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CHEMICAL CONSTITUENTS FROM THE FRUITING BODIES OF THE BASIDIOMYCETE *ALBATRELLUS* SP.

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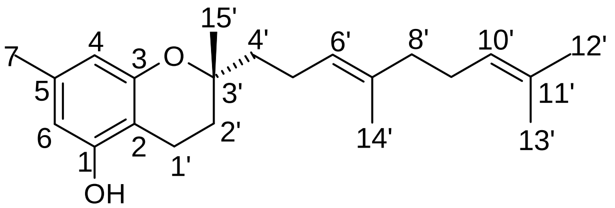
Abstract – A new prenylated orcinol derivative, namely albatrellutin [3'*R*-(7',11'-dimethylnona-6',10'-dienyl)-3',7-dimethyl-chroman-1-ol] (**1**), together with eight known compounds was isolated from ethyl acetate extract of the fruiting bodies of the basidiomycete *Albatrellus* sp. The structure of this new compound was completely elucidated using a combination of 2D NMR techniques (COSY, NOESY, HMQC and HMBC) and HR-EI-MS analyses. The known compounds were elucidated by comparison of their spectroscopic and physical data with those reported in the literatures.

For millennia, mushrooms have been valued by humankind as an edible and medical resource. The chemical constituents produced by macrofungi have been investigated in the past decades.[1,2] A variety of pharmacologically active molecules associated with diverse structural types of terpenoids, steroids, sphingolipids, perylenequinones, polysaccharides, and heterocyclic compounds, which exhibited antitumor, anti-inflammatory, cytotoxic, enzyme inhibitory, antiviral, neuritogenic, antibiotic and immunomodulating effects, have been discovered.[1-4]

Albatrellus sp (Albatrellophorales). is an inedible mushroom that mainly grows under broadleaf and coniferous trees. The aqueous extract of *Albatrellus* sp. was shown to have antitumor effects on mice-bearing carcinoma *S*-180 in Chinese folk medicine.[5] To our knowledge, however, the chemical work of this mushroom has not been previously reported. As part of our ongoing study of biologically active metabolites from higher mushrooms,[6,7] further investigation was carried out to search for the bioactive compounds from this mushroom *Albatrellus* sp., which resulted in the isolation of nine compounds. In this paper, we wish to report the isolation and structural characterization of a novel prenylphenol derivative, albatrellutin (**1**), together with eight known compounds including three prenylphenols, two pyrazinol derivatives and three steroids from specimens of *Albatrellus* sp. and their neurite outgrowth effect on PC12 cells.

Albatrellutin (**1**) was isolated as colorless oil. It was assigned as a molecular formula of $C_{22}H_{32}O_2$, with seven degrees of unsaturation, on the basis of its HREIMS at *m/z* 328.2232 $[M]^+$ (calcd. 328.2238) and NMR data. The IR spectrum indicated the presence of hydroxyl (3379 cm^{-1}) and aromatic ring (1636, 1517, and 1466 cm^{-1}) functionality. Detailed analysis of the ^1H, ^{13}C NMR and HSQC spectroscopic data only disclosed 22 carbon signals, which were classified as five methyl groups, six methylenes, four methines, seven quaternary carbons (three of which are oxygenated ones [δ 154.5, 154.3, and 75.6], ten sp^2 carbons including two trisubstituted olefinic units (δ 124.0 (d, C-6'), 135.1(s, C-7'), 124.3(d, C-10'), 131.3(s, C-11'). These data accounted for all ^1H and ^{13}C NMR resonances and required the compound to contain bicycles including one benzene ring. The ^1H NMR spectrum of **1** displayed two *meta*-coupled aromatic protons at δ 6.26 (1H, *d*, $J=2.1$ Hz, H-6) and 6.18 (1H, *d*, $J=2.1$ Hz, H-4) and one aromatic methyl proton at δ 2.16 (CH_3-7), suggesting the presence of a 1,2,3,5-tetrasubstituted aromatic ring [δ 108.8 (C-6, d), 101.6 (C-4, d), 154.5 (C-1, s), 154.3 (C-3, s), 138.2 (C-5, s), and 112.2 (C-2, s)].



