The Synthesis and Hydrolytic Stability of 1-Glucopyranosylimidazoles

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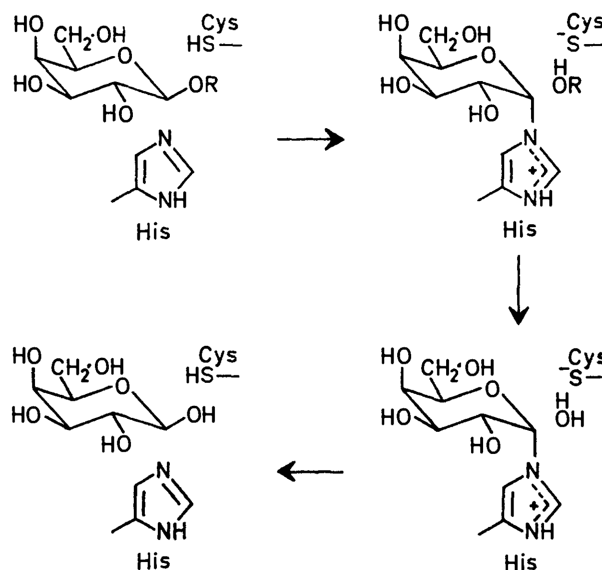
The synthesis of 1-α- and -β-D-glucopyranosylimidazoles *via* their tetra-acetates and their structural characterisation are described. The sugar–base linkage in these glycosylamines has been found to be extremely resistant to cleavage under a variety of hydrolytic conditions. The significance of this 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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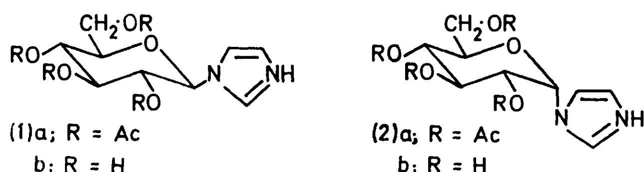
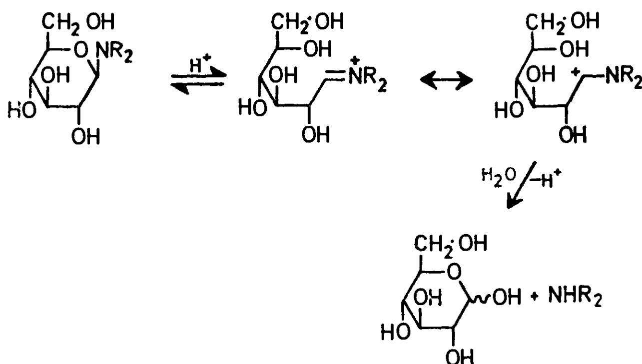
by treatment with methanolic ammonia. No trace of the α-anomer was detected in the reaction mixture; this material was obtained by a procedure used by Todd and his co-workers [14] for the synthesis of 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole. This method, in which an excess of free imidazole was 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.



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.

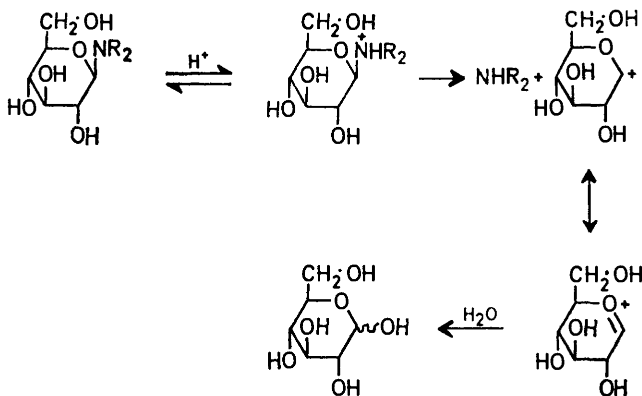
We believe that the resistance to acid hydrolysis of the *N*-glycosylimidazoles may be rationalised by consideration of possible mechanisms of hydrolysis of glycopyranosylamines.[15] Two types of mechanism may be distinguished, the first of which involves the formation of a Schiff base and is followed 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

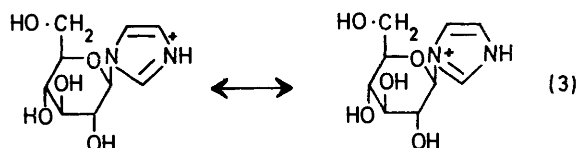
would be lost. This mechanism requires either a hydrogen atom or a lone pair of electrons on the glycosylamine nitrogen atom.

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



(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 $C_9H_{14}N_2O_5$: 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 $C_9H_{14}N_2O_5$: 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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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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