Determination of the Anomeric Configuration in Glycosides of 3-Deoxy-2-aldulosonic Acids by Circular Dichroism and *X*-Ray Crystallography

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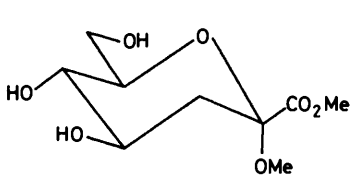
Methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate was obtained by acid-catalysed esterification and glycosidation of the free 2-aldulosonic acid. The molecular geometry of the glycosidic ester was determined by *X*-ray crystallography, and indicated both that the molecule was the α-anomer and that the ring oxygen and the carboxy-group were very nearly coplanar. The c.d. spectrum showed a negative n ⟶ π* transition band centred at 224 nm. The c.d. spectra of a number of methyl glycopyranosides derived from 6-, 7-, and 8-carbon 3-deoxy-2-aldulosonic acids demonstrated that all the compounds believed to be α-glycosides had negative maxima, and all those believed to be β-glycosides had positive maxima around 220 nm independent of their $^2C_5$ or $^5C_2$ conformation. The only known furanoside, believed to be a β-anomer, also had a positive maximum. The chirality of the anomeric carbon atom of this class of compound can thus be conveniently established by determining the sign of the Cotton effect at 220 nm.

GLYCOSIDICALLY bound 3-deoxy-2-aldulosonic acids have been shown to be present in macromolecules of bacterial origin.[1] The chemistry of these compounds is relatively obscure and, because of the absence of an anomeric proton, the chirality of their glycosidic bond is particularly difficult to establish. Thus, except for sialosides for which a specific α-glycosidase is known, the chirality of this bond has to be established by indirect means, such as ^{13}C n.m.r. spectroscopy which has been successfully used by Jennings and his colleagues[2] to elucidate the structure of the capsular polysaccharide of *Neisseria meningitidis*, serotype 29e, and also by Unger *et al.*[3] who established the chirality of the anomeric carbon by measuring the heteronuclear $^3J_{3ax\text{-}H,C\text{-}1}$ coupling constant of both anomeric methyl glycosides of 3-deoxy-D-*manno*-2-octulopyranosidonic acid.

It has been observed that the circular dichroism (c.d.) curves of the α- and β-methyl glycosides of *N*-acetylneuraminic acids[4-6] are different from those of the derived methyl esters. The difference was attributed to the influence of the carboxylic acid or ester groups. However, because of the interference of the acetamido-chromophore, the phenomenon was somewhat difficult to interpret in terms of the chirality of the glycosidic bond. Rees and his colleagues[6] have recently applied Listowsky's planar rule[7,8] to the correlation of the signs of observed Cotton effects with molecular geometry, *i.e.* to determine the chirality of the glycosidic bond in complex sialosides. This rule states that groups on opposite sides of a plane defined by the carboxy-group have opposite chiroptical effects. Owing to the lack of interference from acetamido-groups in glycosides of simple 3-deoxy-2-aldulosonic acids, the correlation of their c.d. curves with their anomeric configurations was expected to be simple and straightforward: accordingly, the application of this method to the determination of the anomeric configuration of 3-deoxy-2-aldulopyranosides was attempted. For the sake of simplicity methyl glycosides were chosen as models. Moreover, since from previous studies[4] it has been concluded by Rees *et al.*[6] that esterification had no major influence on the c.d. curves of neuraminic acid derivatives, and in order to eliminate the strong pH-dependence of c.d. curves of free acids,[9] the methyl esters of the methyl glycosides, rather than the free acids, were used in this study.

Application of Listowsky's rule[7,8] requires precise knowledge of the plane of the carboxy-group in relation to the rest of the molecule, in the present case in relation to the pyranose ring. According to the planar rule, groups of atoms lying to the right of the plane of the carboxy-group when viewed from C-1 along the RO_2C–C-2 bond make a positive contribution, whilst those to the left make a negative contribution to the c.d. curve. In the solid state the preferred conformation of the carboxy-group is such that it is almost in an eclipsed position: in acetic acid, eclipsing occurs with an H atom, in propionic and similar acids it is with C-3, and in α-hydroxy-acids it is with the oxygen atom.[10] In glycosides of 2-deoxy-2-aldulosonic acids, since one carbon atom (C-3) and two oxygen atoms (the glycosidic and the ring oxygens) are bonded to the carbon atom that carries the carboxy-group, the relationship of the plane of the carboxy-group to that of the pyranose ring had to be determined accurately. Since it is known that in crystals of β-*N*-acetylneuraminic acid ($^2C_5$ conformation) this plane passes through the ring oxygen,[11] and that in α-hydroxy-acids the eclipsing of the carbonyl function invariably occurs with the hydroxy-oxygen, the conformation in which the carbonyl function eclipses C-3 can be neglected for 3-deoxy-2-aldulosonic acids. In the case of *N*-acetylneuraminic acid, eclipsing of either the ring or the glycosidic oxygen atom leads, in terms of Listowky's rule, to the same prediction: a positive c.d. band for β-glycosides and a negative band for α-glycosides,[6] but only because of the presence of a substituent ($CHOH\text{–}CHOH\text{–}CH_2OH$) on C-6 of the pyranose ring; as a result, for the α-anomer, even in the rotamer in which the glycosidic oxygen is eclipsed, a large number of

groups appear on the negative side of the plane both in the $^5C_2$ and the $^2C_5$ conformations. In pyranosides of 2-hexulosonic acids there is no substituent on C-6 and in 2-heptulopyranosides the substituent is small; as a consequence, application of the planar rule to this type of compound gives no unequivocal result (Figure 1). Indeed, the ring-oxygen-eclipsed and the glycoside-oxygen-eclipsed rotamers are not equivalent in terms of the number of groups to the right and to the left of the plane defined by the carboxy-group. Hence the chirality of the glycosidic bond cannot be established unless the eclipsed oxygen atom is identified. In order to determine whether the ring-oxygen-eclipsed or the glycosidic-oxygen-eclipsed rotamer was present in 3-deoxy-2-aldulosonic acids, and to establish whether the α- or the β-anomer was formed in the Fischer glycosidation procedure, methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate (1) was synthesised and its structure determined by *X*-ray crystallography.



