Isolation and Characterization of New Bitter Diterpenoids from the Basidiomycete *Sarcodon scabrosus*

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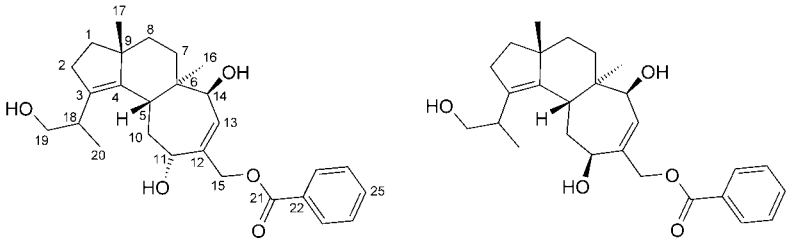
The novel cyathane-type diterpenoids scabronine G and H (**1** and **2**, resp.) were isolated from the fruiting bodies of the basidiomycete *Sarcodon scabrosus* together with four known compounds, allocyathin B_2 (**3**), sarcodonin A (**4**), sarcodonin G (**5**), and scabronine F (**6**). Their structures were determined by spectroscopic means, including 2D-NMR (HMBC, HMQC, ROESY, ^{1}H,^{1}H-COSY).

Introduction. – *Sarcodon scabrosus* is a mushroom belonging to the family Thelephoraceae and has a strongly bitter taste. Diterpenoids, sarcodonins A–H and scabronines A–F, have previously been isolated from this mushroom as the bitter principles [1–3]. All these diterpenoids possess a cyathane skeleton consisting of angularly condensed five-, six-, and seven-membered rings and show stimulating activity on nerve-growth-factor (NGF) synthesis *in vitro*. In continuing our studies on basidiomycete-derived bioactive secondary metabolites, we investigated the chemical constituents of the mushroom *Sarcodon scabrosus* from Yunnan, China. This report describes the structural elucidation of two new compounds, named scabronines G and H (**1** and **2**, resp.).

Results and Discussion. – The $CHCl_3$-soluble fraction of the EtOH extract from the fruiting bodies of *S. scabrosus* was subjected to repeated chromatography to give compounds **1** and **2**[1]) (*Fig. 1*).



1[1]) **2**

Fig. 1. *Structures of scabronines G* (**1**) *and H* (**2**)

[1]) Arbitrary numbering; for systematic names, see *Exper. Part*.

Compound **1** was obtained as an optically active oily solid. High-resolution ESI-MS (pos.) gave a pseudomolecular-ion peak at *m/z* 463.2471 ($C_{27}H_{36}O_5Na^+$; calc. 463.2460). Further spectral data (^{1}H- and ^{13}C-NMR (*Table 1*) ^{1}H,^{1}H-COSY (*Table 1*), HMQC, HMBC (*Table 1*), and ROESY) established the structure and relative configuration of **1** as shown in *Fig. 1*.

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **1**. δ in ppm, J in Hz; arbitrary numbering[1]).

	δ(C) (DEPT)	δ(H)	^{1}H,^{1}H-COSY (selected)	HMBC (selected)
CH_2(1)	37.6 (CH_2)	1.32 (*m*)		C(17)
CH_2(2)	28.6 (CH_2)	2.15 (*m*)		
C(3)	143.2 (C)			
C(4)	134.5 (C)			
H–C(5)	39.9 (CH)	2.95 (br. *d*, J = 11.3)		
C(6)	42.1 (C)			
CH_2(7)	32.2 (CH_2)	1.20 (*m*, H_α), 2.10 (*m*, H_β)		
CH_2(8)	36.3 (CH_2)	1.46 (*m*)		C(4)
C(9)	49.4 (C)			
CH_2(10)	33.4 (CH_2)	2.20 (*m*)	H–C(11)	
H–C(11)	74.1 (CH)	6.02 (*m*)	CH_2(10)	
C(12)	142.1 (C)			
H–C(13)	127.1 (CH)	5.82 (*d*, J = 6.8)	H–C(14)	C(11), C(15)
H–C(14)	75.5 (CH)	3.90 (*d*, J = 6.8)	H–C(13)	C(5), C(7), C(12)
CH_2(15)	62.7 (CH_2)	4.05 (*d*, J = 12.8), 4.02 (*d*, J = 12.8)		
Me(16)	16.4 (Me)	0.72 (*s*)		C(5)
Me(17)	24.4 (Me)	1.02 (*s*)		C(1)
H–C(18)	34.9 (CH)	2.85 (*m*)	CH_2(19), Me(20)	C(4)
CH_2(19)	65.7 (CH_2)	3.35 (*m*), 3.28 (*m*)	H–C(18)	
Me(20)	15.6 (Me)	0.86 (*d*, J = 6.7)	H–C(18)	
C(21)	166.4 (C)			
C(22)	129.8 (C)			
H–C(23), H–C(27)	129.4 (CH)	7.91 (*t*, J = 7.2)	H–C(24), H–C(26)	
H–C(24), H–C(26)	128.2 (CH)	7.32 (*t*, J = 7.2)	H–C(23), H–C(27), H–C(25)	
H–C(25)	133.0 (CH)	7.46 (*t*, J = 7.2)		

