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Sesquiterpenes and other constituents from *Achillea wilsoniana*

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From the methanol extract of the whole plant of *Achillea wilsoniana*, 23 compounds were isolated. Their structures were elucidated by spectroscopic methods and chemical transformations. Three of them are new: 4E, 10E-9β-hydroxy-3-(2-methylbutyroyloxy)-germacra-4,10(1)-diene-12,6α-olide (**1**), 4E,10E-3-(2-methylbutyroyloxy)-germacra-4,10(1)-diene-12,6α-olide (**2**) and 1β,6α-dihydroxy-10β-methyl-5αH,7αH-eudesm-4-one (**3**). In addition, 1β-hydroxy-α-xyperone (**5**) and 9β-acetoxy-3-(2-methylbutyroyloxy)-germacra-4,10(1)-diene-12,6α-olide (**1a**) exhibited effective antibacterial activity against *Staphylococcus aureus*.

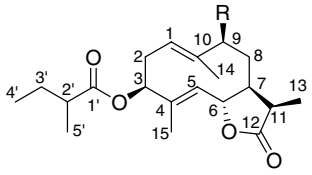
1. Introduction

The *Achillea* genus (Compositae) consists of about 200 species widely distributed in the temperate zone. There are ten species of *Achillea* distributed in China and three of them are used in traditional Chinese medicine with antibacterial, anti-inflammatory and anti-allergic properties. *Achillea wilsoniana* Heimerl ex Hand.-Mazz is a perennial herbaceous plant growing mainly in West China. Its whole plant has been used as a traditional Chinese medicine for detoxifcation, detumescence, hemostasia and acesodyne (Shi and Fu 1983; Xie and Yu 1996). We present the isolation and structural elucidation of the chemical constituents from the whole plant of this species and the antibacterial activity of some of the compounds **1**, **1a**, **5**, **11**, **22**.

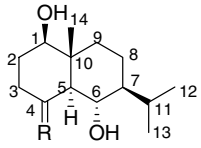
2. Investigations, results and discussion

