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Aromadendrane-type sesquiterpene derivatives and other constituents from *Erigeron acer*

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Received July 4, 2005, accepted August 8, 2005

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Pharmazie 62: 312–315 (2007) *doi: 10.1691/ph.2007.4.5641*

A new aromadendrane-type sesquiterpene derivative (**1**) and a new diterpene acetylarabinoside (**7**), together with twelve known compounds, were isolated from the whole plants of *Erigeron acer*, which can relieve tooth-aches and arthritic pains. Their structures were elucidated by spectroscopic methods and chemical methods.

1. Introduction

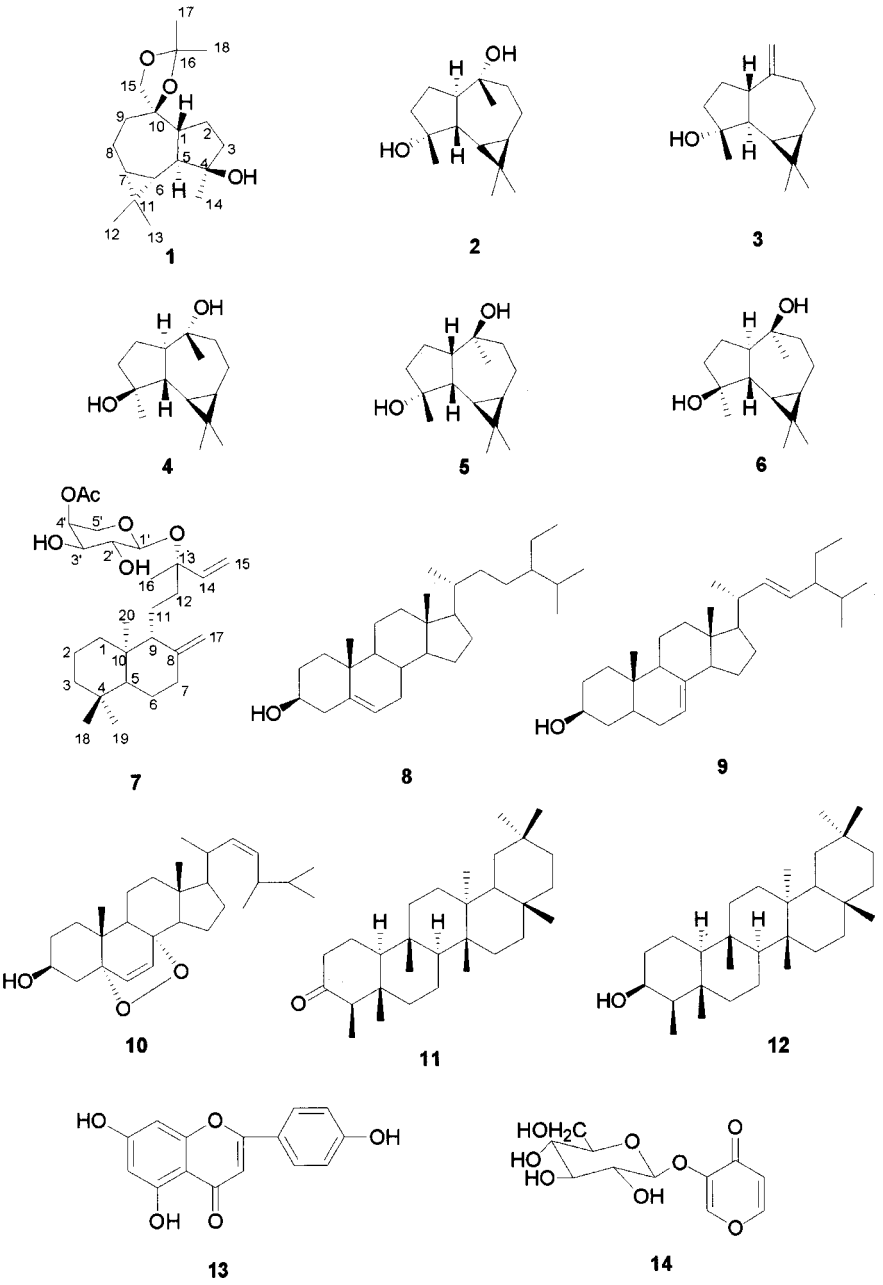
The genus *Erigeron* (Compositae) is widespread in Asia, North America and Europe. There are about 35 species distributed in China (Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, 1985), some of which have a long history of application in Chinese folk medicine (Outline of New China Herbals, 1990). Sesquiterpenes have been reported as the main constituents in the genus by a Japanese research group (Iijima et al. 2003) and our group (Li et al. 2005). In the course of our continuing research to this genus, we investigated *Erigeron acer*, which can relieve tooth-aches and arthritic pains (Pieroni et al. 2004) and has never been studied previously. In this paper, we describe the isolation and structural elucidation of the chemical constituents from the whole plant of this species.

