A polymer of 8-*O*-glucosylated 2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (Kdn) in the cell wall of *Streptomyces* sp. VKM Ac-2090

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The title polymer of Kdn was detected in biological object for the first time.

2-Keto-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (Kdn) was first found in the form of an α-2,8-linked oligomer containing 6–7 residues in rainbow trout eggs.[1] Later, Kdn was found in heterooligosaccharides of animal tissues[2,3] and as a constituent of the capsular heteropolysaccharide of *Klebsiella ozaenae* serotype K4.[4]

We have detected polyKdn in the cell wall of *Streptomyces* sp. VKM Ac-2090 isolated from scab lesions potato.

The polymers were extracted from the cell wall as described previously,[5] and their structure was examined using NMR spectroscopy.

The ^{13}C NMR spectrum of the polymers (Figure 1) displayed the Kdn-containing polysaccharide as the major cell wall anionic polymer, along with several glycerol teichoic acids. The ^{1}H and ^{13}C NMR spectra of the predominant component of the polymer mixture were completely assigned using 2D homonuclear ^{1}H/^{1}H COSY, TOCSY and ROESY and heteronuclear ^{1}H/^{13}C HSQC (Figure 2) and HMBC experiments. The residue of nonulosonic acid was identified with Kdn in accordance with the coupling constants in the ^{1}H NMR spectrum.[6] The upfield chemical shift of H-3$_{eq}$ (2.205 ppm) was in agreement with the β-configuration of the Kdn residue.[4] The second sugar residue in the disaccharide repeating unit of the polymer was identified as the terminal β-glucopyranose (β-Glc*p*) based on the chemical shifts and coupling constants of the ^{1}H and ^{13}C NMR spectra. The ROESY spectrum (Figure 3) revealed correlation peaks for the anomeric protons of Glc*p* and H-8, H-9 and H-9′ of Kdn.

The upfield chemical shift of C-9 and the downfield chemical shift of C-8 in the Kdn residue in comparison with that of non-substituted sugar (Table 1) allowed one to conclude that β-Glc*p* was bonded with Kdn by the 1→8 linkage. The presence of a correlation peak of H-1(Glc*p*)/C-8(Kdn) in the HMBC spectrum is consistent with this conclusion, too. The downfied shift of C-4 by 2 ppm in the ^{13}C NMR spectrum of the β-Kdn residue

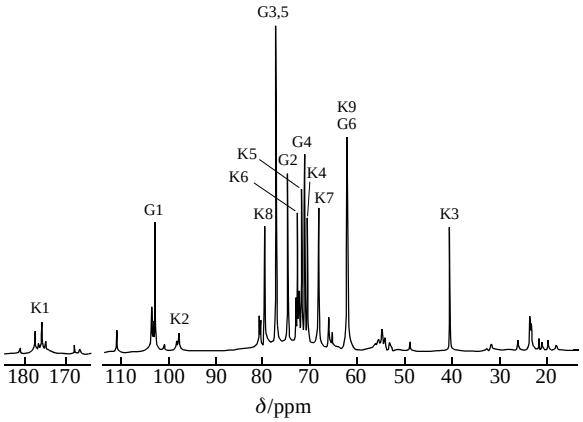


Figure 1 ^{13}C NMR spectrum of the polymers of the cell wall of *Streptomyces* sp. VKM Ac-2090. Designations refer to the numbers of carbon atoms in the Kdn (K) and glucopyranose (G) residues.

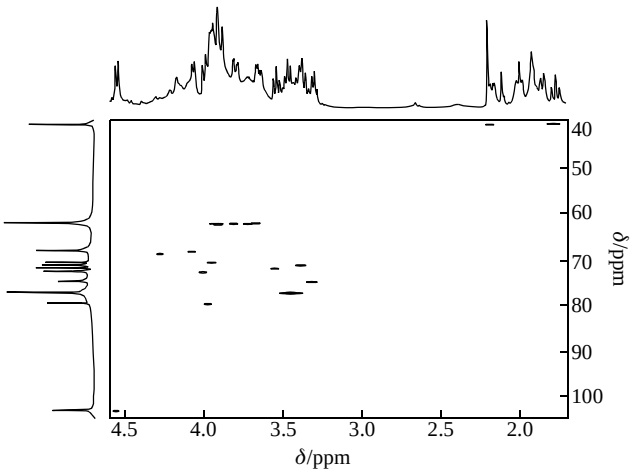


Figure 2 HSQC spectrum of the Kdn-containing polysaccharide of the cell wall of *Streptomyces* sp. VKM Ac-2090. The corresponding parts of the ^{1}H and ^{13}C NMR specta are displayed along the horizontal and vertical axes, respectively.

Table 1 ^{1}H NMR data (D_2O, 303 K, acetone: 2.225 ppm) for β-Kdn[6] and for the polysaccharide of *Streptomyces* sp. VKM Ac-2090 cell wall.

