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CARBOHYDRATE RESEARCH

Crystal and molecular structure of allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosidonate)-monohydrate

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Abstract

The α-(2→8)-linked Kdo disaccharide derivative allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosidonate)-monohydrate $C_{19}H_{28}O_{15}Na_2 \cdot H_2O$, $M_r = 542.32$, is orthorhombic, $P2_12_12_1$ with $a = 9.229(1)$, $b = 12.036(1)$, $c = 21.671(1)$ Å, and $Z = 4$. The structure was solved by direct methods and refined to $R = 0.040$ for 2677 observed reflections. The torsion angles about the (2→8)-glycosidic bond are stabilized by an intramolecular hydrogen bond between the carboxylate group at the anomeric carbon atom of the terminal Kdo residue and the hydroxyl group O-17 of the second Kdo moiety.

Keywords: Chlamydia; Disaccharide; Kdo ; α-(2→8)-Glycosidic linkage; Crystal structure

1. Introduction

Chlamydiae are pathogenic, obligatory intracellular parasites which cause deseases in animals and humans [1]. They possess, in their outer membrane, a lipopolysaccharide which constitutes one of the major surface antigens of Chlamydia harboring a carbohydrate epitope which is shared by the whole genus. The epitope is composed of a trisaccharide of 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) of the sequence α-Kdo*p*-(2→8)-α-Kdo*p*-

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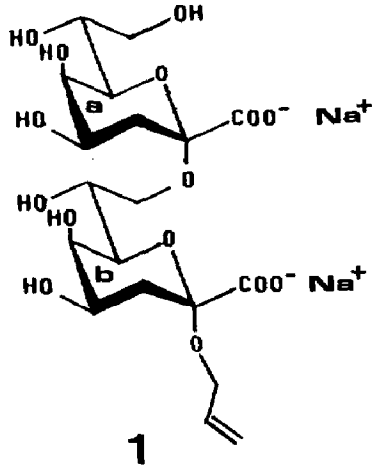
(2→4)-Kdo in which the (2→8)-linked disaccharide portion represents the immunodominant part [2]. The primary structure of the Kdo trisaccharide has been determined [3], it has been chemically synthesized [4], and monoclonal antibodies of high affinity have been prepared against it [5]. However, only preliminary data have been obtained on the conformation of this unique carbohydrate antigen [6]. We report here on the crystal structure of a Kdo disaccharide and provide new data on the stereochemistry of the α-(2→8)-glycosidic bonding.

2. Experimental

The α-(2→8)-linked Kdo disaccharide derivative **1** was synthetized as previously described [4]. Suitable crystals were obtained from aq EtOH at room temperature by adding



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dry EtOH in portions during several weeks to a solution of **1** (63 mg) in H_2O (0.3 mL) until a final volume of 1.5 mL was reached; mp 284–287°C (dec). Anal. Calcd for $C_{19}H_{28}Na_2O_{15} \cdot H_2O$: C, 40.72; H, 5.39. Found: C, 40.20; H, 5.23. The crystal data and details of the intensity-data collection and structure determination are given in Table 1. The data were not corrected for absorption effect. The structure was solved with SHELX76 [7], and refined anisotropically for non-H atoms. All H atoms were located in difference Fourier maps and refined with isotropic thermal parameters. The final positional parameters are given in Table, the description of the internal geometry of the (2→8)-glycosidic linkage is shown in Table 3, and the co-ordination of the sodium ions and the hydrogen bond geometry are given in Tables 4 and 5, respectively. Fig. 1. shows the chemical structure and numbering of atoms.

3. Results and discussion

The conformation of the molecule is shown in Fig. 1. A least square superposition of the ammonium Kdo [8] pyranose ring atoms on those of **1** gives a root-mean-square difference

