THE PREPARATION AND PROPERTIES OF SILICON-CARBOHYDRATE COMPOUNDS

by

FONG LUNG CHOW

A thesis presented for the degree of Doctor of Philosophy in the University of Aston in Birmingham.

October, 1981.

SUMMARY

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Silicon is widely distributed in plant and animal organisms and it has been reasonably well established that the element is structurally bound in biopolymers such as pectin. However the major problem in studying the biological role of silicon is that virtually nothing is known of how silicon is bound within plant and animal polymers. The rather sketchy evidence which exists indicates that in nature silicon is bonded to carbohydrate systems. The aim of the present project was to obtain further information on the bonding of silicon in natural systems.

Attempts were made to prepare compounds of the type $(CH_y)_4Si$ which might serves as models for natural silicon derivatives. Two main modes of approach to these syntheses were followed. The first was to react unprotected monosaccharides with new and modified silylating agents, the second mode involved the reaction of protected monosaccharide derivatives with standard silylating agents. Many modification of these two major approached were investigated. In a number of cases there was evidence that products of the required type had been prepared. These compounds however were generally highly labile and proved difficult to characterise.

A further role to natural silicon derivatives was studied that was the degradation of a variety of pectins, pectins are known to have a fairly high silicon content and it was hoped that degradation and separation would yield specific silyl organic compounds.

Key Words: Silicon, Carbohydrates.

ACKNOWLEDGMENTS

I would like to express my thanks to Dr. A.W.P. Jarvie and the late Dr. A. Holt for their supervision and encouragement of this work; the technical staff of the Chemistry Department especially Mr. E. Hartland and Mrs. V. Clenton for the spectra and analyses which they provided.

Finally, I would like to thank my parents for their financial support.

To Dr. A. Holt

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

Silicon is the second most abundent element in the earth's crust; the average content amounts about 28% by weight. Inorganic silicon compounds such as silica and silicates form the basis of most of the rocks forming the lithosphere. In the atmosphere, silicon is present in very minute quantity as dust of cosmic and terrestial origin. The silicon content in the hydrosphere, mainly in form of dissolved silica(silicic acid) is also very small.

Today there is no doubt that silicon compounds play a significant role in the biosphere. Silicon occurs, at least in trace amounts, in most plant and animal tissues. It plays a particularly important role for many organisms at a lower stage of evolutionary development, such as diatoms and gastropods. It also plays an important role in higher plants and animals, although in the majority of cases the content of silicon is relatively small.

1.1. PHYSICAL PROPERTIES OF CARBON-ELEMENT AND SILICON-ELEMENT BONDS.

Both, carbon and silicon are members of Group IV of the periodic table. In spite of this relationship, there are not only similarities but also striking differences between them. Carbon and silicon differ in the size of their atom (covalent radii: C=77pm; Si=117pm), their electronegativities (Pauling's scale: C=2.5; Si=1.8)[(a)], and the energies of their outershell electrons (electronic configuration: C= $1s^2 2s^2 2p^2$; Si= $1s^2 2s^2 2p^6 3s^2 3p^2$). Because of the position of silicon in the third row of the periodic table, the chemistry of this element is influenced by the availability of empty 3d orbitals which are not greatly higher in energy than the silicon 3s and 3p orbitals. The

availability of low lying 3d orbitals to silicon and the possibility of their involvment in bond formation has been used to explain the easy formation of 5- and 6-coordinated silicon complexes, and the unexpected physical properties, stereochemistry, and chemical behaviour of a number of 4-coordinated silicon compounds.

According to the electronegativities of carbon, silicon and the elements(El) H,N,O,S,F, and Cl the polarisation of the covalent C-El bonds is qualitatively the same as that of the corresponding Si-El bonds, with only two exceptions: the polarisation of the Si-H bond ($Si^{\delta+}-H^{\delta-}$) is opposite to that of the C-H bond ($C^{\delta-}-H^{\delta+}$), and the polarisation of Si-S bond ($Si^{\delta+}-S^{\delta-}$) is opposite to that of the C-S bond which is nearly without polarity. The Si-El bonds always exhibit a higer degree of polarisation than the corresponding C-El bonds. In the Si-C bond system the silicon is the more positive partner.[b]

Whereas carbon is able to form stable $(p-p)\pi$ double bonds and triple bonds with carbon itself, nitrogen and oxygen, leading to coordination numbers 2 and 3 at the carbon atom, silicon cannot build up such bonds. Although the existance of monomeric species[c] such as SiO and SiNH has been established under specific conditions, and $(p-p)_{\pi}$ bonded intermediates have been postulated in a number of reactions, no stable silicon analogues of alkenes, alkynes, aldehydes, ketones, etc. have been isolated so far.

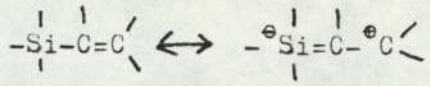
In contrast to carbon, silicon is able to increase its coordination number from 4 to 5- and 6 ($sp^3 \rightarrow sp^3d \rightarrow sp^3d^2$) by one or two additional $(p \rightarrow d)_{\sigma}$ donor bonds, 5- and 6-coordinated silicon compounds appear often as reaction intermediates. In several cases such compounds are isolable.[d]

The availability of empty 3d orbitals of silicon is used to explain further difference between carbon compounds and the Si-analogues: Trisilylamine $N(SiH_3)_3$ was found to have a planar Si-N[e] skeleton in contrast to the carbon analogue trimethylamine $N(CH_3)_3$ which is pyramidal. The structure of the silicon compound was rationalized by assuming that the N atom forms σ-bonds in a trigonal plane leaving the lone pair of electron in a pure p orbital of nitrogen at right angles to their plane. The electron density from this p orbital is donated into the vacant 3d orbitals of the silicon atoms. Not only the geometry, but also the weak donor ability of the N atom in $N(SiH_3)_3$ is in agreement with the involvment of this lone pair in bonding to silicon.

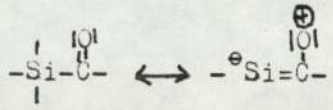
Further evidence on $(p\rightarrow d)_\pi$ bonding has been obtained by studying the behaviour of the pairs R_3COH/R_3SiOH[f,g] and $(R_3C)_2NH/$[h,i] $(R_3Si)_2NH$ as acids. The acidities of the -OH and -NH group should be decreased for the silicon compounds as compared with those of the analogous carbon compounds because of the greater electronegativity of the carbon atom. However, silanol and secondary silylamines were found to be more acidic than the carbon analogues. These results can be explained by additional $(p\rightarrow d)_\pi$ back donation from nitrogen and oxygen to silicon, leading to an increased s-character in the σ-orbitals of nitrogen and oxygen, because of the changing of hybridization at N and O from sp^3 to sp^2.

Silicon can also form partial double bonds $[\sigma + (p\rightarrow d)_\pi]$ to carbon. However, such $\tilde{\pi}$- interaction are only possible, if the carbon atom is a part of an unsaturated group (sp^2 or sp hybridization), eg. in phenyl, vinyl and keto groups, etc.[j,k] This is shown

by the following resonance structures of vinylsilanes(I) and α-silylketones(II) :



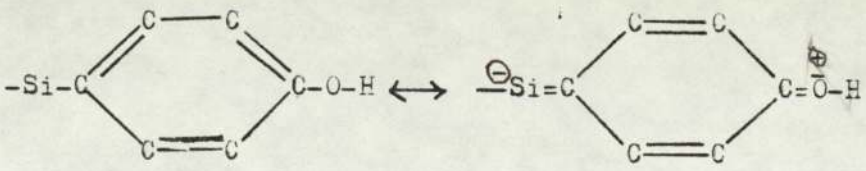
(I)



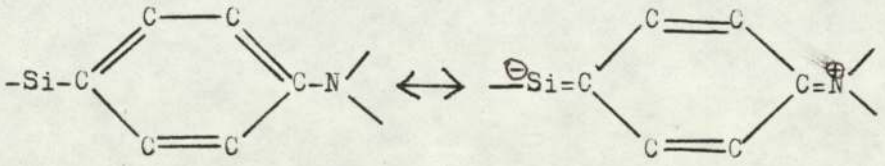
(II)

Spectroscopic data of α-silylketones and the chemical behaviour of vinylsilanes (addition reactions opposite to Markownikoff's rule) are in agreement with this $(p \rightarrow d)_{\pi}$ model.

Silicon bound to a phenyl group can also influence the bond system by additional $(p \rightarrow d)_{\pi}$ back donation from carbon to silicon.[1] p-Trimethylsilyl-substituted benzoic acid shows a greater acidity than expected from inductive effects. Furthermore, p-trimethylsilyl aniline shows a decreased basicity as compared with that of the non-substituted compound. This behaviour can be described by the following resonance structures (III) and (IV) :[m,n]



(III)



(IV)

However, influence on functional groups in organic compounds by a silicon atom is also possible without $(p \rightarrow d)_{\pi}$ interaction. In examples $(CH_3)_3ElCH_2COOH$ and $(CH_3)_3ElCH_2NH_2$ $(El=C,Si)^{(o,p)}$ the silicon compound is the weaker acid and the stronger base. These effects are explained by a σ-electron release from the silicon compounds, caused by the lower electronegativity of silicon.

1.2. CHEMICAL PROPERTIES OF CARBON-ELEMENT AND SILICON-ELEMENT BONDS

Because of the big electronegativity differences, there is strong tendency of Si-El bonds to undergo heterolytic fission. The splitting can be achieved by a nucleophilic attack on silicon and by an electrophilic attack on its partner, or by a more or less concerted action of both types of attack. Under normal conditions the Si-C bond is relatively stable to chemical attack, comparable with the C-C linkage. Only in special cases is reactivity of the Si-C bond increased drastically, eg. ß-functional organosilicon compounds[1]:

i. $(CH_3)_3Si-CH_2-CHOH-CH_3 \xrightarrow{[H_3O^{\oplus}]} (CH_3)_3SiOH + CH_2=CH-CH_3$

ii. $(CH_3)_3Si-CH_2-CO-CH_3 \xrightarrow{C_2H_5OH} (CH_3)_3SiOC_2H_5 + CH_3-CO-CH_3$

iii. $(CH_3)_3Si-CH_2-COO-C_2H_5 \xrightarrow{HCl} (CH_3)_3SiCl + CH_3-COO-C_2H_5$

iv. $(CH_3)_3Si-CH_2CN \xrightarrow{H_2O} (CH_3)_3SiOH + CH_3CN$

Because of the reverse polarisation the Si-H bond behaves chemically quite differently from the analogous C-H bond. The Si-H

linkage can easily be cleaved by water, leading to the corresponding silanols (or disiloxanes) and hydrogen:

v. $$Si\text{-}H + H_2O \xrightarrow{(OH)^{\ominus}} Si\text{-}OH + H_2$$

This reaction is acid/base catalysed: alkali catalyses Si-H hydrolysis more effectively than acid.

The silicon-halogen bonds have a very high ionic character, and they are very resistant towards homolytic fission, whereas they are easily cleaved by means of ionic agents. The primary step of most of the reactions based on Si-halogen cleavage seems to be a nucleophilic attack on the silicon atom, which is able to increase its coordination number from 4 to 5 and 6. The reactivity of silicon halide with respect to hydrolytic cleavage reactions decreases in the order I > Br > Cl > F. The reactivity of Si-halogen bonds is drastically increased as compared with that of the analogous C-halogen bonds. For example, CCl_4 is stable against water, while $SiCl_4$ reacts vigorously. Si-O and Si-N bonds also show a high tendency to undergo heterolytic fission and they have little resistance towards solvolysis in alcohol and water. eg.

vi. $$R_3SiOR' + H_2O \longrightarrow R_3SiOH + HOR'$$

vii. $$R_3SiNR'_2 + H_2O \longrightarrow R_3SiOH + HNR'$$

Such hydrolytic reactions normally take place slowly under neutral conditions.

1.3. SILICON IN PLANTS AND ANIMALS

Silicon was first detected in living organisms at the end of the 18th century. In 1789 Abildgaard wrote about the presence of silicon in sponges. The first statement in print concerning the presence of silicon in plants occurs in 1790.

Silica gel is the most prevalent form of silicon in plants constituting 90 to 95% of the total Si in the rice plant, but the element is also present in other forms.[2] Infrared studies[3] gave no evidence of any absorption bands due to organosilicon compound in the rice plant; however, the results did not exclude the possibility that a minute amount of silicon might exist in the form of organic compounds. There are reports that part of the silica in plants is strongly bound to the cellulose framework.[4]

In some plants silicon is apparently distributed rather uniformly between shoots and roots, while in other plants it may accumulate to a greater extent in the shoots than in the roots. Occasionally, it may even be present at a higher concentration in the roots than in the shoots. In plants where the overall content of silicon is high, then Si appears to occur mostly in aerial parts. Species variation in silicon content is considerable. Cereal grains high in "husk" or fibre such as oats are very much richer in silicon than low-fibre grains such as wheat.[5]

Plants take up different amounts and proportions of silicon from culture solution, depending on the species and the concentration of dissolved silicic acid present. The amount of silica present in the plants increases in direct proportion to the amount of silicic acid dissolved in the soil solution. Rye and sunflower plants[6] gave good evidence for the passive uptake of silicic acid in the

transpiration stream and its subsequent distribution throughout the plant following the transpiration stream.[2,7] However, some experiments carried out with rice plant support the idea that silicic acid can be absorpted independently of the rate of water absorption.[8,9]

By rigid exclusion of silica from the culture solutions and environment it has been possible to demonstrate improved growth in several plant species from addition of silicates.[10] Using plastic containers,[11,12] Si-deficient rice plants developed necrosis in their leaves and were retarded in growth, Si-deficient rice shoots exhibited reduced fertility. It has also been shown[9] that Si promotes the growth of rice plants. Both the roots and shoots were longer in the presence of Si and the grain yield was greater.

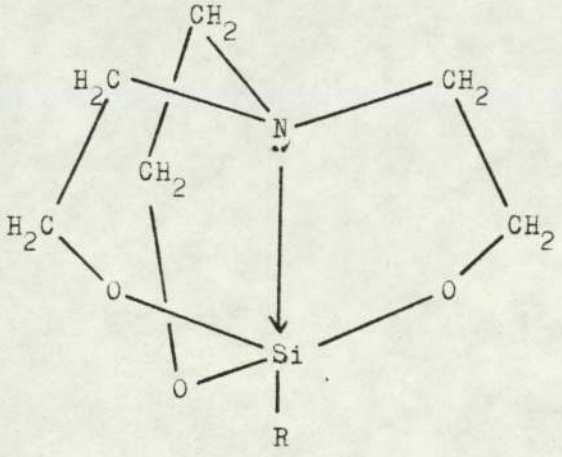
Interest in the silicon content of animal tissues and fluids began with the work of Gonnerman[13], who carried out analyses of many tissues many years ago. Improvement in analytical techniques[14] has revealed consistently high levels of silicon in animal tissues and fluids. Even in the fetal tissue the normal range has been reported as 40-400 ppm SiO_2 on the dry basis, compared with 50-1000 ppm for normal adult tissues[15]. In the fetus the lowest values occur in the lung and highest in the muscles, whereas in adult human the reverse position holds, no doubt due to the inhalation of dust, as with aluminium. The blood of man and other species averages approximately 5mg Si/litre and this level is not increased by the inhalation of silica dust, by the ingestion of silicates in the food, or even by injection[16]. Among the human tissues examined, epidermis and hair contained the largest concentrations.

1.4. NATURALLY OCCURRING ORGANOSILICON COMPOUNDS

Numerous attempts have been made to extract and identify organic silicon compounds from tissues, but in no case has a pure substance been isolated and chemically characterized[17,18]. Silicon "esters" of lipids such as cholestrol lethicin, and choline have been described after extraction of tissues by ethanol-ether. Holt and Yates[19] demonstrated that such products are artifacts, obtainable in vitro by treating these compounds with soluble silicic acid. More recently[20], it has been claimed that by using NH_4PF_6 as complexing agent, ß-thujaplicine-silicon-hexafluorophosphate has been isolated from Thuja plicata. It has been concluded that silicon-thujaplicine complex is present in Thuja plicata.

1.5. BIOACTIVE ORGANOSILICON COMPOUNDS

In 1963, Voronkov and co-workers[21] showed that organosilicon compounds of the general formula $RSi(OCH_2CH_2)_3N$ named by themselves as "silatranes" had very high and specific biological activity. Silatranes can be described as caged compounds (V) with penta-coordinated silicon and tetracoordinated nitrogen.



Silatrane (V)

Physical investigations have given evidence of an intramolecular N- Si coordinative bond. However, a critical analysis[22] of earlier spectroscopic data on silatranes as well as the use of new experimental results, showed that the degree of transannular interaction between the silicon and nitrogen was formerly exaggerated. The electron density transfer from the nitrogen atom to silicon is not so great as was previously considered and does not exceed 0.1-0.2 e.

The most prominent biological feature of silatranes is the remarkable mammalian toxicity exhibited by their 1-aryl derivatives (Table 1)[23].

Table 1

LD_{50} values of some of 1-substituted silatranes[$RSi(OCH_2CH_2)_3N$] [i.p., white mice]

R	LD_{50} mg/kg	R	LD_{50} mg/kg
C_6H_5	0.33	$ClCH_2$	2800
p-CH_3-C_6H_4	0.20	CH_2=CH	3000
p-Cl-C_6H_4	1.70	HC C	3000
cyclo-C_6H_{11}	150	C_2H_5O	3000
C_6H_5-CH_2	1115	C_6H_5O	3200
CH_3	3000		

Some of the silatranes are several times more toxic than widely known poisons such as hydrocyanic acid and strychnine. At the same time 1-aryl silatranes are almost harmless for cold-blooded animals, plants, and microorganisms. For example, frog is very resistant to 1-phenyl silatrane; doses of 30-40 mg/kg have no effect.

The toxicity of silatranes varies within extremely wide limits, being determined mainly by the nature of the substituents R. While 1-aryl silatranes are very toxic, 1-alkyl, 1-vinyl, and 1-ethinyl are practically non-toxic.

The high toxicity of 1-arylsilatranes is already used commercially in the U.S.A. Marketing of 1-(p-chlorophenyl)silatrane as a rodenticide began in 1971[24]. It is noted for its high primary toxicity and for its rapid and complete detoxification in the bodies of the poisoned rodents, so that their corpses are harmless for other animals.

However, 1-(chloromethyl)silatrane and 1-(ethoxy)silatrane have been investigated extensively by Voronkov and co-workers. Animal assays showed that both compounds cause complete healing after treatment of wounds. Clinical tests with 1-(chloromethyl)-silatrane ointments, carried out in USSR hospitals, confirm these findings. In addition, ointments containing 1-(chloromethyl)-silatrane and 1-(ethoxy)silatrane lead to an increase of hair growth. Extensive clinical tests with both compounds as possible drugs for the treatment of different types of alopecia have also been carried out in the USSR with positive results.

A large number of organosilicon compounds with high and specific biological activity has been synthesized and investigated pharmacologically and toxicologically in the last years. Research in the field of biological active organosilicon compounds can be

differentiated into three main areas.

(1) Synthesis and biological investigation of silylated derivatives of well known bioactive organic compounds.

Most of the silyl derivatives of drugs, described in literature till now, are trimethylsilyl compounds containing a Si-O, Si-N or Si-C bond between the Si atom and the drug. The silylated drugs are more soluble in non-polar solvent than the parent compounds. This effect has been shown for several silyl derivatives of drugs, such as the local anaesthetic lidocaine[25], the antiseptic thymol[26], the antipyretic and analgesic paracetamol[27]. The trimethylsilyl groups are readily hydrolysed by water, blocking of polar centres of a drug by silylation and its subsequent gradual hydrolysis in the body fluid can offer a simple and interesting route for the development of novel prodrugs[28], which penetrate easily across lipophilic membranes and liberate the parent drug by hydrolysis. In contrast to O- and N-silylated compounds, C-silylated drugs are relatively stable against hydrolytic decomposition. For this reason, they have no importance so far as prodrugs. However, C-silylated drugs can also exhibit remarkable biological activities[29].

(2) Synthesis and biological investigation of organosilicon compounds, having no organic analogues at all, or having organic analogues with unknown biological activity.

Typical examples are silatranes and organosiloxanes. The properties of silatranes have already been mentioned.

Organopolysiloxanes(silicones) are very important materials for therapeutic applications because of their good physicochemical properties and their inertness to biochemical processes. For example

silicones are employed as ointment (for burns), prosthetic materials (eg. replacement of blood vessels), and plastic surgery.

Organopolysiloxanes are also used as ingredients of topical cosmetic formulation, because of their blandness and their capacity to impart water repellency and lubricity to treated surfaces. Compounds, used in this field of application, are mainly low molecular weight organopolysiloxanes.

However, not all the siloxanes are inert to biochemical processes[30]. Of all the siloxanes studies, 2,6-cis-diphenyl-hexamethylcyclotetrasiloxane has the greatest biological activity. Although its toxicity is low (LD_{50} 5000mg/kg), this compounds has potent hormone like activity similar to that of such known estrogens as estradiol benzoate. Administration of this siloxane to rats, mice, rabbits, rhesus monkeys and dogs results in marked changes in the genital organs. This action is species-dependent, guinea pigs do not show the same response. The unique hormonal activity of siloxanes is limited to methyl-aryl substituted, linear di- and trisiloxanes and cyclic tri- and tetrasiloxanes. In general, at least one aryl silicon group is necessary for activity. The substitution of an alkyl group for a phenyl group either decreases the activity markedly or eliminated it entirely. Cyclosiloxanes are more active than linear siloxanes. The biological activity is correlated with high stereospecificity; 2,6-cis-diphenyl-hexamethyl-cyclotetrasiloxane being approximately 100 times more potent than the 2,6-trans-isomer. Clinical trials in a Swedish hospital on patients with poorly differentiated prostatic carcinoma with 2,6-cis-diphenyl-hexamethyl-cyclotetrasiloxane(Cisobitan®), yielded promising results[31].

(3) Synthesis and biological investigation of organosilicon compounds, having analogous sila-substituted structures of organic compounds with well known bioactivity(sila-pharmaca).

The synthesis of pharmacological active compounds, mostly drugs, in which a characteristic carbon atom was replaced by a silicon, had to follow different routes of preparation. In practice all cases investigated showed that sila analogues exhibited bioactive effects. Mostly the differences between the C/Si pair were negligible. In several cases the sila-pharmaca turned out to be superior; in lower grade of toxicity, in the possibility of "self-destruction" by surrounding water, in positive inotropic effect on the cardiac muscles. Two examples of sila-pharmaca activity are given:

(a) Drugs of the general formula $RC_6H_5COH(CH_2)_n-R'$ have been used for antihistaminic, antiemetic, spasmolytic effects and even against Parkinson disease. As SiOH groups exhibit a far higher acidity than C-OH groups the corresponding sila analogues were synthesized and investigated[32,33]. Preliminary pharmacological tests showed the sila derivatives exhibited strong pharmacological activity. In most cases their effects surpassed those of the carbon parent compounds by factors of 2-10.

(b) Derivatives of benzhydrylether are outstanding for their wide spectrum of pharmacological activities. The sila analogues show nearly the same spasmolytic activity, but are hydrolysed in physiological solutions(Tyrode's) within 15-30 minutes. In contrast certain carbon parent compounds are still active after two days[34,35].

1.6. PHYSIOLOGICAL SIGNIFICANCE OF SILICON COMPOUNDS IN ANIMALS

In 1967, in an extensive review[18] of the biological properties of silicon compounds, it was stated that "although traces of silica are found in all animal tissues, there is no evidence that there is any biological need for silicon in the higher animals". However, subsequently it has been reported that silicon is essential for growth and general development[36a,b] of higher organisms. Using all plastic isolators that excluded the element from the environment, growth of rats is reduced by 30-35% when silicon deficient amino-acid diet are fed, and bone deformations develop[37,38]. Dietary supplements of silicate prevent these symptoms. Similar findings have been reported for chicks[39,40]. Silicon, moreover, is necessary in rats for normal pigment formation in the enamel of incisors[41].

In vitro studies based upon electron microprobe analysis had shown the unique localization of silicon in active calcification sites in young bone[42]. In the earliest stage of calcification in these sites, when the calcium content of the preosseous tissue is very low, there is a direct relationship between silicon and calcium. As a result of these findings it has been suggested that silicon is associated with the calcium in an early stage of bone formation[43]. Further in vivo experiments with rats have also shown a relationship between silicon and calcium in bone formation[44]. It was demonstrated that dietary silicon increases the rate of mineralization; this was particularly apparent on a low calcium diet. A relationship also has been established between Si, Mg, and F in the formation of bone in the chick[45].

Dietary deficiency studies have also produced evidence of

silicon role in connective tissue metabolism. Skeletal and other abnormalities involving glycosaminoglycans in formation of the cartilage matrix and connective tissue were found to be associated with silicon deficiency in the chick[46,47]. Chicks in the silicon-deficient groups have thinner legs and smaller combs in proportion to their size. A role for silicon in glycosaminoglycan metabolism is further supported by the finding that silicon is a component of animal glycosaminoglycans and their protein complexes[48]. Similar results on isolated glycosaminoglycans which included some research reference standards, have also been reported[49]. Silicon has also been reported[50] to be a bound component of collagen as a result of analyses of a variety of collagens obtained from different sources.

Silicon which bound to glycosaminoglycans, polyuronides, pectin and aliginic acid[49], was not dialyzable, did not react with ammonium molybdate, was not liberated by autoclaving or 8M urea, and was stable against weak alkali and acid. Strong alkali and acid hydrolysed the Si-polysaccharide bond. Enzymatic hydrolysis of hyaluronic acid or pectin did not liberate silicic acid, but lead to products of low molecular weight still containing silicon in bound form. It was concluded that Si was present as a silanolate i.e. an ether (or ester like) derivative of silicic acid, and that R_1-O-Si-O-R_2 or R_1-O-Si-O-Si-O-R_2 bridges might play a role in the structural organisation of glycosaminoglycans and polyuronides.

1.7. AIM OF PRESENT WORK

There is currently interest in the presence and possible function of silicon in living organisms, it has been reasonably well established that the element is structurally bound in biopolymers

such as pectin and mucopolysaccharide.

A major problem in studying the biological role of silicon is that virtually nothing is known of exactly how silicon is bound within plant and animal polymers. The rather sketchy evidence which existed suggested that in nature the most common and desirable bond of silicon is with carbohydrate. It was our aim to carry out studies to determine the structure of these silicon carbohydrate derivatives.

Two modes of approach were used:

(a) The major approach: Attempts were made to synthesize compound in which Si is bound to four carbohydrate molecules.

(b) The minor approach: Methods were examined for the isolation of silicon derivative from biological polymer systems.

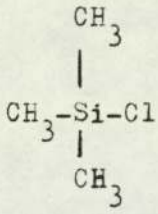
CHAPTER TWO

SILYLATION OF ORGANIC COMPOUNDS

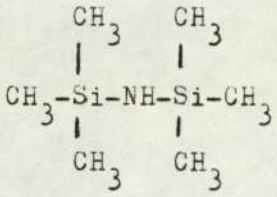
SILYLATION OF ORGANIC COMPOUNDS

2.1. INTRODUCTION

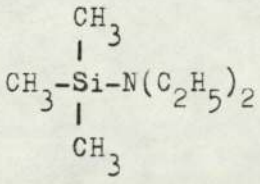
Silylation[51] is the introduction of the silyl group into a molecule, usually by substitution for active hydrogen, occasionally in replacement of the metal compound of a salt. The silyl group referred to is trimethylsilyl(Me_3Si-). The reagents that have been used for this purpose are derivatives of trimethylsilane(Me_3SiH). The common ones are as follow:



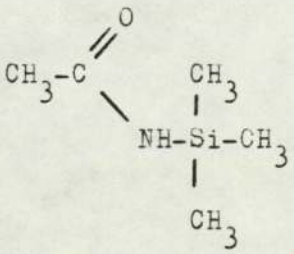
Trimethylchlorosilane(TMCS)



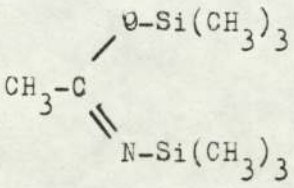
Hexamethyldisilazane(HMDS)



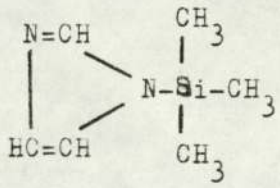
N-Trimethylsilyldiethylamine (TMSDEA)



N-Trimethylsilylacetamide



N,O-Bis(trimethylsilyl)acetamide (BSA)



N-Trimethylsilylimidazole (TSIM)

The replacement of active hydrogen by the silyl group reduces the polarity of the compound and decreases the possibilities of hydrogen bonding. Consequently, where there is marked intermolecular hydrogen bonding in the parent compound the silylated derivative is usually more volatile. Further, stability is enhanced upon silylation by reduction in a number of reactive sites with active hydrogen.

The advantages of volatility and stability imparted by silylation make the process a natural tool for gas-phase purification and analysis. The derivatives are simple and conveniently prepared. In many cases the reactants are mixed at room temperature and the reaction is complete in a few minutes. The silylated products generally can be distilled, and the silyl compounds can be readily hydrolysed to recover the original substance. The only disadvantage of silylation is the need for dry conditions and the high sensitivity of some of the products to moisture.

