Pyranoside Derivatives of 3-Deoxyald-2-ulosonic Acids

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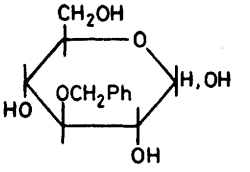
At pH 11.5 in 0.2M-sodium tetraborate solution and in the presence of Ni^{2+}, 2-*O*-benzyl-D-arabinose reacts with oxaloacetic acid to give a 50—60% yield of 5-*O*-benzyl-3-deoxyoct-2-ulosonic acids (mixed D-*gluco*- and D-*manno*-isomers). The methyl esters of these were separated and the α-methyl glycoside of the D-*manno*-isomer was prepared. The conformation of the compounds was established by n.m.r. spectroscopy at 250 MHz. It was observed that esterification of the carboxy-group greatly increases the stability of the glycosidic bond of 3-deoxy-D-*manno*-octulosonic acid while the benzyl substituent in position 5 exerts only a weak stabilising effect. 2-*O*-Benzyl-D-glyceraldehyde, when treated with oxaloacetic acid in the presence of Ni^{2+} at pH 7 in the absence of borate, yields 5-*O*-benzyl-3-deoxyhex-2-ulosonic acids, the predominant D-*erythro*-isomer being isolated as its methyl ester.

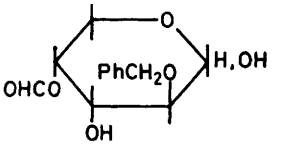
3-DEOXYALD-2-ULOSONIC ACID containing macromolecules have been isolated from various micro-organisms. Thus a 3-deoxyhex-2-ulosonic acid is the main component of the capsular polysaccharide of *Azotobacter vinelandii* [1] while 3-deoxy-D-*manno*-octulosonic acid is a ubiquitous component of endotoxins of gram negative bacteria.[2] It can be expected that upon methanolysis of such heteropolymers 3-deoxyald-2-ulosonic acids will be transformed into methyl glycosides which could be identified as the volatile acetate or methyl ether derivatives by g.l.c–m.s. For the unambiguous identification of these and, in particular, of that of the ring size, well defined furanose and pyranose derivatives of 3-deoxyoct-2-ulosonic and 3-deoxyhex-2-ulosonic acids were required. Also, while it is generally admitted that the glycosidic bond of 3-deoxyald-2-ulosonic acids is very easily cleaved by treatment with 0.1M acetic acid at 100 °C,[3] it has been observed [4] that the glycosidic bond of the phosphorylated 3-deoxyoct-2-ulosonic acid present in the endotoxin of *Bordetella pertussis* was quite resistant to such treatment and required 0.25M-mineral acid to be cleaved. It became, therefore, of interest to synthesise simple glycosides of 3-deoxyald-2-ulosonic acids to make them available for further studies.

It has been observed during the synthesis [5] of a methyl furanoside of 3-deoxy-D-*manno*-oct-2-ulosonic acid that glycoside formation with methanol and an acidic catalyst generally led to complex mixtures from which well defined glycosides could not be readily isolated, the only exception being 3-deoxy-D-*arabino*-hept-2-ulosonic acid which gave a methyl pyranoside. As in these mixtures furanosides appeared to predominate, the synthesis of pyranosides of 3-deoxyaldulosonic acids was attempted using intermediates which had position 5 temporarily blocked.

Both 2-*O*-methyl-D-arabinose [6] and 2-*O*-benzyl-D-arabinose-5-phosphate [7] when condensed with oxaloacetate gave 3-deoxyoct-2-ulosonic acid derivatives substituted at position 5; accordingly in the present study 2-*O*-benzylarabinose was chosen as starting material for the synthesis of the 5-*O*-benzyl ether of 3-deoxyoct-2-ulosonic acids, this protecting group being removable in conditions in which the methyl glycoside of the ulosonic acid could reasonably be expected to be stable. 2-*O*-Benzylarabinose was prepared from 1,2:5,6-di-*O*-isopropylidene-D-glucose by the method of Schwarz and MacDougall [8] except that the isopropylidene groups were removed from the intermediate 3-*O*-benzyl ether of diacetone glucose not by hydrolysis with mineral acid,[9] but by treatment with a strongly acidic ion exchange resin; this led to a pure product exempt of glucose. Treatment of the benzyl ether (1) with one molar equivalent of sodium periodate gave 2-*O*-benzyl-4-*O*-formyl-D-arabinose (2) in quantitative yield. Condensation of this compound with oxaloacetic acid at pH 11 failed; hardly any thiobarbiturate [10] positive material was detectable in the mixture. When (in view of the report [11] that 2-amino-2-deoxyaldoses gave better yields of sialic acids if the condensation was carried out in the presence of borate) the reaction was performed in 0.2M-sodium tetraborate solution, the yield appeared to be *ca.* 10%, as compared to 6% in the absence of borate. However, when the condensation was carried out in the presence of Ni^{2+} (ref. 12) and borate, yields of 50—60% of 3-deoxyoct-2-ulosonate were obtained as judged by the thiobarbiturate reaction, pure ammonium 5-*O*-benzyl-3-deoxyoct-2-ulosonate being used as the standard for the estimation. It is noteworthy that no condens-



(1)



