COPPER AND VANADIUM COMPLEXES ~~WITH~~ OF POLYHYDROXY-COMPOUNDS.

REACTIONS OF ~~ENAMINES WITH~~ CARBOHYDRATE DERIVATIVES, WITH ENAMINES

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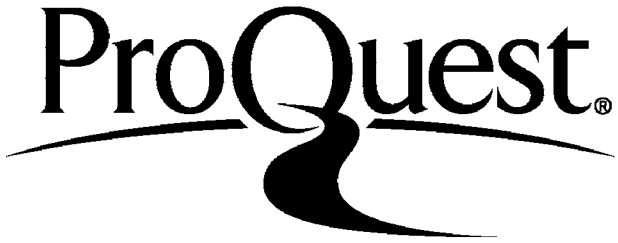

ABSTRACT

Certain polyhydroxy-compounds were found to form cationic complexes with basic copper acetate $Cu(CH_3COO)_2CuO6H_2O$ and the relationship between the conformations of these compounds and their ionophoretic mobilities in basic copper acetate solution was studied. It was established that the mobilities of the reduced glucose disaccharides varied according to whether the glucose units were α- or β-linked, thus affording a method for determining the configuration of the glucosidic linkage in the original disaccharide.

The complexing of polyhydroxy-compounds with the oxy-anions present in acidified solutions of sodium metavanadate, $NaVO_3$, and sodium orthovanadate, $Na_3VO_4,14H_2O$, was explored. Some correlations were drawn between the conformations of cyclic and acyclic polyols and their ionophoretic mobilities in sodium metavanadate solution. From polarimetric and absorptiometric measurements, the stoicheiometry of a number of complexes was determined, while the nature of the complexing vanadate ion was investigated by potentiometric and conductimetric titrations of solutions of sodium metavanadate and orthovanadate containing D-glucitol and D-mannitol. The number of hydroxyl groups in D-glucitol involved in the complexing was derived from periodate

oxidation of the complex.

The polarimetric method developed to establish the stoicheiometry of complexes between sodium metavanadate and polyhydroxy-compounds was applied to the equilibrium complexes formed between sodium tungstate and some cyclic sugars.

The preparation of a sugar derivative in which the sugar is linked directly, by a carbon-carbon bond, to a cyclic ketone was undertaken. The synthesis was achieved by acylation of an enamine of the cyclic ketone by an acid chloride. Various analytical techniques were used to establish the structure of the product. Other methods of forming such derivatives through the intermediate participation of enamines are briefly discussed.

ACKNOWLEDGMENTS

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Contents

Copper and Vanadium Complexes with Polyhydroxy-Compounds

I. INTRODUCTION

The electronic configurations of copper and vanadium ions, in common with those of other transition metals, comprise a partially-filled set of d-orbitals[1] which confers, amongst other characteristic properties, the ability to form coordination complexes[2]. In such aggregates, the central metal ion is surrounded by ligands, where a ligand may be defined as any atom or molecule which is directly attached to the metal ion and can be regarded as bonded to it[3]. The most common types of ligand are mono- or poly-atomic negative ions or electrical dipoles like water, ammonia or carbon monoxide, in which a lone pair of electrons is oriented towards the central metal ion. These electrons associated with the ligand are able to enter the vacant d-orbitals of the transition metal ion leading to complexes whose stereochemistry is determined both by the number of ligands and the relative energy levels of the resulting hybridised orbitals.

Complex-formation is not restricted merely to singly-coordinated donor molecules; it is possible for a ligand to consist of one molecule with several functional groups, which can be coordinated simultaneously to the same metal ion.

Such a complexing agent is known as a chelating ligand, of which ethylene diamine, $NH_2CH_2CH_2NH_2$, and acetylacetone, $CH_3COCH_2COCH_3$, are well-known examples[3]. The importance of ligands of this type lies in the fact that they form more stable complexes than are obtained with equivalent single ligands under the same conditions and can indeed replace them[3]. In particular, cis-glycols are quite good complex-forming agents although simple alcohols show little tendency to form complexes in aqueous solutions[4].

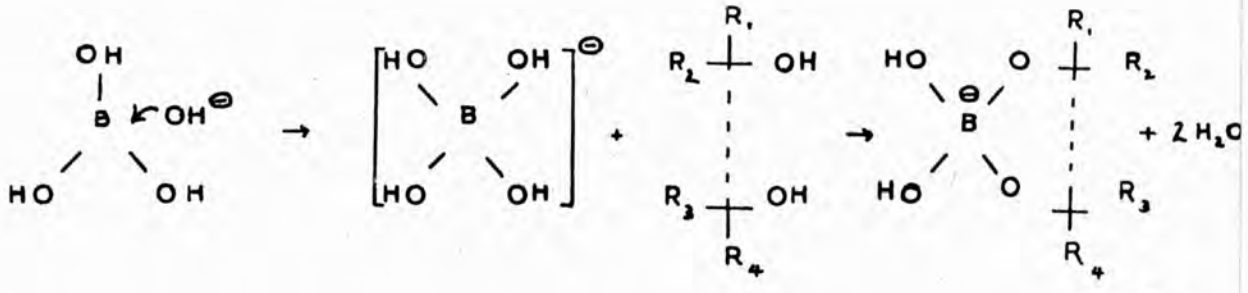
Knowing this property of cis-glycols, it is a short step to the consideration of the complexing behaviour of polyhydroxy-compounds which comprise a number of adjacent glycol groups. A polyhydroxy-compound may indeed be considered as a polydentate chelating ligand which can be expected to coordinate with a transition metal by replacing single OH ligands already present around the metal by OR ligands. Such metal-hydroxyl bonds occur both in basic metal salts, compounds intermediate in structure between normal salts and oxides or hydroxides, and in certain inorganic oxy-acids in which the metal is incorporated in the anion.

Inorganic oxy-acids can be divided into three classes[5]: simple oxy-acids, formed by the lighter strongly electronegative elements like chlorine; complex oxy-acids formed

by heavier weakly electronegative amphoteric elements such as tellurium and antimony; and poly-acids which are formed by the elements of Groups VA and VIA of the Periodic Table. Both the second and third classes of oxy-acids furnish examples of ions which can be coordinated with polyhydroxy-compounds. Thus the salts of complex oxy-acids include stannates, which have already been shown to form complexes with carbohydrates[6], while the last group includes polyvanadates, polymolybdates and polytungstates, derived from the relevant ortho-compound by condensation through oxygen "bridges" to polymeric species on the acidification of their aqueous solutions. The anions of these poly-acids are able to form negatively-charged complexes with polyhydroxy-compounds[7].

Although, since the present work deals mainly with copper and vanadium complexes with carbohydrates, the transition metals only have been mentioned so far, there are of course other elements that, in forming species in which coordination around the central atom is completed by hydroxyl ions, can also complex with polyhydroxy-compounds. Boron, which complexes with polyhydroxy-compounds in the form of the borate anion[8], is an example of such an element. The trigonal boron atom sp^2 hybridised in boric acid becomes

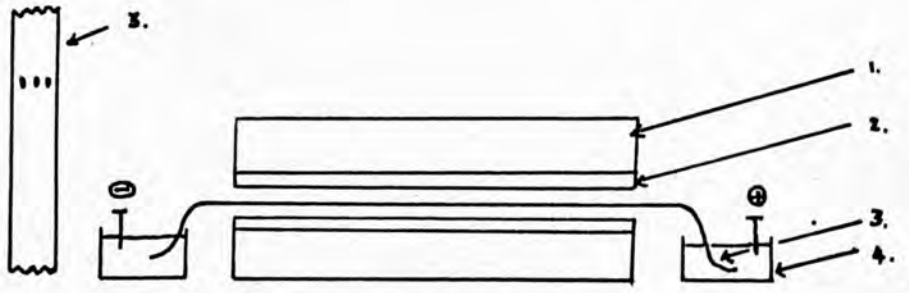
a tetrahedral sp^3 hybridised atom in the borate anion, by the coordination to it of a hydroxyl ion. The interaction of a polyhydroxy-compound with the borate anion can be regarded as the replacement of the OH ligand by an OR ligand followed by the reaction of a further pair of hydroxyl groups to form a B-O-R group with the elimination of water.



Eqn. I.1.

The particular value of the study of complex-formation between metals and carbohydrates lies in its application to the ionophoretic and ion-exchange resin separation of these polyhydroxy-compounds. Ionophoresis is the term given to the migration of charged species under an applied electrical potential. In the initial investigations, the potential was applied across a solution[9], but it is now more usually applied across an inert support, generally paper, impregnated with an electrolyte. The species, in a volatile solvent, each transferred on to a small area of paper as a discrete

spot, migrate to the appropriate electrode at speeds dependent upon their degree of dissociation into charged entities, their solubility in the electrolyte, and to some extent their molecular size. If a molecule has an inherent charge, it will move in the electrolyte under the applied potential. If, on the other hand, the molecule is neutral, the formation of an ionizable complex is necessary to cause it to migrate. This complex-formation has been used extensively for the ionophoresis of carbohydrates: solutions of sodium germanate[10], sodium stannate[6] and sodium borate[8] being examples of the complexing agents employed. In many instances it has been possible to correlate the structural features of the polyhydroxy-compounds with their rate of migration, thereby increasing the analytical use of the method[7].

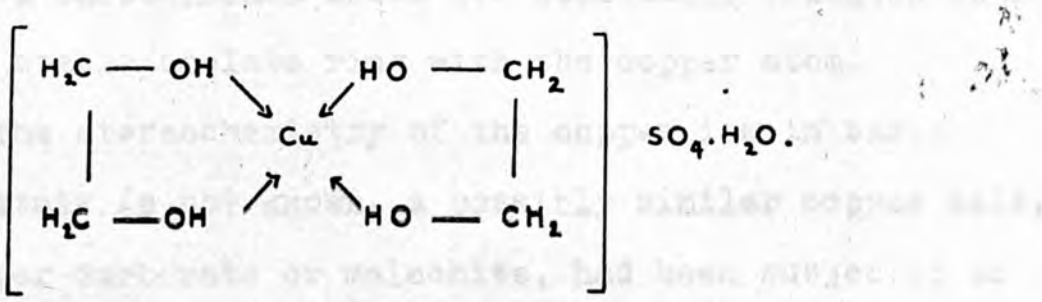


1. WATER-COOLED ALUMINIUM PLATES, PRESSED TOGETHER.
2. INSULATING SHEETS OF POLYTHENE.
3. STRIP OF FILTER PAPER.
4. ELECTROLYTE.

Fig. I.1. Diagrammatic representation of ionophoresis apparatus and prepared paper.

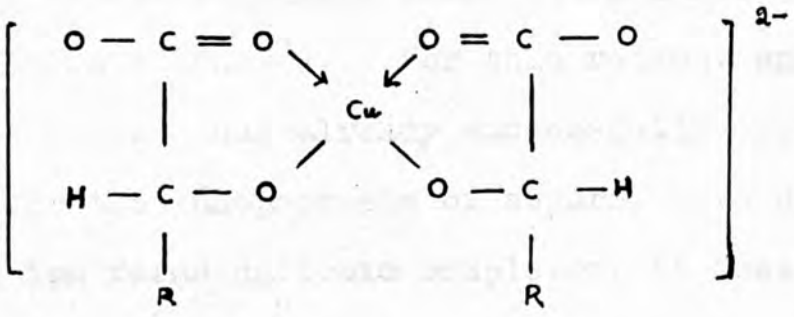
Since the inorganic coordination chemistry of copper and vanadium, resembling to some extent that of lead and molybdenum respectively, suggested that complexing with carbohydrates might occur, it was decided to investigate the potential application of an appropriate copper salt and polymeric vanadate to the ionophoretic separation of poly-hydroxy-compounds.

Reeves[11] demonstrated the ability of copper, in a cuprammonium ion, to complex with carbohydrates. The complexes formed were, however, uncharged and therefore would not undergo ionophoresis. But complex-formation between copper and polyhydroxy-compounds is not confined to the cuprammonium solutions used by Reeves: ethylene glycol is believed to complex with the tetrahydrated ion in copper sulphate to give the following compound[12] (I)..



(I)

while the copper complexes in Fehling's solution are considered to be of the form[13] (II).



II.

The question is to find an appropriate copper compound which not only complexes with carbohydrates, but forms charged complexes.

The ion required must contain a co-ordinated copper atom and the ligands must be replaceable by certain hydroxyl groups of a carbohydrate which are sterically oriented so as to form a stable chelate ring with the copper atom. Although the stereochemistry of the copper ion in basic copper acetate is not known, a possibly similar copper salt, basic copper carbonate or malachite, had been subjected to X-ray, crystallographic investigation[14] and found to contain a copper ion linked to hydroxyl groups, the distance between the oxygen atoms of which was virtually the same as

the inter-oxygen distance in a threo-1,2-diol group in the staggered planar zig-zag conformation of an acyclic polyol[7]. (The assumption of a planar zig-zag conformation is based on the X-ray crystallographic data obtained for the gluconate[15] and arabinonate ions[16]). For this reason, and also because Frahn and Mills[17] had already successfully used basic lead acetate for the ionophoresis of sugars, with certain of which the lead ion forms cationic complexes, it seemed worthwhile to establish the value of basic copper acetate as a complexing agent.

Meanwhile vanadium, analogously to molybdenum and tungsten, is known to form polymeric species on the acidification of an aqueous solution of the orthovanadate. These include the di- or pyro-vanadate, the metavanadate which, depending on pH and concentration may be either a tri- or tetravanadate, and the hexavanadate[18] and decavanadate[19]. Frahn and Mills[17] had initiated the use of solutions of sodium metavanadate for the ionophoretic separation of polyhydroxy-compounds, and the investigation was extended in an attempt to discover the nature of the complexing ion and the fundamental stereochemical features of the polyhydroxy-compound necessary for complexing to occur.

II. Complexes of Polyhydroxy-compounds with Basic Copper Acetate: Results and Discussion.

Certain polyhydroxy-compounds, on ionophoresis in basic copper acetate ($Cu(CH_3COO)_2CuO.6H_2O$) solution migrate towards the cathode, showing that they form cationic complexes in this electrolyte. The optimum pH for migration is that of a freshly-prepared solution, namely 5.1 to 5.3, when D-glucitol has a mobility, under the conditions described in the Experimental, of 2.21×10^{-1} $cm^2 sec^{-1} v^{-1}$. In more acidic solutions the complexes break down while an increase in pH renders the acetate unstable, copper hydroxide being precipitated. [Expt. 1].

The rate of migration of a number of polyhydroxy-compounds was measured relative to that of D-glucitol the mobility of which was taken as unity, and those compounds with relative mobilities less than 0.1 were considered to be non-migrating. [The relative mobility $M_g(Cu) = \frac{\text{distance moved by compound (cm)}}{\text{distance moved by D-glucitol (cm)}}$. The non-migrating marker used was 5-hydroxymethylfurfural].

None of the cyclic monosaccharides, disaccharides or cyclitols tested migrated in this electrolyte, which means that if complexes were formed, they were uncharged. A table of these compounds is given below (Table II.1).

Table II.1. Cyclic compounds having M_g(Cu). less than 0.1.

D-glucose	Kojibiose	Methyl-α-D-glucopyranoside
D-galactose	Sophorose	Methyl-α-D-mannopyranoside
D-mannose	Nigerose	Methyl-α-D-ribopyranoside
D-gulose	Laminaribiose	Methyl-β-D-ribopyranoside
D-xylose	Maltose	Methyl-α-D-lyxopyranoside
L-arabinose	Cellobiose	1,6-anhydro-β-D-galactopyranose
D-ribose	Isomaltose	1,6-anhydro-β-D-gulopyranose
D-lyxose	Gentiobiose	1,6-anhydro-β-D-mannopyranose
Fructose		1,6-anhydro-β-D-altropyranose
Sorbose		
Sucrose		
Epi-inositol		
Myo-inositol		
(+)-inositol		

The investigation of the optical rotation of a cyclic sugar in a solution of basic copper acetate would probably determine whether or not there is any complex-formation. From the composition of the complex, and the enhancement of rotation of a number of substituted and unsubstituted sugars, it should then be possible to make a logical deduction of the number and orientation of the hydroxyl groups necessary

to form a complex. Initial rotation measurements were frustrated by the instability of the copper salt which renders the solution optically dense in a few hours, and some method of stabilising the solution must be found before this investigation can be pursued.

The ionophoretic mobilities of a number of pentitols and hexitols were measured. The rate of migration of the unsubstituted polyols was in general proportional to the number of αT-diol groupings present in the molecule, [Barker and Bourne nomenclature[20]]: iditol moves considerably faster than allitol. On this assumed correlation, however, it would be expected that galactitol would migrate more rapidly than does D-glucitol. This same anomaly is evident in measurements performed using basic lead acetate solution, where the mobilities relative to ribose are L-iditol, 0.57 > D-glucitol, 0.47 > galactitol, 0.32 > allitol, 0.09.[17]

Table II.2. The Mobilities of unsubstituted Hexitols and Pentitols in Basic Copper Acetate.

Compound	$\underline{M}_s(\underline{Cu})$	Number of $^{\alpha T}$-diol groups
Iditol	1.15	2.
D-glucitol	1.00	2.
Galactitol	0.93	2.
D-altritol	0.85	1.
D-mannitol	0.83	1.
Allitol	0.17	0.
Xylitol	1.10	2.
L-arabitol	0.85	1.
Ribitol	0.12	0.

In order to establish the minimum number of hydroxyl groups required for complexing, several diols and butane-1,2,4-triol were investigated ionophoretically (see Table II.3.). None showed any migration, but it could be argued that the energy released on the formation of the complex is insufficient to compensate for the change in entropy on going from a diol with virtually free rotation about the carbon-carbon bond to a more rigid coordinated structure. It is interesting to note, however, that Frahn and Mills[17] found that no compound containing a single pair of hydroxyl groups migrated in basic lead acetate solution.

Table II.3. The Mobilities of some Diols, Triols and Tetritols in Basic Copper Acetate.

Compound	M_s(Cu)	Compound	M_s(Cu)
Propane-1,2-diol	0	3,3-dimethyl-1,5-diol	0
cis-butane-2,3-diol	0	Butane-1,2,4-triol	0
trans-butane-2,3-diol	0	Glycerol	0
Butane-1,3-diol Propane-1,3-diol	0	Erythritol	0
Butane-1,4-diol	0	Threitol	0.20

Ionophoresis of certain substituted hexitols and pentitols gave results generally in accordance with the interpretation of mobilities already suggested i.e. that the rate of migration is proportional to the number of αT-diol groupings present, though anomalies become evident.

3-O-Methyl-D-glucitol has no αT-diol group present and its mobility is zero. 2-Deoxy-D-arabino-hexitol (2-deoxy-D-glucitol) on the other hand still has an αT-diol group present on carbon atoms 3 and 4, and if this is sufficient requirement for migration, its mobility would be expected to be greater than that of D-glucitol since it is a smaller molecule.

Table II.4. The Mobilities of some substituted Hexitols and Pentitols in Basic Copper Acetate.

Compound	M_s(Cu)	Number of αT-diol groups
3-O-methyl-D-glucitol	0.00	0
3-O-methyl-L-gulitol	0.00	1
2-O-methyl-D-mannitol	0.00	1
1,2-di-O-methyl-D-mannitol	0.00	1
2,5-O-methylene-D-mannitol	0.00	1
2-deoxy-D-arabino-hexitol	0.10	1
1,6-dideoxy-L-mannitol	0.14	1
1-deoxy-D-arabitol	0.20	1
1,6-dideoxy-galactitol	0.30	2
1-deoxy-D-xylitol	0.83	2
6-deoxy-L-galactitol	0.85	2
1-O-methyl-L-gulitol	0.93	2
2-deoxy-L-xylo-hexitol	1.12	2

3-O-Methyl-L-gulitol (4-O-methyl-D-glucitol) has a reduced mobility not explained by the above correlation, unless the methyl group sterically hinders the approach of the copper ion to the adjacent hydroxyl group, which, from consideration of molecular models, is feasible.

2-Deoxy-L-xylo-hexitol (5-deoxy-D-glucitol) and 1-O-methyl-L-gulitol (6-O-methyl-D-glucitol) have mobilities the order of magnitude of which can be explained readily by the difference in size of the molecules from that of D-glucitol, but 2-O-methyl- and 1,2-di-O-methyl-D-mannitol do not migrate, although an αT-diol group is still present in each molecule. The methyl group on carbon atom 2 is cis to the hydroxyl group on carbon atom 3, where cis refers to the Fischer projection formula,[21] and is therefore unlikely to interfere sterically with complex-formation. On the other hand, the lack of mobility of 2,5-O-methylene-D-mannitol might well be due to a slight distortion of the planar zig-zag chain to form the methylene ring leading to an O-O distance which precludes the formation of a complex.

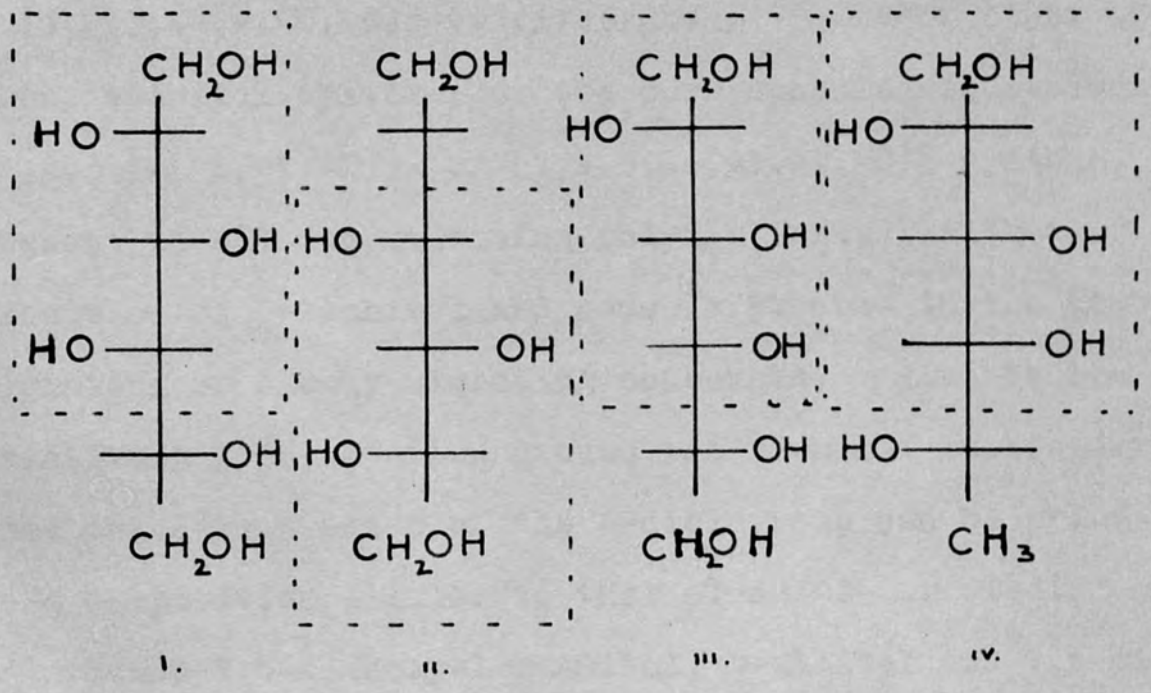
It is however difficult to explain, in terms of the necessity simply for an αT-diol grouping, why 1,6-dideoxy-L-mannitol does not migrate faster than D-mannitol and why the mobility of 1-deoxy-D-altritol is less than that of D-altritol. On the basis of the evidence given above it seems that an αT-diol group is essential for strong complex-formation to occur, but is not in itself sufficient.

Since the possession of an αT-diol group does not necessarily enable a polyhydroxy-compound to complex, the possibility that more than two hydroxyl groups are

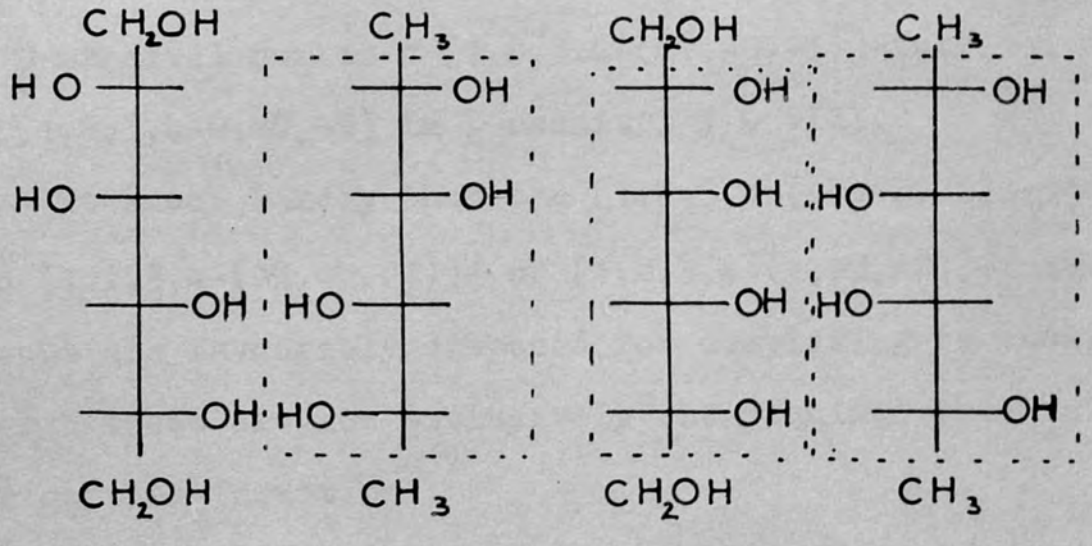
participating in the reaction was considered.

The [1,2,3-(a,aT)]-configuration (Barker & Bourne nomenclature. c.f. Angus & Weigel[22]) of three hydroxyl groups is present in D-glucitol but not in 2-deoxy-D-glucitol. If this were the required configuration, it would explain the reduced mobility of the latter compound. It also has the merit that it provides a reason for the difference in mobility between L-arabitol and 1-deoxy-D-arabitol and similarly the drop in mobility of D-altritol on conversion to 1-deoxy-D-altritol. But D-mannitol does not possess such a triol system, and the mobility of galactitol would again be anomalous, as it possesses two [1,2,3-(a,aT)]-triol systems, if the primary hydroxyl groups are included, equivalent to the two [1,2,3(aT, aT)]-triol systems of iditol. A further objection to the above correlation is that an equivalent spatial disposition of hydroxyl groups is contained in certain cyclic sugars, for example D-gulose and D-mannose, which do not migrate.

Finally it was considered whether four hydroxyl groups may be necessary for strong complexing to occur. Examples of compounds which migrate are iditol, 2-deoxy-L-xylo-hexitol, D-altritol and 6-deoxy-L-galactitol, whose configurations are represented below I-IV. The first pair contain a [1,2,3,4-(a, aT, aT)]-tetritol group while the second contain



I. I ditol. II. 2-deoxy-L-xylo-hexitol. III. D-altritol. IV. 6-deoxy-L-galactitol



V. D-mannitol. VI. 1,6-dideoxy-L-mannitol. VII. D-allitol. VIII. 1,6-dideoxy-galactitol.

a [1,2,3,4-(a,aT,aC)]-tetritol group. One or other of these tetritol systems, or the corresponding equivalent [1,2,3,4-(aT,aT,aT)]- or [1,2,3,4-(aT,aT,aC)]-systems, is present in all the migrating polyols tested, with the exception of D-mannitol and none is present in the non-migrating or slowly-migrating compounds. (Due to the relatively free rotation around the terminal carbon-carbon bond the oxygen atoms of the a-diol group can be orientated to a disposition similar to that of an aC- or aT-diol group).

Since 1,6-dideoxy-L-mannitol, D-allitol and 1,6-dideoxy-galactitol move only slowly, the mobility of D-mannitol can be due neither to a [1,2,3,4-(aC,aT,aC)]-tetritol nor to a [1,2,3,4-aT,aC,aC]-tetritol simulated by [1,2,3,4-(aT,aC,a)] in D-mannitol nor to a [1,2,3,4-(aT,aC,aT)]-tetritol simulated by [1,2,3,4-a,aC,aT] in D-mannitol V - VIII.

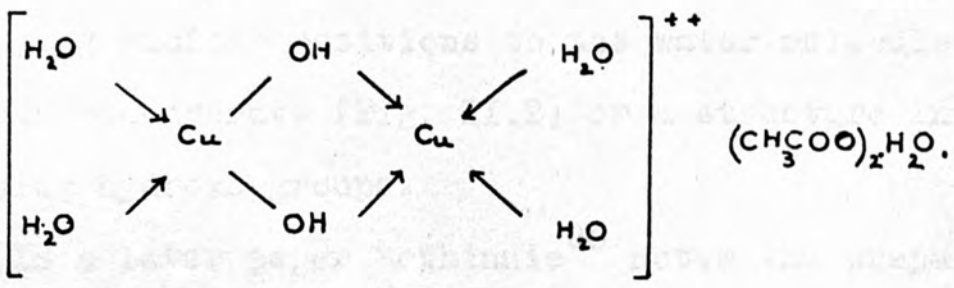
It seems likely that the [1,2,3,4-(aT,aT,aT)]-tetritol and [1,2,3,4-(aT,aT,aC)]- or [1,2,3,4-(a,aT,aC)]- tetritol groups are favourably disposed for complexing to occur, though these are not exclusively the required orientations for complex-formation.

The next consideration is the type of coordination around the copper atom in basic copper acetate, which must be examined in order to determine whether such tetritol groupings

could act as ligands. There has been no report in the literature on the environment of the copper atom in basic copper acetate, but a crystallographic investigation of basic copper carbonate[14] (malachite $Cu_2(OH)_2CO_3$) has revealed that the copper ion is bonded, in a square planar configuration, to two hydroxyl groups and two oxygen atoms at Cu-O distances of approximately 2.0Å, while a further pair of hydroxyl groups, at distances approximately 2.4Å or 2.7Å complete a distorted octahedral coordination group, Fig. II.2. The inter-oxygen distance of one oxygen and hydroxyl group in the square plane at the centre of which is the copper atom is about $2\sqrt{2}$ or 2.8Å, a value close to the distance, 2.82Å, between oxygen atoms in an αT-diol group in the planar zig-zag conformation of an acyclic polyol.[7]

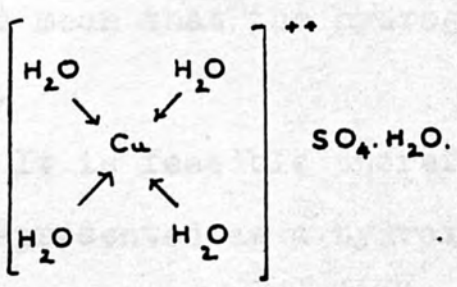
It is not possible to fit four hydroxyl groups of the polyol round a formally isolated copper atom, even if octahedral coordination is allowed, while maintaining the Cu-O distances inferred by the crystalline structure. But the compound is of interest in that it suggests that the O-O distance in a square planar configuration of hydroxyl groups around a copper atom is of the same order of magnitude as the inter-oxygen distance in an αT-diol group in an acyclic polyol.

A possible structure for basic copper acetate, by analogy with basic lead acetate[7], might be that represented below:



IX

The one uncoordinated molecule of water is analogous to that present in cupric sulphate[21], ($CuSO_4.5H_2O$).



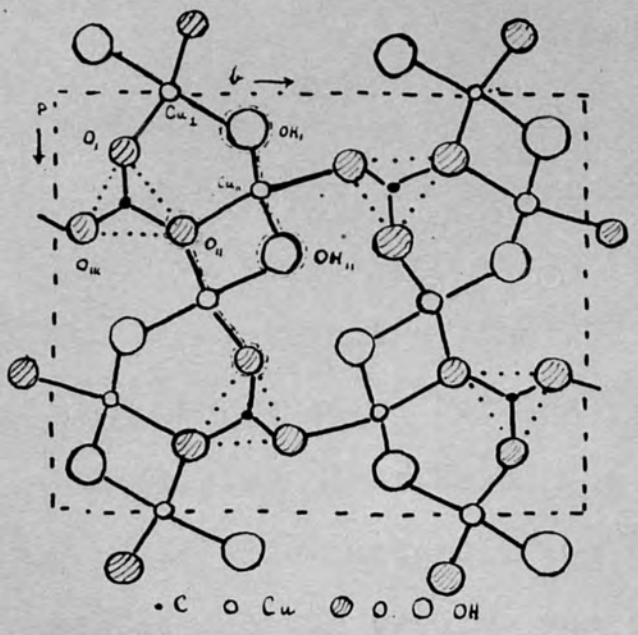
X

It is known that copper does form dimeric coordination compounds in which the copper atoms may be linked through

hydroxo- or alkoxo-bridges. McWhinnie[24] deduced from the magnetic moment of $[(2\text{-ampy})_2(OH).Cu.ClO_4]_2$, where 2-ampy is the 2-aminopyridyl ligand, that the compound is a dimer; either a "copper acetate" structure with the hydroxo-groups occupying similar positions to the water molecules in copper acetate monohydrate (Fig. II.2) or a structure involving two bridging hydroxo-groups.

In a later paper McWhinnie[26] notes the preparation of alkoxo-bridged complexes of the type $[(2\text{-ampy})_2Cu(OR)]_2(NO_3)_2$ from the reaction of copper nitrate trihydrate and 2-aminopyridine in methanol and ethanol, and states that complexes of the form $[(2\text{-ampy})_2Cu(OR)]_2X_2$ are believed to be oxygen-bridged dimers. In $[(2\text{-ampy})_2Cu(OH)]_2(NO_3)_2$ the CuO_2Cu system is probably planar, but it is suggested that the splitting of the OH stretching band in the infra-red spectrum could mean that the hydrogen atoms are not coplanar with the ring.

It is feasible therefore that basic copper acetate could be represented as a hydroxo-bridged dimeric compound. A somewhat distorted dimeric ion of this type could, from consideration of molecular models, complex with the [1,2,3,4-(αT,αT,αT)]- or [1,2,3,4-(α,αT,αT)]-tetritol group. A projection of this dimer on to a plane may be drawn as XI.



The broken lines from Cu atoms represent bonds to O or OH in adjacent unit cells (broken circles).

Cu_I { O_I, O_{II}, OH_I, OH_{II} } 1·98 Å; { O'_I, O'_{III} } 2·71 Å

Cu_{II} { O_{II}, O_{III}, OH_I, OH_{II} } 2·01 Å; { OH'_{II}, OH'_I, OH'_{II} } 2·41 Å

Fig. II.1. The structure of basic copper carbonate (malachite.).

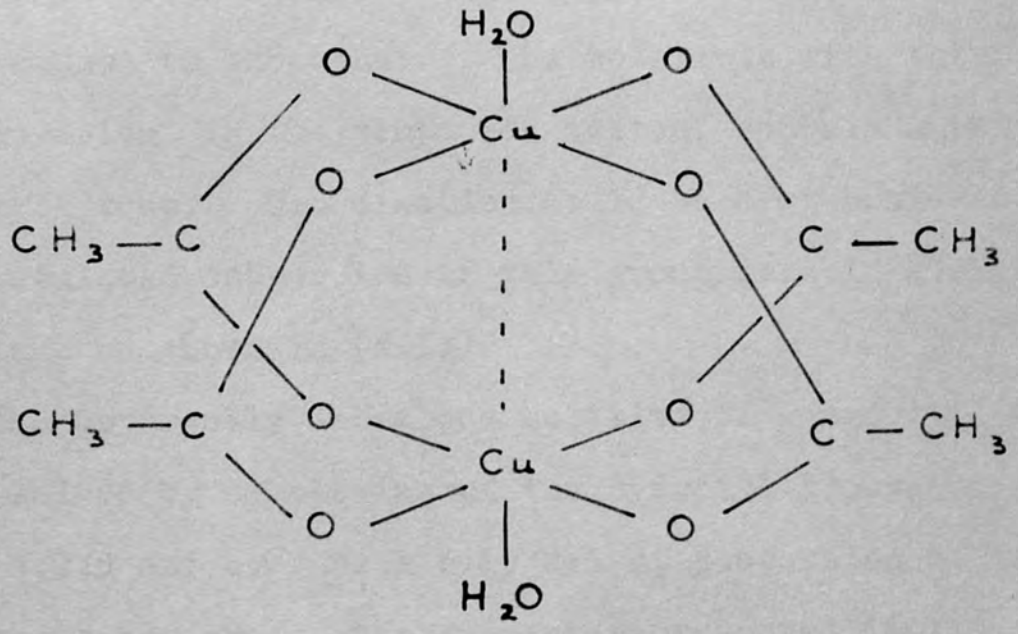


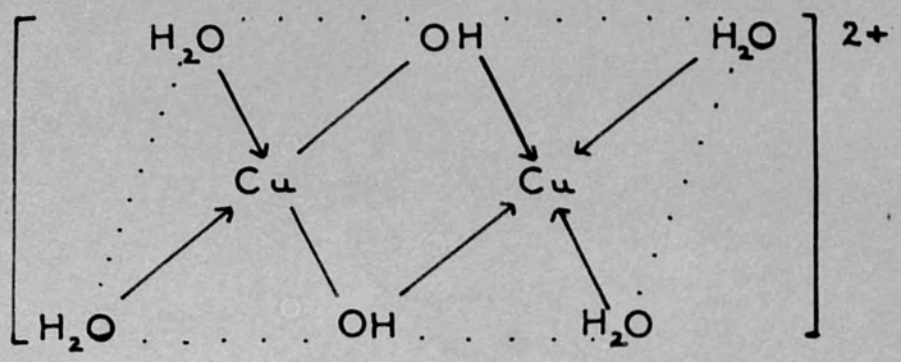
Fig II 2. Copper acetate monohydrate $Cu_2(CH_3COO)_4 . 2H_2O$.

though in fact the copper atom must be slightly above the plane of the oxygen atoms to maintain the Cu-O-R angle the same as that of the ROH group of the original polyol, when complexed. For the [1,2,3,4-(αT,αT,αT)]-tetritol the complex might be of the form (XII).

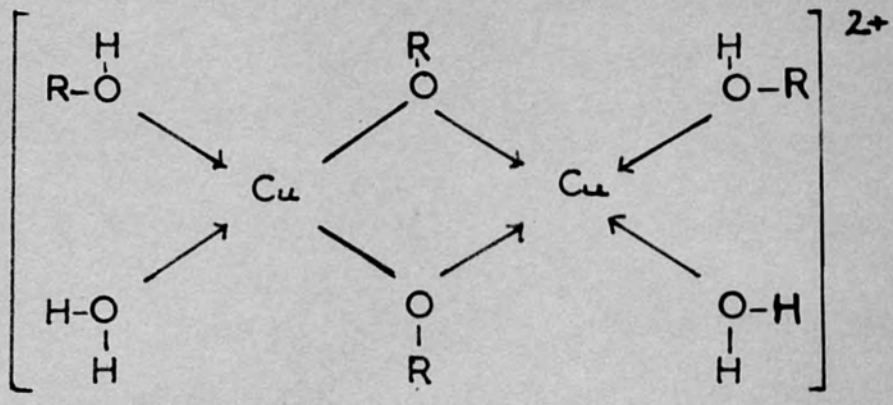
In this instance, two water molecules have been replaced by OR groups, and the hydroxo-bridges by alkoxo-bridges.

The second type of complexing tetritol postulated, the [1,2,3,4-(αT,αT,αC)] or [α,αT,αC]- system, cannot coordinate all four hydroxyl groups to the dimeric ion unless one hydroxo-bridge is broken and the plane containing the oxygen atoms linked to one copper atom is rotated slightly with respect to the other. The tetritols with this configuration, in the compounds tested, contain a primary hydroxyl group. Coordination would be best achieved with the postulated copper ion if this group were in a skew position, as shown in (XIII).

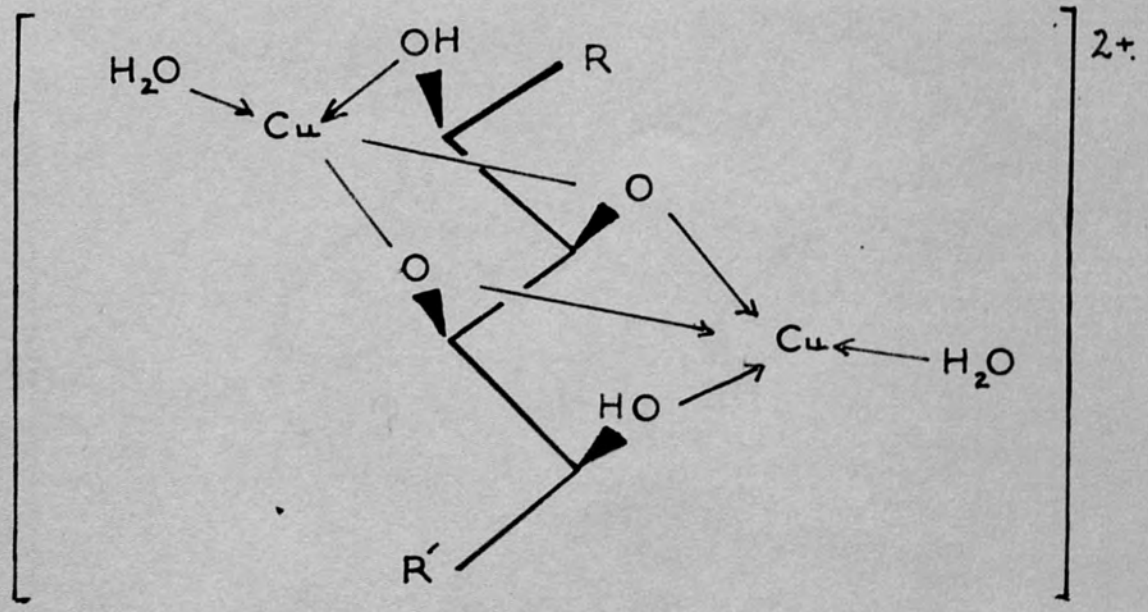
The apparently anomalous mobility of D-mannitol could be explained by complexing of the hydroxyl groups on carbon atoms 1,2,5 and 6. With only slight distortion of the planar zig-zag chain, the complex represented in XIV is possible.


H2O
OH
H2O
2+
Cu
Cu
H2O
OH
H2O

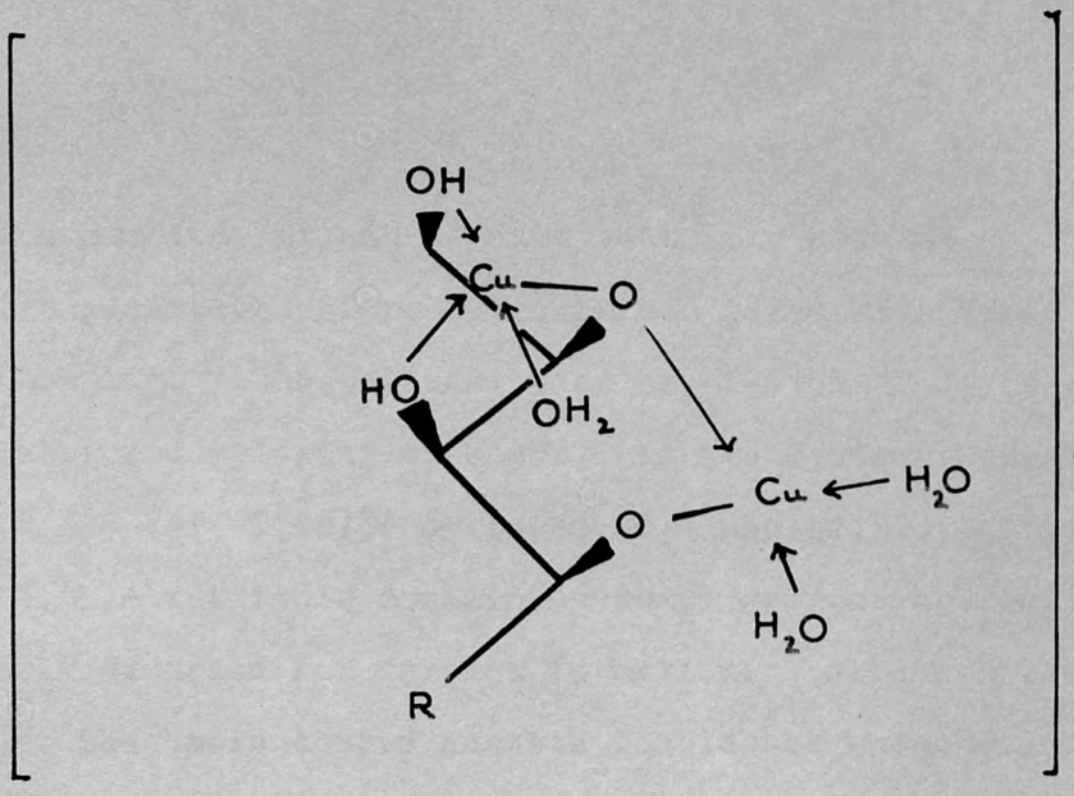
XI (a)


H
R-O
R
O
H
O-R
2+
Cu
Cu
H-O
H
O
R
O-H
H

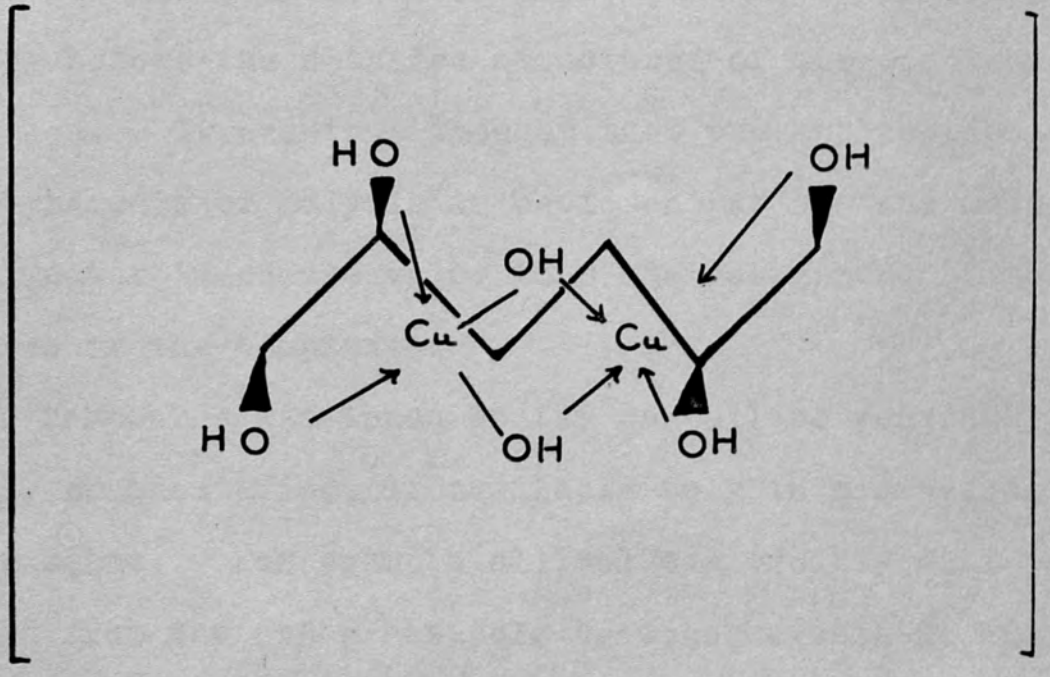
XI (b.)


H2O
OH
R
Cu
O
O
Cu
H2O
HO
R'
2+

XII



XIII.



XIV.

To summarise, it can be said that many acyclic polyhydroxy-compounds form cationic complexes with basic copper acetate. The possession of an αT-diol group is essential for complexing to occur, and two systems, namely the [1,2,3,4-(αT,αT,αT)]- or (α,αT,αT)- and [1,2,3,4(αT,αT,αC)] or (α,αT,αC)- tetritols contain hydroxyl groups particularly favourably disposed for complex formation. Although the nature of the basic copper acetate ion is not known exactly, it is possible to speculate that it is of a dimeric hydroxo-bridged type, but in view of the uncertainty of the structure of the complexing cation derived from basic copper acetate, and the ratio of copper to polyol in a complex which may not be the same for all polyols, it is clear that more studies are necessary before the detailed structures of the complexes can be assigned. It was thus thought that the application of the ionophoresis of polyols in basic copper acetate solution was of greater immediate value than the assignment of the structures of the complexes.

The compounds mentioned so far can all be reasonably resolved, or identified, if available only in micro-quantities, by other means. For example allitol can equally well be separated from the other hexitols by ionophoresis in basic lead acetate[17], while D-mannitol and D-glucitol separate more completely in sodium metavanadate[17]. Allitol and

D-mannitol separate in arsenite[17], while ionophoresis in phenylboronate solution[27] resolves mixtures of L-arabitol and xylitol or ribitol and xylitol; L-arabitol and ribitol migrate differently in sodium germanate[10]. But basic copper acetate ionophoresis has a special application to disaccharide molecules.

Although micro-methods are available for determining the position of the glycosidic linkage to the reducing group of an oligosaccharide, it is more difficult to ascertain the configuration of such a link. Table II.5. shows that all the D-glucopyranosyl-D-glucitols or L-gulitols (i.e. the reduced disaccharides of D-glucose) except laminaribiitol and sophoritol migrate during ionophoresis in basic copper acetate solution, and that each pair of configurational isomers, i.e. α- and β-isomers, can be resolved.

Table II.5. The Mobilities of the D-glucopyranosyl-D-glucitols and L-gulitols in basic copper acetate.

Compound	M_s(Cu)	Link
Kojibiitol	0.18	α1 → 2
Sophoritol	0.00	β1 → 2
Nigeritol	0.29	α1 → 3
Laminaribiitol	0.00	β1 → 3
Maltitol	0.25	α1 → 4
Cellobiitol	0.07	β1 → 4
Isomaltitol	0.50	α1 → 6
Gentiobiitol	0.88	β1 → 6

By the application of ionophoresis in sodium borate, sodium molybdate and finally basic copper acetate, it is possible to determine the position and configuration of the link to the reducing group in a disaccharide of glucose.

The 1 → 3 and 1 → 6 linked disaccharides can be separated from the 1 → 2 and 1 → 4 linked disaccharides by ionophoresis in borate[8]. The disaccharides can then be reduced by sodium borohydride to the corresponding substituted D-glucitol or L-gulitol and subjected to ionophoresis in sodium molybdate which will further separate the 1 → 3 and 1 → 6, and the 1 → 2 and 1 → 4 linked compounds. A final separation of the α- and β-isomers is achieved by ionophoresis in basic copper acetate. The procedure is summarised in Table II.6 below.

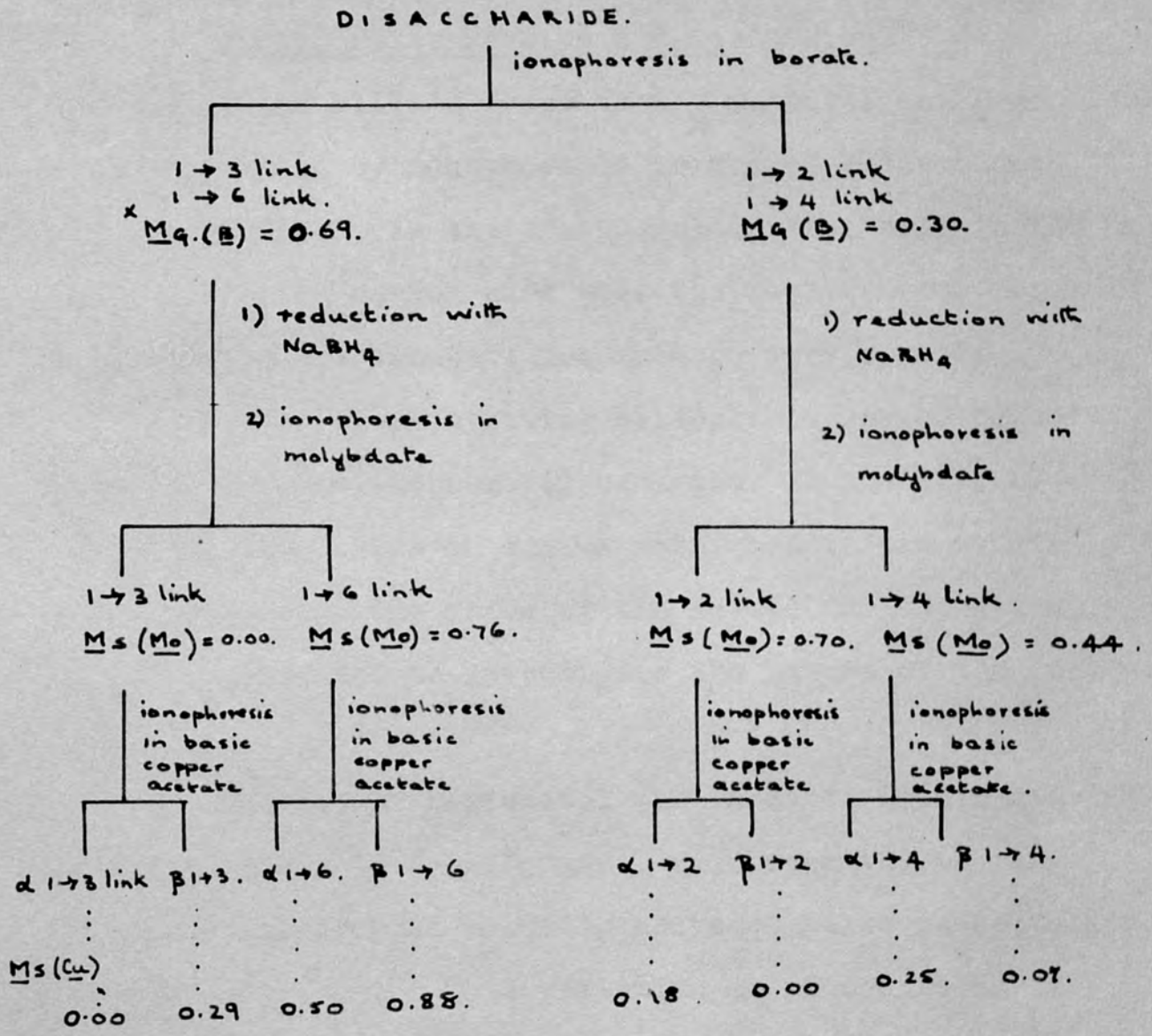
The differentiation between α- and β-linked D-glucopyranosyl-D-glucitols or L-gulitols by basic copper acetate is believed to be due to complexing between the ion and hydroxyl groups on both the cyclic and acyclic parts of the molecule. Since neither the α- nor β-tertiary butyl glucosides migrate in the electrolyte, complexing is unlikely to be facilitated merely by possible modifications of the conformation of the glucopyranose ring caused by the large D-glucitol substituent on carbon atom 1.

Higher homologues of the D-glucopyranosyl-D-glucitols and L-gulitols could probably be separated by the same method, although the increased molecular size would reduce the mobility of the compounds, and separations would take correspondingly longer to achieve. The method might profitably be applied to disaccharides of other sugars, for example mannose. The reducing end of a mannopyranosyl-D-mannose is substituted in the 1 → 2 and 1 → 3 linked disaccharides such that only the 4,6 borate complex can be formed, while in the 1 → 4 and 1 → 6 linked disaccharides, the 2,3 or the 1,2 complex is possible.

By analogy with methyl α- and β-D-mannopyranosides, which complex fairly readily with borate[8], their difference in mobility being attributed to the greater case of formation of the 2,3-complex, which is not hindered by a cis-related methoxyl group in the α-anomer, than the 4,6-complex which is not hindered in the β-anomer[8], it would be expected that the 1 → 4- and 1→ 6-linked disaccharides would migrate faster than the 1 → 2- and 1 → 3-linked disaccharides.
The borohydride reduction of the disaccharides, followed by molybdate ionophoresis would then further separate the sugars, 2-O-mannopyranosyl-D-mannitol has an M_s(Mo) value of 0.80, while 3-O-α-D-mannopyranosyl-D-mannitol has a mobility of M_s(Mo) = 0.[7] The 1 → 4 linked substituted polyol would be expected to have a mobility M_s(Mo) between 0.46 and 0.80 while that of the 1 → 6 linked

mannopyranosyl-D-mannitol would probably again be circa 0.80. The mannose disaccharides can therefore most probably be resolved according to the position of the link and may be separable into α- and β-anomers by ionophoresis in basic copper acetate, as are the disaccharides of glucose.

Table II.6. The separation of D-glucopyranosyl-D-glucoses by ionophoresis in sodium borate, sodium molybdate and basic copper acetate solutions.



× MG (B) is mobility relative to glucose, in borate.

III. The Complexes of polyhydroxy-compounds with the oxy-anions of vanadium.

I) Ionophoretic Results and Discussion.

Frahn and Mills[17] found that D-mannitol and D-glucitol can be separated by ionophoresis in sodium metavanadate, $NaVO_3$, solution. As the two polyols differ only in the configuration on carbon atom two, the requirements for complexing of the vanadate ion must be very specific. For this reason other striking separations may be expected to occur, so the ionophoretic behaviour of a number of polyhydroxy-compounds in sodium metavanadate was studied, both to ascertain the value of the method for separating these compounds and to investigate the nature of the complex formed.

The mobility of D-glucitol was found to attain a steady maximum of 5.0×10^{-5} cm.^{2} sec.$^{-1}$ v.$^{-1}$ over the pH range 7.6 to 8.6 but dropped markedly above or below these values. [Fig. I.1.Expt. 3]. This variation in pH occurs during ionophoresis, presumably due to slowly-reversible changes in the constitution of the vanadate solution, reflected in the deepening colour, from lemon-yellow to orange, in the neighbourhood of the anode. The mobility of D-glucitol did not vary appreciably however, whether the solution was freshly prepared, when the pH was 8.6, or whether the pH had decreased slightly after use.

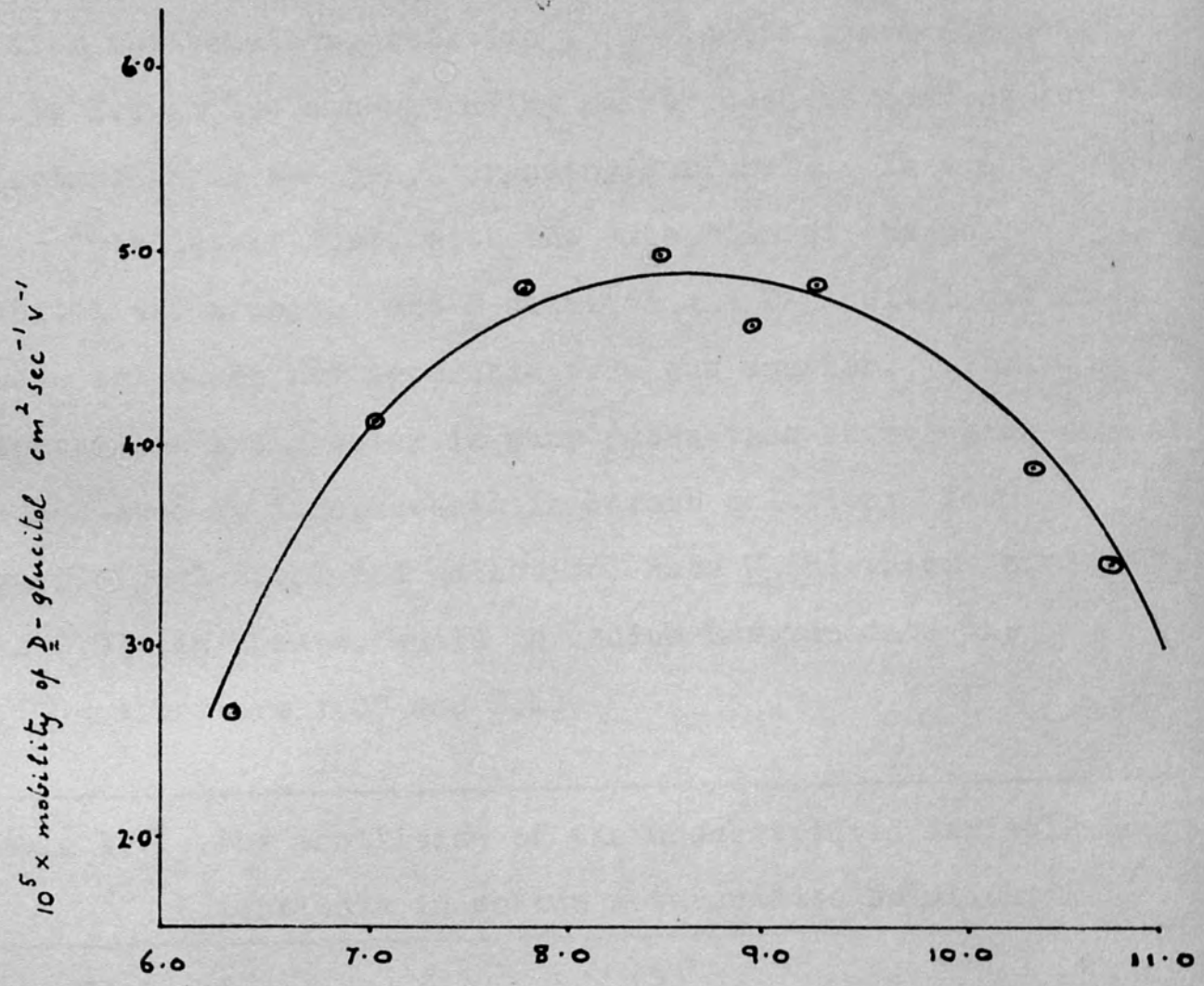


Fig. I.1 The effect of change in pH on the mobility of D-glucitol in sodium metavanadate solution.

The mobilities, $\underline{M}_S(\underline{V})$ of the hexitols and pentitols in sodium metavanadate, relative to D-glucitol, are given in Table I.1. The non-migrating marker used to correct for electroosmosis was 5-hydroxymethylfurfural. It can be seen immediately that, with the exception of the pairs ribitol and arabitol and D-altritol and D-glucitol any of these compounds are separable from one another. The separations are greater in many cases than those which can be achieved by ionophoresis in borate solution: for example D-glucitol and galactitol have $\underline{M}_G(\underline{B})$ values of 0.83 and 0.97[7] in borate, while in sodium metavanadate the $\underline{M}_S(\underline{V})$ values are 1.00 and 0.48.

Table I.1. The mobilities of the unsubstituted hexitols and pentitols in sodium metavanadate solution.

Compound	$\underline{M}_S(\underline{V})$
Iditol	1.50
D-Glucitol	1.00
D-Altritol	0.98
Allitol	0.70
Galactitol	0.48
D-Mannitol	0.40
Xylitol	0.60
D-Lyxitol	0.36
L-Arabitol	0.32
Ribitol	0.31

Substitution of the hydroxyl groups of the hexitols and pentitols in general reduces their mobility (Table I,2), but provided that four adjacent hydroxyl groups remain, complexing still occurs. This simple correlation is insufficient to account for the complexing, however, as 3-deoxy-L-gulitol (4-deoxy-D-glucitol), with an $M_s(V)$ = 0.34, does not contain four adjacent hydroxyl groups.

Table I.2. The mobilities of the substituted hexitols and pentitols in sodium metavanadate solution.

Compound	$M_s(V)$
2-deoxy-L-gulitol	1.15
1-O-methyl-L-gulitol	0.68
1-deoxy-D-altritol	0.67
2-deoxy-D-galactitol	0.54
1-deoxy-D-xylitol	0.48
1-deoxy-D-talitol	0.37
1,6-dideoxy-D-altritol	0.34
1,6-dideoxy-galactitol	0.34
1,2-di-O-methyl-D-mannitol	0.34
6-deoxy-L-galactitol	0.33
3-deoxy-L-gulitol	0.33
1-deoxy-D-arabitol	0.26

Table I.2 (continued).

