SOME CARBOHYDRATE DERIVATIVES OF FERROCENE

A Thesis submitted by

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a candidate for the

Degree of Doctor of Philosophy

September 1960

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ABSTRACT.

Some carbohydrate derivatives of ferrocene have been synthesised with regard to their possible use as water-soluble sources of iron in biological systems.

Ferrocenylmethyl and ferrocenylethyl β-D-glucopyranosides have been prepared by the Koenigs-Knorr method. The former was also prepared by a modified Fischer synthesis.

Ferrocenylmethyl β-D-glucopyranoside was rapidly hydrolysed by acid at a rate comparable with the rates for glucofuranosides. An analysis of the properties of ferrocene and the factors influencing the rates of hydrolysis of glycosides indicated a significant polar assistance from the ferrocenylmethyl substituent to the rate determining heterolysis of hydrolysis.

A Friedel-Crafts reaction between ferrocene and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide did not yield the desired glucosyl ferrocene derivative. As an alternative approach to the preparation of the glucosyl ferrocene derivative:- cyclopentadienylmagnesium bromide was reacted with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide to give 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)cyclopenta-1,3-diene but an attempted condensation of this product with ferric chloride failed to give the desired ferrocene derivative.

Ferrocenemonoaldehyde condensed with D-glucitol to give 2,4-O-ferrocenylidene-D-glucitol. Two isomeric products were isolated

and the evidence suggested that they were related as diastereoisomers.

Ferrocenemonoaldehyde condensed with D-mannitol to give 1(?),3-O-ferrocenylidene-D-mannitol and 1,3:4,6-di-O-ferrocenylidene-D-mannitol.

The acetals were very acid labile and the constitutional factors influencing the rates of hydrolysis were analysed.

Acetylferrocene was reacted with D-mannitol under a variety of conditions but no evidence for ketal formation was obtained.

The optical rotatory dispersion of ferrocenylmethyl β-D-glucopyranoside and 2,4-O-ferrocenylidene-D-glucitol was investigated.

An organic two- ~~reversed~~ phase paper chromatographic procedure was found suitable for the fractionation of some ferrocene derivatives.

ACKNOWLEDGMENTS.

It is my pleasure to thank Professor E.J.Bourne for his interest and help and Dr.J.B.Pridham for supervising the work.

I would also like to thank Dr.J.B.Brimacombe, Dr.A.B.Foster and Dr.J.Majer for their assistance with the isotope experiments and Dr.C.A.Bunton for a generous sample of calibrated ^{18}O-enriched water.

Acknowledgment is made to Professor W.Klyne for optical rotatory dispersion measurements.

I am indebted to Aspro-Nicholas Ltd. for provision of a grant which made the work possible.

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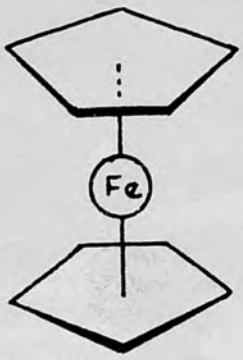
INTRODUCTION

Ferrocene [dicyclopentadienyliron (I)] is an organometallic compound consisting of two cyclopentadienyl radicals bound to an iron atom. It was first prepared by Kealy and Pauson[1] who showed that it displayed properties similar to those of benzene. X-ray crystallographic examination has shown that ferrocene has a pentagonal antiprism structure.[2-5]



(I)

Cyclopentadienyl radicals combine with many other transition elements to form compounds which are structurally analogous to ferrocene.

Ferrocene (I) shows remarkable stability to reducing reagents, alkalies, acids (in the absence of oxygen) and addition reagents e.g. maleic anhydride.[6] It undergoes electrophilic substitution, however, under noticeably mild conditions and it is in this respect much more reactive than benzene.[7] Ferrocene in the presence of mild oxidising agents is converted to the positively charged ferricinium ion[2] ($C_{10}H_{10}Fe^{+}$)(II) which gives a blue solution in water. Stronger oxidising agents will completely destroy the ferrocene nucleus.

The structure and aromaticity of ferrocene have been described in terms of valence bond and molecular orbital theories.[4,8,9] As a simplified general approach the aromatic stability of ferrocene may

be considered as arising from the sharing by each cyclopentadienyl radical of an electron contributed by the iron atom. This, however, gives too local a picture since the aromaticity must be related to the system as a whole. Cyclopentadienyl anions ($C_5H_5^-$) are known to exhibit aromatic stability which is due to the completion of the aromatic sextet by the extra electron.

In the molecular orbital description the bonding results from the possession by the cyclopentadienyl radicals of molecular orbitals with the same elements of symmetry as the 3d, 4s and 4p orbitals of iron. In this scheme all ten $2p_z$ electrons in the cyclopentadienyl radicals are simultaneously bound to the metal and the aromaticity is therefore derived in a complicated way.

An attempt to give the same bonding scheme using the valence bond method 'leads to a very unwieldy picture'.[10] Basically the binding forces are formulated in terms of pairing schemes between the $2p_z$ orbitals of the cyclopentadienyl radicals and the $3d_{xz}$ and $3d_{yz}$ metal orbitals.

Kuch[11] and Fischer[3] have proposed an alternative valence bond description in which the metal orbitals are hybridised to form six orbitals in a trigonal antiprism arrangement. These overlap with the cyclopentadienyl π-orbitals which are combined to form three equivalent orbitals at 120° intervals, thus producing six electron pair bonds for which ten electrons are contributed by the cyclopentadienyl radicals and two by the iron atom.

This approach and the molecular orbital method differ most in the extent to which the 4s and the 4p orbitals of the iron atom are involved in the bonding.

Suitable metallocene derivatives would be a novel method of presenting iron or other transition element to biological systems. Metallocenes are insoluble in aqueous solution and therefore polyhydroxy derivatives of these compounds would be required for biological studies. For this reason it was decided to prepare carbohydrate derivatives of ferrocene for pharmacological tests. Various derivatives were possible and the following types of linkages between the two systems were considered.

(a) glycosidic :- by condensation of an alcohol of ferrocene with an acetohalogeno-sugar (R" Br).

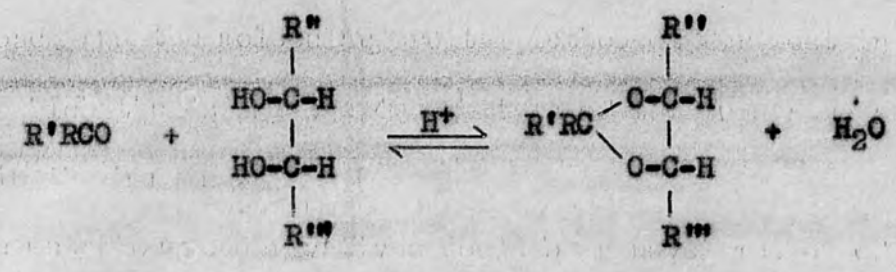
$$R'OH + R''Br \xrightarrow{Ag_2O} R''OR' + HBr$$

(b) glycosyl :- by Friedel-Crafts or Grignard reactions between ferrocene and an acetohalogeno-sugar (R''Br).

$$R'H + R''Br \xrightarrow{AlCl_3} R'R'' + HBr$$

$$R'MgBr + R''Br \longrightarrow R'R'' + MgBr_2$$

(c) acetal or ketal :- by condensation of ferrocenemonoaldehyde or acetylferrocene with a hexitol.



where R' = $C_{10}H_9Fe$ and R = H, $C_{10}H_9Fe$ for a ketal
and R' = $C_{10}H_9Fe$ and R = H for an acetal.

For all the ferrocene compounds listed above analogous benzene derivatives were known and it was considered that a comparison of the properties of the two series would give some information regarding the fundamental characteristics of the ferrocene system e.g. reactivity and the importance of steric and electrical factors in its reactions.

In this work the two main hindrances were, firstly, the need to prepare all the ferrocene derivatives required in the syntheses and, secondly, the solubility difficulties encountered in reacting a solid hydrophilic material (carbohydrate) with a solid hydrophobic material (ferrocene derivative).

DISCUSSION

Synthesis and Properties of some Glycosides containing Ferrocene.

(a) Ferrocenylmethyl β-D-glucopyranoside.

In 1893 Fischer[12] demonstrated that aldoses condensed with alcohols in the presence of mineral acids to yield crystalline products called glycosides. These reactions and products have since been extensively investigated.

Hexoses react with aryl or alkyl alcohols to give, depending on the conditions, glycosides with either furanosyl or pyranosyl rings. The aglycone may be attached in the α or β position at the anomeric carbon centre. (Fig.1).

Earlier in 1879, Michael[13] had produced phenolic glucosides by reacting phenols with 2,3,4,6-tetra-O-acetyl-α-D-glucosyl chlorides. This method was extended by Koenigs and Knorr[14] who allowed 2,3,4,6-tetra-O-acetyl-α-D-glucosyl bromide, silver carbonate and the alcohol to react under anhydrous conditions. Subsequent workers have investigated the advantages of using inert, low boiling solvents, alternative condensing agents, catalysts and internal desiccants. Silver salts have been most generally employed as condensing agents but some success has also been obtained using zinc, cadmium and mercury salts.[15] The traces of water formed during the reaction have been effectively removed with anhydrous calcium sulphate (Drierite) and yields have been much improved by using Drierite in conjunction with small quantities of iodine.[16]

Synthesis and Properties of some Glucosides containing Ferrocene.

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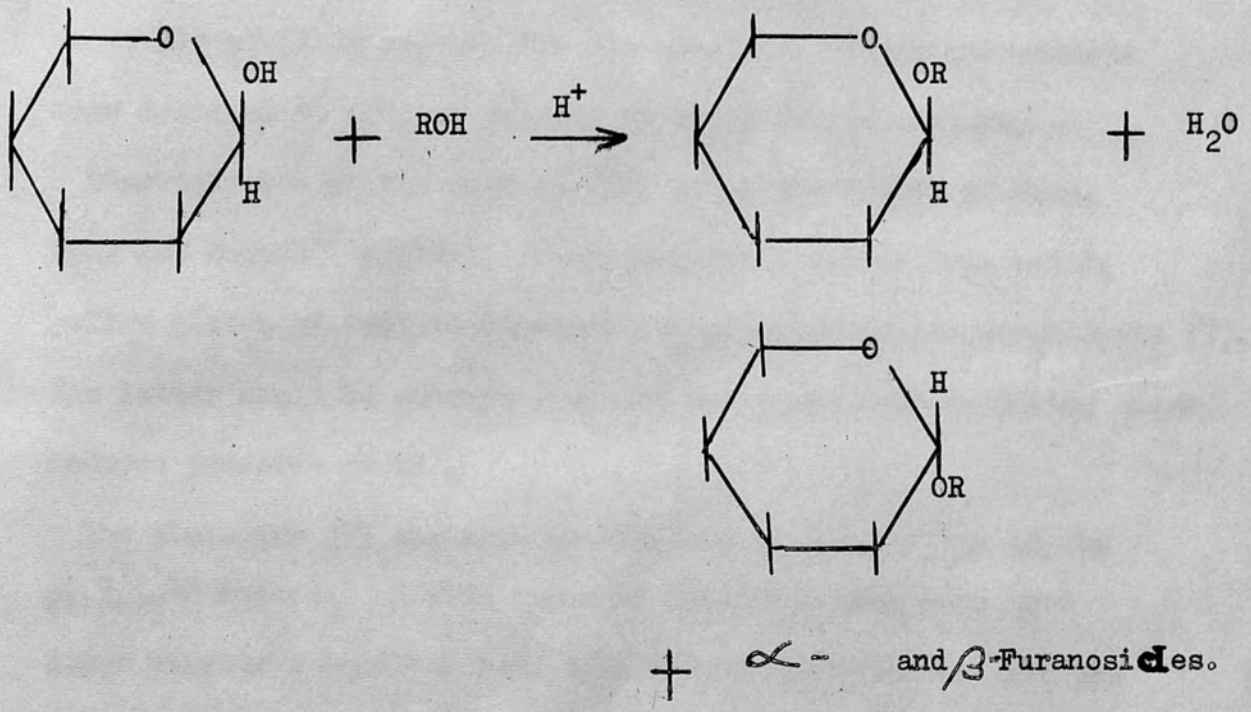
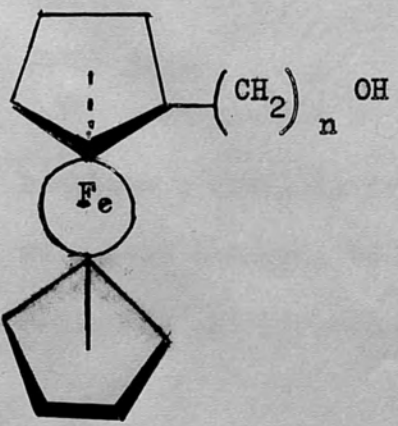
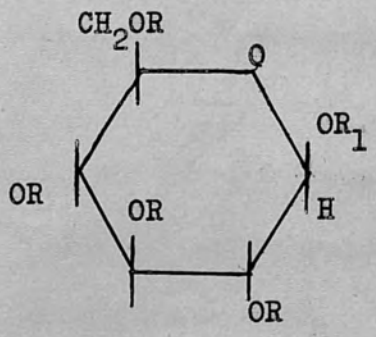
FIG. 1. FORMATION OF GLUCOSIDES (FISCHER)





(III) n=1

(IIIa) n=2



(IV) R_1 = FcnCH$_2$. R = CH$_3$CO

(V) R_1 = FcnCH$_2$. R = H

(VI) R_1 = FcnCH$_2$CH$_2$. R = H

The condensation of hydroxymethylferrocene (III) with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in methylene chloride in the presence of silver oxide and Drierite gave a 55% yield of ferrocenylmethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (IV). The poor yield given by initial experiments was improved by using a large excess of silver oxide and a carefully purified solvent for the reaction. These precautions were designed to prevent acidity in the reaction mixture.

Deacetylation of the acetate (IV) using the method of Ness, Hahn and Hudson[17] yielded, after recrystallisation from water, yellow plates of ferrocenylmethyl β-D-glucopyranoside monohydrate (V). The latter could be converted to the anhydrous form by drying under reduced pressure at 80°.

The glucoside (V) was also synthesised by a variation of the Fischer[12] method. A wide range of catalysts have been used since Fischer's original work with dilute hydrochloric acid and these have been mainly organic acids, acid salts and cation-exchange resins[18]. The use of p-toluenesulphonic acid in the alcoholysis of sugar derivatives has also been reported by Hickinbottom[19] and Combs *et al.*[20]

The condensation of two solids, as in the present example, requires a suitable inert solvent. D-Glucose and hydroxymethylferrocene were both observed to be soluble in dimethylformamide. Thus D-Glucose and hydroxymethylferrocene on heating in dimethylformamide

in the presence of p-toluenesulphonic acid gave a small yield of ferrocenylmethyl β-D-glucopyranoside (V). The latter was isolated from the reaction mixture on paper chromatograms.

The structure of the glucoside (V) from the Koenigs-Knorr preparation was confirmed by periodate oxidation, methylation and enzymic hydrolysis. Preliminary experiments had indicated that the glucoside was very unstable in acid solution and in this respect resembled a furanoside rather than a pyranoside.

Periodate oxidation of the glucoside (V).

Initial experiments showed that potassium metaperiodate in addition to reacting with the vicinal glycol groups also degraded the ferrocene nucleus. The oxidation of ferrocene with periodate liberated ferric ions and acidic products but no formaldehyde could be detected with the dimedone reagent.

The presence of iron was also found to affect the consumption of periodate in various ways. This was shown by measuring the uptake of periodate by the arsenite-iodine method[21,22] in the four systems described below.

(a) Aqueous potassium metaperiodate.

(b) Aqueous potassium metaperiodate and dioxan.

(c) Aqueous potassium metaperiodate and ferrous sulphate.

(d) Aqueous potassium metaperiodate, dioxan and ferrous sulphate.

In each case the initial periodate concentration was identical. The concentration of dioxan used was 20% (v/v) with respect to water

and in (c) and (d) identical concentrations of ferrous ion were employed. The number of molecules of periodate consumed was calculated with reference to the concentration of ferrous ion. The results are given in Table 1.

Table 1

	(a)	(b)	(c)	(d)
Time (hr.)	Periodate consumed (moles.)	Periodate consumed (moles.)	Periodate consumed (moles.)	Periodate consumed (moles.)
1	0	0	1.15	1.89
3	0	0	1.15	1.90
8	0	0	1.10	2.00
11	0	0	1.10	-
15	0	0	1.15	2.15
18	0	0	1.15	2.19

In (c) and (d) a yellow-brown, flocculent precipitate appeared which gave a positive test for iron (ferric) and a weak positive test for periodate ions.

The uptake in (c) was more than sufficient to convert Fe(II) to Fe(III) since, by the relationship shown below, only 0,5 mole. of periodate are required for this purpose.

$$2Fe^{++} + IO_4^- + 2H^+ \longrightarrow 2Fe^{+++} + IO_3^- + H_2O.$$

$$2\left[Fe^{++}\right] \equiv \left[IO_4^-\right]$$

It was found that a determination of periodate after removal of the precipitate gave the same value as when the precipitate was present.

To account for the results it is likely that part of the periodate was removed as a ferric salt which was only slightly soluble in water and even less soluble in aqueous dioxan solution.

Lang and Faude[23] have described the special oxidising properties of periodate-Fe(II) systems and the increasing periodate consumption in (d) when compared with (c) could thus be accounted for by the oxidation of dioxan in the presence of iron.

Thus periodate, in the presence of carbohydrate derivatives of ferrocene and a solvent, can be consumed as follows :-

(a) Oxidation of the ferrocene nucleus.

(b) Oxidation of glycol groups.

(c) Oxidation of the solvent.

(d) Possible precipitation as an insoluble ferric salt.

Fig. 2 shows the uptake of periodate by ferrocene and hydroxymethylferrocene. The observed uptake of ca. 10.5 moles. of periodate seems reasonable, assigning 0.5 mole. for the oxidation of Fe(II) to Fe(III) and 1 mole. for the breaking of each carbon-carbon bond. Hydroxymethylferrocene gave an uptake of 13 moles. The deviation from a predicted uptake of 11.5 moles. is attributed to factors (c) and (d). In Fig.2 it is seen that the substituted ring of hydroxymethylferrocene is rapidly attacked with the expected uptake

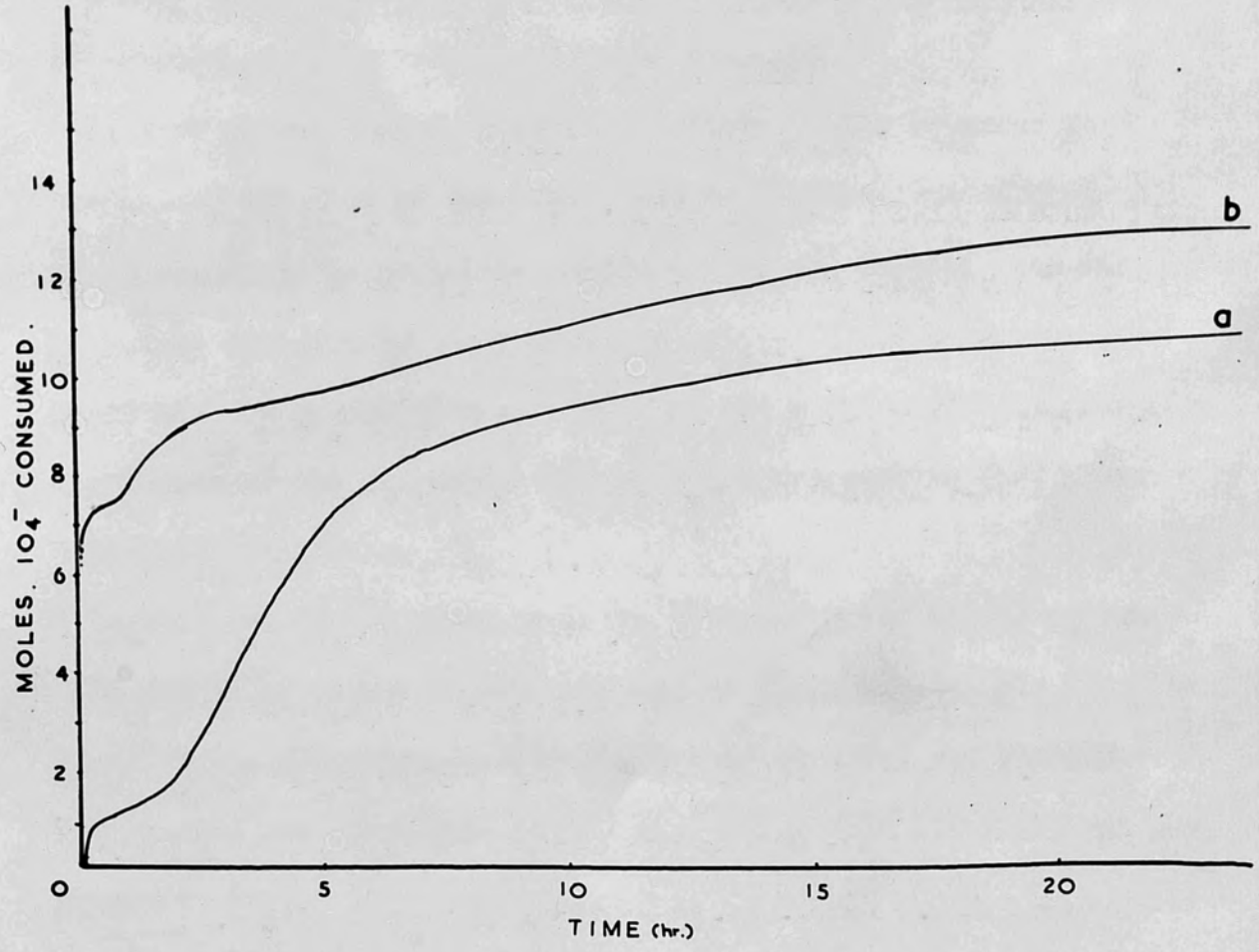


FIG. 2. Uptake of periodate by : (a) ferrocene (b) hydroxymethylferrocene. with 0·02M–KIO_4 at 25°

of ca. 7 moles. of periodate. This is followed by a slow oxidation of the second cyclopentadienyl ring.

Ferrocene after the rapid initial oxidation to the ferricinium ion (uptake of 0.5 mole.) undergoes a slow breakdown. It is probable that factors (c) and (d) give rise to errors in an absolute determination of periodate uptake of 0-1 mole.

In view of the many complicating factors liable to arise in confirming the size of the sugar ring in ferrocenylmethyl-β-D-glucopyranoside by periodate oxidation, it was decided that the periodate consumed by the glucosidic moiety of the glucoside (V) would best be established by comparing the uptakes of equimolar quantities of the glucoside (V) and its tetra-acetate (IV) under identical conditions.

In this way it was shown that the glucosyl group of (V) consumed 2.05 moles. of periodate and produced no formaldehyde.(Fig 3). The formaldehyde was determined by the method of O'Dea and Gibbons.[24] The results are consistent with a glucopyranoside structure for the glucoside (V).

Methylation of the glucoside (V).

Methylation of the glucoside (V) using the procedure described by Kuhn, Trischmann and Loew[25] followed by hydrolysis of the resulting syrup gave a crystalline specimen of 2,3,4,6-tetra-O-methyl-glucose. The latter co-chromatographed with an authentic specimen.(Fig. 4).