From the methanol extract of the whole plant of *Achillea wilsoniana*, 23 compounds were isolated. **1**, **2** and **3** are new compounds. The known compounds: 1β,6α-dihydroxy-eudesm-4(15)-ene (**4**) (Ohmoto et al. 1987), 1β-hydroxy-α-xyperone (**5**) (Sanz and Marco 1990), 2E,4E-N-isobutyl-2,4-decadienamide (**6**) (Yasuda et al. 1981), 2E,4E-undeca-2,4-diene-8,10-diynoic acid isobutyamide (**7**) (Hofer et al. 1986), 2E,4E,8Z-N-isobutyl-2,4,8-decatrienamide (**8**) (Hofer et al. 1986), 2E,4E-undeca-2,4-diene-8,10-diynoic acid piperidide (**9**) (Hofer et al. 1986), 2E,4E-1-piperidin-1-yl-deca-2,4-dien-1-one (**10**) (Hofer et al. 1986), 4-(1-hydroxy-1-methyl-ethyl)-cyclohex-1-enecarbaldehyde (**11**) (Carman and Garner 1996), β-sitosterol (**12**) (Greca et al. 1990), stigmast-5-ene-3β,7α-diol (**13**) (Greca et al. 1990), 7β-methoxy-stigmast-5-ene-3β-ol (**14**) (Zhu et al. 2000), saringosterol (**15**) (Ikekawa et al. 1996), stigmast-4-en-6β-ol-3-one (**16**) (Greca et al. 1990), 3β,5α,8α-trihydroxy-campest-6,22-diene (**17**) (Gao and Jia 1997), daucosterol (**18**) (Kuo and Yeh 1997), sitoindoside I (**19**) (Luo et al. 2001), cycloart-5-ene-3β,25-diol (**20**) (Vermes et al. 1991), sesamin (**21**) (Marcos et al. 1990), 6-acetyl-3,6′-diferuloyl-sucrose (**22**) (Hiroko et al. 1986), 1,2-dilinolenyl-3-*O*-β-D-galactopyranosyl-sn-glycerol (**23**) (Baruah et al. 1983) were identified by direct 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 gum. The molecular formula of **1** was determined as $C_{20}H_{30}O_5$ by the molecular ion peak at m/z = 350 in the EIMS spectrum and the ^{13}C NMR and DEPT data (Table 2). The degree of unsaturation was 6. Its IR (KBr) spectrum showed bands at 3459 (OH), 1766 (γ-lactone ring), 1732 (ester) and 1673 (double bonds) cm^{-1}. In the ^{1}H NMR, five methyl groups appeared at δ 1.72 (3 H, brs), δ 1.48 (3 H, brs), δ 1.24 (3 H, d, J = 7.8 Hz), 1.17 (3 H, d, J = 6.9 Hz), 0.92 (3 H, t, J = 7.5 Hz), three methines connected to oxygen appeared at δ 5.16 (1 H, dd, J = 10.8, 5.7 Hz), δ 4.77 (1 H, dd, J = 9.9, 9.9 Hz) and δ 4.11 (1 H, dd, J = 8.7, 3.9 Hz). ^{13}C NMR showed two trisubstituted olefinic groups δ 140.6 (C), δ 125.5 (CH) and δ 137.1 (C), δ 125.6 (CH) corresponding to the signals δ 5.12 (1H, dd, J = 11.7, 4.8 Hz) and δ 4.70 (1 H, d, J = 9.9 Hz) in the ^{1}H NMR. All the signals of ^{1}H and ^{13}C NMR are indicating that **1** possesses a germacranolide skeleton bearing two substituents on C-3 and C-9. The assignments were made by means of chemical shifts, coupling patterns, two dimensional NMR spectrometry (^{1}H-^{1}H COSY and HMBC) and by comparison of their ^{1}H and ^{13}C NMR spectra (Tables 1, 2) with those of sintenin which was found in *Achillea micrantha* (Hatam et al. 1992). Except for the germacranolide signals in the ^{1}H NMR spectrum, the remaining peaks showed a 2-methylbutyroyloxy group (2-MeBu) at δ 2.39 (1 H, tq, J = 6.9, 6.9 Hz), 1.70 (1 H, m), 1.53 (1 H, m), 1.17 (3 H, d, J = 6.9 Hz), 0.92 (3 H, t, J = 7.5 Hz). The regiochemistry of the two substituents was determined by an HMBC experiment (Fig.). The carbonyl carbon at δ 175.6 (C-1′) presented the HMBC correlations with the 2-MeBu methine (δ 2.39) and H-3 (δ



1. R = OH
1a. R = AcO
2. R = H



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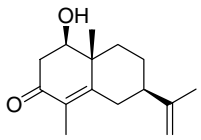
4. R = CH_2



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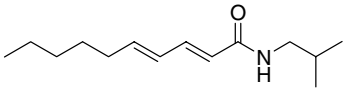

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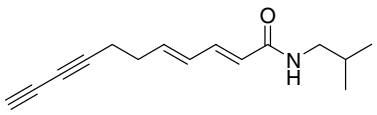

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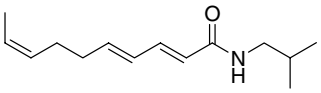

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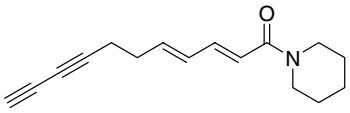

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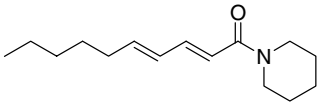

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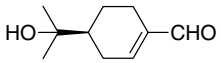

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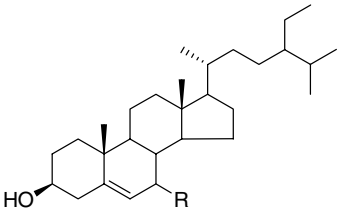
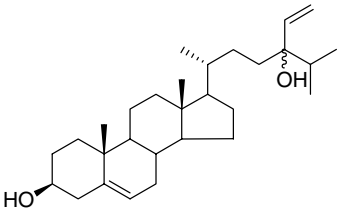


