Furanose Derivatives of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid

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Acylation of methyl 3-deoxy-D-*manno*-oct-2-ulosonate leads essentially to furanose derivatives, whereas acetylation of the free acid or of its ammonium salt gives pyranose derivatives. Upon treatment with HBr in acetic acid, the pentabenzoate of methyl 3-deoxy-D-*manno*-oct-2-ulofuranosonate yielded a crystalline 2-bromo-derivative which, when treated with MeOH–Ag_2CO_3 was transformed into a single methyl furanoside of undetermined anomeric configuration. Reaction of methyl 3-deoxy-D-*manno*-oct-2-ulosonate with MeOH in the presence of an acidic catalyst gives a mixture of pyranoside(s) and furanosides separable by g.l.c.; they can be identified by the characteristic fragments at *m/e* 217 for the furanosides and *m/e* 158 for the pyranoside(s).

3-DEOXYALD-2-ULOSONIC acids frequently occur in nature: 3-deoxy-pentulosonic and -hexulosonic acids are intermediates in carbohydrate metabolism in bacteria, aromatic amino acids are formed from 3-deoxy-D-*arabino*-heptulosonic acid, while 3-deoxy-D-*manno*-octulosonic acid and *N*-acetylneuraminic acid as well as the two lower homologues of the group have been identified in macromolecules present in the bacterial cell envelope. Furthermore *N*-acetylneuraminic acid is a ubiquitous component of glycoproteins. It has been assumed that in endotoxins 3-deoxy-D-*manno*-octulosonic acid possesses a pyranose structure [1] which was shown [2] by ^{13}C n.m.r. to be present in the meningococcal 29e polysaccharide.

In connection with structural studies on the endotoxin of *Bordetella pertussis* in which two differently substituted 3-deoxyoct-2-ulosonic acid units are present whose glycosidic bonds are cleaved at very different rates,[3] it became necessary to obtain well defined glycosides of 3-deoxy-D-*manno*-octulosonic acid. As it was reasonable to expect that upon methanolysis of whole endotoxin, methyl glycosides, easily identifiable by g.l.c.–m.s. would be formed, the action of methanol in the presence of an acidic catalysis (HCl or acidic ion exchange resin) on ammonium 3-deoxy-D-*manno*-octulosonate was examined. It was found both by g.l.c. and t.l.c. that methyl esters of three different glycosides were formed simultaneously. Analysis of the acetylated products by g.l.c.–m.s. established that the two glycosides having the shorter retention times were furanosides (1) while the slower moving glycoside was a pyranoside (2) (Table 1). The furanosides are characterised and differentiated from the pyranoside by fragments at *m/e* 217 and 157. The former, formed by cleavage of the C(5)–C(6) bond, represents C(1)—(5) and not the isomeric C(6)—(8) fragment; this was established by using CD_3OH for the glycosidation–esterification procedure: *m/e* 217 was then shifted by 6 a.m.u. to 223, the intensity of which was 30% of that of the base peak, *m/e* 375 ($M - CO_2CH_3$). The fragment at *m/e* 157 is formed from *m/e* 217 by loss of acetic acid. In the pyranoside cleavage of the exocyclic C(6)–C(7) bond does not give rise to a prominent primary fragment. The characteristic fragment of the pyranoside is at *m/e* 158, probably arising by cleavage of the C(5)–C(6) and C(2)–O(6) bonds and loss of acetic acid (Scheme 1); in the mass spectra of derivatives formed with CD_3OH *m/e* 158 was shifted to 164 which indicated the presence of both C(1) and C(2) in that fragment. It is noteworthy that the β-pyranoside of *N*-acetylneuraminic acid methyl ester (aglycone

TABLE 1

Fragment ions present in the mass spectra of peracetylated methyl glycosides of 3-deoxy-D-*manno*-octulosonic acid methyl esters (1) and (2) and of peracetylated methyl glycosides of 3-deoxyhexulosonic acid methyl esters (6)—(8); all *m/e* values >85 with intensities ≥5% of the base peak are given

