A New Meroterpenoid Pigment from the Basidiomycete *Albatrellus confluens*

by **Xiao-Long Yang**[a])[b]), **Cheng Qin**[c]), **Fei Wang**[a])[b]), **Ze-Jun Dong**[a]), and **Ji-Kai Liu***[a])

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China (phone: +86 871 5216327; fax: +86 871 5150227; e-mail: jkliu@mail.kib.ac.cn)

[b]) Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China

[c]) Kunming Medical College, Kunming 650204, P. R. China

A new farnesyl phenol named (+)-(*R*)-grifolinone C (**1**) has been isolated from the fruiting bodies of the basidiomycete *Albatrellus confluens*. (+)-(*R*)-Grifolinone C (**1**), a dimeric meroterpenoid, is accompanied by albatrellin (**2**), grifolinone B (**3**), grifolin (**4**), and grifolinone A (**5**). Albatrellin (**2**) exhibited cytotoxic activity against HepG2 human lung carcinoma cells with IC_{50} value of 1.55 μg ml^{-1}. The structures were established on the basis of spectral evidence (IR, 1D- and 2D-NMR, and MS analyses).

Introduction. – About 14,000 species of mushrooms are now known in the world. They comprise vast and yet largely untapped sources of powerful new pharmaceutical products. Yunnan Province, southwest of China, is one of the areas with the richest and diverse bioresources in the world, based on its unique geo-environment, diverse geomorphology, and three differentiations of climate. We have been interested in the biologically active substances present in untapped and diverse source of mushrooms from Yunnan Province [1].

A previous chemical investigation of *Albatrellus* sp., which belongs to the Scutigeraceae family, has revealed that they are abundant sources of grifolin and its isomer neogrifolin [2]. These compounds and their derivatives possess interesting biological activities: anti-oxidative [2], antimicrobial [3–5], tyrosinase inhibition [6], promotion of melanin synthesis by B16 melanoma cells [7], activity on human and rat vanilloid receptor 1 (VR1) [8][9], inhibition of tumor-cell growth by inducing apoptosis *in vitro* [10], TNF-α inhibition, anti-HIV activities, and inhibition of nitric oxide production in RAW 264.7 cells [2][5][11].

As a continuing research of bioactive metabolites from *Albatrellus* sp. [3][10][12–14], the chemical constituents of the fruiting bodies of *Albatrellus confluens* were investigated. This report deals with the isolation, structure elucidation, and preliminary biological-activity evaluation of these meroterpenoids.

Results and Discussion. – Compound **1** was obtained as a red powder. The molecular formula of **1** was determined to be $C_{44}H_{60}O_5$ on the basis of HR-ESI-TOF-MS ($[M-H]^-$ at m/z 667.4373; calc. for $C_{44}H_{59}O_5$: 667.4362). This was confirmed by the ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*), in which the signals of all H- and C-

Table 1. *^{13}C-NMR Data* (100 MHz, CD_3OD) *of Compound* **1**. δ in ppm.

Position	δ(C)	Position	δ(C)	Position	δ(C)	Position	δ(C)
1	188.7 (*s*)	12	27.6 (*t*)	1′	114.3 (*s*)	12′	27.9 (*t*)
2	141.6 (*s*)	13	125.6 (*d*)	2′	157.0 (*s*)	13′	125.5 (*d*)
3	144.0 (*s*)	14	136.0 (*s*)	3′	114.5 (*s*)	14′	135.8 (*s*)
4	185.4 (*s*)	15	40.9 (*t*)	4′	135.4 (*s*)	15′	41.0 (*t*)
5	154.0 (*s*)	16	27.8 (*t*)	5′	109.8 (*d*)	16′	27.5 (*t*)
6	137.0 (*s*)	17	125.4 (*d*)	6′	153.7 (*s*)	17′	125.4 (*d*)
7	13.0(*q*)	18	132.0 (*s*)	7′	20.0 (*q*)	18′	132.0 (*s*)
8	23.5 (*t*)	19	26.0 (*q*)	8′	23.8 (*t*)	19′	26.0 (*q*)
9	122.1(*d*)	20	17.8 (*q*)	9′	124.5 (*d*)	20′	17.8 (*q*)
10	137.0 (*s*)	21	16.3 (*q*)	10′	135.3 (*s*)	21′	16.3 (*q*)
11	40.9 (*t*)	22	16.1 (*q*)	11′	41.0 (*t*)	22′	16.1 (*q*)

Table 2. *^{1}H-NMR Data* (400 MHz, CD_3OD) *of Compound* **1**. δ in ppm, J in Hz.

