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Note

The anomeric configurations of the two ammonium (methyl 3-deoxy-D-*manno*-2-octulopyranosid)onate salts (methyl α- and β-ketopyranosides of KDO)

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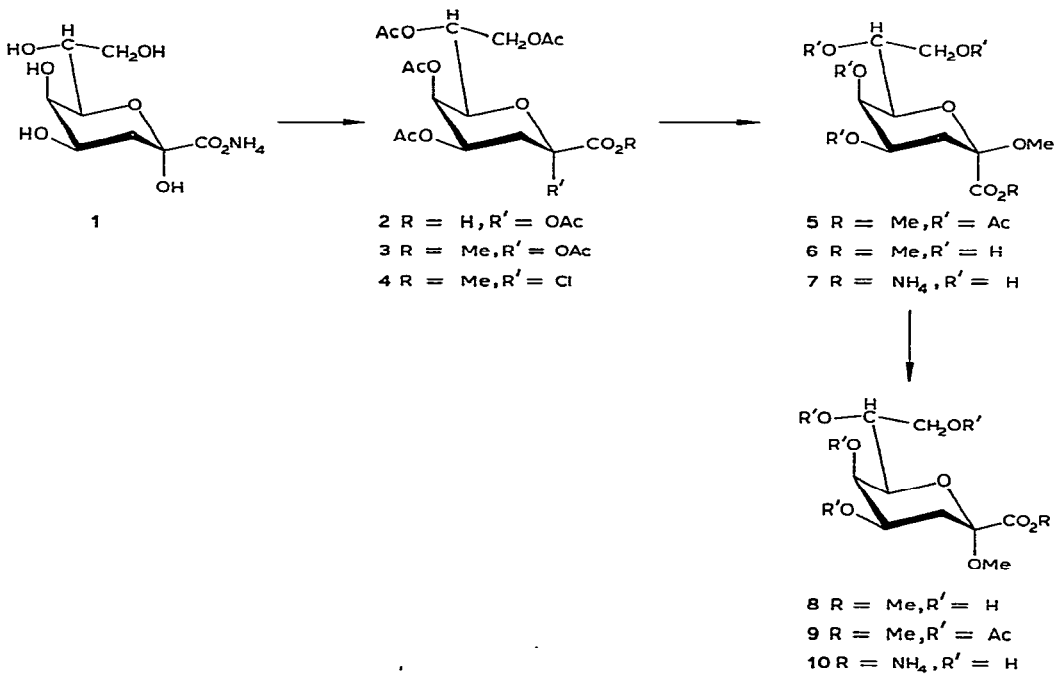
3-Deoxy-D-*manno*-octulosonic acid (KDO) is a constituent of the "core" segment of the Gram-negative bacterial lipopolysaccharide[1] and is also contained in exopolysaccharides from *Escherichia coli*[2] and *Neisseria*[3] species. Recently, Bhattacharjee *et al.*[4] presented evidence for the structure and conformation of KDO residues in the exopolysaccharide from *Neisseria meningitidis* serogroup 29e. These authors synthesized the methyl α- and β-ketopyranosides of KDO (as the sodium salts corresponding to **10** and **7**, respectively) essentially by the procedure that Kuhn *et al.*[5] reported for the synthesis of the analogous *N*-acetylneuraminic acid keto-



pyranosides. Assigning the anomeric configurations of **10** and **7** by direct comparison of the ^{13}C-n.m.r. spectra with those of the analogous *N*-acetylneuraminic acid keto-pyranosides, Bhattacharjee *et al.*[4] concluded that the KDO residues in the *Neisseria* polysaccharide are present as 2,6-pyranosidonates in the $^{1}C_{4}$(D) conformation, and have the β-D configuration[4].

Whereas the assignments of Bhattacharjee *et al.*[4] are primarily based on comparisons of the ^{13}C-n.m.r. chemical shifts of KDO derivatives with those of *N*-acetylneuraminic acid derivatives, we now report direct evidence for the structures, anomeric configurations, and conformations of **10** and **7**.

