Two New Clerodane-type Diterpenoids from *Gomphostemma microdon*

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Two new clerodane-type diterpenoids, along with seven known compounds have been isolated from the aerial parts of *Gomphostemma microdon*. All structures were established on the basis of spectroscopic analyses, including application of MS, UV, IR, 1D and 2D NMR techniques.

Key words: *Gomphostemma microdon*, Clerodane Diterpenoids, *Microdon* A, *Microdon* B Diterpenes

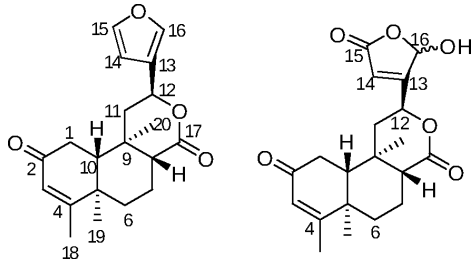
Introduction

The genus *Gomphostemma* is a member of the Labiatae family and comprises about forty species, which are distributed throughout tropical and subtropical areas. Sixteen species are found in China. *Gomphostemma microdon*, a herb plant, is widely distributed in the south and southwest of China, which has been used as a menoxenia drug as well as for the treatment of cough and for eliminating phlegm by the local people [1, 2]. Various medicinal uses of the genus *Gomphostemma* have been reported in Yunnan Province of China. However, phytochemical research on the genus showed only a few types of sterols and terpenoids [3]. Moreover, the absence of chemical reports on *G. microdon* further prompted us to study it.

In this paper, we report the isolation and structure elucidation of two new clerodane-type diterpenoids, named *microdon* A (**1**) and B (**2**) (Fig. 1), together with seven known compounds, phytol (**3**) [4], betulinic acid (**4**) [5], 3-taraxeranol (**5**) [6], 4′,5-dihydroxy-7-methoxyflavone (**6**) [7], 5-hydroxy-2-methoxybenzoic acid (**7**) [8], 3-hydroxystigmast-7-en-11-one (**8**) [9] and apigenin (**9**) [10] from the methanol extract of aerial parts of the title plant.

Results and Discussion

Compound **1** has a molecular formula $C_{20}H_{25}O_4$ derived from HRMS ((+)-ESI) at *m*/*z* = 329.1774 (calcd. 329.1752 for $C_{20}H_{25}O_4$) in combination with ^{1}H and ^{13}C NMR data (Table 1). The IR spectrum of **1** revealed the presence of a furan ring (1504, 1022, 872 and 817 cm^{-1}) [11] and of α,β-unsaturated ketone group functionalities (1652 cm^{-1}) which were supported by ^{1}H NMR signals at δ_H = 7.40 (1H, s), 7.38 (1H, d, *J* = 1.4 Hz), and 6.37 (1H, d, *J* = 1.4 Hz) and the UV absorption band at 243 nm [12], respectively. The ^{1}H NMR spectrum showed 3 tertiary methyls at δ_H = 1.13, 1.17, and 1.89. Additionally, the ^{13}C NMR spectrum showed 20 carbon signals, 5 quaternary carbons (δ_C = 198.2, 171.3, 170.9, 38.7, 36.3), 7 methines (δ_C = 143.5, 139.0, 125.3, 108.1, 71.5, 50.9, 50.2), 3 methylenes (δ_C = 42.8, 34.2, 17.8), and 3 methyls (δ_C = 18.4, 18.1, 14.1). The above NMR data have shown that **1** has the clerodane diterpenoid skeleton. Comparison of the ^{13}C and ^{1}H NMR spectra with those of tinophyllone [13] suggested that both planar structures were similar except that the methyl ester group changed to methyl. This proposal was supported by the HMBC correlations between δ_H = 1.89 (3H, s) with δ_C = 125.3 (C-3, d), 170.9 (C-4, s), 198.2 (C-2, s) and



1 **2**

Fig. 1. Chemical structures of **1** and **2**.

