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New sesquiterpenes from *Ligularia macrophylla*

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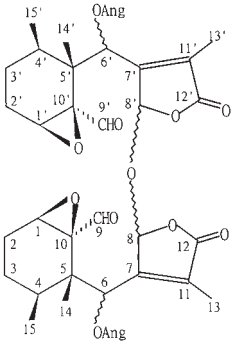
From the roots of *Ligularia macrophylla*, a bisesquiterpene, ligumacrophyllal (**1**), and an eremophilane sesquiterpene, ligumacrophyllatin (**2**) were isolated. Their structures were elucidated by 2D-NMR herein. Additionally, seven known compounds (**3**–**9**) were afforded from the plant, and compound **3** was found as natural product for the first time.

1. Introduction

The roots of *Ligularia macrophylla* (Ledeb.) DC. (Compositae), are used in Chinese traditional medicine for the treatment of tracheitis phthisis, hemoptysis, cough and asthma [1, 2]. There have been several reports about *L. macrophylla* and many compounds such as fatty acids, fatty oil, polyenes, polyacetylenic compounds, pyrrolizidine alkaloids and eremophilane sesquiterpenes have been isolated [3–5]. The extract from the roots of *L. macrophylla* with the mixture solvent (petroleum ether/Et_2O/MeOH) was found to have some antibacterial activity, against *Pasteurella multocida*, *Staphylococcus aureus* and *Escherichia coli* etc. This attracted us to study the chemical constituents of *L. macrophylla*, a plant growing in the Xinjiang region of China. This paper reports the isolation of two new sesquiterpenes, a bisesquiterpene, ligumacrophyllal (**1**), and an eremophliane sesquiterpene, ligunacrophyllatin (**2**). In addition, seven known compounds (**3**–**9**) were obtained. Compound **3** was found for the first time as natural product.



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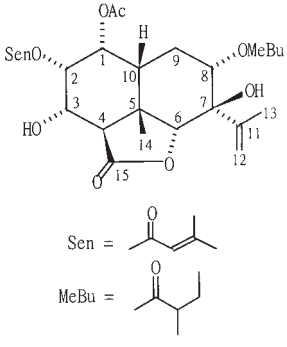

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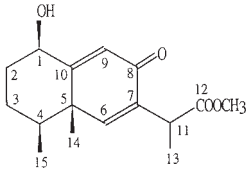

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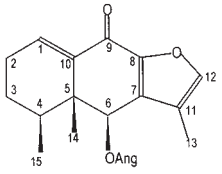

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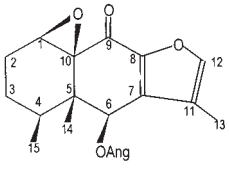

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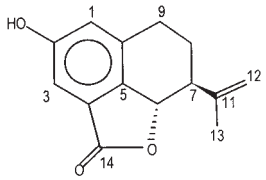

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2. Investigations, results and discussion

From the petroleum ether-Et_2O-MeOH (1 : 1 : 1) extract of the roots, six sesquiterpenes were isolated. Four sesquiterpenes (**2**–**5**) are eremophlia-type as those reported [5] but **1** was a new type sesquiterpene dimer isolated firstly from the plant. Known compounds **4**–**6** were tested in an antibacterial bioassay. Compound **6** showed activity against *Pasteurella multocida* as mentioned above but **4** and **5** obviously had no effect. The new compounds are now under study.

