APPLICATIONS OF BORON TRICHLORIDE

IN CARBOHYDRATE CHEMISTRY.

A Thesis submitted by

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ABSTRACT

The reactions of boron trichloride and boron tribromide with a wide variety of carbohydrate and polyol derivatives have been investigated.

These boron halides were used for the deacylation and dealkylation of mono-, di- and poly-saccharides, the last two types being converted into the monosaccharide constituents. Similarly boron trichloride effected the fission of triphenylmethyl ethers giving the parent monosaccharide or polyol. However, toluene-p-sulphonic esters were found to be stable to treatment with boron trichloride.

With the exception of D-fructose and L-sorbose all the monosaccharides which were investigated were stable to treatment with boron trichloride and boron tribromide. D-Fructose and its derivatives were converted into 5-hydroxymethylfurfuraldehyde, which was isolated and characterised.

In general unsubstituted disaccharides were not attacked by boron trichloride, but were cleaved by boron tribromide to give the constituent monosaccharides or 5-hydroxymethylfurfuraldehyde if fructose units were present.

Cyclic acetals and ketals, glycosides and 1, 6-anhydro hexoses all gave the parent monosaccharide on treatment with boron trichloride. However, 2, 3-anhydro sugars appeared to give chlorodeoxy derivatives.

Boron trichloride was found to have application as a synthetic agent also. The complex obtained by treatment of methyl α - D - glucoside with boron trichloride was a successful glucosylating agent. On condensation with alcohols and phenols, D-glucose and benzene, glucosides, disaccharides and glucopyranosylbenzene were obtained. Of the eleven possible glucose disaccharides all except the three trehaloses were isolated and characterised.

A mechanism was proposed for reactions of this type.

ACKNOWLEDGMENTS

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CONTENTS

INTRODUCTION

Boron trichloride is a powerful Lewis acid which co-ordinates with a wide range of active centres in organic molecules. These complexes frequently undergo further reaction due to the high polarizability of the boron-chlorine bond. For this reason boron trichloride is more reactive than boron trifluoride. The latter readily forms a wide range of co-ordination compounds, but these in general do not react further. Boron trifluoride is therefore an excellent catalyst but is not a successful degradative re-agent.

In the field of carbohydrate chemistry boron trifluoride has been used for the anomerisation of acetylated glycosides[1] and fully acetylated reducing sugars.[2] It has also been used as a catalyst for the condensation of polyacetylated sugars with phenols in the preparation of glycosides.[3]

Although boron trichloride has been used extensively in organic chemistry, mainly by Gerrard et.al.,[4] there are no reports of its use in carbohydrate chemistry. However, at the commencement of the work reported in this thesis, work was in progress on the re-action of boron trichloride with the cyclic acetals and ketals of the hexitols.[5] These were found to yield the parent hexitol in high yield, when treated with boron trichloride.

The reactions of boron trichloride with organic compounds, containing similar groups to those present in

the carbohydrates and their derivatives which are to be investigated, are now considered.

The products of reaction of boron trichloride and alcohols are dependent upon the relative proportions of the reagents present.[6] Borates, chloroboronates and dichloroboronites are formed when 3, 2 or 1 mole. of alcohol respectively are reacted with 1 mole. of boron trichloride,

$$BCl_3 + nROH \longrightarrow (RO)_n BCl_{3-n} + nHCl \qquad \text{Equ.1.}$$

where n = 1, 2 or 3.

Primary and secondary alcohols when reacted with boron trichloride in the ratio 3 : 1 give the corresponding borate (n = 3, Equ.1.), but benzyl alcohol[7] and tert-butanol[6] when reacted in these proportions give the alkyl chloride and boric acid (Equ.2.).

$$BCl_3 + 3\,R'OH \longrightarrow 3\,R'Cl + B(OH)_3 \qquad \text{Equ.2.}$$

Further reaction of the borates and chloroboronates with boron trichloride occurs to give chloroboronates and dichloroboronites respectively[8,9] (Equ.3 and 4.).

$$BCl_3 + 2\,B(OR)_3 \longrightarrow 3\,(RO)_2\,BCl \qquad \text{Equ.3.}$$

$$BCl_3 + (RO)_2\,BCl \longrightarrow 2\,ROBCl_2 \qquad \text{Equ.4.}$$

At room temperature chloroboronates readily decompose to give alkyl chloride, trialkyl borate and boric oxide[9] (Equ.5.).

$$3\,(RO)_2\,BCl \longrightarrow B(OR)_3 + 3RCl + B_2O_3 \qquad \text{Equ.5.}$$

A similar decomposition of dichloroboronites is

possible (Equ.6.), but this occurs less readily.[8]

$$3\ ROBCl_2 \longrightarrow BCl_3 + 3RCl + B_2O_3 \qquad \text{Equ.6.}$$

Under diminished pressure disproportionation of chloroboronates and dichloroboronites occurs according to the following equations.

$$2\ (RO)_2BCl \longrightarrow B(OR)_3 + ROBCl_2 \qquad \text{Equ.7.}$$

$$2\ ROBCl_2 \longrightarrow (RO)_2BCl + BCl_3 \qquad \text{Equ.8.}$$

All boron derivatives of alcohols when treated with aqueous methanol give the free alcohol.[10] This is a most useful procedure for regeneration of the alcohol from borates, chloroboronates or dichloroboronites.

The reactions of boron trichloride with alcohols are slightly modified when vicinal hydroxyl groups are present. Ethylene glycol is a compound of this type. The products again vary according to the relative proportions of ethylene glycol and boron trichloride present. Under various conditions ethylene dichloroboronite $Cl_2.BOCH_2.CH_2.OBCl_2$, ethylene chloroboronate $(CH_2O)_2.BCl$, diethylene ethylene diborate $(CH_2.O)_2\ BOCH_2CH_2\ OB(OCH_2)_2$, ethylene ethylene borate $(CH_2O)_2\ BO.CH_2.CH_2.OH$ and tri-2-hydroxyethyl borate $B.(OCH_2.CH_2.OH)_3$ are formed.[11] Unlike the dialkyl and diaryl chloroboronates, ethylene chloroboronate was very stable. In general, it is found that cyclic chloroboronates are much more stable than straight chain chloroboronates. However, methanolysis of the cyclic chlorobo-

ronate will release the original polyhydroxy compound.

Boron trichloride effects the fission of mixed ethers[12,13,14] to give an alkyl chloride and a dichloroboronite (Equ.9.).

$$ROR' + BCl_3 \longrightarrow R'Cl + ROBCl_2 \qquad \text{Equ.9.}$$

Many of the dichloroboronites, as already discussed, are not stable at room temperature.

The fission of mixed ethers does not give rise to chlorides from both groups of the ether. Alkyl-aryl ethers always give alkyl chloride and aryl dichloroboronite. This is in agreement with the general rule that fission of the carbon-oxygen bond takes place at the carbon atom capable of the greatest electron attainment. Therefore, mixed dialkyl ethers always give the chloride of the more electron releasing of the two groups. Diaryl ethers do not react with boron trichloride.[15] Diglyme, $CH_3 (OCH_2 CH_2)_2 OCH_3$, reacts with boron trichloride[16] to give initially methyl chloride and a dichloroboronite, $CH_3 (OCH_2 CH_2)_2 OBCl_2$.

The mechanism proposed by Gerrard for the fission of mixed ethers with boron trichloride involves the formation of a carbonium ion by an S_N1 reaction as in Equ.10.

$$ROR' + BCl_3 \rightleftharpoons \underset{\cdot\cdot}{ROR'}\!\!:\!BCl_3 \longrightarrow R'^{(+)} + Cl^{(-)} + ROBCl_2$$

$$R'^{(+)} + Cl^{(-)} \longrightarrow R'Cl \qquad \text{Equ.10.}$$

The fact that the more electron releasing group is always found in the alkyl halide supports this mechanism. Further

evidence is obtained from a study of the racemisation and rearrangement of some alkyl groups in the alkyl chloride products.

The reactions in boron trichloride-cyclic ether systems have also been investigated.[10] Interaction of equimolecular proportions of tetrahydropyran or tetrahydrofuran and boron trichloride at -80° produces a solid 1:1 complex. The tetrahydropyran-boron trichloride complex is more thermally stable that the tetrahydrofuran-boron trichloride complex, but both decompose when heated according to the following equation.

$$3\ (\overline{CH_2)_n}O.\ BCl_3 \longrightarrow 3Cl(CH_2)_n \cdot OBCl_2 \rightarrow 3Cl(CH_2)_n Cl + (BOCl)_3$$

Equ.11.

where n = 4 or 5.

However, when boron trichloride and a cyclic ether are reacted together in the proportions 1:6 and excess ether removed, ring opening occurs. After methanolysis of the products tetrahydropyran gives 4 (4' - chlorobutoxy) butan-1-ol (Equ.12.).

$$BCl_3 + 6\ (\overline{CH_2)_4}O \rightarrow \left[Cl(CH_2)_4O(CH_2)_4O\right]_3\ B \xrightarrow{MeOH}$$

$$3Cl.(CH_2)_4O.(CH_2)_4.OH$$

Equ.12.

Some monomers and higher polymers are also present. Tetrahydropyran gives a mixture of 5 (5'-chloropentoxy) pentan-1-ol and 5-chloro-pentan-1-ol.

The mode of decomposition of carboxylic esters with

boron trichloride is dependent upon the nature of the alcohol residue.[17,18] With derivatives of primary alcohols (~~R'OH in Equ.13~~) acyl-oxygen fission occurs, giving a dichloroboronite and an acyl chloride (Equ.13).

$$RCOOR^1 + BCl_3 \longrightarrow RCOCl + R^1OBCl_2 \qquad \text{Equ.13.}$$

Methanolysis of this product gives the free alcohol. Treatment of esters of secondary and tertiary alcohols with boron trichloride results in alkyl-oxygen fission (Equ.14) with the formation of alkyl chloride, acyl chloride and dichlorodiacetyl diborate. This is probably due to the instability of the dichloroboronites of the secondary and tertiary alcohols.

$$3\ RCOOR^1 + 2\ BCl_3 \longrightarrow RCOOBClOBClOCOR + RCOCl + 3R^1Cl \qquad \text{Equ.14.}$$

Boron tribromide has not been used as a degradative reagent in the carbohydrate field. However, like boron trichloride it has been used for the fission of simple alkyl and alkyl aryl ethers.[19] When boron tribromide and the ether are reacted in the proportions of one mole. of boron tribromide to three moles. of ether, the overall reaction may be expressed by Equ. 15.

$$3\ ROR' + BBr_3 \longrightarrow (RO)_3B + 3R'Br \qquad \text{Equ.15.}$$

Hydrolysis of the borate gives an alcohol or a phenol and boric acid (Equ.16).

$$(RO)_3\ B + 3H_2\ O \longrightarrow 3ROH + H_3BO_3 \qquad \text{Equ.16.}$$

When reacted with boron tribromide mixed alkyl aryl ethers always give alkyl bromide and phenol.

One Lewis acid, namely aluminium chloride, has been used widely in the carbohydrate field, but not as a degradative reagent. It has been used in three quite different types of reaction.

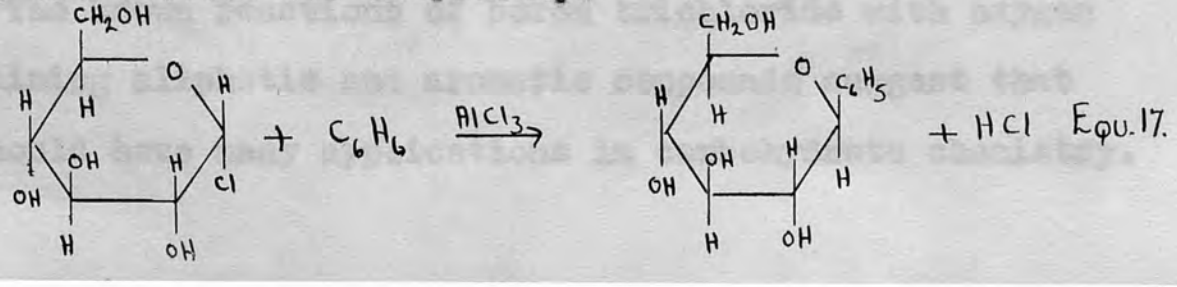
The first type of reaction is that in which an acetylated sugar is converted into a glycosyl chloride. In many reactions of this type a mixture of aluminium chloride and phosphorus pentachloride was used. In 1901 von Arlt[20] and Skraup and Kremann[21] showed that penta-O-acetyl-D-glucose and penta-O-acetyl-D-galactose were converted into the corresponding tetra-O-acetyl-D-glycosyl chlorides by a mixture of aluminium chloride and phosphorus pentachloride. The same mixture of reagents was used to convert 6-chloro-6-deoxy-1,2,3,4 - tetra-O-acetyl-β-D-glucose into 6-chloro-6-deoxy-2,3,4 - tri-O-acetyl-α-D-glucosyl chloride.[22] The solvent was chloroform, but acetyl chloride has also been used as a solvent in this type of reaction.[23] Bonner showed that aluminium chloride alone is capable of converting a fully acetylated monosaccharide into the corresponding acetylated glycosyl chloride.[24]

While investigating the reaction of aluminium chloride with octa-O-acetyllactose in chloroform solution, Kunz and Hudson found in addition to the expected product, hepta-O-acetyl-α-D-lactosyl chloride, a second product.[25] This was later shown to be a derivative of 4-O-β-D-galacto-pyranosyl-α-D-altrose.[26] Inversion of configuration had taken

place at $C_{(2)}$ and $C_{(3)}$ of the glucose portion of the lactose molecule.

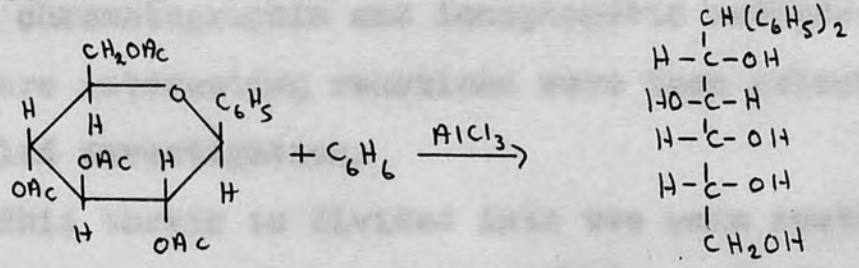
A further example of inversions of configuration with carbohydrate molecules using aluminium chloride was obtained by Hudson.[27] He found that fully acetylated cellobiose on treatment with aluminium chloride gave hepta-O-acetyl-α-D-cellobiosyl chloride and the corresponding derivative of 4-O-β-D-glucopyranosyl-α-D-altrose. Inversion of configuration had again taken place at $C_{(2)}$ and $C_{(3)}$ of the reducing glucose residue.

The third type of reaction in which aluminium chloride has been used in carbohydrate chemistry is the catalytic glycosylation of aromatic hydrocarbons. Hurd and Bonner performed a Friedel-Crafts reaction between aromatic hydrocarbons and tetra-O-acetyl-α-D-glucosyl chloride with aluminium chloride as catalyst.[28] As aluminium chloride reacts with the ester functions of tetra-O-acetyl-α-D-glucosyl chloride in the presence of benzene, at least 5 moles. of aluminium chloride are required. The theoretical amount is 8 moles. in addition to the catalytic amount required for the glycosylation reaction. When 5 moles. of aluminium chloride are used the product, after acetylation, is tetra-O-acetyl-β-D-glucopyranosylbenzene. In Equ.17. the O-acetyl groups are omitted for simplicity.



Equ. 17.

However, when the theoretical amount of aluminium chloride is used a second product is formed, namely, 1,1-diphenyl-1-deoxy-D-glucitol hydrate. This product is also obtained by heating a benzene solution of tetra-O-acetyl-β-D-glucopyranosylbenzene with aluminium chloride (Equ.18.)



Equ.18.

Recently aluminium chloride has been used to effect fission of cyclic acetals and ketals of hexitols.[29]

Titanium tetrachloride has important applications in carbohydrate chemistry. It has been used to effect fission of certain types of anhydro rings.[30,31]

2,3,4-Tri-O-acetyl-1,6-anhydro-β-D-glucose gives 2,3,4-tri-O-acetyl-α-D-glucosyl chloride when heated with excess titanium tetrachloride in chloroform. Zemplen and Csuros found that titanium tetrachloride had no effect on 1,6-anhydro-2,3,4-tri-O-benzoyl-β-D-glucose. Penta-O-benzoyl-β-D-glucose was however, converted by this reagent into 2,3,4,6-tetra-O-benzoyl-α-D-glucosyl chloride.

Like boron trifluoride, titanium tetrachloride has been used for the anomerisation of acetylated glycosides.[32]

The known reactions of boron trichloride with oxygen containing aliphatic and aromatic compounds suggest that it should have many applications in carbohydrate chemistry.

As no reactions of boron trichloride with carbohydrates and their derivatives had been reported previously, a general survey of the reactions of boron trichloride with many different types of carbohydrate derivatives was undertaken. Initially the products were identified by paper chromatographic and ionophoretic methods. Some of the more interesting reactions were then selected for detailed investigation.

This thesis is divided into two main sections. Degradative reactions are described and discussed in the first section and details of synthetic reactions of three different types are reported in the second section.

SECTION 1.

Degradative Reactions of Boron Trichloride.

Reactions of Boron Trichloride with Carbohydrate Derivatives.

Since boron trichloride co-ordinates readily with electronegative centres in the solvent molecule, it was found that few solvents were suitable for use in this type of reaction. Dichloromethane was found to be the most suitable as it is an inert and easily removable solvent. n-Pentane[6] and tetrahydropyran[16] have been used by other workers in similar systems.

The carbohydrate derivatives were dissolved or suspended in dichloromethane and cooled to -80° before addition of boron trichloride, since at this temperature boron trichloride (b.p. 12°) is a liquid,[33] which can be handled with ease. The reaction mixture was then allowed to warm from -80° to room temperature under anhydrous conditions. Carbohydrate derivatives which were initially insoluble in dichloromethane or were precipitated from solution at -80°, frequently dissolved as the reaction mixture warmed to room temperature. The derivatives which remained insoluble were usually recovered unchanged at the end of the reaction. Excess boron trichloride, dichloromethane and any volatile products were removed after the reaction mixture had been maintained at room temperature for 16-18 hr. under anhydrous conditions. The residue was treated with either commercial methanol, which contains a small amount of water, or an aqueous suspension

of silver carbonate. In most cases the products were analysed by paper chromatography using at least three different solvent systems and by paper ionophoresis in borate buffer at pH.10.

Demethylation of Carbohydrate Derivatives with Boron Trichloride.

Cleavage of mixed ethers can be achieved by reaction with boron trichloride.[13] The initial products of reaction are alkyl chloride and a dichloroboronite (Equ.9.). As already mentioned decomposition of the dichloroboronite may occur. However, methanolysis of any boron derivative of an alcohol gives the free alchhol. Similarly, boron tribromide may be used for ether cleavage.[19]

By analogy with these results, it should be possible to remove alkyl and related groups from a substituted carbohydrate molecule by treatment with boron trichloride, followed by methanolysis of the dichloroboronite formed.

Methyl α-D-glucoside, the simplest compound of this type, reacted with boron trichloride to give glucose and some higher saccharides (Table 2.). The glycosidic limk however, is known to be acid labile.[34] An O-methyl group in a non-reducing position of glucose (i.e. 2,3,4 or 6) may be removed only by more drastic treatment.[35,36] The most usual method is to heat the methylated compound with hydrobromic acid in a sealed tube at 100°. Reaction at this temperature freqaently leads to extensive decomposition.[37]

2,3,4,6-Tetra-O-methyl-D-glucose yielded D-glucose as the principal product on treatment with boron trichloride under a variety of reaction conditions (Expt.7.). When the reaction time at room temperature was less than 1 hr.

the partially methylated products predominated. The products were identified by paper chromatography and paper ionophoresis by comparison with authentic compounds. The mono-O-methylglucoses were isolated by paper chromatographic separation on sheets of thick filter paper (Whatman No.3) eluted with solvent system 1 (Expt.1.), and then separated by ionophoresis in borate buffer at p.H.10. (Expt.9.). The mono-O-methylglucoses had M_G values of 0.21 and 0.81 with values of 0.26 and 0.82 in a duplicate experiment. This indicated the presence of either or both 2-O-methyl-D-glucose (M_G 0.23) and 4-O-methyl-D-glucose (M_G 0.24), and of either or both 3-O-methyl-D-glucose (M_G 0.82) and 6-O-methyl-D-glucose (M_G 0.82).[38] Similarly a mixture of di-O-methylglucoses was obtained (Expt.9.). It seems unlikely therefore that boron trichloride can be used for selective demethylation. This is confirmed by the fact that on treatment with boron trichloride methyl 4,6-O-benzylidene - 2, 3-di-O-methyl-α-D-glucoside gives in addition to glucose, both 2-O-methyl-D-glucose and 3-O-methyl-D-glucose (Expt.10.).

In order to eliminate the formation of partially methylated products two treatments with boron trichloride are usually required (Expt.10.). Boron tribromide effects almost complete demethylation in a single treatment (Expt. 8.).

A wide range of methylated monosaccharides were reacted with boron trichloride as in Expt.5. In each case

the corresponding monosaccharide was obtained as the principal product (Table 1.). Other products were frequently present in trace amounts only. With few exceptions these secondary products were partially methylated derivatives and were identified chromatographically by comparison with authentic compounds. When all the partially methylated sugars were not available for comparison, as in the case of derivatives of arabinose and mannose, the R_G values suggested this type of compound.[39]

Methylated amylopectin and methylated cellulose on reaction with boron trichloride gave glucose with some mono- and di-O-methylglucoses (Table 1.).

From Table 1. it will be seen that the di-O-methylsucroses did not yield both the constituent monosaccharides as the principal products when reacted with boron trichloride. In this case glucose and 5-hydroxymethylfurfuraldehyde were the main products. Fructose and unchanged di-O-methylsucroses were present as secondary products. The formation of 5-hydroxymethylfurfuraldehyde from fructose and other ketoses and their derivatives is a standard feature of their reaction with boron trichloride or boron tribromide. This reaction is discussed in detail later.

2,3,4,6-Tetra-O-benzyl-D-glucose gave glucose as the chief product on treatment with boron trichloride. Trace quantities of a product having an $R_{Glucose}$ value of 2.1 in solvent system 1 (Expt.1.) were also formed.

TABLE 1

Substance	Principal Products	Other Products *
2,3,5-Tri-O-methyl-L-arabinose	Arabinose	Oligosaccharides, 2.7+, 3.8+.
2,4-Di-O-methyl-L-arabinose	Arabinose	1.4+.
Methyl 2,3,4-tri-O-methyl-α-L-fucoside	Fucose	0.91+ (R_F value).
2-O-Methyl-L-fucose	Fucose	Oligosaccharides, 2-O-methylfucose+.
2,3,4,6-Tetra-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose	Galactose, rhamnose	0.88+ (R_F value).
Methyl 2,4-di-O-methyl-β-D-galactoside	Galactose	Mono-O-methyl galactoses.
2,3,4,6-Tetra-O-methyl-D-glucose	Glucose	Mono-, di- and tri-O-methylglucoses.
2,3,6 - Tri-O-methyl-D-glucose	Glucose	Mono - and di - O-methylglucoses, 0.77+ (R_G value).
Methyl 2,3-di-O-methyl-α-D-glucoside	Glucose	Mono -O-methyl-glucoses
3-O-Methyl-D-glucose	Glucose	3-O-methylglucose+
2,3,4,6-Tetra-O-methyl-D-mannose	Mannose	0.37, 0.52, 0.61+, 0.74, 0.93 (R_G values).

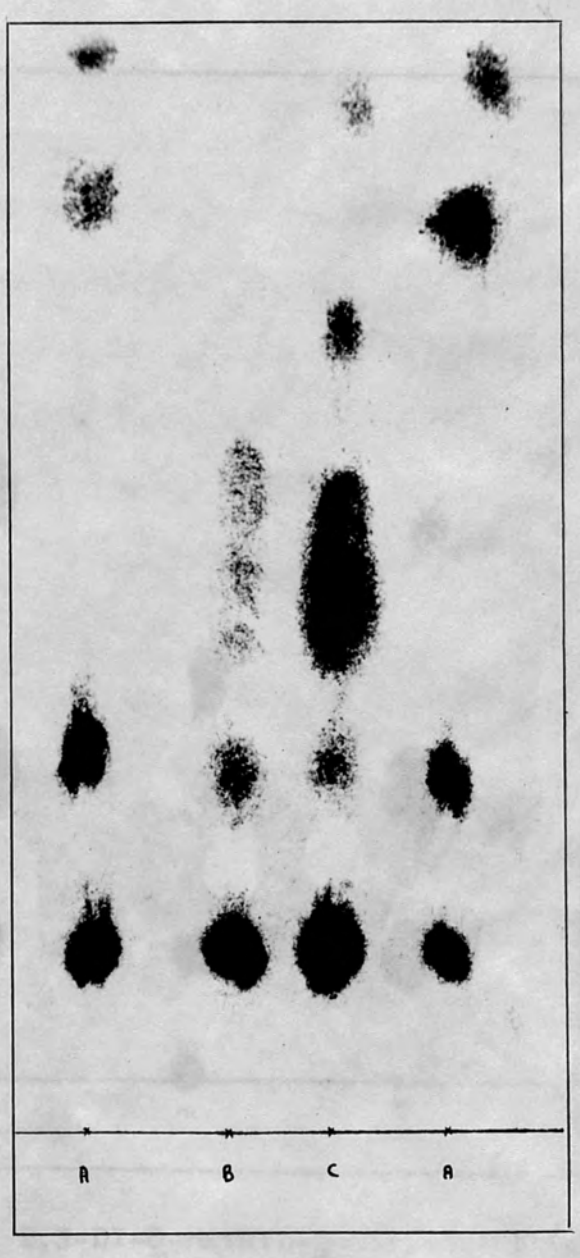
3,4-Di-O-methyl-D-mannose monohydrate	Mannose	0.40,0.56 (R_G values)
2,3,4-Tri-O-methyl-D-xylose	Xylose	-
3-O-Methyl-D-xylose	Xylose	-
Methylated Amylopectin	Glucose	Mono-and di-O-methylglucoses
Methylated cellulose	Glucose	Mono-and di-O-methylglucoses.
2,3,4,6-Tetra-O-benzyl-D-glucose	Glucose	2.1+
Di-O-methylsucroses	Glucose 5-hydroxymethyl-furfuraldehyde	Fructose+ di-O-methylsucroses+

* Unless otherwise stated unidentified products are indicated by R_X values in solvent system 1 (Expt.1.), where X refers to the principal products in each case.

\+ Trace amounts only present.

FIGURE 1

THE REACTION BETWEEN BORON TRICHLORIDE AND 2,3,4,6-TETRA-O-METHYL-D-GLUCOSE.

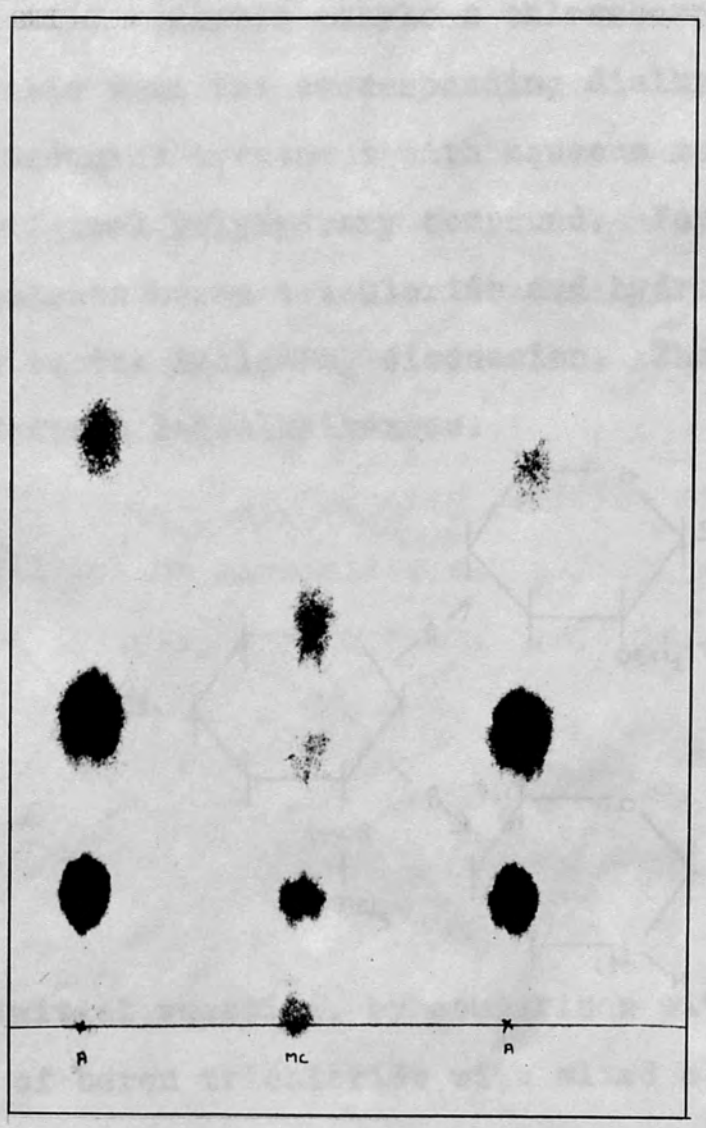


A. . . . 2,3,4,6-TETRA-, 2,3,6-TRI-, 3-O-METHYL-D-GLUCOSE.

B,C. . . . PRODUCTS OF REACTION OF BORON TRICHLORIDE AND 2,3,4,6-TETRA-O-METHYL-D-GLUCOSE.

FIGURE 2

THE REACTION BETWEEN BORON TRICHLORIDE AND METHYL CELLULOSE

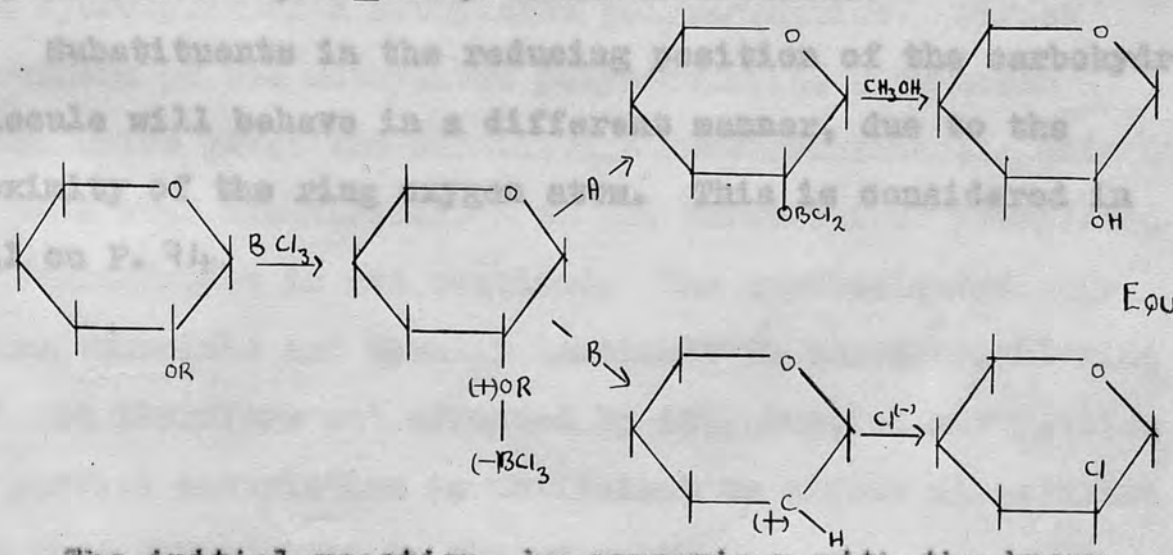


A. . . . 2,3-DI-O-METHYL-D-GLUCOSE; 3-O-METHYL-D-GLUCOSE AND GLUCOSE

MC. . . . PRODUCTS OF REACTION OF BORON TRICHLORIDE AND METHYL CELLULOSE.