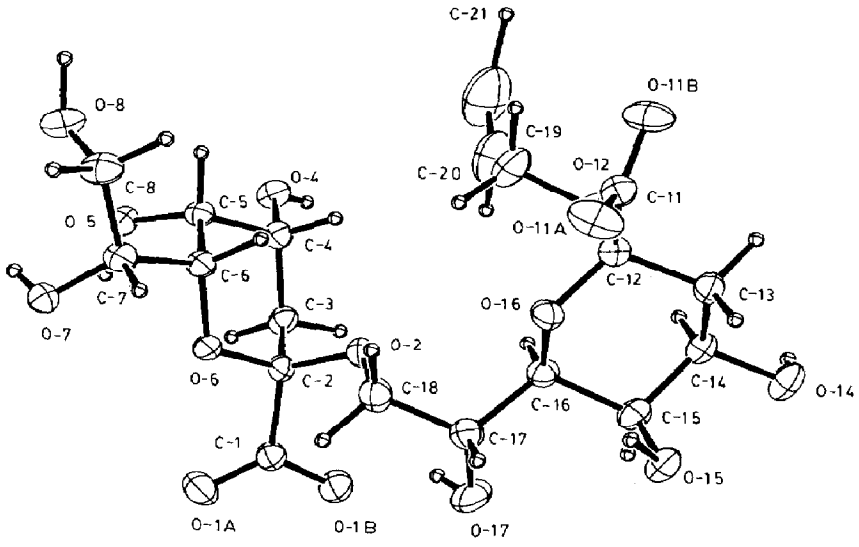

Fig. 1. Perspective view and atom labelling of allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosidonate)-monohydrate. The thermal ellipsoids are at 50% probability. H-atoms are represented by spheres of arbitrary size.

of 0.023 and of 0.028 Å for the atoms of units **a** and **b**, respectively. This indicates that both pyranose rings adopt a $^5C_2$ chair conformation as already noticed for a methyl ketoside of Kdo [9]. The ring pucker places the carboxylate groups in an equatorial position. Including the non-hydrogen axial and equatorial substituents of ammonium Kdo, the superposition on unit **a** and **b** yields a root-mean-square difference of 0.084 and 0.068 Å for unit **a** and **b**, respectively, suggesting a rather identical conformation. The two most significant variations for units **a** and **b** are: (*i*) similar to the methyl ketoside of Kdo [9], the C-1–O-1B bond is synperiplanar to C-2–O-2 whereas in the case of ammonium Kdo the carboxyl bond nearly eclipses the C-2–O-2 bond. This was thought to be a likely result of the presence of a

Table 1
Crystallographic Data for **1** (Enraf–Nonius CAD4 diffractometer)

Crystal dimensions (mm)	0.1×0.2×0.2
Space group	$P2_12_12_1$
Cell dimensions (Å) *a*, *b*, *c*	9.229(1), 12.036(1), 21.671(1)
Cell volume Å^3	2406.43
Z	4
F(000)	1136
Calculated density (g/cm^{-3})	1.5
λ, Cu*Kα* (Å)	1.5406
μ (cm^{-1})	13.83524
$2\Theta_{max}$ (°)	148.6
Number of reflections measured	2783
Number of reflections observed ($F_o > 4\sigma(F_o)$)	2677
Number of refined parameters	373
Final residual factors: R, R_w	0.0401, 0.0462

Table 2
Fractional positional parameters ($\times 10^4$) and equivalent isotropic displacement coefficients ($Å^2$) for non-H atoms (U_{eq}) and isotropic displacement coefficient for H-atoms (U_{iso}). The standard deviations are given in parentheses. $U_{eq} = 1/3\ \Sigma_i\Sigma_j U_{ij} a_i^* a_j^* a_i a_j$

