole (table 11). Triethylamine also appears to be less of a quencher than cyclohexylamine, although a low concentration of

triethylamine such as 0.04 M produces a small reduction in the fluorescence intensity as seen from spectra 14 and 15.

Quinuclidine produced greater shifts than triethylamine with indole, 5-methylindole and 2,3-dimethylindole. Like triethylamine it had no effect on the emission of 1,2,3-trimethylindole. Triethylenediamine, showed an almost identical effect to quinuclidine (tables 12 and 13) and spectra 16 and 17.

Acetic acid was found in general to produce no red shift, with the exceptions of 6,7-dimethyl- and 6-methyl-1,2,3,4-tetrahydrocarbazole. It did however act as an efficient quencher. (table 14 and spectra 18-22). Shifts (nm) and fluorescence emission maximum (nm) listed in tables 1-14, are correct to within $\pm$ 2 nm.

Table 1

Red shift (nm) caused by n-butanol

n-butanol M	Indole	2,3-cyclodecamethyleneindole	2-Butyl-3-propylindole	2,3-Dimethylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydro-carbazole	6-Methyl-1,2,3,4-tetrahydro-carbazole	6-n-butyl-1,2,3,4-tetrahydro-carbazole	6-Methoxy-1,2,3,4-tetrahydro-carbazole	6,8-Dimethyl-1,2,3,4-tetrahydro-carbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	1,2-Dimethylindole	1,3-Dimethylindole	3-Methylindole
maximum emission (nm) at OM	303	317	321	319	305	307	311	305	312	327	312	321	316	317	328	316	325	329	313	322	311
0.04	1	8	10	10	4	3	11	0	0	0	0	3	5	3	2	2	2	2	1	1	9
0.08	2	15	13	15	10	7	23	0	0	0	0	7	13	13	3	4	3	4	1	1	15
0.12	4	18	18	19	14	10	27	0	0	0	0	11	18	16	5	7	5	7	1	4	19
0.16	6	20	19	22	17	14	30	0	0	0	0	13	22	19	5	11	6	11	4	2	21
0.20	8	21	20	24	20	16	31	0	0	0	0	19	24	21	6	14	6	13	6	3	22

Table 2

Red shift (nm) caused by methanol

Methanol M	Indole	2,3-Cyclodeca-methyleneindole	2,3-Dimethylindole	2-Butyl-3-propylindole	7-Methylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm)	303	317	318	321	305	314	322
at OM							
0.04	1	6	10	3	0	0	2
0.08	3	12	15	5	0	1	2
0.12	5	17	21	7	0	2	2
0.16	7	19	23	9	0	5	2
0.20	8	24	25	11	0	6	6

Table 3

Red shift (nm) caused by cyclohexanol

Cyclohexanol M	Indole	2,3-Cyclodeca-methyleneindole	2,3-Dimethylindole	7-Methylindole	2-Butyl-3-propylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm)	303	317	319	305	321	313	322
at OM							
0.04	1	7	9	0	8	0	2
0.08	4	13	13	0	14	0	2
0.12	5	15	19	0	16	2	2
0.16	6	17	21	0	18	2	2
0.20	7	19	22	0	19	4	3

Table 4

Red shift (nm) caused by borneol

Borneol M	Indole	2,3-Cyclodecamethyleneindole	7-Methylindole	1,2-Dimethylindole	1,3-Dimethylindole	1,2,3-Trimethylindole
Maximum emission (nm) at OM	302	317	305	314	323	329
0.04	2	7	0	1	1	1
0.08	4	14	0	2	1	4
0.12	5	15	0	2	2	5
0.16	5	17	0	3	2	7
0.20	6	18	0	3	3	9

Table 5

Red shift (nm) caused by tert-butanol

Tert-butanol M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Butyl-3-propylindole	7-Methylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm) at OM	302	317	319	321	305	313	322
0.04	2	7	9	8	0	1	0
0.08	3	12	13	11	0	2	1
0.12	5	13	16	14	0	2	2
0.16	5	16	18	16	0	3	2
0.20	7	18	21	17	0	3	3

Table 6

Red shift (nm) caused by 3-ethyl-3-pentanol

3-ethyl-3-pentanol M	Indole	2,3-Cyclodecamethylene-indole	2-Butyl-3-propylindole	7-Methylindole	1,2,3-Trimethylindole	1,2-Dimethylindole	1,3-Dimethylindole
Maximum emission (nm) at OM	303	317	321	303	329	313	322
0.04	1	7	6	0	2	1	1
0.08	2	11	10	0	3	2	1
0.12	3	15	14	0	4	3	2
0.16	3	17	15	0	4	3	2
0.20	4	18	-	0	6	3	3

Table 7

Red shift (nm) caused by acetonitrile

Acetonitrile M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Butyl-3-propylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene -1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	1,2-Dimethylindole	1,3-Dimethylindole	3-Methylindole
Maximum emission (nm) at OM	302	317	319	321	305	307	311	305	313	327	312	320	316	317	327	316	327	329	312	323	311
0.04	1	8	9	8	2	2	8	0	0	0	0	3	6	5	2	1	2	5	4	2	5
0.08	2	10	12	10	5	3	13	0	0	0	0	7	10	11	3	2	6	10	8	4	10
0.12	3	13	14	12	7	7	16	0	0	0	0	11	14	14	3	4	7	13	12	7	11
0.16	3	15	17	15	9	7	19	0	0	0	0	15	17	18	4	5	7	15	14	8	14

Table 8

Red shift (nm) caused by tetrahydrofuran

Tetrahydrofuran M	Indole	2,3-Cyclodecamethyleneindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
Maximum emission (nm) at OM	302	317	306	311	305	312	327	312	321	317	316	327	316	325	329	311
0.04	0	6	2	6	0	0	0	0	3	5	5	2	0	0	0	6
0.08	0	8	2	9	0	0	0	0	5	7	9	4	0	0	0	7
0.12	0	9	4	11	0	0	0	0	7	8	10	4	0	0	0	9
0.16	0	10	4	11	0	0	0	0	8	10	12	4	0	0	0	9
0.20	0	11	4	12	0	0	0	0	9	11	12	4	0	0	0	9

Table 9

Red shift (nm) caused by Pyrrolidine

Pyrrolidine M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-Butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
Maximum emission (nm) at OM	303	318	319	305	307	311	304	312	327	313	320	317	316	329	317	325	329	311
0.04	3	5	5	4	3	7	3	2	1	3	2	5	4	0	1	0	0	5
0.08	3	8	8	7	5	13	3	2	1	4	4	10	12	2	2	0	0	9
0.12	4	10	11	7	7	14	4	3	2	5	7	13	14	2	5	0	0	11
0.16	5	12	13	9	8	19	4	3	2	5	9	15	14	6	6	0	0	14
0.20	5	12	15	11	9	19	4	3	2	5	9	15	14	6	7	0	0	14

Table 10

Red shift (nm) caused by Cyclohexylamine

Cyclohexylamine M	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyl-1,2,3,4-tetrahydrocarbazole	6-n-butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole
Maximum emission (nm) at OM	303	317	318	306	311	303	312	327	312	320	317	316	327	317	325	329	312
0.04	3	9	9	3	10	2	2	0	3	4	8	7	3	1	0	0	10
0.08	4	12	12	5	16	3	2	0	3	7	10	11	4	2	0	0	11
0.12	5	13	14	7	17	3	2	0	4	9	14	13	4	3	0	0	12
0.16	5	14	14	8	19	6	4	0	4	12	14	13	4	5	0	0	14
0.20	6	14	14	9	19	6	4	0	5	13	15	15	4	6	0	0	14

Table 11

Red shift (nm) caused by triethylamine

Maximum emission (nm) at OM	303	317	319	305	306	311	305	312	327	312	320	317	316	327	325	329	311	316
	Indole	2,3-Cyclodecamethyleneindole	2,3-Dimethylindole	2-Methylindole	2-Tert-butylindole	Tryptophol	7-Methylindole	5-Methylindole	5-Methoxyindole	5-Fluoroindole	6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	6-Methyll,2,3,4-tetrahydrocarbazole	6-n-Butyl-1,2,3,4-tetrahydrocarbazole	6-Methoxy-1,2,3,4-tetrahydrocarbazole	8,9-Cyclotrimethylene-1,2,3,4-tetrahydrocarbazole	1,2,3-Trimethylindole	3-Methylindole	6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole
Triethylamine M																		
0.04	3	2	5	1	0	7	0	0	0	2	3	5	4	2	0	0	11	1
0.08	3	6	7	2	0	12	0	0	0	2	7	8	6	2	0	0	12	2
0.12	3	7	8	4	0	14	0	0	0	3	11	11	12	3	0	0	12	2
0.16	3	9	9	6	0	14	0	0	0	3	13	13	12	3	0	0	13	2
0.20	3	10	10	7	0	14	0	0	0	3	16	13	12	3	0	0	13	2

Table 12

Red shift (nm) caused by quinuclidine (ABCO)

quinuclidine M	Indole	2,3-Dimethyl-indole	1,2,3-Trimethyl-indole	5-Methyl-indole
Maximum emission (nm) at OM	304	320	329	312
0.04	5	10	0	2
0.08	6	13	0	2
0.12	6	14	0	3
0.16	6	14	0	3
0.20	6	14	0	3

Table 13

Red shift (nm) caused by Triethylenediamine (DABCO)

Triethylene-diamine M	Indole	2,3-Dimethyl-indole	1,2,3-Trimethyl-indole	5-Methyl-indole
Maximum emission (nm) at OM	304	318	329	312
0.04	3	10	0	2
0.08	4	12	0	2
0.12	5	14	0	3
0.16	5	14	0	3
0.20	5	14	0	3

Table 14

Red shift (nm) caused by Acetic Acid

Acetic acid	Maximum emission at OM	0.04	0.08	0.12	0.16	0.20
Indole	303	0	0	0	0	0
2,3-Cyclodecamethyleneindole	317	0	0	0	0	0
2,3-Dimethylindole	319	0	0	0	0	0
Tryptophol	311	0	0	0	0	0
7-Methylindole	305	0	0	0	0	0
5-Methylindole	312	0	0	0	0	0
5-Methoxyindole	327	0	0	0	0	0
5-Fluoroindole	312	0	0	0	0	0
6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole	320	0	2	2	2	2
6-Methyl-1,2,3,4-tetrahydrocarbazole	318	1	2	2	2	2
6-n-butyl-1,2,3,4-tetrahydrocarbazole	317	0	0	0	0	0
6-Methoxy-1,2,3,4-tetrahydrocarbazole	327	0	0	0	0	0
8,9-Cyclomethylene-1,2,3,4-tetrahydrocarbazole	325	0	0	0	0	0
1,2,3-Trimethylindole	329	0	0	0	0	0
3-Methylindole	311	0	0	0	0	0

2.8 General Discussion

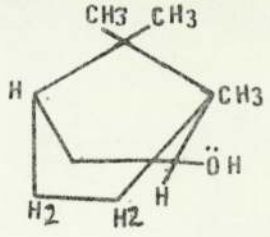
From the previous work on exciplexes, alcohols[16] have been used in the studies of the exciplexes of indole and indole derivatives. In this work methanol, n-butanol and sterically hindered alcohols such as, cyclohexanol, tert-butanol, borneol and 3-ethyl-3-pentanol were used to study structural and electronic requirements for indole exciplex formation. Alcohols may donate a proton as well as lone pair electrons. In contrast acetonitrile, of high dielectric constant[79] should be able to donate the lone pair electrons particularly readily due to the absence of steric hindrance effect. The red shift produced by hydroxylated solvents may arise by several mechanisms. One could be the result of proton transfer. Therefore, acetic acid an efficient proton donor was used to check this idea. Or the red shift may be due to interaction with oxygen in the excited state without transfer of proton. Tetrahydrofuran was used to examine this effect. The amines were chosen as stronger electron donors to show their effects on the exciplex formation. Cyclohexylamine (primary amine), Pyrrolidine (secondary amine), and triethyl amine (tertiary amine) were selected to distinguish between those amines which can act as proton donors and those which can only act as electron donors. Steric effects were examined using quinuclidine and triethylenediamine.

A consideration of the electronic and steric structure of some solvents tested is necessary to partly explain their effectiveness or otherwise, in interacting with the indole derivatives.

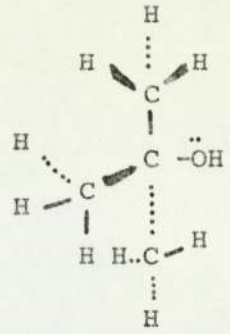
$CH_3-CH_2-CH_2-CH_2-\ddot{O}H$

n-butanol

XXII

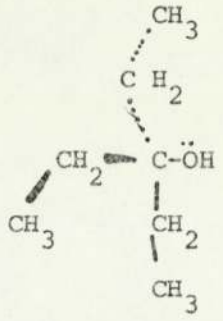


XXIII borneol



tert-butanol

(XXIV)



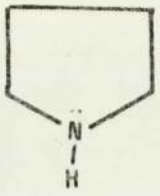
3-ethyl-3-pentanol

(XXV)

$CH_3-C\equiv N:$

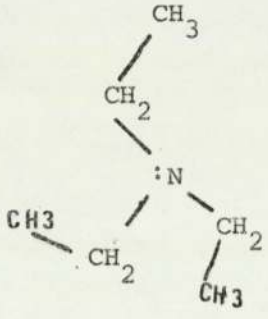
acetonitrile

(XXVI)



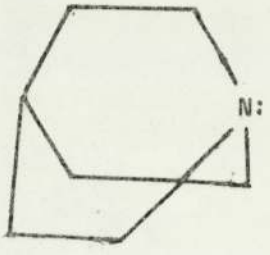
pyrrolidine

(XXVII)



triethylamine

(XXVIII)



quinuclidine

(XXIX)

It can be expected that in the alcohol series structure such as (XXIII) and (XXV) will have greater hindrance for access to their lone pairs than tert-butanol (XXIV) and n-butanol (XXII). Similarly in the amine group, it can be expected that triethylamine (XXVIII)will have hindrance for access to its lone pair in comparison with say quinuclidine (XXIX), cyclohexylamine or pyrrolidine (XXVII).

Triethylamine is known not to form a complex with BF_3 which contrasts with other amines such as quinuclidine and triethylenediamine. This has been attributed due to the effect of the spatial requirement of the "floppy" ethyl groups [82].

On the other hand acetonitrile (XXVI) should be free of steric hindrance effects about its lone pair and may be expected to show exciplex formation which is less dependent upon the indole structure.

The indole derivatives used in this work have been mostly alkylated derivatives. The methyl substituted indoles were chosen to examine electronic effects of the position of substitution on exciplex formation. The indoles, such as 2,3-cyclodecamethyleneindole, 2-tert-butylindole and 2-butyl-3-propylindole, were used to determine steric requirements of exciplex formation. Finally indoles such as 5-methoxy, 5-fluoroindole and tryptophol were studied to examine the effect of hetero atoms and their inductive effects in exciplex formation. The results of this work showed that the substitution in the pyrrole ring was effective in producing a red shift while substitution in benzene ring did not produce any red shift. Therefore it was decided to use derivatives containing substituents in both the pyrrole and benzene rings to evaluate their effects on exciplex formation.

2.8.1. Effect of nature and position of indole substituents upon exciplex formation

Examination of the results obtained from the different indoles with any one polar solvent show the following effects. Most of the 2- or 3-alkyl or 2,3-dialkyl indoles show 10-24 nm red shifts with all the alcohols,

acetonitrile,pyrrolidine, cyclohexylamine, triethylamine,quinuclidine, triethylenediamine and tetrahydrofuran. In general the red shift is greater for these indoles than for the unsubstituted indole itself. The notable exception being 2-tert-butylindole which in general shows much smaller to smaller red shifts with the polar solvents.

This is particularly noticeable in its interaction with triethylamine where it does not appear to form an exciplex (spectra 23). Likewise its interactions with tetrahydrofuran and acetonitrile show reduced red shifts in comparison to the other 2- and, or 3- alkylated indoles (spectra 24 and 25). Its reduced interaction with acetonitrile (table 7) is to be noted in that the latter appears to be less sensitive to indole structure in its ability to form exciplexes.

It appears therefore that the presence of 2- or 3- or both 2- and 3- alkyl substituents enhances exciplex formation. However the reduced red shifts in the emission of 2-tert-butylindole and of 2-methylindole strongly suggest that a steric requirement exists for exciplex formation. Thus the 2,3-positions of the indole play a major role in the exciplex formation. This is further strengthened by observation that in general, 2,3-dimethylindole shows greater red shifts in comparision with 2,3-cyclodecamethyleneindole with the alcohols.

It is also possible that these results are compatible with the operation of a hyperconjugative effect in the excited state indole exciplex structure. In the case of 2-tert-butylindole no hyperconjugation is possible. If this were the only effect it may be expected that it would exciplex to a similar extent as indole itself. This is not the case as can be seen from its interactions with n-butanol and tetrahydrofuran, which demonstrate that the bulky tert-butyl groups does have an enhancing effect on exciplex production.

In marked contrast can be seen the effect of N-alkylation of the indole system upon exciplex formatation. Comparision of 2-methyl, 3-methyl and 2,3-dimethylindole interactions, with the alcohols, the amines and tetrahydrofuran with the interactions of the same N-alkylated derivatives and indole itself shows in many instances a change from a moderate to strong exciplex for the former indoles to very weak or no exciplex formation for the latter compounds. It could be concluded from these observations that this result is due to an electronic effect and/or the absence of hydrogen bonding due to the lack of an N-H group in the N-methyl derivatives. However it is seen that 1,2,3-trimethylindole shows stronger exciplex formation with n-butanol, borneol, 3-ethyl-3-pentanol and acetonitrile than indole itself. It is therefore unlikely that hydrogen bonding due to the presence of an indolic NH group plays any part in exciplex association. This has been previously reported[60]. That the result is not entirely due to an electronic effect may be concluded from the fact that acetonitrile forms exciplex with the 2,3-alkylindoles almost to the same extent as it does with N-alkylated compounds. Likewise all the amines and tetrahydrofuran appear not to give exciplexes with the N-alkylindoles whereas they do with the corresponding NH indoles.

It would appear that a steric effect is again of major importance in the production of the exciplex. This is demonstrated by the interaction of 3-ethyl-3-pentanol with 2,3-cyclodecamethyleneindole and 1,2,3-trimethylindole (table 6 and spectra 26, 27). The latter compound shows a red shift of 4-6 nm in comparison to the 17-18 nm obtained for the former. Only the structurally more compact acetonitrile is able to form exciplexes with the N-alkyl indoles almost as readily as with N-unsubstituted derivatives.

Substitution of the 5- or 7- positions of the indole by methyl groups is found to prevent exciplex formation with all alcohols, acetonitrile, tetrahydrofuran and triethylamine. Further, consideration suggests that

of the two possible factors which produce this the determining one in these instances is an electronic effect. This is concluded from the fact that whereas 5-methylindole shows no exciplex formation with the alcohols, acetonitrile, triethylamine and tetrahydrofuran the same compound when alkylated in the 2,3-positions forms relatively strong exciplexes with these solvents. This is clearly demonstrated by the emission data of 6-methyl-1,2,3,4-tetrahydrocarbazole (tables 1, 7, 8 and 11 and spectra 28-31 and 32-35 respectively). That a steric effect is also present is seen from the behaviour of 6-n-butyl-1,2,3,4-tetrahydrocarbazole with the same solvents. This compound shows similar red shifts, though of slightly reduced magnitude, than the corresponding 6-methyl derivative. Hence the blocking effect of alkyl groups in the benzene ring is completely overcome by the presence of 2,3-dialkyl substitution. It is difficult to see how any effect other than an electronic inductive type can explain these observations. To further ascertain this hypothesis the interaction of 5-methoxy and 5-fluoroindole with polar solvents was studied. With the exception of pyrrolidine and cyclohexylamine all the other polar solvents did not form exciplexes with these two indoles. However when 5-methoxyindole was alkyl substituted in the 2,3-positions, as in 6-methoxy-1,2,3,4-tetrahydrocarbazole then exciplex formation took place. The shifts observed were however only small to moderate with the largest occurring with n-butanol which showed a 6 nm red shift. These red shifts observed for 6-methoxy-1,2,3,4-tetrahydrocarbazole were considerably smaller than those for the 6-methyl-1,2,3,4-tetrahydrocarbazole. This result would appear to be in keeping with the presence of only an electron pushing or donating effect due to the 6-methyl groups and that of the presence of an electron pushing mesomeric effect and an opposing electron withdrawing effect due to the methoxy group.

It is also found in general that dialkyl substitution in the 6,7- and 6,8-positions of tetrahydrocarbazoles shows smaller red shifts than found for 6-alkyltetrahydrocarbazole with polar solvents. This is demonstrated by 6,7-, 6,8-dimethyl and 6-methyl- and 6-n-butyl-1,2,3,4-tetrahydrocarbazoles respectively. The former pair of compounds show 0-19 nm shifts while the latter molecules show 11-24 nm shifts (tables 1,7, 8 and 9-11 and spectra 7-9, 32-38). This may be due to the greater electron pushing effect of the dialkyl groups which reduces the interaction leading to the exciplex formation.

The results are consistent with the production of the largest shifts with tryptophol with alcohols, acetonitriles, tetrahydrofuran and amines (tables 1,7-11). This may be due to presence of the hydroxyl group which may facilitate the exciplex formation between the polar solvents and the indole system of tryptophol.

2.8.2. Effect of nature and structure of solvent upon exciplex formation

n-Butanol appears to be very effective in exciplex formation, however it produces greater red shifts in the 2-,3- alkyl -and 2,3-dialkylindoles as compared with other indoles (table 1). It does not produce red shifts with 5- and 7- substituted indoles. Methanol showed similar behaviour to n-butanol (table 2), but consideration of the result of 2-butyl-3-propylindole with methanol shows a 11 nm shifts as compared with 20 nm with n-butanol; this is thought to be due to the reduced solubility of methanol in cyclohexane in the presence of this indole. The red shifts produced by methyl and n-butyl alcohol are seen to be generally slightly greater than those obtained with cyclohexanol and the alcohols XXIII-XXV, (tables 1-6 and spectra 39-42 and 26).

Comparison of the shifts produced by methanol and cyclohexanol and the alcohols XXIII-XXV show a consistently smaller shift due to the latter compounds. These alcohols are more bulky than methanol and approach to their oxygen atoms is likely to be subject to some steric hindrance due to the presence of ring structures of "floppy" alkyl groups. This

effect is again seen in the emission spectra of 2,3-cyclodecamethyleneindole which shows smaller shifts with cyclohexanol and tert-butanol than does 2,3-dimethylindole (table 3-6 and spectra 40, 42-44). This may be due to some steric hindrance from the bulky 2,3-cyclodecamethylene ring system which further reduces such interaction. This strongly suggests that a steric requirement exists for exciplex formation. Tetrahydrofuran in general shows smaller shifts than alcohols (table 8). This could be due to its smaller dielectric constant[79], which reduces the magnitude of such interaction. Acetonitrile (XXVI) which is a compact polar molecule, might be expected to show less dependence upon indole structure for exciplex formation and the results obtained indicate that this is so (table 7). The only exceptions are 2-tert-butylindole and N-alkylated compounds because interaction with the former showed reduced red shifts in comparison with other 2-3-alkylindoles and the interaction with the latter compounds produced greater shifts than produced by alcohols.

The red shifts produced by pyrrolidine (XXVII) appear to be smaller in 2- or 3- alkyl, 2,3-dialkyl, N-alkylindoles and in indole itself in comparison with those produced by alcohols and acetonitrile. But pyrrolidine appears more effective in producing shifts in 5- or 7-alkylindoles than the alcohols. The shifts showed by 5-methyl, 5-methoxy, 5-fluoro and 7-methylindole clearly demonstrate this effect (table 9 and spectra 11). Cyclohexylamine shows similar shifts to pyrrolidine with the indoles tested (table 10). Pyrrolidine is also found to quench the fluorescence intensity of most of the indoles (spectra 8-11 and 45-47). In comparison with other polar solvents pyrrolidine is the most effective quencher. Triethylamine (XXVIII) produces smaller red shifts than cyclohexylamine and pyrrolidine in the indoles tested (tables 9-11), and it does not show red shifts with 5- or 7-alkylindoles (table 11). Likewise, triethylamine appears to be much poorer quencher than pyrrolidine (spectra 14,15, 48 and 49). Although the lowest concentration of triethylamine,

such as 0.04M produces a small quenching effect, higher concentrations do not cause any further significant quenching. The smaller shifts produced by triethylamine than other amines could be the result of the presence of "floppy" ethyl groups which reduce the interaction with indoles. This is clearly demonstrated by quinuclidine (XXIX) and triethylenediamine which produce shifts in indole, 2,3-dimethylindole and 5-methylindole greater than triethylamine (tables 11-13 and spectra 16,17, and 49). In general interactions of amines with indoles either produced marked red shifts or a marked quenching effect on the fluorescence intensity. The shifts are greater with the 2- or 3- alkyl or 2,3-dialkyl substituted indoles (tables 8-11 and spectra 8-11). The behaviour of triethylamine, quinuclidine and triethylenediamine with indoles suggests that a steric requirement exists for the exciplex formation between indoles and amines. Acetic acid did not show any marked shifts with indole derivatives with the exception of a small shift of 2 nm for 6-methyl- and 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole (table 14). It did however appear to be an efficient quencher for all the indoles tested (spectra 18-22). This effect may be due to formation of non-fluorescent protonated indolenine[96] in the excited state.

2.8.3. The Nature of Indole Exciplexes

The red shifts in the fluorescence of indole and some of its derivatives which occur in nonpolar-polar solvent mixtures at low polar solvent concentrations, where bulk solvent properties are only slightly changed, have been interpreted in several ways. The most generally accepted[16,19,21,22] view is that the large red shifts arise from the formation of a complex between the solvent and the excited state indole system. The exact mechanism of formation, the geometrical requirements, factors influencing exciplex stability and chemical nature of the indole exciplexes is not understood. To date only a small number of indole/exciplexes have been observed and only limited studies have been done. It was the purpose of the present

study to examine the dependence of exciplex formation or lack of it on the type of substituents present on the indole system and the type and structure of the polar solvent. In this way it was hoped a better understanding could be obtained about the nature of the exciplex in terms of the factors mentioned above.

Examination of the complexation of methyl substituted indoles with primary alcohols such as n-butanol and methanol in cyclohexane solution shows there is marked dependence of exciplex formation upon position of the methyl group. Thus it is seen that 2- and 3-methylindole show large red shifts, and when both 2 and 3 positions are occupied then even larger red shifts result. On the other hand the presence of a methyl group on either the 5,7, or 1 positions prevents any red shift being observed. This is in contrast to the reports [16,22] that 1-methylindole and 5-methylindole form exciplexes with polar molecules. It is to be noted that in these reports there is no data given showing the extent of the red shift observed and in the case of 1-methylindole the concentration of alcohol in pentane solution was taken up to 1.1M commencing with 0.33M as the first concentration used. In the present study alcohol or other polar solvent concentration never exceeded 0.20M and commenced at 0.04M. This was done to ensure that the bulk solvent properties of the cyclohexane were little changed by the addition of the polar solvents. Under these conditions no red shift is observable for 5- and 7-methylindoles. It is highly likely that in the published work the presence of approximately 1.0M polar solvent in the nonpolar solvent can no longer be assumed to have no effect on the bulk properties. There is no doubt that the presence of a 5-alkyl group on the indole nucleus has a marked blocking effect on exciplex formation as concluded from the complete absence of any red shift in the fluorescence of such compounds. This result could arise from either an electronic or a steric effect or a combination of both exerted by the presence of a 5- or 7-methyl group. In order to gain an insight as to which of these two effects was operative a number of di- and tri-alkylated indoles were examined. It was found that the presence of 2,3 -

disubstitution in some cases, completely cancelled the effect of the 5- or 7-methyl group and red shifts were observed equal in magnitude to those for indoles with only 2,3-diakylsubstitution. Clearly the blocking effect of a 5-methyl group does not arise from steric effects inhibiting exciplex formation. This can be taken as quite certain for even in the case of a 5-n-butyl-2,3-dialkylindole a large red shift was obtained.

It appeared therefore that alkyl groups affect exciplex formation by virtue of their electronic effect which is that of positive electron induction into the indole nucleus. To test this hypothesis complexation of 5-methoxy and 5-fluoroindole was examined. Both compounds were found to give no red shift with most of the polar solvents tested. The result obtained for 5-methoxyindole was confirmed in a recent publication[54]. It was also found that if the 5-methoxyindole was also 2,3-dialkyl substituted then exciplex formation was restored though the red shift obtained was less than for the 5-methyl-2,3-dialkylindole. At first sight there appears to be a difficulty in understanding the blocking of complexation by two groups as unlike as methyl and fluoro or methoxyl. It is however known that the methoxyl group has an electron donating effect which results from the combination of an electron withdrawing inductive effect and an electron donating mesomeric effect. Consequently if only an overall electronic effect determines whether an exciplex forms or not then clearly both the methyl and methoxyl groups as well as the fluoro group possess the same property. The magnitude of this may differ between the groups and so may account for the smaller red shift observed for the 2,3-dialkyl-5-methoxyindole in which the strong electron withdrawing effect inductive of the oxygen attenuates its mesomeric electron donating effect.

It appeared therefore that an electron donating effect from the direction of the 5,6 and 7-positions of indole, that is from the benzene ring side, towards the pyrrole ring resulted in inhibition of complexation. The same result however was found to hold if the 1-position was alkylated.

This observation was at first surprising since it would be expected that the electron inductive effect of 1-methyl group would be in approximately the opposite direction to that of a 5-methyl group and so might have been expected to result in a greater red shift. It was therefore decided to study whether a steric effect was also operative in exciplex formation. Examination of the red shifts produced by 2- and 3-methylindoles and also those of 1,2-and 1,3-dimethylindoles shows that the 3-substituted compounds give a significantly greater red shift. This taken with the effect of 1-alkyl substitution mentioned earlier suggested that a steric requirement based on the 1 and 2 positions of the indole may be operative in exciplex formation. Consequently 2-tert-butylindole was prepared as a model compound in which steric hindrance to approach to the 2-position would be expected to be present. Indeed it was found that the red shifts obtained with polar solvents for this compound were significantly smaller than the shifts observed for 2-methylindole with the same solvents. There appears therefore to be a steric requirement for exciplex formation for indoles. It cannot be concluded definitely that this is the case because substitution by a 2,3-decamethylene ring or 2-butyl-3-propyl groups did not show any marked reduction in the red shift when compared to 2,3-dimethylindole. Therefore if there is a geometrical requirement it is unlikely to be very strongly defined. The presence of a geometrical requirement was further tested by using alcohols in which the hydroxyl group was placed in a slightly sterically hindered environment. Therefore the alcohols cyclohexanol, tert-butanol, borneol and 3-ethyl-3-pentanol were used in combination with some indole derivatives.

Examination of tables 1 to 6 show that there is a small but consistent reduction in the red shift obtained with the above alcohols and 2,3-cyclodecamethyleneindole.

Since it had been suggested[55] that the exciplex may arise from a charge-transfer interaction it was decided to see whether amines would

produce exciplexes and whether their interaction differed in any way from the alcohols. It was considered that if the exciplex formation involved the use of a "lone pair" of electrons then the amines could be expected to be more reactive than alcohols in this capacity. A primary, secondary and tertiary amine was chosen in the form of cyclohexylamine, pyrrolidine and triethylamine. This choice was based on the considerations that in general tertiary amines have lower oxidation potentials than primary and secondary amines[97] and therefore may act as more efficient donors in charge-transfer complexes and also there may be an involvement of the -NH group in the exciplex formation.

Examination of the results obtained with these three amines show that both the primary and the secondary amines give red shifts with most of the indoles that also interact with the alcohols. Triethylamine, the tertiary amine, however is seen not to give exciplexes as readily as the other two amines. This is seen by examination of table 11 and comparison with tables 9 and 10. As mentioned earlier, triethylamine is known to be a sterically hindered amine in reactions involving its lone pair. It was therefore considered that its complexation was reduced as a result of the spatial requirements of its ethyl groups. To test this hypothesis the complexation of quinuclidine (azobicyclooctane), and triethylenediamine (diazobicyclooctane) with indoles was studied. These two tertiary amines were found to complex with indole, 2,3-dimethyl- and 5-methylindole more efficiently than did triethylamine. A further observation on the behaviour of the azobicyclooctane is that it apparently gives the maximum red shift at lower concentrations with no further shift in emission at higher concentrations. This is in contrast to the other amines and polar solvents.

A further feature found in the interaction of amines with the indoles is the marked quenching effect of cyclohexylamine and pyrrolidine and its absence in the case of triethylamine and quinuclidine and triethylenediamine. The latter three amines behaved analogously to the alcohols in that generally

only a small quenching effect was observed and in many cases either no quenching or enhancement of fluorescence intensity took place. Clearly the primary and secondary amines provide some energy conversion mechanism not present in the tertiary amine or alcohols. It is possible that this involves the NH group which is present in the former but not the latter. If this is so then there is some difference between the NH group of amines and the OH group of alcohols which accounts for the non-quenching of exciplex emission by alcohols. The other differences between alcohol and amine exciplexes of indoles was the observation of a small red shift for 5- and 7-methyl for 5-fluoroindoles with cyclohexylamine and for 5- and 7-methyl)-, 5-fluoro- and 5-methoxyindole with pyrrolidine. With triethylamine only the 5-fluoroindole shows a small red shift. Also the presence of 1-methyl or alkyl group on the indole has a blocking effect to exciplex formation with the amines studied. For example, 1,2,3-trimethylindole and 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole with n-butanol give a 13 and 6 nm red shift respectively. The same two compounds give no red shift at all with any of the amines.

It is concluded that amines interact with indoles to form exciplexes which are generally of slightly lower stability than the exciplexes with alcohols. This conclusion is drawn from the fact that the red shifts with amines are less than those found with alcohols. Like the alcohols the amines appear to show a steric requirement for the complexation. Furthermore with primary and secondary amines the complexation generally results in quenching which implies the possibility of an effect either not present or present to a reduced extent with the alcohols. And lastly it is found that whereas substitution in the 5- and 7-positions of the indole nucleus by overall electron pushing groups is effective in inhibiting exciplex formation with alcohols it is seen that in the case of amine-indole exciplexes it is the presence of alkyl groups on the 1-position that is most effective in preventing exciplex formation. With a view to

help resolve the mechanism and nature of exciplex formation three other polar solvents were tested. Acetic acid was chosen to test the function of protons in such complexation. From the spectra 18-22, it can be seen that acetic acid is an effective quencher of the emission of all the indoles examined and from table 14 it is clear that the quenching is not accompanied by an red shift. This can be taken to demonstrate that the exciplexs do not arise from a proton transfer between the polar solvent and the indole. Indeed it may be that proton transfer takes place from primary and secondary amines and accounts for their quenching effect on the emission of indoles.

Tetrahydrofuran was tested to see what function if any the hydroxyl group of alcohols plays in exciplex formation. Comparison of tables 8 and 1 shows that tetrahydrofuran always shows red shifts much reduced from those with butanol. Also it does not show any shift with those indoles which give only an 8-9 nm shift with 0.20M n-butanol. Clearly the presence of a hydroxyl group is important in exciplex formation.

Finally examination of table 7 and comparison with the results of n-butanol in table 1, demonstrates that acetonitrile complexes with all the same indoles as does the alcohol. The red shifts obtained at 0.16M acetonitrile, owing to its reduced solubility in cyclohexane, are generally less than those given with n-butanol.

The exceptions are the indoles with 1-methyl or 1-alkyl substituents which show slightly greater red shifts with acetonitrile. This is in contrast to the results obtained with the amines where no complexation is observed.

What is the nature of the indole exciplex and its mechanism of formation?. The original suggestion[53] that the indole exciplexes were charge-transfer complexes between excited state indole and the polar solvent is difficult to visualise. It is not obvious which should be

the donor and the acceptor molecule in an indole-alcohol interaction. If the indole acts as the donor, as may be expected from other observations[77,76,78] then it is not clear how the alcohol can act as the acceptor. By analogy with amine-aromatic charge-transfer complexes[83] it could be argued that just as the amines generally act as donor molecules then the alcohols would also act as the donor partner of an indole-complexation. If this is the case then again it is not at all clear why and how the excited state indole acts as an acceptor complex. It may well be that in the excited state indole there is a charge-transfer from say the pyrrole ring system into the benzene ring which enables the pyrrole ring to play the part of an acceptor molecule in a charge-transfer interaction. Furthermore, if the exciplex was entirely or even largely the result of charge-transfer interaction it may be expected that amines would form stronger complexes than alcohols with indoles. This would be reflected in the larger red shifts produced. From the experimental work it is seen that this is not the case, since the amines do not give larger red shifts than the alcohols and indeed do not show any marked variation in themselves as donor molecules. For it might be expected that the oxidation potential of say quinuclidine, a tertiary amine, is less than that of cyclohexylamine, a primary amine, and so might produce a greater shift in the spectrum as has been found for some exciplexes[54]. Comparison of the shifts produced by those two amines with indole and 2,3-dimethylindole tables 10, 12 and 13, show identical red shifts in both cases. If on the other hand the indole residue acts as donor it is not clear why 2-methylindole or 3-methylindole forms an exciplex with a greater red shift than indole itself with polar solvents and yet 5- or 7-methylindole does not form any exciplex at the same conditions. Similarly how does 2,3-disubstitution of a 5-methylindole restore complexation, or why does 5-methylindole not form exciplexes with alcohols but it does form weak exciplexes with amines?