Compound **1** displayed characteristic IR bands at 1766, 1727, 1690 and 1645 cm^{-1}, which suggested the possible presence of an α, β-unsaturated γ-lactone, conjugated ester and ketone or aldehyde groups. The ^1H NMR spectrum (Table 1) gave the signals of two aldehyde groups at quaternary carbon (δ_H 8.69, 8.67, each 1 H, s), two angeloyls (δ_H 6.12, 6.08, each 1 H, qq; 1.96, 1.94, each 3 H, dq; 1.89, 1.88, each 3 H, dq) and two epoxy groups (δ_H 3.49, 2 H, d 1.6). Except for these sets, the six methyl protons at the high field could be divided into two groups, which showed similarities to eremophilanolide (δ_H 2.18, 6 H, brs, H-13/13′; 1.12, 6 H, s, H-14/14′; 0.76, 0.73, each 3 H, d, H-15/15′). The corresponding ^{13}C NMR and DEPT spectra of **1** exhibited 40 carbons in pairs including ten methyls, four methylenes, ten methines and six quaternary carbons. Apart from ten carbon signals of two angeloyloxy groups, the others just constituted the skeleton of a dimer of two identical sesquiterpene units. The quasi-molecular ion peak at m/z 739 $[M + 1]^+$ and the half-molecular ion peak at m/z 379 $[M + H\text{-}360]^+$ in FABMS as well as the strong fragment peak at m/z 538 $[M\text{-}2 \times AngOH]^+$ in EIMS further confirmed the conclusion. Thus, **1** was regarded as a sesquiterpene dimer and its molecular formula was established as $C_{40}H_{50}O_{13}$ with sixteen degrees of unsaturation. The ^1H and ^{13}C NMR spectra of the sesquiterpene unit were similar to those of aldehyde from *Senecio zoellneri* and *Sencio macrotis* [6, 7]. The difference is that the 1,10-double bond in the aldehyde reported was replaced by an epoxy in the unit of **1**. The location of the epoxy of was further confirmed by the HMBC correlations of C-10 with H-2 and C-5 with H-1. This skeleton was confirmed by 2D NMR experiments. The ^1H, ^1H COSY spectrum revealed two parts from H-1 to H-15 (H-1/H-2/H-3/H-4/H-15) and from H-13 to H-6 and H-8 (two long range couplings). The two parts were connected by HMBC correlations of H-6 with C-4,C-10 and C-11, H-9 with C-5, and H-8 with C-11, C-12, which suggested the existence of the left ring and the right lactone in the tricyclic structure of the eremophilanolide. Furthermore, the absence of HMBC correlations from H-9 to C-7 and from H-8 to C-10 showed that the middle ring of the eremophilanolide was broken between C-8 and C-9. The correlations from H-1 to the aldehyde carbon

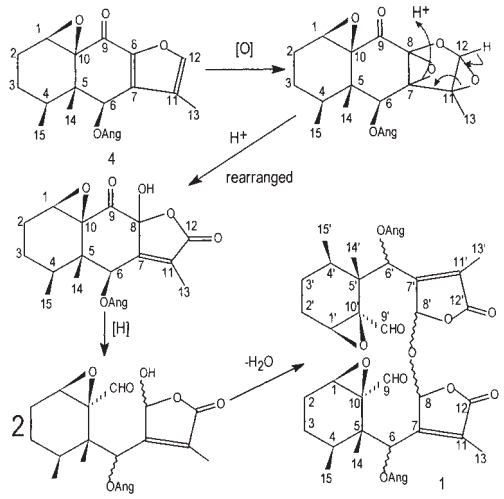
Table 1: ^{1}H NMR and ^{13}C NMR data of compound 1 (^{1}H NMR, 400 MHz, ^{13}C NMR, 100 MHz, $CDCl_3$, TMS, δ, ppm)[a,b]

No.		^{1}H NMR	^{13}C NMR
1		3.49 (1 H, d, 1.6)	59.6 (CH)
1′		3.49 (1 H, d, 1.6)	59.5 (CH)
2α		1.26 (1 H, m)	24.5 (CH_2)
2β		1.38 (1 H, m)	
2′α		1.26 (1 H, m)	24.4 (CH_2)
2′β		1.38 (1 H, m)	
3α		1.41 (1 H, m)	23.7 (CH_2)
3β		1.89 (1 H, m)	
3′α		1.42 (1 H, m)	23.6 (CH_2)
3′β		1.88 (1 H, m)	
4		2.22 (1 H, m)	31.7 (CH)
4′		2.22 (1 H, m)	31.7 (CH)
5		–	43.1 (C)
5′		–	43.0 (C)
6		6.45 (1 H, brs)	72.6 (CH)
6′		6.42 (1 H, brs)	72.3 (CH)
7		–	161.0 (C)
7′		–	160.6 (C)
8		5.87 (1 H, brs)	97.8 (CH)
8′		5.82 (1 H, brs)	97.5 (CH)
9		8.69 (1 H, s)	197.1 (CH)
9′		8.67 (1 H, s)	197.0 (CH)
10		–	64.6 (C)
10′		–	64.5 (C)
11		–	126.6 (C)
11′		–	126.6 (C)
12		–	169.2 (C)
12′		–	169.1 (C)
13		2.18 (3 H, brs)	13.0 (CH_3)
13′		2.18 (3 H, brs)	12.7 (CH_3)
14		1.12 (3 H, s)	15.6 (CH_3)
14′		1.12 (3 H, s)	15.6 (CH_3)
15		0.76 (3 H, d, 7.2)	18.3 (CH_3)
15′		0.73 (3 H, d, 7.2)	18.3 (CH_3)
OAng	1	–	166.8, 166.4 (C)
	2	–	126.7, 126.5 (C)
	3	6.12, 6.08 (each 1 H, qq, 7.2, 1.2)	140.9,140.4 (CH)
	4	1.96, 1.94 (each 3 H, dq, 7.2, 1.0)	16.0, 15.8 (CH_3)
	5	1.89, 1.88 (each 3 H, dq, 1.4, 1.2, 1.0)	20.6, 20.6 (CH_3)

