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Caryose: a carbocyclic monosaccharide from *Pseudomonas caryophylli* [1]

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Abstract

The complete structure of the first example of a carbocyclic monosaccharide, named caryose (4,8-cyclo-3,9-dideoxy-L-*erythro*-D-*ido*-nonose) and obtained from the lipopolysaccharide fraction of *Pseudomonas caryophylli*, is reported. The structure and the relative stereochemistry were inferred by chemical and spectroscopic analysis, and the absolute configuration was elucidated by the Exciton Chiral Coupling method.

Keywords: Caryose; 4,8-Cyclo-3,9-dideoxy-L-*erythro*-D-*ido*-nonose; *Pseudomonas caryophylli*; Deoxy sugar; Carbocyclic sugar

1. Introduction

Pseudomonas caryophylli is a phytopathogenic bacterium responsible for the wilting of carnations (*Dianthus caryophyllus* L.) [1,2]. Recently, the complete structure of the novel monosaccharide caryophyllose, obtained from the lipopolysaccharide (LPS) of this bacterium, was described [3,4].

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In this work the structure and the complete stereochemistry of another novel monosaccharide, isolated from the same source, is described.

2. Experimental

General.—The ^{1}H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, with a Bruker AM 400 spectrometer equipped with a dual probe, in the FT mode. The ^{13}C and ^{1}H chemical shifts were measured in C_6D_6 and in D_2O using 1,4-dioxane (67.4 ppm) and TSP (sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$), respectively, as external standards. DEPT experiments were performed using a polarisation transfer pulse of 135° and a delay adjusted to an average C,H coupling of 160 Hz. H,H COSY and NOE experiments were performed with standard COSY-45 and NOEDIFF sequences, respectively, under the following conditions: the time domain in f_1 was 512 w and in f_2 2K; the spectral width was 1200 Hz. The NOESY experiment was performed with a NOESYPH sequence under the above conditions, using a mixing time of 400 ms and a recycling delay of 2 s. IR spectra were recorded in $CHCl_3$ on a Perkin–Elmer 1760 Fourier-transform spectrometer. UV spectra were recorded in MeOH on a Perkin–Elmer Lambda 7 instrument. Optical rotations were determined on a Perkin–Elmer 141 polarimeter. CD spectra were obtained in MeOH on a Jasco 710 instrument. Mass spectra were recorded with a VG ZAB HF instrument equipped with an FAB source. TLC was carried out on Silica Gel F_{254} (Merck). All compounds were revealed by spraying with a saturated solution of Cr_2O_3 in concentrated H_2SO_4 followed by heating at 120 °C for 15 min.

Methanolysis of LPS fraction.—A sample of the LPS fraction (100 mg) was treated with HCl–MeOH as reported [4]. The usual workup gave a crude mixture (84 mg) which was purified by preparative TLC (0.2 M NaOAc-impregnated plates; 5 : 1 : 1 *n*-BuOH–EtOAc–0.03 M H_3BO_3). The fraction with R_f 0.4 (10 mg) was extracted to give **1a**, as a colourless syrup; $[\alpha]_D$ +81° (*c* 1.75, H_2O); ^{1}H NMR: see Table 1; ^{13}C NMR: see text.

Acetylation of **1a**.—A sample of **1a** (2 mg) was acetylated with 1 : 1 pyridine–Ac_2O (300 μL) at 120 °C for 20 min, to give **1b** (2 mg), which was characterised essentially by ^{1}H NMR as reported in Table 1; FAB-MS: see text; IR: 3580 cm^{-1} ($CHCl_3$).

Acid hydrolysis of **1a**.—A sample of **1a** (6 mg) was hydrolysed with 2 M CF_3CO_2H (250 μL) at 120 °C for 45 min, to give a mixture of **2a**, **2b**, and **2c** (6 mg); $[\alpha]_D$ +9.5° (*c* 1.5, H_2O); ^{1}H NMR: see Table 3.