Atom	x	y	z	U_{eq}/U_{iso} ($\times 10^3$)
C-1	−2224(3)	6645(2)	3680(1)	25(1)
O-1A	−1777(2)	7381(2)	3334(1)	43(1)
O-1B	−3456(2)	6618(2)	3934(1)	34(1)
C-2	−1245(1)	5612(2)	3799(1)	21(1)
O-2	−1256(2)	5304(1)	4430(1)	26(1)
C-3	−1805(1)	4601(2)	3442(1)	25(1)
H-3A	−1977(3)	4832(2)	2966(1)	50(1)
H-3B	−2818(3)	4333(2)	3642(1)	50(1)
C-4	−721(1)	3645(2)	3470(1)	22(1)
H-4A	−670(3)	3389(2)	3947(1)	51(1)
O-4	−1172(2)	2727(2)	3103(1)	28(1)
H-4	−1900(60)	2520(40)	3175(22)	62(2)
C-5	779(3)	4015(2)	3256(1)	21(1)
H-5A	1567(3)	3368(2)	3337(1)	51(1)
O-5	844(2)	4178(2)	2605(1)	26(1)
H-5	380(40)	488(30)	2473(19)	42(1)
C-6	1218(3)	5031(2)	3638(1)	21(1)
H-6A	1310(3)	4654(2)	4089(1)	52(1)
O-6	167(2)	5902(1)	3597(1)	22(1)
C-7	2660(3)	5594(2)	3490(1)	25(1)
H-7A	2849(3)	6179(2)	3860(1)	45(1)
O-7	2589(2)	6233(2)	2938(1)	28(1)
H-7	2870(50)	5940(30)	2680(19)	43(1)
C-8	3961(3)	4821(2)	3521(2)	33(1)
H-8A	3891(3)	4330(2)	3937(2)	51(1)
H-8B	4939(3)	5314(2)	3531(2)	51(1)
O-8	4017(2)	4098(2)	2006(1)	37(1)
H-8	4680(60)	3400(40)	3045(21)	65(1)
C-11	715(3)	3566(2)	6602(1)	25(1)
O-11A	1498(3)	4370(2)	6735(1)	40(1)
O-11B	1003(2)	2568(2)	6715(1)	37(1)
C-12	−786(3)	3790(2)	6308(1)	23(1)
O-12	−1227(2)	2915(2)	5912(1)	28(1)
C-13	−1933(3)	3816(2)	6820(1)	26(1)
H-13A	−1628(3)	4419(2)	7165(1)	53(1)
H-13B	−2016(3)	3005(2)	7030(1)	53(1)
C-14	−3386(3)	4137(2)	6540(1)	26(1)
H-14A	−3701(3)	3527(2)	6200(1)	51(1)
O-14	−4493(2)	4154(2)	7006(1)	34(1)
H-14	−4940(8)	3620(50)	6860(30)	90(2)
C-15	−3242(3)	5264(2)	6224(1)	26(1)
H-15A	−4256(3)	5507(2)	6015(1)	48(1)
O-15	−2919(2)	6114(2)	6657(1)	30(1)
H-15	−1620(60)	6250(50)	6690(30)	82(2)
C-16	−2079(3)	5153(2)	5723(1)	25(1)
H-16A	−2491(3)	4545(2)	5400(1)	52(1)
O-16	−720(2)	4816(2)	5991(1)	26(1)

Table 2 (continued)

Atom	x	y	z	U_{eq}/U_{iso} ($\times 10^3$)
C-17	−1787(4)	6254(2)	5385(1)	31(1)
H-17A	−1262(4)	6825(2)	5698(1)	47(1)
O-17	−3153(3)	6697(3)	5193(1)	52(1)
H-17	−3240(50)	6390(40)	4820(21)	58(1)
C-18	−725(3)	6138(2)	4856(1)	31(1)
H-18A	319(3)	5889(2)	5033(1)	51(1)
H-18B	−622(3)	6924(2)	4620(1)	51(1)
C-19	−304(4)	2692(3)	5395(1)	42(2)
H-19A	88(4)	3462(3)	5200(1)	52(2)
H-19B	602(4)	2186(3)	5536(1)	52(2)
C-20	−1206(5)	2085(5)	4932(2)	61(2)
H-20A	−2124(5)	2535(5)	4749(2)	52(3)
C-21	−1030(8)	1115(5)	4728(3)	86(4)
H-21A	−1700(60)	810(40)	4349(22)	67(1)
H-21B	80(8)	190(5)	4920(30)	120(30)
Na-1	517(1)	7545(1)	2957(1)	30(1)
Na-2	1902(1)	6131(1)	7010(1)	35(1)
O-1W	204(3)	2156(2)	8181(1)	38(1)
H-1W	−170(60)	2600(40)	8550(23)	73(2)
H-2W	360(50)	2490(30)	7913(20)	46(1)

methyl group on the anomeric carbon [9]; (*ii*) a different conformation about the C-6–C-7 was found in unit **a** where C-5–C-6 and C-7–C-8 are in the *gauche* orientation, whereas in the structure of ammonium Kdo, as well as in unit **b**, these bonds are *trans* to one another. This is presumably the result of crystal packing contacts. Interestingly, the solution conformation of unit **b**, as indicated by the coupling constant $^3J_{6,7}$ of the disaccharide **1** ($J_{6,7} \approx 9.0$ Hz), is different from that observed with higher oligosaccharides containing the *Chlamydia* specific trisaccharide epitope α-Kdo*p*-(2→8)-α-Kdo*p*-(2→4)-α-Kdo*p* ($J_{6,7} \approx 4.0$–5.1 Hz)

The C-2–O-2–C-18 bond angle is 115.5 (2)° and the C–O bond distances are 1.417(3) and 1.450(3) Å, respectively. The striking feature of this glycosidic linkage is the presence of an intramolecular hydrogen bond between one carboxylate atom of the unit **a** and the hydroxyl group (O-17–H-17) of the second unit **b** which donates a proton. The hydrogen atom H-17 lies at a distance of 2.41 Å from the glycosidic oxygen O-2 suggestive of a three-center hydrogen bond [10] (Table 5). This H-bond makes the glycosidic linkage rigid and reduces the flexibility around the O-2–C-18 bond. It places the two pyranose rings at an angle of ca. 60°. As the torsion angles indicate, the C-2–O-2 bond is synclinal to C-18–C-17 and O-2–C-18 is synperiplanar to C-17–C-16.