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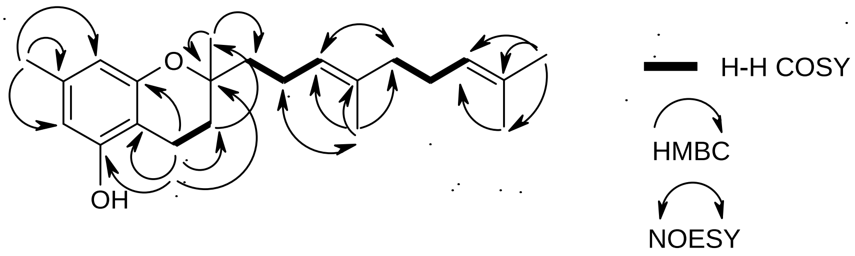
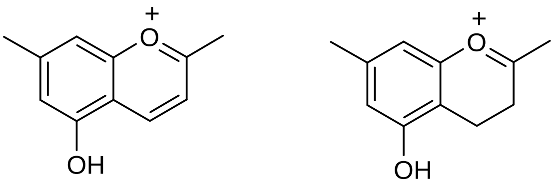


Figure 1. Selected 2D NMR correlations of compound **1**.



m/z 175 *m/z* 177

Figure 2. Major EIMS ion peaks of compound **1**.

In the HMBC experiments (Figure 1), the aromatic methyl proton at δ 2.16 (CH_3-7) showed that long-range correlations with the carbon signals at δ 101.6 (C-4, d), 138.2 (C-5, s), and 108.8 (C-6, d); likewise, two aromatic proton signals at δ 6.18 (H-4) and 6.26 (H-6) showed that correlations with the carbon signal at δ 19.2 (C-7, q), indicating the aromatic methyl to be positioned at C-5; the proton signal of the methyl proton at δ 1.28 (3H, s, H-15′) showed correlations with the carbon signals at δ 75.6 (C-3′) and 39.6 (C-4′); δ 1.68-1.86 (H-2′) showed cross-peaks with C-3′ and C-15′ (δ 24.0); and δ 2.52-2.55 (H-1′) showed cross-peaks with the carbon signals at δ 154.5 (C-1), 112.2 (C-2), and 154.3 (C-3). From this evidence, the presence of a 1,5-disubstituted chromane ring (benzopyran moiety), and one hydroxyl connected to C-1 was postulated, as supported by two characteristic fragments at *m/z* 175 (46% of relative intensity) as well as at *m/z* 177 due to loss of a side chain ($C_{11}H_{21}$), which comprises a geranyl group ($C_{10}H_{17}$), from a molecular ion in the EIMS of **1** (Figure 2). The presence of the side chain was deduced from the ^{1}H-^{1}H COSY correlations of H_2-4′ to H-6′ and H_2-8′ to H-10′, HMBC correlations of H_{13}-14′ to C-6′, C-7′, and C-8′, H_{13}-12′ to C-10′, C-11′, and C-13′, and NOESY correlations of H_2-5′ to H_3-14′ and H-6′ to H_2-8′ (Figure 1). The ^{1}H and ^{13}C NMR spectral data of **1** were very similar to those of confluentin (**2**),[8] except for two methylenes [δ 22.2 (C-1′), 31.0 (C-2′)] in **1** instead of two olefinic methines in **2**, thus indicating compound **1** to be the hydrogenation product of **2**. The NOESY spectrum established that the C-6′/7′ double bond of **1** is *E*, due to the correlations of H-5′ and CH_3-14′, H-9′, and H-8′. The absolute stereochemistry at the asymmetric carbon (C-3′) of **1** was determined as *R* by comparison with the specific optical rotations of **2** and daurichromene D (=13′-hydroxyconfluentin) isolated from the plant *Rhododendron dauricum*[9]: ($[\alpha]_D^{20}$ -2° (c 1.0, $CHCl_3$) for **1**; $[\alpha]_D^{20}$ -1° (*c* 0.75, $CHCl_3$) for **2**; $[\alpha]_D^{20}$ -26.0 (*c* 0.10, MeOH) for daurichromene D[9]). Consequently, the structure of **1** was established as 3'*R*-(7',11'-dimethylnona-6',10'-dienyl)-3',7-dimethyl-chroman-1-ol, which was named albatrellutin (**1**).

