Pergamon

Tetrahedron 54 (1998) 14597–14602

TETRAHEDRON

Phallusides, New Glucosphingolipids from the Ascidian *Phallusia fumigata*[1]

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Received 6 August 1998; revised 15 September 1998; accepted 1 October 1998

Abstract

The ascidian *Phallusia fumigata* contains a series of new glucosphingolipids named phallusides 1-4 (**1-4**). The structures were elucidated by spectroscopic methods and chemical degradations. Phallusides 1-3 (**1-3**) contain the uncommon sphingoid base 2-amino-9-methyl-D-*erythro*-(4*E*,8*E*,10*E*)-octadeca-4,8,10-triene-1,3-diol. This is the first report of glucosphingolipids isolated from ascidians.

Keywords: Glycolipids, marine metabolites, biologically active compounds.

Glycosphingolipids are a large group of biomolecules containing two basic structural units: a sugar and a ceramide. The hydrophobic ceramide portion involves a sphingoid base and an amide-linked fatty acyl chain [1]. In general, glycosphingolipids have exhibited a wide range of biological functions which might be related to the amphipathic nature of the molecule [2].

In the marine environment, glycosphingolipids have been reported as constituents of algae, sponges, echinoderms, and fungi [3,4]. However to our knowledge these group of compounds had not been reported to occur in ascidians (tunicates) and only a related base, assumed to derive from a diterpenic acid and serine, has been described as a metabolite from an unidentified tunicate of the genus *Aplydium* [5].

As a part of our research project directed toward the search for biologically active metabolites from marine invertebrates of the southern coast of Spain, we collected the mediterranean ascidian *Phallusia fumigata* Grübe (Ascidiidae), sampled off Tarifa (Cádiz, Spain). *P. fumigata* had been previously reported to contain (*R*)-2,6-dimethylheptyl sulfate [6]. Our specimens afforded a series of four new glucosphingolipids which we have named phallusides 1-4 (**1-4**).

[1]This research was partially presented in the 1st Euroconference on Marine Natural Products. Athens, Greece, November 1997.

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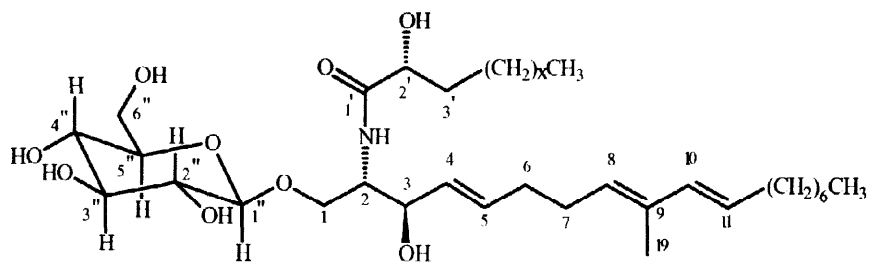
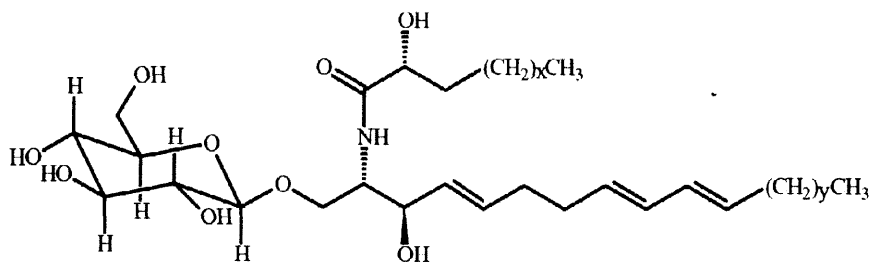
0040-4020/98/$ - see front matter
PII: S0040-4020(98)00917-X

Specimens of *Phallusia fumigata* (61.8 g dry wt) were collected by hand using SCUBA and immediately frozen. The medium polar fraction of an acetone-methanol extract was chromatographed on silica gel. Purification of selected fractions using reversed phase HPLC allowed isolation of four glucosphingolipids: phalluside 1 (**1**, 0.019% dry wt), phalluside 2 (**2**, 0.011% dry wt), phalluside 3 (**3**, 0,006% dry wt), and phalluside 4 (**4**, 0.003% dry wt).