Compound	t_A \ *m/e*	43	99	115	127	139	146	153	157	158	167	171	181	188	195	200	213	217	255	273	375
		Intensity (%)																			
(1)	2.6	67	6	6	10	10	17	56	36			16	16	8	24		28	44	10	7	100
	2.8	58		5	7	11	6	45	37			13	10		17		15	26	9	8	100
(2)	3.1	53	6	7	5	9		48		14	5	10	13		15	6	18		9	5	100

	t_A \ *m/e*	43	102	115	130	139	152	156	163	164	170	174	184	194	198	206	216	223	258	276	378
		Intensity (%)																			
OCD_3 analogue of (1)	2.6	80			6	6	17	49	46			10	8	8	13		15	37	8	5	100
	2.8	71			5	7	7	51	43			10	6		14		15	34	9	5	100
OCD_3 analogue of (2)	3.1	65	7	5		6		45		14	5	9	7		10	5	14		10	5	100

	t_A \ *m/e*	43	97	111	129	139	146	157	171	217	231	259
		Intensity (%)										
(6)	0.6	100	12	46	12	10	7	23	7	6	37	
(7)	0.6	100	25	68	21	25	31	22	13	8	58	5
	0.65	100	28	66	22	17	14	25	11	8	57	
(8)	0.75	100	29	88	29	21			8		70	

TABLE 2

Fragment ions present in the mass spectra of methyl (penta-*O*-acetyl-3-deoxy-D-*manno*-oct-2-uloson)ates; all *m/e* values >85 with intensities ≥5% of the base peak are given

m/e	Intensity (%) for compound (9)	Fragment ion	Intensity (%) for compound (10)
43	100		78
103	7		
115	6		9
127	7		
131			7
139	11	$M - COOCH_3 - 3 \times AcOH - 2 \times CH_2CO$	9
145	7		
155	21	M – C(8)–C(7) $- 2 \times AcOH - CH_2CO$	100
156	14	$M - 2 \times AcOH - AcOH - CH_2CO - 2 \times CH_2CO$	
167	9		7
180	14	$M - 2 \times AcOH - 2 \times AcOH - CH_2CO$	
181	23	$M - COOCH_3 - 3 \times AcOH - CH_2CO$	20
197	6	M – C(8)–C(7) $- 2 \times AcOH$	23
198	25	$M - 2 \times AcOH - AcOH - 2 \times CH_2CO$	
199	10	$M - COOCH_3 - 2 \times AcOH - 2 \times CH_2CO$	
208	7		
217	6	C(8)–C(7)–C(6)	
240	52	$M - 2 \times AcOH - AcOH - CH_2CO$	27
241	10	$M - COOCH_3 - 2 \times AcOH - CH_2CO$	9
245	8	M – C(8)–C(7)–C(6)	
259	9		
282	12	$M - 3 \times AcOH$	9
283	8	$M - COOCH_3 - 2 \times AcOH$	
301	95	$M - COOCH_3 - AcOH - CH_2CO$	9
302	12		
361	15	$M - COOCH_3 - CH_2CO$	
403		$M - COOCH_3$	8

axial) is easily obtained when *N*-acetylneuraminic acid is treated with methanol and an acidic ion exchange resin.[4,5]

As all efforts to separate the isomeric glycosides on a preparative scale failed, the formation of a well defined glycoside *via* a fully protected 2-halogeno-derivative of 3-deoxy-D-*manno*-octulosonic acid was next attempted. Acetylation of methyl 3-deoxy-D-*manno*-octulosonate with acetic anhydride and pyridine or sodium acetate gave a mixture of at least three peracetylated octulosonate derivatives from which a crystalline peracetate could be isolated in very low yield.

In view of the difficulties encountered in the preparation of a well defined peracetate of methyl 3-deoxy-D-*manno*-octulosonate and the instability of the derived 2-bromo-compound we turned to benzoate esters known to lead to more stable halogeno-derivatives. The reaction of methyl 3-deoxy-D-*manno*-octulosonate with benzoyl chloride in pyridine gave an amorphous pentabenzoate in good yield which, upon treatment with HBr in acetic acid yielded a crystalline 2-bromo-derivative in 40% yield. The ^1H n.m.r. spectrum (Figure 1) of this compound (11) at 250 MHz appeared to agree with a pyranose structure with the sugar in the *C*1 conformation, except that $J_{3,4}$ was smaller (8 Hz) than the equivalent constants $J_{3ax,4ax}$ (11.5—12.8 Hz) reported [6,7] for *N*-acetylneuraminic acid derivatives or that (11 Hz) measured for the methyl glycoside of methyl 3-deoxy-D-*arabino*-heptulosonic acid [8-10] and even smaller (but comparable, 8 *versus* 9) than in the case of benzyl 2-deoxy-D-*arabino*-hexopyranoside.[11] Treatment of this bromide with methanol in the presence of silver carbonate gave a methyl glycoside as a syrup in quantitative yield. As judged by the $[\alpha]_D$ values the same glycoside was

