STUDIES IN CARBOHYDRATES

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by

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T. D. Audichya

June 1968 T. D. Audichya

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STUDIES IN CARBOHYDRATES

PART : I

STANNIC CHLORIDE CATALYSED SYNTHESIS OF ARYL-α-GLYCOSIDES

CHAPTER : I

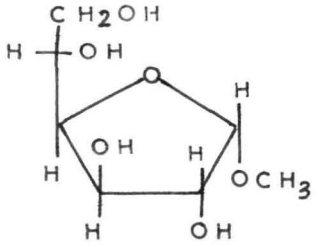
A REVIEW ON THE SYNTHESIS OF GLYCOSIDES

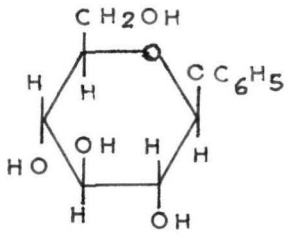
SUMMARY

This Chapter gives a review of the different methods used for the synthesis of glycosides.

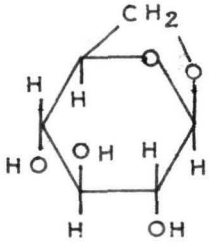
A REVIEW ON THE SYNTHESIS OF GLYCOSIDES

Glycosides are derivatives of cyclic forms of the monosaccharides produced by the reaction of the hemiacetal hydroxyl group with an alcoholic or a phenolic hydroxyl group of another organic molecule with the elimination of a molecule of water. Depending upon the reaction conditions employed and several other factors, glycosides are obtained either as a furanoside or a pyranoside in the α-(I) or the β-(II) anomeric forms or as a mixture of both of these forms. Derivatives of the cyclic forms of monosaccharides produced by the reaction of the hemiacetal hydroxyl group with primary hydroxyl group of the same sugar are also possible. These intramolecular glycosides are, however, classified as glycosans (III).





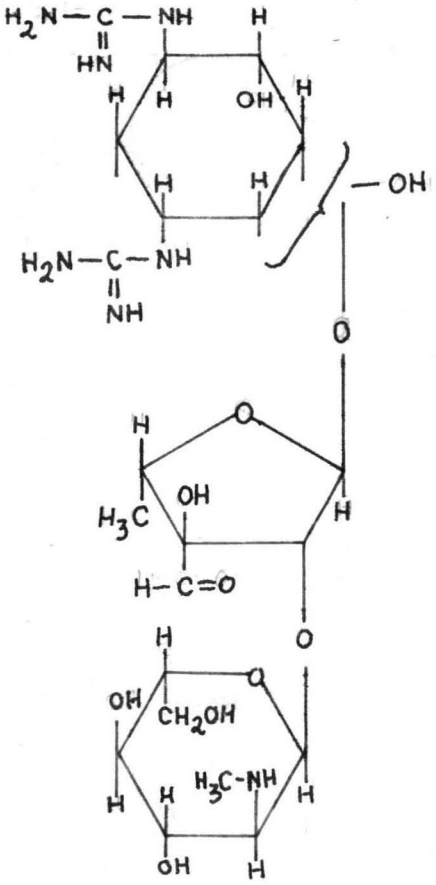
I : METHYL α-D-GLUCOPYRANOSIDE , II : PHENYL β-D-GLUCOPYRANOSIDE



III : GLUCOPYRANOSAN (1,6)

Many naturally occurring organic compounds are present in plants as glycosides. These include the glycosides of flavanoids and carotenoids, cardiac glycosides, saponins, glycosidic alkaloids of the solanum species, aryl glycosides including those of vanillin and coumarin, cyanogenetic glycosides of amygdalin type and the

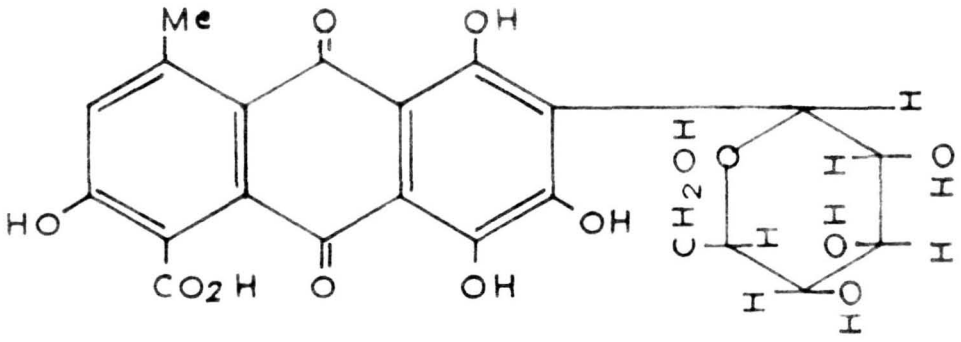
hydroxyanthraquinone glycosides. The important antibiotic steptomycin[1], isolated from the culture of the soil organism, Streptomyces griseus, is composed of three glycosidically linked sugar derivatives, streptidine (diguanidino-scyllo-inisitol), streptose (5-deoxy-3-formyl-L-lyxose) and N-methyl-L-glucosamine.



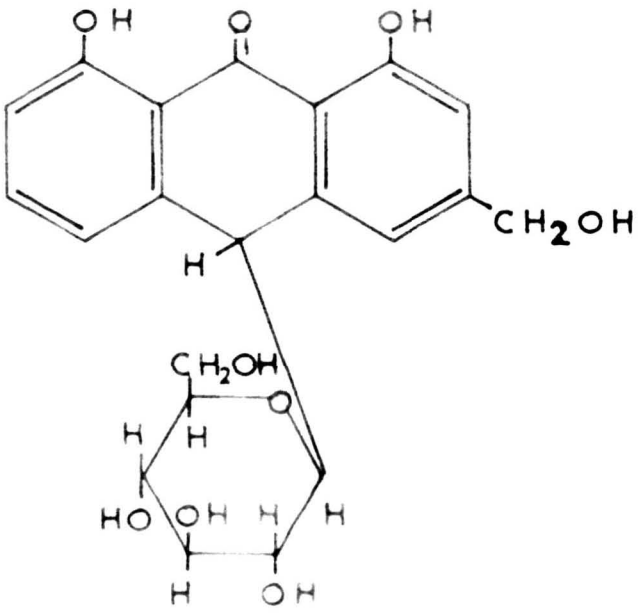
Streptomycin

A biologically important group of complex glycosides consisting of cerebrosides, glycolipids and gangliosides, occur in the brain, nerve tissue and spleen of animals[2].

Disaccharides may also be regarded as glycosides in which the hemiacetal hydroxyl group of a monosaccharide is linked to one of the alcoholic hydroxyl groups of the same or another monosaccharide. Linking up of two molecules of D-glucopyranose in this manner



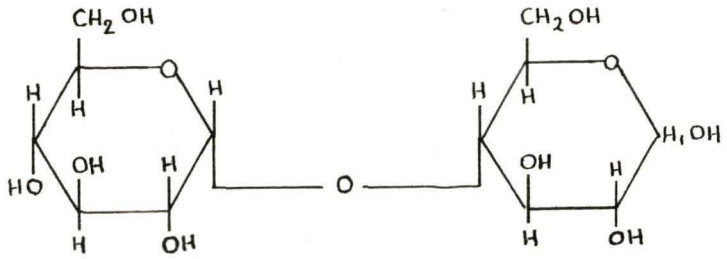
VI : CARMINIC ACID



VII : BARBALOIN

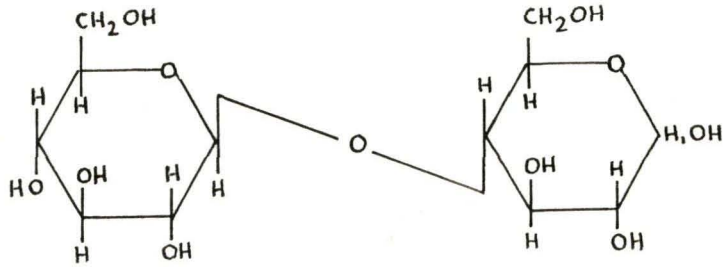
at C(1) and C(4) positions lead to the formation of the two anomeric disaccharides, maltose (IV) and cellobiose (V)

IV



IV MALTOSE (4-O-α-D-GLUCOPYRANOSYL-D-GLUCOSE)

V



V CELLOBIOSE (4-O-β-D-GLUCOPYRANOSYL-D-GLUCOSE)

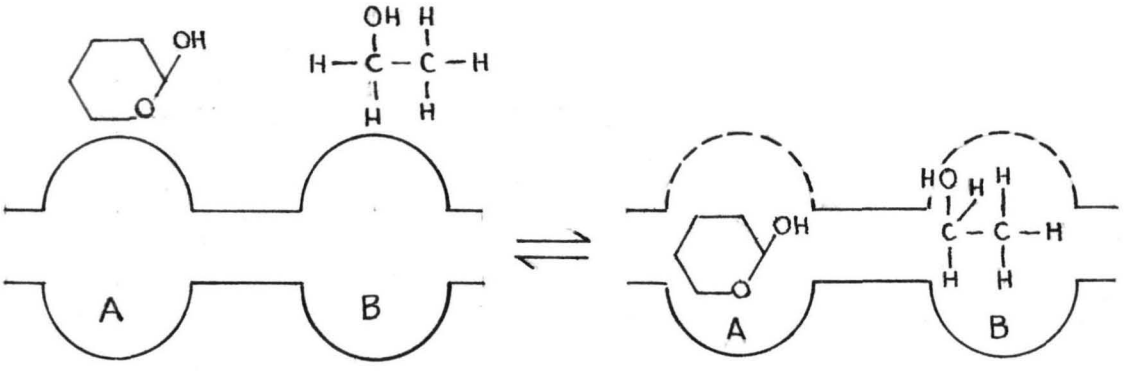
Oligosaccharides and polysaccharides are the examples of further extension of this type of glycosidation.

A few glycosides have been found in nature in which the sugar and the aglycone parts are linked through a carbon-carbon bond instead of a carbon-oxygen bond. Examples of this type of C-glycosides are carminic acid[3] (VI) and barbaloin[4] (VII). Several C-glycosides have been recently synthesised[5].

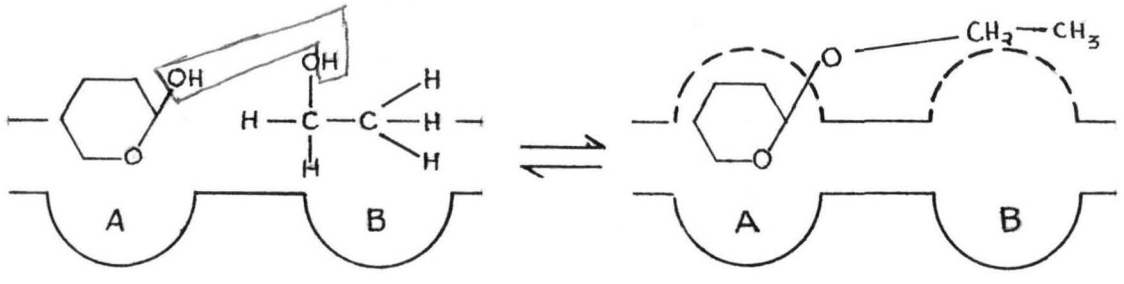
In view of frequent occurence of glycosides in nature, numerous conjectures have been made as to their functions in plants. Glycosides may serve as reserve deposits of sugar[5], particularly in seeds. They may also act as controls for osmotic pressure and as stabilisers of labile aglycones[6]. It is believed, however, that most of the glycosides are formed in the plants in the process of detoxication of undesirable

end products of plant metabolism. For example, potato tubers or wheat plants when exposed to ethylene chlorohydrin produce 2-chloroethyl-β-glucosides[7]. Many phenols and alcohols when injected into animals are detoxicated as glycosides of glucuronic acid and can be recovered from their urine[7]. This procedure is often used for the preparation of this type of glycosides. The biological method has also been utilized for the resolution of racemic compounds. Thus, dl-menthol forms more of d-menthol glucosiduronic acid than the l-isomer[8].

Synthesis of glycosides in nature proceeds through an enzymic process. It has been postulated[9] that the enzyme molecules have two distinct neighbouring areas, represented by A and B in the following figures, which are involved in the synthesis.



VIII IX



X XI

The sugar molecule is adsorbed on area A (VIII) which exhibits extreme specificity, whereas, area B (VIII)is capable of absorbing aglycones (alcohols or phenols) of many types. In the first stage the sugar and the aglycone are adsorbed in the areas A and B (IX) respectively of the enzyme. Next, a molecule of water is eliminated and the glycoside bond is formed (X). The resulting glycoside then detaches itself from the enzyme surface (XI).

The first laboratory synthesis of a glycoside was reported by Michael[10] in 1879. Since then, many synthetic methods have been developed for the preparation of glycosides, but very few of them are of general applicability. Many of these methods are restricted to certain types of aglycones and often affected by the type of monosaccharides employed. It may be stated that the synthesis of almost any individual glycoside, irrespective of the method employed, has its optimum conditions different from those for the preparation of other glycosides even of the same series.

The different methods of synthesis of glycosides have been reviewed by Conchie et. al.[11] in 1956 and later by Štůnek[12] et. al. in 1962[12]. These methods can be conveniently classified under the following heads:

1. Glycosidation with free sugars
2. Glycosidation with acyl glycosyl halides
3. Glycosidation with fully acetylated sugars
4. Glycosidation with thioacetals
5. Glycosidation with orthoesters
6. Trans-glycosidation
7. Non-catalytic glycosidation.

A short account of these methods including further recent developments is given in the following paragraphs:

I. Glycosidation with free sugars

In the synthesis of glycosides it is imperative to protect the alcoholic hydroxyl groups of the monosaccharide in order to prevent it from competing with the reactant aglycone molecule. Fischer[13] however was successful to employ free sugars for glycosidations of lower alcohols without any side reaction involving another molecule of sugar. In fact, this method is the most elegant one for the synthesis of methyl glycosides. In this method a suspension of a monosaccharide in the anhydrous alcohol is treated with dry hydrogen chloride. This method cannot be applied for the glycosidation of disaccharide due to its alcoholysis leading to the formation of the glycosides of the constituent monosaccharides.

Fischer glycosidation may lead to the formation of either or both of the anomeric glycosides. Moreover, the glycoside may be obtained either as a pyranoside or a furanoside according to the reaction conditions employed. Thus, the reaction under reflux conditions with higher concentration of hydrogen chloride favours the formation of pyranosides, whereas, use of lower concentrations of the acid under mild temperature conditions favour the formation of furanosides. This reaction, therefore, may lead to the formation of four different types of glycosides namely the pyranoside and the furanoside in the α-as well as the β-anomeric forms.

The glycosides formed in the Fischer reaction usually separate readily from the reaction mixture. This may, however, be further

facilitated by use of inert solvents such as benzene[14].

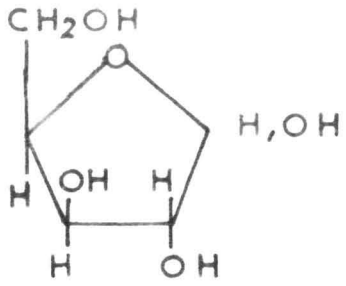
Spontaneous heating up of the reaction mixture during the introduction of hydrogen chloride into the suspension of the sugar in absolute methanol was utilized by Valentin[15] for the synthesis of pure ethyl- ∝ -D-mannopyranoside. It is interesting to note that Fischer method of glycosidation leads to the formation of otherwise difficulty accessible ∝ -anomeric alkyl glycosides as the major product[16,17].

Cation exchange resin in the H^+ -form have also been used successfully as catalysts[17] in the place of hydrogen chloride in Fischer's synthesis with the added advantage that the resin can be easily removed from the reaction mixture by filtration and regenerated for further use. The isolation of the glycosides formed thus becomes very easy. Good yields of methyl- ∝ -D-glucopyranoside, methyl- ∝ -D-mannopyranoside and methyl ∝ -L-rhamnopyranoside have been obtained by following this modified procedure. This method has been used under controlled conditions to prepare the anomeric D-fructofuranosides.

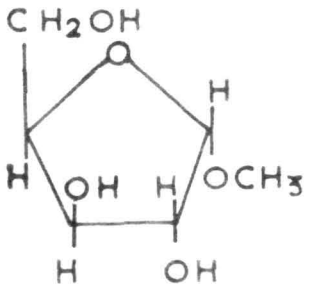
Bishop and Cooper[18] have recently made an exhaustive study of the Fischer reaction for the preparation of methyl glycosides of some pentoses. From an examination of the reaction mixture at various stages of glycosidation by gas-liquid chromatography, they arrived at the conclusion that the reaction in these cases proceed through the following successive stages:

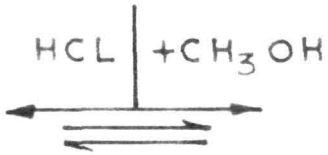
a. Formation of furanosides

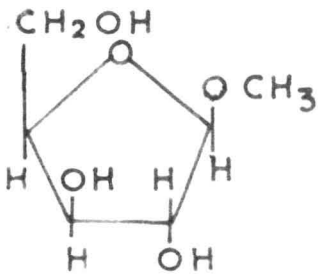
b. Anomerisation of furanosides



D - XYLOFURANOSE

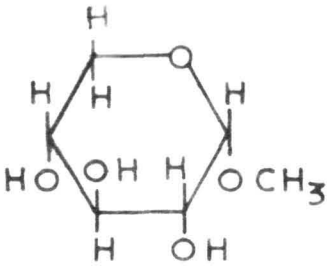


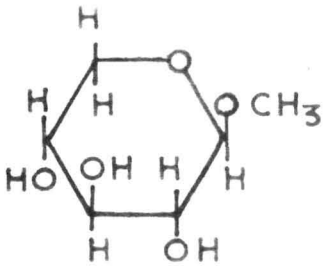




METHYL ∝ - D-XYLOFURANOSIDE

METHYL β - D - XYLOFURANOSIDE





METHYL ∝ - D-XYLOPYRANOSIDE

METHYL β - D - XYLOPYRANOSIDE

FISCHER REACTION

DIAGRAM NO. 1

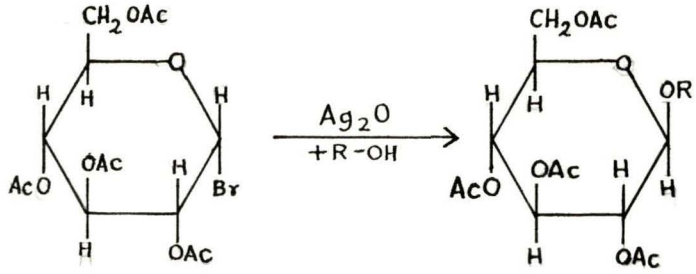
c. Conversion of furanosides into pyranosides

d. Anomerisation of pyranosides (see diagram No.1).

2. Glycosidation with O-acylglycosyl halides

An alcohol or a phenol condenses readily with O-acylglycosyl halides in the presence of an excess of silver carbonate or silver oxide to give acyl derivatives of the corresponding glycosides. This method of glycosidation, known as Koenigs-Knorr method[19-21], has found extensive application for the preparation of various alkyl and aryl glycosides. The silver compounds used, act as acid acceptors and thereby speed up the reaction and prevent deacetylation of the resulting acetyl glycosides.

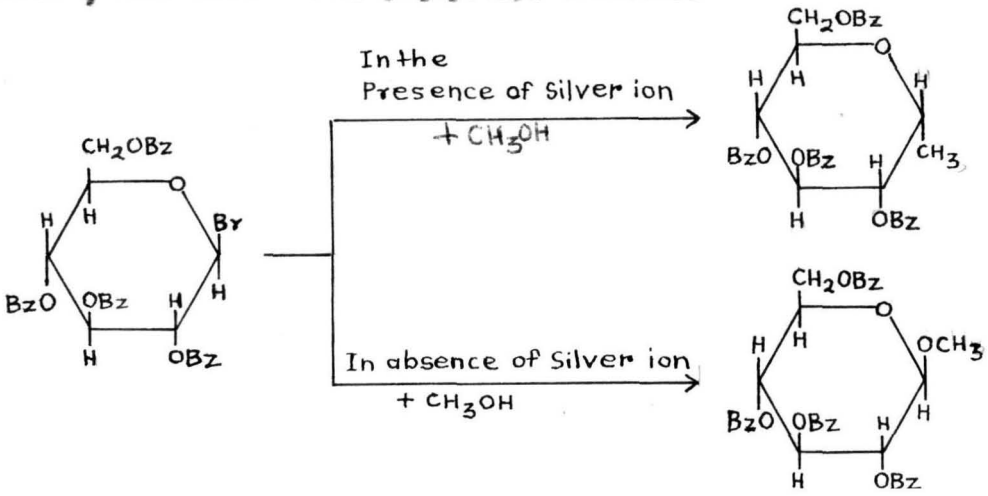
This reaction is, in most cases, accompanied by Walden inversion at C(1) resulting in the formation of acetylated β -D-alkyl/aryl glycosides from the α -O-acetyl-D-glycosyl halides. In this reaction the starting material is usually the easily accessible α -O-acetylglycosyl halide. The O-acetylated glycosides obtained, therefore have the β -configuration. The anomeric α -D-glycoside can also be prepared by this method when the corresponding β -D-glycosyl halide is available. Thus, 2,3,4,6 tetra-O-acetyl- β -D-glucosyl bromide (XII) gives the corresponding tetra-O-acetyl- α -D-glucoside (XIII).



XII XIII

In this reaction, the O-acetylglycosyl halide is usually dissolved in some dry inert solvent such as methylene dichloride, benzene or chloroform. Silver oxide or silver carbonate and the aglycone are then added to the solution. An increased yield has been obtained by adding catalytic amounts of iodine and anhydrous calcium sulphate. The reaction mixture often needs heating for completion of the reaction. The resulting O-acetylated glycoside can be easily and quantitatively deacetylated by Zemplén's[22] catalytic method using small amounts of sodium methoxide in methanol. Some of the nitro phenyl glycosides were previously known only in the form of acetates since the very labile nitrophenyl-O-glycosyl bond could not stand the usual methods of deacetylation. Free glycosides of this series have been recently prepared by Hengstenberg[23] by using trimethyl silylglycosyl bromides. The protective trimethyl silyl-groups can be easily removed at room temperature with a solution of glacial acetic acid (2.5%) in methanol-water mixture (1:1).

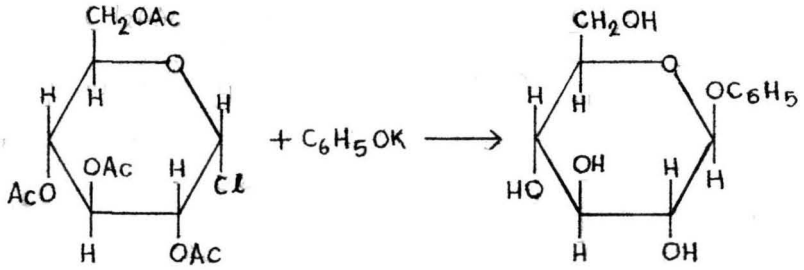
Although, O-acetylglycosyl bromides do not react with aglycones in the absence of a catalyst, the O-benzoylglycosyl bromides react readily without any catalyst to give good yields of the O-benzoylated glycosides under these conditions without any inversion[24]. In the usual Koenigs-Knorr reaction the expected inversion takes place, however, also with O-benzoylglycosyl bromides.



Several other acid acceptors such as alkali hydroxide, quinoline and mercury salts have also been used successfully as condensing agent in this reaction. They are detailed in the following paragraphs:

(a) Alkali hydroxide as the condensing agent

The first ever synthesis of a glycoside was carried out by Michael[10] by reacting O-acetylglycosyl chloride with a phenol in the presence of an ethanolic alkali hydroxide. In this reaction, in addition to the replacement of a halogen atom at C(1) by a phenoxy group with Walden inversion as usual, all the acetyl groups of the sugar are also simultaneously eliminated. Thus, the reaction between 2,3,4,6-tetra O-acetyl-α-D-glucopyranosyl chloride (XIV) and potassium phenoxide yields the free phenyl-β-D-glucopyranoside (XV).



XIV XV

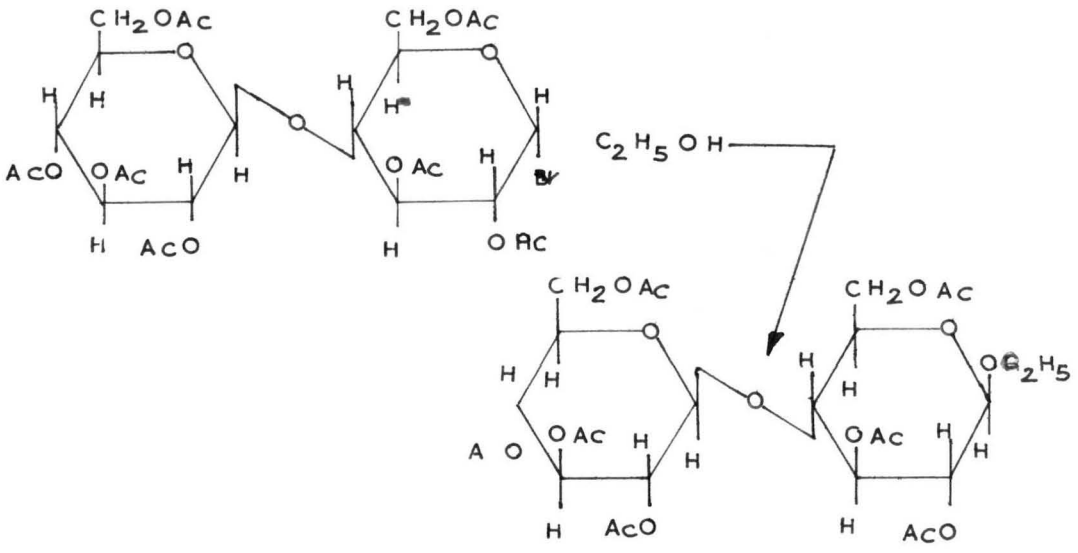
This method has been improved later by preventing deacetylation by using aqueous ethanol or acetone in the place of absolute ethanol and also by employing acetyl-O-glycosyl bromides instead of chlorides as originally used. This method is generally useful for the preparation of aryl glycosides and good results are obtained as a rule with negatively substituted phenols[12]. Recently this method has been used by Debruyne[25] in the synthesis of a number of aryl-β-D-xylopyranosides.

(b) Pyridine or quinoline as the condensing agent

Fischer et. al.[26] observed that pyridine or quinoline can also act as acid acceptors as well as solvents in the glycosidation of an alcohol or a phenol with O-acetylglycosyl bromides. This method usually provides a mixture of the anomeric-glycosides. At higher reaction temperatures, this method has been found to give acetylated glycosides without Walden inversion. Thus, the difficultly accessible α-anomeric glycopyranoside and methyl- α-D-fructopyranoside[27] have been obtained from the corresponding O-acetylated- α-D-glycosyl halides in this manner.

(c) Mercury salts as condensing agent

An elegant modification of Koenigs-Knorr reaction was first made by Zemplén[11] by replacing the costly silver compounds by the cheaper mercury salts as condensing agents. He found that mercuric acetate and mercuric bromide could bring about this glycosidation reaction successfully. Thus, hepta-O-acetyl- α-cellobiosyl bromide (XVI) on treatment of ethanol in presence of mercuric acetate gives the corresponding ethyl hepta-O-acetyl- β -cellobioside (XVII).



The Zemplén modification of Koenigs-Knorr reaction often proceeds without Walden inversion particularly when carried out in inert solvents or without a solvent. Some α-anomeric glycosides which are not easily obtainable by other methods have been prepared in this manner. Thus, good yield of p-tolyl tetra-O-acetyl-α-D--galactopyranoside was obtained by this procedure.

Helferich[12] recommended the use of mercuric cynide in this reaction as the condensing agent. This catalyst, however, may lead to the formation of per O-acetylglycosyl cynides.

3. Glycosidation with fully acetylated sugars

Successful glycosidation of fully acetylated sugars was first described by Helferich[28-31], who used anhydrous zinc chloride or p-toluene sulphonic acid as the condensing agent in the absence of any solvent. The advantage of this method is the use of more readily accessible and more stable fully acetylated sugars in the place of acylglycosyl halides. The more reactive acetoxy group at C(1) is replaced by the aglycone group under the conditions of the reaction which leads usually to the formation of both of the anomeric glycosides. The proportions of anomers, however, have been found to be largely influenced by the reaction conditions and the nature of the catalyst used.

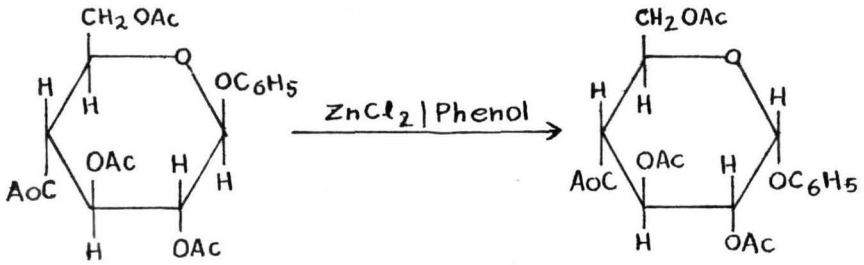
Glycosidation reactions in which fully acetylated sugars were used in the presence of various condensing agents are described in the following paragraphs:

(a) Anhydrous zinc chloride as the condensing agent

In the presence of anhydrous zinc chloride without any solvent, fully acetylated sugars react with a phenol to give a mixture of the two anomeric glycosides in which the α-anomer predominates. Higher temperatures (120^0 - 140^0) and longer periods of heating favour the formation of α-anomeric glycosides.

The anomeric configuration of the glycosides formed in this reaction does not depend on the configuration of the reacting acetylated sugar and, therefore, results of glycosidation with this catalyst are unpredictable[32].

It is believed, however, that irrespective of the anomeric configuration of the acetylated sugar used as the starting material, zinc chloride initially produces the β-anomeric glycoside which later anomerises giving a mixture of the two anomeric glycosides. Thus, it has been found that when phenyl tetra-O-acetyl- β-D--glucopyranoside (XVIII) is heated with zinc chloride and phenol it changes predominently into the α-anomer[33] (XIX)



XVIII XIX

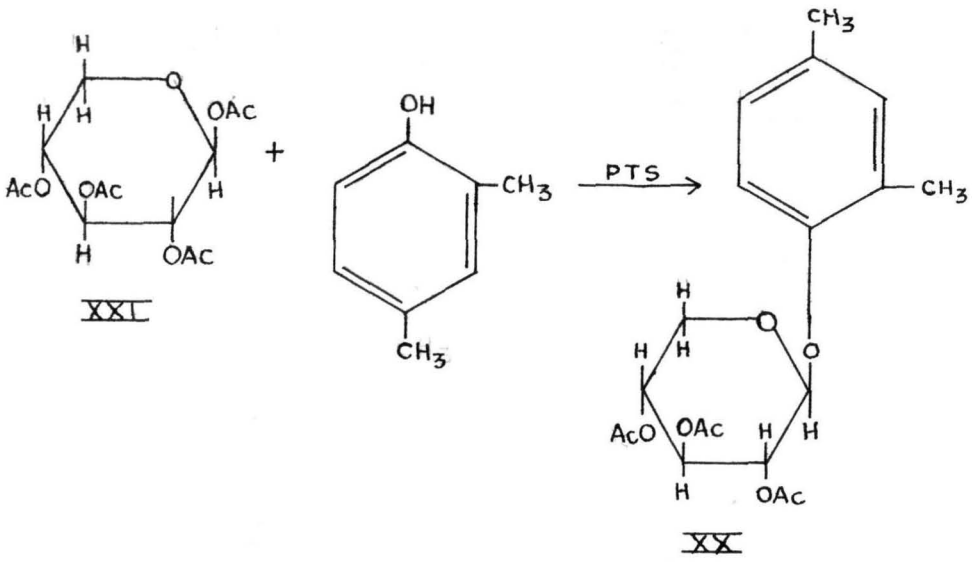
32

A number of modifications have been made in this method of glycosidation to accommodate a large number of sugars and phenols. One of the more recent modification suggested by Trevelyan[34] for

the synthesis of phenyl tetra-O-acetyl- α -D-glucopyranoside and p-nitrophenyl tetra-O-acetyl- α -D-glucopyranoside makes use of lower reaction temperature (120^0) and an atmosphere of nitrogen.

(b) p-Toluenesulphonic acid as the condensing agent

Reaction of phenols with fully acetylated sugars in the form of a melt in the presence of p-toluene sulphonic acid[28-31] as the condensing agent has found wide applicability particularly for the synthesis of aryl-β -glycosides. In this method, lower temperatures (100 - 120^0) and short duration of reaction give better yields. This method has been recently employed[25] for the preparation of acetylated β -D-xylopyranosides (XX) of various substituted phenols from tetra--O-acetyl- β -D-xylopyranose (XXI)



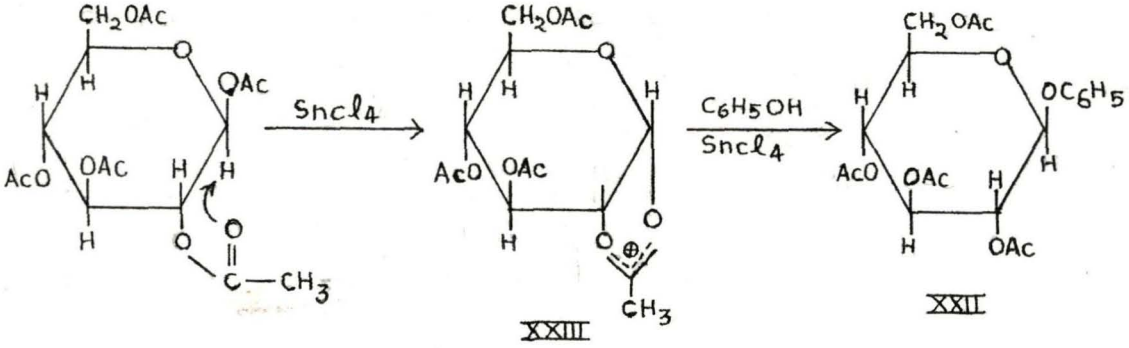
XXI XX

This reaction is not successful with some phenols and particularly ortho substituted phenols[35]. Although the β -anomers are formed as a

rule in this method, exceptions have been found with 2- or 3-deoxy--tetra-O-acetyl- β -D-galactopyranose which yield the α -anomeric glycosides[12,36].

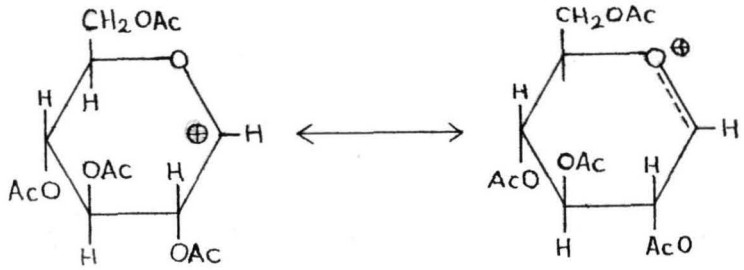
(c) Anhydrous stannic chloride as the condensing agent

Lemieux and Shyluk[37] prepared methyl and phenyl tetra-O-acetyl- β -D-glucopyranosides (XXII), by condensing penta-O-acetyl- β -D--glucopyranose respectively with methanol and phenol in benzene in the presence of anhydrous stannic chloride. According to these workers, the formation of the β -anomeric glycosides was favoured on account of the prominent role played by the carbonium ion (XXIII) formed by elimination of the acetoxy anion at C(1) and stabilized by anchimeric assistance[38] of the acetoxy group at C(2).



In the course of glycosidation of phenol and ortho nitro thiophenol with penta-O-acetyl- β -D-galactopyranose and penta-O--acetyl- β -D-glucopyranose respectively under the conditions prescribed by Lemieux and Shyluk (loc. cit), it was observed by Bose and Ingle[39] of this Laboratory that a considerable proportions of the α -anomeric glycosides are also formed in this reaction. This observation was initially based on examination of the glycosi--dation products by thin-layer chromatography (TLC) and was subsequently confirmed by actual isolation of the anomeric glycosides

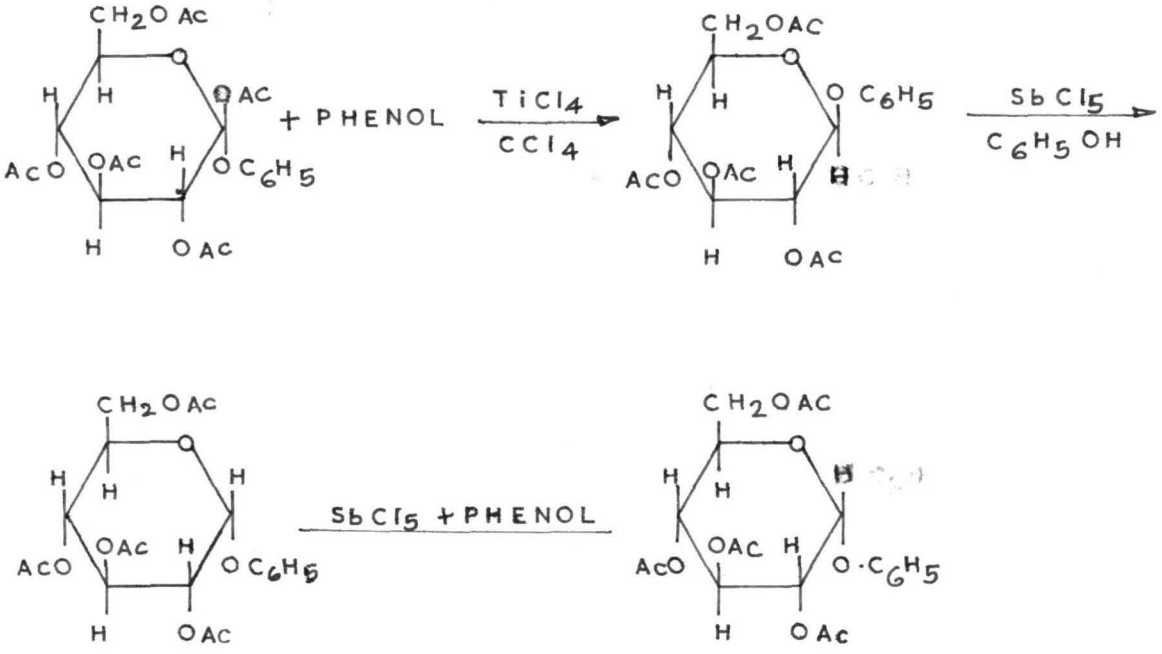
in fairly good yields either by fractional crystallisation or preparative layer or column chromatography. These authors also observed that two anomeric glycosides are formed in more or less equal amounts and postulated that the glycosidation reaction probably takes place through the carbonium ion (XXIV) stabilised by resonance with oxonium ion (XXV). Stannic chloride, therefore, proved to be a suitable condensing agent for the preparation of aryl- α -glycosides and thioglycosides from the fully acetylated sugars.



XXIV XXV

(d) Titanium tetra chloride as the condensing agent

Karasawa et. al.[40] have synthesised a number of acetylated aryl- β -D-glycosides in good yields by condensing phenols with fully acetylated sugars in carbon tetrachloride in the presence of anhydrous titanium tetrachloride. These authors observed that phenyl tetra-O-acetyl- β -D-glucopyranoside (XXVI) could be anomerised with antimony pentachloride in the presence of phenol to give the α -anomer to an extent of 25% under these conditions. They found that pure phenyl tetra-O-acetyl α -D-glucopyranosides (XXVII) did not give any β -anomer by a similar treatment.



Besides these four catalysts a number of others[11] have also been used to a limited extent from time to time. These include anhydrous ferric chloride[41], anhydrous aluminium chloride[42], boron trifluoride[43], phosphorus chloride[44] and sulphuric acid[45].

Ferric chloride as the condensing agent

Anhydrous ferric chloride has been used by Zemplén[41] as the condensing agent for the preparation of ethyl hepta-O-acetyl-∝--cellobioside from octa-O-acetyl-∝-cellobiose. It was postulated that ferric chloride formed a complex with the fully acetylated sugar which ultimately yielded the glycoside.

Aluminium chloride as the condensing agent

Anhydrous aluminium chloride[42] has also been used as a condensing agent for the glycosidation of azophenols such as p-phenyl azophenol and p-(3-nitrophenyl) azophenol.

Boron trifluoride as the condensing agent

Trace of boron trifluoride has been used by Bretschneider and Beran[43] as the condensing agent for the condensation of penta-O-acetyl-β-D-glucose at the room temperature in benzene. The same reaction in other solvents required more of boron trifluoride.

Phosphorus oxychloride as the condensing agent

Phosphorus oxychloride has been successfully used by Bembry and Powell[44] for the preparation of several aryl-β-D-glycosides such as phenyl tri-O-acetyl-β-D-xyloside, α-naphthyl tetra-O-acetyl-β-D--glucoside, O-phenyl-phenyl tetra-O-acetyl-β-D-glucoside, phenyl tetra-O-acetyl-β-D-fructoside. It has also been used as the catalyst for the preparation of tetrahydroxy cannabinyl tetra-O--acetyl-β-D-glucoside in which the phenol was available in small quantities.

Sulphuric acid as the condensing agent

Sulphuric acid has been successfully used by Helferich et. al.[45] for the preparation of vanillin-β-D-glucoside, phenyl-α/β-D--glucosides and phenyl-α/β-D-galactoside, but it has not found favour as a condensing agent as it brings about degradation of the compounds as well.

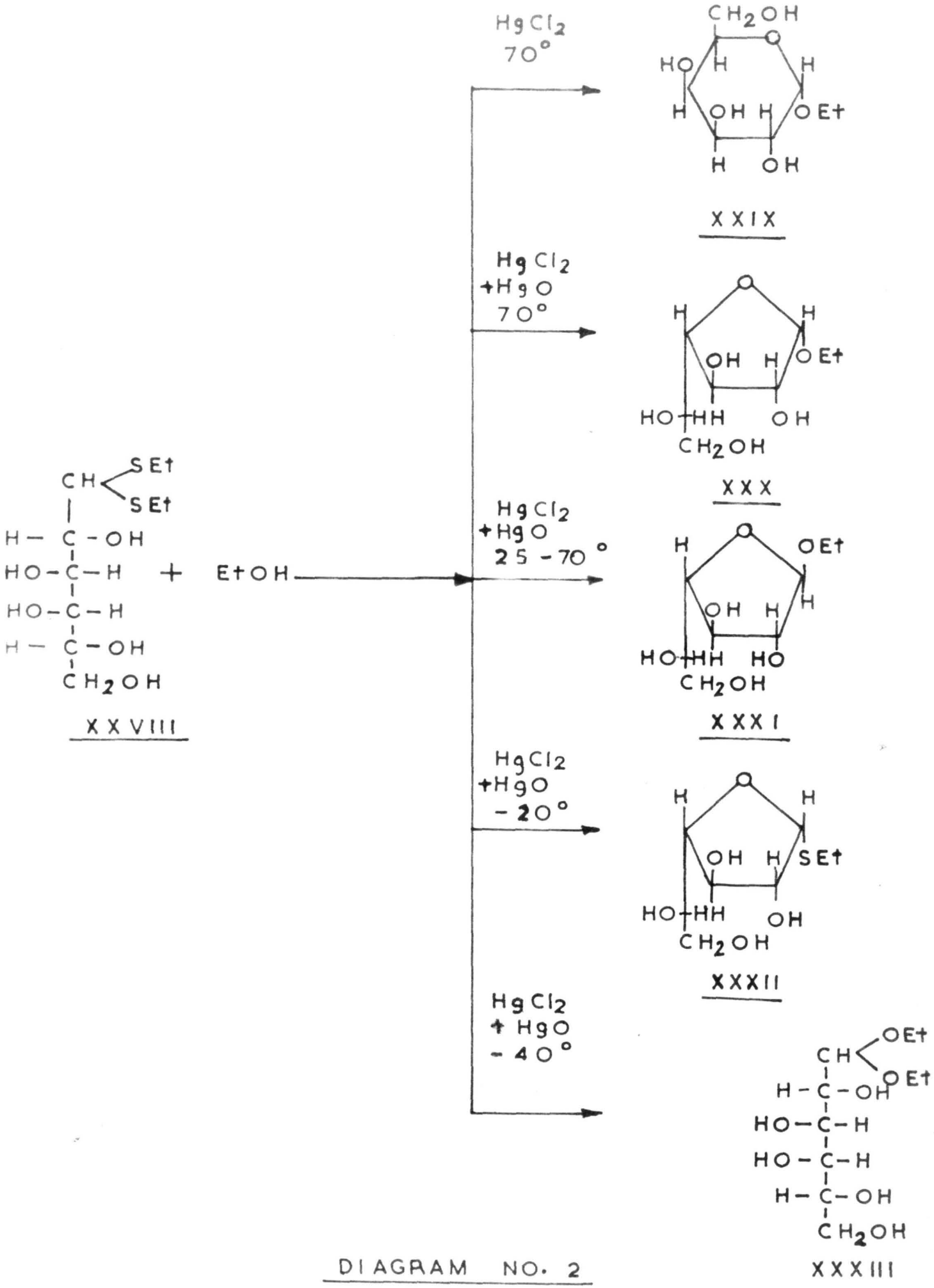

HgCl2
70°
HgCl2
+HgO
70°
HgCl2
+HgO
25 - 70°
HgCl2
+HgO
- 20°
HgCl2
+HgO
- 40°
EtOH
XXVIII
XXIX
XXX
XXXI
XXXII
XXXIII

DIAGRAM NO. 2

4. Glycosidation with thioacetals

Dialkyl- and di-aralkyl thioacetals of monosaccharides, which are open-chain derivatives and can be easily prepared by the action of thioalcohols on sugars in the presence of hydrogen chloride were successfully used by Pacsu[46] for the preparation of alkyl glycosides. This reaction between dithioacetal and alcohol is catalysed by mercuric chloride. This method was considerably improved later by employing lower temperatures and incorporating yellow mercuric oxide in reaction mixture for immediate neutralisation of hydrogen chloride formed in the reaction[11,12].

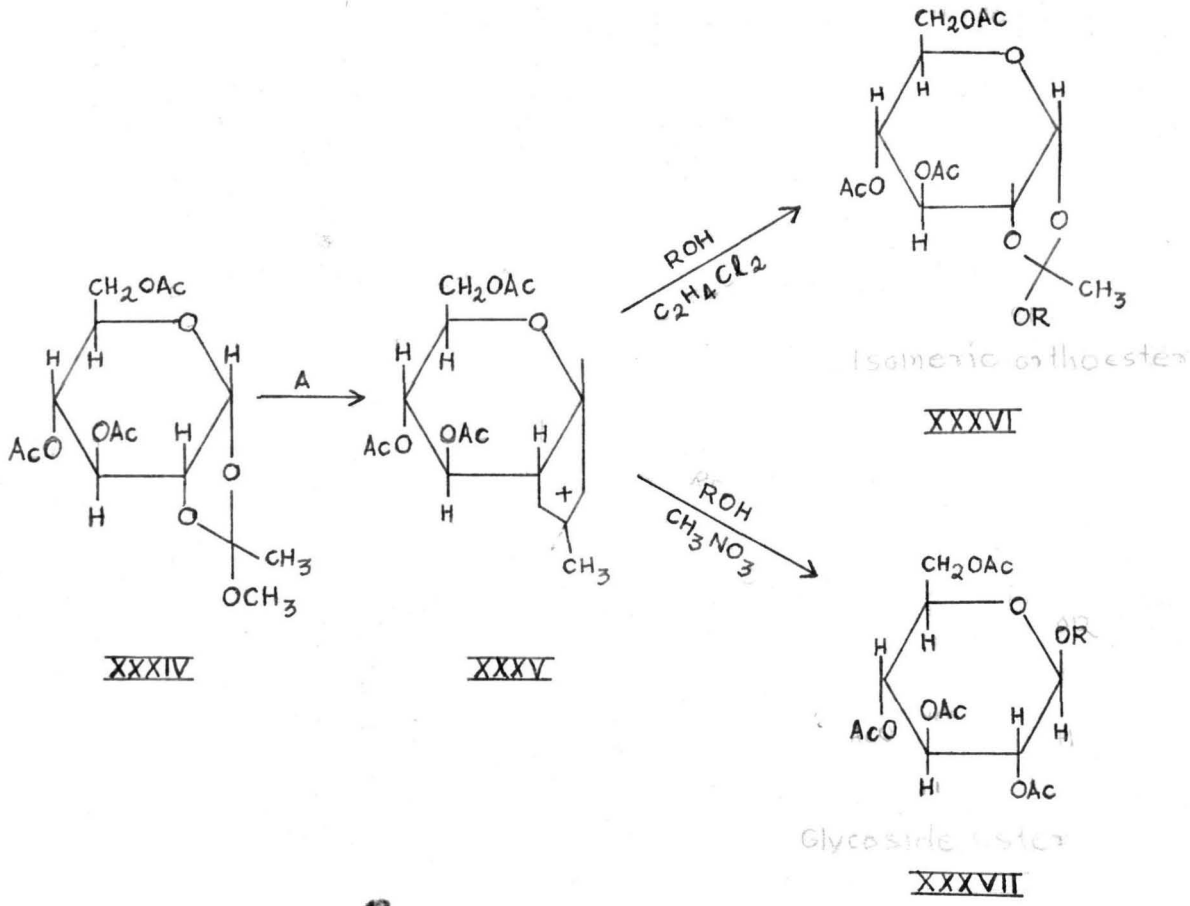
The structure of the final product obtained in this reaction very much depends upon the reaction conditions. Thus D-galactose diethyl dithioacetal (XXVIII) gives ethyl-α-or β-D-galacto furanoside (XXIX) and (XXX), ethyl-α-D-galactopyranoside (XXXI), ethyl thio-α-D-galactofuranoside (XXXII), or acetal (XXXIII) according to the reaction conditions described in the schematic diagram below: (see diagram No. 2)

5. Glycosidation with ortho esters

An entirely new approach for the synthesis of glycosides has been recently described by Kochetkov et. al.[47,48] in which a 1,2-ortho ester sugar acetate (XXXIV), is reacted with an aglycone under the catalytic influence of mercuric bromide and traces of p-toluene sulphonic acid in nitromethane medium. This method was extended subsequently for glycosidation of a wide range of non-aromatic aglycones. The glycosides prepared by this method include cholesterol glucoside, disaccharides, oligosaccharides and aliphatic glycosides.

This reaction is specific with respect to the amount of catalysts used and nature of the solvent employed. The size of the sugar ring (furanose or pyranose) does not affect the glycosidation. Different types of sugars such as hexoses, pentoses, deoxy-sugars and disaccharides were found to exhibit the same activity towards glycosidation by this method.

The reaction proceeds according to the scheme outlined below:



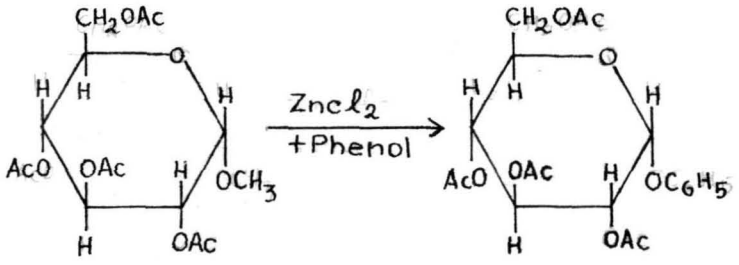
XXXIV XXXV XXXVI XXXVII

These workers[43] also developed a convenient method for the preparation of the ortho esters of sugars, used as starting materials in this reaction, by the action of an alcohol on acylglycosyl halides in ethyl acetate medium.

6. Transglycosidation

Under certain conditions it is possible to exchange the aliphatic aglycone by a different aglycone either aliphatic or aromatic. This process, known as trans-glycosidation, may take place with or without any change in configuration depending on the conditions of the reaction. Thus, in methanol containing hydrogen chloride, ethyl-α-D-glucopyranoside is transformed to methyl-β-D-glucopyranoside, whereas, both methyl and benzyl-α-D-fructopyranosides yield benzyl-β-D-fructofuranoside when the reaction is carried out in benzyl alcohol[49].

Under the conditions of the Helferich method methyl tetra-O-acetyl-α-D-glucopyranoside XXXXII was converted into the difficulty accessible phenyl tetra-O-acetyl-α-D-glucopyranoside XXXXIII by reacting with phenol in the presence of zinc chloride[33]. This method is particularly useful for the preparation of aryl-α-D-glycosides of mannose, arabinose and altrose whose acetylated methyl-α-D-glycosides are more easily available than the corresponding fully acetylated sugars[11].



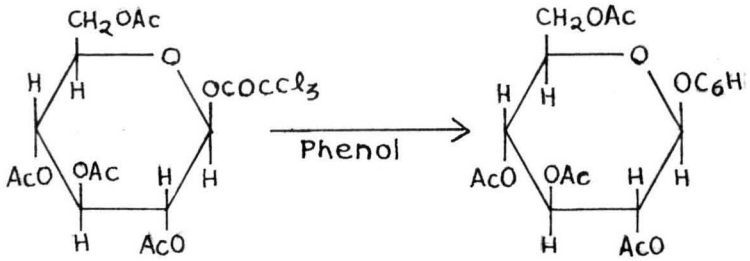
XXXXII XXXXIII

Bonner et. al.[50] found that methyl-α-D-glucoside and phenyl-α-D-glucoside formed complexes with boron trichloride. These complexes were found to exchange aglycone parts readily with other alcohols and phenols and thus constitute an alternative method of trans-glycosidation. Although this method directly yields the free glycoside and is suitable for glycosidation of alkali or acid labile sugars, it is accompanied

by many side reactions necessitating purification by fractionation.

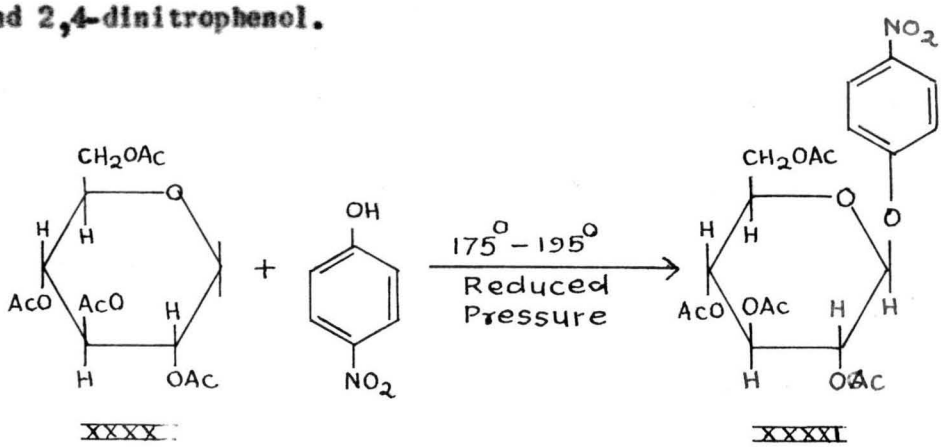
7. Non-catalytic glycosidation

Helferich et. al.[50] carried out the first successful non-catalytic glycosidation to obtain phenyl tetra-O-acetyl- β -D-glucopyranoside (XXXIX) by heating 1-O-trichloro acetyl, 2,3,4,6-tetra-O-acetyl- β -D-glucopyranose (XV) with phenol. The polarity of the trichloroacetyl group at C(1), induced it to leave the sugar moiety to be immediately replaced by the phenoxy group.



XXXVIII XXXIX

More recently Sato et. al.[52,53] have reported successful non-catalytic thermal condensation of fully acetylated sugars with phenols, purines and carboxalic acids, to obtain good yields of acetylated- β -D-glycosides. These authors synthesised- β -D-glucoside tetraacetates (XXXI) of p-nitrophenol and m-nitrophenol but, failed in the case of O-nitrophenol and 2,4-dinitrophenol.



XXXX XXXXI

Non-catalytic glycosidations between benzoylglycosyl halides[24] and phenols were also successful and has been described under the Koenigs-Knorr reaction. In 1966, a similar non-catalytic glycosidation of phenol with 1,2,4,6-tetra-O-acetyl-3-deoxy- β -D-glucopyranose has been reported by Antonakis[35].

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CHAPTER : II

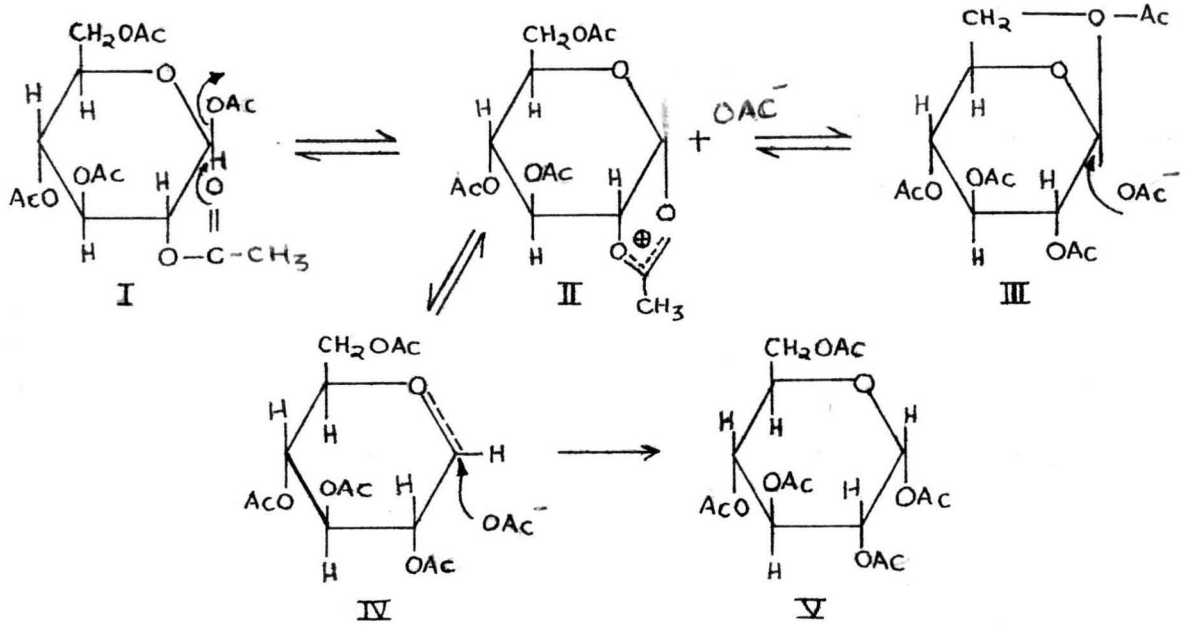
STUDIES IN THE VARIABLES OF STANNIC CHLORIDE CATALYSED GLYCOSIDATION.

