DOI: 10.1002/ejoc.201001203

Abiespiroside A, an Unprecedented Sesquiterpenoid Spirolactone with a 6/6/5 Ring System from *Abies delavayi*

Xian-Wen Yang,[a,b] Su-Mei Li,[b] Yong-Li Li,[a] Jia-Han Xia,[a] Liang Wu,[a] Yun-Heng Shen,[a] Jun-Mian Tian,[a] Ning Wang,[c] Yonghong Liu,[b] and Wei-Dong Zhang*[a]

Keywords: Natural products / Terpenoids / Spiro compounds / Lactones / *Abies delavayi* / Nitric oxide

Abiespiroside A (**1**), a unique sesquiterpenoid spirolactone with a 6/6/5 ring system, was isolated from *Abies delavayi* Franch. The structure was established as (1*R*,5*R*,6*R*,7*S*,9*R*)-6,9α-epoxy-9,15-bisabolanolide-5-*O*-β-D-glucopyranoside by extensive analysis of various spectroscopic data and further confirmation by the single-crystal X-ray diffraction. It showed potent inhibitory activity against the production of nitric oxide in RAW264.7 macrophages stimulated by lipopolysaccharides.

Introduction

2(5*H*)-Furanones are not only one of the commonly found structural units in natural products,[1] but also important intermediates in organic synthesis.[2] Compounds containing such moieties have been considered to possess diverse forms of bioactivity, such as antiinflammatory, antitumor, antibacterial, and insecticide activity.[3] Thus, much attention has been focused on the efficient and diverse syntheses of this moiety.[4] However, spiro-2(5*H*)-furanone moieties are rarely obtained from natural sources, especially from sesquiterpenoids.

Abies dalavayi Franch. var. *delavayi* are tall trees occurring exclusively in highlands of about 3,300–4,000 m high in the northwest of the Yunnan and southeast of the Tibet provinces of China.[5] Previously, a novel sesquiterpenoid was discovered from this plant with potent inhibitory effect against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7.[6] Further investigations on *A. delavayi*, however, resulted in the isolation of a unique sesquiterpenoid spirolactone, abiespiroside A (**1**) (Figure 1). In this communication, the isolation and structure elucidation of **1** is reported together with its bioactivity against LPS-induced NO production in RAW264.7 macrophages.

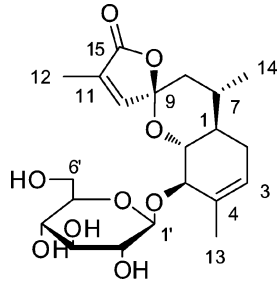

Figure 1. Structure of abiespiroside A (**1**).

Results and Discussion

Monoclinic crystals of abiespiroside A (**1**) were assigned the molecular formula $C_{21}H_{30}O_9$ as evidenced by the HR-ESI-MS (negative) peak at *m*/*z* = 425.1821 $[M - H]^-$ (calcd. for $C_{21}H_{29}O_9$, 425.1812), implying seven unsaturation indices of the molecule. Its IR spectrum displays absorption bands characteristic of hydroxy (3478 cm^{-1}), carbonyl (1772 cm^{-1}), and olefinic bonds (1444 and 1381 cm^{-1}). The ^1H NMR spectrum shows two olefinic protons [δ_H = 6.95 (d, *J* = 1.2 Hz, 1 H, 10-H), 5.50 (d, *J* = 4.2 Hz, 1 H, 3-H) ppm], two oxymethine groups [δ_H = 4.21 (d, *J* = 7.5 Hz, 1 H, 5-H), 3.95 (dd, *J* = 11.4, 7.5 Hz, 1 H, 6-H) ppm], two doublet methyl groups at olefinic bonds [δ_H = 1.88 (d, *J* = 1.8 Hz, 3 H, 12-Me), 1.81 (d, *J* = 0.6 Hz, 3 H, 13-Me) ppm], and one doublet methyl at the sp^3 carbon atom [δ_H = 0.96 (d, *J* = 6.6 Hz, 3 H, 14-Me) ppm]. In addition, another oxymethine at δ_H = 4.47 (d, *J* = 7.8 Hz, 1 H) ppm, and six

