PYRROLO[2,3-b]PYRIDINES AS POTENTIAL ANTI-TRYPANOSOMAL AGENTS

BY

PHILLIP GANGITEMILE KANYANGA KIGODI

A thesis presented for the degree of

DOCTOR OF PHILOSOPHY

IN THE

University of Aston in Birmingham

February 1983

Department of Pharmacy

University of Aston in Birmingham

The University of Aston in Birmingham

1H-Pyrrolo[2,3-b]pyridines as Potential Anti-trypanosomal Agents

A Thesis Submitted for the Degree of Doctor of Philosophy,
by P.G.K. Kigodi, February 1983

Summary

A brief review of African human trypanosomiasis, the disease caused by the protozoa T.b. gambiense and T.b. rhodesiense in man, the classification and the life cycle of these protozoa has been given. The metabolic pathway of glucose, the amino acids threonine, proline, tryptophan, tyrosine and phenylalanine by these two protozoa has been discussed. Control methods of the disease by chemotherapeutic and chemoprophylactic agents as well as the recent research efforts to develop suitable chemotherapeutic agents has been given.

The synthesis, chemical properties and the biological properties of 1H-pyrrolo[2,3-b]pyridines have been briefly reviewed and the rationale for the preparation of 1H-pyrrolo[2,3-b]pyridines for the evaluation as potential anti-trypanosomal agents discussed.

The preparation of 1H-pyrrolo[2,3-b]pyridine derivatives and the screening of a selected number of 1H-pyrrolo[2,3-b]pyridines as well as 2'-N-methyl tryptophan against T.b. brucei has been described.

The possible mass spectral fragmentation pathways of a selected number of 1H-pyrrolo[2,3-b]pyridine derivatives have been postulated.

Key words: Trypanosomiasis (African), Chemotherapy, 1H-pyrrolo-[2,3-b]pyridines.

ACKNOWLEDGEMENTS

The author wishes to thank Professor D.G. Wibberley for the kind supervision and encouragement throughout the course of this work. Grateful thanks are also extended to Professor M.F.G. Stevens for valuable advice and discussion; Dr. D. Evans of Medical Protozoology, London School of Tropical Medicine and Hygiene for his assistance and discussion during the biological screening work; Professor B. Ferry for his assistance in setting up screening facilities in the Department of Pharmacy; and Professor P.R. Khonje for suggesting the topic and screening one of the compounds.

The technical assistance of Mr. P. Lowe, Mr. J. Chalton, Miss K. Enshor and Mr. P. Behan is appreciated.

The author acknowledges the permission by Hodder & Stoughton to reproduce figures 2(a) and (b), 3, 4(a) and (b) from Introduction to Animal Parasitology by J.D. Smyth.

To my wife Hellen,

my daughter Nguvila Tumaini,

and my son Mufwimi Faraja

ABBREVIATIONS

The abbreviations as used in the text of this thesis are as follows;

Biochemical

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CNS	Central nervous system
DHAP	Dihydroxyacetone phosphate
FAD	Flavin adenosine dinucleotide
FFA	Free fatty acid(s)
F-6-P	Fructose-6-phosphate
F-1, 6-diP	Fructose-1, 6-diphosphate
GAP	Glyceraldehyde-3-phosphate
GP	Glycerol-3-phosphate
GPO	sn-Glycerol-3-phosphate oxidase
NAD^+	Nicotinamide adenosine dinucleotide (oxidised)
NADH	Nicotinamide adenosine dinucleotide (reduced)
TCA	Tricarboxylic acid cycle

INDEX

		Page
INTRODUCTION		
I	Trypanosomiasis	1
	A. Historical Perspective of Trypanosomiasis	1
	B. The Disease	3
	C. Classification of Life Cycle of Trypanosomes	10
	D. Metabolism	18
	1. Carbohydrate Metabolism	19
	2. Lipid Metabolism	23
	3. Amino Acid Metabolism	24
	E. Control, Host Immunity, Chemotherapy and Chemoprophylaxis	30
	1. Antigenic Variation and Host Immunity	30
	2. Chemotherapy and Chemoprophylaxis	33
	3. Recent Advances: GPO Inhibition	38
II	1H-Pyrrolo[2,3-b]pyridines	46
	A. Synthesis	46
	B. Chemical Properties	52
	C. Biological Properties	55
DISCUSSION		57
I	Aims and Objectives	58
II	Screening of Selected 1H-Pyrrolo[2,3-b]pyridines Against T.b. brucei	59
III	A. Preparation of 1H-Pyrrolo[2,3-b]pyridines	61
	B. Chemical Properties of 1H-Pyrrolo[2,3-b]pyridines	63
IV	Mass Spectra of Fragmentation Patterns of Selected 1H-Pyrrolo[2,3-b]pyridines	77

Page

EXPERIMENTAL 107

I Screening of Selected 1H-Pyrrolo[2,3-b]pyridines Against T.b. brucei 108

II Synthesis of 1H-Pyrrolo[2,3-b]pyridines

Bibliography 162

LIST OF TABLES

TABLE		Page
1	Number of Recorded New Cases of Human Trypanosomiasis, Africa 1968 - 1974	5
2	Quantities of Aromatic Amino Acid Catabolites Present in Urine of Mice Uninfected and Infected with T.b. gambiense for 72h.	29
3	Summary of Results of Testing Compounds Against T.b. rhodesiense in Mice	41
4	Screening of Selected 1H-Pyrrolo[2,3-b] - Pyridine Derivatives Against T.b. brucei (LIMP 1164, TREU 667 and 8/18)	110
5	Infectivity Inhibition Testing with Selected 1H-Pyrrolo[2,3-b] pyridine Derivatives Against T.b. brucei (8/18)	112

LIST OF FIGURES

FIGURE		Page
1.	Possible Roles of Trypanosomal Phospholipases and Free Fatty Acids in the Pathogenesis of the Trypanosomiases	7
2.	Trypanosome (a) Typical Features (b) Ultra Structure	15
3.	Developmental Stages of Trypanosomatid Flagellates and Descriptive Terminology	16
4.	(a) Life Cycle and (b) Mitochondria Activity of Trypanosomes	17

INTRODUCTION

I TRYPANOSOMIASIS

A. Historical Perspective of Trypanosomiasis

African human trypanosomiasis, or sleeping sickness, is a parasitic disease caused by the protozoa of the genus Trypanosoma. Transmission of the disease is effected by the tsetse fly species of the genus Glossina. The geographical distribution of the disease parallels that of its vector - the Glossina. The latter is found between latitudes 20°N and 20°S in areas of more than 500 mm annual rainfall. Rivers may extend the range of the riverine G. palpalis into more arid zones. The pupa of Glossina cannot stand desiccation and the tsetse fly is not found in drier areas.

The disease has caused untold suffering of people through its debilitating effect and a high death rate. Within the first half of this century, two epidemic outbreaks have been described.[1] During the first epidemic between 1896 and 1906, it was estimated that half a million people died in the Congo Basin alone. Further to the east, the Busoga district in Uganda lost 300,000 of its inhabitants while the neighbouring Buvuma islands of Lake Victoria lost 57,000 people. Evacuation of the remaining 22,000 people from these islands in 1909 probably prevented a total disaster from happening.

In West Africa, owing to delayed opening up of the hinterland, no figures of the first epidemic are available, except for the island of Principe where the epidemic had killed all its inhabitants but 350 by 1907.

These figures are only estimates as no hospitals were in existence then for proper diagnosis and all the cases were not recorded. There were no effective antitrypanosomal drugs then; and the first

one, atoxyl (13) came in to clinical use in 1905.

The second epidemic, extending right across the continent from west to east, followed twenty years later. In Gambia the number of reported cases rose from twenty to one thousand between 1925 and 1935. Upper Volta's figures rose from 32,000 in 1934 to 80,000 in 1939, while Dahomey had reported 10,000 cases by 1934. In Northern Nigeria the epidemic spread rapidly from 1927 when 400 cases were recorded to 18,000 by 1930 and by 1940 a total of 300,000 patients had been treated by mobile teams in the field. Cameroon had reported as many as 2,000 cases by 1926 while in the Congo Basin a total of 64,000 people were treated between 1920-23.

In western Tanzania between Lake Rukwa in the south and Kahama in the north 3,000 cases were reported in 1934 and of 24,000 cases reported in Maswa between 1922 and 1946 about 50% of whom died.

By 1926 two new drugs, tryparsamide (14) and suramin (12) had been introduced into clinical use - and from about 1940 epidemic outbreaks had been controlled and the number of cases dropped to about 400 - 700 per year in West Africa, though in some endemic areas an incidence of 2% was still prevalent.[2] In the whole of Eastern and Southern Africa the number of cases dropped to less than 2,000 per year[3] from about 1955. Zaire fared differently, for by 1958 it had an incidence of 0.02% and this had changed to 15% by 1964, due to internal strife in that country.[4]

Potentially about 35 million people in Africa are at risk of the infection today and the danger posed by an unchecked spread of trypanosomiasis over a large area of Africa is now well known. A

number of methods aimed at controlling or eradicating the disease have been attempted but none have had lasting success. The situation has not changed significantly since the 1950s and in some countries there are indications that the disease is on the increase again; though not reaching epidemic levels. Table 1 below indicates the trend of the disease in recent years.[5]

For more than twenty five years, no new drugs have been introduced to fight the disease. Increased resistance to those now in use is causing concern among medical and veterinary workers and WHO. Recently WHO launched a multidisciplinary programme for study and research on the disease with a long term objective of developing means of effective containment or ultimate eradication.[6]

The review and discussion below is confined to African human trypanosomiasis, although some of the points raised are equally valid for trypanosomiasis of domestic animals. It is also worth noting here that the two diseases, human and domestic animals trypanosomiases together account for the exclusion of more than ten million square kilometres of land from settlement and economic development.[7,8,9]

B. The Disease

Two clinical forms of African human trypanosomiasis are recognnised. The disease found in East Africa and the countries bordering the Zambezi river takes an acute course and is transmitted by G. morstans, the tsetse fly that inhabits the savannah woodlands. This disease is called East African or Rhodesian trypanosomiasis. The main hosts of the disease are wild animals such as bushbucks. Man is infected when hunting, farming, fishing or collecting firewood[10] and the trypanosome responsible for the disease is

T.b. rhodesiense (see C, for nomenclature). Its nosodeme, or clinical variant T.b. gambiense causes the Gambian or West African disease the range of which extends from the west coast to the western shores of Lakes Tanganyika and Victoria in the east. It runs a more chronic course and is transmitted by the riverine G. palpalis. Zones of overlap between the two diseases in terms of vector and geographical distribution do exist.

The onset of the Rhodesian disease may be indicated by appearance of the trypanosome chanchre, a hard swelling localised at the site of infection within a period of five to fifteen days of the bite by the infected tsetse fly. The trypanosomes then move to invade the lymph nodes, blood and other tissues. This is followed by bouts of fever and indications of liver and spleen enlargement appear. These symptoms are not specific and are often mistaken for malaria and even influenza. Typical neurological symptoms are less well marked.

The Gambian disease has a longer incubation period followed by irregular fevers, anaemia, splenic, hepatic and lymph node enlargement. As the disease progresses the patient shows signs of lethargy, neurological involvement including headache, hyperesthia, lassitude, loss of muscular control, somnolence, coma and finally death. The pathological lesions that eventually kill the human host are largely unknown and are only speculated upon.

Experimentally infected animals may have as many as $2 - 3 \times 10^9$ trypanosomes/ml blood,[11] but even this large number of trypanosomes in the blood and the tissues do not seem to cause extensive damage. Experts on the subject have failed to pinpoint a single lesion that

Table 1 Number of recorded new cases of human trypanosomiases Africa 1968-74

Country	Total population in millions	1968	1969	1970	1971	1972	1973	1974
Western Africa								
Dahomey	3	17	18	11	22	-	157	410
Ghana	10	181	160	166	135	-	156	69
Guinea	3.7	379	351	357	425	38	55	31
Guinea Bissau	0.5	-	-	-	-	-	46	11
Ivory Coast	5	376	176	148	-	144	136	87
Mali	5.5	356	221	231	190	193	388	188
Niger	4.4	9	0	4	0	1	0	0
Nigeria	79	944	599	393	529	498	331	132
Senegal	4.2	43	16	16	-	17	10	2
Togo	2.1	36	84	14	57	23	25	21
Upper Volta	5.8	164	262	145	114	69	75	78
Central Africa								
Cameroon	6.2	219	217	229	125	103	297	476
C.A.R.	1.7	19	22	63	175	112	91	79
Chad	4	61	50	47	18	17	15	54
Congo	1	134	73	59	172	49	42	91
Gabon	1	84	49	59	38	419	32	47
Zaire	25	3357	4957	6152	5124	4126	-	-
Eastern and Southern Africa								
Botswana	0.7	36	37	59	272	-	-	-
Burundi	4	65	12	78	75	114	-	-
Ethiopia	26	28	173	44	12	1	-	-
Kenya	13	43	20	16	26	7	13	-
Malawi	5	1	0	0	2	0	-	-
Rwanda	4	21	20	69	35	13	-	-
Sudan	15	-	-	8	2	22	125	287
Tanzania	15	403	530	564	569	612	477	488
Uganda	11	90	56	81	184	160	37	-
Zambia	4.5	80	69	127	200	394	391	-
Total		7148	8172	9140	8501	7126	2899	2551

(-) indicates figures not available.

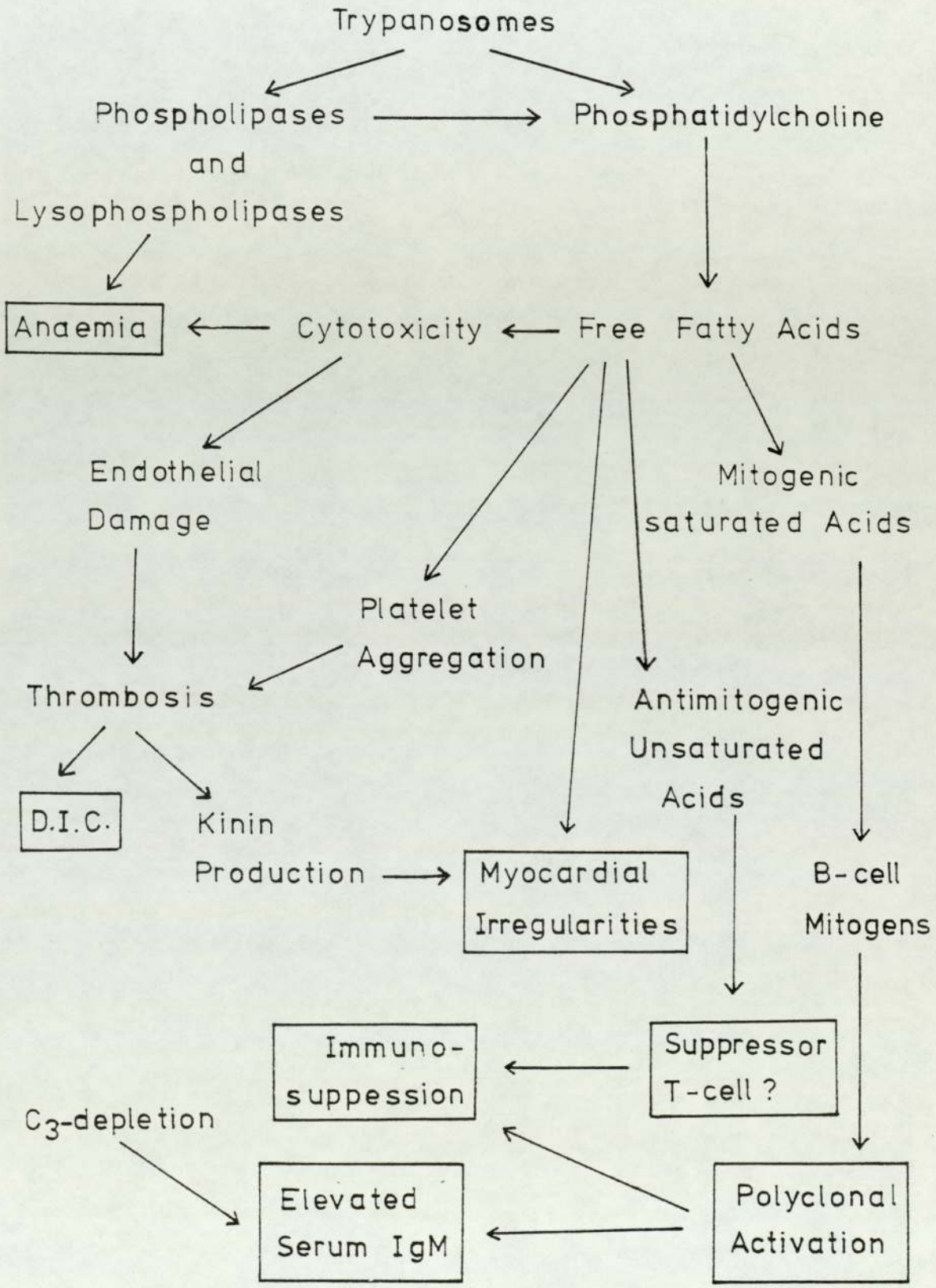
would be lethal to the host and consequently suggested that the combined effect of metabolic products of trypanosomes, repeated abuse of the immune system by the rise of successive antigenically different populations, release of a number of pharmacologically active substances, changes in blood clotting, deposition of immune complexes in the kidney and the direct immune suppression that accompanies the infection may, taken together, have a significant role in the fate of the host. The identity of some of these metabolic products, their effects, and the evidence in support of the effect of the pharmacologically active substances released by trypanosomes - both live and dead - is now available and is described in more detail below.

In vitro trypanosomes consume 50-100% of their dry body weight of glucose per hour,[11,12] and in vivo the end product of glucose breakdown, pyruvate is converted into lipids and stored in fibroblasts. The amount of pyruvate produced by the trypanosomes is five times that produced by the host. Persistent high levels of pyruvate divert more fibroblasts which cease producing collagen tissues. The breakdown of connective tissues that occurs following infection may be due in part to this activity.

When T.b. brucei and T. congolense are autolysed at 20°C for 8-24h significant amounts of phospholipase A_1 and lysophospholipase are produced.[13,14,15] These two enzymes sequentially degrade phosphatidylcholine to give free fatty acids (F.F.A.) and lysophosphatidylcholine which is further broken down to more F.F.A. and glycerolphosphorylcholine.

The free fatty acids thus produced have a variety of activities and the diagram (Fig. 1) below summarises some of the main ones.

Figure 1. Possible Roles of Trypanosomal Phospholipases and Free Fatty Acids (F.F.A.) in the Pathogenesis of the Trypanosomiases.[15]



Free fatty acids can haemolyse erythrocytes, and of those produced palmitic, stearic and linoleic acids each constitute 25% and the haemolytic activity lies with linoleic acid. It is theoretically possible that when a large number of trypanosomes are dying they could release sufficient lipases to produce large amounts of F.F.A. to saturate albumin and lyse erythrocytes in vivo. Repetition of this process as wave after wave of parasitaemia passes could in part contribute to the anaemia observed in infected host. Apart from the haemolytic ability linoleic acid can cause immuno suppression by acting directly on lymphocytes. Further, linoleic acid can disrupt the structures of spleen and lymph nodes which in a chronic disease could result in the atrophy of these organs. Free fatty acids produce these effects through their detergent-like property by inserting themselves on cell membrane causing perturbations that result in prevention of cell division. In addition, linoleic acid is a precursor of prostaglandins. Prostaglandins can cause immune suppression by controlling lymphocyte activity or regulating cyclic nucleotide levels.

When trypanosomes are lysed they produce lysosomes containing both acid phosphatases and cathesin D. These enzymes produce toxic products by acting on plasminogen and kininogen to release plasmin and kinins in large amounts in the host circulation. In the microvascular locations these could lead to significant local endothelial damage and changes in vascular permeability could occur.

Trypanosomes catabolise tryptophan, tyrosine and phenylalanine to give potentially toxic products (see section D). These products may contribute to the symptoms seen in trypanosomal infection. One of these products, indole-3-ethanol when injected in pharmacological

doses in mice, produces sleep-like states as well as a drop in body temperature.[16] This finding suggests that indole-3-ethanol (and 5-hydroxyindole-3-ethanol) produced within the central nervous system (C.N.S.) may be directly involved in the lethargy observed during an infection with African trypanosomes.[17] Further, indole-3-ethanol suppresses humoral immune response in doses similar to those causing sleep states. Since the sleep-like behavioural states, change in body temperature and immune suppression resemble symptoms in humans chronically infected with trypanosomes, it has been hypothesized that indole-3-ethanol is the metabolic toxin produced by these organisms.[15]

The mechanism by which indole-3-ethanol exerts these effects is unknown. In vitro it has been observed to lyse human erythrocytes.[18] Hence indole-3-ethanol produced within the C.N.S. could combine with cell membrane and alter the permeability of synaptic membranes leading to changes in nerve impulse transmission which may lead directly to behavioural changes and other systemic effects such as immunosuppression. The relative amounts produced in vivo has been calculated. A suspension of 10^8 trypanosomes produced 167pg of indole-3-ethanol, 170 pg of indole-3-acetic acid and 184 pg indole-3-lactic acid per hour. At peak parasitaemia the average amount in an infected mouse would be 3.2 mg/kg and this quantity is consistent with the expected levels to account for the above changes.

Apart from the direct effects of the above catabolites, the depletion of host tryptophan, tyrosine and phenylalanine by trypanosomes would have grave consequences. Diversion of tryptophan from the kynurenine pathway leading to niacin and serotonin would give rise to niacin deficiency and decreased brain levels of serotonin. Low brain serotonin level could lead to behavioural

depression and interference in sleep patterns.[19] Diversion of tyrosine from the catecholamine pathway would lead to changes in body temperature, blood pressure, sleep patterns and produce the neuropsychiatric syndrome of African trypanosomiasis.[16,20] Phenylpyruvate, the product of phenylalanine transamination can inhibit a number of pathways including synthesis of adrenaline in the adrenals, breakdown of tyrosine to acetoacetate and tyrosine incorporation in the liver as well as inhibition of hexokinase, 6-phosphogluconate dehydrogenase and glucose incorporation to brain macromolecules.[20] All these changes could contribute to the disease of trypanosomiasis.

Terminally infected animals show hypoglycemia which is not due to glucose or glycogen depletion. It is caused by the near absence of glucose-6-phosphatase, the enzyme responsible for gluconeogenesis. A trypanosome-produced inhibitor of this enzyme has been detected.[11]

Although any one of the above factors may not produce the lethal lesions, taken together they may account for the death of the host. In the absence of conclusive evidence as to the extent each factor contributes towards the death of the host from uncomplicated trypanosomiasis, the broad conclusion that the sum total of the effects of these factors account for the death of the host stands. Very often, however, such hosts die of secondary infections such as pneumonia.

C. Classification and Life Cycle of Trypanosomes

Since the genus Trypanosoma was first described by Gruby in 1843 many trypanosomes have been discovered and assigned specific status in this genus. Assignment of specific status, however, was not done

on the basis of some standard rules or application of uniform criteria. This criteria for assigning any organism to a particular taxan was left to the author's judgement.[21] Soon misunderstanding and confusion arose among medical and veterinary workers on one hand and systematic taxanomists on the other.

The two clinical forms of trypanosomiasis were considered as being caused by two different organisms which were assigned the specific names Trypanosoma gambiense Dutton, 1902 and Trypanosoma rhodesiense Stephens & Fantham, 1910. But these two organisms were morphologically indistinguishable from Trypanosoma brucei Plimer & Bradford, 1899 which infects domestic and wild animals but not man. Medical and veterinary workers could not achieve an overall view of the problem of trypanosomiasis and thus co-ordinate the fight against the disease so long as they held the view that three different organisms existed.

An acceptable taxanomic scheme ought to satisfy formal taxanomic requirements and clearly meet the practical needs of medical and veterinary workers in the field. No such system was available until 1966 when Hoare[22] proposed a trinomial system which combined biological and morphological criteria as the best compromise. This proposal gained general acceptance. Under this system mammalian trypanosomes were divided into two broad sections according to the position in which they completed their cyclical development in the vector. In section stercoraria the development of the trypanosome is completed in the posterior station (hind gut) of the vector and its transmission is contaminative. The causative agent of chagas' disease is in this category. In section salivaria, developmental completion of the parasite is achieved in the mouth parts (salivary

glands & proboscis) i.e. anterior station and transmitted by inoculation. The trypanosomes of the human sleeping sickness and domestic animals disease also come under this category.

The use of the descriptive term 'section' and subgeneric separation in the genus Trypanosoma by Hoare[22] was aimed at rationalising the hitherto complex classification of mammalian trypanosomes while at the same time trying to conform with formal taxa. Hence no emphasis was given to the subgeneric name which was considered optional. Thus in this view the revised systematics of trypanosomes causing sleeping sickness became Trypanosoma (Trypanozoon) gambiense and Trypanosoma (T.)rhodesiense. The morphologically identical trypanosome causing nagana in domestic animals was designated Trypanosoma (T.) brucei.

However, this subgeneric separation did not resolve the 'complex brucei group' completely. For medical and veterinary practical purposes it was still inconvenient to retain the specific status. Further, the Rhodesian disease shows more virulence in the northern part of its range, i.e. East Africa, than in the south. In the Luangwa - Zambezi area it is more benign, its incubation period longer and microscopically the organisms show smaller refractile granules. It was argued by Ormerod[23] that a specific rank for the 'brucei group' would necessitate the setting up of a fourth species to separate these two varying forms of the Rhodesian disease. But such a move would lead to more confusion as the Rhodesian disease would refer to the disease further to the north of the region it was originally described. A more appropriate way of resolving the problem would be to demote the former 'species' to strains or demes,[23] but Hoare[21] suggested that when evolutionary relationship in the

'brucei' group is taken into account a subspecific status may be more appropriate. This problem was left unresolved.

Despite the success of this trinomial system into clarifying evolutionary relationship within the salivarian trypanosomes and the substantial simplification of the description and discussion, more recent WHO[24] proposals have called for a more unified, flexible classification of all salivarian trypanosomes. These current proposals seek to distinguish those purely operational terms used to describe the laboratory history of the material and characteristic terms related to Linean classification. Though provisionally retained, the descriptive term salivaria may not be available in future; for it occupies a position not allowed by taxanomic rules. It may become the new subspecies designation replacing the current four i.e. Duttonella, Trypanozoon, Pycnomonas and Nannomonas which would be redundant or withdrawn altogether.

Given the definition of salivarian trypanosome species[24] as "an assemblage of organisms that can be distinguished from other species by one or more stable, discontinuous morphological characters" and that the subgeneric designation becomes unspecific in Trypanozoon a single species is recognised by the 1978 WHO[24] proposals: Trypanosoma brucei Plimmer & Bradford 1899, under it are included five subspecies; thus:

Trypanosoma brucei brucei Plimmer and Bradford, 1899

Trypanosoma brucei rhodesiense Stephens and Fantham, 1910

Trypanosoma brucei gambiense Dutton, 1902

Trypanosoma brucei evansi Steel, 1885

Trypanosoma brucei equiperdum Doflein, 1901

Usually the names are not cited in full but abbreviated to T.b. brucei;

T.b. rhodesiense etc.

Under microscopic examination trypanosomes exhibit very simple features and the more important of them are summarised in the sketch below (Fig. 2(a)). The length of trypanosomes varies between 15-30μ and the width is 1.5-3μ

Trypanosomes undergo a complex life cycle, both in man and in the tsetse fly vector. These cycles are also accompanied by morphological changes for which specialised descriptive terms are used. In the past, these terms were derived from the genera in which the corresponding stages were the most characteristic. However, association of developmental stages with certain genera is inappropriate and misleading. Such usage implies more similarity with the genera and the stage than exists and rules for changing taxa on revision do not govern descriptive terms. Hoare and Wallace[25] in 1966 suggested a new terminology based on the position of the flagellum in the cell. The characteristic stages and the new terms are given in the sketch (Fig.3), (obsolete terms in brackets).

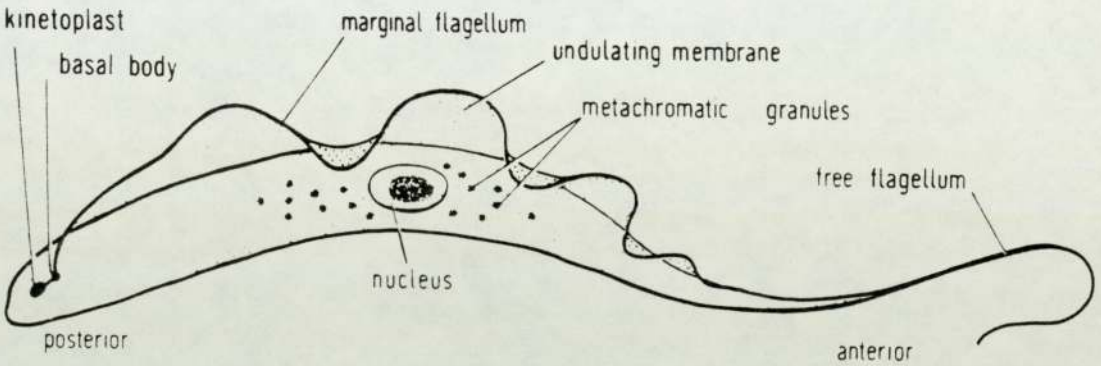
Following a bite by an infected tsetse fly, the metacyclic trypomastigote forms may develop a local lesion at the site of the bite which takes the form of a hard chancre persisting for about a fortnight. From this lesion the parasites spread to invade other organs. In the mammalian host, in general, the developmental cycle is characterised by the appearance of three morphological forms; differing in body thickness and the size of the flagellum. The following terms are used to describe these mammalian forms.[26]

1) Slender form: long and thin with a free flagellum

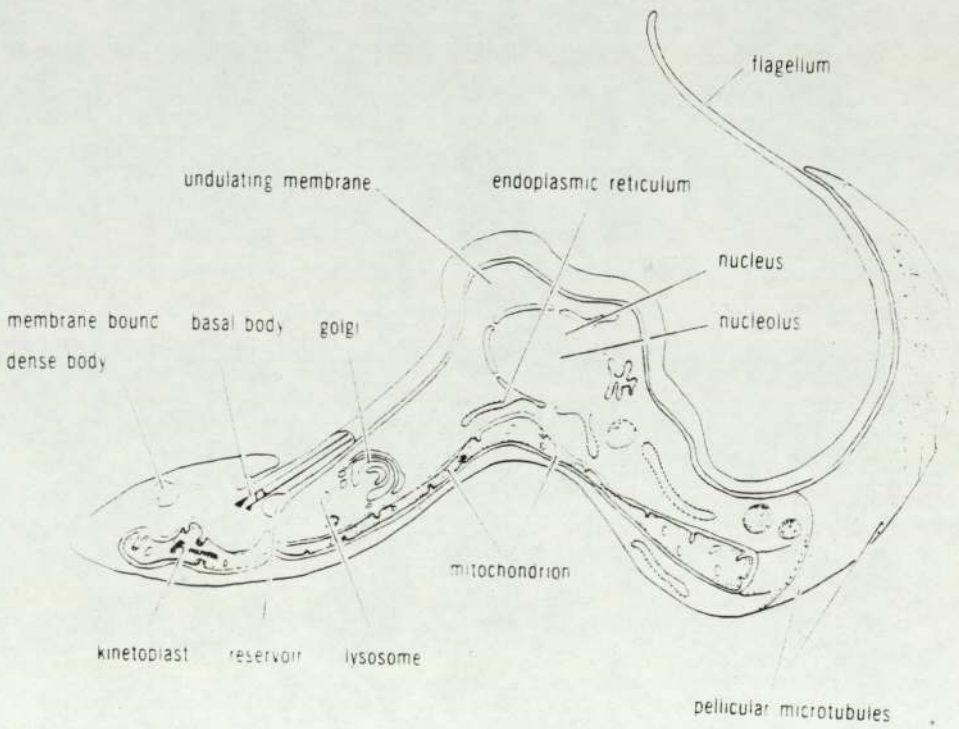
2) Stumpy form: short and thick, typically no flagellum

Figure 2 Trypanosome (a) Typical Features

(b) Ultra Structures

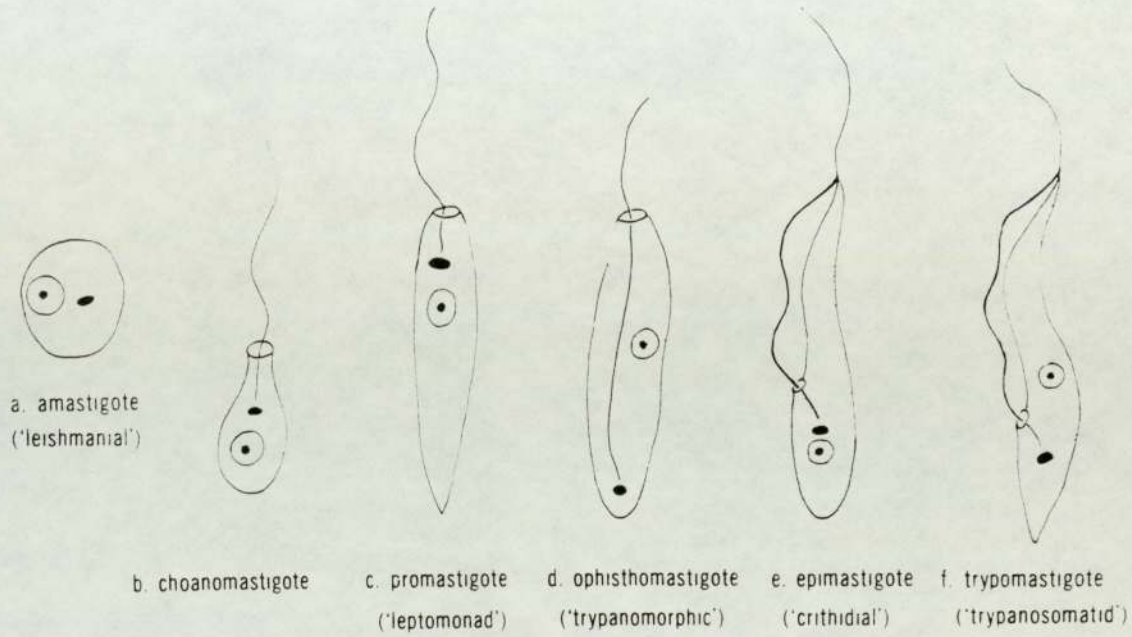


a



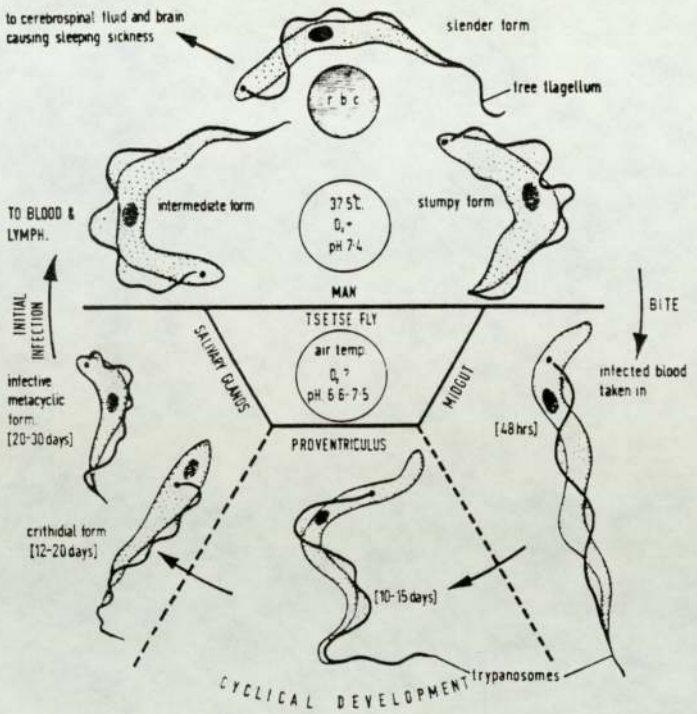
b

Figure 3 Developmental Stages of Trypanosomatid Flagellates and Descriptive Terminology

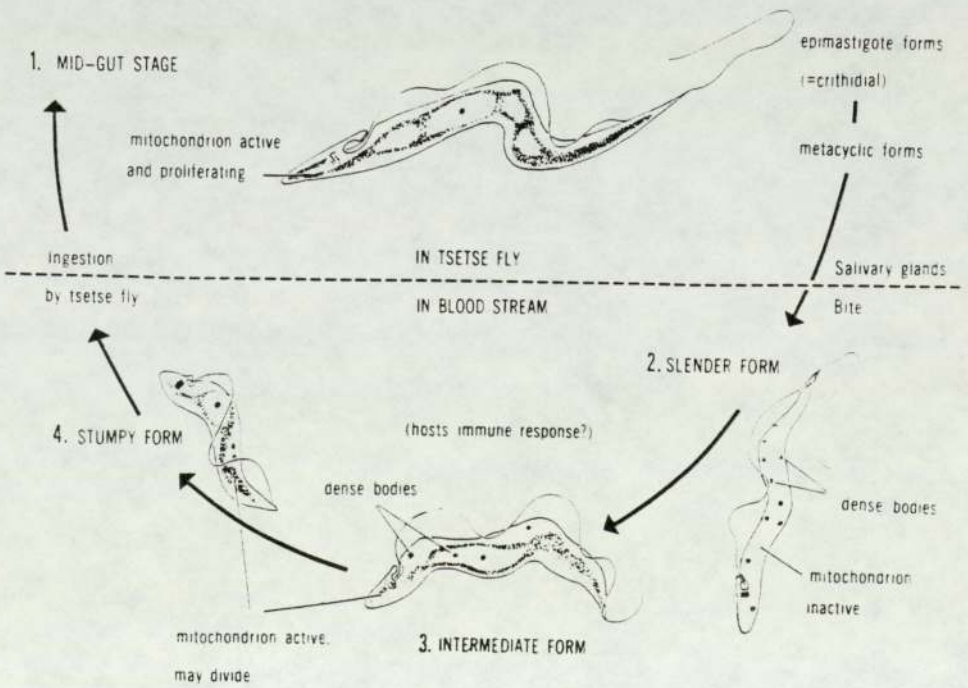


a) Amastigote (Leishmanial). Rounded forms, no flagellum.
b) Chaomastigote. Barley corn form, kinetoplast antenuclear Flagellum from funnel-shape reservoir, emerges anteriorly.
c) Promastigote (Leptomonad). Elongated body, Antenuclear kinetoplast flagellum arise near it, emerges anteriorly.
d) Opisthomastigote (Trypanomorphic). Postnuclear kinetoplast flagellum arise near it pass through body, emerges anteriorly
e) Epimastigote (Crithidial). Juxta nuclear kinetoplast, flagellum arise near it emerging on side of body, runs along short undulating membrane.
f) Trypomastigote (Trypanosomatid). Post-nuclear kinetoplast, flagellum arise near it runs along undulating membrane to emerge anteriorly.

Figure 4 (a) Life Cycle and (b) Mitochondria Activity



a



b

3) Intermediate form: moderately thick with a free flagellum.

These morphological variations were erroneously regarded as a manifestation of polymorphism,[27] but since the variations are continuous rather than distinct and exist in clonal infections, they cannot have a genetic basis.[23,28] They are termed pleomorphic or clonal pleomorphs.

The life cycle of the parasites in the mammalian and insect vector is diagrammatically shown ~~below~~ above (Fig.4). Multiplication in the mammalian host continues with long slender forms predominating initially, these then give rise to the intermediate forms which are eventually transformed into short stumpy forms. The transformation into short stumpy forms is accompanied by a switch over to an active mitochondrial respiration with a partially functional tricarboxylic acid (T.C.A.) cycle. When a tsetse fly takes a blood meal it will suck in all three forms, but only the short stumpy form develops in the tsetse fly midgut.[28]

In the tsetse fly the parasites start development in the endoperitrophic cavity of the midgut, then move through the ectoperitrophic space and proventriculus into the salivary glands. During the process they change from short stumpy into lo.g, thin forms called epimastigote and eventually reach the final metacyclic trypomastigote stage which is the infective form to man.

D. Metabolism

Knowledge of the metabolic pathways of trypanosomes and especially the enzyme systems that catalyse the different reactions has been difficult to ascertain and is still largely fragmentary. One

of the most vexing problems in probing these pathways and their enzymes has been the failure to develop a suitably defined culture medium to duplicate the entire life cycle of trypanosomes in vitro. The second problem arises from the fact that being digenic, the trypanosomes must express different nutritional requirements as they undergo cyclic development in the vertebrate host and the insect vector. For instance the culture forms (from tsetse fly midgut) derive their energy from proline and threonine metabolism rather than glucose.[28,29] Thus trypanosomes are able to 'switch off' some enzyme activity in one host and express different ones in another. The most remarkable is the supression of the T.C.A. cycle in the long slender forms in a mammalian host.[30,31] The mechanism which sets the changes in motion is unknown though the sequence of some of these changes is now known. The short stumpy forms show partial activity of the T.C.A. cycle,[28,30] probably a pre-adaptive preparation for transfer to the insect vector.[28]

1) Carbohydrate Metabolism

Blood stream forms of African trypanosomes are entirely dependent on glycolysis for their energy production.[32,33] Lacking stores of their own, glycolytic substrates must be exogenously supplied in the form of glucose, fructose, mannose or glycerol.[34] These blood stream forms lack cistae and a functional T.C.A. cycle in the mitochondrion.[28]

Under aerobic conditions the classical Embden-Meyerhof glycolytic pathway produces a net two moles of ATP while under anaerobic conditions, such as low oxygen tension only a net one mole of ATP is produced.[28] Equations 1 and 2 illustrate the process.[35]

Aerobically:

$$\text{Glucose} + 2\,ADP + 2Pi + O_2 \longrightarrow 2\,\text{pyruvate} + 4H_2O + 2ATP$$
$$(\text{Glycerol} + ADP + Pi + O_2 \longrightarrow \text{pyruvate} + 3H_2O + ATP) \quad \Big\} \ldots 1$$

Anaerobically:

$$\text{Glucose} + ADP + Pi \longrightarrow \text{pyruvate} + \text{glycerol} + H_2O + ATP \ldots 2$$

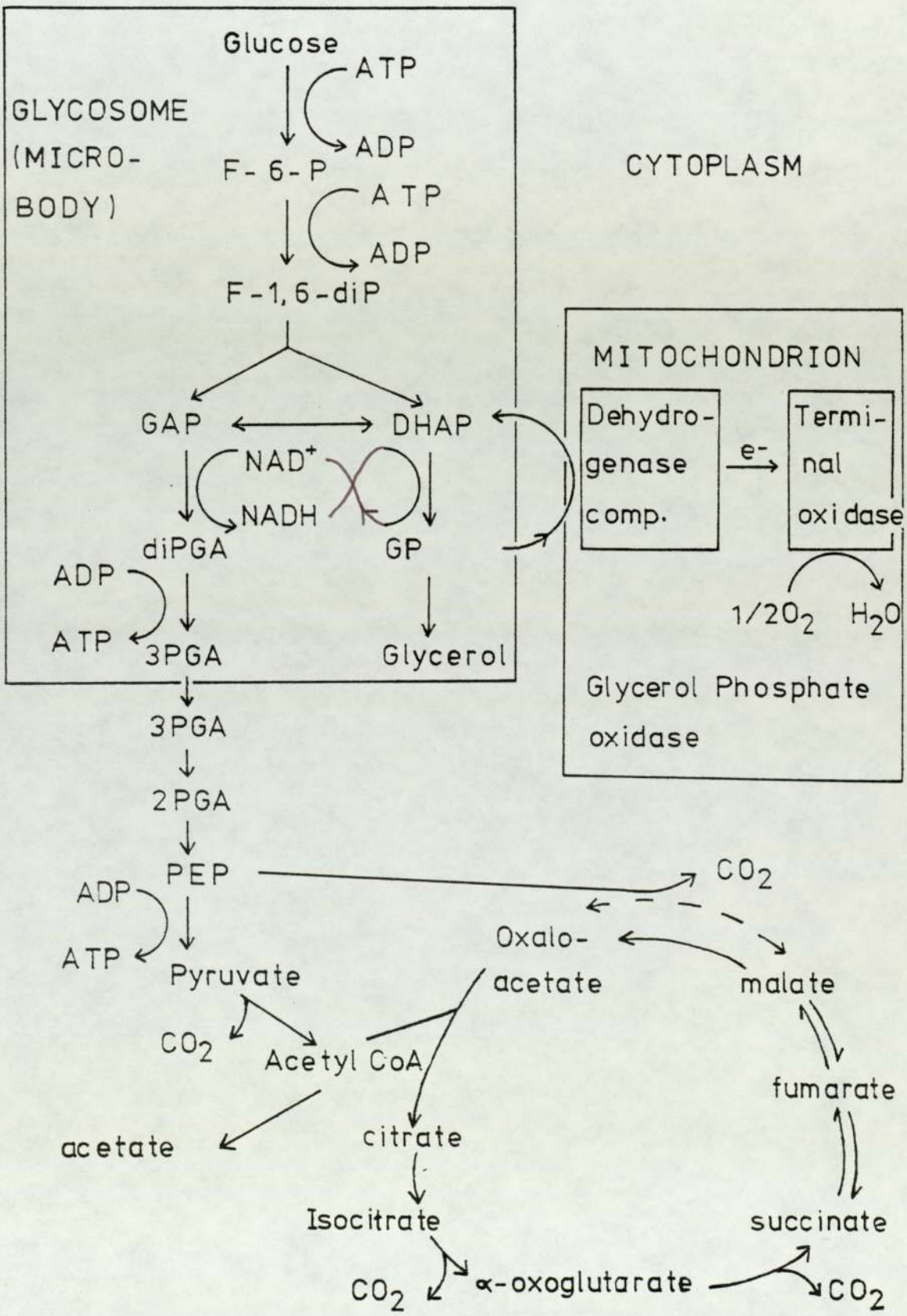
The glycolytic sequence is outlined in scheme 1 below.[30,34,36]

Trypanosomes show a remarkable degree of specialization and compartmentation. The glycolytic enzymes and other enzymes are not just uniformly dispersed throughout the cytoplasm, but are localised in three compartments; the glycosome (microbody), mitochondrion, and the cytoplasm.[34,37,38,39]

The NADH produced during the conversion of glyceraldehyde - 3 - phosphate to 1,3 - diphosphoglyceraldehyde (scheme 1) is re-oxidised via a glycerol phosphate shuttle catalysed by the concerted effect of two enzymes; the glycosomal NAD^+ dependent sn - glycerol - 3 - phosphate dehydrogenase and the mitochondrial sn glycerol - 3 - phosphate oxidase.[30,34] This oxidase enzyme is unique to trypanosomes; for it is not inhibited by mammalian terminal cytochrome oxidase inhibitors such as cyanide, azide, amytal, antimycin A or carbon di-oxide.[30,31,32] Its composition includes iron, thiol groups, copper and FAD.[40,41] The re-oxidation of NADH has been suggested to take place in two steps, thus:-[30]

SCHEME 1

Aerobic and Anaerobic Glycolysis of T.b.brucei and the T.C.A. Cycle of Midgut forms.



