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Ganotheaecolin A, a Neurotrophic Conjugated Ergosterol with a Naphtho[1,8-*ef*]azulene Scaffold from *Ganoderma theaecolum*

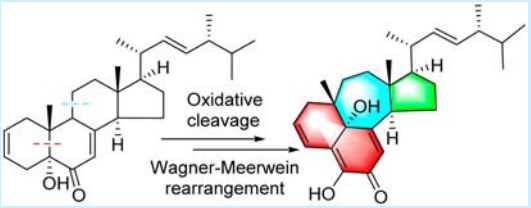
Qi Luo,[†,‡] Zhu-Liang Yang,[§] Yong-Ming Yan,[†] and Yong-Xian Cheng*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany and [§]Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P.R. China

[‡]University of Chinese Academy of Sciences, Beijing 100049, P.R. China

Supporting Information

ABSTRACT: Ganotheaecolin A (**1**), a novel ergosterol with a rare naphtho[1,8-*ef*]azulene ring system, was isolated from the fruiting bodies of *Ganoderma theaecolum*. Its structure was determined by spectroscopic data and computational methods. Compound **1** represents a 6/6/7/5-fused carbon skeletal ergosterol typically formed by Wagner−Meerwein rearrangement, whose plausible biosynthetic pathway was briefly discussed. Finally, the neurotrophic activity of **1** was examined using PC12 cells.



Ganoderma fungi have been widely used as traditional medicines in China, Japan, Korea, and other Asian countries for centuries.[1] Previous studies on this genus revealed the presence of triterpenoids, polysaccharides, meroterpenoids, alkaloids, nucleotides, steroids, fatty acids, proteins/peptides, and trace elements,[1−4] which possess various pharmacological activities including immunomodulation, antitumor, antiinflammatory, antifibrotic, antidiabetic, hepatoprotective, antibacterial, antiviral, antioxidative, antiaging, and sleep-promoting properties.[1−9] *Ganoderma theaecolum* is a mushroom commonly seen in Chinese herbal medicine markets. It is normally considered to have functions similar to those of *G. lucidum* in treating a broad range of disorders in China.[10] In spite of the important medicinal values of *G. theaecolum*, investigations on this species are scarce. As a part of our continuing studies on *Ganoderma* fungi, we investigated the chemical components of the title species, resulting in the isolation of ganotheaecolin A (**1**, Figure 1) with an unprecedented naphtho[1,8-*ef*]azulene ring system, representing a novel 6/6/7/5-fused carbon skeletal ergosterol. Inspired by the traditional medicinal uses of *Ganoderma* for neurological disorders, we found **1** to be a small-molecule neurotrophic factor mimetic.

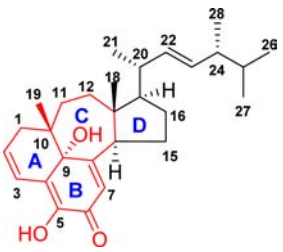


Figure 1. Chemical structure of **1**.

Ganotheaecolin A (**1**),[11] obtained as a yellow gum, possesses a molecular formula $C_{28}H_{40}O_3$ deduced from its HRESIMS, ^{13}C NMR, and DEPT spectra, having 9 degrees of unsaturation. The ^1H NMR spectrum (Table 1) of **1** contains six methyl signals including two methyl singlets, four methyl doublets, and five olefinic protons. The ^{13}C NMR and DEPT spectra show 28 carbons ascribed to six methyls, five aliphatic methylenes, 10 methines (five sp^2 and five sp^3), and seven quaternary carbons (one ketone, three sp^2 including one oxygenated, and three sp^3 including one oxygenated). These spectral data prompted us to conclude that **1** might be an ergostane derivative.[12] The $^1H-^1H$ COSY spectrum (Figure 2) of **1** shows the presence of H-1/H-2/H-3, H-11/H-12, H-14/H-15/H-16/H-17/H-20/H-22/H-23/H-24/H-25/H_3-26, H-20/H_3-21, H-24/H_3-28, and H-25/H_3-27. The planar structure of **1** was mainly determined with HMBC data. HMBC correlations of H_3-19/C-1, C-9 (δ_C 73.9), C-10, H-1/C-9, C-10 (δ_C 41.1), H-2/C-4 (δ_C 126.4), and H-3/C-9 imply the existence of ring A. HMBC correlations of 5-OH (δ_H 6.48)/C-4, C-5 (δ_C 140.7), C-6 (δ_C 180.9), and H-7 (δ_H 6.24, s)/C-5, C-8 (δ_C 165.7), and C-9 suggest that rings A and B are fused by sharing a C_4-C_9 bond. The presence of ring C is supported by the HMBC observations of H-11/C-9, C-10, C-13, H-12/C-10, C-13, C-14, and H-14/C-7, C-8, C-9, C-13. With these data in hand, the pivotal motif (rings A−C) of compound **1** were unambiguously clarified. In addition, HMBC correlations of H_3-18/C-12, C-14, C-17, H-16, H-17/C-13 indicate the presence of ring D. Thus, the planar structure of **1** was deduced as shown in Figure 2.