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Table 1. NMR data of **1** and **2** in $CDCl_3$ solution (500 MHz for ^1H and 100 MHz for ^{13}C, δ in ppm, J in Hz). Assignments are based on DEPT, HSQC and HMBC spectra.

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	2.39 (2H, dd)	34.2(t)	2.63 (2H, dd)	34.4(t)
2	–	198.2(s)	–	199.2(s)
3	5.71 (1H, s)	125.3(d)	5.74 (1H, s)	125.7(d)
4	–	170.9(s)	–	171.0(s)
5	–	38.7(s)	–	36.8(s)
6	1.39 (1H, m)	34.2(t)	1.41 (1H, m)	34.6(t)
	1.98 (1H, m)		2.00 (1H, m)	
7	1.69 (1H, m)	17.8(t)	1.68 (1H, m)	18.3(t)
	2.15 (1H, overlap)		2.15 (1H, overlap)	
8	2.16 (1H, overlap)	50.2(d)	2.16 (1H, overlap)	50.6(d)
9	–	36.3(s)	–	
10	1.83 (1H, t, J = 9.0)	50.9(d)	1.87 (1H, m)	51.2(d)
11	2.24 (1H, dd, J = 13.5, 5.5 Hz)	42.8(t)	1.65 (1H, overlap)	40.9(t)
	1.65 (1H, d, J = 13.5 Hz)		2.24 (1H, overlap)	
12	5.50 (1H, dd, J = 11.2, 5.7 Hz)	71.5(d)	5.46 (1H, m)	72.8(d)
13	–	125.2(s)	–	166.0(s)
14	6.37 (1H, d, J = 1.4 Hz)	108.1(d)	6.22 (1H, br s)	117.7(d)
15	7.38 (1H, d, J = 1.4 Hz)	143.5(d)	–	169.8(s)
16	7.40 (1H, s)	139.0(d)	6.09 (1H, br s)	97.5(d)
17	–	171.3(s)	–	172.2(s)
18	1.89 (3H, s)	14.1(q)	1.93 (3H, s)	14.3(q)
19	1.17 (3H, s)	18.1(q)	1.18 (3H, s)	18.5(q)
20	1.13 (3H, s)	18.4(q)	1.14 (3H, s)	18.7(q)

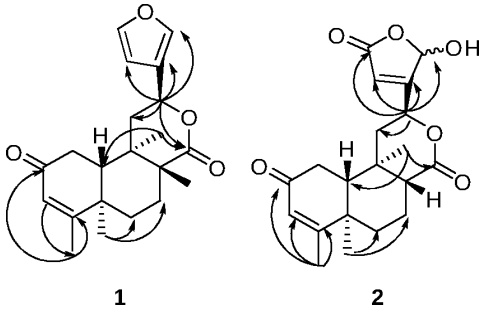

Fig. 2. Key HMBC correlations of **1** and **2**.

18.1 (C-19, s) (Fig. 2). On the basis of HMBC, HSQC experiments, all carbons and protons were unambiguously assigned. Thus, we named it as *microdon* A.

The relative configuration of **1** was deduced by a ROESY experiment. Crosspeaks from H-1 (δ_H = 2.39, 2H, dd) to H-19 and H-20, and from H-12 to H-20 indicated that these atoms are at the same side in α-orientation. ROESY correlations of H-10/H-8 and the absent correlation of H-19/H-10 suggested that H-10 and H-8 are β-oriented [13, 14]. Thus, the relative configuration of *microdon* A is as represented in Fig. 3.

