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# SYNTHESIS OF METHYL PYRANOSIDES AND FURANOSIDES OF 3-DEOXY-D-*manno*-OCT-2-ULOSONIC ACID (KDO) BY ACID-CATALYSED SOLVOLYSIS OF THE ACETYLATED DERIVATIVES

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## ABSTRACT

Treatment of methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosonic acid, or its methyl ester, with refluxing methanolic 0.1M hydrogen chloride for 16 h gave 95% of methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate. Acetylation of the methyl ester of 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO) gave mainly methyl 2,4,6,7,8-penta-*O*-acetyl-3-deoxy-α,β-D-*manno*-oct-2-ulofuranosonate. Treatment of this mixture with methanolic 0.02M hydrogen chloride at room temperature gave methyl (methyl 3-deoxy-α,β-D-*manno*-oct-2-ulofuranosid)onate and the corresponding 4-acetates which were isolated by reverse-phase column chromatography of their 7,8-*O*-isopropylidene derivatives. Confirmation of the position of the isopropylidene group was obtained by acetylation to give methyl (methyl 4,6-di-*O*-acetyl-3-deoxy-7,8-*O*-isopropylidene-α,β-D-*manno*-oct-2-ulofuranosid)onate. The furanose anomers were differentiated primarily by $J_{3,4}$ values (α ~6.1 Hz, β ~2.2 Hz). The anomeric configuration in the furanose series has been assigned on the basis of optical rotation.

## INTRODUCTION

In connection with structural studies of lipopolysaccharides of Gram-negative bacteria, reference glycosides of 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO) were required. Published procedures[1,2] for the synthesis of the methyl pyranosides of KDO involve the treatment of the glycosyl chloride of methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-oct-2-ulopyranosonate with methanol and silver carbonate to give methyl (methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-D-*manno*-oct-2-ulopyranosid)onate, from which the corresponding α-anomer can be obtained by acid-catalysed methanolysis.

Little attention has been given to the methyl furanosides of KDO. There has been a suggestion[3] that KDO may exist as a furanoside in the lipopolysaccharide of *Bordetella pertussis*, but only one reference furanoside, methyl (methyl 4,6,7,8-tetra-*O*-benzoyl-3-deoxy-D-*manno*-oct-2-ulofuranosid)onate, has been synthesised[4] and designated β on the basis of c.d. data[5].

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We now report an improved synthesis of KDO and routes to the methyl α-pyranoside and furanosides by solvolysis of the acetylated derivatives.

## RESULTS AND DISCUSSION

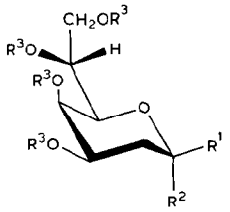
Several approaches to the synthesis of KDO have been developed[6–8], but methods based on the Cornforth procedure[9,10], although not achieving optimum yield, are simpler and more commonly used. These methods involve the coupling of oxaloacetic acid with D-arabinose followed by decarboxylation. The reaction consumes base and is slow below pH 11. Hence, base is added to maintain pH 11, but excessively basic conditions lead to side reactions. The procedure is simplified when sodium carbonate buffer is used and consistently improved yields (36–40%) of pure product are obtained.

In an attempt to produce the methyl α-pyranoside of KDO by a more direct route, mercuric salts[11,12] were used as catalyst for the reaction of the glycosyl bromide of methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-oct-2-ulopyranosonate with methanol. The lack of participating groups at C-3 indicates that attack by a methoxyl group should occur equally well at either the α- or β-face, especially when the anomeric carbonium ion is stabilised by the strongly complexing anions of the mercuric salts[11]. This procedure gave the methyl β-pyranoside (**5**) and a glycal (**19**). The glycal has been synthesised by the reaction of the glycosyl chloride of methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosonate with silver(I) diphenyl phosphate[13] and by the reaction of methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosidonate (**3**) with trimethylsilyl triflate[14]. However, in our hands, the bromide underwent spontaneous elimination.

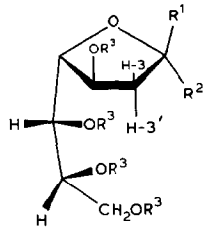
As a first attempt to synthesise the methyl furanosides of KDO, methyl 2,4,6,7,8-penta-*O*-acetyl-3-deoxy-β-D-*manno*-oct-2-ulofuranosonate was converted into the corresponding bromide. The $^1$H-n.m.r. spectrum of the crude bromide showed the presence of large amounts of the furan **20**. Treatment of the crude bromide with methanol in the presence of mercuric salts gave a single product, the $^1$H-n.m.r. spectrum of which was consistent with the furan structure **20**, indicating that a double elimination had occurred. Evidently, the anomeric bromides are not likely to be satisfactory intermediates for synthesis of methyl glycosides of KDO.

