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Three types of sesquiterpenes from rhizomes of *Atractylodes lancea*

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ABSTRACT

Eight sesquiterpenes, including four guaiane-types containing an interesting epoxy unit (**1**–**4**), a rare tricyclic carbon skeleton-type (**5**) and three eudesmane-types (**6**–**8**), along with five known compounds, were isolated from rhizomes of *Atractylodes lancea*. The structures and relative configurations of **1**–**8** were determined by analysis of spectroscopic data, and the absolute configuration of **8** was assigned by application of the CD technique. Compounds **1**, **2** and **4** were evaluated for their cytotoxic effects against P388 and A549 cells, but all were inactive. Possible biosynthetic pathways for sesquiterpenes (**1**–**8**) were discussed.

1. Introduction

The genus *Atractylodes* DC, belonging to the Compositae family, consists of about seven species in the world, of which five are widely distributed in China (Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, 1997). The rhizomes of *Atractylodes lancea* have been used as an important crude drug against rheumatic diseases, digestive disorders, night blindness and influenza (Xiao, 2002; Jiangsu College of New Medicine, 1977) and were listed in Shen-nong-ben-cao-jing, the first Chinese pharmacopoeia written in the Han dynasty (Editor Committee of National Chinese Medical Management Bureau Chinese Herbs, 1999). In the Korean and Japanese pharmacopoeias, the rhizomes of *A. lancea* were prescribed in traditional medicine as diuretic and gastric drugs (Kitajima et al., 2003).

Previous phytochemical investigations into *A. lancea* showed the presence of polyacetylenes (Kano et al., 1989; Marion et al., 1997; Resch et al., 2001), sesquiterpenoids (Chen et al., 1997; Ding et al., 2000; Resch et al., 1998; Zhang et al., 1998) and sesquiterpene glycosides (Yahara et al., 1989; Kitajima et al., 2003). Our interest in identification of sesquiterpene constituents from the family of Compositae (Wu et al., 2004; Li et al., 2005; Liu et al., 2006) prompted us to conduct a detailed chemical investigation into the rhizomes of *A. lancea*. Eight new sesquiterpenes were obtained from this plant, which belong to guaiane-types (**1**–**4**), a rare tricyclic carbon skeleton-type (**5**) and eudesmane-types (**6**–**8**). In addition, five known compounds, named (1*E*)-atractylodin (**9**) (Resch et al., 2001), β-eudesmol (**10**) (Angelopoulou et al., 2001), atractylenolide II (**11**) (Resch et al., 1998), atractylenolide III (**12**) (Bohlmann et al., 1980), and erythro-(1,3*Z*,11*E*)-tridecatriene-7,9-diyne-5,6-diyl diacetate (**13**) (Resch et al., 2001) were identified by direct comparison of their NMR and MS spectroscopic data with those reported in the literature. Compounds **1**, **2** and **4** were tested for cytotoxicity against murine leukemia (P388) and human lung carcinoma (A549) cells by reason that a similar compound guaianediol was reported as having the corresponding activities (Sayed and Hamann, 1996). Here, we report the isolation, structural elucidation and possible biogenetic relationships of compounds **1**–**8**, as well as results of the cytotoxicity evaluation of compounds **1**, **2** and **4**.

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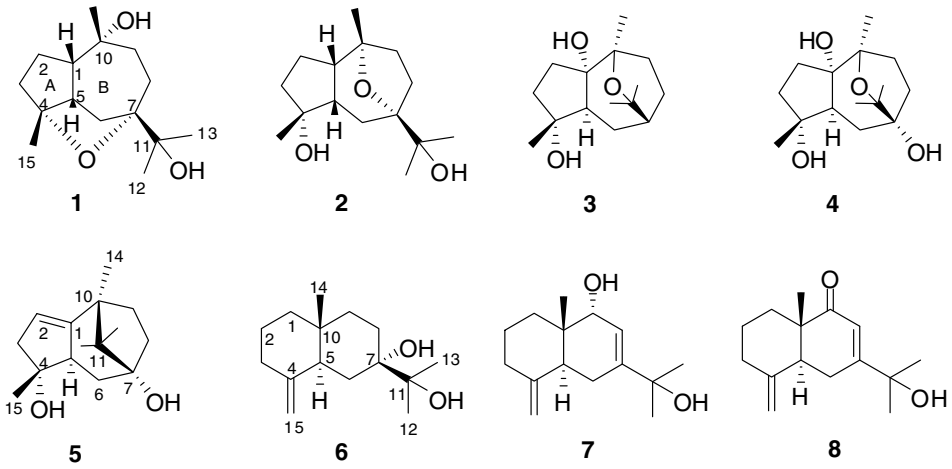


2. Results and discussion

Compound **1** was obtained as a colorless oil and its molecular formula, $C_{15}H_{26}O_3$ was deduced from HRESIMS for the $[M+H]^+$ peak at *m/z* 255.1960 (calcd for $C_{15}H_{27}O_3$, 255.1955). Its IR spectrum showed an absorption band at ν_{max} 3376 cm^{-1}, indicating the presence of hydroxyl groups. The ^1H NMR spectrum of **1** displayed signals for four methyl groups at δ_H 1.17 (s), 1.23 (s), 1.30 (s), and 1.33 (s), which were characteristic of a sesquiterpene. The ^{13}C NMR spectrum showed 15 carbon resonances which were classified by DEPT experiments into four primary, five secondary, two tertiary and four oxygenated quaternary carbon signals (δ_C 94.3, 92.6, 75.6 and 73.4). With three oxygen atoms and three degrees of unsaturation, **1** apparently is a bicyclic sesquiterpene with an epoxy bridge.