2.1.1. GENERAL METHODS FOR SILYLATION

(1) Trimethylchlorosilane(TMCS) based procedures

(a) TMCS alone (without base)

This method has been used with alcohols, phenols and a few acids. TMCS is refluxed many hours with the compounds[52].

$$ROH + Me_3SiCl \longrightarrow ROSiMe_3 + HCl$$

(b) with ammonia

Ammonia gas is passed rapidly into a cold stirred mixture of TMCS and substrate until an excess persists. With NH_3 (or any other acid acceptor) the reaction is rapid and practically

complete when run cold.

$$ROH + Me_3SiCl + NH_3 \longrightarrow ROSiMe_3 + NH_4Cl$$

(c) with bases

The use of a base other than NH_3 as acid acceptor with TMCS as silyl donor is common in preparative work. Pyridine and triethylamine are frequently used bases. TMCS is added slowly to a stirred solution of a hydroxy or amino compound in at least one mole of the base intended as acid acceptor. The reaction is rapid and often complete when heat evolution stops. Sometimes heat is then applied to complete the reaction[53]. The charge is filtered from the base hydrochloride and distilled.

(d) with salts

Metal salts of thiols, alcohols, carboxylic acids and amines yield silyl derivative with TMCS. This method is useful for thiols.

$$C_3H_7SNa + Me_3SiCl \longrightarrow C_3H_7SSiMe_3 + NaCl$$

(e) with formamide in pyridine and hexane[54]

The pyridine accepts the HCl as usual from the TMCS reaction, then catalyses its consumption by formamide.

$$C_5H_5N\cdot HCl + HCONH_2 \longrightarrow C_5H_5N + NH_4Cl + CO$$

The final reaction charge is in two phases with the product cleanly in the hexane layer.

(2) Hexamethyldisilazane(HMDS) based procedures

(a) HMDS alone, elimination of ammonia

Alcohols, phenols, amines and acids may be silylated by refluxing with excess HMDS to constant charge temperature, cessation of

NH_3 evolution or solution of starting material. The reaction has been particularly useful with aliphatic alcohols and phenols. This reaction is catalysed by acid and hindered by alkali[52].

$$2ROH + Me_3SiNHSiMe_3 \longrightarrow 2ROSiMe_3 + NH_3$$

(b) HMDS with acid catalyst

The addition of a small amount of acidic catalyst usually increases the rate or degree of silylation by HMDS. TMCS is most commonly used. The most used method of silylation was developed by Sweeley[55] who employs an optimum HMDS/TMCS ratio of 2:1, pyridine was the solvent.

$$3ROH + Me_3SiNHSiMe_3 + Me_3SiCl \longrightarrow 3ROSiMe_3 + NH_4Cl$$

Clearly, TMCS is not a true catalyst for HMDS as:

i. the effect is proportional to the amount taken

ii. it is an effective silyl donor alone

iii. it is at least partially consumed; NH_4Cl formed

Further proof of the consumption of TMCS lies in the results of mixed silylation reported by VandenHeuvel[56a] and Supina [56b].

(3) Silylamine methods

Silylamines have been used for the silylation of alcohols, amines, amino acids, amides and ureas.

$$ROH + R'NHSiMe_3 \longrightarrow ROSiMe_3 + R'NH_2$$

the reaction is run hot and completed by expulsion of the amine $R'NH_2$.

(a) N-trimethylsilyldiethylamine(TMSDEA)

This reagent has been the most used of the silyamines. It has been extensively applied to amino acids[57,58]. All functional groups - amino, carboxylic, hydroxyl, and sulphydryl are silylated. When a moderate excess of TMSDEA is heated with the substrate, diethylamine distils. The amount distilled indicates the progress of the reaction. As with HMDS, yields with TMSDEA are improved by the addition of an acid catalyst[59].

(b) N-trimethylsilylimidazole(TSIM)

This reagent has only recently been used for the silylation of hydroxyl group[60]. This reagent is relatively tolerant to moisture. In combination with HMDS, this reagent has been found particularly effective in trimethylsilylating syrups containing a high proportion of D-fructose[61]. Although there are several instances in which TSIM has been used alone[62,63,64] it has been reported to cause ghost peaks on polyester columns; this was attributed to the cleavage of some polyester linkages under the basic influence of the imidazole[65].

(4) Silylamide methods

(a) N-trimethylacetamide

This was first synthesized from trimethylsilyl-t-butylamine and acetamide in 1959[66]. This silylacetamide has been used in the molten state in the silylation of sugars[67]. The acetamide formed was removed by filtration and the excess reagent and product were distilled.

(b) N,O-bis(trimethylsilyl)acetamide[BSA]

The amides are among the most powerful silyl donors[68,69]. BSA

was first described by Birkofer, Ritter and Giessler[70] and its structure have been well established[71]. BSA has been used extensively to silylate amides, amino acids and even sterically hindered phenols, carboxylic acids and enols. The power of BSA as a silyl donor is increased by the addition of TMCS[72]. BSA is a more satisfactory silylating agent than the combination HMDA/TMCS since it is less destructive to sensitive compounds. BSA has been found to silylate L-ascorbic acid completely[73], and to react with tertiary hydroxy groups in steroids[68,74]. In the silylation of amino sugars, BSA was not sufficiently effective even at 70°, but a mixture of HMDS, TMCS and BSA gave complete N- and O-trimethylsilylation in 30 minutes at 20°. In contrast a mixture of HMDS and TMCS gave only O-trimethylsilylation. A wide variety of acidic compounds has been silylated, and the preferred procedures is to treat the sodium, calcium, or barium salt, as a suspension in pyridine with BSA and TMCS[75,76], this gives the trimethylsilylester of the O-trimethylsilyl derivative.

2.1.2. APPLICATION OF SILYLATION TO ORGANIC COMPOUNDS

The earliest example of silylation was that of Sauer[77], who silylated methanol and ethanol with TMCS and pyridine to yield trimethylmethoxysilane and trimethylethoxysilane respectively.

The general order of silylation of proton-active acceptor is: alcohol ($1^{\circ} > 2^{\circ} > 3^{\circ}$) > phenol > carboxylic acid > amine ($1^{\circ} > 2^{\circ}$) > amide. Steric considerations are probably responsible for the observed order of reactivity of the alcohol. The tertiary alcohol group is not even silylated by HMDS alone but only in boiling DMF/DMSO the reaction proceeds[78]. Higher charge temperature with the high boiling solvents may be a factor here.

The first silylated sugar was reported in 1956[54,79] when pentasilylglucose was obtained. Langer, Pantages and Wender[80] had observed the advantages of silyl ethers for glc.Silylated sugars are usually more volatile than the parent compounds and can be analysed by glc. It was the methyl ethers of carbohydrates which were studied by glc in 1958[81]. The separation of fully methylated methyl glucopyranoside was described. Fully methylated sucrose was also shown to be sufficiently volatile for application of glc[82]. In contrast to the ease with which methyl group is placed in a carbohydrate, the removal of methyl groups is difficult. In fact the reaction conditions required for the removal of methyl group may often destroy the mother compound. Therefore the original material is difficult to be recovered. The silyl group is much more readily removed, this gives it an advantage over the methyl group as protective function.

Early attempts at separation of silylated sugar derivatives by glc were made by Hedgley and Overend[83]. A few years later, a simple technique for derivatization, by HMDS and TMCS in pyridine, and glc was developed and applied successfully to over a hundred sugar compounds by Sweeley and co-workers[56].

Further improvement in the preparation of trimethylether of carbohydrates and in their separation by glc have been reported[84a], and the method has been extended to oligosaccharides up to the tetramers and to complex glycoside[84b].

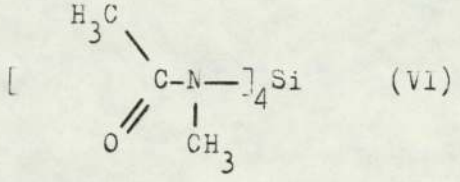
The completion of the silylation can be shown by obtaining on glc a single peak from a pure anomer, or two peaks from a known mixture of two components. If not all hydroxyl groups are silylated, many peaks will result from partial silylation products.

Trimethylsilylation is adversely affected by moisture, and therefore, hydrolyzates should be evaporated to dryness as completely as possible. However, if silylation is catalysed by trifluoroacetic acid, a moderate proportion of water may be tolerated[85,86] but, extra peaks may be obtained from partly silylated derivatives[87].

2.2. THE REACTIONS OF METHYL α-D-GLUCOPYRANOSIDE WITH THE SILYLATING AGENTS

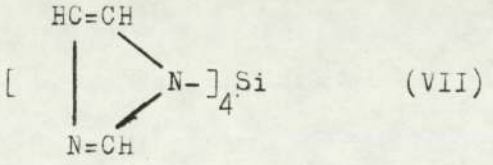
Because of the success of BSA and TSIM as silylating agents, our original approach to the problem of synthesising compounds of the type - $Si(CH_y)_4$ [whereas CH_y is a monosaccharide molecule], was to prepare analogues of these compounds which might be used for this purpose.

Routes to the preparation of tetra(N-methylacetylamino)silane(VI)



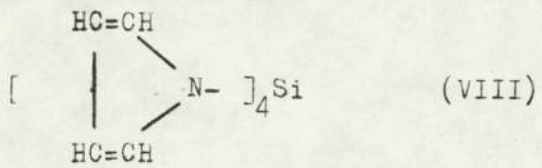
(VI)

and tetra(1-iminazolyl)silane(VII) were investigated.



(VII)

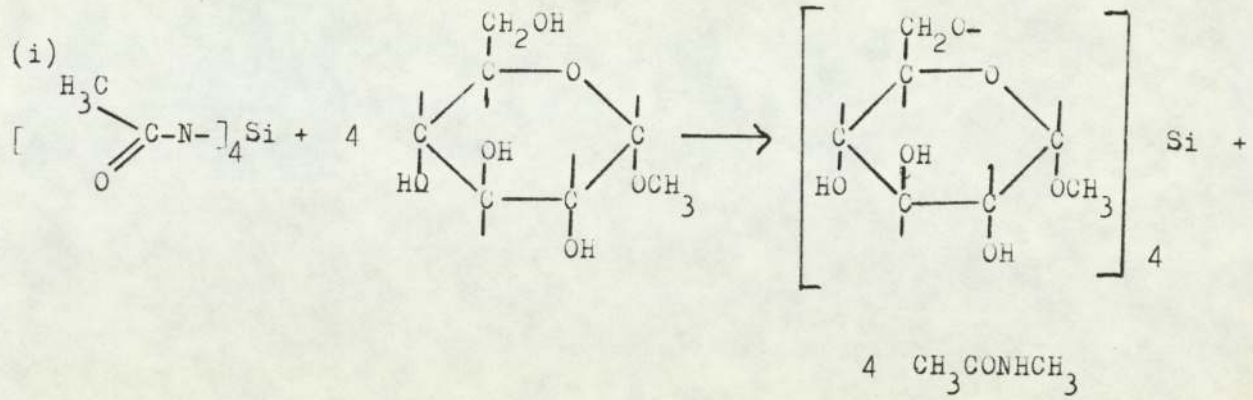
The pyrryl analogue of (VII), tetra(1-pyrryl)silane(VIII) which was previously prepared by Reynolds[88] was also synthesised



(VIII)

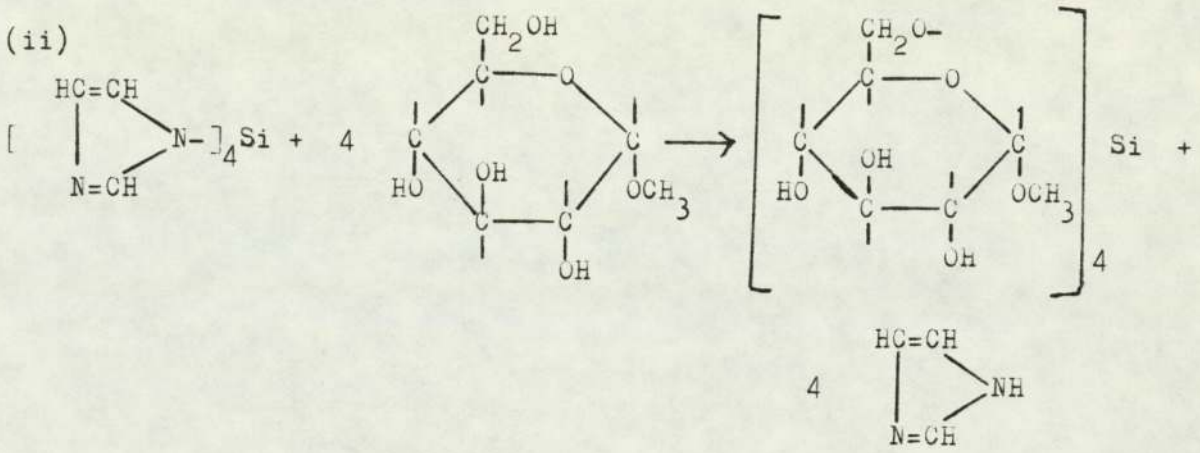
As the primary OH group of carbohydrates is more reactive than the secondary OH group(Chapter 3), we hoped that these three compounds might react with methyl ∝-D-glucopyranoside(IX) as follows:

(i)

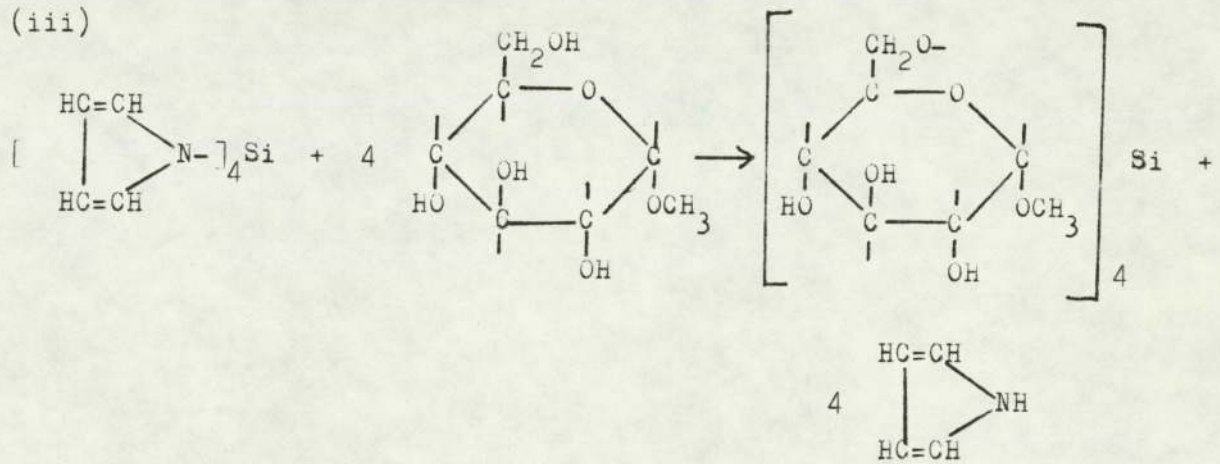


4 $CH_3CONHCH_3$

(ii)



(iii)



(1) Preparation of tetra(N-methylacetylamino)silane(VI)

The compound(VI) was prepared by reaction of $SiCl_4$ with N-methylacetamide in the presence of triethylamine to pick up the hydrogen chloride which was produced and prevent the back reaction.

$$SiCl_4 + 4CH_3CONHCH_3 \xleftrightarrow{Et_3N} (CH_3CONCH_3)_4Si + Et_3NHCl$$

(VI)

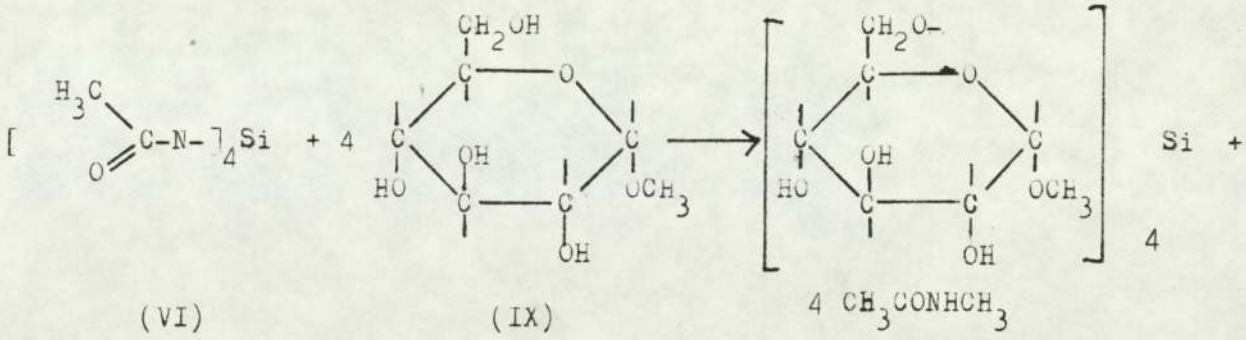
A product was obtained which gave relatively satisfactory IR and NMR analyses for the required material. The IR did indicate some -NH bands suggesting that the product was contaminated with starting material or triethylamine hydrochloride. The NMR of N-methylacetamide showed one doublet at τ7.15-7.2 and a singlet at τ7.9 of equal intensity. The doublet is assigned to $N-CH_3$ and arises from coupling with the N-H. The singlet is assigned to the CH_3 of the acetyl group. Two singlets were expected from the expected product since replacement of the N-H by silicon splitting of the $N-CH_3$ should not be observed. The NMR spectrum of the reaction product showed one singlet at τ7.15 as would be expected for the $N-CH_3$, but the CH_3 signal in the acetyl group has shifted upfield to τ 8.3 which is unexpected and difficult to explain.

When this reaction product was reacted with anhydrous methanol, tetramethoxysilane and N-methylacetamide were obtained. These were the expected products from the reaction of tetra(N-methylacetylamino)silane with methanol.

$$(CH_3CONCH_3)_4Si + 4\ CH_3OH \longrightarrow Si(OCH_3)_4 + 4\ CH_3CONHCH_3$$

From all the evidence obtained it was concluded that tetra(N-methylacetylamino)silane was synthesised, but the product was not pure, and because of its instability no attempts were made to purify it.

(2) Reaction of tetra(N-methylacetylamino)silane(VI) with methyl ∝-D-glucopyranoside(IX) in 1:4 molar ratio.



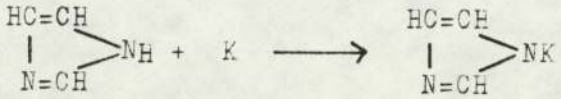
(VI) (IX) 4 $CH_3CONHCH_3$

When the reaction mixture was added to ether, an oil separated out, after stirring for some time the oil solidified. TLC analysis showed two spots one strong and one weak, the strong spot had the same R_f value as methyl ∝-D-glucopyranoside(IX) and the weak spot corresponded to triethylamine hydrochloride. The IR spectrum was very similar to that of methyl ∝-D-glucopyranoside. There was an extra band at 1605cm^{-1} in the product from the DMF reaction, this disappeared on chromatographing the material on silica gel column. In the products from both the DMF and pyridine reactions the band at 1050cm^{-1} was much stronger than in the starting material, this could be due to the Si-O bond. The products were analysed for Si, however the silicon content varied from batch to batch of material. On hydrolysis of the reaction product a gelatine liked product was obtained probably a silicate. Although our results indicate that silicon was present in our product, the spectral and TLC results together with the inconsistency of the silicon analysis suggests not the formation of a silicon carbohydrate compound but that the isolated material was methyl ∝-D-glucopyranoside contaminated with a siloxane. An alternative explanation is that a product was formed but was so unstable that it hydrolysed on chromatographing. However

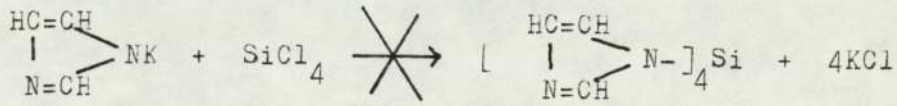
since a definite purifiable product could not be obtained no further work was done on the reaction.

(3) Preparation of tetra(1-iminazoiyl)silane

The initial preparation of 1-potassium imidazole was successful as all the potassium was consumed.



However, no reaction was occurred when silicon tetrachloride was added to the 1-potassium imidazole, unlike the reaction between 1-potassium pyrrole and silicon tetrachloride which is highly exothermic. It was concluded that 1-potassium imidazole do not react with $SiCl_4$ under these conditions.



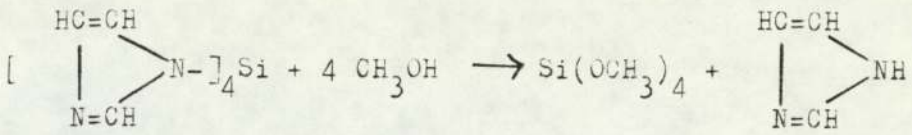
(4) Preparation of tetra(1-pyrryl)silane

Two methods of preparation were attempted.

(a) The initial preparation of 1-potassium pyrrole was not successful as solid potassium was still present when the reaction was stopped. The reaction between 1-potassium pyrrole and $SiCl_4$ was not carried out because potassium reacts with $SiCl_4$ explosively.

(b) A very small quantity of reaction product was obtained by the Grignard reaction. The reaction product had a similar melting point to tetra(1-pyrryl)silane because the yield of the product was so low no IR and NMR spectra were obtained, however when this

product was refluxed in methanol, tetramethoxysilane was obtained

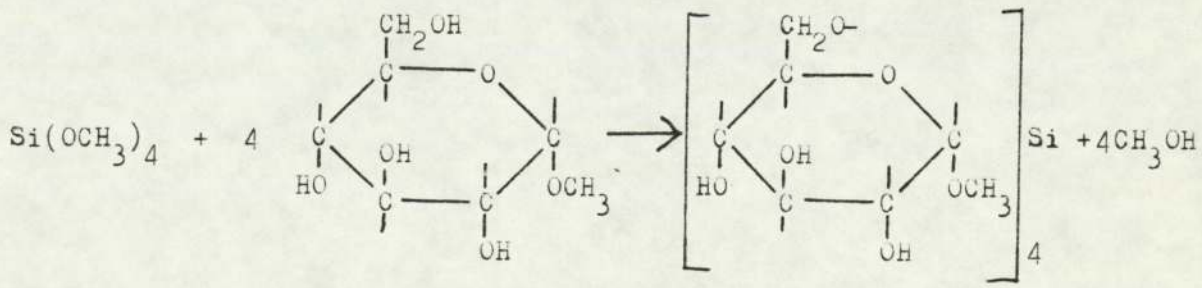


These were the expected products from the reaction of tetra(1-pyrryl)silane with methanol and it was concluded from this result that tetra(1-pyrryl)silane had been successfully synthesized. However, since so much expensive pyrrole was required to give such low yields of product, this procedure was not repeated.

2.3. THE REACTION OF METHYL α-D-GLUCOPYRANOSIDE WITH TETRAMETHOXY SILANE

Tetramethoxysilane was prepared as standard reference for the previous experiments. Since alkoxysilane reacts with alcohol readily, the reaction of tetramethoxysilane with methyl α-D-glucopyranoside was investigated.

It was hoped that the reaction between tetramethoxysilane and methyl α-D-glucopyranoside will proceed according to the equation:



A product was obtained from the reaction. TLC analysis showed one spot which had the same R_f value as methyl α-D-glucopyranoside. The IR and NMR spectra were very similar to the methyl α-D-glucopyranoside. However, one extra IR band at 1650cm^{-1} in the product from the DMF reaction was observed. The spectra did not show any sign of Si-OCH_3 grouping, since the cleavage of every Si-OCH_3 bond will produce one mole of methanol, failure to detect the methanol from the reaction product by glc was difficult to explain.

The silicon content of this reaction product was varied from batch to batch(2.5-4.2%). This product was again readily hydrolysed by water to a gelatinous material probably a silicate. When this product was put through a silica gel column, its silicon content was drastically reduced, and the product obtained after chromatographing was identified as methyl α-D-glucopyranoside by IR and m.p. studies.

The products obtained from this reaction were very similar to those obtained from the reaction between the aminosilanes with glucoside. Therefore no further work was carried out.

CHAPTER THREE

CARBOHYDRATES

CARBOHYDRATES

3.1. INTRODUCTION

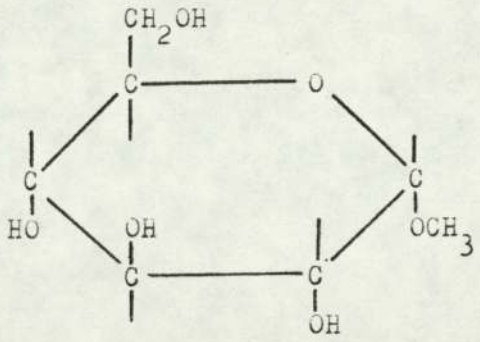
The carbohydrates comprise one of the major groups of naturally occurring organic materials. The structural material of plants is mainly the polysaccharide cellulose and the related hemicellulose, accompanied by smaller proportions of a phenolic polymer(lignin).

In the case of the higher animals, the principal structural material is protein, not carbohydrates, and the animal carbohydrates are found both loose and in combination with proteins as well as with other materials.

As far as is known, the carbohydrate in all living cells are the central source for the supply of energy needed for mechanical work and chemical reactions. Nucleic acids(polymeric carbohydrate derivatives) control the biosynthesis of proteins and the transfer of genetic informations.

The term "carbohydrate" cannot be defined with exactitude, an oversimplified but possibly acceptable definition of carbohydrate is that they are composed of the polyhydroxy aldehydes, ketones, alcohols, acids, their simple derivatives, and their polymers having polymeric linkages of the acetal type.

3.2. RELATIVE REACTIVITIES OF HYDROXY GROUPS IN CARBOHYDRATES(MONO-SACCHARIDES



Methyl ∝-D-glucopyranoside (IX)

It is apparent from the diagram that (IX) contains two kinds of hydroxyl groups, (i) A primary OH group at C-6 and (ii) three secondary OH groups at C-2,3,4. All of these groups are in equatorial positions, therefore their reaction are sterically less hindered than those of axial groups.

The relative reactivities of the primary and secondary OH groups in monosaccharides are briefly discussed below.

3.2.1. ETHERIFICATION

The differentiation of primary and secondary OH groups by the use of the sterically demanding reagent chlorotriphenylmethane (trityl chloride) is well known[89a]. The rates of reaction of trityl chloride with several characteristic compounds are given[89b] in Table II, which also illustrates the effect of the trityl chloride concentration. With an 8-fold excess, the primary OH group of the galactose derivative reacts 220 times as rapidly as the secondary OH of the glucose derivative. However, the difference in reactivity between the primary OH group of the sorbose derivative and the secondary OH group of glucose derivative is only 34 times.

Table II

Rate of reaction of sugar derivatives with trityl chloride

Substance	Excess of trityl chloride	k x 100
1,2:3,4-Di-O-isopropylidene-D-galactpyranose	4-fold	14
	8-fold	36
2,3:4,6-Di-O-isopropylidene-L-sorbofuranose	4-fold	5.2
	8-fold	5.5
1,2:5,6-Di-O-isopropylidene-D-glucofuranose	4-fold	0.12
	8-fold	0.16

Similar differences in reactivity for functions other than hydrogen in the primary and secondary sites are also observed. For example, methyl-2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside can be prepared in good yield by the preferential methanolysis of the trimethylsilyl residue on the C-6 position of the corresponding fully substituted trimethylsilyl derivative of methyl α-D-glucopyranoside[90].

3.2.2. ESTERIFICATION

Compton[91] obtained the 6-tosyl derivatives of methyl α-D- and ß-D-glucopyranoside with tosyl chloride and pyridine. Selective mesylation has also been shown to occur in the same way. One of the most important of the properties of tosyl esters is the ease of cleavage of those derived from primary alcohols. In this regard the formation of an iodo compound by reaction with sodium iodide in acetone has had wide application[92]. Replacement of secondary tosyloxy groups does occur[93,94], however, the conditions necessary to effect reaction were more drastic. 1,2-O-isopropylidene-α-D-glucofuranose reacts with methyl octadecanoate at 180° in the

presence of K_2CO_3 to afford the 6-octadecanoate[95].

The greater steric availability of this necessarily terminal function may be a factor in determining the greater reactivity of the primary OH group over those of the other secondary OH groups as indicated.

3.3 THE RING CLEAVAGE OF ANHYDRO SUGARS

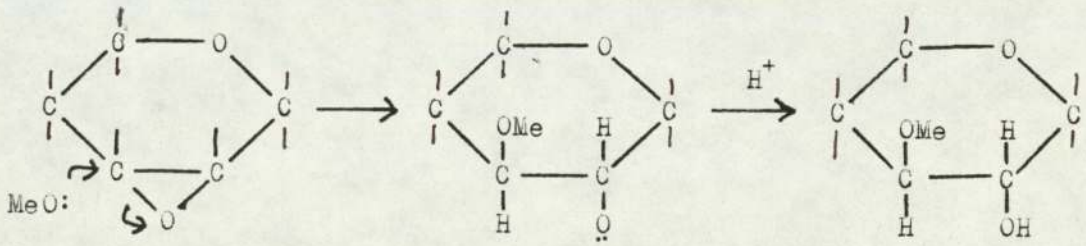
The anhydro sugars and their derivatives are inner ethers formed by the intramolecular elimination of the elements of water from two alcoholic hydroxyl groups with the formation of a heterocyclic anhydro ring. Compounds with the 3-membered(ethylene oxide or epoxy type) and 5-membered(tetramethylene oxide or hydrofuran type) heterocyclic rings are the most common and useful.

