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Sesquiterpenes, lignans and other constituents from *Saussurea macrota*

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From the methanol extract of the whole plant of *Saussurea macrota* Franch, 21 compounds were isolated. Their structures were elucidated by spectroscopic methods and X-ray crystallography. Two of them are new: 3α-hydroxy-11αH-guaia-4(15),10(14)-diene-12,6α-olide (**1**) and 7′-hydroxyiso-lappaol A (**11**). Compound **2** is reported as a natural compound for the first time. In addition, the compounds **12** and **13** showed significant antitumor activity against Bel-7402 and HO-8910 cells. Some of the compounds exhibited weak antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

1. Introduction

The *Saussurea* genus (Compositae) consists of about 400 species distributed throughout the world. More than 300 species of *Saussurea* are widely distributed in China and about ten of them are used as traditional Chinese medicine for treatment of rheumatoid arthritis, injuries from falls, gynecology, and anesthesia (Ren and Yang 2000). In our previous papers, we reported sesquiterpenonds, flavonoids and lignans from *S. involucrate* (Li et al. 1985; Jia et al. 1988; Li and Jia 1989), *S. japonica* (Jia et al. 1991), *S. hieracioides* (Liu et al. 1989) and *S. parviflora* (Yang et al. 2003) collected from northwest China. In continuation of our research on *Saussurea* plants, we now report the isolation and structural elucidation of the chemical constituents from the whole plant of *S. macrota* collected from northwest China, too. In addition, the antitumor and antibacterial activity test of compounds **11**, **12**, **13**, **14**, **15** and **16** are described.

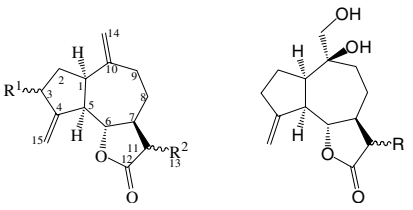
2. Investigations, results and discussion

From the methanol extract of the whole plant of *Saussurea macrota*, 21 compounds were isolated. **1** and **11** are new compounds. Compound **2** is reported as a natural compound for the first time. (Tan et al. 1990) previously obtained the acetylated derivative of compound **4**, but no data of **4** was reported. Here, we sumplement the spectrum data of compound **4**. The known compounds: 3α-hydroxy-11βH-guaia-4(15),10(14)-diene-12,6α-olide (**2**) (Mashayoshi et al. 1989), 3β-hydroxy-11αH-guaia-4(15),10(14)-diene-12,6α-olide (**3**) (Bohlmann and Chen 1982), 10β,14-dihydroxy-11αH-guaia-4(15)-ene-12,6α-olide (**4**) (Tan et al. 1990), 10β,14-dihydroxy-11βH-guaia-4(15)-ene-12,6α-olide (**5**) (Tan et al. 1990), 11αH-dihydro-dehydrocostuslactone (**6**) (Neves et al. 1999), caryolane-1,9β-diol (**7**) (Heymann et al. 1994), clavane-2β,9α-diol (**8**) (Delgado et al. 1984), 1β,6α-dihydroxyeudesm-4(15)-ene (**9**) (Ohmoto et al. 1987), taraxast-20-ene-3β,30-diol (**10**) (Dai et al. 2001), lappaol A (**12**) (Kaoru et al. 1993; Ichihara et al. 1979), matairesinol (**13**) (Kaoru et al. 1993), arctiin (**14**) (Omar 1978), pinoresinol (**15**) (Kensuke et al. 1991), syringaresinol (**16**) (Vermes et al. 1991), egonol (**17**) (Talapatra et al. 1978a), coniferaldehyde (**18**) (Tibor et al. 1980), sinapaldehyde (**19**) (Talapatra et al. 1978b), stigmast-5ene-3β,7α-diol (**20**) (Greca et al. 1990), isoscopoletin (**21**) (Hiroki et al. 1984) were identified by comparison of their spectral data (MS, ^{1}H NMR and ^{13}C NMR) with those reported in the literature.