An improved understanding may be obtained if it is accepted that there are at least two different classes of exciplexes[52], and that not all exciplexes are the result of charge-transfer interaction. It had been found[53] that N,N-dimethyl-3-(1-naphthyl)propylamine formed an intramolecular exciplex of a charge-transfer type. At room temperature the emission is entirely due to this exciplex with λmax about 430 nm in benzene. On addition of a small amount of acetonitrile, dioxane, or ethanol to this solution it was found that a second emission band appeared, red shifted from the original exciplex emission. This was ascribed due to the formation of a weak exciplex resulting from a stoichiometric dipole-dipole stabilised complex between an excited species and one or more polar molecules. Thus there is a distinction between weak and strong exciplexes. The latter are charge-transfer complexes with their characteristic properties such as a strong dependence of λmax of fluorescence on the difference between the oxidation and reduction potentials of the donor and acceptor,respectively, in non-polar solvents. Similarly, a linear correlation[98] has been found between the donor ionisation potential and the fluorescence quenching of the acceptor and because of the polarity of the strong exciplex it is very sensitive to the polarity of the solvent[54].

Weak exciplexes[52,53] are seen as resulting from the dipole-dipole interaction between the excited molecule and one or more polar molecules. If the excited state molecule has a large dipole moment which is well localised then it may form a weak exciplex with one or more molecules having a polar group. The presence of the polar molecule may attenuate the field of the localised charge and possibly in some cases undergo further change to form a partial charge-transfer complex or a strong exciplex with complete electron transfer. Although there may be little requirement for a single preferred geometry in a weak exciplex, the dipole-dipole interactions may produce an induction of charge in the polar partner

by the excited state molecule and result in some stereochemical direction. Evidence has been found for geometrical requirements in the case of intramolecular exciplexes[99]. The dipolar nature of the weak exciplex will invalidate the use of the Lippert-Mataga[42] equation in calculating the dipole moment of the excited state. This problem was encountered by Lumry et al[49] who could not calculate a dipole moment sufficiently large to account for the red shift of indole emission at low alcohol concentrations[16]. Likewise the emission λmax will not depend strongly upon the ionisation potentials and electron affinities for weak exciplexes. However substituents on the complexing partners may well exert strong effects upon the stability of the exciplex. The properties of weak exciplexes are therefore likely to differ from those of the strong, charge-transfer exciplexes. It is possible that weak exciplexes in some cases are the precursor of strong exciplexes.

It is on the basis of weak exciplexes formed by the dipole-dipole interaction between indoles and polar molecules that the complexation observed in this work can be best interpreted. It follows that the direction and the magnitude of the excited state dipole of indole and its derivatives will be the most important factor which will decide whether an exciplex forms or not.

Studies of the direction of the excited state indole and methylindoles have been made although only a small number of literature reports are available. Thus Tyutyulkov and Dietz[112] have calculated that excited state dipole direction of indole is as shown in the figure 2.

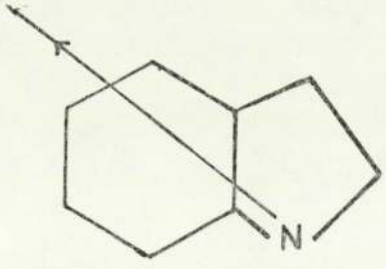


Figure 2

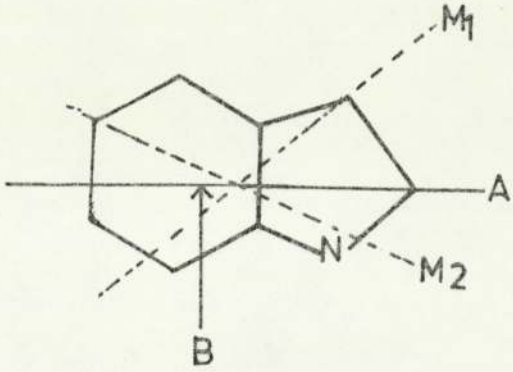


Figure 3

More recently Lombardi et al[113] using the optical Stark Effect determined that the dipole moment of the indole in the lowest singlet $\pi \rightarrow \pi^*$ state lies at 26° from the A molecular axis as shown in fig. 3. They were unable to say which direction the dipole took and therefore presented the two possible orientations for it as M_1 or M_2. The magnitudes of the dipole moments for the excited state indoles have not been investigated to any large extent and so only a few reports are given. For example Mataga et al[42] estimated that the change in the dipole moment for indole from the ground state to the excited state was 5D.

Gladchenko et al[114] estimated that the excited states indole dipole moment was 5.6D and its direction was along the short axis of the molecule. Kawski[115] has calculated dipole moments for indole, 1-methyl-,2-methyl-, 1,2- and 2,3-dimethylindole in the ground state and excited state. He gives the changes in the dipole between the ground and excited states to be of the order of 2-3D with only a small difference in this between indole and methylindoles. Finally Lumry et al[54] in a discussion on the lack of exciplex formation by 5-methoxyindole estimates that the dipole change for this molecule on excitation is only of the order of 1.1D.

The magnitude of the dipole moment of the molecule can be expected to be attenuated by the presence of substituents. The direction of the attenuation and its degree will be dependent upon the position of the substituent with respect to the direction of the dipole moment and the specific electronic effect exerted by the substituent. The resultant direction and magnitude of the interaction of two dipole moments can be obtained by vector addition. For example the resultant magnitude and direction of the interaction of two vectors $\bar{X}$ and $\bar{Y}$ is given by $\overline{xy}$ as shown in fig. 4.

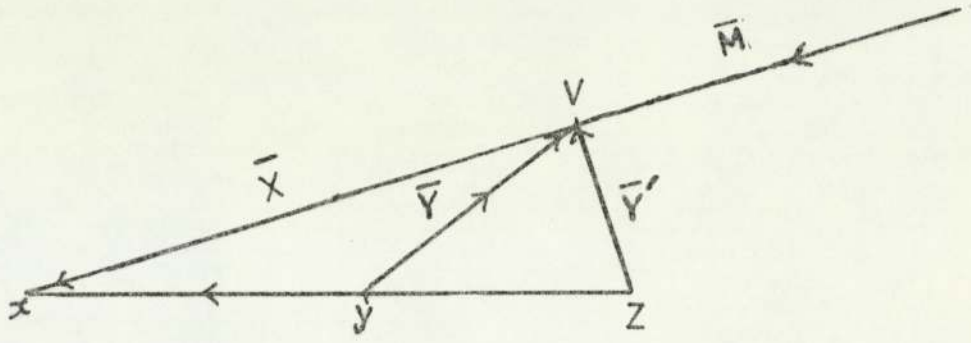


Figure 4

It is readily seen that provided the angle between $\bar{X}$ and $\bar{Y}$ is less than 60° the result is to give a dipole moment of lower magnitude than $\bar{X}$. At all angles greater than 60° the result will be a greater dipole moment given the directions of the interacting moments are as in $\bar{X}$ and $\bar{Y}'$ in the fig.4. In this instance the magnitude of the moment becomes $\bar{xz}$. If the interacting moments are exactly parallel as given by $\bar{X}$ and $\bar{M}$ then the result and moment is the sum of the two moments $(\bar{X} + \bar{M})$, and the direction is unchanged. This analysis can be applied in the case of the effect of position of substitution by alkyl groups (or positive electron inductive groups) on the magnitude of the dipole moment of the excited state indole.

Drawing fig. 5 on a larger scale it is possible to see the effect of a methyl group substituted at different positions on the magnitude of the excited state dipole moment. The direction of the excited state dipole moment which gives the greatest agreement with the observed data is that given by Ml. The assumption is made here that reduction in the magnitude of the excited state dipole moment will reduce the tendency for the formation of a weak exciplex and may in some instances prevent complexation altogether.

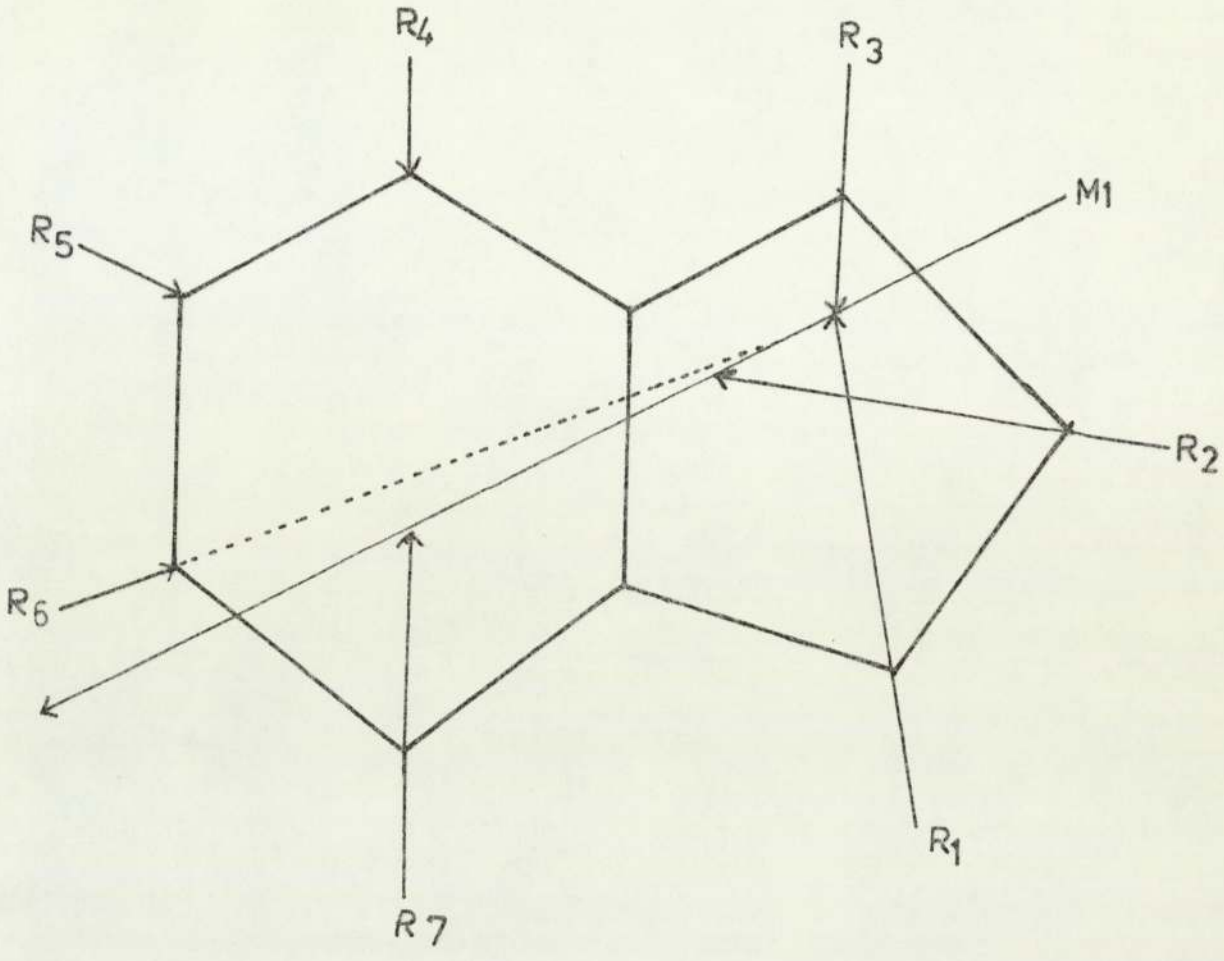


Figure 5

It can now be seen that depending on the exact angles of direction of the interacting moments alkyl groups at position 1,5,6 and 7 can easily reduce the magnitude of the excited state dipole. Methyl groups at positions 5 and 6 appear to be particularly favourably directed for this purpose. But likewise positions 1 and 7 exert an opposing dipole and so reduce the excited state dipole. On the other hand it is seen that positions 2 and 3 and probably 4 will all reinforce the excited state dipole and thus produce a greater overall polarity in the excited molecule.

This analysis appears to fit broadly all the experiments observed in

this work on the exciplex formation between polar solvents and position of substitution of the indole system. Some of the more subtle differences which have been observed between the behaviour of specific solvents and particular type of indole substituents may arise as a result of one or two other factors. For example, the direction of orientation of the polar solvent molecule in the exciplex may differ according to the structure of the polar solvent molecule. It is possible that in the case of alcohols the direction is dictated by the polarity of the $(R)\!-\!\overset{-}{O}\!-\!\overset{+}{H}$ bond. If this is the case then the group R will lie in the exciplex in the general direction of the 2,3-positions of the indole if the exciplex geometry is taken to be one where the two interacting molecules take up positions close to one another and parallel to the directions of their dipole moments. In the case of acetonitrile if the polarisation of the $(R)\!-\!\overset{+}{C}\equiv\overset{-}{N}$ is taken as the important factor then the R group will tend to lie towards the benzene ring of the indole molecule. The same will apply for tertiary amines. The primary and secondary amines would be expected to behave as the alcohols but may show some tendency to adopt positions in between those of the alcohols and acetonitrile. The effect of these orientations may be one which induces a change in the direction of the dipole moment of the excited state indole and hence may result in a change in magnitude of the dipole moment so that differences such as the weak complexation of 5-methyl- and 5-fluoroindole may take place with cyclohexylamine but not with n-butanol. Similarly the 1-alkyl substituted indoles may not form an exciplex with the amines but will complex with alcohols. An alternative explanation may rest in the fact that the weak exciplex is not one due to a pure dipole-dipole interaction and that there is indeed a contribution from a charge-transfer interaction. The degree of charge transfer will depend upon the usual factors and will vary with the structure of the indole

and the polar solvent molecule. Such differences are known to exist for complexation between different partners. If the generalised wavefunction ψ of a bimolecular, excited state complex is given by Mataga et al[59].

$$\psi = \Sigma_i aiAi^*s + \Sigma_i biASi^* + CA^+ S^- + dA^- S^+$$

where ai, bi, c, d are coefficients and Ai*, Si* refer to various neutral and excited singlet states of the partners A and S_i then it is found that for excimer formation a = b and c = d. However for aromatic hydrocarbon-amine complexes a > b and d > c.

It may be that in the case of the amine-indole exciplexes there is a greater contribution of the charge-transfer interaction with a resultant quenching being observed with the primary and secondary amines. This however would leave unexplained the lack of quenching of fluorescence by the tertiary amines. This particular result may be better accommodated by the presence of another mechanism in the case of primary and secondary amines which involves the presence of the H atom of the NH group. As a result of some degree of charge-transfer interaction between the indoles and the primary and secondary amines the N-H group may acquire a strong acid character with the effect of providing a proton source. The exciplex would then lose proton which would be picked by the excess of amine molecules and thus provide a route for deactivation of the excited state.

That other structural features in the indoles may play a part in complexation can be deduced from the interactions of tryptophol with polar solvents. This indole was selected to see whether the presence of an hydroxyl group in the molecule would result in an intramolecular exciplex which would show no further red shifts on the addition of polar solvent molecules. It was found that tryptophol in fact shows the largest red shifts of all indoles with polar solvents as seen from tables 1,7,8,9 and 10. This may arise from the presence of the hydroxyl group in the tryptophol which enhances the interaction between this indole and the polar molecules due to its own dipole moment and its hydrogen bonding properties. It may also

be that a weak intramolecular exciplex is formed which due to its greater polarity shows a stronger interaction with polar solvents and so produces the larger red shifts.

This work has further illustrated the variety of complexation reaction available to indole derivatives. It has produced a possible explanation for some of the observed interactions of indoles and polar solvents in non-polar solutions and the dependence of this interaction upon the structures of the partners. At the same time it has revealed the further need for a study of the complexation of indoles with polar molecules if the fluorescence properties of proteins are to be more clearly understood. For presumably the tryptophan residue which often is largely the source of protein fluorescence is subject to a variety of environmental interactions within the protein structures.

2.9 EXPERIMENTAL

2.9.1. Materials and Methods

All indoles and solvents were checked for purity prior to use by ultraviolet spectroscopy. Indole and 5-fluoroindole (Koch-Light Lab.); 2,3-dimethylindole; 3-methylindole; 2-methylindole and 5-methoxyindole (Aldrich Chemical Co. Ltd.); 5-methylindole (I.C.N. pharmaceutical) and 7-methylindole (Fluka A.G.-chemischefabrik), were pure industrial preparations. 2,3-Cyclodecamethyleneindole; 8,9-cyclotrimethylene-1,2,3,4-tetrahydrocarbazole and 6,7-dimethyl-1,2,3,4-tetrahydrocarbazole (gifts from Dr. Britten) were recrystallized from methanol. 1,3-Dimethylindole and tryptophol (gifts from Dr. Britten) were very pure. 1,2-Dimethylindole (gift from Dr. Britten) was purified by vacuum sublimation, and 1,2,3-trimethylindole; 6-methyl-1,2,3,4-tetrahydrocarbazole; 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole; 2-tert-butylindole; 6-n-butyl-1,2,3,4-tetrahydrocarbazole; 2-butyl-3-propylindole; 6-methoxy-1,2,3,4-tetrahydrocarbazole, were made and their purity checked in the laboratory.

Cyclohexane was purified by shaking with conc. H_2SO4 and distilled over activated charcoal. Methanol, n-butanol, cyclohexanol and tert-butanol, were purified by distillation with potassium hydroxide. Acetonitrile was purified by distillation and pyrrolidine by distillation over zinc powder. Tetrahydrofuran was purified by distillation over lithium aluminium hydride.

The free base of quinuclidine Hcl(ABCO) was liberated[100] by combining very concentrated aqueous solutions of the hydrochloride with potassium hydroxide, the solid amine was removed by filtration, dried in a vacuum desiccator, dissolved in ether, filtered and the ether removed by vacuum distillation. Borneol, triethylenediamine, acetic acid, triethylamine and 3-ethyl-3-pentanol were pure industrial preparations.

The ultraviolet absorption for most of the above solvents gave a 100% transmission at 200-400 nm. A concentration of 25×10^{-5} M indole in cyclohexane was used to check the ultra-violet absorption spectra.

This was too high for fluorescence work and was therefore diluted to 5×10^{-5}M.

The ultra-violet spectra were recorded on a Pye Unicam 8000 Spectrophotometer and an Aminco-Bowman spectrofluorometer and a Hewlett Packard 7035B X-Y recorder was used to obtain the fluorescence emission spectra at room temperature.

The slit settings on the instrument were:

Excitation wavelength	285 nm
Excitation inner slit	3 mm
Excitation outer slit	3 mm
Emission inner slit	2 mm
Emission outer slit	0.5 mm
Photomultiplier slit	1 mm
Amplifier setting at	0.3
Amplifier sensitivity	36

The above settings were used for all the experiments except in the case of 1,2-dimethylindole when the photomultiplier slit was changed to 0.5 mm.

All indoles were at 5×10^{-5} M concentration in cyclohexane solutions containing 0.04, 0.08, 0.12, 0.16 and 0.2M of the alcohols, amines, acetonitrile, tetrahydrofuran and acetic acid as the polar solvents. Emission spectra were recorded in the above solvents and the shift of maximum emission in each case is listed in the results. Some of the emission spectra are included in the appendix.

2.9.2. Synthesis of Indole Derivatives

The starting materials used were pure industrial preparations. Ultra-violet (UV) and Infra-red (IR) spectra were recorded on Pye Unicam SP 8000 and SP 200 spectrophotometers respectively. Gas liquid chromatography was used to check the purity of 1,2,3-trimethylindole using a Perkin Elmer F11 with the following conditions.

column	3% OV1
oven temperature	150°C
injection temperature	2
N_2 pressure	21 lb/in^2
H_2 pressure	20 lb/in^2
air pressure	19 lb/in^2
chart speed of recorder	5 mm/min.

1) 1,2,3-Trimethylindole

Hedney and Ley[101] described N-alkylation of indole and pyrroles in dimethylsulphoxide, and made 1-benzylindole from indole and benzylbromide. The same procedure has been used for making 1,2,3-trimethylindole from 2,3,-dimethylindole and methyliodide.

Procedure

Dry dimethyl sulphoxide (200 ml) was added to pot. hydroxide (22.4 gm) pellets and the mixture stirred for 15-20 mins. 2,3-Dimethylindole (14.52 gm "0.1 mol") was then added and the mixture was stirred for 3/4 hour. Methyliodide (28.382 gm "12.5 ml," 0.2 mol) was added and the mixture was cooled briefly and stirred for a further 3/4 hour. Water (200 ml) was added, the mixture was extracted with ether (3 x 100 ml), and each extract was washed with water (3 x 50 ml). The combined ether layer was dried using $Cacl_2$ and a small quantity of anhydrous sod. sulphate, the ether was removed under reduced pressure. The red liquid was purified by vacuum distillation to yield a pale yellow, clear, oily liquid of 1,2,3-trimethylindole 14 gm (88%). It has a melting point 18°C[102].

2) 2-Tert-butylindole

The Fischer indole synthesis[103] was used for making 2-tert-butylindole from methyl-tert-butyl-Ketone with polyphosphoric acid.

Procedure

To a mixture of (5 gm) methyl-tert-butyl-Ketone and (5 ml) phenylhydrazine, which was first heated on water bath for 10 minutes,

about (20 gm) of polyphosphoric acid was added. The mixture was stirred and warmed gently on oil bath until 155°C (at which point there is a sudden rise in temperature). The mixture was maintained at about 155°C until the temperature began to drop spontaneously and then the mixture was cooled with water and 100 ml of water was added. This was then extracted with ether (4 x 60 ml) and each extract was filtered through a layer of the same quantity of anhydrous sod. sulphate. The combined dried ether extracts yield the indole. The ether was evaporated to yield an oily brown product 5 gm (58%). The product was purified by vacuum distillation. Yellow waxy crystals were produced which when recrystallized from methanol gave colourless crystals which become darker with time and gave a melting point of 60-63°C. Reference melting point was 65-69°C(104).

3) 2-Butyl-3-propylindole

This compound has not previously been made, it was synthesised by the same method as 2-tert-butylindole, using (6.3 gm) dibutyl-ketone, (5 ml) phenylhydrazine and about (20 gm) polyphosphoric acid. The sudden rise in temperature in this reaction was at 145°C. A brown thick liquid was produced after evaporation of the ether, which was purified by vacuum distillation to yield a pale yellow, oily, clear liquid with a characteristic odour, 8.7 gm (79.8%). The compound was checked by IR (thin film), which shows peaks at 3400 cm^{-1} and 740 cm^{-1} due to the indole N-H and CH(1,2-disubstituted) benzene ring respectively. The picrate of this compound gave chocolate brown needles with a melting point of 90-94°C.

4) 2-Decyl-3-nonylindole

This compound has also not previously been made. It was prepared by the method of preparation of 2-tert-butylindole, using (5 gm) didecyl-ketone, (1.6 ml) phenylhydrazine and about (6.6 gm) of polyphosphoric acid. The beginning of the reaction was a sudden rise in temperature at 155°C. A gummy orange product was produced after evaporation of ether, with 6.2 gm (69.9%) yield. Two recrystallizations from methanol yielded orange

amorphous crystals, with a melting point of 50-55°C. The compound is unstable when left in ordinary conditions and should be kept under nitrogen.

5) 6-Methyl-1,2,3,4-tetrahydrocarbazole

This compound was synthesised in two stages. The first stage being the synthesis of p-tolylhdrazine HCl and then cyclization to get the carbazole.

(i) p-tolylhydrazine HCl was made by the Fischer method[105], which was modified to avoid the precipitation of sod. chloride. This method gives after recrystallization the hydrochloride of the hydrazine in the pure state.

Procedure

P-toluidine (7.1 gm) was dissolved in 150 ml water, and 12 ml conc. HCl was added. The solution was cooled in iced water with mechanical stirring and a solution of sod. nitrite (4 gm $NaNo_2$ in 15 ml H_2O) was added. When the addition was complete the diazotised solution was poured into the sod. sulphite solution (37.5 gm $Na_2SO3. H_2O$).

The mixture has a red/orange colour which after some time (one hour at least) became more yellowish. Then 4.5 ml glacial acetic acid and a little zinc dust was added and the solution was warmed on a water bath for 3/4 hour - 1 hour. This was filtered and 225 ml conc. HCl was added to the filtrate and the solution cooled in iced water. White crystals deposited which were filtered with suction and washed with dil. Hcl and dried in a vacuum disiccator. The yield was 4 gm (38%) of p-tolylhydrazine HCl with a melting point of 234-237°C.

(ii) Cyclization of p-tolylhydrazine Hcl to produce the tetrahydrocarbazole

The preparation of 1,2,3,4-tetrahydrocarbazole was reported by Rogers and Croson[106], when they cyclised phenylhydrazine Hcl with cyclohexanone.

Procedure

A mixture of (1.69 gm) cyclohexanone and (7.2 gm) of acetic acid,

contained in 250 ml three necked round bottomed flask, equipped with reflux condenser, and a slip-sealed stirrer, was heated under reflux and stirred while (3.16gm) p-tolylhydrazine Hcl was added in one hour. The mixture was heated under reflux for an additional hour and poured into a beaker and stirred by hand until it solidified. It was then cooled to about 5°C and filtered with suction. The filter cake was washed with 2 ml water and with 2 ml of 75% ethanol and the crude solid was vacuum dried. The yield was 2.8 gm (75.45%); when recrystallized from methanol white crystals were formed with a melting point of 138-142°C. Reference melting point 142-3°C.[107]

6) 6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole

2,4-Dimethylaniline was used as a starting material to prepare 2,4-dimethylphenylhydrazine Hcl which after cyclization yielded the 6,8-dimethyl-1,2,3,4-tetrahydrocarbazole.

2,4-Dimethylphenylhydrazine Hcl was prepared by the same method (i) as for 6-methyl-tetrahydrocarbazole using 2,4-dimethylaniline as a starting material. The product of yellow crystals had a melting point of 149-152°C. Cyclization of the above product was carried out as method (ii) with some changes.

Procedure

A mixture of (9.8 gm) of cyclohexanone and (36 gm) acetic acid contained in 250 ml three necked round bottom flask equipped with reflux condenser, and a slip-sealed stirrer,was heated under reflux and stirred while (17.1 gm) of 2,4-dimethylphenylhydrazine Hcl was added during 1 hour. The mixture was heated under reflux for an additional hour, cooled to about 5°C to give a thick brown liquid, which was purified by vacuum distillation to give a pale yellow oil. This when recrystallized from methanol-petroleum ether (boiling point 80-100°C), gave white crystals with a melting point of 92-95°C. Reference melting point was 92-94°C.[108].

7) 6-n-Butyl-1,2,3,4-tetrahydrocarbazole

p-n-Butylaniline (9.94 gm) was used as a starting material to prepare p-n-butylphenylhydrazine Hcl by the same method (i) as for 6-methyl-tetrahydrocarbazole; the product of p-n-butylphenylhydrazine HCl gave yellow crystals with a melting point of 80-85°C and a yield of 11.2 gm (83.79%). Cyclization of this product was carried out as in method (ii) in 6-methyl-tetrahydrocarbazole, using (10.02 gm) of p-n-butylphenylhydrazine HCl, (5 gm) cyclohexanone and (18 gm) acetic acid. The crude solid yielded 8.2 gm (75.3%) of violet crystals. When recrystallized from methanol, pale violet crystals were formed which were dissolved in ether and shaken with conc. HCl. The ethereal layer was washed with 20% HCl, and filtered through anhydrous sod. sulphate. The ether was removed under reduced pressure and grey crystals were formed. It had a melting point of 100-104°C. Reference melting point of 102-104°C[109]

8) 6-Methoxy-1,2,3,4-tetrahydrocarbazole

This compound was prepared as in mthod (ii) in 6-methyl-1,2,3,4-tetrahydrocarbazole using p-methoxy-phenylhydrazine HCl as a starting material with the following changes.

Procedure

A mixture of (2.5 gm) of cyclohexanone and (9 gm) acetic acid was heated until boiling. The mixture was transferred to a 250 ml three necked, round bottom flask equipped with reflux condenser and a slip-sealed stirrer. This was heated under reflux and stirred while (4.4 gm) p-methoxy-phenylhydrazine HCl was added during 1 hour. The mixture was heated under reflux for an additional hour, cooled to about 5°C and filtered with suction. The filter cake was washed with (2.5 ml) water and then with (2.5 ml) of 75% ethanol. The product was light brown, with a yield of 4.6 gm (81.3%). This was twice recrystallized from methanol which gave light tan needles with a melting point of 94-98°C, and a reference melting point of 93-107°C.[110]

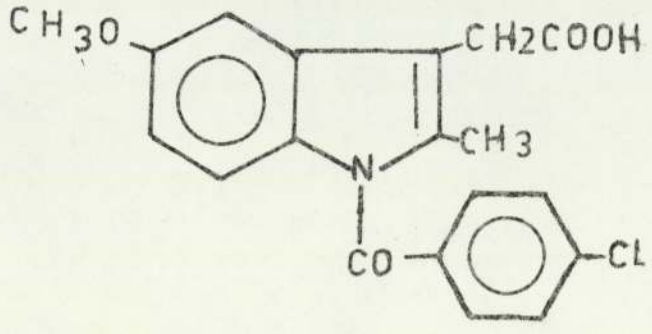
9) Many attempts to prepare 5,8-dimethyl-1,2,3,4-tetrahydrocarbazole and 7,8-dimethyl-1,2,3,4-tetrahydrocarbazole from 2,5-dimethylaniline and 2,3-dimethylaniline respectively, were unsuccessful due to the diazo-coupling effect.[111]

3. FLUOROMETRIC ANALYSIS OF 5-METHOXYINDOLES

3.1. Introduction

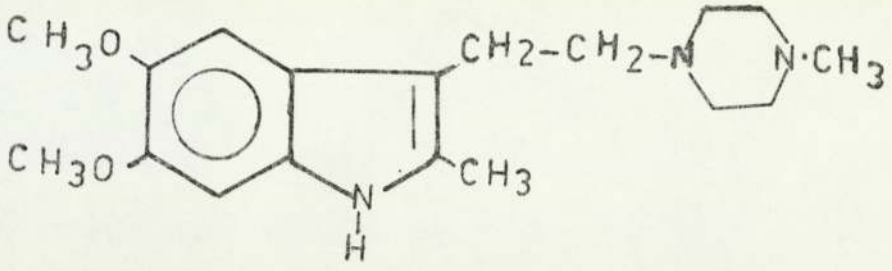
Fluorimetry is used widely in the analysis of drug compounds.[116]. The native fluorescence of 5-methoxyindoles is sufficiently intense and specific to permit its use for quantitative assay purposes. The procedures for analysis have been based on their native fluorescence in acidic, neutral or alkaline solutions. The fluorescence intensities and emission wavelengths vary with the pH of the solutions[18]. The compounds chosen for the purpose of analysis were, Indomethacin (XXX) [1-(P-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid] an anti inflammatory drug[12], oxypertine (XXXI) (1-[2-(5,6-dimethoxy-2-methyl-3-indolyl)ethyl]-phenylpiperazine), which is a psychosedative indole derivative with a tranquilizing effect[117], and the biologically important[118] compounds 5-Hydroxyindole-3-acetic acid (XXXII) and 5-Hydroxytryptophan (XXXIII).

Indomethacin



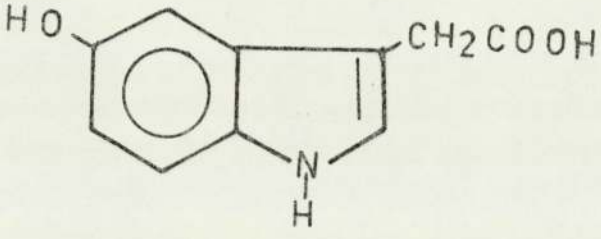
(XXX)

Oxypertine



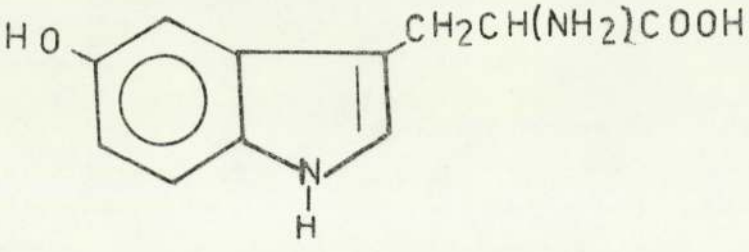
(XXX1)

5-Hydroxyindole-3-acetic acid



(XXX11)

5-Hydroxytryptophan



(XXX111)

Quantitative determinations of these compounds were carried out to investigate the possibility of using fluorimetry as a general method for the analysis of all 5-methoxyindoles. Udenfriend and co-workers[18] reported that in a neutral or slightly acid solution, 5-Hydroxyindoles fluoresce at 330 nm when excited at 295 nm, but when the acidity is increased by the addition of Hcl, the 330 nm fluorescence decreases and a new fluorescence peak appears at 550 nm. In concentrated acidic solutions the 550 nm fluorescence is maximal and the 330 nm fluorescence is extremely low. This shift of fluorescence from the ultraviolet to the visible, with increasing acidity is completely reversible and is not accompanied by a noticeable change in the absorption spectrum. Chemically induced fluorescence may also be used for analysis of compounds which do not show native fluorescence under the above conditions.

Shore and co-workers[119] reported a fluorescence assay procedure for histamine, based on the reaction of the amine with o-phthalaldehyde (OPT) in alkaline solution. Under the same conditions negative results were obtained with a number of compounds, including serotonin[120]. Later Curzon and Giltrow[121] studied the reactivity of various aromatic compounds with amino acids under acidic conditions, and found that aromatic 1,2-dialdehydes reacted with amino acids such as tryptophan to yield coloured products. However, the use of o-phthalaldehyde in the reaction may yield a fluorescent product, and therefore the sensitivity of the determination may be increased.

A comparison of these determinations with gas chromatography was carried out, as it was believed that the latter might be more sensitive.

Quantitive analysis was carried out to determine detection limits by native fluorescence, the o-phthalaldehyde reaction and gas chromatography.

The detection limit[122] is defined as the concentration which gives a reading equal to 2 x standard deviation of a series of (at least) ten determinations at or near the blank level. The following equations were used to determine the standard deviation.

mean (average) $\bar{x} = \frac{\Sigma x}{n}$

where x is the recorded value and n is the number of recorded values.

standard deviation (σ) $= \sqrt{\frac{\Sigma x^2 - n\bar{x}^2}{n-1}}$

3.2 Native Fluorescence

Native fluorescence of 5-Hydroxyindoles has been used for their quantitative analysis for several years[120]. Holet and Hawkins[123] estimated indomethacin in serum by its native fluorescence in alkaline solution (phosphate buffer pH8), and this estimation was used to study indomethacin absorption. It was reported[116] that indomethacin produces a strong fluorescence in 0.1 N NaoH at 408 nm when excited at 295 nm. Aurther et al[124] have determined indomethacin in ethanol by its native fluorescence at 410 nm when excited at 328 nm.

Native fluorescence had been used to determine 5-Hydroxyindole-3-acetic acid and 5-Hydroxytryptophan in brain tissue[118,125].

Table 15 shows the results obtained for the determination of these compounds by their native fluorescence. The acidic solution of 5-Hydroxyindole-3-acetic acid shows two emissions when activated at 295 nm, one at 340 nm and the other at 545 nm. The lower limit determined at the former emission was 0.1 ug/ml and at the latter emission 0.01 ug/ml. Linearity over the range of 0.1-0.6 ug/ml and 0.01-0.05 ug/ml respectively was achieved (Fig. 6 and 7).

Table 15

Detection limit by native fluorescence

Compound	solvent	detection limit ug/ml	excitation wavelength nm	emission wavelength nm
	8 NHcl	0.1	295	340
5-Hydroxyindole	8 NHcl	0.01	295	545
-3-acetic acid	0.1N NaoH	0.01	360	460
	ethanol	0.002	295	340
	8NHcl	0.01	295	340
5-Hydroxytryptophan	8 NH cl	0.004	295	540
	ethanol	0.001	295	340
oxypertine	ethanol	0.002	295	350
Indomethacin	0.1N NaoH	0.01	310	375

The alkaline solution of 5-Hydroxyindole-3-acetic acid was found to be weakly fluorescent when excited at 295 nm, but showed strong fluorescence at 460 nm when excited at 360 nm. The minimum limit determined was 0.01 ug/ml. A linear relation between concentration over the range of 0.01-0.1 ug/ml and fluorescence intensity was obtained (fig. 8). In contrast the lower limit determined of 5-Hydroxyindole-3-acetic acid in a neutral solution of ethanol was 0.002 ug/ml at 340 nm when activated at 295 nm, and linearity over the range of 0.002-0.005 ug/ml was achieved (fig. 9).