Both the IR and NMR spectroscopic data of the phallusides were quite similar. The IR absorptions at 3400 cm^{-1}, 1650 cm^{-1}, and 1540 cm^{-1} indicated the presence of hydroxyl and amide groups. The ^1H and ^{13}C NMR spectra of compounds **1-4** were diagnostic. The ^{13}C NMR signals at δ 105.7 (d) together with the ^1H NMR doublets around δ 4.9 were assigned to the anomeric methines of β-glucopyranosides moieties which, in addition, gave rise to the carbon signals at δ 78.6 (d), 78.5 (d), 75.2 (d), 71.6 (d), and 62.7 (t), and to a series of proton signals in the ^1H NMR at δ 4.5-3.8. The *N*-alkylamide functionalities were ascertained by the ^{13}C NMR signals at δ 175.7 (s) and 54.6 (d). These common spectroscopic features together with the presence of a series of aliphatic and olefinic signals suggested that the phallusides 1-4 were four glucosphingolipids with slight structural differences.





Compound **1** was isolated as an amorphous solid of molecular formula $C_{41}H_{75}NO_9$ as indicated by the HR FABMS. By methanolysis of **1** using aq HCl-MeOH, methyl 2-(*R*)-hydroxyhexadecanoate and a methyl glucopyranoside anomeric mixture were isolated. The methyl ester had the molecular formula $C_{17}H_{34}O_3$ as indicated by the HR FABMS. Its structure was established using ^1H NMR spectrocopy and upon comparison of the optical rotation value ($[\alpha]_D^{25}$ -3.5°, $CHCl_3$) with that described for the *R*-enantiomer [7]. In addition the optical rotation value of the methyl glucopyranoside anomeric mixture ($[\alpha]^{25}_D$ +72.0°, MeOH) allowed to define the sugar as the D-isomer [8].

The glucose and the hydroxyacid account for $C_{22}H_{40}O_7$. The remaining $C_{19}H_{35}NO_2$ unit was assigned to the long chain base. A careful inspection of the ^1H and ^{13}C NMR spectra of **1** together with the correlations established using the COSY and HETCOR experiments indicated

that the base present in **1** was 2-amino-9-methyl-(4*E*,8*E*,10*E*)-octadeca-4,8,10-triene-1,3-diol. Thus, the correlations observed in the COSY spectrum between the H-2 signal at δ 4.80 (m, 1H) both with the H-3 signal at δ 4.75 (m, 1H) and with the H-1 signals at δ 4.71 (dd, J = 10.3 and 5.9 Hz, 1H) and 4.23 (m, 1H) defined the 2-amido-1,3-dioxygenated fragment, whereas the correlations observed between the olefinic proton signals of H-4 at δ 6.00 (dd, J = 15.4 and 6.0 Hz, 1H) with the H-3 signal and of H-8 at δ 5.49 (br t, J = 6.7 Hz, 1H) with the methyl proton signal at δ 1.76 (s, 3H), together with the long-range coupling observed between the H-8 signal and the H-10 vinylic proton signal at δ 6.20 (d, J = 15.6 Hz, 1H), were useful to define the positions of the double bonds. Furthermore, comparison of the spectroscopic data of **1** with those described for the ophidiacerebrosides isolated from the sea star *Ophidiaster ophidianus* [8] and for the agelasphins isolated from the sponge *Agelas mauritanus* [9] supported that the long chain base of phalluside 1 (**1**) had the same carbon skeleton and stereochemistry. In particular, the doublets at δ 72.3 assigned to C-3 and at δ 54.6 assigned to C-2, supported the proposed stereochemistry at C-2 and C-3. Therefore structure **1** was proposed for phalluside 1.