The known compounds, confluentin (**2**),[8] grifolin (**3**),[9-12] neogrifolin (**4**),[8,12] emeheterone (**5**),[13] 3,6-dibenzyl-2-hydroxy-5-methoxy-pyradine (**6**),[14] ergosterol (**7**),[6] ergosterol peroxide (**8**)[7] and ergosta-4,6,8 (14), 22-tetraen-3-one (**9**),[7] were also isolated from the same fungus. Their structures were identified by comparison of their physicochemical and spectroscopic data (IR, EIMS, NMR) with the published data.

The effects of compounds **1-9** on neurite outgrowth from PC12 cells were evaluated according to previously reported procedures.[15] Only compounds **1** and **2** had very weak neurite outgrowth effect on PC12 cells in the presence of NGF (20 ng/mL) at concentrations of 50 μM.

The prenylated orcinol natural products exist widely in terrestrial plants,[9,12,16] while compounds of this group are shown to be rarely found in macrofungi.[8,17,18] Interestingly, two prenylated orcinol derivatives, confluentin (**2**) and grifolin (**3**), have been isolated from the Chinese medicinal plant *Rhododendron dauricum*.[9] Grifolin (**3**) was also isolated from the plant *Peperomia galioides*.[12] In addition, grifolin and its related metabolites also showed various activities in biological systems. For instance, confluentin, grifolin and neogrifolin were found to possess the CNS activities (modulation of vanilloid and dopamine D1 receptors).[8] Grifolin, a potential antitumor natural product from the funus *Albatrellus confluens*, inhibits tumor cell growth by inducing apoptosis *in vitro*;[17] neogrifolin was a tyrosinase inhibitor.[18] Additionally, confluentin and grifolin inhibited compound 48/80-induced histamine release from rat peritoneal mast cells.[9] Compound **6** was reported to promote melanin synthesis by B16 melanoma cells.[14]

EXPERIMENTAL

General experimental procedures. Melting points were obtained on an XRC-1 apparatus and uncorrected. Optical rotations were measured on a Perkin Elmer Model 341 polarimeter. The NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 instruments with TMS as an internal standard. Mass spectra were recorded on a VG Auto Spec-3000 spectrometer (Micromass, UK) and Thermo Finnigan LCQ Advantage. The IR spectra were obtained in KBr pellets with a Bio-Rad FTS-135 infrared spectrophotometer.

Column chromatography (CC) was performed over silica gel (200–300 mesh, Qingdao Marine Chemical

Ltd., Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Silica gel 60 F254 TLC plates (Qingdao Marine Chemical Ltd., Qingdao, China) and RP-18 F_{254} TLC plates (Merck, Darmstadt, Germany) were used for analyses. Spots were visualized by spraying with 10% H_2SO_4 in EtOH, followed by heating.

Fungal material. The fresh fruiting bodies of *Albatrellus* sp. were collected in the summer of 2001 from the rural area near the city of Kunming, Yunnan, China and identified by Ms. X. H. Wang, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan. A voucher specimen is deposited at the Herbarium of the Kunming Institute of Botany, China.

Extraction and Isolation. The dried and powdered fruiting bodies (2 kg) of *Albatrellus* sp. were extracted three times with MeOH at rt. The combined solvent was concentrated in vacuo to give a brown extract. The crude extract was partitioned between H_2O and petroleum ether, and then AcOEt successively. The AcOEt-soluble extract (47.7 g) was separated by CC on silica gel with petroleum ether/acetone (100:0-0:100, v/v) gradient elution to give 7 fractions.