The ^{1}H-NMR spectrum of **1** showed signals for one secondary and two tertiary Me groups at δ 0.86 (*d*, J = 6.7 Hz), and 0.72 and 1.02 (each *s*, each 3 H), respectively. The secondary Me and a CH_2 group at δ(H) 3.35 and 3.28 (2*m*) were coupled with a CH proton at δ(H) 2.85 (*m*), demonstrating the presence of an isolated system CH(Me)CH_2OH. ^{1}H-NMR Signals of 5 aromatic H-atoms at δ(H) 7.32–7.91 and six signals in the ^{13}C-NMR between (δ(C) 128.2 and 133.0, besides a carbonyl signal at δ(C) 166.4, were consistent with a benzoate moiety. Moreover, the ^{13}C-NMR of **1** showed two O–CH and one O–CH_2 signals δ(C) 75.5 and 74.1, and δ(C) 62.7, resp.), and signals of a tetrasubstituted δ(C) 143.2 and 134.5) and trisubstituted C=C bond (δ 142.1 (C) and 127.1 (CH)). Based on these partial structures, the construction of the molecular framework was deduced from ^{1}H,^{1}H COSY, HMQC, and HMBC experiments. The HMBC correlations C(2) (δ 28.6)/H–C(18) (δ 2.85), C(5), C(7), and C(12) (δ 39.9, 32.2, and 142.1)/H–C(14) (δ 3.90), C(17) (δ 24.4)/CH_2(1) (δ 1.32), C(4) (δ 134.5)/CH_2(8) (δ 1.46) and H–C(18) (δ 2.85), C(5) (δ 39.9)/Me(16) (δ 0.72), and C(11) and C(15) (δ 74.1 and 62.7)/H–C(13) (δ 5.82) were consistent with the gross structure shown in *Fig. 2*. The ROESY correlations H_β–C(5)/H_β–C(7) and H_β–C(5)/H–C(11) indicated that these H-atoms were situated on the same side. In addition, the ROESY correlations H_α–C(7)/H–C(14), CH_2(8)/Me(16) and H–C(13)/Me(16) confirmed the proposed relative configuration of **1**.

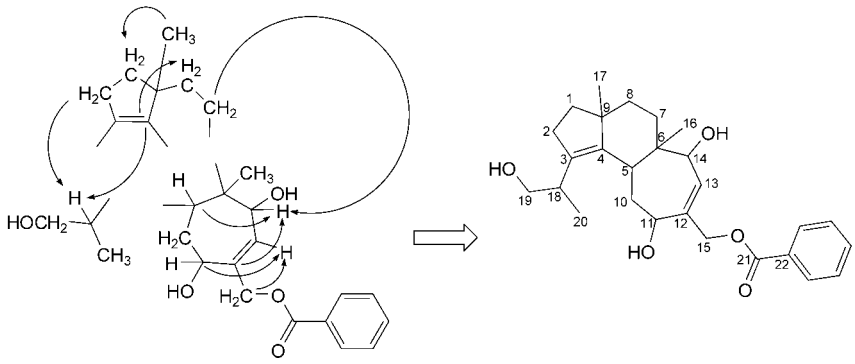


Fig. 2. *HMBC Correlations and planar structure of scabronine G* (**1**)