Compound **2** was isolated as an amorphous solid. The molecular formula, $C_{43}H_{79}NO_9$, was obtained from the HR FABMS measurement and indicated that **2** contained an additional C_2H_4 moiety upon comparison with that of phalluside 1 (**1**). Methanolysis of **2** gave rise to a methyl glucoside anomeric mixture identical to that obtained from **1** and a different methyl ester. Its molecular formula $C_{19}H_{38}O_3$ obtained from HR FABMS, together with the optical rotation value ($[\alpha]_D^{25}$ -3.6°, $CHCl_3$) [10] indicated that the fatty acid portion in phalluside 2 (**2**) was 2-(*R*)-hydroxyoctadecanoic acid. All these data are in agreement with the proposed structure for phalluside 2 (**2**).

Compound **3** was isolated as an amorphous solid and the HR FABMS indicated a molecular formula of $C_{42}H_{77}NO_9$. The additional CH_2 unit upon comparison with the structure of phalluside 1 (**1**) was similarly located in the fatty acid portion because of the isolation of methyl 2-(*R*)-hydroxyheptadecanoate [11] in the methanolysis of **3** and upon observation of the general resemblance of the remaining data of **3** with those of the pallusides 1 and 2 (**1**, **2**) dicussed above. Therefore structure **3** was proposed for phalluside 3.

Compound **4** was the minor glucosphingolipid isolated from *Phallusia fumigata*. The molecular formula, $C_{42}H_{77}NO_9$, was obtained from the HR FABMS measurement, and indicated that **4** was an isomer of phalluside 3 (**3**). The NMR spectrocopic data of **4** clearly indicated that its structure lacked the methyl group at C-9 as ascertained by the absence of the ^{13}C NMR signal at δ 12.8 (q) and the presence of six doublets in the olefinic carbon region attributable to three disubstituted double bonds. A careful analysis of the COSY spectrum enabled us to locate the double bonds at 4, 8, and 10 positions and to propose *E* geometries upon observation of the allylic methylene carbon signals at δ 32.9 (t), 32.7 (t) and 32.6 (t) [12]. Because both compounds are isomers the additional difference in the structure of **4** upon comparison with that of **3** must be the number of methylenes either of the fatty acid or of the saturated portion of the base. However, the small amount of compound **4** isolated from *P. fumigata* prevented the methanolysis reaction from being carried out and therefore the structure of **4** could not be completely defined. The stereochemistry is proposed as depicted in formula **4** upon observation

of the optical rotation value ($[\alpha]_D^{25}$ +12°, *n*-PrOH) and ^{13}C NMR data similarities with those of the phallusides 1,2, and 3 (**1** - **3**) discussed above. An unbranched $\Delta^{4,8,10}$ long chain base had been previously reported in a ceramide from the octocoral *Acabaria ondulata* [13].

Structurally related ophidiacerebrosides [8] have been described to show strong cytotoxicity against L 1210 leukaemia cells *in vitro* [8] and the agelasphins [9] have shown *in vivo* antitumour activity against B 16 murine melanoma although being inactive in *in vitro* tests. Phalluside 1 and 2 (**1**, **2**) were tested against P-388 suspension culture of mouse lymphoid neoplasm and the monolayer cultures of human lung carcinoma (A 549), human colon carcinoma (HT 29), and human melanoma (MEL 28) being both inactive with ED_{50} values over 10 μg/mL in all cases.

Experimental Section

General: Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR and UV spectra were recorded on Perkin-Elmer 881 and Phillips PU 8710 spectrophotometers, respectively. ^1H NMR and ^{13}C NMR spectra were carried out on a Varian Unity 400 at 400 MHz and 100 MHz respectively using C_5D_5N or $CDCl_3$ as solvent. Chemical shifts for ^1H and ^{13}C spectra are referenced to solvent peaks: $\delta_{H/C}$ 8.71/149.9 for C_5D_5N and 7.26/77.0 for $CDCl_3$. Assignments with identical superscripts may be interchanged. Mass spectra were recorded on a VG Autospec spectrometer. In High Performance Liquid Chromatography separations LiChrosorb RP-18 was employed using a differential refractometer. All solvents were spectral grade or were distilled from glass prior to use.