SUMMARY

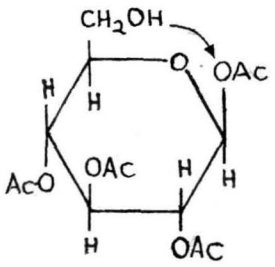
This Chapter gives an account of studies in the different variables of the stannic chloride catalysed glucosidation of phenol, such as, the effects of (1) the quantity of the diluent (benzene) used, (2) temperature and period of reaction, (3) relative proportion of the reactants and (4) catalyst concentration for optimisation of the reaction conditions under which it is possible to obtain either of the anomeric glucosides in the pure state.

STUDIES IN THE VARIABLES OF STANNIC CHLORIDE CATALYSED GLYCOSIDATION

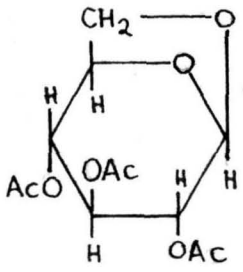
In 1928, Pacsu[1] observed that anhydrous stannic chloride in absolute chloroform solution catalysed the anomerisation of sugar acetates and alkyl glycosides. Similar anomerisations were also observed when titanium tetrachloride[2] or boron trifluoride[3] was used. The stannic chloride catalysed anomerisations were studied in more details later by Lemieux and Brice[4], who proposed a possible mechanism of this transformation. According to these authors, anomerisation of penta-O-acetyl-β-D-glucopyranose (I) by the catalyst involves initially a rapid dissociation of the bond between the C(1) carbon atom and the acetoxy group followed by participation of the C(2) acetoxy group to form a resonance stabilized carbonium ion (II) in which the lactol carbon atom has an α-configuration. Rearrangement of the carbonium ion (II) to the ion (III) or (IV) is possibly facilitated by the approach of the acetoxy anion and results in recombination of carbonium ions and the anion to the highly stable α-acetate (V).



Attempts to anomerise 1,2, 3,4,tetra-O-acetyl- β -D-glucopyranose (VI) with stannic chloride in chloroform solution, however, led to the formation of the internal glucoside, tri O-acetyl-D-glucosan (1,5) - β - (1,6) (VII) instead of anomerisation at C(1). This observation indicated the possibility of use of stannic chloride as a catalyst in glycosidation reactions.

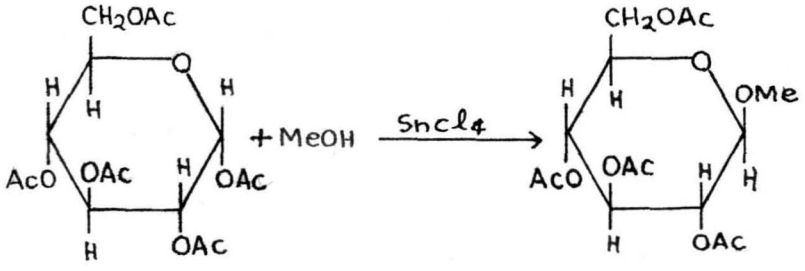


VI



VII

With this object in view, Lemieux and Shyluk[5] carried out the reaction between penta O-acetyl- β -D-glucopyranose (VIII) and methanol in the presence of stannic chloride in benzene solution and were successful in obtaining the expected methyl tetra-O-acetyl- β -D-glucopyranoside (IX).



VIII IX

Use of half a mol of stannic chloride per mol of methanol at 40° gave an optimum yield of 50-60% of this glycoside. The percentage of methyl tetra-O-acetyl- β -D-glucopyranoside present in the total reaction product, as determined by the isotopic dilution analysis, agreed very nearly with the methoxy content of

the total reaction product. This indicated that very little of the α-anomeric glucoside was formed in this reaction. The other by-product of this reaction showing a positive optical rotation was presumed to be acetochloro-, α-D-glucose (16.4%) on the basis of its conversion to methyl tetra-O-acetyl- β -D-glucopyranoside by the Koenigs-Knorr method of glycosidation.

This reaction was also extended for the synthesis of phenyl tetra-O-acetyl- β -D-glucopyranoside which was obtained in about 40% yield.

According to Lemieux and Shyluk[5] the formation of the β -anomeric glucoside was favoured in this reaction on account of the prominent role played by the carbonium ion (II) generated by the elimination of the acetoxy anion at C(1) of penta-O-acetyl- β -D-glucopyranose (I), as has already been discussed in connection with the anomerisation reactions catalysed by stannic chloride.

Bose and Ingle[6] of this Laboratory, however, detected the presence of considerable amounts of phenyl tetra-O-acetyl- α -D--glycopyranosides by thin layer chromatography of the reaction products obtained in the course of the preparation of phenyl tetra-O--acetyl- β -D-glycopyranosides by the procedure described by Lemieux and Shyluk[5]. The relative proportions of the two anomers of the aryl glycosides formed in the reaction were found to depend on several factors including concentration of the acetylated sugar and duration of the reaction. Under the conditions described by Lemieux and Shyluk[5] for the preparation of phenyl tetra-O-acetyl- β -D-glucopyranoside, Bose and Ingle[6] found that the α and β -anomers were formed in the

proportion of 3:7 approximately and the pure β-anomer was found to crystallise out from ethanolic solution of the reaction products as the first fraction and the mixture of the anomers present in the mother liquor could be separated by column or preparative layer chromatography on silica gel. They also found that under similar conditions, penta-O-acetyl- β -D-galactopyranose gave a mixture of phenyl tetra-O--acetyl α -and β -D-galactopyranosides in the proportion of 6:4 and in this case the pure α -anomeric galactoside acetate crystallises out as the first crop. The reaction between acetylated sugar and phenol in presence of stannic chloride, therefore, constituted a new method for the syntheses of acetylated aryl α-glycosides.

These findings of Bose and Ingle[6] clearly indicated that the anchimeric assistance[7] of the C(2) acetoxy group leading to the exclusive formation of β-anomeric glycosides, cannot be considered to be a major significant factor in this reaction. On the other hand, in view of the formation of almost equal quantities of the two anomeric glycosides, these authors considered that the formation of the carbonium in (IV) by elimination of the acetoxy anion at C(1) would be the major contributing factor in this reaction.

This new approach for the syntheses of aryl α -D-glycosides has now been studied in greater details in order to have a better understanding of the reaction. For this purpose, the glucosidation of phenol with penta-O-acetyl- β -D-glucopyranose was chosen as the model reaction and the effects of following different variables were studied in details:

1. Effect of dilution with an inert solvent
2. Effect of temperature and period of reaction
3. Effect of relative proportion of the reactants
4. Effect of concentration of the catalyst.

Effect of dilution with an inert solvent

Considerable differences have been observed[8,9] in the effect of polar solvents in glycosidation reactions. Non-polar solvents, however, are known to have no such effect[10]. Lemieux et. al.[4,5] used the non-polar solvent benzene in their stannic chloride catalysed glucosidations. The same solvent was also used in the present studies.

Preliminary thin-layer chromatographic (TLC) studies of a few stannic chloride catalysed glucosidations of phenol using different amounts of benzene as diluent, revealed that even under identical reaction conditions the amount of benzene used actually showed a profound influence on the proportion of the anomeric glucosides formed in these reactions. This observation which was not studied by the earlier workers was, therefore, followed up by a systematic study of the reaction between penta-O-acetyl-β-D-glucopyranose and phenol in the molar proportion of 1:2 in the presence of 1 mol of the catalyst, anhydrous stannic chloride, at 95° for a period of 10 minutes using varying amounts of benzene as a diluent.

TABLE I

Effect of change in dilution of benzene on the reaction among penta-O-acetyl-β-D-glucopyranose (10 millimol) -phenol (20 millimol) and $SnCl_4$ (10 millimol) at 95° ± 1°

S.No.	Volume of benzene used (ml)	Percentage yield of the reaction product	Melting point of the reaction product °C	Relative amounts of phenyl tetra-O-acetyl glucopyranosides formed*	
				α-anomer	β-anomer
1	300	35	124-125	0-5	95-100
2	150	35	118-119	5-10	90- 95
3	100	35	93- 95	30-35	65- 70
4	25	32	96-97	40-50	50- 60
5	10	30	111	70-80	20- 30
6	5	30	114	90-95	5- 10
7	2.5	25	114	95-100	0- 5
8	0.0	10	114	95-100	0- 5

* some tarry matter was also formed

Table I gives the results of a series of eight such experiments which reveal that the use of an excess of benzene as diluent leads to the almost exclusive formation of the β-anomeric glucoside. A gradual decrease in the amount of the diluent, however, leads to the formation of proportionately increased amounts of the α-anomeric glucoside in the mixture of the anomeric glucosides formed. With very small amounts of the diluent or in its absence, the α-anomeric

glucoside was found to be almost exclusively formed in the reaction. The TLC of these reaction products showed, in addition to the anomeric phenyl tetra-O-acetyl-α/β-D-glucopyranoside, some unreacted glucose pentaacetate (when a large volume of the diluent is used) and some degradation products. The relative amounts of phenyl tetra-O-acetyl--glucopyranosides shown in the Table I are based on eye-estimation from the relative darkness and area of the TLC spots. Some of the eye-estimates were varified by PLC on silica gel and found to be within $\pm$ 5 units of error. In these experiments, crystallisation of phenyl tetra-O-acetyl- α-and β-D-glucopyranosides is not complicated by the presence of the decomposition products.

Effect of temperature and period of reaction

Effects of temperature and period of reaction were studied together for the reaction between penta-O-acetyl- β-D-glucopyranose (1 mol), phenol (2 mols) in the presence of stannic chloride (1 mol) and benzene. Aliquots were taken out at different intervals from reactions carried out at different temperatures, and after isolation, the products were examined by TLC. The eye estimated relative intensities of the different TLC spots were taken as a measure of the relative concentrations of the various products formed in the reaction. Table II gives the results of a series of such experiments carried out for different periods at various temperatures between 0° and 100°.

TABLE II

Effect of temperature and period of reaction on the reaction between penta-O-acetyl-β-D--glucopyranose (10 millimol) and phenol (20 millimol) in the presence of stannic chloride (10 millimol) and benzene (5 ml)

S.No.	Temperature °C	Time	Relative amount existing in reaction mixture of			
			Phenyl tetra-O-acetyl-D-glucopyranoside		Penta-O-acetyl-β-D-glucopyranose	Other reaction products
			α-anomer	β-anomer		
1	2	3	4	5	6	7
1		5 min	45-50	15	5-10	20-25
2		15 min	50-60	0-5	-	40-45
3	100	30 min	40	-	-	65
4		1 hr	25	-	-	75
5		2 hr	20	-	-	80
6		5 hr	5-10	-	-	90-95
1		5 min	40	30	20	10
2		15 min	50-55	15	10	20
3	70	30 min	45	-	-	50
4		1 hr	35	-	-	65
5		2 hr	15-20	-	-	85
6		5 hr	0- 5	-	-	95

1	2	3	4	5	6	7
1		5 min	25	60	5	10
2		15 min	30	60	-	10
3		30 min	40	45	-	15
4		45 min	45	40	-	15
5		1 hr	50	30	-	20
6	50	1½ hr	50	30-25	-	20-25
7		2 hr	50	25	-	25
8		2½ hr	60	20	-	25
9		5 hr	65	5	-	30
10		7 hr	60	-	-	40
11		9 hr	58	-	-	50
1		15 min	25	35-40	25-30	10
2		30 min	40	45	5	10
3		45 min	45	30	-	25
4		1 hr	50	50	-	25
5		2 hr	45	20	-	35
6	40	5 hr	45	10	-	40
7		15 hr	50	-	-	50
8		40 hr	50	-	-	55
1		6 hr	20	70	5	5
2		20 hr	35	50	-	15
3	20	40 hr	50	25	-	25
4		70 hr	65	-	-	35
5		90 hr	55	-	-	45

1	2	3	4	5	6	7
1		20 hr	25	65	5	5
2		40 hr	25	65	-	10
3	0	70 hr	30	50	-	20
4		90 hr	30	50	-	20
5		120 hr	30	50	-	20

It will be evident from Table II that at higher temperatures such as 100° the α-anomeric glucoside is the only anomer seen on the TLC plate after some time. But with an increase in the time of reaction more and more of resinous products are formed, evidently at the expense of the α-anomer. The formation of the unwanted by-products were less in the reactions carried out at lower temperatures for longer periods. Thus, whereas practically no phenyl tetra-O-acetyl-α-D-glucopyranoside remains in the products of reaction carried out for 5 hrs at 100° in which TLC places show mostly the decomposition products, in reaction carried out at 40° for 15-40 hrs, TLC shows the presence of almost equal amounts of the α-anomeric glucoside and the decomposition products and no β-anomer. In all these experiments use of only 5 ml of benzene per 10 millimol of the sugar pentaacetate gave best results.

It will be clear from Table II that heating of the reaction mixtures for short periods at higher temperatures leads to almost exclusive formation of the α-anomeric glycoside. These high temperature reactions are however difficult to control and often lead to unsatisfactory yields owing to the formation of large

quantities of unwated by-products and consequent difficulties in the isolation of the pure glycoside by crystallisation.

Better results are obtained when the reaction rates are slowed down by bringing down the reaction temperatures to 40 to 50°, and increasing the period of reaction to 7 to 15 hr. Room temperature reactions (20 - 30°) require a longer period of up to 48 hours for completion. Under these conditions the formation of the by-products is less and crystallisation of the phenyl- α -D-glucoside easier.

Effect of relative proportions of the reactants

It was thought of interest at this stage to see whether the proportion of phenol to glucose pentaacetate has any effect on the yields of two anomeric glucosides. 5 - 0.25 parts of phenol per part of glucose pentaacetate in mols were tried. The reactions were carried out at the room temperature (mean 25°) using 5 - 0.25 millimols of phenol per millimol of glucose pentaacetate for periods up to 70 hr following the progress of the reactions by TLC.

Although Lemieux and Shyluk[5] used two mols of phenol to one mol of glucose pentaacetate in the stannic chloride catalysed glucosidation reactions, the present series of experiments described in Table III established that the use of these two reactants in equimolar proportion gives the best results. Moreover, with more costly phenols use of large proportions (as used by Lemieux[5] and Helferich[11]) will be uneconomical and their removal from the reaction mixture will be laborious.

TABLE III

The effect of change of concentration of phenol at 25°

S.No.	Ratio of phenol with glucose penta acetate	Time in hr	Relative amounts existing in reaction mixture of			
			Phenyl tetra-O-acetyl -D-glucopyranoside		Penta-O-acetyl β-D-glucopyranose	Other by-products
			α-anomer	β-anomer		
1		6	5	80	10	5
2	5:1	20	10	75	5	10
3		45	15	70	-	15-20
1		6	20	70	10	15
2		20	30	45	5	20
3	2:1	45	50	25	-	25
4		70	60	-	-	40
1		6	30	60	5	5
2		20	40	65	-	5
3	1:1	45	70	20	-	10-15
4		70	70	-	-	30
1		6	20	30	45	5
2	0.5:1	20	35	15	40	10
3		45	45	5	40	20
1		6	15	10	70	5
2	0.25:1	20	25	5	65	5
3		45	20	1	65	10

Effect of concentration of the catalyst

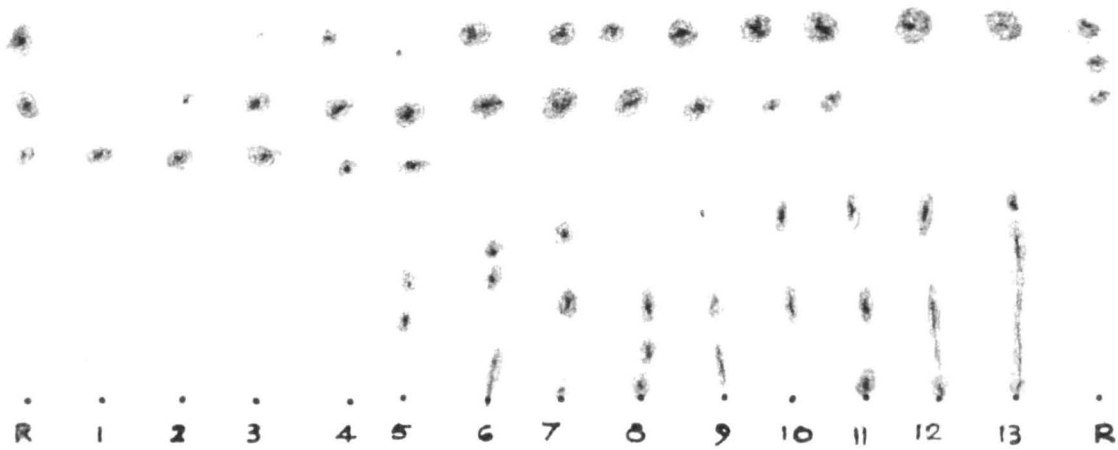
The other variable studied in these reactions was the proportion of the catalyst, stannic chloride. These reactions were also carried out at the room temperature (mean 25^{0}). Keeping the proportion of the reactants equimolar, the proportion of stannic chloride was varied from 5 mols to 0.25 mols. Use of two mols to 0.5 mol of stannic chloride per mol of the reactants seemed to have little effect on the overall yield of the α-anomeric glucoside formed. The proportion of the β-anomer, however, seemed to increase with decrease in the proportion of stannic chloride. The results of these studies are given in Table IV.

TABLE IV

Effect of change of catalyst concentrations at 25°

S.No.	Ratio of stannic chloride and phenol	Time in hr	Relative amounts existing in reaction mixture of			
			Phenyl tetra-O-acetyl- D-gluco-pyranoside		Penta-O-acetyl-β-D-glucopyranose	Other by-products
			α-anomer	β-anomer		
1		6	40	55	-	5
2	5:1	20	60	20	-	20
3		45	65	-	-	35
1		6	40	55	-	5
2	2:1	20	60	25	-	15
3		45	70	-	-	30
1		6	35	60	1	5
2	1:1	20	50	35	-	15
3		45	70	5	-	25
1		6	30	60	5	5
2	0.5:1	20	40	65	-	5-10
3		45	70	20	-	10-15
1		6	15	60	20	5
2	0.25:1	20	30	40	10	15
3		45	45	35	-	20

PLATE — I



Progress of glucosidation between phenol and penta-O-acetyl β -D-glucopyranose at 20°.

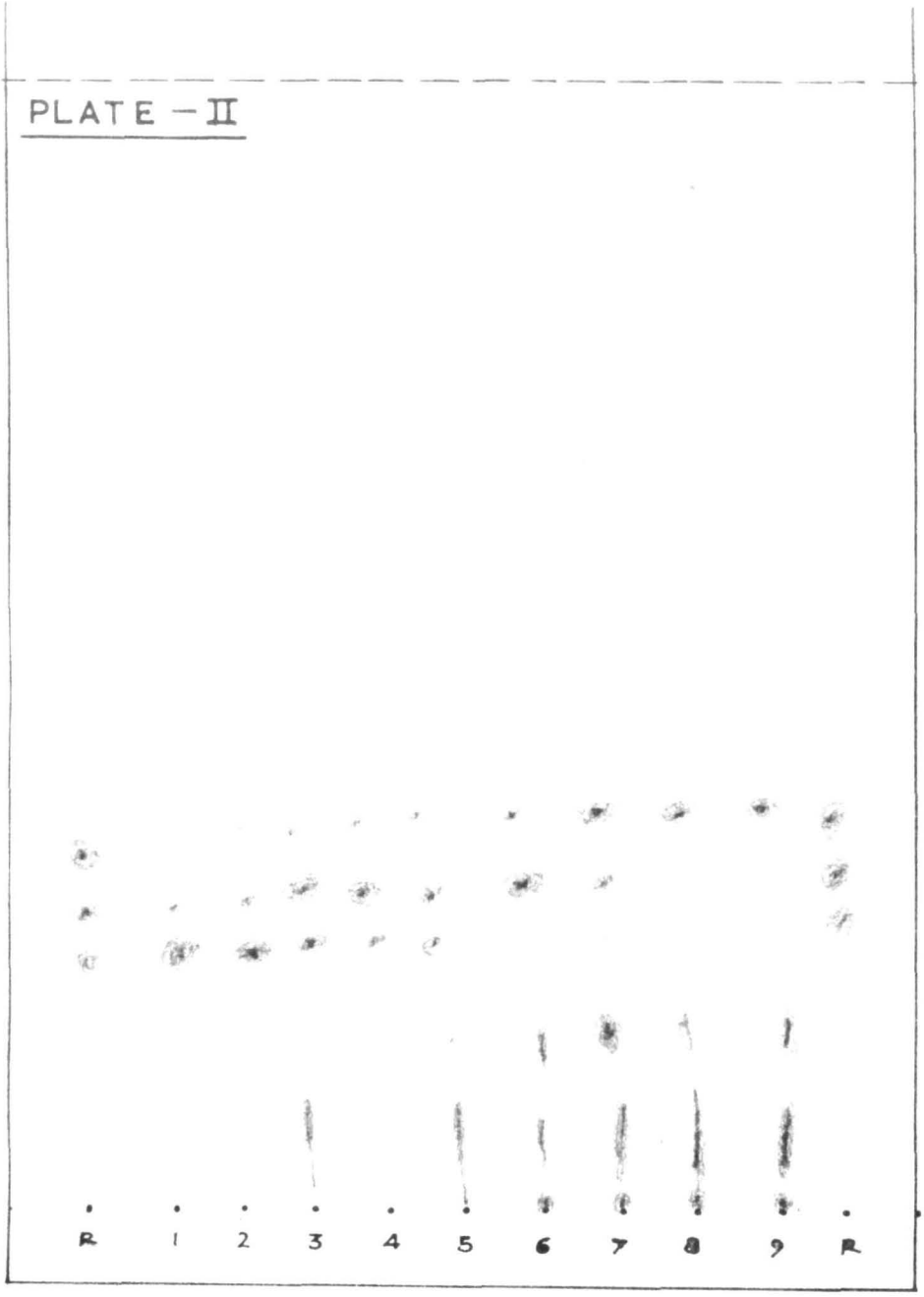
Solvent system A

R. Mixture of penta-O-acetyl β -D-glucopyranose, phenyl tetra-O-acetyl β -D-glucopyranoside and phenyl tetra-O-acetyl α -D-glucopyranoside

1-4. Reaction mixtures taken after 5, 15, 30 and 60 minutes.

5-13. Reaction mixtures taken after 2, 5, 10, 15, 25, 35, 50, 65 and 80 hours

PLATE – II



Progress of glucosidation between phenol and penta-O-acetyl β-D-glucopyranose at 30°.

Solvent system: A

R. Mixture of penta-O-acetyl β-D-glucopyranose phenyl tetra-O-acetyl- β-D-glucopyranoside and phenyl tetra-O-acetyl- α-D-glucopyranoside.

1-4. Reaction mixtures taken after 5, 15, 30 and 60 minutes.

5-9. Reaction mixtures taken after 2, 4, 6, 8 and 10 hours.

From these detailed studies of the different variables in stannic chloride catalysed glucosidation of phenol, it has now been established that equimolar proportions of the reactants should be used for obtaining best yields of phenyl-α-glucosides in the presence of equi-or bi-molar proportion of stannic chloride at temperatures between 25°-40° for a period which can be better established from the TLC data of the pilot experiments. ~~Diluent~~ Dilution should be low if the α-glucosides are required as end products. (Use of excess of the diluent such as benzene in these reactions lead to almost exclusive formation of the β-glucosides.) Use of excess of the diluent (benzene) leads to almost exclusive formation of the phenyl-β-D-glucoside, whereas, use of small quantities of the diluent or its complete absence, or the other hand, leads to almost exclusive formation of phenyl-α-D-glucoside. These studies, therefore, have established for the first time, the optimum conditions for the stannic chloride catalysed synthesis of either of the anomeric phenyl-D-glucosides. The conditions are likely to be of general applicability for the synthesis of other glycosides also.

The significance of these factors and their bearingson the possible mechanisms of stannic chloride catalysed glucosidations will be discussed in Chapter IV of this part of the thesis.

The following TLC plates give a clear picture of the stannic chloride catalysed glucosidation reactions under the optimum conditions. (See diagram) ~~No.~~)

EXPERIMENTAL

All reactions were conducted under anhydrous conditions. All melting points were recorded on a Kofler-block and are uncorrected.

Concentrations were carried out at a temperature of 50° under reduced pressure.

Thin-layer chromatography was performed over silica gel plates (no binder) in the following solvent systems:

(1) Petroleum ether (40°-60°) and methyl ethyl ketone (3:1)

(2) Petroleum ether (40°-60°) and acetone (3:1)

Sulphuric acid was used as the spray reagent.

Constant bath temperatures were thermostatically regulated to within ± 1°C.

Reagents

Thiophene-free benzene was prepared according to the directions given in Practical Organic Chemistry[12] and was stored over fresh sodium wire.

Penta acetyl-β-D-glucopyranose (M.P. 135°) was prepared by acetic anhydride - sodium acetate method and was crystallised from alcohol[12].

Anhydrous stannic chloride ('Riedel') was used in these experiments.

Phenol was distilled under reduced pressure and stored in a desiccator.

Methods

Effect of variation of dilution

Penta-O-acetyl- β -D-glucopyranose (3.9 gm, 10 millimol) was added to a mixture of phenol (1.9 gm, 20 millimol), stannic chloride (1.1 ml, 10 millimol approximately) and required volume of thiophene-free benzene and the mixture was refluxed for ten minutes, allowed to cool to room temperature and diluted with benzene to a volume of 300-325 ml. The benzene solution was washed thoroughly with cold water, and then with saturated aqueous sodium bicarbonate solution followed by more of water and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure yielded a syrup which was stirred with ethanol (10 ml) and cooled at -5°C for 10-15 hr. The resulting crystalline product was collected by filtration and washed with cold ethanol (0.5 ml). The yield, melting point, and other relevant data are given in Table I.

Effect of variation of temperature and period of reaction

Penta-O-acetyl- β -D-glucopyranose (0.39 gm, 1 millimol) was added to a mixture of phenol (0.19 gm, 2 millimol), stannic chloride (0.1 ml, 1 millimol) and thiophene-free benzene (0.5 ml). Six such different flasks containing this mixture were kept at temperatures of 100°, 70°, 50°, 40°, 20° and 0° respectively. Aliquots of reaction mixtures were withdrawn at intervals and immediately treated with a mixture of benzene and saturated aqueous bicarbonate solution. The benzene layer was concentrated and dried over anhydrous sodium sulphate and spotted on TLC plates which were developed in solvents (1) and (2), sprayed with conc. sulphuric acid and heated at 110°. The TLC results (eye-estimates) are given in Table II.

Effect of variation of proportion of phenol

Solutions of different quantities of phenol in thiophene free benzene (0.5 ml) containing stannic chloride (0.1 ml, 1 millimol) were prepared and penta-O-acetyl- β -D-glucopyranose (0.39 gm, 1 millimol) was added to each solution. All these reaction mixtures were kept at 20°. Aliquots of these reaction mixtures were withdrawn at different intervals and processed as described in the preceeding paragraph. Eye-estimates of the TLC data thus obtained are given in Table III.

Effect of variation of proportion of phenol

Solutions of phenol (0.9 gm, 10 millimol) in thiophene-free benzene (5 ml) containing different quantities of stannic chloride were prepared and penta-O-acetyl- β -D-glucopyranose (3.9 gm, 10 millimol) was added to each solution. All these reaction mixtures were kept at 20°. Aliquots of these reaction mixtures were withdrawn at different intervals and processed as described earlier. Eye-estimates of the TLC data thus obtained are given in Table IV.

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CHAPTER : III

STANNIC CHLORIDE CATALYSED SYNTHESIS OF SOME ARYL-α-GLYCOSIDES

SUMMARY

The general applicability of the stannic chloride catalysed glycosidation reaction for the syntheses of various acetylated aryl-α-D-glucopyranosides as well as of acetylated-β-D-glucopyranosides under two different sets of conditions is described in this Chapter. Use of stannic chloride in bringing about anomerisation of acetylated aryl-β-D-glucopyranosides is also described.

STANNIC CHLORIDE CATALYSED SYNTHESES OF SOME ARYL α-AND β-GLYCOSIDES

Although Lemieux[1] described the preparation of only one aryl β-glycoside, namely, that of phenyl β-D-glucopyranoside as acetate by the reaction between penta-O-acetyl- β-D-glucopyranose and phenol as early as in 1953, no further account of preparation of any other aryl glycoside by this method was available so far in literature. The recent observations of Hose and Ingle[2] that under favourable conditions a mixture of aryl α-and β-glycosides can be prepared by this method has increased the potentiality of this method for the syntheses of aryl glycosides.

The conditions under which the α- or the β-anomeric glucosides can be preferentially obtained in this reaction have already been established for phenyl α-and β-D-glucopyranosides as described in the preceding Chapter. The present Chapter aims to establish the general applicability of the optimum conditions already established for phenyl glucosides for the syntheses of various other aryl α- and β-glucosides by actual syntheses of several anomeric pairs of these glucosides. The preparation of a few aryl tetra-O-acetyl- α-D-glucosides by anomerisation of the corresponding β-D-glucoside acetates is also described in this Chapter.

For the synthesis of the aryl-α-D-glucoside prefertially according to the present established method, one mol of fully acetylated β-D-glucopyranose is mixed with one mol each of phenol[a] and anhydrous stannic chloride along with benzene (250 ml approx.) The mixture is kept under anhydrous conditions either at the room

temperature or heated up to 50° with occasional shaking. The time required is 8 to 70 hr depending on the reaction temperature and the nature of the phenol used. The same reaction can also be carried out at higher temperatures for a comparatively shorter periods. The progress of the reaction is followed by TLC. The reaction is continued until the last traces of the aryl tetra-O-acetyl- β -D-glucopyranoside disappear. The tetra-O-acetyl- ∝-D-glucosides prepared by this general procedure are listed in Table I. The corresponding free glucosides were obtained by catalytic deacetylation of the glucoside acetates with sodium methoxide[3]. These are listed in Table II.

The glucoside acetates of ortho substituted phenols were found to be more difficult to crystallise. The acetylated glucosides in such cases, were either purified on preparative-layer chromatography (PLC) and then crystallised. Alternatively, the free glucosides were obtained by deacetylation of the uncrystallisable acetates with sodium methoxide. These free glucosides ~~could be~~ crystallised either from syrup or from methanol or a mixture of methanol-ether, without any difficulty.

This elegant synthesis of aryl-∝-D-glucosides appears superior to the zinc chloride catalysed glycosidation i.e. Helferich reaction[4], which often leads to the formation of a considerable proportion of the β -anomeric glycosides and undesirable decomposition products. Stannic chloride catalysed glycosidation reaction for the synthesis of aryl- ∝ -glycosides on the other hand gives several advantages which are enumerated below:

1. The reaction can be carried out at the room temperature
2. Temperature of reaction ($< 50°$) is low and does not cause charring

3. Excess of phenol is not required as recommended in Helferich reaction and thus it can be employed for the preparation of glucosides of rare and costly phenols.

The starting material in this method is the sugar acetate which gives it advantages over the Koenigs-Knorr[5-7] method. The reactions were all monitored by the thin-layer chromatography to check the amount of the β -anomeric glucosides formed as well as the completion of reaction.

The general applicability of this method was established by the syntheses of seven aryl-α -D-glucosides described here and of several other aryl- α -glycosides and thioglycosides by Bose et. al.[2].

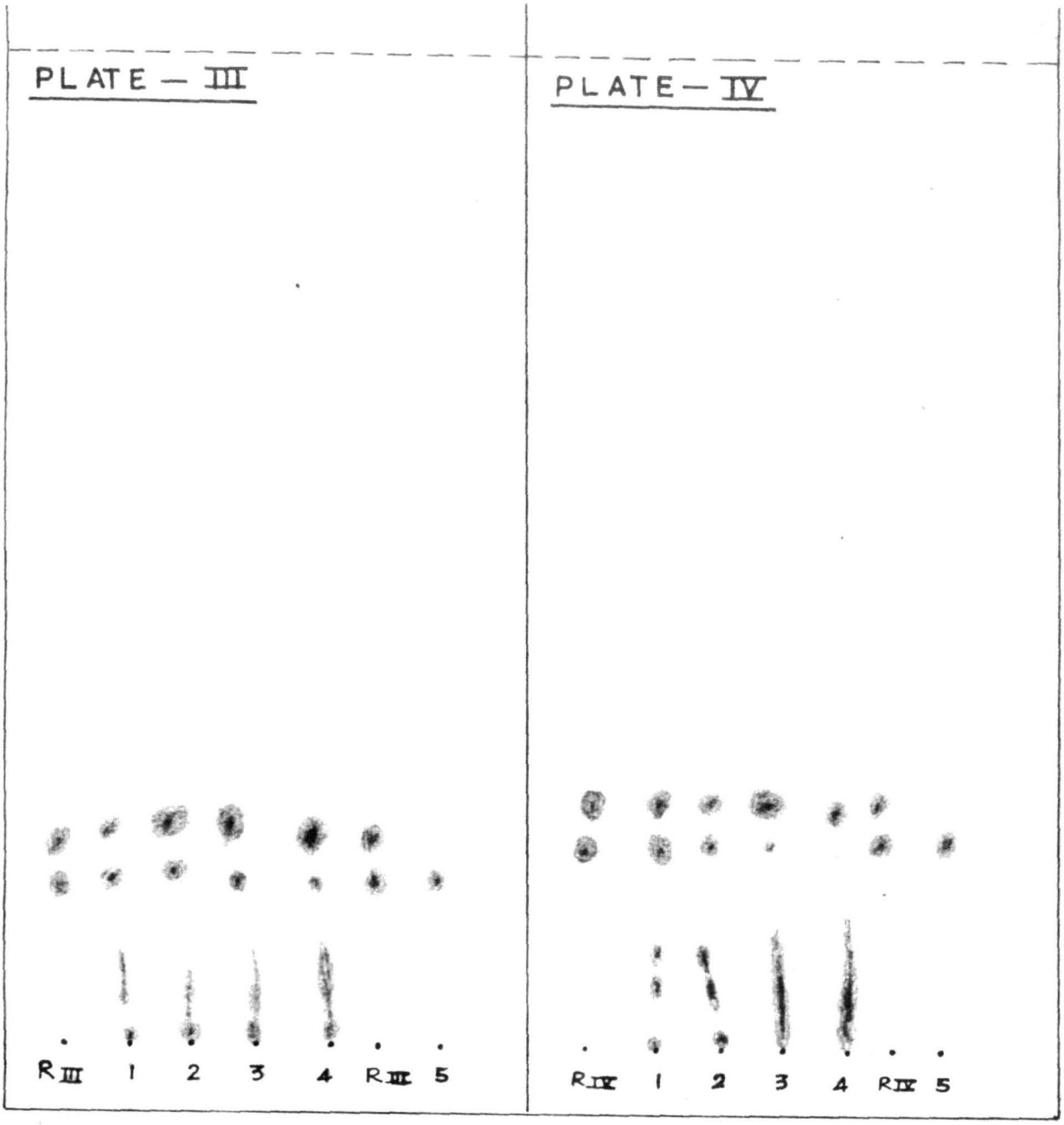
Lindberg's[8] studies on the rates of anomerisation of some acetylated alkyl glycosides led Lemieux et. al.[1] to presume that the success of the stannic chloride catalysed glycosidation is uncertain and will depend on the nature of the phenol used in the reaction. These authors, prepared only one aryl-glycoside, namely phenyl tetra-O--acetyl- β -D-glucopyranoside, and, therefore, their presumptions were not supported by experimental evidence. The present work, however, has established that the stannic chloride glucosidation reaction is an excellent general method not only for the preparation of the difficultly accessible aryl- α -glucosides but also for the general synthesis of aryl- β -glucosides as well under suitable condition such as high dilution and use of larger proportion of the phenol as described in the earlier Chapter. The general applicability of this method has been verified in the course of the present work by synthesising seven acetylated aryl- β -D-glucosides which are listed in Table I by this procedure. The corresponding free glucosides were obtained

by catalytic deacetylation of the acetylated glucosides with sodium methoxide and are listed in Table II.

The only phenol which failed to react under these conditions was o-nitrophenol. This is probably due to the strong chelation of the phenolic hydroxyl group with the nitro group in the ortho position, reducing the reactivity of the phenolic group towards glucosidation. This reaction resulted only in the anomerisation of the penta-O-acetyl-β-D-glucopyranose.

Anomerisation of aryl glycosides was regarded as an uncommon phenomenon by Lindberg[9] and Lemieux[1]. It is, however, known that alkali brings about anomerisation of 2,4 dinitrophenyl-β-D-glucopyranoside but not of other nitrophenyl glycosides[9]. Anomerisation of only one aryl tetra-O-acetyl-β-D-glucopyranoside is reported in literature with the acid catalysts, zinc chloride[10] and antimony penta chloride[11] respectively. In view of these, it was considered of interest to study the possibility of the acid catalyst, stannic chloride, acting as an anomerisation catalyst.

When a mixture of an aryl tetra-O-acetyl-β-D-glucopyranoside the corresponding phenol and excess of stannic chloride in a little benzene was heated at 40°-50° for about 20 hr under anhydrous conditions, a gradual conversion of the β-anomeric glucoside into the α-anomer was noticed and was followed up by thin-layer chromatographic analysis of reaction aliquots withdrawn at different intervals. The reaction was continued until the mixture did not show any β-anomer. The α-glucoside acetate was isolated and characterised. Use of more of stannic chloride and less of phenol appeared to lead to a better anomerisation reaction.



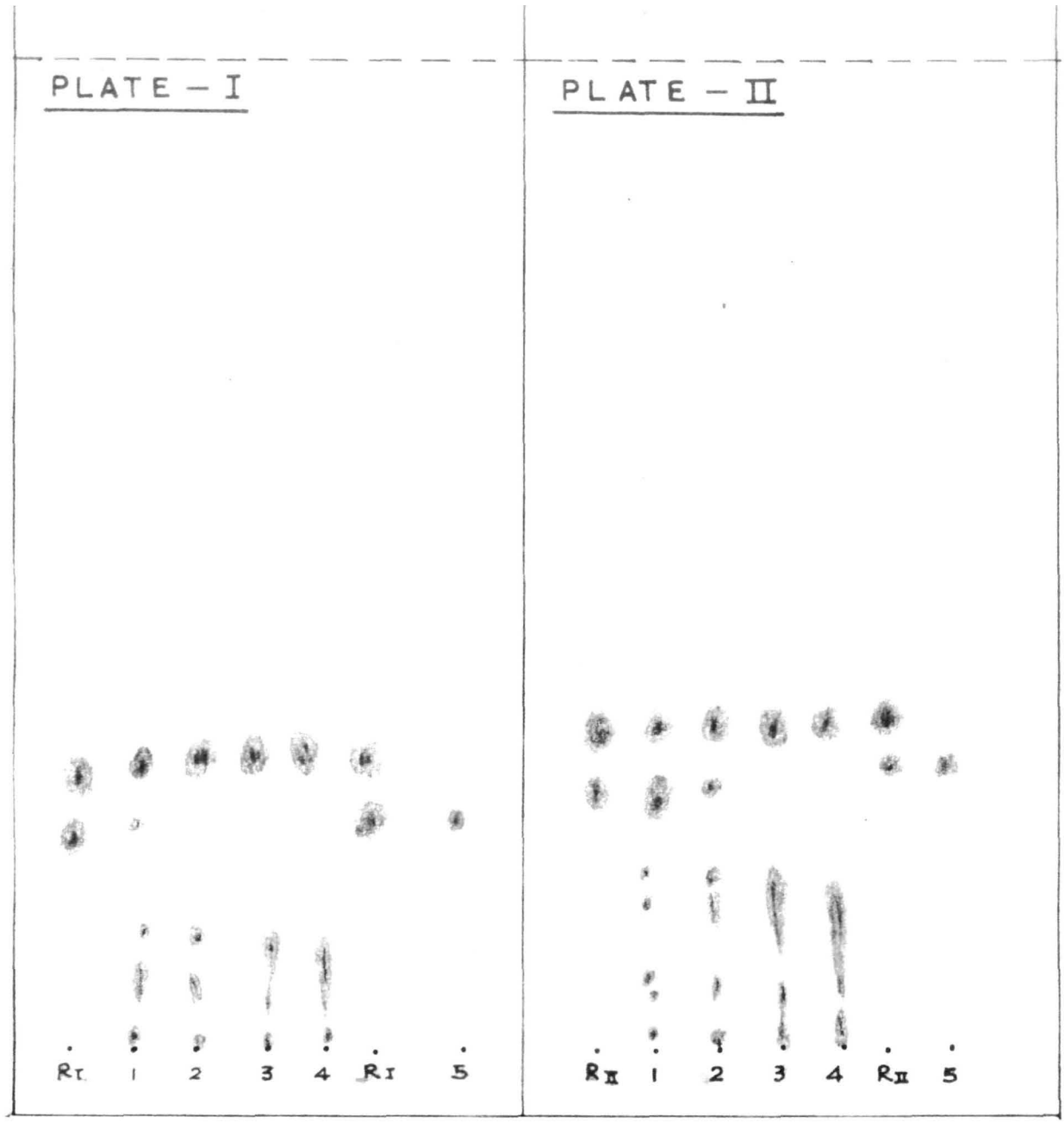
Progress of stannic chloride catalysed anomerisation of p-phenyl phenyl tetra-O-acetyl- β D-glucopyranoside (Plate-III) and o-chlorophenyl tetra-O-acetyl β -D-glucopyranoside (Plate-IV) at 50°

Solvent system: A

R_{III}. Mixture of α -and β -p-phenyl phenyl tetra-O-acetyl D-glucopyranosides.

R_{IV} . Mixture of α -and β o-chlorophenyl tetra-O-acetyl D-glucopyranosides.

1-5 . Reaction mixtures taken after 6, 20, 40, 50 and zero hour respectively.



Progress of stannic chloride catalysed anomerisation of phenyl tetra-O-acetyl- β -D-glucopyranoside (Plate-I) and p-chlorophenyl tetra-O-acetyl β -D-glucopyranoside (Plate-II) at 50°

Solvent system: A

R_I. Mixture of α -and β -phenyl tetra-O-acetyl-D-glucopyranosides

R_{II}. Mixture of α -and β p-chlorophenyl tetra-O-acetyl D-glucopyranosides

1-5. Reaction mixtures taken after 6, 20, 40, 50 and zero hour respectively.

Table III gives a list of aryl tetra-O-acetyl β-D-glucosides anomerised in this manner.

TABLE III

S.No.	Aryl tetra-O-acetyl-β-D-glucopyranoside used for anomerisation	M.P.	Aryl tetra-O-acetyl-α-D-glucopyranoside obtained by anomerisation	M.P.	Yield
1	Phenyl tetra-O-acetyl-β-D-glucopyranoside	124°-125°	Phenyl tetra-O-acetyl-α-D-glucopyranoside	113°-114°	26%
2	p-Chlorophenyl tetra-O-acetyl-β-D-glucopyranoside	124°	p-Chlorophenyl tetra-O-acetyl-α-D-glucopyranoside	101°-102°	21.6%
3	p-Phenyl-phenyl tetra-O-acetyl-β-D-glucopyranoside	155°-156°	p-Phenyl-phenyl tetra-O-acetyl-α-D-glucopyranoside	169°	31.2%
4	o-Chlorophenyl tetra-O-acetyl-β-D-glucopyranoside	141°	o-Chlorophenyl tetra-O-acetyl-α-D-glucopyranoside	79°	11.5%

It was also observed that phenyl tetra-O-acetyl-α-D-glucopyranoside is comparatively more stable and does not anomerise into the corresponding β-anomer when subjected to the same treatment. Therefore anomerisation of aryl tetra-O-acetyl-β-D-glucopyranoside appears to be irreversible. This helps the progressive accumulation of the α-anomer, although accompanied by side reactions, which adversely affect the yields. It may be noted that in these anomerisation reactions the presence of phenol is essential. Only stannic chloride cannot bring about these anomerisations.

From these observations it is evident that anomerisation of an aryl-β-D-glucoside acetate to the α-anomer is possible under the conditions of stannic chloride catalysed syntheses of α-D-glucopyranosides. It is very probable, therefore, that in this glucosidation reaction anomerisation plays a role towards the formation of the α-D-glucoside. This is depicted in the diagram for the progress of glucosidation at 20° and 50° in Chapter II, Part I.

The possible mechanism of the stannic chloride catalysed anomerisation and glycosidation reactions will be discussed in the next Chapter.

TABLE I

Physical properties of aryl tetra-O-acetyl-D-glucopyranosides prepared by stannic chloride catalysis

S. No.	Aglucone	Configuration	M.P. °C	$[\alpha]_D^{30}$ in chloroform	Yield in per cent	Analysis Found C	Analysis Found H	Analysis Calculated C	Analysis Calculated H	Spectral data (d)	Remark & Reference
1	Phenol	α	113°	+162.20° C, 1.00	32.0	56.80	5.91	56.59	5.65	IR, Mass spm.	7, 13, 14
		β	124°	- 21.80° C, 0.90	35.5	56.62	5.65	56.59	5.65	IR, Mass spm.	7, 13, 14
2	p-Methyl phenol	α	—	-	-	-	-	-	-	- -	(a) 16
		β	123°	- 21.40° C, 2.09	39.9	57.01	6.11	57.40	5.94	IR -	19
3	p-Chloro phenol	α	102°	+ 166° C, 1.44	26.8	52.11	4.82	52.20	5.22	IR, Mass spm.	(b)
		β	124°	- 20.50° C, 1.0	33.5	52.15	4.91	52.20	5.22	IR, Mass spm. PMR	13, 21
4	p-Nitro phenol	α	113°	+ 201.0° C, 1.10	26.0	51.34	5.30	51.07	5.10	IR, Mass spm. PMR	10, 13
		β	174°	- 21.0° C, 1.0	26.7	51.21	5.17	51.07	5.10	IR, Mass spm. PMR	10, 22
5	p-Phenyl phenol	α	169°	+ 198.55° C, 1.38	36	62.81	5.86	62.40	5.60	IR, Mass spm. PMR	(b)
		β	155°	- 12.30° C, 1.98	29	61.19	5.89	62.40	5.60	IR, Mass spm.	(b)
6	6-Chloro phenol	α	78°	+ 128.40° C, 1.23	3	52.43	4.80	52.20	5.22	IR -	(b), (c)
		β	141°	- 45.90° C, 1.29	25	52.25	4.85	52.20	5.22	IR -	13, 21
7	o-Methyl phenol	α	98°	+ 130.0° C, 0.77	4.5	56.98	5.85	57.40	5.94	IR -	(c) 18
		β	141°	- 25.1°	24.4	57.01	6.23	57.40	5.94	IR -	24

(a) Reported uncrystalline, (b) reported for the first time (c) isolated by preparative silica gel layer chromatography (Syrup)

(d) various spectra are given in the Chapter V.

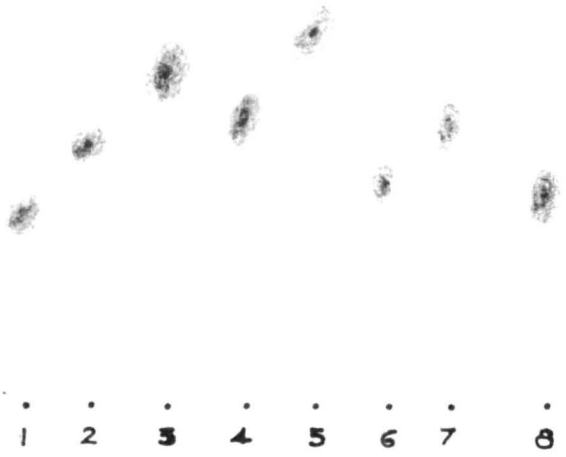
TABLE II

Physical properties of aryl-D-glucopyranosides prepared by deacetylation[a],[b] of the corresponding tetra acetates reported in Table I

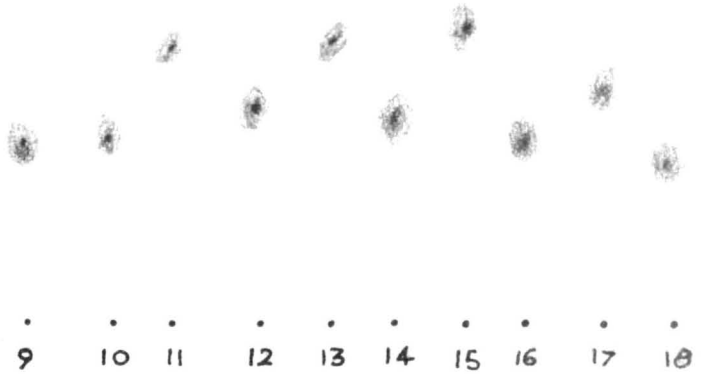
S. No.	Aglycone	Configuration	M.P. °C	$[\alpha]_D^{30}$ in solvent	Yield in per cent	Analysis Found C	Found H	Calculated C	Calculated H	Spectral data[d]	Remark & Reference
1	Phenol	α	176°	+ 179.90° C, 1.03 water	23.4	55.03	6.11	56.10	6.10	IR, PMR	(a), 7, 13, 15
		β	172°	- 74.0° C, 1.01 water	24.5	56.27	5.81	56.10	6.10	IR, PMR	(a), 7, 13, 15
2	p-Methyl phenol	α	192°	+ 176° C, 1.10 water	23.0	57.62	6.86	57.77	6.66	IR, PMR	(b), 13, 16
		β	180°	- 66.6° C, 1.0 water	25.1	57.62	6.88	57.61	6.66	IR, PMR	(a) 13, 20
3	p-Chloro phenol	α	201°	+ 125.89° C, 0.39 dioxan	20.8	50.05	5.65	49.63	5.19	IR, PMR	(a), (c)
		β	175°	- 66.01° C, 1.0 methanol	23.0	49.34	5.19	49.63	5.19	IR, PMR	(a) 13, 21
4	p-Nitro phenol	α	214°	+ 228.01° C, 1.0 methanol	10.0	47.46	5.63	47.80	4.98	IR, PMR	(a) 17
		β	166°	- 100.0° C, 0.99 water	17.2	47.62	5.27	47.80	4.98	IR, PMR	(a) 10,23
5	p-Phenyl phenol	α	226°	+ 221.93° C, 0.42 dioxan	22.5	64.81	6.15	65.06	6.10	IR, PMR	(a), (c)
		β	207°	- 5.45° C, 0.55 dioxan	17.5	64.95	6.28	65.06	6.10	IR, PMR	(a), (c)
6	o-Chloro phenol	α	150°	+ 86.90° C, 0.42 dioxan	10.1	49.75	5.60	49.34	5.19	IR, PMR	(a), (c)
		β	164°	- 66.02° C, 0.99 methanol	16.8	49.43	5.29	49.34	5.19	IR, PMR	(a), (c),21
7	o-Methyl phenol	α	122°	+ 155.0° C, 1.0 water	14.6	57.95	6.56	57.77	6.66	IR, PMR	(b), 13, 18
		β	166°	- 68.04°	19.5	57.93	6.77	57.77	6.66	IR, PMR	(a), 20

(a) Crystalline tetra acetate was deacetylated, (b) the reaction products containing tetra acetates were deacetylated as the tetra acetates were not crystallising, (c) reported for the first time, (d) various spectra are given in Chapter V and (e) yields mentioned express the final yields obtained from penta-O-acetyl-β-D-glucopyranose of aryl-D-glucopyransides at the end of both the steps i.e. preparation of tetra acetates and their deacetylations.

PLATE—V



PLATE—VI



Mobilities of different anomeric aryl tetra-O-acetyl-D-glucopyranosides on thin layer of silica gel in solvent system: A.

Plate-V and VI together

1, 8, 9, 18. Penta-O-acetyl- β -D-glucopyranose
2. Phenyl tetra-O-acetyl- β -D-glucopyranoside
3. Phenyl tetra-O-acetyl- α -D-glucopyranoside
4. p-Chlorophenyl tetra-O-acetyl- β -D-glucopyranoside
5. p-Chlorophenyl tetra-O-acetyl- α -D-glucopyranoside
6. o-Chlorophenyl tetra-O-acetyl- β -D-glucopyranoside
7. o-Chlorophenyl tetra-O-acetyl- α -D-glucopyranoside
10. p-Nitrophenyl tetra-O-acetyl- β -D-glucopyranoside
11. p-Nitrophenyl tetra-O-acetyl- α -D-glucopyranoside
12. o-Methylphenyl tetra-O-acetyl- β -D-glucopyranoside
13. o-Methylphenyl tetra-O-acetyl- α -D-glucopyranoside
14. p-Methylphenyl tetra-O-acetyl- β -D-glucopyranoside
15. p-Methylphenyl tetra-O-acetyl- α -D-glucopyranoside
16. p-Phenyl phenyl tetra-O-acetyl- β -D-glucopyranoside
17. p-Phenyl phenyl tetra-O-acetyl- α -D-glucopyranoside

EXPERIMENTAL

All reactions described in this Chapter were carried out under anhydrous conditions and all evaporations were carried out under reduced pressure at 40° to 50°.

Melting points were recorded on a Kofler bloc and uncorrected values are given.

Silica gel (without binder) was used in Thin Layer Chromatography (TLC). The thin-layer plates were prepared by spreading silica gel slurry in ethanol (1:2 w/v) on clean glass plates (10 x 15 cm) with the help of an applicator. The plates were dried in air, heated for 1 hr at 110° and cooled in a desiccator before use.

Plates (25 x 25 cm) for preparative layer chromatography were also prepared similarly.

TLC was carried out in closed vessels containing one of the following solvent systems:

1. Petroleum ether (40° to 60°), methyl ethyl ketone (3:1 v/v)
2. Petroleum ether (40° to 60°), acetone (3:1 v/v)
3. Benzene, ethyl acetate (7:3 v/v)
4. Toluene, ether with traces of dimethyl sulphoxide (2:1 v/v)

Reagents

Penta-O-acetyl-β-D-glucopyranose was prepared according to A Text Book of Practical Organic Chemistry[12] using pure D-glucose. Melting point of the final product was 135° (twice recrystallised from ethanol).

Phenols were distilled before use and were of analytical quality. Stannic chloride used was anhydrous and pure.

Benzene used in the reaction was made thiophene free[12] and was finally dried over sodium wire. Distilled benzene was used for other purposes.

Synthesis of aryl-α-glucosides

Phenyl tetra-O-acetyl-α-D-glucopyranoside[7,13,14]

Phenol (1.9 gm, 20 millimol) and penta-O-acetyl-β-D-glucopyranose (7.8 gm, 20 millimol) were dissolved in benzene (5 ml). After addition of stannic chloride (2.2 ml, 20 millimol), the mixture was kept at the room temperature (30° to 35°) for 60 hr with occasional shaking. Aliquots of the reaction mixture were withdrawn, and examined on TLC for the spot corresponding to phenyl tetra-O-acetyl-β-D-glucopyranoside.

After complete disappearance of the β-anomer, the reaction mixture was triturated with benzene in the presence of saturated aqueous sodium bicarbonate solution (0.5%). The benzene layer was washed twice with cold aqueous alkali (1%) and thrice with water and dried over anhydrous sodium sulphate. Evaporation under reduced pressure yielded a syrup which crystallised from ethanol (20 ml) on cooling (-10°). The crystals were filtered, washed with a little ethanol and dried. The pure product was obtained as colourless needles, m.p. 113°, $[\alpha]_D^{30}$ + 162.2° (C, 1.0 chloroform) Literature[14] gives m.p. 114°, $[\alpha]_D$ + 166° (chloroform). Yield 2.82 gm (32.1%).

Found: C, 56.80; H, 5.91. Calculated for $C_{20}H_{25}O_{10}$, C, 56.59; H, 5.65 per cent.

Phenyl-α-D-glucopyranoside[7,13,15]

The crystalline phenyl tetra-O-acetyl-α-D-glucopyranoside (2.8 gm) was deacetylated with sodium methoxide solution (0.2 mol, 5 ml) in methanol at the room temperature. After 5 hr deacetylation was found to be complete (TLC) and the reaction mixture was diluted with methanol (20 ml) and decationised with Amberlite IR-120 (H^+) resin. The decationised solution was evaporated to a syrup and crystallised from ethanol-ether mixture in colourless needles, m.p. 176°, $[\alpha]_D^{30}$ 179.9° (C, 1.03 water). Literature[15] gives m.p. 173°-174° $[\alpha]_D$ + 180.8 (water). Yield 2.46 gm (23.4%).

Found: C, 56.63; H, 6.10. Calculated for $C_{12}H_{16}O_6$, C, 56.10; H, 6.11 per cent.

p-Methyl phenyl tetra-O-acetyl-α-D-glucopyranoside

p-Cresol (2.1 gm, 20 millimol) was condensed with penta-O-acetyl-β-D-glucopyranose (7.8 gm, 20 millimol) in benzene (5 ml) in the presence of stannic chloride (2.2 ml, 20 millimol) at the room temperature for 60 hr. The product was purified in the usual manner but could not be crystallised from any of the solvents like, n-propanol, ether, benzene, pet. ether or their mixtures. Nisizawa[16] also obtained it as a non-crystalline syrup.

p-Methylphenyl-α-D-glucopyranoside[16]

The syrupy p-methylphenyl tetra-O-acetyl-α-D-glucopyranoside (7.5 g) was deacetylated with sodium methoxide solution (0.2 mol, 5 ml) in methanol at the room temperature for 4 hr. The reaction mixture was cooled at 0° and the resulting crystalline solid was collected and

recrystallised from methanol, m.p. 192-193°, $[\alpha]_D^{30}$ + 176.0° (C, 1.10 water). Literature[16] gives m.p. 190-191°, $[\alpha]_D$ + 178° (water). Yield 1.2 gm (23%).

