A New Ceramide from the Basidiomycete *Russula cyanoxantha*

Jin-Ming Gao[a,b], Ze-Jun Dong[a], and Ji-Kai Liu*[,a]

[a]Laboratory of Phytochemisty, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204, People's Republic of China, and [b]College of Life Science, Northwest Science & Technology University of Agriculture and Forestry, Yangling, Shaanxi 712100, People's Republic of China

ABSTRACT: A new phytosphingosine-type ceramide (**1**) was isolated along with nine other compounds—5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol, 5α,8α-epidioxy-(24*S*)-ergosta-6-en-3β-ol, (24*S*)-ergosta-7-ene-3β,5α,6β-triol, (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol, inosine, adenine, L-pyroglutamic acid, fumaric acid, and D-allitol from the ethanol and chloroform/methanol extract of the fruiting bodies of the basidiomycete *Russula cyanoxanotha* (Schaeff.) Fr. The structure of (**1**) was established as (2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino) octadecane-1,3,4-triol by means of spectroscopic and chemical methods.

Paper no. L8628 in *Lipids 36*, 175–180 (February 2001).

The family Russulaceae is one of the largest in the subdivision Basidiomycotina in Whitthaker's Kingdom of Fungi and comprises hundreds of species (1). Although secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have well been investigated, the *Russula* mushrooms have received less attention, notwithstanding the larger number of existing species (2). Recently some new terpenoids from *Russula* species have been reported (2,3).

The fruiting bodies of *Russula cyanoxantha* have long been used as foods and medicinal agents in China; an extract of its fruiting bodies has been shown to be active against tumors (4). In a preceding paper, we reported on two sphingadienine-type glucocerebrosides isolated from *R. ochroleuca* (5). In a continuation of our study on the bioactive metabolites of the higher fungi in Yunnan Province, the chemical constituents of *R. cyanoxantha* collected at Ailao Mountains in Yunnan Province of the People's Republic of China were investigated. Ten compounds were isolated from the fruiting bodies of *R. cyanoxantha*, and the structure of **1** was established as (2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino) octadecane-1,3,4-triol. The present report deals with the isolation and structure elucidation of the new ceramide (**1**) from the $CHCl_3$-soluble fraction of the EtOH and $CHCl_3$/MeOH extract of the fruiting bodies of this fungus by repeated column chromatography (CC).

EXPERIMENTAL PROCEDURES

Chromatographic and instrumental methods. Melting points were obtained on an XRC-1 apparatus (Sichuan, People's Republic of China) and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). The nuclear magnetic resonance (NMR) spectra (^1H, ^{13}C NMR and two-dimensional NMR) were recorded on Bruker AM-400 and DRX-500 NMR instrument (Karlsruhe, Germany) at 500 MHz for ^1H and 100 MHz for ^{13}C NMR; tetramethylsilane was used as an internal standard and coupling constants were represented in Hertz. Mass spectra were carried out with a VG Autospec3000 mass spectrometer (VG, Manchester, England). Infrared (IR) spectra were obtained in KBr pellets on a Bio-Rad FTS-135 infrared spectrophotometer (Bio-Rad, Richmond, CA). Gas chromatography–mass spectrometry (GC–MS) was performed on a Finnigan 4510 GC–MS spectrometer (San Jose, CA) employing the electron impact (EI) mode (ionizing potential 70eV) and a capillary column (30 m × 0.25 mm) packed with 5% phenyl-dimethylsilicone on HP-5 (Hewlett-Packard, Palo Alto, CA). Helium was used as carrier gas; column temperature 160–240°C (rate of temperature increase: 5°C/min).

Materials. CC was conducted over silica gel (200–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China). Thin-layer chromatography (TLC) analysis was carried out on plates precoated with silical gel F_{254} (Qingdao Marine Chemical Ltd.). Reversed-phase chromatography was carried out on LiChroprep® RP-8 (40–63 μm) (Merck, Darmstad, Germany).

Fresh fruiting bodies of *R. cyanoxantha* were collected from Ailao Mountains of Yunnan Province in August 1998 and identified by Prof. P.G. Liu and X.H. Wang (Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China). A voucher specimen is deposited at the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences, People's Republic of China.

Extraction and isolation. Dried fruiting bodies (300 g) of *R. cyanoxantha* were extracted with 95% EtOH (1.2 L × 3), followed by extraction with $CHCl_3$/MeOH (1:1, vol/vol) at

*To whom correspondence should be addressed. E-mail: jkliu@mail.kib.ac.cn

Abbreviations: CC, column chromatography; DMSO, dimethyl sulfoxide; EI–MS, electron impact–mass spectrometry; FAB–MS, fast atom bombardment–mass spectrometry; GC–MS, gas chromatography–mass spectrometry; HMBC, heteronuclear multiple bond connectivity; HR–EI–MS, high resolution–electron impact–mass spectrometry; IR, infrared spectrometry; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography.

