

Note

X-Ray structure of methyl (methyl 4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-2-octulopyranosid)onate, an α -D-glycoside of 3-deoxy-D-manno-2-octulosonic acid (KDO)

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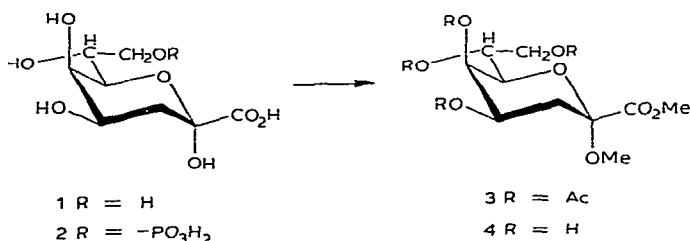
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3-Deoxy-D-manno-2-octulosonic acid (KDO, **1**) occurs as a constituent of all Gram-negative, bacterial lipopolysaccharides investigated so far¹. The first evidence regarding its structure was reported by Levin and Racker² who demonstrated the enzyme-catalyzed formation, from D-arabinose 5-phosphate and phosphoenol pyruvate, of a compound later identified as 3-deoxy-D-manno-2-octulosonic acid



8-dihydrogenphosphate (KDO 8-phosphate, **2**). These authors assumed that the configuration of the three, chiral carbon-atoms of D-arabinose 5-phosphate (C-2–C-4) was retained in **2** (corresponding to C-5–C-7). Levin and Racker² did not attempt to assign the configuration at C-4 of **2**. Heath and Ghalambor³ demonstrated, for the first time, that KDO is both the product of dephosphorylation of **2**, and a constituent of the lipopolysaccharide from *Escherichia coli*. They tentatively assigned the D-manno configuration to **1**, because the rate of periodate consumption suggested that OH-4 and -5 were in *cis* position. A similar argument had previously been used by Preiss and Ashwell⁴ to distinguish between 3-deoxy-D-erythro-2-hexulosonic and

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3-deoxy-D-*threo*-2-hexulosonic acids. Ghalambor and Heath⁵ confirmed the D-*arabino* configuration at C-5-C-7 of **1** by demonstrating the enzymic cleavage of **1** (to give D-arabinose and pyruvic acid) by a KDO aldolase from *Aerobacter cloacae*. Ghalambor *et al.*⁶ degraded the product of reduction of **1** by borohydride (a mixture of 3-deoxy-D-*glycero*-D-*talo*- and -D-*galacto*-octonic acids) with ceric sulfate⁷, to give a 2-deoxyheptose that Perry⁸ synthesized from D-mannose, thus demonstrating that it (and, indeed, **1**) has the D-*manno* configuration⁹. Williams and Perry¹⁰ utilized 2-deoxy-D-*manno*-heptose for a synthesis of **1**, and a similar synthesis was reported shortly thereafter by Charon *et al.*¹¹.

Whereas the primary structure and configuration of **1** is thus well established, the anomeric configuration of its methyl ketosides was investigated only recently, by comparing the ¹³C-n.m.r. spectra with those of analogous derivatives of N-acetylneuraminic acid¹². Conclusive evidence was obtained from the ¹H-¹³C heteronuclear coupling-constants, in spin-coupled ¹³C-n.m.r. spectra, of the appropriate methyl glycoside carboxylic acid salts¹³. The ultimate proof confirming the KDO structure by X-ray crystallography has so far been lacking and, in the present communication, we report the X-ray structure of methyl (methyl 4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-*manno*-2-octulopyranosid)onate (**3**), an α -D-glycoside of **1**.

EXPERIMENTAL

Compound **3** was prepared¹³ by standard acetylation of the methyl glycoside methyl ester **4**, which had been obtained¹³ by acid-catalyzed anomerization of the methyl β -D-glycoside, similarly to the procedure described by Bhattacharjee *et al.*¹². Crystallization from an ether solution by slow addition of petroleum ether yielded two crystalline forms. The bulk of the material crystallized as tetragonal platelets [space group P4₁ 222, *a* 10.17 Å and *c* 43.01 Å, and *D_x* (for *Z* 8) 1.30] which were far too small for a complete crystal structure analysis. After several weeks, however, a single, 1-mm long, well-formed crystal appeared in the filtered-off mother liquor. All crystallographic investigations described in the following paragraphs were performed with a 0.25 × 0.25 × 0.25-mm fragment of this crystal. For crystallographic data, see Table I.

TABLE I

CRYSTALLOGRAPHIC DATA

Crystal system	Tetragonal lattice, Laue group 4/m; 0, 0, and 1 only present for 1 = 4n
Space group	P4 ₁ (No. 76)
<i>Z</i>	4
Cell constants from diffractometer data	<i>a</i> 12.998(3) Å <i>b</i> 13.035(3) Å
<i>D_M</i> (floatation in a KI solution)	1.308
<i>D_x</i>	1.315

Intensities were measured with an automated 4-circle diffractometer (Stoe STADI 4) with graphite-monochromatized MoK_α -radiation (λ 0.71069 Å) using the following conditions: $\omega/2\theta$ -scan; scan width (2 θ): 2° ; constant measuring time for all reflections: 50 s for the integration of the intensity, 2×12.5 s for the backgrounds; periodical (every 50 reflections) re-measurement of two standard-reflections (1,0,6 and 3,6,0). All reflections with positive indices and $2\theta \leq 47^\circ$ were recorded. Averaging of symmetry-equivalent reflections yielded 1729 independent observations, 863 of which were significantly above background [$|F_o| > 3\sigma(|F_o|)$]. No absorption correction was applied [$\mu(\text{MoK}_\alpha)$ 1.201 cm^{-1}].

