SYNTHESIS AND PROPERTIES OF N-GLYCOSYLIMIDAZOLES

A Thesis submitted by

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ABSTRACT

In a model study concerned with the mechanism of glycosidase action, six N-glycosylimidazoles and five N-(tetra-O-acetyl-glycosyl) imidazoles have been symthesized by the condensation of the appropriate tetra-O-acetyl-glycosyl halide with imidazole.

These compounds have been characterised by their elemental analysis, optical rotation and NMR spectra. In all cases the β-anomeric compounds predominated over those with α-configuration in the reaction products.

Analysis of ^{1}H NMR data was carried out on a first order basis, and also by non-iterative and iterative calculation procedures in order to establish the conformations of the N-glycosylimidazoles. The analysis revealed that under certain conditions, in four compounds the sugar pyranose ring is distorted from the normal $^{4}C_{1}$ conformation. This distortion has been interpreted in terms of the operation of the reverse anomeric effect.

The hydrolytic stabilities of 1-α- and β-D-glucopyranosylimidazoles have been examined in water, pH 3.0 sodium formate buffer, 0.56M formic acid, 6N-hydrochloric acid, 10N-sulphuric acid and 1N NaOH. Optical rotation measurements, paper chromatography and analysis for estimation of glucose failed to detect any evidence of hydrolysis. This result is strong evidence against the nucleophilic intervention of a histidinyl side chain in glycosidase action. Hydrolytic cleavage was also attempted with solutions of (yeast) α- and (almond) β-glycosidases, and no glucose was released under conditions in which standard compounds methyl α-D-glucoside and cellobiose were hydrolysed significantly.

The ORD/CD curves of N-glycosylimidazoles and a series of p-nitrophenyl-glycosides have been examined as an aid to establishing anomeric configurations for N-glycosylimidazoles. In general anomeric pairs of compounds show ORD/CD curves of opposite sign, but some exceptions have been observed in the case of N-glycosylimidazoles.

1)-α- and β-D-glucopyranosylimidazoles were found to possess inhibitory properties towards the enzymes (yeast) α-glucosidase, (almond) β-glucosidase and lysozyme. These properties have been investigated in detail. The α-anomer shows an unexpectedly high degree of inhibitory behaviour.

To my (late) Father and my Mother

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CHAPTER 1

General Introduction

Enzymes which catalyze the cleavage of glycosidic linkages have been the subject of much study. An enzyme of this type, namely, lysozyme was the first enzyme to have its three-dimensional structure determined by x-ray crystallographic studies. These enzymes catalyze the transfer of a glycosyl moiety to a suitable acceptor, which becomes the aglycone of a newly formed glycoside. The reaction may be formally represented[1] as

$$G - OR' + R^2O - H \xrightarrow[\text{transferase}]{\text{Glycosyl}} G - OR^2 + R'O - H$$

when $R^2 = H$; the reaction describes the hydrolysis of G - OR'.

A major objective of the present work on these (and other) enzymes is to formulate the mechanisms, i.e. sequence of steps by which the overall reactions proceed. Only then can an understanding of the catalytic power of enzymes be obtained.

There is a large amount of literature in which it is suggested that glycosyl transferases possess essential imidazole (from histidine) and/or carboxyl (from asparatic and glutamic acid) residues at the enzyme active sites. Enzymes for which this has been proposed are shown in Table 1.1.

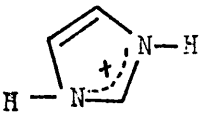
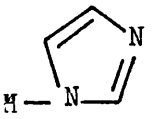
The evidence based on studies of variation of enzyme activity with pH for the presence of the active site functional groups should be regarded as insufficient because interactions in an enzyme molecule may cause the pK of a particular group to differ significantly from its value in the free amino acid, and also several groups have approximately the same pK-value. Thus the enzyme activity change with pH may not be a consequence of the

Table 1.1

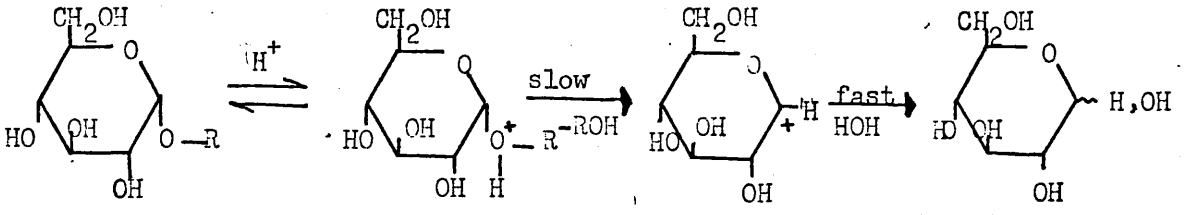
Enzyme	Evidence
α-amylase	variation of kinetic parameters with pH[2,3-6]
β-amylase	variation of kinetic parameters with pH[2,7-8]
α-glucosidase	variation of kinetic parameters, photo-oxidation inactivation studies.[9]
β-glucosidase	isolation of inositol-enzyme complex.[19]
Sucrose phosphorolylase	isolation and properties of glucosyl enzyme.[11]
Glucamylase	CO_2^- reagent.[18]
Lysozyme	x-ray;[20] titration,[15] CO_2^- reagent.[14,16,17]
Potato phosphorylase	variation of kinetic parameters with pH.[12,13]

state of an active site.[21] Furthermore the multistep nature of enzyme catalysed reactions means that a change of activity with pH does not necessarily reflect the direct involvement of a particular group in a reaction with the substrate.

The function of the imidazole and carboxyl groups has not been precisely defined in most cases, but in principle they could act as catalytic groups as either nucleophiles, general bases, or general acids. If one adopts the widely but not wholly accepted proposition that the course of an enzyme reaction should be describable in terms of known physical organic principles, then some appreciation of the non-enzyme catalyzed cleavage of glycosides is necessary before the mode of intervention of enzyme active site functional groups can be assessed.

Group	Nucleophile	General acid	General base
Imidazole			
carboxylic group	$-CO_2^-$	$-CO_2H$	$-CO_2^-$

Hydrolysis catalyzed by glycosidases resembles acid catalyzed hydrolysis of simple alkyl and aryl glycosides, in that cleavage of the glycosyl (C-1)-oxygen bond occurs. Like the analogous hydrolysis of acetals, glycoside hydrolysis shows specific acid catalysis; except where R is an aryl residue, base catalysis is absent. The intermolecular specific acid catalyzed hydrolysis of glycosides is generally formulated as shown below (Scheme 1.1).

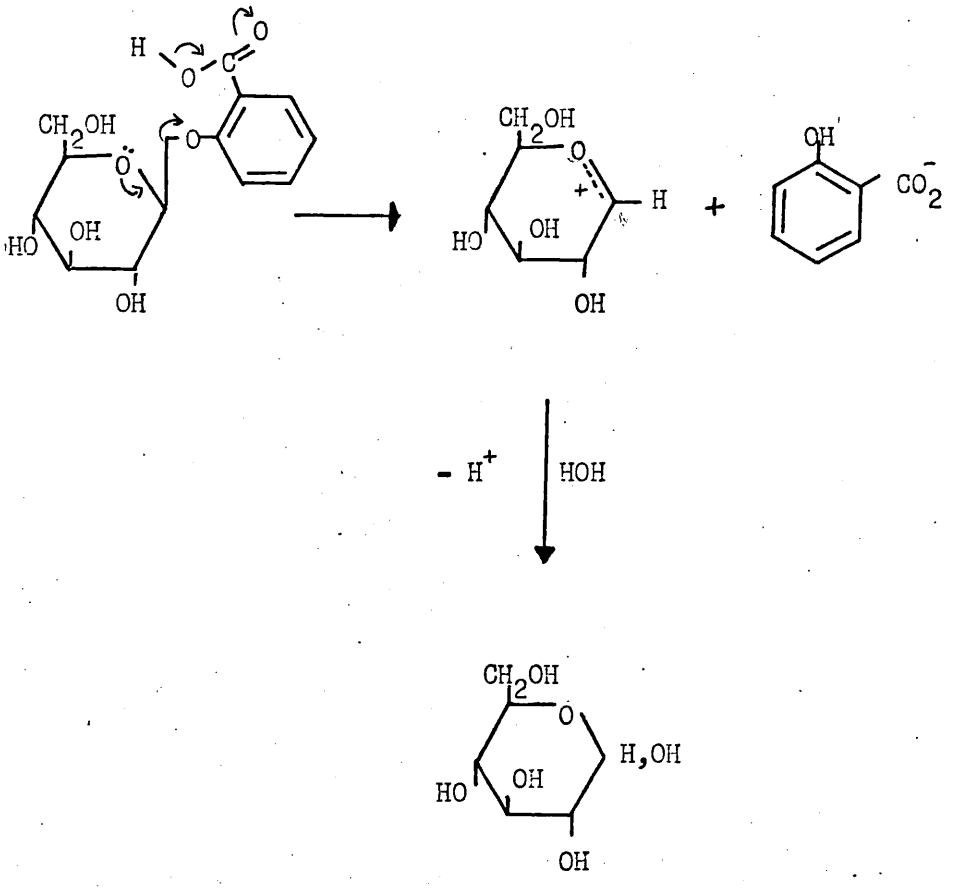


Scheme 1.1

Intermolecular base catalysis in the hydrolysis of aryl glycosides proceeds by a variety of mechanisms according to the structure of the glycosides. These mechanisms may be classified according to whether they involve (i) intramolecular neighbouring group participation by a hydroxyl group (ii) bimolecular displacement at the aromatic carbon atom (iii) bimolecular displacement at the anomeric carbon.

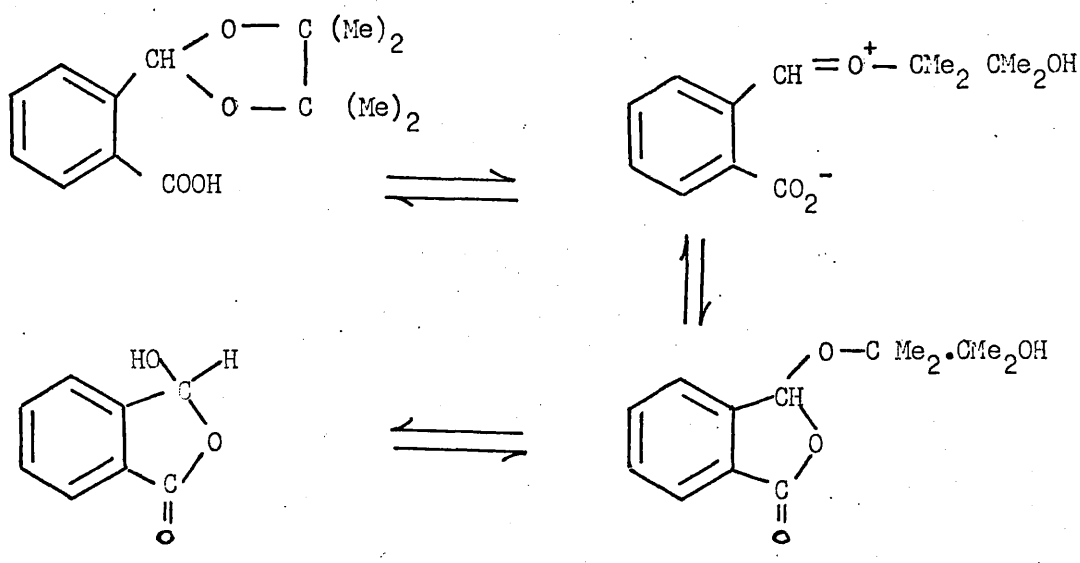
Intermolecular general acid catalysis, general base catalysis, or nucleophilic catalysis have not been established in a model system.

General acid catalysis has probably been included in every mechanism of glycosidase action. This mode of catalysis has so far only been observed in aryl glycosides and acetals. An example of general acid catalysis is found in the hydrolysis of 2-carboxyphenyl-β-D-glucoside, where an unionized carboxyl group provides intramolecular general acid catalysis,[22] as shown in Scheme 1.2.



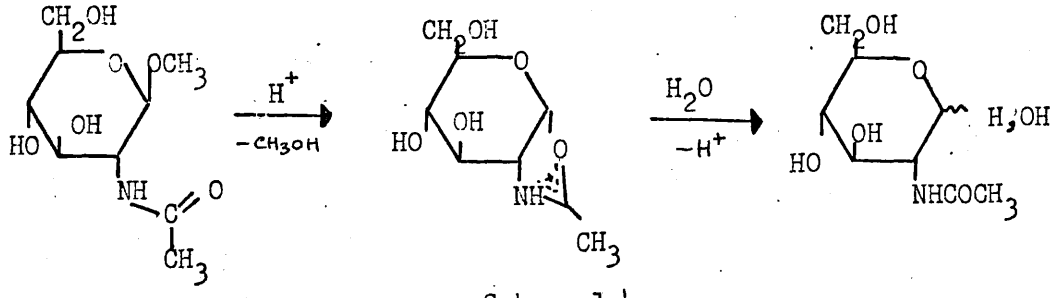
Scheme 1.2

Intramolecular nucleophilic catalysis has been established in the hydrolysis of 2-(o-carboxyphenyl)-4,4,5,5-tetramethyl-1,3-dioxolan in water.[23] (Scheme1.3).



Scheme 1.3

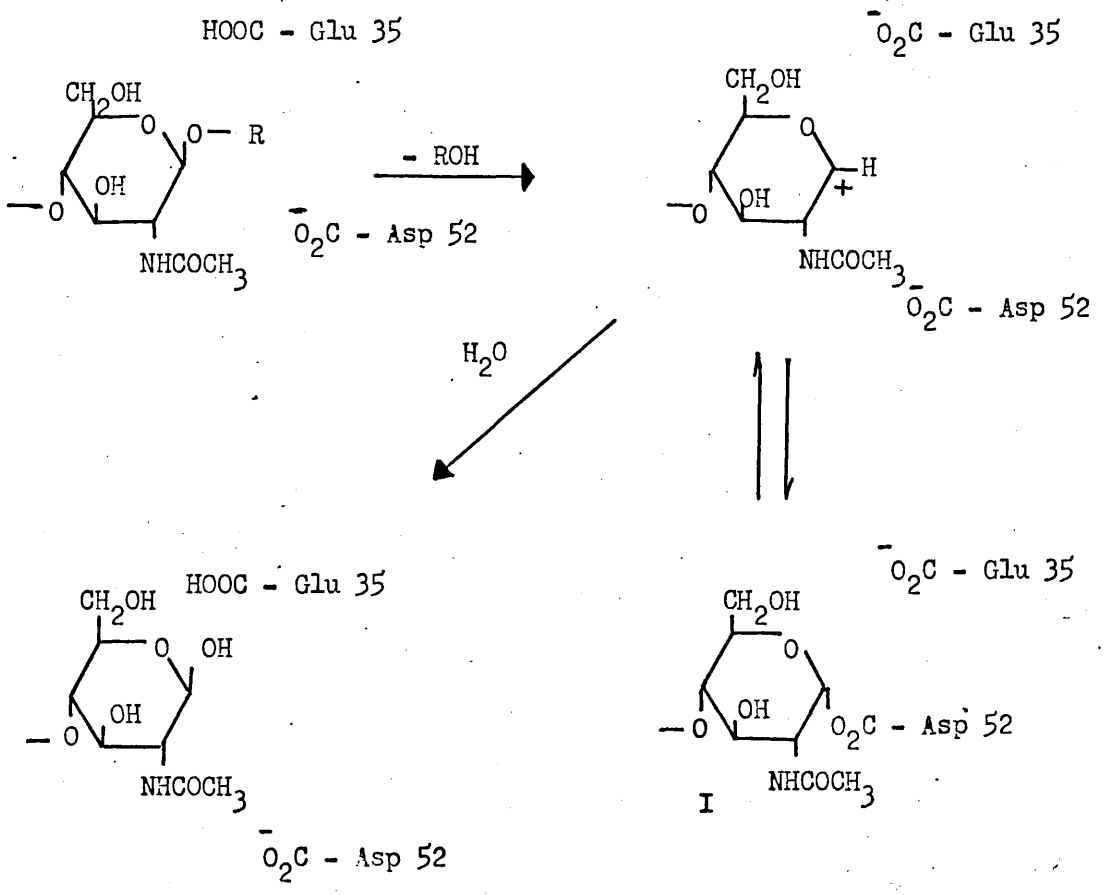
Another example of this type of catalysis, which at one time was thought to be important in lysozyme action,[24,25] involves intramolecular nucleophilic participation by an acetamido group (Scheme1.4).



Scheme 1.4

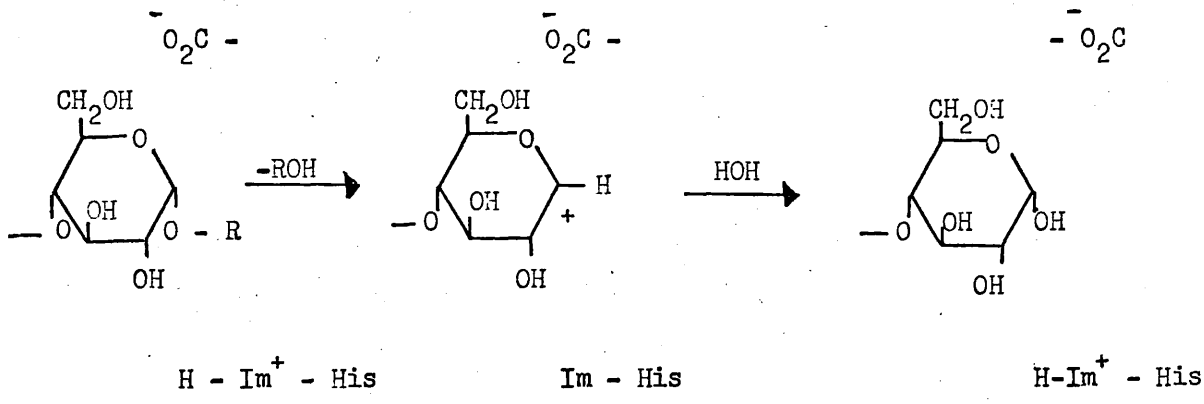
The proposed mechanisms of enzyme catalyzed glycosyl cleavage reactions and the interaction of active site functional groups may be considered in the light of this data available for model compounds. Three examples are presented to illustrate this.

1. Lysozyme. In the case of lysozyme there is evidence that the carboxylic acid group of glutamic acid 35 has an abnormally high pK (5.9)[15] and would be mainly un-ionized in the pH region 6-9 and therefore able to provide general acid catalysis,[24,25,26] analogous to the intramolecular general acid catalysis discussed earlier. The carboxylic group of Aspartic acid 52 has a normal pK (4.5)[15] and could therefore act in its ionized form either (i) as a nucleophilic catalyst to form a glycosyl enzyme intermediate[24,25] (I) or (ii) to stabilize the glycosyl cation by an electrostatic interaction.[26] (Scheme 1.5)



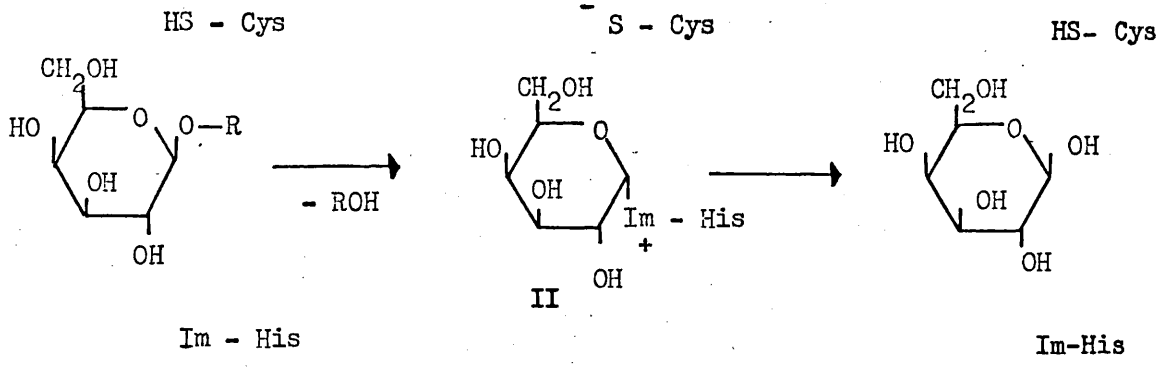
Scheme 1.5

2. α-amylase. There has been much speculation on the nature of the catalytic groups of amylases mainly based on the variation of the kinetic parameters with pH. It appears that sulphydryl groups[7] and N-terminal amino groups[27] are not necessary for catalytic action and the groups proposed are imidazolium and carboxylate.[28] The protonated imidazole group is visualized as acting as a general acid catalyst, and the carboxylate group as stabilizing the developing glucosyl cation electrostatically through ion-pair formation. (Scheme 1.6).



Scheme 1.6

3. β-galactosidase. The variation of enzyme activity with pH indicated a dependence on the ionization of two groups of pKa = 6.67 and 8.90 which were considered to be imidazole and sulphydryl. It was suggested that the unprotonated imidazole group acts as a nucleophile and the sulphydryl group as a general acid;[29] the reaction involving a galactosyl-enzyme intermediate (Scheme 1.7).



Scheme 1.7

Nucleophilic participation by an enzyme functional group must necessarily lead to the formation of a glycosyl-enzyme intermediate of the type I or II.

One general approach to the investigation of these proposals is the study of the properties of compounds which can be considered as models of I and II. This approach has been applied in this thesis through the study of N-glycosylimidazoles.

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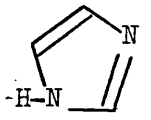
CHAPTER 2

SYNTHESIS OF N-GLYCOSYLIMIDAZOLES

INTRODUCTION

The central theme of the present investigation is the synthesis of N-glycosylimidazoles in order that their stability and conformational properties may be studied. The heterocyclic portion of these glycosylamines has been referred to as glyoxaline,[1] iminazole, 1,-3-diazole and imidazole.[2] Imidazole (1), which is the term used most frequently, indicates a five membered heterocyclic ring system containing an imino group and a tertiary nitrogen, and will be used throughout this thesis.

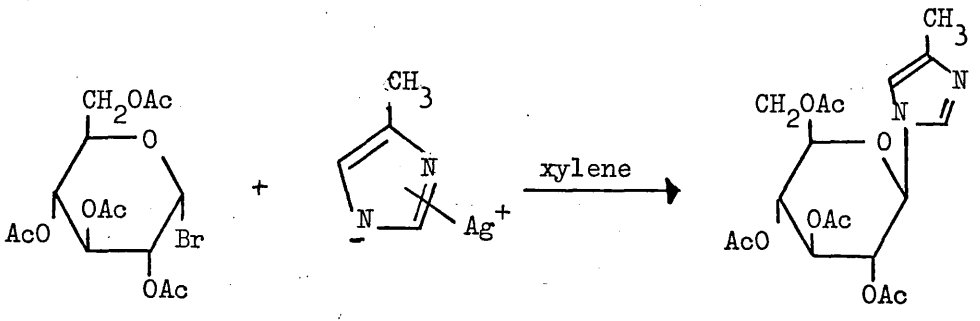


(1)

Several reviews[3-8] on the chemistry of the so-called imidazole nucleosides and nucleotides have appeared in the literature. A review[9] by Townsend lists seventy-one imidazole and substituted imidazole nucleosides and nucleotides of which forty-five bear a furanosyl and twenty-six a pyranosyl sugar moiety. These compounds have been synthesized by various methods, viz., condensation of heavy metal salts of imidazole and substituted imidazoles with acylglycosyl halides; ring closure of glycosylamines; and acid catalysed fusion reactions between glycosyl penta-acetate and a suitable derivative of the base. It is noteworthy that the nucleosides and nucleotides listed possess without exception the β-configuration at the anomeric carbon atom.

The first reported chemical synthesis of an imidazole nucleoside was accomplished in connection with the elucidation of the site of glycosidic

attachment of purine nucleosides isolated from nucleic acids, by the condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide (2) and the silver salt of 5(4)-methylimidazole (3), in xylene to give a crystalline 4-methyl-1[10]-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl) imidazole (4) in 11% yield.



(2) (3) (4)

This type of condensation with subsequent deacetylation was used by Bergmann and Heimhold[11] to synthesize 1[9]-D-glucopyranosylimidazole (5) and 1[9]-D-rhamnopyranosylimidazole (6) in yields of 6.1% and 8% respectively.

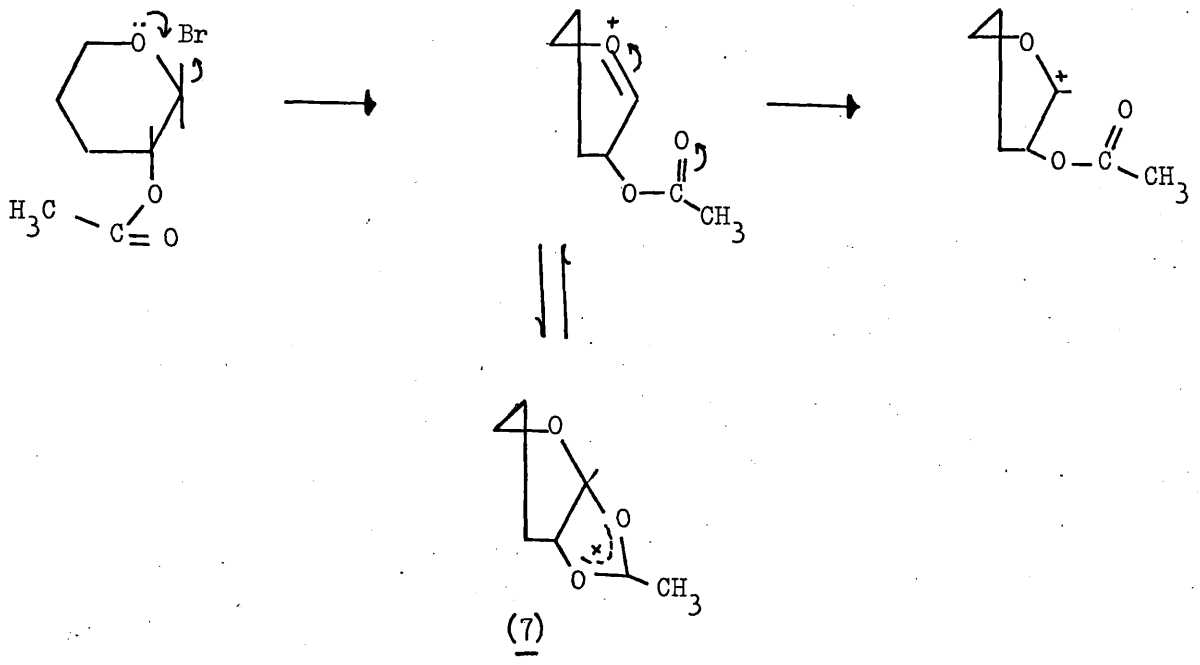
(5) (6)

These authors however did not establish the configurations at the anomeric carbons. More recently two groups of workers have reported the synthesis and characterization of both anomers of N-glucopyranosyl

and N-mannopyranosyl imidazoles. Lemieux and Saluja[12,13] have synthesised the N-glycosylimidazoles by the condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide with imidazole in the presence of tetra-n-butylammonium bromide in yields of 20% and 25% for the α- and β- anomers respectively. They were able to characterise and establish the anomeric configuration of their products mainly by nuclear magnetic resonance (NMR) spectroscopy. The NMR results were used to study the operation of the "anomeric effect" and "reverse anomeric effect" in these N-glycosylimidazoles, and this aspect will be discussed in greater detail in Chapter 3. Another group of workers in Poland, Jański and Sokolowski[14,15] have carried out investigations of N-glycosylimidazoles derived from D-glucose, D-mannose and D-galactose. These authors have been able to isolate the β-anomers, and this configurational assignment was supported by optical rotation studies. Our own studies discussed in Chapter 4 of the optical rotation of these glycosylamines, have shown this to be an unreliable method of establishing anomeric configuration. These authors however established the pyranoside structures of their glycosides by periodate oxidation. In 1971, they were able to substantiate the assignment of the β-configuration of their products by NMR spectroscopy.[15] These authors however failed to notice any conformational changes in their N-glycosylimidazoles.

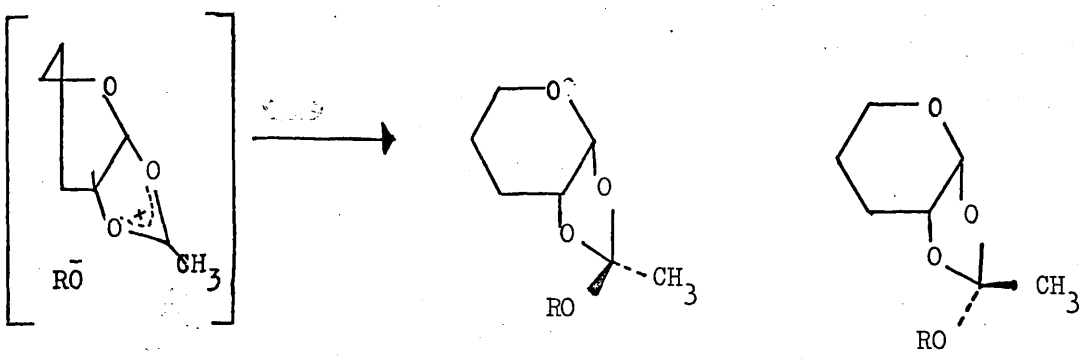
Of the many reactions which permit the synthesis of glycosides from suitably protected glycosylhalides, the Koeings-Knorr[16] reaction is one of the most widely used for the synthesis of O-glycosides and in particular of oligosaccharides. In its original form the reaction consisted of treating a fully acetylated glycosyl halide with an alcohol, dissolved in an inert solvent, in the presence of either silver carbonate or silver oxide. In the broadest terms it can be taken that those

glycosyl halides that bear an acyloxy group at C-2, which is cis to the 1-halide normally react with inversion of configuration, whilst the corresponding trans halides react with retention of configuration. This sterospecificity arises because the acetoxy group at C-2 participates in the reaction by the formation of a 1,2-cyclic carboxonium cation (7) and guiding the incoming nucleophile into the 1,2-trans position.[17]



(7)

This method is therefore most readily applied to the synthesis of 1,2-trans glycosides. An additional complication of the Koenigs-Knorr reaction arises due to the attack of the incoming nucleophile on the 1,2-cyclic ion (7) giving rise to diastereoisomeric ortho esters (8) and (9).[20]



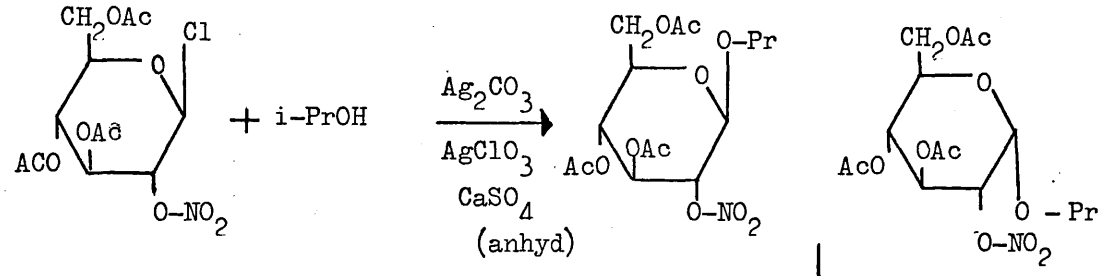
(8) (9)

Since acylglycosyl halides are generally prepared under anomerising conditions the thermodynamically more stable α- compounds are by far the more common in the glucose, mannose and galactose series, and these would then normally react to give the β-glycosides. The chemical synthesis of α- compounds has always proved difficult in the past, as a consequence of this and of the need to prepare α-glycosides, recent developments have made available several new procedures which have been reviewed extensively in the literature,[21-24] some of which are discussed here.

It has been demonstrated on many occasions that mercuric salts in polar solvents such as nitromethane or acetonitrile favour the formation of α- glycosides.[25,26] Ferrier and Prasad[27] were able to obtain α- and β-xylosylxyloses in 25% and 4% yields respectively, by the condensation of 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide with benzyl-β-D-xylopyranoside 2,4-phenyl boronate in anhydrous nitromethane containing mercuric cyanide. In this case it may be noted that a non participating group is present on C-2. The condensation of acylglycosyl halides with the mercuric (II) acetate complex of 4(5)-nitroimidazole in nonpolar solvent as toluene is reported by Gughelnis and Vegin[28] to give 19%

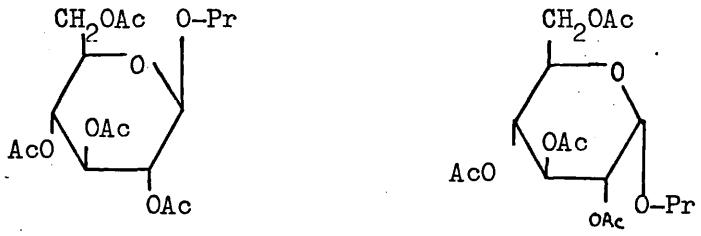
yield of 4-nitro-1-(α-D-arabinopyranosyl)imidazole.

Another approach involves the use of β-chlorides carrying a non-participating group at C-2. Wolfrom, Pittet and Gillman[29] introduced the use of stable crystalline 3,4,6-tri-O-acetyl-2-O-nitro-β-D- gluco-pyranosyl chloride (10) in a modified Koenigs-Knorr reaction. In 1963 Wolfrom et al.[30] reacted isopropyl alcohol and 3,4,6-tri-O-acetyl-2-O-nitro-β-D-glucopyranosyl chloride.



(10)

catalytic reduction and acetylation

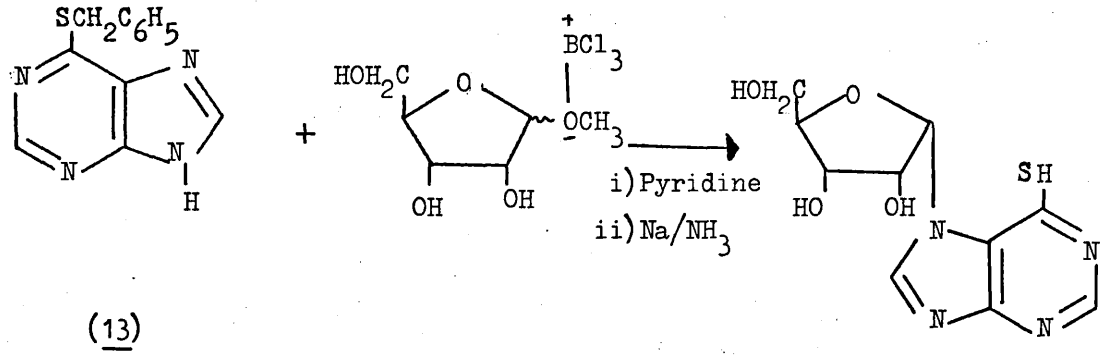


(11) (12)

The alcohol served as solvent and reactant, a 4.7% yield of crystalline isopropyl tetra-O-acetyl-β-D-glucopyranoside (11) and 35% yield of the crystalline α-D-anomer (12) were obtained.

Results of appreciable interest have been obtained with benzylated glycosyl halides which are devoid of a participating group at C-2, and hence allow the synthesis of α-D-glycosides.[31]

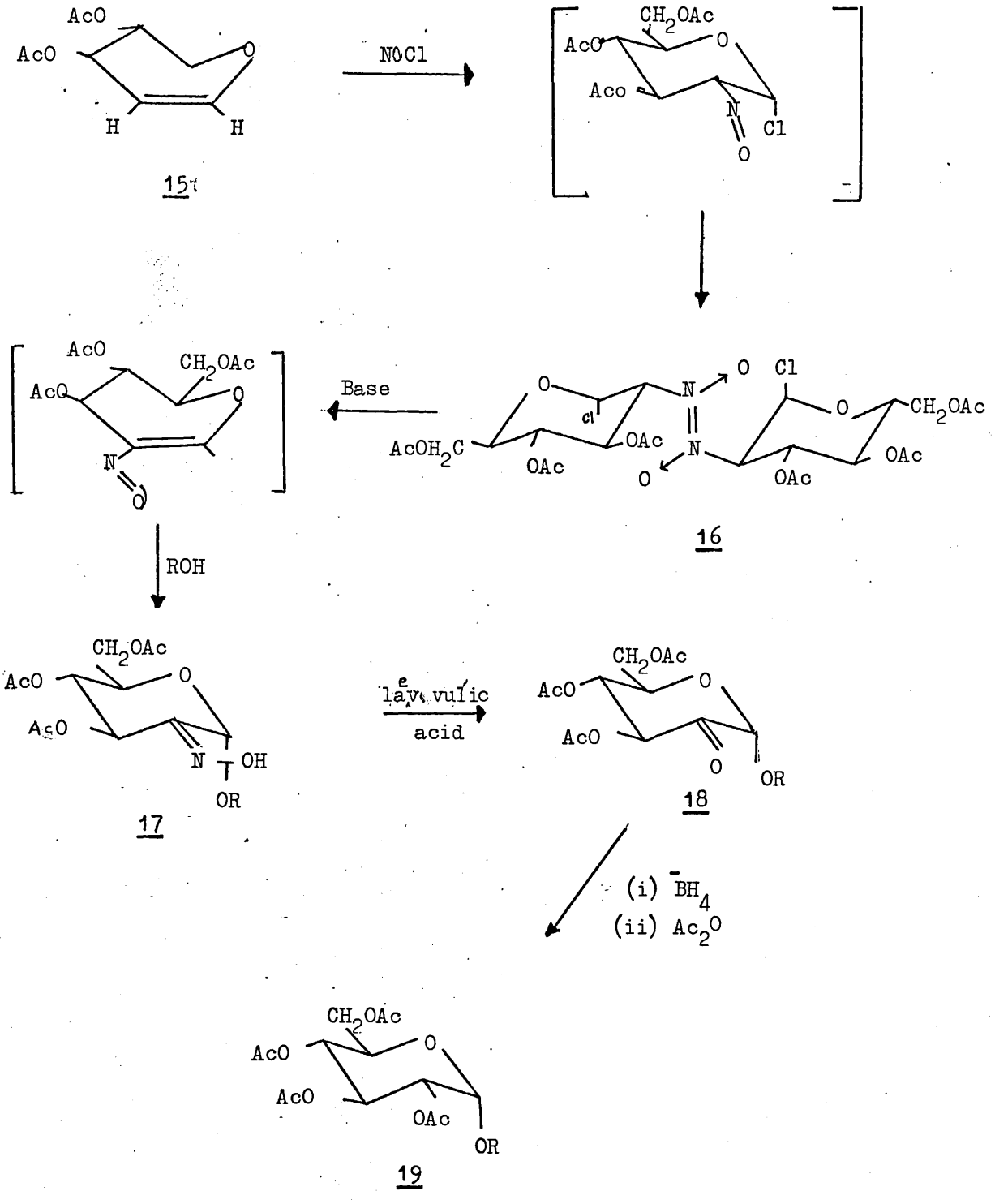
The use of methyl α-D-glucoside-borontrichloride reagent has also been reported in the synthesis of α-D-glycosides, although a mixture of α- and β-anomers are commonly obtained. Bonner, Bourne and McNally[32] studied the reactions of methyl α-D-glucoside-boron trichloride reagent with alcohols, phenols and monosaccharides, and were able to obtain mixtures of α- and β-anomers. Furukawa et al.[33] were however able to apply this method successfully towards the synthesis of purine α-D-ribonucleosides.



(13) (13a)

6-benzylthiopurine (13) on refluxing with the borontrichloride complex afforded 9-α-D-ribofuranosyl-6-thiopurine (13a) in 53% yield.

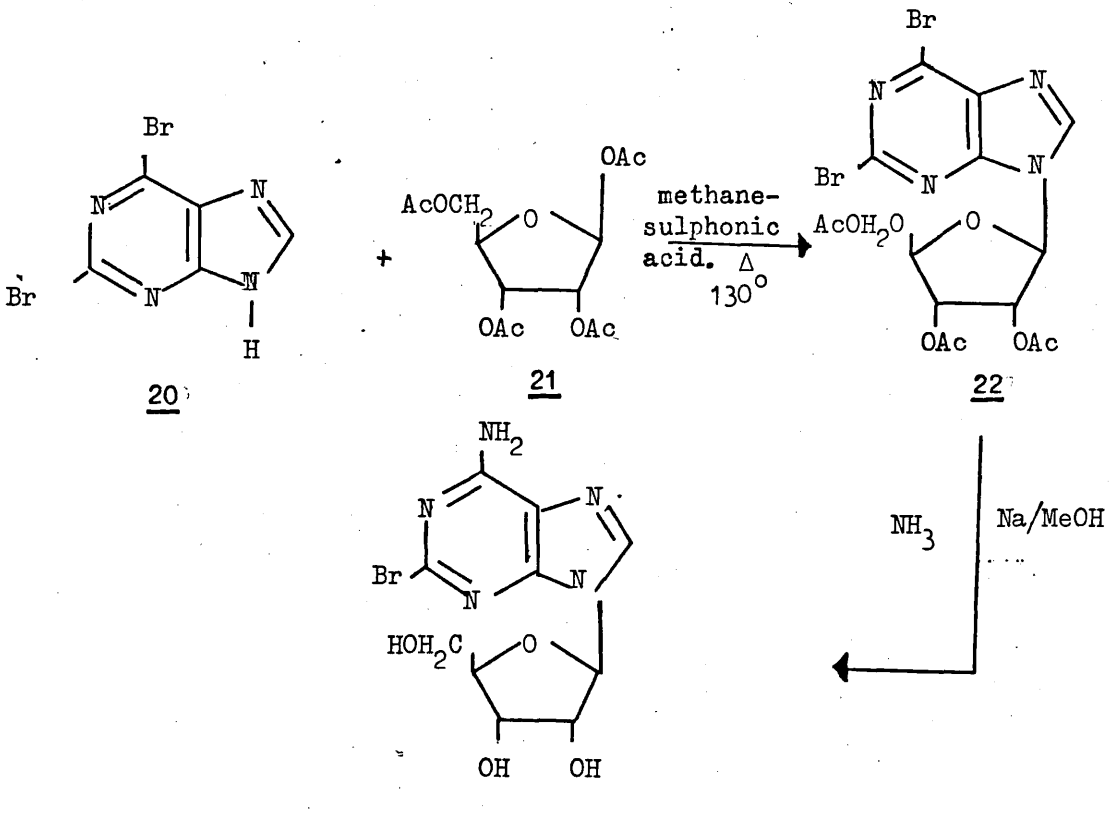
Glycosides have also been prepared from glycals, and work with nitrosyl chloride in particular has proved profitable since via this route have come new syntheses of α-D-glucosides and 2-amino-2-deoxy-α-D-glucosides.[34-37] Addition of nitrosyl chloride to tri-O-acetyl-D-glucal (15) gave the dimeric adduct (16) which then reacted with alcohols or phenols in N,N-dimethylformamide to give the corresponding 2-oximino-α-D-glucosides (17).



