A Novel Sesquiterpene from the Basidiomycete *Boletus calopus*

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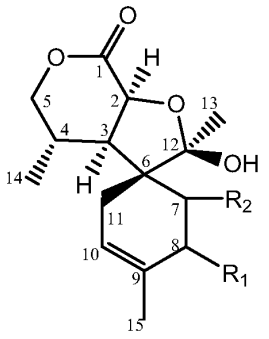
A novel sesquiterpene named cyclopinol (**1**) together with two related known compounds, cyclocalopin A (**2**) and *O*-acetylcyclocalopin A (**3**), has been isolated from the basidiomycete *Boletus calopus*. The structure of compound **1** was established on the basis of spectral methods (MS, IR, 1D, and 2D NMR experiments).

Key words: *Boletus calopus*, Basidiomycete, Sesquiterpene

Introduction

Mushrooms have proved to be a rich source of secondary metabolites with unusual structures as well as interesting biological activities. Despite their potential for drug development, few bioactive metabolites have been reported from mushrooms as compared with higher plants and microbes. We have screened biologically active and chemically novel compounds from the higher fungi of Yunnan Province in China [1 – 3]. In the course of our continuing research, a novel sesquiterpene named cyclopinol (**1**), together with two related known compounds cyclocalopin A (**2**) and *O*-acetylcyclocalopin A (**3**), has been isolated from the basidiomycete *Boletus calopus* (Fig. 1). *Boletus* spp. are known to produce a variety of amino acid analogs such as 2-amino-4-hydroxypentanoic acid [4] and 2-amino-4-methyl-5-hexenoic acid [5], prenylated phenolics such as asiaticusin [6], peptaibols such as boletusin [7], benzoquinones such as boviquinone [8], thelephoric acid precursors such as cyclovariegatin [9], polyene dicarboxylic acids such as boletocrocin [10], macrolide phenolics such as ornatipolide [11], and hydroxylated pulvinic acids such as xerocomic acid and pulviquinone [12]. In this paper, we present the isolation and structure determination of the title compound **1**.



1 R_1 = •-OH R_2 = •-OH
2 R_1 = =O R_2 = •-OH
3 R_1 = =O R_2 = •-OAc

Fig. 1. The structures of compounds **1** – **3**.

Results and Discussion

Compound **1** was obtained as colorless needles and had the molecular formula of $C_{15}H_{22}O_6$ as determined by analysis of ^{1}H and ^{13}C NMR spectral data (Table 1), which was verified by HRMS ((+)-ESI (*m/z* = 303.1202, calcd. 303.1208 for $[M-H_2O+Na]^+$), requiring five degrees of unsaturation. The ^{13}C NMR and DEPT spectra (Table 1) revealed the presence of one ester carbonyl group at δ = 170.5, two sp^2 carbons at δ = 134.2 and 123.0, three oxygenated methine carbons at δ = 74.3, 75.5 and 80.1, one oxygenated methylene carbon at δ = 72.4, one oxygenated quaternary carbon at δ = 116.6, as well as three methyl (δ = 17.1, 21.9, 24.4), one methylene (δ = 26.3), two methine (δ = 29.4, 49.0) carbons, and one quaternary carbon (δ = 56.0). These observations, in combination with the molecular formula, indicated the presence of three OH groups and three rings in **1**.

Comparison of the ^{13}C NMR data of **1** with those of cyclocalopin A (**2**) [13] implied that they shared the same planar structure except for the absence of a carbonyl group signal at δ = 199.3 and the presence of the signal of an oxygenated methine assigned to C-8 (δ = 80.1) in **1**. This assignment was in accord with the observation of a remarkable upfield shift of C-10 from