Position	δ(H)	Position	δ(H)
7	1.77 (*s*)	5′	6.22 (*s*)
8	3.14 (*d*, J=7.2)	7′	1.85 (*s*)
9	5.16 (*t*, J=7.2)	8′	3.33 (*d*, J=7.8)
11	1.97–1.88 (*m*)	9′	5.23 (*t*, J=7.8)
12	2.02–2.07 (*m*)	11′	1.97–1.88 (*m*)
13	5.05–5.07 (*m*)	12′	2.02–2.07 (*m*)
15	1.97–1.88 (*m*)	13′	5.05–5.07(*m*)
16	2.02–2.07 (*m*)	15′	1.97–1.88 (*m*)
17	5.05–5.07 (*m*)	16′	2.02–2.07(*m*)
19	1.65 (*s*)	17′	5.05–5.07 (*m*)
20	1.57 (*s*)	19′	1.65 (*s*)
21	1.75 (*s*)	20′	1.57 (*s*)
22	1.56 (*s*)	21′	1.75 (*s*)
		22′	1.56 (*s*)

atoms were present. The presence of two partial structures was confirmed by comparisons of their spectral data with those of compounds **3** and **4** (*Fig. 1*). For the first partial structure, ^{13}C-NMR data of compound **1** from C(8) to C(22) were identical with those of compound **4** [2], while ^{13}C-NMR data from C(1) to C(7) were identical with those of compound **3** [11], indicating the presence of a 1,4-benzoquinone moiety carrying an intact farnesyl chain, as well as a Me and an OH substituent in compound **1**. This was further confirmed by the absorption bands at 651 and 283 nm in the UV, and at 3406 cm^{-1} (OH), and 1650 and 1638 cm^{-1} (C=O) in the IR spectra. The quinone is linked to C(8), based on HMBC correlations (*Fig. 2*) between H–C(8) and C(1) (δ(C) 188.7(*s*)), C(5), and C(6). The second partial structure provided NMR-spectral data very similar to those of grifolin from C(1) to C(22). Finally, the linkage between two monomers was clearly detected from C(2) to C(3′) by the HMBC correlations: H–C(7) and C(3′); H–C(7′) and C(2). All of the open-chain C=C bonds were (*E*)-configurated, as deduced by comparison of the NMR data with those reported in [2][11]. Accordingly, compound **1** was determined to be a dimeric meroterpenoid.

