An Unusual Nitrogenous Terphenyl Derivative from Fruiting Bodies of the Basidiomycete *Sarcodon scabrosus*

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A new nitrogenous metabolite with *p*-terphenyl core, sarcodonin δ (**1**), together with two known *p*-terphenyl metabolites (**7, 8**), was isolated from the fruiting bodies of the basidiomycete *Sarcodon scabrosus*. The structures of these compounds were elucidated by spectral and chemical methods.

Key words: Sarcodon scabrosus, Basidiomycete, Sarcodonin δ, *p*-Terphenyl Derivatives

Introduction

Occurrence of *p*-terphenyl derivatived in Basidiomycete fungi has been investigated by Jaegers *et al.* [1] who reported the isolation of leucomelone and protoleucomelone from fruiting bodies of *Boletopsis leucomelaena*. Then, Takahashi *et al.* [2] reported three other *p*-terphenyl derivatives showing an inhibitory effect on 5-lipoxygenase from the same species. Later, curtisians A-Q as new free radical scavengers from *Paxillus curtisii* [3 – 5], leucomentin-5 and -6 from *Paxillus panuoides* [6, 7], kynapcin-12 from *Polyzellus multiplex* [8], ganbajunins A-G from *Thelephora ganbajun* [9, 10], aurantiotinin A from *Thelephora aurantiotincta* [11], thelephantins A-H from the same species [12 – 13], and thelephorin A from *Thelephora vialis* [14] were reported.

Sarcodon scabrosus is a mushroom belonging to the family Thelephoraceae and has a strongly bitter taste. Diterpenoids, sarcodonins A-H, scabronines A-F, scabronines L and M, have previously been isolated from this mushroom as the bitter principles [15 – 18]. These diterpenoids show stimulating activity towards the nerve growth factor (NGF)-synthesis *in vitro*. Two

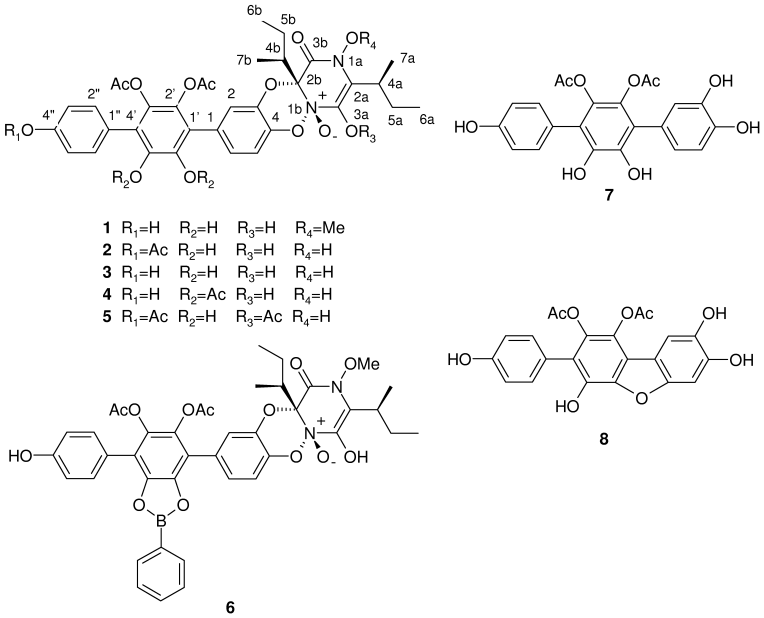

Fig. 1. The structures of compunds **1 – 8**.

previously unreported antibiotic metabolites, 2',3',4"-triacetoxy-3,4,5',6'-tetrahydroxy-*p*-terphenyl and 2', 3'-diacetoxy-3,4,5',6',4"% -pentahydroxy-*p*-terphenyl, and a nitrogenous terphenyl derivative (sarcodonin, **2**), have been isolated from the basidiomycete *Sarcodon leucopus*, respectively [19, 20]. Sarcodonin (**2**), an unique polyhydroxy-*p*-terphenyl pyrazinediol dioxide conjugate, is moderately active against KB and P-388 cells [20]. Recently, three compounds of this type (**3**–**5**, sarcodonins α, β, and γ, respectively) with different acetylation patterns of the hydroxyl groups were isolated from fruiting bodies of the basidiomycete *Sarcodon leucopus* [21].

