The Deamination of Pyranose Amines. Part II.[1] Methyl 2-Amino-2-deoxy-α-D-mannopyranoside

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The deamination of methyl 2-amino-2-deoxy-α-D-mannopyranoside with nitrous acid gives methyl 2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose and 2-*O*-methyl-D-glucose in a ratio of *ca.* 2 : 1. The disputed mechanism for the deamination of 2-amino-2-deoxy-D-mannose is further discussed.

THE deamination of glycosides of 2-amino-2-deoxypyranoses in which the amine function is equatorial results in ring contraction and concomitant cleavage of the glycosidic linkage, a reaction which is useful in structure elucidation.[2] The reaction pathway for the corresponding pyranosides in which the amine function is axial is of special interest † because the deamination of free 2-amino-2-deoxypyranoses in which the amino-group is axial is anomalous in that little rearrangement occurs. We report here a study of the deamination of methyl 2-amino-2-deoxy-α-D-mannopyranoside (1), and comment on the mechanism of the deamination of the corresponding free sugar.

Hydrolysis of the known methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside [3b] in aqueous acetic acid gave in high yield the acetate salt of the amine glycoside (1), which was converted into the crystalline amine (1) by treatment with anion-exchange resin. Deamination of the amine in dilute aqueous acetic acid (pH 3—4) with sodium nitrite gave two major products which were separated by preparative paper chromatography. Methyl 2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (2) was isolated as a syrup in 50% yield, and its structure was established by the

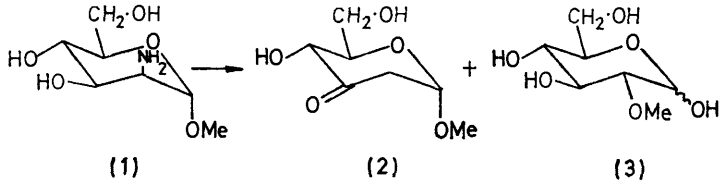
† After this work was completed the deamination of 6-*O*-(2-amino-2-deoxy-β-D-mannopyranosyl)-D-glucose and the corresponding alditol was reported to give substitution and rearrangement products.[3a]

[1] Part I, N. M. K. Ng Ying Kin, J. M. Williams, and A. Horsington, *J. Chem. Soc.* (*C*), 1971, 1578.

[2] *Cf.*, *e.g.*, (*a*) A. B. Foster, E. F. Martlew, and M. Stacey, *Chem. and Ind.*, 1953, 825; (*b*) D. C. Ellwood, M. V. Kelemen, and J. Baddiley, *Biochem. J.*, 1963, **86**, 213.

[3] (*a*) S. Hase and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **72**, 1117; S. Hase, Y. Tsuji, and Y. Matsushima, *ibid.*, p. 1549; (*b*) S. T. K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Tetrahedron*, 1970, **26**, 3653.

n.m.r. spectrum. The anomeric proton gave a quartet owing to coupling with the adjacent methylene group ($J_{1.2eq}$ 1·5, $J_{1.2ax}$ 4·5 Hz), and a characteristic [4,5] long-range coupling was observed between the axial 2- and 4-protons ($^4J_{2.4}$ 1 Hz). Acetylation of the 3-ulose gave a homogeneous diacetate, which gave a satisfactory n.m.r. spectrum, and reaction with semicarbazide gave a crystalline semicarbazone with physical constants close to the literature values.[6]



The second major product, 2-*O*-methyl D-glucose (3), isolated in 27% yield, was identified by comparison with an authentic sample [7] by g.l.c., n.m.r., and paper chromatography. When the deamination reaction mixture was deionised with Amberlite MB3 resin, and then analysed quantitatively by g.l.c. of the trimethylsilyl ethers using glucitol as internal standard, the yields of 3-ulose (2) and 2-*O*-methyl D-glucose (3) were found to be 42 and 22%, respectively. N.m.r. analysis also gave a 2 : 1 ratio for (2) and (3). Fractionation of the deionised mixture gave the 3-ulose (28%) and 2-*O*-methyl-D-glucose (15%); thus the resin treatment lowered the recovery of products.