Found: C, 57.62; H, 6.86. Calculated for $C_{13}H_{18}O_6$, C, 57.7; H, 6.66 per cent.

p-Chlorophenyl tetra-O-acetyl- α-D-glucopyranoside

Condensation of p-chlorophenol (2.6 gm, 20 millimol) with penta-O-acetyl- β -D-glucopyranose (7.8 gm, 20 millimol) in benzene (5 ml) in the presence of stannic chloride (2.2 gm, 20 millimol) at the room temperature for 60 hr and working up of the reaction product in the usual manner gave a syrup which on repeated crystallisation from ethanol gave colourless needles, m.p. 102°, $[\alpha]_D^{30}$ + 166° (C, 1.44 chloroform). This is a new compound. Yield 2.49 gm (26.8%).

Found: C, 52.11; H, 4.82, d, 7.51, $C_{20}H_{24}O_{10}Cl$ requires, C, 52.20; H, 5.22, d, 7.50 per cent.

p-Chlorophenyl- α -D-glucopyranoside

Deacetylation of p-chlorophenyl tetra-O-acetyl- α-D-glucopyranoside (2.2 gm) with sodium methoxide solution (0.2 mol, 2 ml) at the room temperature for 2 hr and the reaction mixture was diluted with pet. ether (40°-60°) (2 ml) and cooled when the pure product crystallised in colourless needles, m.p. 201° $[\alpha]_D^{30}$ + 125.89° (C, 0.39 dioxan). This is a new compound. Yield 1.24 gm (20.8%).

Found: C, 50.05; H, 5.65, d, 11.51, $C_{12}H_{15}O_6Cl$ requires, C, 49.63; H, 5.19, d, 11.55 per cent.

p-Nitrophenyl tetra-O-acetyl- α-D-glucopyranoside[10,13]

Condensation of p-nitrophenol (2.8 gm, 20 millimol) with penta-O-acetyl- β-D-glucopyranose (7.8 gm, 20 millimol) in benzene (5ml) in the presence of stannic chloride (2.2 gm, 20 millimol) followed by subsequent working up as usual gave a syrup which crystallised from ethanol. Recrystallisation from the same solvent gave the pure product as colourless needles, m.p. 113°, $[\alpha]_D^{30}$ + 201° (C, 1.0 chloroform). Literature gives, m.p. 113°, $[\alpha]_D$ + 200° (chloroform). The yield was 2.42 gm (26%).

Found: C, 51.34; H, 5.30. N, 2.86 Calculated for $C_{20}H_{24}O_{12}N$, C, 51.07; H, 5.107 N, 3.0 per cent.

p-Nitrophenyl-α-D-glucopyranoside[17]

Deacetylation of p-nitrophenyl tetra-O-acetyl- α-D-glucopyranoside (2.4 gm) with sodium methoxide solution (0.2 mol, 2 ml) at the room temperature for ½ hr gave a yellow solution which deposited crystals of the free glucoside when the solution was cooled to -10° and treated with a drop of glacial acetic acid to remove the yellow colour. The product was thus obtained in colourless needles, m.p. 214° $[\alpha]_D^{30}$ + 228.01 (C, 1.0 methanol). Literature[17] gives m.p. 216-217° $[\alpha]_D$ + 227.9° (methanol). The yield was 1.2 gm (10%).

Found: C, 47.48; H, 5.63. N, 4.32 Calculated for $C_{12}H_{15}O_8N$, C, 47.80, H, 4.98 N, 4.35 per cent.

p-Phenyl-phenyl tetra-O-acetyl- α -D-glucopyranoside

Condensation of p-phenyl phenol (3.4 gm, 20 millimol) with penta-O-acetyl- β -D-glucopyranose (7.8 gm, 20 millimol) in benzene (5 ml) in the presence of stannic chloride (2.2 ml, 20 millimol) at the room temperature for 90 hr and subsequent working up in the usual manner gave a syrup which on repeated crystallisation from ethanol gave colourless needles, m.p. 169-170°, $[\alpha]_D^{30}$ + 198.55° (C, 1.38 chloroform). This is a new compound. The yield was 3.52 gm (36%).

Found: C, 62.81; H, 5.86. $C_{26}H_{28}O_{10}$ requires, C, 62.40; H, 5.60 per cent.

p-Phenyl-phenyl-α-D-glucopyranoside

p-Phenyl-phenyl tetra-O-acetyl- α -D-glucopyranoside (3.5 gm) was dissolved in a warm mixture of benzene and methanol (10 ml each) and kept with sodium methoxide (0.2 mol, 10 ml) at the room temperature for 2 hr. Resulting free glucoside separated out and was recrystallised from ethanol when the pure product was obtained in colourless needles, m.p. 226°, $[\alpha]_D^{30}$ + 221.9° (C, 0.32, dioxan). The yield was 2.1 gm (22.5%). This is a new compound.

Found: C, 64.81; H, 6.15. $C_{18}H_{20}O_6$ requires, C, 65.06; H, 6.10 per cent.

o-Chlorophenyl tetra-O-acetyl-α-D-glucopyranoside

This was prepared by a procedure similar to that used for the p-chloro-isomer. The resulting syrup failed to crystallise from the

common organic solvents. The material was then purified by preparative layer chromatography on silica gel (solvent C) to remove trace impurities which interfere with the crystallisation. The material so obtained, crystallised easily from ethanol in colourless needles, m.p. 78°, $[\alpha]_D^{30}$ + 128.4° (C, 1.23 chloroform). It is a new compound. The yield was 0.3 gm (3%).

Found: C, 52.43; H, 4.80. $C_{20}H_{24}O_{10}Cl$ requires, C, 52.20; H, 5.22 per cent.

o-Chlorophenyl-α-D-glucopyranoside

The crude syrupy o-chlorophenyl tetra-O-acetyl-α-D-glucopyranoside (7.5 gm) was used for deacetylation in the usual manner. The free glucoside crystallised from a mixture of methanol-ether in colourless needles, m.p. 150-151°, $[\alpha]_D^{30}$ + 86.90 (C, 0.42 dioxan). This is a new compound. The yield was 0.6 gm (10%).

Found: C, 49.75; H, 5.60. $C_{12}H_{15}O_6Cl$ requires, C, 49.34; H, 5.91 per cent.

o-Methylphenyl tetra-O-acetyl-α-D-glucopyranoside[35]

This was prepared by a procedure followed for the p-isomer described earlier. This product was also obtained as a syrup which could not be crystallised from the common organic solvents and was purified by preparative layer chromatography on silica gel. The purified material crystallised readily from ethanol in colourless needles, m.p. 93°, $[\alpha]_D^{30}$ + 130.0° (C, 0.77 chloroform). Literature[35] does not mention the m.p. and gives $[\alpha]_D$ + 156° (water). The yield was 0.38 gm (4.5%).

Found: C, 56.98; H, 5.85. Calculated for $C_{21}H_{27}O_{10}$, C, 57.40; H, 5.94 per cent.

o-Methylphenyl- α -D-glucopyranoside[13,18]

This was prepared by the sodium methoxide deacetylation of the crude syrupy acetate (7.6 gm). The pure product crystallised from methanol in colourless needles, m.p.122°, $[\alpha]_D^{30}$ + 155.0° (C, 1.0 water). Literature[18] gives m.p. 120-122°, $[\alpha]_D$ + 156° (water). The yield was 0.73 gm (14.6%).

Found: C, 57.93; H, 6.77. Calculated for $C_{13}H_{18}O_6$, C, 57.77; H, 6.66 per cent.

Synthesis of aryl- β -glucosides

Phenyl tetra-O-acetyl- β -D-glucopyranoside[1,13,14]

Phenol (3.76 gm, 40 millimol) and penta-O-acetyl- β -D-glucopyranose (7.8 gm, 20 millimol) were dissolved in benzene (350 ml). Stannic chloride (2.2 ml, 20 millimol) was added to the solution and the mixture was refluxed for ten minutes. The reaction mixture was cooled and washed respectively with water, saturated solution of sodium bicarbonate and water and dried over anhydrous sodium sulphate. Evaporation of the benzene solution under diminished pressure gave a syrup which was dissolved in warm ethanol (20 ml) when the required product crystallised in colourless needles, m.p.124-125°, $[\alpha]_D^{30}$ - 21.8° (C, 0.90 chloroform). Literature [1,14] gives m.p. 120-122°, $[\alpha]_D$ - 22° (chloroform). The yield was 3.2 gm (35.5%).

Found: C, 56.80; H, 5.91. Calculated for $C_{20}H_{25}O_{10}$, C, 56.59; H, 5.65 per cent.

Phenyl-β-D-glucopyranoside[13, 14]

Deacetylation of tetra-O-acetyl-β-D-glucopyranoside (3.2 gm) taken in methanol (30 ml) with sodium methoxide in methanol (0.2 mol, 5 ml) for 1 hr followed by decationisation with Amberlite IR-120 (H^+) resin, gave the free glycoside which crystallised from n-propanol in colourless needles, m.p. 172°, $[\alpha]_D^{30}$ - 74.0° (C, 1.01 water). Literature[14] gives m.p. 172-173°, $[\alpha]_D$ - 73.3° (water). The yield was 0.99 gm (24.5%).

Found: C, 56.27; H, 5.81. Calculated for $C_{12}H_{16}O_6$, C, 56.10; H, 6.10 per cent.

p-Methylphenyl tetra-O-acetyl-β-D-glucopyranoside[19]

Condensation of p-cresol (4.3 gm, 40 millimol) with penta-O-acetyl-β-D-glucopyranose (7.8 gm, 20 millimol) in benzene (350 ml) in the presence of stannic chloride (2.2 ml, 20 millimol) at reflux temperature for 10 minutes, followed by usual working up gave a syrup which crystallised from ethanol (20 ml) in fine long needles, m.p.123°, $[\alpha]_D^{30}$ - 21.4° (C, 2.09 chloroform). Literature[19] does not record any m.p. or optical rotation. The yield was 3.3 gm (39.9%).

Found: C, 57.01; H, 6.11. Calculated for $C_{20}H_{27}O_{10}$, C, 57.4; H, 5.94 per cent.

p-Methylphenyl-β-D-glucopyranoside[13,20]

The crystalline p-methyl tetra-O-acetyl-β-D-glucopyranoside (3.0 gm) was deacetylated by sodium methoxide in methanol (0.2 mol, 5 ml)

at the room temperature for 1 hr, the crystalline material was filtered after keeping the reaction mixture at - 10° for some time. Recrystallisation from methanol gave the pure product in colourless needles, m.p.180°, $[\alpha]_D^{30}$ - 68.0° (C, 1.0 water). Literature[20] gives m.p. 178-179.5°, $[\alpha]_D$ - 67.7° (water). The yield was 1.2 gm (25.17%).

Found: C, 57.62; H, 6.88. Calculated for $C_{13}H_{18}O_6$, C, 57.77; H, 6.66 per cent.

p-Chlorophenyl tetra-O-acetyl- β -D-glucopyranoside[13,21]

Condensation of p-chlorophenol (5.2 gm, 40 millimol) with penta-O-acetyl- β -D-glucopyranose (7.8 gm, 20 millimol) in benzene (360 ml) in the presence of stannic chloride (2.2 ml, 20 millimol) at reflux temperature for 10 minutes, followed by subsequent treatment gave a syrup which was crystallised from methanol in colourless needles, m.p. 124°, $[\alpha]_D^{30}$ - 20.5° (C, 1.0 chloroform). Literature[21] gives m.p. 124° $[\alpha]_D$ - 20° (chloroform). The yield was 2.85 gm (31.5%).

Found: C, 52.15; H, 4.91. Cl, 7.42 Calculated for $C_{20}H_{24}O_{10}Cl$, C, 52.20; H, 5.22 Cl, 7.50 per cent.

p-Chlorophenyl-β -D-glucopyranoside[13,21]

Deacetylation of p-chlorophenyl tetra-O-acetyl- β -D-glucopyranoside (2.79 gm) with sodium methoxide (0.2 mol, 5 ml) in methanol for 1 hr at the reflux temperature followed by dilution of the reaction mixture with methanol (3 ml) and light petroleum (6 ml) and cooling to - 10° gave the pure glucoside in colourless needles, m.p. 175-176° $[\alpha]_D^{30}$ - 66.01° (C, 1.0 methanol). Literature[21] gives m.p.174-175° $[\alpha]_D$ - 65.5°.

Found: C, 49.34; H, 5.19; Cl, 11.38. Calculated for $C_{12}H_{15}O_6Cl$, C, 49.63; H, 5.19; Cl, 11.55 per cent.

p-Nitrophenyl tetra-O-acetyl-β-D-glucopyranoside[10,22]

Condensation of p-nitrophenol (5.6 gm, 40 millimol) with penta-O-acetyl-β-D-glucopyranose (7.8 gm, 20 millimol) in benzene (400 ml) in the presence of stannic chloride (2.2 ml, 20 millimol) at reflux temperature for 10 minutes, followed by subsequent treatment as usual, resulted in a syrup which crystallised from methanol in colourless needles, m.p. 174° $[\alpha]_D^{30}$ - 21.0° (C, 1.0 chloroform). Literature[10,22] gives m.p. 174-175° $[\alpha]_D$ - 20.17° (chloroform). The yield was 2.5 gm (26.7%).

Found: C, 51.21; H, 5.17; N, 3.03. Calculated for $C_{20}H_{24}O_{12}N$, C, 51.07; H, 5.17; N, 3.0 per cent.

p-Nitrophenyl-β-D-glucopyranoside[10,23]

p-Nitrophenyl tetra-O-acetyl-β-D-glucopyranoside (2.5 gm) was deacetylated at the room temperature for 1 hr with sodium methoxide (0.2 mol, 10 ml) in methanol. The separated free glucoside was filtered after keeping at -10° for 12 hr. The crude product crystallised from methanol. The yellowish product on washing with a little alcohol containing glacial acetic acid gave colourless needles, m.p. 166°, $[\alpha]_D^{30}$ - 100.0° (C, 0.42 water). Literature[10,23] gives m.p. 164-165°, $[\alpha]_D$ - 99.0° (water). The yield was 1.01 gm (17.2%).

Found: C, 47.62; H, 5.27; N, 4.42. Calculated for $C_{12}H_{15}O_8N$, C, 47.8; H, 4.98; N, 4.35 per cent.

p-Phenyl phenyl tetra-O-acetyl-β-D-glucopyranoside

p-Phenyl phenol (6.8 gm, 40 millimol) and penta-O-acetyl-β-D-glucopyranose (7.8 gm, 20 millimol) were dissolved in benzene (350 ml). Stannic chloride (2.2 ml, 20 millimol) was added to the solution and the mixture was refluxed for 10 minutes. After cooling, the reaction mixture was washed with water, saturated sodium bicarbonate solution and water successively and dried over anhydrous sodium sulphate. Removal of the solvent under diminished pressure yielded a syrup which crystallised on trituration with warm ethanol. It gave colourless needles, m.p. 155-156°, $[\alpha]_D^{30}$ - 12.3° (C, 1.95 chloroform). It is a new compound. The yield was 2.9 gm (29%).

Found: C, 62.09; H, 5.89. Required for $C_{26}H_{28}O_{10}$, C, 62.40; H, 5.60 per cent.

p-Phenyl phenyl-β-D-glucopyranoside

The crystalline p-phenyl phenyl tetra-O-acetyl-β-D-glucopyranoside (2.9 gm) was dissolved in hot benzene (8 ml) and sodium methoxide (0.2 mol, 6 ml) solution in methanol was added. The mixture was heated under reflux for 1 hr. On slow cooling a white solid separated out, which crystallised from ethanol in colourless needles, m.p. 207-208°, $[\alpha]_D^{30}$ - 5.45° (C, 0.55 dioxan). It is a new compound. The yield was 1.2 gm (17.5%).

Found: C, 64.95; H, 6.28. Calculated for $C_{18}H_{20}O_6$, C, 65.06; H, 6.10 per cent.

o-Chlorophenyl tetra-O-acetyl- β -D-glucopyranoside[13,21]

It was prepared in the same manner as the para isomer from o-chlorophenol (5.2 gm, 40 millimol) and penta-O-acetyl- β -D-glucopyranose (7.8 gm, 20 millimol). The crude product obtained as a syrup, crystallised from ethanol in colourless needles, m.p. 141°, $[\alpha]_D^{30}$ - 45.9° (C, 1.29 chloroform). Literature[21] gives m.p. 141-143°, $[\alpha]_D$ - 46.5° (chloroform). The yield was 2.3 gm (25.0%).

Found: C, 52.25; H, 4.85. Calculated for $C_{20}H_{24}O_{10}Cl$, C, 52.20; H, 5.22 per cent.

o-Chlorophenyl- β -D-glucopyranoside[13,21]

Deacetylation of o-chlorophenyl tetra-O-acetyl- β -D-glucopyranoside (2.3 gm) with sodium methoxide (0.2 mol, 7 ml) in methanol at the room temperature for 5 hr and evaporation of the decationised reaction mixture gave a syrup which crystallised from n-propanol in colourless needles, m.p.164-165°, $[\alpha]_D^{30}$ - 66.02° (C, 0.99 methanol). Literature[21] gives m.p. 163-164°, $[\alpha]_D$ - 65.8° (methanol). The yield was 0.84 gm (16.8%).

Found: C, 49.34; H, 5.19. Calculated for $C_{12}H_{15}O_6Cl$, C, 49.63; H, 5.19 per cent.

o-Methylphenyl tetra-O-acetyl- β -D-glucopyranoside[13,24]

This was prepared in the same manner as described for the p-isomer from o-cresol (4.3 gm, 40 millimol) and penta-O-acetyl β -D-glucopyranose (7.8 gm, 20 millimol). The crude product obtained as a syrup crystallised from methanol in colourless needles,

141°, $[\alpha]_D^{30}$ - 25.1° (C, 1.0 chloroform). Literature[24] gives m.p.141°, $[\alpha]_D$ - 25.4° (water). The yield was 2.5 gm (24.4%).

Found: C, 57.01; H, 6.23. Calculated for $C_{20}H_{27}O_{10}$, C, 57.4; H, 5.94 per cent.

o-Methylphenyl-β-D-glucopyranoside[13,20]

Deacetylation of o-methylphenyl tetra-O-acetyl-β-D-glucopyranoside (2.5 gm) with sodium methoxide solution (0.2 mol, 6 ml) in methanol at the room temperature for 4.5 hr, followed by deeationisation and removal of solvent gave a syrup which crystallised from a mixture of acetone and petroleum ether (40-60°) in colourless needles, m.p. 166°, $[\alpha]_D^{30}$ - 68.04° (C, 1.0 water). Literature[20] gives m.p. 163-164.5°, $[\alpha]_D$ - 68.7° (water). The yield was 1.0 gm (20%)

Found: C, 57.93; H, 6.77. Calculated for $C_{13}H_{18}O_6$, C, 57.77; H, 6.66 per cent.

Attempted glucosidation of o-nitrophenol

o-Nitrophenol (5.6 gm, 40 millimol) and penta-O-acetyl-β-D-glucopyranose (7.8 gm, 20 millimol) were dissolved in benzene (350 ml). Stannic chloride (2.2 ml, 20 millimol) was added to the solution and the mixture was refluxed for ten minutes. The reaction mixture was worked up as usual. The dry syrup crystallised from ethanol in yellow needles, m.p. 43° (mixed m.p. with o-nitrophenol, m.p.43°, undepressed). The mother liquor was extracted with pet. ether (40-60°) to remove o-nitrophenol and then evaporated to dryness. The crystallisation of the residual material from benzene-pet. ether mixture yielded a

yellowish solid which was recrystallised to a colourless product, m.p. 111°, $[\alpha]_D^{30}$ + 119° (C, 1.02 chloroform).

This product (m.p. 110°, $[\alpha]_D^{30}$ + 119) was found to be identical with penta-O-acetyl- α -D-glucopyranose by direct comparison. On deacetylation with sodium methoxide D-glucose m.p. and m.m.p. 146°, $[\alpha]_D^{30}$ + 52.7° was obtained.

Anomerisations

Anomerisation of phenyl tetra-O-acetyl- β -D-glucopyranoside[9,10]

By a procedure similar to that used in the preparation of phenyl tetra-O-acetyl- β -D-glucopyranoside, the β -anomer was transformed into the α -anomer. A mixture of phenyl tetra-O-acetyl- β -D-glucopyranoside (3 gm), phenol (0.3 gm) and stannic chloride (1 ml) was heated at 50° ± 1° for 20 hr. Examination of reaction aliquots by TLC indicated complete disappearance of the β -anomer at the end of this period. The reaction mixture was processed as usual and the purified reaction product was crystallised from ethanol in colourless needles, m.p. 113-114°, $[\alpha]_D^{30}$ + 162.5° (C, 1.0 chloroform). Its IR spectrum was superimposable with that of authentic phenyl tetra-O-acetyl- α -D-glucopyranoside. Literature[7,14] gives m.p. 114°, $[\alpha]_D$ + 168° (chloroform). The yield was 0.77 gm (26.0%).

Anomerisation of p-chlorophenyl tetra-O-acetyl- β -D-glucopyranoside

A mixture of p-chlorophenyl tetra-O-acetyl- β -D-glucopyranoside (2.5 gm) p-chlorophenol (0.25 gm) stannic chloride (1 ml) and benzene (3 ml) was heated at 50° ± 1° for 20 hr. The complete disappearance of β -anomer at the end of this period was confirmed by TLC.

The reaction mixture was worked up in the usual manner. The product crystallised from ethanol in colourless needles, m.p. 101-102°, $[\alpha]_D^{30}$ + 166.1° (C, 1.44 chloroform). IR spectrum was superimpossable with that of authentic p-chlorophenyl tetra-O-acetyl-α-D-glucopyranoside. The product was found to be identical with p-chlorophenyl tetra-O-acetyl -α-D-glucopyranoside (described earlier). The yield was 0.54 gm (21.6%).

Anomerisation of p-phenyl phenyl tetra-O-acetyl- β -D-glucopyranoside

A mixture of p-phenyl phenyl tetra-O-acetyl-β-D-glucopyranoside (2.5 gm), p-phenyl phenol (0.5 gm), stannic chloride (1 ml) and benzene (3 ml) was heated at 50° for 20 hr. The reaction product was worked up as usual and crystallised from ethanol in colourless needles, m.p.169-170°, $[\alpha]_D^{30}$ + 198.01° (C, 1.4 chloroform). IR spectrum was superimpossable with that of authentic p-phenyl phenyl tetra-O-acetyl- α -D-glucopyranoside. The product was found to be identical with p-phenyl phenyl tetra-O-acetyl- -α-D-glucopyranoside described earlier. The yield was 0.8 gm (31.24%).

Anomerisation of o-chlorophenyl tetra-O-acetyl- β -D-glucopyranoside

A mixture of o-chlorophenyl tetra-O-acetyl-β-D-glucopyranoside (3.5 gm), O-chlorophenol (0.5 gm), stannic chloride (1 ml) and benzene (3 ml) was heated at 50° for 20 hr and the reaction product was processed as usual. The resulting syrup could not be crystallised and the α-D-glucoside acetate was obtained by preparative layer chromatography of the syrup. The isolated product was recrystallised from ethanol in colourless needles, m.p. 78°, $[\alpha]_D^{30}$ + 128.5° (C, 1.22 chloroform). IR spectrum was superimpossable with that of o-chlorophenyl tetra-O-acetyl- α -D-glucopyranoside. The product was found to be identical

with o-chlorophenyl tetra-O-acetyl- ∝ -D-glucopyranoside described earlier. The yield was 1.0 gm (11.5%).

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CHAPTER : IV

STUDIES IN THE MECHANISM OF STANNIC CHLORIDE CATALYSED GLYCOSIDATION

SUMMARY

Different carbonium ions have been postulated to be formed by the action of Lewis acids on acetylated sugars in glycosidation and anomerisation reactions. The conditions under which these different ions are likely to be predominent in stannic chloride catalysed glucosidation of phenols are discussed.

A new stannic chloride-phenol complex has been isolated under the reaction conditions of stannic chloride catalysed glucosidation. This complex, in the presence of stannic chloride has been found to be responsible for the anomerisation of acetylated aryl glucosides, which cannot be brought about by stannic chloride alone. The presence of the complex or free phenol (presumably for the formation of the complex) together with stannic chloride is necessary for bringing about anomerisation.

The anomerisation reaction of aryl glucosides, is irreversible and has been established to be an intermolecular reaction. This is unlike the anomerisation of alkyl glycosides which is reversible and intramolecular.

PROBABLE MECHANISM OF STANNIC CHLORIDE CATALYSED GLYCOSIDATION

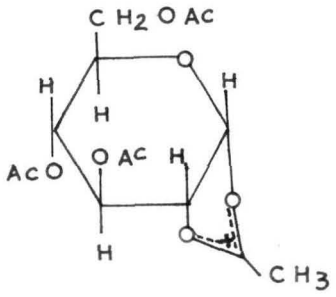
Glucosidations described in this dissertation are mainly restricted to the reactions at the room temperature (20^{0} - 40^{0}), between equimolar quantities of penta-O-acetyl- β -D-glucopyranose and a phenol in the presence of equimolar quantity of anhydrous stannic chloride as a catalyst and a small quantity of benzene as a diluent. Aryl tetra-O-acetyl- α -D-glucopyranosides are the main products of the reaction under these conditions together with small quantities of the corresponding- β -anomeric glucosides and some unidentified by-products.

A probable mechanism of this reaction in the light of our observations and recent developments in the subject is discussed in three successive parts of this chapter. The first part deals with the action of stannic chloride on penta-O-acetyl- β -D-glucopyranose and the possible role of the different carbonium ion intermediates postulated so far on the configuration of the glucoside formed. The second part describes the anomerisation of aryl tetra-O-acetyl- β -D-glucopyranosides into the corresponding α -glucosides, and the significance of this anomerisation reaction on the course of the glucosidation reaction. The third part describes the results of our attempts to find out the nature of the by-products from their chemical reactions, analytical data and spectral characteristics.

Action of stannic chloride on penta-O-acetyl- β -D-glucopyranose

According to Lemieux and Brice[1], addition of stannic chloride

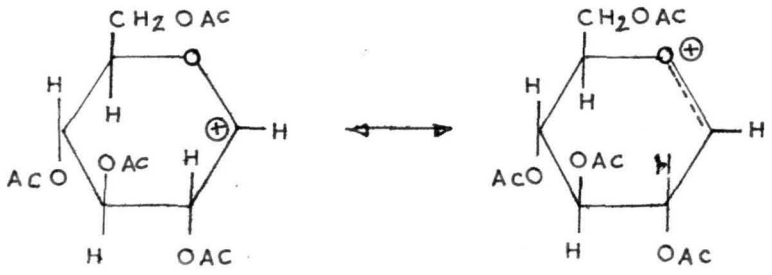
to penta-O-acetyl- β -D-glucopyranose results in the dissociation of the bond between C(1) and the acetoxy group attached to it. This dissociation is favoured by the neighbouring C(2)-acetoxy group by what is termed as anchimeric assistance[2], which favours the formation of the carbonium ion (I). Under the prevailing acidic environment, the carbonium ion (I) reacts with an alkoxy or an aryloxy group, with inversion of configuration leading to the formation of acetylated β -D-glucopyranoside as the main product[3].



I

Bose and Ingle[4] recently observed that considerable amounts of anomeric acetylated aryl- ∝ -D-glycosides are also formed under the conditions of glycosidation described by Lemieux and Shyluk[3]. Moreover, they found that glycosidations with penta-O-acetyl- β -D-galactopyranose lead to the formation of the acetylated ∝-anomeric aryl galactosides as the major product. Based on these results Bose and Ingle contended that the anchimeric assistance of the C(2)-acetoxy group cannot be considered to be a significant factor in this reaction and proposed that the carbonium ion (II)

formed by elimination of the acetoxy anion at C(1) and stabilised by resonance with the oxonium ion (III) to be a major contributing factor in the formation of the anomeric glycosides in stannic chloride catalysed glycosidations.



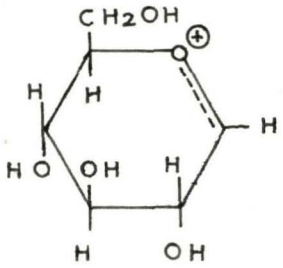
II III

Lindberg et. al.[5] have also criticised Lemieux's postulation of anchimeric assistance in the course of their studies in the anomerisation of acetylated alkyl glycosides in strong acids.

Lemieux and Brice[1], however, agree that the anomerisation of penta-O-acetyl- β -D-glucopyranose by the action of stannic chloride cannot be explained by postulating the carbonium ion (I) as the intermediate and proposed the formation of the carbonium ion (III) which can lead to the formation of the D-glucose pentaacetates with either α or β configuration.

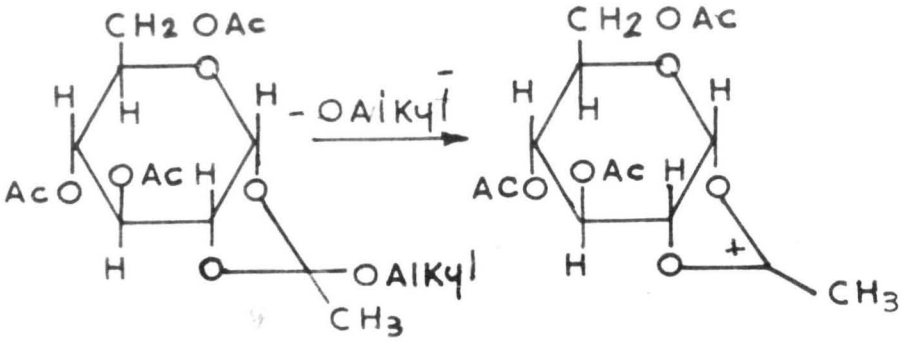
Capon[6] has recently proved the existance of the carbonium ion (IV), which is nothing but deacetylated carbonium ion (III), and postulated it to be the intermediate in the anomerisation of methyl glucosides in the presence of methyl sulphonyl chloride and

methanol. This observation provides a strong support to the possibility of formation of the carbonium ion (III) also in the stannic chloride catalysed glycosidation reactions.



IV

Kochetkov et. al.[7] have recently developed a new method of glycosidation using 1,2-O-alkyl ortho esters as starting materials and a mixture of mercuric bromide and p-toluenesulphonic acid as catalyst, the reactions being carried out in polar solvents such as nitrobenzene. These authors envisage the formation of the carbonium ion (VI) from the ortho ester (V) which then undergoes a nucleophilic attack either on the electrophilic lactol carbon atom giving rise to a new ortho ester (VII) or on the glycosidic centre C(1) resulting in the formation of glycoside (VIII). They presumed that $HgBr_2$ formed a complex anion such as $HgBr_3^-$ in the polar solvent, which then formed a close ion pair (IX) with the cation (VI), thus, shielding the electrophilic lactol carbon atom (IX) and directing the reaction to glycosidation at C(1) leading to the formation of β-anomeric glycosides. The formation of the close ion-pair is, however, inhibited when the anion is present in low concentrations



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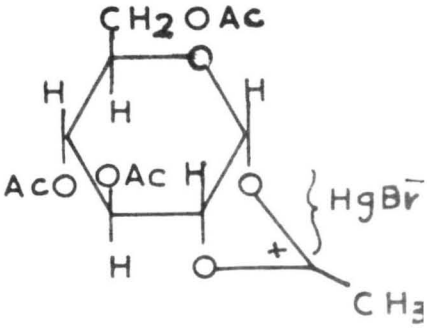

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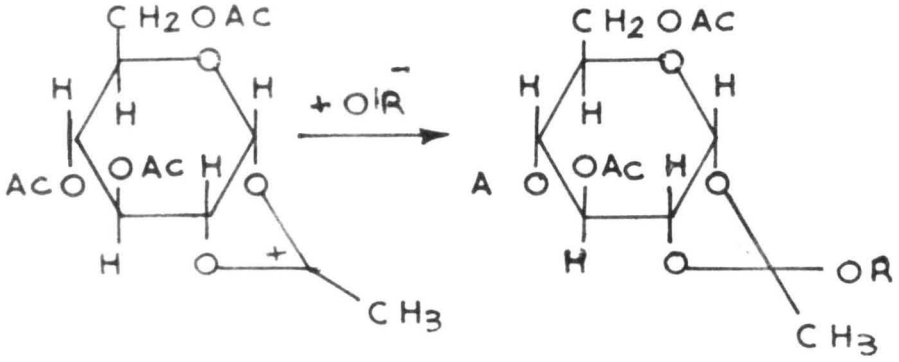
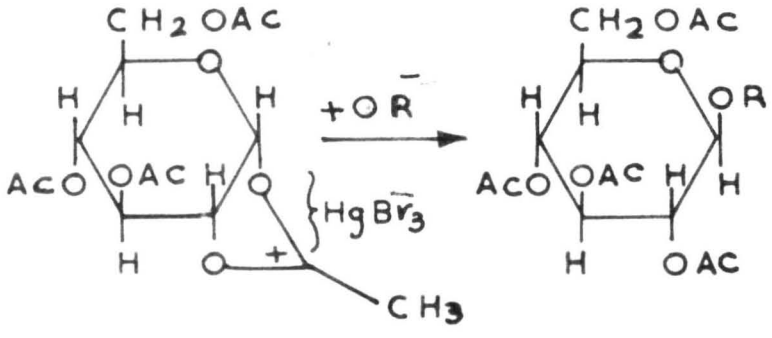


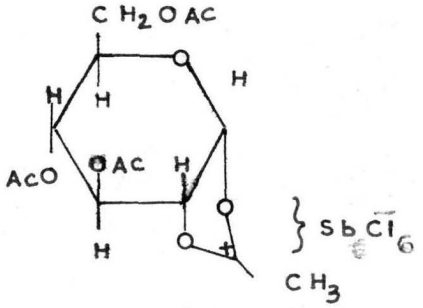
VI VII



IX VIII

or when catalysts such as p-toluene sulphonic acid or mercuric acetate are used or when the reactions are carried out in a media of low polarity. This leads to a nucelophilic attack on the electrophilic lactol carbon (VI) resulting in the formation of the ortho ester (VII).

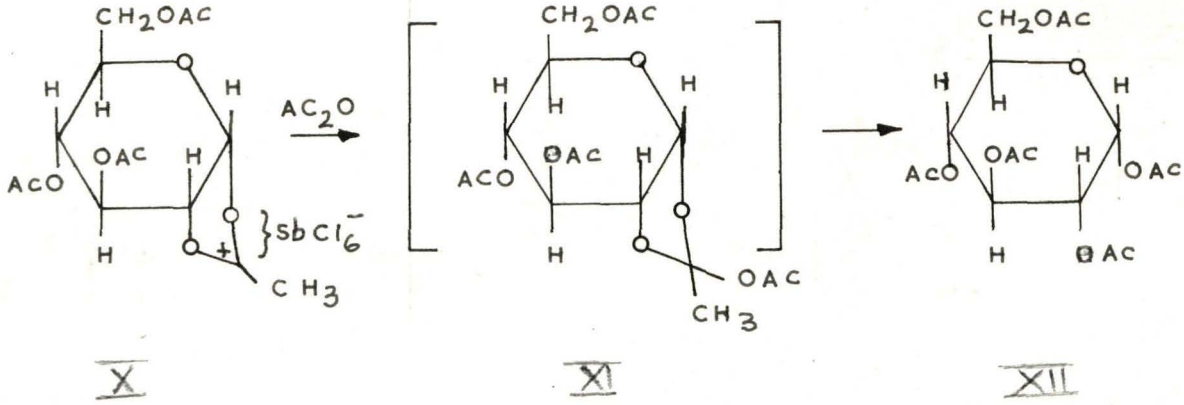
Heyns et. al.[8,9] have recently isolated the ~~stable~~ cation (VI) as its stable crystalline salt or ion pair (X) by reacting either of the anomeric acetyl glucosyl chloride with antimony pentachloride. The cation of type (VI) was also found to be formed by the action of antimony pentachloride on penta-O-acetyl- β -D-glucopyranose, penta-O-acetyl- β -D-galactopyranose and tetra-O-acetyl- β -D-xylopyranose respectively, but the structure of the anions of the ion-pairs in these cases has not been definitely established.



X

The cation (VI) named as the 3,4,6-tri-O-acetyl- α -D-glucopyranose 1,2-acetoxonium cation[9] by Heyns, is regarded as the intermediate in a series of reactions at C(1) viz. formation of acetylated - β -glycosides from acetylated - β -glycosyl halides, the rearrangement of orthoesters into β -glycosides and the anomerisation of peracetylated aldoses, acetyl glycosyl halides and acetylated alkyl glycosides.

Under the prevailing acid conditions of acetylation with acetic anhydride and acetic acid of 3,4,6, tri-O-acetyl- ∝ -D-glucopyranose 1-2-acetoxonium antimony hexachloride (X), produced penta-O-acetyl- ∝ -D-glucopyranose (XII). The reaction proceeds with the cis-opening of a acetoxonium ring probably through the intermediate (XI). This was supported from the results of reactions in which deuterated acetic anhydride was used.



These authors further pointed out that the cation (VI) can stabilise itself by delocalisation of the positive charge at the electrophilic lactol carbon atom in the oxonium ring. The structure of this self-stabilised cation (VI) will be identical with that of the carbonium ion (I) proposed by Lemieux and such a carbonium ion has been already shown to result in the formation of β -glucosides in stannic chloride catalysed glucosidation by trans-opening of the oxonium ring.

From these observations, it is evident that both of the carbonium ions (I) and (VI), which are easily interconvertible, play

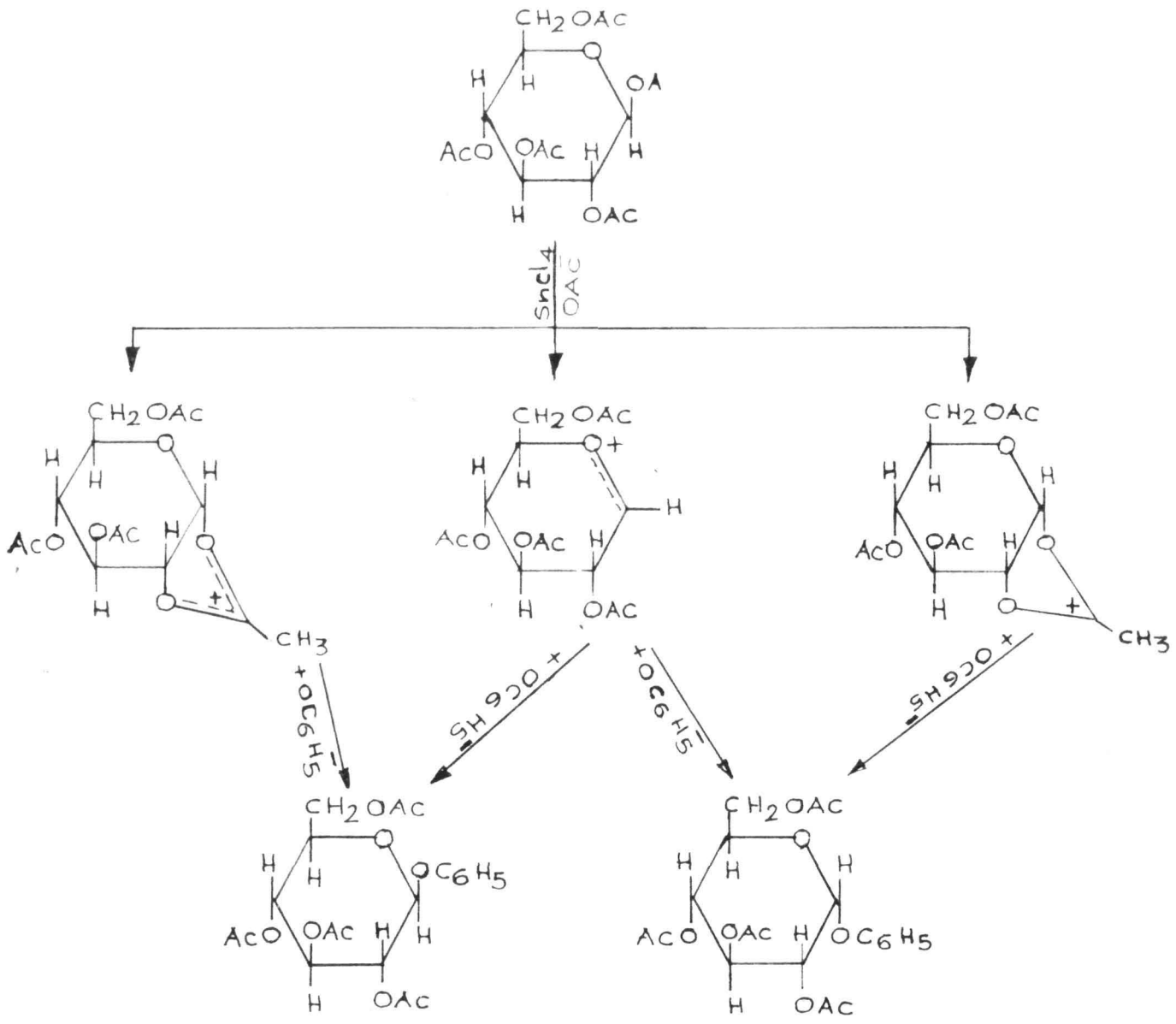


DIAGRAM NO. 1.

important roles in the stannic chloride catalysed glucosidation reactions. Under high dilution conditions, the stable carbonium ion (I) would be predominant leading to the formation of β-anomeric glucosides[3] by trans- opening of the oxonium ring. On the other hand, under higher concentration conditions, an ion-pair of the type (X) formed from the carbonium ion (VI) having a localised charge and stabilized by some sort of protecting anion derived from stannic chloride would be predominant and would lead to the formation of the α-anomeric glucosides by cis- opening of the oxonium ring[8].

In addition to these, the carbonium ion (II) stabilized by resonance with the oxonium ion (III) is also likely to play an important part in this glucosidation reaction. The results of the stannic chloride catalysed glucosidation of phenols with penta-O-acetyl- β -D-glucopyranose can thus be explained by the sequence of reactions as shown in the adjoining diagram(1).

Anomerisation of aryl tetra-O-acetyl-β-D-glucopyranosides

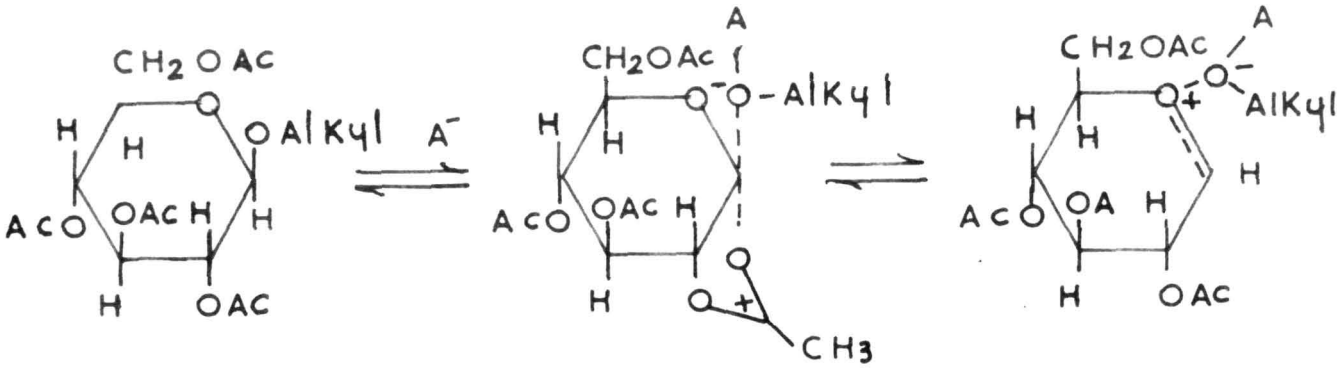
A careful study of the reaction between penta-O-acetyl- β -D-glucopyranose and phenol in the presence of stannic chloride and small quantities of benzene at 50°, at various stages by thin-layer chromatography on silica gel, revealed that both of the anomeric phenyl tetra-O-acetyl-D-glucopyranosides were formed in perceptible amounts within the first 15 to 30 min. At the stage when penta-O-acetyl- β -D-glucopyranose was completely used up (3 to 4 hr), the ratio between the α-and the β-anomeric glucosides was found to be approximately 3:7. Further progress of the reaction resulted in

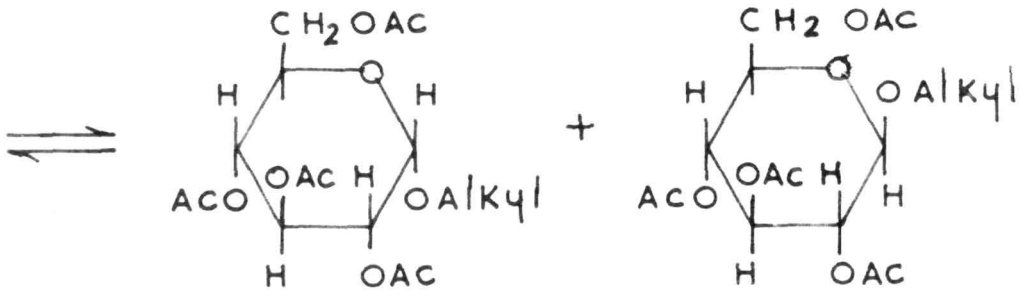
changes in the proportion of the two glucosides in favour of the α-anomer. In 8 to 10 hr the spot due to the β-glucoside almost disappeared and the spot due to the α-anomer become prominent.

These observations indicate that after the initial glucosidation reaction is over, the β-glucoside slowly anomerises irreversibly into the α-anomer. To confirm this, a mixture of phenyl tetra-O-acetyl β-D-glucopyranoside, phenol and stannic chloride and a small quantity of benzene was heated at 50^0 and the progress of reaction was followed by TLC. The formation of the α-anomeric glucoside was first observed after 3 to4hr. In about 20 hr the spot due to the starting material i.e. the β-glucoside disappeared with a concommitant increase in the intensity of the spot due to the α-anomer (Plate I).

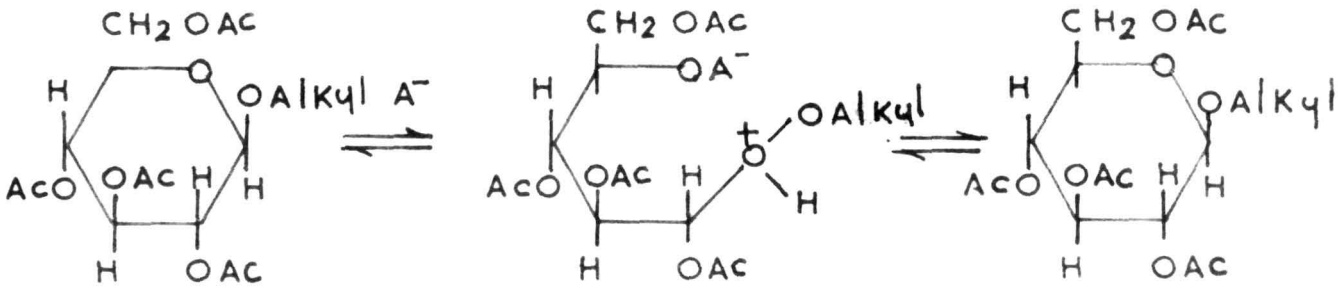
Thus, in the first step in the stannic chloride catalysed glucosidation of phenol, both of the anomeric glucosides are simultaneously formed in the ratio 7:3 in favour of the β-anomer. This comparatively faster initial reaction is followed by a slow process of anomerisation of the β-anomer irreversibly to the more stable α-anomer. Experimental verification of the fact that the rate of anomerisation is very slow also confirms that the formation of the α-anomer in the first stage of the reaction is direct and not through the process of anomerisation.

In this reaction all of the carbonium ions (I), (II) and (VI) may be present and the relative proportion of the α-anomer formed in the initial stages of the reaction depends on the relative abundance of the three ionic species.





MECHANISM PROPOSED BY LEMIEUX



MECHANISM PROPOSED BY LINDBERG

INTRAMOLECULAR ANOMERISATION OF ACETYLATED ALKYL GLYCOSIDES

DIAGRAM 2.

The results of the experiments carried out to study the anomerisation of aryl tetra-O-acetyl- β -D-glucopyranosides into the corresponding α -anomers have already been reported in Chapter III (Part I) of this dissertation. The anomerisation in the case of acetylated alkyl glycosides appears to proceed intramolecularly by either or both of the pathways proposed by Lemieux[10] and Lindberg[5] as shown in the adjoining diagram (2).

Neither of these two intramolecular reaction mechanisms proposed for the anomerisation of acetylated alkyl-β -D-glycosides appear to be operative in the anomerisations of acetylated aryl-β -D-glucopyranosides. Differences which have been observed between the anomerisations in the alkyl and the aryl series are detailed below:

1. Attempts to anomerise phenyl tetra-O-acetyl-α -D-glucopyranoside under the usual conditions described for the anomerisation of the corresponding β -anomer resulted in the formation of some degradation products. Most of the α -glucoside remained unchanged and no positive evidence of the formation of the β -glucoside was obtained (Plate I & II).

2. Attempts to anomerise phenyl tetra-O-acetyl-β -D-glucopyranoside with stannic chloride in the presence of small quantities of benzene in the absence of phenol, for 40 hr resulted also in the formation of some degradation products. Most of the β -glucoside remained unchanged and no evidence of the formation of the α -anomer was obtained.

Similar results as in (2) were also observed when stannic chloride was excluded from the reaction mixture.

PLATE — I

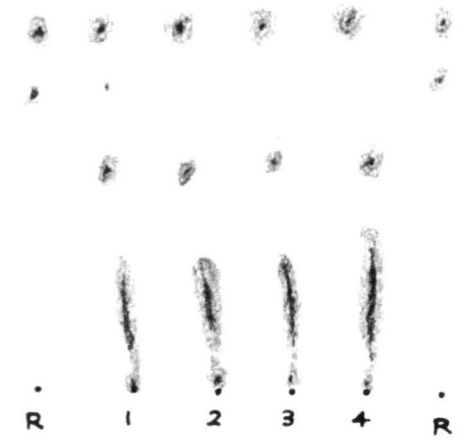
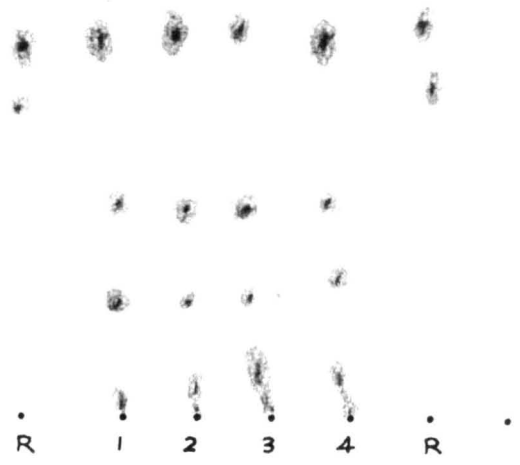


PLATE — II



Progress of anomerisation of phenyl tetra-O-acetyl-β D-glucopyranoside (Plate-I) and phenyl tetra-O-acetyl α -D-glucopyranoside (Plate-II) under similar reaction conditions

Solvent system: A

Plate I and II together

R. Mixture of anomeric α and β phenyl tetra-O-acetyl D-glucopyranosides.

1-4. Reaction mixture taken after 5, 10, 20 and 40 hr

These and earlier observations on anomerisation[14,15] lead to the following conclusions:

(a) Unlike most of the alkyl glycosides the anomerisation of phenyl tetra-O-acetyl- β -D-glucopyranoside into the corresponding α -anomer is irreversible.

(b) The presence of stannic chloride as well as of phenol is essential for the anomerisation reaction to take place, this is unlike the anomerisation of alkyl glycosides where the presence of only the catalyst but not of the alcohol is necessary for the reaction[10-13].

These observations and the earlier work reported on anomerisation of phenyl tetra-O-acetyl - β -D-glucopyranoside by mixtures of zinc chloride and phenol[14] as well as antimony pentachloride and phenol[15] suggest that the anomerisation reactions studied by us are brought about jointly by stannic chloride and phenol in the form of a complex. The possibility of formation of such a complex was, therefore, thoroughly investigated.

Formation of stannic chloride - phenol complex

The possibility of the formation of a stannic chloride - phenol complex under the conditions prevailing in the stannic chloride catalysed glucosidation reactions was investigated. On keeping a mixture of equimolar quantities of stannic chloride and phenol in a small quantity of benzene at 40° for 48 hr, a buff coloured hygroscopic amorphous solid was obtained. This amorphous compound was stable at room temperature

under anhydrous conditions. It analysed for stannic dichloro diphenoxide, $SnCl_2(OC_6H_5)_2$.

Although some stannic chloride - alcohol complexes have been recently described my Malhotra et. al.[16,17] similar complexes with phenols have not been described so far. Analogous titanium tetrachloride - phenol complexes, such as, $TiCl_3(OC_6H_5)$, $TiCl_2(OC_6H_5)_2$, $TiCl(OC_6H_5)_3$ and $Ti(OC_6H_5)_4$ are however known for many years[18-21]. Reeves[12] earlier reported the formation of bis-o-nitro phenyl-dichloro-titanate i.e. $TiCl_2[OC_6H_4NO_2(o)]_2$ in titanium-tetra chloride catalysed glycosidation reaction. This strongly supports our present observation on the formation of the similar stannic dichloro diphenoxide. The possible role of this complex in glucosidation reactions was then investigated.

Anomerisation and glucosidation in the presence of stannic dichloro diphenoxide

No anomerisation was observed (TLC) when a mixture (a) containing phenyl tetra-O-acetyl- β -D-glucopyranoside (one mol) and stannic dichloro diphenoxide (half mol) was kept with small quantities of benzene at 40°. Most of the starting material remained unchanged and only some degradation products were found to be formed. Similarly no glucosidation was observed to take place when a mixture (b) containing penta-O-acetyl- β -D-glucopyranose (one mol) the complex (one mol) and small quantities of benzene was heated at 40°. In the presence of phenol (one mol), the reaction mixture (a) showed no change but the reaction mixture (b) revealed the formation of phenyl tetra-O-acetyl- β -D-glucopyranoside. On the other hand,

PLATE — III

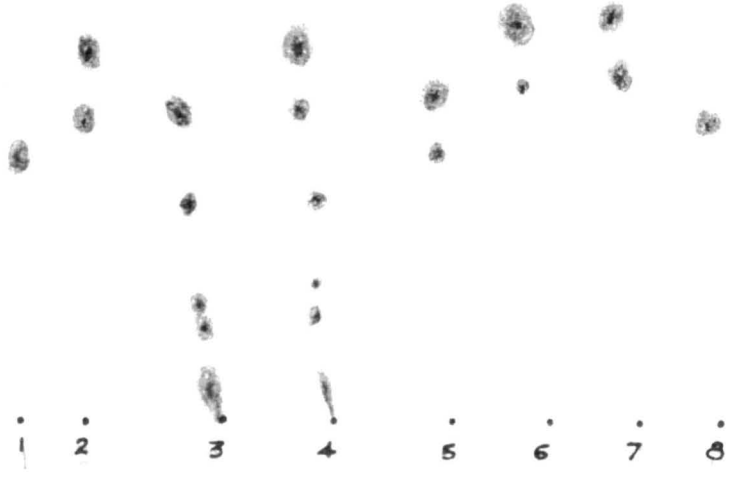
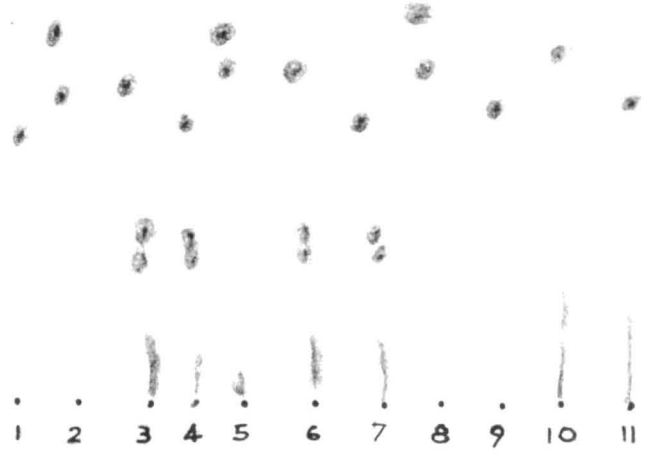


PLATE-IV



Solvent system: A

Plate-III

1, 8. Penta-O-acetyl β-D-glucopyranose
2, 7. Mixture of α and β anomeric phenyl tetra-O-acetyl-D-glucopyranosides
3. Product of reaction between reaction mixture (a) and phenol for 48 hr
4. Product of reaction between reaction mixture (a) and stannic chloride for 48 hr
5. Product of reaction between reaction mixture (b) and phenol for 48 hr
6. Product of reaction between reaction mixture (b) and stannic chloride for 40 hr

Plate-IV

1, 2. Same as in Plate-III
3. Product of anomerisation in the presence of $Sn(OC_6H_5)_2Cl_2$ only
4. Product of glucosidation in the presence of $Sn(OC_6H_5)_2Cl_2$ only
5. Product of anomerisation in the presence of $SnCl_4$ only
6. Product of glucosidation in the presence of $SnCl_4$ only
7. Product of anomerisation in the presence of phenol only
8. Product of glucosidation in the presence of phenol only

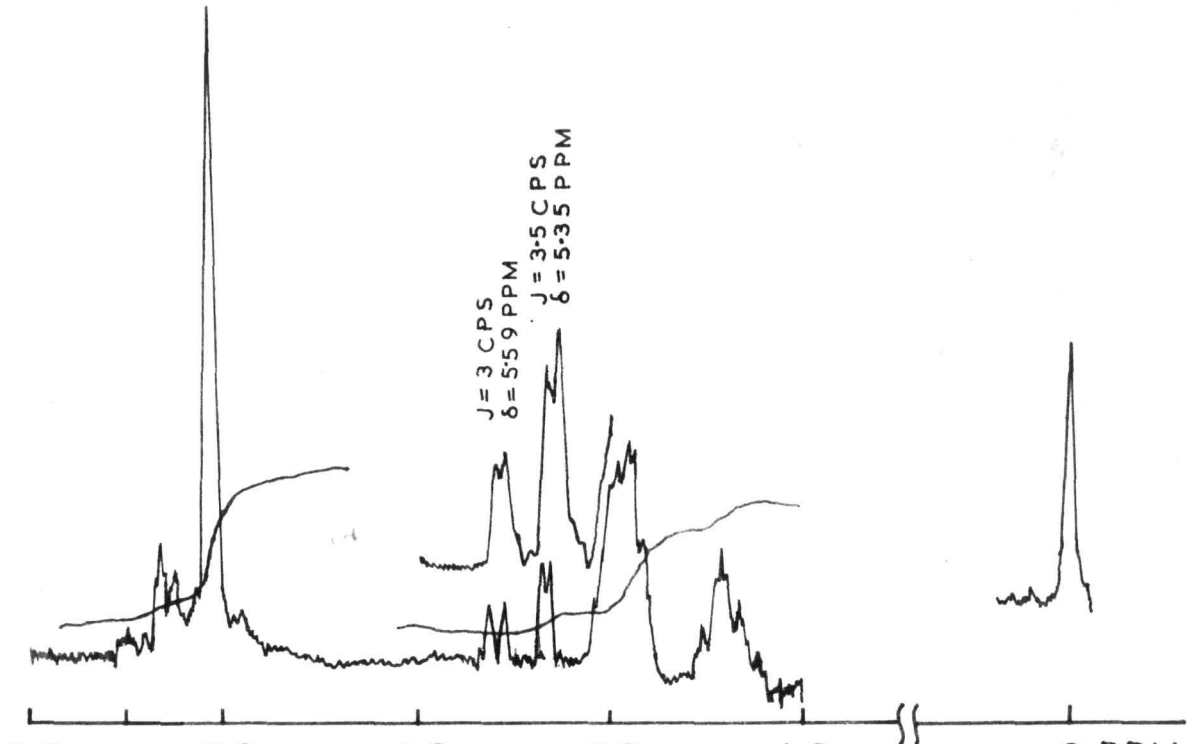
in the presence of stannic chloride (one mol) the reaction mixture (a) showed the formation of phenyl tetra-O-acetyl- α -D-glucopyranoside and the reaction mixture (b) showed the formation of both of the anomeric phenyl tetra-O-acetyl-D-glucosides (Plate III & IV).

