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# EMPIRICAL $^{13}$C-N.M.R.-CORRELATIONS BETWEEN THE *Escherichia coli* K 13 AND LP 1092 CAPSULAR POLYSACCHARIDES AND MODEL OLIGOSACCHARIDES CONTAINING D-RIBOSE AND 3-DEOXY-D-*manno*-2-OCTULOSONIC ACID

ANDRÁS NESZMÉLYI,
*Central Research Institute for Chemistry of the Hungarian Academy of Sciences, H-1025 Budapest (Hungary)*

PAUL KOSMA,
*Institut für Chemie der Universität für Bodenkultur Wien, A-1180 Vienna (Austria)*

RUDOLF CHRISTIAN, GERHARD SCHULZ, AND FRANK M. UNGER
*Sandoz Forschungsinstitut Wien, A-1235 Vienna (Austria)*

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## ABSTRACT

The proton-decoupled, Fourier-transform, $^{13}$C-n.m.r. spectra of the two anomeric sodium (methyl 3-deoxy-7-*O*-β-D-ribofuranosyl-α- and β-D-*manno*-2-octulopyranosid)onates, of the two anomeric sodium [methyl 3-deoxy-7-*O*-(2-*O*-β-D-ribofuranosyl-β-D-ribofuranosyl)-α- or -β-D-*manno*-2-octulopyranosid]onates, and of methyl 2-*O*-β-D-ribofuranosyl-β-D-ribofuranoside have been recorded. The constitutions of these compounds correspond to repeating units and partial structures of the capsular polysaccharides from *Escherichia coli* K 13, K 20, K 23, and LP 1092 strains. The $^{13}$C-n.m.r.-line patterns of these oligosaccharide derivatives and the corresponding polysaccharides show striking differences dependent upon the anomeric configurations of the KDO residues. These differences may be used for the identification, by visual or computer-assisted pattern analysis, of the anomeric configurations of KDO-residues in oligo- or poly-saccharides. Thus, it was confirmed that the KDO residues in the K 13, K 20, and K 23 polysaccharides have the β anomeric configuration, whereas those in the LP 1092 polysaccharide have the α anomeric configuration.

## INTRODUCTION

Several n.m.r.-spectroscopic approaches have been explored toward the identification of the anomeric configurations of 3-deoxy-D-*manno*-2-octulopyranosylonic acid (KDO) residues in oligo- and poly-saccharides. The parameters determined for this purpose include the $^{1}$H-n.m.r.-chemical shifts[1–9] of the (equatorial) H-3, the $^{13}$C-n.m.r.-chemical shifts[10–12] of the (carboxylate) C-1 or the (anomeric)

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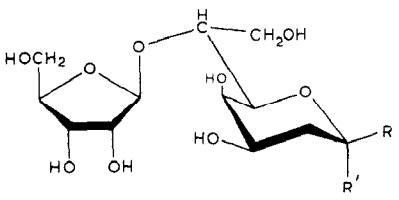
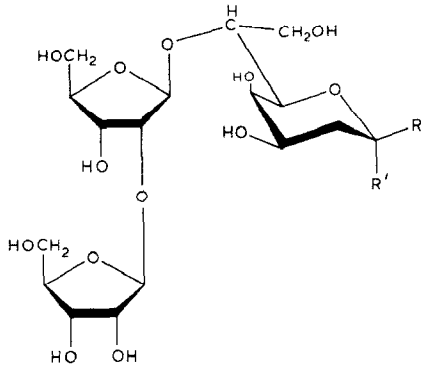
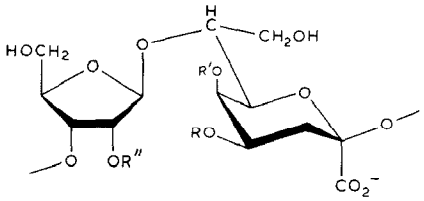
C-2, and the three-bond, heteronuclear coupling constants $^3J_{H\text{-}3a,C\text{-}1}$ as observed in proton-coupled, $^{13}$C-n.m.r.-spectra[1,2,4,5]. In addition, empirical correlations between the shift values of H-4 in the $^1$H-n.m.r.-spectra of ketosidic, *O*-acetylated KDO-derivatives show that, for the $\alpha$-ketosides, the shift values ($\delta$) of the H-4 signals are significantly larger (by ~0.4 p.p.m.) than for the $\beta$-ketosides[13]. We are reporting herein characteristic differences between the $^{13}$C-n.m.r.-spectra of the pair of model disaccharides **1** and **2**, and of the pair of model trisaccharides **3** and **4**, each pair differing only in the anomeric configurations of the respective KDO residues[14].



