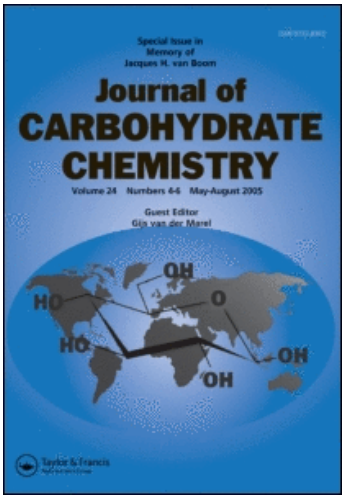

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Conformations of Ammonium 3-Deoxy-D-*manno*-2-octulosonate (KDO) and Methyl α- and β-Ketopyranosides of KDO: X-Ray Structure and ^{1}H NMR Analyses

George I. Birnbaum[a]; René Roy[b]; Jean-Robert Brisson[a]; Harold J. Jennings[a]
[a] Division of Biological Sciences National Research Council of Canada, Ottawa, Ontario, Canada [b] Ottawa-Carleton Chemistry Institute Chemistry Department, Ottawa University, Ottawa, Ontario, Canada

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CONFORMATIONS OF AMMONIUM 3-DEOXY-D-MANNO-2-OCTULOSONATE (KDO) AND METHYL α- AND β-KETOPYRANOSIDES OF KDO: X-RAY STRUCTURE AND ^{1}H NMR ANALYSES[1‡]

George I. Birnbaum,[+*] René Roy,[#*] Jean-Robert Brisson,[+] and Harold J. Jennings[+]

[+]Division of Biological Sciences
National Research Council of Canada
Ottawa, Ontario, Canada K1A 0R6

and

[#]Ottawa-Carleton Chemistry Institute
Chemistry Department
Ottawa, University
Ottawa, Ontario, Canada K1N 9B4

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ABSTRACT

Ammonium 3-deoxy-D-manno-2-octulosonate monohydrate (KDO) crystallizes in the orthorhombic space group $P2_12_12_1$, and the cell dimensions are a = 6.9700(4) A, b = 7.7230(4) A, c = 23.4067 (12) A. X-ray intensity data were measured on a diffractometer, and the structure was determined by direct methods. Least-squares refinement, which included all hydrogen atoms, converged at R = 0.034 for 1526 observed reflections. The pyranose ring exists in an almost perfect $^5C_2$ (D) chair conformation. The COO^-, 4-OH and 6-$CHOHCH_2OH$ groups are in equatorial orientation, while the 2-OH and 5-OH groups are axial. The solution conformations of the ammonium salts of methyl α- and β-ketopyranosides of KDO were determined by high-resolution ^{1}H NMR spectroscopy. The confor-

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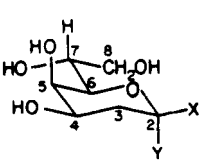
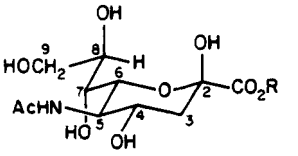
mation of the ethylene glycol side chain in the α-methyl glycopyranoside of KDO was found to be indistinguishable from that in the solid state. However, the solution conformation of the side chain is different in the β-anomer, possibly indicative of an intramolecular hydrogen bond between the 8-OH and carboxylate groups.

INTRODUCTION

3-Deoxy-D-manno-2-octulosonic acid (KDO, 1) is present in the inner-core region of all Gram-negative bacterial lipopolysaccharides (LPS).[2] It also occurs as part of the repeating unit of many acidic exopolysaccharides (K antigens) of Escherichia coli[3] and Neisseria[4] species. Its presence has also been recently detected in the polysaccharides of higher plant cell walls.[5] The LPS form the outer layer of the cell walls of Gram-negative bacteria without being covalently linked to the murein layer or the inner (cytoplasmic) membrane. The polysaccharide chain linked to the outer-core region of LPS extends into the surrounding media. Because of their mobility, LPS, or endotoxins, are secreted into the infected organisms where they are highly toxic, causing fever (pyrogenicity) and the so-called septic shock. Since they appear at the cell surface, KDO-containing polysaccharides and LPS can trigger the host's immune system to produce specific antibodies.[6] In this regard, a KDO-specific monoclonal antibody recognizing LPS has been described.[7] Recent investigations[8,9] of LPS have also demonstrated that only one KDO residue is found in the main chain with one or two other KDO molecules being linked α-(2→4) to it. The α-anomeric configuration between the main chain KDO unit and the glucosamine disaccharide portion of lipid A has been demonstrated in the LPS from a heptoseless mutant of Salmonella minnesota Re 595.[10] Because of the pathogenic character of the species in which KDO is found, it is of interest to study the physical properties of KDO in more detail. Also, because of its many similarities with sialic acid (5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid, N-acetyl-neuraminic acid, NeuAc, 4), its 2-nonulosonic acid counterpart,[11] it is worthwhile to compare their overall conformations

both in aqueous solution and in the solid state. This information was available in part for NeuAc but was still lacking for KDO.





