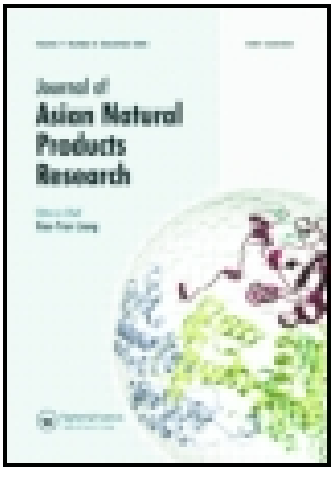

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A new bisabolane-type sesquiterpenoid from the fermentation broth of fungus Antrodiella gypsea

Zhen-Zhu Zhao[ab], Li-Qiang He[ab], He-Ping Chen[ab], Zheng-Hui Li[a], Ze-Jun Dong[a], Tao Feng[a] & 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 650201, China

[b] University of Chinese Academy of Sciences, Beijing 100049, China

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A new bisabolane-type sesquiterpenoid from the fermentation broth of fungus *Antrodiella gypsea*

Zhen-Zhu Zhao[ab], Li-Qiang He[ab], He-Ping Chen[ab], Zheng-Hui Li[a], Ze-Jun Dong[a], Tao Feng[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 650201, China;* [b]*University of Chinese Academy of Sciences, Beijing 100049, China*

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Studies of the fermentation broth of fungus Antrodiella gypsea led to the isolation of a new bisabolane-type sesquiterpenoid that was named gypseatriol (**1**), together with the known compound 2,10-dodecadiene-1,6,7-triol (**2**). The structure of this new metabolite was assigned by analysis of 2D NMR and HR-EI-MS. Absolute configuration was assigned by single crystal X-ray diffraction analysis. Compound 1 was evaluated for its antifungal activity on Candida albicans.

Keywords: Basidiomycete; *Antrodiella gypsea*; bisabolane; sesquiterpenoid

1. Introduction

Antrodiella gypsea is a higher fungus which can cause wood-decaying and belongs to basidiomycetes [1]. Previous studies of genus *Antrodiella* manily focused on taxonomy [1,2], biodegradation [3,4] and biotransformation [5]. However, few researches were carried on the investigation of secondary metabolites from cultural broth or fruiting bodies of this genus. Therefore, our team studied the constituents of *Antrodiella albocinnamomea* by fermentation and found some interesting compounds [6,7]. In continuation of our ongoing investigation on structurally interesting and biologically active natural products from *Antrodiella sp.*, we studied the EtOAC extract of culture of *A. gypsea*, which resulted in the isolation of a new bisabolane-type sesquiterpenoid that was named gypseatriol (**1**) and a known linear sesquiterpene 2,10-dodecadiene-1,6,7-triol (**2**) (Figure 1). The absolute structure of the new compound was determined by X-ray diffraction analysis.

2. Results and discussion

Compound **1** was isolated as colorless crystals (petroleum ether/acetone). The molecular formula of **1** was determined to be $C_{15}H_{24}O_3$ on the basis of HR-EI-MS at *m/z* 252.1713. The 1D NMR spectra displayed signals of a *p*-substituted benzene ring, three oxygenated sp^3 carbons, one methylene, one methine, and four methyls, of which one was doublet, others were singlets. These data exhibited similarities with those of the known compound bisacumol (**1a**) [8], which revealed compound **1** possessing bisabolane sesquiterpenoid skeleton. The notable difference between **1** and **1a** was a double bond at δ_C 128.8 and 133.7 was oxygenated to two hydroxy-substituted carbons at δ_C 76.3 (d) and 74.3 (s),

*Corresponding author. Email: fengtao@mail.kib.ac.cn; jkliu@mail.kib.ac.cn

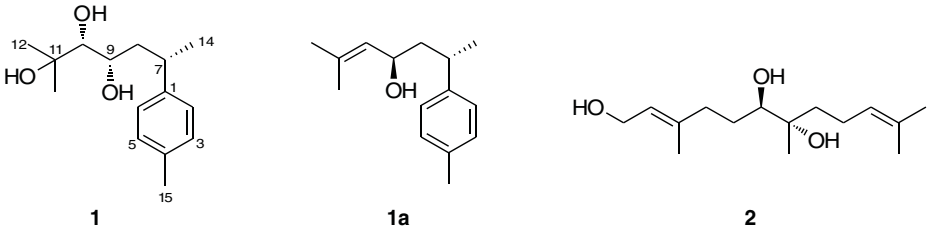

Figure 1. Structures of compounds **1** and **1a**.

respectively. The above assignments were further supported by the HMBC correlations from Me-12 (δ_H 1.16, s)/Me-13 (δ_H 1.26, s) and H-9 (δ_H 3.91, br. t) to C-10 (δ_C 76.3)/C-11 (δ_C 74.3), and from H-8 (δ_H 1.93–1.98 and 1.81–1.85, m) to C-10 (δ_C 76.3) (Figure 2). This enabled completion of the gross structure of **1**, for which we proposed the name gypseatriol.