**1** R = $CO_2^-Na^+$, R′ = OMe
**2** R = OMe, R′ = $CO_2^-Na^+$



**3** R = $CO_2^-Na^+$, R′ = OMe
**4** R = OMe, R′ = $CO_2^-Na^+$



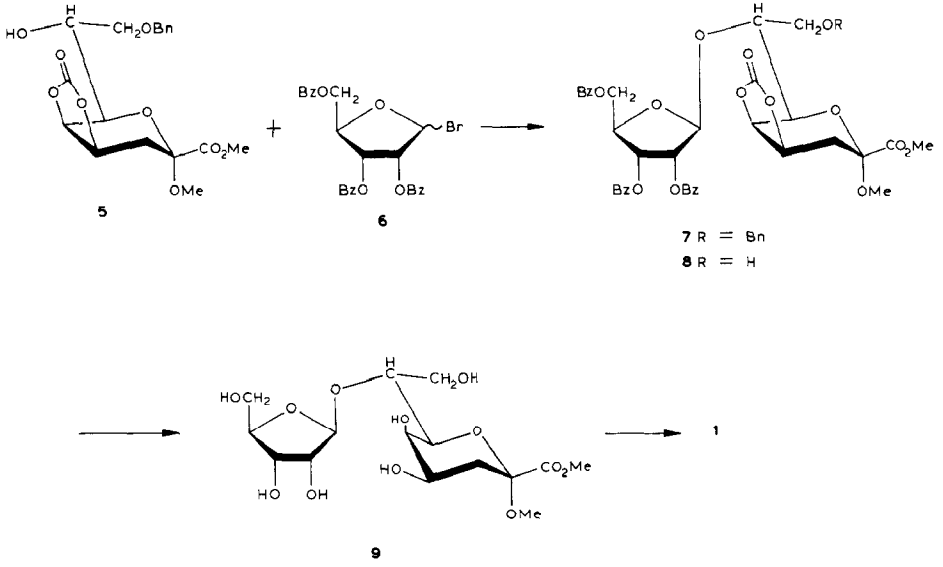
Scheme 1. Backbone structure common to the capsular polysaccharides from *E. coli* strains K 13, K 20, and K 23. The unsubstituted structure corresponds to the K 23-antigen[9]. The K 13-antigen is acetylated at residue O-4 (R) or -5 (R′) of the KDO residue[15], and the K 20-antigen is acetylated at position O-2 (R″) of the ribofuranosyl residue.

The resulting spectral differences are sufficiently pronounced to be diagnostic of the anomeric configurations of KDO residues in oligosaccharides or regular polysaccharides. The reliability of this criterion is demonstrated by its use to confirm independently the $\beta$-D anomeric configuration of the KDO residues in the *O*-deacetylated K-13-antigen (Scheme 1) of *Escherichia coli*, as previously identified[4]. The usefulness of such pattern analyses[16] is exemplified by the conclusive assignment of the $\alpha$ anomeric configuration of the KDO residues in a capsular polysaccharide[17] from *E. coli* strain LP 1092.

After completion of this work, an investigation based on circular-dichroism spectroscopy[18] has similarly assigned the $\alpha$ anomeric configuration to the KDO residues of the LP 1092 polysaccharide(s). The use of c.d.-spectroscopy for this purpose had been advocated previously by Charon and Szabó[19].

## RESULTS AND DISCUSSION

*O-Deacetylated K 13-antigen.* — The model disaccharide derivative **1** was synthesized from the previously described[14] methyl (methyl 8-*O*-benzyl-4,5-*O*-carbonyl-3-deoxy-$\alpha$-D-*manno*-2-octulopyranosid)onate (**5**). In analogy to our previous work[14], **5** was glycosylated at O-7 with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**6**) in the presence of silver trifluoromethylsulfonate (triflate), essentially under the modified[14] conditions of Hanessian and Banoub[20], to give crystalline **7**. Subsequent hydrogenolysis in the presence of palladium catalyst afforded the crystalline alcohol **8** in quantitative yield. Finally, **8** was subjected to Zemplén saponification to give the methyl ester **9** as a glass which was in turn saponified, by the action of 0.2M sodium hydroxide, into **1** in quantitative yield.