1 X = CO_2^-, Y = OH

2 X = CO_2^-, Y = OCH_3

3 X = OCH_3 Y = CO_2^-

4 R = H

5 R = CH_3

EXPERIMENTAL

Ammonium 3-deoxy-α-D-manno-2-octulopyranosonate (1) was prepared by the aldol condensation[12] of oxalacetic acid and D-arabinose (Aldrich, Milwaukee, WI) at pH 11.0 as described by Unger.[2] Crystals of the monohydrate were obtained from hot 85% aqueous ethanol (100 mg/4 mL); mp 123-125 °C, lit.[2] mp 121-124 °C. Anal. Calcd for $C_8H_{17}NO_8 \cdot H_2O$: C, 35.17; H, 7.01; N, 5.13. Found: C, 35.43; H, 7.12; N, 5.06.

Ammonium (methyl 3-deoxy-D-manno-2-octulopyranosid)onates (2 and 3) were prepared by the methods of Bhattacharjee et al.[4] and Unger et al.[13] with slight modifications. These glycosides and all the intermediates had physical properties (mp, $[\alpha]_D$, microanalyses, ^1H and ^{13}C NMR spectra) in accord with published values.[4,13]

X-ray Analysis of Ammonium 3-Deoxy-D-manno-2-octulosonate Monohydrate

Precession photographs of a colorless crystal, measuring 0.50 × 0.50 × 0.55 mm, showed systematic absences corresponding to the space group $P2_12_12_1$. The crystal was mounted on an Enraf-Nonius CAD-4 diffractometer, and the following data were obtained: a = 6.9700(4) A, b = 7.7230(4) A, c = 23.4067(12) A, V = 1259.96 A^3,

ρ_c = 1.44 g cm^{-3}, $\underline{Z}$ = 4 (20°C; Cu Kα_1, λ = 1.54056 Å); $\underline{F}$(000) = 576, μ(Cu Kα) = 11.1 cm^{-1}.

Unit cell parameters were determined from a least-squares refinement of 25 high-order ($51<\theta<70°$) reflections. Intensities were measured with Ni-filtered Cu Kα radiation, using $\omega/2\theta$ scans with variable scan ranges and speeds. Three standard reflections were monitored at regular intervals; their intensities decreased by <3% during the data collection. Both hkl and hk$\bar{l}$ reflections were measured, and their net intensities were averaged. There were 1544 unique reflections with $2\theta \leq 152°$ of which only three had $\underline{I}<3\sigma(\underline{I})$ and were considered unobserved. The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary.

The structure was determined by direct methods with the aid of the computer program MULTAN78.[14] Of the 12 starting sets subjected to tangent refinement, the solution with the highest combined figure of merit yielded an $\underline{E}$ map on which all non-hydrogen atoms could be located. Refinement was carried out by the block-diagonal least-squares procedure. Anisotropic temperature parameters were used for non-hydrogen atoms, while hydrogen atoms, located on difference Fourier maps, were refined with isotropic parameters. The scattering factors were taken from the "International Tables for X-Ray Crystallography,"[15] and the oxygen curve was corrected for anomalous dispersion. Throughout the refinement the function $\Sigma\underline{w}(|\underline{F}_o|-|\underline{F}_c|)^2$ was minimized, and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages: $\underline{w} = \underline{w}_1 \cdot \underline{w}_2$, where $\underline{w}_1 = 1$ for $|\underline{F}_o|<7$, $\underline{w}_1 = 7/|\underline{F}_o|$ for $|\underline{F}_o| \geq 7$, $\underline{w}_2 = \sin^2\theta/0.9$ for $\sin^2\theta \leq 0.9$, and $\underline{w}_2 = 1$ for $\sin^2\theta > 0.9$. This scheme made the average values of $\underline{w}(\Delta\underline{F}^2)$ independent of $|\underline{F}_o|$ and $\sin^2\theta$. After the final cycle the average parameter shift equalled 0.14σ and the largest 0.60σ. Fifteen strong, low-angle reflections suffered from secondary extinction effects and were given zero weights. The final conventional residual index $\underline{R}$ is 0.034, and the weighted index $\underline{R}_w$ is 0.041 for 1526 reflections. The atomic coordinates are listed in Table 1.