In view of the known quantitative formation of the methyl α-pyranoside of KDO from the β-pyranoside during acid-catalysed solvolysis[1,2], this method was investigated as a means to produce the methyl α-pyranoside more directly. However attempts to prepare the glycosides by using ammonium KDO gave intractable products.

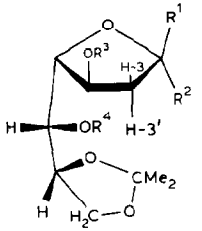
Glycosides have been formed from 1-*O*-acylglycoses by acid catalysis with methanesulphonic acid[15]and toluene-*p*-sulphonic acid[16], and from acetylated sugars by using mercuric cyanide[17], zinc chloride[18], boron trifluoride–etherate[19], toluene-*p*-sulphonic acid[20], trimethylsilyl trifluoromethanesulphonate[21], and methanolic hydrogen chloride[22]. Loss of non-anomeric acyl groups does not occur when the reactions are of short duration[17–20].



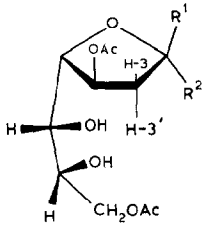
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 2 | COOH | OAc | Ac |
| 3 | COOMe | OAc | Ac |
| 4 | OAc | COOMe | Ac |
| 5 | OMe | COOMe | Ac |
| 6 | COOMe | OMe | H |



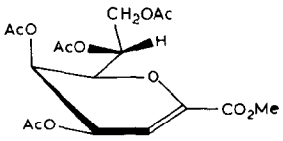
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 7 | COOMe | OAc | Ac |
| 8 | OAc | COOMe | Ac |
| 17 | COOMe | OMe | H |
| 18 | OMe | COOMe | H |



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 9 | COOMe | OMe | H | H |
| 10 | OMe | COOMe | H | H |
| 11 | COOMe | OMe | Ac | H |
| 12 | OMe | COOMe | Ac | H |
| 13 | COOMe | OMe | Ac | Ac |
| 14 | OMe | COOMe | Ac | Ac |



| | $R^1$ | $R^2$ |
|---|---|---|
| 15 | COOMe | OMe |
| 16 | OMe | COOMe |



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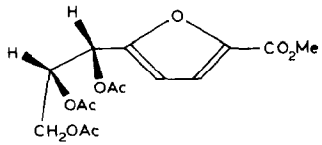

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Dry, refluxing, methanolic 0.1M hydrogen chloride converted methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosonic acid (**2**), or its methyl ester (**3**), into methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)-onate (**6**) in greater than 95% yield. Forcing conditions were used to maximise the yield of the thermodynamically more stable α-anomer[1,2]. The single-pot reaction

provides a route to the α-series of KDO in two, almost quantitative, steps. Attempts to synthesise the corresponding β-anomer using methanolic 0.02M hydrogen chloride at room temperature gave a complex mixture, and very low yields of purified product were obtained.

Entry to the furanose series was achieved by acetylation of the methyl ester of KDO. The original report[4] indicated that only one acetylated furanose methyl ester was isolated but, in our hands, the α,β-pyranose (**3** and **4**) and α,β-furanose (**7** and **8**) forms were obtained and t.l.c. indicated the presence of ~70% of furanose derivatives. The furanose and pyranose derivatives could be separated by column chromatography, as could the α- and β-furanose derivatives, but this was not essential for subsequent synthesis. The furanose derivatives have very similar $^1$H-n.m.r. spectra and the presence of the furanose ring system is confirmed by the downfield position of the H-6 signal relative to that of H-5 (Table I). The $^{13}$C-n.m.r. spectrum of methyl 2,4,6,7,8-penta-*O*-acetyl-3-deoxy-β-D-*manno*-oct-2-ulofuranosonate (**8**) (Table II) is similar to that previously reported[4], but our assignment of resonances, based on selective proton decoupling, differs. The $^1$H- and $^{13}$C-n.m.r. spectra of **3** are in accord with previous results[1,2], and the $^1$H-n.m.r. spectrum of the β-anomer **4** contains a characteristic pseudo triplet for H-3 and a chemical shift separation of 0.15 p.p.m. between the signals of H-3 and H-3′.

Solvolysis of a mixture of the furanose penta-acetates **7** and **8** with methanolic 0.02M hydrogen chloride, conditions selected to minimise the formation of the more-stable pyranose derivatives, gave (n.m.r. data of the crude product) a mixture of the methyl furanosides together with some 4-*O*-acetyl derivatives.

Neither the methyl α- and β-furanosides nor the corresponding acetylated derivatives could be separated by chromatography. The tetrabenzoates were resolved on a microgram scale by reverse-phase h.p.l.c., but preparative separation was impracticable because of the low solubility of the derivatives in the eluant.