A stereoscopic drawing of the crystal structure, viewed along the *a*-axis is shown in Fig. 2. All the oxygen atoms, except the ring oxygen O-12, participate in the hydrogen bond network. The structure contains two independent sodium ions. The first one Na1 shows a six-fold oxygen coordination, whereas the second sodium has a five-fold coordination involving a distorted trigonal bipyramide. The Na1–O and Na2–O distances average

Table 3
Selected bond lengths, bond angles, and torsion angles around the (2→8)-glycosidic linkage

Bond lengths (Å)	
C-1–C-2	1.558(3)
O-6–C-2	1.419(3)
C-2–O-2	1.417(3)
O-2–C-18	1.450(3)
C-18–C-17	1.515(4)
C-17–C-16	1.537(4)
Bond angles (°)	
O-1B–C-1–C-2	115.4(2)
O-1A–C-1–C-2	118.7(2)
O-6–C-2–O-2	111.6(2)
C-1–C-2–O-2	111.4(2)
C-2–O-2–C-18	115.5(2)
O-2–C-18–C-17	109.1(2)
C-18–C-17–C-16	113.2(2)
Torsion angles (°)	
O-1A–C-1–C-2–O-2	137.6(2)
O-1B–C-1–C-2–O-6	−167.5(2)
O-6–C-2–O-2–C-18	58.1(3)
C-1–C-2–O-2–C-18	−60.8(3)
C-2–O-2–C-18–C-17	131.9(3)
O-2–C-18–C-17–C-16	−56.2(3)
O-2–C-18–C-17–O-17	66.2(3)
C-18–C-17–C-16–C-15	175.6(2)

2.44(12) and 2.34(9) Å, and fall in the expected range of Na–O distances for sodium ions having a co-ordination number of six and five, respectively [11].

The crystal packing, projected along the *a*-axis, is dominated by the co-ordination of the sodium ions and hydrogen bonds involving the water molecule. The Kdo unit **a** has its pyranose ring almost parallel to the *a*–*b* layer.

Table 4
Geometry of the sodium co-ordination. The distances are given in Å. O-1A and O-11A are carboxylate oxygens, O-15, O-4, O-7, O-5, and O-8 are hydroxyl oxygen atoms, O-6 is a ring oxygen, and O-1W the water oxygen. Symmetry code: (I) x,y,z; (II) $-x+1/2,-y,z+1/2$; (III) $-x,y+1/2,-z+1/2$; (IV) $1/2+x,-y+1/2,-z$

Na1···O-1A$^{(I)}$	2.28
Na1···O-15$^{(IV+b+c)}$	2.32
Na1···O-4$^{(III)}$	2.39
Na1···O-6$^{(I)}$	2.44
Na1···O-7$^{(I)}$	2.48
Na1···O-5$^{(III)}$	2.63
Na2···O-11A$^{(I)}$	2.23
Na2···O-1A$^{(IV+b+c)}$	2.29
Na2···O-8$^{(II+b)}$	2.34
Na2···O-1W$^{(III+c)}$	2.34
Na2···O-5$^{(II+b)}$	2.48

Table 5
Geometry of the hydrogen-bonding pattern and short contacts. The distances are given in Å and the angles in degrees. For the symmetry codes, see Table 4. The weak hydrogen bonds, which are a minor component of the three- or four-center bonds, are listed in the lines below that of the major hydrogen-bond component. All the minor components are intramolecular hydrogen bonds

