Paxillamide: a Novel Phytosphingosine Derivative from the Fruiting Bodies of *Paxillus panuoides*

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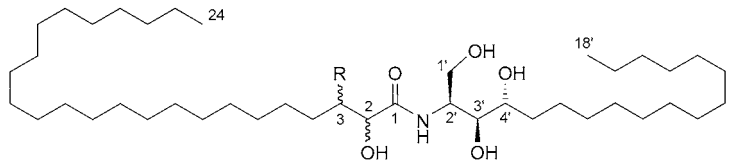
The new phytosphingosine-type ceramide **1**, named paxillamide (=2,3-dihydroxy-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadecyl]tetracosanamide), was isolated from the $CHCl_3$/MeOH extract of the fruiting bodies of the Basidiomycete *Paxillus panuoides*, and its structure was elucidated by spectroscopic and chemical methods.

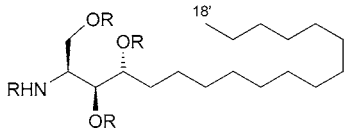
Introduction. – The fungi of the genus *Paxillus* belonging to the family Paxillaceae (basidiomycete) are widely distributed on decayed pine trees in East Asia and North America [1]. Previously, *p*-terphenyl leucomentins (so-called 'coloring matter') produced by *Paxillus panuoides* have been isolated [2–4] and found to be free-radical scavengers [2–5] with interesting neuroprotective properties [6].

In the course of our research on biologically active metabolites from higher fungi, we recently described a series of non-terphenyl compounds of *Paxillus panuoides* [7][8] isolated from *Thelephora ganbajun* [9][10] and named ganbajun A–G, as well as two sphingolipids from *Armillaria mellea* [11] and *polyporus ellisii* [12]. The present report deals with the structure elucidation of the new ceramide **1**, named paxillamide, which was isolated from the fruiting bodies of *P. panuoides* collected in Yunnan Province, China.



1 R = OH
4 R = H

Trivial atom numbering



2 R = H
3 R = Ac

Results and Discussion. – Compound **1** was isolated as a white amphorous powder. Its molecular formula was determined as $C_{42}H_{85}NO_6$ by high-resolution EI-MS, giving rise to *m/z* 699.6358 (M^+; calc. 699.6377). The IR spectrum revealed OH and NH absorptions (3350–3218 cm^{-1}), a secondary amide group (1548 and 1620 cm^{-1}), and aliphatic chains (722 cm^{-1}). The ^{1}H-NMR spectrum (see *Table*) of **1** showed the presence of two terminal Me groups at δ(H) 0.80 (*t*, *J* = 7.7 Hz), ten CH_2 groups at δ(H) 1.25–1.30 (br. *s*), and an amide H-atom at δ(H) 8.64 (*d*, *J* = 8.8 Hz). The ^{13}C-NMR (DEPT) spectrum (*Table*) of **1** indicated two Me, 34 CH_2, five CH groups, and one C=O group at δ(C) 174.0. Four methine C-atoms (δ(C) 73.0, 73.7, 76.5, 76.7) were oxygenated, and the remaining one (δ(C) 53.1) was connected to a N-atom. Moreover, one CH_2 group (δ(C) 61.9) was also oxygenated. The ^{1}H-NMR spectrum of **1** showed the characteristic signals of five HO–C*H* resonances at δ(H) 4.31 (*m*), 4.37 (*dd*, *J* = 6.5, 4.6 Hz), 4.80 (*d*, *J* = 5.6 Hz), 4.43 and 4.50 (2*dd*, *J* = 10.6, 4.4 Hz each), and 4.59 (*m*), as well as an N–CH group at δ(H) 5.14 (*m*)).

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**. At 400 and 100 MHz, resp., in (D_5)-pyridine; δ in ppm, *J* in Hz. Trivial atom numbering (see chemical formula).

	δ(C)	δ(H)	^{1}H,^{1}H COSY [a])
C(1)	174.0		
H–C(2)	76.7	4.80 (*d*, *J* = 5.6)	H–C(3)
H–C(3)	73.7	4.59 (*m*)	H–C(2), H–C(4)
CH_2(4)	32.5	1.95–2.00 (*m*)	H–C(3)
CH_2(5)	23.0	1.95–2.00 (*m*)	
CH_2(6) to CH_2(22)	29.7–32.2	1.25–1.30 (*m*)	
CH_2(23)	22.8		
Me(24)	14.3	0.80 (*t*, *J* = 7.7)	
NH		8.64 (*d*, *J* = 8.8)	H–C(2′)
CH_2(1′)	61.9	4.50 (*dd*, *J* = 10.6, 4.4), 4.43 (*dd*, *J* = 10.6, 5.2)	H–C(2′)
H–C(2′)	53.1	5.14 (*m*)	NH, H–C(1′), H–C(3′)
H–C(3′)	76.5	4.37 (*dd*, *J* = 6.5, 4.6)	H–C(2′), H–C(4′)
H–C(4′)	73.0	4.31 (*m*)	H–C(3′), H–C(5′)
CH_2(5′)	34.3	1.95–2.00 (*m*)	
CH_2(6′)	26.7	1.70 (*m*)	
CH_2(7′) to CH_2(16′)	29.7–32.2	1.25–1.30 (*m*)	
CH_2(17′)	22.8		
Me(18′)	14.3	0.80 (*t*, *J* = 7.7)	

[a]) Diagnostic signals only.