Compound **1** was obtained as colorless crystal. The molecular formula of **1** was determined as $C_{15}H_{20}O_3$ by the molecular ion peak at $m/z = 248$ in the EIMS spectrum and the ^{13}C NMR and DEPT data (Table 1). Its IR (KBr) spectrum showed bands at 3457 (OH), 1751 (γ-lactone

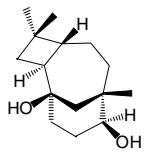
Table 1: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT data of compound 1 ($CDCl_3$, TMS, δ, ppm)

H	δ_H	C	δ_C	DEPT
1	3.05 (m)	1	43.6	CH
2	1.87 (m)	2	39.5	CH_2
	2.15 (ddd, J = 13.5, 6.2, 4.2 Hz)			
3	4.67 (t, J = 6.2 Hz)	3	74.6	CH
		4	154.5	C
5	3.05 (m)	5	49.6	CH
6	3.98 (t, J = 9.5 Hz)	6	85.1	CH
7	1.90 (dddd, J = 10.0, 9.5, 8.0, 4.2 Hz)	7	39.3	CH
8	2.42 (dddd, J = 12.7, 4.0, 4.0, 4.2 Hz)	8	28.8	CH_2
	1.35 (dddd, J = 12.7, 4.0, 10.0, 10.0 Hz)			
9	1.95 (ddd, J = 12.8, 10.0, 4.0 Hz)	9	37.7	CH_2
	2.54 (ddd, J = 12.8, 4.0, 4.0 Hz)			
		10	149.3	C
11	2.69 (dq, J = 8.0, 8.0 Hz)	11	45.3	CH
		12	179.8	C
13	1.15 (d, J = 8.0 Hz)	13	11.4	CH_3
14	4.74 (brs)	14	112.8	CH_2
	4.90 (brs)			
15	5.34 (brs)	15	112.3	CH_2
	5.44 (brs)			



1 $R^1 = \alpha$-OH, $R^2 = \beta$-Me
2 $R^1 = \alpha$-OH, $R^2 = \alpha$-Me
2a $R^1 = \alpha$-OAc, $R^2 = \alpha$-Me
3 $R^1 = \beta$-OH, $R^2 = \beta$-Me
3a $R^1 = \beta$-OAc, $R^2 = \beta$-Me
6 R^1 = H, $R^2 = \beta$-Me

4 R = β-Me
5 R = α-Me



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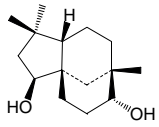

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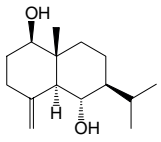

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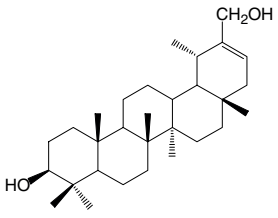

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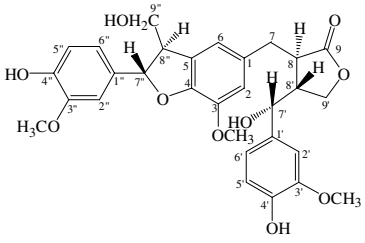


