Vibratilicin: a Novel Compound from the Basidiomycete *Cortinarius vibratilis*

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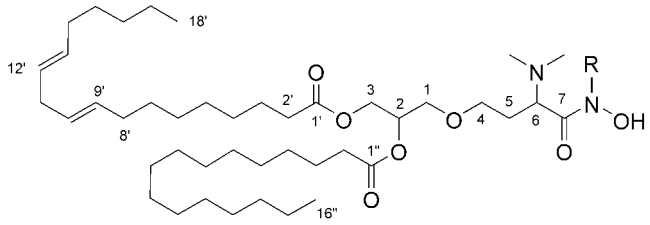
A novel N-containing compound, vibratilicin (= 3-[3-(dimethylamino)-4-(hydroxyamino)-4-oxobutoxy]-2-(palmitoyloxy)propyl (9*E*,12*E*)-octadeca-9,12-dienoate; **1**), was isolated from the fruiting bodies of the basidiomycete *Cortinarius vibratilis* Fr. Compound **1** is a representative of the rare natural products containing hydroxamic acid moieties, and can be viewed as a derivative of neoengleromycin (**2**). Also isolated from the same fungus were five known compounds: ergosta-5,7,22-trien-3β-ol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol, *p*-anisic acid, *N*-(2-hydroxyhexadecanoyl)-4-sphingenine, and (4*E*,8*E*)-2-*N*-(2′-hydroxypalmitoyl)-1-*O*-(β-D-glucopyranosyl)-9-methyl-4,8-sphingadienine. Their structures were determined mainly spectroscopically, including 2D-NMR techniques (HMBC, HMQC, ^{1}H,^{1}H-COSY).

Introduction. – *Cortinarius* (Cortinariaceae) is one of the largest genus in the subdivision Basidiomycotina, kingdom of fungi, comprising hundreds of species and being widely distribute in the world [1]. From the fruiting bodies of *Cortinarius* species, a large number of toxins and/or pigments, including cyclic polypeptides [2], bipyridyl compounds [3], several types of anthraquinone derivatives and chromogenic triterpenoids [4][5], have been isolated and characterized. Most studies on the chemical constituents of *Cortinarius* have been focused on toadstools in Europe and Australia. In our previous papers, we reported three new cerebrosides, cortenuamides A – C, isolated from the fruiting bodies of the basidiomycetes *Cortinarius tenuipes*, chemical constituents from *Cortinarius umidicola* [6–8]. In continuing our studies on basidiomycete-derived bioactive secondary metabolites, we investigated the chemical constituents of the inedible mushroom *Cortinarius vibratilis*. This report describes the structural elucidation of a new compound named vibratilicin (**1**), together with five known compounds.



1 R = H
2 R = Et

Arbitrary numbering

Results and Discussion. – Vibratilicin (= 3-[3-(dimethylamino)-4-(hydroxyamino)-4-oxobutoxy]-2-(palmitoyloxy)propyl (9*E*,12*E*)-octadeca-9,12-dienoate; **1**) was obtained as a yellow oily solid. The FAB mass spectrum (negative mode) showed a quasi-molecular ion peak at *m/z* 735 ($[M-1]^-$). High-resolution electrospray-ionization mass spectrometry (ESI-MS; positive mode) indicated a molecular formula of $C_{43}H_{80}N_2O_7$ (*m/z* 736.5961 (M^+; calc. 736.5965)). FAB-MS experiments showed a series of characteristic fragment ions at *m/z* 676 ($[M-CH_2NO_2]^-$), 473 ($[M-C_{18}H_{31}O]^-$), 437 ($[M-CH_2NO_2-C_{16}H_{31}O]^-$), and 413 ($[M-CH_2NO_2-C_{18}H_{31}O]^-$). This fragmentation pattern was rationalized according to the following *Figure*.

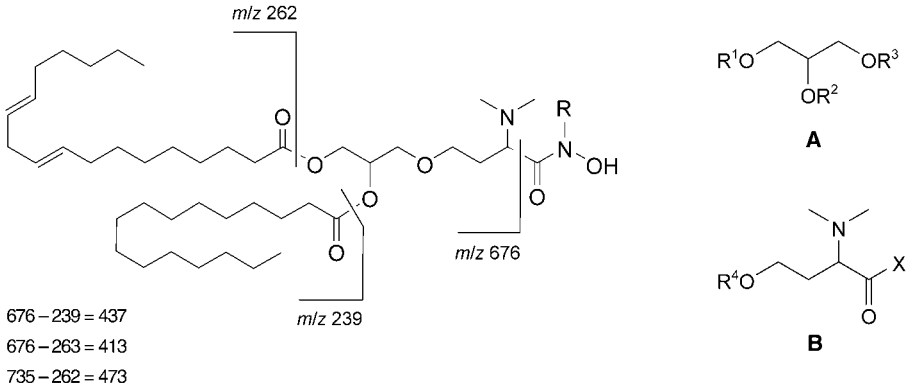


Figure. *MS Fragmentation of vibratilicin* (**1**), *and partial structures* **A** *and* **B**