TABLE 1

Final Atomic Parameters and Their Standard Deviations[a]

atom	x	y	z	U_{eq}/U_{iso}
C1	61784(23)	58423(19)	39748(6)	211
C2	60893(22)	39352(19)	37576(6)	200
O2	76662(18)	35308(15)	34174(5)	264
C3	60489(26)	26806(20)	42673(6)	243
C4	55810(27)	8499(19)	40682(6)	252
O4	53722(28)	-2041(15)	45643(5)	381
C5	37347(25)	8406(19)	37138(6)	239
O5	21211(21)	13627(17)	40414(6)	338
C6	39849(24)	21127(19)	32213(6)	213
O6	43314(16)	38287(13)	34423(4)	204
C7	22232(24)	22263(22)	28309(6)	250
O7	19719(21)	5782(18)	25672(5)	338
C8	25390(30)	35763(26)	23680(8)	346
O8	9456(25)	37049(22)	19928(6)	426
O9	47340(19)	64333(16)	42250(6)	306
O10	77242(19)	66356(16)	39051(6)	305
NA	61078(21)	2126(20)	57402(6)	281
OW	51628(28)	62225(25)	54478(7)	522
HO2	764(4)	420(4)	3154(12)	15(6)
H3e	741(5)	268(5)	4446(14)	28(8)
H3a	510(4)	306(3)	4549(10)	5(5)

(continued)

atom	x	y	z	U_{eq}/U_{iso}
H4	660(3)	43(3)	3827(10)	1(5)
HO4	514(4)	-119(4)	4448(12)	15(6)
H5	357(3)	-28(3)	3575(10)	3(5)
HO5	171(6)	46(5)	4214(16)	33(8)
H6	503(4)	176(3)	3002(10)	1(5)
H7	117(4)	247(4)	3063(11)	8(5)
HO7	115(5)	-4(4)	2739(12)	16(6)
H8 (pro-S)	269(5)	473(4)	2533(13)	17(7)
H8'(pro-R)	367(5)	320(4)	2142(13)	22(7)
HO8	100(6)	305(5)	1765(15)	29(8)
HA1	628(4)	129(4)	5841(13)	17(6)
HA2	705(4)	-33(4)	5815(12)	16(6)
HA3	581(6)	10(5)	5391(15)	33(8)
HA4	520(5)	-16(5)	5963(15)	29(8)
HW1	491(6)	629(5)	5075(15)	32(8)
HW2	410(6)	665(6)	5585(17)	40(9)

[a]The coordinates of the non-hydrogen atoms were multiplied by 10^5 and U_{eq} by 10^4; the z coordinates of the hydrogen atoms were multiplied by 10^4 and the other parameters by 10^3.

Lists of anisotropic temperature parameters and observed and calculated structure factors are available from the first author.

NMR Analyses

The ammonium salts (3 mg each) of methyl glycosides of KDO (2 and 3) were exchanged 3 times at room temperature in deuterium oxide (D_2O, 99.7%, Merck Sharp & Dohme). The samples were lyophilized and were then dissolved in 0.4 mL of 99.7% D_2O. They were analyzed in 5 mm NMR tubes (Wilmad Glass Co. Inc.) at pH 7.0 (pD 7.4). The ^1H NMR spectra were recorded at 300 ± 0.5 K on a Bruker AM-500 spectrometer in the FT mode with resolution enhancement, using the Lorentzian-Gaussian transformation method of Ernst.[16] The parameters used were 8K data points for a S.W. of 2 kHz and a 90° pulse of 9.3 μs; 64 scans were used. Acetone (1%) was used as an internal reference (δ = 2.225 ppm). The spectral assignments were made by performing two-dimensional homonuclear shift correlated experiments (COSY), using the standard software supplied by Bruker (D1SB85 850510.0). The chemical shifts and coupling constants of the proton resonances were obtained from a spin simulation of the observed spectra. The calculations were performed on an Aspect 3000 computer using the Bruker software PANIC.

RESULTS AND DISCUSSION

X-ray Analysis of the Ammonium Salt of 1

Molecular Dimensions. The C-C bond lengths (Figure 1) in the pyranose ring range from 1.524(2) to 1.537(2) A. These values and the differences between them are quite normal. The shorter C7-C8 distance reflects the relatively larger thermal motion of these atoms. The C6-O6 bond length agrees very well with the value (1.444 A) calculated for α-D-aldopyranoses,[17] but the O6-C2 distance is 0.012 A longer, presumably reflecting the increased substitution at the anomeric carbon atom. The C1-C2 bond is significantly longer than the corresponding ones in other

