510. *Synthesis of 2-Amino-2,6-dideoxy-L-mannose (L-Rhamnosamine).**

By J. S. Brimacombe and M. C. Cook.

Platinum-catalysed oxidation of methyl α-L-rhamnopyranoside yields methyl 6-deoxy-α-L-*arabino*-hexopyranuloside, which, on sequential oximation, catalytic reduction, *N*-acetylation, and hydrolysis with hydrochloric acid, yields 2-amino-2,6-dideoxy-L-mannose hydrochloride and the epimer, 2-amino-2,6-dideoxy-L-glucose hydrochloride.

Recent publications [1-3] have shown that amino-sugars can be conveniently synthesised by way of keto-sugar intermediates, since the oxime derived therefrom can be reduced to yield a mixture of epimeric amino-sugars. Catalytic reduction of oximes or hydrazones attached to cyclohexane or pyranose rings usually leads to a preponderance of the epimer having an axial amino-group in the stable chair form.[2,4] This permits syntheses of amino-sugars [2,3] which are not readily accessible by other methods, and we now report the synthesis of 2-amino-2,6-dideoxy-L-mannose hydrochloride.

The platinum-catalysed oxidation of methyl α-L-rhamnopyranoside gave a syrupy product, in approximately 20—25% yield, which was identified as methyl 6-deoxy-α-L-*arabino*-hexopyranuloside † since it gave L-rhamnose and 6-deoxy-L-glucose (L-quinovose) on reduction and acidic hydrolysis. The selective, catalytic oxidation of axial hydroxyl

* Preliminary communication, *Chem. and Ind.*, 1963, 1281.

† The name is based on Rule 7 of the U.S.–British Rules of Carbohydrate Nomenclature.

[1] Overend, *Chem. and Ind.*, 1963, 342; Collins and Overend, *ibid.*, 1963, 375.

[2] Lindberg and Theander, *Acta Chem. Scand.*, 1959, **13**, 1226.

[3] Brimacombe and How, *J.*, 1963, 3886.

[4] Posternack, *Helv. Chim. Acta*, 1950, **33**, 1597; Anderson and Lardy, *J. Amer. Chem. Soc.*, 1950, **72**, 3141.

groups in cyclitol and pyranose derivatives is now well exemplified, and Heyns and Paulsen [5] recently reviewed this subject. Thus, on the assumption that axial hydroxyl groups are selectively oxidised, methyl α-L-rhamnopyranoside is oxidised preferentially in the 1C conformation [6] at the axial C-2 hydroxyl group. Under these conditions, methyl 4,6-*O*-ethylidene-α-D-mannopyranoside is oxidised in the sterically favoured C1 conformation at the axial C-2 hydroxyl group, to yield, after mild acidic hydrolysis, methyl α-D-*arabino*-hexopyranuloside.[7]

Treatment of methyl 6-deoxy-α-L-*arabino*-hexopyranuloside with hydroxylamine afforded a syrupy oxime, in high yield, which was reduced with hydrogen over a platinum catalyst to give a mixture of epimeric amino-sugar glycosides. Chromatography on Dowex-50 (H^+), after *N*-acetylation and acidic hydrolysis of the product mixture, effected only a partial separation of the amino-sugar hydrochlorides. Visual examination of paper chromatograms of the acidic hydrolysate, prior to fractionation on the resin, did not reveal a significantly large preponderance of one of the epimers. The pure amino-sugar hydrochloride, which crystallised from the first fractions eluted from the resin, was assigned the structure 2-amino-2,6-dideoxy-L-mannose hydrochloride by the following evidence. It gave a positive response in the Elson–Morgan reaction [8] and afforded a component with chromatographic and electrophoretic properties indistinguishable from those of 5-deoxy-L-arabinose on oxidative deamination with ninhydrin,[9] signifying an original *gluco*- or *manno*-configuration. The physical constants {m. p. 180° (decomp.), $[\alpha]_{Hg}^{22}$ +26° (final)} of the amino-sugar hydrochloride were distinct from those reported [10] for 2-amino-2,6-dideoxy-L-glucose hydrochloride {m. p. 173—175°, $[\alpha]_D^{22}$ −53° (final)}, and its infrared spectrum, *X*-ray diffraction pattern, and chromatographic properties readily differentiated it from those of authentic 2-amino-2,6-dideoxy-D-glucose hydrochloride.[11]

Subsequent fractions eluted from the resin were combined with the mother-liquors from the foregoing crystallisation, and, after neutralisation and concentration, were chromatographed on thick filter papers. This procedure afforded additional amounts of 2-amino-2,6-dideoxy-L-mannose hydrochloride and another crystalline sugar, identified as 2-amino-2,6-dideoxy-L-glucose hydrochloride by comparison of its melting point, chromatographic properties, and infrared spectrum with those of the crystalline D-enantiomorph.[11] The yields of amino-sugars obtained showed that the *manno*- and *gluco*-epimers, formed on reduction of the oxime, were in the approximate ratio of 2·4 : 1. With less-flexible ring systems [3] the higher yield of the amino-sugar possessing an axial amino-group is even more marked.

