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Tetrahedron Letters 49 (2008) 3042–3044
Tetrahedron Letters

Abiesanol A, a novel biflavanol with unique six connective hexacyclic rings isolated from *Abies georgei*

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Received 18 December 2007; revised 11 March 2008; accepted 14 March 2008
Available online 16 March 2008

Abstract

A novel biflavanol with unique six connective hexacyclic rings, abiesanol A (**1**), was isolated from the aerial parts of *Abies georgei* together with four known flavanols. The structure of new compound was elucidated mainly by the analysis of 1D and 2D NMR spectroscopic data. In addition, single-crystal X-ray diffraction analysis was adopted to confirm the structure of abiesanol A (**1**). In biological assay for inhibitory activities against LPS-induced nitric oxide (NO) production in RAW264.7 macrophages, abiesanol A (**1**) showed potent effects at 50 μg/mL with the inhibition rate of 43.0%. Under the same concentration, abiesanol A (**1**) did not show any cytotoxicity against RAW264.7 macrophages.
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Keywords: *Abies georgei*; Pinaceae; Biflavanol; Abiesanol A; Nitric oxide (NO); RAW264.7 macrophages

Abies is an important genus of the Pinaceae family. There are about 50 species occurring in the highlands of Asia, Europe, North and Middle America, and North Africa.[1] Due to their diverse biological activities, much attention has been paid to this genus.[2] Plants of *Abies georgei* Orr are arbores occurring exclusively in the northwestern Yunnan and the southwestern Sichuan Provinces, China.[1] In our current research for bioactive plants from the natural sources, the EtOAc extract of *A. georgei* showed potent anti-inflammatory activities in vivo and in vitro. Thus, further investigation was carried out to search for the bioactive compounds from this plant, which resulted in the isolation of a new compound (abiesanol A, **1**) and four known flavanols (**2**–**5**). Abiesanol A (**1**) was a novel biflavanol with unique six connective hexacyclic rings by cyclization on C3–*O*–C5′, C4–C4′, and C3′–*O*–C5. This Letter reports the isolation and structural elucidation of abiesanol A (**1**), the inhibitory activity of compounds **1**–**4** against LPS-induced NO production in RAW264.7 macrophages, and the cytotoxicity of **1** against RAW264.7 macrophages.

The EtOAc-soluble fraction of the aerial parts of *A. georgei*[3] was subjected to column chromatography over silica gel, ODS, and Sephadex LH-20, as well as preparative TLC to afford five flavanols,[4] including a novel biflavanol, abiesanol A (**1**), and four known ones. By comparison of their physical and spectroscopic data with the published data,[5–9] the known compounds were identified as (−)-Epiafzelechin (**2**), afzelechin (**3**), (+)-catechin (**4**), and ethyl (+)-cyanidan-3-ol-8-carboxylate (**5**), respectively.

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doi:10.1016/j.tetlet.2008.03.065

Abiesanol A[10] (**1**) was assigned the molecular formula of $C_{30}H_{22}O_{10}$ in the positive HRESIMS at *m/z* 565.1172 $[M+Na]^+$, indicating 20 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3379 cm^{-1}) and aromatic ring (1636, 1517, and 1466 cm^{-1}). However, its ^1H and ^{13}C NMR spectroscopic data of **1** (Table 1) only exhibited 15 carbon signals including a 1,4-substituted phenyl [δ_H 7.47 (2H, d, $J = 8.4$ Hz, H-12,16), 6.85 (2H, d, $J = 8.4$ Hz, H-13,15)], a 1,3,4,5-substituted phenyl [δ_H 5.84 (1H, d, $J = 2.4$ Hz, H-6), 5.74 (1H, d, $J = 2.4$ Hz, H-8)], an oxygenated methine [δ_H 5.10 (1H, s, H-2); δ_C 83.9 (d, C-2)], a sp^3 methine [δ_H 3.56 (1H, s, H-4); δ_C 31.2 (d, C-4)], and an oxygenated quarternary carbon [δ_C 92.5 (s, C-3)]. Therefore, compound **1** should be a complete symmetric structure. In the HMBC experiment, the correlations traced from two methines suggested the presence of a flavanol fragment (Fig. 2). Since **1** had 20 degrees of unsaturation, while two symmetric flavanol moieties simply accounted for eighteens', abiesanol A (**1**) should bear another two rings. This indicated two spatially close hydroxy groups at C-5/5′ should participate in the cyclization. Since C-4/4′ were not oxygenated methines, the hydroxy groups should be attached to C-3/3′, while C-4 to C-4′. As such, abiesanol A (**1**) was connected as shown in Figure 1. The relative configuration was determined mainly by NOESY correlation of H-2/2′ to H-4/4′, which established that H-2/4 and/or H-2′/4′ were cofacial. However, the relative structure of **1** could not be deduced easily because of the configurations in C-3/3′. Fortunately, triclinic crystals of **1** were obtained in MeOH–H_2O for X-ray crystallographic analysis,[11] which confirmed the relative configuration of abiesanol A (**1**) (Fig. 3).