Deoximation of (17) using levulic acid gave the ketoglucoside (18) which on subsequent reduction with sodium borohydride and on acetylation gave the α-D-glucoside (19) almost stereospecifically.

Using this method phenyl- and α-naphthyl-tetra-O-acetyl-α-D-gluco-pyranosides were obtained in 70% and 40% yields respectively. Yields of α-anomers of nearly 80% were reported by the same group of workers[34-37], using primary, secondary and tertiary alcohols as aglycones.

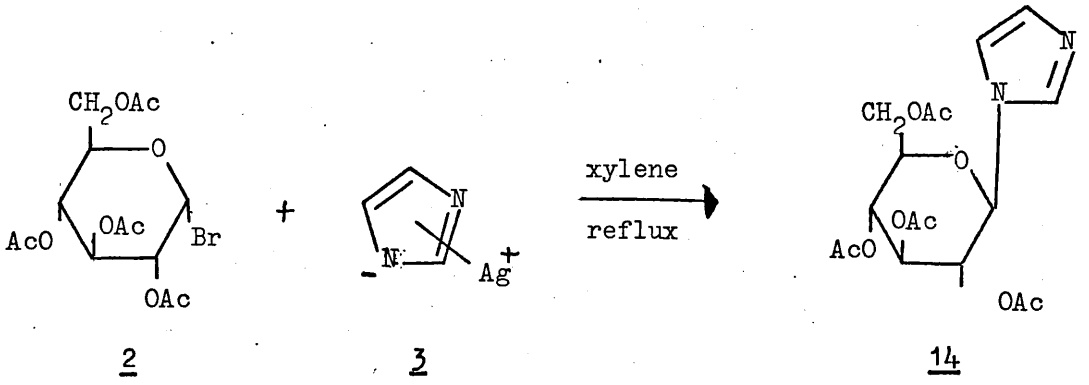
A recent advance in the field of nucleoside synthesis was the advent of acid catalysed fusion reaction. This technique was first used in the area of nucleoside synthesis with purines to produce N-glycosylpurines[38] and has been subsequently applied to various other heterocyclic systems. The preparation of 2-bromo adenosine (23) illustrates this procedure. Fusion of 2,6-dibromopurine (20) in vacuo with 1,2,3,5-tetra-O-acetyl-β-D-ribose (21) in presence of toluene-p-sulphonic acid, affords the acetylated D-ribofuranosyl-9H-purine (22) in 48% yield; treatment of (22) with methanolic ammonia simultaneously deacetylates the sugar residue and replaces the bromine atom (preferentially at C-6) by an amino group to give 2-bromoadenosine (23).[39]



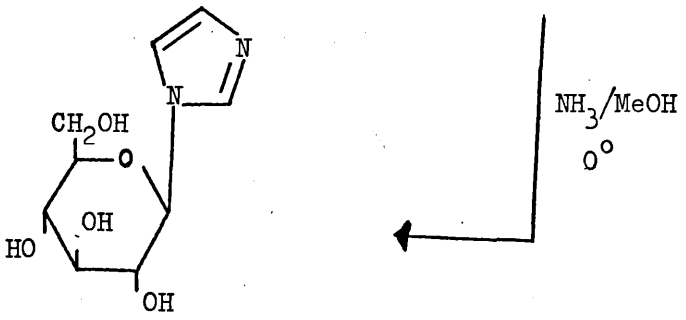
A yield of 7.8% of 9-α-D-glucopyranosyl adenine by the fusion reaction of 2,3,4,6-tetra-O-acetyl-1-O-trichloroacetyl-α-D-gluco pyranose and benzamidopurine in presence of toluene p-sulphonic acid has also been reported.[40]

Results and Discussion

In this work the preparation described by Bergmann and Heimhold[11] outlined below was repeated, when a crystalline compound (14) was



2 3 14



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readily obtained whose melting point 205-206° was in good agreement with that reported and the elemental analysis were consistent with the structure (14). The specific rotation of -7.3° also was in good agreement with that reported. The NMR spectrum of (14) in $CDCl_3$ showed a multiplet at 4.65 τ for the methine protons of the gluco-pyranose ring. It was therefore not possible to establish the anomeric configuration of (14). The four signals due to the acetyl

methyl protons at 7.93, 7.95; 8.00 and 8.14 τ are in the region (7.88 - 8.03 τ) normally found for equatorially situated acetyl methyl protons on a 6-membered ring.[41] The O-deacetylated compound (5) m.p. 215-216°, specific rotation +13.6°, gave a NMR spectrum in D_2O which showed the anomeric proton H-1' at 4.16 τ with a spacing of 8.4 Hz. It is well known that vicinal coupling constants are dependent upon molecular conformation i.e. the dihedral angle between the hydrogen substituents.[42,43] A coupling constant of 8.4 Hz for H-1' corresponds to a torsional angle of about 180° between the C-1'-H and C-2'-H bonds which requires (5) to possess the β-configuration.

No trace of the α-anomer was detected in the reaction mixture following the procedure of Bergmann and Heimhold, hence an alternative method was needed to synthesise the α-anomer required for this work.

The following methods were attempted unsuccessfully.

(a) Use of methyl α-glucoside-boron trichloride complex.

Following the experimental procedure of Bonner et al.[32] and Furukawa,[33] an attempt was made to condense excess imidazole with the boron trichloride complex of methyl α-D-glucoside in chloroform. On addition of imidazole to the complex a white solid precipitated (imidazole-hydrochloride). After refluxing for about two hours the solution was added to water. The two layers were separated, the aqueous layer was then treated with IR-4B OH^- resin to remove any $MeOH,BCl_3$ and concentrated to a small volume. Thin layer chromatography and paper chromatography revealed no formation of glycosyl-imidazoles in the aqueous as well as in the chloroform extracts. An attempt was also made to effect condensation in the presence of pyridine which acts as an acid acceptor, but this also proved unsuccessful.

(b) Fusion method.

β-glucose penta-acetate and imidazole were melted together at 130-135° under reduced pressure, chloroacetic acid was added and the reaction mixture heated for one hour. The reaction mixture was then extracted with chloroform. The chloroform extract, after repeated washing with water, was dried and concentrated to dryness to give a dark syrup which, on crystallisation from propan-1-ol, gave β-glucose penta-acetate.

(c) Condensation of sugar with a base in the presence of phenyl polyphosphate.

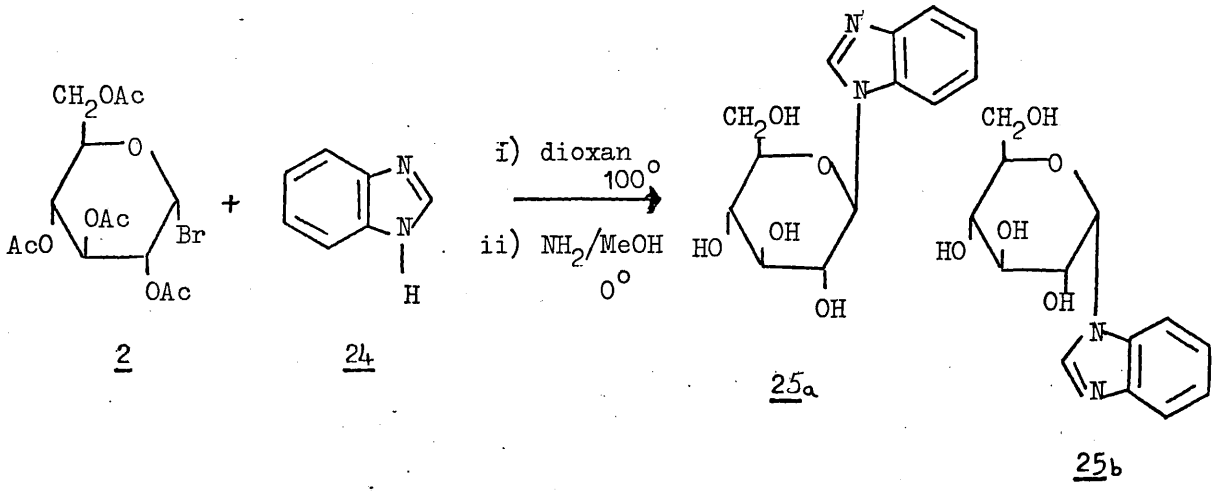
The method developed by Schramm and co-workers[57,58] was attempted, when glucose, imidazole and phenyl polyphosphate were heated in N,N-dimethylformamide. No trace of glycosylimidazoles were detected in the reaction mixture.

(d) Condensation of Dimeric tri-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (16) and imidazole.

Following the experimental conditions developed by Lemieux et al.[34-37] for the synthesis of (16), (16) and imidazole were refluxed in dichloromethane to obtain the oxime which was difficult to purify. The NMR spectrum of the oxime in DMSO-d_6 showed a singlet at 2.1 due to C=NOH proton. The IR spectrum showed a weak absorption at 1660 cm^{-1} attributable to the C=N stretching vibration. The oxime was then treated with laevulinic acid and extracted with chloroform. The chloroform extracts were concentrated to dryness to give a syrup which was then reduced with sodium borohydride in tetrahydropyran. After the usual work-up for removing boric acid, the residual solution showed no traces of any glycosylimidazoles. In another experiment the aqueous

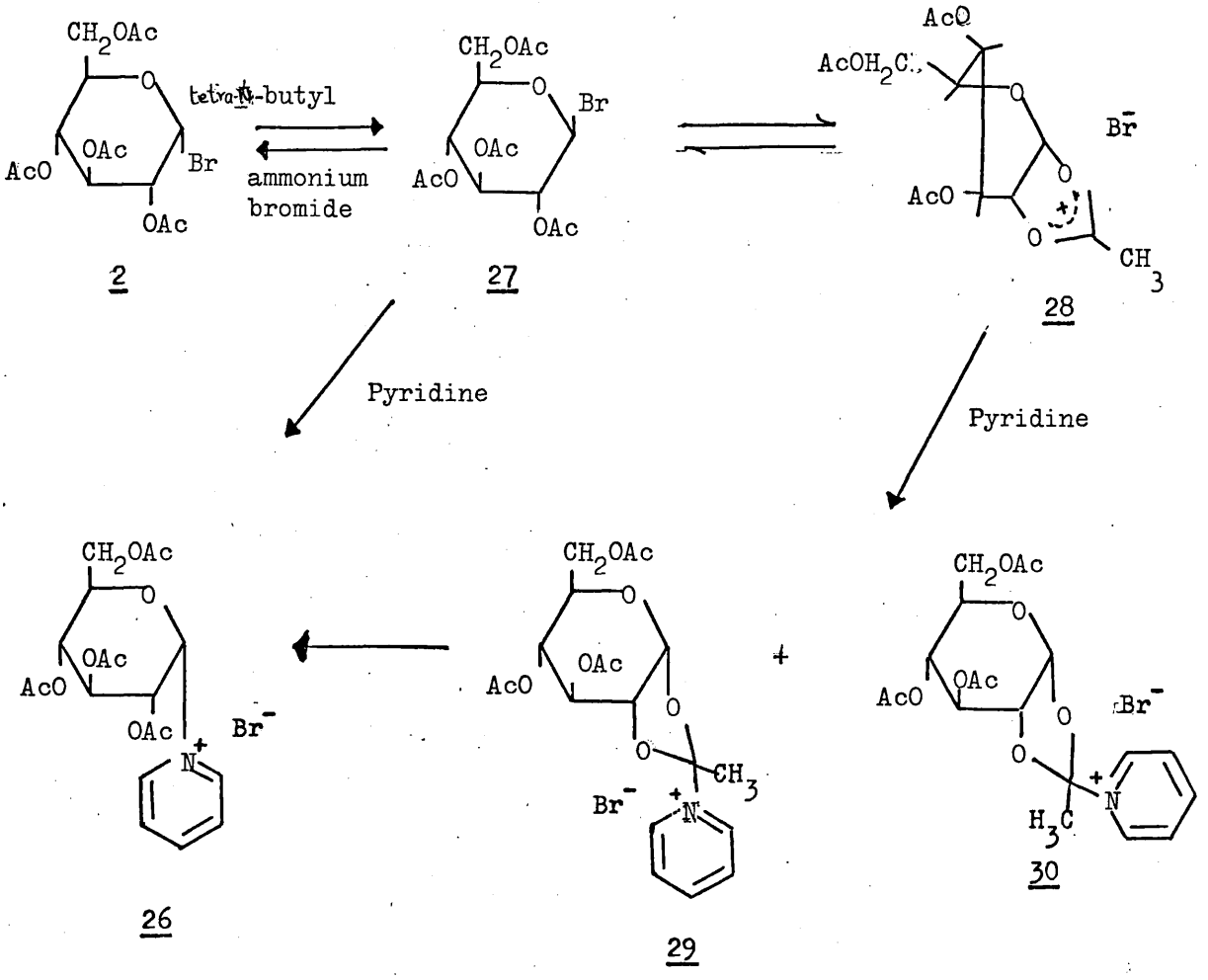
layer obtained after the treatment of laevulinic acid and sodium borohydride was analysed for glycosylimidazoles, no formation of these compounds was detected.

Todd and co-workers[44] in 1953 reported the synthesis of 1-D-glucopyranosylbenzimidazoles by the condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide and benzimidazole (24) in dioxan. Yields of 3.7% and 21% of the α- and β-anomers respectively were obtained. The same reaction was subsequently used by Foster et al.[45] in the synthesis of 1-glycosylbenzimidazoles.



In 1965, Lemieux and Morgan[46] reported that when tetra-O-acetyl-α-D-glucopyranosyl bromide (2) was reacted with pyridine a 3:2 mixture of the anomeric N(tetra-O-acetyl-D-glucopyranosyl) pyridinium bromides were formed in which the α-anomer predominated. The same reaction when carried out in the presence of tetra-n-butylammonium bromide afforded

the α-anomer (26) in over 90% yield.

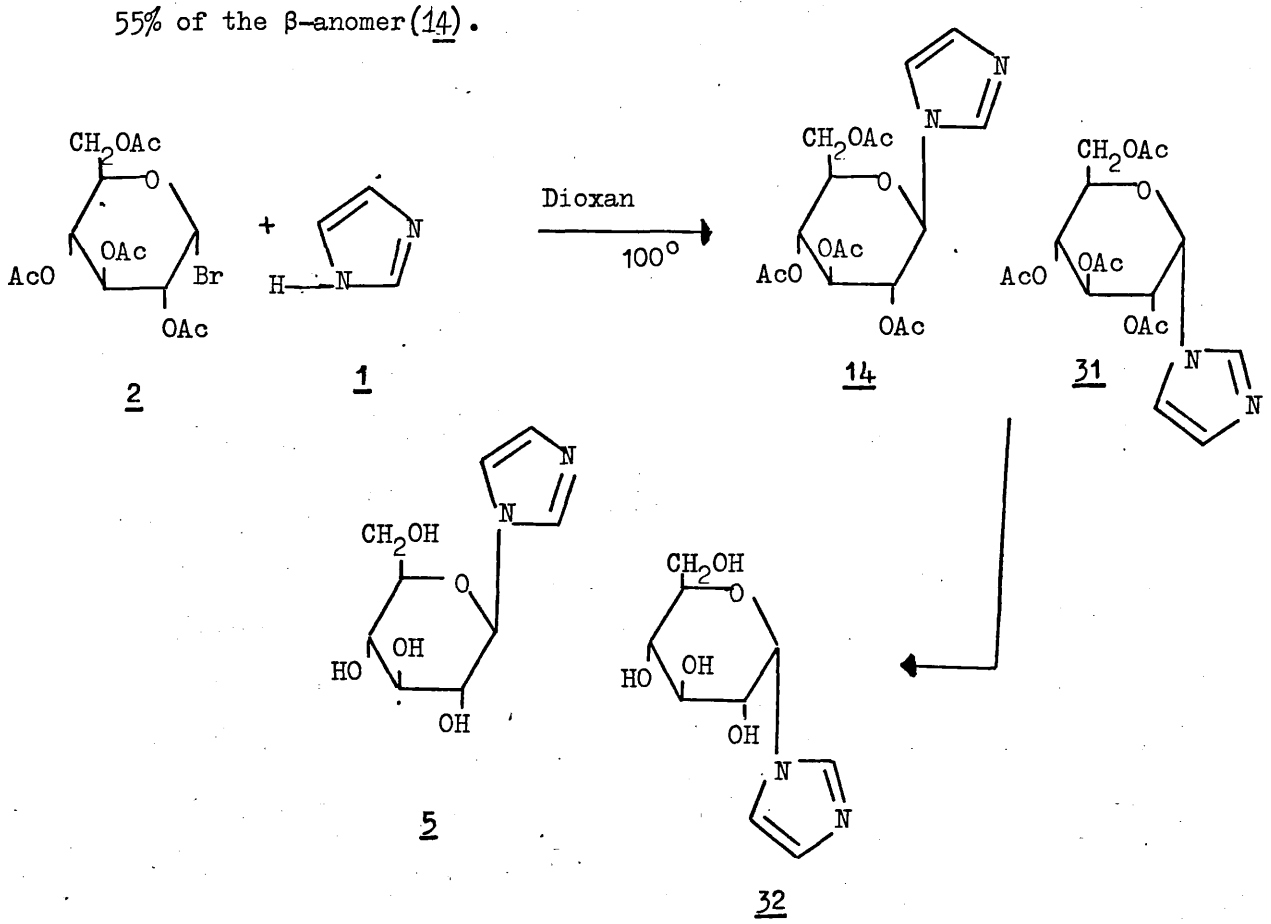


These authors concluded that the initial presence of the bromide ion in the reaction mixture led to a rapid equilibrium of the starting material α-bromide (2) with its β-anomer (27) and that the thermodynamically less stable and more highly reactive β-bromide was the source of N-(tetra-O-acetyl-α-D-glucopyranosyl) pyridinium bromide (26).

The acetoxonium ion (28) in pyridine can be in equilibrium with the orthoamide type intermediates (29) and (30). Rearrangement of (29) and (30) was proposed to lead to the formation of the α-anomer (26).[46,47,13] This proposed mechanism could be extended to explain the formation of an anomeric mixture of glucopyranosylbenzimidazoles when free benzimidazole rather than a silver salt is used in the reaction, where the Br^- ion is quickly removed by Ag^+.

In our hands the addition of tetraethylammonium bromide gave a reaction mixture which was more difficult to purify and did not lead to a greatly increased yield, this method was therefore abandoned.

Following the experimental conditions of Todd and co-workers,[44] the condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide (2) and imidazole (1) in dioxan at 100° afforded about 2.4% of the 1'-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)imidazole (31) and 55% of the β-anomer (14).



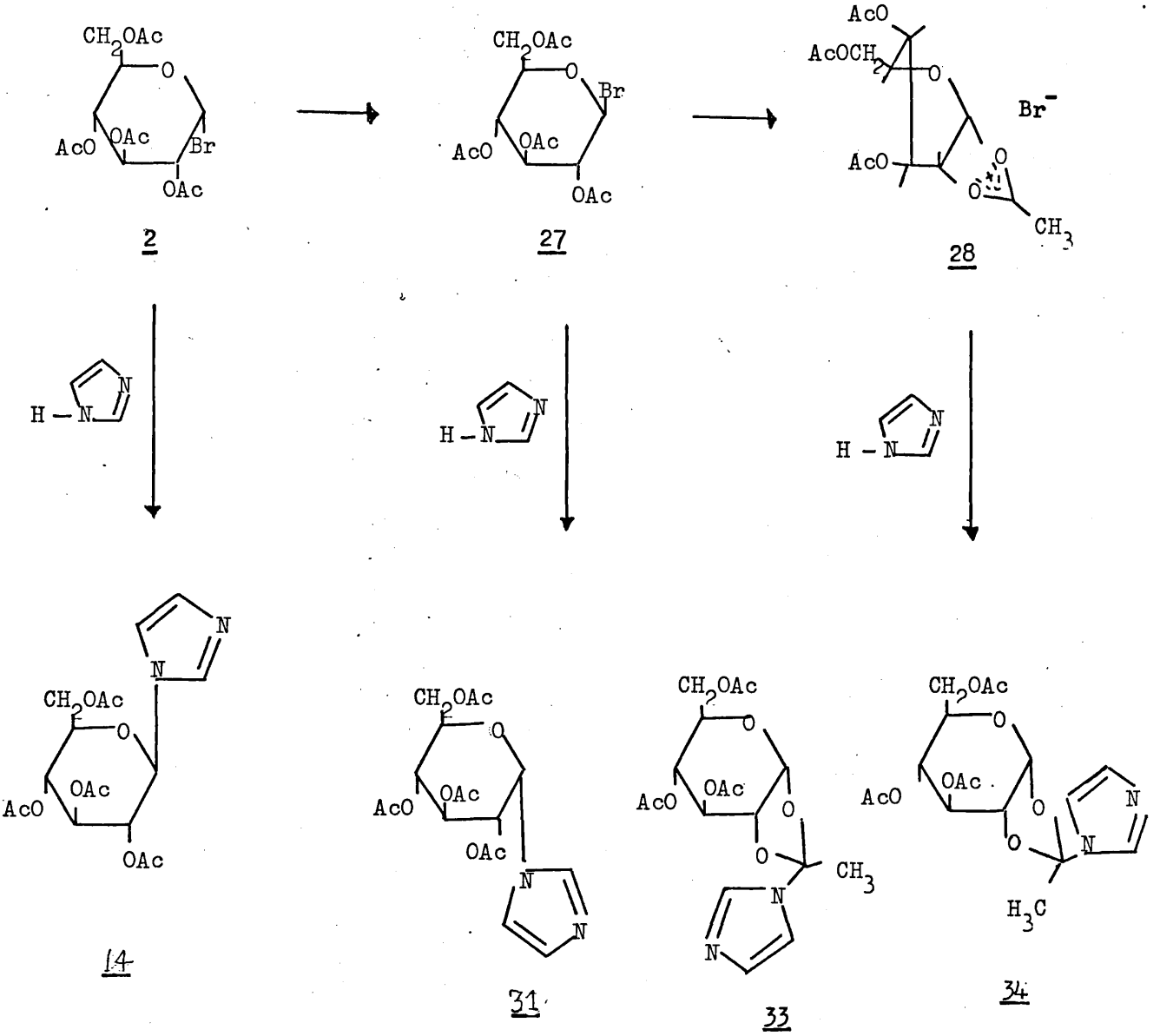
The β-anomer was characterised by its melting point, optical rotation, elemental analysis and by comparison of its NMR spectrum with that obtained by the method of Bergmann and Heimhold.

The NMR spectrum of the α-anomer (31) is reproduced in Fig. 3.1 page 79. The parameters are presented in Table 3.9-10, page 102-3 and will be discussed in greater detail in Chapter 3. The chemical shift of the anomeric proton of 3.89 τ and the coupling constant of 5.1 Hz are in accord with the α-configuration. The specific rotation of +118° of (31) as compared to -7.3° for (14) is consistent with this anomeric configuration. The NMR spectrum Fig. 3.5 page 83 of the O-deacetylated α-anomer (32) shows the anomeric proton at 3.86 τ with a spacing of 5.7 Hz as compared to 4.16 τ for the β-anomer (5). It is generally accepted that an axial proton will absorb at about 0.5 ppm higher field than its equatorial counterpart.[43,48]

Several detailed studies of the solvolyses of glycosyl halide derivatives have been undertaken and have led to the conclusion, that reaction usually occurs by way of an S_N1 mechanism, although inversions are frequently observed and S_N2 character is known to intrude under some conditions, notably in solvents of low polarity and in the presence of strong nucleophiles.[49-53]

The work of Rhind-Tutt and Vernon [50] suggests that in the absence of neighbouring group participation, α-D-glucopyranosyl halide derivatives react with predominant inversion. Thus the formation of 1'-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)imidazole (14) could result from a nucleophilic attack of imidazole on the α-bromide (2) with inversion of configuration at the anomeric centre. As mentioned earlier, in the presence of bromide ion a rapid equilibrium between the α-bromide (2) and the β-bromide (27) is established and a nucleophilic attack by imidazole on the β-bromide could furnish 1'-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)imidazole (31).

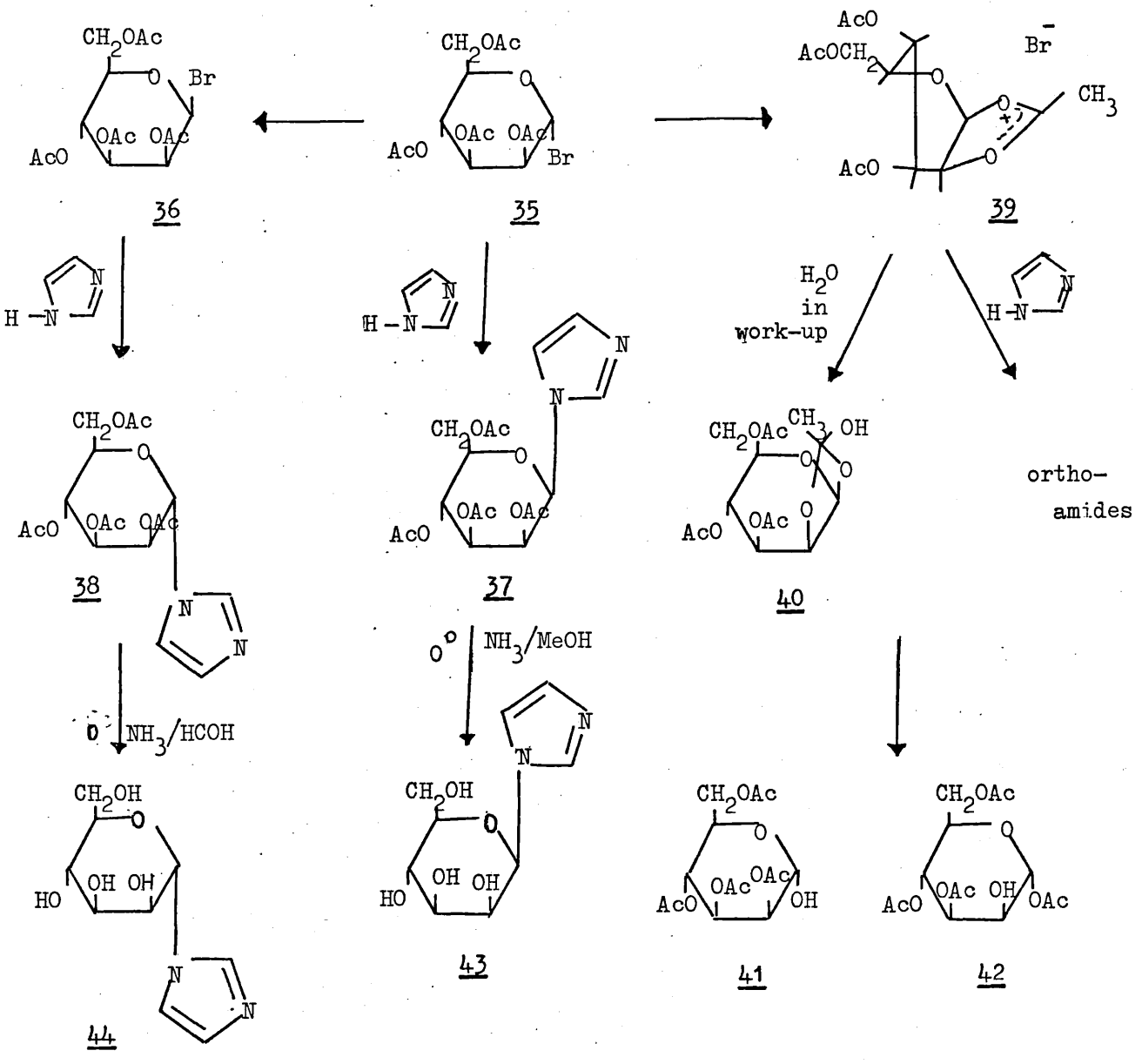
The β-bromide (27) can also be expected to yield readily the 1,2-acetoxonium ion (28) which would react with imidazole to give orthoamides (33) and (34), provided the liberated hydrogen bromide is captured by a suitable base such as imidazole itself. It has been proposed that the orthoamides are the major products of the reaction of glycosyl halide and imidazole only if the reaction is carried out at room temperature.[13]



One of the objectives of the present work, as already indicated, was to study the conformational properties of the N-glycosylimidazoles.

The manno- and galacto- compounds were synthesised according to the experimental procedure followed for the gluco compounds i.e. by refluxing the appropriate glycosyl halide with two molar proportions of imidazole in dry dioxan.

The explanation as to how the products of the reaction of tetra-O-acetyl-α-D-mannopyranosyl bromide (35) and imidazole arise, follows a similar line of reasoning to that developed for the gluco series.



By the application of the trans rule and of nucleophilic participation of the C-2 acetoxy group, one would expect the α-product to predominate. In the absence of neighbouring group participation α-D-mannopyranosyl halide derivatives yield anomeric mixtures of products of nucleophilic attack at C-1.[59] The yield (4.7%) of 1-(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl) imidazole (38) was lower than that of the β anomer (37) (25%). We believe this violation of the "trans rule" to be due to absence of participation of the C-2 acetoxy group (39) and the decreased stability of the α-anomer (38) resulting from the operation of the reverse anomeric effect[54] (Chapter 3).

A somewhat interesting observation was the isolation of a compound whose elemental analysis and NMR spectrum suggested structure (41) or (42).

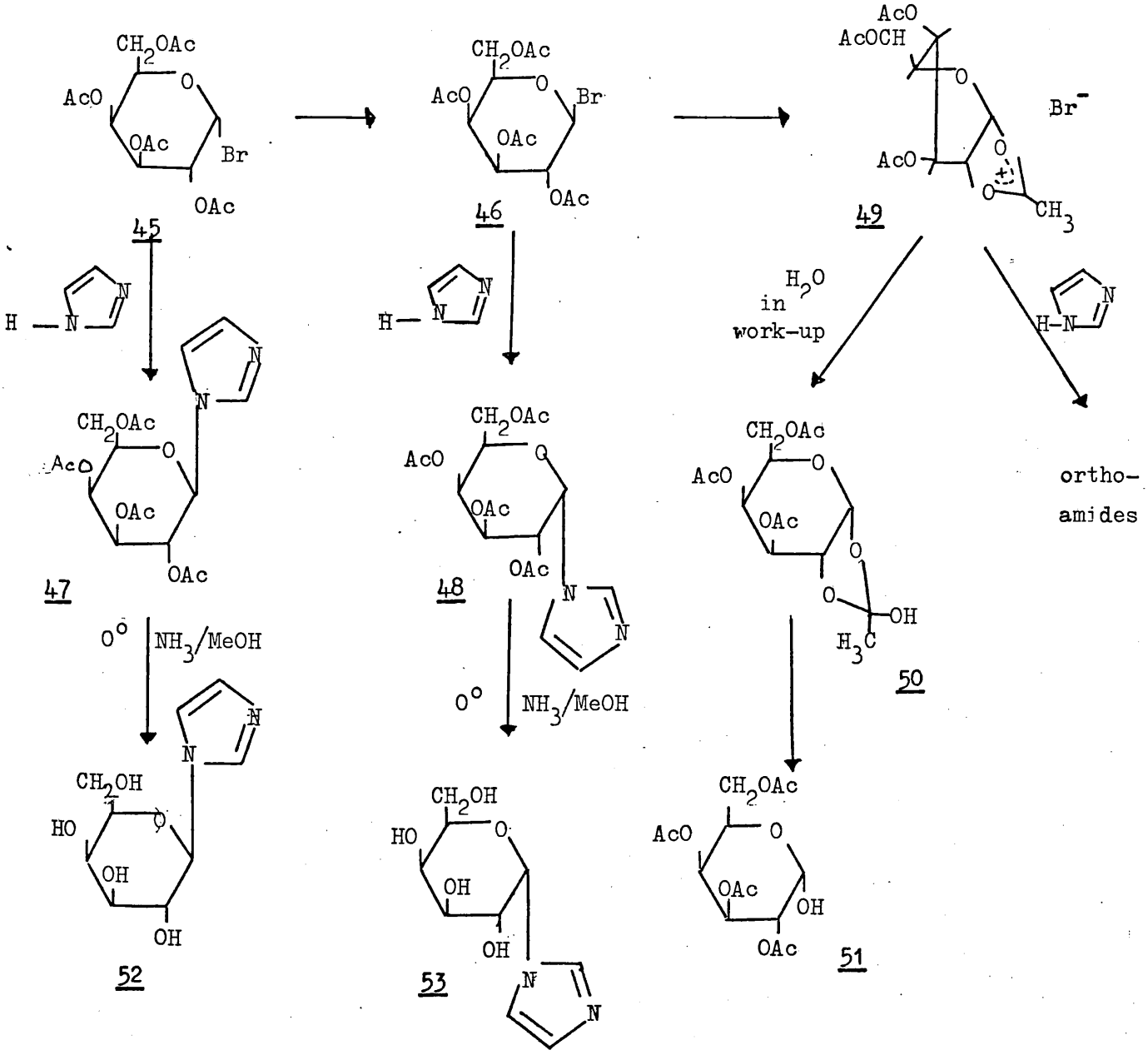
The identity of 1-(2',3',4',6'-tetra-O-acetyl-β-D-mannopyranosyl)-imidazole (37) was established by its elemental analysis, and NMR parameters are given in Tables 3.11,12 page 104-5; compound (37) had a specific rotation of -14.55°.

1-(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl) imidazole (38) possessed a specific rotation of +37.7° which is in accord with the α-configuration. The NMR spectrum is reproduced in Fig. 3.11 and the parameters are given in Tables 3,17,18, page 110-11.

The O-deacetylated compound, 1-β-D-mannopyranosyl imidazole (43) had a specific rotation of +27.48° and the NMR parameters are presented in Tables 3, 13,14, page 106-7.

1-α-D-mannopyranosylimidazole (44) possessed a specific rotation of +63.7° and its NMR spectral data are presented in Tables 3.15,16, page 108-9.

The reaction of tetra-O-acetyl-α-D-galactopyranosyl bromide (45) with imidazole in dioxan afforded 1-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)imidazole (47) in 43.7% yield and an impure sample of 1-(2',3',4',6'-tetra-O-acetyl-α-D-galactopyranosyl) imidazole (48) which after repeated attempts at purification on a silica column did not analyse correctly. The possible source of impurity could be the presence of the tetra acetate (51). It was however possible to purify the O-deacetylated α-anomer (53) on a basic ion-exchange resin column.



The 1-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)imidazole (47) had a specific rotation of +11.43°. NMR parameters of 47 (Tables 3.19-20, page 112-13) shows a coupling constant of 9.1 Hz for the anomeric proton. A coupling constant of this magnitude corresponds to a dihedral angle of about 180° which requires the galactosylamine to possess a β-configuration. The O-deacetylated β-anomer (52) had a specific rotation of +51.02°.

1-α-D-galactopyranosylimidazole (53) had a specific rotation of +130.16° and the NMR spectral data Table 3.23-24, page 116-17, support the assignment of the α-configuration at the anomeric carbon.

EXPERIMENTAL

1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)imidazole (14).

The compound was prepared according to the method of Bergmann and Heimhold[11] by the condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide prepared according to the method of Lemieux[56] and freshly prepared silver salt of imidazole in anhydrous xylene at reflux temperature for 4h. The mixture was filtered hot and allowed to stand for 12 h at 0° when a crystalline solid deposited which was filtered, washed well with water and dried under vacuo. Thin layer chromatography using benzene-methanol (9:1) (v/v) showed this to be a single component. Crystallisation with propan-1-ol gave 43% yield of (14) m.p. 205-206° (lit.[11], 205-208°), $[\alpha]_D^{21}$ - 7.3° (c 1.5 in $CHCl_3$) [lit.[11], -9° (c 0.7 in $CHCl_3$)], $\lambda_{max.}$ 214 nm (log ε 3.58).

Elemental analysis calcd. for $C_{17}H_{22}O_9N_2$:

C = 51.25; H = 5.56; N = 7.03%

Found C = 51.13; H = 5.41; N = 7.19%.

1-β-D-glucopyranosylimidazole (5).

Compound (14) on deacetylation in methanolic ammonia at 0° for 45 min. and after crystallisation from propan-1-ol gave (55%) yield of (5). Paper chromatography using n-butanol-ethanol-water (40:11:19; v/v) showed this to be a single component.

m.p. 215-216° (lit.[11] 217-218°; lit.[14], 218-220°), $[\alpha]_D^{21}$ +13.6° (c 1.0 in H_2O) [lit. 12° (c 0.7 in H_2O)]. $\lambda_{max.}$ 210 nm (log ε 3.63).

Elemental analysis cald. for $C_9H_{14}O_5N_2$:

C = 46.95; H = 6.12; N = 12.16%

Found C = 47.14; H = 6.24; N = 12.05%.

Compound (5) consumed 1.76; 1.97 and 1.99 moles of periodate (4.60 mol. equiv. originally present) (Theor. 2.0) after 1, 5 and 6 h respe c tively, and gave no formaldehyde and 0.84 mole of formic acid.

1'-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)imidazole (31).

Tetra-O-acetyl-α-D-glucopyranosyl bromide (32.9 g. 0.080 moles) and imidazole (12.0 g, 0.176 moles) were dissolved in dioxan (80 ml; dried over sodium) and heated under reflux for 4 h. The mixture was filtered and after addition of xylene (30 ml) kept at 0° for 12 h. A solid was separated by filtration, washed several times with water and dried in vacuo. Thin layer chromatography (benzene-methanol 9:1) showed two major components to be present. Extraction of this solid with dry methanol gave a mixture (4.0 g) richer in the faster moving component. Chromatography of this mixture on a column (100 x 4 cm) of silica gel (500 g), eluting with benzene-methanol (9:1) followed by thin layer chromatography gave the anomeric tetra-acetates (2.0 g, 6.3%) of the α-anomer and (1.5 g) of the β-anomer. Crystallisation of (31) from ethanol gave pure sample m.p. 162-163° (lit.[13], m.p. 172-173°): $[\alpha]_D^{21}$ +118° (c 1.5 in $CHCl_3$) [lit.[13], $[\alpha]_D$ +111°]. $\lambda_{max.}$ 223 nm (log ε 3.10).

Elemental analysis calcd. for $C_{17}H_{22}O_9N_2$:

C = 51.25; H = 5.56; N = 7.03%

Found C = 51.42; H = 5.72; N = 6.83%

1'-α-D-glucopyranosylimidazole (32)

A mixture (6g) richer in (31) was deacetylated in methanolic ammonia at 0° to yield a mixture (3.6 g) of 1-α and 1-β-D-glucopyranosyl-imidazoles. The mixture dissolved in 10 ml of deionized carbon dioxide free water was applied on a column (50 x 3 cm) Dowex 1-X8 (OH^-) resin and eluted with deionized carbon dioxide free water. Fractions (10 ml) were analysed by paper chromatography [development with butan-1-ol-ethanol-water (40:11:19)] to give 0.9 g (yield 2.4%) of the α anomer. (R_f of α anomer 0.32; R_f of β anomer 0.24). The α anomer resisted attempts at crystallisation. $[\alpha]_D^{21}$ + 104° (c, 0.53 in H_2O) [lit.[13], $[\alpha]_D$ + 104°]. $\lambda_{max.}$ 220 nm (log ε 3.20).

Elemental analysis calcd. for $C_9H_{14}O_5N_2$:

C = 46.95; H = 6.12; N = 12.16%

Found C = 46.83; H = 6.19; N = 12.02%.

Compound (32) consumed 1.05; 1.56 and 2.04 moles of periodate (4.60 moles originally present) after 1,5 and 8 h respectively and gave no formaldehyde and 0.96 mole of formic acid.

1'-(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl)imidazole (38).

Tetra-O-acetyl-α-D-mannopyranosyl bromide (32.88 g; 0.080 moles) prepared according to the method of Talley[55] was allowed to react with imidazole (12.0 g; 0.176 moles) under the conditions described for the synthesis of (31). After the reaction the solvent was removed under reduced pressure to give a dark brown syrup (27 g).

Thin layer chromatography (benzene-methanol (9:1) showed the presence of an anomeric mixture of the tetra-O-acetyl-mannopyranosyl imidazoles along with several other impurities. The syrup was then taken up in chloroform and the chloroform extract washed several times with water, dried over anhydrous sodium sulphate, and concentrated under reduced pressure to give a syrup which was dissolved in chloroform and applied (9 g) on a silica gel column (100 x 4 cm) and eluted with benzene-methanol (9:1). Fractions containing the faster moving component were combined and concentrated to dryness to under reduced pressure to give a syrup. This syrup on examination by thin layer chromatography in ethyl acetate followed by visualisation by iodine vapours showed two well resolved spots only one of which was revealed by spraying with 2% ethanolic sulphuric acid. The syrup (3.0 g) was then applied on another silica gel column and eluted with ethyl acetate to give (1.5 g; 4.7% yield) of (38). (38) resisted several attempts at crystallisation but solidified under high vacuum.

$[\alpha]_D^{21}$ + 37.7° (c 1.35 in $CHCl_3$)[lit., $[\alpha]_D$ + 35°].

$\lambda_{max.}$ 225 nm (methanol) (log ε 3.15).

Elemental analysis calcd. for $C_{17}H_{22}O_9N_2$:

C = 51.25; H = 5.56; N = 7.03%

Found C = 51.45; H = 5.70; N = 6.84%.

Fractions containing the impurity were combined and concentrated to dryness to give 1.0 g of white solid . The IR (KBr disc) spectrum of this solid showed a strong absorption at 3500 cm^{-1} suggesting the presence of an hydroxyl group. The NMR spectrum (in $CDCl_3$) shows signals at 8.12, 7.97 and 7.92, 7.86 τ due to the four acetyl methyl protons. On the

basis of its elemental analysis. The compound appears to be tetra-O-acetyl-D-mannose.

Elemental analysis calcd. for $C_{14}H_{20}O_6$:

C = 48.27; H = 5.78%

Found C = 48.36; H = 5.83%.

1 -α-D-mannopyranosylimidazole (44)

(44) was obtained by deacetylation of (1.5 g 38) in methanolic ammonia at 0°. Removal of the solvent at room temperature under reduced pressure gave 0.9 g (4.7% yield) of syrup which solidified to a fine powder under high vacuum. Paper chromatography using butan-1-ol-ethanol-water (40:11:19) of this substance showed it to be a single spot (R_f 0.235) $[\alpha]_D^{21}$ + 63.7° (c 0.45 in H_2O) [lit.[13], $[\alpha]_D$ + 60°].

$\lambda_{max.}$ 215 nm (water) (log ε 3.50).

Elemental analysis calcd. for $C_9H_{14}O_5N_2$:

C = 46.95; H = 6.12; N = 12.16%

Found C = 46.74; H = 6.21; N = 11.99%.