The structure of **1** was further established through a variety of two-dimensional NMR spectroscopic techniques. Two partial structures A and B were determined by analysis of ^1H–^1H COSY and HMQC spectra (Fig. 1). A was defined on the basis of the correlations from the methylene protons H-3 to H-2, with the spin system continuing from H-2 through the methine proton H-1 to H-5, and then to the methylene protons H-6. B was assigned due to the correlations between the methylene protons H-9 and H-8. The linkage of the two structural fragments A and B with quaternary carbons was achieved by analysis of the HMBC correlations (Fig. 2): H-15 (δ_H 1.33, s) with C-4 (δ_C 94.3), C-3 (δ_C 39.5) and C-5 (δ_C 54.0); H-14 (δ_H 1.30, s) with C-10 (δ_C 75.6), C-1 (δ_C 53.6) and C-9 (δ_C 34.0); H-5 (δ_H 2.31) with C-4 (δ_C 94.3), C-6 (δ_C 32.2) and C-7 (δ_C 92.6); H-6 with C-4 (δ_C 94.3), C-7 (δ_C 92.6) and C-8 (δ_C 29.7), which indicated that two methyl groups were connected to C-4 and C-10, respectively. The other two methyl groups were determined to be geminal at C-11 connected with C-7 based on mutual HMBC correlations to each other and correlations from H-12 and H-13 (δ_H 1.17 and 1.23) to the oxygen substituted C-7 (δ_C 92.6) and C-11 (δ_C 73.4) (Fig. 2). Thus, **1** was determined as a guaiane-type sesquiterpene oxygenated at C-4, C-7, C-10, and

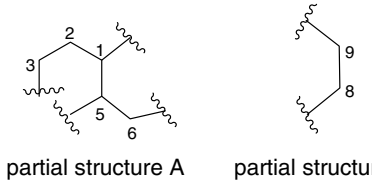


Fig. 1. Partial structures of **1** were deduced from ^1H–^1H COSY and HMQC.

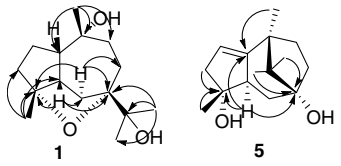


Fig. 2. Selected HMBC (H → C) correlations of compounds **1** and **5**.

C-11. An epoxy bridge was deduced to be at C-4 and C-7 through comparing the ^{13}C NMR spectroscopic data of **1** with those of known guaiane sesquiterpenes with either 4-hydroxyl or 7-hydroxyl moieties. The resonances of C-4 (δ_C 94.3) and C-7 (δ_C 92.6) of **1** were evidently shifted downfield compared with the C-4 (δ_C 81.1) of teuclatriol (Bruno et al., 1993) and C-7(δ_C 79.8) of aokumanol (Itokawa et al., 1987), whereas the signals of C-10 (δ_C 75.6) and C-11 (δ_C 73.4) were coincident with those of the known 1α-H, 4α-H, 5α-H, 7α-H, 10α, 11-dihydroxuguaiane (Bittner et al., 1994). So, the structure of **1** was established.

The relative stereochemistry of **1** was determined by examination of coupling constants and NOE difference spectra. The A/B ring system was *cis*-fused due to $J_{1,5}$ = 7.8 Hz (Aknin et al., 1998; Bittner et al., 1994), which was confirmed by NOE between H-5 and H-1 (2.12%). Additional NOEs were observed between H-5 and H-15 (1.60%), H-14 and H-1 (1.96%), H-14 and H-8β (2.23%), and H-6β and H-12 (1.92%). These correlations suggested that the hydroxyl at C-10 and the epoxy bridge at C-4 and C-7 were on the same side of the ring system. Thus, the structure of **1** was finally determined as 4α,7α-epoxyguaiane-10α,11-diol.

The HRESIMS of **2** gave the same molecular formula $C_{15}H_{26}O_3$ as **1** for the $[M+NH_4]^+$ ion at *m/z* 272.2221 (calcd for $C_{15}H_{30}NO_3$, 272.2220). The IR spectrum showed that **2**, like **1**, also contained hydroxyl groups (3398 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data of **2** were very similar to those of **1**, indicating that **2** should be an isomer of **1**. The ^1H–^1H COSY and HMBC spectra showed that **2** was also a guaiane-type sesquiterpene oxygenated at C-4, C-7, C-10 and C-11. Further comparison of their ^{13}C NMR spectroscopic data showed that the resonances of C-4 and C-7 as well as the adjacent C-5 and C-6 of **2** were shifted upfield to δ_C81.2, 85.5, 49.0 and 24.5 from δ_C 94.3, 92.6, 54.0 and 32.2 of **1**, whereas the signals of C-10 was shifted downfield to δ_C 82.0 from δ_C 75.6 of **1**. These data indicated that the position of the epoxy bridge in **2** was different from **1** and was instead at C-7 and C-10. The structure of **2** was finally determined by further comparison with known compounds 5-hydroxy-6, 9-epoxyguaiane (Somyote et al., 2003) and orientalol E (Peng et al., 2003). The relative configuration of **2** was

determined to be the same as **1** based on examination of the coupling constants and the results of NOE experiments. So, compound **2** was assigned as 7α,10α-epoxyguaiane-4α,11-diol.