The rearrangement which gave the pyranosid-3-ulose is similar to those observed for other monocyclic cyclohexane [8] and pyranoside [4] derivatives which contain an equatorial hydroxy-group vicinal to an axial amino-group. The 1,2-shift of the methoxy-group is a significant competing reaction pathway. A similar methoxy-group migration occurred in a closely related reaction, the brominolysis of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-iodo-α-D-mannopyranoside.[6] That no substitution occurs to give methyl α-D-glucopyranoside is noteworthy. The deamination of two 2-amino-2-deoxy-β-D-mannopyranosides, in which the aglycones were D-glucose and D-glucitol, has recently been reported [3a] to give the substitution products (β-D-glucopyranosides), but extensive rearrangement also occurred to give products which were tentatively identified as 2-deoxy-D-*erythro*-hexopyranosid-3-uloses. The detection, in each case, of a small amount of a compound corresponding to the free aglycone can be attributed to a hydride shift from C-1 of the mannopyranose ring. The deaminations of 2-amino-2-deoxy-D-mannose and 2-amino-2,6-dideoxy-L-talose give as major products D-glucose [9] and 6-deoxygalactose,[10b] respectively. We consider these reactions to be anomalous in that the major product results from substitution (with inversion) rather than from rearrangement. To account for this anomaly and for the fact that glucose is formed stereospecifically [4] from 2-amino-2-deoxy-D-mannose, we suggested [4] that the reaction involved participation of the anomeric hydroxy-group to give a 1,2-epoxide (4), which would presumably be hydrolysed readily under the conditions of the reaction [*cf.* reactivity of the triacetate of (4)].[10b] Horton and Philips have argued that the predominance (57%) of the β-anomer of 2-amino-2-deoxy-D-mannose hydrochloride in aqueous solution is inconsistent with this mechanism, and they favour the substitution mechanism.[9b] However, we disagree with these authors for the following reasons. Although the β-anomer of 2-amino-2-deoxy-D-mannose hydrochloride predominates in aqueous solution, the rates of *N*-nitrosation (the rate-determining step in deaminations [11]) of the α- and β-anomers may, and probably, do, differ. We suggest that 2-amino-2-deoxy-α-D-mannose is nitrosated more rapidly than the β-anomer, in which the C-1 and C-2 substituents are *cis*-related (*i.e.* $k_\alpha > k_\beta$ in the Scheme). An analogy is provided by the relative rates of deamination of methyl 2-amino-2-deoxy-α- and β-D-glucopyranosides,[2a] the slower reaction of the α-anomer in this case being attributed to steric effects resulting from the *cis*-relationship of the substituents at C-1 and C-2. The anomerisation of the hydrochloride of 2-amino-2-deoxy-D-mannose is unusually rapid, and it has been suggested that this is due to the ability of the $^+NH_3$ group to protonate the anomeric or ring oxygen atom.[12] It is likely that the nitrosoamine/diazonium ion intermediates anomerise at 'normal' rates which are much less than their rate of conversion into products,* and so the major product of the reaction would originate from the α-anomer.†

Similar competing pathways operate in the deamin-

* *Cf.* 2-acetamido-2-deoxy-α-D-mannose; a 2% solution in M-acetic acid took 30 min. to mutarotate to equilibrium at 25°.

† Thus, the position of the anomeric equilibrium of the amine hydrochloride is unimportant because of the rapid anomerisation, and this also holds if, as is less likely, the nitrosoamines anomerise more rapidly than they are converted into products.

[4] N. M. K. Ng Ying Kin and J. M. Williams, *Chem. Comm.*, 1971, 1123.

[5] P. M. Collins D. Gardiner, S. Kumar, and W. G. Overend, *J.C.S. Perkin I*, 1972, 2596.

[6] R. U. Lemieux and B. Fraser-Reid, *Canad. J. Chem.*, 1964, **42**, 539.

[7] W. E. Dick, jun., *Carbohydrate Res.*, 1972, **21**, 255.

[8] M. Cherest, H. Felkin, J. Sicher, F. Sipos, and M. Tichy, *J. Chem. Soc.*, 1965, 2513; T. Posternak, *Helv. Chim. Acta*, 1950, **33**, 1597.

[9] (*a*) P. A. Levene, *J. Biol. Chem.*, 1919, **39**, 69; (*b*) D. Horton and K. D. Philips, *Carbohydrate Res.*, 1972, **21**, 417.