Compound **2** showed in its HRMS ((+)-ESI) spectrum a molecular ion peak at m/z = 361.1666 $[M+H]^+$ in accordance with the formula $C_{20}H_{24}O_6$. The IR spectra of **2** indicated the presence of a hydroxyl

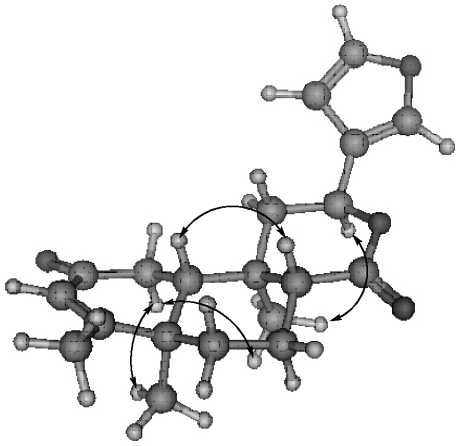

Fig. 3. Key ROESY correlations of **1**.

group (3323 cm^{-1}) and of α,β-unsaturated carbonyl (1653 cm^{-1}) functionalities. Careful analysis of the ^{13}C and ^1H NMR spectra of **2** implied that this compound might be an analog of **1**. However, they differ in the chemical shifts of the β-furan ring. The ^{13}C NMR signals [δ_C = 169.8 (s), 166.0 (s), 117.7 (d)] suggested the existence of another α,β-unsaturated carbonyl which was supported by the HMBC correlation between H-12 at δ_H = 5.46 (1H, m) with δ_C = 117.7 (d), 166.0 (s), and 169.8 (s). In addition, the acetal group [δ_C = 97.5 (d)] also showed correlation with H-12. So a γ-hydroxy-α,β-unsaturated γ-lactone was indicated.

Experimental Section

General

Optical rotations were measured with a Horiba SPEA-300 spectropolarimeter. All melting points were measured on an XRC-1 apparatus and are uncorrected. One- and two-dimensional NMR experiments were performed on Bruker AM-400 MHz and DRX-500 MHz NMR spectrometers with tetramethylsilane as the internal standard. MS ((+)-ESI) and HRMS ((+)-ESI) spectral data were obtained on a API Qstar Palsar I mass spectrometer. IR spectra were measured on a Brucker Tensor 27 spectrometer with KBr pellets. UV spectra were taken on a Shimadzu 2401PC spectrophotometer. RP-18 silica gel (40 – 65 μm) was bought from Merck, Germany. Silica gel (200 – 300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qindao Marine Chemical factory, Qindao, P. R. China. Compounds were detected under UV (254 nm and 365 nm) before spraying with an anisaldehyde sulfuric acid solution followed by heating.

Plant material

The aerial parts of *Gomphostemma microdon* were collected from Xishuangbanna, Yunnan Province, People's Republic of China, in April 2007. Its identity was confirmed by Mr. Jing-yun Cui, and a voucher specimen (NO. 200704) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dried and milled sample (10 kg) was soaked in 95 % MeOH (40 L × 3) under reflux (48 h × 3), and the solvent was evaporated. The residue was partitioned between EtOAc and H_2O to give an EtOAc-soluble fraction. This fraction (150 g) was chromatographed on a prepacked silica gel (2.0 kg, 200 – 300 mesh) column, using a mixture of $CHCl_3$-Me_2CO [from $CHCl_3$-Me_2CO (10 : 1) to $CHCl_3$-Me_2CO (1 : 1)], to give eight fractions (I – VIII). Fraction II (10.3 g) was submitted to silica gel (200 g) and eluted with petroleum ether-$CHCl_3$ (8 : 1, 4 : 1, 1 : 1), to yield subfraction A. Subfraction A was purified by silica gel and eluted with petroleum ether-Me_2CO to afford compound **3** (938.5 mg). Fraction III (12.0 g) was submitted to silica gel (300 g) and eluted with petroleum ether-Me_2CO (12 : 1, 10 : 1, 8 : 1, 4 : 1), to yield subfractions B–D. Subfraction B was purified on Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1), to yield compound **4** (618.7 mg). Subfraction C was purified by silica gel and eluted with petroleum ether-EtOAc to afford compound **5** (38.5 mg). Subfraction D was applied on RP-18 using MeOH-H_2O, to give compounds **6** (51.3 mg) and **7** (33.6 mg). Fraction IV (19.0 g) was submitted to silica gel (350 g) and eluted with $CHCl_3$-Me_2CO (8 : 1, 4 : 1, 2 : 1), to give subfractions E–F. Subfraction E was purified by recrystallization from cold MeOH to give compound **1** (485.6 mg) and compound **2** (18 mg). Subfraction F was first separated on RP-18 using MeOH-H_2O and then on Sephadex LH-20 (MeOH-H_2O = 9 : 1), to give compounds **8** (162.7 mg) and **9** (58.3 mg).