1 -(2',3',4',6'-tetra-O-acetyl-β-D-mannopyranosyl)imidazole (37)

Fractions containing the slower moving component obtained during the synthesis of (38) were combined and concentrated to dryness to give a syrup which solidified under high vacuum to obtain 2.7 g (25% yield) of (37). Thin layer chromatography of this substance using benzene-methanol (9:1) showed it to be a single component. $[\alpha]_D^{21}$ -14.55° (c 0.98 in $CHCl_3$) [lit.[15], $[\alpha]_D$ -16.0°]

$\lambda_{max.}$ 225 nm (methanol) (log ε 3.251).

Elemental analysis calcd. for $C_{17}H_{22}O_9N_2$:

C = 51.25; H = 5.56; N = 7.03%

Found C = 51.37; H = 5.72; N = 6.84%

1 -β-D-mannopyranosylimidazole (43)

Deacetylation of (37) (5 g) in methanolic ammonia at 0° gave, after removal of the solvent, (3.0 g) of (43) (16% yield). Paper chromatography using butan-1-ol-ethanol-water (40:11:19) showed it to be a single component (R_f =0.201). $[\alpha]_D^{21}$ + 27.48° (c 0.60 in H_2O) [lit.[13], $[\alpha]_D$ + 27°].

$\lambda_{max.}$ 210 nm (water) (log ε 3.00).

Elemental analysis calcd. for $C_9H_{14}O_5N_2$:

C = 46.95; H = 6.12; N = 12.16%

Found C = 46.84; H = 6.08; N = 12.02%.

1'-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)imidazole (47)

Following similar procedure to that adopted for the synthesis of (38); tetra-O-acetyl-α-D-galactopyranosyl bromide (82.0 g; 0.199 moles) prepared according to the method of Lemieux,[56] and imidazole (30 g; 0.441 moles) in dioxan at 100° for 4 h. Following a similar work up procedure to that adopted for the mannosides, 57 g of crude syrup was obtained after removal of the solvent. The syrup extracted in chloroform and the chloroform extracts washed several times with water, dried over anhydrous sodium sulphate and concentrated under reduced pressure. Thin layer chromatography of this syrup in benzene-methanol (9:1) showed the presence of imidazole galactosides. 10 g of this syrup dissolved in chloroform was put on a silica gel column (100 x 4 cm) and eluted with benzene-methanol (9:1). Fractions containing the slower component were

combined and concentrated to dryness under reduced pressure to give s syrup which solidified under high vacuum to give (3.1 g, 43.7% yield) of (47) and which resisted further attempts at crystallisation.

$[\alpha]_D^{21}$ + 11.43° (c 0.60 in $CHCl_3$)

$\lambda_{max.}$ 225 nm (methanol) (log ε 2.96).

Elemental analysis calcd. for $C_{17}H_{22}O_9N_2$:

C = 51.25; H = 5.56; N = 7.03%

Found C = 57.07; H = 5.49; N = 6.92%.

1-β-D-galactopyranosylimidazole (52)

Deacetylation of (47) (5 g) using methanolic ammonia at 0° gave on removal of the solvent a syrup (3 g) (55% yield) which solidified under high vacuum and resisted attempts at crystallisation. Paper chromatography using butan-1-ol-ethanol-water (40:11:19) showed it to be a single component (R_f 0.286) $[\alpha]_D^{21}$ + 51.02° (c 1.6 in H_2O).

$\lambda_{max.}$ 210 nm (water) (log ε = 3.00).

Elemental analysis calcd. for $C_9H_{14}O_5N_2$:

C = 46.95; H = 6.12; N = 12.16%

Found C = 47.05; H = 6.26; N = 12.30%.

1-α-D-galactopyranosylimidazole (53)

Fractions containing the faster moving component obtained from the synthesis of (47) were combined together and concentrated to dryness under reduced pressure to give a syrup (2 g) which was deacetylated with methanolic ammonia at 0°. Removal of the solvent at room temperature afforded a syrup (1.1 g). Paper chromatography using butan-1-ol-ethanol-water (40:11:19) showed it to be contaminated with D-galactose. Application of this syrup on a column of Dowex 1-X8 OH^- resin and eluting

with deionised carbon dioxide free water gave 0.700 g of solid (5% yield) of (53) which resisted attempts at crystallisation. Paper chromatography confirmed this to be a single substance (R_f 0.315). $[\alpha]_D^{21}$ + 130.16° (c 0.60 in H_2O). $\lambda_{max.}$ 214 nm (log ε = 3.530).

Elemental analysis calcd. for $C_9H_{14}O_5N_2$:

C = 46.9; H = 6.12; N = 12.16%

Found C = 46.2; H = 6.19; N = 11.82%.

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CHAPTER 3

APPLICATION OF NMR TO CONFORMATIONAL STUDIES OF N-GLYCOSYLIMIDAZOLES

INTRODUCTION

The study of the conformation of organic molecules originated in the early eighteen-nineties, when Sachse[1] pointed out that six membered ring systems could exist free from bond-angle strain in two puckered forms, (i) the rigid form (chair) (54) and (ii) the flexible form (boat and the skew) (55,56).

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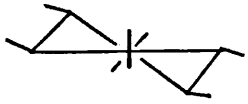

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The first application of this observation in carbohydrate chemistry was made by Sponsler and Dore[2] in their interpretation of x-ray data for ramie-fibre cellulose, who realised that the ideas of chair and boat forms of cyclohexane derivatives could be applied to pyranoid sugar derivatives and that D-glucopyranosyl units of cellulose were probably chair forms. It was Haworth[3] in 1929 who introduced the term "conformation of a ring" to describe various geometric forms of the pyranose ring. Conformations were later defined[4] as the different

arrangements in space of the atoms in a single classical organic structure, the arrangement being produced by the rotation or twisting of normal single bonds.

The effect of conformation on chemical reactivity was first recognised by Hann, Merril and Hudson [74] and was further elaborated by Isbell [5-9] who provided conclusive evidence of the non-planar conformation of a pyranoid ring from an investigation of the oxidation of free sugars by bromine. It was principally through the work of Hassel and Ottar [10] that the actual conformation of a molecule was believed to be determined by the non-bonded interactions of substituent groups. Hassel [11] recognised that cyclohexane is more stable in the chair form than in the boat form, and that the two chair forms of a monosubstituted cyclohexane have different energies, the form having the substituent equatorially attached being thermodynamically more stable than the one having the group axially attached. In an extension of these ideas to pyranoid sugars, Hassel and Ottar [10] were able to predict the favoured chair forms for many sugar derivatives, thereby firmly establishing the technique of conformational analysis in the carbohydrate field.

X-Ray crystal structure analysis is one of the most precise techniques available for determining the conformation of a sugar molecule, as it provides valuable information on bond lengths, dihedral angles, interatomic distances and valency angles. Although aldohexoses have not been extensively investigated by x-ray crystallographers, structures of crystalline α- and β-D-glucopyranosides are known by the application of neutron diffraction [12], and the presence of a pyranoid ring in the chair conformation has been established. An example of the application of

x-ray crystal structure analysis to the study of complex molecules of particular interest is the study of casimidine dihydrochloride in which β-D-glucose in a pyranoid form was shown to adopt a $^4C_1$ conformation.[75] The applications of x-ray crystal structure analysis in carbohydrate chemistry have been the subject of several reviews.[13-15]

The pioneering work on the conformations of sugars in solution was carried out by Reeves[16-24] in studies of the complexes formed from sugars and their derivatives in cuprammonium solutions. He was able to provide evidence that pyranoid sugars exist in chair conformation and that in most cases one of the chair forms is prevalent. Boat or skew conformations were also considered if non-bonded interactions between the substituents became large in the chair conformation. Following Reeves' investigation, conformational analysis of cyclic carbohydrate molecules has been the subject of much study and of several reviews.[4,25-29]

The conformational analysis of pyranoid sugars and their derivatives may be expected to follow the principles established with cyclohexane,[25] and also to exhibit several major points of difference. The symmetry of the pyranoid ring is decreased by the replacement of the methylene group by an oxygen atom[16] which also exerts a stereo-electronic influence on the conformation that differs from that of a methylene group. The free energy contents of the various possible conformers may be considered to be determined by various factors including (i) steric interactions, (ii) bond torsional strain, (iii) bond angle strain, (iv) electronic factors involving interaction of dipoles, (v) effects of solvation and hydrogen bonding and in the solid state, (vi) crystal-lattice forces.[30]

One approach to the conformational analysis of carbohydrates is to calculate the free energy difference between pyranose conformations based on the calculation of free energies, first elaborated by Angyal[31] in 1961. In a comparatively rigid system such as the six membered pyranose ring, the interaction energies can be evaluated and totalled. Such calculations have been carried out successfully on cyclitols[32] and acetylated aldoses.[33] The calculations however, are based on two assumptions namely (i) the pyranose ring has the same geometry as cyclohexane and (ii) the free energies are additive functions of energy terms associated with the presence of non-bonded interactions, that is the occurrence of one interaction in a molecule does not affect the magnitude of another one. Both these assumptions are only approximations and probably cannot be justified.[34] To obtain the conformational free energy of a pyranoid form in one of its chair conformations, the following interaction energies were taken into account: (i) interactions between two syn-axial atoms, other than hydrogen atoms, (ii) between two atoms, other than hydrogen gauche on adjacent carbon atoms, and (iii) the anomeric effect (if present), and totalled. Angyal[34] in this way has calculated the conformational free energies for all the aldohexoses and aldopentoses and shows good agreement between the calculated conformational free energy and the predominant conformations observed experimentally.

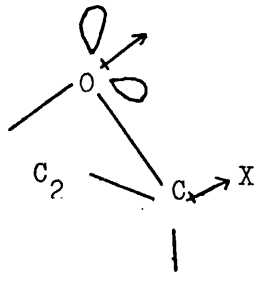
An alternative, theoretically more satisfactory approach, is the use of theoretical models in ab initio conformational analysis that is conformational analysis starting from the laws and particles of matter (nuclei and electrons) and taking into account all interactions without using any emperical parameters.[35] Ab initio SCF-LCAO-MO (self consistent field linear combination of atomic orbitals-molecular orbitals)

calculations of good quality have been able to reproduce total energies of various conformations of a molecule and geometrics.[35] These calculations may be performed on relatively complex systems and satisfactory answers have been obtained in several cases.

Applications of such calculations to conformational analysis in carbohydrate chemistry are relatively unknown. Wolfe et al.[36] in a theoretical study of the anomeric effect have used an ab initio (Hartree-Fock)(one electron scheme) calculation with fluoromethanol as the model compound. Zhdanov[73] has recently determined the favoured conformations of cyclohexanediols and cyclitols by the application of the extended Huckel (MO) (EHMO) method.

Anomeric Effect

Most 1-substituted derivatives of α-D-glucopyranose (and many other sugars) are more stable than the corresponding β-D-anomers, although the former have the bond to the substituent in the axial position and the latter in the equatorial one. This predisposition of a polar substituent at C-1 of a pyranoid ring to assume axial orientation contrary to expectations based merely on steric grounds has been termed the "Anomeric effect" by Lemieux.[33,38] An explanation of this effect was given by Edward[39] in terms of the repulsive interaction between the equatorial carbon-oxygen dipole and the dipole formed as the resultant of the two carbon-oxygen dipoles in the pyranose ring. These dipoles form a small angle when the substituent is equatorial : 57 , and a large one when it is axial, 58 .



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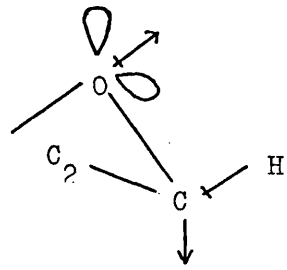

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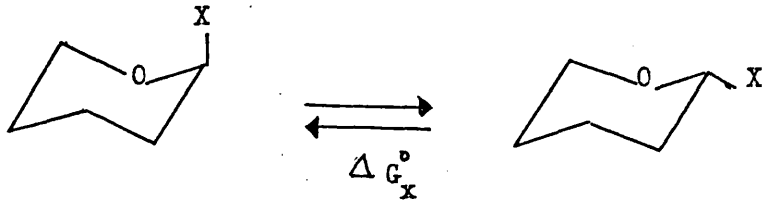
Lemieux and Chu [33] have interpreted the effect in terms of an electrostatic interaction between the C-1 to substituent and C-5-O.5 bonds. This interaction was later alluded [40] to as the "rabbit ear effect" and was considered to arise from a repulsion of the electric dipoles engendered by the parallel disposition of electron pairs occupying non-bonding orbitals of the ring hetero-atom and the electro negative atom bonded directly to the anomeric carbon atom. [40-42] In accordance with the above explanations the anomeric effect varies inversely with the dielectric constant of the solvent and is directly proportional to the magnitude of the dipole moment in the C-1 substituted bond. [30]

At equilibrium in aqueous solution D-glucose contains about 64% of the β-anomer and 36% of the α-anomer, whereas D-mannose contains 67% of the α-anomer and 33% of the β-anomer. [43] This would appear to show that the anomeric effect is greater when the hydroxyl group at C-2 is axial. In less polar media such as methanol an equilibrium mixture of D-glucose contains a higher proportion of the α-anomer [44] because of decreased solvation of dipoles. The free energy difference between an axial and equatorial acetoxy group on a cyclohexane ring is 0.66 kcal mol^{-1} (2.76 kJ mol^{-1}) in favour of the equatorial anomer in carbon-

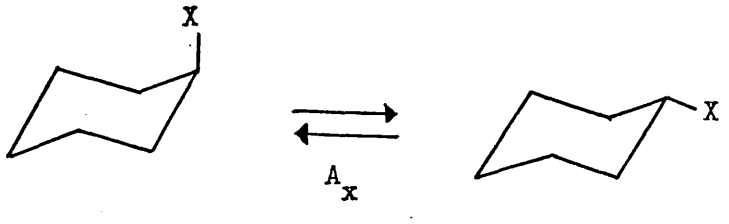
tetrachloride[46] or carbon disulphide.[45] On the other hand the free energy difference between penta-O-acetyl-α-D-glucopyranose and penta-O-acetyl-β-D-glucopyranose is 1.1 kcal mol^{-1} (4.60 kJ mol^{-1}) in favour of the α-anomer in a mixture of acetic anhydride and acetic acid.[48] Similarly, under experimental conditions favouring equilibration the formation of methyl D-glucopyranosides and tetra-O-acetyl-D-glucopyranosyl bromide affords more α-anomer than β-anomer.[44]

Quantitatively the anomeric effect can be expressed[47] as the sum of the free energy difference for the process shown in eq. (1).



eq.-(1)

and the conformational free energy or "A value"[48] for an axial substituent 'X' in cyclohexane as shown in eq. (2).



eq.-(2)

i.e. the anomeric effect = $\Delta G^{\circ}_{X} + A_X$.

Thus in the case of penta-O-acetyl-D-glucopyranose cited above the anomeric effect for the acetoxy group is about 1.8 kcal mol^{-1} (7.5 kJ mol^{-1}). The magnitudes of the anomeric effects for other polar anomeric substituents are given in Table 3.1 .

Table 3.1

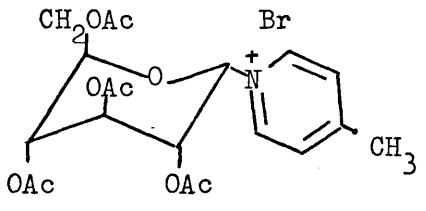
Anomeric substituent	Compound	Anomeric effect		Solvent	Ref.
		kcal mol^{-1}	kJ mol^{-1}		
-OH	D-(glucose)	0.90	3.76	water	30, 34
	D-(mannose)	1.35	5.64	"	
	2-deoxy-D-glucose	1.20	5.02	"	
-OMe	OH(pentose) aldopento-pyranosides	1.20	5.02	1% methanolic HCl	49
	OH(hexose) aldohexo-pyranosides	1.40	5.85	1% methanolic HCl	50
	2-methoxy-4-methyl-tetra-hydropyran	1.30	5.43	p-dioxane	47
		0.90	3.76	aq. methanol	47
-OAc	peracetylated aldohexo - pyranoses	1.50	6.27	1:1 acetic acid - acetic anhydride	38,33
	per acetylated aldopento-pyranose	1.30	5.43	1:1 acetic acid - acetic anhydride	38,33
	2-acetoxy-4-methyl-tetrahydropyran	1.35	5.69	acetic acid	47
-Cl	tetra-O-acetyl-D-glucopyranosyl chloride	2.70	11.29	acetonitrile	51
-Br	tetra-O-acetyl-D-glucopyranosyl bromide	3.20	13.38	2-bromotetra-hydropyran	51

The quantitative defination of anomeric effect assumes that the geometry of the pyranoid ring is similar to that of the cyclohexane ring.

Martin[37] in a recent review presents an account of theoretical and experimental work on the anomeric effect. He estimated the magnitude of this effect by summing the interaction energies of unit bond and lone pair dipoles. Values in good agreement with those observed were obtained.

Reverse anomeric effect

In 1965, Lemieux and Morgan[52] from an NMR spectral study of some pyridinium α-D-glucopyranosides discovered that in N-(tetra-O-acetyl-α-D-glucopyranosyl)-4-methyl-pyridinium bromide (59),



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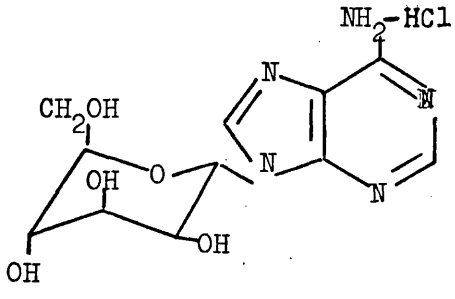
The pyranose ring is strongly distorted from the normal $^4C_1$ conformation. The observed coupling constants (J1',2' = 2.8 Hz; J2',3' = 3.1 Hz; J3',4' = 3.2 Hz; J4',5' = 5.7 Hz) suggested a nearly equatorial orientation for the pyranose ring protons, also the chemical shifts of three of the four O-acetyl protons were indicative of axial orientation. In view of the observed coupling constants,

these authors suggested that the compound appears to have a conformation which is close to the $^{1}C_{4}$ conformation. Such a strong distortion of the pyranose ring from the $^{4}C_{1}$ conformation must arise from powerful non-bonding interactions arising from the 4-methylpyridinium group when in axial orientation at the anomeric centre. Since the "A value" of the benzene ring is believed to be in order of only 2-3 kcal mol^{-1} (8.3 - 12.5 kJ mol^{-1}). The distortion noted in (59) could not have been expected on steric grounds alone. These authors argued that the establishment of a positively charged atom in axial orientation at the anomeric centre must be expected to meet with a strongly destabilising effect (relative to when the group is in equatorial orientation) arising from the electrostatic interactions between C-1 to N and C-5 to O bonds, when the nitrogen and C-5 atoms are in gauche relationship. This phenomenon was further substantiated by Lemieux and Saluja [53] from a study of the effects of protonation and N-methylation of N-glycosylimidazoles.

The reverse anomeric effect has been invoked to explain conformational inversion of other glycosylamine derivatives[54-56] 9-α-D-mannopyranosyladenine hydrochloride (60) and 7-α-D-mannopyranosyltheophylline (61) have been shown to exist in



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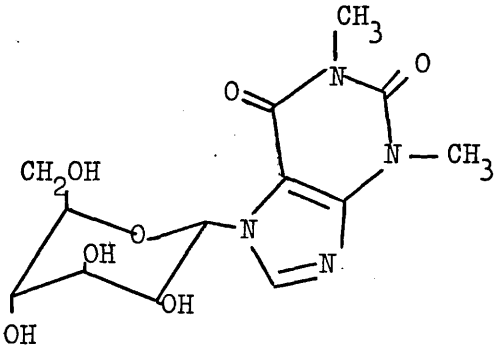

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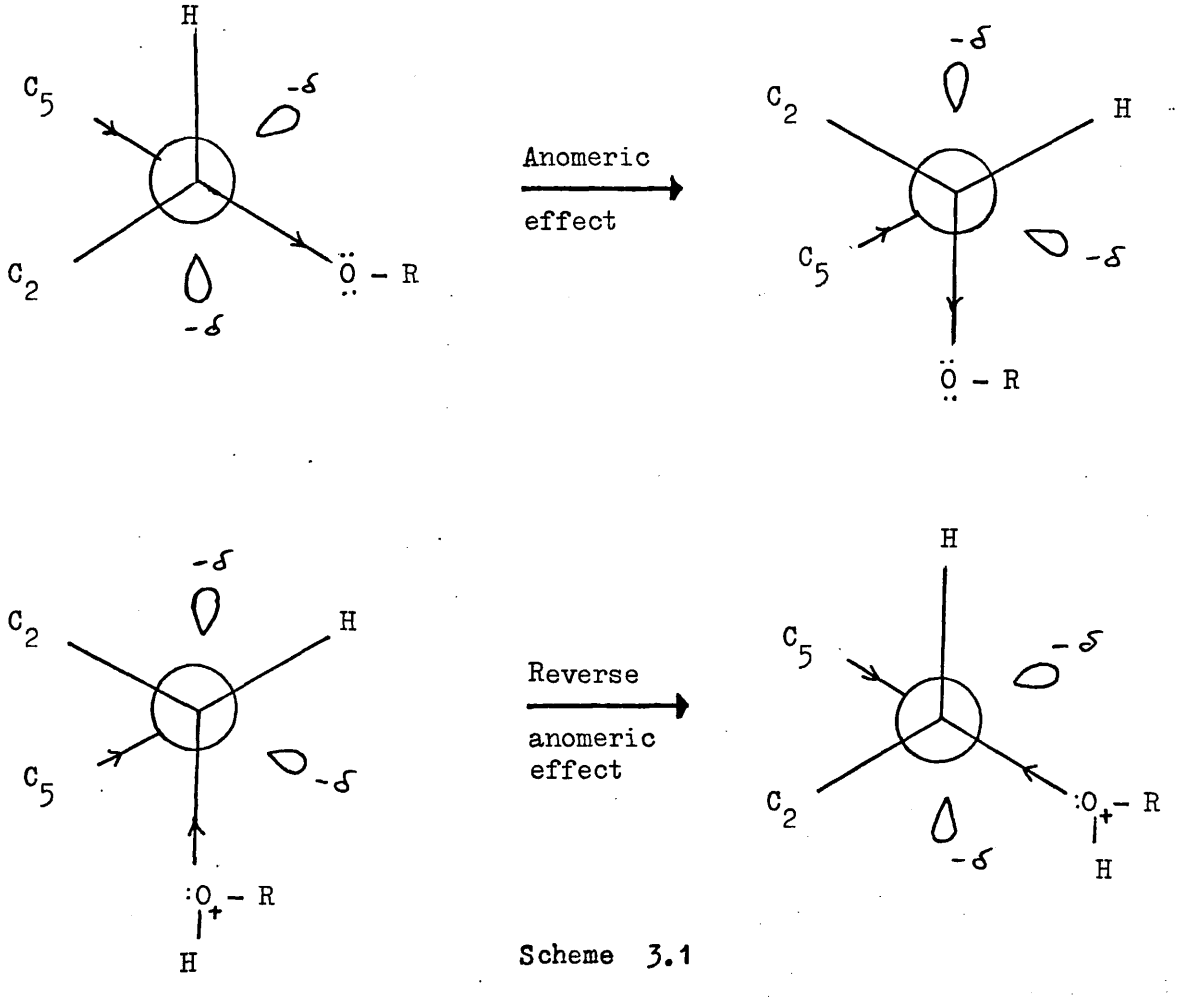
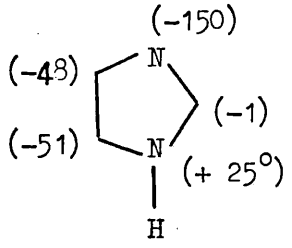
the $^1C_4$ conformation, having the aglycone in the equatorial orientation.[30] The bulkiness of the anomeric substituent was also considered to be a factor in the destabilisation of the conformer having the aglycone attached axially.[30]

The anomeric effect is supposed to arise from a more favourable arrangement of dipole-dipole interactions when the aglycone is in axial orientation, with the reverse anomeric effect the electro positive aglycone would prefer the equatorial position. Scheme 3.1 depicts the possible origins of the anomeric and reverse anomeric effect.[57]



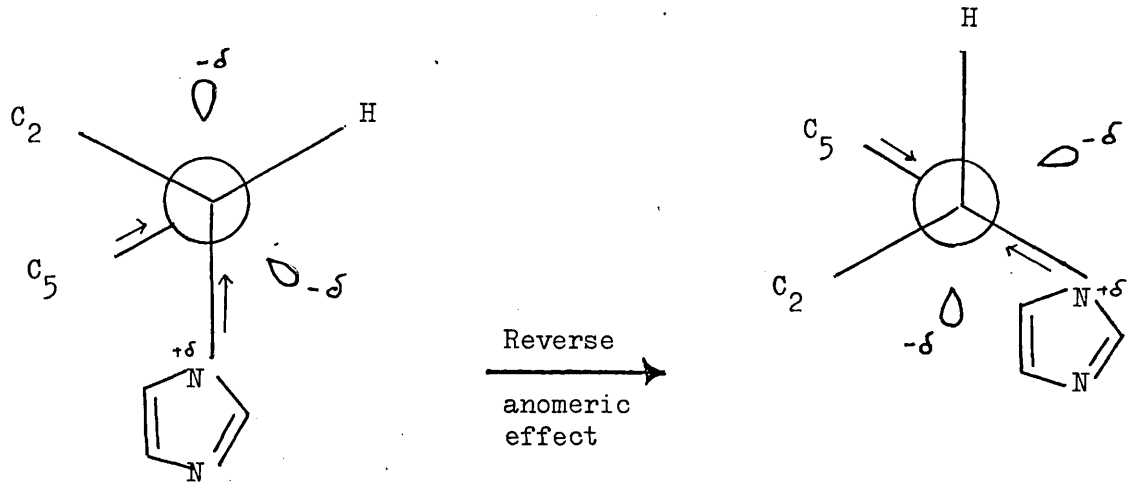
Scheme 3.1

Molecular orbital calculations of the π-electron densities and bond order have been calculated for imidazole and for a number of other heterocyclic ring systems. The general conclusions that arise from these calculations are that imidazole shows some degree of aromatic character and that the bond orders show some similarity to the classical structure. Also, the amino nitrogen which contributes two electrons to the system usually attains a positive charge, while the imino nitrogen atom attains a slight negative charge.[59] The electron densities lead to dipole moments of the correct order of magnitude.[58,60]

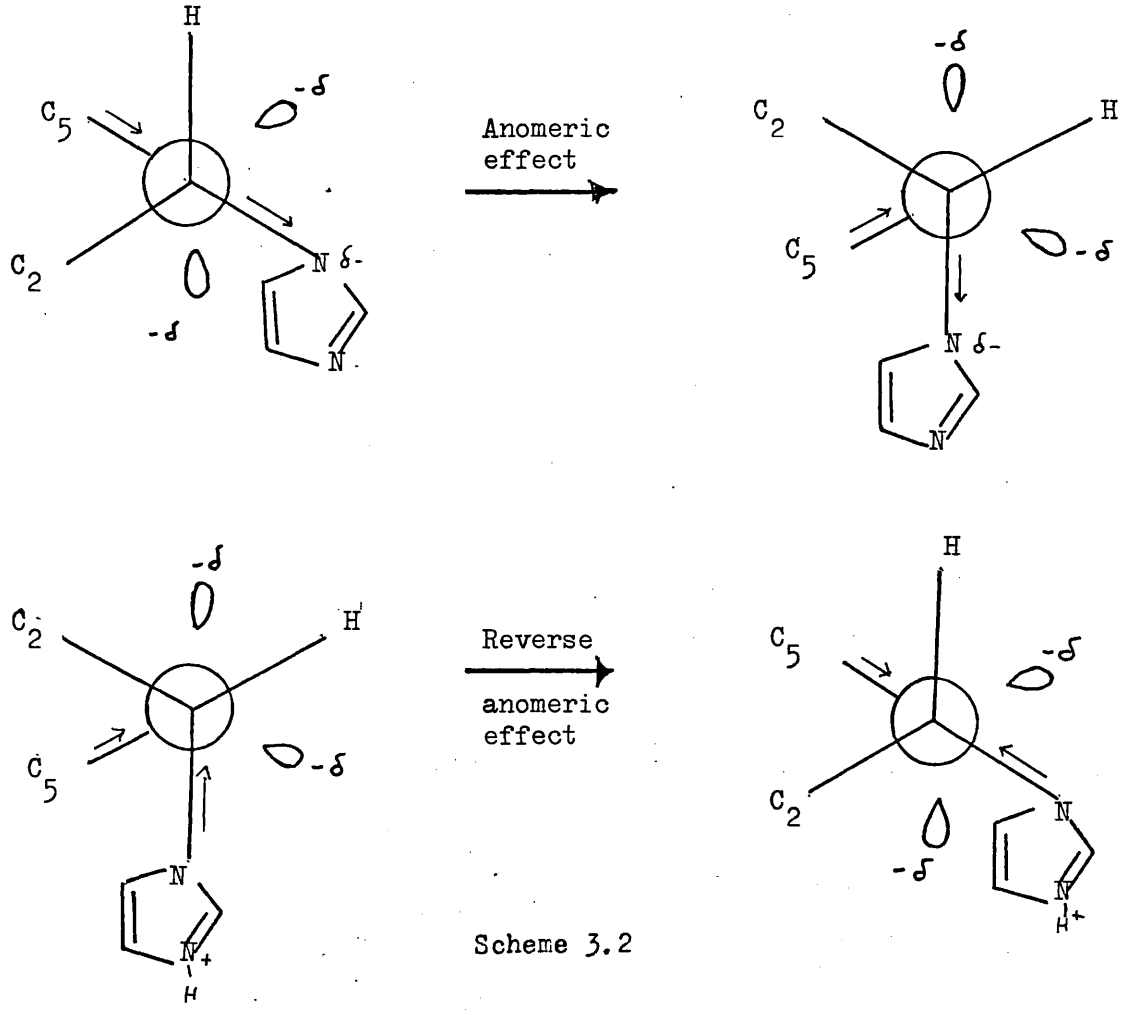


Molecular diagram of imidazole showing net charges

In view of this observation it would appear that the reverse anomeric effect would operate in the unprotonated N-glycosylimidazoles.



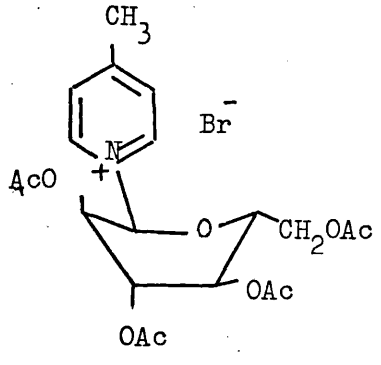
Lemieux [57] however has discussed the anomeric effect and reverse anomeric effect in N-glycosylimidazoles in terms of the development of a full positive charge on the imidazole ring as shown in Scheme 3.2



Scheme 3.2

In view of the dipoles associated with the imidazole ring the presence of a fully protonated imidazole ring may not be necessary for the reverse anomeric effect to be observed. Also, the abnormal conformation noted by Onodera[54-56] in the case of 7-α-D-mannopyranosyl theophylline (61) supports this argument.

The reverse anomeric effect was shown to operate in N-(tetra-O-acetyl-α-D-glucopyranosyl)-4-methyl pyridinium bromide (59), where the pyridinium grouping is forced from the axial to an equatorial orientation. James[61] recently established the conformation of (59) in the crystalline state as boat ($B^{2,5}$) conformation (59).



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The NMR data obtained in case of 59 is consistent with this boat form. The driving force towards this conformation could partially be of steric origin because of the large bulk of the pyridinium group.

To date it has not been possible to separate the steric from dipole-dipole interactions for the structures believed to exhibit the reverse anomeric effect, since in all these cases very substantial steric effects are associated with the axial orientation of the aglycone,

and it has not been ascertained whether or not these are the only important driving forces for a molecule to adopt a conformation in which the aglycone takes up an equatorial orientation.

Wolfe et al.,[36] have in a theoretical study that used an ab initio (Hartree-Fock) calculation carried out a theoretical study of the anomeric effect with fluoromethanol as the model compound.

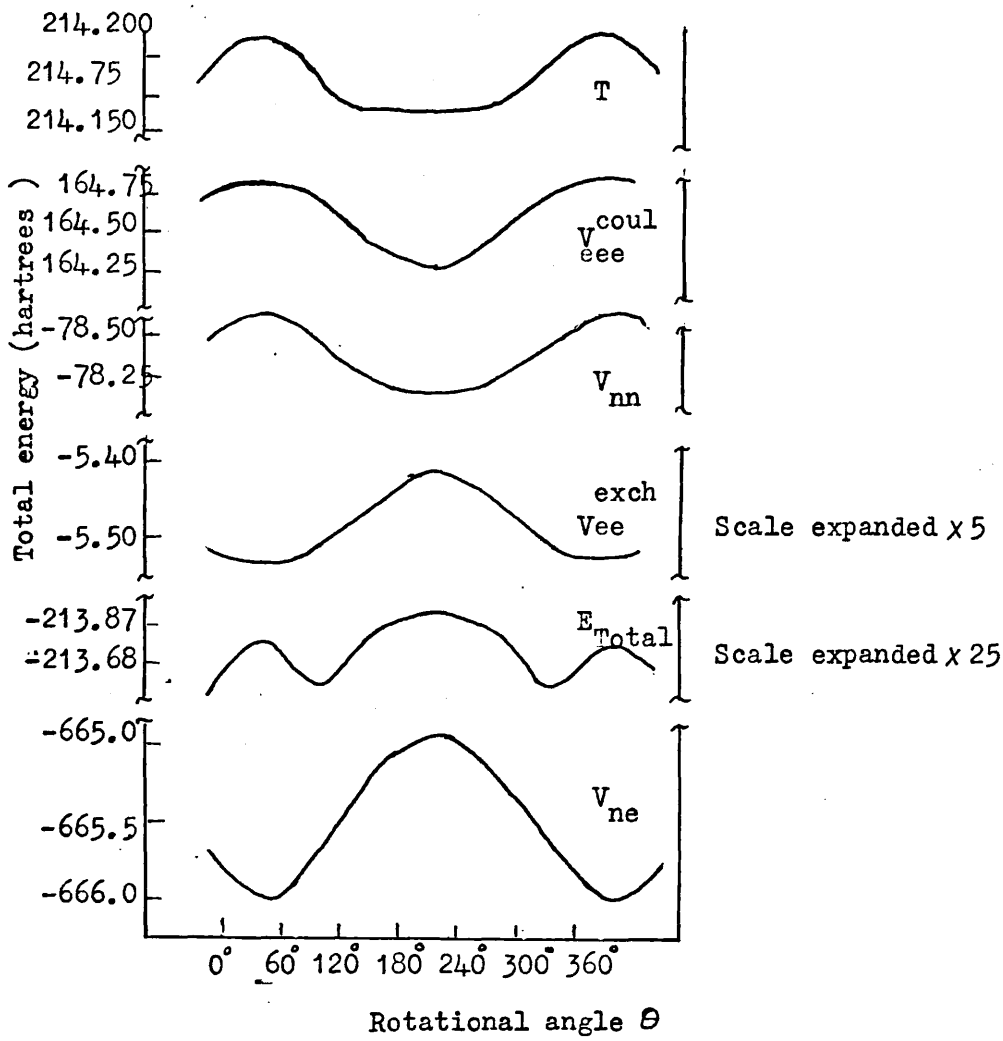

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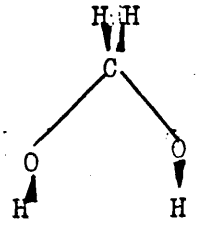
Fig. 62 shows the total energy of fluoromethanol and the energies of the various components as a function of rotation about the carbon-oxygen bond. T = kinetic energy; V_{ee}^{coul} = coulombic repulsion term; V_{nn} = nuclear-nuclear repulsion term; V_{ee}^{exch} = exchange term

and V_{ne} = nuclear-electron attraction term. These authors have been able to separate the electron-electron repulsion term (V_{ee}) into Coulombic (V_{ee}^{coul}) and exchange (V_{ee}^{exch}) terms, thereby permitting the significance of the coulombic term to be assessed. As has been observed in all other ab initio calculations of rotation-inversion barriers, the attraction and repulsion terms have opposite phases. It is clear from Fig. 62 that the form of V_{ee}^{coul} is not distinguishable from that of the other repulsive terms and hence Wolfe et al.[36], concluded that the anomeric effect could not be ascribed in any straightforward way simply to dipole-dipole interaction. This view requires that the coulombic term be equated with the interaction of the net dipoles due to individual atoms or groups, which may not be justified since the latter may include contributions arising from interactions of nuclei. One might suppose that these considerations may also apply to the reverse anomeric effect. More recently Jeffrey, Pople and Radom[76] have used ab initio molecular orbital calculations on methanediol as the model compound. The results found for methanediol suggest, for the sugars, favoured conformations which are consistent with the observed anomeric effect. The relative energies of possible structures of methanediol are shown to be particularly relevant to the favoured conformations associated with the anomeric carbon atom in pyranoses. The minimum energy conformation of methanediol was proposed to be (+SC + SC) (synclinal) (63)



63

These calculations also show that shortenings of the C-O bond of the order of 0.01 to 0.04 Å, relative to methanol, are to be expected, and that bond lengths have a strong conformational dependence.

Since the pioneering work of Lemieux and co-workers[62] in 1958, on the application of high resolution NMR spectroscopy to structural problems in the carbohydrate field, this physical method has developed into one of the most powerful tools for the investigation of conformational aspects of sugars and their derivatives in solution, and the application of this technique has been described in several reviews.[63-66]

NMR spectroscopy yields basically two kinds of data, spin-spin coupling constants and chemical shifts. The relationship between the chemical shift of a proton and its environment in six membered ring systems was investigated by Lemieux and co-workers.[62] Three conclusions were reached that permitted the assignment of conformation to various aldopyranoses and their derivatives viz.

(i) the anomeric hydrogen atom being on a carbon atom attached to two oxygen atoms is strongly deshielded and usually resonates at lower field than other ring protons (ii) axially attached protons usually resonate at higher field (i.e. they are more highly shielded) than equatorially attached protons in chemically similar environment (iii) axial acetyl methyl protons usually resonate at lower field than equatorial acetyl methyl protons. Although a few exceptions have been found, these generalisations are of great use in making configurational and conformational assignments.

Lemieux and co-workers[62] also discovered that vicinal spin-spin coupling constants are dependent upon molecular conformation i.e. the dihedral angle between hydrogen substituents. Karplus[67] rationalised

the change in coupling constant with the dihedral angle by the following relationship obtained from valence bond calculations.

$$J = K \cos^2\phi - C$$

where J is the coupling constant between two hydrogen atoms attached to adjacent carbon atoms at a dihedral angle of ϕ ; K and C are constants (C = -0.28 and K = 8.5 (ϕ 0-90°) and 9.5 (ϕ 90° - 180°). It is important to note that the parameters given apply only to sp^3 hybridized carbon atoms. Later Anet [68] pointed out that vicinal coupling constants are also dependent upon the presence of strongly electronegative or electropositive groups, the carbon-carbon bond length, bond angles and other molecular properties. A better semi-theoretical relationship based on valence bond, σ -electron calculations is given by Karplus[69] later equation

$$J_{HH'} = (A + B \cos \phi + C \cos 2\phi)(1 - m\Delta X)$$

where A, B and C are constants, ϕ is the dihedral angle and ΔX is the difference between the electronegativities of the substituent and hydrogen. Clearly therefore application of the Karplus equation even with the most suitable parameters can give only an approximate estimate of the molecular conformation.

RESULTS AND DISCUSSION

In order mainly to investigate the phenomenon of the reverse anomeric effect an investigation of the conformations of N-glycosylimidazoles under various conditions was undertaken.

The value of the NMR method to provide information regarding the conformations of sugar molecules has the particular merit that the perturbations used for the measurement of resonances have energies several orders of magnitude lower than those required for inducing significant conformational change.[30] The NMR spectra discussed here have been analysed on a first-order basis, and the assignments for the sugar ring protons were checked by double resonance experiments. It is well known that the measured line splittings derived directly from an NMR spectrum may differ from the true J values which are most directly related to conformation. Thus it is important that reliable values be obtained from the spectra. In this work the coupling constants derived from the measured line splittings have been used to calculate spectra using a non-iterative computer programme (UEA NMR basic). In general, the J values which on calculation produced most nearly the observed spectrum are those used in the discussions of conformation.

In order to calculate a theoretical spectrum, it is necessary only to read into the programme (UEA NMR basic) a set of chemical shifts and coupling constants, together with (i) some input data to define the frequency range of interest, (ii) the minimum intensity of a transition of interest and (iii) numbers to identify any different types of nuclei involved. The result of the computation is a table of theoretical frequencies and intensities of spectral lines, which with

the insertion of suitable Lorentzian and Gaussian half line widths (0.03 - 1.0 Hz for protons) provides a trace of the spectrum above a frequency scale.

The observation of a marked similarity between experimental and theoretical spectra affords some evidence that the spectral analysis is valid. If however, the analysis has been made by an inexact method (guessing parameters on the basis of those closely related systems) it may be desirable to refine the approximate parameters by using the iterative part of the computer programme. This is usually done if the trial theoretical spectrum bears a recognizable resemblance to the observed spectrum. The initial parameters together with the set of line assignments obtained by matching lines in the trial spectrum are inserted into the programme; the number of iterations and the sets of parameters to be varied (coupling constants, chemical shifts or both) have to be provided at this stage.

In the iterative programme, corrections to the chosen parameters are so calculated as to minimize the differences between theoretical line frequencies and assigned experimental line frequencies according to a criterion of least squares. The iterations are terminated if the errors of successive iterations differ by less than 1% or if the assigned maximum number of iterations is reached.

Satisfactory fits with experimental NMR spectrum using a non-iterative computer programme (UEA NMR BASIC) were obtained in case of the following compounds.

Compound	Exp. Spec. Fig.	Compu. Spec. Fig.	Tables	
1'-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl) imidazole (31)	3.1	3.2	3.9	3.10
" + TFA	3.3	3.4	3.9	3.10
1'-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl) imidazole (14)	-	-	3.3	3.4
1'-α-D-glucopyranosylimidazole (32)	3.5	3.6	3.7	3.8
" + TFA	3.7	3.8	3.7	3.8
" + NaOD	3.9	3.10	3.7	3.8
1'-(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl) imidazole (38)	3.11	-	3.17	3.18
1'-(2',3',4',6'-tetra-O-acetyl-β-D-mannopyranosyl) imidazole (37)	-	-	3.11	3.12
1'-α-D-mannopyranosylimidazole + TFA	-	-	3.15	3.16
1'-β-D-mannopyranosylimidazole (43)	-	-	3.13	3.14
1'-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl) imidazole (47)	-	-	3.19	3.20

Attempts using an iterative programme (UEA NMR ITERATIVE) for the following compounds produced satisfactory fits.