Collection, Extraction and Purification: The tunicate *Phallusia fumigata* (61.8 g dry weight) was collected by hand using SCUBA off the southern coast of Cádiz in May 1996 and was immediately frozen. The frozen tissue was extracted exhaustively with acetone-methanol (1:1) at room temperature. The filtered solution was evaporated under reduced pressure and the aqueous residue was extracted with Et_2O. The solvent was evaporated to give an oil residue (3.76 g) which was chromatographed on a SiO_2 column using solvents of increasing polarity from hexane to Et_2O, EtOAc, and subsequently mixtures of increasing polarity from $CHCl_3$ to MeOH. Fractions of the general chromatography eluted with $CHCl_3$/MeOH (85:15) were subjected to reversed phase HPLC separation using a preparative LiChrosorb RP-18 column eluted with MeOH/H_2O (98:2) to afford, in order of elution, phalluside 1 (**1**, 12 mg, 0.019% dry wt), phalluside 3 (**3**, 4 mg, 0.006% dry wt), phalluside 4 (**4**, 2 mg, 0.003% dry wt) and phalluside 2 (**2**, 7 mg, 0.011% dry wt). Final purification of each compound was accomplished by HPLC on reversed- phase mode using MeOH/H_2O (95:5).

Phalluside 1 (1): amorphous solid; $[\alpha]_D^{25}$ +7° (*c* = 0.1, *n*-PrOH); UV (MeOH) λ_{max} (lg ε) = 232 nm (4.43); IR (film) 3400, 1650, 1540 cm^{-1}; ^1H NMR (400 MHz) δ 8.36 (d, 1H, *J* = 8.6 Hz, NH), 7.65 (br s, 1H, OH), 7.25 (br s, 1H, OH), 7.17 (br s, 1H, OH), 6.90 (br s, 1H, OH), 6.41 (br s, 1H, OH), 6.20 (d, *J* = 15.6 Hz, 1H, H-10), 6.00 (dd, *J* = 15.4, 6.0 Hz, 1H, H-4), 5.90 (dt, *J* = 15.4, 6.2 Hz, 1H, H-5), 5.65 (m, 1H, H-11), 5.49 (br t, *J* = 6.7 Hz, 1H, H-8), 4.91 (d, *J* = 7.7 Hz, 1H, H-1"), 4.80 (m, 1H, H-2), 4.75 (m, 1H, H-3), 4.71 (dd, *J* = 10.3, 5.9 Hz, 1H, H-1), 4.57 (m, 1H, H-2'), 4.50 (br d, *J* = 10.3 Hz, 1H, H-6"), 4.36 (m, 1H, H-6"), 4.23 (m, 1H, H-1), 4.21 (m, 2H, H-3" and H-4"), 4.03 (m, 1H, H-2"), 3.90 (m, 1H, H-5"), 2.21 (m, 2H, H-7), 2.16 (m, 3H, H-3' and H-6), 2.12 (m, 2H, H-12), 2.00 (m, 1H, H-3'), 1.76 (s, 3H, H-19), 1.75 (m, 2H, H-4'), 1.36 (m, 4H, H-5' and H-13), 1.25 (br s, 28H), 0.86[a] (t, *J* = 6.7 Hz, 3H, H-18),

0.84[a] (t, J = 6.7 Hz, 3H, H-16'); ^{13}C NMR (100 MHz) δ 175.7 (s, C-1'), 135.4 (d, C-10), 134.3 (s, C-9), 132.2 (d, C-4), 132.0 (d, C-5), 130.1 (d, C-8), 128.0 (d, C-11), 105.7 (d, C-1"), 78.6 (d, C-5"), 78.5 (d, C-3"), 75.2 (d, C-2"), 72.5 (d, C-2'), 72.3 (d, C-3), 71.6 (d, C-4"), 70.1 (t, C-1), 62.7 (t, C-6"), 54.6 (d, C-2), 35.7 (t, C-3'), 33.2 (t, C-12), 32.8 (t, C-6), 32.2 (t), 32.1 (t), 30.0 (2xt), 29.7 (2xt), 29.5 (2xt), 28.3 (t, C-7), 25.9 (t, C-4'), 23.0 (t), 22.9 (t), 14.3 (2xq, C-18 and C-16'), 12.8 (q, C-19); FAB HRMS m/z 748.5339 [(M+Na)$^+$, $C_{41}H_{75}NO_9Na$ Δ +0.1 mmu].