Treatment of the furanoside mixture with 2,2-dimethoxypropane gave a complex mixture of products, but reaction with copper sulphate in acetone gave methyl (methyl 3-deoxy-7,8-*O*-isopropylidene-α,β-D-*manno*-oct-2-ulofuranosid)-onate (**9** and **10**) and their 4-acetates (**11** and **12**) which were easily resolved by preparative reverse-phase chromatography. The location of the acetyl group was indicated by the downfield shift (~0.92 p.p.m.) of the H-4 signal in the $^1$H-n.m.r. spectra of **11** and **12** relative to that for **9** and **10** (Table I).

Confirmation of the position of the isopropylidene group was obtained by acetylation of **9**–**12** which gave methyl (methyl 4,6-di-*O*-acetyl-3-deoxy-7,8-*O*-isopropylidene-α,β-D-*manno*-oct-2-ulofuranosid)onate (**13** and **14**). All $^1$H-n.m.r. assignments were confirmed by homonuclear decoupling and the positions of the acetyl groups were indicated by the downfield shift of the signals of H-4 and H-6 relative to those in **9** and **10** (Table I).

Attempted removal of the 7,8-*O*-isopropylidene from **13** and **14**, using trifluoroacetic acid[23] or trifluoroacetic acid in ethyl acetate[24], resulted in extensive decomposition. Treatment of **13** and **14** with copper(II) chloride dihydrate in

TABLE I

PROTON CHEMICAL-SHIFT AND SPIN-COUPLING DATA AT 200 MHz FOR FURANOSE KDO DERIVATIVES[a]

| *Compound* | *Chemical shifts (δ)* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *H-3* | *H-3'* | *H-4* | *H-5* | *H-6* | *H-7* | *H-8* | *H-8'* | $COOCH_3$ | $OCH_3$ | *OAc* | $CMe_2$ |
| **7** | 2.49 | 2.81 | 5 25 | 4.51 | 5.33 | 5.20 | 4.23 | 4 35 | 3.77 | | 2.00–2.08 | |
| **8** | 2.48 | 2.87 | 5 16 | 4 57 | 5 40 | 5 24 | 4.20 | 4 39 | 3 78 | | 2 02–2 11 | |
| **9** | 2.35 | 2.57 | 4.44 | 4 09 | 3 50 | 4 05[b] | [c] | [c] | 3.81 | 3 31 | | 1 34,1.42 |
| **10** | 2.27 | 2.48 | 4.33 | 4.41 | 3 50 | 4 06[b] | [c] | [c] | 3 81 | 3.37 | | 1.33,1.40 |
| **11** | 2.43 | 2.66 | 5.34 | 4 54 | 3 64 | 4 06[b] | [c] | [c] | 3.79 | 3.32 | 1.99 | 1.29,1.35 |
| **12** | 2.28 | 2.54 | 5 26 | 4 54 | 3 68 | 4 08[b] | [c] | [c] | 3.80 | 3.31 | 2.04 | 1.30,1.37 |
| **13** | 2.26 | 2.56 | 5 12 | 4 41 | 5 21 | 4 26 | 3 85 | 4.03 | 3 76 | 3.28 | 1.99,2.08 | 1.30,1 34 |
| **14** | 2 19 | 2.49 | 4 96 | 4 41 | 5.28 | 4.29 | 3.86 | 4.00 | 3.78 | 3 31 | 2.04,2.06 | 1.31,1 36 |
| **15** | 2.45 | 2 68 | 5.38 | 4.65 | 3.65 | 3.90 | 4 25 | 4 45 | 3.83 | 3.35 | 2.03,2.09 | |
| **16** | 2 28 | 2 53 | 5.27 | 4 58 | 3.71 | 3.89 | 4.25 | 4 40 | 3.81 | 3.30 | 2 06,2.09 | |
| **17**[d] | 2.35 | 2 53 | 4.54 | 4.27 | 3 70 | [e] | [e] | [e] | 3.87 | 3 31 | | |
| **18**[d] | 2 19 | 2.49 | 4.46 | 4.34 | 3.73 | [e] | [e] | [e] | 3.80 | 3.34 | | |
| **19** | 7.10 | | 6 49 | | 6.04 | 5.51 | 4.25 | 4 30 | 3.86 | | 1 99–2.07 | |

