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The relative and absolute configurations of stereocenters in caryophyllose

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Abstract

The complete stereochemistry of the monosacccharide 3,6,10-trideoxy-4-*C*-[(*R*)-1-hydroxyethyl]-D-*erythro*-D-*gulo*-decose, named caryophyllose, obtained from the lipopolysaccharide fraction of *Pseudomonas caryophylli* is reported. The relative stereochemistry was inferred by ^{1}H NMR analysis and the absolute configuration was independently elucidated by Mosher's and Exciton Chiral Coupling methods.

Keywords: Caryophyllose; Lipopolysaccharide; Caryophylli; Configuration; Deoxy sugar

1. Introduction

In a preliminary comunication, we recently [1] reported the occurrence of a novel sugar in the acid hydrolysate of the lipopolysaccharide (LPS) fraction of *Pseudomonas caryophylli*, the causal agent of the bacterial wilting of carnation (*Dianthus caryophyllus* L.) [2]. The structure (**1**) of this 12-carbon sugar has a 3,6-dideoxyhexopyranose ring, with a *xylo* configuration, branched at C-4 with a 1,3,4,5-tetrahydroxyhexyl side chain.

In this report we describe the complete stereochemistry of **1**.

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2. Experimental

General.—The ^1H 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 ^1H 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 internal 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. The standard Bruker software (XHCORR) was used for the heteronuclear H,C COSY experiment under the following conditions: the time domain in f_2 was 1K; 64 spectra were collected with 1280 scans; the spectral width was 8000 Hz in the f_2 and 1200 Hz in the f_1 domain; delays were optimised for $^1J_{C,H} = 160$ Hz. Fourier transformation was performed with a shifted sine-bell function in both dimensions. 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 1K; the spectral width was 1200 Hz. 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 in positive mode. 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 concd H_2SO_4 followed by heating at 120°C for 15 min. Solvent systems: A, 5:1:1 *n*-BuOH–EtOAc–0.03 M H_3BO_3; B, 97:3 $CHCl_3$–MeOH; C, 95:5 C_6H_6–EtOAc; D, 4:1 *i*-PrOH–H_2O; E, 14:6:1 $CHCl_3$–MeOH–H_2O; F, 97:3 C_6H_6–EtOAc; G, 85:15:5 EtOAc–MeOH–H_2O.

Methanolysis of LPS fraction.—A sample of LPS fraction (50 mg) was treated with 1 M HCl–MeOH at 80°C for 18 h. Usual workup gave a crude mixture (42 mg) that was purified by preparative TLC (0.2 M NaOAc-impregnated plates; eluent A). Three fractions were eluted: the methyl glycosides of **1** (8 mg, R_f 0.3), **2a** (8 mg, R_f 0.5), and **3a** (5 mg, R_f 0.6). Compound **2a** was a colourless syrup; FAB-MS: $[M + H]^+$ m/z 311, $[M - 32 + H]^+$ m/z 279; ^1H NMR: see Table 1; ^{13}C NMR: see text. Compound **3a** was a colourless syrup; $[\alpha]_D$ −16° (*c* 1, H_2O); FAB-MS: $[M + H]^+$ m/z 279; ^1H NMR: see Table 1; ^{13}C NMR: see text.

Acetylation of **2a** *and* **3a**.—Samples of **2a** (1 mg) and **3a** (1 mg) were treated with 1:1 pyridine–Ac_2O (300 μL) at 120°C for 20 min. Usual workup gave **2b** (1 mg) and **3b** (1 mg), which were characterised essentially by ^1H NMR as reported in Table 1. The FAB-MS spectrum of **2b** showed a pseudomolecular ion at $[M + NH_4]^+$ m/z 538 and two other assignable peaks at $[M - 32 + H]^+$ m/z 489 and $[M - 32 - 60 + H]^+$ m/z 429. Compound **3b** gave a pseudomolecular ion at $[M + H]^+$ m/z 489.

Treatment of **3a** *with NaBH*$_4$.—A sample of **3a** (2 mg) in MeOH was recovered unaltered after treatment with $NaBH_4$ (1 mg) for 1 h at room temperature.

