Two new sesquiterpenoids from *Doellingeria scaber*

Suping Bai[a]*, Xingke Ma[b], Guangzhou Lu[a] and Fulin Yan[a]

[a]*Pharmacy College, Xinxiang Medical University, Xinxiang, Henan 453003, P.R. China*

[b]*Department of Chemistry, Zhoukou Normal University, Zhoukou, Henan 466000, P.R. China*

Examination of *Doellingeria scaber* collected in China afforded six sesquiterpenoids (**1**–**6**) of which two (**1** and **4**) are new. The structures of two new compounds were established by interpretation of spectroscopic data (IR; HR-MS; ^{1}H, ^{13}C and 2D NMR).

Keywords: *Doellingeria scaber*, Compositae, sesquiterpenoids, guaiane, aromadendrane

Doellingeria scaber Thunb (Compositae), a traditional Chinese herb, was previously assigned as an *Aster* species, is widely distributed in China. Its root has been used for treatment of traumatic injury and snake bite.[1] Phytochemical studies on this plant collected in different regions have led to identification of over 20 triterpene saponins.[2] In our effort to find biologically active components from Chinese medicinal plants[3] we found that the roots of *D. scaber* collected from Huanren Prefecture of Liaoning Province, China, afforded two new sesquiterpenoids, *i.e.*, 4α-hydroxy-10α-methoxy-1β-H, 5β-H-guaian-6-ene (**1**) and 4α-hydroxy-10β- methoxy-1β-H, 5β-H-aromadendrane (**4**), together with four known sesquiterpenoids, *i.e.*, guaianediol (**2**), 4β-hydroxy-10α-methoxy-guaian-6-ene (**3**), 4α,10β-aromadendranediol (**5**) and 4α-hydroxy-10β-methoxy-aromadendrane (**6**). In this paper, we report the isolation and structure elucidation of two new compounds and their total ^{1}H and ^{13}C NMR chemical shifts assignments (Fig 1).

The dried and crushed roots of *D. scaber*, which were collected from Huanren Prefecture of Liaoning Province, China, and identified as *Doellingeria scaber* Thunb by Professor Changshan Zhu, Henan Agriculture University, P. R. China, were extracted with petroleum ether (PE)–Et_2O–MeOH (1 : 1 : 1). Silica gel column chromatography gave compounds **1**–**6**.

Compound **1** was obtained as a colourless gum. Its HR–ESI–MS spectrum exhibited an M + Na ion peak at *m/z* 275.2 (calcd. for M + Na 275.1982), corresponding to a molecular formula $C_{16}H_{28}O_2$. Its ^{1}H, ^{13}C and DEPT NMR spectra coupled with the IR spectrum revealed the presence of a trisubstituted double bond [IR: 1648 cm^{-1}; δ_H 5.02 (1H, br s); δ_C 149.5 (s) and 119.9 (d)], a methoxy group [δ_H 3.23 (3H, s); δ_C 48.1 (q) and 78.0 (s)], a hydroxy group [IR: 3324 cm^{-1}; δ_C 82.1 (s)], two tertiary methyl groups connected to oxygen bearing carbons [δ_H 1.09 (3H, s) and 1.44 (3H, s); δ_C 24.7 (q) and 25.4 (q)], an isopropyl group [δ_H 0.95 (3H, d, J = 6.8 Hz), 0.96 (3H, d, J = 6.8 Hz) and 2.23 (1H, qq, J = 6.8 Hz, 6.8 Hz); δ_C 21.2 (q), 21.2 (q) and 37.8 (d)]. With reference to the known structures of sesquiterpenoids reported previously,[4-7] this suggested that compound **1** might be a guaiane sesquiterpenoid with a hydroxy group, a methoxy group and a trisubstituted double bond. Careful comparison of the ^{13}C NMR data of **1** with those of compound **3** revealed that compound **1** could be the diastereomer of **3,** *i.e.*, the hydroxy group and methoxy group were connected to C-4 and C-10 respectively. The location of the 4-OH and 10-OCH_3 was further confirmed by HMBC correlations (Fig. 2) of H-15 with C-3, C-4 and C-5, OCH_3 with C-10, and H-14 with C-1, C-9 and C-10, respectively. In the ^{1}H–^{1}H COSY spectrum of compound **1**, the trisubstituted double bond proton at δ_H 5.02 (1H, br s, H-6) showed a correlation with methine proton at δ_H 2.56 (1H, m, H-5), which in turn correlates with the proton at δ_H 2.76 (1H, m, H-1). These facts suggested the position of the double bond, which was further confirmed by HMBC correlations as shown in Fig. 2. In the NOESY spectrum of **1** (Fig. 3), the double bond proton at δ_H 5.02 (H-6) showed correlations with isopropyl group at δ_H 0.95 (Me-13), two tertiary methyl groups at δ_H 1.09 (Me-14) and δ_H 1.44 (Me-15), the proton at δ_H 2.76 (H-1) showed correlations with H-5, H-14 and OCH_3. These facts suggested that the seven-