Epimerisation [12] of 2-acetamido-2,6-dideoxy-D-glucose and acidic hydrolysis of the products afforded, *inter alia*, a component with chromatographic properties indistinguishable from those of 2-amino-2,6-dideoxy-L-mannose hydrochloride. It is noteworthy that the paper-chromatographic mobilities of L-quinovose and L-rhamnose are reversed in the 2-amino-2-deoxy-analogues, as noted by Kuhn, Bister, and Dafeldecker.[10]

Experimental

Paper chromatograms were run on Whatman No. 1 or 3MM paper (for preparative separations) by downward irrigation with the organic phase of one of the following solvent systems: A, butan-1-ol–ethanol–water (4 : 1 : 5); B, butan-1-ol–acetic acid–water (4 : 1 : 5); C, butan-2-one–acetic acid–saturated boric acid solution (9 : 1 : 1).

Oxidation of Methyl α-L-Rhamnopyranoside.—Optimal conditions for the oxidation were

[5] Heyns and Paulsen, *Adv. Carbohydrate Chem.*, 1962, **17**, 169.
[6] Tipson and Isbell, *J. Res. Nat. Bur. Stand.*, 1960, **64**, 239.
[7] Lindberg, Svensson, Theander, Brimacombe, and Cook, *Acta Chem. Scand.*, 1963, **17**, 930.
[8] Rondle and Morgan, *Biochem. J.*, 1955, **61**, 586.
[9] Stoffyn and Jeanloz, *Arch. Biochem. Biophys.*, 1954, **52**, 373.
[10] Kuhn, Bister, and Dafeldecker, *Annalen*, 1958, **617**, 115.
[11] Morel, *Helv. Chim. Acta*, 1958, **41**, 1501.
[12] Kuhn and Brossmer, *Annalen*, 1958, **616**, 221.

determined in small-scale experiments and, generally, prolongation of the oxidation did not substantially increase the yield of keto-glycoside, but resulted in the formation of secondary products.

A rapid stream of oxygen was bubbled for 6 hr. through a solution of methyl α-L-rhamnopyranoside [13] (3·3 g.) in water (80 ml.) containing a platinum catalyst [14] (2 g.) at 30°. The filtered solution was freeze-dried, taken up in a little water, and applied to a column of IRA-400 resin (HSO_3^- form). Unreacted starting material was washed from the resin with water, and, thereafter, the keto-glycoside was eluted from the column with water containing increasing amounts (5—10%) of acetone. Concentration of the eluate yielded the syrupy methyl 6-deoxy-α-L-*arabino*-hexopyranuloside (0·9 g.), $[\alpha]_D^{20}$ −97° (*c* 2 in H_2O) (in the preliminary communication, *Chem. and Ind.*, 1963, 1281, this rotation was mistakenly given as +97°). Characterisation of the product was achieved by refluxing a sample with Raney nickel [15] in 70% aqueous ethanol, and acidic hydrolysis of the glycosides so produced. Paper chromatograms (solvent C) and electrophoretograms (borate buffer, pH 10) revealed two components indistinguishable in their mobilities from those of L-rhamnose and L-quinovose [*R*(rhamnose) 0·86].

Oximation and Reduction of the Oxime.—A solution of methyl 6-deoxy-α-L-*arabino*-hexopyranuloside (2 g.) in water (60 ml.) was added in portions to a stirred solution of hydroxylamine hydrochloride (7·5 g.) in water (100 ml.) which had been previously adjusted to pH 4. The temperature was kept at 10° by external cooling, and the pH maintained at 4 by addition of 0·1N-sodium hydroxide. After 4 hr. the solution was adjusted to pH 7 and concentrated (<40°) to dryness under reduced pressure. The solid residue was extracted with redistilled butan-1-ol (4 × 50 ml.), and the extract concentrated to *ca.* 100 ml., and reduced with a slight overpressure of hydrogen in the presence of Adams catalyst (0·5 g.) for 20 hr. at room temperature. The filtered solution was concentrated under reduced pressure to yield a syrup (1·4 g.) which did not crystallise. Chromatograms (solvent A) sprayed with ninhydrin reagent [16] revealed the major components as a diffuse spot at *R*(glucosamine) 4—5.