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The $\beta$-D anomeric configuration of the KDO-residues in the *O*-deacetylated K-13-antigen (Scheme 1) has previously been identified[4] by the chemical shift of the equatorial H-3 and the heteronuclear coupling-constant $^3J_{H\text{-}3,C\text{-}1}$ ~5 Hz. The proton-decoupled, Fourier-transform, $^{13}$C-n.m.r.-spectra of sodium (methyl 3-deoxy-7-*O*-$\beta$-D-ribofuranosyl-$\alpha$-D-*manno*-2-octulopyranosid)onate (**1**) and the corresponding, anomeric model-disaccharide derivative, sodium (methyl 3-deoxy-7-*O*-$\beta$-D-ribofuranosyl-$\beta$-D-*manno*-2-octulopyranosid)onate (**2**) have now been recorded.

TABLE I

$^{13}$C-N.M R CHEMICAL SHIFTS OF THE *O*-DEACETYLATED K 13 POLYSACCHARIDE AND OF RELATED MONO- AND DI-SACCHARIDE DERIVATIVES

| *Carbon atom* | *Compound* | | | | |
|---|---|---|---|---|---|
| | **13** | **12** | **1** | **2** | *K 13-D polysaccharide* |
| OMe | 55.92 | 52.29 | 51.47 | 52.36 | |
| 1 | | 174.48 | 176.08 | 174.42 | 174.04 |
| 2 | | 102.06 | 101.31 | 102.06 | 102.36 |
| 3 | | 35.25 | 34.97 | 35.33 | 35.22 |
| 4 | | 68.24 | 66.69 | 68.21 | 68.06 |
| 5 | | 66.17 | 66.85 | 65.95 | 65.98 |
| 6 | | 74.26 | 70.52[a] | 72.89 | 73.03[a] |
| 7 | | 69.91 | 75.32[a] | 75.40[a] | 75.82 |
| 8 | | 64.84 | 59.64 | 60.82 | 59.89 |
| 1′ | 108.72 | | 106.00 | 105.84 | 104.33 |
| 2′ | 74.97 | | 75.70[a] | 75.67[a] | 73.42[a] |
| 3′ | 71.57 | | 70.96[a] | 71.20 | 74.86 |
| 4′ | 83.62 | | 83.39 | 83.47 | 81.91 |
| 5′ | 63.55 | | 62.74 | 63.18 | 63.24 |

[a]Assignment supported by deuterium-induced shift (d.i.s.)-spectroscopy[21].

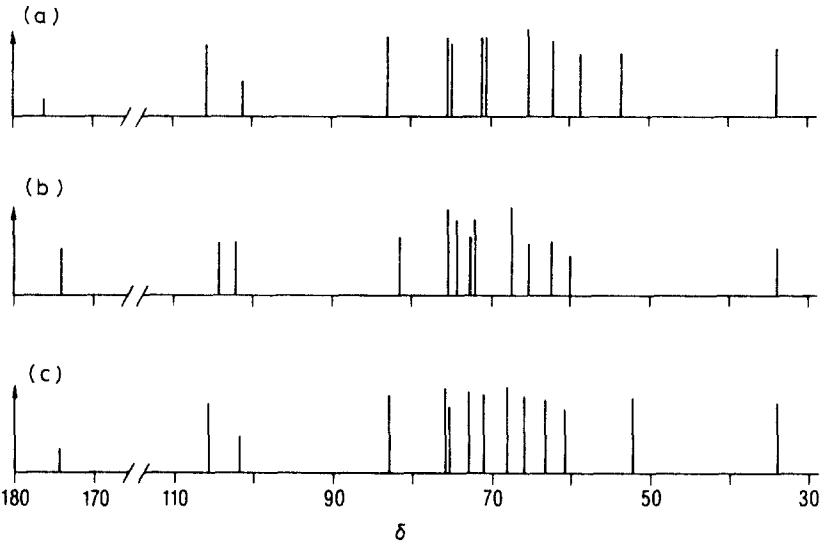
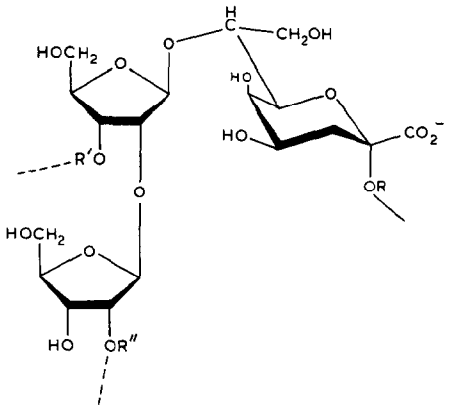