Abbreviations

F-6-P	Fructose-6-phosphate
F-1, 6-diP	Fructose-1, 6-diphosphate
DHAP	Dihydroxyacetone phosphate
GAP	Glyceraldehyde-3-phosphate
diPGA	1,3 diphosphoglyceraldehyde
2PGA	2 & 3-phosphoglyceraldehyde
GP	Glycerol-3-phosphate
PEP	Phosphoenol pyruvate

L - glycerol - 3 - phosphate + O_2 ⟶ DHAP + H_2O_2 3

L - glycerol - 3 - phosphate + H_2O_2 ⟶ DHAP + $2H_2O$ 4

2L - glycerol - 3 - phosphate + O_2 ⟶ 2DHAP + $2H_2O$ 5

However, this is probably an oversimplification since in preparations containing this enzyme no catalase and virtually no activity of H_2O_2 has been detected.[7,37]

Whatever the exact pathway, the coupling of glycolysis to G.P.O. is unique to these organisms and helps them overcome the problem imposed by their lack of lactate dehydrogenase[32] for re-oxidation of NADH. In this way trypanosomes are able to convert 82-100% of glucose to pyruvate.[27,32,33,36]

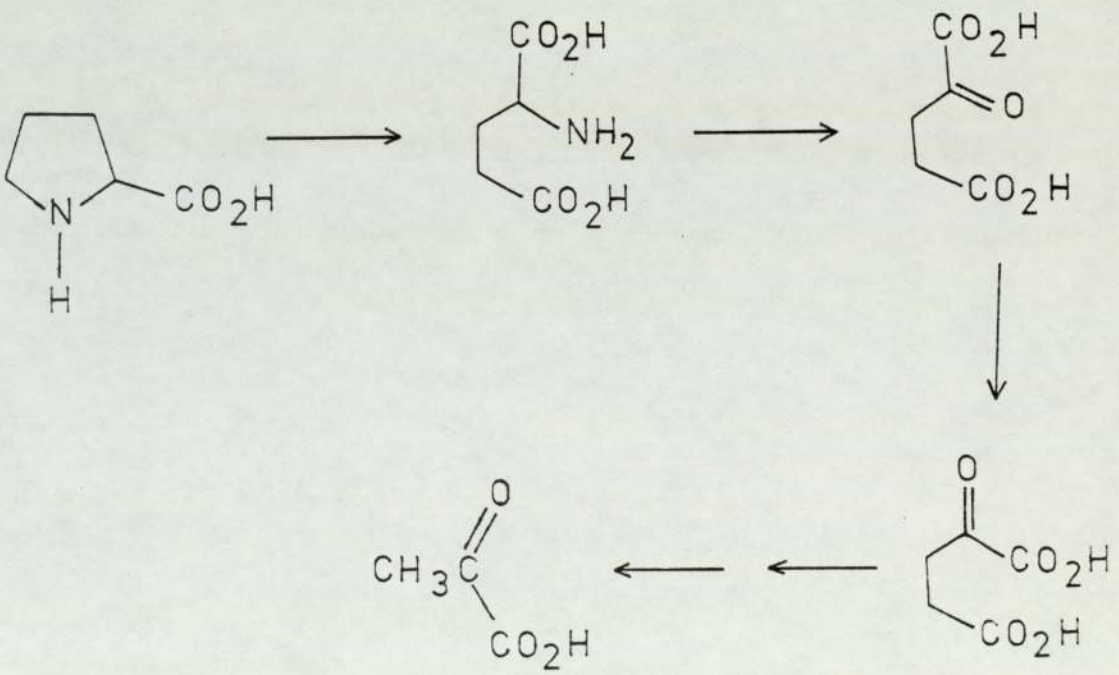
2) Lipid Metabolism

Lipids are unimportant as a source of oxidisable substrate for the supply of energy for trypanosomes. Very little is known about the degradation of lipids in trypanosomes. Only minimal quantities of fatty acids are used by blood stream forms. T.b. rhodesiense utilises 0.008 n moles h^{-1} mg^{-1} of its dry weight of palmitate computed from $^{14}CO_2$ of [1-^{14}C] labelled substrate. This contrasts sharply with utilization of 1500 n moles $h^{-1}mg^{-1}$ dry weight of glucose.[30] Bloodstream forms cannot split tristearyl glycerol and tripalmityl glycerol to furnish fatty acids for oxidative degradation.

3. Amino Acids Metabolism

Synthesis of amino acids by trypanosomes has received little attention.[43] It has been found that in pleomorphic and monomorphic forms of salivarian species 2.5% of their total cell protein content is amino acids; and the main component of these amino acids is alanine which is synthesized from glucose.[30] Other amino acids synthesized by trypanosomes are aspartic acid, glycine, serine and glutamic acid.

More information is available on the catabolism of amino acids. Blood forms of T.b. brucei and T.b. rhodesiense cannot catabolise proline, glutamic acid and alanine. Culture forms of these organisms depend on proline catabolism for energy production. The breakdown steps are outlined below:-



The use of this substrate is an adaptive measure as the insect host depends on this amino acid for its energy source for the flight muscles.

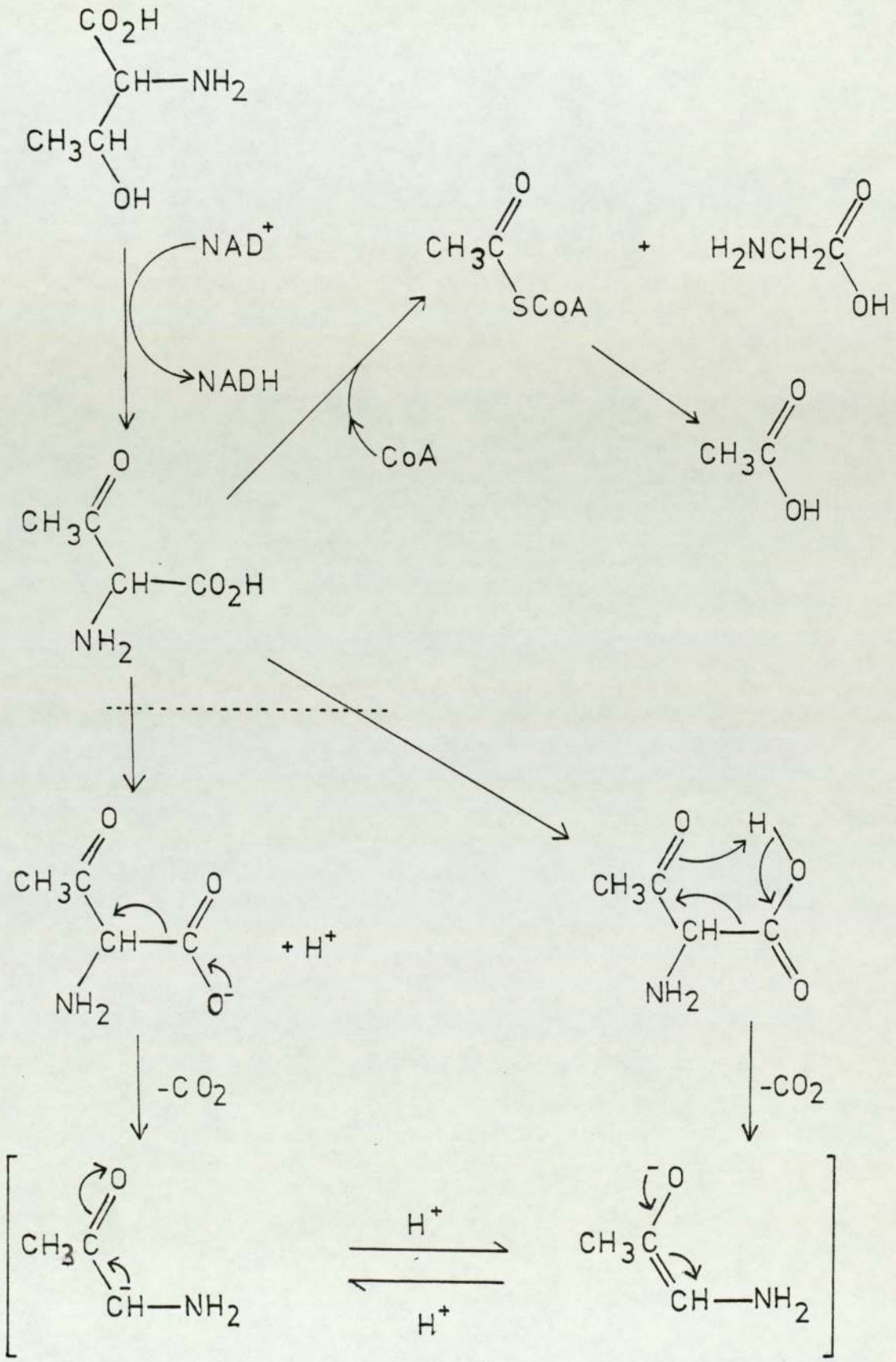
Both blood-stream and culture forms of T.b. brucei show a preference for threonine over glucose as an alternative source of a two-carbon fragment (acetyl) in the generation of Acetyl CoA. In the presence of 30 molar fold excess glucose, threonine contributes about 50% of fatty acyl chain.[29] Acetyl CoA is used in the de novo fatty acid chain elongation. Threonine degradation follows the scheme outlined below (scheme 2).

Investigations carried out by Newport et al[44] in field voles (Microtus montanus) infected with T.b. gambiense revealed a considerable alteration of their free amino acid level composition.

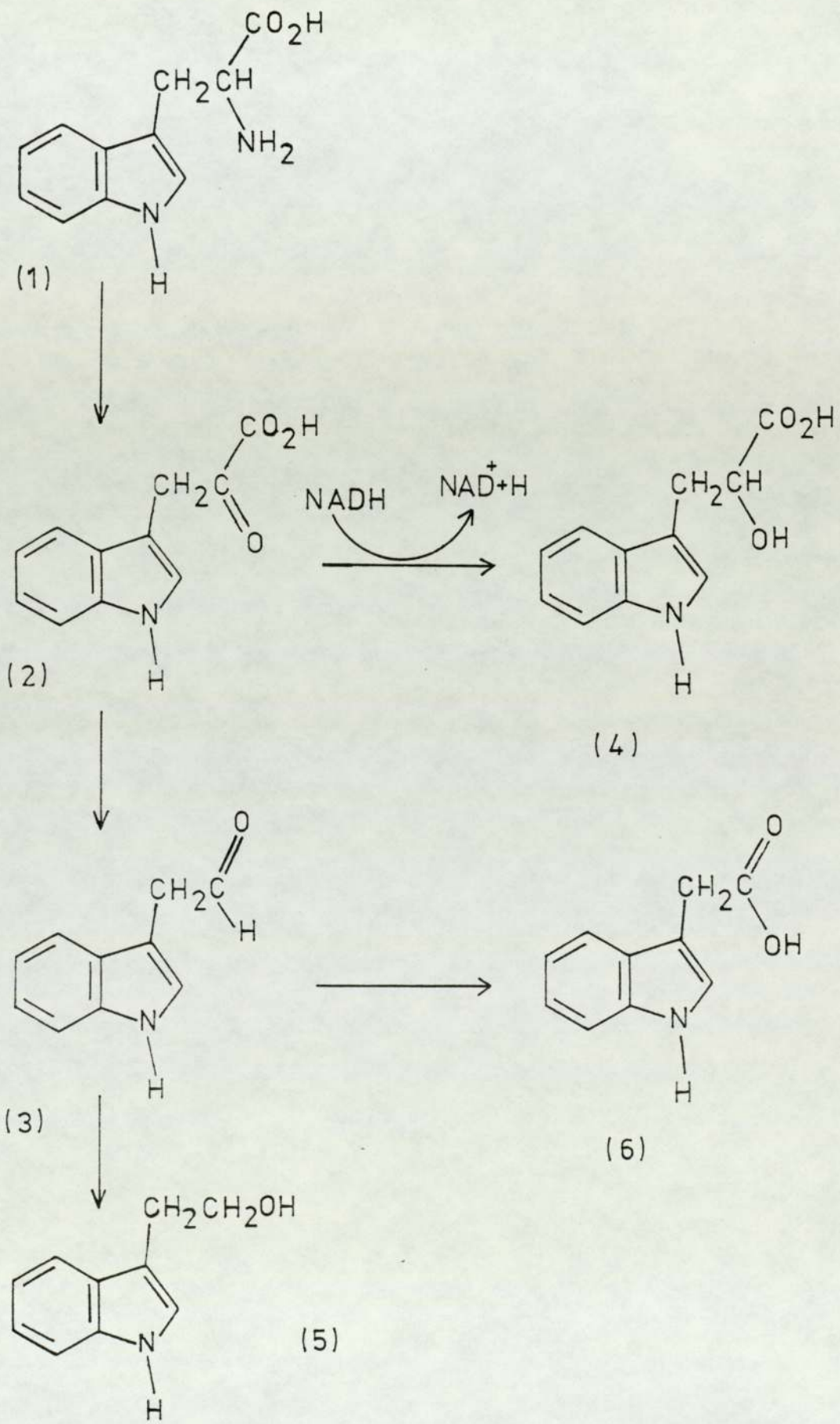
Tryptophan (1) which was reduced below detectable level in the serum was found to be degraded to indole - 3 - lactic acid (4) in the presence of NADH and to 2-(indol-3-yl) ethanol (tryptophol) (5) and indole - 3 - acetic acid (6) in vitro in the absence of added NADH.[17,19] The last two named products accounted for 76% of the metabolites found in vivo using tracer [^{14}C] tryptophan.[19] The scheme below outlines the pathway of tryptophan (1) metabolism.[15,18,45,46] Of the eighteen amino acids examined, four of them; threonine, valine, tyrosine (7) and tryptophan (1) were decreased by about 17-100%. Since in the same voles, adrenal glands were found to have been hypertrophied it was suggested that the alteration may have been due to impairment of the regulatory mechanism at the hormonal level.

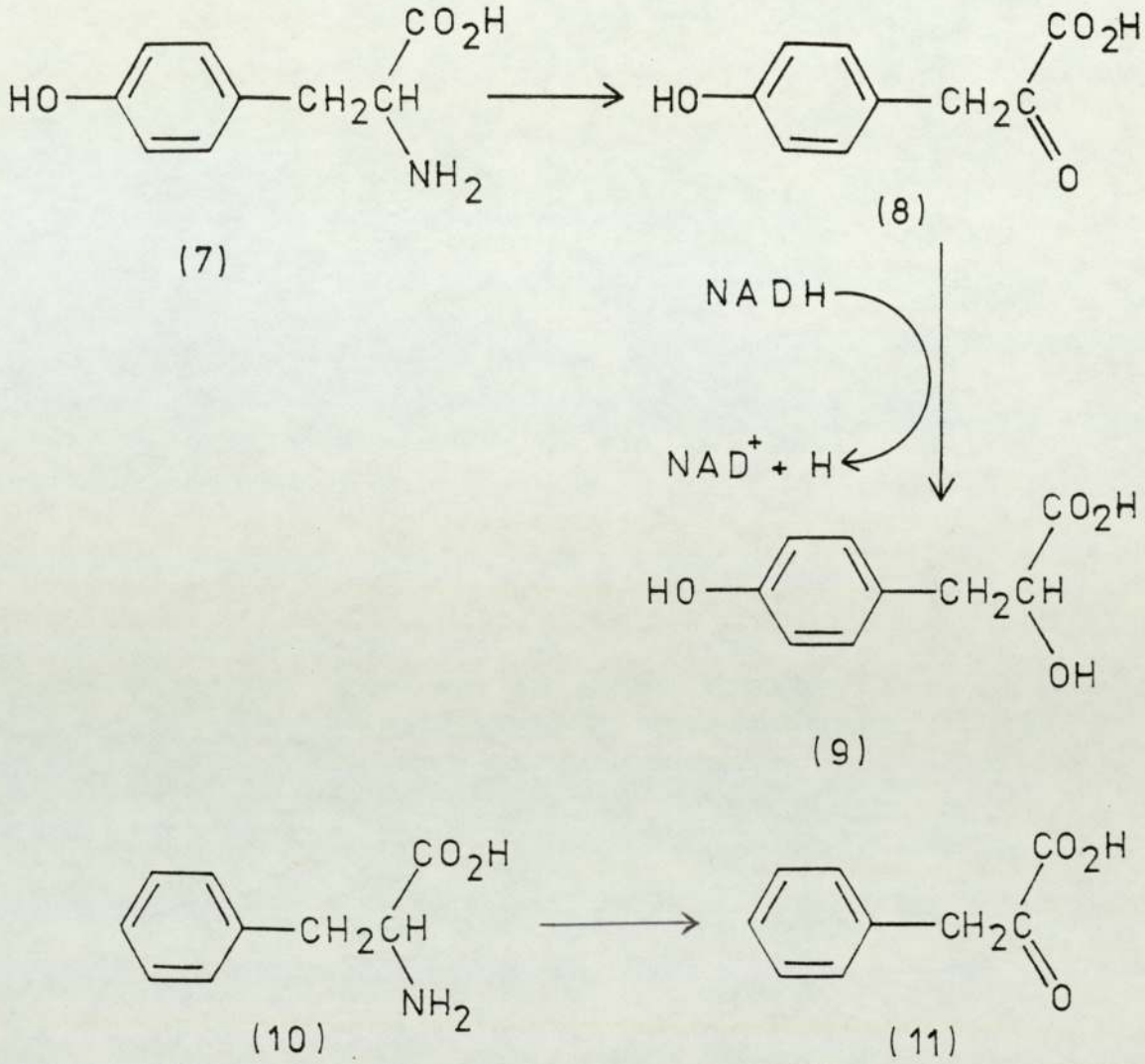
Subsequently it was established that of the four amino acids, significantly reduced in the course of the infection, two were catabolised to various products. Tyrosine with a reduction level of 50% is converted to p-hydroxyphenyl acetate (9) thus:[16]

SCHEME 2


CO2H
CH—NH2
CH3CH
OH
NAD+
NADH
CH3C
O
SCoA
+
H2NCH2C
O
OH
CoA
CH3C
O
OH
CH3C
O
CH—CO2H
NH2
CH3C
O
CH
C
O
NH2
O-
+ H+
-CO2
H
O
CH3C
O
CH
C
O
NH2
-CO2
CH3C
O
CH—NH2
H+
H+
CH3C
O-
CH—NH2

SCHEME 3


CO2H
CH2CH
NH2
N
H
(1)
CO2H
CH2C
O
N
H
(2)
NADH
NAD+H
CO2H
CH2CH
OH
N
H
(4)
O
CH2C
H
N
H
(3)
O
CH2C
OH
N
H
(6)
CH2CH2OH
N
H
(5)



Phenylalanine (10) was only converted to phenylpyruvate (11). Two different transaminase enzymes are involved in the catabolism of these amino acids. The amino group is transferred to α- ketoglutarate. Synthesis of p-hydroxyphenyl acetate (9) may serve to regenerate NAD^+ reduced during glycolysis and threonine catabolism. The enzyme system catalysing the conversion of tryptophan (1) to indole-3-acetic acid (6) and indole-3-ethanol (5) are not sensitive to inhibition by iproniazid, pyrazole and diethyl dithiocarbamate. The corresponding alcohol and aldehyde dehydrogenase enzymes of mammalian origin are sensitive to these inhibitors.[19] Thus the alcohol and aldehyde dehydrogenase enzymes of trypanosomes are different and unique to them.[45]

In table 2 below are shown the quantities of the various catabolites of tryptophan (1), tyrosine (7) and phenyl alanine (11) found in uninfected and infected mice by Hall and Seed.[47]

Table 2 Quantities of Aromatic Amino Acids Catabolites Present in Urine of Mice Uninfected and Infected with T.b. gambiense for 72-80h. Values are Expressed as Average μ Moles Excreted per 8h per Animal (± S.E)

	PPyA	HPPyA	IPyA	ILA	IAA	TOL
Uninfected	0.242	0.181	0.091	0.009	0.126	ND
	(0.008)	(0.007)	(0.007)	(0.001)	(0.003)	
Infected	3.401	1.457	1.703	0.181	0.252	ND
(72-80h)	(0.422)	(0.071)	(0.117)	(0.004)	(0.007)	
Infected/uninfected	14.1	8.1	18.7	20.1	2	-

PPyA = phenylpyruvic acid, HPPyA = hydroxyphenyl pyruvic acid, IPyA = indole - 3 - pyruvic acid, ILA = indole - 3 - lactic acid IAA = indole - 3 - acetic acid, TOL = tryptophol[2-(indole -3- yl)]acetic acid).

The table shows that there was a significant disturbance in the metabolism of aromatic amino acids by infected mice. The significance of tryptophan (1) catabolism to the trypanosomes is that it may be linked to carbohydrate metabolism.[46] The initial step is transamination of the amino group to an α - ketoglutaric acid and subsequently transferring this amino group to pyruvic acid to form alanine. This could be a detoxifying step removing the large intracellular quantities of pyruvic acid produced during glycolysis.[17,45] Further the twenty-fold increase of urinary indole - 3 - acetic acid suggests that this pathway may be important in the redox balance by re-oxidation of NADH formed during glycolysis and threonine

breakdown.

E. Control, Host Immunity, Chemotherapy and Chemoprophylaxis

Over the years since the discovery of the disease and its vectors a number of control programmes have been devised and tried. Most of them have been aimed at disrupting transmission chains by eliminating the vector. The attack on the vector took two forms; direct and indirect measures. The direct measures aimed at killing the tsetse fly at various points in its life cycle. Such measures have included trapping, hand catching, use of predators, release of sterile males and spraying with insecticides. The more popular insecticides have been dichlorodiphenyl trichloromethane (DDT), benzene hexachloride (BHC) (Gammexane), endosulphan (Thiodan), the organophosphorous fenthion (Baytex) and pyrethrum extracts.

Indirect methods concentrated on modifying the environment to render it inhospitable for the tsetse flies. The popular ones have included partial bush clearance to deny the vector resting places, selective killing of wild animals (e.g. bushbuck.) known to act as natural parasite's reservoir on which tsetse flies feed.

Detailed reviews of these control methods including appraisal of the logistical and administrative problems as well as evaluation of the effectiveness of the methods are to be found in the monograph 'The African Trypanosomiases' edited by H.W. Mulligan (1970). These methods will not be discussed further in this thesis.

1) Antigenic Variation and Host Immunity

Precise information on the nature of trypanosomal antigens produced during the course of infection is largely lacking. In the

past, ~~only the aspect involving~~ the multiplicity of antigens, has attracted all the attention. It is well known now that one of the characteristic features of trypanosomal infection is the appearance of new antigenic populations at predictable intervals. The appearance of the new population is preceded by a 'crisis' in which the earlier different antigenic population is destroyed. This destruction of the trypanosomes in the peripheral circulation is caused by interaction with the host antibodies which are released at that time. Only the antigenically different population escape this host immune response and go on to establish the next wave of parasitaemia.

It has been estimated that a single trypanosome is capable of producing over twenty different antigens. The actual number produced depends on the length of time the host lives after the infection.[15,48]

The surface coat of the trypanosome is associated with the variant specific or surface antigen (VSA). This coat consists of glycoproteins about 10-50 nm thick and has an apparent molecular weight of 64000 - 65000 and it accounts for 5 - 10% of the total cell protein.[15,49] Most of the information on antigenic variation has been collected from this glycoprotein. The variant specific antigens show similar sizes, carbohydrate content, but a wide variety of amino acids composition as shown by the N-terminal sequence analysis.[49]

To account for the ability of trypanosomes to alter their antigenic constitution in order to evade the host's immune response a number of hypotheses have been put forward.[50] The variation is suggested arises: (a) due to the influence of the host's antibody release,

(b) by random mutation and selection,

(c) when a dominant strain in the population is eliminated by host antibody, allowing the next most virulent strain to establish the next wave of parasitaemia,

(d) as a result of cell-independent immunochemical antigen-antibody reactions on the cell surface which expose new antigenic determinants.

(e) from genetically coded information within the trypanosome.

There was no conclusive evidence in support of any of them. Recently, however, evidence to support the last mentioned hypothesis (e) has been forthcoming. A specific mRNA responsible for the synthesis of variant specific antigens has been isolated, purified and in vitro shown to produce an antigen (VSA 121) which was identified by gel electrophoresis.[51,52]

A partial characterisation of variant specific antigens has been accomplished by both N- and C- terminal sequences.[49,53] N- terminal amino acid sequencing has shown this part of the glycoprotein to be homogenous but has no homology. The only sequence homology has been found in the C- terminal around the glycosyl groups. In three of the five variants analysed the sequence Asx Xxx Thr/Ser has been found but extensive comparison is not yet possible.

The sugar linkage occurs on aspatic acid or asparagine and the terminal serine. The carbohydrate moieties present are of two kinds; the first contains mannose and glucosamine while the second contains both these as well as galactose.

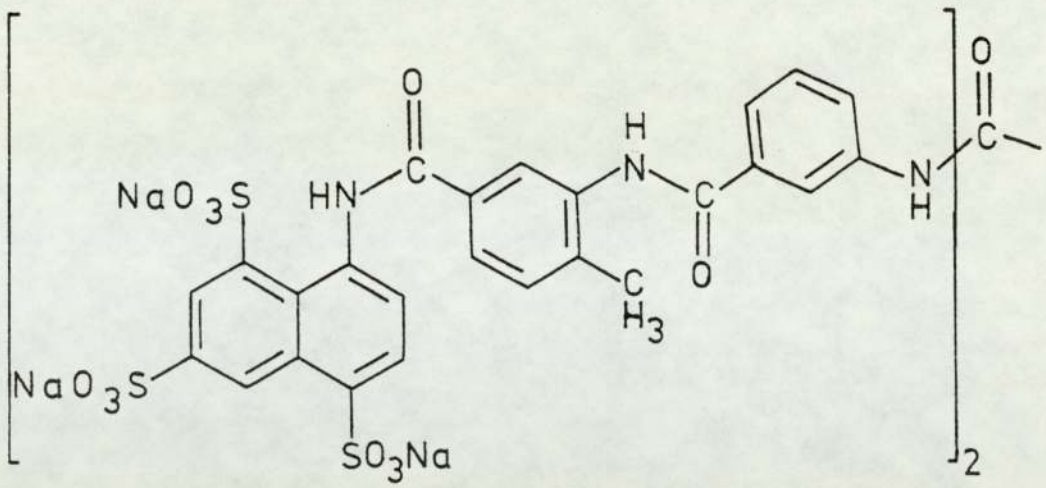
Since sequential cleavage techniques only afford the identification of the initial 30-40 residues of the intact protein, this means that only 6% of the glycoprotein has thus been analysed and much more awaits to be done.

Antigenicity of the glycoproteins was formerly considered to be conferred by the carbohydrate moiety in the C-terminus. However it has been found that purified variant specific glycoproteins from different populations show extensive cross reaction with specific antisera, but showing none of the cross reaction when on intact trypanosome suggested otherwise.[54] Until more information is available, this aspect of specificity must remain in doubt, and, by the same token, control of trypanosomiasis by vaccines derived from these organisms remains more remote.

2) Chemotherapy and Chemoprophylactic agents

Chemotherapy and chemoprophylaxis play an important role in the control of trypanosomiasis and will undoubtedly continue to do so. Unfortunately only a handful of drugs are available for this and all of them have been in use for more than twenty five years and all have serious deficiencies.[6,55]

Among the agents used for chemotherapy and chemoprophylaxis, suramin (12) is the most important. It was introduced in chemotherapy following Ehrlich's pioneering work on antitrypanosomal activity of azo dyes. Investigation of the structural requirements for optimal activity established that the trisulphonated naphthylamine, the m-amino and the p-methyl groups of the benzoyl groups were essential for the activity of the drug.



Suramin (12)

Recent re-investigation of suramin (12) and various residues of the compound has confirmed this earlier observation.[56]

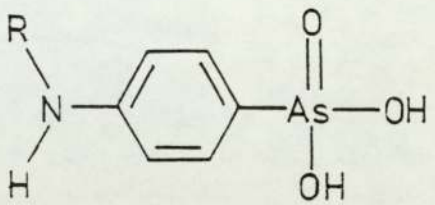
Despite being clinically used for more than fifty years the mode of action of suramin is still largely unknown.[55] Recently it has been shown to bind to two enzymes. It binds competitively to GPO and binds to protein kinase I non-competitively.[40,56,57] It has been suggested that the mode of action of suramin includes inhibition of phosphorylation of regulatory proteins and enzymes of aerobic glycolysis.[34,57]

The shortcomings of suramin include its ineffectiveness in late stage infection and its toxicity which include vomiting, shock and collapse which is occasionally fatal.[58] Other adverse reactions include pruritis, urticaria, popular eruption conjunctivitis, photophobia, oedema, haemolytic anaemia, necrosis at the site of the infection and nephrotoxicity.

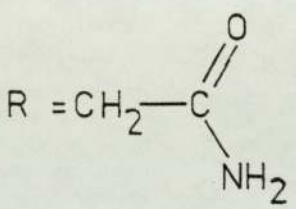
The first arsenical drug to be used against trypanosomiasis was atoxyl (13) in 1905 followed by tryparsamide (14). The active form

of both drugs is the reduced trivalent arsenic produced *in vivo*. To exploit this property and enhance the activity of the drugs, new ones were prepared as melaminyl derivatives containing trivalent arsenic. They have largely replaced atoxyl (13) and tryparsamide (14).

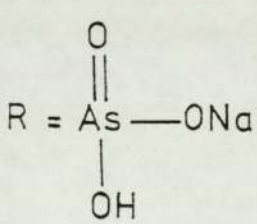
Atoxyl (13) R = H

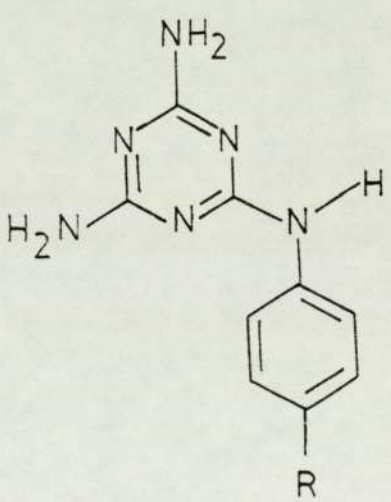


Tryparsamide (14)

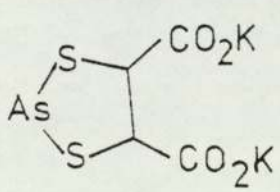


Melarsen (15)

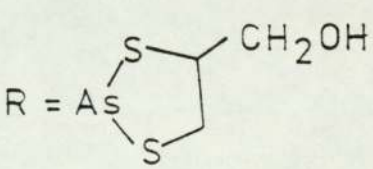




Melarsonyl (16) R =



Melarsoprol (17)

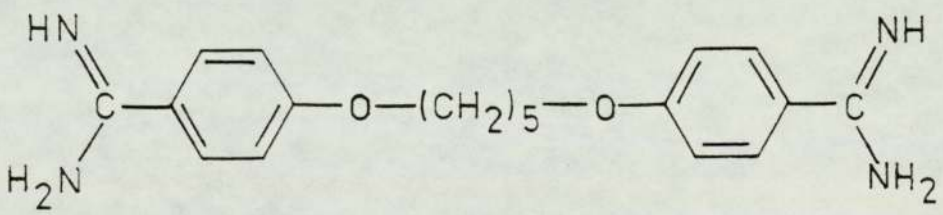


The new drugs were effective in cases resistant to tryparsamide (14); and in addition, melarsoprol (17) is effective in advanced cases of the Rhodesian disease.

The mode of action of arsenical drugs is inactivation of thiol groups of enzymes vital for the survival of the trypanosomes.

Toxic effects of both atoxyl (13) and tryparsamide (14) were the atrophy of the optic nerve[59] while the melaminyl derivatives cause encephalopathic reactions; the first reactive encephalopathy about 13% is reversible and haemorrhagic encephalopathy is a delayed reaction but often fatal.[58]

Of a number of analogs derived from diamidine only pentamidine (18) has come into general use as a prophylactic and curative drug in the Gambian disease. It is available as a methane sulphonate or isethionate salt.



(18)

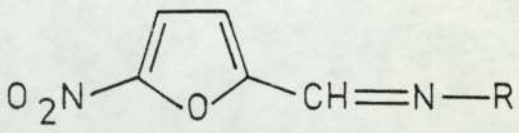
Its mode of action is still unknown in detail. In low concentrations it inhibits amino acid transport as well as DNA and RNA synthesis, but at higher concentration it inhibits respiration.

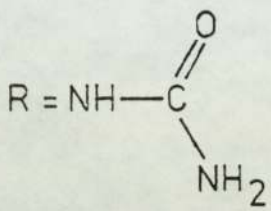
Toxic effects of pentamidine (18) include vasodepression, hypoglycaemia, and histamine liberation. It is retained in the liver, kidney and adrenals for as long as three years and this could lead to cumulative damage occurring.

Derivatives of nitrofuran have been reported to be active in both African and South American trypanosomeases, and in cases

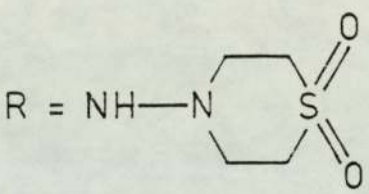
resistant to suramin. Nifurtimox (20) is now in use against South American trypanosomiasis.

Nitrofurazone (19)





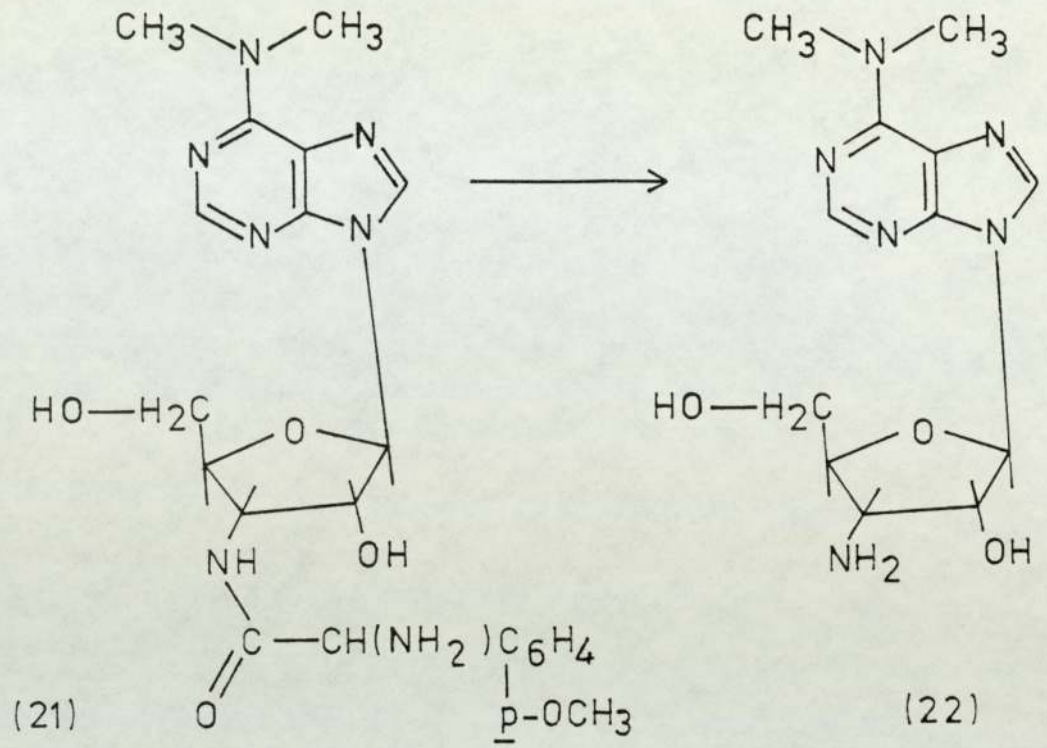
Nifurtimox (20)



They are thought to act as electron acceptors thereby disrupting NAD^+ oxido-reduction.

Toxic reactions include haemolytic anaemia for individuals deficient in glucose-6-phosphate dehydroganase which may be up to 20% among Africans.[55] Other toxic effects include testicular tubules degeneration and neuropathy.

The only antibiotics known to have significant antitrypanosomal activity belong to a group of nucleoside antibiotics of which puromycin (21) is the most important. In vivo puromycin is hydrolysed to give puromycin amino nucleoside (PAN) (22) which is the active compound. Serious nephrotoxicity precluded its ever being used clinically. The toxic substance is thought to be N-demethylated puromycin amino nucleoside which is incorporated into nucleotides through the 5'-OH group.



(21) (22)

Recently it has been shown that the toxicity of puromycin (21) can be eliminated without loss of activity by replacement of 5'-OH by H or Cl.[50] Puromycin (17) acts by interfering with t RNA and terminating polypeptide chain growth in the ribosomes.[56]

3) Recent Advances: GPO Inhibition

In recent years there has been a more rational design of novel agents or specific molecular modification patterned against particular targets, or used to improve activity or exploit known biochemical differences. The results of these investigations have given more insight into the biochemical processes of trypanosomes.

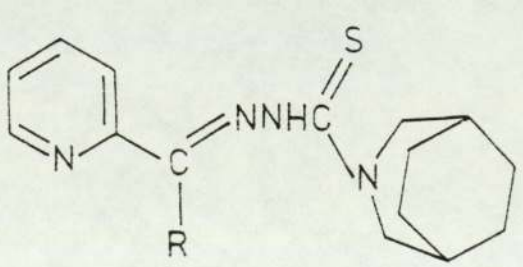
The dependence of blood stream forms on glucose metabolism for the generation of energy and the discovery of GPO linked to glycolysis formed a possible target for selective attack by chemotherapeutic agents. This was realised when it was discovered that the enzyme

was sensitive to inhibition by hydroxamic acids. *In vitro* investigation showed that although salicylhydroxamic acid (SHAM) inhibited GPO, it did not result in the death of the trypanosome cells. Glucose utilisation continued with production of glycerol and ATP as the end products.[32] Combination of SHAM and glycerol was lethal to the cells.[31,33] However *in vivo* results showed that relapse parasitaemia followed on the sixth day after treatment.[12] It was suggested that parasites outside the vascular system had not been affected by the SHAM/glycerol combination since *T. vivax*, a non-extravascular parasite, did not show relapse.[60] However determination of the quantities of the different substrates involved showed that the inhibition by glycerol in the presence of SHAM was progressive and glucose continued to be consumed.[33] It has now been shown that when GPO is inoperative either under SHAM inhibition or anaerobiasis, trypanosomes can dephosphorylate glycerol-3-phosphate by coupling it to the phosphorylation of ADP.[62] Hence glucose consumption continues as summarised by equation 6.

$$\text{Glucose} + \text{Pi} \longrightarrow 2\ \text{PGA} + \text{glycerol} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 6$$

In addition it is not easy to maintain therapeutic levels of SHAM as it is actively excreted by rodents.

From a slightly different standpoint it has been shown that enzymes containing transition metals are highly susceptible to chelation by thiosemicarbazones. It was suggested that GPO which contains iron should be inhibited by thiosemicarbazones of acetyl pyridine. This was borne out experimentally when out of twenty seven derivatives one of them (23, R = H) was found to be active.[63]



(23)

The glycosomal NAD^+ sn-glycerol phosphate dehydrogenase component of GPO requires polyamines (putrescine, spermadine and spermine) to function fully. It has been envisaged that inhibition of polyamine biosynthesis could be therapeutically useful.[64] A specific inhibitor of this pathway, difluoromethylornithine (DFMO), has been tested against T.b. brucei in vivo and initial results indicate that it is effective in doses of over 75 mg/kg.

During re-oxidation of NADH by GPO a small amount of hydrogen peroxide is formed and excreted or difuses out of the cell. Meshnick et al[7] suggested that agents that lead to increased production of hydrogen peroxide, and caused its homolytic cleavage to radicals, and if such agents also reduced the amount of GSH, trypanosomes could be more susceptible to damage by the radicals. This has been shown to be possible, in vitro at least, using naphthoquinone as electron acceptor to increase hydrogen peroxide production, haem to cause radical formation, and melarsen oxide to reduce the level of available GSH. However a compound with these properties for in vivo activity is yet to be developed.