Attempted isopropylidenation of **1a**.—To a solution of **1a** (1 mg) in dry acetone (300 μL) were added 2,2-dimethoxypropane (100 μL) and a trace of Amberlite IR-120 (H^+ form). The reaction mixture was kept at room temperature. After 8 h, further dimethoxypropane (600 μL) was added. After 12 h, **1a** was recovered unchanged [TLC (0.2 M NaOAc-impregnated plates; 5 : 1 : 1 *n*-BuOH–EtOAc–0.03 M H_3BO_3) and ^{1}H NMR].

Acetylation of the mixture of **2a**, **2b**, *and* **2c**.—A sample of the mixture (3 mg) was acetylated as described above. The crude reaction product was purified by TLC (9 : 1 benzene–2-propanol). Two fractions were eluted. The more polar fraction (R_f 0.35; 1

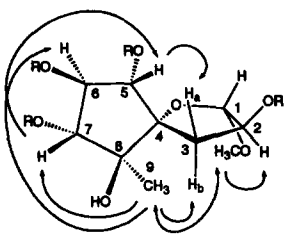
mg) was pure **3**; ^{1}H NMR: see text; IR: 3582 cm^{-1} ($CHCl_3$). The less polar one (R_f 0.40; 1 mg) was a mixture of acetates (^{1}H NMR).

Isopropylidenation of the mixture **2a**, **2b**, *and* **2c**.—To the mixture (3 mg) in dry acetone (600 μL) were added 2,2-dimethoxypropane (200 μL) and a trace of Amberlite IR-120 (H^+ form). The reaction mixture was kept at room temperature. After 8 h, further dimethoxypropane (1.2 mL) was added. After 12 h, TLC (14:6:1 $CHCl_3$–MeOH–H_2O) revealed that no reaction had occurred. The mixture was therefore heated at 65 °C for 3 h. Workup gave a mixture that was resolved by TLC (9:1 $CHCl_3$–MeOH) to yield pure **4a** (1 mg, R_f 0.45) and **5** (2 mg, R_f 0.22), identified by ^{1}H NMR (see Table 4).

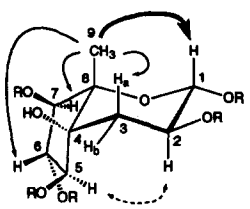
p-*Bromobenzoyl esters* **4b**.—Compound **4a** (1 mg) was treated with dry pyridine (300 μL) and *p*-bromobenzoyl chloride (10 mg) at room temperature for 3 h. After quenching by addition of MeOH, the reaction mixture was evaporated to dryness and purified by preparative TLC (8:2 petroleum ether–AcOEt) to give **4b** (0.7 mg) as a homogeneous solid; ^{1}H NMR ($CDCl_3$) (see Table 4); CD (MeOH): see text.

3. Results and discussion

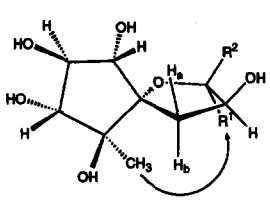
From the methanolysis of the LPS fraction, besides the methyl glycosides of caryophyllose [3], a minor fraction was obtained. The latter, purified by TLC, has now been shown to be the methyl glycoside **1a**.



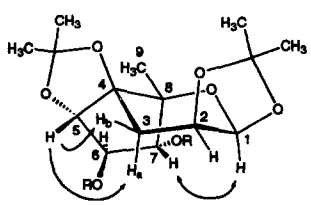
1a R = H
1b R = Ac



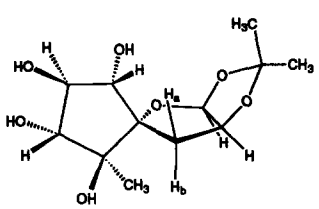
2a R = H
3 R = Ac



2b R^1 = OH, R^2 = H
2c R^1 = H, R^2 = OH



4a R = H
4b R = *p*-BrC_6H_4CO



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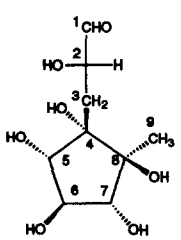