a: Coupling constants in parentheses in Hz;
b: DEPT data in parentheses.

(C-9) and from the aldehyde proton (H-9) to C-1 and C-5 in the HMBC spectrum manifested that C-9 was an aldehyde carbon, which further supported the conclusion. In the ^{1}H, ^{1}H NOESY spectrum, if H-15 was β-oriented, the strong correlations of H-4α with H-1, H-2α, and H-1 with H-2α and H-9 implied that H-1 and H-9 were α-oriented. Therefore, the aldehyde group at C-10 should be α-oriented and the presence of 1β,10β-epoxy was supported. The relative stereochemistry of the β-epoxy can also be explained by the double splitting signal of H-1 ($J_{1\alpha,2\alpha} = 1.6$ Hz). The dihedral angle between H-1α and H-2β was about 90° ($J_{1\alpha,2\beta}$ is nearly zero) due to the existence of 1β,10β-epoxy observed through the molecular model, therefore, the signal of H-1 was not double doublet. H-14β was determined by the obvious correlation of H-14 with H-15. Consequently, the structure of the sesquiterpene unit was elucidated as 6-angeloyloxy-10α-aldehyde-1β,10β-epoxy-8,9-breaking ring eremophila-7(11)-en-8,12-olide. Two units were joined through the 8,8′-ether bond because of the unit being an acetal and the

Scheme



correlations from H-8′ to C-8 and from H-8 and C-8′. Therefore, compound **1** was confirmed as a dimer of 6-angeloyloxy-10α-aldehyde-1β,10β-epoxy-8,9-breaking ring eremophila-7(11)-en-8,12-olide(normal numbering of eremophilane according to the biogenic consideration), named as ligumacrophyllal. The possible biogenic synthesis way of **1** is shown in the Scheme.

For compound **2**, the IR characteristic bands at 3448, 1742, 1719, 1645 and 850 cm^{-1} suggested the presence of hydroxyl, double bands, ester and unsaturated ester carbonyl. The typical signals of acetoxy, senecioyloxy and 2′-methyl-butyryloxy groups could be found in the ^{1}H and ^{13}C NMR spectra (Table 2). The FAB-MS exhibited a quasi-molecular ion peak at m/z 523 $[M + H]^+$, fragments at m/z 505 $[M + H\text{-}H_2O]^+$, 448 $[505\text{-}C_4H_9]^+$, 403 [505-Me-BuOH]$^+$, 349 [448-OSen]$^+$ and basic peak 59 $[OAc]^+$, further revealed the existence of hydroxyl and three ester groups mentioned above. Except for the groups, ^{13}C NMR and DEPT spectra also displayed two methyls (δ_C 19.0 and 16.3), two methylenes (δ_C 33.8 and 115.3, a terminal double bond), seven methines , (δ_C 45.6, 51.4, 69.9, 70.7, 73.4, 74.6 and 76.1, five oxygenated) and four quaternary carbons (bearing an angular methyl at δ_C 56.6, oxygenated at δ_C 72.5, olefinic quaternary carbon at δ_C 153.0 and ester carbonyl at δ_C 176.6). Thus, **2** has a skeleton of 15 carbons, its molecular formula could be established as $C_{27}H_{38}O_{10}$ together with nine unsaturated degrees, four arranged to the esters, one to terminal double bond, one to ester carbonyl and three to the tricyclic structure. ^{1}H, ^{1}H COSY spectrum gave the main partial structure: −CH(8)−CH_2(9)−CH(10)−CH(1)−CH(2)−CH(3)−CH(4)−, which were combined with lactone by the HMBC correlations (C-15/H-3; C-7/H-9, H-12, H-13; C-11/H-8, H-6, C-5/H-3). Hence, compound **2** was a bicyclic sesquiterpene with a lactone. By means of the important correlations of C-15 at δ_C 176.6 with H-3 at δ_H 3.88 and H-6 at δ_H 4.34, the sesquiterpene was confirmed to be eremophila-6,15-olide type sesquiterpene [7]. The position of substituents could be ascertained by a HMBC experiment. The acetoxy group was assigned at C-1 by the correlations of the ester carbonyl (δ_C 170.5, OAc) with the methyl (δ_H 1.98) and H-1 (δ_H 5.35). The 2′-methyl-butyryloxyl group was pointed to C-8 by the correlations of the ester carbonyl (δ_C 174.5, MeBu) with the methyl (δ_H 1.25), the methylene (δ_H 2.18), the methine (δ_H 2.46)

