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Note

Novel teichulosonic acid from cell wall of *Streptomyces coelicolor* M145

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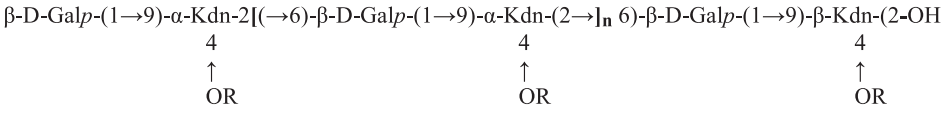
ABSTRACT

The cell wall of *Streptomyces coelicolor* M145, a prototrophic plasmidless (SCP1$^-$ SCP2$^-$) variant of strain *S. coelicolor* A3(2) contains the main glycopolymer represented by Kdn-containing teichulosonic acid with unusual structure which has not been described so far:



where R= H, CH_3 or α-Glc*p*NAc; n = 0 – 5

The minor polymer was found to be a poly(diglycosyl 1-phosphate) with the following repeating unit: -6)-α-Gal*p*-(1→6)-α-Glc*p*NAc-(1-*P*-. The structures of both glycopolymers were established by using a combination of chemical and NMR spectroscopic methods.

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Members of the genus *Streptomyces* (streptomycetes) are the Gram-positive bacteria with high mol % G + C of the DNA, which produce branching vegetative and aerial hyphae bearing long chains of reproductive spores.[1] Streptomycetes are the most abundant natural source of various bioactive secondary metabolites and are therefore of great interest for medicine and industry. Representatives of the genus *Streptomyces* are widely used in genetic research related to antibiotic production, bacterial physiology, and cell differentiation.[2]

Our previous studies showed a large diversity of anionic polymers in the cell wall of *Streptomycetes* species, and numerous novel structures of teichoic and teichuronic acids were revealed.[3–8] In addition, a natural Kdn-polymer (teichulosonic acid) was discovered for the first time.[9] In the present work, we report an unusual heteropolymer that is a Kdn-containing teichulosonic acid, along with a poly(diglycosyl 1-phosphate), found in the cell wall of *S. coelicolor* M145, a prototrophic plasmidless (SCP1$^-$ SCP2$^-$) derivative of strain A3(2). M145 is genetically the best known actinomycete strain and considered a model organism for various aspects of microbial biology,[2,10] such as cell division, hyphal growth[11], and antibiotic resistance.[12] In this respect, our results provide new details on the chemical composition of M145 cell wall and thus broaden an understanding of this model microbe.

Stepwise extraction of the freeze-dried cell wall with 10% trichloroacetic acid (4 °C, 3 × 24 h each) resulted in preparations that were used in structural studies of the anionic glycopolymers. The yield was ca 15% of the cell wall dry mass. The freeze-dried preparations were severally used in composition and NMR analyses. Their similarity allowed us to combine them for the further chemical and NMR spectroscopic analyses.

The compositions of acid hydrolysates (2 M HCl, 100 °C, 3 h) of the preparations obtained and the cell wall itself were studied by paper electrophoresis and paper chromatography, they were found to be qualitatively identical.

Hydrolysis afforded the following products: inorganic phosphate, two phosphorous esters with the mobility m_{Grop} 0.21 and 0.45 (electrophoresis) and monosaccharides: galactose and amino sugar (chromatography).

The results suggested the presence of phosphate-containing polymers. Judging by the number of identified phosphate esters their content in the cell wall of *S. coelicolor* M145 was small.

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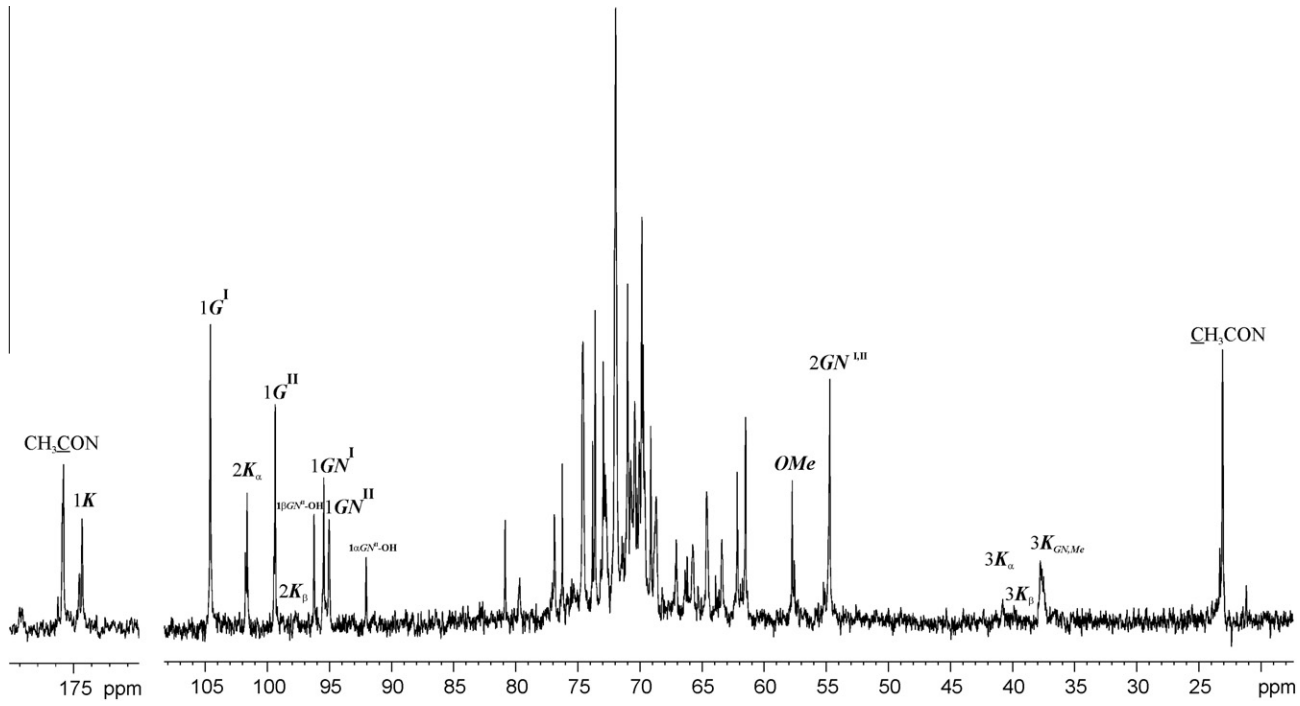