Compound **2** was obtained as an optically active powder. High-resolution ESI-MS (pos.) gave an pseudomolecular-ion peak at *m/z* 463.2464 ($C_{27}H_{36}O_5Na^+$; calc. 463.2460). The NMR spectra of **2** were similar to those of **1** but on TLC (silica gel), **1** was less polar than **2**. Interpretation of the spectral data (^{1}H- and ^{13}C-NMR (*Table 2*),

Table 2. *^{1}H- and ^{13}C-NMR Data* (CD_3OD) *of* **2**. δ in ppm, J in Hz; arbitrary numbering[1]).

	δ(C) (DEPT)	δ(H)	^{1}H,^{1}H-COSY (selected)	HMBC (selected)
CH_2(1)	39.2 (CH_2)	1.40 (*m*)		C(17)
CH_2(2)	30.0 (CH_2)	2.30 (*m*)		
C(3)	143.7 (C)			
C(4)	136.1 (C)			
H–C(5)	41.4 (CH)	2.78 (br. *d*, *J* = 11.2)	CH_2(10)	
C(6)	43.8 (C)			
CH_2(7)	33.0 (CH_2)	1.20 (*m*, H_α), 1.90 (*m*, H_β)		
CH_2(8)	38.0 (CH_2)	1.50 (*m*)		C(4)
C(9)	50.6 (C)			
CH_2(10)	37.0 (CH_2)	2.20 (*m*)	H–C(11)	
H–C(11)	71.6 (CH)	4.72 (*m*)	CH_2(10)	
C(12)	141.2 (C)			
H–C(13)	131.6 (CH)	5.87 (*d*, *J* = 6.8)	H–C(14)	C(6), C(11), C(15)
H–C(14)	76.5 (CH)	4.02 (*d*, *J* = 6.8)	H–C(13)	C(5), C(7), C(12)
CH_2(15)	67.2 (CH_2)	4.93 (*d*, *J* = 12.8), 4.90 (*d*, *J* = 12.8)		C(13), C(21)
Me(16)	17.3 (Me)	0.83 (*s*)		C(5), C(7), C(14)
Me(17)	25.0 (Me)	1.03 (*s*)		C(1), C(4)
H–C(18)	36.5 (CH)	3.03 (*m*)	CH_2(19), Me(20)	C(2), C(4)
CH_2(19)	67.4 (CH_2)	3.49 (*dd*), 3.35 (*dd*)	H–C(18)	C(3)
Me(20)	16.4 (Me)	0.97 (*d*, *J* = 6.7)	H–C(18)	
C(21)	167.8 (C)			
C(22)	128.6 (C)			
H–C(23), H–C(27)	130.6 (CH)	8.03 (*t*, *J* = 7.2)	H–C(24), H–C(26)	
H–C(24), H–C(26)	129.6 (CH)	7.46 (*t*, *J* = 7.2)	H–C(23), H–C(27), H–C(25)	
H–C(25)	134.2 (CH_2)	7.58 (*t*, *J* = 7.2)		

^{1}H,^{1}H-COSY (*Table 2*), HMQC, and HMBC (*Table 2*)) revealed that **2** had the same gross structure as **1**. However, a detailed comparative study of the NMR and ROESY data of **1** and **2** suggested that **2** was the 11-epimer of **1** (*Fig. 1*)

The NMR signals of C(10), C(11), C(13), C(15), and H–C(11), and CH_2(15) of **1** and **2** were observed at different positions. The ROESY correlations H_α–C(7)/H–C(14), CH_2(8)/Me(16) and H–C(13)/Me(16) of **2** indicated that these H-atoms were situated on the same side. The correlation H_β–C(5)/H–C(11) was not observed in the case of **2**.

Comparison of the physicochemical properties with the reported data allowed us to identify compounds **3**–**6**, isolated from the same fungus, as allocyathin B_2 [4], sarcodonin A, sarcodonin G [1], and scabronine F [3], respectively (*Fig. 3*).