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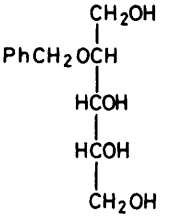
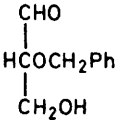


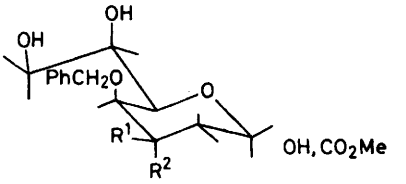
(3)



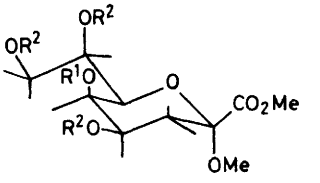
(4)

ation appeared to take place at pH 7. The mixed (epimeric at C-4) 2-*O*-benzyl-3-deoxyoct-2-ulosonic acids were recovered from the reaction mixture by anion-exchange chromatography and isolated as ammonium salts. Simultaneously any unchanged 2-*O*-benzyl-arabinose was also recovered and subsequently re-used. Analysis [7] of the mixed isomers revealed that the ratio of the D-*manno*- to D-*gluco*-isomers was 3 : 2. These were separated after conversion into the methyl esters (5) and (6) which were obtained from the ammonium or sodium salts by treatment with methanol and IR 120 (H^+) resin at room temperature. The methyl esters of 5-*O*-benzyl-3-deoxy-D-*manno*- and -D-*gluco*-oct-2-ulosonic acids could then be isolated in the crystalline state. The ^1H n.m.r. spectrum indicated that for both isomers the *C*1 ($^5C_2$) conformation was favoured: the system (well resolved at 250 MHz) given by H-3 and H-3′, was compatible with the AB part of an ABX system whose coupling constants J_{AX} and J_{BX} were 11.5 and 5 Hz for the D-*manno*-isomer in accordance with the postulated 180 and 60° angles, while they were both quite small (between 0 and 2 Hz) for the D-*gluco*-isomer in agreement with the postulated 60° angles.

Upon refluxing of the methyl ester of 5-*O*-benzyl-3-deoxy-D-*manno*-octulosonic acid with methanol in the presence of IR 120 H^+ resin, a methyl glycoside (7) was formed. As in this case H-3 and H-3′, having identical chemical shifts, gave a doublet instead of the AB part of an ABX system, the conformation of this glycoside could not be determined by ^1H n.m.r. spectroscopy. The α-configuration was tentatively assigned to the anomeric centre because (*a*) Fischer's glycoside synthesis



(5) R^1 = OH, R^2 = H
(6) R^1 = H, R^2 = OH

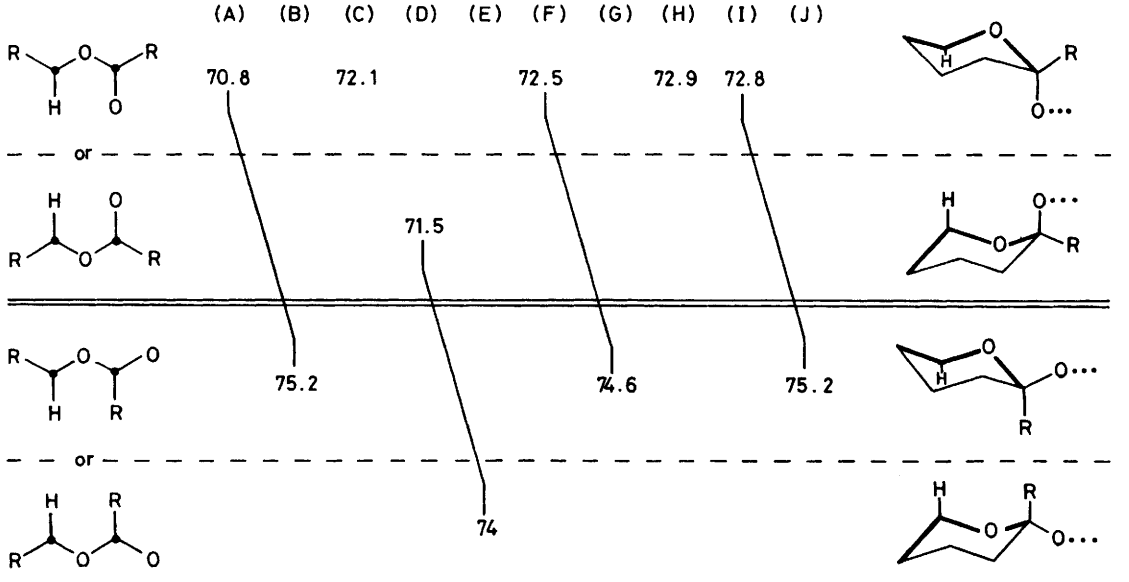


(7) R^1 = CH_2Ph, R^2 = H
(8) R^1 = R^2 = H
(9) R^1 = Ac, R^2 = Me
(10) R^1 = R^2 = Me

usually leads to the thermodynamically more stable isomer, *i.e.* the α-anomer for a homomorph of D-galactose (anomeric effect), and (*b*) the ^{13}C n.m.r. spectrum of the compound is very similar to that of the β-methyl glyco-

