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Note

On the presence of 4-O-(1-carboxyethyl)-mannose in the capsular polysaccharide of *Rhodococcus equi* serotype 3

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Abstract

4-O-[(S)-1-carboxyethyl]-D-mannose and 4-O-[(R)-1-carboxyethyl]-D-mannose were synthesized and their acetylated lactones (**1**) and (**2**) prepared and characterized by 1 and 2D NMR spectroscopy and mass spectrometry. NMR spectra of (**1**) were identical to those of the lactone of a monosaccharide isolated from the exocellular polysaccharide of the cyanobacterium *Cyanospira capsulata*. The same derivative of a monosaccharide isolated from the capsular polysaccharide of *Rhodococcus equi* serotype 3, identified as 4-O-[(S)-1–carboxyethyl]-D-mannose [W. B. Severn and J. C. Richards, *Can. J. Chem.*, 70 (1992) 2664–2676], shows instead NMR spectra different from those of both lactones (**1**) and (**2**). This proves that 4-O-(1-carboxyethyl)-mannose is not among the constituent sugars of *R. equi* capsular polysaccharide. As a consequence, the nature of the sugar obtained from *R. equi* must be reinvestigated. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: 4-O-(1-carboxyethyl)-mannose; Absolute configuration; *Rhodococcus equi*; Capsular polysaccharide, structure; *Cyanospira capsulata*; Exopolysaccharide, structure

In two recent publications [1,2] we reported on the chemical structure of the exocellular polysaccharide from the cyanobacterium *Cyanospira capsulata* (CC-EPS). Among the sugar constituents of CC-EPS was 4-O-[(S)-1-carboxyethyl]-D–mannose (4-S-Lac-D-Man). Determination of the absolute configuration of Man entailed removal of the lactic acid moiety by BBr_3 [3] and GC analysis of the TMS derivative of the glycoside of optically pure 2-butanol [4]. To establish the configuration of the chiral α carbon of lactic acid we used the method devised by Severn and Richards [5,6]. In this general approach, lactic acid forms a cyclic ester with one of the free hydroxyl groups of the sugar; thus, the lactyl group is conformationally blocked, and NOE contacts between the α proton or methyl of lactic acid on one side and the sugar protons on the other one allow determination of the (R) or (S) configuration of the lactyl moiety, provided that the configuration of the sugar is known.

In Fig. 1(a)–(d) the ^{1}H NMR spectrum of the acetylated lactone of 4-S-Lac-D-Man (**1**) isolated

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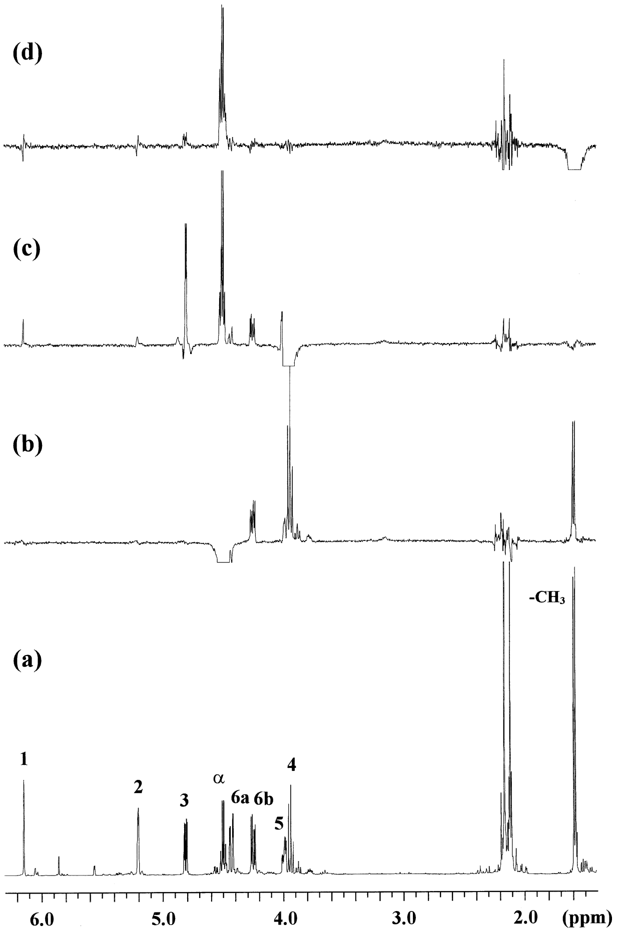
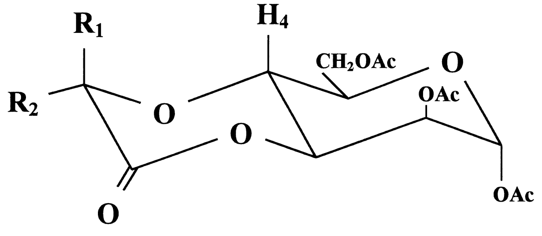