	Exp. Spec. Fig.	Compu. Spec. Fig.	Tables	
1'-(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl) imidazole + TFA	-	3.13	3.17	3.18
1'-α-D-galactopyranosylimidazole + TFA	3.14	3.15	3.23	3.24

In the case of 1'-α-D-galactopyranosylimidazole attempts using an iterative programme to produce a satisfactory computed spectrum was unsuccessful in spite of varying various parameters permitted by the programme. One particular feature in the computed spectrum attributable

to protons 5, 6 and 7 possessing an ABX character did not match with the experimental spectrum. Details of the various attempts made to obtain a satisactory fit are given below. An example of the output obtained from an interative programme is shown on pages 118-123.

Iterative run No.	Number of line numbers assigned	Variable parameters (δ ; J)
1	10 (obtained from trial run (non-iterative))	vary all δ and J
2	38 (obtained from trial run No.1)	vary all δ and J
3	38 (obtained from run No. 2)	vary shifts for protons 5',6',7' and vary $J_{4',5'}$, $J_{5',6'}$, $J_{5',7'}$.

On account of the very complex nature of the NMR spectra of 1 -α-D-mannopyranosylimidazole and 1 -β-D-galactopyranosylimidazole satisfactory fits in certain regions of the spectra could not be obtained.

A graphical representation of chemical shifts of the sugar ring protons of acetylated N-glycosylimidazoles is shown in Figs. 3.18-19.

The NMR spectrum (Fig. 3.1) of 1 -(2',3' 4',6'-tetra-O-acetyl-α-D-glucopyranosyl) imidazole (31) shows the anomeric proton downfield of all pyranose ring protons as expected. The coupling constants (Tables 3.9 page 102) observed are in good agreement with those expected for the compound in the normal $^4C_1$ conformation. From the

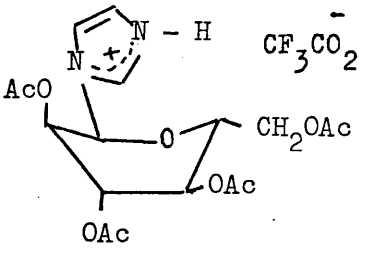
magnitudes of the coupling constants for $J_{2',3'}$ $J_{3',4'}$ and $J_{4'5'}$ which are 10.25, 8.5 and 10.0 Hz respectively, one may infer that these protons are trans-diaxially arranged with respect to one another. The value of 5.5 Hz for $J_{1',2'}$ is higher than that (3.0-3.6Hz) normally found for α-D-glucopyranosides, thus suggesting a small distortion of the chair form of the pyranose ring due to the steric interaction of the imidazole ring. The geminal coupling of 12.5 Hz is characteristic for geminal proton-proton coupling.

The NMR spectrum of the protonated 1'-(2',3',4',6'-tetra-O-acetal-α-D-glucopyranosyl)-imidazole (31) obtained by the addition of trifluoroacetic acid (TFA) to $CDCl_3$ solution is reproduced in Fig.3.3. The imidazole ring on protonation acquires a full positive charge and therefore the more favourable disposition of the dipoles about the anomeric centre should be that with the imidazolium group in the near equatorial orientation, i.e. the compound should exhibit the reverse anomeric effect. The anomeric proton appears as a quartet both in the calculated and observed spectra (Fig.3.4; 3.3). The appearance of a quartet with spacing of 1.5 Hz for the anomeric proton could be explained if H-2' and H-3' are strongly coupled and separated by a small chemical shift (as is evident in this case). then H-1' could be the X of an ABX system, in which case, its theoretical spectrum consists of a quartet and not a doublet as would have been expected on a first-order basis.[63] This type of coupling has been referred to as virtual long range[70] coupling and has been reported previously in the literature.[71]

The coupling constant for protonated (31) Table 2.9 shows $J_{1',2'}$ = 3.0 Hz, thus suggesting a further distortion of the chair form

of the pyranose ring. Furthermore the other coupling constants have changed on protonation from 10.25, 8.5 and 10.0 Hz to 6.25, 8.25 and 8.1 Hz respectively for $J_{2',3''}$, $J_{3',4'}$ and $J_{4',5''}$. On the basis of these coupling constants it is not possible to suggest that the protonated compound exists entirely in the alternate $^1C_4$ conformation.

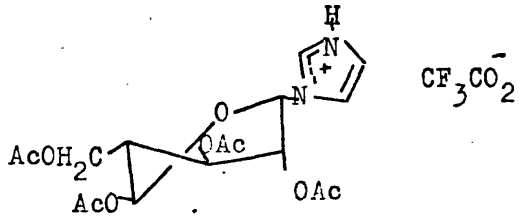
The possibility that the protonated compound adopts a boat conformation as was shown to be the case for N(tetra-O-acetyl-α-D-glucopyranosyl)-4-methyl pyridinium bromide (59) in the solid state may be considered.[61] For the protonated compound to adopt a $^{2,5}B$ conformation (64), the coupling constants for



64

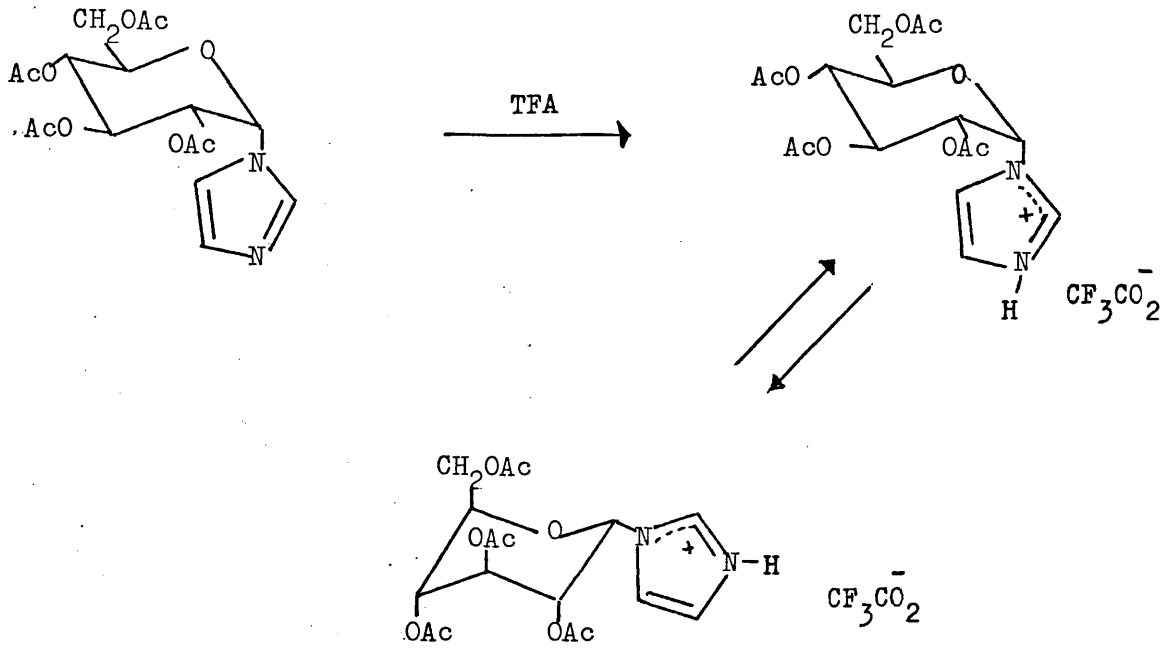
$J_{2',3'}$ and $J_{3',4'}$ should be of nearly the same magnitude but smaller than $J_{4',5''}$, which was observed to be true in the case of pyridinium α-D-glucoside. As can be seen from the coupling constants (Table 3.9 page 102), $J_{3',4'}$ and $J_{4',5'}$ are of the same magnitude, and therefore on this basis the presence of the fully protonated compound in a $^{2,5}B$ conformation can be ruled out. On the same basis one could rule out the possibility of an equilibrium between $^4C_1$ and

$^{2,5}B$ conformations for if this were true, the time averaged coupling constants would be observed and $J_{4',5'}$ would still be larger than $J_{2',3'}$ and $J_{3',4''}$ which is clearly not observed. Similarly the possibility of alternative boat conformations such as $^{3}B_{2,5}$ $^{1,4}B$, $B_{1,4}$, $^{0,3}B$ and $B_{0,3}$ could be eliminated. Alternative twist-boat(S) conformations $^{2}S_{0}$, $^{0}S_{2}$, $^{1}S_{3}$, $^{3}S_{1}$ and $^{5}S_{1}$ were also examined for the protonated compound and the $^{1}S_{5}$ conformation (65) was found to fit reasonably well with the NMR data.



65

Although the existence of the fully protonated compound in the $^{1}C_{4}$ conformation is not supported by the NMR data, a solution may lie in the consideration of an equilibrium between the two $^{4}C_{1}$ and $^{1}C_{4}$ chair conformations. (Scheme 3.3)



Scheme 3.3

However, it is difficult to reconcile exactly the coupling constants with this interpretation, in particular the magnitude of $J_{2',3'}$ (6.25 Hz) appears to be rather low in comparison with $J_{3',4'}$ (8.25 Hz) and $J_{4',5'}$ (8.1 Hz). The invariance of the coupling constants and chemical shifts with temperature further supports the presence of one, rather than two, predominant conformational species.

The magnitudes of the coupling constants (Table 3-7, page 100) obtained from the NMR spectrum of 1-α-D-glucopyranosylimidazole (32)

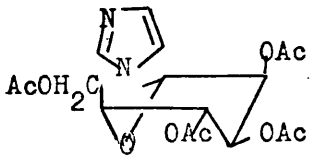
Fig.3.5; strongly suggest that the compound possesses the normal $^4C_1$ conformation. protonation of (32) by addition of TFA produced very little change in coupling constants. A change in the coupling constants would have been expected if the compound underwent any signifcant conformational change, thus suggesting that the reverse anomeric effect is considerably less in the deacetylated compound in aqueous solution than in the acetylated compound in chloroform solution.

Since the pKa of imidazole is 7, in neutral aqueous solution, about 50% of the glycosylimidazole would be expected to be in the protonated form. An attempt was made to eliminate all protonated species by adding NaOD. The NMR spectrum of (32) in NaOD is reproduced in Fig.3.9. The coupling constants (Tables 3-7 page 100) show very little change, as a result there is no evidence for any conformational change.

1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)imidazole (14) and 1-β-D-glucopyranosylimidazole (5) gave NMR coupling constants (Table 3.3; 3.5) whose values were consistent with the normal $^4C_1$ conformation in neutral and acid solution. Protonation of (5) produced an NMR spectrum which showed the signal due to the anomeric proton as a quartet with spacings of 3.0 Hz and 3.5 Hz and this observation was ascribed to the operation of virtual long range coupling, which was not surprising as H-2' and H-3' had near coincidental shifts and were also strongly coupled.

The NMR spectrum of 1-(2',3',4',6'-tetra-O-acetyl-α-D-manno-pyranosyl)imidazole (38) is reproduced in (Fig.3.11). On the basis of the coupling constants (Table 3.17 page 110), the compound (38) appears to have the pyranose ring distorted from the normal $^4C_1$

conformation. The value of $J_{1',2'}$ of 5.1 Hz is much larger than that normally reported for α-D-mannopyranosides which exhibit $J_{1,2}$ values of the order of 1-1.5 Hz [72]. also the magnitudes of $J_{3',4'}$ and $J_{4',5'}$ (6.9 and 5.8 Hz respectively) do not support the $^4C_1$ conformation. The NMR coupling constants do not appear to support the alternate $^1C_4$ conformation. Of the various twist-boat and boat conformations $B_{3,0}$ and $^1S_5$ conformations (65) and (66) appear to reasonably fit the NMR data (Table 3.17.)



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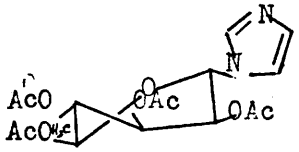

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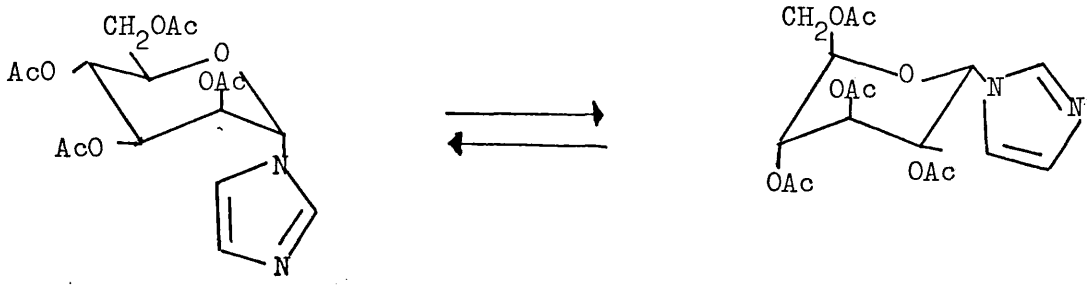
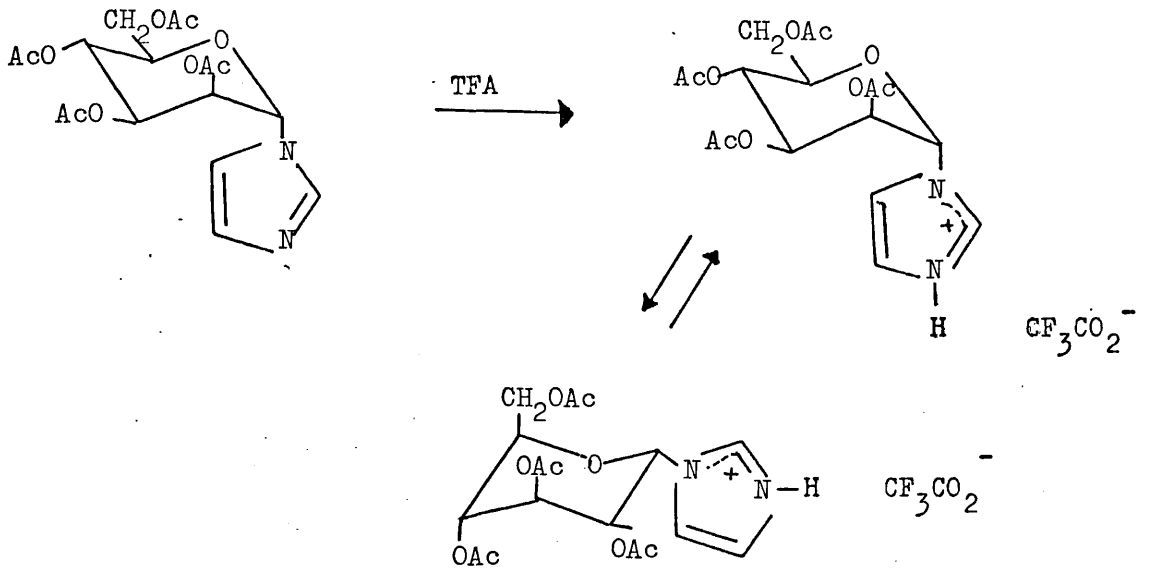
It is well known from the energy profile for the degenerate interconversions of the chair conformers of cyclohexane, that the chair conformations are energetically more stable followed by twist-boat and then the boat conformations, the half-chair being the least stable. In view of this fact it is reasonable to expect for the compound (38) an equilibrium between the alternative chair conformers ($^4C_1 \leftrightarrows {}^1C_4$). The NMR data fit well for an equilibrium of this sort.



38

The evidence that the unprotonated imidazole ring adopts an equatorial orientation proves that the presence of a positive charge on the imidazole ring is not necessary to observe the reverse anomeric effect. This is also consistent with observations on other derivatives of α- D mannopyranoses (60,61)

The values of coupling constants (Table 3.7) obtained from the NMR spectrum of the protonated 1'-(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl)imidazole show that the compound is more highly distorted from the $^4C_1$ conformation on protonation. The coupling constant $J_{1',2'}$ of 7.0 Hz suggests a near trans-diaxial orientation for H-1' and H-2', and $J_{4',5'}$ of 4.25 Hz corresponds to a near gauche orientation for H-4' and H-5'. The NMR data (Table 3.7) fit reasonably well for the adoption of an equilibrium between the two chair conformers, with the $^1C_4$ conformer predominating. (Scheme 3.4)



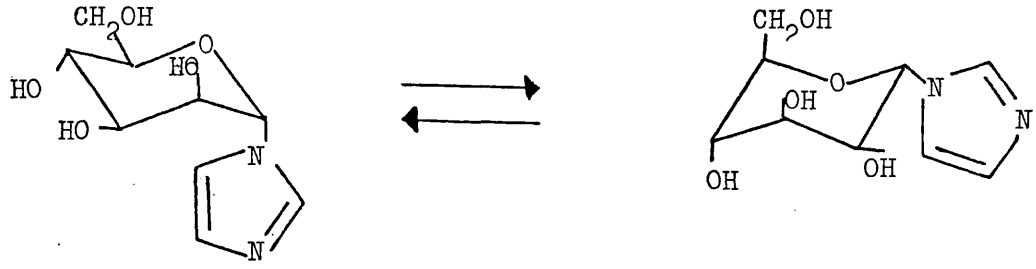
Scheme 3.4

Attempts were made to correlate the NMR data to the six boat and twist-boat conformations for the protonated compound , the data did not fit to any of the twelve conformations, and as a result they could be eliminated.

No abnormal conformational changes were detected in 1-(2',3',4',6'-tetra-O-acetyl-β-D-mannopyranosyl)imidazole (37) and 1-β-D-mannopyranosylimidazole. On the basis of the coupling constants (Table 2.13) the compounds appear to possess the normal $^4C_1$ conformation.

The coupling constants (Table 3.15) derived from the NMR spectrum of 1-α-D-mannopyranosylimidazole (44) do not appear to support the normal $^4C_1$ conformation. The value for $J_{3',4'}$ and $J_{4',5'}$ (5.7 and 6.6 Hz respectively) and much smaller than would have been expected for trans-diaxial protons in the $^4C_1$ conformation. The NMR data (Table 3.15) does not seem to support either the twist-boat or the

boat conformations. However, it seems reasonable to suggest an equilibrium between the two chair conformers $^4C_1$ and $^1C_4$, and the

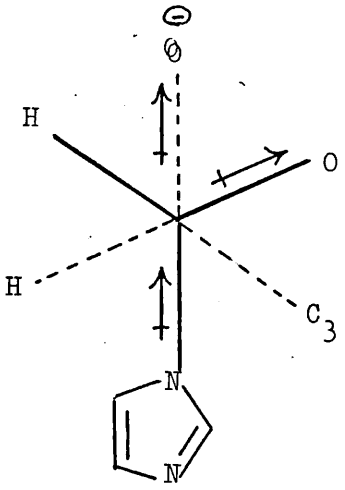


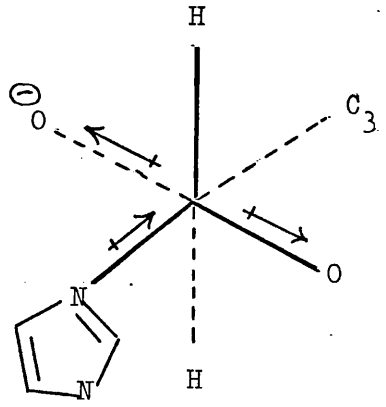
NMR data fit reasonably well for such an equilibrium.

On protonation of (44) the coupling constants $J_{1',2'}$ and $J_{4',5'}$ change from 3.7 and 6.6 Hz to 4.8 and 3.6 respectively, indicating a further distortion of the pyranose ring. The coupling constants (with the exception of $J_{1',2'}$) obtained for the protonated compound have been obtained by deduction as the observed spectrum could not be analysed on a first-order basis. These values were used to obtain a calculated spectrum which did not compare very well with the observed spectrum. Attempts are being made to use an iterative computer programme in order to obtain more reliable coupling constants. Hence rigorous discussions of these coupling constants in terms of twist boat and boat conformations have been avoided.

The NMR spectrum of 1-α-D-mannopyranosylimidazole (44) in NaOD shows $J_{1',2'}$ = 6.5 Hz. This large value of $J_{1',2'}$ could be due to the ionization of the C-2-OH group, which makes the dipole arrangement much more favourable in the $^1C_4$ conformation than in the

normal $^4C_1$ conformation.





α-mannose

$C_1 - C_2$ bond

$^4C_1$

$C_1 - C_2$ bond (C_1 in front)

$^1C_4$

Assuming an equilibrium between the two chair conformers in the mannose series, from the J values observed and making use of $J_{1',2'}$ = 1.5 [75] and 9.0 Hz [52] for diequatorial and diaxial protons respectively and $J_{3',4'}$ = 10.0 (Table 3.11) for diaxial protons in the $^4C_1$ conformation and 3.0 Hz for diequatorial protons in the $^1C_4$ conformation for α-D-mannopyranose as reference standards, the proportions of $^4C_1$ and $^1C_4$ have been estimated approximately.

Table 3.2

Compound	Solvent & pH	% $^4C_1$
α-Man Ac	$(CD_3)_2CO$	49†
α-Man Ac	$(CD_3)_2CO/H^+$	27†
α-Man-OH	D_2O	62†
α-Man-OH	D_2O/H^+	56*

† means derived from $J_{1',2'}$, $J_{3',4'}$ and $J_{4,5}$.

* derived from $J_{1',2'}$

An attempt to estimate the magnitude of the reverse anomeric effect from simple empirical calculations using the data of Table 3.2 was made. However, on account of the very variable 'A' values given in the literature for the various groups, it was decided that figures of any significance could not be obtained.
The data from Table 3.2 appear to be consistent with the fact that the reverse anomeric effect is considerably less in the deacetylated compound in D_2O than in the acetylated compound in $(CD_3)_2CO$.

The energy contributing to greater conformational changes in α-D-manno-compounds as compared to α-D-gluco compounds as seen earlier, would arise (i) from steric preference for the group at C-2 to go equatorial in the alternative conformations (in the case of the α-D-manno compounds) and (ii) the possibly greater reverse anomeric effect. In order to try to separate these two factors a study of 1-α-D-galactopyranosylimidazole was undertaken, unfortunately the acetate of the α-anomer could not be obtained in a pure state.

The magnitudes of the coupling constants (Table 3.23) for 1-α-D-galactopyranosylimidazole suggest that the compound exists predominantly in the normal $^4C_1$ conformation. On protonation there appears no evidence (Table 3.23) for any conformational change as was evident in the case of 1-α-D-mannopyranosylimidazole. This confirms that the polar effect of the substituent at C-2 is greater than the steric effect, i.e. the reverse anomeric effect is greater when the hydroxyl group at C-2 is axial rather than equatorial.

The NMR spectrum of 1 -(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)imidazole (47) and the coupling constants (Table 3.19) support the normal $^4C_1$ conformation for (47). The NMR spectrum of 1'-β-D-galactopyranosylimidazole (52) could not be interpreted on a first-order basis on account of its highly complicated pattern. Attempts are being made to obtain coupling constants by the use of an iterative computer programme. On protonation of (52) by the addition of TFA the anomeric proton appeared as a quartet with a spacing of 1.5 Hz and 1.0 Hz. This would appear to be a further observation of virtual long range coupling arising from the near coincidental shifts of the strongly coupled H-2' and H-3' protons.

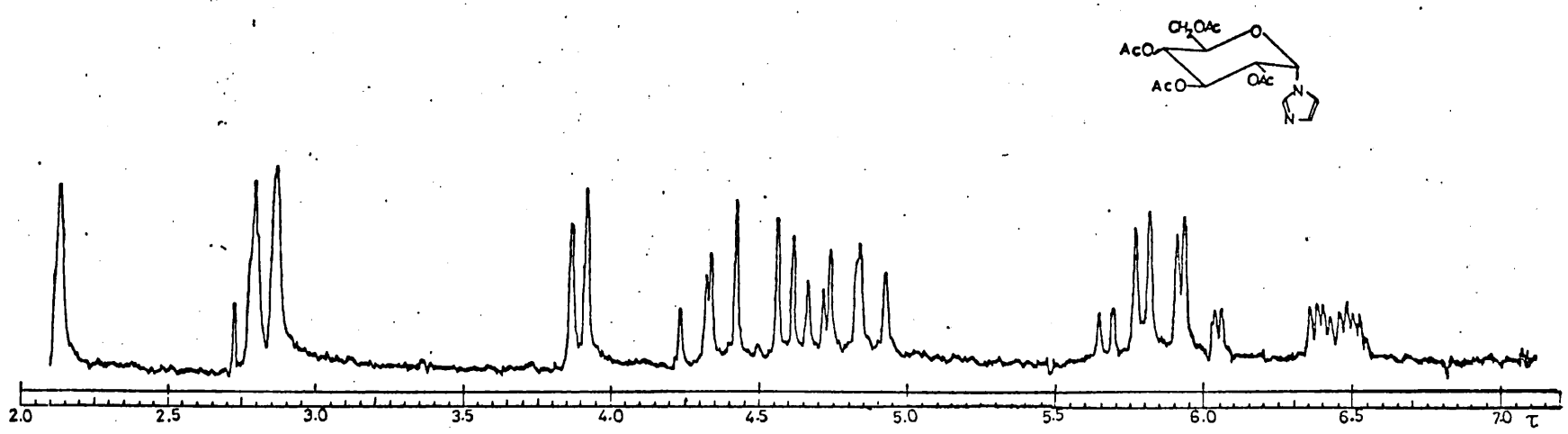


Fig. 3.1 100 MHz NMR spectrum of 1 -(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)-imidazole in $CDCl_3$. (Sweep width 500 Hz)

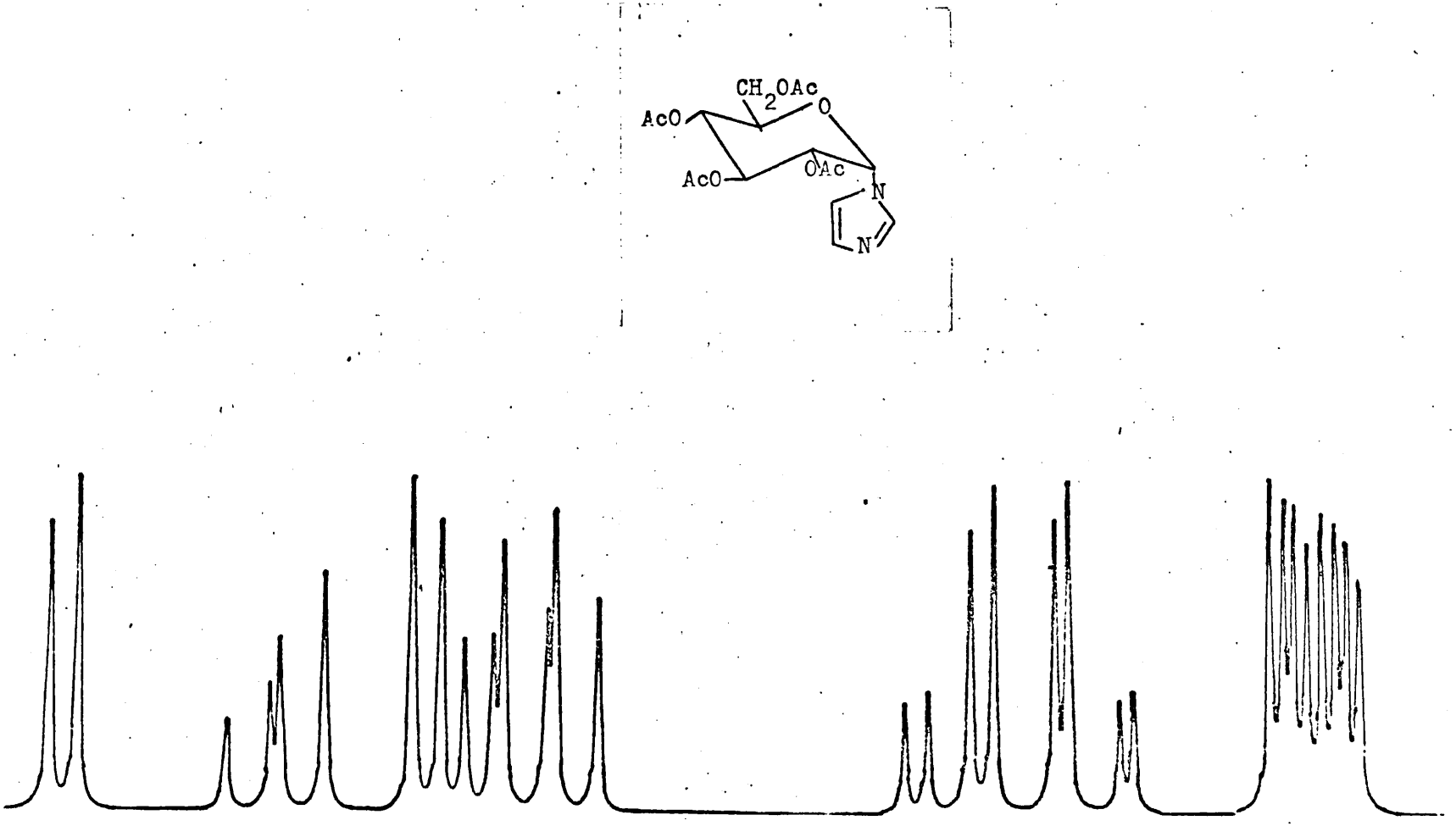


Fig.3.2 Computed NMR spectrum from first order data using a non-iterative program for 1-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)imidazole in $CDCl_3$.
(experimental spectrum 100 MHz)

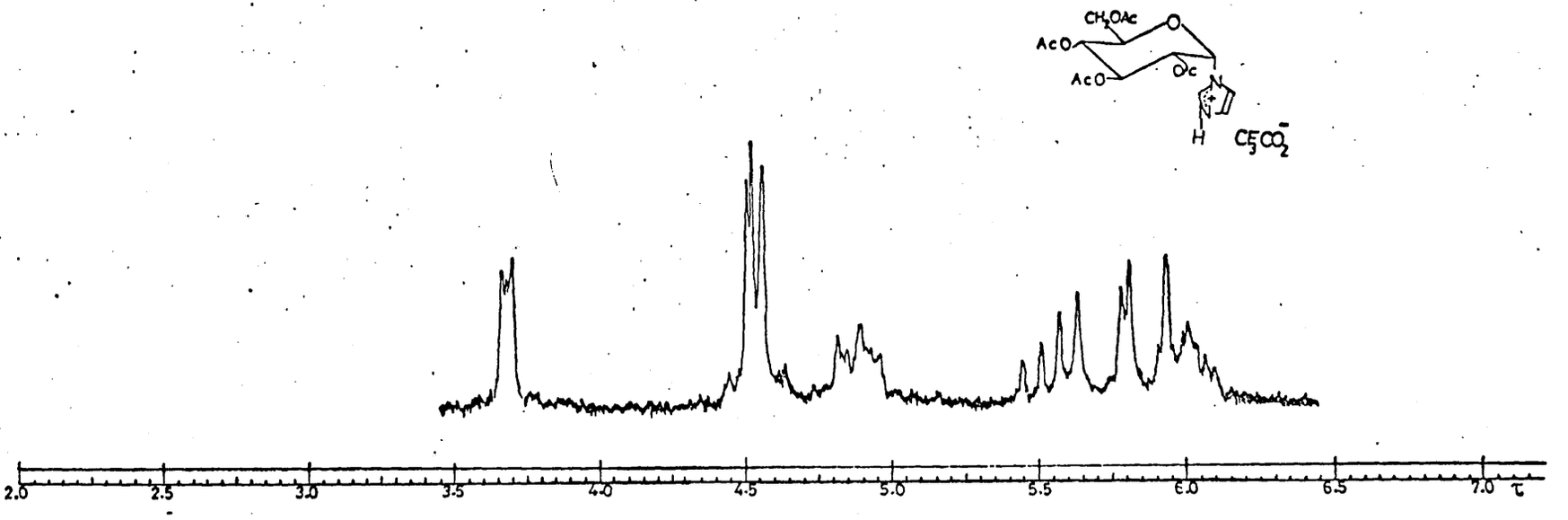


Fig. 3.3 100 MHz NMR spectrum of 1'-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)-imidazole in $CDCl_3$ containing one molar equivalent of TFA. (Sweep width 500 Hz)

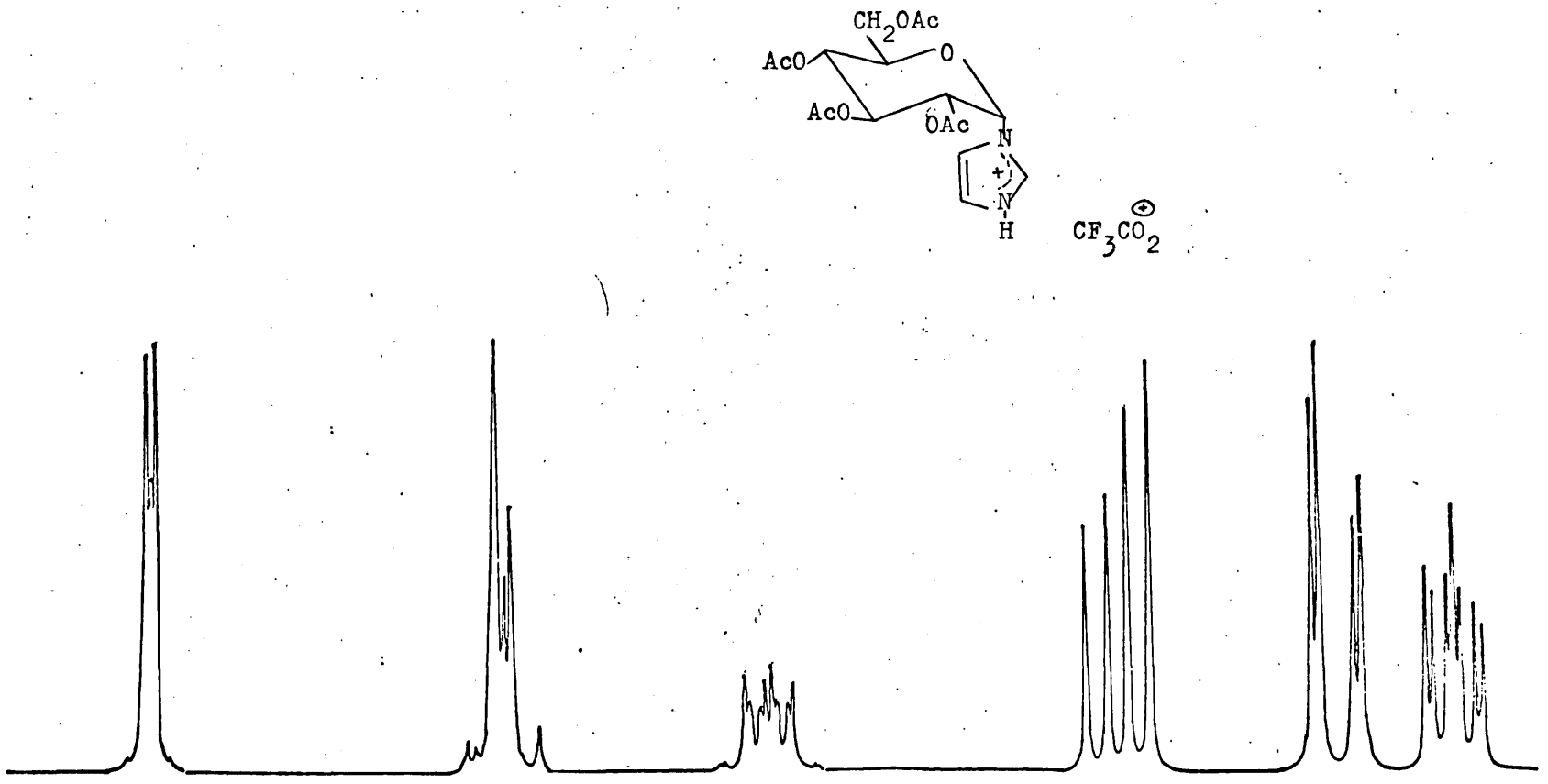


Fig.3.4. Computed NMR spectrum from first order data using a non iterative program for 1-(2',3',4',6'-tetra-O-acetyl-α-D-glucopyranosyl)imidazole in $CDCl_3$ containing one molar equivalent of TFA. (experimental spectrum 220 MHz)

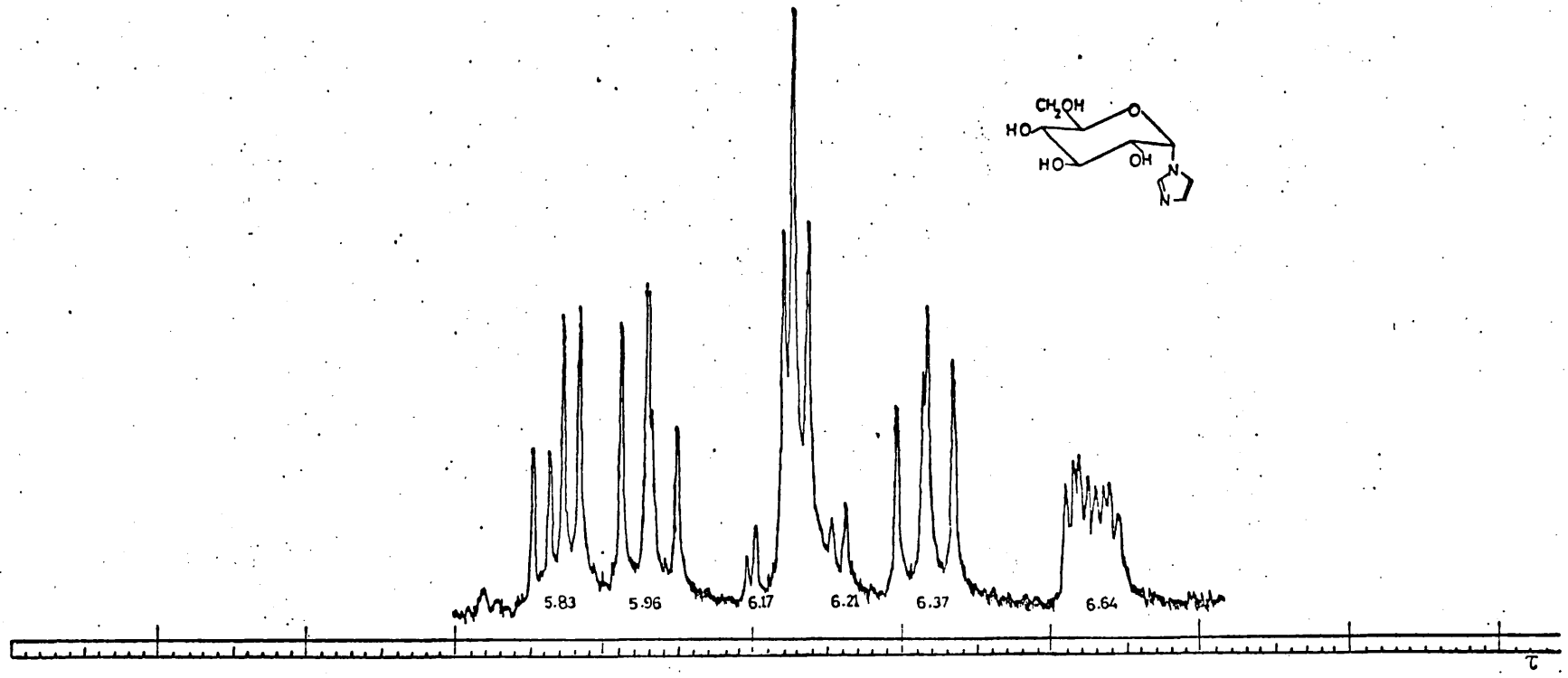


Fig.3.5 220 MHz NMR spectrum of 1'-α-D-glucopyranosylimidazole in D_2O.
(Sweep width 500 Hz)

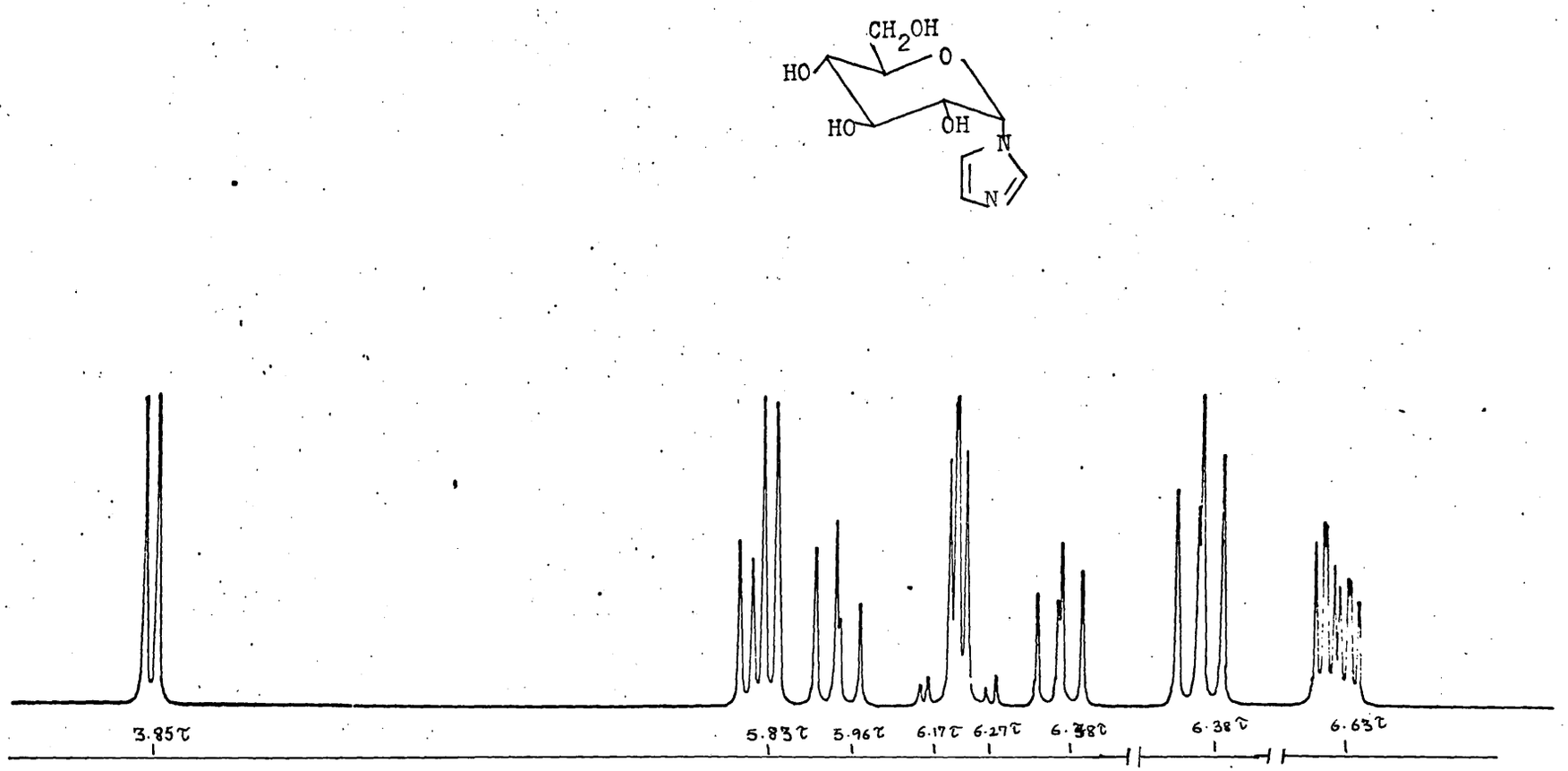


Fig. 3.6 Computed NMR spectrum from first order data using a non-iterative program for 1-α-D-glucopyranosylimidazole in D_2O. (experimental spectrum 220 MHz)

