Mar. Biotechnol. 1, 384–390, 1999

MARINE BIOTECHNOLOGY

Metabolites from the Sponge-Associated Bacterium *Pseudomonas* Species

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Abstract: Quinolones and a phosphatidyl glyceride were isolated from the sponge-associated bacterial strain *Pseudomonas* sp. Structures were elucidated by spectroscopic analysis and chemical transformations.

Key words: *Pseudomonas,* sponge, metabolites, quinolones, bacteria, antimicrobial compounds

Introduction

Marine microorganisms have emerged as a source for the isolation of natural products. The microhabitats for marine bacteria are sediments, animate and inanimate surfaces, and the internal spaces of invertebrate animals. Marine plants and animals are known to have developed highly specific relationships with numerous microorganisms (Tapiolas et al., 1991; Fenical, 1993; Needham et al., 1994; Jansen and Fenical, 1994; Trischman et al., 1994). The surfaces of marine organisms are more nutrient rich than seawater and sediments, and numerous bacterial strains can be observed on these surfaces. Nevertheless, they generally are not considered as true symbionts but rather as nonobligate associates. It is estimated that less than 5% of viable bacterial strains in marine samples ultimately grow under standard culture conditions (Unson et al., 1994).

Bacteria collected from sponges (Stierle et al., 1988; Stierle and Stierle, 1992; Shigemori et al., 1992; Imamura et al., 1993; Jayatilake et al., 1996; Bultel-Poncé et al., 1997) have allowed isolation of antimicrobial compounds, which suggests that these bacteria may play a role in the defense mechanism of these invertebrates. Furthermore, metabolites previously ascribed to invertebrates were later demonstrated to be biosynthesized by bacteria (Eliakov et al., 1991; Stierle et al., 1988; Unson and Faulkner, 1993; Unson et al., 1994).

In a program devoted to the search of bioactive compounds biosynthesized by marine invertebrate-associated bacteria, we studied a bacterial strain collected at the surface of the sponge *Homophymia* sp. (*Porifera*), harvested at Touho, on the east coast of New Caledonia. This gram-negative strain, 1531-E7, was identified as a new *Pseudomonas* species on the basis of 16S DNAr sequence. A voucher specimen is conserved at ORSTOM, Nouméa, New Caledonia.

In this article, we report isolation and structural elucidation of the antimicrobial metabolite compound 4, of other quinolones 1–3 for which other biological activities were demonstrated, and of the unique phosphatidylglycerolipid 5.

Received March 24, 1998; accepted October 20, 1998.

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Materials and Methods

General Experimental Procedures

HRFABMS (positive mode) of compound 5 was measured on a ZAB-SEQ spectrometer in a thioglycerine matrix at the Service Central d'Analyses du CNRS (Lyon); EIMS and CIMS of other compounds, on a Nermag R 10-10. Ultraviolet spectra were obtained in MeOH, using a Kontron-type Uvikon 930 spectrophotometer, and IR spectra were recorded on a Nicolet (Impact 400D) FT IR spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter with a sodium lamp (589 nm) in a 10-cm microcell. Melting points were determined on a hot-plate-fitting light microscope (Reichert).

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AC 300 spectrometer with standard pulse sequences operating at 300.13 MHz and 75.47 MHz, respectively. The chemical shift values are reported as ppm units, and the coupling constants were reported in Hertz.

Silica gel column chromatographies were carried out using Kieselgel 60 (230–400 mesh, Merck). Fractionations were monitored by thin-layer chromatography using aluminium-backed sheets (Si gel 60 F-254, 0.25-mm thick) with visualization under UV (254 and 365 nm). All the solvents were distilled prior to use.

Gas chromatography–mass spectrometry (GCMS) experiments were carried on a Hewlet Packard 6890, on a CP Sil 8 column (Chrompack) (0.25 mm by 50 m). The column was operated at 80°C during 1 minute and then increased at 5°C/min to 290°C, with helium flow rate of 1 ml/min. Methylated acids were analyzed on a Nermag R 10-10 mass spectrometer.

A synthetic sample of lactobacillic acid methyl ester was kindly provided by E. Abou-Mansour from the Australian Institute of Marine Science, Townsville, Australia.