2. Investigations, results and discussion

The dried and powdered whole plant of *Erigeron acer* (5.5 kg) was extracted with acetone 3 times (7 days each time) at room temperature. The extract was evaporated and the residue was chromatographed on silica gel column with petroleum ether (60–90 °C)-acetone gradient in developing ratio. The results of the experiment yielded a new aromadendrane-type sequiterpene named as 4β,10β,15-trihydroxy-aromadendrane-10,15-acetonide (**1**) and a new diterpene acetylarabinoside named as ent-manool-13-*O*-α-L-4-acetylarabinopyranoside (**7**), together with twelve known compounds (**2**–**6**, **8**–**14**). Their structures were determined by spectroscopic and chemical methods.

The structures of the known compounds were identified by comparing their spectral data (MS, IR, ^{1}H and ^{13}C NMR) with those reported in literature as 4α,10α-aromadendranediol (**2**) (Beechan et al. 1978), (+)-spathulenol (**3**) (Duh et al. 1986), 4β,10α-aromadendranediol (**4**) (Anjaneyulu et al. 1995), 4α,10β-alloaromadendranediol (**5**) (Goldsby et al. 1987) and 4β,10β-aromadendranediol (**6**) (Beechan et al. 1978), β-sitosterol (**8**) (Marina et al. 1990), stigmast-7,22-diene-3β-ol (**9**) (Thompson et al. 1972), ergost-6,22-diene-5α,8α-epidioxy-3β-ol (**10**) (Yan et al. 2003), friedelin (**11**) (Shashi et al. 1994) and friedelan-3β-ol (**12**) (Xu et al. 1998), apigenin (**13**) (Yang et al. 2004) and erigeroside (**14**) (Yue et al. 1994).

Compound **1** was obtained as a yellow gum. Its molecular formula was determined to be $C_{18}H_{30}O_3$ by HR-ESI-MS at m/z 317.2084 (calcd for $C_{18}H_{30}O_3Na$ 317.2087). The IR spectrum revealed strong absorption bonds for OH at 3419 cm^{-1} and C–O at 1210, 1153, 1117, 1101 and 1059 cm^{-1}. The appearance of the signals in **1** at δ_H 1.38 and δ_H 1.41 (s, each 3 H) in the ^{1}H NMR spectrum and at δ 108.7, 27.5 and 26.8 in the ^{13}C NMR and DEPT spectra showed that the compound contained a moiety of an acetone ketal. Except for the moiety the ^{13}C NMR and DEPT of **1** showed the presence of 15 carbon signals: three methyls, five methylenes, four methines and three quaternary carbons, which indicated that **1** was a sesquiterpene type compound. Furthermore, the ^{1}H NMR spectrum of the compound exhibited two typical cyclopropyl protons, one at δ 0.40 and the other a lower field δ 0.65 characteristic of 1,5 trans aromadendrane sesquiterpene skeleton (H-1β, H-5α) (Anjaneyulu et al. 1995). It also exhibited two tertiary methyls at δ 1.02 and one tertiary methyl at δ 1.26 connected to an oxygenated carbon. In the HMBC experiment there were the cross peaks between H-14 (δ 1.26) and C-4 (δ 80.6), C-5 (δ 48.7), which indicated that a hydroxyl was attached to C-4. The other cross-peaks were also observed between H-17 (δ 1.38) and C-16 (δ 108.7), C-18 (δ 26.8); H-18 (δ 1.41) and C-16 (δ 108.7), C-17 (δ 27.5); H-15 (δ 3.82, 3.95) and C-10 (δ 86.1), C-16 (δ 108.7), which showed that the acetone ketal was at 10,18-position, thus the planar structure of **1** was determined. The relative stereochemistry of the compound can be deduced by experimental rules: a trans-orientation of the cycolpropane ring (trans between H-6, H-7 and H-5) was assigned because of the ^{13}C NMR spectral data of the geminal dimethyl group on the cyclopropane ring at δ 16.3 and δ 28.5 (Goldsby et al. 1987). The che-