Phalluside 2 (2): amorphous solid; $[\alpha]_D^{25}$ +10° (c = 0.1, n-PrOH); UV (MeOH) λ_{max} (lg ε) = 231 nm (4.52); IR (film) 3400, 1650, 1560 cm^{-1}; ^{1}H NMR (400 MHz) δ 8.35 (d, 1H, J = 8.6 Hz, NH), 7.64 (br s, 1H, OH), 7.24 (br s, 1H, OH), 7.16 (br s, 1H, OH), 6.89 (br s, 1H, OH), 6.39 (br s, 1H, OH), 6.20 (d, J = 15.6 Hz, 1H, H-10), 6.00 (dd, J = 15.5, 5.9 Hz, 1H, H-4), 5.90 (dt, J = 15.3, 6.0 Hz, 1H, H-5), 5.65 (dt, J = 14.6, 7.3 Hz, 1H, H-11), 5.50 (br t, J = 6.8 Hz, 1H, H-8), 4.91 (d, J = 7.7 Hz, 1H, H-1"), 4.80 (m, 1H, H-2), 4.75 (m, 1H, H-3), 4.71 (dd, J = 10.4, 5.8 Hz, 1H, H-1), 4.57 (m, 1H, H-2'), 4.51 (br d, J = 10.1 Hz, 1H, H-6"), 4.35 (br dd, J = 11.7, 5.4 Hz, 1H, H-6"), 4.23 (m, 1H, H-1), 4.21 (m, 2H, H-3" and H-4"), 4.03 (m, 1H, H-2"), 3.90 (m, 1H, H-5"), 2.22 (m, 2H, H-7), 2.18 (m, 1H, H-3'), 2.15 (m, 2H, H-6), 2.11 (m, 2H, H-12), 2.00 (m, 1H, H-3'), 1.80 (m, 1H, H-4'), 1.76 (s, 3H, H-19),1.72 (m, 1H, H-4'), 1.37 (m, 4H, H-5' and H-13), 1.26 (br s, 32H), 0.85[a] (t, J = 6.7 Hz, 3H, H-18), 0.84[a] (t, J = 6.7 Hz, 3H, H-18'); ^{13}C NMR (100 MHz) δ 175.7 (s, C-1'), 135.4 (d, C-10), 134.3 (s, C-9), 132.2 (d, C-4), 132.0 (d, C-5), 130.1 (d, C-8), 128.0 (d, C-11), 105.7 (d, C-1"), 78.6 (d, C-5"), 78.5 (d, C-3"), 75.2 (d, C-2"), 72.5 (d, C-2'), 72.3 (d, C-3), 71.6 (d, C-4"), 70.2 (t, C-1), 62.7 (t, C-6"), 54.6 (d, C-2), 35.7 (t, C-3'), 33.2 (t, C-12), 32.8 (t, C-6), 32.1 (2xt), 30.0 (2xt), 29.6 (t), 29.5 (2xt), 28.3 (t, C-7), 25.9 (t, C-4'), 23.0 (t), 22.9 (t), 14.3 (2xq, C-18 and C-16'), 12.7 (q, C-19); FAB HRMS m/z 776.5631 [(M + Na)$^+$, $C_{43}H_{79}NO_9Na$ Δ +2.2 mmu].