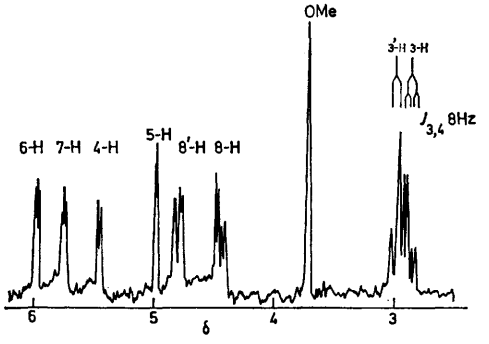


FIGURE 1 The 250-MHz ^1H n.m.r. spectrum (aromatic nuclei omitted) of methyl (4,6,7,8-tetra-*O*-benzoyl-2-bromo-2,3-dideoxy-D-*manno*-oct-2-ulofuranoson)ate (11); assignment of protons was done by irradiation. For coupling constants see Experimental section

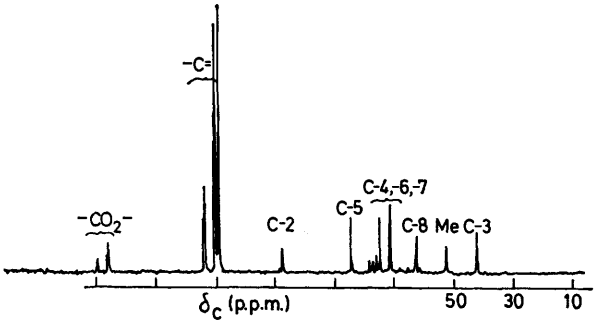
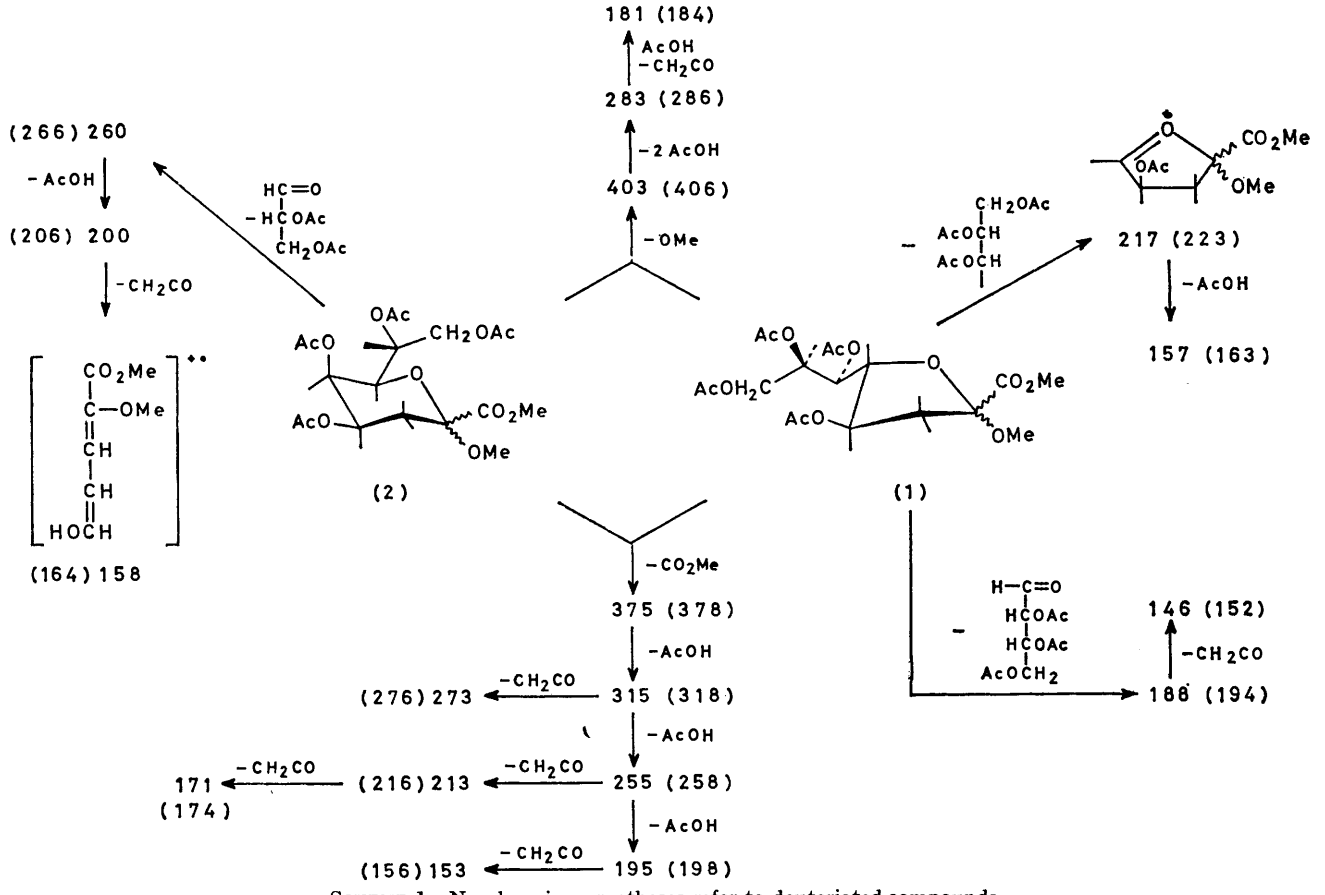