| *Compound* | *Spin couplings (Hz)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $J_{3,3'}$[f] | $J_{3,4}$ | $J_{3',4}$ | $J_{4,5}$ | $J_{5,6}$ | $J_{6,7}$ | $J_{7,8}$ | $J_{7,8'}$ | $J_{8,8'}$[f] |
| **7** | 14 8 | 5.3 | 7.1 | 3 9 | 6.1 | 4.8 | 6.4 | 3.2 | 12 5 |
| **8** | 15.2 | 2 0 | 7.0 | 2 5 | 3.0 | 6.0 | 6 0 | 2.5 | 12 5 |
| **9** | 13.3 | 7 8 | 7.1 | 6 0 | 6.9 | 8.2 | [c] | [c] | [c] |
| **10** | 13.8 | 2 2 | 6.5 | 2.0 | 2.3 | 7.8 | [c] | [c] | [c] |
| **11** | 14.8 | 4.0 | 7.0 | 3 0 | 3 0 | ~9.6 | 6.4 | 5.0 | 8.6 |
| **12** | 14.6 | 2.0 | 7.7 | 2 5 | 1 0 | ~10.0 | 6.8 | 5.2 | 9.2 |
| **13** | 13 9 | 6.2 | 6.9 | 5.2 | 3 4 | 6.2 | 6.4 | 6.0 | 8.4 |
| **14** | 14.8 | 2.5 | 7 9 | 3.6 | 2 5 | 6 5 | 6.2 | 6.1 | 8 6 |
| **15** | 15.0 | 4.5 | 7 5 | 3.0 | 3.0 | 8.0 | 6.0 | 3.0 | 11 5 |
| **16** | 14.9 | 2.0 | 7 7 | 2.5 | 1.0 | 7.8 | 5.0 | 3 0 | 10.0 |
| **17** | 13.9 | 6.5 | 6 7 | 5.5 | 2.4 | [e] | [e] | [e] | [e] |
| **18** | 14.5 | 2 5 | 7.7 | 4 0 | 1.9 | [e] | [e] | [e] | [e] |
| **19** | | 3.4 | | | | 6.5 | 5 6 | 3.8 | 12 4 |

[a]All spectra recorded for solutions in $CDCl_3$ except where indicated. [b]Centre of resonance estimated by decoupling. [c]Congested signals between 4.00–4 10 p p m. [d]Recorded for solutions in $D_2O$. [e]Congested signals between 3 60–3.90 p.p.m. [f]The signs of the geminal couplings were not determined experimentally, but are assumed to be negative

TABLE II

CARBON-13 CHEMICAL-SHIFT DATA AT 50.3 MHz FOR FURANOSE KDO DERIVATIVES[a]

| *Compound* | *Chemical shifts (δ)* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *C-1* | *C-2* | *C-3* | *C-4* | *C-5* | *C-6* | *C-7* | *C-8* | $COCH_3$ | $CMe_2$ | $COOCH_3$ | $OCH_3$ | $CMe_2$ | $COCH_3$ |
| **7** | 167.6 | 103.8 | 41.9 | 72.8[b] | 84.3 | 70.3[b] | 70.4[b] | 61.3 | 169.2–170.2 | | 53.1 | | | 20.6–20.8 |
| **8** | 166.0 | 105.9 | 40.4 | 74.0 | 85.4 | 69.8 | 70 3 | 61.5 | 169.7–170.5 | | 53.1 | | | 20.5–20.9 |
| **9** | 169.3 | 105.5 | 44 2 | 74.3[b] | 89.4 | 71.9[b] | 76.2[b] | 67.6 | | 109.9 | 52.8 | 51.7 | 25.2,26.6 | |
| **10** | 170.2 | 106 7 | 45.7 | 73.9[b] | 89.0 | 73.2[b] | 75.9[b] | 67.2 | | 109.5 | 53.1 | 51.7 | 25.2,26.8 | |
| **11** | 168.7 | 107.1 | 42.6 | 74.8[b] | 86.7 | 72.2[b] | 75.8[b] | 66.9 | 170.4 | 109.4 | 52.8 | 52.5 | 25.4,26.8 | 20.9 |
| **12** | 170.7 | 107.0 | 43.4 | 75.8[b] | 86.2 | 72.9[b] | 75.7[b] | 67.2 | 171.2 | 109.5 | 53.2 | 51.8 | 25.4,26.9 | 21.0 |
| **13** | 169.0 | 106.0 | 41.9 | 74.7[b] | 83.7 | 71.5[b] | 73.0[b] | 66.0 | 169 9,170.0 | 109.4 | 52.7 | 51.8 | 25.5,26.5 | 20.8 |
| **14** | 168.2 | 106.1 | 42.0 | 74.6[b] | 83.4 | 71.5[b] | 73.9[b] | 66.1 | 169.9,170.4 | 109 2 | 52.5 | 51 3 | 25.4,26.5 | 20.7,20.9 |
| **17**[c] | 172.3 | 106.7 | 44 1 | 72.2[b] | 87.3 | 70.7[b] | 70.9[b] | 63.7 | | | 54.3 | 52.3 | | |
| **18**[c] | 171.2 | 107.1 | 44.9 | 72.1[b] | 87.2 | 71.0[b] | 72.1[b] | 63.7 | | | 54.2 | 51.7 | | |

[a]All spectra recorded for solutions in $CDCl_3$ except where indicated. [b]Tentative assignments. [c]Recorded for solution in $D_2O$. Referenced to methanol (49.7 p.p.m.).

ethanol[25] and work-up using sodium hydrogencarbonate caused a 6→8 acetyl migration to give the 4,8-di-*O*-acetyl derivatives (**15** and **16**) as the sole products. The acetyl migration was avoided by using a mixed-bed resin in the work-up. The method was also satisfactory for the deprotection of methyl (methyl 3-deoxy-7,8-*O*-isopropylidene-α,β-D-*manno*-oct-2-ulofuranosid)onate (**9** and **10**). The small amount of transacylation to the ethyl ester could be avoided by using methanol as the reaction solvent.