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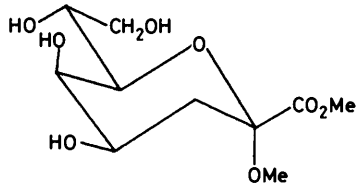

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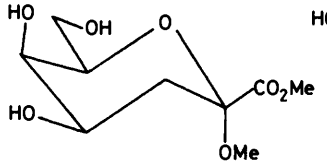

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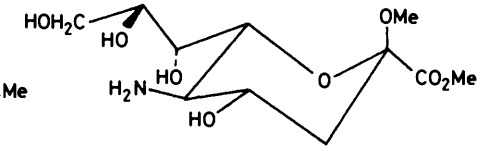

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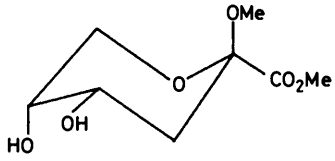

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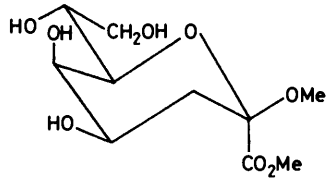

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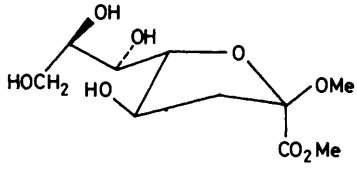

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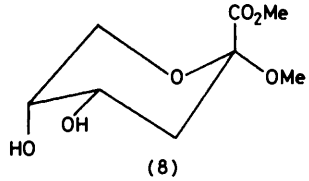

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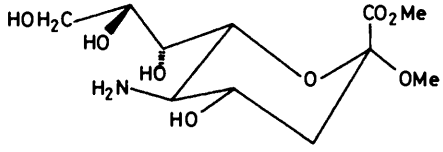


(9)

The molecular structure of compound (1) is represented in Figure 2. Final atomic co-ordinates (Table 1) and bond distances and angles (Tables 2 and 3) are given. The molecule is in the conventional $^5C_2$ chair conformation with the conformational angles around the pyranose

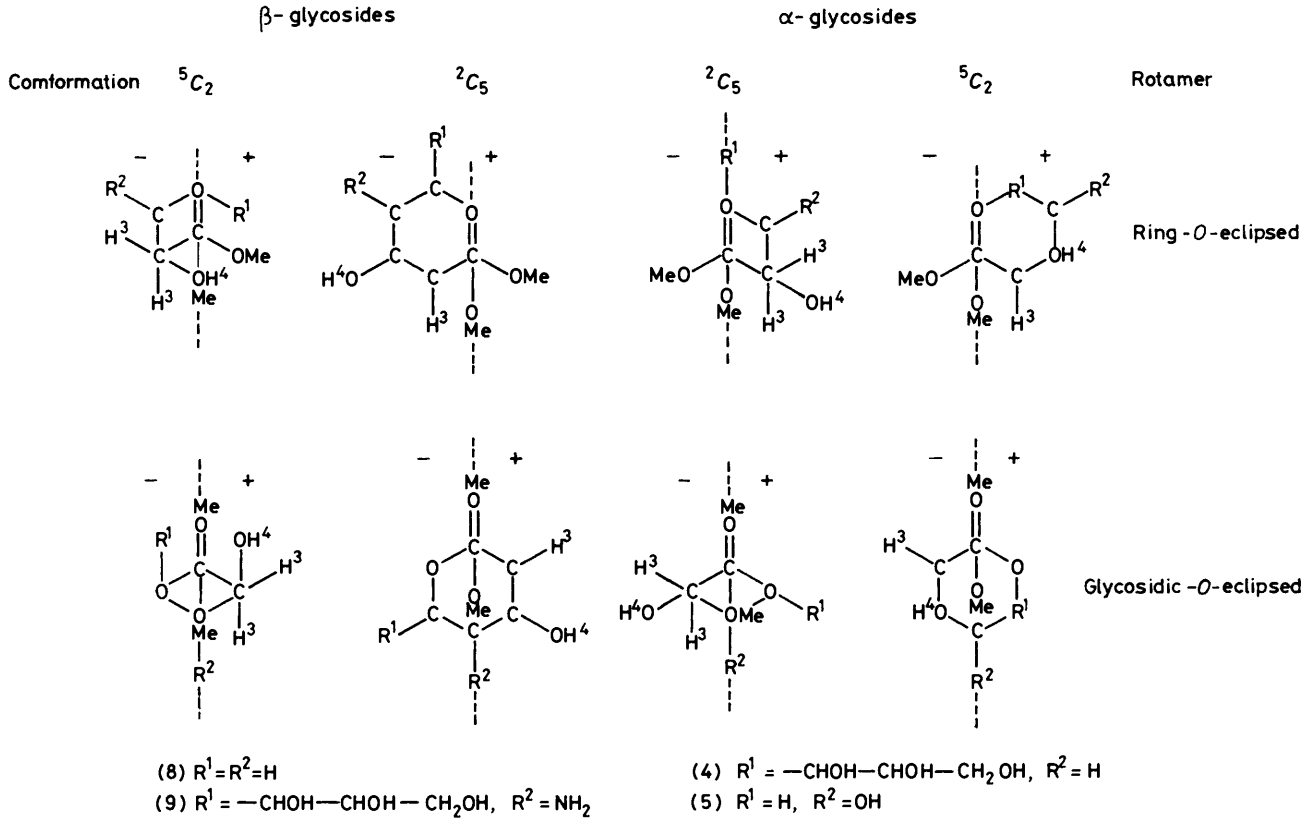


FIGURE 1 Application of the carboxyl planar rule [7,8] to methyl (methyl 3-deoxy-2-aldulosonid)onates: for the ring-oxygen-eclipsed rotamers a positive c.d. curve is predicted for β-glycosides, and a negative c.d. curve for α-glycosides; for all compounds, in both conformations, the predictions are in agreement with the observed values. However, for the glycosidic-oxygen-eclipsed rotamer, both conformers of the hexulosonate (5) lead to a predicted sign opposite to that for the ring-oxygen-eclipsed rotamer, and to that observed experimentally

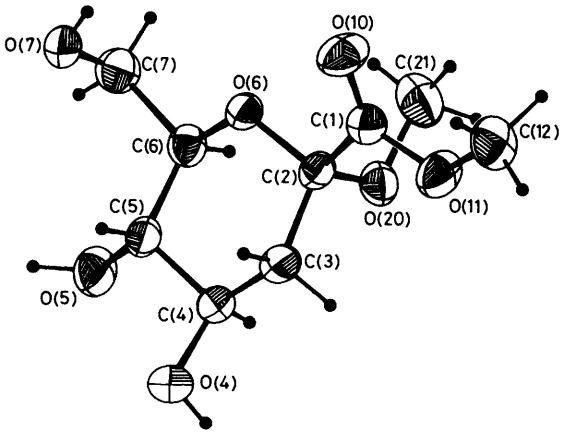


FIGURE 2 Molecular structure and atom-numbering system of compound (1)

ring in the range 50.15—59.8°, as compared with 55.8—61.7° for an ideal pyranose ring.* The torsion angles of the ring can be represented as in equation (1).

$$\text{C-6} \xrightarrow{+59.8} \text{O-6} \xrightarrow{-58.5} \text{C-2} \xrightarrow{+53.1} \text{C-3} \xrightarrow{-50.15} \text{C-4} \xrightarrow{+50.9} \text{C-5} \xrightarrow{-54.7} \text{C-6} \quad (1)$$

* Hypothetical pyranose ring with C—C 1.525 Å, C—O 1.430 Å, bond angle on carbon atoms 109.5°, and that on the ring-oxygen atom 113.3°.