Position	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
1		170.5 (s)		172.6 (s)
2	5.27 (d, 9.7 Hz)	75.5 (d)	4.59 (d, 9.7 Hz)	73.7 (d)
3	2.76 (dd, 9.7, 9.7 Hz)	49.0 (d)	2.21 (m)	46.2 (d)
4	1.97 (m)	29.4 (d)	2.21 (m)	30.6 (d)
5β	4.13 (dd, 11.0, 3.5 Hz)	72.4 (t)	4.07 (dd, 11.0, 2.9 Hz)	72.2 (t)
5α	3.88 (dd, 11.0, 11.0 Hz)		3.83 (dd, 11.0, 11.0 Hz)	
6		56.0 (s)		56.3 (s)
7	4.75 (d, 4.2 Hz)	74.3 (d)	4.27 (s)	76.0 (d)
8	4.29 (d, 4.2 Hz)	80.1 (d)		199.3 (s)
9		134.2 (s)		133.0 (s)
10	5.59 (m)	123.0 (d)	6.72 (m)	144.5 (d)
11α	2.90 (br d, 20.8 Hz)	26.3 (t)	2.72 (ddm, 20.8, 5.7 Hz)	27.8 (t)
11β	2.06 (br d, 20.8 Hz)		2.55 (dd, 20.8, 2.6 Hz)	
12		116.6 (s)		107.6 (s)
13	1.60 (s)	24.4 (q)	1.67 (s)	21.7 (q)
14	0.91 (d, 6.4 Hz)	17.1 (q)	0.77 (d, 6.4 Hz)	16.4 (q)
15	1.72 (m)	21.9 (q)	1.85 (m)	15.1 (q)

Table 1. ^{1}H NMR and ^{13}C NMR data for **1** (400 MHz, C_5D_5N) and **2** (500 MHz, $CDCl_3$); δ in ppm.

δ = 144.5 in **2** to δ = 123.0 in **1**, and further confirmed by cross-peaks from H-8 [δ = 4.29 (d, J = 4.2 Hz)] to C-7, C-9, C-10, and C-15 in the HMBC spectrum of **1** (Fig. 2).

The relative stereochemistry of C-2, C-3, C-4, and C-13 in **1** was the same as that of **2** on the basis of coupling constants, NMR data, and ROESY correlations. The correlations of H-2 with H-8 and of H-3 with H-7 in the ROESY spectrum indicated that H-7 and H-8 possessed α,α-orientations. In the light of the evidence mentioned above, the structure of **1** was assigned to be as shown in Fig. 1.

The structures of the known compounds **2** and **3** were identified as cyclocalopin A (**2**) and *O*-acetylcyclocalopin A (**3**) by spectroscopic analysis and comparison of the spectral data with those reported previously [13] (see Experimental Section and Tab. 1).

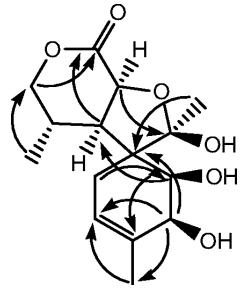


Fig. 2. Key HMBC correlations of compound **1**.

Experimental Section

General

Optical rotation was measured with a Horiba SEPA-300 polarimeter. IR spectra were obtained on a Bruker Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers in C_5D_5N or $CDCl_3$ with TMS as an internal standard. EI-MS were recorded on a VG Autospec-3000 spectrometer, while HRMS ((+)-ESI) were recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200 – 300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10 % H_2SO_4 in ethanol.

Fungal material

The basidiomycete *B. calopus* was collected at Ailao Mountain in the Yunnan Province, China, in July 2001 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited at the Herbarium of Kunming Institute of Botany, CAS.

Extraction and isolation

The fresh fruiting bodies (5 kg) were extracted with 95 % EtOH at r. t. The extracts were evaporated to dryness under reduced pressure and partitioned between water and EtOAc. The concentrated organic layer was dried to give 40 g of extract, which was subjected to silica gel column chromatography, eluting with a $CHCl_3$-MeOH gradient system to give 8 fractions. Fraction II (100 : 1, v/v) was passed through Sephadex LH-20 using $CHCl_3$-MeOH (1 : 1, v/v) as eluent to afford subfractions B_1–B_5. Subfraction B_3 was further purified on a Sephadex LH-20 column ($CHCl_3$-MeOH, 1 : 1, v/v) and by recrystallization from MeOH to yield compound **1** (7.8 mg). Fraction III (50 : 1, v/v) was chromatographed repeatedly over silica gel columns eluted with

petroleum ether/acetone (5 : 1 – 3 : 2, v/v) to afford compounds **2** (10 mg) and **3** (9.3 mg).

