

Benzopyran-4-one Derivatives from the Fungus *Ganoderma applanatum*

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

^b Graduate University of Chinese Academy of Sciences, Beijing 100049, P. R. China

Reprint requests to Prof. Dr. Ji-Kai Liu. Fax: 0086-871-5150227. E-mail: jkliu@mail.kib.ac.cn

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Two new benzopyran-4-one derivatives, applanatins A (**1**) and B (**2**), were isolated from the fruiting bodies of the fungus *Ganoderma applanatum* (Ganodermataceae), along with one known analogue, ganoderma aldehyde (**3**), as well as four known ganoderenic acids. The structures of the new compounds were elucidated on the basis of extensive spectroscopic analysis. The partial assignments of the NMR spectra of **3** were also revised.

Key words: *Ganoderma applanatum*, Benzopyran-4-one Derivatives, Applanatins A and B

Introduction

The fungus *Ganoderma lucidum* is a well-known crude Chinese drug that has been used clinically in China, Japan and Korea for a long time. More than 140 highly oxygenated lanostane-type triterpenoids have been isolated from the fruiting bodies, mycelia and spores of *G. lucidum*, some of them exhibiting a very broad spectrum of biological activities and pharmacological functions [1]. Other *Ganoderma* spp. have also been used in traditional Chinese, Japanese and Korean medicines for the treatment of cancer, hypertension, chronic bronchitis, diabetes, and arteriosclerosis and as a tonic or sedative. In the case of *G. applanatum* (= *Elfvigia applanata*), a series of highly oxygenated lanostane-type triterpenes, such as ganoderenic acids [2, 5, 7], ganoderic acids [2, 7], applanoxidic acids [3, 4] and elfvingic acids [6], have been isolated in addition to a benzopyran-4-one derivative, *i.e.* ganoderma aldehyde [5]. In a continuation of our studies on the bioactive principles from higher fungi in Southwestern P. R. China [9–12], we have conducted a chemical study of *G. applanatum*. Two new benzopyran-4-one derivatives, applanatins A (**1**) and B (**2**), were isolated from the fruiting bodies of this fungus, along with one known analogue, ganoderma aldehyde (**3**), as well as four known triterpenes, ganoderenic acids A (**4**), B (**5**), D (**6**) and G (**7**) (Fig. 1).

In this paper, we describe the isolation and structure elucidation of the new compounds **1–2**, and the revision of the partial NMR spectral assignments of **3**.

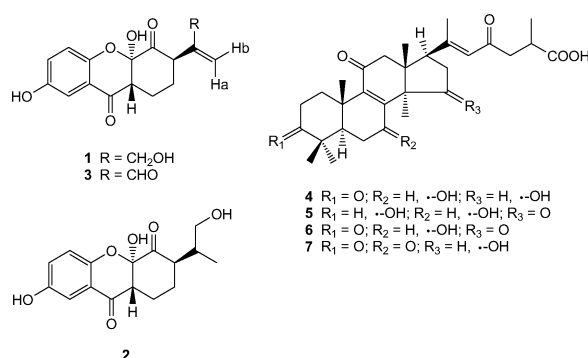


Fig. 1. Molecular formulas of compounds **1–7** isolated from *G. applanatum*.

Results and Discussion

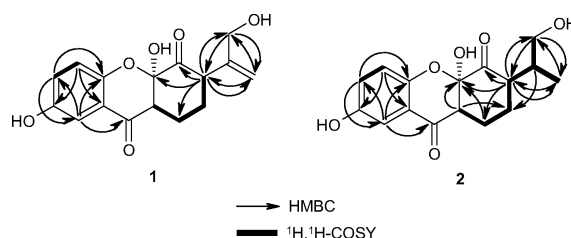
The HRESIMS of applanatin A (**1**) gave a quasi molecular ion at $m/z = 327.0843$ $[M+Na]^+$ corresponding to a molecular formula of C₁₆H₁₆O₆. Analysis of the IR spectrum suggested that the compound contained at least one carbonyl group (1698 cm⁻¹). The ¹H NMR spectrum of **1** displayed signals at $\delta = 6.88$ (d, $J = 2.4$ Hz), 6.94 (d, $J = 8.8$ Hz), and 7.14 (dd, $J = 8.8, 2.4$ Hz), which implied the existence of a trisubstituted phenyl ring. The ¹³C NMR spectrum and DEPT experiment of **1** showed sixteen carbon signals: four methylenes, five methines and seven quaternary carbons, of which the signals at $\delta = 123.1$ (s), 107.8 (d), 167.1 (s), 128.3 (d), 114.6 (d) and 153.8 (s) were attributed to the benzene ring. A quaternary carbon signal at $\delta = 98.4$ was assigned to a hemiketal carbon

