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(±)-Ganoapplanin, a Pair of Polycyclic Meroterpenoid Enantiomers from *Ganoderma applanatum*

Lei Li,[†,‡,⊥] Huan Li,[§,‡,⊥] Xing-Rong Peng,[†] Bo Hou,[†,‡] Mu-Yuan Yu,[†,‡] Jin-Run Dong,[†,‡] Xiao-Nian Li,[†] Lin Zhou,[†] Jian Yang,*[,§] and Ming-Hua Qiu*[,†,‡]

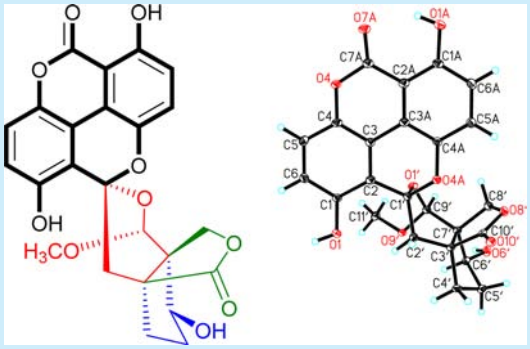
[†]Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‡]Graduate University of the Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[§]Key Laboratory of Animal Models and Human Disease Mechanisms, and Ion Channel Research and Drug Development Center, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, People's Republic of China

Supporting Information

ABSTRACT: (±)-Ganoapplanin (**1**), a pair of novel meroterpenoid enantiomers featuring an unprecedented dioxaspirocyclic skeleton constructed from a 6/6/6/6 tetracyclic system and an unusual tricyclo-[4.3.3.0$^{3',7'}$]dodecane motif, were isolated from *Ganoderma applanatum*. Its structure and absolute configurations were determined by spectroscopic analyses, X-ray crystallography, and ECD (electronic circular dichroism calculations). A plausible biogenetic pathway, involving a key Gomberg−Bachmann reaction, was also proposed for (±)-**1**. Biological studies showed that (±)-**1** and its enantiomers exhibited different inhibitory activities on T-type voltage-gated calcium channels.



Ganoderma applanatum, also called the almighty herb "Lingzhi" (Ganoderma spp.), is one of the most well-known traditional Chinese herbs. It is a valuable medicinal fungus widely distributed in the world[1] and is used to treat various human diseases such as bronchitis, hepatitis, hypertension, tumor diseases, and immunological disorders in Chinese traditional medicine.[2] Modern pharmacological studies have revealed antitumor, immunomodulating, antibacterial, anti-inflammatory, and analgesic activities of *G. applanatum* extracts.[3] The remarkable curative effects of this fungus have attracted great attention in recent decades. Previous research on the chemical constituents of this fungus has led to the isolation of triterpenoids, sterols, meroterpenoids, and other classes of compounds from the liposoluble fraction,[4] some of which are also found to have significant bioactivities including antimicrobial, antitumor, anti-inflammation, antifibrotic, aldose reductase inhibitor, and so on.[5]

Due to fascinating structures and important biological activities, meroterpenoids from *Ganoderma* have attracted broad interest from both natural products and synthetic chemists since 2000. Previous phytochemistry investigations on several species of this genus have led to the isolation of a number of meroterpenoids with diverse biological activities, for example ganomycin I,[6] Cochlearoids A−E,[7] (±)-ganodilactone,[8] and applanatumols A and B,[5c] to name a few. There have also been great efforts toward the synthesis of the meroterpenoids.[9] However, apart from these inspiring reports, the presence and value of meroterpenoids in *Ganoderma* remain largely unexplored.