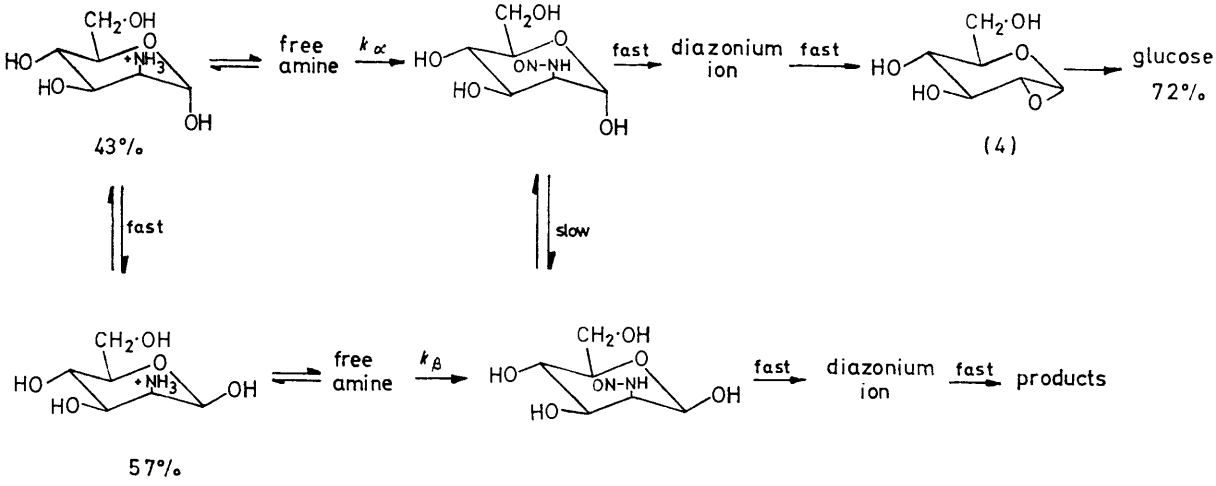
[10] (*a*) S. A. Barker, J. S. Brimacombe, M. J. How, M. Stacey, and J. M. Williams, *Nature*, 1961, **189**, 303; (*b*) N. R. Williams, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 163.

[11] J. H. Ridd, *Quart. Rev.*, 1961, **15**, 418.

[12] D. Horton, J. S. Jewell, and K. D. Philips, *J. Org. Chem.*, 1966, **31**, 3843.

ation of L-1-amino-1-deoxy-*chiro*-inositol (5), which gives the epoxide (6) in 60% yield.[13a] Thus, participation of the hydroxy-group is more favourable than rearrangement to a ketone.

The stereospecific formation of non-rearranged substitution products in the deamination of methyl 4-amino-4,6-dideoxy-2,3-*O*-isopropylidene-α-L-talopyranoside,[13b] cited by Horton and Philips, is not relevant because the absence of rearrangement may be due to the presence of a second fused ring, and the stereospecificity has been attributed to the presence of a substituent which is



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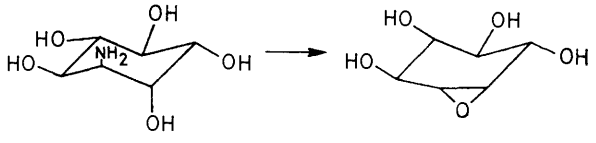
syn-axial to the amino-group. Other *monocyclic* pyranosides containing an axial amino-group undergo deamination to give both epimers of the non-rearranged



(5) (6)

substitution products in addition to rearrangement products.[4]

EXPERIMENTAL

M.p.s were measured on a Kofler hot-stage apparatus. I.r. and n.m.r. spectra were recorded on Perkin-Elmer 257 and Varian HA100 spectrometers respectively. Quoted *J* values are the observed spacings. Microanalysis was performed using an F and M 185 analyser. Optical rotation measurements were made using a Perkin-Elmer 141 polarimeter, and comparisons of materials with authentic compounds were made where possible.

T.l.c. was carried out on Kieselgel G (Merck) plates (0·25 mm thick) in the solvent systems (A) toluene–ether (1 : 1 v/v) and (B) toluene–ethanol (100 : 15 v/v). Spots were detected by spraying with 5% v/v concentrated sulphuric acid in ethanol and heating the sprayed plate to 120 °C in an air oven. Paper chromatography utilized the descending technique on Whatman no. 1 paper in a butan-1-ol–ethanol–water (4 : 1 : 5 v/v) system (organic layer as descending phase; paper pre-equilibrated in solvent vapour). Spots were located with alkaline silver nitrate; [14] R_G = mobility relative to D-glucose. Preparative paper chromatography utilized the same method and solvent system on Whatman no. 3 paper. The bands were detected by spraying the edges of the chromatogram with silver nitrate–sodium hydroxide, and were subsequently eluted with water. G.l.c. was carried out on an F and M 810 chromatograph, (recorder equipped with disc integrator) using a flame ionisation detector and glass columns (240 cm × 0·4 cm int. diam.), packed with 10% silicon rubber U.C.W. 98 on silanized Chromosorb W (60—80 mesh) (argon flow rate 50 cm^3 min^{-1}; column temperature 180 °C; injection port at 240 °C). The compounds examined were converted into their trimethylsilyl ethers [15] prior to injection.