11

ring) and 1637 (double bonds) cm^{-1}. In the ^1H NMR spectrum, one methyl group appeared at δ 1.15 (3 H, d, J = 8.0 Hz), two pairs of exo methylene proton signals appeared at δ 4.74 (1 H, brs), δ 4.90 (1 H, brs), δ 5.34 (1 H, brs), δ 5.44 (1 H, brs), and the ^{13}C NMR showed two oxygenated groups δ 85.1 (CH), δ 74.6 (CH), corresponding to the signals δ 3.98 (1 H, t, J = 9.5 Hz) and δ 4.67 (1 H, t, J = 6.2 Hz) in the ^1H NMR, indicating that **1** possesse the guaianolide skeleton, just like the known 3-hydroxyguaia-4(15),10(14)-diene-12,6α-olides (Mashayoshi et al. 1989; Bohlmann and Chen 1982). By comparison of the ^1H NMR spectrum of **1** with that of **2** (Mashayoshi et al. 1989), the doublet methyl signal was shifted upfield to δ 1.15 from 1.25, and an obvious quintet signal appeared at δ 2.69 (dq, $J_{7,11} = J_{11,13} = 8.0$ Hz). There was no significant difference between the remaining proton signals of the two compounds. So compound **1** was a 11β-methyl lactone indicated by the ^1H NMR signals of H-13 at δ 1.15 (d, J = 8.0 Hz) and of H-11 at δ 2.69 (dq, $J_{7,11} = 8.0$ Hz) while compound **2** was a 11α-methyl lactone indicated by the signals of H-13 at δ 1.25 (d, J = 7.0) and of H-11 at δ 2.23 ($J_{7,11} = 12.5$ Hz) (Mashayoshi et al. 1989; Tan et al. 1990). Thus compound **1** was deduced as 3α-hydroxy-11αH-guaia-4(15), 10(14)-diene-12,6α-olide. This conclusion was verified by X-ray diffraction of **1** (Fig. 1).

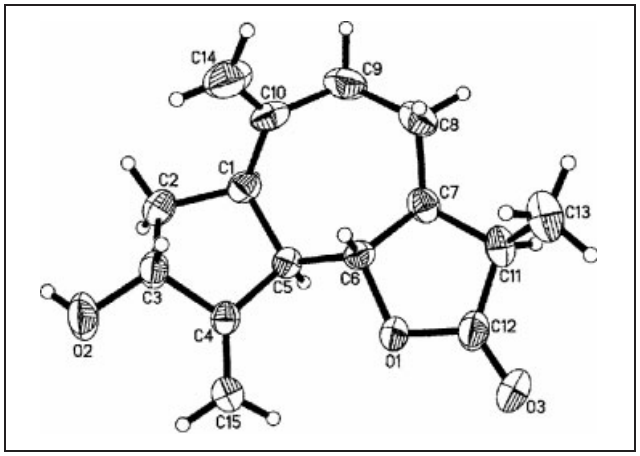
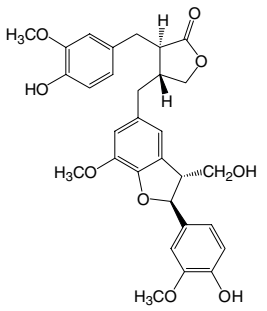


Fig. 1: ORTEP diagram of the crystal structure of compound **1**

Compound **11** was obtained as a gum. The molecular formula of **11** was determined to be $C_{30}H_{32}O_{10}$ by HRESIMS and the ^{13}C NMR and DEPT spectrum data (Table 2). The ^1H NMR spectrum of **11** showed signals due to aromatic rings at δ 6.54–7.05, three methoxy groups at δ 3.81 (3 H, s), 3.78 (3 H, s) and 3.77 (3 H, s), two methylenes connected to oxygen at δ 4.04 (1 H, dd, J = 9.0, 6.9 Hz, H-9′), 3.99 (1 H, dd, J = 9.0, 7.9 Hz, H-9′) and 3.80 (1 H, m, H-9″), 3.74 (1 H, m, H-9″), two methines connected to oxygen at δ 5.54 (1 H, d, J = 6.8 Hz, H-7″) and 4.75 (1 H, d, J = 6.0 Hz, H-



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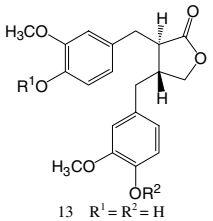
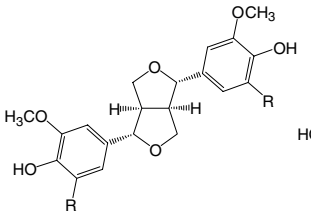
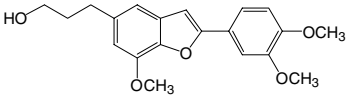