mical shift of C-6 at δ 26.0 suggested that the stereochemistry of CH_3-14 was in an α-orientation when H-5 was α-orientated (Goldsby et al. 1987). The chemical shift of C-15 at δ 68.2, which was at higher field, suggested that the stereochemistry of CH_2-15 was also in an α-orientation (Goldsby et al. 1987; Feliciano et al. 1989), which was further confirmed by NOE correlations between H-5 and H-15, H-14 in NOE different spectrum. So the complete structure of **1** was deduced as 4β,10β,15-trihydroxy-aromadendrane-10,15-acetonide, which should be a natural product rather than an artifact extracting with acetone for it was checked in the methanol extract of the plant.

Compound **7** was obtained as a needle crystal. HR-ESI-MS showed $[M+Na]^+$ at m/z 487.3034 (cacld for $C_{27}H_{44}O_6Na$ 487.3030), indicating a molecular formula $C_{27}H_{44}O_6$, which was supported by ^{13}C NMR and DEPT spectra data. Its IR spectrum displayed the absorption bands for hydroxyl groups (3486, 3429, 1087 cm^{-1}), a carbonyl group (1722 cm^{-1}), olefinyl groups (1641 cm^{-1}). Meanwhile in the low mass region of FAB-MS spectrum a peak at m/z 273 was corresponded to the loss of an acetyl pentose ($C_7H_{11}O_6$). The ^{13}C NMR signals at δ 97.9, 71.4, 70.0, 71.9 and 63.2 were very similar to those of the arabinose moiety of α-arabinopyranoside (Gorin et al. 1975). The $^1H-^1H$ COSY spectrum of **7** showed the correlation points: H-5a′ (3.98, dd, 1 H)/H-5b′ (3.52, brd, 1 H), H-4′ (5.08, brs, 1 H); H-3′ (3.77, brd, 1 H)/H-4′ (5.08, brs, 1 H), H-2′(3.65, dd, 1 H); H-2′ (3.65, dd, 1 H)/H-1′ (4.34, d, 1 H). Furthermore, the signals at δ 171.0, 21.1 and 2.13 indicated that one of the hydroxyls of the arabinose moiety was acetylated. In the ^1H NMR four methyl groups appeared at δ 0.65, 0.79, 0.86 and 1.35 (s, each 3 H), characterisitic methylene signals appeared at δ 4.46, 4.79 and a vinilic group appeared at δ 5.17, 5.25 and 5.79, indicating that **7** was a *ent*-manool diterpene derivative (Urzua et al. 1995). The ^{13}C NMR spectral data of **7** were similar to those of the known compound *ent*-manool-13-*O*-β-4′-acetylxylopyranoside (Urzua et al. 1995), and the obvious difference was the chemical shifts of the moiety of pentose. The arabinopyranoside was confirmed by PC and was determined as L-(+)-configuration by optical rotation ($[\alpha]_D^{20}$ +152° c 0.05, H_2O) after an acid hydrolysis of

7. The $J_{1'2'}$ (7.2 Hz) of **7** suggested a diaxial relationship between H-1′ and H-2′, and α configuration of the anomeric carbon. The chemical shift of H-4′ was at lower field and there was the cross peak of δ_C 171.0/$\delta_{H-4'}$ 5.08 in the HMBC experiment, which showed that the hydroxyl at C-4′ was acetylated. Thus the structure of **7** was determined as *ent*-manool-13-*O*-α-L-4′-acetylarabinopyranoside.

3. Experimental

3.1. Equipment

Silica gel (200–300 mesh) used for column chromatography (CC) and silica GF_{254} (10–40 μm) for TLC were supplied by the Qingdao Marine Chemical factory, Qingdao, P.R. China. TLC were detected at 254 nm or by heating after being sprayed with 5% H_2SO_4 in C_2H_5OH (V/V). M.p.'s were determined on a Kofler hot-stage instrument 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. NMR spectra were recorded on a Varian Mercury-300BB NMR instrument (^{1}H NMR spectra at 300 MHz and ^{13}C NMR at 75 MHz) with TMS as the internal standard and d-$CHCl_3$ as solvents. FAB-MS were measured on ZAB-HS MS instrument and the positive HR-ESI-MS (secondary ion mass spectrometry) were carried out on a Bruker APEX II with glycerol as the matrix.