The only consistent difference between the $^1$H-n.m.r. spectra of the methyl α- and β-furanosides was the $J_{3,4}$ value, which has average values: α 6.1 Hz, β 2.2 Hz (Fig. 1) (Table I). In the β-series, the carboxyl group and the exocyclic chain tend to force the furanose ring into an $E_0$ conformation. The eclipsing of H-3′ and H-4 and a dihedral angle of 115–125° for H-3,4 correspond to estimated coupling constants of 8.2 and 2.2 Hz, respectively[26] (Table I). In the α-series, however, the exocyclic side-chain and the carboxyl group act in opposition to produce a $^2T_3$ conformation. Dihedral angles of 25–35° for H-3′,4 and 145–155° for H-3,4 correspond to estimated coupling constants of 6.0 and 6.8 Hz, respectively (Table I). Also, for a pair of anomers, the signals for H-3,3′,4 are consistently downfield in the α-anomer (Table I).

The $^{13}$C-n.m.r. spectra of the methyl furanosides of KDO do not display any consistent differences based on anomeric configuration (Table II) and are not useful for distinguishing between the anomers.

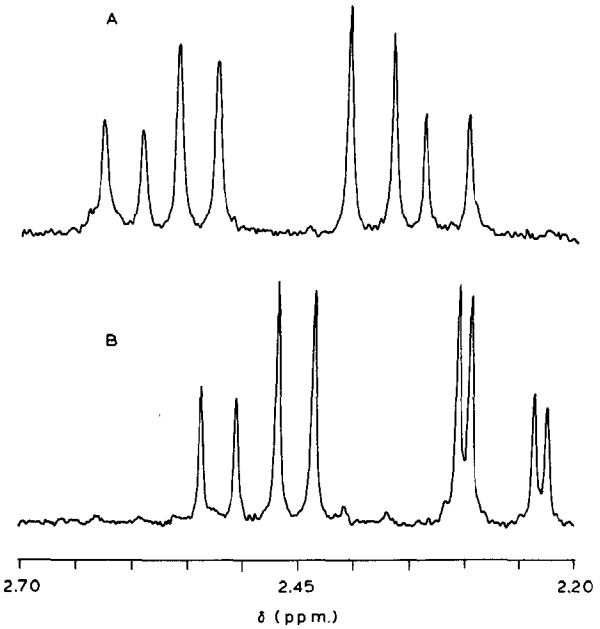


Fig. 1. Typical 200-MHz $^1$H-n.m.r. spectra for H-3 and H-3′ of A, methyl (methyl 3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulofuranosid)onate (**9**); B, the β-anomer **10**.

All the methyl 7,8-*O*-isopropylidene-furanosides of KDO displayed $^{13}C$ signals at $\delta \sim 109.5$, 26.7, and 25.4 (Table II) characteristic of 1,3-dioxolane rings[27]. The progressive acetylation was illustrated by the upfield shift of the signals of the carbon atoms adjacent to the point of acetylation. Thus, the introduction of a 4-*O*-acetyl group in **9** and **10** produces upfield shifts in the C-3 and C-5 resonances (Table II). Similarly, the signals for C-7 and C-5 moved upfield when C-6 was acetylated (Table II).

The assignment of anomeric configurations of the methyl furanosides of KDO rests on their relative optical rotations. The dextrorotory anomer was designated $\alpha$, and the larger $J_{3,4}$ was always associated with this anomer (Table III). The consistent relationship between the optical rotations and coupling constants suggests that it will be possible to assign the anomeric configuration of a furanoside derivative of KDO by inspection of the $^1H$-n.m.r. data, just as inspection of the chemical shift difference between the signals for H-3*a* and H-3*e* enables assignment[13] of the anomeric configuration to pyranose derivatives of KDO.

One previous assignment of the anomeric configuration was based on the $^{13}C$-n.m.r. spectra of 2-deoxy-D-*erythro*-pentoses[28], from which it was predicted that the anomeric carbon of $\beta$-furanose derivatives of KDO would resonate at a higher field. Our results (Table II) indicate that, with one exception (**11** and **12**), this prediction is incorrect.

Another reported approach used the sign of the Cotton effect at ~220 nm to assign the anomeric configuration, but these results are incomplete as only one furanoside was investigated[5]. Moreover, these results are based on the assumption that $\beta$-furanosides are produced by a Koenigs–Knorr reaction as is the case in the pyranose series. Such assumptions are not well founded, as the outcome of Koenigs–Knorr reactions is difficult to predict unless there is clear neighbouring-group participation[11]. The c.d. spectra of methyl (methyl 3-deoxy-$\alpha$-D-*manno*-oct-2-ulofuranosid)onate (**17**) showed a positive Cotton effect at 210 nm, whereas that of the $\beta$-anomer (**18**) exhibited a mixed Cotton effect with a maximum at 226 nm and a minimum at 203 nm. These results are not consistent with the determination of the anomeric configuration by Charon *et al.*[5].