TABLE 1

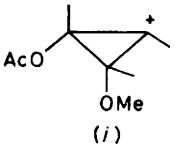
Correlation of the ^{13}C n.m.r. chemical shifts of C-5 (glycosides of aldoses) and C-6 (glycosides of 3-deoxyald-2-ulosonic acid derivatives) with the 1,3-diaxial H–O interaction. (A) Methyl α-D-galactopyranoside; (B) methyl β-D-galactopyranoside; (C) methyl 2-deoxy-α-D-*xylo*-hexapyranoside; (D) methyl (methyl β-D-*N*-acetylneuraminid)onate (unpublished data); (E) methyl (methyl α-D-*N*-acetylneuraminid)onate (ref.13); (F) sodium (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (ref. 14); (G) sodium (methyl 3-deoxy-β-D-*manno*-oct-2-ulopyranosid)onate (ref. 14); (H) methyl (methyl 5-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-inopyranosid)onate (7); (I) methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (8); (J) methyl (methyl 3-deoxy-β-D-*manno*-oct-2-ulopyranosid)onate (unpublished data)



side of sialic acid [13] and to the spectrum of the α methyl glycoside of unsubstituted 3-deoxy-D-*manno*-oct-2-ulosonic acid [14] (*cf.* Table 1). Debenzylation of the methyl glycoside (7) with H_2–Pd gave the amorphous methyl ester–methyl glycopyranoside (8). Although the chemical shifts of H-3 and H-3′ are rather close, a *C*1 conformation ($^5C_2$) can be assigned to the compound on the basis of the 250 MHz ^{1}H n.m.r. spectrum. The ^{13}C n.m.r. spectrum was also in good agreement with that expected for the α-anomer, as judged by comparison with data obtained for sialic acid,[13] for 3-deoxy-D-*manno*-octulosonic acid [14] and for glycosides of 2-deoxyaldoses.

Upon methylation with methyl iodide–silver oxide of the substituted methyl ester–methyl glycoside (7) followed by debenzylation and acetylation, methyl (methyl 5-*O*-acetyl-3-deoxy-4,7,8-tri-*O*-methyl-D-*manno*-oct-2-ulopyranosid)onate (9) was obtained. This compound is expected to be formed from glycopyranosides of 3-deoxy-D-*manno*-oct-2-ulosonic acid bearing glycosidic substituents (rhamnose,[15] heptose [16]) in position 5. The mass spectrum of this compound is described in Table 2 and compared with that of the tetramethyl ether (10). Samples sufficient for g.l.c.–m.s. analysis of the latter were obtained by two independent methods. In the first the benzyl ether (7) was first treated with MeI–Ag_2O, and the permethylated derivative, purified by preparative t.l.c. was hydrogenated and remethylated with the same reagent to yield the fully methylated 3-deoxyoctulosonidonate (10). In the second method, ammonium 3-deoxy-D-*manno*-oct-2-ulosonate was methylated according to Hakomori; [17,18] it has been shown [19] that salts but not the methyl ester of *N*-acetyl neuraminic acid can be methylated by this procedure. The same product (g.l.c.–m.s.) was obtained by both methods.

The presence of an acetoxy-group in compound (9) gives rise to a characteristic ion with *m/e* 116 representing



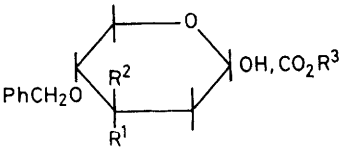
(*i*)

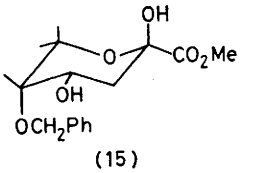
$[AcO{=}CH{-}CH(OMe){=}CH_2]^+$

(*ii*)

a C-4–C-5 fragment as well as an intense ion with *m/e* 129. The latter is probably derived from a G_1 type (*i*) or F_1 type (*ii*) ion (*cf.* ref. 20), which, by loss of keten, leads to *m/e* 87 which is absent from the mass spectrum of the permethylated derivative (10). The presence of the acetoxy-group on C-5 (rather than on C-4) is responsible for the absence of the fragment K_2 (ref. 20) [MeO–CH_2–CH=CH–CH=$\dot{O}$Me or CH_2=CH–CH(OMe)–CH=$\dot{O}$Me, *m/e* 115] in the spectrum of the acetylated compound.

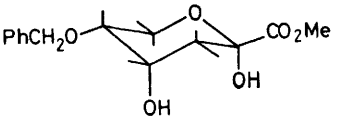
As it has been shown previously [21] that D-glyceraldehyde 2-phosphate, when condensed with oxaloacetic acid at neutral pH gave a good yield of 3-deoxyhex-2-ulosonates phosphorylated at position 5, 2-*O*-benzyl-4-*O*-formyl-D-arabinose (2) was treated with sodium borohydride to yield 2-*O*-benzylarabinitol (3), which, upon oxidation with periodate, gave 2-*O*-benzyl-D-glyceraldehyde [22] (4). Condensation of this aldehyde with the sodium salt of oxaloacetic acid in the presence of Ni^{2+} to yield 5-*O*-benzyl-3-deoxyhexulosonates (11) and (12) proceeded readily at neutral pH in the absence of borate. It appears that in spite of the known [23] tendency of glyceraldehyde to form intermolecular hemiacetals, its 2-*O*-benzyl ether reacts with oxaloacetate as if it were in the free aldehyde form. As the condensation could