room temperature. The combined extracts were concentrated *in vacuo* to give a crude extract, which was partitioned between H_2O and $CHCl_3$ to provide a $CHCl_3$ extract (17 g) and a water-soluble fraction. The $CHCl_3$-soluble fraction was subjected to CC elution with a solvent mixture of petroleum ether/acetone (50:1–1:1, vol/vol) to give 18 fractions. Fraction 4 after crystallization from *n*-hexane furnished 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (73 mg). Fraction 6 subjected to CC (petroleum ether/EtOAc 7:3, vol/vol) afforded 5α,8α-epidioxy-(24*S*)-ergosta-6-en-3β-ol (38 mg) and (24*S*)-ergosta-7-ene-3β,5α,6β-triol (15 mg), respectively. Recrystallization of Fraction 9 from petroleum ether/acetone produced (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (5 mg). Fraction 16 was submitted to repeated CC ($CHCl_3$/MeOH 8:2, vol/vol), and then recrystallized from petroleum ether/acetone (6:4) to give **1** (50 mg). The concentrated H_2O fraction was dissolved in warm MeOH, and the resulting MeOH-soluble fraction (24.2 g) was subjected to CC ($CHCl_3$/MeOH 9:1–4:6, vol/vol) to give 13 fractions. Preparative TLC ($CHCl_3$/MeOH/H_2O 8.5:1.5:0.02, by vol) purification of Fraction 5 afforded inosine (11.2 mg). Fractions 3–4 were further purified by LiChroprep RP-8 CC (using a gradient of 5%–10% MeOH/H_2O, vol/vol, 20 min, ultraviolet detector) afforded adenine (7.6 mg). Fraction 8 was chromatographed over silica gel ($CHCl_3$/MeOH/ H_2O 9:1:0.05, by vol) to yield L-pyroglutamic acid (20 mg). Further CC purification of Fraction 10 gave fumaric acid (21 mg). Fraction 14 afforded D-allitol (6.2 g) after crystallization from MeOH/H_2O.

*(2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino) octadecane-1,3,4-triol (**1**).* White amorphous powder. mp 140–142°C (petroleum ether/acetone); $[\alpha]_D$ = +9.4° (*c* = 0.21, pyridine). IR (KBr) ν 3340, 3220 (OH), 2919, 2850, 2487, 2395,
1619 (N–C=O), 1544 (NH), 1468, 1353, 1068, 1027, 723 cm^{-1}; ^1H and ^{13}C NMR (500 and 100 MHz, pyridine-d_5) see Table 1; high resolution–electron impact–mass spectrometry (HR–EI–MS) *m/z* 683.6407 $[M]^+$ ($C_{42}H_{85}NO_5$ calcd. 683.6427); EI–MS (70 eV) *m/z* (relative intensity %) 683 $[M]^+$ (2), 665 $[M - H_2O]^+$ (11), 651 $[M - CH_2OH - 1]^+$ (5), 456 $[M - CH_3(CH_2)_{13}CHOH]^+$ (13), 439 $[456 - OH]^+$ (18), 409 $[439 - CHOH]^+$ (22), 384 $[CH_3(CH)_{21}CH(OH)CONH_2 + H]^+$ (24), 357 $[M - CH_3(CH_2)_{21} - OH]^+$ (27), 339 (4), 320 (7), 227 (13).

*(2*S*,3*S*,4*R*,2′*R*)-2-(2′-acetoxytetracosanoylamino) octadecane-1,3,4-triacetoxyl (**1a**).* Compound **1** (6.9 mg) was dissolved in pyridine (1.1 mL); the mixture was treated with Ac_2O (1.1 mL) and was left standing overnight at room temperature. The reaction solution was then diluted with 3 mL of water and extracted with EtOAc (3 × 10 mL). The EtOAc extract was washed with brine and dried over Na_2SO_4, then evaporated to dryness under reduced pressure. The residue obtained was subjected to silica gel CC (petroleum ether/ethyl acetate 8:2, vol/vol) to give 5.5 mg of the peracetate (**1a**) as white powder solids. EI–MS (70 eV) *m/z* (relative intensity, %) 851 $[M]^+$ (9), 611 $[M - 4 \times CH_3COOH]^+$ (2), 543 $[M - CH_3(CH_2)_{21} - H]^+$ (85). Positive fast atom bombardment–mass spectroscopy (FAB–MS) *m/z* 853 $[M + 1]^+$ (29); ^1H NMR (400 MHz, $CDCl_3$, in ppm) δ 4.01 (1H, *dd*, *J* = 11.6, 3.1 Hz, 1-Ha), 4.34 (1H, *dd*, *J* = 11.6, 5.4, 1-Hb), 4.44 (*m*, 2-H), 5.10 (*m*, 3-H), 4.95 (*m*, 4-H), 1.82 (*m*, 2H, 5-H_2), 1.63 (*m*, 2H, 6-H_2), 1.25 (28 × CH_2 *brs*), 0.88 (6H, *t*, *J* = 6.5 Hz, 2 × CH_3), 5.10 (*m*, 2′-H), 1.63 (*m*, 3′-H_2), 2.18 (*s*, OAc), 2.09 (*s*, OAc), 2.06 (*s*, OAc), 2.03 (*s*, OAc), 6.61 (1H, *d*, *J* = 9.2 Hz, NH).