13 $R^1 = R^2$ = H
14 R^1 = Glu, $R^2 = CH_3$



15 R = H
16 R = OCH_3



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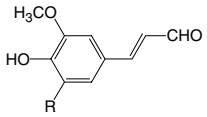
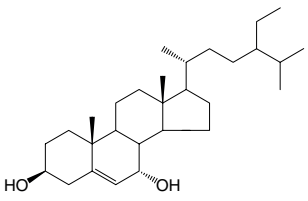


18 R = H
19 R = OCH_3



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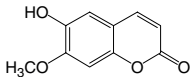

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Table 2: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT data of compound 11 (CD_3COCD_3, TMS, δ, ppm)

H*	δ_H*	C*	δ_C*	DEPT	HMBC correlations
		1	131.2	C	H-7, H-2
2	6.65 (d, J = 1.0 Hz)	2	114.2	CH	H-7, H-6
		3	144.1	C	H-2, OCH_3
		4	147.2	C	H-8″, H-7″, H-2, H-6
		5	129.1	C	H-8″, H-9″, H-7″, H-6
6	6.54 (d, J = 1.0 Hz)	6	118.3	CH	H-7, H-8″, H-2
7	2.79 (dd, J = 14.0, 7.0 Hz)	7	35.1	CH_2	H-2, H-6, H-8, H-8′
	2.95 (m)				
8	2.93 (m)	8	43.0	CH	H-8′, H-7, H-9′, H-7′
		9	178.9	C	H-7, H-8, H-8′, H-9′
		1′	134.7	C	H-7′, H-8′, H-2′, H-6′
2′	6.91 (d, J = 2.0 Hz)	2′	109.8[b]	CH	H-7′, H-6′
		3′	147.6	C	H-2′, H-5′, OCH_3
		4′	146.0	C	H-5′, H-6′, H-2′, Ar-OH
5′	6.77 – 6.82[a]	5′	114.8	CH	H-6′, Ar-OH
6′	6.77 – 6.82[a]	6′	118.9	CH	H-7′, H-5′, H-2′
7′	4.75 (d, J = 6.0 Hz)	7′	73.8	CH	H-8′, H-8, H-9′, H-2′, H-6′
8′	2.70 (m)	8′	45.6	CH	H-7′, H-9′, H-8, H-7
9′	4.04 (dd, J = 9.0, 6.9 Hz)	9′	68.6	CH_2	H-8′, H-8, H-7′
	3.99 (dd, J = 9.0, 7.9 Hz)				
		1″	133.9	C	H-7″, H-8″, H-2″, H-5″
2″	7.04 (d, J = 2.0 Hz)	2″	109.7[b]	CH	H-7″, H-6″
		3″	147.6	C	H-2″, H-5″, OCH_3, Ar-OH
		4″	146.5	C	H-2″, H-5″, H-6″, Ar-OH
5″	6.77 6.82[a]	5″	114.8	CH	H-6″, Ar-OH
6″	6.88 (dd, J = 8.0, 2.0 Hz)	6″	118.9	CH	H-5″, H-2″, H-7″
7″	5.54 (d, J = 6.8 Hz)	7″	87.7	CH	H-8″, H-9″, H-2″, H-6″
8″	3.53 (dt, J = 6.8, 6.0 Hz)	8″	54.2	CH	H-8″, H-9″, H-7″, H-6
9″	3.74 (m)	9″	63.9	CH_2	H-8″, H-7″
	3.80 (m)				
OCH_3	3.81 (s)	OCH_3	55.6	CH_3	
	3.78 (s)		55.6	CH_3	
	3.77 (s)		55.5	CH_3	

* The ^{1}H and ^{13}C NMR spectra data for **11** were assigned based on correlations observed in the ^{1}H-^{1}H COSY and HMBC spectra
[a] overlapped signals
[b] Assignments may be exchangeable each other

7′). The ^{13}C NMR and DEPT spectra of **11** indicating five methylenes, three methines, three aromatic rings, three methoxy groups and the signal of a carbonyl (δ 178.9, s).