Mechanism of Dealkylation of Carbohydrate Derivatives by Boron Trichloride.

Reaction between the unsubstituted hydroxyl groups, and boron trichloride will probably result in the formation of dichloroboronites and cyclic chloroboronates. The latter will resemble ethylene chloroboronate, which is more stable than the corresponding dialkyl chloroboronates. Subsequent treatment with aqueous methanol will give the original polyhydroxy compound. For simplicity reaction between boron trichloride and hydroxyl groups is omitted in the following discussion. The mechanism is considered for a 2-O-alkylhexose.



Equ. 19.

The initial reaction, by comparison with the known reactions of boron trichloride with mixed alkyl ethers, will be the formation of a bond between the ether oxygen and boron atoms. Fission of the carbon-oxygen bond can then occur, followed by the elimination of either alkyl chloride (Equ.19A) or alkoxy dichloroboronite (Equ.19B).

In the former case a carbohydrate dichloroboronite will be formed, which on methanolysis will give the unsubstituted carbohydrate. As the $C_{(2)}$- O bond remains intact there is no possibility of a stereochemical change. In the second case (Equ.19B) fission of the $C_{(2)}$ - O bond will occur. A carbonium ion will be formed and this will subsequently react with a chloride ion to give a chlorodeoxy sugar. There is the possibility of a Walden inversion in this case. As chlorodeoxy compounds are stable to methanolysis[40] reaction B would not give the products found experimentally. Reaction A is therefore the most probable course of reaction between an alkylated carbohydrate and boron trichloride.

Substituents in the reducing position of the carbohydrate molecule will behave in a different manner, due to the proximity of the ring oxygen atom. This is considered in full on P. 94.

Some Applications of Boron Trichloride Demethylation

As there is no evidence of stereochemical change in any of the reactions investigated, this method may be used for the identification of the parent monosaccharides of fully or partially methylated carbohydrates.

It has recently been used successfully in this way for the identification of O - methyl ethers of rhamnose and fucose present in certain glycolipids.[41]

This method is of particular use in the identification of the monosaccharides, which are present in the often complex mixture of methylated monosaccharides, obtained by the hydrolysis of a methylated polysaccharide. Direct treatment of the methylated polysaccharide with boron trichloride gives the constituent monosaccharides. This may be used when identification of the intermediate methylated monosaccharides is not required. The unsubstituted polysaccharides are usually insoluble in boron trichloride and are therefore not affected by it. Partial methylation or partial acetylation is sufficient to effect dissolution and then degradation in the reagent.

A most interesting application is the use by Foster et. al. of an O - methyl ether group as a blocking agent in the preparation of 2 - amino - 2 - deoxy - L - threitol and its removal with boron trichloride.[42] Selective N - acetylation of 2 - amino - 2 - deoxy - 3 - O - methyl - D - glucose

hydrochloride gives 2 - acetamido - 2 - deoxy - 3 - O - methyl - D - glucose, which is then reduced by sodium borohydride to give 2 - acetamido - 2 - deoxy - 3 - O - methyl - D - glucitol. From this by periodate oxidation and reduction 2 - acetamido - 2 - deoxy - 3 - O - methyl - L - threitol is obtained. Acid hydrolysis gives 2 - amino - 2 - deoxy - 3 - O - methyl - L - threitol hydrochloride. This compound is smoothly demethylated by boron trichloride to give 2 - amino - 2 - deoxy - L - threitol hydrochloride.

The same workers found that 2 - amino - 2 - deoxy - D - glucose hydrochloride was unaffected by boron trichloride and that its 3 - O - methyl ether was demethylated without the incursion of side reactions. However, treatment of 2 - acetamido - 2 - deoxy - D - glucose with boron trichloride resulted in some decomposition in addition to de - N - acetylation.

Reactions of Boron Trichloride with Glycosides.

As already mentioned reaction of boron trichloride with methyl α - D - glucoside followed by methanolysis gives glucose as the main product. In addition several oligosaccharides are formed and these suggest the use of boron trichloride as a synthetic agent as well as a degradative one. Coordination of boron trichloride with the glycosidic oxygen atom at $C_{(1)}$ followed by fission of the $C_{(1)}$ - O bond will give a carbonium ion. This ion will be resonance stabilised due to the proximity of the pyranose ring oxygen atom. Further reaction with chloride ion will give glucosyl chloride. This reaction is discussed in detail on p. 94.

In Section 2 details are given of the condensation of the product of reaction of methyl α - D - glucoside and boron trichloride with glucose, benzene, alcohols and phenols to give disaccharides, glucopyranosylbenzene and glucosides.

A variety of glycosides were reacted with boron trichloride as in Expt. 5. The results are reported in Table 2. In each case the product was the parent monosaccharide, with the exception of methyl α - D - fructofuranoside, which gave 5 - hydroxymethylfurfuraldehyde in addition to fructose.

The monosaccharides were identified by paper chromatography using three or four different solvent systems

and by paper ionophoresis in borate buffer at pH 10 (Expt.2). In the case of the glycosides of phenols the corresponding phenol was also identified by paper chromatography using the diazotised nitroaniline spray reagent (Spray 13. Expt. 1.).

Table 2

Substance	Products
Methyl β- D - arabinoside	Arabinose, methyl β- D - arabinoside
Methyl α- D - fructofuranoside	Fructose, 5-hydroxymethylfurfuraldehyde
Methyl α- D - galactoside	Galactose
Methyl β- D - galactoside	Galactose
Methyl α- D - glucoside	Glucose, oligosaccharides+
Methyl β- D - glucoside	Glucose
Phenyl α- D - glucoside	Glucose, phenol
Phenyl β- D - glucoside	Glucose, phenol
Quinol β- D - glucoside (arbutin)	Glucose, quinol
o - Hydroxymethylphenyl - β - D - glucoside (salicin)	Glucose, saligenin
Methyl α- D - mannoside	Mannose, oligosaccharides+
Methyl α- L - rhamnoside	Rhamnose
Methyl β- D - riboside	Ribose
Methyl α- L - xylofuranoside	Xylose

+ Trace amounts only present.

Unless otherwise stated glycosides are in the pyranose form.

Reactions of Boron Trichloride with Cyclic Acetals and Ketals

The reaction between boron trichloride and the carbohydrate cyclic acetals and ketals was found to proceed smoothly to give the parent monosaccharides (Table 3). The notable exception was 2, 3 : 4, 5 - di - O - isopropylidene - D - fructose, which gave 5-hydroxymethylfurfuraldehyde and only a small amount of fructose.

Di - O - isopropylidene pentaerythritol gave pentaerythritol, isolated in 63% yield (Expt. 11).

Table 3

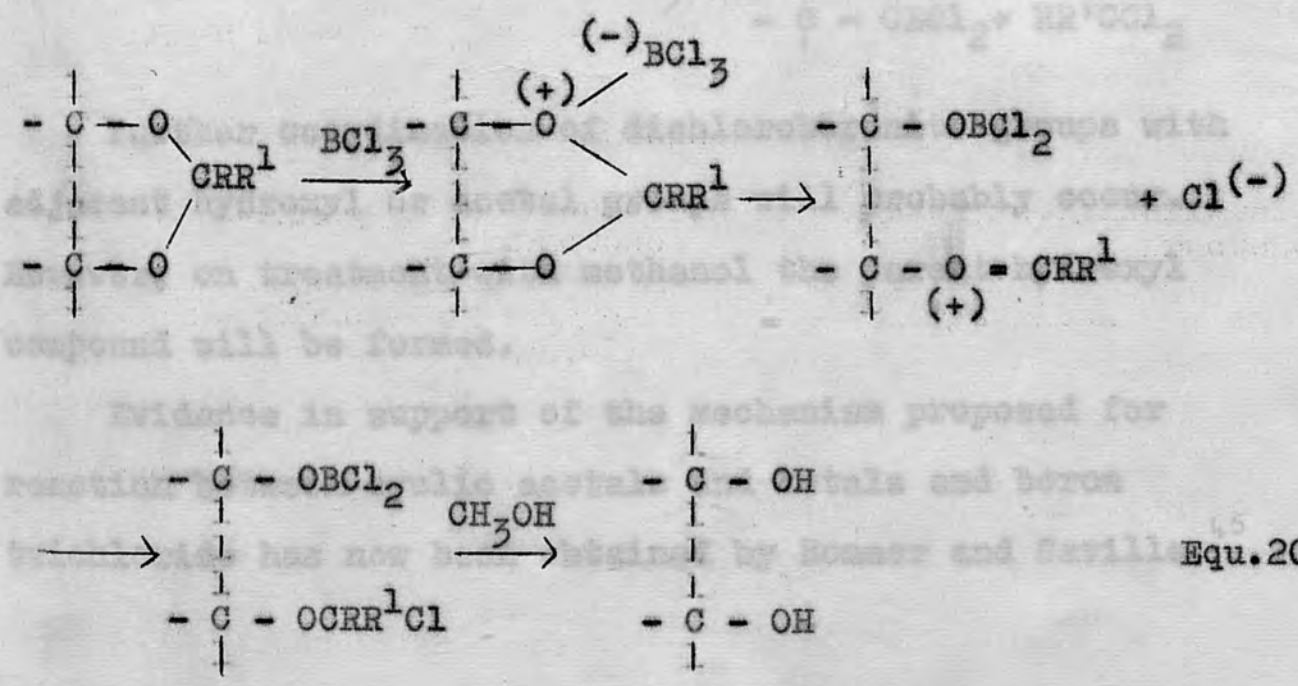
Substance	Products
4,6-O- Benzylidene - D - glucose	Glucose
Methyl 4,6- O - benzylidene - α - D - glucoside	Glucose
1,2 : 5,6 - Di - O - isopropylidene - D - glucose	Glucose
1,2-O - isoPropylidene - D - glucose	Glucose
2,3 : 4,5- Di-O-isopropylidene - D - fructose	5-Hydroxymethylfurfuraldehyde x fructose x
Di-O-isopropylidene pentaerythritol	Pentaerythritol

x Trace amount present.

Mechanism of the Reaction of Boron Trichloride with Cyclic Acetals and Ketals

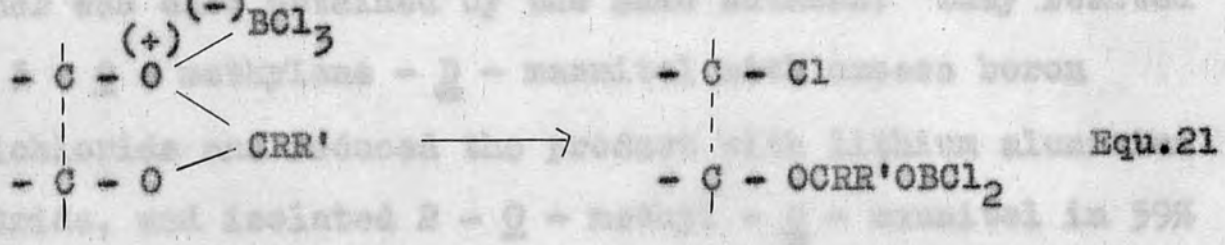
The reaction of boron trichloride with cyclic acetals and ketals will be similar to the known reactions of boron trichloride with aliphatic and cyclic ethers.[10,12,13,14]

Initial coordination of boron trichloride with either of the acetal or ketal ring oxygen atoms is possible. This, by comparison with the ether reactions, will be followed by fission of the carbon - oxygen bond and ring opening will then occur (see Equ. 20). The carbonium ion formed will then react with a chloride ion to give an α - chloro ether. Treatment with aqueous methanol will result in the hydrolysis of both the dichloroboronite[10] and α - chloro ether groups[43,44] to give the free hydroxyl compound.



Equ.20.

Chlorodeoxy compounds would be stable to methanolysis and as no chlorodeoxy compounds were detected ring opening of the type shown in Equ. 21 may be neglected.



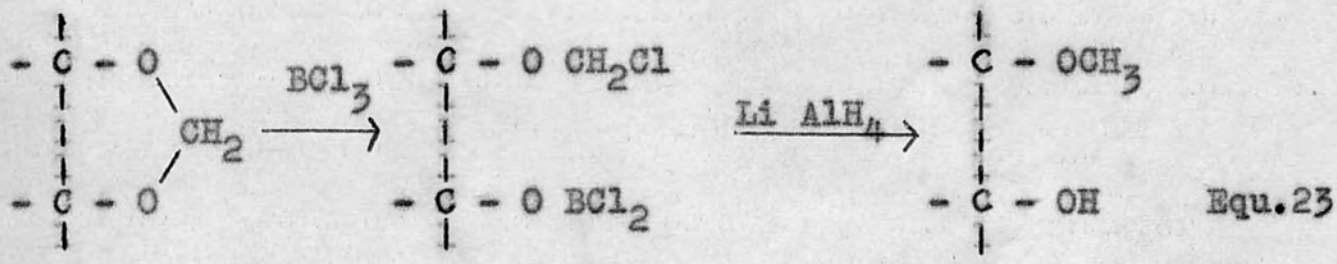
Equ.21

A further reaction which should be considered is that between an α - chloro ether group and excess boron trichloride. The two possible modes of fission would give rise to a chlorodeoxy group or a dichloroboronite (Equ. 22) and for reasons already given the former may be neglected.

Further coordination of dichloroboronite groups with adjacent hydroxyl or acetal groups will probably occur. However, on treatment with methanol the parent hydroxyl compound will be formed.

Evidence in support of the mechanism proposed for reaction between cyclic acetals and ketals and boron trichloride has now been obtained by Bonner and Saville.[45]

They isolated benzylidene chloride from the reaction of boron trichloride and benzylidene derivatives of hexitols. Evidence for the formation of an intermediate α - chloro ether was also obtained by the same authors. They reacted 2, 5 - O - methylene - D - mannitol with excess boron trichloride and reduced the product with lithium aluminium hydride, and isolated 2 - O - methyl - D - mannitol in 59% yield.



Equ.23

Deacetylation of Carbohydrate Derivatives with Boron Trichloride

Treatment of several acetylated mono -, di - and polysaccharides with boron trichloride gave the constituent monosaccharides as the chief products (Table 4). In most cases these were accompanied by partially acetylated products. Complete deacetylation was accomplished by two treatments with boron trichloride.

Octa - O - acetylsucrose gave glucose and 5 - hydroxymethylfurfuraldehyde on treatment with boron trichloride.

Tetra - O - acetylpentaerythritol gave pentaerythritol, which was isolated in 93% yield and was accompanied by trace amounts of partially acetylated products. (Expt. 12).

Table 4

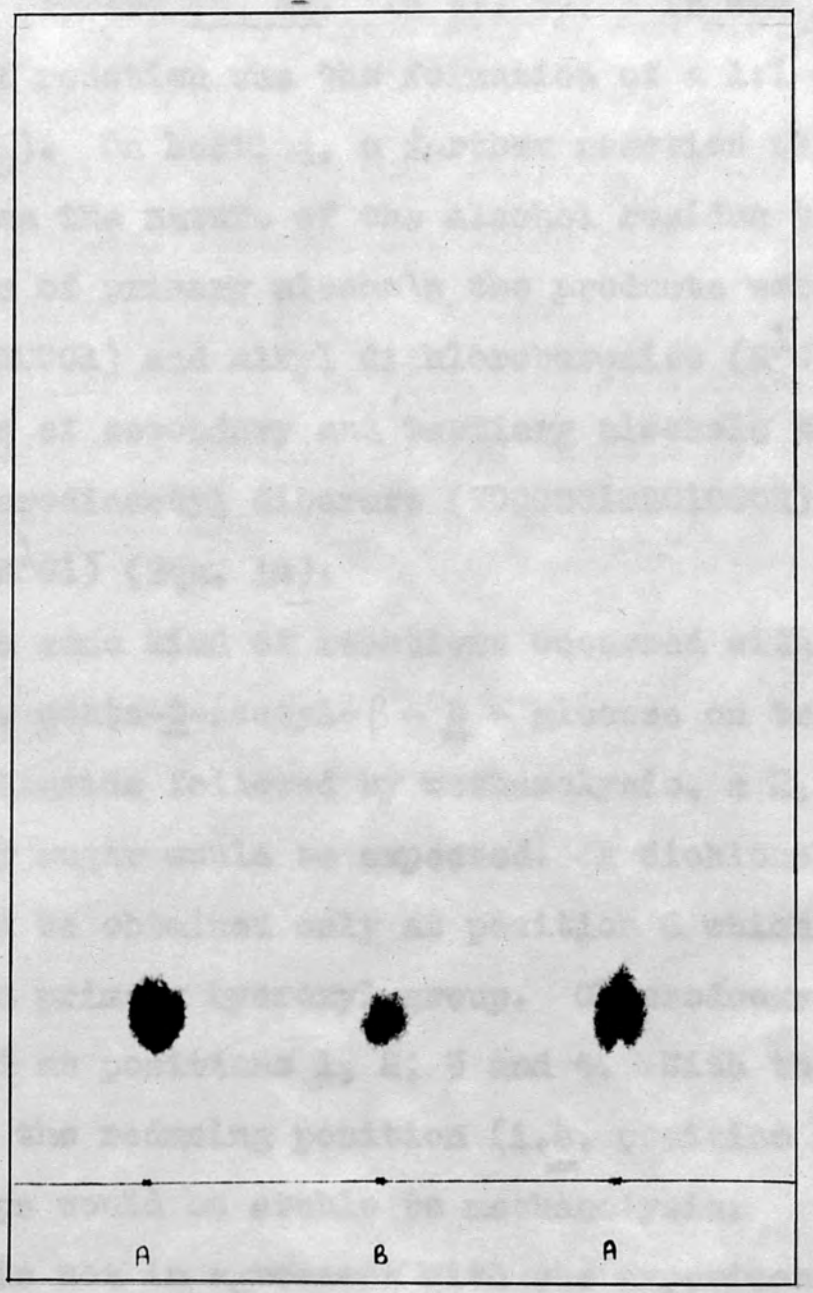
Substance	Principal product	Other products
Octa - O - acetylgentiobiose	Glucose	Gentiobiose, 1.15, 1.5 (solvent 2 Expt. 1).
Penta - O - acetyl -β- D - glucose	Glucose	2.7, 5.0+
Octa - O - acetylsucrose	Glucose, 5 - hydroxy-methylfurfuraldehyde	2.4+
Acetylated amylopectin	Glucose	Oligosaccharides, 1.9, 2.2.
Acetylated cellulose	Glucose	Oligosaccharides, 1.9, 2.2.
Tetra - O - acetylpentaerythritol	Pentaerythritol	1.4+, 1.8+

+ Present in trace amounts.

Unidentified products are indicated by R_X values in solvent system 1 (Expt. 1), where X refers to the principal product in each case.

FIGURE 3

THE REACTION BETWEEN BORON TRICHLORIDE AND 2,3,4,6-TETRA -O-ACETYL-β-D-GLUCOSE.



A. . . . . GLUCOSE

B . . . . . PRODUCT OF REACTION OF BORON TRICHLORIDE AND 2,3,4,6-TETRA-O-ACETYL-β-D-GLUCOSE

Mechanism of Deacetylation by Boron Trichloride

The fission of esters of carboxylic acids has been studied by Gerrard et. al.[17,18] In all cases it was found that the initial reaction was the formation of a 1:1 complex ($RCOOR^1.BCl_3$). On heating, a further reaction which was dependent on the nature of the alcohol residue took place. With esters of primary alcohols the products were the acyl chloride (RCOCl) and alkyl dichloroboronite (R^1OBCl_2) (Equ.13). With esters of secondary and tertiary alcohols the products were dichlorodiacetyl diborate (RCOOBClOBClOCOR) and alkyl chloride (R^1Cl) (Equ. 14).

If the same kind of reactions occurred with an acetylated hexose e.g. penta-O-acetyl-β-D-glucose on treatment with boron trichloride followed by methanolysis, a 2, 3, 4 - tri - chlorodeoxy sugar would be expected. A dichloroboronite group would be obtained only at position 6 which initially possessed a primary hydroxyl group. Chlorodeoxy groups would be obtained at positions 1, 2, 3 and 4. With the exception of that at the reducing position (i.e. position 1) of glucose, these groups would be stable to methanolysis.

This is not in agreement with the experimental results for treatment of acetylated sugars with boron trichloride. The principal product is always the parent sugar, so it appears that all acetyl derivatives of sugars behave in the

same way as derivatives of primary alcohols.

Gerrard proposed that the difference in behaviour between the esters of primary and secondary or tertiary alcohols was due to the instability of the dichloroboronites of the last two.

The behaviour of the sugar derivatives may be due to the formation of dichloroboronites, which have a stability similar to that of the primary alcohols. However, a more feasible explanation is the formation of stable cyclic chloroboronates or borates. These on methanolysis would yield the original hydroxyl compound.

Treatment of Mono - and Disaccharides with Boron Trichloride and Boron Tribromide

Many of the mono - and disaccharides investigated were almost insoluble in dichloromethane and did not become soluble on addition of boron trichloride. There was no evidence of any change after sucrose had been kept in a sealed ampoule with boron trichloride for two years (Expt.13). Similarly, D - glucose remained insoluble when heated under reflux with boron trichloride for 2 hr. at 45° (Expt. 14).

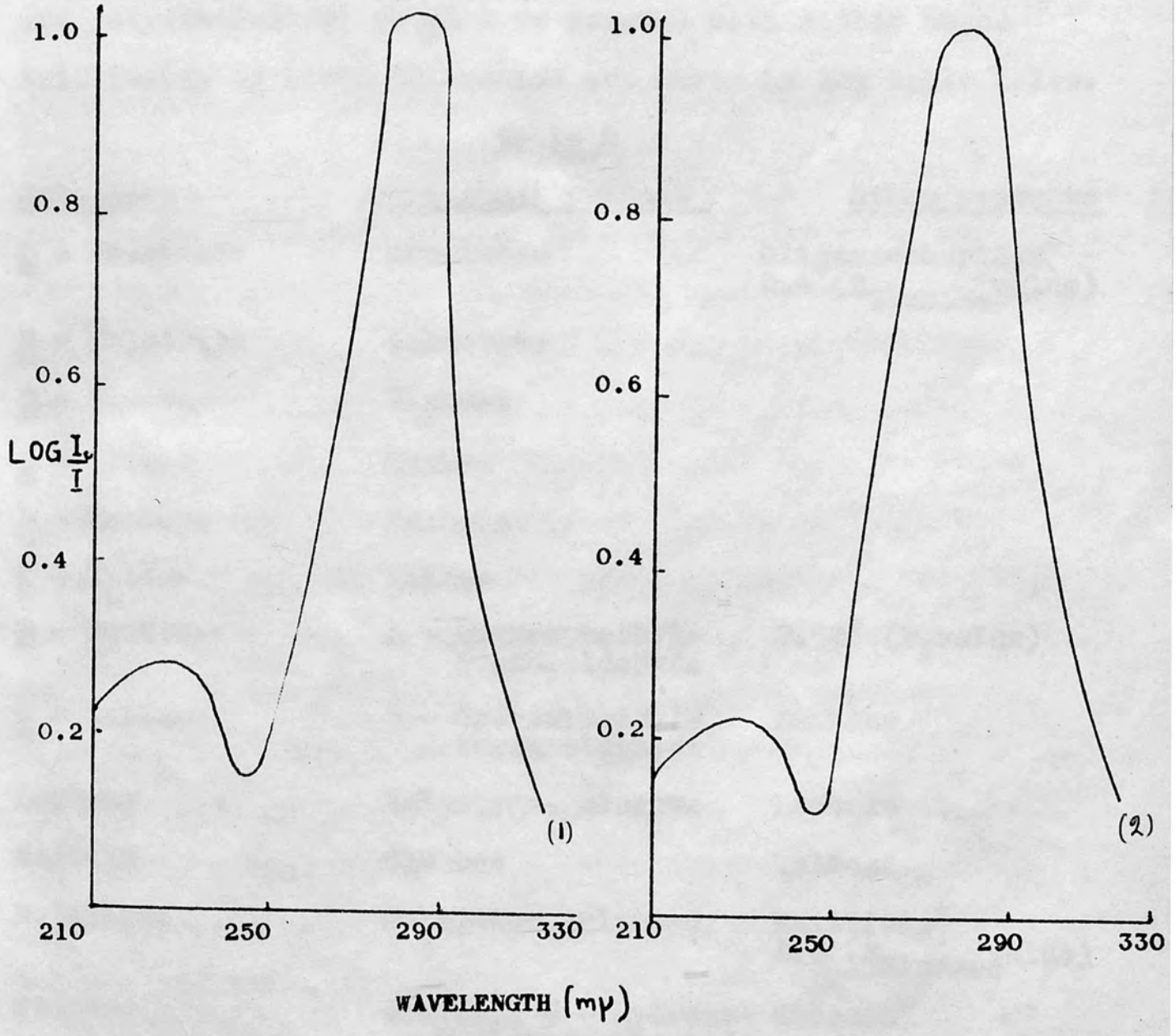
A few of the monosaccharides, e.g. L - arabinose, were soluble in boron trichloride, but were recovered almost unchanged at the end of the reaction. The notable exception was D - fructose, which was decomposed by boron trichloride to give 5 - hydroxymethylfurfuraldehyde (Expt. 16).

The inability of the disaccharides to react with boron trichloride was thought to be due solely to their insolubility. Attempts were made to use dimethyl sulphoxide and dimethyl formamide, in which some of the disaccharides were soluble, as solvents in place of dichloromethane. However, these attempts proved unsuccessful, as the solvents themselves reacted with boron trichloride (Expt. 15).

All the disaccharides which were investigated and L - sorbose reacted with boron tribromide to give the constituent monosaccharides, except where a ketose group was present in which case 5 - hydroxymethylfurfuraldehyde was formed.

FIGURE 4

THE ULTRAVIOLET ABSORPTION SPECTRA OF (1) THE PRODUCT OF REACTION OF D-FRUCTOSE AND BORON TRICHLORIDE AND (2) 5-HYDROXYMETHYL FURFURALDEHYDE



5 - Hydroxymethylfurfuraldehyde was isolated from the reaction of boron trichloride with D - fructose (Expt. 16). Its structure was established by comparison of its ultraviolet spectrum with that of an authentic specimen of 5 - hydroxymethylfurfuraldehyde[46] (as in Expt. 17) and conversion by mild oxidation into 5 - hydroxymethyl - 2 - furoic acid[47] (Expt. 18).

A list of mono- and disaccharides together with a tri - and polysaccharide, which were reacted with either boron trichloride or boron tribromide are given in the table below.

Table 5

Substance	Principal product	Other products
L - Arabinose	Arabinose	Oligosaccharides, 2.4^{+} ($R_{Glucose}$ value).
D - Galactose	Galactose	
D - Glucose	Glucose	
D - Lyxose	Lyxose	
D - Mannose	Mannose	
D - Xylose	Xylose	
D - Fructose	5 - Hydroxymethyl-furfuraldehyde	0.90^{+} (R_{F} value).
L - Sorbose	5 - Hydroxymethyl-furfuraldehyde	Sorbose.
Lactose	Galactose, glucose	Lactose.
Maltose	Glucose	Maltose.
Melibiose	Galactose, glucose	Melibiose, 1.6^{+} ($R_{Glucose}$ value).
Sucrose	Glucose, 5 - hydroxy-methylfurfuraldehyde	Sucrose.

Substance	Principal product	Other products
Turanose	Glucose, 5 - hydroxy-methylfurfuraldehyde	Fructose[+]
Raffinose	Galactose, glucose, 5 - hydroxymethylfurfur-aldehyde	Fructose[+]
Inulin	5 - Hydroxymethyl-furfuraldehyde.	

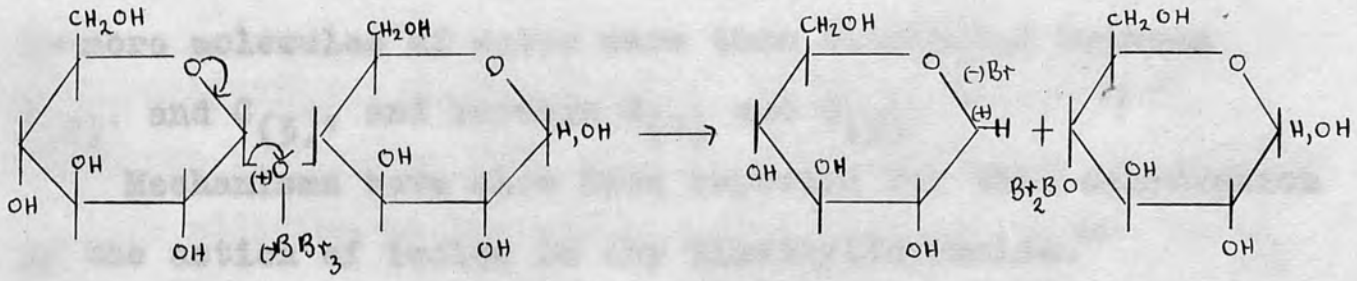
+ present in trace quantity.

Mechanism of the Reaction between Boron Tribromide and Disaccharides.

As the reaction of boron halides with the free hydroxyl groups of a carbohydrate molecule has been discussed elsewhere in this thesis, it will be omitted in the following treatment.

Coordination of boron tribromide with the glycosidic oxygen atom will occur, followed by fission of the bond between $C_{(1)}$ and the glycosidic oxygen atom. A glycosyl cation and a dibromoboronite will be formed. The hemi-acetal character of the glycoside will give rise to resonance stabilisation through the ring oxygen atom adjacent to $C_{(1)}$. The dibromoboronite group will be formed at the position in the second molecule previously participating in the disaccharide linkage. Treatment with methanol will give the two unsubstituted mono-saccharides.

The course of reaction of boron tribromide with maltose is shown in Equ. 24.

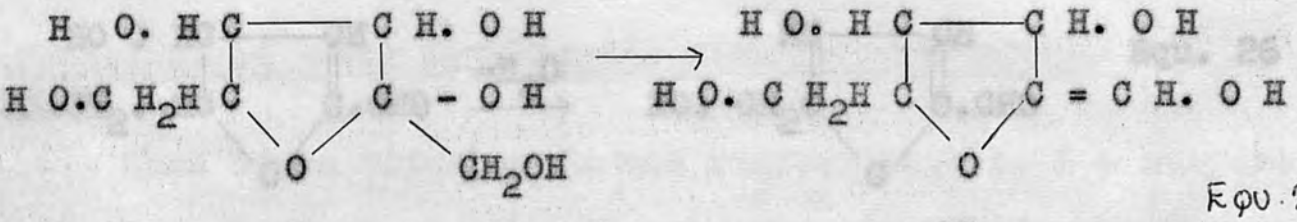


Equ. 24.

Fission of the bond between $C_{(x)}$ where x = 2, 3, 4 or 6 and the glycosidic oxygen atom is less likely to occur as the carbonium ion formed would not be resonance stabilised.

Mechanism of the Reaction between Boron Trichloride and D - Fructose

Kiermayer[48] found that either D - fructose or sucrose could be converted into 5 - hydroxymethylfurfuraldehyde by heating at 120° with 0.3% oxalic acid. Haworth and Jones[49] were able to show that when sucrose was used the 5 - hydroxymethfurfuraldehyde originated solely in the fructose portion of the molecule. They suggested that the process involved the elimination of three molecules of water from the furanose form of fructose (I in Equ. 25). The loss of the first molecule of water gave the intermediate II.



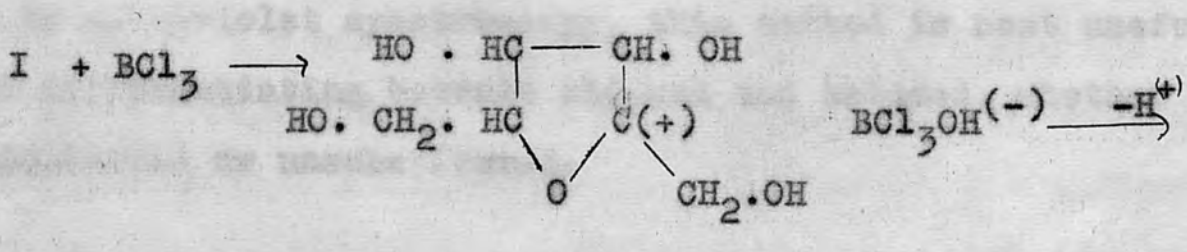
Equ. 25.