RESULTS AND DISCUSSION

Compounds **10** and **7** were prepared by use of procedures analogous to those of Kuhn *et al.*[5], and similar to the work of Bhattacharjee *et al.*[4]. The proton-coupled ^{13}C-n.m.r. spectrum of the crystalline ammonium salt of KDO (**1**) contained the signal for C-1 as a broadened singlet ($^{3}J_{C\text{-}1,CCH\text{-}3a}$ <2 Hz) indicating[6] a gauche (axial–equatorial) relationship between H-3a and the anomeric carboxylate C-1. Thus, **1** in aqueous solution is present preponderantly as the α-D anomer, in agreement with the conclusions that Bhattacharjee *et al.*[4] had drawn from indirect evidence. The proton n.m.r. spectrum of the per-*O*-acetyl free acid **2** was amenable to first-order analysis and is compatible with a pyranosonate structure for **2**. The axial orientation of H-4 and H-6 was deduced from the large couplings with H-3a and H-7, respectively, and the 2,6-pyranosonate ring structure from the chemical shift of H-6. The equatorial orientation of H-5 was indicated by the small couplings to its axial neighbors, H-4 and H-6. The configuration at the anomeric center of **2** could not be deduced from the ^{1}H-n.m.r. spectrum, but it is reasonable to assume that, under the acetylation conditions, it remained the same as that demonstrated for **1**, namely α-D. Moreover, in **2**, the chemical shift of H-3e is very close to that of H-3a, as in the methyl α-D-ketoside (*cf.* **10**)[6].

The n.m.r. spectrum of the per-*O*-acetyl methyl ester[7] (**3**) is similar[8] to that of **2**. The halide **4** was prepared as described by Kuhn *et al.*[5] and converted into the methyl β-D-ketoside[5] **5**. First-order analysis of the spectrum of **5** revealed many similarities to the spectra of **2** and **3**. However, the signal for H-4 occurs at significantly higher field (by 0.4–0.7 p.p.m.) than in the α-D-ketoside derivatives. The signals for H-3a and H-3e are further separated than those of **2**, **3**, or **9**. This greater difference in chemical shifts between H-3a and H-3e is characteristic for the β-D-ketosides of KDO[8]. A similar effect has been previously reported for the analogous derivative of *N*-acetylneuraminic acid[6]. Alkaline methanolysis of **5** gave the ester **6**, which was hydrolyzed into the ammonium salt **7**. In the proton-coupled ^{13}C-n.m.r. spectrum of **7** (in D_2O; *cf.* Fig. 1), $^{3}J_{C\text{-}1,CCH\text{-}3a}$ ~4 Hz indicates a *trans*-diaxial relationship between C-1 and H-3a. This heteronuclear coupling constant definitively establishes the β-D anomeric configuration for **5**, **6**, and **7**. Methanolysis of **6** gave the syrupy methyl ester **8**, which was acetylated into crystalline **9**. The ^{1}H-n.m.r. spectrum of **9** is

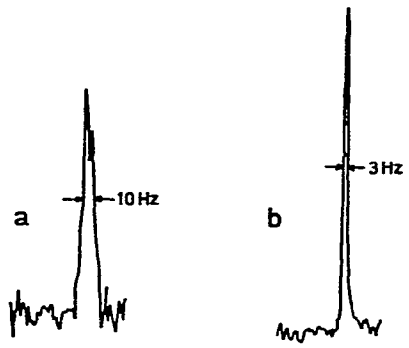


Fig. 1. Proton-coupled ^{13}C-n.m.r. signals due to the carboxylate carbon atoms (C-1) of **7** and **10**. (a) The spectrum of **7** was recorded at a sweep-width of 5500 Hz, resulting, after Fourier transformation, in a digital resolution of 1.35 Hz. The signal for C-1 (at 173.6 p.p.m. from that of tetramethylsilane) shows a spacing of ~4 Hz, with a 10-Hz peak-width at half height. (b) The spectrum of **10** was recorded at a sweep-width of 3500 Hz, resulting in a digital resolution of 0.85 Hz. A sharp signal at 175.1 p.p.m. from that of tetramethylsilane with a 3 Hz peak-width at half height is shown.

similar to the spectra of **2** and **3**. The signals for H-3a and H-3e are barely separated (by 0.04 p.p.m.), which is characteristic for the α-D-ketoside derivatives of KDO[6,8]. Alkaline hydrolysis of **8** gave the amorphous ammonium salt **10**, whose ^{1}H-n.m.r. spectrum (in D_2O) is similar to that of **8**, showing discernible signals at δ 1.79 (dd, 1 H, $J_{3a,3e}$ ~15 Hz, $J_{3a,4}$ ~14 Hz, H-3a) and at δ 2.06 (dd, 1 H, $J_{3e,3a}$ ~15 Hz, $J_{3e,4}$ ~6 Hz, H-3e). In the proton-coupled ^{13}C-n.m.r. spectrum of **10** (in D_2O), the signal for C-1 appears as a sharp singlet, $^3J_{C\text{-}1,CCH\text{-}3a} < 1$ Hz, indicating a gauche (equatorial–axial) relationship between C-1 and H-3a (*cf.* Fig. 1). Compound **10** (as well as **8** and **9**) is thus the α-D anomer[9], as previously suggested by Bhattacharjee *et al.*[4].

