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Note

Formation of methyl (2,7-anhydro-3-deoxy-α-D-*manno*-2-octulofuranos)onate upon methanolysis of 2,4,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosono-1,5-lactone

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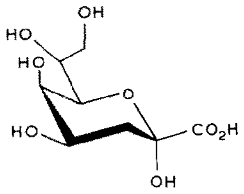
3-Deoxy-D-*manno*-2-octulosonic acid (Kdo, **1**) is an obligatory[1] constituent of the endotoxic lipopolysaccharides that are present on the surface of Gram-negative bacteria. Inasmuch as severance of the hydrophilic and hydrophobic domains of these compounds is usually achieved in acidic conditions, reactions of Kdo in acidic media are of considerable interest for structural analysis. The behaviour of unsubstituted Kdo in aqueous[2–4] or methanolic[5] acid has been described previously. The present study reports the isolation and characterization of the product formed upon acid-catalyzed methanolysis of the peracetylated 1,5-lactone[6] of Kdo, – a by-product formed upon acetylation of Kdo – and an improved synthesis of the peracetate of methyl (3-deoxy-α-D-*manno*-2-octulopyranos)onate[5,7–9] (**3**), the usual[10] starting material for syntheses of pyranose derivatives of Kdo.



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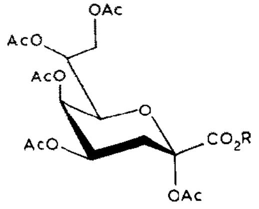

2 R = H
3 R = Me

Preparation of the peracetylated methyl ester **3** from the ammonium salt of Kdo *via* the α-peracetate has been reported repeatedly[5,7–9], but there is some discrepancy as regards the yields. According to one group[9], acetylation of ammonium Kdo affords a quantitative yield of the crystalline peracetylated acid that can be quantitatively transformed into the methyl ester by treatment with diazomethane. Others[5,7,8] did not actually crystallize the peracetate of the acid: instead, the crude material was treated with diazomethane to give a 40–50% yield of the peracetylated methyl ester **3**.

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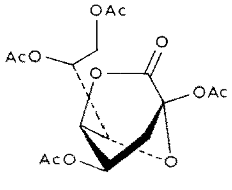
A preparative method is now described that makes use of acetyl chloride: the acetylated methyl ester is obtained crystalline by an uninterrupted sequence in an overall yield of 80%. The conditions specified in the Experimental part are critical and must be strictly followed to obtain the specified yield. Although no systematic work was carried out, it would appear that, in this case, pyridinium salts present in the crude peracetate **2** were mainly responsible for the low yields repeatedly observed for the esterification step with diazomethane. As expected[6] the only detectable by-product (3–5%, eluted after the main product) was the acetylated lactone (**4**).



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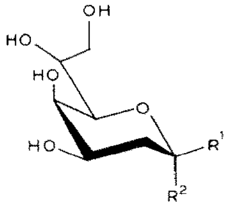
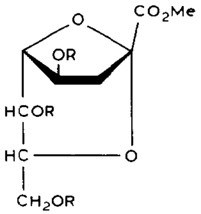


5 $R^1 = CO_2Me, R^2 = OMe$
6 $R^1 = OMe, R^2 = CO_2Me$

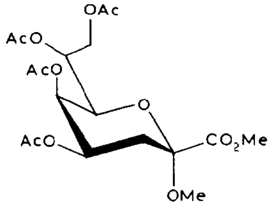
Treatment of the peracetylated methyl ester **3** with methanol–HCl leads to a mixture of the methyl glycosides **5** and **6** from which pure (n.m.r.) methyl (methyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**5**) may be isolated[11] as a syrup in 80% yield. Under the same conditions, the peracetylated lactone **4**, prepared[11] from the ammonium salt[12] of Kdo, afforded a number of products that appeared as three spots upon t.l.c. When separated by column chromatography, the one having the highest R_F value proved to be homogeneous and crystallized (yield 30%). Its elemental analysis agreed with that of an anhydro derivative of methyl 3-deoxyoctulosonate; accordingly an ester-bound Me group (δ 3.85) was identified in its ^{1}H-n.m.r. spectrum. The compound was not affected by periodate and thus had no vicinal diol system. Acetylation gave, as expected, a triacetate: the downfield chemical shifts ($\Delta\delta$ = 0.67 and 1.18) of H-4 and H-6 (identified by decoupling, starting from the easily recognized protons H-3 and H-3′) established that OH-4 and OH-6 were acetylated and, consequently, that the third acetate group was necessarily attached to OH-8. It followed that the compounds were methyl (2,7-anhydro-3-deoxy-α-D-*manno*-2-octulofuranos)onate (**7**) and its 4,6,8-triacetate (**8**). Formation of a 2,7-anhydro-derivative of Kdo upon treatment with mild acid has been reported previously[4], but the compound was apparently not isolated: the proposed structure was based on n.m.r. data and its stability to periodate.