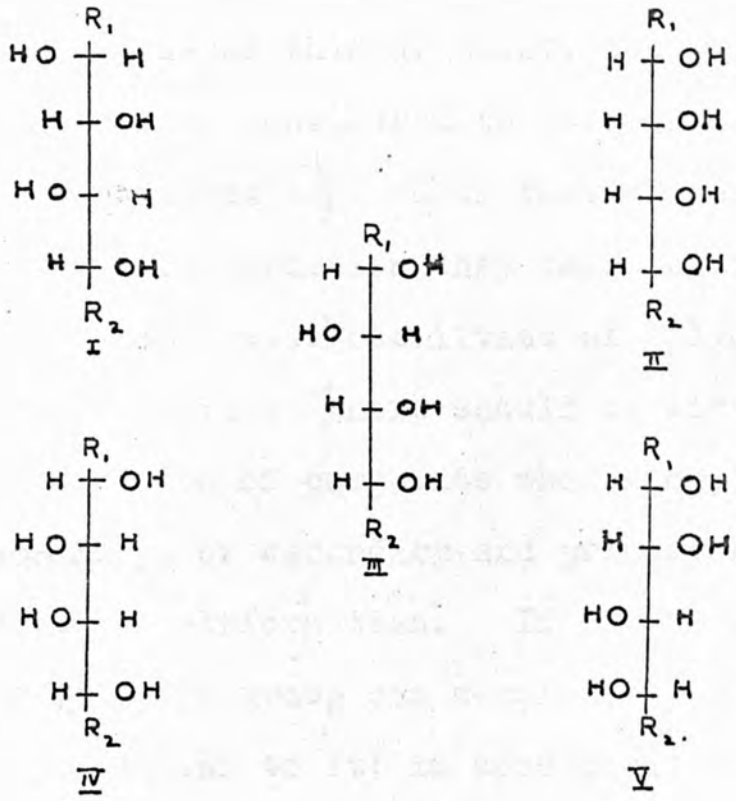
1,6-dideoxy-L-mannitol	0.20
2,5-O-methylene-D-mannitol	0.14
2-deoxy-D-glucitol	0.12
2,5-di-O-methyl-D-mannitol	0.11
3-deoxy-D-glucitol + 3-deoxy-D-mannitol	0.11
1-deoxy-D-lyxitol	0.11
2-deoxy-D-ribitol	0.04

To explain the order of ionophoretic mobilities of polyols in sodium metavanadate, one can start with the premiss that the inter-oxygen distances which occur in a diol or 1,2,3-triol are insufficient by themselves to define the complex formed. This is justifiable since none of the diols or triols tested migrated appreciably, (Table I.3) and wide variations in mobility are shown by compounds which, while comprising more than three adjacent hydroxyl groups, can contain equivalent triol systems.

Table I.3. The Mobilities of some Diols, Triols and Tetritols in Sodium Metavanadate solution.

Compound	$\underline{M}_s(\underline{V})$
Butane-*cis*-2,3-diol	0.08
Butane-*trans*-2,3-diol	0 - 0.35
Butane-1,2,4-triol	0.06
Glycerol	0.00
D-Threitol	0.1 - 0.2
Erythritol	0.13 - 0.21

If four adjacent hydroxyl groups are required for complex formation, the possible orientations which can exist are I (1,2,3,4-aT,aT,aT)-, or (1,2,3,4-a,aT,aT), II (1,2,3,4-aC,aC,aC,)- or (1,2,3,4-a,aC,aC)-, III (1,2,3,4-aC,aT,aT)-, IV (1,2,3,4-aT,aC,aT)- or (1,2,3,4-a,aC,aT)-, V (1,2,3,4-aC,aT,aC)- or (1,2,3,4-a,aT,aC)- tetritols.



I

III

II

IV

V

I is contained in iditol $\underline{M}_S(\underline{V})$ = 1.5, 2-deoxy-$\underline{L}$-gulitol $\underline{M}_S(\underline{V})$ = 1.15, $\underline{D}$-glucitol $\underline{M}_S(\underline{V})$ = 1.0, but also in 1-$\underline{O}$-methyl-$\underline{L}$-gulitol $\underline{M}_S(\underline{V})$ = 0.68 and 1-deoxy-$\underline{D}$-xylitol $\underline{M}_S(\underline{V})$ = 0.48. II is contained in $\underline{D}$-altritol $\underline{M}_S(\underline{V})$ = 0.98, $\underline{D}$-allitol $\underline{M}_S(\underline{V})$ = 0.70, $\underline{D}$-ribitol $\underline{M}_S(\underline{V})$ = 0.31, III in $\underline{D}$-glucitol $\underline{M}_S(\underline{V})$ = 1.0, 1-deoxy-$\underline{D}$-xylitol $\underline{M}_S(\underline{V})$ = 0.48, $\underline{L}$-arabitol $\underline{M}_S(\underline{V})$ = 0.32. IV is contained in 1,2-dimethyl-$\underline{D}$-mannitol $\underline{M}_S(\underline{V})$ = 0.34, galactitol $\underline{M}_S(\underline{V})$ = 0.48, 2-deoxy-$\underline{D}$-glucitol $\underline{M}_S(\underline{V})$ = 0.12 and V in $\underline{D}$-mannitol $\underline{M}_S(\underline{V})$ = 0.40, 1,6-dideoxy-$\underline{L}$-mannitol $\underline{M}_S(\underline{V})$ = 0.20, $\underline{L}$-arabitol $\underline{M}_S(\underline{V})$ = 0.32.

For the purpose of this argument, the primary hydroxyl groups have been considered to rotate freely and their ability to complex equated to that of secondary hydroxyl groups whose conformation they can sustain. By analogy with the ionophoretic mobilities of polyols found in tungstate solutions, there should be little difference in the migration of compounds whose complexing site contains all secondary, or secondary and primary hydroxyl groups in an equivalent conformation. If on the other hand the primary hydroxyl group can complex, by virtue of the rotation peculiar to it, in some position which is intermediate between the cis or trans positions relative to the adjacent hydroxyl group, there should be a correlation between the mobilities of compounds containing the same tetritol incorporating an α group. Neither the [1,2,3,4-α,αC,αC]- nor [1,2,3,4-α,αC,αT]-, nor [1,2,3,4-(α,αT,αC),]- tetritols occasion consistent mobilities in the compounds in which they occur.

From the mobilities cited above, it immediately becomes obvious that no one tetritol group is responsible for complexing. Neither is it possible to say that there is an equilibrium between the complex ion and the tetritol, in which a particular configuration takes precedence over all the others as a complexing agent. If this were so, at least one of the tetritol configurations should correspond

to a definite mobility, irrespective of which compound contains it, when allowance has been made for slight differences in the molecular size of the polyols containing a particular grouping. It might then have been possible to arrange the remaining molecules according to the probability of one tetritol complexing rather than another.

Since four adjacent hydroxyl groups do not necessarily define the complex, further inter-oxygen distances must be considered. The γT-diol occurs in D-altritol, $M_s(V)$ = 0.98, but also in 1-deoxy-talitol, $M_s(V)$ = 0.37, while the γC-diol occurs in D-glucitol, $M_s(V)$=1.0, and in 3-deoxy-D-glucitol, $M_s(V)$ = 0.11. There are other similar examples in the compounds given in the tables. Moreover the smallest ring which could be obtained from such complexing is seven-membered, even if only one vanadium atom is incorporated, so that such complexing is relatively unlikely to occur. The hydroxyl groups further dispersed along the carbon chain suffer increasingly from drawbacks of the instability of the ring which might be formed.

It is valuable to consider the variation in the mobility of a particular hexitol on substitution of the hydroxyl groups and to derive from this, and the mobilities of compounds containing equivalent partial conformations of the hexitol, how many of the hydroxyl groups are essential for

complexing to be of the same type as that shown by the hexitol itself. Although sufficient compounds were not available to derive the necessary data for all the hexitols, some points of interest arise from such an argument.

The $\underline{\underline{M}}_s(\underline{V})$ of unsubstituted $\underline{\underline{D}}$-glucitol is 1.0. The substitution of the C_6 hydroxyl group by a methyl group reduces the mobility to 0.68, which is a reduction which would not be anticipated from the change in size of the polyol molecule. The low mobilities of 2-deoxy- and 3-deoxy-$\underline{\underline{D}}$-glucitol indicate that both these hydroxyl groups are involved in the complexing of $\underline{\underline{D}}$-glucitol, while 2-deoxy-$\underline{\underline{L}}$-gulitol, migrating with an $\underline{\underline{M}}_s(\underline{V})$ = 1.15, suggests that the hydroxyl group on C5 is not involved. From the mobility of $\underline{\underline{L}}$-arabitol, $\underline{\underline{M}}_s(\underline{V})$ = 0.32 it is reasonable to deduce that the C_1 - OH in $\underline{\underline{D}}$-glucitol is essential to produce a mobility of circa 1.0.

By comparison with the mobilities of 1-deoxy-$\underline{\underline{D}}$-altritol, 1-deoxy-$\underline{\underline{D}}$-talitol, 3-deoxy-$\underline{\underline{D}}$-mannitol and 2-deoxy-$\underline{\underline{D}}$-galactitol, it appears that the mobility of $\underline{\underline{D}}$-altritol is dependent upon at least the hydroxyl groups on carbon atoms 1,3,5, and 6.

The hydroxyl groups on C_1 and C_3 of $\underline{\underline{D}}$-allitol are required for a mobility of 0.70 [cf. the mobilities of ribitol and 3-deoxy-$\underline{\underline{D}}$-glucitol], while since the mobility

of xylitol is less than that of iditol, the C_1 - OH of the hexitol must be involved in the complexing.

Galactitol requires the C_1 - OH, C_4 - OH and C_6 - OH groups to complex [cf. the mobilities of 6-deoxy-L-galactitol, 3-deoxy-L-gulitol], while D-mannitol requires the hydroxyl groups on C_1, C_2 and C_3. [cf. the mobilities of 3-deoxy-D-mannitol, and 2-deoxy-D-glucitol].

From this closer study of the mobilities of the hexitols, particularly D-glucitol, it appears that there is a type of complexing peculiar to the hexitols, and probably to certain pentitols, notably xylitol, 2-deoxy-D-galactitol, 1-deoxy-D-altritol, 6-O-methyl-D-glucitol and possibly to tetritols of the (1,2,3,4-αT, αT, αT) type (1-deoxy-D-xylitol) resulting in a higher mobility than is evinced by tetritol groupings alone. If these hexitols and pentitols are temporarily excluded, the remainder of the compounds fall into four well-defined sets: those containing I (1,2,3,4-αC, αC, αT)-, II (1,2,3,4-αT, αC, αT)- and III (1,2,3,4-αC, αT, αC)- tetritols and those containing less than four adjacent hydroxyl groups. The compounds which comprise I and II move with an $\underline{M}_s(\underline{V})$ of the order of 0.3, while those of type III move more slowly: 1,6-dideoxy-L-mannitol $\underline{M}_s(\underline{V})$ = 0.20, 1-deoxy-D-lyxitol

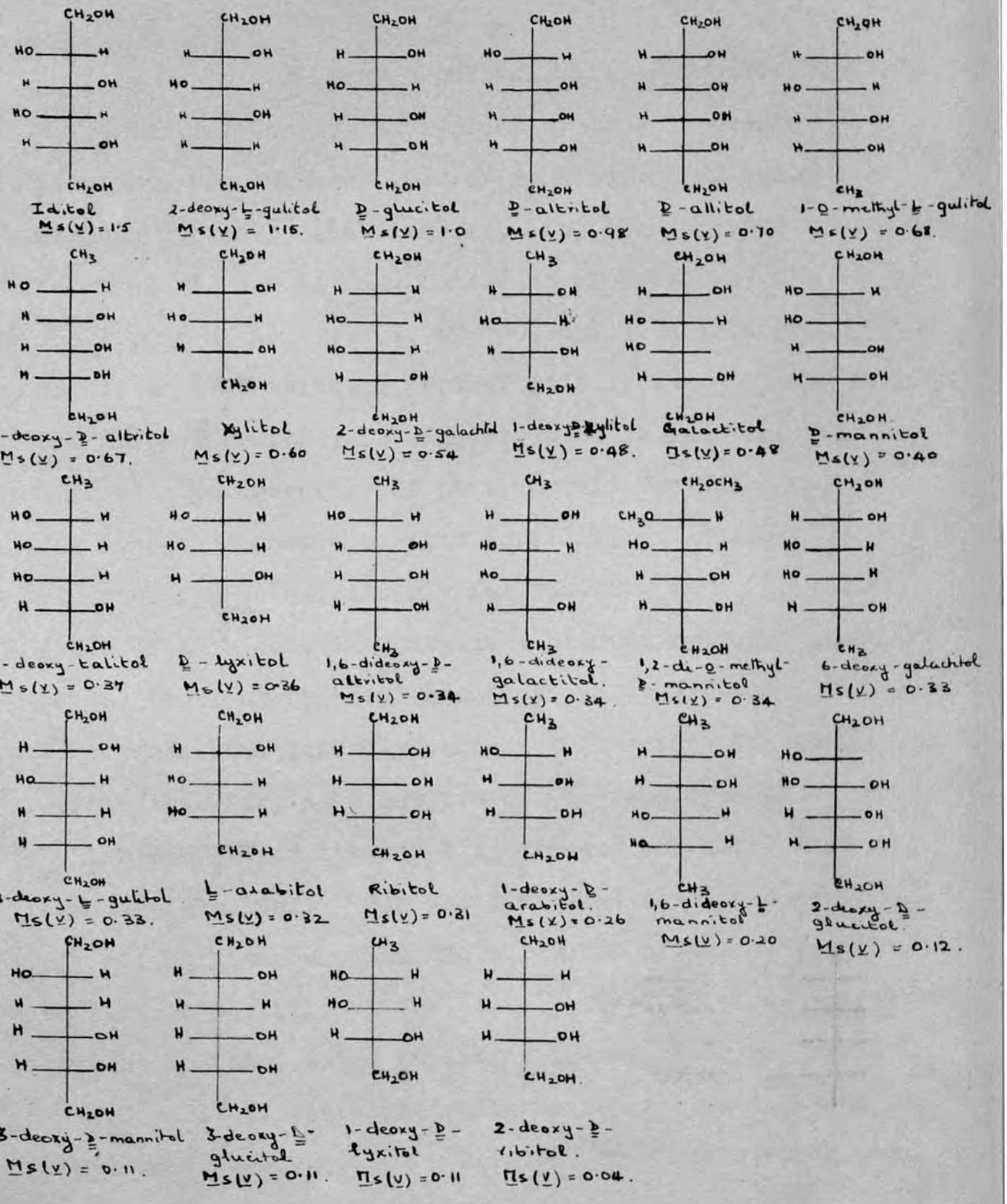


FIG. I. 2. THE CONFIGURATIONS OF POLYOLS MIGRATING ON IONOPHORESIS IN SODIUM METAVANADATE SOLUTION.

$\underline{M}_s(\underline{V})$ = 0.11. 3-Deoxy-L-gulitol, with a mobility of 0.33 suggests that one hydroxyl group of type II can be dispensed with without loss of mobility. If this were so, the virtually identical mobility of compounds containing tetritols I and II may rather be explained by the complexing of the [1,2,4, αT, βC]-triol. This group occurs as a constituent part of iditol, the compound with the highest mobility in vanadate electrolyte, and may well be a fundamental, if partial, unit for the complexing. One exception occurs to this correlation: 2-deoxy-D-glucitol has an anomalously low mobility of $\underline{M}_s(\underline{V})$ = 0.11. Alone of this set, it contains a primary hydroxyl group β to the terminal hydroxyl group of the type I or II tetritol.

D-mannitol probably belongs to the type II complexing rather than forming a complex of the same type as D-glucitol, since its mobility closely resembles that of 1-deoxy-D-talitol. The mobilities of these two compounds are slightly higher than the remainder of the set, a reflection possibly of the (1,2,3,4,5-αC, αC, αT, αC)-pentitol, present in them, which is absent in the rest.

Thus it is postulated that there are two types of complexes: one requires a hexitol, or a pentitol of the type of 1-O-methyl-L-gulitol, 2-deoxy-L-gulitol, 1-deoxy-D-altritol, 2-deoxy-galactitol or -xylitol, and a tetritol of the (1,2,3-αT, αT, αT) type; the other

complexes with (1,2,3,4,5-aC, aC, aT, aC) pentitols, or tetritols other than (1,2,3,4-aT, aT, aT).

The variation in complexing may be due to a different ion or to a different number of polyol molecules being coordinated to the same ion.

Consider first an ion which complexes with D-glucitol. It has just been established that five hydroxyl groups are necessary for this particular complex to be formed. If complexing occurs by condensation the vanadate ion must therefore itself contain at least six hydroxyl groups, so that one is ionisable, giving the complex a negative charge and causing it to migrate. Since vanadium has a co-ordination number of 5, a dimeric (or further condensed) species of some sort must be concerned in the complexing. To pursue this argument it is necessary to turn to the investigation of the types of ion present in solutions of sodium metavanadate.

Meanwhile it is convenient to interpolate here the mobilities of the cyclic sugars and cyclitols found in sodium metavanadate, and to correlate these with the proposals already put forward for the acyclic polyols.

Table I.4. The Mobilities of Inositols and Cyclic Sugars and their derivatives in Sodium Metavanadate Solution.

Compound	M_s(V)	Compound	M_s(V)
D-glucoheptose	0.24	Methyl-β-L-arabino-pyranoside	0
D-glucose	0.1	Methyl-β-L-lyxo-pyranoside	0
Methyl-α-D-gluco-pyranoside	0	Methyl-α-D-xylo-furanoside	0
Methyl-β-D-gluco-pyranoside	0	D-fructose	0.45
		1-O-methyl-D-fructose	0.19
Tertiary butyl-α-D-glucopyranoside.	0	3-deoxy-D-fructose	0.10
Tertiary butyl-β-D-glucopyranoside	0	1,3,4-tri-O-methyl-D-fructose.	0
2-O-methyl-D-glucose	0.1	L-sorbose	0.20 - 0.80
		D-tagatose	0.39
2-deoxy-D-glucose	0.11	Sucrose	0.26
3-O-methyl-D-glucose	0	Turanose	0.26
5-deoxy-D-xylo-hexofuranose	0.79	Maltulose	0.31
		Leucrose	0.45
6-O-methyl-D-glucose	0	Isomaltulose	0.35
6-deoxy-D-glucose	0.10	Epi-inositol	0.1 - 0.26
D-mannose	0	(+)-inositol	0.13
D-gulose.	0.17	Scyllo-inositol	0
D-galactose	0	Muco-inositol	0.12
2-deoxy-D-galactose	0.16		

Table I.4. (continued).

2-O-methyl-D-galactose	0	Allo-inositol	0.11
1,6-anhydro-D-galactose	0	Myo-inositol	0.12
L-fucose	0	Cis-inositol	
1,6-anhydro-altro-pyranose	0.21		
D-ribose	0.40		
Methyl-α-D-ribo-pyranoside	0		
Methyl-β-D-ribo-pyranoside	0		
2-deoxy-D-ribose	0		
L-arabinose	0		
D-lyxose	0.10		
D-xylose	0.10		

From the table it can readily be seen that very few of the cyclic sugars and cyclitols tested show any appreciable mobility in sodium metavanadate solution. The compounds which do migrate fairly fast are 5-deoxy-D-xylo hexafuranose, D-ribose, D-fructose, D-tagatose and L-sorbose and the D-glucopyranosyl-D-fructoses.

Fig. I.3 CONFORMATIONS OF THE CYCLIC SUGARS WHICH DO NOT MIGRATE IN SODIUM METAVANADATE.

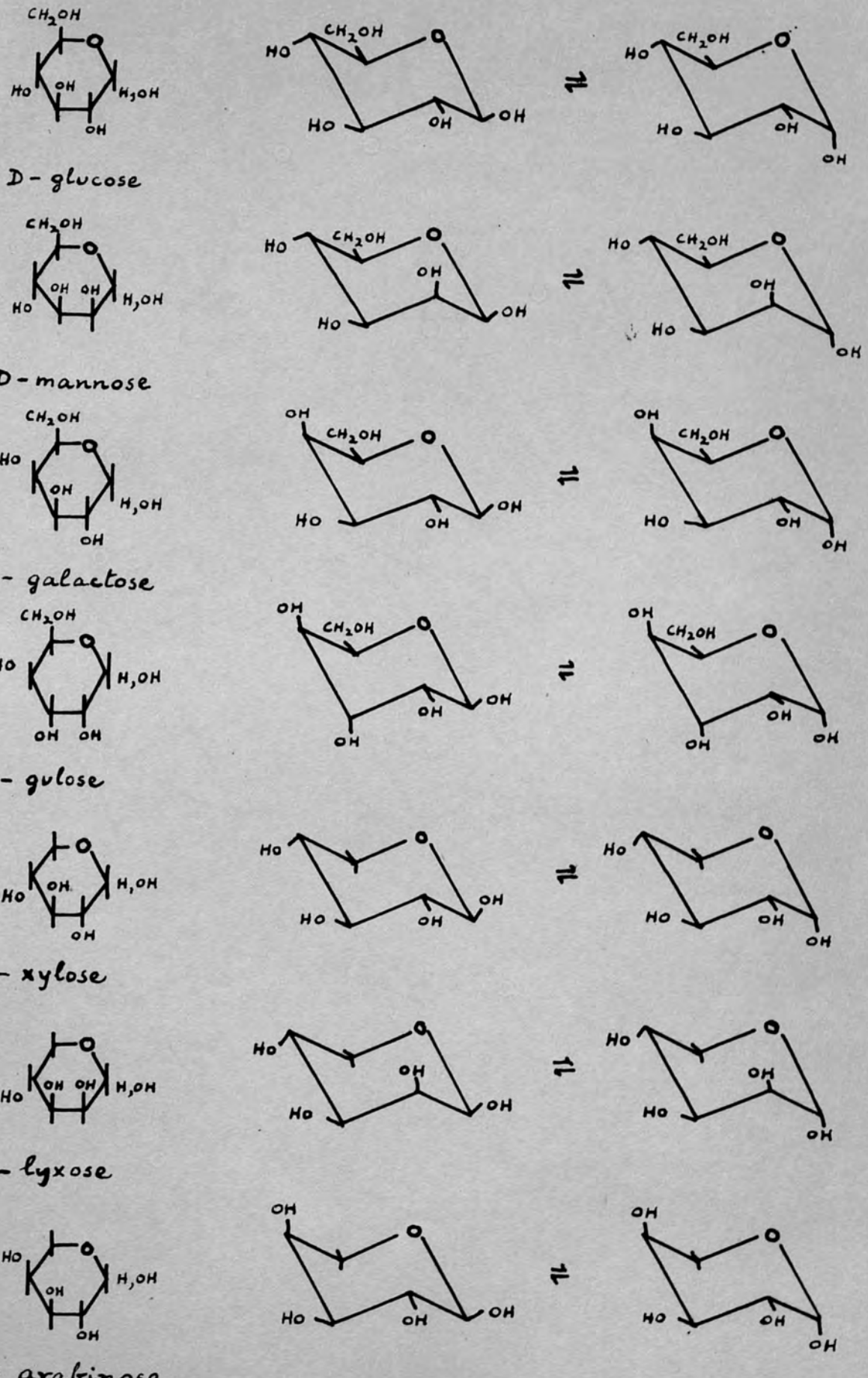
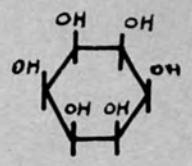


Fig. I.4. THE CONFORMATIONS OF THE INOSITOLS



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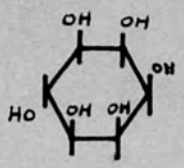

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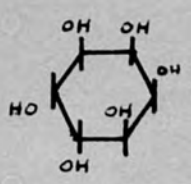
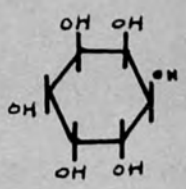


allo



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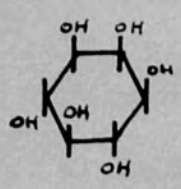
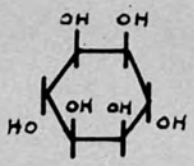
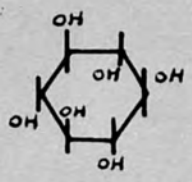


myo



muco



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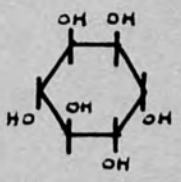

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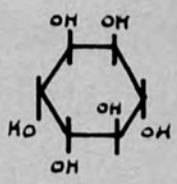
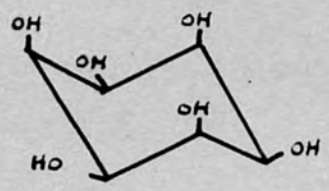
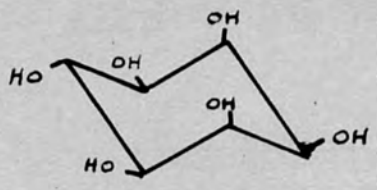


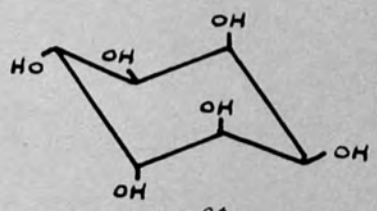
levo



cis



epi



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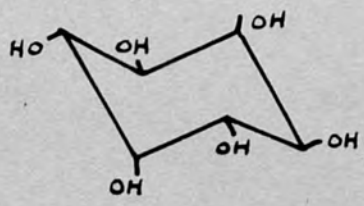
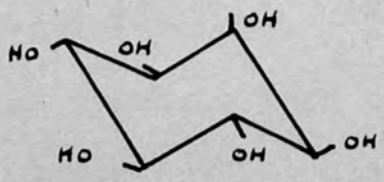
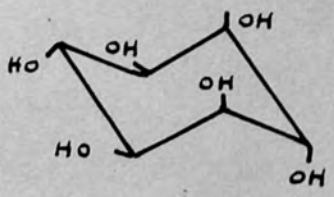


neo



myo



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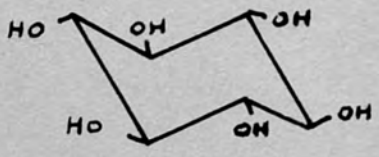
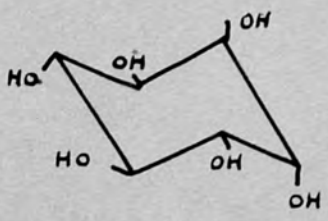
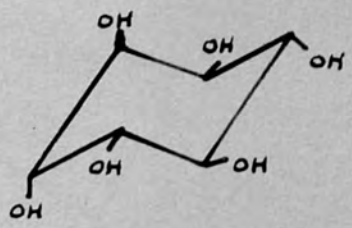


scyllo



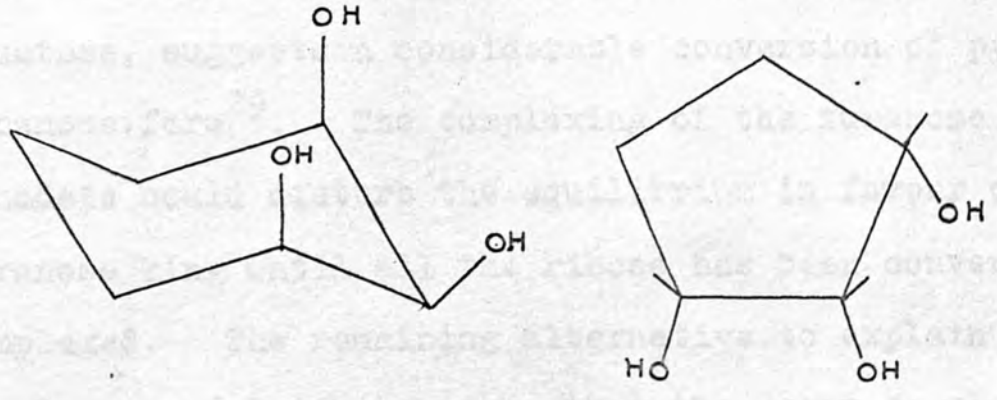
dextro



levo

Consider first the conformation of D-ribose: this sugar can exist in solution as a pyranose ring, a furanose ring or an aldehydo form. The C.1 conformation of α-D-ribopyranose contains a (1,2,3,4-ax,eq,ax,eq)-tetritol which is not evident in the possible conformations of the other sugars listed. In order to check whether such a group is significant for complexing, the mobility of D-talose should be found. However, judging by the mobilities of cis- and epi-inositol this factor can probably be discounted.

The α-D-ribofuranose ring contains a cis,cis-1,2,3-triol on carbon atoms 1,2, and 3 which is not reproduced in D-arabofuranose or D-xylofuranose. The cis, cis-1,2,3-triol is also present in the pyranose form of ribose, lyxose, mannose and gulose but the spatial disposition of the hydroxyl groups is different leading to different oxygen-oxygen distances.



5-deoxy-D-xylo-hexofuranose on the other hand can assume a conformation equivalent to that of α-D-ribofuranose incorporating the hydroxyl groups on C_1, C_2 and C_6. The furanose forms of β-D-lyxose, β-D-mannose and α-D-gulose also contain a cis, cis-1,2,3-triol although these compounds migrate much more slowly in sodium metavanadate solution. If this triol is responsible for the complexing of D-ribose, the argument turns on the relative proportions of furanose form present in solutions of D-ribose, D-lyxose, D-mannose and D-gulose. The complex mutarotation of D-ribose in aqueous solutions suggests a higher proportion of forms other than the pyranose ring present in solutions of this sugar than the other sugars[29]. There is evidence for a much greater amount of the aldehydo form in solutions of D-ribose than in any other sugar, and the study of the rapid mutarotation, the magnitude of the reaction constant, the small activation energy and the influence of pH on the rate of mutarotation, closely resembling the data for fructose, suggests a considerable conversion of pyranose to furanose form[29]. The complexing of the furanose form with vanadate could disturb the equilibrium in favour of the furanose ring until all the ribose has been converted and complexed. The remaining alternative to explain the complexing of D-ribose, i.e. that the sugar complexes in

the aldehydo form which resembles -ribitol, is not borne out exactly by the mobility, which is higher than that of -ribitol, although the aldehydo-sugar would contain the same tetritol group. Also, the suggestion that the furanose ring of D-ribose complexes receives support from the mobilities of some D-glucopyranosyl-D-fructoses, and fructose itself. Thus D-fructofuranose (or pyranose) can contain a triol, equivalent to the cis, cis-1,2,3-triol, on carbon atoms 1,2, and 3 owing to the relative freedom of rotation of the primary hydroxyl group. The same system occurs in D-tagatose and L-sorbose. Leucrose, being a 5-substituted fructose, cannot form a furanose ring. The glucose moiety is not complexing, but the fructopyranose ring again contains the triol on C_1, C_2 and C_3 equivalent to the cis, cis-1,2,3-triol of ribofuranose. The argument can be extended to maltulose but not to turanose or isomaltulose. The latter sugar however contains an O-O distance, spanning the furanose ring between the primary hydroxyl group on C_1 and the secondary hydroxyl group on C_4 equivalent to the $O_1 - O_3$ distance in the cis, cis-1,2,3-triol, while in turanose the hydroxyl groups on C_2 of the glucopyranose ring and C_2 of the fructopyranose ring can be similarly suitably disposed. Sucrose, containing a fructofuranose ring, has the possibility of partial complexing: from C_3-OH to C_6 -OH.

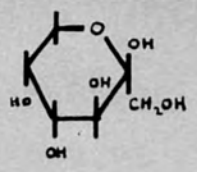
The difference in ease of complexing of the D-glucopyranosyl-D-fructoses is reflected in their mobilities: leucrose migrates noticeably faster than the other disaccharides.

So far the only disaccharides which have been mentioned are the D-glucopyranosyl-D-fructoses. The mobilities of the D-glucopyranosyl-D-glucoses and their reduction products in sodium metavanadate solution were also found (Table I.5.).

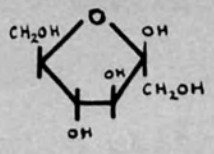
Table I.5. The Mobilities of the D-glucopyranosyl-D-glucoses and their reduction products in sodium metavanadate solution.

Compound	$M_s(V)$	Link	Compound	$M_s(V)$	Link
α,α-trehalose	0	α-1→1			
Kojibiose	2.9	α-1→2	Kojibiitol	0.0	α-1→2
Sophorose	0.13	β-1→2	Sophoritol	0.0	β-1→2
Nigerose	2.9	α-1→3	Nigeritol	0.0	α-1→3
Laminaribiose	0.10	β-1→3	Laminaribiitol	0.33	β-1→3
Maltose	0.26	α-1→4	Maltitol	1.52	α-1→4
Cellobiose	0 - 0.1	β-1→4	Cellobiitol	0.77	β-1→4
Isomaltose	0.1	α-1→6	Isomaltitol	0.76	α-1→6
Gentiobiose	0.1	β-1→6	Gentiobiitol	0.74	β-1→6

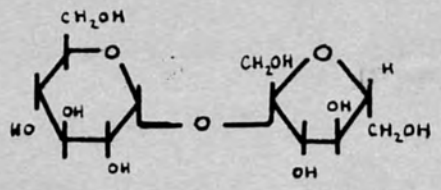
Fig. I. 5. THE CONFIGURATIONS OF FRUCTOSE AND THE D- GLUCOPYRANOSYL - D- FRUCTOSES.



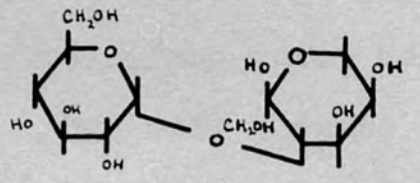
β - D - fructopyranose.



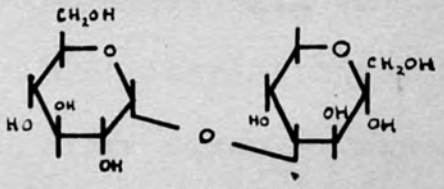
β - D - fructofuranose.



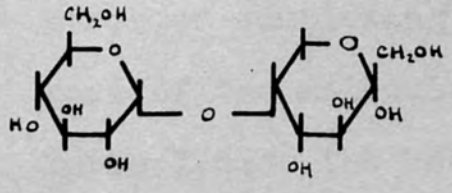
Sucrose



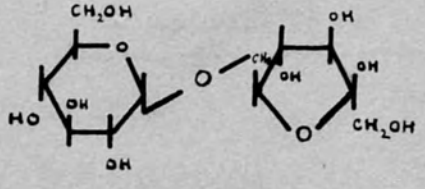
Turanose (3 - O - α - D - glucopyranosyl - D - fructose)



Maltulose (4 - O - α - D - glucopyranosyl - D - fructose)



Leucrose (5 - O - α - D - glucopyranosyl - D - fructose)



Isomaltulose (6 - O - α - D - glucopyranosyl - D - fructose)

Consider first the reducing disaccharides of glucose. Since D-glucose does not migrate on ionophoresis in sodium metavanadate solution neither part of the molecule can be complexing alone. (The possibility that the non-reducing moiety is in an unusual conformation due to the large substituent on C_1, and thus favourably disposed for complexing, can be disallowed since neither α- nor β-tertiary butyl glucoside migrates). Complexing must therefore occur across the link and involve hydroxyl groups from both glucose rings. This is supported by the mobilities of maltose and its homologues which surprisingly increase with the ascent of the homologous series, allowing for retardation of the compound due to its increased molecular size. This effect is not noticeable in the isomaltitol series when it is masked by the obviously stronger complexing involving the substituted D-glucitol part of the molecule.

Table I.6. The Mobilities of the maltose and isomaltitol series in Sodium Metavanadate Solution.

Compound	M_s(V)	Compound	M_s(V)
Maltose	0.26	Isomaltitol	0.76
Maltotriose	0.41	Isomaltotriitol	0.58
Maltotetraose	0.40	Isomaltotetra-iitol	0.56
Maltopentaose	0.44	Isomaltopentaitol	0.47

It is of interest to see whether any interoxygen distances in the disaccharides of glucose correspond to those already postulated as being significant in the complexing of the furanose ring forms of several sugars. In fact the distance d, see figure I.7 below, occurs

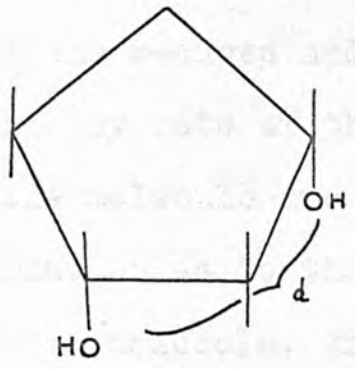


Fig. I. 7.

twice in kojibiose and nigerose, though only once in the other disaccharides. (Only C.1 pyranose conformations considered.)

Among the reduced disaccharides of glucose, two factors may be operating: complexing across the glycosidic link and complexing with the substituted D-glucitol. Neither apparently occurs with kojibiitol, sophoritol or nigeritol. By comparison with 3-deoxy-D-glucitol, complexing with laminaribiitol must take place across the glycosidic link. The mobilities of maltitol and cellobiitol are both markedly greater than that of 3-deoxy-L-gulitol, and those of isomaltitol and gentiobiitol

are greater than that of 1-O-methyl-L-gulitol. The superficial observation of the occurrence of distance d in these compounds cannot be correlated with the mobilities in any simple way, since it is evidenced equally well twice in sophoritol and laminaribiitol. Probably all that can justifiably be concluded from the mobilities of the reduced and non-reduced disaccharides of glucose, at any rate at this stage, is that both moieties of the molecule are involved in the complexing. Without information as to the number of vanadate ions attached to each molecule, which could not be obtained for most because of the scarcity of the material available, or the use of substituted sugars to lessen the number of hydroxyl groups which could complex, it is difficult to further the discussion without becoming purely speculative. Ionophoresis in sodium metavanadate does however augment the separations possible among the disaccharides of glucose, by application of the method already described for basic copper acetate; the two techniques are complementary.

The conclusions which can be drawn from the ionophoretic measurements in sodium metavanadate solution are that the most favourably disposed hydroxyl groups for the complexing of a cyclic sugar are those of a cis, cis-1,2,3-triol of a furanose ring form, or a spatially

equivalent triol. The complexing with acyclic polyols cannot be explained simply but appears to depend to some extent on the number of hydroxyl groups present, the minimum number of adjacent hydroxyl groups being four. Within this broad generalisation, two distinct types of complex seem to have emerged, one of which is typified by D-glucitol the other to some extent by D-mannitol. The further study of the complexes formed requires a knowledge of the stoicheiometry of the reaction between a polyol and the vanadate ion, the derivation of which will be the subject of the next section.

II. The Investigation of the Ratio of Vanadium to Polyhydroxy - Compound in some typical complexes.

At the conclusion of the previous section, it was mentioned that a knowledge of the stoicheiometry of the reaction between the vanadate ion and a polyol was desirable to further the investigation of the type of complex which could be formed between them. Such information can in some instances be derived from polarimetric measurements.

The presence of sodium metavanadate in the aqueous solutions of some polyhydroxy-compounds considerably enhances their optical rotations. For D-glucitol the enhancement occurs over the pH range 4.0 to 8.0 reaching a sharp maximum value at pH 6.0. The increase in rotation of this polyol is remarkable, from $[\alpha]_D^{20} = -1.9^\circ$ in the absence of vanadate to a maximum of $[\alpha]_D^{20} = +400^\circ$ in acidified sodium metavanadate ($NaVO_3$) solution (Fig. II.1 Expt. 4). A lesser, but still very noticeable effect occurs with D-mannitol (Fig. II.2 Expt. 8). Since sodium metavanadate may be derived from sodium orthovanadate (Na_3VO_4) by acidification, it would be predicted that a similar curve would be obtained for the variation with pH of the optical rotation of D-glucitol or D-mannitol in the orthovanadate solution. In fact, an unexpectedly

lower absolute rotation is manifested, in either case, although a maximum is still attained at pH 6.0 (Expts. 5,9].

It is immediately obvious that the pH for the maximum increase in the rotation of D-glucitol (5.8 - 6.0) differs from that at which the D-glucitol complex exhibits maximum ionophoretic mobility i.e. pH 7.6 - 8.6. This is explainable if the ionisation of the remaining hydroxyl groups (attached to the vanadium atom) in the complexes is not complete until the higher pH is reached. At the lower pH, the partially-ionised complex would migrate more slowly.

Enhancement of rotation by the addition of sodium metavanadate is not restricted to the acyclic polyols. The rotation of maltose is also increased, and, though the variation is less dependent on pH, maximum rotation again occurs at pH 6.0. (Fig. II.3 Expt. 15).

(One interesting side-issue of this work is that, particularly for D-glucitol if the pH is maintained rigidly constant, the addition of sodium metavanadate to a solution of a polyol, followed by acidification, could constitute a quick assay method for the polyol. For D-glucitol, the optical rotation of the mixed, acidified solution would yield a sensitive determination of the concentration of polyol present).

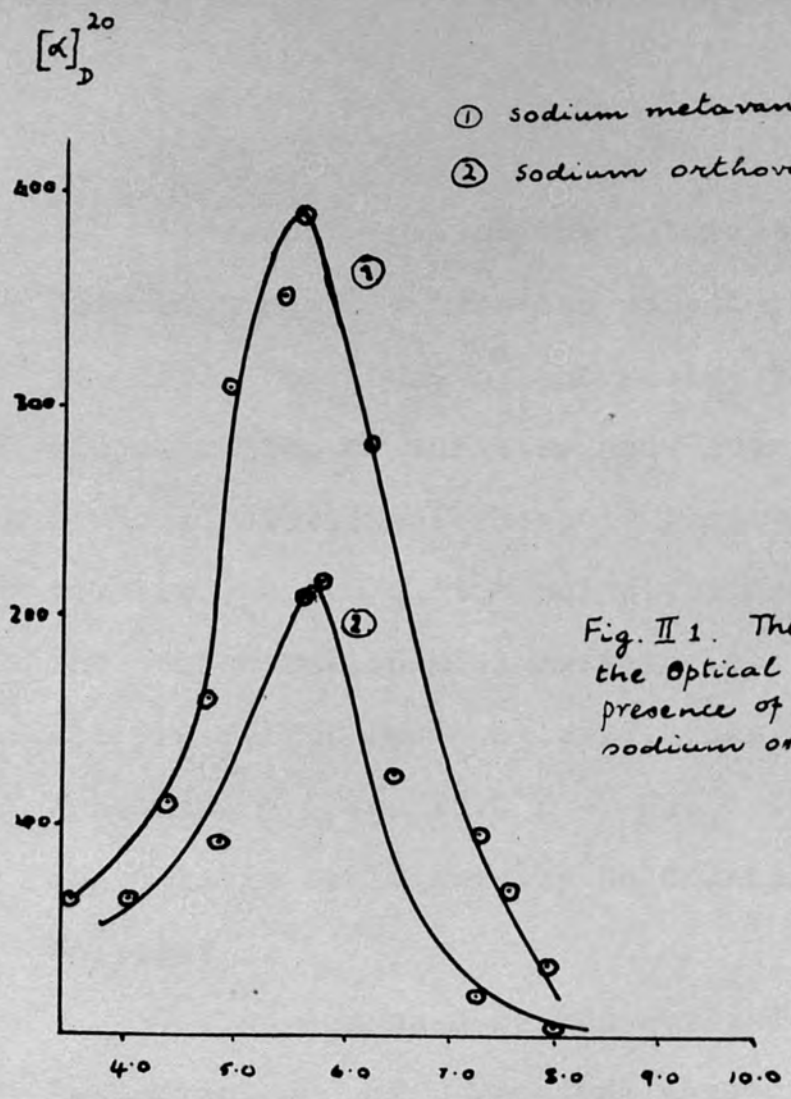


Fig. II 1. The effect of varying the pH on the Optical Rotation of D-glucitol in the presence of sodium metavanadate and sodium orthovanadate.

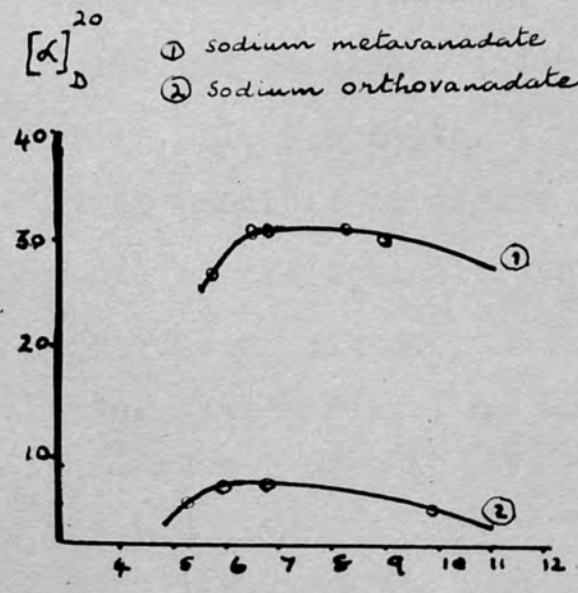


Fig. II. 2. The effect of varying the pH on the Optical Rotation of D-mannitol in the presence of sodium metavanadate and sodium orthovanadate.

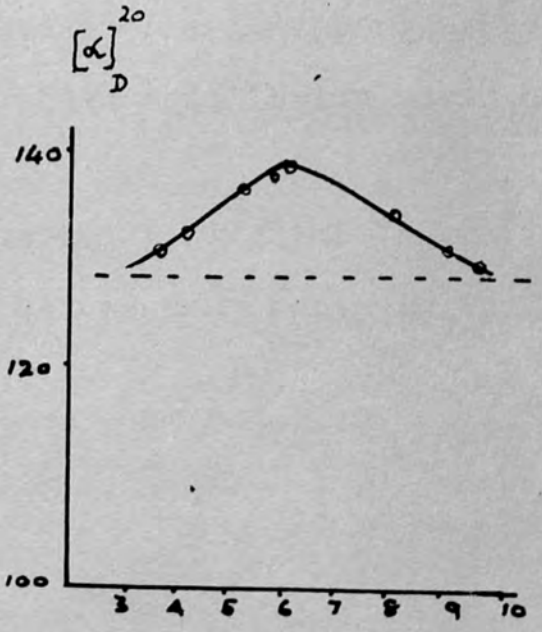


Fig. II. 3. The effect of varying the pH on the Optical Rotation of maltose in the presence of sodium metavanadate

In recent work on the nature of the complexes formed between tungstate ions and polyols, the ratio of tungsten to polyol was found by increasing the relative concentration of tungsten and observing the change in the optical rotation of the polyhydroxy-compound[28]. The rotation, based on the polyol, increased linearly with the concentration of tungstate (see Fig. II.4A - B) until all the polyol was complexed, when the rotation remained constant. (Fig. II.4 B - C). The tungsten/polyol (W/P) ratio could readily be deduced as that corresponding to point B.

The same procedure was applied to solutions of D-glucitol and of D-mannitol each containing varying amounts of sodium metavanadate, but instead of a linear relation, a curve was obtained, indicative of an equilibrium reaction (Figs. II, 5,6 Expts. 7, 10).

It is possible to derive an equation giving the V/P ratio in terms of the optical rotations of the components in the equilibrium mixture.

n Vanadate + Polyol $\rightleftharpoons$ complex.

i.e. $nV + P \rightleftharpoons C$

$$K = \frac{[V]^n [P]}{[C]}$$

Let p be the initial concentration of P

v " " " " V

c " " " " C

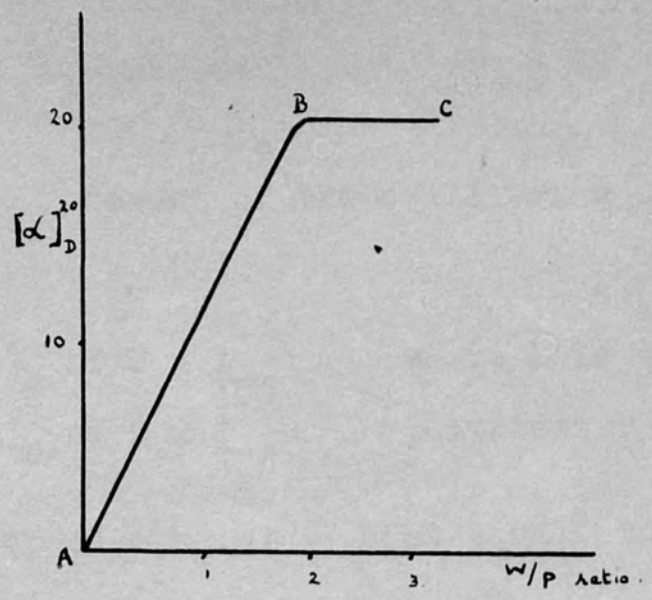


Fig. II 4. The molar ratio of sodium tungstate to D-glucitol.

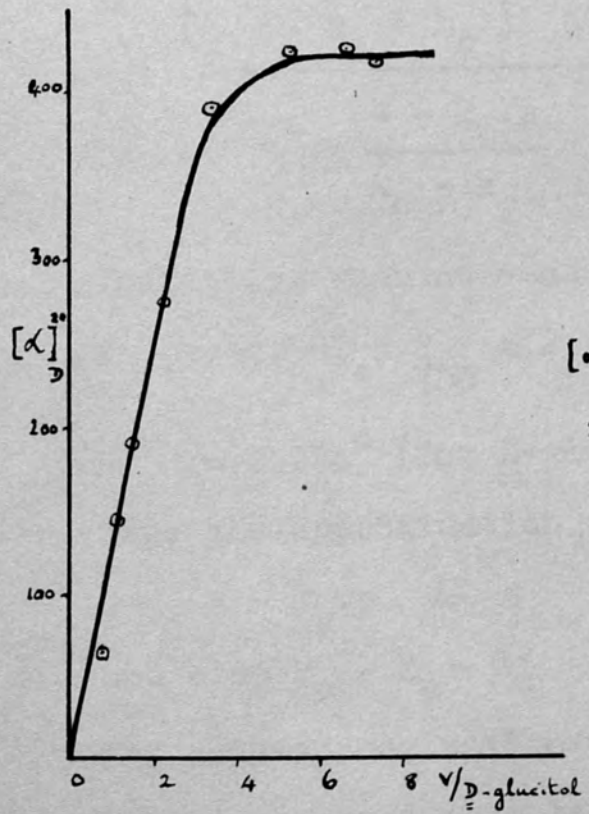


Fig. II.5. The ratio of vanadium to D-glucitol

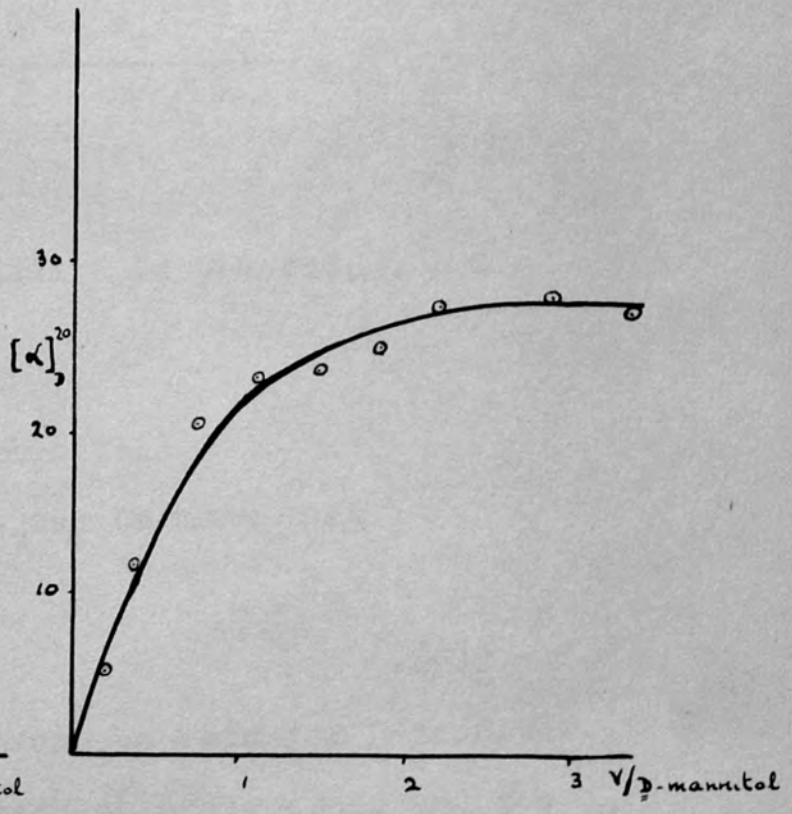


Fig. II 6 The ratio of vanadium to D-mannitol.

$$K = \frac{[v - nc]^n [p-c]}{c} \qquad \text{II.1.}$$

Now, the rotation of the equilibrium solution

$$\alpha = k_p[P] + k_c [C]$$

$k_p = [\alpha]_D$ of P x $\frac{l}{100}$ where l is the length of the

$k_c = [\alpha]_D$ of C x $\frac{l}{100}$ polarimeter tube.

$\alpha = k_p (p-c) + k_c.c.$ at equilibrium

Thus $c = \frac{\alpha - k_p.p}{k_c - k_p}$

Substituting for c in eqn. II.1 :-

$$K = \frac{\left(v - n\,\frac{\alpha - k_p.p}{k_c - k_p}\right)^n \left(p - \frac{\alpha - k_p.p}{k_c - k_p}\right)}{\frac{\alpha - k_p.p}{k_c - k_p}} \qquad \text{II.2.}$$

Initially, when no vanadate is present, c = 0

$k_p.p = -1.98^\circ$ x $\frac{2}{100}$ x 2

$= -0.078^\circ$ (for D-glucitol)

Thus the approximation can be made that

$\alpha - k_p.p \simeq \alpha$

and also $k_c - k_p \simeq k_c$ $([\alpha]_D = 0.4^\circ)$

The same approximation can be made for D-mannitol, if v and p are expressed as molecular proportions and p = 1,

$$K = \frac{(v - n\,a/kc)^n\,(1 - a/kc)}{a/kc}$$

$$= \frac{(v - na')^n\,(1 - a')}{a'} \qquad \text{where } a' = a/kc$$

II.3

In terms of the quantities measured, v = V/p ratio for a given rotation a. k_c is the limiting value of a, i.e. the value of the rotation when the equilibrium has been completely displaced to the right and n is the vanadium to polyol ratio actually present in the complex.

In dealing with equations of this type, an approximation can sometimes be made, when a' is small, such that $1 - a' \rightarrow 1$. The equation is then written

$$K \simeq \frac{(v - na')^n}{a'}$$

For the range of values of a where this assumption is justifiable, a plot of $(v - na')^n$ against a' should be a straight line passing through the origin. This treatment was applied to the values derived from the curve for D-glucitol (Fig. II.5), using different integral values for n. (a corresponds to $[a]_D$ measured: since the polyol concentration remains constant $[a]_D = k'a$ where $K' = \frac{100}{1 \times \text{concentration}}$ and $a' = \frac{[a]_D}{[k_c]_D} = \frac{k'a}{k'kc}$)

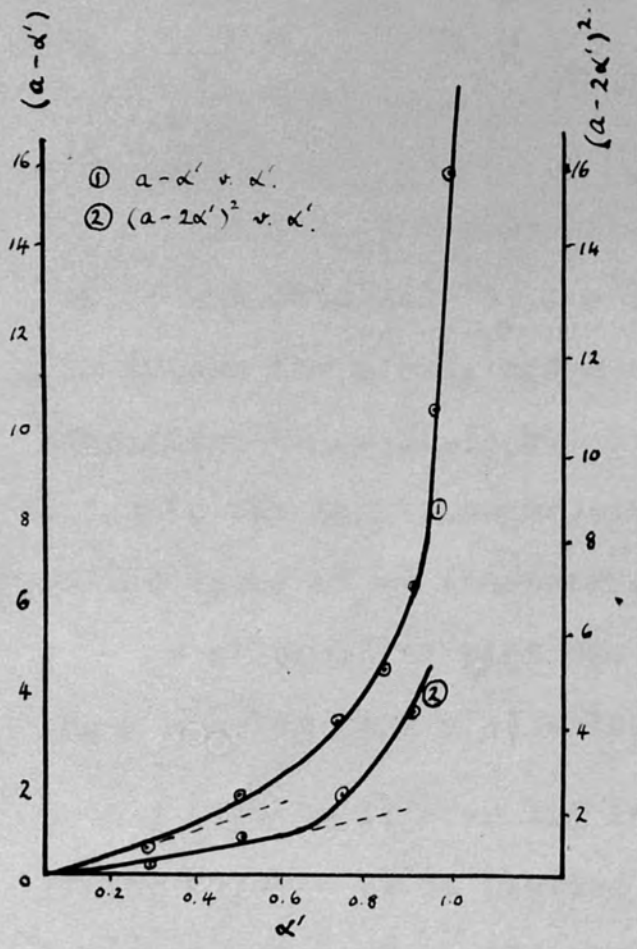
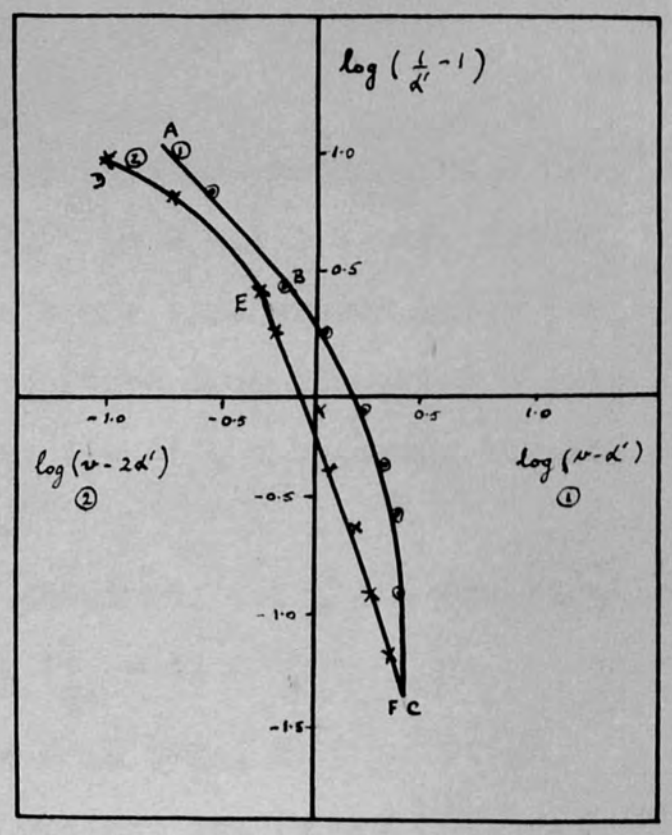


Fig. II 7.



ABC. n = 1. AB: gradient = -1. BC: curve.
DEF n = 2. DE: curve. EF gradient = -2.

Fig II. 8.

Graphs derived from the D-glucitol equilibrium curve.

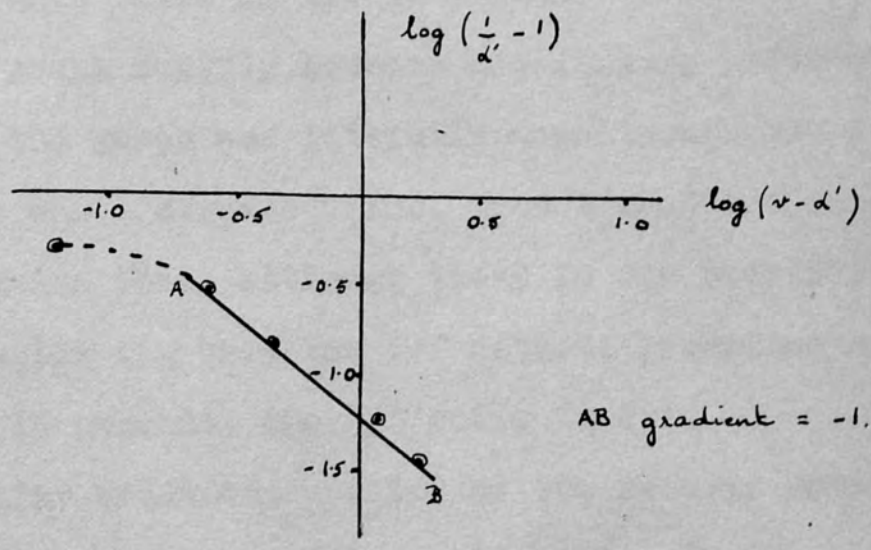


Fig. II.9. Graph derived from D-mannitol equilibrium curve.

For D-glucitol, the best linear relation between $(v - n\alpha')^n$ and α' was obtained for n = 2. (n = 1,2,3,4 were tried). Two graphs for n = 1, and n = 2 are illustrated below for comparison (Fig. II.7) but departure from linearity occurs owing to the innate assumption $1 - \alpha' \rightarrow 1$ becoming invalid as the value of α' increases.

An alternative plot was obtained, using the equation

$$\log K = n \log (v - n\alpha') + \log \left(\frac{1}{\alpha'} - 1\right)$$

or $$\log (1/\alpha' - 1) = -n \log (v - n\alpha') - \log K \qquad \text{II.4}$$

If $\log (1/\alpha' - 1)$ is plotted against $\log (v - n\alpha')$, with an integral value of n, the gradient should be -n. For D-glucitol, (Fig. II.8), it was found that when n = 1, the graph is linear, with gradient -1, for small values of α', i.e. when there is little vanadate present, but after this the graph rapidly becomes non-linear. For n = 2, however, the graph was initially non-linear, but for the most part was a straight line, with a gradient circa -2. This suggests that, although there is the possibility of a 1:1 complex for very low V/P ratios, providing sufficient vanadate is present, the V/P ratio is 2:1.

Similar treatment applied to the results obtained for D-mannitol indicated a 1:1 complex (Fig. II.9).

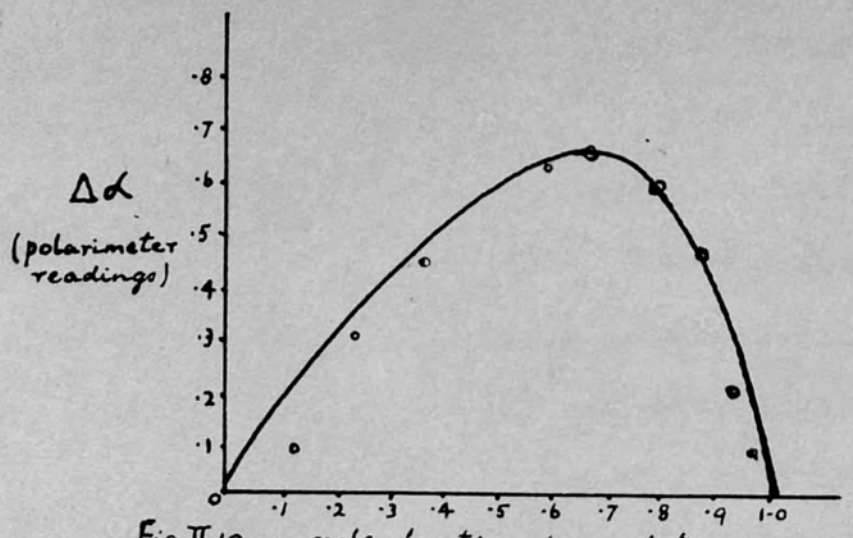


Fig. II.10. The Continuous Variation Plot (Polarimetric) for Vanadium and D-glucitol.

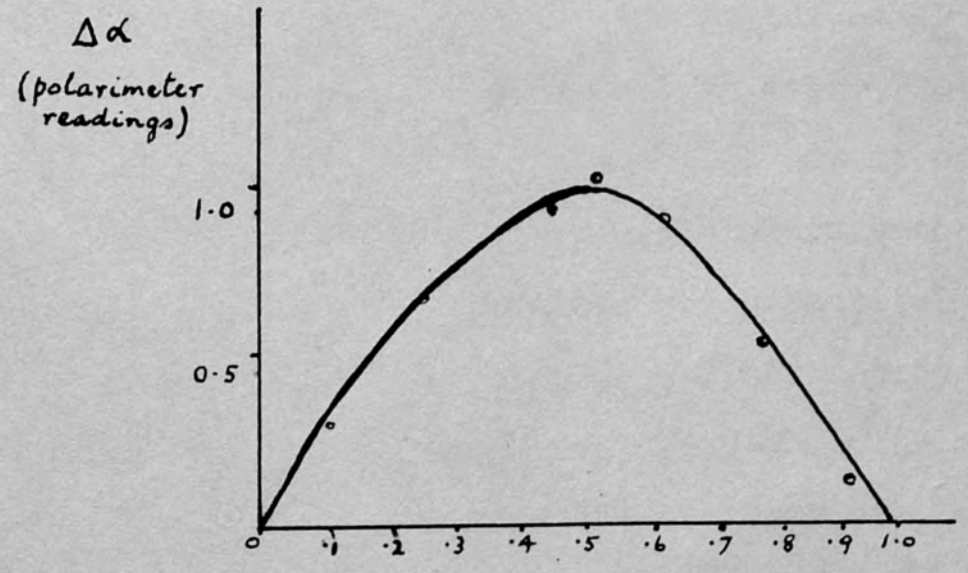


Fig. II.11. The Continuous Variation Plot (Polarimetric) for Vanadium and D-mannitol.