Bioassays

Crude extracts, chromatographic fractions, and pure compounds were assayed in vitro for antimicrobial activity using the agar disk-diffusion technique, against the following microorganisms: *Staphylococcus aureus* (209P) and *Escherichia coli* (RL 65 and 57 b). Extracts and fractions (500 µg to 10 µg, dissolved in 10 µl CH_2Cl_2) were applied on paper disks (6 mm). Cytotoxicity was assayed on KB cells.

Screening for antimalarial activity was performed at the Laboratoire de Biologie Parasitaire, Muséum National d'Histoire Naturelle, Paris, France.

Tests for anti-HIV-1 activity were carried out at ULP/INSERM U74, Institut de Virologie, Strasbourg, France.

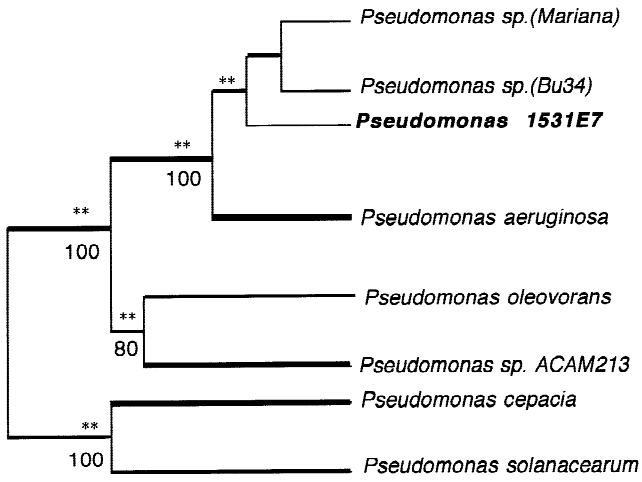


Figure 1. Phylogenetic position of the strain 1531 E7 within a subset of *Pseudomonadaceae* restricted to representative species of *Pseudomonas aeruginosa* cluster and the most related species.

Collection and Cultivation

The sponge *Homophymia* sp. was collected by scuba diving off Touho (New Caledonia) and identified by Prof. C. Lévy, Muséum National d'Histoire Naturelle, Paris. Sponges were immediately rinsed with sterile seawater and brushed. The resultant suspension was then diluted in 10-fold series, and the dilutions were spread on marine agar medium (DIFCO 2216). The best plate was chosen for isolation and further screening on *Staphylococcus aureus, Vibrio anguillarum,* and *Candida tropicalis.*

The bacterial strain isolated was identified as a *Pseudomonas* species. The phylogenetic position of the strain 1531 E7, within a subset of *Pseudomonadaceae* restricted to representative species of *Pseudomonas aeruginosa* cluster and the most related species, is shown in Figure 1. The topology shown is an unrooted tree obtained with the neighbor-joining method. The values (only values above 50% are reported) indicate how these branches are supported by bootstrap analysis. The double asterisks indicate branches that were also found by maximum-likelihood analysis ($p <$ .01). The branches retrieved in the most parsimonious tree are shown by bold lines.

The bacterial strain 1531 E7 was cultivated at 27°C, in 4 × 3-L batch cultures (seawater DIFCO-peptone 18 g/L

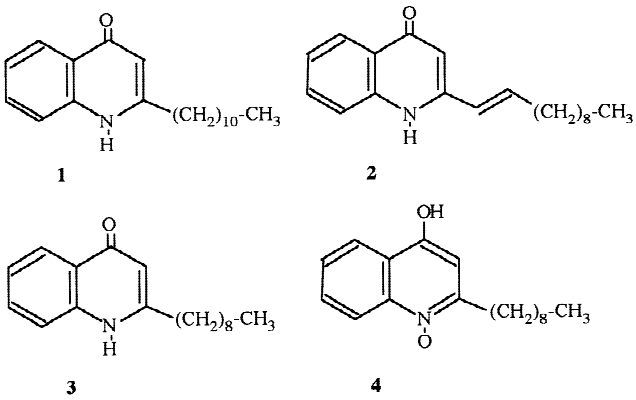


Figure 2. Structure of compounds 1–4.

enriched medium) for 4 days with rotatory shaking. Cells were harvested by centrifugation at 11,000 rpm for 15 minutes.