Fig. 1. Line diagram correlating the proton-decoupled, $^{13}$C-n.m.r.-spectra of: (a) **1**, (b) *O*-deacetylated K 13-polysaccharide, and (c) **2**.

The empirical assignments of the shift values, together with those previously made for the *O*-deacetylated K-13-antigen, are reported in Table I. For a diagram correlating these spectra with that of the *O*-deacetylated K-13 antigen, see Fig. 1. Whereas the shift values of C-2′–5′ (of the respective D-ribofuranosyl residues) of the model

disaccharides **1** (Fig. 1a) and **2** (Fig. 1c) are closely similar, the signals of C-1, -2, -4, and -6 are different, depending upon the anomeric configuration of the respective KDO residues (Table I and Fig. 1). The occurrence, at relatively low fields, of the C-4 and -6 signals of the β-D-ketopyranosides of KDO, as compared to those of the α-D anomers, has been noted previously[10]. It may be explained by the steric compression exerted upon H-4 and -6 by the axially-disposed carboxylate group (C-1) of the β-D anomers. This would result in deshielding of the respective carbon nuclei C-4 and -6, similar to the effect discussed by Sepulchre *et al.*[22]. Analogous observations have been made for derivatives of *N*-acetylneuraminic acid[23]. The spectrum of the *O*-deacetylated K 13 antigen (K 23 antigen) (Fig. 1b) resembles much more closely that of **2** than that of the α anomer **1**, and the spectrum of the polysaccharide may be visualized as an assembly corresponding to a polymerization of the repeating unit **2** (glycosidation shift for the C-3′-signal, +3.66 p.p.m.; β shifts for the C-2′- and -4′-signals, −2.25 and −1.56 p.p.m., respectively; Table I). Thus, consideration of these shift patterns confirms the initial assignment of the β-D anomeric configuration of the KDO residues of the polysaccharide.

*Capsular polysaccharide(s) from Escherichia coli LP 1092.* — Conflicting structural evidences[5,19] have been reported for the K(?)-antigen from the urinary pathogen, *E. coli* LP 1092. The corresponding alternative structures are illustrated in Scheme 2. Irrespective of the proposed alternative modes of polymerization of the repeating units (Scheme 2), the anomeric configuration of the KDO residues could be conclusively identified, as follows: The proton-decoupled, Fourier-trans-




Scheme 2. Alternative structures of the capsular polysaccharide(s) from *E. coli* LP 1092. The initial communication[24] on the structure of this K(?)-antigen reports data compatible with the highly branched structure β-D-Rib*f*-(1→2)-β-D-Rib*f*-(1→7)-KDO*p*-(2→3) . . . (KDO residue linked at O-3′; R = 3′-O-β-D-Rib*f*, R′ = 2-*O*-KDO*p*, and R″ = H), but no conclusion regarding the anomeric configuration of the KDO residues. In an independent investigation, Jennings *et al.*[5] concluded that the LP 1092 polysaccharide has the linear structure β-D-Rib*f*-(1→7)-KDO*p*-(2→2)-β-D-Rib*f*-(1→2)-β-D-Rib*f* . . . (KDO residue linked at O-2″; R = 2′-*O*-β-D-Rib*f*, R′ = H, and R″ = 2-*O*-KDO*p*) and suggested that the KDO residues have the α anomeric configuration. Furthermore, they considered the possibility that two closely similar, but different polysaccharides may have been reported in refs. 24 and 5. This is supported by minor but distinct differences in the $^{13}$C-n.m.r.-spectra[4,5].