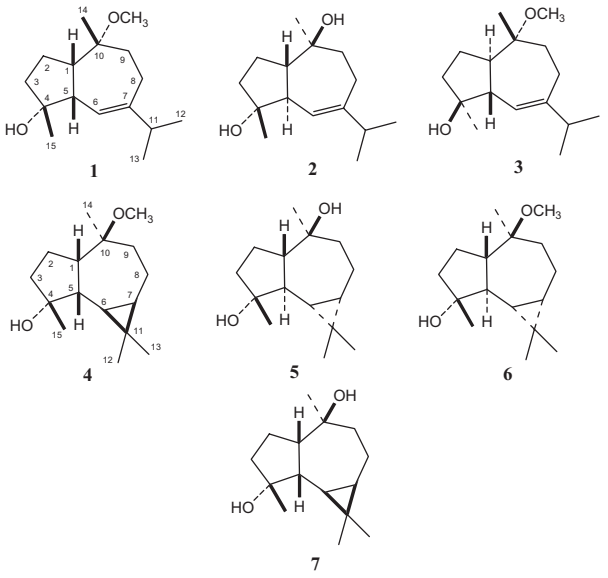

Fig. 1 Molecular structures.

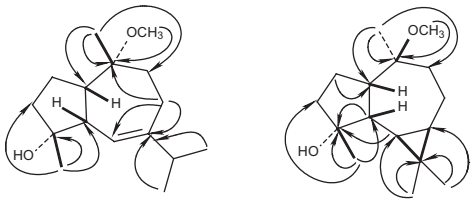

Fig. 2 Key HMBC correlations of **1** and **4**.

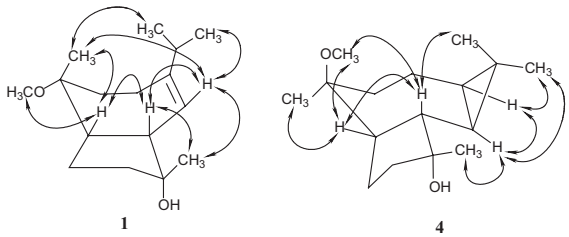

Fig. 3 Key NOESY correlations of **1** and **4**.

* Correspondent. E-mail: baisuping @xxmu.edu.cn.

Table 1 ^{1}H NMR (300 MHz) data of compounds **1**, **4**, **5** and **6** in $CDCl_3$ (δ ppm, *J* Hz)

No	1	4	5	6	7[a]
H-1β	2.76 (1H, m)	2.62 (1H, m)	2.23 (1H, m)	2.00 (1H, m)	2.47 (1H, m)
H-2a	1.61 (1H, m)	1.73–1.87 (1H, m)			
H-2b	1.40 (1H, m)	1.73–1.87 (1H, m)			
H-3a/b	1.64–1.69 (2H, m)	1.61–1.67 (2H, m)			
H-5β	2.56 (1H, m)	1.69 (1H, m)			
H-6	5.02 (1H, br s)	0.00 (1H, dd, 11.2, 8.1)	0.38 (1H, dd, 10.7, 10.2)	0.43 (1H, dd, 10.0, 10.0)	0.00 (1H, dd, 11.0, 9.7)
H-7		0.62 (1H, ddd, 11.2, 9.0, 6.2)	0.59 (1H, ddd, 11.0, 10.2, 6.5)	0.62 (1H, ddd, 11.1, 10.0, 6.2)	0.62 (1H, ddd, 11.0, 9.0, 6.0)
H-8a	2.35 (1H, m)	1.58 (1H, m)		1.84 (1H, m)	
H-8b	1.79 (1H, m)	1.29 (1H, m)		0.86 (1H, m)	
H-9a	1.76 (1H, m)	1.56 (1H, m)			
H-9b	1.37 (1H, m)	0.85 (1H, m)			
H-11	2.23 (1H, qq, 6.8, 6.8)				
Me-12	0.96 (3H, d, 6.8)	1.02 (6H, s)	0.99 (6H, s)	1.04 (6H, s)	1.02 (3H, s)
Me-13	0.95 (3H, d, 6.8)				1.03 (3H, s)
Me-14	1.09 (3H, s)	1.09 (3H, s)	1.14 (3H, s)	1.13 (3H, s)	1.19 (3H, s)
Me-15	1.44 (3H, s)	1.33 (3H, s)	1.21 (3H, s)	1.25 (3H, s)	1.33 (3H, s)
OCH_3	3.23 (3H, s)	3.14 (3H, s)		3.19 (3H, s)	

