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Four New Polycyclic Meroterpenoids from Ganoderma cochlear

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Supporting Information

ABSTRACT: Four pairs of new polycyclic-meroterpenoid enantiomers, ganocins A-C (1-3) possessing a spiro[4,5]decane ring system, along with ganocin D (4) with an eight-membered ring, were isolated from the fruiting bodies of *Ganoderma cochlear*. Their structures were determined by spectroscopic data and X-ray diffraction crystallography. Their anti-AChE activities were evaluated, and a possible biogenetic pathway was also proposed.



T he genus *Ganoderma* (Ganodermataceae) is a basidiomycete white rot fungus mainly distributed in tropical and subtropical areas of Asia. The fungus has been used as a folk medicine to treat and prevent various diseases for centuries, particularly in China, Japan, and Korea.¹ Most of the phytochemical and pharmacological investigations have focused on the ganoderma triterpeniods and polysaccharides.² Our group has been interested in the bioactive constituents of *Ganoderma*³ and was the first to report triterpenoids and the liver-protective activities of *G. cochlear*.⁴ However, several phenolic meroterpenoids including ganomycins A and B,⁵ fornicins A–C,⁶ ganomycin I,⁷ and (±)-lingzhiol with a rotated door structure⁸ from *Ganoderma* were reported, which attracts our attention.

Acetylcholinesterase (AChE), mainly present in the central nervous system (CNS), catalyzes the hydrolysis of neurotransmitter acetylcholine to choline.⁹ This enzyme is related to neurological diseases, such as Alzheimer's disease (AD) and epilepsia.¹⁰ Research has directly demonstrated that *Ganoderma* can enhance memory and protect the nervous system by inhibiting AChE activity.¹¹ Some natural AChE inhibitors (magnolol and ferulic acid) have a phenolic substructure,¹² suggesting that ganoderma meroterpenoids with the phenolic structure may also show anti-AChE activity.

Thus, we studied the total phenolic parts of *G. cochlear*, and four unprecedented polycyclic meroterpenoids, ganocins A-C (1-3) possessing a spiro[4,5]decane substructure, and ganocin D (4), with an eight-membered carbon ring, were isolated. Herein, we report the structural elucidation including absolute configuration analysis, a biogenetic pathway, and bioactive evaluation of 1-4.

The molecular formula of ganocin A (1) was assigned as $C_{21}H_{24}O_4$ by HREIMS ([M]⁺, m/z 340.1669; calcd 340.1679)



with ten degrees of unsaturation. Its IR spectrum showed the presence of an aldehyde group (2962 and 1758 cm⁻¹). The ¹³C NMR spectrum (Table 1) exhibited 21 carbon resonances, corresponding to three methyls, four methylenes, five methines (four aromatic/olefinic methines), eight quaternary carbons (one tetrasubstituted carbon, one carbonyl group, one oxygenated quaternary carbon, and four aromatic/olefinic quaternary carbons), and one aldehyde carbon. The ¹H NMR spectrum (Table 1) showed three typical aromatic signals at δ 7.01 (d, *J* = 2.4 Hz), 6.66 (dd, *J* = 2.4 and 9.0 Hz), and 6.64 (d, *J* = 9.0 Hz), suggesting the presence of a 1,2,4-trisubstituted dihydroxylbenzene substructure (part A in Figure 1), which was similar to that of fornicin C, a meroterpenoid with a 15 carbon side chain.⁶

Similarly, except for the phenol group (part A), the remaining 15 carbons of 1 were representative of four rings based on its 1D-NMR and the degree of unsaturation.

In the ¹³C NMR spectrum of 1, three low-field carbon signals at δ 150.6 (d), δ 139.2 (s), and δ 193.8 (d) were attributed to an α,β -unsaturated aldehyde group (C-2'/C-3'/C-15'), based on the HMBC correlations (Figure 1) of H-2' with C-2, C-3', and C-15'. Meanwhile, the HMBC correlations of H-2' and H-3 with an oxyquaternary carbon (δ 78.1) indicated that the oxyquaternary carbon was located at C-1'. Moreover, the

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Table 1. ¹H and ¹³C NMR Data for Compounds 1-4 (J in Hz)