5-Hydroxytryptophan also shows two emissions in an acid solution (8N Hcl) at 340 nm and 540 nm when excited at 295 nm. The lower limits determined at 340 nm was 0.01 ug/ml ahd at 540 nm was 0.004 ug/ml. Linearity over the ranges of 0.01-0.08 ug/ml and 0.004-0.016 ug/ml

respectively was obtained (fig. 10 and 11). The alkaline solution of 5-Hydroxytryptophan appears to be a very weakly fluorescent even at high concentrations such as 25 ug/ml when scanning between the range 200-600 nm for excitation and emission. In neutral solution of ethanol, in comparison, it seems highly fluorescent at 340 nm when excited at 295, with a detection limit of 0.001 ug/ml, and linearity over the range 0.001-0.01 ug/ml. (fig. 12). Aurther et al[124] reported that indomethacin in ethanol emits at 410 nm when activated at 328 nm. On carrying this out it was found that indomethacin does not show emission at these wavelengths, or for that matter at any excitation and emission wavelengths. Several attempts were carried out to determine the fluorescence of indomethacin in 3NHcl, 5NHcl, 8NHcl and perchloric acid. It has been proposed[123] that indomethacin fluorescence in a phosphate buffer at pH8, but again it was found that such a solution did not fluoresce. However indomethacin appears highly fluorescent in an alkaline solution such as 0.1N NaoH. This is due to the decomposition of indomethacin by strong alkali[126]. The lower limit detected in this case at 375 nm, when excited at 310 nm, was 0.01 ug/ml and linearity over the range of 0.01-0.05 ug/ml was achieved (fig. 13). Oxypertine is insoluble in both acidic and alkaline aqueous solutions, thus the detection limit of oxypertine was determined only in neutral ethanol at 350 nm when activated at 295 nm and was found to be 0.002 ug/ml. A linear relation between fluorescence intensity and concentration over the range of 0.002-0.01 ug/ml was obtained (fig. 14).

From the above investigation it can be concluded that a change in the solvent and pH plays a major role in the native fluorescence determination of the compounds examined. This is because such changes may produce alteration in the emission and excitation wavelengths as well as in the sensitivity. Therefore by careful selection of the solvent media and the excitation and emission wavelengths it may be possible to determine these

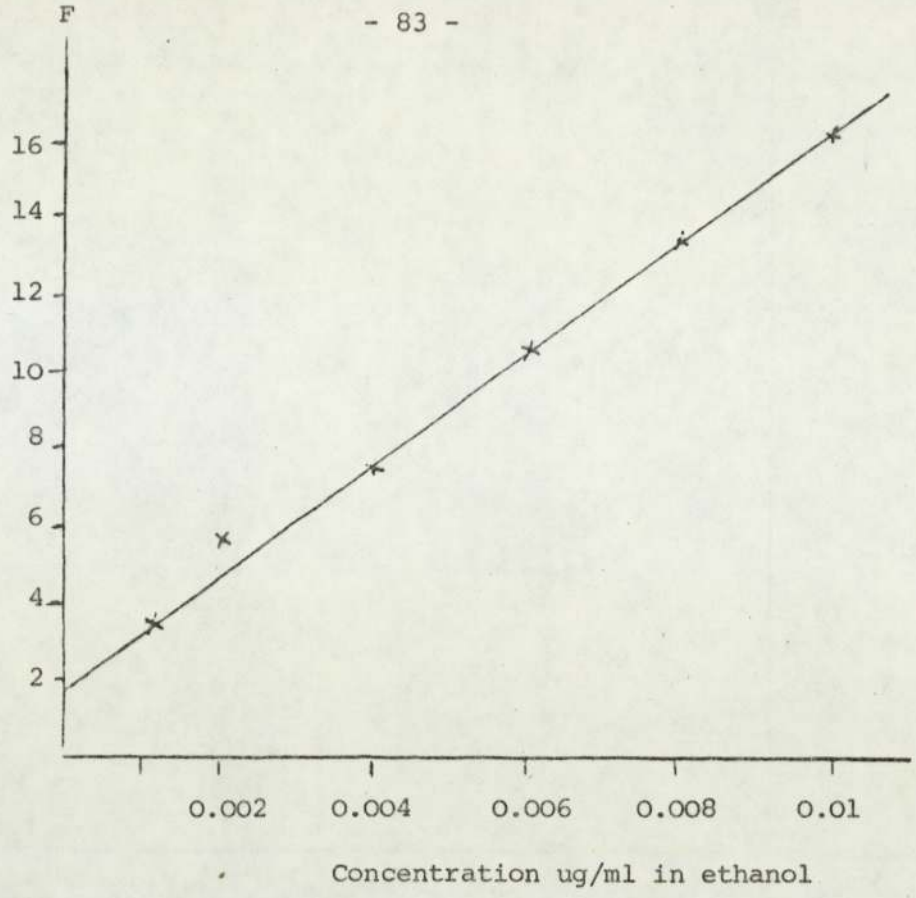


Figure 12. 5-Hydroxytryptophan by native fluorescence

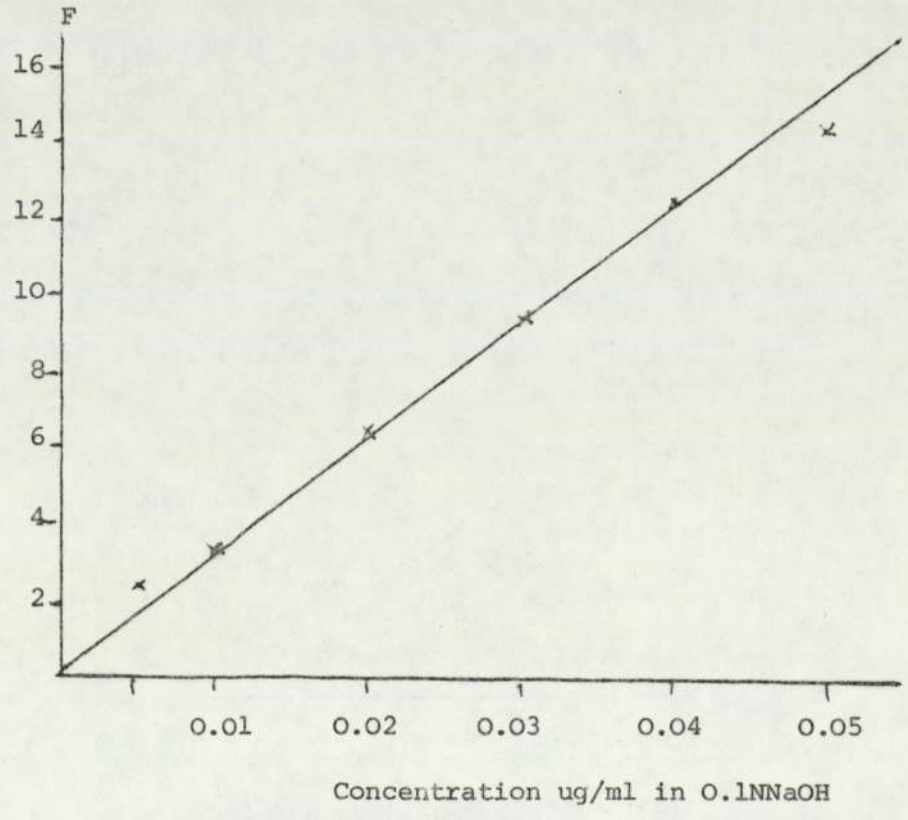


Figure 13. Indomethacin by native fluorescence

substances without interfering from other substances frequently found to be present in the same solutions. This is particularly a problem with biological fluids.

3.3 -O-Phthalaldehyde Reaction Method (OPT)

The compounds shown previously were reacted with o-phthalaldehyde because it was reported[127] that 3- and 5-substituted indoles form highly fluorescent complex with o-phthalaldehyde. Quantitative determinations were carried out by the above reaction, and the results are summarized in table 16.

Table 16

Detection limits by o-phthalaldehyde method

Compound	detection limit ug/ml	excitation wavelength nm	emission wavelength nm
5-Hydroxyindole-3-acetic acid	0.3	360	470
5-Hydroxytryptophan	0.1	360	470
Indomethacin	0.05	360	470
Oxypertine	0.01	380	420

Examination of the detection limits obtained shows the following; 5-Hydroxyindole-3-acetic acid when reacted with o-phthalaldehyde shows a weaker fluorescence in comparison with native fluorescence. The detection limit at 470 nm when excited at 360 nm was 0.3 ug/ml and linearity over the range of 0.3-1ug/ml was achieved (fig. 15), while the maximum detection limit in acid at 340 nm was 0.1 ug/ml. 5-Hydroxytryptophan determination by the OPT method appears to be less sensitive in comparison to the native fluorescence method. The detection limit of 5-Hydroxytryptophan in the o-phthalaldehyde reaction method was 0.1 ug/ml at 470 nm when excited at 360 nm, and linearity relation between fluorescence intensity and

concentration over the range 0.1-0.8 ug/ml was achieved. (fig. 16). The maximum detection limit of 5-Hydroxytryptophan by native fluorescence at 340 nm was 0.01 ug/ml in the acid solution. Similarly indomethacin shows a stronger fluorescence by native fluorescence in comparison with the o-phthalaldehyde reaction method. The detection limit of the former was 0.01 ug/ml at 375 nm while for the latter was 0.05 ug/ml at 470 nm when excited at 360 nm. Finally oxypertine determination also seemed less sensitive with the o-phthalaldehyde reaction than by native fluorescence. The detection limit was 0.01 ug/ml at 520 nm when excited at 380 nm, with a linear relation between fluorescence intensity and concentratoon over the range of 0.01-0.06 ug/ml. (fig. 17). The detection limit by native fluorescence at 350 nm was 0.002 ug/ml.

In general, the native fluorescence method appears more sensitive in comparison with the o-phthalaldehyde reaction. The sensitivity of the former was 3, 10, 5 and 50 times more sensitive than the latter for the compounds 5-Hydroxyindole-3-acetic acid, 5-Hydroxytryptophan, indomethacin and oxypertine respectively. However the o-phthalaldehyde method although less sensitive is useful to determine the above compounds in solution which contain substances that emit at the wavelengths of native fluorescence. The o-phthalaldehyde reaction could be used to shift the wavelength of detection of the emission so that there was no interference from the fluorescence of the other constituents present in the same solution.

3.4 Gas Chromatography

Quantitative analysis of the compounds shown previously, with the exception of indomethacin, was carried out by gas chromatography. Many attempts were made to chromatograph these compounds using stationary phases 3% OV1[128], 4% SE30 and OV17[129]. All were unsuccessful. The compounds are very polar and involatile and require conversion into volatile derivatives. Donike[130] reported that the N-trimethylsilylation rate of the indoles by N-Methyl-N-TMS-trifluoruoacetomide is increased by the addition of a

basic derivative and he suggested using TMS imidazole for this purpose. This was carried out on the compounds and seemed to be unsuitable because it did not give a satisfactory chromatogram. N,O-Bis(trimethylsilyl)-acetamide was used to make the derivatives as it produced sharp non-tailing peaks in the gromatogram. The lower limit of detection for 5-Hydroxyindole-3-acetic acid was 0.266 ug, and linear relation between peak height and sample amount over the range of 0.266 -1.06 ug was achieved (fig. 18). The lower limit for 5-Hydroxytryptophan determination was 2 ug, and linearity over the range 2-10 ug was obtained (fig. 19). Helleberg[131] has described a method in which electron-capture gas liquid chromatography permits the specific determination of serum and urine levels of indomethacin from therapuetic doses. However he was able to detect down to a limit of sensitivity of 50 ng/ml of indomethacin. Quantitative analysis of oxypertine by gas chromatography was carried out by silylating the column with silyl-8. The lower limit determined was10 ug and linear relation between peak height and sample volume over the range of 10-16 ug was achieved (fig. 20).

The above results show that the sensitivity of the gas chromatographic method was poorer than the native fluorescence and o-phthalaldehyde reaction methods. This may be due to the detector used, because the type of detector plays a major part in the sensitivity. Electron capture detection for several indole derivatives is approximately 1000 times more sensitive than a flame ionization detector under the same conditions[132].

Conclusion

It has been found that the sensitivity of quantitative analysis by native fluorescence depends upon the pH of the solvent media. Native fluorescence seems to be more sensitive than the o-phthalaldehyde reaction method which in turn is more sensitive than the gas chromatigraphy method The sensitivity of the latter depends upon the stationary phase of column and the type of detector.

3.5 Experimental

An Aminco-Bowman Spectrofluorometer was used for determining the fluorescence. The compounds analysed were pure industrial preparations; 5-Hydroxyindole-3-acetic acid and 5-Hydroxytryptophan (Aldrich Chemical Co. Inc.,) Indomethacin was from Merk Sharp and Dohme and Oxypertine was obtained from Winthrop Lab. Analar reagents were used where appropriate.

Native Fluorescence

A stock solution of each substance was diluted to known concentrations. Ten measurements of blanks were taken in each determination which were, 8N Hcl in the case of the acidic solution, ethanol in the case of the neutral solution and O.1N NaoH in the case of the alkaline solution.

Excitation and emission wavelengths are listed in table 15. The slit setting on the instrument for all compounds were

Excitation inner slit	3 mm
Excitation outer slit	3 mm
Emission inner slit	2 mm
Emission inner slit	2 mm
Photomultiplier slit	4 mm

O-Phthalaldehyde reaction

O-phthalaldehyde solution was prepared by dissolving 40 mg O-phthalaldehyde in 100 ml absalute methanol (freshly prepared). Ten measurements of the blank were taken in each determination. Excitation, and emission wavelengths are listed in table 16. The reactions were carried out as follows:-

To 1 ml of 0.05 N Hcl containing 1 ug, 1.5 ug, 2.5 ug, 3 ug, 4 ug or 5 ug of 5-Hydroxyindole-3-acetic acid in 6 test tubes respectively and 10 test tubes of blanks, 0.05 ml of the O-phthalaldehyde solution was added. After mixing, 4 ml of 10 N Hcl was added to each tube and after mixing again, the tubes were heated in a boiling water bath for 1 hour. After cooling to room temperature the fluorescence was measured with the following settings.

Excitation inner slit	3 mm
Excitation outer slit	3 mm
Emission inner slit	2 mm
Emission outer slit	2 mm
Photomultiplier slit	4 mm

The same experiment was carried out with 5-Hydroxytryptophan. In the case of oxypertine and indomethacin the settings of the instrument and the solvent were changed as follows:-

To 1 ml of ethanol containing 0.025 ug, 0.05 ug, 0.1 ug, 0.15 ug, 0.2 ug, 0.25 ug or 0.3 ug of oxypertine in 7 test tubes respectively and 10 test tubes of blanks, 0.05 ml of theO-phthalaldehyde solution was added. After mixing, 4 ml of 10N Hcl was added to each tube and after mixing again, the tubes were heated in a boiling water bath for 1 hour. After cooling to room temperature the fluorescence was measured with the following settings.

Excitation inner slit	5 mm
Excitation outer slit	5 mm
Emission inner slit	3 mm
Emission outer slit	5 mm
Photomultiplier slit	4 mm

The same experiment was carried out with indomethacin so that each ml of solution contained either 0.15 ug, 0.25 ug, 0.4 ug, 0.5 ug, or 0.6 ug with the same conditions and setting of the instrument.

Gas Chromatography

All separations were carried out on a F11 Perkin-Elmer Gas Chromatography equipped with flame ionization detector. A Perkin-Elmer 56 Recorder was used to record the separation by the 3% SE30 (100-120 mesh) chrom WAWHMDS column. Derivatization was carried out in reactive vials, and the following methods were used to make the derivatives:-

(i) For 5-Hydroxyindole-3-acetic acid; solutions of 2 mg, 4 mg, 6 mg, 8 mg and 10 mg 5-Hydroxyindole-3-acetic acid in 5 ml acetonitrile were prepared. To 0.2 ml of each above solution in vials and a blank of 0.2 ml acetonitrile in another vial, 0.1 ml N,O-Bis(trimethylsilyl)-acetamide was added. The vials were then heated in a boiling water bath for 2 hours then cooled. The determination was carried out under the following operating conditions:-

Oven temperature	220°C
Injection temperature	310°C
N_2 pressure	25lb/in^2
H_2 pressure	20lb/in^2
Air pressure	20lb/in^2
Sensitivity range	2 x 10^2
Chart speed of recorder	5 mm/min.

(ii) For 5-Hydroxytryptophan; to 0.2 ml of acentonitrile containing either 0.6 mg, 1.2 mg, 1.8 mg, 2.4 mg or 3 mg 5-Hydroxytryptophan in vials with a blank vial containing 0.2 ml of acetonitrile, 0.1 ml N,O-Bis(trimethyl-slilyl)-acetomide was added. The vials were then heated in a boiling water bath for 2 hours and then cooled, and the separation was determined under the following operating conditions:

Oven temperature	235°C
Injection temperature	340°C
N_2 pressure	25lb/in^2
H_2pressure	20lb/in^2
Air pressure	20lb/in^2
Sensitivity range	10 x 10^2
Chart speed of recorder	5 mm/min.

(iii) In the case of oxypertine the column was silylated by injecting 35 ul of silyl-8 with an oven temperature of 200°C. The temperature was kept at 200°C for 10 minutes and then reduced to room temperature. Operating conditions in this case were:

Oven temperature	300°C
Injection temperature	405°C
N_2 pressure	25lb/in^2
H_2 pressure	20lb/in^2
Air pressure	20lb/in^2
Sensitivity range	5 x 10^2
Chart speed of recorder	5mm/min.

Under the above conditions the following amounts; 8 ug, 9 ug, 10 ug, 12 ug, 14 ug, and 16 ug of oxypertine was determined.

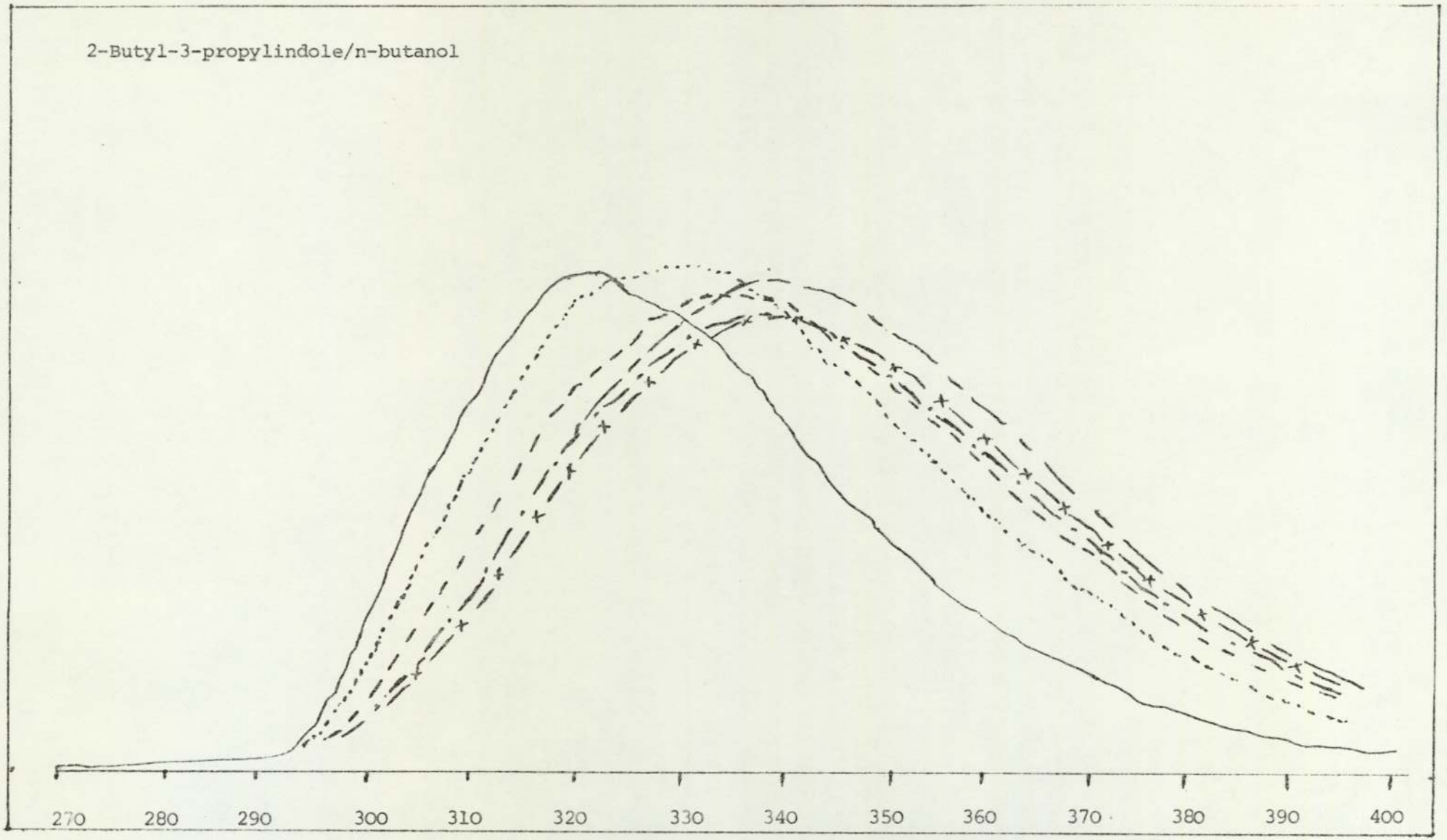
Appendix

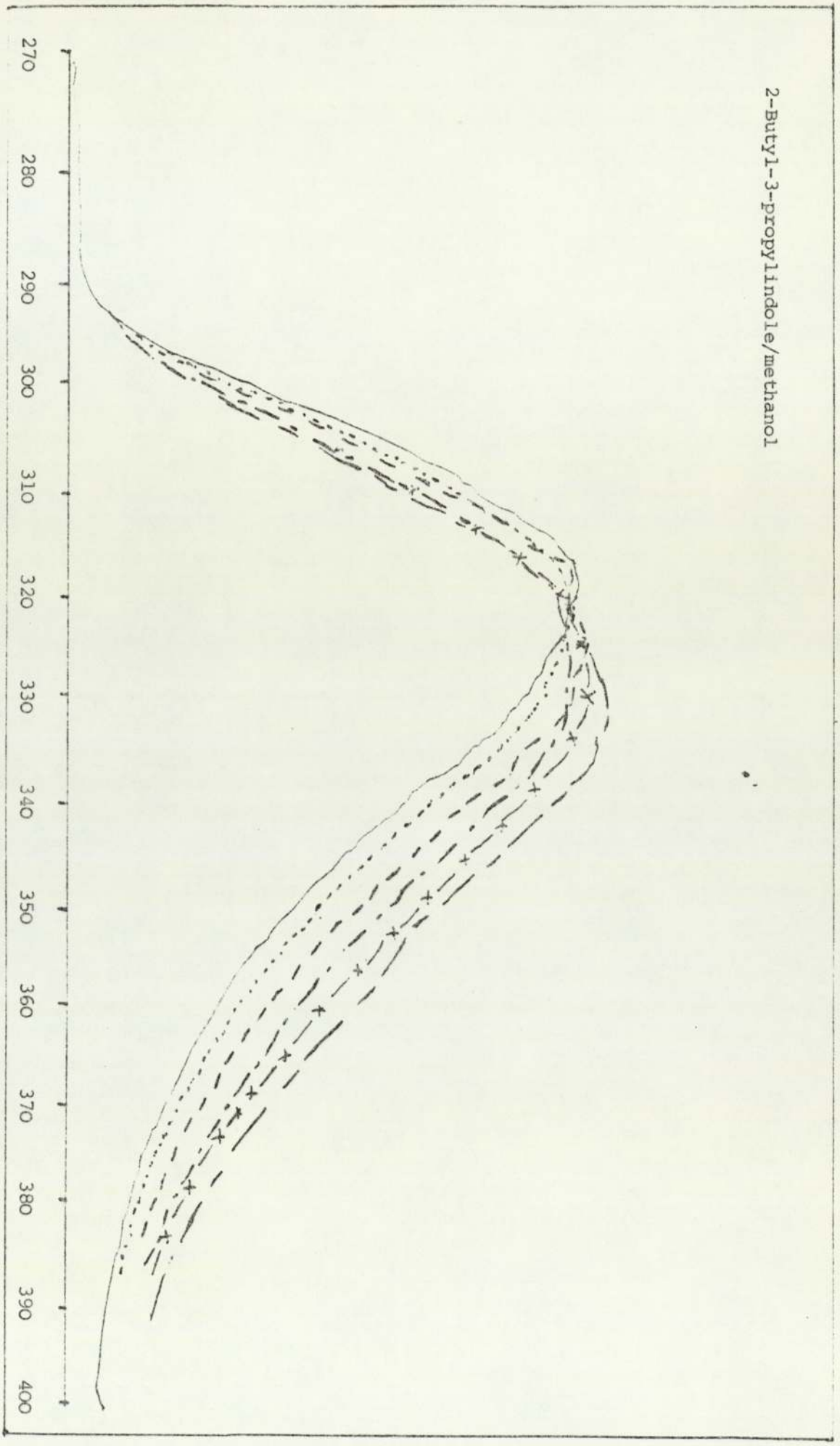
Spectra 1 to 49 are presented of the indoles with the given polar solvent in cyclohexane solution at the polar solvent concentrations of:

(1)	――――――――――――――――――――	0.00M
(2)	....	0.04M
(3)	------------------------------------	0.08M
(4)	.—.—.—.—.—.—.—.—.—.—.—.—.	0.12M
(5)	—x—x—x—x—x—x—x—x—x—x—x—x	0.16M
(6)	――― - ――― - ――― - ――― -	0.20M

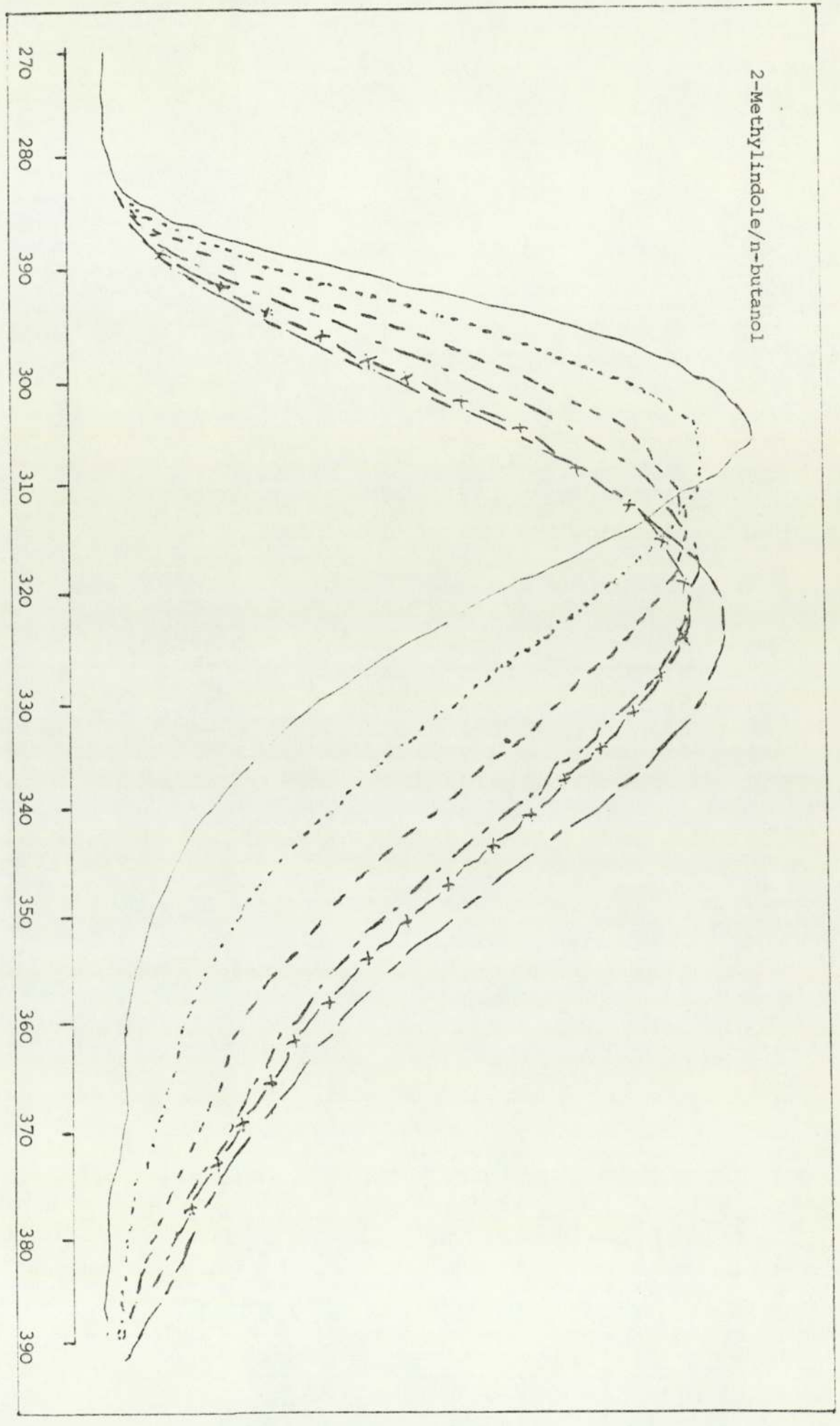
The wavelengths and shifts of wavelength are given in nanometers (nm).

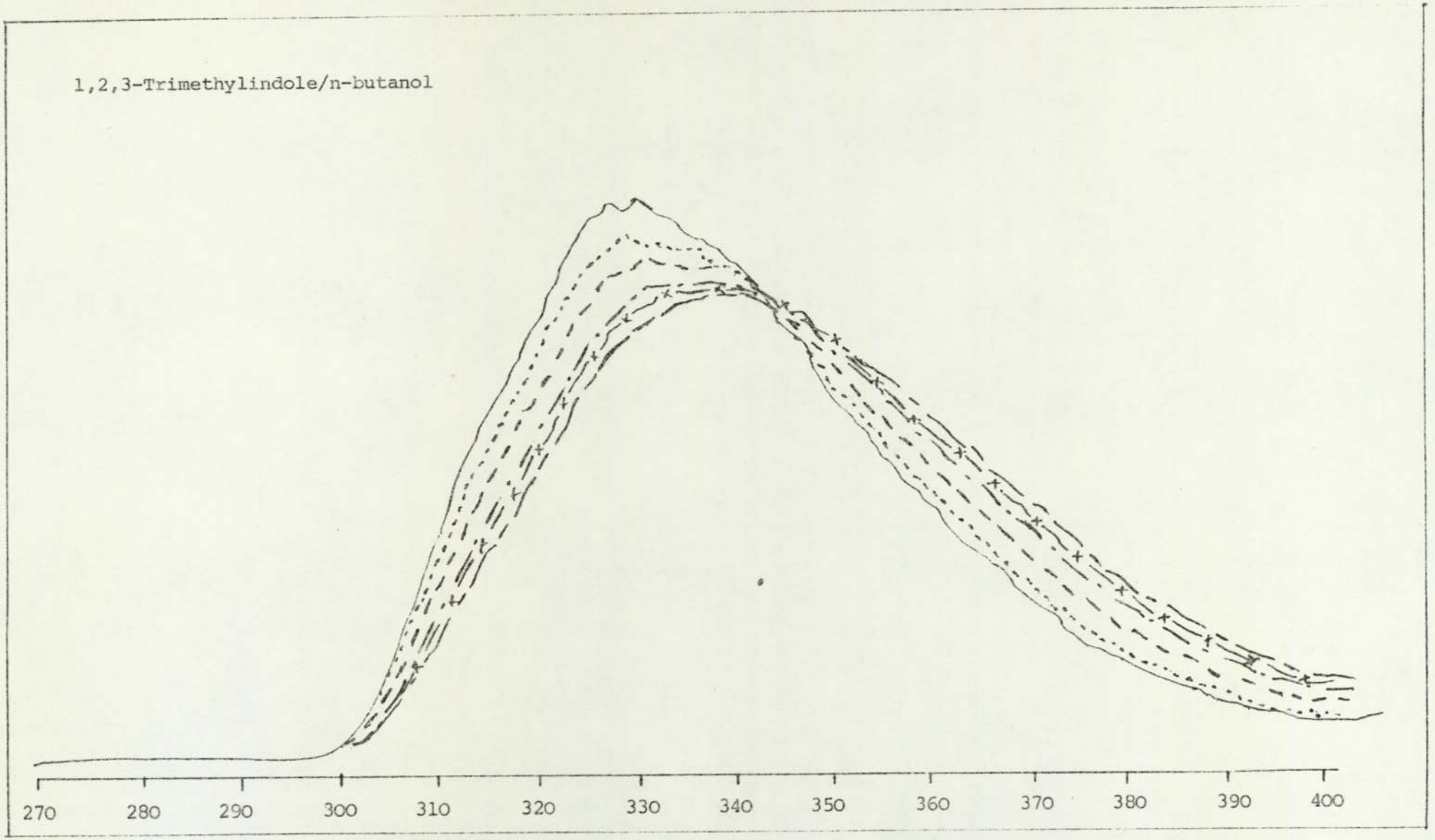
2-Butyl-3-propylindole/n-butanol

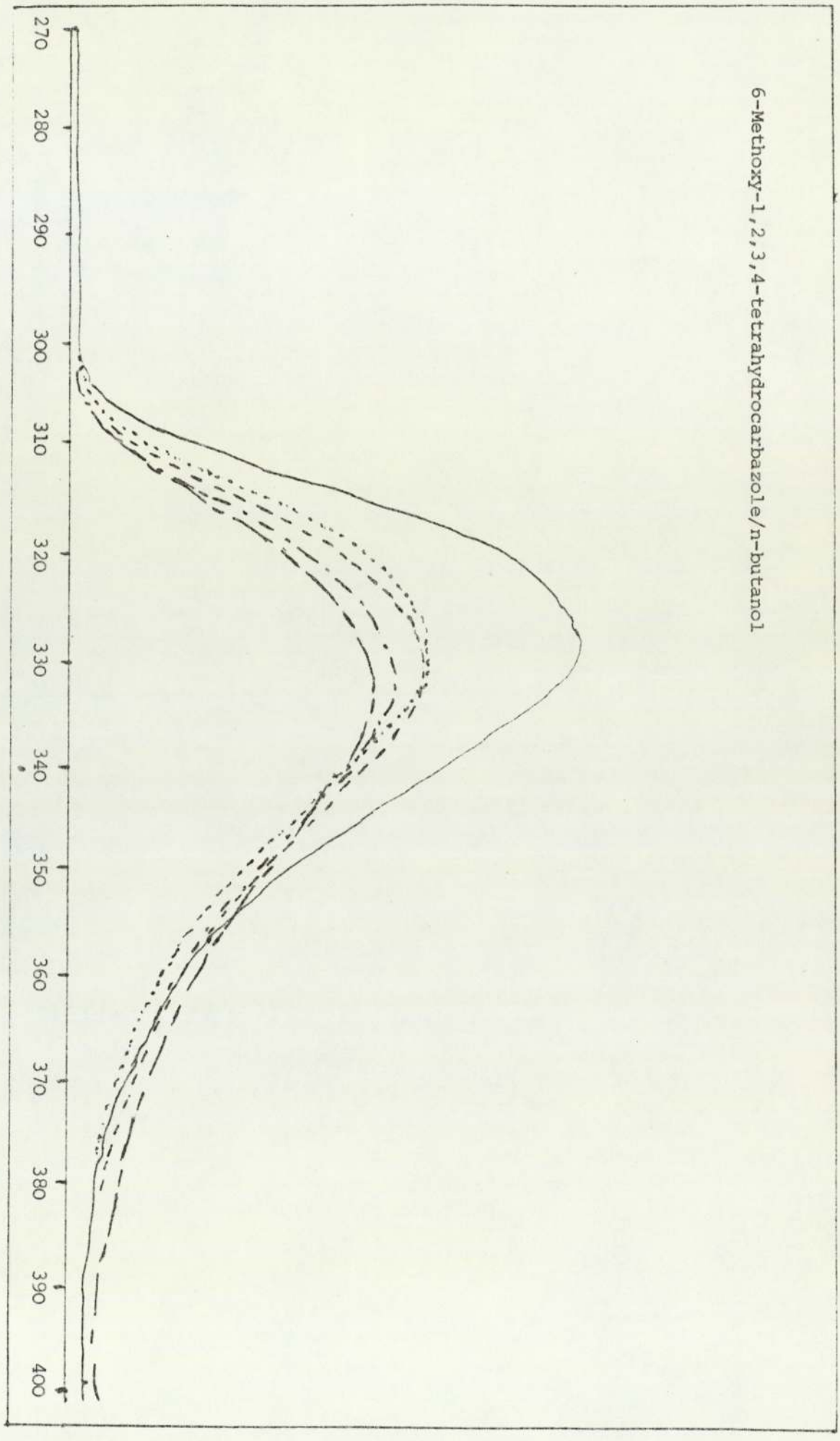


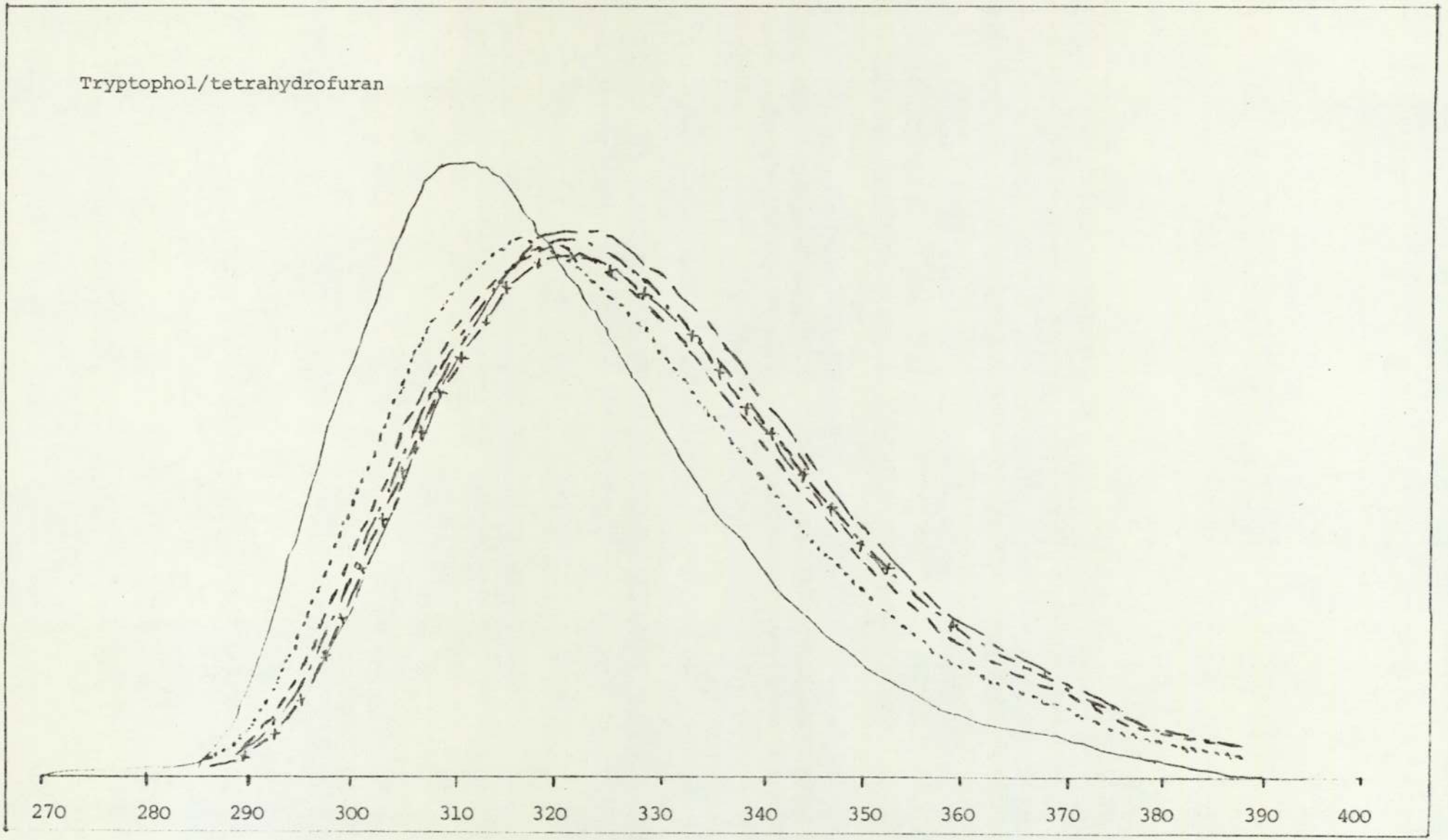

2-Butyl-3-propylindole/methanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