Extraction

The pellet was sonicated and extracted successively with methanol and dichloromethane-methanol (8:2). The organic phase was separated by centrifugation and pooled, and solvents were removed under reduced pressure. The residual gum was successively extracted with dichloromethane and an 8:2 dichloromethane-methanol mixture.

The dichloromethane extract (1.1 g) was fractionated on a silica gel column using a dichloromethane-acetone and then a dichloromethane-methanol gradient. The fractions eluted with dichloromethane-acetone (9:1) to (8:2) were further purified on another silica gel column to obtain compounds 1–4 (Figure 2) and the previously known diketopiperazines, cyclo(S-Pro-R-Leu), cyclo(R-Pro-R-Phe), and cyclo(R-Pro-*R*-Val). The portion (0.5 g) soluble by dichloromethane-methanol (8:2) was repeatedly chromatographed on a silica gel column to give 50 mg of a unique glycerophospholipid, compound 5.

Compound 1

White amorphous solid, mp 132°C; 2-undecyl-4-quinolone (10 mg, 1% dry weight); UV λ^{EtOH}nm (log ε): 213 (3.6), 254 (3.9), 330 (3.4); CIMS $[M + H]^+$ 300 $C_{20}H_{29}NO$; EIMS 70 eV, *m/z* (rel. int.): 299(M^+, 2), 271 (29), 270 (11), 256 (6), 243 (14), 172 (69), 159 (100), 130 (20); IR (NaCl) 3447 cm^{-1}, 2960 cm^{-1}, 2920 cm^{-1}, 2854 cm^{-1}, 1657 cm^{-1}, 1637 cm^{-1}, 1597 cm^{-1}, 770 cm^{-1}; ^{1}H (300 MHz, $CDCl_3$): δ 11.48 (br. s, NH), 8.34 (dd, *J* = 1.3, 8.1, H-5), 7.75 (d, *J* = 8.3, H-8), 7.55 (dd, *J* = 8.0, 8.3, H-7), 7.32 (dd, *J* = 8.0, 8.1, H-6), 6.21 (s, H-3), 2.65 (t, *J* = 8.0, H_2-1′), 1.72 (m, H_2-2′), 1.22 (m, 16 H), 0.83 (t, *J* = 8.1, H_3-11′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 178.88 (C-4), 155.23 (C-2), 140.63 (C-8a), 131.73 (C-7), 125.26 (C-5), 124.96 (C-4a), 123.52 (C-6), 118.50 (C-8), 108.15 (C-3), 34.40 (C-1′), 31.82 (C-2′), 29.66 (C-3′), 29.44 (C-4′), 29.32 (C-5′), 29.24 (C-6′), 29.10 (C-7′), 27.80 (C-8′), 26.48 (C-9′), 22.63 (C-10′), 14.08 (C-11′).

Compound 2

White amorphous solid; mp 131°C; 2-undecen-1′-yl-4-quinolone (10 mg, 1% dry weight); UV λ^{ETOH} nm (log ε): 238 (4.1), 263 (3.4), 316 (3.21), 327 (3.8); CIMS $[M + H]^+$ 298 $C_{20}H_{27}NO$; EIMS 70 eV, *m/z* (rel. int.): 269 (10), 159 (23), 120 (6.5), 83 (100); IR (NaCl) 3440 cm^{-1}, 2960 cm^{-1}, 2927 cm^{-1}, 2848 cm^{-1}, 1637 cm^{-1}, 1591 cm^{-1}, 1545 cm^{-1}, 762 cm^{-1}; ^{1}H (300 MHz, $CDCl_3$): δ 9.22 (br. s, NH), 8.33 (dd, *J* = 1.3, 8.6, H-5), 7.40 (dd, *J* = 1.4, 8.0, H-8), 7.35 (d, *J* = 8.5, 8.6, H-6), 7.31 (dd, *J* = 8.0, 8.5, H-7), 6.29 (s, H-3), 6.21 (d, *J* = 11.8, H-1′), 5.99 (dt, *J* = 7.35, 11.8, H-2′) 2.36 (qd, *J* = 1.60, 7.30, H_2-3′), 1.22 (m, 14 H), 0.83 (5, *J* = 8.1, H_3-11′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 178.90 (C-4), 146.50 (C-2), 139.20 (C-8a), 132.11 (C-7), 126.07 (C-5), 115.00 (C-4a), 123.50 (C-6), 117.23 (C-8), 110.13 (C-3), 141.60 (C-1′), 122.20 (C-2′), 31.74 (C-3′), 29 (C-4′ to C9′), 22.62 (C-10′), 14.07 (C-11′).

