A new guaiane sesquiterpene from *Paraixeris pinnatipartita*

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A new guaiane sesquiterpene, 3α,9α-dihydroxy-11βH-guai-4(15),10(1)-dien-12,6β-lactone, along with five known compounds were isolated from the whole plants of *Paraixeris pinnatipartita*. Their structures were identified on the basis of spectroscopic methods, including IR, EI-MS, HR-ESI-MS, 1D NMR and 2D NMR.

Keywords: compositae, *Paraixeris pinnatipartita*, sesquiterpene, guaiane

The genus *Paraixeris*, which was classified as a subgenus of *Ixeris* before 1942, comprises only 8–10 species which are mainly distributed in the area of East Asia and Southeast Asia with six species occurring in China.[1] The secondary metabolites of this genus have been seldom examined. *Paraixeris pinnatipartita* is an annual plant. Previous phytochemical investigation of this plant have led to the isolation of only a new guaiane-type sesquiterpene lactone glucoside.[2] With the aim of screening the sesquiterpenoids from Compositae plants for cytotoxic activity, we investigated the constituents of whole plants of *Paraixeris pinnatipartita*. We report here the isolation and structural elucidation of a new guaiane sesquiterpene, 3α,9α-dihydroxy-11βH-guai-4(15),10(1)-dien-12,6β-lactone (**1**), as well as five known compounds identified as 11β,13-dihydro-3-epizaluzanin C (**2**),[3,4] lactucine (**3**),[5] β-sitosterol (**4**), 7α-hydroxysitosterol (**5**)[6] and 7α-hydroxystigmasterol (**6**).[7]

Compound **1** was isolated as colourless oil. Its IR spectrum exhibited strong absorption bands characteristic of hydroxyl (3412 cm^{-1}), γ-lactone carbonyl (1766 cm^{-1}) and double bonds (1636 and 1617 cm^{-1}). The HR-ESI-MS showed a quasi-molecular ion peak at *m/z* 282.1707 $[M + NH_4]^+$ (Calcd for $C_{15}H_{24}NO_4$ 282.1700), which established the molecular formula $C_{15}H_{20}O_4$. The ^1H NMR spectrum (Table 1) of **1** showed the signals for a methyl doublet at δ 1.11 (d, J = 6.8), a methyl singlet at δ 1.78 (s) and three oxygenated methines at δ 4.47 (dd, J = 8.0, 7.2), 4.22 (brd, J = 4.8) and 3.69 (brd, J = 7.2), as well as a pair of olefinic singlets at δ 5.18 and 5.09 suggesting an exomethylene. Fifteen carbon signals were observed in ^{13}C NMR spectrum (Table 1), including a lactone carbonyl carbon at δ 178.4, exocyclic double bond carbons at δ 153.7 and 109.3, tetrasubstituted double bond carbons at

Table 1 ^1H, ^{13}C and DEPT data for compound 1 (acetone-d_6, δ in ppm, TMS)[a,b]

Position	δ_H	δ_C	DEPT
1	–	135.7	C
2a	2.77 (dd, J = 15.2, 7.2, 1H)	41.9	CH_2
2b	2.12 (dd, J = 15.2, 8.0, 1H)		
3	4.47 (dd, J = 8.0, 7.2, 1H)	72.5	CH
4	–	153.7	C
5	1.80 (m, 1H)	50.1	CH
6	3.69 (brd, J = 7.2, 1H)	82.5	CH
7	2.57 (m, 1H)	42.0	CH
8a	1.56 (m, 1H)	34.2	CH_2
8b	2.04 (m, 1H)		
9	4.22 (brd, J = 4.8, 1H)	72.6	CH
10	–	133.2	C
11	2.38 (dq, J = 12.0, 6.8, 1H)	47.7	CH
12	–	178.4	C
13	1.11 (d, J = 6.8, 3H)	12.5	CH_3
14	1.78 (s, 3H)	22.5	CH_3
15	5.18 (s, 1H), 5.09 (s, 1H)	109.3	CH_2

[a]Measured at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR.
[b]Assigned by ^1H–^1H COSY and HMBC spectrum.