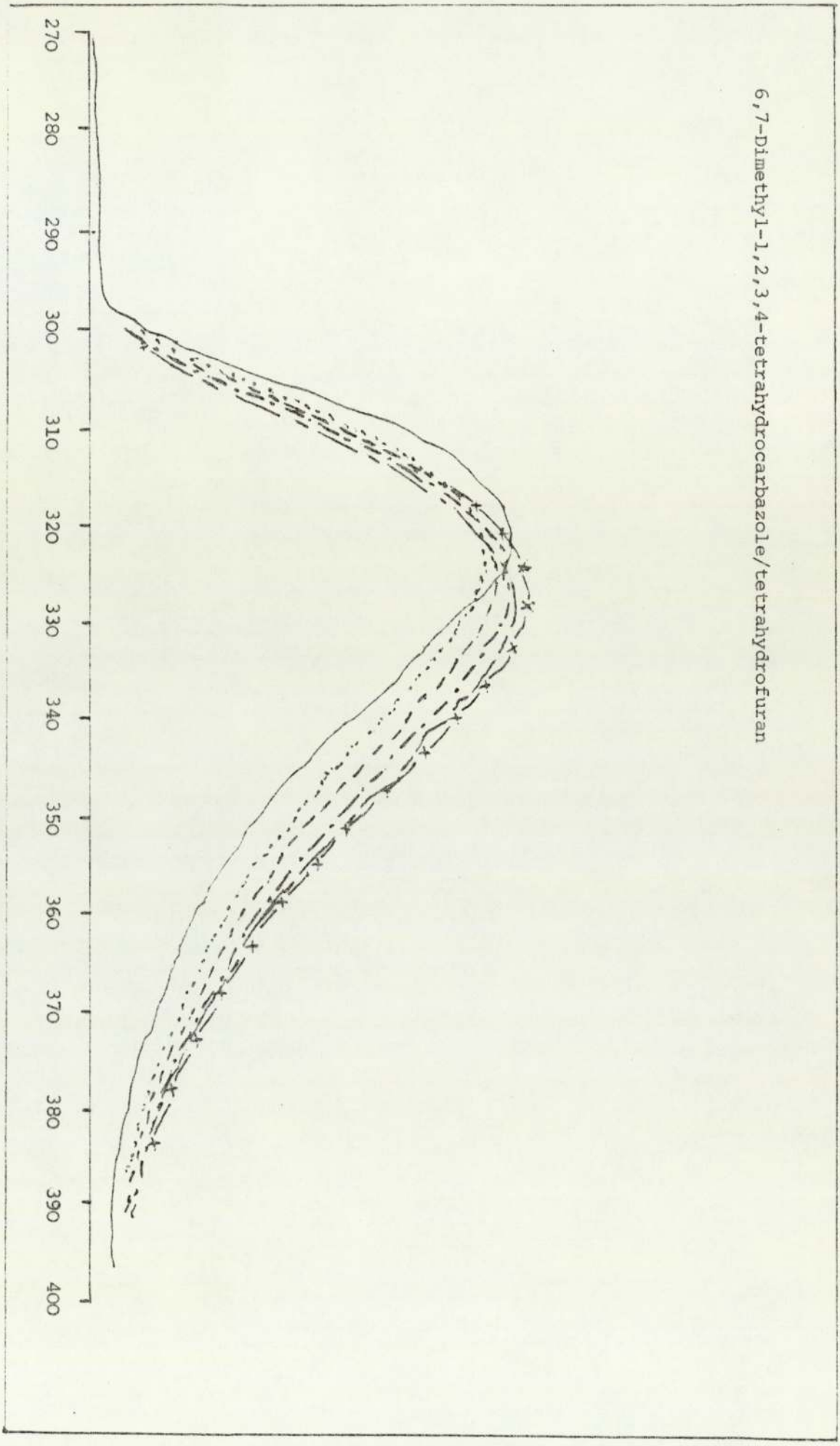
2


2-Methylindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390

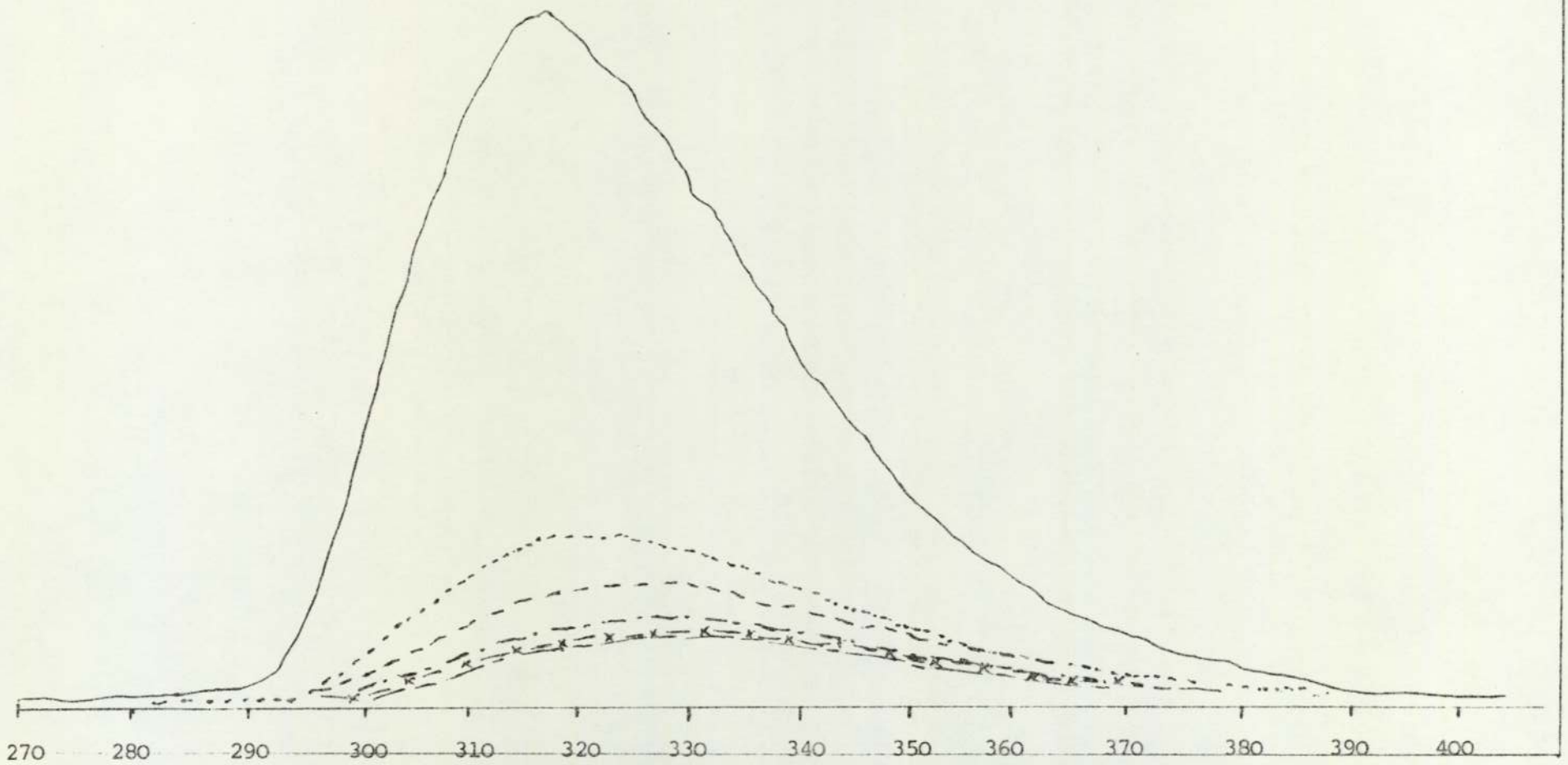

1,2,3-Trimethylindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

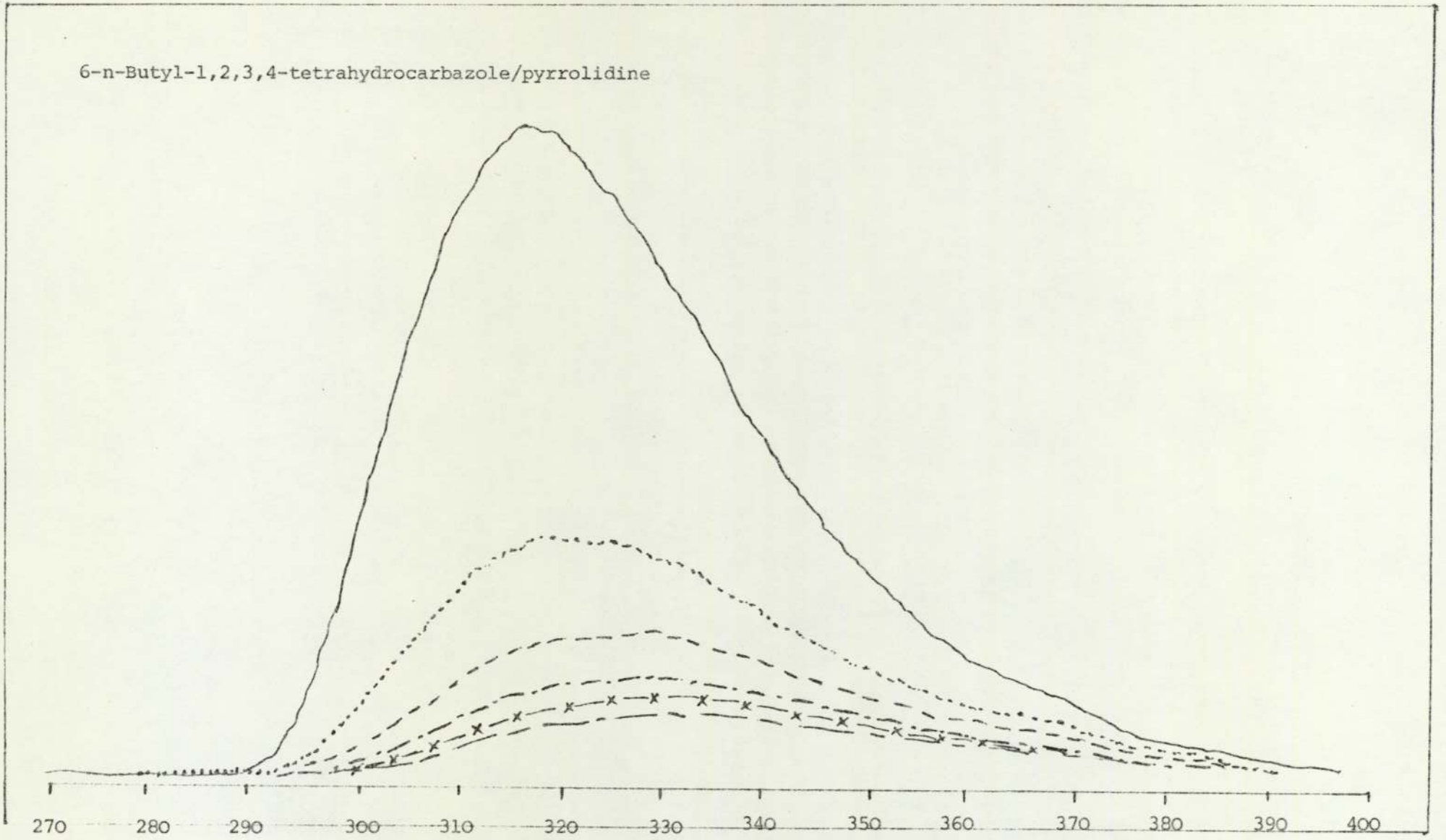

6-Methoxy-1,2,3,4-tetrahydrocarbazole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

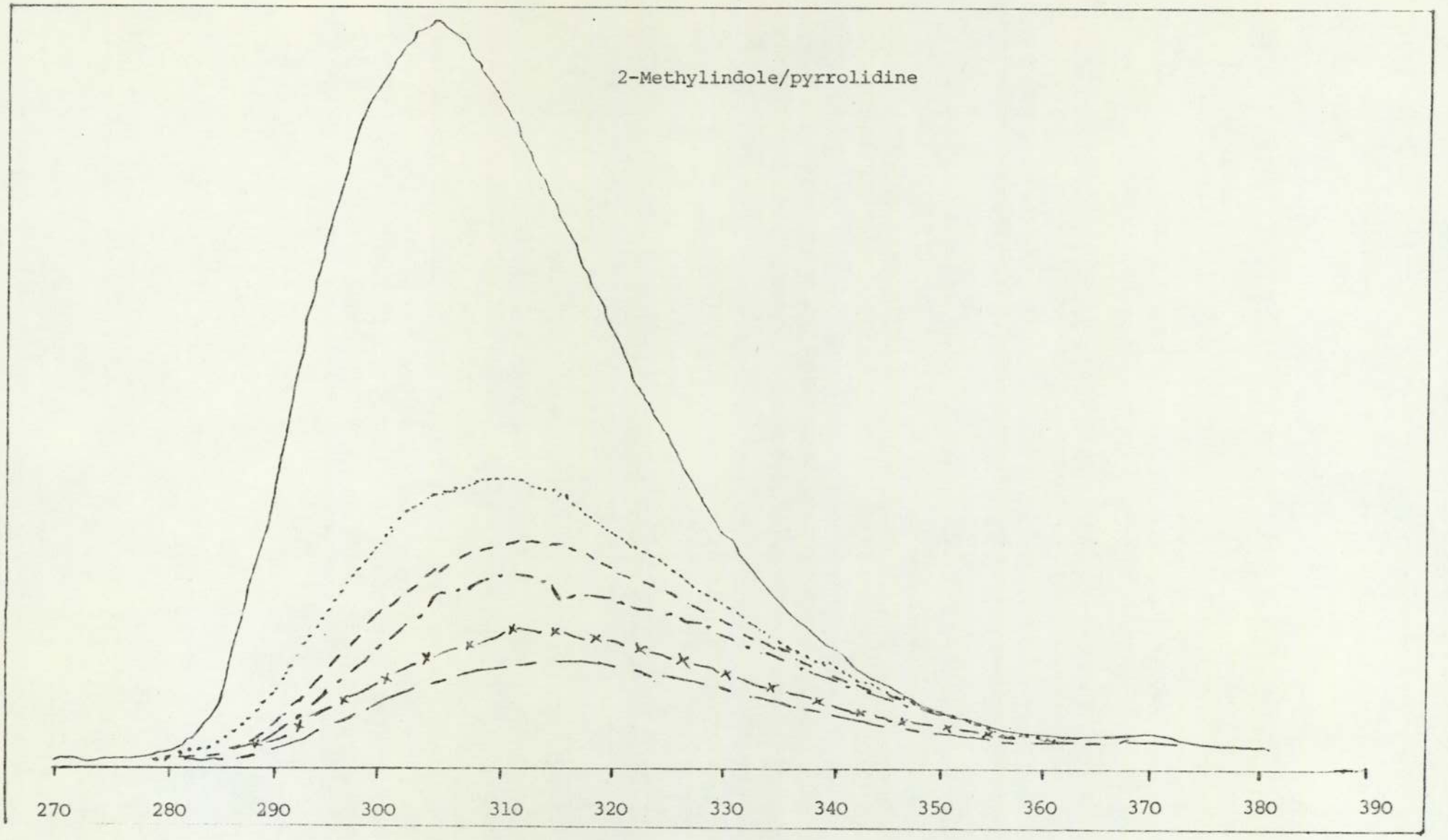

Tryptophol/tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390
400

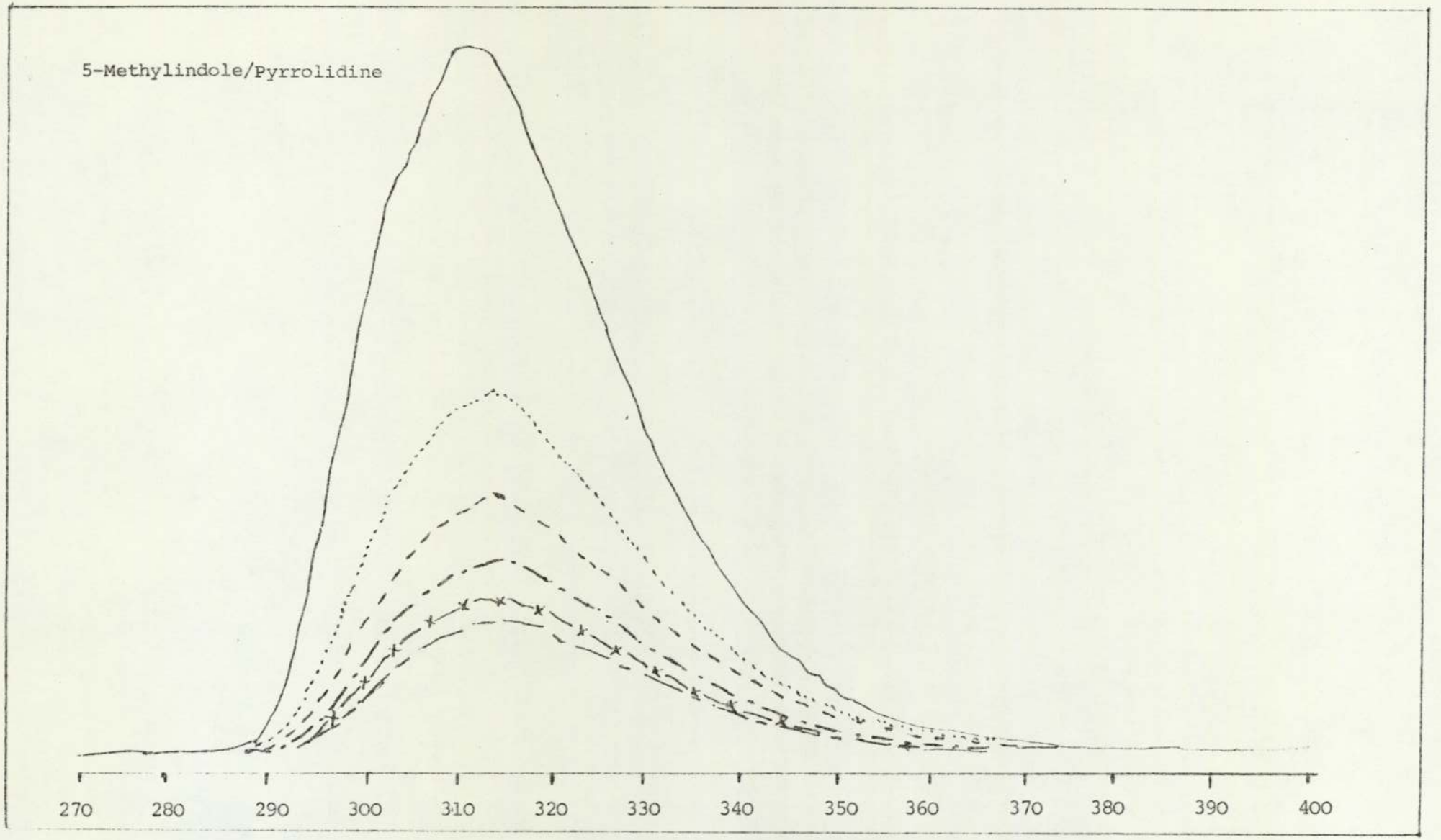

6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole/tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390
400

6-Methyl-1,2,3,4-tetrahydrocarbazole/pyrrolidine

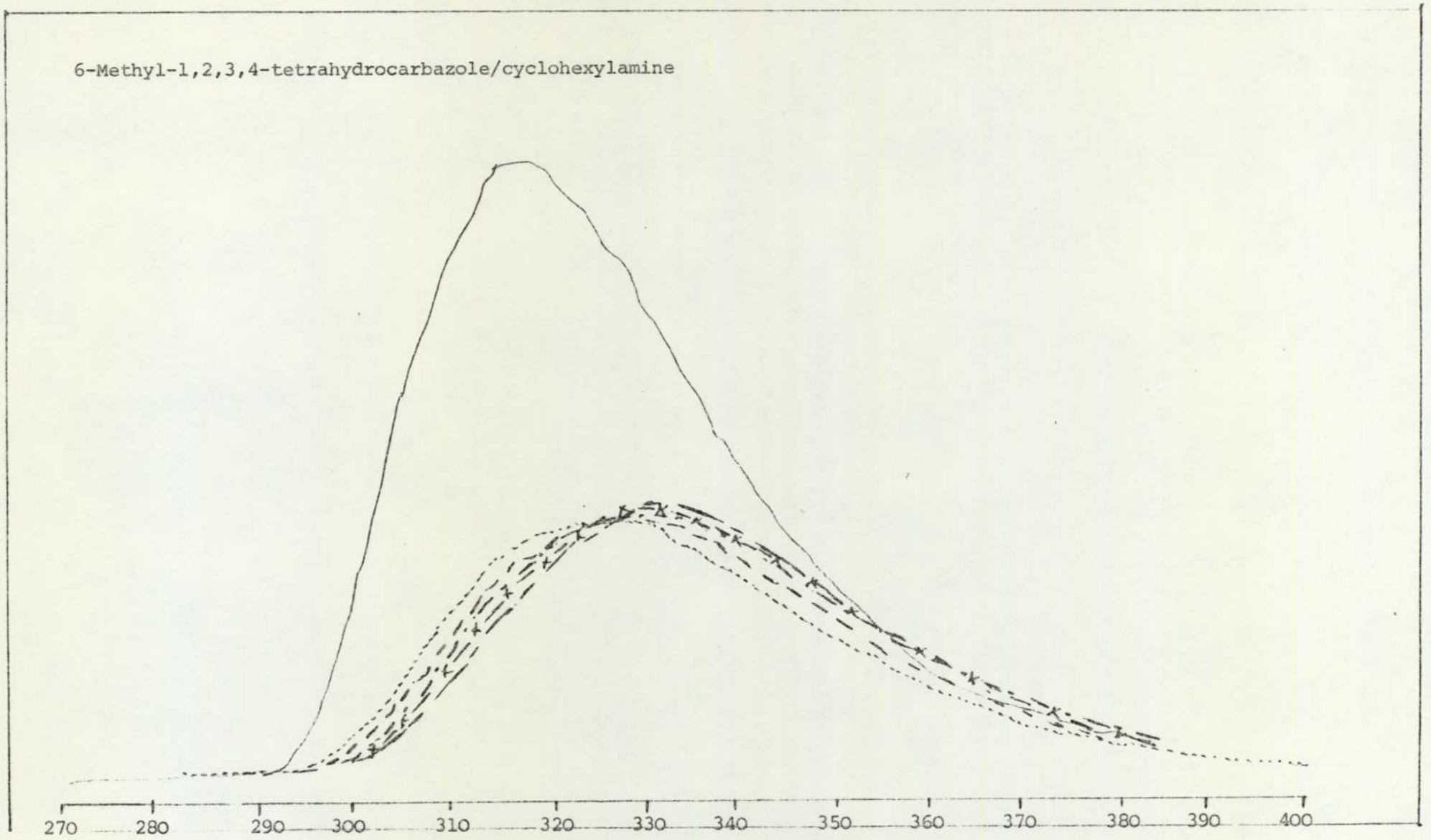


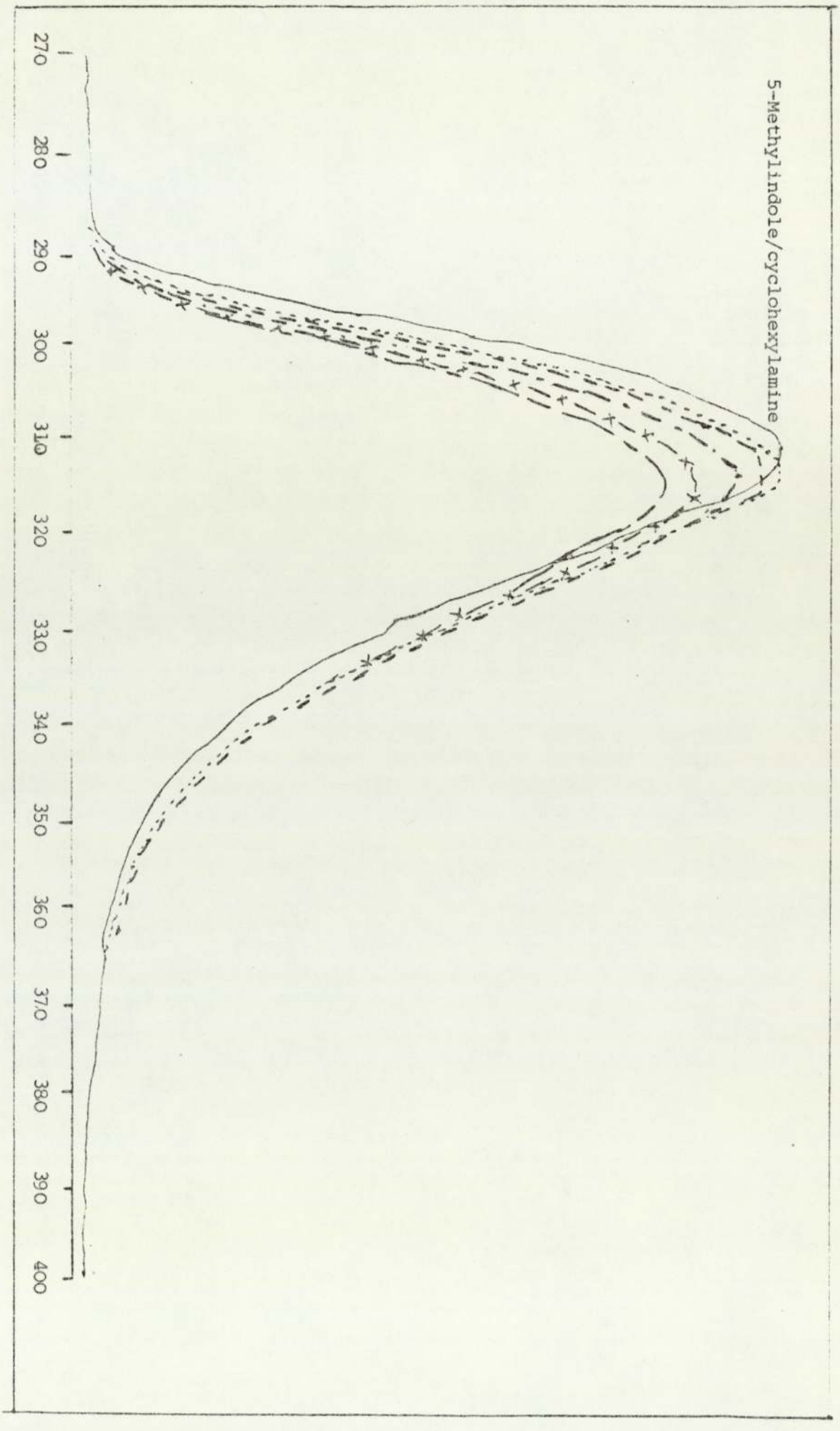

6-n-Butyl-1,2,3,4-tetrahydrocarbazole/pyrrolidine
- 100 -
270
280
290
300
310
320
330
340
350
360
370
380
390
400