The above results were confirmed by measurements of the shift in the absorption edge of solutions of sodium metavanadate in which were dissolved either D-glucitol or D-mannitol (Expts. 18,19). The addition of D-glucitol to an 0.2M sodium metavanadate solution shifted the absorption edge towards a higher wavelength, the maximum difference in absorbance of the two solutions being at 550 mμ. (Fig. II,12). The addition of D-mannitol to an equivalent solution shifted the absorption edge to a lower wavelength, the maximum difference in absorbance being at 470 mμ. (Fig. II.13). By plotting the difference in absorbance, at these wavelengths, in place of Δa in the above experiments, the V/ρ ratio could again be determined. The ratios of vanadium: D-glucitol of 2:1, and vanadium: D-mannitol of 1:1 were thus confirmed (Figs. II 15 & 16, Expts. 26,27).

This method having been established, it could be applied to galactitol on which polarimetric measurements could not be made (Expt. 28). The vanadium: galactitol ratio was found to be 1:1, the continuous variation plot having been obtained from measurements made at 510 mμ. (Fig. II.17). While this work was in progress, a study of the borate: carbohydrate complex was reported[33], using the method of continuous variations. The authors found that for methyl-α-D-galactopyranoside the apparent B/S ratio differed when two different physical methods were used to investigate it.

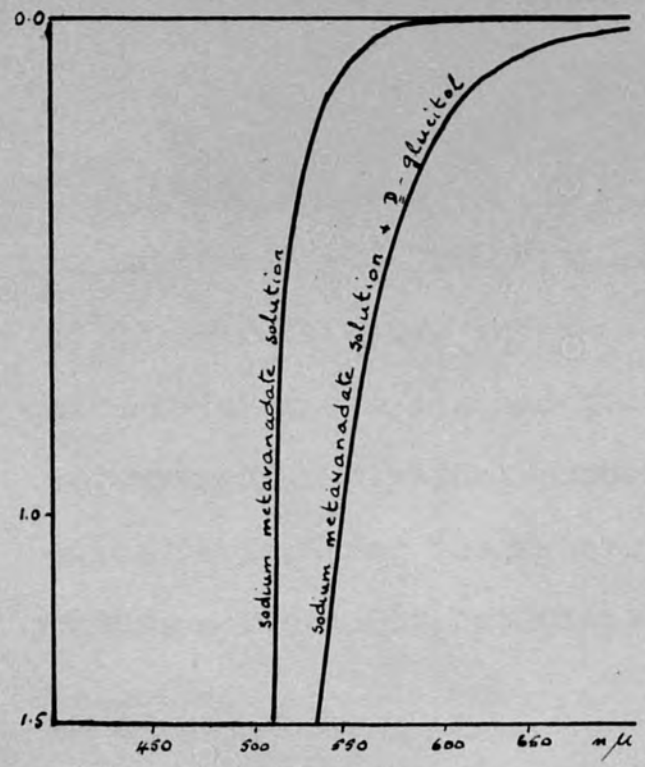


Fig. II. 12. The shift in the absorption edge of an 0·2 M. solution of sodium metavanadate at pH 5·9 by the addition to it of D-glucitol. (0·1 M.).

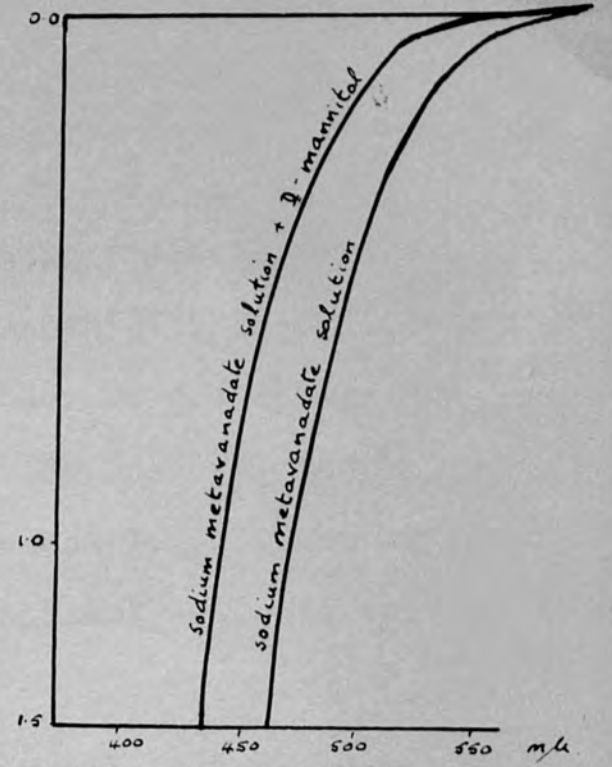


Fig. II. 13. The shift in the absorption edge of an 0·08 M. solution of sodium metavanadate at pH 5·9 by the addition to it of D-mannitol. (0·08 M.).

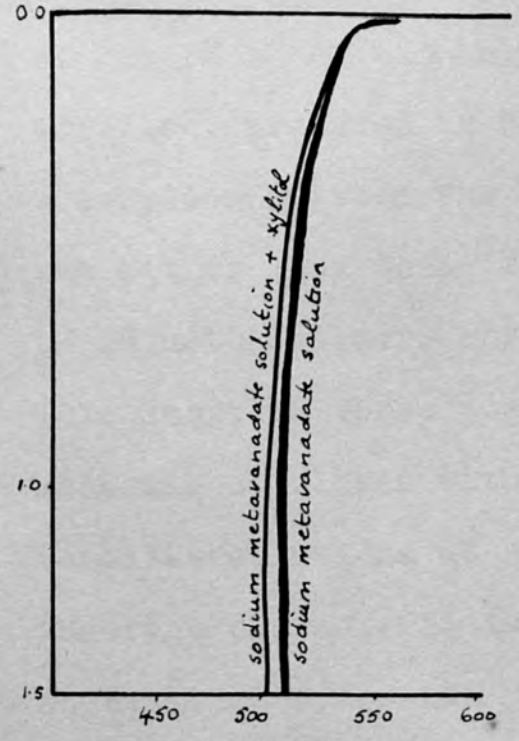


Fig. II. 14. The shift in the absorption edge of an 0·1 M. solution of sodium metavanadate at pH 5·9 by the addition to it of xylitol. (0·1 M).

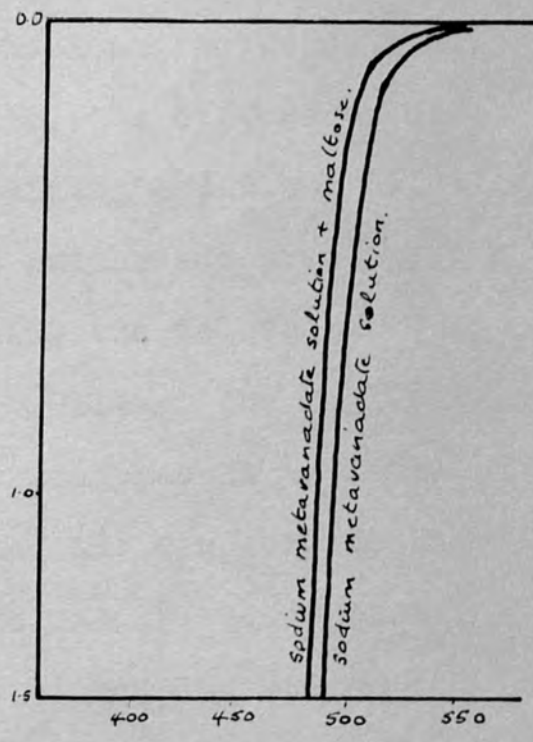


Fig. II. 15. The shift in the absorption edge of an 0·08 M. solution of sodium metavanadate at pH 6·2 by the addition to it of maltose. (0·1 M).

The ratio derived from optical rotation measurements was 1:1, from refractive index measurements 2:1, depending on which type of complex had most influenced the change in the particular physical property. By analogy, the V/p ratios derived for the metavanadate complexes refer to the predominant complex but cannot justifiably be said to be exclusive.

An interesting feature of the D-glucitol measurements was the difference in actual value of the absorbance in the presence and absence of acetate buffer, which was added to the solution to maintain pH 5.9. If there was no adjustment of the pH after mixing the D-glucitol and metavanadate solution, prepared in acetate buffer, the difference in the absorption between the polyol-containing and blank solutions was rather less than if no acetate buffer was present, and pH adjustments were made after mixing the solutions. This suggests three possibilities. Either the D-glucitol molecule complexes with a vanadate ion present in metavanadate solutions at a pH> 6.0, and the complex, as an inherent characteristic, exhibits a maximal absorption shift at pH 6, or the perhaps partial complex at pH> 6.0 is completed at pH 6.0, the further reaction being catalysed by the H^+ ions added. Alternatively the vanadate ion is preformed at pH 6.0 and complexes with D-glucitol, the

maximum absorption shift being merely a consequence of the greater concentration of this ion present at the particular pH, but then no difference would be anticipated when the solution is further acidified after the addition of D-glucitol.

The shift in the absorption edge of solutions of sodium metavanadate occasioned by the addition to them in turn of xylitol, arabitol or ribitol were examined, but were insufficient to allow a continuous variation plot to be attempted (Fig. II.14) Expts. 22,23,24). Likewise, the vanadium/L-arabitol ratio could not be investigated polarimetrically, since the metavanadate caused insufficient change in the rotation of this polyol. (The concentration of vanadium, and therefore of polyol, in these experiments is limited by the colour of the solution which on acidification becomes orange or red. It is not possible to increase indefinitely the difference in rotation by increasing the absolute concentrations).

From polarimetric and absorption measurements as above, the vanadium/maltitol ratio was found to be 1:1 Expts. 13,29. This polyol was chosen since, amongst the readily available reduced glucose disaccharides, it had the highest mobility. It is noteworthy that the V/P ratio differs from that in the

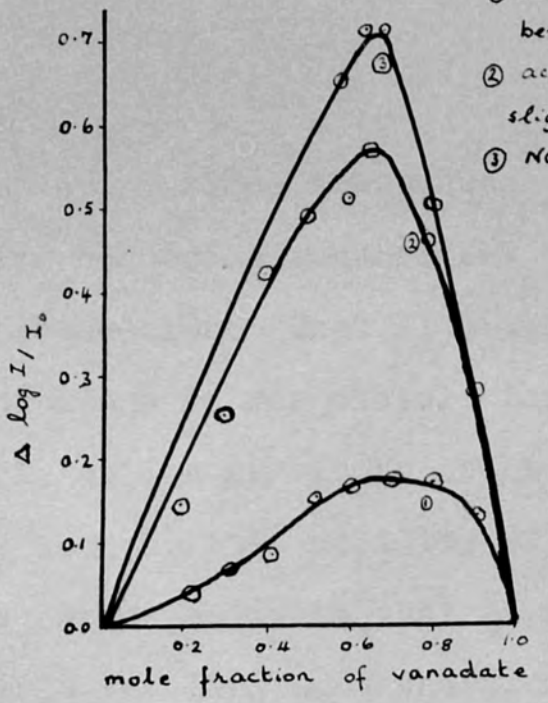


Fig. II. 16. V/P ratio for D-glucitol obtained from absorption measurements.

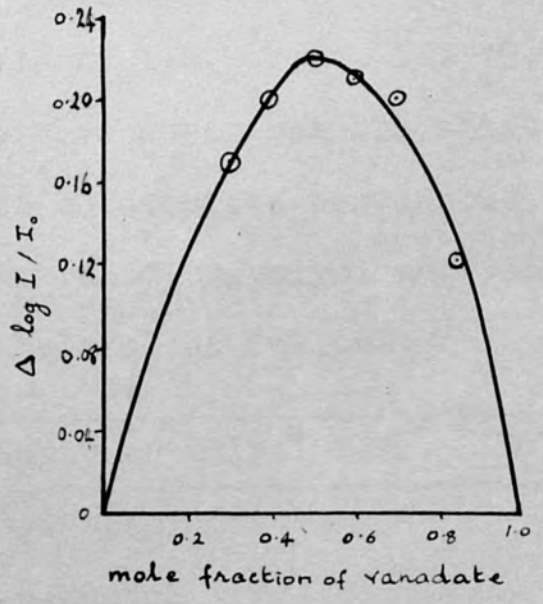


Fig. II. 17. V/P ratio for D-mannitol obtained from absorption measurements.

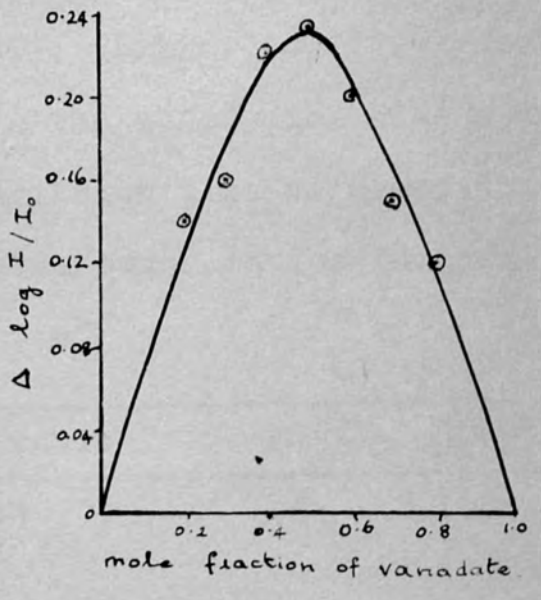


Fig. II. 18. V/P ratio for galactitol obtained from absorption measurements.

D-glucitol complex. Since the presence of the metavanadate ion causes no enhancement of the optical rotation of glucose alone (Expt. 14) the cyclic part of the molecule cannot alone be responsible for the complexing. Complexing could be due merely to the substituted D-glucitol, but if so, the ionophoretic mobility of cellobiitol would be expected to correspond with that of maltitol. Some complexing across the link must therefore be postulated, supporting the deduction made from the ionophoretic measurements alone.

The vanadium: sugar ratio was found polarimetrically in the vanadium-maltose and vanadium-ribose complexes to be 2:1 and 1:2, (Figs. II. 19, 20), (Expts. 16,17). For neither sugar was the shift in the absorption edge sufficient for an accurate continuous variation plot to be attempted. The vanadium/polyol and vanadium/sugar ratios found may be tabulated as follows:

Sugar or polyol	V/p ratio	$M_s(V)$
D-glucitol	2:1	1.00
D-mannitol	1:1	0.40
Galactitol	1:1	0.48
Maltitol	1:1	1.52
Maltose	2:1	0.26
D-Ribose.	1:2	0.40

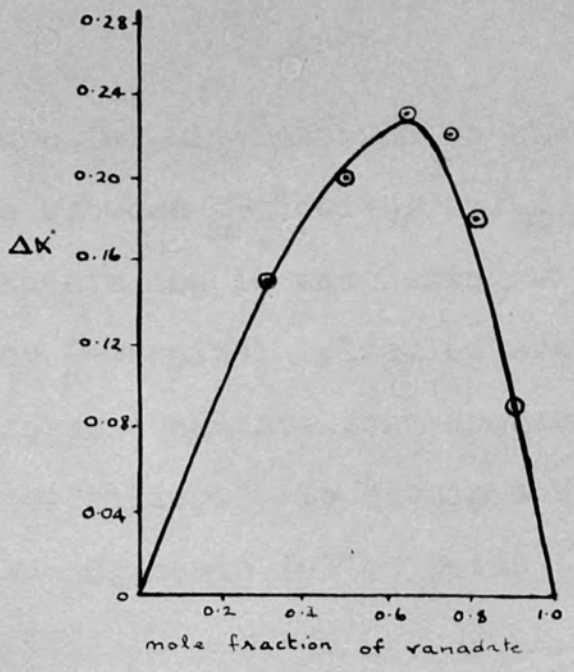


Fig. II. 19. The V/P ratio for maltose obtained polarimetrically.

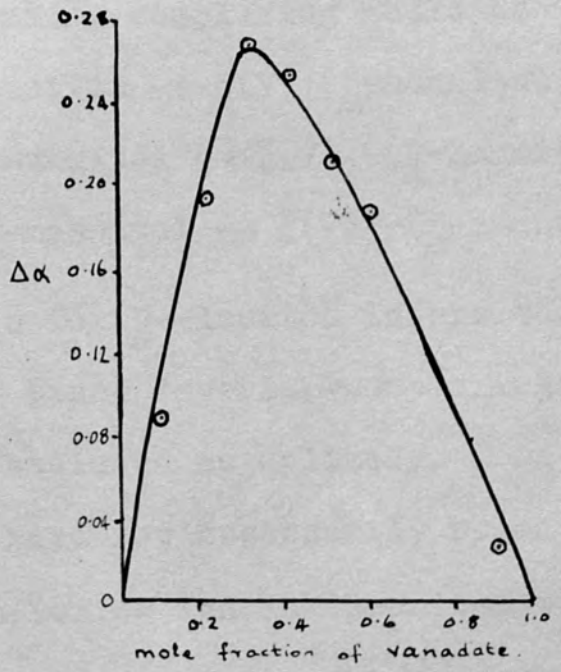


Fig. II 20 The V/P ratio for ribose obtained polarimetrically.

There are two alternatives to explain the difference in V/p ratio between D-glucitol and D-mannitol. Either the same vanadate ion is complexing with both polyols, but twice as many D-mannitol molecules are attached per vanadate ion, or different vanadate ions are employed. Consider the first possibility. To obtain a V/p ratio for D-glucitol of 2:1, complexing could follow paths 1 or 2, or the general eqn.3.

$2(V)^{x-} + \underline{\underline{D}}\text{-glucitol} \rightarrow [(V)\text{-}\underline{\underline{D}}\text{-glucitol-}(V)]^{2x-}$ ... 1

$(V_2)^{y-} + \underline{\underline{D}}\text{-glucitol} \rightarrow [(V)_2\text{-}\underline{\underline{D}}\text{-glucitol}]^{2y-}$ ... 2

$(V_n)^{z-} + n/2\ \underline{\underline{D}}\text{-glucitol} \rightarrow [(V)_n\text{-}\underline{\underline{D}}\text{-glucitol}\ n/2]^{z-}$ ... 3

If the same vanadate ion is complexing with D-mannitol then the corresponding complexing would be

$(V)^{x-} + \underline{\underline{D}}\text{-mannitol} \rightarrow [(V)\text{-}(\underline{\underline{D}}\text{-mannitol})]^{x-}$ ... 1

$(V_2)^{y-} + 2\underline{\underline{D}}\text{-mannitol} \rightarrow [(V)_2\text{-}(\underline{\underline{D}}\text{-mannitol})_2\text{-}]^{y-}$ ... 2

$(V_{n'})^{z-} + n'\ \underline{\underline{D}}\text{-mannitol} \rightarrow [(V)_{n'}\text{-}(\underline{\underline{D}}\text{-mannitol})_{n'}]^{z-}$ ... 3

The V/p ratio for D-glucitol infers that n must be 1 or an even number, since "overlap-complexing" of the (3V) - 3G - (3v) type can be excluded as unlikely. On the other hand n', for D-mannitol, need not necessarily be an even number unless the vanadate complexing ion is identical with that reacting with D-glucitol.

If two molecules of polyol are attached to one vanadate ion, it should be possible to investigate a mixture of two compounds A and B by metavanadate ionophoresis and obtain complexes A-V-A, B-V-B, A-V-B, migrating at different rates. (The argument holds, with a different number of mixed complexes, for any n(P)/n/2(V) complex). In the mixtures tried: maltose + ribose, maltose + D-glucitol, maltose + D-mannitol no third complex was apparent. However, the anticipation of a mixed complex assumes that the ease of complexing with each polyhydroxy-compound is comparable, which may not be justifiable; the complexing with one polyhydroxy-compound may restrict sterically the secondary complexing of the other. Therefore it is not possible to exclude the A-V-A type of complexing for these compounds.

The V/P ratio for ribose bears no immediate resemblance to the other V/P ratios determined. However, this sugar contains the cis, cis-1,2,3-triol, already mentioned in connection with the ionophoretic mobilities measured, which does not occur in the other five compounds. It would be interesting to obtain the V/P ratio of fructose for comparison with that of ribose.

The V/P ratios obtained must be considered against the background of what is already known of the poly-ions which may be present in vanadate solutions, and the further

information gained by physical studies of vanadate solutions containing polyol molecules.

A considerable volume of work has been reported on the constitution of solutions of orthovanadates on acidification, when a number of polymeric species are formed. At this point it is convenient to consider what is already known about such species and how the physical techniques used to determine their composition can be adapted to the investigation of solutions containing polyhydroxy-compounds.

III. The Nature of the ions in Vanadate Solutions.

The main species of vanadate ions occurring in aqueous solution were first classified by Rammelsberg[18] as ortho-(M_3VO_4) and pyro-($M_4V_2O_7$), present in alkaline solutions, meta-(MVO_3), present in neutral solutions and tetra-($M_5V_4O_{11}$) and hexa-(MV_3O_8), present in acidic solutions. Dullberg[34] found that when a solid orthovanadate is dissolved in water an equivalent amount of OH^- is released from which he deduced that the orthovanadate becomes protonated according to the equation:

$$VO_4^{3-} + H_2O \rightleftharpoons HVO_4^{2-} + OH^- \qquad \text{eqn. III. 2.}$$

In more recent potentiometric studies, Britton and Robinson[35] came to the same conclusion. The solubility product

$$[Ag^+]^3 [HVO_4^{2-}][OH^-] = 10^{-24} \qquad \text{eqn. III. 2.}$$

obtained by these workers on titration of an orthovanadate with a silver nitrate solution[36] also supports the formation of the conjugate acid, while in their interpretation of the absorption spectra of vanadium V in strongly alkaline solutions Newman[37] and his coworkers assume that a mixture of VO_4^{3-} and HVO_4^{2-} ions are present.

All the authors cited agree that the orthovanadate condenses first to a di- or pyro-vanadate on acidification.

Dullberg[34] in an early cryoscopic investigation found evidence for a dimeric species. Jander and Jahr[38] also postulate its existence from diffusion data. The solubility of silver pyrovanadate $Ag_4V_2O_7$ in various solutions of sodium vanadate was measured by Souchay[39] who found that initially the solubility product $[Ag^+]^2[HVO_4^{2-}]$ = constant, but that for more concentrated solutions the equation

$$[Ag^+]^4 [V_2O_7^{4-}] = \text{constant} \qquad \text{eqn. III. 3.}$$

was in better agreement with the experimental results. From a spectrophotometric determination of the hydrolysis constant of sodium orthovanadate, Newman[37] was led to conclude that the dimerization

$$2HVO_4^{2-} = V_2O_7^{4-} + H_2O \qquad \text{eqn. III. 4.}$$

takes place, the importance of the pyrovanadate diminishing as the vanadium concentration is decreased. Schwarzenbach and Parissaki's study[40] of the change in the transition point of $Na_2SO_4.10H_2O$ occasioned when small amounts of $Na_4V_2O_7$ are dissolved in the melt confirms Newman's suggestion.[37] According to Russell and Salmon[41], who investigated the interaction of ion-exchange resins with vanadate solutions, the divanadate begins to occur at pH 10.

Further condensation of the pyrovanadate yields a metavanadate but it is at present uncertain whether this is a trimeric or tetrameric species. Cryoscopic measurements by Dullberg[34] indicated the formation of ions containing three vanadium atoms, and a trimer is also postulated by Souchay[42] from the depression of the transition point of fused sodium sulphate by the addition of sodium metavanadate, though Newman[37] questions the validity of an unequivocal decision from the data given. Various ions are suggested by Brintzinger and Wallach[43] in order to interpret their results from the dialysis of solutions of sodium sulphate, at different pH's, in which were dissolved sodium metavanadate or orthovanadate. Their suggestions can be tabulated as follows:

Table II.1. Polyvanadate ions postulated by Brintzinger and Wallach

pH	Postulated ion.
14.0 - 12.2	$[VO_4(H_2O)_6]^{3-}$ or $V_2O_7^{4-}$
11.0 - 10.0	$[V_2O_7(H_2O)_6]^{4-}$ or $V_3O_{10}^{5-}$
8.0 - 7.0	$V_4O_{13}^{6-}$

Russell and Salmon[41] provide evidence for both a trimeric and tetrameric ion, the trivanadate appearing initially at pH 7 and persisting over a wide pH range, whilst the tetravanadate appears at pH 6.5 and exists over a narrow range only. The tetramer is indicated in diffusion measurements[38] and in the determination of the molecular weight of tertiary butyl metavanadate in tertiary butyl chloride[44]. Hazel, McNabb and Santini[45] have suggested a possible structure for a tetravanadate: (Fig. III.1).

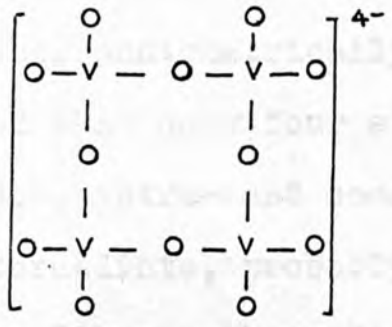


Fig. III.1 A postulated tetravanadate

the polymerization having occurred as follows:

$$H_2V_2O_7^{2-} + H_2V_2O_7^{2-} \rightleftharpoons (VO_3)_4^{4-} + 2H_2O \qquad \text{eqn. III.5.}$$

Cryoscopic studies on vanadate/Glauber salt mixtures[40] yielded evidence for the formation of a tetramer in a pH range in which Brito and Ingri's work[46] indicates a trimer.

Thilo and Schiller[47] attempted to correlate the work of various authors on the vanadate ions present in the so-called "metavanadate range", and concluded that the form of

the ions was dependent both on pH and concentration. Brito and Ingri[48] have recently subjected e.m.f. data from vanadate solutions to computer analysis and support the opinion of the above authors: namely, that both trimeric and tetrameric ions are present in metavanadate solutions, the trimer predominating in dilute solutions of vanadate, whilst the tetramer becomes more important as the concentration is increased. The exact conditions required for the interconversion have not yet been established.

The condensation of vanadate on acidifcation was followed spectrophotometrically by Glemser and Preisler[19] who concluded that only four stable anions were present: the mono-, di-, tetra- and decavanadates, but that an unstable intermediate, probably a dodecavanadate, might also occur. Other authors however have provided some evidence for a hexavanadate ion. Dullberg[34] mentions the existence of an ion containing six vanadium atoms and Souchay and Carpéni[49] postulate the conversion of metavanadate to an orange-coloured hexavanadate according to the equilibrium:

$$2V_3O_9^{3-} + 3H^+ \rightleftharpoons HV_6O_{17}^{3-} + H_2O \qquad \text{eqn. III.6.}$$

The presence of this ion at pH 5 and over a fairly wide pH range is believed to be illustrated by the titration of vanadic acid with ammonia[41]. Richardson and Magee[50], on the other hand, having titrated vanadic acid with sodium hydroxide, state that the shape of the pH curve over the region pH 3 - 6 indicates a continuous shift of equilibrium during this stage, and believe that whilst it is not impossible that the hexavanadate ion is formed at equilibrium, it is doubtful whether it is formed during the initial titration.

In solutions more acidic than those used in the present work, the decavanadate ion is postulated and its existence substantiated by the work of Jahr, Fuchs and Preuss[51], Russell and Salmon[41], Glemser and Preisler[19] and Rossotti and Rossotti[52]. It is also known to exist in certain minerals, for example pascoite and hummerite[53].

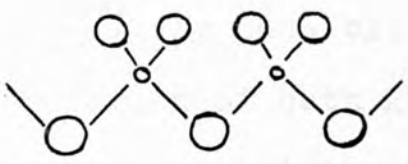
Thus the ions which are or could conceivably be present in the pH range used to investigate the complexes between polyhydroxy compounds and vanadate ions are the ortho-vanadate, the pyrovanadate, the trivanadate and tetravanadate, and the hexavanadate. In theory the dodecavanadate mentioned by Glemser and Preisler[19] could also participate but the V/p ratios obtained (see previous section) suggest that this is unlikely. The inter-oxygen distances in

typical vanadate ions must also be considered to determine whether any such distances correspond to those which are to be found between the hydroxyl groups of an acyclic polyol or a cyclic sugar since without such correspondence the possibility of an ion taking part in complex-formation can be discounted.

From similarities between the Raman spectra of strongly alkaline vanadium V solutions and those of phosphate solutions containing the PO_4^{3-} ion, Siebert[54] has deduced that the simple vanadate ion is tetrahedral. In crystalline $Ni_3(VO_4)_2$,[55] $Co_3(VO_4)_2$,[56] $Mg(VO_4)_2$[56] and $CrVO_4$[57] the oxygen atoms are coordinated round the vanadium atom in more or less distorted but discrete tetrahedra, while Milligan and Vernon[58] showed that fifteen heavy metal orthovanadates possess a body-centred tetragonal unit in which there is a tetrahedron of oxygen atoms around each vanadium atom. Tetrahedral coordination has also been found in ZrV_2O_7 but with pairs of tetrahedra sharing one oxygen corner to form $V_2O_7^{4-}$ groups.[59]

The stereochemistry of the metavanadates quoted in the literature varies between a trigonal bipyramidal five-coordinated structure and a tetrahedral four-coordinated structure depending on whether or not the ion is hydrated. Potassium metavanadate KVO_3 has been subjected

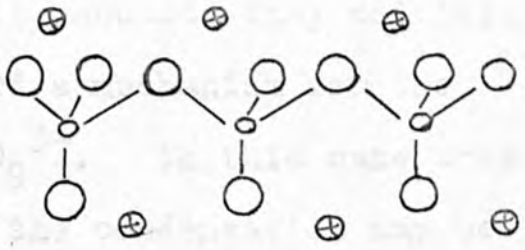
to X-ray analysis[60] and it consists of chains of VO_4 tetrahedra linked together by two shared corners into continuous chains analogous to those of diopside[61]. (Fig. III.2).



O oxygen atoms

o vanadium atoms

Metavanadate in $(VO_3)_n^{n-}$



O oxygen atoms

o silicon atoms

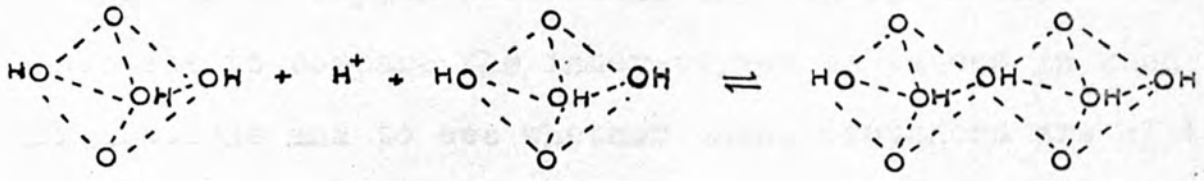
x metal atoms

Diopside $(Ca,Mg)Si.O_3$

Diopside structures have been found also for sodium metavanadate $NaVO_3$[62] and ammonium metavanadate NH_4VO_3.[63] The hydrated potassium metavanadate $KVO_3.H_2O$, on the other hand, is composed of trigonal bipyramids which share edges forming a chain[64]. According to Brito and Ingri[46] it is possible to derive the diopside chain from the staggered trigonal bipyramid by a simple stretching process. These authors compared the relative merits of four- and five-fold coordination of the vanadium atom to explain the course of the condensation of the orthovanadate ion on acidification of its solution. To account for the metavanadate species $V_3O_9^{3-}$ an entity consisting of three VO_4 tetrahedra sharing

corners to form a six-membered ring is postulated, but the authors state that it is difficult to understand the formation of $HV_2O_7^{3-}$ from such tetrahedra. On the other hand, if five-fold coordination is assumed, they maintain that it is possible to put forward a mechanism for the formation of both $HV_2O_7^{3-}$ and $V_3O_9^{3-}$. In this case they postulate that the first step in the condensation may be written.

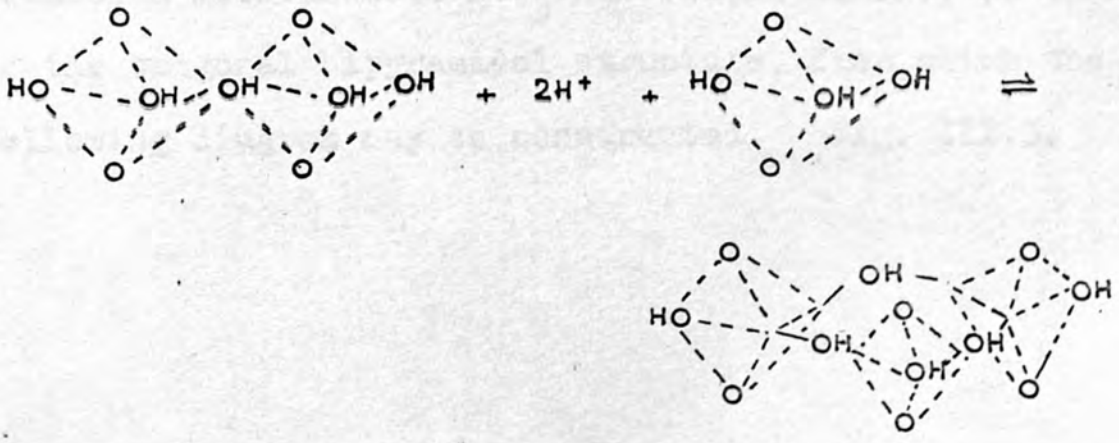
$$VO_2(OH)_3^{2-} + H^+ + VO_2(OH)_3^{2-} \rightleftharpoons (VO_2)_2(OH)_5^{3-} + H_2O$$



eqn. III.7.

and for the reaction

$$(VO_2)_2(OH)_5^{3-} + 2H^+ + (VO_2)(OH)_3^{2-} \rightleftharpoons (VO_2)_3(OH)_6^{3-} + 2H_2O$$



eqn. III.8.

As an alternative to the ring structure, Brito and Ingri[46] also consider that $V_3O_9^{3-}$ may be a chain of units coupled as in KVO_3H_2O but add that it is difficult then to explain why higher complexes e.g. 4- or 5- nuclear species are not detected in vanadate solutions. In fact there is a good deal of evidence for a four-membered species, but so far no indication of a pentanuclear one.

Since it thus appears possible that the vanadate complexing ion at present being considered may be based either on a VO_4 tetrahedron or on a pentagonal bipyramidal coordination of oxygen atoms round the central atom, it is of interest to compare the inter-oxygen distances in such configurations and to see whether these distances are of the same order of magnitude as those between the oxygen atoms of hydroxyl groups of a typical acyclic polyol or sugar, for if complexing is to occur the two distances must be comparable.

Bachmann and Barnes[65] have drawn up a table of the vanadium-oxygen and oxygen-oxygen distances in hydrated potassium metavanadate $KVO_3.H_2O$ (Table III.2), an example of the trigonal bipyramidal structure, from which the following diagram may be constructed. Fig. III.3.

FIG. III.3. THE ENVIRONMENT OF THE VANADIUM ATOM IN $KVO_3.H_2O$.

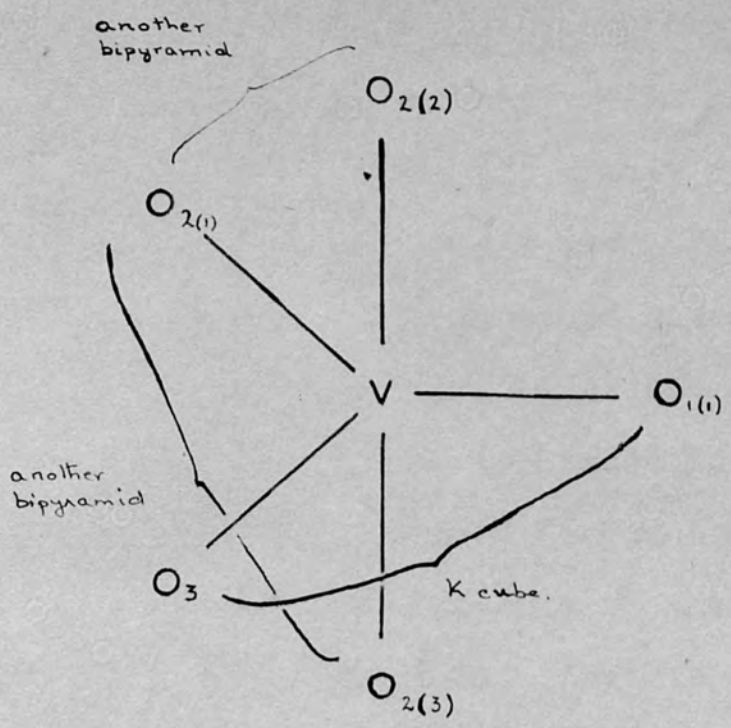


TABLE III.2. THE INTERATOMIC DISTANCES IN $KVO_3.H_2O$

	Å		Å
$V - O_{1(1)}$	1·65	$O_3 - O_{2(1)}$	3·23
$V - O_3$	1·67	$O_{2(2)} - O_{1(1)}$	2·71
$V - O_{2(1)}$	1·97	$O_{2(3)} - O_{1(1)}$	2·71
$V - O_{2(2)}$	1·93	$O_{2(2)} - O_{2(1)}$	2·34
$O_{1(1)} - O_3$	2·65	$O_{2(3)} - O_{2(1)}$	2·34
$O_{1(1)} - O_{2(1)}$	3·27	$O_{2(2)} - O_3$	2·77
		$O_{2(3)} - O_3$	2·77

In this hydrated metavanadate, the chains of trigonal bipyramids are built into a three-dimensional net by sharing edges and corners with almost regular cubic O, HO polyhedra around the potassium atom. In all of the trigonal bipyramids $O_1(1)$ is coordinated to one vanadium only, while $O_2(1)$, $O_2(2)$ and $O_2(3)$ are each shared among three vanadium atoms. O_3 is bonded to one vanadium atom only. The vanadium-vanadium distance across shared edges is 3.12Å and the repeat distance of the double zig-zag chains is approximately 3.6Å. Within the trigonal bipyramid the vanadium atom is in the plane of the equilateral triangle defined by $O_1(1)$ - $O_2(1)$ and O_3; the edges $O_2(2)$ - $O_2(1)$ and $O_2(3)$ - $O_2(1)$ which are shared with adjacent bipyramids are relatively short and the vanadium atom is displaced away from those edges so that the V-$O_2(1)$ distance is relatively long.

Bachmann and Barnes[65] have also estimated the covalent single and double bond radii of vanadium in tetrahedral, five-fold and octahedral coordination and from these the corresponding vanadium-oxygen bond lengths.

While the Bachmann and Barnes paper was in press, the length of one vanadium-oxygen distance in the anhydrous metavanadate KVO_3 was found to be 1.65 to 1.67Å.[46] This presumably corresponds to that of the double bond, by

FIG. III. 4. DIAGRAMMATIC REPRESENTATION OF THE PYROVANADATE ION.

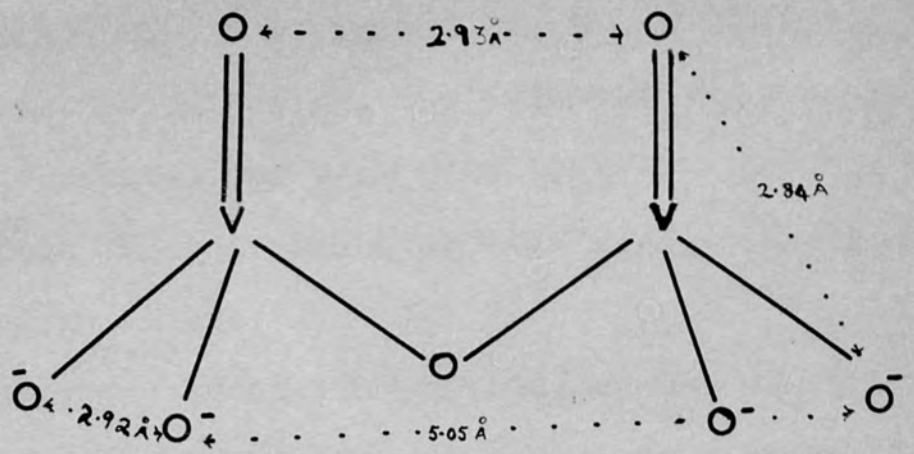


TABLE III. 3. THE ESTIMATED SINGLE AND DOUBLE BOND COVALENT VANADIUM RADII AND THE CORRESPONDING VANADIUM - OXYGEN BOND LENGTHS FOR TETRAHEDRAL, FIVE - FOLD AND OCTAHEDRAL COORDINATION.

A. Without electronegativity correction.

B. With electronegativity correction.

Coordination.	Vanadium radius		V-O bond length.	
	Single bond.	Double bond.	Single bond	Double bond
A. Tetrahedral	1·20 Å	1·09 Å	1·86 Å	1·64 Å
Five-fold	1·24 Å	1·13 Å	1·90 Å	1·68 Å
Octahedral	1·27 Å	1·16 Å	1·93 Å	1·71 Å
B. Tetrahedral	1·20 Å	1·10 Å	1·79 Å	1·57 Å
Five-fold	1·22 Å	1·12 Å	1·81 Å	1·59 Å
Octahedral	1·24 Å	1·13 Å	1·83 Å	1·60 Å

comparison with the estimated lengths. Assuming regular tetrahedral coordination, the following interoxygen distances may be calculated for a hypothetical VO_4 ion: O-O = 2.92Å and 2.84Å. This is assuming that strict single and double bond lengths exist.

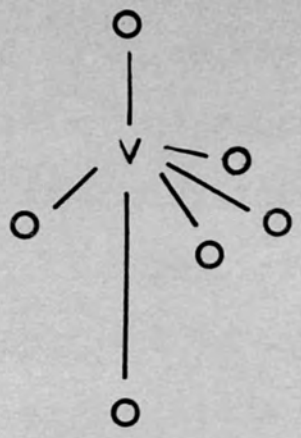
The pyrovanadate ion consists of two VO_4 tetrahedra sharing one oxygen corner and may be diagrammatically represented as in Fig. III.4, in which the interoxygen distances are 2.92, 2.84, 2.93, 5.05Å using the double bond length actually found in the anhydrous metavanadate. If the two vanadium units rotate with respect to one another, other interoxygen distances, varying continuously between 2.93Å and 5.05Å, are introduced. The metavanadate trimer or tetramer could be a continuation of this chain of VO_4^{3-} units.

The possibility that the vanadium atom is octahedrally coordinated to oxygen atoms was inferred in the table above. This eight-fold coordination does occur in the pentoxide V_2O_5, but the type of coordination is very irregular [Fig. III.5]. Orgel[66] suggests that ions with radii such that the octahedral and tetrahedral oxide environments are of comparable stability, of which vanadium is one, often form

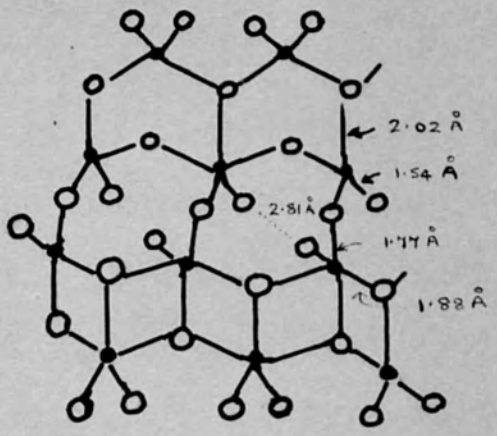
oxides with very unusual structures which can be derived from regular octahedrally coordinated structures by displacement of the metal ion from the centre of an oxide octahedron with only minor displacements of the oxide ions themselves. In vanadium pentoxide the octahedron is so irregular that the metal atom is usually regarded as 5-coordinated.

The metal atom in tungstate and molybdate complexes with polyols is octahedrally coordinated, but the data available on the usual coordination around vanadium, tungsten and molybdenum indicates that the deviation from regular octahedral symmetry is greater for vanadium V than for molybdenum VI and tungsten VI.[66] Although such coordination cannot be categorically excluded for vanadium, it seems more likely that this metal reacts with polyhydroxy-compounds to form complexes in which the vanadium is either tetrahedrally or five-fold coordinated. Assuming that in solution the ions approximately maintain the distances given for the crystals investigated, it appears that both a tetrahedral unit or a pentagonal bipyramidal unit could contain inter-oxygen distances comparable to certain of those occurring in polyhydroxy-compounds.

Both the VO_4^{3-} tetrahedron and the $KVO_3.H_2O$ pentagonal bipyramid contain oxygen-oxygen distances of circa 2.8Å,

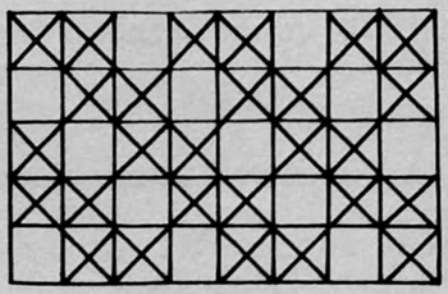


The environment of the V^{5+} ion in V_2O_5.



Layer structure of V_2O_5.

FIG. III. 5. THE STRUCTURE OF VANADIUM PENTOXIDE.



Idealised V_2O_5.

octahedron.

which are close to that of an αT-diol in the zig-zag conformation of an acyclic polyol, i.e. 2.82Å.[7] Ionophoretic measurements have already suggested that this diol has some significance for the complexing, although by itself it is insufficient. In the αC-diol, the oxygen atoms, separated by a distance of 3.65Å[7] could not complex with the oxygen atoms of a monomeric $KVO_3.H_2O$ type of ion without distortion, nor with a monomeric VO_4 tetrahedron. If the VO_4 tetrahedra in, for example, a pyrovanadate are assumed to rotate fairly freely about the linking oxygen atom, an oxygen-oxygen distance of 3.65Å could be produced. Similarly the βT-diol, with an interoxygen distance of 3.43Å[7] might equally well be able to complex. The βC-diol, in which the oxygen atoms are separated by 2.51Å, is less likely to be able to complex.

From ionophoretic measurements, it was established that a cis,cis-1,2,3-triol in a furanose ring is favourably disposed for complexing with an ion in a solution of sodium metavanadate. The cis-1,2-diol interoxygen distance is 2.49Å[38] if the furanose ring is planar, smaller than any equivalent distance in the tetrahedral monomeric or dimeric ion, but may be increased slightly if the ring is puckered. The cis-1,3-diol of a five-membered ring has an interoxygen distance greater than any occurring in a monomeric tetrahedral

or five-coordinated vanadate ion.

From the foregoing, it appears unlikely that the polyhydroxy compounds are complexing with either a monomeric tetrahedral or pentagonal bipyramidal vanadate ion, since of the diols most likely to complex, only the ^{a}T-diol has a corresponding interoxygen distance. Complexing could however be due to a dimeric or further condensed species, though whether this is based on a tetrahedral or five-fold coordinated vanadium atom cannot be decided unequivocally from the data on bond lengths available in the literature.

Before speculating further on the nature of the vanadate complexing species, it is advisable to consider the effect of polyhydroxy-compounds on some of the physical properties of vanadate solutions. Potentiometric and conductimetric studies on vanadate solutions afforded information on their constitution. The presence of a polyol to some extent modifies these properties of the solution and gives an indication of the type of ion involved in the complexing.

IV. The Nature of the Vanadate Complexing Ion.

A Potentiometric Investigations.

Potentiometric titrations depend on the measurement of the change of potential of an indicating electrode, immersed in the titrated solution, as the reagent is added. As a single potential cannot conveniently be measured, a second electrode of constant potential, known as a reference electrode, is employed. When the two electrodes are connected the e.m.f. of the cell so formed can be measured during titration and the observed changes in it are equal to the change of potential of the indicating electrode.

A convenient cell is provided by the hydrogen and potassium chloride electrodes, and the variable quantity measured is the pH, or - log (hydrogen ion concentration), the apparatus constituting the commercially available pH meter.

If the pH of a basic solution is measured while known quantities of strong acid are added to it, and the results plotted as pH versus H^+ added, a curve is obtained with a sharp inflexion at the end point where the base is neutralised. The method can be adapted to the study of the condensation of ions on the acidification of a solution. If the pH is

H^+
plotted against the ratio added/non-condensed ion present initially, a curve is obtained with inflexions corresponding to the completion of the polymerisations. From the H^+/M_xO_y ratios at which these inflexions occur, it is possible to derive the formulae of the condensed species.

Glemser and Preisler[19] titrated sodium orthovanadate (Na_3VO_4) potentiometrically with perchloric acid ($HClO_4$) and obtained inflexions at H^+/VO_4^{3-} ratios of 1.0, 2.0 and 2.6. These they ascribed to the di-, tetra- and decavanadate ions, the first of which was derived from the orthovanadate according to the following equation:

$$2\,VO_4^{3-} + 2H^+ \rightarrow V_2O_7^{4-} + H_2O \qquad H^+/VO_4^{3-} = 1. \qquad \text{eqn. IV. 1.}$$

The ratio of $H^+/VO_4^{3-} = 2$ could correspond to any one of the following reactions:

$$2H^+ + VO_4^{3-} \rightarrow H_2VO_4^- \quad \text{or} \quad VO_3^- + H_2O \qquad \text{eqn. IV. 2.}$$

$$4H^+ + 2VO_4^{3-} \rightarrow H_4V_2O_8^{2-} \quad \text{or} \quad V_2O_6^{2-} + 2H_2O \qquad \text{eqn. IV. 3.}$$

$$6H^+ + 3VO_4^{3-} \rightarrow H_6V_3O_{12}^{3-} \quad \text{or} \quad V_3O_9^{3-} + 3H_2O \qquad \text{eqn. IV. 4.}$$

$$8H^+ + 4VO_4^{3-} \rightarrow H_8V_4O_{16}^{4-} \quad \text{or} \quad V_4O_{12}^{4-} + 4H_2O \qquad \text{eqn. IV. 5.}$$

Glemser and Preisler[19] believe that a tetravanadate is formed,

from absorption measurements performed on the solutions. (These measurements will be discussed more fully later).

The inflexion at $H^+/VO_4^{3-} = 2.6$ was not reproducible,[19] and may correspond to either[19]:

$$5\ V_4O_{12}^{4-} + 8H^+ \rightarrow 2\ V_{10}O_{28}^{6-} + 4H_2O \qquad H^+/VO_4^{3-} = 2 \rightarrow 2{\cdot}4.$$

eqn. IV.6.

or

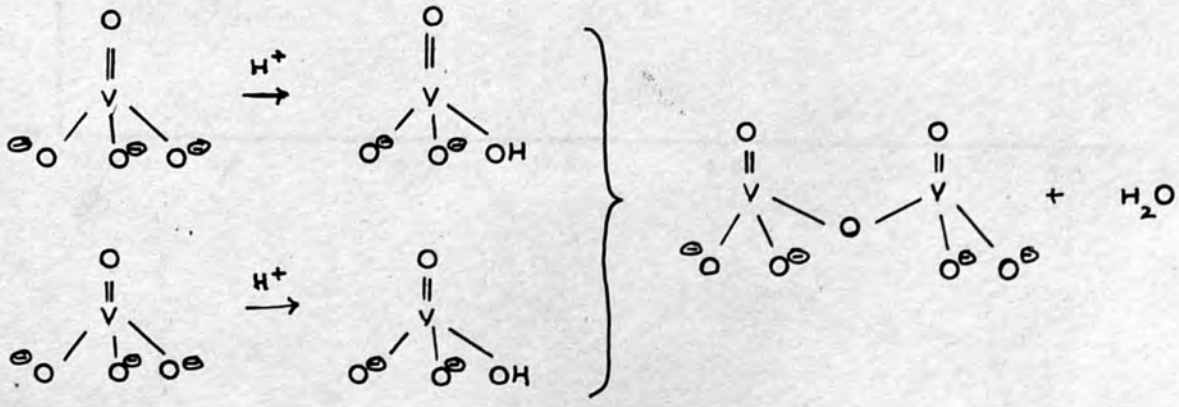
$$5\ V_4O_{12}^{4-} + 12H^+ \rightarrow 2\ H_2V_{10}O_{28}^{4-} + 4H_2O \qquad H^+/VO_4^{3-} = 2 \rightarrow 2{\cdot}6$$

eqn. IV.7.

These workers derived a potentiometric curve from the pH versus H^+/VO_4^{3-} ratio of separate solutions, of the same concentration based on sodium orthovanadate, to which differing volumes of acid had been added. The solutions were allowed to stand several days before the measurements were made. It was found that a mixed acidified solution of sodium orthovanadate and D-glucitol darkens rapidly from the initial yellow, or orange, to green. Presumably the oxidation state of the vanadium is changing. The orthovanadate solution was therefore titrated immediately after its preparation [Expt. 32]. Essentially the same curve is obtained as that of Glemser and Preisler,[19] though the

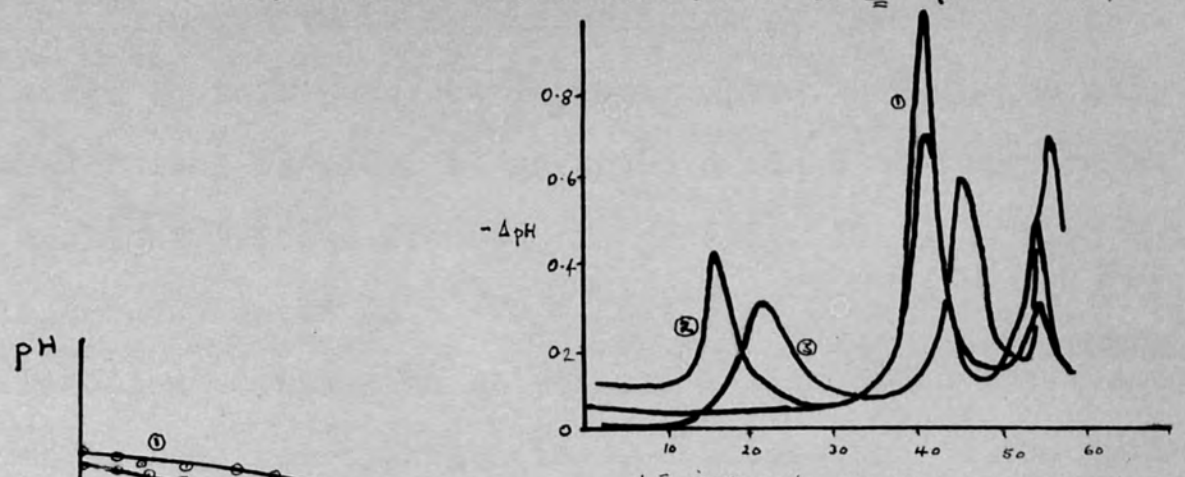
H^+/VO_4^{3-} ratios for the inflexions are slightly higher, probably owing to the equilibrium not having been immediately attained. The experiment was repeated with an orthovanadate solution containing D-glucitol in the V/P ratio of 2, this having been found to be the probable ratio in the complex [Expt. 33]. Owing to the equilibrium nature of the reaction, however, the curve obtained showed little difference from that of the orthovanadate alone (see Fig. IV.1). An excess of D-glucitol (V/P = 16) was therefore used to ensure that the equilibrium was as far as possible in favour of the complexed materials (Fig. IV.1, Expt. 34). The inflexion at $H^+/VO_4^{3-} = 1$, was absent in this curve, suggesting that in such solutions the divanadate is not formed in the usual manner, but the presence of the D-glucitol interrupts the reaction sequence, so that the first condensation occurs at an H^+/VO_4^{3-} ratio of 2.

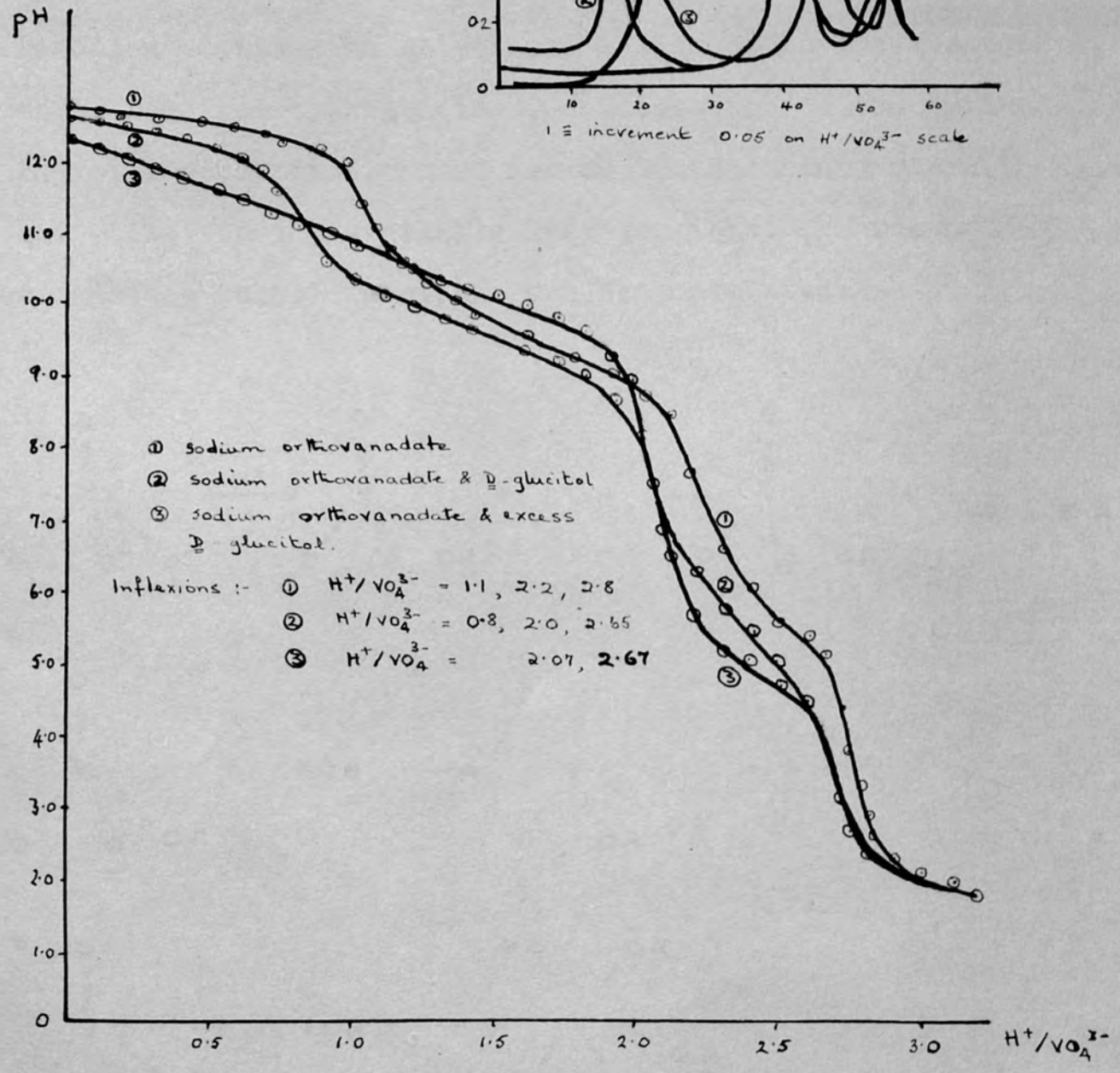
In order for the orthovanadate to condense under normal aqueous conditions, acid must be added. The reaction to form the divanadate can be envisaged as:



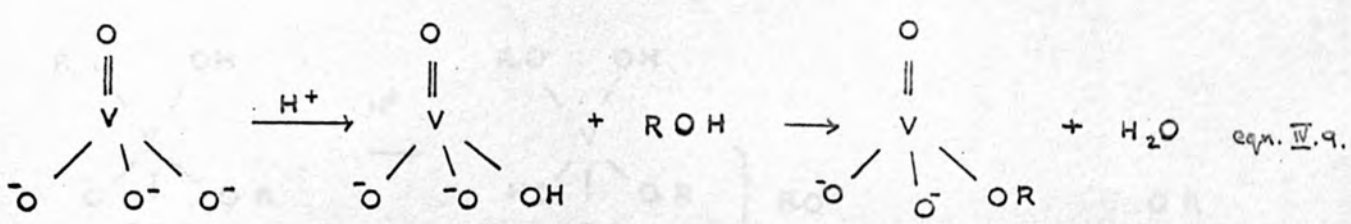
eqn. IV.8.

FIG. IV.1. POTENTIOMETRIC TITRATION OF SODIUM ORTHOVANADATE AND SODIUM ORTHOVANADATE & D-GLUCITOL.

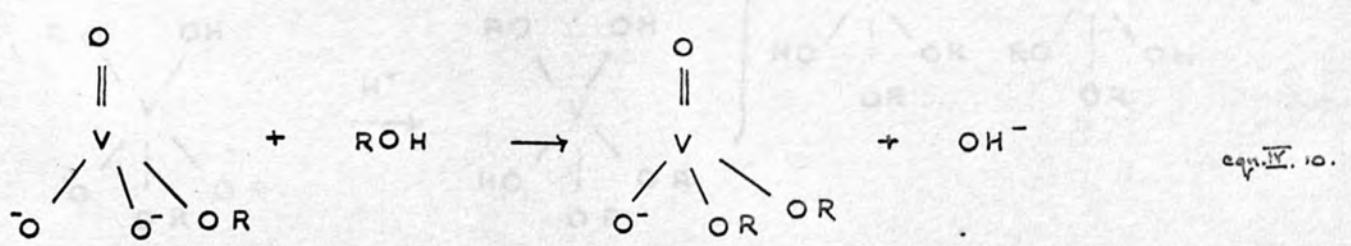




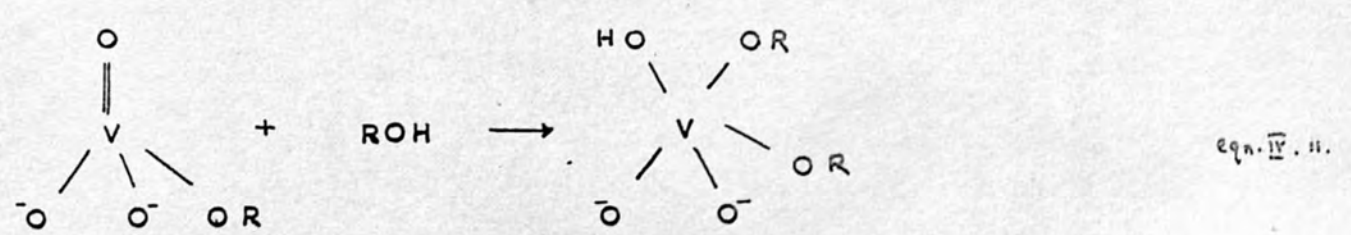
Suppose that, in the presence of the polyol, some stage in this sequence is interrupted. When the polyol D-glucitol is added to an orthovanadate solution there is a slight initial decrease in pH (Fig. IV.1). The pH then decreases until an H^+/VO_4^{3-} ratio of 2 is reached. If a reaction between the polyol and sodium orthovanadate occurs, which requires hydrogen ions, condensation of the vanadate ion will be limited, in so far as the secondary reaction is competing for the available hydrogen ions. Three possible vanadate + polyol reactions can be postulated:



eqn. IV.9.

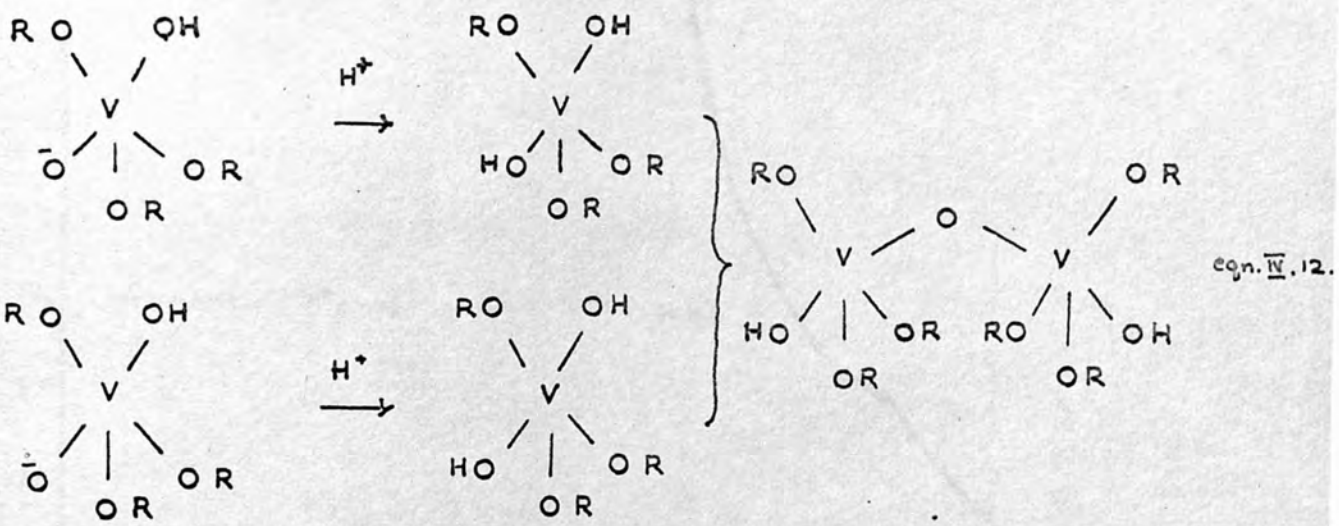


eqn. IV. 10.