FIGURE 2 ^{13}C N.m.r. spectrum of methyl (methyl 4,6,7,8-tetra-*O*-benzoyl-3-deoxy-D-*manno*-oct-2-ulofuranosidon)ate (3)



SCHEME 1 Numbers in parentheses refer to deuteriated compounds

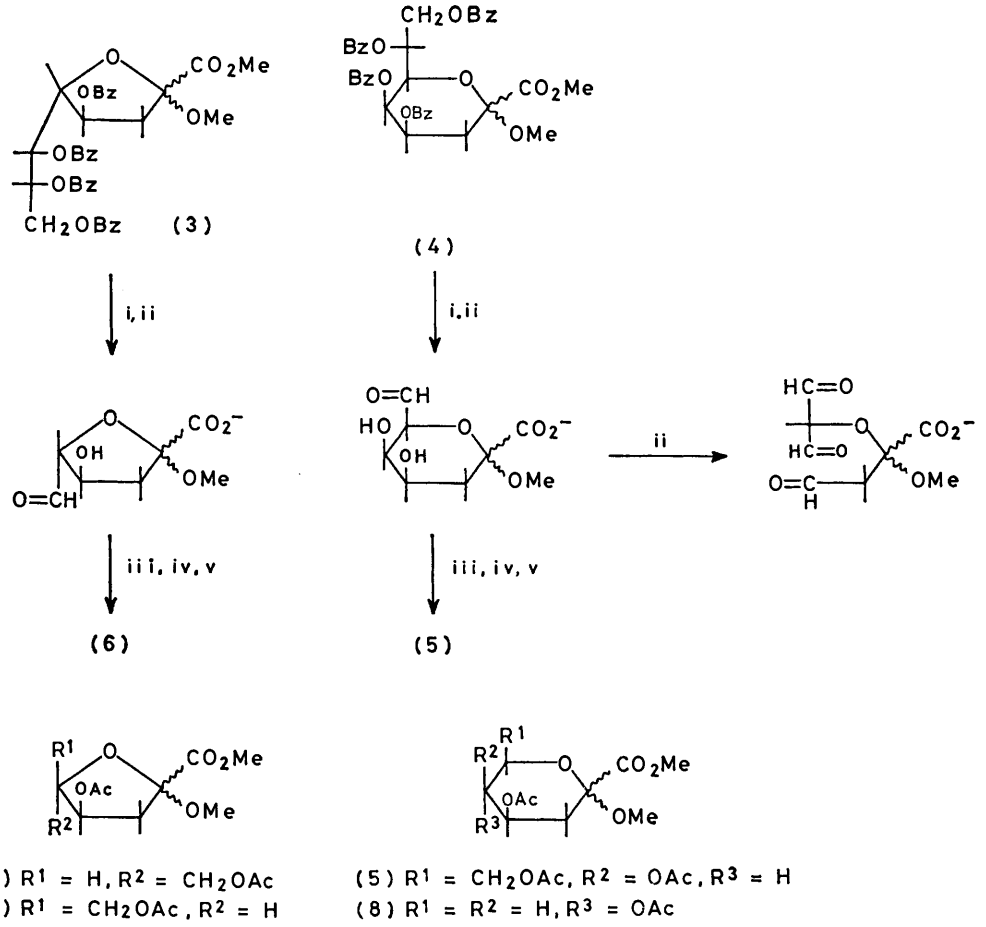
obtained when the methanolysis was carried out in the presence of tetrabutylammonium bromide.[12] The glycoside appeared to be homogeneous by t.l.c. and its ^{13}C n.m.r. spectrum (Figure 2) indicated that it was a pure, single anomer. However, the shift to lower field of most signals compared with those measured for the α- and β-methyl glycopyranosides of 3-deoxy-D-*manno*-octulosonic acid [2] suggested that it was a furanoside rather than the pyranoside. Comparison of the ^{13}C n.m.r. spectrum with that of an authentic sample of methyl (methyl 4,5,7,8-tetra-*O*-benzoyl-3-deoxy-D-*manno*-oct-2-ulopyranosidon)ate (4) prepared from 5-*O*-benzyl-3-deoxy-D-*manno*-oct-2-ulosonic acid definitely established that the methyl glycoside prepared from the benzyl-bromo-derivative was not a pyranoside. The postulated furanoside structure was then established by chemical degradation (Scheme 2): the benzoylated methyl glycoside (3) was debenzoylated and the unsubstituted glycoside was then sequentially treated with periodate and with borohydride. As shown in Scheme 2, pyranosides of 3-deoxyoctulosonic acid (4) would be either destroyed or, if the cleavage of the vicinal diol system on the ring were very slow, transformed into glycosides of 3-deoxyheptulosonic acids (5), whereas furanosides (3) would be transformed into glycosides of 3-deoxyhex-2-ulosonic acids (6). These compounds can be easily distinguished by g.l.c.–m.s. Accordingly, the substance obtained by the periodate–borohydride treatment of the methyl glycoside was acetylated and the material analysed by g.l.c.–m.s.: a single substance was detected which had the same fragmentation pattern (Table 1) as the authentic methyl hexulofuranoside (7) obtained from 3-deoxy-D-*threo*-hexulosonic acid.[8] Comparison (Table 3) of the ^{13}C n.m.r. spectrum of the crystalline methyl