Compound 3

White amorphous solid, mp 134°C; 2-nonyl-4-quinolone (10 mg, 1% dry weight); UV λ^{EtOH}nm (log ε): 214 (3.9), 235 (4.0), 266 (3.0), 315 (3.6), 327 (3.6); CIMS $[M + H]^+$ 272 $C_{18}H_{25}NO$; EIMS 70 eV, *m/z* (rel. int.): 271 (M^+, 29), 172 (69), 159 (100), 130 (21); IR (NaCl) 3434 cm^{-1}, 2960 cm^{-1}, 2927 cm^{-1}, 2854 cm^{-1}, 1637 cm^{-1}, 1552 cm^{-1}, 1439 cm^{-1}, 755 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.08 (br. s, NH), 8.33 (dd, J = 1.3, 8.0, H-5), 7.76 (dd, *J* = 8.5, H-8), 7.57 (dd, *J* = 8.0, 8.5, H-7), 7.30 (dd, *J* = 8.0, 8.5, H-6), 6.20 (s, H-3), 2.64 (t, *J* = 7.5, H_2-1′), 1.68 (m, H_2-2′), 1.25 (m, H_2-3′ to H_2-8′), 0.85 (5, *J* = 7.1, H_3-9′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 178.72 (C-4), 154.34 (C-2), 140.32 (C-8a), 132.16 (C-7), 125.81 (C-5), 125.29 (C-4a), 123.83 (C-6), 118.02 (C-8), 108.68 (C-3), 34.79 (C-1′), 32.22 (C-2′), 29.78 (C-3′), 29.68 (C-4′), 29.63 (C-5′), 29.48 (C-6′), 29.34 (C-7′), 23.01 (C-8′), 14.23 (C-9′).

Compound 4

White amorphous solid, mp 132°C; 2-nonyl-4-hydroxyquinoline *N*-oxide. CIMS $[M + H]^+$ 298 $C_{18}H_{24}NO_2$; EIMS 70 eV, *m/z* (rel. int.): 297 (29), 172 (69), 159 (100), 130 (21); IR (NaCl) 3455 cm^{-1}, 2947 cm^{-1}, 2927 cm^{-1}, 2855 cm^{-1}, 1630 cm^{-1}, 1591 cm^{-1}, 1551 cm^{-1}, 1419 cm^{-1}, 1202 cm^{-1}, 933 cm^{-1}, 755 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$;) δ 8.20 (d, *J* = 8.5, H-5), 8.10 (d, *J* = 8.0, H-8), 7.57 (dd, *J* = 8.0, 8.5, H-6), 7.32 (dd, *J* = 8.0, 8.5, H-7), 6.01 (s, H-3), 2.48 (t, *J* = 7.5, H_2-1′), 1.48 (m, H_2-2′), 1.25 (m, H_2-3′ to H_2-8′), 0.85 (t, *J* = 7.1, H_3-9′); ^{13}C NMR (75 MHz, $CDCl_3$), δ 178.80 (C-4), 152.30 (C-2), 148.80 (C-4a), 139.88 (C-8a), 132.80 (C-8), 125.40 (C-6), 125.21 (C-5), 124.80 (C-7), 118.99 (C-3), 34.22 (C-1′), 31.80 (C-2′), 29.70 (C-3′), 29.40 (C-4′), 29.28 (C-5′), 29.13 (C-6′), 29.13 (C-7′), 22.62 (C-8′), 14.10 (C-9′).

Compound 5

Colorless oil, $[\alpha]_D$ = +6 (C 0.2 $CHCl_3$). HRMS FAB^+ (thioglycerol) $C_{40}H_{78}NO_8P$ observed mass $[M + H]^+$ 732.5465, calculated mass 732.5445.EIMS *m/z* (rel. int.): 731 (M^+, 17), 591 (20); IR (NaCl) 3434 cm^{-1}, 2921 cm^{-1}, 2855 cm^{-1}, 1742 cm^{-1}, 1637 cm^{-1}, 1466 cm^{-1}, 1235 cm^{-1}, 1025 cm^{-1}. ^{1}H and ^{13}C NMR data see Table 2 and text.