Blood stream trypanosomes and tumour cells have certain metabolic similarities. The resemblance was seen when the mode of action of puromycin and its derivative puromycin amino nucleoside was compared in these two systems. The similarities have been summarised by Williamson[65] and Kinnamon et al[66] thus:-

a. Both trypanosomes and tumour cells derive energy from high rates of aerobic glycolysis due to nonfunctional or inefficient mitochondrial system.
b. In both systems the pace-making glycolytic enzymes are hexokinase and pyruvate kinase.

c. In both, the glycolytic pathway may be altered by agents such as arsenical antitrypanosomal agents.

d. Antitumor adenine nucleosides related to puromycin are trypanocidal, they include cordycepin, nucleocidin, tubercidin (7-deaza-adenosine).

e. Adenine or adenosine reverses both the antitumor and the antitrypanosomal activity of the above agents.

On the basis of the resemblances above, it might be expected that compounds with antitumor activity may show anti-trypanosomal activity and vice versa. Forty nine antitumor compounds randomly selected from a list of eighty one were screened against T.b. rhodesiense and six of them showed some activity.[66] Their structures are shown below (Scheme 4).

Following this success, Kinnamon et al[67] reported that they had screened a further 303 compounds against T.b. rhodesiense and found a total of 25 active compounds. The results are summarised in the table below.

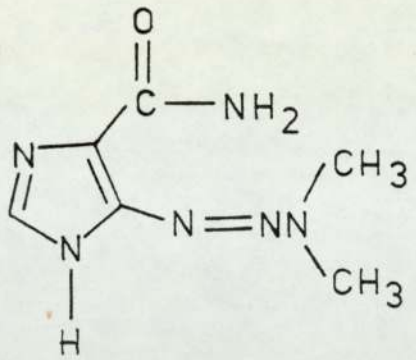
Table 3 Summary of Results of Testing Compounds Against T.b. rhodesiense in Mice in Antitryspanosomal Activity

Anticancer activity	Yes	No	Total
Yes	15	51	66
No	10	227	237
Total	25	278	303

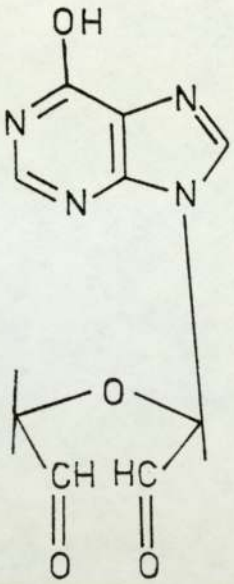
No reports of follow up on these and earlier compounds are available.

The antitrypanosomal activity of arylamidine derivatives of several

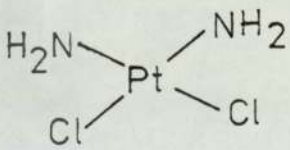
SCHEME 4



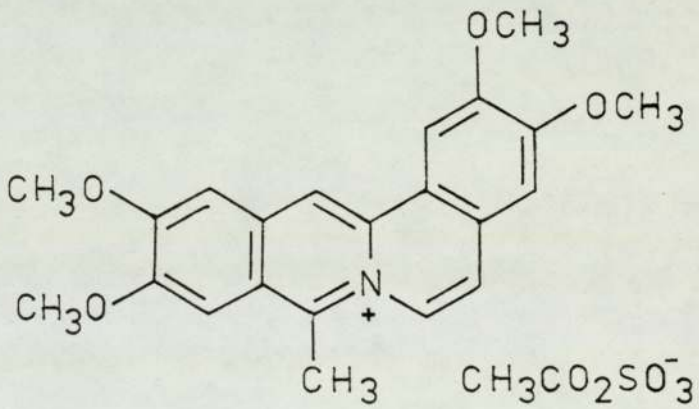
(24) Imidazole -4-carboxamide - 5-(3,3-dimethyl)--1-triazine.



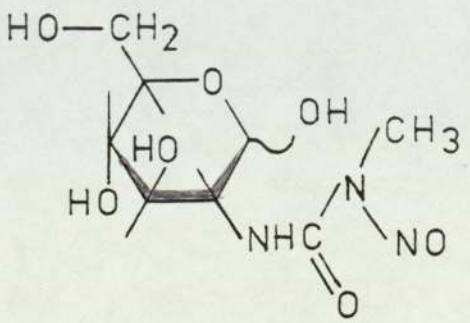
(25) Inosine diglycoaldehyde.



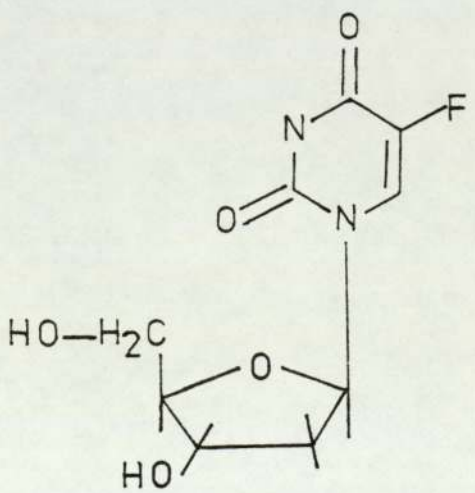
(26) Cis-diamino dichloroplatinum.



(27) Ceralyne sulphate.

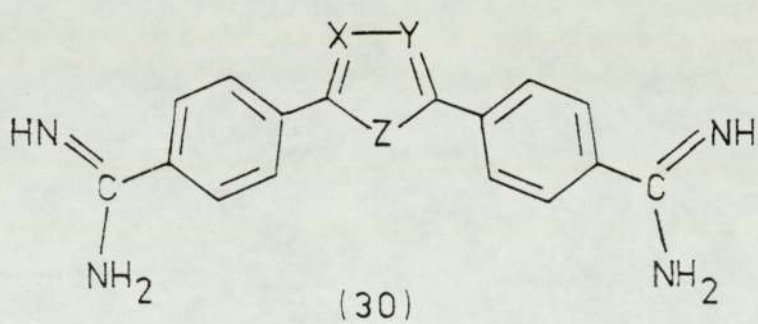


(28) Streptozoticin.



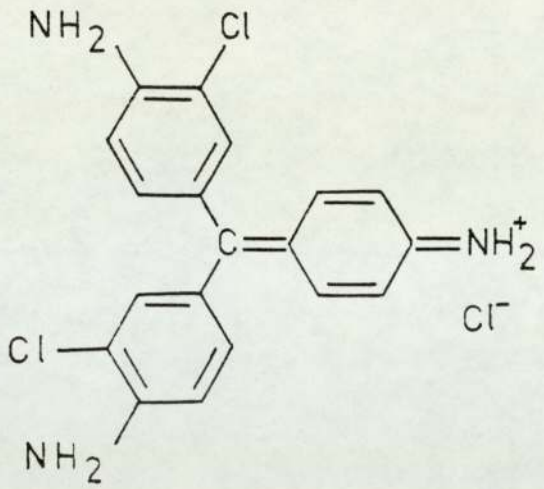
(29) 5-Fluoro-2-deoxyuridine.

ring systems have been investigated by Das and Boykin[68] and Das et al.[69] Their compounds were prepared as 2,5-bis ~~guanyl~~ amidino phenyl derivatives of furan (30, X=Y=CH, Z=O), oxazole (30,X=N, Y=CH, Z=O), thiadiazole (30,X=Y=N, Z=S) and 3,6 bis ~~guanyl~~ amidino phenyl pyridazine (30, X=Y=CH, Z=N=N) derivatives and tested against T.b. brucei. Taking 3,4-dimethyl-2,5-bis (4-~~guanyl~~ amidino phenyl) furan (30, X=Y=CH, Z=O), their best active compound as the standard these workers have made several conclusions. Best activity was observed in furan, oxazole and thiadiazole derivatives with only 4-~~guanyl~~ amidino phenyl as substituents. Cyclic amidines showed increased toxicity and lower activity. Introduction of one methyl group or one chlorine atom in the furan ring enhanced the antitrypanosomal activity but two chlorine atoms reduced the activity of the furan-ring derivatives. Presence of both a methyl group and a chlorine atom in the furan ring did not affect activity significantly.

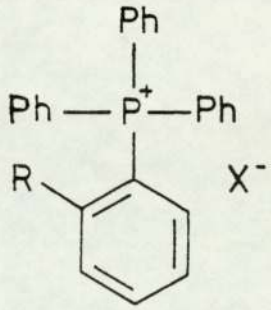


(30)

Triphenyl methane dyes such as tryparsen (31) which are anti-trypanosomal are structurally similar to the Wittig reagents. On the basis of this similarity a number of benzyl triphenyl phosphonium salts were prepared and tested against T.b. rhodesiense.[39] Of the 70 compounds tested, twenty showed activity. Activity was enhanced in the chloro-derivative (32,R=Cl) but dropped by 40% in the methyl compound (32,R=CH_3).



(31)



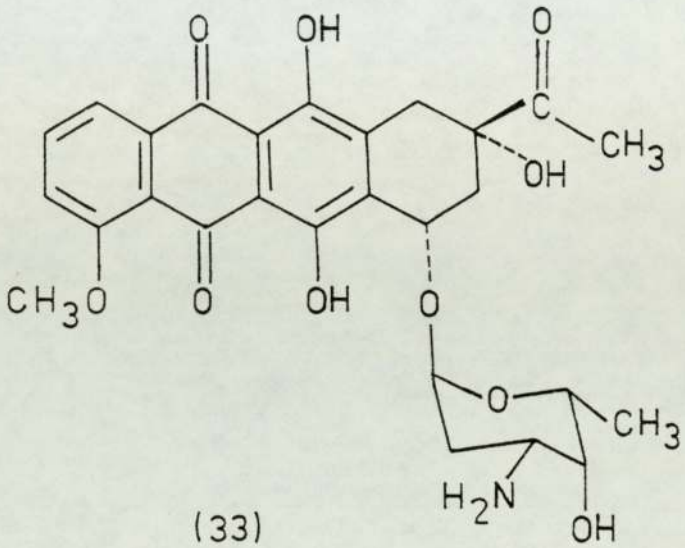
(32)

A recent report by Dunn et al[70,71] indicated that triazines had activity against T.b. rhodesiense though high doses of the order of 400 mg/kg were required.

Intracellular gluta+hione (γ-glutamyl cysteinyl glycine) defends the cell against hydrogen peroxide and free radicals. Depletion of this amino acid should render trypanosomes more susceptible to damage by hydrogen peroxide as they lack catalase. Bradley et al[72] have used buthionine sulfoximine (BSO), a specific inhibitor of glutathione biosynthesis, to deplete trypanosomes of glutathione. Mice infected with T.b. brucei were cleared of parasites when treated with buthionine sulfoximine for 27 hours at 1.5 hourly intervals. The compound is non-specific in its action, and treated animals were hypothermic and died on the third day.

Loss of motility upon incubation of trypanosomes with chemical compounds is used as a marker of inhibition of glycolysis. Loss of infectivity is used as an index of cell division. These two criteria were used by Williamson and Finnigan[73] in 1978 to screen a large number of compounds known to have specific loci of inhibition.

Many of them showed <u>in vitro</u> activity, of which daunorubicin (33), an anthracycline antibiotic, showed the most promising results. It did not show <u>in vivo</u> activity, presumably because it was excreted rapidly. Recently the same workers[74] showed that when daunorubicin (33) is administered as a Schiff base conjugate of glutaldehyde coupled to albumin and ferritin has <u>in vivo</u> activity.

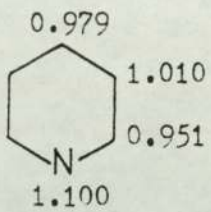


(33)

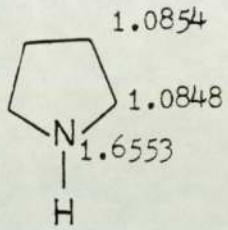
II Pyrrolo[2,3-b]pyridines

A. Synthesis of 1H-pyrrolo[2,3-b]pyridines

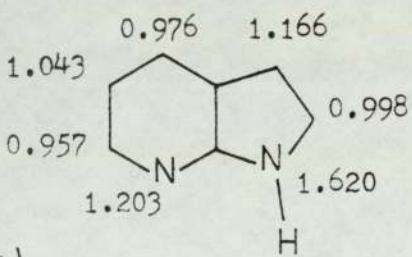
The fusion of the π-electron-deficient pyridine ring (37) and the π-electron-excessive pyrrole ring (38) gives 1H-pyrrolo[2,3-b]pyridine (36) in which the π-electron density is re-distributed as shown (36a). The π-electron distribution in all the three ring-systems and that of indole (39) are shown below.[76,77,78]



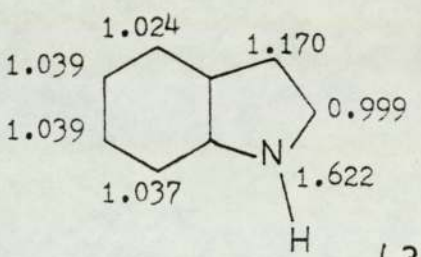
(37)



(38)



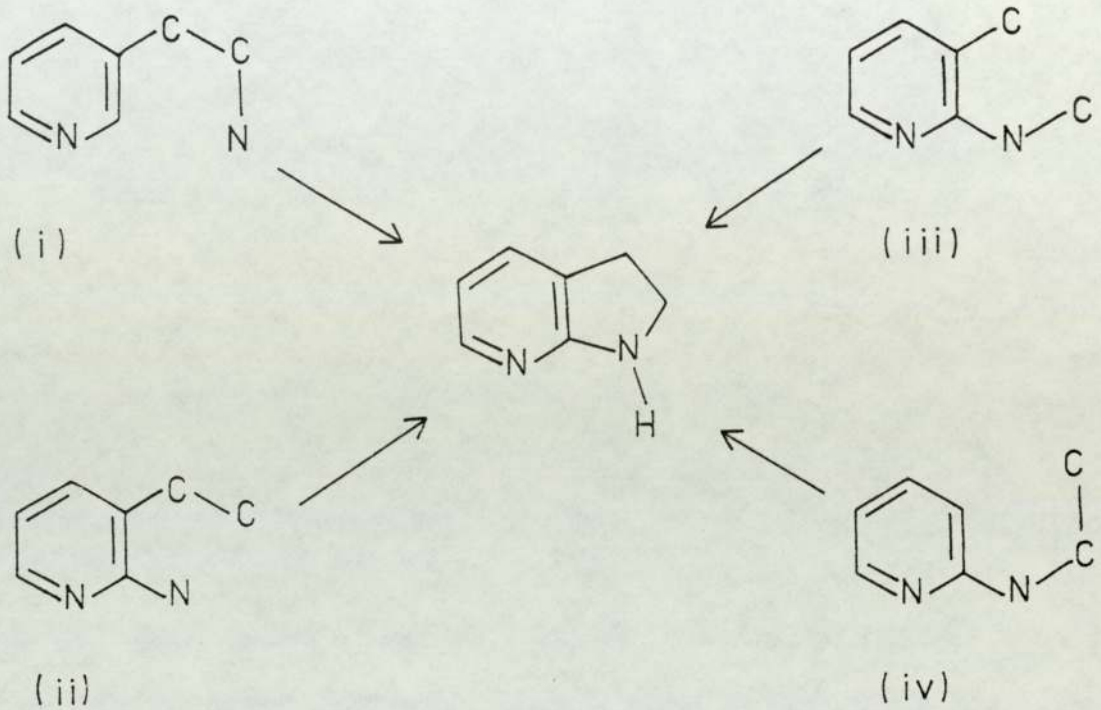
(36a)



(39)

In the 1H-pyrrolo[2,3-b]pyridine (36a) the π-electron density is reduced at positions 1 and 2 and increased at position 3 of the pyrrole ring compared to pyrrole (38) while in the pyridine ring the density of the electrons is reduced at position 4 and increased at positions 5,6 and 7 as compared to the pyridine ring (37). The consequences of this π-electron re-distribution on the chemistry of 1H-pyrrolo[2,3-b]pyridine is significant and is discussed further in relation to the synthesis and properties of this ring-system.

There are four possible ways a pyrrole ring could be formed on the pyridine ring; and all of them have been reviewed by Willette,[79] Yakhontov,[80] and Herbert[81]. The methods are schematically summarised below.

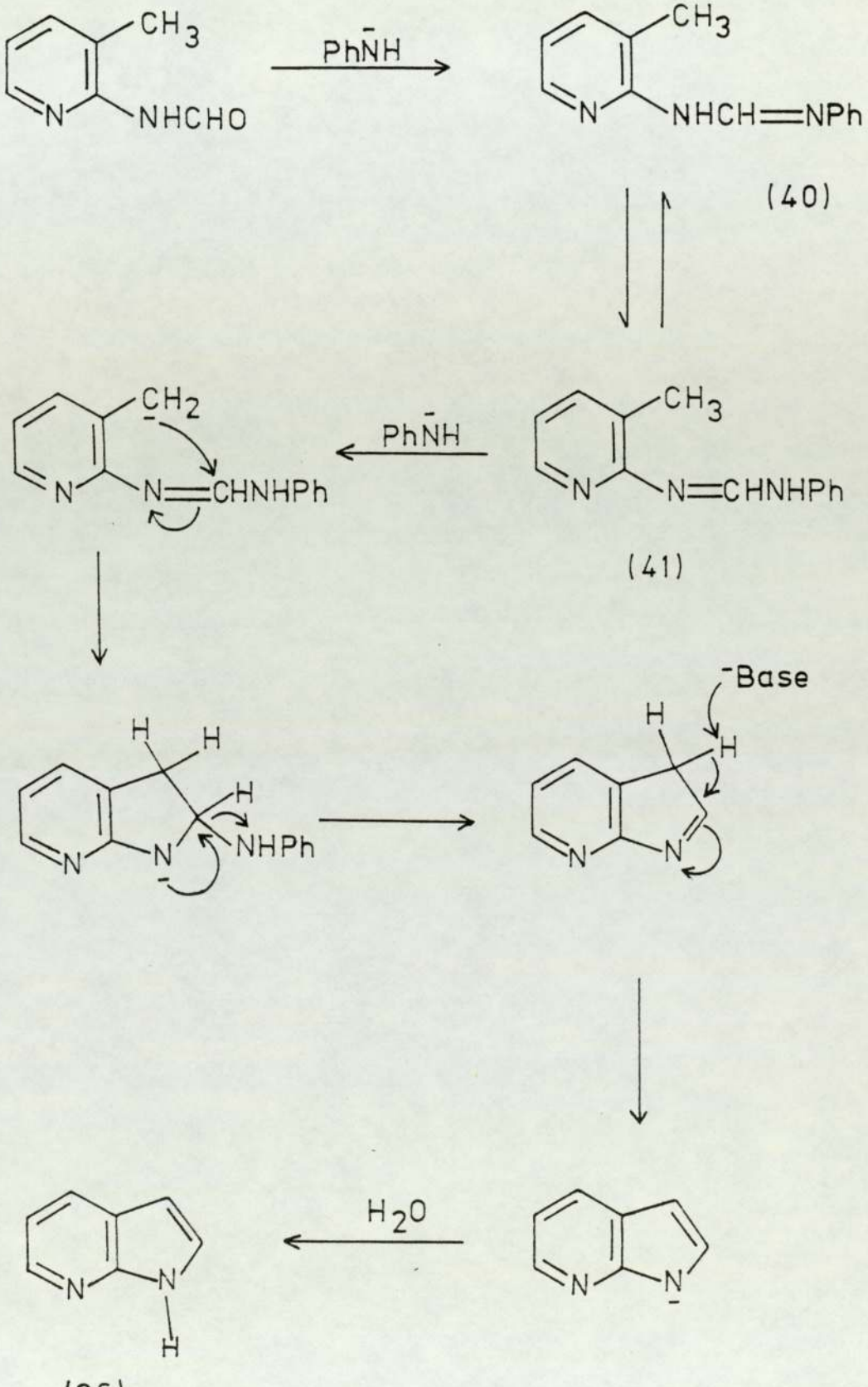


Of the four ring closure methods, types (i) and (ii) have been used to a limited extent only because of the inaccessibility of suitably substituted pyridine starting materials.

Type (iii) is the extension of the Madelung indole synthesis. The method involves the heating at high temperature of 2-amido-3-methyl-pyridine derivatives with a basic catalyst. It is the most useful method for the preparation of the parent ring 1H-pyrrolo[2,3-b]-pyridine (36). It has been widely investigated and was modified by Lorenz et al[82] who proposed the mechanism below (scheme 5) for the cyclisation.

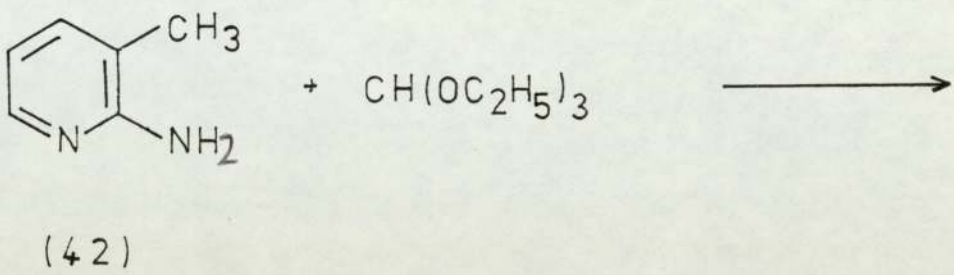
To support this mechanism the formamidine (44) was prepared in

SCHEME 5

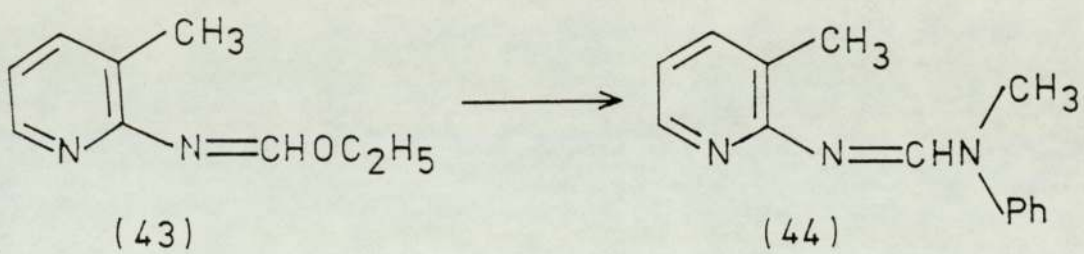


(36)

which the equilibrium (40) $\rightleftharpoons$ (41) was restricted in favour of the cyclisation intermediate (41). When the amidine (44) was cyclised with sodium N-methylanilide as the base, the yield increased from 50-63% to 80%



(42)



(43) (44)

The last method of ring closure (type iv) is exemplified by the application of the Fischer indole synthesis to the cyclisation of 2-pyridylhydrazones. It has had limited success for two reasons. As shown in step three the cyclisation requires a nucleophilic attack by the pyridine π-electrons on the side chain ~~(scheme 6)~~. This attack is less favourable because of the reduced nucleophilic character of the pyridine ring. The second reason is that cyclisation under acid catalysis[80,83,84] involves a protonated nitrogen in the pyridine ring making it more unlikely for the π-electron participation in the nucleophilic step three.

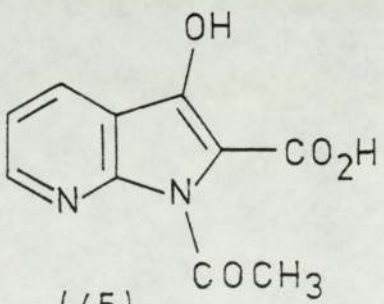
Parrick and co-workers[85,86] found that when normal or Lewis acids were omitted and cyclisation carried out under purely thermal conditions, many of the 2-pyridylhydrazones formed

1H-pyrrolo [2,3-b] pyridines ~~within~~ under electrocyclic conditions in moderate to good yields.

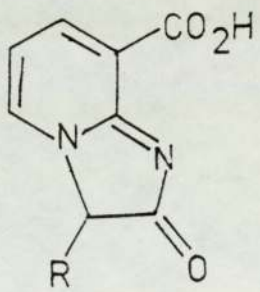
The most successful and widely used methods for the synthesis of the parent ring have involved the construction of the pyrrole ring on the existing pyridine ring. Two features of the π-electron distribution of the pyridine ring (37) have affected the success of the cyclisation reactions. When electrophilic attack at C_3 of the pyridine is involved (as in type iv synthesis) the π-electron is not easily available because of the reduced nucleophilic character of the pyridine ring and high temperature is required to enable cyclisation to take place. Secondly, when type (iii) synthesis is used, the small π-electron excess on C_3 make the hydrogen atoms on the exo-cyclic carbon atom less acidic than benzylic hydrogen atoms in alkyl benzene and only the use of strong bases can remove them to generate the carbanion required for the cyclisation (scheme 5). This requirement for extreme conditions precludes the direct synthesis of most compounds bearing labile substituents which as a result have to be introduced in the formed ring. Further, because of the π -electron distribution of pyridine (37) there is a limitation on the selection of the starting materials for the synthesis to succeed and to avoid formation of unexpected products. Thus Fargher and Furness[83] failed to cause the cyclisation of a number of 2-pyridylhydrazones with zinc chloride or hydrochloric acid as catalysts, and Kelly and Parrick[86] could not cause the cyclisation of 5-nitro-2-pyridyl-hydrazones of cyclohexanone and ethyl ketone under thermal Fischer indolisation.

Unexpected products have been isolated by Yakhontov[80] when

cyclohexanone 2-pyridylhydrazone derivatives were heated with hydrochloric acid to yield pyrido[2,1-c]-as-triazines and when the hydrazones were heated in sealed tubes with boron trifluoride-ethyl etherate in acetic acid at 180°C, to yield triazolo[3,4-a]pyridines. In both cases the expected α-carboline was also isolated. Willette[79] induced 2-amino nicotinic acid to react with chloroacetic acid and treated the resulting intermediate product with acetic anhydride to give a product which he formulated as 1-acetyl-1H-pyrrolo[2,3-b]-pyridine-3-hydroxy-2-carboxylic acid (45). However, Mosby[87] had previously suggested that the reaction of 2-amino-nicotinic acid with chloroacetic acid involved the ring nitrogen atom to give a pyridonimine derivative which under the influence of a mineral acid cyclised to an imidazo[1,2-a] pyridine-3-carboxylic acid(46,R=H). Herbert[81] suggested that the product obtained by Willette was also imidazo[1,2-a] pyridine-3-carboxylic acid (46,R=$COCH_3$) as the 'N-acetyl' group could not be removed by acid hydrolysis.



(45)

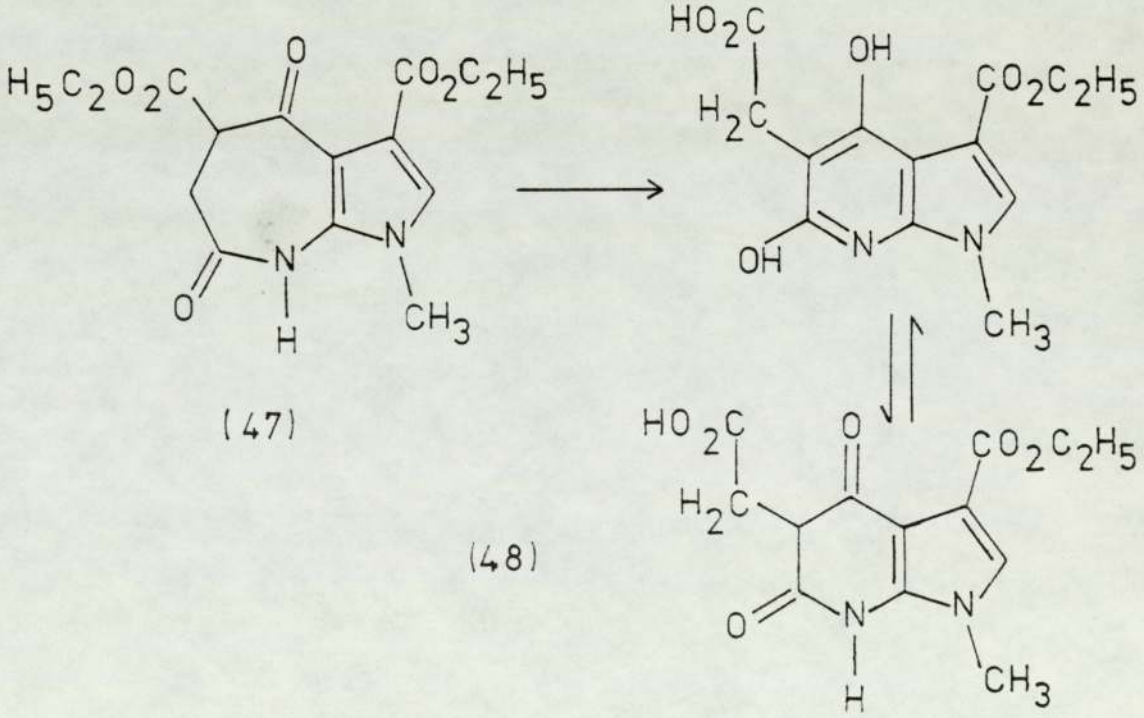


(46)

Since cyclisation involving the formation of the five-membered ring on the pyridine ring to form 1H-pyrrolo[2,3-b]pyridines requires harsh conditions in which only stable substituents such as methyl, ethyl or phenyl can be prepared directly, efforts have been made to prepare compounds with labile substituents by less extreme conditions. Brodrick and Wibberley[88] have reported the successful

preparation of a number of 1H-pyrrolo[2,3-b] pyridines from 2-aminopyrroles. The significant feature of this route is the preparation of compounds with useful substituents such as hydroxy, oxo, nitro, and carboxylate groups on the six membered ring. However, the compounds had a 3-carbonitrile group which proved very resistant to hydrolysis by acidic or basic agents. Similarly, diethyl 2-amino-pyrrole-3,4-dicarboxylate and 2-amino-3-carbonitrile-4-phenylpyrrole could not be caused to cyclise to give 1H-pyrrolo[2,3-b]pyridine derivatives.

Recently Vora et al[89] reported that when 3,5-diethyl-1-methyl-pyrrolo[2,3-b]azepine-4,7-dione-3,5-dicarboxylate (47) was refluxed with hydrochloric acid (0.2M) the azepine ring contracted to give 3-ethoxy carbonyl ~~ethyl~~-1-methyl-1H-pyrrolo[2,3-b]pyridine-4,6-dihydroxy-5-acetic acid (48). ~~3-carboxylate (48).~~



(47)

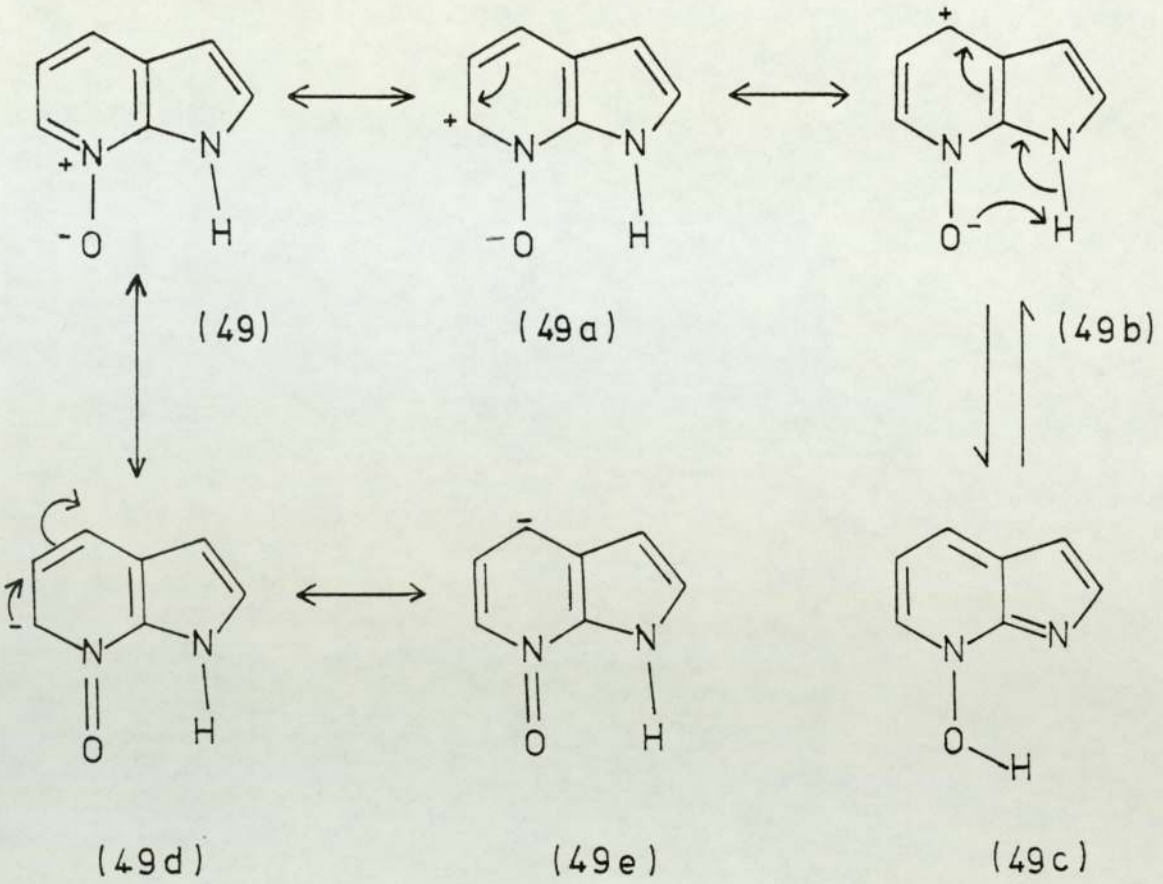
(48)

B. Chemical Properties

Although the π-electron distribution of 1H-pyrrolo[2,3-b] - pyridine (36a) and that of indole (39) resemble each other closely,

the chemical properties of the two ring systems are often dissimilar. The former does not undergo electrophilic substitution in the six-membered ring under normal circumstances and nitration reaction gives only 3-nitro-1H-pyrrolo[2,3-b]pyridine.[90] Nitration of unsubstituted indole does not give 3-nitroindole but an amorphous product of unknown structure[91]. Substitution in the six-membered ring of 1H-pyrrolo[2,3-b]pyridine (36) has been carried out via a 2,3-dihydro derivative in which a 1-nitro product re-arranged under the influence of concentrated sulphuric acid to 1H-2,3-dihydro-5-nitro-pyrrolo[2,3-b]pyridine which was subsequently dehydrogenated and reduced to give the 5-amino-1H-pyrrolo[2,3-b]pyridine.[90]

7-N-oxidation should alter the π-electron distribution in the six-membered ring of 1H-pyrrolo[2,3-b]pyridine (36) and make it more susceptible to substitution. Clark and Parrick[92] treated this compound with hydrogen peroxide (30%) in acetic acid and found that instead of the 7-N-oxidation product, ring opening occurred at the 2-3 bond with the elimination of the C_2 — carbon to give 2-aminonicotinic acid. When α-carboline was treated with the hydrogen peroxide-acetic acid mixture, none of the N-oxide formed; indicating that the ring opening step (where possible) was faster than N-oxidation. Subsequently the same workers[93] treated 1-acetyl-1H-pyrrolo[2,3-b]pyridine with m - chloroperoxybenzoic acid and obtained the 1H-pyrrolo[2,3-b]pyridine-7-oxide (47) in variable yields.



(49) (49a) (49b)

(49d) (49e) (49c)

Attempts to extend the 7-N-oxidation reaction to substituted pyrrolo [2,3-b] pyridines failed.[94] Thus 1-methyl-2,3-diphenyl-1H-pyrrolo [2,3-b] pyridine was treated with m-chloroperbenzoic acid and gave two ring-opening products; 3-benzoyl-2(N-methyl benzoylamino) pyridine and 3-benzoyl-2-(N-methyl benzoylamino)pyridine-1-oxide. Substitution reactions at ring carbon atoms depend on the contribution of the various resonance and tautomeric structures (49) through (49e). Nucleophilic substitution at positions 4 and 6 is favoured by resonance hybrides (49a) and 49b); when the 7-N-oxide (49) was treated with acetic anhydride it gave exclusively the 1-acetyl-6-acetoxy-1H-pyrrolo [2,3-b] pyridine and none of the 4-isomer. However the 7-N-oxide (49) was resistant to nucleophilic substitution with cyanide at 6-position when treated with benzoyl chloride-silver cyanide mixture.

Electrophilic substitution at positions 4 and 5 of (49) should be favoured by the resonance structures (49d) and (49e). However nitration of 1H-pyrrolo[2,3-b]pyridine N-oxide and 3-bromo-1H-pyrrolo-[2,3-b]pyridine N-oxide gave only 3-nitro-1H-pyrrolo[2,3-b]pyridine and none of the 4-isomer.[95] Under acidic nitration conditions the oxygen of the 7-N-Oxide would be protonated leaving a positively charged nitrogen atom which would further deactivate the six-membered ring. The resonance structure (49c) would be the more likely reacting species and in this case the 3-position is the more favourable for the nitration reaction.

C. Biological Properties

1H-Pyrrolo[2,3-b]pyridine derivatives have been tested for activity against a variety of biological systems. Thus 6-amino-2,3-diphenyl-1H-pyrrolo[2,3-b]pyridine had little antimalarial activity when tested against P.lophurae.[96] Verbiscar[97] synthesized a number of 1H-pyrrolo[2,3-b]pyridine derivatives as analogs of quinoline-5--piperidylmethanol which show antimalarial activity and found that 1-(4-chlorobenzyl)-1H-pyrrolo[2,3-b]pyridine-3-α-piperidylmethanol to be weakly antimalarial against P. berghei.

7-Azatryptophan was tested as antimetabolite of the amino acid tryptophan in the protozoan T. pyriformis and found to inhibit the utilisation of tryptophan.[98] Bacteria mutants of E. coli requiring tryptophan and certain virus, T_2-phage were found to incorporate 7-azatryptophan and produce inactive proteins which caused growth to cease at double the population.[99]

Berecz and Godin[100] found that 7-azatryptophan was incorporated

in mammalian proteins. Rats starved of protein incorporated 7-azatryptophan in plasma proteins which were inactive and lead to rapid loss of weight.

Adler and Albert[101] found that 1H-pyrrolo [2,3-b] pyridine when injected in mice caused paralysis and respiratory depression; but it caused relaxation of isolated smooth muscle. It also showed a delayed toxic effect which resulted in the death of the animal about five days after treatment. In rabbits, the compound reduced blood pressure to shock-levels.

A patent[102] appeared in 1965 which described preparation of a number of amidoxime, amidine, and guanidine azaindolylalkyl derivatives which were said to have hypotensive, sedative and psychomotorial stimulating activity. 1H-pyrrolo[2,3-b] pyridine-3-acetamidoxime has been shown to lower blood pressure by depletion of catecholamines from the heart.[103]

2-phenyl-1H-pyrrolo[2,3-b] pyridine was found to be inactive as antihelminthic when tested against Trichostrongyles[104].

Recently it was reported that when the bacterium Cyanobacterium anabaena was incubated with 7-azatryptophan the activity of the enzymes heterocyst and nitrogenase were induced.[105]

DISCUSSION

I Aims and Objectives

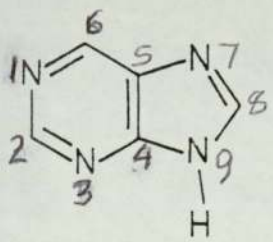
Trypanosomiasis is an important disease in Africa and it has been estimated that about eight million people are infected annually and potentially about 35 million people are at risk of infection. There is an urgent need for the development of new drugs for the treatment of this disease, since no new drugs have been developed for trypanosomiasis for more than thirty years, and those drugs now in use are unsatisfactory.

The problem of trypanosomiasis becomes more serious when it is considered against the background of the fact that pharmaceutical companies which have in the past played an important role in drug development and innovation have largely abandoned research for tropical diseases. Goodwin[75] has suggested that the reason for the decline of research in this area is largely economic; since research leading to introduction of a new drug is expensive and if such a drug was found it would be unprofitable as the people who need it are too poor to afford it.

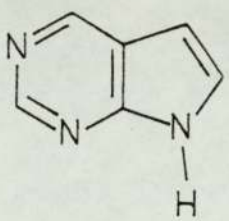
This work was undertaken to prepare derivatives of 1H-pyrrolo[2,3-b]pyridine (36) for evaluation against T.b. brucei. The rationale for making such compounds was that they are 7-azaindoles which are structurally similar to the indole derivative tryptophan (1), which has been found to be rapidly metabolised by trypanosomes (scheme 3 and table 2). Derivatives of 7-azaindole could interfere with utilisation and incorporation of tryptophan and cause damage to trypanosomes.

1H-Pyrrolo[2,3-b]pyridine derivatives bear the same relationship to pyrrolo[2,3-d]pyrimidines (35) as these compounds do to purines (34) i.e. with one ring nitrogen atom less in the five-membered ring of (34). Thus tubercidin (7-deazaadenine) is active against trypanosomes. Derivatives of 1H-pyrrolo[2,3-b]pyridine (36) (1,7-dideazaadenine) could have antitrypanosomal activity.

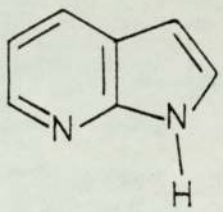
Derivatives of 1H-pyrrolo[2,3-b]pyridine (36) form an entirely new system of compounds that have never been screened before for antitrypanosomal activity. Their investigation could provide a lead compound for further evaluation.



(34)



(35)



(36)

II Screening of Selected 1H-pyrrolo[2,3-b]pyridines against T.b. brucei

The rationale for the synthesis of 1H-pyrrolo[2,3-b]pyridines for screening against T.b. brucei has been given above; and the reasons for selecting particular functional groups are given below. The selection of the compounds and the screening test was carried out before elemental analyses were done. Thus compounds (84), (90) (91) (100) (122) and (123) used in the testing did not give satisfactory analyses. Their identity had been established by

spectroscopic methods (infrared n.m.r. or mass spectra, see experimental section).

Esters and amides hydrolyse slowly at physiological pH. The esters (77), (78), (79), (92) and (123) were prepared with a view to extending their biological half-life which could increase the contact time for biological activity.

The p-chlorophenyl group increases lipid solubility, and the compounds (74), (76), (77), (79), (82), (84), (88), (92), (100), (108), (109) and (b) had this functional group to enhance lipid solubility to increase the chance for biological activity.

Compounds containing the labile imino (C=N) bond, (81), (82) and (84) could conceivably react with important sites of the trypanosomes.

Azatryptophan (121) and its derivatives; the 2 -N-acetyl (122) and the methyl ester (123) as well as 2'-N-methyltryptophan (c) (see table 4 below) were expected to interfere with the utilisation of the amino acid tryptophan which they resemble closely.

The infectivity test establishes whether a given potential drug is incorporated into important trypanosome proteins which then become inactive and lead to the death of the trypanosome. In this test (table 5) two of the compounds (77) and (88) had this effect but did not show activity *in vivo*.

All the compounds screened had no activity. They probably did not bind to important sites in the trypanosomes or did not reach the concentration that would be lethal to the trypanosomes. Higher

doses than those shown in the table killed the test animals within periods varying from 5 minutes to 12 hours. All the compounds appeared to be absorbed from the injection site as indicated by a drowsiness of the test mice within five minutes of the injection which lasted for about ten minutes.