TABLE 3

^{13}C Chemical shifts (expressed on the Me_4Si scale) of furanose and pyranose derivatives of 3-deoxy-D-*manno*-octulosonic acid

Compound	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)	OMe	*CH*$_3$–C	COO	–C=
(3)	169	107	42.5	71.3 *	84.2	71.0 *	74.6 *	62.6	52.4—52.5		165.3—166.0	128.3—133.5
(9)	166.2	106	40.5	70.5 *	85.5	69.9 *	74.1 *	61.6	53.2	20.6—20.8	169.8—170.5	
(4)	167.7	99.5	32.7	68.6 *	67.4 *	65.4 *	69.2	63.1	51.5—52.7		165.1—166.1	128.2—133.2
(10)	166.8	97.5	31.0	67.4 *	65.9 *	64.0 *	69.7	62.2	53.2	20.6—20.9	168—170.5	

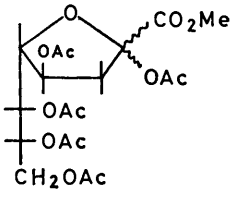
* Tentative assignments.

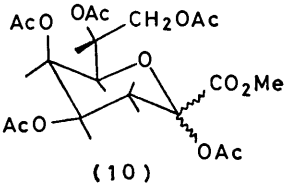


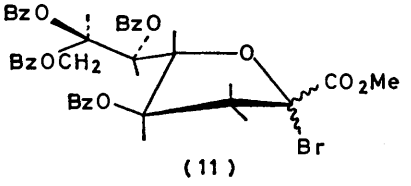
(6) R^1 = H, R^2 = CH_2OAc (5) R^1 = CH_2OAc, R^2 = OAc, R^3 = H
(7) R^1 = CH_2OAc, R^2 = H (8) R^1 = R^2 = H, R^3 = OAc

SCHEME 2 Reagents: i, OH^-; ii, IO_4^-; iii, BH_4^-, iv, CH_2N_2; v, Ac_2O–AcONa

penta-*O*-acetyl-3-deoxy-D-*manno*-octulosonate (9) (prepared by acetylation of methyl 3-deoxy-D-*manno*-octulosonate and mentioned above) with that of methyl (methyl tetra-*O*-benzoyl-3-deoxy-D-*manno*-oct-2-ulo-


(9)


(10)


(11)

furanosidon)ate (3) clearly established that this peracetate too, had a furanose structure. Indeed, the chemical shifts of the authentic pyranose (4) are quite different from those of the furanose derivatives (3) and (9), but are close to those of the crystalline methyl penta-*O*-acetyl-3-deoxy-D-*manno*-octulosonate (10) prepared from the free acid by the method of Unger *et al.*,[13] and thus confirm the pyranose structure of the latter (Table 3).

In cases described previously, the peracetylated methyl ester of 3-deoxy-D-*manno*-octulosonic acid was prepared by acetylation of the free acid [14] or its ammonium salt [2,13] followed by treatment with diazomethane; this procedure led to pyranose derivatives. It appears from the present work that the reverse sequence, esterification of the carboxy-function followed by acetylation or benzoylation, gives mainly furanose derivatives.