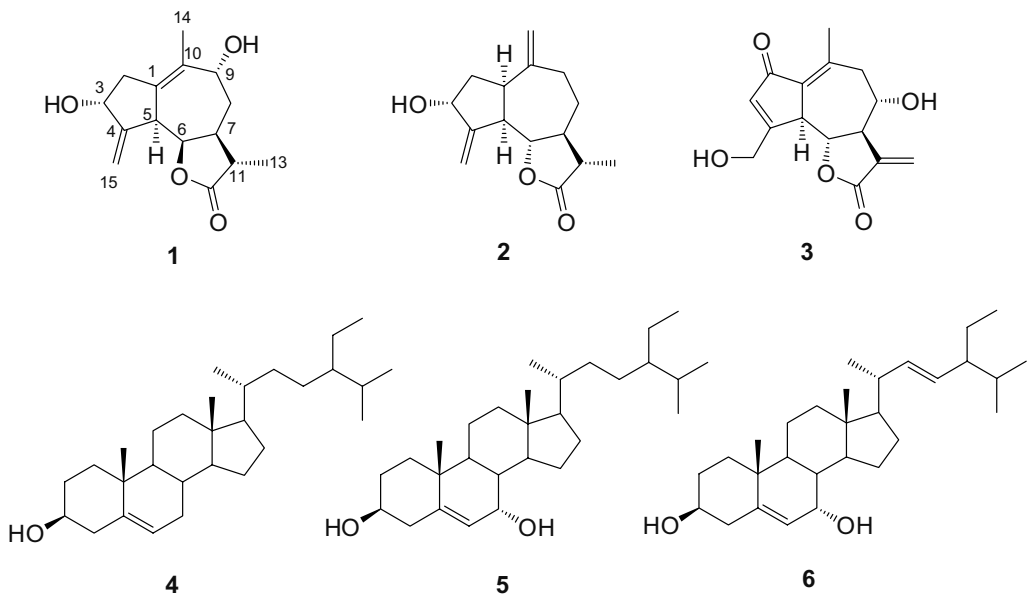

Fig. 1 The structures of compounds **1–6**.

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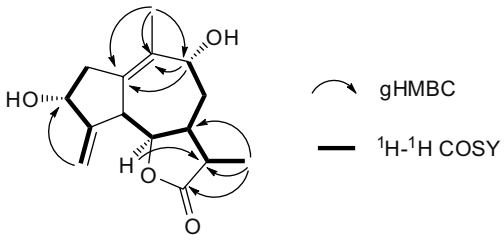

Fig. 2 The key gHMBC and ^{1}H–^{1}H COSY correlations of compound **1**.

δ 135.7 and 133.2, and three oxygenated methylene carbons at δ 82.5, 72.5 and 72.6. Comparison of the above data with those reported in the literature,[4] sugguested that the structure of **1** was very similar to 3α-hydroxy-11βH-guai-4(15),10(1)-dien-12,6α-lactone except for the presence of a more hydroxyl group and the configuration of lactone.

Careful examination of the ^{1}H–^{1}H COSY and gHMBC spectrum allowed the unambiguous assignment of all signals (Table 1 and Fig. 2). The correlations between H-15/H-3, H-3/H-2α,β and H-2α/H-2β in the ^{1}H–^{1}H COSY spectrum suggested a hydroxyl at C-3 and an exocyclic double bond located between C-4 and C-15. The positions of double bond between C-1 and C-10, and the hydroxyl at C-9 could be deduced from the HMBC correlations between CH_3-14/C-1, CH_3-14/C-10, CH_3-14/C-9, H-9/C-1 and H-9/C-10 (Fig. 2). Furthermore, the ^{1}H–^{1}H COSY spectrum exhibited correlations between CH(H-9)–CH_2(H-8)–CH(H-7)–CH(H-6)–CH(H-5) (Fig. 2), which further confirmed the positions of hydroxyl attached to C-9 and the γ-lactone.

The smaller coupling constants of H-6 (brd, J = 7.2) with H-5 and H-7 established the *cis*-diaxial relationship of H-5, H-6 and H-7. Since H-7 in naturally occurring guaianolactones has an α-orientation, the stereochemistry of both H-5 and H-6 were considered to be α-orientation.[8,9] The larger coupling constant between H-11 ($J_{11,7}$ = 12.0 Hz) and H-7 revealed an α-methyl group at C-11.[10] The NOESY correlation observed between H-6 and CH_3-13 also suggested the α-orientation of CH_3-13. The coupling constants of H-3 (dd, J = 8.0, 7.2 Hz) together with the absence of a NOESY correlation between H-3 and H-5 confirmed the α-configuration of hydroxyl group at C-3.[11] The broad doublet of H-9 at δ_H 4.22 (brd, J = 4.8 Hz) in ^{1}H NMR spectrum suggested the α-orientation of hydroxyl group at C-9.[12] Accordingly, the structure of compound **1** was assigned as 3α,9α-dihydroxy-11βH-guai-4(15),10(1)-dien-12,6β-lactone.