[a] Department of Natural Product Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China
Fax: +86-21-81871244
E-mail: wdzhangy@hotmail.com
[b] Key Laboratory of Marine Bio-resources Sustainable Utilization, Guangdong Key Laboratory of Marine Materia Medica, and RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, No. 164 West Xingang Road, Guangzhou 510301, China
[c] Luxembourg Public Research Center for Health (CRP-SANTE), 84, Val Fleuri, 1526 Luxembourg

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201001203.

proton signals ranging from δ_H = 3.0 to 3.8 ppm suggest the presence of a glucopyranose. These signals were all observed in the ^{13}C NMR spectrum as four methine peaks [δ_C = 149.0 (d), 124.8 (d), 83.2 (d), 80.7 (d) ppm], three methyl peaks [δ_C = 10.4 (q), 18.8 (q), 20.1 (q) ppm], and one peak for a glucose moiety [δ_C = 104.4 (d), 78.3 (d), 77.5 (d), 71.2 (d), 62.5 (t) ppm]. In addition, the ^{13}C NMR spectrum showed eight more carbon signals, including two methylene, two methine, and four quaternary carbon peaks due to a carbonyl group [δ_C = 173.7 (s) ppm], two olefinic quaternary carbon atoms [δ_C = 133.0 (s), 135.4 (s) ppm], and one hemiketal carbon atom [δ_C = 106.5 (s) ppm]. Altogether, the ^1H, ^{13}C, and Distortionless Enhancement of Polarization Transfer (DEPT) NMR spectra of **1** exhibited 21 carbon atoms with six from a glucopyranose moiety and the other fifteen from a sesquiterpene unit.

According to the correlations on the ^1H–^1H COSY spectrum of **1**, two fragments were deduced as C-1/C-2/C-3/C-13(4),C-5/C-6/C-1/C-7/C-8(14) and C-10/C-12(11) (Figure 2). In the Heteronuclear Multiple Bond Coherence (HMBC) spectrum, correlations of H_3-12 to C-10,11,15, H_3-14 to C-1,7,9, and 10-H to C-8,9 (Figure 2) suggested the presence of a cyclopentene lactone. In addition, 1-H′ (δ_H = 4.47 ppm) was correlated to C-5 [δ_C = 83.2 (d)], which implied that the glucopyranose group was attached to the C-5 position of the aglycon. Since the molecule bears seven unsaturation degrees, while one cyclopentane lactone, two olefinic bonds, a cyclohexane, and a glucose account for six, there should be another ring in the structure. Taking the molecular formula into account, the planar structure of **1** could then be constructed as shown in Figure 1 by the connection of C-6 to C-9 via an ether bond.

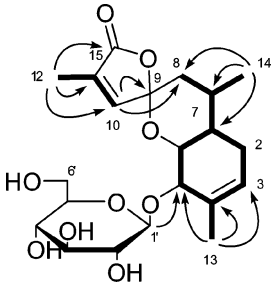

Figure 2. Key ^1H–^1H COSY (bold) and HMBC (arrow) correlations of **1**.

On the basis of the coupling constants of 5-H/6-H, 6-H/1-H, and 1-H/7-H (J = 7.5, 11.4, and 10.8 Hz, respectively), 5-H/1-H and 6-H/7-H were supposed to be cofacial. This could be further supported by the strong NOESY correlations of 1-H to 5-H, 14-H_3, and 6-H to 7-H (Figure 3).

However, the stereochemistry of cyclopentene lactone could not be clarified because 10-H was correlated to both 8-Hα and 8-Hβ in the NOESY experiment, which prevented the establishment of the relative configuration of **1**. Thus, further solid evidence, such as X-ray diffraction, was necessary. Fortunately, monoclinic crystals of **1** were obtained in a mixed solution of $CHCl_3$/MeOH/H_2O and an X-ray crystallographic analysis was realized, which confirmed without doubt the relative stereochemistry of abiespiroside A (**1**) (Figure 4).