Methyl 2-Amino-2-deoxy-α-D-mannopyranoside Acetate Salt.—A solution of methyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside [3b] (168 mg) in 60% aqueous acetic acid (9 ml) was heated for 1 h on a boiling water bath.[16] The solution was concentrated under reduced pressure to a gum and the last traces of acetic acid and benzaldehyde were removed by redissolving the gum in water (5 ml) and re-evaporating under reduced pressure. This process was repeated until the final gum did not smell of either acetic acid or benzaldehyde. When stored under vacuum the gum crystallised (145 mg, 96%); recrystallisation from ethanol–petroleum (b.p. 60—80°) gave needles of the acetate salt, m.p. 117—119°, $[\alpha]_D^{20}$ +136·5° (H_2O); $\nu_{max.}$ (KBr) 3300br (OH str), 1550, and 1410 cm^{-1} (broad, CO_2^-); τ (D_2O; $Me_3Si[CH_2]_3SO_3Na$ reference) 8·10 (3H, s, ^-OAc), 6·58 (3H, s, OMe), and 5·10 (1H, d, *J* 1·5 Hz, H-1). The crystals were deliquescent and accurate elemental analysis proved impossible.

Methyl 2-Amino-2-deoxy-α-D-mannopyranoside (1).—A

[13] (*a*) S. J. Angyal and J. S. Murdoch, *Austral. J. Chem.*, 1969, **22**, 2417; (*b*) A. K. Al-Radhi, J. S. Brimacombe, and L. C. N. Tucker, *Chem. Comm.*, 1970, 1250.

[14] W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, 1950, **166**, 444.

[15] C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, 1963, **85**, 2497.

[16] P. Sinaye, M. D. A. Halford, M. S. Choudhary, P. H. Gross, and R. W. Jeanloz, *J. Biol. Chem.*, 1972, **247**, 391.

solution of the acetate salt (145 mg) in carbon dioxide-free deionised water (2 ml) was passed through a column of Amberlite CG-400(OH^-) resin (8 g). The column was eluted with carbon dioxide-free deionised water and the eluate was concentrated under reduced pressure at 30—35 °C to a syrup which was then pumped under high vacuum for several hours. The resulting gum slowly crystallised (yield 89 mg, 80%); recrystallisation from ethanol–pentane gave the *amine*, m.p. 143—145°; $[\alpha]_D^{24}$ +122° (H_2O); τ ([$^2H_5$]pyridine; after D_2O exchange) 6·68 (3H, s, OMe) and 5·01 (1H, d, *J* 1 Hz, H-1) (Found: C, 43·85; H, 7·95; N, 7·3. $C_7H_{15}NO_5$ requires C, 43·5; H, 7·85; N, 7·25%). The derived syrupy *N*-acetate had $[\alpha]_D^{23}$ +47° (H_2O) {lit.,[17] $[\alpha]_D^{24}$ +50° (H_2O)}.

Deamination of Methyl 2-Amino-2-deoxy-α-D-mannopyranoside.—(i) The amine (119 mg, 0·62 mmol) was dissolved in aqueous M-acetic acid (3·7 ml), and sodium nitrite (169 mg, 2·45 mmol) was added in small portions. The solution was then kept at ambient temperature for 2 h, neutralised (to pH 6) with aqueous sodium hydrogen carbonate, and concentrated to *ca.* 1 ml under reduced pressure at 25—30 °C. Paper chromatography of the product mixture displayed two major spots (R_G 1·75 and 3·5); the product of higher mobility gave a bright yellow spot when sprayed with 2,4-dinitrophenylhydrazine in ethanolic phosphoric acid.[18] The mixture was fractionated by preparative paper chromatography. Elution of the band of highest mobility (R_F 0·58) afforded a colourless homogeneous syrup (54 mg, 50%), identified as methyl-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose; $\nu_{max.}$ (film) 3400br (OH str.) and 1720 cm^{-1} (C=O str.); τ ([$^2H_5$]pyridine; after D_2O exchange) 7·35 (1H, q, $J_{1,2eq}$ 1·5, $J_{2ax\,2eq}$ 14 Hz, H-2*eq*), 7·05 (1H, oct, $J_{1.2ax}$ 4·5, $J_{2ax.4}$ 1 Hz, H-2*ax*), 6·76 (3H, s, OMe), 5·87 (1H, m, H-5), 5·68 (2H, m, H-6 and H-6′), 5·30 (1H, q, $J_{4,5}$ 10, $J_{2ax,4}$ 1 Hz, H-4), and 4·81 (1H, q, $J_{1.2eq}$ 1·5, $J_{1.2ax}$ 4·5 Hz, H-1) (assignments confirmed by spin decoupling).