These experiments evidently proved that unlike stannic chloride, stannic dichloro diphenoxide is incapable of dissociating the C(1) acetoxy or C(1) phenoxy bond of penta-O-acetyl- β -D-glucopyranose and phenyl tetra-O-acetyl-β -D-glucopyranoside respectively. The carbonium ion produced by such dissociations seems to be the first step towards either anomerisation or glucosidation.

Stannic chloride, which alone can anomerise acetylated alkyl glucosides[5,10], was found to be unable to anomerise phenyl tetra-O-acetyl- β -D-glucopyranoside though it can dissociate C(1)-OR bond (where R = aglucone) in both of these cases. This indicates that the mechanism of anomerisation of acetylated aryl glycosides is different from that of acetylated alkyl glycosides. The anomerisation of acetylated alkyl glycosides has been proved to be intramolecular reaction[5,10]. The results of the reactions especially that of the mixture (a) in presence of stannic chloride indicate that the anomerisation as well as glucosidation reactions in the aryl series are both intermolecular reactions. This will now be confirmed by further observations.

Anomerisation of phenyl tetra-O-acetyl β -D-glucopyranoside by stannic chloride in presence of ortho-chlorophenol.

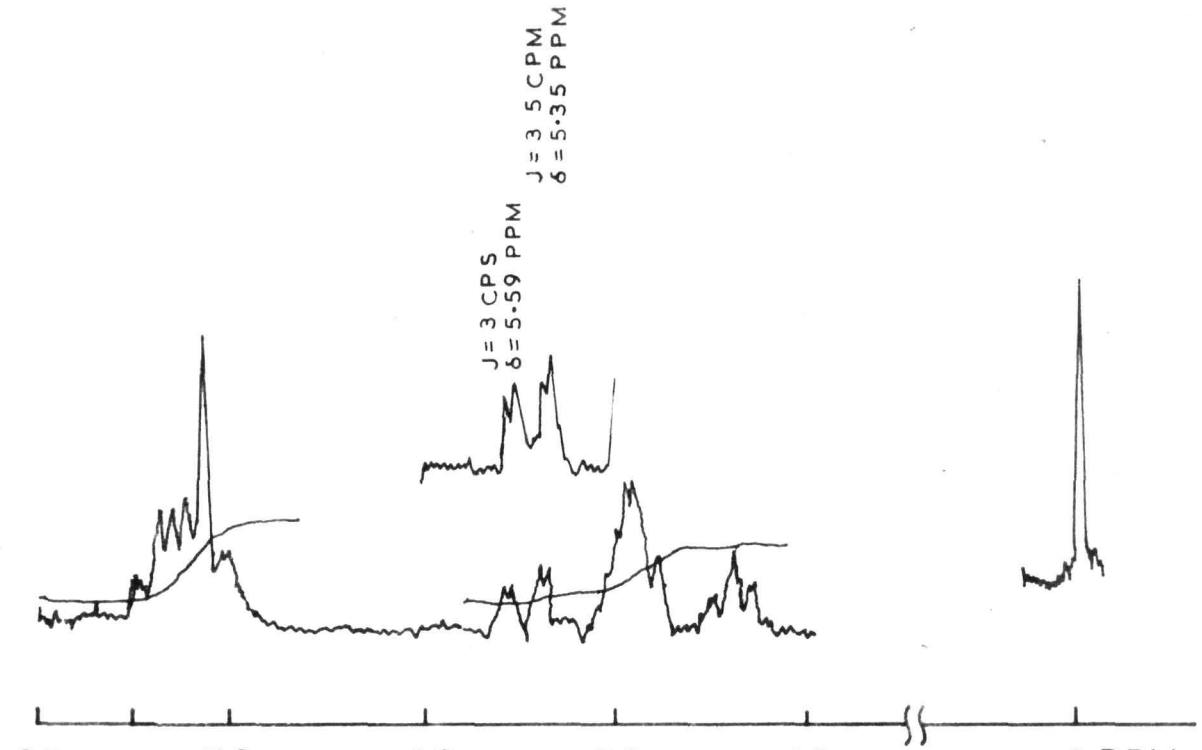
A mixture of equimolar quantities of phenyl tetra-O-acetyl-β -D-glucopyranoside, stannic chloride and ortho-chlorophenol in



PHENYL – ∝ – D – GLUCOPYRANOSIDE

AND

ORTHO – CHLORO PHENYL ∝ – D – GLUCOPYRANOSIDE [AUTHENTIC MIXTURE]

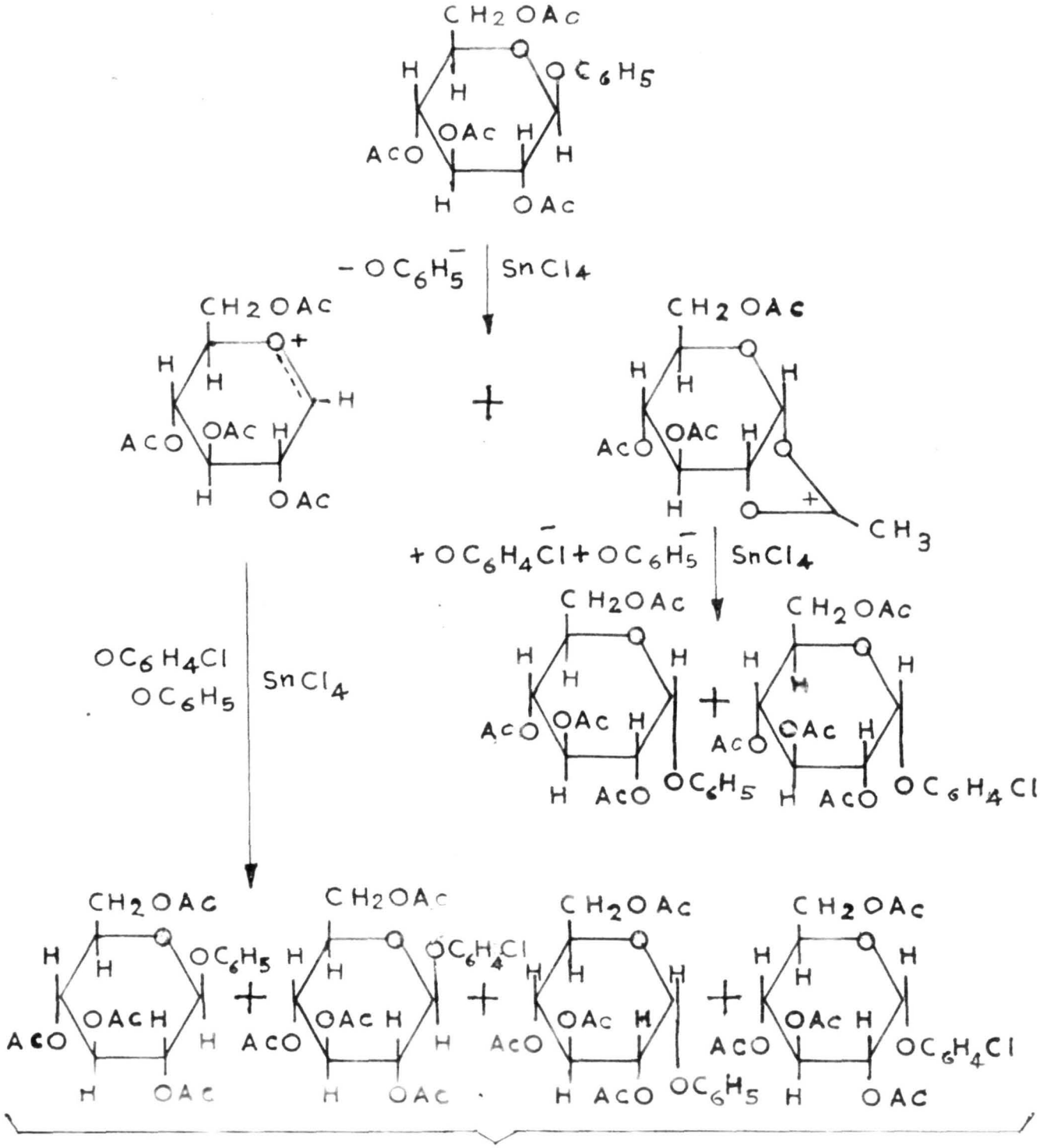


PHENYL-∝ - D - GLUCOPYRANOSIDE

AND

ORTHO - CHLORO PHENYL ∝ – D-GLUCOPYRANOSIDE [REACTION PRODUCT]

DIAGRAM 3



STANNIC CHLORIDE CATALYSED INTERMOLECULAR ANOMERISATION OF PHENYL TETRA-O-ACETYL β-D GLUCOPYRANOSIDE IN PRESENCE OF ORTHO CHLORO PHENOL

DIAGRAM 4

small quantities of benzene was kept at 40° for 48 hr and the reaction product was deacetylated and the PMR spectrum of the deacetylated product in DMSO solution was recorded. The two signals of C(1) protons were found at 5.35 and 5.65 ppm. We found that C(1) proton signal of phenyl- ∝ -D-glucopyranoside appears at 5.35 ppm and that of ortho-chlorophenyl- ∝ -D-glucopyranoside appears at 5.65 ppm (Chapter V, Part I). A mixture of these two pure glucosides showed C(1) proton signals clearly at 5.35 and 5.65 ppm. (Diagram 3). This proves beyond doubt that in this reaction during the anomerisation of the β -glucoside to the ∝ -glucoside ortho-chlorophenyl radical also takes part in the reaction leading to the formation of ortho-chlorophenyl- ∝ -D-glucoside, which support the view that the anomerisation reaction in the aryl glucosides takes place by intermolecular pathway which is shown in the adjoining diagram(4).

Discussions

The observation that no anomerisation or glucosidation takes place when tetra-O-acetyl- β -D-glucopyranoside or penta-O-acetyl- β -D-glucopyranose respectively are treated with stannic dichloro diphenoxide [reaction mixtures (a) and (b) (page 86) respectively], indicates the stannic complex is incapable of dissociating C(1)-acetoxy or C(1)-phenoxy bond under these conditions. In the presence of phenol, however, penta-O-acetyl- β -D-glucopyranose reacts with the stannic complex to give tetra-O-acetyl- β -D-glucopyranoside, indicating that phenol as a polar solvent probably helps the disproportionation of the stannic complex to yield small quantities of stannic chloride necessary to dissociate the C(1)-acetoxy bond to yield the carbonium ion (I) which leads to the formation of the

β-anomeric glucoside.

If, however, stannic chloride is added to the reaction mixture (a), the formation of the carbonium ion (VI) becomes possible due to its stabilization by an anion derived from stannic chloride. This should lead to the formation of the α-anomeric glucoside which is actually observed under these conditions. Since no phenol is added, the phenoxy anion required for this glucosidation must be derived from the stannic complex. The carbonium ions (II), (III) may also bring about the results observed.

The reaction between penta-O-acetyl-β-D-glucopyranose, the stannic complex and stannic chloride, however, leads to the formation of both of the anomeric glucosides. This can be explained by postulating not only the stabilised carbonium ion (VI) but also of (II), (III) as well. The carbonium ion (I) is not expected to be formed under these conditions as delocalisation of the charge on the lactol carbon atom to form the carbonium ion (I) should not take place in presence of excess of stannic chloride in a concentrated solution. This observation strongly supports the postulation of Bose and Ingle about the role of the carbonium ions (II), (III) on stannic chloride catalysed glycosidations.

The observation of Bose and Ingle about the differences in the proportion of the two anomeric glycosides formed from pentaacetates of D-glucose and D-galactose respectively, also indicate that the nature of the sugar evidently plays an important part on the relative abundance or stability of the different carbonium ions.

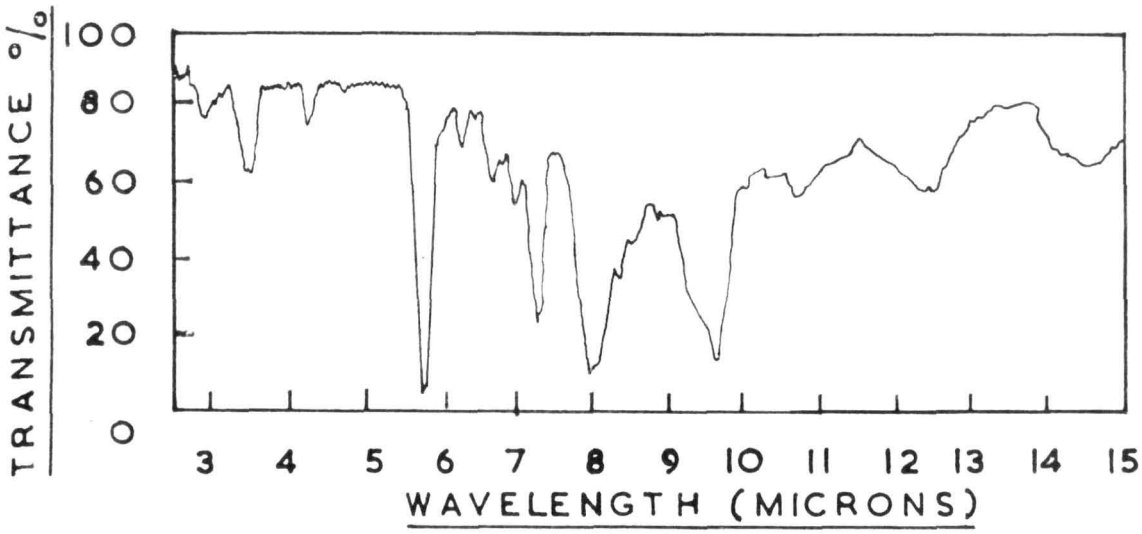
Investigations on the nature of the main by-product formed in the stannic chloride catalysed glucosidation reaction.

Thin-layer chromatograms of the reaction products obtained at various stages of stannic chloride catalysed glucosidation reaction of phenol, always show the presence of a number of slower moving trailing spots. The most prominent of these spots was found to be the one which had the least mobility. This product was isolated in an impure state by fractionating the total reaction product on a silica gel column. Even after repeated purifications by chromatography the product still gave some trailing on TLC. It was obtained as a yellow solid, m.p. 78-79°. It analysed for C, 55.78; H, 6.06 and did not leave any residue on combustion. The fully acetylated phenyl-D-glucoside requires C, 56.25; H, 5.65 and the free glucoside requires C, 56.56; H, 5.25 per cent respectively. The product was soluble in water and in most of the organic solvents like ethanol, acetone, ether, chloroform, carbon tetrachloride, dioxan, DMF and DMSO.

The compound appeared to be unsaturated in the tetranitromethane test. It added up bromine when treated with chloroform solution. The brominated product analysed for Br, 9.9 per cent.

Infrared spectrum of the compound showed the presence of hydroxyl group (3300 cm^{-1}), acetoxy group (1700 cm^{-1}) and an aromatic ring (1500 and 1600 cm^{-1}). Ultra violet spectrum also showed the presence of an aromatic ring. The IR and UV spectral observations were confirmed by its PMR spectrum.

Mass spectrum of the compound gave the heaviest ion peak at m/e 278. It is difficult to say whether this is the molecular

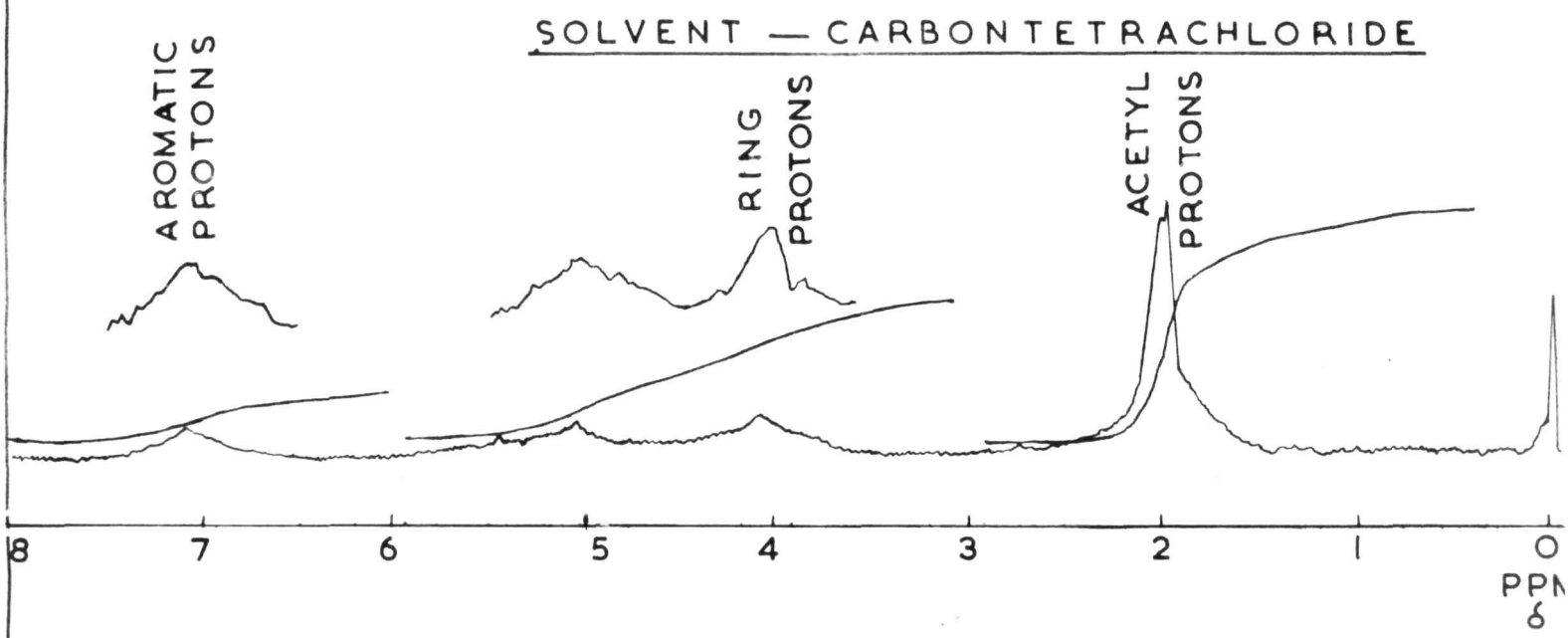


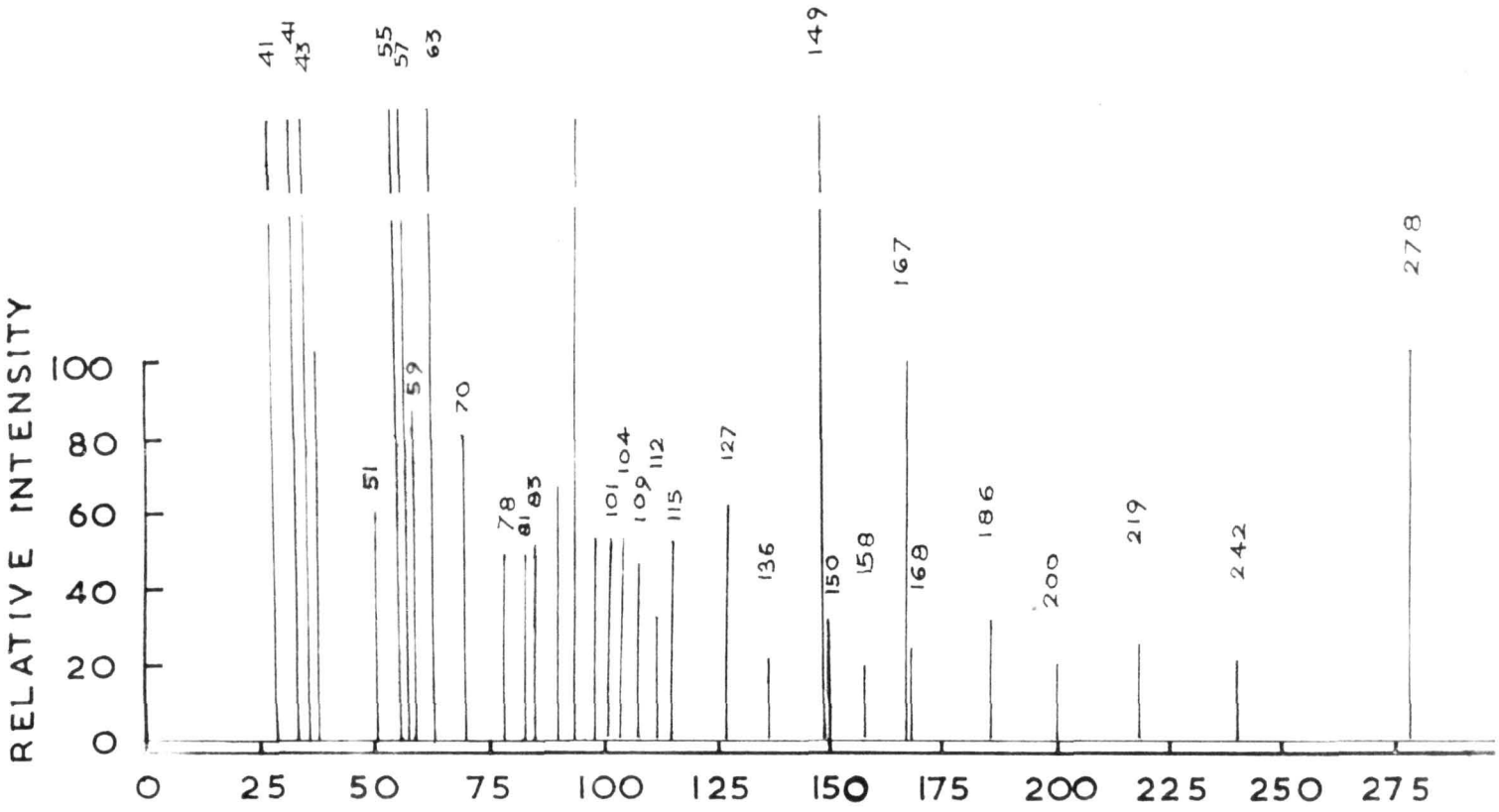
INFRA RED SPECTRUM OF THE MAIN BY PRODUCT FORMED IN STANNIC CHLORIDE CATALYSED GLUCO-SIDATION REACTION

SOLVENT :— CHLOROFORM

DIAGRAM 5

PROTON MAGNETIC RESONANCE SPECTRUM OF THE MAIN BY PRODUCT FORMED IN STANNIC CHLORIDE CATALYSED GLUCOSIDATION REACTION





MASS SPECTRUM OF THE MAIN BY PRODUCT FORMED IN STANNIC CHLORIDE CATALYSED GLUCOSIDATION REACTION

DIAGRAM 6

ion peak. Infrared, proton magnetic resonance and mass spectra of this compound are shwon in the adjoining diagrams (5 & 6).

On acetylation of the product with acetic anhydride and pyridine as well as acetic anhydride and sodium acetate gave a compound, m.p. 74-75° which did not show appreciable change in TLC mobility as compared with the starting material. Its infrared spectrum still showed the presence of free hydroxyl group.

These data are inconclusive for the prediction of the precise nature of this compound or its role, if any, in the glucosidation reaction. It is possible that it is a degradation product or a by-product of the glucosidation reaction.

EXPERIMENTAL

The methods described for glucosidation and anomerisation in this Chapter were carried out according to the procedure already described in Chapter III.

Attempted anomerisation of phenyl tetra-O-acetyl-α-D-glucopyranoside

A mixture of equimolar quantities of phenyl tetra-O-acetyl -α-D-glucopyranoside, phenol, and stannic chloride in a little benzene was kept at 40° for 40 hr. The TLC analysis of the product proved it to be a mixture of mainly unreacted α-D-glucoside and some degradation products.

Attempted anomerisation of phenyl tetra-O-acetyl -β-D-glucopyranoside without using phenol.

A mixture of equimolar quantities of phenyl tetra-O-acetyl -β-D-glucopyranoside and stannic chloride in a little benzene was kept at 40° for 40 hr. The TLC analysis of the product proved it to be a mixture of mainly unreacted β-D-glucoside and some degradation products.

Attempted anomerisation of phenyl tetra-O-acetyl β-D-glucopyranoside without using stannic chloride.

A mixture of equimolar quantities of phenyl tetra-O-acetyl -β-D-glucopyranoside and phenol in a little benzene was kept at 40° for 40 hr. The TLC analysis of the product proved it to be a mixture of mainly unreacted β-D-glucoside and some degradation products.

Anomerisation of phenyl tetra-O-acetyl- β -D-glucopyranoside

A mixture of equimolar quantities of phenyl tetra-O-acetyl -β-D-glucopyranoside, phenol and stannic chloride in a little benzene was kept at 40° for 40 hr. The TLC analysis of the product proved it to be a mixture of mainly ∝-D-glucoside and some degradation products.

Preparation of stannic dichloro diphenoxide

A mixture of phenol (1.9 gm), stannic chloride (1 ml) and benzene (1 ml) was kept under anhydrous conditions at the room temperature (25-30°). After about 48 hr some buff coloured solid appeared in the reaction mixture. After 50 hr the reaction mixture was diluted with benzene (1 ml) and pet. ether (60-80°) (1 ml) and cooled to 0°. The solid was rapidly filtered and washed with pet. ether. The solid was again dissolved in a mixture of benzene and pet. ether and cooled to 0°. The solid obtained was filtered and thoroughly washed with pet. ether-benzene (20:1) and dried in a desiccator under reduced pressure.

The chocolate coloured solid, m.p. 110-112°, was an amorphous powder and was highly hygroscopic. The yield of the material was 0.1178 gm. It did not move on TLC in solvents A, B and C.

Analysis: Found C, 34.26; H, 4.04, and Sn, 30.8

Calculated for Sn $(C_6H_5O)_4$, C, 58.3; H, 4.8; and Sn 24.8

Sn $(C_6H_5O)_3Cl$, C, 50.0; H, 3.48; and Sn 27.2

Sn $(C_6H_5O)_2Cl_2$, C, 38.4; H, 2.6; and Sn 31.5

Sn $(C_6H_5O)Cl_3$, C, 22.6; H, 0.01; and Sn 37.1

percent.

The analysis agrees with that of stannic dichloro diphenoxide approximately.

Anomerisation and glucosidation in presence of $SnCl_2$ $(C_6H_5O)_2$ and phenol.

A mixture of phenyl tetra-O-acetyl -β -D-glucopyranoside (1 millimol), phenol (1 millimol) and $SnCl_2$ $(C_6H_5O)_2$ (0.5 millimol) and a little benzene was kept at 40° for 40 hr. The reaction product was found to consist of mainly unreacted β -D-glucoside along with some degradation products (TLC).

When the same reaction was carried out with penta-O-acetyl-β -D-glucopyranose, the reaction product obtained was found to be a mixture consisting of mainly phenyl tetra-O-acetyl- β -D-glucopyranoside and some degradation products. No α -D-glucoside was noticeable in the reaction product.

Anomerisation and glucosidation in presence of stannic dichloro diphenoxide and stannic chloride.

A mixture of phenyl tetra-O-acetyl -β -D-glucopyranoside (1 millimol), stannic chloride (1 millimol) and stannic dichloro diphenoxide (0.5 millimol) and a little benzene was kept at 40° for 40 hr. The TLC of the reaction product indicated the presence of a mixture of mainly phenyl tetra-O-acetyl- α -D-glucopyranoside and traces of unreacted β -D-glucoside and degraded products.

Similarly, when the same reaction was carried out with penta-O-acetyl- β -D-glucopyranose, the reaction product obtained was found to be a mixture containing mainly phenyl tetra-O-acetyl α -D-glucopyranoside and traces of β -D-glucoside and some degradation products (TLC).

Anomerisation of phenyl tetra-O-acetyl-β-D-glucopyranoside in presence of ortho chlorophenol and stannic chloride

A mixture of phenyl tetra-O-acetyl-β-D-glucopyranoside (0.7 gm), ortho-chlorophenol (0.5 gm), stannic chloride (1 ml) and benzene (0.2 ml) was kept at 40°. The progress of anomerisation was followed by TLC until phenyl tetra-O-acetyl-β-D-glucopyranoside disappeared from the reaction mixture (48 hr). After 48 hr the mixture was diluted with benzene (100 ml) was washed free of phenol and stannic chloride with water and sodium bicarbonate solution. The resulting solution was dried over anhydrous sodium sulphate and freed of the solvent. The resulting syrup could not be resolved distinctly on TLC. The syrup was, therefore, deacetylated with methanolic solution of sodium methoxide (0.1 N, 10 ml) at the room temperature. Complete deacetylation was confirmed by absence of TLC mobile component in the reaction mixture. The deacetylated reaction mixture was decationised by passing through a column of Amberlite IR-120 (H^+) resin and the resulting neutral solution was evaporated to a syrup. PMR spectrum of the syrup in DMSO was recorded and the pattern of signals of C(1) protons in the region 5.35 to 5.65 ppm was found to be identical with the pattern of C(1) proton signals in the PMR spectrum of a mixture of authentic samples of phenyl-α-D-glucopyranoside and ortho chlorophenyl-α-D-glucopyranoside in DMSO.

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CHAPTER : V

CONFORMATIONAL ANALYSIS OF ARYL GLUCOSIDES BY INFRA RED NEUCLEAR MAGNETIC RESONANCE AND MASS SPECTRAL STUDIES

SUMMARY

Studies in the proton magnetic resonance spectra of the free aryl glucosides and mass spectra of their acetates and infrared spectra of both free aryl glucosides and their acetates are presented in this Chapter as evidences for the recognition of the anomeric configuration.

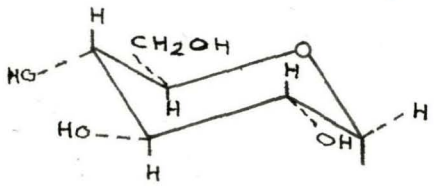
CONFORMATIONAL ANALYSIS OF ARYL GLYCOSIDES BY INFRA RED, PROTON MAGNETIC RESONANCE AND MASS SPECTRAL STUDIES

Although the Haworth representation of the cyclic structures of sugars is satisfactory for general purposes, it leaves much to be desired when the exact spatial arrangements of the hydrogen atoms and hydroxyl groups about the different carbon atoms of the sugar ring, i.e., their conformations, are to be assigned.

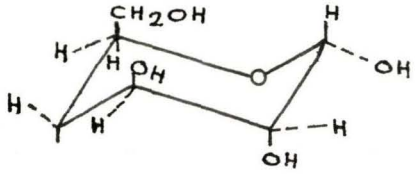
The application of conformational analysis is of great usefulness in carbohydrate chemistry on account of the fact that all the sugars belong to a few families of diastereoisomers and within one such family, they differ only in their steric arrangement and these differences can be accounted for only by conformational factors. Different chemical and physical properties[1], reaction mechanism[2] and specific enzyme activities[3] of various sugars and their derivatives could be explained and predicted if these conformational factors could be exactly evaluated.

The pioneering work in the conformations of sugars was carried out by Reeves[1]. Of the two different possible chair conformations and six different boat conformations possible for the pyranose ring, it is reasonably certain that the pyranoid sugars exist in chair conformations.

The two chair conformations possible for each anomeric sugar have been designated by Reeves as the C1 and 1C forms. These two conformations for each of the α-and the β-D-glucopyranoses are shown in Figures I and II.

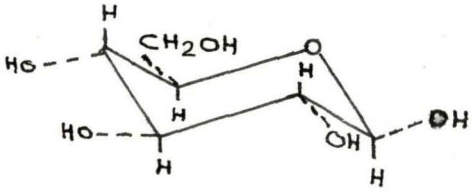


C1

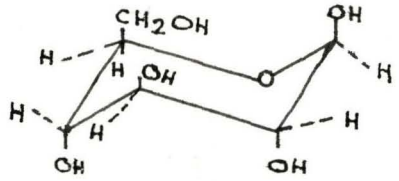


1C

I. α-D-GLUCOPYRANOSE



C1



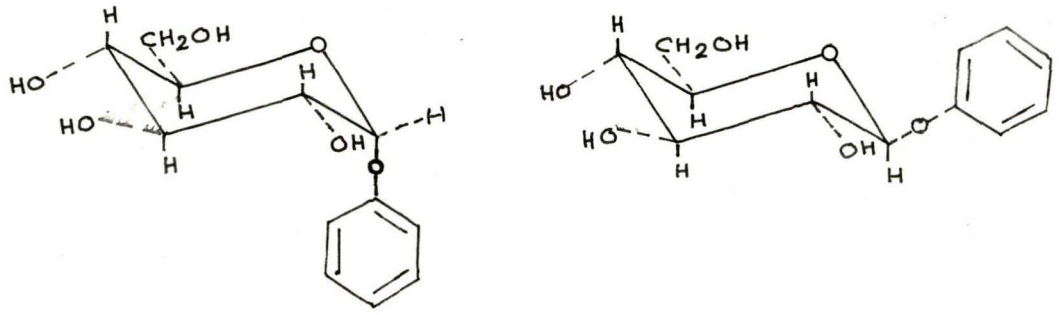
1C

II. β-D-GLUCOPYRANOSE

Since these two forms are interconvertible, the one which is more stable will predominate. It is well known that a molecule assumes a conformation in which the heavier groups are mostly in equatorial positions. In deciding which of the conformation C1 or 1C is more stable for a particular sugar, a number of factors called instability factors or ratings were introduced[4-7]. These instability factors[8] can be used to predict which of the conformations would be more stable for an α-and β -anomeric sugar. If we consider α-D-glucopyranose in 1C and C1 forms, the numerical value for instability ratings for the C1 form is only 1, whereas that for the 1C form is as high as 8. Therefore, the α -anomer must exist in the C1 form only. Similarly if we take the β -anomer, the instability

rating for the Cl form is 0 and for the 1C form, 6.5. Therefore, the β-D-glucopyranose should also exist in the Cl form.

Similarly, the α-and β-phenyl-D-glucopyranosides should also exist in the more stable Cl conformation, although in the α-form the heavy-aryloxy is in the axial position in this conformation.



III. PHENYL-α-D-GLUCOPYRANOSIDE, IV. PHENYL-β-D-GLUCOPYRANOSIDE

It is possible to give configurational assignments to a pair of anomeric glycosides on the basis of isorotational rules of Hudson[9,10]. The favoured conformation of a glycopyranoside is also predictable from the rotational and conductometric behaviour of its cuproammonium solution[1].

In this Chapter an attempt has been made to correlate infrared, PMR and mass spectral characteristics of pairs of anomeric aryl glucosides with their conformations.

Infrared spectroscopy

Infrared spectroscopy is being used extensively for the recognisation and analysis of the structural units in unknown organic compounds or for the confirmation of their presence in known structures[14,16]. For the interpretation of an infrared spectrum, one has to rely on the empirical data which have accumulated so far, relating infrared absorption bands with structural characteristics. This correlation is often difficult due to the shifts in the absorption bands owing to the influence of adjoing structures. A very careful analysis and correlation of known data is therefore necessary to arrive at conclusions helpful for the elucidation of the structure of an organic molecule.

Infrared spectral studies have been usefully employed for elucidation of structures of carbohydrates[17,18]. Thus, a pair of anomeric sugars were found by Kuhn[19] and Barker et. al.[20] to show certain differences in their infrared spectra.

From a comparative study of the infrared spectra of pairs of anomeric methyl glycosides of D-glucose, D-mannose and D-galactose Barker et. al.[20] concluded that the absorption band in the region. 11.75 - 12.10μ (850-830 cm^{-1}) can be attributed to the anomers having the α-configuration.

As a result of further studies of the infrared spectra of some fully methylated glucosides, reducing methyl ethers of sugars, oligosaccharides, polysaccharides and their acetates, Barker et. al.[21] assigned 11.8 $\pm$ 0.03μ (844 $\pm$8cm^{-1}) region for the α-D-glucose configuration band. This absorption band was not observed in the

corresponding β -anomers.

From an examination of three anomeric pairs of phenyl glycosides, House and Whistler[22] pointed out that the α -anomers absorb at the 11.61 - 11.73μ region. This is very near to the value assigned by Barker et. al.[20,21] for the α -D-glucose configuration. They also found that the corresponding β -anomers absorb within the short range of 12.15 - 12.25μ region. In this region there is no absorption band for the α-anomers; moreover the β -anomers were found to have no absorption in the 11.61 - 11.73μ region.

In the course of the present work, infrared spectral characteristics of seven pairs of anomeric aryl glycosides and their acetates (except for one pair of anomers) have been studied with a view to find out the influence, if any, of the aryl substituent on the positions of the bands assigned by the earlier workers for the α -and β -configurations. Table I, lists the characteristic absorption bands of these compounds.

TABLE I

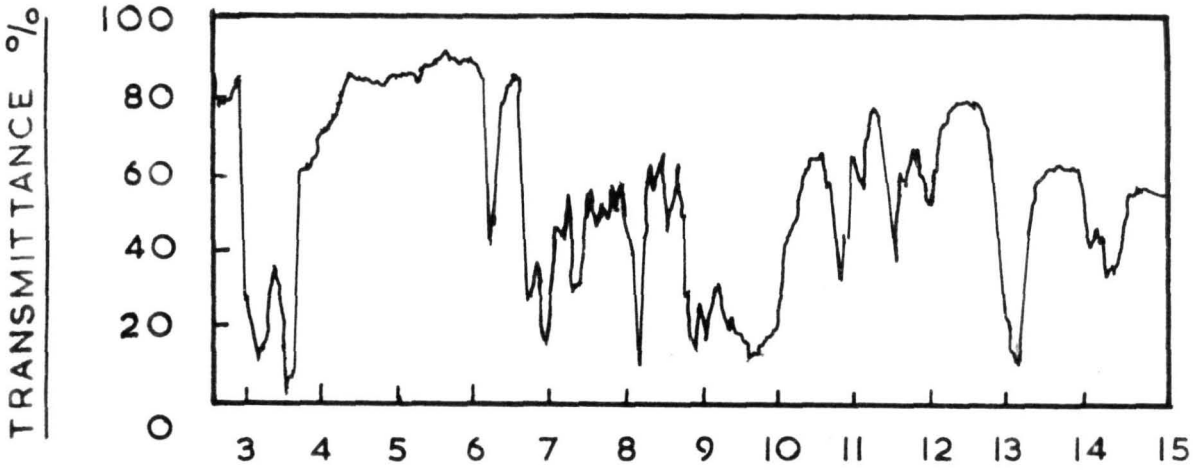
Bands in the infrared spectra of some aryl-D-glucopyranosides and their tetraacetates.

S. No.	Compound	Absorption band in the region of		
		12.15 to 12.25 μ [22]	11.50 to 11.55 μ (11.61 to 12.1 μ [20,21]	8.70 μ (new region)
1	2	3	4	5
1	Phenyl-α-D-glucopyranoside	No band	11.55	8.70
2	o-Methyl phenyl-α-D-glucopyranoside	No band	11.55	8.70
3	o-Chloro phenyl-α-D-glucopyranoside	No band	11.55	8.70
4	p-Methyl phenyl-α-D-glucopyranoside	No band	11.50	8.70
5	p-Chloro phenyl-α-D-glucopyranoside	No band	11.55	8.70
6	p-Phenyl phenyl-α-D-glucopyranoside	No band	11.55	8.70
7	p-Nitro phenyl-α-D-glucopyranoside	No band	11.55 (13.90)	No band
8	Phenyl-β-D-glucopyranoside	12.15	No band	No band
9	o-Methyl phenyl-β-D-glucopyranoside	No band	No band	No band
10	o-Chloro phenyl-β-D-glucopyranoside	No band	No band	No band
11	p-Methyl phenyl-β-D-glucopyranoside	No band	No band	No band
12	p-Chloro phenyl-β-D-glucopyranoside	No band	No band	No band
13	p-Phenyl phenyl-β-D-glucopyranoside	No band	No band	No band

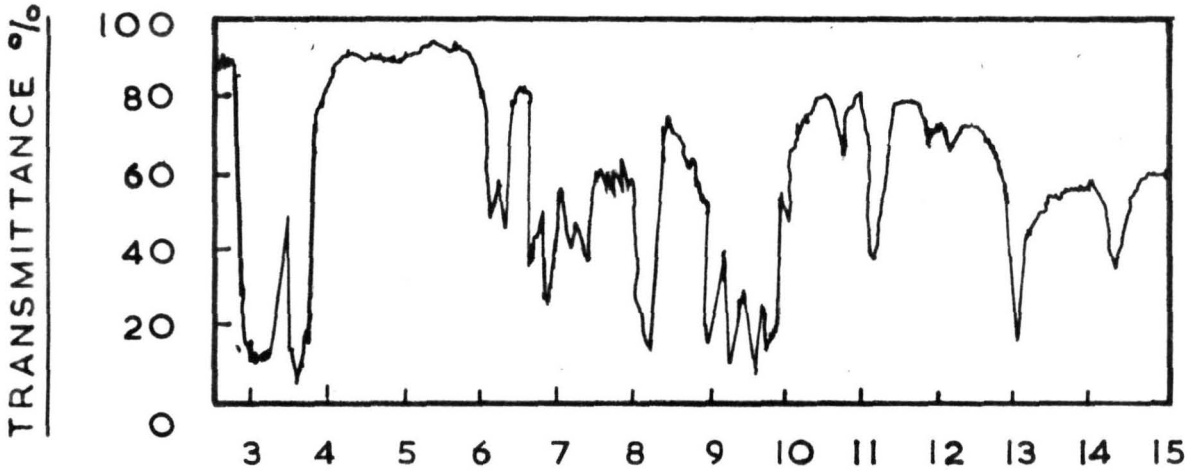
Contd..

TABLE I (contd)

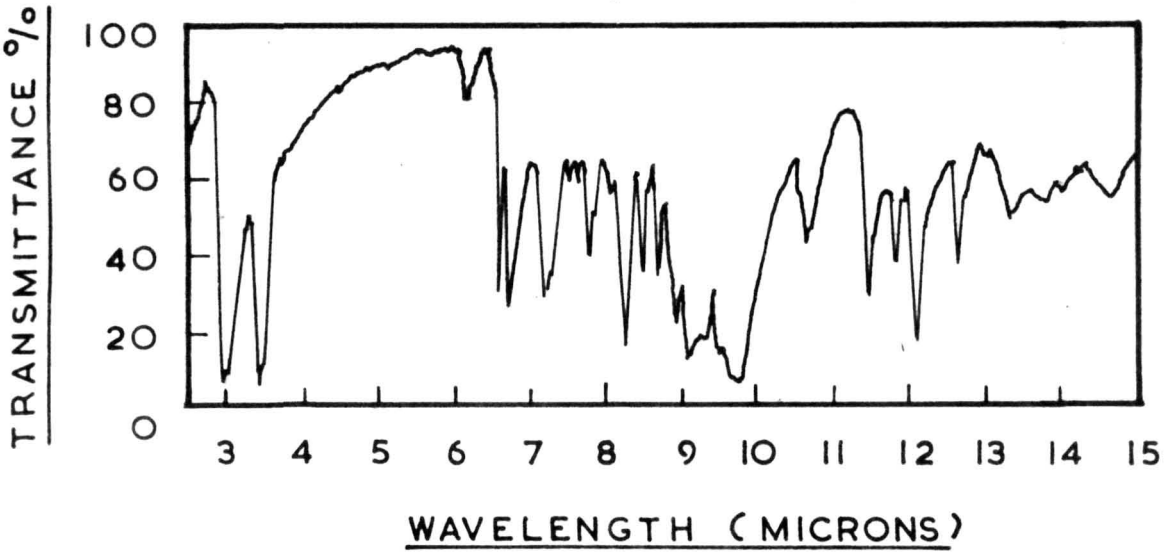
1	2	3	4	5
14	p-Nitro phenyl-β-D-glucopyranoside	No band	11.55	8.70
		Absorption band in the region of 10.40 - 10.45 μ		
15	Phenyl tetra-O-acetyl-α-D-glucopyranoside		10.45	
16	o-Methyl phenyl tetra-O-acetyl-α-D-glucopyranoside		10.40	
17	o-Chloro phenyl tetra-O-acetyl-α-D-glucopyranoside		10.40	
18	p-Chloro phenyl tetra-O-acetyl-α-D-glucopyranoside		10.40	
19	p-Phenyl phenyl tetra-O-acetyl-α-D-glucopyranoside		10.40	
20	p-Nitro phenyl tetra-O-acetyl-α-D-glucopyranoside		10.40	
21	Phenyl tetra-O-acetyl-β-D-glucopyranoside		No band	
22	o-Methyl phenyl tetra-O-acetyl-β-D-glucopyranoside		10.40	
23	o-Chloro phenyl tetra-O-acetyl-β-D-glucopyranoside		10.40	
24	p-Phenyl phenyl tetra-O-acetyl-β-D-glucopyranoside		No band	
25	p-Nitro phenyl tetra-O-acetyl-β-D-glucopyranoside		No band	



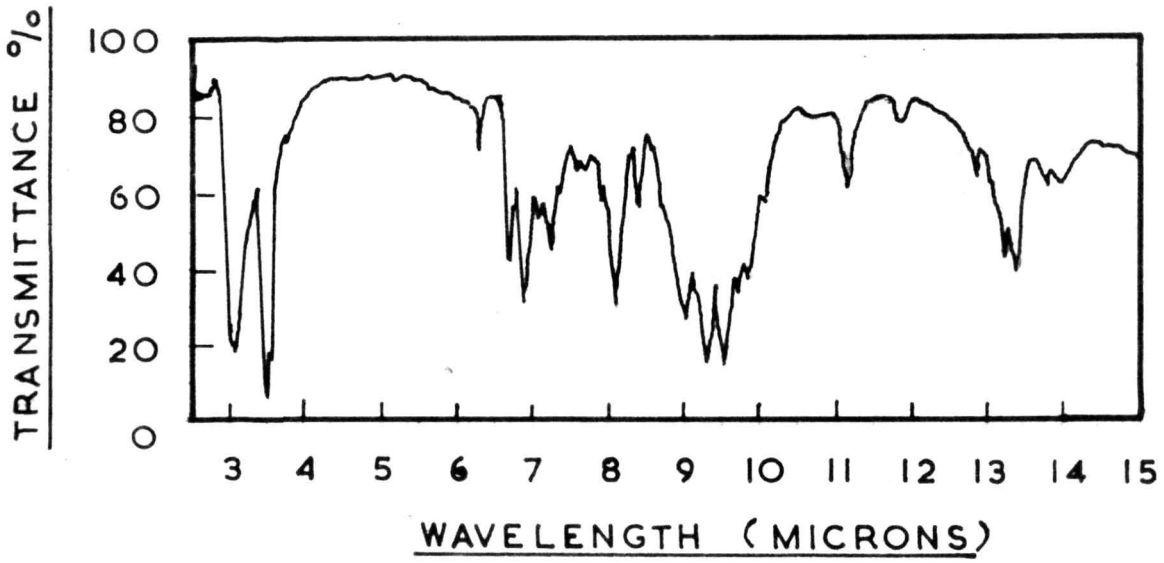
PHENYL ∝ - D - GLUCOPYRANOSIDE



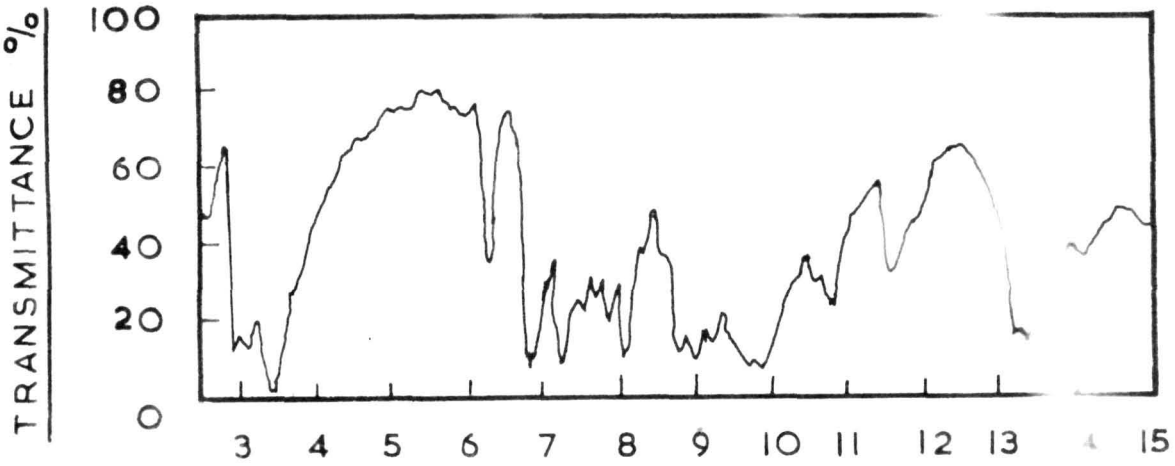
PHENYL β - D - GLUCOPYRANOSIDE



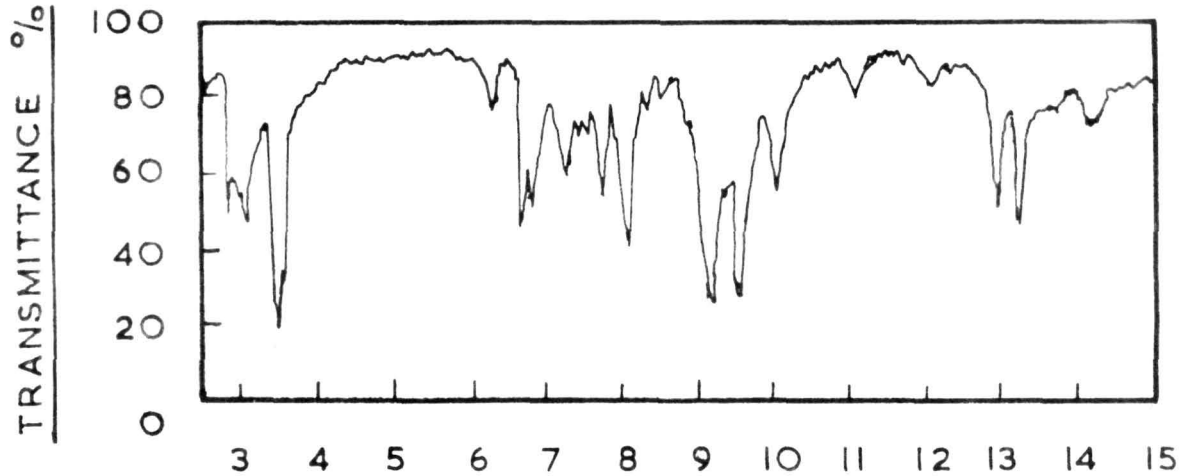
ORTHO - METHYL - PHENYL
∝ - D - GLUCOPYRANOSIDE



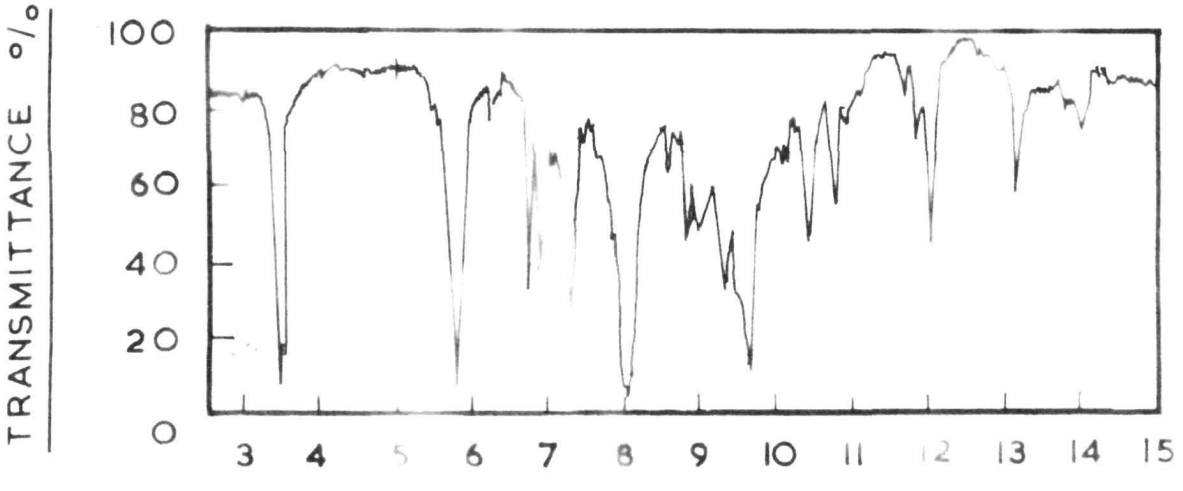
ORTHO - METHYL - PHENYL
β - D - GLUCOPYRANOSIDE



ORTHO - CHLORO - PHENYL
α - D - GLUCOPYRANOSIDE

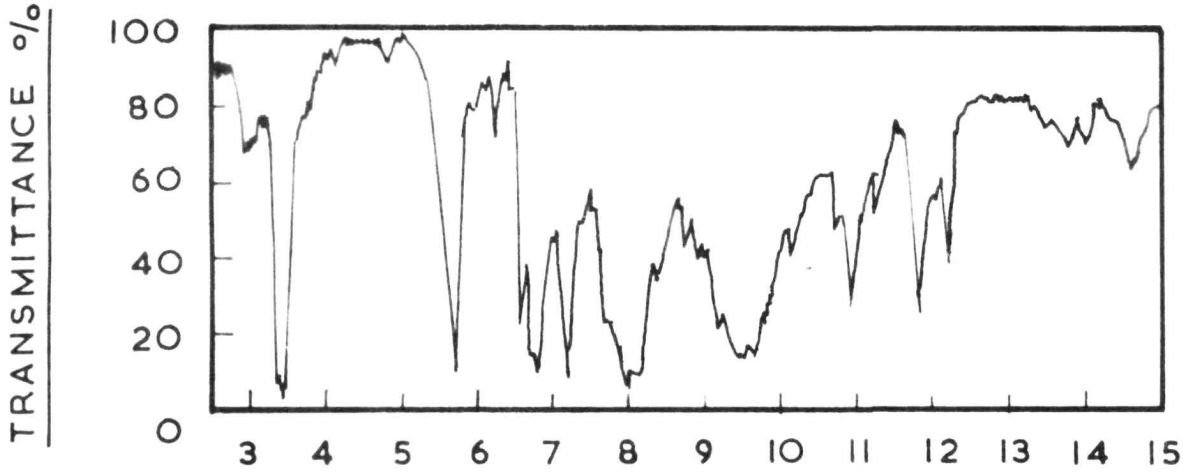


ORTHO - CHLORO - PHENYL
β - D - GLUCOPYRANOSIDE



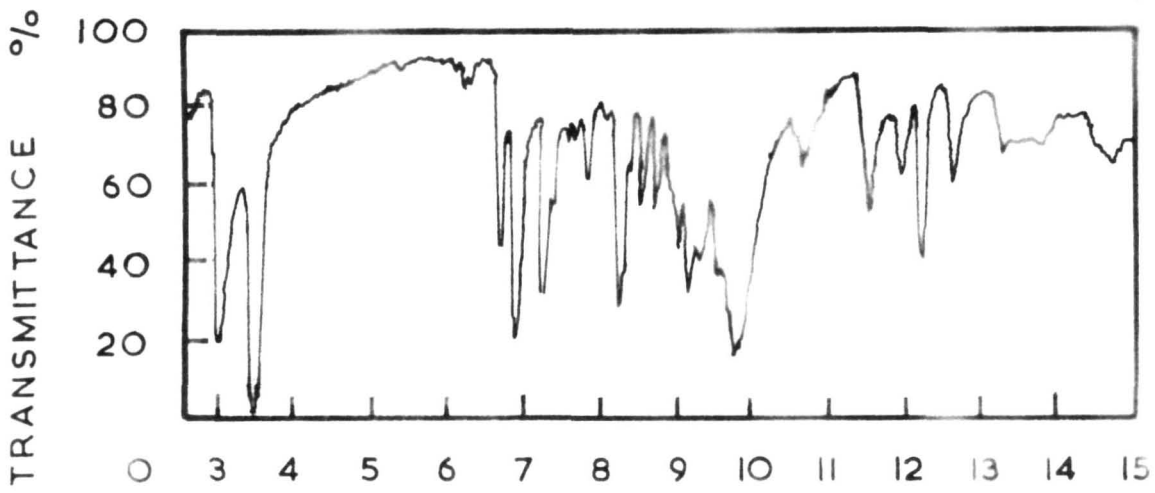
PARA - CHLORO PHENYL - TETRA - O - ACETYL

α - D - GLUCOPYRANOSIDE

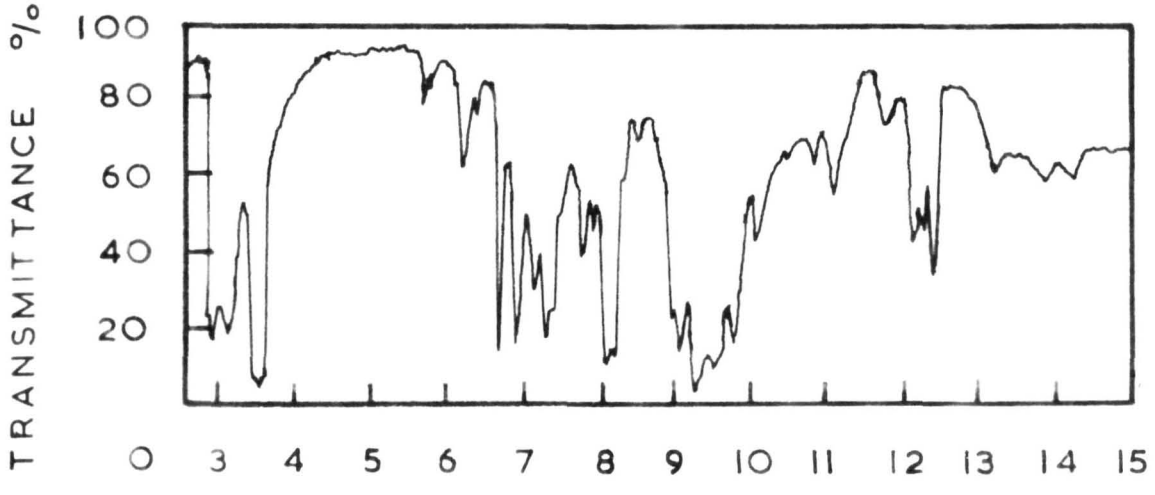


PARA - CHLORO - PHENYL - TETRA - O - ACETYL

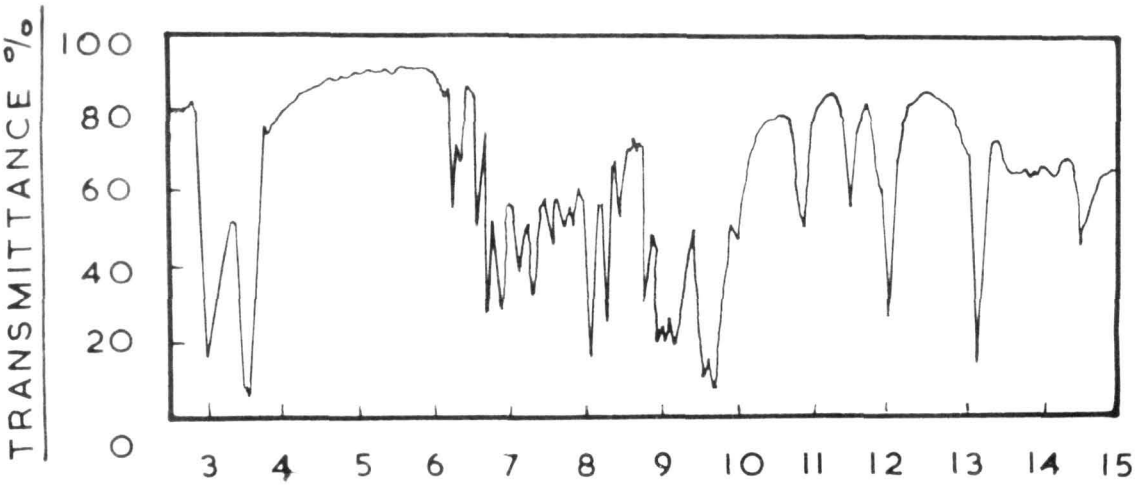
β - D - GLUCOPYRANOSIDE



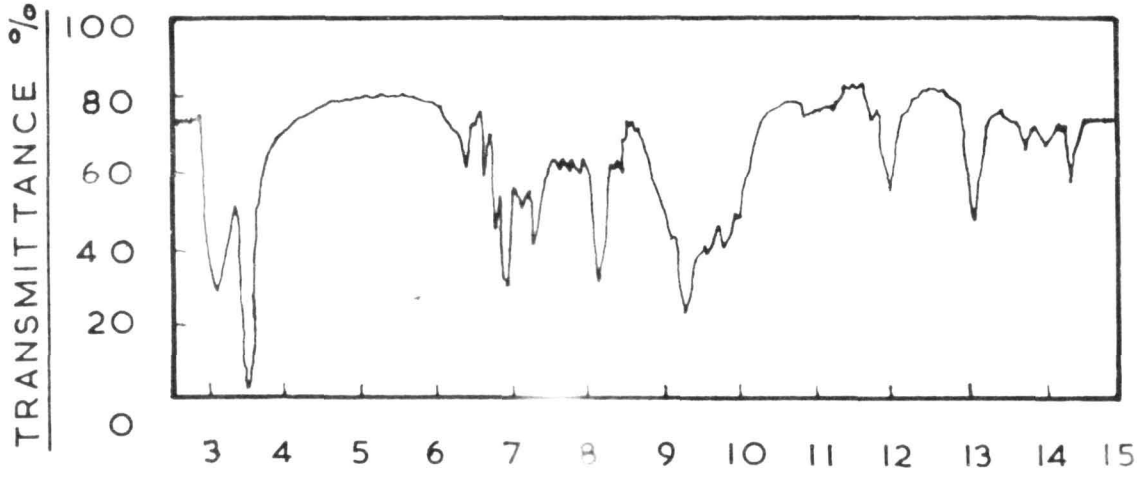
PARA - METHYL PHENYL
∝ - D - GLUCOPYRANOSIDE



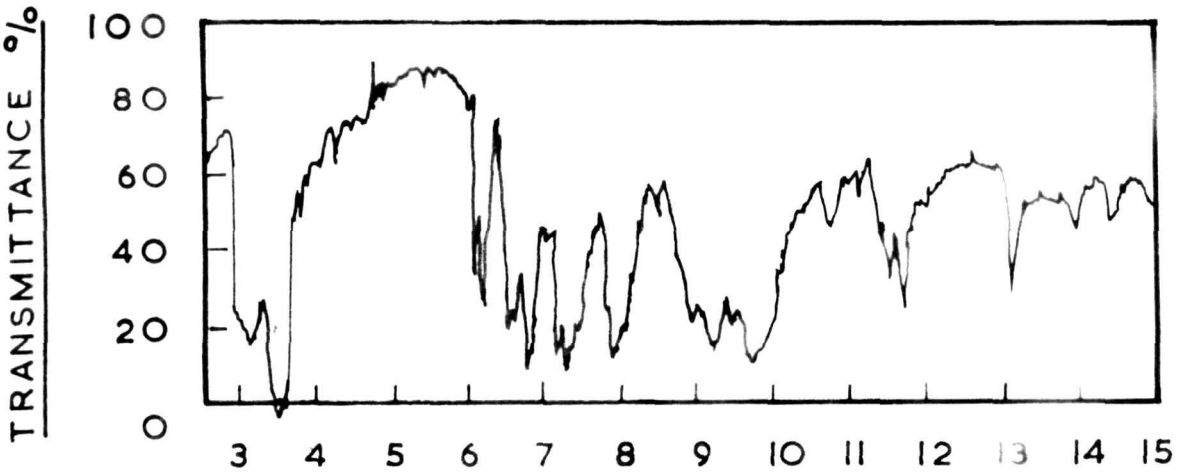
PARA - METHYL PHENYL
β - D- GLUCOPYRANOSIDE