III

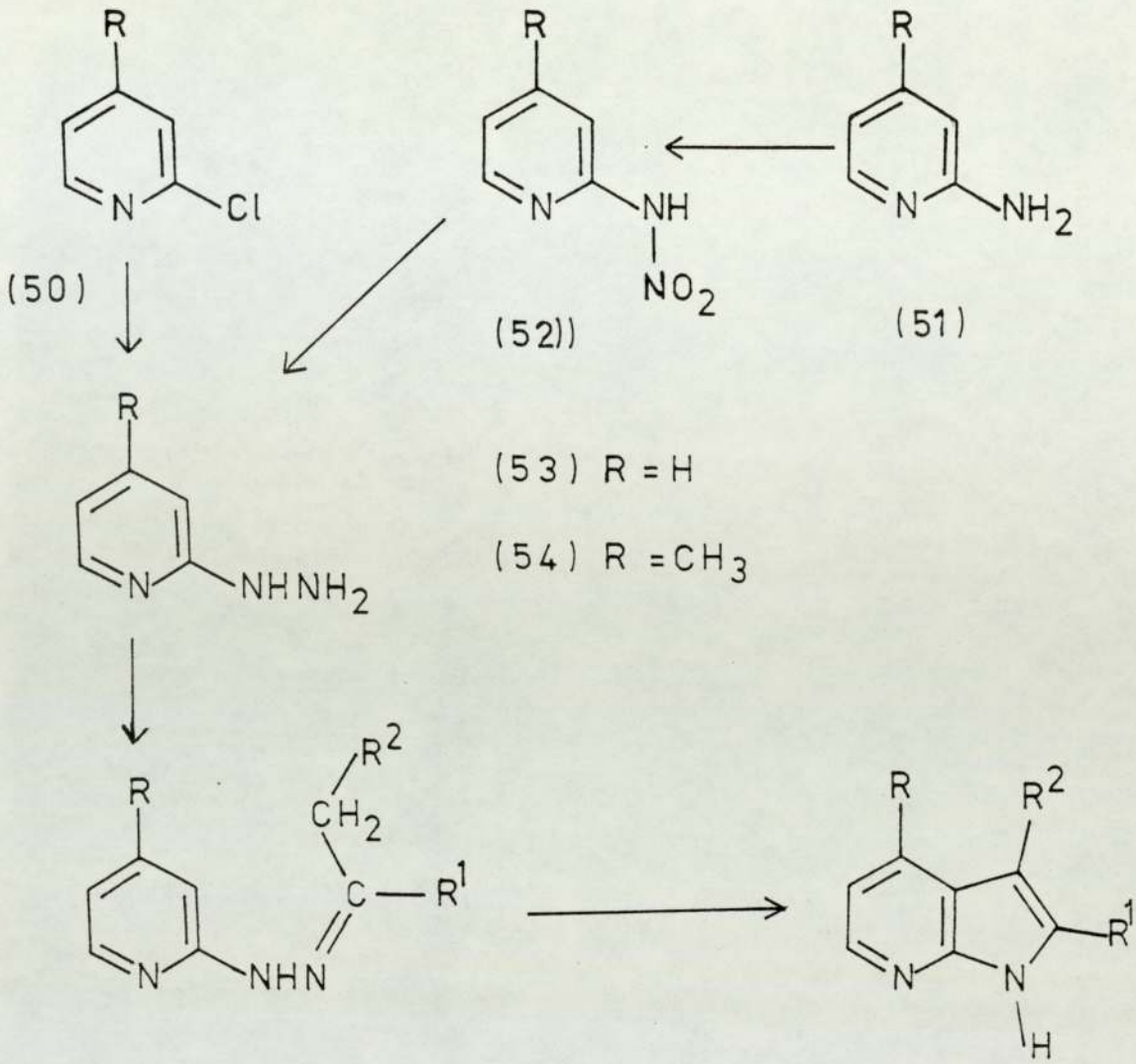
A. Preparation of 1H-Pyrrolo [2,3-b] pyridines

Two approaches were adopted in this study in order to prepare derivatives of 1H-pyrrolo [2,3-b]pyridines for subsequent screening for antitrypanosomal activity against T.b. brucei. In the first approach 1H-pyrrolo [2,3-b]pyridines with stable substitutents were prepared directly; and in the second approach substituents that were too labile for direct synthesis were introduced into the 1H-pyrrolo [2,3-b] pyridines.

Of the four methods commonly used for the formation of 1H-pyrrolo [2,3-b]pyridine by the construction of the five-membered ring on the existing pyridine ring (Section II, A) two were selected for use, namely the Madelung synthesis (type (iii)), and the Fischer indole synthesis type (iv).

2-Pyridylhydrazine (53) was prepared by treatment of 2-chloropyridine (50, R = H) with hydrazine hydrate.[83] 4-Methyl-2-pyridylhydrazine (54) was prepared by nitration of 2-amino-4-methylpyridine (51, R = CH_3) followed by the reduction of the 2-nitramino-4-methylpyridine (52, R = CH_3) intermediate.[106] The 2-pyridylhydrazines were converted into the 2-pyridylhydrazones of p-chloroacetophenone (55), (56), p-chloropropiophenone (57), 3 , - 4 -dimethylacetophenone (58), 3,4-dimethoxyacetophenone (59), and propionaldehyde (60).

SCHEME 7



(55) $R = R^2 = H$ $R^1 = C_6H_4$ -p-Cl (61)

(56) $R = CH_3$ $R^1 = C_6H_4$-p-Cl $R^2 = H$ (62)

(57) $R = H$ $R^1 = C_6H_4$ -p-Cl $R^2 = CH_3$ (63)

(58) $R = R^2 = H$ $R^1 = C_6H_3$—3,4-di CH_3 (64)

(59) $R = R^2 = H$ $R^1 = C_6H_3$—3,4-di OCH_3 (65)

(60) $R = R^1 = H$ $R^2 = CH_3$ (66)

The 2-pyridylhydrazones (55), (56), (57), (58), and (80) were cyclised into the 1H-pyrrolo[2,3-b]pyridines (61), (62), (63), (64) and (66) by heating under reflux in triethylene glycol (trigol) for periods of time which varied from 4 hours to 7 days. 3,4-Dimethoxyacetophenone 2-pyridylhydrazone (59) gave only tarry material which contained none of the expected product (65).

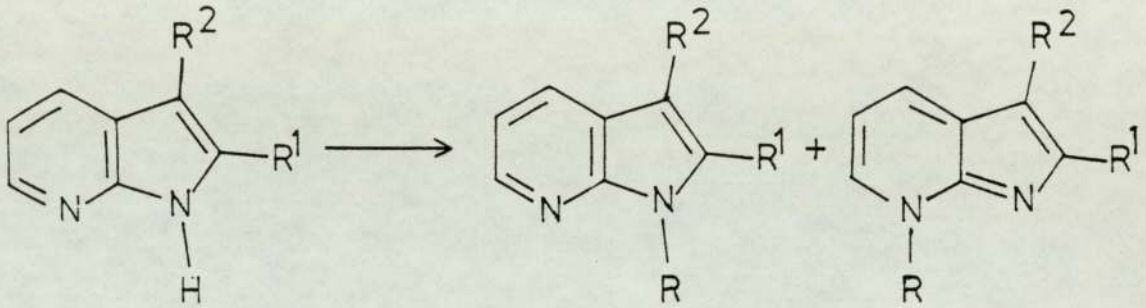
Since the yield of 2-p-chlorophenyl-1H-pyrrolo[2,3-b]pyridine (61) prepared by the Fischer method was low (9.6%) an attempt was made to prepare it by the Madelung method in the hope of improving the yield; since 2-phenyl-1H-pyrrolo[2,3-b]pyridine has been prepared by this method in 60% yield.[106] 2-p-Chlorobenzamido-3-methylpyridine was heated under reflux with sodium N-methylanilide but gave only tars. Under similar reaction conditions, 2-p-methoxybenzamido-3-methylpyridine and 2-phenylacetamido-3-methylpyridine have been reported to fail to cyclise.[106]

Although commercially available, the parent compound 1H-pyrrolo-[2,3-b]pyridine was successfully prepared for use by the modified Madelung method in 50-70% yield.[82]

B. Chemical Properties of 1H-Pyrrolo[2,3-b]pyridines

The 1H-pyrrolo[2,3-b]pyridines (36), and (63) were treated with base (NaH, K_2CO_3 or KOH) and the alkylating agents p-chlorobenzyl chloride or iodomethane to give 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (67), 1-p-chlorobenzyl-2-p-chlorophenyl-3-methyl-1H-pyrrolo-[2,3-b]pyridine (69), and 2-p-chlorobenzyl-1,3-dimethyl-1H-pyrrolo-[2,3-b]pyridine (71) as the major products, and 7-p-chlorobenzyl-7H-pyrrolo[2,3-b]pyridine (68), 7-p-chlorobenzyl-2-p-chlorophenyl-3-methyl-7H-pyrrolo[2,3-b]pyridine (70), and 2-p-chlorophenyl-3,7-

dimethyl-7H-pyrrolo [2,3-b] pyridine (72) as the minor products. The minor products (68), (70) and (72) were separated from the major products by extraction into dilute acetic acid or dilute hydrochloric acid.[97] The infrared spectra and n.m.r. spectra of the derivatives (67) - (71) were consistent with the postulated structures (see experimental section). The analytical data of 2-p-chlorophenyl-3,7-dimethyl-7H-pyrrolo[2,3-b] pyridine (72) indicated that some hydrate had been formed during recrystallisation (from aqueous methanol).



(36) $R^1 = R^2 = H$

(63) $R^1 = C_6H_4\text{-}p\text{-}Cl$, $R^2 = CH_3$

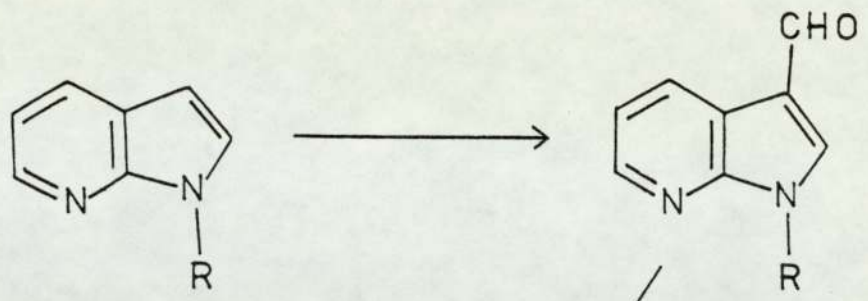
(67), (68) $R = CH_2C_6H_4\text{-}p\text{-}Cl$, $R^1 = R^2 = H$

(69), (70) $R = CH_2C_6H_4\text{-}p\text{-}Cl$, $R^1 = C_6H_4\text{-}p\text{-}Cl$, $R^2 = CH_3$

(71), (72) $R = R^2 = CH_3$, $R^1 = C_6H_4\text{-}p\text{-}Cl$

1H-Pyrrolo[2,3-b] pyridine-3-carbaldehyde (73) was prepared by the treatment of 1H-pyrrolo[2,3-b] pyridine (36) with hexamethylene tetramine in dilute acetic acid.[97] The same compound was reported by Robison and Robison[107] who had prepared it from 3-N, N-dimethylaminomethyl-1H-pyrrolo[2,3-b] pyridine on a small scale. This method could not be adapted to large scale preparation for further

SCHEME 8

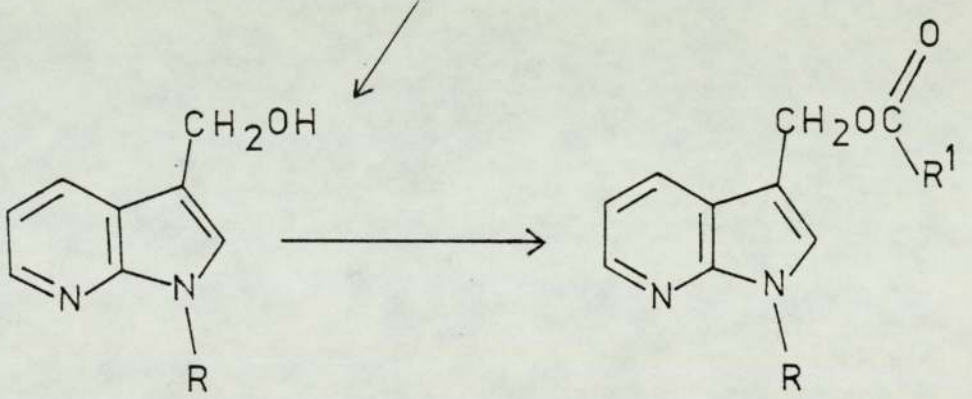


(36) R = H

(67) R = $CH_2C_6H_4$–p–Cl

(73) R = H

(74) R = $CH_2C_6H_4$–p–Cl



(75) R = H

(76) R = $CH_2C_6H_4$–p–Cl

(77) R = H

R^1 = C_6H_4–p–Cl

(78) R = $COCH_3$

R^1 = CH_3

(79) R = $CH_2C_6H_4$–p–Cl

R^1 = CH_3

reactions. 1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (67) was readily converted into 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (74) by the Vilsmeir formylation method. The infrared, n.m.r. and mass spectra for both these aldehydes were consistent with the postulated structures.

These two aldehydes were easily reduced by sodium borohydride[108] to the 3-methanol derivatives, 1H-pyrrolo[2,3-b]pyridine-3-methanol (75) and 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-methanol (76). For both compounds the infrared, n.m.r. and mass spectra were consistent with the proposed structures.

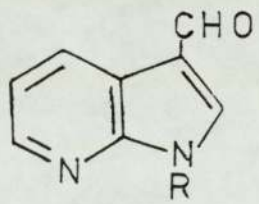
Treatment of 1H-pyrrolo[2,3-b]pyridine-3-methanol (75) with p-chlorobenzoyl chloride in the presence of pyridine gave the ester 3-p-chlorobenzoyloxymethyl-1H-pyrrolo[2,3-b]pyridine (77). This structural assignment is consistent with the infrared spectrum of the compound which showed no OH-stretch at 3150 cm^{-1} and had an intense peak at 1709 cm^{-1} characteristic of ester carbonyl stretch.[109, 110]

When 1H-pyrrolo[2,3-b]pyridine-3-methanol (75) was treated with acetic anhydride both the pyrrole nitrogen and the OH group were acetylated to yield 3-acetoxymethyl-1-acetyl-1H-pyrrolo[2,3-b]-pyridine (78). The infrared spectrum showed the ester C=O stretch at 1735 cm^{-1} and the amide C=O stretch at 1690 cm^{-1}. The n.m.r. and the mass spectra were consistent with this structural assignment (see experimental section).

Treatment of 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-methanol with acetic anhydride gave 3-acetoxymethyl-1-p-chlorobenzyl-

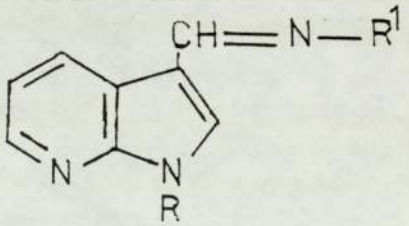
1H-pyrrolo[2,3-b]pyridine (79). In this compound, the C=O stretch appeared at 1722 $cm.^{-1}$ This assignment confirms the assignment of compound (78) above.

The aldehydes, 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (73) and 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (74) condensed with hydrazine, semicarbazide hydrochloride and thiosemicarbazide to give the hydrazones (80) and (84) the semicarbazone (82) and the thiosemicarbazones (82) and (83) in high yield. Spectroscopic data supported the postulated structures (80) - (83) (see experimental section). An analytically pure sample of 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde hydrazone (84) was not obtained. The compound decomposed when recrystallisation from ethanol or dioxan was attempted. The infrared spectrum of the crude compound showed an intense C=N stretch peak at 1625 $cm.^{-1}$ The hydrazone had the high melting point characteristic of these compounds.



(73) R = H

(74) R = $CH_2C_6H_4$-p-Cl



(80) R = H $R^1 = NH_2$

(81) R = H $R^1 = NHC(=S)NH_2$

(82) R = $CH_2C_6H_4$-p-Cl
$R^1 = NHC(=O)NH_2$

(83) R = $CH_2C_6H_4$-p-Cl
$R^1 = NHC(=S)NH_2$

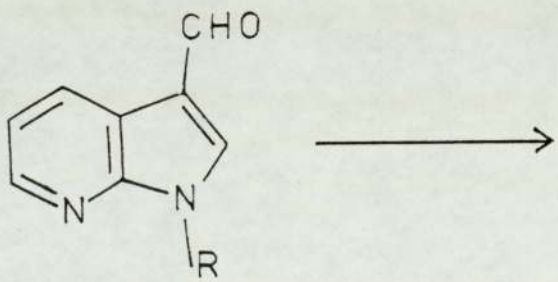
(84) R = $CH_2C_6H_4$-p-Cl $R^1 = NH_2$

(85) R = H $R^1 = C_6H_4$-p-Cl

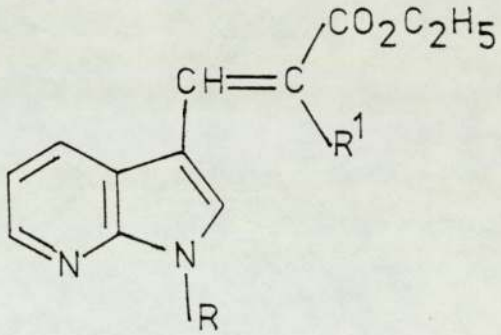
1H-Pyrrolo[2,3-b]pyridine-3-carbaldehyde condensed with p-chloroaniline in the presence of hydrochloric acid to give the imine

(85) in very high yield. Its infrared spectrum had an intense C=N stretch peak at 1655 $cm.^{-1}$ The mass spectrum showed the molecular ion at m/e 255.

The aldehyde (74) condensed readily with ethyl cyanoacetate in the presence of catalytic amount of piperidine to give ethyl 2'-cyano-3'-(1-p-chlorobenzyl 1H-pyrrolo[2,3-b]pyrid-3-yl)acrylate (86). The infrared spectrum showed a C = O peak at 1700 $cm.^{-1}$ A similar condensation product, ethyl 2'-ethoxycarbonyl-3'-(1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyrid-3-yl)acrylate (87) was obtained when the aldehyde (74) was treated with diethyl malonate. An analytically pure sample of this compound was not obtained. The infrared, n.m.r. and mass spectra were consistent with the postulated structure (see experimental section).



(74) R = $CH_2C_6H_4$-p-Cl



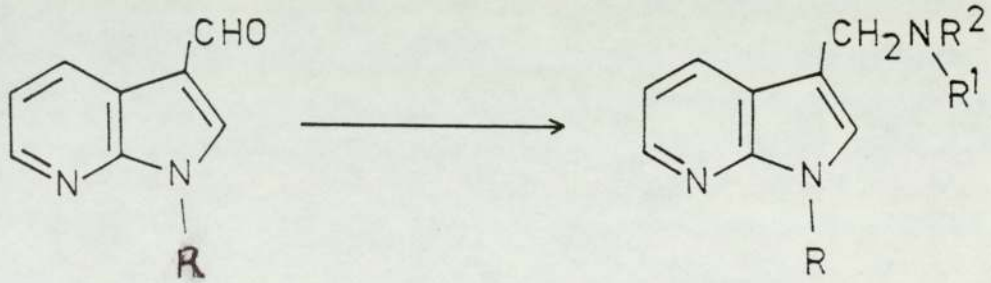
(86) R = $CH_2C_6H_4$-p-Cl
R^1 = CN

(87) R = $CH_2C_6H_4$-p-Cl
R^1 = $CO_2C_2H_5$

It had been hoped that ethyl 2'-cyano-3-(1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyrid-3-yl)acrylate (86) could be catalytically

hydrogenated at the side chain C = C bond and the cyano group of the resulting intermediate compound be converted into a primary amine. However, upon hydrogenation with Adam's catalyst a yellow oil which could not be induced to solidify was obtained. The reaction was not pursued further.

Borch and co-workers[111] have reported a convenient reductive amination of ketones and aldehydes utilising cyanohydridoborate anion in acidic medium. By the use of this method, the aldehydes, 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (73), and 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (74) were reductively aminated to N-p-chlorophenyl-1H-pyrrolo[2,3-b]pyrid-3-ylmethylamine (88), 1-p-chlorobenzyl-N-methyl-N-phenyl-1H-pyrrolo[2,3-b]pyrid-3-ylmethylamine (89), 2 -(1H -pyrrolo[2,3-b]pyrid-3-yl)methylaminopropanoic acid (90), and N,N-dimethyl-1H-pyrrolo[2,3-b]pyrid-3-ylmethylamine (91). The infrared, n.m.r. and mass spectra were consistent with the postulated structures (see experimental section). The last two compounds (90) and (91) were not obtained in analytically pure form.



(73) R = H

(74) R = $CH_2C_6H_4$-p-Cl

(88) R = R^1 = H
R^2 = C_6H_4-p-Cl

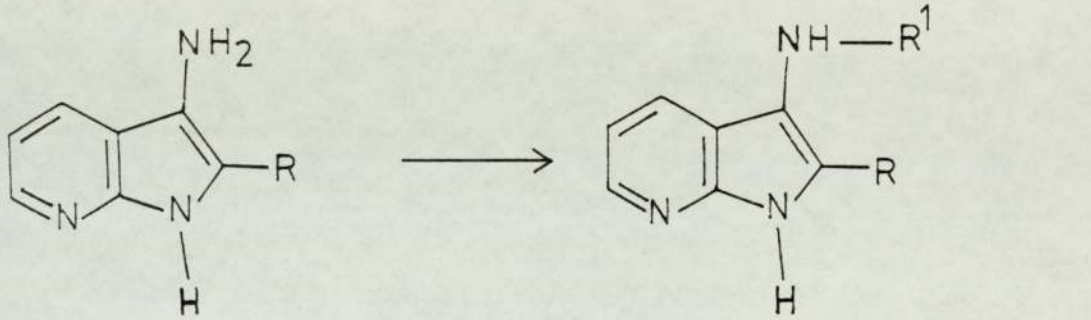
(89) R = $CH_2C_6H_4$-p-Cl
R^1 = CH_3 R^2 = C_6H_5

(90) R = R^1 = H R^2 = $CHCH_3$ / CO_2H

(91) R = H R^1 = R^2 = C_2H_5

The imine (85) was reduced by sodium borohydride to the amine form which was identical with the amine (89) prepared directly.

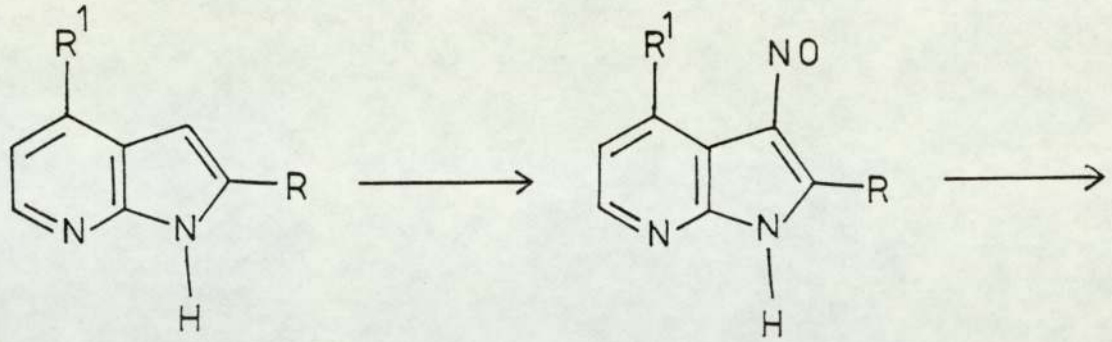
The amine, 2-p-chlorophenyl-3-amino-1H-pyrrolo[2,3-b] pyridine (99) was treated with ethyl acetoacetate under reductive amination conditions to give 2-p-chlorophenyl-3-(2' methyl ethoxycarbonyl-ethyl)amino-1H-pyrrolo [2,3-b] pyridine (92). The infrared spectrum showed C = O stretch at 1722 cm^{-1} and the molecular ion m/e 357 was observed in the mass spectrum.



(99) R = C_6H_4-p-Cl (92) R = C_6H_4-p-Cl

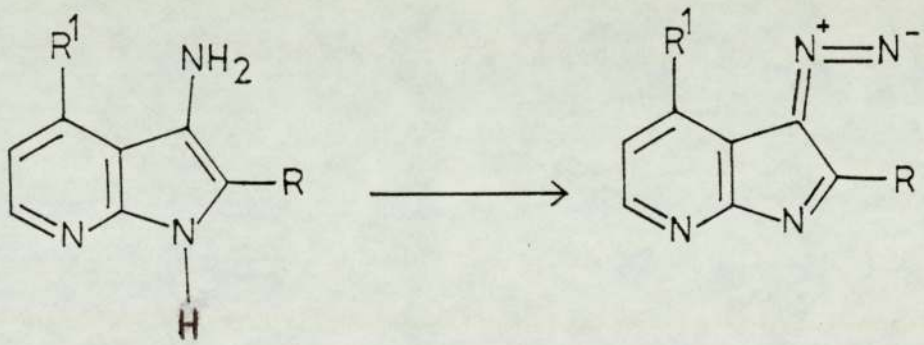
$R^1 = CH(CH_3)CO_2C_2H_5$

2-p-Chlorophenyl-1H-pyrrolo[2,3-b]pyridine (61) and 2-p-chlorophenyl-4-methyl-1H-pyrrolo[2,3-b]pyridine (62) were treated with nitrous acid to give the nitroso compounds (95) and (96) ~~intermediates~~ which were reduced to ~~give~~ the 3-amino derivatives (99) and (100). An analytically pure sample of the amine (100) was not obtained. The 3-amino derivatives were treated with nitrous acid to give the diazo derivatives (102) and (103). The $C=N^+=N^-$ ~~$N^+=N^-$~~ stretch was observed at 2125 cm^{-1} for (102) and 2110 cm^{-1} for (103) in the infrared spectra. An analytical sample for the diazo compound (103) was not obtained.



(61) R = C_6H_4-p-Cl R^1 = H (95)

(62) R = C_6H_4-p-Cl R^1 = CH_3 (96)

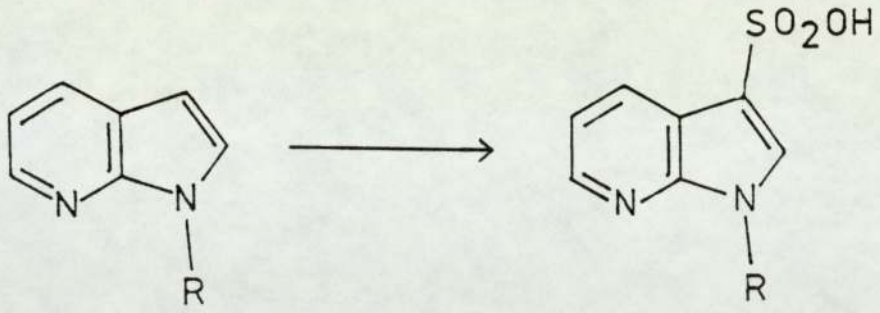


(99) R = C_6H_4-p-Cl R^1 = H (102)

(100) R = C_6H_4-p-Cl R^1 = CH_3 (103)

Chlorosulphonation of 1H-pyrrolo[2,3-b]pyridine (36), and 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (67) with chlorosulphonic acid gave the 3-sulphonyl chlorides (104) and (105) as unstable, colourless powders. These in turn were converted into the derivatives (106) - (112). The infrared, n.m.r. and mass spectra of the compounds (106) - (109) were consistent with the postulated structures (see experimental section). 3-Sulphamoyl-1H-pyrrolo-[2,3-b]pyridine (110) was not obtained in analytically pure form.

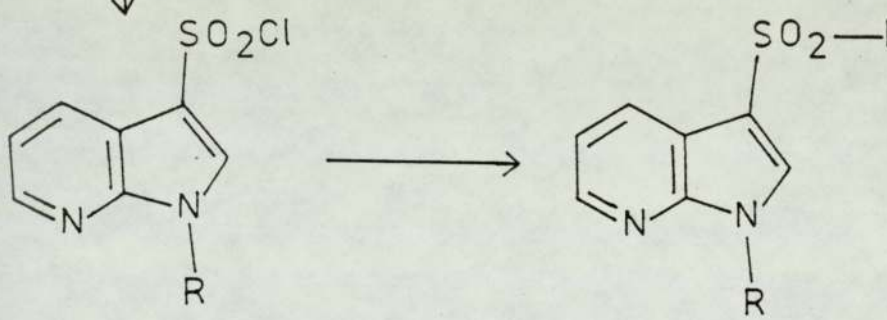
SCHEME 9



(36) R = H

(67) R = $CH_2C_6H_4$-p-Cl

(113) R = H



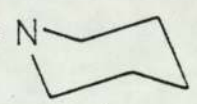
(104) R = H

(105) R = $CH_2C_6H_4$-p-Cl

(106) R = H $R^1 = NHC_6H_5$

(107) R = H
$R^1 = N(CH_3)C_6H_5$

(108) R = $CH_2C_6H_4$-p-Cl
R^1 = 

(109) R = $CH_2C_6H_4$-p-Cl
R^1 = 

(110) R = H $R^1 = NH_2$

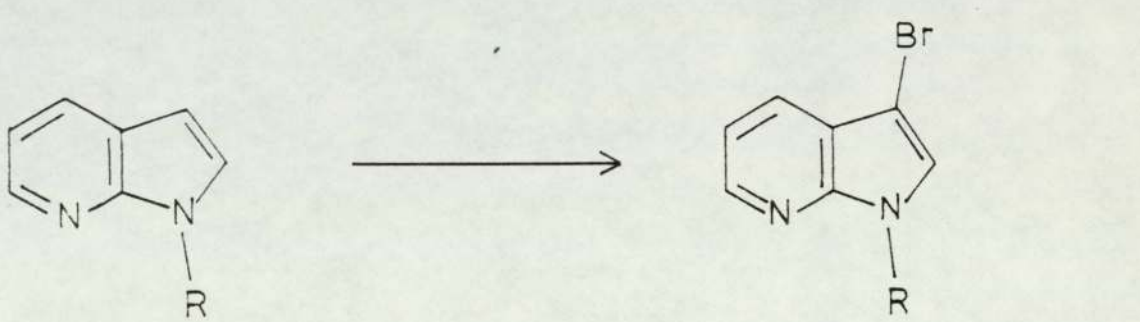
(111) R = H $R^1 = NHNH_2$

(112) R = H
$R^1 = N(CH_2CH_3)_2$

The mass spectrum of the crude compound showed the molecular ion at m/e (197). 3-Hydrazinosulphonyl-1H-pyrrolo[2,3-b] pyridine (111) was not obtained in analytically pure form. The molecular ion was not seen in the mass spectrum. The infrared and n.m.r. spectra were consistent with the postulated structure (see experimental section). 3-N,N-Diethylaminosulphonyl-1H-pyrrolo[2,3-b] pyridine (112) was not obtained in analytically pure form. The infrared, n.m.r. and mass spectra were consistent with the postulated structure (see experimental section).

1H-Pyrrolo[2,3-b] pyridine (36) reacted with disulphuric acid to give 1H-pyrrolo [2,3-b] pyridine-3-sulphonic acid (113) in high yield. Nuclear magnetic resonance spectroscopy indicated that substitution had occurred at 3-position. The mass spectrum showed a parent peak at m/e 198; but an analytically pure sample was not obtained. Sulphonation of indole using pyridinium sulphate lead to 3-substitution.[112]

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b] pyridine (67) was treated with bromine to yield 3-bromo-1-p-chlorobenzyl-1H-pyrrolo [2,3-b] pyridine (114) in high yield.

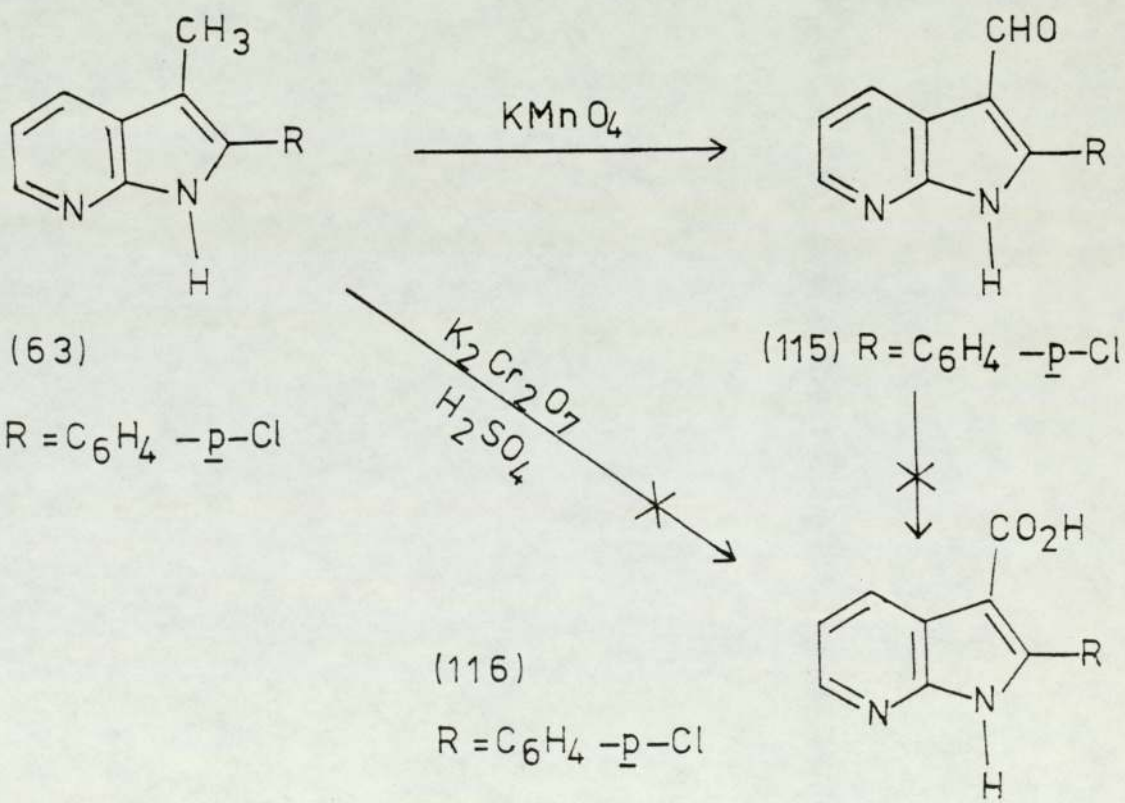


(67) R = $CH_2C_6H_4$-p-Cl (114) R = $CH_2C_6H_4$-p-Cl

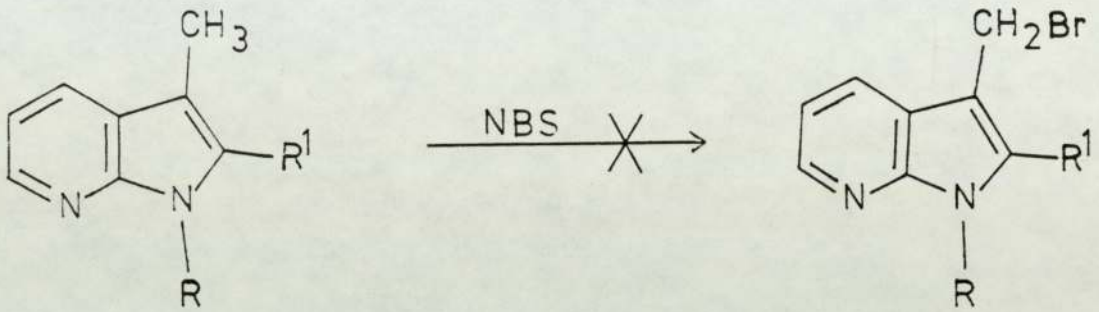
It was expected that the 3-methyl group in 2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b] pyridine (63) could be converted into a

carboxylic acid group by oxidising agents. Treatment of this compound with potassium permanganate yielded the carboxaldehyde derivative (115). Its infrared spectrum showed a strong C = O stretch at 1658 cm^{-1}. In the mass spectrum the M^+ peak at m/e 256 was seen and the M^+-1 peak formed the base peak. This fragmentation pattern is characteristic of aldehydes.[109] The aldehyde was not obtained in analytically pure form.

Further oxidation of this aldehyde (115) or treatment of (63) with potassium dichromate in concentrated sulphuric acid resulted in the degradation of the compounds and only p-chlorobenzoic acid was isolated.



N-Bromosuccinimide in the presence of a peroxy acid readily brominates benzylic protons.[113] Attempts to effect bromination of 2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine (63) or the 1-substituted isomers (69) and (71) with N-bromosuccinimide were unsuccessful.



(63) R = H $R^1 = C_6H_4-p-Cl$ (117)

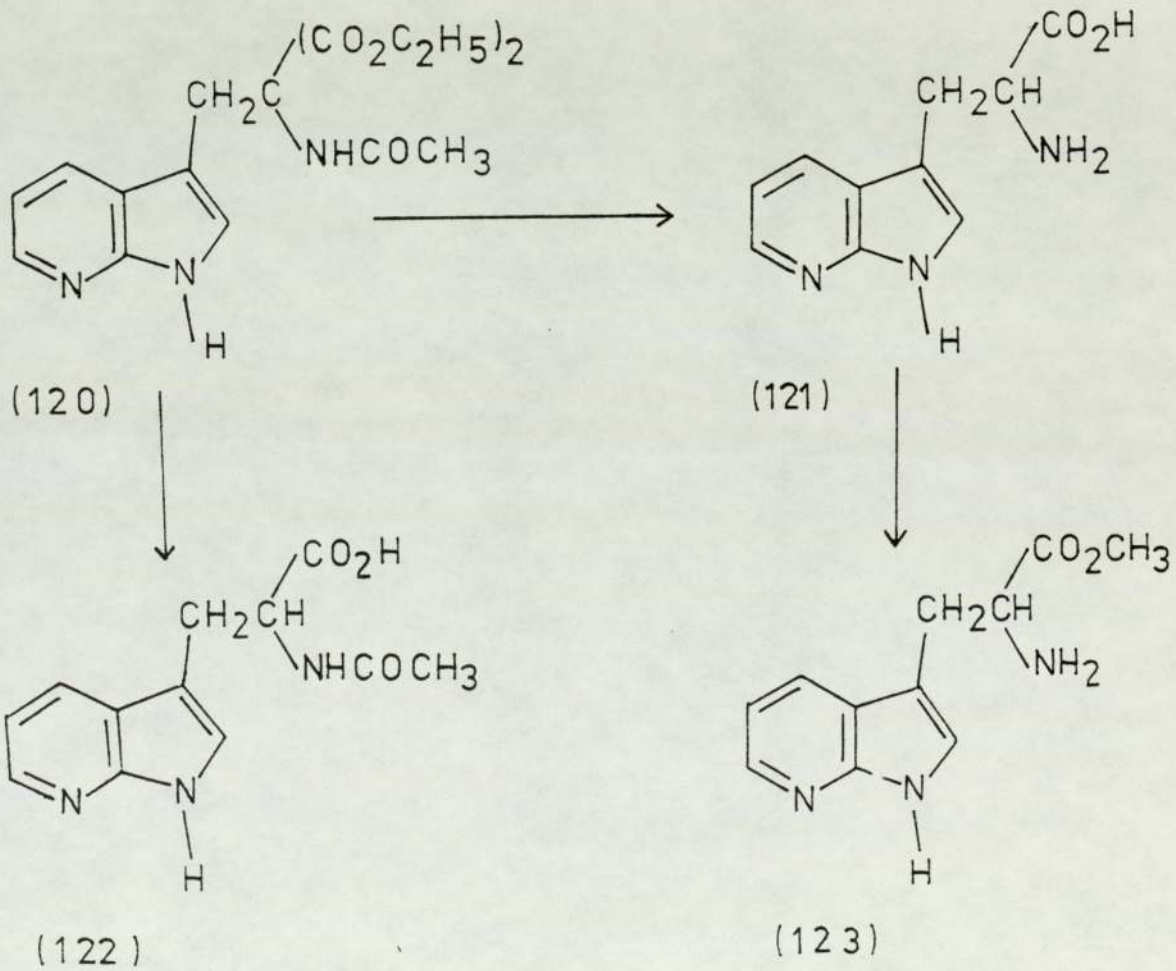
(69) $R = CH_2C_6H_4-p-Cl$ $R^1 = C_6H_4-p-Cl$ (118)

(71) $R = CH_3$ $R^1 = C_6H_4-p-Cl$ (119)

2'-Amino-3-(1H-pyrrolo[2,3-b]pyrid-3-yl)-propanoic acid (7-azatryptophan) (124) was prepared by the method of Robison and Robison.[107] 2'-acetamido-3-(1H-pyrrolo[2,3-b]pyrid-3-yl)-propanoic acid (2-acetamido-7-azatryptophan) (121) was prepared by the base hydrolysis of ethyl 2'-ethoxycarbonyl-2'-acetamido-3-(1H-pyrrolo[2,3-b]pyrid-3-yl) propanoate (120) in good yield. The infrared spectrum of (122) showed the C=O stretch at 1712 cm^{-1} and amide C=O peak at 1625 cm^{-1}. The mass spectrum showed the molecular

ion peak at m/e 247. However an anlytically pure sample was not obtained.

Methylation of 2 amino-1H-pyrrolo[2,3-b]pyrid-3-yl-3'-propanoic acid (121) in methanol yielded the ester, methyl 2 -amino-1H-pyrrolo [2,3-b]pyrid-3-yl-3'-propanoate (123). Its infrared spectrum showed an intense C=O peak at 1740 $cm.^{-1}$ The n.m.r. and mass spectra were consistent with the postulated structure (see experimental section). An analytically pure sample was not obtained.



IV Mass Spectral Fragmentation Patterns of Selected 1H-Pyrrolo-[2,3-b]pyridines.

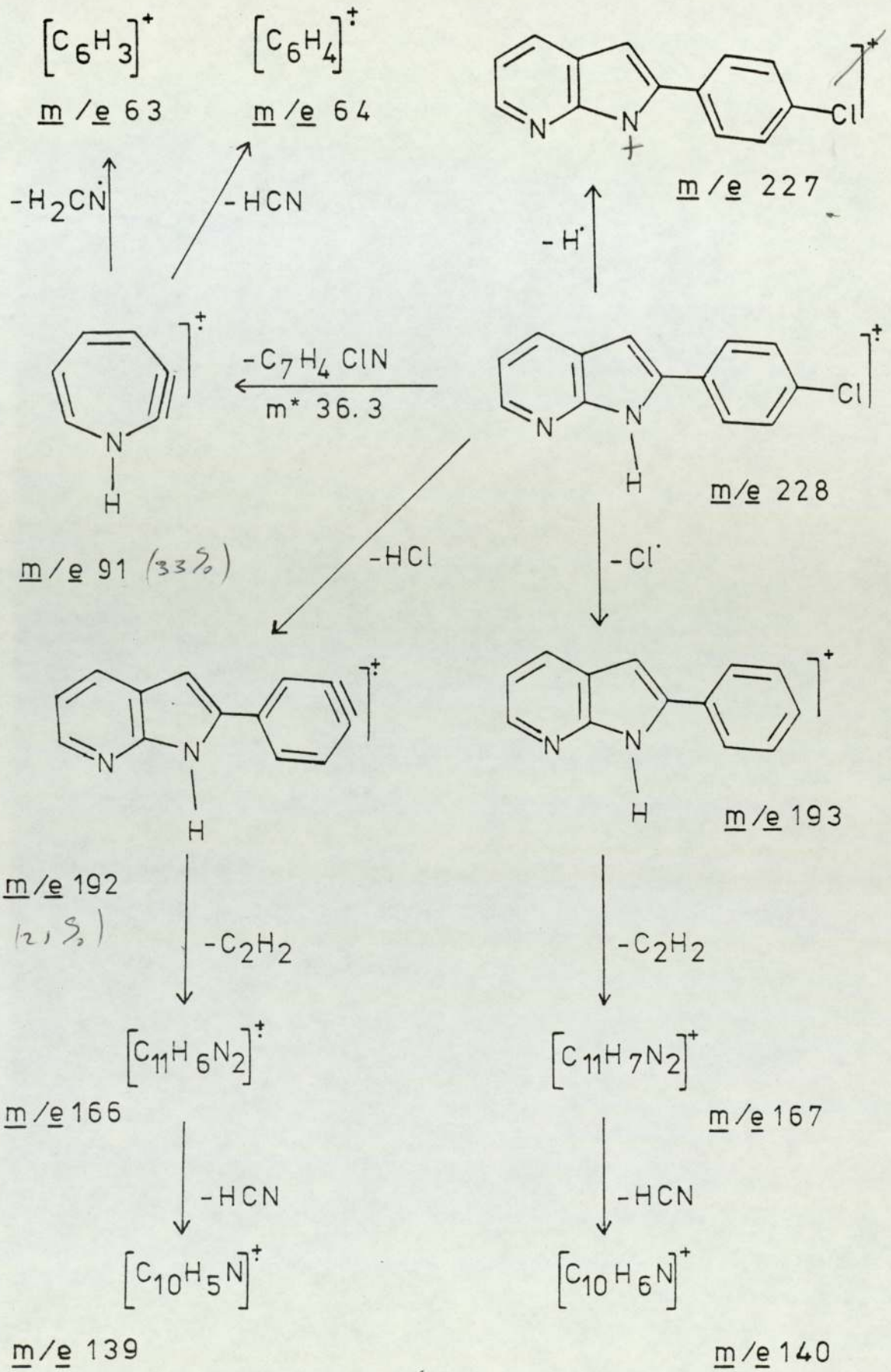
2-p-Chlorophenyl-1H-pyrrolo [2,3-b] pyridine (61).