(15)

(11) R^1 = OH, R^2 = R^3 = H
(12) R^1 = R^3 = H, R^2 = OH
(13) R^1 = OH, R^2 = H, R^3 = Me
(14) R^1 = H, R^2 = OH, R^3 = Me



(16)

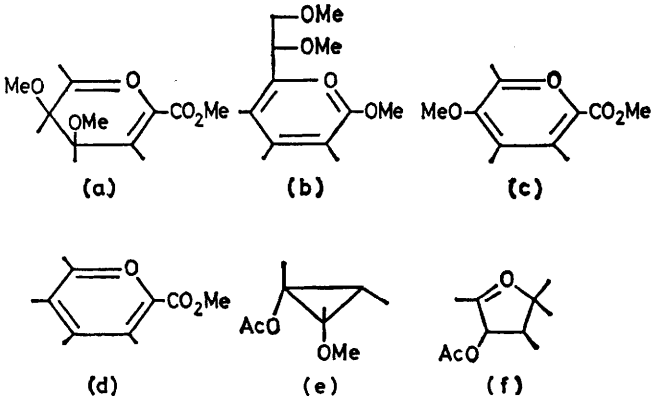
not be monitored by the thiobarbiturate test, the 5-*O*-substituted hexulosonates being unable to form the fragment OHC–CH_2–CO–CO_2H (' β-formyl pyruvate ') the reaction was arbitrarily stopped after 5 h by addition of ethanol; the ethanol-soluble sodium salts of the 5-*O*-benzyl-3-deoxyhexulosonic acids were recovered by decantation from the precipitate formed. The crude mixture contained 75% of the D-*erythro*- (11) and 25% of the D-*threo*- (12) isomer when analysed as alditol acetates [7] by g.l.c. after catalytic removal of the benzyl group. As in the case of octulosonates the mixed Na-salts were transformed into methyl esters and separated. Methyl 5-*O*-benzyl-3-deoxy-D-*erythro*-hexulosonate (13) crystallised, whereas the D-*threo*-isomer (14) did not. Freshly prepared solutions of methyl 5-*O*-benzyl-3-deoxy-D-*erythro*-hex-2-ulosonate in chloroform displayed ^{1}H n.m.r. spectra similar to that given by methyl 5-*O*-benzyl-3-deoxy-D-*manno*-octulosonate (5) as regards resonance of H-3 and H-3′ indicating a 1*C* ($^2C_5$) conformation with OH-4 equatorial; however, after about a week, two resonance systems were detected for H-3 and H-3′, the second reflecting a conformation with OH-4 axial. The slow equilibration of the β-epimer in the 1*C* ($^2C_5$) conformation (15; OH-4 equatorial, OCH_2Ph axial) with the α-anomer in the *C*1 ($^5C_2$) conformation (16; OH-4 axial, OCH_2Ph equatorial) is likely to explain this observation.

Compounds (7) and (8) were used to evaluate the influence of (*a*) esterification of the carboxy-group and (*b*) 5-*O*-substitution of the glycoside on the rate of acid-catalysed hydrolysis of the glycosidic bond of 3-deoxy-D-*manno*-oct-2-ulosonic acid. As it was not feasible to follow the cleavage of this bond by the usual colorimetric methods,[10,24] ^{14}C-labelled methanol was used as

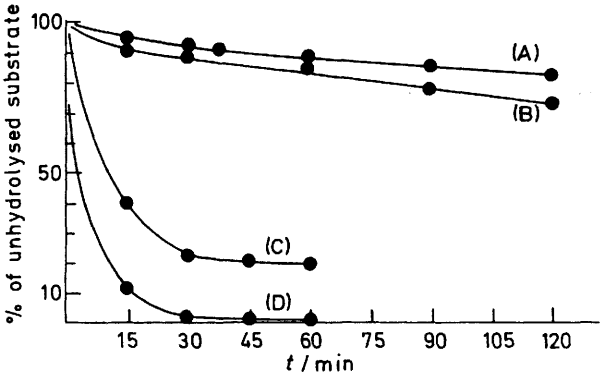
TABLE 2

Characteristic fragments of the mass spectra of methyl (methyl-5-*O*-acetyl-3-deoxy-4,7,8-tri-*O*-methyl-D-*manno*-oct-2-ulopyranosid)onate (9) and methyl (methyl-3-deoxy-4,5,6,7,8-tetra-*O*-methyl-D-*manno*-oct-2-ulopyranosid)onate (10)