For the geometry of the $\Delta^{22(23)}$ double bond, the large coupling constant of H-22 (J = 15.3 Hz) suggests it to be the *E*-form. The relative configuration of **1** was assigned by ROESY

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Table 1. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR Data of 1 (δ in ppm, J in Hz)

no.	δ_H [a]	δ_C [a]	δ_H [b]	δ_C [b]
1a	2.54 dd (18.7, 5.0)	39.8	2.54 dd (18.6, 5.1)	40.8
1b	1.74 m		1.68 dd (18.6, 5.5)	
2	6.03 m	132.5	5.94 m	132.5
3	6.62 d (10.1)	120.4	6.61 d (9.9)	121.9
4		126.4		130.2
5		140.7		142.5
6		180.9		183.0
7	6.24 s	124.9	6.13 s	126.1
8		165.7		167.1
9		73.9		74.4
10		41.1		41.8
11a	2.32 t (13.6)	33.1	2.33 t (13.7)	34.0
11b	0.98 m		0.99 m	
12a	1.97 m	33.9	1.98 m	35.1
12b	1.35 m		1.39 m	
13		43.4		44.2
14	3.44 dd (12.1, 7.2)	48.2	3.50 dd (12.2, 7.3)	49.1
15a	1.77 m	27.1	1.79 overlap	27.8
15b	1.63 m		1.61 m	
16a	1.60 m	27.1	1.79 overlap	28.2
16b	1.44 m		1.46 m	
17	1.61 m	56.7	1.64 m	57.9
18	0.79 s	19.8	0.82 s	19.2
19	0.64 s	18.8	0.62 s	20.1
20	2.14 m	40.5	2.18 m	41.8
21	1.04 d (6.7)	22.0	1.07 d (6.7)	22.4
22	5.21 dd (15.3, 7.8)	134.9	5.26 dd (15.2, 7.8)	136.4
23	5.25 dd (15.3, 7.2)	133.1	5.27 dd (15.2, 7.2)	134.0
24	1.86 m	43.1	1.87 m	44.5
25	1.48 m	33.2	1.49 m	34.4
26	0.84 d (6.8)	20.2	0.87 d (6.8)	20.5
27	0.82 d (6.8)	20.0	0.85 d (6.8)	20.1
28	0.92 d (6.8)	17.8	0.95 d (6.8)	18.2
5-OH	6.48 s			
9-OH [c]	5.33 s			

[a] In $CDCl_3$. [b] In methanol-d_4. [c] In DMSO-d_6.

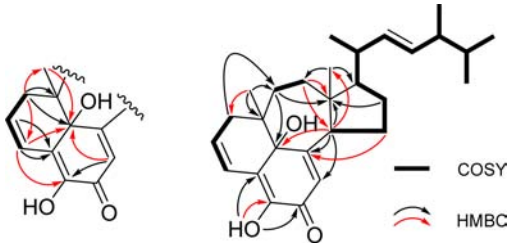

Figure 2. Key COSY and HMBC correlations of 1.

data (Figure 3). In the ROESY spectrum, the correlations between 9-OH (in DMSO-d_6)/Ha-1, Ha-11, H_3-19/Hb-1, Hb-11, H_3-18, H_3-18/H-20, and H-14/Ha-11, H-17 are observed, indicating the α-orientation for 9-OH, H-14, and H-17 and the β-form for CH_3-18 and CH_3-19. In general, the absolute configuration determination of C-20 and C-24 in the flexible side chain is rather challenging. X-ray diffraction is a direct method to solve the absolute configuration of 1; however, the gummy nature and the limited amount of 1 prohibits cultivating a qualified crystal for 1 or its derivative even though we have tried to prepare the acetate of 1 (data not shown). Under these

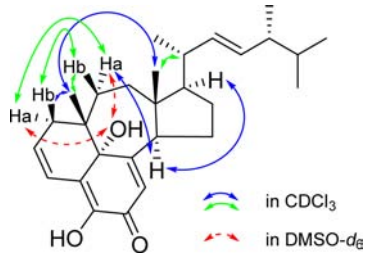

Figure 3. Key ROESY correlations of 1.