The ring is most puckered at O-6 and least puckered at C-5; the same general trend has been observed for a wide range of pyranosides.[12,13] Atoms C-2, -3, -5, and -6 are nearly coplanar (Table 4).

The conformation about the exocyclic C-6–C-7 bond is *gauche–gauche* (Figure 3), in agreement with results often found for other pyranose derivatives. The *gauche–gauche* conformation is characterised by the angle ϕ (O)–(O) = O-6–C-6–C-7–O-7 = −59.1°, the theoretical value [14] being ϕ (O)–(O) = −60°.

The steric arrangement around the anomeric carbon C-2 is shown in Figure 3 as a Newman projection along the axis C-1–C-2. The glycoside has the α-configuration, the methoxycarbonyl group is in the equatorial position, and the carbonyl oxygen and the oxygen atom O-6 of the ring are eclipsed: O-10–C-1–C-2–O-6 = +4.4°. The chain of the methoxycarbonyl group, O-10–C-1–O-11–C-12, is nearly coplanar with the chain C-7–C-6–O-6–C-2 (Table 4). The glycosidic *O*-methyl group is antiparallel to the ring bond C-2–C-3 (Figure 3, Newman projection along C-2–O-20; Table 4). The methoxycarbonyl group excepted, lengths of carbon–oxygen bonds vary from 1.408 to 1.442 Å. The distance C-2–O-6, 1.408 Å, is significantly shorter than the average, the difference being more than ten times the standard error (0.002 Å), perhaps due to the coplanarity

of O-6, C-2, C-1, and O-10. The carbon bond-angles, interior to the pyranose ring, vary from 110.6 to 111.8°, the exterior angles from 106.4 to 114.2°. The oxygen bond-angles (113.7° in the ring, 113.4° for the glycosidic bond) are in good agreement with values found for glucopyranosides.

TABLE 1

Atomic co-ordinates (× 10^4 for non-hydrogen atoms and × 10^3 for hydrogen atoms). The mean values of e.s.d.s for *x/a*, *y/b*, and *z/c* are 0.0001, 0.0001, and 0.0003 for non-hydrogen atoms, and 0.002 for hydrogen atoms

Atom [a]	*x/a*	*y/b*	*z/c*
C(1)	11 446	3 666	5 502
C(2)	10 886	4 447	4 152
C(3)	10 584	3 878	2 201
C(4)	9 891	4 549	1 005
C(5)	9 106	4 930	2 356
C(6)	9 482	5 472	4 275
C(7)	8 744	5 786	5 805
O(4)	9 504	3 910	−616
O(5)	8 558	5 701	1 276
O(6)	10 113	4 757	5 331
O(7)	8 261	4 876	6 594
O(10)	11 222	3 346	7 151
O(11)	12 214	3 377	4 537
C(12)	12 824	2 611	5 535
O(20)	11 431	5 357	3 617
C(21)	11 767	5 950	5 344
$H_a(3)$	1 112	372	133
$H_b(3)$	1 029	317	260
H(4)	1 018	520	42
H(5)	874	429	271
H(6)	984	613	391
$H_a(7)$	904	620	688
$H_b(7)$	832	622	511
$H_a(12)$	1 316	229	458
$H_b(12)$	1 249	203	620
$H_c(12)$	1 327	302	648
$H_a(21)$	1 212	552	630
$H_b(21)$	1 124	633	605
$H_c(21)$	1 228	648	484
H(O4)	988	382	−148
H(O5)	795	544	141
H(O7)	855	458	753

[a] Throughout Tables 1—4 and the supplementary data, C(1) (*etc.*) refers to the atom designated C-1 (*etc.*) throughout the text and Figures. H(O4) (*etc.*) refers to the hydrogen atom bonded to atom O(4) (*etc.*).

The molecular packing (Figure 4) is determined by a set of intermolecular helicoid hydrogen bonds. The spirals of strong hydrogen bonds are extended in the direction *c*, and involve the ring hydroxy-group H–O-5 and the side-chain group H–O-7. The parameters are as follows (equation 2).

$$\text{O-5–H}\ (x, y, z)\ \ldots\ \text{O-7}\ (3/2 - x, 1 - y, -1/2 + z) = 2.723(2)\ \text{Å} \quad (2)$$

The hydrogen-bond geometry is given by equations (3)—(6).

$$\text{O-5}\ \ldots\ \text{H-O–5} = 0.93(3)\ \text{Å} \quad (3)$$

$$\text{O-5}\ \ldots\ \text{O-7} = 2.723(2)\ \text{Å} \quad (4)$$

$$\text{O-5–H}\ \ldots\ \text{O-7} = 1.80(3)\ \text{Å} \quad (5)$$

$$\text{O-5–H}\ \ldots\ \text{O-7} = 170.7° \quad (6)$$

TABLE 2

Bond distances (Å) and angles (°) involving non-hydrogen atoms (estimated standard deviations in parentheses)

C(1)–C(2)	1.535(3)	C(4)–O(4)	1.433(2)
C(1)–O(10)	1.194(3)	C(5)–C(6)	1.522(3)
C(1)–O(11)	1.323(2)	C(5)–O(5)	1.422(2)
C(2)–C(3)	1.521(3)	C(6)–C(7)	1.510(3)
C(2)–O(6)	1.408(2)	C(6)–O(6)	1.442(2)
C(2)–O(20)	1.412(2)	C(7)–O(7)	1.417(3)
C(3)–C(4)	1.514(3)	O(11)–C(12)	1.445(2)
C(4)–C(5)	1.511(3)	O(20)–C(21)	1.430(3)
C(2)–C(1)–O(10)	125.8(2)	C(5)–C(4)–O(4)	108.2(1)
C(2)–C(1)–O(11)	109.4(2)	C(4)–C(5)–C(6)	110.6(2)
O(10)–C(1)–O(11)	124.7(2)	C(4)–C(5)–O(5)	109.4(2)
C(1)–C(2)–C(3)	110.2(1)	C(6)–C(5)–O(5)	108.4(1)
C(1)–C(2)–O(6)	105.6(1)	C(5)–C(6)–C(7)	114.2(2)
C(1)–C(2)–O(20)	110.3(1)	C(5)–C(6)–O(6)	110.7(1)
C(3)–C(2)–O(6)	111.0(1)	C(7)–C(6)–O(6)	106.4(1)
C(3)–C(2)–O(20)	108.4(1)	C(6)–C(7)–O(7)	112.6(2)
O(6)–C(2)–O(20)	111.2(1)	C(2)–O(6)–C(6)	113.7(1)
C(2)–C(3)–C(4)	111.8(2)	C(1)–O(11)–C(12)	117.9(2)
C(3)–C(4)–C(5)	111.1(2)	C(2)–O(20)–C(21)	113.4(1)
C(3)–C(4)–O(4)	109.9(2)		

The c.d. curve of this compound has a negative band centred at 224 nm as predicted by the planar-symmetry rule applied to the projection of the ring-oxygen-eclipsed rotamer (Figure 5).