TABLE II

$^{13}$C-N M R CHEMICAL SHIFTS OF THE CAPSULAR POLYSACCHARIDE FROM *E. coli* LP 1092 AND OF RELATED MONO-, DI-, AND TRI-SACCHARIDE DERIVATIVES

| *Carbon atom* | *Compound* | | | | |
|---|---|---|---|---|---|
| | **10** | **11** | **3** | **4** | *LP 1092 polysaccharide* |
| OMe | 56.3 | 51.42 | 51.55 | 52.42 | |
| 1 | | 176.11 | 176.06 | 174.44 | 175.62 |
| 2 | | 101.29 | 101.41 | 102.19 | 100.58 |
| 3 | | 34.87 | 35.07 | 35.42 | 35 19 |
| 4 | | 66.77 | 66.79 | 68.30 | 66.64[a] |
| 5 | | 67.11 | 66.97 | 66.09 | 66.64[a] |
| 6 | | 72.16 | 70 65[b] | 73.02 | 70.56[b] |
| 7 | | 70.16 | 75.62[b] | 75.78 | 75.22 |
| 8 | | 63.89 | 60 02 | 61 13 | 59.68 |
| 1′ | 107.67 | | 104.79 | 104.78 | 104 32 |
| 2′ | 81.31 | | 82.26 | 82.00 | 82.93 |
| 3′ | 71.10 | | 70.49[b] | 70.69 | 70.41[b] |
| 4′ | 84.12 | | 83.64 | 83.76 | 83 57 |
| 5′ | 63 44 | | 62.63 | 63.09 | 62.47 |
| 1″ | 108.64 | | 108.79 | 108.70 | 107 67 |
| 2″ | 75 40 | | 75.40[b] | 75.44 | 76.76 |
| 3″ | 71.66 | | 71.64[b] | 71.71 | 71.33[b] |
| 4″ | 83.89 | | 83.87 | 83.92 | 84 61 |
| 5″ | 63.76 | | 63.70 | 63.80 | 63.33 |

[a]Signal not resolved. [b]Assignment supported by d.i s.-spectroscopy[21].

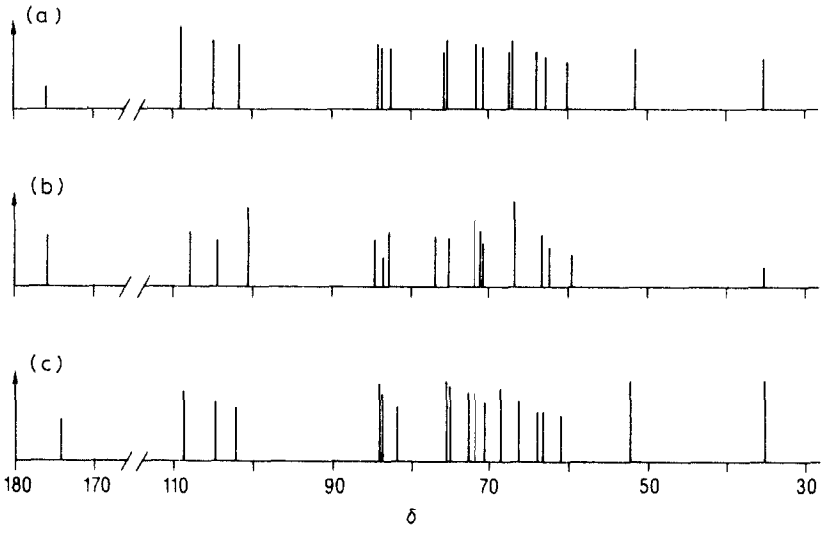
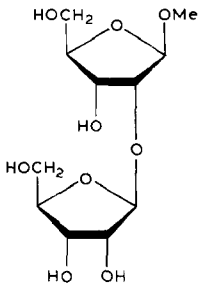
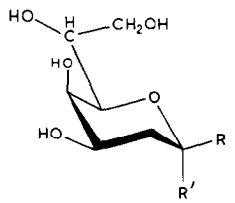



Fig. 2. Line diagram correlating the proton-decoupled, $^{13}$C-n.m.r.-spectra of: (a) **3**, (b) capsular polysaccharide[17] from *E. coli* LP 1092, and (c) **4**.