The mass spectrum of 1H-pyrrolo [2,3-b] pyridine (36) has been reported.[113] The molecular ion formed the base peak and showed fragmentation by loss of HCN or H· in 50 and 9% relative abundance.

It has been proposed that aryl substituents in indoles[114] and 1H-pyrrolo [2,3-b]pyridines[113] stabilised the ring systems and the molecular ions formed the base peaks. This effect has been observed in the mass spectra of the 1H-pyrrolo[2,3-b] pyridines (61), (62), (63) and (71) which contained a 2-p-chlorophenyl substituent. All four compounds had base peaks due to molecular ions. All four compounds showed degradation due to the initial expulsion of HCl, Cl· or H· from the molecular ions.

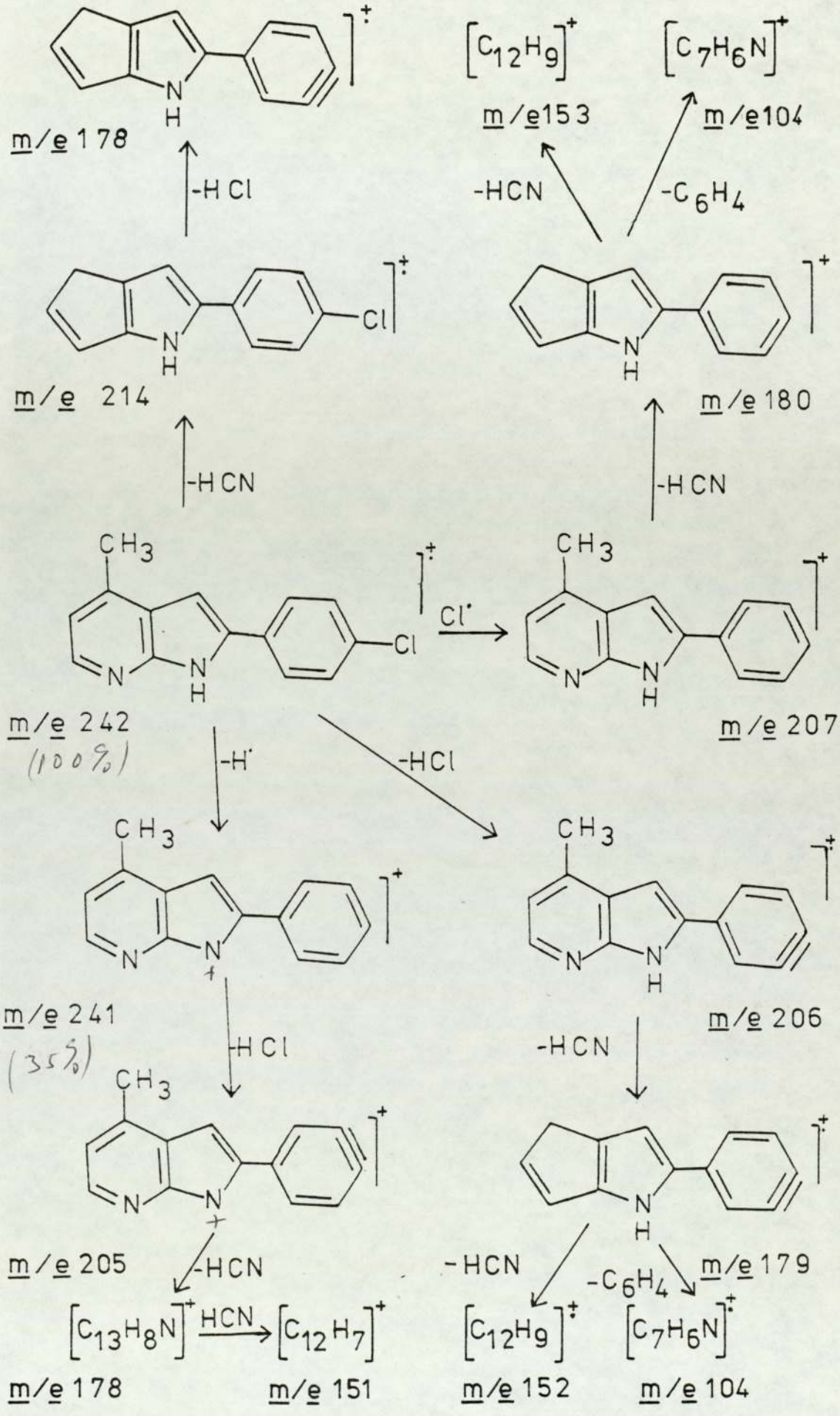
In the case of 2-p-chlorophenyl-1H-pyrrolo[2,3-b] pyridine (61) the loss of HCl and/or Cl· was followed by the loss of HCN from the ion m/e 192 or 193. The loss of hydrogen atom occurred in 7% abundance. This compound showed a significant fragmentation pathway involving the expulsion of C_7H_5ClN (m^* 36.3) to give a peak at m/e 91. This ion probably has the ring-expanded dedihydroazatropylium structure. The ion at m/e 91 degraded further by the loss of HCN and H_2CN to give peaks at m/e 64 and 63.

Some of the structural formulae in the following pages are speculative representations.


[C6H3]+
m/e 63
[C6H4]+
m/e 64
-H2CN·
-HCN
m/e 227
-H·
-C7H4ClN
m* 36.3
m/e 91 (33%)
m/e 228
-HCl
-Cl·
m/e 192
(12%)
m/e 193
-C2H2
-C2H2
[C11H6N2]+
m/e 166
[C11H7N2]+
m/e 167
-HCN
-HCN
[C10H5N]+
m/e 139
[C10H6N]+
m/e 140

2-p-Chlorophenyl-4-methyl-1H-pyrrolo[2,3-b] pyridine (62)

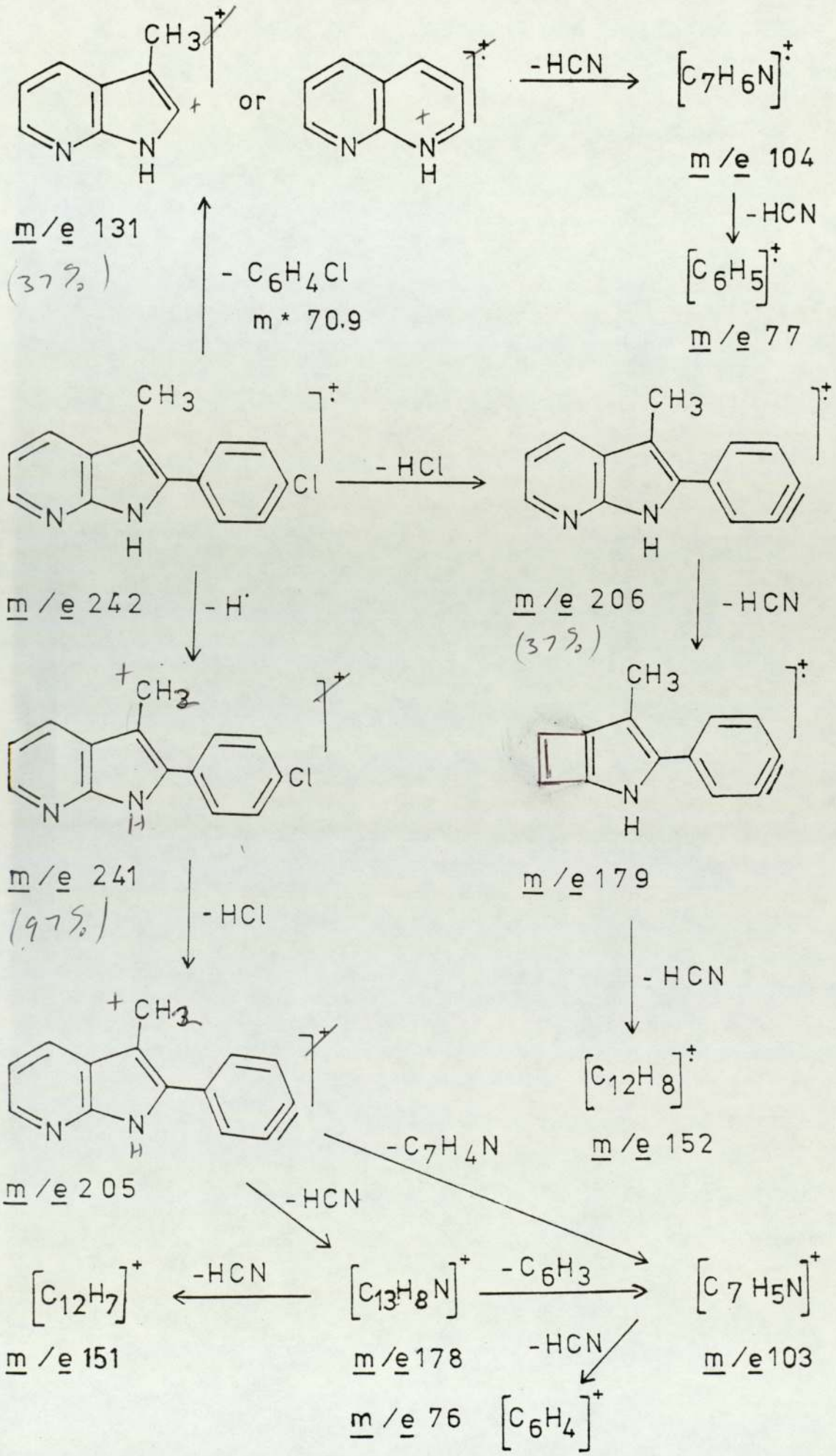
2-p-Chlorophenyl-4-methyl-1H-pyrrolo[2,3-b]pyridine (62) lost $H^{\bullet}$ to give a peak at m/e 241. The molecular ion m/e 242 and the ion at m/e 241 lost HCl followed by 2HCN molecules to give peaks at m/e 206 and 205, 179 and 178 and 152 and 151 respectively. The other route that operated in this compound involved the loss of $Cl^{\bullet}$ to give a peak at m/e 207; this ion then fragmented further as described for the M^{+} and $(M-H)^{+}$ ions. This compound showed a peak of 5% intensity due to direct loss of HCN from the molecular ion.


m/e 178
-HCl
m/e 214
-HCN
m/e 242
(100%)
-H·
-HCl
m/e 241
(35%)
-HCl
m/e 205
-HCN
[C13H8N]+ HCN [C12H7]+
m/e 178
m/e 151
[C12H9]+
[C7H6N]+
m/e153
m/e104
-HCN
-C6H4
m/e 180
-HCN
Cl·
m/e 207
m/e 206
-HCN
-HCN
-C6H4
m/e 179
[C12H9]+
[C7H6N]+
m/e 152
m/e 104

2-p-Chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine (63)

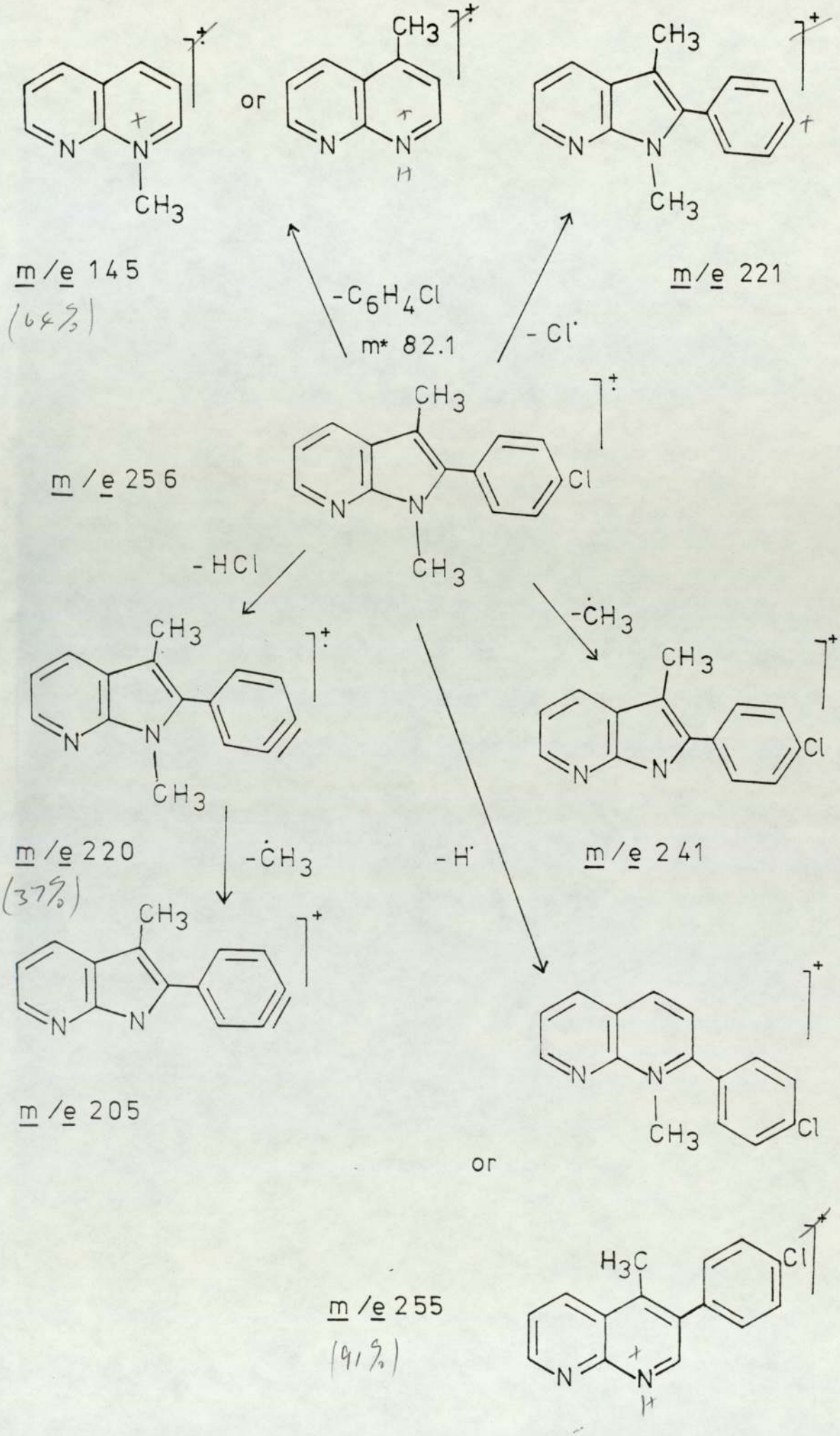
The dominant feature in the spectrum of this compound after the molecular ion was the $(M - H)^+$ peak which had 92% relative abundance. The molecular ion and the $(M - H)^+$ ion both then expelled HCl to give peaks at m/e 206 and 205 followed by the loss of HCN to m/e 179 and 178. The ion m/e 205 also lost C_7H_4N to give the peak at m/e 103.

This compound showed a facile cleavage of the p-chlorophenyl radical to give a peak at m/e 131 (M^* 70.9). This ion has been suggested to have the ring-expanded naphthyridinium structure.[113] This is supported by the lack of the peak at m/e 116 resulting from the loss of $CH_3^{\bullet}$. This ion degraded by the sequential loss of two HCN molecules to give the peaks at m/e 104 and 77.


CH3
or
-HCN
[C7H6N]+
m/e 104
-HCN
[C6H5]+
m/e 77
m/e 131
(37%)
- C6H4Cl
m* 70.9
CH3
Cl
- HCl
m/e 242
- H·
m/e 206
(37%)
-HCN
CH3
Cl
m/e 241
(97%)
- HCl
CH3
m/e 179
-HCN
[C12H8]+
m/e 152
CH3
m/e 205
-C7H4N
-HCN
[C12H7]+
m/e 151
-HCN
[C13H8N]+
m/e 178
-C6H3
[C7H5N]+
m/e 103
-HCN
m/e 76
[C6H4]+

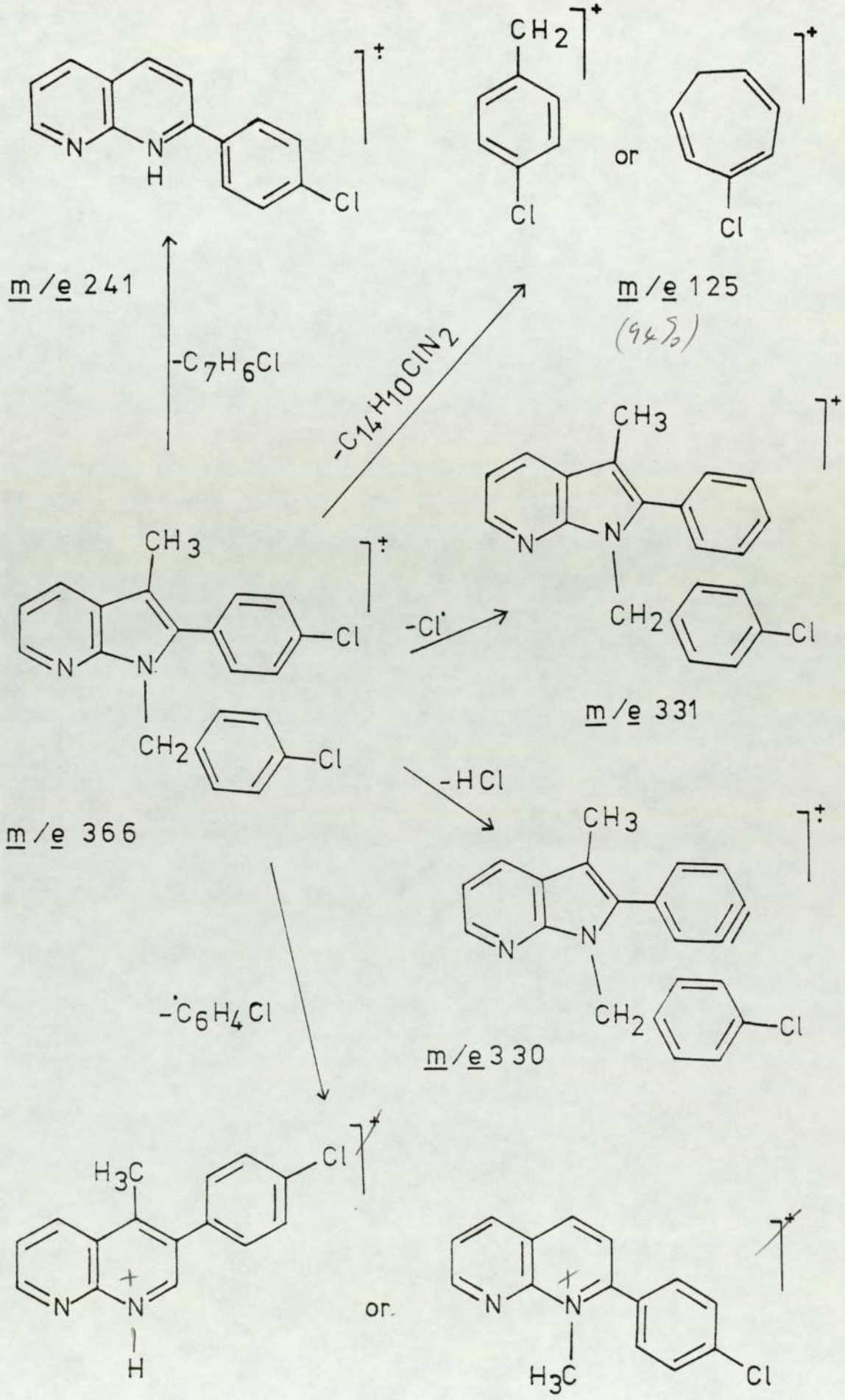
2-p-Chlorophenyl-1,3-dimethyl-1H-pyrrolo[2,3-b] pyridine (71).

This compound showed a very intense $(M-H)^+$ peak. Both the molecular ion and $(M-H)^+$ ion lost HCl to give peaks at m/e 220 and 219 respectively. The molecular ion lost Cl· to give a peak at m/e 221. Both the molecular ion and the ion at m/e lost $CH_3^\cdot$ to give peaks at m/e 241 and 205 respectively; presumably the N-CH_3 is lost more easily than the C-CH_3. The loss of p-chlorophenyl radical was very easy giving a peak at m/e 145 (M^* 82.1) in 64% relative abundance.


or
m/e 145
(64%)
-C6H4Cl
m* 82.1
- Cl·
m/e 221
m/e 256
- HCl
-·CH3
m/e 220
(37%)
-·CH3
- H·
m/e 241
m/e 205
or
m/e 255
(91%)

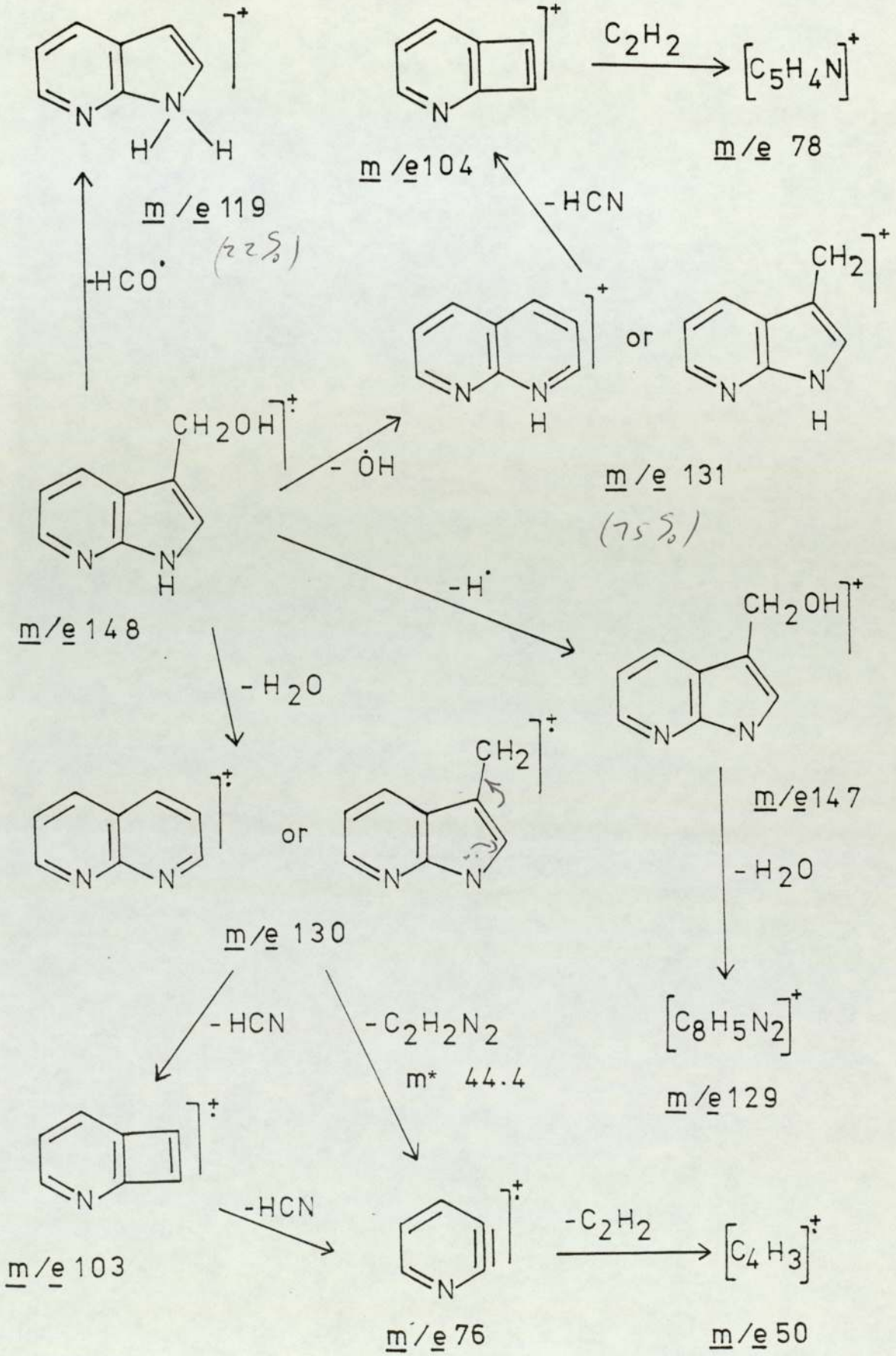
1-p-Chlorobenzyl-2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b] pyridine (69)

The loss of the p-chlorophenyl radical was the dominant feature of the spectrum of this compound and $(M-C_6H_4Cl)^+$ was the base peak. The second most important initial degradation pathway involved the cleavage of the $N-CH_2C_6H_4$-p-Cl bond to give a peak at m/e 125. The ion with this peak presumably exists as the ring expanded chlorotropylium ion. This compound did not show the $(M-H)^+$ ion peak. The other routes involving the loss of HCl or Cl· operated to a less extent. The direct loss of the p-chlorobenzyl radical from the molecular ion gave the peak at m/e 241 in intensity of 15%.


m/e 241
-C7H6Cl
-C14H10ClN2
m/e 125
(94%)
or
-Cl·
m/e 331
m/e 366
-HCl
m/e 330
-·C6H4Cl
m/e 255
or

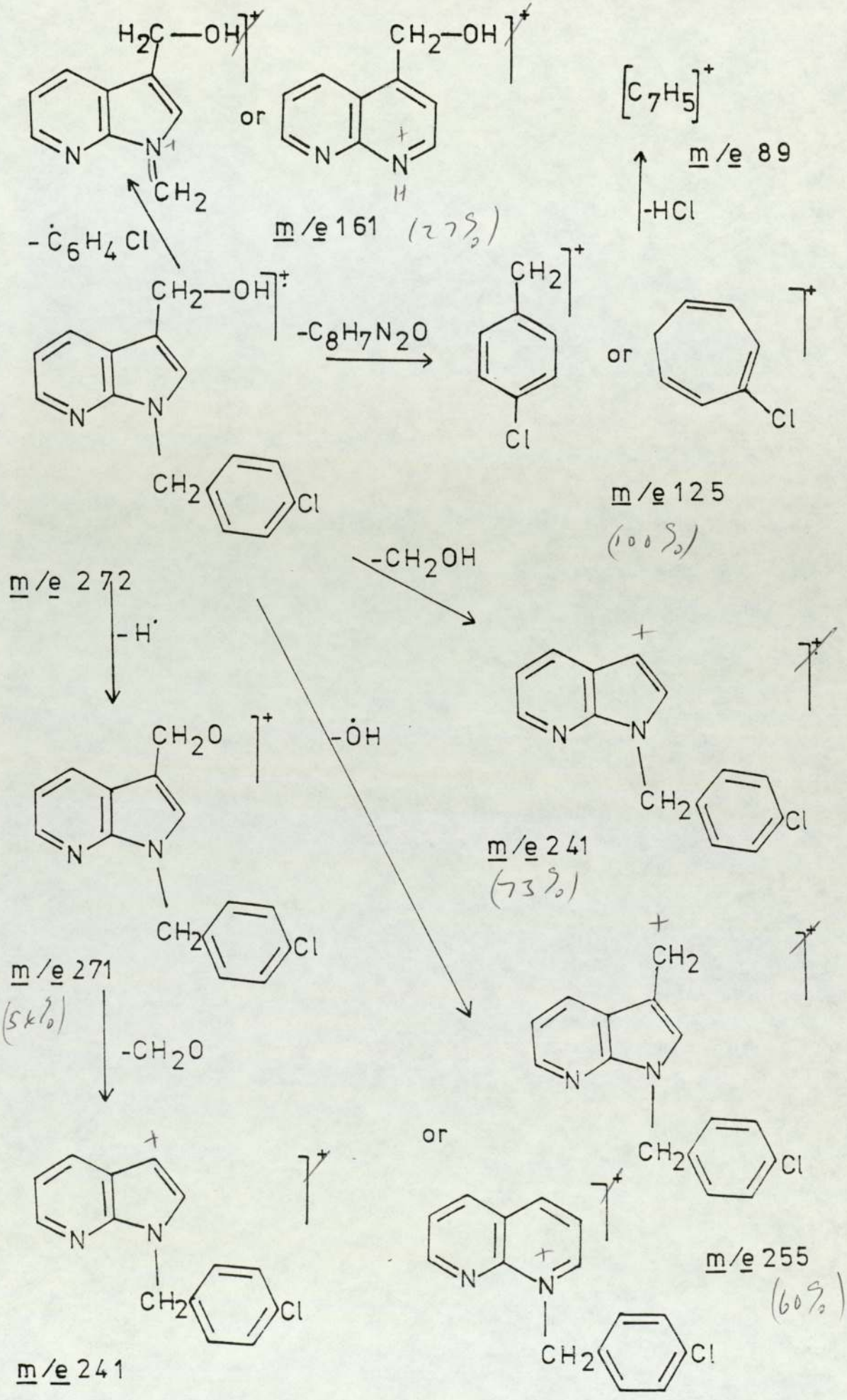
1H-Pyrrolo[2,3-b]pyridine-3-methanol (75)

1H-pyrrolo[2,3-b]pyridine-3-methanol showed fragmentation by the expulsions of H_2O, $OH^{\cdot}$ and $H^{\cdot}$ in 100, 75 and 18% intensities. The ion at m/e 130 degraded by the direct loss of $C_2H_2N_2$ (M^* 44.4) to m/e 76 and also by the sequential expulsion of two HCN molecules; while the m/e 131 ion sequentially lost HCN and C_2H_2 to m/e 104 and 78. A rearrangement reaction involving two proton-transfer to the ring resulted in the expulsion of CHO to give a peak at m/e 119.


m/e 119
(22%)
-HCO·
m/e104
C2H2
[C5H4N]+
m/e 78
-HCN
or
CH2
m/e 131
(75%)
CH2OH
- ·OH
m/e 148
-H·
-H2O
m/e147
-H2O
or
m/e 130
-HCN
-C2H2N2
m* 44.4
[C8H5N2]+
m/e129
m/e 103
-HCN
-C2H2
[C4H3]+
m/e 76
m/e 50

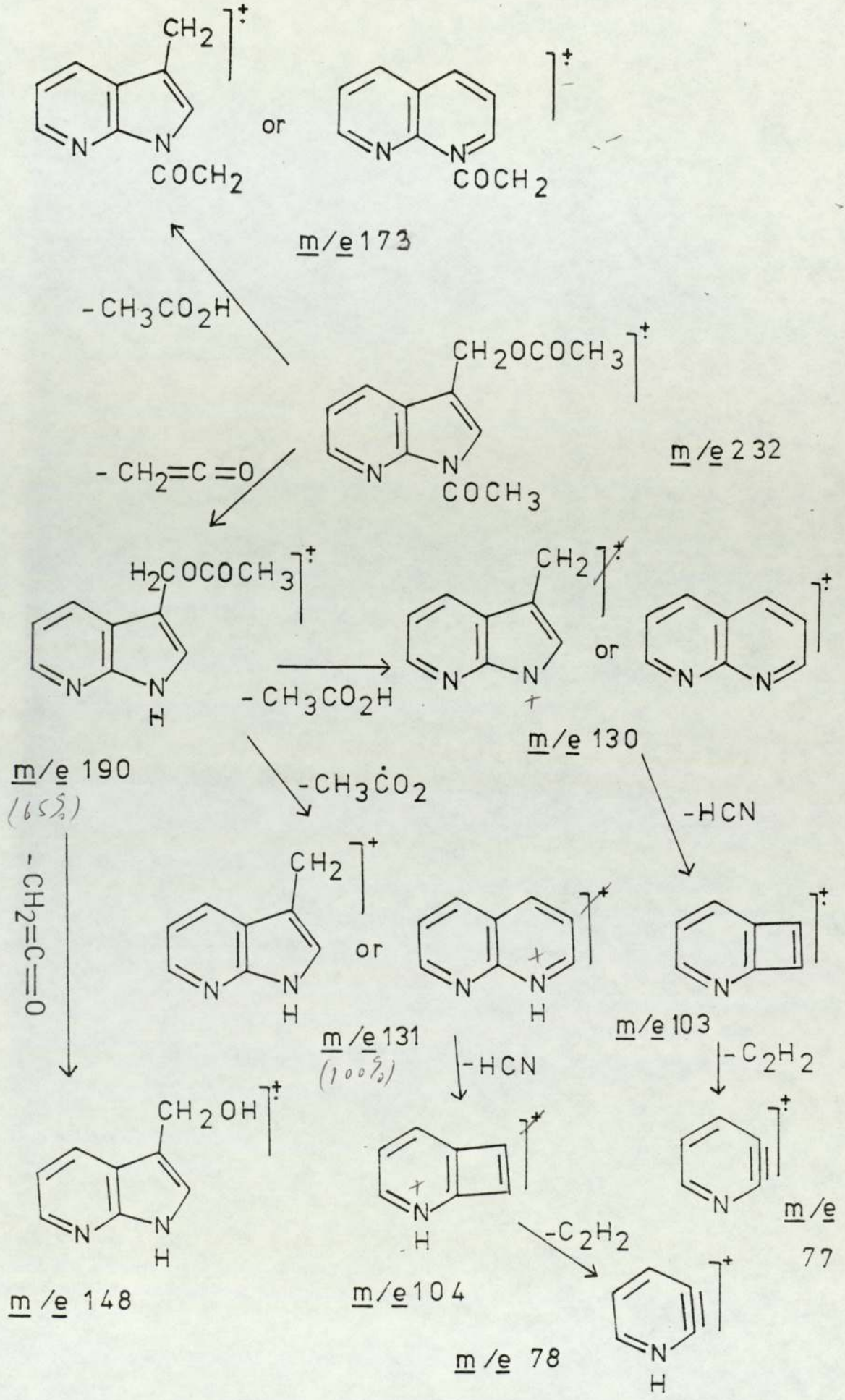
1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-methanol (76)

The base peak in this compound resulted from the cleavage of the $N-CH_2$ bond to give the peak at m/e 125. The other routes involved the expulsion of OH', H' and 'CH_2OH radicals to give peaks at m/e 255, 271 and 241 with 60, 54 and 72% intensities respectively. The other process involved the loss of the p-chlorophenyl radical to give m/e 161 with intensity of 27%.


m/e 161 (27 %)
m/e 89
-HCl
-C6H4Cl
-C8H7N2O
m/e 125
(100 %)
m/e 272
-H·
-CH2OH
-·OH
m/e 241
(73 %)
m/e 271
(54 %)
-CH2O
m/e 241
m/e 255
(60 %)

3-Acetoxymethyl-1-acetyl-1H-pyrrolo[2,3-b]pyridine (78)

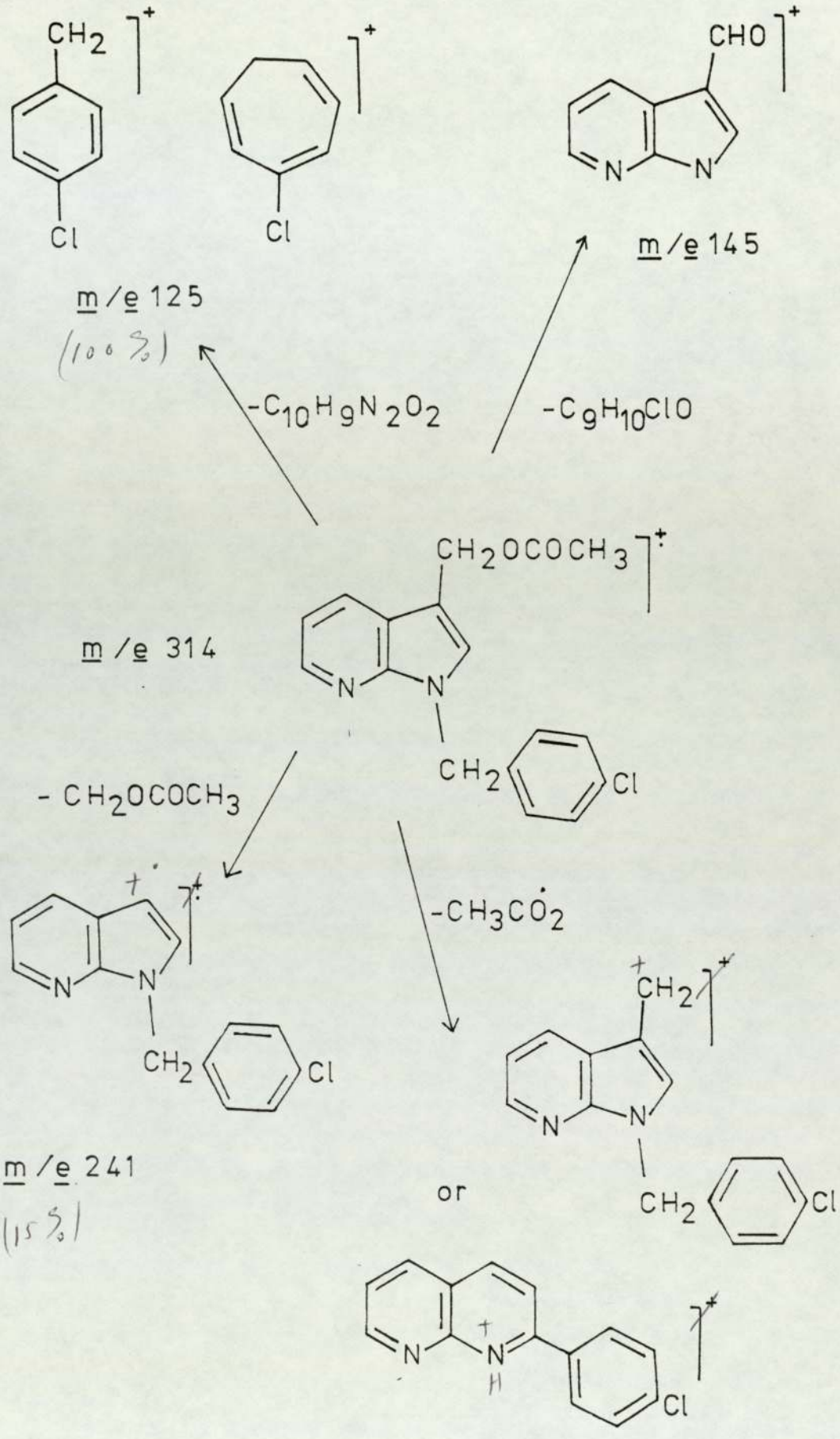
The initial fragmentation of this compound involved the expulsion of ketene molecule ($CH_2 = C = O$) to give a peak at m/e 190. This ion fragmented further by the loss of another ketene molecule and acetyl radical as well as acetic acid molecule to give peaks at m/e 148, 131 and 130 respectively. The ion at m/e 131 formed the base peak. Both the ions at m/e 131 and 130 sequentially expelled HCN and C_2H_2 to give peaks at m/e 104 and 103, 78 and 77 respectively.


CH2
COCH2
or
COCH2
m/e 173
-CH3CO2H
CH2OCOCH3
COCH3
m/e 232
-CH2=C=O
H2COCOCH3
-CH3CO2H
CH2
or
m/e 130
m/e 190
(65%)
-CH3CO2
-HCN
-CH2=C=O
CH2
or
m/e 131
(100%)
m/e 103
-HCN
-C2H2
CH2OH
m/e 148
m/e 104
-C2H2
m/e 77
m/e 78

3-Acetoxymethyl-1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (79)

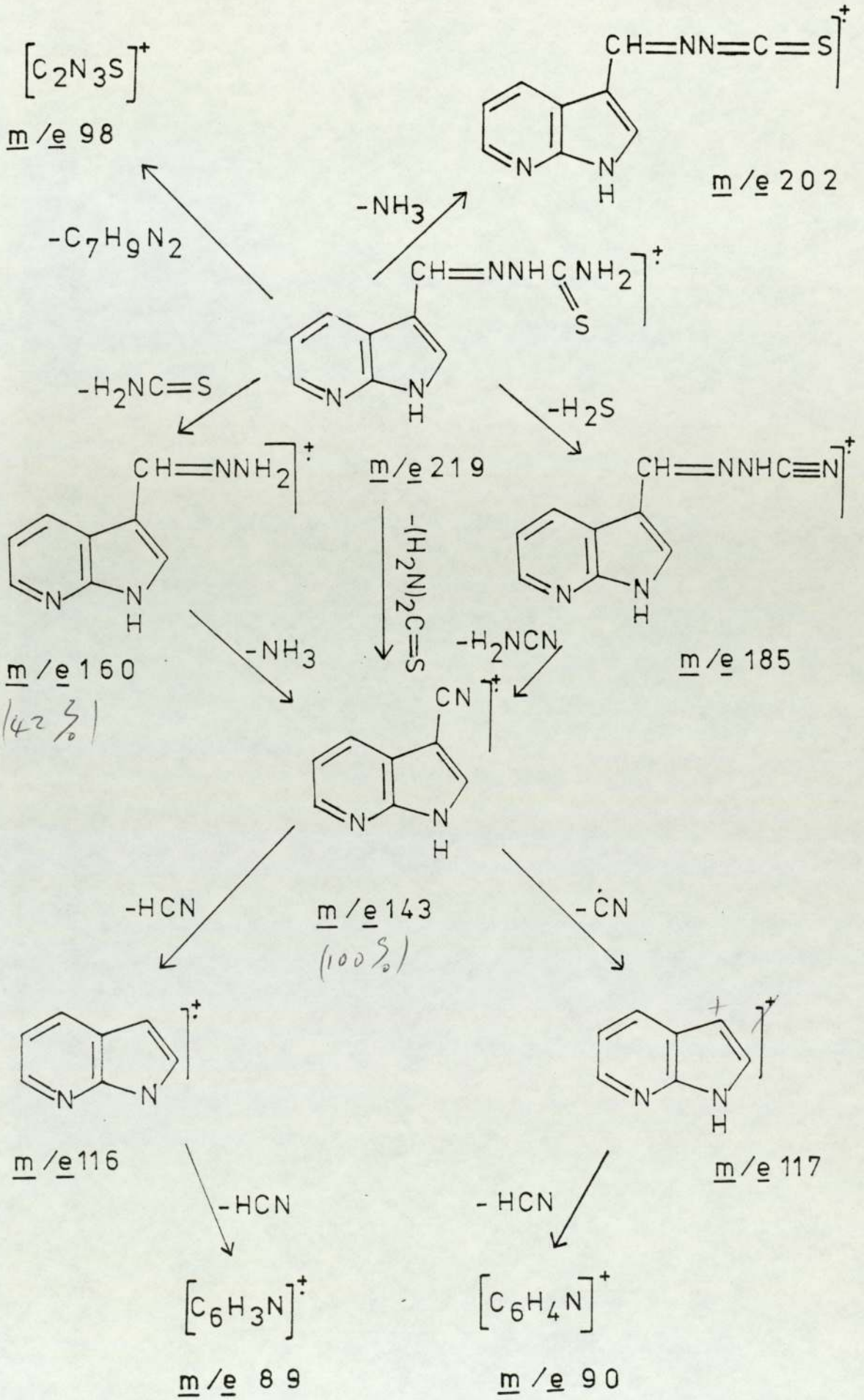
This 1-substituted ester, like the methanol derivative (76) from which it was prepared had a base peak at m/e 125 due to the chlorotropylium ion formed by the cleavage of the N-CH_2 bond.