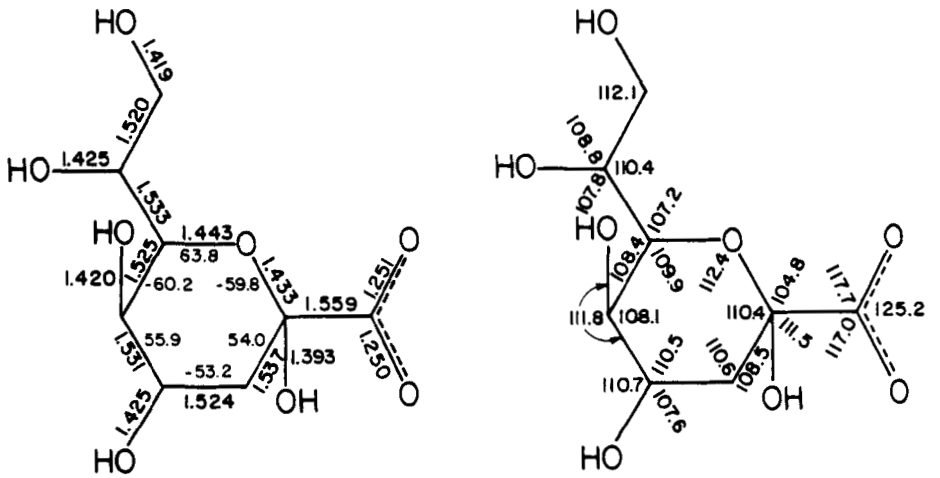


FIG. 1. (Left) Bond distances (Å) and endocyclic torsion angles (deg). Their estimated standard deviations (esd's) are 0.002 Å and 0.2°, respectively. (Right) Bond angles (deg); their esd's are 0.11-0.16°.

structures with a carboxyl or an ester group attached to the anomeric carbon atom.[18-24] Of the five C-OH bonds, four have distances in the range 1.419-1.425 Å while at the anomeric carbon atom the C-O bond is 0.03 Å shorter. This phenomenon was observed long ago by Kim and Jeffrey.[25]

The average bond angle in the ring at the carbon atoms is, as usual, close to 110°,[26] but the angle at O6 is 1° smaller than average.[17,26] The O6-C2-O2 angle is identical, within experimental error, to the previously determined mean value in α-D-aldopyranoses.[17] All angles at C6 are in very good agreement with those in other pyranosides with equatorial alkyl substituents at that atom.[18,19,22-24,27,28] On the other hand, the angles involving the exocyclic -OH groups at C4 and C5 vary greatly from structure to structure in order to optimize the geometry of the hydrogen bonds in which they are involved.

Molecular Conformation

The conformation of the molecule is shown in Figures 2 and 3. The pyranose ring can be described by the Cremer and Pople[29] puckering coordinates: $q_2 = 0.0442$, $q_3 = 0.5835$, $Q = 0.585$ A, $\phi = 290.2°$, $\theta = 4.3°$. The low values of q_2 and θ and the near-equivalence of q_3 and Q indicate an almost perfect $^5\underline{C}_2$ ($^4\underline{C}_1$) chair. The value of θ shows that the slight distortion is in the direction of a boat. This ring pucker is preferable to $^2\underline{C}_5$ ($^1\underline{C}_4$) since it places the bulky carboxylate and ethylene glycol groups, as well as the -OH group at C4, in equatorial orientations. Given the configuration of the substituents in KDO, we can compare its ring conformation with that of galactopyranoses. A survey of those structures[26] revealed average endocyclic torsion angles in the range 53.8-62.4°. Another survey[30] found significant differences between sugars with equatorial and axial anomeric groups. In the latter group, which included various pyranoses and pyranosides, the experimentally determined range was 54.3-59.5°. As can be seen in Figure 1, this range is significantly larger in KDO, and the ring is more puckered. Furthermore, there are significant differences between pairs of torsion angles, usually related by an approximate mirror plane normal to the ring and passing through C4 and O6. It is remarkable, however, that the Cremer-Pople coordinates are in very good agreement with values obtained from molecular mechanics calculations for methyl α-D-galactopyranoside: $Q = 0.562$ A, $\phi = 293.0°$, $\theta = 3.6°$.[30] Two features distinguish KDO from most other sugars: the presence of a carboxyl group at the anomeric carbon atom and the absence of an -OH group at C3. These features are also present in a derivative of KDO,[22] a galacto-2-nonulopyranoside,[23] an arabino-2-heptulopyranoside,[24] as well as in sialic acid[18] and its methyl ester.[19] X-ray analyses of these compounds revealed ring torsion angles in ranges as narrow as 55-59° and as wide as 50-63°. It does not appear, therefore, that these two features exert a significant influence on the conformation of the six-membered

