This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new polyoxygenated cyclohexenes from *Uvaria kweichowensis*

Qiong-Ming Xu[ab]; Yan-Li Liu[a]; Zhong-Mei Zou[b]; Shi-Lin Yang[ab]; Li-Zhen Xu[b]
[a] College of Pharmacy, SooChow University, Su Zhou, China [b] Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medicinal Sciences, Beijing, China

To cite this Article Xu, Qiong-Ming , Liu, Yan-Li , Zou, Zhong-Mei , Yang, Shi-Lin and Xu, Li-Zhen(2009) 'Two new polyoxygenated cyclohexenes from *Uvaria kweichowensis*', Journal of Asian Natural Products Research, 11: 1, 24 — 28
To link to this Article: DOI: 10.1080/10286020802410714
URL: http://dx.doi.org/10.1080/10286020802410714

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research
Vol. 11, No. 1, January 2009, 24–28

Taylor & Francis
Taylor & Francis Group

Two new polyoxygenated cyclohexenes from *Uvaria kweichowensis*

Qiong-Ming Xu[ab], Yan-Li Liu[a], Zhong-Mei Zou[b], Shi-Lin Yang[ab]* and Li-Zhen Xu[b]

[a]*College of Pharmacy, SooChow University, Su Zhou, China;* [b]*Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medicinal Sciences, Beijing, China*

(*Received 24 March 2008; final version received 11 June 2008*)

Two new polyoxygenated cyclohexenes, kweichowenols C and D, were isolated from the leaves of *Uvaria kweichowensis*, and their structures were established on the basis of their spectral data. The two new compounds showed some antitumor activity by the MTT assay.

Keywords: Annonaceae; *Uvaria kweichowensis*; leaves; cyclohexenes; kweichowenol C; kweichowenol D

Downloaded At: 18:46 22 January 2011

1. Introduction

During the past two decades, the genus *Uvaria* has been found to be a rich source of polyoxygenated cyclohexenes, some of which exhibited antitumor, antileukemic, or antibiotic activities [1,2]. Our previous studies have resulted in the isolation of a series of polyoxygenated cyclohexenes from *Uvaria grandiflora* and *Uvaria tonkinensis* var. *subglabra* [3,4]. *Uvaria kweichowensis* is an herb used to cure inflammation and tumor in the southwest area of China. During the course of our investigation for antitumor agents from the chloroform extract of the leaves of *U. kweichowensis*, besides kweichowenols A [5] and B [5], two new crystalline compounds, kweichowenols C (**1**) and D (**2**) were obtained. We now report the isolation and structural elucidation of the two new compounds, as well as the evaluation of their antitumor activities.

2. Results and discussion

Kweichowenol C (**1**) was obtained as white needles. The molecular formula was determined to be $C_{23}H_{22}O_7$ by HR-ESI-MS, which showed quasi-molecular ion peaks at *m/z* 411.1436 $[M + H]^+$, 433.1267 $[M + Na]^+$, and 449.1012 $[M + K]^+$. The IR spectrum of compound **1** suggested the presence of hydroxyl group(s) (3587, 3454, 1115, and 1090 cm^{-1}), ester group(s) (strong absorption at 1722 cm^{-1}) and monosubstituted phenyl ring(s) (1601, 1454, and 710 cm^{-1}). The UV spectrum gave absorption maxima at λ 230, 274, and 280 nm, indicating the presence of one or two benzoyl groups.

The most informative evidence for the structural elucidation of compound **1** was derived from its ^1H NMR spectral data, which, together with those of other related compounds, are summarized in Table 1. The aromatic proton signals between δ 7.3 and 8.1 (10H, m) confirmed the presence of two benzoyl moieties, while the ethidine moiety in the structure was indicated by the proton signals at δ 1.24 (3H, d) and 5.26 (1H, q). The ^1H NMR spectrum also showed two olefinic proton signals at δ 5.87 (2H, m) and a carbinyl proton signal (secondary alcohol) at δ 4.40. The two benzoxyl groups appeared to be located at C-3 and C-6 based upon the downfield shifts of H-3 at δ 5.59 and H-6 at δ

*Corresponding author. Email: yangshilin@suda.edu.cn.

ISSN 1028-6020 print/ISSN 1477-2213 online
© 2009 Taylor & Francis
DOI: 10.1080/10286020802410714
http://www.informaworld.com

Table 1. [1]H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of compounds **1** and **2** ($CDCl_3$, δ ppm).