FIG. 3 . OXIDATION OF a. AND b. WITH 0·01M-KIO_4 AT 25°

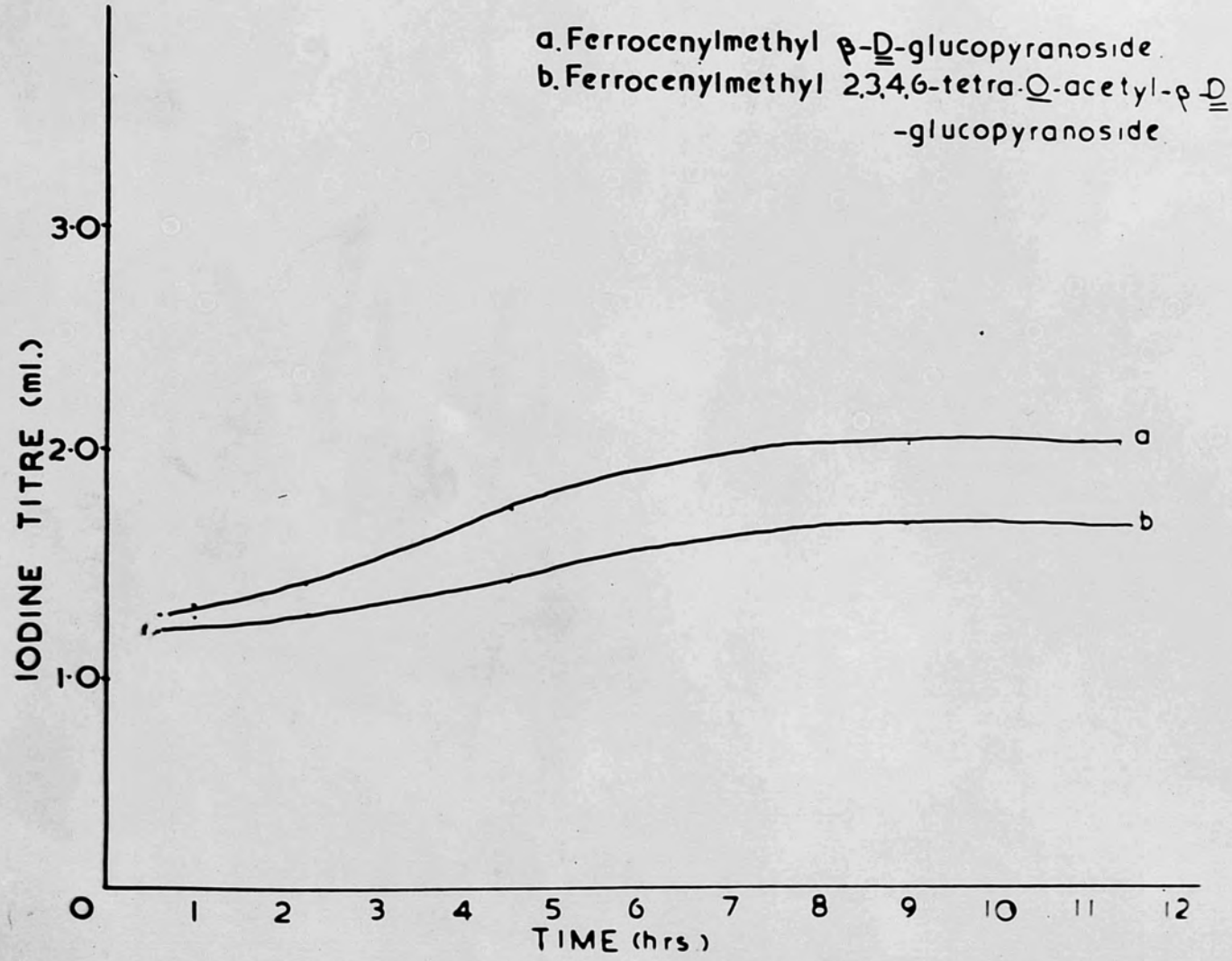


FIG. 4. METHYLATION AND HYDROLYSIS OF FERROCENYLMETHYL β-D-GLUCOPYRANOSIDE.

A. Ferrocenylmethyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside.
B. same - after hydrolysis.
C. 2,3,4,6-Tetra-O-methyl-β-D-glucopyranoside.

Enzyme hydrolysis.

The glucoside (V) was hydrolysed by almond emulsin to give glucose and hydroxymethylferrocene, thus confirming a β-pyranose structure.

Other evidence which may be adduced in favour of a β-pyranoside structure for the glucoside (V) is :-

(a) The compound had a small specific optical rotation, - 37.7°.

(b) The glucoside was immobile on a paper electrophoretogram using borate buffer (pH 10). Glucofuranosides are mobile under these conditions owing to the presence of vicinal cis hydroxyl groups.[26]

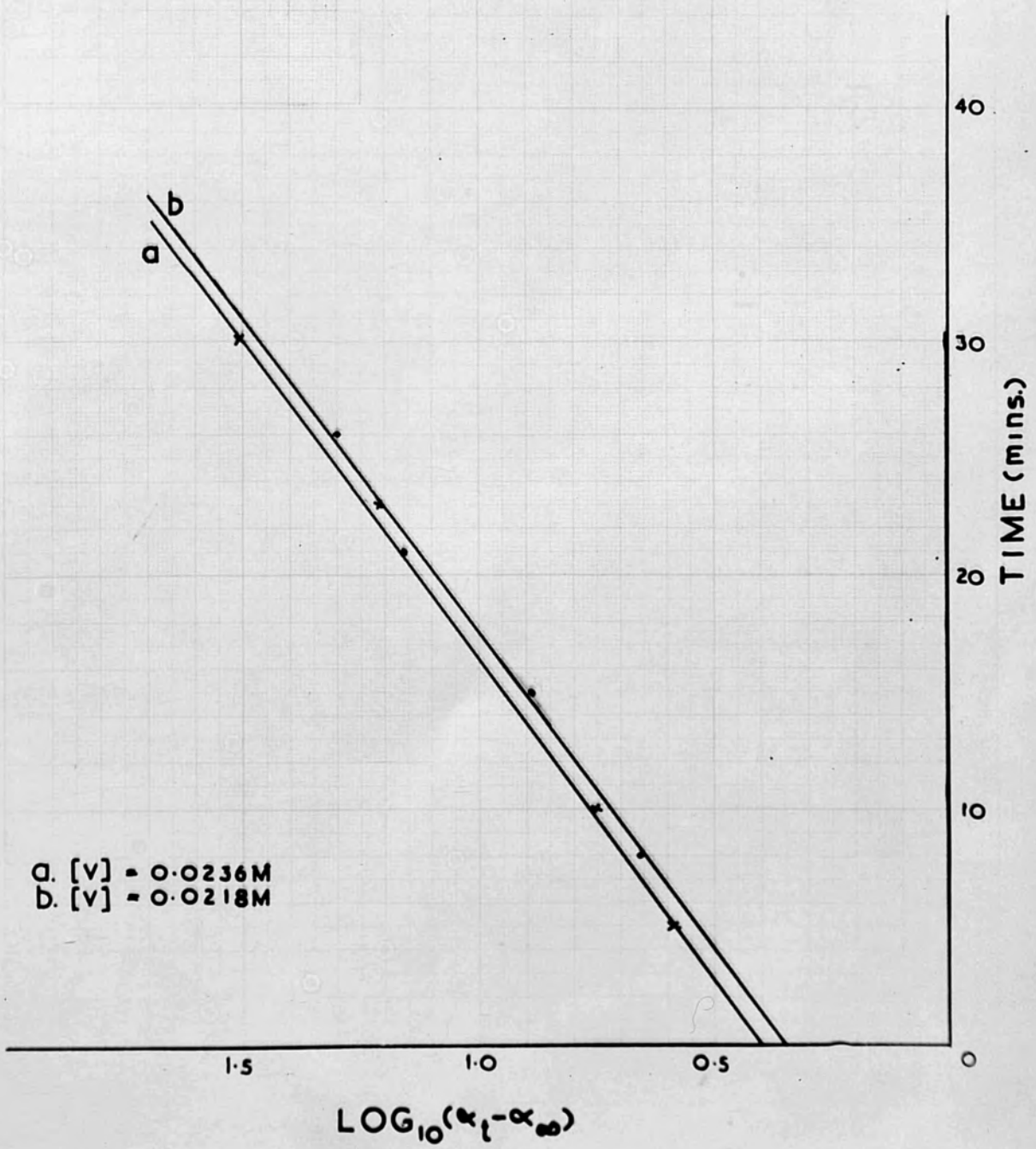
Mechanism of the acid hyrolysis of the glucoside (V).

The glucoside (V) was rapidly hydrolysed by 0.05N sulphuric acid at 25° giving hydroxymethylferrocene, bis(ferrocenylmethyl)ether (VII) and glucose. The first order rate constant for the hydrolysis was 8.6×10^{-2} min.$^{-1}$ (Fig.5).

The unusually high rate of hydrolysis under these conditions resembles the value for the hydrolysis of a glucofuranoside rather than a glucopyranoside. For example, the first order rate constants in 0.01N hydrochloric acid at 95-100° for methyl α-D-glucopyranoside and methyl α-D-glucofuranoside are 0.025×10^{-2} min.$^{-1}$ and 4.5×10^{-2} min.$^{-1}$ respectively.[27]

Bunton and his co-workers[28] have provided kinetic evidence to show that the acidic hydrolysis of α- and β- phenyl and methyl glucopyranosides proceeds by the rate-determining decomposition of

FIG. 5. 1st ORDER RATE PLOTS FOR HYDROLYSIS OF FERROCENYLMETHYL β-D-GLUCOPYRANOSIDE(V) IN 0·05 N-H_2SO_4 AT 25°



the conjugate acid which is formed by an initial fast and reversible protonation.(Fig. 6). The duality of mechanism arises from the possibility of protonation occurring at the cyclic oxygen or the glycosidic oxygen.

Bunton et al. also demonstrated, with the use of O-18 enriched water, that hydrolysis of all four compounds proceeded with hexose-oxygen fission.(Fig. 7). In cases where R could form a stable carbonium ion, it was suggested that hydrolysis with alkyl- or aryl-oxygen bond fission could occur.

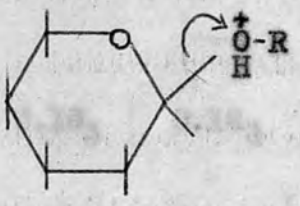


Fig. 7.

Ferrocenylmethyl β-D-glucopyranoside (V) was hydrolysed with dilute sulphuric acid in the presence of O-18 enriched water and the products were combusted to carbon dioxide and analysed by mass spectrometry.[29,30] The isotopic analyses showed that the hydrolysis occurred predominantly with hexose-oxygen fission (Table 2). The small proportion of aryl-oxygen fission would imply that the ferrocenylmethyl carbonium ion has a limited stability.

Bunton et al. established that the rate of hydrolysis of gluco-pyranosides depends primarily on two main factors :- (a) the equilibrium concentration of the conjugate acid and (b) the structural, enviromental and electrical factors influencing the rate of breakdown of this acid.

FIG. 6. MECHANISM FOR THE HYDROLYSIS OF GLYCOSIDES.

Table 2.

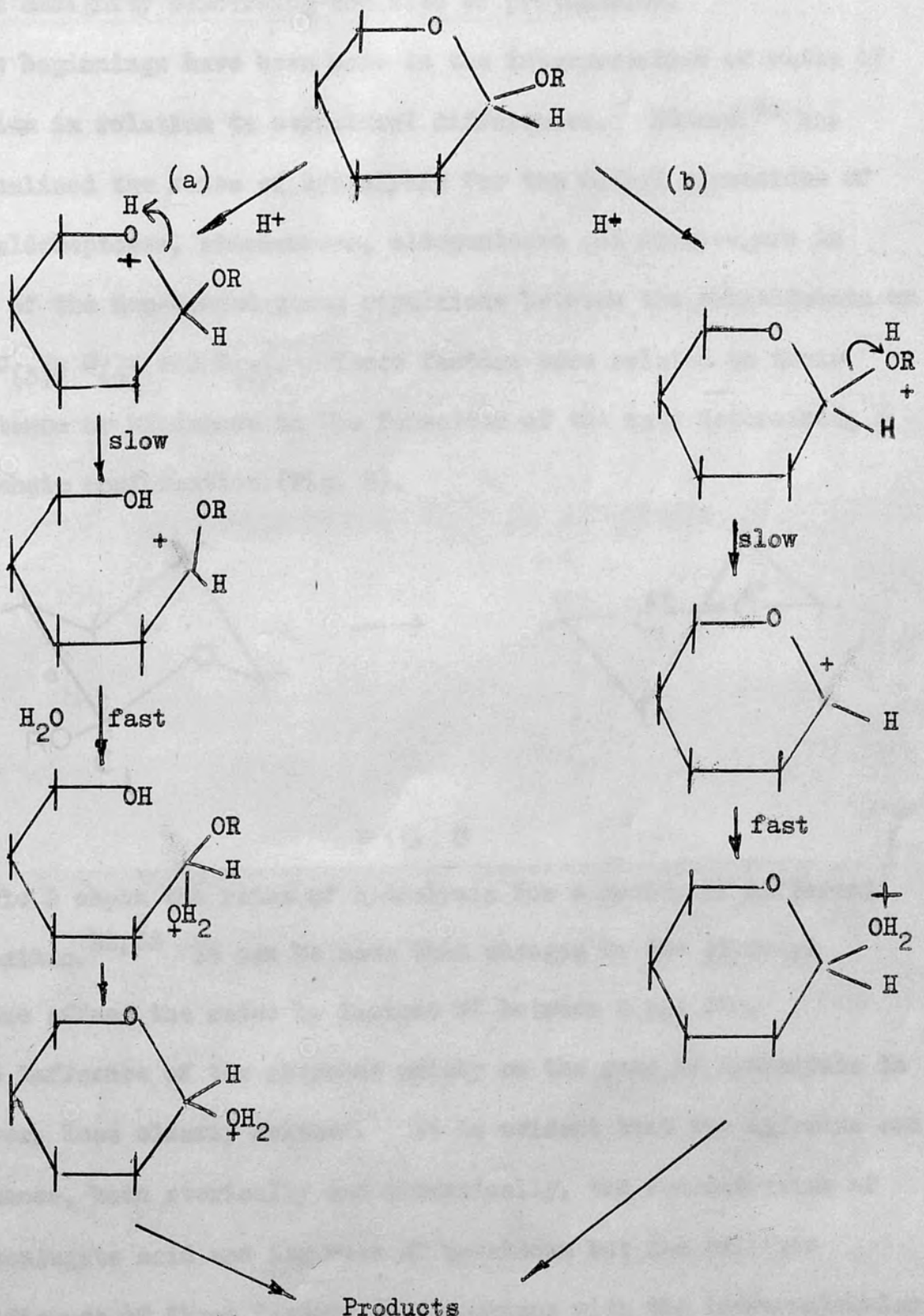
Product	$FcnCH_2OH$	$(FcnCH_2)_2O$	Glucose[a] (exptl.)	Glucose (control)	$FcnCH_2OH$ (control)	Water
$\frac{C^{16}O^{\prime}_2}{C^{16}O^{18}O}$	137	189	51.6	263	263	42.5
Abundance[b] (atom %)	0.35_0	0.25_5	0.92_7	0.18_3	0.18_3	1.11_2
Excess abundance[c] (atom %)	0.16_7	0.07_2	0.74_4	normal	normal	0.92_9

a, Calculated with reference to the $C_{(1)}$ hydroxylic oxygen only, since no enrichment was found with the control glucose.

b. ^{18}O atom % abundance is given by $\frac{100}{2R+1}$ where R is the ratio $\frac{C^{16}O^{\prime}_2}{C^{16}O^{18}O}$.

c. The excess abundance is the total abundance minus the normal abundance (i.e. 0.18_3%)

FIG. 6. MECHANISM FOR THE HYDROLYSIS OF GLYCOSIDES.



A more detailed analysis of the steric and electrical effects of the aglycone and the glucosyl residues on the hydrolysis is complicated by the ambiguity concerning the site of protonation.

Some beginnings have been made in the interpretation of rates of reaction in relation to structural differences. Edward[31] has rationalised the rates of hydrolysis for the methyl glycosides of some aldoheptoses, aldohexoses, aldopentoses and deoxysugars in terms of the non-bonded group repulsions between the substituents on $C_{(2)}$, $C_{(3)}$, $C_{(4)}$ and $C_{(5)}$. These factors were related to their assistance or hindrance in the formation of the rate determining half-chair conformation.(Fig. 8).

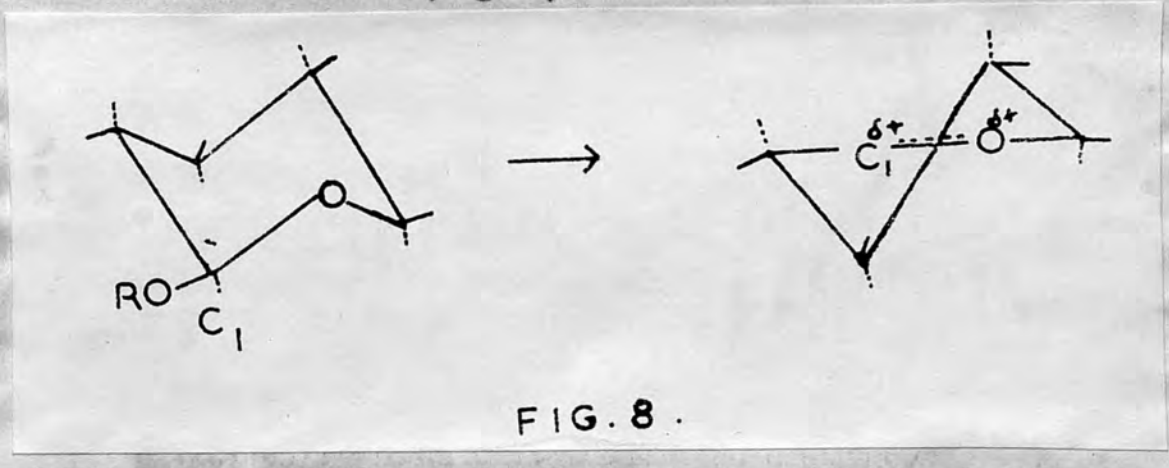


FIG. 8.

Table 3 shows the rates of hydrolysis for a number of different glycosides.[32,33] It can be seen that changes in the glycosyl residue affect the rates by factors of between 5 and 500.

The influence of the aglycone moiety on the rate of hydrolysis is however, less clearly defined. It is evident that the aglycone can influence, both sterically and electrically, the concentration of the conjugate acid and its rate of breakdown but the relative significance of these factors in comparison with the intra-molecular

Table 3.

Rates of hydrolysis in 0.01N-HCl at 95-100°.

Pyranosides.	$10^5 k_1$ (min.$^{-1}$)
Methyl β-D-glucoside	30
Methyl α-D-glucoside	25
Methyl β-D-mannoside	10
Methyl β-D-galactoside	23
Methyl β-D-rhamnoside	125
Furanosides	
Methyl α-D-glucoside	4500
Methyl α-D-mannoside	1500

Rates of hydrolysis in N-HCl at 20°.

Pyranosides.	
Methyl 2-deoxy-α-D-glucoside	1320
Methyl 2-deoxy-α-D-glucoside amide	660

repulsions within the glycosyl unit is uncertain.

Heidt and Purves[34] have concluded that the relative rates depend primarily on the structure of the sugar residue rather than the structure and properties of the aglycone. This assertion was based on kinetic studies with a rather limited range of glycosides i.e. methyl, benzyl and phenyl glycosides only. These general conclusions of Heidt and Purves could only be justified had the experimental data included results for glycosides with aglycones showing a greater structural variation and a more varied electrical behaviour e.g., glycosides of t-butanol, neopentyl alcohol and triphenylmethanol.

Nath and Rydon[35] showed that the introduction of electron-repelling groups into the aromatic ring of phenyl β-D-glucopyranoside increased the lability of the glucoside to acid. These effects, however, were comparatively small. Electronic displacement in conjugated systems is favoured when the neighbouring atoms are coplanar thus giving the π-electron orbitals maximum overlap.[36] Edward[31] suggested that the bulky phenyl group of the phenyl glucosides produces a strained system, the conjugative displacement between the glycosidic oxygen and the aromatic ring thus being reduced. The absence of strong electrical effects in these compounds studied by Nath and Rydon is, therefore, not surprising.

Little work has been published on the electrical and steric behaviour of the ferrocene nucleus. However from the chemistry which has been described in the literature it is possible to draw certain

general conclusions.

Ferrocene will undergo electrophilic substitution with great ease and acylation can be achieved under conditions which have no affect on benzene.[37] Mercuration proceeds readily when ferrocene is treated with mercuric acetate.[38]

Electrophilic substitution involves the attack by a positively charged agent on a substrate. The reaction involves the transfer of the charge and the formation to a lesser or greater degree of some reaction intermediate which subsequently breaks down.[39] (Fig. 9)

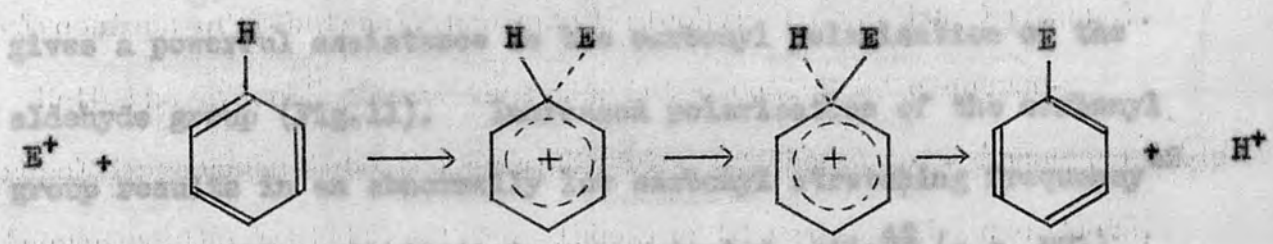


Fig. 9

It is well known that reactivity in electrophilic substitution is related to the electron availability at the reaction site. This is shown by the enhanced rate of reaction of benzene derivatives containing an electron donating substituent.[40] The reactivity of ferrocene in electrophilic substitution is equivalent to that of a highly activated benzene derivative and is therefore probably a function of its capacity to supply electrons to any given site and to form a favourably low energy transition state with the electrophile.

Ferrocene, therefore, acting as either a substrate or a substituent in another system, would be expected to exhibit a positive inductive

(or inductomeric) or mesomeric (or electromeric) effect, depending on the circumstances.

Evidence which may be adduced in favour of these proposed influences is :-

(a) A ferrocenyl group bound to a phenyl ring acts as a strong o- and p- directing agent.[41] Such properties are expected from an electron donating substituent.[40] (Fig.10).

(b) The distinctly basic properties of ferrocenemonoaldehyde[42] which are explained on the basis that the ferrocene nucleus gives a powerful assistance to the carbonyl polarisation of the aldehyde group (Fig.11). Increased polarisation of the carbonyl group results in an abnormally low carbonyl stretching frequency[42] and a reversible solubility in concentrated acids[42] (e.g. HCl). These properties are also found in perinaphthenone (VIII), a carbonyl derivative of perinaphthene[43] (IX). In close analogy to ferrocene, perinaphthene forms a stable positively charged perinaphthenylium ion (X).