Compound **3** was an isomer of **2**, as deduced from the same molecular formula $C_{15}H_{26}O_3$, this being established from the HRESIMS and EIMS data. A close comparison of the ^{13}C NMR spectroscopic data of **3** with **2** showed that the resonance of C-1 was shifted downfield to δ_C 91.5 from δ_C 47.1 of **2**, and the C-7 signal was shifted upfield to δ_C 36.4 from δ_C 85.5 of **2**. This could be attributed to the positions of a hydroxyl group and an epoxy bridge of **3** different from those of **2**. Further comparison of the spectroscopic data of **3** with those of hanamyol (10β,11β-epoxyguaiane-1α-ol) (Itokawa et al., 1984), isolated from *Alpinia japonica* and whose structure determined by X-ray crystallographic analysis, indicated that the hydroxyl group was attached to C-1 and the epoxy bridge was formed between C-10 and C-11. The aforementioned interpretation was further confirmed by analysis of the HMBC spectrum of **3**. In the HMBC experiment, long-rang correlations between the following protons and carbons were observed: H-15 (δ_H 1.21, s) and C-5 (δ_C 54.4), C-4 (δ_C 84.3), C-3 (δ_C 38.1); H-14 (δ_H 1.11, s) and C-10 (δ_C 75.3), C-9 (δ_C 26.1), C-1 (δ_C 91.5); H-5 (δ_H 1.93, m) and C-4 (δ_C 84.3), C-7 (δ_C 36.4), C-6 (δ_C 32.1); H-6 (δ_H 1.94, m) and C-7 (δ_C 36.4), C-8 (δ_C 38.1), C-4 (δ_C 84.3); H-12 and H-13 (δ_H 1.18 and 1.30, each s) and C-7 (δ_C 36.4), C-11 (δ_C 73.5).

The relative stereochemistry of **3** should be identical with that of hanamyol by comparing their ^1H NMR chemical shifts and coupling constants. The NOE experiments apparently confirmed this conclusion: irradiation of H-7 enhanced the signals of H-5 (1.52%) and H-14 (1.23%). So, **3** possessed a *cis*-fused A/B ring system and the epoxy bridge at C-10 and C-11 was β-oriented. Thus, the structure of **3** was elucidated as 10β,11β-epoxyguaiane-1α,4α-diol.

The molecular formula of compound **4** was determined by HRESIMS at *m*/*z* 293.1727 $[M+Na]^+$ as $C_{15}H_{26}O_4$, indicating three degrees of unsaturation, like **1**, **2** and **3**. The IR, ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) were almost identical with those of **3**, except for the presence of one more hydroxyl group, which suggested that **4** was an analog of **3**. In the HMBC spectrum, the proton signals at δ_H 1.18 (H-12) and 1.30 (H-13) showed correlations with two oxygen-bearing quaternary carbon resonances at δ_C 76.2 (C-11) and 72.3 (C-7), implying that the hydroxyl group was located at C-7. The relative configurations at C-1, C-4, C-5, C-7, and C-10 of **4** were consistent with those of **3** as assigned by NOE difference spectra: irradiation of H-5 enhanced the resonance of H-14 (1.63%), and irradiation of H-15 enhanced the signal of H-12 (1.05%). The structure of **4** was determined as 10β,11β-epoxyguaiane-1α,4α,7α- triol.

The ^1H and ^{13}C NMR spectra of compound **5** differed markedly from those of **1**–**4**: two tertiary methyl proton signals (H-12 and H-13) were evidently shifted upfield at δ_H 0.68 and δ_H 0.92, and two quaternary carbon resonances (C-10 and C-11) were also shifted upfield at δ_C 46.6 and δ_C 46.7, which suggested that **5** was another skeleton-type sesquiterpene with a direct connection between C-10 and C-11 rather than through an epoxy bridge. Its ^1H–^1H COSY spectrum was very useful since three spin systems were detected with the first fragment running from an olefinic proton H-2 to H-3, the second connecting H-5 to H-6 and the third from H-8 to H-9. Analysis of the HMBC spectrum indicated that these three fragments were mutually joined eventually merging into the tricyclic carbon skeleton sesquiterpene found in patchoulene (Büchi et al., 1961). Furthermore, olefinic carbons at δ_C 150.2 and 115.1 were assignable to C-1 and C-2, and two oxygenated carbons at δ_C 81.2 and 81.6 to C-4 and C-7 by means of analysis of its HMBC spectrum (Fig. 2) due to clear correlations: H-5, H-14/C-1; H-3, H-5, H-15/C-4; and H-12, H-13/C-7.

In NOE difference experiments, irradiation of H-5 enhanced the resonances of H-14 (2.35%) and H-3α (1.64%), and irradiation of H-15 enhanced the signals of H-3β (2.25%) and H-12 (1.36%), indicating that the two hydroxyl groups attached to C-4 and C-7 were in the α-orientation. Thus, the structure of compound **5** was elucidated as 1-patchoulene-4α,7α-diol.