12. R = H
13. R = α-OH
14. R = β-MeO



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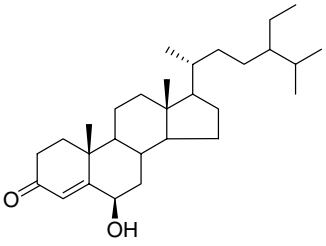

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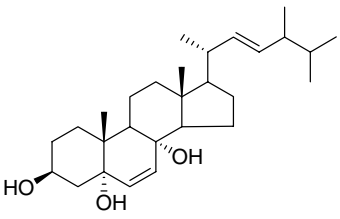

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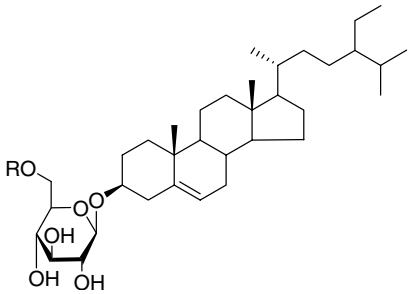
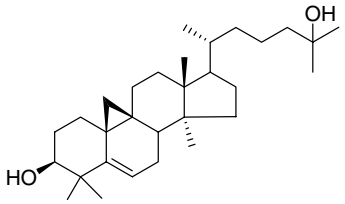
5.16), indicating the 2-MeBu group was attached on C-3. Therefore, the hydroxy group should be on C-9. The configuration at C-6 as H-β and C-11 as methyl-β was determined by means of measured J values (Hatam et al. 1992). In order to confirm the relative stereochemistry of **1**, a NOE difference experiments was conducted for **1**. Irradiation at H-2α in a one dimensional NOE difference experiment produced positive NOE on H-1α (6%) and on H-3α (6%); irradiation at H-9α produced a strong NOE on H-1α (14%) and weaker enhancement on H-7α (6%) and on H-8α (4%); irradiation at Me-15β produced NOE on H-6β (3%). Thus, the structure of **1** has been determined.

In the usual manner using acetic anhydride and pyridine, the monoacetate of **1** was obtained (**1a**). Due to the very good correspondence of the ^{1}H NMR data of the germacranolide skeleton of **1a** and sintenin (Hatam et al. 1992), the conclusion about the structure of **1** was confirmed.

Compound **2** was obtained as colorless gum. The molecular formula of **2** was determined to be $C_{20}H_{30}O_4$ by the molecular ion peak at m/z = 334 in the EIMS spectrum and the ^{13}C NMR and DEPT data (Table 2). The degree of unsaturation was 6. Its IR (KBr) spectrum showed bands at 1774 (γ-lactone ring), 1732 (ester) and 1670 (double bonds) cm^{-1}. ^{1}H and ^{13}C NMR spectra (Tables 1, 2) indicating that the structure of **2** was similar to that of **1**



18. R = H
19. R = $CH_3(CH_2)_{16}CO$



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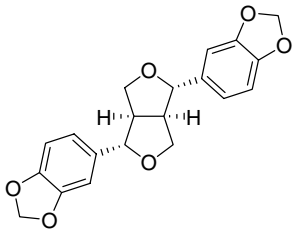

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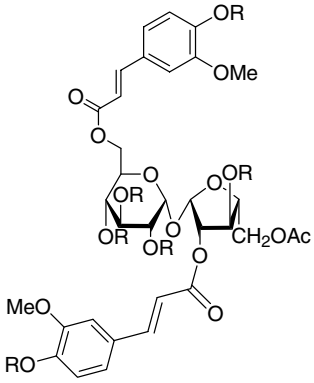

22. R = H
22a. R = Ac

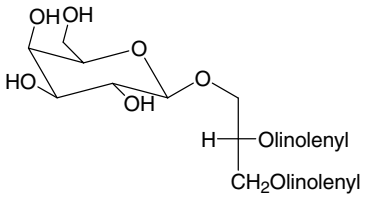

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Table 1: ^{1}H NMR (300 MHz) data of 1, 1a and 2 ($CDCl_3$, TMS, δ, ppm)