[a]Data from ref. 10.

membered ring of **1** might adopt a boat-conformation and the two rings of **1** might possess a *cis*-junction, the hydroxyl and methoxyl groups both adopt α-orientation. The relative stereochemistry of compound **1** was determined as shown in Fig. 3, and the structure of **1** was established as 4α-hydroxy-10α-methoxy-1β-H, 5β-H-guaian-6-ene. The complete ^{1}H and ^{13}C NMR chemical shifts assignments were listed in Tables 1 and 2.

Compound **4** was obtained as colourless gum. Its HR–ESI–MS spectrum exhibited an M + NH_4 ion peak at *m/z* 270.24 (calcd. for M + NH_4 270.2428), corresponding to a molecular formula $C_{16}H_{28}O_2$. The ^{1}H NMR spectra of new compound **4** and known compounds **5**, **6** and **7** (Table 1) exhibited the characteristics of an aromadendrane skeleton,[8-12] showing the cyclopropyl proton signals in high field between δ_H 0.00 to 0.62. In the ^{1}H NMR spectra of compound **4** and **7**, one of the two cyclopropyl protons appeared at higher field δ_H 0.00 and the other at lower field δ_H 0.62, typical of a 1,5 *cis* aromadendrane skeleton.[8-10] The ^{1}H NMR spectrum of compound **4** also showed two tertiary methyl groups at δ_H 1.02 (6H, s), two more tertiary methyl groups connected to oxygenated quaternary carbons at δ_H 1.09 (3H, s) and 1.33 (3H, s), and one methoxy group at δ_H 3.14 (3H, s). The ^{13}C NMR (Table 2) spectrum of compound **4** revealed one quaternary carbon at high field at δ_C 18.5, two oxygenated quaternary carbons at δ_C 78.3 and 82.1 and one methoxy carbon at δ_C 48.2 in support of the above functionalities, suggesting compound **4** might be an aromadendrane with one hydroxy group and one methoxyl group. With respect to the structures of compounds **6** and **7**, the position of hydroxy group and methoxy group ether were located at C-4 or at C-10.

The ^{13}C NMR spectral data of compound **4** agreed closely with those of compound **7** reported in the literature[9,10] except for the difference in the α,β–carbon to the methoxy group or hydroxy group in comparing compound **4** to **7**. The change in the chemical shifts of carbon C-1 (–3.2), C-9 (–4.5), C-10 (+ 4.2), C-14 (–7.3) observed between compound **4** and **7** located the methoxy group at C-10 in compound **4**, while the values of chemical shift of C-3, C-4, C-5 and C-15 almost remained the same in both compounds, proposing the hydroxy group at C-4 in compound **4**. The HMBC correlations of compound **4** (Fig. 2) further confirmed the position of the methoxy group and the hydroxy group. The relative stereochemistry of **4** was determined from the NOESY spectrum as shown in Fig. 3, a 1,5 *cis*-junction, an β–orientation of the cyclopropyl group, 4α-hydroxy and 10β-methoxy were assumed. Compound **4** was 4α-hydroxy-10β-methoxy-1β-H, 5β-H-aromadendrane, *i.e.*, the diastereomer of known compound **6**. The complete ^{1}H and ^{13}C NMR chemical shifts assignments were listed in Tables **1** and **2**.