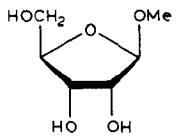
form, $^{13}$C-n.m.r.-spectra of the chemically synthesized[14] repeating-unit trisaccharides sodium [methyl 3-deoxy-7-*O*-(2-*O*-β-D-ribofuranosyl-β-D-ribofuranosyl)-α- (**3**) and -β-D-*manno*-2-octulopyranosid]onate (**4**), as well as the one of methyl 2-*O*-β-D-ribofuranosyl-β-D-ribofuranoside (**10**), were recorded, and the empirical assignments of the shift values are reported in Table II. A line diagram (Fig. 2) correlates these spectra (Figs. 2a and 2c, respectively) with that (Fig. 2b) of the capsular polysaccharide from *E. coli* LP 1092 (Scheme 2), and shows that the spectrum of the LP 1092 polysaccharide resembles that of **3** (Fig. 4c). The anomeric configuration of the KDO residues of the LP 1092 polysaccharide is thus identified as α-D, in accord with the suggestion[5] and conclusions[18] of Jennings and assoc. Together with the identification of the α anomeric configuration of the KDO-residues in lipopolysaccharide (LPS) from *E. coli*[11,12] and *Salmonella* strains[25,26], this finding establishes that, in some natural polysaccharides, KDO residues may have the α, in others the β anomeric configuration. At the same time, earlier speculations on the exclusive occurrence of β-ketosides of KDO[3] are disproved.




**10**




**11** R = $CO_2^-Na^+$, R' = OMe
**12** R = OMe, R' = $CO_2^-Na^+$




**13**

*Glycosylation shifts and β shifts associated with the linkages β-D-Ribf-(1→7)-α- or -β-KDO and β-D-Ribf-(1→2)-β-D-Ribf.* — The information assembled in Tables I and II enables the delineation of empirical glycosylation and β shifts associated with the linkages of β-D-ribofuranosyl groups to O-7 of KDO and to O-2 of β-D-ribofuranosyl residues. Glycosylation of O-7 of KDO residues with β-D-ribofuranosyl groups resulted in $\Delta\delta$ +5.3 ±0.2 of the C-7 signals. When O-2 of a β-D-ribofuranosyl group was in turn glycosylated with a β-D-ribofuranosyl group, a corresponding $\Delta\delta$ +5.7 ±0.2 was observed (Table III). Glycosylation with a β-D-ribofuranosyl group at O-7 of a KDO residue affected the resonances of the adjacent C-8 and -6, resulting in $\Delta\delta$ values of −4.0 ±0.3 (C-8) and −1.4 ±0.3 (C-6). The resonances of C-1 and -3 of a β-D-ribofuranosyl group, upon glycosylation with a β-D-ribofuranosyl group at O-2, showed β shifts of −1.1 ±0.1 (C-1) and −0.5 ±0.1 (C-3).

TABLE III

GLYCOSIDATION- AND β-SHIFTS

| *Compound* | *C-6* | *C-7* | *C-8* |
|---|---|---|---|
| **2** | 72.89 | 75.40 | 60.82 |
| **12** | 74.26 | 69.91 | 64.84 |
| Difference | −1.37 | +5.49 | −4.02 |
| **4** | 73.02 | 75.78 | 61.13 |
| **12** | 74.26 | 69.91 | 64.84 |
| Difference | −1.24 | +5.87 | −3.71 |
| **1** | 70.52 | 75.32 | 59.64 |
| **11** | 72.16 | 70.16 | 63.89 |
| Difference | −1.64 | +5.16 | −4.25 |
| **3** | 70.65 | 75.62 | 60.02 |
| **11** | 72.16 | 70.16 | 63.89 |
| Difference | −1.51 | +5.46 | −3.87 |
| | *C-1* | *C-2* | *C-3* |
| **10** | 107.67 | 81.31 | 71.10 |
| **13** | 108.72 | 74.97 | 71.57 |
| Difference | −1.05 | +6.34 | −0.47 |
| **3** | 104.79 | 82.26 | 70.49 |
| **1** | 106.00 | 75.70 | 70.96 |
| Difference | −1.21 | +6.56 | −0.47 |
| **4** | 104.78 | 82.00 | 70.69 |
| **2** | 105.84 | 75.67 | 71.20 |
| Difference | −1.06 | +6.33 | −0.51 |

EXPERIMENTAL

*General methods.* — See ref. 14.