Experimental

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded with a Bruker Vertex 70 FT-IR spectrometer. ^{1}H, ^{13}C NMR (DEPT) and 2D NMR were recorded on a Bruker AVANCE 400 spectrometer with TMS as internal reference. HR-ESI-MS spectra were obtained on a Bruker APEX II spectrometer. Silica gel (200–300 and 300–400 mesh) which was used for column chromatography (CC) was supplied by the Qingdao Marine Chemical Factory in China. The purity of the samples were checked on TLC (silica gel, GF_{254} and C-18) under UV light at 254 nm or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

Plant material

The whole plants of *Paraixeris pinnatipartita* were collected from Kunyu Mountains, Weihai, People's Republic of China in September 2007. The specimens were identified by Associate Prof. Hong Zhao (Marine College, Shandong University at Weihai). A voucher specimen (No. KY2007008) was deposited in the Herbarium of Laboratory of Botany, Marine College, Shandong University at Weihai.

Extraction and isolation

The dried whole plants of *Paraixeris pinnatipartita* (1.6 kg) were extracted with 95% EtOH three times (5 days each time) at room temperature The extract was concentrated *in vacuo* to afford a residue (138 g). This residue was partitioned between H_2O and petroleum ether (b.p. 60–90 °C), $CHCl_3$ and n-BuOH, successively. The $CHCl_3$ soluble fraction was concentrated to yield a residue (38 g). This residue was subjected to a silica gel column chromatography (200–300 mesh, 500 g) with a gradient of hexane–acetone (10 : 1, 5 : 1, 3 : 1) as eluent to afford three frations (A–C). Fraction A (hexane–acetone 10 : 1, 11.5 g) was separated by silica-gel column chromatography with hexane-acetone (12 : 1) as eluent and repeated low pressure column chromatography (silica gel 300–400 mesh) to afford **4** (178 mg), **2** (16 mg) and the mixture (21 mg) of **5** and **6**. Fraction B (hexane–acetone 5 : 1, 4.1 g) was subjected to a silica gel column with hexane-acetone (6 : 1) as eluent and purified by TLC to yield compound **1** (12 mg) and **3** (4 mg).

3α,9α-Dihydroxy-11βH-guai-4(15),10(1)-dien-12,6β-lactone (**1**): $C_{15}H_{20}O_4$, Colourless oil. $[\alpha]^{18}_D$ – 1.2° (*ca* 1.08, acetone). IR (KBr) cm^{-1}: 3412 (OH), 1766 (C=O), 1636 (C=C), 1617 (C=C). HR-ESI-MS: *m/z*: 282.1707 ($[M + NH_4]^+$, $C_{15}H_{24}NO_4^+$; Calcd 282.1700). ^{1}H, ^{13}C NMR and DEPT data see Table 1.

11β,13-Dihydro-3-epizaluzanin C (**2**): $C_{15}H_{20}O_3$, colourless oil. EI-MS: *m/z*: 248, 233, 230, 219, 202, 187, 157, 105, 91, 79, 77 67, 55, 41. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (s, 1H, H-15), 5.30 (s, 1H, H-15), 4.94 (s, 1H, H-14), 4.91 (s, 1H, H-14), 4.53 (t, 1H, H-3, J = 7.6 Hz), 4.12 (dd, 1H, H-6, J = 10.0, 9.2 Hz), 1.14 (d, 3H, CH_3-13, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 43.3 (C-1), 38.6 (C-2), 73.6 (C-3), 153.2 (C-4), 49.7 (C-5), 83.6 (C-6), 39.3 (C-7), 28.7 (C-8), 36.0 (C-9), 148.9 (C-10), 46.3 (C-11), 179.6 (C-12), 11.4 (C-13), 113.3 (C-14), 111.3 (C-15).

Lactucine (3): C15H16O5, white powder. FAB-MS: *m/z:* 277 [M + H]+. 1H NMR (400 MHz, DMSO-d6): δ 6.26 (s, 1H, H-3), 6.11, 5.99 (brs, 1H respectively, H-13), 5.46 (d, 1H, 8-OH, J = 7.6 Hz), 5.30 (dd, 1H, 15-OH, J = 5.1, 4.5 Hz), 4.62 (dd, 1H, H-15, J = 18.6, 5.1 Hz), 4.27 (dd, 1H, H-15, J = 18.6, 4.5 Hz), 3.77 (dd, 1H, H-16, J = 10.2, 9.6 Hz), 3.71 (d, 1H, H-5, J = 9.6 Hz), 3.74 (m, 1H, H-8), 2.31 (s, 3H, CH3-14). 13C NMR (100 MHz, DMSO-d6): δ 132.5 (C-1), 195.0 (C-2), 132.9 (C-3), 169.4 (C-4), 48.5 (C-5), 81.3 (C-6), 57.0 (C-7), 67.1 (C-8), 48.9 (C-9), 147.2 (C-10), 138.6 (C-11), 175.6 (C-12), 122.2 (C-13), 21.7 (C-14), 61.9 (C-15).

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