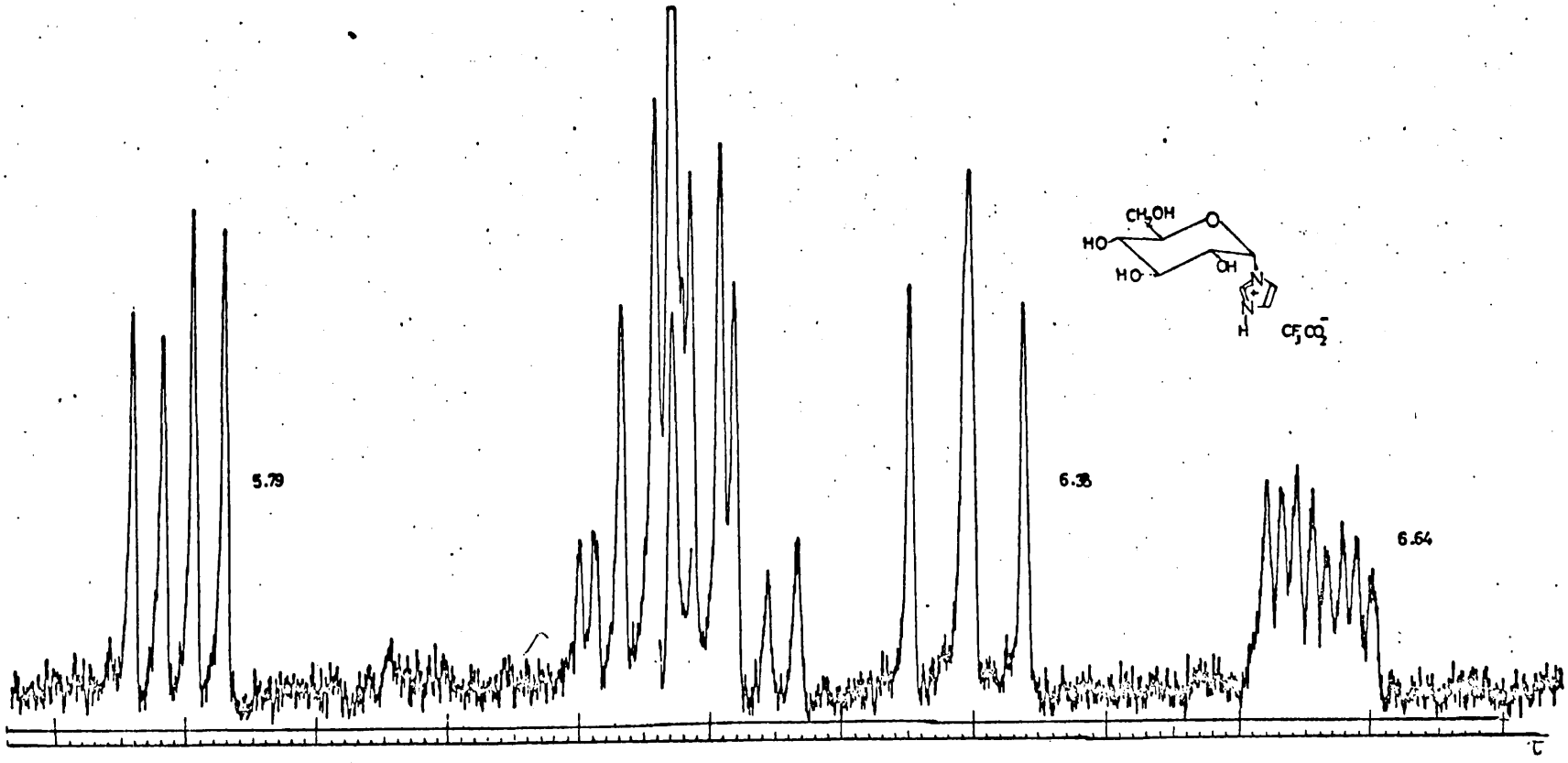


Fig. 3.7 220 MHz NMR spectrum of 1-α-D-glucopyranosylimidazole in D_2O containing one molar equivalent of TFA. (Sweep width 250 Hz)

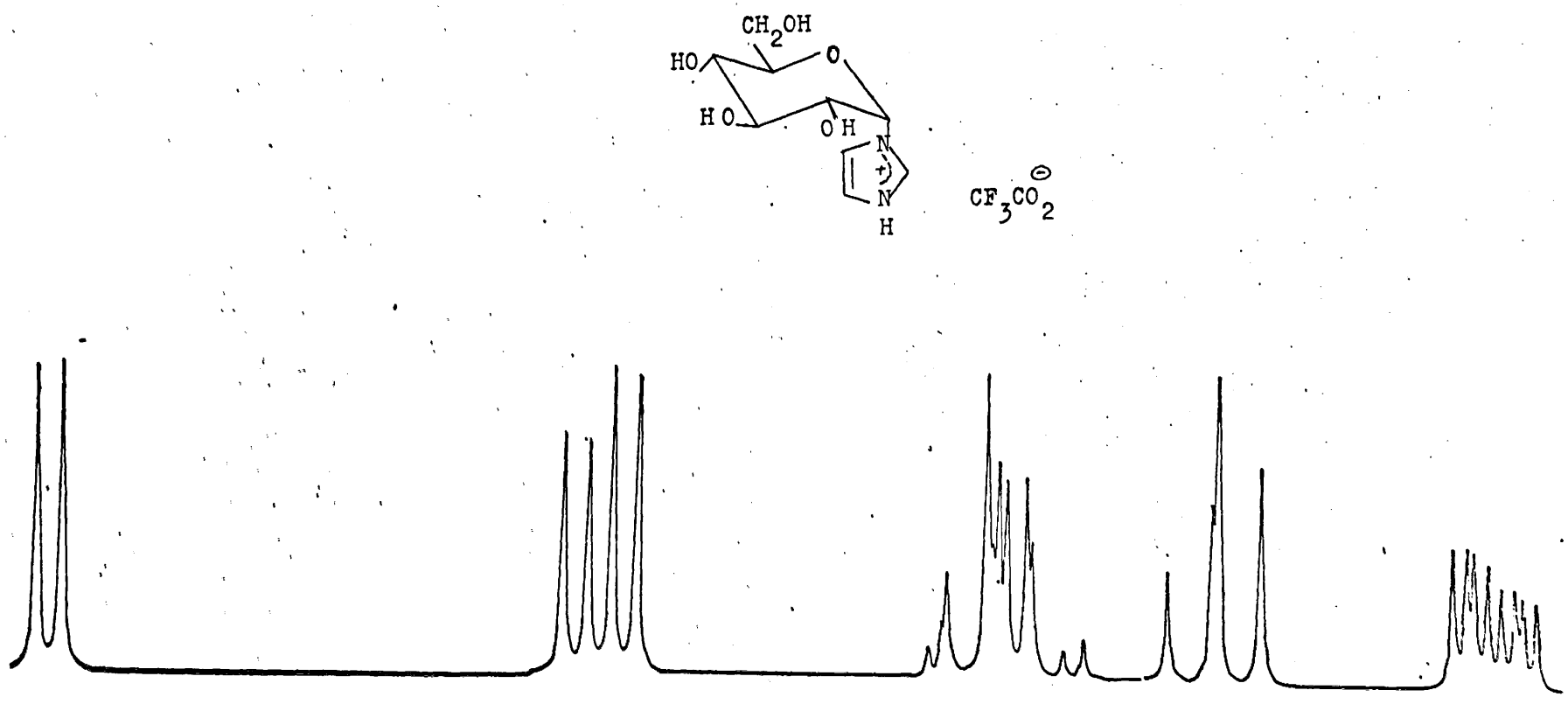


Fig. 3.8 Computed NMR spectrum from first order data using a non-iterative program for 1-α-D-glucopyranosylimidazole in D_2O containing one molar equivalent of TFA. (experimental spectrum 220 MHz)

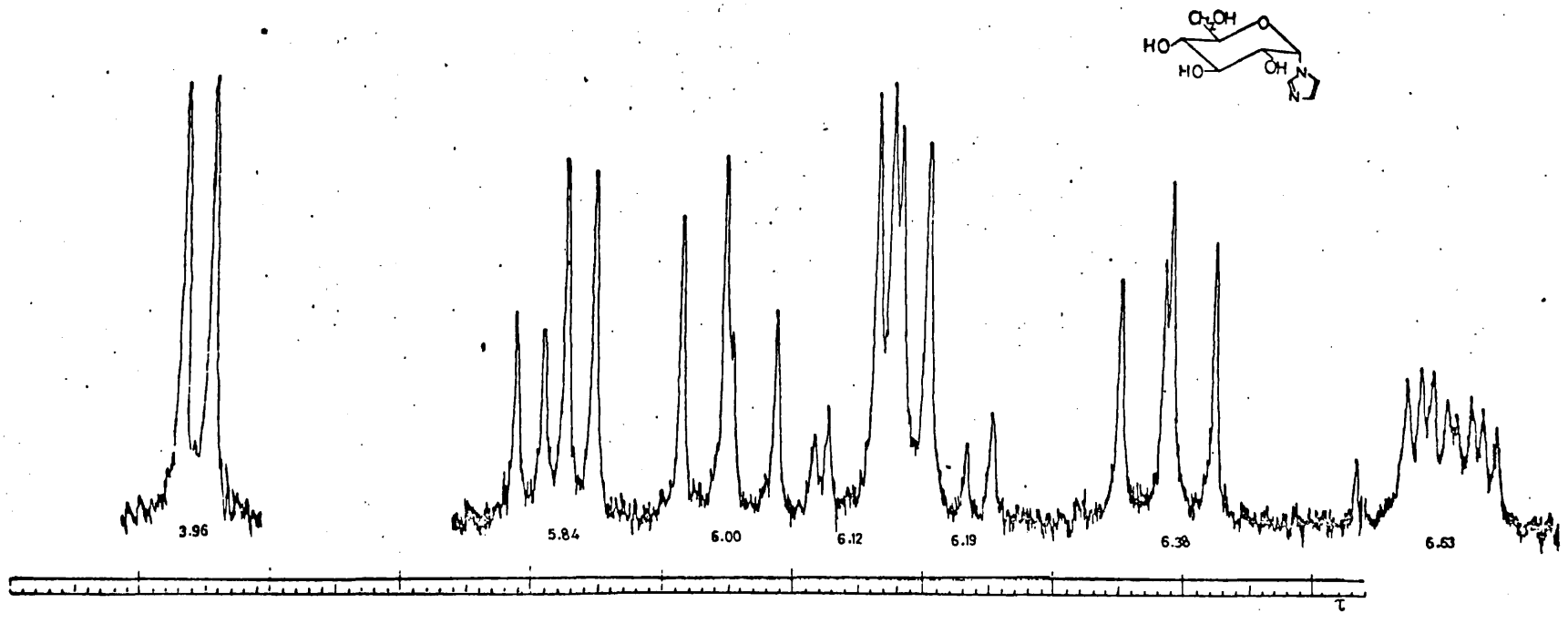
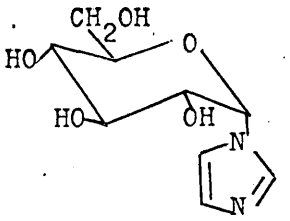


Fig.3.9 220 MHz NMR spectrum of 1 -α-D-glucopyranosylimidazole in 0.1N NaOD solution. (Sweep width 250 Hz)



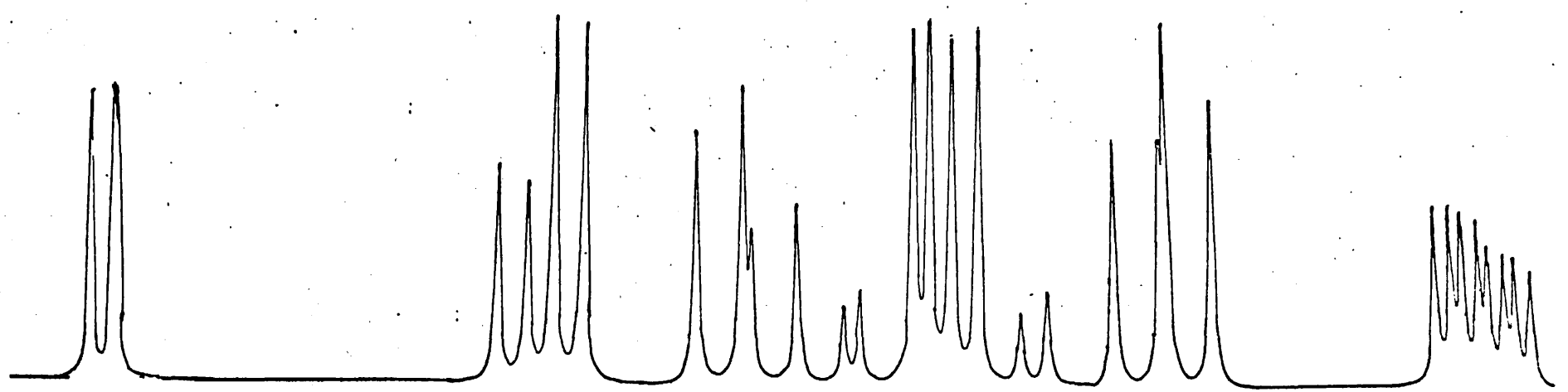


Fig. 3.10 Computed NMR spectrum from first order data using a non-iterative program for 1 -α-D-glucopyranosylimidazole in 0.1N NaOD solution. (experimental spectrum 220 MHz)

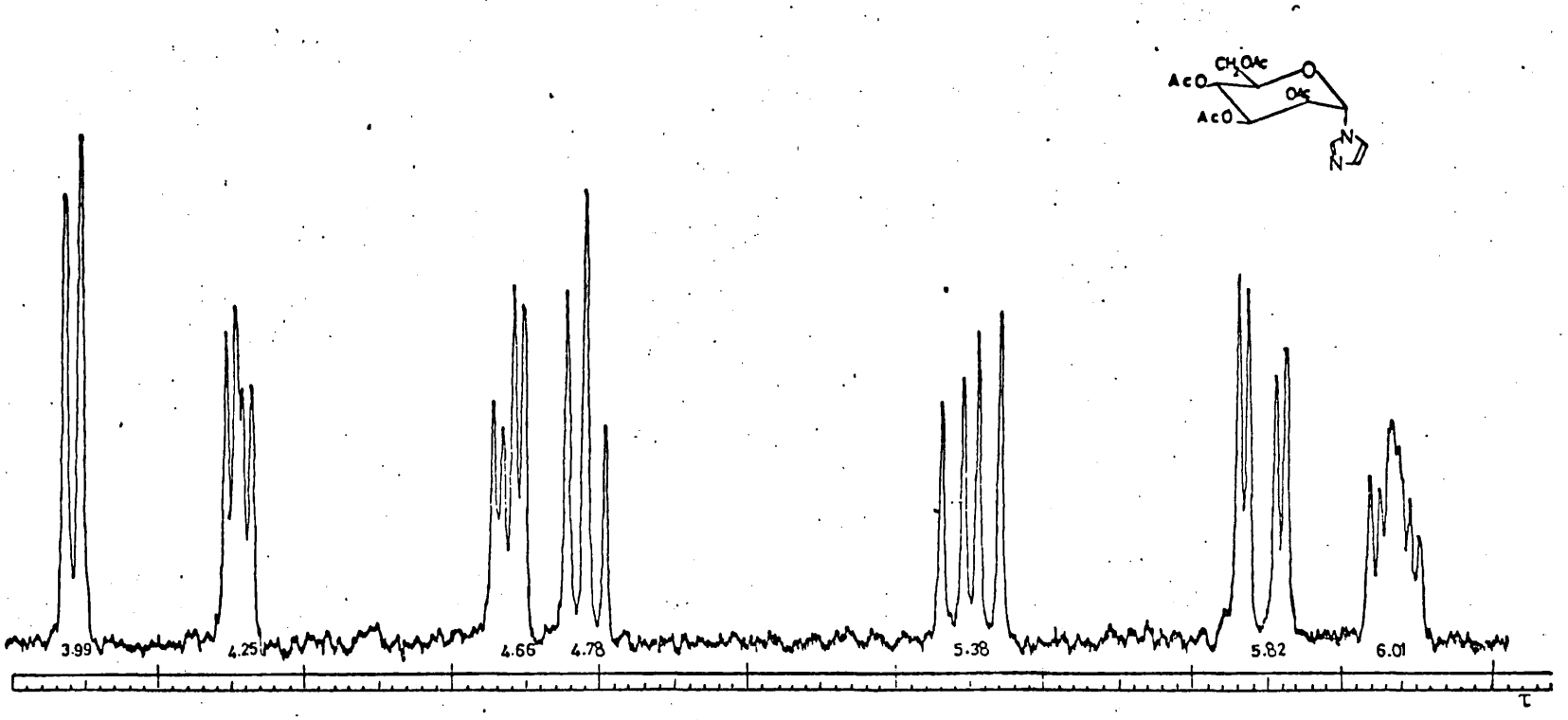


Fig.3.11 220 MHz NMR spectrum of 1 -(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl)imidazole in $(CD_3)_2CO$. (Sweep width 500 Hz)

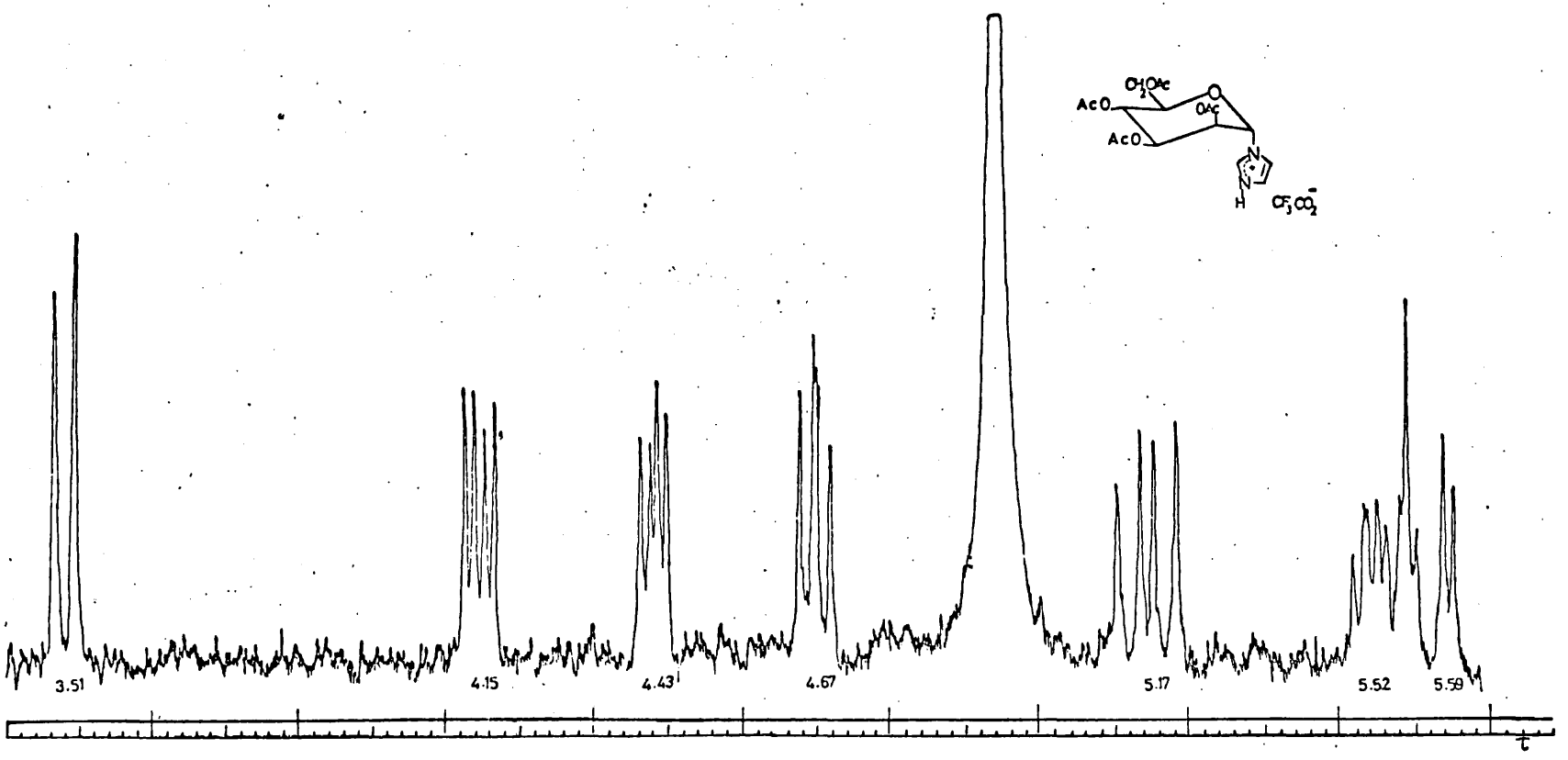


Fig 3.12 220 MHz NMR spectrum of 1 -(2',3',4',6'-tetra-O-acetyl-α -D- mannopyranosyl)-imidazole in $(CD_3)_2CO$ containing one molar equivalent of TFA. (Sweep width 500 Hz)

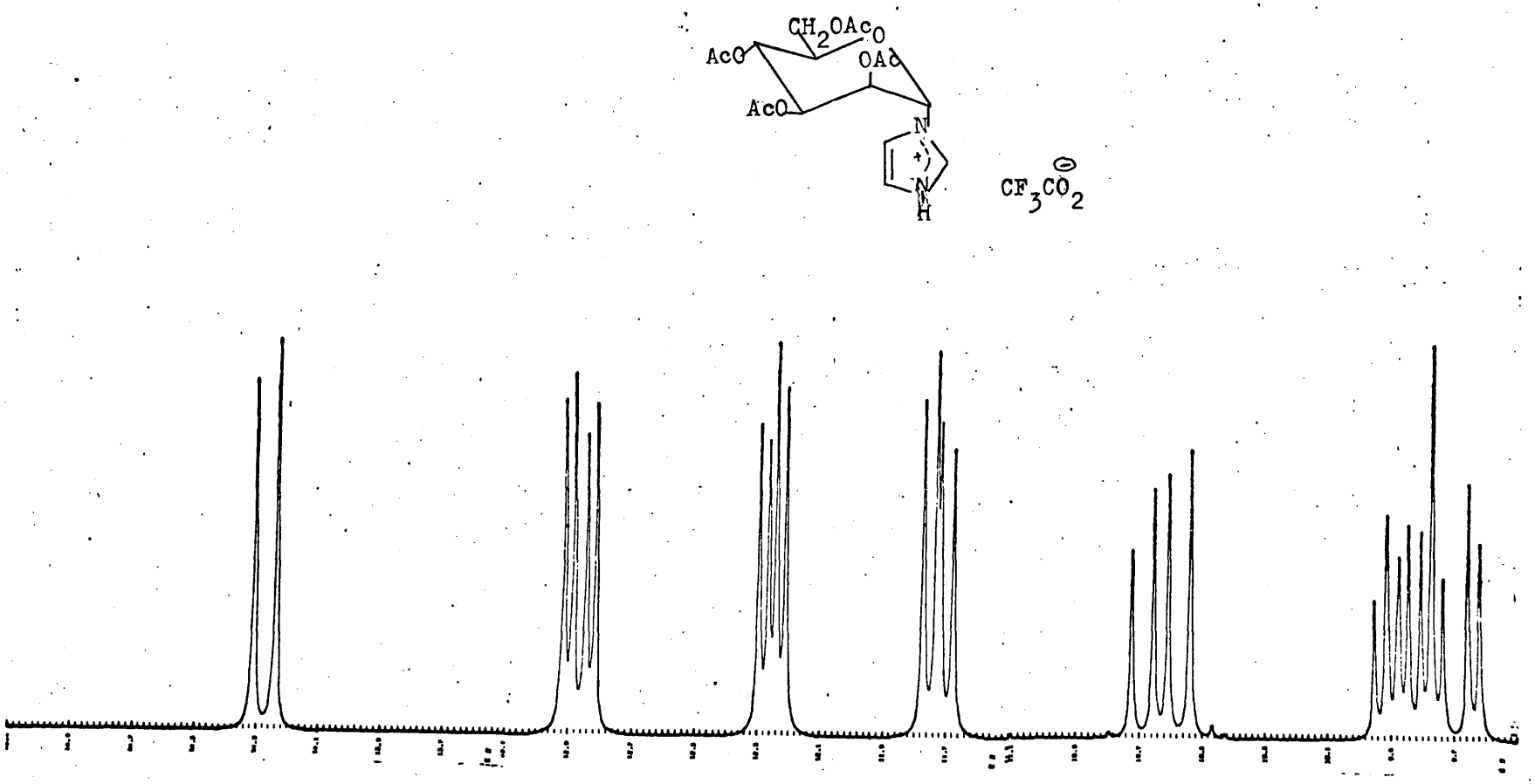


Fig.3.13 Computed NMR spectrum from first order data using an iterative program for 1 -(2',3',4',6'-tetra-O-acetyl-α-D-mannopyranosyl)imidazole in $(CD_3)_2CO$ containing one molar equivalent of TFA. (experimental spectrum 220 MHz)

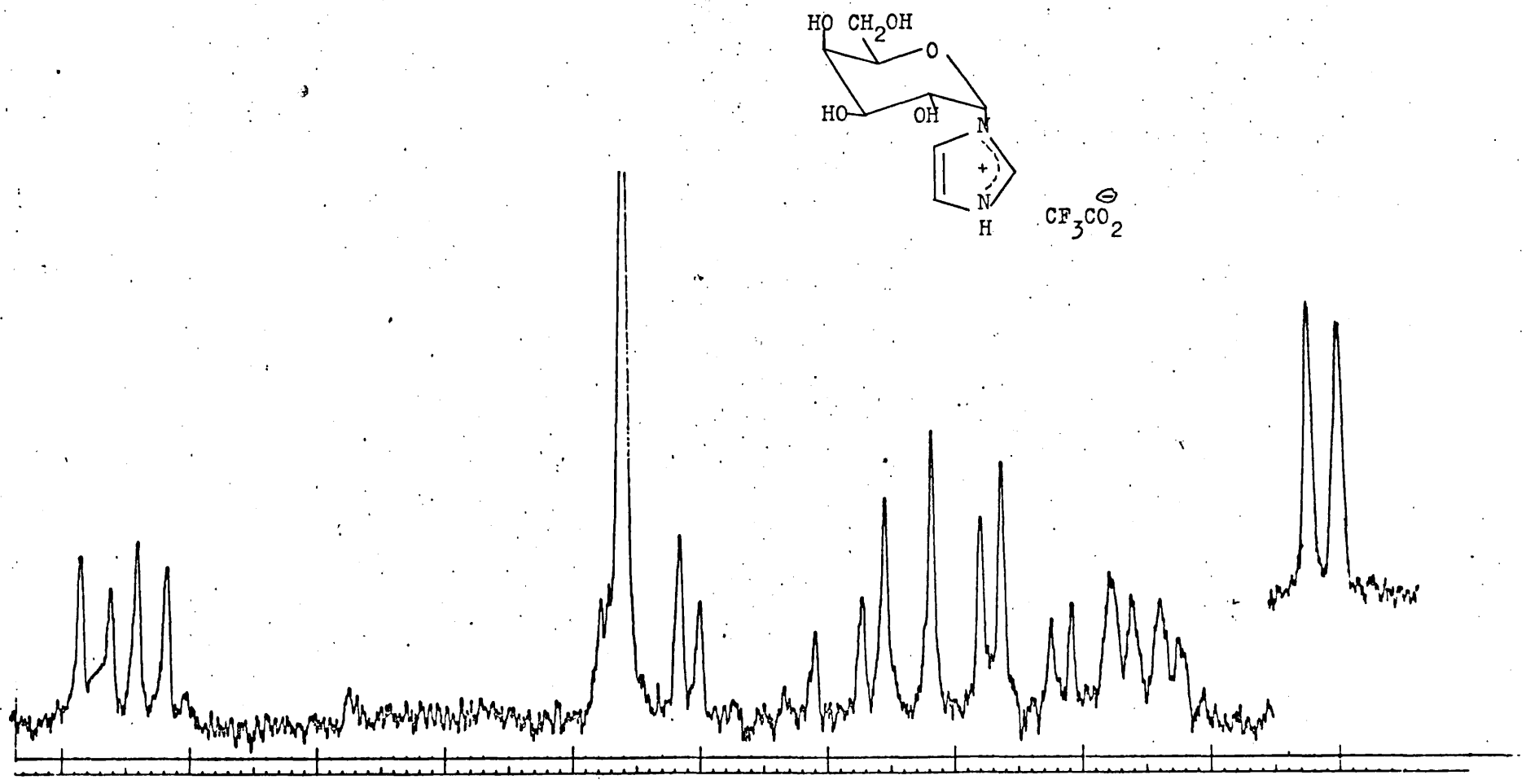


Fig.3,14 220 MHz NMR spectrum of 1-α-D-galactopyranosylimidazole in D_2O containing one molar equivalent of TFA. (Sweep width 250 Hz)

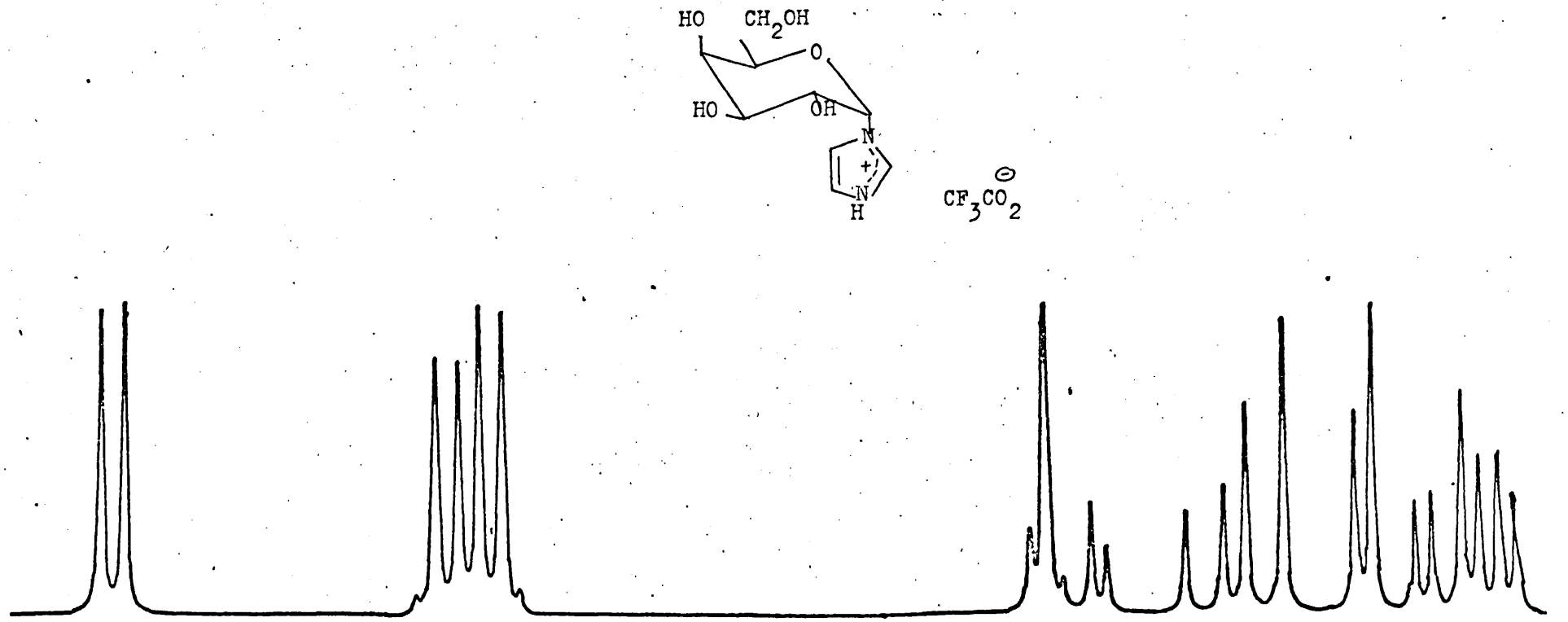


Fig.3.15 Computed NMR spectrum from first order data using an iterative program for 1-α-D-galactopyranosylimidazole in D_2O containing one molar equivalent of TFA. (experimental spectrum 220 MHz)

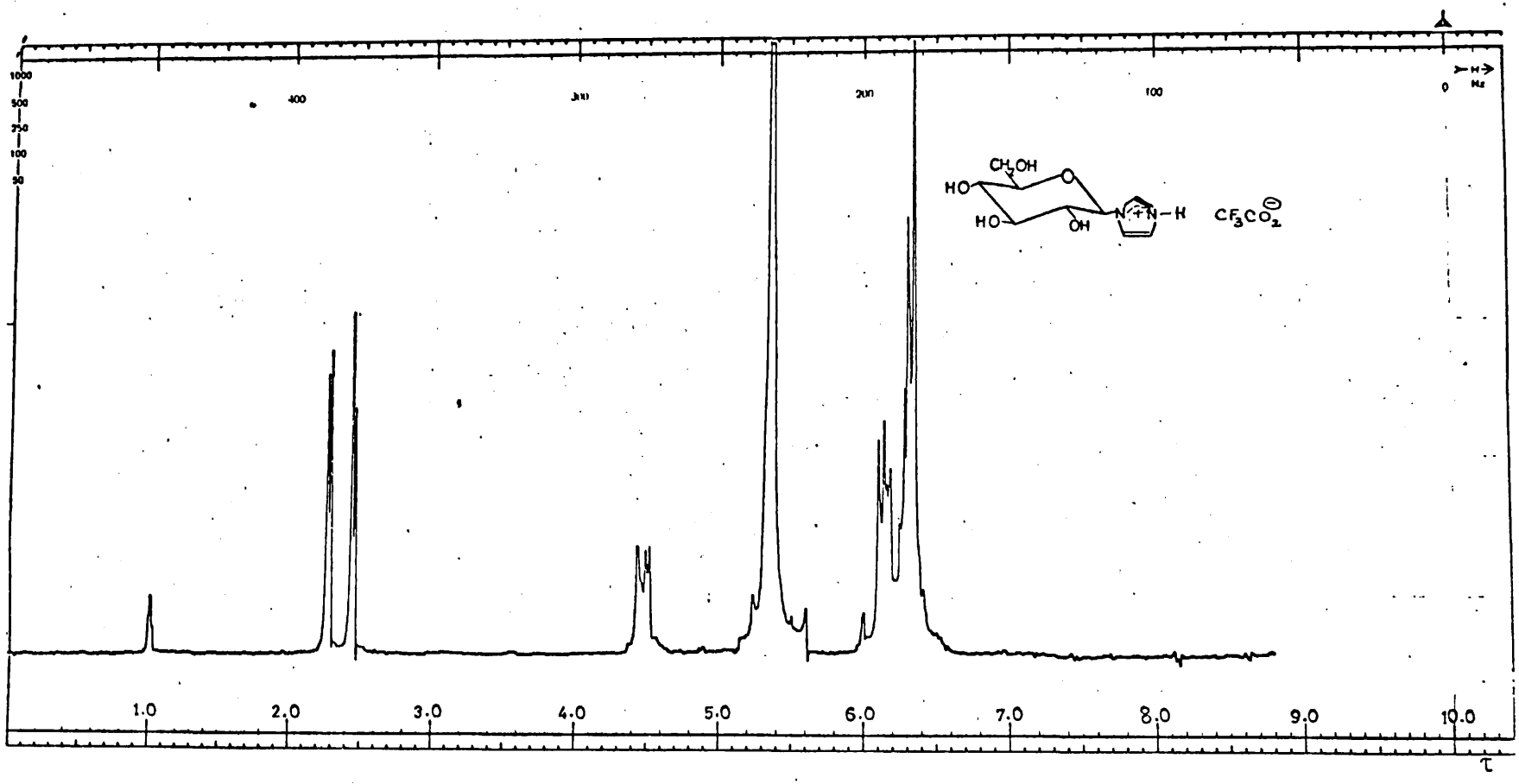


Fig3.16:100 MHz NMR speotrum of 1-β-D-glucopyranosylimidazole in D_2O containing one molar equivalent of TFA. (Sweep width 1000 Hz)

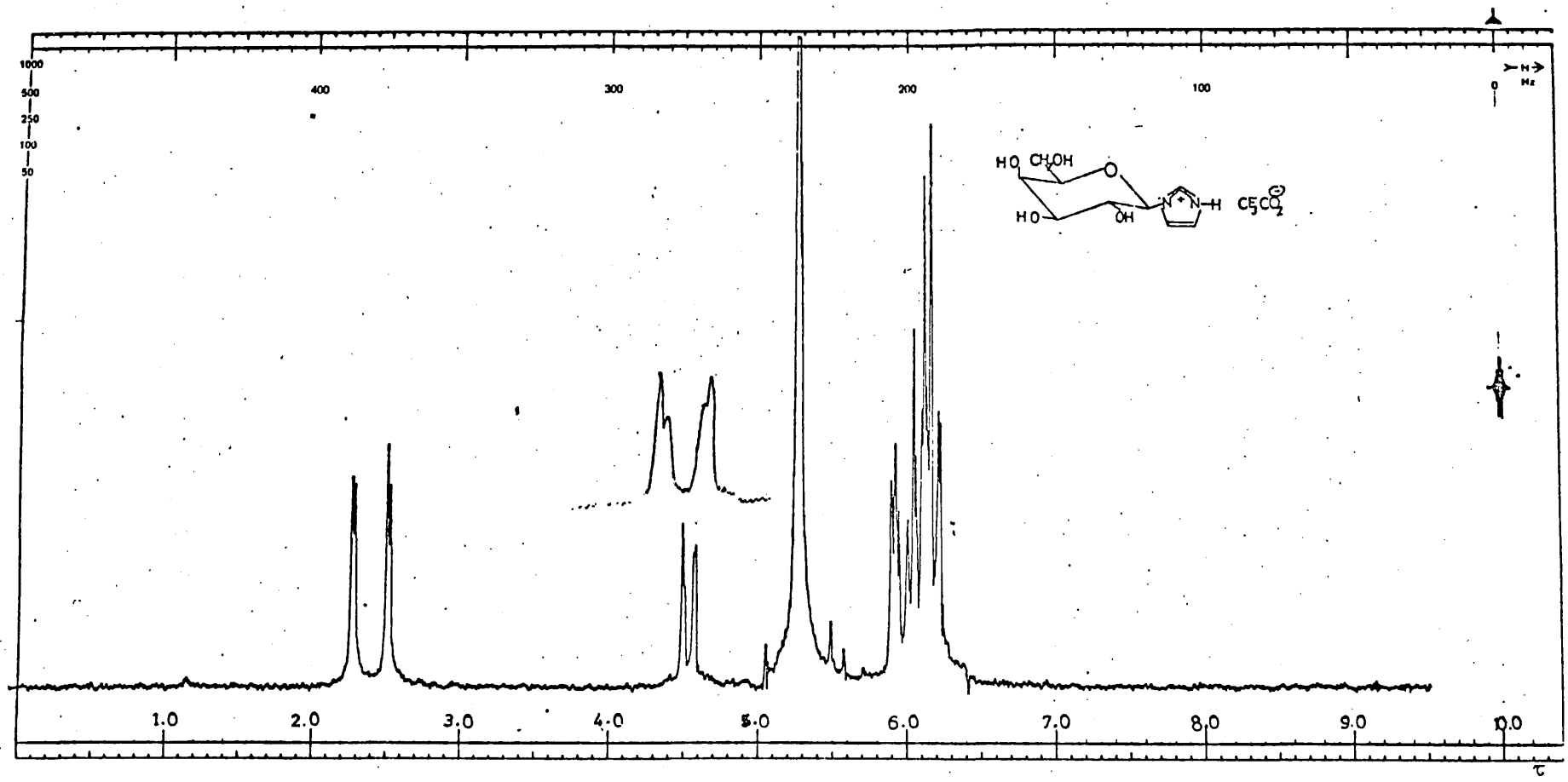
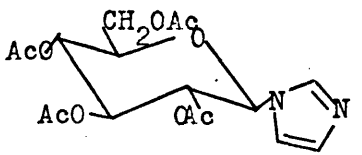


Fig3.17 100 MHz NMR spectrum of 1 -β-D-galactopyranosylimidazole in D_2O containing one molar equivalent of TFA. (Inset sweep width 250 Hz)

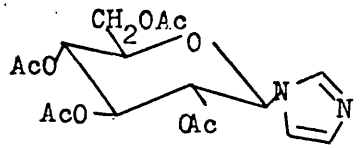
TABLE 3.3



^1H NMR COUPLING CONSTANTS (Hz) FOR 1-(2′,3′,4′,6′-TETRA-O-ACETYL-β-D-GLUCOPYRANOSYL)-IMIDAZOLE

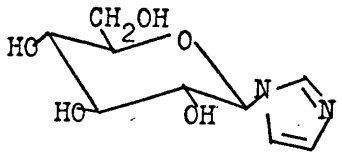
Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
$CDCl_3$	m	m	m	9.1	4.7	2.7	13.0

TABLE 3.4



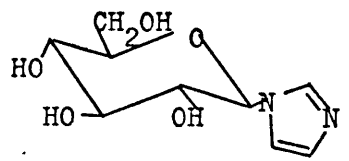
CHEMICAL SHIFTS (τ) OF 1-(2',3',4',6'-TETRA-O-ACETYL-β-D-GLUCOPYRANOSYL) IMIDAZOLE

Solvent	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	H-7'
$CDCl_3$	4.66	4.66	4.66	4.66	5.88	6.06	5.66

TABLE 3.5 

^1H NMR COUPLING CONSTANTS (Hz) FOR 1-β-D-GLUCOPYRANOSYL IMIDAZOLE

Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
D_2O	8.5	m	m	m	m	m	m

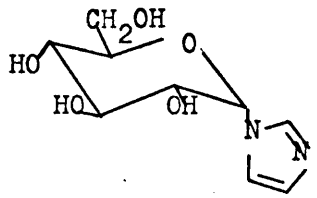
TABLE 3.6 

CHEMICAL SHIFTS (τ) 1-β-D-GLUCOPYRANOSYLIMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
D_2O (100 MHz)	4.66	m	m	m	m	m	m
D_2O (100 MHz) + TFA	4.48	m	m	m	m	m	m