Interestingly, though abiesanol A (**1**) is highly symmetric, it is not a mesomer because it has no symmetric plane, no symmetric center, nor S4 rotation–reflection symmetric axis. Actually, abiesanol A (**1**) is a *C*2 axis-symmetric compound with a large screw-shaped configuration. According to *helix theory* by Yin,[12,13] compounds bearing a large screw-shaped configuration should have a large optical

Table 1
^1H and ^{13}C NMR spectroscopic data for abiesanol A (**1**) in CD_3OD

Position	δ_H[a]	δ_C[b]
2,2′	5.10 s	83.9 d
3,3′		92.5 s
4,4′	3.56 s	31.2 d
5,5′		156.3 s
6,6′	5.84 (d, 2.4)	96.3 d
7,7′		159.0 s
8,8′	5.74 (d, 2.4)	96.7 d
9,9′		154.1 s
10,10′		95.6 s
11,11′		127.2 s
12,16,12′,16′	7.47 (d, 8.4)	132.1 d
13,15,13′,15′	6.85 (d, 8.4)	115.5 d
14,14′		158.9 s

[a] Recorded at 600 MHz. *J* in Hertz within parentheses.
[b] Recorded at 75 MHz.

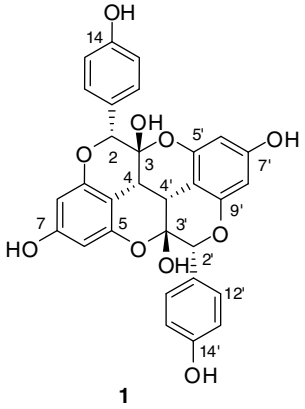

Fig. 1. Chemical structure of abiesanol A (**1**).

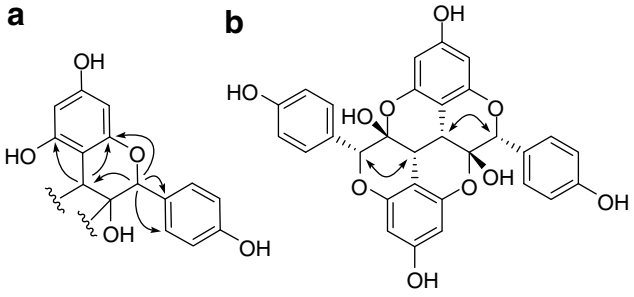

Fig. 2. Key HMBC (a) and NOESY (b) correlations for abiesanol A (**1**).

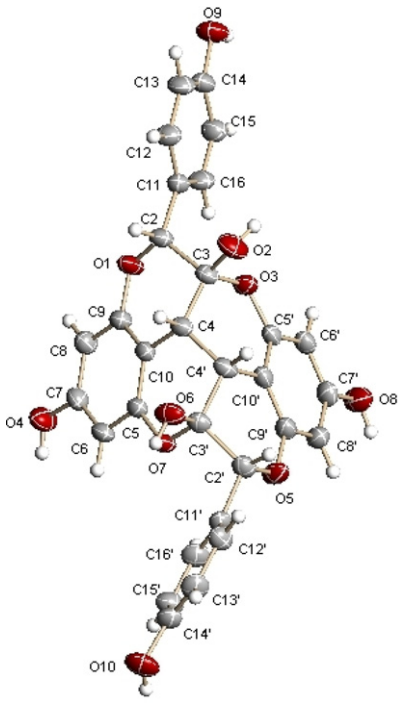

Fig. 3. X-ray crystal structure of abiesanol A (**1**).

Table 2
Effect of compounds **1**–**4** on LPS-induced NO production in RAW264.7 macrophages ($n = 4$, means ± SD)

Groups	Dose	Inhibition rate (%)
Aminoguanidine	25 μM	50.0
Abiesanol A (**1**)	50 μg/mL	43.0
	25 μg/mL	2.0
	10 μg/mL	0
Compounds **2**–**4**	50 μg/mL	0
	25 μg/mL	0
	10 μg/mL	0

Aminoguanidine: positive control.

rotation value. This was further confirmed by the large optical rotation value ($[\alpha]_D^{20}$ −210) of abiesanol A.

Inflammatory process involved three relevant targets including arachidonic acid (AA) metabolite pathways, nitric oxide (NO) and NF-kB.[14] The inhibition of NO release may be effective as a therapeutic agent.[15] Therefore, the ability of four isolates (**1**–**4**) to inhibit LPS-stimulated NO production was measured in RAW264.7 macrophages according to the reported protocol.[16] The results showed that LPS could significantly increase the NO production ($p < 0.01$), while abiesanol A (**1**), in a concentration of 50 μg/mL, exhibited remarkable inhibitory activity against the increase of NO production with the inhibition rate of 43% (Table 2). In another experiment by MTT assay, abiesanol A (**1**) did not show any cytotoxicity on RAW264.7 macrophages under the same concentration.