The results of our *X*-ray analysis substantiate the prediction that, since 3-deoxy-D-*arabino*-2-heptulosonic acid is a homomorph of 2-deoxy-D-*arabino*-hexose, the Fischer glycosidation procedure that was used to give the

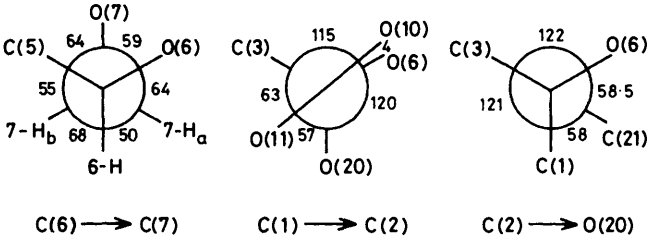


FIGURE 3 Newman projections (angles in °) for compound (1)

methyl glycoside would be expected to yield mainly the pyranoside with an *axial* glycoside bond. It therefore follows that, in the analogous case of 3-deoxy-D-*manno*-2-octulosonic acid, a homomorph of 2-deoxy-D-*lyxo*-hexose, if the compound is blocked at position 5 to prevent furanoside formation,[15] the main product of the Fischer glycosidation procedure should also be the α-pyranoside with an axial glycoside bond, *i.e.* compound (2), and its c.d. curve should have a negative maximum around 220 nm: this is, in fact, the case (Figure 5). As expected, methyl (methyl 3-deoxy-α-D-*lyxo*-2-heptulopyranosid)onate (3) also has a c.d. curve with a negative maximum (Figure 5).

For both the *arabino*-heptulosidonate (1) and the *manno*-octulosidonate (2) the 400 MHz ^{1}H n.m.r. spectrum unequivocally established that in aqueous solution the conformation was $^5C_2$, *i.e.* the same as found by *X*-ray crystallography for the heptulosidonate (1); their ^{13}C n.m.r. spectra are similar to that of the octulosidonate (2).[2,3] The related α-methyl glycoside of neuraminic

acid methyl ester, as well as α-neuraminyl-lactitol,[6] both of whose anomeric configurations are known from enzyme studies, also have c.d. curves with negative maxima, although in solution they both have the $^2C_5$ conformation.

On the other hand, the c.d. curve of methyl (methyl 3-deoxy-D-*manno*-2-octulopyranosid)onate, prepared *via* the 2-halogeno-derivative [2,16] and therefore expected to be the β-glycoside (6) had, as predicted by the application of Listowsky's rule, a positive maximum at 225 nm, similar to those of the β-methyl glycoside of neuraminic acid [6] and its methyl ester (Figure 6).

Application of Fischer's glycosidation method to 5-*O*-benzyl-3-deoxy-D-*erythro*-2-hexulosonic acid,[15,17] followed by hydrogenolysis of the benzyl group, gave a methyl ester–methyl glycoside whose c.d. curve had a positive maximum at 225 nm and was thus a β-glycoside, to which we assigned structure (5). Its 400 MHz ^{1}H n.m.r. spectrum clearly established ($J_{3ax,4ax}$ 12 Hz) that the compound had the $^2C_5$ conformation. The formation of a β-glycoside from a homomorph of 2-deoxy-D-*erythro*-pentose under Fischer's conditions is not unexpected: [18] in the $^5C_2$ conformer the α-glycoside with a favourable anomeric effect has an unfavourable 1,3-diaxial interaction with the hydroxy-group on C-3; this group is absent in the $^2C_5$ conformer of the β-glycoside which also possesses a favourable anomeric effect.

The only synthetic furanose derivative of this class of compound, methyl (methyl 4,6,7,8-tetra-*O*-benzoyl-3-deoxy-D-*manno*-2-octulofuranosid)onate, was obtained [19] *via* the 2-bromo-derivative and was, therefore, expected to be a β-glycoside. The c.d. spectrum of the debenzoylated glycoside had a positive maximum at 207 nm, in agreement with the prediction made by application of Listowsky's rule and the present work. It thus appears that, in the absence of interfering chromophores, the absolute configuration of the anomeric carbon of glycosides of 3-deoxy-2-aldulosonic acids can be easily estab-

TABLE 3

Distances (Å) and angles (°) involving hydrogen atoms (estimated standard deviations in parentheses)

C(3)–H$_a$(3)	0.98(2)	O(5)–H(O5)	0.93(3)
C(3)–H$_b$(3)	1.00(2)	O(7)–H(O7)	0.83(2)
C(4)–H(4)	0.98(2)	C(12)–H$_a$(12)	0.89(3)
C(5)–H(5)	0.97(2)	C(12)–H$_b$(12)	0.96(2)
C(6)–H(6)	0.99(2)	C(12)–H$_c$(12)	1.02(2)
C(7)–H$_a$(7)	0.97(2)	C(21)–H$_a$(21)	0.96(3)
C(7)–H$_b$(7)	0.94(2)	C(21)–H$_b$(21)	1.01(3)
O(4)–H(O4)	0.80(2)	C(21)–H$_c$(21)	1.04(3)
C(2)–C(3)–H$_a$(3)	111(1)	O(7)–C(7)–H$_b$(7)	108(1)
C(2)–C(3)–H$_b$(3)	107(1)	H$_a$(7)–C(7)–H$_b$(7)	110(2)
C(4)–C(3)–H$_a$(3)	109(1)	C(4)–O(4)–H(O4)	109(2)
C(4)–C(3)–H$_b$(3)	109(1)	C(5)–O(5)–H(O5)	104(2)
H$_a$(3)–C(3)–H$_b$(3)	109(2)	C(7)–O(7)–H(O7)	112(2)
C(3)–C(4)–H(4)	111(1)	O(11)–C(12)–H$_a$(12)	108(2)
C(5)–C(4)–H(4)	107(1)	O(11)–C(12)–H$_b$(12)	112(1)
O(4)–C(4)–H(4)	109(1)	O(11)–C(12)–H$_c$(12)	110(1)
C(4)–C(5)–H(5)	107(1)	H$_a$(12)–C(12)–H$_b$(12)	105(2)
C(6)–C(5)–H(5)	110(1)	H$_a$(12)–C(12)–H$_c$(12)	108(2)
O(5)–C(5)–H(5)	111(1)	H$_b$(12)–C(12)–H$_c$(12)	114(2)
C(5)–C(6)–H(6)	110(1)	O(20)–C(21)–H$_a$(21)	115(2)
C(7)–C(6)–H(6)	108(1)	O(20)–C(21)–H$_b$(21)	110(2)
O(6)–C(6)–H(6)	106(1)	O(20)–C(21)–H$_c$(21)	108(2)
C(6)–C(7)–H$_a$(7)	108(1)	H$_a$(21)–C(21)–H$_b$(21)	110(3)
C(6)–C(7)–H$_b$(7)	107(1)	H$_a$(21)–C(21)–H$_c$(21)	100(3)
O(7)–C(7)–H$_a$(7)	111(1)	H$_b$(21)–C(21)–H$_c$(21)	113(3)