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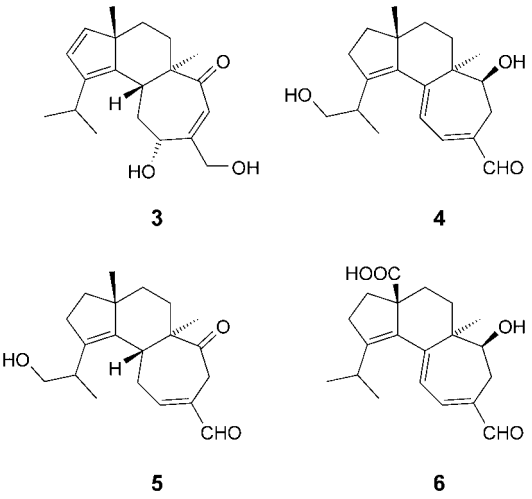

Fig. 3. *Structures of allocyathin B* (**3**), *sarcodonin A* (**4**), *sarcodonin G* (**5**), *and scabronine F* (**6**)

Experimental Part

General. Column chromatography CC. Optical rotations: *Horiba SEPA-300* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer;KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers, $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. MS: *VG Auto-Spec-3000* and *API QSTAR-Pulsar-i* spectrometer; *m*/*z* (rel. int.).

Mushroom Material. The fresh fruiting bodies of *Sarcodon scabrosus* were collected at Ailao Mountain of Yunnan Province, P. R. China, in July 2003. The botanical identification was made by Prof. *Mu Zang*, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen was deposited in the Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and Isolation. The entire freshly collected fruiting bodies of *S. scabrosus* (dry weight after extraction 150 g) were immersed in 95% EtOH and left at r.t. for several days. Then the EtOH extract was decanted and evaporated. The residue was extracted with $CHCl_3$ (4×). The extract (70 g) was fractionated by CC (silica gel, petroleum ether/acetone 9:1, 8:2, 7:3, and 6:4). *Sarcodonin G* (**5**; 12 mg) and *scabronine F* (**6**; 10 mg) were obtained by prep. TLC ($CHCl_3$/MeOH 9:1) of *Fr. 1* (eluted with petroleum ether/acetone 6:4). *Fr. 2* (eluted with petroleum ether/acetone 6:4) was submitted for further purification by reversed-phase CC

(*RP-8*, MeOH/H_2O 8:2): *scabronine G* (**1**; 10 mg), *scabronine H* (**2**; 200 mg), *allocyathin* B_2 (**3**; 50 mg), and *sarcodonin A* (**4**, 150 mg).

Scabronine G (= rel-*(3a*R,*5a*R,*6S*,*9*R,*10a*R*)-2,3,3a,4,5,5a,6,9,10,10a-Decahydro-8-(hydroxymethyl)-1-(2-hydroxy-1-methylethyl)-3a,5a-dimethylcyclohept[e]indene-6,9-diol*; **1**): Red oil. $[\alpha]_D^{20} = -18.53$ ($c = 0.2$, $CHCl_3$). UV ($CHCl_3$): 207.8, 233.6. IR (KBr): 3430, 2931, 2867, 1713, 1452, 1375, 1315, 1275, 1177, 1116, 1027, 714. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 463.2471 ($C_{27}H_{36}O_5Na^+$; calc. 463.2460).

Scabronine H (= rel-*(3a*R,*5a*R,*6S*,*9S*,*10a*R*)-2,3,3a,4,5,5a,6,9,10,10a-Decahydro-8-(hydroxymethyl)-1-(2-hydroxy-1-methylethyl)-3a,5a-dimethylcydohept[e]indene-6,9-diol*; **2**): Yellow powder. M.p. 165 – 168° (MeOH). $[\alpha]_D^{20} = -9.08$ ($c = 0.3$, MeOH). UV (MeOH): 204.4, 228.0. IR (KBr): 3421, 2934, 2867, 1719, 1452, 1373, 1315, 1272, 1176, 1114, 1016, 712. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 463.2464 ($C_{27}H_{36}O_5Na^+$; calc. 463.2460).

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