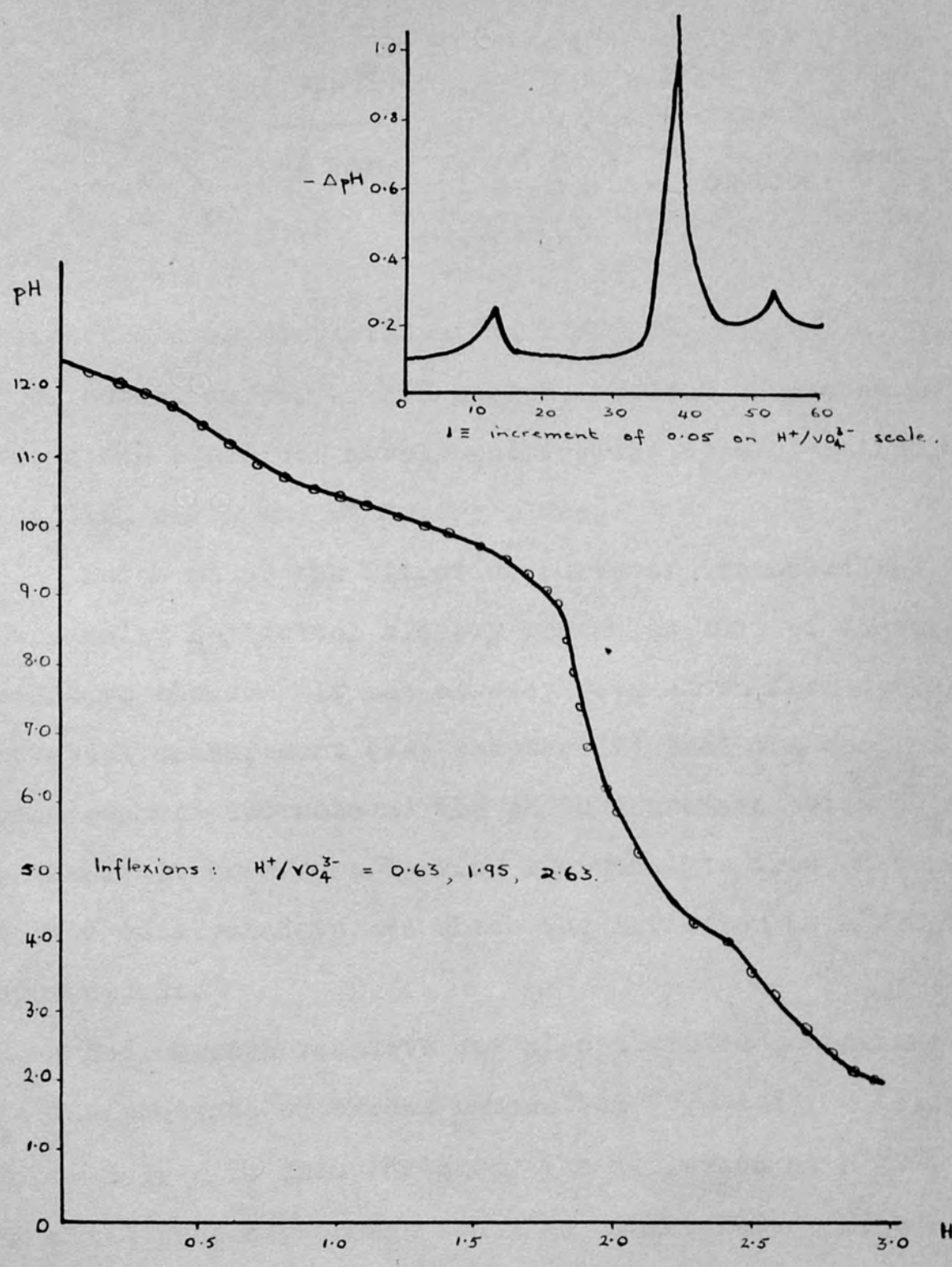
eqn. IV. 11.

In one of these reactions hydroxyl ions would be released, which would mask the decrease in pH due to vanadate polymerisation, while there may be a slight increase in hydrogen ion concentration as a result of the third reaction, depending on the relative ionisation of a V-OH and R-OH hydrogen atom. While some or all of these reactions are taking place an equilibrium may be set up which is reflected in a slight but steady decrease in pH. The second H^+ ion provided by the acid could then be used, as before, to condense the orthovanadate to a divanadate, but at a much lower pH than usual.

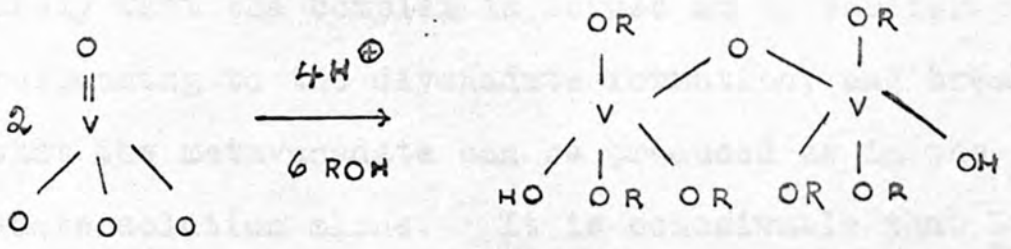


eqn. IV.12.

FIG. IV 2. POTENTIOMETRIC TITRATION OF SODIUM ORTHOVANADATE IN THE PRESENCE OF EXCESS D-MANNITOL.



The overall reaction is then



which would be completed at an H^+/VO_4^{3-} ratio of 2. [The actual number of ROH groups involved cannot be deduced, the above equations merely outline the type of secondary reaction which may be taking place].

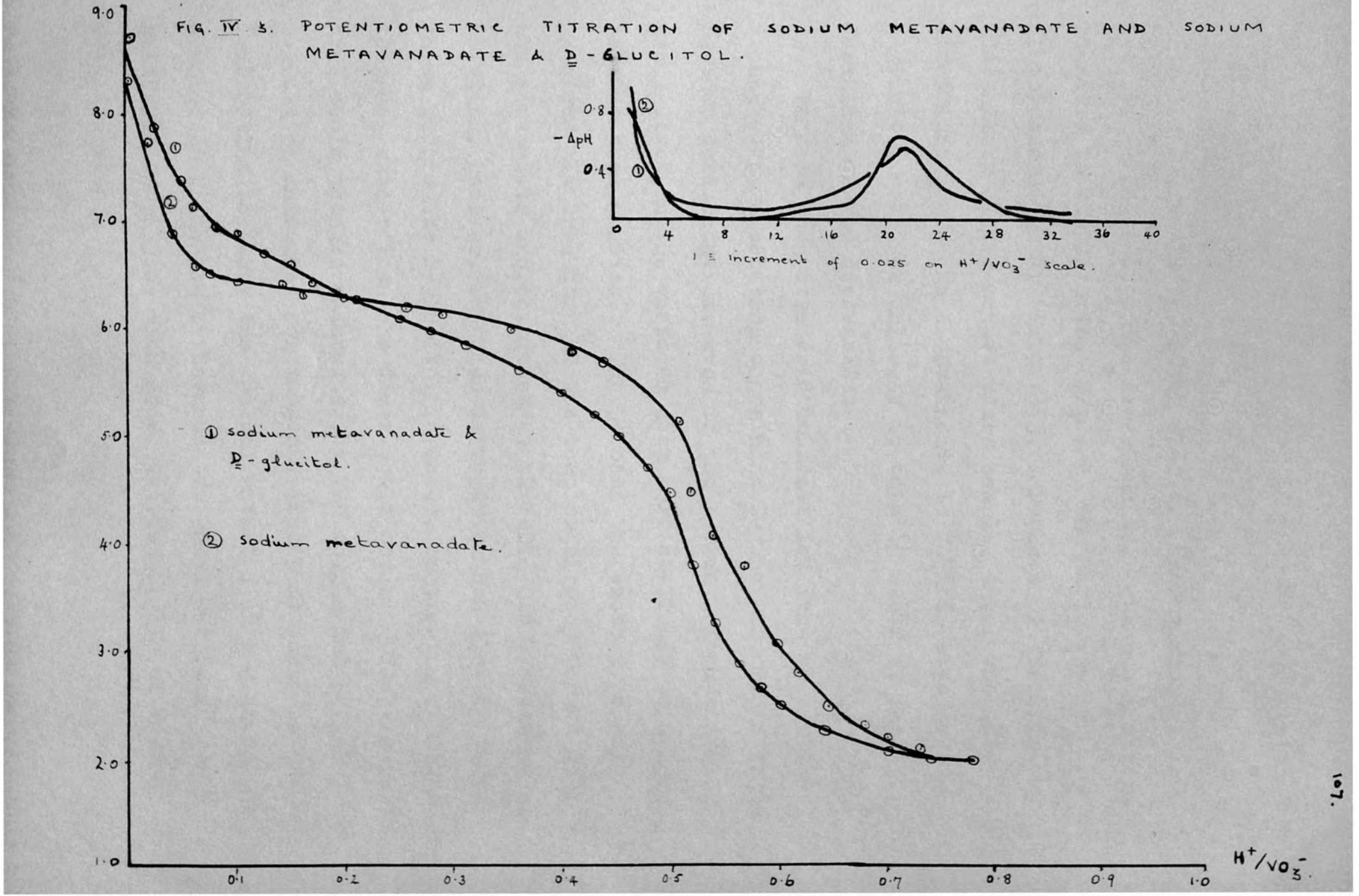
Below pH 5. the titration curve of the solutions containing D-glucitol closely resembles that of the orthovanadate alone. It has already been shown from optical rotation measurement (see chapter II) that the complex becomes more unstable as the pH is decreased below 5, and probably it breaks up leaving the vanadate free to condense to the deca-vanadate, to which the inflexion at $H^+/VO_4^{3-} = 2.6$ corresponds.

Sodium orthovanadate was also titrated potentiometrically in the presence of excess D-mannitol (V/P = 5). (Fig. IV.2, Expt. 35). In this instance, the inflexion at $H^+/VO_4^{3-} = 1$ is still present, though somewhat diminished. If a similar sequence of reactions is postulated as for D-glucitol, the continued inflexion could be due to incomplete complexing.

Since maximal optical rotation occurs at pH 6.0, it seems unlikely that the complex is formed at an earlier pH, corresponding to the divanadate formation, and breaks down so that the metavanadate can be produced as in the orthovanadate solution alone. It is conceivable that D-mannitol could complex with the divanadate, after its formation, in such a way as not to prevent its condensation to the metavanadate. To maintain a V/P ratio of 1, two molecules of D-mannitol would have to be attached to the divanadate ion.

If D-glucitol complexes solely with a divanadate ion, it is difficult to explain the greater enhancement of rotation of D-glucitol by acidified solutions of sodium metavanadate than by acidified sodium orthovanadate. The above postulates to interpret the potentiometric titrations in sodium orthovanadate start with the orthovanadate ion which will not be available in sodium metavanadate solutions. The complexing with the already partially condensed vanadate ion in sodium metavanadate solutions may not be identical with that occurring in orthovanadate solutions and the coincidence of the pH for maximum rotation may be only fortuitous. Potentiometric titration of sodium metavanadate (Fig. IV.3 Expt. 30) shows an inflexion at the H^+/VO_3^-, ratio of 0.6, which corresponds to an ion $6H^+ : 10VO_3'$ or $H_6V_{10}O_{30}$.

FIG. IV. 3. POTENTIOMETRIC TITRATION OF SODIUM METAVANADATE AND SODIUM METAVANADATE & D-GLUCITOL.



This may be rewritten, after Glemser and Preisler[19], as $H_2V_{10}O_{28} + 2H_2O$, i.e. the decavanadate. When D-glucitol is added to the solution (V/p = 2) the inflexion is diminished slightly (Expt. 31]. Because of the known instability of the metavanadate complex under the acid conditions where the decavanadate is formed, even excess D-glucitol would be unlikely to totally restrict the formation of the decavanadate.

To form the tetrameric vanadate ion from the orthovanadate, in a solution containing D-glucitol, would entail further acidification, even if the hydroxyl groups left uncomplexed on the vanadium atom were available to undergo further condensation. The process therefore probably involves the breakdown of the complex already formed with the divanadate and completed at pH 6.0. In a metavanadate solution, however, in which aggregation has already taken place before the D-glucitol is added, complexing with the tetramer rather than the dimer could occur. The V/p ratio of 2 would then be maintained by the complexing of two D-glucitol molecules per vanadate ion. It would be interesting to study the optical rotation of D-glucitol in a solution of sodium orthovanadate, which had been pre-acidified to pH 9.0, then added to the polyol, on further acidification.

B Conductimetric Investigations.

The change in the conductivity of an aqueous solution of sodium orthovanadate, to which acid is being added, can be used to determine the degree of condensation of the vanadate ions. Britton and Robinson[35] reported that the equilibrium was set up slowly in the conductimetric titration of sodium orthovanadate with acid, (c.f. the potentiometric titration of Glemser and Preisler[19]), and in consequence a curve with no definite breaks is obtained. This result was confirmed (Fig. IV.4 Expt. 36).

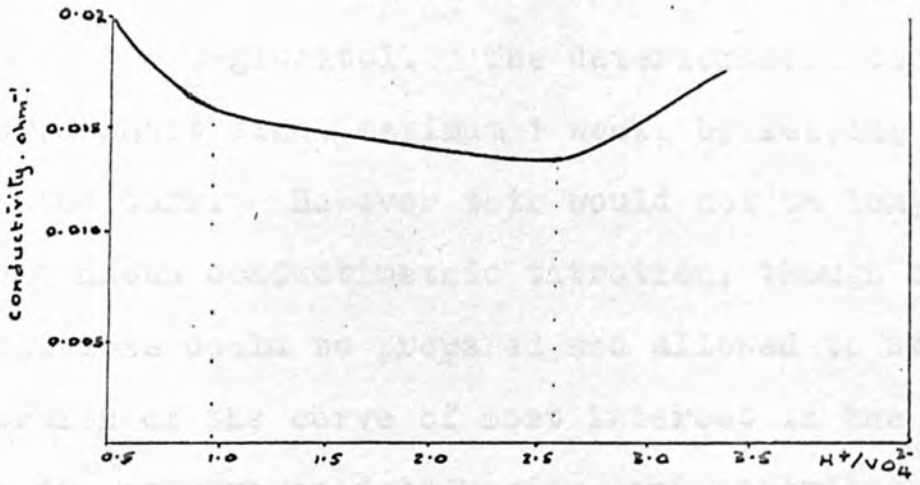


Fig. IV. 4. Conductimetric titration of sodium orthovanadate.

By extrapolation, breaks can be seen to occur at H^+/VO_4^{3-} ratios of 1.0 and 2.6. But there is no inflexion corresponding to the H^+/VO_4^{3-} ratio of 2.0 which would be expected. The above workers left the vanadate solutions for some weeks before taking measurements, or alternatively

heated them to establish the equilibrium more rapidly. Neither method is practicable for solutions containing a polyol. On standing for several days and on heating, solutions containing sodium metavanadate and D-glucitol darken appreciably. It has been noted by Magee and Richardson that vanadic acid is reduced by mannitol and glucose to form green precipitates.[50] Glucose forms a green reduction product on heating with sodium metavanadate, (the effect was noted on drying ionophoretograms), and some similar change in the oxidation state of vanadium, reflected by the green colouration of the solution on standing, is caused by D-glucitol. The deterioration can be prevented for a short time, maximum 1 week, by keeping the solutions in the dark. However this would not be long enough for a continuous conductimetric titration, though separate solutions could be prepared and allowed to stand. But the portion of the curve of most interest is the metavanadate to di- or pyrovanadate region, and according to Jander and Jahr[38], equilibrium results immediately if sodium metavanadate is titrated with sodium hydroxide.

Sodium metavanadate was titrated conductimetrically with sodium hydroxide in the presence and absence of excess D-mannitol (Expts. 38, 37). In the absence of the polyol, definite breaks were noted at OH'/VO_3' ratios of 1.0 and 2.0.

$$2VO_3^- + 2OH^- \rightarrow V_2O_7^{4-} + H_2O \qquad OH^-/VO_3^- = 1. \qquad \text{eqn. IV. 14.}$$

$$2VO_3^- + 4OH^- \rightarrow 2VO_4^{3-} + 2H_2O \qquad OH^-/VO_3^- = 2. \qquad \text{eqn. IV. 15.}$$

The addition of D-mannitol depressed the conductivity of the solution slightly. The inflexion corresponding to the di- or pyrovanadate $V_2O_7^{4-}$ ion was still present at OH^-/VO_3^- $\simeq$ 1.0. This is in accordance with the potentiometric titration of sodium orthovanadate in the presence of D-mannitol and suggests that either D-mannitol can complex with both the pyrovanadate and the metavanadate, or that the complex with the metavanadate is not sufficiently strong to prevent its breakdown so that some pyrovanadate may be formed from uncomplexed metavanadate. The inflexion due to the orthovanadate, at OH^-/VO_3^- = 2.0 has been eliminated (Fig. IV.5).

A similar result was obtained for solutions of sodium metavanadate containing D-glucitol (Expt. 39): the pyrovanadate inflexion reappeared at OH^-/VO_3^- = 1.0, but the orthovanadate inflexion disappeared (Fig. IV.5). In considering these results, it is of interest to compare the change in optical rotation of D-glucitol on acidification

of a solution containing the polyol and sodium metavanadate, and on rebasification. The maximum rotation on alkalisation is much lower, (Fig. IV.6 Expt. 6) but the enhancement of rotation persists to a higher pH. This difference could be explained by postulating the complexing of D-glucitol with a transient intermediate which is formed on the acidification of sodium metavanadate solution and stabilised in the solution by the polyol, but which is not formed on alkalisation.

When this presumed polyanion is not present, the D-glucitol might complex in the same manner as does D-mannitol, yielding similar conductimetric titration curves. To determine whether this argument is feasible, the V/P ratio for D-glucitol in conditions unfavourable for the formation of such an intermediate, i.e. at a higher pH or after alkalisation of an acidified solution, should be investigated.

The possibility of deriving a value conductimetrically for the V/P ratio in the galactitol complex was considered, since this ratio had been found from absorptiometric measurements but cannot be confirmed polarimetrically. The conductivity of a given aqueous solution of sodium metavanadate should change on the addition of the polyol, provided that a complex with a different charge is formed,

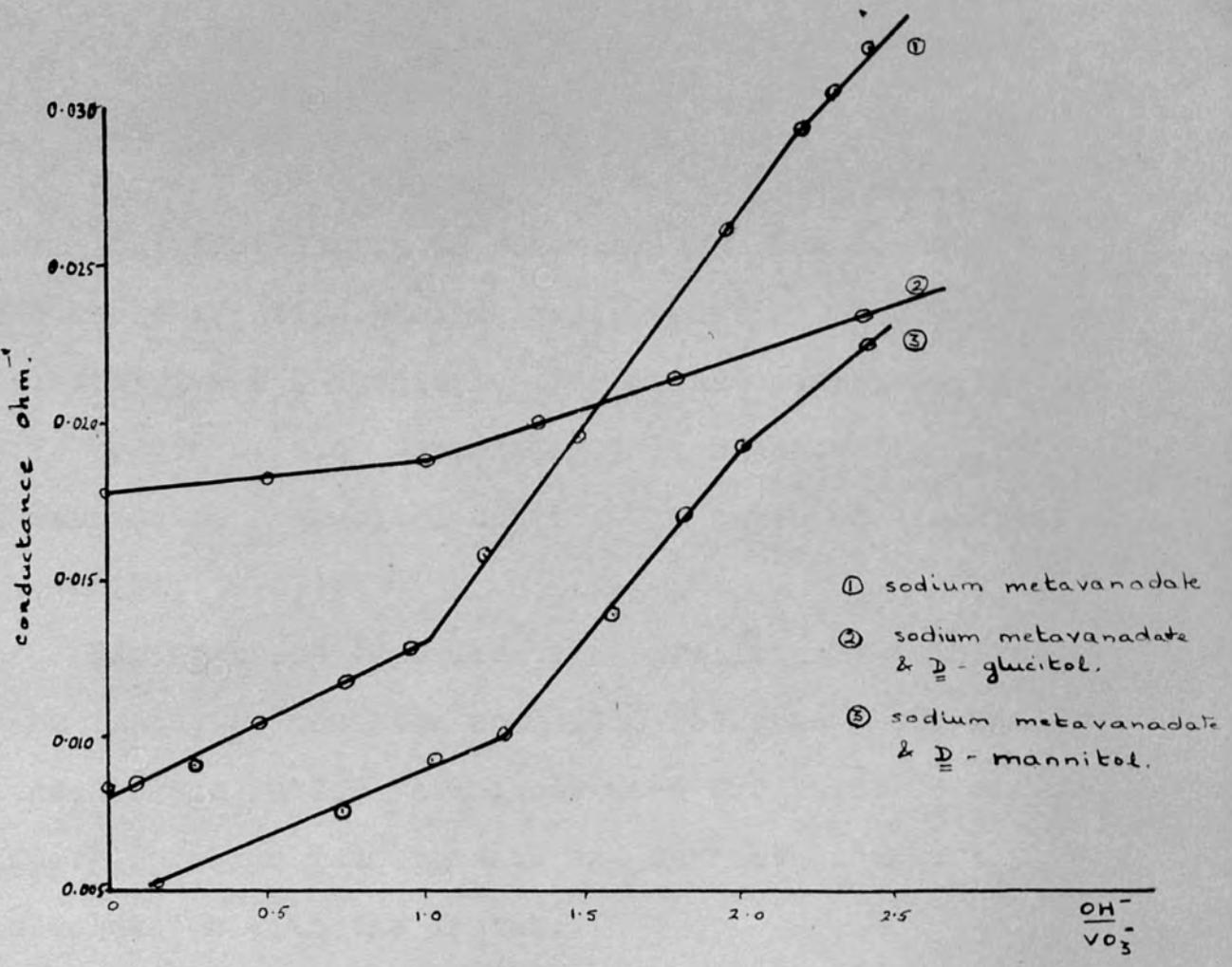


Fig. IV. 5. Conductimetric titrations of sodium metavanadate solutions.

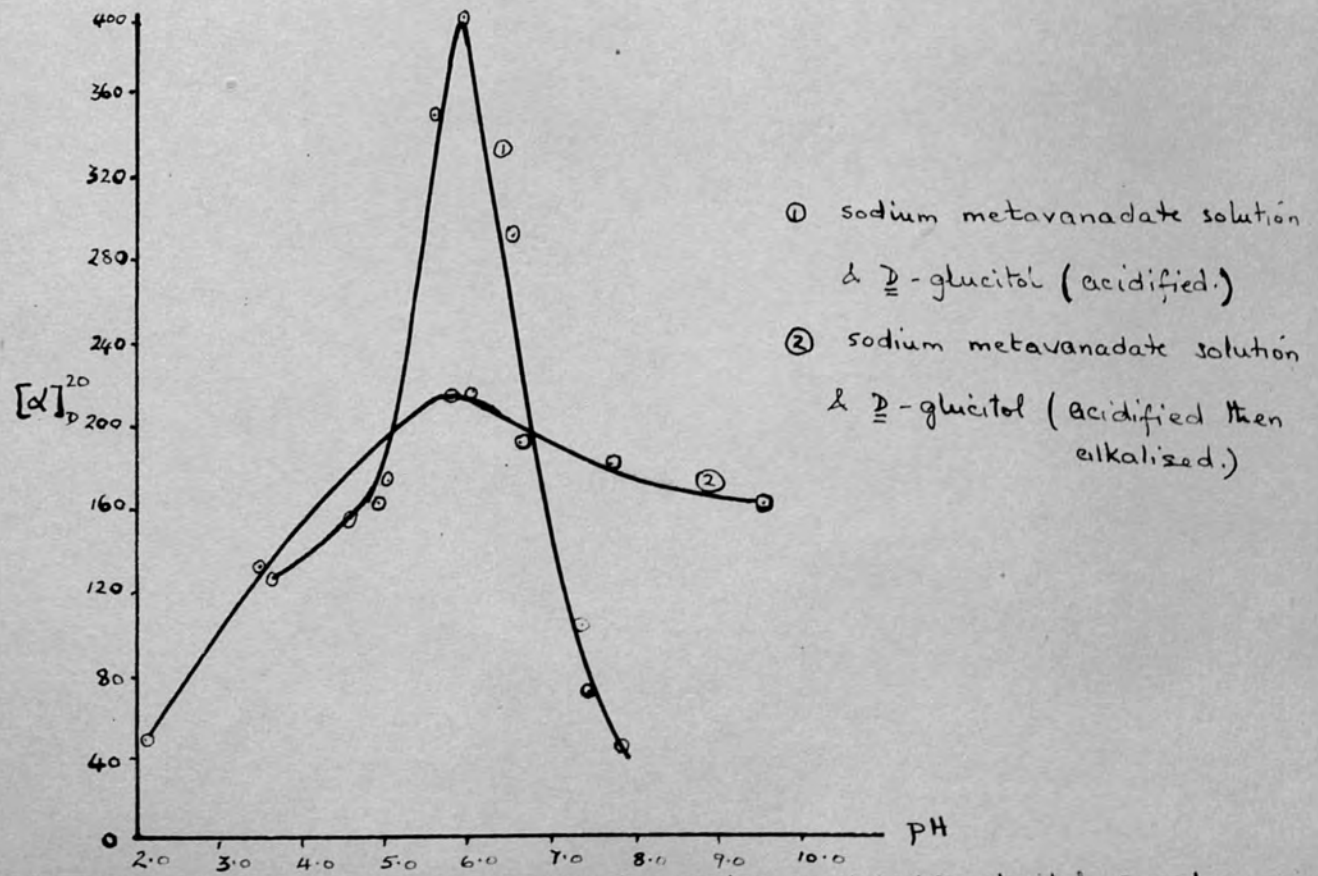


Fig IV. 6. The effect of alkali on the metavanadate | D-glucitol complex.

until the equilibrium is reached, when the conductivity of the whole solution should remain constant. For galactitol and D-mannitol, this steady state was reached at a V/P ratio of 1. Surprisingly it was not finally attained for D-glucitol until a P/V ratio of 1 (Expts. 40, 41,42) (Fig. IV.7).

During these experiments no preliminary acidification took place, so that the solutions resemble those used in ionophoresis rather than those used for polarimetric and absorptiometric measurements and may not necessarily be correlatable with the latter.

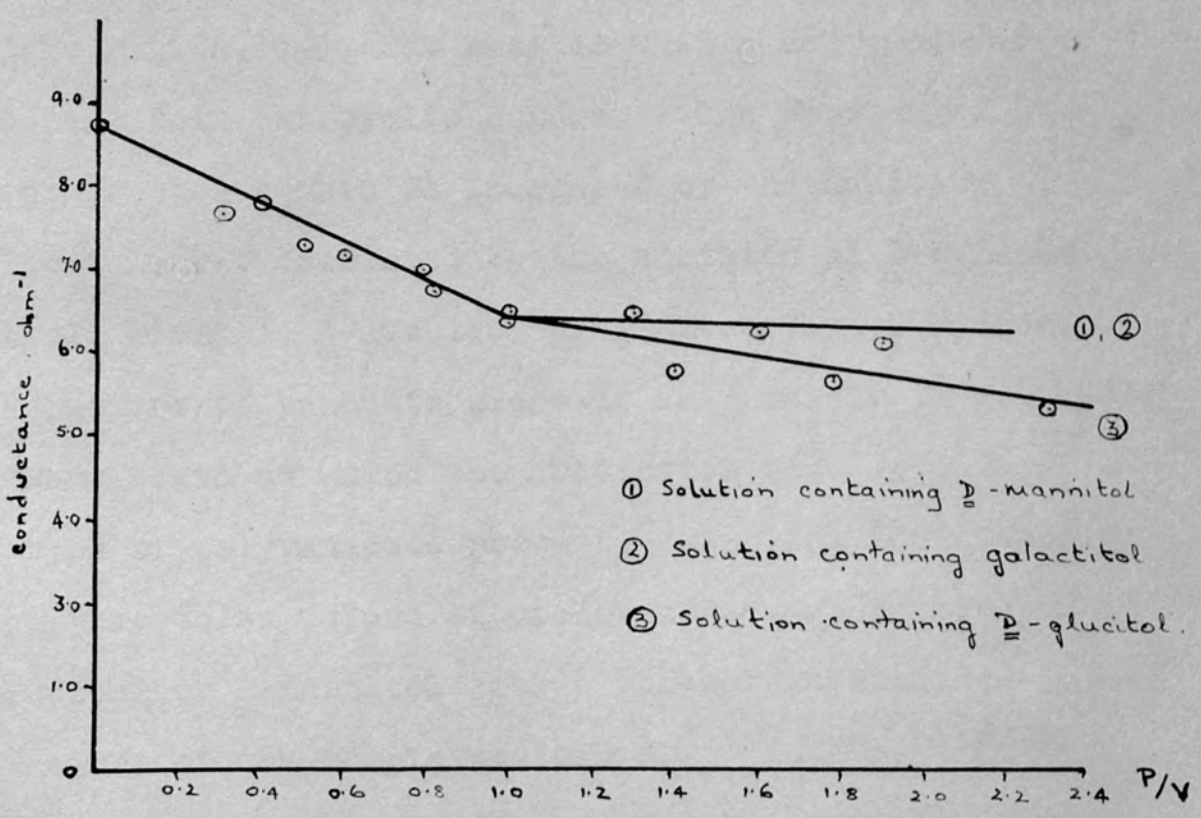


FIG. IV.7. V/P ratios by conductimetric measurements.

C Absorptiometric Investigations.

It has been mentioned earlier (Chapter II), that the addition of certain hexitols to a metavanadate solution causes an appreciable shift in the absorption edge in the visible region, and that smaller shifts are produced by some pentitols and cyclic sugars. The wavelength at which the absorption ceases is increased by the addition of D-glucitol, but decreased by the addition of D-mannitol, xylitol, ribitol, L-arabitol and maltose and D-ribose. In solutions of vanadate alone it is possible to correlate the wavelength at which the absorption edge appears with the type of polyvanadate present. Application of the same principles to solutions of orthovanadate containing D-glucitol or D-mannitol yields further information about the nature of the complexes formed.

Glemser and Preisler[19] traced the absorption edges of solutions of sodium orthovanadate at different acidities, and found that these occurred in three distinct regions of wavelengths (see Fig. IV. 8).

Particular wavelengths within these three distinct groups were chosen, and the extinction at each wavelength was plotted against the H^+/VO_4^{3-} ratio, the values being derived from the absorption curves.[19]

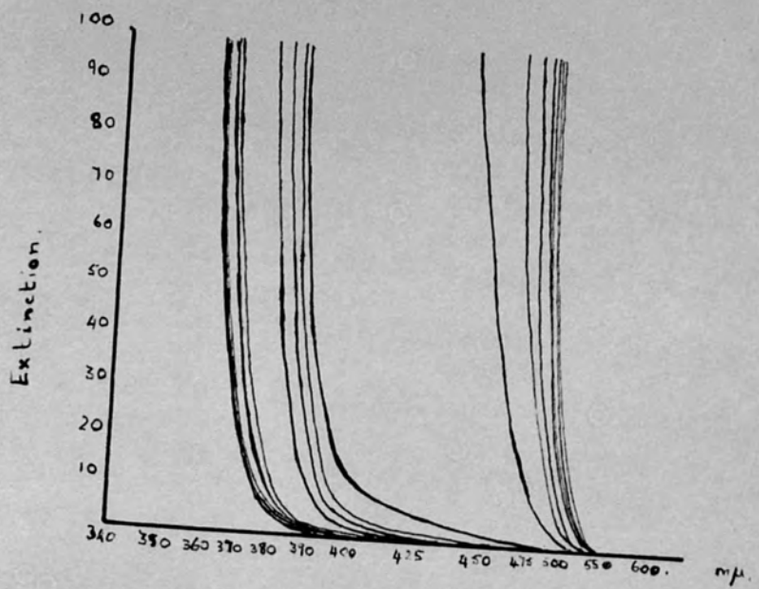


Fig IV. 8 The dependence of the absorption on H^+/VO_4^{3-}

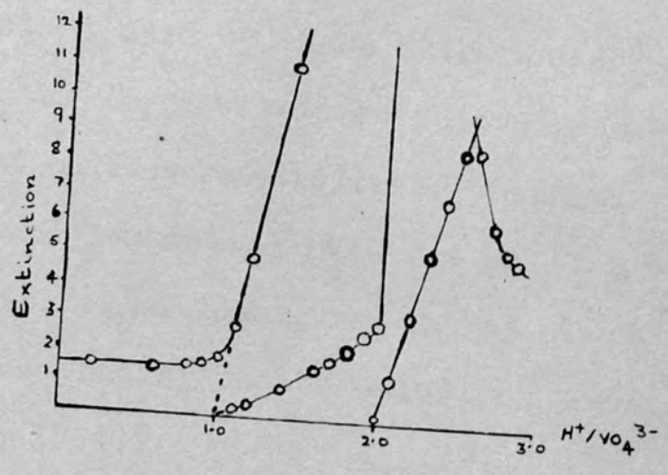


Fig. IV. 9. The dependence of the extinction of vanadate solutions on H^+/VO_4^{3-}.

The three inflexion points occur at H^+/VO_4^{3-} = 1.0, 2.0, 2.6, corresponding to the di- or pyrovanadate in $V_2O_7^{4-}$, the metavanadate ion and the decavanadate $V_{10}O_{28}^{6-}$ ion. Glemser and Preisler[19] then considered the particular absorption edges for the solutions containing acid equivalent to H^+/VO_4^{3-} = 0, 1, 2 and 2.4. The frequency at which the extinction value was 0.5, for each of these absorption edges, was plotted against n_v, the degree of polymerisation of the orthovanadate, known except for the metavanadate. From the resultant graph, the metavanadate was concluded to be tetrameric.

The method used by these authors was employed [Expts. 44, 45] to investigate solutions of sodium orthovanadate, at different acidities, containing excess D-glucitol and D-mannitol [V/P = 6 and V/P = 5 respectively). The absorption edges are shown in Fig. IV 10 a,b,c, the numbers corresponding to the solutions given in the table below (Table IV.1).

Fig. IV.10.a. Spectra of sodium orthovanadate solutions of different acidities.

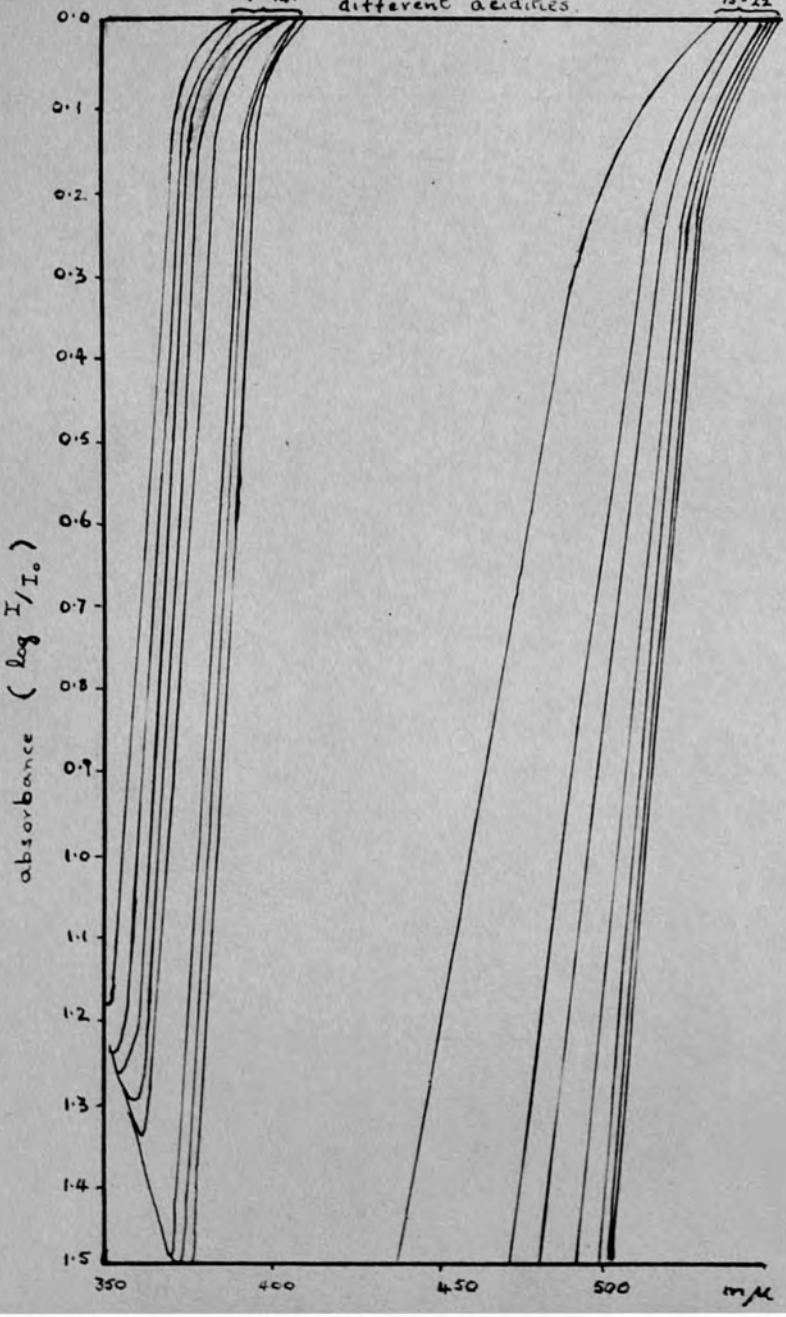


Fig. IV.10.b. Spectra of sodium orthovanadate solutions containing L-glucitol.

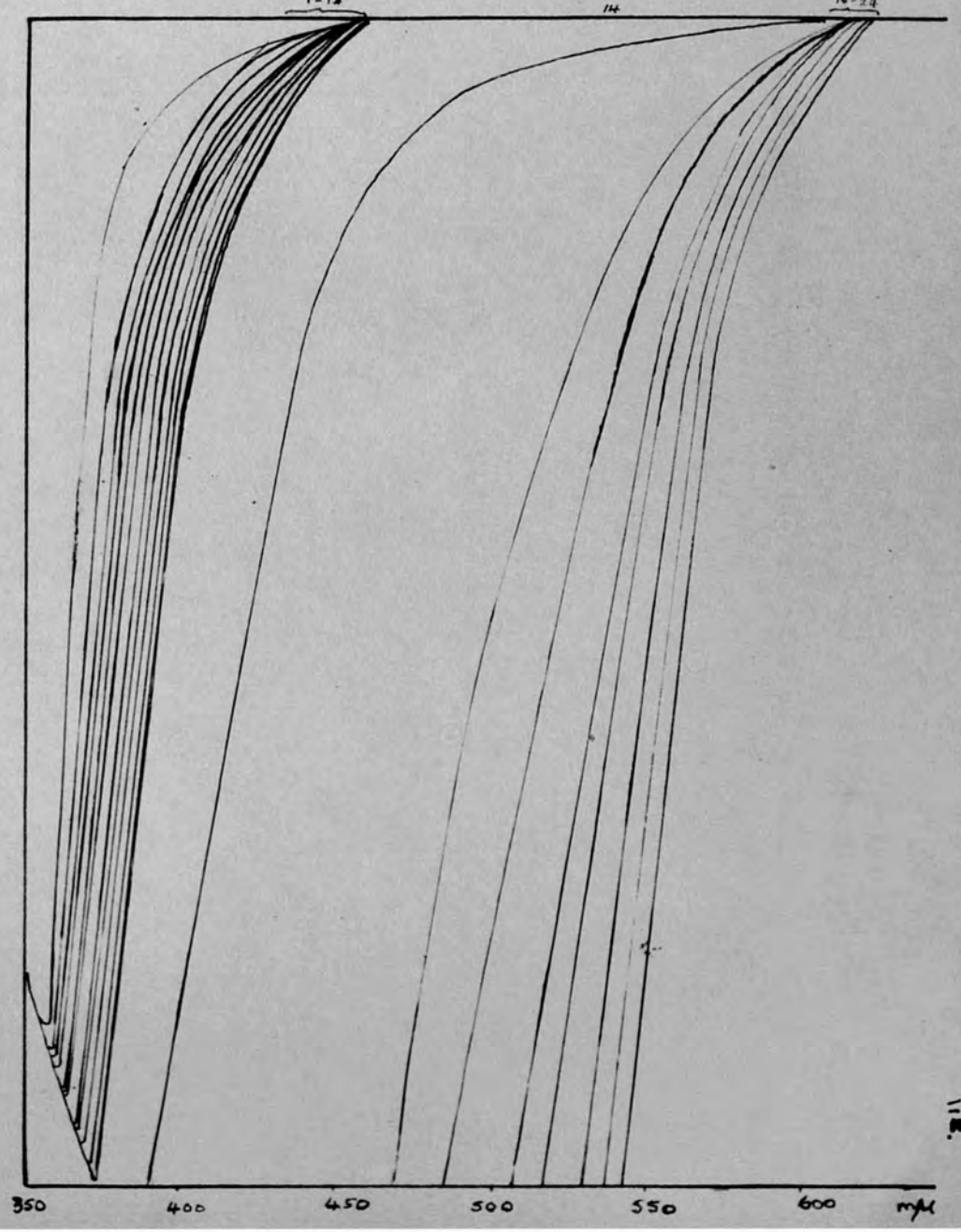


Fig. IV. 10(c). Spectra of sodium orthovanadate solutions, at different acidities, containing D - mannitol.

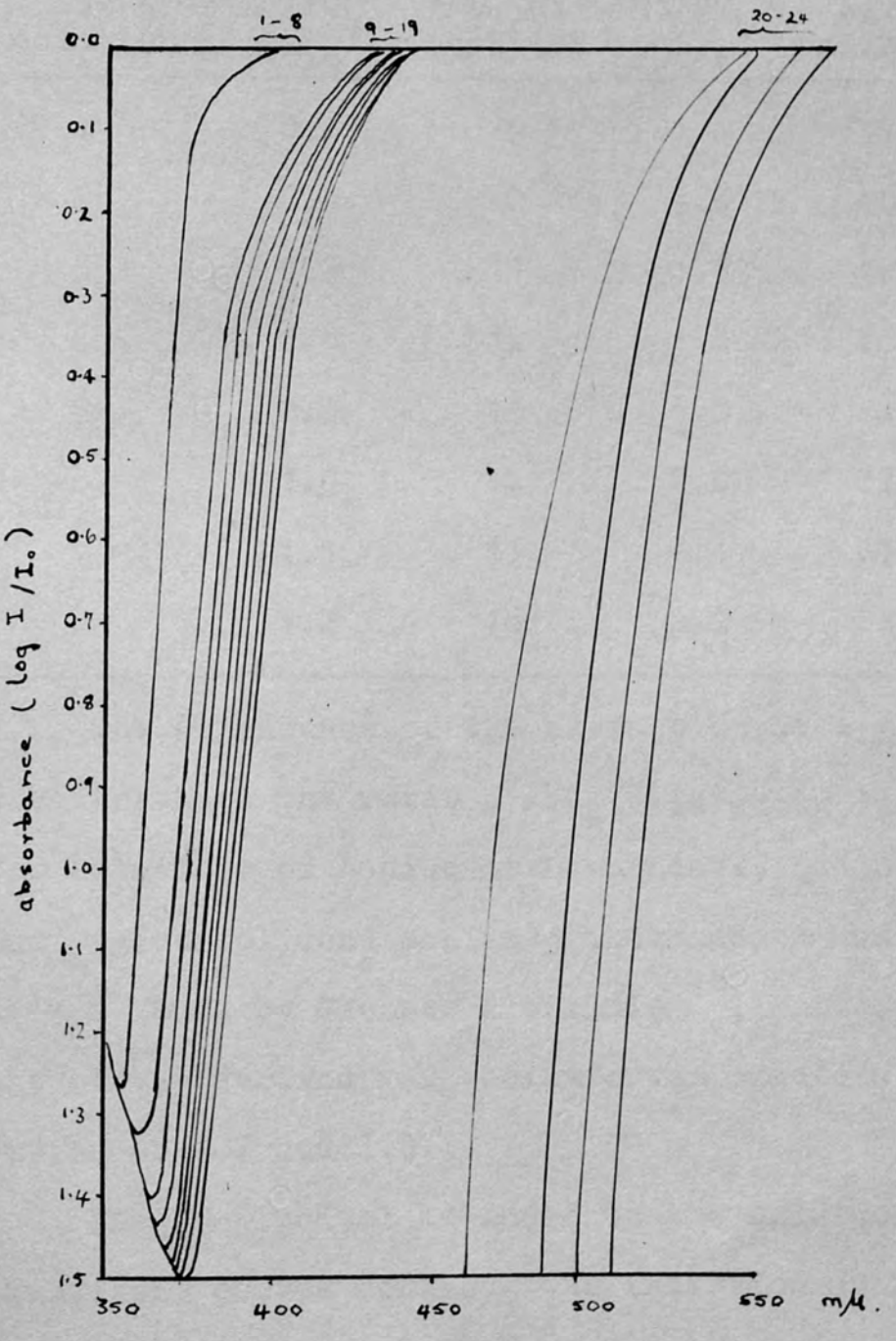


Table IV.1 H^+/VO_4^{3-} ratio for solutions of sodium orthovanadate, sodium orthovanadate & D-glucitol, sodium orthovanadate & D-mannitol.

No. of solution	H^+/VO_4^{3-} ratio	No. of solution	H^+/VO_4^{3-} ratio	No. of solution	H^+/VO_4^{3-} ratio
1	0.2	9	1.4	17	2.3
2	0.4	10	1.6	18	2.4
3	0.6	11	1.7	19	2.5
4	0.8	12	1.8	20	2.6
5	0.9	13	1.9	21	2.7
6	1.0	14	2.0	22	2.75
7	1.1	15	2.1	23	2.8
8	1.2	16	2.2	24	2.85

The dependence of the extinction at a particular wavelength on the ratio H^+/VO_4^{3-} is shown in Figs. 11,12,13, for solutions of sodium orthovanadate, sodium orthovanadate and D-glucitol, and sodium orthovanadate and D-mannitol. As was found by Glemser & Preisler[19], inflexions occur in the curves derived for sodium orthovanadate at H^+/VO_4^{3-} ratios of 1,2 and 2.6.

When D-glucitol is added to the solution, and the equivalent curves derived, the inflexion at $H^+/VO_4^{3-} = 1.0$ is less marked, and the curves at the wavelengths which include this inflexion appear to tend towards a second

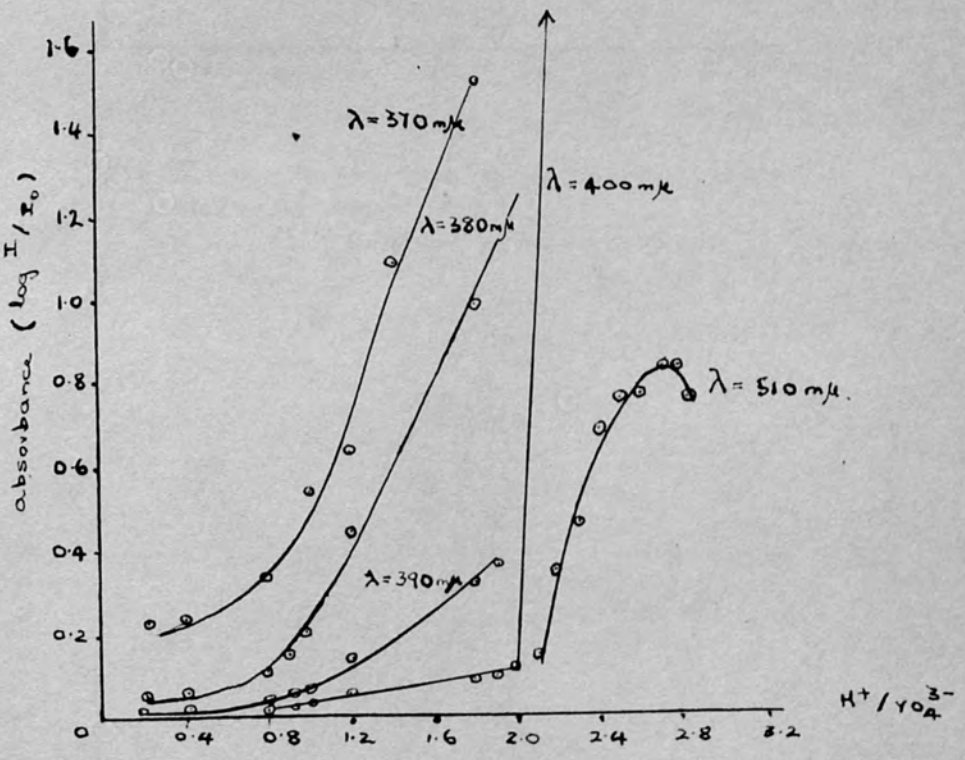
inflexion at $H^+/VO_4^{3-} = 2$. The marked inflexion at $H^+/VO_4^{3-} = 2.0$ is again present for a number of wavelengths, and the inflexion at $H^+/VO_4^{3-} = 2.6$ also remains.

The conclusion which can be drawn is that, at low acidities, some equilibrium is set up, including an ion for which the H^+/VO_4^{3-} ratio is 1, but that as the acidity is increased, such that $H^+/VO_4^{3-} = 2.0$ an ion with a different absorption is produced. The presence of the decavanadate is probably, as in the potentiometric titrations, due to the breakdown of the complex with D-glucitol.

The curves derived for solutions containing D-mannitol show inflexions at $H^+/VO_4^{3-} = 1.0$, and there is some evidence for an inflexion at $H^+/VO_4^{3-} = 2.8$. The inflexion at $H^+/VO_4^{3-} = 2.0$ is entirely absent. Complexing with D-mannitol, in an acidified solution of sodium orthovanadate, therefore seems to involve an ion with $H^+/VO_4^{3-} = 1.0$, and by analogy with the orthovanadate solution, this may be a divanadate ion. By comparison with the potentiometric titration, some absorptiometric evidence for an H^+/VO_4^{3-} ratio of 2.0 might have been expected, since the inflexion at this ratio is still present in the curve obtained potentiometrically. It can only be supposed that the reaction which involves a sharp

Fig. IV. 11. Data from absorption spectra.

(i) sodium orthovanadate.



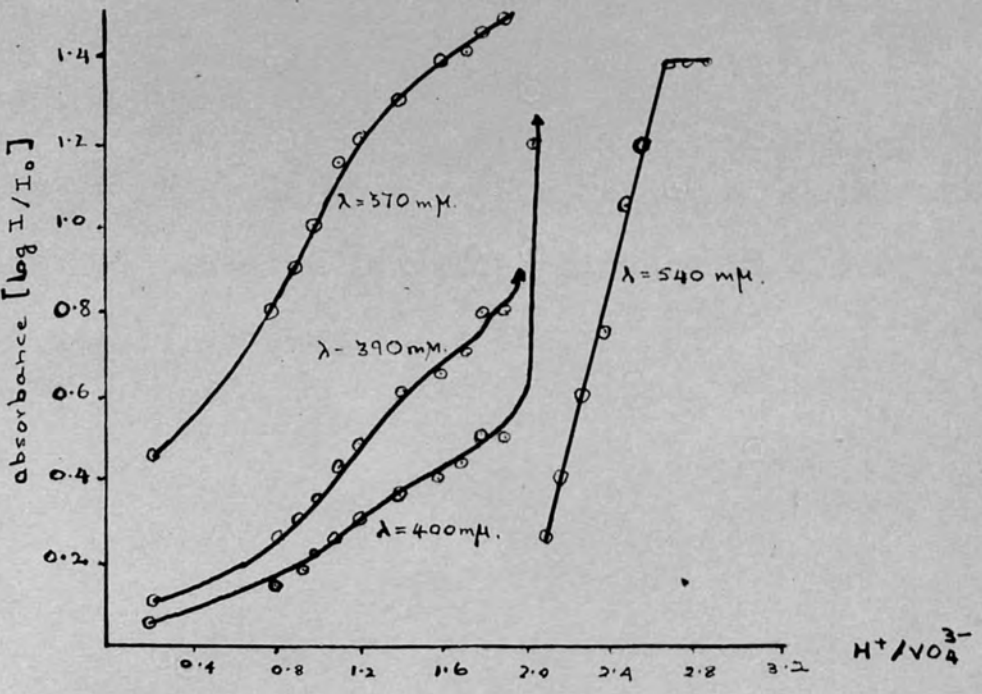


Fig IV . 12. Data from absorption spectra.

(2). sodium orthovanadate & D-glucitol.

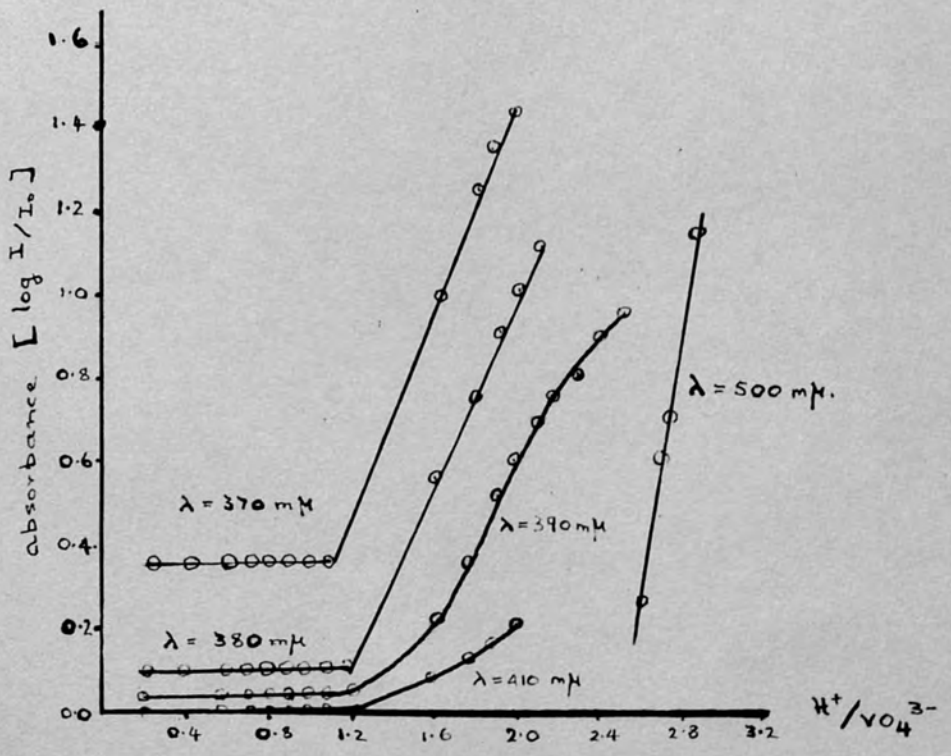


Fig IV . 13. Data from absorption spectra.

(3) Sodium orthovanadate & D - mannitol.

drop in pH at H^+/VO_4^{3-} = 2.0 is not a condensation to a metavanadate ion which differs in absorption from the di- or pyro-vanadate ion.

V. The Nature of the Complex between D-glucitol and Sodium Metavanadate : Periodate Oxidation.

Since the interpretation of the changes in physical properties of vanadate solutions caused by the addition of polyols is complicated by the equilibria present in such solutions, some properties of the actual vanadate : polyol entity were next considered : firstly, its reaction with sodium metaperiodate and secondly the infra-red spectrum of the isolated complex.

Sodium metaperiodate cleaves the α-glycol groups in an inositol, an acyclic polyol or a cyclic sugar[69]. In D-glucitol the susceptibility to cleavage is in the order $\alpha_{trans} > \alpha_{cis} > \alpha$. Of the two α_{trans} and α groups, the 3,4 and 5,6 groups are cleaved more readily and products are formed according to the following table[70]:

Table V.1. Periodate oxidation of D-glucitol

Carbon-carbon bond	Type of glycol	Product obtained
1,2	α	D-arabinose
5,6	α	L-xylose
1,2. 5,6	α,α	Formaldehyde
2,3	αT	D-erythrose
4,5	αC	L-threose
2,3. 4,5	αT,αC	Glycolaldehyde
3,4	αT	Glyceraldehyde

The presence of a complexing agent such as vanadate in the solution of D-glucitol should alter the course of the periodate oxidation in that some of the hydroxyl groups of the polyol will be coordinated to the vanadate ion and will therefore not be available to form a cyclic ester intermediate with the oxidising agent. From the difference in the products obtained on periodate oxidation of D-glucitol in the presence and absence of vanadate, it should be possible to deduce which hydroxyl groups are not involved in the vanadate complex. D-glucitol was used since from ionophoretic measurements it appeared to form a relatively strong complex with vanadate.

The D-glucitol-sodium metavanadate complex has maximum stability, according to polarimetric investigations, at pH 5.9, which is not the optimum pH for periodate oxidation[69]. The experiments were carried out at this pH and also at pH 8. In the latter case the solution containing D-glucitol and sodium metavanadate was first adjusted to pH 5.9 to preform the complex, and then to pH 8. Sodium metaperiodate was added to the solutions, buffered with sodium bicarbonate, in ratios of periodate : D-glucitol ranging from 1:1 to 5:1. The major component in each instance, revealed by paper chromatography, was D-glucitol. In the solution containing periodate in the ratio 5:1, trace quantities of xylose, threose and glycollaldehyde, were

detected chromatographically, suggesting slight preferential cleavage between C_4 and C_5 and between C_5 and C_6 of D-glucitol.

That D-glucitol was the major component may be explained in two ways. Either the metavanadate complexes in such a manner that the polyol portion of the complex does not possess adjacent free hydroxyl groups, or else the periodate is for some reason prevented from attacking the D-glucitol by interaction with the uncomplexed vanadate ions. If sodium metaperiodate is added to a solution of sodium metavanadate, some condensation of the latter occurs, reflected in the deepening colour, but this effect must be due only to the pH of the periodate solution, as, if the metavanadate is previously buffered by the addition of sodium bicarbonate no such colour change occurs. If, however, the periodate added to the metavanadate is back-titrated with arsenite and iodine solutions, it becomes evident that some periodate has been used up in reacting with the metavanadate. Further evidence[71] for an equilibrium reaction between sodium metaperiodate and sodium metavanadate comes from the estimation of periodate, in solutions containing varying amounts of metavanadate, by the weight of the dimedone derivative of formaldehyde produced from the formaldehyde liberated by the remaining

periodate from ethylene glycol added to the solution. The weight of the dimedone derivative decreases as the V/IO_4 ratio increases but an equilibrium is reached. Provided that the complex remains stable, it seems surprising that virtually no products from the degradation of D-glucitol are obtained, even if some of the periodate is rendered ineffective by reaction with the uncomplexed metavanadate, unless the complex does involve sufficient hydroxyl groups that no free α-glycol groups remain. This is supported by the ionophoretic measurements in sodium metavanadate, where apparently only the C_5-OH group can be substituted without loss of mobility.

VI. The Isolation of Complexes and their Infra-red Spectra.

It is known that the vanadium-oxygen bonds in polyvanadates have characteristic stretching frequencies[72]. An attempt was therefore made to isolate the complexes formed between sodium metavanadate and certain poly-hydroxy compounds as crystalline solids in order to investigate their infra-red spectra. The complexes were however very soluble in water and even when isolated as freeze-dried solids were hygroscopic rapidly absorbing moisture from the atmosphere. Attempted precipitation of the complex between D-glucitol and sodium metavanadate by the addition of methanol to the aqueous solution resulted in the decomposition of the complex, a polyvanadate solid being precipitated from the solution, which solid contained no polyol. (Ionophoresis of this material in sodium metavanadate or molybdate solutions after treatment with acid and subsequent deionisation did not reveal the presence of a polyol). An orange crystalline polymetavanadate was obtained from an acidified solution of sodium metavanadate alone, at pH 6.0. Since the complexes could not be obtained crystalline, freeze-dried solid complexes were isolated by the evaporation of aqueous solutions containing the appropriate ratio of vanadium to D-glucitol, D-mannitol or maltose, the solutions having been previously acidified using an ion-exchange resin, Amberlite IR 120 (H^+).

The formation of covalent linkages between vanadium and oxygen in the various vanadate[5], together with the relatively light atomic weight of the metal leads to the appearance of vanadium-oxygen fundamental vibrations in the rock-salt region of the infra-red spectrum[72]. From investigations on both vanadium oxy-trichloride $VOCl_3$ [73] and vanadium pentoxide V_2O_5 [72] the band arising at 1035 cm^{-1} has been assigned to a V-O stretching motion. That at 1020 cm^{-1} has been correlated with a V-O stretching motion, the bond being shorter in this case, while the 825 cm^{-1} band occurring in V_2O_5 is also due to V-O stretching but the bond is longer. Siebert[74] studied the infra-red spectrum of the orthovanadate ion in aqueous solution and found that bands occurred at 870, 345, 825, 480 cm^{-1}, while Frederickson and Hansen[72] postulate an infra-red absorption/structure correlation which can be represented (Fig. VI.1):-

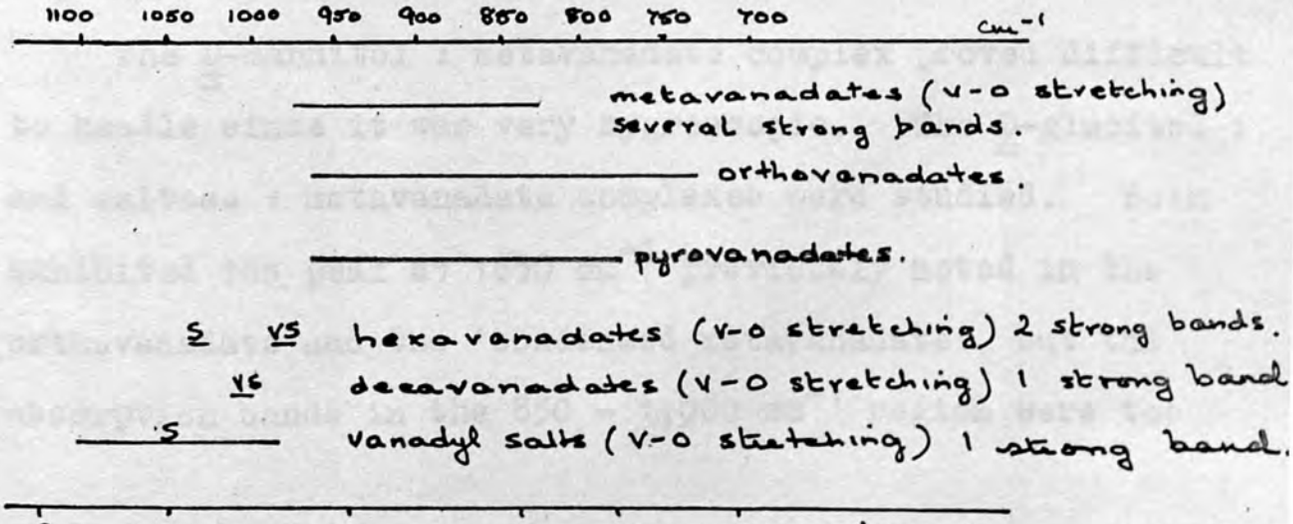


Fig. VI. 1. Infra-red absorption/structure correlation for vanadates.