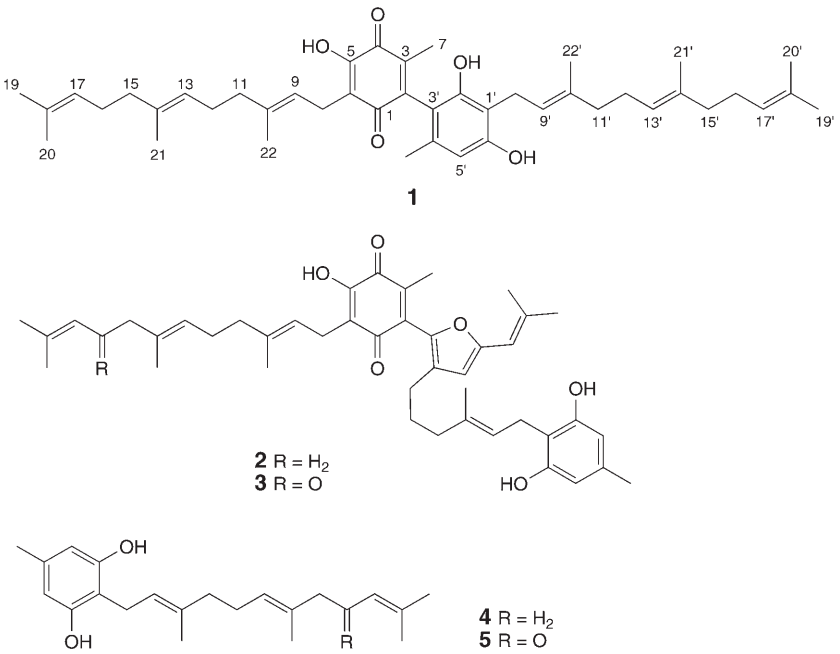


Fig. 1. *Structures of compounds* **1**–**5**

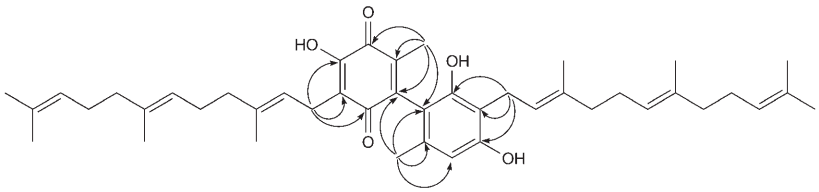


Fig. 2. *Key HMBC correlations of compound* **1**

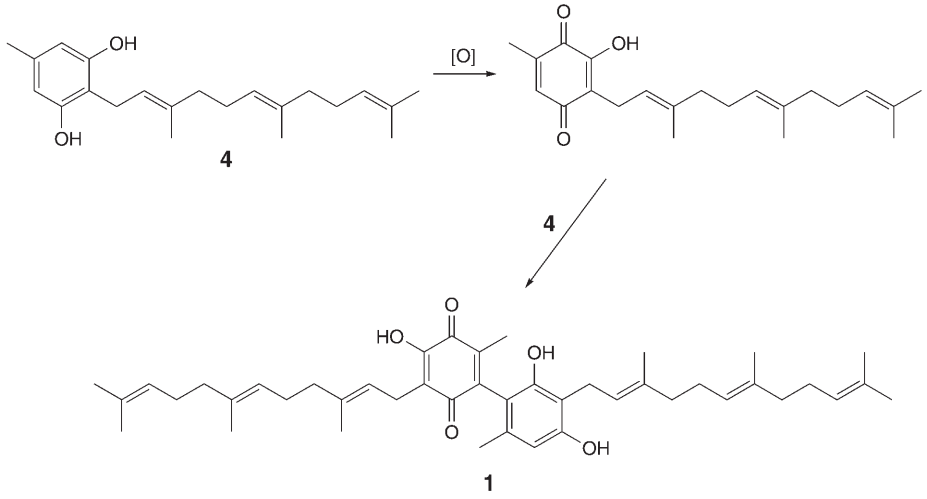
Comparison of the $[\alpha]_D$ value of compound **1** ($[\alpha]_D^{26.6} = +26.1$ ($c = 0.2$, acetone)) with that of the related compound (+)-(*R*)-2,4,2′,4′-tetrahydroxy-6,6′-dimethylbiphenyl ($[\alpha]_D^{25} = +39.4$ ($c = 0.5$, acetone)) indicated the (*R*)-configuration for compound **1** with the same chiroptical properties [15]. Finally, compound **1** was determined to be (+)-(*R*)-grifolinone C (**1**; for the systematic name, *cf. Exper. Part*).

Compound **2** was obtained as a purple oil. HRESI-TOF-MS of **2** indicated a molecular formula of $C_{44}H_{56}O_6$ ($[M-H]^-$ at *m*/*z* 679.3982, calc. for $C_{44}H_{55}O_6$: 679.3998) with 17 degrees of unsaturation. The ^{1}H- and ^{13}C-NMR data of **2** were very similar to those of **3** [11], suggesting that compound **2** possesses the same substitution pattern. The distinct difference between **2** and **3** is that the oxo group at C(16) of **3** (δ(C) 200.4 (*s*, C(16))) is absent in **2** (δ(C) 26.5 (*t*, C(16)); δ(H) 2.05 (*m*)). The HMBC spectra of compound **2** exhibited the following key correlations: H–C(7) → C(2), C(3), C(4), C(3′); H–C(8) → C(1), C(5), C(6); H–C(7′) → C(3′), C(5′); H–C(15′) →

C(14′), C(16′), C(21′); H–C(17′)→C(15′), C(16′), C(18′). The linkage between the two monomers was clearly detected from C(2) to C(3′) by the HMBC correlations, *i.e.*, H–C(7) and C(3′). All of the open-chain C=C bonds were (*E*)-configurated as deduced by comparison of the NMR data with those reported in [2][11]. Finally, compound **2** was determined to be albatrellin, which has been published as a new compound very recently by *Steglich et al.* [16].

The structure of (+)-(*R*)-grifolinone C (**1**) suggests its formation from two components, one derived from grifolin (**4**) and the other from the oxidized product of grifolin (*Scheme*).

Scheme. *Proposed Biosynthetic Route to Compound* **1**



Preliminary biological assay of (+)-(*R*)-grifolinone C (**1**) and albatrellin (**2**) showed that albatrellin (**2**) exhibited cytotoxic activity against HepG2 human lung carcinoma cell with an IC_{50} value of 1.55 μg ml^{-1} (positive control DDP with an IC_{50} value of 0.28 μg ml^{-1} and (+)-(*R*)-grifolinone C (**1**) no activity under the same conditions).