As shown in the *Table*, the ^{1}H- and ^{13}C-NMR (DEPT) spectra of **1** confirmed the presence of two fatty acid fragments, and detailed ^{1}H,^{1}H-COSY experiments suggested the partial structures **A** and **B**. Considering the molecular formula and the key HMBC correlations (*Table*), the structure of **1** could be identified. The molecule contains a glycerol and a hydroxamic acid moiety, and can be viewed as a derivative of neoengleromycin (**2**)[1]), which has been isolated from the fungus *Engleromyces goetzii* [9]. The only difference between **1** and **2** is an additional Et group on the hydroxamic acid N-atom in **2**.

By spectral and physicochemical comparison with literature data, we identified five more compounds from the same fungus: ergosta-5,7,22-trien-3β-ol [10], 5α,8α-epidioxyergosta-6,22-dien-3β-ol [11], *p*-anisic acid (=4-methoxybenzoic acid), *N*-(2-hydroxyhexadecanoyl)-4-sphingenine[2]) [12], and (4*E*,8*E*)-2-*N*-(2′-hydroxypalmitoyl)-1-*O*-(β-D-glucopyranosyl)-9-methyl-4,8-sphingadienine[3]) [13].

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[1]) Or *vice versa*.

[2]) 4-Sphingenine is the trivial name for 2-aminooctadec-4-ene-1,3-diol.

[3]) Systematic name: 2-hydroxy-*N*-[(3*E*,7*E*)-2-hydroxy-1-(hydroxymethyl)-8-methylheptadeca-3,7-dien-1-yl]-hexadecanamide.

Table. *^{1}H- and ^{13}C- NMR Data of Vibratilicin* (**1**). In C_5D_5N; δ in ppm, J in Hz. Arbitrary numbering (see chemical formula).

	δ(C)	δ(H)	^{1}H,^{1}H-COSY	HMBC (selected)
CH_2(1)	69.9	3.73 (*m*)	H–C(2)	CH_2(3), CH_2(4)
H–C(2)	70.8	5.57 (*m*)	CH_2(1), CH_2(3)	
CH_2(3)	63.2	4.66 (*dd*, *J* = 11.9, 3.5)	H–C(2)	CH_2(1)
		4.45 (*dd*, *J* = 11.9, 3.5)		
CH_2(4)	69.1	3.90, 3.98 (2*m*)	CH_2(5)	CH_2(1), H–C(6)
CH_2(5)	29.0	2.29, 2.39 (2*m*)	H–C(6), CH_2(4)	
H–C(6)	77.1	4.10 (*dd*, *J* = 10.9, 2.6)	CH_2(5)	CH_2(4), Me_2N
C(7)	168.1			CH_2(5)
Me_2N	51.6	3.39 (*s*)		H–C(6)
C(1′)	173.4[a])			CH_2(3), CH_2(3′)
CH_2(2′)	34.5[b])	2.43 (*m*)	CH_2(3′)	
CH_2(3′)	25.3	1.70 (*m*)	CH_2(2′)	
CH_2(4′–7′)	29.4–30.0	1.25–1.40 (*m*)		
CH_2(8′)	27.5	2.12 (*m*)	H–C(9′), H–C(10′)	H–C(10′)
H–C(9′)	128.5	5.50 (*m*)	CH_2(8′), CH_2(11′)	CH_2(11′)
H–C(10′)	128.5	5.50 (*m*)	CH_2(11′), CH_2(8′)	CH_2(8′)
CH_2(11′)	26.1	2.92 (*t*, *J* = 5.4)	H–C(9′), H–C(10′), H–C(12′), H–C(13′)	H–C(9′), H–C(13′)
H–C(12′)	130.5	5.50 (*m*)	CH_2(11′), CH_2(14′)	CH_2(14′)
H–C(13′)	130.5	5.50 (*m*)	CH_2(11′), CH_2(14′)	
CH_2(14′)	27.5	2.12 (*m*)	H–C(12′), H–C(13′)	
CH_2(15′)	29.4–30.0	1.25–1.40 (*m*)		
CH_2(16′)	31.7	1.25–1.40 (*m*)		
CH_2(17′)	22.9[c])	1.25–1.40 (*m*)		
Me(18′)	14.2	0.88 (*t*, *J* = 6.5)		
C(1″)	173.1[a])			H–C(2), CH_2(3″)
CH_2(2″)	34.3[b])	2.43 (*m*)	CH_2(3″)	
CH_2(3″)	25.3	1.70 (*m*)	CH_2(2″)	
CH_2(4″–13″)	29.4–30.0	1.25–1.40 (*m*)		
CH_2(14″)	32.1	1.25–1.40 (*m*)		
CH_2(15″)	22.8[c])	1.25–1.40 (*m*)		
Me(16″)	14.2	0.88 (*t*, *J* = 6.5)		