*$^{13}$C-N.m.r. spectroscopy.* — $^{13}$C-N.m.r. spectra were recorded with a Bruker WH 250 instrument at 62.9 MHz for solutions in deuterium oxide at 24° using 32 k of memory and a spectral width of 12 kHz. The instrument was operated in the F.t. mode with complete proton-decoupling. Chemical shifts ($\delta$) are given from the signal of tetramethylsilane whose resonance frequency was set at 67.40 p.p.m. upfield from an external signal of 1,4-dioxane in deuterium oxide. D.i.s. (deuterium-induced shift)-spectra were recorded as described in ref. 21.

*Methyl [methyl 8-O-benzyl-4,5-O-carbonyl-3-deoxy-7-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-α-D-manno-2-octulopyranosid]onate* (**7**). — A solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[27] (**6**; 500 mg) in dichloromethane (5 mL) was added dropwise at −25° during 5 min into a suspension of **5** (210 mg), silver

trifluoromethylsulfonate (257 mg), Drierite (300 mg), and molecular sieves 4A (200 mg) in dichloromethane (5 mL) under dry nitrogen. After 20 min, pyridine (0.2 mL) was added. The mixture was diluted with dichloromethane (25 mL), and washed with water, 5% aqueous $Na_2S_2O_3$, and water. The organic layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in dry toluene (20 mL), the solution evaporated to dryness, and the residue purified on a column of silica Lichroprep $Si_{60}$ (40–60 $\mu$m) (Merck Lobar prefabricated column, size B, 310 × 25 mm; 5:1 toluene–ethyl acetate) to afford **7** (290 mg, 66%), colorless crystals, m.p. 121–122° (ethyl acetate–pentane), $[\alpha]_D^{20}$ +30.3° (*c* 1.0, chloroform); $R_F$ 0.62 (2:1 toluene–ethyl acetate); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.82 (dd, 1 H, $J_{3a,3e}$ ~16.0, $J_{3a,4}$ ~3.5 Hz, H-3*a*), 2.69 (dd, 1 H, $J_{3e,4}$ ~4.0 Hz, H-3*e*), 3.16 (s, 3 H, $CH_3O$), 3.65 (dd, 1 H, $J_{8a,8b}$ ~11.0, $J_{8a,7}$ ~5.0 Hz, H-8a), 3.8 (s, 3 H, $CO_2CH_3$), 3.82–3.88 (m, 2 H, H-8b,6), 4.25 (ddd, 1 H, $J_{8b,7}$ ~2.0, $J_{7,6}$ ~9.0 Hz, H-7), 4.52 (dd, 1 H, $J_{5'a,5'b}$ ~12.0, $J_{5'a,4'}$ ~5.0 Hz, H-5'a), 4.68–4.77 (m, 4 H, H-4,4', $CH_2O$), 4.84 (dd, 1 H, $J_{5'b,4'}$ ~4.0 Hz, H-5'b), 4.98 (dd, 1 H, $J_{5,4}$ ~9.0 Hz, H-5), 5.49 (d, 1 H, $J_{1',2'}$ ~1.0 Hz, H-1'), 5.72 (dd, 1 H, $J_{2',3'}$ ~5.0 Hz, H-2'), 5.86 (dd, 1 H, $J_{3',4'}$ ~7.5 Hz, H-3'), 7.15–7.65 (m, 14 H) and 7.87–8.10 (m, 6 H, arom.).

*Anal.* Calc. for $C_{44}H_{42}O_{16}$: C, 63.9; H, 5.1. Found: C, 63.8; H, 5.1.