Phalluside 3 (3): amorphous solid; $[\alpha]_D^{25}$ +9.4° (c = 0.2, n-PrOH); UV (MeOH) λ_{max} (lg ε) = 233 nm (4.29); IR (film) 3400, 1650, 1540 cm^{-1}; ^{1}H NMR (400 MHz) δ 8.36 (d, 1H, J = 8.8 Hz, NH), 7.65 (br s, 1H, OH), 7.24 (br s, 1H, OH), 7.18 (br s, 1H, OH), 6.90 (br s, 1H, OH), 6.40 (br s, 1H, OH), 6.20 (d, J = 15.5 Hz, 1H, H-10), 6.00 (dd, J = 15.3, 6.4 Hz, 1H, H-4), 5.90 (dt, J = 15.4, 6.1 Hz, 1H, H-5), 5.65 (dt, J = 14.6, 7.3 Hz, 1H, H-11), 5.50 (br t, J = 6.4 Hz, 1H, H-8), 4.90 (d, J = 7.7 Hz, 1H, H-1"), 4.80 (m, 1H, H-2), 4.76 (m, 1H, H-3), 4.71 (dd, J = 10.3, 6.1 Hz, 1H, H-1), 4.58 (m, 1H, H-2'), 4.51 (br d, J = 11.1 Hz, 1H, H-6"), 4.36 (m, 1H, H-6"), 4.23 (m, 1H, H-1), 4.22 (m, 2H, H-3" and H-4"), 4.03 (m, 1H, H-2"), 3.90 (m, 1H, H-5"), 2.22 (m, 2H, H-7), 2.16 (m, 3H, H-3' and H-6), 2.11 (m, 2H, H-12), 2.02 (m, 1H, H-3'), 1.80 (m, 1H, H-4'), 1.76 (s, 3H, H-19),1.72 (m, 1H, H-4'), 1.37 (m, 4H, H-5' and H-13), 1.27 (br s, 30H), 0.85[a] (t, J = 6.7 Hz, 3H, H-18), 0.84[a] (t, J = 6.7 Hz, 3H, H-18'); ^{13}C NMR (100 MHz) δ 175.7 (s, C-1'), 135.4 (d, C-10), 134.3 (s, C-9), 132.2 (d, C-4), 132.0 (d, C-5), 130.1 (d, C-8), 128.0 (d, C-11), 105.7 (d, C-1"), 78.6 (d, C-5"), 78.5 (d, C-3"), 75.2 (d, C-2"), 72.5 (d, C-2'), 72.3 (d, C-3), 71.6 (d, C-4"), 70.1 (t, C-1), 62.7 (t, C-6"), 54.6 (d, C-2), 35.7 (t, C-3'), 33.2 (t, C-12), 32.8 (t, C-6), 32.1 (2xt), 30.1 (t), 30.0 (t), 29.6 (t), 29.5 (2xt), 28.3 (t, C-7), 25.9 (t, C-4'), 23.0 (t), 22.9 (t), 14.3 (2xq, C-18 and C-16'), 12.8 (q, C-19); FAB HRMS m/z 762.5474 [(M + Na)$^+$, $C_{42}H_{77}NO_9Na$ Δ +2.2 mmu].

Phalluside 4 (4): amorphous solid; $[\alpha]_D^{25}$ +12° (c = 0.2 n-PrOH); UV (MeOH) λ_{max} (lg ε) = 231 nm (4.36); IR (film) 3400, 1650, 1540 cm^{-1}; ^{1}H NMR (400 MHz) δ 8.35 (br d, 1H, J = 9.2 Hz, NH), 7.65 (br s, 1H, OH), 6.89 (br s, 1H, OH), 6.39 (br s, 1H, OH), 6.13 (m, 2H, H-9 and H-10), 5.98 (dd, J = 15.5, 5.9 Hz, 1H, H-4), 5.89 (m, 1H, H-5), 5.65 (m, 2H, H-8 and H-11), 4.90 (m, 1H, H-1"), 4.80 (m, 1H, H-2), 4.75 (m, 1H, H-3), 4.71 (dd, J = 10.3, 5.9 Hz, 1H, H-1), 4.58 (m, 1H, H-2'), 4.51 (m, 1H, H-6"), 4.36 (m, 1H, H-6"), 4.23 (m, 1H, H-1), 4.22 (m, 2H, H-3" and H-4"), 4.03 (m, 1H, H-2"), 3.91 (m, 1H, H-5"), 2.19 (m, 1H, H-3'), 2.17 (m, 4H, H-6 and H-7), 2.06 (dd, J = 14.0, 7.0 Hz, 2H, H-12), 2.00 (m, 1H, H-3'), 1.72 (m, 2H, H-4'), 1.35 (m, 4H, H-5' and H-13), 1.26 (br s, 30H), 0.86[a] (t, J = 6.7 Hz, 3H, H-18), 0.84[a] (t, J = 6.7