	1^b		2^a		3 ^{<i>a</i>}		4^a	
	$\delta_{ m H}$	$\delta_{ m C}$	$\delta_{ m H}$	$\delta_{ m C}$	$\delta_{ m H}$	$\delta_{ m C}$	$\delta_{ m H}$	$\delta_{ m C}$
1		146.5 (s)		148.4 (s)		149.7 (s)		146.9 (s)
2		129.9 (s)		120.4 (s)		123.4 (s)		122.3 (s)
3	7.01 d (7.4)	113.4 (d)	7.65, d (3.0)	109.9 (d)	7.66, d (3.0)	111.4 (d)	7.14, d (2.4)	109.0 (d)
4		151.2 (s)		151.7 (s)		152.6 (s)		152.3 (s)
5	6.64, m	116.1 (d)	7.20, dd (3.0, 9.0)	121.9 (d)	7.16, dd (3.0, 9.0)	122.1 (d)	7.03, dd (2.4, 9.0)	115.8 (d)
6	6.64, m	119.5 (d)	6.93, d (9.0)	119.4 (d)	6.91, d (9.0)	118.9 (d)	6.97, d (9.0)	116.1 (d)
1'		78.1 (s)		152.6 (s)		154.5 (s)	3.82, t	46.0 (d)
2'	6.62, m	150.6 (d)	6.94, s	120.9 (d)	6.99, s	122.5 (d)	2.29, m	27.4 (t)
3′		139.2 (s)		198.0 (s)		198.8 (s)		212.0 (s)
4′	2.37, m; 2.18, m	19.1 (t)	2.78, m; 2.55, m	33.6 (t)	2.59, m; 2.49, m	34.6 (t)	2.25, m	24.8 (t)
5'	2.07, m; 1.66, m	30.8 (t)	1.86, m; 1.57, m	33.9 (t)	1.84, m; 1.66, m	30.9 (t)		127.7 (s)
6′		60.7 (s)		51.3 (s)		52.2 (s)		133.8 (s)
7'		88.7 (s)		88.5 (s)		90.6 (s)		80.1 (s)
8'	2.07, m; 1.57, m	39.5 (t)	2.10, m; 1.91, m	37.7 (t)	2.62, m; 2.30, m	28.5 (t)	2.35, m; 1.78, m	48.4 (t)
9′	1.69, m	24.0 (t)	2.23, m; 1.89, m	28.2 (t)	2.05, m; 1.85, m	34.5 (t)	2.49, m; 2.33, m	37.5 (t)
10′	2.39, m	62.0 (d)	3.11, t	53.0 (d)		134.5 (s)	5.06, m	133.8 (d)
11'		84.7 (s)		145.8 (s)		126.6 (s)		131.2 (s)
12'	1.17, s	25.8 (q)	1.46, s	22.5 (q)	1.53, s	18.8 (q)	1.67, s	27.5 (q)
13'	1.30, s	32.5 (q)	4.79, s; 4.70, s	114.3 (t)	1.34, s	23.1 (q)	2.60, br s	36.7 (t)
14'	1.42, s	23.9 (q)	1.23, s	18.9 (q)	1.21, s	17.4 (q)	1.54, s	24.8 (q)
15'	9.40, s	193.8 (d)						

^{*a*}Measured in C₅D₅N. ^{*b*}Measured in CDCl₃. 1D NMR spectra (δ) were measured at 400 (100) MHz for 1 and at 600 (150) MHz for 2–4. The assignments were based on ¹H–¹H COSY, ROESY, HSQC, and HMBC experiments.



Figure 1. Key HMBC, $^1\text{H}{-}^1\text{H}$ COSY, and ROESY correlations of (±)-1.



Figure 2. X-ray crystallographic structure of 1a.



Figure 3. Key HMBC, ${}^{1}H-{}^{1}H$ COSY, and ROESY correlations of (\pm) -2.



Figure 4. Key HMBC, $^1\text{H}{-}^1\text{H}$ COSY, and ROESY correlations of (±)-4.

observed HMBC correlations from H-2', H₂-4', and H₂-5' to C-3' and a quaternary carbon (δ 60.7), together with the ¹H–¹H COSY correlations of H₂-4'/H₂-5', confirmed that C-1' is connected with C-6' (δ 60.7) to form a cyclohex-1-ene-1carbaldehyde substructure (B ring) in **1**.