4,1′:3′,5′-Di-O-isopropylidene derivatives **4a** *and* **4b**.—To an α/β mixture of the **1** methyl glycosides (3 mg) in dry acetone (300 μL) were added 2,2-dimethoxypropane (100 μL) and a trace of Amberlite IR-120 (H^+ form). The mixture was kept for 40 min

under stirring at room temperature. The workup gave a mixture that was resolved by preparative TLC (eluent B) to yield the pure α anomer (**4a**, 2 mg) and the corresponding β anomer (**4b**, 1 mg). ^{1}H NMR data for **4a** and **4b**: see Table 1.

Preparation and purification of Mosher diesters of **4a**.—To a solution of **4a** (1 mg) in CCl_4 (10 μL) were sequentially added pyridine (10 μL) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (2 μL). The mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of MeOH and the solvents were removed by codistillation with toluene. The crude mixture was purified by preparative TLC (eluent C) to give the (*S*)-Mosher-2,4′-diester [(*S*)-MTPA-**4a**]. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, **4a** gave the (*R*)-Mosher-2,4′-diester [(*R*)-MTPA-**4a**]. Both yields were higher than 90%. ^{1}H NMR data for (*S*)-MTPA-**4a** and (*R*)-MTPA-**4a** are shown in Table 2.

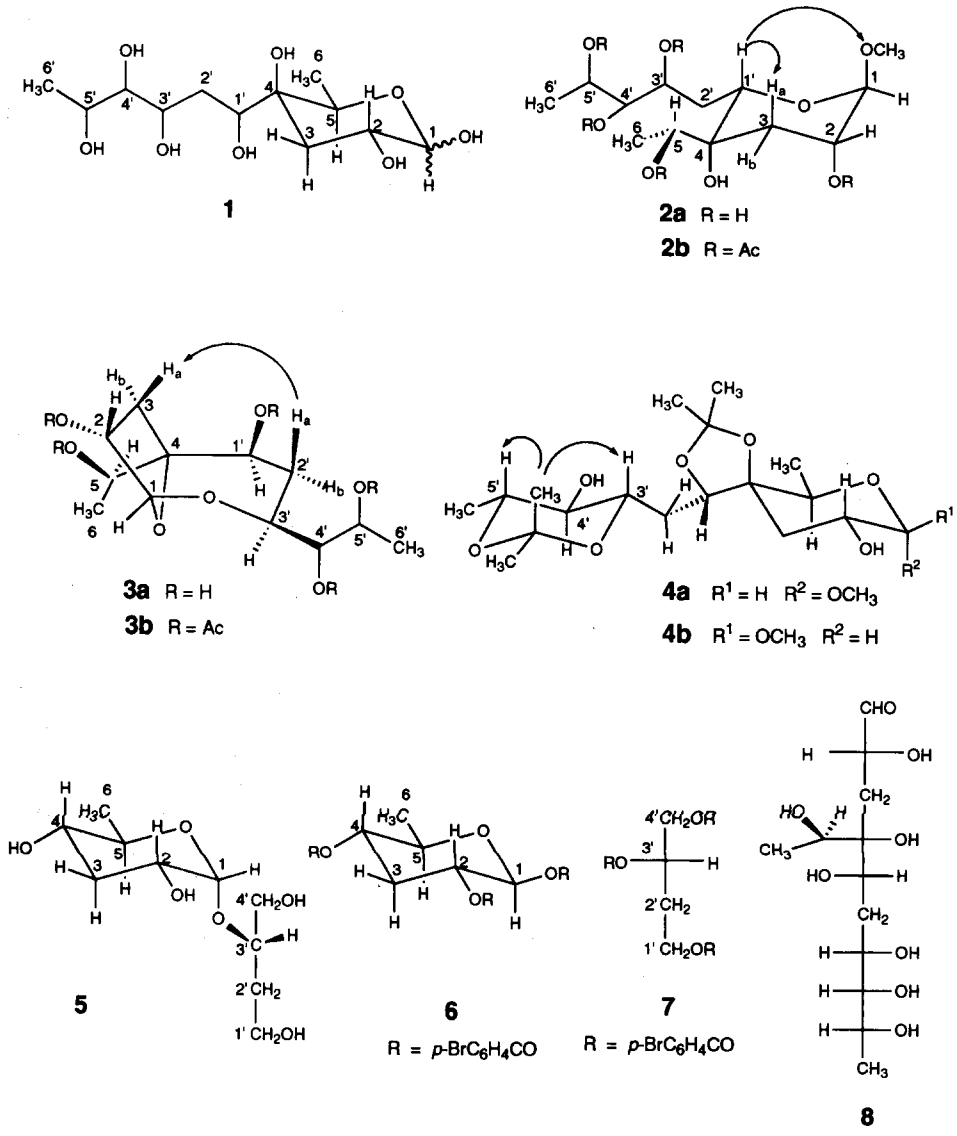
Periodate degradation of LPS fraction.—LPS fraction (50 mg) was subjected to periodate degradation. The oxidation was performed with 0.1 M $NaIO_4$ (12.5 mL) at 6°C in the dark under stirring and monitored by TLC (eluent D). After 48 h the reaction was quenched with ethylene glycol and the crude mixture was reduced at room temperature for 1 h by addition of $NaBH_4$ (50 mg). The mixture was neutralised with glacial acetic acid, lyophilised, and then purified on a Biogel P2 (Bio-Rad, 90 × 1.5 mm i.d.) column, using MilliQ water as eluent. The main fraction group was collected (14 mg) and further purified by preparative TLC (eluent E) to yield **5** as a homogeneous oil (8 mg); $[\alpha]_D$ +90° (*c* 0.4, H_2O); FAB-MS: $[M + H]^+$ *m/z* 237. ^{1}H and ^{13}C NMR data: see Table 3.