The ^{1}H and ^{13}C NMR spectra data for **11** were assigned based on correlations observed in the ^{1}H-^{1}H COSY and HMBC spectra. The NMR spectra data of **11** were similar to those of isolappaol A (Kaoru et al. 1993) except for the methine signal connected to oxygen at δ 4.75 (1 H, d, J = 6.0 Hz, H-7′), which correlated with H-8′ resonating at δ 2.70 (1 H, m) in the ^{1}H-^{1}H COSY spectrum. In the HMBC spectrun (Table 2) of **11**, the correlations were as follows: δ_H 4.75 (H-7′) with δ_C 45.6 (C-8′), 68.6 (C-9′), 109.8 (C-2′), 118.9 (C-6′) and 134.7 (C-1′); δ_H 5.54 (H-7″) with δ_C 54.2 (C-8″), 63.9 (C-9″), 109.7 (C-2″), 118.9 (C-6″), 129.1 (C-5), 133.9 (C-1″) and 147.2 (C-4); δ_H 3.53 (H-8″) with δ_C 63.9 (C-9″), 87.7 (C-7″), 118.3 (C-6), 129.1 (C-5), 133.9 (C-1″) and 147.2 (C-4); δ_H 2.70 (H-8′) with δ_C 35.1 (C-7), 43.0 (C-8), 68.6 (C-9′), 73.8 (C-7′) and 134.7 (C-1′). The above correlations were in agreement with the structure, the hydroxy group was connected to C-7′. Thus, the structure of **11** has been determined as 7′-hydroxyisolappaol A. However, the ^{1}H NMR signal of H-7″ of **11** was a doublet (J = 6.8 Hz), suggesting that H-7″ and H-8″ were in a *trans*-relationship (Kaoru et al. 1993; Ichihara et al. 1979). Furthermore, a NOESY experiment was performed to determine the spatial relationships of **11**, which gave the correlations between δ 4.75 (H-7′) and 2.70 (H-8′), between δ 4.75 (H-7′) and 2.95 (H-7_a), between δ 2.93 (H-8) and 2.79 (H-7_b) and between δ 2.70 (H-8′) and 2.95 (H-7_a). The NOESY (illustrated by arrows in Fig. 2) clearly revealed the presence of a *cis* relationship between H-7′ and H-8′ and *trans* relationships between H-8 and H-8′ and between H-7″ and H-8″. In this way, the stereochemical structure for the new sesquilignan was deduced.

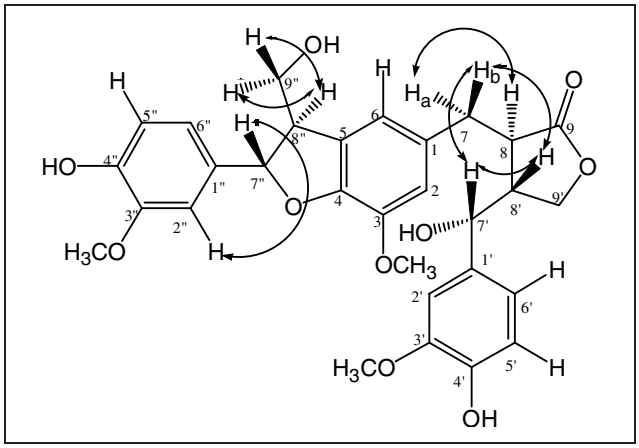

Fig. 2: Key NOESY correlations for compound **11**

Table 3: Antitumor activity of compounds

Compound	HL-60	Bel-7402	HO-8910
11	73.75 ± 7.02	73.96 ± 7.05	30.86 ± 2.45
12	3.45 ± 0.38	5.30 ± 0.46	5.76 ± 0.50
13	2.85 ± 0.25	7.93 ± 1.91	7.02 ± 1.07
14	>100	>100	>100
15	32.13 ± 2.66	53.79 ± 6.50	44.52 ± 5.05
16	>100	>100	>100
Vincristine	6.73 ± 1.52	25.9 ± 3.4	20.7 ± 1.9