3.3.1. THE SCISSION OF ETHYLENE OXIDE TYPE RING

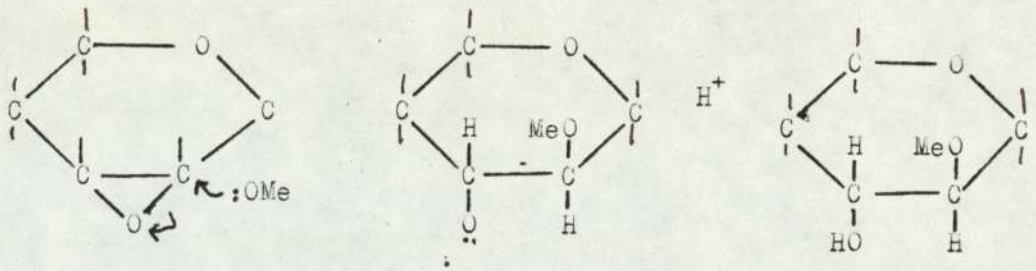
(1) Alkaline systems

The scission of an anhydro ring by alkaline reagents usually sodium methoxide in methanol, may be considered in terms of the exchange of anions on a carbonium cation. The scission, however, is an intermolecular reaction and the displacing anion is methoxyl ion. The exchange may be represented, in generalized form, as follow:

Scheme A



Scheme B

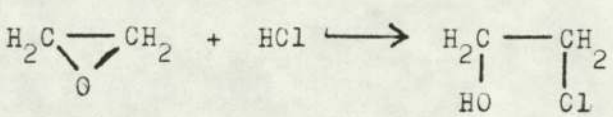


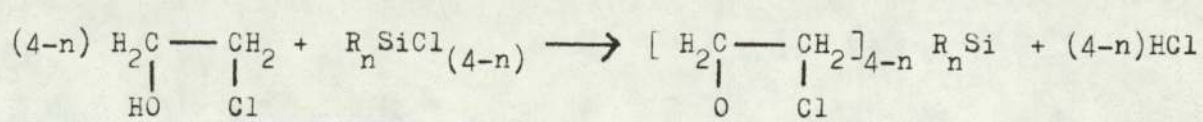
It is obvious that (i) there are two carbon centres at which the anion exchange might occur, (ii) inversion of configuration will occur only on that carbon atom which develops cation reactivity, (iii) the carbon at which inversion occurred is labeled by the displacing anion(OMe^-) becoming attached to it. Two configurationally different sugars are therefore produced by the ring scission of an anhydro sugar and the latter is itself different in configuration from either. The relative proportion of the two sugars formed is dependent upon the configuration on the anhydro sugar as a whole, on the substituent groups in it and upon the nature of the reagent used.

(2) ACID SYSTEMS

The examples of ring cleavage by acid recorded in the literature indicate that the mechanism of the reaction probably does not differ essentially from that of alkaline scission, already mentioned.

Reaction of chlorosilane with ethylene oxide may be due to prelimary addition of HCl(usually present in traces in all silane) followed by reaction of chlorosilane with chlorohydrin so formed[96].





The reactions of (i) 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose, (ii) methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside with Me_3SiCl were to be investigated. Therefore the ring cleavage of these two compounds are briefly mentioned.

(i)5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose

Ring cleavage of this compounds is effected by a wide variety of acid and alkaline reagents, the chief product being in each case a 6-substituted derivative of D-glucofuranose[97]. A few examples are given in Table III.

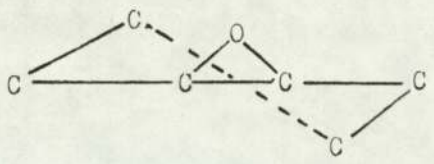
Table III

Ring cleavage of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose

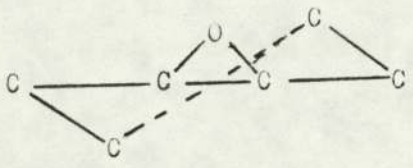
Reagent	product(substituent for OH on C-6 of glucose
Hydrogen sulphide + $Ba(OH)_2$	-SH
Halogen acid + pyridine + Ac_2O	-Cl, Br, I
Carboxylic acid(RCOOH)	-OOCR
Ammonia	$-NH_2$
Sodium alkoxide NaOR	-OR

(ii) Methyl 2,3-anhydro-4,6-O-benzylidene-α-L-allopyranoside

The pyranose ring has been shown to be conformationally very similar to the cyclohexene ring[98], and the 2,3-anhydro-pyranose is likewise analogous to cyclohexene oxide. Cyclo-hexene oxide has been shown[98] to exist in two half-chair conformations A and B:

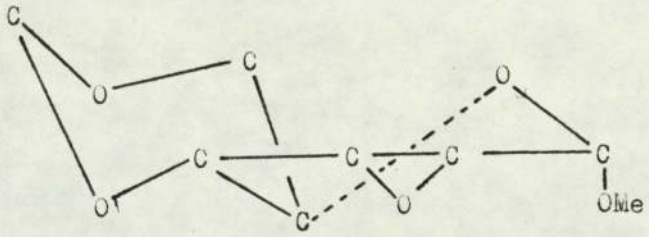


(A)



(B)

Therefore for pyranose, attack on both sides of the epoxide form is possible and two products would be expected. However, because of the fused benzylidene ring, methyl 2,3-anhydro-4,6-benzylidene-α-D-allopyranoside can only exist in one conformation form(C)



(C)

Due to the steric hindrance only one principal product would[99] be expected. Exception to this generalization are opening by acidic reagent in which the acetal blocking group is removed before the epoxide is opened[100].

3.4. CHARACTERISTIC OF SILICON-CHLORINE BOND AND ITS REACTION WITH HYDROXYL GROUP

Due to the great difference between the electronegativity of silicon and chlorine(Pauling's scale: Si=1.8, Cl=3.0) the Si-Cl bond has considerable ionic character. The Si-Cl bond is easily cleaved by means of ionic agents, especially by nucleophile(the Si is usually the one to be attacked), the reaction rates usually increase on going from monohalogenosilane to tetrahalogenosilane,

i.e. with decreasing electron density on silicon atom.

Organohalogenosilanes are easily hydrolysed by water to give silanol(even in neutral condition),

$$SiCl + H_2O \longrightarrow Si\text{-}OH + HCl$$

silanols are generally very prone to condensation of the silanol groups to the siloxane bond,

$$2\ Si\text{-}OH \longrightarrow Si\text{-}O\text{-}Si + H_2O$$

this reaction is catalysed by acid and base.

3.5. PROTECTION OF ALCOHOLIC HYDROXYL GROUPS

Only three classes of protective groups and their ease of introduction, stability under the required reaction conditions, and ease of removal are discussed.

3.5.1. ETHERS

(a) Benzyl ethers

The benzyl group has been used very widely for the protection of hydroxyl functions in sugar chemistry. The most common procedure for the benzylation of alcohols involves heating the substrate, often at an elevated temperature, with an excess of benzyl chloride and powdered KOH[101]. Diluents such as benzene, toluene and dioxan have sometimes been added.

Benzyl ethers are unaffected by alkali; they are also stable enough to acidic hydrolysis to withstand the conditions normally required for the removal of isopropylidene and benzylidene groups, and for methyl glucosides to be converted into the free sugars. Benzyl ethers are also stable to a number of other reagents

including sodium periodate, lead tetraacetate, and lithium aluminium hydride, however, they are readily cleaved in neutral solution at room temperature by catalytic hydrogenolysis, usually in the presence of a palladium catalyst.

(b) Trimethylsilyl ethers

The trimethylsilyl group has been used very widely to protect the hydroxyl functions of carbohydrates, and thereby reduce their polarity. Trimethylsilyl ethers are often distillable, and are especially susceptible to analysis by glc, they may also be analysed by mass spectrometry. An important feature of the trimethylsilyl group is the ease of removal it can be generally removed[51] by heating a solution of the protected intermediate in aqueous alcohol, under reflux. Partial trimethylsilyled sugar derivatives, such as trimethylsilyl 2,3,6-tri-O-trimethylsilyl-α-D-glucopyranoside have been prepared[102] by catalytic hydrogenolysis of the corresponding benzyl ethers.

3.5.2. ESTER OF CARBOXYLIC ACID

(a) Acetates

Acetates have been used more frequently than other esters for the protection of the alcoholic hydroxyl groups of sugar. Acetylation has usually been affected with acetic anhydride in pyridine solution at room temperature, but also with acetic anhydride in conjunction with sodium acetate and certain acidic catalysts(such as $ZnCl_2$, HCl, H_2SO_4 and $HClO_4$). There are reported cases of acetyl migration in sugar chemistry,eg. methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside isomerise to the 2,3,6-triacetate in warm aqueous solution[103].

Acetate esters are most readily solvolysed under basic conditions, but acid-catalysed solvolysis has also been used. The most commonly

used deacetylation procedures are ammonolysis with NH_3/CH_3OH and methoxide ion catalysed methanolysis.

3.5.3. CYCLIC ACETALS AND KETALS

(a) Methylene acetals

Numerous methylene acetals, especially of sugar alcohols have been prepared. Methylene acetals may be cleaved by acidic hydrolysis[104], acetolysis and by treatment with BCl_3. The us of the methylene protecting group is somewhat limited by its comparative resistance to acidic hydrolysis.

(b) Ethylidene acetals

Ethylidene acetals have been prepared by the acid-catalysed reaction between glycols and acetaldehyde, 1,1-dimethoxyethane or paraldehyde. Formation of an ethylidene derivative leads to the of a new chiral centre, and it is thus theoretically possible for a mixture of diastereoisomers to be obtained.

The ethylidene protecting group is stable under a wide range of neutral and basic conditions. Ethylidene protecting groups have been removed both by acidic hydrolysis[105] and acetolysis.

(c) Benzylidene acetals

Benzylidene derivatives have been prepared by reaction between glycols and benzyaldehyde in the presence of acid catalysts. The benzylidene protecting group is stable under most non-acidic conditions. However, it is readily removable by catalytic hydrogenation[106]. The benzylidene group is much more labile to acidic hydrolysis than the methylene group[107].

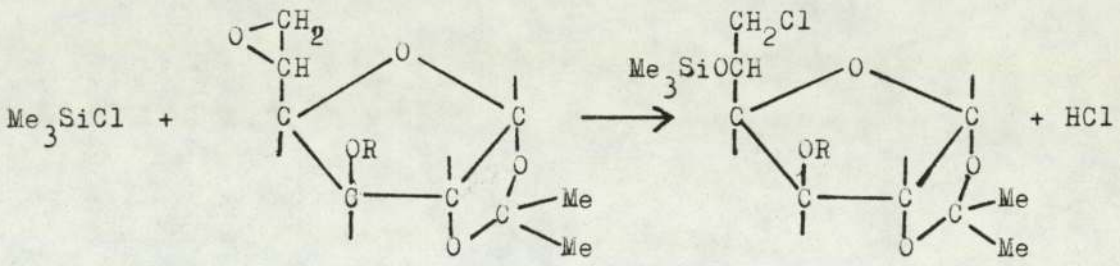
(d) Isopropylidene ketals

Isopropylidene derivatives have been prepared by allowing glycols to react with acetone in the presence of acid catalysts.

The isopropylidene group has one significant advantage over the ethylidene and benzylidene groups is that its use does not lead to the introduction of a new chiral centre, and hence to the possibility of mixture of diastereoisomers. The isopropylidene protecting group owes its wide use to its ease of introduction, its stability in most neutral and basic media, and its lability under comparatively mild conditions of acid hydrolysis.

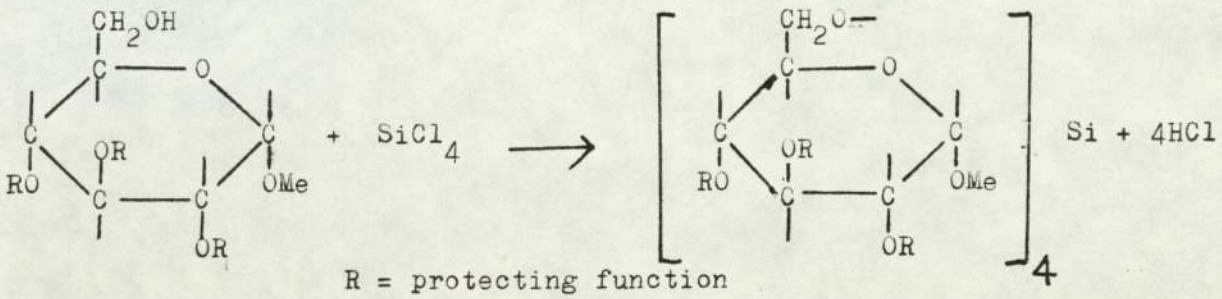
3.6. REACTION OF CARBOHYDRATES WITH Me_3SiCl AND $SiCl_4$

Because of the unsuccessful silylation of the carbohydrates in the previous experiments, two more approaches to the preparation of the compound of the type $Si(CH_y)_4$ [CH_y is a monosaccharide molecule] were attempted. One was involving carbohydrate containing a oxirane ring and having all its OH group being blocked by protecting functions, the ring opening of the oxirane by chlorosilane was then investigated. eg.



R = protecting function

The other method was to block all the OH groups of the monosaccharide with the exception of one OH group with protecting functions. The unprotected OH function was to be used for coupling with chlorosilane. eg.



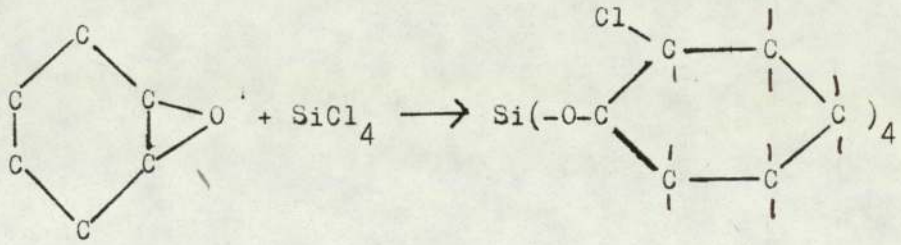
R = protecting function

The reactions of various organohalogenosilane with oxiranes have been well documented[108,109]. A number of these reactions were repeated by ourselves.

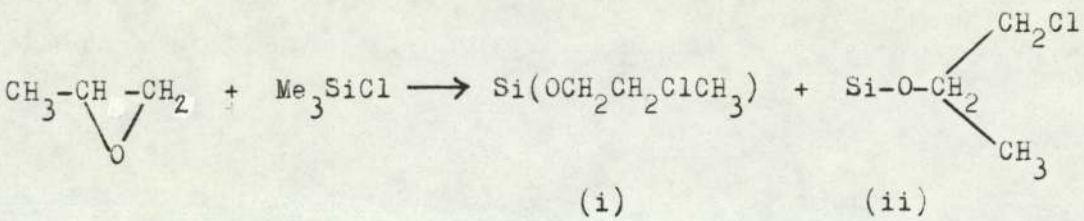
(a) $H_3C-CH(O)CH_2 + Me_3SiCl \longrightarrow Me_3Si(OCH_2CH_2ClCH_3)$

(b) $H_3C-CH(O)CH_2 + SiCl_4 \longrightarrow Si(OCH_2CH_2ClCH_3)_4$

(c)



Our experimental results were in agreement with the previous observation. Although two modes of ring opening are possible for propylene oxide, only one product was obtained (i).



(i) (ii)

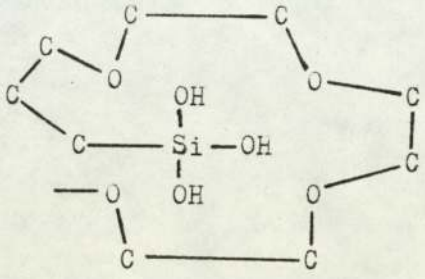
The size and the conformation of cylcohexene oxide is very similar to hexopyranose and since it is possible to attach four cyclohexoxy molecules to one silicon atom, it should be possible to attach four monosaccharide molecules to one silicon atom.

The main difficulty in these two approaches would be the removal of the protective function, the problem was to find protective functions which could be removed under conditions which would leave the Si-O bond intact. The cleavage of Si-O bond is strongly catalysed by acids and alkalis. Base accelerates the reaction much more efficiently than acids do but since it proceeds by a nucleophilic attack of the hydroxyl anion on silicon, the reaction rate is strongly dependent on steric situation on the silicon[110,111] atom and on the electronic effects of its substituents. The acid-catalysed hydrolysis[112] the first step is protonation of the alkoxy oxygen, is substantially less sensitive to the steric and electronic effects[110], therefore, proceeds rather smoothly even in the case of sterically hindered compounds[111].

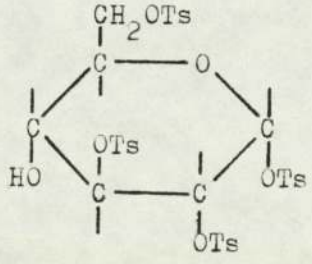
It was hoped that the Si-O bonds would be very sterically hindered in molecules of the type $Si(CH_y)_4$, because of the large groups that they would be more resistant to cleavage and might be stable under conditions which caused cleavage of Si-O bonds in less sterically hindered structures. For example: (i) t-butyldimethyl-silyl-derivatives of the hydroxyl[113] function are more stable to protic conditions and solvents than generally used trimethylsilyl derivatives. Hydrolysis proceeds on the order of 10,000 times more slowly. (ii) Organosilane triols are normally very unstable in solution, but certain triols with general formula

$$CH_3O(CH_2CH_2O)_nCH_2CH_2CH_2Si(OH)_3 \qquad n=1,2,3,6,7$$

have showed to display outstanding stability in aqueous solution. It has been suggested that the stability arises from full shielding of the silanol groups by intramolecular hydrogen bonding with the ether-oxygen atoms. A possible configuration was also suggested[114].



Si-O bond is stable towards catalytic hydrogenation eg. partially trimethylsilylated sugar derivatives such as



Ts = Me_3Si

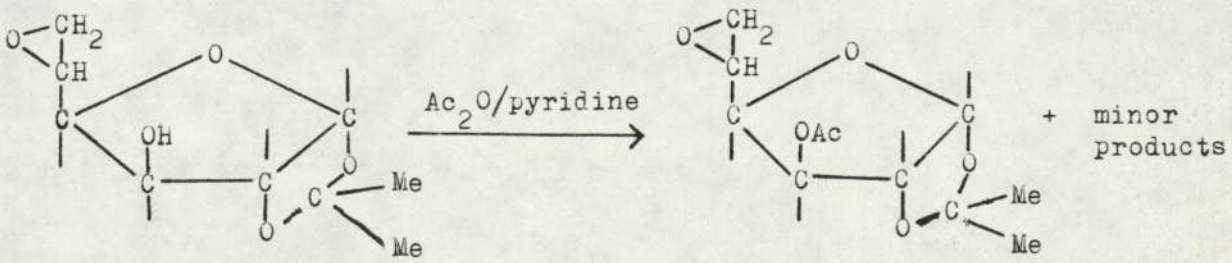
have been prepared[102] by catalytic hydrogenolysis of the corresponding benzyl ethers. Although it has been known that Si-O bond is unstable under basic and acidic conditions, when methyl 2,3,4,6-tetra-O-trimethylsilyl-α-D-glucopyranoside was treated with catalytic amount of K_2CO_3(or AcOH)/MeOH at 0°, only the 6-OH function was selectively unblocked[115]. Therefore protective groups which are susceptible to acid and base, and catalytic hydrogenation were employed and their cleavage under aqueous conditions was investigated even though it was well known that Si-O bond was normally unstable in aqueous media.

3.6.1. REACTION OF ANHYDRO SUGARS WITH Me_3SiCl

(1) Reaction of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose with Me_3SiCl

The cleavage of oxirane ring by Me_3SiCl is not a very common method for the formation of an Si-O bond. The OH groups at C-1,2 of this furanose were protected by isopropylidene group, the OH groups are usually regenerated from such systems by refluxing in dilute acid for several hours. However, the Si-O bond will not be stable under such extreme condition, and this reaction was carried out simply to investigate the opening of oxirane ring by Me_3SiCl.

An attempt was made initially to protect the OH group(C-3) with an acetyl function, this method of acetylation gave very promising results from IR and NMR analyses. However, TLC studies indicated at least four components were present in the reaction product. One of the component(R_f0.71) was assumed to be the expected product - 3-O-acetyl-5,6-anhydro-1,2-O-isopropylidene-α-D-gluco-furanose, as this component gave positive result to NaI test. While other two components R_f0.61 and 0.55 must be the side-products obtained from the opening of the oxirane ring during the acetylation, they did not respnnse to NaI test. The fourth component was identified as unreacted starting material , they both have same R_f value(0.38).

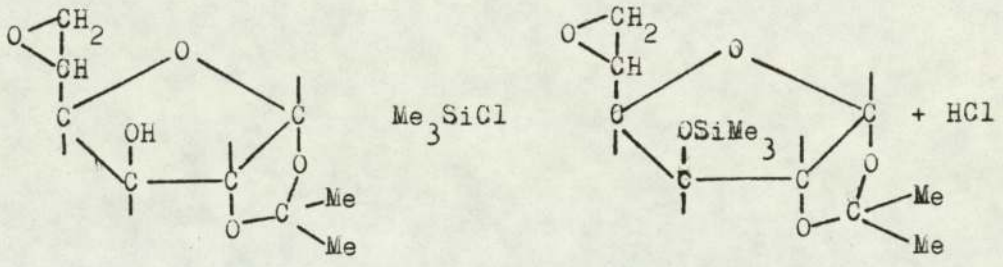


The separation of the components by column chromatography proved to be impossible.

Since the acetylation of the OH(C-3) was not very successful, it was decided to use a trimethylsilyl group as a protecting function. The silylation and the subsequent ring opening were completed in two stages. The possible routes for these reactions are as follows:

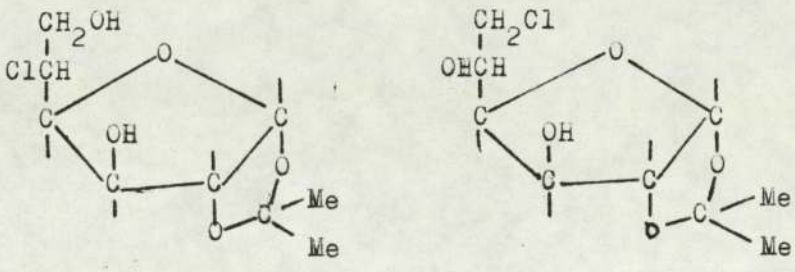
(i) The initial reactions involved the ring opening and the coupling of Me_3SiCl with the OH(C-3).

(a) Me_3SiCl will couple with the OH(C-3) to yield (X)



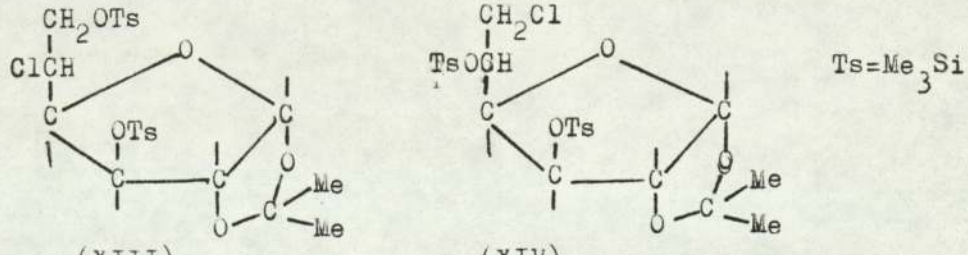
(X)

(b) Traces of HCl are always present in chlorosilane, this HCl could attack the oxirane ring to form chlorohydrins (XI)(XII)



(XI) (XII)

(c) The compounds (XI) and (XII) could react with any Me_3SiCl which might still present to yield (XIII) and (XIV) respectively:



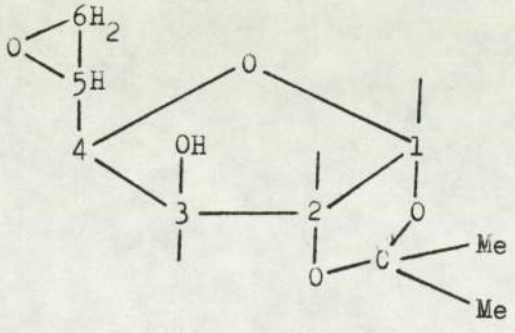
(XIII) (XIV)

(d) Compound(X) could also give (XIII) and (XIV) through the ring opening and then the subsequent silylation of the OH groups.

(ii) As Me_3SiCl group is very susceptible to acid attack and since the last reactions were carried out under acidic condition, there was a strong possibility that the Me_3SiCl group might have been liberated from the reaction products(XIII)(XIV). In order to protect the Me_3SiCl group and also to ensure complete silylation, further silylation by Me_3SiCl in pyridine was carried out.

When the final silylation product was examined by glc only one component was detected, this indicated that either (XIII) or (XIV) was formed from the reaction. The structure of the product was established by NMR($CDCl_3$).

The NMR data of the reaction product is listed in Table IV together with that for 5,6-anhydro-1,2-isopropylidene-α-D-gluco-furanose[116](XV).



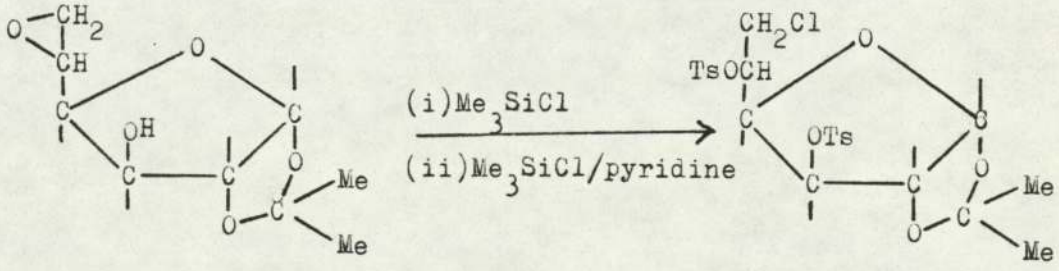
(XV)

Table IV

NMR data of the reaction product obtained from the silylation of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose(XV)

5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose(XV)			Reaction product		
τ	proton assigned	intensity	τ	proton assigned	intensity
4.0-4.1	H_1	2	4.0-4.1	H	2
5.5-5.6	H_2	2	5.5-5.6	H	2
5.7-5.8	H_3	2	5.7	H	2
5.9-6.0	H_4	2	5.8	H	2
6.5-6.7	H_5	2	6.1-6.3	H_5&H_6&$H_{6'}$	6
7.0-7.2	H_6&$H_{6'}$	5			
8.6-8.7	Me_2C	12	8.5-8.7	Me_2C	12
			9.8	Me_3Si	33

It is clearly seen that the chemical shift for the C-6 proton has moved downfield by nearly 1 ppm. This cannot be caused by the presence of Me_3Si group at C-6, this must be due to the presence of the more electronegative Cl atom. Therefore the product must be 6-chloro-6-deoxy-1,2-O-isopropylidene-3,5-di-O-trimethylsilyl-α-D-glucofuranose(XIV).

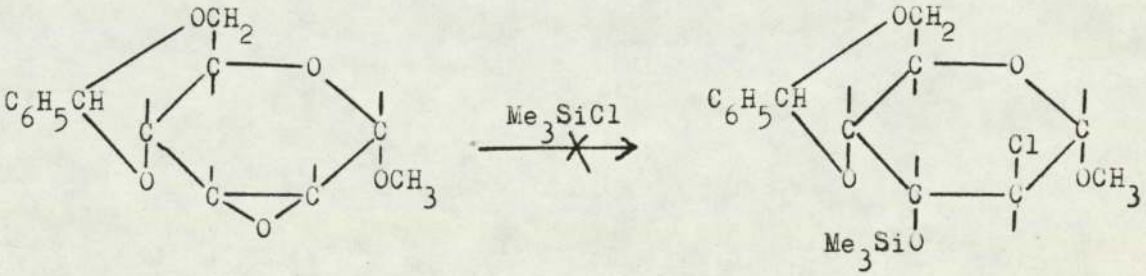


(XV) (XIV) Ts=Me_3Si

This reaction was attempted merely to establish that this type of ring opening represented a suitable reaction for the introduction of a silyl group into a carbohydrate molecule but because of the stability of the isopropylidene group and the difficulty in removing it, this was not considered a suitable system for further investigation and other systems with more labile leaving group were then studied.

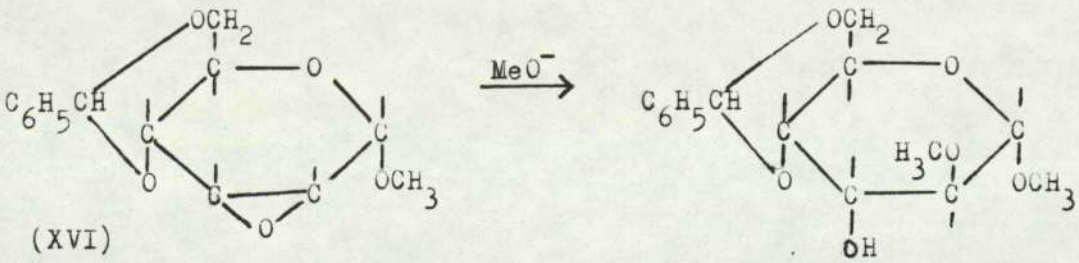
(2) The reaction of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside(XVI) with Me_3SiCl

This benzylidene group is considered to be more labile than isopropylidene group and also it can readily be removed by catalytic hydrogenation[106]. Therefore the reaction of (XVI) with Me_3SiCl was investigated.



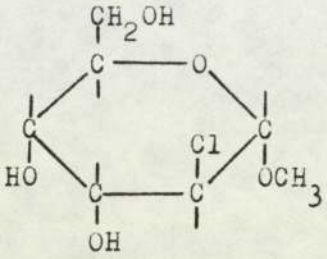
(XVI)

Because of the presence of the fused benzylidene ring, this anhydro alloside(XVI) can only exist in one conformation form[98] and also due to the steric hindrance only one principal product would be expected from the ring opening[99]. For example, when (XVI) was treated in boiling sodium methoxide solution, methyl 4,6-O-benzylidene-2-O-methyl-α-D-altropyranoside was formed.