Table 2: ^{1}H NMR and ^{13}C NMR data of compound 2 (^{1}H NMR, 400 MHz, ^{13}C NMR, 100 MHz, $CDCl_3$, TMS, δ, ppm)[a, b, c]

No.	^{1}H NMR	^{13}C NMR
1	5.35 (1 H, dd, 4.4, 2.0)	76.1 (CH)
2	5.56 (1 H, brdd, 3.2, 2.0)	74.6 (CH)
3	3.88 (1 H, d, 10.4)	70.7 (CH)
4	1.52 (1 H, d, 10.4)	45.6 (CH)
5	–	56.6 (C)
6	4.34 (1 H, brs)	73.4 (CH)
7	–	72.5 (C)
8	5.37 (1 H, dd, 8.0, 3.5)	69.9 (CH)
9β	2.86 (1 H, ddd, 14.4, 3.7, 2.0)	33.8 (CH_2)
9α	2.62 (1 H, t, 8.0)	
10	2.68 (1 H, ddd, 8.0, 6.8, 4.4)	51.4 (CH)
11	–	153.0 (C)
12	5.63 (1 H, brs)	115.3 (CH_2)
12′	5.39 (1 H, brs)	
13	2.16 (3 H, brs)	19.0 (CH_3)
14	1.36 (3 H, s)	16.3 (CH_3)
15	–	176.6 (C)
OSen 1′	–	166.1 (C)
2′	5.17 (1 H, qq, 1.5)	114.1 (CH)
3′	–	139.3 (C)
4′	1.52 (3 H, brs)	16.4 (CH_3)
5′	1.49 (3 H, brs)	11.8 (CH_3)
MeBu 1′	–	174.5 (C)
2′	2.46 (1 H, m)	41.4 (CH)
3′	2.18 (2 H, m)	26.3 (CH_2)
4′	1.25 (3 H, d, 7.2)	16.2 (CH_3)
2′-Methyl (5′)	1.08 (3 H, t, 7.6)	11.9 (CH_3)
OAc C=O	–	170.5
CH_3	1.98 (3 H, s)	21.2

a: Coupling constants in parentheses in Hz;
b: Assignments from ^{1}H, ^{1}H COSY and HMQC experiments;
c: DEPT data in parentheses.

and H-8 (δ_H 5.37). The senecioyloxy group was arranged at C-2 by the correlations of the ester carbonyl (δ_C 166.1, OSen) with the methine (δ_H 5.17) and H-2 (δ_H 5.56). There should be two hydroxyl groups apart from the lactone and ester groups. The location of one was at C-3 by the correlations of ^{1}H, ^{1}H COSY about the proton at δ_H 3.88 mentioned above. The other one was at C-7 on the basis of the existence of an oxygenated quaternary carbon, and in particular, the multiple HMBC correlations of C-7 (C-7/H-9, H-12, H-13) could manifest the conclusion. The relative stereochemistry of **2** was defined according to the coupling constants and chemical shift of the H-14. Firstly, if H-14 was assigned to β-orientated, H-10 and H-14 should have a *cis*-relationship, i.e. H-10 should be also β-orientated due to the downfield shift of H-14 (δ_H 1.36, >0.90) [8]. Furthermore, $J_{1,10}$, $J_{1,2}$, and $J_{2,3}$ were all small (4.4, 2.0, 3.2 Hz, respectively), indicating that H-1, H-2 and H-3 were all β-orientated. Finally, H-4 was determined as α-orientated due to the big $J_{3,4}$ (10.4 Hz). The configuration of **2** was supported by the correlations in the ^{1}H, ^{1}H NOESY spectrum (H-2/H-14, H-1/H-3/H-10). In addition, NOESY correlations of H-6 with H-14 and H-8 could be observed, which concluded H-6 and H-8 were closed in space as β-orientated. The β-orientation of the hydroxyl group at C-7 was deduced from the NOESY correlation between H-4 and H-12. Thus, compound **2** was confirmed as 1α-acetoxy-3α,7β-dihydroxy-8α-(2′-methyl)-butyryloxy-2α-senecioyloxy-eremophila-11(12)-en-6α,15β-olide, named as ligumacrophyllatin.