TABLE 4

Mean-plane calculations. Deviations (Å) of atoms from least-squares planes defined by the equation $Ax + By + Cz - D = 0$. Atoms included in the least squares calculation are denoted by an asterisk

Plane	Coefficient A	B	C	D
I	0.4199	0.7214	−0.5507	9.0435
II	−0.5110	−0.7662	−0.3896	−13.2917
III	−0.8212	0.5700	−0.0256	−9.8364

I		II		III	
C(2) *	−0.007(2)	C(7) *	−0.079(3)	C(3) *	−0.005(2)
C(3) *	0.009(2)	C(6) *	0.062(2)	C(2) *	0.004(2)
C(5) *	−0.008(2)	O(6) *	0.002(1)	O(20) *	0.006(1)
C(6) *	0.008(2)	C(2) *	0.025(2)	C(21) *	−0.005(2)
C(4)	0.615(2)	C(1) *	0.005(2)		
O(6)	−0.625(1)	O(10) *	0.052(2)		
		O(11) *	−0.041(2)		
		C(12) *	−0.027(3)		

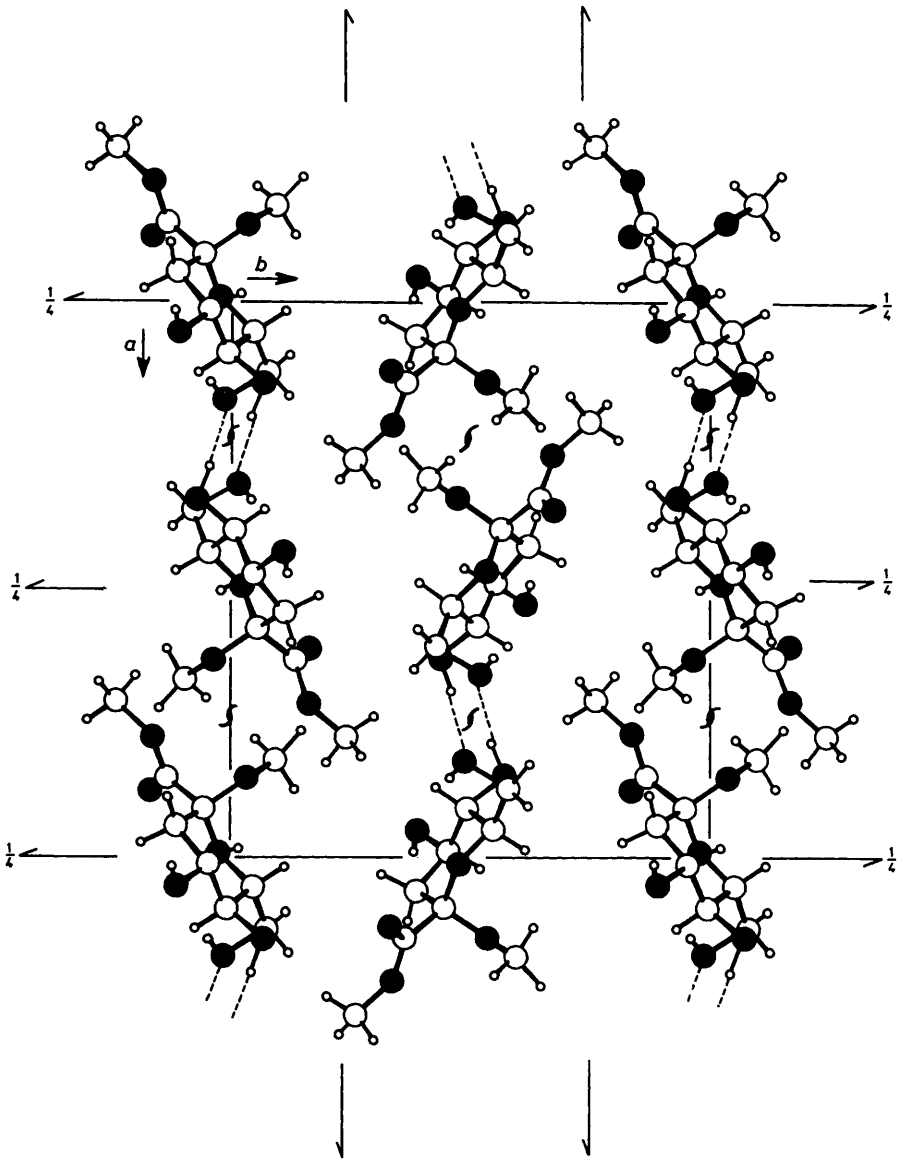


FIGURE 4 A view of the molecular packing of compound (1) along *c*. Dotted lines illustrate the hydrogen-bonding scheme. ○ = Carbon, ● = oxygen

lished by determining the sign of the Cotton effect associated with the n ⟶ π* transition of the carboxy-group.

EXPERIMENTAL

General Methods.—Evaporations were carried out under reduced pressure at <40 °C. Products were dried *in vacuo* over P_2O_5 at room temperature; solutions in organic solvents were dried with Na_2SO_4. M.p.s were determined on a Kofler hot-plate. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. C.d. spectra were obtained with a Dichrograph II (Roussel–Jouan, Paris) instrument. Thin-layer chromatography (t.l.c.) was performed on silica-gel (F 1500 LS_{254}, Schleicher and Schüll) plates; compounds were located with a spray of 10% sulphuric acid in ethanol and the plates were then heated. ^1H N.m.r. spectra at 250 MHz were recorded on a CAMECA instrument while those at 400 MHz were obtained on an instrument built by Dr. E. Kan and his colleagues in the Institut d'Electronique, Université de Paris-Sud, Orsay; tetramethylsilane (in organic solvents) and acetone (in water) were used as internal standards. ^{13}C N.m.r. spectra were recorded on a Brucker WP-60 spectrometer operating at 15.08 MHz in the Fourier-transform mode.

X-*Ray Crystal Structure of Compound* (1).—A crystal of size 0.25 × 0.25 × 0.50 mm was used. Crystal data: $C_9H_{16}O_7$, M = 236. Orthorhombic, space group $P2_12_12_1$, a = 14.409(3) Å, b = 12.302(3) Å, c = 6.547(2) Å; U = 1 160 Å^3, D_c = 1.35 g cm^{-3}; $F(000)$ = 504, λ = 1.5418 Å. Data collection: a set of three-dimensional data was collected on a four-circle diffractometer with graphite-monochromated Cu-K_α radiation. The $\theta/2\theta$ scan technique was employed. A scan speed of 0.22° s^{-1} was used with a