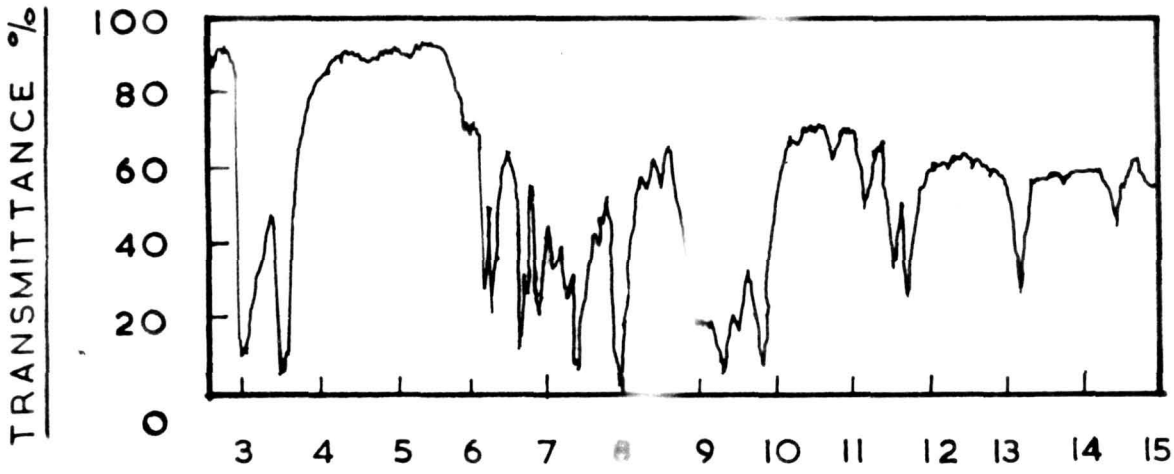
PARA - PHENYL - PHENYL
∝ - D - GLUCOPYRANOSIDE



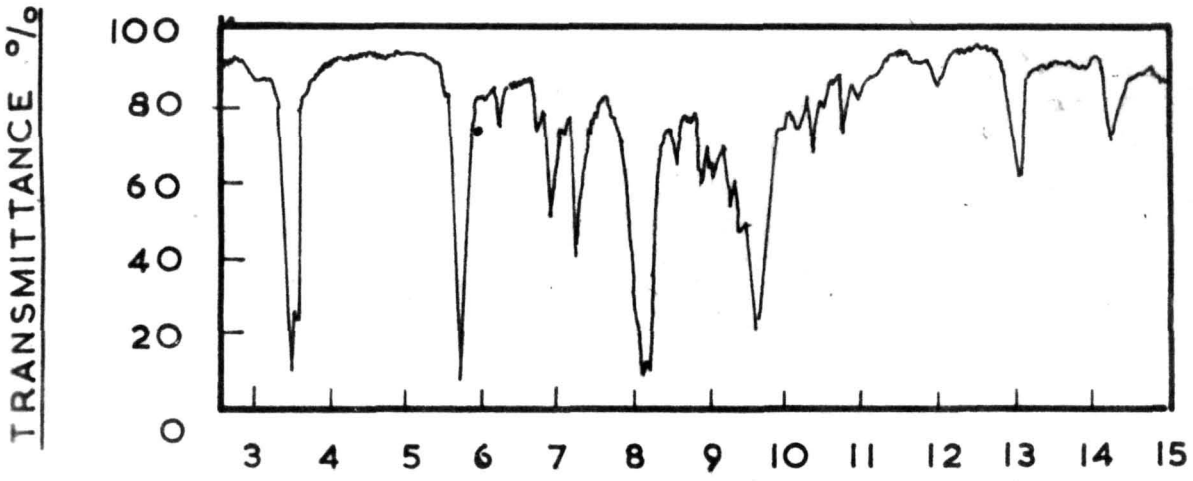
PARA - PHENYL - PHENYL
β - D - GLUCOPYRANOSIDE



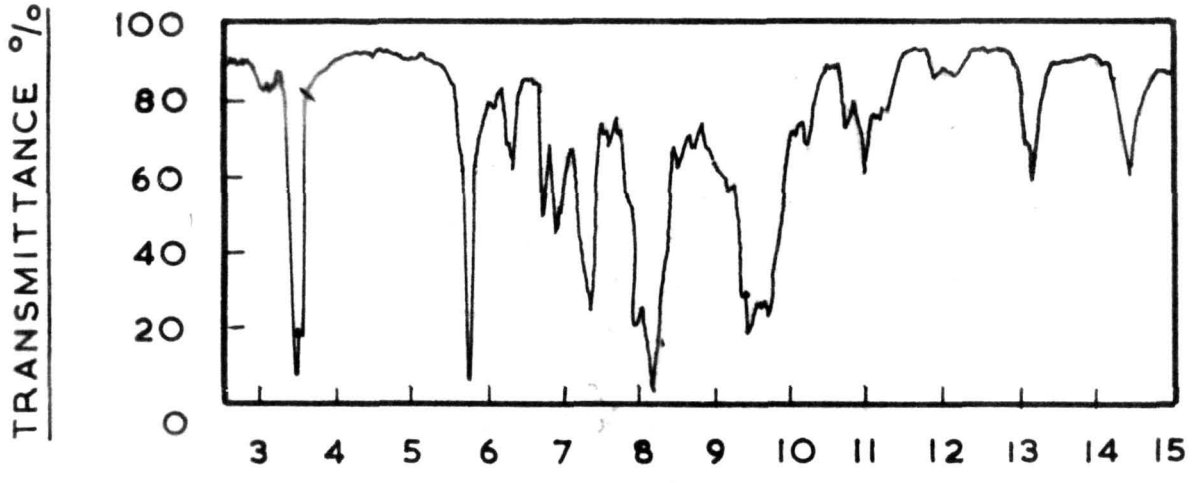
PARA - NITRO - PHENYL
α - D - GLUCOPYRANOSIDE



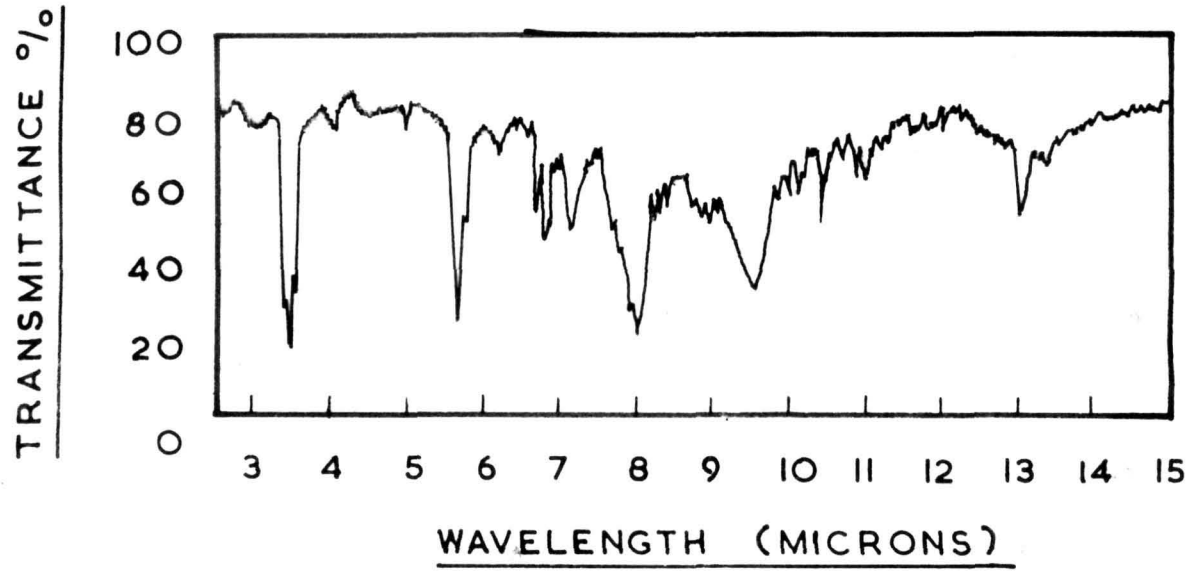
PARA - NITRO - PHENYL
β - D - GLUCOPYRANOSIDE



PHENYL-TETRA - O - ACETYL
α - D - GLUCOPYRANOSIDE

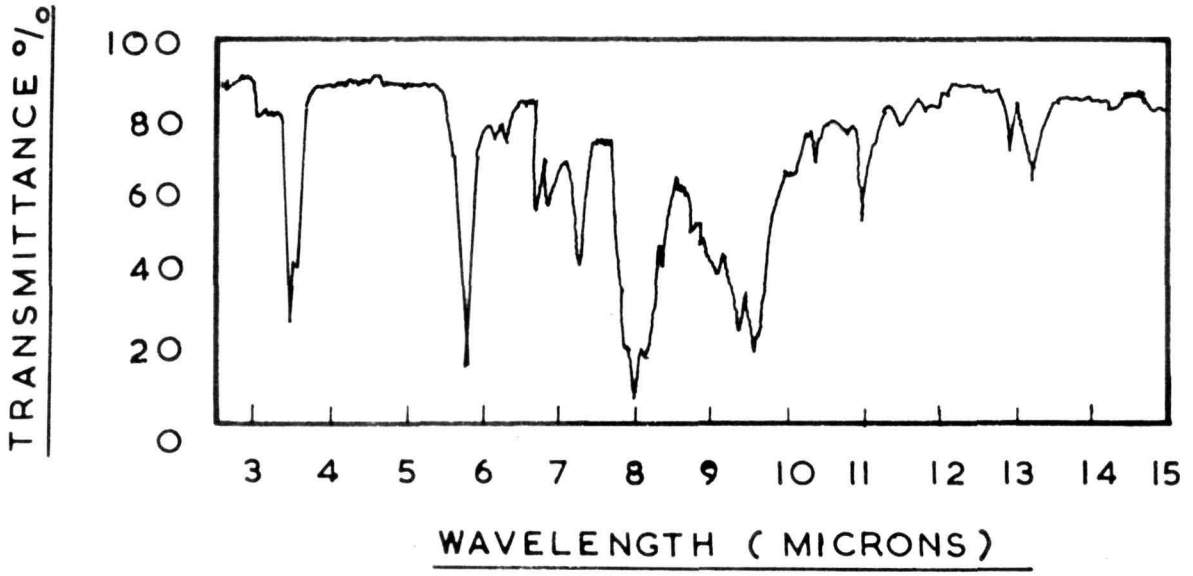


PHENYL-TETRA - O - ACETYL
β - D - GLUCOPYRANOSIDE



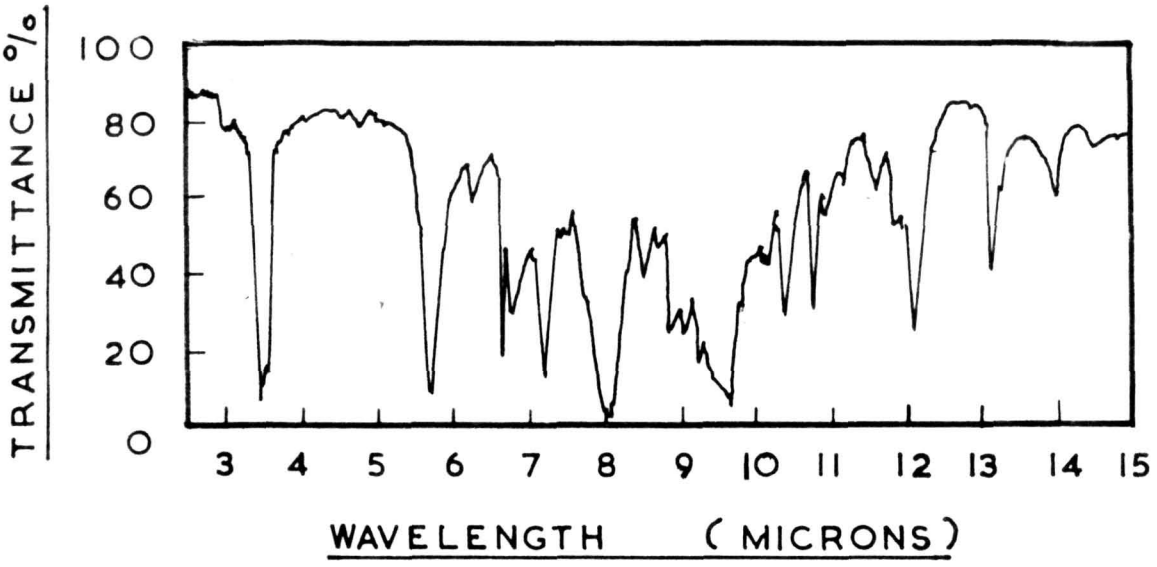
ORTHO - METHYL - PHENYL TETRA - O - ACETYL

∝ - D - GLUCOPYRANOSIDE

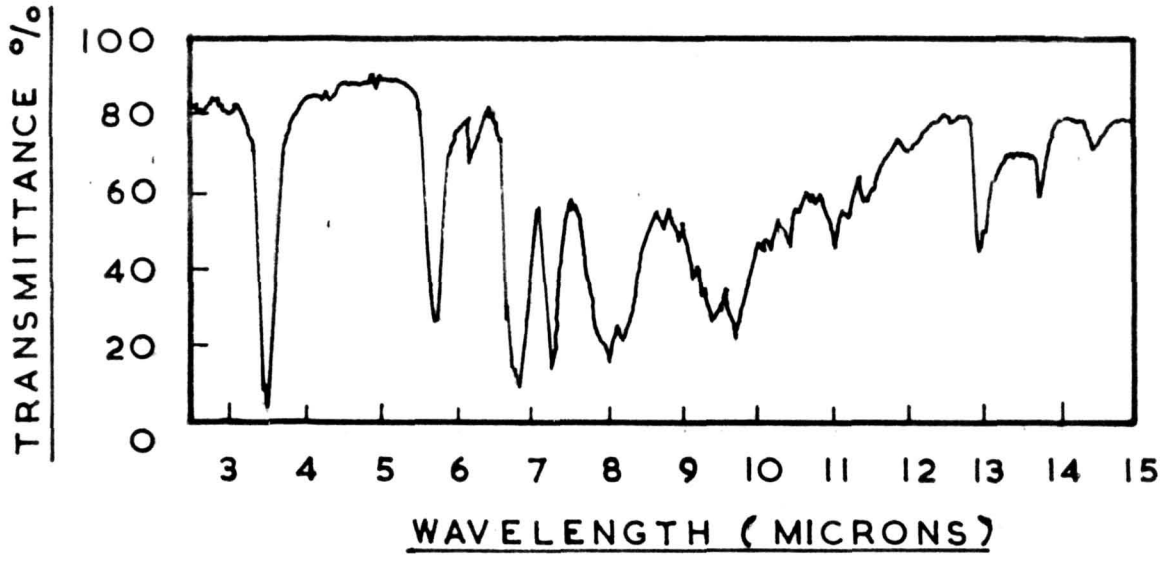


ORTHO- METHYL - PHENYL TETRA - O - ACETYL

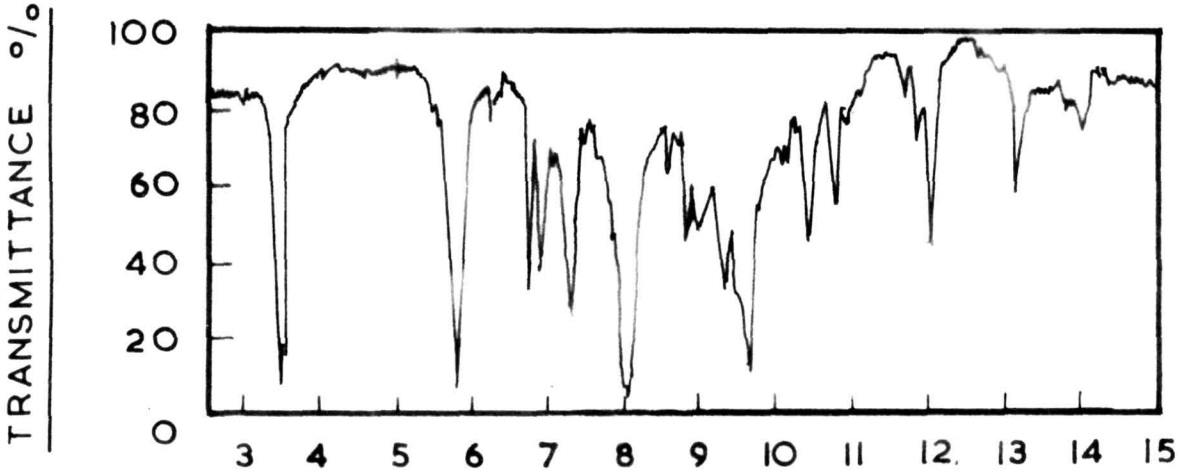
β - D - GLUCOPYRANOSIDE



ORTHO-CHLORO PHENYL TETRA-O-ACETYL
α-D-GLUCOPYRANOSIDE

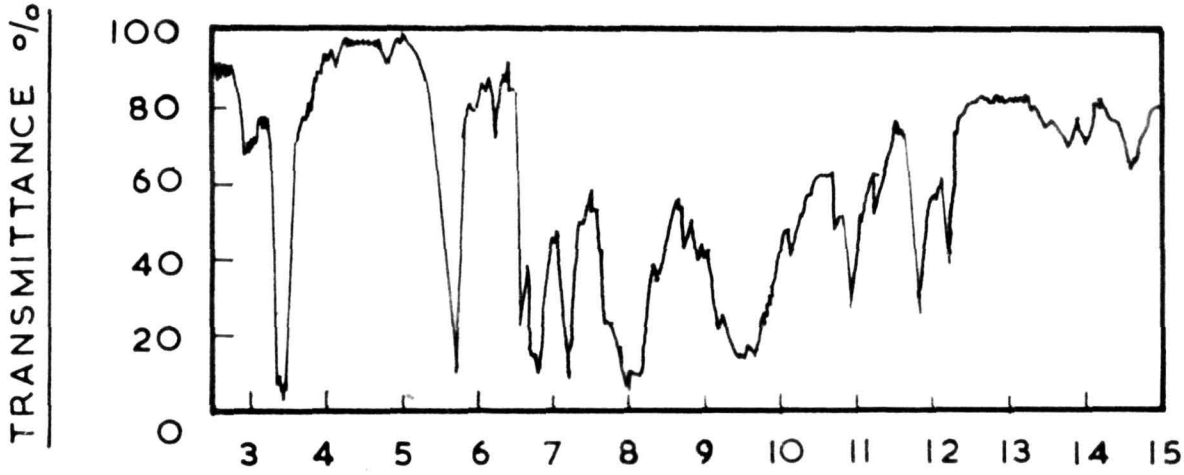


ORTHO-CHLORO PHENYL TETRA-O-ACETYL
β-D-GLUCOPYRANOSIDE



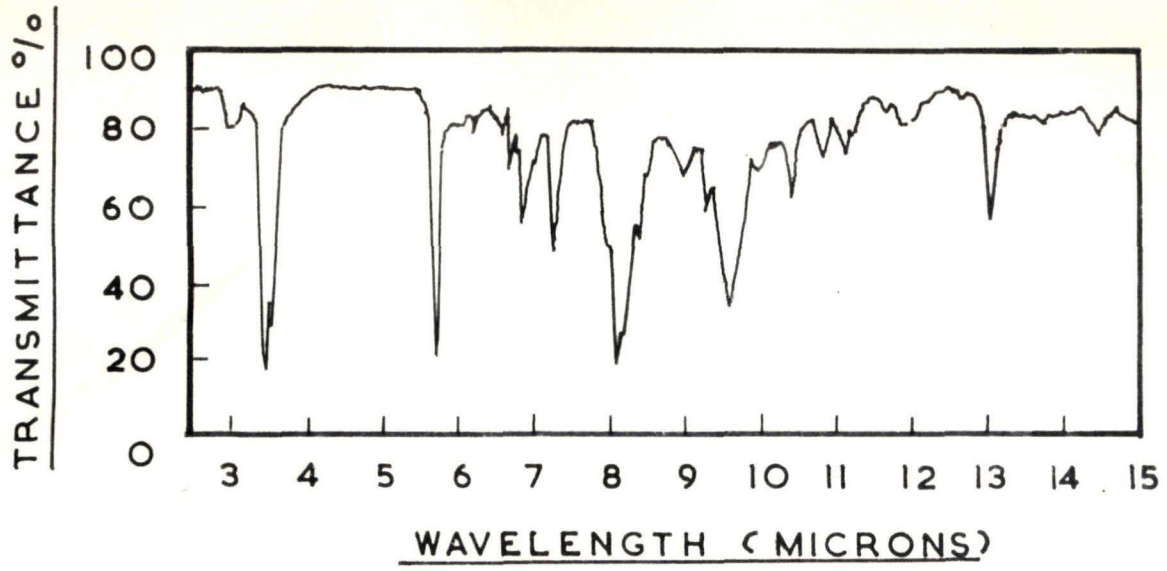
PARA - CHLORO - PHENYL TETRA - O - ACETYL

α - D - GLUCOPYRANOSIDE

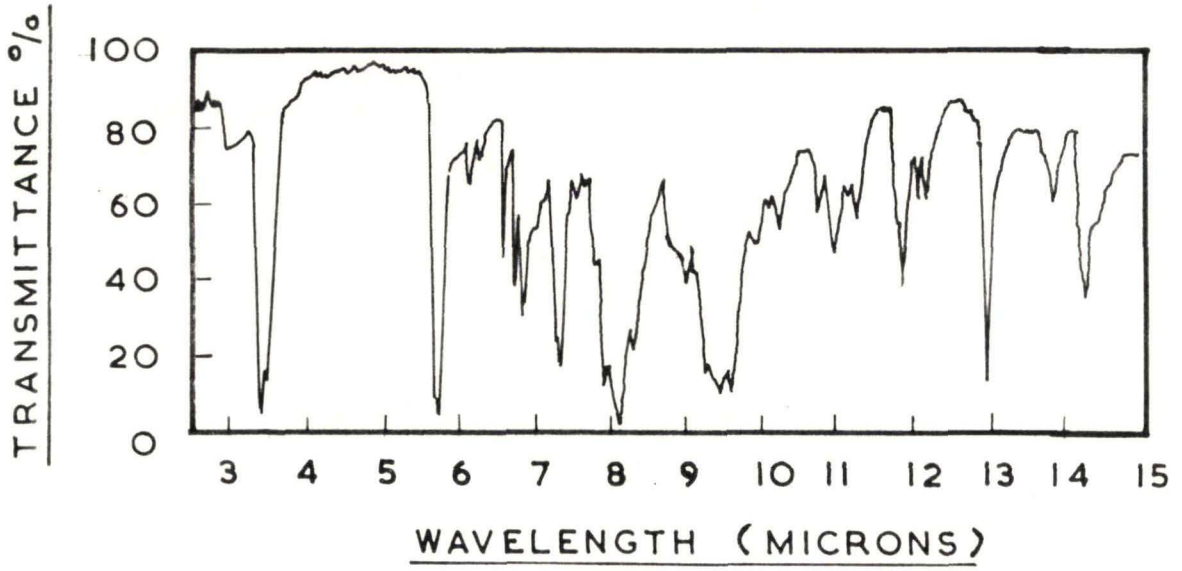


PARA - CHLORO - PHENYL TETRA - O - ACETYL

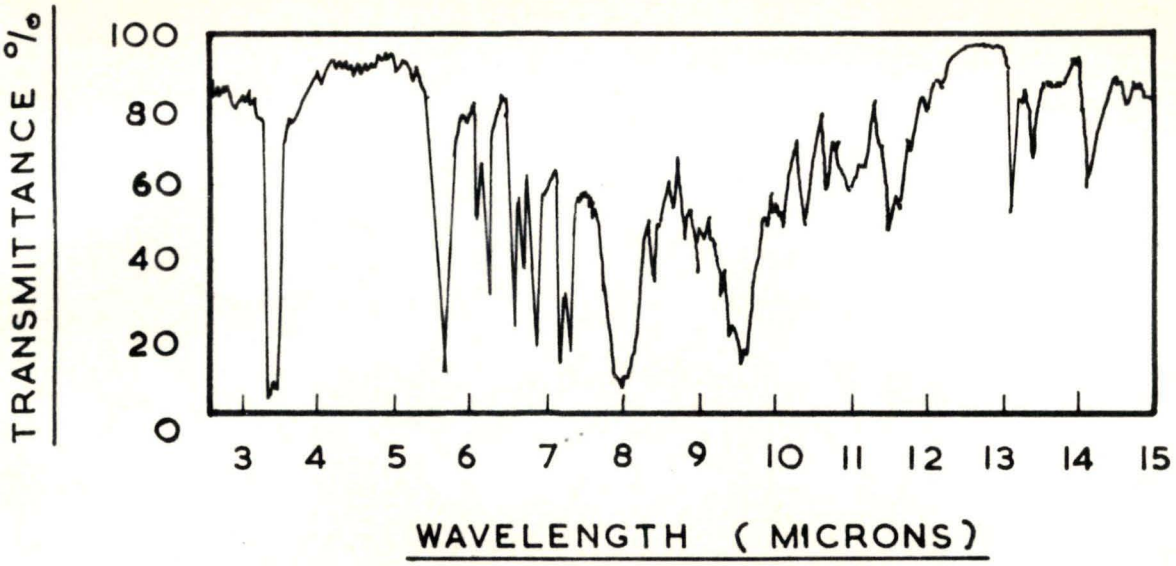
β - D - GLUCOPYRANOSIDE



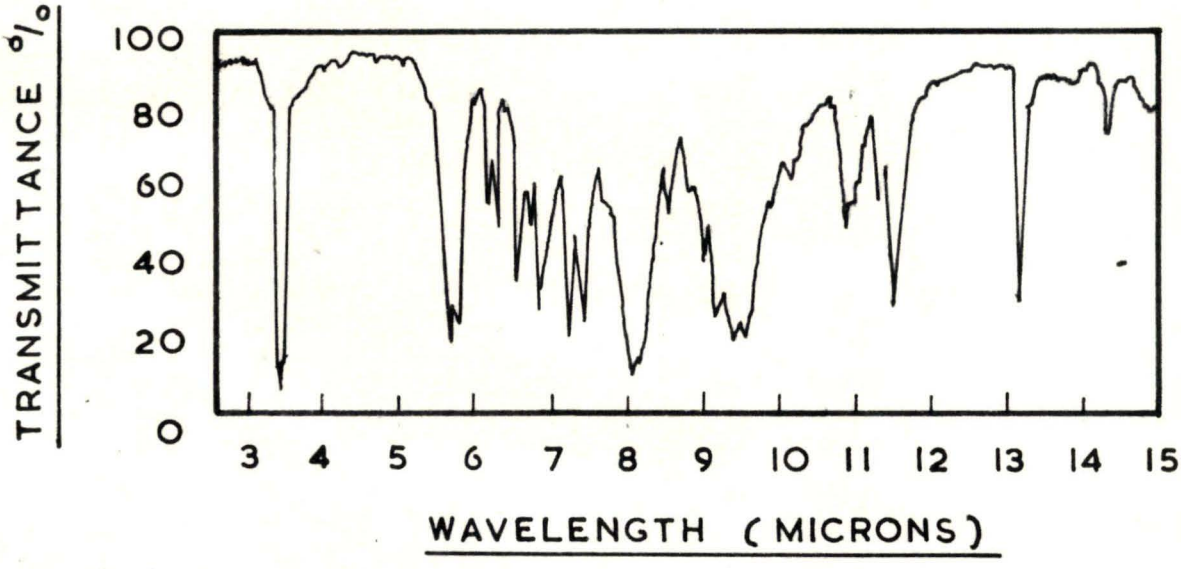
PARA-PHENYL-PHENYL TETRA-O-ACETYL
∝-D-GLUCOPYRANOSIDE



PARA-PHENYL-PHENYL TETRA-O-ACETYL
β-D-GLUCOPYRANOSIDE



PARA - NITRO - PHENYL TETRA - O - ACETYL
∝ - D - GLUCOPYRANOSIDE



PARA - NITRO - PHENYL TETRA - O - ACETYL
β - D - GLUCOPYRANOSIDE

These studies reveal that except p-nitro phenyl- for -D-gluco-pyranoside, all of the aryl-α -D-glucopyranosides studied absorb in the narrow region of 11.50 - 11.55μ, which is very near to the region assigned to the sugars having α -D-configuration by earlier workers. The β -anomers do not have any band in this region. Another characteristic low intensity band at 8.70μ , not mentioned by earlier workers has been observed by us for the α -anomeric glucosides. This band is again absent in the spectra of the β -anomers.

Spectra of anomeric p-nitrophenyl-D-glucopyranosides were exceptional. The band at 11.55 was present in the spectra of both of the anomers while the minor band at 8.70μ was also not seen in the case of both the anomers. The α -anomer showed an additional high intensity band at 13.90μ where the β -anomer did not have any absorption. The presence of the nitro group appears to be responsible for this anamolous behaviour.

The observation of House and Whistler[22] about the characteristic region of absorption of the β -phenyl glycosides in the 12.15 - 12.25 region could not be substantiated for any of the β -anomeric glucosides studied by us except for phenyl β -D-glucopyranoside which showed a weak absorption band at 12.15μ. The infrared spectral studies of the acetates of alkyl glycosides were made by Barker et. al.[20,21]. They observed that the acetates of the α -anomeric alkyl glycosides also absorb in the same region of 11.75 - 12.10μ characteristic of the α -anomers. They did not assign any band for the acetates of β -anomeric alkyl glycosides.

In our studies of acetates of aryl- α -D-glucosides, this observation could not be substantiated. Both of the anomeric glucosides in the series were found to have absorption bands in the 11.75 - 12.10 μ region and, therefore, they were not of any diagnostic value.

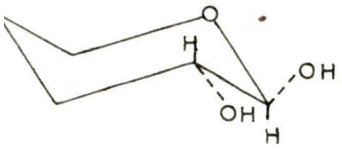
We observed that the acetates of the α -D-glucopyranosides had a characteristic absorption band in the narrow region of 10.40 - 10.45 μ not shown by the corresponding β -anomers. This band has not been reported earlier. Exceptional behaviour was, however, observed for acetates of the o-substituted aryl-D-glucosides as both of the anomers showed absorption band in this region. Therefore, this region of 10.40 - 10.45 is of diagnostic value for acetylated aryl-α-D-glucosides in which the aryl group is not o-substituted.

PMR spectroscopy

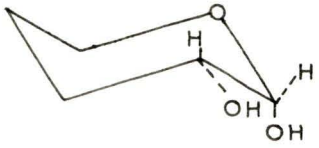
Proton magnetic resonance spectroscopy is a powerful physicochemical tool which has found to be immensely useful in recent years for the investigation of the structures of organic molecules in respect of the location and disposition of various hydrogen atoms present in the molecule[24].

One of the main features in the determination of a fine structure by PMR spectra pertains to the coupling of spin of a hydrogen with that of the neighbouring hydrogen atoms. The coupling is pronounced for protons situated on adjacent carbon atoms and

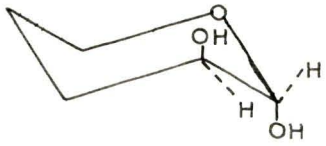
becomes weak with the increase in the distance between the two protons. Coupling between two protons gives rise to a doublet signal and that among three protons signals a quadruplet or a triplet. The splitting of a signal is expressed in terms of coupling constant (J) in cycles per second (cps). For two protons situated on adjacent carbon atoms such as C(1) and C(2) of a carbohydrate molecule, the following spatial arrangements are possible:



V. DI-AXIAL



VI. EQUATORIAL-AXIAL

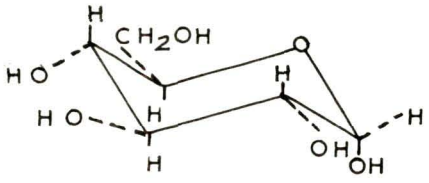


VII. DI-EQUATORIAL

The coupling constant of the two di-axial protons as in (V) is considerably greater than those between the two protons in equatorial-axial or di-equatorial dispositions as in the (VI) and (VII) respectively[1,2].

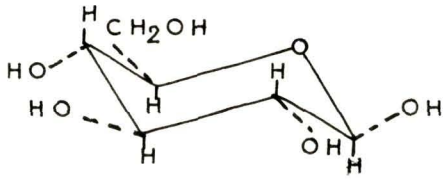
These facts are nicely illustrated from the values of coupling constants of two anomeric sugars such as α-D-glucopyranose (VIII) and β-D-glucopyranose (IX). In the former the two ring protons at C(1) and C(2) are in equatorial-axial disposition and show a coupling constant, J = 3 cps only, whereas in β-D-

glucopyranose these two protons are in di-axial-disposition and show a coupling constant J = 8 cps is quite high. Similar PMR data obtained for many sugars and sugar derivatives are in complete agreement with these findings.[25-30]



α - D - GLUCOPYRANOSE

VIII

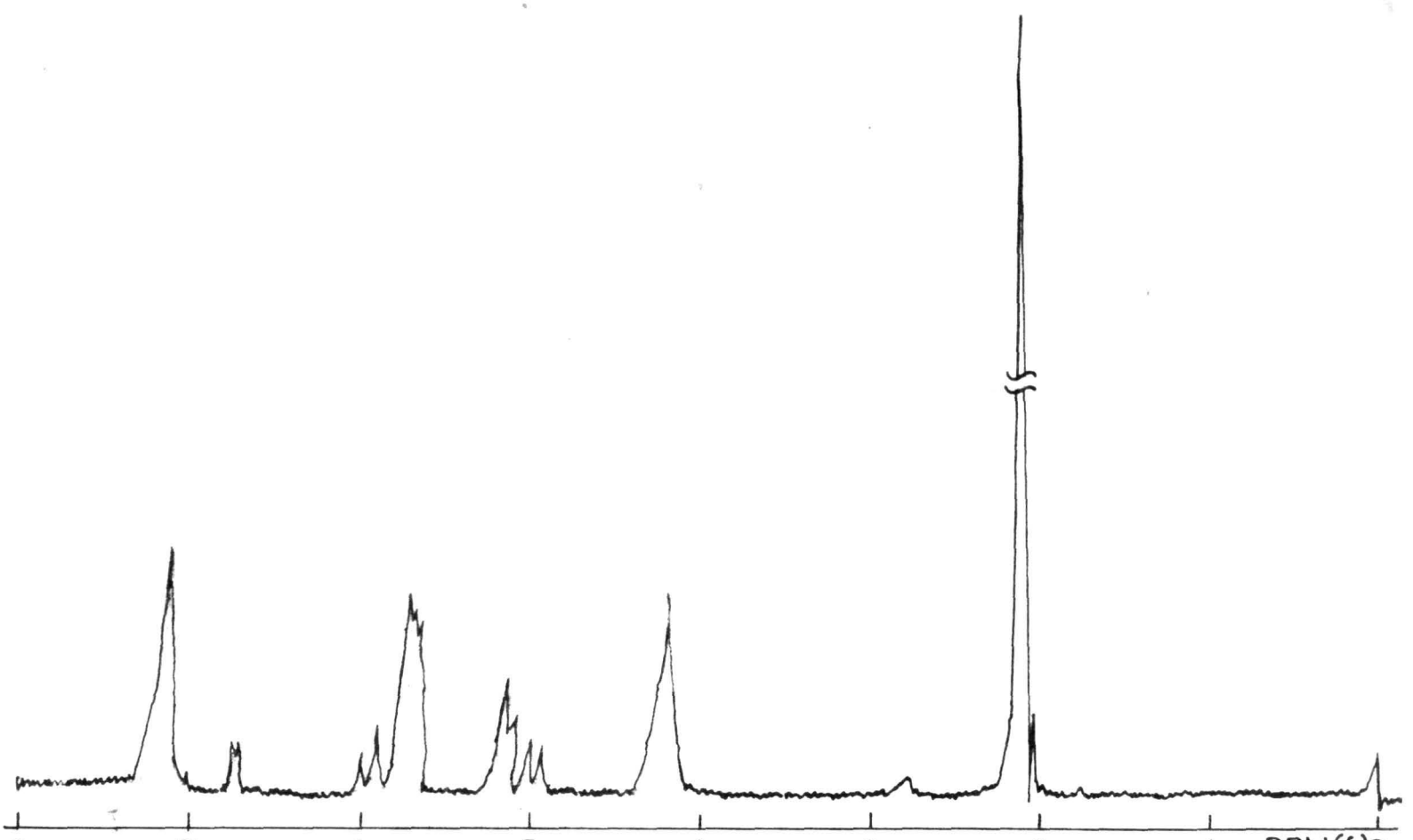


β - D - GLUCOPYRANOSE

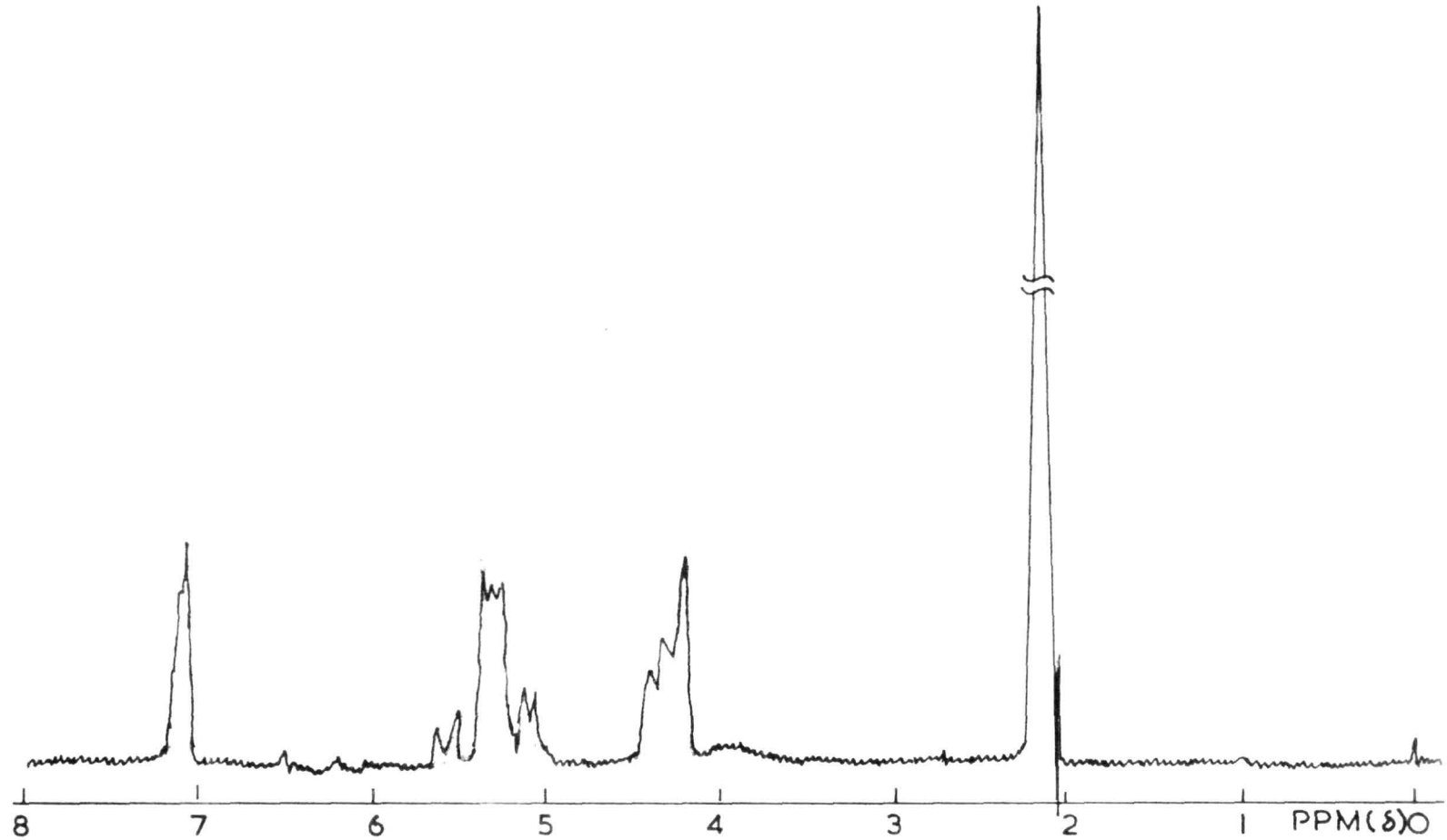
IX

The resonance frequencies of nuclei in different structural environments are different and are expressed in terms of chemical shifts (δ) in either parts per minute (ppm) or tau (τ) scale. A proton has a definite chemical shift and therefore its signal appears at a particular place in the spectrum. Thus, aromatic protons give signals around 7.2 ppm. Chemical shift of a proton is markedly influenced by a change in the electronegativity around it; an increase in electronegativity shifts the signal towards the lower field, whereas, a decrease shifts the signal towards the higher field.

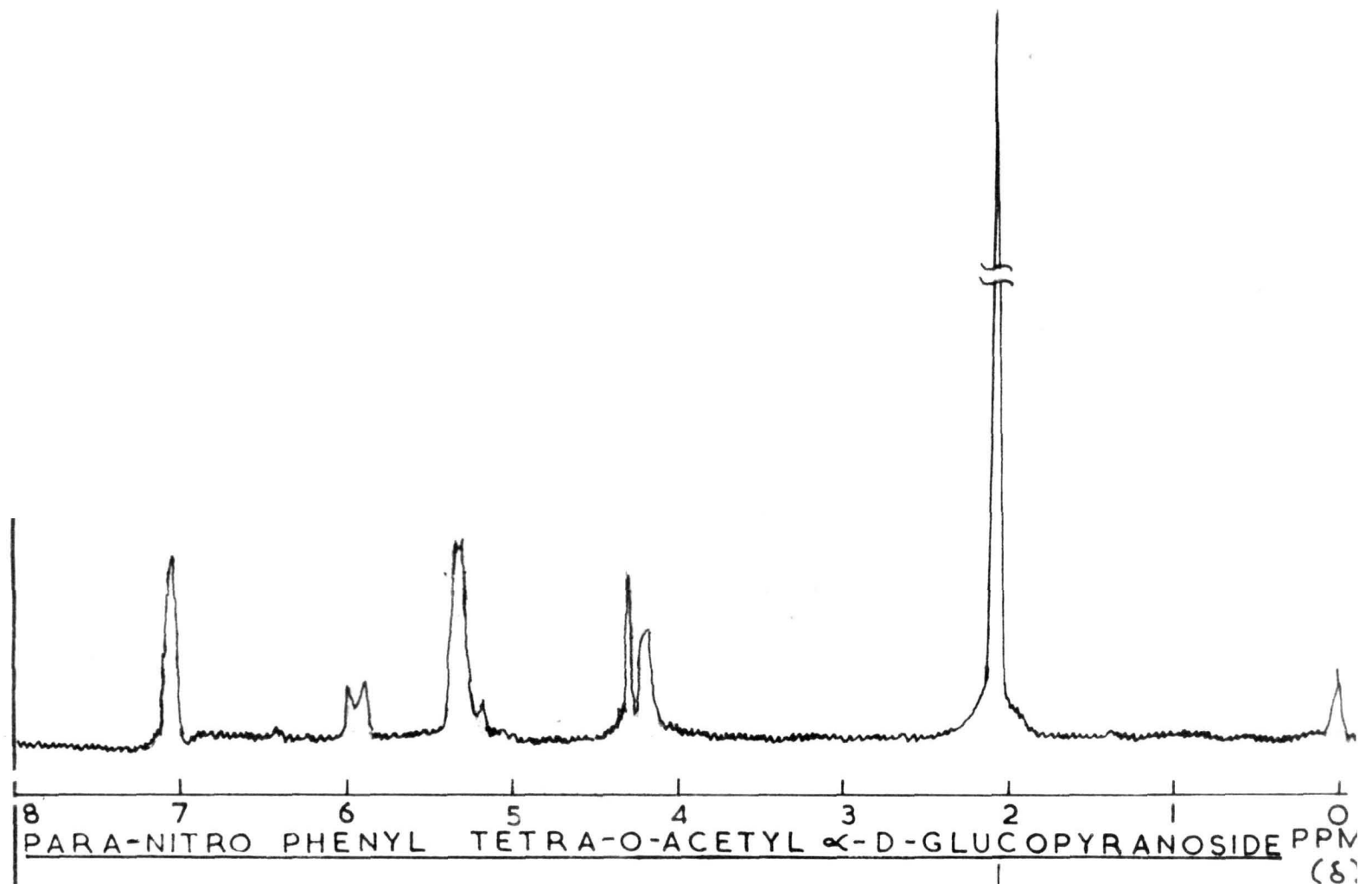
In carbohydrates, the proton at C(1) is the only proton attached to a carbon situated between two oxygen atoms. This proton has, therefore, a more electronegative surrounding than the other



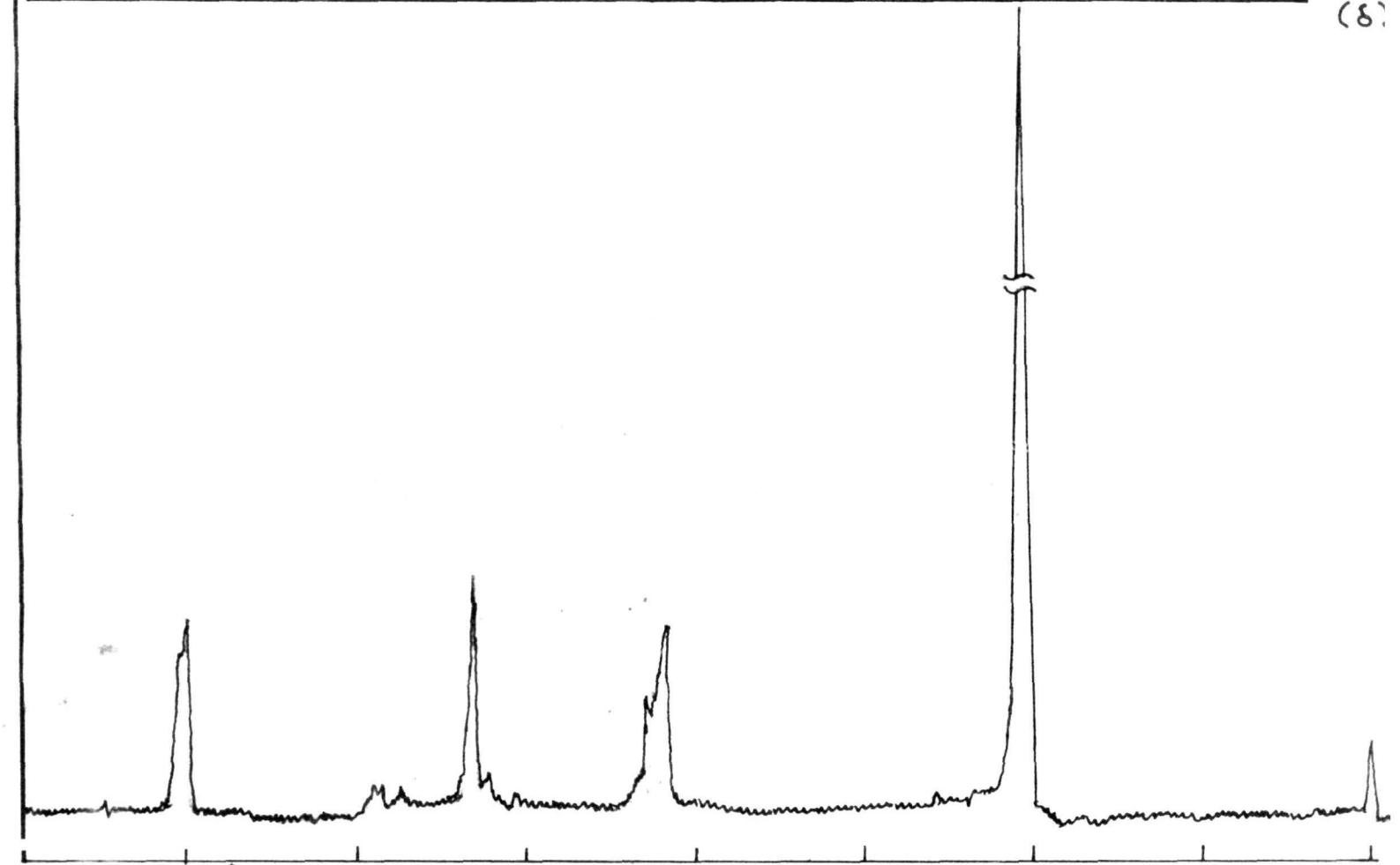
PARA-CHLORO PHENYL TETRA-O-ACETYL-α-D-GLUCOPYRANOSIDE



PARA-CHLORO PHENYL TETRA-O-ACETYL β-D-GLUCOPYRANOSIDE



PARA-NITRO PHENYL TETRA-O-ACETYL α-D-GLUCOPYRANOSIDE



PARA-NITRO PHENYL TETRA-O-ACETYL β-D-GLUCOPYRANOSIDE

protons in the molecule and, therefore, its signal appears at a lower field than those of other ring protons. The environmental influence of the group attached to C(1) may often shift the C(1) proton signal to such an extent that it can no longer be distinguished from the signals of other protons. Thus, the C(1) proton signal in the PMR spectra of α-D-glucopyranose and methyl-α-D-glucopyranoside in dimethyl sulphoxide and of aryl tetra-O-acetyl-β-D-glucopyranosides in $CDCl_3$ are eclipsed by other proton signals[27,28].

The first attempt to distinguish between anomeric acetates of aryl glycosides by PMR spectroscopy was made in 1967 by Tanaka and Tsuzuki[27]. They found that none of the many acetates of β-anomeric aryl glycosides studied by them gave any recognisable H_1 signal. Out of four acetates of aryl-α-glycosides studied by them only two gave distinct H_1 signals.

We studied the spectra of two pairs of acetates of aryl-α-D-glucosides none of which gave any distinct H_1 signal. It was thought of interest, therefore, to study the PMR spectra of the free aryl-α-D-glucosides to ascertain whether any distinct H_1 signals of diagnostic value are given by them.