Table 1. ^1H and ^{13}C NMR data for applanatins A (**1**) and B (**2**), and ganoderma aldehyde (**3**) in CD_3OD .

No.	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		98.4 (s)		99.6 (s)		97.1 (s)
2	3.33 (m)	56.6 (d)	3.18 (dd, 9.2, 8.1)	60.4 (d)	3.34 (m)	59.2 (d)
3		204.7 (s)		206.5 (s)		205.5 (s)
4		123.1 (s)		123.4 (s)		123.6 (s)
5	6.88 (d, 2.4)	107.8 (d)	6.91 (d, 2.4)	108.2 (d)	6.85 (d, 1.7)	108.2 (d)
6		167.1 (s)		167.1 (s)		166.5 (s)
7	7.14 (dd, 8.8, 2.4)	128.3 (d)	7.13 (dd, 8.8, 2.4)	127.7 (d)	7.04 (dd, 8.8, 1.7)	127.5 (d)
8	6.94 (d, 8.8)	114.6 (d)	6.96 (d, 8.8)	114.5 (d)	6.80 (d, 8.8)	114.3 (d)
9		153.8 (s)		153.5 (s)		153.4 (s)
10	2.09 (m), 2.14 (m)	28.0 (t)	2.05 (m)	28.8 (t)	2.14 (m)	28.8 (t)
11	2.07 (m)	30.6 (t)	1.98 (m), 1.72 (m)	28.6 (t)	2.06 (m)	30.3 (t)
12	2.96 (dd, 9.8, 7.3)	53.9 (d)	2.37 (m)	51.5 (d)	3.42 (dd, 8.8, 7.8)	47.0 (d)
13		204.5 (s)		206.4 (s)		210.3 (s)
14		145.9 (s)	1.61 (m)	36.1 (d)		148.3 (s)
15	4.93 (br d, 1.5, H_a)	113.4 (t)	0.95 (d, 6.8)	15.3 (q)	6.18 (br s, H_a)	137.8 (t)
16	5.07 (br d, 1.5, H_b)				6.49 (br s, H_b)	
	3.79 (br s)	65.4 (t)	3.01 (dd, 10.8, 7.3)	67.3 (t)	9.25 (s)	195.2 (d)
			3.15 (dd, 10.8, 5.9)			

with two oxygens attached. In the HSQC spectrum, one methylene carbon at $\delta = 113.4$ correlated with two protons at 4.93 (br d, 1.5) and 5.07 (br d, 1.5), which is characteristic of a terminal methylene. In the ^1H , ^1H COSY spectrum (Fig. 2), proton 10-H ($\delta_{\text{H}} = 2.09$, 2.14) coupled with 11-H ($\delta_{\text{H}} = 2.07$), which in turn were correlated with 2-H ($\delta_{\text{H}} = 3.33$) and 12-H ($\delta_{\text{H}} = 2.96$), respectively. The correlations from $\delta_{\text{H}} = 6.88$ to $\delta_{\text{C}} = 204.7$ in the HMBC spectrum suggested that the ketone carbonyl group was located at the position of C-4. The spectral data of **1** were very similar to those of ganoderma aldehyde (**3**) which has been reported previously from the same species [5], and the obvious NMR differences between compounds **1** and **3** are as follows: the aldehyde signals [$\delta_{\text{H}} = 9.25$ (s), $\delta_{\text{C}} = 195.2$ (d)] of **3** are absent and replaced by a methylene group [$\delta_{\text{H}} = 3.79$ (br s), $\delta_{\text{C}} = 65.4$ (t)] attached to an oxygen in **1**. The structure of **1** was therefore elucidated as shown in Fig. 1, named applanatin A. Considering the biogenetic relationship and the key ROESY correlations (see Experimental Section), the relative stereochemistry of this molecule was deduced to be the same as that of published ganoderma aldehyde (**3**).