Donor	Acceptor	d(D–H)	d(D···A)	d(H···A) [a]	Angle (D–H···A) [a]
O-4–H-4	O-11B$^{(IV-a+c)}$	0.74	2.66	1.94 (1.74)	162 (159)
O-5–H-5	O-14$^{(II-a+b+c)}$	0.98	2.70	1.75 (1.76)	160 (159)
	O-7		3.04	2.80 (2.80)	94 (94)
	O-6		3.05	2.74 (2.73)	99 (100)
O-1W–H-1W1	O-1B$^{(II-a+b)}$	1.02	2.73	1.78 (1.84)	152 (153)
O-17–H-17	O-1B	0.89	2.74	1.95 (1.89)	148 (147)
	O-2		2.94	2.41 (2.39)	118 (116)
O-14–H-14	O-4$^{(IV-a+c)}$	0.83	2.75	1.98 (1.84)	156 (153)
O-8–H-8	O-11B$^{(IV+c)}$	1.04	2.78	1.76 (1.83)	164 (164)
O-15–H-15	O-1W$^{(III+c)}$	1.21	2.82	1.73 (1.93)	148 (152)
	O-16		2.94	2.45 (2.41)	102 (107)
O-7–H-7	O-11A$^{(II+b-c)}$	0.71	2.83	2.16 (1.92)	158 (157)
	O-8		2.89	2.56 (2.49)	111 (105)
	O-5		3.04	2.83 (2.80)	100 (95)
O-1W–H-1W2	O-7$^{(II+b)}$	0.72	2.86	2.43 (2.34)	120 (113)

[a] Values after the D–H distance were normalized to 0.97 Å and are given in parentheses.

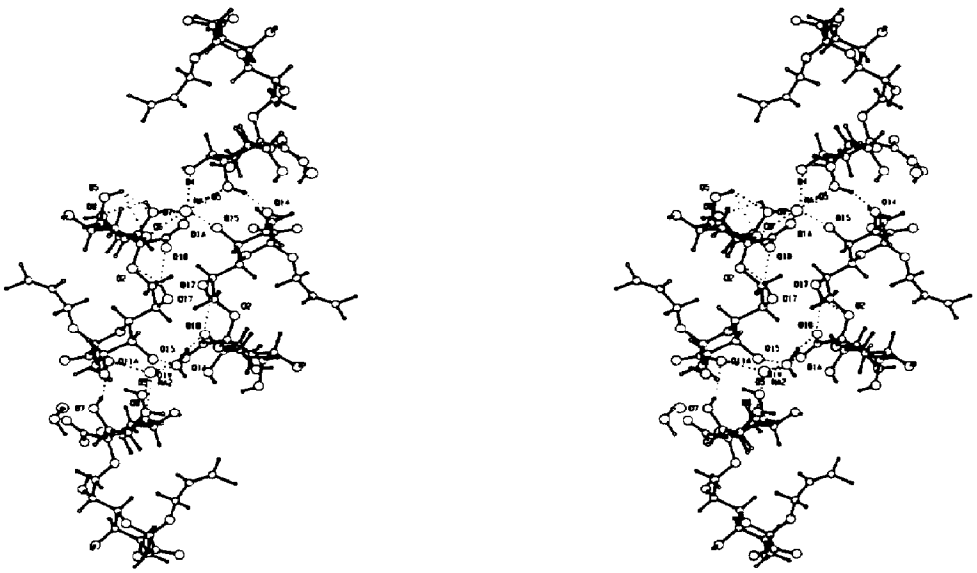

Fig. 2. Stereoview of the unit cell of **1** along the *a* axis (the *c*-axis is vertical). Dotted lines indicate hydrogen bonds or sodium coordination.

Acknowledgements

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References

[1] J. Schachter, *Curr. Top. Microbiol. Immunol.*, 138 (1988) 109–139.
[2] H. Brade, L. Brade, and F.E. Nano, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 2508–2512.
[3] O. Holst, L. Brade, P. Kosma, and H. Brade, *J. Bacteriol.*, 173 (1991) 1862–1866.
[4] P. Kosma, R. Bahnmüller, G. Schulz, and H. Brade, *Carbohydr. Res.*, 208 (1990) 37–50.
[5] Y. Fu, M. Baumann, P. Kosma, L. Brade, and H. Brade, *Infect. Immun.*, 60 (1992) 1314–1321.
[6] K. Bock, J.U. Thomsen, P. Kosma, R. Christian, O. Holst, and H. Brade, *Carbohydr. Res.*, 229 (1992) 213–224.
[7] G.M. Sheldrick, SHELX76, A Program for Crystal Structure Determination, University of Cambridge, UK, 1976.
[8] G.I. Birnhaum, R. Roy, J.-R. Brisson, and H.J. Jennings, *J. Carbohydr. Chem.*, 6 (1987) 17–39.
[9] C. Kratky, D. Stix, and F.M. Unger, *Carbohydr. Res.*, 92 (1981) 299–304.
[10] G.A. Jeffrey and W. Saenger, *Hydrogen Bonding in Biological Structures*, Springer-Verlag, Berlin, 1991, pp 136–146.
[11] I.D. Brown, *Acta Crystallogr., Sect. B*, 44 (1988) 545–553.