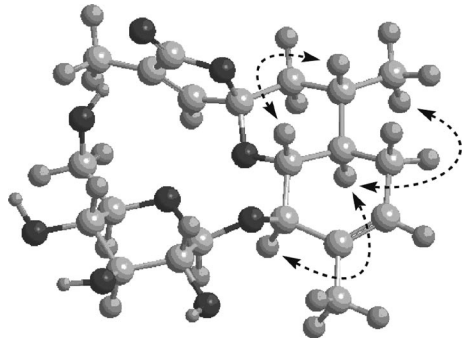

Figure 3. Key NOESY correlations of **1**.

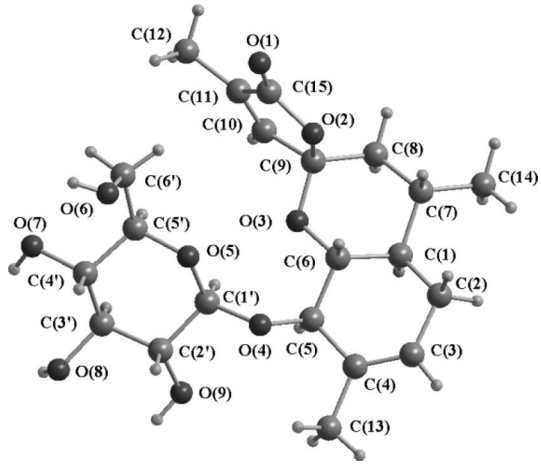

Figure 4. X-ray structure of abiespiroside A (**1**).

By acid hydrolysis of compound **1**, the hexose was proved to be D-glucopyranose, which was detected with TLC by comparison with an authentic sample, and the configuration was determined by measurement of the optical rotation value. The β-anomeric configuration for the glucose was judged from its large coupling constant ($^3J_{H1',H2'}$ = 7.8 Hz).[7] According to the IUPAC sequence rule,[8] the configurations of the five chiral centers at C-1/C-5/C-6/C-7/C-9 were deduced as *R*/*R*/*R*/*S*/*R*, respectively.

The inhibition of nitric oxide (NO) release was regarded as a therapeutic effect for many kinds of inflammatory diseases, such as arthritis. Therefore, the ability of abiespiroside A (**1**) to inhibit LPS-stimulated NO production was measured in RAW264.7 macrophages according to the previously described protocol.[9] Abiespiroside A exhibits a significant effect at 100 μg/mL with an inhibition rate of 35.0%, compared to 51.2% for aminoguanidine (the positive control) at 25.0 μM.

Conclusions

Abiespiroside A (**1**), a unique sesquiterpenoid spirolactone with a novel 6/6/5 ring system, was isolated from *Abies*

delavayi. The absolute configuration of the compound was elucidated unambiguously according to various spectroscopic data, single-crystal X-ray diffraction analysis, and acid hydrolysis.

Experimental Section

General Procedures: NMR spectra were recorded by using a Bruker Avance 600 NMR spectrometer with TMS as internal standard. ESI-MS measurements were performed with an Agilent LC/MSD Trap XCT spectrometer (Waters) and HR-ESI-MS with a Q-TOF micro mass spectrometer. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. IR spectra were recorded with a Bruker Vector-22 spectrometer by using KBr pellets. Materials for column chromatography (CC) were silica gel (Huiyou Silical Gel Development Co. Ltd., Yantai, China), Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (YMC, USA).

Plant Material: The aerial parts of *A. delavayi* were collected in Dali city of Yunnan Province, China, and identified by Prof. L. S. Xie of Kunming Institute of Botany, Chinese Academy of Sciences. A herbarium specimen is deposited in the School of Pharmacy, Second Military Medical University, China (herbarium No. 2006-07-0101).