Fraction 3 was subjected to silica gel CC using petroleum ether/$CHCl_3$ (100:0-1:1, v/v). Subfraction eluted with petroleum ether/$CHCl_3$ (5:1) gave compound **2** (23 mg). Fraction 4 contained a crude crystalline, which was purified by Sephadex LH-20 CC (MeOH) to afford **7** (13 mg); the remaining parts were fractionated by a silica gel CC using petroleum ether/acetone (15:1 -5:1, v/v) to provide three pure compounds **9** (4 mg; petroleum ether/acetone, 15:1), **6** (15 mg; petroleum ether/acetone,10:1), and **3** (137 mg; petroleum ether/acetone, 5:1).

Fraction 5 produced crude crystals, which were separated by a silica gel column with $CHCl_3$/acetone (95:5, v/v), followed by purification on Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) column providing **5** (54 mg); the remaining parts were chromatographed over a silica gel column with $CHCl_3$–MeOH (99:1-80:20, v/v) to furnish three pure compounds: **1** (25 mg; $CHCl_3$–MeOH, 99:1), **4** (248 mg; $CHCl_3$–MeOH, 95:5), and **8** (11 mg; $CHCl_3$–MeOH, 90:10).

Albatrellutin [3'*R*-(7',11'-dimethylnona-6',10'-dienyl)-3',7-dimethyl-chroman-1-ol] (1): colorless oil; $[\alpha]_D^{20}$ -2° (c 1.0, $CHCl_3$); IR(KBr) ν_{max}: 3379, 1636, 1517, 1466, 1076 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.26(1H, d, H-6, J=2.1Hz), 6.18(1H, d, H-4, J=2.3Hz), 5.08-5.14(2H, m, H-6', H-10'), 2.52-2.55(2H, m, H-1'), 2.16(3H, s, H-7), 2.04-2.13(4H, m, H-5', H-9'), 1.97-1.99(2H, m, H-8'), 1.68-1.86(4H, m, H-2', H-4'), 1.66(3H, s, H-12'), 1.60(3H, s, H-13'), 1.60(3H, s, H-14'), 1.28(3H, s, H-15'). ^{13}C-NMR(100 MHz, $CDCl_3$) δ: 154.5(s, C-1), 112.2(s, C-2), 154.3(s, C-3), 101.6(d, C-4), 138.2(s, C-5), 108.8(d, C-6), 19.2(q, C-7), 22.2(t, C-1'), 31.0(t, C-2'), 75.6(s, C-3'), 39.6(t, C-4'), 19.4 (t, C-5'), 124.0(d, C-6'), 135.1(s, C-7'), 39.1(t, C-8'), 26.6(t, C-9'), 124.3(d, C-10'), 131.3(s, C-11'), 25.6(q, C-12'), 17.6(q, C-13'), 15.9(q, C-14'), 24.0(q, C-15'). EI-MS (70 eV, %) *m/z*: 329$[M+H]^+$(1), 328$[M]^+$, 313$[M-CH_3]^+$ (2), 191$[M-C_{10}H_{17}]^+$(3), 177$[M-C_{11}H_{19}]^+$(10), 175$[M-C_{11}H_{19}-2H]^+$(46), 149$[M-C_{13}H_{23}]^+$ (6), 137$[M-C_{14}H_{24}+H]^+$(100), 121[M-$C_{15}H_{27}$]$^+$(8), 69[C_5H_9]$^+$(11); HREIMS at *m/z* 328.2232 [M]$^+$ (calcd. $C_{22}H_{32}O_2$ 328.2238).

Neurite outgrowth-promoting activity. The effects of compounds on neurite outgrowth from PC12 cells were evaluated in the presence or absence of NGF (20 ng/mL) at concentrations of 20 and 50 *M*m according to previously reported procedures.[15]

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