H	δ_H (**1**)	δ_H (**1a**)	δ_H (**2**)
1	5.12 (dd, J = 11.7, 4.8 Hz)	5.25 (dd, J = 12.0, 4.8 Hz)	4.90 (dd, J = 11.8, 4.7 Hz)
2	2.53 (ddd, J = 11.7, 5.7, 4.8 Hz) 2.33 (ddd, J = 11.7, 11.7, 10.8 Hz)	2.56 (ddd, J = 12.0, 6.0, 4.8 Hz) 2.31 (ddd, J = 12.0, 12.0, 10.8 Hz)	2.49 (ddd, J = 12.8, 6.2, 4.7 Hz) 2.27 (ddd, J = 12.8, 11.8, 10.6 Hz)
3	5.16 (dd, J = 10.8, 5.7 Hz)	5.19 (dd, J = 10.8, 6.0 Hz)	5.19 (dd, J = 10.6, 6.2 Hz)
5	4.70 (brd, J = 9.9 Hz)	4.75 (brd, J = 10.0 Hz)	4.81 (brd, J = 9.6 Hz)
6	4.77 (dd, J = 9.9, 9.9 Hz)	4.77 (dd, J = 10.0, 10.0 Hz)	4.76 (dd, J = 9.6, 9.6 Hz)
7	2.26 (m)	2.33 (m)	2.16 (m)
8	1.86 (m)	1.87 (m)	1.81 (m) 1.69 (m)
9	4.11 (dd, J = 8.7, 3.9 Hz)	5.13 (dd, J = 7.8, 6.2 Hz)	2.37 (m) 2.04 (m)
11	2.71 (dq, J = 7.8, 7.8 Hz)	2.74 (dq, J = 8.0, 8.0 Hz)	2.68 (dq, J = 8.0, 8.0 Hz)
13	1.24 (d, J = 7.8 Hz)	1.24 (d, J = 8.0 Hz)	1.23 (d, J = 8.0 Hz)
14	1.48 (brs)	1.50 (brs)	1.44 (brs)
15	1.72 (brs)	1.73 (brs)	1.71 (brs)
2′	2.39 (tq, J = 6.9, 6.9 Hz)	2.42 (tq, J = 7.2, 7.2 Hz)	2.41 (tq, J = 7.2, 7.2 Hz)
3′	1.70 (m) 1.53 (m)	1.71 (m) 1.51 (m)	1.68 (m) 1.52 (m)
4′	0.92 (t, J = 7.5 Hz)	0.92 (t, J = 7.6 Hz)	0.92 (t, J = 7.6 Hz)
5′	1.17 (d, J = 6.9 Hz)	1.18 (d, J = 7.2 Hz)	1.18 (d, J = 7.2 Hz)
CH_3CO		2.04 (s)	

Table 2: ^{13}C NMR (75 MHz) and DEPT data of 1, 1a and 2 ($CDCl_3$, TMS, δ, ppm)

C	**1** δ_C	**1** DEPT	**1a** δ_C	**1a** DEPT	**2** δ_C	**2** DEPT
1	125.5	CH	125.5	CH	124.3	CH
2	31.2	CH_2	31.3[a]	CH_2	32.3	CH_2
3	78.3	CH	78.0	CH	78.9	CH
4	137.1	C	136.4	C	137.5	C
5	125.6	CH	128.0	CH	126.3	CH
6	79.6	CH	79.4	CH	80.2	CH
7	45.7	CH	45.7	CH	49.3	CH
8	33.5	CH_2	31.2[a]	CH_2	25.5	CH_2
9	79.2	CH	80.6	CH	41.1	CH_2
10	140.6	C	137.6	C	139.0	C
11	40.5	CH	40.4	CH	41.2	CH
12	179.2	C	178.7	C	179.8	C
13	10.8	CH_3	10.9	CH_3	11.2	CH_3
14	10.9	CH_3	11.7[b]	CH_3	16.6	CH_3
15	12.4	CH_3	12.4	CH_3	12.7	CH_3
1′	175.6	C	175.5	C	175.8	C
2′	41.1	CH	41.2	CH	41.5	CH
3′	25.6	CH_2	26.3	CH_2	27.0	CH_2
4′	11.5	CH_3	11.6[b]	CH_3	11.9	CH_3
5′	16.5	CH_3	16.5	CH_3	16.9	CH_3
$CH_3\underline{C}O$			169.8	C		
$\underline{C}H_3CO$			21.2	CH_3		

[a, b] Assignments may be exchangeable each other

except for the disappearance of the hydroxy attached to C-9. There was no other difference between **1** and **2**.

