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Note

Synthesis of methyl 4-amino-4-deoxy-β-L-arabinopyranoside and its deamination with nitrous acid

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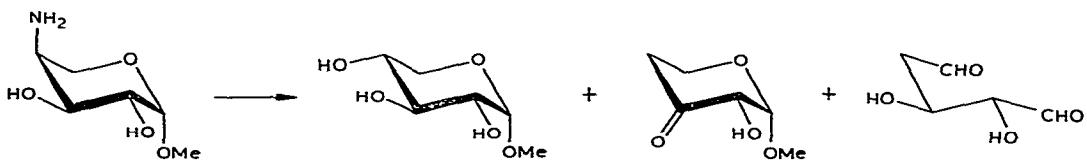
4-Amino-4-deoxy-L-arabinose has been found in Lipid A isolated from several Gram-negative bacteria[1] and in the lipopolysaccharides from some *Vibrio cholerae* strains[2]. The corresponding methyl α-pyranoside and derivatives have been prepared[3,4] by multistep synthesis. We now report a facile synthesis of methyl 4-amino-4-deoxy-β-L-arabinopyranoside (**3**) and some deamination experiments with nitrous acid.

Methyl β-L-arabinopyranoside (**1**) was oxidised with bromine in water at pH 6.5, as devised by Larm *et al.*[5]. In this procedure, the axial HO-4 of **1** is preferentially oxidised. After treatment with methoxyamine hydrochloride and chromatography on silicic acid, methyl β-L-*threo*-pentopyranosid-4-ulose (*E*)-*O*-methyloxime[6] (**2**) was isolated in ~40% yield. Catalytic hydrogenation of **2**, followed by purification of the product on Dowex-50(NH_4^+) resin eluted with an ammonia gradient, then gave **3** in ~80% yield. The ^{13}C-n.m.r. spectrum of **3**, measured in carbon dioxide-free deuterium oxide under nitrogen, showed six signals corresponding to C-1 to C-5 and one *O*-methyl group. If these precautions were not taken, multiple peaks were observed due, most probably, to partial formation of the carbamic acid derivative of **3**. Similar results have been obtained in n.m.r. studies of aminoglycoside antibiotics[7]. The configuration at C-4 of **3** was evident from the ^1H-n.m.r. spectrum of the fully acetylated derivative, which showed $J_{4,5eq} \simeq J_{4,5ax} \simeq 2$ Hz, indicating H-4 to be equatorial.

Hydrolysis of **3** with acid followed by reduction with sodium borohydride and acetylation gave only traces of products in g.l.c. analysis, in agreement with studies of the acidic hydrolysis of glycosides of other 4-amino-4-deoxy sugars[8] in which it was found that the free sugars formed polymeric pyrrole derivatives.

When a mixture of **3** and mannitol (internal standard) was treated with nitrous acid, followed, in sequence, by reduction with sodium borodeuteride, hydrolysis with acid, reduction with sodium borohydride, and acetylation, the products were shown (g.l.c. and g.l.c.–m.s.) to be the acetates of xylitol, arabinitol, and 4-deoxy-*threo*-

and *-erythro*-pentitol (both compounds labelled with deuterium at C-3) in the proportions 1:trace:3:4. The products accounted for ~40% of the starting material. If the reaction mixture was acetylated after the reduction with sodium borodeuteride and the hydrolysis step was omitted, the acetates of methyl α-D-xylopyranoside and two methyl 4-deoxypentosides were found in the approximate proportions 3:4:10; only traces of 4-deoxypentitol acetates were found in this experiment. In separate experiments, it was found that >90% of **3** had been consumed after 15 min. These results may be interpreted as follows: the pentoside (**4**) is formed by nucleophilic attack of water at C-4, while the 4-deoxypentosides are formed *via* hydride migration from C-3 (**5**) or C-5 (**6**). Analogous products were formed on deamination[9] of methyl 4-amino-4-deoxy-α-D-galactopyranoside. The origin of the traces of arabinitol acetate found in the first experiment is not clear. Migration of a hydrogen atom from C-5 should be followed by cleavage of the glycosidic linkage. As essentially no 4-deoxypentitols were formed when the hydrolysis step was omitted and since the 4-deoxypentitols obtained in the experiment with a hydrolysis operation contained no deuterium at C-1, it is concluded that hydride migration takes place almost exclusively from C-3 (see below). Compound **5** gives methyl 4-deoxy-α-D-*threo*- and *-erythro*-pentoside-*3-d* on reduction with sodium borodeuteride. The moderate, total yield of deamination products could, in part, be explained by the loss of some of the volatile products during work-up and/or the destruction of the labile uloses (*e.g.*, **5**) under the acidic reaction conditions used in the deamination.



3 4 5 6

The deamination reactions discussed above may contribute to future studies of natural products containing 4-amino-4-deoxy-L-arabinopyranosyl residues or similar sugar residues.