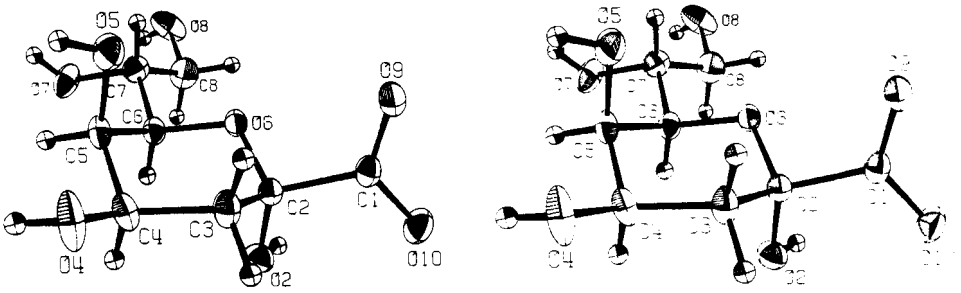


FIG. 2. Stereoscopic view of the KDO anion. The ellipsoids correspond to 50% probability.

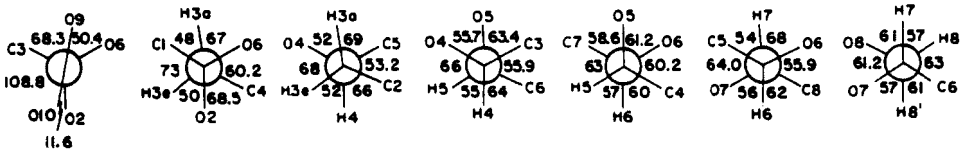


FIG. 3. Newman projections along (left to right) C1-C2, C2-C3, C3-C4, C4-C5, C5-C6, C6-C7, and C7-C8.

ring. However, it should be borne in mind that these structures represent different configurations.

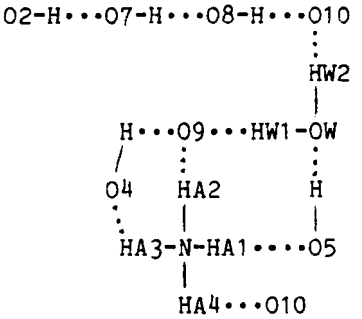
The conformation of the carboxyl group depends on whether or not the anomeric hydroxyl group is methylated. If not, the C=O (or C O) bond almost eclipses C2-O2. In KDO the O10-C1-C2-O2 torsion angle is 11.6°, in sialic acid,[18] its methyl ester,[19] and in 2-keto-L-gulonic acid[20] these angles are 12.4, 25.6, and 7.9°, respectively. The best overlap, 6.6°, is found in the methyl ester of D-threo-2,5-hexodiulosonic acid.[21,31] This overlap must be stabilized by an electrostatic attraction between the proton of the anomeric hydroxyl group and the carbonyl oxygen. In the three structures in which this hydroxyl group is methylated[22-24] the conformation of the carboxy group is such that its C=O bond is syn periplanar or anti periplanar to C2-O6.

The molecule adopts a conformation about C6-C7 which is dictated by steric factors. Of the three rotamers with staggered

bonds, the observed one has a C7-H bond which is in a 1,3-syn diaxial (peri) orientation with respect to C5-O5; in the other two rotamers it would have been either a C-O or a C-C bond. The same orientation is observed in a KDO derivative.[22] In that structure, as well as in the present one, the conformation about C7-C8 is such that C7-O7 and C8-O8 are in the preferred gauche orientation,[32] but in sialic acid and its methyl ester these bonds are trans to one another. This is not surprising since the stabilization provided by the gauche effect is rather weak (0.66 kcal/mol) for a C-O/C-O interaction[33] and can be easily overcome by other forces, e.g., hydrogen bonds.

Hydrogen Bonding and Packing

In addition to the five hydroxyl groups in KDO which can participate in hydrogen bonds, the ammonium ion and the water molecule can donate additional six protons. In fact, all of them do take part in a hydrogen bonding network which can be represented schematically as follows:



The distances and angles in these hydrogen bonds are listed in Table 2. The O-H and N-H bond lengths were normalized to 0.97 and 1.04 A, respectively, the mean values obtained from neutron diffraction analyses. The corrected H···A distances reflect more accurately the strengths of these hydrogen bonds. These distances show that the six bonds in which protons are donated to carboxylate oxygen atoms are weaker (average $H\cdots O_{corr}$ distance

