

# 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.

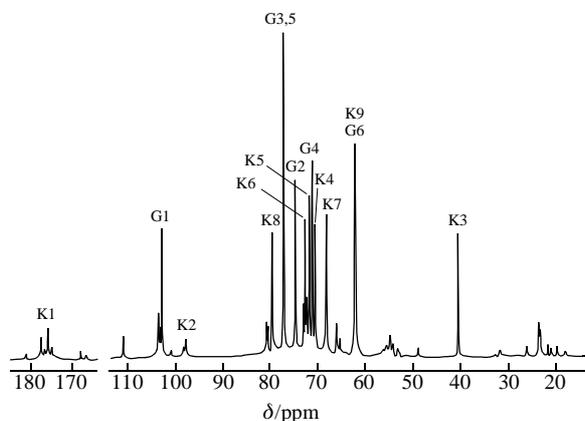
2-Keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid (Kdn) was first found in the form of an  $\alpha$ -2,8-linked oligomer containing 6–7 residues in rainbow trout eggs.<sup>1</sup> Later, Kdn was found in heterooligosaccharides of animal tissues<sup>2,3</sup> and as a constituent of the capsular heteropolysaccharide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

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

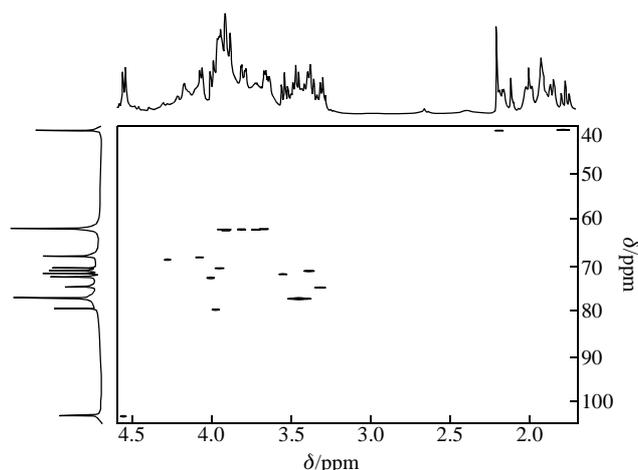
The polymers were extracted from the cell wall as described previously,<sup>5</sup> and their structure was examined using NMR spectroscopy.

The <sup>13</sup>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 <sup>1</sup>H and <sup>13</sup>C NMR spectra of the predominant component of the polymer mixture were completely assigned using 2D homonuclear <sup>1</sup>H/<sup>1</sup>H COSY, TOCSY and ROESY and heteronuclear <sup>1</sup>H/<sup>13</sup>C HSQC (Figure 2) and HMBC experiments. The residue of nonulosonic acid was identified with Kdn in accordance with the coupling constants in the <sup>1</sup>H NMR spectrum.<sup>6</sup> The upfield chemical shift of H-3<sub>eq</sub> (2.205 ppm) was in agreement with the  $\beta$ -configuration of the Kdn residue.<sup>4</sup> The second sugar residue in the disaccharide repeating unit of the polymer was identified as the terminal  $\beta$ -glucopyranose ( $\beta$ -Glc<sub>p</sub>) based on the chemical shifts and coupling constants of the <sup>1</sup>H and <sup>13</sup>C NMR spectra. The ROESY spectrum (Figure 3) revealed correlation peaks for the anomeric protons of Glc<sub>p</sub> 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  $\beta$ -Glc<sub>p</sub> was bonded with Kdn by the 1 $\rightarrow$ 8 linkage. The presence of a correlation peak of H-1(Glc<sub>p</sub>)/C-8(Kdn) in the HMBC spectrum is consistent with this conclusion, too. The downfield shift of C-4 by 2 ppm in the <sup>13</sup>C NMR spectrum of the  $\beta$ -Kdn residue



**Figure 1** <sup>13</sup>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.



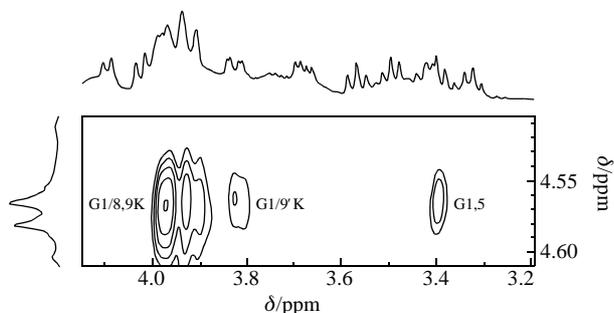
**Figure 2** HSQC spectrum of the Kdn-containing polysaccharide of the cell wall of *Streptomyces* sp. VKM Ac-2090. The corresponding parts of the <sup>1</sup>H and <sup>13</sup>C NMR spectra are displayed along the horizontal and vertical axes, respectively.

**Table 1** <sup>1</sup>H NMR data (D<sub>2</sub>O, 303 K, acetone: 2.225 ppm) for  $\beta$ -Kdn<sup>6</sup> and for the polysaccharide of *Streptomyces* sp. VKM Ac-2090 cell wall.

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

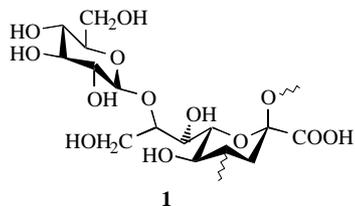
**Table 2** <sup>13</sup>C NMR data (D<sub>2</sub>O, 303 K, acetone: 31.45 ppm) for  $\beta$ -Kdn<sup>6</sup> and for the polysaccharide of *Streptomyces* sp. VKM Ac-2090 cell wall.

Residue	Chemical shifts, $\delta$ /ppm								
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
$\beta$ -Kdn <sup>6</sup>	174.2	96.02	39.19	68.51	70.67	71.06	69.27	72.29	63.86
$\rightarrow$ 4)- $\beta$ -Kdn- (2 $\rightarrow$ 8)	176.0	97.9	40.5	70.5	71.7	72.6	68.05	79.5	61.9
$\beta$ -Glc <sub>p</sub> -(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  $^1\text{H}$  NMR spectrum 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  $\beta$ -Kdn revealed the 2 $\rightarrow$ 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(Glcp)/H-8(Kdn) and H-1(Glcp)/H-9(Kdn) (Figure 3) and relatively large in module negative  $\beta$ -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  $\beta$ -D-configuration of the glucopyranose residue<sup>7,8</sup> **1**.



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

The  $\beta$ -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  $\beta$ -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. Mamyán 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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