EXPERIMENTAL

General methods. — Concentrations were performed under reduced pressure. For chromatography, Merck silica gel was used. G.l.c. was performed with a Perkin–Elmer 990 instrument fitted with a 3% OV-225 column. For g.l.c.–m.s., a Varian MAT 311-SS 100 m.s.-computer system was used. N.m.r. spectra were recorded with a JEOL FX-100 instrument. Chemical shifts are recorded in p.p.m. downfield from internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^{1}H-n.m.r. in D_2O), internal tetramethylsilane (^{1}H-n.m.r. in $CDCl_3$), or external tetramethylsilane (^{13}C-n.m.r. in D_2O). All compounds were chromatographically pure (t.l.c.), and their ^{1}H-n.m.r. spectra were in accordance with the proposed structures.

*Methyl β-*L*-threo-pentopyranosid-4-ulose* (E)-O-*methyloxime* (**2**). — Methyl β-L-arabinopyranoside (11 g) in water (0.3 L) was added to a solution of bromine (28 g) in water (1.1 L). The mixture was stirred at 30–35°, and the pH value was kept at 6.5 by titration with M sodium hydroxide. When most of the bromine had been consumed (~1.5 h), the pH was adjusted to 5.0 with 4M hydrochloric acid, and the solution was concentrated to 250 mL. Methoxyamine hydrochloride (20 g) was added, the pH was adjusted to 4.5 with 4M sodium hydroxide, and the solution was kept at 50° while the pH value was maintained at 4.5 by titration with M sodium hydroxide. After 2.5 h, the solution was neutralized and concentrated to dryness. The residue was extracted with boiling ethanol, to give crude **2**. After chromatography on a column (5 × 30 cm) of silica gel with chloroform–methanol (9:1), compound **2** was obtained as a chromatographically homogeneous syrup (5.1 g, 40%), $[\alpha]_D^{21}$ +142° (*c* 1.0, water). ^{13}C-N.m.r. data (D_2O): 55.5 (C-5), 56.9 (OCH_3), 63.0 (N-OCH_3), 70.0 (C-3), 74.1 (C-2), 100.7 (C-1), and 156.2 (C-4). These data are in good agreement with those reported[6] for a solution of **2** in Me_2SO.

*Methyl 4-amino-4-deoxy-β-*L*-arabinopyranoside* (**3**). — Compound **2** (268 mg) was dissolved in ethanol (50 mL), and triethylamine (3 mL) and 10% palladium-on-charcoal (0.2 g) were added. The mixture was stirred under hydrogen overnight, filtered, and concentrated to dryness. The residue was purified on a column (3 × 40 cm) of Dowex-50(NH_4^+) resin eluted with an ammonia gradient (0→2.5%) (800 mL), to give **3** as a chromatographically homogeneous syrup (173 mg, 76%), $[\alpha]_D^{22}$ +185° (*c* 0.4, water). ^{1}H-N.m.r. data (D_2O, 85°), *inter alia*: δ 4.73 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 3.38 (s, 3 H, OMe), and 3.16 (unresolved m, 1 H, H-4). ^{13}C-N.m.r. data (D_2O): 51.8 (C-4), 56.5 (OMe), 63.0 (C-5), 69.1 (C-2/3), 69.8 (C-3/2), and 101.0 (C-1).

The acetate of **3** was obtained by treatment with acetic anhydride–pyridine. ^{1}H-N.m.r. data ($CDCl_3$), *inter alia*: δ 1.92, 1.96, 2.01 (s, 3 × 3 H, 3 Ac), 3.29 (s, 3 H, OMe), 3.41 (dd, 1 H, H-5ax), 3.91 (dd, 1 H, H-5eq); $J_{5ax,5eq}$ −12, $J_{4,5ax} \simeq J_{4,5eq} \simeq 2$ Hz. In g.l.c., the acetate showed T_{Man} (retention time relative to mannitol hexa-acetate) 0.71 (OV-225 at 190°), and the mass spectrum[10] showed, *inter alia*, ions (relative intensities in parentheses) at *m/e* 43 (100), 156 (3), 170 (30), 193 (2), and 258 (2).

Deamination of **3**. — Compound **3** (20 mg) and mannitol (10 mg) were dissolved in 33% acetic acid (2 mL), and 5% aqueous sodium nitrite (2 mL) was added. After 15 min at room temperature, aqueous ammonia (2.5%, 0.1 mL) was added and the solution was concentrated to dryness. The residue was dissolved in water (2 mL) and treated with sodium borodeuteride (50 mg). After work-up, half of the reduced material was acetylated with acetic anhydride–pyridine and the product analysed by g.l.c. on an OV-225 column at 160°. The acetates of methyl α-D-xylopyranoside and two methyl 4-deoxypentosides showing T_{Xyl} (retention time relative to xylitol penta-acetate) 0.19, 0.063, and 0.052 were obtained in the proportions 3:10:4. The identity of the compounds was deduced from their mass spectra[10]. The other half of the reduced material was subjected to hydrolysis with 0.25M trifluoroacetic acid (100°, 18 h), reduction with sodium borohydride, and acetylation with acetic anhydride

and pyridine. G.l.c.[11] and m.s.[12] on an OV-225 column at 190° revealed the acetates of xylitol, arabinitol, and 4-deoxy-*threo*- and -*erythro*-pentitol (both compounds labelled with deuterium at C-3) (T_{Man} 0.43, 0.35, 0.22, and 0.19, respectively) in the ratios 1 : trace : 3 : 4.

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