	1		2	
	δ_C	δ_H	δ_C	δ_H
1	84.8		75.6	
2	73.8	4.40 d (8.4)[a]	76.7	4.21 d (8.4)
3	74.1	5.59 dd (8.4, 2.4)	74.0	5.78 dd (8.4, 2.4)
4	129.6	5.87 m	128.6	5.85 m
5	127.4	5.87 m	127.3	5.87 m
6	73.3	5.84 d (2.4)	75.8	5.80 m
7	65.6	4.39 d, 4.26 d (8.4)	62.7	4.17 d, 4.11 d (12.0)
8	103.8	5.26 q (4.2)		
9	19.7	1.24 d (4.2)		
Ar	120–140	7.3–8.1 m	120–140	7.3–8.1 m
Carbonyl	166.0, 166.7		166.3, 166.7	

[a]Data in parentheses are *J* values (in Hz).

Downloaded At: 18:46 22 January 2011

5.84, while the ethidine group was established at C-1 and C-7 on the basis of the downfield shifts of H-7 at δ 4.39 and 4.26. The elucidation of compound **1** was supported by the existence of the fragment ion peaks at *m/z* 294 and 116 resulted from the typical retro-Diels–Alder (RDA) cleavage of the molecule of compound **1** in the EI-MS spectrum. In comparison with the ^{1}H NMR spectral data of uvarigranol F [6], the coupling constant $J_{2,3}$ of 8.4 Hz suggested that H-2 and H-3 were favorably located at axial position. In addition, $J_{5,6}$ of 2.4 Hz suggested that the benzoxyl group at C-6 was likely located at the pseudo-equatorial position [7]. The proposed structure of compound **1** was verified by the HMBC experiment (Figure 1). The relative stereochemistry of compound **1** was further established from NOESY interaction. The interactions between H-3 (δ 5.59) and H-7 (δ 4.39 and 4.26) indicated that they were on the same side of the molecule, while that between H-2 (δ 4.40) and H-6 (δ 5.84) suggested that H-2 and H-6 were on the other side. The interactions between H-8 (δ 5.26) and H-2 (δ 4.40) indicated that the relative stereochemistry of the methyl located at C-8 was α-configuration. The CD spectrum of compound **1** exhibited a split curve centered at λ 227 nm with a positive Cotton effect at λ 235 (+4.62) and a negative Cotton effect at λ 219 (−9.86), indicating the clockwise manner for the orientation of the two benzoxyl

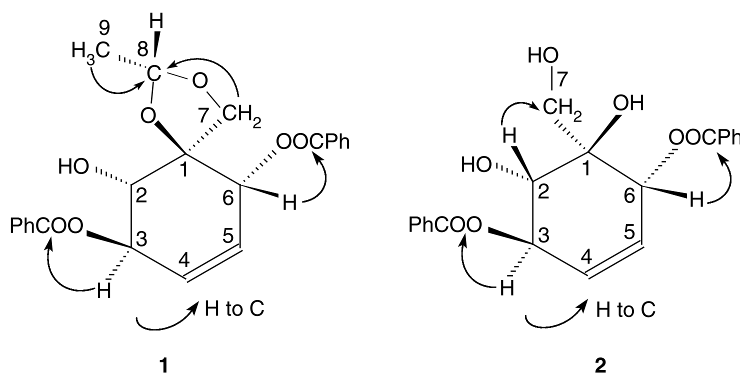

Figure 1. Key HMBC correlations of compounds **1** and **2**.

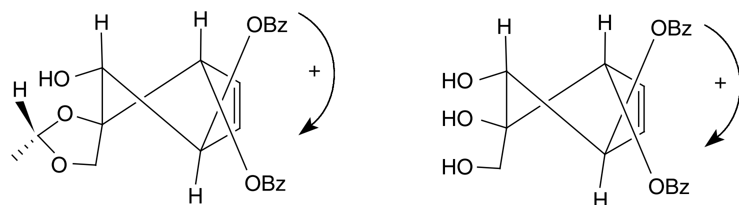

Figure 2. The chirality of the dibenzoates of compounds **1** and **2**.

groups at C-3 and C-6 (Figure 2); thus the absolute configuration was established as 1*R*, 2*S*, 3*R*, 6*R*, 8*R* [8].

In order to determine whether compound **1** was naturally occurring in the leaves of *U. kweichowensis*, the leaves were extracted with $CHCl_3$, and the extract was chromatographed over silica gel column, the fraction eluted by petroleum ether–EtOAc (85:15–82.5:17.5) was subjected to HPLC analysis. The HPLC experiment demonstrated the presence of kweichowenol C in the $CHCl_3$ extract of the leaves of *U. kweichowensis*. Such a result indicated that compound **1** occurred naturally, rather than as a consequence of the utilization of acetone during the separation procedure.