I II

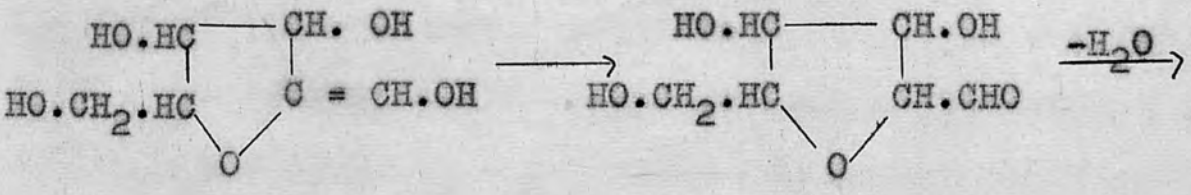
Two more molecules of water were then eliminated between $C_{(2)}$ and $C_{(3)}$, and between $C_{(4)}$ and $C_{(5)}$.

Mechanisms have also been reported for this dehydration by the action of iodine in dry dimethylformamide.[50]

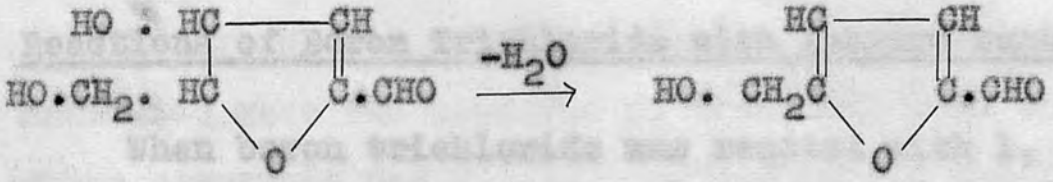
A similar scheme may be formulated for the interaction of D - fructose and boron trichloride. This is based on the initial formation of a carbonium ion at $C_{(2)}$ (III in Equ. 26). Elimination of a proton at $C_{(1)}$ then follows giving an aldehyde group (IV). This aids the elimination of two more molecules of water between $C_{(2)}$ and $C_{(3)}$ and between $C_{(4)}$ and $C_{(5)}$ giving 5 - hydroxymethylfurfuraldehyde, (V.)



III.



IV.



Equ. 26

V.

Some Applications of the Reaction of Boron Trichloride and Boron Tribromide with Mono -, Di - and Higher Saccharides

In addition to the use of boron trichloride and boron tribromide for the degradation of di -, tri - and higher saccharides, there is one other important application. As may be seen from the results given in the various tables aldoses survive unchanged after treatment with either boron trichloride or boron tribromide. Ketoses and ketose derivatives are decomposed by these boron halides to give 5 - hydroxymethylfurfuraldehyde. As 5 - hydroxymethylfurfuraldehyde may be easily detected by paper chromatography or by ultraviolet spectroscopy, this method is most useful for differentiating between aldoses and ketoses, whether substituted or unsubstituted.

Reactions of Boron Trichloride with Anhydro Sugars

When boron trichloride was reacted with 1, 6 - anhydro sugars as in Expt. 5. the parent mono-saccharide was invariably the principal product. Oligosaccharides were the only other products obtained.

The behaviour of the two 2, 3 - anhydro sugars investigated was found to be quite different. In the case of the 2, 3 derivatives no detectable quantities of the parent sugars were obtained. Reaction did however, take place and two products were obtained in each case. (Table 6). The most probable explanation is the formation of chlorodeoxy derivatives, which unlike the 1 - chlorodeoxy sugars, would not be hydrolysed by aqueous methanol.[40] Unfortunately the chlorodeoxy sugars were not available for comparison.

A similar type of reaction is that between hydrochloric acid and 2, 3 - anhydro sugars. Methyl 2, 3 - anhydro - 4, 6 - O - benzylidene - α - D - alloside with hydrochloric acid gave methyl 2 - chloro - 2 - deoxy - α - D - altroside and methyl 3 - chloro - 3 - deoxy - α - D - glucoside by substitution with inversion at $C_{(2)}$ and $C_{(3)}$ respectively.[51] The latter predominated. Similarly, methyl 2, 3 - anhydro - 4, 6 - O - benzylidene - α - D - mannoside gave methyl 3 - chloro - 3 - deoxy - α - D - altroside as the chief product with hydrochloric acid[52] and methyl 2, 3 - anhydro - β - D - riboside gave methyl 3 - chloro - 3 - deoxy - β - D - xyloside.[53]

Methyl 2, 3 - anhydro - 4, 6 - O - benzylidene - α - D - mannoside gave two products on treatment with boron trichloride. These compounds had $R_{Mannose}$ values of 2.3 and 2.5 in solvent 1 (Expt. 1). The former gave a yellow colour with the diphenylamine spray reagent[93] and this is characteristic of a 2 - substituted reducing sugar. A green-blue colour, characteristic of a 3 - or 6 - substituted reducing sugar, was obtained with the second product. The two products are probably 2 - chloro - 2 - deoxy - D - glucose and 3 - chloro - 3 - deoxy - D - altrose.

Table 6.

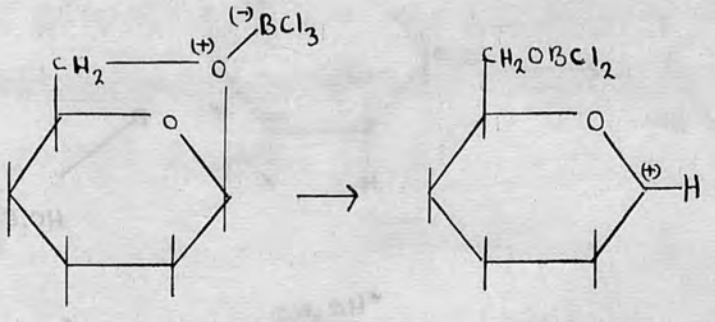
Substance	Principal product	Other products
1, 6 - Anhydro - β - D - galactopyranose	Galactose	Oligosaccharides.
1, 6 - Anhydro - α - D - galactofuranose	Galactose	Oligosaccharides.
1, 6 - Anhydro - β - D - glucopyranose	Glucose	Oligosaccharides.
1, 6 - Anhydro - β - D - gulopyranose	Gulose	Oligosaccharides.
1, 6 - Anhydro - β - D - mannopyranose	Mannose	Oligosaccharides.
Methyl 2, 3 - anhydro - 4, 6 - O - benzylidene - α - D - mannoside	2.3, 2.5 ($R_{mannose}$ values)	4.7+
Methyl 2, 3 - anhydro - β - L - riboside	0.7, 1.7 (R_{Ribose} values).	-

+ Trace quantity present.

Mechanism of the Reaction of Boron Trichloride with Anhydro Sugars

Coordination of boron trichloride with the anhydro ring oxygen atom will occur in the first instance. This will be followed by ring opening.

In the case of the 1, 6 - anhydro compounds fission of the bond between $C_{(1)}$ and the oxygen atom will occur giving a dichloroboronite group at $C_{(6)}$ and a resonance stabilised carbonium ion at $C_{(1)}$. (Equ. 27).



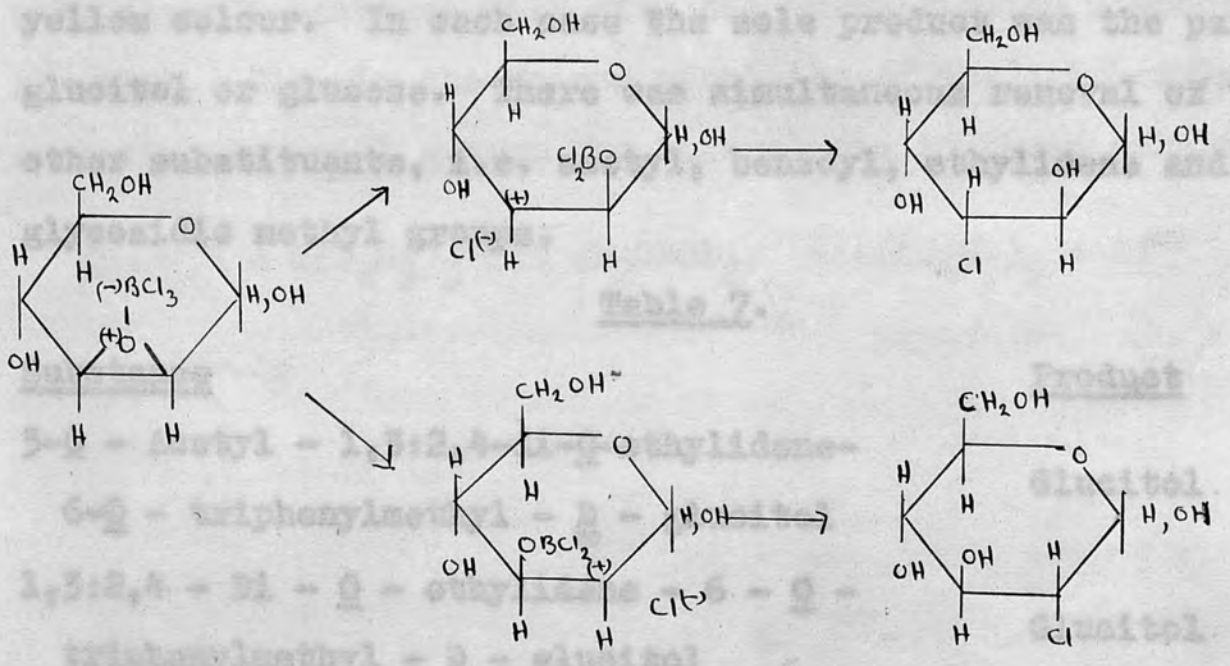
Equ. 27.

I

The pyranose ring oxygen atom will influence the direction of fission. If fission of the $C_{(6)}$ - O bond occurred a less stable carbonium ion would be formed. Hydrolysis of the intermediate compound (I in Equ. 27), or the glycosyl chloride derived from it, with methanol will give the parent monosaccharide. In all the reactions with 1, 6 - anhydro sugars oligosaccharides were obtained as secondary products. These will be formed by reaction of the complex (I in Equ. 27) or the glycosyl chloride derived from it, with the free hydroxyl

(or dichloroboronite) groups of other molecules.

After coordination of boron trichloride with the oxygen atom of the epoxide ring of the 2, 3 - anhydro compounds, fission of either the $C_{(2)}$ - O or $C_{(3)}$ - O bonds will occur. Reaction of the carbonium ions formed with chloride ions, probably with inversion of configuration, will give 2 - chloro - 2 - deoxy - or 3 - chloro - 3 - deoxy - sugars (Equ. 28), which are stable to methanolysis.



Equ.28.

Conformational factors may well decide the direction of bond fission in this type of coordination complex.

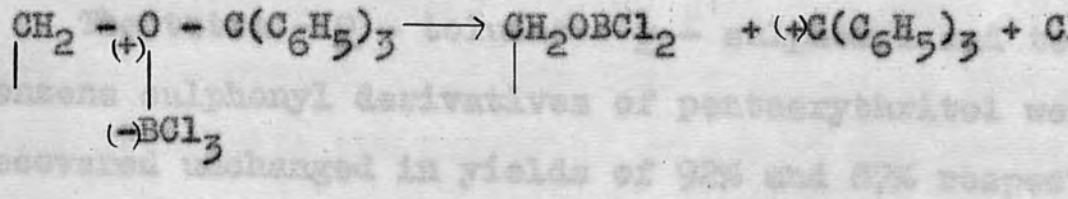
Reaction of Boron Trichloride with Triphenylmethyl Ethers

The triphenylmethyl ethers of glucitol and glucose were reacted with boron trichloride as in Expt. 5. The results are recorded in Table 7. Reaction was probably instantaneous, as immediately on addition of boron trichloride at -80° a deep yellow coloration was noted. This is characteristic of the production of triphenylmethyl ions.[54] Addition of methanol after removal of excess boron trichloride discharged the yellow colour. In each case the sole product was the parent glucitol or glucose. There was simultaneous removal of the other substituents, i.e. acetyl, benzoyl, ethylidene and glycosidic methyl groups.

Table 7.

Substance	Product
5-O - Acetyl - 1,3:2,4-di-O-ethylidene-6-O - triphenylmethyl - D - glucitol	Glucitol.
1,3:2,4 - Di - O - ethylidene - 6 - O - triphenylmethyl - D - glucitol	Glucitol.
Methyl 2,3,4 - tri - O - acetyl - 6 - O - triphenylmethyl - α - D - glucoside	Glucose.
Methyl 4-O- acetyl - 2,3- di-O-benzoyl - 6-O - triphenylmethyl - α - D - glucoside	Glucose.
1,2,3,4 - Tetra - O - acetyl - 6 - O - triphenylmethyl - β - D - glucose	Glucose.

The reaction of boron trichloride with triphenylmethyl ethers will proceed through coordination of boron trichloride with the ether oxygen atom. This will be followed by fission of the bond between the ether oxygen atom and the triphenylmethyl group as shown in Equ. 29. This will result in the formation of a dichloroboronite group on the carbohydrate residue. In the case of the compounds investigated this will be at $C_{(6)}$ of the glucose or glucitol molecule. Treatment with aqueous methanol will give the unsubstituted glucose or glucitol. Clearly the unique stability of the triphenylmethyl carbonium ion will govern the heterolysis in these ethers.[54]



Equ.29.

Reaction of Boron Trichloride with Sulphonic Esters.

The sulphonic esters which were investigated, with the exception of the glycerol derivative, were stable to the attack of boron trichloride (Table 8).

2,5 - Di - O - toluene - p - sulphonyl - D - mannitol was obtained in yields of 96.5% and 88.9% after treatment of 2,5-di-O-toluene - p - sulphonyl - D - mannitol and 1,3 : 4,6 - di - O - ethylidene - 2, 5 - di - O - toluene - p - sulphonyl - D - mannitol respectively with boron trichloride (Expts. 19 and 20). This product was also obtained from the di - O - methylene derivative (Expt. 21).

The tetra - O - toluene - p - sulphonyl and tetra - O - benzene sulphonyl derivatives of pentaerythritol were also recovered unchanged in yields of 92% and 87% respectively (Expts. 22 and 23).

Glucose derivatives containing O - benzylidene, α - methyl, O - acetyl and O - isopropylidene groups in addition to mono - O - toluene - p - sulphonyl groups on treatment with boron trichloride each gave as the main product a compound with an $R_{Glucose}$ value of 5.7 in solvent system h (Expt. 1). This is presumably a mono - O - toluene - p - sulphonyl - D - glucose.

3 - O - Methanesulphonyl - D - glucose was not affected by treatment with boron trichloride.

α - Toluene - p - sulphonylglycerol gave glycerol (30% yield, identified as the tribenzoate) together with unchanged starting material when reacted with boron trichloride.

Tipson[55] states that in general a secondary sulphonyloxy group is more stable to a given chemical reagent than is a primary sulphonyloxy group. This may account partially for the anomalous behaviour of the glycerol derivative. However, as 1,2,3,4 - tetra - O - acetyl - 6 - O - toluene - p - sulphonyl - β - D - glucose, which also contains a primary sulphonyloxy group, does not give glucose on treatment with boron trichloride, this cannot be the whole explanation.

The sulphonic esters are the only compounds found so far which are recovered unchanged from solution in boron trichloride, with the exception of one or two unsubstituted monosaccharides. This may be due to the formation of coordination complexes which do not react further, or to a total inability to coordinate due possibly to some stereochemical factor. This should prove a valuable method for the preparation of particular sulphonic esters. Any of the groups, which may be removed by boron trichloride e.g. O - methyl, can be used as blocking agents. In this way sulphonic ester groups may be introduced into almost any position in the polyol or carbohydrate molecule.

Table 8

Substance	Principal product	Other products
Methyl 4,6-O- benzylidene - 2,3-di-O - toluene - p - sulphonyl - α - D - glucoside	7.7 ($R_{Glucose}$ value)	Glucose$^+$
Methyl 4,6-O - benzylidene - 2 - O - toluene - p - sulphonyl - α - D - glucoside	5.7 ($R_{Glucose}$ value)	Glucose$^+$
1,2:5,6 - Di - O - isopropylidene-3-O - toluene - p - sulphonyl - D - glucose	5.7 ($R_{Glucose}$ value)	-
1,2,3,4 - Tetra - O - acetyl - 6 - O - toluene p - sulphonyl - β- D - glucose	5.7 ($R_{Glucose}$ value)	-
3- O - Methanesulphonyl - D - glucose	3-O- Methanesulphonyl - D - glucose	-
1,3:4,6 - Di - O - ethylidene - 2,5-di - O - toluene - p - sulphonyl - D - mannitol	2,5 - Di - O - toluene - p - sulphonyl - D - mannitol	0.39$^+$ (R_F value).

Substance	Principal product	Other products
1,3:4,6 - Di - O - methylene - 2,5 - di - O - toluene - p - sulphonyl - D - mannitol	2,5 - Di - O - toluene p - sulphonyl - D - mannitol	0.39[+] ($R_F$value)
2,5 - Di - O - toluene - p - sulphonyl - D - mannitol	2,5 - Di - O - toluene - p - sulphonyl - D - mannitol	-
1,3:2,4 - Di - O - ethylidene - 5,6 - di - O - toluene - p - sulphonyl - D - glucitol	0.79 ($R_F$value)	Glucitol[+]
Tetra - O - toluene - p - sulphonyl- pentaerythritol	Tetra - O - toluene - p - sulphonylpentaerythritol	-
Tetra - O - benzenesulphonyl- pentaerythritol	Tetra - O - benzene- sulphonylpentaerythritol	-
α - Toluene - p - sulphonylglycerol	Glycerol and α- toluene - p - sulphonylglycerol	
Benzene sulphonamide	Benzene sulphonamide	-

Reaction of Boron Trichloride with Other Carbohydrate Derivatives

The reaction of boron trichloride with the compounds which are listed in Table 9 has been investigated.

Pentaerythritol tetrabromide was recovered in 72% yield after treatment with boron trichloride (Expt.24). No other product could be detected.

D - Glucosamine hydrochloride was insoluble in both boron trichloride and boron tribromide. Its inability to react may be due to this insolubility. Similarly dipotassium glucose - 1 - phosphate was not attacked by boron trichloride in which it was insoluble.

Nitrocellulose gave glucose as the principal product on treatment with boron trichloride.

Table 9

Substance	Principal product	Other products
Pentaerythritol tetrabromide	Pentaerythritol tetrabromide	-
D - Glucosamine hydrochloride	D - Glucosamine hydrochloride	-
Dipotassium glucose - 1 - phosphate	Dipotassium glucose - 1 - phosphate	
Nitrocellulose	Glucose	0.38, 2.1, 3.6+ ($R_{Glucose}$ values)

+ Present in trace quantity.

Summary

The reactions of boron trichloride and boron tribromide with approximately ninety different carbohydrates, polyols and their derivatives have been investigated. The results are summarised below.

(1) On treatment with boron trichloride or boron tribromide followed by methanolysis the following types of compound yielded the parent monosaccharides or polyols,

(a) O - Methyl, O - benzyl and O - triphenylmethyl ethers

(b) Carboxylic esters

(c) Glycosides

(d) Cyclic acetals and ketals

(e) Mono - , di - and polysaccharides

(f) 1,6 - Anhydro hexoses.

The only exceptions, which have been found so far, are ketoses and their derivatives, which are decomposed to furfuraldehyde derivatives. Unsubstituted polysaccharides are insoluble and so do not react.

(2) 2,3 - Anhydro sugars on treatment with boron trichloride followed by methanolysis do not give the parent sugar. The products are probably chlorodeoxy derivatives.

(3) Sulphonic esters survive unchanged on treatment with boron trichloride followed by methanolysis.

SECTION 2.

Synthetic Reactions of Boron Trichloride

Glycosylation Reactions.

The formation of oligosaccharides as secondary products in some of the reactions discussed in Section 1, suggests that boron trichloride can function as a synthetic agent with certain carbohydrate derivatives. This effect was observed particularly in the demethylation of methyl-α-D-glucoside, when oligosaccharides were formed in addition to glucose. This reaction is discussed on P. 94. and the formation of a glucosyl cation or the glucosyl chloride derived therefrom is postulated. The oligosaccharides probably result from condensation of this cation with itself or with unreacted methyl α-D-glucoside. This glucosyl cation (or glucosyl chloride) therefore acts as a glucosylating agent and should condense with suitable substrates.

Alcohols and phenols, glucose and benzene have been used as substrates. The expected products were obtained, namely glycosides, disaccharides and glucopyranosylbenzene respectively. Details of these reactions are given in this Section.

GLUCOSIDES

INTRODUCTION.

The methods for glycoside synthesis in which the free sugar is condensed directly with an aglycan are rather limited in applicability. Most of these methods are based on the Fischer synthesis[56] and are used for the condensation of monosaccharides with the lowest aliphatic alcohols in the presence of hydrogen ions. These methods cannot be used with a phenolic aglycon. Hydrogen chloride in an inert solvent is the catalyst most commonly used. More recently cation exchange resins have found application.[57] These are particularly useful as they can be filtered off at the end of the reaction. The Fischer synthesis gives a mixture of α and β isomers, the ratio of which cannot be altered.

The first recorded preparation of phenyl β-D-glucoside by Michael[58] in 1879 utilised the reaction between tetra-O-acetylglucosyl chloride and potassium phenoxide. In general O-acetylglycosyl bromides are more reactive than the corresponding chlorides.

Koenigs and Knorr[59] developed a method for glycoside synthesis in which an O- acetylglycosyl bromide or chloride is condensed with an aglycon in the presence of a suitable catalyst. This method may be applied equally to the synthesis of glycosides of alcohols and phenols. One isomer usually predominates. As a Walden inversion occurs at the

reducing position of the carbohydrate molecule in many of these reactions, the glycoside which is formed has the opposite configuration to that of the glycosyl halide used. However, the catalyst has some influence on this inversion.[60] In the presence of silver salts or silver oxide there is almost invariably a Walden inversion, but in the presence of mercuric salts, although there is still a tendency for a Walden inversion to occur either isomer may be formed. Various organic bases[61] and alkali in aqueous acetone[60] have also been used as catalysts. In place of the O- acetylglycosyl halides in the Koenigs and Knorr reaction the corresponding O-benzoylglycosyl bromides have also been used.[62]

Another method for glycoside synthesis is that in which polyacetylated sugars are condensed with phenols in the presence of a catalyst.[63] The most common catalysts used are anhydrous zinc chloride and toluene-p-sulphonic acid,[64] but several others including boron trifluoride[3] and stannic chloride[65] have also been used. With the exception of zinc chloride, which gives the α isomers, the catalysts favour the formation of the β isomers. The yields of both isomers may be increased by performing the reaction in vacuo and removing the liberated acetic acid. A similar reaction is that between methyl tetra-O-acetyl-α-D- glucoside, phenol and zinc chloride in acetic acid and acetic anhydride. The product

is phenyl tetra-O-acetyl-α-D-glucoside.[63]

The final method which is to be considered is the rearrangement of O-acetyl-β-glycosides to the corresponding O-acetyl-α-glycosides. Most of the catalysts, which were used in the synthesis of glycosides from polyacetylated sugars, appear to bring about this reaction. Pacsu used titanium tetrachloride[32] and although this is suitable for the anomerisation of alkyl glycosides, it is less successful for the anomerisation of phenolic glycosides. The transformation of phenyl tetra-O-acetyl-β-D-glucoside into the α isomer has been accomplished by the use of zinc chloride and phenol.[63] In some cases boron trifluoride has been found preferable to titanium chloride as a catalyst.[1]

The Synthesis of Glycosides using Boron Trichloride

A new method for glycoside synthesis, based on the reaction of boron trichloride with other more readily obtainable glycosides, is reported in this Section. Methyl α-D-glucoside, which can be obtained commercially, was found to be a very useful starting reagent.

Various glycosides were prepared on a small scale and the products were identified by paper chromatographic and ionophoretic methods. In addition benzyl glucoside, catechol glucoside and phenyl glucoside have been prepared on a larger scale by the reaction of the corresponding aglycon with a methyl α-D-glucoside-boron trichloride complex. These glucosides were isolated and characterised. A mechanism for this type of glycoside synthesis was also proposed.

The methyl α-D-glucoside - boron trichloride complex used in the glucoside syntheses was prepared in the following way. A suspension of methyl α-D-glucoside in dichloromethane was reacted with boron trichloride at -80°, and then allowed to attain room temperature. At this stage methyl α-D-glucoside became soluble in the reaction mixture, which was allowed to stand overnight at room temperature in order to ensure complete reaction. After removal of excess solvent and boron trichloride under diminished pressure at room temperature the complex was ready for use.

The Synthesis of Benzyl Glucoside

The first method for the synthesis of glycosides which was attempted using the methyl α-D-glucoside-boron trichloride complex was a modification of the Koenigs and Knorr reaction, in which the complex was used to replace the O-acetylglycosyl bromide.

Benzyl β-D-glucoside was prepared by this method (Expt.25). The methyl α-D-glucoside-boron trichloride complex was shaken with benzyl alcohol and silver oxide for 24 hr. at room temperature. After removal of the silver oxide and silver salts by filtration, the filtrate was examined paper chromatographically. This analysis showed the presence of four products, two of which were present in trace amounts only. The two principal products were benzyl glucoside and glucose. The two secondary products were methyl α-D-glucoside and a product which could not be identified at this stage, but was probably another monosaccharide, as it had an R_F value very similar to that of glucose. These products were separated on a 'Celite' column (Expt.3a), which was eluted with solvent system 8 (Expt.1).

The glucose fraction (0.1 g., 12% yield) (Expt.28) was contaminated with a small quantity of a second component. This contamination was probably slight as the specific rotation $[\alpha]_D^{20}$ + 52.5° (c = 2 in water) at equilibrium was identical with the accepted value for D-glucose of +52.5°.[66]

The glucose was identified by paper chromatography in solvent systems 1, 3 and 4 (Expt.1) and by paper ionophoresis in borate buffer at pH 10 (Expt.2). Using spray reagents which were specific for ketoses a positive reaction was given by the contaminant. It was indistinguishable from fructose by paper chromatography in solvent systems 1, 3 and 4 (Expt.1) and by paper ionophoresis in borate buffer at pH 10 (Expt.2).

One fraction yielded methyl α-D-glucoside (0.004 g.), presumably unreacted starting material (Expt.27).

The yield of benzyl glucoside isolated initially as a pure specimen was 25% (Expt.26). However, this yield was increased to 37% by further purification of other fractions, which also contained traces of a compound with an $R_{GLUCOSE}$ value of 2.9 (solvent 1, Expt.1). Insufficient of this material was obtained to facilitate identification. By comparison with Expt.40. it seems quite probable that this was 1, 6-anhydro-β-D-glucose.

The benzyl glucoside was obtained as a pale yellow oil, which proved difficult to crystallise, but eventually crystallisation from ethyl acetate proved successful. The melting point and carbon and hydrogen content were consistent with benzyl β-D-glucoside. Acetylation with acetic anhydride and pyridine gave benzyl 2, 3, 4, 6 -tetra-O-acetyl-β-D-glucoside (Expt.26). The free glucoside was indistinguishable from benzyl β-D-glucoside by paper chromatography in solvent systems 1, 3 and 4 (Expt.1) and by paper ionophoresis in borate buffer at pH 10 (Expt.2) using several spray reagents. It

was hydrolysed completely to glucose and benzyl alcohol by Amberlite IR-120 (H^+) resin and by almond β-glucosidase. The latter experiment proved the presence of the β configuration. No trace of the α isomer could be detected. This is consistent with the product to be expected from a modified Koenigs and Knorr reaction in the presence of silver oxide when one isomer is always found to predominate in the reaction products.

To ensure that no transglucosidation took place in the absence of boron trichloride a control experiment (Expt.29a) was performed in which methyl α-D-glucoside, dichloromethane, benzyl alcohol and silver oxide were reacted in the same proportions and under the same conditions as in Expt.25. The only product was unchanged methyl α-D-glucoside. When a similar experiment (Expt.29b) was performed in the presence of hydrochloric acid, methyl α-D-glucoside was again the only product. Similarly, when D-glucose, dichloromethane, benzyl alcohol and silver oxide were reacted together (Expt.30) D-glucose was the only product.

The Synthesis of Phenolic Glucosides

An attempt was made to prepare phenyl glucoside by the same modification of the Koenigs and Knorr reaction as was used for the preparation of benzyl β-D-glucoside (Expt.31). The reaction was worked up as in Expt.25 and the products examined by paper chromatography using solvent system 1

(Expt.1). No phenyl glucoside could be detected. The only products were glucose and phenol. Presumably glucose was formed by the hydrolysis of the glucosyl chloride derivative formed by reaction of boron trichloride with methyl α-D-glucoside. As no phenyl glucoside was formed, it seemed probable that this glucosyl chloride derivative was insufficiently reactive to condense with phenol.

In general the glucosyl bromide derivatives are more reactive than the corresponding chlorides, so the modified Koenigs and Knorr reaction was repeated using boron tribromide in place of boron trichloride. With phenol as the substrate five products were detected:- glucose, phenyl glucoside, phenol, oligosaccharides and a product with an $R_{GLUCOSE}$ value of 2.4 (solvent system 1, Expt.1). The last two were present in trace quantities only.

The possibility of preparing phenyl glucoside from the methyl α-D-glucoside-boron trichloride complex under different reaction conditions was now considered. A series of experiments all based on the use of this complex were performed.

The complex was reacted with:-

(a) phenol in dichloromethane for 2 days at room temperature (Expt.33i).

(b) phenol and sodium hydroxide in dichloromethane for two days at room temperature (Expt. 33ii).

(c) phenol and silver oxide in dichloromethane with heating for 2 hr. at 50° on a water bath (Expt.33iii).

(d) phenol in dichloromethane with heating for 2 hr. at 50° on a water bath (Expt.33iv).

All the reactions were successful. In each case the products were phenyl glucoside, glucose, oligosaccharides and smaller amounts of one or two other products, which were not identified. The last experiment was selected for further investigation as the yield of phenyl glucoside appeared to be high.

A small scale experiment showed that this method could be used for the synthesis of catechol glucoside also (Expt.34). Catechol glucoside and phenyl glucoside were both prepared on a larger scale by this method and the products were isolated (Expts.35 and 39).

The Synthesis of Catechol Glucoside

A solution of catechol in dichloromethane was added to the methyl α-D-glucoside-boron trichloride complex and the solution was heated under reflux for 2 hr. (Expt.35). Dry ether was added to this solution until no further precipitation occurred and the precipitate was stirred with solvent system 1 (Expt.1). Part of the solid remained insoluble and this was shown to be a boron residue, which contained no carbohydrates other than a trace of glucose. This residue was not investigated further. Paper chromatographic analysis of the solution showed that the principal products present were oligosaccharides, glucose, catechol glucoside and catechol.

These products were separated using a cellulose column (Expt.3b).

One fraction yielded D-glucose (0.05 g., 1.8% yield) which was obtained as a crystalline specimen and characterised (Expt.37). This glucose is formed by hydrolysis of unreacted glucosyl chloride during the later stages of the experiment.

The oligosaccharide fraction was shown to consist mainly of disaccharides, although trisaccharides were also present. These disaccharides were not investigated in detail, but were, however, shown to consist of a mixture of several of the glucose disaccharides (Expt.38). Presumably the disaccharides were formed by the condensation of the glucosyl chloride with itself, or possibly with unreacted methyl glucoside. As all the hydroxyl groups of the glucose residue are free or present as dichloroboronites or chloroboronates, with the exception of the one at the reducing position ($C_{(1)}$), all possible glucose disaccharides except those of the trehalose type could be present. Even the trehaloses cannot be excluded as the presence of moisture would hydrolyse some of the glucosyl chloride to glucose. This could then react with glucosyl chloride to give any of the eleven possible glucose disaccharides. However, some positions will possibly be more sterically favourable for reaction than others, so all the eleven disaccharides may not necessarily be present.