Enzymatic Hydrolysis of Compound 5

The substrate solution (10 mg in 2 ml of diethyl ether/anhydrous methanol 95:5) was placed in a 5-ml volumetric flask, and 25 µl of PLA2 (*Crotalus adamanteus*) enzyme preparation was added. After vigorous shaking for 30 seconds, the reaction was allowed to proceed for 10 minutes at room temperature. Then the reaction was stopped by addition of ethanol to a total volume of 5 ml. The solvents were removed under reduced pressure, and the residual gum was chromatographed on a silica gel column using a dichloromethane-methanol gradient. The fraction eluted with dichloromethane-methanol (6:4) yielded 2 mg of lysophosphatidylglycerol (see Figure 4).

Alkaline Hydrolysis of Compound 5

The substrate solution (10 mg in 2 ml KOH 5% in MeOH) was stirred 12 hours at room temperature. The resulting mixture was acidified and extracted with dichloromethane. The crude extract was subjected to a separation on a silica gel column to afford, after elution with hexane-ethylacetate (9:1), a mixture of two methyl esters, the palmitic ester (*m/z* 270) and the lactobacillic one (*m/z* 295), which were analyzed by GCMS.

Results and Discussion

The dichloromethane-soluble fraction was fractionated on a silica gel column using a dichloromethane-acetone and then a dichloromethane-methanol gradient. The fractions extracted with dichloromethane-acetone were further purified on another silica gel column, to obtain 4 UV absorbing compounds 1–4 and the previously known diketopiperazines, cyclo(S-Pro-R-Leu), cyclo(R-Pro-R-Phe), and cyclo(R-Pro-R-Val) (Adamczeski et al., 1995). About 90% of all gram-negative bacteria produce diketopiperazines when grown in nutrient-rich media (Fenical, 1993).

The ^{1}H NMR spectrum registered in $CDCl_3$ of compound 1 (10 mg, 1% dry weight) was in agreement with a quinolone derivative, and identified as 2-undecyl-4-quinolone; compound 2 (10 mg, 1% dry weight), as the 2-undecen-1′-yl-4-quinolone; compound 3 (10 mg, 1% dry weight), as the 2-nonyl-4-quinolone; and compound 4 (1 mg, 0.1% dry weight), as the 2-nonyl-4-hydroxyquinoline *N*-oxide (Wratten et al., 1977). Compounds 1–4 were submitted to bioassays on KB cells, *Plasmodium falciparum,* and HIV-1. Results are summarized in Table 1. Quinolone 1 is active against *Plasmodium falciparum* (ID_{50} 1 µg/ml) and against HIV-1 (ID_{50} 10^{-3} µg/ml) but shows neither cytotoxic nor antimicrobial activity. Quinolone 2 exhibits a mild cytotoxicity (KB cells). Quinolone 4 shows antimicrobial activity against *S. aureus* and cytotoxicity toward KB cells with an IC_{50} less than 2 µg/ml.

Machan et al. (1992) reported the presence of 2-heptyl-4-quinoline *N*-oxide and 2-heptyl-4-quinolone from clinical isolates of *Pseudomonas aeruginosa* which inhibited the growth of *S. aureus* and other gram-positive bacteria. In a marine pseudomonad, Wratten et al. (1977) found that 2-alkyl-4-hydroxyquinolines (2-pentyl; 2-heptyl; 2-nonyl), derived through reduction of their *N*-oxide homologues, were also produced but were less active against *S. aureus* than the *N*-oxide derivatives.

The fraction soluble by dichloromethane-methanol (8:2) contains a unique glycerophospholipid 5 (50 mg, 5% dry weight). The molecular formula $C_{40}H_{78}NO_8P$ was determined by high-resolution mass measurement: observed mass $[M + H]^+$ 732.5465; calculated mass $[M + H]^+$ 732.5445. In the ^{1}H NMR spectrum registered in $CDCl_3$/

Table 1. Biological Activity of Compounds 1–4

Compound	*P. falciparum* ID_{50} (μg/ml)	KB cells IC_{50} (μg/ml)	HIV-1 ID_{50} (μg/ml)	*S. aureus* Ø inhibition
1	1	> 10	10^{-3} (3.10^{-9} M)	Inactive
2	3.4	5	Inactive	Inactive
3	4.8	NT	Inactive	Inactive
4	NT	< 2	NT	20 mm/20 μg