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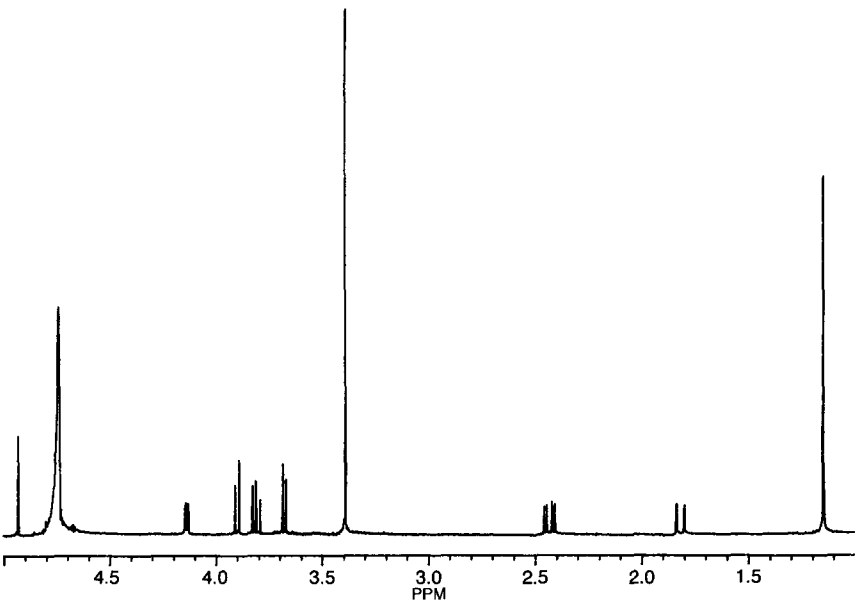

Fig. 1. ^{1}H NMR (400 MHz) spectrum of **1a** in D_2O.

The ^{13}C NMR *on*-resonance spectrum of **1a** showed ten signals, whose nature was defined on the basis of a DEPT experiment. The signals displayed were: one at δ 113.0 assignable to an anomeric carbon, probably in the furanose form owing to its low-field chemical shift; four for carbinolic methine carbons at δ 85.5, 83.3, 80.8, and 76.9; two for tertiary carbinolic carbons at δ 95.9 and 82.4; one for a methylene carbon at δ 38.5; one for a methyl carbon at δ 19.7; and one methoxyl signal at δ 57.9. The ^{1}H NMR

Table 1
^{1}H NMR chemical shifts (δ) and apparent coupling constants in parentheses (Hz) of compounds **1a** and **1b** at 30 °C

Proton	**1a** [a]	**1b** [b]
1	4.93 s	4.96 s
2	4.13 d (5.4)	5.07 d (6.8)
3a	1.82 d (14.8)	1.93 d (14.7)
3b	2.43 dd (14.8; 5.4)	2.87 dd (14.7; 6.8)
5	3.90 d (8.2)	5.31 d (4.4)
6	3.81 dd (8.2; 6.1)	5.20 dd (5.9; 4.4)
7	3.68 d (6.1)	5.00 d (5.9)
CH_3	1.15 s	1.30 s
OCH_3	3.39 s	3.42 s
OAc		2.14 s; 2.14 s; 2.10 s; 2.08 s
OH		2.52 s

[a] Measured in D_2O (δ from external reference TSP).
[b] Measured in $CDCl_3$ (δ from solvent signal at δ 7.27).

spectrum (Fig. 1, Table 1) showed, in agreement with the presence of two unprotonated carbons, two unconnected series of J_{vic}-scalar coupled protons, one due to the fragment –CHOH–CHOH–CHOH– and the other due to the fragment –CHOH–CH_2–. The latter appeared correlated to the anomeric methoxyl group by NOE measurements (see below).