Our research group has already reported a number of meroterpenoids from *Ganoderma*.[10] Continuing the search for structurally diverse and biologically interesting metabolites from this genus, we turned our focus toward *G. applanatum*. This effort resulted in the isolation of (±)-ganoapplanin (Figure 1), characterized by an unprecedented dioxaspirocyclic skeleton constructed from a 6/6/6/6 tetracyclic system and an unusual tricyclo[4.3.3.0$^{3',7'}$]dodecane motif. It is particularly noteworthy that (±)-**1** possesses a biphenyl motif, a spirocyclic

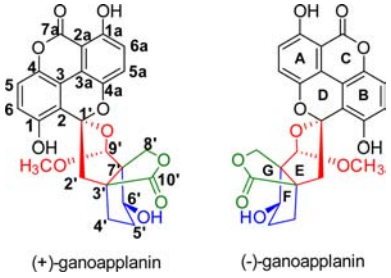


Figure 1. Structures of (+)-**1** (1′*R*,3′*S*,6′*S*,7′*R*,9′*S*) and (−)-**1** (1′*S*,3′*R*,6′*R*,7′*S*,9′*R*).

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ring system, and a bridged ring system. These features are reported for the first time in the meroterpenoid family.

Herein, we describe the isolation and structural elucidation of (±)-**1**, a hypothetical biogenetic pathway for (±)-**1**, and its ability to inhibit T-type voltage-gated calcium channels.

(±)-Ganoapplanin (**1**), obtained as a gray-white powder, had the molecular formula $C_{24}H_{20}O_{10}$ with 15 degrees of unsaturation based on analysis of its HRESIMS ($[M + Na]^+$, m/z 491.0944; calcd 491.0954). Its IR spectrum showed the presence of hydroxyl (3432 cm^{-1}) and ester carbonyl (1706 cm^{-1}). The ^1H NMR spectrum (Table 1) of (±)-**1** showed four

Table 1. ^1H (800 MHz) and ^{13}C (200 MHz) NMR Data of (±)-1 in C_5D_5N (δ in *ppm*, J in Hz)

position	δ_C, type	δ_H, mult (J, Hz)
1a	156.2, C	
2a	104.4, C	
3a	119.6, C	
4a	140.6, C	
5a	124.4, CH	7.46, d (8.8)
6a	117.4, CH	7.13, d (8.8)
7a	164.4, C	
1	152.0, C	
2	113.7, C	
3	116.5, C	
4	142.6, C	
5	118.0, CH	7.27, d (8.9)
6	120.0, CH	7.28, d (8.9)
1′	98.9, CH	
2′	37.6, CH_2	3.30, d (14.0), 4.47, d (11.4)
3′	54.3, C	
4′	36.6, CH_2	2.54, m, 1.87, m
5′	33.4, CH_2	2.20, m, 1.86, m
6′	74.9, CH	5.12, dd (9.1, 6.1)
7′	49.3, C	
8′	67.5, CH_2	5.53, d (9.0), 4.48, d (9.3)
9′	100.7, CH	4.89, s
10′	184.0, C	
OCH_3	55.8, CH_3	3.36, s
OH		10.84, brs
OH		12.59, brs

doublets (δ_H 7.46, d, J = 8.8 Hz, H-5a; δ_H 7.13, d, J = 8.8 Hz, H-6a; δ_H 7.28, d, J = 8.9 Hz, H-6 and δ_H 7.27, d, J = 8.9 Hz, H-5) in the downfield region, suggesting the presence of two 1,2,3,4-tetrasubstituted benzene rings. The ^{13}C NMR and DEPT spectra (Table 1) of (±)-**1** showed 24 carbon resonances ascribed to one methoxyl, four methylenes (one oxygenated), six methines (four aromatic/olefinic and two oxygenated), and 13 quaternary carbons (two ester carbonyls, eight aromatic/olefinic, one oxygenated, and two aliphatic). The above-mentioned data indicated that (±)-**1** was an aromatic meroterpenoid.

