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Note

A novel 4-C-branched sugar from the lipopolysaccharide of the bacterium *Pseudomonas caryophylli*

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Pseudomonas caryophylli is the causal agent of the bacterial wilt of carnation (*Dianthus caryophyllus* L.) [1]. We are interested in the structural determination of the O-chain of the cell wall lipopolysaccharide of this bacterium. This paper describes a preliminary result concerning the isolation of a novel branched sugar carrying a C_6-chain at position 4.

The lipopolysaccharide (LPS), obtained from the cell wall by phenol–water extraction, was purified by gel filtration on Bio-Gel A-15m and acid-hydrolyzed. The ^1H NMR spectrum (Fig. 1 and Table 1) of the main product isolated from the hydrolysate showed signals at δ 5.15 (d, 3.5 Hz) and 4.56 (d, 8.2 Hz) assignable to α- and β-anomeric protons, in addition to signals assignable to methylene and methyl protons, suggesting a dideoxy sugar structure. The assignments indicated in Table 1 were inferred from a COSY experiment that also revealed the interruption of the scalar connectivities at position 4. The ^{13}C *on*-resonance and DEPT NMR spectra showed two anomeric carbons at δ 98.5 ($^1J_{C,H}$ 160 Hz, β anomer) [2] and 91.6 ($^1J_{C,H}$ 174 Hz, α anomer) [2],

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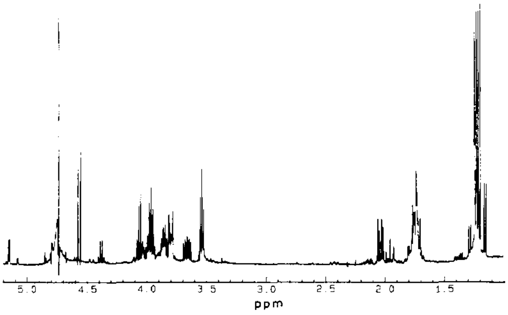


Fig. 1. ^1H NMR (400 MHz) spectrum of **1** in D_2O.

twelve carbinolic methine carbons (78.3–65.2 ppm), two carbinolic quaternary carbons (75.3 and 75.2 ppm), four aliphatic methylene carbons (35.3–30.3 ppm), and four methyl carbons (17.4–13.0 ppm). These data suggested the product to be an α/β mixture of a branched sugar possessing structure **1**.

By treatment of **1** with MeOH–HCl an α/β mixture of methyl glycoside **2** was

Table 1
^1H NMR (400 MHz) data of compounds **1**, **2**, and **3** [a]

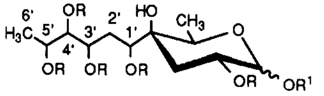
H	**1** [b]		**2** [b]		**3** [c]
	α anomer	β anomer	α anomer	β anomer	β anomer
1	5.15 d (*3.5*)	4.56 d (*8.2*)	4.72 d (*3.7*)	4.32 d (*8.0*)	5.90 d (*8.3*)
2	4.02 [d]	3.66 ddd (*11.5; 8.2; 5.2*)	4.03 [e]	3.68 ddd (*11.5; 8.0; 5.5*)	5.36 ddd (*12.7; 8.3; 5.9*)
3*eq*	1.80 [d]	2.04 dd (*13.2; 5.2*)	1.78 [e]	2.03 dd (*13.3; 5.5*)	2.44 dd (*12.7; 5.9*)
3*ax*	1.96 t (*13.2*)	1.74 [d]	1.93 t (*12.5*)	1.71 [e]	1.85 t (*12.7*)
5	4.38 q (*6.5*)	4.06 q (*6.5*)	4.21 q (*6.5*)	4.06 q (*6.6*)	3.67 q (*6.3*)
6-H_3	1.15 d (*6.5*)	1.20 d (*6.5*)	1.18 d (*6.5*)	1.22 d (*6.6*)	1.26 d (*6.3*)
1′	3.79 m		3.79 m		5.21 dd (*11.2; 1.9*)
2′-H_2	1.74 [d]		1.71 [e]		2.38 ddd (*14.2; 11.7; 1.9*) 1.96 ddd (*14.2; 11.2; 2.4*)
3′	3.85 m		3.85 m		5.24 ddd (*11.7; 3.4; 2.4*)
4′	3.54 m		3.53 t (*6.0*)		5.43 dd (*6.3; 3.4*)
5′	3.96 dq (*6.5; 6.5*)		3.96 dq (*6.5; 6.0*)		5.18 dq (*6.3; 6.3*)
6′-H_3	1.28 d (*6.5*)	1.23 d (*6.5*)	1.23 d (*6.5*)		1.12 d (*6.3*)
1-OCH_3			3.45 s	3.57 s	
-$OCOCH_3$					1.81 s, 1.72 s, 1.66 s, 1.65 s (6 H), 1.64 s

[a] Assignments are based on H,H COSY experiments. Apparent coupling constants are given in parenthesis (*Hz*).