Generally speaking, it is difficult to determine the absolute configuration of a linear triol by using NMR experiments including compound **1**. While the most direct way to ascertain the absolute configuration of a compound is by X-ray diffraction method. Thus, a single crystal cultivation experiment was carefully and successfully performed. Fortunately, a single crystal X-ray diffraction experiment not only confirmed the planner structure of compound **1** as elucidated above but also determined the absolute stereochemistry to be *7S*,*9S*,*10R* (Figure 3).

The known compound 2,10-dodecadiene-1,6,7-triol (**2**) was identified by spectroscopic analysis [9]. Compound **1** was evaluated for its antifungal activity on *C. albicans*. However, it exhibited no significant inhibitory activity (*c* 128 μg/ml, inhibition rate 10.6%).

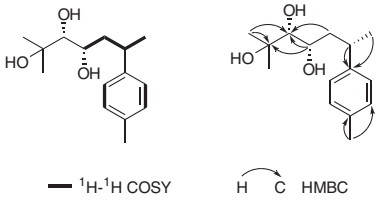

Figure 2. Key 2D NMR correlations of **1**.

3. Experimental

3.1. General experimental procedures

Melting points were measured on an X-4 microscopic melting point meter (Yuhua Instrument Company, Gongyi, China). Optical rotation was obtained on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV spectrum was recorded on Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan). ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance ? 600 MHz spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany). HR-EI-MS was measured on Waters Xevo TQ-S and Waters Autospec Premier P776 mass spectrometers (Waters, Milford, MA, USA). X-ray diffraction was performed on an APEX II·DUO spectrophotometer (Bruker AXS GmbH, Karlsruhe, Germany). Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) was used for column chromatography. Medium Pressure Liquid Chromatography (MPLC) was performed on a Büchi Sepacore System equipping with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan). Preparative High Performance Liquid Chromatography (Prep-HPLC) was performed on an Agilent 1260 liquid chromatography system

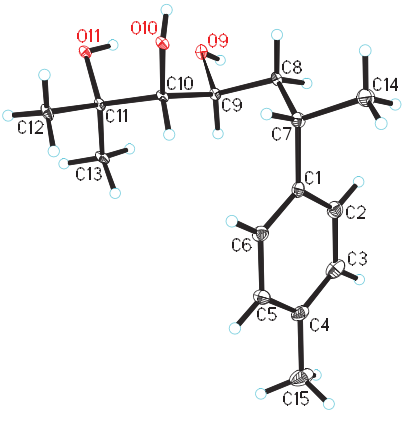

Figure 3. X-ray structure of **1** revealing absolute configuration.

equipped with a Zorbax SB-C18 column (5 μm, 9.4 mm × 150 mm) (Agilent Technologies, Santa Clara, CA, USA).

3.2. *Fungi material*

The fungus *A. gypsea* was collected in Changbai Mountain National Nature Reserve, Jilin Province, China in July 2009, and identified by Prof. Zhu-Liang Yang (Kunming Institute of Botany). A voucher specimen of *A. gypsea* was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (No. HFC 20090825). The culture medium to ferment this fungus consisted of glucose (5%), peptone from porcine meat (0.15%), yeast powder (0.5%), KH_2PO_4 (0.05%) and $MgSO_4$ (0.05%). Five hundred 500-ml Erlenmeyer flasks each containing 350 ml of above-mentioned culture medium were inoculated with *A. gypsea* strains, respectively. Then they were incubated on rotary shakers at 24 °C and 150 rpm for 25 days in dark environment.

3.3. *Extraction and isolation*

The culture broth (20 L) of *A. gypsea* was filtered, and the filtrate was extracted four times with ethyl acetate (EtOAc). Meanwhile, the mycelia were extracted by $CHCl_3$/MeOH (1:1) for three times. The EtOAc layer, together with the mycelia extraction, was concentrated under reduced pressure to afford a crude extract (7.0 g). Then this residue was subjected to Sephadex LH-20 column chromatography ($CHCl_3$:MeOH; 1:1) to decolor, followed by MPLC eluting with MeOH/H_2O

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of **1** and **1a** (δ in ppm, J in Hz, $CDCl_3$).

