DOI: 10.1002/cjoc.201200011

A New Guaiane-type Sesquiterpene with 15 Known Compounds from *Wikstroemia scytophylla* Diels†

Jiang, Hezhong[a,c](蒋合众) Ma, Qingyun[b](马青云) Huang, Shengzhuo[a](黄圣卓)
Liang, Wenjuan[a](梁文娟) Wang, Pengcheng[a](王鹏程) Hu, Jiangmiao[a](胡江苗)
Zhou, Jun[a](周俊) Zhao, Youxing*[,b](赵友兴)

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China*
[b] *Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan 571101, China*
[c] *School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan 610031, China*

A new guaiane-type sesquiterpene, wiksphyllamin A (**1**) with C-10 and C-11 connected, along with fifteen known compounds were isolated from the stems of *Wikstroemia scytophylla* Diels. All these compounds were isolated for the first time from *Wikstroemia scytophylla*. The structures of **1**—**16** were identified using spectroscopic methods.

Keywords guaiane-type sesquiterpene, *Wikstroemia scytophylla* Diels, wiksphyllamin A

Introduction

Wikstroemia scytophylla Diels, a shrub in the family Thymelaeaceae, is regionally distributed in the provinces of Yunnan, Sichuan and Tibet in China. There is no report about the chemical constituent and bioactivity of *W. scytophylla*. *Wikstroemia indica*, the same genus with *W. scytophylla*, has long been used as a traditional crude drug for the treatment of pneumonia, rheumatism, and bronchitis in China.[1] *Wikstroemia indica* has many functional constituents including flavonoids, coumarins and lignans.[1-3] This paper deals with the isolation and structure elucidation of a new compound, wiksphyllamin A (**1**) on the basis of ^{1}H and ^{13}C NMR, DEPT spectroscopic studies, as well as 2D-NMR such as COSY, ROESY, and HSQC.

Results and Discussion

The investigation of the stems of *W. scytophylla* led to the isolation of sixteen compounds (**1**—**16**), including a new guaiane-type sesquiterpene named wiksphyllamin A (**1**). All these compounds were isolated for the first time from *W. scytophylla*, and the structures (Scheme 1) of **1**—**16** were identified as wiksphyllamin A (**1**), 4-hydroxy-benzaldehyde (**2**),[4] 4-hydroxybenzoic acid (**3**),[5] 4-hydroxy-3-methoxy-benzaldehyde (**4**),[6] (*E*)-coniferaldehyde (**5**),[7] 3-(3,4-dihydroxyphenyl)-(*E*)-2-propenoic acid heptyl ester (**6**),[8] eicosanyl (*E*)-caffeate (**7**),[9] docosyl-3,4-dihydroxy-transcinnamate (**8**),[10] daphnodorin B (**9**),[11] wikstrol A (**10**),[12] isolariciresinol (**11**),[13] acacetin (**12**),[14] afzelechin (**13**),[15] (−)-farrerol (**14**),[16] ayapanin (**15**),[17] isodaphnoretin (**16**),[18] respectively, by analysis of MS and NMR data, and comparison with those in the literature.

Compound **1** was obtained as a white powder with m.p. 185—187 ℃ and $[\alpha]_D^{16.7}$ +69.3421 (c=0.152, CH_3OH). The molecular formula was deduced as $C_{15}H_{20}O_4$ through the analysis of HR-ESI-MS (m/z= 287.1265 $[M+Na]^+$, calcd for $C_{15}H_{20}O_4Na$ 287.1259), indicating 6 degrees of unsaturation. The IR spectrum showed absorption bands at 3463, 1751 and 1720 cm^{-1}, which were indicative of hydroxy groups, carbonyls bonds. The ^{13}C NMR (DEPT) (see Table 1) spectrum of **1** showed 15 carbon resonances, including two ketone carbonyls (δ_C 216.6 and 215.1), an aldehyde carbonyl (δ_C 206.4), a hydroxylated quaternary carbon (δ_C 81.5), two quaternary carbons (δ_C 60.5 and 43.1), three methines, three methylenes, and three methyl groups (δ_C 10.7, 14.8 and 19.2). Detailed inspection of the NMR data of **1** revealed this compound to be a guaiane-type sesquiterpene. And the skeleton of **1** was very similar to that of a known compound, 7-hydroxy-8-oxo-11(1→10)-abeo-1-patchoulene-12-al,[19] except for the occurrence of signals due to a ketone carbonyl [δ_C 216.6 (C-2)],

* E-mail: zhaoyx1011@163.com; Tel.: 0086-0898-66989095; Fax: 0086-0898-66989095
Received January 6, 2012; accepted March 11, 2012; published online June 1, 2012.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cjoc.201200011 or from the author.
† Dedicated to Professor Jun Zhou on the occasion of his 80th birthday.