p-*Bromobenzoyl esters* **6** *and* **7**.—Compound **5** (2 mg) was hydrolysed with 2 M CF_3CO_2H at 120°C for 45 min. The acid was evaporated and the residue was treated with dry pyridine (300 μL) and *p*-bromobenzoyl chloride (10 mg) at room temperature for 3 h. The reaction was quenched by addition of MeOH, the solvent evaporated, and the residue purified by preparative TLC (eluent F) to give **6** (0.7 mg) and **7** (0.5 mg) as homogeneous solids. Compound **6**, ^{1}H NMR ($CDCl_3$): δ 8.0–7.5 (15 H, aromatic protons), 6.12 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.39 (ddd, 1 H, $J_{2,3eq}$ 5.4, $J_{2,3ax}$ 11.2 Hz, H-2), 4.98 (ddd, 1 H, $J_{4,3eq}$ 4.9, $J_{4,3ax}$ 10.7, $J_{4,5}$ 9.3 Hz, H-4), 4.04 (dq, 1 H, $J_{5,6}$ 6.3 Hz, H-5), 2.89 (dt, 1 H, $J_{3ax,3eq}$ 12.2 Hz, H-3*eq*), 2.02 (q, 1 H, H-3*ax*), 1.37 (d, 3 H, H-6). CD: see text. Compound **7**, ^{1}H NMR ($CDCl_3$): δ 7.9–7.5 (15 H, aromatic protons), 5.69 (m, 1 H, H-3′), 4.65–4.42 (m, 4 H, H-4′ and H-1′), 2.32 (m, 2 H, H-2′). CD (MeOH): 248 nm ($\Delta\varepsilon$ − 3.5).

Methanolysis of **5**.—Compound **5** (3 mg) was treated with 1 M HCl–MeOH at 80°C for 2 h. Usual workup gave a crude mixture (3 mg) that was purified by preparative TLC (eluent G) to give the mixture of methyl 3,6-dideoxy-α- and -β-D-*ribo*-hexopyranoside (paratoside){2 mg; R_f 0.3 (EtOAc) [3]; ^{1}H NMR (D_2O): δ 4.21 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1β), 4.57 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1α); lit. [4]: β anomer, δ 4.24 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1)} and 1,2,4-butanetriol (1 mg, TLC behaviour and ^{1}H NMR spectrum identical with those of an authentic sample).

3. Results and discussion

The relative stereochemistry of the side-chain stereocentres of **1** was established by the structural analysis of two products correlated to **1** (see Scheme 1) and obtained by



Scheme 1. The numbering for **2–7** refers to that of **1**. The arrows indicate NOEs.

methanolysis of the LPS fraction. Treatment of **1** with MeOH–HCl gave, besides the previously described [1] α/β mixture of the methyl glycosides of **1**, the isomeric methyl glycoside **2a** and the intramolecular glycoside **3a**.