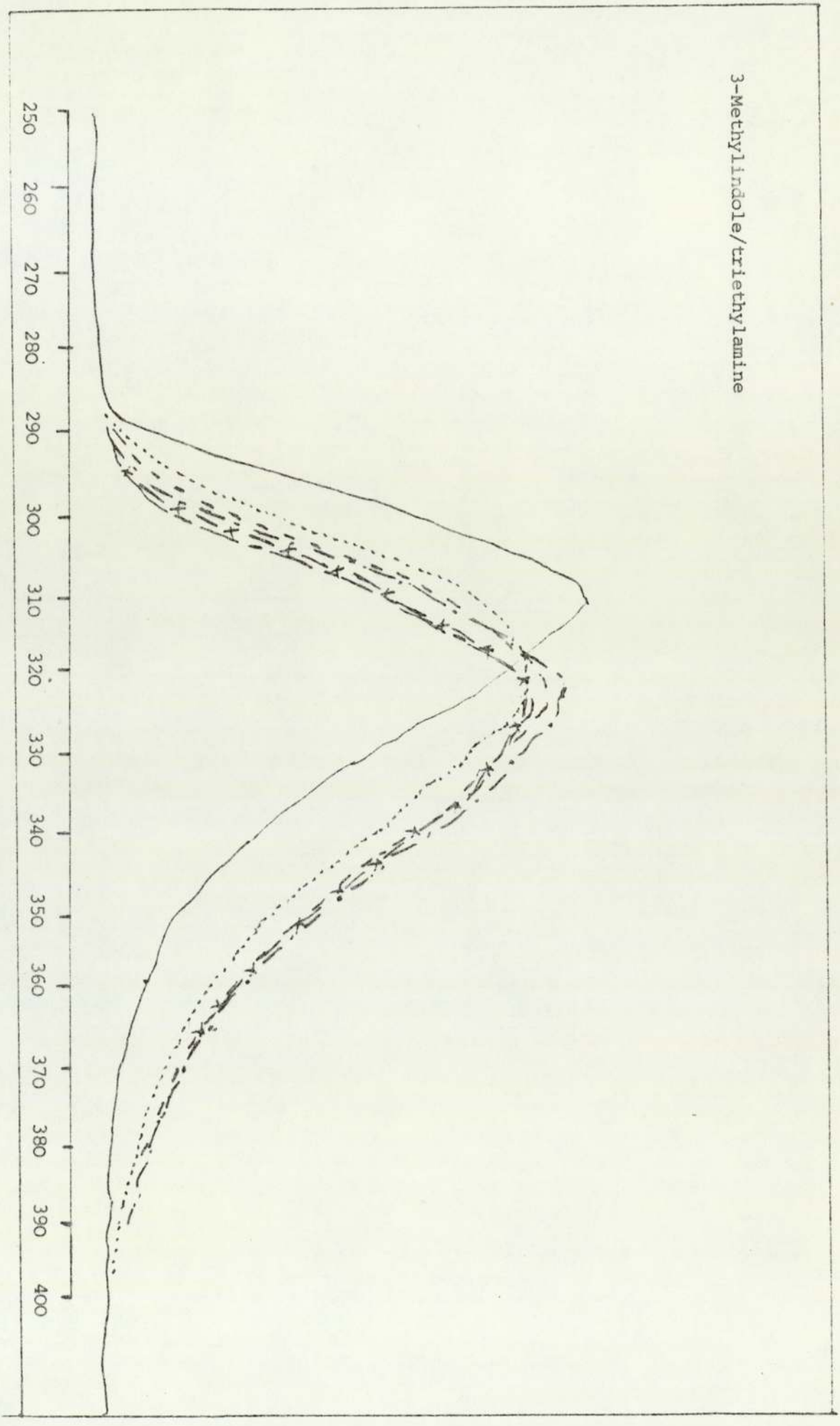

2-Methylindole/pyrrolidine
270
280
290
300
310
320
330
340
350
360
370
380
390

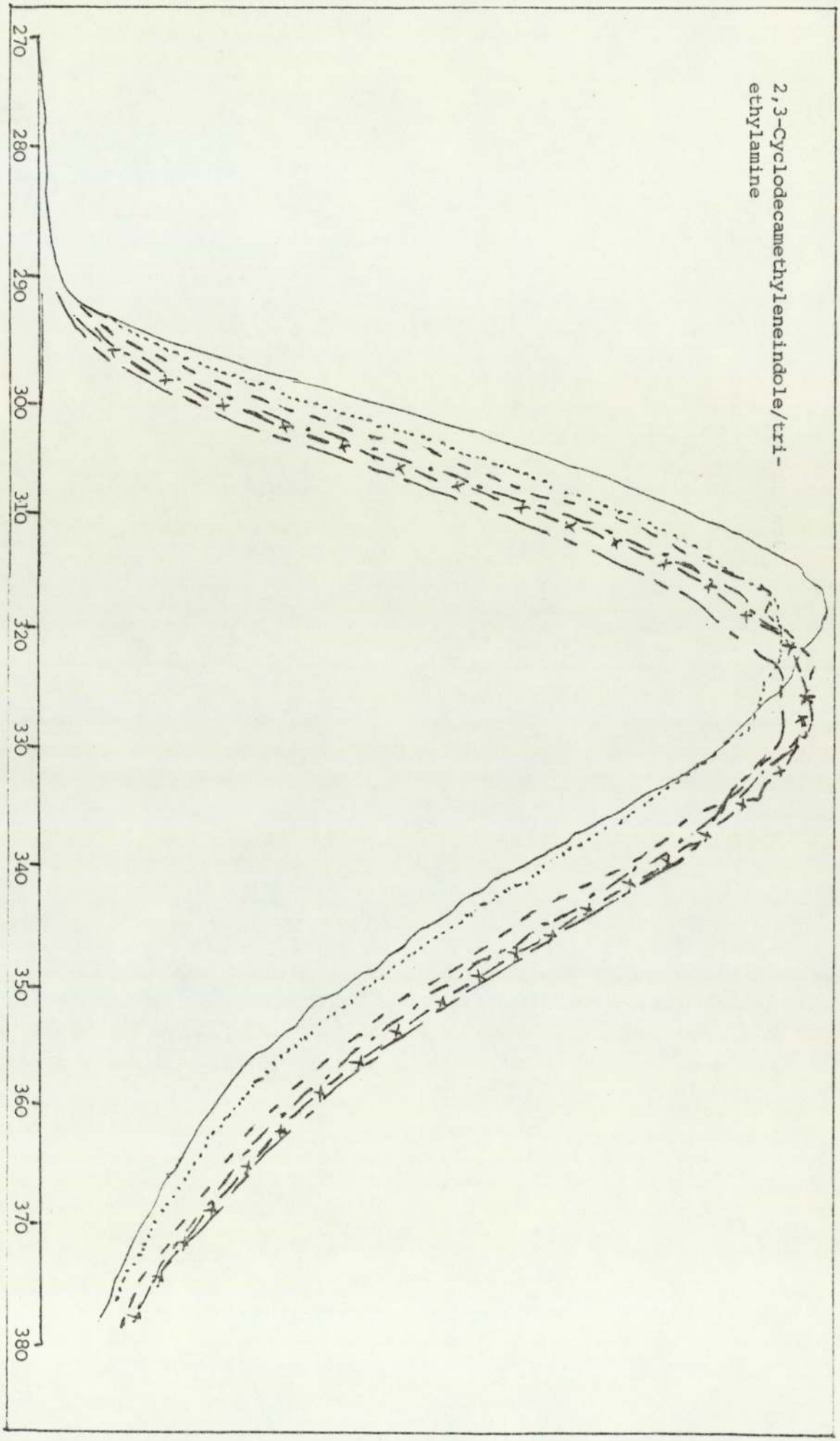

5-Methylindole/Pyrrolidine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

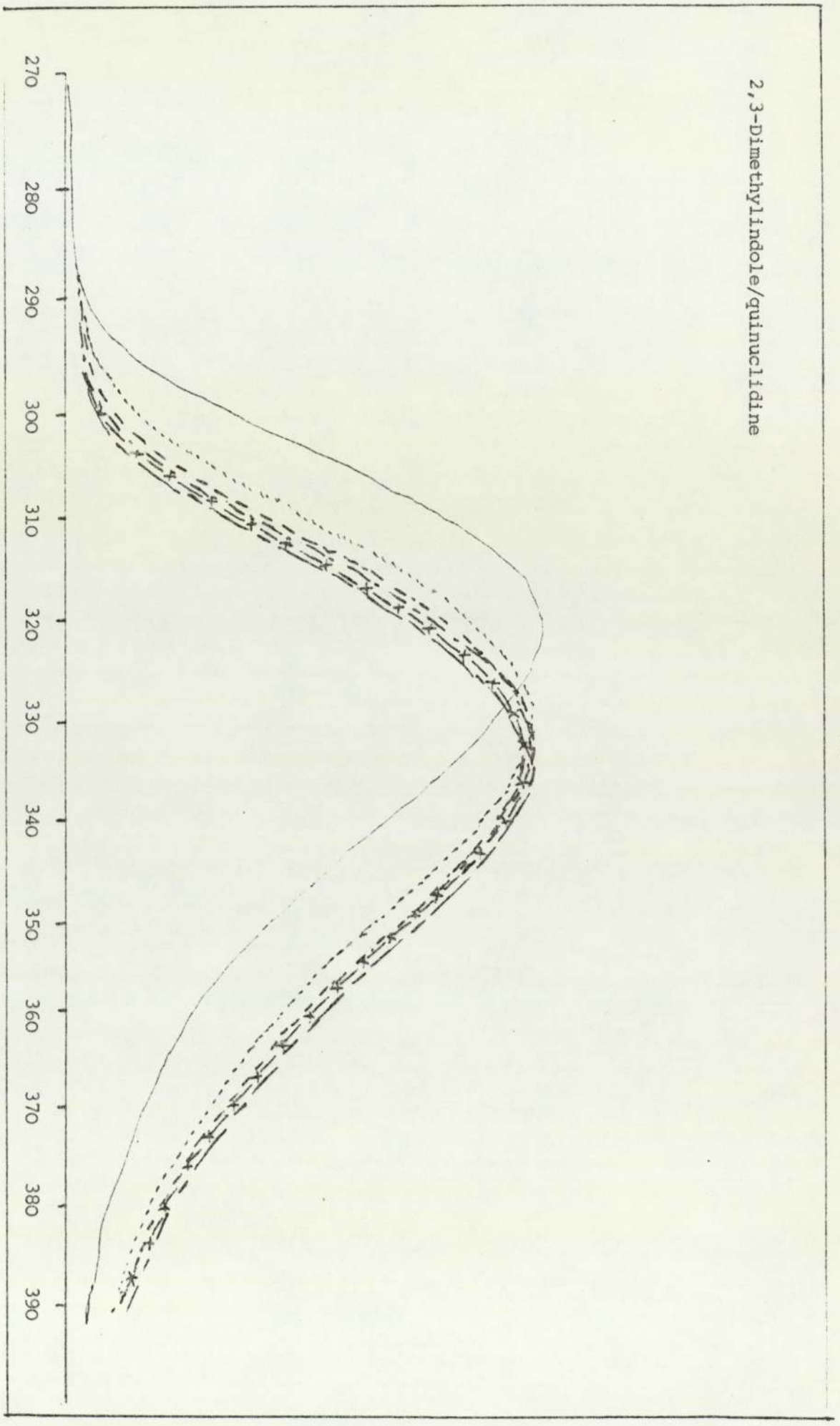
6-Methyl-1,2,3,4-tetrahydrocarbazole/cyclohexylamine



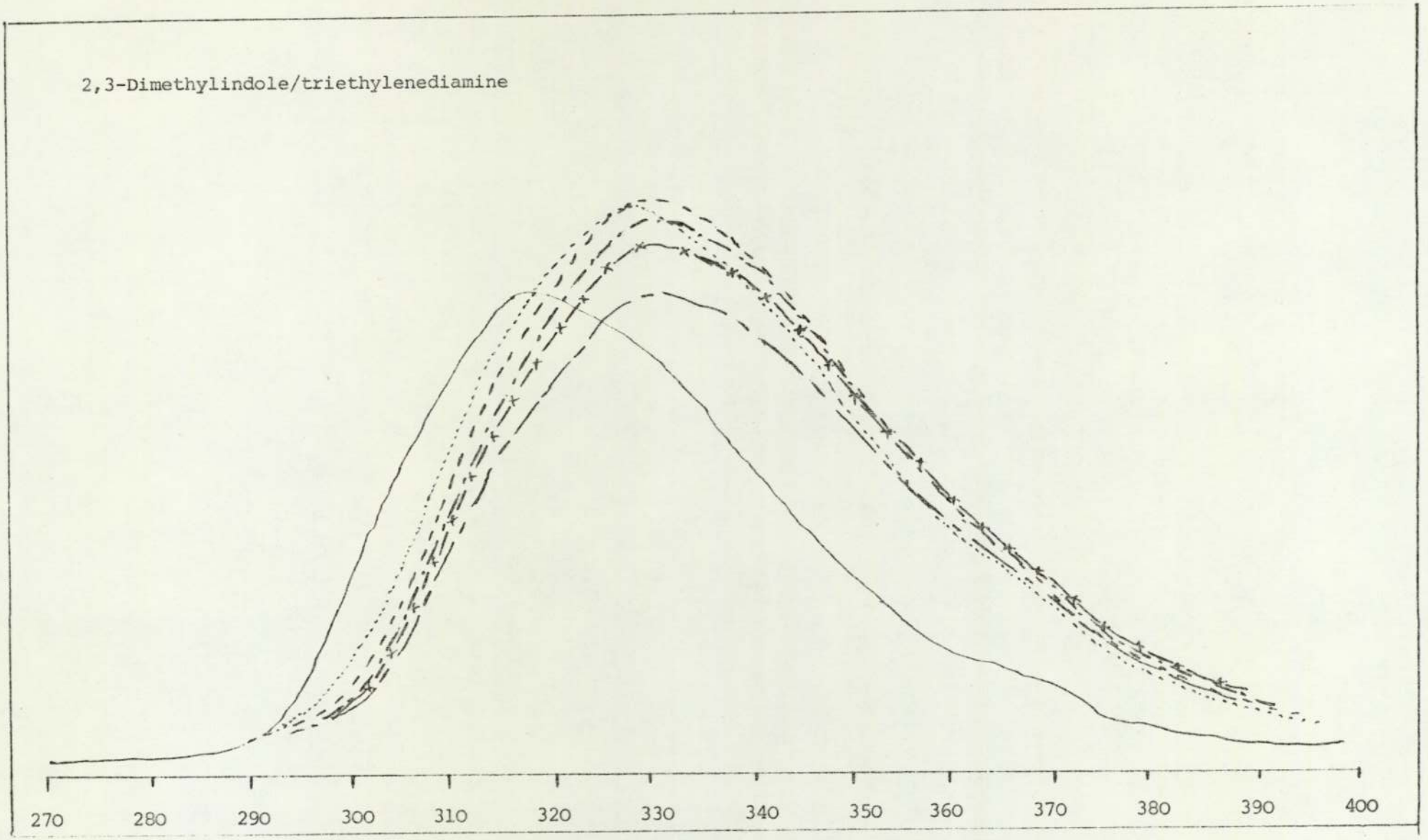

5-Methylindole/cyclohexylamine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

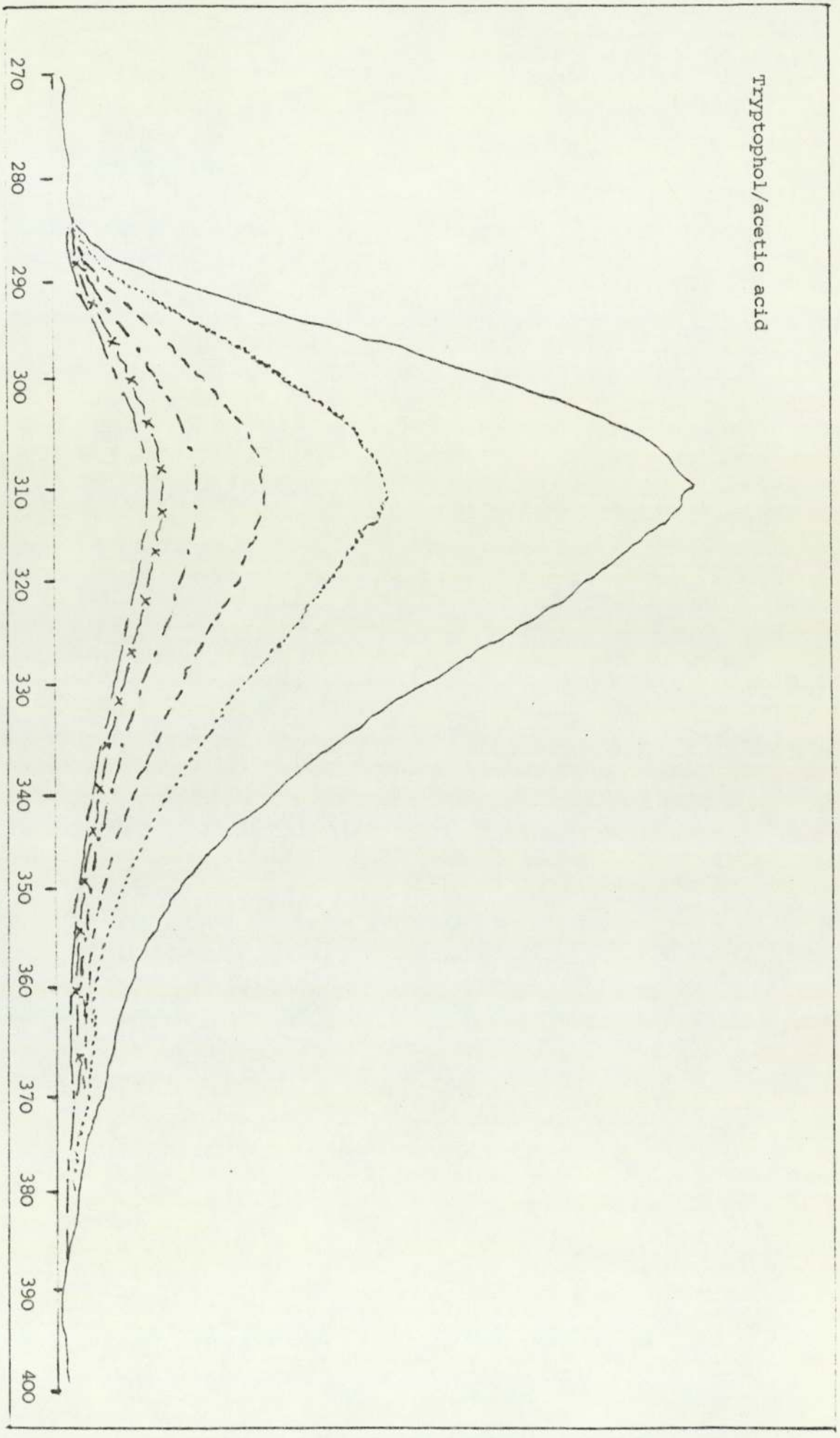

3-Methylindole/triethylamine
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400

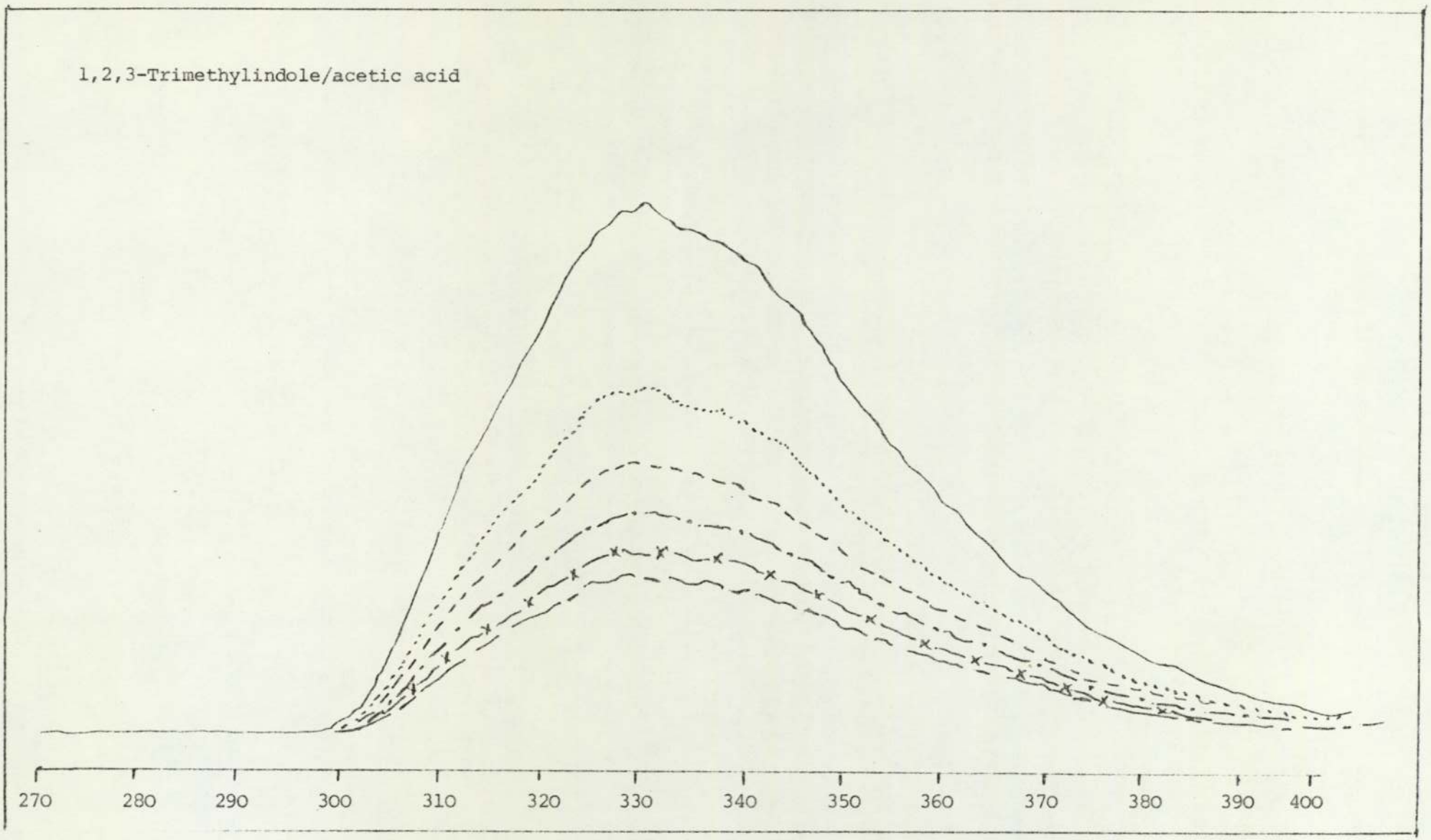

2,3-Cyclodecamethyleneindole/tri-
ethylamine
270
280
290
300
310
320
330
340
350
360
370
380


2,3-Dimethylindole/quinuclidine
270
280
290
300
310
320
330
340
350
360
370
380
390

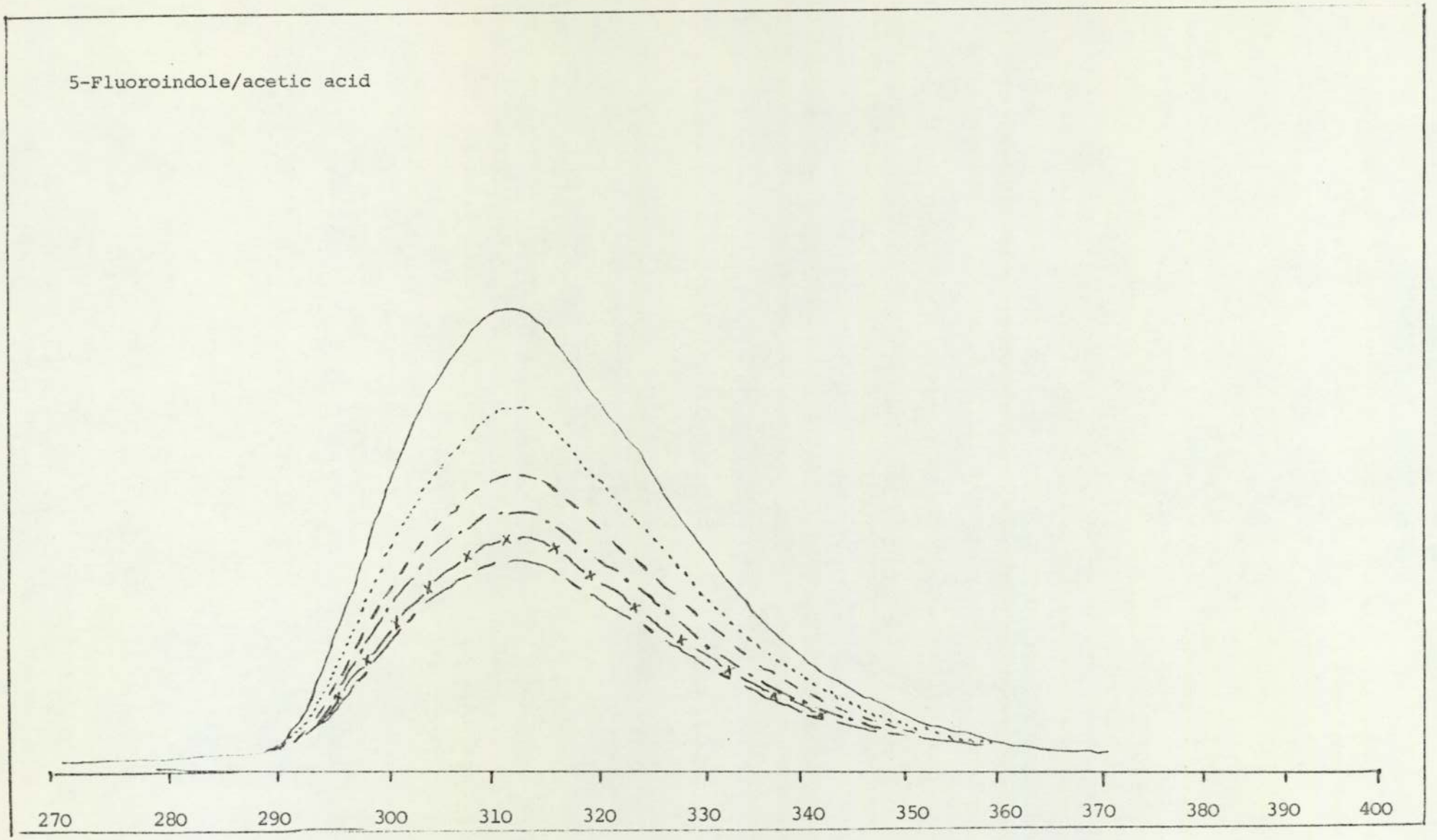
2,3-Dimethylindole/triethylenediamine

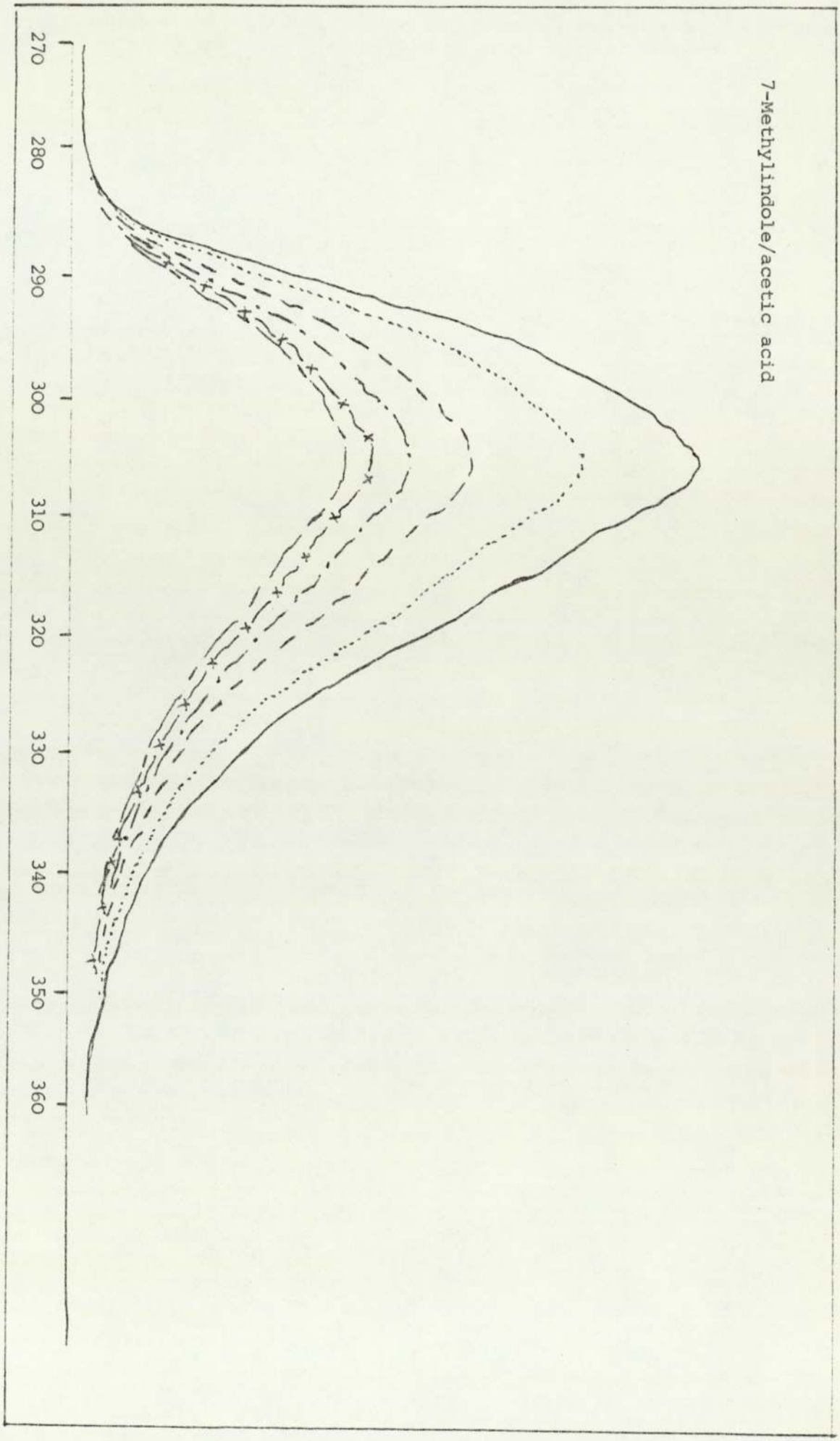



Tryptophol/acetic acid
270
280
290
300
310
320
330
340
350
360
370
380
390
400

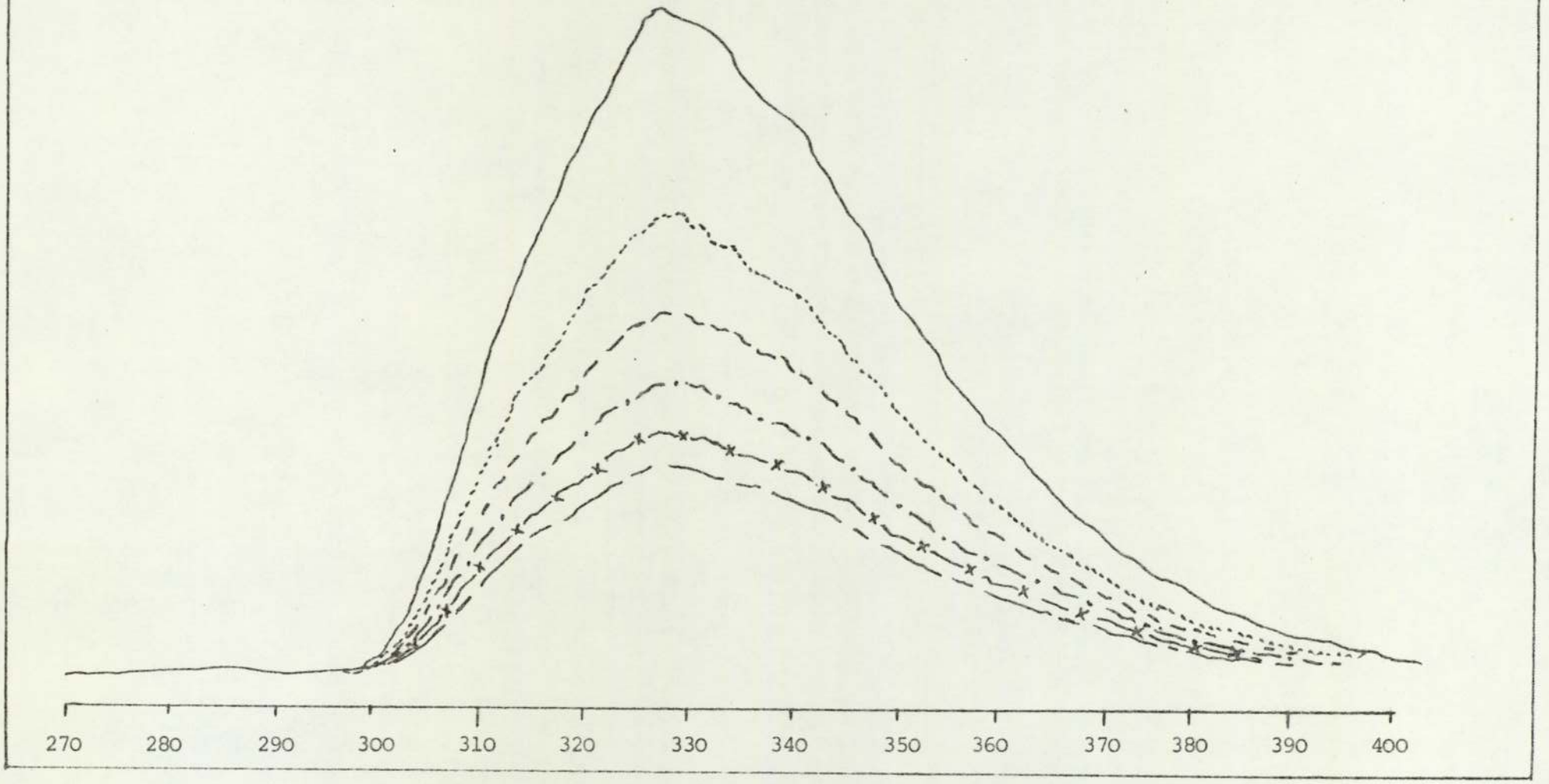

1,2,3-Trimethylindole/acetic acid
270
280
290
300
310
320
330
340
350
360
370
380
390
400