(c) Further evidence of the electrical influence of a ferrocenyl substituent is found in a comparison of the strengths of benzoic and ferrocenemonocarboxylic acids.[44]

Acid	$10^{-7} K_a$
Benzoic	2.4
Ferrocenemonocarboxylic	1.8

The strength of an acid should be increased by electron withdrawal

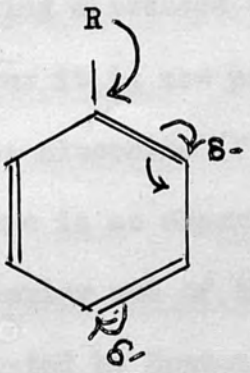


FIG. 10

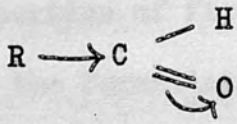
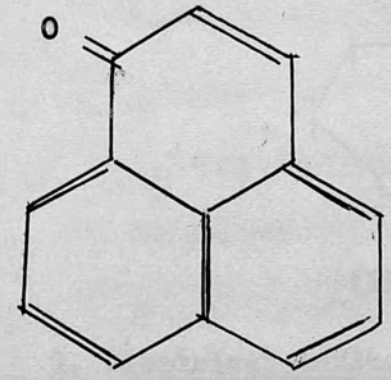
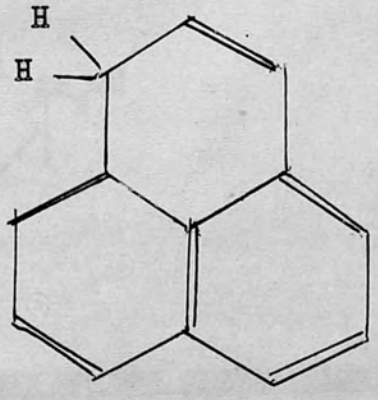


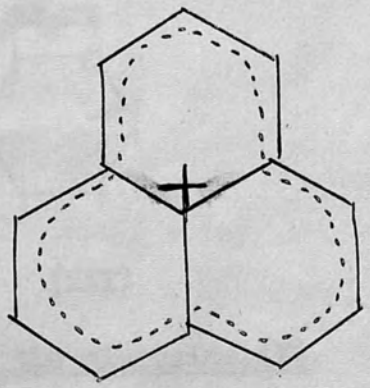
FIG. 11



(V111)



(1X)

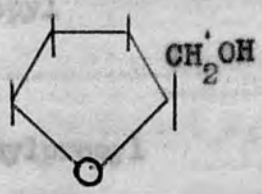


(X)

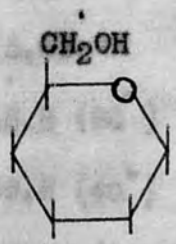
from the ionising group since this process makes the detachment of the proton easier. The electron donating properties of the ferrocenyl substituent in ferrocenemonocarboxylic acid will tend to oppose the ionisation and thus benzoic is the stronger acid.

Having discussed the important influences of the ferrocene nucleus it is now possible to interpret the high rate of hydrolysis of the glucoside (V) in terms of the known properties of ferrocene.

There is no experimental evidence to support the formation of any particular one of the two possible protonated species which were suggested by Bunton et al.(Fig.6). However, the formation of the conjugate acid (Fig.6b) by the protonation of the cyclic oxygen atom may be obstructed by hydrogen bonding between this atom and the hydrogen atom on the hydroxyl group at $C_{(6)}$ of the glucosyl moiety. Infra-red measurements have shown that 2-hydroxymethyl-tetrahydrofuran (XI) and 2-hydroxymethyl-tetrahydropyran (XII) show complete intramolecular hydrogen bonding.[45]



(XI)



(XII)

1. Electrical influences on the protonation of the glucoside.(V).

One important influence of the ferrocenylmethyl group will be in increasing the basicity of the glycosidic oxygen and thus stabilising

the conjugate acid by relay of powerful inductive and inductomeric effects. These influences would also tend to favour the acyclic oxygen in the competing demands of the cyclic and acyclic oxygens for the proton. This should lead to an increase in the equilibrium concentration of the conjugate acid and therefore an increased rate of hydrolysis.

In support of this hypothesis the results of Veibel and co-workers ~~Frederiksen~~[46,47] (Table 4) show that the rates of hydrolysis of glucosides increase as the inductive effect of the aglycone increases i.e. tertiary > secondary > primary.

Table 4.

Rate of hydrolysis in 0.5N-HCl at 70°*

Glucoside.	$10^4 k_1$ (min.$^{-1}$)
Methyl	1.51
Propyl	1.86
Isopropyl	3.09
1-Ethylpropyl	5.05
t-Butyl	42.8 (50°)
1,1-Dimethylpropyl	39.6 (40°)
Neopentyl	6.44 (in N acid)
p-Hydroxyphenyl	12.4

* except where otherwise stated.

Some further support is given by Nath and Rydon[35] who found that electron donating substituents in the aromatic ring of phenyl β-D-

glucopyranoside increased the rate of acidic hydrolysis but these effects for the reasons given earlier (p.29) were small.

Skrabal and Eger[48] showed that for acetals of general formula $R_1R_2'C(OR)_2'$, which in many ways resemble glycosides, as R becomes more electropositive the rate of acidic hydrolysis increases.

It would be an oversimplification to interpret the observed results in terms of these electrical influences alone as in some cases steric factors are important and although an increase in the electropositivity of the aglycone will favour the protonation , it will hinder the bond fission and some estimation of the relative importance of these factors must be made.

2. Electrical factors influencing the bond breaking step.

Fig.12 shows that the heterolysis (b) will be favoured by an increase in the electronegativity of R.

The evidence indicates, however, that the most important factor in determining the bond fission is the assistance (process a) of the neighbouring ring oxygen and that the direct influence of the aglycone R in this respect may be much less important .

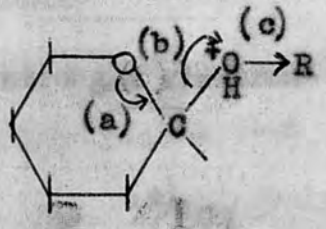
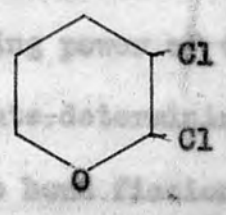


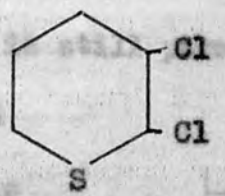
Fig. 12.

Newth and Phillips[49] have demonstrated the importance of the cyclic oxygen atom in assisting the heterolysis in an adjacent bond. Thus the high reactivity of the O-acylglycosyl 1-halides was compared with that of an α-halogeno-ether system where the inductive polarisation

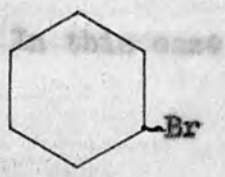
of the C-Cl bond is greatly facilitated by electromeric release from the oxygen. These workers also showed that the rate of solvolysis of 2,3-dichlorotetrahydropyran (XIII) was greater than that of 2,3-dichlorothiocyclohexane (XIV) which was very much greater than that of cyclohexyl bromide (XV).



XIII



XIV



XV

Sulphur being less polarisable than oxygen is expected to give less assistance.

Skrabal and Skrabal[50] found that the extra ethoxyl group of diethyl acetal when compared with diethyl ether increases the rate of acidic hydrolysis by a factor of 10^{11}.

It was also found that acetals possessing strongly electron-donating groups, R, (i.e. groups likely to form stable carbonium ions, e.g., t-butyl, neopentyl and α-phenylethyl)[51,52] were hydrolysed in acid with 'acyl-oxygen' bond fission (a) (Fig.13).

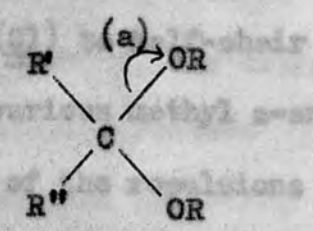


Fig 13.

If the R groups had had a strong influence on the bond fission, then 'alkyl-oxygen' fission might have been expected in these examples. Thus the influence of the group R on the actual bond breaking mechanism does not seem to be large compared with the neighbouring oxygen atom and this principle would apply equally well

to the closely analogous glycosidic derivatives.

These discussions have related for the two reasons given (p.33 & 34) to the protonated species involving the glucosidic oxygen atom. If, however, the alternative species (Fig.6a) were to be taken into account then the correlation of rate of hydrolysis with the electron donating power of the aglycone is still permissible. In this case the rate-determining step would be the bond fission (a) and this would be assisted by the electron displacements shown in Fig.14. This assistance to process (a) would be shown by strongly electron donating aglycone groups.

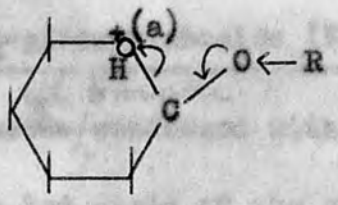


Fig. 14.

3. Steric factors in the rate determining step.

In the mechanisms of acidic hydrolysis which have been discussed the hexose becomes a carbonium ion in the rate determining step and consequently is converted from the chair (C1) to half-chair form.[31] (Fig.8). The relative stabilities of various methyl α-and β-glycosides have been accounted for in terms of the repulsions operating between the substituents in the hexose ring (p.27).

In a comparison of the steric effects influencing the rates of hydrolysis of glucosides with different aglycone groups there are two basic ways in which the steric factor can intervene.

A bulky aglycone can produce a primary steric effect causing

molecular instability by internal steric pressure and a secondary steric effect arising from twisting and strain which in unsaturated systems would influence the conjugative displacements.

A comparison of 'Catalin' models (see adjoining photograph) of benzyl β-D-glucopyranoside and ferrocenylmethyl β-D-glucopyranoside suggests that there is no significant steric pressure in either molecule.

Acidic hydrolysis of ferrocenylethyl β-D-glucopyranoside (VI).

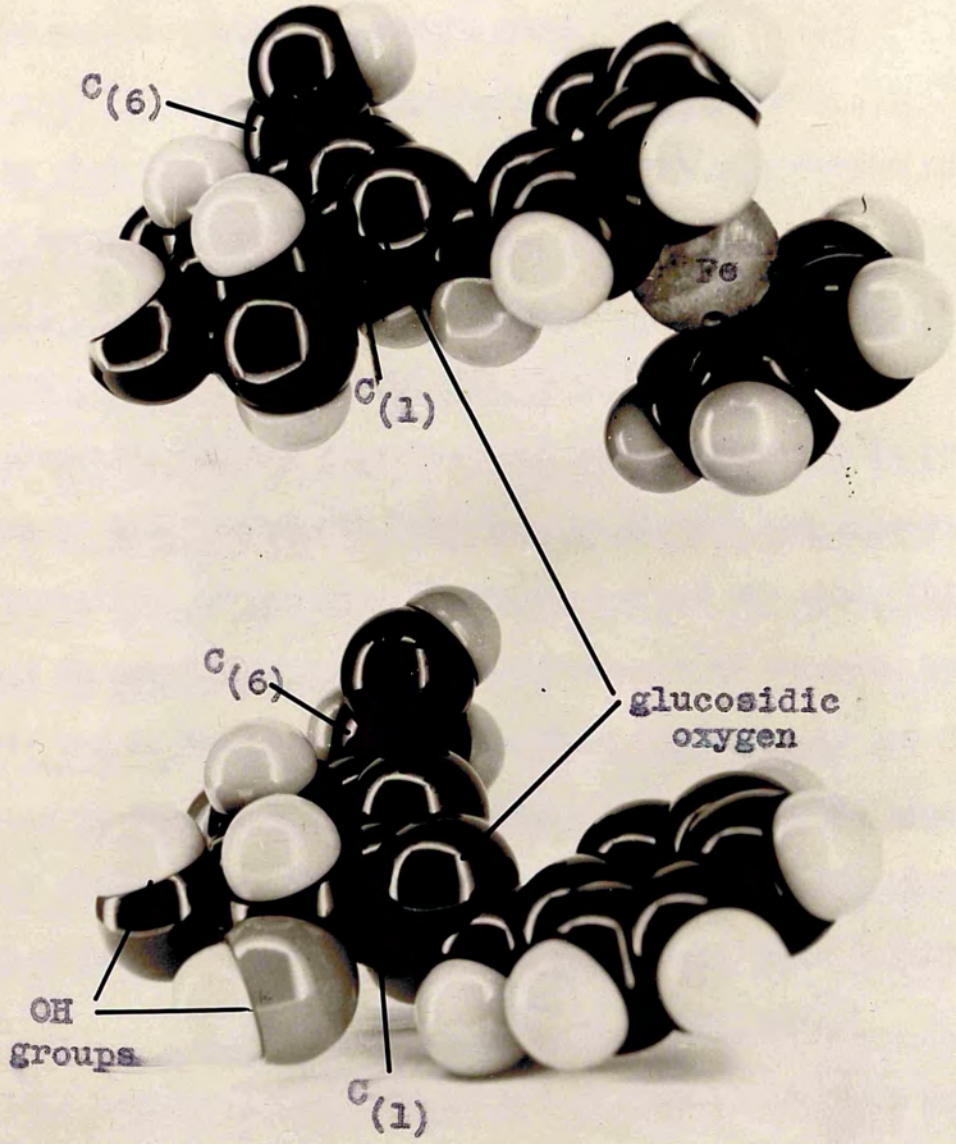
2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide condensed with ferrocenylethyl alcohol (IIIa) to give a low yield of the glucoside (VI).

It was observed that when the ferrocenyl group was removed from the glucose residue by an additional methylene group, as in this glucoside, the compound showed normal acid stability and was not hydrolysed at room temperature by 0.05N sulphuric acid.

This stability is to be expected as the extra methylene group would effectively prevent any electrical factors being relayed from the aglycone to the glycosidic oxygen.[53] However, the extra methylene group would also relieve excessive steric pressure in the molecule and the stability cannot be accepted as unambiguous evidence in favour of an electrical influence being the main cause of the acid lability of ferrocenylmethyl β-D-glucopyranoside (V).

Conclusions.

Strongly electron donating aglycone groups, such as ferrocenyl-



Top. Ferrocenylmethyl β-D-glucopyranoside.

Bottom. Benzyl β-D-glucopyranoside.

methyl, should increase the rate of hydrolysis by increasing the concentration of the conjugate acid.

The influence of R (the aglycone) on the bond breaking process is considered small compared with the electromeric assistance from the neighbouring ring oxygen atom.

The model (Catalin) of ferrocenylmethyl β-D-glucopyranoside suggests that steric factors are not critical in determining the rate of hydrolysis of this compound.

Formation of Bis(ferrocenylmethyl) ether (VII).

The glucoside (V) was hydrolysed at room temperature in the presence of 0.05N sulphuric acid to give glucose and approximately equal quantities of hydroxymethylferrocene and the ether (VII).

It was observed that hydroxymethylferrocene in suitable organic solvents and in the presence of traces of mineral acid was also converted to the ether (VII). In warm water (60°), the alcohol (III) together with a trace of hydrochloric acid, gave an 80% yield of the ether (VII). This work confirmed the results of Hauser and Cain.[54]

The acid catalysed formation of ethers from alcohols is well known.[55] The reaction may proceed by S_N1 or S_N2 mechanisms depending on the alcohol used (Fig.15A). Tertiary alcohols are found to react via the S_N1 route and primary alcohols via the S_N2 route.

The facile formation of bis(ferrocenylmethyl) ether under the mild

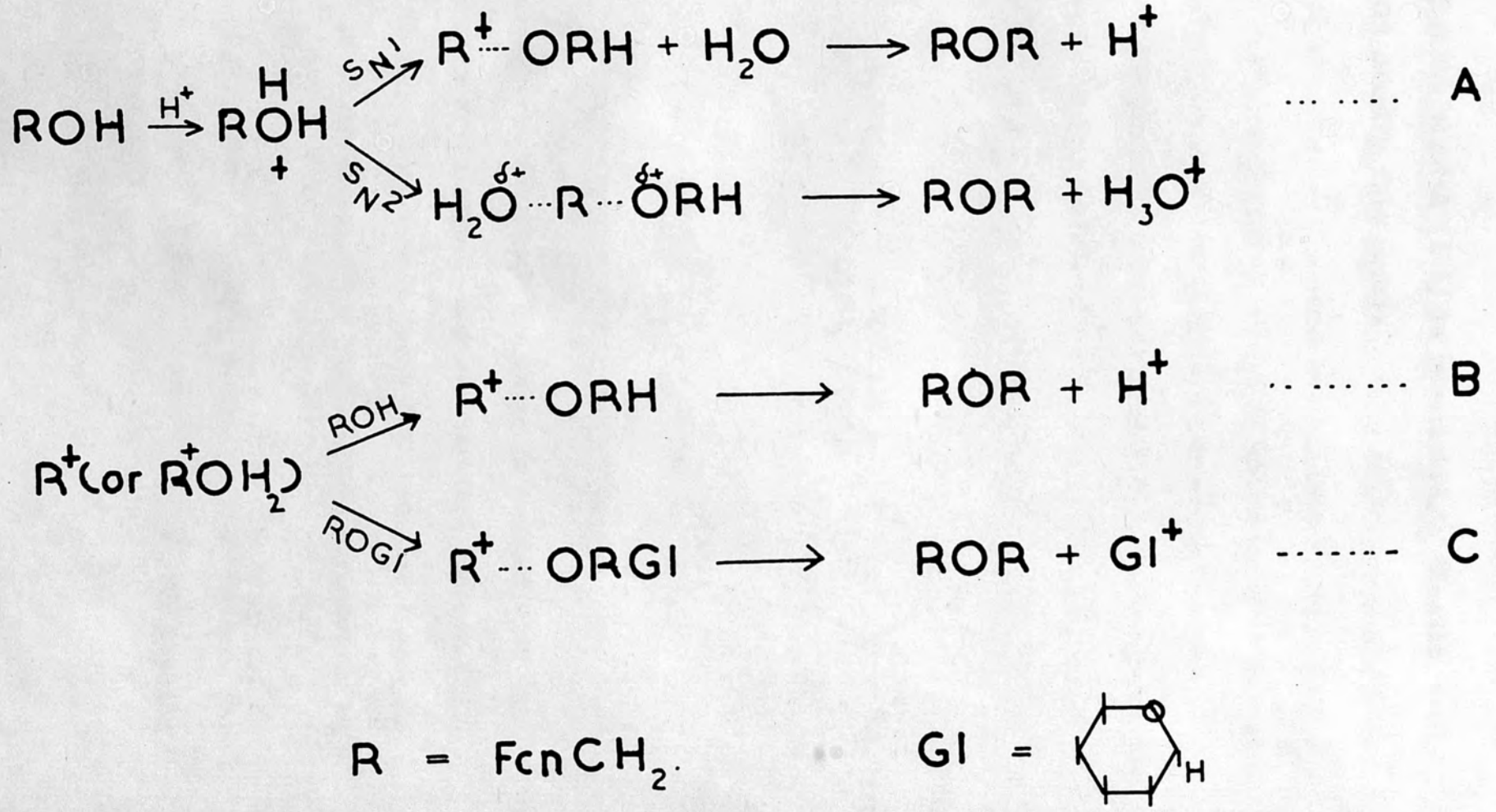
conditions described is characteristic of a tertiary alcohol and implies that the ferrocenylmethyl carbonium ion possesses some stability. The stabilisation is presumably achieved by an electron displacement which partially distributes the charge over the whole ferrocene system, a feature which is implied by other properties and reactions of ferrocene derivatives (p.31).

Two mechanisms may be postulated for the formation of the ether (VII) from the glucoside (V) (Fig.15. B and C). In mechanism B the ferrocenylmethyl carbonium ion (possibly hydrated $FcnCH_2^+.OH_2$) reacts with a further molecule of the alcohol (III) (already liberated from the glucoside) to give the ether. In mechanism C the substrate is the glucoside (V) itself. It is probable that the ether can be produced either as part of the hydrolytic mechanism or as a side reaction of the alcohol (III).

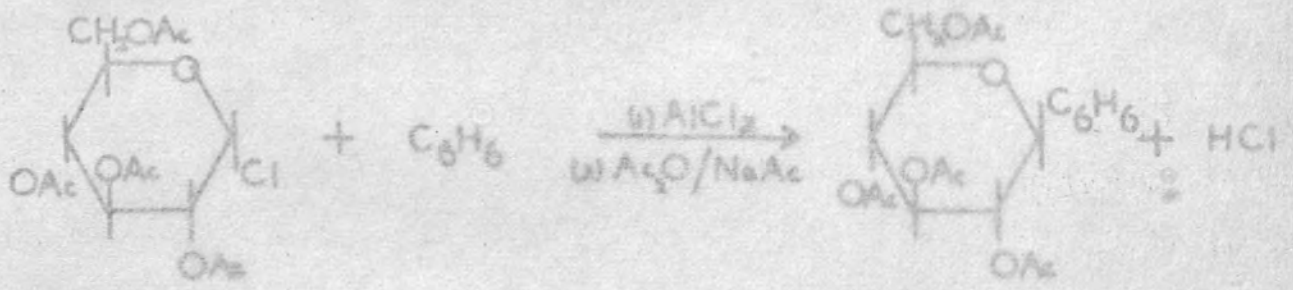
In a control experiment where hydroxymethylferrocene was treated with acid under exactly the same aqueous conditions as in the hydrolysis of the glucoside only traces of the ether (VII) were produced. This could be traced to the insolubility of the alcohol (III) in water at room temperature. It is possible that mechanism B can still operate if the alcohol (III) exists for a finite time in solution after its liberation from the glucoside.

The O-18 enrichments unfortunately do not distinguish between the two reaction mechanisms. The results show that the mechanism produces an unenriched ether, which is compatible with either

FIG. 15.


ROH
H+
ROH+
SN1
R+ ··· ORH + H2O
ROR + H+
A
SN2
H2Oδ+ ··· R ··· δ+ORH
ROR + H3O+
R+(or R+OH2)
ROH
R+ ··· ORH
ROR + H+
B
ROGI
R+ ··· ORGI
ROR + GI+
C
R = FcnCH2.
GI =
H

B or C, providing the alcohol (III) is unenriched. Kinetic experiments would clarify this point.

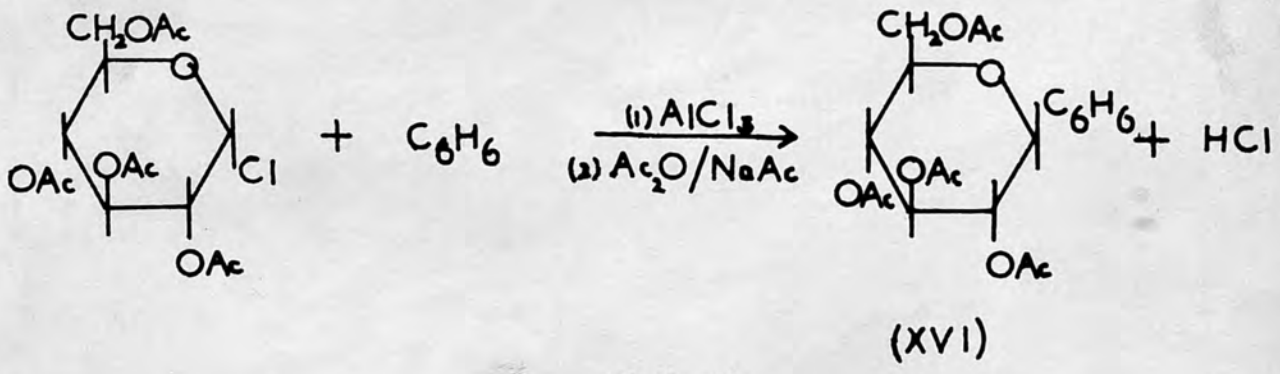


(XVI)

FIG. 16

The Glucosylation of Ferrocene and Cyclopentadiene.