The planar structures of (±)-**1** were constructed mainly based on the results of 2D NMR experiments (Figure 2). The presence of a cyclopenta[*c*]furolactone moiety was established by the observed $^1H-^1H$ COSY correlations of H-4′/H-5′/H-6′, together with the HMBC correlations of H-4′/C-3′, C-7′, C-6′, C-5′, and C-10′; of H-5′/C-3′, C-7′, C-6′, and C-4′; of H-6′/C-3′ C-4′, C-5′, C-8′, and C-10′; and of H-8′/C-10′,C-3′, C-6′, and C-7′.[11] Meanwhile, the E ring (Figure 1) was defined by the HMBC correlations of OCH_3 (δ_H 3.36)/C-2′, C-9′; of H-9′/C-1′, C-3′, C-7′, and C-8′; and of H-2′/C-1′, C-3′, C-7′, and C-10′. The formation of ring E allowed (±)-**1** to possess a rotated-door moiety in part I. Furthermore, the HMBC correlations of H-5/C-3, C-4, and C-1 and of H-6/C-2, C-4, and C-3 suggested the presence of a benzoyl group, and the key HMBC correlations of H-2′/C-3 and H-6/C-1′ were also observed, which clearly indicated that the rotated-door moiety was connected to the benzoyl group via C-1′. Thus, sufficient evidence was provided to confirm the existence of the part I fragment in (±)-**1**.

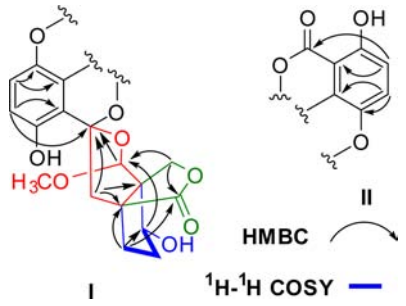

Figure 2. Key HMBC and $^1H-^1H$ COSY correlations of (±)-**1**.

Additionally, the remaining 7 carbons of (±)-**1** were representative of part II on the basis of the characteristic 1D NMR data (Table 1) and a series of HMBC correlations of H-5a/C-3a, C-4a, C-1a, and C-3 and of H-6a/C-2a, C-4a, C-1a, and C-7a (δ_C 164.40). Thus, apart from 13 degrees of unsaturation occupied by part I and part II, the remaining two degrees of unsaturation indicated that two additional rings existed in (±)-**1**. Because of the absence of relevant 2D NMR data, the planar structure of (±)-**1** could not be defined accurately. However, with the help of the single crystal (Figure 3) of (±)-**1** obtained from the C_5H_5N/CH_3OH solvent system, we finally confirmed the skeleton of (±)-**1**.

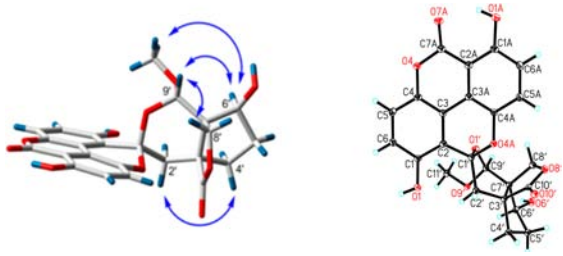

Figure 3. Key ROESY correlations and X-ray crystallographic of (±)-**1**.

Due to its structural complexity, the relative configurations at the chiral centers in (±)-**1** were not assigned by analysis of ROESY experiments (Figure 3). The X-ray diffraction analysis of (±)-**1** showed that (±)-**1** had the space group $C2/c$, indicating the racemic nature of the crystal. However, (±)-ganoapplanin was found to be optically active, with the optical rotation $[\alpha]^{25}_D$ −15.4 (0.08, MeOH), so (±)-**1** was most likely a mixture of two enantiomers in unequal amounts.[12] Subsequent HPLC of (±)-**1** on a chiral stationary phase led to the separation of two enantiomers, (+)-**1** and (−)-**1**, with a ratio of 46:54 (Figure S12), which had opposite CD curves and optical rotations. Furthermore, the ECD spectrum (Figure 4) calculated for (1′*R*,3′*S*,6′*S*,7′*R*,9′*S*)-**1** agreed well with that measured for (+)-**1**. Thus, the absolute configurations of (±)-**1** were unambiguously assigned.