CD_3OD (4:1), signals for one methine and four methylenes were observed in the δ 5.19 to 3.14 ppm region, as well as characteristic signals at δ 2.28 (t), 1.25 (broad s), and 0.86 (t) for acyl long chain. Signals characteristic of a cyclopropyl moiety consisting of three signals in the area δ 0.62 to −0.36 ppm, corresponding to the two methines and the methylene, were coupled to two methylenes and suggested the presence of one unit of lactobacillic acid or an analogue. A COSY experiment allowed us to assign protons H-1, H-2, and H-3 corresponding to the glycerol moiety and showed that the third methylene (δ 4.06 ppm) was coupled only to another one located at δ 3.14 ppm, suggesting the presence of a phosphatidylethanolamine moiety, in agreement with the chemical shift values (Kohama et al., 1994; Batrokov and Nikitin, 1996). A ^{31}P NMR 121-MHz experiment was carried out and allowed us to observe a signal at δ − 0.6 ppm, which confirmed the presence of a phosphatidyl group and the identification of a glycerophospholipid (Figure 3).

The ^{13}C NMR spectrum indicated the presence of signals for two carbonyl groups (δ 173.51 and 173.21 ppm) for two acyl long chains, one methine and three methylenes in the δ 62–70 ppm region (-OCH_2-CH(OR)-CH_2OR'; (P)-O-CH_2-), a methylene signal located at 40.33 ppm in agreement with the presence of a -CH_2NH_2, long chain aliphatic carbons (δ 29.0 ppm), a methyl signal (δ 14.10 ppm), the cyclopropyl signals with a methylene resonating at δ 10.91 ppm, and the two methine groups at δ 15.74 ppm. Direct ^1H–^{13}C (HMQC) and long-range ^1H–^{13}C (HMBC) correlations allowed assignments of all protons and carbons of the molecule (Table 2).

In the FAB^+ mass spectrum, we observed a peak at *m/z* 732 for $[M + H]^+$, and the fragmentation at *m/z* 591 represents the loss of the phosphatidylethanolamine part.

To determine the position of the two different fatty acid substituents, an enzymatic hydrolysis of compound 5 was performed using PLA2 from *Crotalus adamanteus,* which catalyzes the hydrolysis of the fatty acyl ester at the sn-2 position of phospholipids (Wells and Hanahan, 1969). The resulting lysophosphatidylglyceride was purified on a silica gel column using a dichloromethane-methanol gradient. The fraction eluted with dichloromethane-methanol (6:4) yielded 2 mg of compound 6 (Figure 4). The ^1H NMR spectrum clearly showed the lack of the lactobacillic acid moiety; the other signals of the molecule remaining unchanged, except shielding of the signal for H2 (δ 5.19 to 4.20 ppm). Hence, we were able to assign the sn-1 position for the palmitic acid and the sn-2 position for the lactobacillic acid. This is in accordance with the biosynthesis of cyclopropane fatty acids: the cyclopropane ring is built up by methylation on the double bond of the unsaturated fatty acid by the cyclopropane–fatty acid synthetase (Hildebrand and Law, 1964). Most often in gram-negative bacteria, saturated fatty acids occupy the sn-1 position and unsaturated fatty acids the sn-2 position (Hildebrand and Law, 1964).