For these studies we used dimethyl sulphoxide (DMSO) as the solvent, since the aryl glucosides were not readily soluble in D_2O. Although DMSO, as a PMR solvent, suffers from the shortcomings that it exhibits its own proton signals over a wide range below 4 ppm and thus, permits only the region beyond 4 ppm for the study of the proton signals of the solute, it is known however, that the C(1) proton

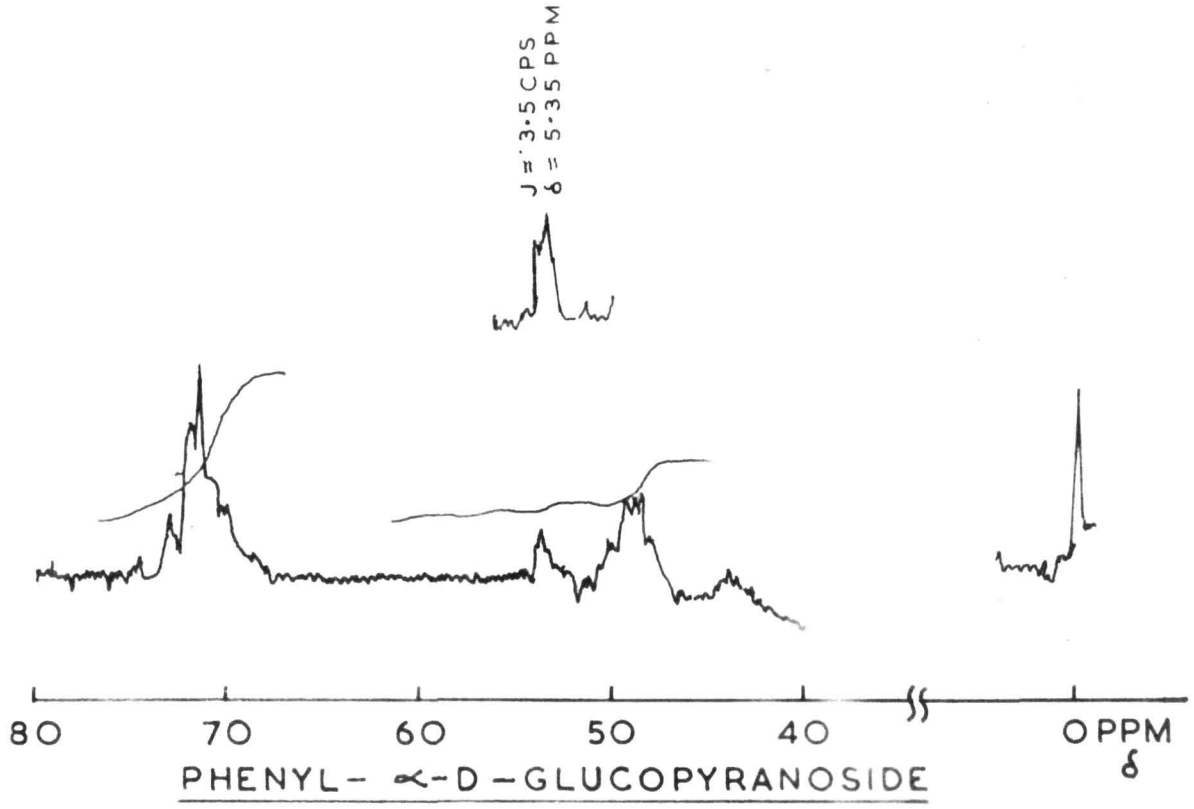
signal of methyl- β -D-glucopyranoside just narrowly escaped being eclipsed by the DMSO proton signals. The presence of an aryl group at C(1) was expected to shift the C(1) proton signal downfield due to anisotropic effect[24,27] so that DMSO was still expected to be useful for the present studies.

This was fully substantiated by the PMR spectra of seven aryl α-D-glucosides studied in the course of the present work. The chemical shift and coupling constant values of the α-glucosides are given in Table II.

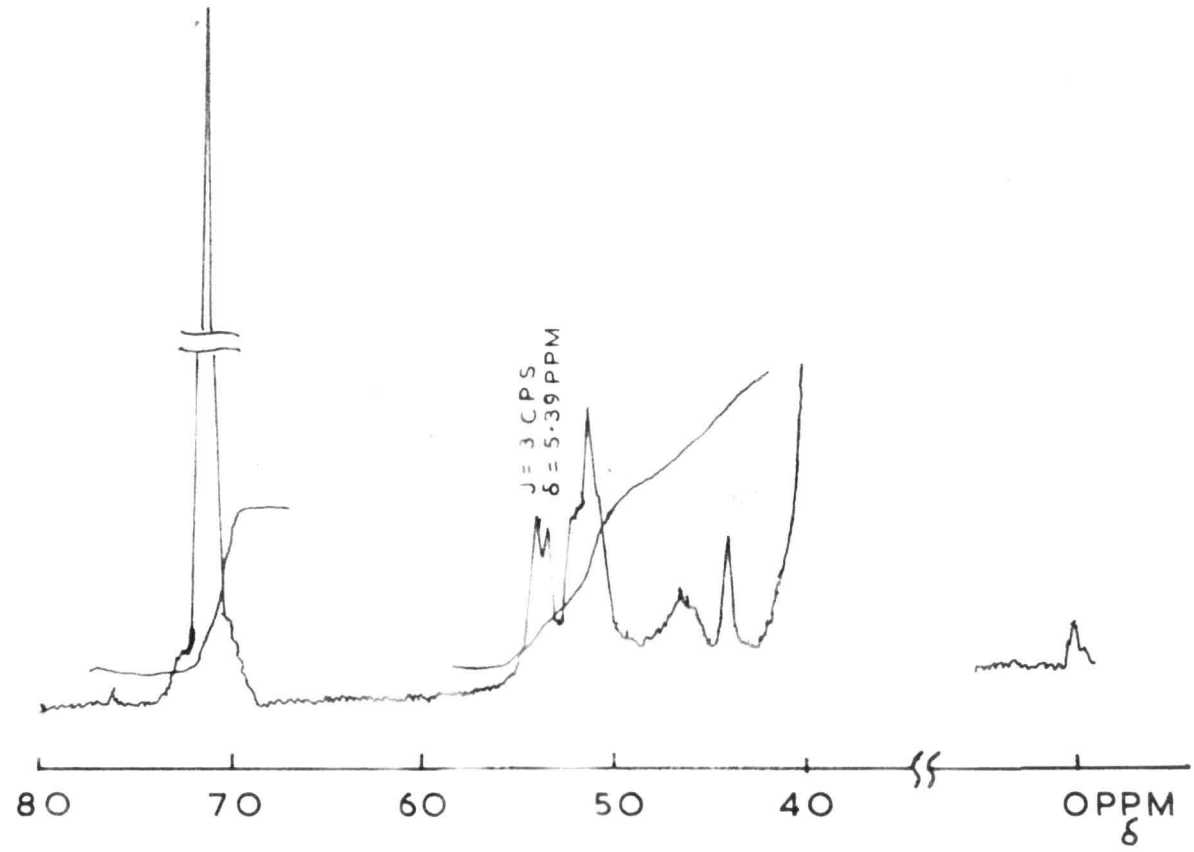
TABLE II

Proton magnetic resonance spectral data of aryl-α-D-glucopyranosides

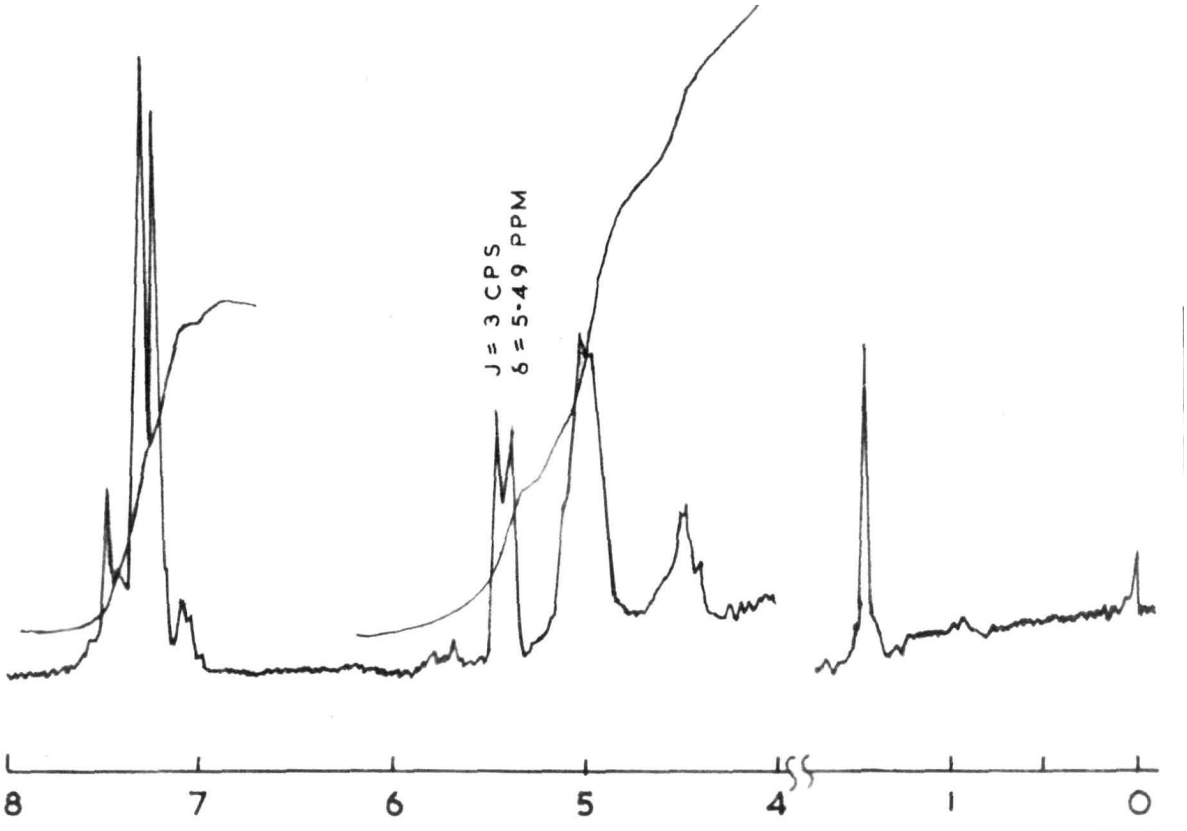
S. No.	Name of the compound	Characteristics of the anomeric proton	
		Coupling constant (J) cps	Chemical shift ( ) ppm
1	Phenyl- α -D-glucopyranoside	3.5	5.35
2	p-Methylphenyl- α -D-glucopyranoside	3.0	5.39
3	p-Chlorophenyl- α -D-glucopyranoside	3.0	5.40
4	p-Nitrophenyl- α -D-glucopyranoside	3.0	5.65
5	p-Phenyl phenyl- α-D-glucopyranoside	3.0	5.45
6	o-Chlorophenyl- α -D-glucopyranoside	3.0	5.59
7	o-Methylphenyl- α -D-glucopyranoside	3.0	5.38



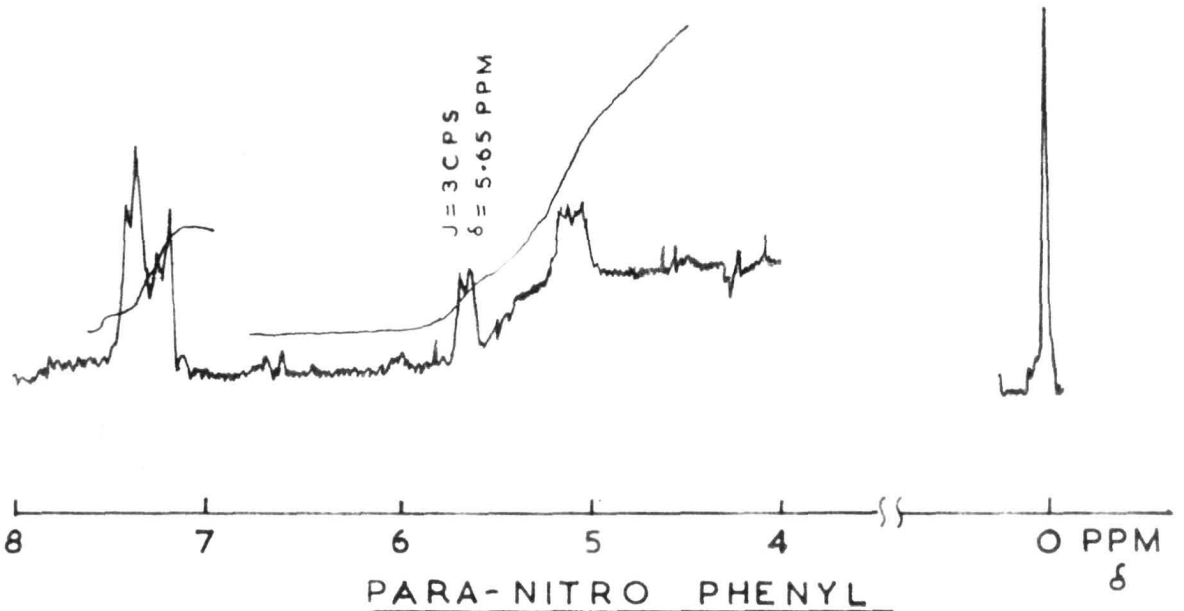
PHENYL- ∝-D-GLUCOPYRANOSIDE



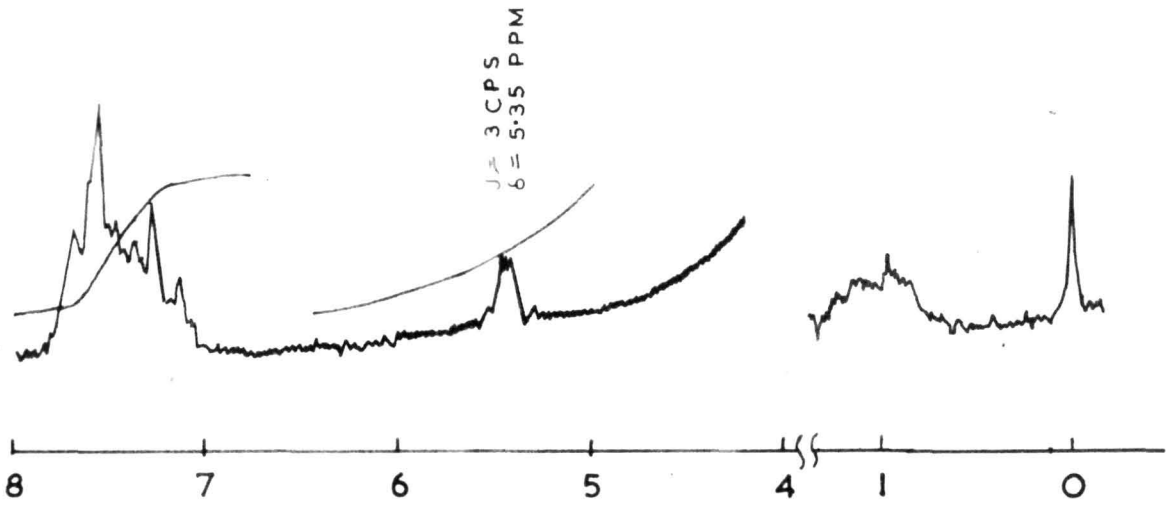
PARA-METHYL - ∝-D-GLUCOPYRANOSIDE



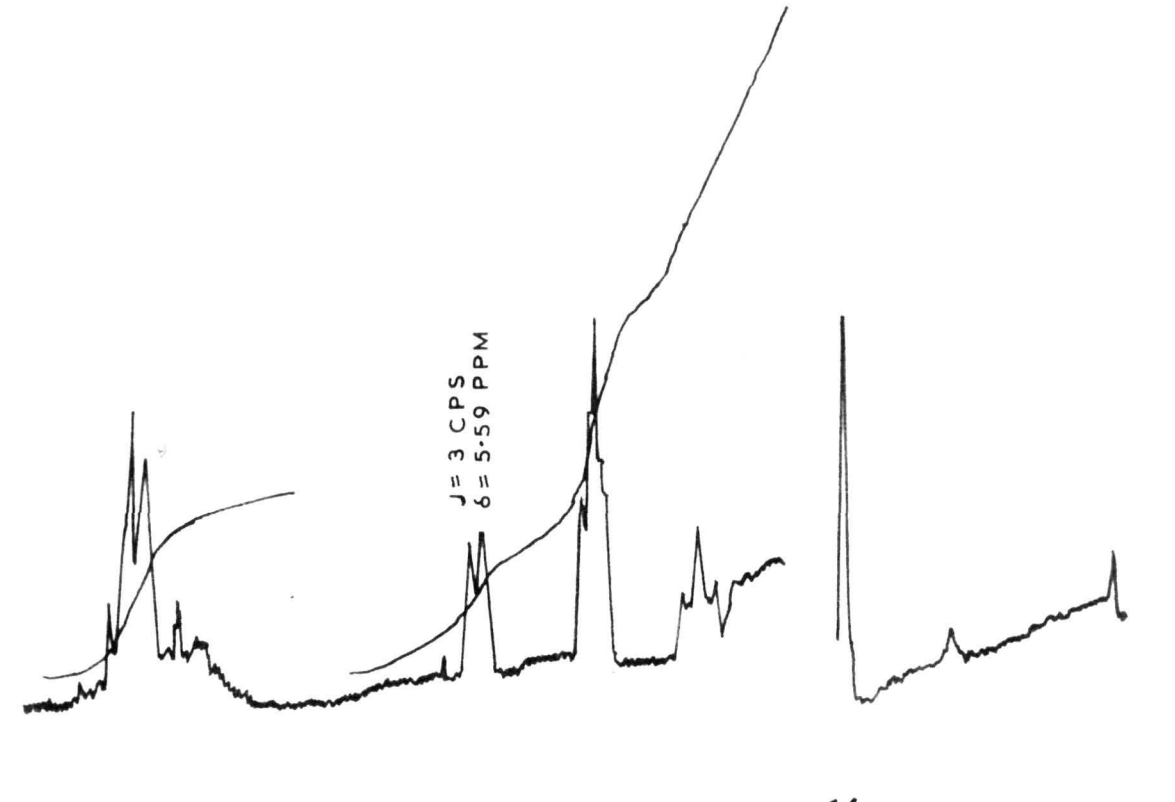
PARA-CHLORO PHENYL
α - D - GLUCOPYRANOSIDE



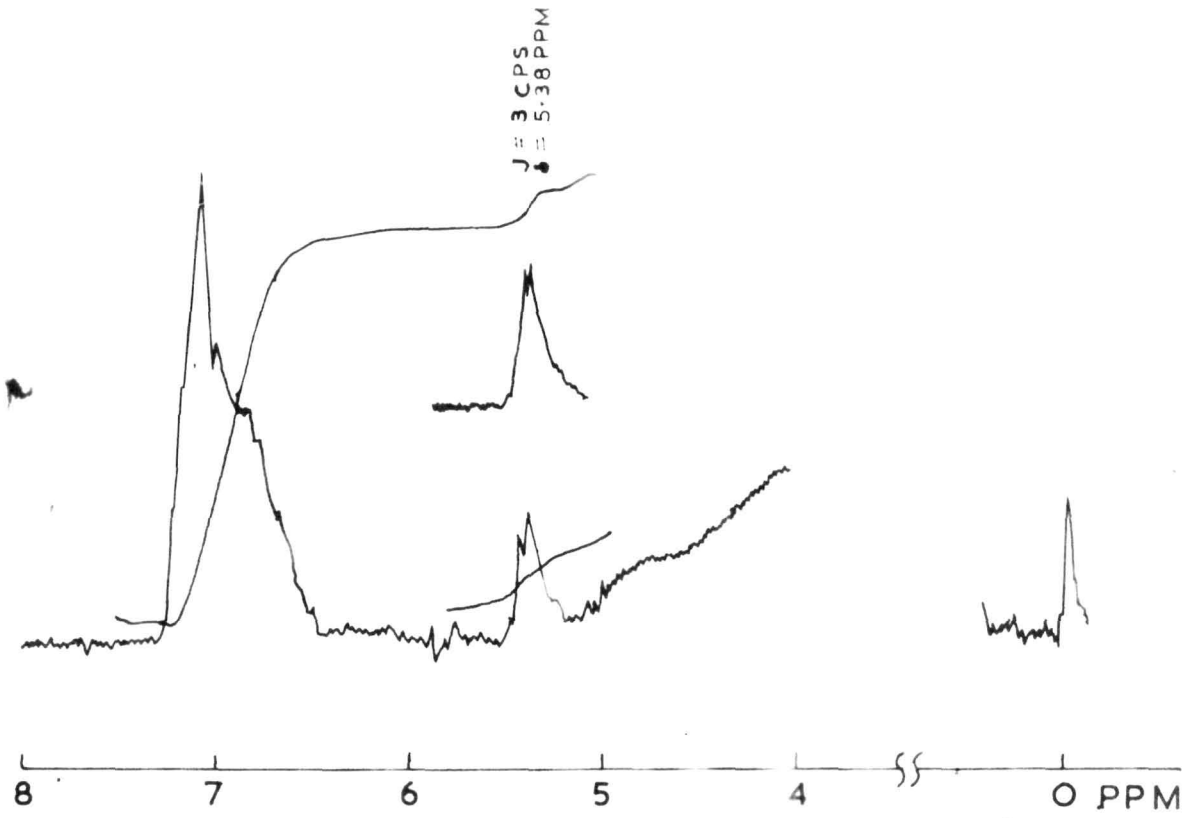
PARA-NITRO PHENYL
α - D - GLUCOPYRANOSIDE



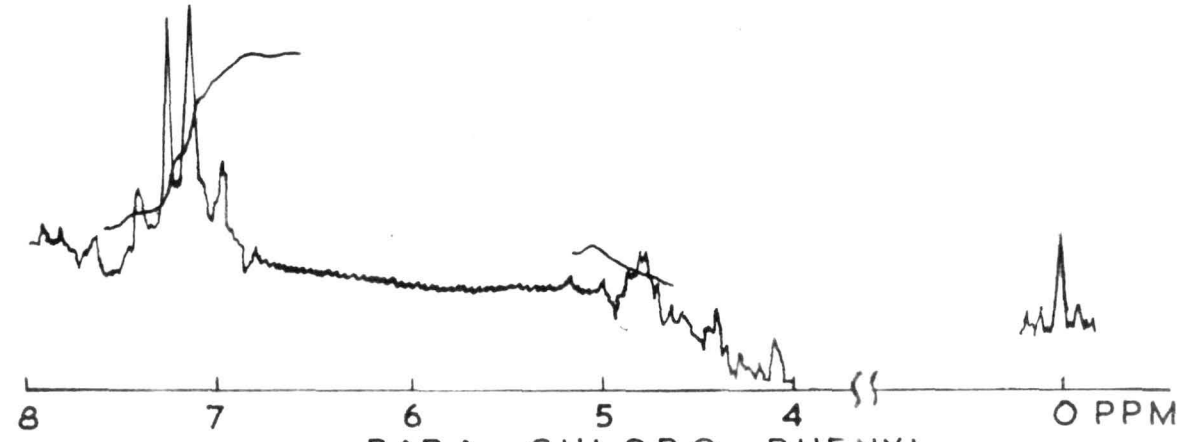
PARA-PHENYL PHENYL
∝-D-GLUCOPYRANOSIDE



ORTHO-CHLORO PHENYL
∝-D-GLUCOPYRNOSIDE



ORTHO - METHYL PHENYL
α - D - GLUCOPYRANOSIDE



PARA - CHLORO PHENYL
β - D - GLUCOPYRANOSIDE

The H_1 signals of all of these α-D-glucosides appeared as doublets having a characteristic coupling constant J = 3 cps and were situated in a short range of 5.35 to 5.65 ppm.

Aryl-β-D-glucosides, on the other hand, have the protons at C(1) and C(2) in axial-axial dispositions and, therefore, are expected to give signals in the region shifted 50 cps upfield in the region nearabout 4.85 to 5.15 ppm. These signals therefore fall in the region of hydroxyl proton signals in the range of 4.48 to 4.95 ppm[26] as well as the H_2, H_3 and H_4 signals with which it overlaps and are, therefore, not of any diagnostic value.

An exceptional behaviour was observed in the case of p-phenyl phenyl tetra-O-acetyl-α-D-glucopyranoside in which the H_1 signal appeared as a triplet or unresolved quartet at 5.45 ppm. This would indicate α long range coupling[25] with the C(3) proton or an aromatic proton of the diphenyl group. The chemical shift however, is consistent with the aryl-α-D-glucopyranoside structure discussed earlier. The question of pyranose ring going to IC conformation from usually stable Cl conformation was also considered, but rejected as the chemical shift of H_1 was not in conformity with the IC structure.

Mass spectrometry

The usefulness of mass spectrometry as a technique for the determination of structure and stereochemistry of a wide variety of organic molecules has been demonstrated in recent years[31-36]. The first application of this technique in the field of carbohydrates was reported by Reeds et. al.[37] in 1958. Biemann[38], in 1963, demonstrated for the first time the usefulness of mass spectrometry in the elucidation of structure and stereochemistry of fully acetylated sugars and acetylated methyl glycosides. This technique has been widely used in recent years for the determination of structures and, in a few cases, stereochemistry of various sugars and sugar derivatives[39-44].

Kochetkov et. al.[45] have employed successfully the mass spectrometric analysis for the identification of partially methylated sugars which are essential for structural analysis of complex polysaccharides. Heyns et. al.[46] employed this technique for the identification of stereoisomers and the size of rings of permethylated pentoses. Positions of groups such as amino[42], isopropylidene[44] and di-ethylene di-thio acetal[43] in substituted sugar molecules have been recently assigned from mass spectral studies. The structures of some isopropylidene sugars have been assigned on the basis of their mass spectral fragmentation patterns[43,44].

Not much attention has been paid so far on the mass spectral studies of the anomeric configurational and stereochemical aspects

of the glycosidic linkage. In 1958, Reed et. al.[37] measured the appearance-potential of $C_6H_{11}O_5^+$ ion derived from α-and β-methyl glucopyranosides and observed a slightly lower appearance-potential in the case of the α-anomer. This was considered to have resulted from a weakening of the α-glycoside bond as compared with that of the β-glycoside. In view of the small differences reported and the difficulties inherent in the determination of accurate appearance-potentials, the technique seems to be of little practical applicability for assignment of anomeric configurations.

Pearl and Darling[47] studied the mass spectral fragmentation of acetylated sugars and acetylated glycosides and observed that the primary fragment is generated by detachment of the substituent at C(1) and no molecular ion peak was obtained. Haslam[48] also examined the mass spectra of some acetylated glucosides with a view to elucidate their structures.

In his studies of the fragmentation patterns of the anomeric penta-O-acetyl-D-glucopyranoses, Biemann[38] observed appreciable differences in the intensities of the M-59 i.e. M-CH_3COO ion (m/e 331). This difference was caused by a crowding of the heavy groups in the α-anomer which causes the α-glycosidic linkage to be less stable to electron impact as compared to the β-glycosidic linkage, although α-glycosidic bond is regarded as more stable chemically.

This observation has been confirmed by us from a study of the mass spectral fragmentation pattern of four pairs of anomeric acetylated aryl glucosides. The fragmentation patterns observed are detailed in Table III. These patterns are in general agreement with those obtained by Haslam [48] for the acetylated aroyl aryl glucosides.

TABLE III

Mass spectral data of aryl tetra-O-acetyl-D-glucopyranosides

m/e	Percentage intensities of the peaks of aryl-D-glucosides							
	I	II	III	IV	V	VI	VII	VIII
1	2	3	4	5	6	7	8	9
332	11.2	6.8	8.1	6	11.3	7.2	56.6	5.4
331	78	48	46	40	82.6	67.2	41	32
229	-	7	-	-	5	5	-	-
211	-	7	-	-	-	-	5	-
200	-	15.2	-	-	-	-	-	-
187	-	7.2	-	-	-	-	-	-
170	8.5	8.2	10	75	10.8	10	75	65
169	100	100	100	100	100	100	100	100
158	-	8.4	-	-	-	-	-	-
157	-	30	-	-	-	-	-	-
145	8.1	18.8	8.3	5.2	7.4	7	5.2	6.5
140	-	-	-	13.3	-	11.5	13.3	14
139	8.1	13.4	10	13	7.2	10	13	11.2
131	-	-	-	-	6	7	-	-
128	-	9	-	-	-	-	-	-
127	21.4	24	-	20	20	28.6	20	20
115	8.5	58	10	13.3	6	7.8	16	10
109	70	52	84.7	60	43.4	70	60	42.5
103	5.6	23.6	6.9	-	8.5	6	-	-
98	8.2	31	-	8.3	-	-	-	-

Contd...

TABLE III (Contd)

1	2	3	4	5	6	7	8	9
97	-	22	9	-	6	9.3	-	7.4
94	38.1	22	-	-	-	-	-	-
81	5.5	13.8	7.1	6.6	-	7	-	6
73	-	12.4	-	-	-	-	-	-
69	-	8.2	-	-	-	-	-	-
42	45.4	>100	>112	>100	>100	147	89	169

List of the aryl tetra-O-acetyl-D-glucopyranosides

I Phenyl tetra-O-acetyl- α-D-glucopyranoside

II Phenyl tetra-O-acetyl- β-D-glucopyranoside

III p-Chlorophenyl tetra-O-acetyl- α-D-glucopyranoside

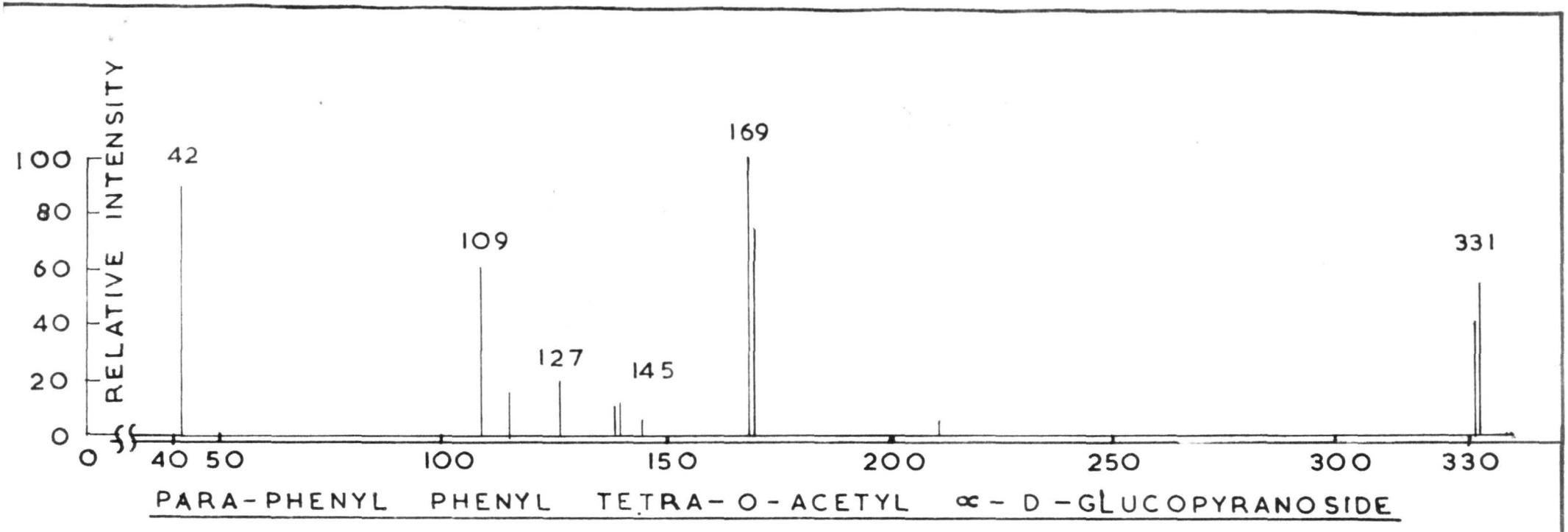
IV p-Chlorophenyl tetra-O-acetyl- β-D-glucopyranoside

V p-Nitrophenyl tetra-O-acetyl- α-D-glucopyranoside

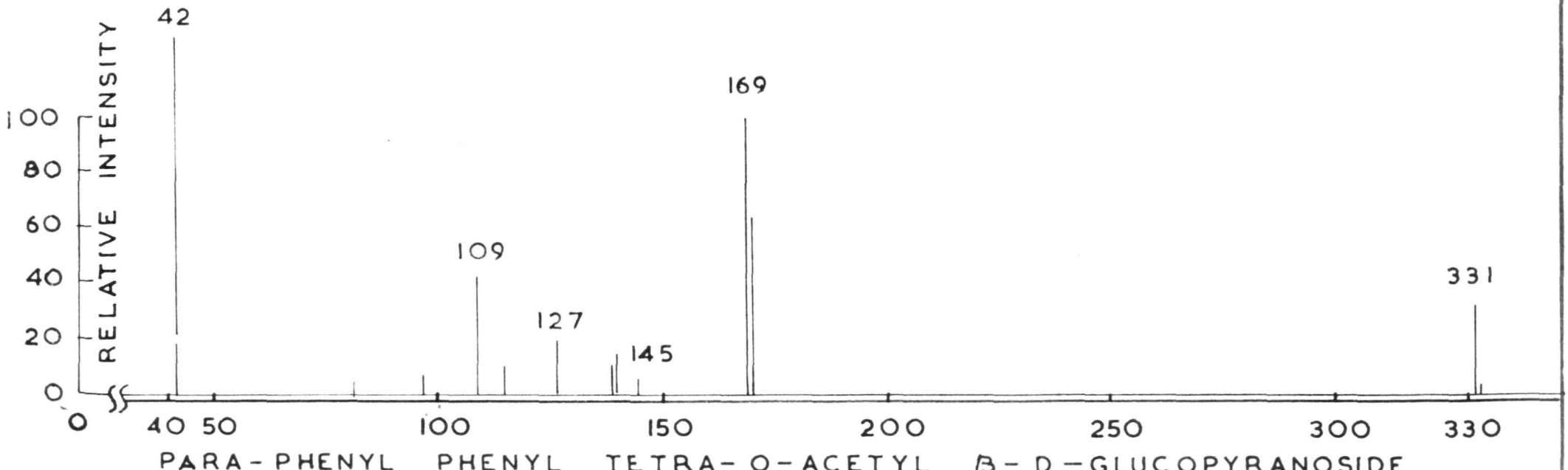
VI p-Nitrophenyl tetra-O-acetyl- β-D-glucopyranoside

VII p-Phenyl phenyl tetra-O-acetyl- α-D-glucopyranoside

VIII p-Phenyl phenyl tetra-O-acetyl- β-D-glucopyranoside



PARA-PHENYL PHENYL TETRA-O-ACETYL α-D-GLUCOPYRANOSIDE



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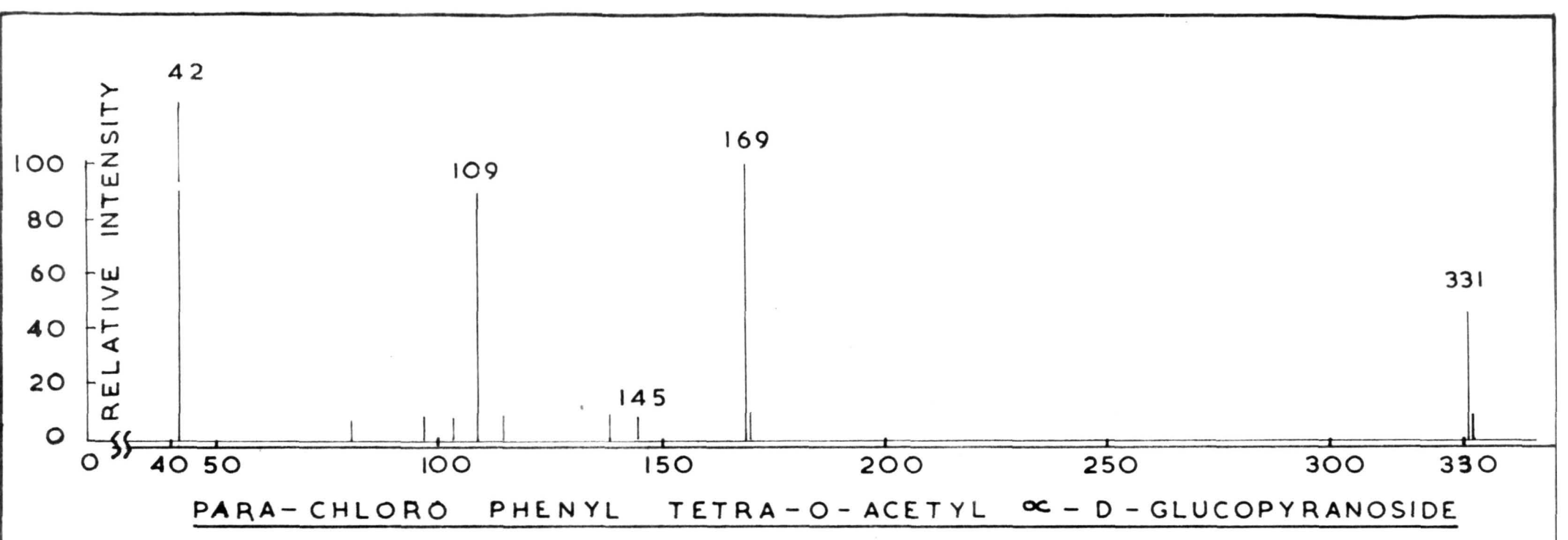
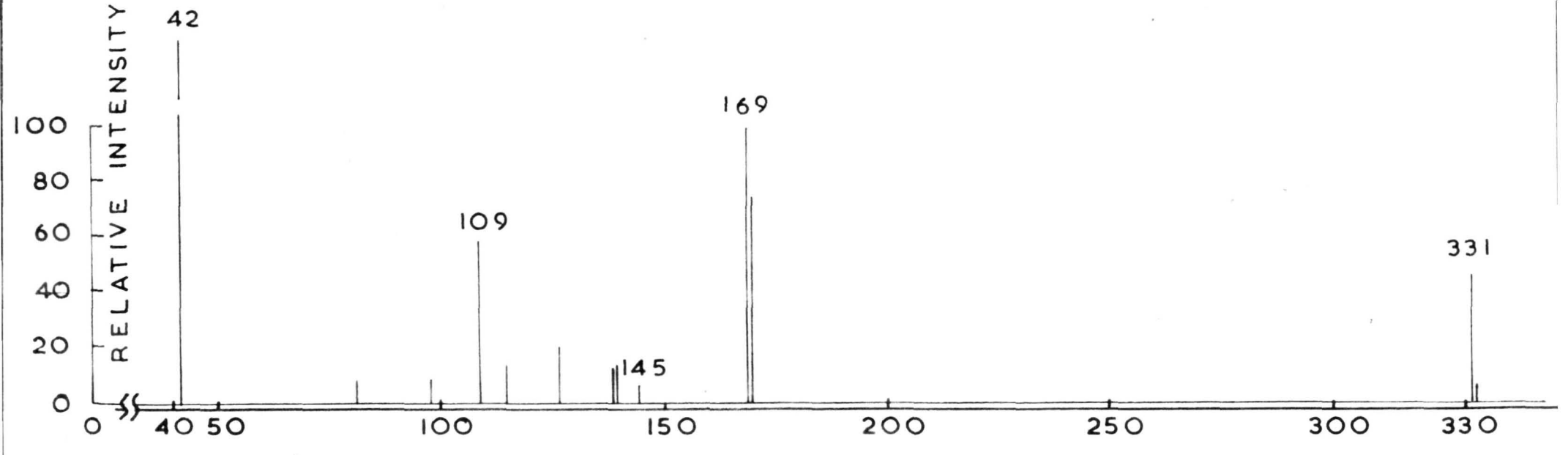


PARA-CHLORO PHENYL TETRA-O-ACETYL ∝-D-GLUCOPYRANOSIDE



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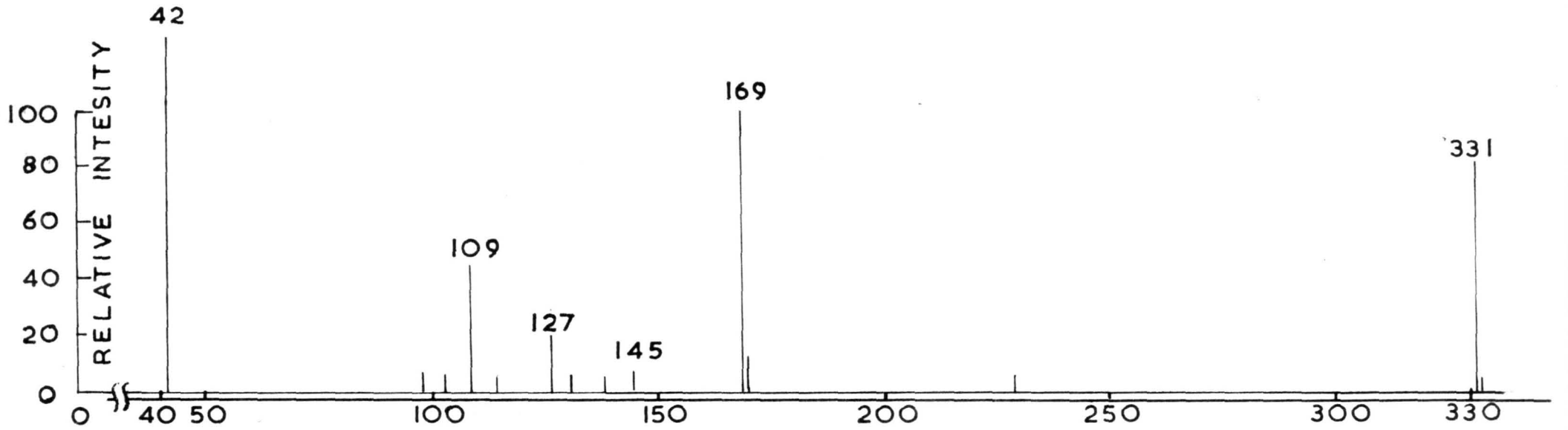
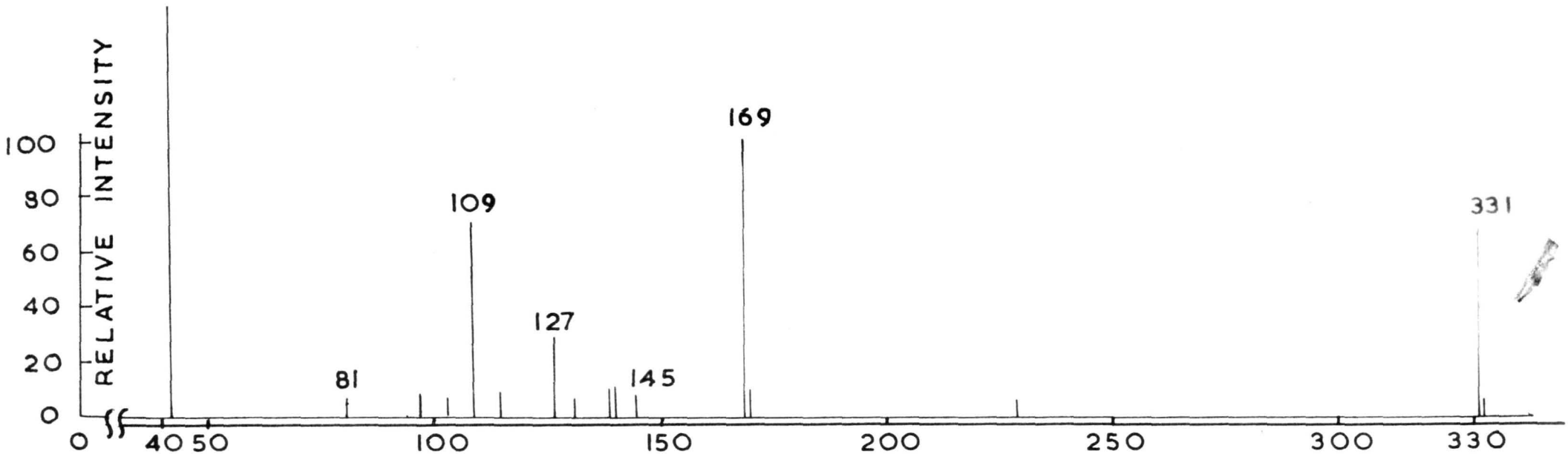
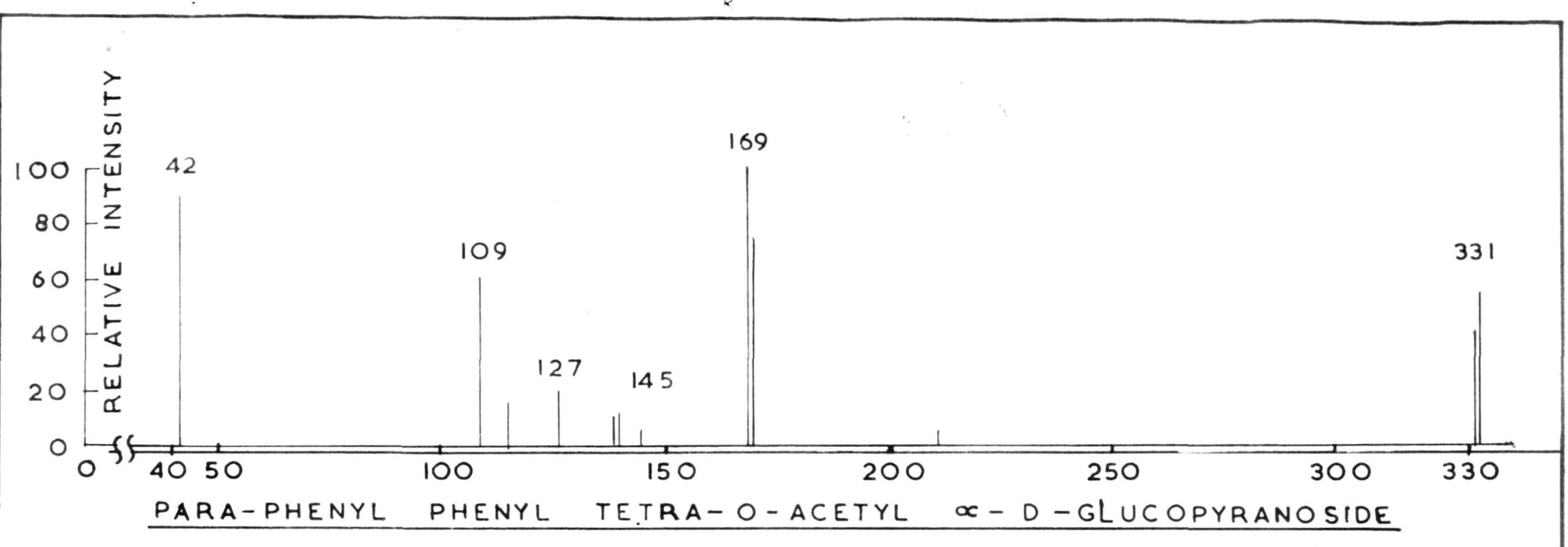


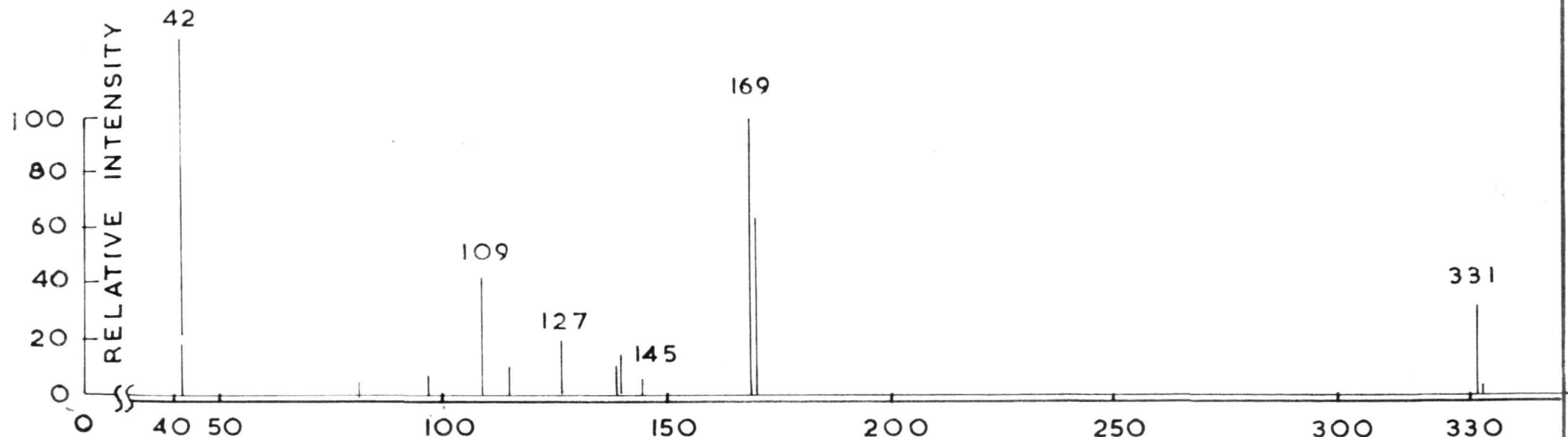
PARA — NITRO PHENYL TETRA — O — ACETYL ∝ - D — GLUCOPYRANOSIDE



PARA - NITRO PHENYL TETRA - O - ACETYL β - D - GLUCOPYRANOSIDE



PARA-PHENYL PHENYL TETRA-O-ACETYL ∝-D-GLUCOPYRANOSIDE



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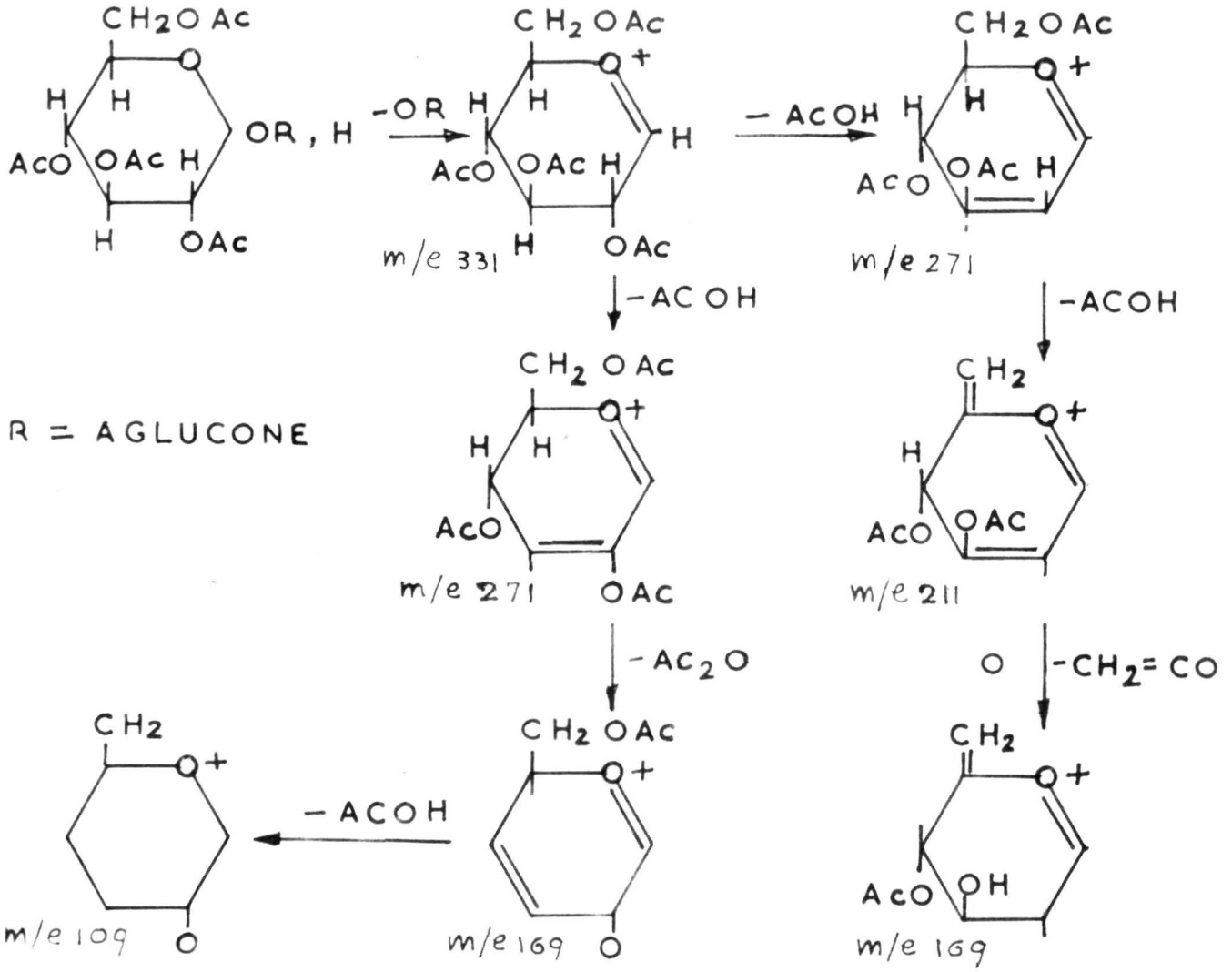
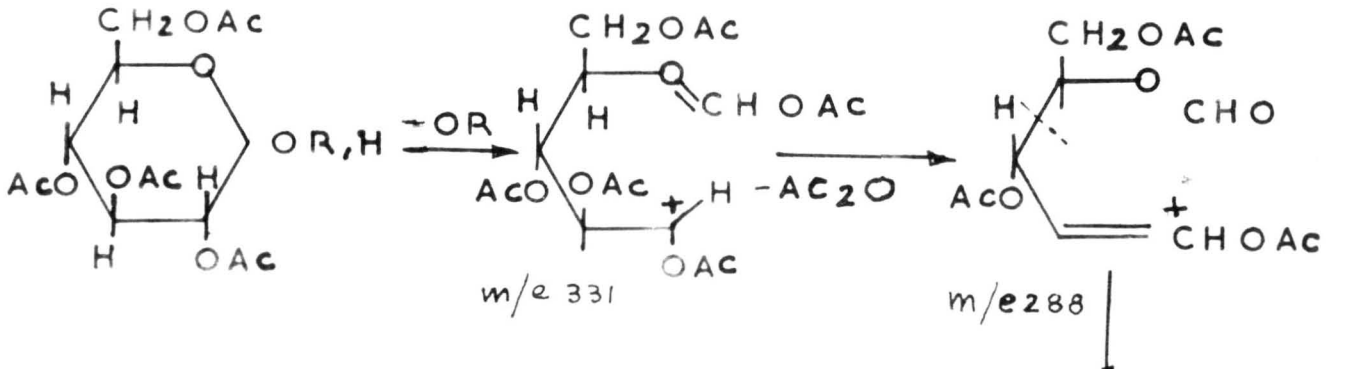


SCHEME - A



$AC-O-CH=CH-\overset{+}{C}H-OAc$ + $AcOH_2C-CH=O-CHO$

m/e 157 m/e 131

$AcO-\overset{+}{C}H=CH-OH$

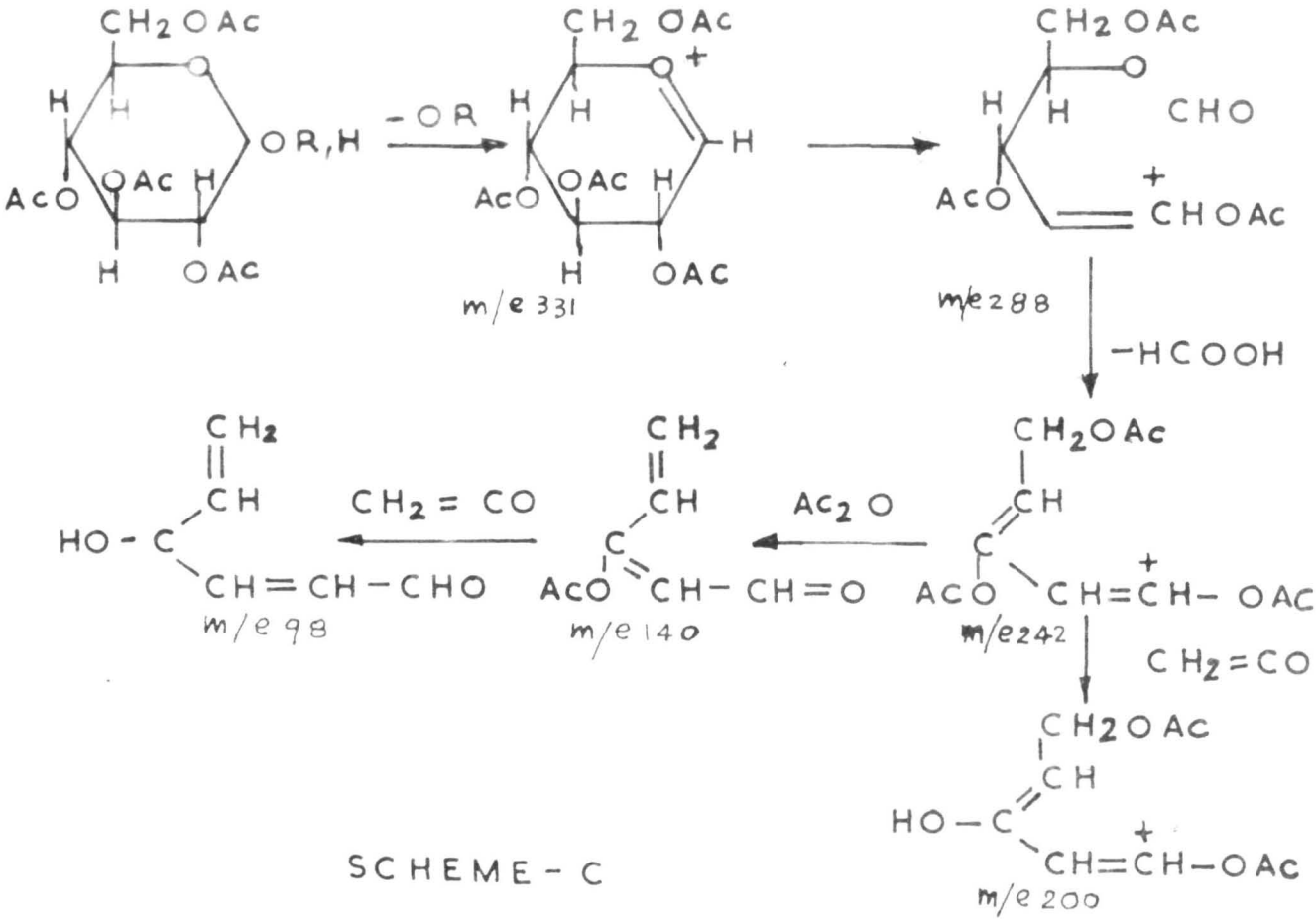
m/e 115

$HO-CH=CH-OH$

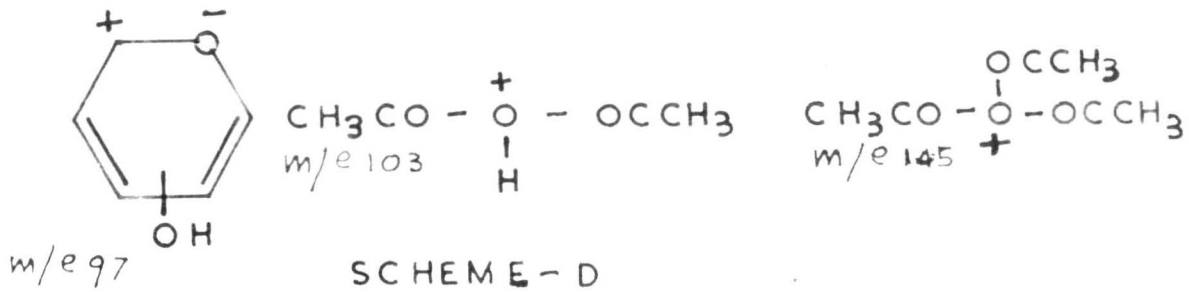
m/e 78

SCHEME - B

FRAGMENTATION OF ARYL TETRA - O - ACETYL D-GLUCOPYRANOSIDE

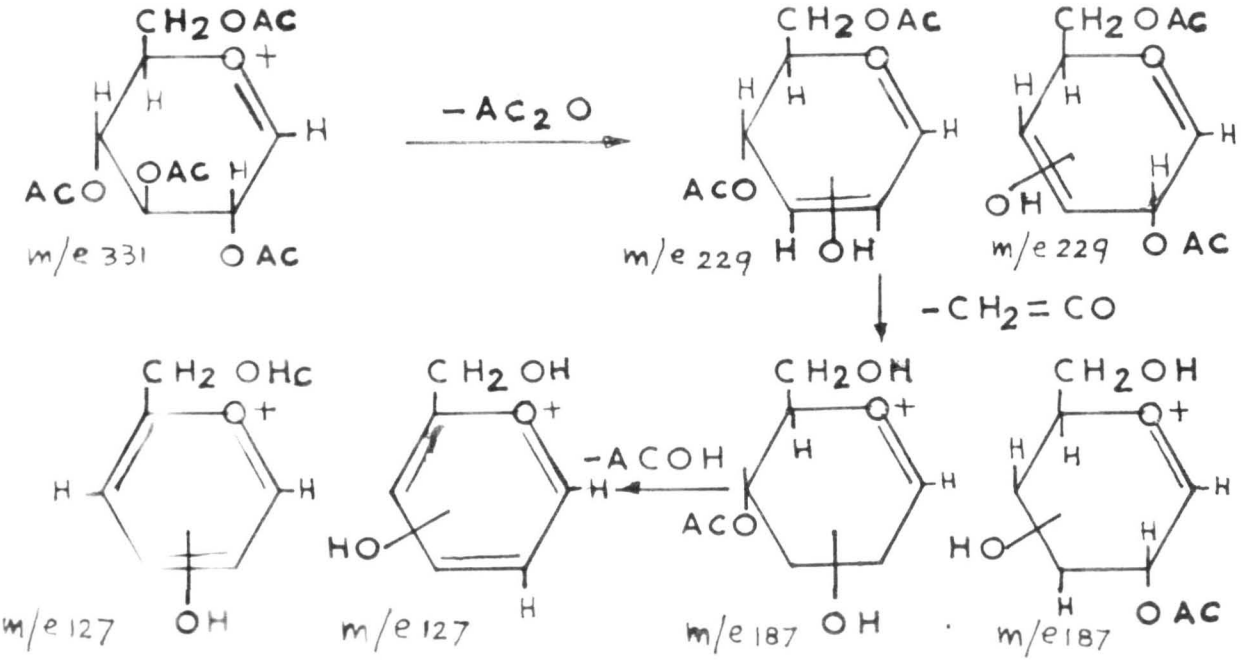


SCHEME - C



SCHEME - D

SCHEME - E



The loss of the aglucone part in the four pairs of acetylated glucosides (Table III) results in the formation of the first major ion peak at m/e 331. There is a considerable difference in the intensities of the m/e 331 ion of the two anomeric pairs, the α-anomers give a more intense peak. This is of significant diagnostic value when relatively small quantities of these anomeric pairs are available for study.