Table 1
^1H NMR spectroscopic data for compounds **1**–**8** in $CDCl_3$

	1[a]	**2**[b]	**3**[a]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]
1α	–	–	–	–	–	1.34 *m*	1.30 *m*	1.31 *m*
1β	2.15* *m*	1.88 *m*	–	–	–	1.65 *m*	1.99 *m*	2.05 *m*
2α	1.68 *m*	2.08 *m*	2.54 *ddd* (14.4, 8.4, 6.0)	2.52 *ddd* (13.5, 12.3, 2.7)	5.07 *br d* (4.2)	1.26 *m*	1.58 *m*	1.62 *m*
2β	1.82 *m*	1.86 *m*	1.78 *m*	1.84* *m*	–	1.41 *m*	1.58 *m*	1.37 *m*
3α	1.76 *m*	2.05 *m*	1.92 *m*	1.91* *m*	2.52 *br d* (16.2)	2.31 *br d* (12.9)	2.35 *ddd* (14.4, 10.5, 3.0)	2.38 *m*
3β	2.17 *m*	1.96 *m*	1.71 *m*	1.72 *m*	2.36 *dd* (16.2, 4.2)	2.05* *m*	1.96 *m*	2.03 *m*
5	2.31 *br t* (7.8)	2.73 *dd* (11.4, 7.8)	1.93* *m*	1.92* *m*	2.83 *dd* (12.0, 7.8)	2.19 *br d* (9.0)	2.15 *dd* (9.6, 4.5)	2.73 *dd* (9.9, 4.2)
6α	2.55 *br d* (12.6)	2.02* *m*	1.94* *m*	2.03* *m*	1.62 *m*	1.47 *m*	2.03 *dd* (13.5, 4.5)	1.96* m
6β	2.36 *ddd* (12.6, 7.8, 1.8)	1.90* *m*	1.70 *m*	1.58* *m*	1.71 *m*	1.55 *m*	2.15* *m*	2.41 *dd* (11.7, 9.9)
7α	–	–	1.34 *m*	–	–	–	–	–
8α	1.70 *m*	1.45 *m*	1.71 *m*	1.92* m	1.64 *m*	1.52 *m*	5.93 *dd* (5.4, 2.1)	6.06 *d* (2.1)
8β	1.81 *m*	1.40 *m*	1.44 *m*	1.80 *m*	1.86 *m*	1.52 *m*	–	–
9α	1.58 *br dd* (13.2, 9.6)	1.84 *m*	1.43 *m*	1.75 *m*	1.50 *m*	1.52 *m*	3.62 *d* (5.4)	–
9β	1.86 *m*	1.96 *m*	1.80 *m*	1.68 *m*	1.82 *m*	1.74 *m*	–	–
12	1.17 *s*	1.20 *s*	1.18 *s*	1.17 *s*	0.68 *s*	1.26 *s*	1.38 *s*	1.43 *s*
13	1.23 *s*	1.23 *s*	1.30 *s*	1.34 *s*	0.92 *s*	1.26 *s*	1.37 *s*	1.43 *s*
14	1.30 *s*	1.23 *s*	1.11 *s*	1.13 *s*	1.02 *s*	0.69 *s*	0.66 *s*	0.91 *s*
15α	–	–	–	–	–	4.40 *br s*	4.63 *br s*	4.71 *br s*
15β	1.33 *s*	1.29 *s*	1.21 *s*	1.30 *s*	1.29 *s*	4.71 *br s*	4.85 *br s*	4.95 *br s*

Coupling constant values (in parentheses) are in Hz.
[a] Spectra obtained at 600 MHz in $CDCl_3$.
[b] Spectra obtained at 300 MHz in $CDCl_3$.
* Signals overlapped are labeled with multiplicity.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**8** in $CDCl_3$

	1[a]	**2**[b]	**3**[a]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]
C-1	53.6 *d*	47.1 *d*	91.5 *s*	90.4 *s*	150.2 *s*	41.3 *t*	37.0 *t*	36.0 *t*
C-2	29.8 *t*[c]	31.5 *t*	29.2 *t*	28.9 *t*	115.1 *d*	23.4 *t*	23.4 *t*	22.8 *t*
C-3	39.5 *t*	30.5 *t*	38.1 *t*	37.9 *t*	49.0 *t*	36.8 *t*	34.1 *t*	32.7 *t*
C-4	94.3 *s*	81.2 *s*	84.3 *s*	84.3 *s*	81.2 *s*	150.8 *s*	147.5 s	148.2 *s*
C-5	54.0 *d*	49.0 *d*	54.4 *d*	54.2 *d*	51.4 *d*	44.1 *d*	38.6 *d*	45.8 *d*
C-6	32.2 *t*	24.5 *t*	32.1 *t*	32.1 *t*	34.4 *t*	29.3 *t*	24.9 *t*	25.4 *t*
C-7	92.6 *s*	85.5 *s*	36.4 *d*	72.3 *s*	81.6 *s*	76.2 *s*	150.1 *s*	167.0 *s*
C-8	29.7 *t*[c]	33.9 *t*	38.1 *t*	37.1 *t*	34.8 *t*	26.4 *t*	118.9 *d*	120.3 *d*
C-9	34.0 *t*	39.2 *t*	26.1 *t*	27.2 *d*	33.7 *t*	36.2 *t*	72.8 *d*	202.3 *s*
C-10	75.6 *s*	82.0 *s*	75.3 *s*	75.1 *s*	46.6 *s*	35.4 *s*	38.5 *s*	45.7 *s*
C-11	73.4 *s*	73.2 *s*	73.5 *s*	76.2 *s*	46.7 *s*	74.5 *s*	72.8 *s*	72.8 *s*
C-12	24.6 *q*	23.6 *q*	28.0 *q*	24.4 *q*	17.2 *q*	24.7 *q*	29.1 *q*	28.8 *q*
C-13	26.1 *q*	23.8 *q*	29.1 *q*	25.2 *q*	18.4 *q*	24.6 *q*	29.1 *q*	28.7 *q*
C-14	33.8 *q*	24.6 *q*	26.4 *q*	25.9 *q*	16.7 *q*	15.2 *q*	16.5 *q*	15.2 *q*
C-15	28.3 *q*	28.9 *q*	19.2 *q*	24.2 *q*	25.4 *q*	105.1 *t*	107.0 *t*	108.1 *t*