*Cyclopinol (**1**)*

Colorless oil. – $[\alpha]_D^{25.4}$ = −0.7 (*c* = 0.3, MeOH). – IR (KBr): ν = 3439, 2959, 2925, 2855, 1742, 1632, 1382, 1107, 1050, 984 cm^{-1}. – ^1H NMR and ^{13}C NMR see Table 1. – FAB MS: *m/z* = 281 $[M+H–H_2O]^+$. – HRMS ((+)-ESI): *m/z* = 303.1202 (calcd. 303.1208 for $C_{15}H_{20}O_5Na$, $[M-H_2O+Na]^+$).

*Cyclocalopin A (**2**)*

Colorless crystals, m. p. 153 °C. – $[\alpha]_D^{26.5}$ = −4.6 (*c* = 0.28, MeOH). – IR (KBr): ν = 3448, 2956, 2926, 2852, 1716, 1676, 1642, 1384, 1135, 1092, 1012 cm^{-1}. – ^1H NMR and ^{13}C NMR see Table 1. – EI MS (70 eV): *m/z* (%) = 296 (1.6) $[M^+]$, 279 (7), 278 (19) $[M^+–H_2O]$, 254 (9), 238 (1), 237 (18), 219 (10), 208 (9), 191 (6), 179 (11), 161 (100), 151 (20), 147 (16), 121 (36). – HRMS ((+)-ESI): *m/z* = 319.1148 (calcd. 319.1157 for $C_{15}H_{20}O_6Na$, $[M+Na]^+$).

*O-Acetylcyclocalopin A (**3**)*

Colorless crystals, m. p. 176 °C. – $[\alpha]_D^{15.1}$ = −11.7 (*c* = 0.14, MeOH). – IR (KBr): ν = 3385, 2989, 2926, 2854, 1727, 1692, 1450, 1413, 1384, 1371, 1232, 1153, 1087, 927, 861 cm^{-1}. – ^1H NMR (400 Mz, C_5D_5N): δ = 0.68 (d, *J* = 6.5 Hz, 3H, H-14), 1.68 (s, 3H, H-13), 1.79 (m, 3H, H-15), 2.22 (s, 3H, H-17), 2.41 (m, 1H, H-4), 2.58 (dd, *J* = 10.5, 10.5 Hz, 1H, H-3), 2.72 (dd, *J* = 20.8, 2.6 Hz, 1H, H-11β), 2.90 (ddq, *J* = 20.8, 4.3, 1.5 Hz, 1H, H-11α), 3.89 (dd, *J* = 11.2, 11.2 Hz, 1H, H-5α), 4.05 (dd, *J* = 11.2, 3.4 Hz, 1H, H-5β), 5.00 (d, *J* = 10.5 Hz, 1H, H-2), 5.85 (s, 1H, H-7), 6.62 (m, 1H, H-10). – ^{13}C NMR (100 Mz, C_5D_5N): δ = 171.9 (C-1), 74.0 (C-2), 48.0 (C-3), 31.0 (C-4), 72.0 (C-5), 56.0 (C-6), 78.1 (C-7), 193.8 (C-8), 133.7 (C-9), 144.9 (C-10), 28.9 (C-11), 107.1 (C-12), 20.9 (C-13), 16.5 (C-14), 15.3(C-15), 170.1 (C-16), 21.3 (C-17). – EI-MS (70 eV): *m/z* (%) = 320 (0.5) $[M^+–H_2O]$, 278 (3), 236 (18), 219 (6), 207 (20), 161 (100), 133 (14). – HRMS ((+)-ESI): *m/z* = 361.1278 (calcd. 361.1263 for $C_{17}H_{22}O_7Na$, $[M+Na]^+$).

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