In the presence of tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,5-octanedione)europium(III) [Eu(fod)$_3$], the signal for H-6 of methyl (methyl 4-*O*-acetyl-3-deoxy-7,8-*O*-isopropylidene-$\alpha$,$\beta$-D-*manno*-oct-2-ulofuranosid)onate (**11** and **12**) was shifted rapidly downfield, indicating binding of HO-6 (ref. 29). The signal for MeO-2 of **11** moved downfield at a rate greater than that of the methyl ester, consistent with the $\alpha$ configuration, and greater movement of the methyl ester resonance occurred with **12**. Similarly, with methyl (methyl 4,8-di-*O*-acetyl-3-deoxy-$\alpha$,$\beta$-D-*manno*-oct-2-ulofuranosid)onate (**15** and **16**), the methyl ester signal of **15** moved downfield more rapidly than that of MeO-2 which is not consistent with the $\alpha$ configuration. With **16**, there was no difference in the rates of downfield movement of the signals for the methyl ester and MeO-2. Thus, the use of shift reagents did not indicate the configurations of the furanosides.

TABLE III

ANOMERIC CONFIGURATION OF FURANOSE DERIVATIVES OF KDO BASED ON OPTICAL ROTATIONS

| *Compound* | $[\alpha]_D$ *(degrees)* | $J_{3,4}$ *(Hz)* | *Compound* | $[\alpha]_D$ *(degrees)* | $J_{3,4}$ *(Hz)* |
|---|---|---|---|---|---|
| *α Anomer* | | | *β Anomer* | | |
| **7** | +96 | 5 3 | **8** | +6 | 2.0 |
| **9** | +15 | 7.8 | **10** | −16 | 2.2 |
| **11** | −7 | 4.0 | **12** | −102 | 2.0 |
| **13** | +19 | 6.2 | **14** | −24 | 2.5 |
| **17** | +4 | 6.5 | **18** | −49 | 2.5 |

The consistent relationship between coupling constants and anomeric configurations (Table III) permits[28,30] assignment of species present in solutions of ammonium KDO. The major furanose form with $J_{3,4}$ 3 Hz and $J_{3',4}$ 7 Hz (ref. 30) is the β-anomer, and smaller amounts of the α-isomer are present. Furthermore, the major furanose form of the reducing residue of a KDO disaccharide isolated from a *Salmonella godesberg* Re mutant[30] is also β.

## EXPERIMENTAL

*General.* — Melting points were determined with a Leitz Wetzler microscope heating-stage model 150. Optical rotations were recorded with either a Polax or Perkin–Elmer 141 polarimeter. N.m.r. spectra were recorded with a Varian XL-200 n.m.r. spectrometer; $^{13}C$ chemical shifts were determined for aqueous solutions relative to internal MeOH (δ 49.7 relative to external $Me_4Si$). Microanalyses were carried out by the Australian Microanalytical Service.

T.l.c. was performed on Kieselgel 60 $F_{254}$ (Merck) with detection by charring with sulphuric acid. Kieselgel 60 (Merck) was used for column chromatography. Reverse-phase column chromatography was performed by using a Lobar-B column (31 × 2.5 cm) (Merck) packed with LiChroprep RP-8 (40–63 μm).

Anhydrous methanol was prepared by refluxing over magnesium and iodine, and anhydrous acetone by stirring with calcium sulphate. Anhydrous copper sulphate was prepared by drying at 140° for several days. Diazomethane was prepared from *p*-tolylsulphonylmethylnitrosamide[31].

*Shift reagent experiments.* — To a solution of the derivative (10 mg) in $CDCl_3$ (1 mL) was added $Eu(fod)_3$ (~1 mg) stepwise up to 10 mg. The $^1H$-n.m.r. spectrum was measured after each addition.

*Ammonium 3-deoxy*-D-manno-*oct-2-ulosonate* (**1**). — Crystalline **1** was synthesised from D-arabinose and oxaloacetic acid by a modification of the standard method[9,10]. To a solution of sodium carbonate (8.2 g, 77.36 mmol) in water (40 mL) was added D-arabinose (15.53 g, 103.50 mmol) followed by the slow addition of oxaloacetic acid (5 g, 37.85 mmol). The solution was adjusted to pH 11 with 10M sodium hydroxide, stirred for 90 min, then acidified (pH 1–2) with Amberlite IR-

120 ($H^+$) resin, filtered, and neutralised with ammonia. The KDO was isolated by column chromatography, using a column (60 × 3.5 cm) of CG-400 ($HCO_3^-$) resin which was washed with water (3 L) and then eluted with 0.5M ammonium hydrogencarbonate (4 L). The eluate was concentrated under reduced pressure to 50 mL and then freeze-dried. Crystallisation of the residue from water–ethanol gave **1** (3.60 g, 37%), m.p. 118–122°, $[\alpha]_D^{25}$ +32° (*c* 2, water); lit.[10] m.p. 121–123°, $[\alpha]_D^{27}$ +42.3° (*c* 1.7, water).

