Neoengleromycin, a Novel Compound from *Engleromyces goetzii*

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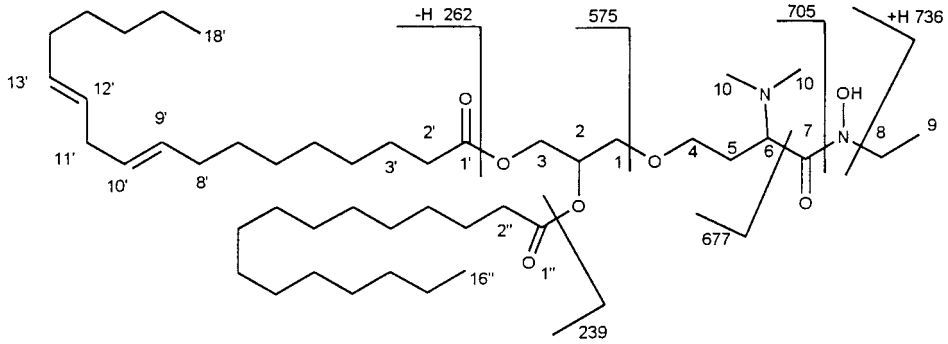
A novel N-containing compound, neoengleromycin (**1**) was isolated from the fruiting bodies of the ascomycete *Engleromyces goetzii* together with two known cytochalasins: cytochalasin D (**2**) and 19,20-epoxycytochalasin D (**3**). Structures were established by spectroscopic (including 2D-NMR) and chemical means. Neoengleromycin (**1**) possesses the rare structure of an *N*-substituted hydroxamic acid and represents a new type of natural products.

Introduction. – The fungus *Engleromyces goetzii* belongs to the Hypocreaceae and grows on the bamboo of high mountains. It has been used as a folk remedy against infection and cancer diseases in the area around Tibet of China including Yunnan and Sichuan Provinces [1]. Little attention has been paid to the chemical constituents of this inedible fungus. As one part of our search for bioactive metabolites of the higher fungi in Yunnan Province [2–7], the chemical constituents of *E. goetzii* collected at Lijiang of Yunnan were investigated. One new compound named neoengleromycin (**1**) was isolated from the fungus *E. goetzii*, together with two known cytochalasins, cytochalasin D (**2**) and 19,20-epoxycytochalasin D (**3**). This report deals with the characterization and structure elucidation of these three compounds.

Results and Discussion. – Compound **1** was obtained as oily solid. High-resolution EI-MS of **1** indicated a molecular formula of $C_{45}H_{84}N_2O_7$ (M^+ at *m*/*z* 764.6339, calc. 764.6279) with 5 degrees of unsaturation and showed a significant ion peak at *m*/*z* 677.5954 ($[M - C_3H_5NO_2]^+$, calc. 677.5958). Compound **1** also exhibited characteristic fragment peaks at *m*/*z* 736, 705, 575, 438, 414, 396, 336, 313, 262, and 239. The pattern of fragmentation is rationalized in *Fig. 1*. On methanolysis, compound **1** yielded two fatty acid methyl esters. These methyl esters were identified as methyl hexadecanoate and methyl (9*E*,12*E*)-octadeca-9,12-dienoate by GC-MS analysis and their structures established by comparison of the ^{1}H-NMR and MS data with those reported [8][9]. As shown in the *Table*, the ^{1}H- and ^{13}C-NMR (DEPT) spectra of **1** confirmed the presence of two fatty acids, and detailed ^{1}H,^{1}H-COSY experiments suggested the presence of the partial structures shown in *Fig. 2*. Considering the molecular formula and the key HMBC correlations (*Table*), the connection of these units was established as shown in **1** (*Fig. 3*). Compound **1** contains a glycerol and an *N*-substituted hydroxamic acid moiety; it has similar partial structures as 'hydroxamic acid antimycotic antibiotics (HAAA)' [10][11], but is quite different. The absolute configuration at C(2) and C(6) of **1** and bioassay tests for antifungal activity are in progress and will be reported in the future.