The other products of the methanolysis were methyl furanosides and pyranosides of Kdo, not readily separated on a preparative scale. Column chromatography of the acetylated (1:1 acetic acid–pyridine, 3 h, room temperature), crude mixture of products formed upon methanolysis of the peracetylated lactone **4**, yielded methyl (methyl -4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate[9] (**9**, 19%) and an inseparable mixture consisting of the triacetate **8**, 21%), the peracetates of methyl (methyl 3-deoxy-β-D-*manno*-2-octulofuranosid)onate (**11**, 10%), and the correspond-



7 R = H

8 R = Ac



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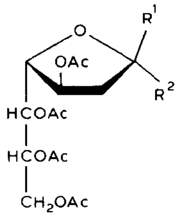

10 $R^1 = CO_2Me, R^2 = OMe$

11 $R^1 = OMe, R^2 = CO_2Me$

TABLE I

^{1}H-N.m.r. data for **10** and **11**

Chemical shifts							
δ	*H-3*	*H-3'*	*H-4*	*H-5*	*H-6 H-7*	*H-8*	*H8'*
10	2,26	2.58	5.11	4.40	5.20–5.40	4.1–4.2	4.42
11	2.17	2.52	4.96	4.34	5.20–5.40	4.1–4.2	4.42
Spin couplings							
J (Hz)	*3,3'*	*3,4*	*3',4*	*4,5*	*5,6*	*7,8'*	*8,8'*
10	14.0	6.0	8.0	5.5	3.5	2.5	13
11	14.5	2.5	8.6	4.0	2.5	2.5	12

ing *a* anomer (**10**, 6%), which were identified and quantified by ^{1}H-n.m.r spectroscopy (Table I); assignments were made by taking into account published data[13]. It is noteworthy that the bulk of product formed upon methanolysis of the acetylated lactone (**4**) consists of furanose derivatives of Kdo.

EXPERIMENTAL

General methods. — Solvents were removed in a vacuum <45°. Column dimensions are given in cm. Column chromatography was carried on with silica gel (Merck 60, 70–230 mesh), t.l.c. on Merck 60 F_{254} silica gel plates. Compounds were detected with sulphuric acid (10% in EtOH) and charring. Melting points were determined on a Kofler block and are uncorrected. N.m.r. data refer to internal Me_4Si (*δ* 0.00).

*Methyl (2,4,5,7,8-penta-*O*-acetyl-3-deoxy-a-*D-manno*-2-octulopyranos)onate* (**3**). — A mixture of AcCl (13 mL) and dry CH_2Cl_2 (20 mL) was added dropwise to a stirred solution of ammonium 3-deoxy-*a*-D-*manno*-2-octulopyranosonate (5.6 g, 20 mmol) in anhydrous pyridine (40 mL) kept at −15°. When addition was completed the stirred mixture was allowed to reach room temperature in ~3 h; stirring was maintained for another 2 h. Anhydrous CH_2Cl_2 (40 mL) and diethyl ether (100 mL) were added, solids were filtered off and washed first with 2:1 anhydrous diethyl ether–CH_2Cl_2 (60 mL), and then with diethyl ether (40 mL). The volume of the pooled filtrate and washings was completed to 400 mL with anhydrous diethyl ether and the mixture was kept overnight in a closed vessel at room temperature. The precipitate was filtered off and the filtrate evaporated. Toluene (3 × 400 mL) was evaporated from the residue which was then dissolved in 3:1 anhydrous diethyl ether–CH_2Cl_2 (400 mL). A solution of diazomethane in anhydrous ether was added to the solution until the yellow colour of the reagent persisted; solvents were then removed. Upon column (26 × 4.5) chromatography (1:1 EtOAc–cyclohexane) of the residue, the title compound (7.7 g, 83%) was eluted first (crystalline solid from diethyl ether, m.p. 157–159°; lit.[5] m.p. 155°; lit.[7] m.p. 155–156°; lit.[8] m.p. 152–153°; lit[9] m.p. 155–158°) followed by the acetylated lactone **4**, m.p. 123–125°, 350 mg, 4.5%; lit.[6] m.p. 123–125°.