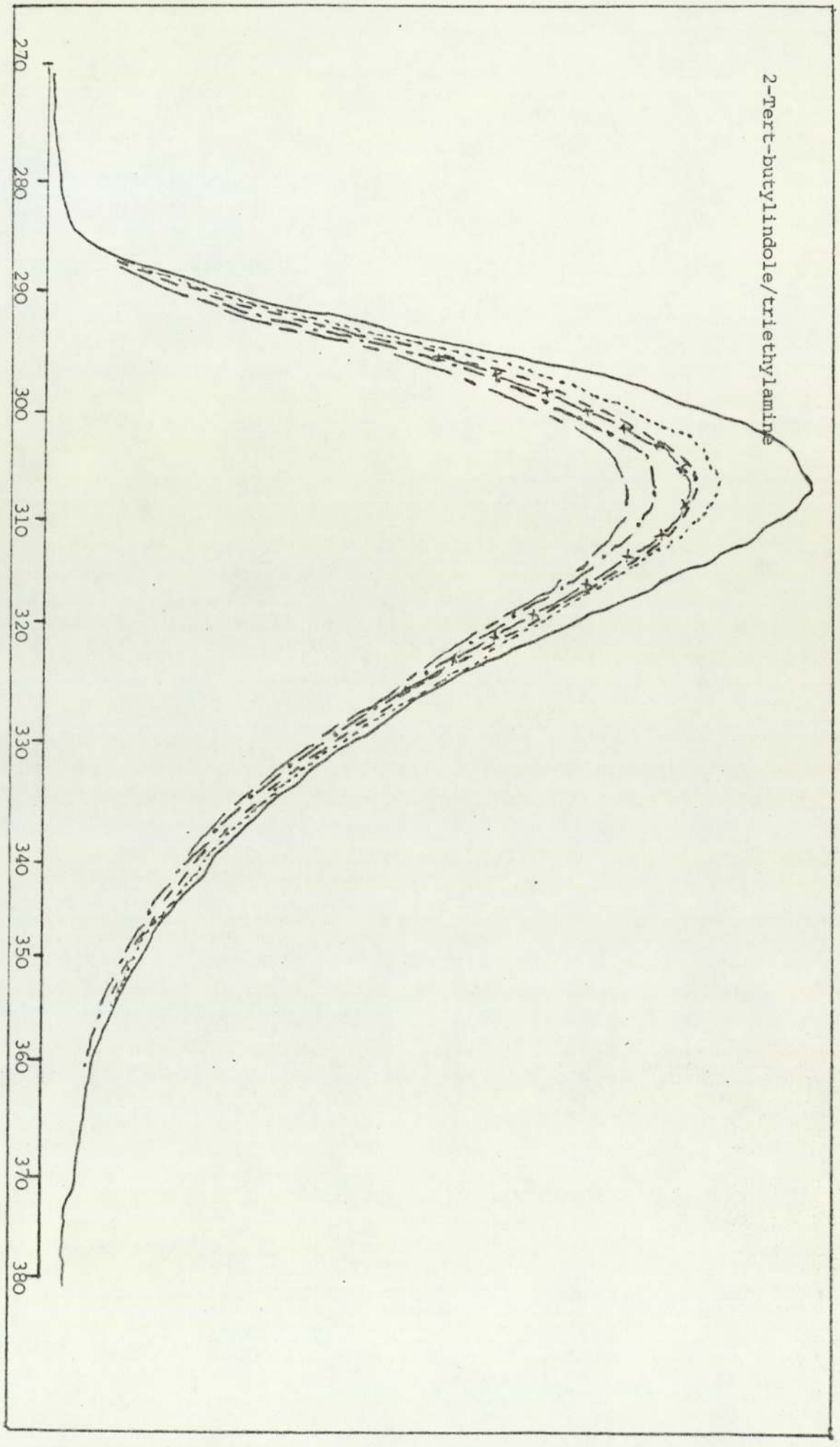
5-Fluoroindole/acetic acid

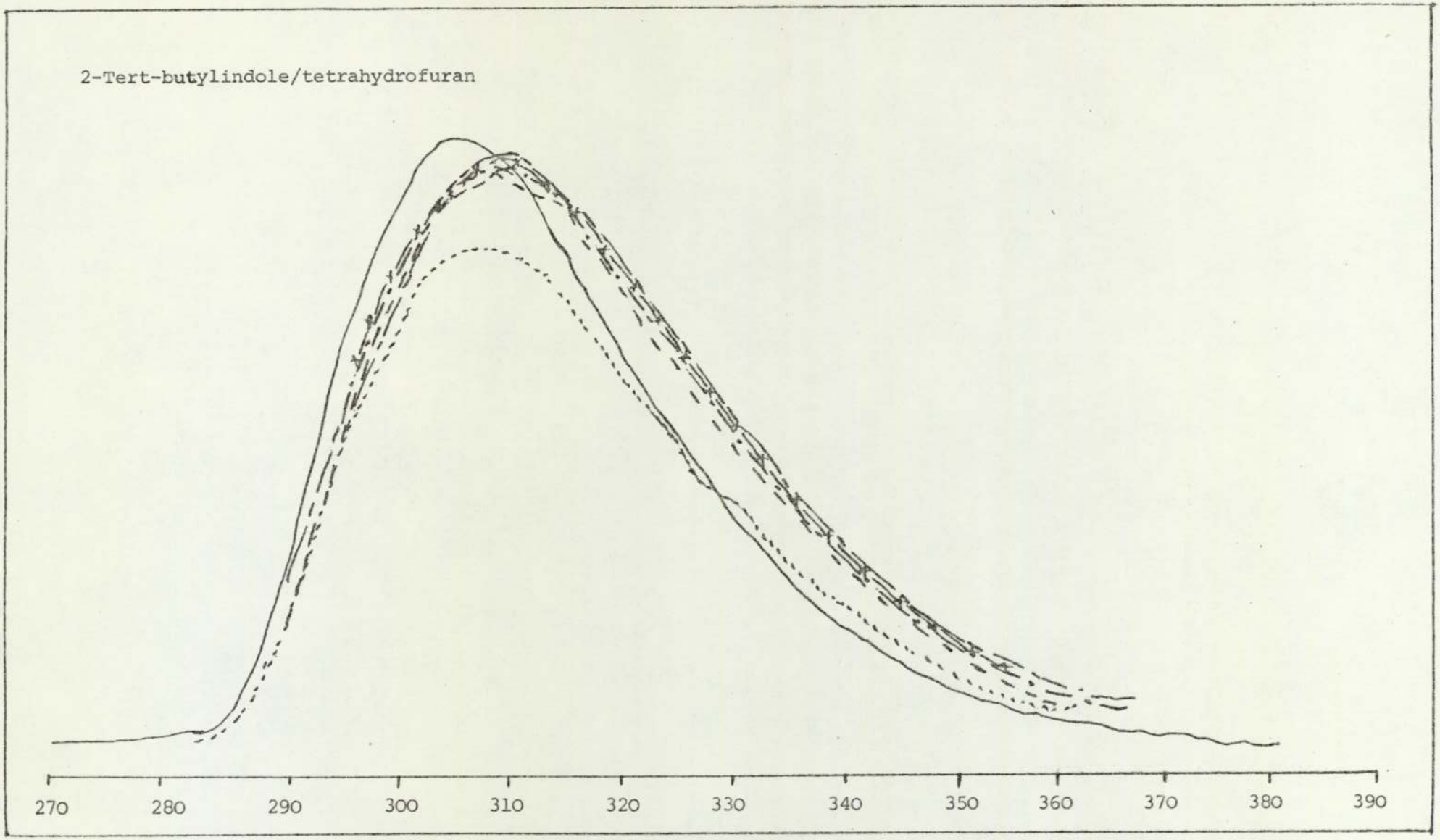


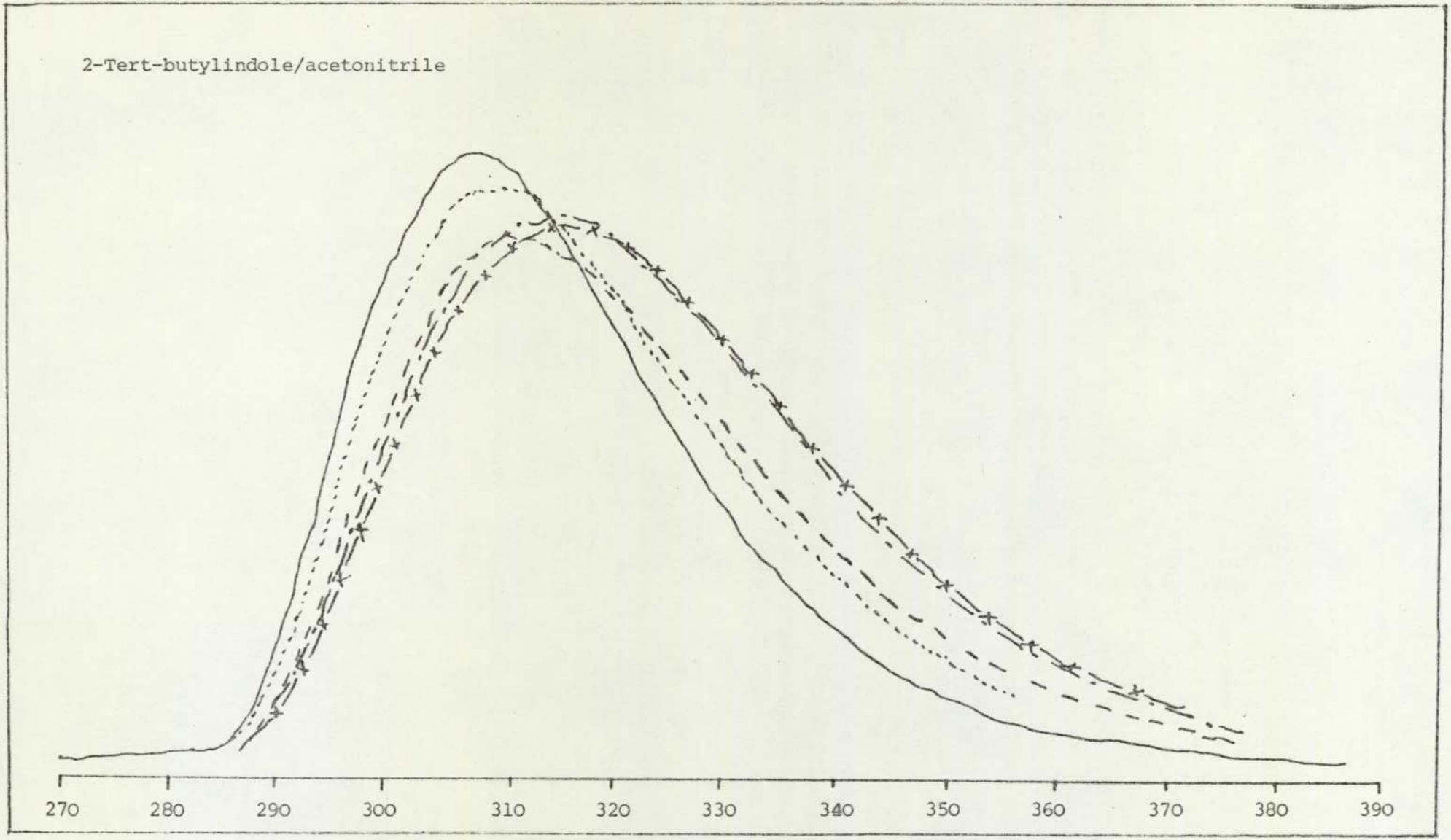

7-Methylindole/acetic acid
270
280
290
300
310
320
330
340
350
360

8,9-Cyclotrimethylene-1,2,3,4-tetrahydro-carbazole/acetic acid

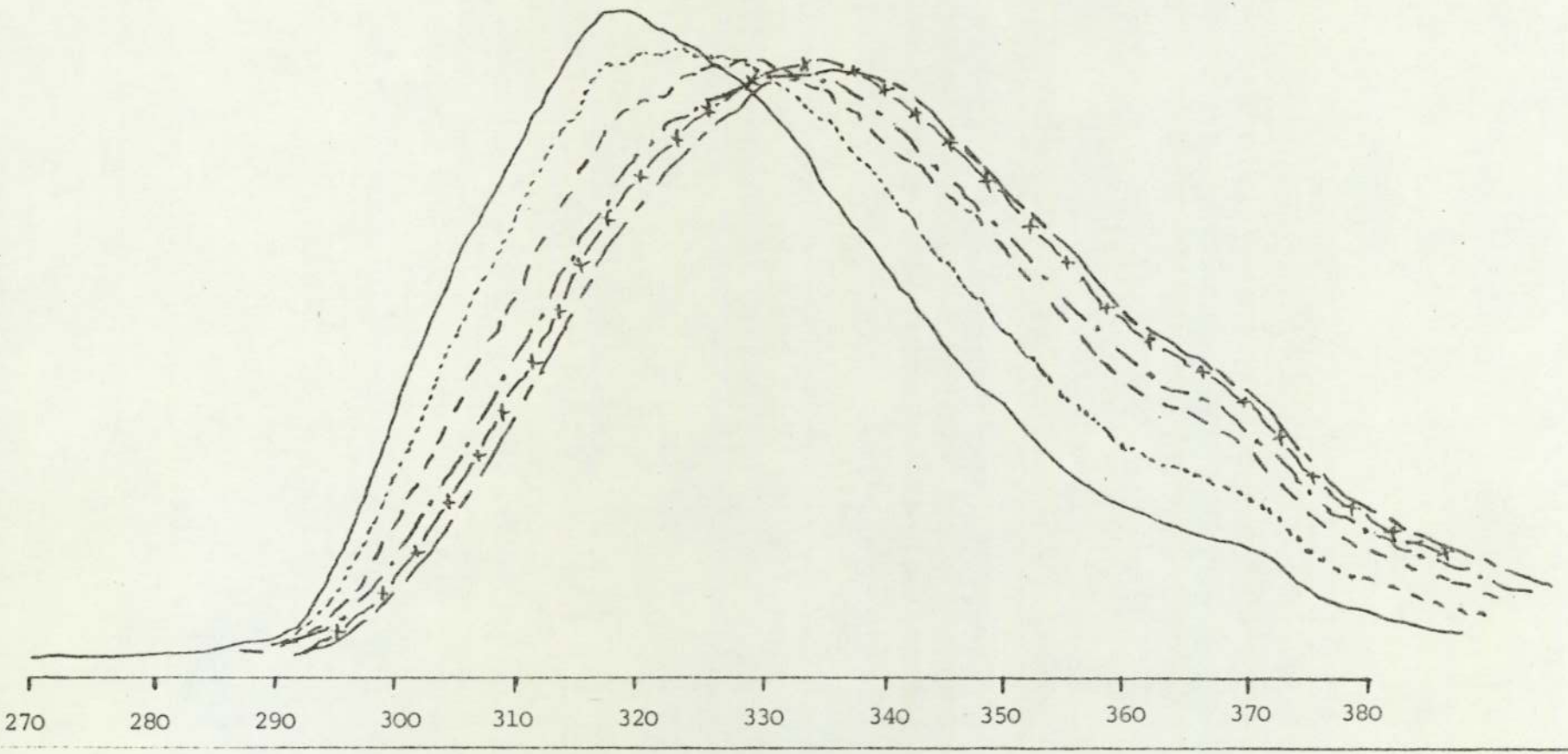



2-Tert-butylindole/triethylamine
270
280
290
300
310
320
330
340
350
360
370
380

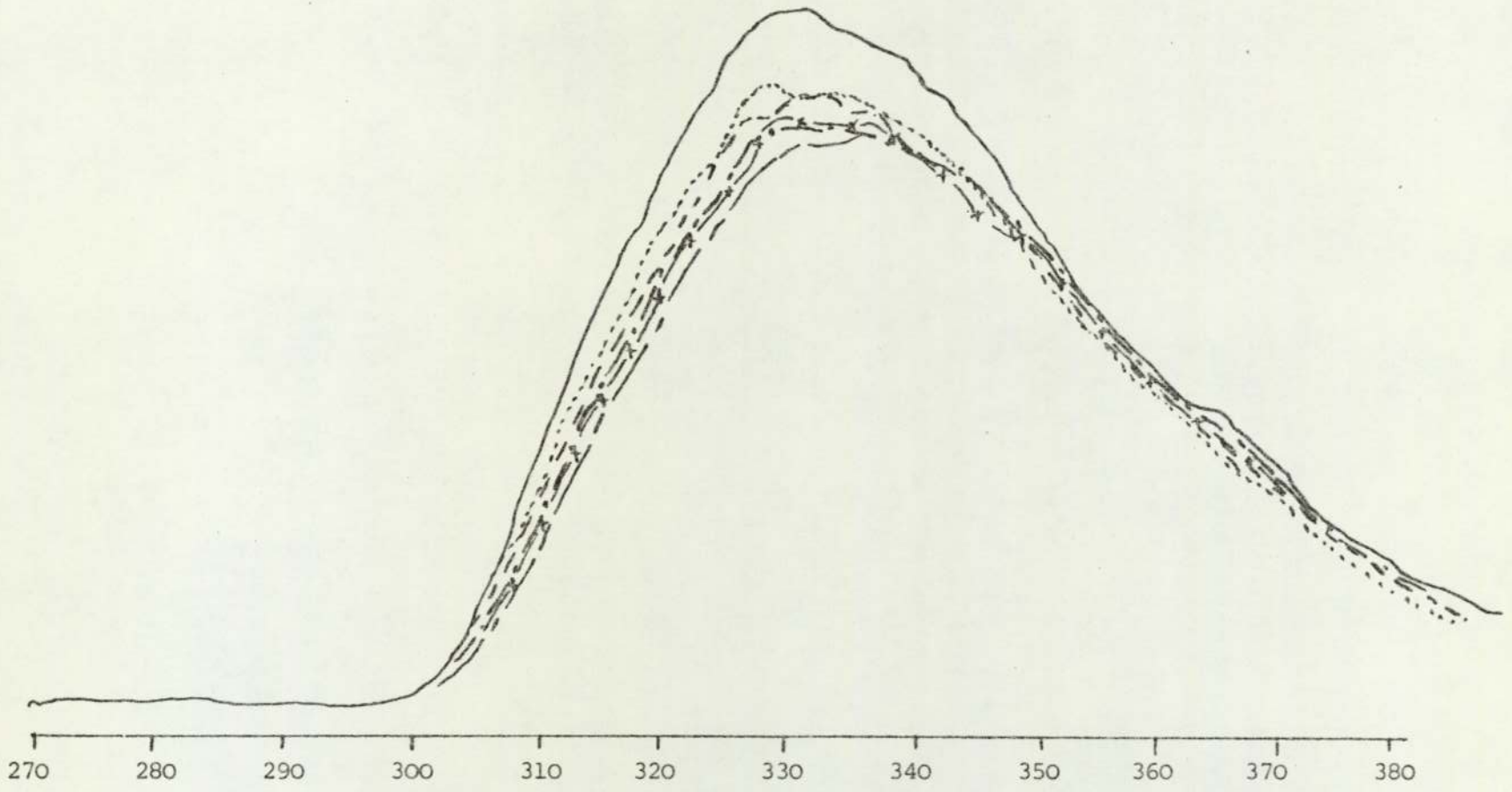

2-Tert-butylindole/tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390

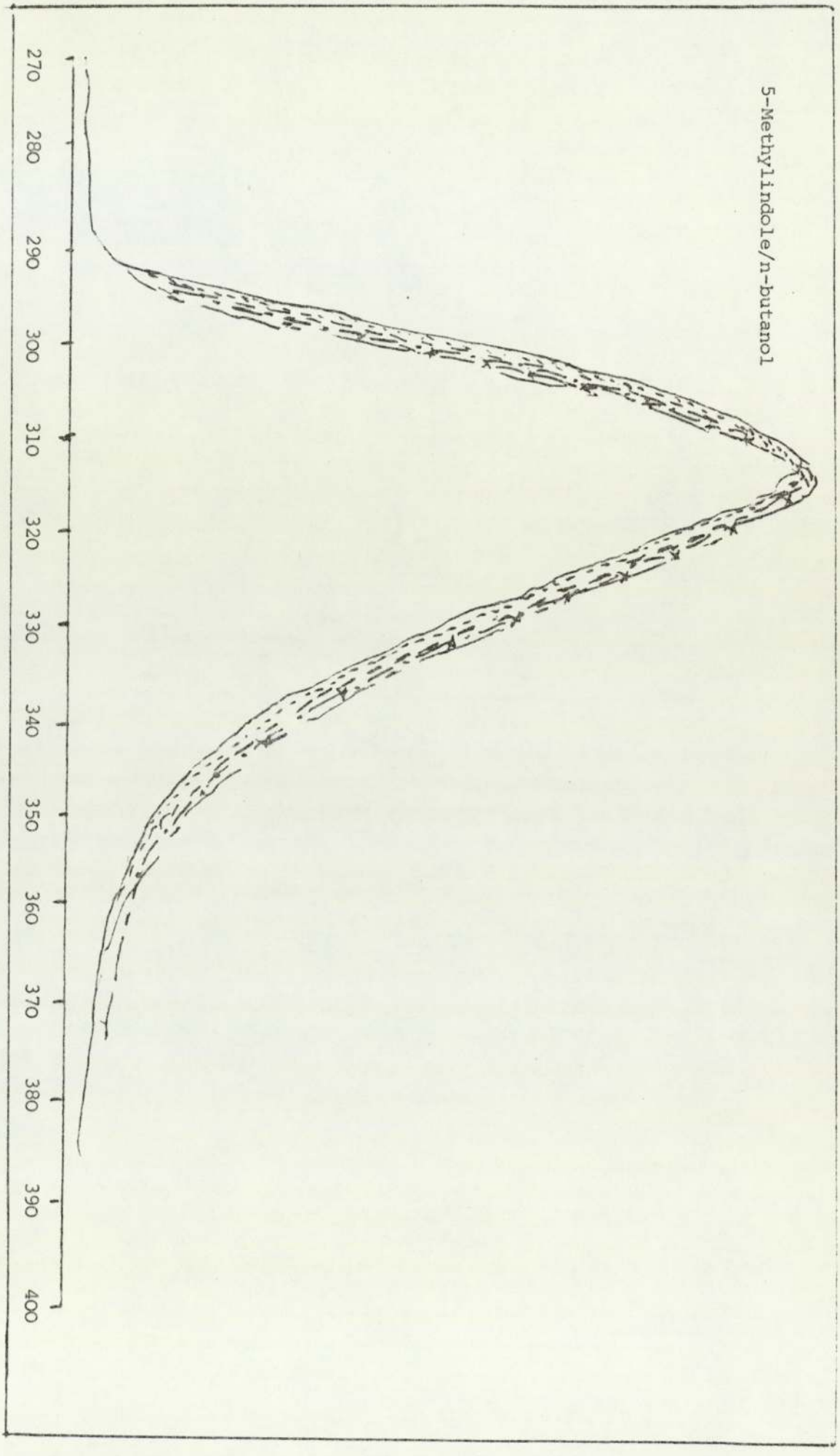

2-Tert-butylindole/acetonitrile
270
280
290
300
310
320
330
340
350
360
370
380
390

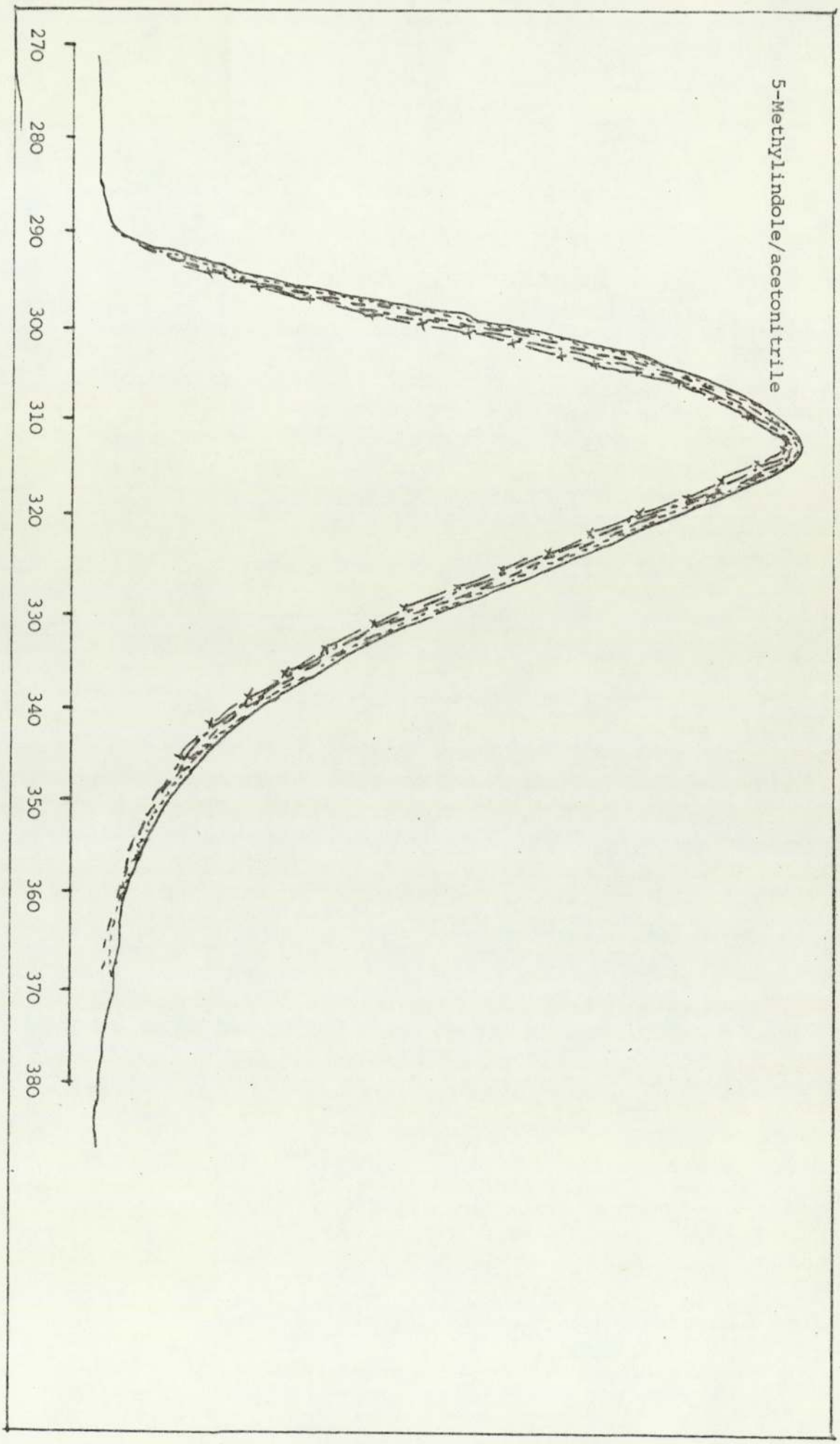
2,3-Cyclodecamethyleneindole/3-ethyl-3-pentanol

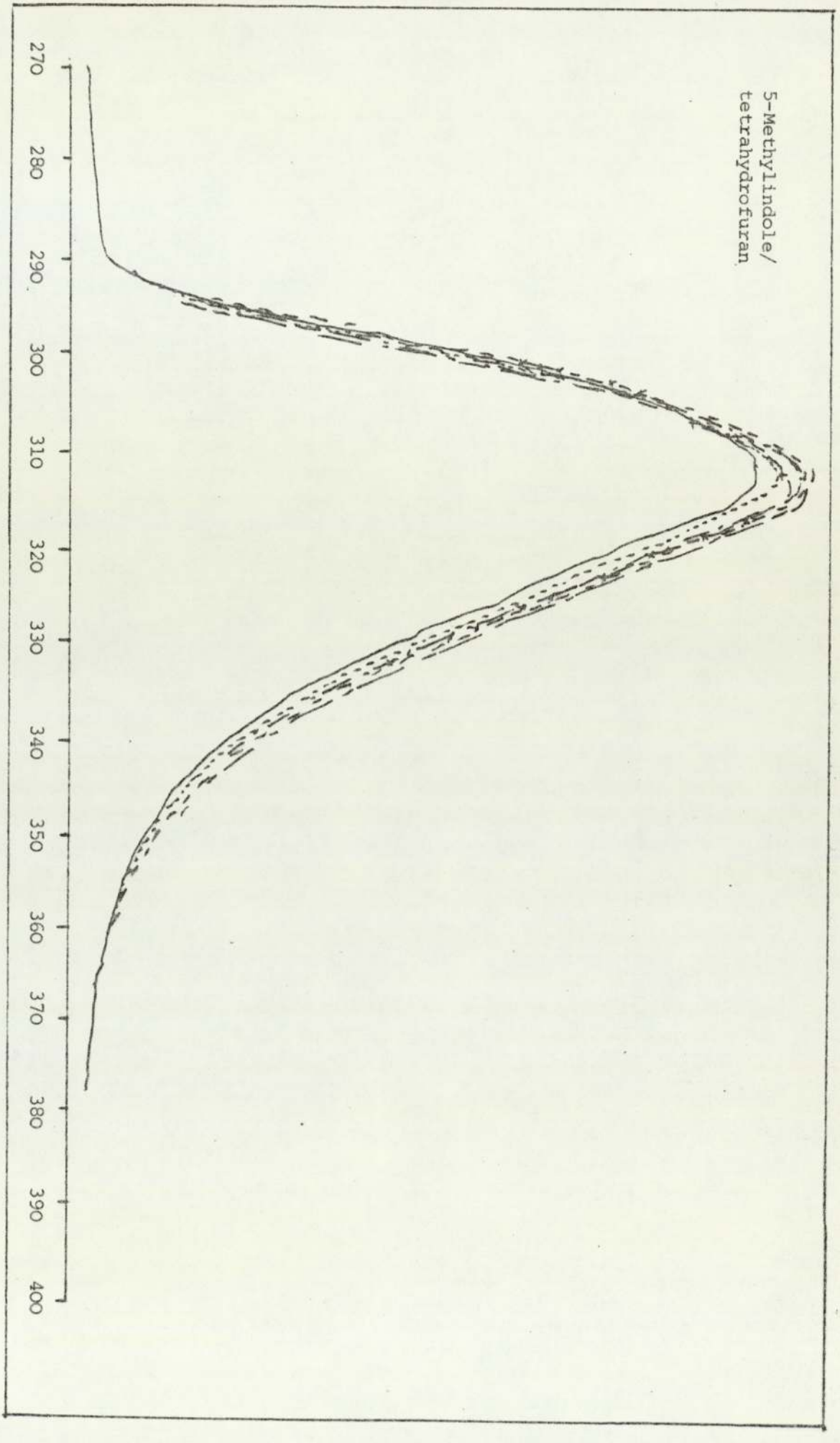


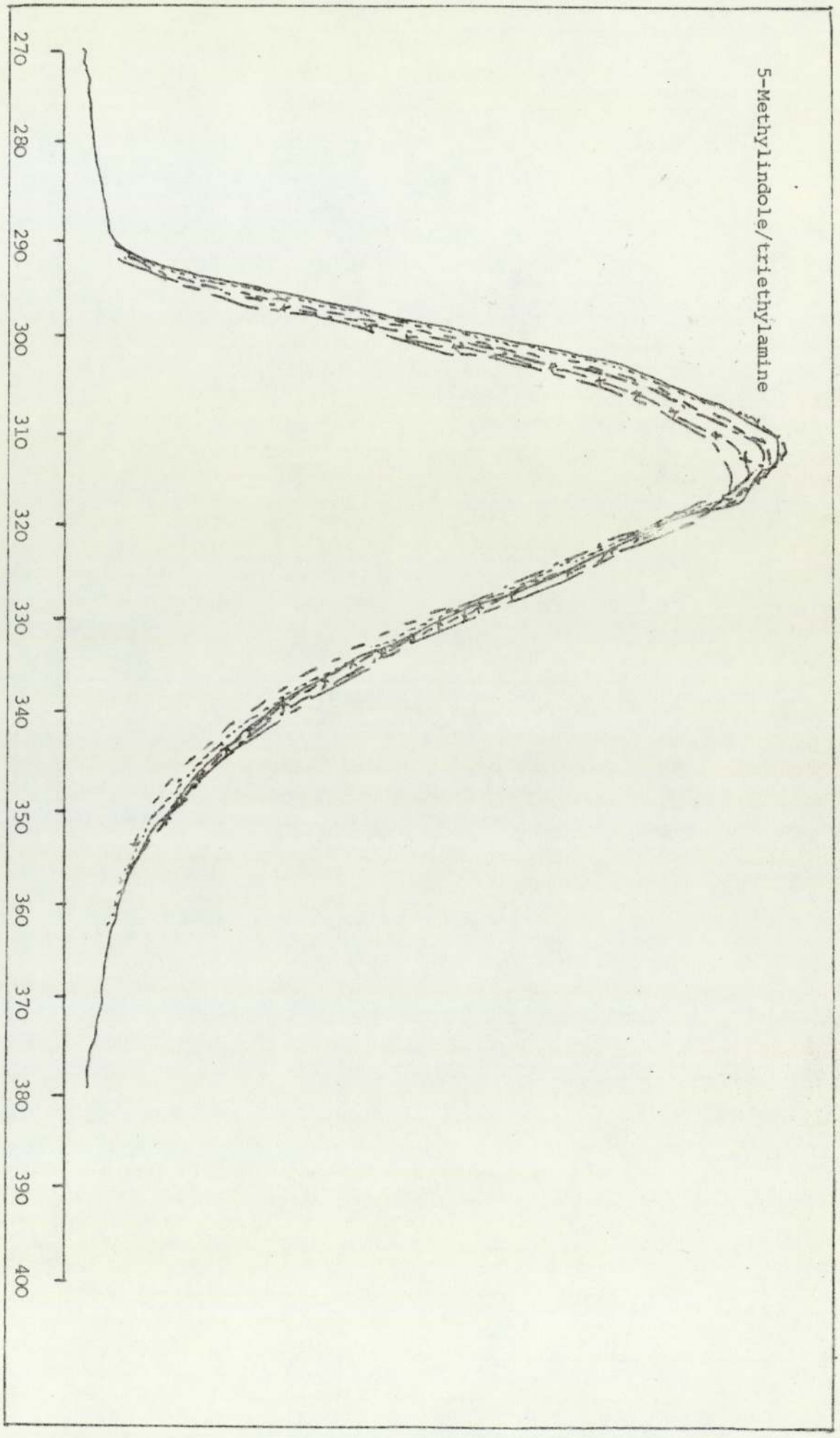
1,2,3-Trimethylindole/3-ethyl-3-pentanol

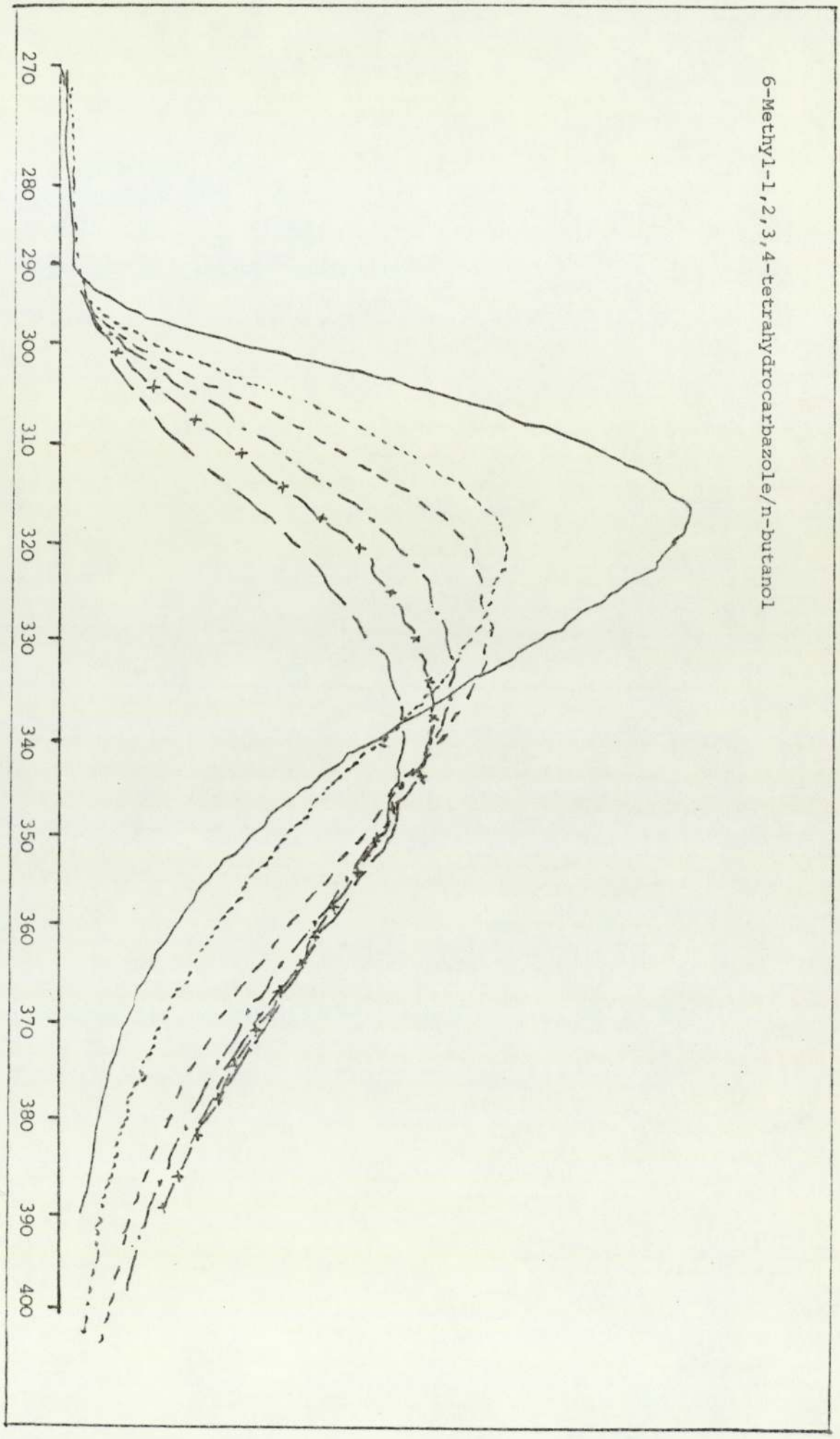


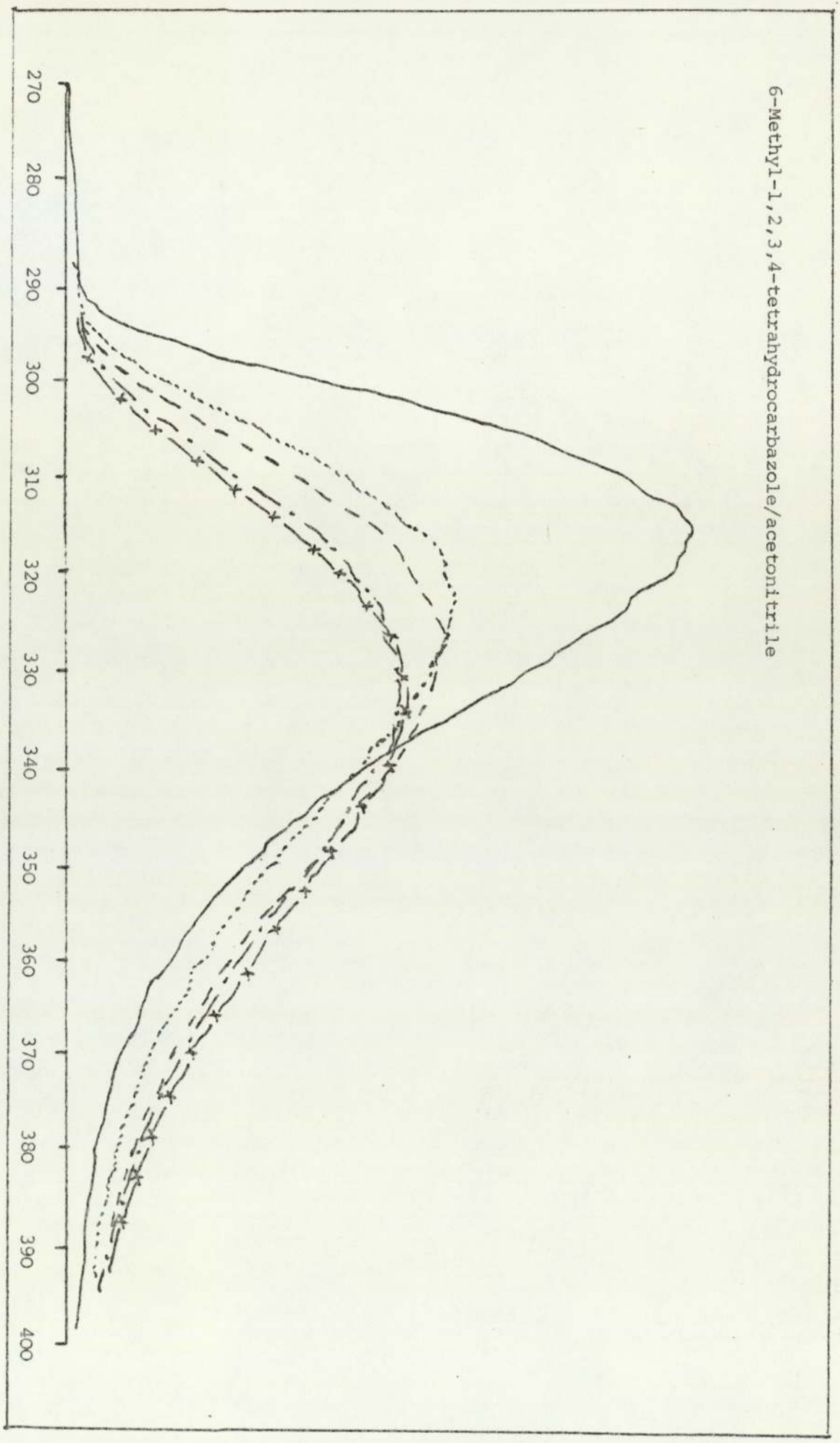

5-Methylindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400


5-Methylindole/acetonitrile
270
280
290
300
310
320
330
340
350
360
370
380

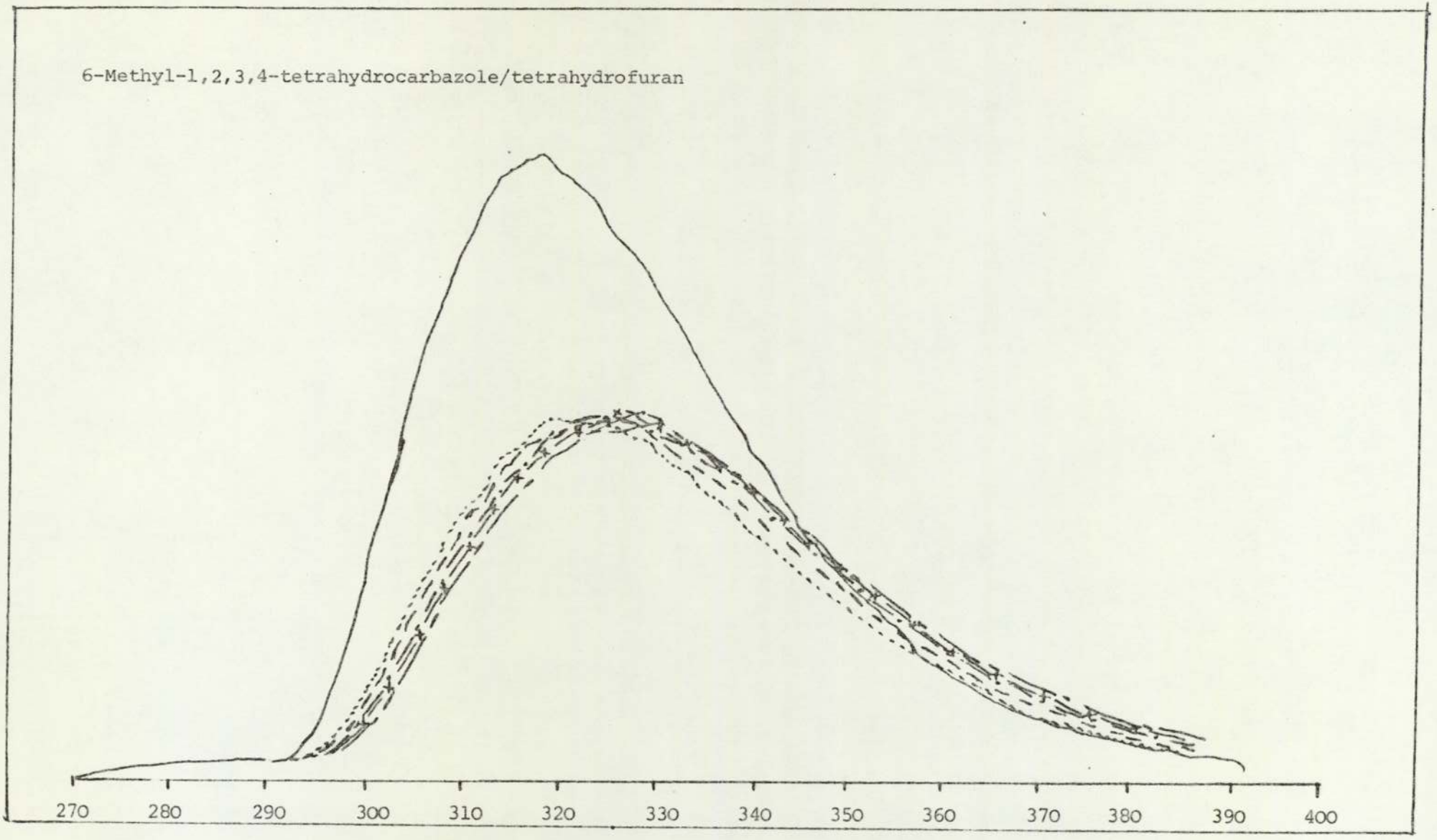

5-Methylindole/
tetrahydrofuran
270
280
290
300
310
320
330
340
350
360
370
380
390
400