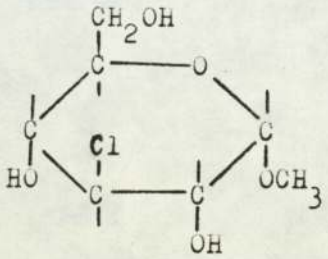


(XVI)

However, if the acetal blocking group is removed before the epoxide is opened, different mode of ring opening will occur.[100] . For example, on treatment of this anhydro alloside(XVI) with HCl(2 mole) give methyl-2-chloro-2-deoxy-α-D-altropyranoside(XVII) and methyl 3-chloro-3-deoxy-α-D-glucopyranoside(XVIII) with the latter predominating.



(XVII)



(XVIII)

After refluxing the anhydro alloside(XVI) with Me_3SiCl in THF for 58 hours, a mixture of products were obtained with the following R_f values(TLC-methanol:benzene/5:95) (i)0.79

(ii)0.71

(iii)0.44

(iv)0

The first compound was identified as the unreacted anhydro alloside(XVI) by having same R_f value. The last component was identified as methyl α-D-glucopyranoside by IR and TLC studies. The detection of this compound after 10 hours of refluxing indicated that some of the benzylidene groups have been removed before the cleavage of the ring and the subsequent ring opening gave (XVIII) which further changed to methyl α-D-glucopyranoside(IX) by neighbouring group effect.

It was very difficult to elucidate the structure of compound (iii)(R_f0,44). This compound appeared to contain a benzylidene and a hydroxyl group from the IR and NMR spectra. The NMR spectrum showed

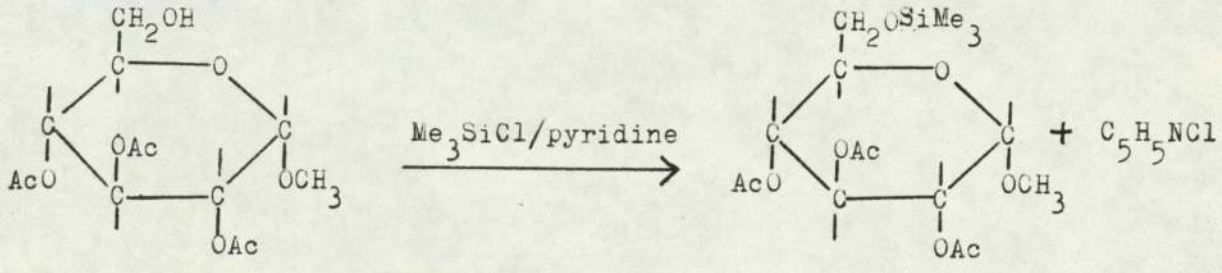
no sign of Me_3Si grouping, furthermore the intensity of the bands at 1250 and 850cm^{-1} in the IR spectrum were too weak to be related to Me_3Si group. The 750cm^{-1} band could be assigned to C-Cl bond. The broad OH band indicated strong hydrogen bonding. Since benzaldehyde was detected during the isolation of this compound(iii) the apparent benzylidene absorption could well arise from traces of benzaldehyde associated with the product. Since it was impossible to isolate the compound(ii)(R_f0.71), no assignment of its structure was possible.

The ring opening of this anhydro sugar did not prove successful for the introduction of a silyl group into a carbohydrate molecule.

3.6.2. THE REACTIONS OF CARBOHYDRATES WITH PROTECTIVE FUNCTIONS WITH Me_3SiCl AND $SiCl_4$

Since the reactions between anhydro sugar and Me_3SiCl did not produced very promising results, another approach was employed. This was to block all the OH groups of the monosaccharide with the exception of one OH group with protective functions. The unprotected OH function was to be used for coupling with chlorosilanes. Five kinds of protective function have been used and the reactions of their carbohydrate derivatives with chlorosilanes are discussed below.

(1) The reactions of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside(XIX) with Me_3SiCl

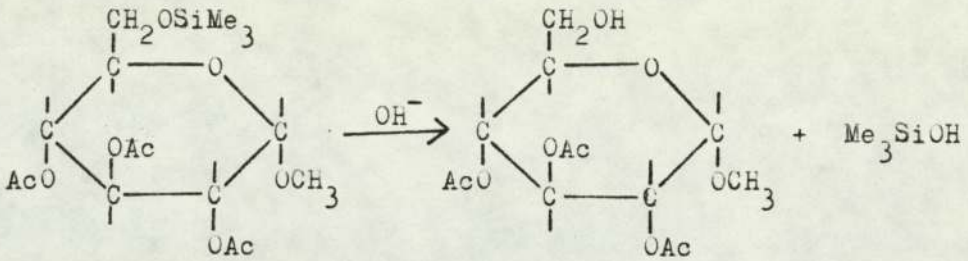


(XIX)

Ac=CH_3COO

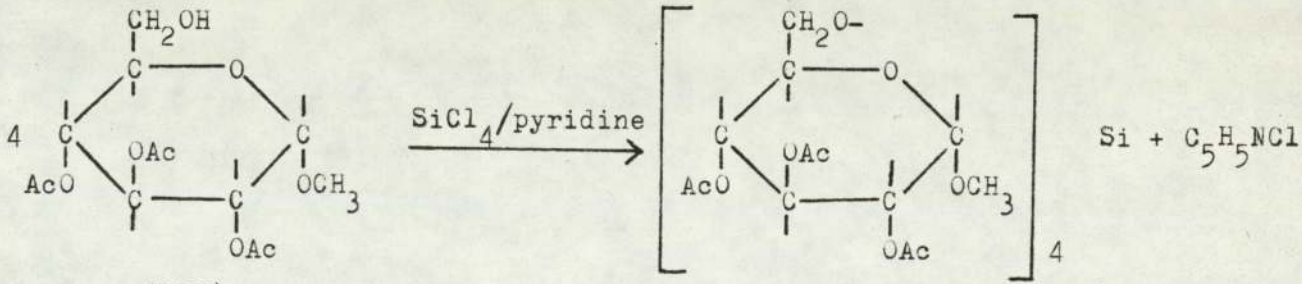
The reaction was carried out in the presence of pyridine, on working up a product was obtained. TLC indicated a single product with R_f 0.74, GLC also detected only one component. NMR spectrum indicated that trimethylsilyl group(τ 9.8) was present and no OH group present. IR spectrum confirmed the absence of OH group and the presence of Me_3SiCl group.

All the analytical results indicated that methyl 2,3,4-tri-O-acetyl-6-O-trimethylsilyl-α-D-glucopyranoside have been prepared. However, this reaction product proved to be extremely unstable in aqueous solution.



(XIX)

(2) The reaction of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside(XIX) with $SiCl_4$



(XIX)

When the reaction product was examined by TLC, three components were detected with the following R_f values(ethyl acetate:ethanol:benzene/ 10:5:40)- (i)0.79 (ii)0.69 (iii)0.61. The third component had the same R_f value as (XIX) and was assumed to be the unreacted starting material. The first component was assumed to be the reaction product. This

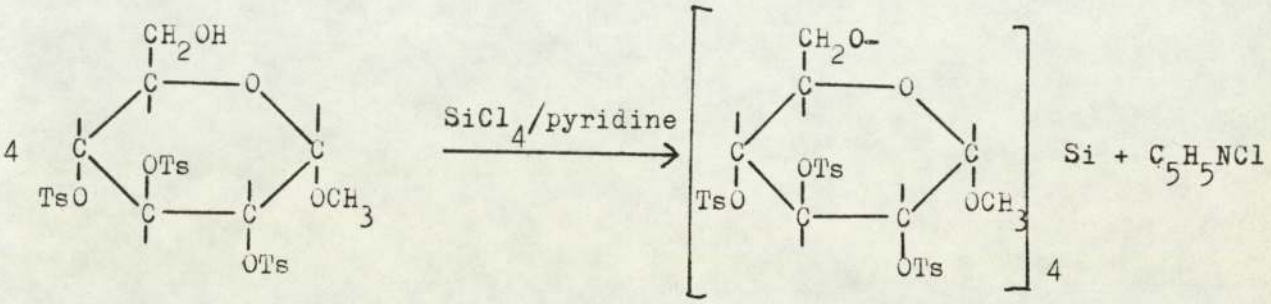
compound was readily hydrolysed in boiling aqueous ethanol which would support our conclusion that this was the silylated compound as the Si-O bond is readily cleaved in hot aqueous alcohol. Unfortunately, it was not possible to separate each individual component by column chromatography. Therefore the structure of the reaction product could not be established. It has been reported[103] that methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside will isomerize to methyl 2,3,6-tri-O-acetyl-α-D-glucopyranoside in hot aqueous solution. When methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside was refluxed in aqueous ethanol, two components were detected by TLC, one corresponded to the starting material(R_f0.61) and the other had R_f0.69 which must be the 2,3,6-triacetyl derivative. Same result was obtained when the reaction products were refluxed in aqueous alcohol, therefore the second compound(0.69) obtained from the silylation must be methyl 2,3,6-tri-O-acetyl-α-D-glucopyranoside.

Enzymatic hydrolysis of the acetyl group

Acetate esters are readily solvolysed under both basic and acidic conditions, unfortunately the same conditions cause cleavage of the Si-O bond. In order to retain the Si-O bond and remove the acetate groups rather more specific methods of cleavage had to be sought. There are a number of enzymes which are known to cleave acetate groups. Two enzymes, acetylesterase and esterase were tried without success. It was thought that the presence of the aglycon group(CH_3 group at C-1) at the reducing end of the carbohydrate could be inhibiting the function of the enzymes, attempts to remove this aglycon group with α-amylase and α-glucosidase were also unsuccessful.

(3) The reaction of methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside(XX) with $SiCl_4$

Because of the unsuccessful attempts to isolate the reaction product and the removal of the acetyl group, it was decided to use trimethylsilyl group as protective function and the reaction between the trimethylsilylated glucoside with $SiCl_4$ was investigated.



(XX) Ts=Me_3Si

When the reaction product was examined by TLC two components were detected, comparison with an authentic sample indicated that one of the component was the unreacted starting material (R_f0.52) while the other(R_f0.93) was assumed to be the reaction product. This reaction product was separated from the mixture by column chromatography. Although good elemental analysis results were obtained for the product no conclusion could be drawn from them since the calculated elementals analysis for the reaction product [assumed to be tetrakis-(methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranosyl-6-O-)silane] and the starting material methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside were very close to each other. However, the melting point of the reaction product(73-74°) and the starting material(98-100°) was quite different.

The IR spectrum of the product did not show any sign of OH absoption band. However, the NMR spectrum showed a small peak (τ8.5)

which was similar to that of the starting material. The original ^{29}Si NMR spectra of the reaction product and that of the starting material were very similar. But a new peak was observed in the product after 24 hours in $CDCl_3$ which suggesting that the product was undergoing decomposition in the $CDCl_3$, while the starting material was stable under the same condition. This new peak(16.8ppm) probably arose from the decomposition of the reaction product through the cleavage of the Si-O bond at C-6. One would assume that $CDCl_3$ contained DCl and would be responsible for the decomposition of the reaction product in the following manner:

$$-\overset{|}{\underset{|}{Si}}-\overset{\oplus}{}\overset{\ominus}{O}- + D^{\oplus}CCl_3^{\ominus} \longrightarrow -\overset{|}{\underset{|}{Si}}-CCl_3 + DO-$$

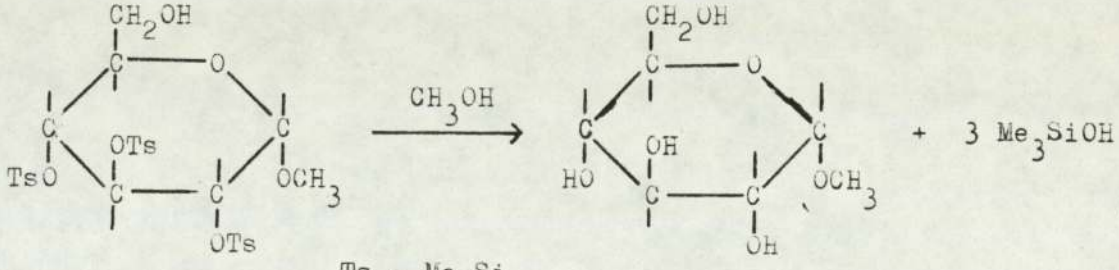
If this was the case it would be expected that the original reaction product should give one more Si signal than the starting material but this was not observed. One possible explanation is that since the central silicon(C-6) is attached to four carbohydrate molecules which could have shielded this silicon atom from the applied radio frequency resulting in no NMR(^{29}Si) signal. And its presence would only be detected when it was liberated from the carbohydrate molecules. Therefore the ^{29}Si NMR indicated the probability of the presence of a silicon at C-6 in the reaction product.

Although the NMR showed the presence of a OH group, the IR did not show any sign of OH absorption and the TLC also showed only one component was present in the reaction product, the NMR result was not conclusive. The IR and TLC and probably the ^{29}Si NMR and the elemental analytical results all favoured tetrakis-(methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranosyl-2-O-)silane as the product.

Since our aim was to prepare a compound in which one silicon was attached to four carbohydrate molecules through four Si-O bonds, in order to study its ^{29}Si NMR spectroscopic data it was necessary to have a reference compound and $(CH_3CH_2O)_4Si$ was chosen. The chemical shift of tetraethoxysilane(^{29}Si) was -6.4ppm which indicated that this silicon atom is more positive than the silicon in tetramethylsilane (TMS). Unfortunately our reaction product did not give any silicon signal other than the Me_3Si grouping therefore no comparison could have been made with tetraethoxysilane.

Methanolysis of the trimethylsilyl grouping

Attempts were made to remove the trimethylsilyl grouping by alcoholysis. The effect of boiling methanol on methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside was first examined. The hydrolysis was completed within 2 hours. When the reaction solution was analysed by GLC no hexamethyldisiloxane(retention distance 17mm) was detected but instead one peak(retention distance 10mm) was observed. This peak could arise from trimethylsilanol.

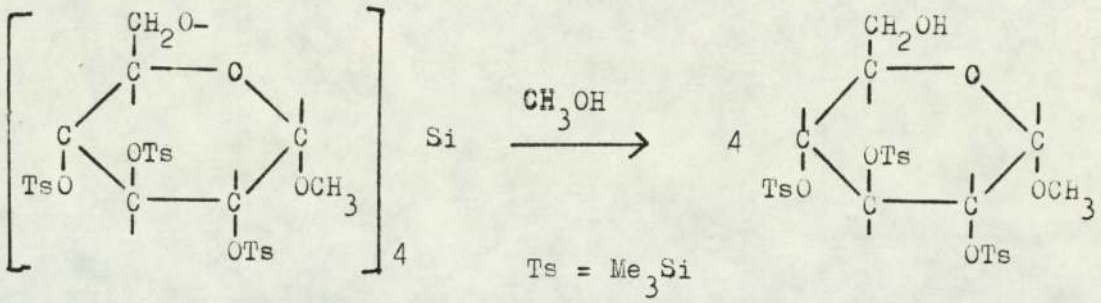


Ts = Me_3Si

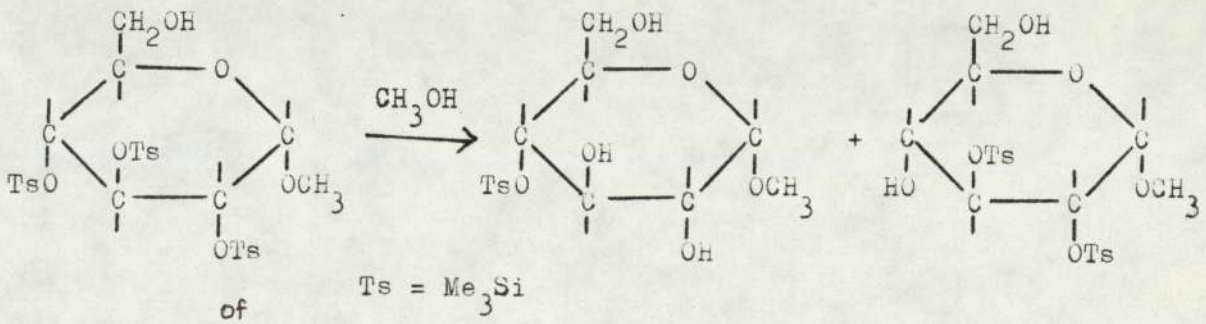
When diluted sulphuric acid was added to the reaction solution, the trimethylsilanol peak(10mm) disappeared and instead a peak for hexamethyldisiloxane appeared. This was expected as trimethylsilanol is readily converted to hexamethyldisiloxane in acidic media.

$$2\ Me_3SiOH \xrightarrow{H^+} Me_3SiOSiMe_3 + H_2O$$

When tetra-(methyl 2,3,4-tri-O-trimethylsilyl-α-D-gluco-pyranosyl-2-O-)silane was treated in boiling aqueous methanol, methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside was detected after 90 minutes(TLC). The detection of methyl 2,3,4-tri-O-trimethyl-silyl-α-D-glucopyranoside indicated that the cleavage of Si-O bond occurred initially at C-6 and this also agreed with the results obtained from the ^{29}Si NMR. These results showed that the primary alcohol is more reactive than the secondary alcohol in monosaccharide.



AT least four new components were detected by TLC after 150 minutes methanolysis, the separation of each component by column chromato-graphy proved to be impossible. It was assumed that these four products were partially hydrolysed trimethylsilyl-glucopyranoside derivatives, eg:

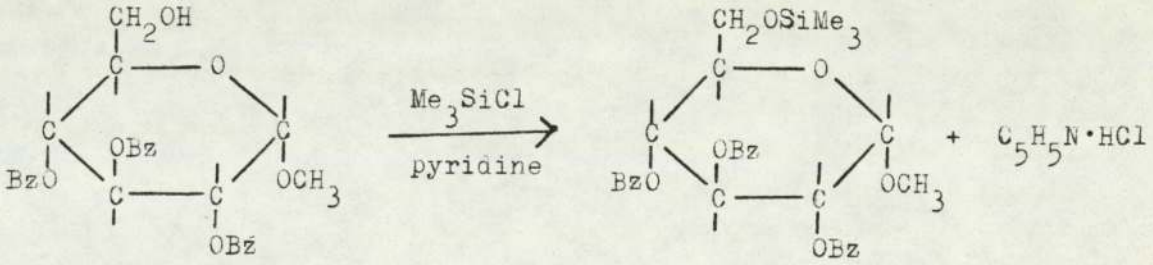


The detection of these components indicated that the Me_3Si groups attached to the secondary alcohol were being cleaved randomly.

(4) The reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside(XXI) with Me_3SiCl

It had been shown from the previous experiment that the Si-O bonds were readily cleaved in aqueous methanol particularly for those attached to primary alcohol, therefore another method had to be employed in order to keep the Si-O bond intact. Benzyl ether is known to be readily cleaved in neutral solution at room temperature by catalytic hydrogenation, it was decided to use benzyl group as protective function and the reaction between benzylated glucoside and chlorosilane was investigated.

The initial preparation of the starting material methyl 2,3,4-tri-O-benxyl-α-D-glucopyranoside was successful but the yield was very low(23%). This compound has a fairly low melting point(50-51°) which could explain why it took so long to crystallize out. The NMR spectrum of this compound gave a small singlet at τ 8.4 definitely arising from the OH group and this was confirmed by IR which showed a typical OH absorption band at 3480cm^{-1}. The observed elemental analytical result also agreed with the theoretical values.



Bz = $C_6H_5CH_2$-

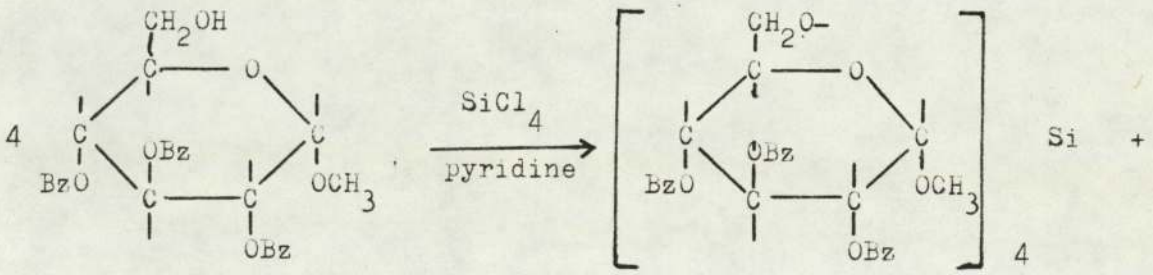
(XXI)

The reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with Me_3SiCl produced a mixed products, two components were detected by TLC. One of the component(R_f0.29) corresponded to the starting

material when compared with an authentic sample while the other(R_f0.83) must be the reaction product. This product was isolated by column chromatography. However, traces of starting material were also detected (TLC) in the purified product(there was possibility that the product was undergoing decomposition on the silica gel column).

Analysis of the product by IR gave bands at 1250,850 and 750cm^{-1} characteristic of Me_3Si group and no evidence of OH. These were confirmed by NMR which showed no signal at τ8.4 but a peak corresponded to Me_3Si group at τ9.8 was recorded. The observed elemental analysis was in agreement with the value calculated for methyl 2,3,4-tri-O-benzyl-6-O-trimethylsilyl-α-D-glucopyranoside. Therefore it was concluded that methyl 2,3,4-tri-O-benzyl-6-O-trimethylsilyl-α-D-glucopyranoside had been synthesized.

(5) <u>The reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with $SiCl_4$</u>



Bz = $C_6H_5CH_2$- $C_5H_5N\cdot HCl$

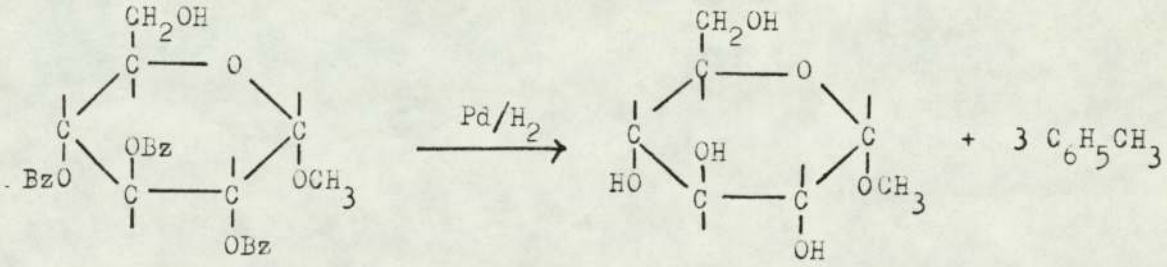
Analysis of the reaction product by TLC gave two spots (i)R_f0.72 (ii)R_f0.30. The second spot was shown to be the unreacted starting material when compared with an authentic sample therefore the first spot must be the reaction product. However, attempts to isolate this compound by column chromatography proved to be unsuccessful. The material collected from the column separation gave two further spots

on TLC analysis which strongly indicated that the product had decomposed on the column.

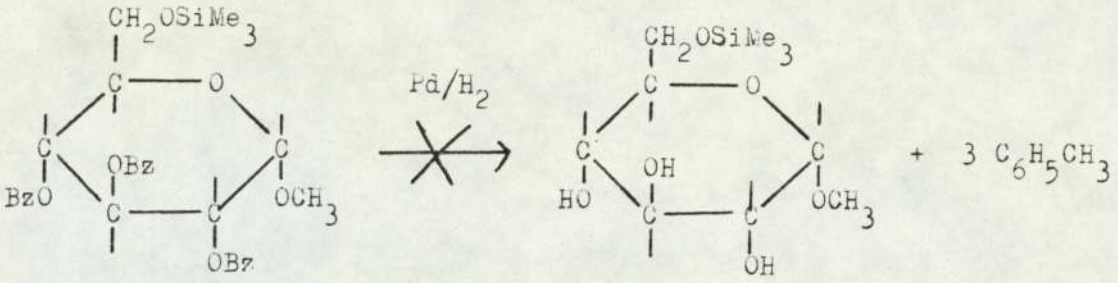
Catalytic hydrogenolysis of the benzyl groups

Attempts were made to remove the benzyl groups by catalytic hydrogenolysis. It is well established that benzyl protective group can be removed by catalytic hydrogenation whereas the available evidence suggested that the Si-O bond will not cleave under the same conditions.

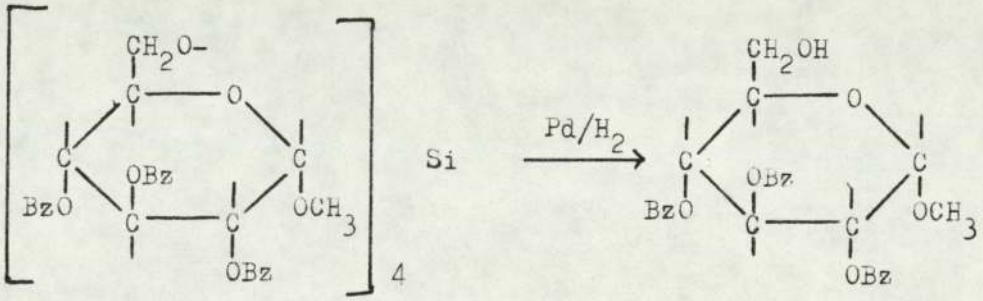
The catalytic hydrogenolysis of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside in anhydrous ethanol was first examined. Analysis of the reaction solution by TLC indicated the hydrogenation was slow, it took about 18 hours for the complete removal of all the benzyl group.



The catalytic hydrogenolysis of methyl 2,3,4-tri-O-benzyl-6-O-trimethylsilyl-α-D-glucopyranoside was then investigated in ethyl acetate. Under this condition the reaction occurred more rapidly than in ethanol and trimethylsilyl group proved to be more labile than the benzyl group. There has been no report of cleavage of the Si-O bond by catalytic hydrogenation. There is one report[102] claiming that a partially trimethylsilylated sugar derivative can be prepared by catalytic hydrogenolysis of the corresponding benzyl ether.

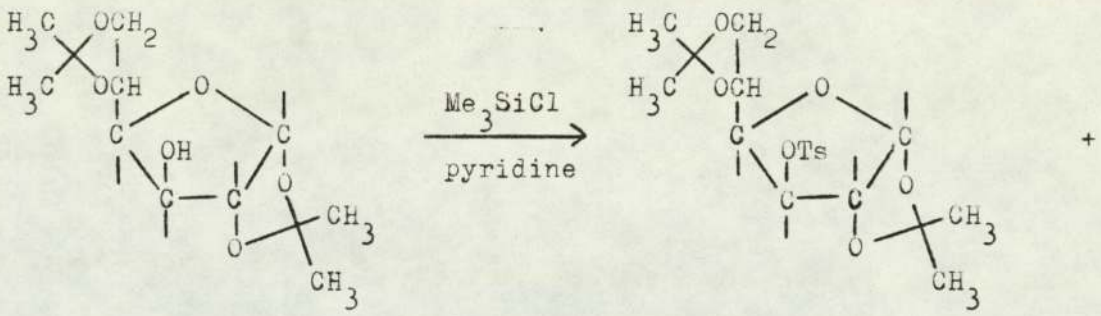


Although the Me_3Si-O bond had been cleaved by hydrogenolysis it was hoped that the more sterically hindered Si-O bonds might survive and in such systems specific cleavage of benzyl groups would occur. The product obtained from the reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with $SiCl_4$ was showed to be contaminated with the starting material, nevertheless the hydrogenolysis in ethanol was still carried out. It is known that boiling aqueous alcohol cleaves the Si-O bond, however, it was hoped that use of anhydrous ethanol as solvent might not induce the cleavage of the Si-O bond. But the detection of only methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside after 8 hours hydrogenolysis indicated that the Si-O bond was again being preferentially cleaved under these conditions. After 12 hours of hydrogenolysis the benzyl group was still detectable by NMR and IR.



(6) The reaction of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (XXII) with Me_3SiCl

From the previous few experiments it had been shown that the Si-O bond at C-6 is relatively more reactive than those at the other position. Therefore attempts were made to synthesize compounds in which the Si-O bonds were formed at position other than at C-6. The reactions of this isopropylidene glucose with Me_3SiCl and $SiCl_4$ had been reported.[117] However, these reactions were repeated by using a slightly different method.



(XXII)

$C_5H_5N \cdot HCl$ Ts = Me_3Si

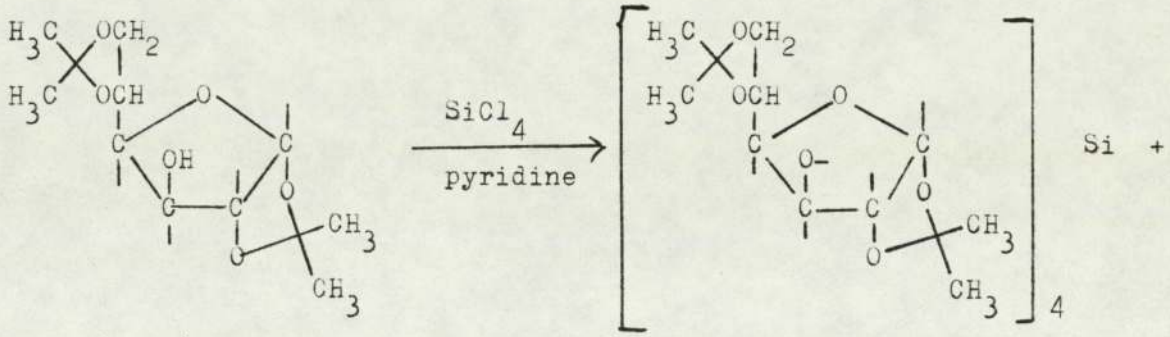
The product was isolated from the unreacted starting material by column chromatography. There was no sign of decomposition of the product on the column.