575-239=336
575-262=313
677-239=438
677-263=414
677-263-H_2O=396

Fig. 1. *Fragmentation of* **1** *in the MS*

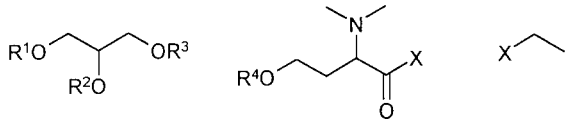
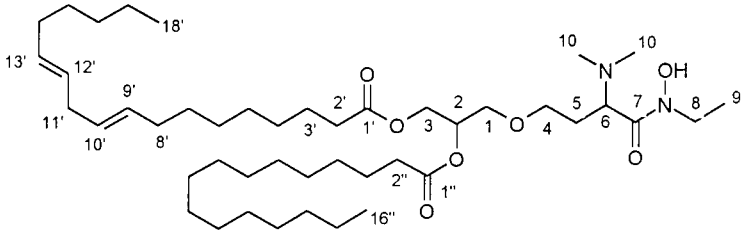
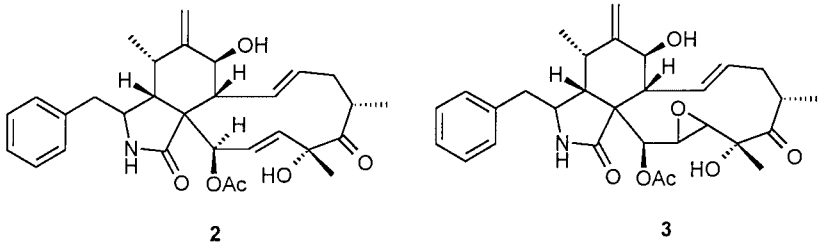


Fig. 2. *Partial structures of compound* **1**



1 (arbitrary numbering)



2 **3**

Fig. 3. *Structures of neoengleromycin* (**1**), *cytochalasin D* (**2**) *and 19,20-epoxycytochalasin D* (**3**)

Table. *^{1}H- and ^{13}C-NMR Data* (C_5D_5N) *of* **1**. δ in ppm, J in Hz; arbitrary numbering (see *Fig. 3*).

	δ (C) (DEPT)	δ (H)	^{1}H,^{1}H COSY	HMBC (selected)
CH_2(1)	69.9(CH_2)	3.72 (*m*)	H–C(2)	CH_2(3), CH_2(4)
H–C(2)	70.8(CH)	5.57 (*m*)	CH_2(1), CH_2(3)	
CH_2(3)	63.3(CH_2)	4.67 (*dd*, *J* = 10.2, 3.2)	CH_2(2)	CH_2(1)
		4.47 (*dd*, *J* = 10.2, 4.9)	CH_2(2)	CH_2(1)
CH_2(4)	68.9(CH_2)	3.88 (*m*)	CH_2(5)	CH_2(1)
CH_2(5)	28.7(CH_2)	2.35 (*m*)	H–C(6), CH_2(4)	
H–C(6)	76.9(CH)	4.18 (*dd*, *J* = 8.0, 3.5)	CH_2(5)	CH_2(4), Me(10)
C(7)	169.5(C)			CH_2(5)
CH_2(8)	42.3(CH_2)	3.12 (*m*)	Me(9)	
Me(9)	12.0(Me)	1.41 (*t*, *J* = 6.8)	CH_2(8)	
Me(10)	51.8(2 Me)	3.46 (*s*)		H–C(6)
C(1′)	173.5(C)			CH_2(3), CH_2(3′)
CH_2(2′)	34.6(CH_2)	2.40 (*s*)	CH_2(3′)	
CH_2(3′)	25.4(CH_2)	1.65 (*m*)	CH_2(2′)	
CH_2(4′) to CH_2(7′)	29.4–30.6(CH_2)	1.25–1.35 (*m*)		
CH_2(8′)	27.6(CH_2)	2.10 (*m*)	H–C(9′), H–C(10′)	
H–C(9′)	128.5(CH)	5.50 (*m*)	CH_2(8′), CH_2(11′)	CH_2(11′)
H–C(10′)	128.5(CH)	5.50 (*m*)	CH_2(8′), CH_2(11′)	CH_2(8′)
CH_2(11′)	26.1(CH_2)	2.92 (*m*)	H–C(9′), H–C(10′)	
			H–C(12′), H–C(13′)	
H–C(12′)	130.3(CH)	5.50 (*m*)	CH_2(11′)	
H–C(13′)	130.3(CH)	5.50 (*m*)	CH_2(11′)	CH_2(11′)
CH_2(14′) to CH_2(17′)	29.4–30.6	1.25–1.35 (*m*)		
Me(18′)	14.3(Me)	0.85 (*t*, *J* = 6.5)		
C(1″)	173.3(C)			H–C(2), CH_2(2″)
CH_2(2″)	34.4(CH_2)	2.45 (*t*, *J* = 7.2)		
CH_2(3″) to CH_2(15″)	29.4–30.6(CH_2)	1.25–1.35 (*m*)		
Me(16″)	14.3(Me)	0.85 (*t*, *J* = 6.5)		