The following important conclusions can be derived from these studies:

1. When the relative intensities of the M-aglucone (m/e 331) peak in case of the α-anomers are compared with each other, it is observed that a decrease in the intensity of m/e 331 peak takes place as the structure, of aglucone changes in the following order; p-nitrophenyl, phenyl, p-chlorophenyl and p-phenyl-phenyl. The same order was observed in the series of acetylated aryl- β -D-glucosides.

In view of the observations made by Reed[37] and Biemann[39], in the present context, more intensity of the m/e 331 peak indicates less stability of the glucosidic bond. Therefore the order of stability of glucosidic bond in anomeric aryl tetra-O-acetyl-D-gluco-pyranosides with the change in substituent in the para position of the phenyl group (aglucone) is as follows;

$$-NO_2 < -H < -Cl < -C_6H_5$$

This should have been minimum for the unsubstituted phenoxy group. The stability of glucosidic bond, therefore, does not depend only on the electron density at the glucosidic oxygen atom which in turn

depends mainly upon the nature of the substituent in para position of the phenoxy group, but also on the steric factors.

2. The differences in relative intensities of m/e 331 peak of the α-and β-anomers of a pair is found to vary with the change in the structure of aglucone part of aryl tetra-O-acetyl-D-glucopyranosides. The differences observed are 30%, 15.4%, 9% and 6% for pheny, p-nitro pheny, p-phenyl phenyl and p-chloropheny groups respectively.

The difference observed in the intensities of m/e 331 peak in the α-and β-anomers of the four pairs were expected to be of more or less of the same magnitude. These studies clearly indicate that the configuration of aglucone (axial or equatorial) cannot be considered to be the only significant factor responsible for the intensity of the m/e 331 peak. Several other factors such as bulk and heaviness of the substituent in the aglucone part and distortion of the bond angle, interactions between different substituents of the aglucone and glucose ring contribute to the instability of the α-glucosidic bond under the electron impact.

EXPERIMENTAL.

Materials

All aryl glucopyranosides and their acetyl derivatives used in the present work were of analytical purity.

Procedures

(a) Infrared spectra

The compounds were handled by the nujol-mull technique and the infrared spectra were recorded on a Perkin-Elmer Infrared Spectrophotometer, calibrated with polystyrene.

(b) Proton magnetic resonance spectra

The PMR spectra were recorded by means of a Varian A-60 spectrometer, operating at a fixed radio frequency of 60 Mc/sec. The measurements of aryl-D-glucopyranosides spectra were carried out with 15-20% (w/v) solutions in dimethyl sulphoxide and that of aryl-tetra-O-acetyl-D-glucopyranosides were carried out in 15-20% (w/v) solutions in chloroform at the room temperature (20-30°). Tetramethylsilane was used as the internal reference.

(c) Mass spectra

The mass spectra were recorded with a CEC 21-1103 mass spectrometer, equipped with a heated inlet system operated at 100°; ionizing potential 70 ev., ionizing current 50 μA. The sample (0.5 to 1.0 mg) was sublimed from a glass tube into the reservoir (2 lit).

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PART : II

INVESTIGATIONS ON THE STRUCTURE OF TERMINALIA TOMENTOSA GUM

CHAPTER : I

MODERN METHODS IN STRUCTURAL STUDIES OF POLYSACCHARIDES

SUMMARY

A review of the applications of gas-liquid chromatography, proton magnetic resonance and mass spectrometry in structural studies of polysaccharides is presented.

MODERN METHODS IN STRUCTURAL STUDIES OF POLYSACCHARIDES

Structural investigation of an organic compound of low molecular weight will, in most cases lead to the assignment of a complete and generally acceptable structure. For polysaccharides, however, this summit is far beyond reach. Even in the case of cellulose, one of the simplest and most studied polysaccharides, there are different opinions among organic chemists or expressed more carefully, there are uncertainties as to the finer details of its structure. It is not unusual for different group of workers to obtain divergent results with the same type of polysaccharide isolated from the same biological source. This can be explained by variations of biological material, but imperfections in the methods used are probably of at least equal importance. This situation is not unusual in the development of a scientific field, and should stimulate a search for new or improved methods.

Polysaccharide chemistry at present is passing through a fast developing era. The progress is mainly because of new methods introduced in this field. Methylation remains a conventional method for structural analysis of polysaccharides. The value of this old method has become greater as a result of improvements in the methylation procedures. Recently, there has been renewed interest in developing improved methods for methylation of polysaccharides. Attempts have been made to improve yields and also to reduce the number of repetitive treatments required with the classical Haworth[1] and Purdie[2] techniques. Kuhn and co-workers[3], Srivastava and co-workers[4], and Hakomori[5] have used dimethyl

formamide and dimethyl sulphoxide as solvents for polysaccharides and different methylating agents such as methyl iodide-barium oxide, dimethyl sulphate-sodium hydride and methyl iodide-sodium hydride respectively. Following these techniques permethylation of polysaccharides has become an easy task[6-8].

Gas-liquid chromatography (GLC) has been used extensively for the qualitative detection and quantitative estimation of a wide variety of highly volatile organic compounds such as acids, hydrocarbons, alcohols, and esters[9]. Compounds of low volatility can also be analysed by this technique if they can be readily converted to derivatives with sufficient volatility. This has been illustrated by the separation of amino acids as their methyl esters[10].

McInnes, Ball, Cooper and Bishop[11] for the first time in 1958 reported the application of GLC to the separation of carbohydrate derivatives. They found that methyl ethers of the methyl glycosides of the heat labile monosaccharides were sufficiently stable and volatile to be analysed by GLC. This technique has been subsequently extended to the separation of fully and partially methylated methyl glycosides by Bishop and Cooper[12], Kircher[13], Klein and Barter[14] and Gee and Walker[15] and has been employed by Adams and Bishop[16-19] and their collaborators for the analysis of the cleavage products from methylated oligo- and polysaccharides. Recently, Aspinall has established the GLC procedure for the identification of the methanolysis products of methylated polysaccharides which eventually provides a large amount of structural informations with very small quantities of the material. The use of GLC in this field seems to be

increasing at a surprising speed[20-25]. GLC data are no substitute for classical methods of structural determination, yet when supported by independent evidence such as paper partition chromatography and ionophoresis of hydrolysates and derived glycitols together with periodate and lead tetraacetate oxidation data, they provide strong and elaborate evidence for the structure of polysaccharides[26].

In addition to this important application for the identification of methanolysis products derived from methylated polysaccharides, the GLC technique also provides rapid and accurate methods for the quantitative estimations of the component sugars obtained by hydrolysis of a heteropolysaccharide without actual separation of the individual sugars. In one of these methods[27], the sugars present in the hydrolysate are converted to their trimethyl silyl derivatives which are then quantitatively estimated by GLC. This procedure, however has some limitations owing to the formation of anomeric sugar derivatives making sometimes the interpretation of the results rather difficult. In another method[19,28,29], the sugars in the hydrolysate are reduced in the aqueous phase with sodium borohydride to the corresponding alditols which are then acetylated with acetic anhydride - pyridine. Both these steps give quantitative conversions and the resulting alditol acetates can be quantitatively estimated by GLC.

The identification of sugar derivatives by GLC, however, requires a set of authentic samples, the problem becoming difficult if these are not available. The mass spectra[30,31] of permethylated sugars and their deutero analogues prompted a novel approach to this problem. The partially methylated

monosaccharides obtained as methanolysis products of a methylated gum or a polysaccharide, are subjected to deuteromethylation and the fragmentation patterns obtained in the mass spectrum of the product are compared with those of similar deuteromethylated sugars. Identification of certain fragments in the spectrum of the compound indicates the positions of the trideuteromethyl groupings and consequently the free hydroxyl groups in the starting partially methylated monosaccharides.

The advantages of this new approach are obvious. It uses only readily available permethyl glycosides as authentic samples, enables identification of very small amounts of substances (1 mg) and provides a basis for standardization of the analysis of partially methylated monosaccharides.

Reyns[32] have recently established the mass spectral fragmentation pattern of fully acetylated monosaccharides and development of this approach will further open up an alternative method to methylation studies of polysaccharides. It will perhaps be possible to identify the partially acetylated sugars obtained on acetolysis of acetylated polysaccharides.

Proton magnetic resonance spectroscopy is at present one of the most trusted and useful tools for studying the conformation of the complex molecules in solution. Conformation assignment in carbohydrates

such as mono-, di-, oligo- and polysaccharides is based on the orientation (axial or equatorial) of the anomeric protons at the C(1) and O(1) which is reflected by the chemical shift and splitting correlations of the corresponding signals in the spectra.

For deciding the nature of glycosidic linkage classical methods known are the enzymic hydrolysis[33] and application of Hudson's isorotation rule[34]. It can also be obtained now by PMR spectroscopy. This tool is gaining importance rapidly for studies in stereochemical aspects of monosaccharides[35,36], disaccharides[36], oligosaccharides[37] and polysaccharides[38-41]. The valuable information obtained in this field is illustrated by the data already available and shown in Table I.

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CHAPTER : II

NATURE OF THE CONSTITUENT SUGARS AND URONIC ACID OF THE TERMINALIA TOMENTOSA GUM

SUMMARY

An account of the occurrance, isolation and purification of Terminalia tomentosa gum and identification and estimation of the constituent monosaccharides and uronic acid is given in this Chapter.

NATURE OF SUGARS AND URONIC ACID OF TERMINALIA TOMENTOSA GUM

Terminalia[1] is the family of gum yielding plants, which include Terminalia catappa, T. belerica, T. tomentosa, T. macroptera, T. stuhlmannii, T. chebula, T. arjuna and T. superba species. Trees of the Terminalia species (N.O. Combretaceae) grow in tropical Africa, Australia, Sikkim, Nepal and most parts of India especially Deccan and Himalayan tracts[2-4]. Terminalia tomentosa, the most important member of Terminalia family, yields timbers which are used as a cheap substitute of teak wood[2]. Besides timber, other useful parts of the plant are the bark and leaves. The bark[1,2], is used as an ayurvedic medicine, as a mild diuretic and a potent cardiac stimulant. The leaves[1,2] are relished by silk worms (Antheroea patheo).

Terminalia tomentosa popularly known as Asan in North India, Ain in Maharashtra and Gujarat, Sadar in Central India, Karrupu manita maran in Madras, Tembaru in Kerala, Nalla-maddichettu in Andhra and Piasal in Bengal, exudes a gel-like fluid which dries up to a light-yellow to amber-coloured gum used as a pergative[1,2] and as an adhesive. This gum, available in abundance, constitutes an important part of the Indian gums. This gum is inferior[3,4] to medium grades of ghatti, karaya or tragacanth gums in adhesive or thickening properties. It has been estimated that about 500 tons of this gum can be collected annually from the forests of Maharashtra state alone[5]. The chemical nature of this gum has not been investigated so far.

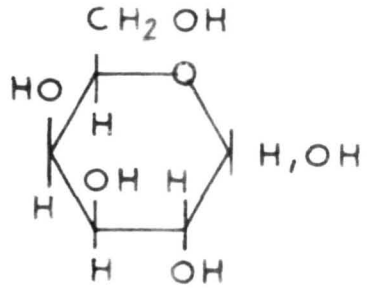
The gum used in the present investigations was isolated from the fresh exudate collected from the Terminalia tomentosa trees of the

forests of Maharashtra state in post-monsoon period. The exudate was a highly viscous gel and had a great tendency to swell in water. This tendency is usually ascribed to 1→4 linked uronic acid moieties[6] in the polysaccharides. The fresh gel reduced Fehling's solution and was found to contain free D-galactose, L-arabinose and D-glucuronic acid. The procedure for obtaining the gum in a purified and solid state from the exudate has been detailed in the experimental part.

The purified gum, $[\alpha]_D^{30}$ + 35.3° (C, 0.17 water) had an equivalent weight of 714 (determined by potentiometric titration) and sulphated ash content of 0.304%. The equivalent weight is found to be quite on the higher side as compared with the value obtained from the molar ratio of the sugars (463.1). It was obtained as a hygroscopic amorphous powder containing no nitrogen, sulphur, halogen, methoxy ~~and~~ or acetoxy groups. The component neutral sugars and the uronic acid were separated from the dilute sulphuric acid hydrolysate of the gum by the barium salt method[6]. The neutral sugars were identified as D-galactose, D-xylose, L-arabinose and L-rhamnose by paper chromatography and ionopharesis in borate buffer by enclosed strip technique[7], and by isolation of individual sugars by cellulose column-chromatography, followed by preparation of their characteristic derivatives.

The uronic acid component was identified as D-glucuronic acid by paper chromatography and its identity was confirmed by conversion of the barium uronate to D-glucose through the sodium borohydride reduction of its methyl ester methyl glycoside followed by hydrolysis[8].

The percentage of the constituent sugars of the gum, as determined by a quantitative separation of the uronic acid component from the



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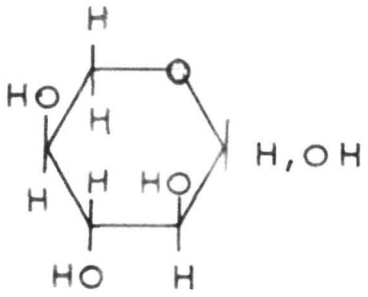

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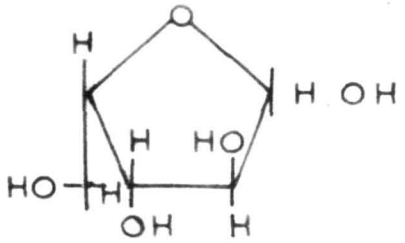

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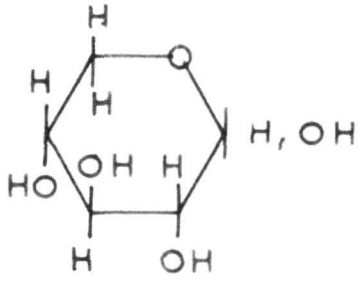

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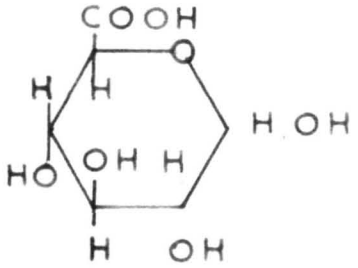

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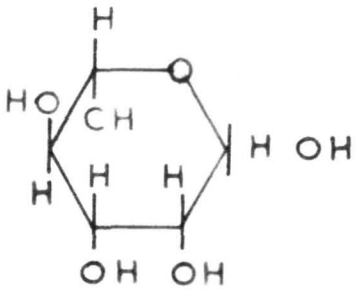

L RHAMNOSE

CONSTITUENT MONOSACCHARIDES OF TERMINALIA TOMENTOSA GUM

hydrolysate of the gum as its barium salt followed by separation of the individual neutral sugars on paper-strips and spectrophotometric estimation of the eluates using phenol-sulphuric acid reagent[9] were as follows:

D-galactose, 30.3; L-arabinose, 21.5; D-xylose, 14.4; L-rhamnose, 1.44 and D-glucuronic acid, 33.7 percent. In the same order their molar ratio calculates approximately to 21:15:10:1:23. The equivalent weight of the gum calculates to 463.1. Katha[10], Peach[11] and Sapote[12] are reported to be composed of the same sugars and uronic acid although in different proportions.

EXPERIMENTAL

Unless otherwise stated all concentrations were carried out under reduced pressure at bath temperature of 45°. All optical rotations are equilibrium values. All melting points are uncorrected and were determined on a Kofler block.

Paper chromatography was done by the descending technique on Whatman. 3 MM and Whatman No.1 papers at the room temperature in the following solvent systems.

A. n-Butanol-ethanol-water	4:1:5 organic phase
B. Ethyl acetate-pyridine-water	10:4:3 organic phase
C. n-Butanol-acetic acid-water	4:1:5 organic phase
D. Ethyl acetate-acetic acid-formic acid-water	18:3:1:4 organic phase
E. Ethyl acetate-acetic acid-formic acid-water	18:8:3:9 organic phase
F. Ethyl acetate-acetic acid-pyridine-water	5:3:5:1 organic phase

Electrophoresis was carried out on Whatman No.3 paper in borate buffer (pH 10) according to Foster's technique[7].

Spray-reagents[6] used were silver nitrate-alkali, p-anisidine hydrochloride and aniline hydrogen phthalate.

Isolation of the gum from the fresh exudate and its purification

The fresh exudate (5 kg) was homogenised by stirring with water (5 lit) at 60° for 1.5 hr and was freed of coarse particles of bark and leaves by passing the solution through a cloth. The resulting solution

(pH 7) was centrifuged to remove finer extraneous insoluble matter and the crude gum was precipitated by pouring it slowly into ethanol (25 lit) under stirring. The precipitated gum was filtered under suction through a filter paper, washed with acetone and dried in vacuo at 60°. It was obtained as a highly hygroscopic brown powder giving sulphated ash (11.7%) which on qualitative spectrographic analysis showed the presence of magnesium, iron, silicon and traces of calcium. The yield of the crude gum was 50 g (5% on the weight of the fresh exudate).

The crude gum (100 g) was purified by dissolving it in sodium hydroxide (700 ml, 1 N) and precipitating it by pouring the solution in acidified ethanol (3 lit) under stirring. On repeating this procedure five times, the gum became water soluble and showed a specific rotation of $[\alpha]_D^{30}$ + 34.2° (water) which did not change after one more precipitation. As the ash content of the gum was still high (3.5%), it was decationised by passing its aqueous solution (1%) through Amberlite IR-120 (H^+) resin. The resulting acidic solution on freeze-drying gave the pure gum in the form of a white powder, $[\alpha]_D^{30}$ + 35.3° (C, 0.17 water), and sulphated ash, 0.304 percent.

No acidity was found to develop during homogenisation of the fresh exudate (pH 7) with water at 60° for 1.5 hr. Under these conditions, no autohydrolysis of the gum solution is expected. The free sugars identified in the aqueous ethanolic solutions by paper chromatography of the concentrates after separation of the acidic and neutral components, were found to be galactose, arabinose and glucuronic acid. The concentration of the glucuronic acid was too low in the original aqueous-ethanolic solution to show any appreciable acidity.

Identification and characterisation of the component sugars of the pure gum.

The pure gum (10 g) was heated with dil. sulphuric acid (1 lit, 2 N) on a boiling water bath for 30 hr. The resulting solution was neutralised with barium carbonate and the filtrate from this was concentrated at 50° under reduced pressure to a syrup which was separated into the methanol soluble neutral sugars (7.0 g) and the methanol insoluble barium uronate (4.0 g) fraction. The neutral sugar fraction was examined by paper chromatography in the solvent systems (A) and (B) and was found to consist of galactose, arabinose, xylose and rhamnose by comparing their mobilities with those of the authentic reference sugars. The identity of these sugars were also confirmed by ionophoresis of this fraction in borate buffer (pH 10) along with the reference sugars. A small part of the barium uronate fraction was dissolved in water and decationised by passing through a column of Amberlite IR-120 (H^+) resin and examined by paper chromatography in the solvent systems C, D, E and F along with authentic samples of glucuronic and galacturonic acids and of glucurone. It was found to contain glucuronic acid and glucurone, the latter being an artifact formed from glucuronic acid by the action of the acidic resin or heating during concentration.

For further confirmation of identity of the sugars, the neutral sugar fraction (5 g) was chromatographed on a cellulose column (No.123, Carl Schleicher and Schull, 40 x 2.4 cm), packed by the slurry method and developed with n-butanol half saturated with water[13]. The fractions (50 ml) were collected in an automatic fraction collector and examined by paper chromatography. The results are shown in Table I.

TABLE I

Fractions	Sugar(s) detected
33 - 44	Rhamnose
44 - 48	Rhamnose and Xylose
49 - 52	Xylose
53 - 85	Xylose and Arabinose
86 - 98	Arabinose
99 - 150	Arabinose and Galactose
151 - 195	Galactose

The individual sugars were obtained in the pure state by removing the solvent from the appropriate fractions under reduced pressure and crystallising from suitable solvents. They were also characterised through their derivatives. The specific rotations of the sugars recorded are their equilibrium values.

The rhamnose fraction crystallised from n-butanol as L-rhamnose monohydrate, m.p. 89°, $[\alpha]_D^{29}$ + 8.6° (C, 1 water). It did not depress the m.p. of an authentic sample of the sugar [lit.[14] L-rhamnose monohydrate, m.p. 90-92.5°, $[\alpha]_D$ + 8.0° (water)]. It gave a yellow colour with p-anisidine hydrochloride on paper.

The D-xylose fraction could not be crystallised. The syrup showed $[\alpha]_D^{29}$ + 20° (C, 1 water). [lit.[8] $[\alpha]_D$ + 18.8° (water)]. It was characterised through the dibenzylidene dimethyl acetal derivative[15] m.p. and mixed m.p. with authentic sample, 209-10°, and both had identical infrared spectra.

The L-arabinose fraction crystallised from ethanol m.p. and mixed m.p. with authentic sample, 156-157°, $[\alpha]_D^{29}$ + 108.0° (C, 1 water) and both had identical infrared spectra [lit[16] m.p. 160°, $[\alpha]_D$ + 104.5° (water)]. It was characterised through the p-nitroanilide derivative[17], m.p. and mixed m.p. 210°, and both had identical infrared spectra.

The D-galactose fraction crystallised from ethanol, m.p. and mixed m.p. with authentic sample, 167°, $[\alpha]_D^{29}$ + 80.1° (C, 1 water) and both had identical infrared spectra [lit[18] m.p. 167°, $[\alpha]_D$ + 80.2° (water)]. It was characterised through the p-nitroanilide derivative[17] m.p. and mixed m.p. with authentic sample, 205°, and both had identical infrared spectra.

The barium uronate fraction was dried at 40-45° <u>in vacuo</u> (Found Ba, 25.96, required for $(C_6H_9O_7)_2$ Ba, Ba, 26.23 percent). The dried barium salt (3.0 g) was refluxed with methanolic hydrogen chloride (4%) for 5 hr, neutralised with silver carbonate and solvent removed from the filtrate under reduced pressure. The resulting syrup was dissolved in water (2 ml) and reduced with sodium borohydride (0.5 g) for 1 hr. The excess of borohydride was decomposed with acetic acid and the cations and anions removed with Amberlite IR-120 (H^+) and Amberlite IRA-400 ($CO_3^{=}$) resins respectively. The resulting solution containing the methyl glycoside was concentrated under reduced pressure and hydrolysed with a small amount of dil. sulphuric acid (1.5 N) for 4 hr. The filtrate obtained after neutralisation with barium carbonate was evaporated under reduced pressure. The resulting syrup, found to contain only glucose by paper chromatography, was crystallised from dil. ethanol. The product had m.p. 140° and did not depress the m.p. of D-glucose and both had identical infrared spectra.

Estimation of the individual monosaccharides

The pure gum (0.5665 g) was hydrolysed with dil. sulphuric acid 56 ml, 2 N) for 30 hr on a boiling water bath. The hydrolysed solution was neutralised with barium carbonate, centrifuged and the precipitate was washed thrice with water. The residue obtained after removing water under reduced pressure from the solution and washings, was dissolved in water (0.25 ml) and poured into methanol (50 ml). The precipitated barium uronate was filtered, washed with methanol (5 x 50 ml) and dried in vacuo at 50° until constant weight. The weight of barium uronate (0.2576 g) corresponded to 33.7% uronic acid content of the gum.

The combined methanolic solution and methanol washings containing the neutral sugars was centrifuged for 1.5 hr and the clear solution was distilled under reduced pressure to remove the solvent completely. A portion (0.0603 g) of the residual material (0.4029 g) was resolved into the component sugars on six Whatman No.1 sheets developed with the solvent system (A). The individual sugars were located by developing narrow strips from the middle and both vertical ends of the papers. The strips containing the bands of the different sugars were eluated out quantitatively with water and the volume made up to 500 ml in each case. The estimation of the individual sugars was carried out by mixing 1 ml aliquots with 1 ml of the phenol-sulphuric acid reagent[9] and measuring the absorption at 490 mμ with a Beckmann DU spectrophotometer. The sugar content of each fraction was then calculated from standard absorption curves prepared with weighed amounts of the reference sugars. The results of these estimations are shown in Table II.

TABLE II

Sugar	Absorption at 490 mμ (corrected)	Amount of sugar present (γ)	% of sugar in the gum
D-Galactose	0.146	28.5	30.3
L-Arabinose	0.176	23.5	21.5
D-Xylose	0.096	13.5	14.4
L-Rhamnose	0.010	1.4	1.44

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CHAPTER : III

PARTIAL HYDROLYSIS OF TERMINALIA TOMENTOSA GUM

SUMMARY

Partial hydrolysis of Terminalia tomentosa gum produced the degraded gum with the release of some mono-, di-, and oligosaccharides. Two of the disaccharides released were assigned the structures of 3-O-β-D-galactopyranosyl-D-galactose and 3-O-β-D-galactopyranosyl-L-arabinose respectively. Further mild hydrolysis of the degraded gum produced a mixture of monosaccharides and two aldobiouronic acids which were assigned the structures of 3-O-β-D-glucuropyranosyl-D-galactose and 4-O-α-D-glucuronopyranosyl-D-galactose respectively.

PARTIAL HYDROLYSIS OF TERMINALIA TOMENTOSA GUM

Determination of the constituent sugar units is one of the first and most fundamental operation in establishing the nature of a polysaccharide. Complete hydrolysis of a polysaccharide into the constituent monosaccharides is usually effected by acid hydrolysis and the individual sugars are then identified and estimated by established methods[1,2]. Partial hydrolysis of a polysaccharide into di-, tri- and oligosaccharides is usually brought about by autohydrolysis or acid-, alkali- or enzymic hydrolysis. This may also be effected by acetolysis of acetylated polysaccharide or methanolysis of methylated polysaccharide. Elucidation of the structures of the products of partial hydrolysis provide details about the linkage between monosaccharides constituting the parent polysaccharide.

In general, dilute acids are used for the partial hydrolysis of polysaccharides. Partial acid hydrolysis is effected by treatment of a polysaccharide with dilute sulfuric acid solution (usually 1 to 2 N) at low temperatures, (20-30°), for long periods[3] or at higher temperatures, (90-100°), for short periods[4]. For more dilute acids (0.01 N - 0.5 N), higher temperatures (90-100°) and long periods of heating are needed. Use of higher concentrations of the acid or of the polysaccharide in solution usually lead to the formation of degradation products and unpredictable artifacts[2].

The partial hydrolysis of the purified Terminalia tomentosa gum was brought about by treating it with dil. sulphuric acid (0.01 N)

at the room temperature (20-30°) for 24 hr followed by heating on a boiling water bath for 7 hr. Paper chromatography of the aliquots of the solution withdrawn at regular intervals showed the pattern of releasing sugars. D-xylose and L-arabinose were released first, and was followed by further release of these pentoses as well as of D-galactose. There was a gradual increase in the reducing power of the hydrolysing solution which became constant after 6 hr. Heating was, therefore, discontinued after a period of 7 hr. The degraded gum was obtained from the hydrolysed solution by precipitation with ethanol and purified by repeated precipitation (yield 35%). The structure of the degraded gum will be discussed in the next Chapter.

The filtrate obtained after removal of the degraded gum was found to contain D-xylose (6 parts), L-arabinose (3 parts), D-galactose (3 parts), L-rhamnose (0.5 part) and barium glucuronate (1 part) and a few di- and higher oligosaccharides. The ease with which D-xylose and L-arabinose units are liberated in the initial stages of hydrolysis indicate that these sugars are present as end groups. The release of a large amount of D-xylose in the initial stage also indicates that the D-xylose units are mainly attached through acid-vulnerable linkages.

The fact that only 35% of the gum survives the mild acid hydrolysis suggests that the other sugars are also attached through acid-labile linkages. This has been confirmed by studies on the methylation of the gum reported in a subsequent Chapter of this thesis.

Terminalia tomentosa gum seems to have structural similarities

with Cholla[3], Cherry[6] and Peach[6] gums which on either autohydrolysis or acid hydrolysis at the room temperature are reported to yield D-xylose and L-arabinose. The results were interpreted to indicate that D-xylose residues in the gums occupy terminal positions.

Structure of the disaccharides obtained by partial hydrolysis of the gum

The monosaccharides present in the hydrolysate after removal of the partially degraded gum could be almost completely removed on a cellulose column. The remaining di- and oligosaccharides were further separated on paper in two different disaccharides (A and B) and a mixture of three very closely and very slow-moving (5-7 cm in seven days in solvent A) oligosaccharides present in small amounts. The two disaccharides were ~~also~~ obtained in comparatively small amounts and their methylation studies could not be undertaken. Structures of these two disaccharides A and B were arrived at mainly by a comparison of their properties with known disaccharides[7].

Disaccharide A

This disaccharide was obtained as a solid, m.p. 157-158°, $[\alpha]_D^{30}$ + 64° (water). Owing to paucity of material no attempt was made to crystallise it. It gave only D-galactose on acid hydrolysis and its mobility on paper in solvent A established it to be a galactobiose.

Determination of nature of the glycosidic linkage between the two monosaccharide units of a disaccharide is a difficult task when only small quantities of the disaccharides are available. Charlson and Perlin[9] suggested that results of lead tetra-acetate

oxidation not catalysed by potassium acetate may provide useful information for differentiating between various linkage types in disaccharides. These authors found that 1 ⟶ 6 linked disaccharides rapidly consume more than two mols of lead tetra-acetate within the first few minutes and the consumption remains practically at the same level up to thirty minutes. 1 ⟶ 4 Linked disaccharides on the other hand had a levelled consumption of 1.8 mols of the oxidant in thirty minutes. In the same period, all of the 1 ⟶ 3 linked disaccharides studied by these authors had a levelled consumption of only one mol in thirty minutes. The disaccharide A was found to consume one mol of lead tetraacetate within thirty minutes. This strongly indicates that it is a 1⟶3 linked disaccharide. The melting point and the optical rotation of disaccharide A agreed with that of known 3-O- β -D-galactopyranosyl-D-galactose, m.p.159-160.5°, $[\alpha]_D^{23}$ + 62° (water). Therefore the same structure has been assigned to it.

This disaccharide has been obtained from the partial hydrolysis products of several galactose containing polysaccharides, such as, Acacia arabica[10], Acacia pycnanth[11], Acacia senegal[12], Anogeissus latifolia[13], Anogeissus schimperi[14], Virgilia orabeids[5] and Khaya senegalensis[15] gums.

Disaccharide B

This disaccharide was obtained as a solid m.p. 199-202° $[\alpha]_D^{30}$ + 63.3° (water). No attempt was made to crystallise this compound also as the quantity available was small. On acid hydrolysis the disaccharide gave D-galactose and L-arabinose. On lead tetraacetate oxidation it consumed 1.4 mol of the oxidant in 30 minutes, which is

slightly higher than that theoretically required for a 1⟶3 linkage[9], but less than that required for a 1⟶4 or 1⟶6 linkage. Only D-galactose was detected in the acid hydrolysate of the lead tetraacetate oxidised product and the expected tetrose could not be detected. Only galactose could be detected as the reducing sugar when a solution of the sodium borohydride reduced disaccharide was hydrolysed. These indicate that L-arabinose is the reducing end of this disaccharide. Comparison of its m.p. and optical rotation with those of the known D-galactosyl-L-arabinoses, the disccharide was found to be identical with known 3-O-β-D-galacto-pyranosyl-L-arabinose[3,13], m.p. 200-201°, $[\alpha]_D$ + 64° (water). The presence of 3-O-substituted L-arabinose in the gum as confirmed by identification of 2,5 di-O-methyl-L-arabinose as a product of hydrolysis of the methylated gum (Chapter V, Part II) further supports the structure of this disaccharide.

This disaccharide has been frequently reported as one of the partial hydrolysis products of several polysaccharides such as Cholla[3], Virgilio oraboids[5], Ghatti[16], Anogeissus schimperi[14] and Combretum leonance[4] gums.

Structure of the aldobiouronic acids obtained by hydrolysis of the partially degraded gum.

In order to obtain valuable informations about the acid hydrolysis resistant glycosidic linkages, Combretum leonence gum[4] was subjected to a two-stage partial acid hydrolysis. With the same object in view, degraded Terminalia tomentosa gum was subjected to a further acid hydrolysis under more drastic conditions (0.25 N-

sulphuric acid on a boiling water bath for 25 hr). After this period, the reducing power of the hydrolysed solution was found to remain, more or less, constant. The acidic and neutral sugars were separated from the hydrolysate by the barium salt method[1]. The neutral sugars were identified, D-galactose (4 parts), D-xylose (1 part), L-arabinose (5 parts), and L-rhamnose (traces). The acidic fraction was found to contain D-glucuronic acid and two aldobiouronic acids C and D, the structures of which are discussed in the following paragraphs:

Aldobiouronic acid C

This acid was isolated in the form of a syrup, $[\alpha]_D^{30}$ + 58.02° (water). Its mobility on paper in solvent E suggested it to be an aldobiouronic acid. On complete acid hydrolysis it gave D-galactose and D-glucuronic acid. Its methyl ester methyl glycoside consumed 2.84 mol of periodate which is in agreement with a 1→4 linkage (theoretical consumption 3 mol). The original compound gave a blue colour with diphenylamine-aniline reagent which is a positive test for 1→4 linkage[18]. Owing to the high positive optical rotation of this compound, α-anomeric configuration was assigned to its glycosidic linkage. The optical rotation of 4-O-β-D-glucuropyranosyl-D-galactose[7] is $[\alpha]_D$ + 15° (water). The structure assigned to this aldobiouronic acid was, therefore, 4-O-α-D-glucuropyranosyl-D-galactose.

This aldobiouronic acid is not very common among glucuronic acid containing polysaccharides. It has been isolated from Neem[19], Acacia karoo[20], Acacia arabica[10] and Tragacanth gums[21].

Aldobiouronic acid D

This acid was also obtained as a syrup, $[\alpha]_D^{30}$ + 11.76° (water).

Its mobility on paper in solvent E suggested it to be an aldobiouronic acid. On complete acid hydrolysis it also gave D-galactose and D-glucuronic acid. Its methyl ester methyl glycoside consumed 1.76 mol of periodate which is in agreement with a 1→3 linkage (theoretical consumption 2 mol). The aldobiouronic acid gave a negative diphenylamine-aniline test[18] indicating the absence of a 1→4 linkage. In view of high positive optical rotation of α-linked aldobiouronic acids[7], a β-anomeric configuration was assigned to the glycoside linkage of this aldobiouronic acid since it had positive but low optical rotation. All the data presented are in agreement with 3-O-β-D-glucopyranosyl-D-galactose structure for this compound. This aldobiouronic acid has not been reported in literature so far.

The partially degraded Terminalia tomentosa gum was found to be completely degraded by the second stage acid treatment. The absence of any neutral di-and oligosaccharides in this hydrolysis suggests that the glycosidic linkage between two monosaccharide units is unable to withstand such hydrolysis conditions. The linkages which survived the second hydrolysis are those bearing glucuronic acid units. Absence of D-glucuronic acid dimer and oligomer suggested the absence of glucuronan chains in the structure of the degraded gum.

EXPERIMENTAL

Partial acid hydrolysis of Terminalia tomentosa gum

Purified *Terminalia tomentosa* gum (20 gm) was allowed to swell in dilute sulphuric acid (0.01 N, 2 l) at the room temperature for 24 hr. The homogeneous thin jelly was heated on a boiling water bath to effect the partial hydrolysis. Aliquots of the hydrolysed solution were withdrawn at intervals and the sugars released were noted. Gradual changes in the reducing power was estimated by the iodometric method and the results are recorded in Table I.

TABLE I

The change in reducing power of the hydrolysing solution with the progress of hydrolysis of Terminalia tomentosa gum.

S.No.	Time hr	Vol. of aliquot used in titration (ml)	Vol. of thiosulphate solution (0.05 N) used to titrate excess iodine (ml)	Sugars present
1	0	4	60.8	-
2	1	4	60.4	Traces of pentose(s)
3	2.5	4	58.5	L-arabinose + D-xylose
4	4.5	4	58.3	L-arabinose + D-xylose
5	6.0	4	57.7	L-arabinose + D-xylose + D-galactose
6	7	4	57.7	L-arabinose + D-xylose + D-galactose + L-rhamnose + D-glucuronic acid

After 7 hr, when the reducing power did not increase further, heating was discontinued. The solution was cooled to room temperature and added in a thin stream to ethanol (10 l) under stirring. The precipitated degraded Terminalia tomentosa gum was separated by filtration. It was purified by repeated precipitation and finally dried under reduced pressure. The yield of the degraded gum was 7.7 gm (35% approx.). The filtrate was neutralised with barium carbonate, and the resulting neutral solution was filtered and evaporated to a thin syrup (500 ml). Paper chromatographic examination of the syrup in solvent A revealed the presence of D-xylose (6 parts), L-arabinose (3 parts), D-galactose (3 parts), L-rhamnose (0.5 part), barium glucuronate (1 part), two disaccharides and three higher oligosaccharides. Most of the monosaccharides were removed from 200 ml of the syrup on a cellulose column (40 x 2.4 cm) using n-butanol half-saturated with water as the eluent. The mixture of the di-and the oligosaccharides collected from the column was further resolved on Whatman 3 MM and No.1 papers in solvent A and the individual sugars were extracted out with water. Two pure disaccharide fractions were collected and the higher oligosaccharides obtained in trace amounts were not further examined.

Identification of the disaccharides

Disaccharide A (3-O- β -D-galactopyranosyl-D-galactose)

The disaccharide (9.5 mg) was obtained in the form of chromatographically pure colourless solid, m.p. 157-158°, $[\alpha]_D^{30}$ + 64.0° (C, 0.75 water). It had R gal. values of 0.50 and 0.66 in solvents A and B respectively. Hydrolysis of the

disaccharide with sulphuric acid (1 N) gave only D-galactose (detected by paper chromatography). 3-O- β -D-galactopyranosyl-D-galactose isolated from cholla gum[3] is reported to have m.p. 159-160.5°, $[\alpha]_D^{23}$ + 62.0° (C, 1.0 water).

The galactobiose (5 mg) was dissolved in a mixture of water (0.5 ml) and glacial acetic acid (0.5 ml) and oxidised with a freshly prepared solution of lead tetraacetate[8,9] in glacial acetic acid (2 ml). Aliquots (0.2 ml) were withdrawn at intervals of 10 minutes, 20 minutes and 30 minutes respectively and treated with stop solution (10 ml) composed of potassium iodide (10 gm) and sodium acetate (50 gm) in water 100 ml. The amount of unconsumed oxidant was determined by titrating the liberated iodine with sodium thiosulphate solution (0.005 N). The value of thiosulphate used in the titration for all the reaction aliquots was 0.84 ml (constant) while that for the blank was 1.31 ml. This calculates lead tetraacetate consumption 1.05 mol.

After 30 min, the excess oxidant was precipitated as lead oxalate, with a solution of oxalic acid in glacial acetic acid (10%). The solution was filtered and the solvent was completely removed from it. The residue was dissolved in water and passed through a column of Amberlite IR - 120 (H^+) resin to remove traces of lead. The acidic solution (containing residual oxalic acid) was concentrated to a small volume (10 ml) and heated on a boiling water bath for 6 hr. The hydrolysate was evaporated to dryness under reduced pressure and the residue was extracted with methanol. The extract was found to contain D-galactose and D-lyxose (paper chromatography).

Disaccharide B (3-O- β -D-galactopyranosyl-L-arabinose)

The disaccharide (35 mg) was obtained as a chromatographically pure solid, m.p. 199-202°, $[\alpha]_D^{30}$ + 63.3° (C, 0.71 water). It had R gal. values 0.63 and 0.76 in solvents A and B respectively. On hydrolysis with sulphuric acid (1 N) it produced D-galactose and L-arabinose (paper chromatography). This disaccharide isolated from Cholla[3] and Anogeissus schimperi[13] gums is reported to have m.p. 200-201° and $[\alpha]_D$ + 64.0° (C, 0.48 water).

Sodium borohydride reduction of the disaccharide (4 mg) followed by acid hydrolysis produced a syrup in which only D-galactose was detected (by paper chromatography).

Lead tetraacetate oxidation of this disaccharide (5.0 mg) was carried out as described earlier. The consumption of the oxidant was found to be constant when the aliquots after 10 minutes, 20 minutes and 30 minutes were tested. The value of lead tetraacetate consumption calculates to 1.4 mol.

Partial acid hydrolysis of degraded Terminalia tomentosa gum

Degraded Terminalia tomentosa gum obtained in the first hydrolysis described earlier was found to be pure (paper chromatography showed no contamination of D-glucuronic acid or any aldobiouronic acid). The degraded gum (2 gm) was dissolved in dilute sulphuric acid (0.25 N, 200 ml) and hydrolysed by heating on a boiling water bath. Aliquots of the hydrolysed solution were withdrawn at intervals and the changes in reducing powers were recorded. The results are shown in Table II.

TABLE II

The change in reducing power of hydrolysing solution with the progress of hydrolysis of degraded Terminalia tomentosa gum

S.No.	Time (hr)	Vol. of aliquot used for titration (ml)	Vol. of thiosulphate solution required to titrate excess iodine (ml)
1	2	3	63.8
2	4	3	63.0
3	7.5	3	61.0
4	9.5	3	60.5
5	14	3	60.1
6	17	3	59.4
7	24	3	59.0
8	27	3	59.0
9	30	3	59.0

After 30 hr when the reducing power did not increase further, heating was discontinued. The solution was cooled to the room temperature, neutralised with barium carbonate and filtered. The clear filtrate was concentrated to a thin syrup (20 ml) and poured into ethanol (200 ml) under stirring. The precipitated barium salts of the acidic sugars were filtered. The filtrate showed the presence of D-galactose (4 parts), L-arabinose (5 parts), D-xylose (1 part), L-rhamnose (traces) and barium glucuronate (2 parts). ~~(detected by~~(paper chromatography). No neutral oligosaccharide was detected in the filtrate.

The precipitated barium salt was dissolved in water and the solution was passed successively through columns of Amberlite IR - 120 (H^+) and IR - 45 (OH^-) resin. The second column was washed with excess of water until the effluent gave negative anthrone test for sugars. This column was eluted with formic acid of increasing concentrations (0.5 - 10%, total 1.8 l) and 36 fractions were collected. These fractions were mostly mixtures and were resolved into three pure acidic fractions on Whatman No.1 paper in solvent E.

Identification of acidic fractions

D-glucuronic acid

The uronic acid (300 mg) ran parallel to an authentic sample of D-glucuronic acid on paper in solvents D, E and F. On sodium borohydride reduction it gave D-glucose (m.p. and m.m.p. 148°). Its infrared spectrum was superimposable with that of pure D-glucose.

Aldobiouronic acid C (4-O-α-D-glucuropyranosyl-D-galactose)

This aldobiouronic acid (37 mg) was obtained as a chromatographically pure syrup, $[\alpha]_D^{30}$ + 58.02° (C, 1.2 water) and had R gal values of 0.68 and 0.56 in solvent E and F and R galacturonic acid values of 0.74 and 0.50 in solvents E and F respectively. Optical rotation of 4-O-α-D-glucuropyranosyl-D-galactose has not been reported in literature[7] and the β-anomer, 4-O-β-D-glucuropyranosyl-D-galactose is reported to have $[\alpha]_D$ + 15°. These data are in agreement with an α-1 $\longrightarrow$ 4 linkage for the aldobiouronic acid C.

On hydrolysis with sulphuric acid (2 N) it produced D-galactose and D-glucuronic acid (paper chromatography). The

aldobiouronic acid (8.9 mg) was converted into its methyl ester methyl glycoside[24] by keeping with methanolic hydrochloric acid (2%) for 12 hr at the room temperature. The ester glycoside was oxidised with sodium periodate solution (0.01517 mol, 10 ml) in the dark. Aliquots of the oxidising solution (2 ml) were withdrawn at intervals of 8 hr and 24 hr and diluted to 500 ml with water. The concentrations of periodate were determined spectrophotometrically[25] by measuring the absorption at 223 mμ. This procedure was adopted to determine the periodate consumption of methyl ester methyl glycoside of 3-D-xylose-L-arabinofuranoside uronic acid from arabinoxylan of Rye flour[26].

TABLE III

The results of the spectrophotometric analysis of the periodate oxidation of the methyl ester methyl glycoside of aldobiouronic acid C.

S. No.	Solution in cell	Cell error	Absorption of cell content	Corrected absorption
1	Water	0	0	0
2	KIO_3 (MW 214.01) solution (0.0032%)	-20	105	125
3	* $NaIO_4$ (MW 213.92) solution (0.003248%)	- 5	620	625
4	Diluted reaction mixture after 8 hr	+26	420	394
5	Diluted reaction mixture after 24 hr	+25	418	393

* Sodium meta periodate solution was standardised by titrating it against sodium arsenite solution (0.26154 mol).

From these data the absorption corresponding to periodate consumed in the reaction comes to 294 and periodate consumption of the methyl ester methyl glycoside calculated to 2.844 mols (required theoretically for a 1⟶4 linkage, 3 mol).

This aldobiouronic acid also showed characteristic blue colour (though slightly masked by brown colour due to the uronic acid) with diphenylamine-aniline-phosphoric acid reagent for the 1⟶4 linkage[18].

Aldobiouronic acid D (3-O-β-D-glucopyranosyl-D-galactose)

This aldobiouronic acid (30 mg) was obtained as a chromatographically pure syrup, having R galacturonic acid values of 0.69 and 0.66 in solvents E and F respectively. It had $[\alpha]_D^{30}$ + 11.76° (C, 1.7 water). The low positive optical rotation indicated a β-configuration for the glycosidic linkage.

On hydrolysis with sulphuric acid (2 N) it produced D-glucuronic acid and D-galactose. The aldobiouronic acid was converted into its methyl ester methyl glycoside by treatment with methanolic hydrogen chloride (2%). The periodate consumption of the resulting ester glycoside was estimated spectrophotometrically as described in the case of aldobiouronic acid C.

TABLE IV

The results of spectrophotometric analysis of periodate oxidation of methyl ester glycoside of aldobiouronic acid D.

S. No.	Solution in cell	Cell error	Absorption of cell content	Corrected absorption
1	Water	0	0	0
2	KIO_3 solution	-29	65	94
3	$NaIO_4$ solution	+22	660	638
4	Diluted reaction mixture after 8 hr	-24	450	474
5	Diluted reaction mixture after 24 hr	-32	441	473

From these data periodate consumption of the methyl ester methyl glycoside of the aldobiouronic acid calculates to 1.757 (required theoretically for a 1$\longrightarrow$3 linkage, 2 mols). The periodate consumption value and negative diphenyl amine-aniline-phosphoric acid-reagent test for 1$\longrightarrow$4 linkage[18] suggest a 1$\longrightarrow$3 linkage for the aldobiouronic acid D.

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CHAPTER : IV

STRUCTURE OF THE DEGRADED GUM

SUMMARY

A tentative structure is assigned to the degraded Terminalia tomentosa gum on the basis of its periodate oxidation, products of its partial and complete hydrolysis, as well as products of hydrolysis of the methylated degraded gum.

STRUCTURE OF DEGRADED TERMINALIA TOMENTOSA GUM

In the preceding Chapter it has been shown that Terminalia tomentosa gum can be degraded stepwise, with the release of monosaccharides as well as acidic and neutral di- and oligo saccharides.

The partial acid hydrolysis of Terminalia tomentosa gum resulted in the preferential release mainly of D-xylose residues together with small quantities of D-galactose, D-glucuronic acid and L-arabinose and traces of L-rhamnose and formation of degraded Terminalia tomentosa gum, $[\alpha]_D^{26}$ + 126.6° (C, 0.3 water).

The degraded gum on complete hydrolysis gave D-galactose, L-arabinose, D-xylose, L-rhamnose and D-glucuronic acid approximately in the molar proportion of 19:19:5:1:16 respectively. The barium salt of the degraded gum contained barium 12.21% which corresponds to an equivalent weight of 493.9.

Terminalia tomentosa gum like ghatti[1-3], Acacia sundera[6], Anogeissus schimperi[7] and Chloroxylon swietenia[8] gums, produced the degraded gum containing L-arabinose units. Hirst et. al.[1-3,7] indicated that L-arabinose units are present in the degraded ghatti gum in the pyranose as well as the furanose forms. Most of the L-arabinofuranose units are present in the degraded gum as end groups while L-arabinopyranose and rest of the L-arabinofuranose units are present in the interior connected by 1 → 3 linkages. Aspinall et. al.[5] have established the presence in degraded ghatti gum of 1 → 3 and 1 → 2 linked arabinose in pyranose as well as furanose forms by gas-liquid chromatographic study of the

hydrolysed methylated gum and of the periodate oxidised gum. Degraded Terminalia tomentosa gum has also been shown to possess a similar arrangement of L-arabinose units in the interior but a different arrangement for the non-reducing end groups through similar methylation and periodate oxidation studies.

Partial acid hydrolysis of the degraded Terminalia tomentosa gum, yielded two aldobiouronic acids (C and D) which were assigned the structure of 3-O- β -D-glucuronosyl-D-galactose and 4-O- α -D-glucuronosyl-D-galactose respectively, indicating the probable modes of linkages between D-galactose and D-glucuronic acid units in the degraded gum.

Methylation of the degraded gum and hydrolysis of the methylated product

The degraded gum was methylated by Srivastava's[9] procedure using dimethyl sulphate, solid sodium hydroxide in dimethyl sulphoxide. A single methylation gave a chloroform soluble product, which, however, showed a strong hydroxyl peak in the infrared spectrum and was, therefore, a partially methylated product. Four further methylations of this product by Purdie's[10] procedure using methyl iodide and silver oxide were necessary to obtain the completely methylated degraded gum showing no absorption in the infrared spectrum ν(3300-3400 cm^{-1}) and having a methoxyl content of 38.23%. Hydrolysis of the completely methylated degraded Terminalia tomentosa gum gave a mixture of neutral and acidic methylated sugars. The neutral sugars were fractionated by paper chromatography and the following sugars were either fully identified or their presence inferred from R_G value, optical rotation, chromatographic identification of the sugar after demethylation or colouration with p-anisidine hydrochloride. They are: 2,3,4,6-tetra-,

2,4,6-tri-, 2,6-di- and 2,4-di-O-methyl-D-galactose, 2,3,5-tri-, 2,3,4-tri-, 2,5-di- and 3,5-di-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-D-xylose and 2,3,4-tri-O-methyl-rhamnose.

All of these methylated sugars were found to be present in the products of methanolysis of the methylated undegraded gum by gas chromatographic analysis.

In addition to these, two monomethyl D-galactoses probably substituted in positions 2 and 6 respectively were also detected (paper chromatography).

The acidic methylated sugars were freed from the contaminated neutral methylated sugars by ion-exchange resins, and were then reduced with lithium aluminium hydride[11]. Subsequent hydrolysis of the reduced product gave two neutral sugars which were identified as 2,3,4-tri- and 2,3-di-O-methyl-D-glucoses which were characterised through their crystalline derivatives. This shows the presence of 2,3,4-tri and 2,3-di-O-methyl-D-glucuronic acids in the hydrolysate of the methylated degraded gum.

The isolation of 2,4,6 tri-O-methyl-D-galactose from the hydrolysis products of the methylated degraded gum as the only tri-O-methyl derivative of D-galactose indicated that D-galactose residues in the degraded gum are mainly linked through 1 → 3 linkages. The presence of 2,3,6-tri-O-methyl-D-galactose could not, however, be detected in the hydrolysate, although its presence was expected in view of the isolation of 4-O-α-D-glucuronosyl-D-galactose (aldobiouronic acid D) from the partial hydrolysis of the degraded gum.

The backbone of the degraded gum

Hydrolysis of the degraded gum gave two aldobiouronic acids (C and D) composed of D-glucuronic acid and D-galactose connected by 1 $\xrightarrow{\beta}$ 3 and 1 $\xrightarrow{\alpha}$ 4 linkages respectively. No residual unhydrolysed gum remained under these conditions. This establishes that the backbone of the degraded gum is composed of these two aldobiouronic acid units. The proportion of galactose to glucuronic acid (19:16) in the degraded gum supports this view.

The isolation of only one di-O-methylated glucuronic acid, viz., 2,3-di-O-methyl-D-glucuronic acid from the products of hydrolysis of the methylated degraded gum establishes that the galactose units are connected to glucuronic acid units by only 1 → 4 linkages.

The isolation of 2,4,6-tri, 2,4-di- and 2,6-di-O-methyl-D-galactose from the products of hydrolysis of the methylated degraded gum in the proportion of 80:20:3 indicates clearly that aldobiouronic acid C (1 → 3) comprises the backbone. Since no 2,3,6-tri-O-methyl-D-galactose could be detected, the number of 1 → 4 linkage in the backbone as indicated by the isolation of the aldobiouronic acid D as well as of 2,6-di-O-methyl-D-galactose must be very small.