*2,4,5,7,8-Penta-*O*-acetyl-3-deoxy-α-*D-manno-*oct-2-ulopyranosonic acid* (**2**). — Ammonium KDO (**1**; 2.0 g, 7.84 mmol) was acetylated in the usual way[1,2]. After 18 h, the pyridine was removed by azeotropic distillation with water under reduced pressure. The crude product was dried over KOH and $H_2SO_4$ for 2 days. The residue was dissolved in methanol (100 mL), passed slowly through a column (2.5 × 18 cm) of Amberlite IR-120 ($H^+$) resin, and concentrated to dryness under reduced pressure. An aqueous solution of the residue was freeze-dried to give amorphous **2** (3.51 g, 100%), $[\alpha]_D^{25}$ +99° (*c* 2.2, chloroform).

*Anal.* Calc. for $C_{18}H_{24}O_{13}$: C, 48.21; H, 5.40. Found: C, 47.87; H, 5.55.

*Methyl (methyl 3-deoxy-α-*D-manno-*oct-2-ulopyranosid)onate* (**6**). — To a solution of **2** (1.76 g, 3.92 mmol) in methanol (95 mL) were added methyl acetate (5 mL) and acetyl chloride (0.5 mL). The solution was boiled under reflux for 16 h, cooled, neutralised with Amberlite IR-45 ($HCO_3^-$) resin, filtered, and concentrated to dryness under reduced pressure. The $^1$H-n.m.r. spectrum showed that the product (1.05 g, 100%) contained 95% of **6**. The product was eluted from a Lobar-B column (31 × 2.5 cm) with methanol–water (1:9, 500 mL) at 1.8 mL/min (5-mL fractions). Fractions 31–40 were combined and concentrated to dryness under reduced pressure, and an aqueous solution of the residue was freeze-dried to give **6** (381 mg, 38%), $[\alpha]_D^{25}$ +108° (*c* 1.3, water); lit.[1] $[\alpha]_D^{20}$ +76° (*c* 1.8, methanol).

*Methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-α,β-*D-manno-*oct-2-ulofuranosonate* (**7** *and* **8**). — The methyl ester of KDO was acetylated[4]. T.l.c. (2:3 ethyl acetate–hexane) of the crude product (3.38 g, 93%) revealed at least four components ($R_F$ 0.84, 0.74, 0.62, and 0.54). Elution of the mixture from a column (1.5 × 25 cm) of Kieselgel 60 with ethyl acetate–hexane (3:17, ~4 L) gave the α-pyranose (**3**; 94 mg, 2.6%), β-pyranose (**4**; 27 mg, 0.75%), α,β-furanose (**7** and **8**; 825 mg, 22.8%), and the α-furanose derivative (**7**; 48 mg, 1.3%), $[\alpha]_D^{26}$ +96° (*c* 0.7, chloroform).

*Anal.* Calc. for $C_{19}H_{26}O_{13}$ (**7**): C, 49.35; H, 5.67. Found: C, 49.25; H, 5.59.

Crystallisation of the α,β-furanose mixture from ethyl acetate–hexane gave the β-furanose derivative **8** (118 mg, 4%), m.p. 121–123°, $[\alpha]_D^{26}$ +42° (*c* 1.65, chloroform); lit.[4] m.p. 123–125°, $[\alpha]_D^{20}$ +4° (*c* 1, methanol).

*Anal.* Found: C, 49.47; H, 5.71.

Further amounts of derivatives **3**, **4**, **7**, and **8** could be obtained by rechromatography.

*Methyl (methyl 3-deoxy-7,8-*O*-isopropylidene-α,β-*D-manno-*oct-2-ulofuranosid)onate* (**9** *and* **10**) *and methyl (methyl 4-*O*-acetyl-3-deoxy-7,8-*O*-isopropylidene-α,β-*D-manno-*oct-2-ulofuranosid)onate* (**11** *and* **12**). — To a solution of the

foregoing α,β-mixture (**7** and **8**) (825 mg, 1.79 mmol) in anhydrous methanol (95 mL) were added methyl acetate (5 mL) and acetyl chloride (0.100 mL) to produce 0.02M hydrogen chloride. The mixture was kept at 23° for 2 days, then neutralised with Amberlite IR-45 ($HCO_3^-$) resin, filtered, and concentrated under reduced pressure. To a solution of the crude methyl furanosides (464 mg, 98%) in dry acetone (60 mL) were added anhydrous copper sulphate (1.03 g, 6.46 mmol) and water (0.100 mL). The mixture was stirred for 5 days at room temperature and then centrifuged, and the residue was dispersed in acetone and re-centrifuged. The combined acetone supernatants were concentrated to dryness under reduced pressure and the crude product was dried over potassium hydroxide.