TABLE 2

Distances and Angles for Hydrogen Bonds

			distances, Å			angles, deg
D	A	A at	D···A	H···A	H···A_c	D-H···A
O2-H···O7		1-x,1/2+y,1/2-z	2.806	2.01	1.85	168
O7-H···O8		$\bar{x}$,-1/2+y,1/2-z	2.700	1.86	1.74	170
O8-H···O10		1-x,-1/2+y,1/2-z	2.798	2.11	1.90	156
NA-HA1···O5		1/2+x,1/2-y,1-z	2.785	1.92	1.76	168
O5-H···OW		-1/2+x,1/2-y,1-z	2.698	1.86	1.76	164
OW-HW1···O9		x,y,z	2.882	2.00	1.92	172
OW-HW2···O10		-1/2+x,3/2-y,1-z	2.814	2.03	1.94	150
NA-HA2···O9		1/2+x,1/2-y,1-z	2.830	2.06	1.83	163
NA-HA3···O4		x,y,z	2.818	1.97	1.78	174
O4-H···O9		x,-1+y,z	2.752	1.93	1.78	175
NA-HA4···O10		-1/2+x,1/2-y,1-z	2.879	2.09	1.94	150

1.89 Å) than those in which the acceptor oxygen atom also acts as a donor (average H···O_{corr} distance 1.78 Å). This observation is in qualitative agreement with the results of a survey of crystal structure analyses of carbohydrates and indicates the cooperative effect in the solid state.[34] The fact that the anomeric hydroxyl group is the only one which does not act as a hydrogen bond acceptor is in line with previously made predictions[35] and observations.[36]

It is interesting to note that the ammonium salt of KDO crystallizes as a hydrate and that the same is true of sialic acid

and its methyl ester. The above-mentioned survey[34] revealed that monosaccharides rarely crystallize as hydrates (4 out of 43). However, carbohydrates with carboxyl groups were not included in that survey and, as can be seen above, both water protons in the present crystal structure are donated to carboxylate oxygen atoms. The coordination of the water molecule is approximately trigonal. In sialic acid dihydrate[18] both protons of one of the water molecules are also donated to carboxyl oxygens while in the monohydrate of the methyl ester[19] one of the water protons is bonded to the carbonyl oxygen of the N-acetyl group. Figure 4 shows the packing of the molecules in the crystal lattice.

^{1}H NMR Analyses

Because efficient antigen-antibody binding is associated with a precise conformation of the epitopes (antigenic determinants) on the surface of the antigens, it is crucial to determine the overall solution conformation of a particular binding site. In contrast to NeuAc,[11,37] natural KDO glycosides exist in both anomeric configurations. They are in the α form in the inner part of LPS[8-10] and in the K antigen of E. coli LP 1092,[38] while the β-anomeric configuration is present in the K antigens of N. meningitidis serogroup 29e[4] and of many E. coli strains.[3] Solutions of free KDO show a more complex tautomeric equilibrium than sialic acid. ^{13}C NMR spectroscopy revealed[39] ~60% and 11% of the α and β pyranose anomers, respectively, and 20% and 9% of the furanose α and β anomers (tentative). Brade et al.[9] recently found, by ^{1}H NMR spectroscopy, that the distribution of tautomers appears to be ~65% for the α-pyranose form, ~2% or less for the β-anomer, and 25% and 8% for the major and minor furanose forms. The observed variations may reflect differences in the extent of equilibration.

In the present study, we assigned unambiguously all the proton chemical shifts by two-dimensional homonuclear shift-correlated experiments (COSY-90). As seen from the ^{1}H NMR spectral data of the ammonium salts of 2 and 3, there is a

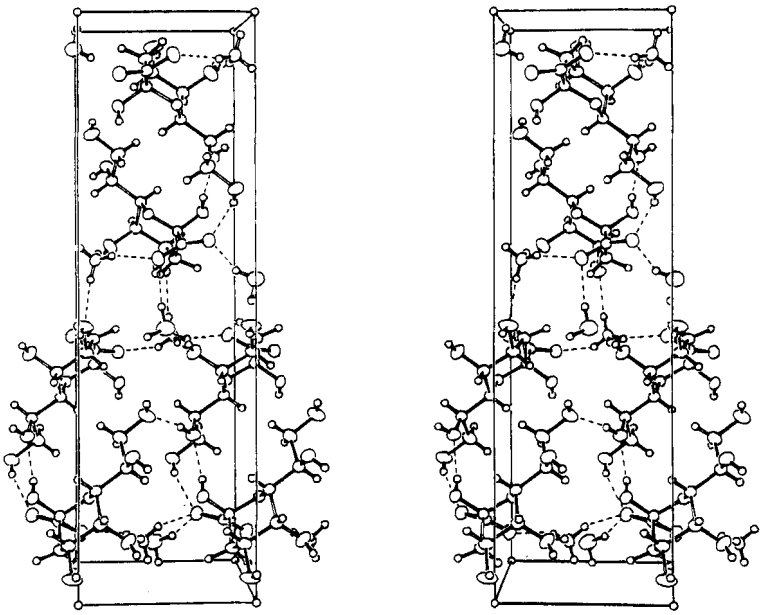


FIG. 4. Stereoscopic view of the molecular packing in the crystal; the directions of the axes are x →, y ⊙, z +. Dotted lines indicate hydrogen bonds.

