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Pachastrissamine, a Cytotoxic Anhydrophytosphingosine from a Marine Sponge, *Pachastrissa* sp.

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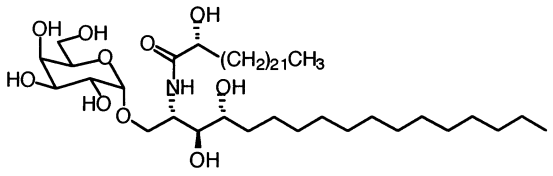
An anhydrophytosphingosine named pachastrissamine (**3**) has been isolated as a cytotoxic principle of a sponge, *Pachastrissa* sp., and the structure including the absolute configuration determined by spectroscopic and chemical analysis.

Sphingolipids are ubiquitous as components of cell membranes. Some unusual sphingolipids have been described from marine organisms. An example is a series of α-galactoceramides, agelasphins (e.g. **1**), exhibiting potent in vivo antitumor activity but no in vitro cytotoxicity, from the sponge *Agelas mauritianus*.[1] This discovery led Natori and co-workers to the development of a synthetic anticancer agent (coded KRN7000, **2**), which is now under clinical trial.[2] In our study of bioactive compounds from coral reef organisms of Okinawa we recently found a new sphingosine derivative, pachastrissamine (**3**), having significant cytotoxicity from a sponge, *Pachastrissa* sp. (family Calthropellidae). In this paper we describe the isolation, structure elucidation, and activity of **3**.

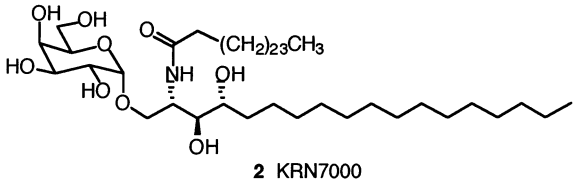
A wet sample of the sponge was extracted with acetone. After concentration the resulting aqueous suspension was extracted with EtOAc to give a crude oil. The oil showed moderate cytotoxicity (IC_{50} 0.25 μg/mL against P388). Bioassay-guided separation of the oil over silica gel followed by preparative TLC (silica gel) and cation-exchange resin gave an active constituent, pachastrissamine (**3**). A pure sample of **3** exhibited cytotoxicity at a level of IC_{50} 0.01 μg/mL against P388, A549, HT29, and MEL28 cell lines.

The molecular formula $C_{18}H_{37}NO_2$ was determined by HREIMS (*m*/*z* 299.2833, Δ +1.0 mmu) and also by HR-FABMS (*m*/*z* 300.2887, Δ −1.5 mmu). The ^{1}H NMR spectrum exhibited signals for five protons in the region δ 3.7−4.3, methylene protons at δ 1.65 (2H) and 1.29 (24H), and methyl protons at δ 0.87, indicating that **3** contains a long alkyl chain and a sugar-like moiety. Analysis of COSY and HMBC spectra revealed the connectivity among the downfield signals and led to a 2-alkyltetrahydrofuran substituted with an amino and a hydroxyl group. The alkyl group was deduced as an *n*-dodecanyl group from the formula and NMR data. The remaining problem was to place the amino and hydroxyl groups on the tetrahydrofuran ring. Since it was not immediately apparent from the NMR data, it was solved by differential acetylation of **3**. When **3** was treated with acetic anhydride and pyridine at 0 °C for 10 min, a monoacetylation product (**4**) was obtained. On the other hand, the treatment of **3** with the same reagents at room temperature for 8 h gave a diacetyl product (**5**). Analysis of the NMR data of these derivatives (see Experimental Section for the assignment) revealed that the amino and hydroxyl groups were located at C-2 and C-3, respectively. The relative configuration of **3** was elucidated by NOE observation (H-1a/H-2, H-2/H-3, H-3/H-4, H-2/H-4) on **5**, which indicated the cis relationship of all of the substituents. To determine the absolute configuration, MTPA monoamides (**6**, **7**) were prepared by treating **3** with MTPA, DCC, and DMAP. From the NMR data of **6** and **7**, the Δδ values were shown to be negative for H-1a (−0.06) and H-1b (−0.08) and positive for H-3 (+0.06), and H-4 (+0.01), indicating the *S* configuration for C-2.[3] Thus, the absolute configuration of **3** was determined as 2*S*, 3*S*, and 4*S*.