The ^{1}H and ^{13}C NMR spectra indicated that **2a** was mainly a single anomer. In particular, the ^{13}C NMR spectrum showed a total of 13 strong signals, corresponding to an anomeric carbon (δ 100.1), six carbinolic methine carbons (δ 78.6, 70.8, 69.8, 69.3,

68.5, 68.2), one tertiary carbinolic carbon (δ 74.1), one methoxyl group (δ 55.4), two methylene carbons (δ 32.0, 29.3) and two methyl carbons (δ 17.5, 16.0). The FAB-MS spectrum in positive mode showed a pseudomolecular ion peak $[M + H]^+$ at m/z 311, in agreement with structure **2a**. The indication that **2a** could be the isomer of the methyl glycosides of **1** with the pyranose ring closed at the 1′ position arose from the ^{1}H NMR spectrum (Table 1). Actually, among the signals of side-chain protons, only the H-1′ signal appeared particularly shifted (0.5 ppm) with respect to those of the methyl glycosides of **1** [1].

Confirmatory evidence was obtained from its acetate **2b**, whose ^{1}H NMR spectrum (Table 1) did not show any acetylation downfield shift for the H-1′ signal, indicating that it was not geminal to a hydroxyl group. The occurrence of five acetyl signals in the ^{1}H NMR spectrum and the presence of a tertiary hydroxyl group, revealed by absorption at 3587 cm^{-1} in the IR spectrum, were in accord with structure **2b**.

The small values of $J_{2,3ax}$ and $J_{2,3eq}$ for both **2a** and **2b** indicated the equatorial orientation of H-2, suggesting a ring conformation opposite to that of the methyl glycosides of **1**, probably due to the equatorial orientation of both chains at C-1′ and C-4.

As for the configuration at C-1′, the NOEs measured on **2a** for the methoxyl group and for H-3a, occurring at 2.01 ppm upon irradiation of H-1′, indicated the axial orientation for H-1′ and therefore the S^* configuration[1]. The axial orientation of H-3a at δ 2.01 was confirmed by the W-coupling observed for the geminal H-3b at δ 1.77, proved by the increase of this signal upon irradiation of H-1.

The ^{13}C NMR spectrum of **3a** showed, by *on*-resonance and coupled and uncoupled DEPT experiments, 12 signals, assignable to one anomeric carbon at δ 104.6, six carbinolic methine carbons at δ 76.5, 76.2, 70.4, 69.5, 69.3, and 67.9, one quaternary carbon at δ 92.2, two aliphatic methylene carbons at δ 36.5 and 33.0, and two methyl carbons at δ 17.1 and 16.7. The FAB-MS spectrum in positive mode of **3a** displayed a pseudomolecular peak $[M + H]^+$ at m/z 279, which, together with the ^{13}C NMR data, suggested the molecular formula $C_{12}H_{22}O_7$ and, therefore, a bicyclic structure for the compound. The absence of a methoxyl group and the occurrence of a signal in the anomeric range suggested an intramolecular acetal function, whose furanose ring could be inferred by the very large value of the $^1J_{C\text{-}1,H\text{-}1}$ ($^1J_{CH}$ 181 Hz) and by the finding of a quaternary carbon at low field (δ 92.2) [5]. The remote possibility that a hemiacetal structure could be produced under the methanolysis conditions was ruled out since **3a** was not reduced by treatment with $NaBH_4$.

The assignment of the ^{1}H NMR signals (Table 1, Fig. 1) of **3a** and of its acetate **3b** allowed us to identify the 3′ position as that involved in the intramolecular acetal bond, as only its signal (δ 3.88), among those of the carbons bearing an oxygen atom, was unshifted by acetylation, indicating that H-3′ was not geminal to a hydroxyl group. The

[1] The descriptor used for the relative configuration was in accordance with the rules of *Chemical Abstracts* (see E.L. Eliel and S.H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, 1994, p 119). It was relative to the lowest numbered chiral centre of the polyhydroxyaldehyde acyclic form of **1**, i.e., C-2, for which the R^* configuration was assigned.