As may be seen from Table 18 products with R_F values of 0.30, 0.40 and 0.47 (solvent system 1, Expt.1) were also present. These three compounds gave a positive reaction with the diazo-

tised nitroaniline spray reagents. They could not be identified however, as they were present in trace amounts only.

The principal product of the reaction was a mixture of catechol α-D-glucoside and catechol β-D-glucoside (2.4 g., 57% yield). Only catechol β-D-glucoside was available for comparison. This mixture of catechol glucosides was indistinguishable from authentic catechol β-D-glucoside by paper chromatography using three solvent systems and by paper ionophoresis with borate buffer at pH 10 (Expt.36). It was partially hydrolysed to glucose and catechol by both almond β-glucosidase and α-glucosidase showing the presence of a mixture of α and β anomers. The specificity of the enzyme solutions was tested under identical conditions. The m.p., carbon and hydrogen content and specific rotation value were consistent with the presence of a mixture of catechol α-D-glucoside and catechol β-D-glucoside. No attempt was made to separate these two anomers, but phenyl α-D-glucoside and phenyl β-D-glucoside were separated using a charcoal-'Celite' column (Expt.44).

The Synthesis of Phenyl Glucoside

Phenyl glucoside was synthesised by a method identical with that used for the synthesis of catechol glucoside. Paper chromatographic analysis using the silver nitrate-sodium hydroxide spray reagent showed that a mixture of products consisting principally of oligosaccharides, glucose

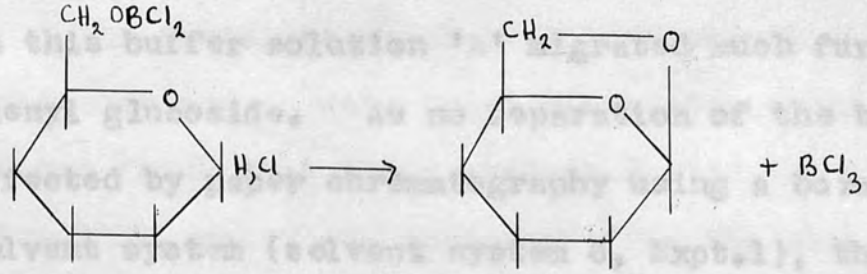
and phenyl glucoside was present. Separation of the products was effected using a cellulose column (Expt.3b) eluted with solvent system 1 (Expt.1).

One fraction yielded D-glucose (0.04g., 0.9% yield), which was obtained as a crystalline sample and characterised (Expt.41).

A mixture of disaccharides was obtained (Expt.42) and these had a similar chromatographic and ionophoretic behaviour to that of the mixture of disaccharides obtained in the catechol glucoside synthesis.

A further fraction yielded a small quantity (12 mg.) of 1, 6-anhydro-β-D-glucose (Expt.40), which was obtained as a crystalline specimen and characterised. Under certain alkaline conditions[67,68] phenyl β-D-glucoside is known to give 1, 6-anhydro-β-D-glucose, but not normally under acidic conditions. However, 1, 6-anhydro-β-D-glucose is sometimes obtained as one of the acid reversion products of D-glucose.[69] In this case it seems more probable that the 1, 6-anhydro-β-D-glucose arose from the internal condensation of the intermediate glucosyl chloride derivative. This derivative is unlikely to have a free hydroxyl group at $C_{(6)}$. Reaction of the hydroxyl groups with boron trichloride would yield dichloroboronite or cyclic chloroboronate groups. Ring closure can then occur by elimination of boron trichloride if a dichloroboronite group is present at $C_{(6)}$, and by elimination

of hydrogen chloride if $C_{(6)}$ is unsubstituted (Equ.30).



Equ.30.

However, by analogy with the formation of 1, 6-anhydro-β-D-glucose from phenyl β-D-glucoside, the reaction may proceed via a 1, 2-anhydro-sugar intermediate.

On concentration of the fractions containing phenyl glucoside a white solid (6.0 g., 91% yield based on $C_{12}H_{16}O_6$) was obtained. Further examination showed this solid to be a mixture of phenyl glucoside and a second component 'A', which had an R_g value similar to that of phenyl glucoside in various chromatographic solvent systems. The component 'A', unlike phenyl glucoside, gave a positive reaction with the diazotised nitroaniline spray reagent. Both components gave positive reactions with the silver nitrate-sodium hydroxide spray reagent, but in order to obtain a similar effect a far greater concentration of phenyl glucoside than of 'A' was required. This later caused some difficulty in detecting small quantities of phenyl glucoside mixed with 'A'. However, the difficulty was resolved recently by the introduction of the potassium periodatocuprate spray reagent, [98] which may be used for the detection of trace quantities of glucosides.

A separation of phenyl glucoside and 'A' was obtained by paper ionophoresis in borate buffer at pH 10 (Expt.2). In this buffer solution 'A' migrated much further than phenyl glucoside. As no separation of the two could be effected by paper chromatography using a borate containing solvent system (solvent system 6, Expt.1), the difference in migratory power is probably due to the presence of an ionisable group in 'A'.

Various methods of separation of phenyl glucoside and 'A' were attempted (Expt.43) and partial success was obtained using a charcoal-'Celite' column (Expt.44). Only a small proportion (15%) of the material was recovered from the column. This is quite a common occurrence with aromatic compounds, which are frequently irreversibly adsorbed onto charcoal.[39] Insufficient material 'A' was obtained to facilitate identification at this stage.

Each fraction obtained from the charcoal-'Celite' column separation, which contained phenyl glucoside was analysed carefully by paper chromatography using the silver nitrate-sodium hydroxide spray reagent. It was noted that the fractions, which were eluted from the column first, reduced the silver nitrate reagent more slowly than the other fractions. As phenyl α-D-glucoside reacts more slowly with this reagent than phenyl β-D-glucoside, it appears that a separation of the two anomers was obtained. This is in agreement with the

known order of elution of glycosides from charcoal-'Celite' columns, as the β-anomer is more strongly adsorbed.[70]

The fractions which contained phenyl α-D-glucoside were combined as were those containing phenyl β-D-glucoside. It was found that the α-anomer formed 77% of the phenyl glucoside, which was recovered from the column. This may not be due entirely to the predomination of the α-anomer in the original mixture. Since so much of the material was irreversibly adsorbed, it is possible that as the β-anomer is more strongly adsorbed on charcoal than the α-anomer, more of the β-anomer may have been retained on the column.

Phenyl α-D-glucoside was obtained as a crystalline specimen (Expt.45). It was hydrolysed to glucose and phenol by hydrochloric acid (0.1N) and by α-glucosidase, but was not hydrolysed by almond β-glucosidase. This confirms the presence of the α-configuration. It had m.p., mixed m.p. and specific rotation identical with those of an authentic specimen of phenyl α-D-glucoside, from which it was indistinguishable by paper chromatography using three solvent systems and by paper ionophoresis in borate buffer at pH 10 (Expt.2).

A similar scheme was used for the identification of phenyl β-D-glucoside (Expt.46), which was hydrolysed by almond β-glucosidase, but not by α-glucosidase, confirming the presence of the β-configuration.

A direct separation of phenyl glucoside and 'A' seemed most difficult. However, a separation of 'A' from a mixture of glucose, phenol and 'A' would be comparatively easy by cellulose column chromatography. So several methods for the hydrolysis of the phenyl glucoside component were attempted.

Boron trichloride was the first reagent used to effect this hydrolysis (Expt.47). A preliminary paper chromatographic analysis of the products showed the presence of 'A', phenol and glucose. As the yield of 'A' was so low being only 2.5% of the original mixture, it was feared that the boron trichloride treatment had resulted in some decomposition of 'A'.

Hydrolysis with hydrochloric acid (Expt.48) gave a yield of 'A' of 18%, but this was subsequently shown to contain some phenyl glucoside (Expt.50).

Preliminary investigation of 'A' by paper chromatography and paper ionophoresis indicated the presence of a phenolic group. With the diazotised nitroaniline spray reagent a positive reaction was obtained. In an attempt to confirm the presence of this phenolic group an examination of the ultraviolet absorption spectra of both phenyl β-D-glucoside and 'A' was carried out in the range 250-300 mμ. The ultraviolet absorption bands of phenolic compounds in ethanol are displaced to longer wavelengths, when the solutions are made alkaline.[71]

As the specimen of 'A' obtained by acidic hydrolysis was thought to contain some phenyl glucoside a preliminary investigation of the ultraviolet absorption spectrum of phenyl β-D-glucoside was performed. Absorption bands were obtained at 268 and 275 mμ and addition of potassium hydroxide (0.2%) in ethanol had no effect on the position of these bands (Fig. 5).

With this specimen of 'A' absorption bands were obtained at 268 and 275 mμ. However, on addition of alkali a shift of the bands to 293 mμ was observed, but a shoulder was still present at 275 mμ (Fig. 6). This displacement of the absorption band is consistent with the presence of an unsubstituted phenolic group. A table of data for phenols and phenolic glucosides,[72] with and without a free phenolic group, is given below for comparison.

Table 10

Compound	Before addition of alkali λ_{max} (mμ)	After addition of alkali λ_{max} (mμ)
Phenol	273	289
p-Hydroxybenzoic acid	254	280
o-Hydroxybenzyl β-D-glucoside	274	297
p-Hydroxybenzyl β-D-glucoside	279	290
o-Hydroxymethylphenyl β-D-glucoside	268	268
p-Hydroxymethylphenyl β-D-glucoside	264	266
2, 3-Dihydroxyphenyl β-D-glucoside	279	290

FIGURE 5

THE ULTRAVIOLET ABSORPTION SPECTRUM OF PHENYL β-D-GLUCOSIDE

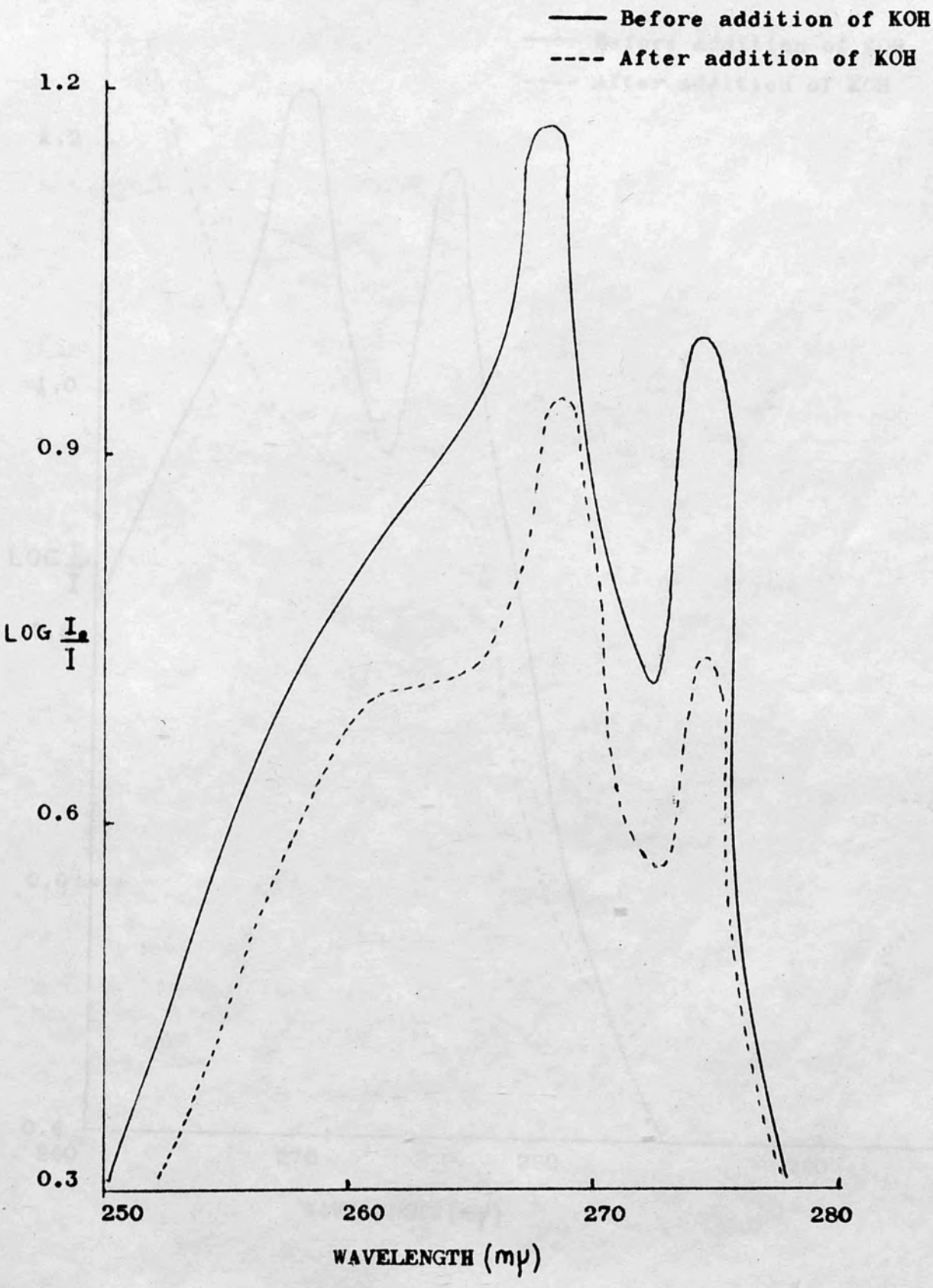
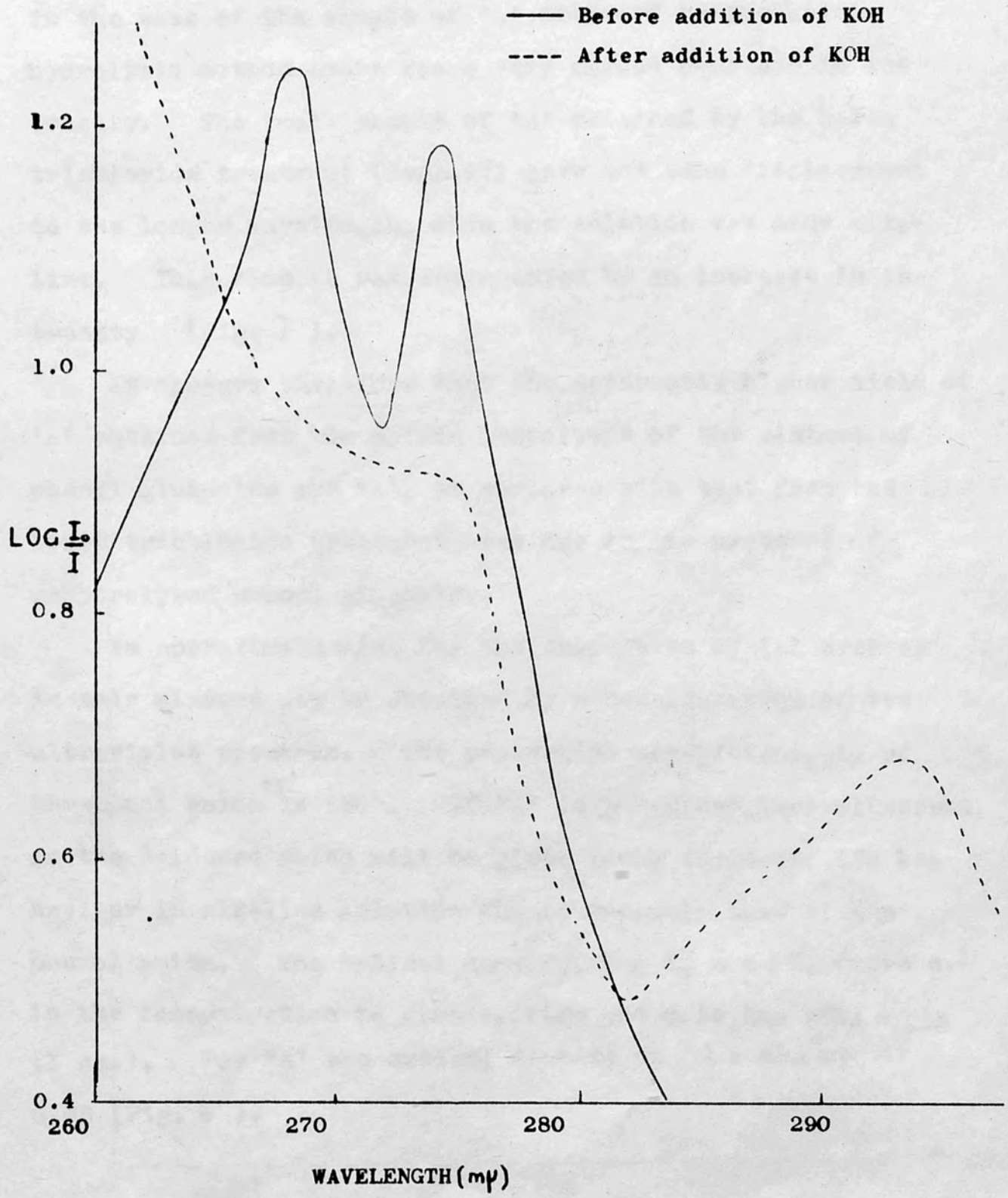


FIGURE 6

THE ULTRAVIOLET ABSORPTION SPECTRUM OF 'A'



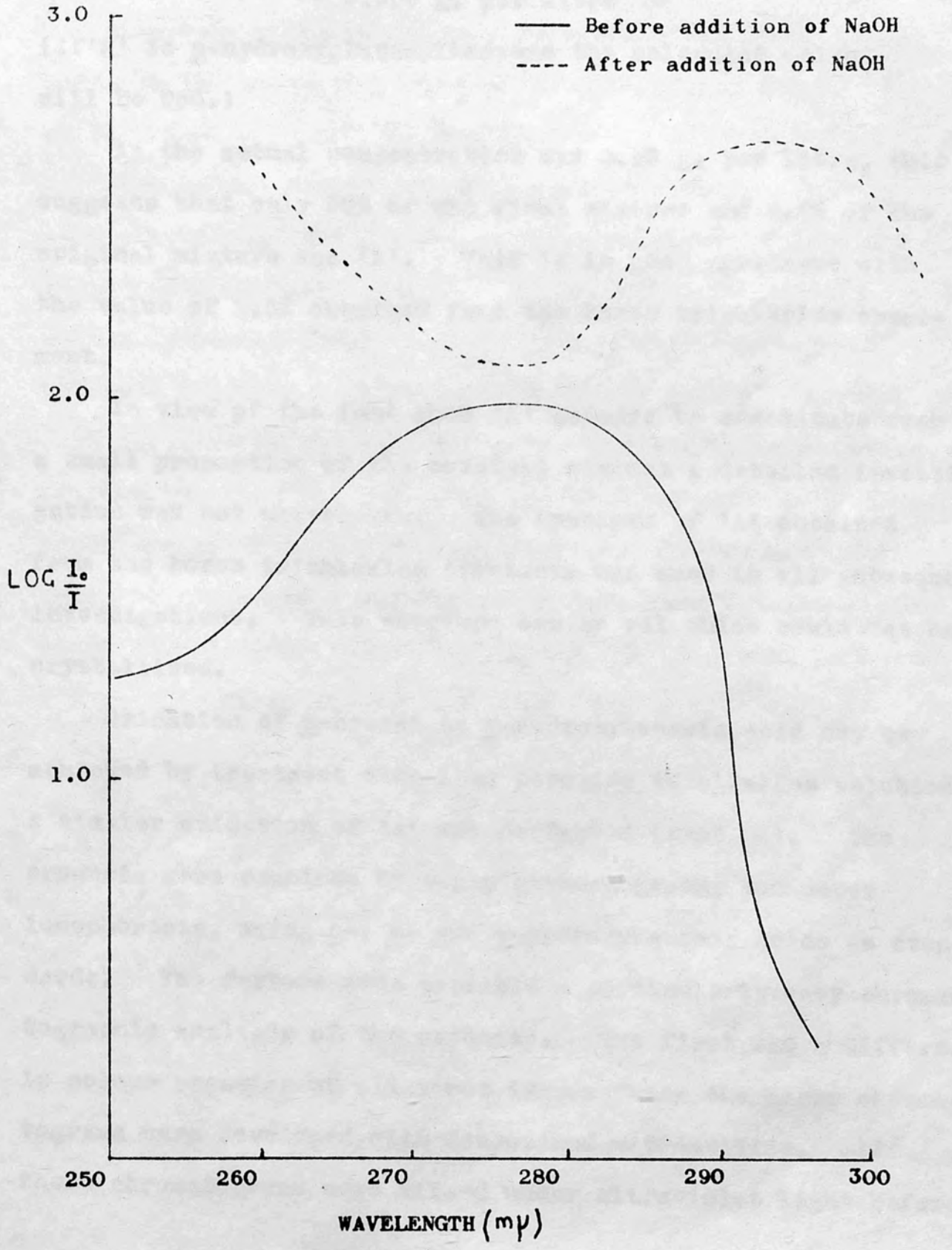
In nearly every case an increase in the intensity of absorption is obtained at the longer wavelength. However, in the case of the sample of 'A' obtained by the acidic hydrolysis method there was a very marked decrease in intensity. The small sample of 'A' obtained by the boron trichloride treatment (Expt.47) gave the same displacement to the longer wavelength, when the solution was made alkaline. This time it was accompanied by an increase in intensity (Fig. 7).

It appears therefore that the apparently higher yield of 'A' obtained from the acidic hydrolysis of the mixture of phenyl glucoside and 'A', as compared with that from the boron trichloride treatment, was due to the presence of unhydrolysed phenyl glucoside.

An approximate value for the proportion of 'A' present in this mixture may be obtained by a consideration of its ultraviolet spectrum. The extinction coefficient, ε, of the phenol anion[73] is 2600. If 'A' is p-hydroxyglucosylbenzene, as the evidence which will be given later suggests, its behaviour in alkaline solution should resemble that of the phenol anion. The optical density, $\log \frac{I_o}{I} = c \varepsilon d$, where c is the concentration in g.mole.litre and d is the cell width (1 cm.). For 'A' the optical density at $\lambda = 293$ mμ is 0.68 (Fig. 6).

FIGURE 7

THE ULTRAVIOLET ABSORPTION SPECTRUM OF 'A'



Therefore, $c = \frac{0.68.256}{2600}$ g. per litre

$= 0.066$ g. per litre

(If 'A' is p-hydroxyglucosylbenzene the molecular weight will be 256.)

As the actual concentration was 0.32 g. per litre, this suggests that only 20% of the final mixture and 3.6% of the original mixture was 'A'. This is in good agreement with the value of 2.5% obtained from the boron trichloride treatment.

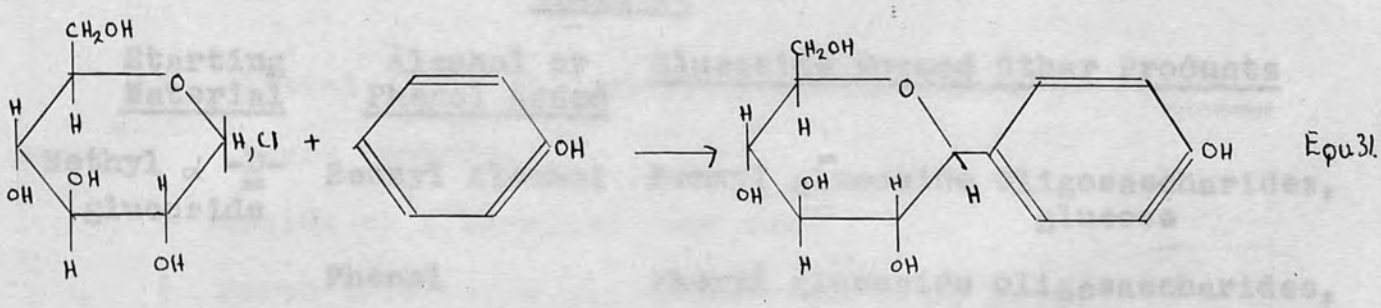
In view of the fact that 'A' appears to constitute such a small proportion of the original mixture a detailed investigation was not undertaken. The specimen of 'A' obtained from the boron trichloride treatment was used in all subsequent investigations. This specimen was an oil which could not be crystallised.

Oxidation of p-cresol to p-hydroxybenzoic acid may be achieved by treatment with lead peroxide in alkaline solution.[74] A similar oxidation of 'A' was performed (Expt.51). The products were examined by paper chromatography and paper ionophoresis, using o-, m- and p-hydroxybenzoic acids as standards. Two factors made possible a particularly easy chromatographic analysis of the products. The first was a difference in colour reaction of all three isomers when the paper chromatograms were developed with diazotised nitroaniline. If these chromatograms were viewed under ultraviolet light before

being sprayed a distinct difference in appearance of the p-hydroxybenzoic acid and the o- and m-hydroxybenzoic acids was observed. The products were analysed by paper ionophoresis in borate buffer at pH 10 and acetate buffer at pH 5.

The products were found to consist principally of p-hydroxybenzoic acid, but some o-hydroxybenzoic acid and phenol were also present.

This result shows that 'A' has an oxidisable group in the para position to a phenolic hydroxyl group. After a consideration of the starting materials and reaction conditions by which 'A' was obtained, the most probable structure would appear to be p-hydroxyglucopyranosylbenzene. This could be formed by a Friedel-Crafts reaction between a glucosyl chloride derivative and phenol (Equ.31) with boron trichloride as catalyst.



Equ 31.

This type of reaction between the methyl α-D-glucoside-boron trichloride complex and benzene is discussed later in this section. The small amount of o-hydroxybenzoic acid found in the oxidation products was probably due to the

presence of o -hydroxyglucopyranosyl-benzene.

Preliminary attempts to prepare 'A' by the action of boron trichloride on a solution of phenol in dichloromethane proved unsuccessful (Expt.52). This suggests the necessity for the presence of a glucose residue. 'A' could not be prepared by the rearrangement of phenyl α -D-glucoside using boron trichloride (Expt.53).

The Synthesis of Other Glucosides

A list of glucosides which were prepared by this new boron trichloride method is given below (Table 11). These preparations were carried out on a small scale (Expt.54) and in most cases the products were not isolated. These products were identified by paper chromatography and paper ionophoresis using a wide range of solvent systems and spray reagents and several buffer solutions. The significance of some of these reactions is discussed on P. 99.

Table 11

Starting Material	Alcohol or Phenol Added	Glucoside Formed	Other Products
Methyl α -D-glucoside	Benzyl Alcohol	Benzyl glucoside	Oligosaccharides, glucose
	Phenol	Phenyl glucoside	Oligosaccharides, glucose, 1,6-anhydro- β -D-glucose
	Catechol	Catechol glucoside	Oligosaccharides, glucose *

	Quinol	Arbutin ≠	Oligosaccharides, glucose
	Resorcinol	Resorcinol ≠ glucoside	Oligosaccharides, glucose
	Saligenin	o-Hydroxybenzyl glucoside, salicin	Oligosaccharides, glucose
	Pyrogallol	2,3- and 2,6- Dihydroxyphenyl glucosides	Glucose
Methyl β-D-Glucoside	Benzyl alcohol	Benzyl glucoside	Glucose
Phenyl α-D-glucoside	Methanol	Methyl glucoside	Oligosaccharides, glucose
	Catechol	Catechol glucoside	Glucose ⁎
Phenyl β-D-glucoside	Methanol	Methyl glucoside	Oligosaccharides, glucose
Arbutin	Methanol	Methyl glucoside	Glucose
Maltose	Methanol	Methyl glucoside	Glucose, maltose‖

⁎ Several other unidentified products were present in trace amounts.

≠ Yields of glucosides were low.

‖ Boron tribromide was used in place of boron trichloride.

It was particularly easy to distinguish between 2, 3- and 2, 6- dihydroxyphenylglucoside. With the diazotised nitroaniline spray reagent the former gave a Prussian blue

colour and the latter a yellow-green colour. In addition there was a substantial difference in their mobilities in various solvent systems. Using paper ionophoresis in borate buffer at pH 10 M_{SA} values of 0.58 and 0.45 were obtained for 2, 3- and 2, 6- dihydroxyphenylglucoside respectively. Ionophoresis in molybdate buffer at pH 5 was a more sensitive test as M_{SA} values of 0.70 and 0.00 were obtained for 2, 3- and 2,6- dihydroxyphenylglucoside respectively. The latter does not possess the configuration, which is required to enable the formation of a complex with molybdate.

Mechanisms for the Reaction between Methyl α -D-Glucoside and Boron Trichloride

There is almost certainly some reaction between boron trichloride and the hydroxyl groups of the methyl α -D-glucoside molecule. Dichloroboronites or chloroboronates are most probably formed. However, this reaction has been neglected in the following treatment, as it has no direct bearing on the mechanism of the glucoside synthesis.

Boron trichloride may co-ordinate at two oxygen centres in the methyl α -D-glucoside molecule, i.e. at the glycosidic oxygen atom of the methoxyl group or at the oxygen atom of the pyranose ring. Subsequent treatment of both of these co-ordination complexes with water and an alcohol or phenol (ROH) will now be considered.

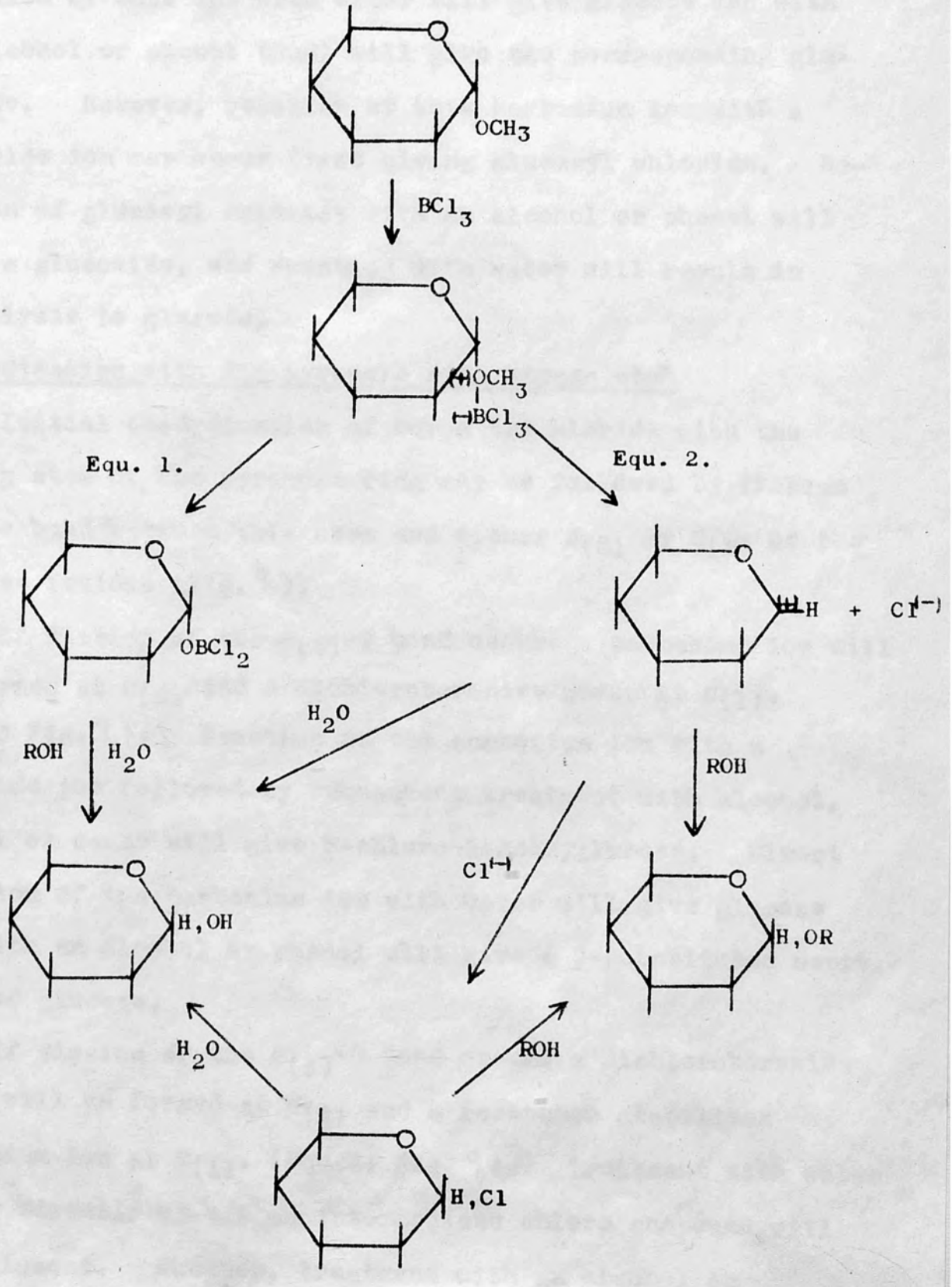
Co-ordination with the glycosidic oxygen atom

After initial co-ordination of boron trichloride with the glycosidic oxygen atom reaction may proceed in one of two ways (Fig. 8).