Subsequently, the presence of CH_2 -8'/ CH_2 -9'/CH-10' moiety was deduced by the ${}^{1}H^{-1}H$ COSY correlations. In the HMBC spectrum of 1, H₂-8', H₂-5', and H₃-14' (δ 1.42, s) showed the HMBC correlations with an oxyquaternary carbon (δ 88.7), which indicated that C-7' was the oxyquaternary carbon. Meanwhile, only H-10' showed the HMBC correlations with another oxyquaternary carbon (δ 84.7) and two methyls (δ 25.8, δ 32.5), suggesting a 2-oxyisopropyl group was located at C-10'. Importantly, the key HMBC correlations of H₂-8' and H-10' with C-6' and C-7' were observed. Thus, we unambiguously deduced that a five-membered ring (part C) and B ring formed a spiro[4,5] decane ring system.

Apart from the above-mentioned two rings, another two rings were finally determined as 1,7'-epoxy and 1',11'-epoxy rings, based on its formula weight and degrees of unsaturation (Figure 1).

The ROESY correlations of $H_3-14'/H_2-5'/H-10'$ indicated that CH_3-14' , CH_2-5' , and H-10' were on the same face. Furthermore, the single-crystal X-ray diffraction of acetylated





derivative of 1 (Figure 2) showed that acetyl ganocin D (1a) was a pair of enantiomers. Thus, the single-crystal X-ray diffraction experiment of 1a performed by using Cu K α radiation confirmed 1a as 1'R,6'R,7'R,10'R and 1'S,6'S,7'S,10'S.

Ganocin B (2) was obtained as a yellow powder with a molecular ion peak at m/z 310.1564 [M]⁺ in HREIMS, coinciding with the molecular formula $C_{20}H_{22}O_3$. A comparison of 1D NMR spectroscopic data between 2 and 1 showed that 2 also had a 1,2,4-trisubstituted dihydroxylbenzene substructure and a spiro [4,5] decane ring system, which was further supported by its 2D NMR spectra (Figure 3). However, the ¹³C NMR spectrum of 2 showed 20 carbons, with one less carbon than 1. Obviously, in the 1D NMR spectra of 2, an α_{β} unsaturated ketone (δ 152.6; δ 120.9, and δ 198.0) was observed, instead of the aldehyde group signal in 1. We speculated that the B ring of 2 was a cyclohexenone moiety and the α_{β} -unsaturated ketone was attributable to C-1', C-2', and C-3'. This was confirmed by the HMBC correlations of the olefinic proton (δ 6.94, s) with C-2, the olefinic quaternary carbon (δ 152.6), and the carbonyl group and C-6'; of H-3 and H_2 -5' with the olefinic quaternary carbon; and of H_2 -4' and H_2 -5' with the carbonyl group and C-6'. Additionally, a terminal double bond (δ 4.79, s, δ 4.70, s; δ 114.3 and δ 145.8) was assigned to C-11' and C-13' by the HMBC correlations of the olefinic protons at δ 4.79 (s) and δ 4.70 (s) with CH₃-12' (δ 22.5) and C-10' (δ 53.0). Thus, the planar structure of 2 was established.

The ROESY correlations of H₃-14/H₂-5/H-10' suggested that CH₃-14', C-6', and C-10' had the same relative configuration (Figure 3). Its optical rotation value ($[\alpha]^{20}_{D}$ +1.8) indicated a racemic nature, and the subsequent chiral

resolution of **2** by HPLC afforded the anticipated enantiomers, **2a** and **2b**, which were opposite in terms of their CD curve and $[\alpha]_D$ spectra ($[\alpha]^{20}_D$ +117.9 and $[\alpha]^{20}_D$ -104.6) (see Supporting Information (SI)). Therefore, **2** was deduced to be 6'R,7'R,10'R and 6'S,7'S,10'S.