Fig. 1. (a) ^{1}H NMR spectrum recorded at 500 MHz in $CDCl_3$ of the acetylated lactone (**1**) of 4-O-[(S)-1-carboxyethyl]-D-mannose isolated from the exopolysaccharide of *Cyanospira capsulata* and NOE difference spectra obtained presaturating (b) H-α, (c) H-4 and (d) methyl group.

from CC-EPS and NOE difference spectra are shown. Presaturation of the α proton at 4.496 ppm [Fig. 1(b)] leads to signal enhancement for H-4 at 3.932 ppm and the methyl group at 1.577 ppm, whilst presaturation of H-4 [Fig. 1(c)] causes signal enhancement for H-α, but not for the methyl. The spectrum in Fig. 1(d), showing the effect of presaturating the methyl signal, confirms that there is no NOE contact between this group and H-4. Knowing that Man belongs to the D configuration, these data establish that the chirality of the 1-carboxyethyl substituent is (S). Severn and Richards report [5] the proton spectrum of what they believe to be the same compound. However, the spectrum they show is different from that of Fig. 1(a), and the ^{13}C chemical shifts they report [5,6] are also different from those shown here in Table 1.



(1): $R_1 = H_\alpha$ $R_2 = -CH_3$

(2): $R_1 = -CH_3$ $R_2 = H_\alpha$

In order to solve this contradiction, 4-S-Lac-D-Man was synthesized as described by Kochetkov et al. [7], who used the synthetic route to unequivocally prove the structure of this substituted Man, which was found for the first time in the extracellular polysaccharide from *Mycobacterium lacticolum* strain 121. In this synthesis commercially available 1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose in anhydrous 1,4-dioxane was reacted with (R)-2-chloropropanoic acid methyl ester in the presence of NaH (see Experimental). After deprotection of the sugar, the reaction mixture was treated as described in [5] to obtain (**1**). Preparative HPLC on a C_{18} column afforded two compounds in a molar ratio of approximately 3:2 (based on UV detector response at 205 nm). FAB mass spectra in NaCl doped 3-nitrobenzylalcohol showed pseudomolecular MNa^+ ions at m/z 383 and an intense fragment at m/z 301 (A1-type cleavage, [8]) for both compounds. A pseudomolecular ion at m/z 367 was obtained when using LiCl doped thioglycerol, confirming a molar mass of 360 a.m.u. 18 eV EI mass spectra were also recorded. The molecular ion was absent in these spectra and abundant fragments are found (see Experimental); no difference was noticeable between the EI mass spectra of the two HPLC fractions. The minor component gave ^{1}H and ^{13}C NMR spectra identical to that of Fig. 1(a) and Table 1 and was therefore identified as the acetylated lactone (**1**). ^{1}H–^{13}C long range correlation spectra (HMBC) of the two reaction products showed in both cases the presence of cross peaks between C-4 of Man and H-α of lactic acid and between H-4 of Man and C-α of lactic acid, proving that the lactyl substituent is linked to position 4 of Man. Gated decoupling ^{13}C NMR spectra showed a $^{1}J_{H1,C1}$ of 178.4 Hz for both compounds, establishing the α configuration at C-1. The ^{1}H spectrum and NOE difference spectra of the major component are shown in Fig. 2(a)–(d), and Table 2 reports ^{1}H and ^{13}C NMR assignments.

Table 1
NMR data[a] for acetylated lactone of 4-O-[(S)-1-carboxyethyl]-D-mannose (**1**)

Mannose ring	1	2	3	4	5	6a	6b
^{1}H ppm	6.138	5.200	4.808	3.932	3.985	4.426	4.245
^{3}J (Hz)	1.7	3.3	9.8	9.6	2.4; 5.2	12.2 (^{2}J)	–
^{13}C ppm	90.28	68.05	75.48	68.30	70.27	62.05	–
1-carboxyethyl group							
	–CH_3	α–CH	–CO–				
^{1}H ppm	1.577	4.496	–				
^{3}J (Hz)	6.9	–	–				
^{13}C ppm	18.21	73.97	168.44				
Acetyl group linked to							
	O-1	O-2	O-6				
–CH_3							
^{1}H ppm	2.155	2.155	2.107				
^{13}C ppm	20.73	20.73	20.68				
–CO–	167.74	169.38	170.63				

[a] Chemical shifts are expressed in ppm from internal TMS for ^{1}H and $CDCl_3$ (77 ppm) for ^{13}C. ^{1}H chemical shifts and coupling constants were refined by spectrum simulation and iteration.

