Nitrogen-containing Carbohydrate Derivatives. Part XXXI.† Synthesis of Derivatives of 2,6-Diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranose: Confirmation of the Structure of Nebrosamine

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The synthesis of methyl 2,6-diacetamido-2,3,6-trideoxy-4-*O*-mesyl-α-D-*ribo*-hexopyranoside is reported: its comparison with a derivative of nebrosamine has confirmed the structure of that natural product.

METHANOLYSIS of per-*N*-acetyltobramycin gave, among other products, a derivative of nebrosamine, a new diaminohexose, which was identified, mainly by n.m.r. spectroscopy, as 2,6-diamino-2,3,6-trideoxy-D-*ribo*-hexopyranose.[1] The configuration at C-5 and the absolute stereochemistry were assigned by analogy with the 2,6-diamino-sugars found in the kanamycins and in gentamicin A.[1] The assigned structure was thus not proved beyond doubt and required confirmation. This has now been achieved by synthesis of a suitable derivative.

Syntheses of nebrosamine can be devised on paper from either D-glucose or 2-amino-2-deoxy-D-glucose. Although the latter compound has the advantage of a 2-amino-group in the required configuration, D-glucose was chosen as the starting material since the introduction of both the 2-amino- and the 3-deoxy-function into this compound in good yield had been reported.[2,3] 2-Amino-2,3-dideoxyhexoses have been prepared from 2-amino-sugars, but yields were poor. The synthesis is shown in the Scheme.

An important intermediate in the synthesis of the nebrosamine derivative (11) was methyl 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*ribo*-hexopyranoside (7), the preparation of which has been reported by two groups.[2,3] The two routes to this compound were essentially the same, involving the preparation and subsequent reduction of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2-hydroxyimino-α-D-*erythro*-hexopyranoside (5). This particular method was developed because the only previous route to compound (7) and its C-2 epimer[4] gave low yields.

Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside (2) was prepared from the 2-mesylate (1) by the improved procedure of Creasey and Guthrie,[5] and reduced with lithium aluminium hydride as previously described[2,6] to give methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (3).

The oxidation of compound (3) with dimethyl sulphoxide, forming the ketone (4), has been reported to give yields of 70[3] and 80%.[2] When we attempted the oxidation with dimethyl sulphoxide and acetic anhydride, however, only poor yields were obtained, even after rigorous drying of the reagents as described by the Canadian group.[2] It was recently reported that when oxidation of methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside (12) was attempted with the same reagent, only poor yields of ketone[4] were obtained, and substantial amounts (30—40%) of the 2-*O*-methylthiomethyl derivative (13) were isolated. An alternative method of preparing the ketone (4) was therefore sought.

Ruthenium tetroxide readily converts secondary alcohols into the corresponding ketones and has been successfully applied to a number of carbohydrates.[7] Jones and his co-workers have reported a method whereby the ruthenium tetroxide is produced *in situ* and which requires only catalytic amounts of ruthenium dioxide dihydrate.[8] Application of this method to the alcohol

† Part XXX, R. D. Guthrie and J. A. Liebmann, preceding paper.

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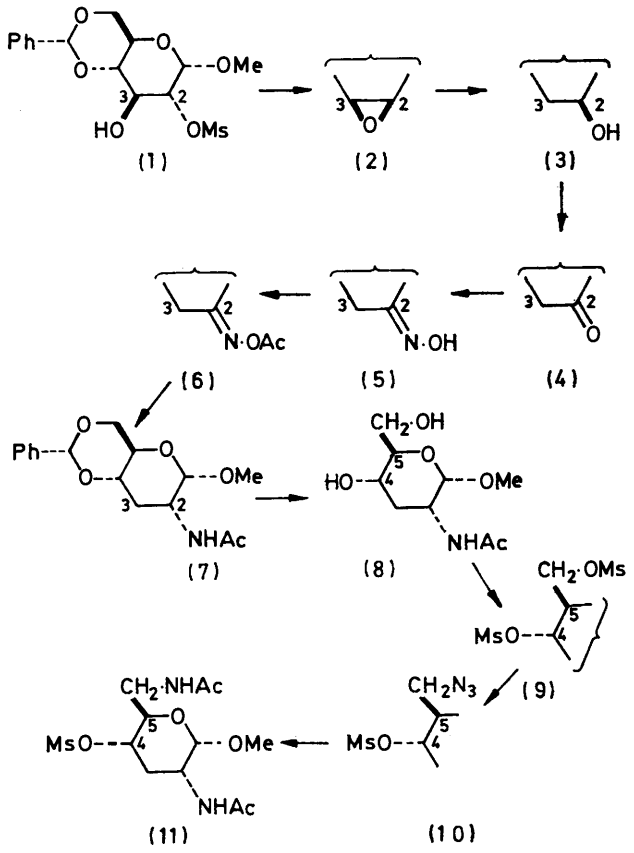
[5] S. E. Creasey and R. D. Guthrie, *Chem. Comm.*, 1971, 801.