Residue	Chemical shifts, δ/ppm and coupling constants, *J*/Hz								
	H-3$_{ax}$	H-3$_{eq}$	H-4	H-5	H-6	H-7	H-8	H-9	H-9′
β-Kdn[6]	1.80	2.23	4.02	3.56	4.01	3.88	3.73	3.63	3.88
	$J_{3a,3e}$ 12.0	$J_{3e,4}$ 5.0	$J_{3a,4}$ 12.0	$J_{4,5}$ 9.0	$J_{5,6}$ 9.0	$J_{6,7}$ 1	$J_{7,8}$ 8.5	$J_{9,9'}$ 11	$J_{8,9'}$ 2
								$J_{8,9}$ 5.5	
→4)-β-Kdn-	1.80	2.20	3.98	3.57	4.02	4.09	3.99	3.94	3.83
(2→8)	$J_{3a,3e}$ 13.0	$J_{3e,4}$ 5.0	$J_{3a,4}$ 12.3	$J_{4,5}$ 9.6	$J_{5,6}$ 9.6	$J_{6,7}$ 1.2	$J_{7,8}$ 8.5	$J_{9,9'}$ 12.5	$J_{8,9'}$ 3.9
								$J_{8,9}$ 3.1	
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′		
β-Glc*p*-(1	4.58	3.33	3.50	3.38	3.42	3.82	3.68		
	$J_{1,2}$ 7.9	$J_{2,3}$ 8.8	$J_{3,4}$ 8.8	$J_{4,5}$ 8.7	$J_{5,6}$ 1.7	$J_{6,6'}$ 12.1	$J_{5,6'}$ 5.7		

Table 2 ^{13}C NMR data (D_2O, 303 K, acetone: 31.45 ppm) for β-Kdn[6] and for the polysaccharide of *Streptomyces* sp. VKM Ac-2090 cell wall.

Residue	Chemical shifts, δ/ppm								
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
β-Kdn[6]	174.2	96.02	39.19	68.51	70.67	71.06	69.27	72.29	63.86
→4)-β-Kdn-	176.0	97.9	40.5	70.5	71.7	72.6	68.05	79.5	61.9
(2→8)									
β-Glc*p*-(1	102.8	74.7	77.1	71.1	77.1	61.9			

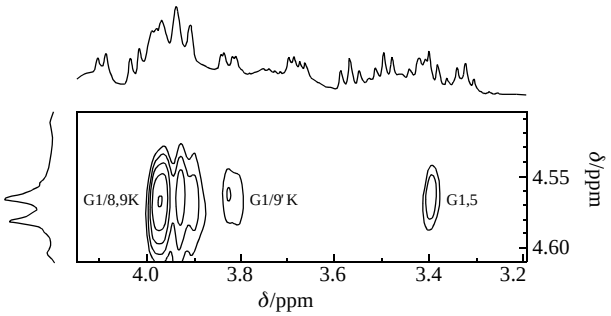
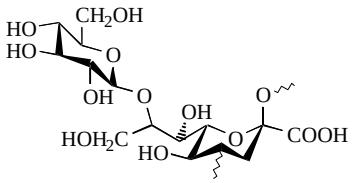


Figure 3 Part of the ROESY spectrum of the Kdn-containing polysaccharide of the cell wall of *Streptomyces* sp. VKM Ac-2090. The corresponding parts of the ^{1}H NMR spectum are displayed along the horizontal and vertical axes. Designations refer to the numbers of protons in the Kdn (K) and glucopyranose (G) residues.

compared to that of non-substituted β-Kdn revealed the 2→4 linkage for the polysaccharide chain. The signals of the terminal monosaccharide residues were not detected. This fact allows us to suggest a high molecular mass of the polymer. Both of the comparable NOE correlation peaks H-1(Glc*p*)/H-8(Kdn) and H-1(Glcp)/H-9(Kdn) (Figure 3) and relatively large in module negative β-effect of glycosylation for C-9 of the Kdn residue were in agreement with the D-*glycero*-D-*galacto*-configuration of Kdn on the assumption of the β-D-configuration of the glucopyranose residue[7,8] **1**.



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Until now, a polymer of Kdn has been found neither in procaryotic nor eucaryotic cells.

The β-configuration of the glycoside bond in this natural sugar has not been reported previously. This communication is the first report on the polyKdn with the β-configuration of the glycoside bond.

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References

1 D. Nadano, M. Iwasaki, S. Endo, K. Kitajima, S. Inoue and Y. Inoue, *J. Biol. Chem.*, 1986, **261**, 11550.
2 M. Muhlenhoff, M. Eckhardt and R. Gerardy-Schahn, *Curr. Opin. Struct. Biol.*, 1998, **8**, 558.
3 F. A. Troy, *Glycobiology*, 1992, **2**, 5.
4 Yu. A. Knirel', N. A. Kocharova, A. S. Shashkov, N. K. Kochetkov, V. A. Mamontova and T. F. Soloveva, *Carbohydr. Res.*, 1989, **188**, 145.
5 Yu. M. Kozlova, G. M. Streshinskaya, A. S. Shashkov, L. I. Evtushenko and I. B. Naumova, *Biokhimiya*, 1999, **64**, 805 [*Biochemistry* (*Engl. Transl.*), 1999, **64**, 671].
6 C. Auge and C. Gautherton, *J. Chem. Soc., Chem. Commun.*, 1987, 859.
7 G. M. Lipkind, A. S. Shashkov, S. S. Mamyan and N. K. Kochetkov, *Carbohydr. Res.*, 1988, **181**, 1.
8 A. S. Shashkov, G. M. Lipkind, Yu. A. Knirel and N. K. Kochetkov, *Magn. Reson. Chem.*, 1988, **26**, 735.

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