circumstances, the absolute configurations at C-20 and C-24 were established on the basis of the diagnostic chemical shifts of H_3-21 and H_3-28. It was reported that the doublets of H_3-21 of 20R are relatively upfield shifted (δ_H 1.28 for 20S, δ_H 1.13 for 20R).[13,14] Therefore, 1 was assigned as the 20R form due to H_3-21 at δ_H 1.04 in 1. In contrast, the previous reports indicated that the doublet of the H_3-28 of 24R form appears slightly downfield compared to that of the 24S form (for example, δ_H 0.912 for 24R, δ_H 0.819 for 24S).[15,16] In the present study, H_3-28 resonating at δ_H 0.919 suggests 1 to be 24R-configured. This conclusion was further confirmed by comparison of the ^{1}H and ^{13}C NMR data of the side chain (C_{22}−C_{28}) of 1 with those of (24R)-ergosterol,[17] which was confirmed by single-crystal X-ray diffraction (Table S1, Supporting Information).

To our knowledge, 24R-form ergosterols from natural origins are very common.[12,17] In contrast, those with the 24S form were mainly chemically synthesized,[15,18−20] in accordance with our present finding. As a result, the absolute configuration of 1 at the side chain was assigned. To further clarify the absolute configurations of chiral carbons in the rings, ECD (electronic circular dichroism) calculations were therefore carried out toward (9R,10S,13R,14R,17R,20R,24R)-1 or (9S,10R,13S,14S,-17S,20R,24R)-1. The results disclose that the weighted ECD spectrum of (9R,10S,13R,14R,17R,20R,24R)-1 agrees well with the experimental one (Figure 4), suggesting the absolute

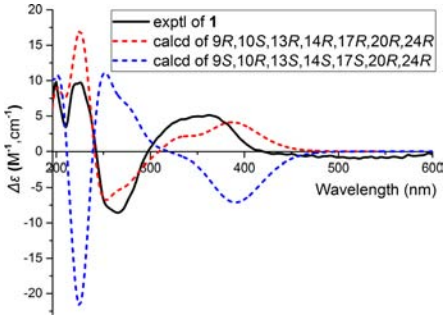

Figure 4. Comparison of the calculated ECD spectra for 9R,10S,13R,14R,17R,20R,24R and 9S,10R,13S,14S,17S,20R,24R at the B3LYP/6-311G(d,p) level with the experimental spectrum of 1 in MeOH, σ = 0.27 eV, shift = +0 nm.

configuration of 1 to be 9R,10S,13R,14R,17R,20R,24R. Considering that 1 bears a new carbon backbone, the calculations of ^{13}C NMR chemical shifts of (9R,10S,13R,14R,-17R,20R,24R)-1 at the MPW1PW91/6-311+G(2d,p) level with the PCM in MeOH were finally performed. The results show that the correlation coefficient (R^2) between the calculated and experimental data from linear regression analysis is 0.99773 (Figure 5), and the corrected mean absolute error (CMAE) is 2.1 ppm (Table S5, Supporting Information), supporting the

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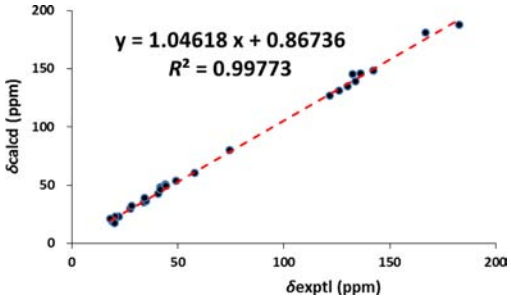
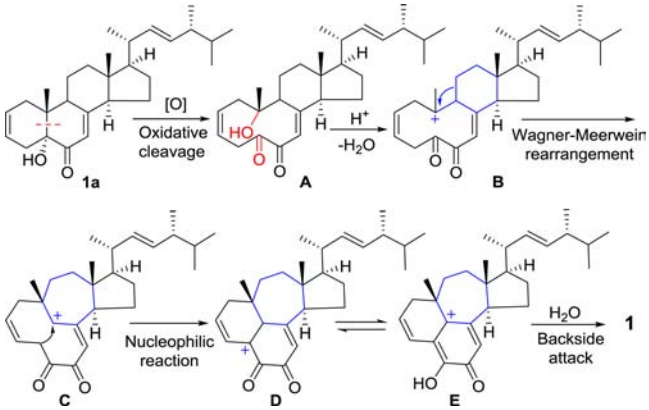