[a] Spectra obtained at 150 MHz in $CDCl_3$.
[b] Spectra obtained at 75 MHz in $CDCl_3$.
[c] Signals may be exchanged.

The HRESIMS of **5** gave the highest mass ion peak at *m*/*z* 219.1743, compatible with the formula $C_{15}H_{23}O$ (calcd for 219.1744), corresponding to the loss of H_2O of $C_{15}H_{25}O_2$, which further confirmed the above deduction.

Compound **6** showed a quasi molecular ion $[M+Na]^+$ at *m*/*z* 261.1827 in its HRESIMS, compatible with the molecular formula $C_{15}H_{26}O_2$ (three degrees of unsaturation). The characteristic features of the ^1H and ^{13}C NMR spectra, such as an angular methyl group (δ_H 0.69, s; δ_C 15.2), suggested that **6** was an eudesmane-type sesquiterpene (Todorova and Tsankova, 1999; Sun et al., 2004; Okasaka et al., 2006). A terminal double bond was located between C-4 (δ_C 150.8) and C-15 (δ_C 105.1), as shown by the HMBC correlations between H-15 (δ_H 4.40) and C-3 (δ_C 22.4) and C-5 (δ_C 44.1). In addition, the proton signal at δ_H 1.26 (6H, s, H-12, H-13) correlated with the carbon resonances at δ_C 76.2 and 74.5, which clearly indicated that two hydroxyl groups were assigned to C-7 and C-11, respectively. The relative configuration of **6** was also determined via NOE difference spectra. Assuming H-14 to be β-oriented, as is the case for most natural eudesmanes isolated from higher plants, the irradiation of H-14 enhanced the signal of H-12(13) (1.29%) indicating H-14, H-12 and H-13 to be β-oriented. NOEs were also observed between H-5 and H-3α, and H-6α, which indicated that the junction of the eudesmane rings was *trans*-fused. Accordingly, the structure of **6** was unambiguously elucidated as eudesm-4(15)-ene-7α,11-diol.

Compound **7** was found to have the molecular formula $C_{15}H_{24}O_2$ on the basis of the HRESIMS for $[M+Na]^+$ ion at *m*/*z* 259.1669 (calcd for $C_{15}H_{24}O_2Na$, 259.1669), accounting for four degrees of unsaturation. The NMR spectra (Tables 1 and 2) suggested that compound **7** also possessed an eudesmane skeleton, like **6**, with the exception that signals for an additional double bond were apparent. Comparison of the ^{13}C NMR and HMBC spectra of **7** with **6** showed that a hydroxyl group could be accommodated at C-9, and one more double bond than **6** was located between C-7 (δ_C 150.1) and C-8 (δ_C 118.9). Irradiation of H-9 (δ_H 3.62) enhanced the resonance of H-14 (δ_H 0.66) in the NOE difference spectrum, indicating that the hydroxyl group was α-oriented if H-14 was assumed to be β-oriented. The structure of **7** was thus elucidated as eudesm-4(15),7-diene-9α,11-diol.

The molecular formula of compound **8** was assigned as $C_{15}H_{22}O_2$ by its HRESIMS for $[M+H]^+$ at *m*/*z* 235.1692 (calcd for $C_{15}H_{23}O_2$, 235.1693). The presence of an α, β-unsaturated ketone unit was supported by the IR (1742 cm^{-1}) and UV (λ_{max} 224.8 nm) spectra, which was also confirmed analysis of the by NMR spectroscopic data. According to the ^1H and ^{13}C NMR spectra (Tables 1 and 2), **8** was very similar to **7**, the only difference occurring at the ketone group located at C-9, which was confirmed by the HMBC correlations of H-8 (δ_H 6.06) and H-14 (δ_H 0.91) with C-9 (δ_C 202.3). So, **8** is a 9-dehydro-derivative of **7** (Scheme 1), named as eudesm-4(15),7-diene-11-ol-9-one.