*Methyl 2-(*R*)-hydroxytetracosanoate (**1b**).* Compound **1** (28 mg) was refluxed with 2.2 mL of 0.9 mol/L HCl in 82% aqueous methanol at 80°C for 16 h. The reaction mixture was extracted with petroleum ether, and the petroleum ether layer was concentrated and chromatographed using silica gel (petroleum ether/ethyl acetate 9:1–7:3, vol/vol; ratios changed as 9:1, 8:2, 7:3) to give a methyl ester of fatty acid (**1b**) as

TABLE 1
^1H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectral Data[a] for Compound 1 in Pyridine-d_5

Atom no.	^{13}C in ppm (*J* in Hz)	^1H in ppm (*J* in Hz)	^1H-^1H COSY selected	HMQC selected	HMBC selected
1	62.14 (*t*)	4.52 (*dd*, 10.6, 4.5) 4.43 (*dd*, 10.6, 5.2)	H-2	H-1	H-2, 3
2	53.10 (*d*)	5.12 (*m*)	NH/H_2-1/H-3	H-2	H-1′, 1, 3
3	76.89 (*d*)	4.35 (*dd*, 6.5, 4.0)	H-2/H-4	H-3	H-1, 2, 4, 5
4	73.12 (*d*)	4.28 (*m*)	H-3/H-5	H-4	H-2, 3, 5, 6
5	34.23 (*t*)	1.93 (*m*)		H-5	H-3, 4, 6
6	26.66 (*t*)	1.70 (*m*)			
7–17	29.63–32.16 (*t*)	1.25–1.41			
18	14.28 (*q*)	0.86 (*t*, 6.7)		H-18	
1′	175.37 (*s*)				
2′	72.56 (*d*)	4.62 (*dd*, 7.6, 4.0)	H-3′	H-2′	H-1′, 3′, 4′
3′	35.75 (*t*)	2.24, 2.04 (*m*)	H-2′/H-4′	H-3′	H-2′, 4′
4′	25.86 (*t*)	1.76 (*m*)	H-3′	H-4′	H-2′, 3′
5′–23′	29.63–32.16 (*t*)	1.25–1.41			
24′	14.28 (*q*)	0.86 (*t*, 6.7)		H-24′	
NH		8.57 (*d*, 8.8)	H-2		H-1′

[a]COSY, correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond connectivity.

white solid, which was subjected to GC–MS. The result showed that **1b** was a methyl 2-hydroxytetracosanoate which displayed major ion peaks at *m/z* 398 $[M]^+$, 339 $[M - 59]^+$; the GC retention time was 35 min. $[\alpha]_D = 4.5°$ (*c* = 0.83, $CHCl_3$); EI–MS (70 eV) *m/z* 398 $[M]^+$; ^1H NMR (400 MHz, $CDCl_3$, in ppm) δ 4.19 (1H, *dd, J* = 4.2, 7.4 Hz, H-2), 3.79 (3H, *s*, OCH_3), 2.74 (1H, *bs*, OH), 1.76 (1H, *m*), 1.63 (1H, *m*), 1.10–1.25 (40 H, *m*), and 0.88 (3H, *t, J* = 7.0 Hz, CH_3).

*2-Acetoamino-1,3,4-triacetoxyoctadecane (**1c**).* The aqueous methanol layer was neutralized with saturated Na_2CO_3 and concentrated to dryness, and then heated with Ac_2O/pyridine (1:1) for 1.5 h at 70°C. The reaction mixture was diluted with H_2O and extracted with EtOAc. The residue of the EtOAc fraction was chromatographed using silical gel (*n*-hexane/EtOAc 8:2, vol/vol) as eluent to furnish an acetate (**1c**) of the long-chain base as a white solid. $[\alpha]_D = +10.9°$ (*c* = 0.67, $CHCl_3$); EI–MS (70 eV) *m/z* (relative intensity %): 486 $[M + 1]^+$ (1), 426 $[M + 1 - HOAc]^+$ (2), 366 $[M + 1 - 2 \times HOAc]^+$ (9), 305 $[M - 3 \times HOAc]^+$ (24.5), 245 $[M + 1 - 4 \times HOAc]^+$ (0.5); ^1H NMR (400 MHz, $CDCl_3$) δ 5.97 (1H, *d, J* = 9.2 Hz, NH), 5.10 (1H, *dd, J* = 8.5 Hz, 3.1 Hz, 3-H), 4.93 (1H, *dt, J* = 9.8, 3.1 Hz, 4-H), 4.47 (1H, *m*, 2-H), 4.29 (1H, *dd, J* = 11.6, 4.3 Hz, 1-Ha), 4.00 (1H, *dd, J* = 11.6, 3.1 Hz, 1-Hb), 2.08 (3H, *s*, 3-OAc), 2.05 (6H, *s*, 1-OAc, 4-OAc), 2.03 (3H, *s*, HNAc), 1.12–1.70 (26H, *m*), 0.88 (3H, *t, J* = 6.1 Hz, CH_3).

*5α,8α-Epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol.* Colorless crystals, mp 182–184°C, $[\alpha]_D = -34°$ (*c* = 0.6, $CHCl_3$); IR (KBr) ν: 3525, 3309, 2957, 2873, 1653, 1459, 1377, 1046, 1029, 985, 970, 969, 935, 858 cm^{-1}; EI–MS (70 eV) *m/z* (relative intensity %): 428 $[M]^+$ (5), 410 (4), 396 (100), 363 (35), 271 (7), 251 (14), 152 (30), 107 (22), 81 (43), 69 (63); ^{13}C NMR (100 MHz, $CDCl_3$, in ppm) δ 34.7, 30.2, 66.5, 37.0, 82.1, 135.4, 130.8, 79.4, 51.2, 37.0, 23.4, 39.4, 44.6, 51.7, 20.6, 28.6, 56.3, 12.9, 18.2, 39.7, 20.9, 135.2, 132.4, 42.8, 33.1, 19.9, 19.6, 17.6; ^1H NMR (400 MHz, $CDCl_3$, in ppm) δ 3.94 (1H, *m*, H-3), 6.22 (1H, *d, J* = 8.5 Hz, H-6), 6.48 (1H, *d, J* = 8.5 Hz, H-7), 0.86 (3H, *s*, H_3-18), 1.06 (3H, *s*, H_3-19), 0.97 (3H, *d, J* = 6.6 Hz, H_3-21), 5.11 (1H, *dd, J* = 15.3, 8.0 Hz, H-22), 5.19 (1H, *dd, J* = 15.1, 7.5 Hz, H-23), 0.83 (3H, *d, J* = 5.0 Hz, H_3-26), 0.82 (3H, *d, J* = 5.0 Hz, H_3-27), 0.89 (3H, *d, J* = 5.3 Hz, H_3-28). The above spectral data were in accord with those reported.