Figure 1. ^{13}C NMR spectrum of preparation from cell wall of *Streptomyces coelicolor* M145. Arabic numerals refer to the atoms in the sugar residues as designated in Table 1.

Table 1
^{13}C and ^{1}H NMR data of the cell wall polymers of *Streptomyces coelicolor* M145

Polymer Residue		C-1 *H-1*	C-2 *H-2*	C-3 *H-3 (H-3e,3a)*	C-4 *H-4 (H-4a,4b)*	C-5 *H-5*	C-6 *H-6 (H-6a,6b)*	C-7 *H-7*	C-8 *H-8*	C-9 *H-9*
Polymer I										
→6)-β-D-Gal*p*-(1→	(**G^{I}**)	104.7	72.1	73.8	69.9	74.6	64.7			
		4.45	*3.56*	*3.65*	*3.93*	*3.77*	*3.93, 3.53*			
→9)-α-Kdn-(2→	(**K$_{\alpha}$**)	174.8	101.6	40.8	71.5	70.9	74.7	68.9	70.1	72.1
				2.68, 1.68	*3.57*	*3.57*	*3.69*	*3.98*	*4.05*	*4.25, 3.85*
→9)-α-Kdn-(2→	(**K$_{\alpha,GN}$**)	174.5	101.5	37.8	76.3	69.1	74.7	68.9	70.1	72.1
4)				*2.72, 1.63*	*3.70*	*3.69*	*3.69*	*3.98*	*4.05*	*4.25, 3.85*
↑										
α-D-Glc*p*NAc-(1	(**GNI**)	95.5	54.8[a]	72.1	71.1	73.0	61.6			
		5.03	*3.93*	*3.80*	*3.53*	*3.93*	*3.81, 3.81*			
→9)-α-Kdn-4-OMe-(2→	(**K$_{\alpha,Me}$**)	174.6	101.6	37.6	80.9[b]	69.8	74.7	68.9	70.1	72.1
				2.86, 1.55	*3.33*	*3.63*	*3.69*	*3.98*	*4.05*	*4.25, 3.85*
Oligomers from polymer I										
β-D-Gal*p*-(1→	(**G$_{t}$**)	104.6	72.1	73.9	69.8	76.3	62.2			
		4.43	*3.56*	*3.66*	*3.92*	*3.69*	*3.78, 3.75*			
→9)-β-Kdn-(2-OH	(**K$_{\beta}$**)	174.2	97.2	40.0	70.0	71.4	72.9	68.8	70.5	72.9
				2.23, 1.80	*4.00*	*3.59*	*3.99*	*4.02*	*3.91*	*4.15, 3.84*
→9)-β-Kdn-(2-OH		176.1	97.2	36.9	75.7	69.3	72.8	68.8	70.5	72.9
4)	(**K$_{\beta,GN}$**)			*2.27, 1.75*	*3.98*	*3.72*	*3.99*	*4.02*	*3.91*	*4.15, 3.84*
↑										
α-D-Glc*p*NAc-(1	(**GN^{I}**)	95.6	54.8[a]	72.1	71.0	73.0	61.6			
		5.03	*3.92*	*3.77*	*3.53*	*3.95*	*3.83, 3.83*			
→9)-β-Kdn-4-OMe-(2-OH	(**K$_{\beta,Me}$**)	176.1	97.4	36.8	79.6[b]	69.9	73.0	68.8	70.5	72.9
				2.43, 1.68	*3.71*	*3.66*	*3.99*	*4.02*	*3.91*	*4.15, 3.84*
Polymer II										
-6)-α-Gal*p*-(1→	(**G^{II}**)	99.4	69.6	70.6	69.9	70.8	65.9			
		5.00	*3.83*	*3.92*	*4.11*	*4.12*	*4.02, 3.97*			
→6)-α-Glc*p*NAc[b]-(1-*P*-	(**GN^{II}**)	95.3	54.8	72.2	70.4	72.8	66.4			
		5.47	*3.98*	*3.75*	*3.71*	*4.03*	*4.12, 3.70*			

[a] $\underline{CH_3}\underline{\underline{C}}ON$ at $\delta_{\underline{C}}$ 23.4 and $\delta_{\underline{\underline{C}}}$ 175.8–176.1.
[b] OMe at δ_C 57.8 and δ_H 3.45.

The structures of the cell wall polymers of *S. coelicolor* M145 were established by NMR spectroscopic methods.

The ^{13}C NMR spectrum of the preparation (Fig. 1, Table 1) was typical of a non-regular polymer or a mixture of polymers. It contains *inter alia* signals of different integral intensities in the anomeric carbon resonance region at δ 92.2–104.7, including signals for quaternary carbons at δ 97.2–97.4 and 101.5–101.6 (data of the APT spectrum, not shown).

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