Scheme 1 Structures of compounds **1**—**16**

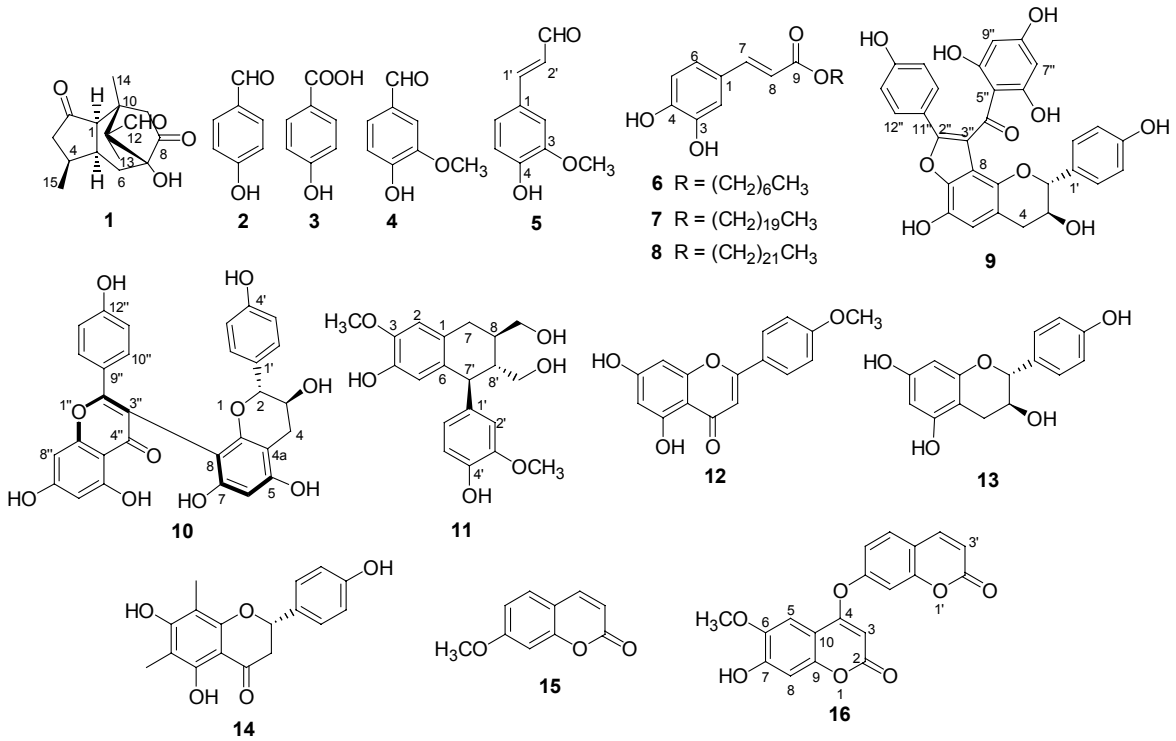


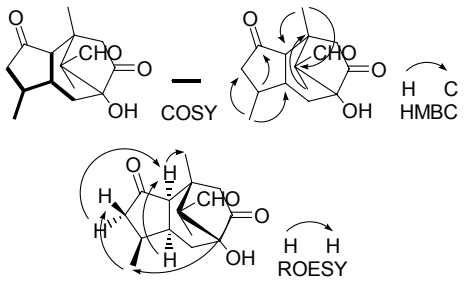
Table 1 ^{1}H NMR and ^{13}C NMR data of **1** in $CDCl_3$

No.	δ ^{13}C	δ ^{1}H
1	60.1, d	2.06 (d, J=7.9 Hz, 1H)
2	216.6, s	—
3	44.6, t	2.43—2.40 (m, 1H) 2.27—2.26 (m, 1H)
4	31.3, d	2.31—2.28 (m, 1H)
5	36.7, d	2.47—2.44 (m, 1H)
6	30.4, t	1.79 (dd, J=13.4, 13.4 Hz, 1H) 1.62 (dd, J=13.4, 7.4 Hz, 1H)
7	81.5, s	—
8	215.1, s	—
9	49.8, t	2.23 (s, 2H)
10	43.1, s	—
11	60.5, s	—
12	206.4, d	9.61 (s, 1H)
13	10.7, q	1.26 (s, 3H)
14	19.2, q	1.58 (s, 3H)
15	14.8, q	1.07 (d, J=6.4 Hz, 3H)

and a methine group [δ_C 60.1 (C-1), δ_H 2.06 (1H, d, J=7.9 Hz)], instead of two olefinic carbons at C-1 and C-2 in 7-hydroxy-8-oxo-11(1→10)-abeo-1-patchoulene-12-al.