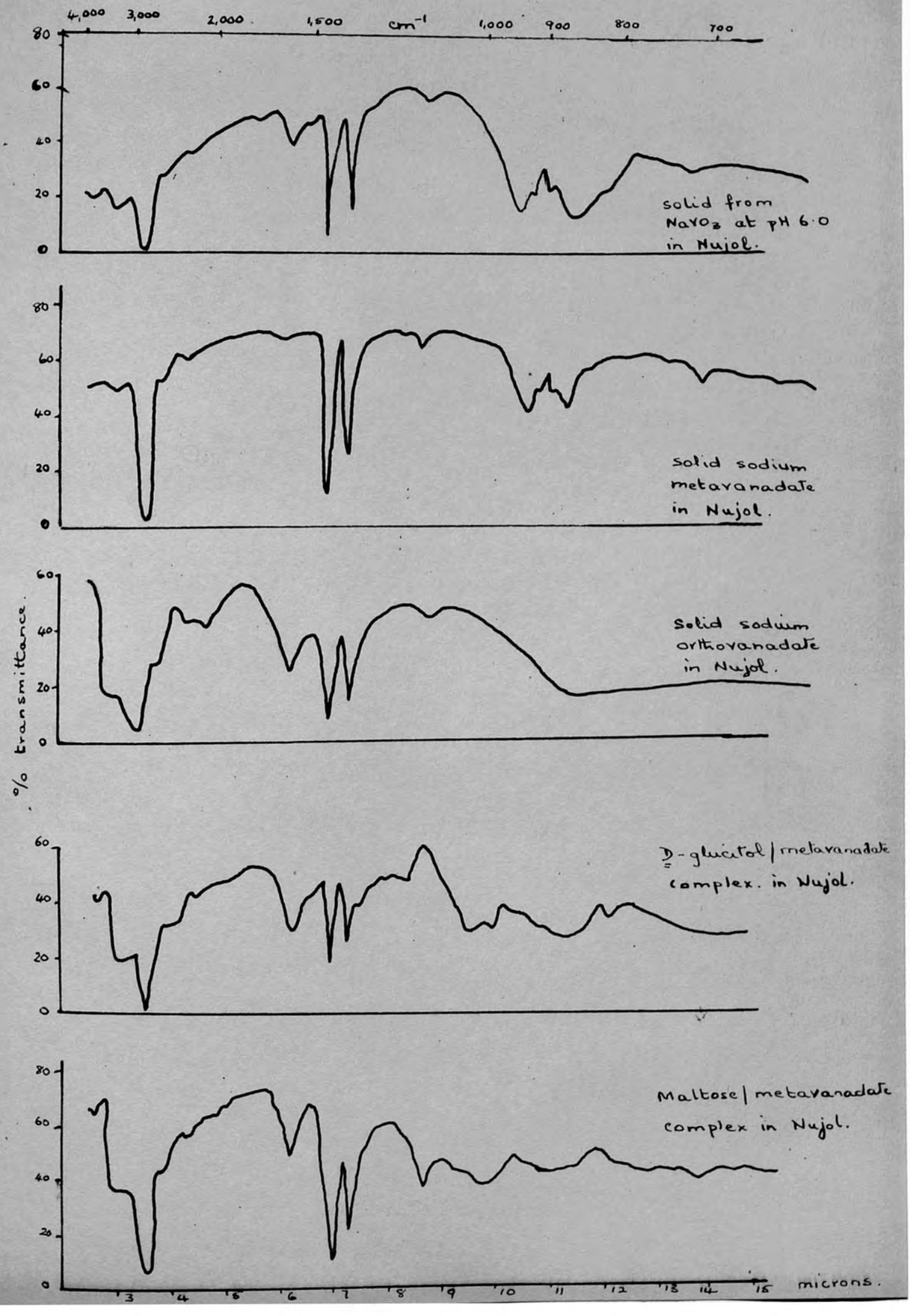
It was decided to investigate the infra-red spectra of the freeze-dried complexes to see if any correlation could be found between those obtained for different polyol- or sugar-vanadate complexes and the metavanadate itself.

The spectra of solid sodium orthovanadate and sodium metavanadate as mulls in Nujol differ noticeably (Fig. VI 2 and 3). The orthovanadate has a pronounced peak at 1650 cm^{-1} which is absent in sodium metavanadate, but appears again in the 'condensed metavanadate' obtained as orange crystals from solutions at pH 6.0. The most marked difference is in the 850-1,000 cm^{-1} region. At the resolution obtainable, the sodium metavanadate and the 'condensed metavanadate' show an almost identical pattern of peaks at 890, 910, 930, 950 cm^{-1} and 880, 920, 930, 970 cm^{-1} respectively, whilst the orthovanadate absorbs almost completely uniformly over this wavelength region.

The D-mannitol : metavanadate complex proved difficult to handle since it was very hygroscopic. .The D-glucitol : and maltose : metavanadate complexes were studied. Both exhibited the peak at 1650 cm^{-1} previously noted in the orthovanadate and the 'condensed metavanadate', but the absorption bands in the 850 - 1,000 cm^{-1} region were too

broad for any correlation with the previous spectra to be made. The spectra do not exclude the particip ation of a "complex metavanadate", of the type isolatable from solutions at pH 6.0, in the formation of the polyol - vanadate entity. The spectra of some pyrovanadates should also be studied.

Fig. VI 2-6. Infra-red spectra of sodium orthovanadate and metavanadate.



VII. Complexes between vanadate polyanions and polyhydroxy-compounds : conclusion.

All the measurements so far performed on solutions of sodium orthovanadate or metavanadate containing polyhydroxy-compounds indicate a rather complex equilibrium, in which more than one vanadate ion may participate, complicating the interpretation of the results. The stoichéiometric ratios determined, though probably limited to that complex which most affects the physical property being used for the investigation, yield information peculiar to the polyols studied, but generalisations cannot be made simply. Some evidence for different types of complex dependent upon the concentration of the vanadate solution has accrued from the detailed study of the D-glucitol complex, which may obscure to some extent correlations between the ionophoretic measurements and physical measurements. However, sufficient indication of the structural features of polyols which are favourable for complexing can be derived from the ionophoretic measurements performed to enable this electrolyte to be used for the reasonably predictable separation of polyhydroxy-compounds.

The physical measurements performed on solutions of sodium orthovanadate and metavanadate containing D-glucitol or D-mannitol reflect the complexity of the equilibria.

From both potentiometric and absorptiometric investigations, on solutions containing D-glucitol, an equilibrium appears to be first established involving ions with H^+/VO_4^{3-} ratios of 1 and 2, giving way on further acidification to a predominance of the ion for which $H^+/VO_4^{3-} = 2$. D-mannitol seems rather to stabilise an ion with $H^+/VO_4^{3-} = 1$. Conductimetric measurements and the comparison of the optical rotation of D-glucitol in solutions of sodium metavanadate acidified, or acidified then realkalised, suggest the possible formation of a complex with a transient intermediate produced on acidification. Periodate oxidation indicates that, for D-glucitol at least, probably no α-glycol groups remain uncomplexed.

There are several methods by which the study of the complexes between polyhydroxy-compounds and the oxy-anions of vanadium could be further developed. Howarth and Richards[75] have recently used the nuclear magnetic resonance properties of the V^{51} atom to study the equilibria in aqueous vanadate solutions, assigning the chemical shifts obtained to the various polyvanadate anions. One interesting aspect of their results is that the colourless species, like the VO_4^{3-} ion, have narrow resonances, suggesting that the basic unit is a VO_4^{3-} tetrahedron, whereas the coloured species have broader resonances which

are consistent with a less symmetrical electrical environment for the vanadium nucleus. Since the chemical shift obtained depends basically on the electrons surrounding the nucleus; complexing with a polyol might well alter the shift corresponding to the particular vanadate polyanion concerned and thus define it.

Diffusion experiments, in which the criterion is the size of the ion involved (cf. ref. 38) or molecular weight determinations by centrifugal precipitation methods[76] where again the actual mass of the molecule is important, might yield valuable results. Any direct molecular weight determination, by a depression of freezing point for example, is limited by the presence of uncomplexed materials, if not by other ions. The molecular weight of the metavanadate alone, as given in the literature, varies considerably according to the methods employed for its determination.[34,39,44,77]

In a recent study of borate solutions, Lormeau and Ahond[78] deduced from potentiometric measurements the number of molecules of sugar bonded to a borate ion, a method which could be employed to confirm the ratios found for vanadium : sugar.

It has already been established that solutions of sodium metavanadate have a certain value for achieving

ionophoretic separations. It would be worthwhile to develop the technique of vanadate complexing for ion-exchange and paper chromatography. Preliminary investigations have shown some slight separation on a vanadate form of resin, though the complexing is rather readily reversible. The initial experiments on vanadate-impregnated paper indicate that some separations can be achieved, and when the optimal conditions have been established, the technique may well supplement those already available to the carbohydrate chemist.

IV. 1. Complexes between tungstate oxy-anions and some sugars.

Many polyhydroxy-compounds migrate on ionophoresis in acidified solutions of sodium molybdate[79,80] and tungstate[81,22]. From a study of the mobilities of cyclitols, aldohexoses and aldopentoses and their derivatives in sodium molybdate at pH 5.0[79], it was established that sugars and other six-membered cyclic polyhydroxy-compounds form complexes only if they possess a (1(ax), 2(eq), 3(ax))-triol system in one of their conformations, or a spatially equivalent system of hydroxyl groups[79].

Thus, of eleven cyclitols examined, only myo-inositol, allo-inositol and epi-inositol migrated, their mobilities being related to the stability of the conformations containing the required disposition of hydroxyl groups, while D-mannose, D-gulose and D-ribose furnished examples of migrating aldoses.[79] The mobility of 1,6-anhydro-β-D-mannopyranose was of interest since it indicated complex-

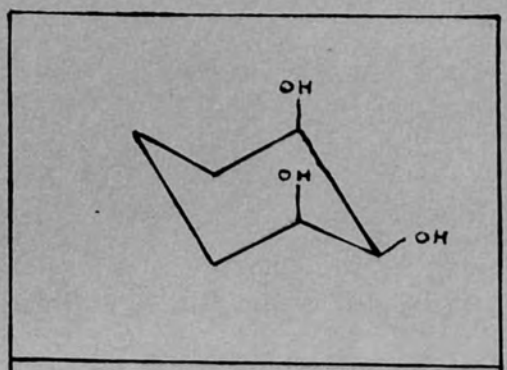


Fig I 1 [1(ax), 2(eq), 3(ax)]-triol system

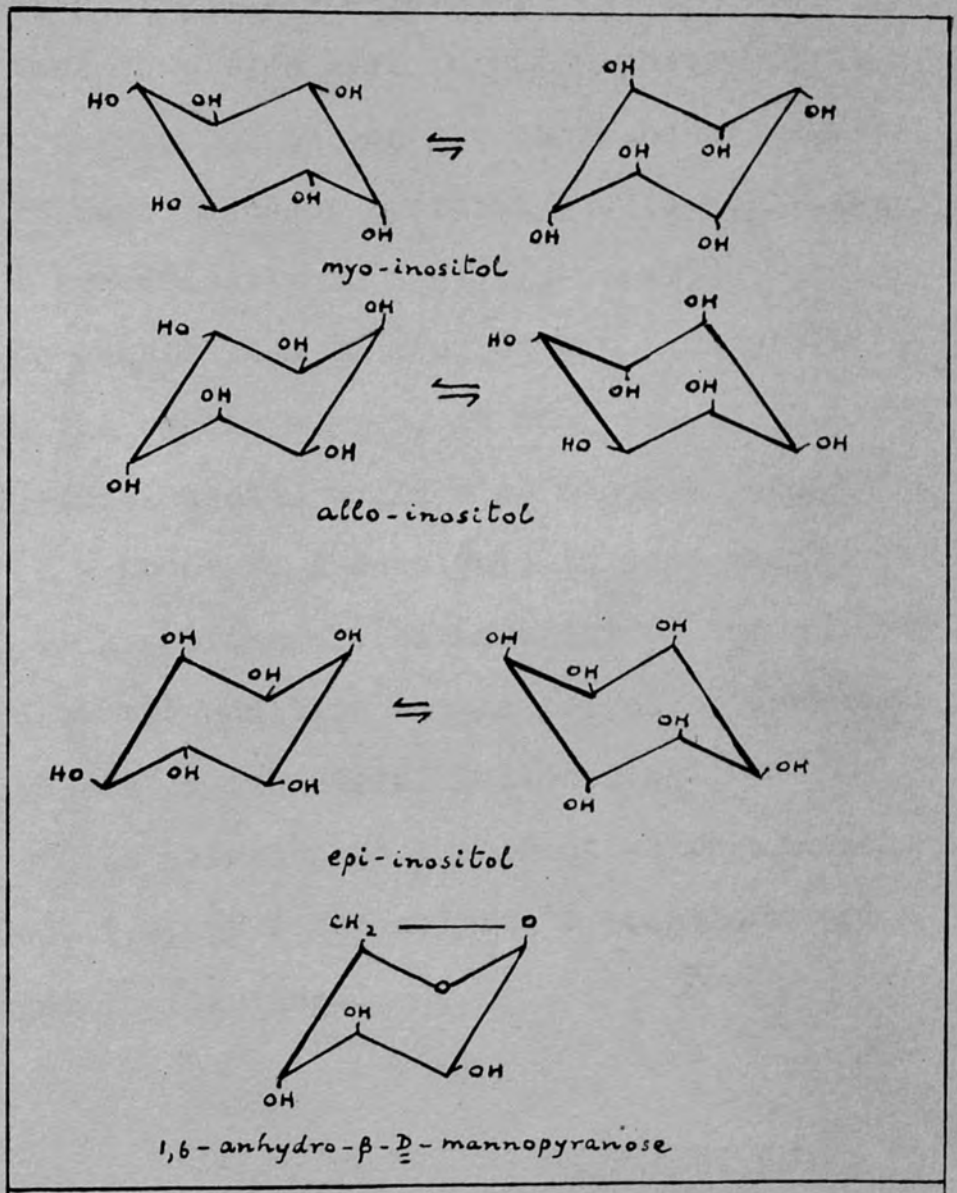


Fig. I 2.

Some cyclic compounds migrating in sodium molybdate

formation by hydrogen-bonding between a molybdenum-hydroxyl group and the oxygen atom of the anhydro-ring[79].

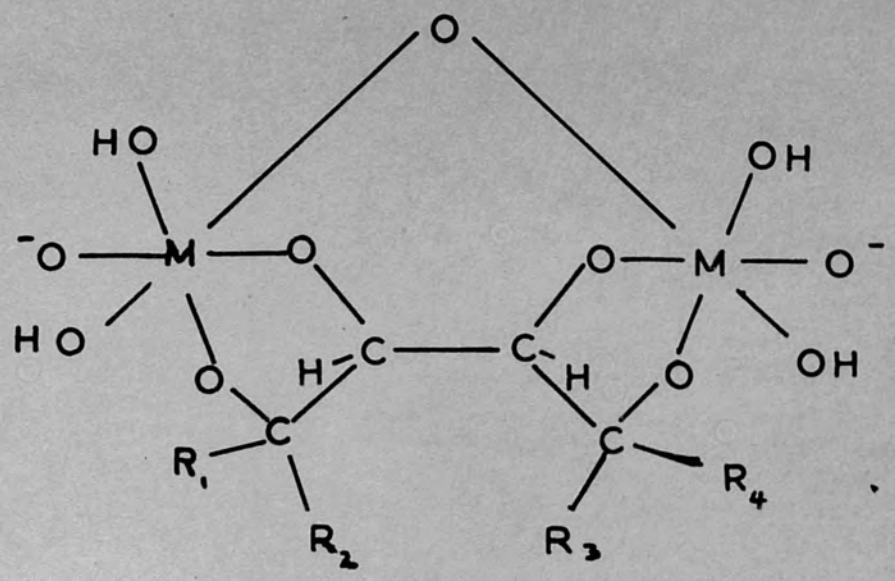
Ionophoretic measurements in sodium tungstate solutions acidified to pH 5.0 indicated that the same type of tridentate complexes were formed with tungstate[81]. Acyclic polyhydroxy-compounds, on the other hand, were found to require four adjacent hydroxyl groups, or three hydroxyl groups specifically in a [1,2,3-(α,αT)]-configuration to enable them to complex with tungstate[22]. By analogy with the cyclic sugars, it was assumed that the [1,2,3-(αT,αT)]-triol system would also complex readily[22]. The M/P ratio (M = Mo or W, P = polyol) in some complexes was found[80,28] by measuring the enhancement of the optical rotation caused by the addition to the polyol of increasing amounts of molybdate or tungstate, followed by acidification of the solution to the pH at which maximal changes occurred, i.e. pH 2 for molybdate solutions and pH 5.5 for tungstate solutions.

The graphs obtained[80,28] showed that D-glucitol and D-mannitol form a complex in which two molybdenum or two tungsten atoms are incorporated for each polyol molecule, while a 4-substituted D-glucitol, 3-O-β-D-glucopyranosyl-L-gulitol forms a complex in which the M/P ratio is 1:1.

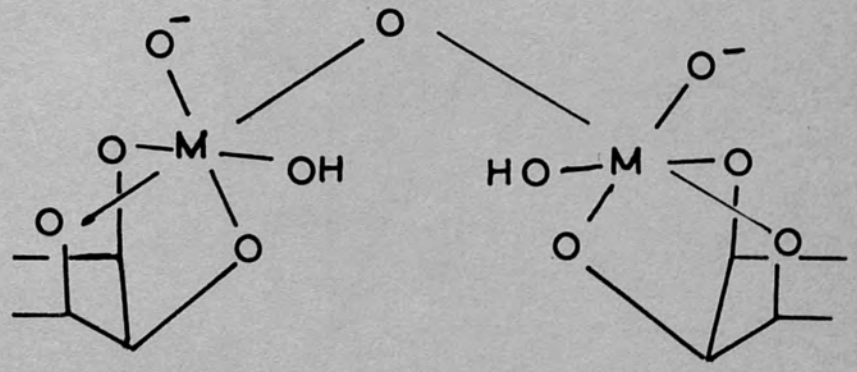
The nature of the complexing ion was investigated by potentiometric studies on tungstate and molybdate solutions in the presence and absence of several complex-forming polyhydroxy-compounds[22]. In all cases a strong inflexion occurred at an H^+/MO_4^{2-} ratio of circa 1.0 due to the ditungstate or dimolybdate ion $W_2O_7^{2-}$ or $Mo_2O_7^{2-}$ and hence these ions were concluded to be the complexing agents[22]. The conclusions were supported by conductimetric measurements[22].

Since the ditungstate or dimolybdate ion is the complexing agent and the ratio of tungsten or molybdenum to D-glucitol is 2:1, it was deduced that the D-glucitol complex, and complexes similarly formed from four adjacent hydroxyl groups have the structure I below[28], while acyclic polyols possessing the 1,2,3-(α,αT)-triol system complex as in II[28], which would explain the M/P = 1 in these.

It was logical to assume that the complexes formed by the migrating cyclic sugars had the structure represented in II, but the M/polyol ratio had yet to be confirmed.



I.



II.

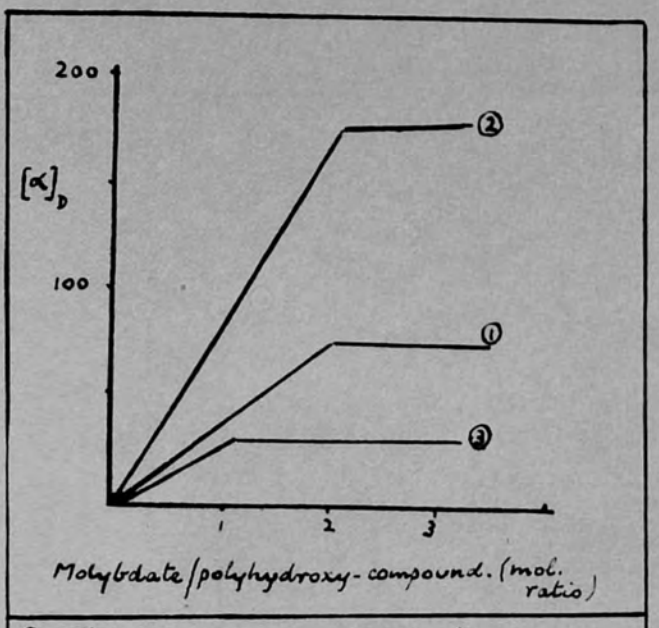


Fig. I 3. Molar ratio of molybdate to some polyhydroxy-compounds.
① D-glucitol ② D-mannitol
③ 3-O-β-D-glucopyranosyl-L-gulitol

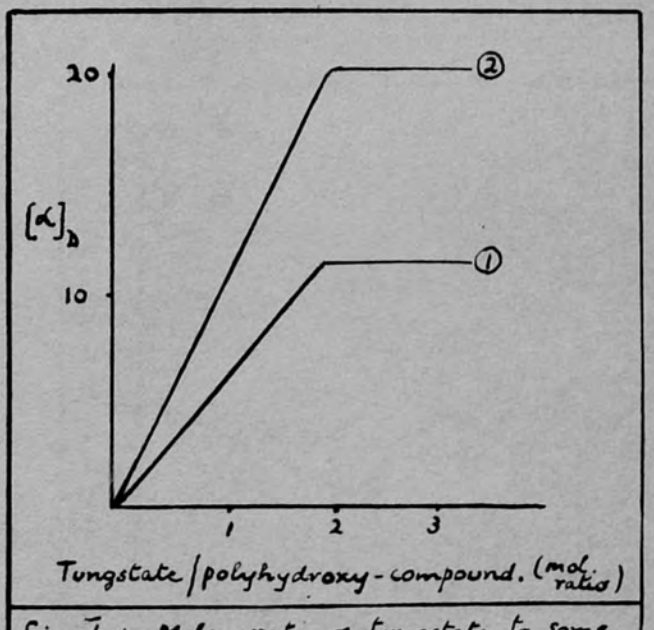


Fig I 4. Molar ratio of tungstate to some polyhydroxy-compounds.
① D-glucitol ② D-mannitol

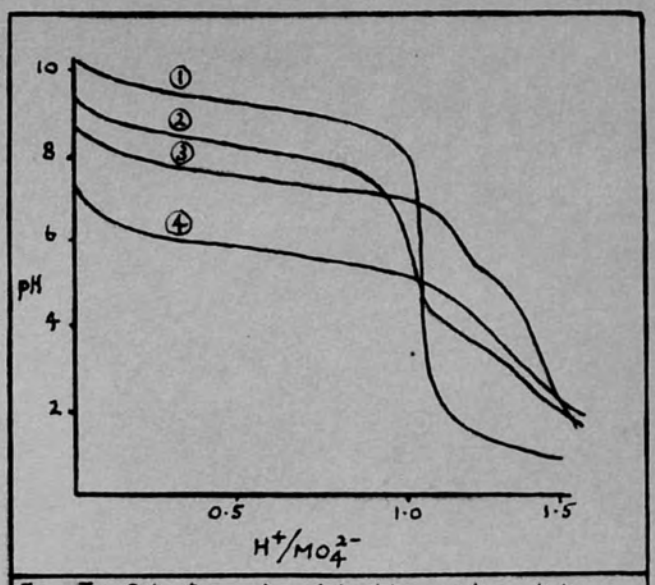


Fig. I5. Potentriometric titrations of tungstate and molybdate solutions.
① sodium tungstate + D mannitol
② sodium molybdate + D mannitol
③ sodium tungstate ④ sodium molybdate

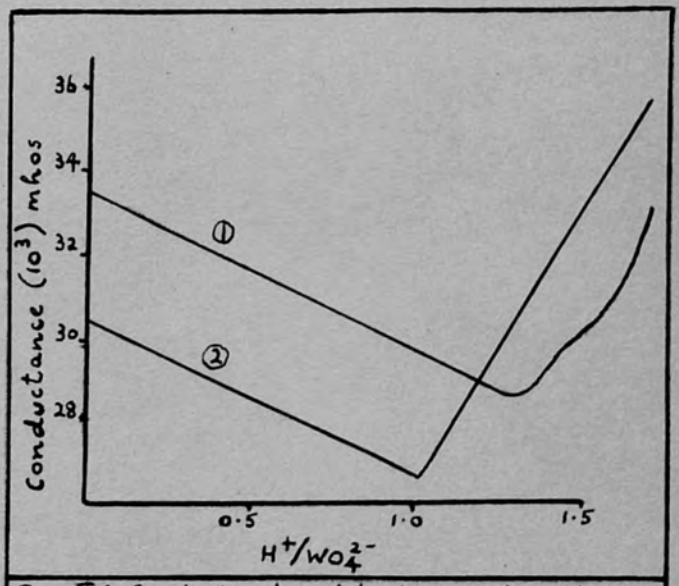


Fig I6. Conductimetric titration of tungstate solutions
① sodium tungstate
② sodium tungstate + D-mannitol

D-mannose and D-ribose exhibit maximal changes in specific rotation in tungstate solutions at pH 5.9 and 6.5 respectively,[82] which rotation is affected by the relative concentration of the sugar and inorganic oxy-anion. The results therefore differ from those obtained for acyclic polyols, in which a constant rotation value is attained, and are instead typical of an equilibrium reaction. It is tedious to derive the M/polyol ratio in such a reaction from the method used previously for D-glucitol, (cf. reactions of polyhydroxy-compounds with sodium metavanadate), and a continuous variation method was therefore employed. The concentrations of the sugar and tungstate was varied and the difference in optical rotation between each solution and a solution containing the same concentration of sugar alone was plotted against the mole fraction of tungstate. Minima occurred at a mole fraction of 0.5 for both D-mannose and D-ribose, [Expts. 53 and 54], indicating a 1:1 ratio of sugar to tungsten as was to be expected. While the work was in progress, the same ratio was reported for complexes formed with molybdate[83]. It is reasonable to conclude that all cyclic compounds containing the (1(ax), 2(eq), 3(ax))-triol system form complexes in which two sugar molecules are bridged by the dimeric tungstate or molybdate ion.

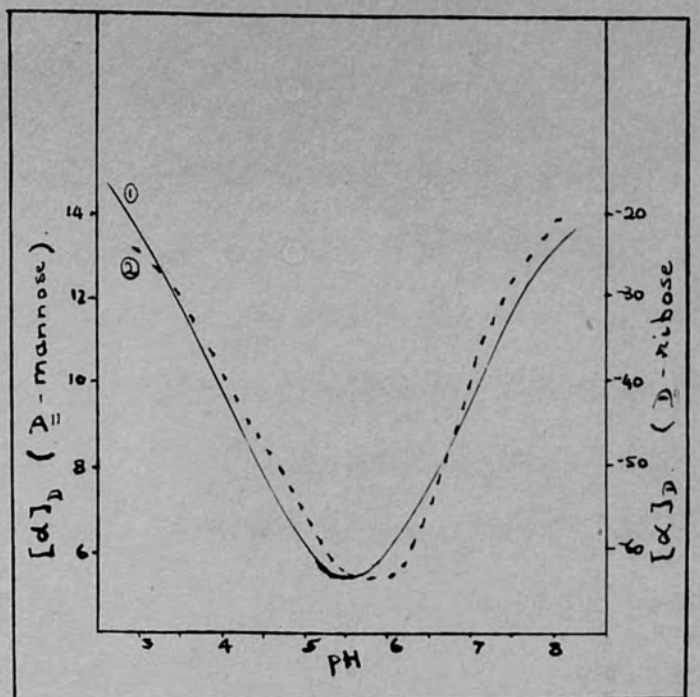


Fig. I.7. Change in rotation of D-mannose and D-ribose in tungstate with pH.
① D-mannose. ② D-ribose

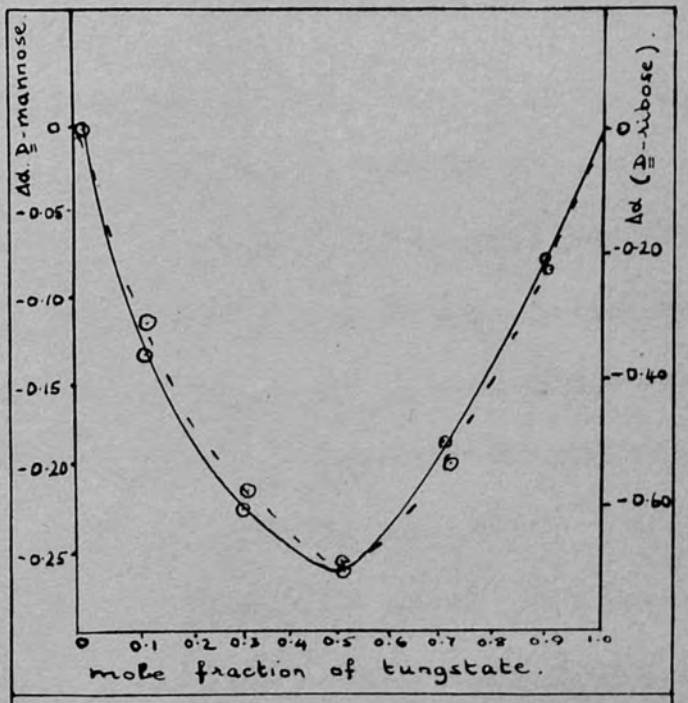


Fig. I.8. Continuous variation plot for D-mannose and D-ribose in tungstate.
① D-mannose ② D-ribose.

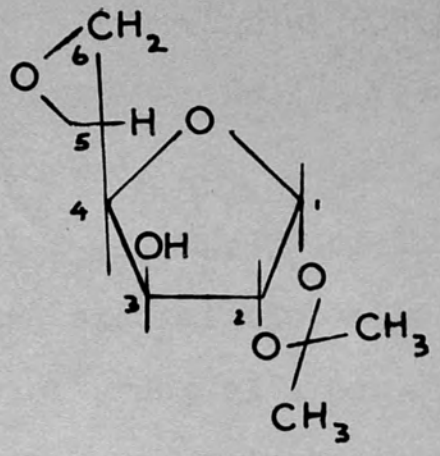
That compounds containing three hydroxyl groups in the required spatial disposition are bridged by the dimeric ion is rather neatly illustrated by ionophoresis in sodium molybdate. In the ionophoresis of mixtures of two maltodextrinols M_1 and M_2 in molybdate, three distinct complexes separate in which either two M_1 molecules, two M_2 molecules, or one M_1 and one M_2 molecule are linked through the oxy-anion[22]. Similarly, a mixture of 3-O-α-D-glucopyranosyl-L-gulitol and D-gulose separates into three distinct components with mobilities Ms(Mo) = 0.48, (3-O-α-D-glucopyranosyl-L-gulitol), 1.1, (D-gulose) and 0.90 (mixed complex).

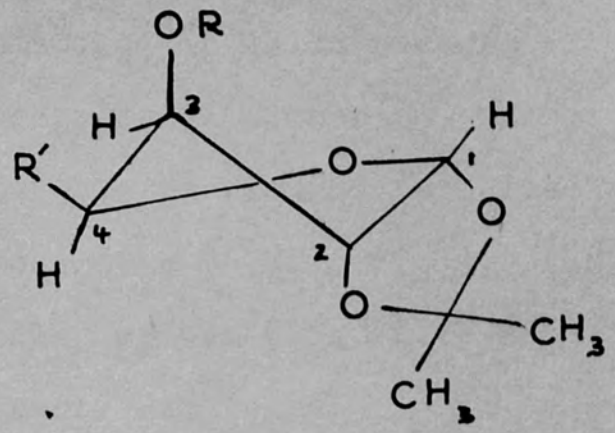
It has been shown already that cyclic sugars possessing a (1(ax), 2(eq), 3(ax))-triol system complex with molybdate and tungstate[79,81]. An interesting extension to this correlation is afforded by the study of the mono-deoxy derivatives of D-glucose. Of these, only the 5-deoxy-D-xylohexose migrates on ionophoresis in sodium molybdate and tungstate, with mobilities of Ms(Mo) = 0.70 and Ms(W) = 0.30. This sugar can exist in aqueous solution as the aldehydo, septanose or furanose form, and of the two ring forms the latter is the more likely. The aldehydo form could complex since the hydroxyl groups on carbon atoms 2,3 and 4 are spatially equivalent to a

(1(ax), 2(eq), 3(ax))-triol system. The same groups are however present in D-glucose which shows no tendency to complex.

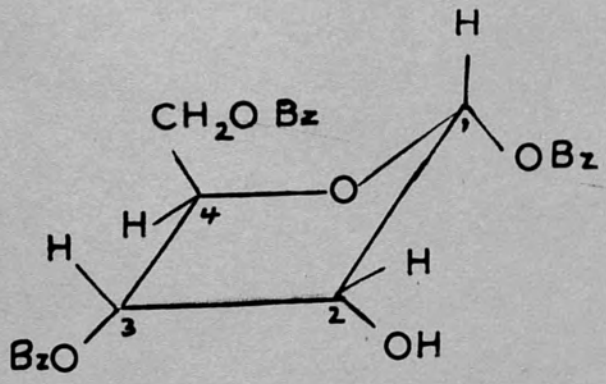
The inter-oxygen distances of the hydroxyl groups of a planar furanose ring of D-glucose would not be equivalent to the required triol system. But evidence has been accumulating that the five-membered ring is in fact puckered. Abraham, McLauchlan, Hall and Hough[84] have studied the proton magnetic resonance spectrum of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose III and concluded that the C(2) and C(3) centres are displaced below and above the plane containing the other ring members.

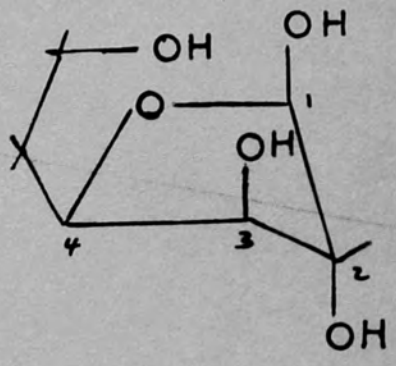
The method was then extended[85] to some ribofuranose derivatives which did not possess an isopropylidene substituent. The spectrum of 1,3,5-tri-O-benzoyl-α-D-ribofuranose[85] suggested that the molecule adopted an "envelope" conformation IV in which four of the ring atoms are in a plane while the fifth is out of the plane.


CH2
O
6
5
H
O
4
1
OH
3
2
O
O
CH3
CH3


OR
H
3
R'
H
4
O
1
H
O
2
O
CH3
CH3

III


H
CH2O Bz
1
OBz
O
4
H
H
H
3
2
BzO
OH

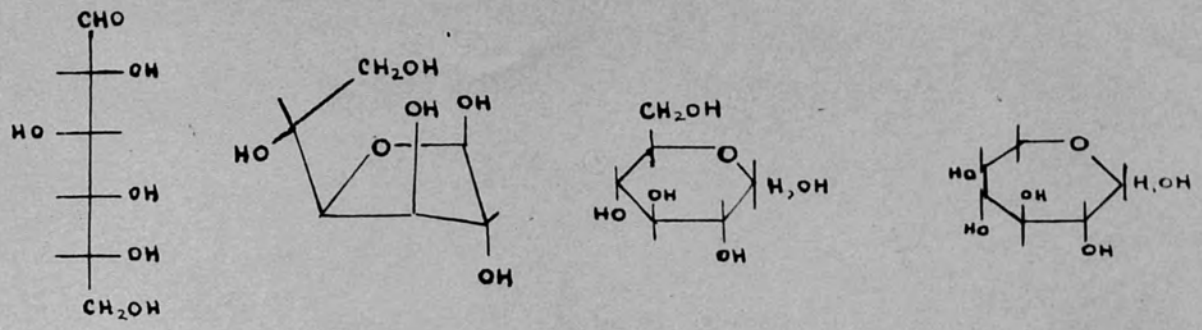

OH
OH
O
1
OH
4
3
2
OH

IV V

If a similar conformation, V, is adopted by the 5-deoxy-D-xylo-hexofuranose molecule, in which carbon atom 2 is out of the plane, the hydroxyl groups on carbon atoms C_1, C_2, C_6 of its β-anomer can be brought into the same spatial disposition as those of a (1(ax), 2(eq), 3(ax))-triol group of a six-membered ring system (Fig. I.1) and it must be in this form that the sugar complexes.

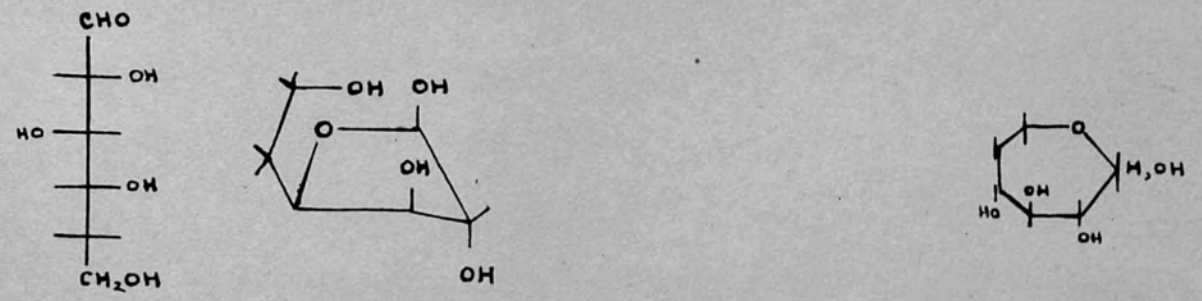
Thus ionophoresis in tungstate or molybdate solution distinguishes between the aldehydo, furanose and pyranose forms of D-glucose, and incidentally provides evidence for the buckling of the furanose ring. The method might well be applied to distinguish between the various conformations of other appropriate sugars.

D - GLUCOSE. $M_s(M_o) = 0$. $M_s(W) = 0$.



aldehydo furanose pyranose Septanose

5 - DEOXY - D - XYLO - HEXOSE. $M_s(M_o) = 0.70$. $M_s(W) = 0.30$.



aldehydo furanose Septanose.

FIG. XI.9. THE CONFIGURATIONS OF D - GLUCOSE AND 5 - DEOXY - D - XYLO - HEXOSE.

Part II

Reactions of enamines with carbohydrate derivatives

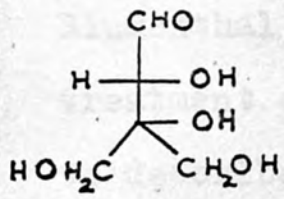
I. Introduction

Before 1944, the only branched-chain sugars known were apiose and hamamelose, the one as a flavone glycoside of the parsley plant, the other occurring in the bark of witch-hazel[91]. Since then, partly owing to the interest in antibiotic substances containing them, branched-chain sugars in which H - C - OH has been modified[92] to R - C - OH or H - C - R have become the subject of a rapidly expanding field of study. The structures of several naturally occurring sugars such as apiose[93-96] hamamelose[92,97], streptose[98,99] (from streptomycin) and noviose[100,101] (from novobiocin) have been investigated and confirmed by synthesis. Important general methods for the synthesis of branched-chain sugars have also been developed, as for example the action of Grignard reagents on methyl oxo-glycosides[102] or the treatment of an oxo-sugar with diazomethane followed by alkaline hydrolysis or reduction with lithium aluminium hydride[103]. Branched-chain amino sugars have also been synthesised[104].

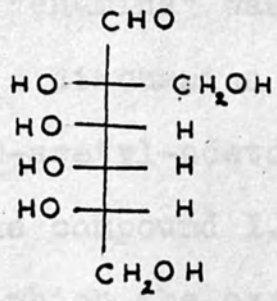
In the compounds prepared to date, the side-chain R, directly linked to a carbon atom of the fundamental sugar is acyclic : often a methyl or hydroxymethyl group[97], sometimes an unsaturated olefinic group[97], and in a recent case an $-(OH)CHCH_3$ group[105]. It was decided that it would

be of interest to modify a sugar by linking directly to it, through a carbon atom a saturated homocyclic system such as cyclohexanone. The compound so prepared would be the first of a new series of carbohydrate derivatives, and if cyclisation of the sugar moiety by hemiacetal formation with the cyclohexanone occurred, decalin-type compounds which would be of conformational interest might be produced.

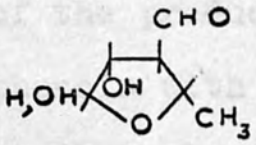
The method which comes immediately to mind for producing such a derivative, an aldol condensation[106] between an acyclic sugar such as glyceraldehyde and the ketone cyclohexanone can be rejected owing to the possibility of autocondensation[107] of the cyclohexanone and dimerisation[108] of the glyceraldehyde under the conditions appropriate for such a condensation. In seeking a more specific method for linking the sugar and a homocyclic system, the methods of preparation of alkyl and acyl derivatives of cyclohexanone reported in the literature were studied and the production of such compounds by the intermediate formation of enamines[109] appeared as a relatively simple and successful technique. In order to explore the potentialities of the reaction of enamines with carbohydrates it is necessary first to digress into the chemistry of enamines.



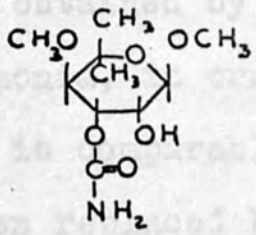
D - apiose



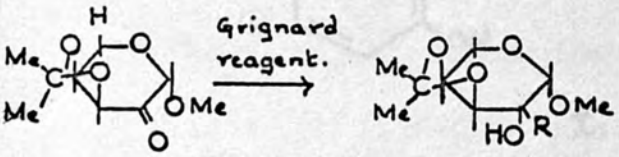
L - hamamelose



streptose



Methyl 3-O-carbamoyl-α-novioside



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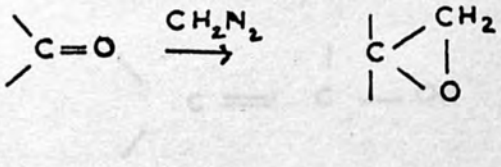
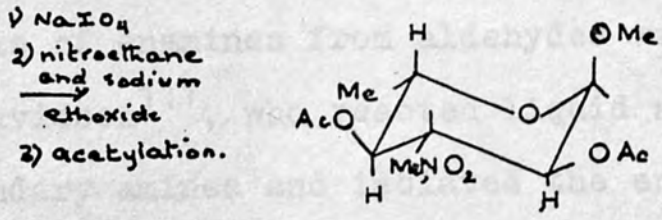


(103)

Methyl α-L-rhamnopyranoside



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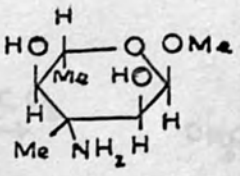
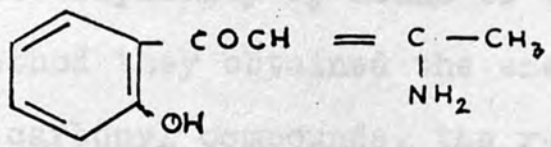


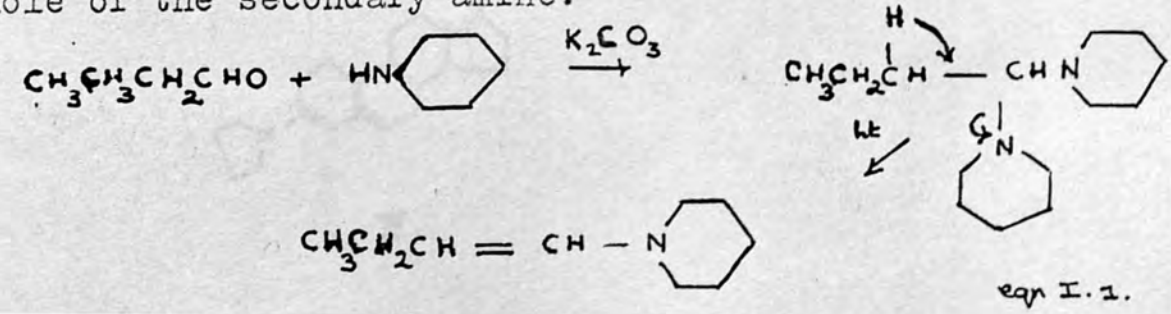
The term "enamine" was first coined by Wittig and Blumenthal[110] in a discussion of the products obtained by the treatment of O-acetyl-acetophenols with ammonia, in order to describe the compound I. The molecule is comparable to an enol in which the oxygen atom has been replaced by



I.

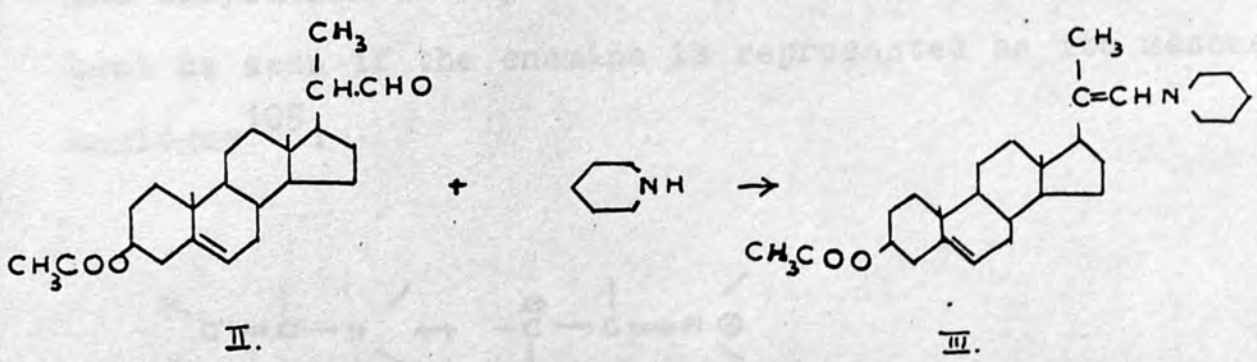
a nitrogen atom, and the term "enamine" has since been generalised to include all α,β-unsaturated amines[109].

The synthesis of enamines from aldehydes was initiated by Mannich and Davidsen[111], who reacted liquid aldehydes with cyclic secondary amines and isolated the enamines produced by fractional distillation. Various pressures were selected in order to facilitate the splitting out of one mole of the secondary amine:

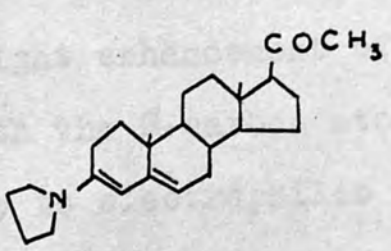


eqn I.1.

The yields of enamines derived from ketones by this method were poor, but Herr and Heyl[112,113] developed a more versatile technique, in which a carbonyl compound is refluxed with an excess of a secondary amine in benzene under nitrogen, and the water formed in the reaction removed azeotropically by means of a water separator. By this method they obtained the enamines of several steroidal carbonyl compounds, the reaction proceeding equally well for aldehydes and ketones. Thus 3β-acetoxybis-nor-5-cholenaldehyde (II) gave 22-(N-piperidyl) bis-nor-5,20(22)-choladien-3β-ol acetate (III) in 84% yield[112], while the C_3-steroidal ketone progesterone was converted to its pyrrolidine enamine (IV) in 90% yield[113].

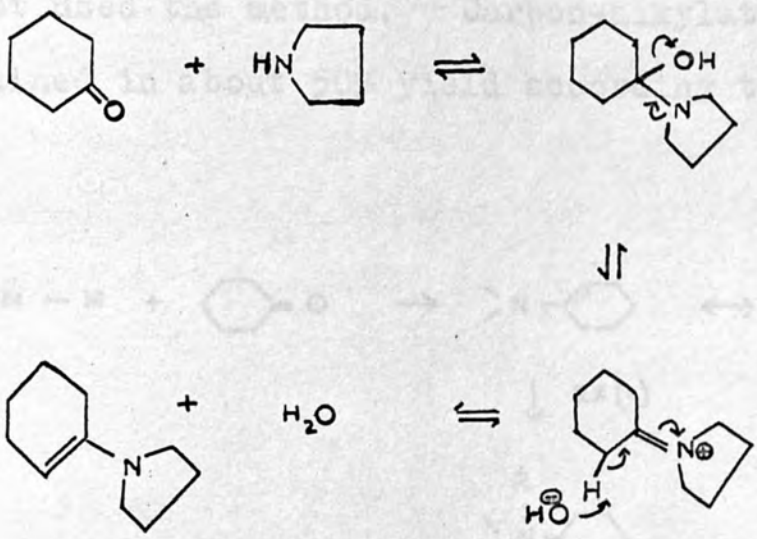


II. III.



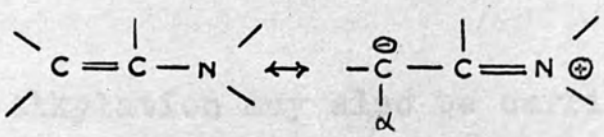
IV.

The procedure was extended by Stork[114] and his coworkers to many other ketones, among them cyclohexanone, the reaction of which with pyrrolidine can be formulated as:[109]



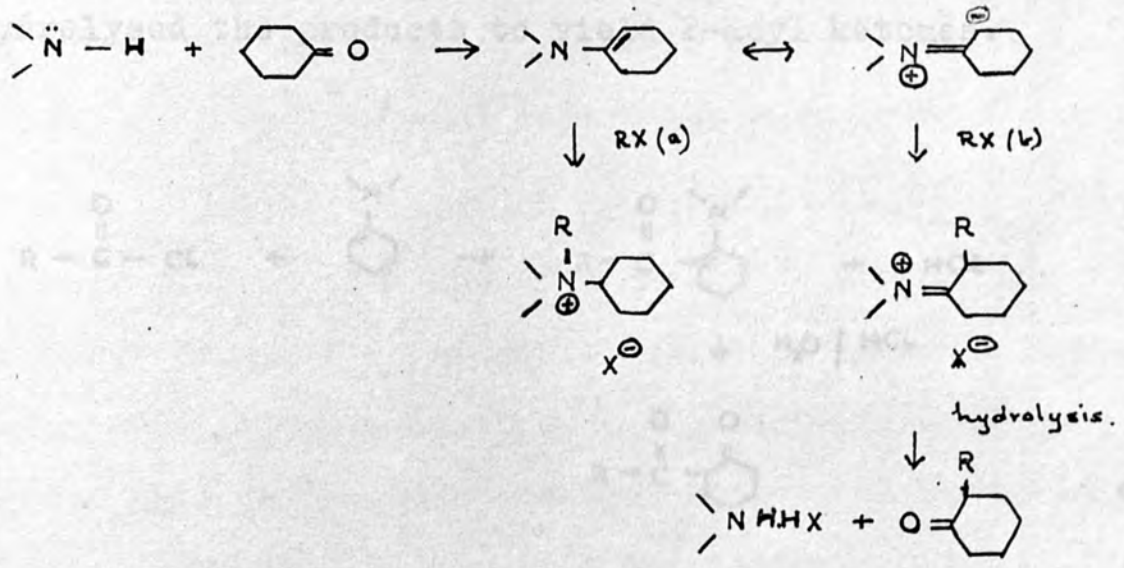
eqn. I.2.

The value of forming an enamine as an intermediate in the alkylation or acylation of a carbonyl compound can best be seen if the enamine is represented as its mesomeric analogue[109]:-



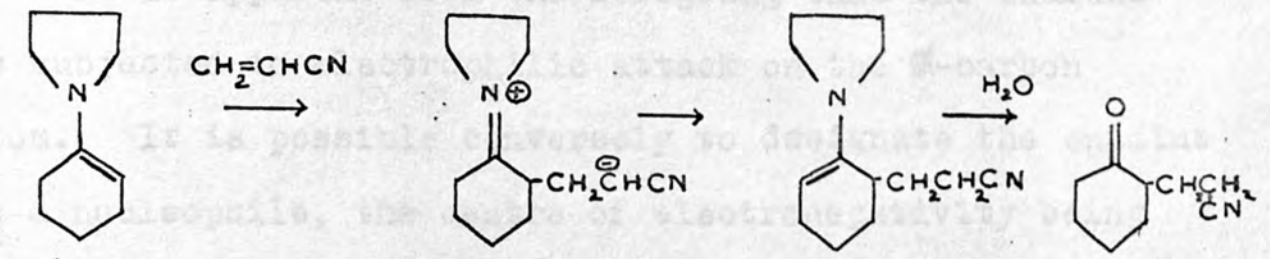
There is a slight enhancement of electromegativity associated with the α-carbon atom which will increase its susceptibility to electrophilic attack.

Several types of electrophile have been reacted successfully with enamines. For example, the alkylation of enamines by alkyl halides has been the subject of a number of communications[109], since Stork and his coworkers first used the method. Carbon-alkylated products are obtained in about 50% yield according to mechanism (b) below:



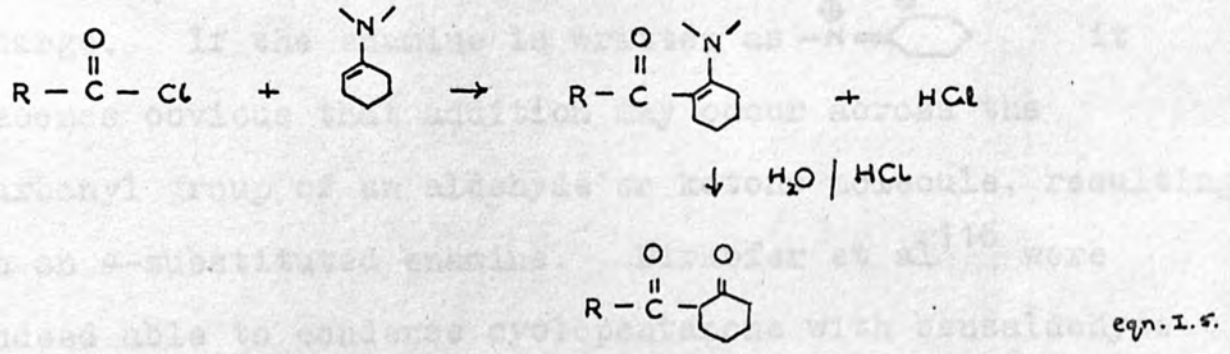
eqn. I. 3.

Alkylation may also be carried out with activated olefins such as α,β-unsaturated nitriles, esters, ketones and aldehydes. For such compounds the undesirable N-alkylation of the enamine is reversible and the yields of C-alkylated products are generally higher[109]. The reaction is illustrated by an enamine of cyclohexanone and acrylonitrile[109].



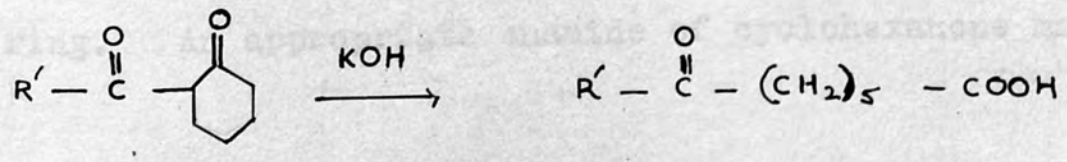
eqn. I.4.

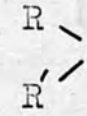
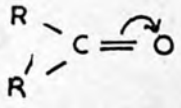
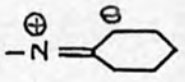
Enamines may likewise be subjected to electrophilic attack by an acyl group. Hünig et al[115] have treated the enamines of cyclic ketones with acid chlorides, then hydrolysed the products to yield 2-acyl ketones.



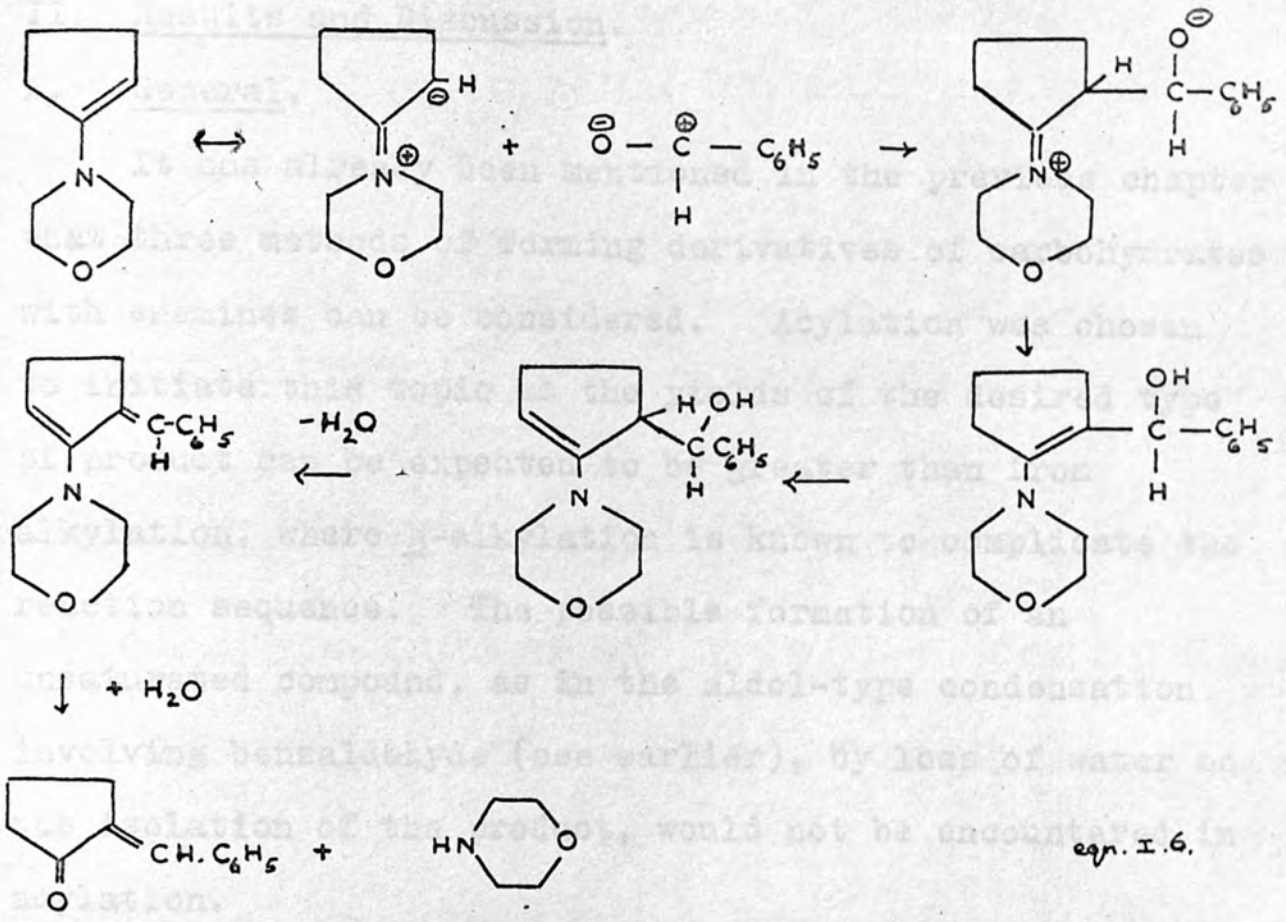
eqn. I.5.

These workers finally ruptured the cyclohexanone ring with alkali to form acyclic keto-carboxylic acids[115].



It is apparent from the foregoing that the enamine is subjected to electrophilic attack on the α-carbon atom. It is possible conversely to designate the enamine as a nucleophile, the centre of electronegativity being associated with the α-carbon atom. The addition reactions of enamines to carbonyl compounds may then be considered. The carbonyl group  undergoes several reactions, notably with hydroxylamine, hydrazines and semicarbazide which can be explained in terms of an initial electromeric shift  that leaves the carbon atom with a $\delta+$ charge. If the enamine is written as  it becomes obvious that addition may occur across the carbonyl group of an aldehyde or ketone molecule, resulting in an α-substituted enamine. Birkofer et al[116] were indeed able to condense cyclopentanone with benzaldehyde via the morpholine enamine of the former.

From the outline of enamine chemistry given above, it can be seen that there are three main lines of approach to forming a carbohydrate derivative in which cyclohexanone is linked directly to the carbon chain or ring. An appropriate enamine of cyclohexanone may be



eqn. I.6.

treated with a sugar halide (alkylation) or with a sugar acid chloride (acylation) or with an aldehydo-sugar (condensation). The present work is concerned with the formation of carbohydrate derivatives by acylation of the morpholine enamine of cyclohexanone.

II. Results and Discussion.

A. General.

It has already been mentioned in the previous chapter that three methods of forming derivatives of carbohydrates with enamines can be considered. Acylation was chosen to initiate this topic as the yields of the desired type of product can be expected to be greater than from alkylation, where N-alkylation is known to complicate the reaction sequence. The possible formation of an unsaturated compound, as in the aldol-type condensation involving benzaldehyde (see earlier), by loss of water on the isolation of the product, would not be encountered in acylation.

The essential feature of acylation is the attachment to a substrate of a group $R - \overset{|}{C} = O$ which is derived from an acylating agent[117]. The mechanism of acylation of a hydrocarbon involves heterolysis of the C-X bond in the acylating agent RCOX to produce the acylium ion $RCO^{\oplus}$ which is then generally attached to the hydrocarbon by either substitution (eqn. 1) or addition (eqn. 2)[117].

$$-\overset{|}{C}-\overset{|}{C}-H + RCOX \longrightarrow -\overset{|}{C}-\overset{|}{C}-COR + HX \qquad \text{eqn II.1.}$$

$$-\overset{|}{C}=\overset{|}{C}- + RCOX \longrightarrow -\overset{|}{\underset{X}{C}}-\overset{|}{\underset{COR}{C}}- \qquad \text{eqn II.2.}$$

The acylium ion may be produced either from the dissociation of an acid halide into $RCO^{\oplus}X^{\ominus}$, or by the reaction of trifluoroacetic anhydride with a carboxylic acid, for when a carboxylic acid is dissolved in trifluoroacetic anhydride the following equilibria are established[118]: and from the infra-red spectra of acetic

$$RCOOH + (CF_3CO)_2O \overset{k_1}{\rightleftharpoons} RCOOCOCF_3 + CF_3COOH \quad \text{eqn. II.3.}$$

$$RCOOH + RCOOCOCF_3 \rightleftharpoons (RCO)_2O + CF_3COOH \quad \text{eqn. II.4}$$

$$(RCO)_2O \quad (CF_3CO)_2O \rightleftharpoons 2\,RCOOCOCF_3 \quad \text{eqn. II.5.}$$

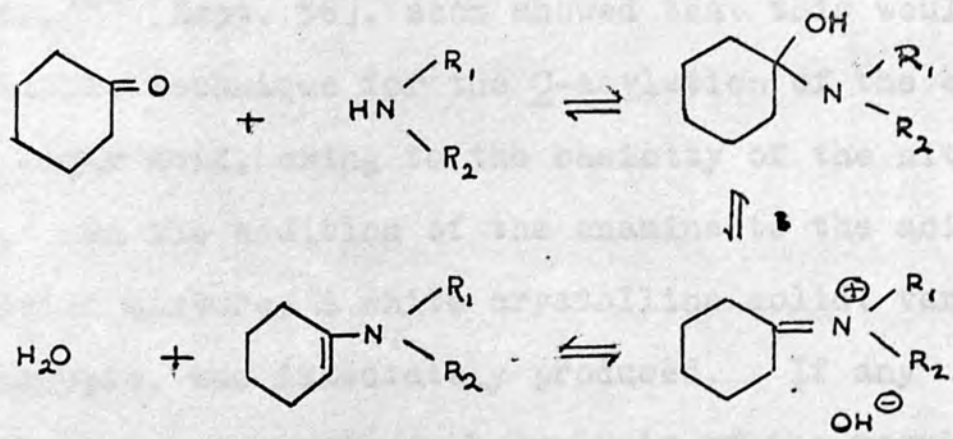
acid/trifluoroacetic anhydride mixtures the equilibrium is strongly in favour of the unsymmetrical anhydride, which can then ionise as follows[118]:-

$$CH_3COOCOCF_3 \rightleftharpoons CH_3CO^{\oplus} + CF_3COO^{\ominus} \quad \text{eqn. II.6.}$$

Acylations by this method generally require only mild conditions[118].

From the foregoing it may be seen that there are two types of acylating system whose potentialities can be explored, namely the use of an acid chloride or an acid/anhydride mixture.

Consider now the enamine. An enamine may be derived, in theory, from any secondary amino-groups and a carbonyl compound. In practice, the cyclic amines generally produce enamines more rapidly and in better yield than the acyclic ones[114]. Pyrrolidine, piperidine and morpholine have been the most frequently used[109]. For the formation of the enamine, pyrrolidine gives a slightly higher reaction rate than the more weakly basic morpholine, which in turn reacts more rapidly than piperidine[114]. The difference in rate between pyrrolidine and piperidine, in reacting with cyclohexanone, is ascribed by Stork and his coworkers[114] to the different rate of dehydration, (step B).



eqn. II.7.

