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Eudesmane derivatives and other constituents from *Saussurea parviflora*

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Abstract

A survey of the whole plant of *Saussurea parviflora* afforded three compounds 11,12,13-trihydroxy-4(15),8-eudesmadiene-9-one, eudesman-8β,12-olide-1-*O*-β-D-glucoside and 1β,3β-dihydroxyursa-9(11),12-diene-3-octadecanoate, as well as 13 known compounds. Their structures were elucidated on the basis of spectral evidence, especially by using NMR spectroscopic techniques. In addition, encelin exhibited effective antitumor activity on L02, SMMC-7721 and HO-8910 cells.

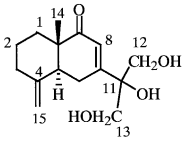
Keywords: *Saussurea parviflora*; Compositae; Eudesmane derivatives; Antitumor activity

1. Introduction

More than 10 *Saussurea* species have long been used in Chinese folk medicine, since they are efficacious in relieving internal heat or fever, harmonizing menstruation, invigorating blood circulation, stopping bleeding, alleviating pain and increasing energy (Jiangsu College of New Medicine, 1976). The chemical constituents and the antitumor activity of compounds from the species *Saussurea parviflora* are described in the present paper.

2. Results and discussion

From *Saussurea parviflora*, two eudesmane derivatives (**1** and **2**) and an ursane triterpene (**3**) were obtained together with 13 known compounds: 4α,15-dihydroencelin (**4**) (Bohlmann et al., 1984), (−)-arctigenin (**5**) (Sazuki et al., 1982), (+)-arctigenin (**6**) (Sazuki et al., 1982), matairesinol-4′-*O*-β-D-glucoside (**7**) (Rahman et al., 1990), isoscopoletin (**8**) (Tsukamoto et al., 1984), quercetin (**9**) (Batterham and Highet, 1964), penduletin (**10**) (Barbera et al., 1986), lupeol (**11**) (Mahato and Kundo, 1994), encelin (**12**) (Quijano et al., 1991), β-sitosterol (**13**), β-sitosterol glucoside (**14**) and tectograndinol lactone (**15**) (Horst and Zlona, 1977) and glyceryl palmitate (**16**) (Bohlmann and Knou, 1979).



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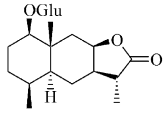

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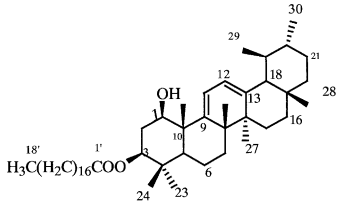

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The pharmacological studies showed that encelin (**12**) has strong antitumor activity. The inhibition of compounds **12** and **7** on the survival of three tumor cell lines, human hepatocytes L02, human hepatoma cell SMMC-7721 and human ovarian neoplasm cell HO-8910, were studied. The survival rates of cells were determined by applying the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method. The half inhibition concentration (IC_{50}) of encelin (**12**) for

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L02 cell was 1.47±0.01 μg/ml, for SMMC-7721 cell 0.57±0.26, and for HO-8910 cell 0.85±0.06. However, **7** had no inhibition on the survival of the three tested tumor cells even when the concentration of **7** was as high as 534 and 666 μg/ml.