*Methyl (2,7-anydro-3-deoxy-α-*D-manno-*2-octulofuranos)onate* (**7**). — A solution of the peracetylated lactone (**4**, 1.7 g, 4.38 mmol) in M methanolic HCl was kept refluxing for ~2 h. When the reaction appeared not to progress any more (monitored by t.l.c., 4:1 $CHCl_3$–MeOH, ~2 h) three spots (R_F 0.39, 0.47, and 0.56) were detected. The acid solution was neutralized (IR45, OH^-), filtered, and evaporated. Column (13 × 3.5) chromatography (EtOAc–PhMe–EtOH, 70:15:5, 90 mL; 70:15:10, 95 mL; and 70:15:15, 400 mL) gave, as the first product eluted the anhydride **7** (300 mg, 30%); m.p. 148–150° (EtOH–EtOAc); $[\alpha]_D^{20}$ +69.7° (*c* 1, MeOH); ^{1}H-n.m.r. (CD_3OD, 250 MHz): δ 2.25 (ddd, 1 H, $^2J_{3,3'}$ 14.5, $^3J_{3,4}$ 2.5, $^4J_{3,5}$ 0.7 Hz, H-3), 2.70 (dd, 1 H, $^3J_{3',4}$ 7 Hz, H-3′), 3.45 (m, $^3J_{6,7}$ 10, $^3J_{7,8}$ 6, $^3J_{7,8'}$ 2.5 Hz, H-7), 3.65 (dd, 1 H, $^2J_{8,8'}$ 12 Hz, H-8), 3.67 (dd, 1 H, $^3J_{6,5}$ 4.5 Hz, H-6), 3.78 (dd, 1 H, H-8′), 3.85 (s, 3H, CO_2CH_3), 4.27 (m, 1 H, H-5), and 4.65 (dd, 1 H, H-4).

Anal. Calc. for $C_9H_{14}O_7$: C, 46.1; H, 6.0. Found: C, 46.1; H, 6.1.

*Methyl (4,6,8-tri-*O*-acetyl-2,7-anydro-3-deoxy-α-*D-manno-*2-octulofuranos)onate* (**8**). — Acetic anhydride (250 μL) was added to a solution of the 2,7-anhydro compound **7** (17 mg, 0.07 mmol) in anhydrous pyridine (250 μL) and the mixture was kept for 24 h at room temperature. Solvents were removed, and dry toluene was evaporated twice from the residue. Column (6.2 × 2.4) chromatography (2:3 EtOAc–cyclohexane) gave the triacetate (23 mg, 90%) as a colourless oil; ^{1}H-n.m.r. ($CDCl_3$, 250 MHz): δ 2.04, 2.08, 2.10 (3 s, 3 × 3 H, 3 × CH_3CO), 2.45 (ddd, 1 H, $^2J_{3,3'}$ 15, $^3J_{3,4}$ 3, $^4J_{3,5}$ 1 Hz, H-3), 2.75 (dd, 1 H, $^3J_{3',4}$ 7.5 Hz, H-3′), 3.85 (m, 1 H, H-7), 3.85 (s, 3 H, CO_2CH_3), 4.18 (m, 2 H, H-8 and H-8′), 4.65 (d, 1 H, $^3J_{5,6}$ 4-5 Hz, H-5), 4.85 (dd, 1 H, $^3J_{7,6}$ 10 Hz, H-6), and 5.32 (dd, 1 H, H-4).

Anal. Calc. for $C_{15}H_{20}O_{10}$: C, 50.0; H, 5.5. Found: C 50.2; H, 5.4.

RERERENCES

1 F. M. Unger, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 323–388.
2 B. A. Dmitriev, L. V. Backinowsky, and N. K. Kochetkov, *Dokl. Akad. Nauk. SSSR*, 193 (1970) 1304–1307.
3 D. Charon and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, (1973) 1175–1179.
4 P. A. McNicholas, M. Batley, and J. W. Redmond, *Carbohydr. Res.*, 165 (1987) 17–22.
5 D. Charon and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, (1979) 2369–2374.

6 D. Charon, F.-I. Auzanneau, C. Mériene, and L. Szabó, *Tetrahedron Lett.*, 28 (1897) 1393–1397.
7 M. A. Ghalambor, E. M. Levine, and E. C. Heath, *J. Biol. Chem.*, 28 (1966) 3207–3215.
8 A. K. Bhattacharjee, H. J. Jennings, and C. P. Kenny, *Biochemistry*, 17 (1978) 645–651.
9 F. M. Unger, D. Stix, and G. Schulz, *Carbohydr. Res.*, 80 (1980) 191–195.
10 P. Kosma, C. Kratky, and H. Paulsen, *A. C. S. Symp. Ser.*, 231 (1983) 121–140.
11 F.-I. Auzanneau, D. Charon, L. Szabó, and C. Mérienne, *Carbohydr. Res.*, 179 (1988) 125–136.
12 C. Hershberger, M. Davis, and S. B. Binkley, *J. Biol. Chem.*, 243 (1968) 1585–1588.
13 P. A. McNicholas, M. Batley, and J. W. Redmond, *Carbohydr. Res.*, 146 (1986) 3207–3215.