Compound (9)				Compound (10)	
m/e	Relative intensity (%)			Relative intensity (%)	*m/e*
318	Trace	$M - 32$			
305	100	$M - 45$			
291	47	$M - 59$	$M - 32$	Trace	290
287	8	$M - 31$—32			
277	15	$M - 15 - CH_2 - CHOMe$	$M - 59$	70	263
245	13	$M - 31 - 32 - 42$; $305 - 60$	$M - 45$—32	5.9	245
			$M - 89$	6.8	233
				6.8	231
217	8	$277 - 60$			
213	19	$M - 45 - 60 - 32$; $M - 31 - 32 - 42 - 32$			
			$M - 89 - 32$ (a)	23	201
199	18	$M - 59 - 32 - 60$ (b)		27	199
				6.8	189
				3.4	184
				4	175
173	12			7.7	173
171	22				
				1.2	170
			(c)	19	169
167	21	$M - 59 - 32 - 60 - 32$	$M - 59 - 32 - 32 - 32$	10	167
				13	160
				52	157
145	8			14	145
143	17		$175 - 32$	32	143
				13	141
139	10	(d)			
				20	133
129	27	(e)		10	129
				9	128
127	11	(f)			
117	8			18	117
116	8	C-4→C-5			
			$MeO-CH_2-CH=CH-CH=\overset{+}{O}Me$	14	115
				100	101
89	10	C-7–C-8		41	89
			$MeO-CH=CH-OMe$	19	88
87	22	$129 - 42$			
85	12			18	85
75	27			93	75
74	10	$116 - 42$			
			$Me\overset{+}{O}=CH-CH=O$	18	73
			$101 - CH_2$	16	71
59	15			44	59
45	37			93	45
43	28				



the aglycone, the carboxy-group being esterified first with non-radioactive methanol. That no labelling was introduced by exchange into the carboxy-group during the glycosidation procedure (carried out with $^{14}CH_3OH$–HCl) was ascertained by treating the radioactive glycosides (7) and (8) with base followed by evaporation when no loss of radioactivity was observed. Thus the hydrolysis of the glycosidic bond could be measured by the loss of radioactivity from samples treated with acid, neutralised, and brought to dryness. Results obtained when the hydrolyses were carried out with 0.1M-HCl at



Kinetics of the hydrolysis of the methyl glycopyranosides of (A) the methyl ester of 5-*O*-benzyl-3-deoxy-D-*manno*-oct-2-ulosonic acid, (B) the methyl ester of 3-deoxy-D-*manno*-oct-2-ulosonic acid, (C) sodium 5-*O*-benzyl-3-deoxy-D-*manno*-oct-2-ulosonate, and (D) sodium 3-deoxy-D-*manno*-oct-2-ulosonate in 0.1M-HCl at 80 °C

80 °C (Figure) clearly establish that esterification of the carboxy-group stabilises the glycosidic bond of 3-deoxy-D-*manno*-oct-2-ulopyranosidonic acid while the substituent benzyl group in position 5 exerts a slight stabilizing effect for both the ester and the free acid.

EXPERIMENTAL

Evaporations were carried out *in vacuo* at 40 °C. Products were dried *in vacuo*. Solutions in organic solvents were dried with Na_2SO_4. Melting points were determined on a Kofler hot-plate. Optical rotations were determined with a Perkin-Elmer model 141 polarimeter. T.l.c. was performed on silica gel (F 1500 LS_{254}, Schleicher and Schüll) and compounds were located by spraying with 10% sulphuric acid in ethanol and heating. ^1H N.m.r. spectra at 250 MHz were obtained on a CAMECA instrument, using Me_4Si as internal standard. ^{13}C N.m.r. spectra were recorded on a Bruker WP-60 spectrometer operating at 15.08 MHz in the Fourier-transform mode.

3-O-*Benzyl*-D-*glucose* (1).—Sodium hydride (5 g; 50% in oil), previously washed with dry hexane, was added portionwise to a solution of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (10 g, 0.04 mol) in dry *NN*-dimethylformamide. Benzyl chloride (15 ml) was added dropwise during 15 min and the mixture was left overnight. After cautious addition of methanol (10 ml), the solvents were removed at 55 °C and 20 mmHg. The residue was partitioned between chloroform (200 ml) and water (20 ml), and the organic layer was washed with water (2 × 50 ml), dried, and concentrated. The resulting syrup was dissolved in aqueous ethanol (1 : 1 v/v) and stirred for 3 h at 90 °C with Dowex 50 resin (H^+, 20 ml), the hydrolysis being monitored by t.l.c. (benzene–ethyl acetate–methanol 7 : 3 : 2 v/v/v). The resin was filtered off and after evaporation of solvents, the residue was crystallised from ethyl acetate (20 ml). The benzyl ether (8 g, 77%) had m.p. 137—140 °C (lit.,[25] 130—140, 138—141 °C); R_F (t.l.c., above solvent) 0.37—0.38.

2-O-*Benzyl*-4-O-*formyl*-D-*arabinose* (2).—Compound (1) (21.6 g, 0.08 mol) was added portionwise to a stirred solution of sodium metaperiodate (200 ml, 0.43M) during 30 min. The mixture was kept for 1 h at room temperature, then extracted with ethyl acetate (4 × 150 ml). The formate ester (18 g, 86%) which crystallised from the dried and concentrated solution had m.p. 134—139 °C (lit.,[8] 120—130 °C); R_F [t.l.c. (benzene–ethyl acetate–methanol 7 : 5 : 2 v/v/v)] 0.69; after alkaline hydrolysis, 0.48.