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, *Qingdao Marine Chemical Inc.*, P. R. China) and *Sephadex LH-20* (*Amersham Biosciences,* Sweden); fractions were monitored by TLC, and spots were visualized by heating silica-gel plates sprayed with 10% H_2SO_4 in EtOH. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} in nm (log ε). IR Spectra: *Tensor 27* with KBr pellets; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Bruker AV-400* and *DRX-500* spectrometers with TMS as an internal standard. MS: *VG Autospec-3000* spectrometer; in *m*/*z*; HR-ESI-TOF-MS: *API QSTAR Pulsar 1* spectrometer.

Mushroom Material. The fresh fruiting bodies of *A. confluens* were collected at Nujiang, Yunnan Province, China in July 2006 and identified by *J.-K. L.*, Kunming Institute of Botany, Chinese Academy of Sciences (CAS).

Cell Line and Culture. HepG2 cell line was grown in RPMI-1640 medium (GIBCO) supplemented with 10% heat-inactivated bovine serum, 2 nM L-glutamine, 10^5 IU/l penicillin, 100 mg/l streptomycin, and 10 mM *Hepes*, pH 7.4. Cell was kept at 37° in a humidified 5% CO_2 incubator.

Cell Growth Inhibition Assay. Growth inhibition of compounds **1** and **2** on HepG2 cell was measured by the microculture tetrazolium (MTT; see below) assay with minor modifications [17–19]. Briefly, adherent HepG2 cell was seeded into 96-well microculture plates and allowed to adhere for 24 h before drug addition, while suspended cells were seeded just before drug addition. HepG2 cell line was exposed to compounds **1** and **2** at 0.01, 0.1, 1, 10, and 100 mg/l concentrations for different periods (adherent cell for 72 h, suspended cell for 48 h), and each concentration was tested in triplicate. At the end of exposure, 20 µl of 5 g/l MTT (= 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide; *Sigma Chemical Co.*) was added to each well, and the plates were incubated for 4 h at 37°; then, 'triplex soln. (10% SDS-5% i-BuOH-0.012M HCl)' was added and the plates were incubated for 12–20 h at 37°. The optical density (OD) was read on a plate reader at a wavelength of 570 nm. Media and DMSO control wells, in which compounds **1** and **2** were absent, were included in all the experiments in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition } [\%] = [OD_{control} - OD_{treated}/OD_{control}] \times 100\%$$

Compounds **1** and **2** against HepG2 human lung carcinoma cell were expressed as IC_{50} values and calculated by LOGIT method.

Statistical Analysis. All experiments were repeated three times, and the results are expressed as means ± SEM. The data were analyzed by *t* test using SPSS software (12.0 versions). The differences were considered statistically significant at $p > 0.05$.

Extraction and Isolation. The fresh fruiting bodies of *A. confluens* (3 kg) were firstly extracted three times with acetone at r.t. After removal of the solvent by evaporation, the residues (200 g) were suspended in H_2O, and then extracted with AcOEt. The AcOEt extract was evaporated to give a brown extract (70 g), which was subjected to CC (silica gel; $CHCl_3$/MeOH (100 : 0, 98 : 2, 95 : 5, 90 : 10, 80 : 20, 50 : 50 (*v*/*v*)) to afford six *Fractions* (*A*–*F*). The *Fr. A* (7 g) eluted with $CHCl_3$/MeOH (100 : 0) was further purified by repeated CC (silica gel; petroleum ether/acetone, 90 : 10), then subjected to repeated *Sephadex LH-20* chromatography ($CHCl_3$/MeOH 1 : 1) to afford compound **4** (30 mg). Further purification by prep. TLC (petroleum ether/acetone, 4 : 1), repeated *Sephadex LH-20* chromatography ($CHCl_3$/MeOH 1 : 1), and prep. TLC (petroleum ether/AcOEt 6 : 1) afforded compound **1** (17 mg). The part eluted with petroleum ether/acetone 80 : 20 was subjected to repeated CC (silica gel; petroleum ether/AcOEt 5 : 1), *Sephadex LH-20* chromatography ($CHCl_3$/MeOH 1 : 1), and prep. TLC (petroleum ether/acetone 3 : 1) to afford compound **3** (18 mg). The *Fr. B* (15 g) eluted with $CHCl_3$/MeOH 98 : 2 was further subjected to repeated CC (silica gel; petroleum ether/AcOEt 10 : 1, 5 : 1), prep. TLC (petroleum ether/acetone 2 : 1), and *Sephadex LH-20* chromatography ($CHCl_3$/MeOH 1 : 1) to afford compounds **5** (4 mg) and **2** (17 mg).