Compound **1** was a colorless gum. The IR spectrum revealed absorptions of α,β-unsaturated enone (1655 cm^{-1}) and hydroxyl groups (3450, 3434, 3321 cm^{-1}). The molecular formula $C_{15}H_{22}O_4$ was deduced from HRESIMS spectrum at *m/z* 267.1596 ($[M+H]^+$, calc. 267.1591) and the corresponding ^1H NMR, ^{13}C NMR and DEPT data (Tables 1 and 2). ^1H NMR, ^{13}C NMR and DEPT spectra indicated a terminal double bond at δ_H 4.93 (*br s*) and 4.69 (*br s*), δ_c 147.78 and 108.48; a methyl δ_H 0.88 (*s*), δ_C 15.28; an α, β-unsaturated carbonyl group δ_H 6.13 (*s*), δ_C 206.06, 162.52 and 123.59; and an R-group (Hong et al., 1998) containing a quaternary carbon atom linked with a hydroxyl group δ_C 78.01 and two hydroxymethyl groups δ_H 3.73, 3.76 (*d*, 9.0 Hz) and 3.77, 3.79 (*d*, 8.4 Hz), δ_C 65.78 and 65.61. Five degrees of unsaturation, two of which were attributed to the α, β-unsaturated carbonyl group and one to the terminal double bond, implied the presence of a bicyclic carbon skeleton. An angular methyl δ_H 0.88 (*s*), δ_C 15.28 and a quaternary carbon atom δc 45.97 suggested that **1** had a eudesmane skeleton and the angular methyl group should be β-orientated (Hong et al., 1998; Mahmoud et al., 1984). The positions of the terminal double bond and carbonyl group were determined to be 4(15) and C-9, respectively, via HMBC which showed the cross peaks: C-15 with H-5 and H-3; C-4 with H-5 and H-3; and C-9 with Me-14. In the ^1H NMR spectrum, H-8 gave a singlet at δ_H 6.13, indicating that the carbonyl and R-groups should be at C-9 and C-7, respectively. A double doublet at δ_H 2.47 (J=10.0, 5.0 Hz, H-5) suggested that H-5 should be in a α-orientation.

Compound **2** displayed a strong IR band at 1751 cm^{-1}, suggesting the presence of a lactone functionality. The NMR spectroscopic data (Tables 1 and 2) showed that **2** had a β-D-glucose moiety (Gong, 1985). The molecular formula $C_{21}H_{34}O_6$ was deduced from HRESIMS spectrum that exhibited a molecular ion at *m/z* 421.2315 ($[M+Li]^+$, calc. 421.2414) and 437.2097 ($[M+Na]^+$, calc. 437.2151) together with the NMR spectral data (Tables 1 and 2). Five degrees of unsaturation, two of which were attributed to the lactone ring and one was caused by pyranose glucose ring, indicated the presence of a bicyclic sesquiterpene lactone carbon skeleton. An angular methyl δ_H 0.96 (*s*) suggested that **2** belonged to a eudesmane backbone and that the angular methyl should be in a β-orientation (Nagasampagi et al., 1981). The presence of a 1β-glucosyl group was established by HMBC, which showed the cross peak of C-1 with Me-14 and by ^1H NMR spectrum, in which H-1 gave a double doublet at δ_H 3.16 (J=10.0, 4.0 Hz). A cross peak of H-9α with H-7 in the NOESY spectrum showed that H-7 should be in a α-orientation. At δ_H 2.90, the *ddd* peak with three small coupling constants ($J_{ae}=J_{9\alpha\text{-}8}$=3.5 Hz, $J_{ee}=J_{9\beta\text{-}8}$=2.0 Hz, $J_{ae}=J_{7\text{-}8}$=5.2 Hz) showed that the lactone ring

Table 1
^1H NMR spectral data for compounds **1** and **2** (400 MHz)[a,b]

H	1	2
1 α	1.41 *ddd* (13.2, 3.2, 2.0)	3.16 *dd* (10.0, 4.0)
1 β	1.52 *br dd* (13.2, 2.2)	
2	1.71 *m*	1.33 *m*
3	1.93–1.98 *m*	1.43 *m*
4		1.64 *m*
5α	2.47 *dd* (10.0, 5.0)	1.54 *ddd* (13.2, 5.0, 3.2)
6α	2.34 *dd* (13.2, 5.0)	1.30 *ddd* (13.6, 5.0, 2.0)
6β	2.36 *dd* (13.2, 10.0)	1.38 *ddd* (13.6, 13.2, 12.0)
7		2.18 *m*
8	6.13 *s*	2.90 *ddd* (5.2, 3.5, 2.0)
9α		1.95 *dd* (14.4, 2.0)
9β		1.35 *dd* (14.4, 3.5)
11		1.74 *dq* (7.0, 7.2)
12	3.79 *d* (8.4)	
12′	3.77 *d* (8.4)	
13	3.76 *d* (9.0)	1.16 *d* (7.2)
13′	3.73 *d* (9.0)	
14	0.88 *s*	0.96 *s*
15	4.93 *br s*	0.91 *d* (7.2)
15′	4.69 *br s*	
Glu-H′		4.36 *d* (7.6)

[a] Spectra of compound **1** was recorded in $CDCl_3$, and that of compound **2** was obtained in CD_3OD.
[b] Values in parentheses are coupling constants in Hz.