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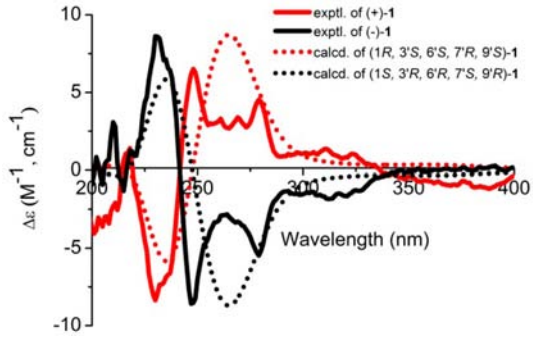
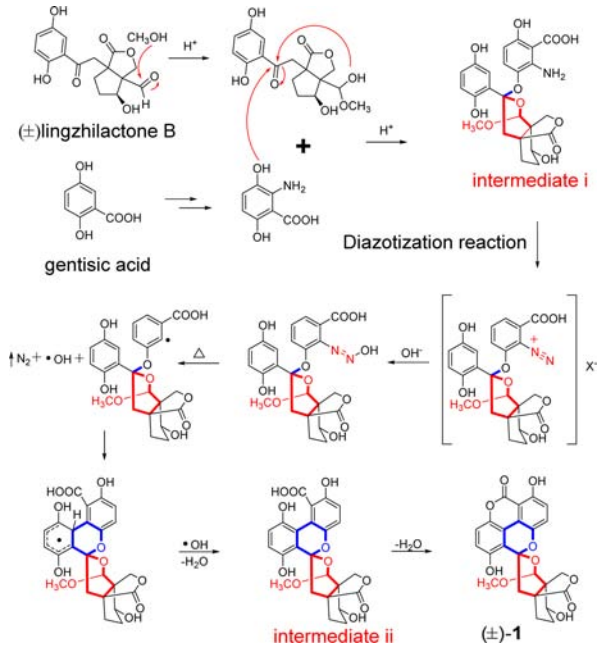


Figure 4. Calculated and experimental ECD spectra of (+)-**1** and (−)-**1** at the TDDFT/B3LYP/6-311++G(d,p) level.

As indicated in Figure 2, the structures of (±)-ganoapplanin (**1**) appeared to be composed of two parts including a polycyclic meroterpenoid fragment (I) and a 2,5-dihydroxybenzoyl fragment (II). On the basis of a prudent analysis, we proposed that (±)-**1** was derived from 2,5-dihydroxybenzoic acid[13] and (±)-lingzhilactone B,[14] which was derived by a route that was a hybrid of the shikimic acid and mevalonic acid. In the biosynthetic pathway (Scheme 1), (±)- lingzhilactone B

Scheme 1. A Plausible Biogenetic Pathway for (±)-1



undergoing two successive nucleophilic additions would give a key intermediate i, which would further be transformed into intermediate ii through a diazotization reaction and a Gomberg−Bachmann reaction.[15] Finally, (±)-**1** was obtained via an intermolecular esterification. Of note, in addition to (±)-**1**, a series of meroterpenoids from *Ganoderma* were isolated as a pair of enantiomers,[11] which made us deduce that biogenetic mechanisms for creating distinct meroterpenoids enantiomers might be widely expressed in this genus.

Ganoderma species are well-known for their ability to promote health and longevity and have been utilized as an adjuvant for central nervous system (CNS) disorders in traditional Chinese medicines.[16] Low voltage-activated T-type calcium currents are observed in many central and peripheral neurons and are involved in various CNS disorders such as insomnia, neuropathic pain, and Parkinson's disease.[17] Thus, T-type voltage-gated calcium channels (TTCCs) have been regarded as an attractive therapeutic target.[18] On the basis of the medicinal effects of *Ganoderma* and the physiological and pathological functions of TTCCs, we decided to test the effects of (±)-**1** from *G. applanatum* on TTCCs.