EXPERIMENTAL

General methods. — ^{13}C-N.m.r. spectra were recorded on a Bruker WH 90 instrument, at a resonance frequency of 22.63 MHz, using an 8K memory and a pulse time of 2 μsec (90° pulse, 5.8 μsec). To retain the nuclear Overhauser effect, the protons were subjected to broad-band decoupling for 5 sec between acquisition time and the following pulse.

*Ammonium 3-deoxy-α-*D-manno-*2-octulopyranosonate* (**1**). — The crystalline ammonium salt of KDO was obtained in 23–28% yields according to Hershberger *et al.*[10] (lit.[4] yield 6–8%), m.p. 122–125°, $[\alpha]_D^{20}$ +39° (*c* 2, water; equil.); lit.[10] m.p. 121–123°, $[\alpha]_D^{27}$ +42.3° (*c* 1.7, water).

*4,5,7,8-Tetra-*O*-acetyl-3-deoxy-α-*D-manno-*2-octulopyranosonic acid* (**2**). — Acetylation of **1** with acetic anhydride–pyridine, in the presence of 4-dimethylaminopyridine[9], gave a quantitative yield of the crystalline carboxylic acid **2**, m.p. 155–160°, $[\alpha]_D^{20}$ +114° (*c* 0.84, chloroform); ^{1}H-n.m.r. (benzene-d_6): δ 2.38 (dd, 1 H, $J_{H\text{-}3a,3e}$ ~13.5 Hz, $J_{3a,4}$ ~13 Hz, H-3a), 2.46 (dd, 1 H, $J_{3e,3a}$ ~13.5 Hz, $J_{3e,4}$ ~4 Hz, H-3e),

4.08 (dd, 1 H, $J_{6,7}$ ~9.7 Hz, $J_{6,5}$ ~1.5 Hz, H-6), 4.20 (dd, 1 H, $J_{8',8}$ ~12.5 Hz, $J_{8',7}$ ~4.5 Hz, H-8'), 4.55 (dd, 1 H, $J_{8,8'}$ ~12.5 Hz, $J_{8,7}$ ~2.5 Hz, H-8), 5.42 (ddd, 1 H, $J_{7,6}$ ~9.7 Hz, $J_{7,8}$ ~2.5 Hz, $J_{7,8'}$ ~4.5 Hz, H-7), 5.44 (ddd, 1 H, $J_{4,3a}$ ~13 Hz, $J_{4,3e}$ ~4 Hz, $J_{4,5}$ ~3 Hz, H-4), and 5.62 (dd, 1 H, $J_{5,4}$ ~3 Hz, $J_{5,6}$ ~1.5 Hz, H-5); lit.[10] m.p. 98–103°.

Anal. Calc. for $C_{18}H_{24}O_{13}$: C, 48.2; H, 5.4. Found: C, 48.2; H, 5.4.

Methyl 4,5,7,8-tetra-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosonate* (**3**). — Treatment of **2** with diazomethane gave the previously known[7] ester **3**, in quantitative yield, so that the overall yield of **3** from oxaloacetate was 23–28% by our procedure; lit. yields, 2.2–3.5% (ref. 4) and 0.8% (ref. 7); m.p. 155–158° (from ethanol), $[\alpha]_D^{20}$ +87.1° (*c* 0.81, chloroform); lit.[7] m.p. 155–156°, $[\alpha]_D^{20}$ +109.7 ±0.5° (*c* 1.387, methanol).