The NMR spectrum showed no signal at τ 7.2-7.3 corresponding to the OH group but the signal at τ 9.9 indicated the presence of Me_3Si group. The ^{13}C NMR spectrum also showed the presence of the Me_3Si group. These observations were comfirmed by IR which showed no characteristic OH band and the bands at 1250, 850 and 750cm^{-1} proved the presence of the Me_3Si group. These results established that the compound 1,2:5,6-di-O-isopropylidene-3-O-trimethylsilyl-α-D-glucofuranose had been synthesized.

Methanolysis of the isopropylidene group

Isopropylidene group can be removed by acidic methanolysis,[118] attempts were made to hydrolysis this grouping at C-5,6 with catalytic amounts of acetic acid. The Me_3Si group of methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside is known to be quite stable to catalytic amount of acetic acid,[90] it was hoped to remove the isopropylidene group at C-5,6 of the reaction product using catalytic amounts of acetic acid and leaving the Me_3Si group intact. However, the experimental results showed that it took 36 hours to remove the isopropylidene group and about 3 hours to hydrolyse the Me_3Si group. Similar results were obtained when sulphuric acid was used. No attempt was made to hydrolyse the isopropylidene group at C-1,2 as this will require more drastic conditions.

(7) The reaction of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose(XXII) with $SiCl_4$



(XXII)

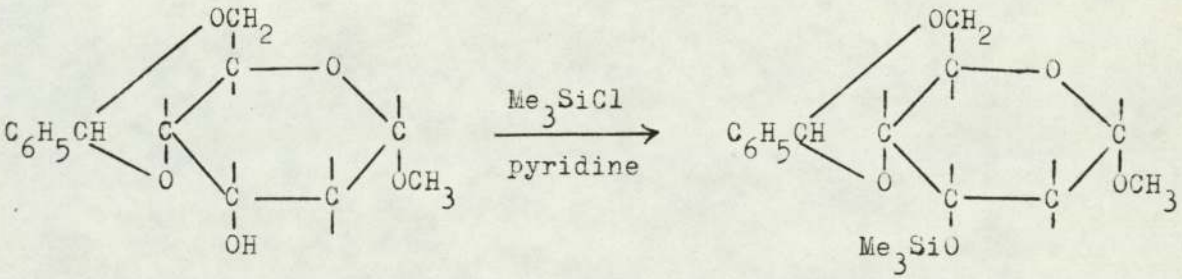
$C_5H_5N \cdot HCl$

The product was isolated from the unreacted starting material by column chromatography. ^{29}Si NMR did not show presence of silicon in the product and the observed optical rotation is some 10 times the literature value which are difficult to explain. However, IR, NMR and TLC and also the elemental analysis all favoured tetrakis-(1,2:5,6-di-O-isopropylidene-α-D-glucofuranosyl-3-O-)silane as product.

(8) The reaction of methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside(XXIII) with Me_3SiCl

Because of the difficulty in removing the isopropylidene group, a more labile protective function was required and benzylidene acetals group was chosen. This acetal group can also be cleaved by catalytic hydrogenolysis.[106] Although we have shown that silicon attached to primary alcohol is quite reactive, those attached to the secondary alcohol are quite stable. Therefore attempts were made to react chlorosilanes with carbohydrates containing one free secondary alcohol group.

The reaction of methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside with Me_3SiCl was first studied.



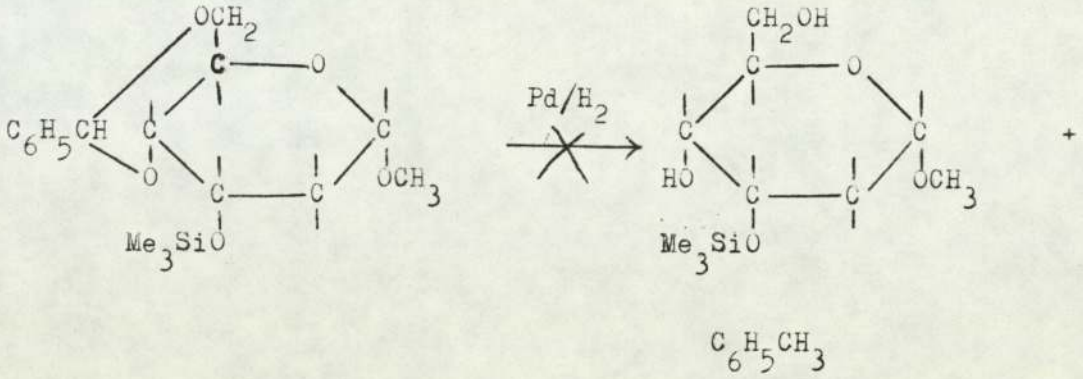
(XXIII)

+ $C_5H_5N \cdot HCl$

A pure reaction product was isolated from the reaction mixture by column chromatography. The IR showed no evidence of OH group between 3700-3200cm^{-1} but bands at 1250, 850 and 750cm^{-1} proved that Me_3Si group to be present. The NMR spectrum confirmed the presence of Me_3Si group(τ 9.8), however, a multiplet at τ7.9-8.0 which arose from an OH group was observed and this is not consistant with the IR observation. This OH group could originate from the residue methanol associated with the product. It was concluded that methyl 4,6-O-benzylidene-2-deoxy-3-O-trimethylsilyl-α-D-ribo-hexo-pyranoside

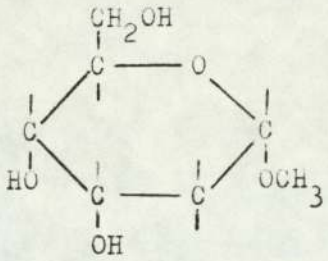
had been synthesized and its catalytic hydrogenolysis was investigated.

Catalytic hydrogenolysis of methyl 4,6-O-benzylidene-2-deoxy-3-O-trimethylsilyl-α-D-ribo-hexo-pyranoside



The catalytic hydrogenation was carried out in ethyl acetate and after 6 hours four spots were detected by TLC with the following R_f values: (i)0.90 (ii)0.57(iii)0.48 (iv)0.

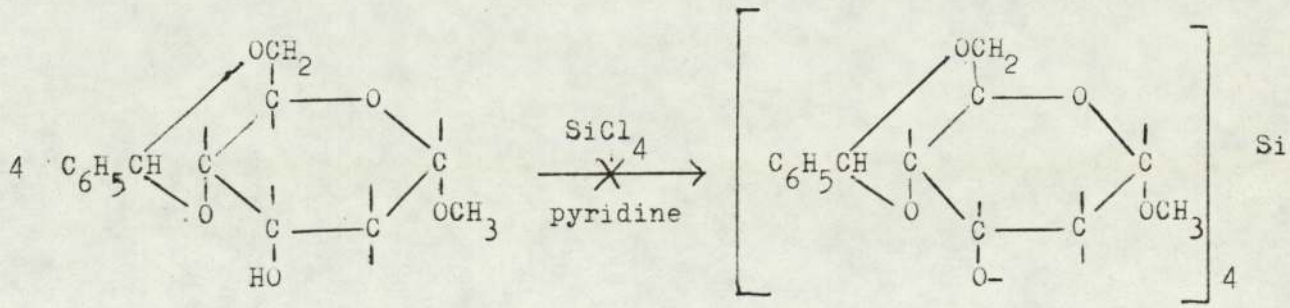
It was shown by comparison with an authentic samples that (i) corresponded as the starting material and(iii)to methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside. The compound (ii) could not be identified with certainty. After further 8 hours of hydrogenation compounds (i) and (iii) could not be detected but four different spots were revealed(TLC). These four spots had the following R_f values (different solvent from the last analysis): (iv)0.65 (v)0.56 (vi)0.39 (vii)0.32. After a total of 20 hours hydrogenation only (vi) and (vii) were detected by TLC. The attempted separation of these two compounds by column chromatography yielded only (vii). This compound seemed to be the final hydrogenated product. It was recrystallized from ether/petroleum ether60-80°/ethanol and is most likely to be methyl 2-deoxy-α-D-ribo-hexo-pyranoside(XXIV).



(XXIV)

this compound was highly hygroscopic and the melting point could not be determine with certainty, about 88-90°(rapid heating). This compound gave a positive test with thiobarbituric acid which also favours this conclusion. This result also shown that Me_3Si was removed by catalytic hydrogenation but because of the difficulty in separating the individual hydrogenated product it was impossible to say whether it was the Me_3Si group or the benzylidene group was the first to be removed.

(9) <u>The reaction of methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside with $SiCl_4$</u>

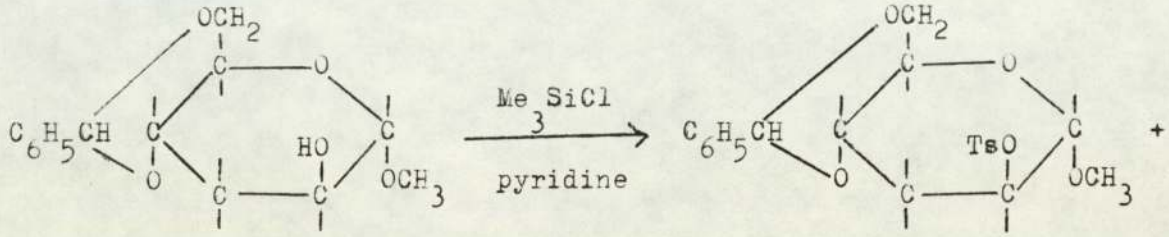


+ $C_5H_5N \cdot HCl$

A complex mixture of products were obtained when the reaction product was examined by TLC. No attempt was made to isolate each individual component. The failure to prepare this desired compound could be due to the bulky size of the benzylidene group which inhibits the attachment of four carbohydrate molecules to one silicon atom through the C-3 position.

(10) The reaction of methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside(XXV) with Me_3SiCl

Because of the possibility of the steric hindrance, (XXV) was tried in which the free OH group was in the C-2 position, hopefully this compound would serve our purpose.



(XXV)

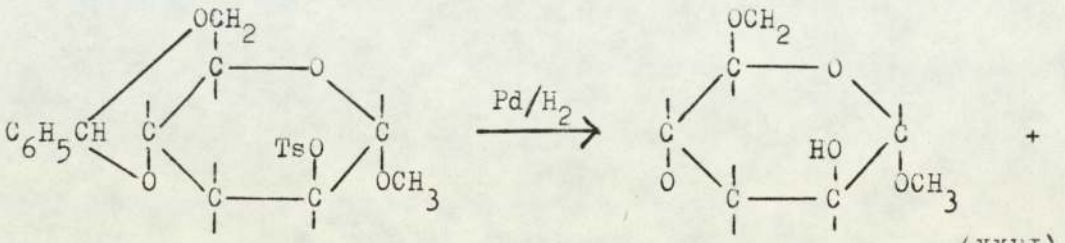
$C_5H_5N \cdot HCl$ Ts = Me_3Si

A single product was obtained from the above reaction(TLC R_f 0.80). IR analysis indicated that the compound contained no OH group and the bands at 1250, 850 and 750cm^{-1} established the presence of the Me_3Si group, these observations were all confirmed by NMR. The spectral results established with certainty that methyl 4,6-O-benzylidene-3-deoxy-2-O-trimethylsilyl-α-D-arabino-hexo-pyranoside had been prepared.

The catalytic hydrogenolysis of methyl 4,6-O-benzylidene-3-deoxy-2-O-trimethylsilyl-α-D-arabino-hexo-pyranoside

The catalytic hydrogenolysis of methyl 4,6-O-benzylidene-3-deoxy-2-O-trimethylsilyl-α-D-arabino-hexo-pyranoside was investigated, only one spot was detected after 18 hours of hydrogenation. The IR of this product showed a strong absorption band corresponded to the OH group and three weak bands at 1750, 1250 and 850cm^{-1}. These bands probably arose from the presence of traces of ethyl acetate. The NMR spectrum showed no sign of benzylidene and Me_3Si groups. The most likely

interpretation of these results is that the benzylidene and the Me_3Si groups have been removed and that the final product is probably methyl 3-deoxy-α-D-arabino-hexo-pyranoside(XXVI). This result was similar to that obtained from the hydrogenation of methyl 4,6-O-benzylidene-2-deoxy-3-O-trimethylsilyl-α-D-ribo-hexo-pyranoside.



(XXVI)

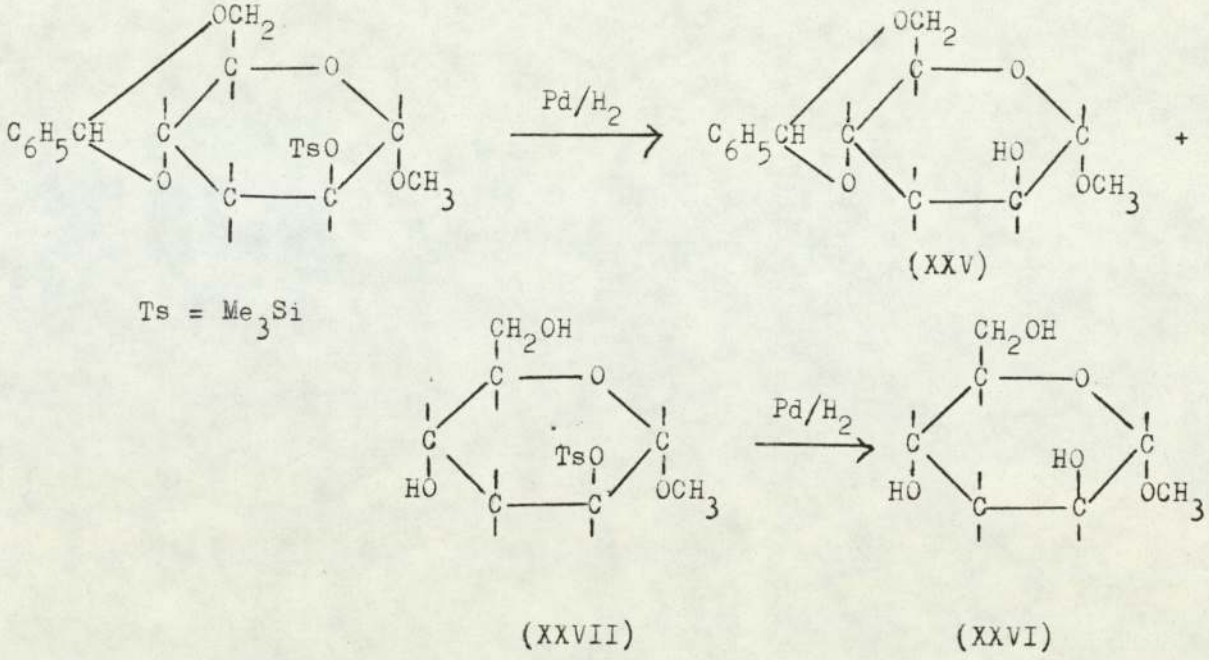
Ts = Me_3Si $C_6H_5CH_3$

The hydrogenation was repeated in ethyl acetate(AR) for a shorter period to see if any specificity could be introduced into the reaction. after 6 hours, four spots were detected by TLC with the following R_f values: (i)0.95 (ii)0.75 (iii)0.65 (iv)0.50. It was shown by comparison with an authentic samples that (i) was corresponded to the starting material while (iii) was to methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside. The last(iv) had the same R_f value as the assumed methyl 3-deoxy-α-D-arabino-hexo-pyranoside(XXVI). As the hydrogenolysis of methyl 4,6-O-benzylidene-3-deoxy-2-O-trimethylsilyl-α-D-arabino-hexo-pyranoside can only yield three products:-

(a)Methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside(XXV)

(b)Methyl 3-deoxy-2-O-trimethylsilyl-α-D-arabino-hexo-pyranoside(XXVII)

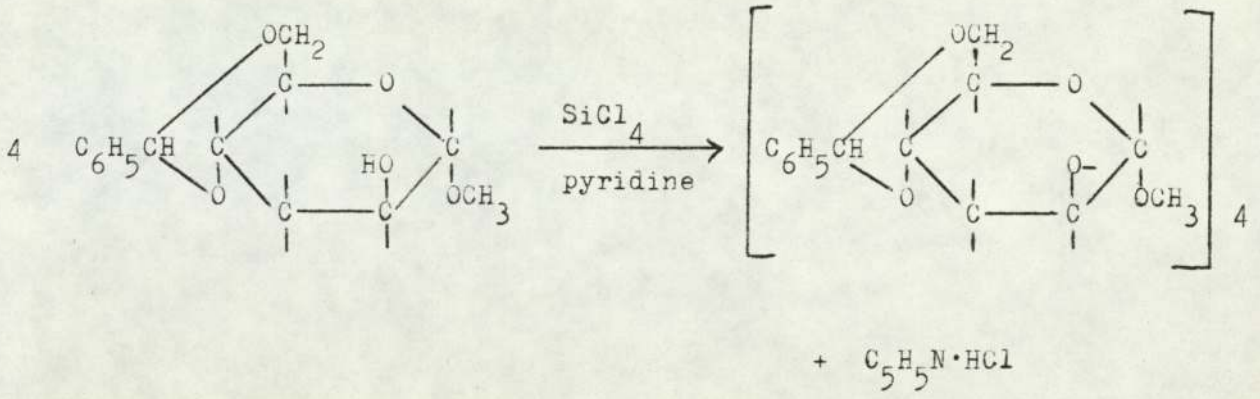
(c)Methyl 3-deoxy-α-D-arabino-hexo-pyranoside(XXVI)



Therefore the component (ii) must be the methyl 3-deoxy-2-O-trimethylsilyl-α-D-arabino-hexo-pyranoside(XXVII). Although (XXVII) contained only two OH group and (XXV) contained one, the presence of Me_3Si group in (XXVII) caused this compound to be more labile than (XXV). Even using shorter reaction time it would appear that the cleavage of benzylidene and Me_3Si groups both occur, suggesting that these reaction have similar rates.

The hydrogenolysis was repeated in absolute ethanol, in this solvent longer time(40 hours) was required for the complete hydrogenolysis(only methyl 3-deoxy-α-D-arabino-hexo-pyranoside was present). However, similar results were obtained(four components by TLC) when the reaction was carried for a shorter period(10 hours). The separation of the mixture by column chromatography proved to be impossible therefore the identification of (XXVII) could not be confirmed.

(11) The reaction of methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside with $SiCl_4$



+ $C_5H_5N \cdot HCl$

The pure product was isolated from the reaction mixture by column chromatography. The NMR showed a broad multiplet at τ7.7-8.1 indicating the presence of an OH group. ^{29}Si NMR did not record any silicon signal. However, the presence of OH group was not confirmed by the IR studies which showed no absorption band between 3700-3200cm^{-1} a broad band between 1150-1050cm^{-1} was present this could arise from Si-O bond.

The exact structure of this compound could not be elucidated with certainty but we assumed that this product is tetrakis-(methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranosyl-2-O-)silane and its behaviour on catalytic hydrogenolysis was examined.

The catalytic hydronenolysis of tetrakis-(methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranosyl-2-O-)silane

When the hydrogenation was carried out in ethyl acetate only methyl 3-deoxy-α-D-arabino-hexo-pyranoside(XXVI) was detected after 10 hours. However, four components were detected by TLC after 3 hours in absolute ethanol with the following R_f values: (i)0.88 (ii)0.81 (iii)0.55 (iv)0.48. The spot(i) was corresponded to the starting material, (iii) to methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-

hexo-pyranoside and (iv) to methyl 3-deoxy-α-D-arabino-hexo-pyranoside while the nature of (ii) was uncertain. The observation of methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside showed that the Si-O bond had been broken. No attempt was made to separate the individual compounds.

The successful reaction between the 3-deoxy arabinopyranoside and $SiCl_4$ indicated that steric hindrance played a important part in restricting the reaction between the 2-deoxy ribopyranoside with $SiCl_4$.

CHAPTER FOUR

PECTINS AND PECTIC ENZYMES

PECTINS AND PECTIC ENZYMES

4.1. INTRODUCTION

Pectins or pectic substances[119,120] are found universally in the primary cell walls and intercellular layers of land plants. They are most abundant in soft tissues such as rind of citrus fruit(30%), sugar beet pulp(25%) and apple(15%), but are also present although in only small proportion in woody tissues.

The term pectic substances is used generally to refer to the group of complex plant polysaccharides in which D-galacturonic acid is the principal constituent, and the term pectin is used in relation to the gel forming, water soluble polysaccharides. Polysaccharides in which a proportion of the galacturonic acid residues are present as methyl esters are designated pectinic acid(pectinate), and those devoid of ester groups as pectic acid(pectate). Although D-galacturonic acid is the main sugar constituent of the pectic substances, various proportions of other sugars including D-galactose, L-arabinose, D-xylose, L-rhamnose, L-fucose and traces of 2-O-methyl-D-xylose and 2-O-methyl-L-fucose are usually present as constituents.

The carboxyl groups of pectins are partially esterified with methanol, and in some cases the hydroxyl groups are partially acetylated. Due to a considerable variation in the degree of polymerization and in the kind, amount, and distribution of substituents, probably no two macromolecules of a pectin preparations are identical.

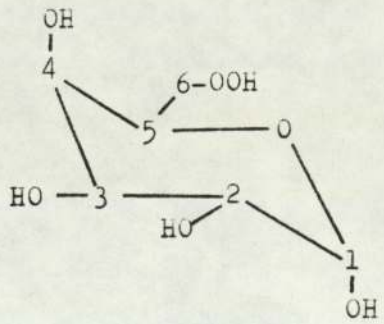
Three types of homopolysaccharide: L-arabinan, D-galactan and D-galacturonan have been recognized among the pectic substances, but most frequently the native macromolecules are heteropolysaccharides and in most cases the acidic polysaccharides contain natural sugars as internal constituents.

Of the three homosaccharides, only D-galacturonan will be discussed.

D-galacturonans as the sole acidic polysaccharide constituent of pectic complexes are of infrequent occurrence; and the polysaccharide from sunflower head is probably the only authenticated example. Structural investigation on degraded galacturonans[121,122] from controlled acid hydrolysis(or methanolysis) of pectic acid by the methylation procedure, the characterization of galacturonobiose and the triose formed on partial enzymic hydrolysis[123] and also studies on degraded galacturonans[124,125,126] have provided evidence for the presence of linear chain of (1→4)-link α-D-galacturonic acid residues.

4)-α-D-GalpA-(1→4)-α-D-GalpA-(1→4)-α-D-GalpA-

The pyranose ring of D-galacturonic acid occurs mainly in the chair 1 form (C1) as shown in the figure (XXVIII).



(XXVIII)

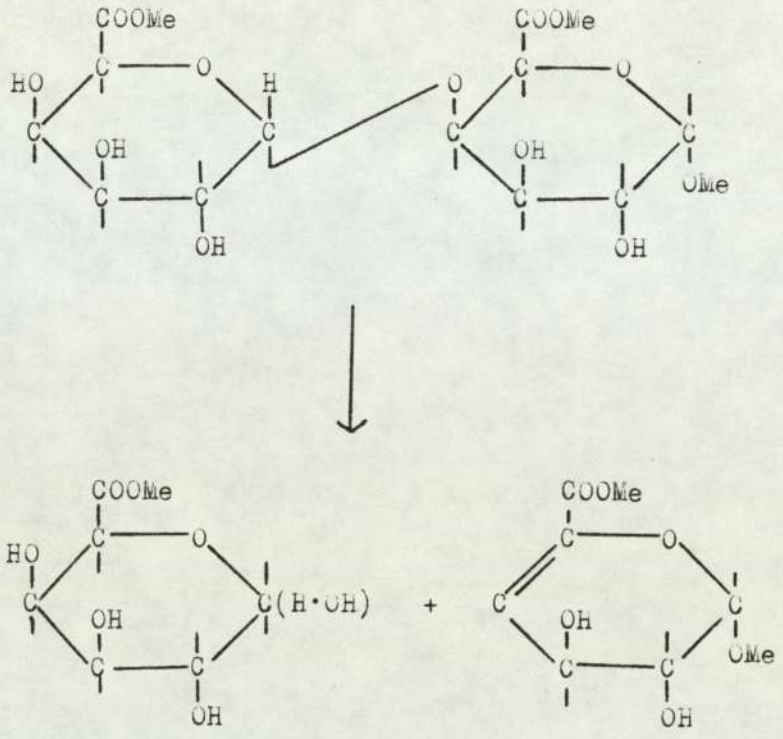
As both hydroxyl groups of D-galacturonic acid at the carbon atoms 1 and 4' are in axial position, the resulting polymer belongs to the trans-1,4'-polysaccharides. The free rotation at the glycosidic linkages is thereby hindered. Hence the pectin macromolecule may be considered a chain with restricted flexibility.

The majority of pectic acids or pectinic acids contain various proportions of neutral sugar constituent. That these sugars are integral constituents of acidic polysaccharide is indicated by the failure of fractionation by precipitation methods and by ion-exchange chromatography to separate the polysaccharide into acidic and neutral components. Direct evident for L-rhamnose as an integral constituent in several pectic acids has been obtained[127,128]. Other natural sugar residues in pectins are probably attached as side chains. Evidence concerning the nature of these subunits and their mode of attachment to the interior chains is still fragmentary.

4.2. DEGRADATION OF PECTINS

(1) Alkali

Pectins in which the D-galacturonic acid residues are esterified are degraded on treatment with alkali. The key reaction involves cleavages of glycosidic linkage by a ß-elimination mechanism. In aqueous solution this reaction take place side by side with saponification, the latter giving pectate, which are relatively stable to cold alkali. The ß-elimination reaction has been clearly demonstrated in the reaction of methyl-4-O-(methyl-α-D-galactopyranosiduronic acid)-(methyl-α-D-galactopyranosid-)uronate with methanolic sodium methoxide to give methyl-galacturonate and methyl-(methyl-4-deoxy-ß-L-threo-hex-4-enopyranosid-)uronate[129]. The reaction mechanism is being shown in scheme C :



Scheme C

The formation of unsaturated hexuronic acid derivatives is readily detected by the intense UV absorption at about 230nm. Degradation of pectins by the ß-elimination mechanism also takes place[130] in hot, aqueous phosphate buffer at pH 6.8.

(2) Acids

Pectic substances undergo many changes through the catalytic action of the hydrogen ion, hydrolysis of ester and glycosidic linkages, dehydration, decarboxylation, cyclization, etc. Although these reactions occur more or less simultaneously, one can favour a particular reaction by appropriate conditions. A decrease in pH favours markedly the saponification of the methyl and acetyl esters; an increase in temperature accelerates especially the hydrolysis of the glycosidic linkages.

(3) Ion exchangers

Pectic substances are rather stable toward anion exchangers in the OH-form or cation exchangers in the H-form. Since the pectic macromolecules can hardly diffuse into the network of highly cross-linked polyelectrolytes, only the external groups of the ion-exchange resin can react with pectin. The methyl ester of polygalacturonic acid is saponified extremely slowly by anion-exchangers, whereas the methyl ester of monogalacturonic acid is easily saponified. Similarly, anion exchangers absorb mono- and oligo-galacturonic acids, whereas high polymer pectins are hardly absorbed. Between pectins and cation-exchangers, however, an exchange of counterions can take place. Ion exchangers, especially mixed bed exchangers, are therefore, useful for the purification of pectin from electrolytes[131].

(4) Heavy-metal ions

D-galacturonic acid can easily be decarboxylated in the presence of heavy-metal ions such as Al^{+++}, Cu^{++}, Ni^{++} and Zn^{++} under very mild conditions, eg. in water at pH 5 and at 70° to 100°[132].

(5) Oxidizing agents

Pectic substances are easily destroyed by oxidation. The oxidation products are not known. Pectin is vigorously attacked by chlorine dioxide[133]. After oxidation by periodic acid which specifically splits 1-2 glycols no polymer with aldehyde groups at C-2 and C-3 can be isolated, unknown secondary reactions bringing about a rapid breakdown of the pectin molecules[134,135].

(6) Reducing agents

The methyl ester of methylated polygalacturonic acid has been reduced to the methylated galactan by $LiAlH_4$[136]. Pectin has been partially reduced by $NaBH_4$ in aqueous solution[137].

4.3. PHYSICAL PROPERTIES OF PECTINS

(1) Solubility

Pectins are soluble in water, formamide, ethylene diamine, and warm glycerol, but insoluble in most organic solvents. The ease of dissolving in water increases with decreasing length of the pectin molecule. Highly polymeric pectic acids are water insoluble. The partial of complete esterification at the secondary hydroxyl groups induce water solubility. High substitution of the alcoholic groups (eg. acyl derivatives) results in derivatives insoluble in water, but soluble in organic solvents.

(2) Acidity

The carboxyl groups of pectin can be titrated with alkali to a point of equivalent at about pH8. Pectins do not have a well defined dissociation constant. The calculated "apparent constant" for pectin is usually less than the constant of monogalacturonic acid(3.25×10^{-4} at 19°.

(3) Gelation properties

High molecular pectins form thermo-reversible hydrogels even at low concentrations. The gel formation is considered to be due to a crosslinking of the polysaccharide molecules to a three-dimensional network by secondary valence bonds. The gelation process is considered to be very specific, intimately related to the structure of the polysaccharide, since out of the very great number of polysaccharides, only a few show gel forming properties. The restricted flexibility of the chain molecules and the equatorial position of the hydroxyl and carboxyl groups are probably essential for the gelation properties of pectin. The formation of the gel network is

considered to occur through the formation of hydrogen bonding. Substituents at these hydroxyl groups change the structure of the specific junction zones, and therefore, influence the crosslinking process. A small number of acetyl groups inhibit gelation, probably by disturbing the formation of junction zones. Highly acetylated pectic acid, however, forms gels under suitable conditions, probably by forming a new regular molecule surface.

(4) Coagulation properties

Pectin is precipitated from an aqueous solution by organic solvents, such as alcohol or acetone. By rapid precipitation with alcohol at 0°, a partially crystallized precipitate is formed as evidence by X-ray examination, pectin slowly precipitated with alcohol, however, is amorphous.