*5α,8α-Epidioxy-(24*S*)-ergosta-6-en-3β-ol.* Colorless crystals, mp 143–145°C, IR (KBr) ν: 3372, 2957, 2874, 1650, 1465, 1379, 1047, 1029, 956, 935, 859 cm^{-1}; EI–MS (70 eV) *m/z* (relative intensity %): 430 $[M]^+$ (24), 412 (41), 398 (100), 379 (17), 365 (49), 339 (25), 271 (7), 251 (9), 152 (57), 107 (30), 95 (40), 81 (51), 69 (46); ^{13}C NMR (100 MHz, $CDCl_3$, in ppm) δ 35.8, 30.2, 66.5, 39.1, 82.2, 135.4, 130.8, 79.5, 51.2, 37.0, 23.5, 39.1, 44.8, 51.6, 20.7, 28.2, 56.4, 12.6, 18.2, 39.5, 18.8, 33.6, 30.6, 39.5, 31.5, 17.7, 20.5, 15.5; ^1H NMR (400 MHz, $CDCl_3$, in ppm) δ 3.95 (1H, *m*, H-3), 6.22 (1H, *d, J* = 8.5Hz, H-6), 6.49 (1H, *d, J* = 8.5 Hz, H-7), 0.77 (3H, *s*, H_3-18), 0.89 (3H, *s*, H_3-19), 0.86 (3H, *d, J* = 5.4 Hz, H_3-21), 0.75 (3H, *d, J* = 6.8 Hz, H_3-26), 0.75 (3H, *d, J* = 6.7 Hz, H_3-27), 0.88 (3H, *d, J* = 6.5 Hz, H_3-28). The above spectral and physical data were in agreement with those reported.

*(24*S*)-Ergosta-7-ene-3β,5α,6β-triol.* Colorless crystals, mp 235–237°C, $[\alpha]_D = 69.4°$ (*c* = 0.16, pyridine). IR (KBr) ν: 3441 (OH), 2958, 2871, 1657, 1465, 1382, 1050, 1031, 969, 940 cm^{-1}; negative FAB–MS *m/z* 585 $[M + 153]^-$; EI–MS (70 eV) *m/z* (relative intensity %): 414 (100), 399 (53), 396 (72), 381 (71), 287 (12), 269 (18), 251 (27), 105 (31), 95, 81, 69; ^{13}C NMR (100 MHz, pyridine-d_5, in ppm) δ 32.6, 33.8, 67.6, 42.0, 76.1, 74.3, 120.4, 141.6, 43.8, 38.1, 22.4, 40.1, 43.9, 55.2, 23.5, 28.2, 56.5, 12.3, 18.8, 37.0, 19.3, 34.0, 31.2, 39.4, 31.8, 17.8, 20.7, 15.7; ^1H NMR (400 MHz, pyridine-d_5, in ppm) δ 4.83 (1H, *m*, H-3), 3.03 (2H, *dd, J* = 12.2, 12.2 Hz, H_2-4), 4.33 (1H, *bd, J* = 5.1 Hz, H-6), 5.74 (1H, *bd, J* = 5.1 Hz, H-7), 0.63 (3H, *s*, H_3-18), 1.53 (3H, *s*, H_3-19), 0.97 (3H, *d, J* = 6.8 Hz, H_3-21), 0.85 (3H, *d, J* = 6.8 Hz, H_3-26), 0.79 (3H, *d, J* = 6.8 Hz, H_3-27), 0.78 (3H, *d, J* = 6.8 Hz, H_3-28). The above data were identical with those reported.

*(22*E*,24*R*)-Ergosta-7,22-dien-3β,5α,6β-triol (= cerevisterol).* Colorless crystals, mp 224–227°C; EI–MS (70 eV) *m/z* (relative intensity %): 430 $[M]^+$ (7), 412 $[M - H_2O]^+$ (18), 394 $[M - 2H_2O]^+$ (26), 379 $[M - 2H_2O - CH_3]^+$ (12), 376 $[M - 3H_2O]^+$ (4), 305 $[M - C_9H_{17}]^+$ (3), 269 $[M - 2H_2O - C_9H_{17}]^+$ (6), 251 $[M - 3H_2O - C_9H_{17}]^+$ (13), 107 (25), 95 (36), 81 (45), 69 (53); ^1H NMR (400 MHz, $CDCl_3$, in ppm) δ 5.30 (1H, *bd, J* = 4.9 Hz, H-7), 5.21 (1H, *dd, J* = 15.2, 7.0 Hz, H-23), 5.16 (1H, *dd, J* =15.2, 7.8 Hz, H-22), 4.06 (1H, *m*, H-3), 3.60 (1H, *bd, J* = 4.9 Hz, H-6), 0.57 (3H, *s*, H_3-18), 1.06 (3H, *s*, H_3-19), 1.00 (3H, *d, J* = 6.6 Hz, H_3-21), 0.89 (3H, *d, J* = 6.9 Hz, H_3-28), 0.82 (3H, *d, J* = 6.6 Hz, H_3-26), 0.80 (3H, *d, J* = 6.5 Hz, H_3-27). The above spectral data agree with the literature values.