Compound **3** was isolated as colorless gum, its IR, EI-MS, ^{1}H and ^{13}C NMR spectra were the same as those of the methyl ester derivative of eremorphilane, thus, it was elucidated as 1β-hydroxy-6(7), 9(10)-dien-8-on-eremophila-12-acid-methyl ester. It was found as a natural product for the first time [9]. Compound **4** was obtained as colorless stick and was determined as neoadenostylone shown on the basis on its IR, MS and ^{1}H NMR data identical to those of in the literature [10]. A CD spectrum of **4** indicated its positive Cotton effect at 298 nm and a negative Cotton effect at 320 nm ($CHCl_3$), which is firstly reported here. Its ^{13}C NMR and DEPT data are also reported herein for the first time. An epoxy group was found in the ^{1}H and ^{13}C NMR spectra of compound **5** instead of an 1,10-double bond in compound **4**. The ^{1}H NMR spectrum of **5** was completely the same as that of 6β-angeloyloxy-1β,10β-epoxy-9-on-furaneremophilane [11], and its IR, MS, CD and ^{13}C NMR data further confirmed this conclusion. Hence, its structure was determined as shown. Compound **6**, 2-hydroxyplatyphyllid, was identified by comparison of spectral data (^{1}H and ^{13}C NMR) with those reported in the literature [12]. Ursolic acid (**7**), β-sitosterol (**8**) and daucosterol (**9**) were determined on the basis of their spectral data by direct comparison with authentic samples.

3. Experimental

3.1. Apparatus

Optical rotations: Perkin-Elmer 241 polarimeter; IR: Nicolet 170SX FT-IR instrument; EI-MS or FAB-MS: VG-ZAB-HS spectrometer (at 70 ev); ^{1}H NMR (400.13 MHz, $CDCl_3$), ^{13}C NMR (100.16 MHz, $CDCl_3$), 2D NMR (^{1}H,^{1}H COSY, HMQC, HMBC, ^{1}H,^{1}H NOESY): Bruker AM-400 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard; M.p.: Kolfler m.p. determining meter (uncorr.); Silica gel (120–160 mesh/200–300 mesh) for CC. and silica GF254 for TLC were supplied by the Qingdao Marine Chemical Factory.

3.2. Plant material

Ligularia macrophylla (Ledeb) DC was collected in the Kashikar region of south Xinjiang of China, in August 1997. It was identified by Prof. Guanmian Shen from the Xinjiang Institute of Biology and Pedology of Chinese Academy of Science. A voucher specimen (No. 970802) is deposited in the herbarium of our institute.

3.3. Extraction and isolation

The air-dried roots of the plant (350 g) were powdered and extracted four times (6 days per time) at room temperature with petroleum ether-Et_2O-MeOH (1 : 1 : 1). The extract was concentrated under reduced pressure to yield residue (22 g), which was isolated on a silica gel column (eluted with petroleum ether-Me_2CO from 30 : 1 to 1 : 3) to give four fractions. Fraction 1 (7 g) was chromatographed over a silica gel column (eluted with petroleum ether-Me_2CO from 30 : 1 to 10 : 1) to obtain **4** (60 mg), **5** (80 mg) and **8** (90 mg). Fraction 2 (6 g) was isolated over a silica gel column (eluted with $CHCl_3$-MeOH from 30 : 1 to 15 : 1) to get **6** (20 mg) and a crude constituent. The crude constituent was further purified by preparative TLC (silica gel) with $CHCl_3$-MeOH (25 : 1) as development to afford **1** (20 mg) and **3** (15 mg). Fraction 3 (3 g) was subjected to a silica gel column (eluted with petroleum ether-EtOAc from 4 : 1 to 1 : 1) to give **2** (15 mg). Fraction 4 was applied to a silica get column (eluted with $CHCl_3$-MeOH from 10 : 1 to 1 : 1) to afford **7** (50 mg) and **9** (60 mg).