4.4. PECTIC ENZYMES[138]

There are three general classes of enzymes known to effect the modification and/or depolymerization of pectins - namely, pectin ester hydrolases, D-galacturonanases and pectate lyases.

The ester hydrolase catalysed the hydrolysis of the methyl ester group in pectins. Pectate lyases catalyses the cleavage of α-D-(1→4) glycosidic bond of D-galacturonan by the mechanism of ß-elimination. The mechanism of ß-elimination had been previously discussed in the alkali degradation of pectin(p.80).

The third kind, D-galacturonanases will be discussed in more details, as this enzyme had been employed in this work.

D-galacturonanases catalyse the hydrolytic cleavage of the glycosidic α-D-(1→4) bonds of nonesterified D-galactopyranosiduronic residue, and hence, pectic acid, and galacturonans having a low degree of esterification are the substrate of preference. The prefixed endo- and exo- used in connection with D-galacturonanases denote a random or a terminal splitting pattern of the glycosidic bonds.

Random splitting of internal bonds of the D-galacturonan chain , which is catalysed by endo-D-galacturonanase , results in a pronounced diminution in the viscosity of a substrate solution at a low degree of the splitting of glycosidic bonds. The primary reaction-products are higher oligo-D-galactosiduronates.

Although the decrease in viscosity and the liberation of oligo-D-galactosiduronates as reaction products are the feature common to the activity of all endo-D-galacturonanases, the mode of action of these enzymes is not identical. Differences in the ratio of lowering of viscosity to the number of hydrolysed glycosidic bonds observed for various endo-D-galacturonanases indicates differences in their mode of action.

The preferred substrate of all endo-D-galacturonanases are the high-molecular D-galacturonans. The rate of splitting of the glycosidic bonds decreases with the shortening of the substrate chain[139].

Exo-D-galacturonanases catalyse the hydrolytic cleavage of the terminal α-D-(1→4) bonds of D-galacturonan chains, releasing D-galactopyranuronic acid as the product. In contrast with endo-D-galacturonanases, exo-D-galacturonanases also split the di(D-galactosiduronate), which is thus a suitable substrate for differentiation of the activity of these two enzymes.

Endo- and exo-D-galacturonanases are produced by most plant pathogens, such as Aspergillus and Penicillium etc. In micro-organisms, the production of D-galacturonanases is influenced by the conditions of cultivation, in particular by the composition of the culture medium, and by other factors.

4.5. ENZYMATIC DEGRADATION OF PECTIN

The silicon content of a wide variety of natural systems was investigated by Schwarz[49] who found that silicon values varied from system to system, subsequently he investigated the enzymatic degradation of pectin by pectinase. The experiment results showed that the enzymatic degradation breakdown of pectin did not convert the bound silicon into free, directly-reacting silicic acid. After nearly complete enzymatic hydrolysis of pectin by pectinase only 6% of the silicon initially bound to the pectin matrix was released as free form. The breakdown products were low molecular weight complexes to which the silicon was firmly bound.

Our aim was to follow the enzymatic degradation of pectin and attempt the isolation of these low molecular weight complexes to which the silicon was firmly bound.

It is clearly demonstrated from the Table(VII)that the amount of bound silicon in pectin varies from one source to another. This could be due to the method of preparation. Purified pectin(8000 µg/g) was used in all our experimental work. The increased silicon(1900 µg/g) content after purification could be due to

(i) contamination by ethanol during purification, or that

(ii) the ethanol removed most of the impurities which contain very low amount of silicon.

Silicon analysis established that the silicon content of ethanol was about 37μg/100cm^3, since the density of ethanol at 20° is 0.79 therefore the silicon content of ethanol was about 0.5μg/g. After the purification of pectin the ethanol extract contain 37μg/g of silicon. Therefore we can conclude that the ethanol had removed the impurities which contain very low amount of silicon.

Only 4.7% of the silicon initially bound to the pectin matrix was found in free form after nearly complete enzymatic hydrolysis of pectin by pectinase. The main portion of silicon must be still firmly attached to the carbohydrate. The result agreed with the results obtained by Schwarz.

Attempts were made to isolate the silicon containing product by chromatography.

(i) Paper chromatography

D-galacturonic acid was detected in the hydrolysed product by comparising the R_f value with that of authentic D-galacturonic acid. Only poor separation of other components could be achieved, probably due to the presence of buffer salt.

(ii) TLC

The hydrolysed products were chromatographed on cellulose plate, using alkaline $KMnO_4$ as visualising reagent. However, this reagent is only sensitive to reducing sugars, although the polysaccharides usually have a terminal reducing group, the relative proportion of these terminal group is probably too small to influence the reaction greatly. Therefore it would be expected that the detection of low molecular weight complexes would be very difficult. The less specific reagent ethanol/sulphuric acid could not be used because its reacts with cellulose.

Three components were detected in the hydrolysed product.

(a)R_f0.43: The higher R_f value indicated that this compound probably have lower molecular weight than D-galacturonic acid, and therefore was unlikely to be the silicon complexes. It was conceivable that this compound was one of the natural "reducing" sugar always present in the pectin. The pectinase used is an endo-galacturonanase which has random splitting pattern, therefore the natural sugar being released during the hydrolysis varies from one experiment to another. The failure to detect this compound in another identical experiment added support to this hypothesis.

(b)R_f0.26: This component corresponded to D-galacturonic acid.

(c)R_f0.15: The detection of this component after 1 hour hydrolysis and subsequently failure to be detected after 2 hours of hydrolysis indicated that this component was probably an oligo-galacturonate.

The results obtained from the TLC(cellulose) were slightly different from the paper chromatography. But the only detectable product using either techniques were D-galacturonic acid or its derivatives. It may have been that our detection reagents were not sufficiently sensitive to detect low level of silicon complexes.

(iii) Gel filtration

The low molecular weight silicon complexes could have been trapped inside the column, only D-galacturonic acid managed to pass through the column and then being detected. However, those low molecular weight silicon complexes could be insensitive towards all the mentioned detection methods.

We established that enzymatic degradation of pectin only liberated small alount of Si(4.7%) from the main matrix. The majority of Si was remained attached to the degraded matrix, and is probably presence as low molecular weight complexes. These complexes could not be detected by our methods. Therefore other methods for detection are required.

EXPERIMENTAL

Melting points and boiling points quoted in this section are uncorrected. Dried solvents are used in all experiment.

Gas Liquid Chromatography(GLC) analyses were performed on a Pye Unicam GCD instrument using an SE 30 silicone gum column(9ft. x 1/8 in. o.d.) with nitrogen as a carrier gas at a flow rate of 50cm^3/min. Temperature for individual separations are recorded in text.

Nuclear Magnetic Resonance(NMR) were recorded at 60MHz/sec using Varian Associates A-60A spectrometer. When the word NMR was used in the text it is referred as ^{1}H NMR. The chemical shift was expressed in τ. Nuclei other than ^{1}H are also used, ^{13}C NMR spectra were recorded at 22.5MHz/sec using Jeol-FX90Q spectrometer; ^{29}Si NMR spectra were recorded at 17.76MHz/sec using Jeol-FX90Q spectrometer. The chemical shift was expressed in ppm in both cases. Tetramethylsilane(TMS) was used as an internal reference in all cases.

Infra-red(IR) spectra were obtained using a Perkin-Elmer Infracord 237 and the interpretation of absorption bands were based on the text of Bellamy.[140]

Thin Layer Chromatography(TLC) analyses were performed on precoated silica gel or cellulose plates. Ascending one-dimentional chromatographic method was used. Solvents for individual compound are recorded in the text. Ethanolic sulphuric acid was used as developing agent on silica gel plate. Alkaline potassium permanganate solution used for cellulose plates. The plates were supplied by CamLab.

Paper Chromatography analyses were performed on Whatman No.4 paper using descending method. Solvents and developing agents for individual separations are recorded in the text.

Column Chromatography was performed on silica gel(60-120mesh) using a glass column(2x30cm). Solvents for individual separations are recorded in the text.

Gel Permeation was performed on Sephadex(G10 or G100) using a glass column(2x30cm). Solvents for individual separations are recorded in the text.

Elemental Analyses of the compounds were carried out by Perkin-Elmer 240B Elemental Analyser.

Reagents:

Ether unqualified refers to diethyl ether

Water refers to distilled water

Me_3SiCl refers to trimethylchlorosilane

$SiCl_4$ refers to silicon tetrachloride

General technique:

Evaporation of solvents was carried out using a rotary evaporator the vacuum for which was provided by a water pump.

5.1. THE REACTIONS OF METHYL α-D-GLUCOPYRANOSIDE WITH SILYLATING AGENTS

(1) The preparation of tetra(N-methylacetylamino)silane

N-methyl-acetamide(29g, 0.4 mole), triethylamine($80cm^3$) were added to dry dichloromethane($300cm^3$) in a three-necked round-bottom flask fitted with condenser, dropping funnel(both closed with $CaCl_2$ tubes), thermometer and mercury sealed stirrer, the reaction flask was cooled in an ice-salt bath to 0°. Freshly distilled $SiCl_4$($11cm^3$, 0.1 mole) diluted in dry ether($20cm^3$) was added from the dropping funnel at such a rate that the reaction temperature was kept below 5°. The reaction mixture was stirred vigorously during the entire reaction period. After the addition of the silane the solution was stirred for further 15 minutes. The solid mass which had been precipitated during the reaction was filtered off under nitrogen. The filtrate was concentrated under reduced pressure at a temperature not greater than 30°. The syrup crystallized readily as needles and was washed several times with dry ether. The product was stored at about -4°; yield 12.5g(40%).

NMR (C_6H_6 solution):-

(i) N-methyl-acetamide: Doublet τ 7.1-7.2 (intensity 18)
Singlet τ 7.9 (intensity 18)

(ii) Reaction product:-

a. Freshly prepared: Singlet τ 7.15 (intensity 12)
Singlet τ 8.3 (intensity 12)

b. After 1 week : Singlet τ 7.7

IR (KBr disc):-

(i) Reaction product: Broad peak between 1200-1000cm^{-1}

Elemental analysis (%):-	Si	C	H
Found	7.6	43.3	6.9
Calculated	8.9	45.6	7.6

The reaction of the reaction product with anhydrous methanol

The reaction product(2g) was refluxed in anhydrous methanol($20cm^3$) 3 hours. When this reaction was examined by glc, $Si(OCH_3)_4$ was detected. The solution was concentrated under reduced pressure to a thick syrup which solidified upon standing.

NMR (C_6H_6 solution):- Doublet τ 7.1-7.2 (intensity 18)

Singlet τ 7.9 (intensity 18)

IR (KBr disc):-

(i) Product: Bands at 3300,3100,1650,1560cm^{-1}

(ii) N-methylacetamide: The spectrum was identical as (i)

(2) The reaction of methyl α-D-glucopyranoside with tetra(N-methyl-acetylamino)silane in 4:1 molar ratio

Tetra(N-methylacetylamino)silane(1g, 0.003 mole) and anhydrous methyl α-D-glucopyranoside(2.5g, 0.013 mole) in dry DMF/pyridine were stirred at 80°for 6 hours. During this stage the reaction solution had turned to dark brown in colour. Some crystals crystallized out when the reaction solution was cooled down, the crystals were filtered off and analysed. The filtrate was poured into dry ether($100cm^3$), the brownish oily material solidified upon stirring.

(i) Reaction product:-

IR (KBr disc): Bands at 1650 and 1050cm^{-1}, the first band was only observed when DMF was used as solvent.

NMR (D_2O solution):- Singlet τ 6.4 (intensity 14)

Multiplet τ 5.9-6.2 (intensity 16)

TLC (silica gel)(n-butanol:acetone:water/4:5:1) :-

Two spots were detected: a. R_f0.60

b. R_f0.42

Reference : methyl α-D-glucopyranoside R_f0.61

Silicon analysis:- Calculated- Si 3.5%

Found- the silicon content varied from different batches of product (2.5-4.6%)

Stability in water: An aqueous solution of the reaction product(10%) was readily turned to gel within 24 hours.

(ii) Unknown crystals:-

NMR (D_2O):- Quartet τ 6.5-6.85 (intensity 19)

Triplet τ 8.5-8.7 (intensity 29)

IR (KBr disc):- Bands at 2735,2680,2500 and 1650cm^{-1}

Both the NMR and IR spectra are identical to an authentic sample of triethylamine hydrochloride spectra.

Halogen determination (sodium fusion test)

Both this unknown and the reaction product were showed to contain chlorine.

TLC (silica gel)(n-butanol:acetone:water/4:5:1):

Only one spot was detected with R_f0.42

(3) <u>The preparation of tetra(1-iminazolyl)silane</u>

<u>The preparation of 1-potassium imidazole</u>

Clean potassium(7.8g, 0.2 mole) was placed in dry benzene(200cm^3) in a two-necked flask with condenser(closed with $CaCl_2$ tube). The suspension was gently warmed until the potassium had melted. Pre-dessicated imidazole(17g, 0.25 mole) was added to the solution

in small portions over a period of 30 minutes, during the addition of imidazole a large volume of gas was given off(careful). The reaction mixture was being stirred during the entire process. After the addition of imidazole, the reaction was refluxed 2 hours. The light blue solid which was floating at the top of the solution was collected by filtration and washed with dry ether several times and stored over P_2O_5 under vacuum; yield 8g(30%).

The reaction of 1-potassium imidazole with $SiCl_4$

(i) 1-potassium imidazole(15g, 0.14 mole) was suspended in dry benzene(100cm^3) with stirring in a flask fitted with condenser, dropping funnel(both closed with $CaCl_2$ tubes), thermometer. The reaction mixture was cooled down to about 5° in an ice bath. Freshly distilled $SiCl_4$(4cm^3, 0.04 mole) diluted in dry ether(5cm^3) was added from the dropping funnel dropwise. There was no change in reaction temperature during the addition of the silane. It was then refluxed 2 hours and the solid was filtered off and the filtrate was concentrated under reduced pressure but nothing was collected.

(ii) Same procedure as (i) except xylene was used instead of benzene and the initial silylation was carried out at 35°. However, no significant product was collected.

(4) The preparation of tetra(1-pyrryl)silane[88,141]

The preparation of 1-potassium pyrrole

Clean potassium(4.9g, 0.13 mole) was melted in hot benzene(150cm^3) in a three-necked flask fitted with a stirrer, condenser, dropping funnel(both closed with $CaCl_2$ tubes). Freshly distilled pyrrole (8.7cm^3, 0.13 mole) in dry benzene(20cm^3) was added dropwise from the dropping funnel. During the addition of pyrrole large volume of gas was given off(careful). The reaction mixture was refluxed 2 hours

and left overnight. There remained a light blue solid and some metallic droplets were floating at the top of the solution. Therefore more freshly distilled pyrrole($2cm^3$, 0.03 mole)(total amount of pyrrole used in this reaction was $10.7cm^3$, 0.16 mole) was added and further refluxed 2 hours. However, metallic potassium was still present and the experiment was abandoned.

The reaction of magnesium-iodo-pyrrole with $SiCl_4$

To $200cm^3$ of an ether solution of methylmagmesium iodide, freshly prepared from methyl iodide($18.7cm^3$, 0.3 mole) and magnesium turning (7.4g, 0.3 mole), was added freshly distilled pyrrole($17cm^3$, 0.25 mole). The solution was refluxed 15 minutes. To this cooled solution 0° a solution of freshly distilled $SiCl_4$($7cm^3$, 0.06 mole) diluted in dry ether($10cm^3$) was added at such a rate that the reaction temperature was kept below 5°. It was then refluxed 2 hour and left overnight. The solid which precipitated out was filtered off and the filtrate was evaporated under reduced pressure to leave behind a solid black cake. This black cake was transferred to a Soxhlet extractor and extracted with petroleum ether($120-160^\circ$) for 5 hours. Tetra(1-pyrryl)-silane was collected as colourless needles; yield 0.35g; m.p. $165-167^\circ$ (rapid heating).

The reaction of tetra-(1-pyrryl)silane with anhydrous methanol

Tetra(1-pyrryl)silane(0.3g) was refluxed in anhydrous methanol ($10cm^3$) for 3 hours. Tetramethoxysilane[$Si(OCH_3)_4$] was detected from the reaction product by glc.

5.2. THE REACTION OF METHYL α-D-GLUCOPYRANOSIDE WITH TETRAMETHOXYSILANE

(1) The preparation of tetramethoxysilane[142]

Freshly distilled $SiCl_4$ ($57cm^3$, 0.5 mole) in dry ether ($200cm^3$) in a three-necked flask fitted with condenser, dropping funnel (both closed with $CaCl_2$ tubes), thermometer and a magnetic stirrer, was cooled in a ice-salt bath to 0°. To this well stirred solution anhydrous methanol ($80cm^3$, 2moles) was added from the dropping funnel at such a rate that the temperature was kept below 5°. After the addition of methanol the solution was refluxed 2 hours. Anhydrous triethylamine ($20cm^3$) was added to remove the last traces of hydrogen chloride. The precipitate was filtered off and the filtrate was distilled under atmospheric pressure. Fraction between 118-122° was collected; yield $20cm^3$ (26%).

NMR (neat):-

Singlet τ 6.5

IR (neat):-

Bands at 1100 and 830cm^{-1}

(2) The reaction of methyl α-D-glucopyranoside with tetramethoxysilane in 4:1 molar ratio

Anhydrous methyl α-D-glucopyranoside (5.1g, 0.026 mole) and tetramethoxysilane ($1cm^3$, 0.0065 mole) were refluxed in dry DMF or pyridine ($10cm^3$) for 6 hours. The reaction product was recovered by the addition of dry ether ($100cm^3$). The solvent was removed by filtration and the solid collected was dried over $CaCl_2$.

NMR (D_2O solution):- Multiplet τ 6.1-6.4 (intensity 9)

Singlet τ 6.58 (intensity 9)

IR (KBr disc):- The spectrum was very similar to that of methyl α-D-glucopyranoside except the intensity and sharpness of some of the lines between 1400-1200cm^{-1} were different for our product. However, one extra band was observed 1650cm^{-1} when DMF was used as solvent.

TLC (silica gel)(n-butanol:acetone:water/4:5:1)

One spot was revealed R_f 0.60

Reference: methyl α-D-glucopyranoside R_f 0.61

Silicon analysis:-

Calculated - Si 3.5%

Found - the silicon content varied from different batches of product (2.5-4.2%)

Stability in water: An aqueous solution of the product(10%) was readily turned to gel within 24 hours.

GLC (80°):-No methanol was detected from the reaction solution.

Melting point:- The exact melting point could not be obtained as the product only chared around 245-265° this value also varied from different batches of product.

5.3. THE REACTIONS OF OXIRANES WITH Me_3SiCl and $SiCl_4$

(1) The reaction of propylene oxide with Me_3SiCl[108]

Propylene oxide(35cm^3 0.5 mole) was placed in a three-necked flask fitted with mercury sealed stirrer, thermometer, dropping funnel and condenser(both closed with $CaCl_2$ tubes). Me_3SiCl(63cm^3, 0.5 mole) was added from the dropping funnel at such a rate that the temperature of the reaction mixture was kept below 32°. After the addition of silane, the mixture was heated to 40° for 8 hours and

left overnight at room temperature. The product (2-chloropropoxy-silane) was collected by vacuum distillation; b.p. 24-26°(14mm); yield 30cm^3.

NMR (C_6H_6 solution):- Multiplet τ 6.0-6.5 (intensity 1)
Doublet τ 8.7-9.0 (intensity 3)
Singlet τ 9.8 (intensity 9)
multiplet τ 6.7-6.9 (intensity 2)

IR (neat):- Bands at 1250,850,750cm^{-1}

(2) The reaction of propylene oxide with $SiCl_4$

Propylene oxide(30cm^3, 0.43 mole) was placed in a three-necked flask fitted with mercury sealed stirrer, thermometer, dropping funnel and condenser(both closed with $CaCl_2$ tubes). A solution of freshly distilled $SiCl_4$(11cm^3, 0.1 mole) in dry ether(20cm^3) was added from the dropping funnel at such a rate that the reaction temperature was kept below 32°. This reaction was highly exothermic and external cooling was required. After the addition of silane the reaction was worked up as last experiment. Tetra(2-chloropropoxy)silane was collected at 164-165°(4mm.); yield 17cm^3.

NMR (C_6H_6 solution):- Multiplet τ 5.5-6.2 (intensity 3)
Doublet τ 6.6-6.8 (intensity 4)
Doublet τ 8.7-8.9 (intensity 7)

Elemental analysis(%)

	C	H
Calculated	35.8	6.0
Found	35.6	6.4

(3) The reaction of cyclohexene oxide with $SiCl_4$[109]

Freshly distilled $SiCl_4$($1cm^3$, 0.009 mole) in dry ether($20cm^3$) were placed in a three-necked flask fitted with mercury sealed stirrer, thermometer, condenser and dropping funnel (both closed with $CaCl_2$ tubes). Cyclohexene oxide($3.6cm^3$, 0.036 mole) in dry ether($5cm^3$) was added from the dropping funnel at such a rate that the reaction temperature was kept below 32°, external cooling was necessary. After the addition of silane the reaction mixture was heated at 65° for 10 hours and left overnight. The solution was washed three times with water, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue solidified upon standing over P_2O_5 under vacuum.

NMR ($CHCl_3$ solution):- Multiplet τ 5.9-6.3 (intensity 16)
Multiplet τ 7.7-8.8 (intensity 65)

5.4. THE REACTIONS OF ANHYDRO SUGARS WITH Me_3SiCl

(1) The preparation of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose[118,143]

Anhydrous α-D-glucose(100g, 0.56 mole) was stirred vigorously in A.R. grade acetone($2000cm^3$) in an ice-bath. Concentrated sulphuric acid($80cm^3$) was added in $15cm^3$ portion at 15 minutes intervals, while maintaining the temperature below 10°. After the addition of the acid, the vigorous stirring was continued for 5 hours, allowing the temperature to rise to room temperature. The solution was cooled again with an ice-bath, and 50% NaOH(123g of NaOH in $150cm^3$ of water) was added slowly with stirring to near neutrality, a small amount of solid sodium hydrogen carbonate was added to maintain the solution

at near neutrality. After standing overnight, the salts were filtered off and the filtrate was concentrated under reduced pressure to a thick syrup which solidified upon standing. The solid was dissolved in warm $CHCl_3$ and the solution was extracted with water twice. The $CHCl_3$ solution was then washed with water, and the water was washed with $CHCl_3$. The respective water and $CHCl_3$ solutions were combined. The solutions were concentrated under reduced pressure to thick syrups. The $CHCl_3$ fraction yield 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose which was recrystallized from cyclohexane; yield 115g (80%) m.p. 108-110°. While the water fraction gave 1,2-O-isopropylidene-α-D-glucofuranose; yield 15 g(12%); m.p. 158-159°.

Spectroscopic data of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose

NMR ($CDCl_3$ solution):- Doublet τ 4.0-4.1 (intensity 2)
Doublet τ 5.4-5.5 (intensity 2)
Multiplet τ 5.6-6.0 (intensity 10)
Doublet τ 7.2-7.3 (intensity 2)
Two doublets τ 8.4-8.8 (intensity 23)

IR (KBr disc):- Sharp band at 3400cm^{-1} and two sharp bands at 1380-1360cm^{-1}

1,2:5,6-di-O-isopropylidene-α-D-glucofuranose(4.5g) was dissolved in methanol(23.4cm^3), treated with 23.4cm^3 of 0.8% H_2SO_4, and allowed to stand 22 hours. The solution was then neutralized with barium carbonate, boiled and filtered. The filtrate was evaporated under reduced pressure. Colourless needles of 1,2-O-isopropylidene-α-D-glucofuranose were formed by crystallization from methanol-ether; yield 2g (53%); m.p. 159-161°.

NMR ($CDCl_3$ solution):- Doublet τ 4.0-4.1 (intensity 2)
Multiplet τ 5.4-6.1 (intensity 13)

Multiplet 7.1-7.3 (intensity 8)

Doublet 8.4-8.8 (intensity 25)

IR (KBr disc):- Broad band at3550-3000cm^{-1}, two sharp bands at 1380-1360cm^{-1}.

1,2-O-isopropylidene-α-D-glucofuranose(9g) was dissolved in dry pyridine(50cm^3), and the solution was cooled to about 5°. p-Toluenesulphonyl chloride(7g) dissolved in cold chloroform(15cm^3) was added carefully over a period of 15 minutes, and the solution was stirred for 12 hours. The solvent was removed under reduced pressure at a temperature not greater than 40°, and the residue dissolved in $CHCl_3$. The $CHCl_3$ solution was washed first with cold diluted sulphuric acid, then with sodium hydrogen carbonate and finally with water, dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The residue was dissolved in warm ether and benzene was added until a slight turbidity developed, and the mixture was kept in a refrigerator(4°). 1,2-O-isopropylidene-6-O-p-toluenesulphonyl-α-D-glucofuranose crystallized out; m.p. 106-108°: yield 7.5g (40%).

10 g of this compound was dissolved with warming in dry $CHCl_3$ (25cm^3), and to this cooled solution was added dry methanol(15cm^3) containing clean sodium(0.75g) dissolved in it. The gelatinous mixture was stirred vigorously in an ice-bath. After 15 minutes, water was added to dissolve the sodium p-toluenesulphate which had separated. The $CHCl_3$ layer was separated, and the aqueous layer was extracted once with $CHCl_3$. The combined $CHCl_3$ solutions were dried over anhydrous magnesium sulphate, filtered, and the filtrate was concentrated under reduced pressure to give a solid which was recrystallized from benzene; yield 3g(56%) of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose; m.p. 131-133° $[\alpha]_D$ -25° (H_2O).

NMR ($CDCl_3$ solution):-	Doublet	τ 4.0-4.1	(intensity	2)
	Doublet	τ 5.5-5.55	(intensity	2)
	Singlet	τ 5.7-5.8	(intensity	2)
	Multiplet	τ 5.9-6.1	(intensity	2)
	Multiplet	τ 6.5-6.7	(intensity	2)
	Multiplet	τ 7.0-7.2	(intensity	7)
	Doublet	τ 8.6-8.8	(intensity	18)

Tests for anhydro sugar containing an oxirane ring

(i) A solution of 1m.mole of the sugar epoxide in water, aqueous acetone, or aqueous dioxane is treated with neutralized 0.2 mole sodium thiosulphate solution containing phenolphthalein. Development of a pink coloration, occurring slowly at room temperature or more rapidly on warming indicates the presence of an epoxide[144].

(ii) A 5% solution of NaI in N-butanol containing 0.01% methyl red has been used as a spraying reagent for detecting epoxide on a chromatogram[145]. Epoxide will produce a yellow spot on a red background.

This 5,6-anhydro glucose gave positive results to these two tests.

Acetylation of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose

5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose(5g) was acetylated by treatment with pyridine($20cm^3$) and acetic anhydride ($10cm^3$) at room temperature for 24 hours. The reaction mixture was poured into cracked ice, the brownish liquid was taken up by $CHCl_3$ ($50cm^3$). The aqueous layer was separated and extracted once with $CHCl_3$. The combined $CHCl_3$ solutions were washed three times with cold water, dried over anhydrous sodium sulphate, filtered, the filtrate was

concentrated under reduced pressure to a thick syrup. The last traces of pyridine was removed by repeated evaporating of toluene.

NMR ($CDCl_3$ solution):- The spectrum was very similar to the original 5,6-anhydro sugar with one extra peak at τ 7.9 (intensity 9).

IR (neat):- Broad band at 1750cm^{-1} and also a broad band at 3500cm^{-1}

TLC (silics gel)(methanol:benzene/5:95)

Four spots were revealed (a) R_f 0.38

(b) R_f 0.55

(c) R_f 0.62

(d) R_f 0.71

Reference: 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose

R_f 0.38

Both (a) and (d) gave positive result to NaI test.

(2) <u>The reaction of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose with Me_3SiCl</u>

5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose(1g) in dry 1,4-dioxan(10cm^3)was added to a flask fitted with condenser and dropping funnel(both closed with $CaCl_2$ tubes) and a magnetic stirrer. Me_3SiCl(2cm^3) diluted in dry 1,4-Dioxan(5cm^3) was added dropwise over a period of 15 minutes. After the addition of silane, the mixture was refluxed for 6 hours and left overnight. Dry pyridine(2cm^3) was then added and followed by a further addition of Me_3SiCl(1cm^3). The reaction mixture was again refluxed for another 2 hours. The solid was filtered off and the filtrate was concentrated under reduced pressure. The last traces of pyridine were removed by repeatedly evaporating with dry toluene. During this stage more precipitate

came down, the syrup was diluted with dry ether(10cm^3), filtered and again concentrated under reduced pressure. The residue was kept over P_2O_5 under vacuum; yield 1g(57%).

GLC (250°): Only one peak was recorded.

IR (neat): No absorption bands between 3500-3000cm^{-1}

Three sharp bands at 1250,850,750cm^{-1}

NMR ($CDCl_3$ solution):

Doublet	τ	4.1-4.2	(intensity 2)
Doublet	τ	5.5-5.6	(intensity 2)
Singlet	τ	5.8	(intensity 2)
Multiplet	τ	5.85-6.0	(intensity 2)
Multiplet	τ	6.2-6.3	(intensity 6)
Doublet	τ	8.5-8.8	(intensity 12)
Singlet	τ	9.8	(intensity 17)

Test for 6-deoxy hexose[146]

To 1 cm^3 of a solution containing 50μg or more of a methylpentose in a test tube are added with cooling 4.5 cm^3 of a mixture of 1 volume of water and 6 volumes of H_2SO_4. The mixture is then warmed to 20-22° for few minutes, held for either 3 or 10 minutes in an boiling water bath, and finally cooled in tap water. To the cold solution 0.1 cm^3 of 3% aqueous cysteine hydrochloride is added with shaking. A greenish yellow colour appears and remains practically unchanged for 24 hours. The extend of colour developed in the cysteine reaction depends upon the time of heating. The green yellow colour is characteristic for methylpentose(10 minutes). For this time period, pentose,hexoses,hexuronic acid give a pink colour. Hexoses show a yellow colour(3 minutes), the intensity decreases rapidly.