The glucosylation of aromatic hydrocarbons was first successfully achieved by Hurd and Bonner.[56] Benzene was allowed to react with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl chloride in the presence of 5 moles. of aluminium chloride to give, after extraction and reacetylation of the product, a low yield of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl benzene (XVI).



(XVI)

FIG. 16

Under these conditions the aluminium chloride reacted with the ester groups to give acetophenone, the deacetylated product (XVI) and tars. It is known that esters react with aromatic compounds in the presence of aluminium chloride producing an arylated or an acylated derivative (Fig.17).

$$RH + R'COOR'' \xrightarrow{AlCl_3} RCOR' + R''OH \quad \ldots (1)$$

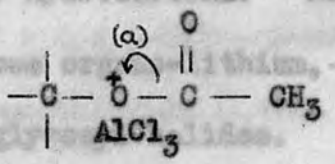
$$RH + R'COOR'' \xrightarrow{AlCl_3} RR'' + R'COOH \quad \ldots (2)$$

Fig. 17.

When R'' is derived from a carbohydrate (open chain or ring form) either a product with free hydroxyl groups and a ketone [reaction (1)] will be formed or an alkylated derivative and an acid [reaction (2)]. The second reaction will account for the hydrocarbons of varying composition (tars) which are produced. When attempting to glucosylate a hydrocarbon with an acetohalogeno sugar it is, therefore, necessary to add sufficient aluminium chloride in order that the reaction at the anomeric carbon may compete with those at the ester groups.

Aluminium chloride is a Lewis acid with a strong affinity for electron pairs and the mechanism of the above reactions presumably involves the intermediate (XVII).

Bond fission (a) would produce an acetylium ion which would then react to give a ketone.



(XVII)

Dilke, Eley and Sheppard[57] after studying the heat of mixing of various organic compounds with aluminium chloride, have proposed that the donor properties of the carbonyl group are greater than those of the ether oxygen but the evidence is otherwise conflicting. The proposed mechanism (XVII) seems to account for the observed features of the reaction but it is necessarily a simplification.

The formation of the intermediate (XVII) was supported in this present study by the fact that 2,3,4,6-tetra-O-acetyl-α-D-gluco-

pyranosyl bromide with excess aluminium chloride in an inert solvent, such as chloroform or methylene chloride, formed a complex, soluble in the organic solvent, which was composed of one molecule of the glucopyranosyl bromide and four molecules of aluminium chloride.

It is, therefore, very probable that one molecule of aluminium chloride is co-ordinated to each of the four acetyl functions in the glucopyranosyl halide and subsequent reaction can then proceed as indicated in (XVII).

The use of Grignard reagents[58] in these glucosylation reactions, in most cases, gives rise to higher yields although reaction between the Grignard reagent and the ester functions still occurs.

Other organo-metallic reagents in addition to Grignard reagents have been used for the glycosylation of hydrocarbons. Hurd and Holysz[59] studied the reactions of various organo-lithium,-sodium and-cadmium compounds with acetylated glycosyl halides. In many cases the expected products were obtained in satisfactory yields. More recently organo-zinc compounds have been used.[60] Organo-cadmium and-zinc reagents were particularly suitable as they did not attack the ester groups.

Ferrocene does not form Grignard reagents under the usual conditions[61] and the organo-metallic compounds with zinc and cadmium are as yet unknown.

However, ferrocene is more easily acylated by the Friedel-Crafts method than benzene[42] and a wide range of acyl and alkyl ferrocene

derivatives have been prepared by this reaction. The reaction can lead to monosubstitution or hetero-annular disubstitution depending on the molar ratios of the reactants. An excess of aluminium chloride leads to disubstitution and an equimolar ratio of the reactants leads to monsubstitution. Rosenblum and Santer[62] have shown that ferrocene forms a complex salt under the conditions of the Friedel-Crafts reaction which analysed as $(C_{10}H_{10}Fe.\ AlCl_3.\ HCl)_n$.

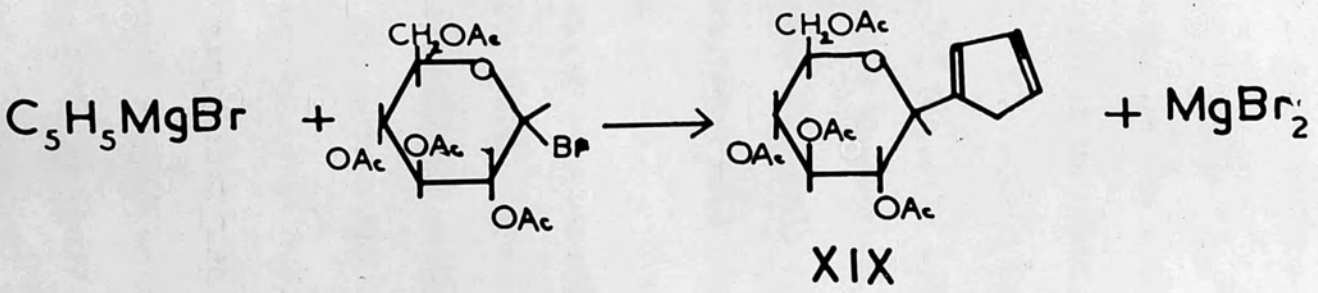
Attempted preparation of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl ferrocene (XVIII).

The reaction between ferrocene and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide was carried out using a variety of conditions but the required glucosyl ferrocene (XVIII) could not be isolated. In one case (using an initial ratio of 2,3,4,6-tetra-O-acetyl-α-D-glucosyl bromide (1 mol.), aluminium chloride (5 mol.) and ferrocene (5 mol.) with carbon disulphide as a solvent), the orange colour of the alkaline extracts of the reaction mixture implied that some reaction had occurred but there was insufficient to permit identification of the product(s).

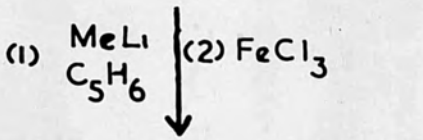
1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)cyclopenta-1,3-diene (XIX).

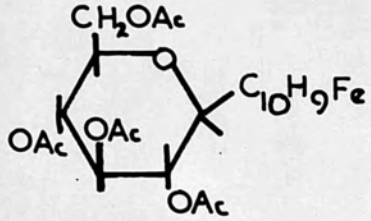
An alternative route for the formation of the glucosyl ferrocene compound (XVIII) via the cyclopentadiene derivative (XIX) was considered. This approach had been successful with many aromatic derivatives of ferrocene. (Fig.18).

$$C_2H_5MgBr + C_5H_6 \longrightarrow C_5H_5MgBr + C_2H_6$$



XIX





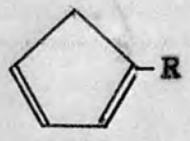
XVIII

FIG. 18.

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)cyclopenta-1,3-diene was prepared by reacting cyclopentadienylmagnesium bromide with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in ether.

Two products with the same constitution as (XIX) could be isolated using slightly different reaction conditions. Compound (XIX[A]) m.p. 120-122°, $[\alpha]_D^{24}$ -19°, λ_{max} 247 mμ (log ε 3.7) was isolated in low yield by preparation of the cyclopentadienyl Grignard reagent in benzene and subsequent reaction of this compound with the glucosyl halide in ether. The second compound (XIX[B]), m.p. 126-128°, $[\alpha]_D^{21}$ -27°, λ_{max} 247 mμ (log ε 3.8) was obtained in higher yield using ether as the solvent throughout. Both compounds [A] and [B] gave adducts with maleic anhydride of the same melting point. Sufficient material for structural analysis could be obtained only in the case of compound [B].

A molecular weight determination suggested that [B] was a monomer with the structure (XX). This was confirmed by spectroscopic evidence. The spectrum of [B] showed two major peaks at 206 mμ (log ε 3.3) and 247 mμ (log ε 3.8). (Fig. 19)



XX

The former is associated with carbonyl ester absorptions and the latter represents a bathochromic shift of 7 mμ from that of cyclopentadiene, which is characteristic of saturated substituents (e.g. methyl groups) in the 1- position of diene systems.[63] Booker, Evans and Gillam[64] have shown that, for the cyclohexadienes, a methyl

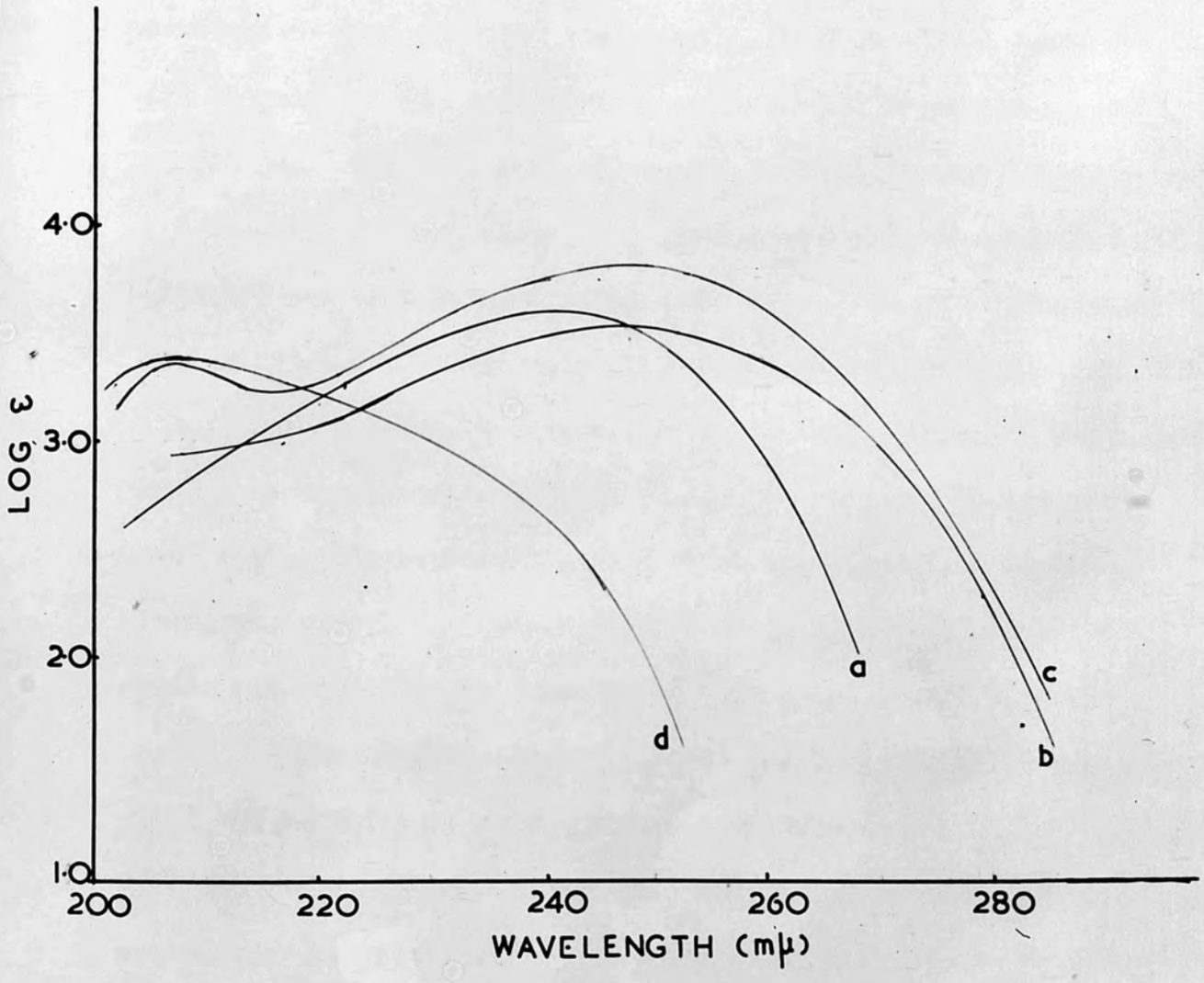


FIG. 19. Absorption spectra of :-

(a) cyclopentadiene. (b) 1-methylcyclopenta-1,3-diene.

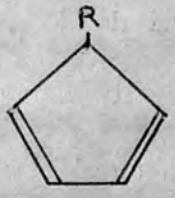
(c) 1-(2,3,4,6-tetra-O-acetyl-β-D-glucosyl)cyclopenta-1,3-diene.

(d) 2,3,4,6-tetra-O-acetyl-β-D-glucosyl cyclopentane.

group on the 1- position produces a bathochromic shift of 7 mμ and a methyl group on the 2- position a 3 mμ shift. The spectra of cyclopenta-1,3-diene and 1-methylcyclopenta-1,3-diene[65] (shown in Fig.19) support these observations. Dimerised cyclopentadiene derivatives with no other absorbing group show a peak ($\log \varepsilon$ 2·4) at 210 mμ only.[65] The infra-red spectrum of XIX[B] showed a peak at 890 $cm.^{-1}$ which is indicative of a β-glucopyranosyl configuration.[66]

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)cyclopenta-1,3-diene (XIX[B]) was hydrogenated using Adams' catalyst in ethyl acetate giving 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl cyclopentane (XXI) with a hydrogen uptake equivalent to 1.9 double bonds. Oxidation of the cyclopentane derivative (XXI) with alkaline permanganate gave cyclopentanecarboxylic acid which was identified by its infra-red spectrum. Deacetylation of (XXI) gave a syrup which would not crystallise. The R_F of this deacetylated product using a butanol/ethanol/water solvent was 0.52, which is consistent with the expected β-D-glucopyranosyl cyclopentane.

Since the compounds [A] and [B] both showed very similar spectral properties in the U.V. and I.R. regions it was assumed that they were not isomers of cyclopentadiene as shown by (XX), (XXII) and (XXIII).



XXII

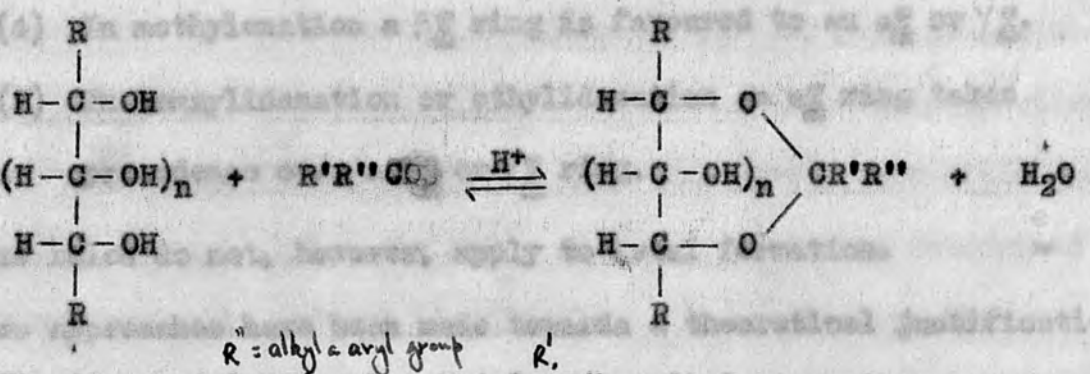
XXIII

It therefore seemed probable that they were related as stereoisomers or merely as different crystalline modifications. Infra-red spectral analysis did not suggest that [A] and [B] were α- and β- anomers and the small difference in the optical rotations of these compounds tended to confirm this.

All attempts to condense the cyclopentadiene derivative (XIX[B]) with ferric chloride and cyclopentadiene to produce the ferrocene derivative (XVIII) failed. Pauson[67] reported that highly substituted phenyl derivatives of cyclopentadiene did not yield the corresponding ferrocene derivatives and ascribed these observations to steric factors. A similar state of affairs may exist with glucosyl derivatives of cyclopentadiene.

Acetals and Ketals containing Ferrocene.

The condensation of polyhydric alcohols with aldehydes and ketones has been well studied and the subject has been described in detail in a review by Barker and Bourne.[68] Carbonyl compounds condense under acidic conditions with polyols to form cyclic derivatives containing five-, six-, or seven-membered ring systems known as acetals (from aldehydes) and ketals (from ketones)



R" = alkyl or aryl group R'

Fig 20. Acetal, R' = H ; Ketal, R" = alkyl or aryl group.

Under favourable conditions more than one ring system will be added and the product will be a diacetal or a triacetal.

Despite the numerous variable factors which influence the formation of acetals and ketals of the polyhydric alcohols Hann and Hudson[69, 70] were able to formulate simple rules rationalising the observed results. These rules were later extended by Barker and Bourne[71] to cover all cases of acetal formation. The system of nomenclature adopted by these authors is briefly as follows:-

(1) α, β and γ are used to signify the relative positions of the two hydroxyl groups involved in the ring formation, along the carbon chain. α will thus represent adjacent hydroxyl groups

β for a 1,3 relationship etc.

(2) Cyclisation of two cis hydroxyls is represented by C and cyclisation of two trans hydroxyls by T. These are only required when the two hydroxyls are secondary groups. The rules state that from the point of view of ease of ring formation :-

(1) The first preference is for βC.

(2) The second preference is for β.

(3) The third preference is for α, αT, βT or γT.

(4) In methylenation a βT ring is favoured to an αT or γT.

(5) In benzylidenation or ethylidenation an αT ring takes precedence over a βT or γT ring.

These rules do not, however, apply to ketal formation.

Two approaches have been made towards a theoretical justification of these rules.

Barker, Bourne and Whiffen[72] assumed that the most suitably situated hydroxyls would react preferentially because the least distortion of the carbon chain was required and hence the least activation energy for the reaction. This presupposed that the geometrical configuration in the transition state was the same as in the product.

By calculating the O-O distance in the O-C-O acetal group and the various O-O distances between the hydroxylic oxygens along the carbon chain they were able to show that a minimum amount of distortion results when a βC ring is formed by 1,3-dioxo cyclisation.

The rules were examined in relation to the amount of distortion of the carbon chain and the bond rotations necessary for the ring formation. In certain cases the resulting staggered or eclipsed valency relationships of the adjacent carbon atoms were considered and this, it was suggested, accounted for the preferential formation of β rather than α rings.

These rules have also been examined, but in less detail, from the standpoint of conformational analysis.[73] In this case it was assumed that the preferred ring structure was the most stable one, that is to say, the one in which the least non-bonded interaction occurred.[74] This followed from the rule that for a reversible reaction at equilibrium the ratio of the products are determined thermodynamically and not kinetically. By considering the numbers of pairs of eclipsed bonds and the spatial orientation and separation of the groups in the various ring systems the analysis accounted for the empirical rules.

The two approaches should be considered as complementary rather than opposed.

The condensation of ferrocenemonoaldehyde with D-glucitol and D-mannitol.

Ferrocenemonoaldehyde (XXIV) has been reacted with ethylene glycol to give ferrocenyl-1,3-dioxolan[42] but no other acetal condensations with this aldehyde have been reported.

In this present study it has been shown that the aldehyde (XXIV) reacts with D-glucitol giving 2,4-O-ferrocenylidene-D-glucitol

and with D-mannitol giving 1(?),3-O-ferrocenylidene-D-mannitol and 1,3:4,6-di-O-ferrocenylidene-D-mannitol.

The evidence in the case of the monoacetal of D-mannitol does not permit an unambiguous assignment of structure and indicates a 1,3 or a 2,3 ring. However, the 2,3 ring in D-mannitol represents the extremely unfavourable cC configuration and is for this reason excluded.

It can be seen that these structures conform to the predictions of the rules for acetal formation.

2,4-O-ferrocenylidene-D-glucitol (XXV).

The solid monoaldehyde (XXIV) was allowed to react with D-glucitol in dimethylformamide (or ethanol) at 70°, in the presence of a phosphorous pentoxide catalyst, to give a low yield of 2,4-O-ferrocenylidene-D-glucitol [X] (m.p. 170-173°, $[\alpha]_D^{24}$ - 70°).

When the reaction was carried out in a benzene/dimethylformamide (1:1, v/v) solvent at 110-120° and the products fractionated on alumina using ethanol as an eluant, a second 2,4-O-ferrocenylidene-D-glucitol [Y] (m.p. 196-198°, $[\alpha]_D^{24}$ -95°) was produced. Both acetals were found to contain 1 mol. of ferrocenemonoaldehyde and D-glucitol.

Compound [Y] could be recrystallised from ethanol without change but crystallisation from boiling water (pH 6) produced compound [X]. Recrystallisation of [X] from ethanol produced no change.

Periodate oxidation studies showed that both compounds [X] and [Y]

consumed 1 mole. of periodate and formed 1 mole. of formaldehyde but no formic acid was produced. The rates of periodate uptake and formaldehyde liberation for compound [X] are shown in Fig.21. The values were obtained by comparing the relative uptakes of the acetal and its tetra-acetate as described earlier (p.19).

With both forms ([X] and [Y]) of the acetal it was found that the rate of periodate oxidation of the glycol groups was much greater than the oxidation of the ferrocene moiety. It was, therefore, possible to determine the absolute uptake of oxidant using the acetal without reference to its acetate. Curve C (Fig. 21) shows the uptake of periodate by the acetal [Y].

Acidic hydrolyses of the periodate-oxidised acetals [X] and [Y] yielded L-xylose which was characterised by paper chromatography and as the dimethylacetal dibenzylidene derivative (cf. Breddy and Jones[75]).

Acetal [X] when acetylated with acetic anhydride in pyridine gave a tetra-acetate (m.p.112-113°) which on deacetylation with sodium in methanol gave the monoacetal [X] (m.p. 169-172°).

Acetal [Y] when acetylated by the same method afforded a tetra-acetate (m.p. 110-111°) which was deacetylated to re-form acetal [Y] (m.p. 197-198°).

Gravimetric and infra-red analysis showed that acetal [X] was not a hydrate of acetal [Y] (water of crystallisation gives rise to a peak at 1645±5 cm.$^{-1}$)[76]. It is, therfore, possible that the two acetals are related as diastereoisomers or as polymorphic modifications.