In addition, the spectrum contained one methyl singlet at δ 1.15, indicating a methyl group on an unprotonated carbon, and one of a methoxyl group at δ 3.39. The assignment of almost all of the protons was based on a COSY experiment. Further support for the structure of **1a** came from its acetate derivative **1b**, whose ^{1}H NMR spectrum (Table 1) showed the introduction of four acetoxyl groups and a singlet at δ 2.52, assigned to a hydroxyl group on the basis of a transfer saturation experiment and of exchange with D_2O. The absorption at 3580 cm^{-1} in the IR spectrum of **1b** confirmed the presence of an unacetylated hydroxyl group localised, very probably, on a tertiary carbon. The molecular weight of **1b** was established by FABMS spectra, in the positive mode, performed with and without addition of NaCl. The latter spectrum showed only the pseudomolecular ion peak $[M + NH_4]^+$ at m/z 436 whereas the former displayed only the pseudomolecular ion peak $[M + Na]^+$ at m/z 441, confirming a molecular weight for **1b** of 418. This result, together the ^{13}C data of **1a**, suggested for **1b** the molecular formula $C_{18}H_{26}O_{11}$, which indicated a bicyclic structure. The occurrence in **1a** of an oxygen-bearing unprotonated carbon shared between two pentacyclic rings was suggested by the low-field chemical shift value of the signal at δ 95.9 [5].

As far as the relative stereochemistry of **1a** was concerned, this was established by means of NOE measurements (Table 2), determined by NOESY and confirmed, in some cases, by NOE difference experiments.

The NOEs, indicated by arrows on formula **1a**, established the *cis* relationships around the furanose ring between the substituents CH_3-9, CH_3O-1, H-2, and H-3b (δ 2.43), on one side, and between H-5 and H-3a (δ 1.82), on the other side. The relative orientation of H-2, *cis* and *trans* with respect to H-3b and H-3a, respectively, was in agreement with the values of its coupling constants with the H-3 protons. For the cyclopentane ring, NOEs established the *cis* relationships between CH_3-9 and H-6, on one side, and H-5 and H-7, on the other side. An NOE was also observed between

Table 2
NOEs [a] measured by NOESY for **1a** in D_2O at 30 °C

Proton	1	2	3a	3b	5	6	7	CH_3	OCH_3
1		x							xx
2			xx	xxx					x
3a		xx		xxx	xx				
3b		xx	xxx					x	
5							x		
6									
7					x				
CH_3				x		x	x		x
OCH_3	xx	x						xx	

[a] The intensities of the NOE effects are indicated as: x = weak, xx = medium, and xxx = strong.

Table 3
^{1}H NMR chemical shifts (δ) and apparent coupling constants in parentheses (Hz) of the equilibrium mixture **2a**, **2b**, and **2c**, and of acetate **3** at 30 °C

Proton	**2a** [a]	**2b** [a]	**2c** [a]	**3** [b]
1	4.81 d (8.1)	5.35 s	5.33 d (3.9)	6.26 d (6.4)
2	3.57 ddd (11.7; 8.1; 5.6)	4.15 dd (1.7; 5.4)	4.27 dt (7.4; 3.9)	5.23 ddd (5.4; 6.4; 8.8)
3a	1.67 dd (13.2; 11.7)	1.87 dd (14.7; 1.7)	1.89 dd (13.9; 7.4)	1.76 dd (14.1; 8.8)
3b	2.31 dd (13.2; 5.6)	2.55 dd (14.7; 5.4)	2.52 dd (13.9; 7.4)	2.20 dd (14.1; 5.4)
5	4.05 d (8.5)	3.94 d (8.3)	3.89 d (7.6)	5.80 d (6.4)
6	4.01 dd (8.5; 4.9)	3.85 [c]	3.84 [c]	5.67 dd (6.4; 4.9)
7	3.67 d (4.9)	3.72 d (6.1)	3.69 d (4.9)	5.71 d (4.9)
9	1.34 s	1.22 s	1.18 s	1.46 s
OAc				1.67; 1.63; 1.62; 1.61; 1.52

[a] Measured in D_2O (δ from external reference TSP).
[b] Measured in C_6D_6 (δ from solvent signal at δ 7.15).
[c] Overlapped signals.