During the initial hydrolysis of the reaction product by the sulphuric acid, the colour of the solution turned to dark brown and the addition of cysteine hydrochloride did not cause any change of colour.

(3) The preparation of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside[147]

A mixture of anhydrous methyl α-D-glucopyranoside(120g, 0.62 mole) and powdered zinc chloride(90g), and benzaldehyde(freshly vacuum distilled)($300cm^3$) were vigorously stirred in a flask fitted with mercury sealed stirrer and condenser(closed with $CaCl_2$ tube) for 24 hours. The mixture was poured slowly, with stirring, into $200cm^3$ of ice cold water; the oily material gradually solidified upon stirring. The mixture was refrigerated overnight. Petroleum ether $60-80^\circ$($200cm^3$) was added and stirred for 30 minutes to remove excess benzaldehyde, and the product was separated on a Buchner funnel. The solid material was transferred to a clean flask and stirred with another portion of petroleum ether for 30 minutes. This was repeated twice and the product finally washed twice with cold water($300cm^3$). The product was air dried overnight; yield 105g (60%) of crude methyl 4,6-O-benzylidene-α-D-glucopyranoside

To a solution of crude methyl 4,6-O-benzylidene-α-D-glucopyranoside(50g) in dry pyridine($300cm^3$) was added 50% excess of p-toluenesulphonyl chloride(100g), and the mixture was stirred at room temperature for 4 days. The mixture was poured onto cracked ice, where upon the ditosyl compound crystallized readily. $CHCl_3$($400cm^3$) was then added to dissolve the ditosyl compound, the aqueous layer was separated. The chloroform solution was washed twice with cold

water(200cm^3), followed with three times of cold diluted sulphuric acid. The chloroform solution was then washed successively with small portions of cold water, aqueous sodium hydrogen carbonate, and water and finally dried over anhydrous sodium sulphate. A small amount of activated charcoal was added, the solid was filtered off and the filtrate was concentrated under reduced pressure to a thin syrup, ether was added to effect crystallization. Methyl 4,6-O-benzylidene-2,3-di-O-p-toluenesulphonyl-α-D-glucopyranoside was recrystallized from chloroform-ether; needles, m.p. 147-149°, $[\alpha]_D$ +10° ($CHCl_3$), yield 84g (46%).

A solution of the ditosyl compound (50g) in dry chloroform(600cm^3) was cooled in ice, and a cold solution of anhydrous methanol(250cm^3) containing clean sodium(9g) was added. The mixture was kept in the refrigerator for 4 days and then 1 day at room temperature with occasional shaking. The solution was diluted with water; the chloroform layer was separated, and the aqueous layer was extracted once with addition portion of chloroform(50cm^3). The combined chloroform solutions were washed with water, dried over anhydrous sodium sulphate, filtered, and the filtrate was concentrated under reduced pressure. The product crystallized readily and was filtered and washed with dry ether; yield 10g (78%). Methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allo-pyranoside was recrystallized from chloroform-ether, m.p. 197-199°, $[\alpha]_D$ +136.5° ($CHCl_3$).

NMR ($CDCl_3$):-

Multiplet	τ 2.6-2.7	(intensity	15)
Singlet	τ 4.45	(intensity	3)
Doublet	τ 5.1	(intensity	3)
Multiplet	τ 5.6-6.2	(intensity	16)
Singlet	τ 6.6	(intensity	10)

IR (KBr disc):- Bands at 1250 and 750 cm^{-1}

(4) The reaction of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allo-pyranoside with Me_3SiCl

The 2,3-anhydro-alloside(3g, 0.011 mole) was dissolved in dry THF($200cm^3$) at 35° in a flask fitted with condenser, dropping funnel (both closed with $CaCl_2$ tubes), thermometer and a magnetic stirrer. Me_3SiCl($1.6cm^3$, 0.013 mole) in dry THF($5cm^3$) was added from the dropping funnel dropwise, there was no change of reaction temperature during the addition of the silane. The solution was refluxed for 10 hours. TLC(silica gel; methanol:benzene/5:95) revealed two spots with the following R_f value: (i)0.81 (ii)0; the R_f value for the anhydro-alloside was 0.81.

The reaction was further refluxed 48 hours. Fours spots were detected by TLC with the following R_f values: (i)0.79 (ii)0.71 (iii)0.44 (iv)0.

Anhydrous triethylamine($5cm^3$) was added to the reaction solution and immediately a precipitate started to come down. The solid was filtered off and the filtrate was concentrated under reduced pressure to a syrup which solidified upon standing.

NMR ($CDCl_3$ solution):- No peak was recorded between τ9.5 and τ10.

IR (neat):- Weak absorption bands at 1250,850 and 750cm^{-1}.

Separation of the reaction product by column chromatography

The reaction product(1g) was dissolved in $CHCl_3$($2cm^3$) and put through a silica gel column using $CHCl_3$ as eluant. After five fractions were collected($50cm^3$ each), methanol was used as eluant and three more fractions($50cm^3$) were collected.The solvents were evaporated under reduced pressure to a thin syrup and examined by TLC.

$CHCl_3$: Three spots were detected from all five fractions with the following R_f values(i)0.79 (ii)0.71 (iii)0.44

Methanol: Only one spot was detected at the base line.

The methanol fractions were combined and concentrated to a thick syrup and kept over P_2O_5 under vacuum. IR(neat) spectrum was obtained and appeared very similar to that of methyl α-D-glucopyranoside. When this product was examined by TLC(silica gel; n-butanol:acetone: water/4:5:1), it had the same R_f value as methyl α-D-glucopyranoside 0.61.

The $CHCl_3$ fractions were combined and concentrated to a thick syrup and chromatographing on silica gel column using $CHCl_3$ as eluant. Ten fractions($10cm^3$) were collected and examined by TLC(methanol-benzene). The first two fractions gave one faint spot at R_f0.85 and these two fractions were combined and concentrated under reduced pressure. The IR(neat) spectrum of this syrup was very similar to that of benzaldehyde.

The last three fractions showed only one spot R_f0.45 and they were combined and concentrated under reduced pressure and stored over P_2O_5 under vacuum, the syrup solidified upon standing.

IR (neat):- Broad band at 3600-3000cm^{-1}

Bands at 1250(very weak), 850(medium)and 750(very strong)cm^{-1}

Broad band between 1150-1000cm^{-1}

NMR ($CDCl_3$ solution):- The spectrum was very similar to that of the 2,3-anhydro-alloside except there was one broad multiplet at τ8.2-8.4. There was no signal around τ9.5-10.

The other five fractions contained two components at R_f0.78 and R_f0.72, the separation of these two components was not successful by column chromatography.

5.5 THE REACTIONS OF CARBOHYDRATES WITH PROTECTIVE FUNCTIONS WITH Me_3SiCl AND $SiCl_4$

(1) The preparation of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside[148,149]

A solution of anhydrous methyl α-D-glucopyranoside(53g,0.27 mole) in dry pyridine(250cm^3) was treated with triphenylchloromethane(trityl chloride)(74g, 0.27 mole) for 16 hours at room temperature and then for 2 hours at 40°. The solution was poured into ice-water. The thick syrup solidified after vigorous stirring. The pyridine was removed from the solid product, most of it by trituration with water and the rest by extraction of a chloroform solution of the product with cold diluted sulphuric acid; the chloroform solution was washed with cold water, aqueous sodium hydrogen carbonate, again with water and finally dried over anhydrous magnesium sulphate. After filtering off the magnesium sulphate the filtrate was concentrated under reduced pressure to yield methyl 6-O-trityl-α-D-glucopyranoside as a glass, yield 90g(77%).

This trityl glucoside(90g) was acetylated by treating with pyridine(300cm^3) and acetic anhydride(180cm^3) for 24 hours at room temperature. The solution was poured into ice-water, the thick syrup solidified after some vigorous stirring. The product was taken up by chloroform and the chloroform solution was washed with cold water, cold dilute sulphuric acid, aqueous sodium hydrogen carbonate, again with water and finally dried over anhydrous magnesium sulphate. The solid was filtered off and the filtrate was concentrated under reduced pressure to a syrup. Methyl 2,3,4-tri-O-acetyl-6-O-trityl-α-D-glucopyranoside crystallized on trituration with a little methanol and recrystallized from acetone-ether-petroleum ether(60-80°); yield 86g(98% calaculated from the original glucopyranoside),m.p. 134-136°

Methyl 2,3,4-tri-O-acetyl-6-O-trityl-α-D-glucopyranoside(4g, 0.01 mole) was dissolved in glacial acetic acid($8cm^3$) by gentle warming. This solution was cooled in ice cold water and before crystallization of acetic acid set in, ice cold hydrogen bromide in acetic acid(45% w/v; 1.8g HBr)($1.2cm^3$) was added with vigorous stirring. The trityl bromide precipitated out in few seconds and was filtered off in a glass funnel. The filtrate was poured into ice-water and the solid was taken up by chloroform($100cm^3$). The chloroform solution was washed with water twice and dried over anhydrous sodium sulphate, filtered, and the filtrate was concentrated under reduced pressure to a syrup. This syrup was taken up by ether and petroleum ether($60-80^\circ$) was added until the solution became cloudy. Upon rubbing with glass rod methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside crystallized out, recrystallized from ether-petroleum ether; yield 1g(33%), m.p. $109-111^\circ$, $[\alpha]_D+147^\circ$ ($CHCl_3$).

NMR ($CDCl_3$ solution):-

Multiplet	τ 4.3-4.6	(intensity 2)
Multiplet	τ 4.8-5.2	(intensity 7)
Multiplet	τ 6.0-6.4	(intensity 7)
Singlet	τ 6.6	(intensity 7)
Broad multiplet	τ 7.4-7.7	(intensity 2)
Doublet	τ 7.9-8.0	(intensity 21)

IR (KBr disc):- Sharp band at $3500cm^{-1}$

Very strong band at 1750,1250 and $1050cm^{-1}$

(2) The reaction of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside with Me_3SiCl

A solution of triacetyl glucoside(7.5g, 0.02 mole) in dry pyridine($50cm^3$) in a flask fitted with condenser, dropping funnel (both closed with $CaCl_2$ tubes), thermometer and magnetic stirrer was cooled to 0° in an ice-salt bath, Me_3SiCl($3cm^3$, 0.02 mole) in dry ether($5cm^3$) was added from the dropping funnel at such a rate that the reaction temperature was kept below 4°. After the addition of silane, the mixture was refluxed for 2 hours. It was then diluted with dry ether($50cm^3$) and left overnight. The solid was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup and stored over P_2O_5 under vacuum.

Two spots were revealed when the reaction product was examined by TLC(silica gel; methanol:benzene/1:9) with the following R_f values: (i)0.74 (ii)0.45, the second spot had same R_f value as the starting material.

Column separation of the reaction product

The compound having R_f0.74 was isolated from the reaction products by column chromatography using silica gel as packing and methanol:benzene/1:9 as eluant. The product(syrup) was kept over P_2O_5 under vacuum.

NMR ($CDCl_3$ solution):-

Multiplet	τ 4.3-4.6	(intensity	2)
Multiplet	τ 4.8-5.0	(intensity	2)
Singlet	τ 5.0	(intensity	2)
Multiplet	τ 5.1-5.3	(intensity	2)
Multiplet	τ 6.1-6.4	(intensity	5)
Singlet	τ 6.6	(intensity	5)

Doublet	7.9-8.0	(intensity 14)
Singlet	9.8	(intensity 17)

IR (neat):- No absorption band between 3600-3100cm^{-1}

Three strong bands at 1250,850 and 750cm^{-1}

Stability test of the reaction product

To a solution of the reaction product(0.5g) in acetone(5cm^3), water was added with stirring until the sugar began to separate out. A few drops of acetone were added to redissolve the sugar. The solution was kept in a well-stoppered flask and left at room temperature for 24 hours. Only one spot(R_f0.45) which had the same R_f value as methyl-2,3,4-tri-O-acetyl-α-D-glucopyranoside was detected by TLC(silica gel; methanol:benzene/1:9).

(3) The reaction of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside with $SiCl_4$

The carbohydrate(11.4g, 0.036 mole) in dry pyridine(50cm^3) in a flask fitted with condenser, dropping funnel(both closed with $CaCl_2$ tubes), thermometer and a magnetic stirrer was cooled to 0° in an ice-bath. Freshly distilled $SiCl_4$(1cm^3, 0.009 mole) in dry ether (5cm^3) was added from the dropping with stirring at such a rate that the reaction temperature was kept below 5°. The reaction mixture was refluxed for 2 hours after the addition of the silane. It was then diluted with dry ether(50cm^3), the solid was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup which solidified upon standing under vacuum.

Three components were revealed by TLC(silica gel; ethyl acetate: ethanol:benzene/10:5:40) with the following R_f value (i)0.79 (ii)0.69 (iii)0.61. The R_f value for the starting material was 0.61.

Attempts to separate each component by column chromatography were not successful. IR and NMR spectroscopic data were not obtained for the mixed products.

Ethanolysis of the reaction products

The reaction mixture(1g) were dissolved in ethanol(15cm^3) and water was added until the solution became turbid, this turbidity was removed by further addition of few drops of ethanol. The solution was refluxed for 2 hours. It was then analysed by TLC(silica gel):

(a) Solvent: methanol:benzene/1:9

Only one spot(R_f 0.45) was observed which had same R_f value as the tri-acetyl glucoside.

(b) Solvent: ethyl acetate:ethanol:benzene/10:5:40

Two components were detected with the following R_f value (i)0.69 (ii)0.62. The R_f value for the tri-acetyl glucoside in this solvent system was 0.61

Stability of methyl 2,3,4-tri-O-acetyl-α-D-gluco-pyranoside in aqueous ethanol

The reaction conditions were the same as for previous experiment. Two components with the following R_f values were detected by TLC (silics gel: ethylacetate:ethanol:benzene/10:5:40) (i)0.69 (ii)0.61. The second component had the same R_f value as the original tri-acetyl glucoside.

Enzymatic hydrolysis of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside

(i) Acetylesterase(E.C. No.3.1.1.6.)

From orange peel partial purified suspension in 2.5 molar $(NH_4)_2SO_4$ solution, pH6.5, containing 0.1 molar sodium oxalate;

2 unit/mg; activity:one unit will produce 1.0 μ mole of acetic acid from triacetin per minute at pH 6.5 at 35°. Methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside(0.05g) in water(10cm^3, the glucopyranoside was only slightly soluble) and acetylesterase(5mg, 10 units) were incubated at 25° for 4 days. It was then analysed by TLC against an authentic sample of methyl α-D-glucopyranoside, however, no methyl α-D-glucopyranoside was detected from the hydrolytic product. When the reaction temperature was raised to 35° the same result was obtained.

(ii) Esterase(E.C. No. 3.1.1.1.)

From porcine liver, suspended in 3.2 molar $(NH_4)_2SO_4$ solution at pH 8, 100 units/mg, one unit will hydrolyse 1.0 μmole of ethyl butyrate to butyric acid and ethanol per minute at pH 8 at 25°.

The hydrolysis of the tri-acetyl glucoside was carried out at 25° ofr 2 days, no methyl α-D-glucopyranoside was detected by TLC.

Enzymatic hydrolysis of methyl α-D-glucopyranoside

(i) α-amylase(hog pancrease)

1 mg will liberate approximately 15mg of maltose from starch in 3 minutes at pH 6.9 at 20°.

A solution of methyl α-D-glucopyranoside(1g) in water(5cm^3) with α-amylase(20mg) were incubated at 20° for 2 days. However, D-glucose could not be detected from the hydrolytic product by TLC.

(ii) α-glucosidase(E.C. No. 3.2.1.20.)

Type I from yeast partially purified powder, 9.8 units/mg, one unit will liberate 1.0 μmole of D-glucose from p-nitrophenyl α-D-glucopyranoside per minute at pH6.8 at 37°.

A solution of methyl α-D-glucopyranoside(1g) in water(5cm^3) with α-glucosidase(2mg) were incubated at 37° for 2 days. However, D-glucose could not be detected from the reaction by TLC. The optical rotation of the reaction solution after 2 days was $[\alpha]_D$ +158.2°, while $[\alpha]_D$ for D-glucose was +157° (water).

(4) The preparation of methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside[83,90]

Anhydrous methyl α-D-glucopyranoside(29g, 0.15 mole) was dissolved in dry pyridine(250cm^3) in a flask fitted with condenser, dropping funnel(both closed with $CaCl_2$ tubes), thermometer and a magnetic stirrer, the mixture was cooled in a ice-bath to 0°. Freshly distilled Me_3SiCl(114cm^3, 0.9 mole) was added from the dropping funnel at such a rate that the reaction temperature was kept below 5°. After the addition of silane, the reaction mixture was refluxed for 1 hour and left overnight. The solid was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup which yielded crude methyl 2,3,4,6-tetra-O-trimethylsilyl-α-D-glucopyranoside 55g, (76%).

A solution of crude methyl 2,3,4,6-tetra-O-trimethylsilyl-α-D-glucopyranoside(55g) in anhydrous methanol(200cm^3) in a flask was cooled to 0°, and a ice cold solution of potassium carbonate(0.11g, 0.8 mMole in 25cm^3 anhydrous methanol) was added. After stirring for

45 minutes at 0° the carbonate was destroyed by the addition of an equivalent amount of glacial acetic acid($0.1cm^3$) to the reaction solution. Distilled water was then added to the latter with vigorous stirring, until the solution remained cloudy. This turbidity was carefully removed by the addition of a just sufficient amount of methanol, and the resulting solution was placed in a refrigerator at 5°. The crystallization was completed after 7 days and the product (methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside) was recrystallized from cold aqueous methanol; yield 30g(64%), m.p.98-100°, $[\alpha]_D$ +90° ($CHCl_3$).

NMR ($CDCl_3$ solution):-

Multiplet	4.3-4.6	(intensity 2)
Multiplet	4.8-5.2	(intensity 2)
Multiplet	6.0-6.4	(intensity 2)
Singlet	6.6	(intensity 2)
Singlet	8.5	(intensity 2)
Doublet	9.8-9.9	(intensity 45)

^{29}Si NMR($CDCl_3$ solution)(proton decoupled):-

1. 17.6 ppm. (intensity 4611 %)
2. 18.6 ppm. (intensity 7598 %)

IR (KBr disc):-

Sharp band at $3500cm^{-1}$

Three strong bands at $1250, 850, 750cm^{-1}$

Broad band at $1050cm^{-1}$

(5) <u>The reaction of methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside with $SiCl_4$</u>

The glucoside(14.8g, 0.036 mole) was dissolved in dry pyridine ($50cm^3$) in a flask fitted with condenser, dropping funnel(both closed

with $CaCl_2$ tubes), thermometer and a magnetic stirrer was cooled to 0° in an ice bath. A solution of freshly distilled $SiCl_4$($1cm^3$, 0.009 mole) in dry ether($5cm^3$) was added from the dropping funnel at such a rate that the reaction temperatuer did not rise above 5°. After the addition of silane, the reaction was heated to 60° for 1 hour and diluted with dry ether($20cm^3$). The solid was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup. When the reaction was examined by TLC(silica gel; methanol:petroleum ether 60°-80°/4:96) two components were revealed with the following R_f values: (i)0.93 (ii)0.52. The spot(i) had higher intensity of the two, while the spot(ii) had the same R_f value as the starting material.

Column separation of the reaction products

The component(R_f0.93)(white solid) was isolated from the reaction mixture by chromatographing in a silica gel column using methanol: petroleum ether 60-80°/4:96 as solvent; m.p. 73-74°.

Elemental analysis(%):-		C	H
(i) Starting material:	Calculated	46.8	9.3
(ii) Reaction product :	Calculated	46.2	8.9
	Found	45.8	9.3

IR (KBr disc):- No absorption band was recorded between 3600-3200cm^{-1}.

NMR ($CDCl_3$ solution):- The spectrum was very similar to the starting material. A small peak at τ 8.5 which was corresponded to OH grouping was recorded.

^{13}C NMR ($CDCl_3$ solution)(proton decoupled):-

No.	ppm.	intensity %
1.	103.1	979
2.	102.6	3368

3.	81.5	1275
4.	80.1	1477
5.	78.7	1862
6.	78.3	3552
7.	77.1	3588
8.	74.8	3138
9.	65.9	2382
10.	57.7	3032
11.	4.5	5728
12.	4.3	6838
13.	3.8	5819

^{29}Si NMR ($CDCl_3$ solution)(proton decoupled):-

No.	ppm.
1.	17.6
2.	18.6

Another spectrum was obtained after 24 hours.

No.	ppm.
1.	16.8
2.	17.8
3.	18.6

When these peaks were expanded, the first peak(16.8ppm) showed a doublet.

Methanolysis of methyl 2,3,4-tri-O-trimethylsilyl-α-D-gluco-pyranoside

To a solution of methyl 2,3,4-tri-O-trimethylsilyl-α-D-gluco-

pyranoside(0.5g) in methanol(5cm^3), water was added with stirring until the solution remained cloudy. This turbidity was carefully removed by addition of few drops of methanol. The solution was then refluxed and examined by TLC at time intervals.

TLC (silica gel):-

(a) Solvent: methanol:petroleum ether 60-80°/4:96

Only one spot was revealed on the base line after 2 hours of hydrolysis.

(b) Solvent: n-butanol:acetone:water/4:5:1

Only one spot(R_f0.61) was detected.

Reference: methyl α-D-glucopyranoside R_f0.61

GLC (100°):-

The hydrolysed product(after 2 hours of hydrolysis) was examined by GLC. The results are summarized on Table V

Table V

GLC results of methanolysis of methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside

Sources	Materials	Retention distance(mm)
References	1. methanol	6
	2. hexamethyldisiloxane	17
Reaction products	first peak	6
	second peak	10
Reaction products	first peak	6
+ diluted H_2SO_4	second peak	17

Methanolysis of tetrakis-(methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranosyl-2-O-)silane

To a solution of carbohydrate(1.0g) in methanol(10cm^3), water was added with stirring until the solution remained cloudy. This turbidity was carefully removed by addition of few drops of methanol. The solution was then refluxed and examined by TLC at time intervals.

TLC (silica gel)

Solvents: (a) methanol:benzene/2:98

(b) n-butanol:acetone:water/4:5:1

The results are summarized on Table VI

Table VI

TLC results of methanolysis of tetrakis-(methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranosyl-2-O-)silane

Sources	Methanolysis time (minutes)	Solvents(R_f values) (a)	(b)
References			
i.		0	0.6
ii.		0.74	0.85
iii.		0.88	0.85
Reaction products			
1.	30	0.88	
2.	90	0.88	
3.	90	0.74	
4.	150	0.88	0.85
5.	150	0.74	0.61
6.	150	0.48	

7.	150	0.27	
8.	150	0.20	
9.	150	0.13	
10.	150	0	0.61
11.	240	0	0.61

Reference:-

i. Methyl α-D-glucopyranoside

ii. Methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranoside

iii. Tetrakis-(methyl 2,3,4-tri-O-trimethylsilyl-α-D-glucopyranosyl-2-O-)silane.

^{29}Si NMR of tetraethoxysilane

Tetraethoxysilane[$(CH_3CH_2O)_4Si$] had a similar structure to our desired product, that is, one silicon atom was attached to four identical compounds through four Si-O bonds. And in order to study the ^{29}Si NMR chemical shift of this Si-O bonding, tetraethoxysilane was chosen as an reference. A pure sample was obtained through H&W.

IR (neat):-

Broad band at 1100cm^{-1}

NMR (neat):-

Quartet	5.7-6.0	(intensity 4)
Triplet	8.6-8.9	(intensity 6)

^{29}Si NMR (neat)(proton decoupled):-

No.	ppm
1.	-6.4

(6) The preparation of methyl 2,3,4-tri-O-benzyl-glucopyranoside

A mixture of methyl 6-O-trityl-α-D-glucopyranoside(50g) and freshly distilled(under reduced pressure) benzyl chloride(500cm^3) in a flask was heated to 90°. Powdered potassium hydroxide(100g) was added in small portions over a period of 30 minutes; the solution was stirred vigorously during the entire reaction. After the addition of hydroxide, the solution was heated to 130° for 8 hours. It was cooled down to room temperature and shaken with water(200cm^3) to remove the excess hydroxide and potassium chloride formed. The aqueous layer was separated and the non aqueous layer was evaporated under high vaccum, yielded crude methyl 2,3,4-tri-O-benzyl-6-O-trityl-α-D-glucopyranoside as viscous fluid (58g, 72%).

Crude methyl 2,3,4-tri-O-benzyl-6-O-trityl-α-D-glucopyranoside (30g) was dissolved in glacial acetic acid(40cm^3) by gentle warming. The solution was cooled in ice bath and before crystallization of acetic acid set in, ice cold hydrogen bromide in acetic acid(45%w/v) (7.6cm^3) was added with vigorous stirring. The trityl bromide which had been precipitated out within 2 minutes was filtered off in glass filter. The filtrate was poured into ice cold water, the yellowish liquid was taken up by chloroform(50cm^3). The aqueous layer was separated and extracted by $CHCl_3$ once. The combined $CHCl_3$ solutions were washed by water twice and dried over anhydrous sodium sulphate. The solid was filtered off and the filtrate was evaporated under reduced pressure to a thick syrup.

TLC (silica gel)(n-butanol:petroleum ether60-80°/2:98)

Several components were detected, the spot with the lowest R_f value(0.30) but with highest intensity was assumed to be methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside.

Column separation of the reaction products

The reaction products(1g) were first dissolved in petroleum ether60-80° and then chromatographing on a silica gel column using 2% n-butanol in petroleum ether60-80° as solvent. Ten fractions($10cm^3$) were collected, all the fractions were analysed by TLC and the assumed tribenzyl derivative was collected in the first three fractions. The corresponding tribenzyl fractions were combined and concentrated under reduced pressure to a thick syrup. The syrup solidified after one week and the solid was dissolved in ethanol and then water was added until the solution just turned cloudy. It was put into refrigerator for 1 week, methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside crystallized as fine needles; total yield after 6 column chromatography 4.5g (23%); m.p. 50-51°.

IR(KBr disc): Sharp band at $3480cm^{-1}$

Bands at 1600, 1500 and $1450cm^{-1}$

NMR($CDCl_3$ solution):

Singlet	τ 2.7-2.8	(intensity	20)
Multiplet	τ 5.1-5.5	(intensity	10)
Multiplet	τ 6.3-6.5	(intensity	9)
Singlet	τ 6.7	(intensity	5)
Singlet	τ 8.35	(intensity	2)
Singlet	τ 8.4	(intensity	2)

Elemental analysis(%):

	C	H
Calculated:	72.4	6.9
Found:	72.5	7.3

(7) The reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with Me_3SiCl

The procedure for trimethylsilylation of this tribenzyl glucopyranoside was exactly the same as for previous experiments. The final reaction product(yellowish syrup) was examined by TLC(silica gel)(methanol:benzene/2:98) and two components were detected with the following R_f value (i) 0.29 (ii) 0.83. The first component had the same R_f value as the starting material.

Column separation of the reaction mixture

The two components were isolated by column chromatography(silica gel)(methanol:benzene/2:98). The fractions containing the reaction product(tlc R_f value 0.83) were combined and evaporated under reduced pressure to a thick syrup.

IR (neat): No absorption band was recorded between 3600-3200cm^{-1}.
Three strong bands at 1250, 850 and 750cm^{-1}.

NMR($CDCl_3$ solution):

Singlet	τ 2.7	(intensity 24)
Multiplet	τ 5.1-5.5	(intensity 10)
Multiplet	τ 6.3-6.5	(intensity 9)
Singlet	τ 6.65	(intensity 5)
Singlet	τ 9.8	(intensity 20)

Elemental analysis (%):

	C	H	Si
Calculated:	69	7.5	5.2
Found:	70.5	7.2	4.8

The reaction product was assumed to be methyl 2,3,4-tri-O-benzyl-6-O-trimethylsilyl-α-D-glucopyranoside.

Catalytic hydrogenation of methyl 2,3,4-tri-O-benzyl-6-O-trimethylsilyl-α-D-glucopyranoside

Palladium charcoal(10%)(0.5g) was suspended in freshly distilled AR grade ethyl acetate($20cm^3$), and saturated with hydrogen at room temperature for 2 hours. The hydrogen was first passed through an acetone/solid CO_2 trap. Methyl 2,3,4-tri-O-benzyl-6-O-trimethylsilyl-α-D-glucopyranoside(1g) in freshly distilled AR grade ethyl acetate ($5cm^3$) was added. The solution was hydrogenated 4 hours at room temperature and examined by TLC at 2 hourly intervals.
TLC(silica gel)(methanol:benzene/2:98):-

After 2 hours, three spots were revealed with the following R_f values (i) 0.86 (ii) 0.31 (iii) 0 . Reference: methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside R_f 0.31.

After another 4 hours, only one spot was detected at the base line. When another type of solvent(n-butanol:acetone:water/4:5:1) was used one spot(R_f0.61) was detected which had same R_f value as methyl α-D-glucopyranoside. The IR and NMR spectra of this final hydrogenated product were very similar to those of methyl α-D-glucopyranoside.

(8) The reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with $SiCl_4$

The procedure for the silylation of this glucopyranoside was exactly the same as for previous experiment. When the final reaction product was examined by TLC(silica gel)(methanol:benzene/2:98), two spots were revealed with the following R_f values (i) 0.72 (ii) 0.30. The second spot had the same R_f value as the original tribenzyl glucopyranoside.