3.2. Plant material

The whole plant *Erigeron acer* was collected in Zhangxian district, Gansu Province, China and authenticated by Professor G. L. Zhang, School of Life Sciences, Lanzhou University, where a voucher specimen has been deposited.

3.3. Extraction and isolation

The dried and powdered whole plant of *Erigeron acer* (5.5 kg) was extracted with acetone 3 times (7 days each time) at room temperature. The combined extracts were evaporated to dryness (172.5 g) under reduced pressure. The residue was separated by column chromatography over 1800 g silica gel (200–300 mesh) with a gradient of petroleum ether-acetone (1 : 0, 50 : 1, 20 : 1, 10 : 1, 8 : 1, 5 : 1, 2 : 1, 0 : 1). According to the differences in the composition indicated by TLC, 6 crude fractions Fr. 1 (1 : 0 and 50 : 1), Fr. 2 (20 : 1 and 10 : 1), Fr. 3 (8 : 1), Fr. 4 (5 : 1), Fr. 5 (2 : 1) and Fr. 6 (0 : 1) were obtained. From Fr. 1, the crude **11** was deposited and recrystalized in acetone, gave **11** (30 mg); Fr. 2 was repetitive chromatographed (petroleum ether-acetone 20 : 1) on silica gel column to give compounds **8** (102 mg), **9** (36 mg), **12** (8 mg) and crude **3**; **3** (30 mg) was purified by repetitive chromatographed (petroleum ether-EtOAc 20 : 1); Fr. 3 was chromatographed on a silica gel column eluting with petroleum ether-acetone (20 : 1–0 : 1) to give two crude fractions: Fr. 3–1 and Fr. 3–2. Fr. 3–1 was chromatographed on a silica gel column eluting with chloroform-acetone (50 : 1, R_f 0.14) to give **1** (2 mg). Crude **10** was obtained from Fr. 3–2, then purified by PTLC over a silica gel plate using chloroform-acetone (10 : 1) as a developing systems to give **10** (5 mg); Fr. 4 was chromatographed on a silica gel column eluting with gradient of chloroform-acetone (50 : 1–0 : 1) to give crude **2**, **4**, **5** and **6**. **2** (5 mg) was obtained by repetitive chromatographed on a silica gel column eluting with chloroform-acetone (5 : 1); **4** (10 mg) was purified by PTLC over a silica gel plate using chloroform-acetone (3 : 1) as a developing system; crude **5** was repetitive chromatographed on a silica gel column eluting with chloroform-acetone (10 : 1) to yield **5** (5 mg) and **6** (7 mg) was obtained by repetitive chromatographed (chloroform-acetone 3 : 1) on silica gel column. **13** (4 mg) was afforded from Fr. 5 by CC on silica gel with $CHCl_3$-acetone (10 : 1) several times and then purified by PTLC over a silica gel plate using chloroform-acetone (2 : 1) as a developing system. Fr. 5 also a crude fraction containing **7**, which was further purified by chromatography on a silica gel column with petroleum ether-EtOAc 2 : 1 to give **7** (15 mg). **14** (25 mg) as a white powder was obtained from Fr. 6, which were purified by recrystallization with acetone.

3.4. Acid hydrolysis of compound 7

Compound **7** (10 mg) was dissolved in 5 ml MeOH and 5 ml 10% HCl. The mixtures were stirred at room temperature for 5 h. The solvent was vaporized and then pumped at vacuum condition in order to eliminate HCl. The remaining residues were diluted with H_2O (5 ml) and extracted with Ch_2Cl_2. The H_2O layers were evaperated to dryness yielding the monosaccharide (2 mg, $[\alpha]_D^{20}$ +152° c 0.05, H_2O).