The direct loss of the acetoxy and the acetoxymethyl groups gave peaks at m/e 255 and 241. The loss of the p-chlorobenzyl group with a proton transfer to the ring gave the peak at m/e 131. The peak at m/e (9%) was due to the loss of p-chlorobenzyl group and acetaldehyde molecule.


CH2
Cl
Cl
CHO
N
N
m/e 145
m/e 125
(100 %)
-C10H9N2O2
-C9H10ClO
CH2OCOCH3
m/e 314
N
N
CH2
Cl
- CH2OCOCH3
-CH3CO2
N
N
CH2
Cl
m/e 241
(15 %)
or
CH2
N
N
CH2
Cl
N
H
Cl
m/e 255 (69 %)

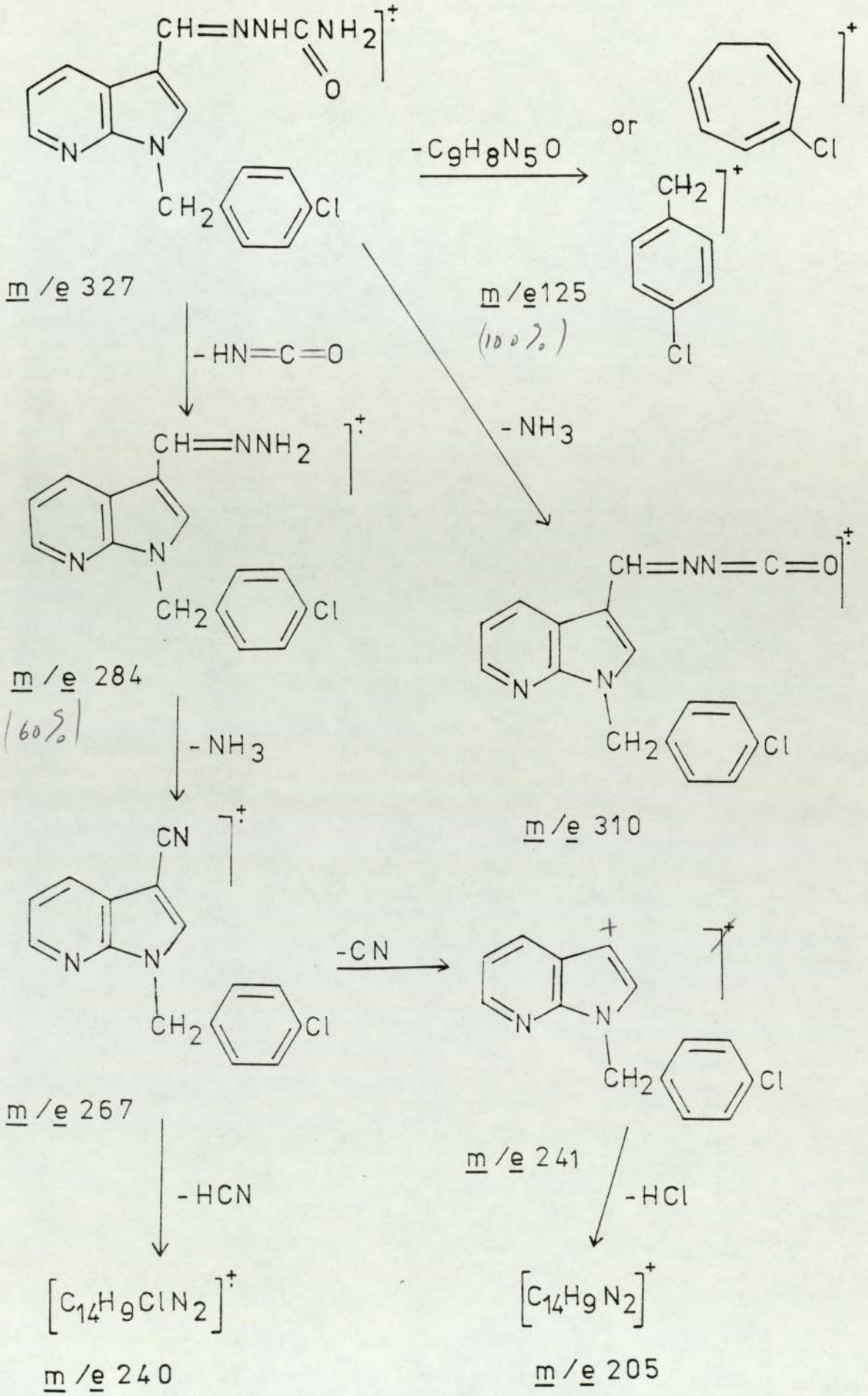
1H-Pyrrolo[2,3-b]pyridine-3-carbaldehyde thiosemicarbazide (81)

This compound showed a base peak at m/e 143 which could presumably have been formed by the cleavage of a molecule of thiourea from the molecular ion. The sequential loss of CH_2NS (m/e 160) and NH_3, as well as that involving H_2S (m/e 185) and CH_2N_2 could give the same ion. The ion m/e 143 degraded by expulsion of HCN and $^{\bullet}CN$ to m/e 116 and 117 followed by HCN to m/e 89 and 90. Loss of NH_3 from the molecular ion gave a peak at m/e 202 (10%).


[C2N3S]+
m/e 98
-C7H9N2
-NH3
CH=NN=C=S
m/e 202
CH=NNHC(=S)NH2
m/e 219
-H2NC=S
-H2S
CH=NNH2
m/e 160
(42 %)
-(H2N)2C=S
CH=NNHC≡N
m/e 185
-NH3
-H2NCN
CN
m/e 143
(100 %)
-HCN
-ĊN
m/e 116
m/e 117
-HCN
-HCN
[C6H3N]+
m/e 89
[C6H4N]+
m/e 90

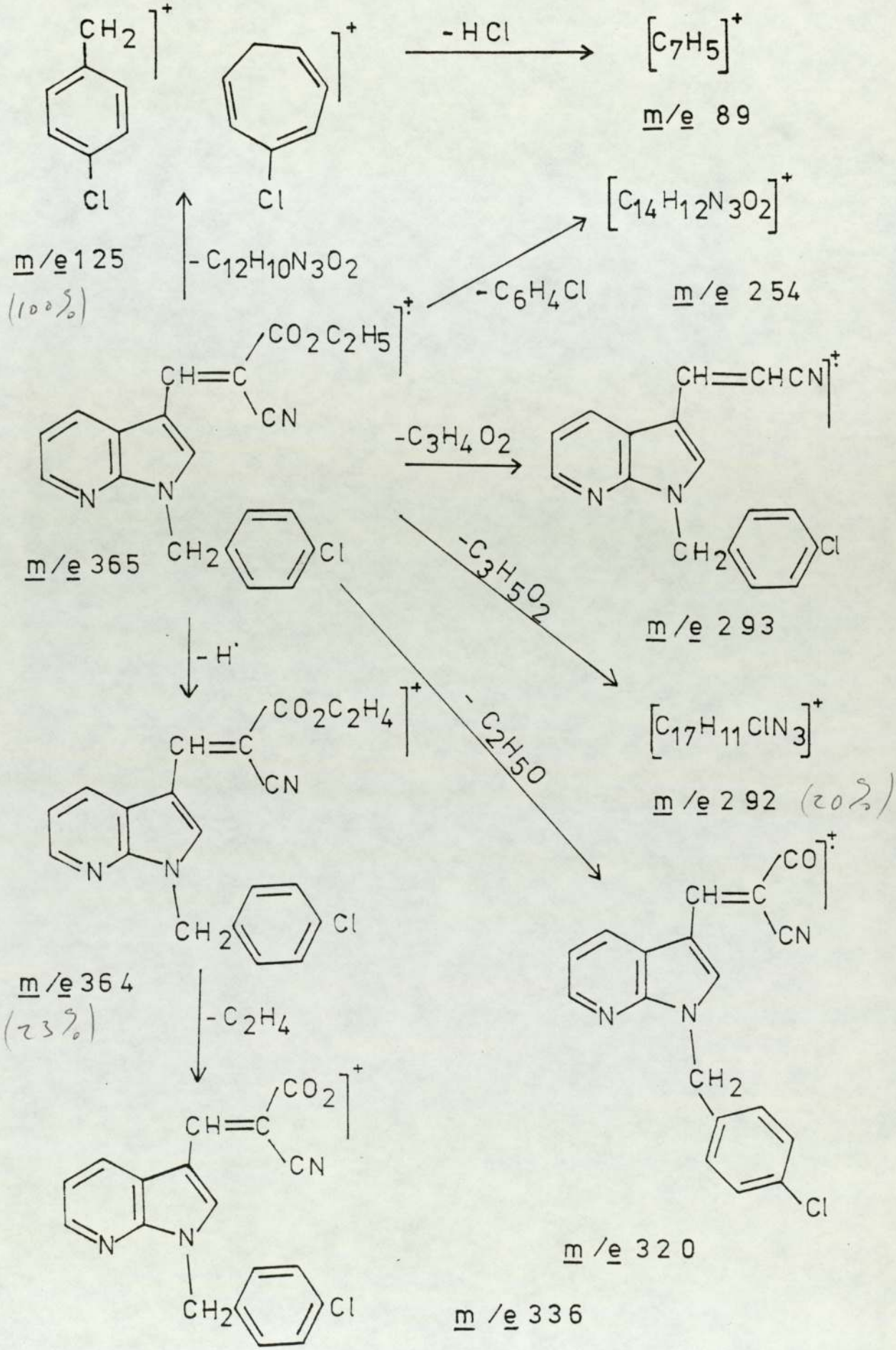
1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde semicarbazide (82)

The base peak of this compound was at m/e 125 resulting from the cleavage of the N-CH_2 bond. The loss of HN=C=O and NCO from the molecular ion gave the peaks at m/e 284 and 285. These ions then lost NH_3 to give peaks at m/e 267 and 268. The loss of an ammonia molecule from the molecular ion to give a peak at 310 did not proceed to a significant extent.


CH=NNHCNH2
O
N
N
CH2
Cl
m/e 327
-C9H8N5O
or
CH2
Cl
m/e 125
(100%)
-HN=C=O
CH=NNH2
m/e 284
(60%)
-NH3
-NH3
CH=NN=C=O
m/e 310
CN
-CN
m/e 267
m/e 241
-HCN
-HCl
[C14H9ClN2]+
m/e 240
[C14H9N2]+
m/e 205

Ethyl 2'-cyano-3'-1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyrid-3-ylacrylate (85)

The base peak in this compound was the molecular ion and the peak due to $N-CH_2$ cleavage at m/e 125 was 90%. The $(M-H)^+$ peak was 23%, and this ion and the molecular ion lost C_2H_4 and C_2H_5 respectively to give a peak at m/e 336. The expulsion of $C_3H_5O_2$ from the molecular ion gave the peak at m/e 292. The peak at m/e 320 was due to the loss of an ethoxy group from the molecular ion. The loss of the p-chlorophenyl radical from the molecular ion was 14% in this compound.

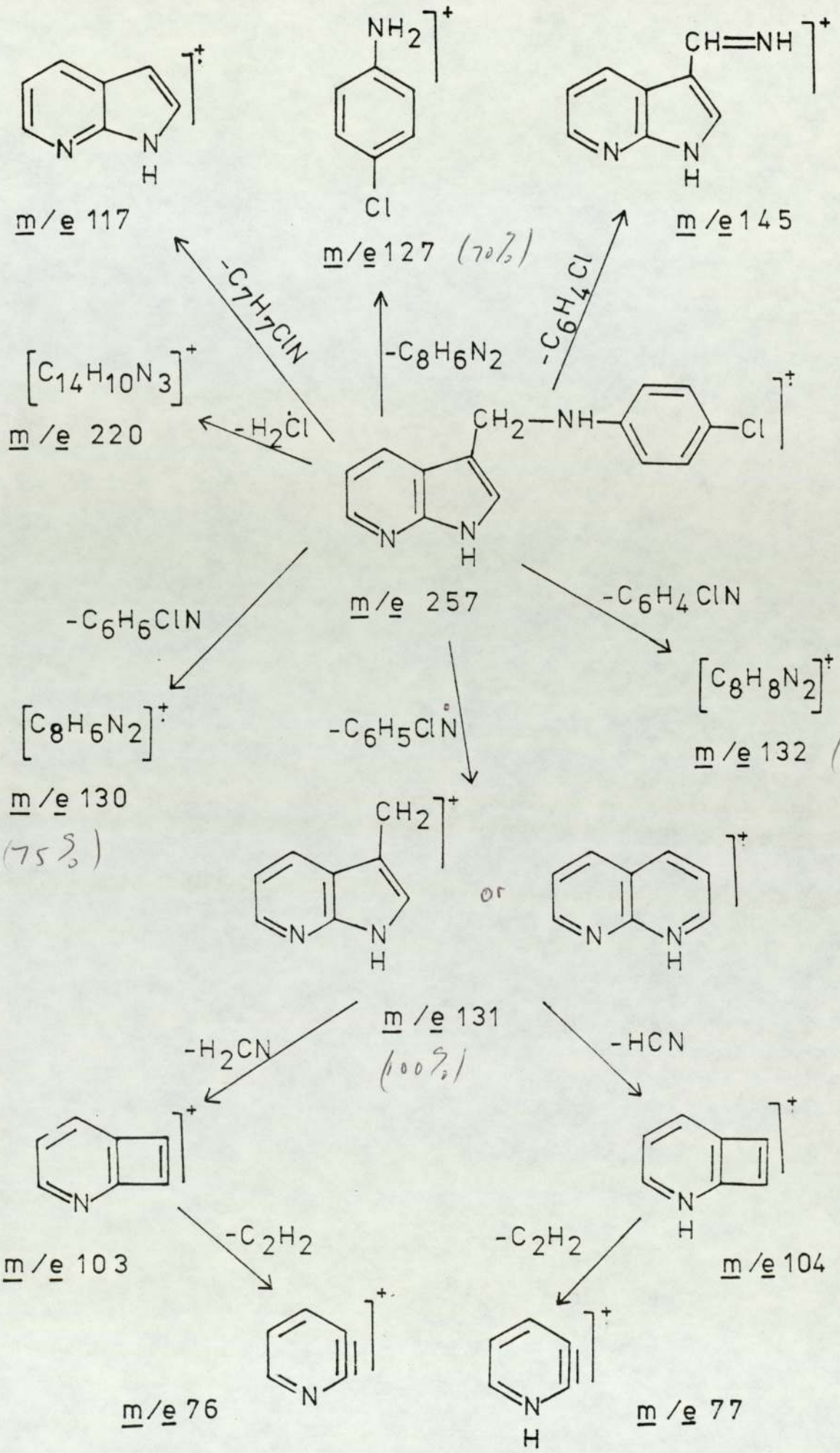

- HCl
[C7H5]+
m/e 89
m/e 125
(100%)
- C12H10N3O2
[C14H12N3O2]+
- C6H4Cl
m/e 254
CO2C2H5
CH=C
CN
m/e 365
- C3H4O2
CH=CHCN
CH2
Cl
m/e 293
- C3H5O2
- C2H5O
- H·
CO2C2H4
m/e 364
(23%)
[C17H11ClN3]+
m/e 292 (20%)
CO
m/e 320
- C2H4
CO2
m/e 336

3-p-Chlorophenyl aminomethyl-1H-pyrrolo[2,3-b] pyridine (88)

The base peak of this compound was at m/e 131 resulting from the expulsion of C_6H_5ClN. The loss of C_6H_6ClN and C_6H_4ClN from the molecular ion gave the peaks at m/e 130 and 132. The molecular ion also lost the p-chlorophenyl radical to give the peak at m/e 145 relative abundance 16%.

The cleavage of the C_3-CH_2 bond resulted in the ion at m/e 117.

The ion m/e 137 fragmented further by the loss of HCN and H_2CN to give peaks at m/e 104 and 103 followed by the loss of C_2H_2 to give m/e 76 and 78 peaks.


m/e 117
m/e 127 (70%)
m/e 145
-C7H7ClN
-C8H6N2
-C6H4Cl
[C14H10N3]+
m/e 220
-H2Cl
CH2—NH—Cl
m/e 257
-C6H6ClN
-C6H5ClN
-C6H4ClN
[C8H6N2]+
m/e 130
(75%)
[C8H8N2]+
m/e 132 (42%)
CH2
or
m/e 131
(100%)
-H2CN
-HCN
m/e 103
m/e 104
-C2H2
-C2H2
m/e 76
m/e 77

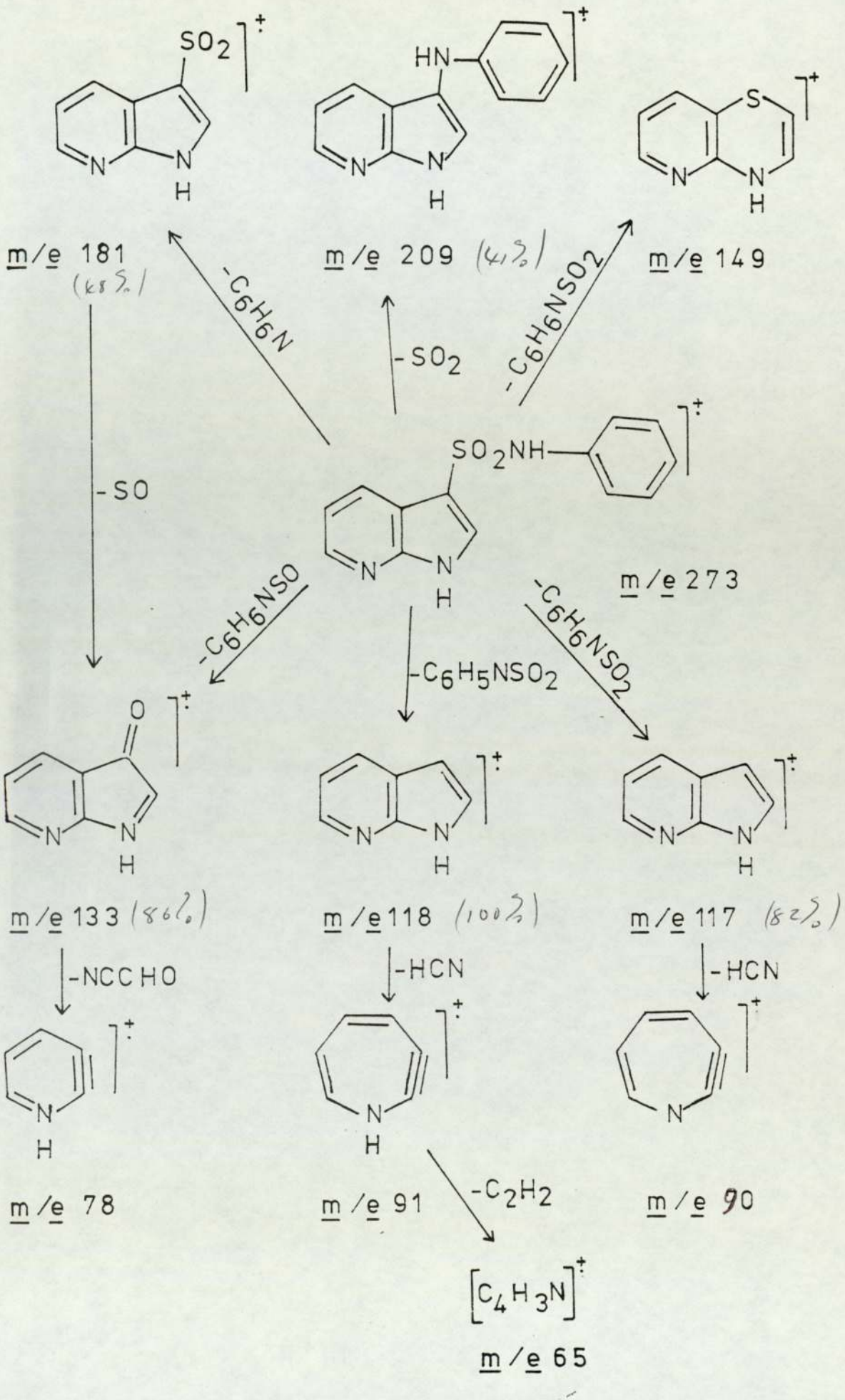
3-N-Phenyl sulphamoyl-1H-pyrrolo[2,3-b]pyridine (106)

The base peak in this compound was due to the loss of $C_6H_5NSO_2^{\cdot}$ to give a peak at m/e 118. This ion then lost HCN to give the peak at m/e 91 followed by the expulsion of C_2H_2 to m/e 65.

A re-arrangement process with transfer of oxygen atom to the ring must operate to give the peak at m/e 133 with a relative abundance of 86%. This ion then loses C_2HNO to give the peak at m/e 78. Another re-arrangement process involved the expulsion of SO_2 to give the peak at m/e 209 with 40% intensity.

The loss of an aniline molecule from the molecular ion gave the peak at m/e 181 and this ion re-arranged and expelled SO to give the peak at m/e 131.

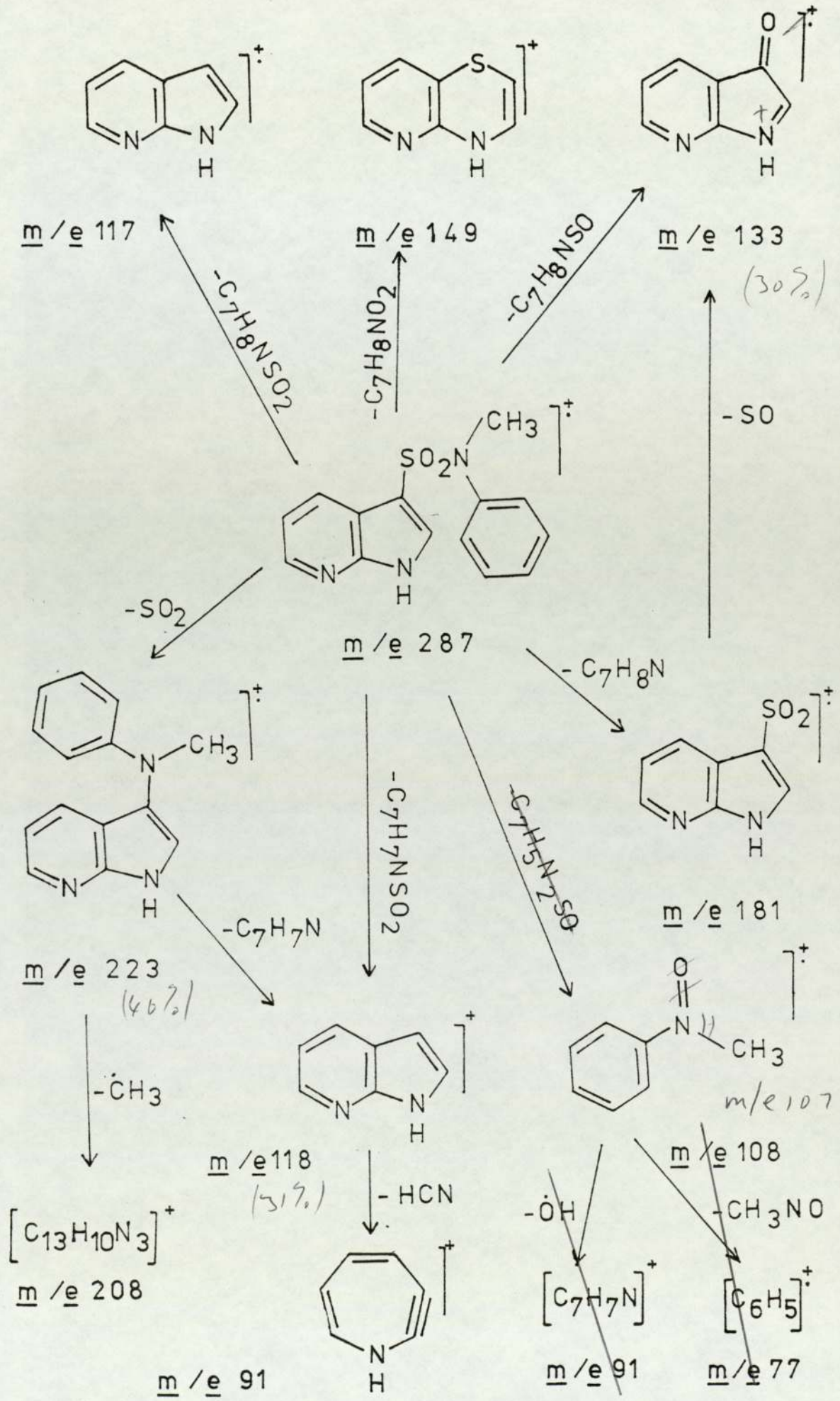
Another re-arrangement process operated in which only the sulphur atom was retained in the ring to give the peak at m/e 149 with 46% intensity. This ion at m/e 149 probably has the ring-expanded structure.


SO2NH
-C6H6N
-SO2
-C6H6NSO2
-SO
-C6H6NSO
-C6H5NSO2
-C6H6NSO2
-NCCHO
-HCN
-HCN
-C2H2
m/e 273
m/e 181 (48%)
m/e 209 (41%)
m/e 149
m/e 133 (86%)
m/e 118 (100%)
m/e 117 (82%)
m/e 78
m/e 91
m/e 90
[C4H3N]+
m/e 65

3-N-Methyl-N-phenylsulphamoyl-1H-pyrrolo[2,3-b] pyridine (107)

The molecular ion in this compound was the re-arrangement ion N-methyl nitrosoaniline at m/e 108.

Like compound (106) above, this compound showed re-arrangement with expulsion of SO_2 to give the peak at m/e 223. Another re-arrangement with oxygen transfer to the ring gave the peak at m/e 133. The peaks at m/e 118 and 117 due to the loss of $C_7H_7NSO_2$ and $C_7H_8NSO_2$ were less important in this compound, so was the loss of N-methyl anilide radical to give the peak at m/e 181.


m/e 117
m/e 149
m/e 133
(30%)
-C7H8NSO2
-C7H8NO2
-C7H8NSO
-SO
m/e 287
-SO2
-C7H8N
m/e 223
(46%)
-C7H7NSO2
-C7H5N2SO
m/e 181
-C7H7N
-·CH3
m/e 118
(31%)
m/e 108
m/e 107 (100%)
-HCN
-·OH
-CH3NO
[C13H10N3]+
m/e 208
m/e 91
[C7H7N]+
m/e 91
[C6H5]+
m/e 77

EXPERIMENTAL

I SCREENING OF SELECTED 1H-PYRROLO[2,3-b] PYRIDINES AGAINST T.b. brucei

II SYNTHESIS OF 1H-PYRROLO[2,3-b]PYRIDINES

The Screening of Selected 1H-Pyrrolo[2,3-b]pyridines against T.b. brucei

Part of the screening programme described below was carried out in the Department of Medical Protozoology at the London School of Tropical Medicine and Hygiene. It was continued and completed in the Department of Pharmacy in the University of Aston in Birmingham. One of the compounds (107) was screened in the Division of Pharmaceutical sciences, University of Dar-es-Salaam, using suramin (12) as a control.

The trypanosome used in the screening, T.b. brucei (LUMP 1164, and TREU 667, and 8/18) is a known suramin sensitive strain; and in the initial screening using compounds of unknown activity, the control treated with suramin is not included.

All the compounds used in the screening had been prepared for the purpose during the course of this work. The compound Abrine (2'-N-methyl-3'-indol -3-ylpropanoic acid) was purchased from Aldrich Chemicals.

Each test compound (10 mg) was dissolved or suspended in propane-1,2-diol (2ml, conc. 5mg/ml). The volume of the mixture to give the required dose was measured out in a small syringe (size 1 ml) and injected into the mouse intraperitoneally. Solutions/ suspensions were made fresh every day (not sterilised).

Male and female Theiller's Original albino mice weighing about 20-45 g were used. Two mice were used per compound. Cryo-preserved trypanosome stabilates of T.b. brucei (LUMP 1164, and TREU 667, and 8/18) supplied by the Department of Medical Protozoology above were

used. The stabilate 8/18 gives an acute infection which is fatal in four to five days. Treatment with a given compound started when parasitaemia was patent; for the chronic forms this was the fifth day and the third day for the acute form (8/18).

During the course of the experiment the parasites were syringe-passaged to other test animals by drawing a little tail blood from a heavily infected mouse and mixing it with a saline-phosphate buffer. This mixture (0.2 ml) was injected intraperitioneally to each of the next test mice. The chronic disease giving stabilates remained unchanged after such passaging.

For the infectivity test, heavily infected mice (with 8/18) were anaesthetized with ether, the thorax opened and all blood from the heart collected into a syringe containing two drops of heparin (2 units/ml) in saline phosphate buffer. The blood was mixed with an equal volume of glucose (21.8 g/l) solution and kept at 25°. The blood mixture (0.9 ml) was mixed with each test compound (0.1 ml, conc. 100 mM in propane-1,2-diol) in turn and incubated at 25° for 1 h. At intervals (0.25, 0.5 and 0.75 h) a drop of the mixture was examined microscopically to ensure parasites were alive. Finally the mixture (0.2 ml) was injected intraperitoneally into a mouse. The mice were examined for about five days.

Screening of Selected 1H-Pyrrolo[2,3-b]pyridine derivatives against T.b. brucei (LUMP 1164, TREU 667 and 8/18)

Table 4

Compound number	T.b. brucei stock designation	Total no. doses given	mg/kg	Curative effect
74	TREU 667	6	25	none
76	TREU 667	3	25	none
77	LUMP 1164	6	25	none
	8/18	2	50	none
78	TREU 667	7	25	none
79	TREU 667	7	25	none
81	LUMP 1164	6	25	none
	8/18	3	25	none
82	LUMP 1164	6	25	none
	8/18	3	25	none
84	LUMP 1164	-	-	
	8/18	3	25	none
88	LUMP 1164	-	-	
	8/18	3	25	none
90	LUMP 1164	6	25	none
	8/18	3	25	none
91	LUMP 1164	6	11.4	none
	8/18	3	12	none
92	LUMP 1164	6	25	none
	8/18	3	25	none
99	TREU 667	6	35	none
100	TREU 667	3	35	none
107		10	50	none
108	TREU 667	7	25	none
121	LUMP 1164	6	50	none
	8/18	3	25	none
122	LUMP 1164	6	25	none
	8/18	3	24	none
123	TREU 667	3	35	none
a*	TREU 667	6	35	none
b*	TREU 667	6	35	none
c*	TREU 667	6	35	none

* These compounds are not discussed in the text of this thesis. They were prepared for the purpose of screening.

a 3-N, N-Dimethylaminomethyl-1H-pyrrolo[2,3-b] pyridine (7-azagramine) was prepared by the method of Robison and Robison[107] in 80% yield.

b 1-p-Chlorobenzyl-3-N,N-dimethylaminomethyl-1H-pyrrolo[2,3-b] pyridine (1-p-chlorobenzyl-7-azagramine) was prepared by the method of Verbiscar[97] in 98% yield.

c. 2'-N-Methyl-3'-indol-3-ylpropanoic acid (2'-N-methyl tryptophan was purchased from Aldrich Chemicals.

Infectivity Inhibition Testing with Selected 1H-Pyrrolo[2,3-b] pyridine Derivatives against T.b. brucei (8/18)

Table 5

Compound number	Weight used (mg)	Vol. of solution (ml)	Conc. (mM)	Conc. in mixture (mM)	Survival of mice
77	28.6	1	100	10	survived
82	32.8	1	100	10	died
88	27.1	1	100	10	survived
90	21.9	1	100	10	died
91	20.3	1	100	10	died
92	32.8	1	100	10	died
122	24.7	1	100	10	died
Blood mixture alone	-	-	-	-	died
Blood mixture with propane-1,2-diol	-	-	-	-	died

Infrared spectra were determined, unless otherwise stated as potassium bromide (KBr) discs with a Unicam SP 200 spectrometer. The most intense peaks in the spectra and those which were easily assignable are recorded.

Nuclear magnetic resonance (n.m.r.) spectra were measured on Varian A60-A and E360-A Spectrometers. Tetramethylsilane was used as the internal standard. All peaks are assigned in terms of δ-values. Abbreviations used in the interpretation of n.m.r. spectra are:-

s = singlet,	d = doublet,	t = triplet,
q = quartet,	m = multiplet,	J = coupling constant.

Mass spectra were determined on A.E.I. MS 9 spectrometer. Percent abundances are shown in parentheses.

Sublimation and reaction temperatures above 100° are those of the external bath, unless otherwise specified. Melting points are uncorrected.

Preparation of 1H-pyrrolo[2,3-b]pyridines

A. By Fischer (thermal) cyclisation

2-Pyridylhydrazine (53, R=H).—

2-Pyridylhydrazine was prepared by the method of Fargher and Furness[83] in yields of 65-72% as pale yellow needles, m.p. 47-48° (Lit.[83] 46°). It was used immediately.

4-Methyl-2-pyridylhydrazine (54, R=CH_3).—

4-Methyl-2-pyridylhydrazine was prepared in two steps from 4-methyl-2-aminopyridine (51, R=CH_3) by the method of Herbert and Wibberley[106] as unstable redish needles and used immediately.

p-Chloroacetophenone 2-pyridylhydrazone (55).—

2-Pyridylhydrazine (20.8g) and p-chloroacetophenone (33.5g) in propan-2-ol (50 ml) were heated under reflux on the waterbath overnight. The mixture was cooled and filtered to give the hydrazone (40.8g, 98.2%), colourless prisms, m.p. 109-111° (from aqueous ethanol).

Found: C, 63.40; H, 4.87; N, 16.99.

$C_{13}H_{12}ClN_3$ required C, 63.67; H, 4.90; N, 17.14%.

ν max. (KBr) 3450(NH), 1600, 1582, 1490, 1435, 1405, 1395 1130, 1095, 840, 780 $cm.^{-1}$

δ ($CDCl_3$) 8.47 (1H, s, N-H)
8.14 (1H, d, J = 5Hz, 6-H)
8.00-7.03 (6H, m, 3, 4, 2', 3', 5', 6' - H)

6.79 (1H, t, $J_{5,6}$ = 5Hz, $J_{5,6}$ = 6Hz, 5-H)
2.08 (3H, s, CH_3).

M^+ 245

m/e 247(6), 246(5), 245(19), 244(6), 233(5), 232(37), 231(34), 230(100), 226(5), 196(5), 195(3), 168(5), 167(3), 153(5), 152(4), 149(6), 146(6), 139(6), 138(12), 137(11), 134(51), 122(11), 111(19), 94(17), 93(55), 78(12), 77(16), 76(22), 75(24), 67(20), 66(25), 57(23), 53(15), 47(22), 39(27), 29(8), 28(28), 27(11).

p-Chloroacetophenone 4-methyl-2-pyridylhydrazone (56).—

4-Methyl-2-pyridylhydrazine (10.0g) and p-chloroacetophenone (13.0g) in propan-2-ol (20 ml) were heated under reflux on a water bath overnight. The mixture was cooled, filtered and washed with petroleum ether (60-80°) to give the hydrazone (10.0g, 47.5%), colourless needles, m.p. 135-136° (from propan-2-ol)

Found: C, 64.62; H, 5.49; N, 16.10.

$C_{14}H_{14}ClN_3$ requires C, 64.86; H, 5.41; N, 16.22%

ν max. (KBr) 3225 (NH), 1610, 1570, 1495, 1440, 1405, 1290, 1290, 1275, 1144, 1102, 1090, 1010, 852, 805cm.$^{-1}$

δ ($CDCl_3$) 8.45 (1H, s, N-H)
8.03 (1H, d, J = 6Hz, 6-H)
7.87 - 6.96 (5H, m, 3,2',3',5',6'-H)

6.65 (1H, d, J = 6Hz, 5-H)

2.34 (3H, s, 4-CH_3)

2.07 (3H, s, CH_3)

p-Chloropropiophenone 2-pyridylhydrazone (57).—

2-Pyridylhydrazine (21.0g) and p-chloropropiophenone (35.0g) in propan-2-ol (50ml) treated as above gave the hydrazone (42.0g, 84.2%), colourless prisms m.p. 69-70° (from ethanol).

Found: C, 64.64; H, 5.48; N, 1616.

$C_{14}H_{14}ClN_3$ requires C, 64.86; H, 5.41; N, 16.22%.

ν max. (KBr) 3350 (NH), 2995 (CH_3), 1602, 1580, 1495, 1450, 1318, 1160, 1142, 1100, 1095, 1015, 960, 842, 780 $cm.^{-1}$

$\delta(CDCl_3)$ 8.68 (1H, s, N-H)

8.20 (1H, d, J = 5Hz, 6-H)

7.54 (6H, m, 3, 4, 2', 3', 5', 6' - H)

6.80 (1H, t, $J_{5,6}$ = 5Hz, $J_{5,4}$ = 6Hz, 5-H)

2.60 (2H, q, J = 8Hz, CH_2)

1.15 (3H, t, J = 8Hz, CH_3).

3,4 Dimethylacetophenone 2-pyridylhydrazone (58).—

2-Pyridylhydrazine (21.0g) and 3,4-dimethylacetophenone in propan-2-ol (50 ml) treated as above gave the hydrazone (44.0g, 95.5%), yellow prisms, m.p. 53-55° (from aqueous ethanol).

Found: C, 75.25; H, 7.21; N, 17.72.

$C_{15}H_{17}N_3$ requires C, 75.31; H, 7.11; N, 17.57%.

ν max. (KBr) 3250 (NH), 2950 (CH_3), 1595, 1575, 1505, 1445, 1330, 1298, 1280, 1125, 766 $cm.^{-1}$

δ ($CDCl_3$) 8.39 (1H, s, N-H)
8.03 (1H, d, J = 5Hz, 6-H)
7.82 - 6.88 (5H, m, 3, 4, 2', 5', 6' - H)
2.32 (6H, s, 3', 4' - CH_3)
2.05 (3H, s, CH_3)

3,4-Dimethoxyacetophenone 2-pyridylhydrazone (59).—

2-Pyridylhydrazine (3.1 g) and 3,4-dimethoxyacetophenone (5.2 g) in propan-2-ol (10 ml) were treated as above to give the hydrazone (8.0g, 98.7%), yellow prisms, m.p. 151-153° (from propan-2-ol).

Found: C, 66.32; H, 6.22; N, 15.25.

$C_{15}H_{17}N_3O_2$ requires C, 66.42; H, 6.27; N, 15.50%.

ν max. (KBr) 3210 (NH), 2960(CH_3), 1600, 1585, 1520, 1445, 1420, 1335, 1300, 1270, 1244, 1225, 1130, 1090, 1020, 774 cm^{-1}.

$\delta(CDCl_3)$ 8.45 (1H, s, N-H)
8.12 (1H, d, J = 4Hz, 6 - H)
7.45 (4H, m, 3, 4, 5, 6' - H)
6.73 (2H, 2', 5' - H)
3.83 (6H, OCH_3)
2.13 (3H, s, CH_3).

Propionaldehyde 2-pyridylhydrazone (60).—

2-pyridylhydrazine (19.2g) in propan-2-ol (20 ml) and propionaldehyde (12.0g) were heated on the waterbath under reflux overnight. The solvent was removed under reduced pressure to give the hydrazone (25.0g, 95%) colourless prisms, m.p. 52-54° (Lit.[86] 54-55.5°).

General method of cyclisation of hydrazones into 1H-pyrrolo[2,3-b] pyridines.

The hydrazone dissolved in triethylene glycol (trigol) was heated under reflux for the stated time, cooled and the mixture poured into water. The product, which separated on standing, was collected and purified by washing with ether and sublimation followed by crystallisation from a suitable solvent.

2-p-Chlorophenyl-1H-pyrrolo[2,3-b]pyridine (61).—

p-Chloroacetophenone 2-pyridylhydrazone (10.0g) in trigol (100 ml) was refluxed under nitrogen atmosphere for 4 h. cooled and poured in water. The dark product was collected and washed with ether followed by sublimation at 230-40°/2mmHg to give the pyrrolopyridine (0.94g, 9.6%) colourless plates m.p. 328° (sublimed slowly from 310°), (from chlorobenzene).

Found: C, 68.08; H, 3.93; N, 12.16.

$C_{13}H_9ClN_2$ requires C, 68.42; H, 3.95; N, 12.28%.

ν max. (KBr) 3450 (NH), 1620, 1590, 1485, 1280, 1094, 815, 775 $cm.^{-1}$

δ(CF_3CO_2H) 8.82 (1H, d, J = 8Hz, 6 - H)
8.43 (1H, d, J = 6Hz, 4 - H)
7.65 (5H, m, 5, 2', 3', 5', 6' - H)
7.14 (1H, s, 3 - H).

M^+ 228

m/e 231(7), 230(39), 229(20), 228(100), 227(7), 194(19), 193(16), 192(21), 167(7), 166(21), 165(7),164(7), 140(7), 139(9), 114(7), 97(9), 96(12), 91(33), 83(12), 73(9), 71(15), 64(9), 63(8), 57(23), 56(7), 55(15), 29(12), 28(67), 27(13).

2-p-Chlorophenyl-4-methyl-1H-pyrrolo[2,3-b]pyridine (62).—

p-Chloroacetophenone 4-methyl-2-pyridylhydrazone (15.0g) in trigol (50 ml) under reflux for 4h gave the crude pyrrolopyridine (3.7g, 26.2%). Sublimed at 222-40°/20 mmHg, colourless prisms

m.p. 295-297° (from butan-1-ol).

Found: C,68.75; H, 4.58; N, 11.56.

$C_{14}H_{11}ClN_2$ requires C, 69.42; H, 4.55; N, 11.55%.

ν max. (KBr) 3450 (NH), 3050, 1596, 1485, 1445, 1390, 1362, 1265, 1250, 1095, 884, 845, 815, 760 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 242

m/e 245(7), 244(39), 243(29), 242(100), 241(35) 214(5), 207(12), 206(15), 205(13), 180(7), 179(5), 178(5), 153(4), 152(7), 151(5), 149(4), 131(4), 121(9), 105(8), 103(13), 89(7), 78(6), 77(11), 76(5), 69(9), 57(13), 51(7), 43(12), 28(5).

2-p-Chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine (63).—

p-Chloropropiophenone 2-pyridylhyrdrazone (42.0g) in trigol (120 ml) under reflux for 24 h gave the pyrrolopyridine (26.7g, 68%), colourless plates m.p. 288-90° (from chlorobenzene). An analytical sample was prepared by sublimation at 250-60/20 mmHg followed by crystallisation from chlorobenzene, m.p. 288-288°.

Found: C, 69.28; H, 4.54; N, 11.55.

$C_{14}H_{11}ClN_2$ requires C, 69.42; H, 4.55; N, 11.57%.

ν max. (KBr) 3475, 3150, 3100, 2900, 1590, 1495, 1470, 1430, 1395, 1295, 1095, 840, 825, 764 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 242

m/e 245(6), 244(38), 243(50), 242(100), 241(97), 207(9), 206(37), 205(78), 179(3), 178(6), 177(4), 152(5), 151(7), 132(4), 131(37), 130(5), 129(23), 104(12), 103(34), 102(22), 89(10), 77(10), 76(15), 75(10), 69(5), 51(8), 44(13), 40(10), 29(3), 28(33), 27(3).

2-(3',4'-Dimethylphenyl)-1H-pyrrolo[2,3-b]pyridine (64).—

3,4-Dimethylacetophenone 2-pyridylhydrazone (44.0g) in trigol (100 ml) under reflux for 7 days gave a tarry product which was washed with several volumes of ether then sublimed to give the pyrrolopyridine (0.2g, 0.54%), colourless prisms, m.p. 185-187° (from ethanol).

Found: C, 80.97; H, 6.40; N, 12.68.