In the EI mass spectrum of **1**, characteristic fragment peaks were observed at *m/z* 472, 442, 400, 355, 325, and 295, which were rationalized according to the pattern shown in the *Figure*.

Upon methanolysis [11][12], compound **1** afforded two products, one of which was methyl 2,3**-** dihydroxytetracosanoate, as evidenced by GC–MS and ^{1}H-NMR analyses. The presence of this unusual fatty acid was also supported by a prominent HR-EI-MS fragment-ion peak at *m/z* 400.3816 ($C_{24}H_{50}NO_3^+$; calc. 400.3791), as well as major fragments at *m/z* 355, 325, and 295 in the EI mass spectrum (*Fig.*). The presence of a key partial structure of the type H_2C(4)–CH(3)(OH)–CH(2)(OH)–C(1)(=O), with

two vicinal OH groups in the fatty acid moiety, was corroborated by cross-peaks between H–C(2) and H–C(3) at δ(H) 4.80 and 4.59, respectively, and by two H–C(4) signals at 1.95–2.00 ppm in the ^{1}H,^{1}H-COSY spectrum (*Table*). The second methanolysis fragment, therefore, had to be a C_{18} long-chain base with three OH groups and an amide NH_2 group, *i.e.*, phytosphingosine (**2**). All these data, thus, revealed that **1** was a phytosphingosine-type ceramide, possessing a 2,3-dihydroxytetracosanoic acid moiety [11][13].

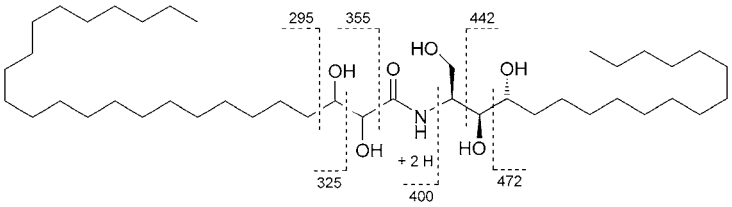


Figure. *Observed key EI-MS fragments* (in *m*/*z*) *of paxillamide* (**1**)

Treatment of the methanolysis product, phytosphingosine (**2**), with Ac_2O afforded a tetraacetylated product whose ^{1}H-NMR and MS data were found to be identical with those of the known compound **3** (=(2*S*,3*S*,4*R*)-2-(acetylamino)octadecane-1,3,4-triyl triacetate [11][13]. The stereochemical assignment was confirmed by the chemical shifts and coupling constants of H–C(1′), H–C(2′), H–C(3′), and H–C(4′) of **1**[1]), which were in good agreement with those reported for the known analog **4** [13][14], as well as for a synthetic derivative of **4** with a slightly shorter *N*-alkyl chain, *i.e.*, (2*R*)-2-hydroxy-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)pentadecyl]tetracosanamide [15]. Thus, the structure of paxillamide (**1**) was established as 2,3-dihydroxy-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadecyl]tetracosanamide.

Together with **1**, compound **4**, which had been obtained earlier from the basidiomycetes *Russula cyanxantha* [13] and *Leccinum extremiorientale* [14], was also isolated from *P. panuoides*, as verified by spectral comparison with the literature data.

Ceramides are cleavage products of sphingolipids, including gangliosides and cerebrosides, and they are involved in various signal-transduction pathways [15]. Extracellular stress induced, *e.g.*, by tumor-necrosis factors and human-immunodeficiency virus (HIV) have been shown to activate sphingomyelinases, which release ceramides to inhibit cell growth and induce apoptosis [16][17]. Because of the importance of ceramides, their chemistry and biology have become a pivotal subject of current lipid research [18–20].

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[1]) Trivial atom numbering.

Experimental Part

General. Silica gel (200–300 mesh) for column chromatography (CC) and silica gel GF_{254} for thin-layer chromatography (TLC) were obtained from *Qingdao Marine Chemical, Ltd.*, China. Melting points (m.p). are uncorrected. Optical rotations: *Horiba SEPA-300* automatic polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrophotometer, KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers (400/100 and 500/125 MHz, resp.); δ in ppm rel. to Me_4Si as internal standard, J in Hz. EI-MS (70 eV): *VG Autospec3000* spectrometer; in *m/z* (rel. %). GC/MS: *Finnigan 4510* apparatus; at 70 eV (EI); capillary column: 30 m × 0.25 mm, packed with 5% phenyl- and 95% methylsilicon on *HP-5*, with He as carrier gas, heating at 5°/min in the range of 160–240°.