2-O-*Benzyl*-D-*arabinitol* (3).—Compound (2) (6.45 g, 0.024 mol) in methanol–water (2 : 5 v/v; 60 ml) was added dropwise to an ice-cold solution of sodium borohydride (3.6 g) in water (45 ml) and the mixture was kept for 30 min at room temperature. Excess of borohydride was destroyed by cautious addition of IR 120 resin (H^+); the solution was passed through a column (3.2 × 44 cm) of the same resin and eluted with water. The combined effluents were passed through a column (4.5 × 20 cm) of Borasorb resin. Water was removed and the crystalline *alditol* (5.25 g, 90%) after recrystallisation from ethyl acetate had m.p. 64°, $[\alpha]_D^{22}$ −6° (*c*, 1.7 in MeOH) (Found: C, 59.6; H, 7.4. $C_{12}H_{18}O_5$ requires C, 59.5; H, 7.4%).

Methyl 5-O-*Benzyl*-3-*deoxy*-D-manno-, *and* -D-gluco-*oct*-2-*ulosonates* (5) *and* (6).—The pH of a cooled solution of oxaloacetic acid (13.2 g, 0.1 mol) in 0.2M-aqueous sodium tetraborate (100 ml) was rapidly adjusted to 11.5 with 5M-aqueous sodium hydroxide, and compound (2) (5.36 g, 0.02 mol) followed by a solution of nickel chloride (1.2 g) in water (2 ml) were added. The reaction mixture was kept at room temperature for 18 h (the pH of the solution being periodically adjusted to 11.5 with 5M-aqueous sodium hydroxide during the first 5 h) at which time the yield of 3-deoxyaldulosonates was usually more than 50% (estimated by the thiobarbiturate reaction on 5 μl of the reaction mixture). The reaction mixture was diluted (250 ml) with water and its pH brought to 4 with IR 120 resin (H^+). The filtered solution was diluted (500 ml) with 1M-aqueous pyridinium acetate (pH 5)–ethanol (1 : 4 v/v) which caused rapid decarboxylation of the excess of oxaloacetic acid. The solution was stirred under vacuum until evolution of gas ceased (*ca.* 30 min), then passed through a column (3.2 × 28 cm) of Lewatit MP 5080 resin (OH^-) (60—150 mesh, Merck) equilibrated with 0.25M-aqueous pyridinium acetate (pH 5)–ethanol (1 : 1 v/v). The elution was carried out using a gradient resulting from a constant volume (1 l) mixing chamber containing 0.25M-aqueous pyridinium acetate (pH 5)–ethanol (1 : 1 v/v) and a reserve chamber (1.2 l) containing 0.5M-aqueous pyridinium acetate (pH 5)–ethanol (1 : 1 v/v). Fractions containing 3-deoxyaldulosonic acids (thiobarbiturate test) were combined and concentrated to dryness. The residue, after being dried (KOH pellets), was dissolved in water and passed through a column (1.4 × 17 cm) of IR 120 resin (H^+). The neutralised (1M-aqueous ammonium hydroxide) effluent was concentrated to give a syrup which was dissolved in ethanol (20 ml), any insoluble material being removed by centrifugation. The crude ammonium salts (4 g) were precipitated by addition of ether [paper electro-

phoresis in 0.1M-pyridinium acetate buffer pH 3.5 showed, besides 5-*O*-benzyl-3-deoxyaldulosonates ($R_{\text{picric acid}}$ 0.7), two major impurities (silver nitrate–sodium hydroxide) having $R_{\text{picric acid}}$ 1.13 and 1.5], and were recovered by centrifugation, suspended in methanol, and stirred overnight at room temperature with dry IR 120 resin (H^+, 25 ml). The filtered solution was concentrated to give a yellow syrup (3.5 g) which contained two major compounds [t.l.c. (chloroform–methanol, 9 : 1 v/v)], the major, R_F 0.23, giving after degradation [7] 3-deoxyglucitol, a product derived from 3-deoxy-D-*manno*-oct-2-ulosonic acid, the other (R_F 0.27) giving 3-deoxygalactitol, the degradation product of the D-*gluco*-isomer. The isomers were separated on a Lobar C (Merck) column using chloroform–methanol (9 : 1 v/v). The crystalline (from chloroform) D-manno-*compound* (5) (1.1 g) had m.p. 123—126 °C, raised to 126—127 °C by two crystallisations (from chloroform), $[\alpha]_D^{20}$ +47.4° (*c* 1.15, methanol); δ (250 MHz) [$(CD_3)_2CO$] 1.88 (1 H, q, H-3eq, $J_{3eq,3ax}$ 12, $J_{3eq,4}$ 4.7 Hz), 2.3 (1 H, t, H-3*ax*, $J_{3eq,3ax} = J_{3ax,4}$ 12 Hz), 3.7 (3 H, s, CH_3), 4.75 (1 H, d, PhC*H*, J_{vic} 11 Hz), 4.96 (1 H, d, PhC*H'*, J_{vic} 11 Hz), 7.3 (m, *m*- and *p*-ArH), and 7.4 (m, *o*-ArH); δ_C (D_2O) 172.6 (C-1), 139.0 (quarternary aromatic), 129.6—129.9 (aromatic CH), 96.7 (C-2), 76.6 (PhCH_2), 75.8 (C-5), 72.6 (C-6), 70.1 (C-7), 67.7 (C-4), 64.1 (C-8), 54.6 (CH_3), and 34.7 p.p.m. (C-3) (Found: C, 56.1; H, 6.4; O, 37.05. $C_{16}H_{22}O_8$ requires C, 56.1; H, 6.4; O, 37.4%). The crystalline (from ethyl acetate) D-gluco-*isomer* (6) had m.p. 104°, $[\alpha]_D^{22}$ +42.6° (*c* 1.45, MeOH), δ (60 MHz) (CD_3Cl) 1.86 (1 H, d, H-3*eq*, $J_{3eq,3ax}$ 14 Hz), 2.46 (1 H, d, H-3*ax*, $J_{3eq,3ax}$ 14, Hz), 3.7 (3 H, s, CH_3), 4.63 (2 H, s, PhCH_2), and 7.3 (5 H, ArH); δ_C (D_2O) 172.4 (C-1), 138.5 (quaternary aromatic) 129.5—129.7 (aromatic CH), 96 (C-2), 76.4 (PhCH_2), 73.9 (C-5), 70.3 (C-7), 68.3 (C-6), 65.3 (C-4), 64 (C-8), 54.4 (CH_3), and 32.7 p.p.m. (C-3) (Found: C, 56.0; H, 6.5. $C_{16}H_{22}O_8$ requires C, 56.1; H, 6.4%).