The ^1H and ^{13}C NMR spectra data for **2** were assigned based on chemical shifts, coupling patterns, two dimensional NMR spectrometry (^1H-^1H COSY and HMBC) and by comparison of their ^1H and ^{13}C NMR spectra (Tables 1, 2) with those of **1**. In the HMBC spectrum of **2**, the correlations were as follows: δ_H 1.44 (H-14), 2.37, 2.04 (H-9), 2.49 (H-2) and 2.27 (H-2) with δ_C 124.3 (C-1); δ_H 1.71 (H-15), 2.49, 2.27 (H-2) and 4.81 (H-5) with δ_C 78.9 (C-3); δ_H 1.71 (H-15) and 5.19 (H-3) with δ_C 137.5 (C-4); δ_H 1.71 (H-15) and 5.19 (H-3) with δ_C 126.3 (C-5); δ_H 1.81, 1.69 (H-8) and 2.68 (H-11) with δ_C 80.2 (C-6); δ_H 1.23 (H-13), 1.81 (H-8) and 1.69 (H-8) with δ_C 49.3 (C-7); δ_H 1.44 (H-14), 1.81, 1.69 (H-8) and 4.90 (H-1) with δ_C 41.1 (C-9); δ_H 1.44 (H-14), 2.37, 2.04 (H-9) and 4.90 (H-1) with δ_C 139.0 (C-10); δ_H 1.23 (H-13) and 2.16 (H-7) with δ_C 41.2 (C-11); δ_H 1.23 (H-13), 2.68 (H-11) and 4.76 (H-6) with δ_C 179.8 (C-12); δ_H 2.68 (H-11) with δ_C 11.2 (C-13); δ_H 2.37, 2.04 (H-9) with δ_C 16.6 (C-14); δ_H 4.81 (H-5) and 5.19 (H-3) with δ_C 12.7 (C-15); δ_H 1.18 (H-5′), 1.68, 1.52 (H-3′) and 5.19 (H-3) with δ_C 175.8 (C-1′). The above correlations were in agreement with the structure. Thus, the structure of **2** has been established.

Compound **3** was obtained as colorless gum. The molecular formula of **3** was determined to be $C_{14}H_{24}O_3$ by the molecular ion peak at m/z = 240 in the EIMS spectrum and the ^{13}C NMR and DEPT data. The degree of unsaturation was 3. Its IR (KBr) spectrum showed bands at 3421 (OH), 1705 (C=O). The ^1H NMR spectrum of **3** showed signals due to two methines connected to oxygen at δ 3.88 (1 H, dd, J = 9.6, 9.6 Hz, H-6) and 3.83 (1 H, dd, J = 11.6, 4.8 Hz, H-1). The ^{13}C NMR and DEPT spectra of **3** indicating four methylenes, five methines, three methyl groups and the signal of a ketone (δ 213.3, s).