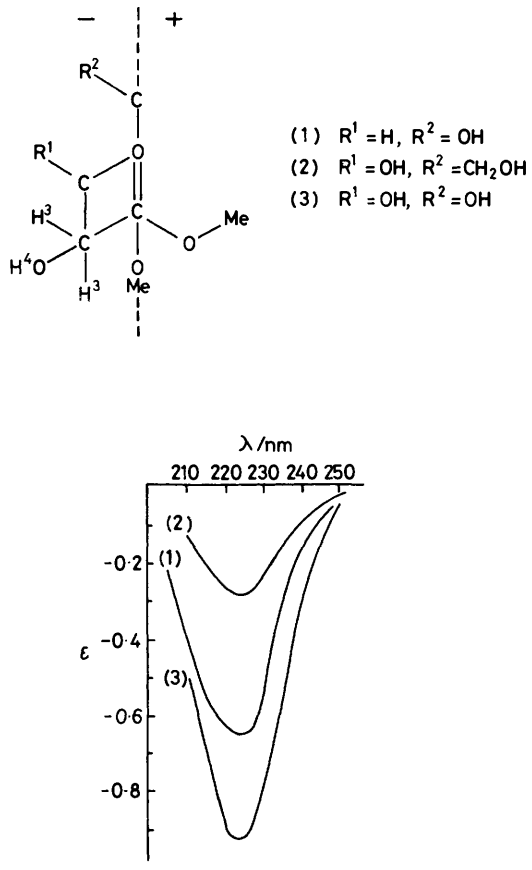
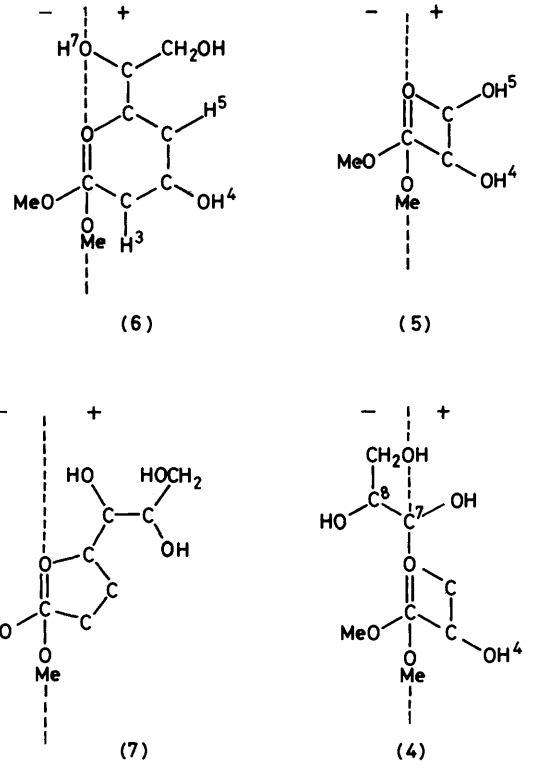


FIGURE 5 Ring-oxygen-eclipsed molecular projections of the α-glycosides (1), (2), and (3) in their experimentally observed conformations, leading to the prediction of negative Cotton effects, and the corresponding experimental c.d. curves

scan width of 1.80°. Each reflection was scanned from one to five times depending on its intensity. The background of every reflection was interpolated using a curve measured once before data collection. The number of measured reflections was 1 075, and the number of observed ones 1 002 [$I > 3\sigma(I)$]. The structure was solved by direct methods (MULTAN program [20]). The positional and anisotropic thermal parameters for non-hydrogen atoms were refined using the full-matrix least-squares method (local version of the ORFLS program [21]). The hydrogen atoms were located on difference Fourier maps. Positional and isotropic thermal parameters were refined. The scattering factors used were those of reference 22. The final discrepancy factors were $R = \Sigma(|F_o| - |F_c|)/\Sigma|F_o| = 2.91\%$, and $R_w = \Sigma_w(|F_o| - |F_c|)/\Sigma_w|F_o| = 3.23\%$ for 1 002 reflections. Observed and calculated structure factors and anisotropic thermal parameters are listed in Supplementary Publication No. SUP 2338 (10 pp.).*

Methyl 3-*Deoxy*-D-arabino-2-*heptulopyranosonate*.—A mixture of ammonium 3-deoxy-D-*arabino*-2-heptulopyranosonate [23] (2.43 g, 10 mmol), Amberlite IR-120 (H^+) resin 15 ml, which had previously been thoroughly washed with methanol, and anhydrous methanol (50 ml) was stirred for 15—20 min. The resin was filtered off and washed with anhydrous methanol, the filtrate and washings were

* For details see Notice to Authors No. 7, *J. Chem. Soc., Perkin Trans. 1*, 1981, Index issue.



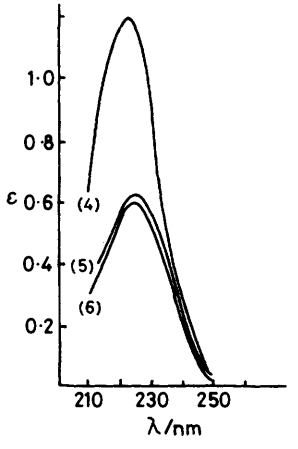


FIGURE 6 Ring-oxygen-eclipsed molecular projections of the β-glycosides (4), (5), (6), and (7) in their experimentally observed conformations, leading to the prediction of positive Cotton effects, and the corresponding experimental c.d. curves for compounds (4), (5), and (6)

combined and the solvent was removed. The residual syrup crystallised spontaneously when kept at 4 °C overnight. The crystals (2.3 g, 97.5%) were filtered off, washed with methanol–diethyl ether (1 : 4), and dried to give the *title ester*, m.p. 92 °C; $[\alpha]_D^{20}$ +43° (*c* 2, water); δ_H (250 MHz) [$(CD_3)_2SO$–$(CD_3)_2CO$ (1 : 1)] 1.7 (1 H, quin, $J_{3ax.3eq}$ 13, $J_{3ax.4}$ 11.5, and $J_{3ax.2\text{-OH}}$ 1.7 Hz, 3_{ax}-H), 2.0 (1 H, dd, $J_{3eq.3ax}$ 13 and $J_{3eq.4}$ 5 Hz, 3_{eq}-H), 3.7 (3 H, s, OMe), and 6.54 (1 H, d, $J_{2\text{-OH}.3ax}$ 1.7 Hz, 2-O*H*); the rest of the spectrum was not analysed (Found: C, 40.2; H, 6.8; O, 53.3. $C_8H_{14}O_7$·

H_2O requires C, 40.00; H, 6.7; O, 53.3%). Acetylation [Ac_2O–pyridine (1 : 1), 24 h, 20 °C] gave a *tetra-acetate* which, when crystallised from diethyl ether–hexane, had m.p. 117 °C; $[\alpha]_D^{20}$ +62° (*c* 2.4, $CHCl_3$) (Found: C, 49.4; H,5.6; O, 44.9. $C_{16}H_{22}O_{11}$ requires C, 49.2; H, 5.6); O, 45.1%.