Methyl (*Methyl* 5-O-*benzyl*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* (7).—Dowex 50 × 8 resin (H^+, 3 g) was thoroughly washed with anhydrous methanol, then equilibrated with anhydrous methanol (5 ml) at 70 °C. The solvent was decanted and replaced by a solution of compound (5) (1.3 g) in methanol (15 ml) and the mixture was stirred at 70 °C for 24 h. The resin was centrifuged off and washed with methanol and the combined supernatants were concentrated to give the *title compound* (1.1 g) which after crystallisation from chloroform–carbon tetrachloride had m.p. 137.5 °C, $[\alpha]_D^{20}$ −73.3° (*c* 1 methanol), t.l.c. $R_{\text{compound (5)}}$ 1.25 (chloroform–methanol, 20 : 3 v/v); δ (250 MHz) [$(CD_3)_2SO$–$(CD_3)_2CO$, 1 : 1 v/v] 1.94 (2 H, d, H-3*eq* and H-3*ax* $J_{3eq,3ax}$ 8 Hz), 3.14 (3 H, s, CO_2CH_3), 3.69 (3 H, s, 2-OCH_3), 4.68 and 4.93 (2 H, 2 d, PhCH_2, $J_{H,H'}$ 11 Hz), 7.32 (5 H, Ph). Upon addition of D_2O, H-4 gave a triplet at δ 4.04; irradiation of this caused the doublet at 1.94 to coalesce to a singlet. δ_C (D_2O) 171.5 (C-1), 100.6 (C-2), 75.6 (C-5), 72.9 (C-6), 70.1 (C-7), 67.5 (C-4), 64 (C-8), 54.7 and 52.4 (CH_3), and 35.3 p.p.m. (C-3) (Found: C, 57.3; H, 6.8; O, 35.95. $C_{17}H_{24}O_8$ requires C, 57.3; H, 6.7; O, 35.95%).

Methyl (*Methyl* 3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* (8).—A solution of compound (7) (0.5 g) in ethyl acetate (30 ml) was hydrogenated in the presence of 10% Pd–charcoal (0.1 g) at 1 atm., the reaction being monitored by t.l.c. The title compound had $R_{\text{compound (7)}}$ 0.34 (chloroform–methanol, 17 : 3 v/v). The catalyst was filtered off and the solvents were removed to give the syrupy title compound (0.427 g) having $[\alpha]_D^{20}$ +96.5° (*c* 0.48, methanol) c.d. (H_2O) negative Cotton effect, $\varepsilon_{224\text{ nm}}$ −0.29; δ (250 MHz) (D_2O) 1.8 (1 H, t, *H*-3*ax*, $J_{3eq,3ax} = J_{3ax,4} = 13$ Hz), 1.9 (1 H, q, H-3*eq*, $J_{3eq,3ax}$ 13, $J_{3eq,4}$ 5 Hz), 3.08 (3 H, CC_2CH_3), and 3.7 (3 H, 2-OCH_3); δ_C (D_2O) 171.6 (C-1), 100.5 (C-2), 72.8 (C-6), 70.1 (C-7), 66.9 (C-5), 66.6 (C-6), 63.9 (C-8), 52.3 and 54.6 (CH_3), and 34.6 p.p.m. (C-3).

Methylation Procedures.—A mixture of compound (7) (150 mg), Ag_2O (200 mg), molecular sieve 4 A (few grains), and MeI (5 ml) was kept overnight at 40 °C in a sealed tube: at this point t.l.c. (EtOAc–hexane, 1 : 1 v/v) showed a major component with R_F 0.53 and minor components with R_F 0.33, 0.26, 0.14, and 0.08. Solids were removed, the solvent evaporated off and the methylation process repeated once. Material with R_F 0.53, recovered from a preparative t.l.c. was hydrogenated (MeOH, 10% Pd–charcoal); t.l.c. ($CHCl_3$–MeOH, 95 : 5 v/v) showed a single product (R_F 0.69). After removal of the catalyst half the material was acetylated [$(AcO)_2O$–pyridine; 1 : 1 v/v; 2 ml]. The solvents were removed by co-distillation with toluene and the residue was purified by preparative t.l.c. ($CHCl_3$–MeOH, 95 : 5 v/v, R_F 0.75); yield *ca.* 60 mg. Upon g.l.c.–m.s. the material gave a single peak (3% SE30 on Varaport 30, stainless steel column 1 800 × 2 mm, 240 °C isothermal; retention time 7—8 min) and the mass spectrum described in Table 2.