Kweichowenol D (**2**) was obtained as white solid. The molecular formula was determined to be $C_{21}H_{20}O_7$ by HR-ESI-MS, which showed quasi-molecular ion peaks at *m/z* 385.1284 $[M + H]^+$, 407.1067 $[M + Na]^+$, and 423.1067 $[M + K]^+$. Compound **2** has similar IR and UV spectra to those of compound **1**. Its ^1H NMR spectrum displayed the signals of two benzoxyl groups (δ 7.3–8.1, 10H, m) and seven protons on a methyl–cyclohexene skeleton (δ 4.2–5.9; the assignment shown in Table 1). Among the latter signals, an AB quartet at δ 4.17 and 4.11 (10.8 Hz) was attributed to the two methylene protons at C-7, similar to those of the known polyoxygenated cyclohexenes.

The HMBC spectrum of compound **2** (Figure 1) indicated that the signal at δ 4.21 was attributed a carbinyl proton at C-2 for the correlation between it with C-7 (δ 62.7). The correlations between H-3 (δ 5.78) and the ester carbonyl at δ 166.7 and between H-6 (δ 5.80) and the ester carbonyl at δ 166.3 indicated that the two benzoxyl groups are located at C-3 and C-6, respectively.

Based upon a careful analysis of the coupling constants and the correlations shown in the NOESY spectrum, the relative stereochemistry of compound **2** was established. The $J_{2,3}$ value of 8.4 Hz revealed that both H-2 and H-3 are located at axial positions. In the NOESY spectrum of compound **2**, interactions between H-3 (δ 5.78) and H-7 (δ 4.17 and 4.11) indicated that they were on the same side of the molecule, while those between H-2 (δ 4.21) and H-6 (δ 5.80) suggested that H-2 and H-6 were on the other side.

The CD spectrum of compound **2** exhibited a positive Cotton effect at λ 235.5 (+3.55) and a negative Cotton effect at λ 218.5 (−6.16), indicating that the two benzoxyl groups were oriented in a clockwise manner. Since the overall relative configuration was known, the absolute configuration of compound **2** was established as 1*R*, 2*S*, 3*R*, 6*R*.

In order to determine whether compound **1** was an acetalation product of compound **2**, compound **1** was hydrolyzed by TFA/H_2O [9], then the hydrolysis product was subjected to HPLC analysis. The HPLC experiment demonstrated the presence of compound **2**. Such a result indicated that compound **1** was an acetalation product of compound **2**.

In the case of bioactivities of compounds **1** and **2**, antitumor activities of them were expressed as IC_{50} values. As determined by MTT assay, the IC_{50} values of compounds **1** and **2** against A549 bronchogenic carcinoma cells were 55 and 22 μg/ml, respectively,

Downloaded At: 18:46 22 January 2011

SK-MES-1 bronchogenic carcinoma cells 58 and 25 μg/ml, and NCI-H446 bronchogenic carcinoma cells 48 and 19 μg/ml. The results suggested that the antitumor activity of compound **2** was a little better than that of compound **1**.

3. Experimental

3.1 General experimental procedures

Metling points were determined using a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a PerkinElmer model 241 polarimeter. UV spectra were measured on a Shimadzu UV-2401 spectrometer. IR spectra were taken on a PerkinElmer 983 G spectrometer. ^{1}H, ^{13}C NMR, and 2D-NMR spectra were recorded on a Varian Inova 600 spectrometer in $CDCl_3$ using tetramethylsilane as internal standard. EI-MS and FAB-MS spectra were determined on a Micromass Zabspec spectrometer. HR-ESI-MS spectra were determined on a Q-TOF2 spectrometer. CD spectra were determined on a JASCO-715 spectrometer. Preparative HPLC was carried out on a column of Phenyl (250 × 9.4 mm i.d., Agilent Zorbax SB-Phenyl; Palo Alto, CA, USA) with a Waters 2996 detector; the flow rate was 3 ml/min and the wave length for detection was 230 nm. MPLC was carried out on a column of silica gel H (460 × 26 mm i.d., Buchi Borosilikat 4.6; Flawil, Switzerland). Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory, Qingdao, China. Precoated plates of silica gel GF_{254} were used for TLC, and detected under UV.

3.2 Plant material

The leaves of *U. kweichowensis* were collected in Guizhou Province of China in November 2002, and identified by Prof. Shou-Quan Lin and Dr Guo-Qiang Li of our institute. A voucher sample is deposited in the Herbarium of the Institute of Medicinal Plant Development, Peking Union Medical College.