Fungal Material. The fresh fruiting bodies of *Paxillus panuoides* were collected at Ailao Mountains, Yunnan Province, P. R. China, in August 1998. A voucher specimen was deposited at the Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The air-dried and powdered fruiting bodies (50 g) of *P. panuoides* were extracted with $CHCl_3$/MeOH 1:1 (3×) at r.t. The combined org. phase was evaporated *in vacuo* to give a deep brown gum. The crude extract was subjected to CC (SiO_2; petroleum ether/acetone gradient). The fraction eluted with petroleum ether/acetone 1:1 afforded pure **1** (6 mg). The fraction eluted with petroleum ether/acetone 6:4 gave pure **4** (26 mg).

2,3-Dihydroxy-N-*[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadecyl]tetracosanamide* (= *Paxillamide*; **1**). White amorphous powder. M.p. 160–162° (petroleum ether/acetone). $[\alpha]_D^{25} = +12.3$ (c = 0.01, pyridine). IR (KBr): 3350, 3218, 2920, 2854, 1620, 1546, 1468, 1358, 1276, 1132, 1109, 1070, 984, 904, 874, 722. ^1H- and ^{13}C- NMR: see the *Table*. EI-MS: 699 (1.5), 682 (5), 681 (4), 651 (6), 668 (3), 472(11), 442 (13), 425 (10), 400 (21), 383 (3), 376 (4), 375 (18), 355 (2), 337 (4), 325 (5), 307 (4), 306 (17), 286 (4), 268 (9), 250 (4), 239 (2), 227 (9), 186 (4), 160 (4), 147 (12), 131 (24), 125 (13), 118 (33), 111 (29), 97 (49), 83 (68), 69 (64), 55 (100). HR-EI-MS: 699.6358 (M^+, $C_{42}H_{85}NO_6^+$; calc. 699.6377).

2-Hydroxy-N-*[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadecyl]tetracosanamide* (**4**). White amorphous powder. M.p. 141–143° (petroleum ether/acetone). $[\alpha]_D = +9.6$ (c = 0.22, pyridine). IR (KBr): 3340, 3220, 2919, 2850, 2487, 2395, 1619, 1544, 1468, 1353, 1068, 1027, 723. ^1H-NMR ((D$_5$)pyridine): 5.12 (*m*); 4.62 (*dd*, J = 7.6, 4.0); 4.52 (*dd*, J = 10.6, 4.5); 4.43 (*dd*, J = 10.6, 5.2); 4.35 (*dd*, J = 6.5, 4.0); 4.28 (*m*). EI-MS: 683 (2, M^+), 665 (11), 651 (5), 456 (13), 439 (18), 409 (22), 384 (24), 357 (27), 339 (4), 320 (7), 227 (13). HR-EI-MS: 683.6407 (M^+, $C_{42}H_{85}NO_5^+$; calc. 683.6427).

Methanolysis and Derivatization. a) Compound **1** (3 mg) was refluxed at 80° for 16 h in 82% aq. MeOH (1.2 ml) containing 0.9M HCl. The mixture was extracted repeatedly with petroleum ether, and the combined org. layers were dried (Na_2SO_4) and evaporated. The resulting residue was purified by CC (SiO_2, petroleum ether/AcOEt 9:1 → 6:4) to afford methyl 2,3-dihydroxytetracosanoate. ^1H-NMR ($CDCl_3$): 4.22 (*dd*, J = 4.2, 5.6); 3.84 (*m*); 3.83 (*s*); 1.47 (*m*); 1.25 (br. *s*), 0.88 (*t*, J = 7.0). EI-MS: 414 (M^+), 355 ($[M-59]^+$).

b) The aq. MeOH phase from the above reaction was neutralized with sat. Na_2CO_3 soln. and concentrated to dryness. The residue was taken up in Ac_2O/pyridine 1:1 and heated at 70° for 90 min. The mixture was diluted with H_2O, extracted with AcOEt, the org. phase was evaporated, and the residue was purified by CC (SiO_2; hexane/AcOEt 8:2) to afford **3**. ^1H-NMR ($CDCl_3$): 5.97 (*d*, J = 9.2); 5.10 (*dd*, J = 8.5, 3.1); 4.93 (*dt*, J = 9.8, 3.1); 4.47 (*m*); 4.29 (*dd*, J = 11.6, 4.3); 4.00 (*dd*, J = 11.6, 3.1); 2.08 (*s*), 2.05 (*s*); 2.05 (*s*); 2.03 (*s*); 1.12–1.70 (br. *s*); 0.88 (*t*, J = 6.1). EI-MS: 486 (1, $[M+1]^+$), 426 (2, $[M+1-\text{AcOH}]^+$), 366 (9, $[M+1-2\text{AcOH}]^+$), 305 (25, $[M-3\text{AcOH}]^+$), 245 (0.5, $[M+1-4\text{AcOH}]^+$).

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