Hz, 3H, H-16'); ^{13}C NMR (100 MHz) δ 175.7 (s, C-1'), 132.9[a] (d, C-8), 132.2[a] (s, C-11), 131.9 (d, C-4), 131.7 (d, C-5), 131.4[b] (d, C-9), 131.0[b] (d, C-10), 105.7 (d, C-1''), 78.6 (d, C-5''), 78.5 (d, C-3''), 75.2 (d, C-2''), 72.5 (d, C-2'), 72.3 (d, C-3), 71.6 (d, C-4''), 70.2 (t, C-1), 62.7 (t, C-6''), 54.6 (d, C-2), 35.7 (t, C-3'), 32.9 (t, C-12), 32.7[c] (t, C-6), 32.6[c] (t, C-7), 32.2 (t), 32.1 (t), 30.0 (2xt), 29.8 (t), 29.6 (t), 29.4 (t), 25.9 (t, C-4'), 23.0[d] (t, C-13), 22.9[d] (t, C-5'), 14.3 (2xq, C-18 and C-16'); FAB HRMS *m/z* 762.5494 [(M + Na)$^+$, $C_{42}H_{77}NO_9Na$ Δ +0.2 mmu].

Methanolysis of phallusides 1, 2, and 3 (1-3). Each sample was treated with 1N HCl in 82% aqueous MeOH (1-3 mL) for 15h at 80°C. The reaction mixture was dried under N_2 and filtered through a small SiO_2 column. Elution with $CHCl_3$ afforded the corresponding fatty acid methyl ester, and elution with $CHCl_3$/MeOH (8:2) gave rise to methyl glucopyranosides (α- and β-anomers, $[\alpha]^{25}_D$ +72.0° in MeOH) in all cases.

Methyl 2-(*R*)-hydroxyhexadecanoate. $[\alpha]_D^{25}$ -3.5° ($CHCl_3$, *c* = 0.2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (m, 1H, H-2), 3.79 (s, 3H, $COOCH_3$), 2.67 (d, *J* = 5.8 Hz, 1H, OH), 1.77 (m, 1H), 1.63 (m, 1H), 1.25 (br s, 24H), 0.88 (t, 6.8 Hz, 3H, C-16); FAB HRMS *m/z* 286.2497 [(M)$^+$, $C_{17}H_{34}O_3$ Δ +1.1 mmu].

Methyl 2-(*R*)-hydroxyoctadecanoate. $[\alpha]_D^{25}$ -3.6° ($CHCl_3$, *c* = 0.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (m, 1H, H-2), 3.79 (s, 3H, $COOCH_3$), 2.67 (m, 1H, OH), 1.77 (m, 1H), 1.63 (m, 1H), 1.25 (br s, 28H), 0.88 (t, 6.8 Hz, 3H, C-18) FAB HRMS *m/z* 315.2871 [(M+1)$^+$, $C_{19}H_{39}O_3$ Δ +2.8 mmu].

Methyl 2-(*R*)-hydroxyheptadecanoate. $[\alpha]_D^{25}$ -3.8° ($CHCl_3$, *c* = 0.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (m, 1H, H-2), 3.79 (s, 3H, $COOCH_3$), 2.67 (br d, *J* = 5.4 Hz, 1H, OH), 1.77 (m, 1H), 1.63 (m, 1H), 1.25 (br s, 26H), 0.87 (t, 6.6 Hz, 3H, C-17); FAB MS *m/z* 301 (M+1)$^+$.

Acknowledgement.

This research was supported by grants from CICYT (Programa Nacional de Ciencia y Tecnología Marinas, MAR98-0834) and Junta de Andalucia (PAI 1081). Cytotoxicity assays were performed through a Cooperation Agreement with Instituto Biomar S.A.

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