Half inhibition concentration IC_{50} (μg/ml)

Table 4: Antibacterial activity of the compounds

Compound	*E. coli*	*B. subtilis*	*S. aureus*
11	+	+	+
12	+	+	−
13	+	−	+
14	+	+	+
15	−	+	+
16	+	+	+
Chloromycinum (100 μg/ml)	+++	+++	+++

Antibacterial circle: +++ > 17 mm; ++ 13−16 mm; + <12 mm.

Compounds **12** and **13** were found to be more active than vincristine against human hepatoma and human ovarian neoplasma cells (Table 3), Some compounds were active against bacteria (Table 4).

3. Experimental

3.1. Apparatus

Melting points were determined on a Kofler melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer M341 polarimeter. ^1H, ^{13}C NMR and 2D NMR spectra were scanned on a Bruker AM-400FT-NMR spectrometer with TMS as internal reference. IR spectra were recorded on a Nicolet 170sx FT-IR spectrometer as a film on KBr plates. EIMS data were obtained on a HP-5988 MS spectrometer. HRESIMS were recorded on a Bruker APEX II mass spectrometer. Silica gel (200-300 mesh) was used for CC and silica GF_{254} for TLC. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

3.2. Plant material

The whole plant of *Saussurea macrota* Franch was collected in 1999, in Zhang County, Gansu Province of China, and was identified by Prof. Guoliang Zhang, Department of Biology, Lanzhou University, China. A voucher specimen (No. 9901) is deposited in Department of Chemistry, Lanzhou University.