significant downfield shift for H3e of 3 (+ 0.37 ppm) associated with the axial orientation of the carboxylate group (Table 3). A similar phenomenon is also present in sialic acid α-glycosides.[11] The axial proton H3a, on the other hand, is insensitive to anomeric variations. Both H6 and H8' of 3 exhibited downfield displacement of ~0.1 ppm, in analogy to their respective carbon signals in the ^{13}C NMR spectra.[4] The upfield shift of H4 (-0.28 ppm) is tentatively attributed to its different anisotropic environment caused by the specific orientation of the carboxylate group in 3. Partial ^{1}H NMR spectra of 2 and 3 are shown in Figure 5.

Table 3 shows a good correlation between the coupling constants of the ring protons of the two anomers (2 and 3). Since the D-manno configuration of KDO is well established,[2] these coupling constants suggest the $^{5}C_{2}$ conformation of both anomers.

TABLE 3

^{1}H Chemical Shifts (in ppm)[a] and ^{1}H-^{1}H Coupling Constants (in Hz)[b] for the Ammonium Salts of 2 and 3[c]

H	δ(H), 2	δ(H), 3	H,H'	$J_{H,H'}$, 2	$J_{H,H'}$, 3
H3e	2.015	2.388	3e,3a	-12.8	-12.3
H3a	1.775	1.776	3e,4	5.3	4.8
H4	4.027	3.754	3a,4	12.2	12.5
H5	4.010	3.956	3e,5	1.0	1.1
H6	3.556	3.640	3a,5	0.3	0.4
H7	3.944	3.924	4,5	3.1	3.2
H8	3.936	3.890	5,6	0.6	1.0
H8'	3.665	3.765	6,7	8.9	9.2
OCH_3	3.145	3.311	7,8	3.0	4.4
			7,8'	7.0	2.8
			8,8'	-12.5	-12.9

[a]Assignments based on COSY experiments and spin simulations. Chemical shifts relative to internal acetone (δ = 2.225 ppm). [b]Refined by spin simulations. [c]In D_2O at pD 7.4.

Furthermore, the recent refinements of the Karplus equation[40] by Haasnoot et al.[41] which take into account the electronegative effects of the hydroxyl substituents in a pyranose ring provide a correlation between coupling constants and torsion angles in chair conformations. As seen from Table 4, the calculated torsion angles of the α-methyl glycoside 2 match very well those obtained from the X-ray analysis of 1. Both analyses clearly indicate an

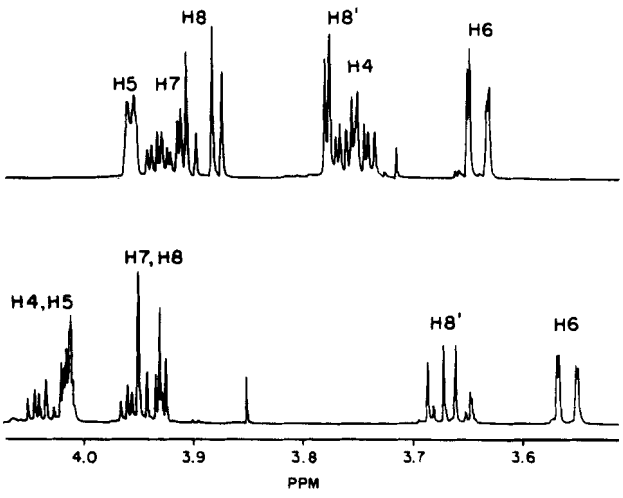


FIG. 5. Partial ^1H NMR spectra of the ammonium salts of 3 (top) and 2 (bottom).

TABLE 4

Coupling Constants (in Hz) and Torsion Angles (in deg) in KDO

H,H	$J_{H,H'}$, 2	τ, 2[a]	τ, 1[b]
3e,4	5.3	-50	-52
3a,4	12.2	175	-172
4,5	3.1	51	55
5,6	0.6	-59	-57

[a]Calculated with the equations of Haasnoot et al.[41] [b]Results of the X-ray analysis.

almost perfect $^{5}\underline{C}_{2}$ chair conformation for 1 and 2. Since the coupling constants of the ring protons of 3 are essentially the same as those of 2 (Table 3), we conclude that the solution conformations of the rings of both 2 and 3 are indistinguishable from that determined by the X-ray analysis of 1.