[6] D. A. Prins, *J. Amer. Chem. Soc.*, 1948, **70**, 3955.

[7] R. F. Butterworth and S. Hannessian, *Synthesis*, 1971, 70.

[8] B. T. Lawton, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1969, **10**, 456.

(3) gave methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*erythro*-hexopyranosid-2-ulose (4) in 63% yield. Apart from the ease of carrying out this reaction, an advantage over the dimethyl sulphoxide method was the absence of evil smelling by-products which are difficult to remove.



SCHEME

Treatment of the ketone (4) with hydroxylamine hydrochloride in aqueous methanol buffered to pH 4 with sodium acetate [3] gave the crude oxime (5) in 86% yield. When recrystallisation of the oxime was attempted as described in the literature [2,3] only a gel was obtained, which was difficult to purify and dry. Acetylation of the oxime in pyridine with acetic anhydride gave methyl 2-acetoxyimino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hexopyranoside (6), which was readily recrystallised from ethanol. The n.m.r. spectrum of this compound contained two anomeric proton resonances in the ratio 1 : 1, and the methoxy-resonance was split. It was concluded that the product (6) was present as a mixture of *syn*- and *anti*-isomers (in the *syn*-isomer the C-1 substituent is shielded by the acetate group; in the *anti*-isomer it is not). This explanation is supported by the fact that methyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose oxime (14) and its acetate (15) have been shown to exist as a mixture of *syn*- and *anti*-isomers.[9] The chemical shifts for anomeric protons of the *syn*- and *anti*-isomers of the two acetoxyimino-compounds (6) and (15) (see Table) were similar (even though the solvents used were different).

Chemical shifts (τ values) for H-1

Compound	*syn*-	*anti*-	Solvent
(6)	4·30	4·83	$CDCl_3$
(14)	4·33	4·78	$(CD_3)_2SO$
(15)	4·20	4·63	C_5H_5N

Reductions of 2-hydroxyimino-glycosides to amines by lithium aluminium hydride,[2,3] by diborane,[2] and by catalytic hydrogenation [3,10,11] have been reported, and applied to the oxime (5).[2,3] Reduction of the acetoxyimino-compound (6) with lithium aluminium hydride in tetrahydrofuran followed by acetylation in pyridine gave the crude acetamido-compound (7), which on fractional crystallisation yielded pure material (38%). Attempted catalytic hydrogenation of the crude oxime (5) over Adams catalyst in acetic anhydride gave, as the only product, the acetoxyimino-derivative (6), contrary to a previous report that the acetamido-compound (7) was obtained almost exclusively, in 86% yield.[3] A variation of this method employing acetic anhydride–acetic acid (7 : 3) as solvent also gave the acetoxyimino-derivative (6); use of absolute ethanol as solvent resulted in no reaction. It is not clear why the catalytic hydrogenation was not successful; other oximes are also reported to be resistant to reduction.