The HRESIMS of **2** gave a quasi molecular ion at $m/z = 329.1000 [\text{M}+\text{Na}]^+$ corresponding to a molecular formula of $\text{C}_{16}\text{H}_{18}\text{O}_6$. The IR spectrum and EIMS suggested that compound **2** possessed the same skeleton as applanatin A (**1**). Comparison of its NMR spectra with those of **1** showed that the signals due to a terminal methylene at $\delta_{\text{H}} = 4.93$, 5.07 and $\delta_{\text{C}} = 113.4$

Fig. 2. Key HMBC and ^1H , ^1H COSY correlations of applanatins A (**1**) and B (**2**).

in **1** were absent, and a methyl signal was observed at $\delta_{\text{H}} = 0.95$ (d, 6.8) and $\delta_{\text{C}} = 15.3$ (q) in **2**, and an additional methine carbon signal also appeared at $\delta_{\text{H}} = 1.61$ (m) and $\delta_{\text{C}} = 36.1$ (d). The above information indicated that the terminal methylene group of **1** was affirmatively hydrogenated in **2**. This inference can be further validated by its ^1H , ^1H COSY and HMBC spectra (Fig. 2), which revealed the key HMBC correlations from 15-H to C-12 and C-16, from 16-H to C-12 and C-15, and from 14-H to C-11. The structure of **2** was therefore elucidated as shown in Fig. 1, named applanatin B.

In the course of scrutinizing the NMR data of our isolated ganoderma aldehyde (**3**) with those reported, we found that the partial NMR assignments of **3** in the original publication should be revised as follows: interchanging the ^1H and ^{13}C NMR assignments between C-2 and C-12, and between C-10 and C-11, as shown in Table 1. The above revision was made on the basis of a careful analysis of the ^1H , ^1H COSY, HSQC and HMBC spectra: the key HMBC correla-

tions from 15-H ($\delta_{\text{H}} = 6.18, 6.49$) and 16-H ($\delta_{\text{H}} = 9.25$) to C-12 ($\delta_{\text{C}} = 47.0$), and the important $^1\text{H}, ^1\text{H}$ COSY correlations between 2-H ($\delta_{\text{H}} = 3.34$) and 10-H ($\delta_{\text{H}} = 2.14$), 10-H and 11-H ($\delta_{\text{H}} = 2.06$), as well as 11-H and 12-H ($\delta_{\text{H}} = 3.42$) were distinctly observed.

Although the secondary metabolites of the family Ganodermataceae have been investigated adequately, to the best of our knowledge, this type of benzopyran-4-one derivatives, such as compounds **1–3**, are only found in this species so far. A series of similar and minor derivatives can be also detected in the MeOH extraction of this fungus by the observation of characteristic UV/vis spectra in HPLC analysis.

Comparison of the physicochemical properties with the reported data allowed us to identify the known polyoxygenated lanostane-type triterpenes **4–7** as ganoderenic acids A, B, D and G [2, 5, 8], respectively, isolated from the same fungus.

Experimental Section

General

Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with a Bruker DRX-500 spectrometer in CD_3OD ($\delta_{\text{H}} = 3.30$ ppm, $\delta_{\text{C}} = 49.00$ ppm) at r.t. EIMS were taken on a Finnigan-MAT 90 instrument, and HRES-IMS was recorded with an API QSTAR Pulsar 1 spectrometer. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Makall Group Ltd., P.R. China) and Chromatorex C-18 (40–75 μm , Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by reversed-phase HPLC (Agilent 1100, Zorbax SB-C-18 column, 5 μm , 4.6 \times 150 mm, 0–100% CH_3CN in H_2O over 15 min, 1 mL min^{-1}).

Fungus material

The fresh fruiting bodies of *G. applanatum* were collected at the southern part of the Gaoligong Mountains in Yunnan Province, People's Republic of China, in August 2006 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, CAS.