(+)-(*R*)-*Grifolinone C* (=*2-{2,4-Dihydroxy-6-methyl-3-[(2*E*,7*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]phenyl}-5-hydroxy-3-methyl-6-[(2*e*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]cyclohexa-2,5-diene-1,4-dione*; **1**). Red powder. $[\alpha]_D^{26.6} = +26.1$ (*c* = 0.2, acetone). UV (MeOH): 651 (2.51), 283 (4.12), 206 (4.96). IR (KBr): 3406 (OH), 1650 (C=O), 1638 (C=O), 1608 (C=C). ^{1}H- and ^{13}C-NMR (CD_3OD): see *Tables 1* and *2*. FAB-MS: 668 (M^-). HR-ESI-TOF-MS: 667.4373 ($C_{44}H_{59}O_5$; calc. 667.4362).

This project was supported by grants from the *Chinese Academy of Sciences* (KSCX1-YW-R-24, KSCX1-YW-R-15) and the *Natural Science Foundation of Yunnan Province* (2005C0052M, 2005C0011R and 2000C0001P).

REFERENCES

[1] J. K. Liu, *Heterocycles* **2002**, *57*, 157.
[2] M. Nukata, T. Hashimoto, I. Yamamoto, N. Iwasaki, M. Tanaka, Y. Asakawa, *Phytochemistry* **2002**, *59*, 731.

[3] D. Q. Luo, H. J. Shao, H. J. Zhu, J. K. Liu, *Z. Naturforsch., C* **2005**, *60*, 50.
[4] L. Zechlin, M. Wolf, W. Steglich, T. Anke, *Liebigs Ann. Chem.* **1981**, *12*, 2099.
[5] T. Hashimoto, D. N. Quan, M. Nukada, Y. Asakawa, *Heterocycles* **2005**, *65*, 2431.
[6] H. Misasa, Y. Matsui, H. Uehara, H. Tanaka, M. Ishihara, H. Shibata, *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1660.
[7] H. Kawagishi, A. Tanaka, K. Sugiyama, H. Mori, H. Sakamoto, Y. Ishoguro, K. Kobayashi, M. Uramoto, *Phytochemistry* **1996**, *42*, 547.
[8] V. Hellwig, R. Nopper, F. Mauler, J. Freitag, J. K. Liu, Z. H. Ding, M. Stadler, *Arch. Pharm.* **2003**, *336*, 119.
[9] V. Szallasi, T. Biro, T. Szabo, S. Modarres, M. Petersen, A. Klusch, P. M. Blumberg, J. E. Krause, O. Sterner, *Br. J. Pharmacol.* **1999**, *126*, 1351.
[10] M. Ye, J. K. Liu, Z. X. Lu, Y. Zhao, S. F. Liu, L. L. Li, M. Tan, X. X. Weng, W. Li, Y. Cao, *FEBS Lett.* **2005**, *579*, 3437.
[11] D. N. Quan, T. Hashimoto, Y. Arakawa, C. Kohchi, T. Nishizawa, G. I. Soma, Y. Asakawa, *Bioor. Med. Chem.* **2006**, *14*, 164.
[12] F. Wang, D. Q. Luo, J. K. Liu, *J. Antibiot*. **2005**, *58*, 412.
[13] W. M. Yang, J. K. Liu, C. Qing, Y. D. Liu, Z. H. Ding, Z. Q. Shen, Z. H. Chen, *Planta Med*. **2003**, *69*, 715.
[14] Z. H. Ding, Z. J. Dong, J. K. Liu, *Helv. Chim. Acta* **2001**, *84*, 259.
[15] M. Hans, S. Willi, *Chem. Ber.* **1968**, *101*, 1510.
[16] B. Koch, W. Steglich, *Eur. J. Org. Chem.* **2007**, 1631.
[17] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[18] M. C. Alley, D. A. Scudiero, A. Monks, M. L.Hursey, M. J. Czerwinski, D. L. Fine, *Cancer Res.* **1988**, *48*, 589.
[19] J. J. Zhou, X. F.Yue, J. X. Han, W. Y. Yang, *Chin. J. Pharm*. **1993**, *24*, 455.

Received April 30, 2007