[a])–[c]) Assignments may be interchanged.

Experimental Part

General. Optical rotations: *Horiba SEPA-300* digital polarimeter. IR Spectra: *Bruker Tensor-27* spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AV-400* (400/100 MHz) and *DXR-500* (500/125 MHz) spectrometers; δ in ppm, J in Hz. MS: *VG Autospec-3000* mass spectrometer; in *m*/*z* (rel. intensities in %).

Fungal Material. The fungus *C. vibratilis* was collected at Ailao Mountain in Yunnan Province, P. R. China, in July 2003. The mushroom was identified by Prof. *Mu Zang*, Kunming Institute of Botany, The Chinese Academy of Sciences. A voucher specimen was deposited at the Herbarium of the Kunming Institute of Botany, The Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered fruiting bodies of *C. vibratilis* (0.74 kg) were extracted successively with $CHCl_3$ (2 ×) and then $CHCl_3$/MeOH 1:1 (3 ×) at r.t. The combined extracts were concentrated to dryness *in vacuo* to afford a syrup (14 g), which was subjected to column chromatography (CC) (SiO_2; $CHCl_3$/MeOH gradient mixtures). *Fraction A* (4.6 g), eluted with $CHCl_3$/MeOH 100:1, was further purified by CC (SiO_2; petroleum ether/acetone 95:5) to give ergosta-5,7,22-trien-3β-ol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol (82 mg), and 5α,8α-epidioxyergosta-6,22-dien-3β-ol (18 mg). *Fraction B* (1.0 g), eluted with $CHCl_3$/

MeOH 80:1, was further purified by CC and prep. TLC ($CHCl_3$/MeOH 60:1) to afford *p*-anisic acid (15 mg). *Fraction C*, eluted with $CHCl_3$/MeOH 50:1, was rechromatographed (SiO_2; $CHCl_3$/MeOH 60:1) to give *N*-(2-hydroxyhexadecanoyl)-4-sphingenine (14 mg). *Fraction D*, eluted with $CHCl_3$/MeOH 15:1, was further purified by repeated CC (SiO_2; $CHCl_3$/MeOH 20:1 and 15:1) to afford both (4*E*,8*E*)-2-*N*-(2′-hydroxypalmitoyl)-1-*O*-(β-D-glucopyranosyl)-9-methyl-4,8-sphingadienine (167 mg) and vibratilicin **1** (38 mg).

*3-[3-(Dimethylamino)-4-(hydroxyamino)-4-oxobutoxy]-2-(palmitoyloxy)propyl (9*E*,12*E*)-octadeca-9,12-dienoate* (= *Vibratilicin*; **1**). Yellow oily solid. $[\alpha]_D^{19.5} = +8.6$ ($c = 1.3$, $CHCl_3$). IR (KBr): 3432, 3011, 2924, 2854, 1737, 1630, 1467, 1384, 1363, 1248, 1179, 1120, 962. ^{1}H- and ^{13}C-NMR: see the *Table*. FAB-MS (neg.): 735 ($[M-1]^-$), 676, 473, 437, 413. EI-MS: 263 (4), 239 (3), 237 (5), 213 (5), 199 (9), 185 (14), 161 (20), 147 (22). HR-ESI-MS (pos.): 736.5961 (M^+, $C_{43}H_{80}N_2O_7^+$; calc. 736.5966).