Comparison of the physicochemical properties with the reported data allowed to identify compounds **2** and **3**, isolated from the same fungus, as cytochalasin D and 19,20-epoxycytochalasin D, respectively. Cytochalasin D (= zygosporin A; **2**) was isolated as the metabolite of *Mytarrhizium anisopliae* and *Hypoxylon terricola* and shows antibiotic and cytotoxic properties similar to those of cytochalasin B [12][13]. The 19β,20β-epoxycytochalasin D was reported as a metabolite of *Xylaria hypoxylon* [14].

Experimental Part

General. CC: Column chromatography. IR: *Perkin-Elmer 577* spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm, J in Hz. MS: *VG Autospec-3000* spectrometer; *m*/*z* (rel. int.).

Mushroom Material. The fungus *Engleromyces goetzii* was collected at Lijiang county in Yunnan Province, P.R. China, in July, 1999. The voucher specimen was deposited at the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and powdered *Engleromyces goetzii* (5 kg) were first extracted twice with $CHCl_3$ and then with $CHCl_3$/MeOH 1:1 three times at r.t. The combined org. phase was evaporated to afford a deep brown gum (240 g). The crude extract was submitted to CC (silica gel, gradient $CHCl_3$/MeOH). The fraction (13 g) eluted with $CHCl_3$/MeOH 98:2 was further purified by CC (silica gel; petroleum ether/

acetone/Et_2NH 7:3:0.5): pure **2** (60 mg) and **3** (9 mg). The fraction (2.1 g) eluted with $CHCl_3$/MeOH 9:1 was further purified by prep. TLC ($CHCl_3$/MeOH/Et_2NH 235:20:4): **1** (33 mg).

Neoengleromycin (=*(9*E*,12*E*)-Octadeca-9,12-dienoic Acid 3-[4-[Ethyl(hydroxy)amino]-3-(dimethylamino)-4-oxobutyl]-2-[(1-oxohexyl)oxy]propyl Ester*; **1**). Oily solid. $[\alpha]_D^{20} = 3.3$ ($c = 3$, $CHCl_3$). IR: 3431, 2926, 2858, 1740, 1735, 1730, 1697, 1365, 1247, 1135, 965. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 764 (7), 736 (25), 705 (40), 677 (97), 575 (7), 438 (7), 414 (5), 396 (10), 336 (10), 313 (37), 262 (38), 239 (18), 116 (45), 84 (100). FAB-MS (pos.): 765 (20), 737 (100).