3.3. Extraction and isolation

The air-dried whole plants of *S. macrota* Franch (6 kg) were pulverized and extracted three times (each time for 7 days) with methanol at room temperature. The extract was concentrated under reduced pressure to yield a residue (424 g), which was suspended in hot H_2O (1000 ml). The H_2O soluble fraction was filtered and extracted with petroleum ether (60−90 °C), EtOAc and BuOH, respectively. The EtOAc extract (85 g) was obtained and subjected to CC separation over silica gel (730 g), eluting with a gradient of petroleum ether-EtOAc (20 : 1, 15 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, 2 : 1, 1 : 1 and 0 : 1) and finally with MeOH. According to differences in composition indicated by TLC, ten fractions were obtained. Compound **6** (20 mg) was purified by silica gel (80 g) column chromatography with $CHCl_3$ as eluant from Fr. 3 (2.0 g, petroleum ether-EtOAc 10 : 1). Fr. 5 (1.1 g, petroleum ether-EtOAc 5 : 1) was separated on CC over silica gel (60 g) with petroleum ether-acetone (7 : 1) gave six subfractions (sfr. $A_1 \rightarrow$ sfr. A_6). Sfr. A_2 (20 mg) was separated by repeated preparative TLC (silica GF_{254}, 10−40 μ) with petroleum ether-Me_2CO (5 : 1, three development) to afford compound **9** (7 mg, $R_f = 0.50$). Sfr. A_4 (25 mg) was separated by preparative TLC with $CHCl_3$−Me_2CO (8 : 1) to afford compound **18** (13 mg, $R_f = 0.50$). Sfr. A_6 (35 mg) was separated by repeated preparative TLC with petroleum ether-EtOAc (1 : 1, two development) to afford compound **10** (8 mg, $R_f = 0.70$). Compound **1** (25 mg), **19** (16 mg) and a mixture (20 mg) of **2** and **3** were obtained from Fr. 6 (2.0 g, petroleum ether-EtOAc 3 : 1) by rechromatographed on a silica gel (80 g) column with petroleum ether-acetone (4 : 1), the mixture of **2** and **3** was acetylated in the usual manner using acetic anhydride and pyridine to give the corresponding monoacetates **2a** (4 mg) and **3a** (4 mg). Fr. 7 (3.1 g, petroleum ether-EtOAc 2 : 1) was further separated by CC over silica gel (90 g) with petroleum ether-acetone (4 : 1) to give eight subfractions (sfr. $B_1 \rightarrow$ sfr. B_8). Sfr. B_3 (30 mg) was separated by CC over silica gel (5 g) with $CHCl_3$−Me_2CO (4 : 1) gave **7** (13 mg) and **8** (6 mg). The residue sfr. B_4 (20 mg) and sfr. B_5 (100 mg) were crystallized from acetone to give **20** (10 mg) and **21** (50 mg), respectively. Sfr. B_7 (20 mg) was separated by preparative TLC with petroleum ether-EtOAc (1 : 1) to afford compound **17** (8 mg, $R_f = 0.50$). Sfr. B_8 (150 mg) was separated by repeated preparative TLC with petroleum ether-EtOAc (1 : 1, two development) to afford compound **13** (35 mg, $R_f = 0.70$) and **15** (58 mg, $R_f = 0.40$). Six subfractions (sfr. $C_1 \rightarrow$ sfr. C_6) was obtained by CC ($CHCl_3$-acetone 8 : 1) over silica gel (80 g) from Fr. 8 (2.7 g, petroleum ether-EtOAc 1 : 1). Sfr. C_2 is compound **16** (136 mg). Sfr. C_5 (500 mg) was separated by preparative TLC with EtOAc to afford compound **12** (30 mg, $R_f = 0.60$) and **5** (19 mg, $R_f = 0.50$). Sfr. C_6 (20 mg) was purified by CC over silica gel (5 g) with petroleum ether-EtOAc (1 : 2) gave compound **4** (7 mg). Fr. 9 (3.7 g, EtOAc) was further chromatographed on a silica-gel (100 g) column with $CHCl_3$-CH_3OH (15 : 1) gave five crude fractions (sfr. $D_1 \rightarrow$ sfr. D_5). Sfr. D_2 (100 mg) was purified by preparative TLC with EtOAc to afford compound **11** (35 mg, $R_f = 0.5$). Sfr. D_5 (500 mg) was separated by preparative TLC with EtOAc-MeOH (9 : 1) to afford compound **14** (195 mg, $R_f = 0.40$).

3.4. 3α-Hydroxy-11αH-guaia-4(15),10(14)-diene-12,6α-olide (**1**)

Colorless crystals, m.p.: 122−124 °C (EtOAc), $[\alpha]_D^{20}$: +31.0° (*c* 2.5 $CHCl_3$); Rf. 0.5 (petroleum ether (60°90 °C)-EtOAc 1 : 1); IR (ν^{KBr}, cm^{-1}): 3457, 1751, 1637; EIMS: *m/z* (rel. int.) = 248 $[M]^+$ (10), 230 $[M\text{-}H_2O]$ (7), 105 (71), 95 (75), 91 (100), 79 (78), 55 (77); ^{13}C NMR data and ^1H NMR data see Table 1.

3.5. 7′-Hydroxyisolappaol A (**11**)

Pale yellow gum, $[\alpha]_D^{20}$: +12° (*c* 2.5 MeOH); Rf. 0.5 (EtOAc); HRESIMS: m/z = 570.2339 $[M + NH_4]^+$ (calcd. For $[C_{30}H_{32}O_{10} + NH_4]^+$: 570.2334); EIMS: *m/z* (rel. int.) = 552 $[M]^+$ (3), 534 $[M\text{-}H_2O]^+$ (9), 522 $[M\text{-}OCH_3]^+$ (6), 516 $[M\text{-}2\,H_2O]^+$ (4), 504 (5), 167 (34), 137 (93), 57 (100), 43 (99); ^{13}C NMR data and ^1H NMR data see Table 2.