The structure was solved with the computer program MULTAN¹⁴ in a straightforward way: 2500 relationships between the 350 strongest E-values entered into the converge-algorithm. Forty eight sets of starting phases were subjected to the tangent

TABLE II

FRACTIONAL ATOMIC COORDINATES^a ($\times 10^4$) IN COMPOUND 3

Atoms	X	Y	Z
C-1	5988 (14)	-323 (17)	5701 (16)
O-11	5416 (17)	-39 (21)	6317 (14)
O-12	6727 (10)	-876 (13)	5921 (10)
C-12	6910 (18)	-1158 (22)	7011 (16)
C-2	5842 (12)	-26 (14)	4577 (14)
O-2	4804 (8)	-121 (10)	4268 (11)
C-21	4360 (15)	-1134 (15)	4370 (20)
C-3	6136 (12)	1072 (13)	4410 (15)
C-4	6134 (12)	1327 (12)	3273 (14)
O-4	6509 (9)	2365 (8)	3132 (13)
C-41	5887 (17)	3075 (16)	2915 (21)
O-41	4974 (15)	2913 (13)	2828 (24)
C-42	6338 (20)	4119 (15)	2795 (22)
C-5	6812 (12)	599 (12)	2698 (14)
O-5	7867 (8)	731 (9)	3046 (9)
C-51	8635 (15)	848 (13)	2349 (16)
O-51	8446 (11)	852 (11)	1417 (11)
C-52	9649 (14)	898 (15)	2782 (17)
C-6	6459 (12)	-483 (12)	2918 (14)
O-6	6484 (8)	-708 (8)	4008 (0)
C-7	7138 (13)	-1337 (13)	2442 (13)
O-7	7035 (8)	-1150 (8)	1339 (9)
C-71	7859 (14)	-1392 (15)	774 (15)
O-71	8630 (10)	-1754 (12)	1093 (11)
C-72	7626 (17)	-1152 (17)	-342 (15)
C-8	6842 (17)	-2397 (15)	2736 (18)
O-8	5767 (12)	-2527 (10)	2547 (11)
C-81	5370 (25)	-3140 (19)	1773 (21)
O-81	6038 (22)	-3599 (17)	1318 (19)
C-82	4326 (24)	-3105 (22)	1679 (26)

^aEstimated standard deviations are in parentheses.

refinement. An E-map, calculated with phases from the solution with the best figures-of-merit, contained 25 peaks in a structurally acceptable pattern. The remaining five non-hydrogen atoms appeared in a $(F_o - F_c)$ -Fourier map. At this point, a calculation of the structure factor gave a figure-of-merit of R 0.26.

After four cycles of full-matrix, least-square refinement¹⁵ with isotropic temperature factors for all atoms (R 0.135), H atoms were included at calculated positions. In view of the high temperature factors of the methyl groups, no attempt was made to observe the hydrogen atoms directly from an $(F_o - F_c)$ -Fourier synthesis. Three additional cycles of block-diagonal, least-squares refinement with anisotropic temperature factors for all non-hydrogen atoms led to convergence at R 0.072*, with H atom contributions included in F_c but not refined. A final $(F_o - F_c)$ -Fourier map showed no peaks higher than $0.4 \text{ e} \cdot \text{\AA}^{-3}$.

DISCUSSION

The atomic coordinates are given in Table II**, the atom numbering and calculated bond lengths, and calculated bond angles, in Fig. 2. Several dihedral angles are reported in Table III. Figure 3 shows a stereo-drawing of the molecule, projected into the plane of atoms C-2-O-4-C-6.

The structure of 3, as determined by X-ray crystallography, confirms the previous data¹⁻¹³ in the following regards. (a) Compound 1 (KDO) has the *D-manno* configuration. (b) The α -D-glycosides of 1 described in the literature^{12,13} have a pyranose ring-structure and a ${}^5C_2(D)$ conformation. (c) The methyl glycoside of 1 showing a heteronuclear coupling-constant ${}^3J_{C-1, CCH-3a} < 1 \text{ Hz}$ in the spin-coupled, ${}^{13}\text{C}$ -n.m.r. spectrum of its ammonium carboxylate¹³ salt is the methyl α -D-glycoside. (d) The quasi-axial orientation of H-7, and the conformation of the side chain in 3, as previously deduced from ${}^1\text{H}$ -n.m.r. data¹³, is also present in crystals of 3.