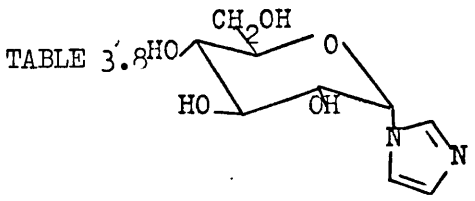
TABLE 3.7

^1H NMR COUPLING CONSTANTS (Hz) FOR 1-α-D-GLUCOPYRANOSYLIMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
D_2O + NaOD	5.25	9.6	8.2	9.2	5.0	2.5	12.1
D_2O	5.5	10.0	8.8	10.0	4.8	2.9	12.5
D_2O + TFA	5.1	10.1	8.9	10.1	4.8	2.4	12.5

TABLE 3.8

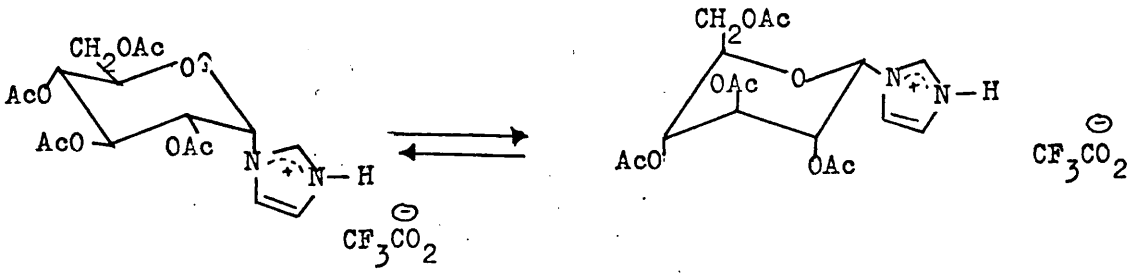


CHEMICAL SHIFTS (τ) OF 1′-α-D-GLUCOPYRANOSYLIMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
D_2O (220 MHz)	3.85	5.83	5.96	6.37	6.64	6.21	6.17
D_2O + TFA (220 MHz)	3.80	5.79	6.16	6.38	6.64	6.20	6.15
NaOD (220 MHz)	3.96	5.84	6.00	6.38	6.63	6.19	6.12

TABLE 3.9

^{1}H NMR COUPLING CONSTANTS (Hz) FOR 1-(2′,3′,4′,6′-TETRA-O-ACETYL-α-D-GLUCOPYRANOSYL)-IMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
$CDCl_3$ (100 MHz)	5.5	10.25	8.5	10.0	4.5	2.5	12.5
$CDCl_3$ + TFA (220 MHz)	3.0	6.25	8.25	8.1	2.3	6.4	12.5

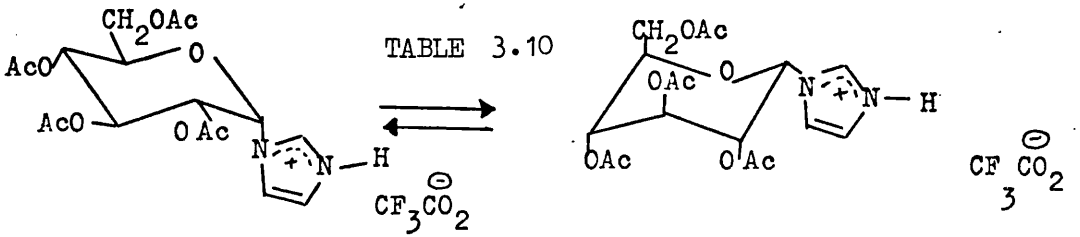


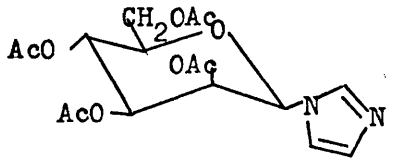
TABLE 3.10

CHEMICAL SHIFTS (τ) OF 1 -(2',3',4',6'-TETRA-O-ACETYL-α-D-GLUCOPYRANOSYL) IMIDAZOLE

SOLVENT	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	H-7'
$CDCl_3$ (100 MHz)	3.89	4.61	4.30	4.80	6.40	5.73	5.98
$CDCl_3$ (220 MHz) + TFA	3.68	4.58	4.55	4.92	6.02	5.87	5.57

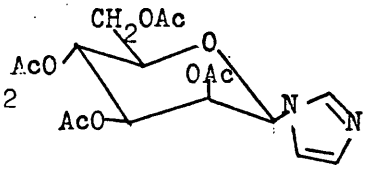
TABLE 3.11

^1H NMR COUPLING CONSTANTS (Hz) FOR 1-(2′,3′,4′,6′-TETRA-O-ACETYL-β-D-MANNOPYRANOSYL)-IMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
$(CD_3)_2CO$	1.4	2.85	9.9	9.7	2.4	4.1	11.75
$CDCl_3$	1.25	2.7	10.0	8.9	5.5	2.5	12.0

TABLE 3.12

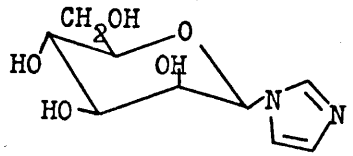


CHEMICAL SHIFTS (τ) OF 1-(2',3',4',6'-TETRA-O-ACETYL-β-D-MANNOPYRANOSYL) IMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
$CDCl_3$ (100 MHz)	4.39	4.49	4.84	4.74	6.12	5.81	5.70
$(CD_3)_2CO$ (220 MHz)	3.82	4.45	4.64	4.68	5.84	5.77	5.75

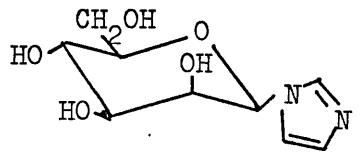
TABLE 3.13

^1H NMR COUPLING CONSTANTS (Hz) FOR 1-β-D-MANNOPYRANOSYLIMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
D_2O	1.1	3.05	6.0	9.25	2.3	5.0	12.5

TABLE 3.14

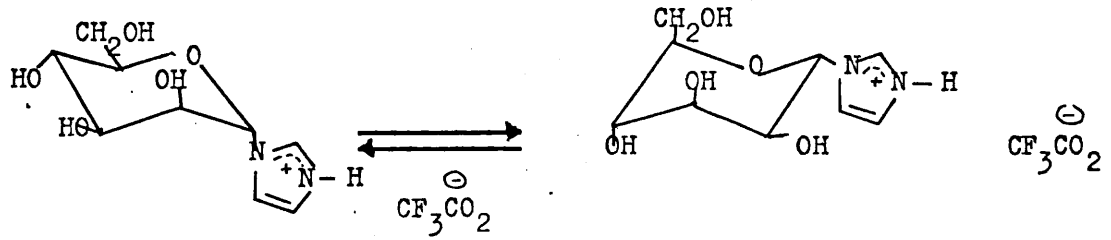


CHEMICAL SHIFTS (τ) of 1 -β-D-MANNOPYRANOSYLIMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
D_2O (220 MHz)	4.28	5.80	6.12	6.25	6.36	6.17	6.07

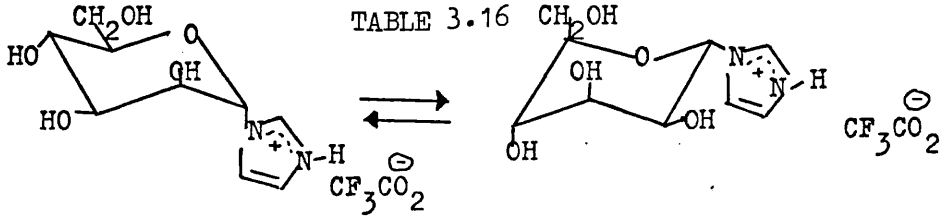
TABLE 3.15

^1H NMR COUPLING CONSTANTS (Hz) FOR 1-α-D-MANNOPYRANOSYLIMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
D_2O	3.7	3.7	5.7	6.6	3.6	6.6	11.75
D_2O + TFA	4.8	2.35	m	3.6	3.6	7.4	m
D_2O + NaOD	6.6	6.6	m	m	m	m	m

TABLE 3.16

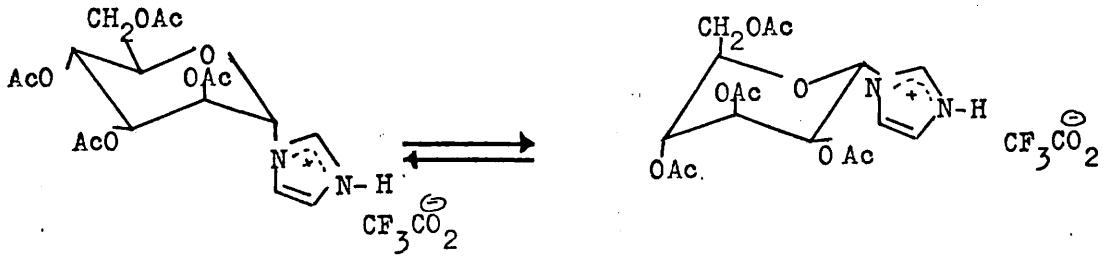


CHEMICAL SHIFTS (τ) OF 1 -α-D-MANNOPYRANOSYLIMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
D_2O (220 MHz)	4.18	5.41	6.05	6.16	6.47	6.1	6.10
D_2O + TFA (220 MHz)	4.02	5.44	m	m	6.28	6.08	6.08

TABLE 3.17

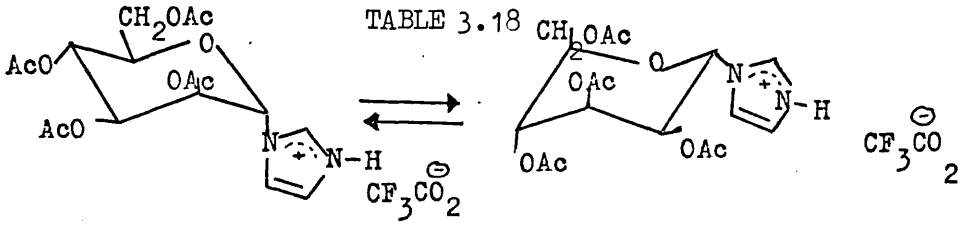
^1H NMR COUPLING CONSTANTS (Hz) FOR 1-(2',3',4',6'-TETRA-O-ACETYL-α-D-MANNOPYRANOSYL)-IMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
$(CD_3)_2CO$	5.2	3.1	6.9	5.8	3.2	7.4	12.4
$(CD_3)_2CO$ + TFA	7.0	3.0	5.5	4.25	3.7	7.4	12.1
$CDCl_3$	m	m	m	4.4	3.6	7.2	12.3
$CDCl_3$ + TFA	6.5	3.0	6.0	4.5	3.2	9.0	12.5
$(CD_3)_2CO$ + TFA	6.68	2.68	5.18	3.93	3.38	7.08	12.1

obtained by using iterative program.

TABLE 3.18

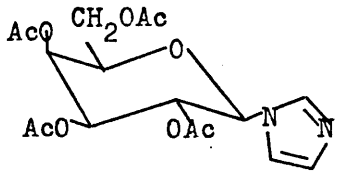


CHEMICAL SHIFTS (τ) OF 1'-(2',3',4',6'-TETRA-O-ACETYL-α-D-MANNOPYRANOSE) IMIDAZOLE

Solvent	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	H-7'
$(CD_3)_2CO$ (220 MHz)	3.99	4.25	4.66	4.78	6.01	5.82	5.38
$(CD_3)_2$ CO + TFA (220 MHz)	3.51	4.15	4.43	4.67	5.52	5.59	5.17
$CDCl_3$ (100 MHz)	4.12	4.12	4.75	4.75	6.14	5.40	5.96
$CDCl_3$ (100 MHz) + TFA	4.02	4.37	4.65	4.87	5.19	5.09	5.80
$(CD_3)_2CO$ + TFA (220 MHz) obtained by using iterative program	3.51	4,16	4,44	4.68	5.20	5.60	5.19

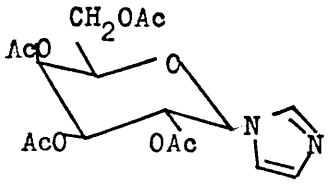
TABLE 3.19

^{1}H NMR COUPLING CONSTANTS (Hz) FOR 1-(2′,3′,4′,6′-TETRA-O-ACETYL-β-D-GALACTOPYRANOSYL)-IMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
$CDCl_3$	9.1	10.2	3.0	4.55	4.5	2.5	13.0

TABLE 3.20

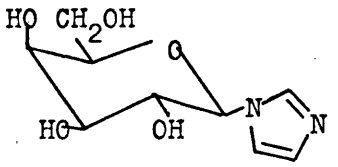


CHEMICAL SHIFTS (τ) OF 1-(2',3',4',6'-TETRA-O-ACETYL-β-D-GALACTOPYRANOSYL) IMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
$CDCl_3$ (220 MH)	4.70	4.48	4.82	4.50	5.85	5.85	5.85

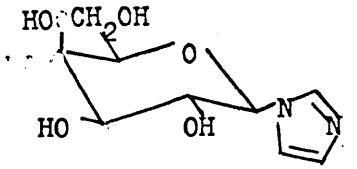
TABLE 3.21

^1H NMR COUPLING CONSTANTS (Hz) FOR 1-β-D-GALACTOPYRANOSYL-IMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
D_2O	m	m	m	m	m	m	m

TABLE
3.22

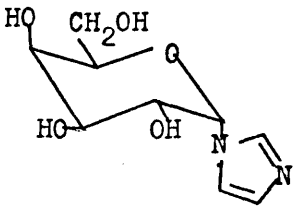


CHEMICAL SHIFTS (τ) OF 1-β-D-GALACTOPYRANOSYLIMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
D_2O (100 MHz)	4.72	(	5.8 - 6.4				)
+ TFA	4.52	(	5.86 - 6.4				)

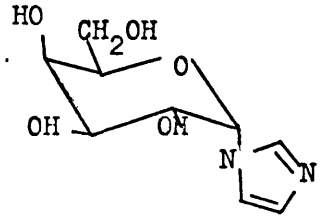
TABLE 3.23

^{1}H NMR COUPLING CONSTANTS (Hz) FOR 1-α-D-GALACTOPYRANOSYLIMIDAZOLE



Solvent	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6a',6b'}$
D_2O	5.55	10.3	3.65	0.1	3.8	7.95	11.9
D_2O + TFA	5.1	9.75	3.5	0	3.55	8.35	12.0
D_2O + TFA obtained by using iterative program	4.69	9.34	3.09	0.4	3.14	7.49	12.0

TABLE
3.24



CHEMICAL SHIFTS (τ) of 1′-α-D-GALACTOPYRANOSYLIMIDAZOLE

Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-7′
D_2O (220 MH_z)	3.93	5.65	5.85	5.96	6.41	6.32	6.23
D_2O + TFA (220 MH_z)	3.76	5.54	5.93	5.98	6.35	6.25	6.12
D_2O + TFA (220 MHz) obtained by using iterative program	3.76	5.54	5.97	5.94	6.35	6.26	6.12

UEANMRITR

CASE 18 1-ALPHA-D-GALACTO-PYR-IMID+TFA, SOL D_2O, 220 MHZ, 250 HZ EXP NN = 7 NWANT = 1 NISO = 1

FREQUENCY RANGE 0.000 TO 2000.000

MINIMUM INTENSITY 0.00100

ISO VALUE	CHEMICAL SHIFT	MULTIPLICITY
1	W(1) = 1374.500	L(1) = 2
1	W(2) = 982.000	L(2) = 2
1	W(3) = 889.000	L(3) = 2
1	W(4) = 896.000	L(4) = 2
1	W(5) = 805.000	L(5) = 2
1	W(6) = 825.000	L(6) = 2
1	W(7) = 854.000	L(7) = 2

COUPLING CONSTANTS

J(1,2) = 5.100
J(1,3) = 0.000
J(1,4) = 0.000
J(1,5) = 0.000
J(1,6) = 0.000
J(1,7) = 0.000
J(2,3) = 9.750
J(2,4) = 0.000
J(2,5) = 0.000
J(2,6) = 0.000
J(2,7) = 0.000
J(3,4) = 3.500
J(3,5) = 0.000
J(3,6) = 0.000
J(3,7) = 0.000
J(4,5) = 0.000
J(4,6) = 0.000
J(4,7) = 0.000
J(5,6) = 3,550
J(5,7) = 8.350
J(6,7) = 12.000

PARAMETER SETS

1

W(2)
W(3)
W(4)
W(5)
W(6)
W(7)

2

J(1,2)
J(2,3)
J(3,4)
J(4,5)
J(5,6)
J(5,7)

ITERATION 0 R M S ERROR = 1.626
ITERATION 1 R M S ERROR = 1.463
ITERATION 2 R M S ERROR = 1.420
ITERATION 3 R M S ERROR = 1.409

CALCULATED ENERGY LEVELS

LEVEL NO.	ENERGY	SPIN STATE	LEVEL NO.	ENERGY	SPIN STATE
-	-	-	-	-	-
-	-	-	-	-	-

REFINED PARAMETERS AFTER 4 ITERATIONS R M S ERROR = 1.406

W(1) = 1374.500
W(2) = 981.246
W(3) = 888.246
W(4) = 895.246
W(5) = 804.246
W(6) = 824.246
W(7) = 853.746

COUPLING CONSTANTS

J(1,2) = 4.699
J(1,3) = 0.000
J(1,4) = 0.000
J(1,5) = 0.000
J(1,6) = 0.000
J(1,7) = 0.000

COUPLING CONSTANTS

J(2,3) = 9.349
J(2,4) = 0.000
J(2,5) = 0.000
J(2,6) = 0.000
J(2,7) = 0.000

COUPLING CONSTANTS

J(3,4) = 3.099
J(3,5) = 0.000
J(3,6) = 0.000
J(3,7) = 0.000

COUPLING CONSTANTS

J(4,5) = -0.401
J(4,6) = 0.000
J(4,7) = 0.000

COUPLING CONSTANTS

J(5,6) = 3.149
J(5,7) = 7.949

COUPLING CONSTANTS

J(6,7) = 12.000

ERROR VECTORS AND STANDARD ERRORS

1.0064 0.0459

STANDARD ERROR = 0.264

-0.0316 1.0027

STANDARD ERROR = 0.362

PROBABLE ERRORS OF PARAMETER SETS

1 0.180
2 0.245

ORDERED SPECTRUM OF NUCLEI WITH ISO VALUE 1

FREQ. NO.	CALC. FREQ.	OBS. FREQ.	D. FREQ.	INTENSITY	LINE NO.	ENERGY LEVELS
-	-	-	-	-	-	-
-	-	-	-	-	-	-

FREQ. NO.	FREQUENCY	INTEN.	LINE NO.	ENERGY LEVELS	CONNECTED TRANSITIONS LABELLED BY FREQUENCY NUMBERS	
					PROGRESSIVE	REGRESSIVE
-	-	-	-	-	-	-
-	-	-	-	-	-	-

FREQUENCY	INTENSITY	CORRESPONDING LINE NUMBERS
-	-	-
-	-	-

SPECTRUM OF NUCLEI WITH ISO VALUE 1

HALF WIDTH = 1.00 C/S

FREQUENCY RANGE 1320.000 to 1400.000

CASE 18 1-ALPHA-D-GAL-PYR-IMID+TFA SOL D_2O, 220 MHZ 250 HZ EXP

LIST OF SHARP MAXIMA AND MINIMA IN THE PLOTTED REGION

MAXIMA		MINIMA	
FREQ.	INT	FREQ.	INT
1372.160	32.717	1374.560	2.788
1376.880	31.913		

THE PLOTTED SPECTRUM HAS BEEN SCALED SO THAT THE MAXIMUM INTENSITY = 1.0

PLOTTED SPECTRUM

FREQUENCY	INTENSITY	FREQUENCY	INTENSITY	FREQUENCY	INTENSITY	FREQUENCY	INTENSITY
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-

END OF JOB

Fig 3.18

CHEMICAL SHIFTS (δ p.p.m.) OF 1-α-D- GLYCOSYL IMIDAZOLES (ACETATES)

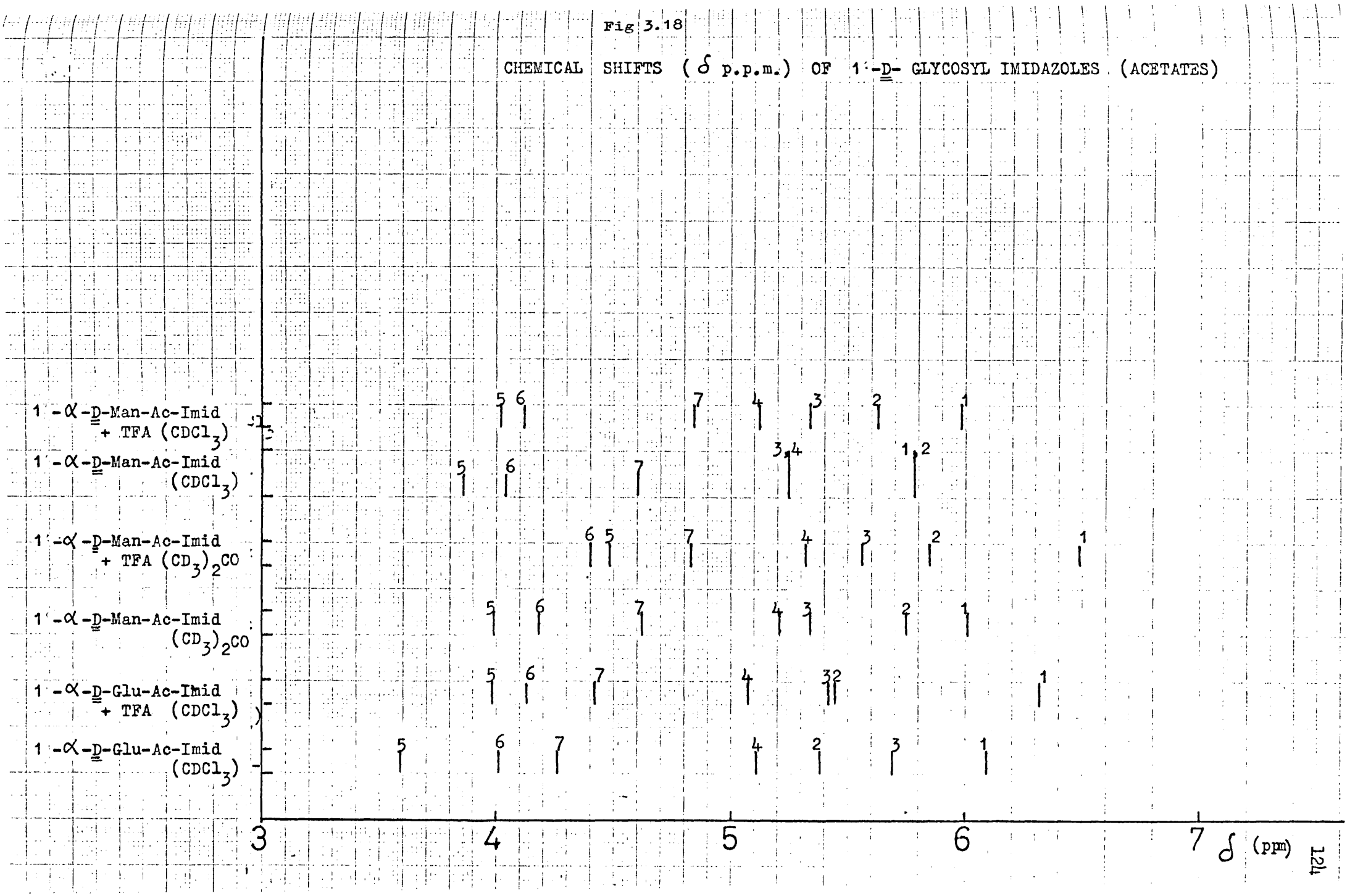
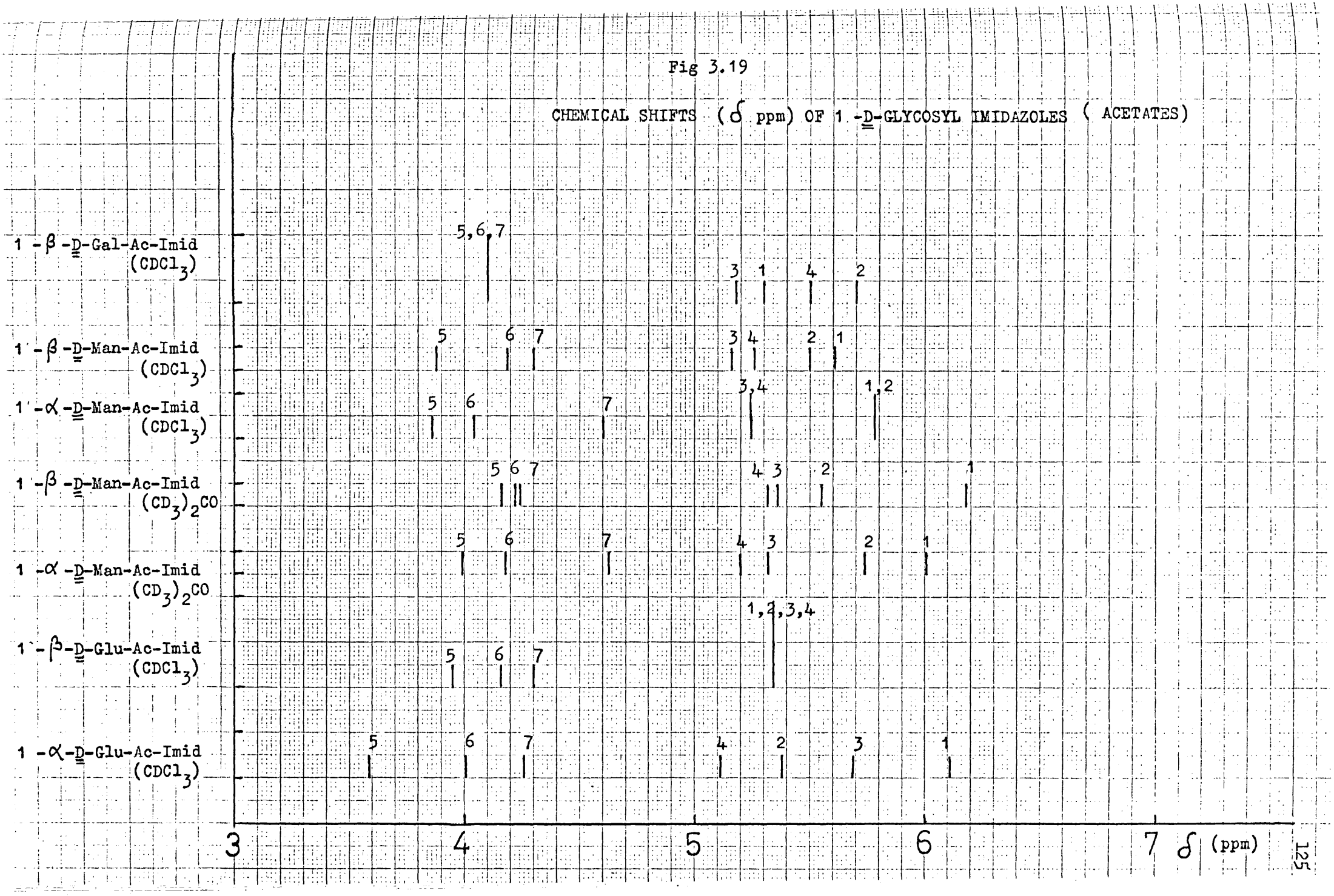


Fig 3.19

CHEMICAL SHIFTS (δ ppm) OF 1 -D-GLYCOSYL IMIDAZOLES (ACETATES)



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CHAPTER 4

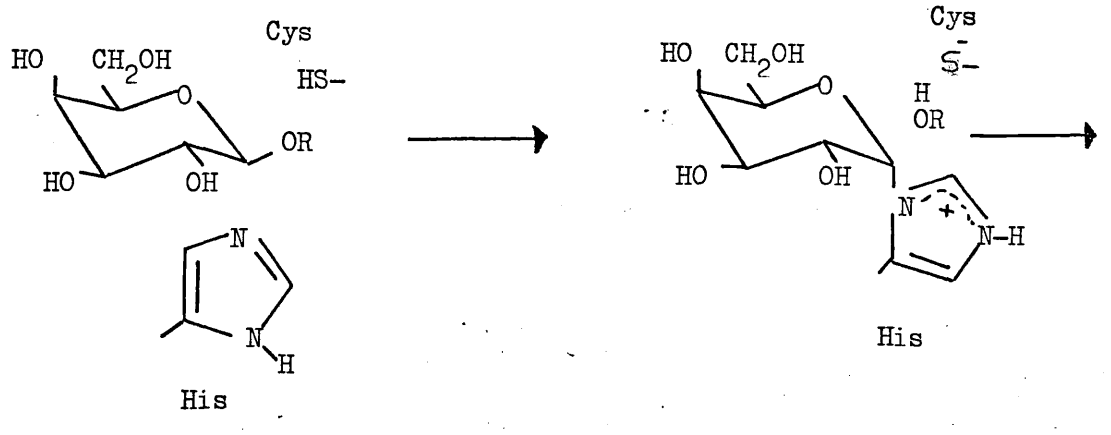
THE STABILITY OF N-GLUCOSYLIMIDAZOLES

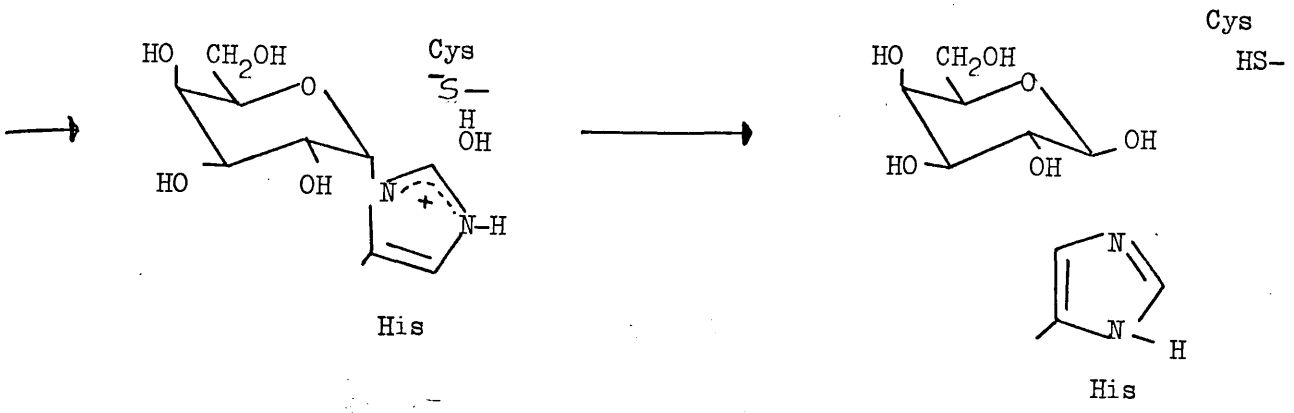
INTRODUCTION

One of the aims of this investigation was the study of the stability of the sugar-base linkage in N-glycosylimidazoles, whose preparation and characterization have been described in Chapters 2 - 4.

Studies of the variation of enzyme activity with pH and photo-oxidation inactivation studies have indicated that a number of glycoside hydrolases and transferases possess essential histidine residues at the enzyme active sites (see Chapter 1). The types of enzyme for which this has been proposed are α-amylase[1-6] β-amylase,[7-10] α-glucosidase,[11] oligo-1,6-glucosidase,[12] phosphorylase[13,14] and β-galactosidase.[15] In the majority of cases it has been suggested that the imidazole ring of the side chain of the active site must be in the protonated, imidazolium form and that this group acts as a general acid catalyst of the glycosyl cleavage reaction.

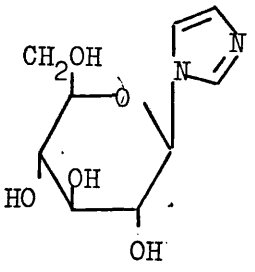
Inhibition experiments and pH-activity studies have demonstrated the presence of two groups, a sulphydryl group and an imidazole group on the active site of E. Coli β-galactosidase. Wallenfels and Malhotra[15] proposed a mechanism for the transfer of a D-galactose residue from galactoside to an acceptor via a neutral imidazole group which acts as a nucleophilic catalyst of the glycosyl cleavage reaction as shown in scheme (4·1)



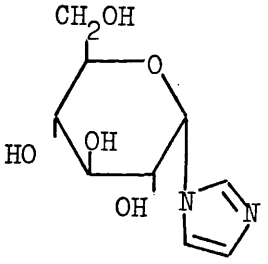


Scheme 4.1

There is little evidence from model studies to support this proposal, although intramolecular nucleophilic assistance of aryl glycoside hydrolysis has been observed.[16] If the mechanism shown in scheme 4.1 is correct, the glycosylimidazolium species would be intermediates and it would therefore be of interest to investigate the stability of the sugar-base linkage in 1-α-D-glucopyranosylimidazole (32) and 1-β-D-glucopyranosylimidazole (5).



5



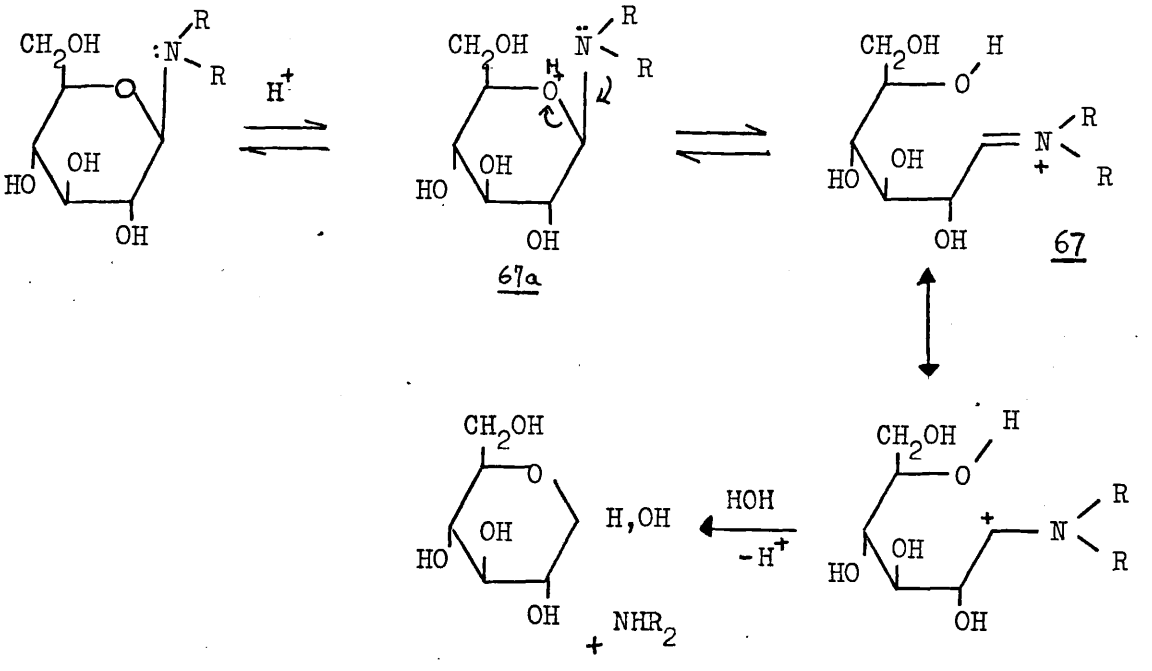
32

Stability of the Sugar-Base Linkage

The susceptibility to hydrolysis of the sugar-base linkage in 1-α and β-D-glucopyranosylimidazole was examined in water, sodium formate buffer (pH 3), 0.565 M-formic acid, 6N-hydrochloric acid, 10N-sulphuric acid and N-sodium hydroxide at 100° for ca. 12 h. Optical rotation measurements, paper chromatography and analysis by use of glucose oxidase reagent did not show any evidence of hydrolysis or anomerization under the

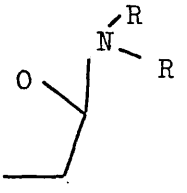
conditions specified. In another experiment 1-β-D-glucopyranosyl-imidazole (5) was recovered and crystallized after being heated under reflux in 5N-sulphuric acid for 6h. Hydrolytic cleavage was also attempted with solutions of the respective α-(yeast) and β-(almond) glucosidases; no glucose was released under conditions under which standard compounds (methyl α-D-glucopyranoside and cellobiose) were hydrolysed significantly.

The resistance to acid hydrolysis of N-glycosylimidazoles may be rationalized by consideration of possible mechanisms of hydrolysis of glycopyranosylamines.[17] Two types of mechanism may be distinguished, the first of which involves the formation of a Schiff base (67) and is followed by those glycosylamines which show mutarotation and are quite readily hydrolysed in acid solutions.(Scheme 4.2)

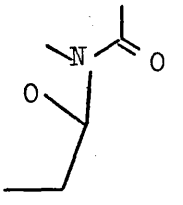


Scheme 4.2

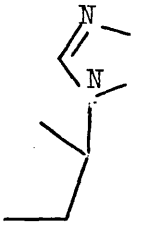
A necessary step in this mechanism in the formation of the Schiff base intermediate and would appear to require electron release by the amino nitrogen. Several workers [18-21] have taken the view that the hydrolysis of nucleosides involves conjugate acid 67a and proceeds with ring opening. The main argument put forward is anology to the mechanism of hydrolysis of glycosylamines, where the ring opening is facilitated by the mesomeric electron release of the amino nitrogen (68).



68



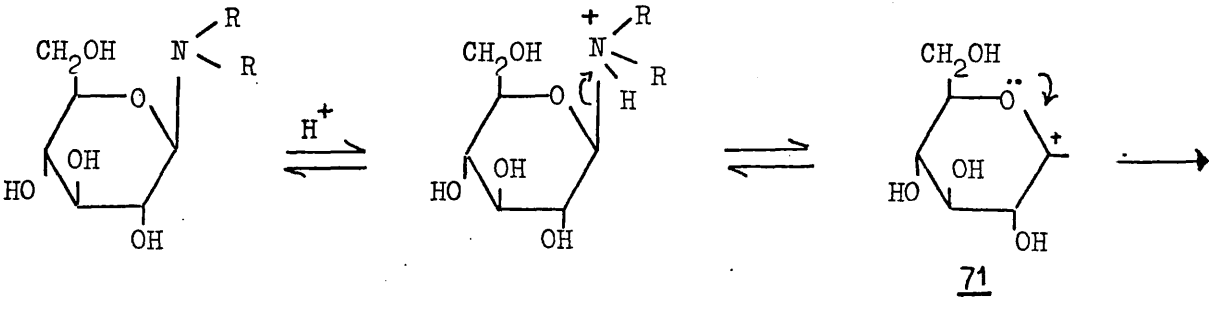
69



70

With nucleosides and glycosylimidazoles the nitrogen atom is not amino but either amido (69) or amidin (70) and would release electrons much less readily [17], also in doing so the aromaticity of the heterocyclic system would be lost and the process is therefore severely restricted in glycosylimidazoles and nucleosides. This mechanism requires either a hydrogen atom or a lone pair of electrons on the glycosylamine nitrogen atom.

The second possible mechanism is characterized by the formation of a glycosyl carbonium ion (71) as shown in scheme 4.3, and is believed to be



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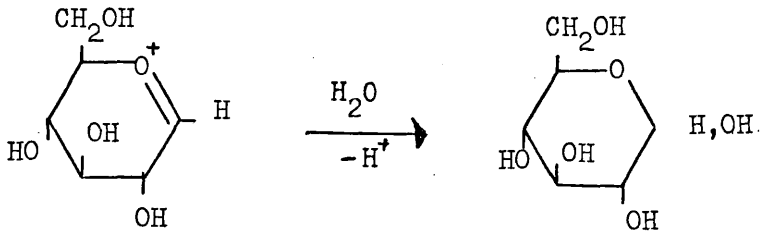
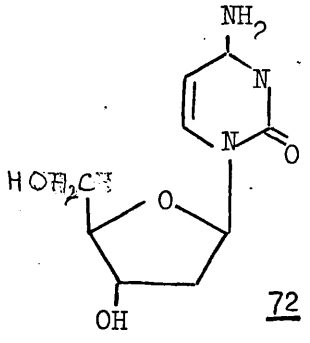


Scheme 4.3

followed for a majority of acetals and a number of nucleosides. Deoxycytidine (72)[22,23] is one of the most thoroughly investigated nucleoside as regards mechanism of hydrolysis and there is evidence



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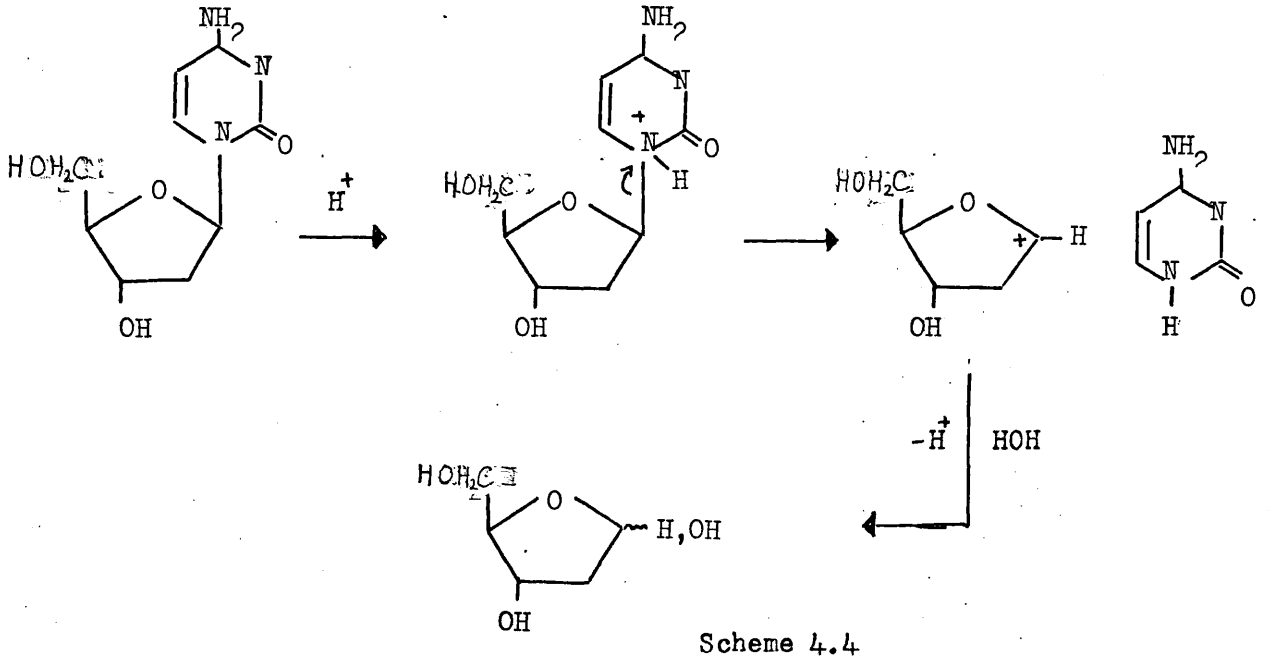
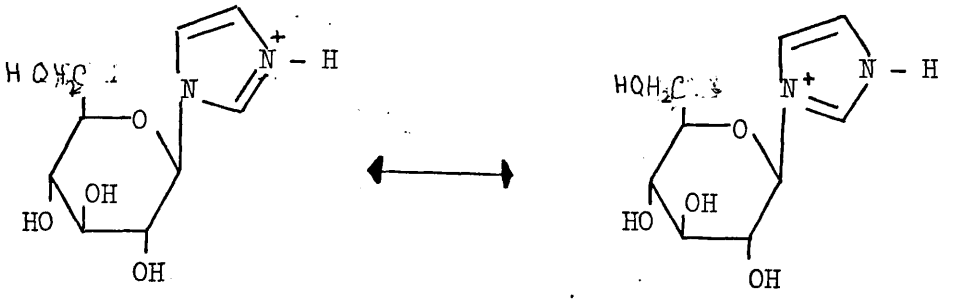
that one of the predominant monoprotonated forms of deoxycytidine : is hydrolysed more rapidly than the unprotonated form since protonation will facilitate the electron flow as shown in scheme 4.4)



Scheme 4.4

The overall rate by the pathway outlined in scheme 4.3 depends in opposite senses on the concentration of the conjugate acid and on the pKa of the leaving group. In the cases of the N-glycosylimidazoles, the imidazole (pKa 7) is too poor a leaving group for breakdown of the resonance-stabilized conjugate acid (72) to occur.