*Inosine (= 1,9-dihydro-9-β-*D*-ribofuranosyl-6H-purin-6-one).* White amorphous powder, mp 213°C (dec.). $[\alpha]_D = -45°$ (*c* = 0.6, H_2O); EI–MS (70 eV) *m/z* (relative intensity %): 268 $[M]^+$ (28), 250 $[M - H_2O]^+$ (8), 237 $[M - CH_2OH]^+$ (41), 178 (49), 164 (99), 135 $[M - \text{ribosyl}]^+$ (100), 108 (38), 73 (16), 55 (19); negative FAB–MS *m/z*: 420 $[M - H + 153]^-$ (100), 266, 188, 134; ^{13}C NMR dimethylsulfoxide [(DMSO)-d_6, 100 MHz, in ppm)] δ 152.3 (C-2, *d*), 149.1 (C-4, *s*), 119.3 (C-5, *s*), 156.1 (C-6, *s*), 139.8 (C-8, *d*), 87.9 (C-1′, *d*), 70.6 (C-2′, *d*), 73.4 (C-3′, *d*), 85.8 (C-4′, *d*), 61.6 (C-5′, *t*); ^1H NMR (DMSO-d_6, 400 MHz, in ppm) δ 5.86 (1H, *d, J* = 6.2 Hz, H-1′), 5.43 (1H, *dd, J* = 4.5, 4.6 Hz, H-2′), 5.19 (1H, *br.d, J* = 4.3 Hz, H-3′), 4.59 (1H, *br.d, J* = 5.3 Hz, H-4′), 3.95–4.13 (2H, *br.dd, J* = 3.2, 3.5 Hz, H_2-5′), 8.33 (1H, *s*, H-8), 8.12 (1H, *s*, H-2), 7.33 (1H, *br.s*, OH). The above data are in agreement with those reported.

Adenine (= 2-aminopurine). White amorphous powder, mp > 338°C (dec.); EI–MS (70 eV) *m/z* (relative intensity %): 135 $[M]^+$ (100), 108 (35), 81 (14), 54 (12); ^{13}C NMR (DMSO-d_6, 100MHz) δ 151.5 (*d*, C-2), 151.9 (*s*, C-4), 116.6 (*s*, C-5), 154.4 (*s*, C-6), 139.8 (*d*, C-8); ^1H NMR (DMSO-d_6, 400 MHz, in ppm) δ 7.62 (*s*, 1H, H-8), 7.59 (*s*, 1H, H-2), 6.59 (*brs*, 1H, NH_2). These data are in agreement with those

reported.

L-Pyroglutamic acid [= (S)-2-pyrrolidone-5-carboxylic acid]. Colorless crystals, mp 156–158°(C (MeOH), $[\alpha]_D$ = –11° (H_2O); EI–MS (70 eV) *m/z* (relative intensity %): 129 $[M]^+$ (34), 101 (18), 84 (82), 56 (100); negative FAB–MS *m/z* 128 $[M - H]^-$; ^{13}C NMR (CD_3OD 100 MHz, in ppm) δ 181.5 (*s*, COOH), 176.1 (*s*, CO), 57.0 (*d*, CH), 30.4 (*t*, C-3), 26.0 (*t*, C-4); ^1H NMR (CD_3OD, 400 MHz, in ppm) δ 6.75 (1H, *s*), 4.24 (1H, *dd, J* = 4.6, 8.6 Hz, 2-H), 2.49 (1H, *m*, 3-H_a), 2.14 (1H, *m*, 3-H_b), 2.31 (2H, *m*, 4-H_2). The above data are in accord with those reported.

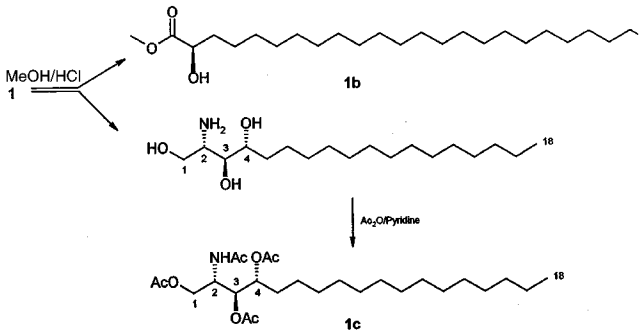
Fumaric acid (= E-butenedioic acid). Leaf-like crystals, mp 210°C (MeOH/$CHCl_3$, sublimation). EI–MS (70 eV) *m/z* (relative intensity %): 116 $[M]^+$ (43), 98 $[M - H_2O]^+$ (100), 88 $[M - CO]^+$ (27), 81 $[M - HO - H_2O]^+$ (20), 72 $[M - CO_2]^+$ (37), 71 $[M - COOH]^+$ (34), 55 (29), 53 (87); ^{13}C NMR (100 MHz, acetone-d_6, in ppm) δ 166.06 (*s*), 134.65 (*d*); ^1H NMR (400 MHz acetone-d_6, in ppm) δ 8.03. The above data are consistent with those reported.