Debenzylidenation of compound (7) with 60% aqueous acetic acid gave methyl 2-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranoside (8) in 97% yield. The rotation of this compound differed from that reported,[2] and microanalysis indicated that no water of crystallisation was present, in contrast to the findings of the Canadian group.[2] The n.m.r. spectrum of (8) in D_2O was, however, in agreement with that reported.[2]

Treatment of (8) with mesyl chloride in pyridine at 0° gave the dimesylate (9), which when treated with sodium azide in dimethylformamide at 80° for 16 h gave the 6-azido-derivative (10) in 77% yield. This was not further purified but immediately reduced catalytically with hydrogen over Adams catalyst in methanol; the resultant amine was acetylated with acetic anhydride in methanol. The product was methyl 2,6-diacetamido-2,3,6-trideoxy-4-*O*-mesyl-α-D-*ribo*-hexopyranoside (11); its i.r. and n.m.r. spectra agreed with the assigned structure and the compound was identical with methyl di-*N*-acetyl-*O*-mesylnebrosaminide,[12] thus confirming the structure previously assigned to nebrosamine.

EXPERIMENTAL

Optical rotations are reported for solutions in chloroform, unless otherwise stated.

Methyl 4,6-O-*Benzylidene*-3-*deoxy*-α-D-erythro-*hexopyranosid*-2-*ulose* (4).—To a solution of the 3-deoxy-sugar (3) (1·2 g) in ethanol-free chloroform (8 ml) and water (8 ml) were added potassium carbonate sesquihydrate (280 mg, 0·48 mol. equiv.), potassium periodate (2·03 g, 2 mol. equiv.)

[9] P. M. Collins, *Chem. Comm.*, 1966, 164.

[10] (*a*) R. U. Lemieux, T. L. Nagabushan, and S. W. Gunner, *Canad. J. Chem.*, 1968, **46**, 405; (*b*) R. U. Lemieux, T. L. Nagabushan, and I. K. O'Neill, *ibid.*, p. 413.

[11] R. U. Lemieux and S. W. Gunner, *Canad. J. Chem.*, 1968, **46**, 397.

[12] K. F. Koch (Eli Lilly and Co.), personal communication.

and ruthenium dioxide dihydrate (30 mg). The mixture was stirred at room temperature for 4 h. Propan-2-ol was added and the solids were filtered off. The chloroform layer was removed and the aqueous layer extracted twice with chloroform. The combined extracts were evaporated to dryness, giving a white solid. Recrystallisation from hexane gave compound (4) (68%), m.p. 112—113°, $[\alpha]_D^{24}$ +105° (*c* 1·15) {lit.,[2] m.p. 114—115°, $[\alpha]_D$ +109° (*c* 2·0); lit.,[3] m.p. 114—115°, $[\alpha]_D$ +101° (*c* 0·3); lit.,[6] m.p. 112—113°, $[\alpha]_D$ +93·8° (*c* 1·2)}.

Methyl 4,6-O-*Benzylidene*-3-*deoxy*-2-*hydroxyimino*-α-D-erythro-*hexopyranoside* (5).—To an ice-cold solution of hydroxylamine hydrochloride (6·25 g) in water (35 ml), previously adjusted to pH 4 with sodium acetate, was added slowly a cold solution of the ketone (4) (7·8 g) in 80% aqueous methanol (435 ml). The mixture was maintained at pH 4 during the addition by the further addition of sodium acetate. The mixture was then stirred at room temperature for 4 h, adjusted to pH 7 with aqueous sodium hydrogen carbonate, and kept at 0° for 16 h. The solids thus obtained were filtered off and dried to give the crude oxime (5) (7·1 g, 86%), m.p. 157—160° (lit.,[2] 138—139°; lit.,[3] 150—157°).

Methyl 2-*Acetoxyimino*-4,6-O-*benzylidene*-3-*deoxy*-α-D-erythro-*hexopyranoside* (6).—Compound (5) (255 mg) was dissolved in anhydrous pyridine (10 ml) and acetic anhydride (1·5 ml). The solution was left overnight, then poured into ice–water (150 ml) and extracted with chloroform (2 × 25 ml). The combined extracts were evaporated to dryness to give the crude product (294 mg, 97%). Recrystallisation from ethanol gave *compound* (6) as a mixture of *syn*- and *anti*-isomers, m.p. 107—110°, $[\alpha]_D$ +65·1° (*c* 0·57); τ (100 MHz) 2·40—3·00 (5H, m, aromatic), 4·38 (0·5H, s, H-1, *syn*), 4·50 (1H, s, PhCH), 4·92 (0·5H, s, H-1, *anti*), 5·78—6·60 (4H, m, H-4,5,6,6′), 6·66 (3H, 2 × s, OCH_3), 6·80—7·78 (2H, m, H-3*ax*,3*eq*), and 7·92 (3H, s, OAc), (Found: C, 59·9; H, 6·2; N, 4·4. $C_{16}H_{19}NO_6$ requires C, 59·8; H, 6·0; N, 4·4%).