Table 2
^{13}C NMR spectral data for compounds **1** and **2** (100MHz[a]

C	1	2
1	32.54	80.69
2	22.70	45.78
3	35.95	24.92
4	147.78	37.53
5	46.32	42.16
6	25.87	25.60
7	162.52	43.94
8	123.59	80.24
9	206.06	41.42
10	45.97	33.64
11	78.01	42.63
12	65.78	182.24
13	65.61	21.62
14	15.28	9.48
15	108.48	8.98
1′		101.57
2′		75.25
3′		78.19
4′		71.75
5′		77.89
6′		62.83

[a] Spectra of compound **1** was recorded in $CDCl_3$, and that of compound **2** was obtained in CD_3OD.

Table 3
^{13}C NMR and DEPT spectral data for compound **3** (100 MHz) ($CDCl_3$)

C	3	DEPT	C	3	DEPT
1	75.56	CH	19	39.37	CH
2	34.49	CH_2	20	39.02	CH
3	76.63	CH	21	31.15	CH_2
4	31.86	C	22	41.26	CH_2
5	48.73	CH	23	27.68	CH_3
6	18.28	CH_2	24	17.77	CH_3
7	34.68	CH_2	25	16.19	CH_3
8	43.19	C	26	17.35	CH_3
9	152.03	C	27	22.90	CH_3
10	41.26	C	28	28.63	CH_3
11	117.66	CH	29	18.57	CH_3
12	123.44	CH	30	21.44	CH_3
13	141.61	C	1′	173.44	C=O
14	44.59	C	2′	34.68	CH_2
15	26.17	CH_2	3′	31.86	CH_2
16	25.09	CH_2	4′–16′	29.1–29.8	CH_2
17	37.98	C	17′	22.62	CH_2
18	57.23	CH	18′	14.04	CH_3

should be at C-8 and H-8 should be α-orientated. The large coupling constant of H-5 with H-6β ($J = 13.2$ Hz) and the small coupling constant of H-5 with H-4 ($J = 3.2$ Hz) showed that H-5 and H-4 should all be α-orientated and thus, Me-15 must be β-orientated. The cross peak of H-8 with Me-13 in the NOESY spectrum suggested that Me-13 should be α-orientated.

Compound **3** displayed a strong IR band at 1731 cm^{-1}, which revealed the presence of an ester carbonyl group. The molecular formula $C_{48}H_{82}O_3$ was deduced from HRESIMS spectrum at *m/z* 707.6284 ($[M+H]^+$, calc. 707.6342) associated with NMR data (Table 3 and Experimental). The octadecanoate moiety was confirmed by the fragments in EIMS spectrum at *m/z* 283, 255, 241, 227, etc. (Cong, 1987) and by the NMR spectroscopic data. In addition, **3** had two double bonds (–C=CH–) on the basis of its ^1H NMR and ^{13}C NMR spectra. Eight degrees of unsaturation, two of which were attributed to the two double bonds and one caused by the ester carbonyl group, implied the presence of a pentacyclic carbon skeleton. The signals for eight methyl groups (Table 3, Experimental) in the NMR spectrum and seven quaternary carbon atoms in the DEPT spectrum showed that it was an ursane triterpene. The positions of two double bonds were determined to be at C-9 (11) and C-12 (13) by comparing the ^1H NMR spectral data of **3** at δ_H 5.48 and 6.52 (each 1H, 6.0 Hz, H-11, H-12) with those of 1β,3β-dihydroxyolean-9(11),12-diene at δ_H 5.45 and 6.51(each 1H, 6.0 Hz, H-11, H-12) (Gonzalez et al., 1987) and by comparing the ^{13}C NMR spectral data of **13** at δ_C 117.65, 152.03, 123.44 and 141.61 with those of known compounds which have two double bonds at C-9 (11) and C-12 (13) (Mahato and Kundu, 1994). The presence of a 1β-hydroxy and a 3β-hydroxy ester was established by comparing the ^1H NMR spectral data of **13** at δ_H 3.96 (1H, *dd*, 11.4, 4.8 Hz, H-1), 4.54 (1H, *dd*, 12.0, 4.4 Hz, H-3) with those of nepetidone at δ_H 3.98 (1H, *dd*, 11.0, 4.8, H-1), 3.59 (1H, *dd*, 12.0, 3.8 Hz, H-3) (Ahmad and Mohammad, 1986) and with those of nepetidone-3,11-diacetate at δ_H 3.68 (1H, *dd*, 10.9, 4.9, H-1), 4.50 (1H, *dd*, 11.9, 4.1 Hz, H-3) (Ahmad and Mohammad, 1986).