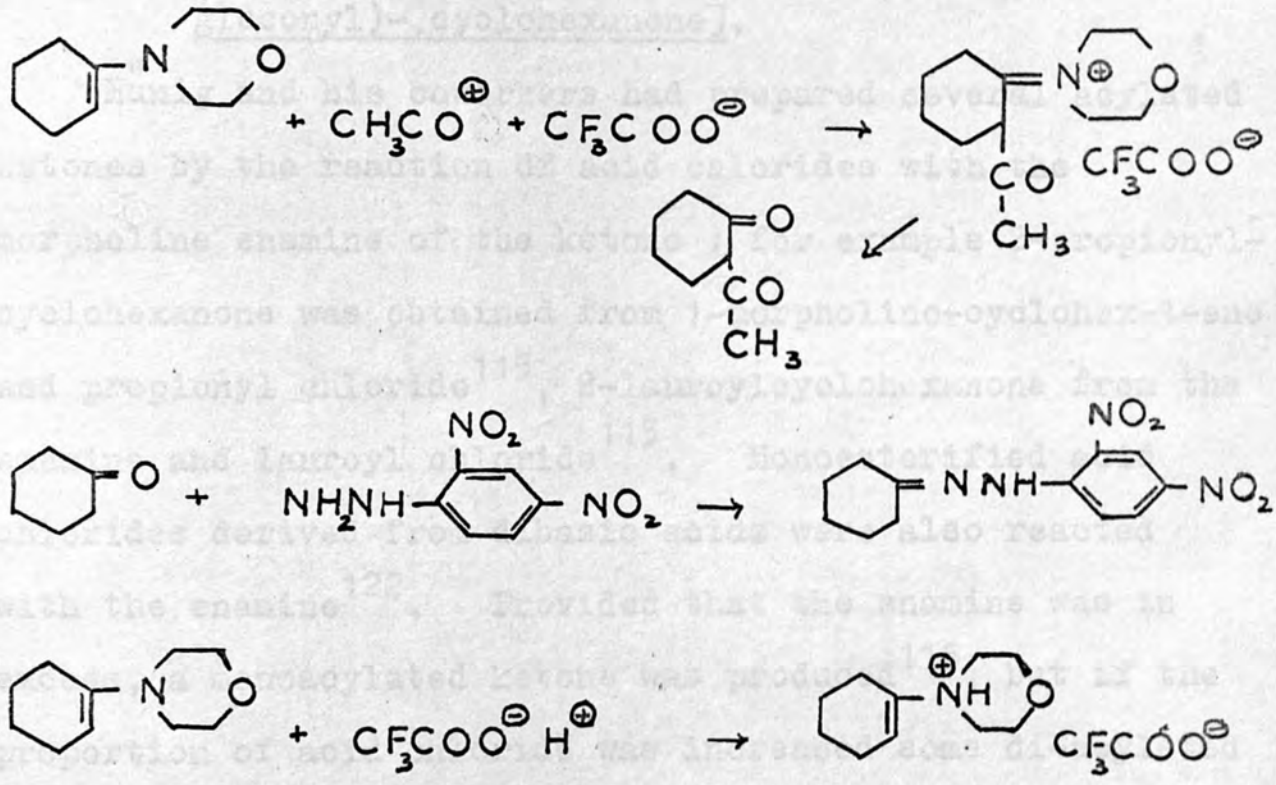
On this basis, the use of the pyrrolidine enamine would be preferred. But Hünig and his coworkers[115] found that morpholine enamines give better yields of acylated ketones than are obtained with pyrrolidine enamines. Since there

is relatively little difference in the ease of formation of the two enamines, Hünig's results were taken to be the deciding factor, and throughout the present work the morpholine enamine of cyclohexanone, or 1-morpholino-cyclohex-1-ene, prepared [Expt. 55] by the method of Hünig, Brenninger, and Lücke[119] was used.

B. The Reaction of 1-morpholino-cyclohex-1-ene with acetic acid - trifluoroacetic anhydride.

Preliminary investigations with the system: 1-morpholino-cyclohex-1-ene, glacial acetic acid and trifluoroacetic anhydride, the latter prepared by distillation of the acid from phosphorus pentoxide after Swarts,[120] [Expt. 56], soon showed that this would not be a suitable technique for the C-acylation of the enamine by a sugar acid, owing to the basicity of the nitrogen atom. On the addition of the enamine to the acid - anhydride mixture, a white crystalline solid, very hygroscopic, was immediately produced. If any C-acylation had occurred, hydrolysis of the resulting product followed by treatment with 2,4-dinitrophenyl hydrazine should have yielded a 2,4-dinitrophenylhydrazone which differed from that cyclohexanone (eqns. below). In fact only the cyclohexanone derivative could be isolated.

[Expts. 57, 58]. Enamines readily form crystalline salts with perchloric acid[121], and it is reasonable to assume that either the $CH_3COO^{\ominus}$ or the $CF_3COO^{\ominus}$ ion had precipitated the enamine as a salt. (eqn. 8).



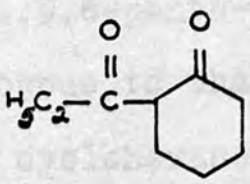
eqn. II. 8.

Attention was therefore turned to the reaction of the morpholine enamine of cyclohexanone with an acid chloride.

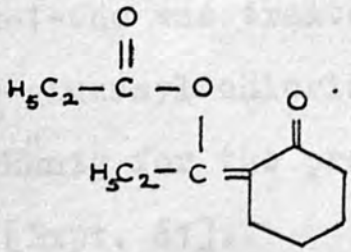
C. The Acylation of 1-morpholino-cyclohex-1-ene with an Acid Chloride.

1. Preparation of 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone, [2-(penta-acetyl-D-gluconyl)-cyclohexanone].

Hünig and his coworkers had prepared several acylated ketones by the reaction of acid chlorides with the morpholine enamine of the ketone : for example 2-propionyl-cyclohexanone was obtained from 1-morpholino-cyclohex-1-ene and propionyl chloride[115], 2-lauroylcyclohexanone from the enamine and lauroyl chloride[115]. Monoesterified acid chlorides derived from dibasic acids were also reacted with the enamine[122]. Provided that the enamine was in excess, a monoacylated ketone was produced[115], but if the proportion of acid chloride was increased some di-acylated product could be obtained. Thus from propionyl chloride and 1-morpholino-cyclohex-1-ene the two products are (I) and (II):



I.

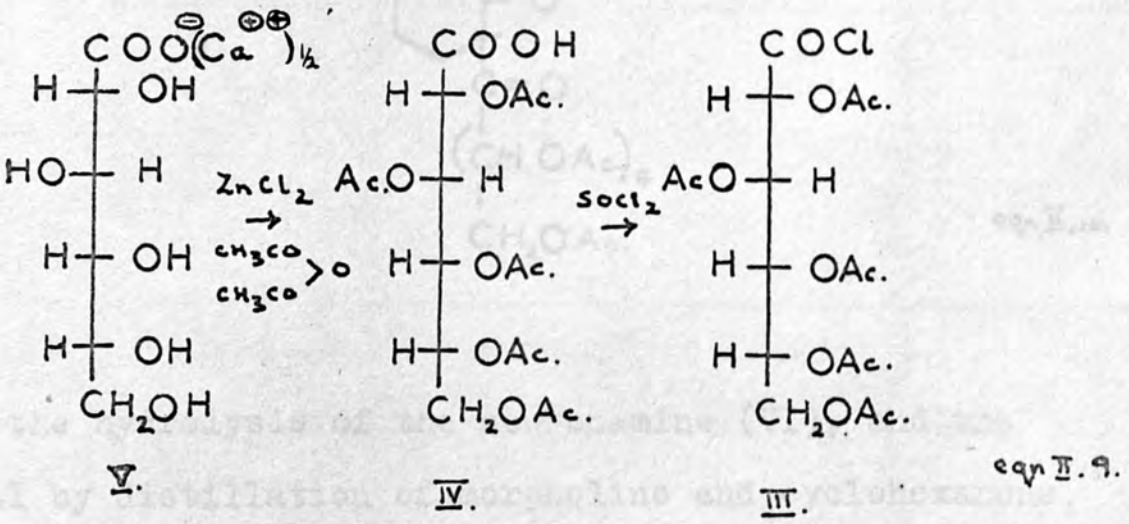


II.

Throughout the following work the enamine was well in

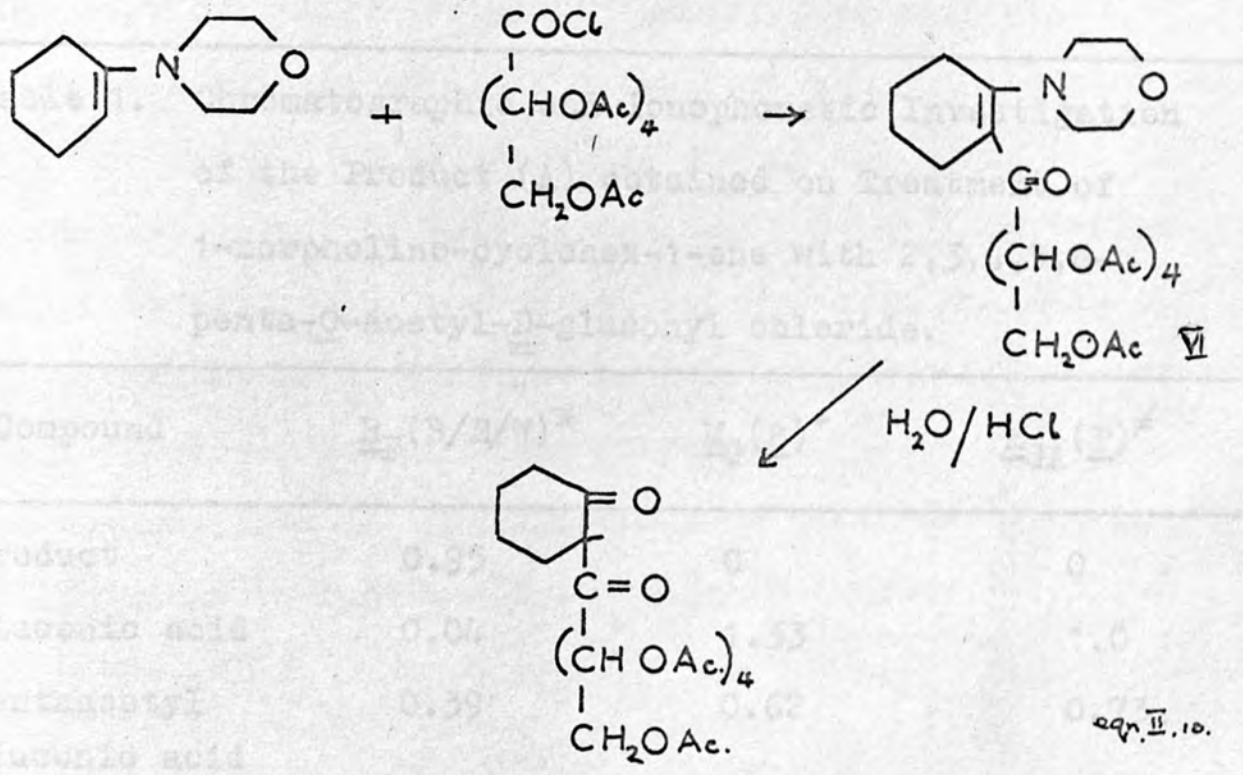
excess of that required for monoacylation.

An appropriate compound from the sugar series, to replace propionyl chloride in the monoacylation reaction, is 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride (III). 2,3,4,5,6-Penta-O-acetyl-D-gluconic acid (IV) can be derived from calcium D-gluconate (V) by the method of Barker[123] [Expt. 59] and converted to the required compound by treatment with thionyl chloride[124]. [Expt.60].



V. IV. III. eqn II.9.

1-Morpholino-cyclohex-1-ene was treated with 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride by a method analogous to that used by Hünig for the preparation of acyl cyclohexanones[115]. [Expt. 61].



eqn. II. 10.

After the hydrolysis of the new enamine (VI), and the removal by distillation of morpholine and cyclohexanone, a syrupy product(A) which could not be distilled at 100°/1 mm remained. Chromatographically this was homogeneous and differed from 2,3,4,5,6-penta-O-acetyl-D-gluconic acid which would have been produced during the hydrolysis from any unreacted 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride. The following table of chromatographic and ionophoretic measurements (Table 1) indicates that the residue (A) is neither 2,3,4,5,6-penta-O-acetyl-D-gluconic

acid, nor D-gluconic acid, nor by inference any partially deacetylated acid.

Table 1. Chromatographic and Ionophoretic Investigation of the Product (A) obtained on Treatment of 1-morpholino-cyclohex-1-ene with 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride.

Compound	R_F(B/E/W)*	M_G(B)+	M_{GA}(P)≠
Product	0.95	0	0
Gluconic acid	0.04	1.33	1.0
Pentaacetyl Gluconic acid	0.39	0.62	0.73

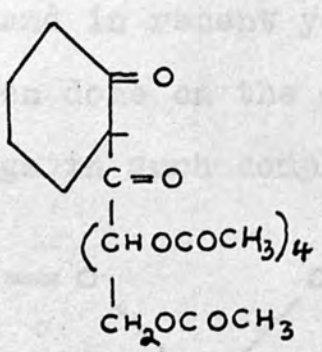
* mobility related to solvent front in solvent 1.

\+ mobility related to that of glucose on ionophoresis in borate solution.

≠ mobility related to that of D-gluconic acid on ionophoresis in phosphate solution.

The product (A) stained on paper with p-anisidine hydrochloride and with 2,4-dinitrophenylhydrazine in hydrochloric acid, giving on the one hand a brown compound, on the other an orange compound. This behaviour is consistent with the structure of the diketo-compound which is expected by analogy with Hünig's work[115].

Recrystallisation of the syrup (A) was attempted from several solvents, namely ether, petroleum ether (fraction boiling at 40-60°), aqueous ethanol and aqueous dioxan, but was achieved by dissolving the syrup (A) in a minimal amount of boiling hexane. On cooling, white crystals separated, the carbon and hydrogen analysis of which agreed well with that calculated for (penta-acetyl-D-gluconyl)-cyclohexanone monohydrate. It is justifiable to assume, from the previous work on enamines, that this compound is in fact 2-(penta-acetyl-D-gluconyl)-cyclohexanone (VII).



VII.

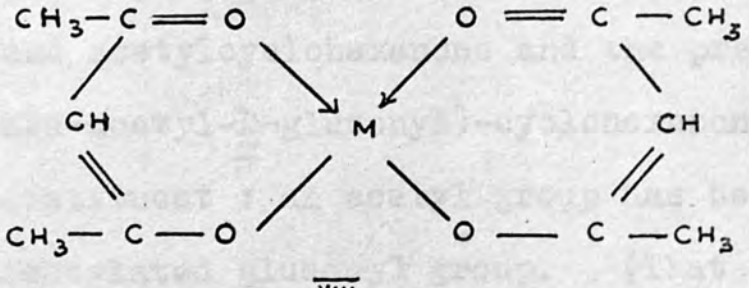
The molecular weight of the compound (VII), determined by the Rast method, was 483, which agrees within experimental error with that calculated for 2-(penta-acetyl-D-gluconyl)-cyclohexanone monohydrate, i.e. 504.

2. Evidence for the Structure of 2-(penta-acetyl-D-gluconyl)-cyclohexanone.

(i) The Copper Complexes of some β-Dicarbonyl Compounds.

A characteristic reaction of 1:3-diketones is the formation of derivatives with heavy metals, e.g. the blue copper compound, soluble in chloroform, the red iron compound, the violet chromium compound b.p.340° and the volatile derivatives of aluminium m.p. 193°, b.p.314° and beryllium m.p. 108°, b.p. 270°, formed by acetylacetone[125].

For a divalent metal the complex can be formulated[125] as (VIII), and in recent years a considerable amount of work has been done on the quasi-aromatic character of the chelate rings in such complexes[126,127].



VIII.

Copper acetylacetonate was prepared from acetylacetone by the method of Borsche[128]. [Expt. 62]. The copper

derivative of 2-acetylcyclohexanone, derived [Expt. 63] from the reaction of acetyl chloride with 1-morpholino-1-cyclohexene, was similarly obtained. [Expt. 64]. The method was extended to 2-(penta-acetyl-D-gluconyl)-cyclohexanone. It was expected that a copper derivative would again be produced, but in fact only starting material was recovered. [Expt. 65].

In order to form the copper chelate, and for the ligand ring to be a quasi-aromatic system, i.e. comprise an acyclic conjugated π-electron system and show chemical properties typical of aromatic systems[126], as with the acetylacetonate, the chelate ring must presumably be planar. It is known that the most energetically favourable conformation of the cyclohexanone ring, though largely dependent on substituents in the ring, is a flattened chair[129]. A major difference between the model compound acetylcyclohexanone and the prepared compound, 2-(penta-acetyl-D-gluconyl)-cyclohexanone, is the size of the substituent : an acetyl group has been replaced by a pentaacetylated gluconyl group. (That the ease of formation of a copper derivative is influenced by steric factors is illustrated by the non-formation of a complex by isopropylbutyryl acetone $CH_3CH_2CH_2\overset{\overset{O}{\|}}{C}-CH_2\overset{\overset{O}{\|}}{C}-CH_2-CH\begin{matrix}CH_3\\CH_3\end{matrix}$ [130])

The inability of the D-gluconyl derivative to form a complex may, then, be due to steric hindrance. Assuming however that in both 2-acetylcyclohexanone and 2-(penta-acetyl-D-gluconyl)-cyclohexanone the acyl group is an equatorial substituent, a not unreasonable assumption from the analogy of non-bonded interactions in cyclohexane, the formation of a planar chelate ring appears, from models, equally likely in either case (see fig. 1 below). Alternatively, the explanation may lie in the relative ease of keto to enol conversion in the two compounds, since the copper complex is obtained from the enol form of the diketone.

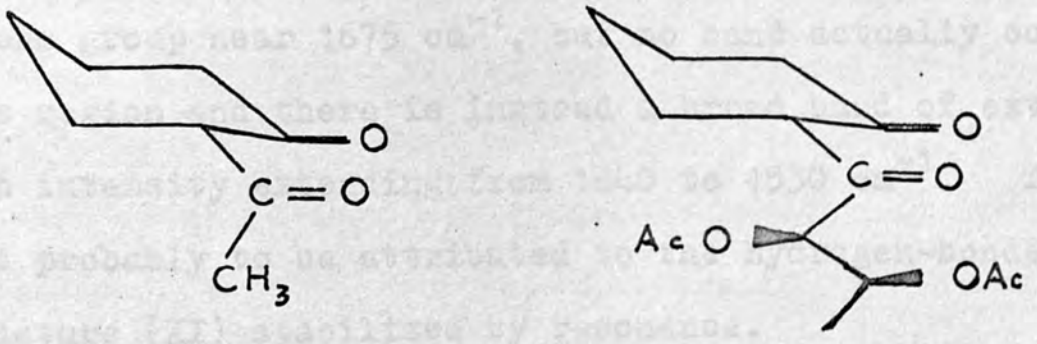
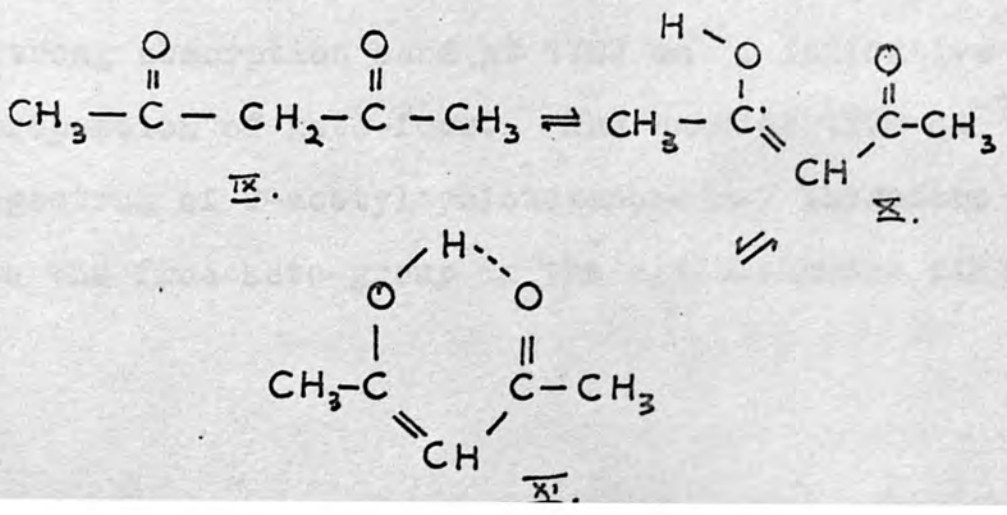


Fig. 2.

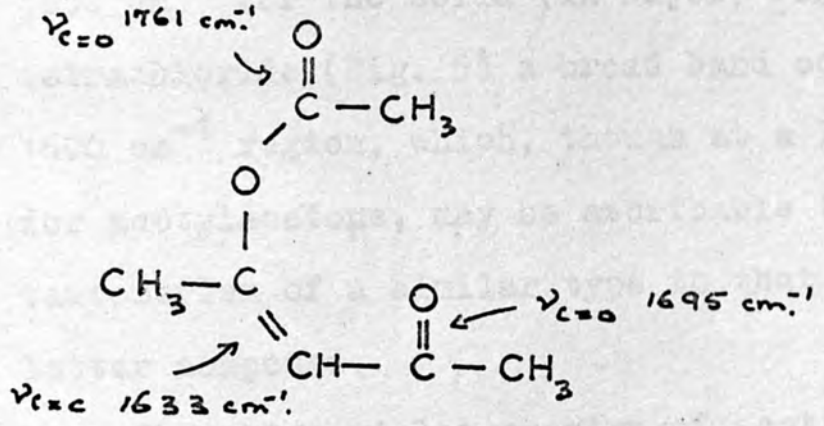
(ii) The Infra-red and Ultra-violet spectra of 2-(penta-acetyl-D-gluconyl)-cyclohexanone.

The infra-red spectrum of cyclohexanone shows a strong carbonyl absorption at 1705 cm^{-1} [Fig. 2].

This is the region in which an absorption from a simple ring ketone would be expected to occur[131]. 2-Acetylchclohexanone, on the other hand, shows a moderately strong absorption at 1700 and 1710 cm^{-1}, but a broad intense absorption in the region 1550 - 1650 cm^{-1}, with a maximum at 1600 cm^{-1}. [Fig. 3]. It is known that the spectra of β-diketones between 1770 and 1600 cm^{-1} can become quite complex, and at least three molecular species may be involved[132]. For liquid acetylacetone Rasmussen, Tunnicliff and Brattain[133] have observed that the ketonic structure (IX) gives an infra-red band of moderate intensity at 1709 cm^{-1}. The simple enol structure (X) would be expected to show the carbonyl absorption of an α.β-unsaturated ketone group near 1675 cm^{-1}, but no band actually occurs in this region and there is instead a broad band of extremely high intensity extending from 1640 to 1530 cm^{-1}. This is most probably to be attributed to the hydrogen-bonded structure (XI) stabilized by resonance.



On acetylation of the hydroxyl group of (X), the conjugated chelate structure (XI) can no longer be formed and it is observed accordingly that the infra-red spectrum of acetonylacetate has infra-red bands at 1761, 1695 and 1633 cm^{-1} which can be assigned to C = O and C = C vibrations as indicated[133] (XII).



XII.

The band at 1710 cm^{-1} for 2-acetylcyclohexanone can therefore be assigned to a simple keto-group, while the broad band with a maximum at 1600 cm^{-1} is probably due to the conjugated chelate structure, as present in acetylacetone. Rasmussen et al[133] also noted that the infra-red spectrum of 5,5-dimethylcyclohexane-1,3-dione contains a moderately strong absorption band at 1702 cm^{-1}, indicative of a good proportion of keto-form. The band at 1700 cm^{-1} in the spectrum of 2-acetylcyclohexanone may therefore be assigned to the free keto-group in the cyclohexanone ring.

Consider now the infra-red spectrum of 2-(penta-acetyl-D-gluconyl)-cyclohexanone (Figs. 4, 5). The absorption of the carbonyl group is masked to a great extent by that due to the acetate groups, at 1730 cm^{-1}. However, it can be discerned as a shoulder on the acetate peak at 1705 cm^{-1}. There is no absorption over the range 1550-1650 cm^{-1} for the solid (in Nujol, Fig. 4) but in carbon tetrachloride (Fig. 5) a broad band occurs in the 1500 - 1600 cm^{-1} region, which, though at a lower frequency than for acetylacetone, may be ascribable to keto-enol tautomerism of a similar type to that occurring in the latter compound.

The ultra-violet spectra of acetylacetone and 2-(pentaacetyl-D-gluconyl)-cyclohexanone are of interest in this connection. Rasmussen *et al.*[133] state that acetylacetone gives an ultra-violet spectrum markedly different from a simple conjugated ketone such as acetophenone, exhibiting a very strong band with a maximum at 2700 Å. Acetylation to acetylacetone acetate converts the absorption from that of the conjugated chelate type to that of a simple conjugated ketone[133], with maxima at 2300 and 3250 Å (Fig. 6). The ultraviolet spectrum of

2-(penta-acetyl-D-gluconyl)-cyclohexanone partially resembles that of acetylacetone (Fig. 6). Two maxima appear, one at 2280Å and one at 2700Å. The maximum at 2280Å occurs also in 1,2,3,4,6-penta-O-acetyl-β-D-glucose and may be attributed to the acetate groups present in either molecule. The maximum at 2700Å suggests a resemblance to acetylacetone. Taken in conjunction, the spectra indicate that 2-(pentaacetyl-D-gluconyl)-cyclohexanone contains a β-dicarbonyl grouping similar to that in acetylacetone, but with some slight modification of the keto-enol tautomerism usually present in this system, which is reflected in the altered frequency of the band in the infra-red. It is conceivable that one of the carbonyl groups is hydrated. The fact that the spectra are not identical is in accordance with the non-formation of the copper complex, the production of which depends upon the enol form[134].

(iii) The Deacetylation of 2-(penta-acetyl-D-gluconyl)-cyclohexanone.

For the further study of the structure of the prepared compound, it was decided to derive the parent sugar by deacetylation, and to subject it to periodate oxidation. During the course of the investigation, an interesting property of the type of compound exemplified by

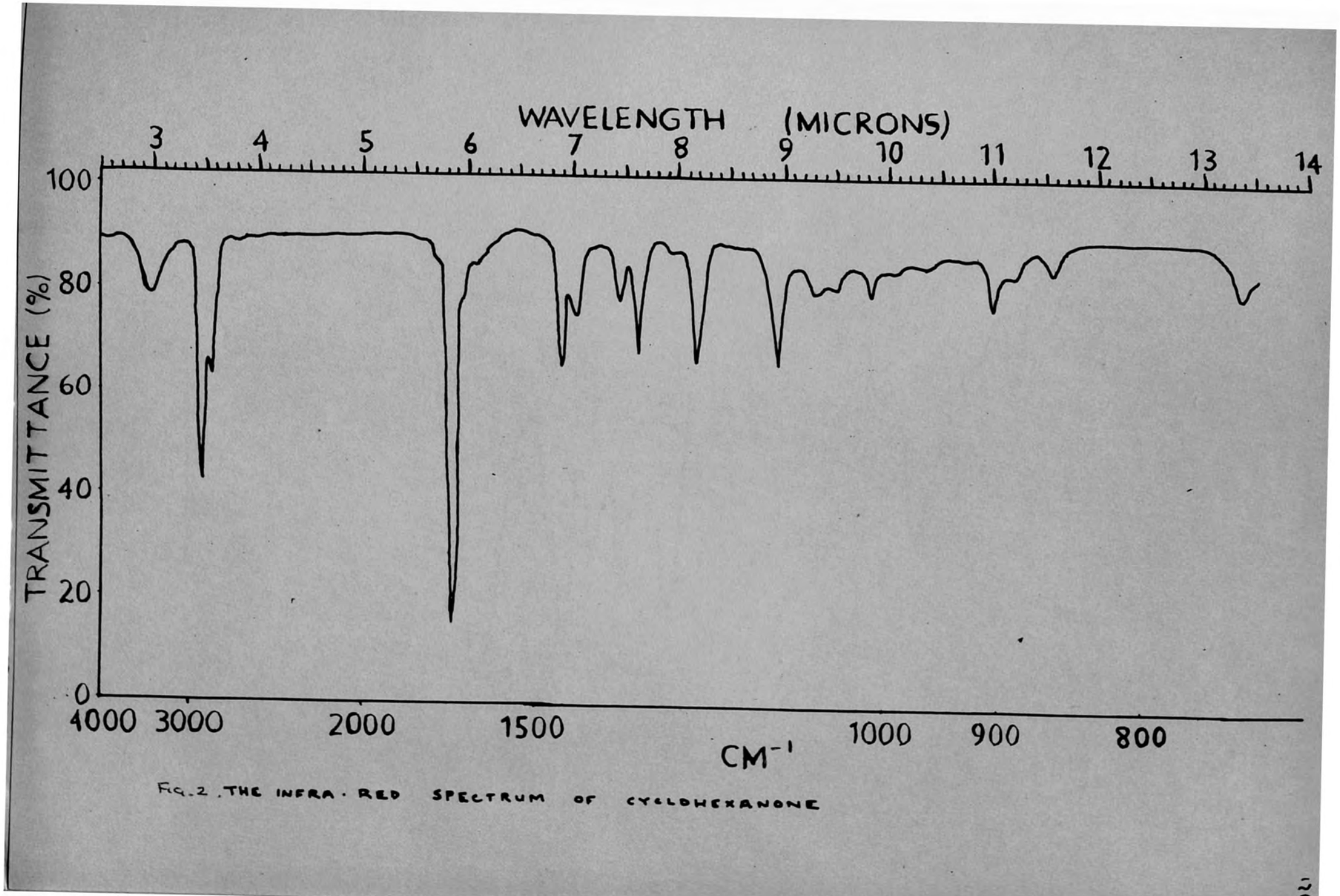


FIG. 2. THE INFRA-RED SPECTRUM OF CYCLOHEXANONE

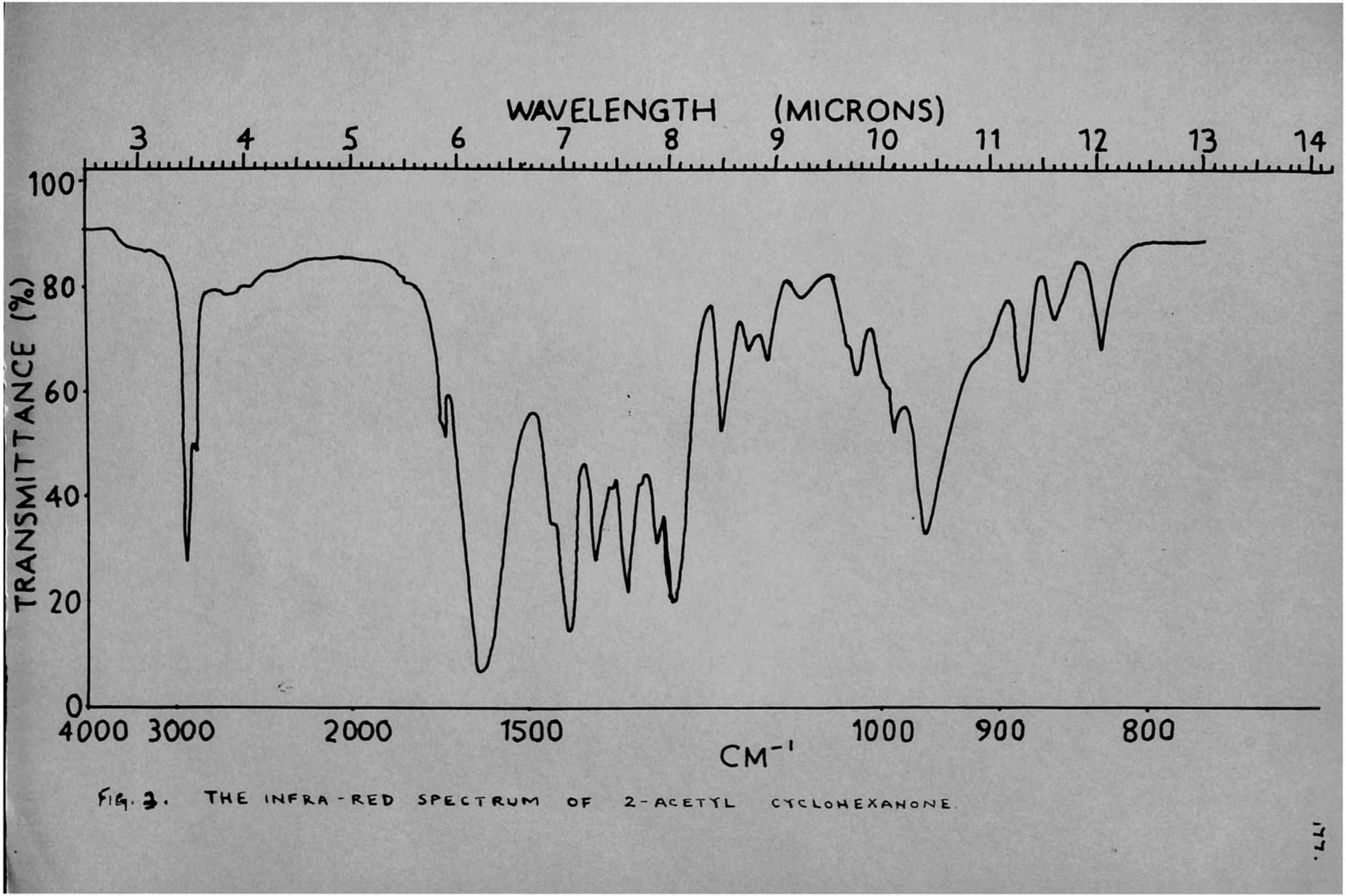


FIG. 3. THE INFRA-RED SPECTRUM OF 2-ACETYL CYCLOHEXANONE.

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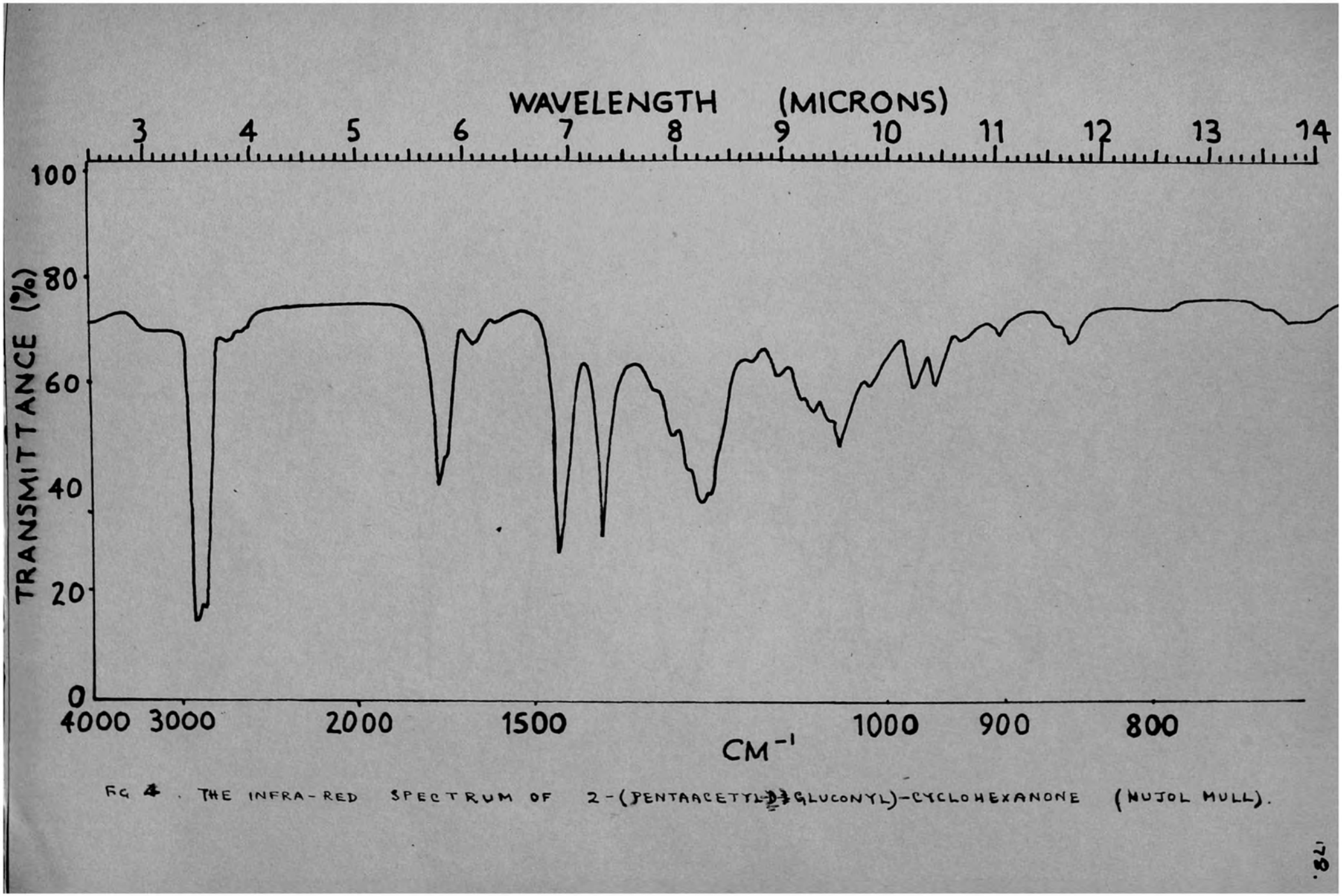

FIG 4. THE INFRA-RED SPECTRUM OF 2-(PENTAACETYL-D-GLUCONYL)-CYCLOHEXANONE (NUJOL MULL).

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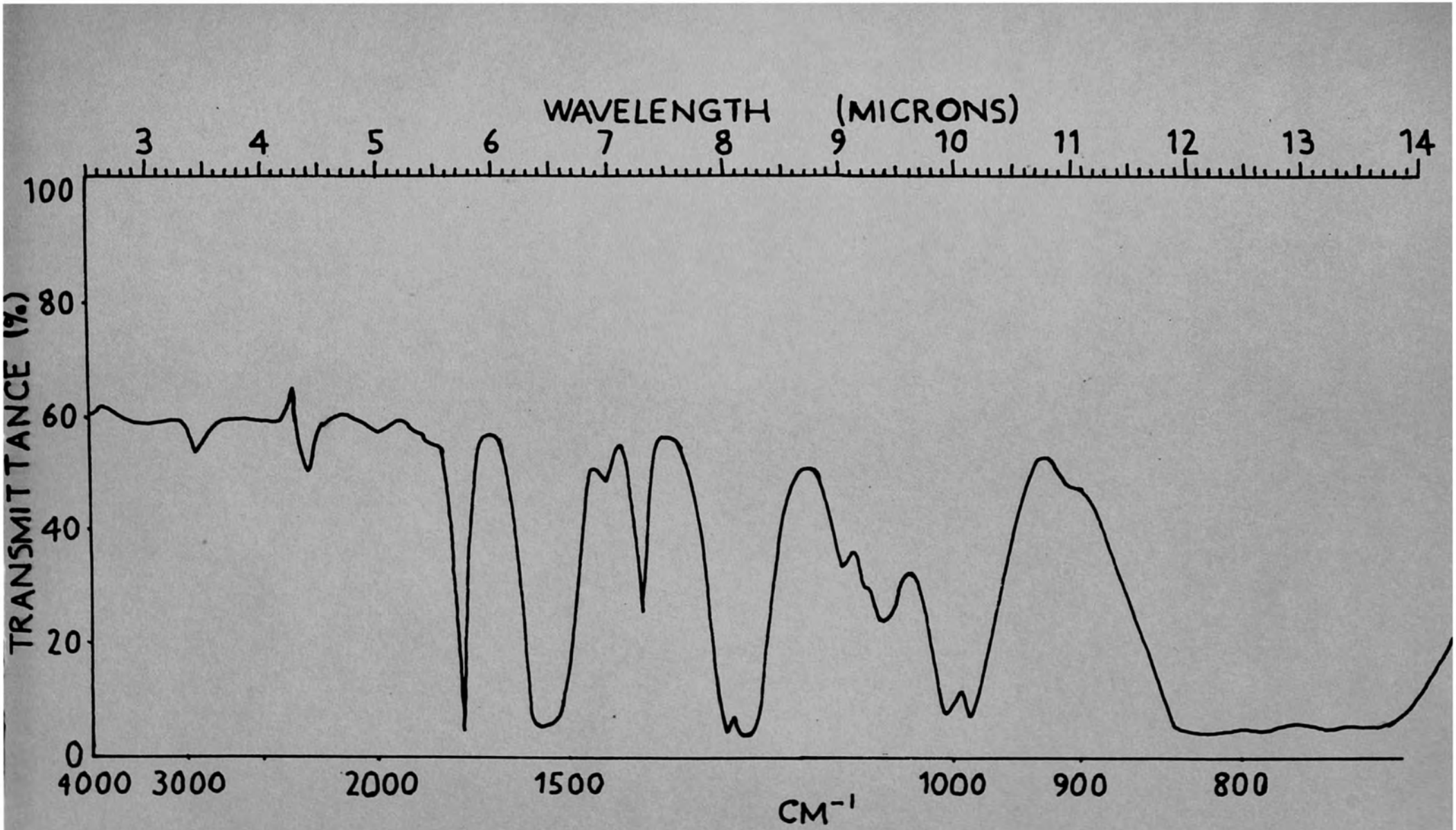
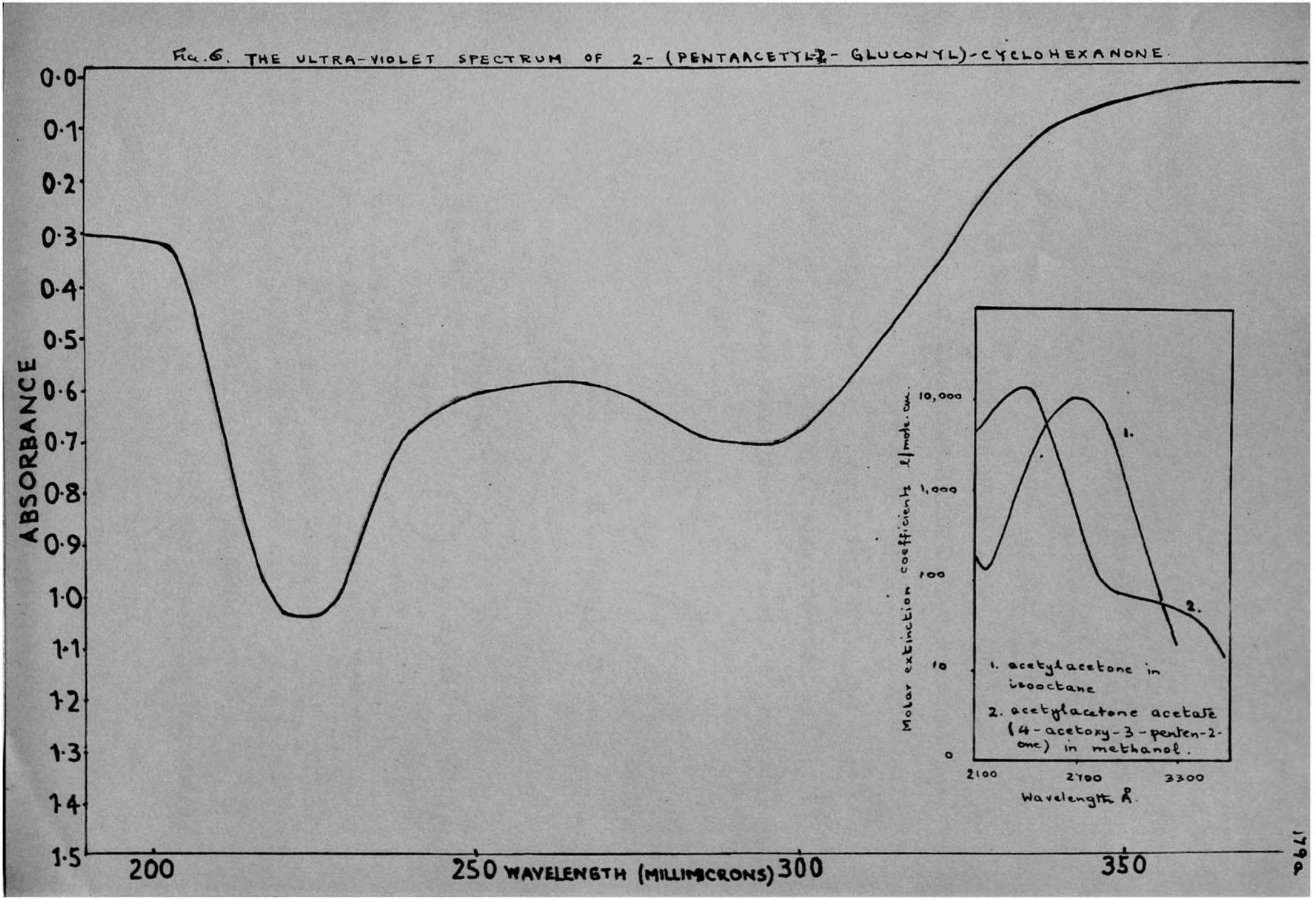


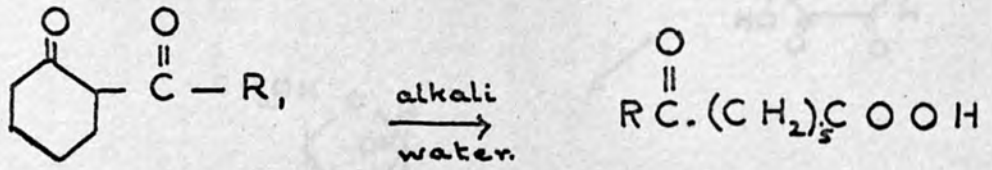
FIG. 5. THE INFRA-RED SPECTRUM OF 2-(PENTAACETYL-D-GLUCONYL)-CYCLOHEXANONE (IN CCl_4.)

Fig. 6. THE ULTRA-VIOLET SPECTRUM OF 2-(PENTAACETYL-β-GLUCONYL)-CYCLOHEXANONE.



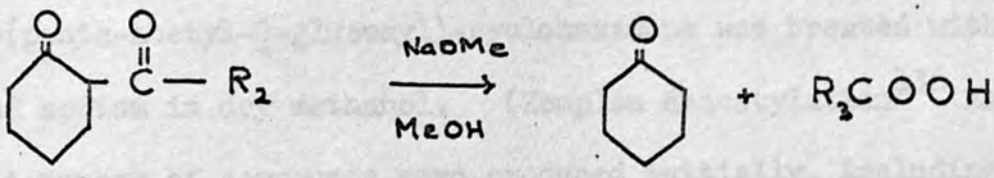
1796

2-(penta-acetyl-D-gluconyl)-cyclohexanone was brought to light, namely the considerable lability of the carbon-carbon bond between the cyclohexanone ring and the sugar residue under mildly alkaline conditions. This lability, in the presence of sodium methoxide or ammonia, was not to be anticipated on the basis of the known properties of 2-alkoxycyclohexanones. In Hünig's work, the cleavage of the substituted cyclohexanone ring by moderately strong alkali was used as a general method for preparing keto-carboxylic acids[115].



R_1 = alkyl.

eqn. II. 11.



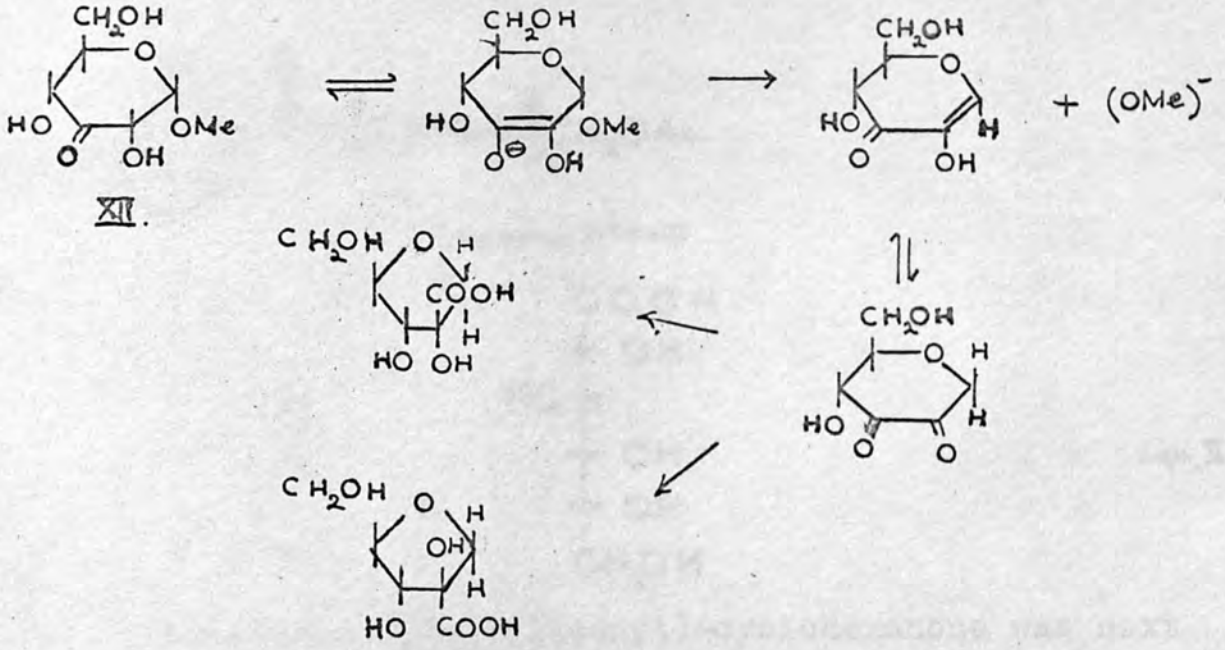
R_2 = acetylated polyol residue.

R_3 = polyol residue.

eqn. II. 12.

The cleavage observed was therefore not only occurring under much milder conditions, but at a different point in the molecule. It is true that the fragmentation and dissolution of oxidised polysaccharides in alkali has generally been ascribed to the presence of carbonyl groups,

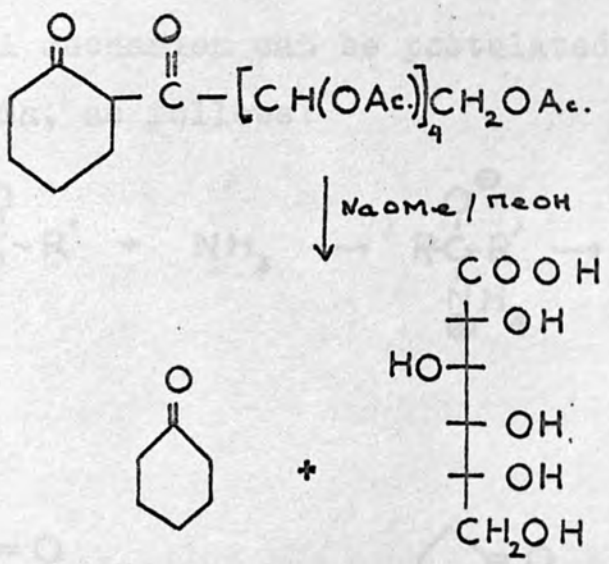
and a keto-sugar such as methyl 3-oxo-β-D-ribo-hexopyranoside (XII) has a half-life of only one minute in saturated lime-water at room temperature[135] so some decomposition after deacetylation might have been proposed. Nevertheless, the following reactions were somewhat unexpected.



XII.

2-(penta-acetyl-D-gluconyl)-cyclohexanone was treated with a trace of sodium in dry methanol. (Zemplen deacetylation[136] Expt. 66). A number of compounds were produced initially, including a compound which co-chromatographed with D-gluconic acid in solvent (1), R_F (B.E.W.)= 0.06, and migrated with D-gluconic acid on ionophoresis in phosphate, $M_{G.A}(P)$ = 1.0, and borate $M_G(B)$ = 1.3, solutions. The final sole product detectable chromatographically after 18 hr. was the material which co-chromatographed with D-gluconic acid. Distillation of the mixture showed that cyclohexanone was also present. This compound was

characterised as its 2,4-dinitrophenylhydrazone. [Expt. 67]. Since no cyclohexanone was initially present, it was obvious that the 2-(penta-acetyl-D-gluconyl)-cyclohexanone molecule had been cleaved by the treatment with sodium in methanol.

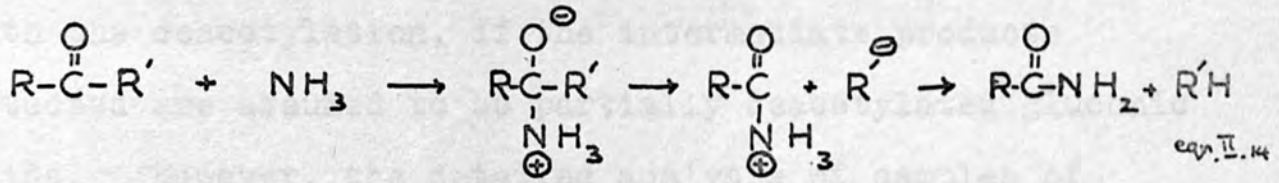


eqn. II. 13.

2-(penta-acetyl-D-gluconyl)-cyclohexanone was next treated with dry ammonia in methanol (Expt. 68). The molecule was again cleaved, this time yielding a crystalline material which behaved chromatographically and ionophoretically exactly as did D-gluconamide prepared from D-glucono-δ-lactone[137]. [Expt. 69]. The crystalline material was indeed shown to be D-gluconamide since admixture with an authentic specimen caused no depression in melting point.

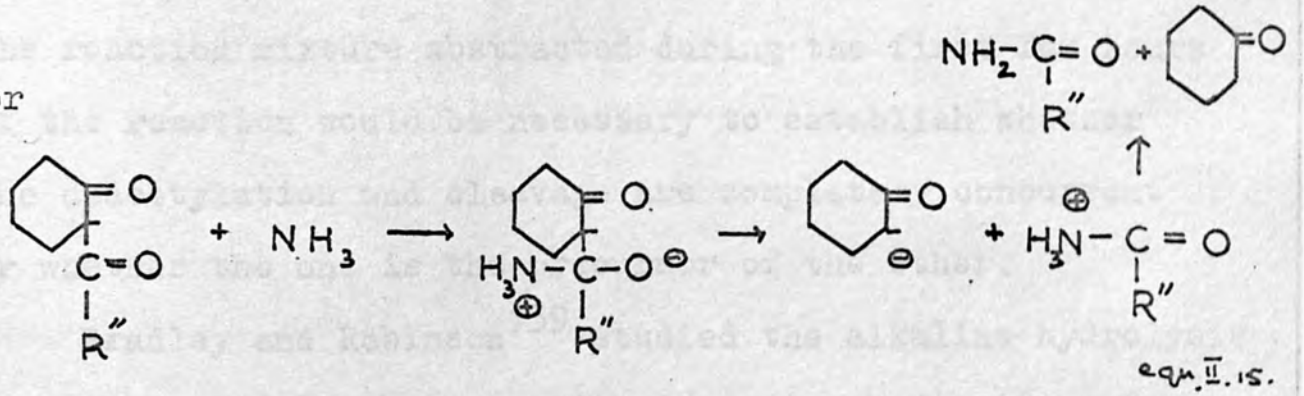
Compound	Solvent	Phosphate	Molybdate	Borate
	R_F	$M_{G.A}$(P)	M_S(Mo)	M_G(B)
D-Gluconamide	0.14	0,1	0.98, 1.11	0.94
Crystalline material	0.14	0,1	0.98, 1.11	0.94

A mechanism can be postulated for the reaction with ammonia, as follows:



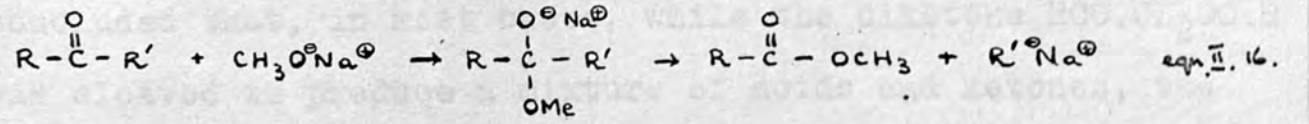
eqn. II. 14

or

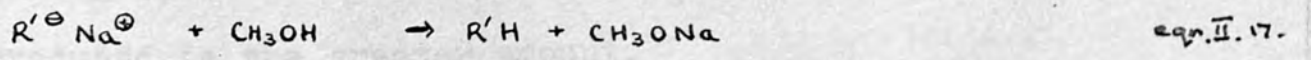


eqn. II. 15.

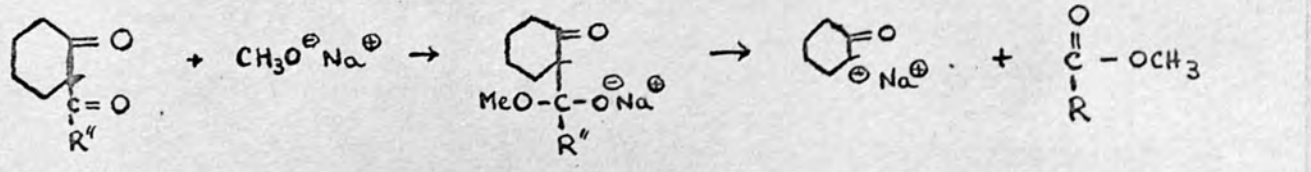
and therefore, for sodium in methanol, by analogy:

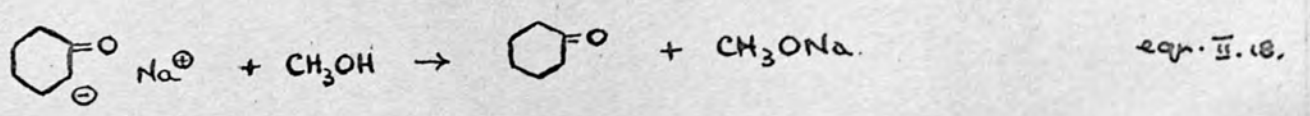


eqn. II. 16.



eqn. II. 17.



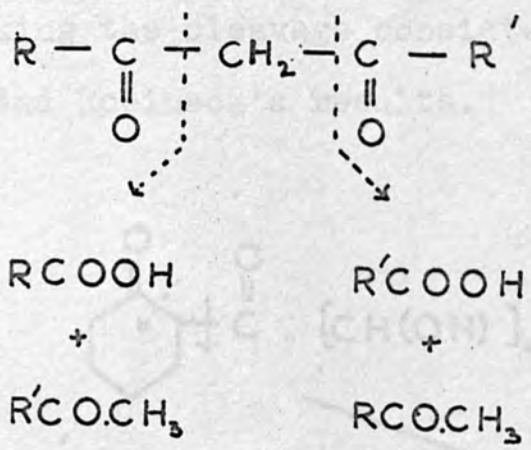


eqn. II. 18.

(Methyl-D-gluconate is hydrolysed fairly readily in water[138] so that D-gluconic acid is actually detected).

The mechanism as it stands gives no indication of why the carbonyl group on C_1 of the side chain rather than that in the cyclohexanone ring should be preferentially attacked. It is also independent of any deacetylation which may be proceeding concurrently. From the data obtained it appears that the cleavage occurs concurrently with the deacetylation, if the intermediate products detected are assumed to be partially deacetylated gluconic acids. However, the detailed analysis of samples of the reaction mixture abstracted during the first few hours of the reaction would be necessary to establish whether the deacetylation and cleavage are completely concurrent or whether the one is the precursor of the other.

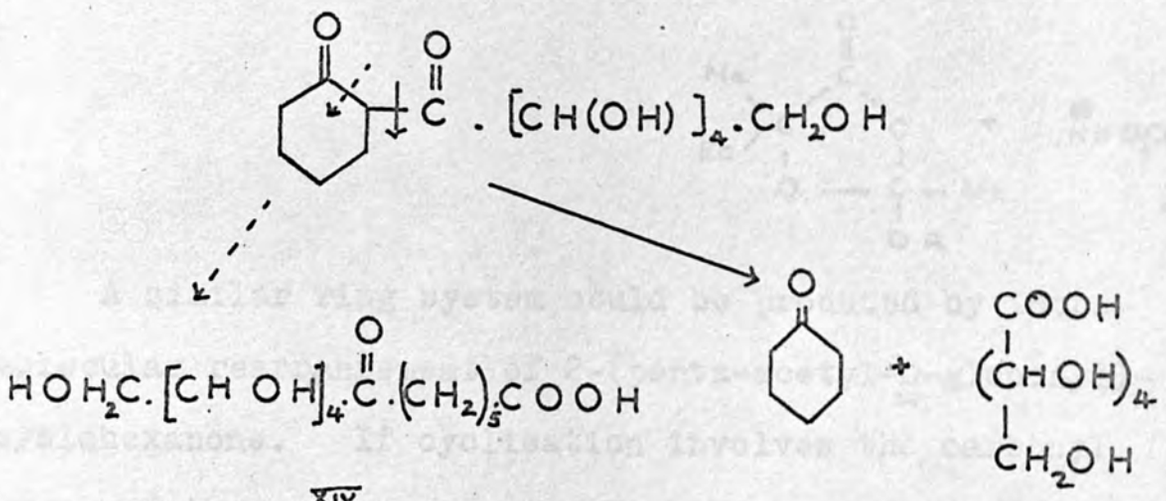
Bradley and Robinson[139] studied the alkaline hydrolysis of a number of β-diketones with 1% sodium hydroxide and concluded that, in most cases, while the diketone $RCO.CH_2CO.R'$ was cleaved to produce a mixture of acids and ketones, the stronger of the two acids which might be formed was produced in the greater amount.



The hydrolytic fission was also characteristic of the diketonic phases and did not occur in the keto-enolic modifications. Kutz and Adkins[140] extended the study to the alcoholysis of β-diketones with sodium ethoxide at 60° and again found increased activity in the compounds incapable of enolization.

The two acids which could be formed from concurrent deacetylation and cleavage of 2-(penta-O-acetyl-D-gluconyl)-cyclohexanone by sodium ethoxide are D-gluconic acid and 6-D-gluconyl-hexanoic acid (XIV). The dissociation constant of D-gluconic acid is quoted[141] as $K = 1.65 \times 10^{-4}$, and, while the dissociation constant of the second acid is not available, to the author's knowledge, it is unlikely to be considerably greater than that of hexanoic (caproic) acid $(CH_2)_5COOH$, which is quoted[142] as 1.44×10^{-5}. Although the results are not directly

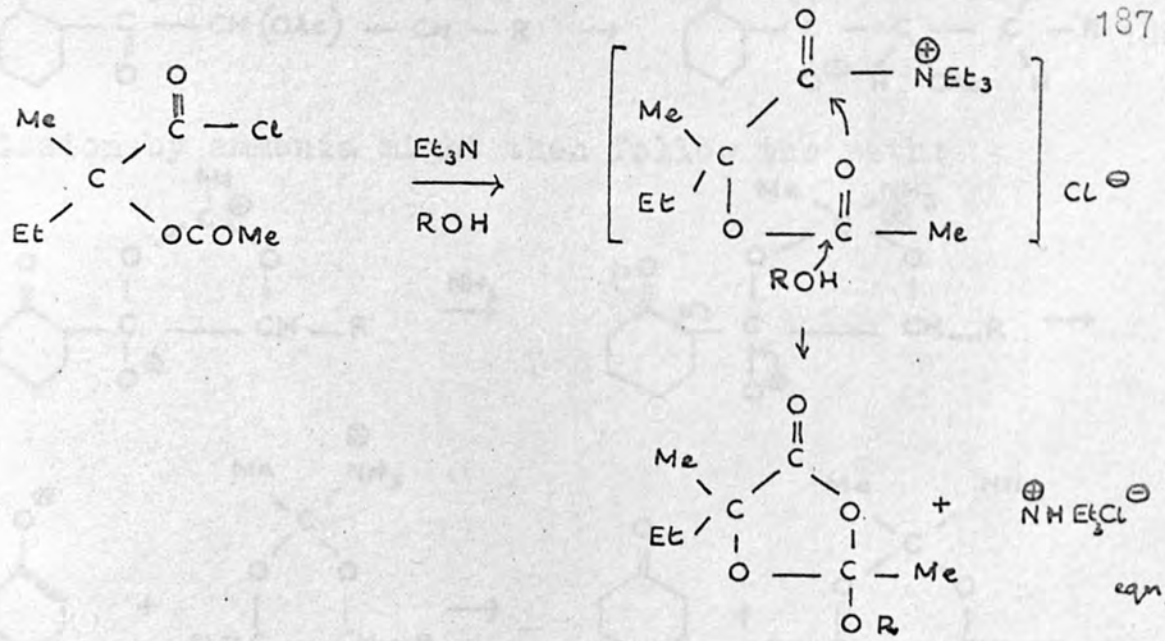
comparable, it appears that D-gluconic acid is the stronger acid, making the cleavage consistent with the majority of Bradley and Robinson's results.