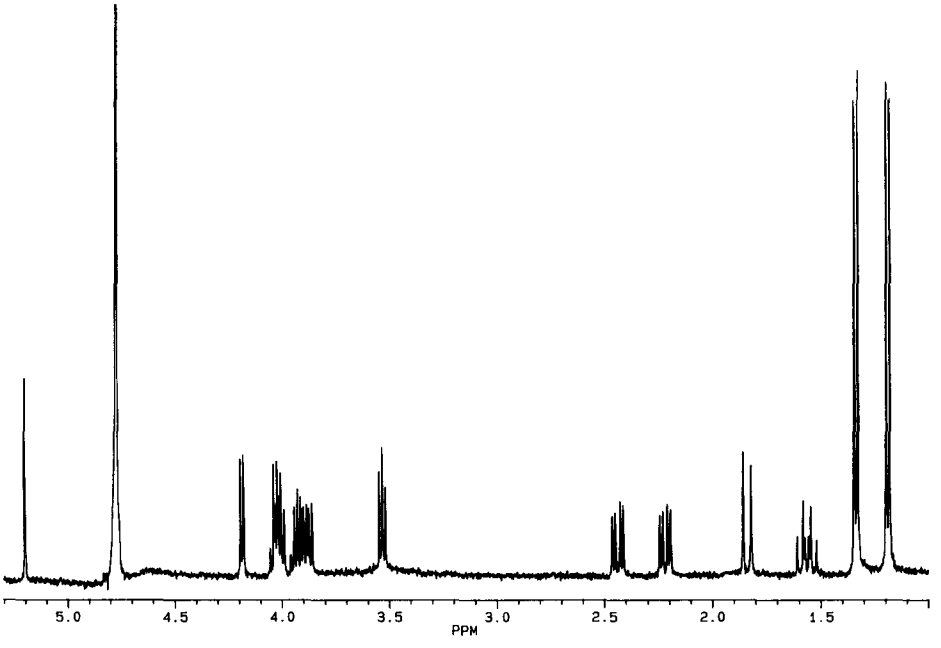


Fig. 1. The ^{1}H NMR (400 MHz) spectrum of **3a** in D_2O.

structure of **3a** was further supported by the presence of five acetyl signals occurring in the ^{1}H NMR spectrum of **3b**.

The conformation depicted in Scheme 1 for **3a** and the relative *S** configuration of C-3′ was defined on the ground of the analysis of ^{1}H NMR signals and of NOE measurement. The NOE measured for the H-3 signal at δ 2.44 upon irradiation of the H-2′ proton at δ 1.56 was in agreement with the conformation depicted in Scheme 1 and with a higher energy boat-like one; the latter was ruled out by molecular mechanics considerations. The appearance of the signal at δ 1.56, indicated as H-2′a, as a double-triplet with large values for the coupling constants (14.6 Hz for the geminal and 10.5 Hz for both the vicinal ones) indicated the *trans* orientation of this proton with respect to H-1′ and H-3′. Accordingly, the other H-2′ indicated as H-2′b, showed, besides the geminal coupling, only the coupling with H-1′, suggesting a dihedral angle of ca, 90° with H-3′. Therefore the C-3′ configuration could be assigned as *S**.

The assignment of H-3a at 2.44 ppm and H-3b at 1.84 ppm was inferred by the NOE of the former with H-2′a and by the absence of any coupling of the latter with H-2. This indicated the *cis* relationship between the H-3a at δ 2.44 and H-2.

In order to assign the relative configurations of H-4′ and H-5′, the α/β mixture of the methyl glycosides of **1** was treated with 2,2-dimethoxypropane and acetone to give the methyl 4,1′:3′,5′-di-*O*-isopropylidene derivatives **4a** and **4b**. Their structures were defined by ^{1}H NMR analysis (Table 1). In particular, in the spectrum of **4b** the H-4′ signal appears as a triplet at δ 2.97, further split by the coupling with the vicinal

Table 1
^{1}H NMR chemical shifts (δ) [a], visible multiplicities, and apparent coupling constants (Hz) of **2a**, **2b**, **3a**, **3b**, **4a**, and **4b**