3.4. Ligumacrophyllal (1)

Colorless gum, $[\alpha]_D^{20}$ +12.80 (c 0.78, $CHCl_3$); Rf 0.46 ($CHCl_3$/MeOH 25 : 1); IR (KBr) ν_{max} cm^{-1}: 2935, 1766 (α,β-unsaturated γ-lactone), 1727 (C=CCOOR), 1690 (CHO), 1645 (C=C), 1455, 1384, 1229, 1150, 1088, 1043, 972, 944, 850; FAB-MS: m/z 739 $[M + H]^+$ (1), 539 $[M + H\text{-}2 \times AngOH]^+$ (1), 379 $[M + H\text{-}360]^+$ (5), 321 $[M + H\text{-}2 \times CHO]^+$ (5), 278 $[379\text{-}OAng]^+$ (13), 263 $[278\text{-}CH_3]^+$ (15), 83 $[C_4H_7CO]^+$ (100), 55 $[C_4H_7]^+$ (85), 43 $[C_3H_7]^+$ (48); EI-MS m/z (int.) 578 $[M\text{-}2 \times HCHO\text{-}AngOH]^+$ (0.5), 538 $[M\text{-}2 \times AngOH]^+$ (1), 523 $[538\text{-}CH_3]^+$ (1), 495 $[523\text{-}CO]^+$ (1), 481 $[523\text{-}C_3H_6]^+$ (0.5), 445 $[481\text{-}2 \times H_2O]^+$ (2), 378 $[M\text{-}360]^+$ (2) (produced by the ether bond broken), 295 $[378\text{-}C_4H_7CO]^+$ (2), 279 $[295\text{-}O]^+$ (3), 250 $[279\text{-}CHO]^+$ (3), 83 $[C_4H_7CO]^+$ (100), 55 $[C_4H_7]^+$ (50); ^{1}H and ^{13}C NMR data see Table 1.

3.5. 1α-Acetoxy-2α-senecioyloxy-3α,7β-dihydroxy-8α-(2′-methyl)-butyryloxy-eremophila-11(12)-en-6α,15β-olide, ligumacrophyllatin (2)

Colorless needles, m.p. 176–178 °C (petroleum ether/EtOAc 3 : 1); $[\alpha]_D^{20}$ +18.3 ($CHCl_3$, c 0.15); Rf 0.14 (petroleum ether/EtOAc 2 : 1); IR (ν_{max}^{KBr}, cm^{-1}): 3448 (OH), 2965, 2928, 1742 (CO2R), 1719 (C=CCO2R), 1645 (C=C), 1462, 1383, 1350, 1251, 1224, 1141, 1039, 896, 850 (C=CH_2); FAB-MS m/z (m-NBA): 523 $[M+H]^+$ (17), 505 $[M+H-H_2O]^+$ (25), 462 $[505\text{-}COCH_3]^+$ (1), 449 $[505\text{-}C_4H_8]^+$ (1), 448 $[505\text{-}C_4H_9]^+$ (1), 403 [505-MeBuOH]$^+$ (1.5), 349 [449-SenOH]$^+$ (70), 97 (61), 73 (13), 59 $[OCOCH_3]^+$ (100); ^{1}H and ^{13}C NMR data see Table 2.

3.6. 1β-Hydroxy-6(7),9(10)-dien-8-on-eremophila-12-acid-methyl ester (3)