	1		**1a**(literature data)
No.	δ_H	δ_C	δ_C
1		143.7, s	144.0, s
2	7.10, br. d, 8.4	126.9, d	126.9, d
3	7.12, br. d, 8.4	129.5, d	129.0, d
4		136.0, s	135.1, s
5	7.12, br. d, 8.4	129.5, d	129.0, d
6	7.10, br. d, 8.4	126.9, d	126.9, d
7	2.77–2.83, m	36.6, d	35.8, d
8	1.93–1.98, m1.81–1.85, m	42.6, t	46.0, t
9	3.91, br. t, 6.7	70.1, d	66.7, d
10	3.15, br. s	76.3, d	128.8, d
11		74.3, s	133.7, s
12	1.16, s	26.3, q	18.0, q
13	1.26, s	27.4, q	25.6, q
14	1.27, d, 6.6	23.1, q	23.0, q
15	2.32, s	21.1, q	20.9, q

(10:90→100:0) to give seven main fractions (A-G). Fraction F (0.7 g) was separated by Sephadex LH-20 column chromatography (MeOH) to afford three subfractions (F1-F3). Subfraction F3 (220 mg) was subjected to Sephadex LH-20 column chromatography (acetone) to afford six fractions (F3a-F3f). Fraction F3c (12 mg) was separated on a Prep-HPLC (25–45%, $CH_3CN–H_2O$, 10 ml/min, 20 min, retention time: 12.25 min) to obtain **1** (2.5 mg). Similarly, 2,10-dodecadiene-1,6,7-triol (**2**) (1.1 mg) was obtained from F3a by using Prep-HPLC (20–40%, $CH_3CN–H_2O$, 10 ml/min, 20 min, retention time: 13.1 min)

3.3.1. *Gypseatriol (**1**)*

Colorless crystals (petroleum ether/acetone). m.p. 156–159°C.$[\alpha]_D^{17}$ + 6.44 (*c* 0.12, MeOH). UV (MeOH) λ_{max} (log ϵ): 212 (3.56), 265 (2.37) nm. For ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data, see Table 1. HR-EI-MS *m/z*: 252.1713 $[M]^+$ (calcd for $C_{15}H_{24}O_3$ 252.1725).

3.3.2. *Single crystal cultivation*

Compound **1** (2.5 mg) was dissolved with 1 ml acetone in a small flat bottle, then added 2 ml petroleum ether drop by drop. The solution was sealed with parafilm and punched two small holes. Then the solution was stored in a quiet and dry condition to evaporate the organic solvent. After several days, colorless needles of compound **1** were successfully cultivated.

3.3.3. *Crystallographic data for gypseatriol (**1**)*

Crystallographic data for cu_gypseatriol_0m: $C_{15}H_{24}O_3$, $M = 252.34$, monoclinic, $a = 35.7229(9)$ Å, $b = 5.7993(2)$ Å, $c = 14.0759(3)$ Å, $\alpha = 90.00°$, $\beta = 92.3790(10)°$, $\gamma = 90.00°$, $V = 2913.56(14)$ Å^3, $T = 100(2)$ K, space group *C*2, $Z = 8$, μ(CuKα) = 0.625 mm^{-1}, 12764 reflections measured, 4073 independent reflections (R_{int} = 0.0514). The final R_1 values were 0.0650 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1811 ($I > 2\sigma(I)$). The final R_1 values were 0.0657 (all data). The final $wR(F^2)$ values were 0.1837 (all data). The goodness of fit on F^2 was 1.066. Flack parameter = 0.3(2). The Hooft parameter is 0.28(8) for 1268 Bijvoet pairs. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1047706). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html(or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK.; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).

3.4. *Antifungal activity*

C. albicans (ATCC 32354) was purchased from Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College; Amphotericin B was purchased from Sigma-Aldrich (St. Louis., MO, USA) as the positive control (*c* = 0.5 μg/ml, inhibition rate = 96.9%). The test was performed in potato dextrose agar (PDA). The samples were dissolved in dimethylsulfoxide (DMSO) and diluted to the highest concentrations (128 μg/ml). A volume of 100 μl aliquot from the stock solutions of the samples initially prepared, was added into the 96-well plates. Then 100 μl of the inoculum was added to achieve a final inoculum concentration of 2×10^5 CFU/ml in each well. The final volume in each well was 200 μl. Negative control and positive control were included in every experiment. Read plate at 625 nm after incubation at 30 °C for 24 hours, and calculate the MIC_{90} (minimal inhibitory concentration of 90% of the fungi). The assay was carried out in duplicate.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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