D-Allitol. Colorless needles, $[\alpha]_D$ = 0° (c = 0.36, H_2O); mp 154.5–156°C (MeOH/H_2O). IR ν_{max}^{KBr} cm^{-1}: 3271, 2959, 2936, 1461, 1377, 1351, 1332, 1304, 1092, 1025; ^1H NMR (400 MHz, DMSO-d_6, in ppm) δ 4.42 (2H, *d, J* = 5.4 Hz, H-1, 6), 4.36 (2H, *t, J* = 5.4 Hz, H-3, 4), 3.46 (2H, *d, J* = 1.8 Hz, H-1, 6), 3.36 (2H, *q, J* = 5.4 Hz, H-2, 5); ^{13}C NMR (100 MHz, pyridine-d_5, in ppm) δ 73.45 (*d*, C-3, 4), 72.33 (*d*, C-2, 5), 65.54 (*t*, C-1, 6); EI–MS (70 eV) *m/z* (relative intensity %): 183 $[M + H]^+$ (36), 146 (15), 133 (70), 115 (26), 103 (73), 93 (53), 85 (46), 74 (84), 73 (100), 61 (89). The above data are consistent with literature values.

RESULTS AND DISCUSSION

The $CHCl_3$-soluble fraction of the EtOH and $CHCl_3$/MeOH extract from the fruiting bodies of *R. cyanoxantha* was subjected to repeated column chromatography to yield (2*S*,3*S*, 4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino) octadecane-1,3,4-triol (**1**).

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D$ +9.4° (*c* = 0.21, pyridine). The HR–EI–MS spectrum of **1** indicated a molecular formula of $C_{42}H_{85}NO_5$ (M^+ 683.6407, calcd. 683.6427). The IR spectrum of **1** revealed the absorption bands of hydroxyls at 3340 and 3220 cm^{-1}, a secondary amide at 1544 and 1619 cm^{-1}, and the long aliphatic chains at 723 cm^{-1}. The ^1H NMR spectrum of **1** showed the presence of two terminal methyls at δ 0.86 ppm (6H, *brt, J* = 6.7 Hz) and methylenes at δ 1.25–1.41 ppm (*ca.* 56H, *brs*), an amide proton signal at δ 8.57 ppm (1H, *d, J* = 8.8 Hz). In the ^{13}C NMR (distortion enhancement by polarization transfer) spectrum of **1** the signals for carbons (1 × C, 4 × CH, 35 × CH_2, 2 × CH_3) were recognized in which the presence of one quaternary carbon at δ 175.37 ppm (CONH, C-1′), four methines at δ 53.10 (CHNH, C-2), 72.56 (CHOH, C-2′), 73.12 (CHOH, C-4), and 76.89 ppm (CHOH, C-3) and a methylene at 62.14 ppm (CH_2OH, C-1) were followed from NMR data. Compound **1** possesses five characteristic signals of protons geminal to hydroxyls at δ 4.28 (1H, *m*), 4.35 (1H, *dd, J* = 6.5, 4.0 Hz), 4.62 (1H, *dd, J* = 7.6, 4.0 Hz), 4.43 (1H, *dd, J* = 10.6, 5.2 Hz), and 4.52 ppm (1H, *dd, J* = 10.6, 4.5 Hz). A sixth signal at low field appeared as a multiplet at δ 5.12 ppm and was assigned as a methine proton vicinal to the nitrogen atom. Therefore, all of the above spectral data revealed that **1** should be a phytosphingosine-type ceramide containing a 2-hydroxy fatty acid (6,7). Furthermore, compound **1** was considered to possess normal- type side chains since the carbon atom signals due to terminal methyl groups were observed at δ = 14.28 (*normal* form) (8) in the ^{13}C NMR spectrum of **1** (Table 1).



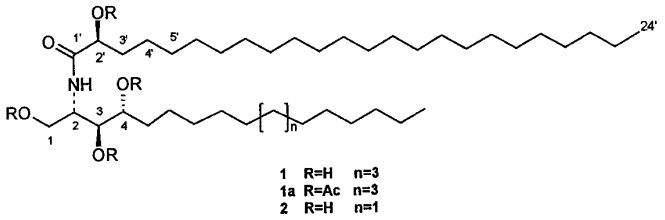
To determine the numbers of hydroxyl groups, compound **1** was acetylated with Ac_2O-pyridine at room temperature to yield the corresponding tetra-acetylated product **1a** which gave prominent peaks at *m/z* 851 $[M]^+$, 611 $[M - 4 \times CH_3COOH]^+$ in the EI–MS. The peracetate **1a** showed four ester methyl proton signals at δ 2.18, 2.09, 2.06, and 2.03 ppm in the ^1H NMR spectrum; thereby the presence of four hydroxyl groups in the original structure of **1** was confirmed.

Acidic methanolysis (6) of **1** with 0.9 N HCl solution in 82% aqueous MeOH yielded a fatty acid methyl ester and a long-chain base (Scheme 1). The fatty acid methyl ester was identified as methyl 2′-hydroxytetracosanoate (**1b**) by the help of GC–MS analysis. The existence of this fatty acyl moiety in **1** was also confirmed by the significant fragment ion peaks at *m/z* 384 $[CH_3(CH_2)_{21}CH(OH)CONH_2 + H]^+$ and 357 $[M - CH_3(CH_2)_{21}(OH]^+$ in the EI–MS. In addition, the ^1H NMR spectrum and optical rotation ($[\alpha]_D$ = –4.5°) of **1b** are in good accord with the data reported in the literature (6),

TABLE 2
^1H NMR Spectral Data[a] and Optical Rotations of Compound 1, Natural Ceramide 2, and Synthetic Ceramide 2 in Pyridine-d_5