XIV.

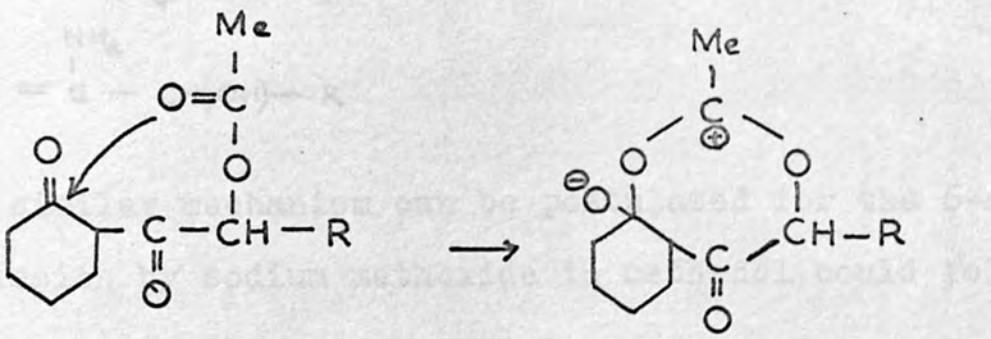
It is noteworthy that neither acetic acid nor acetamide are detectable, by ionophoresis in phosphate solution, or by chromatography in solvent 1 respectively, when 2-acetylcyclohexanone is treated with either sodium in methanol or ammonia in methanol.

If the cleavage occurs before deacetylation, the course of the reaction may be influenced by interaction between the acetyl group on C_2' or C_3' and the carbonyl group on C_1' in 2-(penta-acetyl-D-gluconyl)-cyclohexanone. Mattocks[143] reports the formation of dioxolanones between α-acetoxy-α-methylbutyrylchloride and alcohols in triethylamine



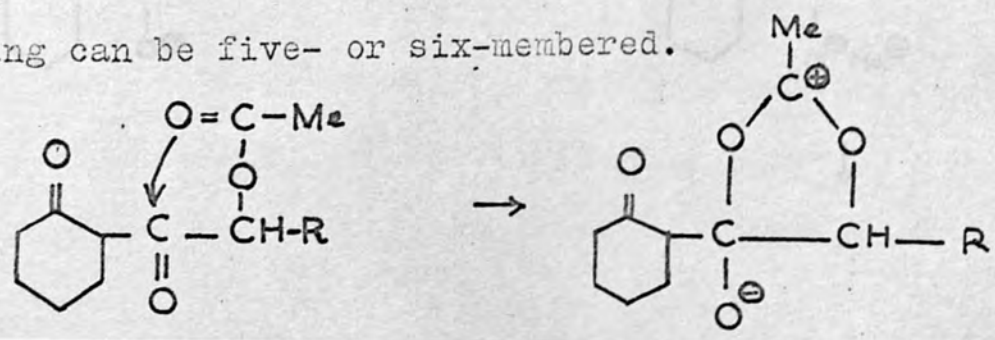
eqn. II. 19.

A similar ring system could be produced by intramolecular rearrangement of 2-(penta-acetyl-D-gluconyl)-cyclohexanone. If cyclisation involves the carbonyl group of the cyclohexanone ring, the smallest ring which can be formed is seven-membered.

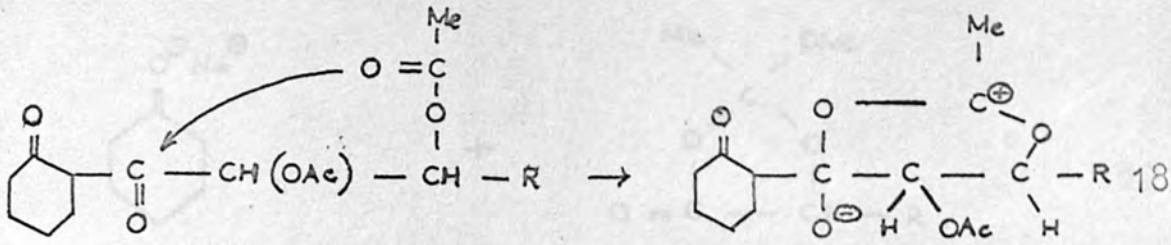


eqn. II. 20.

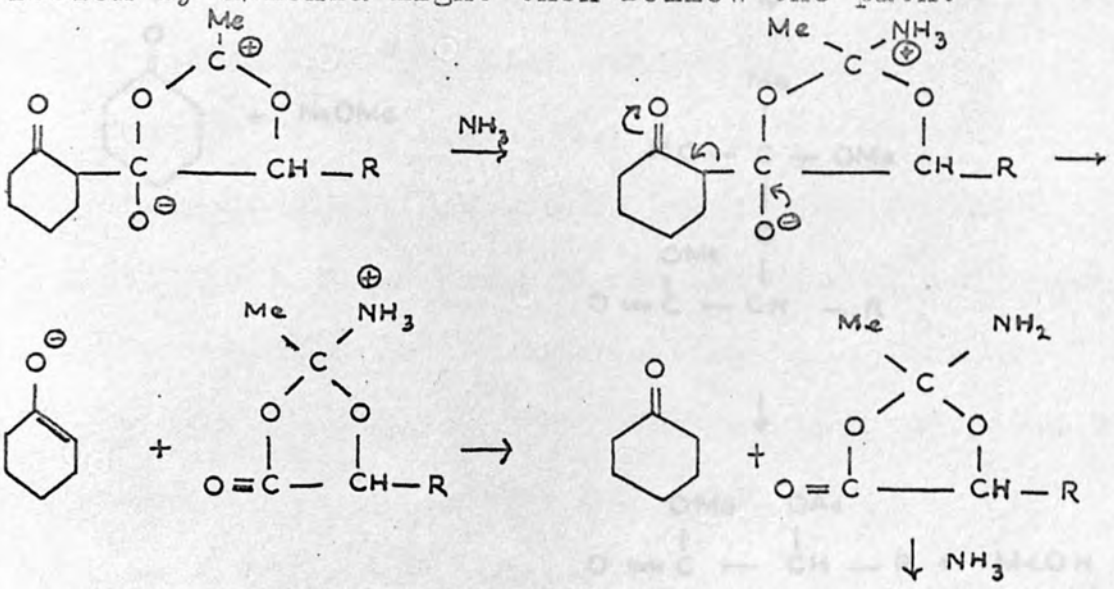
If, on the other hand, the carbonyl group in the sugar residue is participating in the cyclisation the resulting ring can be five- or six-membered.

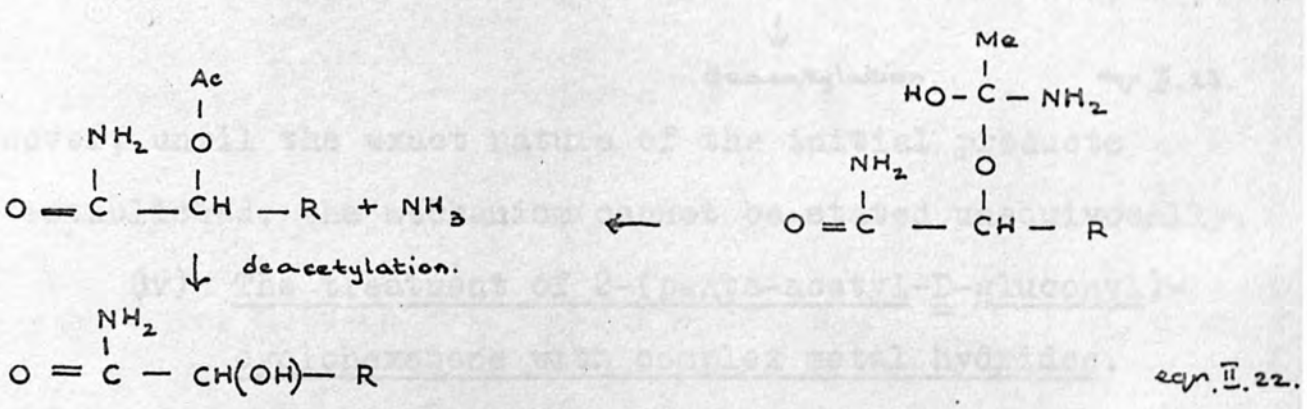


eqn. II. 21.



Fission by ammonia might then follow the path:

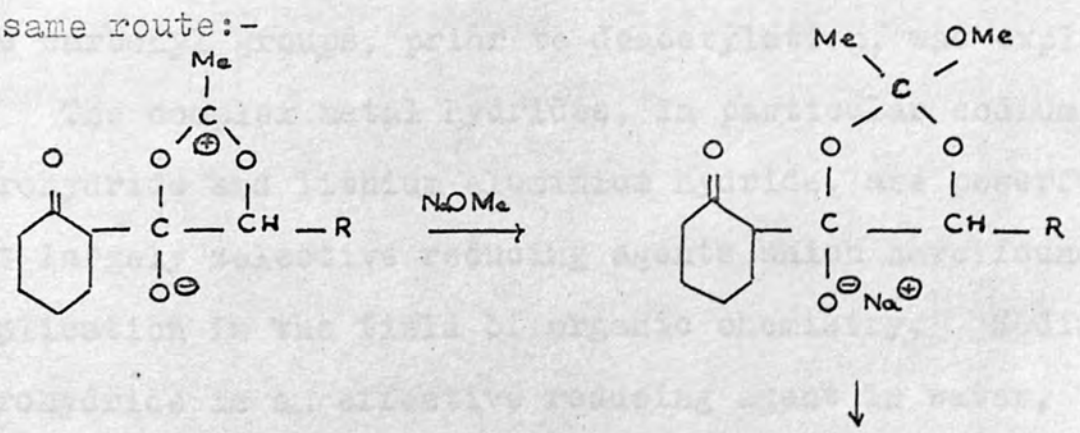


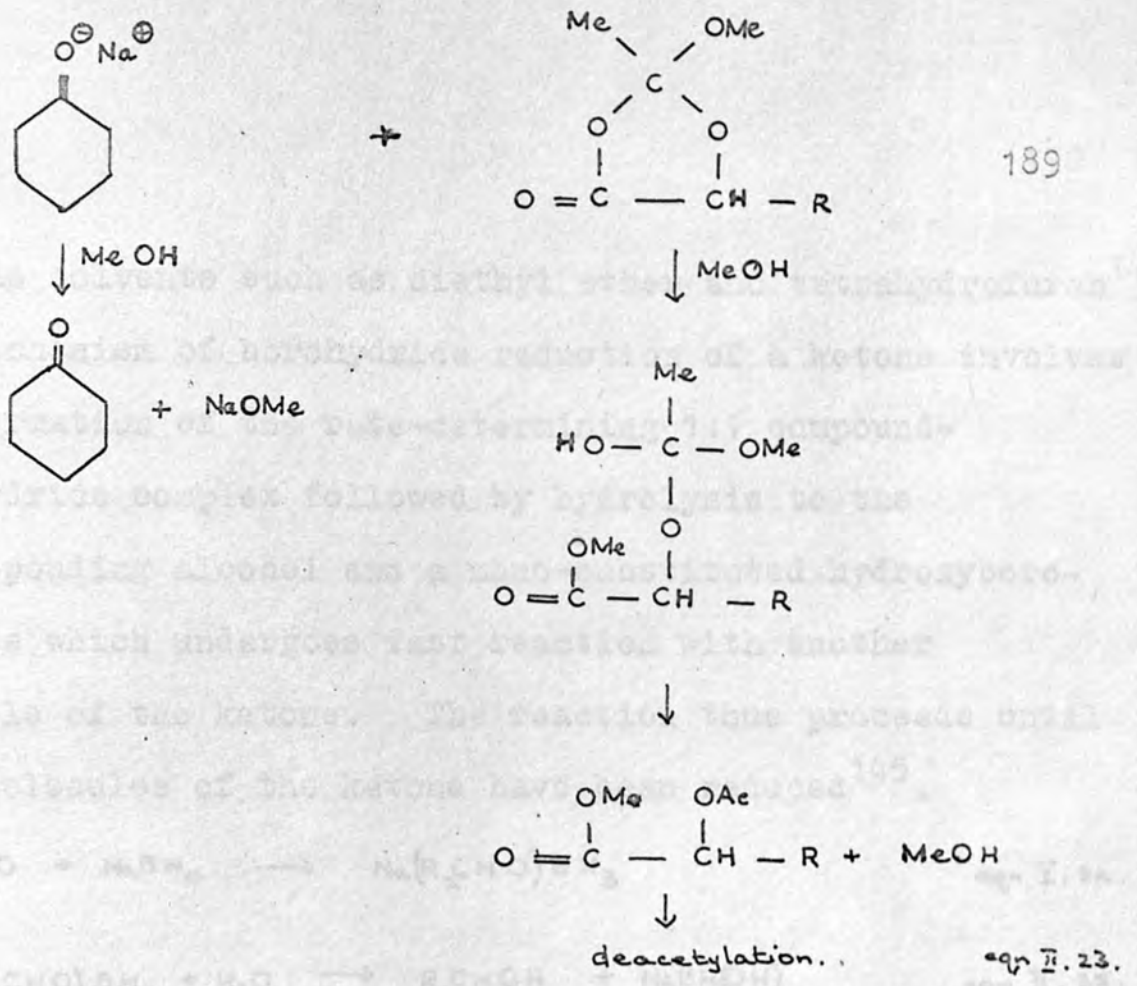


eqn. II.22.

A similar mechanism can be postulated for the 6-membered ring.

Fission by sodium methoxide in methanol could follow the same route:-





eqn II.23.

However, until the exact nature of the initial products is established, the mechanism cannot be stated unequivocally.

(iv) The treatment of 2-(penta-acetyl-D-gluconyl)-cyclohexanone with complex metal hydrides.

Since 2-(penta-acetyl-D-gluconyl)-cyclohexanone had proved to be labile under the mild conditions of deacetylation previously used, the possibility of reducing the carbonyl groups, prior to deacetylation, was explored.

The complex metal hydrides, in particular sodium borohydride and lithium aluminium hydride, are powerful yet largely selective reducing agents which have found wide application in the field of organic chemistry. Sodium borohydride is an effective reducing agent in water, methanol or dioxan solution, but is not very soluble in

organic solvents such as diethyl ether and tetrahydrofuran[144]. The mechanism of borohydride reduction of a ketone involves the formation of the rate-determining 1:1 compound-borohydride complex followed by hydrolysis to the corresponding alcohol and a mono-substituted hydroxyboro-hydride which undergoes fast reaction with another molecule of the ketone. The reaction thus proceeds until four molecules of the ketone have been reduced[145].

$$R_2C{=}O + NaBH_4 \longrightarrow Na(R_2CHO)BH_3 \qquad \text{eqn II. 24.}$$

$$Na(R_2CHO)BH_3 + H_2O \longrightarrow R_2CHOH + NaBH_3(OH) \qquad \text{eqn II. 25.}$$

$$R_2C{=}O + NaBH_3(OH) \longrightarrow Na(R_2CHO)BH_2(OH) \qquad \text{eqn. II. 26.}$$

$$Na(R_2CHO)BH_2(OH) + H_2O \longrightarrow R_2CHOH + NaBH_2(OH)_2 \qquad \text{eqn. II. 27.}$$

$$R_2C{=}O + NaBH_2(OH)_2 \longrightarrow Na(R_2CHO)BH(OH)_2 \qquad \text{eqn II. 28.}$$

$$Na(R_2CHO)B(OH)_3 + H_2O \longrightarrow R_2CHOH + NaBO_2 + 2H_2O \qquad \text{eqn II. 29.}$$

Since the preferential reduction of the keto-groups was required, sodium borohydride, which according to Gaylord does not normally reduce an ester grouping, was chosen[146]. Dale's method[147] in which potassium boro-hydride in water is added slowly to a methanolic solution of the reducible material, was used. [Expt. 70]. Chromatographic investigation of the product in solvent 1

revealed that it was a mixture of substances $\underline{R_F}$(B/E/W) = 0.07 → 0.24, 0.42 and 0.61, while ionophoresis in sodium molybdate solution indicated at least partial deacetylation: $\underline{M_S}$(Mo) = 0.59, 0.70, 0.83, 1.0. The reaction conditions were simulated using 1,2,3,4,6-penta-O-acetyl-β-D-gluco-pyranose [Expt. 72], prepared previously by the acetylation of D-glucose[148] [Expt. 71], as a model compound. A single product was obtained which migrated on ionophoresis in borate solution as did D-glucitol: $\underline{M_G}$(B) = 0.85, indicating that reduction and deacetylation had taken place under the experimental conditions employed. It is reasonable to assume that the borohydride had partially deacetylated the 2-(penta-acetyl-D-gluconyl)-cyclohexanone. Reduction of the keto-groups could also be inferred from the ultra-violet spectrum of the reaction mixture, in which the peak at 270 mμ, initially present, had disappeared. It also had to be established whether the mixture of products obtained from 2-(penta-acetyl-D-gluconyl)-cyclohexanone with sodium borohydride was merely several partially deacetylated, reduced derivatives, or whether any cleavage of the molecule had taken place. Ionophoresis in phosphate solution indicated at least partial cleavage to 2,3,4,5,6-penta-O-acetyl-D-gluconic acid, $\underline{M_{GA}}$(P) = 0.73, and a presumably partially deacetylated acid $\underline{M_{GA}}$(P) = 0.86, though

there was some non-migrating, i.e. neutral material. Clearly this method of reduction was not ideal.

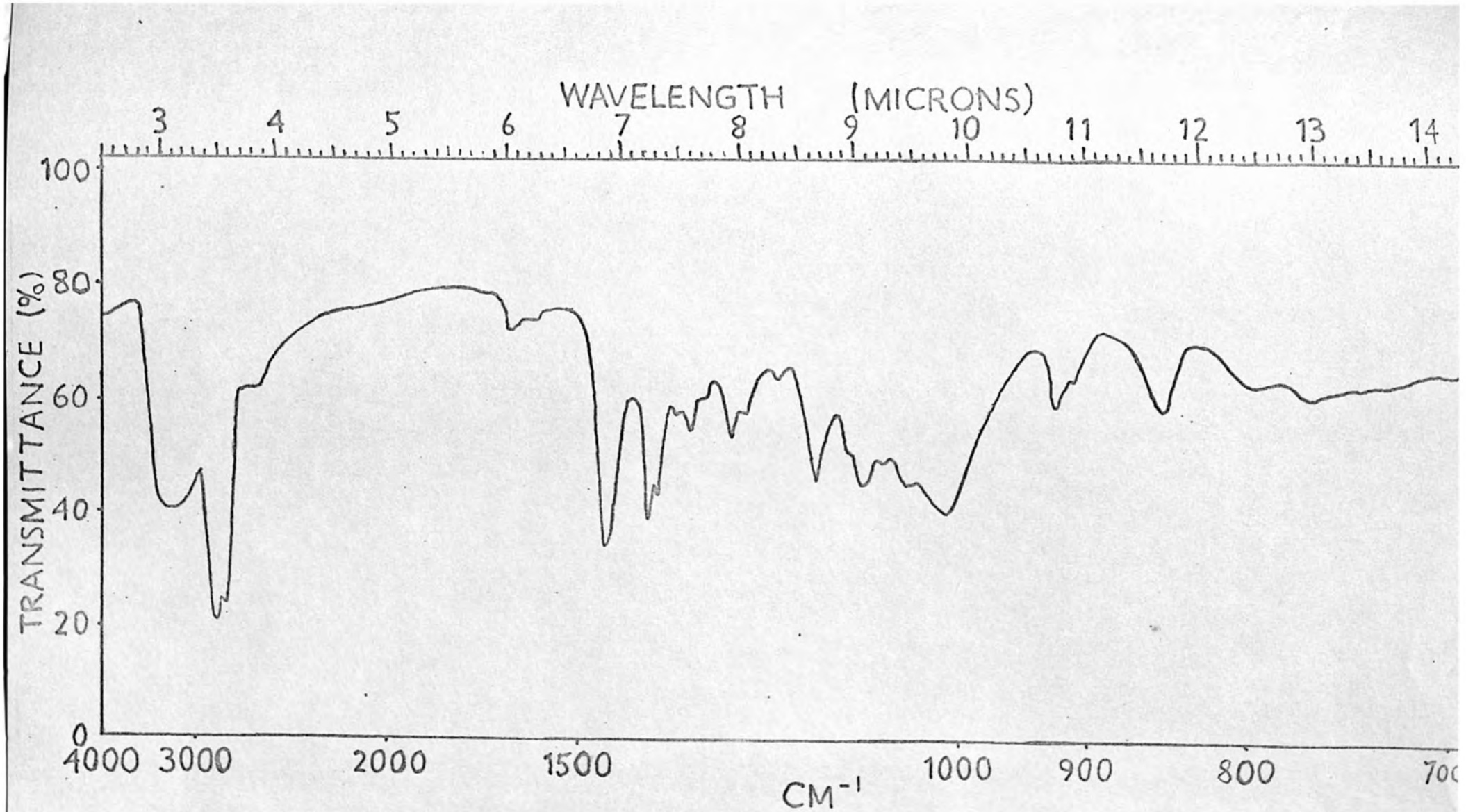
In order to avoid as far as possible any alkalinity before the reduction of the carbonyl groups had taken place, it was decided to employ lithium aluminium hydride in diethyl ether as the reducing agent, although it was realised that the acetyl groups would be removed according to the following equation[149]:

$$2RCOOR' + LiAlH_4 \rightarrow (RCH_2O)_2(R'O)_2LiAl \xrightarrow{H_2O} 2RCH_2OH + 2R'OH + LiAlO_2$$

eqn. V. 30.

This complex hydride had been used successfully by Ness, Fletcher and Hudson[150] to obtain L-glucitol from 2,3,5,6-tetra-O-acetyl-D-gulono-γ-lactone. In their treatment, the lithium aluminium hydride is suspended in dry ether, and the reducible material, in dry ether, is added slowly. An insoluble complex is formed which is hydrolysed by water. The released hexitol is obtained as a syrup, by concentration of the aqueous layer, after deionisation.

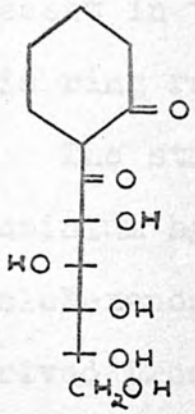
The method was applied to 2-(penta-acetyl-D-gluconyl)-cyclohexanone. [Expt. 73]. After aqueous hydrolysis and ethereal extraction, only a very small amount of syrupy material (B) was obtained. (Most of the complex resisted hydrolysis). The syrupy material (B) after recrystallisation



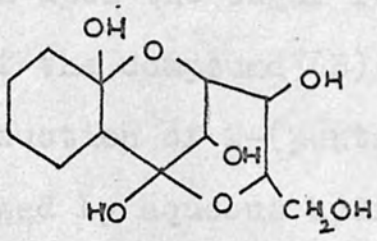
Infra-red spectrum of material B isolated from ether layer after lithium aluminium hydride reduction of 2-(penta-acetyl-D-gluconyl)cyclohexanone, in Nujol.

from n-butanol, melted at 212.5°. It stained with p-anisidine hydrochloride and yielded a 2,4-dinitrophenyl-hydrazine derivative melting at 83-85°. From this evidence it was apparent that at least one of the carbonyl groups was not reduced by lithium aluminium hydride. The infra-red spectrum of the product (B), however, showed virtually no carbonyl absorption, indicating that it was fully deacetylated and suggesting that it may have a hemi-acetal structure, cyclisation having occurred with one of the hydroxyl groups liberated by the above treatment. According to the above evidence there are several possible structures for compound (B). [XV - XXIII; molecular models show that other hemi-acetal modifications of XV containing three fused rings are unlikely]. The spectrum shows relatively strong absorption at 855 cm^{-1}, 910 cm^{-1} and 925 cm^{-1}. All the common sugars having a cyclic structure, and their derivatives, display an absorption[151] at 929 ± 15 $cm.^{-1}$. According to the studies of Barker *et al.*[152-155] it can be assigned in the pyranoses to a ring vibration which includes a considerable contribution from the ring C-O-C antisymmetrical stretching.

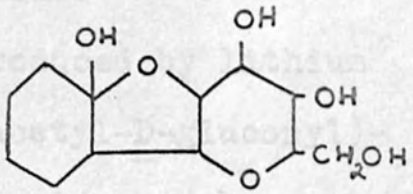
Compounds containing a furanose ring exhibit an absorption[155] at 858 ± 7 cm^{-1}, but other absorptions normally associated with a furanose ring[154], i.e.



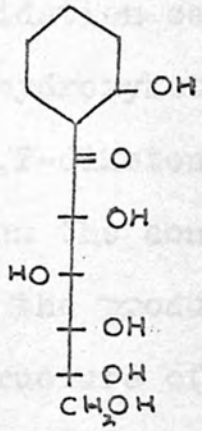
XV



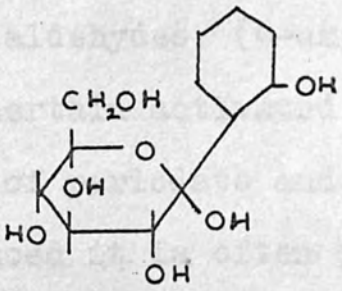
XVI



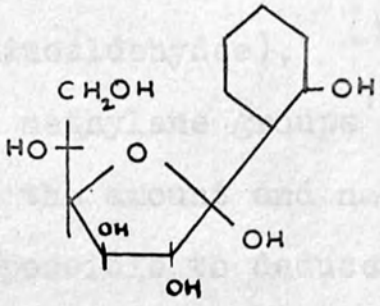
XVII



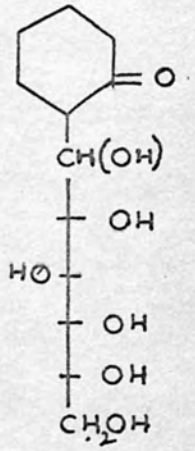
XVIII



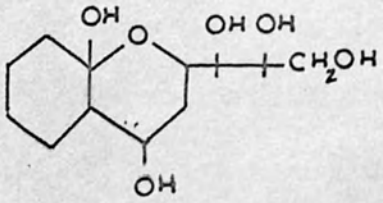
XIX



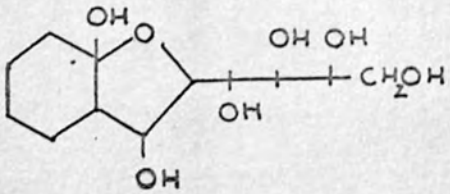
XX



XXI



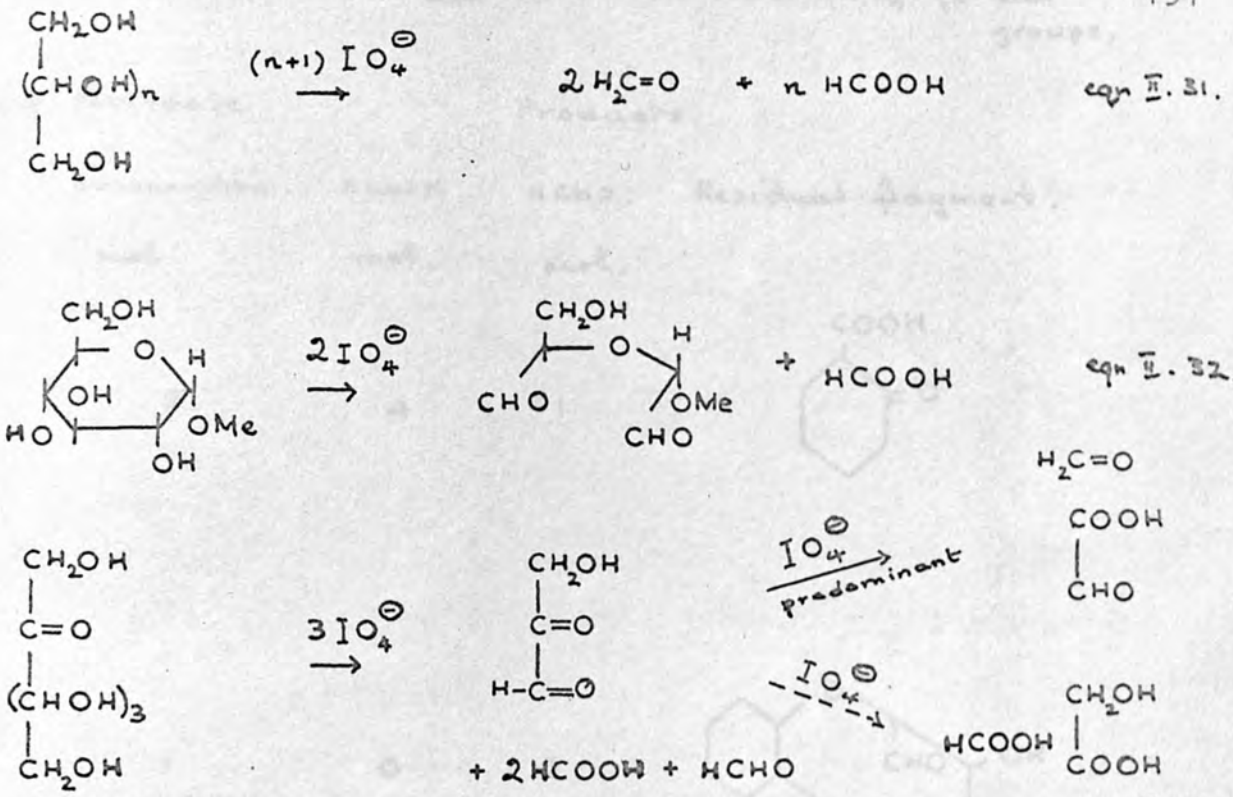
XXII



XXIII

879 ± 7 cm^{-1}, 799 $cm.^{-1}$ are absent from the spectrum of compound (B). The peak occurring at 910 $cm.^{-1}$ is also present in the spectrum of cyclohexanone and may depend upon this ring rather than upon the sugar residue.

The structure of the compound (B) produced by lithium aluminium hydride reduction of 2-(penta-acetyl-D-gluconyl)-cyclohexanone, followed by aqueous hydrolysis, can be derived from a study of its oxidation with sodium meta-periodate, When applied under the proper conditions, the oxidation can be limited to 1,2-glycols, (2-aminoalcohols), α-hydroxyketones and aldehydes, (α-aminoaldehydes), (1,2-diketones) and certain activated methylene groups[156]. From the consumption of periodate and the amount and nature of the products produced it is often possible to deduce the structure of the original polyhydroxy-material. The following equations[156] illustrate the type of reaction which may be involved:-



eqn II. 31.

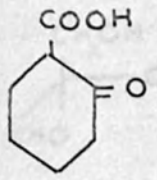
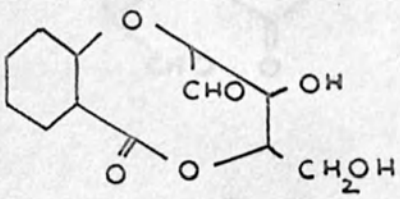
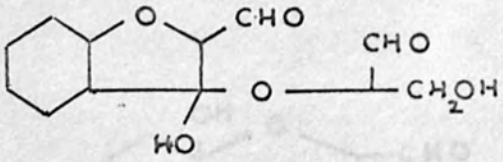
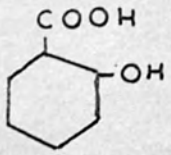
eqn II. 32.

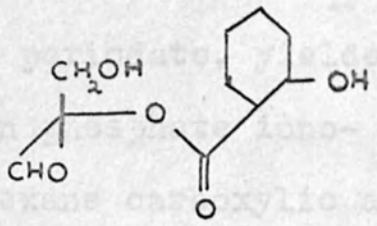
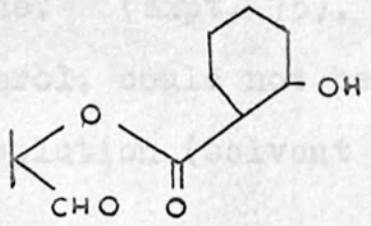
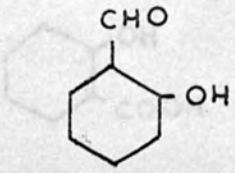
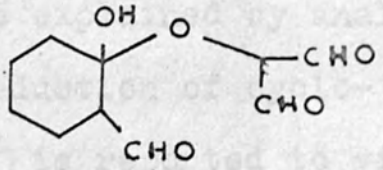
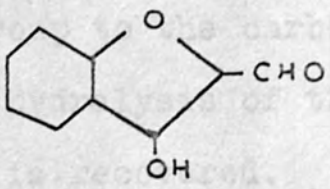
eqn II. 33.

Table 2 shows the expected results of periodate oxidation of compounds with Structures XV - XXIII, when the oxidation is limited to the cleavage of 1,2-diol groupings. In fact, compound (B) consumed fairly rapidly ca. 3 mol. of periodate with the concomitant formation of ca. 2 equivalents of acid. No formaldehyde was produced. [Expt. 74]. According to Table 2 the formation of acids other than formic acid (either directly or after hydrolysis of esters) is accompanied by a periodate consumption > 3 mol. and/or formation of formaldehyde. Thus compound (B) is 1-C-(2'-hydroxycyclohexyl)-D-glucopyranose (XIX). The configuration of the anomeric carbon atom was, however, not ascertained.

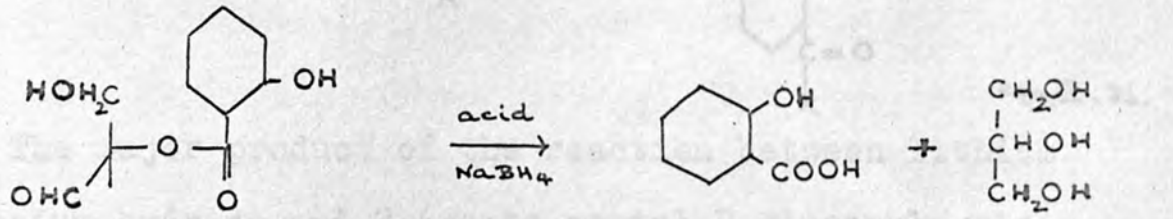
Table 2.

Periodate oxidation of XV - XXIII limited to cleavage of 1,2-diol groups.

Compound	Periodate consumption. mol.	Products. HCOOH mol.	HCHO. mol.	Residual fragment.
XV	5	4	1	
XVI	1	0	0	
XVII	1	0	0	
XVIII	5	4	1	

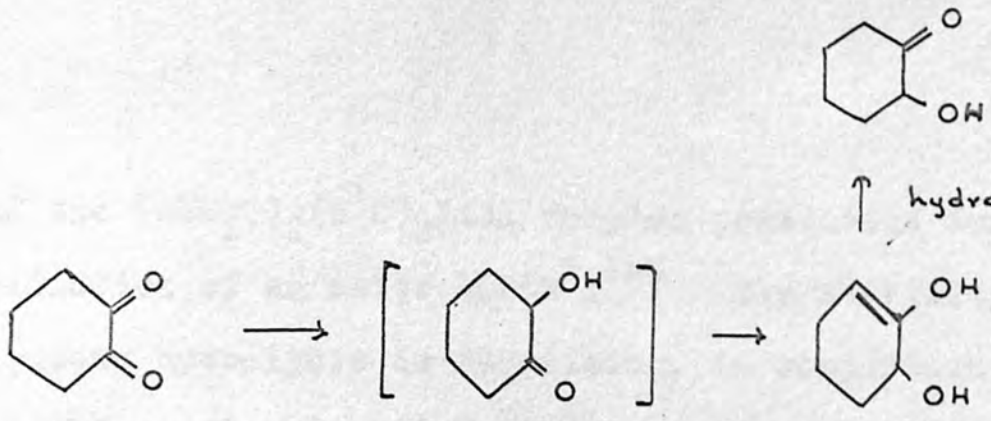
Compound	Periodate Consumption mol.	Products. HCOOH mol.	HCHO mol.	Residual fragment
XIX	3	2	0	
XX	3	1	1	
XXI	5	4	1	
XXII	3	1	1	
XXIII	3	2	1	

The residual fragment of compound (B) was not fully investigated, though acid hydrolysis followed by boro-hydride reduction of the remaining mixture, after deionisation to remove the unreacted periodate, yielded a trace of substance which behaved on phosphate iono-phoresis as did cis-2-hydroxy-cyclohexane carboxylic acid prepared previously from cyclohexanone. (Expt. 75). But the other expected product, glycerol, could not be detected chromatographically in the solution (solvent 1).



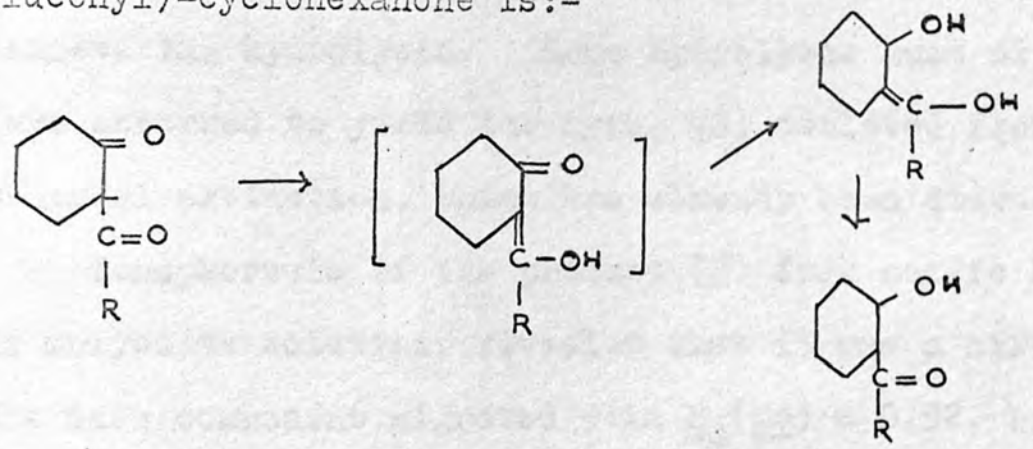
eqn II. 34.

The production of a monoketo compound on reduction with lithium aluminium hydride may be explained by analogy with the lithium aluminium hydride reduction of cyclo-hexane-1,2-dione[157,158], which at 35°. is reported to yield 41% of 2-hydroxy-cyclohexanone and 1 mole of hydrogen gas. The dione is believed to react in the form of the enol ketone, with reduction of the keto group to the carbinol and retention of the enol form. On hydrolysis of the reduction mixture, the keto-carbinol is recovered.



eqn. II. 35.

An analogous series of reactions for 2-(penta-acetyl-D-gluconyl)-cyclohexanone is:-



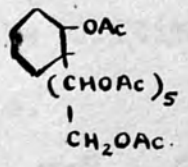
eqn. II. 36.

The major product of the reaction between lithium aluminium hydride and 2-(penta-acetyl-D-gluconylcyclohexanone was a white solid (C), which contained lithium and aluminium. [Expt. 73]. The material dissolved, with considerable effervescence, in dilute hydrochloric acid. After removal of the lithium and aluminium ions by treatment with Amberlite IR 120 (H^+) resin; and precipitation of the chloride as silver chloride by the addition of silver carbonate, the solution can be evaporated to yield a syrup (D) [Expt. 76]. The white solid (C) was presumably a complex between the inorganic salts and the polyhydroxy-compound, something of the form

of the $(RCH_2O)_2(R'O)_2LiAl$ complex postulated for the reduction of an ester RCOOR'.[149] The stability towards aqueous hydrolysis is surprising, in comparison to that of the complex formed during the reduction of 2,3,5,6-tetra-O-acetyl-D-gulono-γ-lactone[150]. where an hour's treatment with water at room temperature was adequate to achieve the hydrolysis. Some hydrolysis must of course have occurred to yield the syrup (B) isolated from the ethereal extraction, which has already been discussed.

Ionophoresis of the product (D) from acidic hydrolysis, in molybdate solution, revealed that it was a mixture. The main component migrated with $M_S(Mo) = 0.92$, but there were other trace components. Treatment of a sample of the mixture with sodium borohydride reduced the number of products to a major component (E) with $M_S(Mo) = 0.91$ and a trace component with $M_S(Mo) = 0$.

The fully reduced product (E) was isolated, as a crystalline heptaacetate (XXV) trihydrate (F). (Expt. 76). (The carbon and hydrogen analysis agrees well with trihydrate, but the acetyl determination corresponds to a monohydrate).

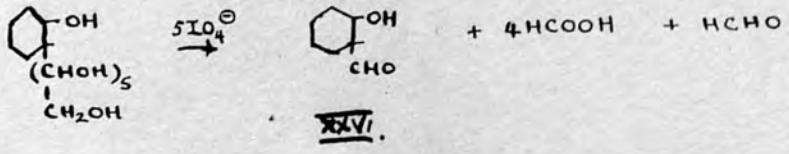


XXV

The relative configurations of the acetyl groups on the new asymmetric carbon atoms have not been established, and (F) may be a mixture of stereoisomers.

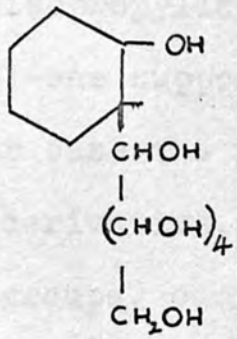
The deacetylated material could not be obtained crystalline, but, as a freeze dried syrup (G), was subjected to periodate oxidation [Expt. 77]. It consumed 5 mol. of periodate and ca. 4 mol. formic acid were produced. The formaldehyde liberated was estimated with chromotropic acid, to avoid, if possible, interference from the 2-hydroxy-cyclohexane aldehyde produced. As the colorimetric determination gave an unrealistic value for the formaldehyde, ca. 60 mol., the second aldehyde (XXVI) produced according to the equation below obviously interfered. It was not known whether (XXVI) would steam distil. However formaldehyde was driven out of the solution by gentle heating and trapped in cooled water. The concentration of aldehyde in this solution corresponded to the formation of ca 1 mol. formaldehyde from compound (G).

The residual reaction mixture, from which the formaldehyde had been removed, was treated with dimedone. A compound (H) was obtained which melted at 155° and depressed the melting point of the dimedone derivative of formaldehyde.



XXVI.

The above results show that compound (G) is a 1-C-(2'-hydroxy-cyclohexyl)-hexitol, (XXVII), in which C_2 - C_5 have the gluco-configuration.

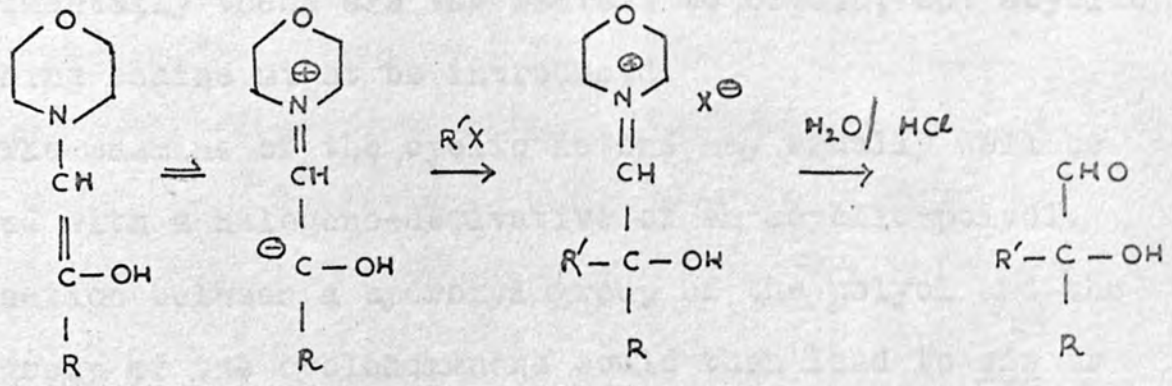


XXVII

(v) Conclusion.

From the experimental data accumulated, it can be concluded that the acylation of an enamine of a cyclic ketone with the acetate of an aldonic acid chloride successfully produces a ketose (as its acetate) in which the alicyclic ring is linked by a carbon-carbon bond to the anomeric carbon atom. The dicarbonyl compounds produced show an interesting fission reaction under mildly alkaline conditions, the mechanism of which deserves further study. Although so far only the D-gluconyl derivative has been synthesised, the method could appropriately be applied to form for example mannonyl, galactonyl, arabinonyl and glyceryl derivatives.

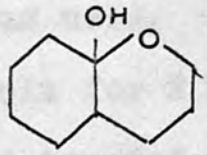
It should also be possible to prepare derivatives of carbohydrates by the condensation of an aldehydo-sugar with an enamine of cyclohexanone. Tentative investigations with monoisopropylidene-D-glyceraldehyde and 1-morpholino-cyclohex-1-ene support this suggestion, though in this particular case the ready dimerisation of the glyceraldehyde derivative, despite the protection of the hydroxyl groups, complicates the desired reaction sequence. An alternative study would be to form the enamine of an aldehydo-sugar and then react this with an alkyl halide to produce branching at C_2.



The alkylation of an enamine by halide can be further developed. An interesting reaction to pursue would

be the treatment of an enamine of cyclohexanone with a sugar halide, such as acetobromoglucose. The major product should be a C_1-carbon glycoside in which the aglycone is of a size sufficient to affect considerably the normal conformation of the pyranose ring. The synthetic method is not limited to a C_1 halide. Branching could theoretically be introduced at any point in the sugar ring where a halogen atom can be substituted. An example which could be investigated is 1,2,3,4-tetra-O-acetyl-6-iodo-D-glucopyranose. Furthermore, the enamine need not be derived from a cyclic ketone, though experimentally these are the easiest to obtain, but acyclic branching chains might be introduced.

The enamine of the cyclic ketone may equally well be treated with a halogeno-derivative of an acyclic polyol. Cyclisation between a hydroxyl group of the polyol and the keto-group of the cyclohexanone could then lead to cis or trans-decalin-type systems of the following fundamental structure:-



In fact, the reaction of enamines with carbohydrate derivatives is capable of considerable variation and could well develop into a fascinating field of study.

Experimental

1. Ionophoresis.

The Shandon High Voltage Electrophoresis apparatus was used. Unless otherwise stated, Whatman No.3 chromatography paper, width 11 cm., was employed. The polyol samples were applied to the paper in 2% solution in aqueous methanol.

Basic Copper Acetate Electrolyte.

B.D.H. technical grade basic copper acetate (50 g.) was dissolved in water (1.l.) by shaking (18 hr. room temp.) to give a solution of pH 5.1 - 5.3. Undissolved material was removed by centrifugation (0.5 hr.) Ionophoresis was carried out for 2 hr. at 4000 volt. The paper was dried in an oven at 120-130° and treated with spray 1. (see below). The non-migrating marker used to correct for electroendosmosis was 5-hydroxymethyl-furfural.

Sodium metavanadate Electrolyte.

A 1.5% solution of sodium metavanadate ($NaVO_3$) in water was used. Ionophoresis was carried out at 3,000 volt for 2 hr. The paper was dried in an oven at 120° and treated with spray 1. (see below). The non-migrating marker was 5-hydroxymethylfurfural.

Sodium molybdate and tungstate electrolytes.

1.5% aqueous solutions of sodium molybdate dihydrate or sodium tungstate dihydrate were adjusted to pH 5.0 by dropwise addition of concentrated sulphuric acid. Ionophoresis was carried out at 4,000 volt for 0.75 hr. The paper was dried at 120-130° and treated with spray 2. (see below). The non-migrating marker was glycerol.

2. Paper chromatography.

Solvent system A: butanol, ethanol, water; 40:11:19 v/v 86

Solvent system B: ethyl acetate, acetic acid, water; 9:2:2 v/v 87

Solvent system C: butanol, acetone, water; 5:3:2 v/v 88

3. Staining Reagents.

Reagent 1: A freshly prepared saturated solution of potassium permanganate in acetone. Sugars and polyols appear as yellow spots on a purple background and fade rapidly[89].

Reagent 2: The dried paper is sprayed with a) a solution of saturated aqueous silver nitrate (2.5 ml.) in acetone (500 ml.) then b) a solution of sodium hydroxide (10g) dissolved in water (50 ml.) and added to methanol (500 ml.). Sugars and polyols appear as brown spots on a yellow background and are fixed by dipping in a dilute solution of ammonia[90].

1 ml. of the D-glucitol solution was added to 10 ml. of the basic copper acetate solution, the pH adjusted to the required value by the addition of anhydrous sodium acetate or dilute hydrochloric acid and the resultant solution made up to 25 ml. with distilled water. The optical rotation was measured at 5461 mμ. The specific rotation $[\alpha]^{20}_{5461}$ is based on the concentration of D-glucitol.

pH of basic copper acetate solution containing D-glucitol.		$[\alpha]^{20}_{5461}$
4.45		16.15°
4.70		21.13°
4.90		22.71°
5.00	HCl added.	44.74°
5.10		56.67°
5.20		49.72°
5.30		58.66°
5.60		54.30°
6.00	Sodium acetate added.	60.6°
7.00		56.7°
Precipitation of copper hydroxide		-

Expt. 2. The effect of pH on the ionophoretic mobility of D-glucitol in sodium orthovanadate solution.

A 1.5% aqueous solution of sodium orthovanadate, $Na_3VO_4.14H_2O$, was prepared. The ionophoretic mobility of D-glucitol in this electrolyte, calculated from the relation

$$\text{mobility} = \frac{\text{distance moved by compound}}{\text{potential gradient} \times \text{time in sec.}} \text{ cm} \quad \text{cm}^2\text{sec}^{-1}\text{v}^{-1}$$

was measured at different pH values, the pH being adjusted by the dropwise addition of concentrated sulphuric acid. Ionophoresis was carried out for 1 hr. at 4,000 volt. using Whatman No.1 chromatography paper, width 11 cm. Glycerol was used as the non-migrating marker.

pH	Ionophoretic mobility of D-glucitol
12.3	1.04×10^{-5} $cm^2 sec.^{-1} v.^{-1}$
12.0	1.6×10^{-5} "
10.7	2.5×10^{-5} "
10.0	2.76×10^{-5} "
9.2	2.98×10^{-5} "
8.2	3.06×10^{-5} "
6.6	2.4×10^{-5} "
6.0	0.9×10^{-5} "

Expt. 3. The effect of pH on the ionophoretic mobility of D-glucitol in sodium metavanadate solution.

Method as for expt. 2 except that the pH values greater than 8.7 were attained by dropwise addition of 2N-sodium hydroxide, and Whatman No.3 paper was used.

pH	Ionophoretic mobility of D-glucitol.
10.7	3.4×10^{-5} cm.^{2}sec.$^{-1}$V.$^{-1}$
10.4	3.9×10^{-5} "
9.6	3.7×10^{-5} "
9.0	4.6×10^{-5} "
8.7	4.4×10^{-5} "
8.0	5.0×10^{-5} "
7.6	4.7×10^{-5} "
7.0	4.1×10^{-5} "
6.4	2.7×10^{-5} "

Expt. 4. Effect of pH on the optical rotation of D-glucitol in the presence of sodium metavanadate, $NaVO_3$

Aqueous solutions of D-glucitol (10%) and sodium metavanadate (5%) were prepared. Aliquots of the meta-

vanadate solution (15 ml.) and the D-glucitol solution (2 ml.) were mixed, the pH adjusted by the dropwise addition of concentrated sulphuric acid, and the resultant solution made up to 100 ml. with distilled water. The optical rotations of D-glucitol in the presence of sodium metavanadate at different pH values were measured at 5890 mμ.

pH	$[\alpha]_D^{20}$
7.90	45°
7.50	70°
7.40	105°
6.55	290°
5.90	395°
5.60	350°
5.30	260°
5.0	173°
4.6	155°
3.6	125°

Expt. 5. The Effect of pH on the optical rotation of D-glucitol in the presence of sodium orthovanadate, $Na_3VO_4\ 14H_2O$.

0.25 M-aqueous solutions of sodium orthovanadate (5.4494 g. 100 ml.) and D-glucitol (4.5545 g. 100 ml.)

were prepared. Sodium orthovanadate solution (7 ml) and D-glucitol solution (1 ml.) were mixed and the pH adjusted to different values with N-sulphuric acid, the resulting solutions being made up to 10 ml. with water. The optical rotations were measured at 5890 mμ.

pH	$[\alpha]_D^{20}$	pH	$[\alpha]_D^{20}$
12.8	0	6.6	131.6°
11.1	0	6.0	219.3°
10.4	0	5.8	210.5°
9.1	0	4.8	140.3°
7.9	8.7°	4.0	127.2°
		2.7	105.2°
7.2	26.3°	1.4	8.7°

Expt. 6. The effect of the addition of alkali on an acidified mixture of D-glucitol and sodium metavanadate solutions.

Aqueous solutions of D-glucitol (10%) and sodium metavanadate (5%) were prepared. For each reading, D-glucitol solution (1 ml.) and sodium metavanadate solution (7.5 ml.) were mixed and the pH adjusted to 2.1 with concentrated sulphuric acid. Mixtures of different pH were obtained by the addition of N-sodium hydroxide and the resulting solutions made up to 10 ml.

with distilled water. The optical rotation of each solution, based on D-glucitol, was measured.

pH	$[\alpha]_D^{20}$	pH	$[\alpha]_D^{20}$
9.6	160°	4.9	164°
7.8	180°	3.5	132°
6.6	192°	2.1	94°
6.1	216°		
6.0	216°		
5.9	216°		

Expt. 7. The effect of the relative concentration of sodium metavanadate on the optical rotation of D-glucitol.

Aqueous solutions of D-glucitol (10%) and sodium metavanadate (5%) were prepared. A constant volume (2 ml) of the D-glucitol solution was mixed with varying volumes of sodium metavanadate solution, the pH being adjusted to 5.9 ± .05 by the dropwise addition of N-sulphuric acid. The pH was noted after the solution had been made up to the total volume (100 ml) and was adjusted where necessary by a drop of acid, the volume error thus introduced being considerably less significant than errors due to a variation in pH. The optical

rotations of the solution, based on the concentration of D-glucitol, were measured.

V/P ratio	$[\alpha]_D^{20}$
0.37	22.5°
0.74	65°
1.11	145°
1.48	190°
1.85	215°
2.28	275°
3.70	390°
5.55	425°
6.65	425°
7.4	420°

Values derived from graph obtained (for explanation of symbols, see discussion).

V/P	limiting $[\alpha]_D^{20}$	$[\alpha]_D^{20}$	$\alpha' = \frac{[\alpha]_D^{20}}{\lim[\alpha]_D^{20}}$	$\frac{1}{\alpha'}$	$2\alpha'$	$(v - \alpha')(v - 2\alpha')$	$\log (v-\alpha')$	$\log (v-2\alpha')(\frac{1}{\alpha'}-1)$	$\log (\frac{1}{\alpha'}-1)$
	425°								
0.25		32°	0.075	13.3	0.15	0.185	0.10	-0.7328 -1	1.0899
0.50		56°	0.132	7.6	0.26	0.37	0.24	-0.4318 -0.6198	0.8195
1.00		115°	0.271	3.64	0.54	0.73	0.46	-0.1367 -0.3372	0.4298
1.50		168°	0.395	2.53	0.79	1.1	0.71	0.0414 -0.1487	0.1847
2.00		235°	0.553	1.81	1.01	1.45	0.99	0.1614 -0.0044	-0.0915
2.50		290°	0.683	1.46	1.37	1.82	1.13	0.2601 0.0531	-0.3372
3.00		330°	0.777	1.28	1.55	2.22	1.45	0.3464 0.1614	-0.5528
3.50		370°	0.871	1.15	1.74	2.63	1.76	0.4200 0.2455	-0.8239
4.00		390°	0.917	1.09	1.83	3.08	2.17	0.4886 0.3365	-1.0458
4.50		405°	0.953	1.05	1.91	3.55	2.59	0.5502 0.4133	-1.3837
5.00		410°	0.965	1.03	1.93	4.03	3.07	0.6053 0.4871	-1.5229

Expt. 8. Effect of pH on the optical rotation of D-mannitol in the presence of sodium metavanadate, $NaVO_3$.

An 0.1M-aqueous solution of D-mannitol and sodium metavanadate (3.0485g. sodium metavanadate, 4.5545g. D-mannitol 25 ml. water) was prepared and acidified by the addition of known volumes concentrated sulphuric acid to different pH values. The optical rotation, based on the concentration of D-mannitol, was found at 5890 mμ.

pH	$[\alpha]_D^{20}$
8.4	28.2
7.6	28.8
6.6	28.4
6.25	29.5
6.0	30.2
5.7	31.2
5.0	27.7
4.5	too strongly coloured.

Expt. 9. Effect of pH on the optical rotation of D-mannitol in the presence of sodium orthovanadate $Na_3VO_4 14H_2O$

A 10% aqueous solution of D-mannitol and a 7.6% aqueous solution of sodium orthovanadate were prepared. Aliquots of

the D-mannitol solution (2 ml.) and the orthovanadate solution (10 ml.) were mixed, the pH adjusted by the addition of concentrated sulphuric acid and the solution made up to 25 ml. with water. The optical rotation, based on the concentration of D-mannitol, was found at 5890 mμ .

pH	$[\alpha]_D^{20}$
12.1	0
10.6	0
9.3	1.6
8.1	1.3
7.4	1.3
6.5	3.9
5.9	4.4
5.3	2.5
2.9	0

Expt. 10. The effect of relative concentration of sodium metavanadate on the optical rotation of D-mannitol.

Aqueous solutions of D-mannitol (10%) and sodium metavanadate (5%) were prepared and the experiment performed as for D-glucitol (Expt. 7).

V/P	$[\alpha]_D^{20}$
0.19	5.0°
0.37	11.3°
0.74	20.0°
1.11	22.5°
1.48	23.1°
1.85	24.4°
2.22	27.2°
2.96	27.5°
3.33	26.8°

Values derived from the graph, for explanation of symbols see text

V/P	lim. $[\alpha]_D^{20}$	$[\alpha]_D^{20}$	α'	$1/\alpha'$	$(v-\alpha')$	$\log (v-\alpha')$	$\log (1/\alpha'-1)$
0.25	27.5°	8°	0.29	3.49	-0.04	-	-
0.50		14°	0.51	1.96	-0.01	-	-
0.75		19°	0.69	1.45	+0.06	-1.2218	-0.35
1.00		21.6°	0.77	1.30	0.23	-0.6383	-0.52
1.50		24°	0.87	1.15	0.63	-0.2007	-0.82
2.00		25.2°	0.92	1.09	1.08	+0.0334	-1.04
2.50		26.2°	0.96	1.04	1.54	0.1875	-1.40

Expt. 11. The derivation of the ratio of vanadium to D-glucitol from a polarimetric continuous variation plot.

0.2M-aqueous solutions of D-glucitol and sodium metavanadate were prepared. Different volumes of these two solutions were mixed such that the total volume was 20 ml. and the pH was adjusted to 5.9±0.05 with N-sulphuric acid. The resulting solution was diluted to 25 ml. with distilled water and the pH again adjusted where necessary. The polarimeter reading minus zero correction was plotted against the mole fraction of vanadate.

mole fraction of vanadate	corr. polarimeter reading	mole fraction of vanadate	corr: polarimeter reading
1.0	0	0.575	0.56
0.95	0.10	0.50	0.58
0.90	0.23	0.40	0.43
0.85	0.53	0.325	0.34
0.80	0.57	0.25	0.20
0.75	0.62	0.15	0.06
0.625	0.62	0.05	0.01
0.60	0.69		

Expt. 12. Derivation of the vanadium : D-mannitol ratio from a polarimetric continuous variation plot.

0.5M-aqueous solution of D-mannitol (9.109g./100 ml.) and sodium metavanadate (6.087g./100 ml.) were prepared. The method was thenceforth the same as for Expt. 9.

mole fraction of vanadate	corr. polarimeter reading
0.9	0.12
0.75	0.60
0.60	0.94
0.50	1.00
0.40	0.96
0.25	0.68
0.10	0.26

Expt. 13. The derivation of the ratio of vanadium : maltitol by a polarimetric continuous variation plot.

Maltitol was prepared by the borohydride reduction of maltose:- To maltose (5g.) dissolved in water (50 ml.) was added poassium borohydride (0.5g.) and the solution was left to stand (18 hr.). The solution was treated with Amberlite IR 120(H^+) resin to remove the potassium ions, then evaporated to dryness several times with methanol. The maltitol was freeze-dried and was shown to be uncontaminated with maltose by ionophoresis in sodium molybdate solution.

0.5M-aqueous solutions of maltitol (0.86g./50 ml.) and sodium metavanadate (0.6087 g./100 ml.) ware prepared. Aliquots of these solutions were taken such that the sum of the volumes was 20 ml., the mixture adjusted to pH 5.9 with 0.1M-sulphuric acid, and made up to 25 ml. with water. The differences in polarimeter readings between such solutions and solutions containing the same concentration of maltitol alone were plotted against the mole fraction of vanadate.

Mole fraction of vanadate	Difference in polarimeter readings
0.3	0.09
0.4	0.12
0.5	0.14
0.6	0.11
0.7	0.10
0.8	0.07

Expt. 14. The effect of pH on the optical rotation of D-glucose in the presence of sodium metavanadate.

0.5M-aqueous solutions of glucose and sodium metavanadate were prepared. Constant volumes of the sodium metavanadate (5 ml.) and glucose (1 ml.) solutions were mixed and made up to 10 ml. after acidification to different pH values with

concentrated sulphuric acid. The optical rotations of the solutions, based on D-glucose were measured.

pH	$[\alpha]_D^{20}$
8.0	54.4°
7.2	55.5°
6.5	54.0°
6.0	55.5°
4.6	55.5°
2.5	54.4°

Expt. 15. The effect of pH on the optical rotation of maltose in the presence of sodium metavanadate.

0.2M-aqueous solutions of maltose (7.206g./100 ml.) and sodium metavanadate (2.44g./100 ml.) were prepared. Volumes of the maltose solution (5 ml.) and metavanadate solution (15 ml.) were mixed, acidified to different pH values with N-sulphuric acid and made up to 25 ml. The optical rotations of the resultant solutions were found.

pH	$[\alpha]_D^{20}$	pH	$[\alpha]_D^{20}$
11.9	128.3°	6.2	136.8°
9.9	134.6°	6.1	137.4°
9.0	136.8°	5.8	134.6°
7.6	138.1°	5.0	131.8°
6.8	137.4°	4.0	130.5°
6.5	140.2°	maltose alone	127.6°

Expt. 16. The derivation of the ratio of vanadium : maltose from a polarimetric continuous variation plot.

An 0.1M-aqueous solution of maltose (3.64g./100 ml.) and an 0.4M-aqueous solution of sodium metavanadate (4.88g./100 ml) were prepared. Volumes of these solutions were taken such that the sum of the volumes was 20 ml., the mixtures acidified to pH 6.1 by dropwise addition of concentrated sulphuric acid, and the resultant solutions made up to 25 ml. with distilled water. The differences in polarimeter readings between such solutions and solutions containing the same concentration of maltose alone were plotted against the mole fraction of vanadate.

mole fraction of vanadate	Difference in polarimeter readings
0.8	0.18
0.73	0.22
0.84	0.16
0.63	0.23
0.50	0.20
0.31	0.15
0.94	0.07
0.90	0.09

Expt. 17. The derivation of the vanadium : D-ribose ratio from a polarimetric continuous variation plot.

0.3M-aqueous solutions of ribose (4.504g./100 ml.) and sodium metavanadate (3.66g./100 ml.) were prepared and the experiment performed as for maltose (Expt. 16).

mole fraction of vanadate	Difference in polarimeter readings
0.9	0.025
0.6	0.175
0.5	0.21
0.4	0.25
0.3	0.265
0.2	0.195
0.1	0.09

Expt. 18. Ultra-violet and visible spectra of sodium metavanadate in the presence of D-glucitol.

0.2M-aqueous solutions of sodium metavanadate and D-glucitol were prepared in sodium acetate buffer at pH 5.9. Sodium metavanadate (12 ml.) was mixed with D-glucitol (8 ml.) and the resulting solution made up to 25 ml. with acetate buffer. The ultra-violet and visible spectra were examined using a freshly-prepared mixture, since the mixed solution tended to darken on standing overnight. Standard spectra of sodium metavanadate, D-glucitol and acetate buffer were obtained for comparison. The experiment was repeated in the absence of acetate buffer.