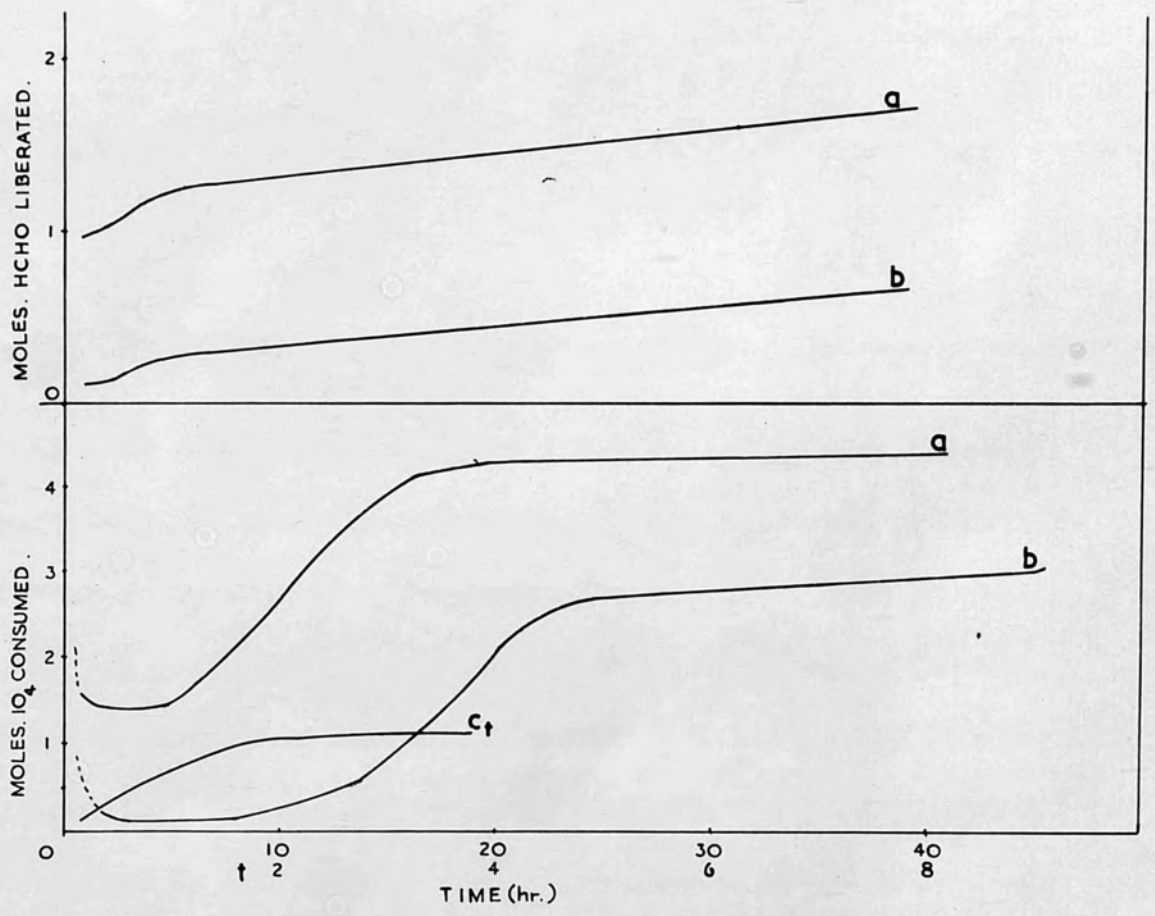
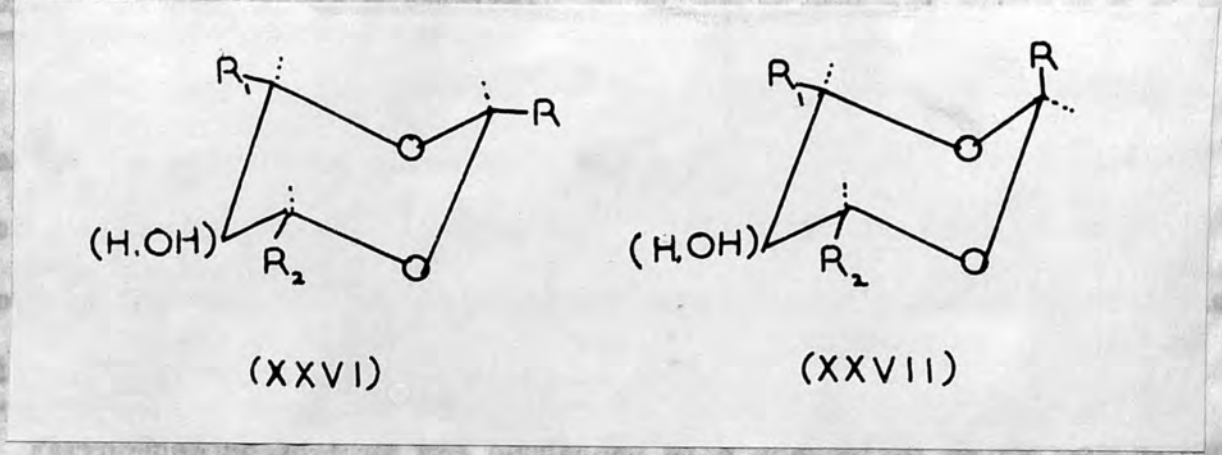


FIG. 21. Uptake of IO_4 and liberation of formaldehyde by a. 2,4-O-ferrocenylidene-D-glucitol [Y] and b. 1,3,5,6-tetra-O-acetyl-2,4-O-ferrocenylidene-D-glucitol.
c. uptake of IO_4^- by 2,4-O-ferrocenylidene-D-glucitol [X].

The condensation of aldehydes of general formula RCHO with polyols to yield five and six-membered cyclic acetals and the concomitant formation of a new asymmetric centre might be expected to give rise to diastereoisomers (XXVI and XXVII).



(XXVI) (XXVII)

If R is a particularly bulky group then repulsive forces between R and other substituents, R_1 and R_2, would undoubtedly favour an equatorial arrangement for R (XXVI).[73] In general only one such product is stable enough to be isolated. However, diastereoisomerism has been observed with 1,3-O-benzylidene-glyceritol[77,78] and the evidence suggests that the phenomenon is exhibited by the 3,4-O-benzylidene derivatives of methyl β-D-arabinopyranoside.[79]

Two 1,3:5,7-di-O-benzylidene derivatives of D-perseitol exist but in this case it has been suggested that this is due to polymorphism.[80] With the two forms of 2,4-O-ferrocenylidene-D-glucitol, the significant difference in specific optical rotation and the

conversion of [Y] to [X] in boiling water (pH 6) could be adduced in favour of diastereoisomerism, in which case [X] would appear the most stable form and, therfore, may have the ferrocenyl group in an equatorial position.

Di-O-ferrocenylidene-D-glucitol.

Chromatographic fractionation of the reaction products resulting after the extraction of the monoacetal (XXV) yielded a small quantity of a probable diacetal of glucitol. Yields of this product could not be improved and insufficient was obtained for complete characterisation.

1(?),3-O-ferrocenylidene-D-mannitol.(XXVIII)

Ferrocenemonoaldehyde was condensed with D-mannitol using phosphorous pentoxide as a catalyst and dimethylformamide as a solvent, to give a low yield of 1,3-O-ferrocenylidene-D-mannitol. The monoacetal (XXVIII) would not crystallise satisfactorily from water or ethanol and it was, therefore, further purified using a cellulose column with butanol/ethanol/water solvent and obtained as a fine yellow powder after freeze-drying from water. This product was chromatographically homogeneous in solvents A and B. Acetylation with acetic anhydride in pyridine gave a crystalline tetra-acetate. Deacetylation of this tetra-acetate and attempted crystallisation of the product from water yielded a gel.

The monoacetal (XXVIII) when oxidised with 0.01M potassium metaperiodate consumed 1.8 moles. of the oxidant and 0.7 moles. of formic acid were liberated. The relative uptakes of the acetal and

its tetra-acetate are shown in Fig.22. It was found that in the case of the two isomers of 2,4-O-ferrocenylidene-D-glucitol, the rate of glycol bond fission by periodate ion was considerably greater than the rate of oxidation of the ferrocene nucleus. With the monoacetal of D-mannitol (XVIII), however, the rates of oxidation of the ferrocenyl and the polyol moieties were comparable.

A preparative oxidation of (XXVIII) followed by hydrolysis of the product with Amberlite IR-120(H^+) resin yielded D-erythrose which was characterised by paper chromatography and paper ionophoresis. Reduction of the tetrose with potassium borohydride gave erythritol but an attempted benzoylation of this tetritol did not yield a crystalline product.

The low values obtained for the periodate uptake and formic acid liberation by the monoacetal (XXVIII) are almost certainly due to the presence of another isomeric acetal since traces of D-arabinose were detectable when the hydrolysed products of the periodate oxidation were analysed on paper chromatograms. This would imply the presence of a 2,4 cyclic acetal; acetals of this structure have not previously been recorded in the case of mannitol.

The results presented here do not permit an unequivocal assignment of a 1,3- structure to the monoacetal; the evidence being also compatible with 2,3-O-ferrocenylidene-D-mannitol.

An attempt to tosylate the monoacetal was unsuccessful. An infra-red examination of the product obtained by periodate oxidation

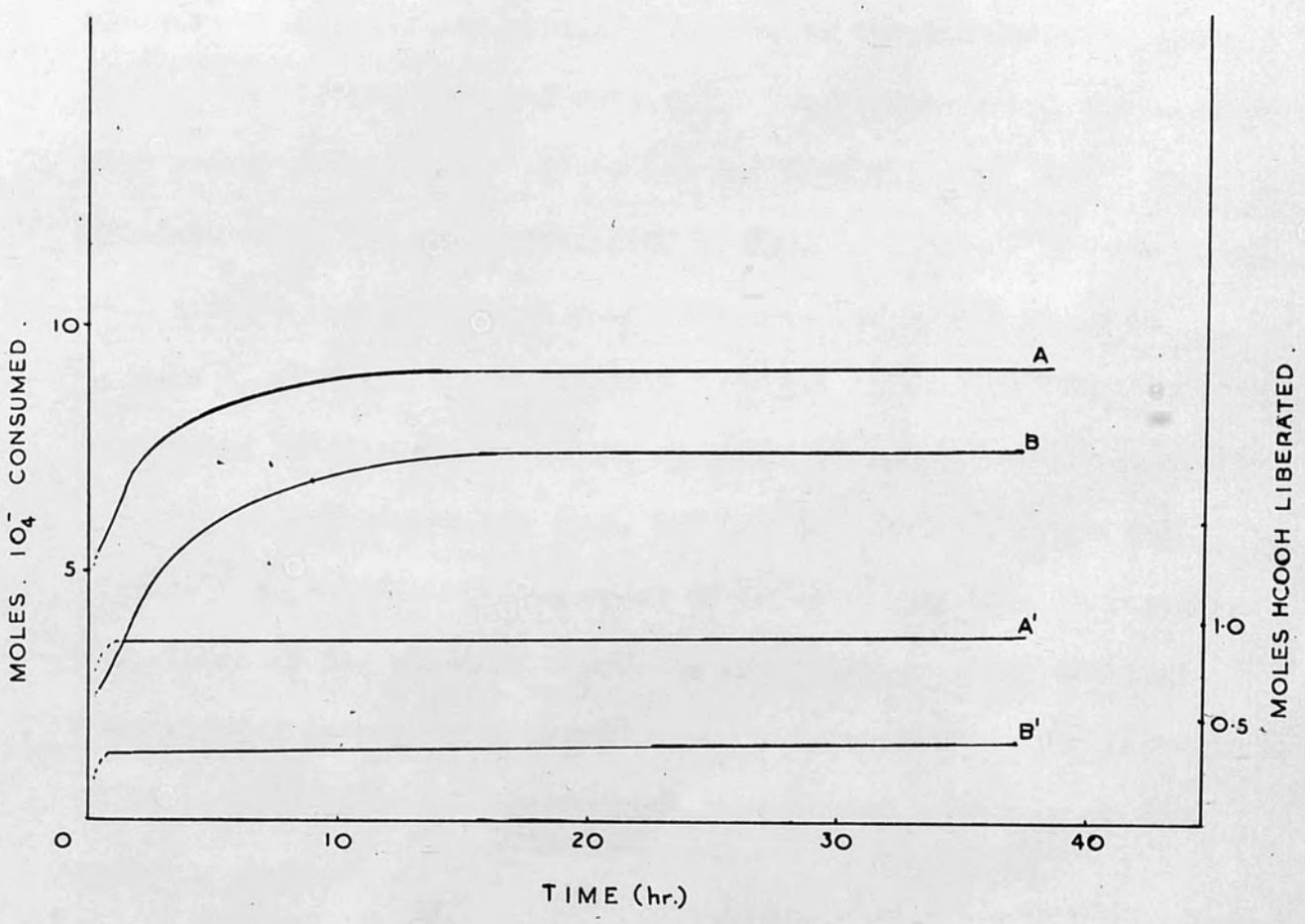


FIG. 22. Uptake of IO_4^- (A,B) and liberation of formic acid (A',B') by 1,3-O-ferrocenylidene-D-mannitol (A curves) and 2,4,5,6-tetra-O-acetyl-1,3-O-ferrocenylidene-D-mannitol (B curves)

of the acetal (XXVIII) (i.e., a monoacetal of D-erythrose) failed to show conclusively that no primary hydroxyl groups were present.

1,3:4,6-di-O-ferrocenylidene-D-mannitol (XXXI).

The diacetal, which was also produced during the preparation of the monoacetal (XXVIII) (p.60), was isolated as its acetate. On hydrolysis this acetate yielded 2 moles. of ferrocenemonoaldehyde, 2 moles. of acetic acid and mannitol. On deacetylating the diacetate with sodium methoxide in chloroform the free diacetal (XXXI) was obtained which was then methylated by Kuhn's method. The resulting methyl ether was hydrolysed with Amberlite IR-120(H^+) resin in aqueous alcohol and the methylated mannitol derivative then acetylated to give a crystalline specimen of 1,3,4,6-tetra-O-acetyl-2,5-di-O-methyl-D-mannitol (m.p. 105-107°). Bourne, Bruce and Wiggins[81] reported a melting point of 107-108° for this derivative. Unfortunately the physical constants for other possible dimethyl tetra-acetyl isomers of D-mannitol are not recorded in the literature so it is impossible to fix the structure incontrovertibly on this evidence alone.

The condensation of acetylferrocene and D-mannitol.

No ketal condensations have been reported in the literature for acetylferrocene. In this present study acetylferrocene was reacted with D-mannitol under a wide variety of conditions but in no case could the desired ketals be detected. It is probable that such ketals would be extremely acid labile and, therefore, very difficult to prepare by the recognised general methods.

Hydrolysis of ferrocenylidene acetals.

It was observed that 2,4-O-ferrocenylidene-D-glucitol and 1,3-O-ferrocenylidene-D-mannitol were hydrolysed rapidly by 0.01N hydrochloric acid at 25°. Under similar conditions 2,4-O-benzylidene-D-glucitol was only very slowly attacked.

The mechanism of the hydrolysis of ketals and acetals has been extensively investigated.[82,83] The kinetic evidence is consistent with a unimolecular mechanism (Fig.23).

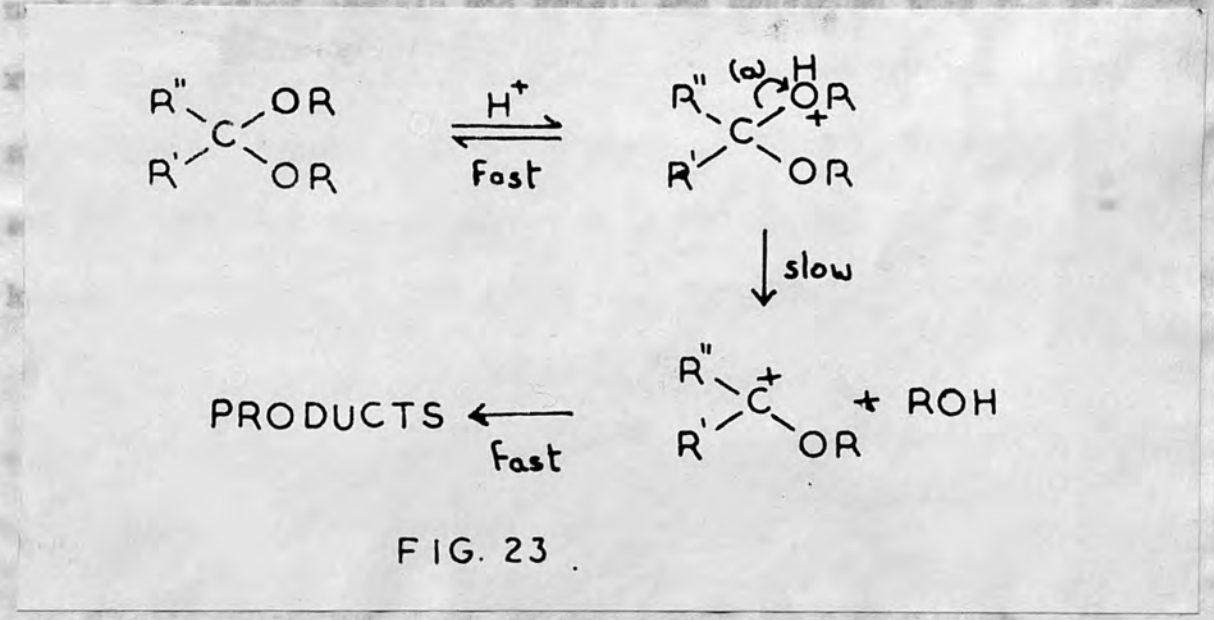


FIG. 23

The rate is determined by the heterolysis (a) of the protonated intermediate. In support of this proposed mechanism, McIntyre and Long[84] found, that the rate of hydrolysis (log k_1) of methylal was proportional to the acidity as measured by the Hammett acidity function.

Several workers[51,52,82] have found that alkyl and aryl groups capable of racemisation or rearrangement can be recovered intact after

hydrolysis thus demonstrating bond fission of the type (a).

Finally, the constitutional influences of R' and R'' groups are readily understood in terms of the mechanism shown in Fig.23 where the carbon atom to which these groups are bound becomes a carbonium ion. Thus it is found that where R' and R'' have electron donating properties the rate of hydrolysis is increased, but with groups with electron withdrawing properties the rate is decreased. Kreevoy and Taft[85] investigated the rates of hydrolysis of a large number of diethyl acetals and ketals and concluded that the relative rates were governed almost entirely by the polar effects of the substituents. Some results are given in Table 5, where R' and R'' represent the substituents in the diethyl acetal or ketal, $R'R''C(OEt)_2$. The rates (k_2) were measured in acetic acid at 25°.

Table 5.

R'	R''	k_2 ($l.mole^{-1}sec.^{-1}$)
H	H	4.13×10^{-5}
CH_3	H	0.248
CH_3	CH_3	7.52×10^{2}
t-C_4H_9	H	0.188
C_6H_5	H	7.07
$C_6H_5CH_2$	H	8.7×10^{-3}
$ClCH_2$	H	1.03×10^{-5}
$HOCH_2$	H	8.47×10^{-4}
$CH_3CH{=}CH$	H	2.98×10^{2}

The small rate difference in changing from a methyl to a t-butyl substituent was ascribed to the lack of hyperconjugation in the latter being compensated by an increased inductive effect.

Studies have also been extended to the cyclic acetals[86,87] and in the case of simple 1,3-dioxan and 1,3-dioxolan derivatives the evidence suggested an identical hydrolytic mechanism to that described for the acyclic compounds (Fig.24).

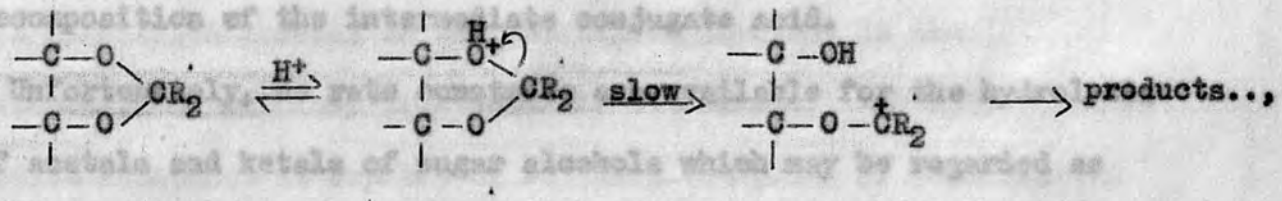


Fig 24.

Recently Kreevoy, Morgan and Taft[88] have considered the steric and ring size effects on the rates of hydrolysis of cyclic ketals of a slightly different type (Fig.25). These workers discussed their results in terms of three main factors which control the rate of breakdown of the protonated intermediate:-

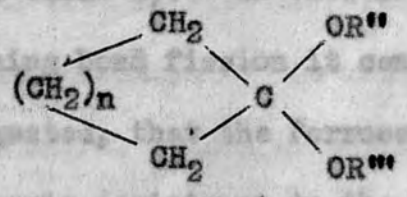


Fig. 25.

(a) Conformational influences in the initial and transition state i.e., whether the formation of the carbonium ion, in which the central carbon atom is planar will result in increased or decreased ring strain or unfavourable intramolecular repulsions.

(b) Steric acceleration from bulky R'' and R''' groups.

(c) The polar assistance to the heterolysis from R'' and R'''.

However, when considering the constitutional effects of R'' and R''' in the same ring system only factors (b) and (c) will be important.

The rates of hydrolysis at 25° for the cyclic acetals of pentaerythritol with formaldehyde, acetaldehyde and acetone are in the ratio 1 : 6,000 : 10,000,000 respectively.[89] These facts are understood on the basis of a +I influence on the unimolecular decomposition of the intermediate conjugate acid.

Unfortunately, no rate constants are available for the hydrolysis of acetals and ketals of sugar alcohols which may be regarded as substituted 1,3-dioxan and 1,3-dioxolan systems. However, for acetals spanning the same hydroxyl groups of the same hexitol, we may state that the rates of hydrolysis are in the following order :- ferrocenylidene > benzylidene > methylene. The rates for benzylidene and methylene correspond, as expected, to the rates for the equivalent diethyl acetals (see Table 5). In view of the polar effects assisting the rate-determining bond fission it can be concluded, as earlier evidence suggested, that the ferrocenyl substituent gives a strong electromeric assistance to the bond fission (Fig.26).

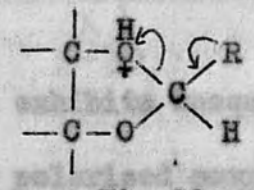


Fig 26.

Optical Rotatory Dispersion with Ferrocene Derivatives.

The phenomenon of optical rotatory dispersion [90, 91] has been known for the past one hundred and fifty years but only in the last ten years, with the development and improvement of the photoelectric spectrophotometer, has the organic chemist begun to regard rotatory dispersion as an important weapon in his analytical armoury.

A simplified account of the principles involved in the phenomenon of rotatory dispersion is as follows. An optically active substance is capable of rotating the plane of polarisation of a beam of linearly polarised light. Linearly polarised light may be regarded as consisting of left and right circularly polarised components. In an optically active material these are transmitted with unequal velocities producing, on recombination, a phase difference and thus a rotation of the plane of polarisation. Since light of different wavelengths travels with different velocities through any given medium the rotation produced by the medium will vary with the wavelength of the incident light. This effect alone in compounds possessing only one asymmetric centre will produce a plain dispersion curve (Fig.27).