Colorless gum, $[\alpha]_D^{20}$ +45.8 ($CHCl_3$, c 0.13); Rf 0.33 ($CHCl_3$/MeOH 25 : 1). IR (ν_{max}^{KBr}, cm^{-1}): 3394 (OH), 2929, 2859, 1737 (CO_2R), 1662, 1628, 1457, 1380, 1259, 1203, 1158, 1096, 1044, 1012; ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 4.55 (1 H, brt, H-1), 1.67 (1 H, m, H-2α), 2.07 (1 H, m, H-2β), 1.55 (1 H, m, H-3α), 1.95 (1 H, m, H-3β), 1.54 (1 H, m, H-4), 6.90 (1 H, brs, H-6), 6.20 (1 H, brs, H-9), 3.74 (1 H, q, 7.2 Hz, H-11), 1.34 (3 H, d, 7.2 Hz, H-13), 1.36 (3 H, s, H-14), 1.12 (3 H, d, 6.6 Hz, H-15), 3.68 (3 H, s, OCH_3); ^{13}C NMR and DEPT δ ppm ($CDCl_3$, 100 MHz): 73.8 (CH, C-1), 34.4 (CH_2, C-2), 25.0 (CH_2, C-3), 41.4 (CH, C-4), 43.6 (C, C-5), 152.4 (CH, C-6), 136.5 (C, C-7), 185.3 (C, C-8), 125.8 (CH, C-9), 163.1 (C, C-10), 38.0 (C, C-11), 177.5 (C, C-12), 18.7 (CH_3, C-13), 16.4 (CH_3, C-14), 16.1 (CH_3, C-15), 52.0 (CH_3, OMe); EI-MS m/z (int): 278 $[M]^+$ (4.0), 264 (1.4), 247 (1.3), 246 (3.5), 235 (1.0), 219 (5.5), 218 (4.3), 202 (9.0), 191 (44), 189 (15.5), 175 (9.0), 173 (7.8), 161 (44), 147 (30.3), 145 (24.7), 135 (30.3), 128 (20.0), 121 (13.6), 119 (23.5), 105 (44.8), 91 (84.4), 77 (63.9), 69 (27.1), 55 (61.4), 43 (100).

3.7. Neoadenostylone (4)

Colorless sticks, m.p. 99.5–100.5 °C (petroleum ether/acetone 8 : 1); $[\alpha]_D^{20}$ −46.7 ($CHCl_3$, c 0.41); IR (ν_{max}^{KBr}, cm^{-1}): 2961, 2932, 1713, 1669, 1624, 1598, 1462, 1412, 1382, 1354, 1256, 1225, 1143, 1043, 985, 931, 896, 857, 796; ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 7.01 (1 H, t, 4.0 Hz, H-1), 2.24 (2 H, m, H-2), 1.44 (2 H, m, H-3), 1.52 (1 H, m, H-4), 6.43 (1 H, s, H-6), 7.39 (1 H, brs, H-12), 1.87 (3 H, brs, H-13), 1.14 (3 H, s, H-14), 0.96 (3 H, d, 7.0 Hz, H-15), OAng: 6.28 (1 H, qq, 7.5 Hz, 1.3 Hz, H-3′), 2.10 (3 H, dq, 7.5 Hz, 1.5 Hz, H-4′), 1.98 (3 H, dq, 1.5 Hz, 1.3 Hz, H-5′); ^{13}C NMR and DEPT δ ppm ($CDCl_3$, 100 MHz): 138.1 (CH, C-1), 27.9 (CH_2, C-2), 25.1 (CH_2, C-3), 37.8 (CH, C-4), 46.6 (C, C-5), 73.8 (CH, C-6), 135.9 (C, C-7), 146.9 (C, C-8), 176.6 (C, C-9), 141.3 (C, C-10), 121.1 (C, C-11), 145.9 (CH, C-12), 8.3 (CH_3, C-13), 15.8 (CH_3, C-14), 17.4 (CH_3, C-15), OAng: 167.0 (C, C-1′), 126.9 (C, C-2′), 141.6 (CH, C-3′), 16.0 (CH_3, C-4′), 20.5 (CH_3, C-5′); EI-MS m/z (int): 328 $[M]^+$ (0.3), 245 $[M\text{-}C_4H_7CO]^+$ (3), 228 [M-AngOH]$^+$ (29), 213 $[228\text{-}CH_3]^+$ (10), 83 $[C_4H_7CO]^+$ (100), 55 $[C_4H_7]^+$ (50); CD ($L^{-1} \cdot mol^{-1} \cdot cm^{-1}$) $\Delta\varepsilon_{296}$: +0.64, $\Delta\varepsilon_{320}$: −0.73.