The other half of the debenzylated material was methylated with MeI–Ag_2O overnight; after working up, the material was purified by t.l.c. ($CHCl_3$–MeOH, 95 : 5). When analysed by g.l.c.–m.s. (the SE30 column at 170 °C increasing 1 °C min^{-1} retention time *ca.* 9 min) it gave a mass spectrum identical to the material obtained from ammonium 3-deoxy-D-*manno*-oct-2-ulosonate described below.

Ammonium 3-deoxy-D-*manno*-oct-2-ulosonate (10 mg) was methylated according to Hakomori [17] by the procedure described by Lindberg *et al.*[18] The product when isolated by preparative t.l.c. ($CHCl_3$–MeOH, 95 : 5 v/v) and analysed by g.l.c.–m.s. gave a single peak and the mass spectrum described in Table 2.

Methyl 5-O-*Benzyl*-3-*deoxy*-D-erythro-*hex*-2-*ulosonate* (13).—An aqueous solution (75 ml) of sodium metaperiodate (5.8 g) was added dropwise to a solution of compound (3) (3 g) in water (10 ml). The mixture was left 30 min at room temperature, then extracted with ethyl acetate (3 × 50 ml). The organic layer was dried and concentrated to yield a syrup (2.5 g) which was dried overnight over potassium hydroxide pellets. An aqueous solution (70 ml) of sodium oxaloacetate (10 g of oxaloacetic acid) at pH 7, and nickel chloride (1.2 g) in water (2 ml) were added to the syrupy 2-*O*-benzyl-triose in ethanol (20 ml). Oxaloacetic acid was added from time to time to maintain the pH of the solution at neutral. After 5 h the mixture was diluted with an equal volume of ethanol when two phases were formed. The lower, green phase was discarded. The upper phase was concentrated to dryness and the residue was dissolved in methanol, any insoluble material being removed by filtration. Solvents were removed and the resulting foam (5 g) was stirred in methanol (60 ml) with dry Amberlite IR 120 H^+ resin at room temperature for 3 h. The filtered solution was treated with ethereal diazomethane until the pH was neutral. (Caution: an excess of diazomethane causes formation of undesirable side-products as revealed by t.l.c. and, consequently, the yield of hexulosonate decreases). The syrup (3.8 g)

remaining after removal of the solvents contained two major products (t.l.c. chloroform–methanol 6 : 4) having R_F 0.32 (Me D-*erythro*-hexulosonate) and R_F 0.26 (the *threo*-isomer) which were separated on a Lobar C column (Merck) previously equilibrated with chloroform containing 3.5% methanol. After crystallisation from ethyl acetate–hexane (1 : 2 v/v) the D-*erythro*-isomer (1 g) had m.p. 114—118 °C, $[\alpha]_D^{20}$ −92° [3 min after dissolution in methanol (*c* 1.65)] and −72° [after 24 h]; δ (250 MHz) (CD_3Cl) 1.96 (1 H, q, H-3*eq* of α anomer in $^5C_2$ or β anomer in $^2C_5$ conformation, $J_{3,3'}$ 12.5, $J_{3,4}$ 5 Hz), 2.12 (1 H, *H*-3*ax* of α anomer in $^2C_5$ or β anomer in $^5C_2$ conformation, $J_{3,3'}$ 14, $J_{3,4}$ 3 Hz), 2.27 (1 H, q, H-3*eq* of α anomer in $^2C_5$ or β anomer in $^5C_2$ conformation, $J_{3,3'}$ 14, $J_{3,4}$ 4 Hz), 2.29 (1 H, t, *H*-3*ax* of α anomer in $^5C_2$ or β anomer in $^2C_5$ conformation, $J_{3,3'}$ 12.5, $J_{3,4}$ 12.5 Hz), 3.8 (3 H, d, CO_2CH_3 of both anomers), 4.48 (1 H, d, H of PhCH_2, J_{vic} 11 Hz), 4.78 (1 H, d, H′ of PhCH_2, J_{vic} 11 Hz); 7.11 (5 H, m, ArH); 3.6, 3.98, and 5 (3 H, complex system, H-4, H-5 and H-6); δ_C (CD_3OD) 171.9 (C-1), 139.9 (quaternary aromatic), 139.2 and 128.8 (aromatic CH), 96.7 (C-2), 76.2 (PhCH_2), 72.4 (C-5), 66.5 (C-4), 62.5 (C-6), 53 (CH_3), and 36 p.p.m. (C-3) (Found: C, 59.6; H, 6.45. $C_{14}H_{18}O_6$ requires C, 59.6; H, 6.4%).

After the above separation had been performed it was found that the separation was highly dependent on the quantity of eluant that was used to equilibrate the column. It was subsequently observed that more reproducible separations were obtained using a stainless steel column (2 × 27 or 2 × 50 cm) packed with silica gel (60 PF_{254}, Merck) and eluting with ethyl acetate–hexane (2 : 1 v/v) under 50 atm.

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