The ring size of ammonium 3-deoxy-D-*manno*-octulosonate in the crystalline state is not known, but as upon dissolution in water rapid mutarotation takes place several molecular species can be expected to be present in solution. The observation [15] that the ammonium salt of 3-deoxy-D-*manno*-octulosonic acid gave two, and the free acid three trimethylsilylated derivatives when treated with bis(trimethylsilyl)trifluoroacetamide–chlorotrimethylsilane in acetonitrile solution is in keeping with this surmise. 3-Deoxy-D-*manno*-octulosonic acid is homorphous with D-galactose and can form furanose

derivatives which, in the C_s conformation, have *quasi*-equatorial substituents only (1). It is therefore not surprising that this acid should readily form furanose derivatives. It is, however, not yet clear why furanose derivatives should be preferentially formed from the ester while the free acid and its ammonium salt give mainly pyranose derivatives.

EXPERIMENTAL

General.—^{13}C N.m.r. spectra reported in Table 3 were recorded on a Bruker WP-60 spectrometer operating at 15.08 MHz in the Fourier-transform mode. Spectra were taken in deuteriochloroform solution with Me_4Si as internal standard. G.l.c. was performed with a Varian Aerograph 2700 instrument equipped with a flame detector: carrier gas, nitrogen; columns, stainless steel; A, 3% SE-30 on Varaport 30 (100—120 mesh) 5 ft × 1/8 in; B, 3% OV-1 on Gaschrom Q (100—120 mesh), 4 m × 1/8 in; C, 2% OV-17 on Chromosorb G.H.P. (80—100 mesh), 2 m × 1/8 in. Retention times (t_A) are given with respect to penta-*O*-acetylarabinitol. Mass spectra were obtained with a Dupont 21-492B double focusing instrument coupled to a Varian Aerograph 2700 instrument equipped with column A. Helium (25 ml min^{-1}) was used as carrier gas, electron beam energy 75 eV; ionising current 250—300 μA; source temperature 250°. T.l.c. was performed on plastic sheets coated with silica gel (F 1500 LS254, Schleicher and Schüll), p.l.c. on glass plates coated with 1.5 mm silica (Merck 60 PF_{254}). Solvents are specified for each compound.

Glycosidation of 3-*Deoxyald*-2-*ulosonates.*—For mass spectrometry methyl glycosides of 3-deoxy-D-*manno*-oct-2-ulosonic and 3-deoxy-D-*threo*-hex-2-ulosonic acids were prepared by boiling a stirred suspension of the anhydrous ammonium salts (20 mg) in anhydrous methanol (5 ml) with dry Amberlite IR-120 (H^+) resin (100 mg) for 22 h. The methyl glycosides were isolated by p.l.c. (7 : 1 : 2, ethyl acetate–benzene–methanol). The mixed methyl glycoside of the octulosonate had R_F *ca.* 0.4—0.5 and those of the hexulosonate had R_F *ca.* 0.6—0.7. The isolated glycosides were acetylated with either 1 : 1 pyridine–acetic anhydride or acetic anhydride–sodium acetate, both at 80° for 1 h. Upon g.l.c. the peracetylated methyl glycosides of 3-deoxy-D-*manno*-octulosonic acid methyl ester had α- and β-furanosides (1), t_A 2.60 and 2.80, α- or β-pyranoside (2), t_A 3.1, on column A at 225°. The peracetylated methyl glycosides of 3-deoxy-D-*threo*-hexulosonic acid methyl ester had α- and β-furanosides (7), t_A 0.6 and 0.65, α- or β-pyranoside (8), t_A 0.75, on column A at 200°.

Methyl (2,4,6,7,8-*Penta*-O-*acetyl*-3-*deoxy*-D-manno-*oct*-2-*ulofuranoson*)*ate* (9).—Amberlite IR 120 (H^+) resin (5 g), thoroughly dried and then washed with anhydrous methanol, was added to a stirred suspension of the ammonium salt of 3-deoxy-D-*manno*-oct-2-ulosonic acid (1 g) in anhydrous methanol (50 ml). Dissolution was complete in 30 min and t.l.c. (7 : 1 : 2 ethyl acetate–benzene–methanol) indicated that most of the starting material was esterified. Solids were filtered off and ethereal diazomethane was added to the filtrate until a faint yellow colour persisted. The solvents were removed and the glassy methyl ester (1 g) was dried (P_2O_5). A sample (0.5 g) was acetylated with acetic anhydride (5 ml) and anhydrous sodium acetate (0.25 g) at 70° for 4 h. After the usual work-up, the syrupy acetate (0.9 g) was chromatographed on a column (17 × 2.4 cm) of silica gel (Merck 60 PF_{254}) and eluted with 2 : 1 ethyl acetate–hexane. The elution was monitored by t.l.c. An apparently homogeneous fraction was isolated (0.3 g) which yielded a crystalline acetate (0.1 g) (ether–hexane) having m.p. 123—125°, $[\alpha]_D^{20}$ +4° (*c* 1, MeOH), R_F (2 : 1 ethyl acetate–hexane) 0.61 (Found: C, 49.2; H, 5.5; O, 44.8. $C_{19}H_{26}O_{13}$ requires C, 49.35; H, 5.6; O, 45.0%). The same compound (m.p. and mixed m.p.) was obtained when acetylation was performed with pyridine instead of sodium acetate, t_A 3.60 on column A (225°), 3.60 on column B (215°), and 6.9 on column C (220°).