Methyl 2-*Acetamido*-4,6-O-*benzylidene*-2,3-*dideoxy*-α-D-ribo-*hexopyranoside* (7).—Lithium aluminium hydride (3·2 g) was added in portions during 10 min to a solution of compound (6) (3·2 g) in anhydrous tetrahydrofuran (350 ml) and the mixture was refluxed for 4 h. The excess of reagent was destroyed with water and the mixture was filtered through Celite 545. The solids were washed with chloroform (2 × 200 ml) and the combined filtrates were evaporated to dryness to give a syrup (3·0 g). The syrup was dissolved in anhydrous pyridine (10 ml) and acetic anhydride (15 ml) was added. Next day the mixture was poured into ice–water (400 ml). The crude product was filtered off, washed with water. and dried. Recrystallisation from ethyl acetate–ether gave compound (7) (1·2 g, 38%), m.p. 245°(subl.), $[\alpha]_D$ +55·5° (*c* 0·95) {lit.,[2] m.p. 263—264°, $[\alpha]_D$ +52° (*c* 1·0); lit.,[3] m.p. 224°(subl.), $[\alpha]_D$ +53·7° (*c* 1·0)}; τ (60 MHz) 2·38—2·83 (5H, m, aromatic), 4·10—4·40 (1H, d, NH, $J_{2,N}$ 9 Hz), 4·48 (1H, s, PhCH), 5·42 (1H, d, H-1, $J_{1,2}$ 3·5 Hz), 5·53—6·51 (5H, m, ring protons), 6·59 (3H, s, OCH_3), 7·73—8·24 (2H, m, H-3-*eq* and -*ax*), and 8·03 (3H, s, NAc).

Reaction of Methyl 4,6-O-*Benzylidene*-3-*deoxy*-2-*hydroxyimino*-α-D-erythro-*hexopyranoside* (5) *with Hydrogen and a Catalyst*.—(*a*) A solution of the crude oxime (5) (400 mg) in acetic anhydride (20 ml) containing Adams catalyst (40 mg) was shaken with hydrogen at room temperature. After 5 h t.l.c. (97% chloroform–methanol) showed the absence of starting material and a new spot at higher R_F value. Anhydrous pyridine (20 ml) was added and the mixture was left overnight. The catalyst was filtered off and the filtrate poured into water and extracted with ethyl acetate. The combined extracts were evaporated to dryness to give solid methyl 2-acetoxyimino-4,6-*O*-benzylidene-3-deoxy-α-D-*erythro*-hexopyranoside (6) (354 mg, 77%) as the only product.

(*b*) Experiment (*a*) was repeated, with a mixture of acetic anhydride (14 ml) and acetic acid (6 ml) instead of acetic anhydride alone. Compound (6) was again the only product isolated.

(*c*) Experiment (*a*) was repeated with absolute ethanol (25 ml) instead of acetic anhydride as solvent. After 5 h only starting material was obtained.

(*d*) A solution of the oxime (500 mg) in absolute ethanol (25 ml) and hydrazine hydrate (0·5 ml) was hydrogenated over 10% palladium–charcoal (100 mg). After 6 h only starting material was observed by t.l.c. (97% chloroform–methanol).