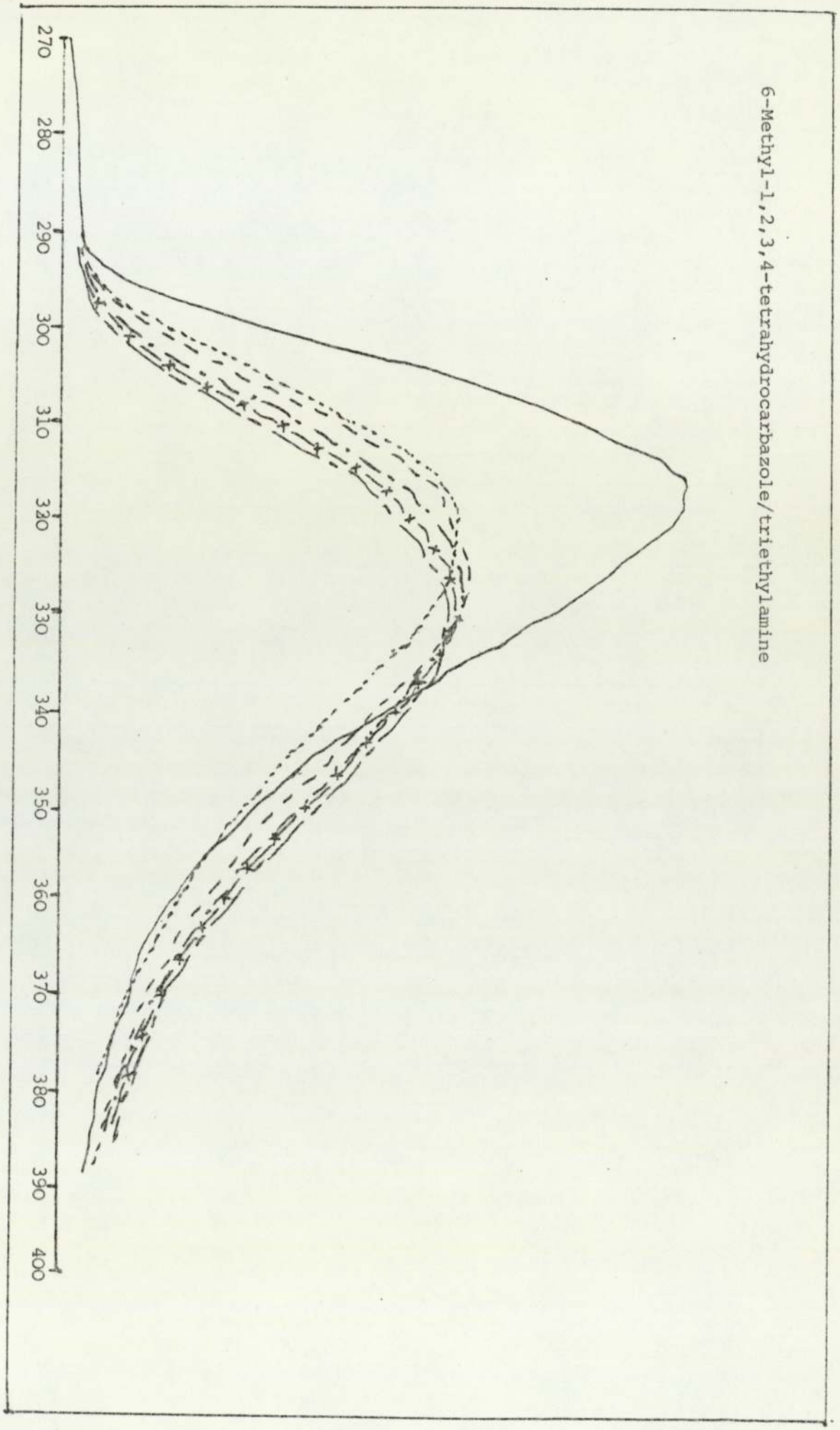

5-Methylindole/triethylamine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

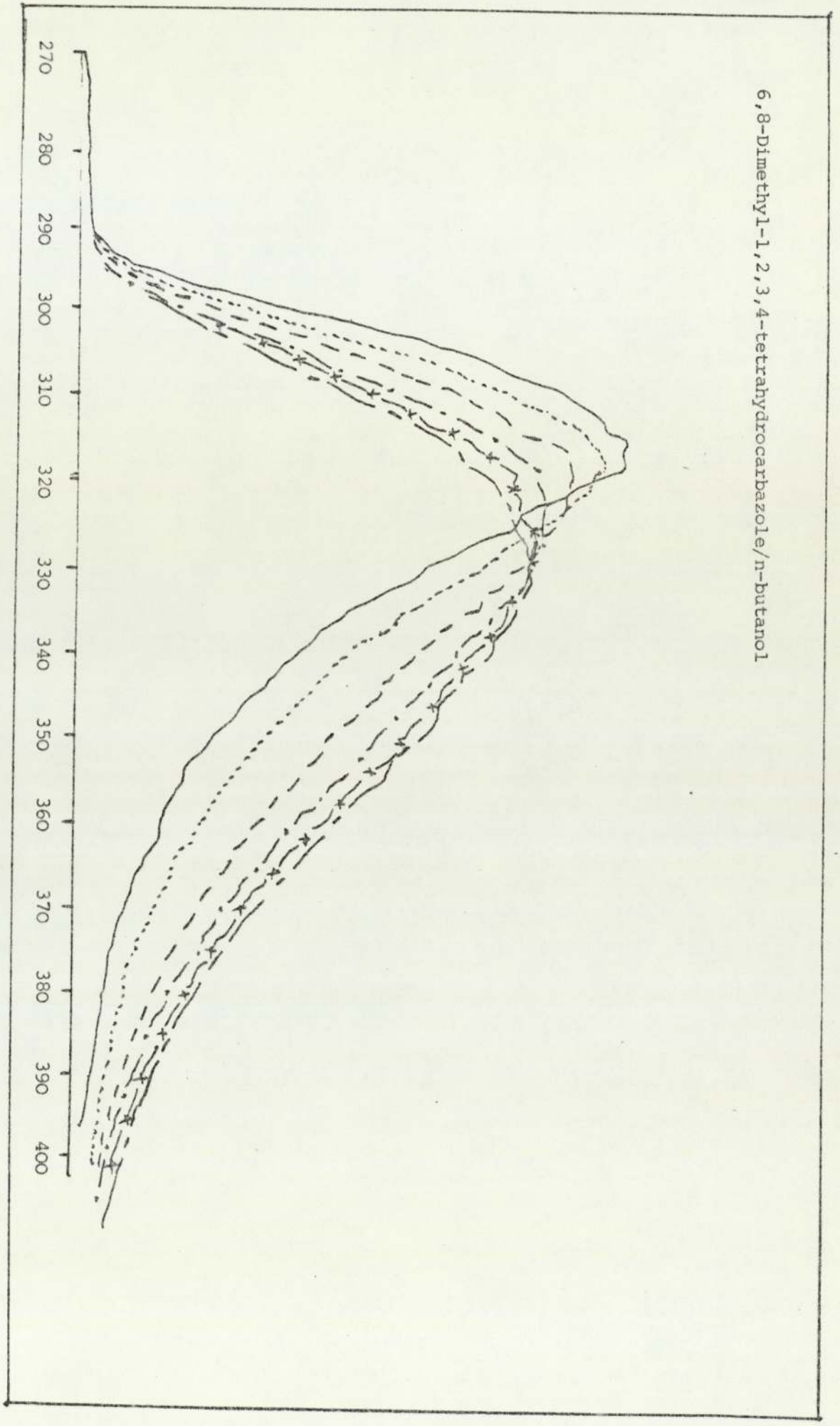

6-Methyl-1,2,3,4-tetrahydrocarbazole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380
390
400


6-Methyl-1,2,3,4-tetrahydrocarbazole/acetonitrile
270
280
290
300
310
320
330
340
350
360
370
380
390
400

6-Methyl-1,2,3,4-tetrahydrocarbazole/tetrahydrofuran

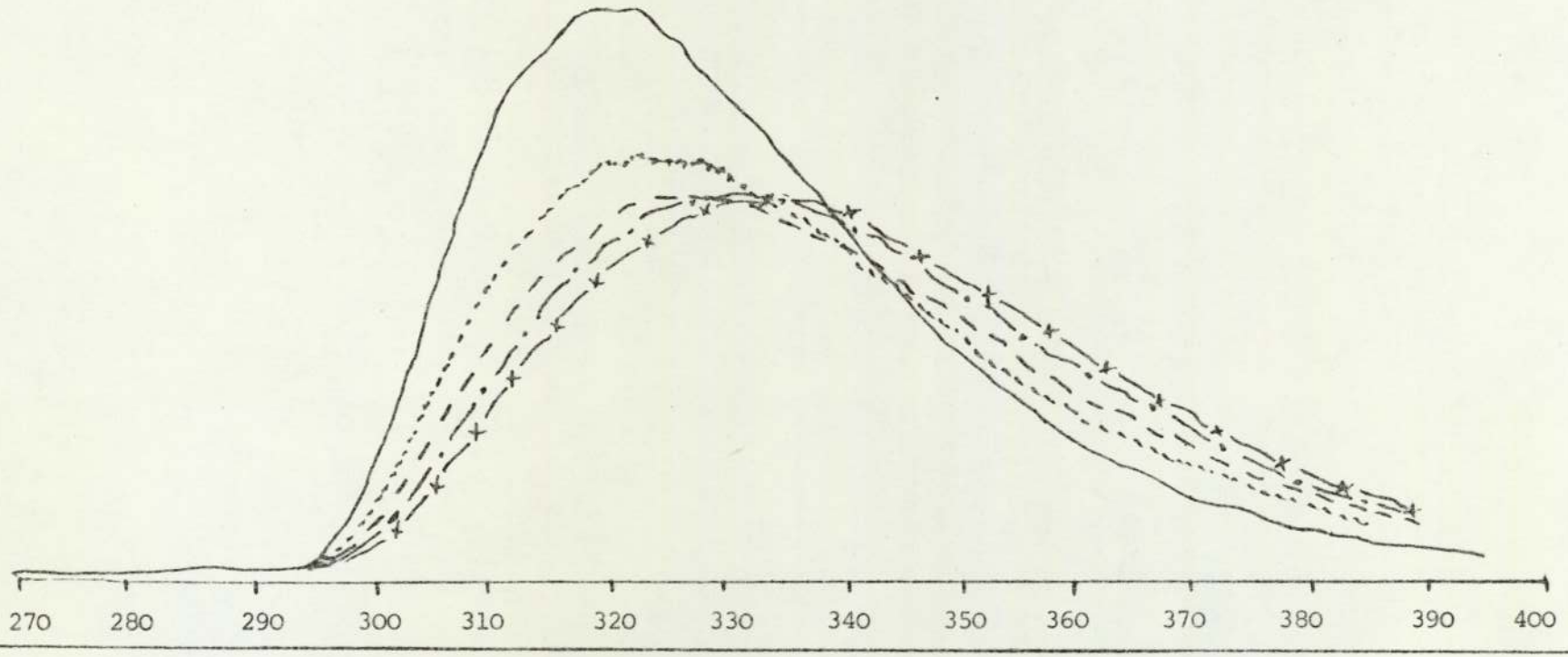



6-Methyl-1,2,3,4-tetrahydrocarbazole/triethylamine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

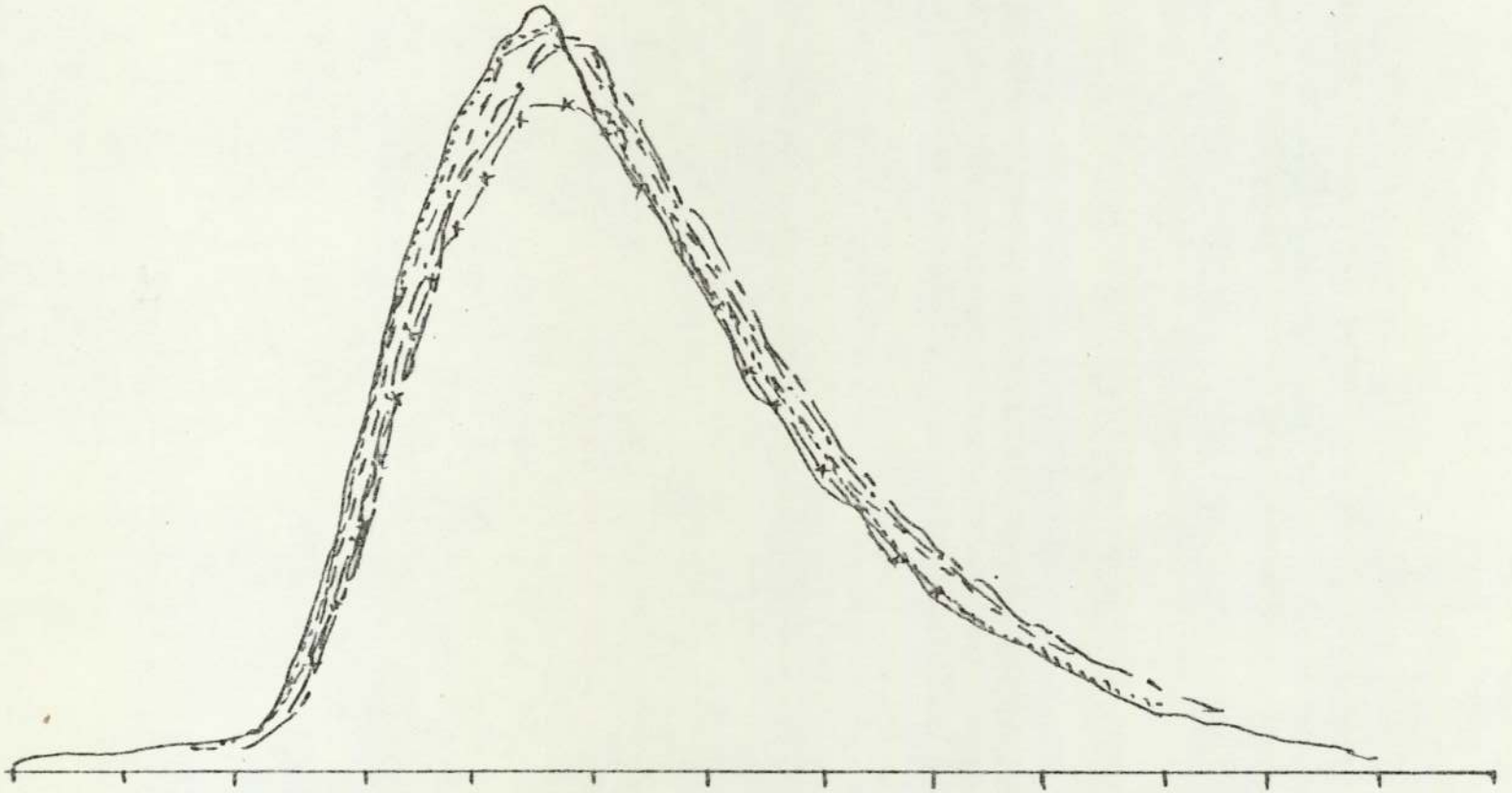


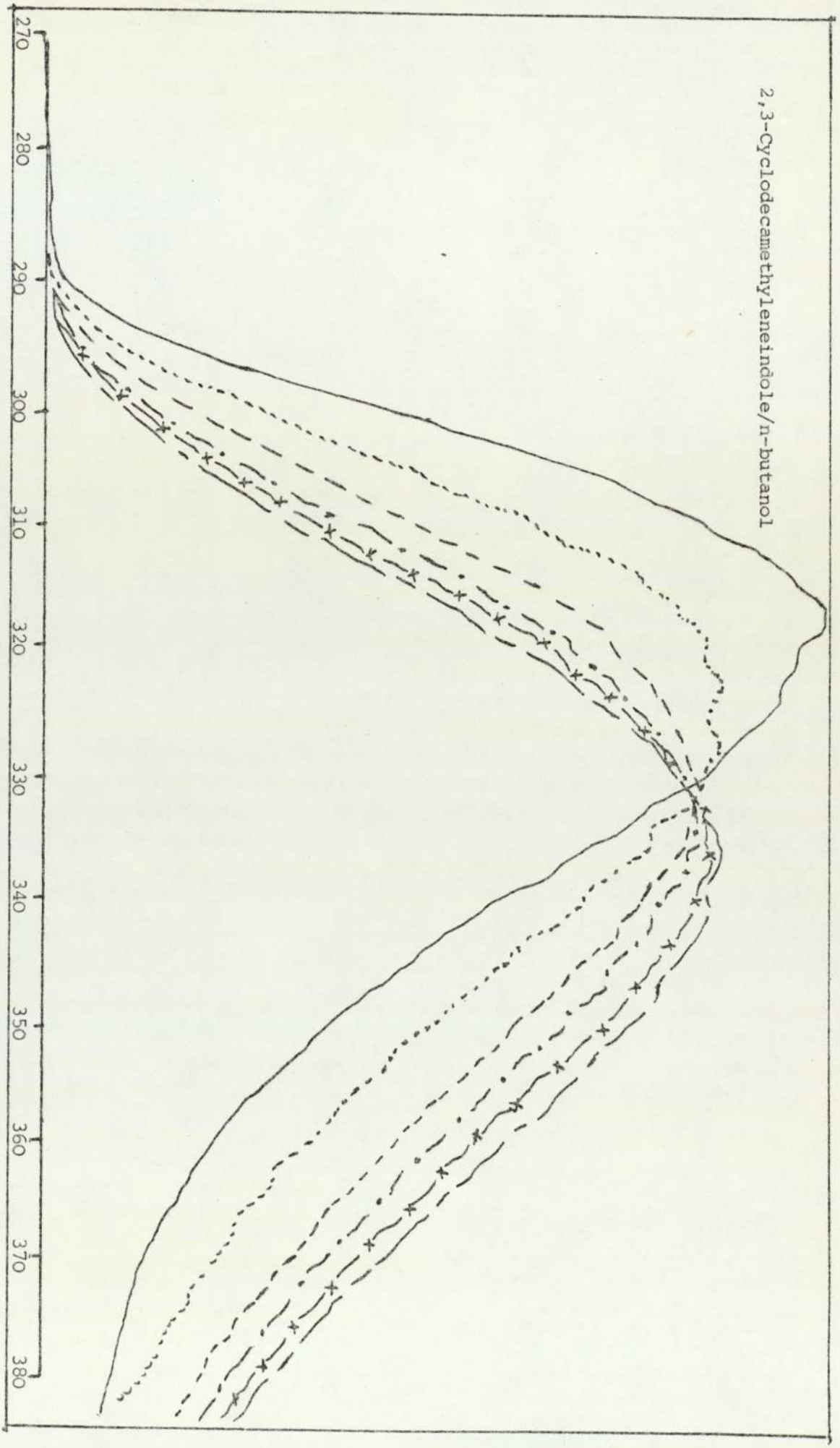
6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole/n-butanol

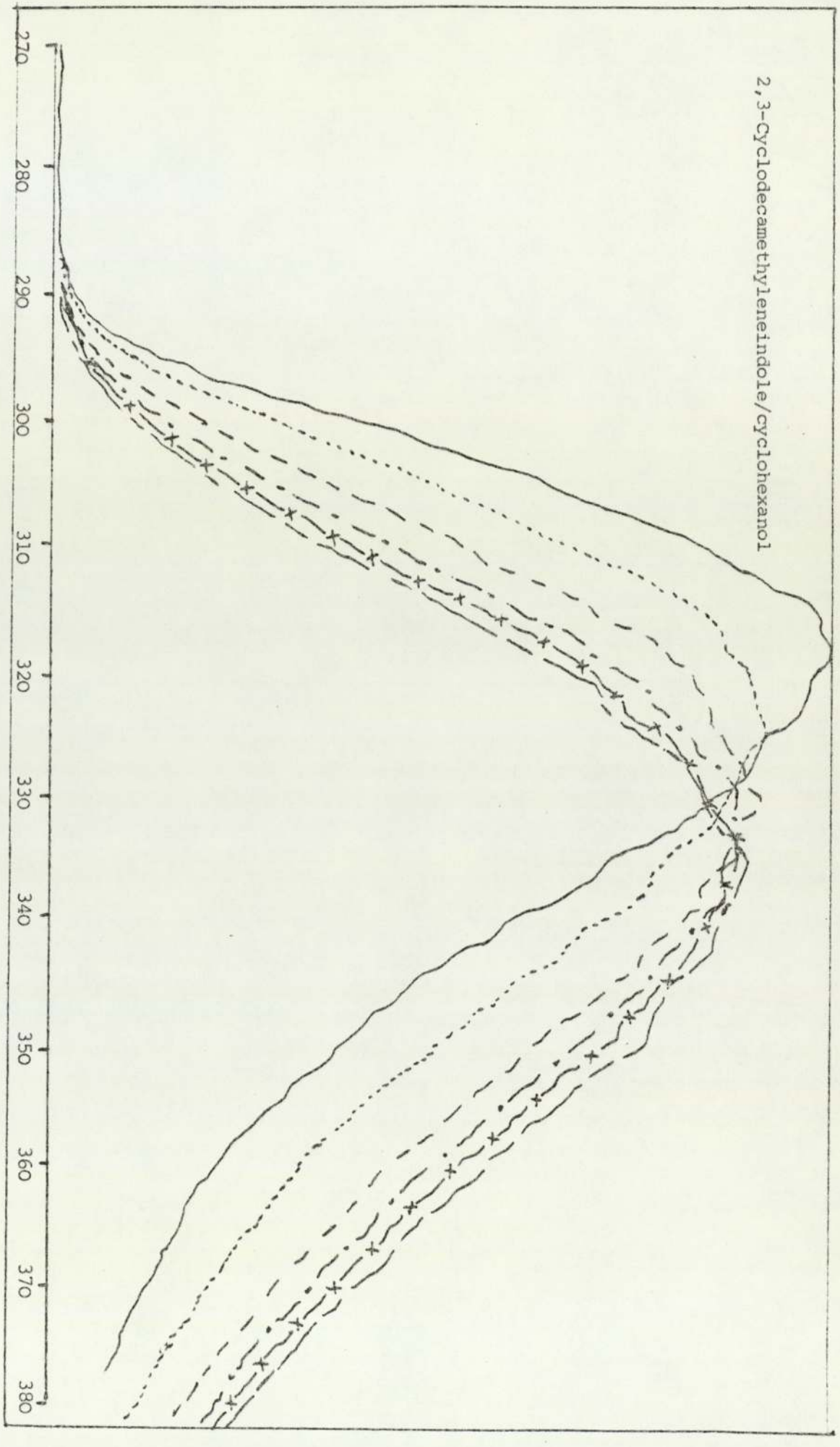
6,7-Dimethyl-1,2,3,4-tetrahydrocarbazole/acetonitrile

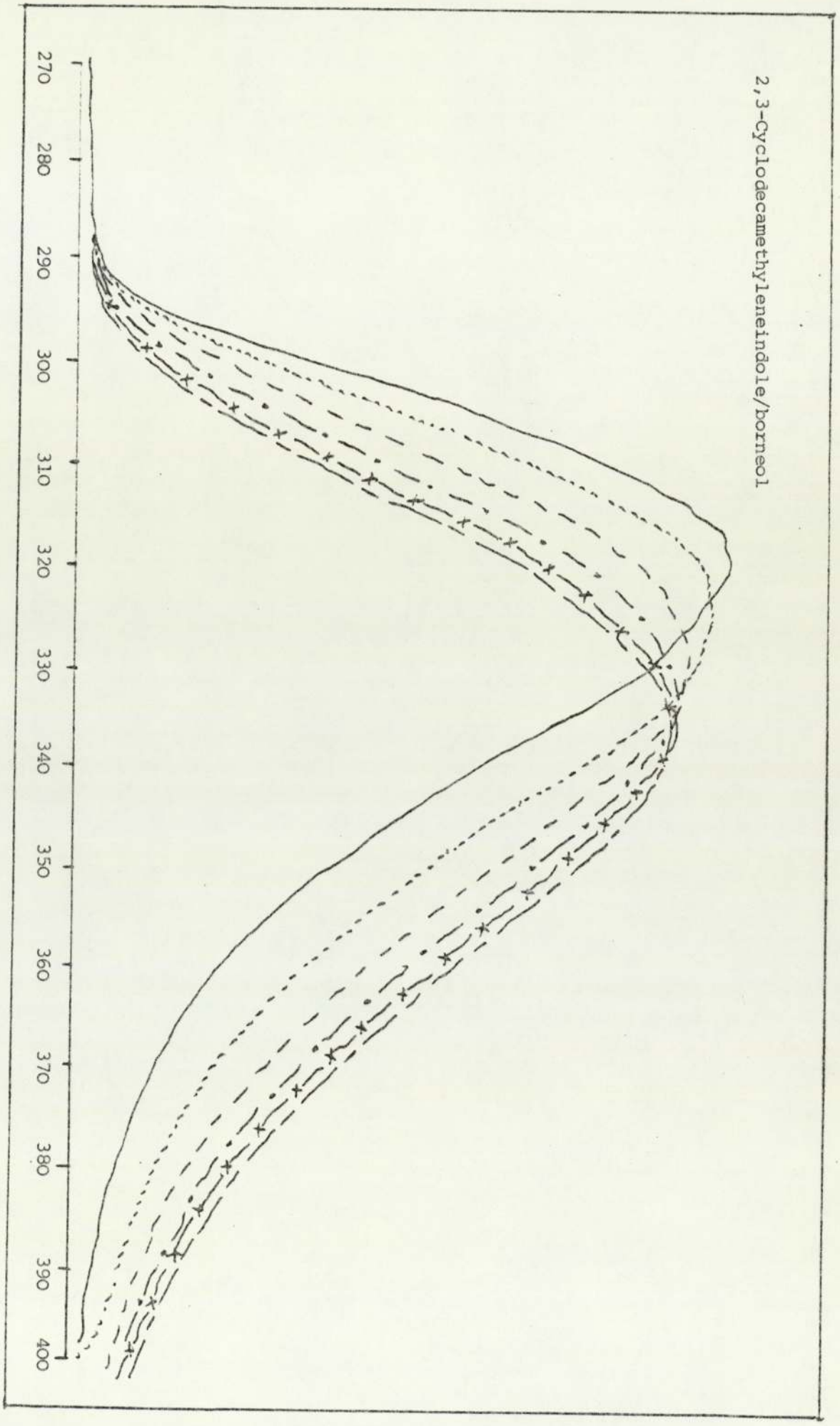


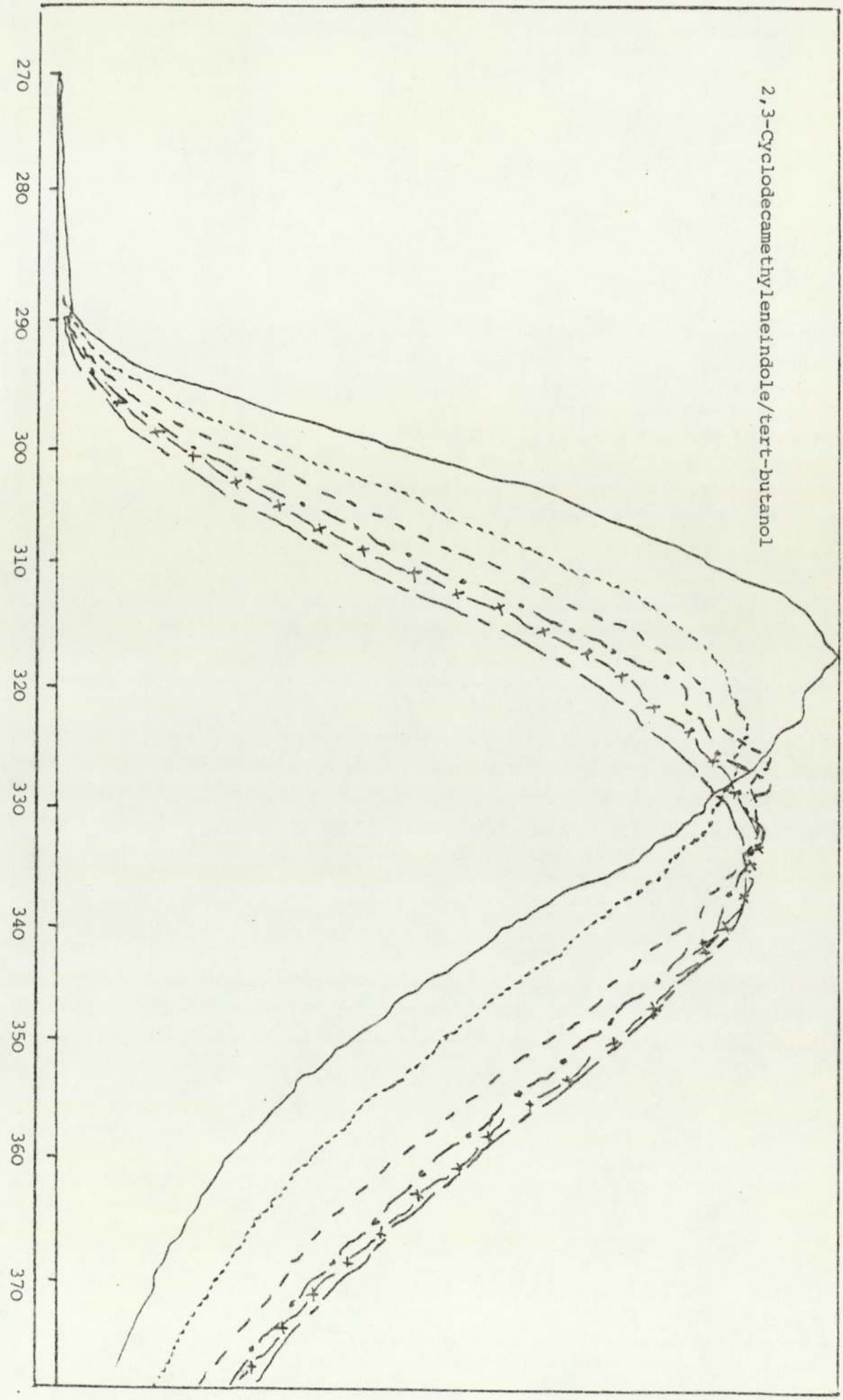
6,8-Dimethyl-1,2,3,4-tetrahydrocarbazole/triethylamine

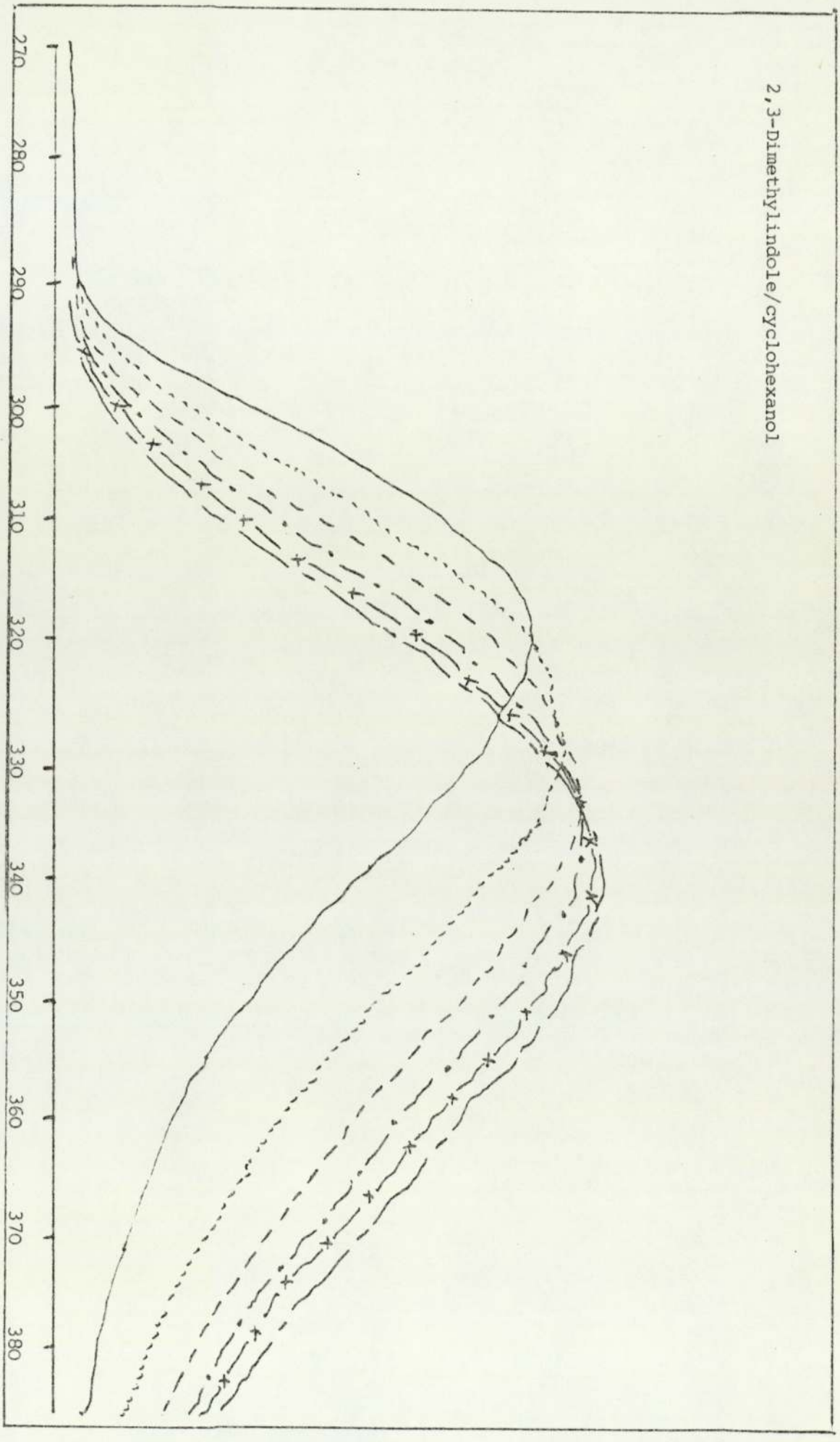


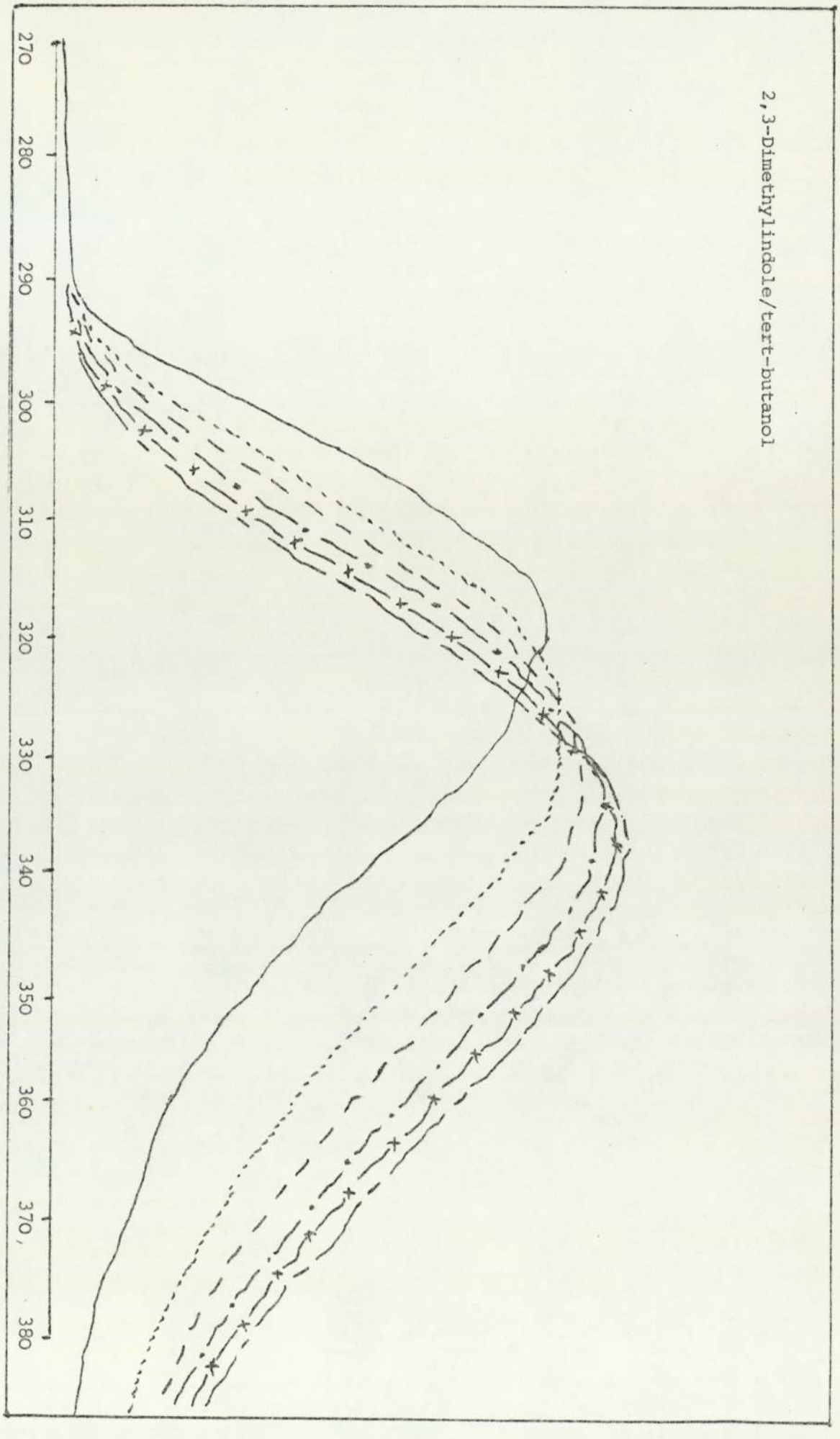

2,3-Cyclodecamethyleneindole/n-butanol
270
280
290
300
310
320
330
340
350
360
370
380