Methyl (*Methyl* 3-*Deoxy*-α-D-arabino-2-*heptulopyranosid*)-*onate* (1).—Amberlite IR-120 (H^+) resin, thoroughly washed with anhydrous methanol and then dried (1 g), was added to a solution of the aforementioned ester (2.2 g, 9.6 mmol) in anhydrous methanol (10 ml). The mixture was boiled under reflux for 18 h, the progress of the glycoside formation being monitored by t.l.c. [ethyl acetate–benzene–methanol (7 : 1 : 2) as developer]. The cooled mixture was filtered and the residue was washed with methanol (2 × 10 ml); the combined filtrate and washings were diluted with benzene (100 ml) and the solvents were removed: the *product* (1) crystallised during concentration. Recrystallisation from methanol–ethyl acetate (5 : 100) gave crystals (1.907 g, 88%) in three successive crops, m.p. 148—149 °C; $[\alpha]_D^{20}$ +65° (*c* 1, MeOH), and a negative Cotton effect: Δε −0.67 at 224 nm; δ_H (250 MHz) (D_2O) 1.53 (1 H, dd, $J_{3ax.3eq}$ 13 and $J_{3ax.4}$ 11.5 Hz, 3_{ax}-H), 2.14 (1 H, dd, $J_{3eq.3ax}$ 13 and $J_{3eq.4}$ 5 Hz, 3_{eq}-H), 3.0 (3 H, s, 2-OMe), 3.25 (1 H, t, *J* 9.4 Hz, 5-H), 3.36 (1 H, o, *J* 9.4, 5.12, and 12.2 Hz, 6-H), 3.60 (1 H, dd, J_{gem} 12.5 and $J_{6,7}$ 5 Hz, 7-H), 3.64 (3 H, s, CO_2Me), 3.68 (1 H, dd, J_{gem} 12.5 and $J_{6,7}$ 2.2 Hz, H-7), and 3.70 (1 H, o, *J* 11.5, 9.4, and 5 Hz, 4-H). [Assignments were made on the basis of irradiation experiments; coupling constants were determined on a product obtained by performing the esterification–glycosidation reactions with CD_3OH.] δ_C (D_2O) 171.6 (C-1), 100.2 (C-2), 40.2 (C-3), 69.4, 71.5, and 75.5 (C-4, -5, and -6), 61.8 (C-7), 54.8 (CO_2-*Me*), and 52.1 p.p.m. (OMe) (Found: C, 45.6; H, 6.9. $C_9H_{16}O_7$ requires C, 45.8; H, 6.8%).

From the syrup (200 mg) remaining after evaporation of the mother-liquor, a further crop (75 mg) of compound (1) was isolated by column chromatography on silica gel [chloroform–methanol (9 : 1) as eluant], along with a mixture {85 mg; R_F (mixture)/R_F [compound (1)] 1.12} of the α- and β-methyl (methyl 3-deoxy-D-*arabino*-2-heptulofuranosid)-onates identified by ^{13}C n.m.r. δ_C (D_2O) 172.5 and 172.0 (C-1), 107.7 and 107 (C-2), 45.6 and 46.5 (C-3), 69.9, 70.5, 71.2 and 71.4 (C-4 and -6), 83.2 and 85.6 (C-5), 64.6 (C-7), 54.6 (CO_2Me), and 52.5 p.p.m. (OMe).

When treated with 0.1M sodium hydroxide (1 mol equiv.), the ester (1) gave *sodium* (*methyl* 3-*deoxy*-α-D-arabino-2-*heptulopyranosid*)*onate* as a solid, $[\alpha]_D^{20}$ +62° (*c* 0.6, water) (Found: C, 34.3; H, 6.1; Na, 8.4. $C_8H_{13}NaO_7 \cdot 2H_2O$ requires C, 34.3; H, 6.1; Na, 8.2%).

When acetylated [acetic anhydride–pyridine (1 : 1), 16 h, 20 °C] the ester (1) gave the crystalline *tetra-acetate*, m.p. 74—75 °C (diethyl ether–hexane) (Found: C, 49.8; H, 6.0. $C_{15}H_{22}O_{10}$ requires C, 49.7; H, 6.1%).

Methyl (*Methyl* 3-*Deoxy*-α-D-lyxo-2-*heptulopyranosid*)*onate* (3).—A solution of sodium periodate (162.4 mg, 0.76 mmol) in water (6 ml) was added to a solution of methyl (methyl 5-*O*-benzyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate [15] (245 mg, 0.69 mmol) in methanol (0.5 ml) and water (1 ml). The mixture was kept at 4 °C for 15 min and was then extracted with ethyl acetate (3 × 10 ml). The dried organic layer was concentrated to give a syrup which was diluted with methanol (5 ml), and the solution was treated with hydrogen (*ca.* 2 bar) and 10% Pd–carbon catalyst (10 mg). When cleavage of the benzyl group was complete [t.l.c.; chloroform–methanol (85 : 15) as developer] (*ca.* 1 h), platinum dioxide catalyst (*ca.* 15 mg) was added and hydrogenation was continued at 50 °C and 5 bar pressure until complete reduction of the aldehyde group was attained (spot test with anilinium oxalate on paper). The mixture was filtered and the filtrate was evaporated to dryness. The *product* (3) (78 mg) crystallised from ethyl acetate upon addition of trace amounts of hexane; it had m.p. 121—122 °C; $[\alpha]_D^{20}$ +96° (*c* 0.2, MeOH), and a negative Cotton effect: Δε −0.94 at 224 nm; δ_C (D_2O) 172.6 (C-1), 100.3 (C-2), 34.6 (C-3), 66.4, 68.4, and 74.5 (C-4, -5, and -6), and 62.6 p.p.m. (C-7) (Found: C, 45 80; H, 6.9. $C_9H_{16}O_7$ requires C, 45.8; H, 6.8%).

Methyl (*Methyl* 3-*Deoxy*-β-D-erythro-2-*hexulopyranosid*)-*onate* (5).—A solution of methyl 5-*O*-benzyl-3-deoxy-D-*erythro*-2-hexulosonate [15,17] (500 mg) in 0.1M hydrochloric acid in anhydrous methanol (10 ml) was kept at 50 °C for 36 h, after which time the reaction appeared to be complete [t.l.c.; ethyl acetate–hexane (80 : 50) as developer]. The mixture was neutralised with silver carbonate and filtered, and the filtrate was evaporated to dryness. The residue, dissolved in ethyl acetate, was treated with hydrogen in the presence of 10% Pd–carbon catalyst (50 mg). The mixture was filtered and the filtrate was evaporated to afford the *title compound* as a syrup (420 mg); $[\alpha]_D^{20}$ −84° (*c* 1.2, MeOH), and a positive Cotton effect: Δε +0.63 at 225 nm; δ_H (400 MHz) (D_2O) 1.76 (1 H, t, $J_{3ax.3eq}$ 12 and $J_{3ax.4}$ 12 Hz, 3_{ax}-H), 1.88 (1 H, dd, $J_{3eq.3ax}$ 12 and $J_{3eq.4}$ 4.5 Hz, 3_{eq}-H), 3.03 (3 H, s, 2-OMe), 3.66 (3 H, s, CO_2Me), 3.7 (3 H, m, 5-, 6-, and 6′-H), and 3.92 (1 H, o, $J_{4.3ax}$ 12, $J_{4.3eq}$ 4.5, and $J_{4.5}$ 3 Hz, 4-H).