Isolation: The air-dried samples (14 kg) were extracted with 85% EtOH. The resin was filtered, and the filtrate was successively partitioned with $CHCl_3$, EtOAc, and *n*BuOH. The EtOAc-soluble extract and the *n*BuOH-soluble extract were combined and chromatographed over a silica gel column eluting with a gradient of $CHCl_3/Me_2CO$ (100:0→0:100) to give eight fractions (Fr. 1–8). Fr. 6 was divided into 11 subfractions (Fr. 6.1–6.11) by RP-MPLC (Büchi) eluting with MeOH/H_2O (5:95–100:0). Fraction Fr. 6.8 (705 mg) was subjected to CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), followed by repeated recrystallization to give abiespiroside A (**1**, 125.0 mg).

Abiespiroside A (1): Monoclinic crystals; m.p. 133–135 °C. $[\alpha]_D^{20}$ = +17.3 (c = 0.34, MeOH). UV (MeOH): λ_{max} (log ε) = 219 (3.80) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3478, 3211, 2975, 2929, 2877, 1772, 1444, 1381, 1270, 1214, 1183, 1096, 1025, 992, 849, 791, 601 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 1. ESI-MS (positive) m/z = 449 $[M + Na]^+$, 875 $[2M + Na]^+$. ESI-MS (negative) m/z = 425 $[M - H]^-$, 851 $[2M - H]^-$. HR-ESI-MS (negative): calcd. for $C_{21}H_{29}O_9$ $[M - H]^-$ 425.1812; found 425.1821.

Crystal Data for Abiespiroside A (1): Colorless monoclinic crystal of $C_{21}H_{30}O_9$. Space group $P2(1)$, a = 9.018(7) Å, α = 90.0°; b = 5.918(4) Å, β = 92.424(9)°; c = 20.075 (14) Å, γ = 90.0°; V = 1070.4(13) $Å^3$, Z = 2; crystal size 0.25 × 0.10 × 0.04 mm. A total of 4850 unique reflections (θ = 2.03–26.01°) were collected by using graphite monochromated Mo-K_α (λ = 0.71073 Å) with a CCD area detector diffractometer. The structure was solved by direct methods (SHELXS-97)[10] and expanded by using Fourier techniques (SHELXS-97). The final cycle of full-matrix least-squares refinement was based on 3781 data, 1 restraint, and 272 variable parameters. Final R values are R_1 = 0.0466, wR_2 = 0.1106 [$I > 2\sigma(I)$]. CCDC-773749contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acid Hydrolysis of Compound 1: Compound **1** (10.0 mg) was resolved in HCl (6%, 5 mL) and kept at 80 °C for hydrolysis. Three hours later, the reaction solution was extracted with EtOAc. The aqueous phase was condensed to afford D-glucose (3.7 mg), which was detected with TLC by comparison with an authentic sample, and its configuration was determined by measurement of its optical rotation value, $[\alpha]_D^{25}$ = +94.7 (c = 0.75, MeOH).

Table 1. ^1H and ^{13}C NMR data for compound **1** in CD_3OD.[a]

No.	δ_H [ppm] (J [Hz])	δ_C [ppm]
1	1.33 (ddt, 11.4, 10.8, 4.8)	42.9 (d)
2	2.34 (ddt, 11.4, 4.8); 1.73 m	29.1 (t)
3	5.50 (d, 4.2)	124.8 (d)
4		135.4 (s)
5	4.21 (d, 7.5)	83.2 (d)
6	3.95 (dd, 11.4, 7.5)	80.7 (d)
7	1.84 m	32.2 (d)
8	1.69 (dd, 13.5, 4.2); 1.66 (d, 13.5)	41.1 (t)
9		106.5 (s)
10	6.95 (q, 1.6)	149.0 (d)
11		133.0 (s)
12	1.88 (d, 1.6)	10.4 (q)
13	1.81 (d, 0.6)	20.1 (q)
14	0.96 (d, 6.6)	18.8 (q)
15		173.7 (s)
1′	4.47 (d, 8.0)	104.4 (d)
2′	3.15 (dd, 9.2, 8.0)	75.6 (d)
3′	3.27 (t, 9.2)	78.3 (d)
4′	3.35 (dd, 9.4, 9.2)	71.2 (d)
5′	3.07 (ddd, 9.4, 4.3, 2.7)	77.5 (d)
6′	3.71 (dd, 11.4, 2.7); 3.60 (dd, 11.4, 4.3)	62.5 (t)

[a] Data were recorded with a Bruker Avance 600 spectrometer; chemical shifts (δ) are given in parts per million with references to the center peak of CD_3OD with δ = 3.30 ppm for ^1H and δ = 49.0 ppm for ^{13}C.