$C_{14}H_{11}ClN_2$ requires C, 81.08; H, 6.31; N, 12.61%.

$\nu_{max.}$ (KBr) 1585, 1484, 1454, 1400, 1278, 804, 764 $cm.^{-1}$

$\delta[(CD_3)_2SO]$ 8.29 (1H, q, $J_{6,5}$ = 6Hz, $J_{6,4}$ = 2Hz, 6 - H)
7.97 (1H, q, $J_{4,5}$ = 8Hz, $J_{4,6}$ = 2Hz, 4 - H)
7.75 (2H, d, J = 7Hz, 2', 6' - H)
7.28 (1H, d, J = 7Hz, s' - H)
7.03 (1H, q, $J_{5,4}$ =8Hz, $J_{5,6}$ = 6Hz, 5 - H)
6.87 (1H, s, 3 - H)
2.30 (6H, s, 3', 4' - CH_3).

3-Methyl-1H-pyrrolo[2,3-b]pyridine (66).—

3-Methyl-1H-pyrrolo[2,3-b]pyridine was prepared by the method of Kelly and Parrick[86] in a yield of 6.7%, colourless needles m.p. 129-131° (Lit.[3] 131.5-133°).

Attempted preparation of 2-(3',4'-dimethoxyphenyl) 1H-pyrrolo [2,3-b]pyridine. (65).—

3,4-Dimethoxyacetophenone 2-pyridylhydrazone (8.0g) in trigol (60 ml) under reflux for 5 days, gave only tars from which none of the required product could be isolated.

B. By the Madelung cyclisation

1H-pyrrolo[2,3-b]pyridine (36).—

Initially this compound was purchased from Aldrich Chemicals, and subsequently it was prepared by the Lorenz et al[82] modified method of the Madelung indole synthesis in yields of 50-70% m.p. 104-105° (Lit.[107] 105-106°).

Attempted preparation of 2-p-chlorophenyl-1H-pyrrolo[2,3-b] - pyridine (61).—

2-p-chlorobenzamido-3-methylpyridine.—

Prepared by the method of Herbert and Wibberley[106] in 94.7% yield. Colourless needles m.p. 174-176° (Lit.[115] 181-182°) (from ethanol).

Following known procedure,[106] the amidopyridine was treated with sodium N-methylanilide under nitrogen and refluxed for 7 min. A dark brown solid was isolated m.p. 100°. Infrared spectroscopy

showed a diminished C=O peak intensity and in the n.m.r. spectrum a singlet was observed at 3.3 . Tars were produced when the time of the reaction was increased to 30 min.

Alkylation of pyrrolo[2,3-b]pyridine at N_1-position: 1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (67).—

Following the procedure used by Verbiscar,[97] sodium hydride (6.47g) was washed with light petroleum and all traces of solvent removed *in vacuo*, and dimethylformamide (150 ml) added to the sodium hydride and the mixture cooled in ice. A solution of 1H-pyrrolo [2,3-b]pyridine (17.5 g) in dimethylformamide was added dropwise. When evolution of hydrogen gas had ceased; p-chlorobenzylchloride (24.2 g) in dimethylformamide was added dropwise and the mixture stirred at room temperature for 3h, and most of the solvent removed *in vacuo*. Water (100 ml) was added to the residue, which was then extracted with ether. The ether extract was extracted with dilute acetic acid, dried (Na_2SO_4) and the ether removed *in vacuo* to give the product (29 g, 81%), colourless needles, m.p. 34-36° (from petroleum ether 60-100°), (Lit.[97] 35-37°).

7-p-Chlorobenzyl-7H-pyrrolo[2,3-b]pyridine (68).—

The acetic acid phase from the above preparation of p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine was basified with dilute sodium hydroxide to yield the product (4.2%) yellow needles, m.p.118-121° (Lit.[97] 120-122).

1-p-Chlorobenzyl-2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b] pyridine (69).—

2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine (5 g), p-chlorobenzyl chloride (3.5 g), and potassium carbonate (3.6 g) in acetone (250 ml) were refluxed and stirred together for 72 h and the solvent removed in vacuo. The residue was washed with petroleum ether (60-80°), suspended in ethyl acetate/water (8:2) and filtered to give starting material (0.5 g). The ethyl acetate phase was extracted with 1M hydrochloric acid (20ml X3), dried (Na_2SO_4) and the solvent removed in vacuo to give the product (3.7 g, 54.4%), colourless plates, m.p. 127-127° (from ethanol).

Found: C, 68.59; H, 4.47; N, 7.60.

$C_{21}H_{16}Cl_2N_2$ requires C, 68.85; H, 4.37; N, 7.65%.

ν max. (KBr) 1492, 1450, 1410, 1339, 1015, 828, 799, 779 $cm.^{-1}$

δ ($CDCl_3$) 8.34 (1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
7.88 (1H, q, $J_{4,5}$ = 8Hz, $J_{4,6}$ = 2Hz, 4 - H)
7.54 - 6.57 (9H, m, 5, benzyl -2,3,5,6; phenyl-2,3,5, 6 - H)
5.31 (2H, s, CH_2)
2.20 (3H, s, CH_3).

M^+ 366 (Cl 35)

m/e 371(4), 370(15), 369(19), 368(65), 367(73), 366(93), 365(73), 352(18), 333(4), 332(9), 331(9), 330(5), 316(8), 258(9), 257(25), 256(16), 255(100), 243(6), 242(8), 241(15), 209(75), 208(8), 206(12), 205(18), 128(13), 127(54), 126(16), 125(94), 105(16), 89(29), 78(11), 77(18), 76(13), 63(13), 57(13), 51(15), 40(33), 29(13), 28(31), 27(13).

7-p-Chlorobenzyl-2-p-chlorophenyl-3-methyl-7H-pyrrolo [2,3-b]-pyridine (70).—

The hydrochloric acid phase from the preparation of the 1-isomer above was basified with dilute sodium hydroxide solution to give the product (2.4 g, 35.3%), orange needles, m.p. 130-131° (from cyclohexane).

Found: C, 68.58; H, 4.54; N, 7.60.

$C_{21}H_{16}Cl_2N_2$ requires C, 68.85; H, 4.37; N, 7.65%.

$\nu_{max.}$ (KBr) 1640 (C=N), 1615, 1498, 1469, 1408, 1360, 1158, 1139, 1090, 840, 750 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

2-p-Chlorophenyl-1,3-dimethyl-1H-pyrrolo[2,3-b] pyridine (71).—

To a mixture of dimethyl sulfoxide (100 ml) and potassium hydroxide (13.5 g) stirred together for 5 min, was added 2-p-chlorophenyl-3-methyl-1H- pyrrolo[2,3-b]pyridine (15 g) and the mixture stirred for 0.45 h. Methyl iodide (17 g) was added and the mixture cooled briefly, and stirred for a further 1 h, then water (200 ml) was added and extracted with ether (2 X 100 ml). The ether layer was extracted with dilute acetic acid, and dried ($CaCl_2$) and the ether removed to give a brown oil which was distilled at 185-190/3 mmHg to give the product (13 g, 81.9%), colourless needles, m.p. 84-85° (from aqueous ethanol).

Found: C,70.07; H, 5.19; N, 10.99.

$C_{15}H_{13}ClN_2$ requires C, 70.31; H, 5.08; N,10.94%.

ν max. (KBr) 1490, 1472, 1462, 1450, 1420, 1405, 1390, 1338, 1305, 1210, 1082, 1010, 850, 830, 763 $cm.^{-1}$

δ ($CDCl_3$) 8.50 (2H, d, J = 7Hz, 4, 6 - H)
7.53 (5H, m, s, 2', 3', 5', 6' - H)
4.14 (3H, s, N - CH_3)
2.37 (3H, s, CH_3)

M^+ 256
259(5)
m/e 258(36), 257(48), 256(100), 255(91), 241(9), 240(4), 221(9), 220(37), 219(32), 218(30), 206(11), 205(22), 146(7), 145(64); 128(6), 110(21), 109(24), 103(14), 89(6), 78(4), 77(7), 76(9), 75(7), 51(7).

2-p-Chlorophenyl-3,7-dimethyl-7H-pyrrolo[2,3-b]pyridine (72).—
The acetic acid extract above was basified with dilute sodium hydroxide and left to stand overnight. The product was collected, pale yellow needles m.p. 143-144° (from aqueous methanol).

The analytical data indicated that some hydrate was formed during recrystallisation.

Found: C, 65.60; H, 4.22; N, 10.15.

$C_{15}H_{13}ClN_2$ requires C, 70.31; H, 5.08; N, 10.94%.

$C_{15}H_{15}ClN_2O$ requires C, 65.69; H, 5.47; N, 10.22%.

ν max. (KBr) 1643 (C=N), 1619, 1488, 1459, 1372, 1300, 1260, 1070, 790, 760 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

Preparation of 1H-pyrrolo[2,3-b]pyridine-3-carbardehyde derivatives

1H-Pyrrolo[2,3-b]pyridine-3-carbardehyde (73).—

Prepared by the method of Verbiscar[97] in 50-55% yield as colourless needles, m.p. 213-215° (Lit.[107] 214.5-215°).

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (74).—

Prepared by method (B) of Verbiscar,[97] in 62.7-85% yield as colourless plates, m.p. 103-105° (Lit.[97] 105-107°).

Reactions of 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde derivatives:

Preparation of 1H-pyrrolo[2,3-b]pyridine-3-methanol derivatives.

1H-Pyrrolo[2,3-b]pyridine-3-methanol (75).—

Following the method of Yakhontov et al[108] a suspension of 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (1 g) in methanol (10 ml) was stirred and treated portionwise with sodium borohydride (0.5 g) and stirred for 0.5 h. The solvent was removed in vacuo and water (10 ml) added to the residue and filtered to give the product (0.75 g, 73%), colourless needles m.p. 176-178°. Analytical sample m.p. 189-190° (from aqueous ethanol).

Found: C, 64.48; H, 5.41; N, 18.49.

$C_8H_8N_2O$ requires C, 64.86; H, 5.41; N, 18.92%.

max. (KBr) 3150(OH), 1546, 1455, 1425, 1369, 1310, 1290, 1005, 782, 762 $cm.^{-1}$

$\delta[(CD_3)_2SO]$ 8.20 (1H, q, $J_{6,5}$ = 4.5Hz, $J_{6,4}$ = 1.5Hz, 6 - H)
8.08 (1H, q, $J_{4,5}$ = 7.5 Hz, $J_{4,6}$ = 1.5 Hz, 4 - H)
7.43 (1H, s, 2-H)
4.73 (1H, s, OH)
3.65 (2H, s, CH_2).

M^+ 148

m/e 149(6), 148(44), 147(19), 132(13), 131(75), 130(100), 129(8), 119(22), 104(22), 103(66), 92(11), 79(11), 78(17), 77(25), 76(94), 75(16), 74(9), 58(16), 51(17), 50(27), 44(56), 29(13), 28(91), 27(9).

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridyl-3-methanol (76).—

To a cooled and stirred suspension of 1-p-chlorobenzyl pyrrolo [2,3-b] pyridine-3-carbaldehyde (1.0 g) in methanol (10 ml) was added portionwise sodium borohydride (0.5 g), and the resulting solution stirred for 0.5 hr, the methanol removed in vacuo at 20°. To the residue was added water (10 ml) and extracted with chloroform (40 ml) and dried (K_2CO_3) and the chloroform removed in vacuo at 30°. The oily residue solidified on cooling in ice to give the product (0.88 g, 87.4%) colourless prisms m.p. 90-91° (from aqueous ethanol).

Found : C, 66.03; H, 4.70; N, 10.19.

$C_{15}H_{13}ClN_2O$ requires C, 66.18; H, 4.78; N, 10.29%.

ν max (KBr) 3300 (OH), 2900, 1602, 1536, 1500, 1460, 1435,

1360, 1345, 1170, 1095, 1005, 810, 781, 775 $cm.^{-1}$

δ ($CDCl_3$) 8.27 (1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
8.02 (1H, q, $J_{4,5}$ = 8Hz, $J_{4,6}$ = 2Hz, 4 - H)
7.12 (6H, m, 2, 5, 2', 3', 5', 6' - H)
5.27 (2H, s, N_1 - CH_2)
4.73 (2H, s, 3 - CH_2)
2.97 (1H, s, OH).

M^+ 272

m/e 274(29, 273(33), 272(79), 271(54) 270(15), 258(6), 257(15), 256(19), 255(60), 244(6), 243(31), 242(17), 241(73), 207(6), 206(15), 205(10), 162(4), 161(27), 160(4), 145(10), 131(8), 128(8), 127(81), 126(23), 125(100), 89(48), 85(15), 77(10), 76(10), 75(8), 71(21), 64(15), 45(59), 29(19), 28(58), 27(48).

Preparation of Esters of 1H-Pyrrolo[2,3-b]pyridine-3-methanol

3-p-Chlorobenzoyloxymethyl-1H-pyrrolo[2,3-b]pyridine (77).—

A mixture of 1H-pyrrolo[2,3-b]pyridine-3-methanol (1.0 g), p-chlorobenzoyl chloride (1.2 g) in pyridine (15 ml) was stirred together for 15 h, water (30 ml) was added and filtered to give the product (1.2 g, 62.1%), colourless plates, m.p. 157-158° (from ethyl acetate).

Found: C, 62.74; H, 3.71; N, 9.65.

$C_{15}H_{11}ClN_2O_2$ requires C, 62.94; H, 3.85; N, 9.79%.

νmax. (KBr) 3150 (NH), 2900, 1709 (C=O), 1595, 1275 (C-O), 1100, 1090, 779, 765 $cm.^{-1}$

No suitable solvent was available for the determination of the n.m.r. spectrum.

M^+ 286

m/e 288(3), 286(8), 158(8), 156(22), 142(5), 141(74), 139(100), 131(25), 130(58), 113(13), 111(36), 104(9), 103(74), 77(14), 76(53), 75(28), 61(16), 60(6), 51(13), 50(23), 45(19), 44(31), 43(50), 29(14), 28(58), 27(9).

3-Acetoxymethyl-1-acetyl-1H-pyrrolo[2,3-b]pyridine (78).—

1H-Pyrrolo[2,3-b]pyridine-3-methanol (0.8 g) in acetic anhydride (1 ml) was heated under reflux on a waterbath overnight. The mixture was cooled in ice, filtered and washed with water to give the product (0.8 g, 63.8%), colourless prisms, m.p. 90-91° (from ethyl acetate).

Found: C, 61.90; H, 5.10; N, 11.97.

$C_{12}H_{12}N_2O_3$ requires C, 62.07; H, 5.17; N, 12.07%.

ν max. (KBr) 1735 (C=O), 1690 (N-C=O), 1565, 1410, 1385, 1345, 1318, 1245 (C-O), 1224, 1050, 1040, 827, 810, 780 cm^{-1}

δ($CDCl_3$) 8.42 (1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
8.05 (2H, m, 2, 4 - H)
7.25 (1H, q, $J_{5,4}$ = 7Hz, $J_{5,6}$ = 5Hz, 5 - H)
5.29 (2H, s, 3 - CH_2)
3.07 (3H, s, N_1 - 2' - CH_3)
2.12 (3H, s, 3-Ac-CH_3).

M^+ 232

m/e 233(4), 232(20), 191(10), 190(65), 173(3), 149(5), 148(25), 137(11), 135(8), 132(30), 131(100), 130(5), 119(5), 104(26), 103(30), 102(9), 92(7), 91(4), 90(3), 79(4), 78(12), 77(19), 76(26), 75(6), 65(6), 64(4), 63(7), 60(11), 52(5), 51(13), 50(11), 45(10), 44(17), 43(45), 42(25), 39(11), 29(6), 28(33), 27(17).

3-Acetoxymethyl-1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (79).— 1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-methanol (1.4 g) in acetic anhydride (1 ml) was treated as above to give the product (1.1 g, 68.1%), colourless needles, m.p. 83-84° (from ethyl acetate).

Found: C, 64.71; H, 4.67; N, 8.79.

$C_{17}H_{15}ClN_2O_2$ requires C, 64.97; H,4.78; N, 8.92%.

ν max. (KBr) 1722 (C=O), 1550, 1455, 1435, 1390, 1370, 1305, 1250 (C-O), 1230, 1175, 1094, 805, 775 cm^{-1}

δ ($CDCl_3$) 8.40 (1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
8.05 (1H, q, $J_{4,5}$ = 7Hz, $J_{4,6}$ = 2Hz, 4 - H)
7.20 (6H, m, 2, 4, 2', 3', 5', 6' - H)
5.44 (2H, s, N_1 - CH_2)
5.22 (2H, s, 3 - CH_2)
2.04 (3H, s, Ac - CH_3).

M^+ 314

m/e 316(9), 315(4), 314(29), 258(6), 257(21), 256(19), 255(49), 254(6), 242(9), 241(15), 145(9), 131(11), 127(38), 125(100), 89(23), 81(6), 77(6), 76(9), 69(13), 45(19), 44(21), 43(26),

29(6), 28(53), 27(4).

Condensation of 1H-Pyrrolo[2,3-b]pyridine-3-carbaldehydes with derivatives of ammonia

1H-Pyrrolo[2,3-b]pyridine-3-carbaldehyde hydrazone (80).—

To the suspension of 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (0.2 g) in ethanol (2 ml) was added hydrazine hydrate (0.1 g) and the mixture warmed on a waterbath for 15 min, cooled, filtered and washed with water to give the product (0.2 g, 91.3%), colourless needles m.p. 320°.

Found: C, 59.59; H, 5.00; N, 34.46.

$C_8H_8N_4$ requires C, 60.00; H, 5.00; N, 35.00%.

ν max. (KBr) 3350 (NH_2), 3100, 2900, 1580, 1529, 1500, 1465, 1425, 1285, 810, 784, 770, 730 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

1H-Pyrrolo[2,3-b]pyridine-3-carbaldehyde thiosemicarbazone (81).—

1H-Pyrrolo[2,3-b]pyridine-3-carbaldehyde (0.23 g) and thiosemicarbazide (0.15 g) in ethanol (2 ml) treated as above gave the product (0.25 g, 72.5%), colourless needles, m.p. 275-277° (decomp) (from dimethylformamide).

Found: C, 49.32; H, 4.08; N, 31.67.

$C_9H_9N_5S$ requires C, 49.32; H, 4.11; N, 31.96%.

ν max. (KBr) 3425 (NH), 3275 (NH), 3125, 3100, 2829, 1609 (C=N), 1580, 1540, 1370, 1280, 1130, 802, 779 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 219

m/e 219(9), 202(10), 185(6), 167(8), 161(5), 160(42), 159(25), 149(31), 146(31), 145(49), 144(32), 143(100), 142(9), 118(10), 117(13), 116(19), 104(9), 98(75), 90(16), 89(16), 74(32), 73(25), 72(44), 58(35), 57(39), 44(25), 43(35), 42(40), 41(25), 40(18), 29(39), 28(25), 27(25).

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde semicarbazone (82).—

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (1 g) and semicarbazide hydrochloride (0.41 g) and sodium acetate (0.3 g) in ethanol (5 ml) treated as above gave the product (0.97 g, 80%), colourless needles m.p. 215-217° (from ethanol).

Found: C, 58.64; H, 4.31; N, 21.51.

$C_{16}H_{14}ClN_5O$ requires C, 58.72; H, 4.28; N, 21.41%.

ν max. (KBr) 3500 (NH), 3400 (NH), 2910, 1690 (C=O), 1575, 1500, 1445, 1425, 1400, 1170, 809, 785 $cm.^{-1}$

$\delta[(CD_3)_2SO]$ 11.29 (1H, s, N - H)
8.74 (1H, d, J = 7.5Hz, 6 - H)
8.42 (2H, m, 3 - CH 4 - H)
8.10 (1H, s, 2 - H)

7.30 (5H, m, 5, 2', 3', 5', 6' - H)

5.53 (2H, s, N_1 - CH_2)

3.42 (2H, s, N - H_2).

M^+ 327 (Cl 35)

m/e 329(11), 328(8), 327(23), 310(9), 287(5), 286(20), 285(16), 284(60), 283(15), 272(5), 271(9), 270(16), 269(27), 268(32), 267(35), 266(29), 256(12), 255(9), 254(15), 143(11), 242(9), 241(23), 240(8), 233(9), 232(11), 231(7), 208(9), 207(7), 206(9), 205(11), 185(9), 173(11), 171(9), 159(12), 156(23), 126(9), 127(39), 125(100), 124(21), 123(61), 111(20), 99(20), 98(29), 97(36), 89(59), 84(53), 83(29), 82(44), 71(49), 69(76), 64(19), 59(39), 45(33), 44(57), 43(44), 41(48), 29(44), 28(44), 27(25).

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b] pyridine-3-carbaldehyde thiosemicarbazone (83).—

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (1 g) and thiosemicarbazide in ethanol (5 ml) treated as above gave the product (1.14 g, 89-8%), pale yellow powder, m.p. 278-280°.

Found: C, 56.02; H, 4.08; N, 20.16.

$C_{16}H_{14}ClN_5S$ requires C, 55.98; H, 4.08; N, 20.41%.

ν max. (KBr) 3402 (NH), 3200 (NH), 3150(NH), 1610 (C=N), 1595, 1573, 1540, 1520, 1449, 1428, 1400, 1370, 1355, 1302, 1165, 809, 780 $cm.^{-1}$

δ[$(CD_3)_2SO$] 11.29 (1H, s, N - H)

8.74 (1H, q, $J_{6,5}$ = 7Hz, $J_{6,4}$ = 1Hz, 6 - H)

8.42 (2H, 4 - H and 3 - CH)

8.10 (H, 2, 2 - H)

7.30 (5H, m, 5, 2', 3', 5', 6' - H)

5.53 (2H, s, CH_2)

3.42 (2H, s, NH_2).

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde hydrazone (84).—

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (0.88 g) and hydrazine hydrate (0.17 g) in ethanol treated as above gave the product (0.8 g, 86.4%) colourless needles m.p. 265°. Melting point affected by recrystallisation from ethanol or dioxan.

An analytically pure sample was not obtained.

ν max. (KBr) 3450(NH), 1625(C=N), 1495, 1445 1425, 1400, 1169, 805, 781, 775 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

The molecular ion was not seen in the mass spectrum.

3-p-Chlorophenyliminomethyl-1H-pyrrolo [2,3-b] pyridine (85).—

A mixture of 1H-pyrrolo [2,3-b]pyridine-3-carbaldehyde (1.46 g), p-chloroaniline (7.6 g), and 5 M methanolic hydrochloric acid (2 ml) in methanol (12 ml) were stirred together for 60 h, then the solvent was removed in vacuo. Water (20 ml) was added to the residue and filtered to give the product (2.4g, 94.1%), pale yellow needles, m.p. 275-278° (from ethanol).

Found: C, 65.54; H, 4.04; N, 16.25.

$C_{14}H_{10}ClN_3$ requires C, 65.88; H, 3.92; N, 16.47%.

ν max. (KBr) 3376(NH), 3050(=C-H), 1655 (C=N), 1595, 1490, 1438, 1370, 1354, 1270, 1210, 1205, 1175, 902, 830, 802 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 255.

m/e 258(9), 257(39), 256(22), 255(100), 230(3), 127(56), 126(15), 125(98), 107(42), 106(51), 89(20), 79(22), 78(14), 77(34), 76(17), 46(36), 45(51), 44(51), 43(49), 40(34), 29(34), 28(51), 27(39).

Condensation of 1H-Pyrrolo[2,3-b] pyridine-3-carbaldehyde with Compounds Containing an Activated Methylene group

Ethyl 2 -cyano-3 -(1-p-chlorobenzyl -1H-pyrrolo[2,3-b] -pyrid-3-yl)acrylate (86).—

A mixture of 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (2.7 g), ethyl cyanoacetate (2.3 g), and piperidine

(10 drops) in ethanol (40 ml) were heated under reflux for 17 h, concentrated to a small volume in vacuo, cooled and filtered to give the product (3.3 g, 90.4%) yellow needles m.p. 161-162° (from ethyl acetate).

Found: C, 65.43; H, 4.24; N, 11.46.

$C_{20}H_{16}ClN_3O_2$ requires C, 65.75; H, 4.38; N, 11.51%.

ν max. KBr 1700 (C=O), 1602, 1588, 1512, 1260 (C-O), 1250, 1215, 1170, 1095, 1018 cm^{-1}

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 365 (Cl 35)

m/e 368(11), 367(33), 366(28), 365(100), 364(23), 339(9), 338(27), 337(27), 336(78), 320(16), 295(6), 294(11), 293(13), 292(20), 291(8), 290(6), 254(11), 127(64), 124(30), 125(100), 99(22), 89(63), 81(27), 69(47), 63(19), 57(25), 55(23), 45(52), 44(61), 41(31), 29(25), 28(48), 27(20).

Attempted preparation of ethyl 2-cyano-3-(1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyrid-3-yl)propanoate

Ethyl 2-cyano-3-(1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyrid-3-yl)acrylate (3.65 g) and Adam's catalyst (platinum oxide) (0.1 g) in ethanol (100 ml) were treated with hydrogen (3 atmospheres) at room temperature for four days. The mixture was filtered and the solvent removed in vacuo to leave a yellow oil that did not solidify on standing.

Ethyl 2'-ethoxycarbonyl-3'-(1-p-chlorobenzyl)-1H-pyrrolo[2,3-b]pyrid-3-ylacrylate (87).—

A mixture of 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (1 g), diethyl malonate (5 ml) and piperidine (10 drops) in ethanol (20 ml) were heated under reflux for 48 h, then ethanol and ethyl malonate removed in vacuo. A yellowish residue solidified on standing to give the product (1 g, 65.5%), colourless plates, m.p. 81-84° (from aqueous ethanol).

The analytical data indicated presence of impurity:

Found: C, 64.91; H, 5.14; N, 7.58.

$C_{22}H_{21}ClN_2O_4$ requires C, 64.08; H, 5.10; N, 6.80%.

ν max. (KBr) 3110, 3000, 2950, 1704 (C=O), 1630, 1535, 1500, 1455, 1405, 1375, 1305, 1285 (C-O), 1202, 1179, 1165, 1140, 804, 780 $cm.^{-1}$

$\delta(CDCl_3)$ 8.42 (1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
8.19 (1H, q, $J_{4,5}$ = 7.5Hz, $J_{4,6}$ = 2H, 4 - H)
7.98 (1H, s, 3' - CH)
7.75 (1H, s, 2H)
7.20 (5H, m, 5, 2', 3', 5', 6' - H)
5.47 (2H, s, N_1 - CH_2)
4.29 (4H, q, J = 6.5Hz, CH_2)
1.29 (6H, t, J = 6.5Hz, CH_3).

M^+ 412

m/e 412(3), 342(8), 341(6), 340(18), 339(4), 313(4), 311(10), 295 (6), 270(10), 269(6), 268(6), 267(4), 241(8), 200(6), 199(29), 154(20), 150(18), 149(10), 141(12), 127(31), 126(63), 125(65), 112(61), 99(4), 97(39), 85(78), 84(100), 83(45),

82(18), 81(20), 71(71), 70(41), 69(67), 58(25), 57(67), 56(63), 55(59), 44(51), 43(74), 42(47), 41(69), 29(57), 27(37).

Reductive Amination of 1H-Pyrrolo[2,3-b]pyridine-3-carbaldehydes

N-p-Chlorophenyl-1H-pyrrolo[2,3-b]pyrid-3-ylmethylamine (88).— A. The method of Borch et al[111] was used with modification on the isolation of the product. To the mixture of 1H-pyrrolo[2,3-b]-pyridine-3-carbaldehyde (0.7 g), and p-chloroaniline (3.8 g) in methanol (12ml) was added 5 M methanolic hydrochloric acid (2 ml) and sodium cyanoborohydride (0.22 g) and the mixture stirred together for 48 h. The solvent was removed in vacuo, and to the residue was added ethanol (2 ml). The solid that separated on standing was filtered off and washed with a little water to give the product (0.8 g, 65.4%), colourless prisms m.p.178-179° (from aqueous ethanol).

Found: C, 65.14; H, 4.66; N, 16.21.
$C_{14}H_{12}ClN_3$ requires C, 65.37; H, 4.67; N, 16.34%.

ν_{max}. (KBr) 3415 (NH), 3150 (NH), 2900, 1595, 1505, 1455, 1425, 1310, 1290, 1244, 819, 775, 765 $cm.^{-1}$

$\delta[(CD_3)_2SO]$ 8.30(1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
8.05 (1H, q, $J_{4,5}$ = 8Hz, $J_{4,6}$ = 2Hz, 4 - H)
7.48 (1H, s, 2 - H)
7.28 - 6.50 (5H, m, 4, 2', 3', 5', 6' - H)
6.15 (1H, t, J = 5Hz, N' - H)
4.39 (2H, d, J = 5Hz, 3 - CH_2)

M^+ 257

m/e 259(12), 258(9), 257(40), 256(19), 255(30), 254(32), 220(7), 219(5), 192(4), 156(5), 146(14), 145(16), 132(42), 131(100), 130(75), 129(65), 128(21), 127(70), 119(11), 118(7), 117(12), 111(16), 104(49), 103(56), 102(18), 100(26), 92(35), 91(16), 90(14), 79(9), 78(28), 77(40), 76(25), 75(25), 65(60), 63(25), 51(26), 50(28), 46(28), 45(51), 43(26), 29(26), 28(49), 27(37).

B. The carbaldehydimine (85) (0.4 g) in methanol (5 ml) was treated with sodium borohydride (0.1 g) and stirred for 0.5h, and filtered to give the product (0.3 g, 74.4%), colourless prisms, (from aqueous ethanol). The melting point was undepressed with the sample above.

1-p-Chlorobenzyl-N-methyl-N-phenyl-1H-pyrrolo[2,3-b]pyrid-3-ylmethylamine (89).—

A mixture of 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (1.4 g), N-methylaniline (3.2 g) in methanol (12 ml), and 5M methanolic hydrochloric acid (2 ml) and sodium cyanoborohydride (0.22 g) was treated as above for 65 h to give the product (1.53 g, 81.7%), colourless needles, m.p. 104-5° (from ethanol).

Found: C, 73.15; H, 5.59; N, 11.68.

$C_{22}H_{20}ClN_3$ requires C, 73.13; H, 5.54; N, 11.63%.

ν max. (KBr) 3050(=C-H), 2950, 2810, 1595, 1510, 1500, 1455, 1432, 1360, 1344, 1304, 1238, 1103, 1090, 1021, 938, 810, 781, 761, 740, 700 $cm.^{-1}$

δ ($CDCl_3$) 8.30 (1H, d, J = 5Hz, 6 - H)

7.80 (1H, d, J = 7.5 Hz, 4 - H)

7.64 - 6.24 (11H, m, phenyl, benzyl 2, 5 - H)

5.28 (2H, s, 1 - CH_2)

4.42 (2H, s, 3 - CH_2)

2.80 (3H, s, N - CH_3)

2'-(1H-Pyrrolo[2,3-b]pyrid-3-ylmethylamino)propanoic acid (90).—

The mixture of 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (2 g), DL-alanine (2 g) in methanol (25 ml), and 5 M methanolic hydrochloric acid (3 ml), and sodium cyanoborohydride (0.5 g) treated as above for 48 h, gave the product (1.4 g, 46.7%) colourless powder, m.p. 244-6° (decomp) (from propanol).

The analytical data indicated presence of impurity:

Found: C, 59.23; H, 5.93; N, 18.68.

$C_{11}H_{13}N_3O_2$ requires C, 60.27; H, 5.94; N, 19.18%.

ν max. (KBr) 3410(NH), 3100(NH_2^+), 1610(C=O), 1588, 1465, 1425, 1400, 1360, 1290, 768 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 219

m/e 220(4), 219(13), 174(11), 172(6), 162(11), 149(11), 148(29), 147(16), 146(33), 145(16), 133(9), 132(58), 131(91), 130(100), 129(16), 119(24), 118(24), 117(11), 104(60), 103(89), 102(15), 92(9), 91(15), 90(15), 79(18), 78(18), 77(31), 76(75), 75(44), 74(71), 65(18), 63(20), 55(35), 51(38),

50(50), 46(44), 45(82), 44(67),43(71), 42(78), 41(62), 40'86), 39(73), 29(58), 28(82), 29(58).

N,N-Dimethyl-1H-Pyrrolo[2,3-b]pyrid-3-ylmethylamine (91).—

A mixture of 1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde (1.45 g), diethylamine (4 g) in methanol (12 ml), and 5 M methanolic hydrochloric acid (2 ml), and sodium cyanoborohydride (0.31 g) treated as above gave the product (1.34 g, 66%), colourless fine needles, m.p. 157-9° (from ethanol).

An analytically pure sample for elemental analysis was not obtained.

ν max. (KBr) 3100, 2955(CH_3), 2950(CH_2), 2855, 1585, 1540, 1460, 1428, 1295, 1116, 1045, 901, 770, 760 $cm.^{-1}$

M^+ 203

m/e 204(5), 203(33), 202(22), 201(73), 200(5), 189(5), 188(28), 186(13), 172(13), 171(8), 170(13), 169(3), 159(15), 158(17), 157(27), 156(42), 146(10), 145(28), 144(32), 143(13), 133(10), 132(60), 131(100), 130(80), 129(12), 119(20), 118(22), 117(13), 104(45), 102(18), 79(11), 78(18), 77(30), 76(63), 73(25), 72(73), 58(57), 56(38), 44(45), 43(48), 29(35), 28(52), 27(52).

2-p-Chlorophenyl-3-(2-methylethoxycarbonylethylamino-1H-pyrrolo-[2,3-b]pyridine (92).—

A mixture of 3-amino-2-p-chlorophenyl-1H-pyrrolo[2,3-b]pyridine (0.2 g) and ethyl acetotoacetate (0.3 g) in methanol (20 ml), and

5M methanolic hydrochloric acid (0.4 ml), and sodium cyanoborohydride (0.1 g) was treated as above to give the product (0.22 g, 74%), yellow plates, m.p. 220° (from propan-2-nol).

Found: C, 63.29; H, 5.68; N, 11.49.

$C_{19}H_{20}ClN_3O_2$ requires C, 63.87; H, 5.60; N, 11.76%.

ν max. (KBr) 3150(NH), 2960, 1722(C=O), 1490, 1290(C-O), 1168, 1092, 770 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 357

m/e 360(5), 359(14), 358(11), 357(33), 355(10), 354(10), 342(6), 311(6), 309(14), 272(19), 271(14), 270(52), 269(11), 268(18), 245(21), 244(38), 243(79), 242(100), 241(89), 230(16), 228(18), 220(16), 208(18), 207(56), 206(40), 205(32), 149(29), 131(32), 129(19), 104(22), 103(32), 102(22), 78(18), 77(21), 76(25), 75(19), 60(30), 57(76), 44(38), 43(60), 42(37) 29(22), 28(41), 27(40).

Preparation of 3-Nitro and 3-Nitroso Derivatives of 1H-Pyrrolo-[2,3-b]pyridines

3-Nitro-1H-pyrrolo[2,3-b]pyridine (93).—

Prepared by the method of Robison et al[90] in a yield of 90%, m.p. 310° (decomp) (Lit [90] 300°).

3-Nitroso-2-phenyl-1H-pyrrolo[2,3-b]pyridine (94).—

Prepared by the method of Herbert and Wibberley[106] in a yield

of 91.3%, m.p. 232-234° (decomp) Lit[106] 234-235°).

2-p-Chlorophenyl-3-nitroso-1H-pyrrolo[2,3-b]pyridine (95).—

2-p-Chlorophenyl-1H-pyrrolo[2,3-b]pyridine (2 g) was suspended in acetic acid (50 ml) and a solution of sodium nitrite (1 g) in water (4 ml) was added dropwise to the stirred suspension. The mixture was stirred a further 0.5 h then water (50 ml) added, and filtered off to give the product (1.8 g, 79.4%) orange needles m.p. 262-4° (decomp) (from methanol).

An analytically pure sample was not obtained.

ν max. (KBr) 3450(NH), 1594, 1490, 1410, 1030, 794 $cm.^{-1}$

2-p-Chlorophenyl-4-methyl-3-nitroso-1H-pyrrolo[2,3-b]pyridine (96).—

A mixture of 2-p-chlorophenyl-4-methyl-1H-pyrrolo[2,3-b]-pyridine (2 g) in acetic acid (50 ml) and sodium nitrite (0.8 g) in water (1 ml) was treated as above to give the product (2.1 g, 93.8%) yellow needles m.p. 225-7° (decomp). It turned green on recrystallisation from acetic acid/water and an analytically pure sample was not obtained.

ν max. (KBr) 1598, 1490, 1370, 1325, 1280, 1090, 1040, 1020 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

Preparation of 3-Amino-1H-pyrrolo[2,3-b]pyridines

3-Amino-1H-pyrrolo[2,3-b]pyridine dihydrochloride (97).—

Prepared by the method of Robison et al[90] in a yield of 95.1%, m.p. 245-50° (decomp) (Lit[90] 253°).

3-Amino-2-phenyl-1H-pyrrolo[2,3-b]pyridine (98).—

Prepared by the method of Herbert and Wibberley[106] in a yield of 93.5%, m.p. 205° (Lit[106] 207-209).

3-Amino-2-p-chlorophenyl-1H-pyrrolo[2,3-b]pyridine (99).—

To 2-p-chlorophenyl-3-nitroso-1H-pyrrolo[2,3-b]pyridine (1.8 g) dissolved in a mixture of ethanol (8 ml) and 2M sodium hydroxide (16 ml) was added sodium dithionite (3.1 g), and the mixture heated under reflux on a waterbath for 10 mins., cooled and filtered to give the product (1.6 g, 94.2%), yellow plates m.p. 278-280° (decomp) from propan-2-ol. An analytical sample was sublimed at 240°/4 mmHg.

Found: C, 64.19; H, 4.24; N, 17.11.

$C_{13}H_{10}ClN_3$ requires C, 64.20; H, 4.12; N, 17.28%.

ν max. (KBr) 3490(NH), 3150(NH), 1620(N-H), 1595, 1495, 1485, 1398, 1095, 840, 788, 762, 748, 710 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 243

m/e 246(6), 245(36), 244(34), 243(100), 242(47), 241(36), 208(21), 207(36), 206(25), 205(13), 181(8), 179(8), 149(13),

131(17), 129(13), 105(25), 104(72), 103(28), 102(21), 97(15), 95(15), 81(28), 79(19), 78(19), 77(19), 71(25), 69(42), 57(42), 55(32), 44(43), 43(36), 29(13), 28(60), 27(11).

3-Amino-2-chlorophenyl-4-methyl-1H-pyrrolo[2,3-b]pyridine (100).—

2-p-Chlorophenyl-4-methyl-3-nitroso-1H-pyrrolo[2,3-b]pyridine (1.8 g) in a mixture of ethanol (8 ml) and 2M sodium hydroxide (16 ml) and sodium diothionite was treated as above to give the product (1.4 g 82%), yellow plates m.p. 280-282° (decomp), (from benzene/propan-2-ol). A sample was purified by sublimation at 240°/2 mmHg. Analytical data indicated presence of slight impurity.

Found: C, 64.72; H, 4.76; N, 15.67.

$C_{14}H_{12}ClN_3$ requires C, 65.37; H, 4.67; N, 16.34%.

$\nu_{max.}$ (KBr) 3450(NH), 3150(NH), 1602, 1388, 1086, 810 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

Preparation of 3-Diazo Derivatives of 1H-Pyrrolo[2,3-b]pyridines

3-Diazo-2-phenyl-3H-pyrrolo[2,3-b]pyridine (101).—

Prepared by the method of Herbert and Wibberley[106] in a yield of 94%, m.p. 196-198° (Lit[106] 195-197° (decomp).

2-p-Chlorophenyl-3-diazo-3H-pyrrolo[2,3-b]pyridine (102).—

3-Amino-2-p-chlorophenyl-1H-pyrrolo[2,3-b]pyridine (0.7 g) in

water (30 ml) and concentrated sulphuric acid (2.25 ml) was stirred and cooled in ice and treated with sodium nitrite (0.6 g) in water (3 ml) and stirred for a further 0.5 h. The mixture was basified with sodium carbonate, filtered and washed with water to give the diazo compound (0.6 g, 92.2%), yellow needles m.p. 192-195° (decomp) (from toluene).

Found: C, 60.86; H, 2.72; N, 21.54.

$C_{13}H_7ClN_4$ requires C, 61.42; H, 2.76; N, 22.05%.

$C{=}N^{+}{=}N^{-}$

ν max. (KBr) 2125($N^{+}{=}N^{-}$), 1464, 1410, 1390, 1360, 1350, 1310, 1092, 802 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

2-p-Chlorophenyl-3-diazo-3H-4-methylpyrrolo[2,3-b]pyridine (103).— 3-Amino-2-p-chlorophenyl-4-methyl-1H-pyrrolo[2,3-b]pyridine (0.7 g) in water (30 ml) and concentrated sulphuric acid (2.25 ml) was treated as above to give the diazo compound (0.6 g, 82.2%), yellow plates m.p. 181.191° (decomp) (from toluene).

An analytically pure sample was not obtained.

$C{=}N^{+}{=}N^{-}$

ν max. (KBr) 2110 ($N^{+}{=}N^{-}$), 1595, 1464, 1407, 1359, 1290, 1090 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

Reactions of 1H-Pyrrolo[2,3-b]pyridine with Chlorosulphuric acid and Disulphuric acid

1H-Pyrrolo[2,3-b]pyridine-3-sulphonyl chloride (104).—

To chlorosulphonic acid (10 ml) stirred and cooled in ice (0-5°) was added portionwise 1H-pyrrolo[2,3-b]pyridine (1 g). The resulting solution was stirred for a further 0.5 h and very cautiously transferred into well stirred ice (200 g). The precipitated product was collected washed with water, and chloroform to give the sulphonyl chloride (1.56 g, 84%), colourless powder m.p. 183-5°. The product turned pink when stored at low temperature and could not be purified for elemental analysis.

ν max. (KBr) 3145, 3005, 2825, 1585, 1500, 1462, 1415, 1374(S=O), 1341, 1280, 1266, 1160, 1124, 808, 780, 682 $cm.^{-1}$

No suitable solvent was available for the determination of the n.m.r. spectrum.

M^+ 216

m/e 218(7), 216(19), 181(30), 152(7), 150(14), 149(8), 133(18), 119(18), 118(100), 117(31), 92(11), 91(64), 90(21), 78(11), 73(11), 69(8), 65(11), 64(74), 63(51), 62(12), 59(10), 55(11), 48(33), 44(20), 43(12), 41(14), 40(13), 39(20), 32(56), 29(6), 28(56), 27(12).

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-sulphonylchloride (105).—

To chlorosulphonic acid (10 ml) stirred and cooled in ice (0°C) was added 1-p-chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (1.2 g) in portions. The resulting solution was stirred for a further 15 min.

and cautiously transferred into stirred ice (200 g), and the precipitate collected and washed with ether to give the product (1.3 g, 77.3%), colourless powder m.p. 155-6°. The sulphonyl chloride turned purple on storage at low temperature and could not be purified for elemental analysis.

ν max. (KBr) 3148, 1518, 1395, 1369,(S=O), 1303, 1205 (S=O), 1165, 1115, 800, 787, 768 $cm.^{-1}$

No suitable solvent was available for the determination of the n.m.r. spectrum.

M^+ 340

m/e 342(8), 341(4), 340(10), 339(3), 307(3), 305(6), 276(4), 275(3), 274(8), 245(5), 244(19), 243(47), 242(7), 241(100), 207(9), 206(15), 205(13), 178(4), 177(5), 131(51), 127(27), 125(54), 103(35), 99(6), 90(16), 89(44), 77(8), 76(8), 75(8), 64(67), 63(19), 38(34), 36(22), 29(9), 28(26), 27(8).