3. Experimental

3.1. General

Melting points were determined on a Kofler melting point apparatus and were uncorrected. NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C on a Bruker FT 400 spectrometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were determined on a Nicolet 170SX spectrometer. HRESIMS were obtained on a Bruker Daltonics APEX II 47e spectrometer. EIMS (70eV) were obtained with a HP-5988 AGC MS apparatus. UV spectra were obtained on a Shanghai UV-240 spectrophotometer. CC utilized silica gel (200–300 mesh) and polyamide (80–120 mesh).

3.2. Plant material

The whole plant of *Saussurea parviflora* was collected in 1998 in Gansu Province of China and identified by Professor Zhang Guoliang, Department of Biology, Lanzhou University, China. A voucher specimen (No. 1998828) is deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried plant material (5 kg) was exhaustively extracted with MeOH (5.0 l for three times, 5 days/time) at room temp. The crude extract (197.7 g) was partitioned between H_2O and petroleum ether (residue 94.5 g), H_2O and EtOAc (23.2 g), and then H_2O and *n*-BuOH (80 g).

The petroleum ether extract was prefractionated by CC on silica gel yielding Fraction A (petroleum ether–Me_2CO 10:1), B (petroleum ether–Me_2CO 8:1), C (petroleum ether–Me_2CO 6:1), D (petroleum ether–Me_2CO 1:1). Fraction A (11.0 g), C (1.2 g) and D (2.2 g) were purified, respectively, by recrystallization to yield **11** (10.0 g), **13** (1.0 g) and **14** (2.0 g). Fr. B (0.2 g) was separated by CC on silica gel eluting with petroleum ether–EtOAC (8:1) and $CHCl_3$–EtOAC (20:1) to yield **3** (15.0 mg), **15** (8.0 mg) and **16** (8.0 mg), respectively.

The EtOAc extract was prefractionated by CC on silica gel yielding Fr. I, II ($CHCl_3$–MeOH 40:1), III, IV ($CHCl_3$–MeOH 30:1), V ($CHCl_3$–MeOH 20:1), VI ($CHCl_3$–MeOH 10:1) and VII ($CHCl_3$–MeOH 6:1). Fr. I (0.1 g) was applied to a silica gel column and eluted with petroleum ether–EtOAC (3:1) to yield **4** (13.0 mg). Fr. II (0.6 g) was purified by recrystallization to yield **12** (500 mg). Fr. III (0.3 g) was subjected to silica gel chromatography (CC) elute with petroleum ether–Me_2CO (4:1) to yield **8** (8.0 mg). Fr. IV (60.0 mg) was applied to a silica gel column, eluted with petroleum ether–Me_2CO (5:1) to yield **1** (8.0 mg). Fr. V (50.0 mg) was further purified by prep. TLC with $CHCl_3$–MeOH (10:1) to yield **10** (5.0 mg). Fr. VI (1.2 g) was separated by CC on silica gel eluting with $CHCl_3$–MeOH (30:1, 20:1, 10:1) to obtain **5** (10.0 mg), **6** (5.0 mg) and **7** (500 mg), respectively. Fr. VIII (60.0 mg) was chromatographed on polyamide column eluting with MeOH–H_2O (1:1) to yield **9** (13.0 mg).