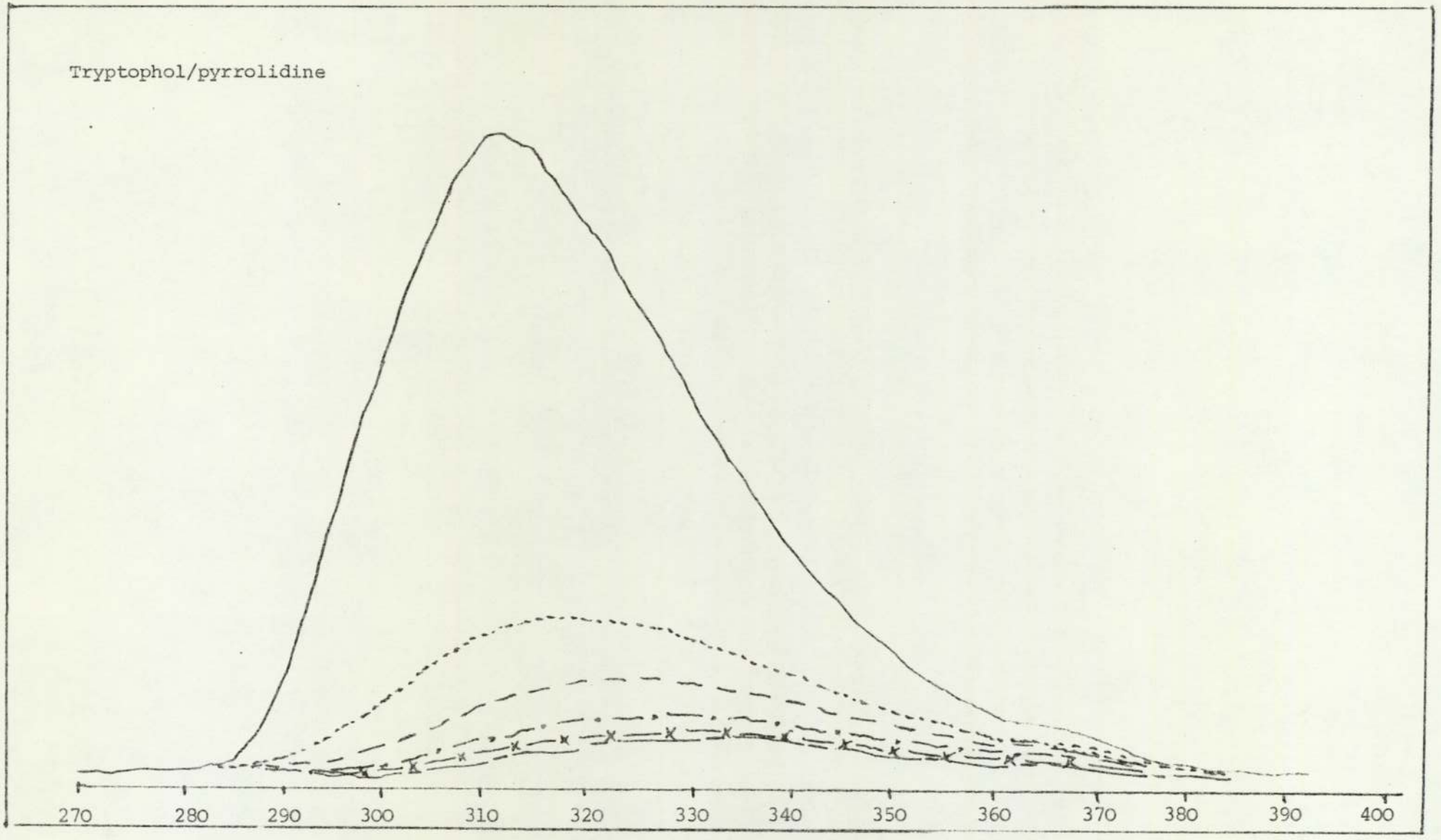

2,3-Cyclodecamethyleneindole/cyclohexanol
270
280
290
300
310
320
330
340
350
360
370
380


2,3-Cyclodecamethyleneindole/borneol
270
280
290
300
310
320
330
340
350
360
370
380
390
400

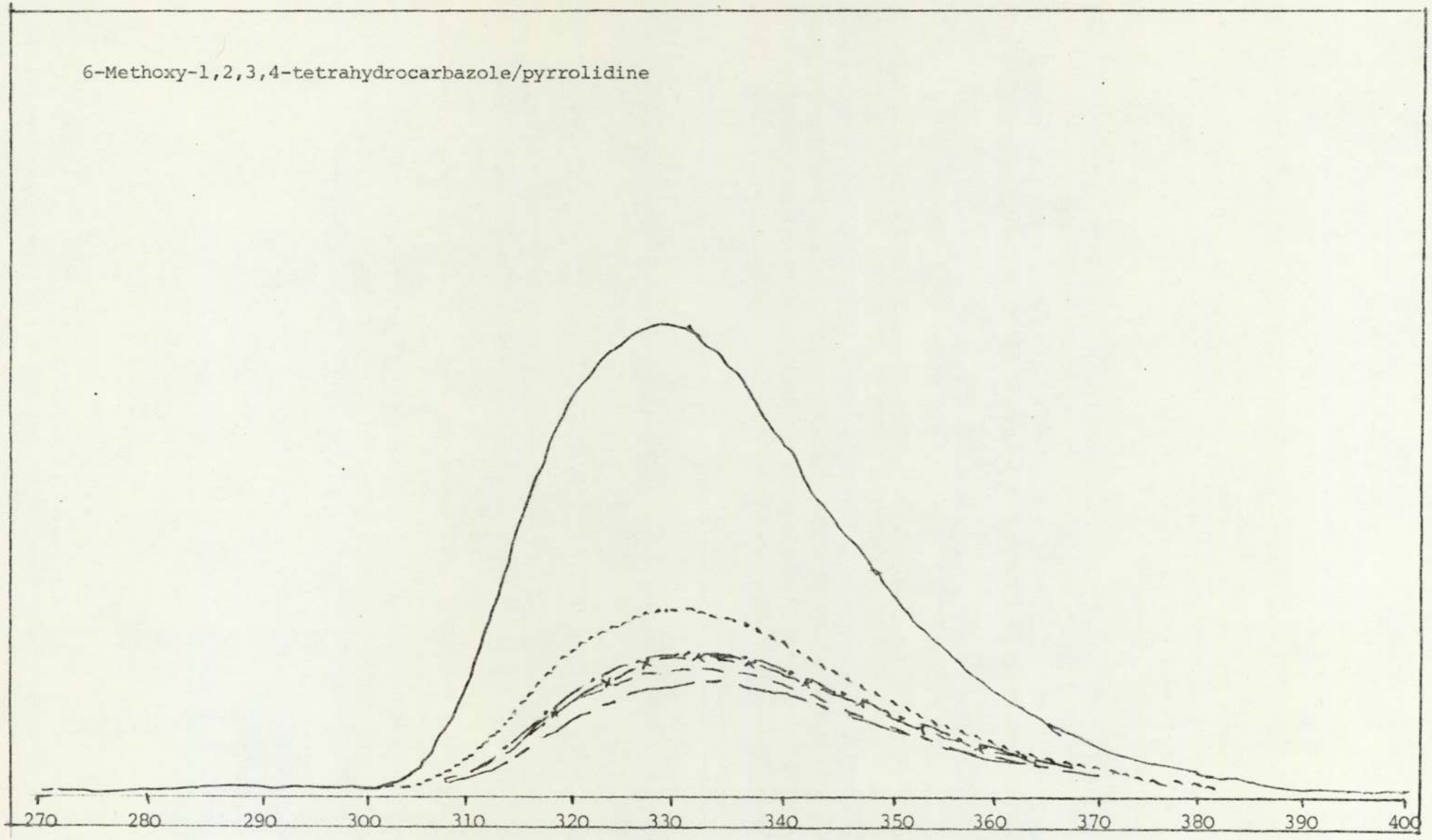

2,3-Cyclodecamethyleneindole/tert-butanol
270
280
290
300
310
320
330
340
350
360
370


2,3-Dimethylindole/cyclohexanol
270
280
290
300
310
320
330
340
350
360
370
380

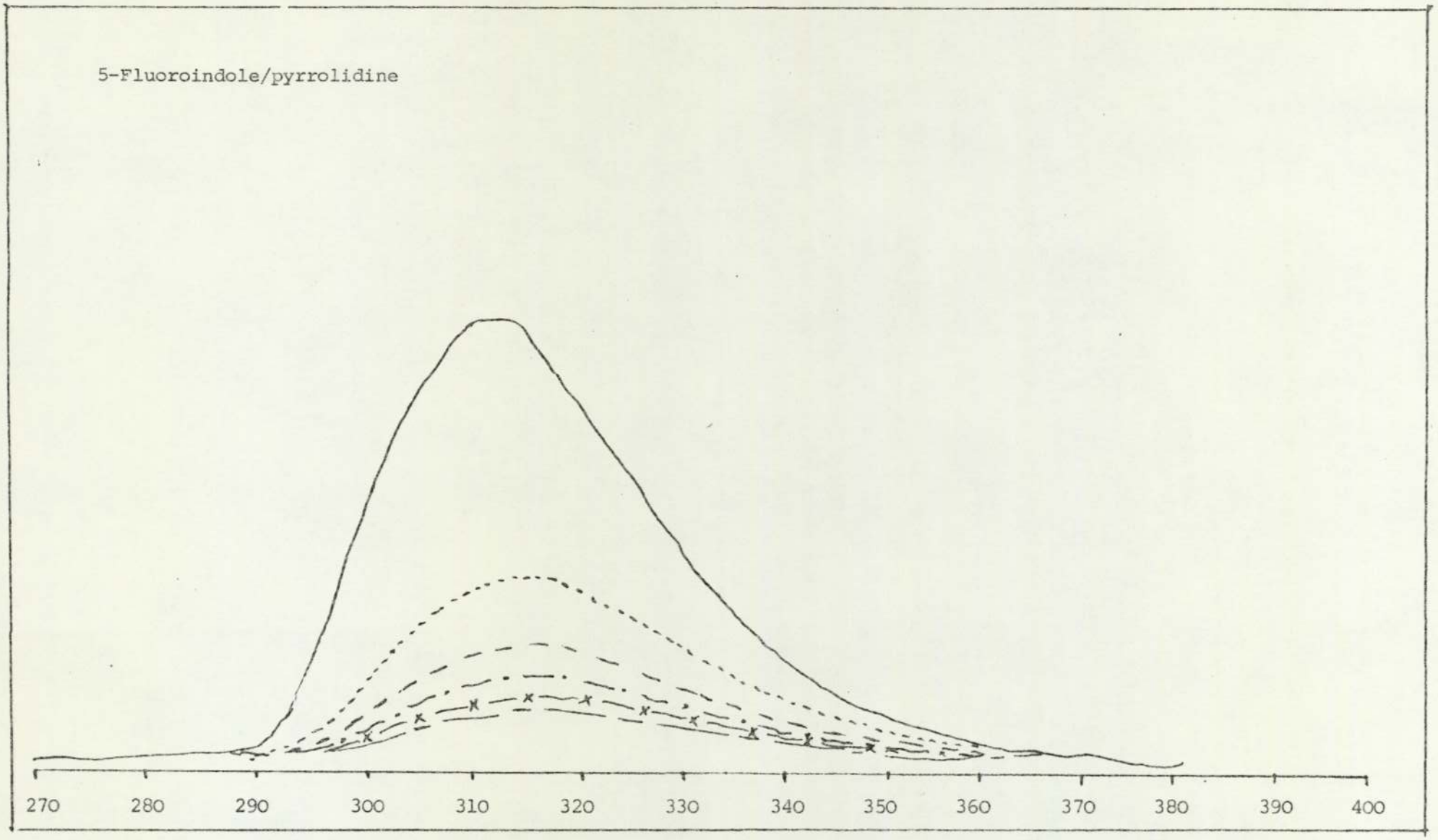

2,3-Dimethylindole/tert-butanol
270
280
290
300
310
320
330
340
350
360
370
380

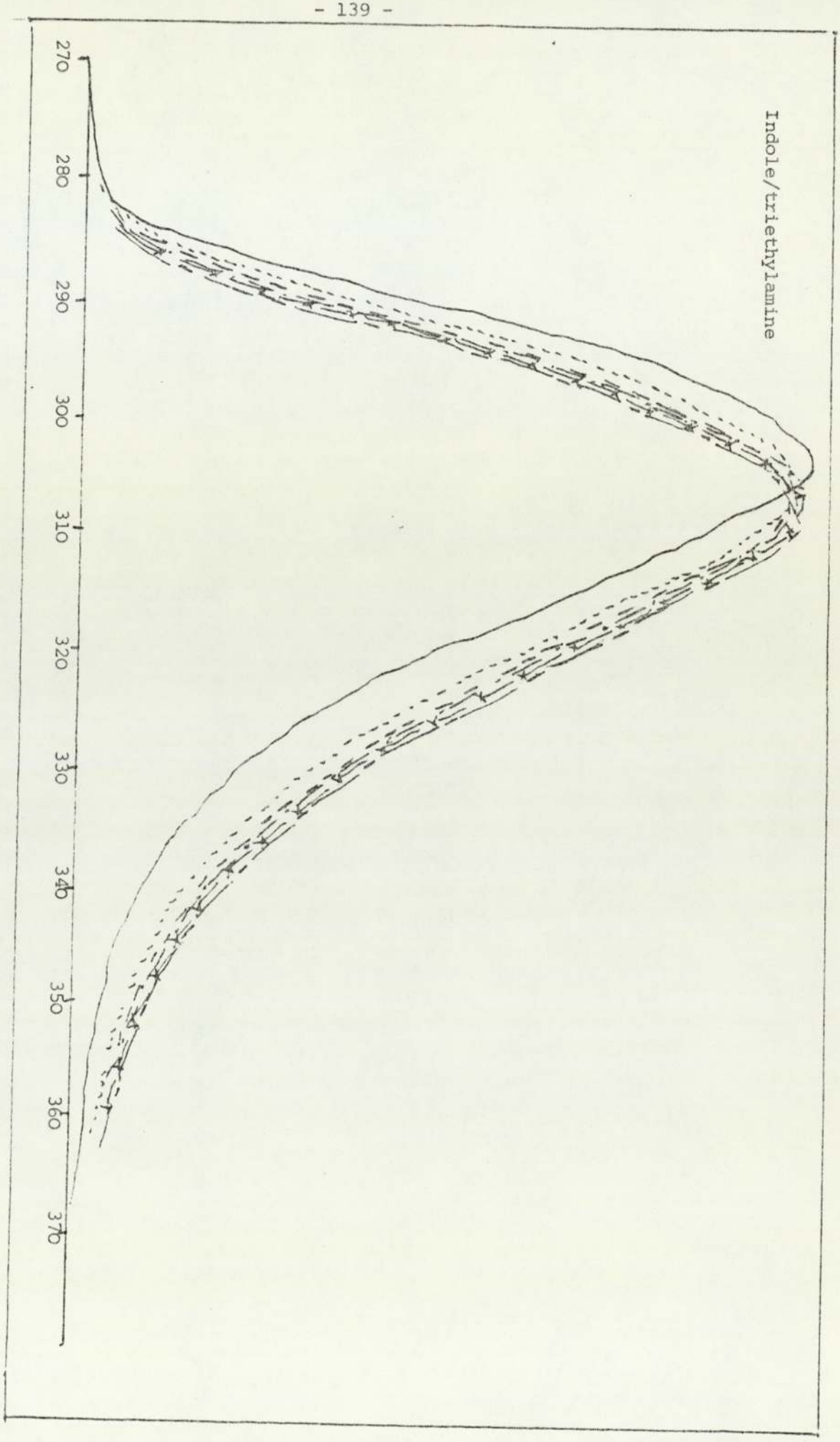

Tryptophol/pyrrolidine
270
280
290
300
310
320
330
340
350
360
370
380
390
400

6-Methoxy-1,2,3,4-tetrahydrocarbazole/pyrrolidine

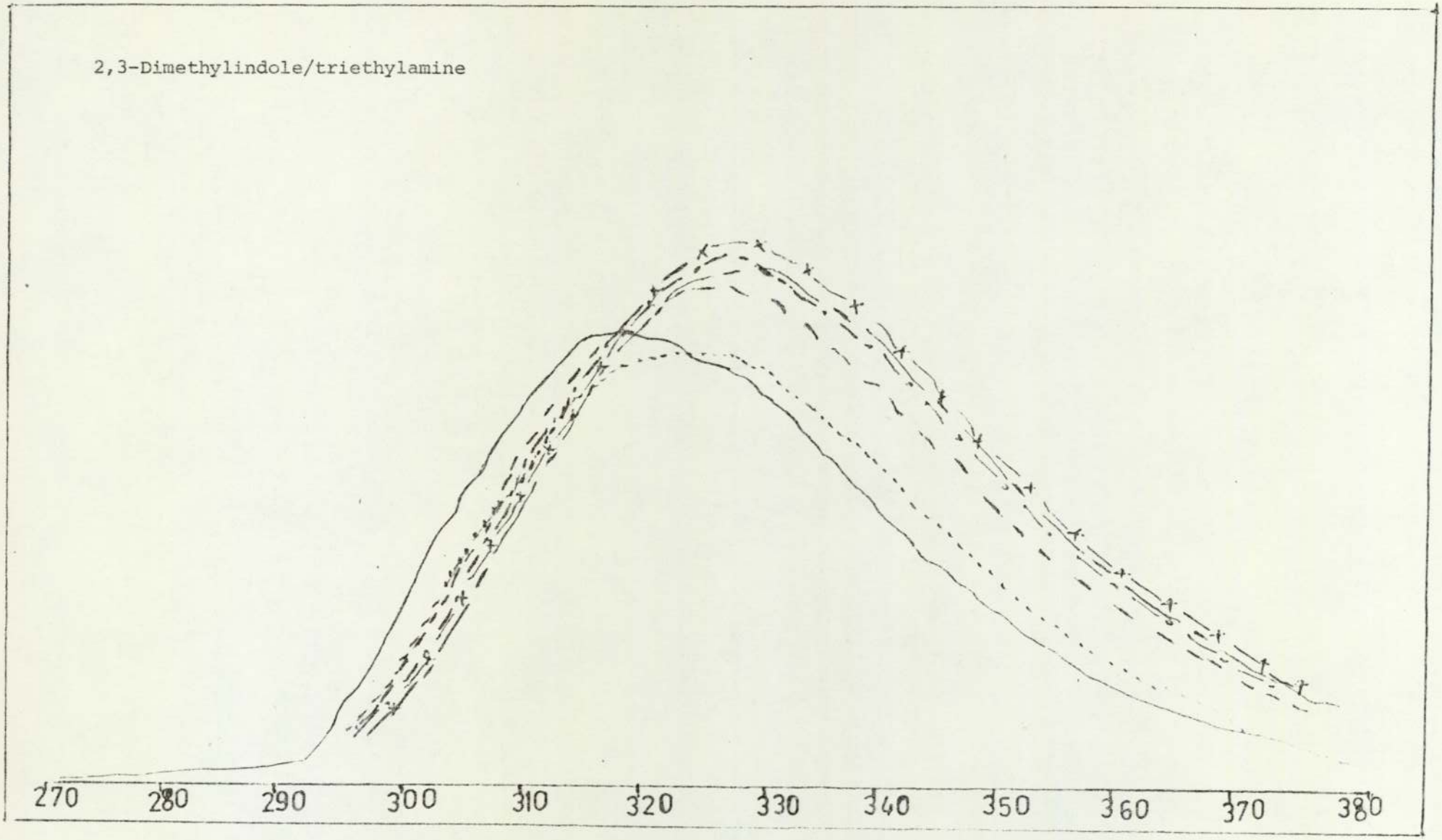


5-Fluoroindole/pyrrolidine




Indole/triethylamine
270
280
290
300
310
320
330
340
350
360
370

2,3-Dimethylindole/triethylamine



BIBLIOGRAPHY

1. P. Pringsheim, "Fluorescence and Phosphorescence", Interscience, London (1965).

2. D.M. Hercules, "Fluorescence and Phosphorescence Analysis", Interscience, London (1966).

3. S. Udenfriend, "Fluorescence Assay in Biology and Medicin", vol.I, Academic Press, London (1962).

4. G.N. Lewis and M. Kasha, J. Am. Chem. Soc., 66, 2100 (1944).

5. M. Kasha, Radiation Res. Suppl., 2, 243 (1960).

6. C.A. Parker and C.G. Hatchard, J. Phys. Chem., 66, 2506, (1962).

7. E.J. Bowen and F. Wokes, "Fluorescence of Solutions", Longmans, Green, London, 1953.

8. S.P. McGlynn, J. Daigre and F.J. Smith, J. Chem. Phys., 39, 675 (1963).

9. L.V.S. Hood and J.D. Wineforder, Anal. Chem., 38, 1922 (1966).

10. J. Nag-Chandhuri, L. Stoessell, and S.P. McGlynn, J. Chem. Phys., 38, 2027 (1963).

11. E.L. Wehry, "Fluorescence", edited by G.G. Guilbault, Edward Arnold Ltd., (London) (1967).

12. C.A. Winter, E.A. Risley and G.W. Nuss, J. Pharmacol. exp. Ther., 141, 369 (1963).

13. A.N. Terenin and V.L. Ermolaev, Trans. Faraday Soc., 52, 1042 (1956).

14. N.J. Turro, "Molecular Photochemistry", W.A. Benjamin, New York (1967).

15. J.B. Birks, "Organic Molecular Photophysics", Wiley, London (1970).

16. M.S. Walker, T.W. Bednar and R. Lumry, J. Chem. Phys., 47, 1020 (1967).

17. R.T. Williams, J. Roy. Inst. Chem., 83, 611 (1959).

18. S. Udenfriend, D.F. Bogdanski and H. Weissback, Science, 122, 972 (1955).

19. M.S. Walker, T.W. Bednar and R. Lumry, Photochem. Photobiol., 14, 147 (1971).

20. R.W. Ricci, Photochem. Photobiol., 12, 67 (1970).

21. M.S. Walker, T.W. Bednar and R. Lumry, "Molecular Luminescence",edited

by E.C. Lim, W.A. Benjamin, Nww York (1969).

22. T.R. Hopkins and R. Lumry, Photochem. Photobiol., 15, 555 (1972).

23. F.W.J. Teale, Biochem. J., 76, 381 (1960).

24. B.L. Van Duuren, Chem. Rev., 63, 325 (1963).

25. W.J. Houlihan, "Indoles" part 1, Wiley London (1972).

26. R.J. Sundberg, "The Chemistry of Indoles", vol. 18, Academic Press., London (1970).

27. J.W. Longworth, "Excited states of proteins and nucleic acids" edited by I. Weinrub and R.F. Steiner, MacMillan, London (1971).

28. J.W. Bridges and R.T. Williams, Biochem. J., 107, 225 (1968).

29. R.W. Cowgill, Biochem. Biophys. Acta, 200, 18 (1970).

30. E.H. Strickland, C. Billups and E. Kay, Bioch.,11, 3657 (1972).

31. L.J. Andrews and L.S. Forster, Photochem. Photobiol., 19, 353 (1974).

32. B.K. Selinger and R.J. McDonald, Aust. J. Chem., 25, 897 (1972).

33. J. Eisnger and G. Navon, J. Chem. Phys. 50, 2069 (1969).

34. R.W. Ricci, J. Chem. Education, 51, 692 (1974).

35. B.L. Van Duuren, J. Org. Chem., 26, 2954 (1961).

36. S.V. Konev, "Fluorescence and Phosphorescence of Proteins and Nucleic acids", Plenum Press, New York (1967).

37. P.S. Song and W.E. Kurtin, J. Amer. Chem. Soc., 91, 4892 (1969).

38. M.S. Walker, T.W. Bednar and R. Lumry, J. Chem. Phys. 45, 3455, (1966).

39. A. Gillam and E.S. Stern "Electronic Absorption Spectroscopy", E.Arnold Ltd., London (1950).

40. E.G. McRae, J. Phys. Chem., 61, 562 (1957).

41. N. Mataga and S. Tsuno, Bull. Chem. Soc. Japan, 30, 368 (1957).

42. N. Mataga, Y. Torihashi and K. Ezumi, Theoret. Chem. Acta. (Berl.) 2, 158 (1964).

43. N. Mataga, Y. Kaifu and M. Koizumi, Bull. Chem. Soc. Japan, 28, 690 (1955).

44. S. Sambursky and G. Wolfsohn. Trans. Far. Soc. 36, 427 (1940).

45. W.B. DeLauder and P H. Wahl, Biochem. Biophys. Acta, 243, 153 (1971).

46. N. Mataga, Y. Kaifu and M. Koizumi, B ull. Chem. Soc. Japan, 29, 465 (1956).

47. J.B. Birks, "Photophysics of Aromatic Molecules", Wiley, London (1970).

48. M. Ottolenghi, Accounts Chem. Res., 6, 153 (1973).

49. R. Lumry, S. Yanari, and F.A. Bovey, Amer. Chem. Soc. Div. Org. Coating Plastics Chem. Preprints 142, 18R (1962).

50. D. Creed and R.A. Caldwell, J. Amer. Chem. Soc., 96, 7369 (1974).

51. N.C. Yang and J. Libman, J. Amer. Chem. Soc., 94, 1405 (1972).

52. E.A. Chandross, "The Exciplex", edited by M. Gordon and W.R. Ware, Academic Press. London (1975).

53. E.A. Chandross and H.T. Thomas, Chem. Phys. Letters, 9, 397 (1971).

54. M.V. Hershberger and R.W. Lumry, Photochem. Photobiol., 23, 391 (1976).

55. J.B. Birks, Nature, 214, 1187 (1967).

56. G.N. Taylor, E.A. Chandross and A.H. Schiebel, J. Amer. Chem. Soc., 96, 2693 (1974).

57. E.A. Chandross and C.J. Dempster, J. Amer. Chem. Soc., 92, 3586 (1970).

58. T. Okada, T. Fujita, M. Kubota, S. Maski, N. Mataga, R. Ide, Y. Sakata and S. Misumi, Chem. Phys. Letters, 14, 563 (1972).

59. N. Mataga, K. Ezumi and T. Okada, Mol. Phys., 10, 201 & 203 (1966).

60. G. Lockwood, A. Thesis of Ph.D University of Aston, Gosta Green, Birmingham (1974).

61. J.W. Longworth, Photochem. Photobiol., 7, 587 (1968).

62. R.J. McDonald and B.K. Selinger, Aust. J. Chem. 24, 1797 (1971).

63. M. Irie, S. Tomimoto and K. Hayashi, J. Phys. Chem., 76, 1419 (1972).

64. R.S. Mulliken, J. Amer. Chem. Soc., 74, 811 (1952), J. Phys. Chem., 56, 801 (1952).

65. R.S. Mulliken and W.B. Person, Annu. Rev. Phys. Chem., 13, 107 (1962).

66. J. Rose, "Molecular Complex", Pergamon, London (1967).

67. C.H. Linschitz and L. Pekkarinen, J. Amer. Chem. Soc., 82, 2411 (1960).

68. M. Itoh, T. Mimura and T. Okamoto, Bull. Chem. Soc. Japan, 47, 1078 (1974).

69. M.A. Slifkin, "Charge Transfer Interactions of Biomolecules," Academic Press. London (1971).

70. L.J. Andrews and R.M. Keefer, "Molecular Complexes in Organic Chemistry", Holden-Day, London (1964).

71. R. Forster and T.J. Thomson, Trans. Farad. Soc., 58, 860 (1962)

72. A. Weller, Pure Appl. Chem., 16, 115 (1968).

73. M.G. Kuzmin and L.N. Guseva, Chem. Phys. Letters, 3, 71, (1969).

74. H. Knibbe, K. Roellig, F.P. Schaefer and A. Weller, J. Chem. Phys., 47, 1184 (1967).

75. H. Beens, H. Knibbe and A. Weller, J. Chem. Phys., 47, 1183 (1967).

76. R. Foster and H. Hanson, Tetrahad., 21, 255 (1965).

77. A. Szent-Gyorgi, I. Isenberg and J. McLaughlin, Proc. Natn. Acad. Sci., 47, 1089 (1961).

78. E. Fujimori, Pro. Natn. Acad. Sci., 45, (1959).

79. R.L. Schneider, Eastman Organic Chemical Bulletin, 47, (1975).

80. J.P. Green and J.P. Malrieu, Pro. Natn. Acad. Sci., 54, 659 (1965).

81. R. Foster and C.A. Fyfe, J. Chem. Soc. (B) 926 (1966).

82. D.J. Gram and G.S. Hammond, "Organic Chemistry", M. Grow Hill Book Comp. Inc., London (1974).

83. T.R. Evans, J. Amer. Chem. Soc., 93, 2081 (1971).

84. C.R. Goldschmidt, R. Potashnik and M. Ottolenghi, J. Phys. Chem., 75, 1025 (1971).

85. D.A. Labianca, G.N. Taylor and G.S. Hammond, J. Amer. Chem. Soc., 94, 3679 (1972).

86. S. Ander, H. Blume, G. Heinrch and D.S. Frohlinde, Chem.Commun.,745 (1968).

87. L.M. Stephenson and G.S. Hammond, Pure Appl. Chem., 16, 125 (1968).

88. H. Ohta, D. Creed, P.H. Wine, R.A. Caldwell and L.A. Melton, J. Amer. Chem. Soc., 98, 2002 (1976).

89. N.J. Turro and R. Engel, J. Amer. Chem. Soc., 91, 7113 (1969).

90. M.T. McCall, G.S. Hammond, O. Yonemitsu and B. Witkop, J. Amer. Chem. Soc., 92, 6991 (1970).

91. I. Weinryb and R.F. Steiner, Biochemistry, 7, 2488 (1968).

92. R.F. Steiner and E.P. Kirby, J. Phys. Chem.,73, 4130 (1969).

93. S.S. Lehrer, Biochemistry, 10, 3254 (1971).

94. A. White, Biochem. J., 71, 217 (1959).

95. R.W. Ricci and K.B. Kilichowske, J. Phys. Chem., 78, 1953 (1974).

96. A.Z. Britten (private communication).

97. R.O. Loutfy and R.O. Loutfy, Can. J. Chem. 50, 4052 (1972).

98. D. Rehm and A. Weller, Ber. Bunsenges Phys. Chem., 73, 834 (1969).

99. R.S. Davidson and K.R. Trethewey, J. Chem. Soc. Comm, 827 (1976); J.H. Borkent, J.W. Verhoeven and Th. J. D E. Boer, 42, 50 (1976).

100. A.M. Halpern, J.L. Roebber and K. Weiss, J. Chem. Phys., 49, 1348 (1968).

101. H. Heaney and S.V. Ley J. Chem. Sci. Perkin, 1, 499 (1973).

102. Dictionary of Organic Compounds, vol. 5, 3178, E. and F.N. Spon Ltd., London (1965).

103. H.M. Kissman, D.W. Farnsworth and B. Witkop, J.Amer. Chem. Soc., 74, 3948 (1952).

104. Bell, Malcolm Rie [1-(carboxyalkyl)indole], Chem. Abst., 72, 66807 m (1970).

105. S. Veibel et N. Hauge, Bull. Soc. Chem., 5, 1506 (1938).

106. C.U. Rogers and B.B. Corson, Organic Synthesis (Collective), vol. 4, 884 (1963).

107. N.K. Kochetkov, N.F. Kucherova and V.P. Evdakov (Derivatives of indole), Chem. Abst., 51, 12883i (1957).

108. G. Anderson and N. Campbell, J. Chem. Soc., 2855 (1950).

109. A. Smith and J.H.P. Utley, J. Chem. Soc., C, 1-5 (1970).

110. A.H. Milne and M.L. Tomlisnoson, J.Chem. Soc., 2789 (1952); E. Campaigne and R.D. Lake, J. of Org. Chem., 24, 478 (1959); A. Smith and J.H. Utley, J.Chem. Soc., C, 1-5 (1970).

111. J.D. Robert and M.C. Caserio, "Basic Principles of Org. Chem.," W.A. Benjamin, California (1964).

112. N. Tyutyulkov, F. Dietz, Bulgarian Academy of Sciences, Comm. Depart. Chem., 2, No. 1, 55 (1969).

113. Chiang-Tung Chang, Chung-Uung Wu, A.R. Muirhead and J.R. Lombardi, Photochem. Photobiol., 19, 347 (1974).

114. L.F. Gladechenko and L.G. Pikulik, Chem. Abst., 67, 432 gge (1967).

115. A. Kawski and G. Jacyno, Bull. Acad. Pol. Sci, 22, 339 (1974); A. Kawski and J. Sepiol, ibid, 20, 707 (1972).

116. N. Strojny and J. Arthur F. de Silva. J. of Chromatographic Science 13, 583 (1975).

117. M.M. Airaksinen, P.J. Neuvonen and I. Jaakonmaki, Biochem. Pharmacol., 19, 2181 (1970).

118. E. Giacalone and L. Valzelli, Pharmacology, 2, 171 (1969).

119. P.A. Shore, A. Burkhalter and V.H. Coh. J. Pharmacol. Exp. Therap., 127, 182, (1959).

120. R.P. Maickel, "Methods of Neurochemistry," vol. 2, edited by Ranir Fried, Marcel Dekker, New York (1972).

121. G. Curzon and J. Giltrow, Nature, 173, 314 (1954).

122. R.L. Pecsok, L.D. Shields, T. Cairns and I.G. McWilliam , "Modern Methods of Chemical Analysis," (2nd ed.) Wiley, London (1968).

123. L.P.J. Holt and C.F. Hawkins, Brit. Med. J., 1, 1354 (1965).

124. J. Arthur F. de Silva, N. Strojny and K. Stika, Analytical Chemistry, 48, 144 (1976).

125. R.G. Wiegand and E. Scherfling, J. Neurochem., 9, 113 (1962).

126. "The Merk Index", 8th ed., Merk and Co., Rahway N.J., 1968.

127. R.P. Maickel and F.P. Miller, Analytical Chemistry, 38, 1937 (1966).

128. D.G. Ferry, D.M. Ferry, P.W. Maller and E.G. McQueen, J. Chromatography, 89, 110 (1974).

129. E. Gelpi, E. Peralta and J. Segura, J. of Chromatographic Science, 12, 701 (1974).

130. M. Donike, Chromatographia, 9, 440 (1976).

131. L. Helleberg, J. Chromatography, 117, 167 (1976).

132. J.L. Brook, R.H. Biggs, P.A. St. John and D.S. Anthony, Analytical Biochemistry, 18, 453 (1967).