H	**2a** (D_2O)	**2b** (C_6D_6)	**3a** (D_2O)	**3b** (C_6D_6)	**4a** (C_6D_6)	**4b** (C_6D_6)
1	4.65 d	4.66 s	5.20 s	5.30 s	4.53 d	3.85 d
	$J_{1,2}$ 1.5				$J_{1,2}$ 3.9	$J_{1,2}$ 7.8
2	3.80 [b]	5.11 t	4.18 d	5.00 d	4.15 ddd	3.91 [b]
		$J_{2,3a} = J_{2,3b} = 3.9$	$J_{2,3b}$ 5.4	$J_{2,3b}$ 6.1	$J_{2,3a}$ 10.2	
					$J_{2,3b}$ 5.9	
3a	2.01 dd	1.85 [b]	2.44 dd	1.75 [b]	1.69 [b]	1.56 dd
	$J_{3a,3b}$ 14.8		$J_{3a,3b}$ 14.6			$J_{3a,3b}$ 13.2
	$J_{3a,2}$ 3.7					$J_{3a,2}$ 11.0
3b	1.77 dd	2.26 dd	1.84 d	2.29 [c]	1.69 [b]	1.93 [c] dd
	$J_{3b,2}$ 3.7	$J_{3b,3a}$ 15.1				$J_{3b,2}$ 4.9
5	3.75 q	5.38 [c]	4.03 q	5.26 q	3.66 q	3.22 q
	$J_{5,6}$ 6.4		$J_{5,6}$ 6.8	$J_{5,6}$ 6.8	$J_{5,6}$ 6.4	$J_{5,6}$ 6.4
6	1.18 d	1.27 d [e]	1.33 d	1.30 d	1.26 d	1.27 d
		$J_{6,5}$ 6.4				
1′	4.28 dd	4.25 dd	4.01 dd	5.18 dd	3.89 dd	3.91 [b]
	$J_{1',2'b}$ 11.3	$J_{1',2'b}$ 11.2	$J_{1',2'b}$ 5.9	$J_{1',2'b}$ 6.1	$J_{1',2'b}$ 10.2	
	$J_{1',2'a}$ 1.8	$J_{1',2'a}$ 2.1	$J_{1',2'a}$ 10.5	$J_{1',2'a}$ 10.7	$J_{1',2'a}$ 2.4	
2′a	1.70 ddd	2.31 ddd	1.56 dt	1.23 [f]	1.78 ddd	1.67 ddd
	$J_{2'a,2'b}$ 14.8	$J_{2'a,2'b}$ 15.1	$J_{2'a,2'b}$ 14.6		$J_{2'a,2'b}$ 14.2	$J_{2'a,2'b}$ 14.6
	$J_{2'a,3'}$ 12.6	$J_{2'a,3'}$ 10.2	$J_{2'a,3'}$ 10.5		$J_{2'a,3'}$ 6.3	$J_{2'a,3'}$ 6.3
						$J_{2'a,1'}$ 2.9
2′b	1.89 ddd	2.49 ddd	2.22 dd	2.29 [c]	2.00 ddd	1.95 [c]
	$J_{2'b,3'}$ 2.0	$J_{2'b,3'}$ 2.5			$J_{2'b,3'}$ 6.3	
3′	3.89 [b]	5.78 dt	3.88 dd	3.90 dd	3.72 dt	3.67 dt
		$J_{3',4'}$ 2.5	$J_{3',4'}$ 6.0	$J_{3',4'}$ 8.3	$J_{3',4'}$ 9.3	$J_{3',4'}$ 9.3
				$J_{3',2'a}$ 9.8		$J_{3',2'b}$ 6.3
4′	3.49 t	5.70 dd	3.53 t	5.42 dd	2.96 t [g]	2.97 t [g]
	$J_{4',3'} = J_{4',5'} = 5.9$	$J_{4',5'}$ 6.7	$J_{4',5'}$ 6.0	$J_{4',5'}$ 3.2	$J_{4',5'}$ 9.3	$J_{4',5'}$ 9.3
5′	3.89 [b]	5.38 [c]	3.93 dq	5.51 dq	3.76 dq	3.73 dq
			$J_{5',6'}$ 6.8	$J_{5',6'}$ 6.4	$J_{5',6'}$ 6.4	$J_{5',6'}$ 6.4
6′	1.19 d	1.30 d [e]	1.19 d	1.13 d	1.40 d	1.36 d
	$J_{6',5'}$ 6.4	$J_{6',5'}$ 6.4		$J_{6',5'}$ 6.4		
Other signals	OCH_3 3.45 s	OCH_3 3.32 s OAc s: 1.57; 1.78; 1.82 [b]; 1.89; 1.96		OAc s: 1.48; 1.56; 1.66; 1.67; 1.74 [b]	OCH_3 3.03 s CH_3 s: 1.52; 1.38; 1.33; 1.17	OCH_3 3.31 s CH_3 s: 1.53; 1.37; 1.32; 1.66