The ^1H and ^{13}C NMR spectra data for **3** were assigned based on correlations observed in the ^1H-^1H COSY and HMBC spectra. The NMR spectra data indicated that **3** was similar to compound **4** (Ohmoto et al. 1987) except for that the exo methylene was replaced by oxygen. At the same time, the chemical shifts of H-1 and H-6 were downfield shifted but their coupling constants were very good correspondence with those of **4**, indicating that the relative stereochemistry of **3** was the same with that of **4**. There was no other difference between **3** and **4**. In the HMBC spectrum of **3**, the correlations were as follows: δ_H 2.41 (H-3) with δ_C 76.8 (C-1), 213.3 (C-4), 29.9 (C-2); δ_H 2.16 (H-5) with δ_C 213.3 (C-4), 66.1 (C-6), 43.6 (C-10), 11.8 (C-14); δ_H 0.93 (H-12) with δ_C 47.7 (C-7), 25.4 (C-11), 16.0 (C-13); δ_H 0.85 (H-13) with δ_C 47.7 (C-7), 25.4 (C-11), 20.8 (C-12); δ_H 0.78 (H-14) with δ_C 76.8 (C-1), 61.7 (C-5), 43.6 (C-10), 35.9 (C-9). The above correlations were in agreement with the structure. The stereochemistry of **3** was determined by comparison of its ^1H NMR signals with those of **4**. The observed coupling $J_{1,2}$ and the chemical shift of H-14 led to the assignment of the stereochemistry at C-1 and C-10. The 6α-orientation assignment of the oxygen function followed from the couplings observed, which were in agreement with those of similar 6α-oxygenated eudesmane. Thus, the structure of **3** has been deduced. At the same time, we obtained this compound from the whole plant of *Erigeron annuus* (L.) (Compositae).

Compounds **1a** and **5** showed antimicrobial activity against *Staphylococcus aureus* (Table 3).

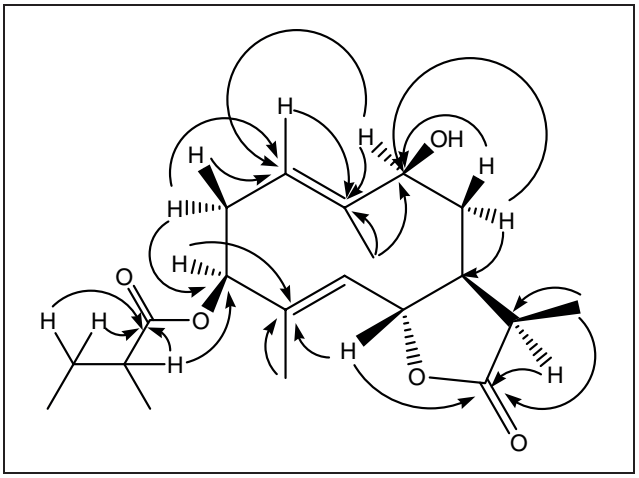

Fig.: H−C long-range correlations observed from the HMBC spectrum of **1** (400 MHz). Most protons are omitted for clarity

Table 3: Antibacterial activity of compounds 1, 1a, 5, 11, 22

Compound	E. coli	B. subtilis	S. aureus
1	−	−	−
1a	−	−	+++
5	−	−	+++
11	−	−	−
22	−	−	−
Chloromycinum (100 μg/ml)	+++	+++	+++
DMSO (4%)	−	−	−

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

3. Experimental

3.1. Apparatus

Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer M341 polarimeter. ^{1}H, ^{13}C NMR and 2D NMR spectra were scanned on Varian Mecury-300BB and 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. 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

Whole plants of *Achillea wilsoniana* Heimerl ex Hand.-Mazz were collected in 2002, in Zhang County, Gansu Province of China, and was identified by Prof. Guoliang Zhang, Department of Biology, Lanzhou University, China. A voucher specimen (No. 0208) is deposited in Department of Chemistry, Lanzhou University.