Figure 5. Regression analysis of experimental versus calculated ^{13}C NMR chemical shifts of (9*R*,10*S*,13*R*,14*R*,17*R*,20*R*,24*R*)-**1** at the MPW1PW91-SCRF/6-311+G(2d,p) level; linear fitting is shown as a line.

structure furnished by 1D and 2D NMR data. Hence, the DFT-^{13}C NMR calculations further secure the rationality of the carbon skeleton of **1**. Of note, **1** possesses one enol group. In general, there should exist a keto−enol tautomerism in **1**. However, the ^{1}H NMR of **1** indicates that only the enol tautomer is present, which might be due to the formation of large conjugate system in the structure of **1** allowing the enol form to be more stable.

To our knowledge, ganotheaecolin A represents to date the first example of a 6/6/7/5-fused carbon skeleton steroid, which aroused our interest in its plausible biogenesis. Analysis of the structure of **1** indicates that it might be formed by oxidative cleavage followed by Wagner−Meerwein rearrangement of (22*E*,24*R*)-5α-hydroxyergosta-2,7,22-trien-6-one (**1a**)[21] (Scheme 1). First, the key intermediate A is generated from

Scheme 1. Plausible Pathway for the Biogenesis of 1



the precursor **1a** by oxidative cleavage. An interesting feature in the biogenesis of **1** is the formation of an 11 (9→10)-*abeo* skeleton by Wagner−Meerwein rearrangement of monomer A.[12,22] Second, the intermediate C could be cyclized via nucleophilic reaction to generate D, which could be transformed to E by further keto−enol tautomerism. Finally, **1** was established by stereospecific backside attack.

Neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease are conditions where cells of the brain or spinal cord degenerate and die, markedly affecting the lives of millions. The lack of effective treatments for these diseases indicates a huge medical need. Neurotrophic factors exemplified as nerve growth factor (NGF) and other members have generated much excitement over the past decade due to their therapeutic potentials in regulating the proliferation, survival, migration, and differentiation of cells in the nervous system.[23] However, the inability of large neurotrophic molecules to cross the blood−brain barrier and adverse effects have limited the clinical usefulness of neurotrophic factors themselves. Small-molecule neurotrophic factor mimetics that could overcome the pharmacokinetic and side-effect barriers of neurotrophins are therefore urgently needed.[24] To date, a number of neurotrophic factor mimetics are undergoing clinical trials.[25] With this context and inspired by the traditional uses of *Ganoderma* in the treatment of neurological diseases,[26,27] compound **1** was evaluated for its neurite outgrowth-promoting activity in PC12 cells (Table S6 and Figure 6) with 5 ng/mL

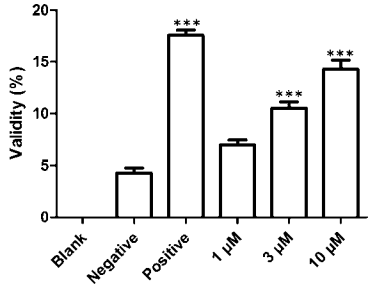


Figure 6. Neurite outgrowth-promoting activity of compound **1** in PC12 cells. Values are expressed as the mean ± SD of three independent experiments. ANOVA, ***p < 0.001 versus negative control.

and 50 ng/mL of NGF as the negative and positive controls, respectively. The results show that **1** could stimulate cell differentiation in a dose-dependent manner and reach a maximum effect at 10 μM, indicating its potential in neurotrophic factor mimetics.

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via Internet at The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00012.

1D, 2D NMR, HRESIMS spectra, computational methods, detailed isolation procedures, and bioassay methods (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +86-871-65223048. E-mail: yxcheng@mail.kib.ac.cn.

ORCID

Yong-Xian Cheng: 0000-0002-1343-0806

Notes

The authors declare no competing financial interest.

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(11) Ganotheaecolin A (**1**): yellow gum; $[\alpha]_D^{19.2}$ +173.2 (*c* 0.2, MeOH); UV (MeOH) λmax (log*ε*) 433 (3.27), 249 (4.11), 202 (3.98) nm; CD (MeOH) Δ*ε*210 +3.43, Δ*ε*225 +9.72, Δ*ε*265 −8.55, Δ*ε*362 +5.12; ESIMS *m*/*z* 423 $[M - H]^-$, HRESIMS *m*/*z* 423.2899 $[M - H]^-$ (calcd for $C_{28}H_{39}O_3$, 423.2905). For ^1H and ^{13}C NMR data, see Table 1.
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