In continuation of 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 a new compound, named sarcodonin δ (**1**), including two known compounds (**7, 8**).

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 afford **1**. High-resolution ESI-MS (pos.) indicated a molecular formula of $C_{35}H_{38}N_2O_{13}$ ($M+Na^+$ at *m/z* 717.2258, calcd. for $C_{35}H_{38}N_2O_{13}Na$ 717.2271) with 18 degrees of unsaturation.

Preliminary ^1H and ^{13}C NMR analysis of **1**, as well as IR and UV data, showed marked similarity with *p*-terphenyl derivative **2** [20]. Complete high-field NMR analysis (400 MHz for ^1H), including two-dimensional experiments (^1H, ^1H-COSY, ROESY, HSQC, HMBC) was performed on **1**. These data, aided by DEPT, allowed the complete signal assignments reported in the Experimental Section (see Table 1). The NMR data for **1** were assigned largely by comparison with the data reported for **2** [20].

From comparison of the proton ^1H and carbon ^{13}C NMR spectra of **1** with those of protoleucomelone obtained in previous work [19], it was apparent that **1** contained a *p*-terphenyl nucleus bearing oxygenated functions at the same positions as in **7**. In addition to the *p*-terphenyl resonances, **1** showed signals attributable to an aliphatic moiety, and to two acetoxy groups located at C-2' and C-3'. This was confirmed by the HMBC correlations of the oxygen-bearing quaternary carbons with the acetate methyl groups. When **1** was treated with phenylboronic acid and the reaction mixture was examined directly by FAB-MS (neg.) in glycerol, an ion observed at *m/z* 872 ($[M+92]^-$) indicated the formation of a phenylboronate **6**, which implied the presence of a pair of vicinal OH groups.

Table 1. ^1H and ^{13}C NMR (CD_3OD) data of sarcodonin δ (**1**).

	δC	δH	^1H-^1HCOSY selected	HMBC selected
1	124.7			H-5
2 (CH)	119.9	7.03 (d, 1.8)		H-6
3	142.6			H-5
4	141.9			H-6
5 (CH)	117.5	7.12 (d, 6.5)	H-6	
6 (CH)	126.6	7.05 (dd, 1.8, 6.5)	H-5	H-2
1'	122.4			H-6
2'	134.7			$COCH_3$
3'	134.8			$COCH_3$
4'	124.7			H-2", 6"
5'	131.6			
6'	131.6			
1"	124.7			H-3", 5"
2", 6" (CH)	132.5	7.16 (d, 8.8)	H-3", 5"	
3", 5" (CH)	116.7	6.84 (d, 8.8)	H-2", 6"	
4"	142.7			H-2", 6"
2a	158.9			H-5a, 7a
3a	158.2			H-4a
4a (CH)	34.7	3.16 (m)	H-5a, 7a	H-6a
5a (CH_2)	27.8	1.40 (m, H-β), 1.54 (m, H-α)	H-4a, 6a	H-7a
6a (CH_3)	12.4	0.87 (t)	H-5a	H-4a
7a (CH_3)	16.3	1.06 (d, 7.0)	H-4a	H-5a
2b	94.5			H- 5b, 7b
3b	160.5			H-4b
4b (CH)	43.9	2.48 (m)	H-5b, 7b	H-6b
5b (CH_2)	25.9	1.52 (m, H-β), 1.90 (m, H-α)	H-4b, 6b	H-7b
6b (CH_3)	12.9	1.00 (t)	H-5b	H-4b
7b (CH_3)	13.7	1.19 (d, 6.8)	H-4b	H-5b
OCH_3	67.9	3.99 (s)		
$COCH_3$	170.5, 170.6 20.3, 20.1	1.93 (s), 1.92 (s)		