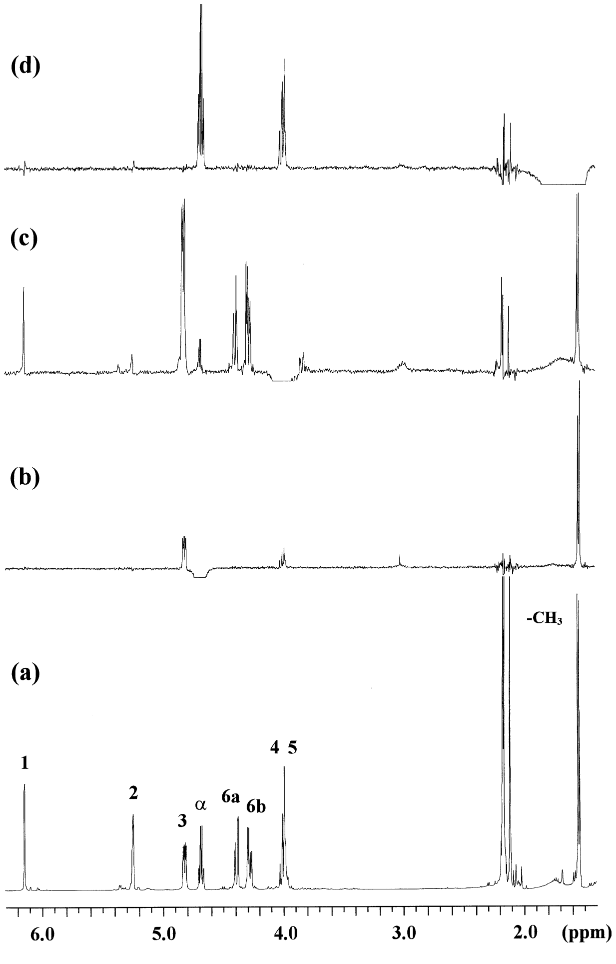


Fig. 2. (a) ^{1}H NMR spectrum recorded at 500 MHz in $CDCl_3$ of the acetylated lactone (**2**) of synthetic 4-O-[(R)-1-carboxyethyl]-D-mannose and NOE difference spectra obtained presaturating (b) H-α, (c) H-4 and (d) methyl group.

The lactic acid α proton at 4.682 ppm shows a strong NOE contact with the α methyl at 1.539 ppm [Fig. 2(b)]. The NOE difference spectrum in Fig. 2(c) is more complex because H-4 and H-5 are almost isochronous and therefore presaturation of H-4 cannot be selective and NOE enhancements which derive from both protons can be found. A strong enhancement of the methyl group is however noticeable. In the spectrum of Fig. 2(d) presaturation of the methyl group generates signal enhancements for H-α and H-4. These data establish that the other product of the synthesis is lactone (**2**). Obtaining both diastereoisomers through partial inversion of configuration of (R)-2-chloropropanoic acid is not reported in [7], but is not surprising in a substrate that may be compared to secondary alkyl halides. It is well known that in these cases SN1 and SN2 mechanisms coexist.

It may be concluded that the sugar isolated by Severn and Richards from the capsular polysaccharide of *Rhodococcus equi* serotype 3 is not 4-O-(1-carboxyethyl)-mannose and that its nature should be reinvestigated.

1. Experimental

*Synthesis and isolation of 4-O-[(*S*)-1-carboxyethyl]-*D*-mannose and 4-O-[(*R*)-1–carboxyethyl]-*D*-mannose.*—4-O-[(S)-1-carboxyethyl]-D-mannose was synthesized as described by Kochetkov

Table 2
NMR data[a] for acetylated lactone of 4-O-[(R)-1-carboxyethyl]-D-mannose (**2**)

Mannose ring	1	2	3	4	5	6a	6b
^1H ppm	6.138	5.247	4.818	4.000	3.974	4.384	4.278
^3J (Hz)	1.7	3.4	9.9	9.7	2.3; 5.3	12.2 (^2J)	–
^{13}C ppm	90.31	67.80	74.78	64.64	70.46	62.06	–
1-carboxyethyl group							
	$-CH_3$	α–CH	–CO–				
^1H ppm	1.539	4.682	–				
^3J (Hz)	7.0	–	–				
^{13}C ppm	17.75	71.41	169.11				
Acetyl group linked to							
	O-1	O-2	O-6				
$-CH_3$							
^1H ppm	2.153	2.163	2.105				
^{13}C ppm	20.72[b]	20.72[b]	20.78[b]				
–CO–	167.72	169.37	170.54				

[a] Chemical shifts are expressed in ppm from internal TMS (0 ppm) for ^1H and $CDCl_3$ (77 ppm) for ^{13}C. ^1H chemical shifts and coupling constants were refined by spectrum simulation and iteration.