Acknowledgments

We acknowledge the Pharmaceutical Analysis Center of Second Military Medical University for measuring the IR and NMR spectra. We also thank the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences for the X-ray diffraction analysis. The work was supported by China Postdoctoral Science Foundation (20070420674), Shanghai Postdoctoral Science Foundation (07R214163), Changjiang Scholars and Innovative Research Team in University (PCSIRT), NCET Foundation, NSFC (30725045), National 863 Program (2006AA02Z338), Shanghai Leading Academic Discipline Project (B906), and in part by the Scientific Foundation of Shanghai China (07DZ19728, 06DZ19717, 06DZ19005).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.03.065.

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3. The aerial part of *A. georgei*, collected from Zhongdian city, Yunnan Province of China in July 2006, was identified by Professor Li-Shang Xie, Kunming Institute of Botany, Chinese Academy of Sciences. A herbarium specimen was deposited in School of Pharmacy, Second Military Medical University, China (herbarium No. 2006-07-016).
4. The air-dried material (22 kg) was finely pulverized and extracted with 80% EtOH under reflux for 3 × 3 h. The combined extracts were concentrated to a small volume in vacuo and then partitioned, successively, with $CHCl_3$ (25 L), EtOAc (40 L), and *n*-BuOH (50 L). The EtOAc extract (282 g) was separated into six fractions (F_1–F_6) by CC on silica gel (100–200 mesh) using a $CHCl_3$–Me_2CO gradient. Fraction F_4 (47.1 g) was subjected to RP-18 CC eluted with gradient MeOH–H_2O to give 13 subfractions (F_{S1}–F_{S13}). From subfraction F_{S1} (5.4 g), repeated CC over LH-20 with $CHCl_3$–MeOH (1:1) and MeOH afforded (+)-catechin (**4**, 2.6 g) and ethyl (+)-cyanidan-3-ol-8-carboxylate (**5**, 4.8 mg). Subfraction F_{S2} (3.6 g) was divided into four parts (P_{S1}–P_{S4}) by CC eluting with $CHCl_3$–MeOH (1:1). (−)-Epiafzelechin (**2**, 48.2 mg) was obtained from P_{S1} by LH-20 with MeOH. P_{S2} (120 mg) and P_{S3} (180 mg) were further separated by repeated preparative TLC with $CHCl_3$–MeOH (5:1) to give afzelechin (**3**, 108.5 mg), respectively. Subfraction F_{S4} was subjected to CC over Sephadex LH-20 with $CHCl_3$–MeOH (1:1), and preparative TLC with $CHCl_3$–MeOH (5:1) to give **1** (26.4 mg).
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10. *Abiesanol A* (**1**): Pale yellow triclinic crystals; $[\alpha]_D^{20}$ −210 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ε): 229 (4.37), 273 (3.23), 312 (2.43); CD (MeOH) $\Delta\varepsilon_{227}$ −300.6, $\Delta\varepsilon_{276}$ +31.5; IR (KBr) ν_{max} 3379, 2914, 1636, 1602, 1517, 1499, 1466, 1446, 1395, 1264, 1140, 1054, 899, 814, 618 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; ESIMS (negative) 541.3 $[M-H]^-$, 577.4 $[M+Cl]^-$; HRESIMS (positive) $[M+Na]^+$ *m*/*z* 565.1172, calcd for $C_{30}H_{22}O_{10}Na$, 565.1111.
11. Pale yellow triclinic crystal of $C_{30}H_{22}O_{10}\cdot 2H_2O$. Space group *P*1, $a = 6.300(5)$ Å, $\alpha = 70.323(15)°$; $b = 8.938(7)$ Å, $\beta = 77.243(15)°$; $c = 12.522(11)$ Å, $\gamma = 76.697(14)°$; $V = 638.2(9)$ $Å^3$, $Z = 1$; crystal size $0.439 \times 0.327 \times 0.211$ mm^3. A total of 3751 unique reflections ($\theta = 1.75$–$27.00°$) were collected using graphite monochromated Mo Kα ($\lambda = 0.71073$ Å) on a CCD area detector diffractometer. The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (SHELXS-97). The final cycle of full-matrix least-squares refinement was based on 2698 data, 8 restraints and 408 variable parameters. Final *R* indicates $R_1 = 0.0352$, $wR_2 = 0.0850$ [$I > 2\sigma(I)$]. Crystallographic data (excluding structure factors) for the structure of abiesanol A (**1**) in this paper have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 665567. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
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