One methoxyl, four methyls, two methylenes, two methines, and four quaternary carbons were assigned to the aliphatic part of the molecule on the basis of the DEPT experiment. ^1H, ^1H-COSY and HMBC correlations established the presence of two distinct units, both possessing a 3-methylpentane skeleton. The unambiguous assignment of the four quaternary carbons C-2α, C-3α, C-2β, and C-3β was based on the integrated analysis of HMBC. The deshielded chemical shifts of these quaternary carbons indicated their direct connection with one (or more) heteroatoms. The three lower-field signals (δ 160.5, 158.9, 158.2) were clearly due to sp^2-hybridized carbons, while the

fourth (δ 94.5) was evidently a strongly deshielded sp^3-hybridized carbon [20]. No HMBC correlations of aromatic quaternary carbons with the protons of methoxyl were observed, so methoxyl was connected directly with *N*-1a.

Comparison of the physicochemical properties with the reported data allowed to identify compounds **7** and **8**, isolated from the same fungus, as 2, 3'-diacetoxy-3,4,4",5',6,-pentahydroxy-*p*-terphenyl [20], and 1,2-diace -toxy-3-(4-hydroxyphenyl)-4,7,8-trihydroxy-dibenzofuran [2].

Experimental Section

General

Optical rotation was determined on a Horiba Sepa-300 polarimeter (Horiba, Tokyo, Japan). ^{1}H, ^{13}C NMR and two-dimensional NMR spectra were recorded on the Bruker DRX-500 (Karlsruhe, Germany) at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR, chemical shifts δ in ppm to TMS as internal standard and coupling constants in Hz. Mass spectra were measured with a VG Autospec 3000 mass spectrometer (VG, England) and API Qstar Pulsar (Applied Biosystems, Foster City, USA), respectively. Infrared (IR) spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer (Bio-Rad, Richmond, CA, USA) in KBr pellets.

Material

Column chromatography was carried out on silica gel (200 – 300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). TLC was carried out on plates precoated with silical gel F_{254} (Qingdao Marine Chemical Ltd., Qingdao, P. R. China).

Fungal material

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

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 *in vacuo*. The residue was extracted with $CHCl_3$ (4 times). The extract (70 g) was fractionated by column chromatography (silica gel, eluted with petroleum ether/acetone 9:1, 8:2, 7:3, 6:4, v/v). The fraction eluted by petroleum ether/acetone (6:4, v/v) was submitted for further purification by Sephadex LH-20 chromatography eluted with MeOH to give two main fractions. Compound **1** (30 mg) was obtained from fraction 1. Fraction 2 was submitted for further purification by reverse phase column chromatography (RP-8, MeOH/H_2O 6:4) to afford compounds **7** (6 mg), **8** (4 mg).

*Reaction of **1** with phenylboronic acid.* **1** (2 mg) in acetone (1 ml) and phenylboronic acid (2 mg) were refluxed at 50 °C for 6 h, and then evaporated. The crude residue was directly measured with FAB-MS (neg.): *m/z* 872 (100) $[M+92]^-$.

*Sarcodonin δ (**1**).* Red amorphous powder. $[\alpha]_D^{20}$ −2.71° (*c* 0.1, MeOH). – UV/vis (MeOH): λ_{max} (lgε) = 260 nm (4.79), 205 nm (4.26). – IR (KBr): $\tilde{\nu}$ = 3433 (OH), 2971, 2878, 1775, 1612, 1523, 1458, 1370, 1276, 1215, 1021, 820 cm^{-1}. – ^{1}H and ^{13}C NMR (CD_3OD) see Table 1. – HR-ESI-MS: *m/z* 717.2258 (calcd. for $C_{35}H_{38}N_2O_{13}Na$ 717.2271). FAB-MS (neg.): *m/z* (%) = 693 (100) $[M-1]^-$, 650 (5) $[M-1\text{-}CH_3CO]^-$.

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