[a] The assignments were performed by H,H COSY and decoupling experiments.
[b,c,d] Overlapped signals in each column.
[e] Interchangeable signals.
[f] Overlapped with impurity.
[g] Split by coupling with HO-4′ (2.7 Hz).

hydroxyl proton, as was confirmed by disappearance of this splitting by D_2O exchange. The NOEs measured for H-3′ and H-5′ on irradiation of the isopropylidene methyl protons at δ 1.37 indicated the chair conformation of the dioxane ring and the *cis*-diaxial orientation for H-3′ and H-5′. The *trans*-diaxial orientation for H-4′ with

Table 2
^{1}H NMR data of (*R*)-and (*S*)-MTPA-**4a**, in C_6D_6, and chemical shift differences, $\Delta\delta_H(\delta_R-\delta_S)$, of protons vicinal to 2 and 4′ ester groups. Apparent coupling constants in parentheses (Hz)

H	δ (*R*)-MTPA-**4a**	δ (*S*)-MTPA-**4a**	$\Delta\delta_H(\delta_R-\delta_S)$
1	5.08d ($J_{1,2}$ 3.4)	4.94 d ($J_{1,2}$ 3.4)	Positive
2	5.57 ddd ($J_{2,3eq}$ 4.9, $J_{2,3ax}$ 12.7)	5.54 ddd ($J_{2,3eq}$ 4.9, $J_{2,3ax}$ 12.7)	
3*ax*	2.36 t ($J_{3ax,3eq}$ 12.7)	2.30 t ($J_{3ax,3eq}$ 12.7)	Positive
3*eq*	1.79 dd	1.86 [a]	Negative
5	3.87 q ($J_{5,6}$ 6.3)	3.76 [b]	
6	1.29 d	1.19 [c]	
1′	3.98 dd ($J_{1',2'a}$ 2.9, $J_{1',2'b}$ 9.8)	3.95 dd ($J_{1',2'a}$ 2.9, $J_{1',2'b}$ 9.8)	
2′a	2.03 ddd ($J_{2'a,3'}$ 9.8, $J_{2'a,2'b}$ 15.1)	1.79 [a]	
2′b	1.71 dd ($J_{2'b,3'}$ 9.8)	1.59 dd ($J_{2'b,3'}$ 9.8)	
3′	4.02 t ($J_{3',4'}$ 9.8)	3.99 t ($J_{3',4'}$ 9.8)	Positive
4′	4.73 t ($J_{4',5'}$ 9.8)	4.74 t ($J_{4',5'}$ 9.8)	
5′	3.68 dq ($J_{5',6'}$ 6.3)	3.78 [b]	Negative
6′	1.01 d	1.19 [c]	Negative
1-OCH_3	3.01 s	3.01 s	
CH_3	1.39, 1.38, 1.18, 1.15 s	1.39, 1.35, 1.25, 1.19 s	
MTPA	7.8–7.7, 7.1–7.0, 3.48 s, 3.50 s	7.8–7.7, 7.1–7.0, 3.36 s, 3.34 s	

[a,b,c] Overlapped signals.

respect to H-3′ and H-5′ was inferred by the large values of the coupling constant (9.3 Hz) with both protons. Therefore the 5′*R** and 4′*R** configurations were established.

On the basis of the data above, the relative configuration of **1** could be defined as 3,6-dideoxy-4-*C*-(*altro*-1,3,4,5-tetrahydroxyhexyl)-*xylo*-hexopyranose.

As far as the absolute configuration was concerned, two approaches were used: the first exploits Mosher's empirical method [6] and the second the absolute method of Exciton Chiral Coupling [7].

The Mosher ester methodology analyses the differences between the proton chemical shifts of (*S*)- and (*R*)-MTPA esters on both sides of the chiral carbinol centres. Since this procedure cannot be applied when more hydroxyl groups are too near, as the phenyl rings of the Mosher ester can interfere each other [8], we applied this procedure to **4**, whose esterifiable hydroxyl groups at the 4′ and 2 positions are sufficiently distant. The reaction was performed with the **4a** isomer since it was available in a higher amount. The ^{1}H chemical shifts of the pertinent protons of (*S*)-MTPA-**4a** and (*R*)-MTPA-**4a** esters and their differences are reported in Table 2.