	1	**2** natural[b]	**2** synthetic[c]
1-Ha	4.52 (*dd*, 10.6, 4.5)	4.53 (*dd*, 10.7, 4.5)	4.52 (*dd*, 10.7, 4.5)
1-Hb	4.43 (*dd*, 10.6, 5.2)	4.43 (*dd*, 10.7, 4.5)	4.43 (*dd*, 10.6, 5.0)
2-H	5.12 (*m*)	5.12 (*m*)	5.12 (*m*)
3-H	4.35 (*dd*, 6.5, 4.0)	4.35 (*dd*, 6.5, 4.6)	4.36 (*dd*, 6.6, 4.6)
4-H	4.28 (*m*)	4.29 (*m*)	4.29 (*m*)
2′H	4.62 (*dd*, 7.6, 4.0)	4.63 (*dd*, 7.6, 3.7)	4.63 (*dd*, 7.6, 4.0)
$[\alpha]_D$	+9.4°	+11.5°	+9.1°

[a]*J* in parentheses, δ in ppm. For abbreviations see Table 1.
[b]Data from Reference 8.
[c]Data from Reference 9.



therefore the absolute configuration at C-2′ in **1b** is also supposed to be *R*. The long-chain base, namely phytosphingosine, is a C_{18} aliphatic amino alcohol unit containing three hydroxyls and an amino group. It was confirmed by treatment of methanolysis product of **1** with Ac_2O/pyridine at 70°C to afford a tetraacetylphytosphingosine, i.e., 2-acetoamino-1,3,4-triacetoxyoctadecane (**1c**). The ^1H NMR spectrum and optical rotation ($[\alpha]_D = +10.9^\circ$) for **1c** was found to be identical to that of the known counterpart (7).

The relative stereochemistry at C-2, C-3, C-4, and C-2′ was proposed as 2*S*,3*S*,4*R*,2′*R*, since the chemical shifts and coupling constants of 1-H, 2-H, 3-H, 4-H, and 2′-H in **1** were in good agreement with those of the natural ceramide, (2*S*, 3*S*,4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino) hexadecane-1,3,4-triol (**2**) isolated from the starfish *Acanthaster planci* (Table 2) (8) and which was confirmed by synthesis (9). The above fact and the comparison of the optical rotations of **1** with compound **2** (natural, $[\alpha]_D = +11.5°$; synthetic, $[\alpha]_D = +9.1°$) (8,9) suggested that **1** has the same absolute configuration as that of the natural one for the core structure like positions 2, 3, 4, 2′ chiral centers. Accordingly, the above evidence led to the establishment of the structure of **1** as (2*S*,3*S*, 4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino) octadecane-1,3,4-triol, whose structure as shown in Scheme 2 was verified by further two-dimensional NMR experiments: ^1H-^1H correlation spectroscopy heteronuclear multiple bond connectivity, and heteronuclear multiple quantum coherence.

Based upon comparison of spectroscopic (MS, IR, ^1H and ^{13}C NMR) and physical data with the literature, the structures of the other nine known compounds were characterized as 5α, 8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (10,11), 5α, 8α-epidioxy-(24*S*)-ergosta-6-en-3β-ol (10), (24*S*)-ergosta-7-ene-3β,5α,6β-triol (12,13), (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (12,14), inosine (15), adenine (5), L-pyroglutamic acid (16), fumaric acid (17), and D-allitol (5), respectively.

The ceramides, cleavage products of various sphingolipids including gangliosides and cerebrosides, are involved in various signal transduction pathways (18). Many extracellular stresses, such as tumor necrosis factors-α and human immunodeficiency virus, have been shown to activate sphingomyelinases that release ceramides which inhibit cell growth and induce apoptosis (19,20). Because of the importance of ceramides, the chemistry and biology of ceramides have been a vital subject of research in recent years (9,21,22).

The occurrence of the ceramide-containing C_{18}-phytosphingosine and an α-hydroxy fatty acid is rather common in the bonding form in mushrooms. More recently, Jennemann *et al.* (23) reported on a series of glycoinositolphosphoceramides possessing this type of ceramide from higher mushrooms (*Agaricus*). However, except for the fact that this type of ceramide (phytosphingosine/α-fatty acid) itself is a normal constituent of the glycosylphosphoinositolcermides of fungi in general, it has been reported previously to occur in the free state only in the fungus *Phellinus pini* (7). This probably represents a precursor of these glycolipids. One functional aspect of the hydroxyl group cluster of this ceramide, especially in the neighborhood of a phosphoinositol, may indeed be to strengthen the structures where it occurs.

These ergostane-type compounds were previously obtained from marine organisms (11–13) and mushroom (10,14). Biogenetically, Δ^6-ergosterol peroxides and Δ^7-polyhydroxysterols seem quite obviously to originate from ergosterol distributed widely in both fungi and marine organisms (12,13). The occurrence of closely related ergostane derivatives and ceramides in taxonomically remote species is interesting and may indicate the connection with a common producer, probably symbiotic microorganisms. The above fact suggests that a close correlation between terrestrial fungi and marine organisms appears to exist, which is of evolutionary value.

ACKNOWLEDGMENTS

We wish to acknowledge financial support from the Natural Science Foundation of Yunnan Province (98C086M, 98C008Z) and National Natural Science Fundation of China (39969005). We are grateful to Xiang-Hua Wang, Profs. Pei-Gui Liu and Da-Gah Ji for their help in this project.