3.3. Extraction and isolation of compounds

The air-dried whole plants of *Achillea wilsoniana* (6.29 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 residue (700 g), which was suspended in hot H_2O (1000 ml). The H_2O soluble fraction was filtered and extracted with EtOAc and BuOH, respectively. The EtOAc extract (168 g) was obtained and subjected to CC separation over silica gel (1500 g), eluting with a gradient of petroleum ether-EtOAc (20 : 1, 15 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, 1 : 1 and 0 : 1) and finally with MeOH. According to differences in composition indicated by TLC, twenty fractions were obtained. Compound **12** (0.8 g) and **21** (80 mg) were deposited from Fr. 3 (petroleum ether-EtOAc 10 : 1, 2.0 g) and Fr. 4 (petroleum ether-EtOAc 8 : 1, 1.0 g) respectively and recrystallized from acetone. Compound **6** (15 mg) was deposited from Fr. 5 (petroleum ether-EtOAc 5 : 1, 1.0 g) and recrystallized from acetone. Fr. 5 was further separated by repeated preparative TLC (silica GF_{254}, 10–40 μ) with petroleum ether-acetone (3.5 : 1, three development) to afford compound **2** (7 mg, $R_f = 0.6$) and compound **10** (1 mg, $R_f = 0.7$). Fr. 6 (petroleum ether-EtOAc 5 : 1, 0.7 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$-acetone 100 : 1 repeatedly to give **8** (10 mg) and **15** (4 mg). Compound **20** (4 mg) was deposited from Fr. 7 (petroleum ether-EtOAc 5 : 1, 1.0 g) and recrystallized from acetone. Fr. 7 was further separated by repeated preparative TLC (silica GF_{254}, 10–40 μ) with $CHCl_3$-acetone (4 : 1, three development) to afford compound **4** (8 mg, $R_f = 0.5$). Compound **14** (2 mg) was purified by silica gel column chromatography with $CHCl_3$-acetone 70 : 1 as eluant from Fr. 8 (petroleum ether-EtOAc 3 : 1, 0.9 g). Compound **16** (4 mg) was purified by silica gel column chromatography with $CHCl_3$-acetone 40 : 1 as eluant from Fr. 9 (petroleum ether-EtOAc 3 : 1, 0.6 g). Fr. 10 (petroleum ether-EtOAc, 3 : 1) was separated on CC over silica gel with $CHCl_3$-acetone 40 : 1 as eluant to give compound **17** (5 mg) and **5** (28 mg). Fr. 11 (petroleum ether-EtOAc 3 : 1, 1.0 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$-acetone 40 : 1 repeatedly to give **11** (8 mg). Fr. 12 (petroleum ether-EtOAc 3 : 1, 0.9 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$-acetone 20 : 1 repeatedly to give **3** (10 mg). Compound **7** (12 mg) was purified by silica gel column chromatography with $CHCl_3$-acetone 20 : 1 as eluant from Fr. 13 (petroleum ether-EtOAc 3 : 1, 2.5 g). Fr. 14 (petroleum ether-EtOAc 1 : 1, 1.1 g) was rechromatographed ($CHCl_3$-acetone 10 : 1) again to afford crude **13**, from which compound **13** was deposited and recrystallized from acetone. Fr. 15 (petroleum ether-EtOAc 1 : 1, 1.2 g) was subjected to column chromatography on silica gel eluted with petroleum ether-EtOAc 1 : 1 repeatedly to give crude **9**, which was then purified again using different solvent system (petroleum ether-acetone 3.5 : 1) to give **9** (5 mg). Fr. 16 (petroleum ether-EtOAc 1 : 1, 1.3 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$-acetone 10 : 1 repeatedly to give **19** (50 mg). Compound **1** (30 mg) was purified by silica gel column chromatography with $CHCl_3$-acetone 8 : 1 as eluant from Fr. 17 (petroleum ether-EtOAc 0 : 1, 0.9g). Compound **1** was acetylated in the usual manner using acetic anhydride and pyridine to give the corresponding monoacetates **1a**. Fr. 18 (petroleum ether-EtOAc 0 : 1, 1.8 g) was separated on CC over silica gel with $CHCl_3$–CH_3OH (20 : 1) gave two subfractions (sfr. 1 and sfr. 2). Sfr. 1 was separated by repeated preparative TLC (silica GF_{254}, 10–40 μ) with $CHCl_3$-acetone (1 : 1) to afford compound **23** (18 mg, $R_f = 0.5$). Sfr. 2 was further separated by column chromatography on silica gel using $CHCl_3$-acetone 1 : 1 as eluant to give **22** (27 mg). Compound **18** (0.6 g) was deposited from Fr. 19 (petroleum ether-EtOAc 0 : 1, 2.0 g) and recrystallized from MeOH.