TTCCs were expressed heterologously in HEK293T cells, and the effects of various compounds on TTCC currents were examined by whole-cell patch clamp. The results (Figure 5)

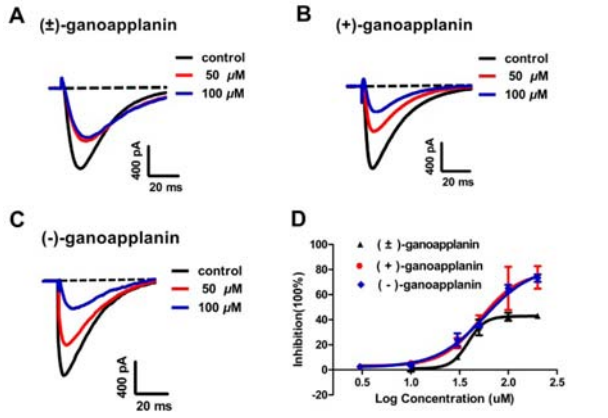


Figure 5. Inhibitory effects of (±)-ganoapplanin, (+)-ganoapplanin, and (−)-ganoapplanin on TTCCs. (A−C) Representative TTCC current traces elicited by 50 ms depolarization to −10 mV from a holding potential (HP) of −80 mV in the absence (control) and presence of different concentrations of the indicated compounds. Zero current is indicated by dashed lines. (D) Dose−response relationship of inhibition on TTCCs by the indicated compounds. Data are presented as mean ± SEM $n \geq 5$ for each concentration. The solid curves represent fits to the Hill equation.

showed that (±)-**1** inhibited TTCCs with an apparent IC_{50} at 36.6 μM. Interestingly, this inhibition was partial, with a maximum inhibition of only 43% (Figure 5D), suggesting that (±)-**1** altered channel gating rather than blocking the pore. Since the enantiomers of substances often have distinct biological activities, for example *R*-/*S*-thalidomide, *R*-/*S*-warfarin, and *R*-/*S*-ketamine,[19] we examined the effects of the enantiomers of (±)-**1** on TTCCs. (+)-**1** and (−)-**1** also inhibited TTCCs, with an IC_{50} of 51.5 and 56.4 μM respectively, which suggested that both enantiomers exerted similar inhibitory effects. The maximum inhibition by (+)-**1** and (−)-**1** was >80%, indicating that the enantiomers were more efficient than (±)-**1** in suppressing TTCC activity. The different potencies and maximum levels of inhibition of (±)-**1** and its enantiomers were noteworthy, but the precise molecular basis for these differences was unclear and remained to be elucidated. Finally, to exclude the possibility that the effects of the compounds were related to cellular toxicity, the same experiments were performed on mock-transfected cells, and all three compounds showed no detrimental effects.

In summary, (±)-ganoapplanin A (**1**) with an unprecedented dioxaspirocyclic skeleton constructed from a 6/6/6/6 tetracyclic system and an unusual tricyclo[4.3.3.0^{3,7}]dodecane motif was isolated from *G. applanatum* for the first time. Notably, the novel molecule provides unique insights into the biosynthetic pathway of meroterpenoids, whose formation is involved in rearrangement reactions of aromatic free radicals. Last but not least, the inhibitory effects of (±)-ganoapplanin and its

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enantiomers are in line with some of the medicinal effects of *Ganoderma*, which improves the possibility of finding more TTCC inhibitors in these species.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03064.

Crystallographic data for **1** (CIF)

1D and 2D NMR spectra of **1**, data for single-crystal X-ray diffraction of **1**, physical constants and spectral data of **1**, detailed experimental procedure, computational and bioassay methods (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: jianyang@mail.kiz.ac.cn.
*E-mail: mhchiu@mail.kib.ac.cn.

ORCID

Ming-Hua Qiu: 0000-0001-9658-1636

Author Contributions

⊥L.L. and H.L. contributed equally.

Notes

The authors declare no competing financial interest.

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