Measurement of NO in LPS-activated Macrophages for Compound 1: RAW 264.7 macrophages were seeded into 24-well cell culture plates (10^5 cells/well). The cells were co-incubated with compound **1** and LPS (1 μg/mL) for 24 h. For the positive control group, the cells were co-incubated with aminoguanidine and LPS. The amount of NO was assessed by determining the nitrite concentration in the culture supernatants with Griess reagent in RAW264.7. The absorbance was read by using a microtiter plate reader at 570 nm.

Supporting Information (see footnote on the first page of this article): 1D and 2D NMR spectra for **1**.

Acknowledgments

Financial support for this work is greatly appreciated from the China Postdoctoral Science Foundation (20070420674), the Shanghai Postdoctoral Science Foundation (07R214163), the Research Network for Applied Microbiology (RNAM), the Center for Marine Microbiology (KSCX2-YW-G-073), the Shanghai Leading Academic Discipline Project (B906), the Scientific Foundation of Shanghai China (09DZ1975700, 09DZ1971500), the Special Program for New Drug Innovation of the Ministry of Science and Technology, China (2009ZX09311-001, 2008ZX09308-005), and the United States Department of Agriculture (USDA) Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009.

[1] T. Murakami, Y. Morikawa, M. Hashimoto, T. Okuno, Y. Harada, *Org. Lett.* **2004**, *6*, 157–160.
[2] X. Cheng, X. Jiang, Y. Yu, S. Ma, *J. Org. Chem.* **2008**, *73*, 8960–8965.

[3] a) V. E. J. Hikawczuk, J. R. Saad, O. S. Giordano, C. Garcia, T. Martin, V. S. Martin, M. E. Sosa, C. E. Tonn, *J. Nat. Prod.* **2008**, *71*, 190–194; b) M. Arno, L. Betancur-Galvis, M. A. Gonzalez, J. Sierra, R. J. Zaragoza, *Bioorg. Med. Chem.* **2003**, *11*, 3171–3177.
[4] a) S. N. Patil, F. Liu, *Org. Lett.* **2007**, *9*, 195–198; b) N. Maulide, I. E. Marko, *Org. Lett.* **2006**, *8*, 3705–3707.
[5] W. J. Zheng, L. G. Fu in *Flora of China*, vol. 7 (Ed.: Z. Y. Wu), Science Press, Beijing, **1978**, pp. 76–77.
[6] X. W. Yang, Y. Ding, X. C. Li, D. Ferreira, Y. H. Shen, S. M. Li, N. Wang, W. D. Zhang, *Chem. Commun.* **2009**, 3771–3773.
[7] X. W. Yang, Y. L. Ma, H. P. He, Y. H. Wang, Y. T. Di, H. Zhou, L. Li, X. J. Hao, *J. Nat. Prod.* **2006**, *69*, 971–974.
[8] J. Rigaudy, S. P. Klesney (Eds.), *IUPAC Nomenclature of Organic Chemistry, Sections A–H*, Pergamon, New York, **1979** (Recommendation for section A, Spiro hydrocarbons).
[9] X. W. Yang, H. W. Zeng, X. H. Liu, S. M. Li, W. Xu, Y. H. Shen, C. Zhang, W. D. Zhang, *J. Pharm. Pharmacol.* **2008**, *60*, 937–941.
[10] G. M. Sheldrick, *Acta Crystallogr. A* **2008**, *64*, 112–122.

Received: August 25, 2010
Published Online: October 19, 2010