[b] Measured at 30°C in D_2O (δ from external reference TSP).

[c] Measured at 30°C in C_6D_6 (δ from solvent signal at δ 7.15).

[d,e] Overlapped signals.



1	R = H	R^1 = H
2	R = H	R^1 = Me
3	R = Ac	R^1 = Ac

obtained. Its ^1H (Table 1) and ^{13}C (Experimental) NMR chemical shifts were completely assigned by COSY and HETCOR experiment and were consistent with structure **2**.

Confirmatory evidence for the structure of the novel sugar was obtained from the spectral data of the acetate derivative **3**, obtained by treatment with pyridine and acetic anhydride. Its ^1H NMR spectrum (Table 1) contained mainly the signals of the β anomer and exhibited six acetyl signals, in agreement with the difficulty of acetylation of the tertiary hydroxyl group, whose signal clearly appeared in the IR spectrum at 3600 cm^{-1}. Consistent with structure **3** were also the ^{13}C data (Experimental) and the positive-ion FABMS spectrum, which showed a pseudomolecular ion $[M + NH_4]^+$ at *m/z* 566.

The *xylo* configuration of the ring was inferred from two NOE experiments, performed with **3**. In fact, enhancement of the signals at δ 3.67 and 1.85, attributed to the H-5 and the *axial* H-3 protons, respectively, was measured by irradiation on the anomeric proton at δ 5.90, indicating the *axial* orientation of the H-5 proton. The *equatorial* orientation of the side chain at C-4 was deduced from the spatial proximity between H-1′ and H-5; irradiation of the latter enhanced the H-1′ signal at δ 5.21.

The EIMS spectrum of the alditol-*1-d* acetate of **1** was measured on a GLC–MS instrument and was also in agreement with the suggested structure (Fig. 2).

The possibility that the monose could possess, differently from **3**, the alternative pyranose form with the C-1′ included in the ring was ruled out by the enhancement of the quartet at δ 4.06, assigned to the β anomer H-5, by irradiation of the β-anomeric proton at δ 4.56.

To the best of our knowledge, yersiniose A and B are the only two 4-C-branched sugars known until now [3,4]. They were both isolated from bacteria and are 3,6-dideoxy-4-*C*-(1-hydroxethyl)-D-*xylo*-hexoses and differ in the configuration of the side chain. In addition, in a bacterial lipooligosaccharide, a new branched 3,6-dideoxy sugar has been recently [5] identified, which at C-4 bears a C_7-chain.

Work is in progress to establish the stereochemistry of **1** and to identify the constitution of the LPS fragment which yields **1** by hydrolysis.

1. Experimental

General.—The ^1H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, with a spectrometer equipped with a dual probe, in the FT mode at 50°C. The DEPT experiment was performed using a polarization transfer pulse of 135° and a delay adjusted to an average C,H coupling of 160 Hz. The standard Bruker software

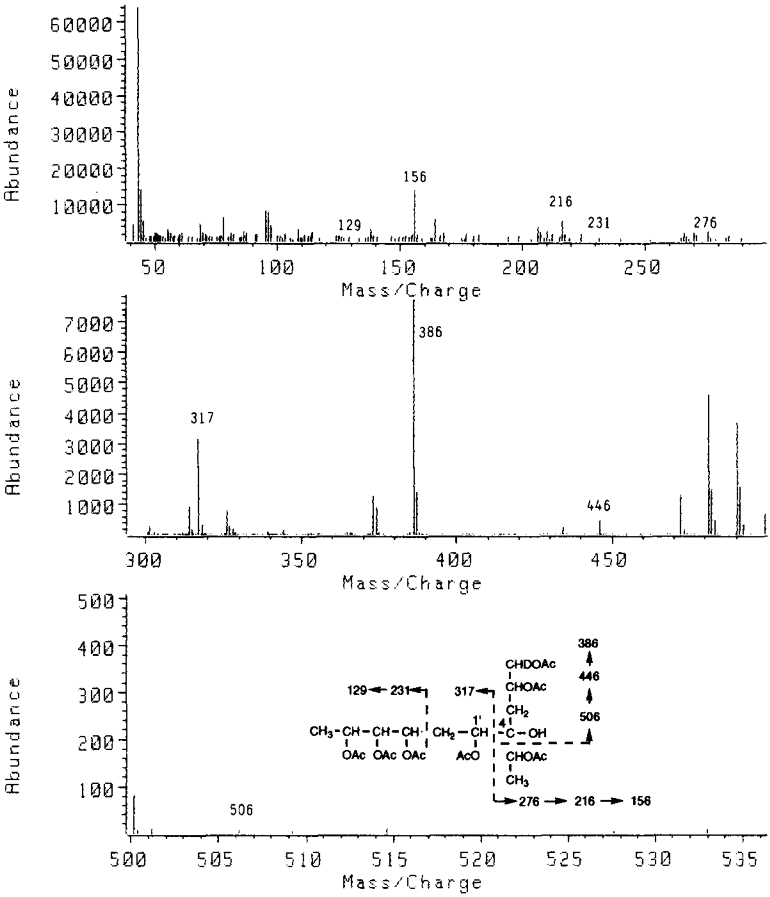


Fig. 2. EIMS spectrum of the alditol-*1-d* acetate of **1**.