N-*Acetylation and Acidic Hydrolysis.*—To a stirred and cooled (0°) solution of the foregoing syrup (1·4 g.) in water (80 ml.) and methanol (5·6 ml.) was added Dowex-1 (CO_3^{2-}) (140 ml.) and redistilled acetic anhydride (2·8 ml.); the reaction was allowed to proceed for 90 min. The combined filtrate and washings were stirred for 10 min. with Amberlite IR-120 (H^+), the solution was filtered, and the resin was thoroughly washed with water. The combined filtrate and washings were freeze-dried, to give a syrup (0·8 g.) which was hydrolysed with 2N-hydrochloric acid (40 ml.) for 6 hr. at 95°. Two major components, *R*(glucosamine) 1·6 and 2·0, of approximately equal intensity were revealed on paper chromatograms (solvent A) sprayed with ninhydrin reagent.[16]

Fractionation on Dowex-50 (H^+) *and on Thick Filter Papers.*—The foregoing hydrolysate was diluted with water (250 ml.) and applied to a freshly regenerated column of Dowex-50 (H^+) (23 × 6 cm.; 200—400 mesh); elution was with 0·3N-hydrochloric acid. Fractions (25 ml.) were collected automatically and portions (1 ml.) of appropriate fractions were analysed for amino-sugars with the Elson–Morgan reagent.[8] Paper chromatograms of the eluate revealed that only a partial separation of the amino-sugars had taken place. The fraction eluted from the column between 3 and 3·5 l. was neutralised with Deacidite-FF (CO_3^{2-}), filtered, and freeze-dried, to give 2-*amino*-2,6-*dideoxy*-L-*mannose hydrochloride* (80 mg.), m. p. 180° (decomp.; browning at 160°) (from methanol–acetone), $[\alpha]_{Hg}^{22}$ +26° (final, *c* 1·75 in H_2O) (Found: C, 36·0; H, 6·8; N, 6·8. $C_6H_{14}ClNO_4$ requires C, 36·1; H, 7·1; N, 7·0%). The paper-chromatographic properties, *X*-ray diffraction pattern, and infrared spectrum of this product were distinct from those of authentic 2-amino-2,6-dideoxy-D-glucose hydrochloride.[11] Subsequent fractions (3·5—4 l.) were neutralised as described previously, and, after concentration, combined with the mother-liquors from the previous crystallisation. The two components in the mixture, *R*(glucosamine) 1·6 and 2·0, were separated on Whatman No. 3MM papers (solvent A) and eluted from the appropriate strips with water. The residue (58 mg.) obtained from the slower-moving fraction had m. p. 180° (decomp.) (from methanol–acetone) and an infrared spectrum indistinguishable from that of the amino-sugar hydrochloride obtained previously. The residue (58 mg.) obtained from the second fraction, *R*(glucosamine) 2·0, had m. p. 172° (corr.) (from methanol–acetone)

[13] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[14] Brimacombe, Brimacombe, and Lindberg, *Acta Chem. Scand.*, 1960, **14**, 2236.
[15] Karabinos and Ballun, *J. Amer. Chem. Soc.*, 1953, **75**, 4501.
[16] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.

(lit.,[10] 173—175°), and its infrared spectrum was indistinguishable from that of the enantiomorphic 2-amino-2,6-dideoxy-D-glucose hydrochloride.[11]

Oxidative Deaminations.—Solutions of 2-amino-2,6-dideoxy-L-mannose hydrochloride (5 mg.) and 2-amino-2,6-dideoxy-L-glucose hydrochloride (5 mg.) in water (0·2 ml.) were severally treated with 2% aqueous ninhydrin containing 4% of pyridine for 1 hr. by essentially the procedure described by Stoffyn and Jeanloz [9] for D-glucosamine hydrochloride. Paper chromatography (solvent A) of the solutions showed one component which was common to both and which was indistinguishable in its chromatographic and electrophoretic properties from 5-deoxy-L-arabinose.

Epimerisations.[12]—A solution of 2-acetamido-2,6-dideoxy-D-glucose (4·7 mg.) in pyridine (1·5 ml.) was treated with finely powdered nickel acetate tetrahydrate (4·7 mg.) at 95° for 2 hr. After addition of ethanol, the solution was evaporated to a syrup, which was hydrolysed with 2N-sulphuric acid (0·5 ml.) at 95° for 1 hr. The neutralised ($BaCO_3$) solution was centrifuged and the supernatant solution, on paper chromatography (solvent A), was shown to contain two components indistinguishable in their chromatographic properties from 2-amino-2,6-dideoxy-L-mannose and 2-amino-2,6-dideoxy-L-glucose.

We thank Professor M. Stacey, F.R.S., and Dr. S. A. Barker for their interest in this investigation, and Dr. C. J. Morel for a gift of 2-amino-2,6-dideoxy-D-glucose hydrochloride. One of us (M. C. C.) thanks the D.S.I.R. for a scholarship.

CHEMISTRY DEPARTMENT, THE UNIVERSITY, BIRMINGHAM 15. [*Received, October 26th,* 1963.]