The first method involves elimination of methyl chloride giving a dichloroboronite group at $C_{(1)}$ of the glucose residue (Equ.1 Fig. 8). Treatment of this compound with water or an alcohol or phenol (ROH) will result in the formation of glucose.

A second type of reaction is the elimination of methoxy boron dichloride by fission of the bond between $C_{(1)}$ and the

FIGURE 8


OCH3
BCl3
(+)OCH3
(-)BCl3
Equ. 1.
Equ. 2.
OBCl2
(+)H + Cl(-)
ROH
H2O
H2O
ROH
Cl(-)
H,OH
H,OR
H2O
ROH
H,Cl

co-ordinated oxygen atom (Equ.2. Fig. 8). In this way a resonance stabilised carbonium ion will be formed. Direct reaction of this ion with water will give glucose and with an alcohol or phenol (ROH) will give the corresponding glucoside. However, reaction of this carbonium ion with a chloride ion may occur first giving glucosyl chloride. Reaction of glucosyl chloride with an alcohol or phenol will give a glucoside, and reaction with water will result in hydrolysis to glucose.

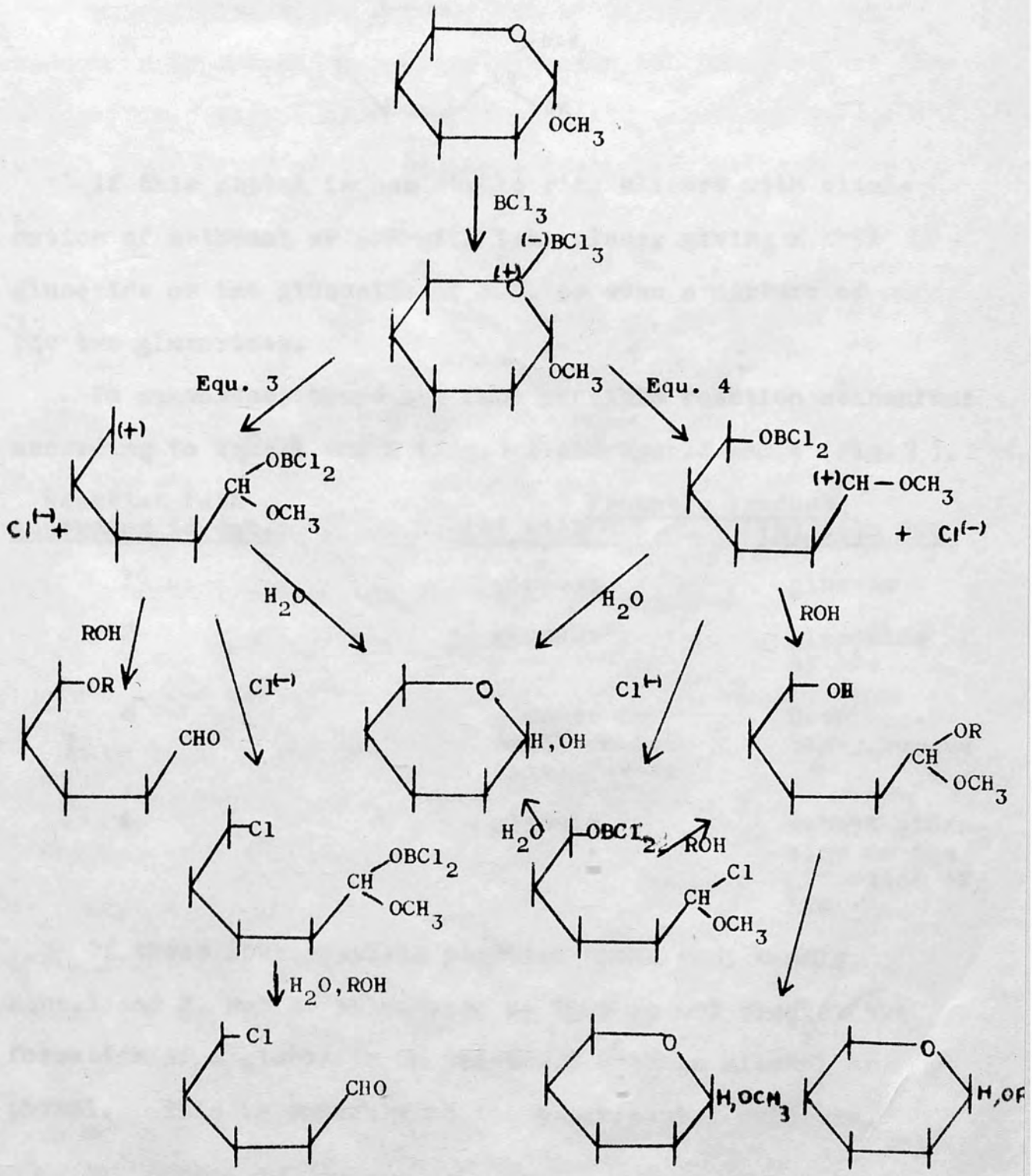
Co-ordination with the pyranose ring oxygen atom

Initial co-ordination of boron trichloride with the oxygen atom of the pyranose ring may be followed by fission of the bond between this atom and either $C_{(5)}$ or $C_{(1)}$ of the glucose residue (Fig. 9).

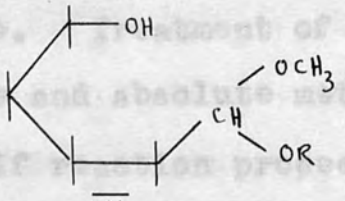
If fission of the $C_{(5)}$-O bond occurs a carbonium ion will be formed at $C_{(5)}$ and a dichloroboronite group at $C_{(1)}$. (Equ.3 Fig. 9). Reaction of the carbonium ion with a chloride ion followed by subsequent treatment with alcohol, phenol or water will give 5-chloro-5-deoxyglucose. Direct reaction of the carbonium ion with water will give glucose and with an alcohol or phenol will give a 5-substituted derivative of glucose.

If fission of the $C_{(1)}$-O bond occurs a dichloroboronite group will be formed at $C_{(5)}$ and a resonance stabilised carbonium ion at $C_{(1)}$. (Equ.4. Fig. 9). Treatment with water either directly or via an intermediate chloro compound will give glucose. However, treatment with an alcohol or

FIGURE 9


OCH3
BCl3
(-)BCl3
(+)
OCH3
Equ. 3
Equ. 4
(+)
OBCl2
CH
OCH3
Cl(-)+
OBCl2
(+)CH — OCH3
+ Cl(-)
H2O
H2O
ROH
ROH
OR
CHO
Cl(-)
H,OH
Cl(-)
OH
OR
CH
OCH3
Cl
OBCl2
CH
OCH3
H2O
OBCl2
ROH
Cl
CH
OCH3
H2O, ROH
Cl
CHO
O
H,OCH3
O
H,OH

phenol (ROH) will result in a more complicated reaction, depending on the stability of the mixed acetal VI.



VI

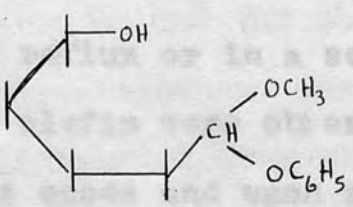
If this acetal is not stable ring closure with elimination of methanol or ROH will take place, giving methyl glucoside or the glucoside of ROH, or even a mixture of the two glucosides.

To summarise, there are four possible reaction mechanisms according to Equs.1 and 2 (Fig. 8) and Equs.3 and 4 (Fig. 9).

Reaction Path according to Equ.	Probable Products (a) with H_2O	(b) with ROH
1	glucose	glucose
2	glucose	glucoside of ROH
3	glucose or 5-chloro-5-deoxyglucose	5-chloro-5-deoxyglucose
4	glucose	methyl glucoside or the glucoside of ROH

Of these four possible reaction paths two, namely Equs.1 and 3, may be eliminated as they do not predict the formation of a glucoside on treatment with an alcohol or phenol. This is contrary to the experimental evidence.

It has been shown experimentally (Expt.39) that treatment of methyl α -D-glucoside with boron trichloride and phenol gives phenyl glucoside. Treatment of phenyl α -D-glucoside with boron trichloride and absolute methanol gives methyl glucoside (Table 11). If reaction proceeds according to Equ.4 (Fig. 9) the mixed acetal in these two cases should be the same (VII).



VII

If this mechanism is valid the glucosides formed from these two reactions should be the same as each other. The products could be methyl glucoside, phenyl glucoside or a mixture of the two. It has been shown experimentally that different glucosides are formed in each case, as has been stated already, so Equ.4 Fig. 9 may be eliminated also. The products resulting from fission of the type shown in Equ.2 Fig. 8 are in accordance with the experimental findings, as on treatment of the methyl α -D-glucoside - boron trichloride complex with water, glucose is formed and on treatment with an alcohol or phenol, the corresponding glucoside is obtained. Similarly, by this method the predicted product of reaction of phenyl α -D-glucoside, boron trichloride and absolute methanol would be methyl glucoside.

There is one other method by which the correct products

might possibly be predicted. This is based on Equ.1 (Fig. 8). Decomposition of dichloroboronites may occur in three ways: [4]

$$3ROBCl_2 \longrightarrow B_2O_3 + BCl_3 + 3RCl$$

$$3ROBCl_2 \longrightarrow B_2O_3 + BCl_3 + 3HCl + 3 \text{ olefin}$$

$$2\ ROBCl_2 \rightleftharpoons BCl_3 + ClB(OR)_2$$

The mode of decomposition was found by Gerrard and Lappert to be dependent on the reaction conditions. At atmospheric or higher pressures under reflux or in a sealed tube reactions giving alkyl halide or olefin were observed. Under reflux, without heating in some cases and upon reduction of pressure the formation of chloroboronate was favoured.

If the dichloroboronite formed in Equ.1 Fig. 8 decomposed with formation of an alkyl halide, glucosyl chloride would be formed. This in turn would react with water to give glucose and with an alcohol or phenol to give a glucoside. However, this type of reaction is not favoured by the reaction conditions, as the first stage of the reaction is performed under reduced pressure. Under these conditions, if decomposition did occur, the most probable product would be a chloroboronate, which would give glucose on treatment with an alcohol or phenol.

It seems, therefore, that the most probable mechanism for the reaction of boron trichloride with methyl α-D-glucoside is that in which initial co-ordination of boron trichloride with the glycosidic oxygen atom is followed by fission of the bond between $C_{(1)}$ and this oxygen atom.

General Conclusions

The new method for glycoside synthesis, which has just been described, should prove useful due to its simplicity and general applicability. For this reason it is of particular use for the preparation of reference compounds for paper chromatographic analysis, when a specific anomer is not required. As this method gives the free glycoside directly it should prove a valuable method for the synthesis of some alkali labile phenolic glycosides. These glycosides can be prepared in the fully acetylated form by the Koenigs and Knorr reaction, but tend to decompose during deacetylation.

However, for the synthesis of a specific anomer the Koenigs and Knorr method is to be preferred.

DISACCHARIDES

Introduction

The Koenigs and Knorr reaction, which has been used so successfully for the synthesis of glycosides may also be used for the synthesis of disaccharides. In this method an O-acetylglycosyl halide is reacted with a monosaccharide, usually in the presence of silver oxide or silver carbonate. In order that specific disaccharides may be formed, the monosaccharide should be substituted in all positions, except the one at which the disaccharide linkage is to be formed. The groups which are used as blocking agents must be groups, which can be removed without destruction of the disaccharide bond. For this purpose O-acetyl groups are particularly useful, as they may be removed by the action of sodium methoxide.

It has been found that the yield of disaccharide in the Koenigs and Knorr reaction is increased by the addition of 'Drierite' and iodine. Iodine is added mainly to accelerate the reaction. 'Drierite' excludes moisture, which would hydrolyse the O-acetylglycosyl halide to give an acetylated monosaccharide. This also prevents the formation of disaccharides of the trehalose type, by reaction of this acetylated monosaccharide with the O-acetylglycosyl halide.

Thereare many examples of the use of the Koenigs and Knorr method for the synthesis of disaccharides, but only

three will be mentioned here. McCloskey, Pyle and Coleman[75] prepared octa-O-acetyltrehalose by the reaction between 2, 3, 4, 6-tetra-O-acetyl-α-D-glucosyl bromide and 2, 3, 4, 6-tetra-O-acetyl-β-D-glucose in the presence of silver carbonate, iodine and 'Drierite'. Similarly, octa-O-acetylgentiobiose was prepared by the reaction of 2, 3, 4, 6-tetra-O-acetyl-α-D-glucosyl bromide with 1, 2, 3, 4-tetra-O-acetyl-β-D-glucose.[76] In this case silver oxide was used in place of silver carbonate. The solvent was alcohol free chloroform. Octa-O-acetylnigerose wasprepared by the reaction between 2, 3, 4, 6-tetra-O-acetyl-α-D-glucosyl bromide and 1, 2, 4, 6-tetra-O-acetyl-β-D-glucose in nitromethane with mercuric cyanide as catalyst.[77] Some acetylated laminaribiose was also formed.

By the use of a molecule, which contained a halogen and a free hydroxyl group Haq and Whelan[78] have prepared some higher saccharides. They treated 2, 3, 4-tri-O-acetyl-α-D-glucosyl bromide in chloroform at 20° with silver oxide, iodine and anhydrous calcium sulphate. The products were 2, 3, 4-tri-O-acetyl-1, 6-anhydro-β-D-glucose and derivatives of gentiobiose, triose and tetraose.

The Synthesis of Disaccharides using Boron Trichloride

As has been mentioned already the formation of oligosaccharides has been observed during the reaction between boron trichloride and certain carbohydrate derivatives. This effect was particularly marked in the case of the reaction of methyl α-D-glucoside with boron trichloride. The oligosaccharide fraction consisted mainly of disaccharides, although some trisaccharides were present.

The first method attempted for the synthesis of disaccharides using boron trichloride was based on the Koenigs and Knorr reaction. The methyl α-D-glucoside - boron trichloride complex was reacted with 1, 2:5, 6-di-O-isopropylidene-D-glucose in the presence of silver carbonate and iodine (Expt.55). However, the products contained a mixture of at least three disaccharides. It seems that the dichloroboronite groups, which are probably present in the methyl α-D-glucoside-boron trichloride complex, may have removed the blocking groups.

The next method to be attempted was similar to that used for the preparation of benzyl ~~alcohol~~ glucoside. The methyl α-D-glucoside- boron trichloride complex was shaken with a solution of D-glucose in the presence of silver oxide (Expt.56). Nitrobenzene was the solvent used.

Preliminary investigation of the crude mixture by paper chromatography suggested that there was some specificity in

the linkage of the disaccharides formed. This was later shown not to be the case. The nitrobenzene solution was extracted with water and after neutralisation and concentration, the aqueous solution was introduced at the top of a charcoal-'Celite' column. Excess glucose was removed first by elution with water. By careful elution of the column with ethanol (1-10%) some separation of the disaccharides was achieved.

In the preliminary identification of the disaccharides by paper chromatography and paper ionophoresis (Expt.57) two spray reagents were found to be particularly useful. The first of these was the diphenylamine spray reagent, which gives different colours according to the type of linkage.[93] The following colours were observed with it for glucose disaccharides:-

Position of linkage	Colour
1, 1	Nil
1, 2	Yellow
1, 3	Grey-green
1, 4	Blue
1, 6	Grey-green

Alkaline triphenyltetrazolium chloride,[92] which reacts with all reducing disaccharides except those substituted at position 2 of the glucose residue, was also a useful spray reagent at this stage.

By combination of the fractions, which contain the same disaccharide or the same mixture of disaccharides as each

other, seven main fractions were found. Assuming the presence of glucose units only, a fact which was later confirmed, it was possible at this stage to identify tentatively the contents of each fraction. Details of the paper chromatographic and ionophoretic results are given in Table 12.

By chromatographic separation of the fractions containing mixtures of disaccharides on sheets of thick paper (Whatman No.3), paper chromatographically pure specimens of five disaccharides were obtained (Expt.58). Where the minor component could not be isolated as a pure specimen the identification scheme was applied as far as possible to the original mixture.

TABLE 12.

Disaccharide Fraction Number		Link Indicated by Paper Chromatography in Three Solvents	Link Indicated by Paper Chromatography of Benzylamine Derivative	Reaction With Triphenyl Tetrazolium Chloride	Colour With Diphenylamine Spray Reagent	Link Indicated By Ionophoresis in Borate Buffer	Conclusion
1	a	1, 6	1, 6	+ve	Grey-green	1,6 or 1,3	1,6
	b	1, 2	1,2 or 1, 4	-ve	Yellow	1, 2	1,2
2	a	1, 4	1, 4	+ve	Blue	1, 4	1,4
	b	1,3 or 1,1 *	-	-	-	-	-
3		1, 4	1, 4	+ve	Blue	1, 4	1,4
4	a	1, 4	1, 6	+ve	Blue	1, 4	1,4
	b	1, 6	1,2 and/or 1,4	+ve	Grey-green	1,6 or 1,3	1,6
	c	1, 2		-ve	Yellow	-	1,2
5	a	1, 6	1, 6	+ve	Grey-green	1,6 or 1,3	1,6
	b	1, 2	-	-ve	Yellow	1, 2	1,2
6		1, 4	1, 4	+ve	Blue	1, 4	1,4
7		1, 3	1, 3	+ve	Grey-green	1,6 or 1,3	1,3

* Present in trace quantity only

The following scheme (Expt.59) was used for the identification of each of the five disaccharides, which were obtained chromatographically pure.

(i) Paper chromatography and paper ionophoresis using isomaltose, gentiobiose, maltose, cellobiose, nigerose, laminaribiose, sophorose and α-α trehalose as standards.

(ii) Hydrolysis.

Glucose was identified as the only product of acidic hydrolysis of each disaccharide. This confirmed the original hypothesis that the disaccharides were composed of glucose units.

Each disaccharide was incubated with almond β-glucosidase and α-glucosidase to determine the anomeric character. The two disaccharides, which were thought to contain a 1, 4 link, were also incubated with glucamylase, which hydrolyses an α-1,4, but not a β-1,4 link.

(iii) Reduction to the corresponding O-glucosyl-D-glucitol.

It has been reported recently[79] that the position of linkage of glucose disaccharides may be determined by examination of the ionophoretic behaviour of the corresponding O-glucosyl-D-glucitols in molybdate buffer at pH 5. The reduced glucose disaccharides fall into three distinct groups according to the type of linkage present (i) 1, 6 and 1, 2 (ii) 1, 4 (iii) 1, 3. Hutson's results are given in Table 13.

Table 13

Compound	M_G value
Isomaltitol	0.8
Gentiobiitol	0.8
Sophoritol	0.9
Maltitol	0.4
Cellobiitol	0.4
Nigeritol	0
Laminaribiitol	0

The only two types of linkage, which cannot be differentiated are the 1, 6 and 1, 2. However, these can be readily distinguished by paper ionophoresis of the disaccharides in borate buffer at pH 10 when M_G values of 0.69-0.75 and 0.24 respectively are obtained.[80]

The results of the identification scheme are given in Table 14, from which it can be seen that the five principal products obtained in Expt. 58 are in actual fact composed of six disaccharides, namely isomaltose, gentiobiose, maltose, cellobiose, nigerose and laminaribiose. The last two were obtained as a mixture.

Table 14

Disaccharide Fraction Number	Link Indicated by Paper Chromatography and Paper Ionophoresis	Product of Acidic Hydrolysis	Action of Almond β-Glucosidase	Action of α-Glucosidase	Action of Glucamylase	M_S Value of Disaccharide Alcohol	Conclusion
1a	1, 6	Glucose	-ve	+ve	-	0.83 ∴ 1,6	Isomaltose
3	1, 4	Glucose	-ve	+ve	+ve	0.50 ∴ 1,4	Maltose
5a	1, 6	Glucose	+ve	-ve	-	0.77 ∴ 1,6	Gentiobiose
6	1, 4	Glucose	+ve	-ve	-ve	0.40 ∴ 1,4	Cellobiose
7	1, 3	Glucose	+ve *	+ve*	-	0.06 ∴ 1,3	Nigerose and Laminaribiose

* Incomplete hydrolysis

The only glucose disaccharides, which have not been found, are therefore those with a 1, 2 or 1, 1 mode of linkage. The paper chromatographic and ionophoretic evidence strongly suggests that the 1, 2 linked disaccharides are present in fractions 1 and 5. It is a recognised fact that the α-anomers are eluted before the β-anomers from a charcoal-'Celite' column.[70,81] Fraction 1 should, therefore, contain kojibiose and fraction 5 sophorose. This was confirmed by acidic and enzymic hydrolysis of the mixtures 1a, 1b and 5a, 5b (Expt.59). Both gave glucose as the sole product of acidic hydrolysis. The former was completely hydrolysed by α-glucosidase, but not by almond β-glucosidase. The latter was not hydrolysed by α-glucosidase, but was completely hydrolysed by almond β-glucosidase.

A disaccharide of the trehalose type could not be identified. This may have been the component which was present together with maltose in fraction 2. However, it was present in trace quantity only and so could not be identified. A table summarising the final result is given below:

Table 15

Disaccharide Fraction Number	Compound	Linkage
1a	Isomaltose	α - 1, 6
1b	Kojibiose	α - 1, 2
2a, 3 and 4a	Maltose	α - 1, 4
4b and 5a	Gentiobiose	β - 1, 6

4c and 5b	Sophorose	β- 1, 2
6	Cellobiose	β- 1, 4
7	Nigerose	α- 1, 3
	Laminaribiose	β- 1, 3

This is consistent with the known order of elution of glucose disaccharides from a charcoal-'Celite' column namely gentiobiose, sophorose, cellobiose and laminaribiose.[81] Also, as already mentioned α-glycosides are eluted before β-glycosides.

The preparation of disaccharides using boron trichloride is somewhat similar to the acid reversion of D-glucose using mineral acids[69] or cation exchange resins[82] in that all possible glucose disaccharides may be formed.

In the acid reversion reaction water is eliminated between two glucose units to form a mixture of di-, tri- and higher saccharides. Thompson et.al.[69] were able to isolate gentiobiose, isomaltose, maltose, cellobiose, sophorose and β-β-trehalose together with 1, 6-anhydro- β-D-glucose from the acid reversion mixtures of D-glucose. Gentiobiose and isomaltose formed almost 90 percent of the disaccharide mixture.

By comparison with the formation of glycosides using boron trichloride, the formation of disaccharides will involve

the elimination of hydrogen chloride, which will be removed by the silver oxide. As no blocking groups were used all eleven glucose disaccharides may be formed theoretically. In actual fact the 1, 6- linked disaccharides predominated, probably due to the greater availability and reactivity of the primary hydroxyl group at $C_{(6)}$ of the glucose molecule, but as may be seen from Table 15 many other glucose disaccharides were also present. As in the preparation of phenyl glucoside (Expt.39) the α-isomers were found to predominate.

GLUCOPYRANOSYLBENZENE

An outline of the method developed by Hurd and Bonner[28] for the catalytic glucosylation of benzene has already been given on P. 15. This method is based on a Friedel-Crafts reaction between tetra-O-acetyl-α-D-glucosyl chloride and benzene in the presence of aluminium chloride.

By its use in the synthesis of glucosides and disaccharides, the complex obtained by reaction of methyl α-D-glucoside with boron trichloride has been shown to be an effective glucosylating reagent. In this section details of its use for the glucosylation of benzene are given.

The methyl α-D-glucoside-boron trichloride complex was reacted with benzene and $AlCl_3$ at 100° for 7 hr. and at room temperature for 16 hr. (Expt.60). Initially a mixture of four compounds was obtained. This mixture was composed principally of glucopyranosylbenzene and glucose, but compounds with $R_{Glucose}$ values of 4.9 and 5.5 were present in trace quantities. However, by extraction with cold chloroform a pure sample of glucopyranosylbenzene (32% yield) was obtained. This compares quite favourably with the Friedel-Crafts reaction of Hurd and Bonner when a yield of 27% was obtained.[28] The glucopyranosylbenzene was acetylated and the product had m.p. and mixed m.p., specific rotation and carbon and hydrogen content in agreement with those of authentic tetra-O-acetyl-β-D-glucopyranosylbenzene.

When the experiment was repeated using methyl α-D-glucoside in place of the methyl α-D-glucoside - boron trichloride complex, the principal product was methyl α-D-glucoside and glucopyranosylbenzene (crude) was obtained in a yield of 11%. (Expt.61).

Glucose was the principal product when the reaction was performed in the absence of aluminium chloride, although glucopyranosylbenzene was also formed (Expt.62). In this reaction oligosaccharides were present among the products. The production of glucose and oligosaccharides in such high yields suggests that glucosyl chloride is being formed during the reaction, but is not reacting with benzene. Unreacted glucosyl chloride could condense with itself, or with methyl α-D-glucoside to give oligosaccharides, or be hydrolysed to glucose in the final stages of the reaction. This inability to react with benzene to any large extent may be due to the fact that boron trichloride is not such a good Friedel Crafts catalyst as aluminium chloride for this system. However, boron trichloride should be an effective catalyst in the more activated phenol system, as a compound which was tentatively identified as p-hydroxy glucopyranosylbenzene was isolated during the synthesis of phenyl glucoside (Expt.39).

The low yield of glucopyranosylbenzene is to be expected when boron trichloride is omitted from the system as aluminium

chloride is not a good demethylating agent. Saville found that aluminium chloride will not demethylate 2, 3, 4, 6-tetra-O-methyl-D-glucose and will only partially demethylate methyl α-D-glucoside.[29] Therefore when the Friedel Crafts reaction is carried out in the absence of boron trichloride insufficient glucosyl chloride will be formed to give a good yield of glucopyranosylbenzene.

It seems, therefore, that both boron trichloride and aluminium chloride are required in order to obtain glucopyranosylbenzene in good yield. By variation of the reaction conditions it should be possible to increase the yield still further.

The methyl α-D-glucoside - boron trichloride complex has therefore proved a successful glucosylating reagent using alcohols, phenols, glucose and benzene as substrates.

EXPERIMENTAL SECTION.

GENERAL TECHNIQUES.

Experiment 1. Paper Chromatography.

Paper chromatography was carried out on Whatman No.1. or No.3. filter paper using the descending solvent technique and the following solvent systems:-

Solvent 1. Butan-1-ol, ethanol and water (4 : 1 : 5, organic phase)

Solvent 2. Propan-1-ol. ethyl acetate and water. (7 : 1 : 2)

Solvent 3. Butan-1-ol, benzene, pyridine and water. (5 : 1 : 3 : 3, organic phase)

Solvent 4. Ethyl acetate, acetic acid and water. (9 : 2 : 2)

Solvent 5. Butan-1-ol, acetic acid and water. (12 : 3 : 5)

Solvent 6. Butan-1-ol, pyridine, water and saturated aqueous boric acid solution. (6 : 4 : 2 : 1)

Solvent 7. Ethyl acetate, pyridine and water (2 : 1 : 2, organic phase)

Solvent 8. Butan-1-ol and water (1 : 1, organic phase)

The rates of movement of the compounds were referred to the movement of a standard substance X giving an R_X value.

$$R_X = \frac{\text{distance travelled by substance}}{\text{distance travelled by X}}$$

In some cases the movement was measured in relation to

the solvent front giving an R_F value.

$$R_F = \frac{\text{distance travelled by substance}}{\text{distance travelled by solvent front.}}$$

The following spray reagents were used to detect the carbohydrates and their derivatives:-

Spray 1. Silver nitrate in acetone and ethanolic sodium hydroxide.[83]

Spray 2. Aniline hydrogen phthalate in butan-1-ol followed by heating at 110°.[84]

Spray 3. p-Anisidine hydrochloride in butan-1-ol followed by heating at 110° .[85]

Spray 4. Trichloracetic acid in ethanol followed after heating at 100° for 2-3 min. by p-anisidine hydrochloride. Ketoses gave yellow colour without further heating and aldoses gave brown colour when reheated to 100° .[86]

Spray 5. α-Naphthol in ethanol and phosphoric acid followed by heating at 110°.[87]

Spray 6. Urea hydrochloride in ethanol followed by heating at 110°.[88]

Spray 7. Urea in phosphoric acid and water saturated butan-1-ol followed by heating at 110° .[89]

Spray 8. Orcinol in water saturated butan-1-ol and trichloracetic acid followed by heating at 100°.[90]

Spray 9. Phloroglucinol in acetone and trichloracetic acid in water.[91]

Spray 10. Triphenyl tetrazolium chloride and sodium hydroxide followed by exposure to a water saturated atmosphere at 40° for 20 mins.[92]

Spray 11. Diphenylamine and aniline in acetone and phosphoric acid followed by heating at 80°.[93]

Spray 12. 2, 4- Dinitrophenylhydrazine in hydrochloric acid.[94]

Spray 13. Diazotised nitroaniline in hydrochloric acid and sodium hydroxide.[95]

Spray 14. Saturated aqueous potassium periodate followed after 10 min. at 25° by p-anisidine hydrochloride.[86]

Spray 15. Anthrone in glacial acetic acid and ethanol and phosphoric acid, followed by heating at 108°.[96]

Spray 16. Ninhydrin in ethanol, colour develops slowly at room temperature.[97]

Spray 17. Potassium periodatocuprate in water followed by rosaniline in acetone.[98]

Experiment 2. Paper Ionophoresis.[80]

Paper ionophoresis was carried out on Whatman No. 3. filter paper in borate buffer (0·2M) at pH. 10·0

using the technique developed byFoster. The polyhydroxy compounds migrated as their negatively charged borate complexes. The rate of migration of the compound was referred to the migration of D-glucose giving an M_G value.

$$M_G = \frac{\text{true distance migrated by substance.}}{\text{true distance migrated by D-glucose.}}$$

2,3,4,6 - Tetra-O-methyl-D-glucose and 1,6 anhydro-β-D-glucose, which do not migrate in borate buffer, were used to correct for electrosmotic flow.

In a few cases other buffer solutions have been used and these are noted in the appropriate places.

Experiment 3. Column Chromatography

(a) 'Celite' Column.[99]

'Celite' was stirred with concentrated hydrochloric acid and left over-night. It was then washed with distilled water until the pH of the washings was the same as that of distilled water and dried at 110° .

The dry 'Celite' was slurried with half its mass of distilled water and solvent system 8 (Expt;1) added until a thin paste was obtained.

The column was fitted with a procelain disc, which was covered with a pad of glass wool. It was then filled with the 'Celite' paste. After washing with solvent system 8. (Expt.1.) the column was ready for use.

(b) Cellulose Column.

Cellulose powder was stirred with acetone and the

slurry poured into a column fitted with a porcelain disc. The column was washed first with acetone and then with solvent system 1.(Expt.1.) A little bromothymol blue was eluted down the column in order to test it for defects causing uneven flow.