Methyl 2-*Acetamido*-2,3-*dideoxy*-α-D-ribo-*hexopyranoside* (8).—Compound (7) (1·15 g) was heated with 60% aqueous acetic acid (15 ml) on a boiling water-bath for 1 h. The solution was cooled and evaporated to dryness to give a white solid (750 mg, 97%), which was recrystallised from ethanol–ether to give *compound* (8), m.p. 208—210°, $[\alpha]_D^{24}$ +139°, (*c* 1·0 in H_2O), $[\alpha]_D^{23}$ +153°, (*c* 0·28 in abs. EtOH) {lit.,[2] m.p. 204—206°, $[\alpha]_D$ +80° (*c* 1·0 in H_2O)}; τ (100 MHz; D_2O) 6·60 (3H, s, OCH_3), 7·87 (1H, m, H-3*eq*, partially obscured by NAc peak), 8·02 (3H, s, NAc), and 8·30 (1H, q, H-3*ax*, total width 35 Hz) (Found: C, 49·3; H, 8·2; N, 6·3. $C_9H_{17}NO_5$ requires C, 49·3; H, 7·8; N, 6·4%).

Methyl 2-*Acetamido*-2,3-*dideoxy*-4,6-*di*-O-*mesyl*-α-D-ribo-*hexopyranoside* (9).—A solution of compound (8) (360 mg) in anhydrous pyridine (5 ml) was cooled in ice–salt. Mesyl chloride (0·3 ml, 0·25 mol. equiv.) was added, and the mixture was kept at 0° for 1 h and then at room temperature for 3 h, diluted with ice–cold water, and evaporated to dryness. The yellow residue was extracted with chloroform and the extracts were evaporated to dryness to give a syrup. The syrup was dissolved in ethanol; on cooling crystals of the *dimesylate* (9) (380 mg, 49%) were obtained, m.p. 159—161°, $[\alpha]_D$ +91·5° (*c* 2·0); τ (100 MHz) 4·38 (1H, d, NH, $J_{2,N}$ 9 Hz), 5·40 (1H, d, H-1, $J_{1,2}$ 3·5 Hz), 5·47—5·97 (3H, m, H-2,4,5), 6·00—6·30 (2H, m, H-6,6′), 6·59 (3H, s, OCH_3), 6·94 (6H, s, $MeSO_2$·O), 7·52 (1H, m, H-3*eq*), 8·03 (3H, s, NAc), and 8·32 (1H, m, H-3*ax*, partially obscured by NAc peak) (Found: C, 35·2; H, 5·9; N, 3·6. $C_{11}H_{21}NO_9S_2$ requires C, 35·2; H, 5·6; N, 3·7%).

Methyl 2,6-*Diacetamido*-2,3,6-*trideoxy*-4-O-*mexyl*-α-D-ribo-*hexopyranoside* (11).—A solution of the dimesylate (9) (380 mg) in anhydrous dimethylformamide (10 ml) containing sodium azide (140 mg, 1·5 mol. equiv.) was stirred at 80° for 16 h, cooled, filtered, and evaporated to dryness. The residue was twice extracted with hot chloroform and the combined extracts were evaporated to dryness to give methyl 2-acetamido-6-azido-2,3,6-trideoxy-4-*O*-mesyl-α-D-*ribo*-hexopyranoside (10) (250 mg, 77%), $\nu_{max.}$ 3280, 2090, 1645, 1545, 1375, and 1180 cm^{-1}.

A solution of compound (10) (240 mg) in methanol containing Adams catalyst (10 mg) was shaken for 4 h under hydrogen. The solution was filtered, acetic anhydride (1 ml) was added, and the mixture was left at room temperature overnight. The solution was evaporated to dryness

to give a white solid, which was recrystallised from ethanol to give *compound* (11) (112 mg, 45%), m.p. 164—165·5°, $[\alpha]_D$ +118° (*c* 0·65 in abs. EtOH); τ (60 MHz; C_5D_5N–D_2O) 5·00—5·76 (4H, m, ring protons), 6·63 (3H, s, OCH_3), 6·74 (3H, s, $MeSO_2$·O), 7·05—7·78 (2H, m, H-3*ax*,3*eq*), 7·88 (3H, s, NAc), and 7·97 (3H, s, NAc) (Found: C, 39·4; H, 6·15; N, 7·2. $C_{12}H_{22}N_2O_7S$ requires C, 38·9; H, 5·95; N, 7·6%). This compound was shown [12] to be identical with methyl di-*N*-acetyl-*O*-mesylnebrosaminide.

We thank the S.R.C. for a studentship (to C. L. B.).

[3/1619 *Received*, 30*th July*, 1973]