Methyl (*methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosid*)-*onate* (**5**). — Conversion of **3** to the halide (**4**) was carried out in quantitative yield, by use of the method of Kuhn *et al.*[5] as previously described by Bhattacharjee *et al.*[4]; $[\alpha]_D^{20}$ +112° (*c* 2.24, dichloromethane); lit.[4] (after chromatography) $[\alpha]_D^{20}$ +138° (*c* 2.8, chloroform). Treatment of **4**, according to Kuhn *et al.*[5] gave **5**, as described by Bhattacharjee *et al.*[4]; $[\alpha]_D^{20}$ +67.8° (*c* 1.08, chloroform); lit.[4] $[\alpha]_D^{20}$ +59° (*c* 5.0, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 2.10 (dd, 1 H, $J_{3a,3e}$ ~12.5 Hz, $J_{3a,4}$ ~12.5 Hz, H-3a), 2.36 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, $J_{3e,4}$ ~5.5 Hz), 4.19 (dd, 1 H, $J_{6,7}$ ~10 Hz, $J_{6,5}$ ~1 Hz, H-6), 4.38 (narrow d, 2 H, H-8, -8'), 4.89 (ddd, 1 H, $J_{4,3a}$ ~12.5 Hz, $J_{4,3e}$ ~5.5 Hz, $J_{4,5}$ ~2 Hz, H-4), 5.17 (m, 1 H, H-7), and 5.26 (dd, 1 H, $J_{5,6}$ ~1 Hz, $J_{5,4}$ ~2 Hz, H-5).

Ammonium (*methyl 3-deoxy-β*-D-manno-*2-octulopyranosid*)*onate* (**7**). — Zemplén saponification of **5** gave the syrupy ester **6** (~100%), $[\alpha]_D^{20}$ +60.4° (*c* 0.99, water), which was saponified with sodium hydroxide, treated with Dowex 50 (H^+) ion-exchange resin, neutralized with aqueous ammonia, and chromatographed on Sephadex G-10 to give an 86% yield of **7**, m.p. 143–145° (from water–ethanol), $[\alpha]_D^{20}$ +47° (*c* 0.5, water); lit.[4] (sodium salt) $[\alpha]_D^{20}$ +47° (*c* 2.0, water). The ^{1}H- and ^{13}C-n.m.r. data[8] are in agreement with those of the literature[4].

Methyl (*methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosid*)-*onate* (**9**). — Treatment of **6** with dry Dowex 50 (H^+) ion-exchange resin or dry hydrogen chloride in methanol[4] gave, after acetylation[9] of the intermediate, crude syrupy **8**, a 91% yield of **9**, m.p. 110° (from ether–petroleum ether), $[\alpha]_D^{20}$ +76.8° (*c* 0.62, chloroform); ^{1}H-n.m.r. (benzene-d_6): δ 2.25 (dd, 1 H, $J_{3a,3e}$ ~12.8 Hz, $J_{3a,4}$ ~12.5 Hz, H-3a), 2.29 (dd, 1 H, $J_{3e,3a}$ ~12.8 Hz, $J_{3e,4}$ ~5 Hz, H-3e), 3.89 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, $J_{6,7}$ ~9.7 Hz, H-6), 4.14 (dd, 1 H, $J_{8',8}$ ~12.5 Hz, $J_{8',7}$ ~5 Hz, H-8'), 4.67 (dd, 1 H, $J_{8,8'}$ ~12.5 Hz, $J_{8,7}$ ~2.5 Hz, H-8), 5.52 (ddd, 1 H, $J_{7,6}$ ~9.7 Hz, $J_{7,8}$ ~2.5 Hz, $J_{7,8'}$ ~5 Hz, H-7), 5.56 (ddd, 1 H, $J_{4,3a}$ ~12.5 Hz, $J_{4,3e}$ ~5 Hz, $J_{4,5}$ ~3 Hz, H-4), and 5.62 (dd, 1 H, $J_{5,4}$ ~3 Hz, $J_{5,6}$ ~1.5 Hz, H-5).

Anal. Calc. for $C_{18}H_{26}O_{12}$: C, 49.8; H, 6.0. Found: C, 49.5; H, 6.1.

Zemplén saponification of **9** gave pure **8** (100%) as a colorless syrup, $[\alpha]_D^{20}$ +80° (*c* 3, water).

Ammonium (methyl 3-deoxy-α-D-manno-2-octulopyranosid)onate (**10**). — Alkaline saponification of **8**, followed by Sephadex G-10 chromatography, as described for **6**, gave a quantitative yield of **10** as a colorless glass, $[\alpha]_D^{20}$ +80° (*c* 1.38, water); lit.[4] (sodium salt) $[\alpha]_D^{20}$ +79° (*c* 0.5, water).

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