Ganocin C (3) has the same molecular formula $C_{20}H_{22}O_3$ established by the $[M]^+$ ion peak at m/z 310.1566 in the HREIMS as compound 2. The 1D NMR spectroscopic data of 3 were similar to those of 2, except that a methyl (δ 23.1, C-13') and two olefinic quaternary carbons (δ 134.5, C-10' and δ 126.6, C-11') in 3 replaced the terminal double bond and a methine in 2, which was confirmed by the HMBC correlations of H₃-12' (δ 1.54, s) and H₃-13' (δ 1.34, s) with two olefinic quaternary carbons and of H2-5', H2-8' and H2-9' with the olefinic quaternary carbon (δ 134.5). Its ROESY spectrum showed an interaction between H₂-5' and H₃-14', suggesting that CH₃-14' and C-5' were ipsilateral. Its optical rotation value $([\alpha]_{D}^{20} - 0.7)$ indicated that 3 could be a pair of enantiomers, which was supported by HPLC analysis on an analytical chiral column, showing two peaks (see SI). Due to only two chiral centers in 3, C-6' and C-7' were assigned as R,R and S,S.

The molecular formula of ganocin D (4) assigned as $C_{20}H_{22}O_3$ by its ion peak at m/z 310.1573 [M]⁺ (calcd 310.1569) in the HREIMS spectrum was also the same as that for compound **3**. However, the chemical shift of the carbonyl carbon was shifted low-field to 212.0 ppm, suggesting the absence of the double bond ($\Delta^{1,2}$) in **4**. This was confirmed by the HMBC correlations (Figure 4) of H-1' (δ 3.82, t), H₂-2' (δ 2.29, m) with C-1 and C-3' and of H-3 with C-1' (Figure 4). Additionally, the observed HMBC correlations of H-1' and H₂-4', with two olefinic quaternary carbons (δ 127.7 and δ 133.8), suggested the existence of C-5'=C-6', which indicated that the quaternary carbon in **4**. From this, we speculated that its C ring was different from that of **3**.

On the basis of the HMBC correlations of methylene protons (δ 2.35, m; δ 1.78, m), H-1' and H₃-14' with C-7' (δ 80.1), the methylene was assigned to C-8'. The ¹H–¹H COSY spectrum deduced the presence of the $-CH_2-CH_2-CH=$ moiety (C-8'/C-9'/C-10'), of which H-10' showed an HMBC correlation with C-11', CH₃-12', and a methylene (δ 36.7). This indicated that the methylene in 4 replaced CH₃-13' in 3. Meanwhile, H₂-13' showed the HMBC correlations with C-4', C-5', and C-6', which confirmed that the C ring of 4 was an eight-membered ring. Thus, the planar structure of 4 was determined as shown in Figure 4.

The ROESY correlations of H_2 -2'/ H_3 -14' indicated that the relative configurations of H-1' and CH₃-14' were reverse (Figure 4). On the basis of its optical rotation value and the chiral HPLC analysis result (see SI), 4 was finally established to be 1'*R*,6'*R* and 1'*S*,6'*S*.

Ganocins A–C (1-3) possessing a spiro[4,5]decane substructure and ganocin D (4) with an eight-membered ring were established to be polycyclic enantiomers. Compared to fornicins A–C, all of them have a 1,2,4-trisubstituted dihydroxylbenzene moiety. We deduced that the B and D rings of 1–4 were formed by the hetero-Diels–Alder reaction of fornicin C. Meanwhile, the prenylated side chain of fornicins A–C could provide appropriate conditions for a free radical reaction. The dienophile may be directed away from diene (*exo* approach) or toward the diene (*endo* approach) to produce a pair of enantiomers,¹³ which also would biosynthetically explain the racemic nature of compounds 1–4. The C ring was

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subsequently derived from the further free radical reactions. Thus, a plausible biogenetic pathway for 1-4 was proposed (Scheme 1).

Research showed that the extracts of *Ganoderma* can decrease AChE to protect the CNS and improve memory.¹¹ In the present study, the evaluation of anti-AChE effects showed that compound 4 had weak anti-AChE activity with an inhibition of 32% (50 μ M). Nevertheless, other compounds are inactive. Compared to natural phenolic AChE inhibitors with a big conjugated system (flavonoids and anthraquinones),¹² compounds 1–4 only had a benzene ring. We deduced that their low conjugation system and coplanarity affected their anti-AChE activity.

ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra of 1–4, the data for single-crystal Xray diffraction of 1a (CIF), $[\alpha]_D$ spectra and CD spectra for 2a and 2b, and in vitro anti-AChE activity of 1–4, together with experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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