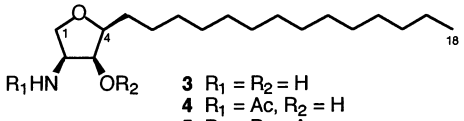
An analogous anhydrophytosphingosine (1,4-anhydro-D-*ribo*-phytosphingosine, **8**) has been reported without stereochemistry as a derivative of a plant metabolite.[4] Later, its absolute structure was assigned by asymmetric synthesis of a compound (**9**) having a shorter side chain.[5−7] More recently, **8** itself has been reported as a synthetic



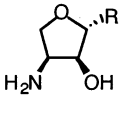
1 Agelasphin 9a



2 KRN7000



3 $R_1 = R_2 = H$
4 $R_1 = Ac, R_2 = H$
5 $R_1 = R_2 = Ac$
6 $R_1 = (S)$-MTPA, $R_2 = H$
7 $R_1 = (R)$-MTPA, $R_2 = H$



8 $R = (CH_2)_{13}CH_3$
9 $R = (CH_2)_{11}CH_3$

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entity.[8] The NMR data for the tetrahydrofuran portion of neither **8** nor **9** were identical with those of **3**.[5−8]

Experimental Section

General Experimental Procedures. Optical rotation was measured on a Jasco DIP-1000 polarimeter. ^{1}H and ^{13}C NMR spectra were taken on a JEOL A-500 instrument. EIMS, ESIMS, and FABMS were measured on a Hitachi M-2500, JEOL JMS-SX102A, and JEOL JMS-700 mass spectrometer, respectively.

Animal Material. The sponge was collected at the depth of 10−30 m by hand using scuba at Funauki Bay, Iriomote Island in May 1998. The sponge was identified by Dr. J. N. A. Hopper, Queensland Museum, Brisbane, Australia. A voucher specimen (QM G317007) has been deposited at the museum.

Extraction and Isolation. The specimen was brought back to the laboratory and kept frozen until extraction. The sponge (100 g, wet weight) was cut and steeped in acetone (0.5 L). The acetone solution was filtered, and the extraction was repeated three times. The combined extracts were concentrated, and the resulting aqueous residue was extracted with EtOAc to give 470 mg of an oil (IC_{50} 0.25 μg/mL). A portion (310 mg) of the oil was chromatographed on silica gel (hexane−EtOAc−MeOH) to give 12 fractions. The ninth fraction (63.0 mg) was further separated by preparative TLC (silica, 1:1 EtOAc−MeOH) into five bands. The sample (20.8 mg) collected from the third band was passed through a cation exchanger (Sep-pak CM, H_2O, MeOH, then MeOH−TFA) to give six fractions. The fraction eluted with MeOH gave compound **3** (8.1 mg). An additional amount (2.0 mg) of **3** was isolated from another fraction.

Pachastrissamine (3): white powder; $[\alpha]_D$ +18° (*c* 0.1, EtOH); FTIR (neat) 3342, 2920, 1556, 1469, 1215 cm^{-1}; ^{1}H NMR (CD_3OD, TFA salt) δ 0.87 (3H, m, H-18), 1.29 (24H, brs, H-6 to H-17), 1.65 (2H, m, H-5), 3.71 (1H, dt, J = 3.4, 7.6 Hz, H-4), 3.79 (1H, dd, J = 5.0, 8.5 Hz, H-1b), 3.88 (1H, m, H-2), 3.91 (1H, dd, J = 7.3, 8.5 Hz, H-1a), 4.25 (1H, dd, J = 3.4, 5.2 Hz, H-3); ^{13}C NMR (CD_3OD, TFA salt) δ 14.4 q (C-18), 23.7 t, 27.2 t, 30.4 t, 30.7 t, 30.8 t, 33.0 t, 29.7 t (C-5), 54.3 d (C-2), 68.9 t (C-1), 70.9 d (C-3), 84.3 d (C-4); COSY H-1a/H-1b,2, H-1b/H-2, H-2/H-3, H-3/H-4,5, H-4/H-5, H-5/H-6; HMBC H-1b/C-2,3,4, H-3/C-1,4, H-5/C-3,4,6, H-6/C-5,7, H-18/C-16,17; ^{1}H NMR (CD_3OD, free base) δ 0.90 (3H, m, H-18), 1.29 (24H, brs, H-6 to H-17), 1.62 (2H, m, H-5), 3.43 (1H, dd, J = 7.9, 8.9 Hz, H-1b), 3.51 (1H, dt, J = 4.3, 8.9 Hz, H-2), 3.81 (1H, dt, J = 2.8, 6.7 Hz, H-4), 3.86 (1H, dd, J = 2.8, 4.3 Hz, H-3), 3.90 (1H, dd, J = 7.6, 7.9 Hz, H-1a); ^{13}C NMR (CD_3OD, free base) δ 14.4 q (C-18), 23.7 t, 27.1 t, 30.5 t, 30.5 t, 30.7 t, 30.8 t, 33.1 t, 30.9 t (C-5), 56.0 d (C-2), 72.1 t (C-1), 73.3 d (C-3), 84.3 d (C-4); EIMS *m*/*z* 299 (M^+, 4), 252 (3), 61 (100 rel %); ESIMS *m*/*z* 300 ($[M + H]^+$); FABMS *m*/*z* 300 ($[M + H]^+$); HREIMS *m*/*z* 299.2833 (calcd for $C_{18}H_{37}NO_2$, 299.2823); HRFABMS *m*/*z* 300.2887 (calcd for $C_{18}H_{38}NO_2$, 300.2903).