Methyl (*Methyl* 3-*Deoxy*-β-D-manno-2-*octulopyranosid*)-*onate* (6).—To a solution of methyl (2,4,5,7,8-penta-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosonate [3,19] in ethanol-free, anhydrous chloroform (5 ml) was added hydrogen bromide in acetic acid (0.2 ml of a 35% w/v solution). After 1 h the acids were removed by co-distillation with anhydrous toluene under reduced pressure, and the residual syrupy bromide (unstable towards silica gel) was dissolved in anhydrous diethyl ether (5 ml). Silver carbonate (1 g), anhydrous $CaSO_4$ (drierite) and then, dropwise, anhydrous methanol (1 ml) were added in turn to the vigorously shaken ethereal mixture. After 30 min all the bromide was transformed [t.l.c.; hexane–ethyl acetate (2 : 1) as developer] into a single product (R_F 0.84). The mixture was diluted with dichloromethane, insoluble solids were filtered off, and the solution was concentrated to give a syrup (250 mg) the n.m.r. spectrum of which indicated a high state of purity. The syrup was dissolved in methanol (5 ml) and to the solution was added 0.25M barium methoxide in methanol (0.05 ml). After 1 h, t.l.c. [chloroform–methanol (85 : 15)] indicated that deacetylation was complete (R_F of product 0.28). The solution was filtered through a pad of silica gel. The neutral eluant was concentrated to dryness to give the *title compound* as an amorphous solid, $[\alpha]_D^{20}$ +64° (*c* 2, MeOH), and a positive Cotton effect; Δε +0.59 at 225 nm; δ_H (400 MHz) (D_2O) 1.78 (1 H, t, $J_{3ax.3eq}$ 13 and $J_{3ax,4}$ 13 Hz, 3_{ax}-H), 2.23 (1 H, dd, $J_{3eq.3ax}$ 13 and $J_{3eq.4}$ 4.5 Hz, 3_{eq}-H), 3.20 (3 H, s, 2-OMe), 3.41 (1 H, d, $J_{6,7}$ 9 Hz, 6-H), 3.54 (1 H, q, $J_{8,8'}$ 13 and $J_{8,7}$ 7 Hz, 8-H), 3.66 (1 H, o, $J_{4.3ax}$ 13, $J_{4.3eq}$ 4.5, and $J_{4.5}$ 3 Hz, 4-H), 3.70 (3 H, s, CO_2Me), 3.75 (2 H, m, 7- and 8′-H), and 3.82 (1 H, d, $J_{5,4}$ 3 Hz, 5-H); δ_C (D_2O) 170.7 (C-1), 100.5 (C-2), 33.86 (C-3), 66.07, 67.47, 75.19, and 70.11 (C-4, -5, -6, and -7), 64.08 (C-8), 54.1 (CO_2Me), and 52.3 p.p.m. (OMe).

Methyl (*Methyl* 3-*Deoxy*-α-D-manno-2-*octulopyranosid*)-*onate* (2).—This compound was obtained as described previously,[15] $[\alpha]_D^{20}$ +96.5° (*c* 0.48, MeOH) {lit.,[3] $[\alpha]_D^{20}$ +80° (*c* 3, water)} and a negative Cotton effect: Δε −0.29 at 224 nm.

Methyl (*Methyl* 3-*Deoxy*-β-D-manno-2-*octulofuranosid*)*onat*-(7).—This compound was prepared from its tetra-*O*-benzoyl derivative [19] by Zemplén saponification with barium methoxide as described for compound (6), followed by preparative t.l.c. [chloroform–methanol (85 : 15) as developer]. Work-up gave the title compound, $[\alpha]_D^{20}$ +13° (*c* 0.54, MeOH), and a positive Cotton effect: Δε +0.26 at 207 nm.

Methyl (*Methyl* 5-*Amino*-3,5-*dideoxy*-β-D-glycero-D-galacto-2-*nonulopyranosid*)*onate* (4).—A solution of the commercial β-methyl glycoside of neuraminic acid (30 mg) in methanol was treated with an amount of diazomethane sufficient to transform it into the methyl ester (t.l.c.). The syrup remaining after removal of the solvent was dissolved in a known amount of methanol, and the c.d. spectrum was measured on an aliquot of this solution. A positive Cotton effect was observed: Δε +1.18 at 222.5 nm.

[2/246 *Received*, 10*th February*, 1982]

REFERENCES

[1] F. M. Unger, *Adv. Carbohydr. Chem. Biochem.*, 1981, **38**, 323.

[2] A. K. Battacharjee, H. J. Jennings, and C. P. Kenny, *Biochemistry*, 1978, **17**, 645.

[3] F. M. Unger, D. Stix, and G. Schulz, *Carbohydr. Res.*, 1980, **80**, 191.

[4] G. Keilich, R. Brossmer, V. Eschenfelder, and L. Holmquist, *Carbohydr. Res.*, 1975, **40**, 255.

[5] H. J. Jennings and R. E. Williams, *Carbohydr. Res.*, 1976, **50**, 257.

[6] L. D. Melton, E. R. Morris, D. A. Rees, and D. Thom, *J. Chem. Soc., Perkin Trans. 2*, 1979, 10.

[7] I. Listowsky, G. Avigad, and S. Englard, *J. Org. Chem.*, 1970, **35**, 1080.

[8] I. Listowsky, *Trans. N. Y. Acad. Sci.*, 1972, 218.

[9] E. R. Morris, D. A. Rees, G. R. Sanderson, and D. Thom, *J. Chem. Soc., Perkin Trans. 2*, 1975, 1418.

[10] J. A. Kanters, J. Kroon, A. F. Peerdman, and J. C. Schoone, *Tetrahedron*, 1967, **23**, 4027.

[11] J. L. Flippen, *Acta Crystallogr., Sect. B*, 1973, **29**, 1881.

[12] M. Berman and S. H. Kim, *Acta Crystallogr., Sect. B*, 1968, **24**, 897.

[13] B. M. Gatehouse and B. J. Poppleton, *Acta Crystallogr., Sect. B*, 1970, **26**, 1761.

[14] M. Sundaralingam, *Biopolymers*, 1968, **6**, 189.

[15] D. Charon and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1971.

[16] F. M. Unger, D. Stix, E. Schwartzinger, G. Schulz, and F. Hammerschmid, *Carbohydr. Res.*, quoted in reference 1.

[17] A. Danan, M. Mondange, S. R. Sarfati, and P. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1275.

[18] S. Furberg, *Acta Chem. Scand.*, 1960, **14**, 1357.

[19] D. Charon and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2369.

[20] G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, 1971, **27**, 368.

[21] W. R. Busing, K. O. Martin, and M. A. Levy, ORFLS Oak Ridge National Laboratory, Report ORNL-TM-305, 1965.

[22] 'International Tables for *X*-Ray Crystallography,' Kynoch Press, Birmingham, 1974, vol. 4, pp. 72—98.

[23] D. Charon and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1175.