The $^{3}\underline{J}_{6,7}$ values (Table 3) are identical, within experimental error, in 2 and 3 and very close to the value (10.1 Hz) calculated for a trans rotamer with the equations of Haasnoot et al.[41] As mentioned above, the trans conformation about the C6-C7 bond is favored on steric grounds. The NMR data are similar to those obtained from the fully acetylated derivatives of 2 and 3.[13,22]

In contrast, the rotamer population about C7-C8 differs in the two compounds, as is evident from the different $^{3}\underline{J}_{7,8}$ and $^{3}\underline{J}_{7,8'}$ values. The population values of the staggered rotamers about C7-C8 (Figure 6) were evaluated from the Karplus equation, assuming a dynamic equilibrium between conformers and using the relationship with six parameters proposed by Haasnoot et al.[41] For each bond one can calculate rotamer populations for both the possible assignments of the AB protons in an ABX system. The results of these calculations are listed in Table 5. As shown previously,[42] it is possible to choose the more likely of the two interpretations by taking into account the results of an X-ray analysis. The solid-state conformation of 1 was found to be gauche^{+}; this conformation can, therefore, be expected to be a

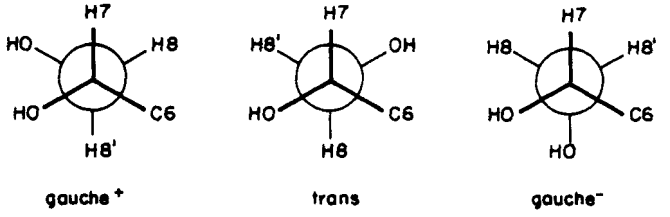


FIG. 6. Staggered rotamers about the C7-C8 bond in KDO.

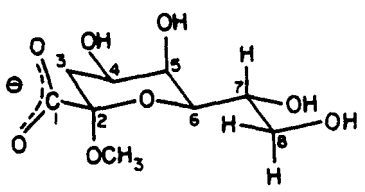
TABLE 5

Populations of Rotamers (in %) about the C7-C8 Bond in 2 and 3[a]

compound	$J_{7,8}$	$J_{7,8'}$	gauche$^+$	trans	gauche$^-$
2	3.0	7.0	62	0	38
	7.0	3.0	0	52	48
3	2.8	4.4	36	-2	66
	4.4	2.8	12	18	70

[a]Calculated with values of (3.1, 10.7 Hz), (10.7, 5.0 Hz), and (2.9, 0.9 Hz) for the coupling constants for the gauche$^+$, trans, and gauche$^-$ rotamers, respectively.[41]

major contributor to the solution conformation of 2. A trans conformation would position O8-H within hydrogen-bonding distance of O6. However, ring oxygens are relatively poor hydrogen bond acceptors,[34] and the trans rotamer is destabilized both by the gauche effect[32] and by making the C8-O8 bond 1,3-syn periplanar to C6-O6. Thus, we conclude that the first of the two assignments is much more likely for both 2 and 3. The difference in gauche$^+$: gauche$^-$ rotamer populations (62:38 in 2 and 35:65 in 3) corresponds to a difference of 0.6 kcal/mol between the two compounds. This preference for the gauche$^-$ rotamer in 3 is in agreement with the postulated hydrogen bonding between O8 and the carboxylate group (Figure 7).[4] As mentioned above, the C=O bond in 3 is very likely to be syn periplanar to C2-O6, bringing the carbonyl oxygen within hydrogen bonding distance of O8. Further studies by ^{1}H and ^{13}C NMR are now in progress to better evaluate these implications. It should also be pointed out that the pH at which the NMR spectra are recorded has to be taken into account because preliminary results indicate shift dependencies due to this parameter. Temperature, solvent, and pH effects are currently under investigation.



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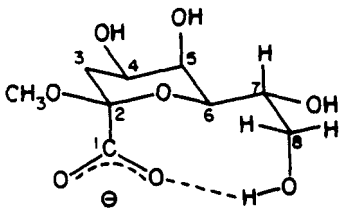

3

FIG. 7. Proposed dominant conformations in aqueous solution (pH 7.0) of 2 and 3.

SUMMARY

The present study shows that a monohydrated crystal of the ammonium salt of KDO (1) crystallizes in the α-anomeric configuration. Sialic acid (4) and its methyl ester (5) both crystallize in the β-anomeric configuration. Thus, both sugars crystallize in the form in which they are most abundant in aqueous solution. We have also investigated the solution conformations of the α- and β-methyl glycosides of KDO (2 and 3). We obtained supportive evidence for a difference in the solution conformation of the ethylene glycol side chain in the two glycosides. It seems obvious to attribute this difference to the axial orientation of the carboxyl group in the β-glycoside anomer of KDO. This supports the previously postulated hypothesis of an internal hydrogen bond between the carboxyl group and 8-OH in 3. In view of the conformational role attributed to the carboxyl groups of KDO[38] and NeuAc[43] in the antigen-antibody interactions at the molecular level, this information becomes of prime importance.

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