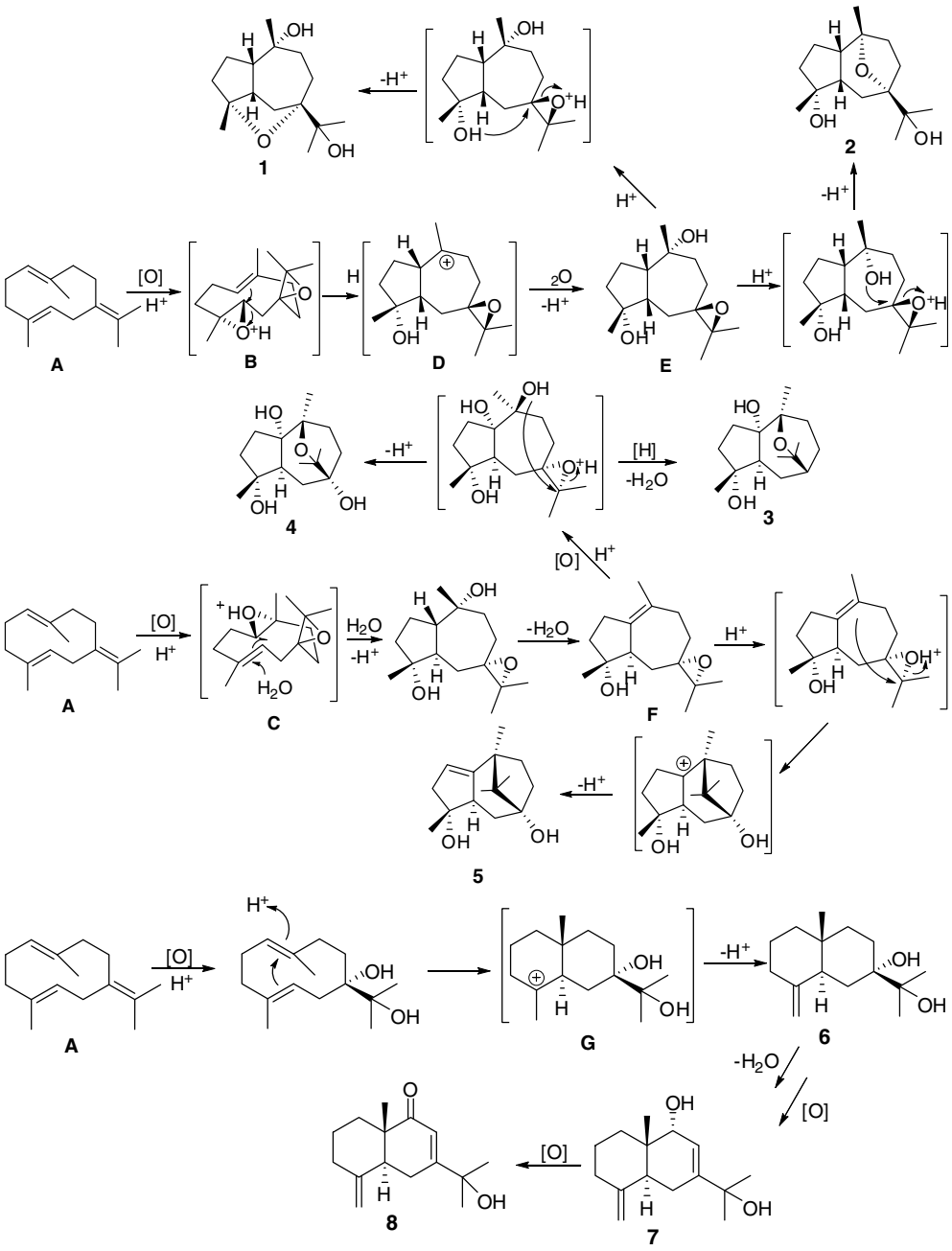
The absolute configuration of **8** was determined by its CD spectrum, in which a positive Cotton effect for $n \rightarrow \pi^*$ was shown at 317 nm [$\Delta\varepsilon_{max}$ + 0.08 (MeOH; *c* 0.5)] and a negative effect for $\pi \rightarrow \pi^*$ at 244 nm [$\Delta\ \varepsilon_{max}$ − 0.62 (MeOH; *c* 0.5)]. Application of the octant rule to **8** (Kirk, 1986) indicated that the configurations at C-5 and C-10 of **8** were 5*R* and 10*S*, respectively.

It has long been recognized that germacrane sesquiterpenes are the biosynthetic precursors of eudesmanes, guaianes and other types of sesquiterpenes (Bülow and König, 2000). Epoxidation of germacrane-type trienes (**A**) leads to either germacrane-4(5), 7(11)-diepoxide (**B**) or germacrane-1(10), 7(11)-diepoxide (**C**). Protonation of the 4,5-epoxide of (**B**) initiates the rearrangement to sesquiterpene with carbocation of guaiane skeleton (**D**), which is attacked by water to give (**E**). Nucleophilic attack of hydroxyl group at either C-4 or C-10 gives rise to the formation of (**1**) and (**2**). Under acidic conditions, germacrane-1(10), 7(11)-diepoxide (**C**) is converted to (**F**) by hydration–dehydration. Compounds **3**, **4**, and **5** are formed from the same intermediate compound (**F**). Protonation of the 1,10-double bond of (**A**) initiates the rearrangement to sesquiterpene with the carbocation of eudesmane skeleton (**G**). In carbocation (**G**), a proton can be eliminated to give the compound **6** and a further oxidation to **6** can take place, resulting in the formation of the compounds **7** and **8**. The possible biosynthetic pathways for new sesquiterpenes **1**–**8** were proposed as shown in Scheme 1.

The antitumor activities against P388 and A549 were tested for compounds **1**, **2** and **4**by reason that their similar guaiane derivatives were reported as having the corresponding activities (Sayed and Hamann, 1996). But it turned out that the result was disappointing and these compounds had no effect for P388 and A549.