Methyl (2,4,5,7,8-*Penta*-O-*acetyl*-3-*deoxy*-D-manno-*oct*-2-*ulopyranoson*)*ate* (10) *by the Method of Unger* et al.[13,*]—4-Dimethylaminopyridine (0.05 g) was added to a stirred mixture of ammonium 3-deoxyoctulosonate (0.3 g), pyridine (4 ml), and acetic anhydride (3 ml). After 40 min, the mixture was homogeneous. After 28 h, it was partitioned between chloroform and cold, dilute sulphuric acid. The organic layer was washed with water, dried (Na_2SO_4), and concentrated to give a crystalline syrup (0.45 g), which was dissolved in methanol (5 ml) and treated with ethereal diazomethane. The crystalline material contaminated with a yellow syrup thus obtained was purified on a column (6 × 2 cm) of silica gel (Merck 60 PF_{254}; 6 : 4 ethyl acetate–hexane). After recrystallisation from ethyl acetate–hexane, the compound (0.33 g) had m.p. 155°, $[\alpha]_D^{20}$ +101° (*c* 1, MeOH) (lit., m.p. 155—158°, $[\alpha]_D$ +87°; [13] m.p. 152—153°, $[\alpha]_D$ +97°; [2] m.p. 155—156°, $[\alpha]_D$ +109.7° [14]), R_F (2 : 1 ethyl acetate–hexane) 0.71, ^{13}C n.m.r. in Table 3, m.s. in Table 2, t_A 3.6 on column A (225°), 3.4 on column B (215°), and 5.8 on column C (220°).

Methyl (4,6,7,8-*Tetra*-O-*benzoyl*-2-*bromo*-2,3-*dideoxy*-D-manno-*oct*-2-*ulofuranoson*)*ate* (11).—Benzoyl chloride (4.5 ml) was added dropwise to a solution of methyl 3-deoxy-D-*manno*-oct-2-ulosonate (1 g) in anhydrous pyridine (7 ml) cooled to 20°. The resulting solid mixture was kept overnight at room temperature, and gave, after the usual work-up, a syrup which was purified on a column (24 × 2.4 cm) of silica gel (Merck 60 PF_{254}), elution being carried out with hexane–ethyl acetate (8 : 2, 100 ml; 7 : 3, 100 ml; 6 : 4, 100 ml), to yield the *pentabenzoate* (1.9 g) as a foam (Found: C, 68.45; H, 4.85. $C_{44}H_{36}O_{13}$ requires C, 68.4; H, 4.7%). To a sample (1.5 g) of this compound in chloroform (4 ml) freshly distilled from P_2O_5 a solution (1.5 ml) of hydrogen bromide in acetic acid (35% w/v) was added and, 2 h later, toluene (10 ml). The solvents were removed *in vacuo* and toluene was evaporated twice from the residue. The *bromide* (0.5 g) which crystallised on addition of ether (15 ml) [a further amount of bromide (0.1 g) was obtained by cautious addition of hexane to the ethereal mother-liquors] had m.p. 119—120°, $[\alpha]_D^{20}$ −77° (*c* 1.5, CH_2Cl_2) (Found: C, 60.8; H, 4.3. $C_{37}H_{31}BrO_{11}$ requires C, 60.7; H, 4.2%) (the bromide is unstable in solution and must be recovered rapidly), δ_H 2.88 (q, $J_{3,3'}$ 16, $J_{3,4}$ 8 Hz, 3-H), 2.99 (d, $J_{3,3'}$ 16 Hz, 3'-H), 3.68 (3 H, s, CO_2Me), 4.45 (q, $J_{8,8'}$ 12.5, $J_{8,7}$ 6 Hz, 8-H), 4.82 (q, $J_{8,8'}$ 12.5, $J_{8',7}$ 3.5 Hz, 8'-H), 4.99 (1 H, 5-H), 5.47 (1 H, 4-H), 5.77 (1 H, 7-H), 6.0 (1 H, q, $J_{6,7}$ 6.4, $J_{6,5}$ 2.4 Hz, 6-H), 7.36 (12 H, m, *m*- and *p*-H), and 7.90 (8 H, m, *o*-H).