*Methyl [methyl 4,5-O-carbonyl-3-deoxy-7-O-(2,3,5-tri-O-benzoyl-β-*D*-ribofuranosyl)-β-*D*-manno-2-octulopyranosid]onate* (**8**). — A solution of **7** (210 mg) in 1:1 methanol–ethyl acetate (30 mL) was hydrogenolyzed in the presence of PdO (80 mg) at atmospheric pressure for 48 h. The mixture was filtered and the filtrate evaporated. The residue was chromatographed on a column of silica gel (size B, 2:1 toluene–ethyl acetate) to afford **8** (190 mg, ~100%), colorless crystals, m.p. 95–97° (dec., from diethyl ether–ethyl acetate), $[\alpha]_D^{20}$ +33.3° (*c* 0.94, chloroform); $^1$H-n.m.r. ($CDCl_3$): 1.88 (dd, 1 H, $J_{3a,3e}$ ~16.0, $J_{3a,4}$ ~3.5 Hz, H-3*a*), 2.16 (dd, 1 H, $J_{8a,OH}$ ~5.5 $J_{8b,OH}$ ~8.0 Hz, OH), 2.70 (dd, 1 H, $J_{3e,4}$ ~3.5, H-3*e*), 3.23 (s, 3 H, $CH_3O$), 3.72 (ddd, 1 H, $J_{8b,8a}$ ~12.5, $J_{8b,7}$ ~4.0 Hz, H-8b), 3.78 (dd, 1 H, $J_{6,7}$ ~9.0, $J_{6,5}$ 1.5 Hz, H-6), 3.81 (s, 3 H, $CO_2CH_3$), 3.96 (ddd, 1 H, $J_{8a,7}$ ~3.0 Hz, H-8a), 4.13 (ddd, 1 H, H-7), 4.51 (dd, 1 H, $J_{5'a,5'b}$ ~12.0, $J_{5'b,4'}$ ~4.5 Hz, H-5'b), 4.73 (ddd, 1 H, $J_{4,5}$ ~8.5 Hz, H-4), 4.75 (ddd, 1 H, $J_{4',5'a}$ ~3.5, $J_{4',3'}$ ~6.5 Hz, H-4'), 4.90 (dd, 1 H, H-5'a), 5.05 (dd, 1 H, H-5), 5.49 (d, 1 H, $J_{1',2'}$ ~1.5 Hz, H-1'), 5.67 (dd, 1 H, $J_{2',3'}$ ~5.0 Hz, H-2'), 5.84 (dd, 1 H, H-3'), 7.36–7.64 (m, 9 H) and 7.95–8.11 (m, 6 H, arom.).

*Anal.* Calc. for $C_{37}H_{36}O_{16}$: C, 60.3; H, 4.9. Found: C, 59.9; H, 4.9.

*Methyl (methyl 3-deoxy-7-O-β-*D*-ribofuranosyl-α-*D*-manno-2-octulopyranosid)onate* (**9**) *and sodium (methyl 3-deoxy-7-O-β-*D*-ribofuranosyl-α-*D*-manno-2-octulopyranosid)onate* (**1**). — A suspension of **8** (104 mg) in dry methanol (20 mL) was treated with 0.2M methanolic sodium methoxide (1 mL) for 18 h at room temperature. The solution was made neutral with Dowex 50 ($H^+$) cation-exchange resin, filtered, and the filtrate evaporated to dryness. The residue was extracted with 5-mL portions of diethyl ether and dried to give **8** (54 mg, ~100%), colorless glass, $[\alpha]_D^{20}$ +27.2° (*c* 1.0, methanol); $^1$H-n.m.r.: (90 MHz, $D_2O$): $\delta$ 1.78–2.12 (m, 2 H,

H-3*a*,3*e*), 3.28 (s, 3 H, $CH_3O$), 3.88 (s, 3 H, $CO_2CH_3$), 3.60–4.35 (m, 11 H, H-4,5,6,7,8a,8b,2′,3′,4′,5′a,5′b), and 5.30 (s, 1 H, H-1′).

A solution of **8** (43 mg) in water (5 mL) was treated with 0.2M aqueous NaOH (0.8 mL) for 2 h at room temperature. The mixture was acidified with Dowex 50 ($H^+$) ion-exchange resin to pH 3, filtered, and the filtrate titrated to pH 7.5 with 20mM NaOH. Lyophilization and subsequent purification of the residue on Bio-Gel P-2 afforded **1** (44 mg, ~100%), colorless glass, $[\alpha]_D^{20}$ +22.5° (*c* 1.2, water); $^1$H-n.m.r. (250 MHz, $D_2O$): $\delta$ 1.78 (dd, 1 H, $J_{3a,3e}$ ~14.0, $J_{3a,4}$ ~12.5 Hz, H-3*a*), 2.03 (dd, 1 H, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 3.18 (s, 3 H, $OCH_3$), 3.54–4.10 (m, 9 H, H-4,5,6,7,8a,8b,4′,5′a,5′b), 4.15 (dd, 1 H, $J_{2',3'}$ ~5.0, $J_{1',2'}$ ~1.0 Hz, H-2′), 4.25 (dd, 1 H, $J_{3',4'}$ ~7.5 Hz, H-3′), and 5.29 (d, 1 H, H-1′).

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