(XHCORR) was used to obtain the heteronuclear H,C COSY experiment under the following conditions: the time domain in f_2 was 1K, 128 spectra were collected with 1600 scans, the spectral width was 10 000 Hz in the f_2 and 2000 Hz in the f_1 domain, delays were optimized for $^1J_{C,H}$ 160 Hz. Fourier transformation was performed with a shifted sine-bell function in both dimensions. The H,H COSY experiment was performed with standard COSY, with a second pulse of 45°.

Total carbohydrates were determined by the phenol–H_2SO_4 method [6]. The alditol acetates were analyzed by GLC–MS, using an SP-2330 capillary column (Supelco, 30 m × 0.25 mm i.d., flow rate 0.8 mL/min, He as carrier gas) at 140°C.

Preparation of cellular lipopolysaccharide.—A crude sample of LPS (306 mg), extracted by the conventional phenol procedure [7] from dry cells (7 g) of *Pseudomonas caryophylli* NCPPB 2151 (National Collection of Plant Pathogenic Bacteria, Harpenden, UK), was chromatographed on Bio-Gel A15m (300 mM triethylamine neutralized to pH 7 with HCl) to yield purified LPS (250 mg).

Hydrolysis of LPS.—A sample (20 mg) of the LPS was hydrolyzed with 0.125 M CF_3CO_2H (100°C, 30 min). After removal of lipids by centrifugation, the crude material (16 mg) was chromatographed on Bio-Gel P2, eluting with water. Fractions were analyzed by TLC (silica gel impregnated with 0.2 M NaOAc; eluent, 5:1:1 n-BuOH–EtOAc–0.03 M H_3BO_3). The main fraction was **1** (4 mg); $[\alpha]_D$ −15° (*c* 3, H_2O, room temperature). ^1H NMR: see Table 1 and Fig. 1. ^{13}C NMR [D_2O at 30°C, δ from external reference TSP (sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$)]: δ 98.5 (β anomer C-1), 91.6 (α anomer C-1), 78.3, 78.2, 75.3, 75.2, 70.2, 69.9, 68.5, 68.2, 68.0, 67.7, 67.0, 65.2 (carbinolic methine carbons), 75.3, 75.2 (carbinolic quaternary carbons), 35.3, 33.0, 32.5, 30.3 (methylene carbons), 17.4, 17.4, 13.2, 13.0 (methyl carbons).

A sample of **1** (200 μg) was converted into the alditol-*1-d* acetate by the usual procedure [8]. GLC–MS: see Fig. 2.

A sample of **1** (2 mg) was treated with 1 M HCl–MeOH at 80°C for 90 min. Usual work-up gave **2** (1 mg). ^1H NMR: see Table 1. ^{13}C NMR [D_2O at 30°C, δ from external 1,4-dioxane (δ_C, 67.4 ppm)]: δ 106.7 (β anomer C-1); 99.6 (α anomer C-1); 78.8 (β and α C-4′), 75.8 (β C-5), 75.6 (β and α C-4), 71.2 and 70.8 (β and α C-1′), 69.6 (β and α C-3′), 60.8 (β and α C-5′), 67.7 (α C-5), 67.5 (β C-2), 65.6 (α C-2), 57.7 (β OCH_3), 55.9 (α OCH_3), 36.2 (β C-3), 33.5 (β and α C-2′), 18.1 (β and α C-6′), 13.6, 13.3 (β and α C-6).

A sample of **1** (1 mg) was treated with 1:1 pyridine–Ac_2O (0.3 mL) at 120°C for 20 min. Usual work-up gave **3** (1 mg). IR ($CHCl_3$): 3600 cm^{-1}. ^1H NMR: see Table 1. ^{13}C NMR (C_6D_6): δ 170.1, 169.6, 169.4, 169.1, 169.0 (carbonyl carbons), 94.2 (β-anomeric carbon), 77.1, 74.7, 69.8, 68.2, 68.0, 67.8 (carbinolic methine carbons), 73.6 (carbinolic quaternary carbon), 34.6, 28.3 (methylene carbons), 20.5, 20.4, 20.2, 16.3, 13.6 (methyl carbons).

Acknowledgments

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