3.3 Extraction and isolation

The dried plant material (5 kg) was extracted three times with 95% EtOH under reflux. The solvent was subsequently dried under reduced pressure to give the residue, which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction was further portioned between petroleum ether and 90% MeOH (V/V). The 90% MeOH fraction (85 g) was chromatographed over silica gel column, which was eluted with petroleum ether–EtOAc gradients to afford 25 fractions (F1–F25). Further purification of F8 through MPLC, using petroleum ether–EtOAc (85:15) as eluent, yielded kweichowenol C (**1**; 15 mg). Kweichowenol D (**2**; 130 mg) was obtained from F15 through RP-HPLC using MeOH–H_2O (80:20) as eluent.

3.3.1 Kweichowenol C (**1**)

White needles, mp 48–49°C. $[\alpha]_{20}^{D}$ −92.6 ($c = 0.001$, $CHCl_3$). UV λ_{max} ($CHCl_3$, nm): 230, 274, 280. IR (KBr; ν_{max}, cm^{-1}): 3587, 3454, 1722, 1601, 1454, 1273, 1115, 710. For ^{1}H and ^{13}C NMR ($CHCl_3$) spectral data, see Table 1. EI-MS *m/z*: 395 (6.0), 294 (4.5), 273 (6.5), 227 (6.5), 203 (9.0), 130 (6.5), 122 (18.6), 105 (100), 77 (35). FAB-MS *m/z* 411 ($[M + H]^+$). HR-ESI-MS *m/z*: 411.1436 $[M + H]^+$ (calcd for $C_{23}H_{23}O_7$, 411.1444).

3.3.2 Kweichowenol D (**2**)

White solid, mp 63–64°C. $[\alpha]_{20}^{D}$ −85.2 ($c = 0.002$, $CHCl_3$). UV λ_{max} ($CHCl_3$, nm): 230, 274, 280. IR (KBr; ν_{max}, cm^{-1}): 3438, 3261, 1728, 1701, 1599, 1583, 1452, 1273, 1115, 710. For ^{1}H and ^{13}C NMR ($CHCl_3$) spectral data, see Table 1. EI-MS *m/z*: 294 (7.5), 273 (9.0), 227 (5.5), 203 (11.5), 130 (8.5), 122 (25.5), 105 (100), 77 (45). FAB-MS *m/z*: 385 ($[M + H]^+$). HR-ESI-MS *m/z*: 385.1284 $[M + H]^+$ (calcd for $C_{21}H_{21}O_7$, 385.1287).

3.4 Antitumor bioassays

To evaluate the antiproliferative effect of compounds **1** and **2** on A549, SK-MES-1, and

Downloaded At: 18:46 22 January 2011

NCI-H446 bronchogenic carcinoma cell lines, the MTT colorimetric assay was performed. The amount of formazan was determined by a photometer at 570 nm. Cells were plated onto 96-well flat-bottomed culture plates at a concentration 5×10^4 cell per well in complete RPMI 1640 culture medium. Twenty-four hours after plating, the medium containing fetal calf serum was removed and test solutions were given to the cells in various final concentrations such as 10, 25, 50, 100, and 200 μg/ml. After incubation with drugs for 24 h, the MTT solution was added to the wells and plates were incubated at 37°C for 4 h. Results were expressed as percentage of the absorbance in control cells compared with those in the drug-treated cells.

Acknowledgements

We express our thanks to Prof. Chao-Yi Deng of the Institute of Forestry of Guizhou Province in China for the collection of the plant material.

References

[1] M. Leboeuf, A.P. Cave, K. Bhaumik, B. Mukherjee, and R. Mukherjee, *Phytochemistry* **21**, 2783 (1982).

[2] M.H.H. Nkunya, H. Weenen, N.J. Koyl, L. Thus, and B. Zwanenburg, *Phytochemistry* **26**, 2563 (1987).

[3] A. Liu, Z.M. Zou, L.Z. Xu, and S.L. Yang, *Chin. Chem. Lett.* **14**, 1144 (2003).

[4] Y.H. Liao, Z.M. Zou, J. Guo, L.Z. Xu, M. Zhu, and S.L. Yang, *J. Chin. Pharm. Sci.* **9**, 170 (2000).

[5] Q.M. Xu, Z.M. Zou, L.Z. Xu, and S.L. Yang, *Chem. Pharm. Bull.* **53**, 826 (2005).

[6] X.P. Pan, D.Q. Yu, C.H. He, and J.J. Chai, *Acta Pharm. Sin.* **32**, 530 (1997).

[7] R.J. Abraham, H. Gottschalck, H. Paulsen, and W.A. Thomas, *J. Chem. Soc.*, 6268 (1965).

[8] N. Harada and K. Nakanishi, *J. Am. Chem. Soc.* **91**, 3989 (1969).

[9] A. Liu, Z.Z. Liu, L.Z. Xu, Z.M. Zou, and S.L. Yang, *Tetrahedron* **60**, 3689 (2004).

Downloaded At: 18:46 22 January 2011