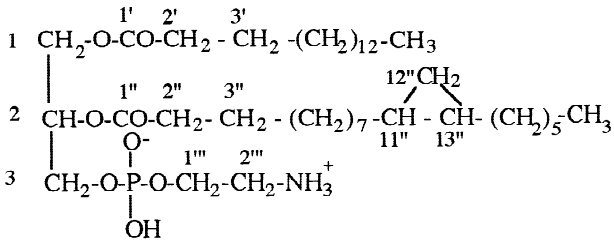


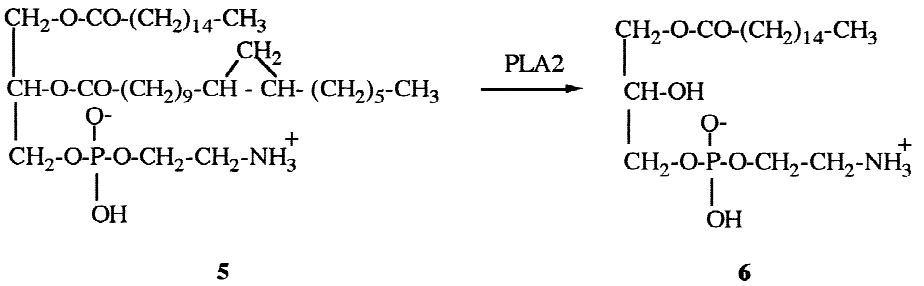
Figure 3. Structure of compound 5.

Finally, the alkaline hydrolysis of the phospholipid was carried out. The resulting mixture was acidified and extracted with dichloromethane. The crude extract was subjected to a separation on a silica gel column to afford, after elution with hexane-ethylacetate (9:1), a mixture of methyl

Table 2. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR Data of Compound 5 (δ ppm, *J* Hz, $CDCl_3/CD_3OD$ (4:1))

Assignments	^{1}H	^{13}C	HMBC	COSY
1	4.35–4.14 (dd, *J* = 9.18, 6.85), 2H	62.55	1′	2
2	5.19 (m), 1H	70.18	1″	1, 3
3	3.94, 3.89, 2H	63.92	—	2
1′	—	173.21*		—
2′	2.28, 2H	34.24	1′, n CH_2	3′
3′	1.57, 2H	32.60	n CH_2	—
n CH_2	1.25 (m), 24 H	29.00	3′, 3″	CH_3
CH_3	0.80 (t), 6H	14.10	n CH_2	n CH_2
1″	—	173.51*		—
2″	2.28, 2H	34.24	1″, n CH_2	3″
3″	1.57, 2H	32.60	n CH_2	2″, n CH_2
1‴	4.06 (t), 2H	62.06		2‴
2‴	3.14 (t), 2H	40.33	—	1‴
11″, 13″	0.62 (m), 2H	15.74	1″	12″, n CH_2
12″	0.54 (dd, *J* = 9.18, 4.32), 1H −0.36 (dd, *J* = 9.03, 4.62), 1H	10.91	11″	11″, 13″

*May be reversed.



5 **6**

Figure 4. Hydrolysis of compound 5 by PLA2 (*Crotalus adamanteus*).

esters. Analysis by GCMS revealed the presence of palmitic (*m/z* 270) and lactobacillic (*m/z* 295) methyl esters. This latter was clearly identified in gas chromatography (GC) by coinjection with synthetic lactobacillic acid methyl ester, completing establishment of structure 5. To the best of our knowledge, this phospholipid has never been described.

Moreover, the crude methanolic extract and compound 5 were compared with 1,2-dihexadecanoyl-sn-glycero-3-phosphatidylethanolamine as a positive control on a thin-layer chromatography plate (Merck Kieselgel 60), eluted by dichloromethane-methanol (6:4), and sprayed with Phospray (Zinzade reagent Supelco, to detect phosphorus), which indicated that compound 5 was the sole phospholipid present in the crude extract.

The proportion of phosphatidylethanolamine found in this strain is in agreement with the data reported in the literature. Oliver and Colwell (1973) showed that the phospholipid compositions of marine and estuarine bacteria differed very little from those of nonmarine organisms, phosphatidylethanolamine and phosphatidylglycerol being the predominant phospholipids, i.e., a phosphatidylethanolamine content of 95% for *Pseudomonas aeruginosa*. A large proportion of phosphatidylethanolamine, 86%, was found in a preparation of the marine pseudomonad *P. perfectomarinus* (Sinha and Gaby, 1964). This pattern may be typi-

cal of the genus. However, these lipids are most often encountered as a mixture of several phosphatidylethanolamines acylated by different fatty acids on both the sn-1 and sn-2 positions.

Acknowledgments

We thank J.P. Brouard for GCMS and Service Central d'Analyse du CNRS (Lyon) for mass measurements, E. Abou-Mansour from the Australian Institute of Marine Science, Townsville, Australia, for the sample of methylic ester of lactobacillic acid, and IFREMER for financial support (ORSTOM/IFREMER agreement 93/5 570 093).

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