Compounds **2**, **3**, **5** and **6** were identified by comparison of their ^{1}H and ^{13}C NMR, MS and IR spectroscopic data with those reported in the literature as guaianediol (**2**),[6,7] 4β-hydroxy-10α-methoxy-guaian-6-ene (**3**),[4,5] 4α,10β-

Table 2 ^{13}C NMR (75 MHz) data of compounds **1–7** in $CDCl_3$ (δ[a] ppm)

No	1[b]	2[b]	3[b]	4[b]	5[b]	6[b]	7[c]
1	47.5, d	50.2, d	47.9, d	50.6, d	56.5, d	52.2, d	53.8, d
2	23.0, t	21.4, t	24.6, t	24.7, t	24.6, t	23.6, t	25.4, t
3	36.7, t	40.4, t	40.5, t	36.9, t	41.3, t	41.1, t	37.6, t
4	82.1, s	80.2, s	80.2, s	82.1, s	80.6, s	80.3, s	81.9, s
5	50.5, d	50.5, d	50.2, d	47.9, d	48.5, d	48.1, d	47.5, d
6	119.9, d	121.2, d	121.1, d	28.9, d	28.9, d	28.1, d	28.8, d
7	149.5, s	149.6, s	149.6, s	25.4, d	26.7, d	26.7, d	25.1, d
8	24.2, t	25.0, t	21.6, t	18.1, t	20.4, t	19.7, t	18.7, t
9	31.9, t	42.5, t	35.5, t	33.5, t	44.6, t	37.5, t	38.0, t
10	78.0, s	75.3, s	79.2, s	78.3, s	75.3, s	78.9, s	74.1, s
11	37.8, d	37.2, d	37.2, d	18.5, s	20.3, s	19.6, s	18.6, s
12	21.2, q	21.3, q	21.2, q	28.5, q	28.5, q	28.7, q	28.5, q
13	21.2, q	21.4, q	21.5, q	16.2, q	16.7, q	16.4, q	16.1, q
14	24.7, q	21.1, q	17.9, q	24.7, q	20.5, q	17.7, q	32.0, q
15	25.4, q	22.4, q	22.4, q	25.6, q	25.1, q	24.4, q	25.1, q
OCH_3	48.1, q		48.7, q	48.2, q		48.2, q	

[a] ^{13}C NMR multiplicities were established by DEPT. [b]Data from the present research. [c]Data from ref. 10.

aromadendranediol (**5**)[8,10] and 4α-hydroxy-10β-methoxy-aromadendrane (**6**).[11]

Experimental

Melting points (uncorrected) were determined on a Kofler melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were taken on a Nicolet 170 SX FT-IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM-300 FT-NMR spectrometer with TMS as internal standard. HR–ESI–MS and EI-MS spectra were obtained on a Bruker APEX II FT-MS and HP 5988 MS spectrometers respectively. Silica gel (200–300 mesh) for column chromatography and silica gel GF_{254} (10-40 μm) for TLC were from Qingdao Marine Chemical Factory, Qingdao, China.

Extraction and isolation procedures

The dried and crushed roots of *D. scaber* (7.5kg) were extracted four times with PE–Et_2O–MeOH (1 : 1 : 1) at room temperature for 7 days. The extract was evaporated and gave 200 g residue after removing the solvent. This residue was separated by silica gel column chromatography (7.0 × 125 cm, 200–300 mesh, 1300 g) with gradient elution of PE–Me_2CO (1 : 0 → 0 : 1) to yield fraction 1–15. Fraction 7 (10:1) was repeatedly subjected to silica gel column chromatography eluting with PE–Me_2CO and cyclohexane–EtOAc to yield two parts. The first part was purified by preparative TLC developing three times with $CHCl_3$–Me_2CO (80 : 1, R_f = 0.3) to give compound **3** (10 mg), the second part was chromatographed on silica gel column developing with CH_2Cl_2–Me_2CO (80:1) to yield compound **6** (25 mg). Fraction 8 (10:1) was repeatedly separated by silica gel column chromatography and eluted with PE–EtOAc and $CHCl_3$–Me_2CO (80 : 1; 50 : 1), and the portion obtained from $CHCl_3$–Me_2CO 50:1 was further separated by preparative TLC developing four times with cyclohexane-isopropanol (20 : 1) to afford compound **1** (6 mg) and compound **4** (6 mg). Fraction 11 (5 : 1) was chromatographed on silica gel column and recrystallised in PE–EtOAc (5 : 1) to give compounds **2** (80 mg) and **5** (10 mg). The structures of two new compounds **1** and **4** were identified on the basis of HR–MS, ^{1}H, ^{13}C and 2D NMR spectroscopic methods. The structures of compounds **2, 3, 5** and **6** were characterised by comparing their m.p., IR, MS, ^{1}H and ^{13}C NMR chemical shifts with those reported in literatures.