(c) Charcoal-'Celite'Column.[100]

Equal volumes of charcoal (B.D.H. decolorising grade) and 'Celite' were mixed together and stirred with concentrated hydrochloric acid. After three hours the mixture was filtered and washed with distilled water until the washings were acid free. The mixture was then stirred with ethanol and left to stand for 3 hrs. Next, it was washed with the same volume of distilled water as was used to remove the hydrochloric acid and stirred into a thick slurry with distilled water. The column was fitted with a porcelain disc, which was covered with thin layers (2-3 cm.thick) of cellulose and 'Celite'. The charcoal-'Celite' mixture was poured in and when it had settled, thin layers of 'Celite' and cellulose were added. After washing with distilled water the column was ready for use.

Degradative Reactions of Boron Trichloride.

Experiment 4. Purification of reagents and solvents.

Boron trichloride (b.p. 12·5°) was supplied in sealed ampoules by B.D.H.Ltd. The ampoules were cooled before opening. The contents were transferred to a previously cooled distillation flask, which was connected by glass tubing to a receiving flask maintained at -80° by an acetone-solid carbon dioxide bath. Moisture was excluded from the system by a silica gel drying tube. Boron trichloride distilled over at room temperature and atmospheric pressure. The distillate was poured quickly into cooled weighed tubes, which were sealed off immediately and re-weighed, giving the mass of boron trichloride in each.

Boron tribromide(b.p. 91.7°) which was supplied by Borax Consolidated Ltd. in sealed ampoules, was distilled before use and stored in sealed tubes.

Commercial methanol was used without further purification.

Dichloromethane was washed with aqueous sodium carbonate(5%) and water and dried over calcium chloride. It was then distilled and the fraction boiling at 39·5 - 41° collected.

All carbohydrate derivatives were chromatographically pure and were dried *in vacuo* over phosphorus pentoxide before use.

Experiment 5. Interaction of carbohydrate derivatives and boron trichloride.

The derivative (1-10mg.) was dissolved or suspended in dichloromethane (1-2ml.) and cooled to -80° in an acetone - solid carbon dioxide bath. Boron trichloride(1-2g.), previously cooled to -80°, was then added to the substrate solution. The reaction mixture was maintained at -80° for 20-30 min. and then allowed to attain room temperature gradually and was maintained at this temperature for 16 hrs. Moisture was rigidly excluded during the reaction. Substances which were initially insoluble in dichloromethane, frequently became soluble in the reaction mixture as the temperature rose from -80° to room temperature.

When a substance remained insoluble it was usually found that no reaction had taken place. After 16 hr. at room temperature any dichloromethane or boron trichloride which remained was removed by evaporation under diminished pressure at room temperature. At this stage a glassy solid usually remained. This was treated by adding either (a) methanol (3 x 3 ml. portions) and evaporating to dryness under diminished pressure at room temperature after each addition or(b) an aqueous suspension of silver carbonate until the solution reached pH 7., filtering off the silver salts and freeze drying the aqueous filtrate. In either case the

residue was dissolved in a small amount of water or methanol and analysed by paper chromatography using at least three different solvent systems and by paper ionophoresis in borate buffer at pH.10 . Several different spray reagents were used. In some experiments the reaction conditions were varied, but these are noted in the appropiate places.

Experiment 6.

Interaction of carbohydrate derivatives and boron tribromide.

The derivative (1-10mg.) was dissolved or suspended in dichloromethane and cooled to between 0° and -45° , before addition of boron tribromide (2g.). The reaction conditions were identical with those given in Expt.5. except that in order to remove excess boron tribromide it was necessary to raise the temperature of the reaction mixture above room temperature.

Experiment 7.

Reaction of boron trichloride with 2,3,4,6 - tetra-O-methyl - D - glucose.

The reactions were carried out as in Expt.5. but the temperature conditions were varied in order to effect different degrees of demethylation.

TABLE 16

Initial Conditions. Temp.	Time min.	Final Conditions. Temp.	Time hr.	Treatment with Ag_2CO_3 or Methanol	Principal Product.	Other Products.
-80°	30	20°	16	Methanol	Glucose.	mono,di-,and tri-o-methyl glucoses.
-45°	20	20°	3	Methanol	Glucose.	mono-,di-,and tri-o-methyl glucoses
-35°	30	20°	0.5	Methanol	tri-o-methyl glucose	di- and tetra-o-methylglucoses.
-55°	30	20°	16	Ag_2CO_3	Glucose.	mono-,di-and tri-o-methyl glucoses.
-60°	20	20°	0·75	Ag_2CO_3	di-o-methyl glucoses	glucose,mono-, tri- and tetra-o-methylglucoses.
-50°	30	50° 80° 20°	1·5 1 16	Ag_2CO_3	Glucose	mono-and di-o-methylglucoses

Experiment 8. Reaction of boron tribromide with 2,3,4,6 tetra-o-methyl-D-glucose.

The reaction was carried out as in Expt.6. with slight variation in the temperature conditions.

Initial Conditions. Temp.	Time min.	Final Conditions. Temp.	Time hr.	Treatment with Ag_2CO_3 or methanol	Principal Product.	Other Products.
-60°	20	20°	16	Ag_2CO_3	Glucose	oligosaccharides.
-77°	10	20°	16	Methanol.	Glucose	0·59(R_g Value)

Experiment 9. Examination of the products of interaction of boron trichloride and 2,3,4,6 - tetra-o-methyl-D-glucose by combined paper chromatography and ionophoresis.

2,3,4,6 - Tetra-o-methyl-D-glucose was reacted with boron trichloride as in Expt.5. The intermediate complex was decomposed with an aqueous suspension of silver carbonate. After removal of the silver salts, the aqueous solution was freeze dried and the residue dissolved in methanol. Chromatograms of this solution were developed with solvent system.1.(Expt.1.) for 13 hr. and guide strips were cut off and sprayed with reagent 2 (Expt.1.). The products detected were glucose (R_G0·14), mono-o-methyl glucoses (R_G0·33) , di-o-methylglucoses (R_G0·58),tri-o-methylglucoes (R_G0·76) and tetra-o-methylglucoses (R_G 1·0). The last two were present in trace amounts only.

The mono-o-methylglucoses were separated by ionophoresis in borate buffer at pH 10 and the paper sprayed with reagent 2 (Expt.1.). Two products were detected with M_G values of 0·21 and 0·81. Values of 0·26 and 0·82 were obtained in a duplicate experiment.

A similar separation of the di-o-methyl glucoses showed the presence of two products with M_G values of 0·08 and 0·53.

Experiment 10. Treatment of methyl 4,6-o-benzylidene-2,3-di-o-methyl-α-D-glucoside with boron trichloride.

The glucoside(0·013g.) was reacted with boron trichloride (1·5g.) as in Expt.5. The complex was decomposed with an aqueous suspension of silver carbonate. After removal of the silver salts, the aqueous extract was concentrated and examined by paper chromatography in several solvent systems and ionophoresis in borate buffer at pH10, results are quoted for chromatography in solvent 1 (Expt.1.)

R_G 0·19 and 0·41

M_G 1·00 and 0·77 and 0·26

The following values were obtained with authentic compounds.

Glucose. R_G 0·19 and M_G 1·00

3-o-Methyl-D-glucose R_G 0·41 and M_G 0·80.

Foster[38] gives the following values:-

Glucose. M_G 1·00

3-o-methyl-D-glucose. M_G 0·82

2-o-methyl-D-glucose M_G 0·23

The powder, obtained after the solution was freeze dried, was dried over phosphorus pentoxide *in vacuo* and then reacted with boron trichloride as before.

One product ($R_{Glucose}$ 1·0, M_G 1·0) was detected.

Experiment 11. Reaction of boron trichloride with di-o-isopropylidene pentaerythritol.

Di-o-isopropylidene pentaerythritol (0·06g.) was dissolved in dichloromethane (2 ml.) cooled to -80° and boron trichloride (1·5g.) added. A deep brown solution was obtained. This solution was maintained at -80° for 30 min. and at room temperature for 16 hr. Excess solvent and dichloromethane were removed under diminished pressure at room temperature. Methanol (3 x 3 ml.) was added and distilled off. A thin oil, which crystallised on addition of chloroform was obtained. On recrystallisation from methanol and chloroform, pentaerythritol(0·024g, 63·5,) with m.p. and mixed m.p. 259-260° was obtained. Paper chromatographic analysis using solvent 1 (Expt.1.) showed no other product in addition to pentaerythritol.

Experiment 12. Reaction of boron trichloride with tetra-o-acetylpentaerythritol.

The acetate(0·125g.)was dissolved in dichloromethane (2ml.) , cooled to -80° and boron trichloride (2g.) added. The reaction conditions were the same as those in the previous experiment. Pentaerythritol(0·052g., 93%) m.p. 258° - 260° and mixed m.p. 260° was isolated.[66] Paper chromatographic analysis showed that the crystals contained only pentaerythritol ($R_{pentaerythritol}$ = 1·0, Solvent 1.(Expt.1.)).

in Partially acetylated products ($R_{pentaerythritol}$ 1·4 and 1·8, solvent 1) were detected in the mother liquors.

Experiment 13. Reaction of boron trichloride with sucrose.

Sucrose (0·1g.) was ground into a fine powder and placed in a test tube. A constriction was made at the neck of the tube and previously cooled boron trichloride (4g.) added. The tube was then sealed off and left at room temperature for 2 years. The sucrose remained insoluble. However, boron trichloride was evaporated off and the experiment completed as in Expt. 5. by the addition of methanol. Chromatograms in several solvent systems with a variety of spray reagents showed the presence of sucrose only.

Experiment 14. Reaction of D-Glucose with boron trichloride.

D-Glucose (0·5 g.) was suspended in trichloromethane(2 ml.) cooled to -80° and boron trichloride (5g.) added. The flask was fitted with a 'cold finger' condenser filled with solid carbon dioxide, and a silica gel drying tube. The reaction mixture was allowed to warm to room temperature and was then heated under reflux for 2 hr. at 45° . Most of the glucose remained insoluble. After decanting off the liquid, the solid was treated several times

with methanol and examined paper chromatographically. Similarly the liquid was evaporated to dryness and treated with methanol to remove the boron residues, before chromatographic analysis. Both fractions showed the presence of no product other than glucose.

Experiment 15. Reaction of boron trichloride with sucrose using (a) dimethyl formamide and (b) dimethyl sulphoxide as solvents.

(a) Sucrose (0·013g.) was dissolved in dimethyl formamide (5ml.) and the solution placed in a test tube. A constriction was made at the neck of the tube and previously cooled boron trichloride (0·5g.) added. The tube was then sealed off and left at room temperature for 3 days. When the tube was opened a black solution and precipitate were obtained. Excess boron trichloride was removed and the black residue was treated with methanol as in Expt. 5. This residue was extracted with water and the solution freeze dried. Chromatographic examination of the solution showed the presence of trace amounts of glucose.

(b) Sucrose (0·010g.) was dissolved in dimethyl sulphoxide (5ml.) and boron trichloride (0·5g.) added at -80° giving a deep yellow solution. The solution was left at room temperature in a sealed tube for 24 hrs. After removal

of excess boron trichloride the residue was treated with methanol as in Expt.5. Chromatographic analysis showed the presence of trace amounts of glucose.

Experiment 16. Reaction of boron trichloride with D-fructose.

Boron trichloride (10g.) was added to a suspension of D-fructose in dichloromethane (5ml.) at -80° . After 30 min. at -80° and 16 hr. at room temperature excess boron trichloride and dichloromethane were removed under diminished pressure at room temperature. The product was neutralised with an aqueous suspension of silver carbonate. The silver salts were filtered off and the aqueous extract freeze dried. On examination by paper chromatography using solvent systems 1,2,3, and 5(Expt.1.) and spray reagents, 1,3,5,6,7,8,9,11,12 and 15 and by paper ionophoresis in borate buffer and pH 10. the principal product was indistinguishable from authentic 5 - hydroxymethylfurfuraldehyde. This product was purified by paper chromatographic separation on sheets of Whatman No.3. filter paper using solvent system.1. (Expt.1.). The papers were viewed under ultraviolet light when the sections containing 5-hydroxymethylfurfuraldehyde were clearly visible. The appropiate sections were cut into thin strips and extracted with ether in a Soxhlet extraction apparatus. Concentration of the extract gave

a syrup (0.4g.) which was shown to be identical with pure 5- hydroxymethylfurfuraldehyde by paper chromatography and paper ionophoresis.

Experiment 17. Examination of the ultraviolet absorption spectrum of 5- hydroxymethylfurfuraldehyde.

D- Fructose (1.0g.) was reacted with boron trichloride (8 g.) as in the previous experiment. The residue obtained after removal of excess boron trichloride was treated with methanol (3 x 10 ml.) and evaporated to dryness. Water was added and the solution made up to 500 ml. (in a volumetric flask). Further dilution was necessary so 1 ml. of this solution was diluted to 100 ml. The ultraviolet spectrum of this solution was then measured on a Hilger Uvispek and compared with that of a solution of authentic 5- hydroxymethylfurfuraldehyde (see Fig. 4). The yield of 5- hydroxymethylfurfuraldehyde was obtained from consideration of the optical density ($\log \frac{I_o}{I}$) at a wave length of 284mμ where the extinction coefficient ε of 5-hydroxymethylfurfuraldehyde is 16,700.

The optical density is given by,

$$\log. \frac{I_o}{I} = \varepsilon \, c \, d ,$$

where c is the concentration in g. mole/litre and d is

the cell width (1 cm).

From the graph,

$$c = \frac{1{\cdot}08.\ 126}{16{,}700} \text{ g./litre}$$

Therefore the yield = $\frac{50.\ 1{\cdot}08.\ 126 \text{ g.}}{16{,}700}$

= 0·41g., (58·2%).

Experiment 18. The oxidation of 5-hydroxymethylfurfuraldehyde.

The aldehyde (0·37g.) obtained in Expt.16. was dissolved in a little water and silver oxide (1g.) added. After cooling the solution in ice water , a solution of sodium hydroxide (1·6g.)in water (15 ml.) was added. The solution was shaken for 30 min, filtered and the precipitate washed with water. Hydrochloric acid was added to the combined filtrate and washings until an acid reaction was obtained with Congo red paper. The filtrate was then extracted with ether. Concentration of the ether extract gave 5-hydroxymethyl-2-furoic acid (0·37, 89%) which was recrystallised from toluene-acetone (1 : 1) to give colourless needle shaped crystals, m.p. and mixed m.p. 165°. (Found: C, 51·1 ; H, 4·5. Calc. for $C_6H_6O_4$: C, 50·7 ; H 4·3%). Reichstein[47] give m.p. 163-166°.

Experiment 19. Interaction of boron trichloride and 2,5 -di-o-toluene-p-sulphonyl-D-mannitol.

The ester (0.228g.) was suspended in dichloromethane (2ml.) cooled to - 80° and boron trichloride (2g.) added. After 30 min. the flask was removed from the cold bath and the solution became homogeneous. Excess solvent and boron trichloride were removed after 18 hr. at room temperature and the residue was treated with methanol (4 x 4 ml.). A white solid separated out and was recrystallised from ethyl acetate giving 2,5-di-o-toluene-p-sulphonyl-D-mannitol (0.220g., 96.5%) m.p. and mixed m.p. 125°. Fletcher et al[101] give m.p. 125-126°. Examination of the mother liquors by paper chromatography in solvent system 1. (Expt.1.) showed the presence of no products other than a trace of 2,5-di-o-toluene-p-sulphonyl-D-mannitol. (R_F 0.86).

Experiment 20. Reaction of boron trichloride with 1,3 : 4,6 - di-o-ethylidene-2,5-di-o-toluene-p-sulphonyl-D-mannitol

The ester (0.10g.) was dissolved in dichloromethane, cooled to -80° and boron trichloride (2g.) added. After 30 min. at -80° and 18 hr. at room temperature excess boron trichloride and dichloromethane were removed under diminished pressure. Methanol

(3 x 5 ml.) was added to the residue and distilled off. Recrystallisation of the solid remaining from ethyl acetate gave 2,5-di-o-toluene-p-sulphonyl-D-mannitol (0·08g.88·9%) m.p. and mixed m.p. 125° and with an R_F value of 0·86 in solvent 1 (Expt.1.). In addition a trace product with R_F value of 0·39 in solvent 1 (Expt.1.) was detected using the potassium periodatocuprate spray reagent.

Experiment 21. Interaction of 1,3 : 4,6-di-o-methylene 2,5-di-o-toluene-p-sulphonyl-D-mannitol and boron trichloride.

The ester (0·10g.) was reacted with boron trichloride (2g.) as in the previous experiment. Attempts to crystallize the products proved unsuccesful. Chromatographic analysis showed the presence of 2,5-di-o-toluene-p-sulphonyl-D-mannitol with R_F value of 0·86 and a second product with R_F value of 0·39 in solvent 1 (Expt .1.).

Experiment 22. Reaction of boron trichloride with tetra-o-toluene-p-sulphonylpentaerythritol.

The ester (0·100g.)was dissolved in dichloromethane (2 ml.) cooled to -80° and boron trichloride (1·5g.) added . The reaction conditions and time were the same as in Expt.20. Recrystallisation of the residue obtained after treatment with methanol gave tetra-o-

toluene-p-sulphonylpentaerythritol (0·092g. 92%) m.p. and mixed m.p. 154·5°. Buchman[102] gives m.p. 154·5-155·5° . No other products could be detected by paper chromatography.

Experiment.23. Reaction of boron trichloride with tetra-o-benzene-sulphonylpentaerythritol.

The ester (0·100g.)was dissolved in dichloromethane(2ml.) cooled to -80° and reacted with boron trichloride (2g.) as in Expt.20. Un-changed tetra -o-benzene-sulphonylpentaerythritol (0·87, 87%) was recovered m.p. and mixed m.p. 103° . Buchman[102] gives m.p. 103° . No other products could be detected by paper chromatography.

Experiment 24. Reaction of boron trichloride with pentaerythritol tetrabromide.

Pentaerythritol tetrabromide(0·072g.) was dissolved in dichloromethane (2ml.), cooled to -80° and boron trichloride (2g.) was added. A white precipitate was formed. After 30 min. the reaction mixture was allowed to warm to room temperature and was maintained at this temperature for 16 hr. Excess solvent and boron trichloride were distilled off and the residue treated with methanol (4 x 3 ml.) in which it was insoluble. The unchanged

pentaerythritol tetrabromide (0·052g, 72%) was filtered off and had m.p. and mixed m.p. 161° [102]. No other products could be detected by paper chromatography.

SYNTHETIC REACTIONS OF BORON TRICHLORIDE.

I Glycosides.

Experiment 25. The preparation of benzyl glucoside by a modified Koenigs and Knorr reaction.

Methyl α-D-glucoside (0·9g.) was suspended in dichloromethane (5 ml.) and the suspension was cooled to -80° in an acetone-solid carbon dioxide bath. Previously cooled boron trichloride (3·3g.) was added and the mixture maintained at -80° for 30 min. It was then allowed to warm gradually to room temperature. As the temperature rose the glucoside became soluble in the reaction solution, which was kept at room temperature for 16 hr. under anhydrous conditions. After removal of any solvent and boron trichloride which remained by evaporation under diminished pressure at room temperature, a pale pink solid was obtained. Benzyl alcohol (6ml., dried over anhydrous magnesium sulphate and redistilled) and silver oxide(4·0g., dried *in vacuo* at 60° in the dark) were added and the reaction mixture was shaken in a darkened flask for 24 hr. Silver oxide and silver chloride were filtered off and the filtrate concentrated

to low bulk.

Chromatograms of the filtrate in solvent system 1 (Expt.1.) showed the presence of four components.

(1) $R_{Glucose}$ = 1·0 glucose.

(2) $R_{Glucose}$ = 1·3 *

(3) $R_{Glucose}$ = 2·0 * methyl α-D-glucoside.

(4) $R_{Glucose}$ = 5·4 benzyl glucoside.

* Present in trace quantities only.

The solution was then slurried with 'Celite' and introduced to the top of a 'Celite' column 50 x 5 cm. (Expt.3a.) which was then eluted with solvent system 8 (Expt.1.) and 50ml. fractions were collected. Each fraction was concentrated on a rotary evaporator and analysed by paper chromatography using solvent system 1 (Expt. 1.) The results are given in the following table.

Table 17.

Fraction.	$R_{Glucose}$ Value of products.
1 - 5	-
6 - 15	5·4, benzyl glucoside
16- 20	5·4, benzyl glucoside
	2·8*, -
21- 35	2·9* -
36- 50	- -
51- 55	2·0, methyl glucoside
56- 60	-
61- 160	1·0, glucose
	1·3*, -

* Present in trace quantities only.

Experiment 26. A detailed investigation of fractions 6 - 15.

Fractions 6 - 15 obtained in Expt 25. were combined and concentrated to give a pale yellow oil (0·3g.). This oil was crystallised from ethyl acetate and identified as benzyl β-D- glucoside by the following scheme.

(i) Paper Chromatography The product was indistinguishable from benzyl β-D- glucoside by paper chromatography in solvent systems 1, 3, and 4

(Expt.1.). A positive reaction was obtained with the silver nitrate-sodium hydroxide spray reagent, but no reaction was obtained with the aniline hydrogen phthalate spray reagent, confirming that the reducing position of the glucose residue was substituted.

(ii) Paper ionophoresis Ionophoretograms of fractions 6 - 15 in borate buffer at pH 10 (Expt. 2.) and sprayed with the silver nitrate-sodium hydroxide spray reagent showed the presence of one product only with an M_G value of 0·12.(Benzyl β-D-glucoside had an M_G value of 0·12).

(iii) Hydrolysis

(a) Resin hydrolysis[103] The glucoside (5mg.) was dissolved in water (1 ml.) and heated with Amberlite 1R - 120 (H^+) resin (0·1g.) in a sealed tube at 100° for 8 hr. After the resin had been filtered off the resulting solution was examined by paper chromatography in solvent systems 1 and 4 (Expt. 1.) and by paper ionophoresis in borate buffer at pH 10. The chromatograms were sprayed with the silver nitrate-sodium hydroxide reagent and in each case one product identical with glucose, $R_{Glucose}$ 1·0, was detected. Similarly the ionophoretogram showed one product with an M_G value of 1·0.

(b) Enzymic hydrolysis.[104] An aqueous solution of the benzyl glucoside was incubated in a sealed capillary tube with an equal volume of almond β- glucosidase solution at 27° for 7 days. This solution was then applied directly to the base line of a chromatogram, which was eluted in solvent system 1 (Expt. 1.) and sprayed with the silver nitrate-sodium hydroxide reagent. The principal product was glucose, but there was a trace of unchanged benzyl glucoside. The specificity of the almond β- glucosidase was checked under these conditions. When an α- glucosidase was used there was no hydrolysis.

(iv) Analysis The oil was recrystallised several times from ethyl acetate and benzyl glucoside m.p. 121° was obtained. Fischer and Helferich[105] give m.p. 123-125°.

Found: C, 57.8 ; H 6.9 : Calc. for C_{13} H_{18} O_6

C, 57.8 ; H 6.7%.

(v) Acetylation Benzyl glucoside (0.1g.), dry pyridine (1 ml.) and acetic anhydride (0.4 ml.) were heated under reflux on an oil bath for 5 min. The solution was then poured into ice water and colourless crystals of benzyl 2,3,4,6-tetra-O-acetyl- β -D- glucoside separated out, m.p. and mixed m.p. 96° Fischer and Helferich[105] give m.p. 96-101°.

Recrystallisation of the oil obtained from fractions 16-20 gave a benzyl β-D-glucoside (0·17g.).

Experiment 27. Further investigation of fractions 51-55.

Fractions 51-55 obtained in Expt. 25 were combined and concentrated to give a colourless oil (0·004g.). The oil was not crystallised, but dissolved in methanol and was analysed by paper chromatography. Its mobility in solvent systems 1, 2 and 4 (Expt.1.) was indentical with that of methyl α-D-glucoside . With the aniline hydrogen phthalate spray reagent a negative result was obtained , but with silver nitrate-sodium hydroxide there was a slow positive reaction. Insufficient material was obtained on concentration of fractions 21-35 to facilitate further examination.

Experiment 28. Further investigation of fractions 61-160.

Fractions 61-160 obtained in Expt.25. when combined and concentrated gave a colourless oil (0·1g.) with $[\alpha]_D^{20}$ + 52·5 (C= 2 in water). Examination of the oil by paper chromatography showed the presence of two components, I and II . The latter was present in trace quantity only.

Component I was identical with D-glucose

having an $R_{Glucose}$ value of 1·0 in solvent systems 1, 3 and 4 (Expt.1.) and an M_G value of 1·0 in borate buffer at pH.10. (Expt.2.).

Component II gave a positive reaction with the urea hydrochloride spray reagent. When chromatograms of component II were sprayed with the trichloracetic acid reagent, heated to 100° for 3 min. and resprayed with p- anisidine hydrochloride reagent a yellow colour was obtained immediately without further heating. Both these tests are specific for ketoses. Component II was indistinguishable from D- fructose by paper chromatography in solvent systems 1, 3, and 4 (Expt.1) and by paper ionophoresis in borate buffer at pH 10 (Expt.2.).

Experiment.29. Attempted preparation of benzyl glucoside from methyl α-D-glucoside in the absence of boron trichloride.

(a) Methyl α-D- glucoside (0·2g.) dichloromethane (1 ml.), benzyl alcohol (1·2 ml., dried and redistilled) and silver oxide (0·8g., dried in vacuo at 60° in the dark) were shaken together for 24 hr. in a darkened flask. The silver oxide and silver chloride were filtered off. Analysis of the resulting solution by paper chromatography in solvent systems 1, 3 and 4 (Expt.1.) showed

the presence of one product only, methyl α -D-glucoside.

(b) Methyl α -D-glucoside (1·0g.) , dichloromethane (5 ml.) benzyl alcohol (6 ml.), concentrated hydrochloric acid (1 ml.) and silver oxide (4·0g.) were shaken together as in the previous experiment. The resulting solution was examined by paper chromatography and only methyl α- D-glucoside was detected.

Experiment 30. Attempted preparation of benzyl glucoside from D- glucose in the absence of boron trichloride.

D- Glucose (0·2g.),dichloromethane (1 ml.), benzyl alcohol (1·2 ml., dried and redistilled) and silver oxide (0·8g. dried *in vacuo* at 60° in the dark) were shaken together for 24 hr. in a darkened flask. Silver oxide and silver chloride were filtered off and the resulting solution analysed by paper chromatography in solvent systems 1, 3, and 4 (Expt.1.). The only product which could be detected was glucose.

Experiment 31. Attempted preparation of phenyl glucoside by a modified Koenigs & Knorr reaction.

Methyl α- D- glucoside (0·2g.) was suspended in dichloromethane (1 ml.) and reacted with boron trichloride

(0.7g.) as in Expt.5. The glassy residue which was obtained was dissolved in dichloromethane (3 ml.) and a solution of dry phenol (0.4g.) in dichloromethane (7 ml.) was added, together with silver oxide (0.6g., dried in vacuo at 60° in the dark). The mixture was then shaken for 48 hr. in a darkened flask. Silver oxide and silver chloride were filtered off and the filtrate examined by paper chromatography in solvent system.1. (Expt.1.) using the silver nitrate-sodium hydroxide spray reagent. No phenyl glucoside was detected. The only products were glucose ($R_{Glucose} \equiv 1.0$) and phenol ($R_{Glucose} \equiv 8.0$) .

Experiment 32. The preparation of phenyl glucoside using boron tribromide.

Methyl α-D-glucoside (0·1g.) was suspended in dichloromethane (1 ml.) and the suspension was cooled to between - 50° and-60° before addition of boron tribromide (0.5g.). The mixture was maintained at this temperature for 20 min. and then at room temperature for 16 hr. under anhydrous conditions. After evaporation to dryness under diminished pressure a solution of phenol (0.4g.) in dichloromethane (10 ml.) was added. This solution was then shaken for 24 hr. with silver oxide (0.5 g., dried in vacuo at 60° in the dark). The insoluble silver salts were removed by filtration

and the filtrate was examined by paper chromatography in solvent system 1. (Expt.1.). Five components were detected, although two were only present in trace amount.

(1) $R_{Glucose}$ = 0·3* oligosaccharides.

(2) $R_{Glucose}$ = 1·0 glucose.

(3) $R_{Glucose}$ = 2·4*

(4) $R_{Glucose}$ = 4·9 phenyl glucoside.

(5) $R_{Glucose}$ = 7·0 phenol.

* Present in trace amount only.

Experiment 33. The preparation of phenyl glucoside using boron trichloride under various reaction conditions.

(i) Methyl ∝ -D- glucoside(0·1g.) was reacted with boron trichloride (0·5g.) as in Expt.5. A solution of dry phenol in dichloromethane (20 ml.) was added to the methyl ∝ -D- glucoside- boron trichloride complex and the solution was kept at room temperature for 2 days. Chromatograms of the solution eluted in solvent system 1 (Expt.1.) showed the presence of five components in addition to phenol.

(1) $R_{Glucose}$= 0·3. oligosaccharides.

(2) $R_{Glucose}$= 1·0 glucose.

(3) $R_{Glucose}$ = 2·2*

(4) $R_{Glucose}$ = 3·3*

(5) $R_{Glucose}$ = 4·9 phenyl glucoside.

* Present in trace amounts only.

Component 3 is most probably methyl α-D- glucoside.

(ii) Methyl α-D- glucoside (0·09g.) was reacted with boron trichloride (0·4g.) as in Expt.5. The glassy residue, which was obtained after removal of excess solvent and boron trichloride, was dissolved in a solution of sodium hydroxide (0·2g.) in dichloromethane (15 ml.) together with phenol (0·4g.). This solution was kept at room temperature for 2 days. By paper chromatographic analysis in solvent system.1.(Expt.1.) using the silver nitrate-sodium hydroxide and potassium periodatocuprate spray reagents it was shown that five compounds were present in addition to phenol. Of these compounds two were present in trace amount only.

(1) $R_{Glucose}$= 0·3. oligosaccharides.

(2) $R_{Glucose}$= 1·0 glucose.

(3) $R_{Glucose}$= 2·0*

(4) $R_{Glucose}$= 2·6*

(5) $R_{Glucose}$= 4·9 phenyl glucoside.

* Present in trace amounts only.

This experiment was repeated and the products analysed

by paper chromatography using solvent system 4 (Expt .1.) and the diazotised nitroaniline spray reagent. In addition to phenol there was one other product. This product gave a deep pink colour with the spray reagent and had an R_F value of 0·64.

An identical result was obtained when the products from Expt.33(i) were examined by paper chromatography using the diazotised nitroaniline spray reagent.

(iii) Methyl α-D-glucoside(0·09g.) was reacted with boron trichloride(0·3g.) as in Expt.5. The resulting solid was heated with phenol (0.5g.), silver oxide (0·2g. dried *in vacuo* at 60° in the dark) and dichloromethane (20ml.) at 50° for 2 hr. The silver oxide and silver chloride were filtered off and the filtrate examined by paper chromatography.
Five substances in additiom to phenol were present. The $R_{Glucose}$ values refer to elution with solvent system 1.(Expt.1.).

(1) $R_{Glucose}$=0·3 oligosaccharides.
(2) $R_{Glucose}$=1·0 glucose.
(3) $R_{Glucose}$=1·7*
(4) $R_{Glucose}$=2·5*
(5) $R_{Glucose}$=4·6 phenyl glucoside.

* Present in trace quantities only.

The spot corresponding to phenyl glucoside showed a strong tendency to streak, suggesting the presence of some other product with a similar $R_{Glucose}$ value.

(iv) Methyl α-D-glucoside (0·09g) was reacted with boron trichloride (0·4g.) as in Expt.5. The glassy residue was obtained after removal of excess solvent and boron trichloride, was heated under reflux at 50° for 2 hr. with a solution of dry phenol (0·5g.) in dichloromethane (20 ml.). Paper chromatographic analysis showed the presence of four components in addition to phenol. The $R_{Glucose}$ values again refer to elution with solvent system 1 (Expt.1.).

(1) $R_{Glucose}$ = 0·3 oligosaccharides
(2) $R_{Glucose}$ = 1·0 glucose
(3) $R_{Glucose}$ = 2·9*
(4) $R_{Glucose}$ = 5·1 phenyl glucoside.

* Present in trace amounts only.

Experiment 34. The preparation of catechol glucoside using boron trichloride.