If, in addition, the medium exhibits unequal absorption towards the left and the right circularly polarised components then the resulting beam will be elliptically polarised. An optically active centre containing a chromophore may thus give rise to anomalous dispersion

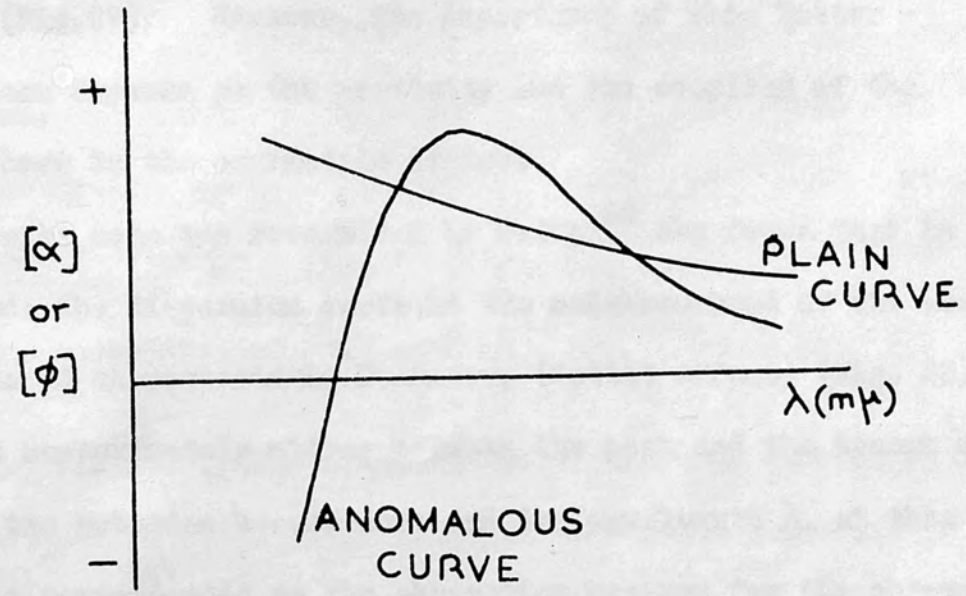

+
[α]
or
[φ]
−
PLAIN
CURVE
λ(mμ)
ANOMALOUS
CURVE

FIG. 26

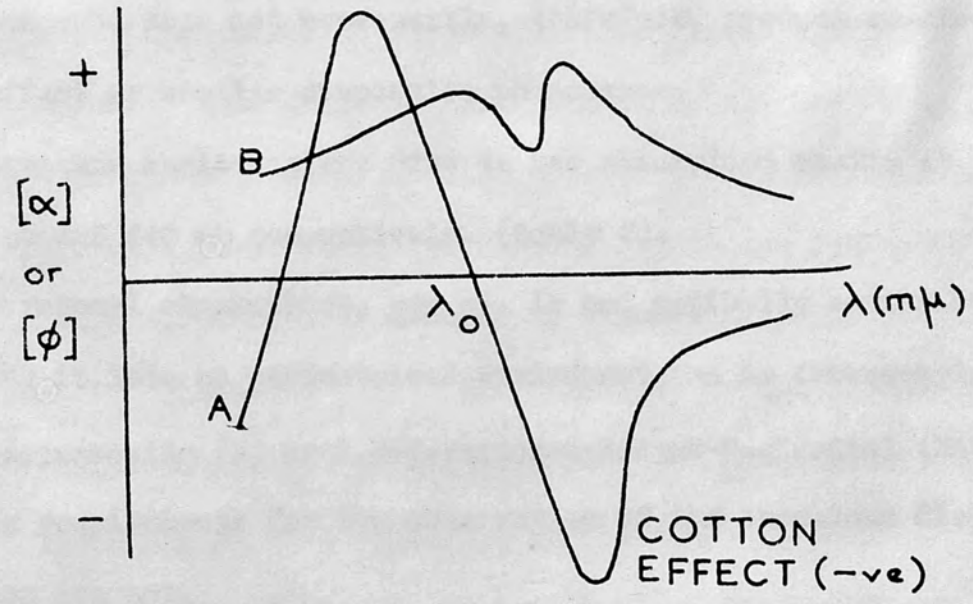

+
[α]
or
[φ]
−
B
A
λo
λ(mμ)
COTTON
EFFECT (-ve)

FIG. 27

curves (Fig.27). However, the importance of this latter phenomenon depends on the proximity and the coupling of the chromophore to the asymmetric centre.

A special case was recognised by Cotton[92] who found that in some compounds the dispersion curve in the neighbourhood of the absorbing band showed characteristic features. (Cotton effect) (Fig. 28). At a point approximately midway between the peak and the trough of the curve, the rotation became zero and the wavelength λ_0 at this position corresponded to the absorption maximum for the chromophore. These particular features are only found when the electronic system producing the absorption band is the only significant contributor to the optical activity.

In cases where this is not so the rotation will not fall or rise to zero although the same type of inflection may occur (curve B Fig. 28). Each absorption maximum in the ultra violet spectrum of an optically active compound does not necessarily, therefore, produce an associated Cotton effect or similar dispersion phenomenon.

The ferrocene nucleus gives rise to two absorption maxima at ca. 320 mμ and 440 mμ respectively. (table 6).

The ferrocenyl chromophore, per se, is not optically active but by introducing it into an asymmetrical environment, as in ferrocenylmethyl β-D-glucopyranoside (V) or 2,4-O-ferrocenylidene-D-glucitol (XXV), the basic requirements for the observation of the anomalous dispersion phenomenon are met.

	Solvent	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Ferrocene.	Hexane	325	50	440	92
Hydroxymethylferrocene.	Hexane	325	58	440	97
Ferrocenylmethyl β-D-glucopyranoside.	95% ethanol	322	78	439	104
2,4-O-ferrocenylidene-D-glucitol.	95% ethanol	323	66	438	104

Table 6. U.V. absorption maxima of some ferrocene derivatives.

The optical rotatory dispersion curves for the glucoside (V) and the monoacetal (XXV) are shown together with the ultra-violet absorption spectra (Fig. 29). It can be seen that the ferrocene chromophore introduces only low amplitude Cotton effects on the dispersion curve of the asymmetric centre. The absorption band at 440 mμ is apparently inactive while the band at 320 mμ gives rise to small inflections on the absorption curve.

The complex nature of curve A probably means that ferrocene has other absorption bands in the appropriate region (i.e. 300-400 mμ) of the spectrum. This could be investigated by the use of different solvents and equipment with greater powers of resolution.

It is surprising that the acetal (XXV) in which the ferrocene is joined directly to an asymmetric centre, shows smaller dispersion effects than the glucoside (V). The proximity of the chromophore is not the only factor controlling this phenomenon, however; the nature of the group is also important with respect to the perturbational forces it directs on to the electron systems at the asymmetric centre.

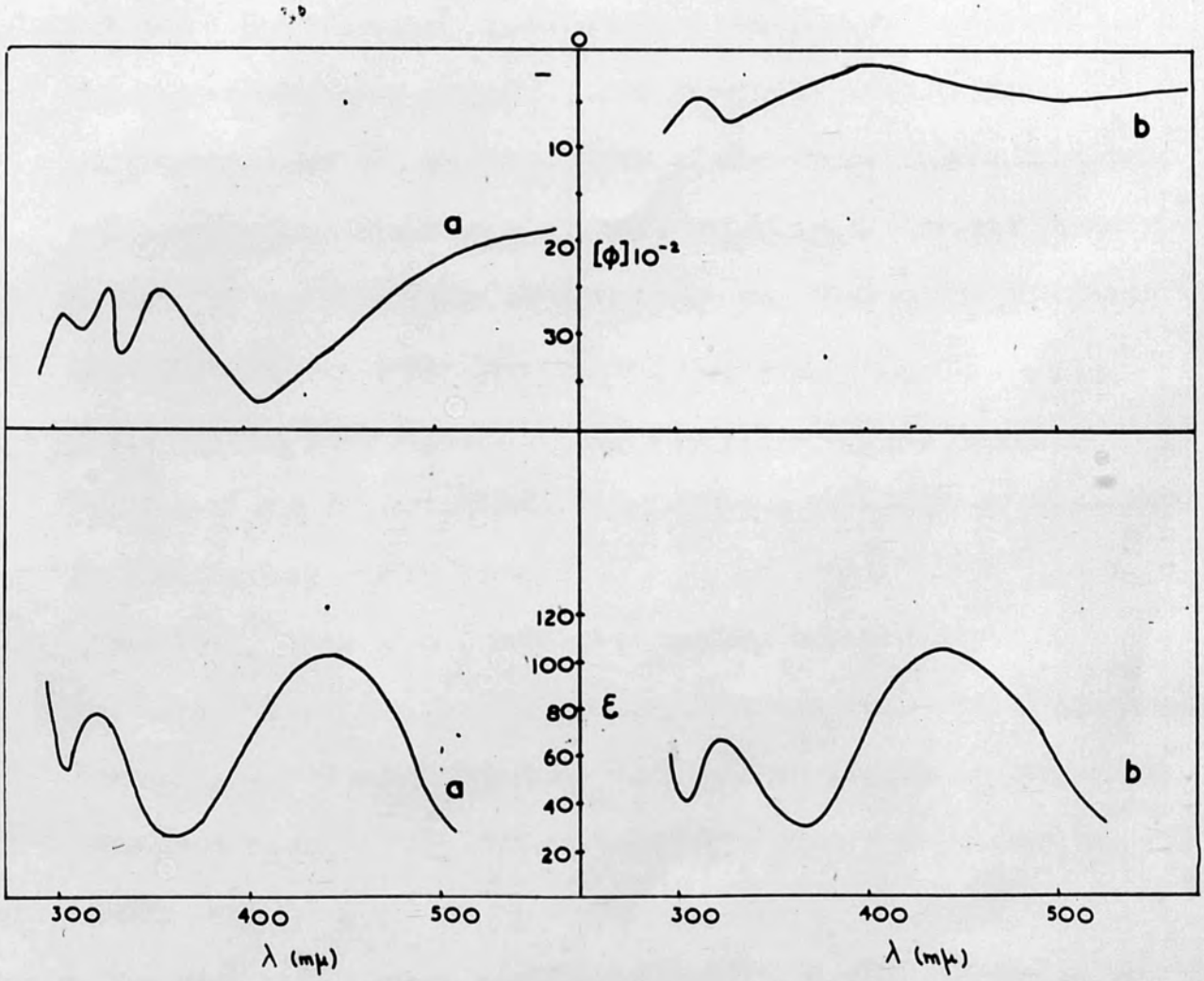


FIG. 29. U.V. absorption spectra (in 95% ethanol) and rotatory dispersion (in methanol) curves for : (a) ferrocenylmethyl β-D-glucopyranoside. (b) 2,4-O-ferrocenylidene-D-glucitol.

Paper Chromatography of Ferrocene Derivatives.

The initial investigations were designed to find a suitable paper chromatographic technique for sugar acetates and the adopted method was later found to be applicable to ferrocene derivatives.

Micheel and Schweppe[93] had described a method for separating sugar acetates using reversed phase paper chromatography on acetylated paper but the resolution of the monosaccharide acetates was poor. Reichstein et al.[94] also described a reversed phase method for the separation of some mono- and di-acetates but their method was not suitable for polyacetates.

The systems investigated during this present study employed 'silicone' and 'vaseline' coated papers and a wide range of solvents. No satisfactory system was found.

Wickberg,[95] soon after, published another method for sugar acetates using a two phase organic solvent system. Dimethylsulphoxide, formamide and dimethylformamide were used as stationary phases and petroleum ether, diethyl ether, isopropyl ether and benzene as mobile phases.

The most satisfactory results were obtained with dimethylsulphoxide as the stationary phase.

It was then discovered that these solvent systems could be employed successfully for the chromatographic resolution of some ferrocene derivatives.[96] The chromatographic behaviour of some ferrocene derivatives, using dimethylsulphoxide as the stationary

phase and petroleum ether (60-80°) saturated with dimethylsulphoxide as the mobile phase, is shown in Table 7 and Fig.30.

Ferrocene compounds are coloured and therefore readily located on paper chromatograms but they are more easily identified as dark red spots by spraying the chromatogram with a solution of potassium thiocyanate (5% w/v in N-HCl) or, alternatively, aqueous sodium periodate (1% w/v) followed by potassium thiocyanate (cf. Goldberg).[97]

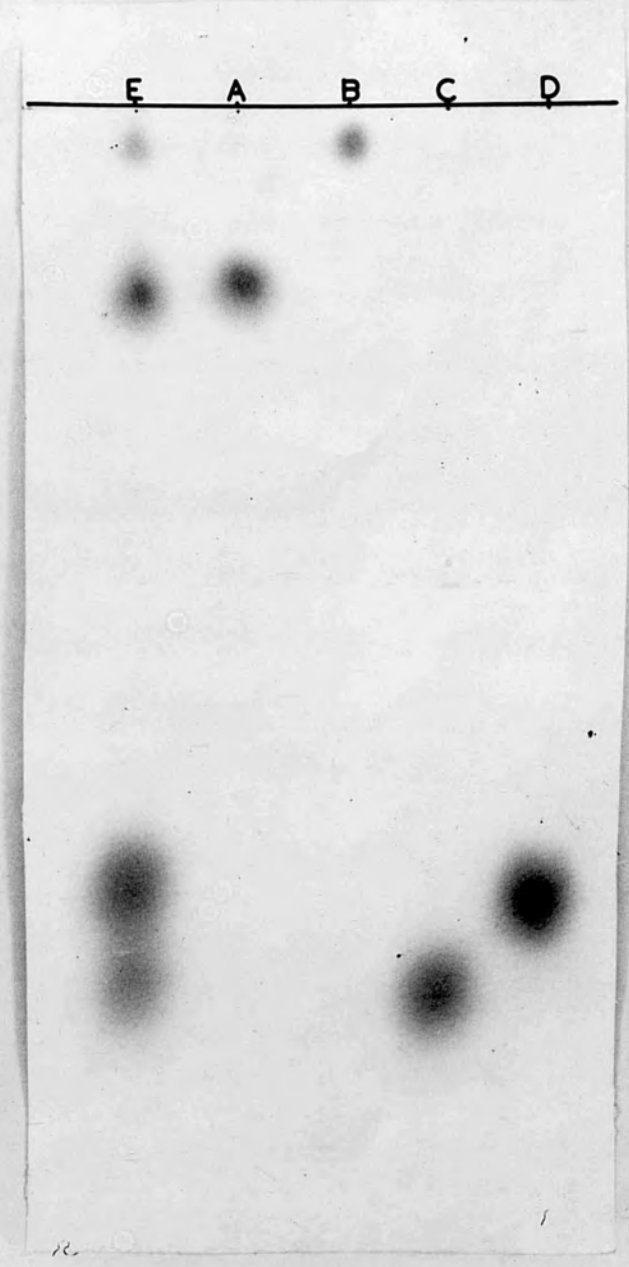
The action of the aqueous acidic solution of potassium thiocyanate alone as a spray reagent could possibly be due to the oxidation of the CNS^- anion (by photo-oxidation or atmospheric oxygen) to thiocyanogen which in turn could react with the ferrocene nucleus liberating the iron and producing red ferric thiocyanate.

Table 7.

Solvent system :- Dimethylsulphoxide/ petroleum ether (60-80°).

Compound	R_F
Ferrocenemonocarboxylic acid	0.05
Hydroxymethylferrocene	0.19
Hydroxyethylferrocene	0.28
Acetylferrocene	0.59
Ferrocenemonoaldehyde	0.62
Ethylferrocoate	0.94
Ferrocene	0.98

FIG. 30. PAPER CHROMATOGRAPHY OF FERROCENE DERIVATIVES.



A. Hydroxymethylferrocene.
B. Ferrocenecarboxylic acid.
C. Acetylferrocene.
D. Ferrocenemonoaldehyde.
E. Mixture of A,B,C,D.

EXPERIMENTAL

General Methods.

All melting points are uncorrected. Boiling points are quoted as vapour temperatures except where otherwise stated.

The volumetric solutions, iodine and sodium hydroxide were standardised against sodium arsenite and potassium hydrogen phthalate respectively. The methods for the determination of periodate, formaldehyde and formic acid were checked against standard substances (e.g. D-glucitol).

Ferrocene was prepared initially by the Grignard procedure[1,98] but finally as reported in Organic Syntheses[99].

The ferrocene derivatives used were synthesised according to the methods described in the following references.

Compound.	Reference.
(Ferrocenylmethyl)trimethylammonium iodide	100,101.
Hydroxymethylferrocene	100,42.
Acetylferrocene	87.
Ferrocenylacetic acid	102.
Hydroxyethylferrocene	102.
Ferrocenecarboxaldehyde	97 (see p.98)
Ferrocenemonocarboxylic acid	105.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide was prepared as described in Organic Syntheses[104].

The alumina used was Spence Type 'H'. Neutral alumina was prepared according to Lederer and Lederer[105].

General Methods.

All melting points are uncorrected. Boiling points are quoted as vapour temperatures except where otherwise stated.

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Ferrocene was prepared initially by the Grignard procedures[I,98] but finally as reported in Organic Syntheses[99].

The ferrocene derivatives used were synthesised according to the methods described in the following references.

Compound.	Reference.
(Ferrocenylmethyl)trimethylammonium iodide	I00,I0I.
Hydroxymethylferrocene	I00,42.
Acetylferrocene	37.
Ferrocenylacetic acid	I02.
Hydroxyethylferrocene	I02.
Ferrocenemonoaldehyde	37 (see p.98)
Ferrocenemonocarboxylic acid	I03.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide was prepared as described in Organic Syntheses[I04].

The alumina used was Spence's Type 'H'. Neutral alumina was prepared according to Lederer and Lederer[I05].

Paper Chromatography.

Solvent.	Reference
A. Butanol-ethanol-water (4:I:5, v/v). Top layer alternatively as single phase solvent;	I06
Butanol-ethanol-water (40:II:I9,v/v)	I07
B. Ethyl acetate-acetic acid-water (9:2:2,v/v)	I06
C. Phenol saturated with water (800:30,w/v). Bottom layer.	I08
D. Butanol-benzene-pyridine-water (5:I:3:2,v/v).	I09
E. Two phase solvent.	96

Whatman No. 3 paper was dipped twice in a solution of dimethylsulphoxide in benzene (20%,v/v) and the benzene removed by heating at 80° for ca. 30 sec. The mixtures for analysis were then applied to the paper chromatogram which was developed with petroleum ether (60-80°) saturated with dimethylsulphoxide. The rate of movement was rapid (20 cm./hr.) and sufficient resolution was obtained in most cases within 2 hr.

Ionophoresis.

I_1. Sodium borate buffer (0.I$\underline{M}$, pH I0). 2 hr. at 2000-3000 volts. II0

I_2. Ammonium molybdate buffer. (0.008$\underline{M}$, pH 5). I hr. at 2000 volts. III

Ferrocenylmethyl 2,3,4,6- tetra-O-acetyl-β-D-glucopyranoside (IV).

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (4.I g.) in dry, freshly distilled methylene chloride (30 ml.) was added slowly to a mixture of hydroxymethylferrocene (3.3 g.), silver oxide (9.2 g.) and calcium sulphate (5.0 g.) in methylene chloride (30 ml.). After shaking for I8 hr. the mixture was filtered, evaporated to leave a viscous residue which was taken up in hot methanol. On cooling this gave a yellow-orange precipitate which on recrystallisation from a benzene-hexane mixture afforded orange crystals of the acetate (IV). (3.0 g.), m.p. I83-I85°, $[\alpha]_D^{24}$ -II.7° (c I.0 in chloroform), (Found : C, 55.0; H, 5.8; Fe, I0.0; Acetyl, 3I.3. $C_{25}H_{30}O_{I0}Fe$ requires C, 55.0; H, 5.5; Fe, I0.2; Acetyl, 3I.5%).

Bis(ferrocenylmethyl)ether.

Fractionation on neutral alumina[I05] of part of the methanolic filtrate (from the above reaction after precipitation of the acetate (IV)) using benzene-hexane (I00:I v/v.) as the eluant yielded hydroxymethylferrocene and yellow crystals of bis(ferrocenylmethyl)ether (from 95% ethanol), m.p. I30-I32°, (Found : C, 63,3; H, 5.47. Calculated for $C_{I7}H_{24}OFe$; C, 63.8; H, 5.35/). (lit.[37] I29-I30°).

Ferrocenylmethyl β-D-glucopyranoside (V).

The acetate (IV) was dissolved in chloroform and deacetylated with 0.2N-NaOMe in methanol at room temperature for 24 hr. The water soluble fraction was freeze-dried and after one recrystallis-

ation from water gave yellow plates of ferrocenylmethyl β-D-glucopyranoside monohydrate (V), m.p. 135-136°, $[\alpha]_D^{22}$ -37.7° (c 1.0 in water), (Found : C, 51.8; H, 6.29; Fe, 14.1. $C_{17}H_{24}O_7Fe$ requires C, 51.5; H, 6.11; Fe, 14.1%).

Fischer Method. - Ferrocenylmethyl β-D-glucopyranoside (V).

Hydroxymethylferrocene (0.1 g.), D-glucose (0.05 g.) and p-toluenesulphonic acid (5 mg.) in dimethylformamide (5 ml.) were heated for 6 hr. at 80-90°. Fractionation of the reaction mixture by chromatography on Whatman No.3 paper using solvent A yielded a small specimen of the glucoside (V), m.p. 133-136°.

An attempt was made to fractionate the reaction mixture after neutralisation with sodium methoxide on acid-washed Celite 116, using water-saturated butan-1-ol as eluant. This procedure would not separate hydroxymethylferrocene and the glucoside but effected a good resolution of glucose and the two ferrocene products.

A synthesis of the glucoside was attempted by substituting Amberlite IR-120(H^+) resin for p-toluenesulphonic acid on the above described preparation. The method was, however, unsuccessful.

Hydrolysis of the Glucoside (V).

(a) With Amberlite IR-120(H^+) resin.

The glucoside (V)(50 mg.), in ethanol, was shaken with the resin for 2 hr. at 80-90°. The resulting glucose and hydroxymethylferrocene were identified by paper chromatography using solvents A and B

(b) With 0.1N-HCl.

The glucoside (V)(50 mg.) in water (9 ml.) was shaken at room temperature with N-HCl (1 ml.) for 1 hr. The solution was extracted with benzene and the extract was chromatographed on alumina using benzene as an eluant. This gave hydroxymethylferrocene, m.p. 75-76° (after one recrystallisation from hexane) and bis(ferrocenylmethyl) ether, m.p. 130-131° (after one recrystallisation from 95% ethanol) in approximately equal quantities.

(c) With emulsin.

Emulsin was prepared as described by Sumner and Somers.[117] The glucoside (V) was incubated with emulsin in water (3 ml.) at 30° for 18 hr. and the products, glucose and hydroxymethylferrocene, characterised by paper chromatography using solvent A.

Methylation of the Glucoside (V).

Silver oxide (0.5 g.) was added over a period of 30 min. to a solution of the glucoside (0.2 g.) and methyl iodide (1 ml.) in dimethylformamide (5 ml.). The mixture was shaken for 18 hr.. It was then centrifuged and the precipitate washed with dimethylformamide/chloroform (1:1, v/v). The combined extracts were mixed with water (30 ml.) and potassium cyanide (0.5g.) and then extracted three times with chloroform (10 ml.) The chloroform extracts were washed with water and dried over magnesium sulphate. After evaporation of the chloroform, the methylation procedure was repeated to give a yellow syrup (0.1 g.). This was mixed with N-HCl (10 ml.) and steam passed through the solution for 30 min.

After cooling, hydroxymethylferrocene was removed from the hydrolysate by extraction with ether. The hydrolysate was then saturated with sodium sulphate and extracted with chloroform (5x20 ml.). The chloroform extract was dried over magnesium sulphate, decolourised with charcoal and evaporated. The resulting colourless syrup was taken up in petroleum ether (40-60°) - ether, (95.5:0.5,v/v) and on standing this solution gave crystals of 2,3,4,6-tetra-O-methyl-glucose (ca. 40 mg.), m.p. 96-97°(lit.[118] m.p. 98°).