Elution of the products of lower mobility followed by deionisation of the eluate with Amberlite MB-3 resin afforded a colourless syrup (32 mg), shown to be 2-*O*-methyl-αβ-D-glucopyranose (containing three minor contaminants) by comparison (g.l.c. retention time, n.m.r. spectrum, and mobility on a paper chromatogram) with an authentic sample.[7]

(ii) The amine (171 mg, 0·89 mmol) was deaminated in M-acetic acid (6 ml) with sodium nitrite (276 mg) as in (i). The neutralised solution was then deionised with Amberlite MB-3 resin and concentrated to a syrup (103·5 mg) under reduced pressure. G.l.c. analysis of the product mixture using glucitol as internal standard indicated it to consist chiefly of the 3-uloside (63%) (composition by weight) and 2-*O*-methyl-αβ-D-glucose (36%), and four minor components. An n.m.r. spectrum of the product mixture ([$^2H_5$]pyridine; after D_2O exchange) displayed OMe singlets at τ 6·76 (uloside) and 6·38 and 6·11 (2-*O*-methyl-αβ-D-glucose). The ratio of the integrals of uloside to 2-*O*-methyl-D-glucose signals was *ca.* 2 : 1. The OMe singlet of methyl β-D-glucopyranoside was absent. Separation of the mixture by preparative paper chromatography afforded the 3-uloside (43 mg, 28%) and 2-*O*-methyl-D-glucose (26 mg, 15%).

Methyl 4,6-Di-O-acetyl-2-deoxy-α-D-erythro-hexopyranosid-3-ulose.—The 3-uloside (28 mg) was dissolved in dry pyridine (0·5 ml) and acetic anhydride (0·5 ml). The solution was kept at ambient temperature for 16 h and then poured into iced water (3 ml). After several hours the solution was extracted with chloroform (3 × 4 ml) and the organic phase was washed in turn with 5N-HCl (2 × 5 ml), saturated sodium hydrogen carbonate solution (2 × 5 ml), and deionised water (2 × 5 ml). Drying ($MgSO_4$) and concentration gave the syrupy diacetate (28·5 mg, 70%), which was homogeneous by t.l.c.; $\nu_{max.}$ (film) 1735 cm^{-1} (C=O str.); τ ($CDCl_3$) 7·90 and 7·85 (each 3H, s, OAc), 7·36 (1H, q, $J_{1.2eq}$ 1·5, $J_{2ax.2eq}$ 14·5 Hz, H-2*eq*) 7·13 (1H. q, $J_{1.2ax}$ 4·5 Hz, H-2*ax*), 6·64 (3H, s, OMe), 5·90—5·55 (3H, m, H-5, H-6, and H-6′), 4·85 (1H, q, $J_{1.2ax}$ 4·5, $J_{1,2eq}$ 1·5 Hz, H-1) and 4·77 (1H, d, $J_{4.5}$ 10·5 Hz, H-4).

Semicarbazone. The 3-uloside diacetate (28·5 mg) was dissolved in ethanol (0·15 ml), and water (0·04 ml) was added, causing slight turbidity. Semicarbazide hydrochloride (19 mg) and anhydrous sodium acetate (27 mg) were then added. The solution was kept at 0—2 °C for 2 h, after which time crystallization had occurred. The crude product (17 mg, 50%) was recrystallised twice from ethanol–water to yield slightly impure semicarbazone (1 mg), m.p. 188—192°; $[\alpha]_D^{24}$ +171 ± 49° (pyridine) {lit.,[6] m.p. 195—196°; $[\alpha]_D^{24}$ +146° after four recrystallisations}.

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[17] S. Beychok, G. Ashwell, and E. A. Kabat, *Carbohydrate Res.*, 1971, **17**, 19.
[18] G. D. Johnson, *J. Amer. Chem. Soc.*, 1951, **73**, 5888.