The guaiane-type sesquiterpene skeleton in compound **1** could be further confirmed through 2D NMR spectra. The ^{1}H-^{1}H COSY spectrum displayed correlations of H-1/H-5, H-6/H-5, H-3/H-4, H-5/H-4 and H-15/H-4, consistent with the fragment shown with bold lines in Scheme 2. The HMBC (Scheme 2) correlations of **1** from H-1 to C-2, C-5, C-6 and C-7, H-5 to C-3, C-4, C-6 and C-7, and H-6 to C-4, C-7 and C-8, also were accord with the guaiane-type sesquiterpene. The linkage between C-10 and C-11 forming the carbon bridge was established by the key HMBC correlations from H-14 to C-10 and from H-13 to C-10. The above evidence allowed the elucidation of the planar structure of **1**. The relative configurations at C-1, C-4, C-5, C-7, C-10, and C-11 in **1** were deduced by a ROESY NMR experiment (Scheme 2) to be similar to those of 7-hydroxy-8-oxo-11(1→10)-abeo-1-patchoulene-12-al.[19] The α-orientations of H-1, H-4, H-15 and C-14 were established by the NOE correlations of H-14/H-1, H-1/H-4, H-1/H-3α, H-15/H-3β and H-15/H-13. Thus, the structure of compound **1** was assigned as shown and this compound has been named as wiksphyllamin A.

Scheme 2 The key 2D NMR correlations of **1**



The *in vitro* cytotoxicity of compound **1** was also tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetra-

zolium bromide (MTT) methods. Compound **1** showed poor cytotoxicity against five tumor cell lines MCF-7, SMMC-7721, HL-60, A-549, and SW480, with IC_{50} values above 40 μmol•L^{-1}, more higher than the positive control Taxol (IC_{50}=0.008 μmol•L^{-1}).

Experimental

General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu double-beam 210A spectrometer. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400, a DRX-500, or AVANCE III-600 spectrometer with TMS as an internal standard. ESI-MS and HR-ESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. EI-MS and HR-EI-MS were recorded with a Waters Autospec Premier. Silica gel (200—300 mesh, Qingdao Marine Chemical Inc., People's Republic of China), RP-18 (40—70 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating after spraying with 10% H_2SO_4 in ethanol.

Plant material

The stems of *Wikstroemia scytophylla* D. were collected in Diqing Tibetan Autonomous Prefecture, Yunnan Province, People's Republic of China, and identified by Dr. L. L. Yue. Voucher specimen (JHZ0002) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and isolation

The dried and powdered stems of *Wikstroemia scytophylla* D. (3.0 kg) were extracted with 95% EtOH under reflux for three times (10 L×3). The extract was concentrated and suspended in water followed by successive partition with petroleum ether (5 L×3), EtOAc (5 L×3), and *n*-BuOH (5 L×3), respectively. The EtOAc extract (320 g) was chromatographed over silica gel (4.9 kg, 200—300 mesh) using a gradient solvent petroleum ether/acetone (100∶1, 98∶2, 95∶5, 90∶10, 80∶20, 70∶30, 60∶40, *V*∶*V*) to afford six fractions. Fraction 2 (35 g) was subjected to silica gel column using a gradient solvent petroleum ether/acetone (4∶1—0∶1) to afford fractions 2-1—2-7. Fraction 2-4 (10.5 g) gel was filtrated on Sephadex LH-20 ($CHCl_3$/MeOH, 1∶1) to give nine subfractions (2-4-1—2-4-9). Fraction 2-4-3 (4.2 g) was subjected to repeated RP-18 (MeOH/H_2O, 60%, 70%, 80%, 90%, and 100%) and Sephadex LH-20 ($CHCl_3$/MeOH, 1∶1) to yield compounds **1** (16 mg), **5** (3 mg) and **6** (12 mg). Fraction 2-4-4 (3.7 g) was further purified on repeated RP-18 (MeOH/H_2O, 70%, 80%, 90%, and 100%) and Sephadex LH-20 ($CHCl_3$/MeOH, 1∶1) to give compounds **7** (6 mg), **8** (6 mg), **12** (40 mg), **14** (336 mg), and **15** (7 mg). Fraction 4 (110 g) was chromatographed on silica gel column using a gradient solvent petroleum ether/acetone (2∶1—0∶1) to give six fractions (4-1—4-6). Fraction 4-3 (26.5 g) was further separated on repeated RP-18 (MeOH/H_2O, 60%, 70%, 80%, 90%, and 100%) and Sephadex LH-20 ($CHCl_3$/MeOH, 1∶1) to afford compounds **2** (4 mg), **3** (7 mg), **4** (5 mg), **13** (450 mg), and **16** (4 mg). Fraction 4-4 (15 g) was separated on silica gel column using a gradient solvent $CHCl_3$/MeOH (10∶1—1∶1, *V*∶*V*) to give seven fractions (4-4-1—4-4-7). Fraction 4-4-1 (261 mg) was further purified on Sephadex LH-20 ($CHCl_3$/MeOH, 1∶1) to give **11** (11 mg). Further purification of 4-4-6 (1.42 g) was submitted to Sephadex LH-20 ($CHCl_3$/MeOH, 1∶1) to yield **9** (19 mg) and **10** (8 mg).