Cytochalasin D (**2**). Colorless needles. ^{1}H-NMR ($CDCl_3$): 7.32 (*m*); 7.26 (*m*); 7.13 (*m*); 6.11 (*dd*, $J = 15.7$, 2.7); 5.62 (*dd*, $J = 15.7$, 9.8); 5.32 (*m*); 5.29 (*s*); 5.13 (*dd*, $J = 15.7$, 2.3); 5.08 (*s*); 4.64 (*s*); 3.80 (*d*, $J = 10.5$); 3.20 (*m*); 2.82 (*m*); 2.73 (*m*); 2.50 (*m*); 2.26 (*s*); 2.15 (*m*); 2.02 (*dd*, $J = 5.1$, 13.0); 1.51 (*s*); 1.19 (*d*, $J = 6.8$); 0.94 (*d*, $J = 6.8$). ^{13}C-NMR ($CDCl_3$): 210.2 (C); 173.7 (C); 169.6 (C); 147.6 (C); 137.2 (C); 134.1 (CH); 132.3 (CH); 130.6 (CH); 129.1 (CH); 128.9 (CH); 127.6 (CH); 127.0 (CH); 114.4 (CH_2); 77.6 (C); 76.7 (CH); 69.8 (CH); 53.5 (CH); 53.3 (C); 49.9 (CH); 46.9 (CH); 45.3 (CH_2); 42.3 (CH); 37.7 (CH_2); 32.6 (CH); 24.2 (Me); 20.8 (Me); 19.4 (Me); 13.6 (Me). EI-MS: 507 (4), 479 (17), 464 (6), 447 (14), 404 (18), 338 (20), 254 (30), 120 (30), 91 (100).

19,20-Epoxycytochalasin D (**3**). White crystalline solid. ^{1}H-NMR ($CDCl_3$): 7.31 (*m*); 7.23 (*m*); 7.15 (*m*); 5.89 (*dd*, $J = 15.6$, 9.8); 5.68 (*ddd*, $J = 15.6$, 9.8, 5.6); 5.50 (br. *s*); 5.26 (br. *s*); 5.05 (br. *s*); 3.99 (*s*); 3.80 (*d*, $J = 10.0$); 3.53 (br. *s*); 3.22 (*m*); 3.13 (*d*, $J = 1.8$); 2.84 (*dd*, $J = 13.4$, 5.0); 2.72 (*dd*, $J = 13.4$, 9.1); 2.62 (*m*); 2.25 (*dd*, $J = 5.2$, 3.3); 2.14 (*s*); 2.08 (*m*); 1.56 (*s*); 1.52 (*s*); 1.18 (*d*, $J = 6.6$); 0.89 (*d*, $J = 6.9$). ^{13}C-NMR ($CDCl_3$): 215.2 (C); 173.5 (C); 169.8 (C); 147.6 (C); 137.2 (C); 133.4 (CH); 131.2 (CH); 129.2 (CH); 129.0 (CH); 127.2 (CH); 114.3 (CH_2); 76.4 (CH); 74.2 (CH); 70.1 (CH); 59.7 (CH); 53.9 (CH); 52.8 (CH); 52.6 (C); 50.8 (CH); 46.6 (CH); 45.2 (CH_2); 42.0 (CH); 37.5 (CH_2); 32.6 (CH); 21.9 (Me); 20.6 (Me); 19.2 (Me); 13.5 (Me). EI-MS: 523 (3), 495 (28), 404 (30), 321 (75), 270 (73), 228 (25), 120 (33), 91 (100).

Methanolysis of **1**. Compound **1** (7 mg) was refluxed with 0.9M HCl (2 ml) in 80% aq. MeOH at 80° for 18 h. The resultant mixture was extracted with hexane, the combined org. layer dried (Na_2SO_4) and evaporated, and the mixture of fatty acid methyl esters purified by CC (silica gel, hexane/AcOEt $9:1 \rightarrow 7:3$) to give the methyl esters of hexadecanoic acid and (9*E*,12*E*)-octadeca-9,12-dienoic acid, which were analyzed by GC/MS.

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