TABLE III

SOME DIHEDRAL ANGLES IN COMPOUND 3^a

Bond	Angle (degrees)
O-6-C-2-C-1-O-11	-165
O-6-C-6-C-7-C-8	53
O-6-C-2-O-2-C-21	58
C-6-C-7-C-8-O-8	52
C-3-C-4-O-4-C-41	-105
C-4-C-5-O-5-C-51	-131

^aEstimated standard deviations, 1.5° .

*Function minimized: $\sum W_i [|F_{oi}| - |F_{ci}|]^2$; $W_i = 1/\sigma(F_{oi})^2$.

**A list of temperature factors is deposited with, and can be obtained from, Elsevier Scientific Publishing Company, BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No BBA/DD/175/*Carbohydr. Res.*, 92 (1981) 299-304.

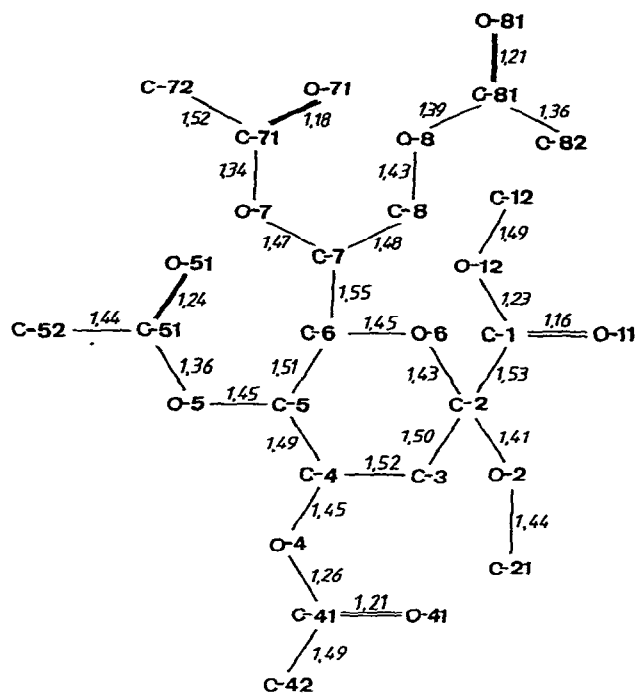


Fig. 1. Bond lengths in compound 3 (estimated standard deviations, 0.025 Å)

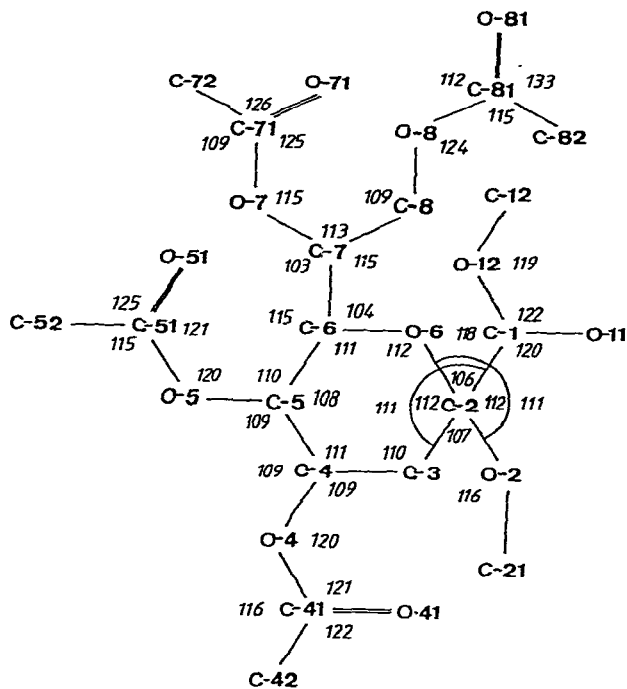
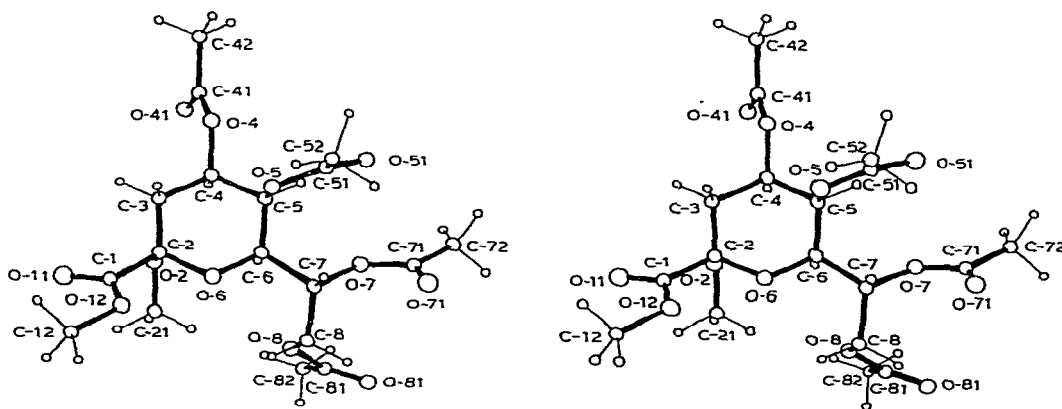


Fig. 2. Bond angles in compound 3 (estimated standard deviations, 1.5 Å).



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