Methanolysis of Methyl (4,6,7,8-*Tetra*-O-*benzoyl*-2-*bromo*-2,3-*dideoxy*-D-manno-*oct*-2-*ulofuranoson*)*ate.*—(*a*) The $[\alpha]_D$ of a solution of bromide (11) (0.03 g) in dry dichloromethane (2 ml) was measured and dry methanol (0.05 ml) was

* We thank Dr. Unger for communicating his results before publication.

added. The rate of solvolysis was followed polarimetrically, $[\alpha]_D^{20}$ at equilibrium + 12°, $t_{1/2}$ 30 min.

(*b*) A solution of bromide (11) (0.06 g) in dichloromethane (2 ml)–anhydrous methanol (0.05 ml)–tetrabutylammonium bromide (0.105 g) had $[\alpha]_D^{20}$ at equilibrium + 10°, $t_{1/2}$ 6 min.

Methyl (*Methyl* 4,6,7,8-*Tetra*-O-*benzoyl*-3-*deoxy*-D-manno-*oct*-2-*ulofuranosidon*)*ate* (3).—To a stirred mixture of freshly prepared silver carbonate (1 g) and Drierite (0.2 g) in anhydrous methanol (5 ml) was added a small crystal of iodine and then, dropwise, a solution of the bromide (11) (0.2 g) in ether (15 ml). The reaction was monitored by t.l.c. (1 : 2 ethyl acetate–hexane). After 1 h, starting material (R_F 0.17) was completely converted into the methyl glycoside (R_F 0.28). Solids were filtered off and washed with ether. Evaporation of the combined filtrate and washings gave a *syrup* (0.167 g) having $[\alpha]_D^{20}$ +13° (*c* 1, CH_2Cl_2); ^{13}C n.m.r. data in Table 3 (Found: C, 67.1; H, 4.4; O, 27.9. $C_{38}H_{34}O_{12}$ requires C, 66.9; H, 4.9; O, 28.2%).

Methyl (*Methyl* 4,5,7,8-*Tetra*-O-*benzoyl*-3-*deoxy*-D-manno-*oct*-2-*ulopyranosidon*)*ate* (4).—Methyl 5-*O*-benzyl-3-deoxy-D-*manno*-oct-2-ulopyranosidonic acid methyl ester (0.5 g) in ethyl acetate (30 ml) was hydrogenated in the presence of 10% palladium on charcoal and the reaction was monitored by t.l.c. (17 : 3 chloroform–methanol) (R_F starting material 0.51, debenzylated product 0.17). After 2 h, the catalyst was filtered off and the solvent was removed to give a syrup (0.427 g) of which a sample (0.15 g) in pyridine (5 ml) was treated overnight at room temperature with benzoyl chloride (1 ml). After the usual working up and column chromatography (column Merck Lobar B; 1 : 1 ethyl acetate–hexane) the *benzoate* (0.22 g), crystallised from ethyl acetate (1 ml) containing sufficient hexane to produce persistant turbidity, had m.p. 169°, $[\alpha]_D^{20}$ −36° (*c* 1, CH_2Cl_2), ^{13}C n.m.r. data in Table 3 (Found: C, 67.2; H, 5.1. $C_{38}H_{34}O_{12}$ requires C, 66.9; H, 5.0%).

Transformation of Methyl (*Methyl* 4,6,7,8-*Tetra*-O-*benzoyl*-3-*deoxy*-D-manno-*oct*-2-*ulofuranosidon*)*ate* (3) *into Methyl* (*Methyl* 4,6-*di*-O-*acetyl*-3-*deoxy*-L-erythro-*hex*-2-*ulofurano-sidon*)*ate* (6).—A solution of compound (3) (40 mg, 0.6 mmol) in methanol (3 ml) was treated for 3 h with a 0.1M solution (3 ml) of sodium hydroxide in 90% methanol, after which time, as shown by paper electrophoresis (Whatman 3 MM; 0.1M-pyridinium acetate; pH 3; alkaline silver nitrate) a single, acidic product ($R_{\text{picric acid}}$ 1) was present. The pH of the solution was adjusted to 5.2 with 0.1M-acetic acid and a cold, aqueous solution (3 ml) of sodium meta-periodate (3 mmol) was added. After 1 h, sodium boro-hydride (1 mmol) in water (0.5 ml) was added and 0.5 h later the cold mixture was diluted with cold methanol (5 ml) and the effluent neutralised with an ethereal solution of diazomethane. The product (6) obtained after removal of the solvents and acetylation of the residue (acetic anhydride–pyridine) was homogeneous on g.l.c., t_A 0.6 (column A, 205°); m.s. data in Table 1.

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