CH_3-9 and the vicinal *trans*-oriented H-7. Therefore, all of the vicinal hydroxyl groups of the cyclopentane ring are in the *trans* relationship. This accorded with the failure to achieve isopropylidenation of **1a**.

In order to characterise the parent monosaccharide, **1a** was hydrolysed with acid. The crude reaction product appeared by TLC to be a mixture, whose ^{1}H NMR spectrum (Table 3, Fig. 2) showed two main anomeric signals at δ 5.35 (s) and 4.81 (d, J 8.1 Hz) and a minor one at δ 5.33 (d, J 3.9 Hz). Starting from these signals, the identification of all the signals of the three components, **2a**, **2b**, and **2c**, of the equilibrium mixture was achieved by COSY and NOE experiments. These data (see below) allowed us also to define the anomeric configuration and the ring sizes of the three component monosaccharides.

As far as the more abundant α-pyranose isomer **2a** is concerned, by irradiation of CH_3-9 at δ 1.34, enhancements of the signals at δ 4.81 (H-1), 1.67 (H-3a), 3.67 (on this basis the signal was assigned to H-7), and 4.01 (H-6) were measured. Further indications were obtained on the corresponding acetate derivative **3**. For this compound, the irradiation of CH_3-9 (δ 1.46) confirmed the above spatial proximity with H-3a (δ 1.76), H-7 (δ 5.71), and H-6 (δ 5.67). In addition, the irradiation of H-2 at δ 5.23 caused a strong increase of the signal at δ 5.80 (H-5), indicating a *cis*-fusion of the two rings. (The NOE contacts measured for either **2a** or **3** are indicated with bold and dotted arrows, respectively; those measured for both **2a** and **3** are indicated with plain arrows.)

For the furanose form **2b**, by irradiation of the methyl protons at δ 1.22, enhancements of the signals at δ 2.55 dd (H-3b), 3.72 d (H-7), and 3.85 dd (H-6) were observed. Since these experimental results are identical to those obtained for **1a**, the same anomeric configuration can be assumed for **2b**.

Finally, the furanose form **2c** was suggested by the close similarity of its ^{1}H chemical shifts with those of **2b**, and confirmed by the NOE enhancement, shown in the formula, found for the anomeric signal at δ 5.33 upon irradiation of the methyl protons at δ 1.18.

The ^{13}C NMR spectrum of the mixture, in agreement with the above data, showed

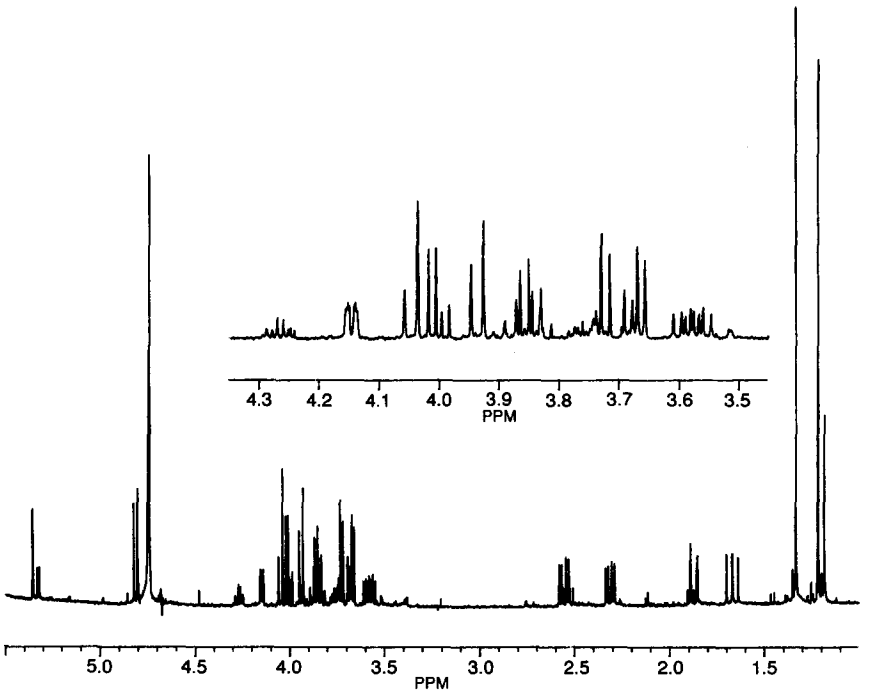

Fig. 2. ^{1}H NMR (400 MHz) spectrum of the mixture of **2a**, **2b**, and **2c** in D_2O.