The *n*-BuOH fraction was prefractionated by CC on silica gel yielding Fr. a (EtOAc–MeOH 10:1), Fr. b (EtOAc–MeOH 8:1), Fr. c (EtOAc–MeOH 6:1), Fr. d (EtOAc–MeOH 4:1). Fr. a (1 g) was further purified by CC on silica gel eluting with $CHCl_3$–MeOH (6:1) to yield **2** (10.0 mg).

3.4. Assays of antitumor activity

3.4.1. Cell culture

Human hepatocytes L02, human hepatoma cell SMMC-7721 and human ovarian neoplasm cell HO-8910 have been cultured with 10% bovine serum at 37° and with 5% CO_2. The survival rates were determined with MTT method (Hussain et al., 1993).

3.4.2. Testing of anti-tumor activity

Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and dispersed in replicate 96-well plates with 4×103 cells/well for 24 h. Compounds **12** and **7** or vincristine (used as a positive control) were then added. After 48-h exposure to the toxins, cell viability was determined by the [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) colorimetric assay by measuring the absorbance at 595 nm with an ELISA reader. Each test was performed in 5 replicate.

3.5. 11,12,13-Trihydroxy-4(15), 8-eudesmadiene-9-one (1)

Colorless gum, $[\alpha]_D^{10}$ +64.9° ($CHCl_3$; *c* 0.15); HRESIMS *m/z* 267.1596 ($[M+H]^+$, calc. 267.1591); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 324 (4.50); IR $\gamma_{max}^{CHCl_3}$ cm^{-1}: 3434, 3321, 1655; for NMR spectra, see Tables 1 and 2.

3.6. Eudesman-8β,12-olide-1-O-β-D-glucoside (2)

White powder, mp 149–152 °C; $[\alpha]_D^{10}$ −37.9° (CH_3OH; *c* 0.2); HRESIMS *m/z* 421.2315 ($[M+Li]^+$, calc. 421.2414), 437.2097 ($[M+Na]^+$, calc. 437.2151); IR γ_{max}^{MeOH} cm^{-1}: 3434, 1751; for NMR spectra, see Tables 1 and 2.

3.7. 1β,3β-Dihydroxyurs-9(11),12-diene-3-octadecanoate (3)

White powder, mp 152–154 °C; $[\alpha]_D^{10}$ +97.7° (CH_3OH; *c* 0.3); HRESIMS *m/z* 707.6284 ($[M+H]^+$, calc. 707.6342); EIMS *m/z* (rel. int.): 706 [M^+] (1), 423 (12), 405 (4), 283 (6), 255 (50), 241 (6), 227 (9), 213 (13), 199 (12), 185 (19), 171 (36), 157 (21), 143 (16), 129 (13), 115 (6), 111 (17), 97 (34), 85 (22), 83 (36), 73 (14), 71 (41), 69 (63), 57 (76), 43 (100); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 250 (4.50); IR γ_{max}^{MeOH} cm^{-1}: 3439, 1731, 2924, 2854; ^1H NMR: 1.16 (3H, s), 0.99 (3H, *s*), 0.93 (3H, *s*), 0.90 (3H, *s*), 0.87 (3H, *s*), 0.82 (3H, *s*), 0.89 (3H, *d*, *J*=2.0 Hz), 0.87 (3H, *d*, *J*=2.0 Hz), 6.52, 5.48 (each 1H, *d*, *J*=6.0 Hz, H-11, 12), 4.54 (1H, *dd*, *J*=12.0, 4.4 Hz, H-3), 3.96 (1H, *dd*, *J*=11.4, 4.8 Hz, H-1), 0.88 (3H, *t*, *J*=2.2 Hz), 1.25 (32H, *m*); for ^{13}C NMR spectra, see Table 3.

Acknowledgements

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