3. Conclusions

The species from the family Compositae have a strong tendency to accumulate sesquiterpene compounds. The obtained results in this paper, in comparison with previous studies, indicated a significant intraspecific similitude: *Atractylodes lancea* growing in different regions contained similar eudesmane and guaiane sesquiterpenes (Kitajima et al., 2003; Chen et al., 1997). At the same time, the results also indicated a significant interspecific and intergenus



Scheme 1. Proposed biosynthesis of the sesquiterpenes.

variability: different species and genus contained different types of sesquiterpenes, such as eremophilanes from *Ligularia muliensis* (Wu et al., 2004), bisabolanes from *Ligularia cymbulifera* (Liu et al., 2006), and eudesmanes and guaianes from *Atractylodes lancea*. Thus, the present study and previous reports on plants of the family Compositae suggests that eudesmanes, guaianes, and other types of sesquiterpenes have the same biosynthetic precursors in the biosynthetic pathway.

4. Experimental

4.1. General procedures

Melting points were determined on an X-4 micro melting point apparatus and are uncorrected. Optical rotations were measured using a Perkin Elmer Model 341. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. UV spectra were measured using a Shimadzu UV-260 spectrometer. The CD spectrum was recorded on an Olis DSM 1000 spectropolarimeter. NMR spectra were recorded on Varian Mercury (600, 400 and 300 BB respectively) with TMS as internal standard and $CDCl_3$ as solvents (^1H and ^{13}C NMR spectroscopic data of compounds **1**–**8** are shown in Tables 1 and 2, respectively). The EIMS were measured on a VG ZABHS MS instrument at 70 eV. The HRESIMS were carried out on a Bruker APEXII with glycerol as the matrix. Silica gel (200–300 mesh) used for column chromatography (CC) and silica GF_{254} (10–40 μ) for TLC were supplied by the Qingdao Marine Chemical factory, Qingdao, PR China. TLC was detected at 254 nm or by heating after spraying with 5% H_2SO_4 in EtOH (V/V).

4.2. Plant material

The rhizomes of *A. lancea* were purchased in the traditional Chinese medicine market, Lanzhou City, Gansu Province, P. R. China, in March, 2005 and identified by Prof. Chengyi Li, worked in Gansu College of Traditional Chinese Medicine. A voucher specimen (No. 2005008) was deposited at the Natural Product Laboratory of the College of Chemistry and Chemical Engineering, Lanzhou University, People's Republic of China.

4.3. Extraction and isolation

The dried rhizomes of *A. lancea* (1800 g) were extracted three times with Me_2CO by heating the suspension together until reflux began. This was continued for 4 h, then the extract combined was evaporated under reduced pressure to yield a residue (180 g). The latter was applied to a silica gel (200–300 mesh, 1200 g, 8.0 × 140 cm) column, eluted successively with a gradient of petrol (60–90 °C)–Me_2CO (40:1, 20:1, 15:1, 10:1, 5:1, 2:1 and 0:1, v/v). According to the differences in composition indicated by TLC, seven crude fractions (A–G) were collected. From fraction A (1 g), compounds **9** (650 mg, R_f 0.55, petroleum ether–EtOAc, 50:1) and **11** (5 mg, R_f 0.50, petroleum ether–EtOAc, 30:1) were obtained by repeated silica gel CC with petroleum ether–EtOAc (from 80:1 to 20:1, v/v). Fractionation of fraction C (1.3 g) by repeated silica gel CC with petrol–EtOAc (from 20:1 to 5:1, v/v) as mobile phase afforded compound **10** (101 mg, R_f 0.40, petroleum ether–EtOAc, 15:1). Fraction D (11.2 g) was applied to a silica gel column eluted in a step gradient manner with petrol–EtOAc (from 10:1 to 0:1, v/v) to afford fractions D1–D3. From fraction D1 (0.95 g), compounds **8** (3 mg, R_f 0.75, petrol–EtOAc, 2:1, v/v), **1** (12 mg, R_f 0.55, petrol–EtOAc, 1:1, v/v), **12** (168 mg, R_f 0.45, petrol–EtOAc, 5:1, v/v), and **13** (3 mg, R_f 0.60, petrol–EtOAc, 7:1, v/v) were obtained by repeated silica gel CC with petrol–EtOAc (from 8:1 to 1:2, v/v). Fraction D2 (0.89 g) was separated by silica gel CC (25 g), eluting with petrol–EtOAc (from 6:1 to 1:2, v/v), then purified by preparative TLC (30 g) with petrol–EtOAc (1:1, v/v, two developments) to yield **7** (2 mg, R_f 0.60, petrol–EtOAc, 1:1, v/v). Subfraction D3 (1.1 g) was applied to silica gel CC, eluted with petrol–EtOAc (5:1 to 1:2, v/v) to give **2**(5 mg, R_f 0.52, petrol–EtOAc, 1:1, v/v). Fraction E (7.2 g) was further purified by silica gel CC (150 g), with elution by petrol–EtOAc (from 5:1 to 0:1, v/v) to afford fractions E1-E2. Separation of fraction E1 (0.97 g) by silica gel CC eluted with petrol–EtOAc (4:1 to 1:2, v/v) to yield **3**(10 mg, R_f 0.51, petrol–EtOAc, 1:1, v/v), **4** (2 mg, R_f 0.55, petrol–EtOAc, 1:1, v/v) and **6** (32 mg, R_f 0.48, petrol–EtOAc, 1:1, v/v). The remainder of E2 (1.12 g) was applied to a silica gel column using $CHCl_3$–EtOAc (from 15:1 to 2:1, v/v) as solvent, and finally purified by preparative TLC (30 g) with petrol–EtOAc (1:2, v/v) to yield **5** (10 mg, R_f 0.68, petrol–EtOAc, 1:2, v/v) as a white solid.