3.8. 6β-Angeloyloxy-1β, 10β-epoxy-9-on-furaneremophilane (5)

Colorless cuboids, m.p. 112–113 °C (petroleum ether/acetone 8 : 1); $[\alpha]_D^{20}$ −18.4 ($CHCl_3$, c 0.41); IR (ν_{max}^{KBr}, cm^{-1}): 2935, 1720, 1687, 1647, 1600, 1532, 1458, 1412, 1382, 1359, 1229, 1143, 1089, 985, 911, 849, 806, 773; ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 3.34 (1 H, d, 4.8 Hz, H-1), 2.04–1.81 (2 H, m, H-2), 1.79–1.45 (2 H, m, H-3), 1.61 (1 H, m, H-4), 6.69 (1 H, s, H-6), 7.44 (1 H, brs, H-12), 1.88 (3 H, brs, H-13), 1.21 (3 H, s, H-14), 0.99 (1 H, d, 7.2 Hz, H-15), OAng: 6.25 (1 H, qq, 7.2 Hz, 1.2 Hz, H-3′), 2.03 (3 H, dq, 7.2 Hz, 1.5 Hz, H-4′), 1.95 (3 H, dq, 1.5 Hz, 1.2 Hz, H-5′); ^{13}C NMR and DEPT δ ppm ($CDCl_3$, 100 MHz): 62.4 (CH, C-1), 19.1 (CH_2, C-2), 24.7 (CH_2, C-3), 31.8 (CH, C-4), 45.1 (C, C-5), 68.5 (CH, C-6), 137.0 (C, C-7), 146.3 (C, C-8), 181.0 (C, C-9), 65.3 (C, C-10), 121.5 (C, C-11), 146.6 (CH, C-12), 8.1 (CH_3, C-13), 15.3 (CH_3, C-14), 16.1 (CH_3, C-15), OAng: 166.9 (C, C-1′), 126.5 (C, C-2′), 141.3 (CH, C-3′), 15.9 (CH_3, C-4′), 20.5 (CH_3, C-5′); EI-MS m/z (int): 344 $[M]^+$ (10), 262 $[M\text{-}C_4H_6CO]^+$ (5), 245 [M-OAng]$^+$ (12), 244 [M-AngOH]$^+$ (10), 228 [244-O]$^+$ (1.5), 229 $[244\text{-}CH_3]^+$ (14), 201 $[244\text{-}C_2H_3]^+$ (20), 109 $[C_4H_7]^+$ (3), 108 $[C_7H_8O]^+$ (0.6), 83 $[C_4H_7CO]^+$ (100), 55 $[C_4H_7]^+$ (60); CD ($L^{-1} \cdot mol^{-1} \cdot cm^{-1}$) $\Delta\varepsilon_{277}$: +2.5, $\Delta\varepsilon_{283}$: −16.7, $\Delta\varepsilon_{295}$: −20.63.

3.9. 2-Hydroxyplatyphyllid (6)

Colorless needles, m.p. 99.5–100.5 °C (petroleum ether/acetone 8 : 1); $[\alpha]_D^{20}$ −66.2 (MeOH, c 0.45); ^{1}H NMR δ ppm (CD_3COCD_3, 400 MHz): 6.97 (1 H, d, 1.8 Hz, H-3), 6.93 (1 H, d, 1.8 Hz, H-1), 5.23 (1 H, d, 10.7 Hz, H-6), 4.91 (1 H, d, 1.2 Hz, H-12), 4.88 (1 H, d, 1.2 Hz, H-12), 3.02 (1 H, dd, 17.9 Hz, 7.5 Hz, H-9β), 2.76 (1 H, d, 1.2 Hz, H-9α), 2.08 (1 H, m, H-8α), 1.94 (1 H, m, H-8β), 2.03 (1 H, m, H-7), 1.82 (3 H, brs, H-13); ^{13}C NMR and DEPT δ ppm (CD_3COCD_3, 100 MHz): 120.7 (C-1), 160.3 (C-2), 108.4 (C-3), 136.1 (C-4), 126.3 (C-5), 80.8 (C-6), 47.6 (C-7), 26.4 (C-8), 27.4 (C-9), 141.1 (C-10), 145.9 (C-11), 121.1 (C-12), 20.5 (C-13), 170.9 (C-14).

3.10. Ursolic acid (7)

Colorless powder, $[\alpha]_D^{20}$ +62.0 (MeOH, c 0.52). Its m.p. and EI-MS data were entirely the same as those reported for ursolic acid.

3.11. β-Sitosterol (8)

Colorless needles, $[\alpha]_D^{20}$ −33.0 ($CHCl_3$, c 0.48). All the data were identical to those of an authentic sample.

3.12. Daucosterol (9)

Colorless powder, $[\alpha]_D^{20}$ −42.1 (C_5H_5N, c 0.52). Its spectral data were fitted to those of an authentic sample.

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