3.5. 4β,10β,15-Trihydroxy-aromadendrane 10,15-acetonide (**1**)

Yellow gum, $[\alpha]_D^{20}$ −12° (c 0.20, CH_3OH). IR (ν_{max}, cm^{-1}, KBr): 3419, 1210, 1153, 1117, 1101 and 1059; ^{1}H NMR and ^{13}C NMR data see Table 1; EL-MS (m/z): 294, 279 (M-15), 236, (M-$C_3H_6O_2$); HR-ESI-MS (m/z): 317.2084, calcd for $C_{18}H_{30}O_3Na$ 317.2087.

Table 1: ^{1}H NMR and ^{13}C NMR data of compound 1

H	δ_H	C	δ_C	DEPT
1	2.03 m	1	54.3	CH
2a	1.62 m	2	26.1	CH_2
2b	1.74 m			
3a	1.50 m	3	41.0	CH_2
3b	1.65 m			
		4	80.6	C
5	1.05 m	5	48.7	CH
6	0.40 dd (9.6, 10.8)	6	26.0	CH
7	0.65 ddd (6.0, 9.6, 11.1)	7	29.0	CH
8a	0.95 m	8	19.9	CH_2
8b	1.77 m			
9a	1.65 m	9	40.5	CH_2
9b	1.98 m			
		10	86.1	C
		11	19.8	C
12	1.02 s	12	16.3	CH_3
13	1.02 s	13	28.5	CH_3
14	1.26 s	14	25.0	CH_3
15a	3.82 d (8.8)	15	68.2	CH_2
15b	3.95 d (8.8)			
		16	108.7	C
17	1.38 s	17	27.5	CH_3
18	1.41 s	18	26.8	CH_3

(δ, ppm, $CDCl_3$, ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz)

3.6. ent-Manool-13-O-α-L-4′-acetylarabinopyranoside (**7**)

Corlorless needle crystal. m.p. 147–148 °C; $[\alpha]_D^{20}$ +46° (c 0.30, CH_3OH). IR (ν_{max}, cm^{-1}, KBr): 3486, 3429, 1087, 1641, 1722, 1258; ^{1}H NMR and ^{13}C NMR data see Table 2; FAB-MS (m/z): 487.4 $[M+Na]^+$, 273.3 (M-$C_7H_{11}O_6$); HR-ESI-MS (m/z): 487.3034, calcd for $C_{27}H_{44}O_6Na$ 487.3030.

Table 2: ^{1}H NMR and ^{13}C NMR data of compound 7

H	δ_H	C	δ_C	DEPT
1	1.68 ∼ 1.76 m	1	39.0	CH_2
2	1.33 ∼ 1.55 m	2	19.3	CH_2
3	1.17 ∼ 1.31 m	3	42.1	CH_2
		4	33.5	C
5	1.01 ∼ 1.09 m	5	55.5	CH
6	1.17 ∼ 1.31 m	6	24.4	CH_2
7a	2.35 dt (9.0, 3.2)	7	38.3	CH_2
7b	1.93 dt (9.0, 3.2)			
		8	148.7	C
9	1.33 ∼ 1.55 m	9	57.2	CH
		10	39.8	C
11	1.01 ∼ 1.09 m	11	17.6	CH_2
12	1.68 ∼ 1.76 m	12	40.5	CH_2
		13	81.3	C
14	5.79 dd (11.1, 18.7)	14	142.1	CH
15a	5.17 brd (18.7)	15	116.4	CH_2
15b	5.25 brd (11.1)			
16	1.35 s	16	22.3	CH_3
17a	4.46 brs	17	106.4	CH_2
17b	4.79 brs			
18	0.86 s	18	33.5	CH_3
19	0.79 s	19	21.7	CH_3
20	0.65 s	20	14.4	CH_3
Arabinose		Arabinose		
1′	4.34 d (7.2)	1′	97.9	CH
2′	3.65 dd (7.2, 8.4)	2′	71.4	CH
3′	3.77 brd (8.4)	3′	70.0	CH
4′	5.08 brs	4′	71.9	CH
5a′	3.98 dd (3, 12.8)	5′	63.2	CH_2
5b′	3.52 brd (12.8)			
Ac		Ac		
		1″	171.0	C
2″	2.13	2″	21.1	CH_3

(δ, ppm, $CDCl_3$, ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz)

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No. 20372029 and No. 20021001) and by the Key Project of Chinese Ministry of Education (No. 104178).

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