Table 4
^{1}H NMR chemical shifts (δ) and apparent coupling constants in parentheses (Hz) of compounds **4a**, **4b**, and **5** at 30 °C [a]

Proton	**4a**	**4b**	**5**
1	5.6 d (5.9)	5.64 d (4.4)	5.89 d (4.4)
2	4.36 ddd (5.9; 3.9; 2.4)	4.32 ddd (4.4; 4.4; 2.4)	4.82 dd (6.4; 4.4)
3a	1.92 dd (15.1; 3.9)	2.22 dd (15.6; 4.4)	2.02 d (14.2)
3b	2.35 dd (15.1; 2.4)	2.48 dd (15.6; 2.4)	2.52 dd (14.2; 6.4)
5	4.15 d (2.4)	4.27 d (2.8)	3.69 d (6.4) [c,d]
6	3.95 [b]	5.41 dd (8.8; 2.8)	3.96 dd (6.4; 2.9) [c]
7	3.95 [b]	5.85 d (8.8)	4.10 d (2.9) [d,e]
9 and CMe_2	1.62 s; 1.48 s; 1.40 s; 1.40 s; 1.33 s	1.60 s; 1.59 s; 1.44 s; 1.36 s; 1.27 s	1.56 s; 1.30 s; 1.32 s
Aromatic		7.89 m; 7.58 m	
OH			3.19 d (2.0); 2.35 bs [2 H]; 1.92 s

[a] Measured in $CDCl_3$ (δ from solvent signal at δ 7.27).
[b] Overlapped signals.
[c] Upon irradiation of hydroxyl protons at δ 2.35.
[d] Interchangeable.
[e] Upon irradiation of hydroxyl proton at δ 3.19.

three anomeric signals occurring at δ 103.6 ($^1J_{C,H}$ 174 Hz), 97.4 ($^1J_{C,H}$ 174 Hz) (assignable to the furanose isomers **2b** and **2c**, respectively), and 94.2 ($^1J_{C,H}$ 167 Hz), attributable to the α-pyranose isomer **2a**.

The absolute configuration was determined by the Exciton Chiral Coupling method [6] applied to **4b**, which is the di-*O*-*p*-bromobenzoyl derivative of **4a**, the latter having been isolated, together with compound **5**, by isopropylidenation of the mixture **2a**, **2b**, and **2c**. The assignments (Table 4) of protons of the compounds **4a** and **4b** were based on the benzoylation shifts, and decoupling and NOE experiments. In particular the NOEs measured for **4b** between H-7 and H-1, by reciprocal irradiation, and at H-3a and H-3b by irradiation of H-5 allowed us to define the *cis*-fusion between the cyclopentane and the pyranose rings, and the conformation of the latter. For **5**, the assignments were based on decoupling experiments and by comparison with ^{1}H NMR data of **1a**.

Compound **4b** displayed a strong first positive Cotton effect at 254 nm ($\Delta\varepsilon = +31.5$) and a negative one at 237 nm ($\Delta\varepsilon = -14.0$), indicating a clockwise arrangement of the two chromophores at the C-6 and C-7 positions.

On the basis of the results above, the structure **6** is defined for this novel monosaccharide. As far as the systematic name in accordance with carbohydrate nomenclature rules is concerned, there is no previous example, to our knowledge, of an analogous carbohydrate compound. We suggest for **6** the name 4,8-cyclo-3,9-dideoxy-L-*erythro*-D-*ido*-nonose, derived from formal cleavage of the C-4–C-8 bond by addition of two hydrogens. We propose the trivial name caryose for **6**.

Acknowledgements

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