The backbone of the degraded gum may, therefore, be represented as follows:

$$\rightarrow G \xrightarrow{1,4} GA \xrightarrow{1,3} G \xrightarrow{1,4} GA \xrightarrow{1,3} G \xrightarrow{1,4} GA \xrightarrow{1,3} G \xrightarrow{1,4} GA \xrightarrow{1,3} G \xrightarrow{1,4} GA \xrightarrow{1,3} G \xrightarrow{1,4} GA \rightarrow \ldots\ldots$$

FIG. 1

Points of branching

It is evident that the branching of the backbone of the degraded gum is only through the galactose units, since no monomethyl glucuronic acid was detected in the hydrolysate of the methylated degraded gum. The branching points and the frequency of branching are clearly indicated by the proportion of the three partially methylated galactoses, viz., 2,4,6-tri-, 2,4-di- and 2,6-di-O-methyl-D-galactoses (80:20:3) and the structure of the two di-O-methyl-D-galactoses isolated from the same hydrolysate. Thus every fifth galactose unit in the backbone is branched at the 6-position and every thirtyfourth galactose unit is branched at the 4-position. The modified structure of the backbone of the degraded gum showing the branching points can be depicted as follows:

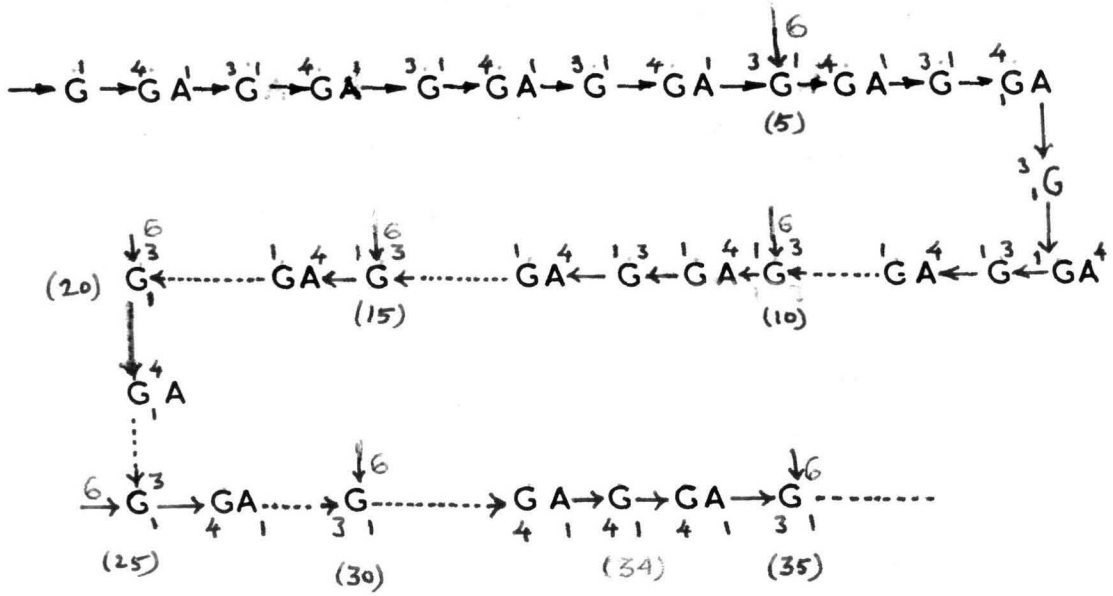


FIG. 2.

Nature of the branches

L-Arabinose, D-galactose and D-glucuronic acid are present in the degraded gum in almost equimolar proportion (19:19:16). In view of the already established fact that aldobiouronic acid C units constitute the backbone of the degraded gum, it is evident that the branches are mainly constituted of L-arabinose chains. Isolation of 2,3,4-tri-O-methyl-L-arabinose from the hydrolysate of methylated degraded gum indicates that arabinopyranose units form the non-reducing ends of some of these branches. A mixture of di-O-methyl arabinoses composed of 2,5-di- and 3,5-di-O-methyl-L-arabinose as indicated from the optical rotation, were also isolated from this hydrolysate as a single fraction. This indicated the presence of 1 → 3 and 1 → 2 linked L-arabinofuranose units in the main part of the branch structures. Although the relative proportion of these two di-O-methyl sugars could not be determined, it is clear that most of the linkages were of 1 → 3 type, since 1 → 2 linkages are less stable to acids than 1 → 3 linkages and since the degraded gum is a product of partial acid hydrolysis of the gum.

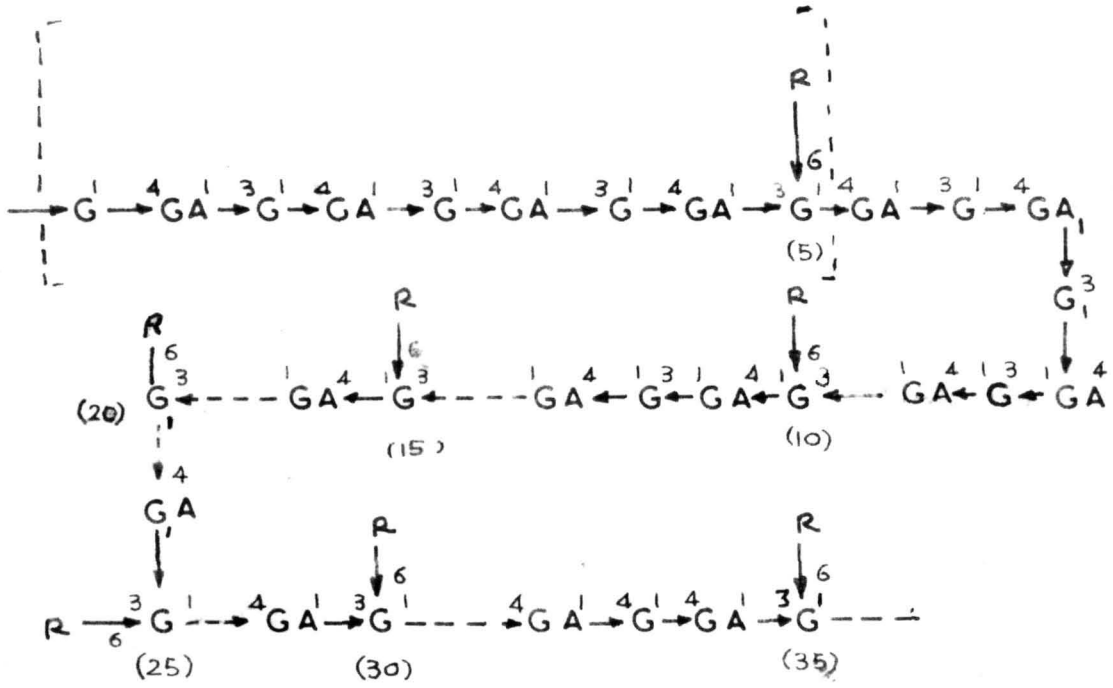
The proportion of D-xylose to L-arabinose in the degraded gum is about 1:4. This indicates that for every four L-arabinose units there is only one D-xylose unit. Since only 2,3,4-tri-O-methyl-D-xylose could be detected in the hydrolysate of the methylated degraded gum, some of the non-reducing ends of the branches would be the D-xylopyranose units. But since this does not account for all of the xylose units, it may be presumed that some of them must be

in other positions of the branches, although this could not be substantiated by experimental evidence. The L-rhamnopyranose units are also likely to be at the reducing ends of the branches as the sugar was isolated as its 2,3,4-tri-O-methyl ether from the hydrolysate of the methylated degraded gum.

It may be mentioned here that the sugars tri-O-methyl-L-arabinose-D-xylose and L-rhamnose may also be formed to some extent as a result of degradation of the side chain of the degraded gum during methylation.

From these observations, the degraded Terminalia tomentosa gum may be assigned the following structure tentatively (Fig.-3)

STRUCTURE OF THE DEGRADED GUM



R $\leftarrow A_f \leftarrow A_f \leftarrow A_f \leftarrow A_f \leftarrow A_p \leftarrow X_p \cdots X_p$ (OR Rh)

G = D-GALACTOSE GA = D-GLUCURONIC ACID

A = L-ARABINOSE X = D-XYLOSE

Rh = L-RHAMNOSE R = SIDE-CHAIN

FIG. 3

The high positive optical rotation ($[\alpha]_D^{26}$ + 126.6°) of the degraded gum as compared to the optical rotation ($[\alpha]_D^{26}$ + 35.3°) of the original gum may be attributed to the reduction in the number of negative rotation contributing linkages and consequent increase in the proportion of the α-D and β-L linkages in the degraded gum.

Degraded cholla gum[12] also similarly shows a positive optical rotation ($[\alpha]_D$ + 46.5°) as compared to the negative optical rotation ($[\alpha]_D$ - 86.2°) of the original gum[14].

The methoxyl content of the methylated degraded gum was found to be 38.23% as compared to the required value of 36.5% based on the structure of the degraded gum (Fig. 3). This higher value may be attributed to the degradation of the polysaccharide under methylation conditions using dimethyl sulphoxide, leading to the formation of considerable quantities of fully methylated monosaccharide units. This is confirmed by unaccountable high yields of tetra-O-methyl-D-galactose, tri-O-methyl-L-arabinose and-D-xylose respectively in the products of hydrolysis of the methylated degraded gum.

Similar observations have been recently reported by Anderson et. al.[13,14] for Acacia gum exudates.

It was thought of interest to find out whether periodate oxidation data of the degraded gum are in agreement with the structure (Fig. 3) assigned to it. The observed values of periodate consumption (0.5731 mol) and formic acid generated (0.2696 mol) per anhydro hexose residue of the degraded gum are, however, not in agreement with the calculated values of periodate consumption based on the assigned structure (Fig. 3) of the degraded gum.

The repeating block of sixteen sugars as shown in the Fig.3 under parenthesis would consume seven mols of periodate and yield one mol of formic acid from the non-reducing end. The observed values for periodate consumed is 9.2 mol and for formic acid liberated is 4.31 mol. The higher observed values can be explained on the basis of alkali degradation and subsequent side reactions of the degraded gum during periodate oxidation and consequent formation of saccharinic acid. Similar higher periodate consumption and formic acid formation has been reported for the cherry gum[15].

EXPERIMENTAL

R_G values of the methylated sugars refer to their rates of movement relative to 2,3,4,6-tetra-O-methyl-D-glucose in solvent A.

Demethylations were carried out by heating with hydroiodic acid[16].

Estimation of constituent monosaccharides of the degraded gum

The pure degraded gum (0.5635 gm) was hydrolysed with dilute sulphuric acid (2 N, 100 ml) on a boiling water bath for 30 hr. The resulting solution was neutralised with barium carbonate, filtered and the filtrate concentrated to a syrup which was repeatedly extracted with methanol. Barium glucuronate obtained as the methanol insoluble residue (0.2447 gm) was found to contain 26% Ba. This corresponds to anhydrouronic acid content of the degraded gum as 29.27%.

The methanol soluble fraction on removal of the solvent gave a mixture of neutral monosaccharides (0.3824 gm). A part of this fraction (31.8 mg) was quantitatively resolved on Whatman No.1 paper in solvent A and the individual sugars were quantitatively eluted and each made up to 250 ml with water. Each of the sugar solution (1 ml) was mixed with aqueous phenol (5%, 1 ml) and sulphuric acid A.R (5 ml). The mixture was cooled to the room temperature and its absorption was recorded at 490 mμ on a Beckman D.U. spectrophotometer[17].

The amounts of the respective sugars were then calculated from the standard absorption curves plotted for each authentic sugar. The results are given in the Table I.

TABLE I

Sugar	Cell error	Absorption	Corrected absorption	Corresponding amount of sugar (γ)	Percentage of the sugar in the degraded gum.
Galactose	0.008	239	231	45	29.80
Arabinose	0.008	204	196	38	24.17
Xylose	0.008	74	66	9.5	5.98
Rhamnose	0.008	16	8	1.2	0.5

Percentage of each anhydro sugar in the degraded gum was calculated as follows:

$$\text{Anhydro galactose} = \frac{45}{(45 + 38 + 9.5 + 1.2)} \times \frac{0.3824}{0.5635} \times \frac{162}{180}$$

$$= 29.80$$

Similarly the percentage of the other sugars were also calculated.

The molar ratios of the sugars calculate roughly to galactose: arabinose: xylose: rhamnose:glucuronic acid:: 19:19:5:1:16.

Methylation of the degraded gum

The degraded gum (2.5 gm) was dissolved in dimethyl sulphoxide (350 ml) and dimethyl sulphate (70 ml) and solid sodium hydroxide (100 gm) were added to the solution in small lots over a period of 20 hr at 5-10° under an atmosphere of nitrogen. The reaction was continued for 40 hr.

The reaction mixture was then heated to decompose the excess of dimethyl sulphate and the excess of alkali was neutralised with sulphuric acid under cooling. The precipitated sodium sulphate was filtered off and the filtrate was poured into excess of water and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The resulting partially methylated degraded gum showing hydroxyl absorption in the infrared spectrum was further methylated with methyl iodide (5 gm) and silver oxide (1.5 gm) under reflux for 10 hr. On repetition of this methylation three times more the fully methylated degraded gum (0.85 gm) was obtained. This material did not show any hydroxyl peak in the infrared spectrum and had a methoxyl content 38.23%

Hydrolysis of the methylated degraded Terminalia tomentosa gum and identification of the hydrolysis products

The methylated degraded gum (0.75 gm) was kept in methanolic sulphuric acid (2%, 50 ml) at the room temperature for 24 hr. The resulting solution was diluted with water (60 ml) and methanol removed under reduced pressure. The resulting solution was further hydrolysed on a boiling water bath for 15 hr, neutralised with barium carbonate, filtered, and the filtrate evaporated to thick syrup. The syrup was repeatedly extracted with chloroform and chloroform containing methanol (10%). The solvents were removed from the combined extract and the resulting solid (0.45 gm)consisting of the neutral methylated sugars were separated into various fractions on Whatman No.1 paper in solvent A.

Fraction 1: (2,3,4-Tri-O-methyl L-rhamnose)

This sugar (5.0 mg) was obtained as a chromatographically homogeneous material. It had R_G 1.08 and gave a brownish-yellow spot with p-anisidinehydrochloride.

Fraction 2: (Mixture of 2,3,5-tri-O-methyl L-arabinose and 2,3,4-tri-O-methyl D-xylose)

This fraction (80 mg) moved as a single spot, R_G 0.96 and gave a pink coloured spot with p-anisidine hydrochloride. It had an optical rotation of $[\alpha]_D^{26}$ - 5° (C, 1.05 chloroform). Demethylation produced arabinose and xylose in a visually estimated ratio of 2:1. The literature values for 2,3,4-tri-O-methyl D-xylose[15,18] are: R_G 0.98 and $[\alpha]_D$ + 48° (C, 0.81) and for 2,3,5-tri-O-methyl-L-arabinose[19] are: R_G 0.95 and $[\alpha]_D$ - 33° (C, 3.9).

It may be mentioned that these two methylated sugars were identified in the methanolysate of the methylated undegraded gum by gas chromatography.

Fraction 3: (2,3,4,6-Tetra-O-methyl-D-galactose)

This fraction (45 mg) had R_G 0.90 and $[\alpha]_D^{26}$ + 105° (C, 0.7 chloroform). It gave D-galactose on demethylation. On refluxing with ethanolic aniline, the corresponding anilide was obtained which crystallised from ethanol in colourless needles, m.p. 190-192°. Literature[18] values are: R_G 0.89, $[\alpha]_D$ + 118° and m.p. of 2,3,4,6-tri-O-methyl-D-galactose-anilide 195°.

Fraction 4: (Mixture of 2,5 di-O-methyl-L-arabinose and 3,5-di-O-methyl-L-arabinose)

This fraction (87 mg) had R_G 0.80 and $[\alpha]_D^{26}$ - 23.3° (C, 0.90 chloroform).

It moved as a single spot and gave pink colour with p-anisidine-hydrochloride. Demethylation produced L-arabinose. Ionophoresis on paper in borate buffer (pH 10) showed this fraction to be a mixture of two sugars. The major compound moved slower. 3,5-Di-O-methyl-L-arabinose is known to move much faster than 2,5-di-O-methyl-L-arabinose in ionophoresis[20,21]. The presence of 2,5 and 3,5 di-O-methyl-L-arabinoses is confirmed by the identification of only these two dimethyl arabinose sugars in the products of hydrolysis of methylated undegraded gum by gas chromatography. The literature value is known only for 3,5-di-O-methyl-L-arabinose. It is known as a syrup which has $[\alpha]_D$ - 12.8° (water)[20] and $[\alpha]_D$ - 15° (methanol)[21]. Very recently the separation of the two sugars by continuous paper electrophoresis and then their crystalline arabonamides are reported by Anderson[22]. The melting points of 2,5- and 3,5-di-O-methyl-L-arabonamides are 124-126° and 143-144° respectively.

Fraction 5: (2,4,6-Tri-O-methyl-D-galactose)

This chromatographically homogeneous fraction (40.2 mg) had R_G 0.75, $[\alpha]_D^{26}$ + 80.5° (C, 0.92, chloroform) and gave a brown spot with p-anisidine hydrochloride. It gave D-galactose on demethylation. On refluxing with ethanolic aniline, it gave an anilide which crystallised slowly from ethanol-ether in colourless needles, m.p. 174-175°. The literature[11] values for 2,4,6-tri-O-methyl-D-galactose are: R_G 0.73, $[\alpha]_D$ + 82°, and the anilide m.p. 174°.

Fraction 6: (2,6-Di-O-methyl-D-galactose)

This fraction (1.5 mg) moved as a single spot R_G 0.60 and gave a brown colour with p-anisidine hydrochloride. Literature[23] gives R_G value of 0.58 for the same sugar.

Fraction 7: (2,4-Di-O-methyl-D-galactose)

This fraction (10 mg) was obtained as a chromatographically pure syrup $[\alpha]_D^{26}$ + 81° (C, 1.01 chloroform). It had R_G value 0.55 and gave a brown colouration with p-anisidine hydrochloride. The literature[23] values for 2,4-di-O-methyl D-galactose are; R_G 0.54 and $[\alpha]_D$ + 64°.

Fraction 8: (Mono-O-methyl-D-galactose)

This fraction (4.1 mg) was moving as a single spot(R_G 0.40) and gave a brown colouration with p-anisidine hydrochloride. The literature value for the R_G of 6-O-methyl-D-galactose is the same.

Fraction 9: (Mono-O-methyl-D-galactose)

This fraction (6 mg) was also moving as a single spot R_G 0.35 and gave a brown colour with p-anisidine hydrochloride similar to the fraction 8. The literature[19,24] value for the R_G of 2,O-methyl-D-galactose is 0.33 or 0.35.

Identification of acidic methylated sugars

The barium salt of the acidic methylated sugars contained traces of neutral sugars (detected chromatographically) as impurities.

The barium salt was dessolved in water and barium ions were removed by passing the solution through a column of Amberlite IR -120 (H^+) resin. The barium free solution was passed through Amberlite IR -45 (OH') resin and the column was washed with large excess of aqueous ethanol to remove neutral methylated sugars. The acidic sugars were then eluted with sodium hydroxide solution (1 N) and sodium ions were removed with Amberlite IR -120 (H^+) resin. The aqueous solution of the free acidic sugars was evaporated to dryness and the acidic sugars were converted into their methylester-methyl-glycosides by refluxing with 3% methanolic hydrochloric acid. The ester glycosides were reduced with lithium aluminum hydride[11] in tetrahydrofuran. The reduced products were hydrolysed with sulphuric acid (1 N) and the hydrolysates were separated into the individual neutral methylated sugars on paper in solvent A. The identification of the neutral methylated sugars is detailed below:

Fraction 1: (2,3,4-Tri-O-methyl-D-glucose)

This fraction (38 mg) was chromatographically pure (R_G 0.85) and gave a brown colouration with p-anisidine hydrochloride. It had $[\alpha]_D^{25}$ + 61.2° (C, 0.55 chloroform). It was converted into its anilide in the usual manner. The anilide crystallised from methanol in colourless needles, m.p. 143°. The literature[2,25,26] values for 2,3,4-tri-O-methyl-D-glucose are; R_G 0.85 $[\alpha]_D$ + 60.8° and m.p. of the anilide 145-146°.

Fraction 2: (2,3-Di-O-methyl-D-glucose)

This chromatographically pure fraction (57.5 mg) moved as a single spot (R_G 0.58) and gave a brown colouration with p-anisidine

hydrochloride. It showed $[\alpha]_D^{26}$ + 56.6° (C, 0.50 chloroform). Its anilide was prepared in the usual manner which on crystallisation from methanol, gave colourless needles, m.p. 131-132°. The literature[2,26,27] values for 2,3-di-O-methyl-D-glucose are: R_G 0.58, $[\alpha]_D$ + 58° and m.p. of the anilide 134°.

Periodate oxidation of degraded Terminalia tomentosa gum

Degraded Terminalia tomentosa gum (0.1035 gm) and sodium meta periodate solution [(0.05 M (approx), 50 ml] were kept at room temperature (20-30°) in the dark. The solution was shaken from time to time and aliquots (2 ml) were withdrawn at different intervals and titrated iodometrically for the periodate content. The titration readings for periodate consumption are given in the Table II.

TABLE II

Time (hr)	Vol of $NaAsO_2$ solution (0.00523 M) in ml used by		Vol. of $NaAsO_2$ solution corresponding to $NaIO_4$ consumed in the reaction
	$NaIO_4$ solution (2 ml) reaction blank	Reaction mixture aliquot (2 ml)	
48	17.4	14.6	2.8
72	17.4	14.5	2.9
100	17.4	14.1	3.1

The periodate consumption per anhydro hexose unit calculated to 0.5731 mol. (100 hr.)

For the estimation of formic acid produced after 100 hr, the oxidised mixture (10 ml) was withdrawn and the unreacted periodate

in it was destroyed with an excess of ethylene glycol. The resulting solution was titrated against standard sodium hydroxide solution (0.005859 N, 5.9 ml). From these data the formic acid produced calculated to 0.26% mol per anhydro hexose unit after applying the correction for the acidity due to the degraded gum.

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CHAPTER : V

THE STRUCTURE OF THE TERMINALIA TOMENTOSA GUM

SUMMARY

A tentative structure is assigned to the Terminalia tomentosa gum on the basis of its periodate oxidation, products of its partial and complete hydrolysis, as well as products of methanolysis and hydrolysis of the methylated gum.

THE STRUCTURE OF THE TERMINALIA TOMENTOSA GUM

Methylation of Terminalia tomentosa gum

Terminalia tomentosa gum could be completely methylated by one methylation according to Srivastava's procedure[1] followed by three successive methylations according to Kuhn's procedure[2]. The fully methylated gum had a methoxyl content of 43.03 per cent and did not show any hydroxyl peak in its infrared spectrum.

Products of methanolysis of the methylated gum

A part of the fully methylated gum was methanolysed with methanolic hydrochloric acid (3%) under reflux and the products of methanolysis were examined by gas chromatography for qualitative and quantitative data. The results are given in Table I.

TABLE I

S. No.	Sugar*	Relative proportion	Retention time	
			Column a	Column b
1	2	3	4	5
1	2,3,4-Tri-O-methyl-L-arabinose	7	1.04 (-)	0.83 (-)
2	2,3,5-Tri-O-methyl-L-arabinose	33	0.56 (0.72)	0.47 (0.59)
3	2,5-Di-O-methyl-L-arabinose	16	1.89 (3.47)	0.70 (1.03)
4	3,5-Di-O-methyl-L-arabinose	12	1.08 (2.55)	0.60 (0.84)
5	2,3,4-Tri-O-methyl-D-xylose	33	0.46 (0.57)	0.45 (0.54)
6	2,3,4,6-Tetra-O-methyl-D-galactose	36	1.80 (-)	1.52 (1.60)
7	2,3,4-Tri-O-methyl-D-galactose	3	7.5 (-)	2.62 (2.89)

* Sugars identified were present as methyl glycosides

Contd..

TABLE I (contd)

1	2	3	4	5
8	2,4,6-Tri-O-methyl-D-galactose	29	4.17 (4.70)	2.08 (2.38)
9	2,3,4-Tri-O-methyl-L-rhamnose	1	0.46 (-)	0.46 (-)
10	2,3-Di-O-methyl-L-rhamnose	1	0.73 (1.01)	0.61 (-)
11	2,3,4-Tri-O-methyl-D-glucuronic acid (methylester)	5	2.53 (3.24)	1.77 (2.21)
12	2,3-Di-O-methyl-D-glucuronic acid (methylester)	6	8.4 (9.31)	2.47 (3.12)
13	2,4-Di-O-methyl-D-galactose	-	-	3.74 (4.4)

Column a; butane 1;4-diol succinate polyester (15%) on celite

Column b; polyphenyl ether [m-bis-(m-phenoxy-phenoxy)] benzene (10%) on celite.

Retention times are quoted relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside as an internal standard. The retention times given in brackets indicate the value for the anomer when present.

Products of hydrolysis of the methylated gum

A part of the fully methylated gum was hydrolysed in two stages with aqueous methanolic sulphuric acid (2%) at the room temperature followed by aqueous sulphuric acid (2%) on a boiling water bath. The products of hydrolysis were separated into neutral and acidic fractions. The acidic fraction was reduced with lithium aluminium hydride[3] and the neutral sugars were isolated in the pure state by paper chromatography and identified. These are listed in Table II.

TABLE II

S. No.	Neutral sugar identified	R_G	Corresponding parent acidic sugar
1	2,3,4-Tri-O-methyl-D-glucose	0.85	2,3,4-Tri-O-methyl-D-glucuronic acid
2	2,3,Di-O-methyl-D-glucose	0.58	2,3,Di-O-methyl-D-glucuronic acid

R_G value refers to the mobility with respect to 2,3,4,6-tetra-O-methyl-D-glucose in solvent A.

The neutral fraction from the hydrolysate was resolved in various fractions (containing pure as well as mixture of sugars) by paper chromatography leading to the identification of the sugars listed in Table III. The identities were derived from R_G values, preparation of derivatives (wherever possible), optical rotation, nature of the sugar obtained on demethylation and colouration with p-anisidine hydrochloride.

TABLE III

S. No.	Sugar	R_G (.)	Colour with p-anisidine hydrochloride	Sugar obtained on demethylation	Derivative
1	2,3,4,6-Tetra-O-methyl D-galactose	0.90	Brown	D-galactose	Anilide m.p. 191°
2	2,4,6-Tri-O-methyl D-galactose	0.75	Brown	D-galactose	Anilide m.p. 174°
3	2,3,6-Tri-O-methyl D-galactose	0.67	Brown	D-galactose	-
4	2,6-Di-O-methyl D-galactose	0.60	Brown	D-galactose	-
5	2,4-Di-O-methyl D-galactose	0.55	Brown	D-galactose	-
6	6-O-Monomethyl D-galactose	0.41	Brown	-	-
7	2-O-Monomethyl D-galactose	0.35	Brown	-	-
8	2,3,4-Tri-O-methyl D-xylose	0.97	Pink	D-xylose	-
9	2,3,5-Tri-O-methyl L-arabinose			L-arabinose	-
10	2,5-Di-O-methyl L-arabinose	0.80	Pink	L-arabinose	-
11	3,5-Di-O-methyl L-arabinose				
12	2,3,4-Tri-O-methyl L-rhamnose	0.89	Brownish yellow	-	-

(.) R_G = Relative mobility (on paper) as compared to TMG

* The components of this mixture were identified from ionophoresis in borate buffer (pH 10)

Periodate oxidation of Terminalia tomentosa gum

The pure gum was oxidised with sodium metaperiodate (0.05 M) at the room temperature in dark and the consumption of the oxidant was found to become constant after 48 hr. The periodate consumed and formic acid formed during this period were 1.050mol and 0.6390 mol respectively per anhydrohexose unit. The oxidised gum gave D-galactose and L-arabinose almost in equal proportion (detected by paper chromatography) on reduction with sodium borohydride followed by hydrolysis.

Tentative structure of the Terminalia tomentosa gum

Terminalia tomentosa gum, containing, D-galactose, L-arabinose, D-xylose, L-rhamnose and D-glucuronic acid in the molar proportion of 21:15:10:1:23, loses 65 per cent of its weight during the mild acid hydrolysis leading to the formation of the degraded gum in which the same sugars are present in the proportion of 19:19:5:1:16 respectively. The molar proportions of the sugars lost in this process as calculated from the molar proportions of these sugars in the gum and degraded gum are in good agreement

with the relative intensities of the spots of the released sugars. From these observations it is possible to conclude that in the process of formation of the degraded gum, the original gum has lost about twentyeight anhydro sugar units comprised of eight galactose, five arabinose, six xylose, little rhamnose and nine glucuronic acid per repeating block of the degraded gum consisting of sixteen anhydro sugar units. These twentyeight anhydro sugar units are to be distributed to each repeating block of the sixteen anhydro sugar units of the degraded gum in conformity with the experimental data to arrive at an approximate structure of the gum.

The arrangement of xylose units

The number of xylose units lost in the process of obtaining the degraded gum is six. The repeating block of the degraded gum structure contains an arabanside chain having one or two xylose units at the end. These six xylose units can possibly be regarded as a linear extension of the xylose terminal of the araban branch of the degraded gum.

The identification of only 2,3,4-tri-O-methyl xylose in the hydrolysis product of the methylated gum can again be explained on the basis of extreme instability of 1—> 4 linked xylopyranose chains, leading to the liberation of the xylose units during methylation and consequent formation of the tri-O-methyl xylose.

There appears to be not many branching from these xylose units since no xylose was found to survive the periodate oxidation of the original gum.

Fig. 1 represents the possible arrangement of xylose units.

FIG. 1. X_p = XYLOPYRANOSE

The arrangement of arabinose units

The five arabinose units which are lost from the original gum in the course of formation of the degraded gum are likely to be attached preferably (Fig. 2) in the form of an araban sub-branch of the araban side chain, rather than (Fig. 3) a linear extension of the araban side chain containing 5 xylose units.

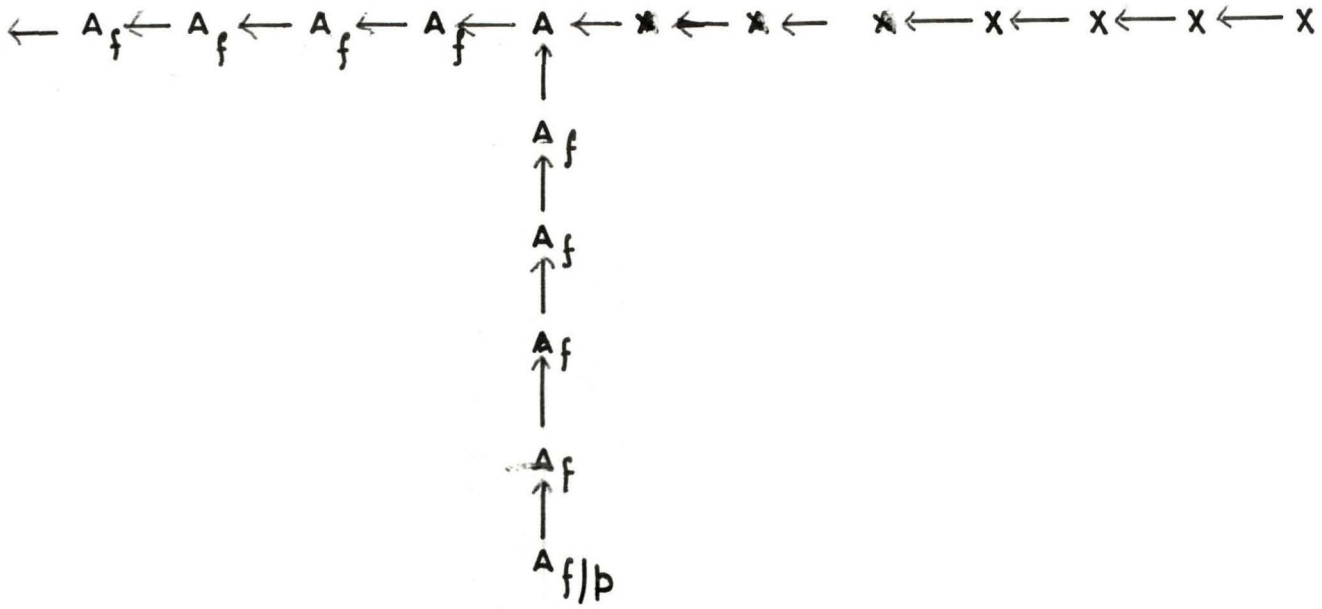

FIG. II

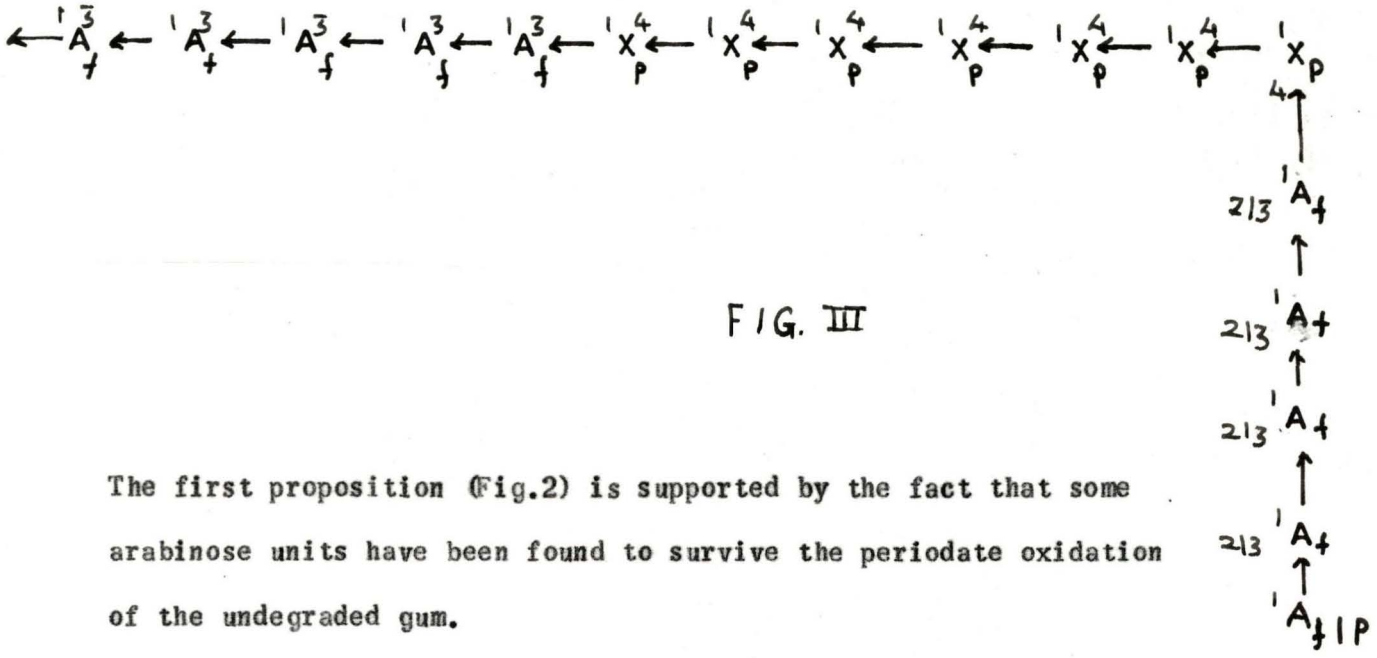

FIG. III

The first proposition (Fig.2) is supported by the fact that some arabinose units have been found to survive the periodate oxidation of the undegraded gum.

Isolation of 2,5 and 3,5-di-O-methyl-L-arabinoses in the hydrolysate of the methylated gum indicates that the arabinose units in the sub-branch are in furanose form and linked through

1 → 2 or/and 1 → 3 linkages. 1 → 3 Linkage is widely distributed in the structure of the degraded gum, therefore, 1 → 2 linkage will be more frequent in the sub-branch.

A small proportion of the arabinose units is present in the pyranose form also,as indicated by the isolation of 2,3,4-tri-O-methyl-L-arabinose.

The arrangement of galactose units

The number of galactose units lost in the process of obtaining the degraded gum is about eight. Almost the same number of units of glucuronic acid (nine) is also lost in the process. The conditions of this hydrolysis, however, is mild enough to warrant any degradation of the aldobiouronic acid components of the backbone to yield these individual sugars. This is supported by the fact that no aldobiouronic acid could be detected in the hydrolysate obtained in the process of obtaining the degraded gum. It appears safe, therefore to conclude that the galactose and glucuronic acid units liberated in the mild hydrolysis of the gum originated from the branches.

Since an araban side chain of the five units remains intact in the degraded gum during mild hydrolysis of the gum, these galactose and glucuronic acid units must have come from the arabinose or xylose sub-branches.

Identification of the disaccharides 3-O- β -D-galactopyranosyl-D-galactose and 3-O- β -D-galactopyranosyl-L-arabinose liberated during the mild hydrolysis of the gum established the presence of galactobiose and galactose units attached to the araban sub-branch probably as

galactan chain rather than single galactose or digalactose units in view of the fact that no monomethyl arabinose has been detected. The galactose units are not likely to be attached to the xylose sub-branch since no galactosyl xylose was isolated. These galactose units are probably 1 → 3 linked in view of the isolation of 3-O-β-D-galactopyranosyl-D-galactose in the products of mild hydrolysis of the gum, and the abundance of 2,4,6-tri-O-methyl-D-galactose as compared to 2,3,4-tri-O-methyl-D-galactose. Isolation of small quantities of 2,4- and 2,6-di-O-methyl-D-galactoses indicate the possibility of some branching of the galactan chain possibly for attachment to rhamnose unit.

Fig. 4 represents the possible arrangement of D-galactose units.

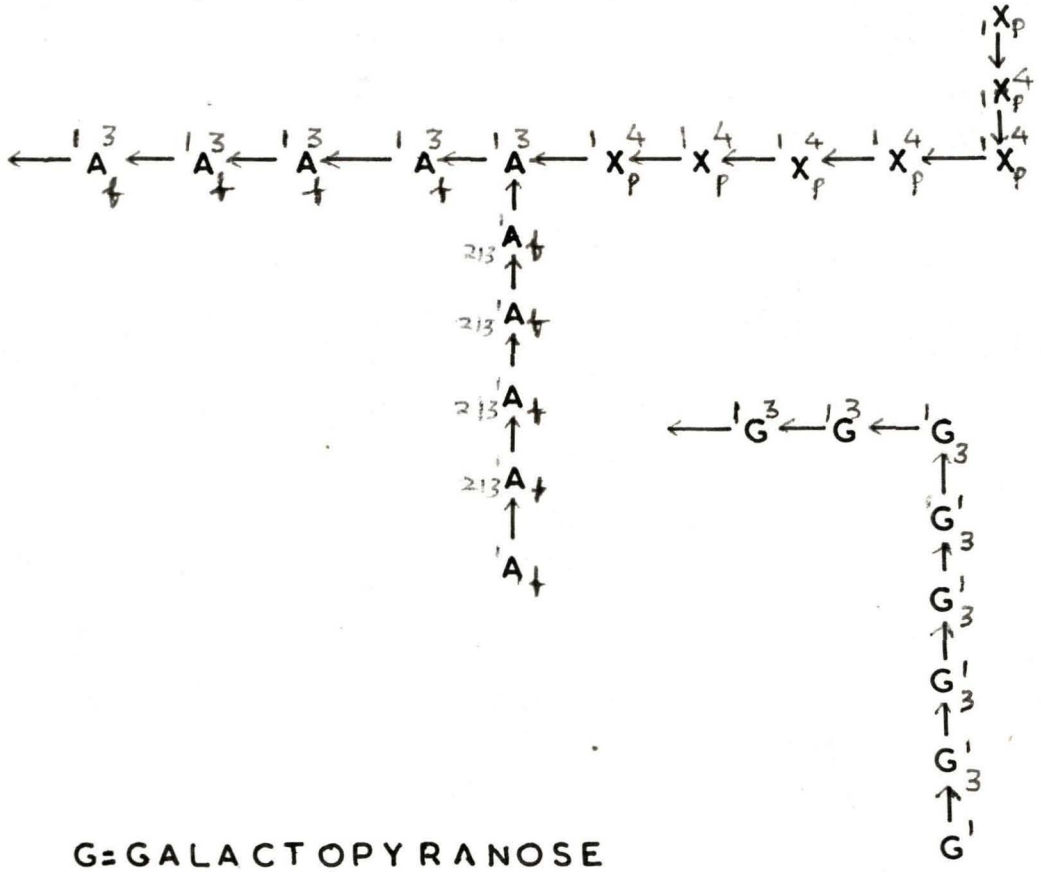

FIG. IV.

The arrangement of rhamnose units

Although the very small number of L-rhamnose units present in the gum are likely to be the non-reducing end groups attached to galactose, arabinose or xylose units in view of the detection of 2,3,4-tri-O-methyl-L-rhamnose, some branching from rhamnose is also indicated by the identification of 2,3-di-O-methyl-L-rhamnose. These branching may consist of single D-glucuronic acid units.

Fig. 5 represents the possible arrangement of L-rhamnose units.

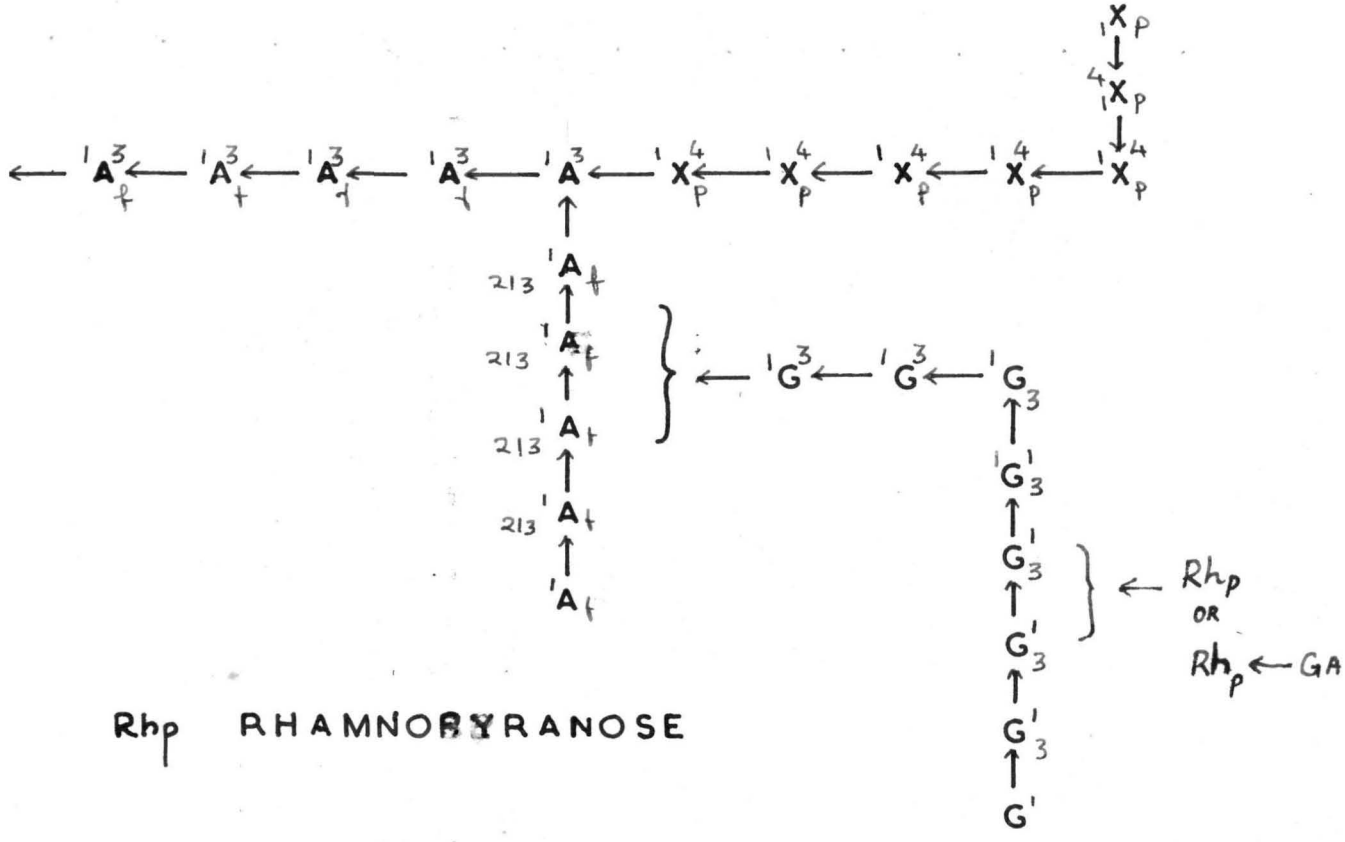

FIG. V

The arrangement of glucuronic acid units

The identification of 2,3,4-tri-O- and 2,3-di-O-methyl-D-glucuronic acids in the products of hydrolysis of the methylated gum can be explained by the backbone structure of the gum which is the same as the already postulated backbone of the degraded gum. It is not possible to arrive at any conclusions about the position of the nine glucuronic acid units liberated in mild hydrolysis except that a few of them may be attached individually to xylose or to arabinose units or to the ends of L-rhamnose units. Uronic acid chains are quite stable under the mild hydrolytic conditions and no diuronic acid was detected, therefore, no polyuronide (glucuran) chain can be envisaged.

Fig. 6 represents the tentative structure of the Terminalia tomentosa gum based on the available data.

EXPERIMENTAL

R_G values of methylated sugars refer to the mobility on paper[4] relative to 2,3,4,6-tetra-O-methyl-D-glucopyranose in solvent A. Demethylation of the methylated sugars was performed with hydroiodic acid[7].

Gas chromatography of the methylated and partly methylated methyl glycosides was carried out using a Pye-Argon chromatograph according to the procedure of Bishop and Cooper[5]. Separations were made on the following columns (120 x 0.5 inner diameter) at gas flow rates 80-100 ml/min : (a) 15% by weight of butan 1-4 diol succinate polyester on acid washed celite (80-100 mesh) at 175° (b) 10% by weight of polyphenyl ether [m-bis-(m-phenoxy phenoxy) benzene] on acid washed celite at 200°. Retention times are quoted relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside as an internal standard.

Methylation of Terminalia tomentosa gum

Purified Terminalia tomentosa gum (5 g) was suspended in freshly distilled dimethyl sulphoxide (450 ml) in a four-necked round-bottomed flask (2 l) fitted with a dropping funnel, a mercury-seal stirrer, an inlet for bubbling nitrogen and a condenser fitted with an outlet for the exit of gas. The gum dissolved in dimethyl sulphoxide on heating at 50-60° on a water-bath for 4 hr under stirring. The solution was then cooled in the ice bath and maintained at 5-10° and nitrogen was bubbled through it. Dimethyl sulphate (170 ml) and sodium hydroxide pellets (250 g) were added in small lots to the reaction flask during a period of 20 hr maintaining the temperature of

the reaction mixture at 5-10°. The reaction mixture was then brought to the room temperature (mean 25°) and the reaction allowed to proceed for a further period of 32 hr. The reaction mixture was heated on a boiling water bath for 1.5 hr to decompose excess of dimethyl sulphate. Water (200 ml) was then added to the cooled mixture in small lots to dissolve the remaining sodium hydroxide pellets, and the solution was neutralised with sulphuric acid (10 N) under cooling (~10°) to a pH of 6 to 6.5. After filtering off the precipitated sodium sulphate, the solution was diluted with a large volume of water and the methylated gum extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and the solvent was completely removed. The resulting partially methylated gum obtained in the form of a syrup (10 g) showed a hydroxyl peak in its infrared spectrum.

The syrup (10 g) was further methylated by refluxing with methyl iodide (15 ml) and silver oxide (5 g) for 10 hr. The reaction mixture was cooled, diluted with chloroform and filtered. The filtrate was dried and freed of chloroform and methyl iodide. The resulting syrup was subjected to two further methylations under these conditions and the completely methylated gum was obtained in the form of a syrup (1.6 g). It did not show any hydroxyl absorption in its infrared spectrum. It had a methoxyl content of 43.03 per cent and $[\alpha]_D^{26}$ - 24.88° (C, 0.884, methanol).

Methanolysis of the methylated gum and identification of the methanolysis products by gas-chromatography

The methylated gum (200 mg) was refluxed with methanolic hydrochloric acid (3%, 100 ml) for 15 hr. The cooled solution was

neutralised with silver carbonate, filtered and examined by gas chromatography. The methanolysis products listed in Table I (page 184) were identified.

Hydrolysis of the methylated gum and identification of the hydrolysis products

The methylated gum (1 gm) was kept with methanolic sulphuric acid (2%, 50 ml) at the room temperature for 24 hr. The solution was diluted with water (60 ml) and methanol removed under reduced pressure. The resulting solution was further hydrolysed by heating on a boiling water bath for 15 hr. The solution was cooled, neutralised with barium carbonate, filtered and the filtrate was evaporated to a thick syrup. The syrup was repeatedly extracted with chloroform and also chloroform containing methanol (10%). On removal of the solvents from the combined extract, the neutral methylated sugars were obtained as a solid (0.67 g). The chloroform and chloroform-methanol insoluble fraction contained the barium salts of the acidic sugars.

The neutral methylated sugars were separated into various fractions on Whatman No.1 paper in solvent A.

The following methylated sugars were identified:

Fraction 1: (2,3,4-Tri-O-methyl-L-rhamnose)

The fraction (15.14 mg) was chromatographically homogeneous. It had R_G 1.05 and it gave a brownish yellow colour with p-anisidine hydrochloride. Its identity was confirmed by gas chromatography of its methyl glycoside.

Fraction 2: (Mixture of 2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose)

This fraction (326.5 mg) moved as a single spot, R_G 0.97 and gave a pink colour p-anisidine hydrochloride. It had $[\alpha]_D^{26}$ + 1.0° (C, 0.5 chloroform). On demethylation it gave L-arabinose and D-xylose (paper chromatography). Literature values for 2,3,5-tri-O-methyl L-arabinose[9] are: R_G 0.95 and $[\alpha]_D$ - 33°, and for 2,3,4-tri-O-methyl-D-xylose[6,7] are: R_G 0.98 and $[\alpha]_D$ + 48°. The two sugars were identified from the retention times of their methyl glycosides in gas chromatography. Further these were separated by GLC and oxidised with bromine water to the corresponding lactones. The arabinolactone had optical rotation $[\alpha]_D^{26}$ - 25° (C, 0.5 water) and xylolactone had optical rotation $[\alpha]_D^{26}$ + 20° (C, 0.45 water). The literature values are: $[\alpha]_D$ - 24° (water) and $[\alpha]_D$ + 21° (water) for 2,3,5-tri-O-methyl arabinolactone[8] and for 2,3,4-tri-O-methyl xylolactone[9] respectively.

Fraction 3: (2,3,4,6-Tetra-O-methyl-D-galactose)

This fraction (95.5 mg) had R_G 0.90 and $[\alpha]_D^{26}$ + 109° (C, 0.81 chloroform). It gave a brown colour with p-anisidine hydrochloride. On demethylation it gave D-galactose. On refluxing with ethanolic aniline it furnished an anilide which slowly crystallised from ethanol-ether in colourless needles, m.p. 191°. Literature[7] values are: R_G 0.89, $[\alpha]_D$ + 118° and m.p. of the anilide 195°.

Fraction 4: (Mixture of 2,5-di-O-methyl-L-arabinose and 3,5-di-O-methyl-L-arabinose)

This fraction (217.7 mg) had R_G 0.80 and $[\alpha]_D^{26}$ - 29.03° (C, 0.93 chloroform). It moved as a single spot and gave a pink

colour with p-anisidine hydrochloride. Ionophoresis on paper in borate buffer of this fraction showed it to be a mixture of two compounds one moving very slow as compared with the other. Demethylation produced L-arabinose. The two sugars were identified by gas chromatography of their methyl glycosides. The two sugars were collected by preparative gas chromatography[12] and each of these was oxidised with bromine-water. The aldonolactones formed were converted to amides with methanolic ammonia. The crystalline amides were identified as 2,5-di-O-methyl-L-arabonamide, m.p. 124° and 3,5-di-O-methyl-L-arabonamide, m.p. 142°. Literature[13] values for the m.p. of 2,5 and 3,5-di-O-methyl-L-arabinomides are 124-126° and 143-144° respectively.

Fraction 5: (2,4,6-Tri-O-methyl-D-galactose)

This sugar (140.6 mg) was chromatographically homogeneous and had R_G 0.75, $[\alpha]_D^{26}$ + 80° (C, 0.9 chloroform) and gave a brown colour with p-anisidine hydrochloride. Demethylation gave D-galactose. On refluxing with ethanolic aniline, it gave an anilide which slowly crystallised from ethanol-ether in colourless needles, m.p. 174°. Literature[14] values are: R_G 0.73, $[\alpha]_D$ + 82° and anilide m.p. 174°.

Fraction 6: (2,3,4-Tri-O-methyl-D-galactose)

This sugar (20.6 mg) was chromatographically homogeneous and had R_G 0.67 and $[\alpha]_D^{26}$ + 100° (C, 1.0 chloroform). It gave a brown colour with p-anisidine hydrochloride. On demethylation it gave galactose. The sugar was identified from the retention time of its methyl glycoside. Literature values[15] for 2,3,4-tri-O-methyl-D-galactose are: R_G 0.680, $[\alpha]_D$ + 100°.

Fraction 7: (2,6-Di-O-methyl-D-galactose)

This fraction (16.3 mg) moved as a single spot (R_g 0.60) and gave a brown colour with p-anisidine hydrochloride. It showed $[\alpha]_D^{25}$ + 88.3° (C, 0.8 chloroform). It gave D-galactose on demethylation (paper chromatography). Literature[14] values for 2,6-di-O-methyl-D-galactose are: R_G 0.58 and $[\alpha]_D$ + 82°.

Fraction 8: (2,4-Di-O-methyl-D-galactose)

This fraction (83.5 mg) moved as a single spot (R_G 0.55) and had $[\alpha]_D^{25}$ + 79° (C, 1.1 chloroform). It gave a brown colour with p-anisidine hydrochloride. On demethylation it gave D-galactose. The literature[14] values are: R_G 0.54, $[\alpha]_D$ + 84°. It was identified from the retention time of its methyl glycoside.

Fraction 9: (6-Mono-O-methyl-D-galactose)

This sugar (5 mg) was obtained as a chromatographically homogeneous (R_G 0.41) fraction. It gave a brown colour with p-anisidine hydrochloride. Literature value of the mobility of 6-mono-O-methyl-D-galactose is R_g 0.60.

Fraction 10: (Mono-O-methyl-D-galactose)

This sugar (10 mg) was isolated as a chromatographically homogeneous (R_G 0.35) fraction. It gave a brown colour with p-anisidine hydrochloride. Its exact identity could not be established.

Fraction 11: (D-Galactose, D-xylose and L-arabinose)

Very small amounts of these sugars (unmethylated) were obtained as a part of the neutral methylated sugar mixture. These sugars were identified by paper chromatography.

Identification of the acidic methylated sugars:

The mixture of barium salts of acidic methylated sugars contained small quantities of 2,4,6-tri- and 2,4-di-O-methyl-D-galactose as impurities. The mixture of barium salts was dissolved in water and barium ions removed by passing the solution through a coloumn of Amberlite IR -120 (H^+) resin. The barium free solution was then passed through a column of Amberlite IR - 45 (OH^-) resin and the column was washed with a large excess of aqueous ethanol to remove the neutral methylated sugars. The acidic sugars were then eluted with sodium hydroxide solution, (1 N) and sodium ions were removed from the eluate with Amberlite IR -120(H^+) resin. The aqueous solution of the free acidic sugars was evaporated to a thick syrup and converted into their methyl ester methyl glycosides by refluxing with methanolic hydrochloric acid (3%). The mixture of ester glycosides was reduced with lithium aluminum hydride in tetrahydrofuran[3]. The reduced products were hydrolysed with sulphuric acid (1 N) and the hydrolysates were separated into the component neutral methyl sugars on paper in solvent A.

The following different fractions were identified:

Fraction 1: (2,3,4-Tri-O-methyl-D-glucose)

This fraction (80 mg) was chromatographically pure (R_G 0.85)

and gave a brown colour with p-anisidine hydrochloride. It had $[\alpha]_D^{25}$ + 60 (C, 0.85 chloroform). It was converted into its anilide in the usual manner. The anilide crystallised from methanol in colourless needles, m.p. 143-145°. Literature values[16-19] for 2,3,4-tri-O-methyl-D-glucose are: R_G 0.85, $[\alpha]_D$ + 60.8 and anilide m.p. 145-146°.

Fraction 2: (2,3-Di-O-methyl-D-glucose)

This fraction (65 mg) moved as a single spot (R_G 0.58) and gave a brown colour with p-anisidine hydrochloride. It gave $[\alpha]_D^{26}$ + 55.3° (C, 0.52 chloroform). Its anilide was prepared in the usual manner. The anilide crystallised from methanol in colourless needles, m.p. 131°. The literature[16,18,19] values are: R_G 0.58, $[\alpha]_D$ + 58° (water) and anilide m.p. 134°.

Periodate oxidation of Terminalia tomentosa gum

Terminalia tomentosa gum (0.0888 gm) was kept with sodium meta periodate solution [0.05 M (approximately), 50 ml] at the room temperature (20-30°) in the dark. The solution was shaken from time to time and aliquots (2 ml) were withdrawn at different intervals and titrated iodometrically for the periodate content. The periodate consumption at various intervals are shown in Table II.

TABLE II

Time (hr)	Volume of $NaAsO_2$ solution (0.00523M) (in ml) used by		Volume of $NaAsO_2$ soln. corresponding to $NaIO_4$ consumed in the reaction	Mols of periodate consumed per anhydro hexose
	$NaIO_4$ solution (2 ml) reaction blank	Reaction mixture aliquot (2ml)		
48	17.4	13.1	4.3	1.026
72	17.4	13.0	4.4	1.050
100	17.4	13.0	4.4	1.050

Thus, Terminalia tomentosa gum consumed 1.050 mol per anhydrohexose unit. For the estimation of formic acid produced after 100 hr, the oxidised mixture (10 ml) was withdrawn and unreacted periodate destroyed with an excess of ethylene glycol. The resulting solution was titrated against standard sodium hydroxide solution (0.005859 M, 12.1 ml). The formic acid produced calculated to 0.6469 mol. After applying a correction for the acidity of the gum, the formic acid produced calculated to 0.6386 mol per anhydro hexose unit.

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