Column separation of the reaction product

The reaction product(1g) was first dissolved in dry ether(5cm^3) and then chromatographing on a silica gel column using methanol:benzene/2:98 as eluant. Six fractions(10cm^3) were collected and they were all analysed by TLC using same solvent. Each of the first four fractions showed presence of four components with the following R_f values: (i)0.73 (ii)0.69 (iii)0.54 (iv)0.31. Further attempts to isolate each individual component were not successful.

Catalytic hydrogenation of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside

The hydrogenation was carried out under the same conditions as previous experiment except absolute ethanol was used instead of ethyl acetate in this case.

Weight of carbohydrate = 0.5g

Weight of catalyst = 0.5g

Absolute ethanol = 20cm^3

Duration of hydrogenation = 7 hours

The resulting mixture was analysed by TLC(silica gel)

(a) Solvent: methanol:benzene/4:96

Four spots were revealed with the following R_f values (i)0.53 (ii)0.28 (iii)0.13 (iv)0; the first component had the same R_f value as an authentic methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside.

(b) Solvent: n-butanol:acetone:water/4:5:1

Three spots were revealed with the following R_f values (i)0.95 (ii)0.72 (iii)0.62; references: methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside R_f0.95, methyl α-D-glucopyranoside R_f0.61. Total time for complete hydrogenation of the benzyl groups was about 18 hours.

Catalytic hydrogenation of the products obtained from the reaction of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside with $SiCl_4$

The hydrogenation was carried out under usual conditions.

Weight of the reaction products = 0.5g

Weight of catalyst = 0.5g

Solvent = absolute ethanol $20cm^3$

Duration of hydrogenation(initially) = 4 hours

The resulting mixture was analysed by TLC(methanol:benzene/4:96), four spots were revealed with the following R_f values (i)0.86 (ii)0.52 (iii)0.12 (iv)0;reference: methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside R_f 0.53.

The reaction mixture was further hydrogenated for 4 hours, four spots were detected by TLC (methanol:benzene/4:96) with the following R_f values (i) 0.52 (ii)0.20 (iii)0.12 (iv)0. More catalyst(0.5g) was added to the solution and hydrogenated for another 4 hours. Same result was obtained. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup and stored over P_2O_5 under vacuum.

IR(neat): Broad band at 3500-3200cm^{-1}

Bands at 1600,1500 and 1450cm^{-1}

NMR($CDCl_3$ solution): Multiplet τ 2.7

Multiplet τ 8.5

The silicon content of this reaction mixture was about 0.5%. Prolonged hydrogenation of the mixture(20 hours) gave only one spot(TLC) which had same R_f value as an authentic sample of methyl α-D-glucopyranoside.

(9) The reaction of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose with Me_3SiCl

1,2:5,6-di-O-isopropylidene-α-D-glucofuranose(4g, 0.015 mole) in dry pyridine($20cm^3$) in a flask fitted with condenser, dropping funnel(both closed with $CaCl_2$ tubes), thermometer and a magnetic stirrer was cooled in an ice bath. A solution of Me_3SiCl($2cm^3$,0.015 mole) in dry ether($5cm^3$) was added from the dropping funnel at such a rate that the reaction temperature was kept below 5^o. After the addition of the silane the reaction mixture was refluxed for 2 hours and left overnight at room temperature, then diluted with dry ether($20cm^3$) and the solid was filtered off and the filtrate was concentrated under reduced pressure. Pyridine was removed by repeated evaporating with toluene. The residue was kept over paraffin wax under vacuum. This viscous liquid solidified after 2 days.

TLC(silica gel)(methanol:benzene/5:95)

Two spots were detected from the reaction product with the following R_f values (i)0.83 (ii)0.52. The second spot had the same R_f value as the starting material.

Column separation of the reaction product

The reaction product(1g) was dissolved in $CHCl_3$($2cm^3$) and chromatographing on a silica gel column using 5% methanol in benzene as eluant. Pure 1,2:5,6-di-O-isopropylidene-3-O-trimethylsilyl-α-D-glucofuranose was obtained as glass after removal of the solvent.

IR(KBr disc): No absoption band between 3500-3200cm^{-1}

Three bands at 1250,850and 750cm^{-1}

Broad band at 1100-1000cm^{-1}

NMR($CDCl_3$ solution): Doublet τ 4.1-4.2 (intensity 2)

Multiplet τ 5.7-6.1 (intensity 12)

Triplet τ 8.6-8.8 (intensity 24)

Singlet τ 9.9 (intensity 20)

^{13}C NMR ($CDCl_3$ solution)(proton decoupled):-

No.	ppm	intensity %
1.	112.2	3472
2.	109.5	3591
3.	105.3	3790
4.	85.2	3799
5.	81.7	3601
6.	79.3	1707
7.	77.2	1880
8.	76.7	3757
9.	75.1	1798
10.	72.5	3802
11.	67.5	3423
12.	27.0	7002
13.	1.0	6793

Methanolysis of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose with (i)acetic acid (ii) sulphuric acid as catalyst

To a solution of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (0.5g) in anhydrous methanol($5cm^3$) was added few drops of (i)glacial acetic acid (ii)concentrated sulphuric acid, the reaction mixtures were left in refrigerator and examined by TLC at time intervals. The expected product from both hydrolysis was 1,2-O-isopropylidene-α-D-glucofuranose.

Time for complete hydrolysis: (i) 36 hours (ii) 14 hours.

Methanolysis of 1,2:5,6-di-O-isopropylidene-3-O-trimethylsilyl-α-D-glucofuranose

Same experiment procedures as previous experiment, however, after 3 hours(glacial acetic acid as catalyst) only 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose was detected by tlc.

(10) The reaction of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose with $SiCl_4$

A solution of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (9.4g, 0.036 mole) in dry pyridine($20cm^3$) in a flask fitted with condenser, dropping funnel(both closed with $CaCl_2$ tubes), thermometer and a magnetic stirrer was cooled in an ice bath to 0°. A solution of freshly distilled $SiCl_4$($1cm^3$, 0.009 mole) in dry ether($5cm^3$) was added from the dropping funnel at such a rate that the reaction temperature was kept below 5°. After the addition of silane, the reaction mixture was refluxed for 2 hours. It was then diluted with dry ether($20cm^3$) and left overnight. The solid was filtered off and the filtrate was concentrated under reduced pressure and the pyridine was removed by repeated evaporating with toluene. The residue solidified after standing over paraffin wax under vacuum. When it was examined by tlc(n-butanol:petroleum ether60-80°/1:8) two spots were revealed with the following R_f values (i)0.80 (ii)0.48. The second spot had the same R_f value as the starting material.

Column separation of the reaction product

The reaction product(1g) was dissolved in $CHCl_3$($2cm^3$) and chromatographing on a silica gel column using n-butanol:petroleum ether60-80°/1:8 as eluant. Six fractions($10cm^3$) were collected and

the solvents were evaporated under reduced pressure to a thin syrup, and left overnight. Crystals(prism) were found in the first three fractions, they were filtered off and washed with ice cold n-butanol and kept over P_2O_5 under vacuum. The R_f of this substance was 0.80 (TLC; n-butanol:petroleum ether60-80°/1:8).

$[\alpha]_D$ -40° ($CHCl_3$) (-4.1° reported)[152]

Elemental analysis(%):-

	C	H	Si
Calculated	54.1	7.1	2.6
Found	54.8	7.7	2.1

IR(KBr disc): No band was recorded between 3600-3000cm^{-1}
Broad band at 1100-1000cm^{-1}

NMR($CDCl_3$ solution):

Doublet	τ 4.1-4.2	(intensity 3)
Doublet	τ 5.2-5.3	(intensity 3)
Doublet	τ 5.4-5.5	(intensity 3)
Multiplet	τ 5.7-6.1	(intensity 12)
Quartet	τ 8.5-8.7	(intensity 42)

^{13}C NMR ($CDCl_3$ solution)(proton decoupled):-

No.	ppm.	intensity %
1.	112.3	1309
2.	109.4	1135
3.	105.1	3108
4.	85.1	3006
5.	81.5	3057
6.	78.5	497
7.	76.5	3199
8.	75.7	370
9.	72.3	3142

10.	67.3	2933
11.	26.9	6122
12.	26.3	3503
13.	25.2	3049

^{29}Si ($CDCl_3$ solution)(proton decoupled):- No signal was recorded.

(11) The preparation of methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside[150]

A solution of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allo-pyranoside(10g) and lithium aluminium hydride(2g) in dry ether(200cm^3) in a flask fitted with double walled condenser(closed with $CaCl_2$ tube) was refluxed for 3 hours. Another 1g of lithium aluminium hydride was added and the refluxing was continued for a further hour. The excess reagent was destroyed by the addition of small amount of cold water. Dilute sulphuric acid was added to dissolve the aluminium salt, the mixture was thoroughly shaken and the etheral layer was separated and washed with aqueous sodium carbonate and then by water, dried over anhydrous sodium sulphate, filtered, and the filtrate was concentrated under reduced pressure to give solid mass. Methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside was recrystallized from ether, yield 4g (40%), m.p. 118-120°, $[\alpha]_D$+152° ($CHCl_3$).

IR(KBr disc): Sharp band at 3500cm^{-1}

NMR($CDCl_3$ solution):

Multiplet	τ 2.6-2.7	(intensity 18)
Singlet	τ 4.4	(intensity 3)
Doublet	τ 5.1-5.2	(intensity 3)
Multiplet	τ 5.6-6.4	(intensity 18)
Singlet	τ 6.6	(intensity 10)
Multiplet	τ 7.0-7.1	(intensity 3)
Multiplet	τ 7.8-8.0	(intensity 6)

(12) The reaction of methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside with Me_3SiCl

The 2-deoxy sugar(2.5g, 0.009 mole) was trimethysilylated under the usual conditions by Me_3SiCl($2cm^3$, 0.016 mole, slight excess). When the final reaction product was examined by TLC(silics gel) (methanol:benzene/2:98), two components were revealed with the following R_f values (i)0.75 (ii)0.41. The second spot had the same R_f value as the original 2-deoxy sugar.

IR(neat): Sharp band(weak) at $3500cm^{-1}$

Three strong bands at 1250, 850 and $750cm^{-1}$

NMR($CDCl_3$ solution):

Multiplet	τ 2.4-2.7	(intensity 9)
Singlet	τ 4.4	(intensity 2)
Triplet	τ 5.1-5.3	(intensity 2)
Multiplet	τ 5.4-6.3	(intensity 7)
Singlet	τ 6.6	(intensity 6)
Multiplet	τ 7.9-8.0	(intensity 3)
Singlet	τ 9.8	(intensity 13)

Catalytic hydrogenation of methyl 4,6-O-benzylidene-3-O-trimethylsilyl-2-deoxy-α-D-ribo-hexo-pyranoside(crude)

The conditions for the hydrogenation were the same as previous experiments.

Weight of carbohydrate = 0.5g

Weight of catalyst = 0.5g

Solvent = ethyl acetate(AR) $20cm^3$

Duration of hydrogenation = 6 hours

When the final reaction mixture was examined by TLC(silica gel) (methanol:benzene/5:95), four spots were revealed with the following

R_f values (i)0.90 (ii)0.57 (iii)0.48 (iv)0

References:- (a) methyl 4,6-O-benzylidene-3-O-trimethylsilyl-2-deoxy-α-D-ribo-hexo-pyranoside R_f 0.90

(b) methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside R_f 0.48

A further 0.5g of catalyst was added to the reaction mixture and hydrogenation was carried out for another 8 hours. It was then examined by TLC(methanol:benzene/2:8) and four spots were revealed with the following R_f values (v)0.65 (vi)0.56 (vii)0.39 (viii)0.32

References:- (a) methy 4,6-O-benzylidene-3-O-trimethylsilyl-2-deoxy-α-D-ribo-hexo-pyranoside R_f 0.95

(b) methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside R_f 0.77

This reaction mixture was further hydrogenated for further 6 hours (total 20 hours),TLC(methanol:benzene/2:8) showed two spots with the following R_f values(ix)0.54 (x)0.31

Column separation of the reaction products from the catalytic hydrogenation

Separation of the hydrogenated products was carried out on a silica gel column using methanol:benzene/2:8 as eluant. Six fractions ($10cm^3$) were collected and examined by TLC(methanol:benzene/2:8) only one spot(R_f 0.33) was detected. The solvent was removed under reduced pressure and the residue was triturated with dry petroleum ether60-80°, recrystallized from ethanol-ether-petroleum ether, m.p. 90°(rapid heating)(hygroscopic).

IR(neat): Broad band at 3500-3000cm^{-1}

No bands at 1250, 850 and 750cm^{-1}

NMR($CDCl_3$ solution): Singlet τ 5.1-5.2 (intensity 3)

Multiplet τ 5.9-6.5 (intensity 20)

Singlet τ 6.6 (intensity 10)

Multiplet τ 7.8-8.1 (intensity 10)

Thiobarbituric acid has been proposed as spraying agent(TLC) for deoxy sugar[151]. Deoxy sugars give a red colour after being treated with this reagent. When this final hydrogenated product(0.33) was sprayed with thiobarbituric acid, a red colour spot was revealed.

(13) The reaction of methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside with $SiCl_4$

To a solution of the 2-deoxy sugar(4.8g, 0.018 mole) in dry pyridine($20cm^3$) in a flask fitted with condenser, dropping funnel (both closed with $CaCl_2$ tubes), thermometer and magnetic stirrer at 35°, a solution of $SiCl_4$($0.5cm^3$, 0.0044 mole)in dry toluene($5cm^3$) was added from the dropping funnel with stirring. The reaction temperature was kept below 35° by external cooling, the mixture was then refluxed for 3 hours and the solution changed to brown in colour. The solution was left overnight and then diluted with dry ether($30cm^3$), the solid was filtered off and the filtrate was concentrated under reduced pressure. The last traces of pyridine was removed by repeated evaporating with toluene. When this reaction product was examined by TLC(methanol:benzene/4:96), five components were detected with the following R_f values (i)0.85 (ii)0.81 (iii)0.67 (iv)0.59 (v)0.28.

Reference: methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside

R_f 0.59

The separation of each individual components by column chromatography was not successful.

(14) The preparation of methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside[147,150]

A solution of methyl 4,6-O-benzylidene-α-D-glucopyranoside (50g, 0.27 mole) in dry pyridine($80cm^3$) in a flask was cooled in an ice bath, and a solution of p-toluenesulphonyl chloride(25g, 0.13 mole) in dry chloroform($50cm^3$) was added with stirring over a period of 30 minutes. The reaction mixture was stirred at room temperature for 24 hours and was then shaken with a mixture of benzene($200cm^3$) and water($200cm^3$). The aqueous layer was separated and extracted once with benzene. The combined benzene solutions were washed with water, cold dilute sulphuric acid, aqueous sodium hydrogen carbonate and finally again with water. During this process, some unchanged benzylidene compound was precipitated out and was filtered off. The benzene extract was dried over anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure to dryness. The residue was dissolved in hot absolute ethanol, methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucopyranoside separated out; yield 42g (36%), m.p.152-154°, $[\alpha]_D$ +62.5° ($CHCl_3$).

IR(KBr disc):- Sharp band at $3500cm^{-1}$

Sharp band $1600cm^{-1}$

NMR($CDCl_3$ solution):-

Doublet	τ 2.1-2.3	(intensity 6)
Multiplet	τ 2.5-2.8	(intensity 20)
Singlet	τ 4.55	(intensity 2)
Doublet	τ 5.1-5.2	(intensity 2)
Multiplet	τ 5.6-6.5	(intensity 16)
Singlet	τ 6.7	(intensity 9)
Doublet	τ 7.3-7.4	(intensity 2)
Singlet	τ 7.6	(intensity 9)

Methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucopyranoside(12g, 0.028 mole) was dissolved in dry methanol(100cm^3) containing clean sodium(0,85g) dissolved in it, and the mixture was refluxed for 4 hours. On cooling, crisp long needles of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside separated. This crystals were recrystallized from absolute ethanol, yield 3.5g (48%),m.p. 146-148°, $[\alpha]_D$ +18° ($CHCl_3$).

IR(KBr disc): Bands at 1250 and 750cm^{-1}

NMR($CDCl_3$ solution):

Multiplet	τ 2.5-2.7	(intensity 14)
Singlet	τ 4.55	(intensity 2)
Singlet	τ 5.1	(intensity 2)
Multiplet	τ 5.7-5.8	(intensity 2)
Multiplet	τ 6.2-6.4	(intensity 6)
Singlet	τ 6.6	(intensity 9)
Doublet	τ 6.8-6.9	(intensity 2)

Lithium aluminium hydride(2g) in dry ether(250cm^3) was mildly refluxed and stirred in a three-necked flask(2000cm^3) fitted with double walled condenser and dropping funnel(both closed with $CaCl_2$ tubes). A solution of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside(5g) in dry ether(350cm^3) was added slowly from the dropping funnel. The reaction was refluxed for 2 hours and further lithium aluminium hydride(1g) was added and reflux continued for 1 hour. The excess reagent was destroyed by addition of small amount of water. Dilute sulphuric acid was added to dissolve the solid aluminium salt, the mixture was thoroughly stirred and the ether layer was separated and washed with sodium carbonate solution and water, finally dried over anhydrous sodium sulphate. The salt was

filtered off and the filtrate was concentrated under reduced pressure to give a solid residue. Methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside was recrystallized from ether-pentane, yield 1.5g (31%) m.p. 109-111°, $[\alpha]_D$ +106° ($CHCl_3$).

IR(KBr disc): Broad band at 3600-3300cm^{-1}

NMR($CDCl_3$ solution):

Multiplet	τ 8.5-8.8	(intensity 18)
Singlet	τ 6.5	(intensity 3)
Singlet	τ 5.5	(intensity 3)
Multiplet	τ 5.7-6.3	(intensity 18)
Singlet	τ 6.6	(intensity 12)
Broad multiplet	τ 7.7-8.1	(intensity 10)

(15) The reaction of methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside with Me_3SiCl

The 3-deoxy sugar(2.6g, 0.01 mole) was silylated by Me_3SiCl(1.5cm^3, 0.012 mole) under the usual conditions. The total time for silylation was 2 hours. The final reaction mixture was diluted with dry ether (20cm^3) and left overnight. The solid was filtered off and the filtrate was concentrated under reduced pressure, pyridine was removed by repeated evaporating with toluene. A thick syrup was left behind and TLC(methanol:benzene/2:98) showed two components were present with the following R_f values (i)0.80 (ii)0.19(very faint). The second spot had the same R_f value as the starting material.

Column separation of the reaction products

The compound having R_f 0.80 was isolated from the reaction products by silica gel column chromatography(methanol:benzene/2:98). The product(syrup) was kept over P_2O_5 under vacuum.

IR(neat): No absorption band was recorded between 3600-3200cm^{-1}

Three bands at 1250,850 and 750cm^{-1}

NMR($CDCl_3$ solution):	Multiplet	τ 2.5-2.7	(intensity 15)
	Singlet	τ 4.5	(intensity 3)
	Singlet	τ 5.5	(intensity 3)
	Multiplet	τ 5.7-6.3	(intensity 18)
	Singlet	τ 6.6	(intensity 10)
	Singlet	τ 9.8	(intensity 24)

Catalytic hydrogenation of methyl 4,6-O-benzylidene-2-O-trimethylsilyl-3-deoxy-α-D-arabino-hexo-pyranoside

The conditions for the hydrogenation were the same as previous experiments.

Weight of carbohydrate = 0.5g

Weight of catalyst = 0.5g

Solvent = ethyl acetate(AR) $20cm^3$

Duration = 18 hours

When the final reaction mixture was examined by TLC (methanol: ethyl acetate:benzene/1:1:3), only one spot at R_f 0.50 was observed. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup and kept over P_2O_5 under vacuum.

IR(neat): Broad band at $3600-3000cm^{-1}$

Band at $1750cm^{-1}$

Bands at 1250 and $850cm^{-1}$

NMR(absolute ethanol): No signal was recorded at τ 2.5 and τ 9.8.

When the time for hydrogenation was reduced to 6 hours, four components were detected by TLC with the following R_f values (i)0.95 (ii)0.75 (iii)0.65 (iv)0.50.

References: methyl 4,6-O-benzylidene-2-O-trimethylsilyl-3-deoxy-α-D-arabino-hexo-pyranoside R_f 0.95

Mehtyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside R_f 0.65

The separation of each individual compound by column chromatography was not successful.

The catalytic hydrogenation of methyl 4,6-O-benzylidene-2-O-trimethylsilyl-3-deoxy-α-D-arabino-hexo-pyranoside was repeated except absolute ethanol was used as solvent. When the reaction mixture was examined by TLC(methanol:ethyl acetate:benzene/1:1:3)after 10 hours of hydrogenation, four spots were revealed with the following R_f values (i)0.95 (ii)0.75 (iii)0.65 (iv)0.50. However, when the hydrogenation was carried out for 48 hours, only one component was detected by TLC R_f0.50.

(16) The reaction of methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside with $SiCl_4$

Methy 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside (7.8g, 0.029 mole) was silylated by freshly distilled $SiCl_4$($0.8cm^3$, 0.007 mole) under the usual conditions for 2 hours. When the reaction mixture was examined by TLC(methanol:benzene/2:98), two components were revealed and their R_f values were as follow (i)0.73 (ii)0.21. The second spot corresponded to the starting material.

Column separation of the reaction products

The products(0.5g) were dissolved in minimum amount of dry ether and then chromatographing on a silica gel column using 2% methanol in benzene as eluant. Six fractions($10cm^3$) were collected and from the first three fractions, the compound of R_f0.73(TLC) was isolated. The three fractions were combined and concentrated under reduced pressure to yield tetrakis-(methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranosyl-2-O-)silane as glass.

Elemental analysis (%):-		C	H	Si
(i) Starting material:	Calculated	63.1	6.8	
(ii) Reaction product :	Calculated	61.8	6.3	2.6
	Found	61.6	6.4	2.0

IR (neat):- No absorption band between 3600-3200cm^{-1}

Broad band at 1150-1050cm^{-1}

Sharp band at 900cm^{-1}

NMR ($CDCl_3$ solution):		
Multiplet	2.6	(intensity 15)
Singlet	4.5	(intensity 3)
Singlet	5.4	(intensity 3)
Multiplet	5.7-6.3	(intensity 16)
Singlet	6.6	(intensity 9)

^{13}C ($CDCl_3$ solution)(proton decoupled):-

No.	ppm.	intensity %
1.	128.3	689
2.	126.1	601
3.	102.1	318
4.	100.1	343
5.	78.5	5006
6.	77.0	4313
7.	75.6	3728
8.	70.4	274
9.	65.1	252

^{29}Si NMR ($CDCl_3$ solution)(proton decoupled):-

No signal was recorded.

Catalytic hydrogenation of tetrakis-(methyl 4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranosyl-2-O)silane

(A) The carbohydrate(0.5g) was hydrogenated under the usual conditions in ethyl acetate(AR) for 10 hours. The final reaction mixture was examined by TLC.

a)Solvent : methanol:benzene/2:98

Only one spot was detected at the base line.

b) Solvent : methanol:ethyl acetate:benzene/1:1:3

Only one spot of R_f 0.52 was detected.

The catalyst was filtered off and the filtrate was concentrated under reduced pressure to a thick syrup and kept over P_2O_5 under vacuum.

IR(neat) : Broad band at 3500-3200cm^{-1}

Band at 1750cm^{-1}

Band at 1250 and 850cm^{-1}

NMR(absolute ethanol) : No signal was recorded around τ 2.5

Multiplet at τ 7.9-8.2

(B) The hydrogenation of this reaction product was carried out under the usual conditions in absolute ethanol for 3 hours. The final reaction mixture was examined by TLC.

a) Solvent: methanol:benzene/2:98

Three spots were revealed with the following R_f values (i)0.73 (ii)0.12 (iii)0.

b) Solvent : methanol:ethyl acetate:benzene/1:1:3

Four spots were revealed with the following R_f values (i)0.88 (ii)0.81 (iii)0.52 (iv)0.48. Further 3 hours hydrogenation yield only one spot R_f 0.52.

The IR spectra of these two hydrogenated products(A and B) were very similar to the spectrum of the final product obtained from the hydrogenation of methyl 4,6-O-benzylidene-2-O-trimethylsilyl-3-deoxy-α-D-arabino-hexo-pyranoside.

5.6 ENZYMATIC DEGRADATION OF PECTIN

Schwarz[49] had shown that enzymatic hydrolysis of pectin did not liberate silicic acid, but lead to products of low molecular weight still containing silicon in bound form. Therefore methods were examined by us for the isolation of silicon derivative from pectin.

Pectins were obtained from different origins and suppliers and their silicon content were analysed.[152]

Table VII

Bounded silicon(μg/g) in pectins

Pectin (supplier)	Silicon μg/g
a. Citrus fruit (H&W)	1400
b. Citrus fruit (Sigma)	1800
c. Apple (Cambrian)	800
d. (i) Apple (BDH)	740
(ii) Apple (BDH)	6100
(iii) As (ii) but purified by ethanol extraction	8000

Purification of pectin[153]

Pectin was purified by extraction twice with 70% v/v ethanol. The initial silicon content of the ethanol was 37μg/100cm^3(0.5μg/g), however, the silicon content of the ethanol was increased to 37μg/g after being used to purify the pectin. The free silicon content of

the purified pectin was 54 μg/g.

Pectinase(E.C. No.3.2.1.15)(Aspergillus niger): 1 unit(1.1unit/mg) will liberate 1 μmole of galacturonic acid from polygalacturonic acid per minute at pH4 at 25°.

Pectin and pectinase in water(25cm^3) at pH4(buffer) was incubated at 25°, and analysed chromatographly at time intervals. The results were summarized on Table VIII and IX.

Table VIII

Enzymatic hydrolysis of pectins

Sample	Weight of pectin(g)	Weight of pectinase(g)	Incubation(hrs)
1.	0.0968	0.0527	½
2.	0.1028	0.0583	1
3.	0.1085	0.0514	2
4.	0.1137	0.0520	3
5.	0.0983	0.0500	4
6.	0.1023	0.0577	12
7.	0.1076	0.0546	24

2cm^3 was taken from each sample and warmed in boiling water for 10 minutes to deactivate the pectinase, then diluted to 50cm^3 and analysed for free silicon.

Table IX

Free silicon obtained from the enzymatic hydrolysis of pectins

Samples	Free silicon µg/g (less 54 µg/g)	% of silicon liberated
1.	335	4.2
2.	316	4.0
3.	374	4.7
4.	396	5.0
5.	406	5.1
6.	366	4.6
7.	410	5.1

Average % of silicon liberated = 4.7

Paper chromatography(Whatman No.4)(ethyl acetate:acetic acid:water/2:1:1)

The developed chromatograms were veiwed under UV light. All the sample showed one spot at R_f0.76, while samples 1-4 showed some spots below 0.76 but their resolution were not good enough to give a well define values.

Reference: D-galacturonic acid R_f0.76

TLC(Cellulose plate)(n-butanol:acetic acid:water/2:1:1)

Developing agent: alkaline $KMnO_4$

The TLC results were summarized on Table VIII

Table X

TLC results of the enzymatic hydrolysis of pectin

Samples	R_f values
1-2	0.15
	0.26
3-6	0.26
7	0.26
	0.43

Reference: D-galacturonic acid R_f 0.26

The enzymatic hydrolysis was repeated but instead of using buffer solution the pH was adjusted by addition of small amount of 0.1 mole sodium hydroxide solution(methyl orange as indicator). The reaction solution was incubated at 25° for 24 hours. The product was analysed by TLC. Two spots of R_f 0.26 and 0.15 were detected. The solution was evaporated to a thick syrup and chromatographing on a Sephadex G15 column using water as eluant. However, only one component of R_f 0.26 was collected after chromatography. Similar result was obtained when Sephadex G100 was used. The compound(R_f0.26) was D-galacturonic acid.

CONCLUSION

A new silylating agent of the type $Si(NR)_4$ was prepared. It appeared that in the reaction of this $Si(NR)_4$ compound and $Si(OR)_4$ compound with monosaccharides that primary and secondary OH groups reacted at very similar rates because of the lack of specificity in these reactions, the products of these reactions were complex and their structure could not be determined.

In the alternative approach in which all the -OH functions were protected with the exception of the -OH to be reacted with silane, the difficulty which arose was the sequent removal of the protective functions. It was found generally that the cleavage of the Si-O bonds accompanied the removal of the protective groups. A number of reactions such as hydrogenation which it appeared from the literature would not cause cleavage of the Si-O bond but would specifically remove the protective function were investigated in detail it was found in contrary to literature observations that regardless of the conditions cleavage of the Si-O bond occurred as rapidly or more rapidly than removal of the protective functions.

The reaction of methyl-4,6-O-benzylidene-3-deoxy-α-D-arabino-hexo-pyranoside with $SiCl_4$ was successful while the reaction of methyl-4,6-O-benzylidene-2-deoxy-α-D-ribo-hexo-pyranoside with $SiCl_4$ was not successful these observations indicated that the introduction of four molecules such as monosaccharide to one silicon is steric dependent.

The enzymatic degradation of pectin was successful to the extent that the long chain complex was broken into several fragments which still contained silicon. But the methods which we had available for

the detection and the identification of such fragments were not adequate.

^{29}Si NMR offered a very powerful tool for the detection of silicon. But the interpretation of spectra was difficult for our complex structures. However, this technique afforded an alternative approach for the detection of silicon to the classical colorimeter and gravimetric analysis which also have their limitations.

We have synthesized three compounds which we are quite certain are all derived from the bonding of one silicon to four carbohydrates molecules. However, all these carbohydrate molecules do not occur naturally and we cannot be certain whether it is possible to attach one silicon atom to four natural occurred carbohydrates.

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