[b] Assignments may be reversed.

et al. [7], with the exception that 1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose in anhydrous 1,4-dioxane was reacted with (R)-2-chloropropanoic acid methyl ester instead of the free acid. The reaction mixture, after quenching with water, evaporation in vacuo to remove 1,4-dioxane, washing with $CHCl_3$, acidification and extraction with $CHCl_3$, was not purified by silica gel chromatography as in [7], but was evaporated in vacuo and treated directly with boiling 3% HCl for 4 h to deprotect the sugar ring and finally neutralized with Ag_2CO_3. The filtered solution was lyophilized, suspended in tetrahydrofuran and reacted overnight with Ac_2O at room temperature. Pyridine was then added and the reaction stirred for 8 h [5]. The mixture was evaporated in vacuo and analyzed by C_{18} HPLC. By isocratic elution with H_2O/CH_3CN 70/30 at 2 mL/min and UV detection at 205 nm two peaks were eluted after 30 min, well resolved from early eluting material. Their intensity ratio was 3:2, with the major component eluting at 35.0 min and the minor component at 37.4 min. The final yield calculated as the sum of the two compounds purified by preparative HPLC was 13%. The chromatography apparatus consisted of a Varian VISTA 5500 pump equipped with a Rheodyne injection valve, a 2050 variable wavelength Varian UV detector and a Phase Sep Spherisorb S5 ODS2 column (10 mm×25 cm).

NMR spectroscopy.—NMR spectra were recorded in $CDCl_3$ on a ^{UNITY}*INOVA* Varian instrument operating at 500 (^1H) and 125 (^{13}C) MHz. NOE difference spectra were acquired with 4928 Hz spectral width, 32 K points, 5 s presaturation period with 50 ms alternation on lines of multiplets [9] at 300 K. Subtraction of a control spectrum (presaturation frequency set at 3 ppm) was performed internally adding alternated FIDs acquired with decoupler set at multiplet and control frequency. ^1H assignments are based on measurement of coupling constants and on the COSY spectra. Observed first order chemical shifts and coupling constants were refined by spectrum simulation and iteration using the PC program CALM (version 2.00, Resonance Co., downloadable free from the Web).^{13}C assignments are based on HSQC spectra acquired with gradient coherence selection [10], 3851 Hz (^1H) and 25,000 Hz (^{13}C) spectral width, 128 increments and 1024 points FIDs. ^{13}C decoupling was attained with GARP and phase sensitivity in F1 by the SRH method. Gradient selected HMBC [11] used 60 or 120 ms period for evolution of long range couplings, same spectral width of HSQC experiment, 256 increments and 2048 points FIDs, magnitude mode in F1. Data matrices were multiplied by shifted sinebell functions in both dimensions and zerofilled twice in F1 before FT.

Mass spectrometry.—FAB mass spectra were recorded in positive ion mode on a double focusing Kratos MS 50 instrument equipped with the Maspec2 data acquisition and processing system (Mass Spectrometry Services Ltd.) and a cesium ion gun (20 KV) FAB ion source. 3-nitrobenzylalcohol saturated with NaCl or thioglycerol saturated with LiCl were used as matrices. CsI was used as a calibrant and spectra acquired at 10 s/decade at resolution 1000 with an acceleration potential of 8 KV. 18 eV EI mass spectra were recorded on a QMD 1000 GC–MS system (Carlo Erba Instruments) equipped with the solid sample direct insertion probe. The quadrupole analyzer, calibrated with heptacosafluorotributylamine, was set to scan from 9 to 500 *m*/*z* at 3 s scan time and 3 s interscan delay, while the sample was heated from 30 to 400 °C at 20 °C/min. 18 eV EI mass spectra of compounds (**1**) and (**2**) showed no molecular ion at *m*/*z* 360, but intense fragment ions were present. Compound (**1**): *m*/*z* 301 (18%, A1-type ion); *m*/*z* 229 (59%, loss of CH_3CHCO_2 from *m*/*z* 301); *m*/*z* 187 (96%, loss of chetene from *m*/*z* 229); *m*/*z* 145 (100%, loss of chetene from *m*/*z* 187); *m*/*z* 127 (44%, loss of H_2O from *m*/*z* 145); *m*/*z* 43 (59%). Compound (**2**): *m*/*z* 301 (20%); *m*/*z* 229 (61%); *m*/*z* 187 (91%); *m*/*z* 145 (100%); *m*/*z* 127 (49%); *m*/*z* 43 (86%).

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