The methylated sugar was also characterised by paper chromatography in solvents A and B.

Estimation of Acetyl Groups in the Acetate (IV).

A ca. 0.05N-HCl solution was standardised with ca. 0.05N-NaOH solution, which itself was standardised against potassium hydrogen phthalate. The sodium hydroxide solution was prepared by dissolving 'Analar' sodium hydroxide (5 g.) in water (5 ml.), leaving 24 hr., decanting and centrifuging. This solution (1 ml.) was added to 'boiled out' water (500 ml.).

Samples of the acetate (5-10 mg.) were weighed out and dissolved in 95% ethanol (4 ml.). 0.05N-NaOH (4 ml.) was added and the mixture left 15 hr. The excess alkali was then titrated with the acid using a methyl orange indicator.

The method was checked with a standard sample of octa-acetyl sucrose.

Periodate Oxidations.

(a) Determination of periodate uptake.

The samples (5-20 mg.) were dissolved in dioxan or ethanol (20 ml.) and the solution was diluted to 50 ml. by addition of 0.02M potassium metaperiodate.

The potassium periodate solution was adjusted prior to mixing so as to give the combined dioxan/periodate mixture an initial pH of 7.

Aliquots (3 ml.) were removed at intervals and saturated sodium bicarbonate (5 ml.), 0.01M sodium arsenite (5 ml.), and 20% potassium iodide (1 ml.) in saturated sodium bicarbonate were added rapidly. After standing 1 hr., a 0.1% sodium starch glycollate solution (0.5 ml.) was added and the excess sodium arsenite titrated with 0.01M iodine solution.

(b) Determination of formic acid.

Aliquots (5 ml.) were removed from the reaction mixture. After addition of ethylene glycol (0.1 ml.) and standing 10 min., the solution was titrated potentiometrically to pH 6.8 with 0.01N sodium hydroxide.

(c) Determination of formaldehyde.

Aliquots (1 ml.) were removed at intervals and the excess periodate destroyed with 0.04M sodium sulphite (1 ml.). Chromotropic acid (9 ml.) was added to 1 ml. of the above solution and then heated on a boiling water bath for 30 min. After cooling, half-saturated thiourea (1 ml.) was added and the absorption at 570mμ was measured using 60% (v/v) sulphuric acid as the blank.

Table 8. Oxidation of ferrocenylmethyl β-D-glucopyranoside (V) and ferrocenylmethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (IV) with 0.01M potassium metaperiodate at 25°.

	Tetra-acetate(IV)		Glucoside(V)	
Time (hr.)	IO_4^- uptake (ml. of I_2)	HCHO liberation (scale rdg.)[a]	IO_4^- uptake (ml. of I_2)	HCHO liberation (scale rdg.)[a]
1.00	1.23	13	1.28	13
2.25	1.24	15	1.37	16
4.50	1.40	12	1.72	13
7.25	1.61	22	2.00	26
9.00	1.65	20	2.02	21
11.00	1.65	-	2.03	-

Difference in titre = 0.38 ml. I_2 = 2.05 mole. IO_4^-

a Spectrophotometer transmission scale reading.

A standard curve was obtained using sorbitol (D-glucitol) as a formaldehyde source.

Rate Measurements.

The acidic hydrolysis at 25° was followed by optical rotation measurements. The glucoside (V) (ca. 0.25 g.) was dissolved in water (25 ml.) and N-H_2SO_4 (1.25 ml.) added. Aliquots (5 ml.) were removed at intervals, neutralised with 0.05N ammonium hydroxide, centrifuged (to remove precipitated ferrocene derivatives) and their optical rotations measured.

The volume of ammonia required for neutralisation and the accuracy of the method was determined in preliminary experiments. The procedure was found to give deviations between theoretical and and experimental values of less than 4%.

The first order rate coefficients were calculated and a plot of time (min.) against $\log_{10}(a_t - a_\infty)$, where a_t is the rotation of the solution at time t and a_∞ rotation of the solution at the completion of the reaction, was constructed.

Table 9. 1st. Order Rate Constants for the Hydrolysis of the Glucoside (V) in 0.05N-H_2SO_4 at 25°.

[V] = 0.0218M.

Time (min.)	8.0	15.0	21.0	26.0	44.0
Rotation (a°)	-0.04	+0.05	+0.11	+0.13	+0.16
k_I (min.$^{-I}$)	0.088	0.083	0.088	0.084	*

[V] = 0.0236M.

Time (min.)	5.0	10.0	23.0	30.0	60.0
Rotation (α°)	-	+0.01	+0.13	+0.16	+0.18
k_1 ($min.^{-1}$)	-	0.095	0.089	0.087	*

* reaction over 90% complete.

Acidic Hydrolysis of the Glucoside (V) in ^{18}O-enriched water.

The isotopically enriched water used in these experiments contained an ca.0.8 atom % excess abundance. It was recovered in each experiment by freeze-drying.

The glucoside (V) (ca.0.4 g.) was dissolved in the isotopically enriched water (30 ml.). N sulphuric acid (1.5 ml.) was added and the reaction mixture left at room temperature for 45 min. The products were then separated between benzene and water.

The aqueous extract was shaken for 15 min. with barium carbonate, filtered, shaken for 15 min. with 'Biodemineralit' (CO_3^{--} form), filtered and freeze-dried. The residue was taken up in boiling methanol (1-2 ml.) and the cloudiness cleared by the addition of a few drops of water. After cooling, isopropanol (1-2 ml.) was added and on refrigerating overnight crystals of α-D-glucose, m.p. 149-152°, were deposited.

The benzene extract was washed with water and dried over potassium carbonate. Chromatography on alumina using benzene as eluant yielded samples of hydroxymethylferrocene (40 mg.), m.p. 74-76° (from hexane) and bis(ferrocenylmethyl) ether (50 mg.), m.p. 130-131° (from 95% ethanol).

Control Experiments.

(a) Hydroxymethylferrocene.

Hydroxymethylferrocene (0.4 g.) was shaken with ^{18}O-enriched water (20 ml.) containing N sulphuric acid (1.0 ml.) for 45 min. Extraction with benzene and chromatography on alumina, as before, yielded hydroxymethylferrocene (0.35 g.), m.p. 75-76°. No significant quantities of the ether could be detected.

(b) β-D-glucose.

β-D-glucose (0.4 g.) was dissolved in ^{18}O-enriched water (20 ml.) containing N sulphuric acid (1.0 ml.) and left at room temperature for 45 min. Treatment as described earlier gave crystals of α-D-glucose (0.2 g.), m.p. 148-149°.

Determination of ^{18}O in the samples.

The method was based on the procedure described by Oita and Conway[30]. The sample (10-20 mg.) was pyrolysed to carbon monoxide on platinised carbon at 900° and then oxidised to carbon dioxide by iodine pentoxide at 120°. The carbon dioxide was collected by freezing out in a liquid nitrogen trap. After allowing the collected sample of carbon dioxide to warm up to room temperature, it was analysed isotopically in a mass-spectrometer.

Apparatus.

The apparatus is shown diagrammatically in Figs.31 and 32. Furnace temperatures were measured by a platinum/platinum-13%rhodium thermocouple to ±10°.

The reducing valve on the nitrogen cylinder A was connected to the over-pressure valve unit B by thick rubber tubing. An orifice-type flowmeter C, calibrated for flow rates of 5-20 ml. per minute, was followed by a drying tube D containing soda-asbestos and Anhydrone (magnesium perchlorate). Connected to the three-way stopcock S_1 was the bypass E which was connected in turn at the other end to the three-way stopcock S_3. The silica pyrolysis tube of 11 mm. external diameter held the platinised carbon and was heated by the micro-combustion furnace H. The U-tube O contained soda-asbestos and Anhydrone. This was followed by the iodine pentoxide tube J, 22 cm. long, and heated by the furnace K to 120 ± 10°. The iodine absorber L, 22 cm. long, and 16 mm. external diameter, contained in order a plug of glass wool (5 mm.), a layer of sodium thiosulphate crystals (8 cm.) a further glass wool plug (5 mm.) and Anhydrone in the remaining space. A similar tube D, 22 cm. long, contained three consecutive thicknesses (6 cm.) of Anhydrone separated by glass wool plugs. The carbon dioxide trap M was surrounded by liquid air in a suitable Dewar flask N.

Procedure.

A steady slow flow of nitrogen (10 ml/min.) was passed through the apparatus. The furnaces H and K were set at 900° and 120° respectively. A sample (20 mg.) was weighed out in a micro-platinum boat and after reversing and increasing the flow of nitrogen through the pyrolysis tube, the sample-inlet joint F was disconnected and the sample boat

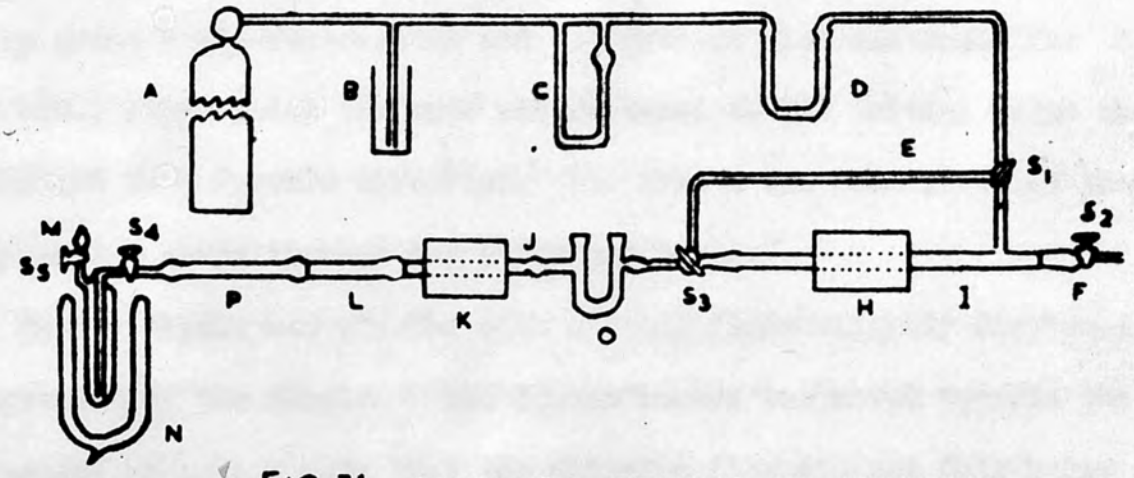

FIG.31 SCHEMATIC DRAWING OF APPARATUS.

A. Nitrogen source
B. Overpressure blow off
C. Orifice-type flowmeter
D. CO_2 and H_2O absorber
E. Bypass
F. Sample-inlet joint
H. Furnace
I. Pyrolysis tube
J. I_2O_5 tube
K. I_2O_5 heater
L. Iodine absorber
M. CO_2 trap
N. Vacuum flask
O. Soda-asbestos/anhydrone tube
P. Anhydrone guard tube
S_1-S_5 Stopcocks

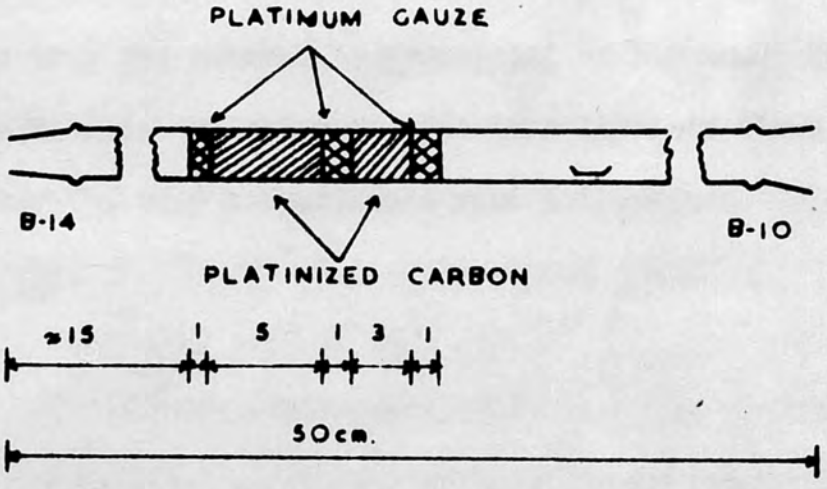

FIG.32 DETAILS OF PYROLYSIS TUBE.

placed about 8 cm. from the furnace by means of a platinum wire. The joint F was reconnected and the reverse flow continued for 5 min., after which the rate was restored to its initial value and changed to a forward direction. The trap M was connected and the apparatus swept through for a further 5 min.

The pyrolysis was started with a small flame slightly displaced upstream of the sample. The Bunsen burner was moved towards the furnace at such a rate that the nitrogen flow did not fall below 7 ml/min. The flame was then gradually increased to its maximum temperature. After keeping it directly under the sample for five minutes, it was moved slowly (2 cm/min.) towards the furnace. The burner was kept adjacent to the furnace for 5 min. The apparatus was then swept through for a further 45 min., after which stopcocks S_4 and S_5 were closed and the trap M removed. The nitrogen remaining in the trap was removed by evacuating to 2-3 mm. The sample of carbon dioxide collected was fed directly into the mass spectrometer after the trap had attained room temperature.

Ferrocenylethyl β-D-glucopyranoside (VI).

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (0.41 g.) in methylene chloride (2 ml.) was added to a mixture of hydroxyethylferrocene (0.33 g.), silver oxide (0.9 g.) and calcium sulphate (0.5 g.) in methylene chloride (3 ml.). After shaking for 24 hr., filtering and evaporating, a non-crystallising gummy residue was obtained. This was then treated with 0.1N sodium methoxide (1 ml.) in methanol for 2 hr. The yellow precipitate which formed was filtered off and after two recrystallisations from water gave yellow crystals of ferrocenylethyl β-D-glucopyranoside (VI), m.p. 174°, $[\alpha]_D^{23}$ - 29° (c. 0.3 in water), (Found : C, 55.3; H, 6.11. $C_{18}H_{24}O_6Fe$ requires C, 55.1; H, 6.16%).

Hydrolysis of the Glucoside (VI).

(a) With emulsin.

A solution of the glucoside (VI) (ca. 1%) in water was incubated with emulsin at 30°. The hydrolysate was separated between chloroform and water. Glucose and hydroxyethylferrocene were identified by paper chromatography in the solvents A and F respectively.

(b)(i) With 0.05N sulphuric acid at 25°.

The glucoside (VI) (20 mg.) was dissolved in water (5 ml.) and 0.1N sulphuric acid (5 ml.) added. Aliquots (1 ml.) were removed at intervals, neutralised with barium carbonate, centrifuged and samples (0.2 ml.) were fractionated by paper chromatography using solvent A. No glucose could be detected even after 12 hr.

(b)(ii) With 0.05N sulphuric acid at 70°.

The experiment was repeated as in (i). Increasing quantities of glucose were detectable with time.

Complex Formation between 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl Bromide and anhydrous Aluminium Chloride.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (0.5 g.) was added to a suspension of aluminium chloride (2.0 g.) in methylene chloride (30 ml.). The mixture was stirred under nitrogen for 1 hr. and then filtered into dilute hydrochloric acid (100 ml.). The layers were mixed thoroughly and separated. The organic layer was washed with water and the aqueous extracts were combined and the aluminium present estimated as alumina[120].

A control experiment was conducted by omitting the acetohalogeno sugar.

Attempted Syntheses of 2,3,4,6-Tetra-O-acetyl-glucosyl Ferrocene (XVIII).

(a) 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (4.1 g., 2 mol.), ferrocene (0.9 g., 1 mol.) and resublimed aluminium chloride (6.7 g., 10 mol.) were stirred and refluxed in carbon disulphide (80 ml.) for 7 hr.

After cooling, the mixture was poured into ice-water (200 ml.) and separated. The organic layer was washed with dilute alkali and the washings added to the water layer. The organic layer was dried over sodium sulphate. Evaporation and recrystallisation of the residue from isopropyl ether gave 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl

bromide (2.5 g.), m.p. 86-88°. Chromatography of the remaining mother liquors on alumina gave ferrocene (0.7 g.), m.p. 173-174°, and some other material (50 mg.) which could not be crystallised.

The aqueous extracts were neutralised, freeze-dried and extracted with boiling pyridine (3x50 ml.). After concentration of the pyridine extracts, acetic anhydride was added and the solution left overnight.

No crystalline products were extracted from the acetylation reaction.

(b) The complex [2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (1 mol.): aluminium chloride (4 mol.)] was preformed using 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (2.0 g.) and aluminium chloride (3.0 g.) in methylene chloride (30 ml.). This solution after stirring 1 hr. was allowed to stand 10 min. and then decanted into a dropping funnel from which it was added slowly to a mixture of ferrocene (5.0 g.) and aluminium chloride (0.7 g.) in methylene chloride (30 ml.). The reaction mixture was stirred and refluxed under nitrogen for 5 hr. After this time it was processed as in (a). None of the desired products was extracted.

(c) 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (2.0 g.) and aluminuim chloride (3.5 g.) were stirred in carbon disulphide (50 ml.) for 30 min. Ferrocene (5.0 g.) in carbon disulphide (20 ml.) were added and the mixture stirred and refluxed for 15 hr. The orange coloured alkali washings indicated some reaction had occurred but processing as in (a) yielded none of the desired product.

1-(Tetra-O-acetyl-glucosyl)cyclopenta-1,3-diene (XIX.A).

Ethylmagnesium bromide from ethyl bromide (18.0 g.) and magnesium turnings (4.0 g.) was prepared in ether (75 ml.). The ether was evaporated off and dry benzene (50 ml.) added. Freshly distilled cyclopentadiene (11.0 g.) in benzene (20 ml.) was added over 15 min. and the mixture stirred and refluxed under nitrogen at 60° for 6 hr.[121] The benzene was removed and ether (50 ml.) added followed by 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (4.1 g.) in ether (25 ml.) during 30 min. The mixture was stirred and refluxed for 4 hr.

It was then cooled, hydrolysed with cold dilute acetic acid (150 ml.) and separated. The ether layer was washed with dilute alkali and water and these washings then combined with the original aqueous layer. The combined aqueous extracts were neutralised, freeze-dried and acetylated [sodium acetate (5.0 g.), acetic anhydride (150 ml.) 2 hr. at 90°]. The hydrolysed acetylation mixture was extracted with ether and the ether extracts dried over sodium sulphate, decolourised with charcoal and evaporated to give a syrup (0.2 g.). This was taken up in isopropanol and on standing gave crystals of the tetra-acetate (XIX.A), which on recrystallisation from isopropanol had m.p. 120-122°, $[\alpha]_D^{24}$ -18.8° (c. 1.0 in chloroform), λ_{max} 247mμ (log ε 3.7), (Found : C, 57.2; H, 6.18; M, 392(Rast); Acetyl, 45.1. $C_{19}H_{24}O_9$ requires C, 57.5; H, 6.11; M, 396; Acetyl, 43.4%)

Maleic anhydride adduct.

The acetate (XlX.A) (0.1 g.) was reacted with maleic anhydride (0.03 g.) in benzene (3 ml.) overnight. Evaporation of the benzene and recrystallisation from a benzene-ethanol mixture yielded the adduct (80 mg.), m.p. 216°, (Found : C, 56.2; H, 5.25. $C_{23}H_{26}O_{12}$ requires C, 55.9; H, 5.30%).

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)cyclopenta-1,3-diene (XlX.B).

Ethylmagnesium bromide was prepared from ethyl bromide (18.0 g.) and magnesium turnings (4.0 g.) in ether (75 ml.). Freshly distilled cyclopentadiene (11.0 g.) in ether (25 ml.) was added over 15 min. and the mixture refluxed and stirred for 12 hr.[122] 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (7.0 g.) in ether (30 ml.) was added over 15 min. and the mixture refluxed and stirred a further 5 hr.

The reaction mixture was cooled and poured on to ice-cold dilute acetic acid (150 ml.) and worked up as described earlier. The freeze-dried product was extracted with pyridine (3x50 ml.) and acetylated by addition of acetic anhydride (40 ml.)-standing overnight. The dark coloured ether extracts from the hydrolysed acetylation mixture, after drying, and evaporating were decolourised with charcoal in ethanol. The yellow syrup (1.5 g.) remaining after evaporation of the ethanol produced crystals of the acetate (XlX.B) which after two recrystallisations from isopropanol had m.p. 126-128°, $[\alpha]_D^{21}$ -27°, (c. 0.6 in chloroform), λ_{max} 247mμ (log ε 3.8),

(Found : C, 57.8; H, 6.55. $C_{19}H_{24}O_9$ requires C, 57.5; H, 6.11%).

Maleic anhydride adduct.

The acetate (XIX.B) (0.1 g.) was reacted with maleic anhydride (0.03 g.) in benzene (3 ml.) overnight. Evaporation of the benzene and recrystallisation from benzene-hexane yielded the adduct (50 mg.), m.p. 216-217°, (Found : C, 56.2; H, 5.24. $C_{23}H_{26}O_{12}$ requires C, 55.9; H, 5.30%).

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl cyclopentane (XXI).

The acetate (XIX.B) (0.2 g.) was dissolved in ethyl acetate ('Analar', 10 ml.) and after addition of Adams catalyst (20 mg.), it was shaken at atmospheric pressure with hydrogen. No further absorption of hydrogen was observed after 2 hr. The uptake was equivalent to 1.9 double bonds.

The solution was filtered and the ethyl acetate evaporated off. The product was recrystallised from ethanol-water (3:2, v/v) to give white crystals of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl cyclopentane, m.p. 90-91°, $[\alpha]_D^{20}$ -15.4° (c 1.2 in methanol), (Found : C, 56.99; H, 7.04. $C_{19}H_{28}O_9$ requires C, 56.97; H, 7.03%) .

Oxidation of the tetra-acetate (XXI).

The tetra-acetate (XXI) (50 mg.) was added to a solution of potassium permanganate (0.2 g.) in water (5 ml.). 10% sodium hydroxide (0.1 ml.) was added and the solution was refluxed for 3 hr. After acidifying with 5N sulphuric acid, the solution was refluxed for 30 min. It was cooled, the permanganate colour discharged with saturated sodium

bisulphite and then extracted with ether. The ether extracts were washed with dilute alkali. The alkaline extracts were then acidified and extracted with ether. The ether extracts were dried over magnesium sulphate. Evaporation of the ether left an oily droplet which showed the same I.R. spectrum as a standard sample of cyclopentanecarboxylic acid.

Attempted Synthesis of 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Ferrocene from the Tetra-acetyl Cyclopentadiene Compound (XIX.B).

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)cyclopenta-1,3-diene (XIX.B) (1.4 g.) in ether (50 ml.) was added dropwise at room temperature to methyl-lithium (0.65 g.) in ether (40 ml.), prepared from lithium metal (0.55 g.) and methyl iodide (5.3 g.).

After stirring for 1 hr. the reaction mixture was cooled to 0° and ferric chloride (1.0 g.) in ether (20 ml.) added. The reaction was stirred overnight, the product poured on to iced dilute hydrochloric acid and then extracted with ether. The ether layer was extracted with dilute alkali and these washings added to the original aqueous extract. The combined aqueous extracts* were neutralised and freeze dried. The freeze-dried residues were extracted with pyridine and acetylated by addition of acetic anhydride.