REFERENCES

1. Whitthaker, R.H. (1969) New Concepts of Kingdoms of Organisms, *Science 163*, 150–160.
2. Vidari, G., Che, Z.L., and Garlaschell, L. (1998) New Nardosinane and Aristolane Sesquiterpenes from the Fruiting Bodies of *Russula lepida*, *Tetrahedron Lett. 39*, 6073–6076.
3. Tan, J.W., Dong, Z.J., and Liu, J.K. (2000) New Terpenoids from Basidiomycetes *Russula lepida*, *Helv. Chim. Acta, 83*, 3191–3197.
4. Gao, J.M., Dong, Z.J., and Liu, J.K. (2000) Constituents of Basidiomycetes *Russula cyanoxantha, Acta Bot. Yunnanica 22*, 85–89.
5. Gao, J.M., Dong, Z.J., Yang, X., and Liu, J.K. (2001) Constituents of Basidiomycetes *Russula ochroleuca, Acta Bot. Yunnanica*, in press.
6. Natori, T., Morita, M., Akimoto, K., and Koezuka, Y. (1994) Agelasphins, Novel Antitumor and Immunostimulatory Cerebrosides from the Marine Sponge *Agelas mauritianus, Tetrahedron 50*, 2771–2784.
7. Lourenco, A., Lobo, A.M., Rodriguez, B., and Jimeno, M.-L. (1996) Ceramides from the Fungus *Phellinus pini, Phytochemistry 43*, 617–620..
8. Inagaki, M., Isobe, R., Kawano, Y., Miyamoto, T., Komori, T., and Higuchi, R. (1998) Isolation and Structure of Three New Ceramides from the Starfish *Acanthaster planci*, *Eur. J. Org. Chem.,* 129–131.
9. Sugiyama, S., Honda, M., Higuchi, R., and Komori, T. (1991) Stereochemistry of the Four Diastereomers of Ceramide and Ceramide Lactoside, *Liebigs Ann. Chem.*, 349–356.

10. Ishizuka, T., Yaoita, Y., Kikuchi, M. (1997) Sterols from the Fruit Bodies of *Grifola frondosa*, *Chem. Pharm. Bull. 45*, 1756–1760.
11. Gunatilaka, A.A.L., Gopichand, Y., Schmitz, F.J., and Djerassi, C. (1981) Minor and Trace Sterols in Marine Invertebrates. 26. Isolation and Structure Elucidation of Nine New 5α,8α-Epidioxy Sterols from Four Marine Organisms, *J. Org. Chem. 46*, 3860–3866.
12. Iorizzi, M., Minale, L., Riccio, R., Lee, J.-S., and Yasumoto, T. (1988) Polar Steroids from the Marine Scallop *Patinopecten yessoensis*, *J. Nat. Prod. 51*, 1098–1103.
13. Madaio, A., Piccialli, V., Sica, D., and Corriero, G. (1989) New Polyhydroxysterols from the Dictyoceratid Sponges *Hippospongia commonis, Spongia officinalis, Ircinia variabilis,* and *Spongionella gracilis*, *J. Nat. Prod. 52*, 952–961.
14. Kawagishi, H., Katsumi, R., Sazawa, T., Mizuno, T., Hagiwara, T., and Nakamura, T. (1988) Cytotoxic Steroids from the Mushroom *Agaricus blazei, Phytochemistry 27*, 2777–2779.
15. Wang, C.Z., and Jia, Z.J. (1996) Purine Nucleosides from *Pedicularis longflora, J. Lanzhou Uni. Nat. Sci. 32(4)*, 87–91.
16. Hao, X.Y., Tan, N.H., and Zhou, J. (2000) Constituents of *Gastrodia elata* in Guizhou, *Acta Bot. Yunnanica 22*, 81–84.
17. Grasselli, J.G., and Ritchey, W.M. (eds.) (1975) *Atlas of Spectral Data and Physical Constants for Organic Compounds,* 2nd edn., Vol. 3, p. 341, CRC Press, Cleveland.
18. Kolter, T., and Sandhoff, K. (1999) Sphingolipids—Their Metabolic Pathways and the Pathobiochemistry of Neurodegenerative Diseases, *Angew. Chem. Int. Ed. Engl. 38*, 1532–1568.
19. Van Veldhoven, P.P., Matthews, T.J., Bolognesi, D.P., and Bell, R.M. (1992) Changes in Bioactive Lipids, Alklyacylglycerol and Ceramide Occur in HIV-Infected Cells, *Biochem. Biophys. Res. Commun. 187*, 209–216.
20. Jayadev, S., Liu, B., Bielawska, A.E., Lee, J.Y., Nazaire, F., Pushkareva, M., Obeid, L.M., and Hannun, Y.A. (1995) Role for Ceramide in Cell Cycle Arrest, *J. Biol. Chem. 270*, 2047–2052.
21. Hannun, Y.A. (1994) The Sphingomyelin Cycle and the Second Messenger Function of Ceramide, *J. Biol. Chem. 269*, 3125–3128.
22. Jiang, H., Huang, X., Nakanishi, K., and Berova, N. (1999) Nanogram Scale Absolute Configurational Assignment of Ceramides by Circular Dichroism, *Tetrahedron Lett. 30*, 7645–7649.
23. Jennemann, R., Bauer, B.L., Bertalanffy, H., Geyer, R., Gschwind, R.M., Selmer, T., and Wiegandt, H. (1999) Novel Glycoinositolphosphosphingolipids, Basidiolipids, from *Agaricus, Eur. J. Biochem. 259*, 331–338.

[Received September 18, 2000, and in revised formNovember 13, 2000; revision accepted November 15, 2000]