Reactions of 1H-Pyrrolo[2,3-b]pyridine-3-sulphonyl Chloride with Ammonia and its Derivatives

3-N-Phenylsulphamoyl-1H-pyrrolo[2,3-b]pyridine (106).—

To amiline (0.54 g) in ethanol (2 ml) was added 1H-pyrrolo-[2,3-b]pyridine-3-sulphonyl chloride (1.26 g) and the mixture warmed on a waterbath for 15 min. cooled in ice and filtered to give the product (1.05 g, 65.8%), colourless plates m.p. 188-191°(from ethanol).

Found: C, 56.94; H, 4.06; N, 15.25.

$C_{13}H_{11}N_3SO_2$ requires C, 57.15; H, 4.03; N, 15.72%.

ν max. (KBr) 3500(NH), 3250(NH), 2850, 1620, 1600, 1580, 1500, 1415, 1340(S=O), 1282, 1160, 1149(S=O), 1120, 800, 762, 775, 720 $cm.^{-1}$

δ $[(CD_3)_2SO]$ 12.61 (1H, s (broad), N - H)
10.28 (1H, s, N' - H)
8.44 (1H, m, 6- H)
8.27 (2H, m, 2,4 - H)
7.50 (6.85 - 6H, m, 5, 2', 3', 4', 5', 6' - H).

M^+ 273

m/e 275(7), 274(14), 273(73), 210(10), 209(41), 208(30), 207(10), 206(4), 183(5), 182(7), 181(48), 180(5), 150(12), 149(10), 139(46), 134(12), 133(86), 119(33), 118(100), 117(82), 111(7), 94(41), 93(43), 92(41), 91(63), 90(70), 84(19), 79(16) 78(35), 77(30), 67(49), 66(40), 65(41), 51(53), 49(41), 44(41), 41(41), 29(14), 28(27), 27(26).

3-N-Methyl-N-phenylsulphamoyl-1H-pyrrolo[2,3-b]pyridine (107).—

To N-methylaniline (0.3 g) in ethanol (2 ml) was added 1H-pyrrolo [2,3-b]pyridine-3-sulphonyl chloride and the mixture warmed on a waterbath for 15 min., cooled in ice and filtered to give the product (0.48 g, 73%), colourless needles m.p. 212-215° (from ethanol).

Found: C, 58.69; H, 4.34; N, 14.54.

$C_{14}H_{13}N_3SO_2$ requires C, 58.54; H, 4.53; N, 14.63%.

ν max. (KBr) 3450, 3150, 2875, 1495, 1455, 1350(S=O), 1280, 1140(S=O), 865, 795, 770, 765, 705, 690 $cm.^{-1}$.

$\delta[(CD_3)_2SO]$ 12.81 (1H, s, N - H)
8.38 (1H, q, $J_{6,5}$ = 4.5Hz, $J_{6,4}$ = 2Hz, 6 - H)
8.08 (1H, s, 2 - H)
7.51 (1H, q, $J_{4,5}$ = 8Hz, $J_{4,6}$ = 2Hz 4 - H)
7.39 - 6.95 (6H, m, 5, 2', 3', 4', 5', 6' - H)
3.19 (3H, s, N' - CH_3).

M^+ 287

m/e 288(3), 287(13), 224(9), 223(46), 222(31), 208(6), 207(6), 182(4), 181(3), 149(8), 133(30), 119(5), 118(31), 117(15), 108(10), 107(100), 106(87), 105(18), 104(21), 91(26), 90(15), 79(39), 78(21), 77(64), 69(15), 65(13), 64(18), 63(17), 29(10), 28(71), 27(15).

1-p-Chlorobenzyl-3-morpholinosulphonyl-1H-pyrrolo[2,3-b] pyridine(108).—

To a suspension of 1-p-chlorobenzyl-1H-pyrrolo [2,3-b] pyridine-3-sulphonylchloride (0.8 g) in chloroform (10 ml) was added morpholine (0.2 g) in chloroform and the mixture stirred together overnight. The solvent was removed in vacuo, and aqueous ethanol added to the residue and filtered to give the product (0.6 g, 65.2%) colourless needles m.p. 128-130° (from aqueous ethanol).

Found: C, 55.65; H, 4.70; N, 10.72.

$C_{18}H_{18}ClN_3SO_3$ requires C, 55.25; H, 4.60; N, 10.74%.

3150(=C-H), 2900 (ν_{H}^{H})

ν max. (KBr) 1521, 1422, 1410, 1401, 1350(S=O), 1260, 1170,

1150(S=O), 1110, 1075, 945, 818, 780, 760, 730 $cm.^{-1}$.

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 391

m/e 394(3), 393(13), 392(10), 391(32), 327(8), 307(15), 306(8), 305(35), 245(17), 244(30), 243(58), 242(63), 241(58), 240(5), 207(5), 206(8), 205(13), 131(28), 127(73), 126(22), 125(100), 89(48), 86(98), 85(40), 71(28), 69(23), 57(56), 56(68), 55(30), 43(38), 29(46), 28(45), 27(38).

1-p-Chlorobenzyl-3-piperidinosulphonyl-1H-pyrrolo[2,3-b]pyridine(109).— 1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine-3-sulphonyl chloride (0.6 g) and piperidine (0.6 g) treated as above gave the product (0.5 g, 88.2%), colourless needles m.p. 128-130° (from aqueous ethanol).

Found: C, 58.07; H, 5.06; N, 10.49.

$C_{19}H_{20}ClN_3SO_2$ requires C, 58.61; H, 5.14; N, 10.80%.

νmax. (KBr) 3140, 2950 (CH_2), 1520, 1340 (S=O), 1330, 1310, 1165, 1145 (S=O), 1115, 935, 810, 722 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 389

m/e 391(5), 390(3), 389(9), 306(5), 305(3), 304(9), 255(5), 244(12), 243(17), 242(35), 241(45), 207(4), 206(6), 205(7),

200(4), 149(6), 131(17), 127(38), 126(11), 125(100), 89(31), 63(8), 57(13), 55(16), 29(11), 28(21), 27(9).

3-Sulphamoyl-1H-pyrrolo[2,3-b]pyridine (110).—

To ammonia solution (1 ml) (35%) in ethanol (2 ml) was added 1H-pyrrolo[2,3-b]pyridine-3-sulphonyl chloride (0.8 g) and warmed on a waterbath for 10 min., cooled in ice and filtered to give the sulphonamide (0.53 g, 72.6%), colourless needles, m.p. 264-266° (decomp).

An analytically pure sample was not obtained.

ν max. (KBr) 3199(NH), 3110, 1580, 1520, 1455, 1410, 1285, 1230(S=O), 1185, 1066, 778 $cm.^{-1}$

No suitable solvent was available for the determination of the n.m.r. spectrum.

M^+ 197

m/e 199(12), 198(15), 197(79), 181(91), 150(18), 149(30), 133(91), 119(27), 118(64), 117(52), 111(36), 97(55), 91(49), 90(39), 85(55), 83(61), 82(58), 81(46), 80(58), 79(27), 71(76), 69(82), 57(100), 56(42), 45(61), 44(100), 43(100), 41(64), 29(39), 28(73), 27(39).

3-Hydrazinosulphonyl-1H-pyrrolo[2,3-b]pyridine (111).—

To hydrazine hydrate (1 ml) in ethanol (2 ml) was added 1H-pyrrolo[2,3-b]pyridine-3-sulphonyl chloride (1.33 g) and the mixture warmed on a waterbath for 10 min. cooled in ice and filtered to give the sulphonylhydrazide (1.74 g, 77.8%), colourless

powder m.p. 215-6° (decomp) washed with water and acetone).

An analytically pure sample was not obtained

ν max. (KBr) 3250(NH), 1590, 1500, 1460, 1420, 1335(S=O), 1319, 1295, 1251, 1140,(S=O), 912, 800, 780, 710, 650 cm^{-1}.

δ[$(CD_3)_2SO$] 8.32 (2H, m, 4, 6 - H)
7.73 (1H, s, 2 - H)
6.96 (4H, m, 5 - H and $NHNH_2$ - H).

The molecular ion was not seen in the mass spectrum.

3-*N*,*N*-Diethylaminosulphonyl-1H-pyrrolo[2,3-b] pyridine (112).—

To diethylamine (0.3 ml) in ethanol (2 ml) was added 1H-pyrrolo[2,3-b] pyridine-3-sulphonylchloride and warmed on a water-bath for 15 min, the solvent removed under reduced pressure to give the product (0.32 g, 91.1%), colourless prisms, m.p. 215-7° (from ethanol).

An analytically pure sample was not obtained.

ν max. (KBr) 2960(CH_3), 2802(CH_2), 2750, 1285, 1225, 1185(S=O), 1070, 105, 780 cm^{-1}.

δ(D_2O) 8.24 (2H, 4, 6 - H)
7.84 (1H, s, 2 - H)
7.22 (1H, q, $J_{5,4}$ = 5Hz, $J_{5,6}$ = 8Hz, 5 - H)
2.92 (4H, q, J = 7Hz, CH_2)
1.13 (6H, t, J = 7Hz, CH_3).

M^+ 253

m/e 253(10), 238(32), 229(6), 228(6), 182(9), 181(69), 149(16), 133(24), 129(10), 119(7), 118(25), 117(15), 111(14), 110(6), 109(14), 99(9), 98(28), 95(20), 91(20), 90(11), 85(28), 84(13), 83(31), 81(25), 74(68), 73(100), 72(94), 71(56), 70(30), 69(45), 60(32), 59(86), 58(94), 57(87), 56(81), 55(56), 45(42), 44(96), 43(92), 42(85), 41(83), 40(56), 39(32), 32(78), 31(51), 30(87), 29(87), 28(87), 29(93).

1H-pyrrolo[2,3-b]pyridine-3-sulphonic acid (113).—

To disulphuric acid (5 ml) cooled in ice to 0-5° was added in portions with stirring 1H-pyrrolo[2,3-b]pyridine (0.5 g). The resulting solution was stirred at room temperature for 0.5h and added to water (20 ml), followed by sodium hydrogen carbonate (2 g), the mixture was cooled briefly and the precipitate filtered off washed with water to give the sulphonic acid (0.074 g, 88.4%), colourless needles, m.p. 324-5° (decomp) (from water).

The analytical data indicated presence of impurity.

Found; C, 41.28; H, 3.16; N, 13.59.

$C_7H_6N_2SO_3$ requires C, 42.42; H, 3.03; N, 14.14%.

ν max. (KBr) 3350, 3105, 3050, 1643, 1357, 1210 (S=O), 1066(S=O), 1011, 798, 761 $cm.^{-1}$

δ (CF_3CO_2H) 9.21 (1H, d, J = 8Hz, 6 - H)

8.40 (1H, s, 2 - H)

7.88 (1H, $J_{5,4}$ = 6Hz, $J_{5,6}$ = 8Hz, 5 - H).

M^+ 198

m/e 199(3), 198(28), 181(7), 134(5), 133(22), 119(45), 118(100), 117(51), 92(30), 91(60), 90(31), 80(16), 79(6), 78(14), 67(14), 65(19), 64(79), 63(49), 62(14), 59(31), 57(14), 48(30), 44(30), 43(14), 41(26), 40(12), 39(27), 32(48), 29(7), 28(4), 27(16).

3-Bromo-1-p-chlorobenzyl -1H-pyrrolo[2,3-b]pyridine (114).—

1-p-Chlorobenzyl-1H-pyrrolo[2,3-b]pyridine (0.8 g) in chloroform (15 ml) was cooled in ice and treated with bromine (0.53 g) in carbon tetrachloride (20 ml). The mixture was extracted with 5% hydrochloric acid (40 ml), then the organic solvent removed in vacuo to leave the product (1.0 g, 94.5%), colourless needles, m.p.75-77° (from carbon tetrachloride/petroleum ether 60-80°).

Found: C, 52.31; H, 2.98; N, 8.51.

$C_{14}H_{10}BrClN_2$ requires C, 52.50; H, 3.13; N, 8.75%.

ν max. (KBr) 1568, 1498, 1440, 1422, 1321, 1300, 1154, 1090, 800, 794, 780, 768, 758 $cm.^{-1}$

δ ($CDCl_3$) 8.42 (1H, q, $J_{6,5}$ = 5Hz, $J_{6,4}$ = 2Hz, 6 - H)
7.90 (1H, q, $J_{4,5}$ = 8Hz, $J_{4,6}$ = 2Hz, 4 - H)
7.22 (6H, m, 2, 5, 2', 3', 5, 6' - H)
5.42 (2H, s, CH_2).

M^+ 320

m/e 324(9), 323(11), 322(30), 321(17), 320(22), 319(20), 242(8), 241(42), 211(5), 209(6), 206(6), 205(6), 143(5),

142(3), 127(31), 126(13), 125(100), 107(6), 99(9), 90(11), 89(33), 77(5), 76(6), 75(5), 63(11), 44(16), 29(6), 28(44), 27(5).

Oxidation of 2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine -3-carbaldehyde

~~3-Carbaldehyde~~-2-p-Chlorophenyl-1H-pyrrolo[2,3-b]pyridine (115).— 2-p-Chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine (2 g) and potassium permanganate (3.1 g) in water (36 ml) were heated under reflux until all the permanganate colour was discharged. The mixture was cooled briefly, and concentrated hydrochloric acid (20 ml) added. The mixture was heated to boil briefly, cooled and filtered to give the carbaldehyde (1.8 g, 85%) colourless plates, m.p. 335-7° (decomp) (from acetic acid).

The analytical data indicated presence of impurity:
Found: C, 64.97; H, 3.61; N, 10.82.
$C_{14}H_9ClN_2O$ requires C, 65.63; H, 3.52; N, 10.94%.

ν max. (KBr) 1658(C=O), 1590, 1465, 1422, 1275(C-O), 775, 760 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 256

m/e 259(5), 258(29), 257(47), 256(75), 255(100), 222(5), 221(27), 220(52), 200(10), 192(10), 191(27), 190(10), 166(8),

165(13), 164(15), 149(5), 144(9), 139(10), 138(8), 137(5), 116(7), 76(23), 84(8), 83(15), 75(8), 60(32), 45(39), 44(16), 43(39), 29(8), 28(40), 27(7).

Attempted preparation of 2-p-chlorophenyl-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid (116).—

A. A stirred mixture of 2-p-chlorophenyl-3-methyl-1H-pyrrolo-[2,3-b]pyridine (2 g) and potassium dichromate (6 g) in water (30 ml) was treated dropwise with concentrated sulphuric acid (14 ml). After the addition of acid was complete the mixture was heated under reflux for 0.5h, cooled and filtered to give colourless plates m.p. 257°. The product was identified as p-chlorobenzoic acid by mass sepctroscopy, M^+ 156.

B. A mixture of ~~3-carbaldehyde~~-2-p-chlorophenyl-1H-pyrrolo[2,3-b]-
-3-carbaldehyde
pyridine (115) (1 g) and potassium permanganate (1.5 g) in water (30 ml) was heated under reflux until the permanganate colour was discharged. The mixture was cooled briefly, then concentrated hydrochloric acid (10 ml) was added, the mixture was heated to boil briefly, cooled and filtered to give none of the expected product but p-chlorobenzoic acid as above.

Attempted preparation of 3-bromomethyl derivatives of 2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine

3-Bromomethyl-2-p-chlorophenyl-1H-pyrrolo[2,3-b]pyridine (117).—

A mixture of 2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]pyridine (4 g), N-bromosuccinimide (3 g) and benzoyl peroxide (0.05 g) in

chlorobenzene (100 ml) was heated under reflux for 17 h (upon heating the mixture dissolved to give a clear solution, then in about 0.25 h a solid separated). The mixture was cooled and filtered to give a yellow compound, m.p. 228°. It turned brown on exposure to air. An n.m.r. spectrum indicated an unchanged 3-CH_3 signal.

When a little of the product was boiled with 2M sodium hydroxide the starting material was recovered.

3-Bromomethyl-1-p-chlorobenzyl-2-p-chlorophenyl-1H-pyrrolo[2,3-b]-pyridine. (118).—

1-p-Chlorobenzyl-2-p-chlorophenyl-3-methyl-1H-pyrrolo[2,3-b]-pyridine (2 g), N-bromosuccinimide (1 g) and benzoylperoxide (0.05 g) in carbon tetrachloride (20 ml) were heated under reflux for 2 h and the solvent removed in vacuo to give a sticky brownish residue. N.m.r. spectroscopic analysis indicated an unchanged 3-CH_3 signal.

3-Bromomethyl-2-p-chlorophenyl-1-methyl-1H-pyrrolo-[2,3-b] pyridine (119).—

2-p-Chlorophenyl-1,3-dimethyl-1H-pyrrolo[2,3-b] pyridine, (2.6 g) N-bromosuccinimide (1.8 g) in chlorobenzene (40 ml) were heated under reflux for 2.5 h then heated further on a waterbath overnight. The solvent was removed in vacuo to leave a sticky brown material. An n.m.r. spectrum indicated an unchanged 3-CH_3 group.

3-(
2-Amino-1H-pyrrolo[2,3-b] pyrid-3-yl) propanoic acid (121).—

Prepared by the method of Robison and Robison[107] m.p.261-262°

(Lit[107] 257-259°).

2 -N-Acetamido-(3-C)-1H-pyrrolo[2,3-b]pyrid-3-yl)-3 -propanoic acid (122).—

Ethyl-2 -N-acetamido-2 -ethoxycarbonyl-3 -(1H-pyrrolo[2,3-b]-pyrid-3-yl) propanoate (6.5 g) in 8% sodium hydroxide (50 ml) were heated under reflux for 3 h, and cooled, then acidified with dilute hydrochloric acid and cooled to 0° to precipitate the product (3 g, 64.8%), colourless needles m.p. 242-244° (washed with hot aqueous ethanol).

An analytically pure sample was not obtained.

ν max. (KBr) 3425(OH), 3300(NH), 1712(C=O), 1625(N-B=O), 1534, 1378, 1250 $cm.^{-1}$

No suitable solvent was available for the determination of an n.m.r. spectrum.

M^+ 247

m/e 247(8), 229(8), 201(9), 189(7), 188(43), 161(7), 160(6), 159(12), 158(8), 142(8), 132(25), 131(100), 130(8), 118(9), 104(15), 92(15), 91(23), 77(10), 71(12), 69(15), 58(18), 56(18), 46(77), 45(100), 44(100), 43(43).

Methyl 2 -amino-(3-C)-1H-pyrrolo[2,3-b]pyrid-3-yl)-3 propanoate (123).—

To a suspension of 2 amino-(3-C)-1H-pyrrolo[2,3-b]pyrid-3-yl)-3 -propanoic acid (1.4 g) in methanol (20 ml) hydrogen chloride gas was bubbled till solution was effected. The solution was heated

under reflux for 6h, the solvent removed *in vacuo* and a minimum amount of water added to the residue and neutralised with 1M ammonia solution to precipitate the product (0.9 g, 60.2%), colourless plates, m.p. 239-241° (from acetone/water).

An analytically pure sample was not obtained.

ν *max.* (KBr) 3180(NH), 2940, 1740(C=O), 1540, 1442, 1290(C-O), 1218, 779 $cm.^{-1}$

$\delta[(CD_3)_2SO]$ 8.26 (1H, d, J = 5Hz, 6 - H)
8.06 (1H, d, J = 8Hz, 4 - H)
7.50 (1H, s, 2 - H)
7.11 (1H, q, $J_{5,6}$ = 5Hz, $J_{5,4}$ = 8Hz, 5 - H)
4.26 (1H, t, J = 5.8Hz, 2' - H)
3.66 (3H, s, CH_3)
3.40 (2H, d, J = 5.8Hz, 3' - H).

M^+ 219

m/e 219(5), 216(6), 160(20), 159(8), 158(6), 157(12), 156(10), 149(5), 143(6), 133(15), 132(62), 131(100), 130(22), 129(6), 105(7), 104(39), 103(15), 102(10), 81(14), 79(17), 78(23), 77(19), 71(14), 69(24), 55(14), 51(14), 44(21), 43(20), 41(18), 39(25), 32(22), 29(10), 28(24), 27(10).

BIBLIOGRAPHY

1. A.J. Duggan, An Historical Perspective. In The African Trypanosomiases, ed. H.W. Mulligan, Allen & Unwin, London, 1970, xli-lxxxviii.
2. D. Scott, The Epidemiology of Gambian Sleeping Sickness. In The African Trypanosomiases, ed. H.W. Mulligan, Allen & Unwin, London, 1970, 614-644.
3. F.I.C. Apted, The Epidemiology of Rhodesian Sleeping Sickness. In The African Trypanosomiases ed. H.W. Mulligan, Allen & Unwin, London, 1970, 645-660.
4. African Trypanosomiasis. Brit. Med. J., 1976, 1298.
5. P. de Raadt, African Sleeping Sickness today. Trans. R. Soc. Trop. Med. Hyg., 1976, 70(2), 114-116.
6. Special Programme for Research and Training in Tropical Diseases. Technical Report TDR/TRY-SWG(1)/77.3. WHO 1977. 1-40.
7. S.R. Meshnick, S.H. Blobstein, R.W. Grady and A. Cerami, An Approach to the Development of New Drugs for African Trypanosomiasis. J. Exp. Med., 1978, 148 569-579.
8. J. Ford, Ideas which have Influenced Attempts to solve the Problems of African Trypanosomiasis. Soc. Sci & Med., 1979, 13(B), 269-275.
9. J.R. Foulkes, Human Trypanosomiasis in Africa. Brit. Med. J., 1981, 283 1172-1174.
10. J.R. Baker, Epidemiology of African sleeping sickness. In Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Ciba Foundation Symposium 20 (New series), Elsevier, Amsterdam, 1974, 29-50.
11. Y. Marciacq and J.R. Seed, Reduced Level of Glycogen and Glucose-6-phosphate in Livers of Guinea-pigs Infected with

Trypanosoma gambiense. J. Infect. Dis., 1970, 121(6), 653-655.

12. A.B. Clarkson, Jr. and F.H. Brohn, Trypanosomiasis: An Approach to Chemotherapy by the Inhibition of Carbohydrate Catabolism. Science, 1976, 194, 204-206.

13. I.R. Tizard, K. Nielsen, A. Mellors and R.K. Assok, Free Fatty Acids and Pathogenesis of African Trypanosomiasis. Lancet, 1977, i 750-751; ii, 91.

14. C.J. Roberts and M.J. Clarkson, Free Fatty Acids, Lysophosphatidylcholine, and Pathogenesis of Trypanosomiasis, Lancet, 1977, i 952-953, C.J. Roberts, I.R. Tizard, A. Mellors and M.J. Clarkson, Lysophospholipases, Lipid Metabolism and the Pathogenesis of African Trypanosomiasis. Lancet, 1977, ii, 1187-1188.

15. I.R. Tizard, K.H. Nielsen, J.R. Seed and J.E. Hall, Biologically Active Products from African Trypanosomes. Microbiological Reviews, 1978, 42(4), 661-681.

16. H.H. Stibbs and J.R. Seed, Metabolism of Tyrosine and Phenylalanine in Trypanosoma brucei gambiense. J. Biochem., 1975, 6 197-203.

17. H.H. Stibbs and J.R. Seed, Chromatographic evidence for the Synthesis of Possible Sleep-mediators in Trypanosoma brucei gambiense. Experientia, 1973, 29, 1563-1565.

18. J.R. Seed, T.M. Seed and J. Sechelski, The Biological Effects of Tryptophol (Indole-3-ethanol): Hemolytic, Biochemical and Behaviour Modifying Activity. Comp. Biochem. Physiol., 1978, 60C, 175-185.

19. H.H. Stibbs, and J.R. Seed, Short-term Metabolism of [^{14}C] Tryptophan in Rats Infected with Trypanosoma brucei gambiense. J. Infect. Dis., 1975, 131(4), 459-462.

20. H.H. Stibbs and J.R. Seed, Elevated Serum and Hepatic Tyrosine Aminotransferase in Voles Chronically Infected with Trypanosoma brucei gambiense. Exp. Parasitol., 1976, 39, 1-396.

21. C.A. Hoare, Evolutionary Trends in Mammalian Trypanosomes, Adv. Parasitol., 1967, 5, 47-91.

22. C.A. Hoare, The Mammalian Trypanosomes of Africa. In African Trypanosomiases ed. H.W. Mulligan, Allen & Unwin, London, 1970, 3-23.

23. W.E. Ormerod Taxanomy of the Sleeping Sickness Trypanosomes. J. Parasitol., 1967, 53(4), 824-830.

24. WHO, Proposals for the Nomenclature of Salivarian Trypanosomes and for the Maintenance of Reference Collections. Bull. WHO., 1978, 56(3), 467-480.

25. C.A. Hoare and F.G. Wallace, Developmental Stages of Trypanosomatid Flagellates: A New Terminology. Nature, 1966, 212, 1385-1386.

26. J.D. Smyth, Introduction to Animal Parasitology. 2nd ed., Hodder and Stoughton, London, 1976, 53-81.

27. J.F. Ryley, Studies on the Metabolism of the Protozoa. 9. Comparative Metabolism of Blood-stream and Culture forms of Trypanosoma rhodesiense. J. Biochem., 1962, 85, 211-223.

28. K. Vickerman, Polymorphism and Mitochondrial Activity in Sleeping Sickness Trypanosomes. Nature, 1965, 208, 762-766.

29. R.A. Klein, In Alternate Metabolic Pathways in Protozoan Energy Metabolisms. Workshop 1, EMPO 2. Parasitol., 1981, 82, 3-5.

30. I.B.R. Bowman and I.W. Flynn, Oxidative Metabolism of Trypanosomes. In Biology of the Kinetoplastida Vol.1, eds.

W.H.R. Lumsden and D.A. Evans, Academic Press, London, 1976, 435-476.

31. A.H. Fairlamb, F.R. Opperdoes and P. Borst, New Approach to Screening Drugs for Activity Against African Trypanosomes. Nature, 1977, 265, 270-271.

32. F.R. Opperdoes, P. Borst and K. Fonck, The Potential Use of Inhibitors of Glycerol-3-phosphate oxidase for Chemotherapy of African Trypanosomiasis. FEBBS Lett., 1976, 62(2) 169-172.

33. F.H. Brohn and A.B. Clarkson, J.R. Quantitative Effects of Salicylhydroxamic Acid and Glycerol on Trypanosoma brucei Glycolysis in vitro and in vivo. Acta Tropica, 1978, 35, 23-33.

34. A.H. Fairlamb, In Alternate Metabolic Pathways in Protozoan Energy Metabolism. Workshop 1, EMPO 3. Parasitol., 1981, 82, 1-3.

35. A.H. Fairlamb and I.B.R. Bowman. Uptake of the Trypanocidal Drug Suramin by Bloodstream Forms of Trypanosoma brucei and its Effect on Respiration and Growth Rate in vivo. Molecular and Biochemical Parasitol., 1980, 1, 315-333.

36. A.B. Clarkson, Jr. and Fitt. Brohn, Trypanosomiasis: An Approach to Chemotherapy by the Inhibition of Carboydrate Catabolism. Science, 1976, 194, 204-206.

37. D.H. Hammond, W.E. Gutteride and F.R. Opperdoes, A Novel Location for two Enzymes of De NOVO Pyridine Bio-synthesis in Trypanosomes and Leishmania. FEBBS Lett., 1981, 128(1), 27-29.

38. K.K. Oduro, I.W. Flynn and I.B.R. Bowman, Trypanosoma brucei: Activities and Subcellular Distribution of Glycolytic Enzymes from Differently Disrupted Cells. Exp. Parasitol., 1980, 50, 123-135.

39. K.E. Kinnamon and E.A. Steck, A New Chemical Series Active Against African Trypanosomes: Benzyltriphenylphosphonium Salts. J. Med. Chem., 1979, 22(4), 452-455.

40. I.B.R. Bowman and A.H. Fairlamb, L-Glycerol-3-phosphate Oxidase in Trypanosoma brucei and the Effect of Suramin. In Biochemistry of Parasites and Host-Parasite Relationships, ed. H. van de Bossche Elsevier, Amsterdam, 1976, 501-507.

41. A.H. Fairlamb and B.R. Bowman, The Isolation and Characterisation of Particular sn-Glycerol-3-phosphate oxidase from Trypanosoma brucei. Int. J. Biochem., 1977, 8, 659-667.

42. A.H. Fairlamb and I.B.R. Bowman, Inhibitor Studies on Particulate sn-Glycerol-3-phosphate oxidase from Trypanosoma brucei. Int. J. Biochem., 1977, 8, 669-675.

43. J. Williamson and R.S. Desowitz. The Chemical Composition of Trypanosomes. Exp. Parasitol., 1961, 11, 161-175.

44. G.R. Newport, C.R. Page, III., P.U. Ashman, H.H. Stibbs and J.R. Seed, Alteration of Free Serum Amino Acids in Voles Infected with Trypanosoma brucei gambiense. J. Parasitol., 1977, 63(1), 15-24.

45. H.H. Stibbs and J.R. Seed, Further Studies on the Metabolism of Tryptophan in Trypanosoma brucei gambiense: Co-factors, Inhibitors and End-products. Experientia, 1975, 31(3), 274-278.

46. J.E. Hall, K.H. Dahm and J.R. Seed, In vitro Tryptophan Catabolism by Trypanosoma (Trypanozoon) brucei gambiense, T.(T.) equiperdum, T. (Hepertosoma) lewisi and T.(H) musculi. Comp. Biochem. Physiol., 1981, 69B, 617-620.

47. J.E. Hall and J.R. Seed, Quantitation of Aromatic Amino Acid Catabolites in Urine of Mice Acutely Infected with Trypanosoma brucei gambiense. Comp. Biochem. Physiol., 1981, 69B, 791-796.

48. J.R. Seed, Antigens and Antigenic Variability of the African Trypanosomes, J. Protozool., 1974, 21(5) 639-646.

49. P.J. Bridgen, G.A.M. Cross and J. Bridgen, N-terminal Amino Acid Sequences of Variant-specific Surface Antigens from Trypanosoma brucei. Nature, 1976, 263, 613-614.

50. P. De Raadt, Immunity and Antigenic Variation: Clinical Observations Suggestive of Immune Phenomena in African Trypanosomiasis. In Trypanosomiasis and Leishmaniasis with Special Reference to Chagas' Disease. Ciba Foundation Symposium 20 (new series), Elsevier Amsterdam, 1974, 199-224.

51. J.S. Cordingly and M.J. Turner, Isolation of Trypanosoma brucei Variant-specific Antigen in RNA by Immunoprecipitation of Polysomes, Molecular & Biochemical Parasitol, 1980, 1, 129-137.

52. S.C. Meritt, Purification and Cell-free Translation of mRNA Coding for a Variant Specific Antigen from Trypanosoma brucei gambiense. Molecular & Biochemical Parasitol, 1980, 1, 151-165.

53. A.A. Holder & G.A.M. Cross, Glycopeptides from Variant Surface Glycoproteins of Trypanosoma brucei. C-terminal Location of Antigenically Cross-reacting Carbohydrate Moieties. Molecular & Biochemical Parasitol. 1981, 2, 135-150.

54. G.A.M. Cross, Cross-reacting Determinants in the C-terminal Region of Trypanosome Variant Surface Antigens. Nature, 1979, 277, 310-312.

55. J. Williamson, Chemotherapy of African Trypanosomiasis. Trop. Dis. Bull., 1976, 73, 531-542.

56. A.H. Fairlamb and I.B.R. Bowman, Trypanosoma brucei: Suramin and other Trypanocidal Compounds Effects on sn-Glycerol-3-phosphate Oxidase. Exp. Parasitol., 1977, 43, 353-361.

57. R.D. Walter, Effect of Suramin on Phosphorylation Reaction in Trypanosoma gambiense. Molecular & Biochemical Parasitol.,

1980, 1, 139-142.

58. F.I.C. Apted, Present Status of Chemotherapy and Chemoprophylaxis of Human Trypanosomiasis in the Eastern Hemisphere. Pharm. Ther., 1980, 11, 391-413.

59. L.P. Walls, The Chemotherapy of Trypanosomiasis. Progr. Med. Chem., 1963, 3, 52-88.

60. R. Vince, H. Lee, A.S. Narang and F.N. Shirota, 5'-Chloropuromycin. Inhibition of Protein Synthesis and Antitrypanosoma Activity. J. Med. Chem., 1981, 24, 1511-1514.

61. D.A. Evans, C.J. Brightman and M.F. Holland, Salicylhydroxamic Acid/Glycerol in Experimental Trypanosomiasis, Lancet, 1977, ii, 769.

62. D.J. Hammond and I.B.R. Bowman, Trypanosoma brucei: The Effect of Glycerol on the Anaerobic Metabolism of Glucose. Molecular & Biochemical Parasitol., 1980, 2, 63-75.

63. R.A. Casero Jr., D.L. Klayman, G.E. Childs, J.D. Scovill and R.E. Desjardins, Activity of 2-Acetylpyridine thiosemicarbazones against Trypanosoma rhodesiense in vitro. Antimicrob. agents & chemother., 1980, 18(2), 317-322.

64. C.J. Bacchi, H.C. Nathan, S.H. Hutner, P.P. McCan and A. Sjoerdsma, Polyamine Metabolism: A Potential Therapeutic Target in Trypanosomes. Science, 1980, 210, 332-334.

65. J. Williamson, Review of Chemotherapeutic and Chemoprophylactic Agents. In African Trypanosomiases, ed. H.W. Mulligan, Allen & Unwin, London, 1970, 125-221.

66. K.E. Kinnamon, E.A. Steck and D.S. Rane, Activity of Antitumor Drugs Against African Trypanosomes. Antimicro. agents and Chemother., 1979, 15(2), 157-160.

67. K.E. Kinnamon, E.A. Steck and D.S. Rane, Anticancer Agents and Antitrypanosomiasis activity in mice. J.N.C.I. 1980, 64(2),

391-394.

68. B.P. Das and D.W. Boykin, Synthesis and Antiprotozoal Activity of 2,5-Bis(4-guanylphenyl) furans. J. Med. Chem., 1977, 20(4), 431-436.

69. B.P. Das, R.A. Wallace and D.W. Boykin, Jr., Synthesis and Antitrypanosomal Activity of Some Bis(4-guanylphenyl)five-and six-membered Ring Heterocycles. J. Med. Chem., 1980, 23, 578-581.

70. A.C. Capomacchia and J.J. Vallner, 1-(p-tolyl)-3-acetyl-3-methyltriazene: A Compound with Activity Against African Trypanosomiasis. J. Pharmaceu. Sci., 1980, 69(12), 1465-1467.

71. W.J. Dunn, M. Greenberg and J. Powers. 1-Aryl-3,3-dialkyl triazenes with Antitrypanosomal Activity. J. Pharm. Sci., 1982, 71(1), 126-127.

72. B.A. Arrick, O.W. Griffith and A. Cerami. Inhibition of Glutathione Synthesis as a Chemotherapeutic Strategy for Trypanosomiasis J. Exp. Med., 1981, 153, 720-725.

73. J. Williamson and T.J. Scott-Finnigan. Trypanocidal Activity of Antitumor Antibiotics and other Metabolic Inhibitors. Antimicrob. agents and Chemother., 1978, 13(5), 735-744.

74. J. Williamson, T.J. Scott-Finnigan, M.A. Hardman and J.R. Brown, Trypanocidal Activity of Daunorubicin and Related Compounds. Nature, 1981, 292, 466-467.

75. L.G. Goodwin, New Drugs for Old Diseases. Trans. R. Soc. Trop. Med. Hyg., 1980, 74(1), 1-7.

76. M.H. Palmer, The Structure and Relations of Heterocyclic Compounds. Edward Arnod Ltd., London, 1967, 12-65.

77. D.T. Clark, A Self Consistent Field Molecular Orbital Treatment of Pyrrole and Pyrrole Anion Including all Valence Electrons.

Tetrahedron, 1968, 24, 4689-4698.

78. D.A. Bochvar, A.A. Bagaturyants, A.U. Tutkevich, L.N. Yakhontov, M. Ya. Uritskaya, D.M. Krasnokutskaya and M.V. Rubtsov, Study of the π-electron Structure of 7-Azaindole by the LCAO MO Method. Bul. Acad. Sci. USSR Div. Chem. Sci. (Eng. Trans.), 1966 327-329.

79. R.E. Willette, Monoazaindoles: The Pyrrolopyridines. In Adv. Heterocyclic Chem., ed. A.R. Katritzky and A.J. Boulton, Academic Press, London, 1968, 9, 27-105.

80. L.N. Yakhontov, The Chemistry of Azaindoles Pyrrolo[2,3-b]-pyridines Russian. Chem. Rev., 1968, 37(7), 551-565.

81. R. Herbert, The Preparation and Properties of 7-Azaindoles. PhD Thesis. Sunderland Polytechnic, 1969.

82. R.R. Lorenz, B.F. Tullar, C.F. Koelsch and S. Archer. A New Indole Synthesis. J. Org. Chem., 1965, 30, 2531-2533.

83. R.G. Fargher and R. Furness, Derivatives of 2-Pyridyl-hydrazine and 2-Quinolylhydrazine. J. Chem. Soc. 1915, 107, 688-699.

84. S. Okuda and M.M. Robison, 7-azaindole. V. Investigations of Alternative Syntheses of the Ring System, J. Am. Chem. Soc., 1959, 81, 740-743.

85. A.H. Kelly, D.H. McLeod and J. Parrick, Thermal Indolization of Arylhydrazones. Can. J. Chem., 1965, 43, 296-301.

86. A.H. Kelly and J. Parrick, 1. Preparation of 7-Azaindoles by Thermal Indolization of 2-Pyridylhydrazones. Can. J. Chem., 1966, 44, 2455-2459.

87. W.L. Mosby, Heterocyclic Systems with Bridgehead Nitrogen Atoms. Part 1. Interscience, New York, 1961, 480-481.

88. A. Brodrick and D.G. Wibberley, 1H-Pyrrolo[2,3-b]pyridines. Part III. A Novel Synthetic Route from 1-Substituted 2-Aminopyrroles. J. Chem. Soc. Perkin I, 1975, 1910-1913.

89. M.M. Vora, C.S. Yi and C. Dewitt Blanton, Jr. Synthesis of Pyrrolo[2,3-b]azepine-4,7-dione derivatives. J. Hetero-cyclic Chem., 1981, 18, 507-510.

90. M.M. Robison, B.L. Robison and F.P. Butler, 7-Azaindole. VI. Preparation of 5- and 6-substituted 7-Azaindoles.

91. R.J. Sunberg, The Chemistry of Indoles. Academic Press, London, 1970, 11-14.

92. B.A.J. Clark and J. Parrick, Diazaindenes (Azaindoles). IV. Oxidation of 1,5- and 1,7-Diazaindenes and a Novel Route to 3-substituted Diazaindenes. Tetrahedron, 1974, 30, 475-478.

93. B.A.J. Clark and Parrick, Diazaindenes (Azaindoles). Part VI. Preparation and Some Properties of 1,7-Diazaindene 7-oxide and 6,7,8,9-Tetrahydro-α-carboline 2-oxide. J. Chem. Soc. Perkin I, 1974, 2270-2274.

94. C.R. Hardy and J. Parrick, Ring Opening or Rearrangement versus N-oxidation in the Action of Peracids upon Pyrrolo[2,3-b] pyridines, Pyrrolo[2,3-b] pyrazines, and Triazolo[1,5-a]- and Triazolo [4,3-a]-pyrazine. Some Chemical and Spectroscopic Properties of the Triazolopyrazines and their N-oxides. J. Chem. Soc. Perkin I. 1980, 506-511.

95. S.W. Schneller and Jiann-Kuan Luo, Synthesis of 4-Amino-1H-pyrrolo[2,3-b]pyridine (1,7-Dideazaadenine) and 1H-Pyrrolo-[2,3-b] pyridin-4-ol (1,7-Dideazahypoxanthine). J. Org. Chem. 1980, 45, 4045-4048.

96. J. Bernstein, B. Strearns, E. Shaw, and W.A. Lott, Derivatives of 2,6-Diaminopyridine. J. Am. Chem. Soc. 1947, 69, 1151-1158.

97. A.J. Verbiscar, Synthesis of 1-p-Chlorobenzyl-7-aza-indole-3--piperidylmethanol as a Potential Antimalarial Agent. J. Med. Chem., 1972, 15'2), 149-152.

98. G.W. Kidder and V.C. Dewey, Inhibition of Tetrahymena pyriformis. Biochem. Biophys. Acta, 1955, 17, 288.

99. A.B. Pardee, V.G. Shore and L.S. Prestidge, Incorporation of Azotryptophan into Proteins of Bacteria and Bacteriophage. Biochem. Biophys. Acta. 1956, 21, 406-407.

100. A. Berecz and C. Godin, The Incorporation of Tryptophan Analogues into Rat Plasma Proteins. Can. J. Biochem. Physiol. 1962, 40, 153-157.

101. T.K. Adler and A. Albert, The Biological and Physical Properties of the Azaindoles. J. Med. Chem., 1963, 6, 480-483.

102. M.R. Bell, Amidoximes, Amidines and Guanidines for Medical Use. Chem. Abstr. 1966, 64, 2098h.

103. M.R. Bell, J.O. Hoppe, H.E. Lape, D. Wood, A. Arnold and W.H. Selberis, Antihypertensive Activity of 7-Azaindole 3-acetaminoxime and Indole-1-acetaminoxime Experientia, 1967, 23, 298-299.

104. M.H. Fisher, G. Schwartzkopf and D.R. Hoff, Azaindoles as antihelminthic Agents. J. Med. Chem., 1972, 15, 1168-1171.

105. P.J. Bottomley, C.V. Baalen and F.R. Tabita, Heterocyst Differentiation and Tryptophan Metabolism in Cyanobacterium anabaena. Arch. Biochem. Biophys., 1980, 203(1), 204-213. Chem. abstr., 1980, 93, 110388.

106. R. Herbert and D.G. Wibberley, Synthesis and Properties of 1H-pyrrolo[2,3-b] pyridines. J. Chem. Soc. C, 1969, 1505-1514.

107. M.M. Robison and B.L. Robison, 7-Azaindole. I. Synthesis and Conversion to 7-Azatryptophan and other Derivatives, J. Am. Chem. Soc., 1955, 77, 457-460.

108. L.N. Yakhontov and M.V. Rubtsov, Pyrrolo[2,3-b] pyridine Derivatives (7-Azaindoles). VIII. Synthesis and Some Reactions of 4-Methyl-1-phenyl-1H-4-methyl-1-phenyl-1H-pyrrolo[2,3-b]-

pyridine-3-carboxaldehyde. J. Gen. Chem. USSR., 1964, 34, 2626-2631.

109. R.M. Silverstein, G.C. Bassler and T.C. Morrill, Spectrometric Identification of Organic Compounds. John Wiley & Sons, New York, 1974.

110. D.L. Pavia, G.M. Lampman and G.S. Kriz, Introduction to Spectroscopy. W.B. Saunders Company, Philadelphia, 1979.

111. R.F. Borch, M.D. Bernstein and H.D. Durst, The Cyanohydridoborate Anion as a Selective Reducing Agent. J. Am. Chem. Soc., 1971, 93, 2897-2904.

112. G.F. Smith and D.A. Taylor, The Sulphonation of Indole and Some of Its Simple Alkyl Derivatives. Tetrahedron, 1973, 29, 669-672.

113. R. Herbert and D.G. Wibberley, 1H-Pyrrolo[2,3-b]pyridines. Part II. Fragmentation of some 1H-Pyrrolo[2,3-b]pyridines induced by Electron Impact. J. Chem. Soc. (B), 1970, 459-463.

114. J.C. Powers, The Mass Spectrometry of Simple Indoles., J. Org. Chem. 1968, 33(5), 2044-2050.

115. R.B. Moffett, A. Robert and L.C. Skaletzky, Antiulcer Agents. p-Aminobenzamido Aromatic Compounds, J. Med. Chem., 1971, 14(10), 963-968.