No crystalline products were obtained. The aqueous extracts* and the products of the acetylation were investigated by U.V. and I.R. spectroscopy for characteristic ferrocene peaks but none were evident.

Preparation of Ferrocenemonoaldehyde.

Ferrocene (54 g.) and phosphorous oxychloride (59 g.) were heated to 50° under nitrogen. N-Methylformanilide (54 g.) was then added over 1 hr. with stirring and the mixture left overnight at room temperature. The viscous dark-red mass was poured on to ice (150 g.) and left 12 hr. with occasional stirring. The hydrolysed mixture was then extracted with chloroform. These extracts were washed with water and then evaporated to give a dark coloured residue which was taken up in hot ethanol (ca. 100 ml.). After boiling with charcoal to remove tars and filtering, the filtrate was condensed to give, after addition of water (20 ml.) and refrigerating for some hours, dark red crystals of ferrocenemonoaldehyde, m.p. 116-118° (lit.[37] 121°).

2,4-O-ferrocenylidene-D-glucitol [X].

D-glucitol (1.4 g.) and ferrocenemonoaldehyde (4.8 g.) in dimethylformamide (5 ml.) were stirred at 70° for 10 hr. with addition of phosphorous pentoxide (0.1 g.) as dessicant and catalyst. The reaction mixture was then neutralised with sodium methoxide and separated between chloroform and water. The aqueous extract was condensed and left at 10° overnight. Two recrystallisations of the product from water gave yellow needles of 2,4-O-ferrocenylidene-D-glucitol [X] ,(0.5 g.), m.p.* 170-173° , $[\alpha]_D^{21}$ -69.8° (c. 0.2 in water), (Found : C, 54.2; H, 6.22. $C_{17}H_{22}O_6Fe$ requires C, 55.9; H, 5.86%).

* The melting point was found to decrease according to the drying treatment given. The figures quoted were recorded after 10 hr. at 60° in vacuo.

1,3,5,6-Tetra-O-acetyl-2,4-O-ferrocenylidene-D-glucitol [X'].

The monoacetal [X] was acetylated with pyridine/acetic anhydride. Recrystallisation from ethanol afforded yellow crystals of the tetra-acetate [X'], m.p. 110-112°, (Found : C, 54.80; H, 5.75; Acetyl, 30.5. $C_{25}H_{30}O_{10}Fe$ requires C, 54.96; H, 5.54; Acetyl, 31.4%).

2,4-O-ferrocenylidene-D-glucitol [Y].

Ferrocenemonoaldehyde (5.4 g.) in benzene (30 ml.) was added to D-glucitol (4.5 g.) in dimethylformamide (30 ml.). After adding p-toluenesulphonic acid (50 mg.), the mixture was stirred and refluxed (ca. 110-120°) for 10 hr. in an apparatus fitted with a Soxhlet filled with calcium chloride. The solvents were then removed *in vacuo* and after neutralising the free acid with a small volume of sodium methoxide, the residue was extracted with hot ethanol. The extracts were condensed and chromatographed on alumina using ethanol as the eluant. The top yellow band was removed from the extruded column and extracted with ethanol. The extracts were condensed and left at 10° for several hours. The product after three recrystallisations gave yellow rosettes of 2,4-O-ferrocenylidene-D-glucitol [Y] , m.p. 196-198°, $[\alpha]_D^{24}$ -95.4 (c 0.1 in water), (Found : C,53.9; H, 5.87. $C_{17}H_{22}O_6Fe$ requires C, 53.9; H, 5.86%).

1,3,5,6-Tetra-O-acetyl-2,4-O-ferrocenylidene-D-glucitol [Y'].

The monoacetal [Y] was acetylated with pyridine/acetic anhydride Two recrystallisations from ethanol gave yellow crystals of the

tetra-acetate [Y'], m.p. 112-113°, (Found : C, 54.76; H, 5.90; Acetyl, 30.8. $C_{25}H_{30}O_{10}Fe$ requires C, 54.96; H, 5.54; Acetyl, 31.4%).

Conversion of Acetal [Y] to Acetal [X].

The monoacetal [Y] was recrystallised from boiling water (pH 6) to give fine yellow needles of the acetal [X], m.p. 169-172°, (mixed m.p. 168-172°). The monoacetal [X] could be recrystallised from methanol unchanged.

Hydrolysis of the Monoacetals [X] and [Y].

The monoacetal (20 mg.) was dissolved in water (20 ml.) and Amberlite IR 120(H^+) resin added. The mixture was heated on a water bath for 30 min. at 60°, cooled, filtered and separated between chloroform and water. The aqueous extract was freeze-dried and the residue examined by paper ionophoresis (using buffer I_1). Spray S_3 showed D-glucitol to be present.

Estimation of Ferrocenemonoaldehyde in the Acetals.

The acetal (2-5 mg.) was dissolved in a volume of ethanol in a 50 ml. volumetric flask. N sulphuric acid (5 ml.) was added and the solution was made up to the mark with ethanol (with cooling). The absorption at 269 mμ was measured against a blank made up by diluting 5 ml. of N sulphuric acid to 50 ml. with ethanol.

A calibration curve for ferrocenemonoaldehyde was obtained under identical conditions. A plot of concentration of the aldehyde against absorption was found to be linear within the range 6-60 μg/ml.

Acetyl Determination in the Acetate Derivatives of the Acetals.

The method described on p.82. was used. It was necessary with these derivatives to reflux the samples for 1-2 hr. in order to effect complete hydrolysis of the ester groups. The solutions were then cooled and titrated as before.

Oxidation of the Acetals [X] and [Y] with Potassium Metaperiodate.

The procedure was essentially that described on p.83. The dioxan/0.02M potassium metaperiodate (2:3, v/v) was adjusted by preliminary experiments to pH 6 and the determination of the uptake of oxidant and the liberation of formic acid carried out in the manner described. For the determination of formaldehyde an initial pH 7.5 was used. The results for the oxidation of 2,4,-O-ferrocenylidene -D-glucitol [Y] and 1,3,5,6-tetra-O-acetyl-2,4-ferrocenylidene-D-glucitol [Y'] are shown in Table 10 and for the oxidation of 2,4-O-ferrocenylidene-D-glucitol [X] are shown in Table 11.

Oxidation of the Monoacetals [X] and [Y] to L-Xylose.

The monoacetal (0.2 g.) was shaken in 0.02M potassium periodate (50 ml., initial pH 7) until it dissolved and the mixture left for 2 hr. The excess periodate was destroyed with ethylene glycol (0.5 ml.) and after addition of 10N sulphuric acid (5 ml.), the solution was heated at 90° for 1 hr. The solution was cooled, extracted with chloroform and neutralised with barium carbonate, washing the precipitate thoroughly with hot water. The aqueous solution and washings were combined and shaken with Biodemineralit (CO_3^{--} form)

Table 10. Oxidation of 2,4-O-ferrocenylidene-D-glucitol [Y] and 1,3,5,6-tetra-O-acetyl-2,4-O-ferrocenylidene-D-glucitol [Y'] with 0.01M potassium metaperiodate at 25°.

	2,4-O-ferrocenylidene-D-glucitol [Y]			1,3,5,6-tetra-O-acetyl-2,4-O-ferrocenylidene-D-glucitol [Y']		
Time (hr.)	IO_4^- consumed. (moles.)	0.01N-NaOH. (ml.)	HCHO liberated. (moles.)	IO_4^- consumed. (moles.)	0.01N-NaOH. (ml.)	HCHO liberated. (moles.)
0.5	2.50	-	0.95	0.78	-	0.11
1.5	1.40		1.00	-	-	0.09
2.0	1.50	-	-	0.20	-	-
3.0	1.40	-	-	0.10	-	-
4.0	1.20	-	1.20	0.10	-	0.26
5.0	1.60	-	-	0.20	-	-
16.0	4.10	0.10	-	1.00	0.02	-
19.5	-	-	1.40	-	-	0.39
22.0	4.30	0.12	-	2.5	0.08	-
24.0	-	-	1.50	-	-	0.44
38.0	-	-	1.70	-	-	0.67
40.0	4.36	0.15	-	2.90	0.12	-
47.0	4.40	0.16	-	3.10	0.14	-

Table 11. Oxidation of 2,4-O-ferrocenylidene-D-glucitol [X] with 0.01M potassium metaperiodate at 25°.

Time. (hr.)	IO_4^- consumed. (moles.)	Formic acid liberated. (pH of solution)
0.25	0.2	-
0.50	0.3	7.1
1.0	-	6.9
2.0	1.1	6.9
3.0	1.0	7.0
4.0	1.1	-

resin for 30 min. after which it was filtered and freeze-dried. Examination of the residue by paper chromatography (described below) showed the presence of L-Xylose.

Solvent system	Spray	Remarks
A	S_1 & S_2	Xylose present R_F = 0.17.
B	S_2	Xylose present; pink spot.
C	S_2	Xylose present, reverses position w.r.t. arabinose as found in systems A & B.

(a) The anhydrous xylose extract was shaken with a solution (3 ml.) of benzaldehyde (9%, w/v) in anhydrous methanolic hydrochloric acid (2.5N) until dissolved. On refrigerating overnight fine white needles of the dimethylacetal dibenzylidene derivative of L-xylose were deposited which were recrystallised from chloroform-methanol, with m.p. 208-210°, $[\alpha]_D^{21}$ +5° (c. 0.9 in chloroform).

This derivative of L-xylose is not reported in the literature but Breddy and Jones report a m.p. 211°, $[\alpha]_D^{20}$ -9° for the same derivative of D-xylose.

Di-O-ferrocenylidene-D-glucitol.

The chloroform extracts from the monoacetal synthesis were dried over potassium carbonate and then chromatographed on alumina using chloroform as the eluant. After elution of the unreacted ferrocenemonoaldehyde, the column was extruded and the faint yellow band at the top removed and extracted with ethanol. Evaporation of

the ethanol and recrystallisation of the residue from ethanol-chloroform gave yellow crystals of di-O-ferrocenylidene-D-glucitol (5 mg.) m.p. 197-202°; hydrolysis of this compound showed the presence of D-glucitol and 2.4 mols. of ferrocenemonoaldehyde.

1,3-O-ferrocenylidene-D-mannitol.(XXVIII).

D-mannitol (7.2 g.) and ferrocenemonoaldehyde (19.2 g.) in dimethylformamide (20 ml.) were stirred at 70° in the presence of phosphorous pentoxide (0.2 g.) for 15 hr. After cooling, the mixture was neutralised with sodium methoxide in methanol and then separated between chloroform and water. The aqueous extract was washed well with chloroform to remove the dimethylformamide and then freeze-dried.

Attempts to purify the monoacetal by recrystallisation of the freeze-dried residue from water produced only a gel. This material shrank on the filter to an amorphous solid which was very soluble in water and appeared somewhat unstable when dried. The freeze-dried material was taken up in butanol-ethanol-water (40:11:19,v/v) to which a trace of ammonia had been added, and chromatographed on a cellulose column using the same solvent as the developer. The yellow band was eluted and the solvent evaporated under reduced pressure. The residue was redissolved in water (pH 7.5) and freeze-dried to give the monoacetal (XXVIII) as a yellow powder (1.5 g.), m.p. ca. 50°, $[\alpha]_D^{24}$ + 37° (c . 1.0 in water), (Found : C, 52.2; H, 5.89. $C_{17}H_{22}O_6Fe$ requires C, 53.9; H, 5.86%).

2,4,5,6-Tetra-O-acetyl-1,3-O-ferrocenylidene-D-mannitol (XXVIX).

The monoacetal (XXVIII) (0.4 g.) was acetylated with pyridine-acetic anhydride. Recrystallisation from aqueous ethanol gave yellow crystals of the tetra-acetate (XXVIX), m.p. 112-113°, $[\alpha]_D^{22}$ +9° (*c* 2.0 in chloroform), (Found : C, 54.5; H, 5.58; $C_{25}H_{30}O_{10}Fe$ requires C, 54.9; H, 5.54%).

2,5-Di-O-acetyl-1,3:4,6-di-O-ferrocenylidene-D-mannitol (XXX).

The chloroform extracts† from the monoacetal (XXVIII) preparation (†see p.105) were dried over potassium carbonate, condensed and chromatographed on alumina using chloroform as the eluant. After the complete elution of the ferrocenemonoaldehyde, the column was extruded and the top yellow section cut out and extracted with ethanol. Evaporation of the ethanol and acetylation with pyridine/acetic anhydride yielded after four recrystallisations from chloroform/ethanol, orange-yellow crystals of the diacetate (XXX) (0.3 g.), m.p. 198-201°, $[\alpha]_D^{24}$ -16° (*c* 1.0 in chloroform), (Found : C, 58.7; H, 5.5; Acetyl, 15.5. $C_{32}H_{34}O_8Fe_2$ requires C, 58.4; H, 5.2; Acetyl, 13.1%).

1,3:4,6-Di-O-ferrocenylidene-D-mannitol (XXXI).

The diacetate (XXX) (0.2 g.) was deacetylated with sodium methoxide (0.02N, 2 ml.) in chloroform (5 ml.) to give after two recrystallisations from chloroform-hexane yellow-orange crystals of the diacetal (XXXI), m.p. 194-196°, $[\alpha]_D^{20}$ + 39.2° (*c* 2.7 in chloroform).

Estimation of Ferrocenemonoaldehyde in the Acetals.

The method described on p.100 was used for both the monoacetal and the diacetal.

Detection of D-mannitol in the Acetals.

The acetals were hydrolysed in aqueous or aqueous ethanolic solution with Amberlite IR 120(H+) resin as described on p.100. Paper ionophoresis in buffer I_1 and spray S_2 showed presence of D-mannitol.

Periodate Oxidation of 1,3-O-ferrocenylidene-D-mannitol (XXVIII) and the Tetra-acetate (XXVIX).

The results of the determination of periodate uptake and the liberation of formic acid for the oxidations are given in Table 12.

Periodate Oxidation of 1,3-O-ferrocenylidene-D-mannitol to D-Erythrose.

The monoacetal (XXVIII) (0.2 g.) was dissolved in water (75 ml.) and potassium metaperiodate (0.2 g.) added. The pH was quickly adjusted to 7.5 with dilute alkali. The mixture was shaken for 2 hr. and filtered. Barium hydroxide (20 ml. 0.04M) was added and after 1 hr., carbon dioxide was bubbled through to precipitate the excess Ba^{++} ions. The solution was then filtered and Amberlite IR 120(H^+) resin added and the solution stirred and heated at 80° overnight. After cooling and filtering, the solution was freeze-dried. The residue was examined by paper chromatography and ionophoresis.

Solvent	Spray	Remarks
A	S_1	Erythrose present

Table 12. Oxidation of 1,3-O-ferrocenylidene-D-mannitol and 2,4,5,6-tetra-O-acetyl-1,3-O-ferrocenylidene-D-mannitol with 0.01M potassium metaperiodate at 25°.

Time	1,3-O-ferrocenylidene-D-mannitol		2,4,5,6-tetra-O-acetyl-1,3-O-ferrocenylidene-D-mannitol	
	IO_4^- consumed (moles.)	HCOOH liberated (moles.)	IO_4^- consumed (moles)	HCOOH liberated (moles.)
0.25	5.4	-	2.5	-
0.50	5.6	1.08	2.7	0.38
1.00	6.1	1.08	-	0.38
2.00	7.3	-	4.4	-
4.00	8.2	1.08	5.9	0.38
5.50	8.5	-	6.5	-
18.00	8.8	-	7.2	-
24.00	9.3	1.08	7.8	0.38
41.00	9.4	-	7.6	-

A	S_2	Erythrose present. (fluorescent under U.V.)
Buffer I_1	S_2	Erythrose present. M_S 0.9 (lit.[111] 0.9)

(a) Reduction of D-erythrose to D-erythritol.

A solution of potassium borohydride (0.1 g.) in water (10 ml.) was added to the freeze-dried residue (from the previous experiment) in water (10 ml.) and the mixture left overnight. The potassium ions were removed by shaking (15 min.) with IR 120(H^+) resin, filtering and evaporating the solution to dryness under reduced pressure. The boric acid was then removed by repeated distillation of the residue with methanol under reduced pressure. Examination of the final residue by paper chromatography in solvents A and B showed the presence of erythritol. Attempts to prepare a crystalline sample of the tetra-benzoate derivative by treatment with benzoyl chloride in pyridine were unsuccessful.

Attempted Tosylation of 1,3-O-ferrocenylidene-D-mannitol.

The monoacetal (XXVIII) (0.4 g.) was dissolved in pyridine (10 ml.) and p-toluenesulphonyl chloride (0.8 g.) was added. The mixture was left at room temperature for 3 days. Slow addition of water (10 ml.) over 1 hr. and standing several hr. produced only a dark gum. The solution was extracted with ether. The ether extracts were washed several times with water and then with cadmium chloride (10%, w/v) and finally dried over magnesium sulphate. Evaporation of the ether produced a gum which would not crystallise.

Methylation of 1,3:4,6-di-O-ferrocenylidene-D-mannitol (XXXI).

The diacetal (XXXI) (0.11 g.) was dissolved in dimethylformamide (3 ml.) followed by methyl iodide (0.5 ml.) and silver oxide (0.5 g.) then added slowly. After shaking for 20 hr., the mixture was centrifuged, the precipitate washed with chloroform-dimethylformamide (1:1, v/v) and the centrifugate and washings separated between chloroform and water. A small quantity of potassium cyanide was added to remove the cloudiness due to silver compounds. The chloroform layer, after washing with water was dried over sodium sulphate and the methylation repeated. The final chloroform extract was evaporated and the crystalline dimethyl diacetal derivative hydrolysed in ethanol-water (2:1, v/v) with Amberlite IR 120(H^+) resin for 3 hr. at 80-90°. After cooling and filtering, the solution was extracted with ether (3x10 ml.), the ether layers washed with water and the washings returned to the aqueous phase, which was then freeze-dried.

A portion of the freeze-dried residue was examined by paper chromatography in solvent A. Sprays S_3 and S_4 showed appreciable quantities of a dimethyl mannitol (presumably 2,5-di-O-methyl-D-mannitol), R_F 0.48, to be present.

The residue was acetylated with sodium acetate in acetic anhydride for 2 hr. at 90° and the hydrolysed reaction mixture extracted with ether. The ether extracts were washed with water, dried and then evaporated to give a syrup. Addition of petroleum ether (60-80°)

-ether (4:1, v/v) to this syrup produced on standing at 10° for several hours, translucent crystal clusters of 1,3,4,6-tetra-O-acetyl-2,5-di-O-methyl-D-mannitol (0.01 g.), which after two recrystallisations had m.p.105-107° (lit.[81] 107-108°).

Attempted Condensation of Acetylferrocene with D-mannitol.

(a) The D-mannitol (0.04 g.) and concentrated sulphuric acid (0.01 ml.) were mixed to form a paste. Acetylferrocene (0.2 g.) was stirred in and the mixture stood for 10 min. It was then poured on to saturated sodium bicarbonate solution and separated between water and chloroform.

The aqueous layer was colourless and paper chromatography in solvents A and B showed only mannitol present. The organic layer exhibited no rotation and gave only acetyl ferrocene on chromatographing the extract on alumina and on paper chromatographs in solvent system F.

(b) Experiment (a) was repeated using phosphoric acid (syrupy) in place of the sulphuric acid but with no greater success.

(c) D-mannitol (0.14 g.) and acetylferrocene (0.63 g.) were shaken for 24 hr. in a 10% zinc chloride solution (10 ml.) in dimethylformamide. The mixture was fractionated and examined as in (a) but revealed only mannitol and acetylferrocene present.

(d) D-mannitol (1.0 g.) acetylferrocene (3.5 g.) and p-toluenesulphonic acid (0.05 g.) were added to dimethylformamide-benzene (1:1 v/v). The mixture was stirred and refluxed using a Soxhlet water extractor for 15 hr. After cooling and neutralising with sodium methoxide the

mixture was separated between benzene and water. Investigation of the fractions as described earlier provided no evidence of ketal formation.

(e) D-mannitol (0.6 g.) and acetylferrocene (2.2 g.) were dissolved in dioxan (20 ml.) with the addition of 60% hydrobromic acid (0.5 ml.) and the mixture shaken for 24 hr. Neutralisation and examination of the fractions as before showed only the starting materials were present.

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Erratum

J. = J. Chem. Soc.

REPRINTED FROM CHEMISTRY AND INDUSTRY, 1959, pp. 996—997

PAPER CHROMATOGRAPHY OF FERROCENE AND ITS DERIVATIVES

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Mixtures of ferrocene derivatives may readily be fractionated by adsorption chromatography, for example on alumina, but partition chromatography, with water as the stationary phase, is generally unsuitable owing to the hydrophobic properties of many of these compounds.

Paper chromatographic methods have now been developed in this laboratory in order to identify ferrocene derivatives in complex reaction mixtures. An organic two-phase solvent system is used, which is essentially the same as that described by Wickberg[1] for the separation of sugar acetates. Strips of Whatman No. 3 paper are dipped twice in a solution of dimethyl sulphoxide in benzene (20% v/v) and the benzene is removed from the papers after each treatment by heating at 80° in an oven for approximately 30 secs. The mixtures for analysis (dissolved in absolute ethanol) are quickly applied to the paper strips which are then developed with light petroleum (b.p. 60–80°) saturated with dimethyl sulphoxide. This solvent has a high rate of movement (20 cm./hour at 20°) and complete resolution of a mixture of ferrocene (I), acetylferrocene (II), ferrocenealdehyde (III), hydroxymethylferrocene (IV) and ferrocenecarboxylic acid (V) can easily be achieved in 2 hours, the R_F values for these compounds being 0·98, 0·62, 0·59, 0·19 and 0·05 respectively.

The majority of ferrocene compounds are highly coloured and are therefore readily located on paper chromatograms. However, for a more positive identification, the chromatograms can be sprayed with a solution of potassium thiocyanate (5% w/v in NHCl) (*cf.* Goldberg[2]). This reagent at room temperature produces an immediate red-brown colouration with compounds (II), (III) and (V) and on heating the chromatograms at 90° for 7–8 minutes compounds (I) and (IV) also give the same colours. Prolonged heating (90° for 15 minutes) causes all the spots to change to yellow-brown. The rate of appearance of the original red-brown colour on the chromatogram can assist in the identification of the derivative. A further useful spray reagent, for the detection of certain ferrocene derivatives, is aqueous sodium periodate solution (1% w/v). Compounds (I), (IV) and (V) are rapidly oxidised by periodate at room temperature to blue-green products; in the case of compound (I), the product is presumably the ferrocinium ion. No colour change is apparent with (II) and (III). If required, the thiocyanate reagent can be applied to chromatograms which have already been sprayed with periodate solution, and this will then give the normal red-brown colour with all the above-mentioned derivatives. With compound (IV) this second colour change is slow and goes via a dark green intermediate.

One of the most difficult compounds to detect on chromatograms which have been developed with the solvent system described is (I) which moves near the solvent front and tends to become dispersed over a large area of paper. It is probably best to rely on the natural colour of this compound for its location and this should be marked as soon as the paper is withdrawn from the chromatography tank.

One of us (A. N. de B.) is indebted to Aspro-Nicholas Ltd., for a research grant.

Received June 15, 1959

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