Ergosta-5,7,22-trien-3β-ol [10]. White needles. M.p. 165–168° ($CHCl_3$). ^{13}C-NMR ($CDCl_3$): 141.4 (C_q); 139.8 (C_q); 135.7 (CH); 132.0 (CH); 119.6 (CH); 116.4 (CH); 70.5 (CH); 55.8 (CH); 54.6 (CH); 46.3 (CH); 42.9 (CH); 42.9 (C_q); 40.8 (CH_2); 40.4 (CH); 39.1 (CH_2); 38.4 (CH_2); 37.1 (C_q); 33.1 (CH); 32.0 (CH_2); 28.3 (CH_2); 23.0 (CH_2); 21.1 (2 CH_2); 19.9 (Me); 19.6 (Me); 17.6 (Me); 16.3 (Me); 12.0 (Me). EI-MS: 396 (25), 378 (3), 363 (19), 337 (17), 271 (33), 253 (20), 69 (100), 55 (53).

5α,8α-Epidioxyergosta-6,22-dien-3β-ol [11]. White needles. M.p. 182–184° ($CHCl_3$). ^{13}C-NMR ($CDCl_3$): 135.4 (CH); 135.2 (CH); 132.4 (CH); 130.7 (CH); 82.1 (C_q); 79.4 (C_q); 66.5 (CH); 56.2 (CH); 51.7 (CH); 51.1 (CH); 44.6 (C); 42.8 (CH); 39.7 (CH); 39.3 (CH_2); 37.0 (CH_2); 37.0 (C); 34.7 (CH_2); 33.1 (CH); 30.1 (CH_2); 28.6 (CH_2); 23.4 (CH_2); 20.9 (Me); 20.6 (CH_2); 19.9 (Me); 19.6 (Me); 18.2 (Me); 17.5 (Me); 12.9 (Me). EI-MS: 428 (9), 410 (17), 396 (55), 363 (21), 271 (100), 251 (30), 69 (100), 55 (34).

p-*Anisic Acid*. Colorless crystals. M.p. 184–186° ($CHCl_3$). ^{1}H-NMR ($CDCl_3$): 7.89 (*dd*, $J = 9.3, 2.5$, 2 H); 6.81 (*dd*, $J = 9.4, 2.3$, 2 H); 3.75 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 168.8 (C_q); 163.4 (C_q); 131.8 (2 CH); 122.5 (C_q); 113.4 (2 CH); 55.2 (Me). EI-MS: 152 (83), 151 (100), 135 (99), 107 (31).

N-*(2-Hydroxyhexadecanoyl)-4-sphingenine* [12]. White powder. ^{1}H-NMR (C_5D_5N): 8.30 (*d*, $J = 8.4$); 6.05 (*dd*, $J = 15.2, 5.6$); 5.98 (*dt*, $J = 15.2, 6.0$); 4.85 (*t*, $J = 5.6$); 4.68 (*m*); 4.60 (*dd*, $J = 7.9, 3.9$); 4.47 (*dd*, $J = 10.8, 4.8$); 4.23 (*dd*, $J = 10.8, 4.4$); 2.23 (*m*); 2.12 (*m*); 2.05 (*m*); 1.80 (*m*); 1.26–1.44 (br. *s*); 0.89 (*t*, $J = 6.8$). ^{13}C-NMR (C_5D_5N): 175.5 (C_q); 132.6 (CH); 132.0 (CH); 73.3 (CH); 72.6 (CH); 62.1 (CH_2); 56.2 (CH); 35.8 (CH_2); 32.8 (CH_2); 32.2 (2 CH_2); 29.6–30.1 (18 CH_2); 25.9 (CH_2); 22.9 (2 CH_2); 14.3 (2 Me). FAB-MS (neg.): 553 (100, M^-).

*(4*E*,8*E*)-2-*N*-(2′-Hydroxypalmitoyl)-1-*O*-(β-D-glucopyranosyl)-9-methyl-4,8-sphingadienine* [13]. White powder. ^{13}C-NMR (CD_3OD): 177.2 (C_q); 136.8 (C_q); 134.8 (CH); 131.0 (CH); 124.8 (CH); 104.6 (CH); 77.9 (CH); 77.8 (CH); 74.9 (CH); 73.1 (CH); 72.8 (CH); 71.5 (CH); 69.8 (CH_2); 62.6 (CH_2); 54.5 (CH); 40.8 (CH_2); 35.9 (CH_2); 33.8 (CH_2); 33.1 (2 CH_2); 30.4–30.8 (13 CH_2); 29.1 (CH_2); 28.7 (CH_2); 26.1 (CH_2); 23.7 (2 CH_2); 16.2 (Me); 14.5 (2 Me). FAB-MS (neg.): 727 (100, M^-), 565 (35). EI-MS: 276 (4), 262 (16), 222 (14), 180 (4).

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