Microdon A (**1**): colorless square crystals (MeOH). – M. p. 174 – 176 °C. – UV($CHCl_3$): λ_{max} = 243 nm. – $[\alpha]_D^{20}$ = +36.8 (c = 0.38, $CHCl_3$). – IR (KBr): ν = 1723, 1652 (C=O) cm^{-1}. – MS ((+)-ESI): *m/z* = 329 $[M+H]^+$. – HRMS ((+)-ESI): *m/z* = 329.1774 (calcd. 329.1752 for $C_{20}H_{25}O_4$, $[M+H]^+$). – ^{1}H NMR (500 MHz, $CDCl_3$), and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data see Table 1.

Microdon B (**2**): colorless square crystals (MeOH). – M. p. 192 – 193.5 °C. – UV($CHCl_3$): λ_{max} = 243 nm. – $[\alpha]_D^{20}$ = −28.7 (c = 0.36, $CHCl_3$). – IR (KBr): ν = 3323 (OH), 1740, 1653 (C=O) cm^{-1}. – MS ((+)-ESI): *m/z* = 361 $[M+H]^+$. – HRMS ((+)-ESI): *m/z* = 361.1666 (calcd. 361.1651 for $C_{20}H_{25}O_6$, $[M+H]^+$). – ^{1}H NMR (500 MHz, $CDCl_3$), and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data see Table 1.

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[1] Kunming Institute of Botany, Jiangsu Institute of Botany, *ZhonghuaBencao Gangyao daiyaojuan*, Shanghai Science and Technology Press, Shanghai, **2005**, pp. 52.

[2] Yunnan Institute of Tropical Botany, Chinese Academy of Science, *Xishuangbanna Daiyaozhi*, Xishuangbanna Health Bureau Press, Xishuangbanna, **1979**, pp. 15.

[3] W. Qiong, S. D. Luo, J. Peng, Y. F. Wang, *Helv. Chim. Acta* **2007**, *90*, 1360 – 1365.

[4] R. A. Goodman, E. Oldfield, A. Allerhand, *J. Amer. Chem. Soc*. **1973**, *95*, 7553 – 7558.

[5] R. T. Aplin,T. G. Halsall, T. Norin, *J. Chem. Soc*. **1963**, *6*, 3269 – 3273.

[6] R. E. Gorbett, S. Coming, *J. Chem., Soc. Perkin* **1972**, 2827 – 2829.

[7] H. Wagner, V. M. Chari, *Tetrahedron Lett.* **1976**, *21*, 1799 – 1802.
[8] M. Andrew, S. Madhavlal, *J. Indian Chem. Soc*. **1931**, *8*, 575 – 580.
[9] S. K. Sharma, M. Ali, *J. Indian Chem. Soc*. **1995**, *72*, 339 – 342.
[10] C. C. Shen, Y. S. Chang, L. K. Ho, *Phytochemistry* **1993**, *34*, 843 – 845.
[11] P. Puebla, J. L. Lopez, M. Guerrero, R. Carron, M. L. Martin, L. S. Roman, A. S. Feliciano, *Phytochemistry* **2003**, *62*, 551 – 555.
[12] R. Maurya, V. Wazir, A.Tyagi, R. S. Kapil, *Phytochemistry* **1995**, *3*, 659 – 661.
[13] S. R. Wilson, L. A. Neubert, J. C. Huffman, *J. Amer. Soc*. **1976**, *9*, 3669 – 3674.
[14] W. Tang, H. Hioki, K. Harada, M. Kubo, Y. Fukuyama, *J. Nat. Prod*. **2008**, *71*, 1760 – 1763.