Methyl α-D-glucoside (0·1g.) was reacted with boron trichloride (0·3g.) as in Expt.5. After removal of excess dichloromethane and boron trichloride a glassy solid was obtained. A solution of catechol

(0·5g.) in dichloromethane (20ml.) was added and this solution was heated gently under reflux on a warm water bath for 2 hr. The products were analysed by paper chromatography using solvent system 1 (Expt.1) and the silver nitrate-sodium hydroxide spray reagent. In addition to catechol four products were detected.

(1) $R_{Glucose}$ = 0·3 oligosaccharides

(2) $R_{Glucose}$ = 1·0 glucose

(3) $R_{Glucose}$ = 2·1*

(4) $R_{Glucose}$ = 5·3 catechol glucoside.

Experiment 35. The preparation of catechol glucoside.

Methyl α -D- glucoside (3g.) was suspended in dichloromethane (20ml.) cooled to -80° and boron trichloride (9g.) added. The reaction mixture was kept at -80° for 30 min. and at room temperature for 16 hr. under anhydrous conditions. Excess dichloromethane and boron trichloride were removed by evaporation under diminished pressure at room temperature. A glassy residue remained. A solution of catechol (6 g.) in dichloromethane (30ml.) was added to the residue. When the initial evolution of gas had ceased the solution was heated under reflux at 50° for 2 hr. Ether (dried over sodium) was added to this solution until no more

solid was precipitated. The precipitate was filtered off, washed thoroughly with ether and then slurried with solvent system 1(Expt.1.). Only part of the precipitate dissolved. The insoluble portion was filtered off and washed with solvent system 1. This solid gave a positive test for boron with turmeric paper and was shown by paper chromatography to contain no carbohydrates other than a trace of glucose. The combined filtrate and washings were analysed by paper chromatography using solvent system 1 (Expt.1) and the silver nitrate-sodium hydroxide spray reagent. Three products were detected in addition to catechol.

(1) $R_{Glucose}$ = 0·2 oligosaccharides

(2) $R_{Glucose}$ = 1·0 glucose

(3) $R_{Glucose}$ = 5·1 catechol glucoside.

The solution was then introduced at the top of a cellulose column 55 x 4·7 cm. (Expt.3b), which was eluted with solvent system 1 (Expt.1.) and 25ml. fractions were collected. Each fraction was examined by paper chromatography and the results are shown in the following table .

Table 18.

Fraction.	R_p values of products detected with, (i)silver nitrate sodium hydroxide.	(ii)diazotised nitroaniline.	Identity of products.
1 - 10	-	-	
11 - 17	0.82	0·81(Brown)	catechol.
	0.61	0·61(pink)	catechol glucoside.
	-	0·47*(pink)	
	-	0·40*(pink)	
18 - 29	-	0·47*(pink)	
	-	0·39*(pink)	
	-	0·30*(pink)	
30 - 45	0·19	-	glucose.
46 - 47	-	-	
48 - 87	0·07	-	oligosaccharides.
88 →	-	-	

* Present in trace quantities only.

Insufficient material was obtained on concentration of fractions 18-29 to facilitate identification of such a complex mixture.

Experiment 36. A detailed investigation of fractions 11-17.

Fractions 11 - 17 obtained in Expt. 35. were combined and concentrated under nitrogen to prevent oxidation. A colourless syrup was obtained. This syrup was crystallised from aqueous methanol and petroleum ether to give colourless crystals (2·4g.) , which were identified as catechol glucoside by the following scheme.

(i) Paper chromatography. The material was indistinguishable from catechol β-D-glucoside by paper chromatography with solvent systems 1, 3 and 4 (Expt.1.). A positive reaction wasobtained with the silver nitrate-sodium hydroxide spray reagent and a negative reaction with the aniline hydrogen phthalate spray reagent, confirming that the reducing position of the glucose residue was substituted. A pink coloration was obtained with the diazotised nitroaniline spray reagent.

(ii) Paper ionophoresis. Ionophoresis of fractions 11-17 in borate buffer at pH 10 (Expt.2.) showed the presence of one product only (detected with the diazotised nitroaniline spray reagent,) having an $M_{\text{Salicylic acid}}$ value of 0.52. Catechol β-D-glucoside also gave a value of 0.52.

(iii) Hydrolysis.

(a) Almond β -glucosidase. An aqueous solution of the glucoside was incubated with an equal volume of almond β -glucosidase in a sealed capillary tube at 35° and 27° for 3 days. These solutions were then applied directly to the base line of several chromatograms which were eluted with solvent system 1 (Expt.1.). The products were detected with the silver nitrate-sodium hydroxide and diazotised nitroaniline spray reagents. The products were glucose, catechol glucoside and catechol. The specificity of the almond β -glucosidase was checked under identical conditions.

(b) α -Glucosidase. The glucoside was incubated with α -glucosidase in a sealed tube at 37° for 2 days. Analysis of the products by paper chromatography showed the presence of glucose, catechol glucoside and catechol. Under these conditions phenyl α-D-glucoside was completely hydrolysed and catechol β -D- glucoside was unchanged.

(iv) Analysis. The syrup obtained from fractions 11-17 was crystallised several times from aqueous methanol and petroleum ether to give catechol glucoside m.p. 132° and $[\alpha]_D^{20}$ + 29° (C = 3 in water). Helferich, Lang and Schmitz-Hillebrecht[106] give catechol β -D- glucoside m.p.

129-133°, $[\alpha]_D^{20}$ - 71° in water.

Found. C, 49.8; H.6.4; Calc. for $C_{12}H_{16}O_7.H_2O$ C, 49·6; H 6·2%.

Experiment 37. A detailed investigation of fractions 30-45.

Fractions 30-45 obtained in Expt.35. were combined and concentrated to low bulk under diminished pressure. Crystals of D- glucose (0·05g.) separated out and were identified as follows.

(i) Paper chromatography. The product was indistinguishable from D - glucose by paper chromatography in solvent systems, 1, 4, and 5 (Expt.1) using a variety of spray reagents. A positive reaction was obtained with the silver nitrate-sodium hydroxide and aniline hydrogen phthalate spray reagents and a negative reaction with the diazotised nitroaniline reagent.

(ii) Paper ionophoresis. The product had an M_G value of 1·0 in borate buffer at pH 10 (Expt.2.).

(iii) Melting point and optical rotation. The solid obtained on concentration of fractions 30-45 was recrystallised several times from methanol and propan-2-ol giving D-glucose m.p. and mixed m.p. 146°, $[\alpha]_D^{20}$ + 52 .2° (c=1·6 in water). Literature values[66] are m.p. 146° and $[\alpha]_D^{20}$ + 52.5° in water.

Experiment 38. Further investigation of fractions 48-87.

Fractions 48-87 obtained in Expt.35 were combined and concentrated to low bulk. As a mixture of several disaccharides were present these fractions were not investigated in detail.

(i) Hydrolysis.

(a) Fractions 48-87 were partially hydrolysed by almond β-glucosidase and α-glucosidase using the same conditions as in Expt. 36. In each case the products were glucose and unchanged disaccharides.

(ii) Paper chromatography. Analysis of fractions 48-87 by paper chromatography using a variety of solvent systems and spray reagents showed the presence of several disaccharides. In particular, using the diphenylamine spray reagent the following result was obtained: -

(1) $R_{Glucose}$ = 0.37, grey-green.
(2) $R_{Glucose}$ = 0.56, yellow.
(3) $R_{Glucose}$ = 0.43, blue.

This spray reagent gives an indication of the linkage of the disaccharides, as different colours are obtained with the various glucose disaccharides[93], namely

1,6 grey green
1,4 blue
1,3 grey-green
1,2 yellow.

(iii) Paper ionophoresis. The solution was examined

by paper ionophoresis in borate buffer at pH10(Expt.2.) using the diphenylamine spray reagent. The following results were obtained:-

(1) M_G = 0·29 yellow-blue

(2) M_G = 0·69 grey-green.

The accepted M_G values[38] are nigerose, laminaribiose and isomaltose 0·69, cellobiose 0·23, maltose 0·32 and trehaloses 0·19 and 0·23. The trehaloses would not be detected by the diphenylamine spray reagent.

Experiment. 39. The preparation of phenyl glucoside.

Methyl α-D-glucoside (5g.) was suspended in dichloromethane(20ml.), cooled to -80° and boron trichloride (15.g.) added. The reaction mixture was kept at -80° for 20 min. and at room temperature for 16hr. before removal of any dichloromethane and boron trichloride which remained. A solution of phenol(9g.) in dichloromethane (25ml) was added to the methyl α-D-glucoside— boron trichloride complex. At first there was a brisk effervescence, but this ceased after a few minutes. The solution was then heated under reflux on a warm water bath for 1½ hr. Next, a solution of phenol (4g.) in dichloromethane was added, but there was no further effervescence and the solution was reheated for 1 hr.

Ether(dried over sodium) was added to the dichloromethane solution until there was no further precipitation. The precipitate was filtered off,washed with ether and then stirred with solvent system.1.(Expt.1.). Most of the solid dissolved and the insoluble portion was filtered off. This residue gave a positive test for boron with turmeric paper and was shown by paper chromatography to contain no carbohydrates other than glucose. The pale brown filtrate was examined paper chromatographically in solvent system 1.(Expt.1.) and three principal products were detected:-

(1) $R_{Glucose.}$ = 0·2 oligosaccharides.

(2) $R_{Glucose.}$ = 1·0 glucose.

(3) $R_{Glucose.}$ = 4·5 phenyl glucoside.

This filtrate was then introduced at the top of a cellulose column 55 x 4·7 cm. (Expt.3b), which was eluted with solvent system.1. (Expt.1.) and 25ml.fractions were collected. These fractions were analysed by paper chromatography. The results are given in the following table.

Table 19.

Fraction.	R_p value of products.
1 - 13	-
14 - 22	0·63 phenyl glucoside. 0·54 -
23-26	0·64 phenyl glucoside 0·53 - 0·35* 1,6-anhydro-β-D-glucose.
27-30.	-
31-33	0·34 1,6-anhydro-β-D-glucose.
34-44	-
45-58	0·12 glucose.
59 - 65 66 - 99 100 -	- 0·05 oligosaccharides. -

* Present in trace quantity.

As fractions 23-26 contained principally the same components as fractions 14-22 they were not investigated further.

Experiment 40. A further investigation of fractions 31-33.

Fractions 31-33 obtained in Expt. 39. were combined and concentrated to give a colourless oil(0·012g.). Crystallisation of the oil fron propan-2-ol gave colourless crystals m.p. 178°. Peat[107] gives 1,6-anhydro-β-D-glucose m.p.178°. The product obtained from fractions 31-33 could not be distinguished from authentic 1,6-anhydro-β-D-glucose by paper chromatography with solvent systems 1, 3 and 4(Expt. 1). when $R_{Glucose}$ values of 3·3 , 2·05 and 3·1 respectively were obtained. The 1,6-anhydro-β-D-glucose was revealed using the silver nitrate-sodium hydroxide spray reagent, as a negative result was obtained with p-anisidine hydrochloride reagent even when viewed under ultra-violet light. Similarly no differentiation was obtained between fractions 31-33 and 1,6- anhydro-β-D-glucose by ionophoresis in borate buffer at pH10, in which neither compound migrated. This enables 1,6-anhydro-β-D-glucose to be used as a base line marker to correct for endosmosis during ionophoresis in borate buffer at pH10. This is particularly useful as unlike the usual marker 2,3,4,6-tetra-o-methyl-D-glucose., 1,6-anhydro-β-D-glucose can be detected with the silver nitrate-sodium hydroxide spray reagent.

Experiment 41. A detailed investigation of fractions 45-58.

Fractions 45-58 obtained in Expt.39. were combined and concentrated to low bulk under diminished pressure. Crystals of D-glucose (0·04g.) separated out and were identified as follows.

(i) Paper chromatography. The product was indistinguishable from D-glucose by paper chromatography in solvent systems, 1, 2 and 4(Expt.1) in which R_F values of 0·13, 0·30 and 0·18 respectively were obtained. D-Glucose was detected using the silver nitrate-sodium hydroxide and p-anisidine hydrochloride spray reagents.

(ii) Paper ionophoresis. Paper ionophoresis in borate buffer at pH10 (Expt.2.) showed the presence of one product. This compound had an M_G value of 1·0 which is identical with that of D-glucose.

(iii) Melting point and optical rotation. The solid obtained on concentration of fractions 45-58 was recrystallised from methanol and propan-2-ol several times giving D-glucose m.p. and mixed m.p. 146° and $[\alpha]_D^{20}$ + 52·9 (c= 1·3 in water). The accepted values[66] are m.p. 146° and $[\alpha]_D^{20}$ + 52·5 in water.

Experiment 42. Further investigation of fractions 66-99.

These fractions contained a mixture of disaccharides and were not investigated in detail. It was

shown by paper chromatography and paper ionophoresis that these disaccharides were very similar to those obtained in the preparation of catechol glucoside by the same method (Expts. 35 and 38). The results obtained by paper chromatography are identical with those given in Expt.38. Similar results were also obtained by paper ionophoresis in borate buffer at pH10. Two products with M_G values of 0·25 and 0 ·65 were obtained. The former gave a blue-yellow colour and the latter a grey-green colour with diphenylamine spray reagent.

Experiment 43. Preliminary investigation of fractions 14-22.

Fractions 14-22 obtained in Expt.39. were combined and concentrated under diminished pressure to give a white solid(6g., m.p. 135-142°). The material was washed with ether and recrystallised from hot water. It then had m.p. 158°, but still contained a mixture of phenyl glucoside and a second component, 'A'. The mixture was examined in the following way.

(i) Paper chromatography.

(a) Silver nitrate-sodium hydroxide spray reagent:- Both components gave a positive reaction with this spray reagent, but 'A' reacted immediately and phenyl glucoside after 5 min.

(b) p-Anisidine hydrochloride spray reagent:- Under normal conditions neither component gave a colour reaction with this reagent. However, one component became visible when viewed under ultraviolet light and had an R_F value of 0·63 (Solvent system 4, Expt.1.). In this system phenyl α-D-glucoside has an R_F value of 0·63 and it gave a similar reaction with p-anisidine hydrochloride.

(c) Diazotised nitroaniline spray reagent:- With this reagent phenyl α-D-glucoside gave no colour reaction and 'A' gave a deep pink colour and had an R_F value of 0·53 (solvent system 4, Expt.1.).

(d) Various spray reagents:- Paper chromatograms were developed with a solvent system containing boric acid (solvent system 6, Expt.1.) and the following spray reagents were used:- silver nitrate-sodium hydroxide, diszotised nitroaniline, sodium periodate- potassium permanganate- benzidine and potassium periodate - p-anisidine. In each case one component only was detected with an R_F value of 0·71 - 0·75. (Phenyl α-D-glucoside and phenyl β-D-glucoside have R_F values of 0·72 and 0·73 respectively).

(ii) Paper ionophoresis. Using borate buffer at pH 10 (Expt. 2.) and the silver nitrate-sodium hydroxide

spray reagent two components were detected with M_G values of 0·14 and 0·70 . In the same buffer solution phenyl α-D-glucoside had an M_G value of 0·14. However, when the diazotised nitroaniline spray reagent was used only one component was detected with an M_G value of 0·70.

(iii) Extraction with chloroform and methanol. The solid obtained from fractions 14-22(Expt.39) was extracted first with chloroform and then with methanol. These extracts were examined by paper ionophoresis in borate buffer at pH10 (Expt.2.) using the silver nitrate-sodium hydroxide spray reagent. Each extract contained two components with M_G values of 0·17 and 0·77. In this experiment phenyl α-D-glucoside had an M_G value of 0·18.

(iv) Charcoal-'Celite' column chromatography. The mixture of phenyl glucoside and 'A' obtained in Expt.39 (0·12g.) was introduced at the top of a trial size charcoal-'Celite' column (14 x 1·6 cm.). The column was eluted with aqueous ethanol (0 - 50%) and 5 ml. fractions were collected. Each fraction was analysed by paper chromatography. A separation of phenyl glucoside and 'A' was effected, so this experiment was repeated on a larger scale (Expt.44).

Experiment 44. The separation of phenyl glucoside and 'A' using a charcoal-'Celite' column.

A mixture of phenyl glucoside and 'A' (2·0g.) obtained in Expt 39 was introduced at the top of a charcoal-'Celite' column, 60 x 4·8 cm. (Expt 30). The column was eluted with aqueous ethanol (0-50%) and 25ml. fractions were collected. Each fraction was analysed by paper chromatography in solvent system 1 (Expt.1.). The results which were obtained are given in the following table.

Table 20.

Fraction.	R_F value of products detected with (a) silver nitrate-sodium hydroxide.	(b) diazotised nitroaniline.
1-221	-	-
222-246	0·63	-
247-262	0·63	-
263-273	-	-
274-310	0·53	0·53
310 -	-	-

A difference in the rate at which fractions 222-246 and 247-262 reduced the silver nitrate spray reagent was

observed. Fractions 222-246 reduced the reagent more slowly than fractions 247-262. A similar difference in this rate was observed between phenyl α -D-glucoside and phenyl β-D-glucoside.

Insufficient material was obtained from fractions 274-310 to facilitate identification of 'A' at this stage.

Experiment 45. A detailed investigation of fractions 222-244

Fractions 222-244 obtained in Expt.44. were combined and concentrated to low bulk under diminished pressure. A white solid (0·2g.)was obtained and after recrystallisation from water it was identified as phenyl α -D-glucoside by the following scheme.

(i) Paper chromatography. The product could not be distinguished from phenyl α-D- glucoside by paper chromatography using solvent systems 1, 2, 3, and 5 (Expt.1.) with which $R_{Glucose}$ values of 5·1, 2·9, 2·7 and 5·9 respectively were obtained. Phenyl α -D-glucoside gave values of 5·1, 2·9, 2·7 and 5·9 and phenyl β -D-glucoside values of 5·1, 3·0, 2·9, and 6·1. The glucosides were detected with the silver nitrate-sodium hydroxide spray reagent. The rate of reaction of the compounds obtained from fractions 222-244 with this reagent corresponded with that given by phenyl α-D-

glucoside and was slower than that given by phenyl β-D-glucoside. No reaction was obtained with the diazotised nitroaniline spray reagent.

(ii) Paper ionophoresis. The product obtained from fractions 222-244 was examined by paper ionophoresis in borate buffer at pH 10 (Expt .2.) using phenyl α-D-glucoside and phenyl β-D-glucoside as standards. The following M_G values were obtained:-

Fractions. 222-244	0·10
Phenyl α-D-glucoside	0·10
Phenyl β-D-glucoside	0·20

(iii) Hydrolysis.

(a) Acidic hydrolysis. The product obtained from fractions 222-244 (0·01g.)was placed in an ignition tube and 0·1 N hydrochloric acid (1 ml.) was added. The tube was then fitted with a long glass capillary tube to act as a condenser and heated on a boiling water bath for 2½ hr. Excess hydrochloric acid was removed by evaporation in a vacuum desiccator over sodium hydroxide. Methanol was added to the residue and the solution obtained was analysed by paper chromatography in solvent system 1 (Expt.1.). The diazotised nitroaniline and silver nitrate-sodium hydroxide spray reagents were used to detect the products. Glucose and phenol were detected.

(b) Almond β- glucosidase hydrolysis.

An aqueous solution of the solid which was obtained from fractions 222-244 was incubated in a sealed capillary tube with almond β- glucosidase at 27° for 24 hr. The only product was unchanged phenyl glucoside. Under identical conditions phenyl α-D- glucoside was not hydrolysed, but phenyl β-D-glucoside was completly hydrolysed to give glucose and phenol .

(c) α-Glucosidase hydrolysis. This was carried out in a similar way to the almond β -glucosidase hydrolysis, but the incubation time was 48hr. at 37° . The glucoside from fractions 222-244 was completely hydrolysed to glucose and phenol, which were identified by paper chromatography. The specificity of the enzyme was checked under identical conditions.

(iv) Melting point and optical rotation. The glucoside was recrystallised several times from water, giving phenyl α-D- glucoside with m.p. and mixed m.p. 170°and $[\alpha]_D^{20}$ + 175° (c = 0·4 in water). Bunton et. al.[108] give phenyl α-D- glucoside m.p. 169-170° and $[\alpha]_D^{20}$ + 181° (c= 0·65 in water).

Experiment 46. A detailed investigation of fractions 248-262.

Fractions 248-262 obtained in Expt 44. were combined

and concentrated to low bulk under diminished pressure. Colourless crystals (0·06g.) were obtained. After recrystallisation from aqueous methanol the product was identified as phenyl β-D-glucoside by the following scheme.

(i) Paper chromatography. The product had the same $R_{Glucose}$ values as phenyl β-D- glucoside in solvent systems, 1, 2, 3, and 5 (Expt.1.), namely 5·1, 3·0, 2·9, and 6·1. Using the same solvent systems phenyl α-D-glucoside gave values of 5·1, 2·9, 2·7, and 5·9. The silver nitrate-sodium hydroxide spray reagent was used to detect the glucosides. Fractions 248-262 reduced this reagent at the same rate as phenyl β-D- glucoside. No reaction was obtained with the diazotised nitroaniline spray reagent.

(ii) Paper ionophoresis. The product obtained from fractions 248-262 was examined by paper ionophoresis in borate buffer at pH10 (Expt.2.) using phenyl α-D-glucoside and phenyl β-D- glucoside as standards. The following M_G values were obtained in this experiment.

Fractions 248-262	0·20
Phenyl α-D-glucoside.	0·14
Phenyl β-D-glucoside	0·20

(iii) Hydrolysis. The acidic and enzymic hydrolyses were carried out under the same conditions as

those used for fractions 222-244 (Expt 45). The products which were identified by paper chromatography are given below.

(a) Acidic hydrolysis -- Glucose and phenol.

(b) β-Glucosidase hydrolysis -- glucose and phenol.

(c) α-Glucosidase hydrolysis -- phenyl glucoside.

(iv) Melting point and optical rotation. The glucoside was recrystallised several times from aqueous methanol giving phenyl β-D- glucoside with m.p. 174-175° and mixed m.p. 174° and $[\alpha]_D^{20}$ - 71° (C= 0·4 in water). Bunton et. al.[108] give phenyl β-D-glucoside m.p. 173·5-174·5° and $[\alpha]_D^{20}$ - 70.7° (c= 2·0 in water).

Experiment 47. Reaction of boron trichloride with the mixture of phenyl glucoside and 'A' obtained from Expt.39.

The mixture of phenyl glucoside and 'A' (4·0g.) obtained from Expt.39. was dissolved in dichloromethane and cooled to -80°. Boron trichloride (10g.) was added and the reaction mixture was kept at -80° for 30 min. and at room temperature for 16 hr. Excess boron trichloride and solvent were evaporated off at room temperature under diminished pressure. Methanol (3 x 10 ml.) was added to the residue and distilled off. The residue was analysed by paper chromatography with

solvent system 1 (Expt. 1.) and found to contain phenol, glucose and 'A'. It was then dissolved in a little solvent 1(Expt.1.) and introduced to the top of a cellulose column 40 x 5 cm. (Expt. 3b.) which was eluted with solvent system 1 and 25 ml. fractions were collected. Each fraction was analysed by paper chromatography and the following result was obtained.

Table 21.

Fraction.	R_F value of products.
1 - 16	-
17 - 18	0·84 phenol.
19 - 32	0·53 'A'
31 - 38	-
39 - 40	0·3* methyl glucoside.
41 - 50	-
51 - 67	0·12 glucose.
68 -	-

* Present in trace quantity only.

Fractions 19 - 32 were combined and evaporated to dryness under diminished pressure giving 'A' (0·1g.).

Experiment 48. Hydrolysis of the mixture of phenyl glucoside and 'A' with hydrochloric acid.

The mixture of phenyl glucoside and 'A' (3·3g.),

obtained in a similar way to that described in Expt.39., was heated with c·1 N hydrochloric acid (50 ml.) at 100° for 3 hr. A little was evaporated to dryness in a vacuum desiccator over sodium hydroxide and examined by paper chromatography. The chromatograms were viewed under ultraviolet light and no unchanged phenyl glucoside was visible. Similarly, using the silver nitrate-sodium hydroxide spray reagent, only glucose, phenol and 'A' could be detected. The remaining solution was then evaporated to dryness in a vacuum desiccator over sodium hydroxide. The residue was dissolved in aqueous methanol and the products separated by paper chromatography on several sheets of thick paper (Whatman No.3.) in solvent system 1. Guide strips were cut from the edge of each sheet and sprayed with the diazotised nitroaniline spray reagent. The sections of the main sheets containing 'A' were then cut out and eluted with methanol. On concentration of the methanol solution, 'A' (0·6g.) was obtained as a pale brown oil. Attempts to crystallise this oil from methanol, chloroform, acetone, acetone-toluene, ether and ether-methanol were unsuccessful.

Experiment 49. Investigation of the ultraviolet absorption spectra of 'A' and phenyl β-D-glucoside.

Ethanolic solutions of 'A' and phenyl β-D-glucoside were

used and the absorption spectra were measured in the range 250-300 mμ (See Fig. 5.).
An aqueous solution of potassium hydroxide (4%) was diluted to 0·2% with ethanol. The spectra were measured again after the solutions of 'A' and phenyl β-D-glucoside were made alkaline with the potassium hydroxide solution (see fig.5).

Experiment 50. Reinvestigation of the sample of 'A' obtained in Expt.48.

The sample of 'A' obtained in Expt. 48 was examined by paper ionophoresis in borate buffer at pH10 (Expt.2.) using several spray reagents. With the silver nitrate-sodium hydroxide[83] and diazotised nitroaniline[95] spray reagents only one product was detected, namely 'A' with an M_G value of 0·70. However, when the potassium periodatocuprate reagent[98] was used two products were detected with M_G values of 0·17 and 0·71. The former was presumably phenyl glucoside. 'A' did not react very well with the reagent.

Experiment 51. The oxidation of 'A' using lead peroxide.

Potassium hydroxide (0·25g.), 'A' (0.03g.) and water (0·5ml.) were heated together on an oil bath to 210-220°. Lead peroxide (0·17g) was then added stirring the solution continuously and heating was continued for 1 hr. Dilute sulphuric acid was added until most of the

alkali was neutralised and the solution was filtered. The filtrate was acidified and a precipitate was formed. The filtrate and this precipitate were extracted with ether and the ether extract examined by paper chromatography and paper ionophoresis, using o-, m- and p- hydroxybenzoic acids as standards.

The following results were obtained, where

$$M_{SA} = \frac{\text{distance migrated by substance}}{\text{distance migrated by o-hydroxybenzoic acid.}}$$

Paper ionophoresis.

(i) Borate buffer pH 10.

	M_{SA} value.
o-hydroxybenzoic acid.	1.00
m-hydroxybenzoic acid	1·07
p-hydroxybenzoic acid	1·25
ether extract	(a) 1·25, (b) 1·0* and (c) 0·26*.

(ii) Acetate buffer pH 5.

	M_{SA} value.
o-hydroxybenzoic acid.	1·00
m-hydroxybenzoic acid	0·83
p-hydroxybenzoic acid	0·74
ether extract	(a) 0·74, (b) 1·0* and (c) 0·1*

Paper chromatography.

(1) Solvent system 1. (Expt.1.)

	R_F value.
o-hydroxybenzoic acid	0·39
m-hydroxybenzoic acid	0·29
p-hydroxybenzoic acid	0·26
ether extract	(a) 0·84 and (c) 0·92*
phenol	0.90

(ii) Solvent system.4. (Expt.1.)

	R_F value.
o-hydroxybenzoic acid	0·92
m-hydroxybenzoic acid	0·89
p-hydroxybenzoic acid	0·90
ether extract.	(a) 0·90 and (c) 0·96*
phenol	0·96

* Present in trace quantity.

The following colour reactions were obtained using the diazotised nitroaniline spray reagents:- o-hydroxybenzoic acid, pink turing to orange after 20 min.; m-hydroxybenzoic acid, bright red-pink; p-hydroxybenzoic acid, dull purple red; phenol, pink; ether extract (a) dull purple-red; (b) pink turning to orange after 20 min.; (c) pink.

Similarly a difference in appearance of the isomers was observed when the papers were viewed under ultra-violet light before spraying. A blue fluorescence was given by o- and m- hydroxybenzoic acids and (b), p-hydroxy benzoic acid and (a) gave a dull brown absorption.

No carbohydrates were detected in the aqueous layer.

Experiment 52. The action of boron trichloride on a solution of phenol in dichloromethane.

Phenol (0.1g.) was dissolved in dichloromethane (2ml.) cooled to -80° and boron trichloride (1·5g.) added.

The solution was kept at -80° for 30 min. and at room temperature for 16hr. before removal of excess boron trichloride and solvent under diminished pressure. The residue was dissolved in dichloromethane and the solution was heated under reflux at 50° for 2 hr. Careful chromatographic analysis of the resulting solution showed the presence of phenol only.

Experiment 53. The reaction of boron trichloride with phenyl α-D-glucoside.

(i) Phenyl α-D- glucoside was reacted with boron trichloride as in Expt.5. The residue after removal of excess boron trichloride was dissolved in nitromethane and heated at 100° for 1½ hr. The products were examined by paper chromatography using solvent system 1 (Expt.1.) and the diazotised nitroaniline spray reagent. In addition to phenol there was one other product (R_F = 0·42).

(ii) Phenyl α-D-glucoside was reacted with boron trichloride at -80°. After 30 min. at -80° the solution was heated to between 40° and 50° for 1 hr. A 'cold finger' condenser filled with solid carbon dioxide was used. The products were analysed by paper chromatography using the silver nitrate-sodium hydroxide

and diazotised nitroaniline spray reagents. Glucose and phenol were present.

Experiment 54. Small scale glycoside synthesis using boron trichloride.

The glycoside (0·01-0·05g.) was suspended in dichloromethane, cooled to -80° and boron trichloride (0·5g.) added. The reaction mixture was kept at -80° for 30 min. and at room temperature for 16 hr. Excess boron trichloride was removed before treatment with the alcohol or phenol. Alcohols (3 x 3ml.) were added to the glycoside-boron trichloride complex and concentrated under diminished pressure. Phenols (GA. 0·5g.) were dissolved in dichloromethane or ether and the solution added to the complex and heated for 1-2 hr. at 50° . In each case the products were examined by paper chromatography using several of the solvent systems and spray reagents listed in Expt.1. and by paper ionophoresis in borate buffer at pH10. and acetate buffer at pH 5.

II Disaccharides.

Experiment 55. Small scale disaccharide synthesis using boron trichloride.

Methyl α-D-glucoside (0.11g.) was suspended in dichloromethane (5 ml.) and cooled to -73° Boron trichloride (1.0g.) was added and the mixture maintained at this temperature for 30 min. The reaction mixture was allowed to attain room temperature and then kept at this temperature for 16 hr. Excess boron trichloride and solvent were removed under diminished pressure at room temperature. The residue was dissolved in dichloromethane (20 ml.) and this solution was then shaken with 'Drierite' (previously heated to 200° for 2 hr.) for 30 min. 1,2 : 5,6 - Di-O-isopropylidene-D-glucose (0.14g.), silver carbonate (0.15g.), iodine (trace) and dichloromethane (10 ml.) were stirred with 'Drierite' in a darkened flask for 30 min. The first solution was added slowly to the di-o-isopropylidene-D-glucose solution and stirring was continued for 4-5 hr. The flask was then shaken for a further 15 hr. The solution was filtered and concentrated and the residue extracted with chloroform and pyridine. These solutions were examined by paper chromatography with solvent 1. (Expt. 1.).

(i) Pyridine extract.

(1) $R_{Glucose}$ = 0·2

(2) $R_{Glucose}$ = 1·0

(3) $R_{Glucose}$ = 0·05*

(4) $R_{Glucose}$ = 0·35*

(5) $R_{Glucose}$ = 6·2 --- detected with diphenylamine spray reagent, but not silver-nitrate-sodium hydroxide reagent.

(ii) Chloroform extract.

(1) $R_{Glucose}$= 1·0

(2) $R_{Glucose}$= 3·0 *

(3) $R_{Glucose}$= 5·4 mono-o-isopropylidene -D-glucose.