Cytotoxic assays

Compound **1** was dissolved with DMSO to a stock concentration of 10 mmol•L^{-1} and then diluted to the required concentrations with the medium. Cytotoxicity of compound **1** against five human tumor cell lines: HL-60 (leukemia), SMMC-7721 (liver carcinoma), A-549 (lung carcinoma), MCF-7 (mammary carcinoma), and SW480 (colon carcinoma) was measured. Briefly, cells were placed in 96-well plates for 12 h before treatment with initial density of 5000 cells/well and continuously exposed to different concentrations (40, 8, 1.6, 0.32, and 0.064 μmol•L^{-1}) of compound for 48 h, with cisplatin (Sigma, USA) as the positive control. Inhibition rates of cell proliferation after compound treatment were determined by MTT method,[20] and IC_{50} was calculated with Reed and Muench method.[21]

Acknowledgement

This work was supported by the Natural Science foundation of Hainan (No. 211020) and Yunnan (No. 2008CD159), the National Nonprofit Institute Research Grant of CATAS-ITBB (No. 110301) and the Fundamental Research Funds for the Central Universities (Nos. SWJTU11BR073, SWJTU11ZT26). The authors are thankful to the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the spectral measurements.

References

[1] Wang, L. Y.; Unehara, N.; Kitanaka, S. *Chem. Pharm. Bull.* **2005**, *53*, 1348.
[2] Wang, L. Y.; Unehara, T.; Kitanaka, S. *Chem. Pharm. Bull.* **2005**, *53*, 137.
[3] Chen, Y.; Fu, W. W.; Sun, L. X.; Wang, Q.; Qi, W.; Yu, H. *Chin. Chem. Lett.* **2009**, *20*, 592.
[4] Magano, J.; Chen, M. H.; Clark, J. D.; Nussbaumer, T. *J. Org. Chem.* **2006**, *71*, 7103.

[5] Sivakumar, S.; Reddy, M. L. P.; Cowley, A. H.; Vasudevan, K. V. *Dalton Trans.* **2010**, *39*, 776.
[6] Pelter, A.; Ward, R. S.; Gray, T. I. *J. Chem. Soc.*, *Perkin Trans. 1* **1976**, 2475.
[7] Moujir, L.; Seca, A. M. L.; Silva, A. M. S.; Barreto, M. C. *Planta Med.* **2008**, *74*, 751.
[8] Uwai, K.; Osanai, Y.; Imaizumi, T.; Kanno, S.; Takeshita, M.; Ishikawa, M. *Bioorg. Med. Chem.* **2008**, *16*, 7795.
[9] Wang, H.; Zhang, X.; Pan, L.; Yang, S.; Ma, Y.; Luo, X. *Nat. Prod. Res. Dev.* **2003**, *15*, 483.
[10] Lee, T. H.; Chiou, J. L.; Lee, C. K.; Kuo, Y. H. *J. Chin. Chem. Soc.* (*Taipei*, *Taiwan*) **2005**, *52*, 833.
[11] Zhang, Q.; Ye, N.; Sun, W. X.; Zhang, K. M.; Jiang, J. Q. *Biochem. Syst. Ecol.* **2008**, *36*, 63.
[12] Liang, S.; Tang, J.; Shen, Y. H.; Jin, H. Z.; Tian, J. M.; Wu, Z. J.; Zhang, W. D.; Yan, S. K. *Chem. Pharm. Bull.* **2008**, *56*, 1729.
[13] Erdemoglu, N.; Sener, B.; Ozcan, Y.; Ide, S. *J. Mol. Struct.* **2003**, *655*, 459.
[14] Wagner, H.; Chari, V. M.; Sonnenbichler, J. *Tetrahedron Lett.* **1976**, 1799.
[15] Wan, S. B.; Chan, T. H. *Tetrahedron* **2004**, *60*, 8207.
[16] Wu, Z. B.; Zhao, Y. Y.; Yang, X. W.; Liang, H. *Chem. Pharm. Bull.* **2009**, *57*, 628.
[17] Poumale, H. M. P.; Amadou, D.; Shiono, Y.; Kapche, G. D. W. F.; Ngadjui, B. T. *Asian J. Chem.* **2011**, *23*, 525.
[18] Zheng, W.; Shi, F. *Acta Pharm. Sinica* **2004**, *39*, 990.
[19] Jacquot, D.; Poeschke, O.; Burger, C. *EP 2285788*, **2009** [*Chem. Abstr.* **2009**, *152*, 37722].
[20] Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
[21] Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493.

(Cheng, F.)