4α-hydroxy-10α-methoxy-1β-H,5β-H-guaian-6-ene (**1**): colourless gum, $[\alpha]_D^{20}$ –6° (*c* 0.6, CH_3Cl). IR (KBr) ν_{max}/cm^{-1}: 3324, 2925, 2858, 1648, 1461, 1368, 1168, 1110, 1087, 1065, 1023, 917, 856. HR–ESI–MS *m/z* 275.1986 (calcd. for $C_{16}H_{28}O_2$ + Na 275.1982). EI-MS *m/z* (rel. int.): 254 (M + 2, 31), 220 (M-CH_3OH, 99), 205 (M-CH_3OH-Me, 57), 202 (34), 192 (33), 187 (48), 177 (65), 162 (35), 119 (30), 107 (28), 91 (31), 85 (39), 43 (100). ^{1}H and ^{13}C NMR data see Tables **1** and **2**.

4α-hydroxy-10β-methoxy-1β-H,5β-H-aromadendrane (**4**): colourless gum, $[\alpha]_D^{20}$ +23° (*c* 0.6, CH_3Cl). IR (KBr) ν_{max}/cm^{-1}: 3407, 2925, 2858, 1459, 1372, 1248, 1166, 1119, 1076, 917, 882. HR–ESI–MS *m/z* 270.2429 (calcd. for $C_{16}H_{28}O_2$ + NH_4 270.2428). EI–MS *m/z* (rel. int.): 254 (M + 2, 38), 220 (M–CH_3OH, 34), 205 (M–CH_3OH–Me, 41), 202 (42), 187 (44), 177 (37), 162 (17), 119 (16), 107 (17), 91 (22), 85 (34), 43 (100). ^{1}H and ^{13}C NMR data see Tables **1** and **2**.

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References

1 Jiangsu New Medicinal College, *The Dictionary of Traditional Chinese Medicines*, Shanghai Science Press, Shanghai, 1995, pp. 641-642.
2 (a) T. Nagao, R. Tanaka and H. Okabe, *Chem. Pharm. Bull.*, 1991, **39,** 1699; (b) T. Nagao, R. Tanaka and H. Shimokawa, *Chem. Pharm. Bull.*, 1991, **39**, 1719; (c) T. Nagao and H. Okabe, *Chem. Pharm. Bull.*, 1992, **40**, 886; (d) T. Nagao, R. Tanaka and Y. Iwase, *Chem. Pharm. Bull.*, 1993, **41**, 659; (e) T. Nagao, Y. Iwase and H. Okabe, *Chem. Pharm. Bull.*, 1993, **41**, 1562.
3 (a) S.P. Bai and L. Yang. *Chemical Lett.*, 2004, **15**, 1303; (b) S.P. Bai, X.L. Jin and L. Yang, *J. Chem. Res. (S)*, 2004, 384; (c) S.P. Bai, Q.Y. Wei, X.L. Jin, Q.X. Wu and L. Yang, *Planta Med.*, 2005, **71**, 764; (d) S.P. Bai and L. Yang, *Acta Crystallogr*, 2005, **E61**, o967.
4 D.T.A. Youssef, W.Y. Yoshida, M. Kelly and P.J. Scheuer, *J. Nat. Prod.*, 2001, **64**, 1332.
5 M.R. Rao, K.V. Sridevi, U. Venkatesham, T.P. Rao, S.S. Lee and Y. Venkateswarlu, *J. Chem. Res. (S)*, 2000, 245.
6 M. Yoshikawa, S. Hatakeyama, N. Tanaka, Y. Fukuda, N. Murakami and J. Yamahara, *Chem. Pharm. Bull.*, 1992, **40**, 2582.
7 K.A. Sayed and T. Hamann, *J. Nat. Prod.*, 1996, **59**, 687.
8 C.M. Beechan, C. Djerassi and H. Eggert, *Tetrahedron*, 1978, **34**, 2503.
9 G. Goldsby and B.A. Burke, *Phytochemistry*, 1987, **26**, 1059.
10 A.S.R. Anjaneyulu, K.S. Sagar and M.J.R.V. Venugopal, *Tetrahedron*, 1995, **51**, 10997.
11 H.J. Liu, C.L. Wu, H. Becker and J. Zapp, *Phytochemistry*, 2000, **53**, 845.
12 C.L. Wu, Y.M. Huang and J.R. Chen, *Phytochemistry*,1996, **42**, 677.