T.l.c. (1:1 ethyl acetate–hexane) of the residue revealed at least three components ($R_F$ 0.77, 0.71, and 0.34). The mixture was eluted from a Lobar-B column (31 × 2.5 cm) with methanol–water (3:7, 800 mL) at 1.8 mL/min (8-mL fractions). Fractions 37–46 were combined and concentrated to dryness, and an aqueous solution of the residue was freeze-dried to give the α-furanose derivative **9** (105 mg, 19%), $[\alpha]_D^{25}$ +15° (*c* 1, methanol).

*Anal.* Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.68; H, 7.18.

Fractions 47–65 were treated in the same way to give the corresponding β-anomer **10** (135 mg, 24%), $[\alpha]_D^{25}$ −16° (*c* 1.6, methanol).

*Anal.* Found: C, 50.74; H, 7.10.

Further elution with methanol–water (11:9, 500 mL) at 1.8 mL/min (5-mL fractions), with combination and concentration of fractions 18–28, gave the α-furanose derivative **11** (69 mg, 11%), $[\alpha]_D^{25}$ −7° (*c* 0.9, methanol).

*Anal.* Calc. for $C_{15}H_{24}O_9$: C, 51.72; H, 6.94. Found: C, 51.56; H, 6.83.

Fractions 29–40 gave the corresponding β-anomer **12** (84 mg, 13%), $[\alpha]_D^{25}$ +103° (*c* 0.7, methanol).

*Anal.* Found: C, 51.62; H, 6.75.

*Methyl (methyl 4,6-di-*O*-acetyl-3-deoxy-7,8-*O*-isopropylidene-α,β-*D*-manno-oct-2-ulofuranosid)onate* (**13** *and* **14**). — Compounds **9**–**12** were acetylated with pyridine, acetic anhydride, and a catalytic amount of 4-dimethylaminopyridine at room temperature; **9** and **11** gave methyl (methyl 4,6-di-*O*-acetyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulofuranosid)onate (**13**) in quantitative yield, $[\alpha]_D^{25}$ +19° (*c* 1.1, methanol); **10** and **12** gave the corresponding β-anomer **14**, $[\alpha]_D^{25}$ −24° (*c* 2.2, methanol).

*Methyl (methyl 4,8-di-*O*-acetyl-3-deoxy-α,β-*D*-manno-oct-2-ulofuranosid)-onate* (**15** *and* **16**). — To a solution of **13** (21 mg) in ethanol (0.5 mL) was added copper(II) chloride (45 mg, 5 equiv.), and the mixture was stirred for 24 h. Sodium hydrogencarbonate (45 mg) was added and the mixture stirred until no more carbon dioxide was evolved (~30 min). Water (0.1 mL) was added and the solution was then diluted with water (0.4 mL) to facilitate precipitation. The solution was filtered and extracted with chloroform (0.5 mL), and the aqueous layer was treated with Chelex-100 resin (Bio-Rad), filtered, and extracted with chloroform (0.5 mL). The combined chloroform solutions were dried ($MgSO_4$), filtered, and concen-

trated to dryness under reduced pressure. The residue was dried at 0.1 mmHg to give **15** (18 mg, 90%). Compound **14** (42 mg) was treated similarly, yielding **16** (32 mg, 85%).

*Methyl (methyl 3-deoxy-α,β-*D*-manno-oct-2-ulofuranosid)onate* (**17** *and* **18**). — Compound **9** (27 mg) was deprotected using the copper(II) chloride dihydrate method but, after 2 days, the reaction was terminated by the addition of sufficient Amberlite MB-3 resin to cause complete discharge of colour. The solution was filtered and concentrated to dryness under reduced pressure. The residue was eluted from a Lobar-B column (31 × 2.5 cm) with methanol–water (3:7, 800 mL) at 2.5 mL/min (8-mL fractions). Fractions 6–9 and 15–24 contained by-products resulting from hydrolysis of the methyl glycoside and formation of the ethyl ester, respectively. Fractions 10–14 contained **17** (11 mg, 46%), $[\alpha]_D^{25}$ +4° (*c* 0.2, water); lit.[5] $[\alpha]_D^{20}$ +13° (*c* 0.54, methanol).

*Anal.* Calc. for $C_{10}H_{18}O_8$: C, 45.11; H, 6.81. Found: C, 45.02; H, 6.70.

Compound **10** (168 mg) was treated similarly to give **18** (40 mg, 27%), $[\alpha]_D^{25}$ −49° (*c* 0.8, water).

*Anal.* Found: C, 44.94; H, 6.63.

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