Wavelength in millimicrons	D-glucitol + sodium metavanadate in acetate buffer. Absorbance.	Wavelength in millimicrons	D-glucitol in acetate buffer Absorbance.
200 → 490	1.5	200 → 225	1.5
500	1.48	230	1.04
510	1.45	240	0.60
520	1.42	250	0.35
530	1.3	270	0.31
540	1.08	280	0.31
550	0.84	290	0.31
560	0.60	200	0.26
570	0.44	310	0.16
580	0.30	320	0.09
590	0.22	330	0.05
600	0.15	350	0.00
650	0.03	et seq.	0.00

Wavelength in millimicrons.	D-glucitol + sodium metavanadate. After standing and darkening in acetate buffer. Absorbance.	Sodium metavanadate in water. Absorbance.	Sodium metavanadate + D-glucitol in water. Absorbance.
200 → 490	1.5	1.5	1.5
500	1.25	1.5	1.5
510	1.0	1.5	1.5
520	0.77	1.03	1.5
530	0.56	0.55	1.5
540	0.37	0.25	1.2
550	0.37	0.11	0.85
560	0.30	0.04	0.60
570	0.24	0.01	0.40
580	0.20	0.00	0.23
600	0.15	0.00	0.12
620	0.15	0.00	0.05
650	0.15	0.00	0.00

Expt. 19. Investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of D-mannitol.

0.2M-aqueous sodium metavanadate (10 ml.) was mixed with 0.2M-aqueous D-mannitol (10 ml.) and the resulting solution adjusted to pH 6.0 by dropwise addition of M-sulphuric acid and made up to 25 ml. with water. The visible spectra

of this solution and the corresponding solution of sodium metavanadate alone were found.

Wavelength in millimicrons.	Sodium metavanadate + D-mannitol. Absorbance.	Sodium metavanadate. Absorbance.
350	1.48	1.48
400	1.48	1.48
430	1.48	1.48
440	1.41	1.48
450	1.19	1.48
460	0.93	1.15
470	0.70	1.15
480	0.51	0.85
490	0.36	0.59
500	0.25	0.39
510	0.15	0.23
520	0.09	0.12
530	0.05	0.06
540	0.03	0.03
550	0.02	0.02
560	0.01	0.01
570	0.00	0.00

Expt. 20. The investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of galactitol.

The spectra of a mixed solution of 0.2M-sodium metavanadate (5 ml.) and 0.2M-galactitol (5 ml.) made up to 25 ml. with water, the pH having been adjusted to 5.9 by the dropwise addition of N-sulphuric acid, and of the corresponding solution of sodium metavanadate alone were traced.

Wavelength in millimicrons	Sodium metavanadate and galactitol. Absorbance.	Sodium metavanadate Absorbance.
480	1.5	1.5
490	1.5	1.5
500	1.5	1.3
510	1.18	0.85
520	0.89	0.60
530	0.70	0.44
540	0.55	0.35
550	0.45	0.30
560	0.38	0.28
570	0.31	0.26
580	0.31	0.26

/contd.

590	0.29	0.26
600	0.27	0.26
610	0.26	0.25
620	0.25	0.25
630	0.25	0.25

Expt. 21. The investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of maltitol.

The visible spectra of a mixed solution of 0.05 M-sodium metavanadate (2.5 ml.) and maltitol (7.5 ml.) in water (25 ml.) adjusted to pH 5.9 with 0.1 N-sulphuric acid and of the corresponding metavanadate solution alone were found.

&&&&

Wavelength in millimicrons.	Sodium metavanadate and maltitol. Absorbance.	Sodium metavanadate Absorbance.
350	1.48	1.48
370	1.48	1.36
390	1.48	0.94
410	1.28	0.70
430	0.91	0.49
450	0.61	0.33
470	0.37	0.18
490	0.18	0.07
510	0.05	0.00
530	0.00	0.00
550	0.00	0.00

Expt. 22. The Investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of L-arabitol.

The visible spectra of a mixed solution of 0.2M-sodium metavanadate (10 ml.) and 0.2 M-L-arabitol (10 ml.) adjusted to pH 6.0 by dropwise addition of N-sulphuric acid, and of the corresponding solution of metavanadate alone were found.

Wavelength in millimicrons.	Sodium metavanadate and arabitol. Absorbance.	Sodium metavanadate Absorbance.
500	1.48	1.48
510	1.25	1.48
520	0.70	0.90
530	0.35	0.50
540	0.18	0.23
550	0.08	0.10
560	0.04	0.04
570	0.01	0.00
580	0.00	0.00
590	0.00	0.00
600	0.00	0.00

Expt. 23. The investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of Xylitol.

Method as for expt. 22.

Wavelength in millimicrons.	Sodium metavanadate and xylitol. Absorbance.	Sodium meta-vanadate. Absorbance.
500	1.48	1.48
510	1.40	1.48
520	0.80	1.00
530	0.42	0.50
540	0.20	0.25
550	0.08	0.10
560	0.04	0.04
570	0.01	0.01
580	0.00	0.00
590	0.00	0.00
600	0.00	0.00

Expt. 24. The investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of ribitol.

Method as for expt. 22.

Wavelength in millimicrons.	Sodium metavanadate and ribitol. Absorbance.	Sodium meta-vanadate.
500	1.5	1.5
510	1.3	1.5
520	0.82	1.0
530	0.50	0.53
540	0.30	0.28
550	0.23	0.22
560	0.18	0.10
570	0.15	0.08
580	0.13	0.07
590	0.12	0.06

Expt. 25. The investigation of the shift in the absorption edge of a solution of sodium metavanadate caused by the addition of maltose.

The visible spectra of a mixed solution of 0.1 M-sodium metavanadate (10 ml.) and 0.1M-maltose (10 ml.) adjusted to pH 6.2 by dropwise addition of N-sulphuric acid and of the corresponding solution of sodium metavanadate alone were found.

Wavelength in millimicrons.	Sodium metavanadate and maltose.	Sodium metavanadate.
480	1.49	1.49
490	1.23	1.49
500	0.80	1.0
510	0.45	0.55
520	0.25	0.30
530	0.10	0.13
540	0.03	0.04
550	0.00	0.00
560	0.00	0.00
570	0.00	0.00

Expt. 26. The absorptiometric investigation of the vanadium : D-glucitol ratio in the metavanadate complex.

(a) Adjustment of pH before mixing.

0.2 M-solutions of sodium metavanadate and D-glucitol were made up in acetate buffer (0.5 M at pH 5.9). At wavelength 550 mμ the difference in absorption readings between a solution containing D-glucitol and sodium metavanadate, and one containing the same concentration of sodium metavanadate alone was measured. For each determination, different volumes of the D-glucitol and metavanadate were

mixed such that the total volume was 10 ml. and the resulting solution made up to 25 ml. with acetate buffer.

(b) Slight adjustment of pH after mixing.

Method as for (a) except that the pH was adjusted slightly, to 5.8, after mixing, with dilute acetic acid.

(c) No acetate buffer present.

Method as for (a) except that aqueous solutions were prepared and the solution was acidified after mixing by the dropwise addition of N-sulphuric acid.

Mole fraction of vanadate.	Difference in absorption readings. (a)	Difference in absorption readings. (b)	Difference in absorption readings. (c)
0.20	0.03	-	0.13
0.30	0.063	0.25	-
0.40	0.082	-	0.42
0.50	0.15	0.49	0.49
0.60	0.16	0.51	0.65
0.65	-	0.58	0.71
0.70	0.167	0.55	0.71
0.80	0.168	0.462	0.50
0.90	0.134	-	0.28

Expt. 27. Absorptiometric investigation of the vanadium : D-mannitol ratio in the metavanadate complex.

Method as for expt. 26(c) except that the final volume to which the solution was made up was 50 ml. and the wavelength at which the measurements were performed was 460 mμ .

Mole fraction of vanadate	Difference in absorption readings
0.30	0.17
0.40	0.20
0.50	0.22
0.60	0.21
0.70	0.20
0.85	0.12

Expt. 28. Absorptiometric investigation of the vanadium : galactitol ratio in the metavanadate complex.

Method as for 26(c) except that readings were taken at 510 mμ .

Mole fraction of vanadate.	Difference in absorption readings
0.2	0.14
0.3	0.16
0.4	0.22
0.5	0.23
0.6	0.20
0.7	0.15
0.8	0.12

Expt. 29. Absorptiometric investigation of the vanadium : maltitol ratio in the metavanadate complex.

0.025 M-aqueous solutions of maltitol and sodium metavanadate were prepared. Volumes of these solutions were taken such that the sum of the volumes was 20 ml., the pH adjusted to 5.9 with 0.1 N-sulphuric acid and the solution made up to 25 ml. Absorption measurements were performed at 410 mμ .

mole fraction of vanadate.	Difference in absorption readings
0.25	0.16
0.30	0.30
0.40	0.50
0.50	0.68
0.60	0.66
0.80	0.40

Expt. 30. The potentiometric titration of sodium metavanadate with sulphuric acid.

An 0.5 M-aqueous solution of sodium metavanadate (3.05g./50 ml.) was prepared. This solution was titrated potentiometrically with 0.9931 sulphuric acid added from a 10 ml. microburette. The pH was plotted against the ratio H^+/VO_3^- where this ratio was obtained from the relation

$\frac{H^+}{VO_3^-}$ = $\frac{\text{ml. acid added x normality of acid}}{\text{millimoles of } NaVO_3 \text{ present}}$

pH	H^+/VO_3^-	pH	H^+/VO_3^-	pH	H^+/VO_3^-
8.35	0	6.40	0.1392	4.5	0.5232
8.25	0.004	6.35	0.1568	4.1	0.5472
8.15	0.0096	6.35	0.1596	3.8	0.5656
8.0	0.0144	6.30	0.1760	3.1	0.6008
7.75	0.0216	6.30	0.1936	2.8	0.6200
7.5	0.0288	6.30	0.2028	2.5	0.6480
7.1	0.036	6.30	0.2328	2.3	0.6792
6.8	0.0396	6.20	0.2616	2.2	0.7040
6.8	0.0504	6.20	0.2736	2.1	0.7352
6.65	0.0520	6.15	0.2888	2.1	0.7640
6.60	0.0624	6.10	0.3056	2.0	0.7840
6.55	0.0696	6.0	0.3288	1.9	0.8168
6.50	0.0760	6.0	0.3552	1.9	0.8476
6.50	0.0848	6.0	0.3824	1.8	0.8952
6.45	0.0936	5.8	0.4184	1.75	0.9424
6.45	0.1016	5.7	0.4424	1.75	0.9840
6.40	0.1136	5.5	0.4784	1.70	1.0184
6.40	0.1256	5.15	0.5172		

Expt. 31. The potentiometric titration of sodium metavanadate with sulphuric acid in the presence of D-glucitol.

The method was as for expt. 30 except that D-glucitol (2.275 g.) had been added to the solution so that the V/P ratio was 2:1.

pH	H^+/VO_3^-	pH	H^+/VO_3^-	pH	H^+/VO_3^-
8.7	0	6.6	0.1368	5.4	0.4048
8.5	0.0072	6.6	0.1488	5.2	0.4304
8.2	0.0152	6.5	0.1616	5.0	0.4512
7.9	0.024	6.4	0.1744	4.7	0.4824
7.7	0.032	6.35	0.1920	4.5	0.4492
7.5	0.0404	6.3	0.2088	3.8	0.5216
7.4	0.0488	6.15	0.2328	3.3	0.5392
7.25	0.0576	6.10	0.2520	2.9	0.5560
7.15	0.0626	6.0	0.2764	2.7	0.5736
7.05	0.0752	5.85	0.3048	2.55	0.5968
7.0	0.0832	5.75	0.3296	2.30	0.6448
6.9	0.0920	5.6	0.3632	2.10	0.6968
6.9	0.1004	5.6	0.3808	2.00	0.7408
6.8	0.1128				
6.7	0.1244				

Expt. 32. The potentiometric titration of sodium orthovanadate $Na_3VO_4\ 14H_2O$ with sulphuric acid.

An 0.25M-aqueous solution of sodium orthovanadate (5.4494g. 50 ml.) was prepared and the method of expt. 28 was followed except that a 50 ml. burette was used and the H^+/VO_4^{3-} ratio was calculated:-

$$\frac{H^+}{VO_4^{3-}} = \frac{\text{ml. acid x normality of acid}}{\text{millimoles of } Na_3VO_4 . 14\ H_2O \text{ present}}$$

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
12.8	0	11.4	1.018	8.5	2.13
12.75	0.016	11.1	1.07	7.7	2.21
12.7	0.035	10.8	1.12	6.6	2.29
12.7	0.070	10.6	1.17	6.3	2.33
12.7	0.115	10.5	1.23	6.1	2.37
12.65	0.149	10.3	1.235	5.8	2.45
12.65	0.205	10.15	1.29	5.6	2.49
12.6	0.256	10.05	1.34	5.5	2.53
12.6	0.315	10.0	1.37	5.4	2.57
12.6	0.354	9.9	1.42	5.15	2.57
12.6	0.442	9.7	1.55	3.8	2.73
12.6	0.518	9.55	1.60	3.3	2.77
12.5	0.565	9.4	1.68	2.9	2.81
12.5	0.601	9.3	1.77	2.5	2.85
12.4	0.701	9.2	1.82	2.4	2.89
12.3	0.726	9.1	1.89	2.2	2.92
12.2	0.885	9.0	1.97	2.05	3.01
12.0	0.967	8.8	2.03	1.95	3.09
				1.8	3.17

Expt. 33. The potentiometric titration of sodium orthovanadate with sulphuric acid in the presence of D-glucitol.

The method used was that of expt. 32 except that D-glucitol (1.147g.) was first added to the vanadate solution, such that the V/P ratio was 2:1.

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
12.7	0.00	10.90	0.84	9.30	1.68
12.6	0.04	10.75	0.88	9.25	1.72
12.6	0.08	10.60	0.92	9.20	1.76
12.6	0.12	10.50	0.96	9.10	1.80
12.55	0.16	10.40	1.00	9.00	1.84
12.55	0.20	10.30	1.04	8.90	1.88
12.50	0.24	10.20	1.08	8.75	1.92
12.50	0.28	10.15	1.12	8.55	1.96
12.45	0.32	10.05	1.16	8.25	2.00
12.40	0.36	10.00	1.20	7.50	2.04
12.40	0.40	9.90	1.24	6.90	2.08
12.35	0.44	9.95	1.28	6.60	2.12
12.30	0.48	9.80	1.32	6.45	2.16
12.20	0.52	9.75	1.36	6.30	2.20
12.20	0.56	9.70	1.40	6.10	2.24
12.10	0.60	9.65	1.44	5.95	2.28
11.95	0.64	9.60	1.48	5.80	2.32
11.85	0.68	9.50	1.52	5.70	2.36
11.65	0.72	9.50	1.56	5.55	2.40
11.40	0.76	9.40	1.60	5.40	2.44
11.15	0.80	9.40	1.64	5.30	2.48

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
5.05	2.52	2.50	2.84
4.80	2.56	2.40	2.88
4.50	2.60	2.30	2.92
4.20	2.64	2.20	2.96
3.75	2.68	2.15	3.0
3.15	2.72	2.10	3.04
2.80	2.76	2.05	3.08
2.60	2.80	2.0	3.12

Expt. 34. The potentiometric titration of sodium orthovanadate with sulphuric acid in the presence of excess D-glucitol.

The method was that of expt. 31 except that excess D-glucitol (17g.) was added.

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
12.35	0	12.00	0.24	11.70	0.48
12.25	0.04	11.95	0.28	11.65	0.52
12.15	0.08	11.90	0.32	11.60	0.56
12.15	0.12	11.90	0.36	11.50	0.60
12.10	0.16	11.80	0.40	11.45	0.64
12.05	0.20	11.75	0.44	11.40	0.68

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
11.30	0.72	10.25	1.44	5.70	2.20
11.20	0.76	10.20	1.48	5.40	2.24
11.15	0.80	10.20	1.52	5.20	2.28
11.10	0.84	10.10	1.56	5.20	2.32
11.0	0.88	10.05	1.60	5.20	2.36
10.95	0.92	10.00	1.64	5.10	2.40
10.90	0.96	9.90	1.68	5.00	2.44
10.85	1.02	9.85	1.72	4.90	2.48
10.80	1.04	9.80	1.76	4.80	2.52
10.75	1.08	9.70	1.80	4.75	2.56
10.70	1.12	9.55	1.84	4.50	2.60
10.65	1.16	9.40	1.88	4.10	2.64
10.60	1.20	9.30	1.92	3.00	2.68
10.55	1.24	9.00	1.96	2.70	2.72
10.55	1.28	8.80	2.00	2.50	2.76
10.45	1.32	8.20	2.04	2.40	2.80
10.40	1.36	6.90	2.08	2.30	2.84
10.35	1.40	6.10	2.12	2.20	2.88
		5.95	2.16	2.10	2.92

Expt. 35. The potentiometric titration of sodium ortho-vanadate with sulphuric acid, in the presence of excess D-mannitol.

The method was that of expt. 31 except that excess D-mannitol (11.5g.) was added to the vanadate.solution.

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
12.50	0	11.00	0.68	10.05	1.36
12.40	0.04	10.90	0.72	10.00	1.40
12.35	0.08	10.85	0.76	9.90	1.44
12.25	0.12	10.80	0.80	9.85	1.48
12.20	0.16	10.70	0.84	9.80	1.52
12.15	0.20	10.65	0.88	9.70	1.56
12.10	0.24	10.55	0.92	9.65	1.60
12.05	0.28	10.50	0.96	9.55	1.64
12.00	0.32	10.50	1.00	9.45	1.68
11.95	0.36	10.40	1.04	9.35	1.72
11.85	0.40	10.40	1.08	9.20	1.76
11.75	0.44	10.35	1.12	9.00	1.80
11.65	0.48	10.30	1.16	8.75	1.84
11.55	0.52	10.20	1.20	8.10	1.88
11.40	0.56	10.20	1.24	6.80	1.92
11.30	0.60	10.15	1.28	6.30	1.96
11.15	0.64	10.10	1.32	6.00	2.0

pH	H^+/VO_4^{3-}	pH	H^+/VO_4^{3-}
5.65	2.04	3.60	2.52
5.35	2.08	3.50	2.56
5.10	2.12	3.35	2.60
4.95	2.16	3.15	2.64
4.80	2.20	2.95	2.68
4.65	2.24	2.80	2.72
4.50	2.28	2.65	2.76
4.35	2.32	2.50	2.80
4.25	2.36	2.35	2.84
4.10	2.40	2.30	2.88
3.95	2.44	2.20	2.92
3.80	2.48	2.15	2.96
		2.05	3.00

Expt. 36. The Conductimetric titration of sodium orthovanadate with sulphuric acid.

An 0.1M-aqueous solution of sodium orthovanadate was prepared (4.3595g.100 ml.). The conductance of this solution was measured after the addition of known volumes of 0.9931N-sulphuric acid.

H^+/VO_4^{3-}	Conductivity x 10^2		H^+/VO_4^{3-}	Conductivity x 10^2		H^+/VO_4^{3-}	Conductivity x 10^2	
0.50	2.12	ohm^{-1}	1.60	1.87	ohm^{-1}	2.65	1.85	ohm^{-1}
0.60	2.12	"	1.65	1.87	"	2.70	1.78	"
0.65	2.09	"	1.70	1.87	"	2.75	1.87	"
0.70	2.07	"	1.75	1.87	"	2.80	1.89	"
0.75	2.02	"	1.80	1.87	"	2.85	1.92	"
0.80	2.02	"	1.85	1.87	"	2.90	1.95	"
0.85	2.00	"	1.90	1.87	"	2.95	1.98	"
0.90	1.94	"	1.95	1.85	"	3.00	2.02	"
0.95	1.83	"	2.00	1.85	"	3.05	2.05	"
1.00	1.83	"	2.05	1.89	"	3.10	2.07	"
1.10	1.909	"	2.10	1.87	"	3.20	2.11	"
1.15	1.89	"	2.15	1.87	"	3.30	2.16	"
1.20	1.89	"	2.20	1.87	"	3.40	2.22	"
1.25	1.76	"	2.25	1.85	"	3.50	2.29	"
1.30	1.87	"	2.30	1.85	"	3.60	2.32	"
1.35	1.87	"	2.40	1.85	"	3.90	2.49	"
1.40	1.87	"	2.45	1.85	"			
1.45	1.85	"	2.50	1.83	"			
1.50	1.87	"	2.55	1.80	"			
1.55	1.78	"	2.60	1.83	"			

Expt. 37. Conductimetric titration of sodium metavanadate with sodium hydroxide.

An 0.1M-aqueous solution of sodium metavanadate (1.22g./100 ml.) was prepared. The conductance of the solution was measured after the addition of known volumes of 1.002N-sodium hydroxide.

$\frac{OH^-}{VO_3^-}$	Conductivity x 10^3	$\frac{OH^-}{VO_3^-}$	Conductivity x 10^3
0	8.46 ohm^{-1}	1.28	17.20 ohm^{-1}
0.08	8.46 "	1.38	18.60 "
0.18	8.64 "	1.48	19.40 "
0.28	9.03 "	1.58	20.35 "
0.38	9.90 "	1.68	21.95 "
0.48	10.48 "	1.78	23.40 "
0.58	10.80 "	1.88	24.75 "
0.68	11.30 "	1.98	26.10 "
0.78	11.70 "	2.08	27.20 "
0.88	12.83 "	2.10	28.35 "
0.98	12.96 "	2.20	29.20 "
1.08	14.76 "	2.30	30.40 "
1.18	15.80 "	2.40	31.55 "
		2.50	32.75 "

Expt. 38. The conductimetric titration of sodium metavanadate with sodium hydroxide in the presence of excess D-mannitol.

The method was that of expt. 37 except that excess D-mannitol (7.3g.) was added.

OH^-/VO_3^-	Conductivity x 10^3	OH^-/VO_3^-	Conductivity x 10^3	OH^-/VO_3^-	Conductivity x 10^3
0	4.68 ohm^{-1}	0.90	9.87 ohm^{-1}	1.90	18.36 ohm^{-1}
0.10	5.26 "	1.00	10.87 "	2.00	19.28 "
0.20	5.97 "	1.10	11.39 "	2.10	20.10 "
0.30	6.77 "	1.20	12.24 "	2.20	20.88 "
0.40	7.12 "	1.30	13.14 "	2.30	21.75 "
0.50	7.71 "	1.40	14.04 "	2.40	22.50 "
0.60	8.21 "	1.50	14.84 "	2.50	23.40 "
0.70	8.75 "	1.60	15.74 "	2.60	24.10 "
0.80	9.25 "	1.70	16.64 "	2.70	24.90 "
		1.80	17.58 "	2.80	25.75 "

Expt. 39. The conductimetric titration of sodium metavanadate with sodium hydroxide in the presence of excess D-glucitol.

The method was that of expt. 37 except that excess D-glucitol was added.

OH^-/VO_3^-	Conductivity x 10^2	OH^-/VO_3^-	Conductivity x 10^2
0	1.67 ohm^{-1}	1.60	2.10 ohm^{-1}
0.10	1.68 "	1.81	2.13 "
0.21	1.70 "	2.00	2.20 "
0.49	1.76 "	2.20	2.27 "
0.59	1.78 "	2.40	2.34 "
0.79	1.84 "	2.60	2.42 "
1.00	1.89 "		
1.20	1.93 "		
1.39	2.00		

Expt. 40. Conductimetric determination of the vanadium : D-mannitol ratio in the metavanadate complex.

An 0.1M-aqueous solution of sodium metavanadate (1.22g./100 ml.) was prepared. Known weights of solid D-mannitol were dissolved in the solution and the conductivity measured after each addition.

P/V	Conductivity x 10^2	P/V	Conductivity x 10^2
0	7.70 ohm^{-1}	1.016	5.36 ohm^{-1}
0.09194	7.42 "	1.144	5.22 "
0.2947	6.66 "	1.337	5.46 "
0.4832	6.33 "	1.587	5.37 "
0.7058	6.26 "	1.697	5.33 "
0.8379	6.05 "	1.928	5.15 "

Expt. 41. Conductimetric determination of the vanadium : galactitol ratio in the metavanadate complex.

*

P/V	Conductivity x 10^2	P/V	Conductivity x 10^2
0	7.50 ohm^{-1}	1.007	5.76 ohm^{-1}
0.1762	7.42 "	1.151	5.47 "
0.3136	7.16 "	1.521	5.37 "
0.5218	6.63 "	1.658	5.26 "
0.6797	6.30 "	1.936	5.11 "
0.8063	6.15 "		

* Method as for expt. 40 using galactitol.

Expt. 42. Conductimetric determination of vanadium : D-glucitol ratio in the metavanadate complex.

*

R/V	Conductivity x 10^2	R/V	Conductivity x 10^2
0.1417	7.30 ohm^{-1}	1.431	4.75 ohm^{-1}
0.3535	6.77 "	1.595	4.72 "
0.4906	6.63 "	1.785	4.65 "
0.6230	6.19 "	1.991	4.46 "
0.8320	5.83 "	2.140	4.44 "
1.054	5.55 "	2.270	4.39 "
1.230	5.22 "	2.530	4.32 "
		2.760	4.30 "

* Method as for expt. 40 using D-glucitol.

Expt. 43. The absorption spectra of solutions of sodium orthovanadate of different acidities.

An 0.1M-aqueous solution of sodium orthovanadate (8.7190g./200 ml.) was prepared. 10 ml. aliquots of this solution were acidified using 0.1002N, or for higher acidities 0.2003N-sulphuric acid, and were made up to 25 ml. with water. The pH of each solution was found initially and after the solutions had stood in the dark for 2 days.

The absorption spectrum of each solution was examined after 2 days. At several specific wavelengths the value of the absorbance for different H^+/VO_4^{3-} ratios was plotted against this ratio.

H^+/VO_4^{3-}	No. of solution	Initial pH	final pH
0.2	1	12.1	12.0
0.4	2	12.05	11.7
	3		
0.8	4	11.45	10.3
0.9	5	11.15	9.95
1.0	6	-	9.85
	7		
1.1	8	10.9	9.6
	9		
	10		
	11		
1.8	12	9.6	8.9
1.9	13	9.5	8.4
2.0	14	9.3	7.5
2.1	15	9.0	6.95
2.2	16	8.7	6.9
2.3	17	8.35	6.7
2.4	18	7.1	6.5

2.5	19	6.5	6.4
2.6	20	5.8	6.0
2.7	21	4.9	5.2
2.75	22	4.8	5.0
	23		
2.85	24	3.8	3.8

Wavelength in millimicrons	Absorbance value Solution 1.	4.	5.	6.
350	1.25	1.28	1.35	1.30
360	0.75	1.00	1.20	1.28
370	0.20	0.32	0.50	0.65
380	0.03	0.07	0.14	0.20
390	0.00	0.00	0.02	0.03
	Solution 8.	12	13	14.
350	1.50	1.50	1.50	1.50
360	1.35	1.50	1.50	1.50
370	1.00	1.50	1.50	1.50
380	0.40	0.90	1.00	1.13
390	0.10	0.30	0.33	0.36
400	0.00	0.05	0.06	0.08

	Solution 15	16	17	18
440	1.46	1.50	1.50	1.50
450	1.20	1.50	1.50	1.50
460	0.95	1.50	1.50	1.50
470	0.71	1.50	1.50	1.50
480	0.52	1.25	1.50	1.50
490	0.34	0.85	1.18	1.50
500	0.21	0.56	0.76	1.14
510	0.10	0.31	0.43	0.65
520	0.04	0.15	0.22	0.35
530	0.00	0.06	0.08	0.15
540	0.00	0.00	0.02	0.05
550	0.00	0.00	0.00	0.00

Expt. 44. The absorption spectra of solutions of sodium orthovanadate, of different acidities, containing D-glucitol.

An 0.1M-solution of sodium orthovanadate (10.8987g./250 ml.) was prepared, containing D-glucitol (30g.). The method of expt. 41 was then followed. The circa 0.2N-acid used for this experiment was 0.1992N.

Wavelength in millimicrone.	Absorbance value			
	Solution 1.	4	5	6
360	1.05	1.30	1.38	1.34
370	0.45	0.80	0.90	1.02
380	0.20	0.42	0.52	0.60
390	0.10	0.25	0.30	0.35
400	0.07	0.15	0.19	0.21
410	0.04	0.09	0.11	0.13
420	0.03	0.06	0.07	0.08
430	0.00	0.03	0.05	0.05
	Solution 7	8	9	10
360	1.39	1.39	1.5	1.50
370	1.12	1.20	0.34	1.40
380	0.70	0.79	0.95	1.03
390	0.43	0.48	0.59	0.65
400	0.25	0.29	0.36	0.42
410	0.15	0.17	0.22	0.25
420	0.09	0.10	0.13	0.15
430	0.05	0.06	0.07	0.08

	Solution 11	12	13
370	1.43	1.48	1.50
380	1.09	1.25	1.25
390	0.70	0.83	0.83
400	0.44	0.50	0.52
410	0.26	0.30	0.32
420	0.15	0.17	0.19
430	0.08	0.10	0.11
440	0.00	0.05	0.06

	Solution 14	Wavelength in millimicrons	Solution 15	16
390	1.48	460	1.50	1.50
400	1.18	470	1.39	1.50
410	0.90	480	1.10	1.50
420	0.69	490	0.88	1.37
430	0.52	500	0.73	1.13
440	0.39	510	0.57	0.90
450	0.29	520	0.45	0.71
460	0.22	530	0.34	0.40
470	0.15	540	0.25	0.28
480	0.12	550	0.18	0.19
490	0.09	560	0.12	0.12
500	0.07	570	0.07	0.07
550	0.05	580	0.04	0.04

Wavelength in millimicrons.	Absorbance 17	18	19	20	21,22,23,24
500	1.50	1.50	1.50	1.50	1.50
510	1.35	1.50	1.50	1.50	1.50
520	1.08	1.30	1.50	1.50	1.50
530	0.83	1.02	1.38	1.25	1.37
540	0.61	0.75	1.04	0.90	1.00
550	0.43	0.53	0.73	0.60	0.70
560	0.30	0.37	0.50	0.42	0.45
570	0.12	0.25	0.34	0.27	0.30
580	0.07	0.16	0.22	0.16	0.17
590	0.03	0.10	0.14	0.08	0.10
600	0.00	0.06	0.08	0.04	0.05
610	0.00	0.04	0.04	0.00	0.00
620	0.00	0.00	0.00	0.00	0.00

Expt. 45. The absorption spectra of sodium orthovanadate solutions, of different acidities, in the presence of excess D-mannitol.

An 0.1M-solution of sodium orthovanadate was prepared, as in expt. 43 and to it was added D-mannitol (20g.). The method of experiment 43 was then followed.

Wavelength in millimicrons	Solutions 1 - 8	10	12	13	14	15
350	1.20	1.33	1.50	1.50	1.50	1.50
360	1.00	1.00	1.25	1.30	1.40	1.47
370	0.35	0.55	0.76	0.90	1.00	1.10
380	0.10	0.30	0.44	0.52	0.62	0.70
390	0.03	0.17	0.25	0.30	0.35	0.41
400	0.00	0.08	0.11	0.16	0.19	0.23
410	0.00	0.04	0.07	0.09	0.10	0.11
420	0.00	0.00	0.02	0.03	0.06	0.06
430	0.00	0.00	0.00	0.00	0.02	0.02

Wavelength in millimicrons	16	17	18	19
360	1.5	1.5	1.50	1.50
370	1.2	1.26	1.50	1.50
380	0.75	0.80	1.37	1.40
390	0.44	0.46	0.92	0.96
400	0.24	0.25	0.56	0.57
410	0.11	0.12	0.32	0.32
420	0.06	0.06	0.12	0.12
430	0.02	0.02	0.08	0.08
440	0.00	0.00	0.03	0.03

Wavelength in millimicrons	20	21	22	24
450	1.50	1.50	1.50	1.50
460	1.20	1.50	1.50	1.50
470	0.88	1.50	1.50	1.50
480	0.60	1.40	1.50	1.50
490	0.40	0.95	1.10	1.50
500	0.26	0.60	0.70	1.10
510	0.14	0.34	0.40	0.65
520	0.07	0.18	0.22	0.36
530	0.04	0.08	0.11	0.19
540	0.00	0.03	0.05	0.05
550	0.00	0.00	0.00	0.04

Expt. 46. The preparation of the solid sodium metavanadate complex from a solution at pH 5.9.

Sodium metavanadate (2.44g.) was dissolved in the minimum of water and the pH was adjusted to 5.9 by the addition of Amberlite I.R.120 (H^+) resin. The resin was filtered off and the solution evaporated to dryness at 40° under reduced pressure, giving a mixture of yellow and orange solids. Some of the solid, (1g.) was redissolved in water and left in the refrigerator. Orange well-defined crystals (0.5g.) separated out after several days.

Expt. 47. The isolation of the sodium metavanadate : D-glucitol complex.

Sodium metavanadate (2.44g.) was dissolved in the minimum of water and D-glucitol (1.922g.) was added such that the V/P ratio was 2/1. The pH of the resultant solution was adjusted to 6.0. by the addition of Amberlite I.R. 120 (H^+) resin. The resin was filtered off and the solution evaporated to dryness, leaving a blood-red viscous syrup, which was freeze-dried (4.4g.). Crystallisation from water was unsuccessful. The addition of methanol or acetone to aqueous solution precipitated a yellow solid which on treatment with acid, deionisation of the resultant solution and ionophoresis in sodium metavanadate solution and sodium molybdate solution was shown to contain no D-glucitol.

Expt. 48. The isolation of the sodium metavanadate : D-mannitol complex.

Sodium metavanadate (2.44g.) was dissolved in the minimum of water. D-mannitol (2.644g.) was added and the resultant solution was acidified to pH 5.9 by the addition of Amberlite I.R.120(H^+) resin. The resin was filtered off

and the orange solution evaporated to dryness. A brown-gold viscous syrup remained (6.08g.) which was freeze-dried to yield a dark-green hygroscopic material.

Expt. 49. The isolation of the sodium metavanadate : maltose complex.

Sodium metavanadate (2.44g.) was dissolved in the minimum of water. Maltose (7.204g.) was added, and the pH adjusted to 6.3 by the addition of Amberlite I.R.120 (H^+) resin. The resin was filtered off and the orange solution was evaporated to dryness yielding a golden-brown viscous syrup (9.65g.) which was freeze-dried to give a yellow material.

Expt. 50. The infra-red spectra of some complexes.

The infra-red spectra of sodium orthovanadate, sodium metavanadate, and the orange complex metavanadate, also of the D-glucitol, metavanadate complex and the maltose metavanadate complex were traced, the solids as mulls in Nujol.

Expt. 51. The partial separation of D-glucose and D-glucitol by ion-exchange chromatography.

I.R.A.400(Cl^-) resin (50 ml. dry) was packed in a column 2 cm. diameter to a depth of circa 20 cm. Aqueous sodium metavanadate solution (5%) was passed through the column

until the column was saturated, i.e. the concentration of vanadate found spectrophotometrically in the eluate, pH adjusted to 6.0, at 500 mμ equalled the initial concentration of 5%. The pH of the eluate was then 8.8.

Sodium metavanadate solution (1%), the pH of which had previously been adjusted to 6.0 by Amberlite I.R. 120(H^+) resin, was passed down the column until the eluate had a constant pH of 6.0.

A mixture of D-glucose and D-glucitol (2 ml. of a 1% solution of each) was placed on the column and the column was eluted with water. 10 ml. fractions were collected.

Both D-glucose and D-glucitol were present in the first 50 fractions, the glucose being mostly removed in the first 5 fractions and completely absent after 50 fractions, whilst the D-glucitol was not absent until after 250 fractions. D-glucose and D-glucitol were identified in the fractions by ionophoresis in sodium molybdate solution after deionisation with Biodeminrolit mixed bed resin.

Expt. 52. Chromatography using vanadate - Impregnated Paper.

Whatman No.1 chromatography paper was impregnated by dipping in aqueous solutions of sodium metavanadate (2.5%) adjusted to different pH values with concentrated sulphuric acid or 1N-sodium hydroxide. The paper was air-dried.

The solvents used were (1) acetone : butanol : water 5:3:1v/v

(2) butanol : pyridine : water 6:4:3v/v

Solvent 1	R glucose			
	pH 9.4	pH 8.0	pH 6.2	pH 5.0
D-glucitol	0.42	0.48	0.43	0.85,1.08
D-mannitol	0.42	0.48	0.54	0.97
Galactitol	0.42	0.41	0.37	0.85
D-Glucose	1	1	1	1
D-Xylose	1.47	1.33	1.33	1.5
D-Ribose	1.05	1.07	1.06	1.55
Sucrose	0.58,1	0.71,1	0.62,1	1.08

Solvent 2	R Glucose	
	pH 8.0	pH 6.0
D-glucitol	0.29	0.33
D-mannitol	0.29	0.45
Galactitol	0.29	0.43
D-Glucose	1	1
D-Xylose	1.45	1.45
D-Ribose	1.1	1.3
Sucrose	1,050	1,050

Expt. 53. Determination of the Ratio of tungsten : D-mannose by continuous variation Polarimetry.

0.5 M-aqueous solutions of D-mannose and sodium tungstate, $Na_2WO_4 . 2H_2O$ were prepared. Volumes of these solutions were taken such that the sum of the volumes was 10 ml., and the resulting solution acidified to pH 6.0 by the careful addition of N-sulphuric acid, then made up to 25 ml. with water. The optical rotations of this solution and a solution containing the same concentration of D-mannose alone were measured and the difference plotted against the mole fraction of tungstate.

Mole fraction of tungstate	Difference in polarimeter readings
0.1	- 0.14
0.3	- 0.23
0.5	- 0.26
0.7	- 0.16
0.9	- 0.05

Expt. 54. Determination of the ratio of tungsten : D-ribose by continuous variation polarimetry.

The method was as for expt. 53 except that the pH was adjusted to 6.7

Mole fraction of tungstate.	Difference in polarimeter readings
0.1	- 0.04
0.3	- 0.35
0.5	- 0.66
0.7	- 0.61
0.9	- 0.32

Expt. 55. 1-Morpholino-cyclohex-1-ene.

A solution of cyclohexanone (147g. 1.5 moles) morpholine (157g. 1.8 moles) and p-toluene sulphonic acid (1.5g.) in dry toluene (30 ml.) was heated to boiling in a flask to which was attached a water separator under a reflux condenser. Separation of water began at once and was continued for 8 hr. After removal of the solvent, distillation under reduced pressure yielded 1-morpholino-cyclohex-1-ene, (180g. 70%) 150-120°/10-14 mm.

Expt. 56. Trifluoroacetic anhydride.

Trifluoroacetic acid (20 ml.) was added to phosphorus pentoxide (24g.) in a flask cooled in ice. The flask was warmed carefully and trifluoroacetic anhydride (10g. 55%) b.p. 42-45°/76 mm., was collected.

Expt. 57. Cyclohexanone-2,4-dinitrophenylhydrazone c.f. Vogel[159]

A mixture of 2,4-dinitrophenylhydrazine (0.5g.) and cyclohexanone (0.25g.) in methanol (25 ml.) was heated to boiling. The mixture was allowed to cool slightly and hydrochloric acid (0.5 ml.) was added. The solution was boiled for 2 min. and set aside to crystallise. The crystals were filtered and washed with a little methanol to give cyclohexanone-2,4-dinitrophenylhydrazone (0.46g., 70%) m.p. 163°.

Expt. 58. The Reaction between Trifluoroacetic Anhydride, Glacial Acetic Acid and 1-morpholino-cyclohex-1-ene.

Trifluoroacetic anhydride (4.0 ml.) was mixed with glacial acetic acid (1.2 ml.) and 1-morpholino-cyclohex-1-ene (3.4 ml.) was added slowly. The solution became yellow and considerable heat was developed. On cooling, a white crystalline solid, m.p. 62-64°, was produced. Hydrochloric acid (10 ml. 17%) was added to the reaction mixture, which was then heated under reflux for 2 hr. The mixture was cooled and extracted twice with chloroform. The chloroform layer (ca. 100 ml.) was washed with distilled water until the pH of the washings was 5.5. The aqueous layer and washings were brought to pH 5.5 with sodium

hydroxide and re-extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulphate, the drying agent was filtered off, and the chloroform removed by distillation under reduced pressure. The residual liquid was distilled and cyclohexanone (1.75 ml.) b.p. 55°/ 22 mm., was recovered. Treatment of this fraction with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid yielded 4.0g.(71% based on enamine) of cyclohexanone-2,4-dinitrophenylhydrazone, m.p. 164° after recrystallisation from methanol. Admixture with authentic cyclohexanone-2,4,-dinitrophenylhydrazone caused no depression in m.p.

Expt. 59. 2,3,4,5,6-penta-O-acetyl-D-gluconic acid.

Calcium gluconate (50g.) was added to a mixture of fused zinc chloride (15g.) in acetic anhydride (250 ml.) cooled to -5°. The suspension was saturated with dry hydrogen chloride while the temperature was kept below 5° by cooling the reaction flask in an ice/salt bath. When saturated, the mixture was left at room temperature for 18 hr., being protected from atmospheric moisture by a calcium chloride tube. The mixture was chilled in an ice-bath* to destroy the excess of acetic anhydride. The mixture was left at 0° for 1 hr. and extracted six times with chloroform,

*. and small pieces of chipped ice were added

(total 1 litre). The extracts were combined and dried over sodium sulphate. After filtration, the chloroform solution was concentrated at 60° to a yellow syrup. The syrup was twice concentrated under reduced pressure at 40° with 500 ml. portions of dry toluene, then dissolved in the minimum of warm toluene. After the solution had stood for several days in the refrigerator, 2,3,4,5,6-penta-O-acetyl-D-gluconic acid [49.92g., 60%] m.p. 110°, was deposited.

Expt. 60. 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride.

2,3,4,5,6-penta-O-acetyl-D-gluconic acid (24.9g.) was dissolved in thionyl chloride (80 ml.) and heated under reflux for 1 hr. Most of the excess thionyl chloride was removed by distillation at 12 mm. The residual thionyl chloride was removed by evacuating a vacuum desiccator, containing the reaction flask over pelleted potassium hydroxide, for 5 hr. at 4 mm. During this time the contents of the reaction flask solidified to yield 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride (26g. 99%) m.p. 60-63°.

Expt. 61. 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone.

1-Morpholino-cyclohex-1-ene (30 ml.) and triethylamine

(20 ml.) in dry chloroform (100 ml.) were warmed with 2,3,4,5,6-penta-O-acetyl-D-gluconyl chloride (26g.) for 2 hr., with stirring, at 40-50°. The mixture was left for 18 hr. at room temperature. Hydrochloric acid (100 ml. 17%) was added and the reaction mixture heated under reflux for 5 hr. After cooling, the chloroform layer was extracted with water until no chloride was present in the extracts (ca. 4.5l.). The combined water extracts were brought to pH 6.0 with N-sodium hydroxide and re-extracted with chloroform. The chloroform extracts were added to the original chloroform layer and the whole solution dried over anhydrous sodium sulphate. After filtration, the chloroform was removed by distillation under reduced pressure. The residual syrup (A), (45g. 52%) could not be distilled at 100°/1 mm., but recrystallisation from hexane gave

2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone monohydrate m.p. 93-94° $[\alpha]_D^{20}$ + 16.3° in methanol (Found: C, 52.35; H,6.32. $C_{22}H_{30}O_{12}.H_2O$ requires C,52.38; H,6.35%).

Expt. 62. Copper Acetylacetonate.

Acetylacetone (7g.) was added to copper acetate, $(CH_3COO)_2.Cu.H_2O$ (7g.) dissolved in warm water (100 ml.). Slate-blue needle-shaped crystals of copper acetylacetonate

(14.1g, 80%), m.p. 238° (sublimed) were precipitated immediately.

Expt. 63. 2-Acetylcyclohexanone*

Acetyl chloride (5g.) was warmed with 1-morpholino-cyclohex-1-ene (24 ml.) and triethylamine (10 ml.) in dry chloroform (80 ml.) for 2 hr. with stirring at 30-40°. The mixture was left for 18 hr. at room temperature. Hydrochloric acid (17%, 80 ml.) was added and the mixture heated under reflux for 5 hr. After cooling, the chloroform layer was extracted with water until no chloride was present in the extracts. The combined water extracts were brought to pH 6.0 with N-sodium hydroxide and re-extracted with chloroform. The chloroform extracts were added to the original chloroform layer and the whole solution dried over anhydrous sodium sulphate. After filtration and removal of the solvent, the residual liquid was distilled under reduced pressure to yield 2-acetylcyclohexanone (7g. 70%), b.p. 108-110°/18 mm.

* The technical assistance of J. Best and G. Sandison is acknowledged.

Expt. 64. Copper 2-Acetylcyclohexanonate.

Copper acetate, $(CH_3COO)_2.Cu.H_2O$., (0.7g.) was dissolved in warm water (10 ml.) and 2-acetylcyclohexanone (0.1g.) was added. Centrifugation afforded copper

2-acetylcyclohexanonate (1.20g. 61%), which, after thorough washing with water and drying under vacuum over phosphorus pentoxide, had m.p. 155°.

Expt. 65. Attempted Preparation of Copper 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanonate.

2-(2',3',4',5',6'-Penta-O-acetyl-D-gluconyl)-cyclohexanone (1g.) was added to a saturated solution of copper acetate $(CH_3COO)_2.Cu.H_2O$ in water (30 ml.). The mixture was heated on a boiling water bath for ca. 1 hr. The solution was decanted from undissolved syrup and left to stand for 18 hr. A white crystalline solid S (0.11g.), m.p. 93-94°, was precipitated and was separated from the mother-liquor by centrifugation.

S (ca. 30 mg.) was shaken with ether and dilute sulphuric acid was added until the solid S had dissolved. No blue colouration developed.

S (ca. 30 mg.) was dissolved in nitric acid and a saturated solution of potassium ferrocyanide was added dropwise. No blood-red colouration was produced. Admixture of S with 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone caused no depression in m.p.

Expt. 66. Treatment of 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone with sodium in methanol.

2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)cyclohexanone (1g.) was dissolved in dry methanol (20 ml.). A trace of sodium in dry methanol (5 ml.) was added. The solution immediately darkened to deep red. The mixture was examined chromatographically in solvent system 1*, and ionophoretically in phosphate and borate solutions. The results are shown below. Evaporation of the methanol under reduced pressure yielded a syrup which resisted recrystallisation from methanol, aqueous methanol and methanol/n-butanol.

Syrup.	Gluconic acid.
M_G(B) = 1.3	M_G (B) = 1.3
$M_{G.A}$(P) = 1.0	$M_{G.A.}$(P) = 1.0
After 1 hr.	
R_p(B.E.W.) = 0.06,0.14,0.16 0.27,0.36,0.41,1	R_p(B.E.W.) = 0.06
After 18 hr.	
R_p(B.E.W.) = 0.06	

* solvent system 1 here and hereafter is butanol/ethanol/water 40:11:19 v/v.

Expt. 67. Isolation and Identification of Cyclohexanone in the Reaction between 2-(2'3',4',5',6'-penta-O-acetyl-D-gluconyl)cyclohexanone and sodium in Methanol.

2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)cyclohexanon

(1g.) was dissolved in dry methanol (20 ml.) and a trace of sodium in dry methanol (5 ml.) was added. The solution was left to stand for 18 hr. The mixture was distilled under reduced pressure (12-14 mm.) and the fractions distilling at 25° (methanol) and 25-30° (cyclohexanone with a little methanol) were collected. The second fraction (25-30°) was added to a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid. The yellow precipitate was centrifuged, washed several times with water, dried under vacuum over phosphorus pentoxide and recrystallised from methanol to give cyclohexanone 2,4-dinitrophenylhydrazone (0.3g., 67%), m.p. and mixed m.p. 160-161°.

Expt. 68. Treatment of 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone with ammonia in methanol.

A solution of 2-(2',3'4',5'6'-penta-O-acetyl-D-gluconyl)-cyclohexanone (1.09g.) in dry methanol (20 ml.) was saturated with ammonia, generated by heating an aqueous solution (sp. gr. 0.88) and dried by passing through quicklime. The mixture was left for 18 hr. at room temperature in a stoppered flask, then investigated

chromatographically in solvent 1 and ionophoretically in borate, molybdate and phosphate solutions. (Results are given in Table p. 183). On standing for 2 days, crystalline D-gluconamide (0.3g., 70%) was obtained. It had m.p. 145°, and on admixture with an authentic specimen (Expt. 69) had m.p. 140-141°.

Expt. 69. D-gluconamide.

D-glucono-δ-lactone (0.18g.) was dissolved in aqueous ammonia (5 ml., sp.gr.0.88) and left to stand in a stoppered flask for 10 min. at room temperature. Ethanol was added to the solution until an incipient cloudiness was produced. A drop of water was added to clarify the solution. After standing several days in the refrigerator, D-gluconamide (0.10g., 50%), m.p. 142°, separated out.

Expt. 70. Treatment of 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone with sodium borohydride.

2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone (1g.) was dissolved in methanol (20 ml.). Potassium borohydride (0.2g.) in water (20 ml.) was added slowly to the methanol solution, with stirring. The mixture was left to stand for 18 hr., treated with Amberlite I.R.120(H^+) resin (to remove the potassium ions) and evaporated to dryness several times with methanol. The pale yellow syrupy residue

was investigated chromatographically in solvent system 1 and by ionophoresis in phosphate and molybdate electrolytes.

R_p(B.E.W.) = 0.07 - 0.24, 0.42, 0.61

M_g(Mo) = 0, 0.59, 0.70, 0.83, 1.0

$M_{g.A.}$(P) = 0.86, 0.73

Expt. 71. 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose.

Anhydrous sodium acetate (4g.) was ground together with dry D-glucose (5g.) To the powdered mixture acetic anhydride (25 ml.) was added. The mixture was heated for 2.5 hr. on a boiling water bath, cooled and poured on to ice. The white solid precipitated was filtered from the ice-cold water and washed with ice-water. The solid was recrystallised twice from ethanol to give 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (8.5g., 60%), m.p. 130°.

Expt. 72. Deacetylation and reduction of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose with sodium borohydride.

1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (1.3g.) was dissolved in cold methanol (70 ml.). Potassium borohydride (0.9g.) in water (10 ml.) was added slowly, and the solution was allowed to stand for 18 hr. The sodium ions and boric acid were removed by treatment with Amberlite I.R.120(H^+) resin and repeated distillation with methanol,

respectively. The residual syrup (1.31g.) was investigated by ionophoresis in borate solution : $\underline{M}_G(\underline{B}) = 0.85$.

Expt. 73. Treatment of 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone with lithium aluminium hydride.

Lithium aluminium hydride (3.35g.) was suspended in ether (500 ml.) previously dried over sodium ribbon. 2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone (5g.) dissolved in dry ether (50 ml.) was added slowly to the suspension. A white solid was precipitated immediately. The stoppered flask was left to stand, with occasional shaking, for 1 hr. at room temperature. A further 200 ml. of ether was then added. The flask was cooled in running water while distilled water was added until all the excess lithium aluminium hydride had been destroyed (500 ml.). The insoluble white material (C) remaining was filtered off (8.1g.). The ethereal and aqueous layers were separated, the aqueous layer extracted with ether (total 500 ml.) and the combined extracts dried over anhydrous sodium sulphate. The ether was removed by distillation under reduced pressure to leave a residual syrup (B), 0.55g. which was freeze-dried. A portion of the syrup was recrystallised from n-butanol to

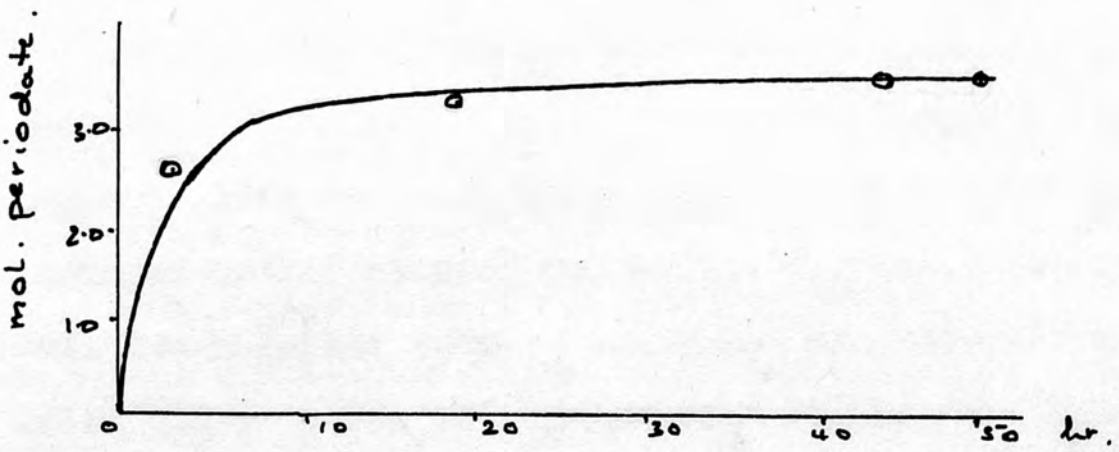
give 1-C-(2'-hydroxycyclohexyl)-D-glucopyranose m.p. 212.5°
Its 2,4-dinitrophenylhydrazone had m.p. 83-85°.

Expt. 74. Periodate Oxidation of 1-C-(2'-hydroxycyclohexyl)-D-glucopyranose.

1. Periodate Uptake.

Sodium metaperiodate (1.0195g.) was dissolved in distilled water (100 ml.). A portion of this solution (40 ml.) was diluted to 50 ml. with water to form a blank solution. A second portion of the initial periodate solution (40 ml.) was added to the freeze-dried solid (B) (0.0461g.) obtained from the ethereal layer in the previous experiment. The resulting solution was made up to 50 ml. The periodate uptake was estimated using 0.01N-solutions of sodium arsenite and iodine. Saturated sodium bicarbonate (20 ml.) and 20% potassium iodide in water (2 ml.) were added to aliquots (5 ml.). Arsenite solution (40 ml.) was added to the mixture and the excess arsenite back-titrated with iodine, using sodium starch glycollate indicator. The results are shown in the table and figure below.

Time. hr.	Arsenite added. ml.	Iodine titre ml.	Arsenite titrated ml.	Periodate uptake mol.
0	40	2.95	37.05	
2	40	11.20	28.80	2.49
30	40	12.00	28.00	2.73
43.5	40	13.65	26.35	3.17
50	40	13.65	26.35	3.17



2. Formic acid liberated.

An aliquot (10 ml.) of the above reaction mixture was titrated potentiometrically with 0.0012N-sodium hydroxide. The end-point was reached with 57 ml. of the sodium hydroxide solution, corresponding to 2.03 mol. of formic acid liberated.

3. Formaldehyde produced.

An aliquot of the reaction mixture (5 ml.) was added to a solution of dimedone (0.065 g.) in water (20 ml.) at 80°. No derivative was produced on cooling.

Expt. 75. cis-2-Hydroxy-cyclohexane carboxylic acid.

(i) Ethyl 2-oxo-cyclohexane carboxylate.[160]

A solution of sodium ethoxide was prepared by cautious addition of sodium (23g.) to anhydrous ethanol (300 ml.) in a 1 litre 3-necked flask equipped with a dropping funnel, mercury-sealed stirrer and reflux condenser carrying a calcium chloride tube. The flask was immersed in an ice/salt bath. When the temperature of the solution had reached 10°, an ice-cold solution of cyclohexanone (98g.) in diethyl oxalate (146g.) was added from the dropping funnel over 0.5 hr., while the solution was stirred vigorously. The ice-bath was retained for 1 hr. The mixture was then stirred at room temperature for ca. 12 hr. The reaction mixture was decomposed by the careful addition of ice-cold dilute sulphuric acid prepared by adding concentrated acid (28 ml.) to ice (218g.). During the neutralisation, the temperature of the mixture was maintained at less than 10° by means of the ice-salt bath. The solution was diluted with cold water to 2 litres. The ethyl 2-oxo-cyclohexylglyoxalate separated as a heavy yellowish oil. The aqueous solution was extracted with portions of benzene (4 x 250 ml.). The crude product was combined with the extracts and the resulting solution washed

with water (2 x 100 ml.). The benzene solution was dried with anhydrous sodium sulphate, and the benzene distilled off at atmospheric pressure on a water bath. The water bath was replaced by an oil bath and the system was gradually evacuated to a pressure of 14 mm., while the oil bath was held at about 90-95°. When all the benzene, unchanged ester and ketone had distilled, the temperature was increased and the fraction distilling over between 110° and 170° at 11/18 mm was collected. The yield was 94g. The distillate was transferred to a 50 ml. distillation flask and a trace of iron powder and some finely-ground soft glass added. (1 mg. iron, 0.5g. glass). The mixture was distilled at 35 mm., the bath temperature being maintained at 155-170°, to yield ethyl 2-oxo-cyclohexane carboxylate (70g. 40%), b.p. 120-138°/35 mm.

(ii) cis-2-Hydroxy-cyclohexane carboxylic acid.[161]

Ethyl 2-oxo-cyclohexane carboxylate (5.1295g.) was hydrogenated in absolute ethanol (5 ml.) over platinic oxide (Adams catalyst, 0.5g.) for 2 days. The catalyst was removed by filtration and the filtrate was distilled to yield ethyl 2-hydroxy-cyclohexane carboxylate (4g.), b.p. 119-120°/18-20 mm. This ester was mixed with hot 25% sodium hydroxide (14 ml.), stirred while cooling, and set

aside overnight at room temperature. The sodium salt which had separated was filtered off, washed with a little ethanol and air-dried. The salt was dissolved in the minimum of water, decomposed with dilute hydrochloric acid (10%) and the solution saturated with ammonium sulphate and extracted with ether. The ether layer was dried and on evaporation gave cis-2-hydroxy-cyclohexane carboxylic acid (2.5g. 58%), m.p. 80° after recrystallisation from ether.

Expt. 76. 1,2,3,4,5,6-hexa-O-acetyl-1-C-(2'-acetoxy-cyclohexyl)-hexitol.

2-(2',3',4',5',6'-penta-O-acetyl-D-gluconyl)-cyclohexanone (1g.) in ether (10 ml.) was added to lithium aluminium hydride (0.67g.) in dry ether (500 ml.). The flask was left to stand for 1 hr. at room temperature. A further 200 ml. of ether was added. Distilled water (500 ml.) was added to the mixture, the flask being cooled in running water. The insoluble white material (c) left was filtered off. The insoluble material was treated with N-hydrochloric acid, in which it dissolved with considerable effervescence. Sufficient acid was added to dissolve all the material (30 ml.). Silver carbonate (10g.) was added to remove the chloride ions. The filtrate was evaporated

under reduced pressure (rotary evaporation) and the residue was extracted twice with methanol. The methanolic solution was evaporated (rotary evaporation) to yield a colourless syrup (D).

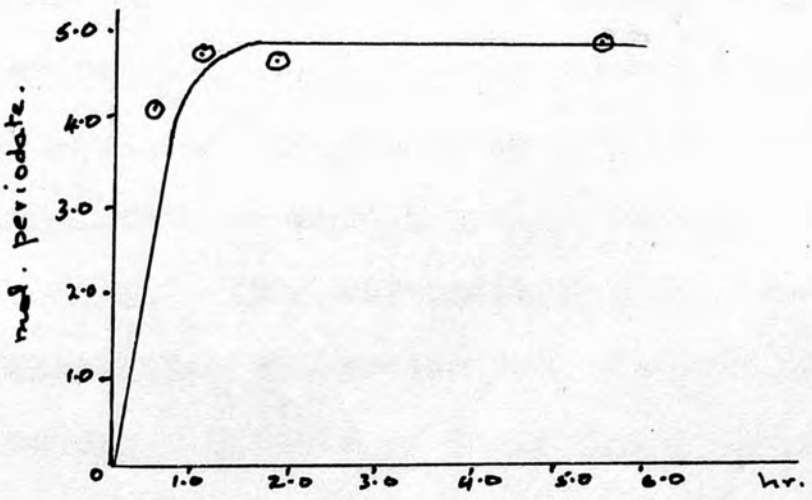
The syrup was dissolved in methanol (10 ml.) and sodium borohydride (0.15g.) in water (10 ml.) was added. The reaction mixture was left for 1 hr. then acidified with dilute acetic acid to destroy the excess of the sodium borohydride and evaporated to dryness by rotary evaporation. The residual syrup (E) was freeze-dried overnight. The residue was shaken with acetic anhydride (15 ml.) containing sulphuric acid (1 ml.) until most of the solid had dissolved[162] then warmed for 10 min. at 50-60°. The reaction mixture was allowed to cool and was poured on to crushed ice. The aqueous mixture was extracted with chloroform (100 ml.). The chloroform layer was dried over sodium sulphate, filtered and evaporated. The residual syrup was recrystallised from aqueous ethanol to yield 1,2,3,4,5,6-hexa-O-acetyl-1-O-(2'-acetoxy-cyclohexyl)-hexitol trihydrate. (0.5g. 40%), m.p. 87-88°. (Found C,49.9; H,6.09. $C_{26}H_{44}O_{17}$ requires C,49.06; H,6.05%. Acetyl content: Found 50.37. $C_{26}H_{40}O_{15}$ requires 50.8%.

Expt. 77. The Periodate Oxidation of 1-C-(2'-hydroxy-cyclohexyl)-hexitol.

1. Periodate uptake.

The material used was the freeze-dried syrup (E) obtained in the previous expt. (Expt. 76) before acetylation. To a solution of 1-C-(2'-hydroxy-cyclohexyl)-hexitol (16.8 mg.) in water (2 ml.) was added 0.7663% aqueous sodium metaperiodate (40 ml.) and the whole made up to 50 ml. with water. The amount of periodate consumed was determined by titration of aliquots (5 ml.) with 0.01N-arsenite and 0.00995N-iodine. A blank solution (time 0 hr.) was obtained by diluting 40 ml. of the above periodate solution to 50 ml. with water. The results are shown in the table and figure below.

Time hr.	Arsenite added ml.	Iodine added ml.	Arsenite titrated ml.	Periodate uptake mol.
0.00	40	11.25	28.75	
0.25	40	16.40	23.60	4.29
0.75	40	16.95	23.05	4.75
1.50	40	16.90	23.10	4.71
5.50	40	17.20	22.80	4.96



2. Formic acid liberated.

An aliquot (10 ml.) of the above reaction mixture was titrated potentiometrically with 0.00117N-sodium hydroxide. The end-point was reached with 37 ml. of the sodium hydroxide solution, corresponding to 3.7 mol. of formic acid liberated.

3. Formaldehyde liberated.[163]

Solutions (100 ml.) of D-glucitol were prepared containing 0.0042, 0.0078 and 0.0019g. of polyol respectively. A portion (1 ml.) of each solution was used to obtain a calibration curve. To each aliquot was added 0.015M-sodium metaperiodate (1 ml.). The mixed solutions were allowed to stand for 2 hr. in the dark. Excess of periodate was

destroyed by the addition of sodium sulphite solution (20% 0.15 ml.). The formaldehyde obtained by oxidation of the D-glucitol was estimated by heating the solutions containing the oxidation product with a solution of chromotropic acid (10 ml.) in a boiling water bath for 30 min. [The chromatropic acid solution was prepared by dissolving the sodium salt (0.5g.) in water (50 ml.) and adding a mixture of concentrated sulphuric acid and water 2:1 v/v (200 ml.)]. After cooling the formaldehyde solution, a half-saturated solution of thiourea was added (0.25 ml.) in order to remove any colour due to free iodine. The absorption was measured at 570 mμ . Water (1 ml.) was added to a portion (1 ml.) of the reaction mixture. The procedure was then repeated as above.

Wt. D-glucitol/ 100 ml.	moles formaldehyde	reading at 570 mμ
0.0042	0.49×10^5	0.103
0.0078	0.87×10^5	0.132
0.0019	0.21×10^5	0.074
formaldehyde solution from reaction mixture.		0.34 (ca 6.0 mol.)

A portion (5 ml.) of the reaction mixture was warmed gently in a distillation flask connected to a U-tube, with

arms of different bore, the narrow arm being nearest the distillation flask. The U-tube contained water (5 ml.) and was cooled in ice. The flask was warmed for 10 min. to drive out the formaldehyde from the solution.

A portion (1 ml.) of the aqueous solution in the U-tube was added to water (1 ml.) and treated with chromotropic acid as before. The absorption reading at 570 m was 0.070, which corresponds to 1.3 mol. formaldehyde. A portion (1 ml.) of the solution remaining in the distillation flask was also treated as above. The absorption reading was 0.270, ie. the combined reading is 0.34, as above.

A portion of the residual solution (3 ml.) was added to a solution of dimedone (0.0065g./2 ml.) at 80°. On cooling, a white crystalline solid, (0.0025g.), m.p.160° was precipitated. Admixture with the dimedone derivative of formaldehyde depressed the m.p. to 140°.

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