(4) $R_{Glucose}$= 7·6 1,2 : 5,6-di-o-isopropylidene-D-glucose.

* Present in trace quantity only.

Experiment 56. Preparation and isolation of disaccharides.

Methyl α-D-glucoside (12g.) was suspended in dichloromethane(10 ml), cooled to -80° and reacted with boron trichloride (50 g.) under the same reaction conditions and time as in Expt.5. The residue, which remained after removal of excess solvent and boron trichloride, was treated with a solution of D-glucose

(12g.) in dry nitrobenzene(50ml.). This solution was then shaken with silver oxide(25g., dried *in vacuo* at 60° in the dark) in a darkened flask. The silver oxide and chloride was filtered off and washed thoroughly with water. The nitrobenzene solution was extracted with water several times. The combined aqueous extract and washings were neutralised with sodium bicarbonate and then concentrated to low bulk on a rotary evaporator at 40°. Chromatograms of this solution with solvent 1(Expt.1.) showed the presence of glucose and oligosaccharides. The concentrated solution was introduced at the top of a charcoal-'Celite' column 88 x 8cm. (Expt.3c) and eluted first with water and then with aqueous ethanol. Fractions of volume 1 litre were collected and analysed by paper chromatography. The following results were obtained:

Fraction.	Products.
1 -2	-
3 - 16	glucose
17 - 33	disaccharides.

Experiment 57. Preliminary identification of the disaccharides present in fractions 17-33.

Each fraction was concentrated to low bulk and

examined by paper chromatography using solvent systems 1, 2 and 4 (Expt.1.) and paper ionophoresis in borate buffer at pH10(Expt.2.). The spray reagents used to detect the disaccharides were chiefly silver nitrate-sodium hydroxide, diphenylamine, triphenyltetrazolium chloride and aniline hydrogen phthalate, Chromatography of the benzylamine derivatives of the disaccharides was also used.[97] The fractions containing the same disaccharide or the same mixture of disaccharides as each other were combined together giving seven main fractions of total mass 1·85g. The individual masses were fraction 1 (0·53g.) ,2 (0·10g.), 3 (0·50g.), 4 (0·20g.), 5 (0·18g.), 6 (0·10g.), 7 (0·24g.). The results are given in Table 12.

Experiment 58. Purification of each disaccharide fraction.

The fractions were freeze dried and as most of the fractions contained a trace of glucose in addition to the disaccharides, they were purified by chromatographic separation on sheets of thick paper (Whatman No.3.) with solvent system 1 (Expt.1.). The elution time was 3 days. Guide strips were cut off and sprayed with the diphenylamine reagent in order to locate the disaccharides. The appropriate sections were then cut out and eluted with water. The resulting solutions were

freeze dried.
In this way chromatographically pure specimens of disaccharides 1a, 3, 5a, 6 and 7 were obtained.

Experiment 59. Identification of the disaccharides.

The following scheme was used for the identification of each disaccharide and the results are recorded in Table 14.

(a)Paper chromatography and ionophoresis.

Chromatographic analysis was carried out using the same solvent systems and the same spray reagents as in Expt. 57, with isomaltose, gentiobiose, maltose, cellobiose, nigerose, laminaribiose, sophurose and trehalose as standards.

(b) Hydrolysis.

(i) Acidic hydrolysis. The disaccharide (2mg.) was put in a small ignition tube fitted with a glass capillary tube to act as a condenser and 1·5 N hydrochloric acid (1 ml.) was added. The solution was then heated on a boiling water bath for 4 hr. Excess hydrochloric acid was removed by evaporation in a vacuum desiccator over sodium hydroxide. The resulting solid was dissolved in the minimum amount of aqueous methanol. Each solution was examined by paper chromatography with solvent systems 1, 2, 4 and 5 (Expt.1)

and by ionophoresis in borate buffer at pH 10 with several monosaccharides as standards. In each case the sole product was glucose.

(ii) Enzymic hydrolysis.[104] The enzymes used were almond β-glucosidase, α-glucosidase, and glucamylase. The last was used only in the case of fractions 3 and 6, which contain a 1,4 link. An aqueous solution of each disaccharide was sealed in a capillary tube with an equal volume of enzyme solution and incubated at the appropriate temperature. In the case of almond β-glucosidase and α-glucosidase the incubation period was 48 hr. at 37° and in the case of glucamylase it was 6 hr. at 50°. After incubation the solutions were applied directly to the base line of paper chromatograms, which were eluted with solvent system.1. (Expt.1.) The specificity of each enzyme was checked under similar conditions.

(iii) Reduction followed by ionophoresis in molybdate buffer.[79] Each disaccharide (2 mg.) was dissolved in water (0·5ml.) and reduced with a solution of potassium borohydride (2 mg.) in water (0·5 ml.) at room temperature for 18 hr. Amberlite IR-120(H+) (0·1g.) was added and the mixture was shaken for a few minutes. The solution was decanted

off, and evaporated to dryness under diminished pressure at room temperature. Dry methanol (3 x 2ml.) was added and the whole evaporated to dryness under diminished pressure. The resulting disaccharide alcohols were analysed by ionophoresis in molybdate buffer at pH 5.

In addition to the results reported in Table 14 the following results were obtained

(i) Fractions 1b, 2a, 4a, 4b, 4c, and 5b, gave glucose as the only product of acidic hydrolysis.

(2) Fractions 1a and 1b were hydrolysed by α-glucosidase, but not by almond β-glucosidase.

(3) Fractions 5a and 5b were hydrolysed by almond β-glucosidase, but not by α-glucosidase.

III Glucopyranosylbenzene.

Experiment 60. The synthesis of glucopyranosylbenzene.

Methyl α-D-glucoside (10.8g) was suspended in dichloromethane (12 ml.) cooled to -80° and previously cooled boron trichloride (31g.) was added. The reaction mixture was kept at -80° for 30 min. and at room temperature for 16 hr. before removal of excess reagents under diminished pressure. Benzene (300 ml., dried over calcium chloride) and aluminium chloride (5g.) were added to the complex. The reaction mixture was heated under reflux with constant stirring on a boiling water bath. Immediately the solution darkened in colour and finally became very dark brown and a thick tar formed. After 7 hr. heating and stirring were discontinued, and the mixture was kept at room temperature for 16 hr. It was then poured into cold distilled water (500 ml.) to decompose the catalyst complex, after which the solution was centrifuged. The dark brown benzene layer was separated off and washed with water and aqueous sodium hydroxide (10%). The combined washings were added to the aqueous layer, which was neutralised with aqueous sodium hydroxide (10%). The precipitated aluminium hydroxide was removed by filtration through a sintered

glass crucible (No.4.) and washed thoroughly with hot water. The combined aqueous extract and washings were concentrated to dryness and the resulting oil extracted with boiling pyridine. On concentration of the pyridine extract a creamy white solid was obtained.

Chromatograms of this solid in solvent system 1 (Expt.1.) showed the presence of four components giving a positive reaction with the silver nitrate-sodium hydroxide spray reagent.

(1) $R_{Glucose}$=1.0. glucose.
(2) $R_{Glucose}$=4.9 *
(3) $R_{Glucose}$=5.5 *
(4) $R_{Glucose}$=6.4 glucopyranosylbenzene.

* Present in trace quantity only.

Extraction of the solid with cold chloroform yielded a pale yellow solution which contained components 2,3, and 4, but not component 1. Glucopyranosylbenzene (4.3g., 32%) was obtained from this solution.

The product was acetylated by stirring on a boiling water bath for 2 hr. with acetic anhydride (30 ml.) and anhydrous sodium acetate (1.5g.). The solution was cooled, poured into water and allowed to hydrolyse overnight. The solution was then extracted with ether and

the extract washed with aqueous sodium bicarbonate (1%), dried over anhydrous sodium sulphate and decolorised by filtration through charcoal. The ether was distilled off and the tetra-O-acetyl-β-D-glucopyranosylbenzene recrystallised three times from propan-2-ol. Colourless needle shaped crystals were obtained which had m.p. and mixed m.p. 155-156° and $[\alpha]_D^{25}$ -17° (c= 0·6 in $CHCl_3$), Hurd and Bonner[24,28] give m.p. 156·5° and $[\alpha]_D^{25}$ -16·4° (c= 0·85 in $CHCl_3$) and $[\alpha]_D^{25}$ -18·6.

Found: C, 58·6 ; H, 6·05 : Calc for $C_{12}H_{12}O_5(OCCH_3)_4$ C,58·8 ; H, 5·9%.

Experiment 61. The preparation of glucopyranosylbenzene from methyl-α-D-glucoside in the absence of boron trichloride.

Methyl-α-D-glucoside (3·5g.) was suspended in dichloromethane (4 ml.) and benzene (100 ml., dried over calcium chloride) and aluminium chloride (1.6g.) were added. The solution was heated under reflux with constant stirring on a boiling water bath. The benzene solution remained colourless for the first 30 min. of the reaction and then became pale yellow and finally, after 7 hr. heating, was light brown in colour. The tar formation was very low. The reaction

was worked up as in the previous experiment. Chromatographic analysis of the solid obtained by extraction with pyridine showed the presence of four components. The products were detected with the silver nitrate-sodium hydroxide spray reagent and the $R_{Glucose}$ values refer to elution with solvent system.1. (Expt.1.).

(1) $R_{Glucose}$ = 1·0 glucose.

(2) $R_{Glucose}$ = 2·1 methyl-α-D-glucoside.

(3) $R_{Glucose}$ = 5·3*

(4) $R_{Glucose}$ = 6·3* glucopyranosylbenzene.

* Present in trace quantity.

The pricipal product was methyl α-D-glucoside. Extraction with cold chloroform gave crude glucopyranosylbenzene (0·5g.; 11%), which was acetylated as in the previous experiment. However, no tetra-o-acetyl-β-D-glucopyranosylbenzene could be detected.

Experiment 62. The preparation of glucopyranosylbenzene in the absence of aluminium chloride.

Methyl α-D-glucoside (2·0g.) was suspended in dichloromethane (2 ml.) and reacted with boron trichloride (6 g.) as in Expt.60. Benzene (60 ml., dried over calcium chloride) was added and the solution

heated under reflux on a boiling water bath for 3 hr. with constant stirring. The solution gradually darkened in colour. The reaction mixture was cooled to -80° and boron trichloride(1 g.) was added and the mixture heated for a further 4 hr. The tar formation was very low. Examination of the solution by paper chromatography using solvent system 1 (Expt.1.) and the silver nitrate-sodium hydroxide spray reagent showed the presence of three components.

(1) $R_{Glucose}$ = 0·3 oligosaccharides.

(2) $R_{Glucose}$ = 1·0 glucose.

(3) $R_{Glucose}$ = 5·9 glucopyranosylbenzene.

The principal product was glucose.

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BORON TRICHLORIDE AS A DEGRADATIVE REAGENT FOR CARBOHYDRATES AND THEIR DERIVATIVES

By S. Allen, T. G. Bonner, E. J. Bourne and N. M. Saville

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In a study of the interaction of Lewis acids with cyclic acetals and ketals of hexitols it has been found that boron trichloride[1] is a valuable reagent for degrading these derivatives to the parent hexitols. In addition, it is effective in demethylation and deacylation of sugar derivatives and in degrading those polysaccharide derivatives which can be brought into solution with this reagent. Demethylation and deacylation usually do not proceed quite to completion in a single treatment but the main product from aldose derivatives is always the parent sugar and the method provides a simple and convenient means of identifying the latter, e.g. 2:3:4:6-tetra-*O*-methyl-glucose, 2:3:6-tri-*O*-methyl-glucose, methyl cellulose, cellulose acetate and amylopectin acetate each give glucose as the main product. Acetals and ketals always regenerate the original sugar or hexitol in high yield.

The possibility of stereochemical changes occurring in the sugar derivatives during reaction is under investigation but results to date indicate that glucose, mannose, galactose and arabinose are essentially unaffected. Fructose and sorbose, however, appear to be almost completely converted to a derivative, which has a much higher R_F value (solvent, butanol: ethanol: water=4:1:5) than either of these ketoses. The disaccharides, lactose, maltose, and sucrose are attacked to only a very slight extent by boron trichloride, probably due to their low solubility in the reagent but in these cases, boron tribromide[2] has been successfully employed to produce the constituent hexoses. From sucrose, only the glucose constituent has been identified, the fructose portion apparently undergoing further reaction, as referred to above.

The experimental procedure is based on that first reported by Gerrard.[1] Redistilled boron trichloride (0·3–0·5 g.) is introduced into a small tube which is sealed off and weighed; the tube is then cooled to −70° prior to opening. About 10 mgm. of the sugar derivative is added to 1–2 c.c. of dry dichloromethane and the contents of the sealed tube added. In some experiments, the dichloromethane was omitted and excess boron trichloride used. The mixture is kept at −70° for 30 minutes, allowed to attain room temperature and then to evaporate overnight under anhydrous conditions before working up. Any remaining solvent and boron trichloride are drawn off under vacuum and about 5 c.c. of aqueous methanol is added to decompose the residue. The methanol is removed under vacuum at room temperature and the solid product investigated by paper chromatography and ionophoresis. An alternative method of treating the residue after removal of the dichloromethane and excess boron trichloride is to shake with an aqueous suspension of silver carbonate.

Larger quantities have been treated in this way, giving products which can be isolated, purified and identified, e.g. mannitol can be obtained in 63% yield from 1:3–2:5–4:6-tri-*O*-methylene-D-mannitol by following the above procedure.

A detailed examination of selected reactions is now being made.

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591. *Dealkylation and Deacylation of Carbohydrate Derivatives with Boron Trichloride and Boron Tribromide.*

By T. G. Bonner, E. J. Bourne, and S. McNally.

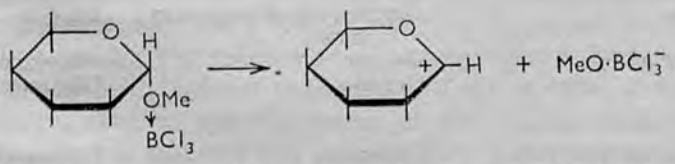
The use of boron trichloride and boron tribromide is described for the deacylation and demethylation of mono-, di-, and poly-saccharide derivatives, the last two types being converted into the monosaccharide constituents.

All the monosaccharides investigated are stable to the reagent, except fructose and sorbose which are both degraded to 5-hydroxymethylfurfuraldehyde.

The oxygen atoms of ethers, esters, and many other derivatives of carbohydrates provide sites for co-ordination with electron-deficient molecules such as Lewis acids. Since some diversity of co-ordinating power can arise in a molecule through variation in polar character and steric environment of these basic oxygen centres there are clearly possibilities for effecting particular reactions at selected sites. Some initial studies with aluminium chloride were only partially successful because this reagent is difficult to separate from the products of reaction. Boron trichloride does not have this disadvantage and has proved to be a versatile reagent in carbohydrate chemistry, as has already been reported briefly.[1] Although it is a gas at room temperature (b. p. 12°), boron trichloride is strongly reactive at temperatures well below its boiling point and can easily be handled in a medium such as a mixture of solid carbon dioxide and acetone (*ca.* −80°).[2] One of its valuable uses is for removal of *O*-methyl groups from methylated sugars which provides a rapid and convenient method of identifying the monosaccharide units in a methylated polysaccharide, or in the methylated monosaccharides derived therefrom. The polysaccharide requires only partial methylation, sufficient to effect dissolution in the reagent, and partial acetylation is equally effective. A survey of this method of converting carbohydrate derivatives into the monosaccharide units present has been carried out for over 60 compounds and forms the main subject of this paper. Cyclic *O*-methylenedioxy-, *O*-ethylidenedioxy-, and *O*-benzylidenedioxy-derivatives of hexitols also react with boron trichloride, giving the unsubstituted hexitol as the main product; the mechanism of this ring opening is the subject of a separate report.[3]

All the monosaccharides so far investigated are unchanged by prolonged contact with boron trichloride, except fructose which, whether free or part of a higher saccharide, is always substantially converted into 5-hydroxymethylfurfuraldehyde; only traces of fructose itself survive.

The formation of oligosaccharides in some reactions indicated that boron trichloride can initiate syntheses in some cases; *e.g.*, with methyl α-D-glucoside co-ordination at the glycosidic oxygen could lead to the formation of a glucosyl cation, the hemiacetal character of a glucoside providing resonance stabilisation through the ring oxygen atom adjacent



to $C_{(1)}$. The ionic complex may undergo conversion into glucosyl chloride and methoxyboron dichloride but under suitable conditions either form should behave as a glucosylating agent. This possibility has been investigated by removing the excess of boron trichloride from the product of its reaction with methyl α-D-glucoside and adding an excess of a suitable substrate. The expected products have been obtained with benzene, phenols,

TABLE. (*Continued.*)

Substance	Principal product	Other products *
Monosaccharides		
L-Arabinose	Arabinose	Oligosaccharide †, 2·4 † ($R_{glucose}$)
D-Galactose	Galactose	
D-Glucose	Glucose	
D-Lyxose	Lyxose	
D-Mannose	Mannose	
L-Rhamnose	Rhamnose	
D-Xylose	Xylose	
D-Fructose	5-Hydroxymethylfurfuraldehyde	0·90 † (R_F)
L-Sorbose	5-Hydroxymethylfurfuraldehyde	Sorbose
Disaccharides		
Lactose	Galactose, glucose	Lactose
Maltose	Glucose	Maltose
Melibiose	Galactose, glucose	Melibiose †, 1·6 † ($R_{glucose}$)
Sucrose	Glucose, 5-hydroxymethylfurfuraldehyde	Sucrose †
Turanose	Glucose, 5-hydroxymethylfurfuraldehyde	Fructose †
Miscellaneous		
Raffinose	Galactose, glucose, 5-hydroxymethylfurfuraldehyde	Fructose †
Inulin	5-Hydroxymethylfurfuraldehyde	
Nitrocellulose	Glucose	0·38, 2·1, 3·6 †
Me 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucoside	Glucose	Mono- and di-*O*-methylglucoses, 0·70 † (R_G)
Me 4,6-*O*-benzylidene-2-*O*-*p*-toluene sulphonyl-α-D-glucoside	5·7 ($R_{glucose}$)	Glucose †

* Unless otherwise stated, unidentified products are indicated by R_x values in butanol–ethanol–water (4 : 1 : 5), where x refers to the principal product in each case. R_G indicates that 2,3,4,6-tetra-*O*-methyl-D-glucose is the reference compound.

† Traces only present. ‡ Probably partially methylated products. [a] Solvent propan-1-ol–ethyl acetate–water (7 : 1 : 2).

which is inert and easily removed. Few solvents are suitable since the reagent co-ordinates readily with any electronegative centre in the solvent molecule and even if there is no subsequent decomposition the complex formed is usually non-volatile. The only other solvents which appear to have found use are n-pentane [2] and tetrahydropyran.[4] Reaction of a substrate with boron trichloride requires a homogeneous environment. This is sometimes achieved without the addition of a solvent, dissolution gradually occurring in the boron trichloride as the temperature rises from *ca.* −80° to room temperature. Where dissolution did not occur, boron tribromide (b. p. 92°) often provided an alternative. Disaccharides, in particular, which were recovered unchanged from prolonged contact with boron trichloride underwent partial dissolution in boron tribromide with the expected scission at the glycosidic linkage. Boron tribromide was also more effective in the reaction with tri-*O*- and tetra-*O*-methyl-D-glucose, almost complete demethylation being effected in a single treatment in both cases, in contrast to the formation of some partly methylated derivatives in the reaction with boron trichloride.

In the general procedure, the reactants and solvent were mixed at *ca.* −80°, allowed to attain room temperature, and left overnight. After removal of excess of boron trichloride and dichloromethane, and treatment of the residue with methanol or silver carbonate, the product was isolated and examined by paper chromatography in several different solvents; where desirable, separation by ionophoresis in a borate buffer was also used. The results reported in detail in the Table are based on a qualitative examination only of 1—10 mg. amounts of substrate.

Methylated monosaccharides form the monosaccharide as the chief product (see Table), and frequently any other formed is only present in traces. Highly methylated sugars that

and glucose, the last forming a number of disaccharides. This synthetic use of boron trichloride is being investigated further.

Reactions with boron trichloride are conveniently carried out in dichloromethane

Substance	Principal product	Other products *
Methylated sugars		
2,3,5-Tri-*O*-methyl-L-arabinose	Arabinose	Oligosaccharides, 2·7 †‡, 3·8 †‡
2,4-Di-*O*-methyl-L-arabinose	Arabinose	1·4 †‡
Me 2,3,4-tri-*O*-methyl-α-L-fucoside	Fucose	0·9 † (R_F)
2-*O*-Methyl-L-fucose	Fucose	Oligosaccharides, 2-*O*-methylfucose †
2,3,4,6-Tetra-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose	Galactose, rhamnose	0·88 † (R_F)
2,3,4,6-Tetra-*O*-methyl-D-glucose	Glucose	Mono-, di-, and tri-*O*-methylglucoses
2,3,6-Tri-*O*-methyl-D-glucose	Glucose	Mono- and di-*O*-methylglucoses, 0·77 † (R_G)
Me 2,3-di-*O*-methyl-α-D-glucoside	Glucose	Mono-*O*-methylglucoses †
3-*O*-Methyl-D-glucose	Glucose	3-*O*-Methyl-D-glucose †
2,3,4,6-Tetra-*O*-methyl-D-mannose	Mannose	0·37, 0·52, 0·61 †, 0·74, 0·93 (R_G) ‡
3,4-Di-*O*-methyl-D-mannose monohydrate	Mannose	0·40, 0·56 (R_G) ‡
Di-*O*-methylsucroses	Glucose, 5-hydroxymethylfurfuraldehyde	Fructose,† di-*O*-methylsucroses †
3-*O*-Methyl-D-xylose	Xylose	—
Methylated amylopectin	Glucose	Mono- and di-*O*-methylglucoses
Methylated cellulose	Glucose	Mono- and di-*O*-methylglucoses
Glycosides		
Me β-D-arabinoside	Arabinose	Me β-D-arabinoside †
Me α-D-fructofuranoside	Fructose, 5-hydroxymethylfurfuraldehyde	—
Me α-D-galactoside	Galactose	—
Me β-D-galactoside	Galactose	—
Me α-D-glucoside	Glucose	Oligosaccharides †
Me β-D-glucoside	Glucose	—
Ph α-D-glucoside	Glucose, phenol	—
Ph β-D-glucoside	Glucose, phenol	—
Quinol β-D-glucoside (arbutin)	Glucose, quinol	—
o-Hydroxymethylphenyl β-D-glucoside (salicin)	Glucose, saligenin	—
Me α-D-mannoside	Mannose	Oligosaccharides †
Me α-L-rhamnoside	Rhamnose	—
Me β-D-riboside	Ribose	—
Me α-D-xylofuranoside	Xylose	—
Acetals and ketals		
4,6-*O*-Benzylidene-D-glucose	Glucose	—
Me 4,6-*O*-benzylidene-α-D-glucoside	Glucose	—
1,2:5,6-Di-*O*-isopropylidene-D-glucose	Glucose	—
1,2-*O*-Isopropylidene-D-glucose	Glucose	—
2,3:4,5-Di-*O*-isopropylidene-D-fructose	5-Hydroxymethylfurfuraldehyde	Fructose †
Acetylated sugars		
Octa-*O*-acetylgentiobiose	Glucose	Gentiobiose, 1·15, 1·5 [a]
Penta-*O*-acetyl-β-D-glucose	Glucose	2·7, 5·0 †
Octa-*O*-acetylsucrose	Glucose, 5-hydroxymethylfurfuraldehyde	2·4 †
Acetylated amylopectin	Glucose	Oligosaccharides, 1·9, 2·2
Acetylated cellulose	Glucose	Oligosaccharides, 1·9, 2·2
Anhydro-sugars		
1,6-Anhydro-β-D-galactopyranose	Galactose	Oligosaccharides
1,6-Anhydro-α-D-galactofuranose	Galactose	Oligosaccharides
1,6-Anhydro-β-D-glucopyranose	Glucose	Oligosaccharides
1,6-Anhydro-β-D-gulopyranose	Gulose	Oligosaccharides
1,6-Anhydro-β-D-mannopyranose	Mannose	Oligosaccharides
Me 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannoside	2·3,2·5 ($R_{mannose}$)	4·7 † ($R_{mannose}$)
Me 2,3-anhydro-β-L-riboside	0·7, 1·7 (R_{ribose})	

One type of sugar derivative which is being investigated further is that containing the toluene-*p*-sulphonyl group. Only a trace of glucose was obtained from methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucoside, the main product having a high R_F value. Unless there is some particular effect exerted by the toluene-*p*-sulphonyl group, the α-methyl and benzylidene groups should be completely removed by the reagent; the product might then be the monotoluene-*p*-sulphonyl ester. If this is the case, it should be possible to demethylate methylated toluene-*p*-sulphonic esters of sugars with retention of the ester group.

Experimental

Materials.—Commercial methanol was used without purification.

Boron trichloride was handled as described elsewhere.[3] Boron tribromide (b. p. 92°) was distilled before use. Dichloromethane was washed with 5% aqueous sodium carbonate and water, dried ($CaCl_2$), and distilled, the fraction boiling at 39·5—41° being collected.

Paper Chromatography and Paper Ionophoresis.—Paper chromatography was carried out on Whatman No. 1 filter paper, with the following solvents (the organic phase being used where two phases form): (1) butan-1-ol–ethanol–water (4 : 1 : 5); (2) propan-1-ol–ethyl acetate–water (7 : 1 : 2); (3) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3), (4) ethyl acetate–acetic acid–water (9 : 2 : 2); (5) butan-1-ol–acetic acid–water (4 : 1 : 5). The sprays most commonly used to detect the sugars and their derivatives were: (1) silver nitrate and ethanolic sodium hydroxide; (2) *p*-anisidine hydrochloride; (3) aniline hydrogen phthalate; (4) urea hydrochloride; (5) diphenylamine, aniline, and phosphoric acid; (6) 2,4-dinitrophenylhydrazine and hydrochloric acid; (7) α-naphthol and phosphoric acid; (8) phloroglucinol and trichloroacetic acid; (9) potassium periodatocuprate.[8]

Paper ionophoresis was carried out on Whatman No. 3 paper in borate buffer (0·2M) at pH 10·0.

Interaction of Sugar Derivatives and Boron Trichloride.—The sugar derivative (1—10 mg.) was dissolved or suspended in dry dichloromethane (1—2 ml.) and cooled in acetone–solid carbon dioxide. Boron trichloride (1—2 g.), cooled to −80°, was then added. The mixture was kept at −80° for 30 min., then allowed to warm to room temperature and kept for 16 hr. under anhydrous conditions. Substances which were insoluble in dichloromethane initially frequently became soluble as the mixture attained room temperature. Any solvent or boron trichloride remaining was removed under diminished pressure at room temperature. The glassy residue was treated by adding either (*a*) methanol (3 × 3 ml.) and evaporating to dryness under diminished pressure after each addition or (*b*) an aqueous suspension of silver carbonate to neutralise the solution, filtering from the insoluble silver salts, and freeze-drying the aqueous filtrate. The residue in either case was dissolved in a small amount of methanol or water and examined by paper chromatography and paper ionophoresis.

Interaction of D-Fructose and Boron Trichloride.—Boron trichloride (10 g.) was added to a suspension of D-fructose (1·6 g.) in dichloromethane at −80°. After 30 min. at −80° and 16 hr. at room temperature excess of boron trichloride and of dichloromethane was removed and the product treated by the silver carbonate method. On examination by paper chromatography (with solvents 1, 2, 3, and 5) and by paper ionophoresis, the principal product could not be distinguished from authentic 5-hydroxymethylfurfuraldehyde. This product was purified by chromatography on several sheets of thick paper (Whatman No. 3) in solvent 1. The papers were viewed under ultraviolet light, which rendered 5-hydroxymethylfurfuraldehyde visible. The appropriate sections were cut into thin strips and extracted with ether (Soxhlet). Concentration of the extract gave a syrup (0·4 g.) which was shown to be pure 5-hydroxymethylfurfuraldehyde by chromatography and by oxidation with silver oxide (1 g.) and sodium hydroxide (1·6 g.) in water to 5-hydroxymethyl-2-furoic acid (0·37 g.), m. p. and mixed m. p. 165°. A solution of the sample in water had the same ultraviolet absorption spectrum as a pure sample of 5-hydroxymethylfurfuraldehyde.[9]

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are not completely demethylated in a single treatment with boron trichloride give the expected partially methylated derivatives which in some cases were identified chromatographically; when authentic compounds were not available for comparison, the R_F values of the products clearly indicated this type of derivative. In the case of the monomethyl product from 2,3,4,6-tetra-*O*-methyl-D-glucose, elution from the paper on which it was separated and examination of the eluted product by ionophoresis established that no selective demethylation had occurred. The M_G values of 0·21 and 0·81 (with values of 0·26 and 0·82 in a duplicate determination) indicate the presence of either or both 2- (M_G 0·23) and 4-*O*-methylglucose (M_G 0·24), and of either or both the 3- (M_G 0·82) and 6-*O*-methylglucose (M_G 0·82).[5] The two methylated polysaccharides gave mainly glucose as expected, with some mono- and di-*O*-methylglucose. Di-*O*-methylsucrose is converted into glucose and 5-hydroxymethylfurfuraldehyde; the latter showed the same chromatographic behaviour as the product obtained on a preparative scale directly from the same treatment of fructose. The structure of this product was established by comparison of its ultraviolet absorption spectrum with that of an authentic specimen and by conversion by mild oxidation into 5-hydroxymethyl-2-furoic acid. Stoicheiometrically, the reaction involves loss of three molecules of water from fructose. Mechanisms have been proposed for this dehydration when brought about by acids[6] and by iodine in dry dimethylformamide.[7] In the latter reaction, the presence of the furanoside ring and of the attached hydroxy- and hydroxymethyl groups at the tertiary carbon atom at position 2 provide a plausible route for the progressive elimination of three molecules of water. A similar scheme for the interaction with boron trichloride could be formulated based on the formation of a carbonium ion at $C_{(2)}$ similar to that of the glucosyl ion (above), followed by elimination of a proton at $C_{(1)}$ to give an aldehyde group; the aldehyde group would assist the elimination of the second and third molecules of water from positions 2,3 and 4,5 by resonance stabilisation through conjugation with the olefinic bonds so formed, as in the iodine reaction.

Glycosides, acetals, and ketals are converted almost exclusively into the parent sugar (accompanied on chromatograms by the corresponding aglycone in the case of the phenyl glucosides), except when a fructose unit is present. Acetylated sugars behave similarly, but small amounts of subsidiary products are formed which appear to be incompletely deacetylated derivatives; oligosaccharides appear in addition with cellulose and amylopectin acetates. Nitrocellulose also gives glucose as the main product, with smaller amounts of other derivatives. The behaviour of 1,6-anhydro-sugars contrasts with that of 2,3-anhydro-compounds. The former react normally with boron trichloride, but neither of the 2,3-anhydro-sugars examined gave detectable quantities of the parent sugar, the chief products in both cases having R_F values well removed from the expected values. Chloro-sugars may be formed with these substrates and this possibility is being examined. No evidence has been obtained that any monosaccharide formed as the principal product in the reaction of boron trichloride with its derivatives itself undergoes detectable degradation with this reagent under the experimental conditions reported in the Table. The effect of boron tribromide was examined when no dissolution in boron trichloride was apparent, but no differences in the type of reactivity of the two reagents were observed. D-Fructose forms 5-hydroxymethylfurfuraldehyde with both reagents; this product is also obtained by the action of boron tribromide on L-sorbose, which is not soluble in boron trichloride.

The disaccharides investigated, together with raffinose, did not dissolve in or react with boron trichloride, but reaction occurred with boron tribromide in all cases, with fission into the expected monosaccharide units; as expected, 5-hydroxymethylfurfuraldehyde appears in the products from sucrose, turanose, and raffinose with only traces of fructose. This was the only product from inulin which, in contrast to the other three substrates, reacts with boron trichloride.

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