4.3.1. 4α,7α-Epoxyguaiane-10α,11-diol (**1**)

Colorless oil, $[\alpha]_D^{21} + 1(CH_3OH, C0.10)$; IR (KBr) ν_{max} cm^{-1}: 3376, 2966, 2924, 1371, 1115; HRESIMS ($[M+H]^+$ *m/z*: 255.1960; calcd. 255.1955); EIMS (probe) 70 eV, *m/z*: 43 (100), 97(53), 177 (79.5), 203 (1.5), 221 (1.4), 236 (0.5), 239 (0.9), 254 $[M]^+$ (0.1); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. 7α,10α-Epoxyguaiane-4α,11-diol (**2**)

Colorless oil, $[\alpha]_D^{21} + 18(CH_3OH, C0.02)$; IR (KBr) ν_{max} cm^{-1}: 3396, 2981, 1368, 1159; HRESIMS ($[M+NH_4]^+$ *m/z*: 272.2221; calcd. 272.2220); EIMS (probe) 70 eV, *m/z*: 43 (100), 95 (57), 177 (5.7), 178 (5.6), 203 (1.3), 221 (1.9), 236 (0.9), 239 (0.3), 254 $[M]^+$ (0.1); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3. 10β,11β-Epoxyguaiane-1α,4α-diol (**3**)

Colorless oil, $[\alpha]_D^{21} + 23(CH_3OH, C0.10)$; IR (KBr) ν_{max} cm^{-1}: 3418, 2978, 2935, 1387, 1114; HRESIMS ($[M-H_2O+H]^+$*m/z*: 237.1849; calcd. 237.1849); EIMS (probe) 70 eV, *m/z*: 43 (93), 84 (100), 86 (61), 110 (25), 149 (39), 179 (15), 254 $[M]^+$ (1); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. 10β,11β-Epoxyguaiane-1α, 4α,7α-triol (**4**)

Colorless oil, $[\alpha]_D^{21} + 3(CH_3OH, C0.07)$; IR (KBr) ν_{max} cm^{-1}: 3427, 3385, 2991, 1380, 1168; HRESIMS ($[M+Na]^+$*m/z* :293.1727; calcd. 293.1723); EIMS (probe) 70 eV, *m/z*: 43 (100), 95 (16), 99 (22), 179 (26), 201 (2.1), 219 (0.8), 237 (1.2), 252 (0.9), 270 $[M]^+$ (0.1); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. 1-Patchoulene-4α,7α-diol (**5**)

Colorless crystals, m.p. 153–154 °C; $[\alpha]_D^{21} + 4(CH_3OH, C0.07)$; IR (KBr) ν_{max} cm^{-1}: 3420, 2923, 1625; HRESIMS ($[M+H-H_2O]^+$*m/z* 219.1743 calcd. 219.1744); EIMS (probe) 70 eV, *m/z*: 43 (100), 91 (25), 97 (13), 107 (22), 135 (20), 175 (13), 193 (19), 221 (4), 236 $[M]^+$ (8); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.6. Eudesm-4(15)-ene-7α,11-diol (**6**)

Colorless oil, $[\alpha]_D^{21} - 26(CH_3OH, C1.00)$; IR (KBr) ν_{max} cm^{-1}: 3426, 2948, 1389; HRESIMS ($[M+Na]^+$ *m/z* 261.1827 calcd. 261.1825); EIMS (probe) 70 Ev, *m/z*: 43 (100), 59 (97), 93 (35), 161 (38), 179 (47), 221 (24), 238 $[M]^+$ (0.9); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.7. Eudesm-4(15),7-diene-9α,11-diol (**7**)

Colorless crystals m.p. 124–125 °C; $[\alpha]_D^{21} - 30(CH_3OH, c0.10)$; IR (KBr) ν_{max} cm^{-1}: 3421, 2924, 1640, 1376; HRESIMS ($[M+Na]^+$ *m/z* 259.1673 calcd 259.1669); EIMS (probe) 70 eV, *m/z*: 43 (100), 59 (76), 91 (43), 145 (44), 160 (20), 203 (8) 218 $[M-H_2O]^+$ (28); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.8. (5R,10S)-Eudesm-4(15),7-diene-11-ol-9-one (**8**)

Colorless oil, $[\alpha]_D^{21} - 8(CH_3OH, c0.25)$; IR (KBr) ν_{max} cm^{-1}: 3425, 2933, 1742, 1234; UV (MeOH) λ_{max} nm ($\log\varepsilon$): 273 (1.77), 225 nm (2.02); HRESIMS ($[M+H]^+$ *m/z* 235.1692 calcd 235.1693); EIMS (probe) 70 eV, *m/z*: 43 (100), 97 (52), 175 (7.4), 193 (5.6), 206 (3.7), 219 (2.7), 234 $[M]^+$ (6); For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

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