On the basis of Mosher's arguments, C-4′ was assigned the *R* configuration, since the sign of $\Delta\delta_H(\delta_R-\delta_S)$ is positive for H-3′, showing relatively less shielding for this proton in (*R*)-MTPA-**4a**, and negative for H-5′ and H-6′, showing relatively less shielding for this side in (*S*)-MTPA-**4a**. Less clear information came from the shifts induced by the ester in the 2-position. Actually, H-3a and H-3b were shifted in opposite directions. This discrepancy and the lack of application, to our knowledge, of Mosher's

Table 3
^1H and ^{13}C NMR chemical shifts (δ) and coupling constants (Hz) of **5** in D_2O [a]

	H	C
1	4.85 d	97.6
	$J_{1,2}$ 3.4	
2	3.78 ddd	
	$J_{2,3a}$ 11.2	67.3
	$J_{2,3b}$ 4.4	
3*eq*	2.09 dt	
	$J_{3eq,3ax}$ 11.2	34.9
	$J_{3eq,4}$ 4.4	
3*ax*	1.71 q	
	$J_{3ax,4}$ 11.2	
4	3.34 ddd	70.3
	$J_{4,5}$ 10.7	
5	3.72 [b]	69.3
6	1.19 d	16.8
	$J_{6,5}$ 6.3	
1′	3.70 [b]	58.7
2′	1.81 m	33.3
3′	3.83 m	77.4
4′	3.65 [b]	64.5

[a] By H,C-COSY experiment.
[b] Overlapped signals.

method to sugars prompted us to confirm the absolute configuration by utilising the absolute method of Exciton Chiral Coupling on a convenient derivative of **1**.

With this aim, we exploited the information gained in the course of our running structural studies of the LPS fraction. The permethylation of this, followed by acid hydrolysis and acetylation, yielded a compound whose structure indicated the presence of solely α-(1 → 3′) interglycosidic linkages in the related lipopolysaccharide. This result suggested that oxidation of the LPS with $NaIO_4$, followed by $NaBH_4$ reduction, would give, almost exclusively, 1′,4′-dihydroxybut-3′-yl 3,6-dideoxy-α-*ribo*-hexopyranoside (**5**), a glycoside of the well-known paratose. This was experimentally verified and the structure of **5** was defined on the basis of a complete ^1H and ^{13}C NMR study (Table 3).

The relative configuration at C-4 of **5** was deduced from the coupling constant values of H-4 (Table 3), which indicated the axial orientation of both H-4 and H-5, in agreement with the paratose structure. To confirm the paratose structure for **5**, this compound was submitted to methanolysis with MeOH–HCl to give a mixture of the methyl α-and β-glycosides of 3,6-dideoxy-*ribo*-hexopyranose (paratose), identified by comparison of the TLC and ^1H NMR data with those reported for the β anomer (see Experimental) [4].

As the coplanar location of the hydroxyl groups at the 4 and 2 positions of **5** was not suitable for application of the Exciton Chiral Coupling method, **5** was hydrolysed and treated with *p*-bromobenzoyl chloride to give the paratose triester **6**, as a single β anomer, and the 1′,3′,4′-butanetriol triester **7**, both identified by ^1H NMR analysis (see

Experimental). These compounds were subjected to CD analysis. Compound **6** displayed a strong first negative Cotton effect at 254 nm ($\Delta\varepsilon - 36.4$) and a positive one at 238 nm ($\Delta\varepsilon + 14.8$), indicating the *R* configuration at C-2.

The sign of the Cotton effect in the CD spectrum of **7** was opposite to that of the authentic *p*-bromobenzoyl triester, of (*R*)-(+)-1,2,4-butanetriol, and allowed us to define the *S* configuration for C-3′.

On the grounds of the results above, the structure **1** can be defined as 3,6-dideoxy-4-*C*-(D-*altro*-1,3,4,5-tetrahydroxyhexyl)-D-*xylo*-hexopyranose, which is the ring form assumed in the *Pseudomonas caryophylli* LPS by the novel branched sugar 3,6,10-trideoxy-4-*C*-[(*R*)-1-hydroxyethyl]-D-*erythro*-D-*gulo*-decose **8**, which we name caryophyllose.

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