72

The fact that most nucleosides are hydrolyzed at measurable rates in acid solutions may be attributed to two features not present in glycosylimidazoles. Firstly, it has been widely observed that the glycofuranosides are hydrolysed more rapidly than the corresponding glycopyranosides, and the mechanisms involving sugar ring opening (A2) process has been suggested.[17] An alternative explanation is that the attainment of the transition state leading to the cyclic oxycarbonium ion is accompanied by more steric strain in the case of pyranosides than in that of furanosides.

Secondly, the pKa values of the bases relevant to the forms of the nucleosides undergoing hydrolysis in acid solutions are almost certainly lower than the pKa value of imidazole, which would therefore be expected to be a poorer leaving group. In support of this 5-amino-1-D-ribo-furanosylimidazole-4-carboxamide (pKa 3.8) is more susceptible to hydrolysis

than the 1-D-ribofuranosyl derivatives of other imidazole derivatives of higher pKa values.[24] 1-D-ribofuranosyl[24] and 1-β-D-galactopyranosyl[25] derivatives of benzimidazole have been shown to be extremely resistant to acid hydrolysis.

The extreme stability of 1-glucopyranosylimidazoles does not support the formation of N-glycosylhistidinyl intermediates in enzyme catalyzed glycoside hydrolysis.

EXPERIMENTAL

Hydrolysis experiments

I. Samples (15 mg) of 1-α and β-D-glucopyranosylimidazole were heated separately in sealed tubes with 10N-sulphuric acid, 6N-hydrochloric acid, 0.565 M-formic acid, sodium formate buffer (pH 3), N-sodium hydroxide and water (2 ml of each, separately) at 100° for 12h. No change in optical rotation from the initial values was detected during this period. After neutralization with IR-120(H^+) and IR-4B(OH^-) resins the solutions were analysed for glucose by use of the glucose oxidase reagent, and by paper chromatography [development with propan-1-ol-ammonia (d. 0.88) (3:1)]. Paper chromatograms were sprayed with glucose oxidase reagent (Worthington Glucostat), silver nitrate - sodium hydroxide reagent or the Pauly reagent (diazosulphanilic acid), no evidence for anomerization or hydrolysis was obtained.

II. 1-β-D-glucopyranosylimidazole (0.5 g) was heated with 5N-sulphuric acid at 100° under reflux for 6h. The solution was neutralized with 5N-sodium hydroxide, concentrated to dryness and extracted with dry methanol.

Crystallization of the extract from propan-1-ol gave a solid (0.1 g) indistinguishable from the starting material (paper chromatography and mixed m.p.).

III. 1-α-D-glucopyranosylimidazole (0.020 g) was incubated at 37° with (yeast) α-glucosidase (0.002%) (Sigma; 2.1 International Units, measured by the rate of hydrolysis of p-nitrophenyl α-D-glucopyranoside) in pH 6.8 phosphate buffer (I 0.05; 20 ml), and samples (1 ml) were analysed for glucose by the glucose oxidase procedure. It was established that the presence of imidazole at the levels anticipated did not invalidate the glucose analysis, and a control experiment was carried out, with methyl α-D-glucopyransodie (0.0194 g) as substrate. No release of glucose from α-D-glucosylimidazole was observed.

1-β-D-glucopyranosylimidazole (0.0115 g) was incubated at 37° with (almond) β-glucosidase (0.002%) (Sigma, 1.5 International Units, measured by the rate of hydrolysis of salicin) in 0.02M-citrate buffer (pH 5.3) (20 ml). A control experiment was carried out with cellobiose as the substrate. No release of glucose from β-D-glucopyranosylimidazole was observed.

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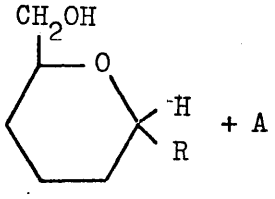
CHAPTER - 5

THE OPTICAL ROTATORY PROPERTIES OF N-GLYCOSYLIMIDAZOLES

INTRODUCTION

Sugars have been among the first organic compounds for which extensive optical rotation data has been accumulated. Therefore the most useful correlations of configurations and conformations of carbohydrates with optical rotatory power has been achieved by comparison of molecular rotations at suitable wavelengths, very often at the sodium D-line (589 nm).

Hudson[1] was able to apply van't Hoff's "principle of optical superposition" successfully to a large number of carbohydrate derivatives in the form of his "isorotation rules", which attempt to correlate the molecular rotation with the configuration at the anomeric centre for a number of pyranoid derivatives. According to the "isorotation rules", the molecular rotations of glycosyl compounds are assumed to be made up of two components, 'A' and 'B'; contribution 'A' is attributed to the anomeric centre and is taken as positive for α-D-and β-L-derivatives (73), and negative for β-D- and α-L derivatives (74) contribution 'B' is supposed to



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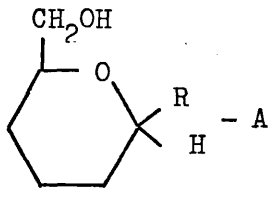


74

α-anomer

$[M_\alpha] = +A +B$

β-anomer

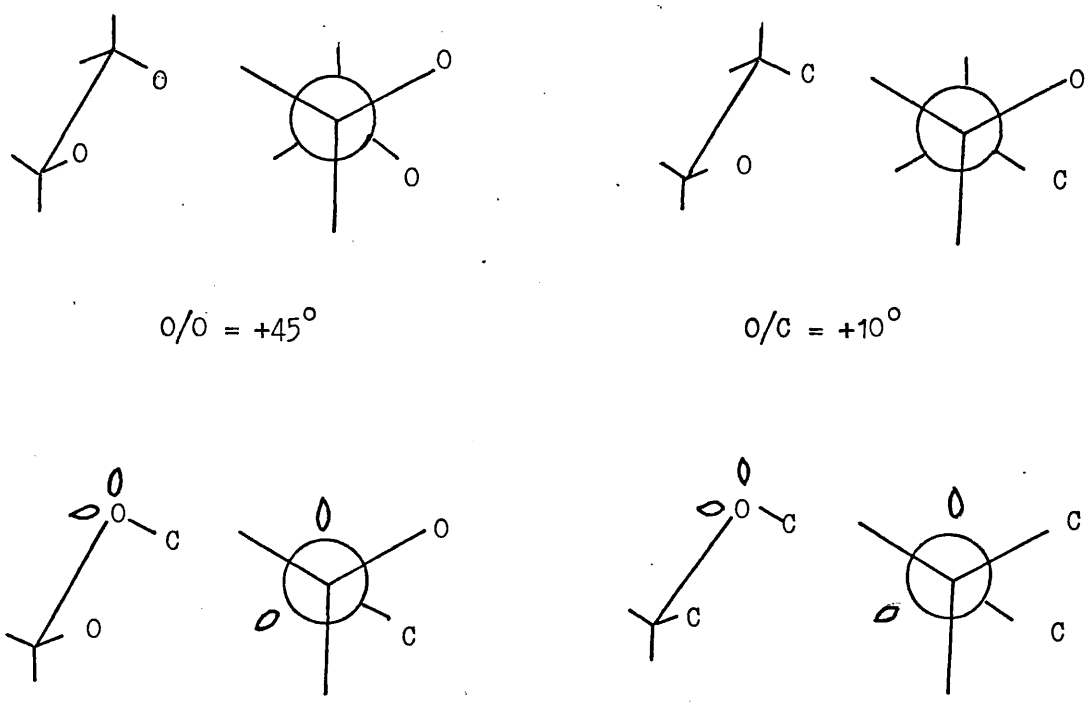
$[M_\beta] = -A +B$

arise from the remainder of the molecule[2]. According to this rule structural changes in one position of the molecule do not appreciably alter the contribution of the other. The values of 'A' and 'B' could be obtained from a knowledge of the rotations of anomeric pair of glycosides.

Whilst these rules give satisfactory values of 'A' and 'B' when comparisons are made on closely related compounds of similar structure and configuration, the values from configurationally different compounds are often unreliable and inconsistent.[2] The isorotation rules have been found to break down completely in several instances where chromophoric groups are close to the anomeric centre. Attempts to apply the principle of superposition to asymmetric centres other than the anomeric centre has not met with much success.[3]

Several empirical approaches[4-6] have been devised for predicting the sign and even the magnitude of the molecular rotation of carbohydrate derivatives. These methods assume that separate conformational units in the molecule contribute independently to the molecular rotation and that the total molecular rotation is obtained by summing the contributions. A more extensive treatment was presented by Brewster[4] who considered a centre of optical activity to be described by a screw pattern of polarizability of the electrons with correlations existing (i) between the handedness of the screw and (ii) between the amount of polarizability and the magnitude of the molecular rotation. The best correlations between the calculated and experimental values were obtained with compounds that do not absorb in the near ultra-violet and have predictable conformations.

Lemieux and Martin[5] have attempted to simplify the rules proposed by Whiffen[6] for predicting the molecular rotation of pyranoid derivatives which exist mainly in one conformation. The simplified rules require only four rotational parameters (Fig. 75) and are considered in terms of only "pairwise interactions",



O/O = +45° O/C = +10°

O/Co = +115° C/Co + 60°

75

that is in terms of the dihedral angle defined by carbon and oxygen substituents, (i) the O/O parameter which refers to oxygen atoms in gauche relationship and bridged by two carbon atoms, (ii) the O/C relationship which refers to an oxygen and a carbon atom in gauche relationship and bridged by two carbon atoms, (iii) the O/C relationship which refers to an oxygen and a carbon atom in gauche relationship and bridged by carbon and oxygen atoms and (iv) the

C/Co relationship which refers to two carbon atoms in gauche relationship and bridged by a carbon and oxygen atom. Using this scheme Lemieux and Martin obtained quite good agreement between the observed and calculated D-line rotations of several carbohydrate derivatives.

The technique developed by Djerassi and co-workers[32] which makes use of optical rotation as a function of wavelength and is usually referred to as optical rotatory dispersion (ORD), has shown some utility in providing configurational and conformational information about carbohydrates.[7-9] The phenomenon of circular dichroism (CD) and optical rotatory dispersion (ORD) are intimately related, the former refers to unequal absorption of right and left circularly polarized light by the optically active medium, while the latter corresponds to the change in optical rotation (unequal refractive indices of medium for right and left circularly polarized light) with wavelength. It is well known that when a molecule is devoid of a chromophore as in the case of a hydroxy compound such as a sugar, then the molecule will usually exhibit a plain curve.[10] Plain curves can be quite useful for configurational assignments and ORD curves of over eighty methyl glycosides and monosaccharides have been measured.[41]

Important applications of ORD measurements have been made on compounds possessing optically active chromophores absorbing in the spectral range under investigation, and exhibiting the phenomenon of Cotton effect.[11] Miles, Robins and Erying[40] have reported an important study in attempting to correlate the ORD/CD data (especially CD data) with the absorption bands associated with the

chromophore and to ascertain the electronic transitions associated with the optical rotatory activity.

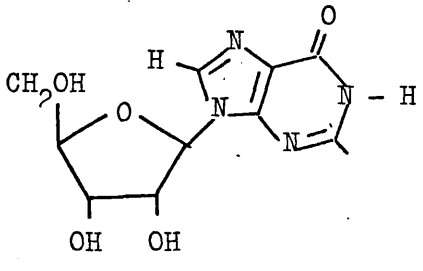
In a recent survey [33] on ORD/CD of aromatic compounds it was noted that very few of the theoretical aspects of Cotton effects attributed to electronic transitions have been investigated. The theories of Condon, Altar and Erying [34] and of Kirkwood[35] provide expressions governing the relationship between optical rotatory strength and chemical structure. It was shown by Erying et al.[34] that optical activity will be generated by a single electron if a perturbation due to the asymmetric force field of surrounding atoms will act on the electron. Some progress along these lines has been made by several investigators employing the one electron theory of optical rotation. Recently Moscowitz [36,37] has used this concept for the calculation of rotational strength of electronic transitions in ketones and steroids and also to explain the enhancement of the Cotton effect when the benzene ring is substituted with oxygen or nitrogen atoms. Erying et al.[38,39] have described an improved method of calculating rotational strengths of electronic transitions attributed to the bases in nucleosides by a bond-bond coupled oscillator theory. These authors suggest that the coupled oscillator theory[12] accounts for most of the observed optical activity in pyrimidine nucleosides.

Because of the importance of molecular conformations in determining biological activity, the conformations of nucleosides and nucleotides have been actively studied over the past decade.[13]

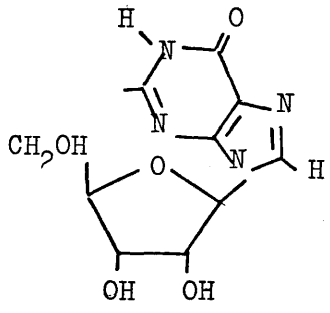
The problem of conformation of nucleosides in solution has usually been divided into two parts, the first part being the determination of the dihedral angles between the substituents (usually hydrogen) on the sugar and the second being the establishment of the relative orientation

of the base and the sugar about the glycosyl bond.[14] Elucidation of the sugar conformation in solution has been approached primarily by the analysis of the NMR coupling constants. The sugar conformation has been thought to have only minor effects on the orientation of the base about the glycosyl bond.[14] The conformational distribution about the glycosyl bond has not been so easily determined and this problem has been approached by the application of several methods.[14] A complete review[43] of the analyses of nucleosides and nucleotides conformationsis available. The application of ORD/CD to this problem has received considerable attention in providing information about the conformation of the base with respect to the sugar ring.

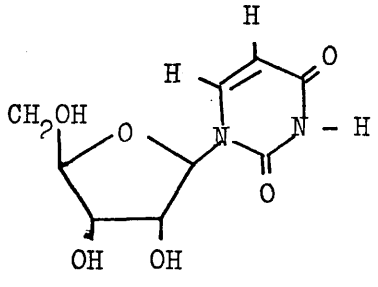
Most workers in this area have interpreted their results in terms of preferred 'anti' conformation (see 76) for both purine and pyrimidine nucleosides in solution.[14]



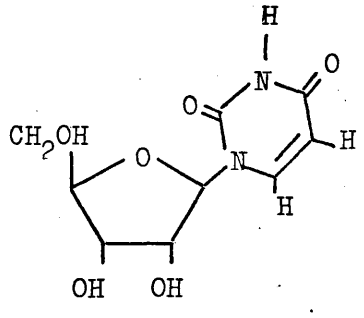
anti



syn



anti



syn

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A number of recent [15-22] measurements have shown that the sign of the Cotton effect of β-purine nucleosides and nucleotides is negative, while the α-anomers give a positive Cotton effect. Pyrimidine derivatives on the other hand have a negative Cotton effect for the α-anomers and a positive Cotton effect for the β-anomers, and therefore do not obey Hudson's isorotation rules.

Ulbricht et al.[16] in a study of some fifty pyrimidine derivatives have been able to show that the variation in the sign of and magnitude of the Cotton effect is due to the changing conformation of the planar pyrimidine ring with reference to the sugar ring. Recently some workers [23,24] have established the sign of the ORD/CD in nucleosides and nucleotides as a function of the sugar-base torsion angle (τ).[25] The Cotton effect is a function of the asymmetry of the environment of the base, or more precisely of the orientation of the transition dipole of the base with respect to the asymmetric centres of the neighbouring sugar moiety. Since there is evidence that the direction of transition dipoles of ordinary purines and pyrimidines with respect to the nitrogen atom linked to the anomeric carbon atom are not very different,[27-29] it is reasonable to expect that the sign of the ORD may reflect primarily the orientation of the base with respect to the sugar. Model building studies have shown that in the case of purine nucleosides neither the 'syn' or 'anti' conformations necessitates very close interatomic distances, while pyrimidine nucleosides are clearly hindered in the 'syn' conformation.[25,26] Studies of ORD of pyrimidine nucleosides led Ulbricht et al.[30,31] to conclude that pyrimidine nucleosides retain the 'anti' conformation in solution. Klee and Mudd[26] are of the opinion that the conformation of the purine nucleosides should be opposite to that of the

pyrimidines in solution since the ORD's are of opposite sign and would be consistent with the experimental transition moments. These considerations would thus support the 'syn' conformationfor ordinary purine nucleosides.

DISCUSSION OF RESULTS

The optical rotation data for N-glycosylimidazoles is summarised in Table 5.1. Optical rotations at the D-line are in accord with the anomeric configuration for compounds 1-11 and Hudsons rules appear to be valid at the D-line as has been observed in the case of purine nucleosides.

The 'A' values obtained for N-glycosylimidazoles by the application of Hudson's isorotation rules are affected by the changes in the rest of the molecule. The 'A' value in the mannose series is considerably lower than in glucose and galactose derivatives of imidazole. Similar observations have been reported for methyl and phenyl glycosides (Table 5.2). The low 'A' value in the mannose series could be attributed to change of configuration at C-2.[2] No significant inference could be drawn from the 'B' values.

It is well known that the sign of the ORD/CD depends not only on the anomeric configuration but also on the orientation of the aglycone with respect to the sugar ring (glycosyl torsion angle) or more precisely on the direction of the transition dipoles associated with the chromophore with respect to the asymmetric centres of the neighbouring sugar moiety. In N-glycosylimidazoles the strong absorption band at 220 nm can be attributed to a $\pi-\pi^*$ transition. The possibility of an $n-\pi^*$ transition is less likely as the UV spectrum of N-glycosylimidazoles and of imidazole is unaffected in this region by changes in pH, and also an $n-\pi^*$ transition is characterized by a low intensity band at 250 nm in the case of free imidazole.[44] The direction of this $\pi-\pi^*$ transition dipole must to a certain extent affect the sign in ORD/CD of these glycosides in the spectral range under investigation. However, no theoretical or experimental information about the transition dipole moment is available.

It has been observed in the case of purine nucleosides that rotation about the glycosyl bond is much more free than in pyrimidine nucleosides, as a result the amplitude of the Cotton effects are generally small in purine nucleosides.[16] On steric grounds alone it should be possible for the imidazole ring to rotate reasonably freely about the glycosyl bond; this would then account in part for the low amplitudes observed in the ORD/CD of the N-glycosylimidazoles.

The signs of the ORD/CD curves of compounds a,c,d and i (Table 5.1) appear to follow a pattern which is in accord with the anomeric configurations. Compounds with α-configuration at the anomeric centre display positive ORD/CD curves, while compounds with β-configuration display negative ORD/CD curves. In order to establish the general nature of this pattern, a search in the literature was made for ORD/CD data of compounds of known anomeric configuration possessing a pyranoside structure and having an optically active chromophore at the anomeric centre. However, no complete set of data for such compounds could be found. It was therefore decided to examine the CD properties of p-nitrophenyl-D-glycosides of known anomeric configuration. The results are summarized in Table 5.3 .

In each case the signs of the CD curves were opposite for each member of an anomeric pair of compounds at each extremum. These compounds were found to possess UV absorption maxima at 300 nm and 220 nm. The extrema in the CD curves were observed more or less corresponding to these maxima. Compounds a, b, g and h (Table 5.3) also showed a third extremum, but again the signs of the curves were opposite for each anomeric pair.

However, in cases of N-glycosylimidazole compounds b, e and g (Table 5.1) the signs do not appear to fit the pattern established. Explanation for this deviation may lie in consideration of glycosyl torsion angle, as previously shown in the case of nucleosides where the glycosyl torsion angle is a function of the sign of the ORD/CD. The glycosyl torsion angle may not be important with p-nitrophenyl-D-glycosides in which the chromophore is symmetrical and is at a distance from the sugar ring than is the imidazole chromophore in N-glycosyl-imidazoles. Possibly the deviations from the pattern arises from restricted rotation about the C-N bond in compounds b, e and h (Table 5.1) in which the substituents at C-1 and C-2 are cis related.

The ORD results of the Polish workers[45] mentioned earlier (Chapter 2) include a plain negative curve for 1'-β-D-glucopyranosyl-imidazole which is in accord with our observation for this compound. The positive CD observed in this work for 1'-β-D-mannopyranosylimidazole (43) is however, at variance with the plain negative curve observed by the above workers.

ORD and CD measurements were carried out on a Cary 60 , Jouan CD 185 and Cary 61 spectropolarimeter. ORD curves were plotted with wavelengths in millimicrons [mu] on the abscissa against molecular rotations [ϕ] on the ordinate. The molecular amplitude [a] is the difference between the molecular rotation at the extremum of longer wavelength and the molecular rotation at the extremum of shorter wavelength divided by 100. CD curves were plotted with lengths in millimicrons [mu] as abscissa against dichroic absorption ($\Delta\epsilon$) as ordinate. The solvent in which the compounds were examined was methanol.

TABLE 5.1

OPTICAL ROTATION DATA FOR N-GLYCOSYLIMIDAZOLES

Compound	$[\alpha]_D$	$[M]_D$	c.d. ($\Delta\epsilon$) or o.r.d. (a)
a) B-Glu.Ac (14)	7.3°	-29.07°	-ve cotton effect (-909)
b) α-Glu.Ac (31)	+118°	+469.99°	-ve c.d. (-0.62)
c) β-Glu.OH (5)	+13.6°	+31.30°	plain -ve curve (o.r.d.) no significant c.d. detected.
d) α-Glu.OH (32)	+100.6°	+231.58°	+ve cotton effect in both o.r.d. (+ 52.3) and c.d. (+ 1.08).
e) β-Man.Ac (37)	-14.55°	-57.95°	+ve c.d. (+ 1.93)
f) α-Man.Ac (38)	+37.7°	+150.15°	+ve c.d. (+ 0.91)
g) β-Man.OH (43)	+27.48°	+63.25°	+ve c.d. (+ 0.59)
h) α-Man.OH (44)	+63.7°	+146.63°	+ve c.d. (+ 0.52)
i) β-Gal.Ac (47)	+11.43°	+45 52°	-ve c.d. (-1.60)
j) β-Gal.OH (52)	+51.02°	+131.27°	no significant dichroism detected
k) α-Gal.OH (53)	+130.16	+299.62°	+ve c.d. (+ 0.50)

TABLE 5.2

APPLICATION OF HUDSON'S ISOROTATION RULES

Compound	$[\alpha]_D$	$[M]_D$	A_{value}	B_{value}
(a) Methyl α-D-glucopyranoside	+ 158.0°	+306.0°		
(b) Methyl α-D-mannopyranoside	+ 82.0°	+151.0°		
(c) Methyl α-D-galactopyranoside	+ 196.0°	+378.0°	184	122
(d) Methyl β-D-glucopyranoside	- 32.0°	- 62.0°	126	24
(e) Methyl β-D-mannopyranoside	- 53.0°	-102.0°	189	189
(f) Methyl β-D-galactopyranoside	+ 0.61°	+ 1.18°		
(g) Phenyl α-D-glucopyranoside	+ 181.0°	+463.0°	323	139
(h) Phenyl α-D-mannopyranoside	+ 114.0°	+291.0°	238	53
(i) Phenyl α-D-galactopyranoside	+ 217.0°	+555.0°	237	227
(j) Phenyl β-D-glucopyranoside	- 72.0°	-184.0°		
(k) Phenyl β-D-mannopyranoside	- 72.0°	-184.0°		
(l) Phenyl β-D-galactopyranoside	- 43.0°	-100.0°		
(m) 1-α-D-glucopyranosylimidazole (32)	+ 100.6°	+231.5°	100	131
(n) 1-α-D-mannopyranosylimidazole (44)	+ 63.7°	+146.6°	41	104
(o) 1-α-D-galactopyranosylimidazole (53)	+ 130.16°	+299.6°	91	208
(p) 1-β-D-glucopyranosylimidazole (5)	+ 13.6°	+ 31.3°		
(q) 1-β-D-mannopyranosylimidazole (43)	+ 27.48°	+ 63.2°		
(r) 1-β-D-galactopyranosylimidazole (52)	+ 51.02°	+ 117.4°		

TABLE 5.3

CD DATA FOR 1-O-NITROPHENYL GLYCOSIDES

Compound	1st extremum		2nd extremum		3rd extremum	
	$\Delta\epsilon$	λ(nm)	$\Delta\epsilon$	λ(nm)	$\Delta\epsilon$	λ(nm)
a) p-nitrophenyl-α-D-glucopyranoside	+1.17	291-8	-0.39	242	+0.88	224
b) p-nitrophenyl-β-D-glucopyranoside	-0.22	313	+0.67	246	-0.78	221
c) p-nitrophenyl-α-D-mannopyranoside	+1.38	291	-	-	+0.88	218
d) p-nitrophenyl-β-D-mannopyranoside	-0.68	305	-	-	-0.98	220
e) p-nitrophenyl-α-D-galactopyranoside	+1.94	296	-	-	+1.14	223
f) p-nitrophenyl-β-D-galactopyranoside	-0.18	318	-	-	-1.16	217
g) p-nitrophenyl-α-D-xylopyranoside	+1.79	293	-0.42	236	+1.05	214
h) p-nitrophenyl-β-D-xylopyranoside	-0.38	336	+0.32	300	-1.21	222

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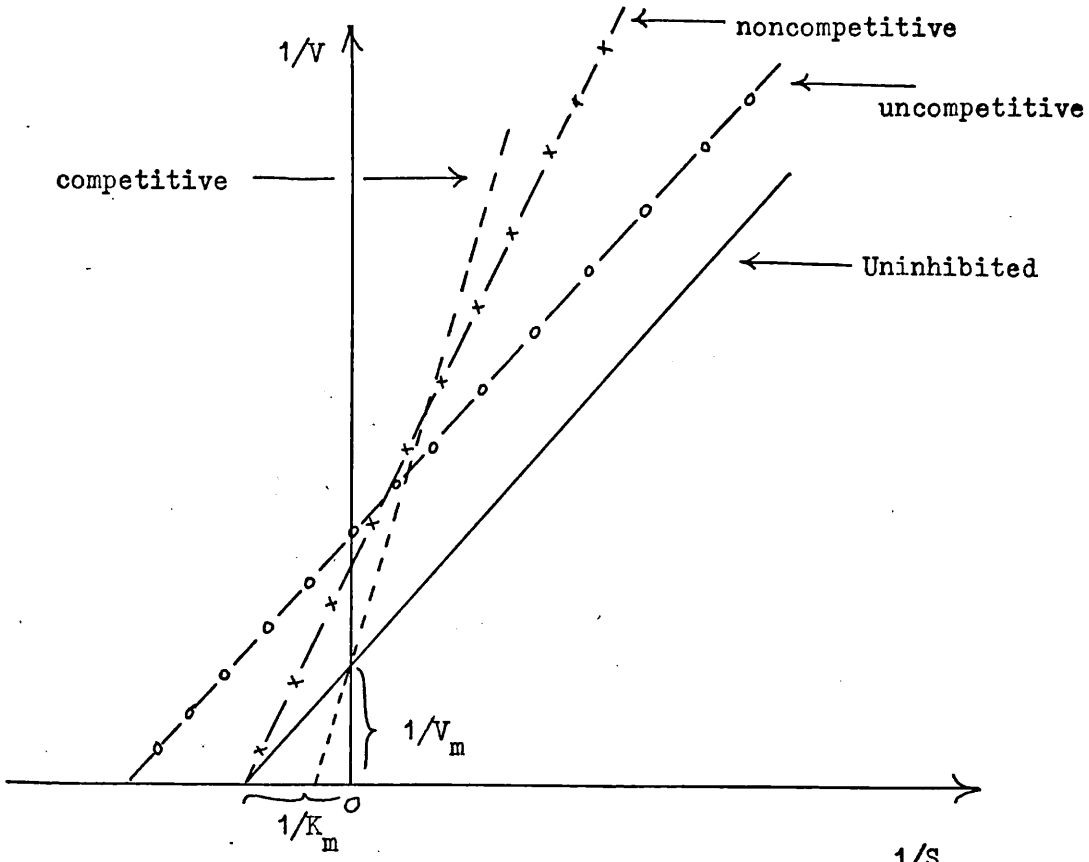
CHAPTER 6

THE ENZYME INHIBITORY PROPERTIES OF N-GLUCOSYLIMIDAZOLES

INTRODUCTION

During the investigation of the hydrolytic stability of the sugar-base linkage in 1-α- and β-D-glucopyranosylimidazoles with solutions of α-(yeast) and β(almond) glucosidases, it was found that the hydrolysis of the enzyme substrates was inhibited by the respective 1'-D-glucopyranosylimidazoles. The exceptional stability of the glycosyl linkage in 1'-D-glucopyranosylimidazoles makes them of particular value for studies of glucosidase-inhibitor interactions.

In practice it is usual to distinguish between various typesof inhibition by consideration of the characteristics of various kinetic plots of experimental data. Of the several methods[1,2] of graphical analysis, the method outlined by Lineweaver and Burk is the most widely used, and the plots obtained are frequently referred to as Lineweaver-Burk plots[3] or reciprocal plots, obtained by plotting the reciprocal of the initial velocity (1/V) of the enzyme catalysed reaction against the reciprocal of the substrate concentration (1/S). The characteristic of these plots for competitive inhibition is that the slopes vary with inhibitor concentration, but the 1/V intercept remains constand and corresponds to the reciprocal of the maximal velocity (1/Vm). The intercept on the horizontal axis corresponds to $-1/Km(1+ I/Ki)$, where Km is the Michaelis constant and Ki is the inhibition constant. For non-competitive inhibition the 1/S intercept remains constant and the slopes vary with inhibitor concentration. For uncompetitive inhibition the slopes remain constant and a series of parallel lines is obtained (see 77).



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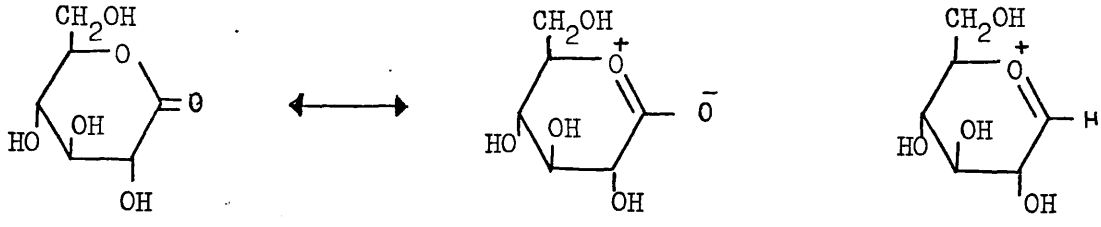
However, kinetic behaviour intermediate between these three types of inhibition may be observed. Since competitive inhibitors do not affect the maximal rate of an enzyme reaction, it is often inferred that they act by occupying the same site on the enzyme usually occupied by the substrate. Non-competitive inhibitors, which alter the maximal rate but not the pseudo binding constant (Km), are often assumed to bind at a site not connected with the substrate and to alter the reactivity of the enzyme-substrate complex. Uncompetitive inhibitors which are supposed to bind only with the enzyme-substrate complex are relatively uncommon.

Reversible inhibitors can be classified into two categories, those which resemble the substrate are termed as substrate analogue

inhibitors and those which resemble a substrate transition state are termed transition state analogue inhibitors. Lactones derived from various hexoses have been studied extensively as inhibitors of glycosidases,[5-8] and they fall into the category of transition state analogue inhibitors. The high affinity of glycosidases for these lactones reflects steric similarity between the lactones and the glucopyransoyl portions of possible transition states in enzymic hydrolysis of glycosides, and these involve intermediates having half-chair conformations. It is well known that D-glucono-1,5-lactone (78) assumes a half chair conformation similar to that of a D-glucopyranosyl cation (79).



78 79

Because of the presence of the simple pyranose ring, the N-glucosylimidazoles, could be regarded as substrate analogue inhibitors of the respective glycosidases. One would therefore expect the 1-α-D-glucopyranosylimidazole to inhibit α-glucosidases and the 1-β-D-glucopyranosylimidazole to inhibit β-glucosidases.

Results and Discussion

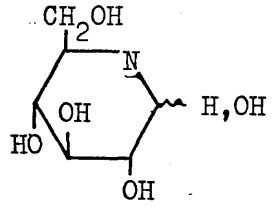
α(yeast)glucosidase:

Double reciprocal plots for the inhibition of hydrolysis of methyl α-D-glucopyranoside catalysed by α(yeast)glucosidase, are shown in Fig.6.3-4; page 167[68] thereby characterising the pattern of inhibition

as competitive. The range of substrate concentration examined was far below that which substrate inhibition was found to occur. Results of the slopes against inhibitor concentration were found to be linear in each case, and thus the inhibition was "linear competitive". The inhibition constants (Ki) obtained from the intercepts of the replots were 0.29mM for 1-α-D-glucopyranosylimidazole and 190 mM for 1-β-D-glucopyranosylimidazole (Table 6.1). Thus, as as expected the yeast enzyme shows a high degree of anomeric specificity. The Ki values obtained for the α-anomer are lower than the values obtained for D-glucose (2.0 mM)[9] and for the potential transition state analogue inhibitor 1-L-1-2-anhydro-myo-inositol (6.9 mM).

β-(almond)glucosidase:

Double reciprocal plots for the inhibition of the hydrolysis of cellobiose catalysed by β-(almond)glucosidase are shown in Fig.6.1-2; page 165-66. Inhibition was competitive except at high relative concentrations of 1-α-D-glucopyranosylimidazole which displayed non-competitive inhibition. In this case the initial slope of the double reciprocal plot was used in the replot. Replots were linear and gave Ki values of 33mM for 1-α-D-glucopyranosylimidazole and 50mM for 1-β-D-glucopyranosylimidazole. These substrate analogue inhibitors give Ki values which are of the same order of magnitude as the Km value (24 ± 2mM) for the substrate cellobiose, but are much less effective than D-glucono-1,5-lactone and nojirimycin (80).[10]



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The lack of anomeric specificity of this enzyme towards 1 -D-glucopyranosylimidazoles is somewhat surprising but not unique. Nitta et al.[11] during an investigation of inhibition of TaKa-amylase 'A' catalysed hydrolysis of phenyl α-D-maltoside by substrate analogue inhibitors found a similar lack of anomeric specificity with methyl α-D-galactopyranoside (Ki 86 mM) and methyl β-D-galactopyranoside (Ki 125 mM), as well as with other glycosides.

Lysozyme:

This enzyme (mol. wt. 14,600) consists of a single polypeptide chain held together by four disulphide residues. The natural function of the enzyme is the lysis of bacterial cell walls which consist of polysaccharide chains cross-linked by peptides of varying length. The polysaccharide chain is made up of disaccharide subunits consisting of β-1,4-linked 2-deoxy-2-acetamido-D-glucopyranosyl and muramyl acid units. Bond cleavage occurs immediately adjacent to the anomeric carbon atom of the muramyl units. This glycosidic cleavage is considered to be the first step in the lysis of the cells and is measured by the reduction in turbidity of a suspension of dried cells of Micrococcus lysodikiticus in aqueous buffer solutions. However, the use of this insoluble substrate is somewhat unsatisfactory because of the difficulty in obtaining reproducible results.

1 -β-D-glucopyranosylimidazole did not inhibit the degradation of the cells by lysozyme, at inhibitor concentrations of up to 69 mM. This result was not unexpected since although small molecules such as 2-acetamido-2-deoxy-D-glucose and the methyl and ethyl glycosides thereof are known to be inhibitory,[12] it is believed that the 2-acetamido[13] or other 2-amino group[14] is necessary for binding.

1 -α-D-glucopyranosylimidazole at 50mM concentration was found to be an uncompetitive inhibitor as shown by a double reciprocal plot Fig.6.5, page 169. Previous workers[12,14] have shown that imidazole and imidazole derivatives in their protonated forms inhibit lysozyme, possibly by the formation of a charge transfer complexes with the tryptophan residues of the enzyme. This proposal has received recent support from an x-ray crystallographic study[15] of the complexes of lysozyme with histamine and histidine. These compounds were proposed to act as competitive inhibitors although the kinetic data do not seem to warrant this.

The greater inhibitory power of 1 -α-D-glucopyranosylimidazole than that of the β-anomer may be illustrative of a general phenomenon where by the anomeric configuration is not of overriding importance except for specific substrates. Ethyl 2-acetamido-2-deoxy-α-D-glucopyranoside has been shown by Davies et al,[12] to be a stronger inhibitor of lysozyme than the β-anomer at pH 6.2.

The results of this work show that 1 -α-D-glucopyranosylimidazole is a more potent inhibitor of glycosidases than the corresponding β-anomer. It is possible that the preferential binding of the α-anomer may arise in part indirectly from the operation of the reverse anomeric effect. Although no evidence for the presence of the alternative $^1C_4$ conformation for 1 -α-D-glucopyranosylimidazole is neutral or acidic aqueous solutions is detectable by NMR spectroscopy, there is evidence that 1 -α-D-mannopyranosylimidazole adopts the alternative $^1C_4$ conformation to a significant extent (Chapter 3). The decreased stability of the α-anomers of this type of compound may account for the greater binding affinity to the enzymes, if binding involes distortion, as has been proposed for lysozyme[17] and other carbohydrases.[18]

In view of the inhibitory property and the extreme stability of N-glucosylimidazoles, these compounds have been tested for biological activity, carried out in collaboration with Dr. P.J.V. Cleare, I.C.I. Plant Protection Ltd.

Compound	Tested for	Result
α-anomer	insecticide	-ve
β-anomer	"	-ve
α-anomer	bacteriocide	-ve
β-anomer	"	-ve
α-anomer	seed dressing	-ve
β-anomer	fungicide	weak activity against one species only.