3.6. 10β,14-Dihydroxy-11αH-guaia-4(15)-ene-12,6α-olide (**4**)

Colorless gum, $[\alpha]_D^{20}$: +15° (c 0.7 $CHCl_3$); Rf. 0.3 (EtOAc); EIMS: *m/z* (rel. int.) = 266 $[M]^+$ (0.4), 248 $[M\text{-}H_2O]^+$ (8), 235 (14), 217 (12), 189 (9), 145 (21), 109 (34), 80 (80), 55 (100); ^1H NMR (400 MHz, $CDCl_3$): δ = 5.18 (brs, H-15), 5.00 (brs, H-15′), 4.15 (t, J = 10.2 Hz, H-6), 3.45 (d, J = 10.8 Hz, H-14), 3.36 (d, J = 10.8 Hz, H-14′), 2.79 (m, H-5, H-7), 2.70 (dq, J = 8.0 Hz, H-11), 2.49 (m, H-3), 2.41 (m, H-3′), 2.37 (m, H-1), 1.85 (m, H-8, H-9), 1.75 (m, H-2, H-2′), 1.65 (m, H-9′), 1.54 (m, H-8′), 1.18 (d, J = 8.0 Hz, H-13); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.96 (C-12), 150.52 (C-4), 109.59 (C-15), 82.21 (C-6), 75.92 (C-10), 68.21 (C-14), 52.03 (C-5), 49.22 (C-1), 42.21 (C-11), 39.55 (C-7), 30.12 (C-9), 30.04 (C-3), 25.48 (C-8), 20.62 (C-2), 11.44 (C-13).

3.7. X-Ray crystallographic analysis of **1**

Crystal data: $C_{15}H_{20}O_3$, formula wt 248.31; T = 291 (2) K; wavelength: 0.71073 Å; crystal system: orthorhombic; space group: P2 (1) 2 (1) 2 (1); unit cell dimensions: a = 8.346 (1) Å, b = 9.951 (1) Å, c = 15.934 (2) Å, α = β = γ = 90, V = 1323.3 (3) $Å^3$, Z = 4; D_c = 1.246 Mg/m^3; range for data collection: $2.41 \le \theta \le 26.90$; limiting indices $0 \le h \le 10$, $0 \le k \le 12$, $-1 \le l \le 20$; reflections collected: 1741, independent reflections: 1719 [R (int) = 0.0073]; refinement method: full-matrix lease-squares on F^2; goodness-of-fit on F^2: 0.970; final R indices [I > 2 sigma (I)]: R1 = 0.0392, wR2 = 0.0778; R indices (all data): R1 = 0.0593, wR2 = 0.0833; largest diff. Peak and hole: 0.146 and −0.109 e. $Å^3$; audit creation method: SHELXL = 97.

3.8. Assays of antitumor activity

The antitumor activity of **11**, **12, 13, 14, 15** and **16** was determined using the sulforhodamine B (SRB) colorimetric assay (Skehan et al. 1990) with three tumor cell lines, i.e. human leukemia HL-60 cells, human hepatoma Bel-7402 cells and human ovarian neoplasm HO-8910 cells with vincristine as a positive control (Table 3). It can be seen from the table that the 50% inhibitory concentration (IC_{50}) values of **12** and **13** are smaller than those of vincristine, especially in the case of Bel-7402 and HO-8910. Thus, **12** and **13** exhibited most effective antitumor activity, especially on Bel-7402 cells and HO-8910 cells.

3.9. Antimicrobial assays

We carried out antibacterial activity assays of compounds **11**, **12**, **13**, **14**, **15** and **16** according to the paper-disk method (Xu et al. 1982). The results indicated that some of the compounds were active against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Table 4).

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