3.4. 4E,10E-9β-Hydroxy-3-(2-methylbutyroyloxy)-germacra-4, 10(1)-diene-12, 6α-olide (1)

Colorless gum, $[\alpha]_{21}^{D}$: +170.6° (c 4.6 $CHCl_3$); Rf. 0.5 (petroleum ether (60–90 °C)-EtOAc 2 : 1); IR (ν^{KBr}, cm^{-1}): 3459 (OH), 2971, 2937, 2877, 1766 (γ-lactone), 1732 (ester), 1673 (double bonds); EIMS: m/z (rel. int.) = 350 $[M]^+$ (1), 266 (2), 248 (3), 175 (5), 166 (9), 149 (5), 121 (6), 109 (2), 85 (76), 83 (56), 57 (100), 55 (39); ^{13}C NMR data and ^{1}H NMR data see Table 2 and Table 1.

3.5. 4E,10E-3-(2-Methylbutyroyloxy)-germacra-4, 10(1)-diene-12, 6α-olide (2)

Colorless gum, $[\alpha]_{21}^{D}$: +121.0° (c 0.7 $CHCl_3$); Rf. 0.5 (petroleum ether (60–90 °C)-EtOAc 5 : 1); IR (ν^{KBr}, cm^{-1}): 2969, 2935, 2877, 1774 (γ-lactone), 1732 (ester), 1670 (double bonds); EIMS: m/z (rel. int.) = 334 $[M]^+$ (1), 250 (6), 232 (2), 177 (2), 151 (3), 139 (4), 121 (3), 107 (4), 85 (64), 57 (100); ^{13}C NMR data and ^{1}H NMR data see Table 2 and Table 1.

3.6. 1β,6α-Dihydroxy-10β-methyl-5αH, 7αH-eudesm-4-one (3)

Colorless gum, $[\alpha]_{21}^{D}$: + 24° (c 0.6 $CHCl_3$); Rf. 0.5 (petroleum ether (60–90 °C)-acetone 6 : 1); IR (ν^{KBr}, cm^{-1}): 3421 (OH), 2955, 2872, 1705 (C=O); EIMS: m/z (rel. int.) = 240 $[M]^+$ (4), 225 (1), 222 $[M-H_2O]^+$ (10), 207 (3), 204 (3), 189 (3), 181 (4), 178 (8), 163 (5), 155 (100), 127 (41), 109 (10), 95 (43), 83 (29), 69 (26), 55 (51), 43 (90); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.88 (dd, J = 9.6, 9.6 Hz, 1 H, H-6), 3.83 (dd, J = 11.6, 4.8 Hz, 1 H, H-1), 2.41 (m, 2 H, H-3), 2.27 (septd, J = 7.2, 2.0 Hz, 1 H, H-11), 2.16 (d, J = 9.6 Hz, 1 H, H-5), 2.13 (m, 1 H, H-2), 1.92 (brddd, J = 12.0, 11.6, 6.8 Hz, 1 H, H-2), 1.88 (m, 1 H, H-8), 1.57 (m, 1 H, H-8), 1.25 (m, 1 H, H-7), 1.25 (m, 2 H), 0.93 (d, J = 7.2 Hz, 3 H, H-12), 0.85 (d, J = 7.2 Hz, 3 H, H-13), 0.78 (s, 3 H, H-14); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 213.3 (C, C-4), 76.8 (CH, C-1), 66.1 (CH, C-6), 61.7 (CH, C-5), 47.7 (CH, C-7), 43.6 (C, C-10), 39.3 (CH_2, C-3), 35.9 (CH_2, C-9), 29.9 (CH_2, C-2), 25.4 (CH, C-11), 20.8 (CH_3, C-12), 18.4 (CH_2, C-8), 16.0 (CH_3, C-13), 11.8 (CH_3, C-14).

3.7. Antimocrobial assays

We carried out antibacterial activity assays of compounds **1**, **1a**, **5**, **11** and **22** according to the paper-disk method (Xu et al. 1982). The results indicated that compounds **1a** and **5** were active against *Staphylococcus aureus* (Table 3).

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