Amide 4. A mixture of **3** (1.3 mg), acetic anhydride (0.44 μL), and pyridine (200 μL) was stirred at 0 °C for 10 min. An excess of the reagents was removed by blowing nitrogen, and the resulting product was dried under vacuum to give **4** (0.8 mg, 54%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, m, H-18), 1.25 (24H, brs, H-6 to H-17), 1.65 (2H, m, H-5), 2.02 (3H, s), 3.59 (1H, dd, J = 7.6, 8.5 Hz, H-1b), 3.77 (1H, dt, J = 3.1, 7.3 Hz, H-4), 4.09 (1H, brt, J = 8.5 Hz, H-1a), 4.10 (1H, m, H-3), 4.57 (1H, dq, J = 4.9, 7.6 Hz, H-2).

Diacetyl Derivative 5. A mixture of **3** (3.0 mg), acetic anhydride (600 μL), and pyridine (600 μL) was stirred at room temperature for 8 h. After removing an excess of the reagents, the resulting residue was dried under vacuum and then purified by HPLC (Si60, 7:3:0.4, hexane−CH_2Cl_2−MeOH) to give compound **5** (1.7 mg, 44%) as a white powder. Compound **5** showed the following data: ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, m), 1.29 (24H, brs, H-6 to H-17), 1.99 (3H, s), 2.17 (3H, s), 3.60 (1H, dd, J = 8.0, 8.5 Hz, H-1b), 3.90 (1H, ddd, J = 3.4, 6.2, 9.2 Hz, H-4), 4.15 (1H, dd, J = 8.0, 8.5 Hz, H-1a), 4.81 (1H, dq, J = 5.5, 8.0 Hz, H-2), 5.38 (1H, dd, J = 3.4, 5.5 Hz, H-3), 5.56 (1H, d, J = 8.0 Hz, NH); ESIMS *m*/*z* 384 ($[M + H]^+$).

(*S*)-MTPA Amide 6. A mixture of **3** (0.7 mg), pyridine (300 μL), (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA, 1.4 mg), 1,3-dicyclohexylcarbodiimide (DCC, 0.9 mg), and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) was stirred at room temperature for 12 h. The reaction mixture was concentrated under nitrogen flow and dried under vacuum. The residue was separated by preparative TLC (Si60, CH_2Cl_2) to furnish (*S*)-MTPA amide **6** (0.5 mg, 42%): white solid; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, m, H-18), 1.25 (24H, brs, H-6 to H-17), 1.61 (2H, m, H-5), 3.43 (3H, s, OMe), 3.59 (1H, dd, J = 7.3, 8.9 Hz, H-1b), 3.78 (1H, dt, J = 2.8, 7.3 Hz, H-4), 4.08 (1H, dd, J = 8.5, 8.9 Hz, H-1a), 4.19 (1H, dt, J = 2.8, 5.5 Hz, H-3), 4.60 (1H, m, H-2), 7.40 (3H, brs, Ph), 7.50 (2H, brs, Ph).

(*R*)-MTPA Amide 7. Compound **7** (0.3 mg) was similarly prepared as above using (*R*)-MTPA: white solid; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, m, H-18), 1.25 (24H, brs, H-6 to H-17), 1.61 (2H, m, H-5), 3.40 (3H, s, OMe), 3.67 (1H, dd, J = 7.3, 9.2 Hz, H-1b), 3.77 (1H, dt, J = 2.8, 6.7 Hz, H-4), 4.12 (1H, m, H-3), 4.14 (1H, dd, J = 8.6, 9.2 Hz, H-1a), 4.60 (1H, m, H-2), 7.40 (3H, brs, Ph), 7.50 (2H, brs, Ph).

Cytotoxicity Testing. Cells were maintained in logarithmic phase of growth in a medium comprised of the following: Eagle's minimum essential medium (MEM) with Earle's balanced salts [with L-Gln (2.0 mM) and nonessential amino acids, without Na_2CO_3] supplemented by 10% fetal calf serum, Na_2CO_3 (10^{-2} M), penicillin G, and streptomycin sulfate. P388 cells (10^4 cells/16 mm well) and A-549, HT-29, and Mel-28 cells (2×10^4 cells/16 mm well) were seeded in 1 mL of the above medium with each concentration of samples. All assays were carried out in duplicate. After 3 days of incubation (37 °C, 98% humidity, 10% CO_2) cells were visually counted against control wells to determine IC_{50}.

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Supporting Information Available: Photo of the sponge *Pachastrissa*. This material is available free of charge via the Internet at http://pubs.acs.org.

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