Extraction and isolation

The fresh fruiting bodies of *G. applanatum* (2.5 kg) were immersed in 12 L of $\text{CHCl}_3/\text{MeOH}$ (1:1, v/v) and left at r.t. for two weeks. Then the extraction was concentrated *in vacuo* to give a brown gum (60.0 g), which was fractionated by silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ gradient elution. a) The fractions (3.5 g) eluted with $\text{CHCl}_3/\text{MeOH}$ (80:20, v/v), mainly containing the benzopyran-4-one derivatives, were further repeatedly separated by a Chromatorex C-18 column. Purification of these fractions using a Chromatorex C-18 column at different gradients and Sephadex LH-20 (pure methanol as eluent), afforded the new compounds: applanatins A (**1**; 30 mg; 25% MeOH in H_2O ; $t_{\text{R}} = 7.5$ min) and B (**2**; 8.5 mg; 21% MeOH in H_2O ; $t_{\text{R}} = 7.9$ min), and one known analogue, ganoderma aldehyde (**3**; 15 mg; 20% MeOH in H_2O ; $t_{\text{R}} = 8.3$ min). b) The fractions (5.0 g) eluted with $\text{CHCl}_3/\text{MeOH}$ (98:2, v/v) were further separated and purified by a Chromatorex C-18 column at different gradients to afford four known triterpenes: ganoderenic acids A (**4**; 45 mg; 45% MeOH in H_2O), B (**5**; 120 mg; 40% MeOH in H_2O), D (**6**; 350 mg; 58% MeOH in H_2O) and G (**7**; 65 mg; 60% MeOH in H_2O).

Applanatin A (1): amorphous powder. – $[\alpha]_{\text{D}}^{16} = -144.1$ ($c = 0.17$, acetone). – UV/vis (MeOH): $\lambda_{\text{max}} = 214, 257, 372$ nm. – IR (KBr): $\nu = 3431, 2957, 2878, 1698, 1630, 1488, 1463, 1385, 1310, 1214, 1135$ cm^{-1} . – ^1H and ^{13}C NMR: see Table 1. – Key ROESY correlation: $15\text{a-H} \rightarrow 12\alpha\text{-H}$, $15\text{b-H} \rightarrow 16\text{-H}$. – MS (EI): m/z (%) = 304 (35) $[\text{M}]^+$, 286 (37), 268 (8), 257 (5), 241 (36), 213 (12), 201 (23), 189 (34), 137 (100), 136 (44). – HRMS ((+)-ESI): $m/z = 327.0843$ (calcd. 327.0844 for $\text{C}_{16}\text{H}_{16}\text{O}_6\text{Na}$, $[\text{M}+\text{Na}]^+$).

Applanatin B (2): amorphous powder. – $[\alpha]_{\text{D}}^{18} = -38.1$ ($c = 0.14$, MeOH). – UV/vis (MeOH): $\lambda_{\text{max}} = 215, 224, 257, 372$ nm. – IR (KBr): $\nu = 3424, 2933, 2880, 1698, 1629, 1580, 1488, 1467, 1395, 1312, 1217, 1080, 1034$ cm^{-1} . – ^1H and ^{13}C NMR: see Table 1. – MS (EI): m/z (%) = 306 (13) $[\text{M}]^+$, 288 (11), 270 (2), 247 (21), 243 (10), 229 (6), 213 (3), 201 (32), 189 (8), 137 (100), 136 (26). – HRMS ((+)-ESI): $m/z = 329.1000$ (calcd. 329.1001 for $\text{C}_{16}\text{H}_{18}\text{O}_6\text{Na}$, $[\text{M}+\text{Na}]^+$).

Ganoderma aldehyde (3): amorphous powder. – UV/vis (MeOH): $\lambda_{\text{max}} = 216, 255, 370$ nm. – ^1H and ^{13}C NMR data: see Table 1. – MS (EI): m/z (%) = 302 (84) $[\text{M}]^+$, 284 (6), 273 (2), 257 (40), 247 (7), 229 (13), 227 (14), 213 (11), 201 (27), 189 (22), 137 (100), 136 (56).

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