TABLE 6.1

INHIBITION OF GLYCOSIDASES BY N-GLUCOSYLIMIDAZOLES

Substrate	Km(mM)	Enzyme	Type of inhibition	Inhibitor	Ki(mM)
α Methyl-glucoside		Yeast α-glucosidase	Competitive	1-α-D-glucopyranosyl imidazole	0.29
"	30±5	"	"	1-β-D-glucopyranosyl imidazole	190
Cellobiose		Almond β-glucosidase	"	1-α-D-glucopyranosyl imidazole	33
"	24±2	"	"	1-β-D-glucopyranosyl imidazole	50
Micrococcus lysodeketicus		Lysozyme	Non competitive	1-α-D-glucopyranosyl imidazole	-
"	-	"	No inhibition	1-β-D-glucopyranosyl imidazole	-

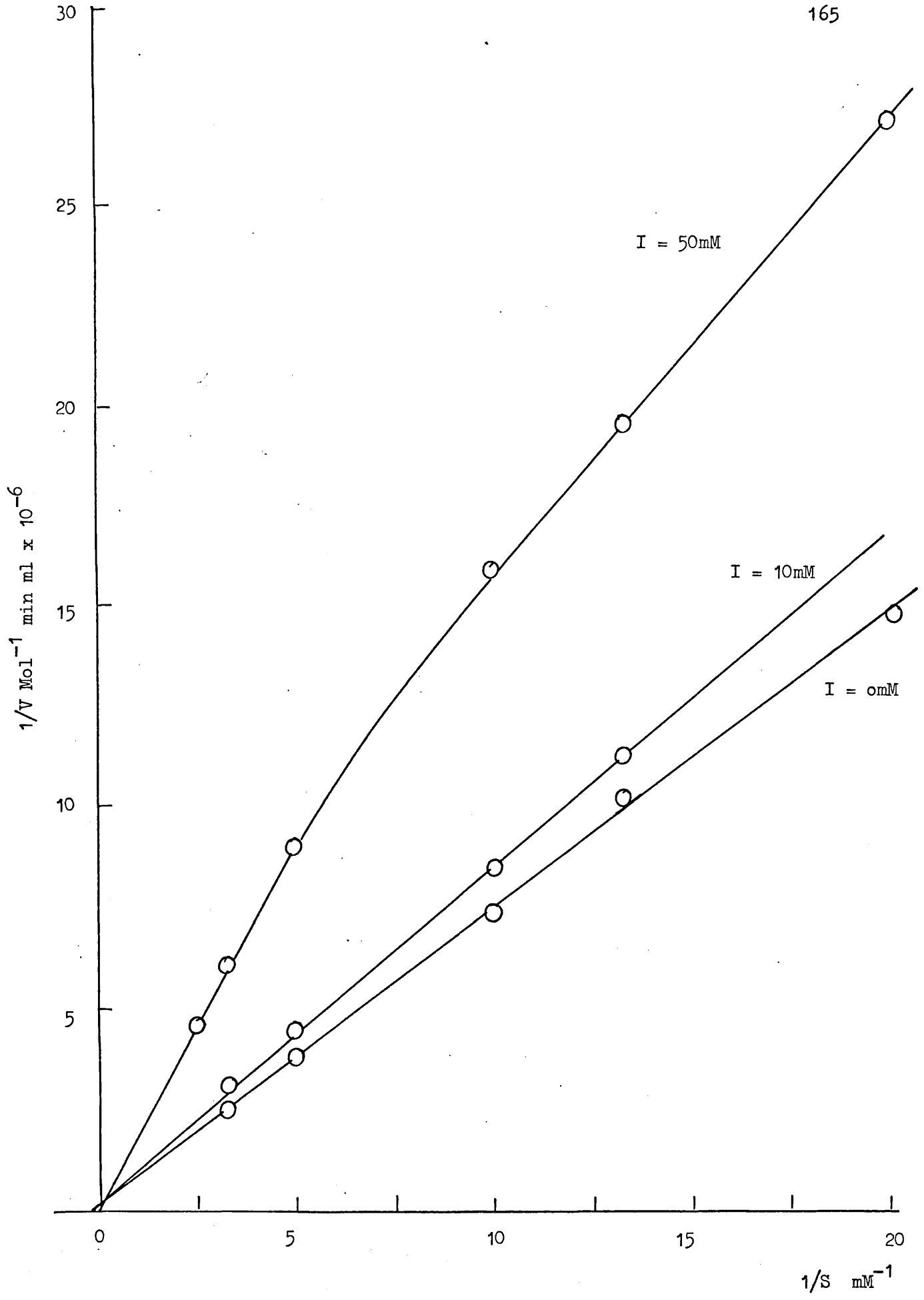

Fig.6.1 Inhibition of β-(almond)glucosidase by 1-α-D-glucopyranosylimidazole

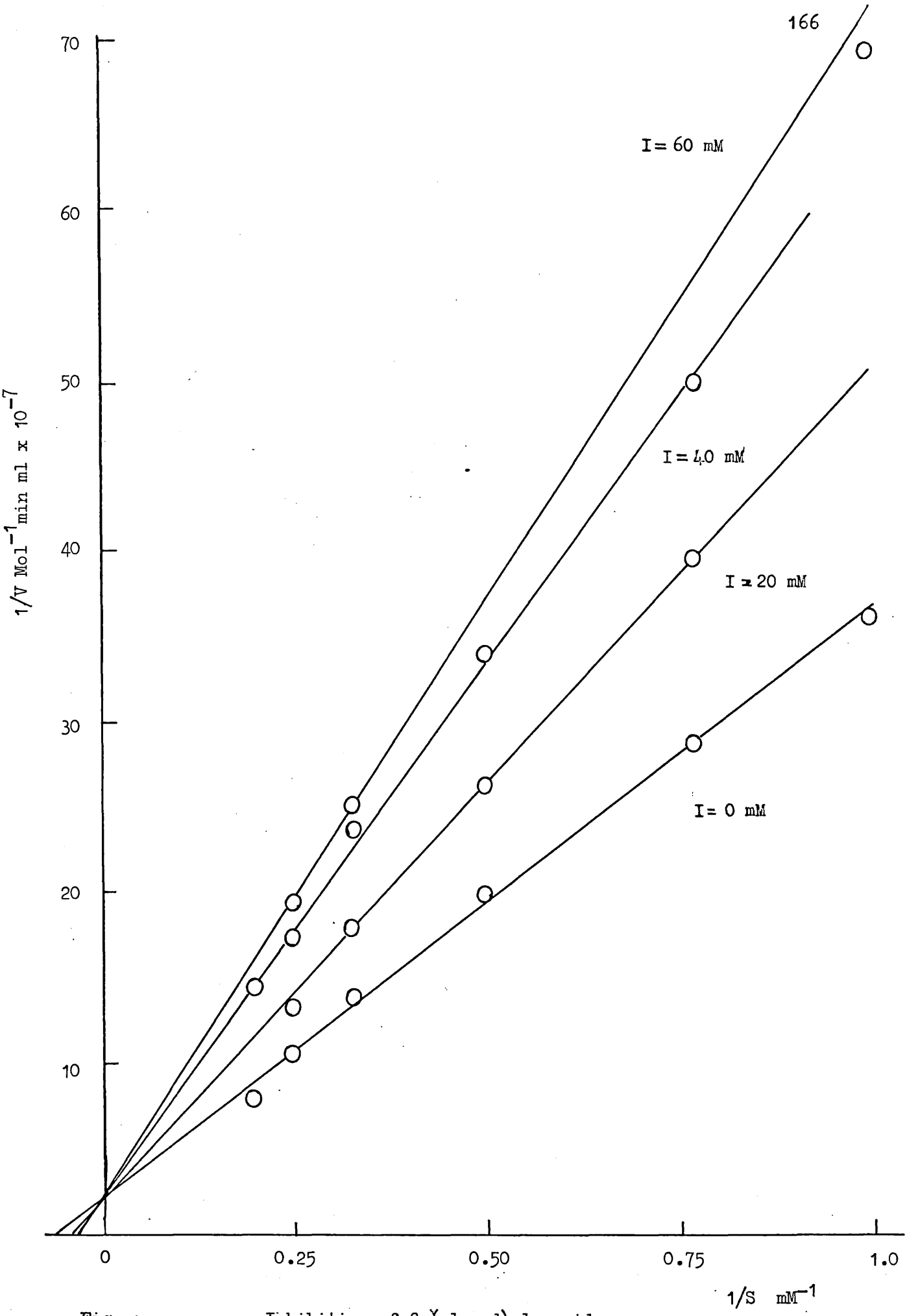

Fig. 6.2 Inhibition of β-(almond) glucosidase by 1-β-D-glucopyranosylimidazole

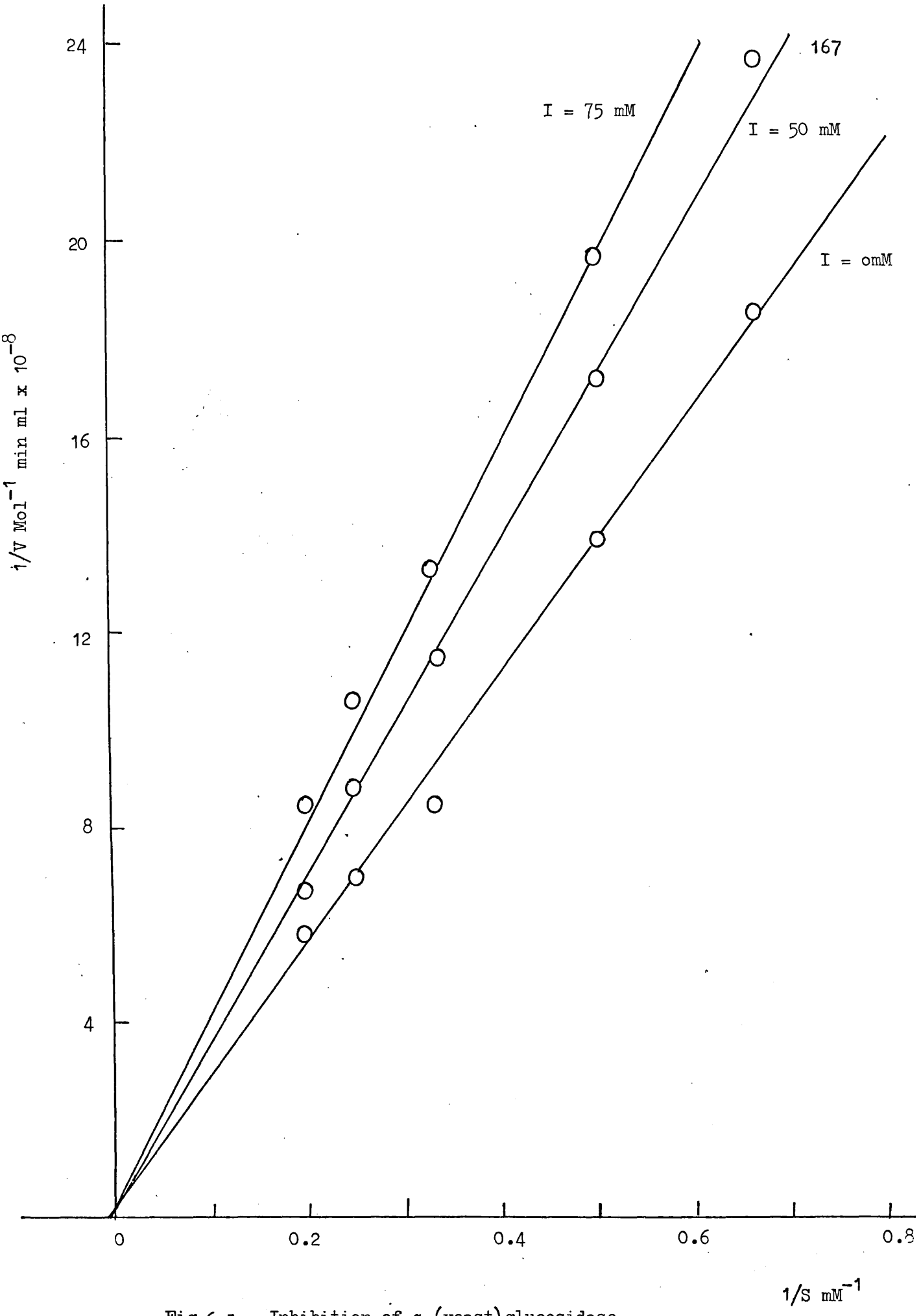

Fig.6.3 Inhibition of α-(yeast)glucosidase by 1 -β-D-glucopyranosylimidazole

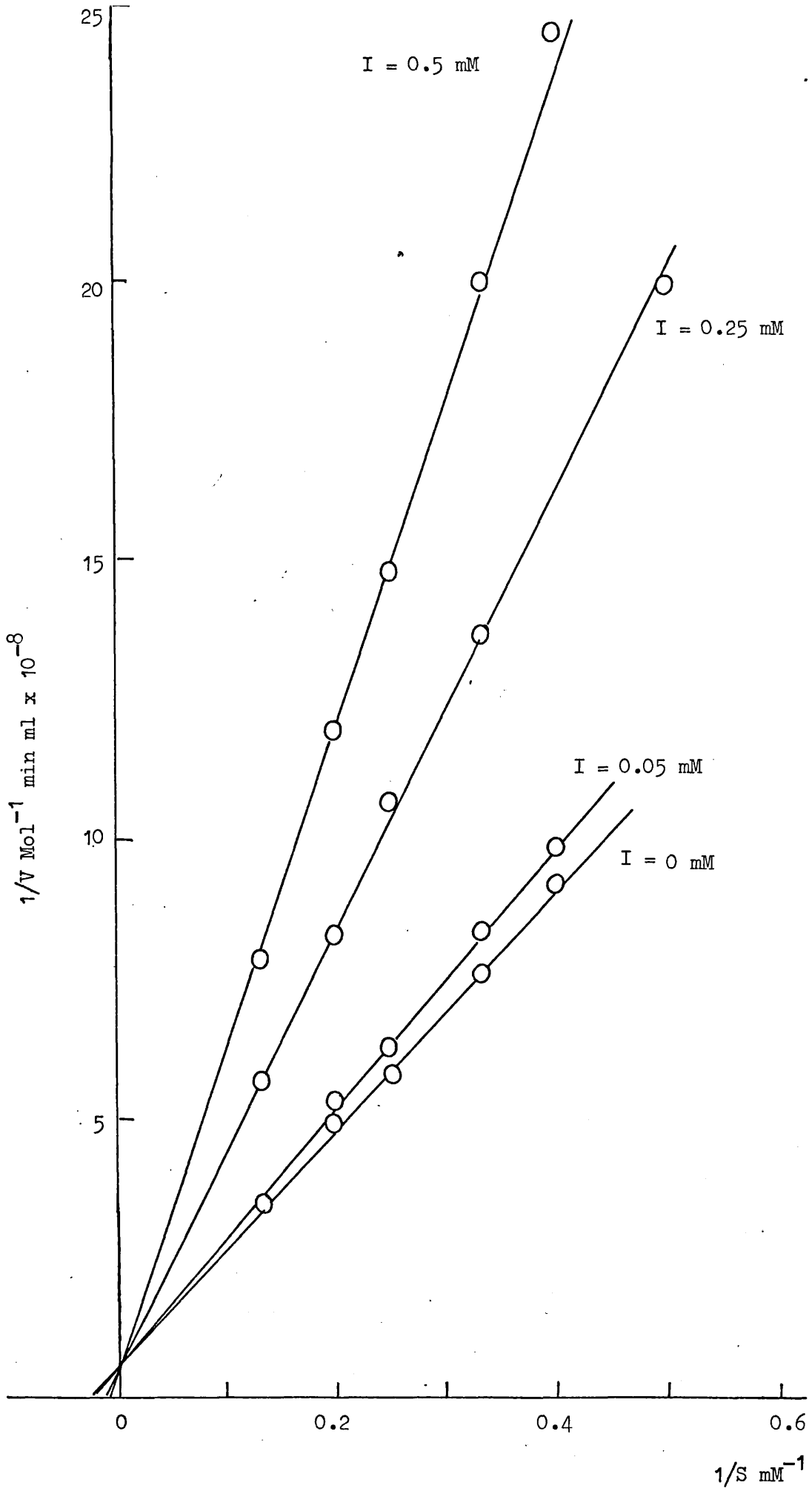

Fig.6.4 Inhibition of α-(yeast) glucosidase by 1 -α-D-glucopyranosylimidazole

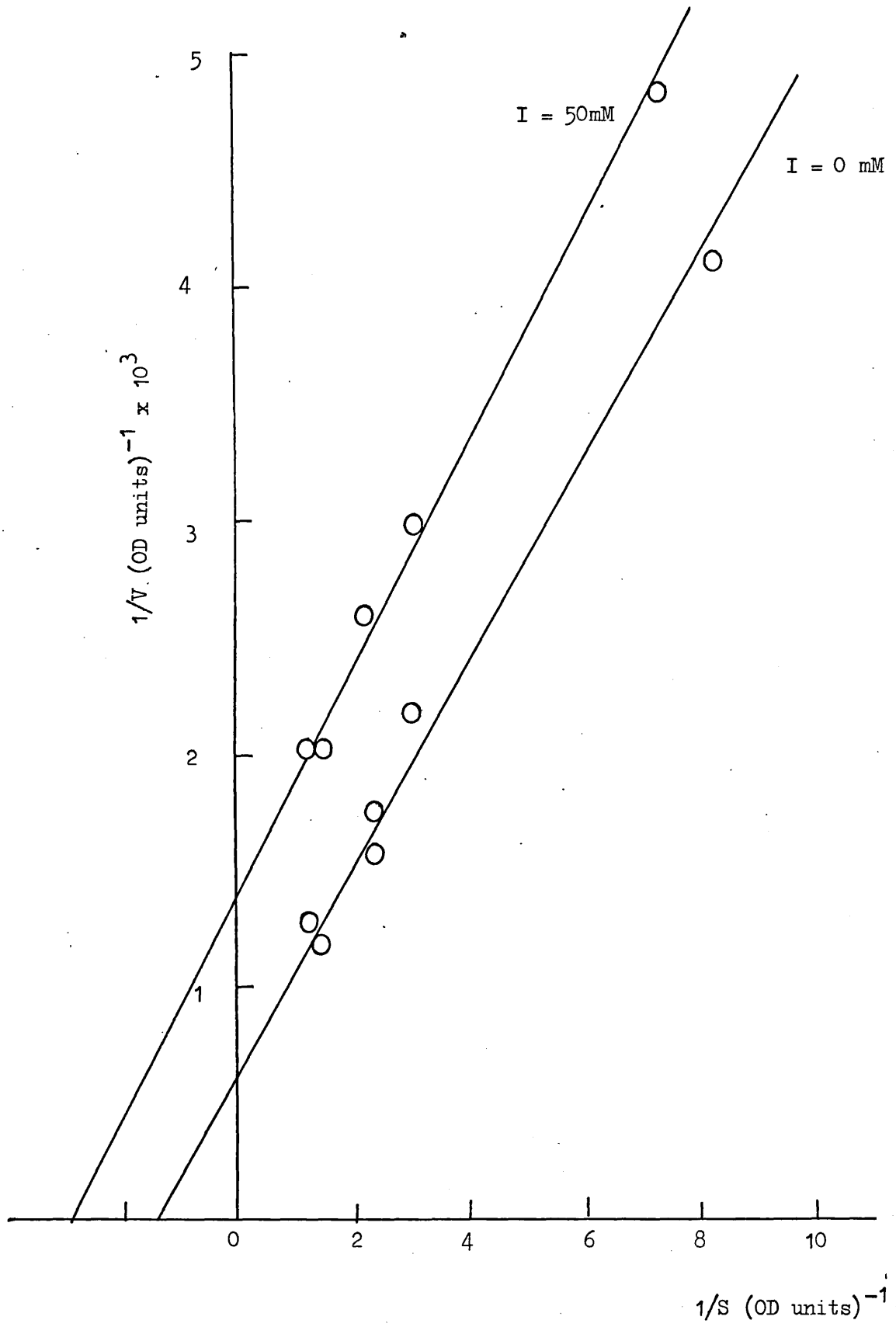

Fig.6.5 Inhibition of lysozyme by 1 -β-D-glucopyranosylimidazole

EXPERIMENTAL

Inhibition of α-(yeast) glucosidase by 1 -α-D-glucopyranosylimidazole

The reaction mixtures contained enzyme (Sigma, 0.002% w/v), substrate methyl α-D-glucopyranoside (2.0 - 7.5 mM) and inhibitor (0 - 0.5 mM) dissolved in pH 6.8 phosphate buffer (I = 0.05) (1 ml). After 120 min. at 37° the reactions were terminated by heating the mixtures at 100° for 2 min, and the glucose liberated was estimated using glucose oxidase reagent.[16]

Inhibition of α-(yeast) glucosidase by 1 -β-D-glucopyranosylimidazole

The reaction mixtures contained enzyme (Sigma, 0.0025% w/v), substrate methyl α-D-glucopyranoside (1.0 - 5.0 mM) and inhibitor (0 - 75 mM) dissolved in pH 6.8 phosphate buffer (I = 0.05) (1 ml). After 120 min. at 37° the reactions were terminated by heating the mixtures at 100° for 2 min, and the glucose liberated estimated using glucose oxidase reagent.[16]

Inhibition of β-(almond) glucosidase by 1 -α-D-glucopyranosylimidazole

The reaction mixtures contained enzyme (Sigma, 0.05% w/v), substrate cellobiose (0.05 - 0.4 mM) and inhibitor (0 - 50 mM) dissolved in pH 5.3 citrate buffer (1 ml). After 120 min. at 37° the reactions were terminated by heating the mixtures at 100° for 2 min, and the glucose determined using glucose oxidase reagent. A blank correction was made for glucose derived non-enzymically from cellobiose.

Inhibition of β-(almond) glucosidase by 1'-β-D-glucopyranosylimidazole

The reaction mixtures contained enzyme (Sigma, 0.0125% w/v), substrate cellobiose (1.0 - 5.0 mM) and inhibitor (0 - 60 mM) dissolved in pH 5.3 citrate buffer (1 ml). After 32 min. at 37° the reactions were terminated by heating the mixtures at 100° for 2 min, and the glucose determined using glucose oxidase reagent.[16] A blank correction was made for glucose derived non-enzymically from cellobiose.

Inhibition of Lysozyme by 1''-α-D-glucopyranosylimidazole

The reaction mixtures contained enzyme (Sigma, 1 μg ml^{-1}), aubstrate Micrococcus lysodikticus cells (Sigma, 22 - 180 μg ml^{-1}) and inhibitor (0 and 50 mM) dissolved in pH 6.2 phosphate buffer (I = 0.067) (2.6 ml) at 20°. The optical density of the mixtures compared to a blank not containing cells was monitored at 450 nm using Pye Unicam SP 1800 spectrophotometer. Initial rates were computed using a least squares curve-fitting programme.

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GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

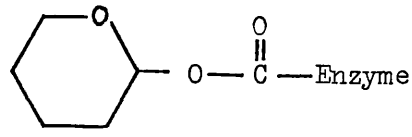
The reaction products obtained by the condensation of the appropriate glycosyl halide and imidazole in dioxan have been mainly with β-configuration at the anomeric carbon atom. In view of high biological activity exhibited by a number of naturally occurring α-glycosides and also on account of the interest in nucleosides with α-configuration, the need for the development of a stereoselective synthesis of α-glycosides in good overall yield is a prime need for future work. The use of dimeric 3,4,6-tri-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride in solvents of different polarity is one of the approaches which could be considered.

The operation of the reverse anomeric effect has been observed in certain derivatives of N-glycosylimidazoles. The phenomenon of reverse anomeric effect has so far been observed only in N-glycosides where the nitrogen atom is a part of a heterocylic aromatic system. It would be interesting to observe this phenomenon in glycosides of other types of structure for e.g. trialkylammonium; such observations would be relevant to the understanding of the reverse anomeric effect.

The application of computer analysis to NMR spectra of carbohydrate derivatives has been shown in this work. The application of such analysis in routine work for checking spectral assignments and obtaining reliable and accurate data from experimental spectra is an attractive technique, although it would appear that a rapid analysis of complex spectra does not necessarily follow.

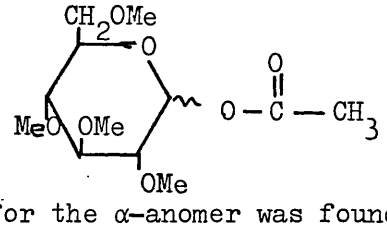
The extreme hydrolytic stability of the glycosidic linkage in N-glucosylimidazoles established in this work does not support nucleophilic intervention of the histidnyl side chain in glycosidase action.

There is a large amount of evidence for the presence of carboxyl groups at the active sites of a number of glycosyl transferases. In many cases evidence is far from conclusive and more work on definite identification of active site functional groups is required. The function of these groups has not been precisely defined, but in principle they could act as nucleophiles, general bases or general acids. The first mechanism would presumably lead to a glycosyl-enzyme intermediate of the type shown below



which would subsequently be hydrolyzed. Studies of the properties of such esters should be relevant to the understanding of the role of the carboxyl groups at the active sites.

In this connection preliminary work on 1-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucopyranosides has been carried out by the



author. The half-life for the α-anomer was found to be 290 min. and for the β-anomer to be 90 min. for ethanolysis in 0.1N-ethanolic-HCl However, ethanolysis in the presence of sodium ethoxide was extremely fast, even at 0°. Both alkyl-oxygen and acyl-oxygen fission appeared to be followed in acid.

The application of ORD/CD data to establishing anomeric configurations for a set of compounds has been demonstrated in this work. The observation that anomeric pairs of compounds containing a chromophoric group at the anomeric carbon atom normally give curves of opposite sign may be of general application.

The inhibitory properties of N-glucosylimidazoles towards glucosidases has been established. Although these inhibitors are attractive from a stability point of view, commercial application may require the development of effective inhibitors of the transition state or irreversible type, rather than of the substrate analogue type.

The Synthesis and Hydrolytic Stability of 1-Glucopyranosylimidazoles

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The Synthesis and Hydrolytic Stability of 1-Glucopyranosylimidazoles

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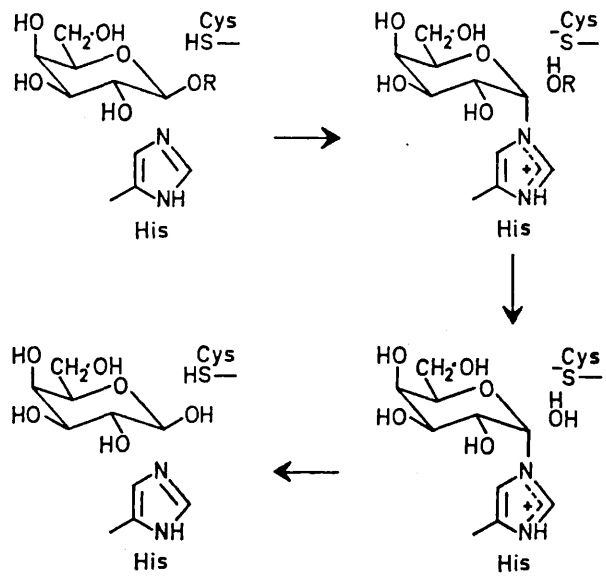
The synthesis of 1-α- and -β-D-glucopyranosylimidazoles *via* their tetra-acetates and their structural characterisation are described. The sugar–base linkage in these glycosylamines has been found to be extremely resistant to cleavage under a variety of hydrolytic conditions. The significance of this finding to the understanding of the mechanisms of nucleoside hydrolyses and of the role of histidine in glycosidase action is discussed.

STUDIES of the variation of enzyme activity with pH and photo-oxidation inactivation studies have indicated that a number of glycoside hydrolases and transferases possess essential histidine residues at the enzyme active sites. The types of enzyme for which this has been proposed are α-amylase,[1] β-amylase,[2] α-glucosidase,[3] oligo-1,6-glucosidase,[4] phosphorylase,[5] and β-galactosidase.[6] In the majority of cases it has been suggested that the imidazole ring of the side chain of the active site histidine residue must be in the protonated, imidazolium, form for enzyme activity, and that this group acts as a general acid catalyst of the glycosyl cleavage reaction. However, such a mechanism is difficult to reconcile with the fact that the hydrolysis of alkyl glycosides is normally specific acid catalysed only, and as would be expected no hydrolysis of methyl α-D-glucopyranoside is detected on refluxing solutions in water, water–dimethyl sulphoxide (1 : 1), and water–hexamethylphosphoramide (1 : 1), each containing a ten molar excess of imidazole and acetic acid.[7] A different role has been proposed [6] for a side chain of histidine at the active site of *E. coli* β-galactosidase, in which a neutral imidazole group acts as a nucleophilic catalyst of the glycosyl cleavage reaction as shown in Scheme 1.† Again there is little evidence from model studies to support this proposal, although intramolecular nucleophilic assistance of aryl glycoside hydrolysis has been observed.[8]

In order to investigate the role of histidine in enzymic glycosyl transfer reactions we have synthesised 1-α- and -β-D-glucopyranosylimidazoles, *via* their tetra-acetates, and studied the stability of the sugar–base linkage under a variety of hydrolytic conditions. The *N*-glycosylimidazoles are also of interest because they may exhibit the reverse anomeric effect,[9,10] and their hydrolytic behaviour is relevant to considerations of mechanisms of nucleoside hydrolysis.

Synthesis.—The synthesis of the 1-β-D-glucopyranosylimidazoles has been described by Bergmann and Heimhold,[11] who did not however establish the structures



SCHEME 1

and anomeric configurations of their products. More recently the synthesis and characterisation of the β-compounds have been reported by Jaskinska and Sokolowski,[12] and of the α- and β-compounds by Saluja.[10]

We have synthesised 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)imidazole by the method of Bergmann and Heimhold from 'acetobromoglucose' [13] and the silver salt of imidazole; the product was deacetylated

† Originally formulated as a front-side displacement process.

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[3] O. B. Jorgensen, *Acta Chem. Scand.*, 1964, **18**, 1115.

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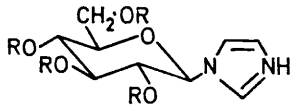
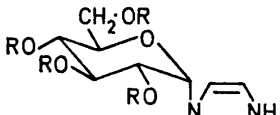
by treatment with methanolic ammonia. No trace of the α-anomer was detected in the reaction mixture; this material was obtained by a procedure used by Todd and his co-workers [14] for the synthesis of 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole. This method, in which an excess of free imidazole was treated with tetra-*O*-acetyl-α-D-glucosyl bromide in dioxan, afforded a mixture of anomers. The anomers were separated by chromatography on silica gel, or after deacetylation, on Dowex 1-X8 (OH^-). The structures of the products [(1a) and (2a)] were established by spectroscopic methods, and in the cases of the deacetylated compounds [(1b) and (2b)] by periodate oxidation (see Experimental section). ^1H N.m.r. coupling constants derived from the measured line splittings were checked by computer calculation; the magnitudes of the values showed that the *N*-glycosylimidazoles adopted the normal D^4C_1 conformation in neutral aqueous solution, as did their acetates in deuteriochloroform.



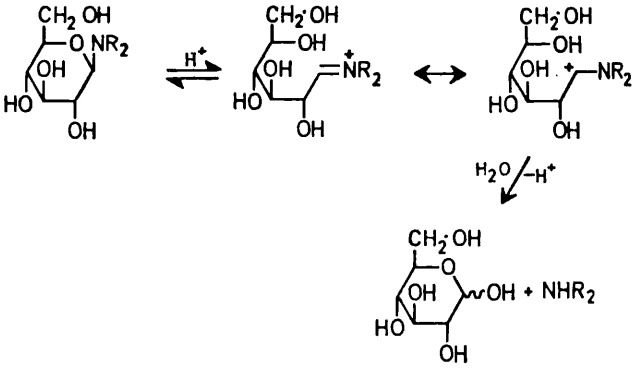
(1)a; R = Ac
b; R = H



(2)a; R = Ac
b; R = H

Stability of the Sugar–Base Linkage.—The susceptibility to hydrolysis of the sugar–base linkage in 1-α- and -β-D-glucopyranosylimidazole was examined in water, sodium formate buffer (pH 3), 0·565M-formic acid, 6N-hydrochloric acid, 10N-sulphuric acid, and N-sodium hydroxide at 100° for *ca.* 12 h. Optical rotation measurements, paper chromatography, and analysis by use of glucose oxidase reagent did not show any evidence of hydrolysis or anomerisation under the conditions specified. In another experiment 1-β-D-glucopyranosylimidazole was recovered and crystallised after being heated under reflux in 5N-sulphuric acid for 6 h. Hydrolytic cleavage was also attempted with solutions of the respective α- (yeast) and β- (almond) glucosidases; no glucose was released under conditions in which standard compounds (methyl α-D-glucopyranoside and cellobiose) were hydrolysed significantly. However the hydrolysis of the standards was inhibited by the respective *N*-glucosylimidazoles, a finding which is at present under further investigation.

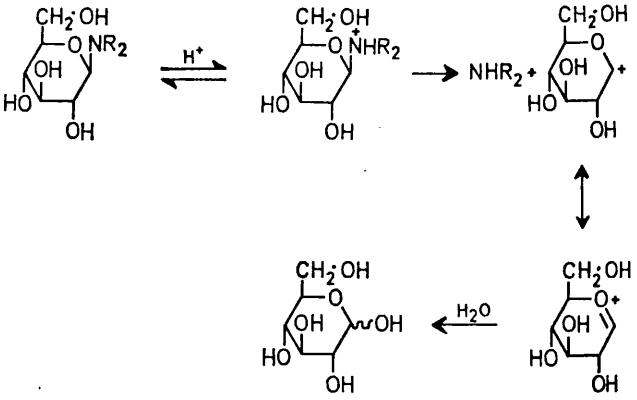
We believe that the resistance to acid hydrolysis of the *N*-glycosylimidazoles may be rationalised by consideration of possible mechanisms of hydrolysis of glycopyranosylamines.[15] Two types of mechanism may be distinguished, the first of which involves the formation of a Schiff base and is followed by those glycosylamines which show mutarotation in acid solution and are hydrolysed quite readily (Scheme 2). A necessary step in this mechanism is the formation of the Schiff base intermediate; this would appear to require electron release by the amino nitrogen atom, a process which is severely restricted in the glycosylimidazoles and in many nucleosides, since the aromaticity of the heterocyclic system



SCHEME 2

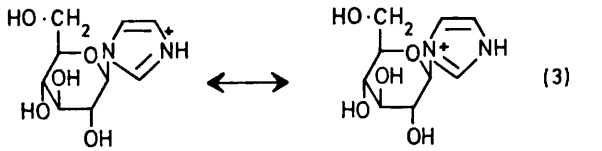
would be lost. This mechanism requires either a hydrogen atom or a lone pair of electrons on the glycosylamine nitrogen atom.

The second possible mechanism (*A*1 hydrolysis) is characterised by the formation of a glycosyl carbonium ion as shown in Scheme 3 and is believed to be followed



SCHEME 3

for the majority of acetals [16] and a number of nucleosides.[17] The overall rate by this pathway depends in opposite senses on the concentration of the conjugate acid and on the pK_a of the leaving group. In the cases of the *N*-glycosylimidazoles it appears that the imidazole



(3)

(pK_a *ca.* 7) is too poor a leaving group for breakdown of the resonance-stabilised conjugate acid (3) to occur.

The fact that most nucleosides are hydrolysed at

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measurable rates in acid solution may be attributed to two features not present in the glycosylimidazoles. Firstly, it has been widely observed that glycofuranosides are hydrolysed more rapidly than the corresponding glycopyranosides, and the operation of an *A*2 process or of mechanisms involving sugar ring opening has been suggested.[15] An alternative explanation is that the attainment of the transition state leading to the cyclic oxycarbonium ion is accompanied by more steric strain in the case of pyranosides than in that of furanosides. Secondly the pK_a values of the bases relevant to the forms of the nucleosides undergoing hydrolysis in acid solution are almost certainly lower than the pK_a value of imidazole, which would therefore be expected to be a poorer leaving group. In support of this, 5-amino-1-D-ribofuranosylimidazole-4-carboxamide (pK_a *ca.* 3·8) is more susceptible to hydrolysis than the 1-D-ribofuranosyl derivatives of a number of other imidazole derivatives of higher pK_a values.[18] However the 1-D-ribofuranosyl[18] and 1-β-D-galactopyranosyl[19] derivatives of benzimidazole (pK_a 5·33)[14] are extremely resistant to acid hydrolysis.

The extreme stability of the 1-glucopyranosylimidazoles appears to mitigate severely against the formation of *N*-glycosylhistidinyl intermediates in enzyme-catalysed glycoside hydrolyses.

EXPERIMENTAL

U.v. spectra (solutions in water or methanol) were recorded with a Perkin-Elmer 137 UV spectrophotometer. N.m.r. spectra were recorded with Varian HA-100D and HR-220 instruments, and analysed on a first-order basis. Assignments for the sugar ring protons were checked by double-resonance experiments and by computer calculation of spectra (program UEA NMR BASIC *). Optical rotations were recorded at ambient temperature with a Perkin-Elmer 141 polarimeter. T.l.c. was performed on silica gel [Polygram SIL-*G* sheets (Macherey–Nagel)]; spots were located with iodine or sulphuric acid.

1-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*imidazole* (1a).—This compound was prepared according to the procedure of Bergmann and Heimhold.[11] Recrystallisation from propan-1-ol gave needles (43%), m.p. 205—206° (lit.,[11] 205—208°), $[\alpha]_D^{21}$ −7·3° (*c* 1·5 in $CHCl_3$) [lit.,[11] −9° (*c* 0·7 in $CHCl_3$)] (Found: C, 51·15; H, 5·4; N, 7·2. Calc. for $C_{17}H_{22}N_2O_9$: C, 51·25; H, 5·55; N, 7·05%), $\lambda_{max.}$ 214 nm (log ε 3·58), τ ($CDCl_3$) 2·38 (1H, s, H-2), 2·92 (2H, s, H-4 and H-5), *ca.* 4·65 (4H, m, H-1′, H-2′, H-3′, H-4′), 5·71 (1H, q, $J_{6a',5'}$ 4·7, $J_{6a',6b'}$ 13·0 Hz, H-6a′), 5·84 (1H, q, $J_{6b',5'}$ 2·7, $J_{6b',6a'}$ 13·0 Hz, H-6b′), 6·05 (1H, octet, $J_{5',4'}$ 9·1, $J_{5',6a'}$ 4·7, $J_{5',6b'}$ 2·7 Hz, H-5′), and 7·93, 7·95, 8·00, and 8·14 (each 3H, s, Ac).

1-β-D-*Glucopyranosylimidazole* (1b).—Compound (1a) was deacetylated with methanolic ammonia at 0° to give 1-β-D-glucopyranosylimidazole (55%), m.p. 215—216° (from propan-1-ol) (lit.,[11] 217—218°; lit.,[12] 218—220°), $[\alpha]_D^{21}$ +13·6° (*c* 1·0 in H_2O) [lit.,[12] 12° (*c* 0·7 in H_2O)] (Found: C, 47·15; H, 6·25; N, 12·05. Calc. for $C_9H_{14}N_2O_6$: C, 46·95; H, 6·1; N, 12·15%), $\lambda_{max.}$ 210 nm (log ε 3·63), τ (D_2O) 1·58 (0·2H, s, partially exchanged H-2), 2·12 (1H, s, H-4), 2·39 (1H, s, H-5), 4·16 (1H, d, $J_{1',2'}$ 8·4 Hz, H-1′), *ca.* 5·7, (6H, m, H-2′ to H-6′). Compound (1b) consumed 1·76, 1·97, and 1·99 mol. equiv. of periodate (4·60 mol. equiv. originally present) (theor. 2·0) after 1, 5, and 6 h, respectively, and gave no formaldehyde and 0·84 mol. equiv. of formic acid.

1-(2,3,4,6-*Tetra*-O-*acetyl*-α-D-*glucopyranosyl*)*imidazole* (2a).—Tetra-*O*-acetyl-α-D-glucopyranosyl bromide[13] (32·9 g) and imidazole (12·0 g) were dissolved in dioxan (80 ml; dried over sodium) and heated under reflux for 4 h. The mixture was filtered and after the addition of xylene (30 ml) kept at 0° for 12 h. A solid was filtered off, washed several times with water, and dried; t.l.c. [benzene–methanol (9 : 1)] showed two major components to be present. Extraction of this solid with dry methanol gave a mixture (4·0 g) richer in the faster-moving component. Chromatography of this mixture on a column (100 × 4 cm) of silica gel (Merck 7734; 500 g) [elution with benzene–methanol (9 : 1)] followed by t.l.c. gave tetra-acetates (2a) (2·0 g, 6·3%) and (1a) (1·5 g). The former (2a) had m.p. 162—163° (from ethanol) $[\alpha]_D^{21}$ +118° (*c* 1·5 in $CHCl_3$) (lit.,[10] m.p. 172—173°, $[\alpha]_D$ +111°) (Found: C, 51·4; H, 5·7; N, 6·85. Calc. for $C_{17}H_{22}N_2O_9$: C, 51·25; H, 5·55; N, 7·05%), $\lambda_{max.}$ 223 nm (log ε 3·10), τ ($CDCl_3$) 2·11 (1H, s, H-2), 2·78 (1H, s, H-4), 2·84 (1H, s, H-5), 3·89 (1H, d, $J_{1',2'}$ 5·1 Hz, H-1′), 4·33 (1H, q, $J_{3',2'}$ 10·1, $J_{3',4'}$ 8·6 Hz, H-3′), 4·64 (1H, q, $J_{2',3'}$ 10·2, $J_{2',1'}$ 5·3 Hz, H-2′), 4·84 (1H, q, $J_{4',3'}$ 8·75, $J_{4',5'}$ 9·9 Hz, H-4′), 5·74 (1H, q, $J_{6a',6b'}$ 12·3, $J_{6a',5}$ 4·6 Hz, H-6a′), 5·99 (1H, q, $J_{6b',6a'}$ 12·45, $J_{6b',5'}$ 2·5 Hz, H-6b′), 6·45 (1H, octet, $J_{5',4'}$ 10·0, $J_{5',6a'}$ 4·4, $J_{5',6b'}$ 2·3 Hz, H-5′), and 7·93 (6H, s), 7·97 (3H, s), and 7·99 (3H, s) (4 × Ac).

1-α-D-*Glucopyranosylimidazole* (2b).—A mixture (6 g) rich in α-tetra-acetate (2a) was deacetylated in methanolic ammonia to yield a mixture (3·6 g) of 1-α- and -β-D-glucopyranosylimidazoles (1b) and (2b). This mixture was applied to a column (50 × 3 cm) of Dowex 1-X8 (OH^-) and eluted with deionised carbon dioxide-free water. Fractions (10 ml) were analysed by paper chromatography [development with butan-1-ol–ethanol–water (40 : 11 : 19)]; fractions 215—240 yielded the α-anomer (2b) (0·9 g, 2·4%), R_F 0·32 [R_F of β-anomer (2a) 0·24], which resisted attempts at crystallisation; $[\alpha]_D^{21}$ +104° (*c* 0·53 in H_2O) (lit.,[10] +104°) (Found: C, 46·85; H, 6·2; N, 12·0. Calc. for $C_9H_{14}N_2O_6$: C, 46·95; H, 6·1; N, 12·15%), $\lambda_{max.}$ 220 nm (log ε 3·20), τ (D_2O) 1·83 (1H, s, H-2), 2·46 (1H, s, H-4), 2·78 (1H, s, H-5), 3·86 (1H, d, $J_{1',2'}$ 5·7 Hz, H-1′), 5·82 (1H, q, $J_{2',1'}$ 5·7, $J_{2',3'}$ 10·0 Hz, H-2′), 5·96 (1H, q, $J_{3',2'}$ 10·0, $J_{3',4'}$ 8·8 Hz, H-3′), 6·12 (2H, m, H-6a′ and -6b′), 6·38 (1H, q, $J_{4',3'}$ 8·9, $J_{4',5'}$ 10·0 Hz, H-4′), and 6·62 (1H, octet, $J_{5',4'}$ 10·0, $J_{5',6a'}$ 4·8, $J_{5',6b'}$ 3·2 Hz, H-5′).

Compound (2b) consumed 1·05, 1·56, and 2·04 mol. equiv. of periodate (4·60 mol. equiv. originally present) (theor. 2·0) after 1, 5, and 8 h, respectively, and gave no formaldehyde and 0·96 mol. equiv. of formic acid.

Hydrolysis Experiments.—(*a*) Samples (*ca.* 0·015 g) of 1-α- and -β-D-glucopyranosylimidazole were heated separately in sealed tubes with 10N-sulphuric acid, 6N-hydrochloric acid, 0·565M-formic acid, pH 3 sodium formate buffer, N-sodium hydroxide, and water (2 ml of each, separately) at 100° for 12 h. No change in optical rotation from the initial values was detected during this period. After

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[19] A. J. Cleaver, A. B. Foster, and W. G. Overend, *J. Chem. Soc.*, 1959, 409.

neutralisation with IR-120(H^+) and IR-4B(OH^-) resins the solutions were analysed for glucose by use of glucose oxidase reagent [20] and by paper chromatography [development with n-butan-1-ol–ethanol–water (40 : 11 : 19) or propan-1-ol-ammonia (*d* 0·88) (3 : 1)]. Paper chromatograms were sprayed with glucose oxidase reagent (Worthington Glucostat), silver nitrate–sodium hydroxide reagent, or the Pauly reagent (diazosulphanilic acid); [21] no evidence for anomerisation or hydrolysis was obtained.

(*b*) 1-β-D-glucopyranosylimidazole (0·5 g) was heated with 5N-sulphuric acid at 100° for 6 h. The solution was neutralised with 5N-sodium hydroxide, concentrated to dryness, and extracted with dry methanol. Crystallisation of the extract from propan-1-ol gave a solid (0·1 g) indistinguishable from the starting material (paper chromatography and mixed m.p.).

(*c*) 1-α-D-glucopyranosylimidazole (0·020 g) was incubated at 37° with yeast α-glucosidase (Sigma; 2·1 International Units, measured by the rate of hydrolysis of *p*-nitrophenyl α-D-glucopyranoside) in pH 6·8 phosphate buffer (*I* 0·05; 20 ml), and samples (1 ml) were analysed by the glucose oxidase procedure. It was established that the presence of imidazole at the levels anticipated did not invalidate the glucose analysis, and a control experiment was carried out with methyl α-D-glucopyranoside (0·0194 g) as substrate. No release of glucose from the α-glucosylimidazole was observed. 1-β-D-glucopyranosylimidazole (0·0115 g) was incubated at 37° with almond β-glucosidase (Sigma; 1·5 International Units, measured by rate of hydrolysis of salicin) in 0·02M-citrate buffer, pH 5·3 (20 ml). A control experiment was carried out with cellobiose (0·0171 g) as substrate. No release of glucose from the β-glucosylimidazole was observed.

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