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TWO NEW SESQUITERPENE LACTONES FROM *Sonchus arvensis*

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*Two new sesquiterpene lactones, 1β,15-diacetoxy-5,7α,6,11β(*H*)-eudesm-3,4-en-6,12-olide (**1**) and 1β-hydroxy-3,4-en-15-*O*-β-glucopyranosyl-5,7α,6,11β(*H*)-eudesman-6,12-olide (**2**), were isolated from* Sonchus arvensis *L. (Asteraceae), together with ten known compounds. Their structures were elucidated through spectroscopic and chemical methods. All these known compounds except oleanolic acid (**11**) were isolated from the genus* Sonchus *for the first time. Selected new compounds were evaluated for antibacterial activity; unfortunately, they showed weak or no activities against* Streptococcus mutans *ATCC 25175.*

Keywords: *Sonchus arvensis* L., Asteraceae, sesquiterpene lactone.

Sonchus arvensis L. (Asteraceae), a traditional Chinese medicine named "Ju Mai Cai," used for the treatment of fever, inflammation, stasis, as well as for detoxication and blood circulation [1], is distributed mainly in the northeast, southwest, and central-south regions of China [2]. Flavonol [3], flavonol glycoside [4, 5], monoacyl galactosylglycerol [6], sesquiterpene lactone [7, 8], quinic acid [7], steroid [9], and phenol [9] were previously isolated from this plant. In continuation of our search for bioactive metabolites from *S. arvensis* L., we have isolated two new sesquiterpene lactones (**1**, **2**) and ten known compounds, viz., 3-*O*-*β*-D-glucopyranosyltaraxasterol (**3**) [10], glucose (**4**), *β*-glucosylbutane (**5**), 5-hydroxymethyl furfural (**6**) [11], santonin (**7**) [12], taraxerol (**8**) [13], acetylated taraxerol (**9**) [14], oleanolic acid (**10**) [15], palmitic acid 2,3-dihydroxypropyl ester (**11**) [16], and linopalmitic acid 2,3-dihydroxypropyl ester (**12**) [17]. All these known compounds except **10** were isolated from the genus *Sonchus* for the first time. Selected new compounds were evaluated for antibacterial activity against the oral pathogen *Streptococcus mutans* ATCC 25175. This paper presents the details of isolation and structural identification of these compounds and their antibacterial activity.

Compound **1** was isolated as a yellow oil, and its molecular formula was determined to be $C_{19}H_{26}O_6$ by HR-ESI-MS (*m/z* found 373.1625 $[M + Na]^+$, calcd 373.1622).The ^{1}H NMR spectrum (Table 1) of **1** showed typical signals of one olefinic proton (δ 5.68, br.s); one oxygenated methylene [δ 4.61 (2H, dd, J = 14, 18 Hz)]; two oxygenated methines [δ 4.90 (dd, J = 7, 10 Hz), 3.98 (dd, J = 9.5, 11.4 Hz)]; four methyls, with the first one (δ 2.04, br.s), the second one (δ 2.04, br.s), the third one (δ 1.21, d, J = 7 Hz), and the last one (δ 0.99, s). The ^{13}C NMR spectrum (Table 1) of **1** indicated the presence of 19 carbons, including three ester carbonyl carbon signals (δ 178.5, 170.5, 170.4), two olefinic carbon signals (δ 131.9, 125.0), four methyl carbon signals (δ 29.2, 29.2, 12.3,12.2), three carbon signals (δ 79.2, 76.2, 66.2) with oxygen functional groups, and a quaternary carbon (δ 39.5). The above information suggests that compound **1** has an eudesmanolide-type skeleton, including two acetyl groups. All these were similar to (1*β*,6*α*)-1,6,14-trihydroxyeudesm-3-en-12-oic acid *γ*-lactone [18], except that **1** had two acetyl groups. This was further confirmed by the cross-peaks in HMBC (Fig. 1a). In the HMBC spectrum, the proton H-1 (δ_H 4.90) correlated with the carbon signals at C-1′ (δ_C 170.4), and the proton H-15 (δ 4.61) correlated with the carbon signals at C-3′ (δ_C 170.5). Thus, the two acetyl groups were located at C-1 and C-15 in **1**. Concerning the relative configuration of **1**, the large coupling constants observed for H-1 with H-2 ($J_{1\alpha,2\beta}$ = 10 Hz), H-6 with H-5 ($J_{6\beta,5\alpha}$ = 11 Hz), and H-7 ($J_{6\beta,7\alpha}$ = 9.5 Hz) allowed the assignment of the relative stereochemisty for H-1 as *α*-oriented and that of the lactone group at C-6 and C-7 as *trans* (6*β*, 7*α*). In the ROESY experiment (Fig. 1b), the cross-peaks between H-5*α* and H-7*α* and H-6*β* and H-14*β* indicated that the A/B ring was *trans*-fused. In the ^{13}C NMR spectrum, the chemical shift value of the methyl group at δ 12.3 is typical for eudesmanolides with an *α* methyl group at C-11 [19], which was further confirmed by the ROESY experiment, which showed cross-peaks between H-7*α* and H-13, and H-9*α*.

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TABLE 1. ^{1}H NMR and ^{13}C NMR Data of Compounds **1** and **2** (500 and 125 MHz, respectively, δ in ppm, TMS as internal standard)

C atom	**1**		**2**	
	δ_H ($CDCl_3$, multiplicity, J/Hz)	δ_C	δ_H (CD_3OD, multiplicity, J/Hz)	δ_C
1	4.90 (dd, J = 7, 10)	76.2	3.62 (dd, J = 7, 10)	78.1
2	2.56 (br.d, J = 18, H_α)	29.2	2.52 (br.d, J = 18.3, H_α)	33.4
	2.09 (m, H_β)		2.05 (m, H_β)	
3	5.68 (br.s)	125.0	5.78 (br.s)	127.0
4		131.9		134.4
5	2.47 (d, J = 11.3)	52.0	2.32 (d, J = 9.9)	50.0
6	3.98(dd, J = 11.9.5)	79.2	4.30 (t, J = 11.5)	82.8
7	2.46 (m)	53.2	2.60 (m)	54.6
8	1.87 (m, H_α)	22.5	1.86 (m, H_α)	23.6
	1.53 (m, H_β)		1.58 (m, H_β)	
9	1.26 (m, H_α)	34.3	1.25 (m, H_α)	36.0
	1.82 (m, H_β)		1.58 (m, H_β)	
10		39.5		41.1
11	2.29 (dq)	40.5	2.33 (dq)	41.8
12		178.5		182.2
13	1.21 (d, J = 7)	12.3	1.16 (d, J = 6.5)	12.6
14	0.99 (s)	12.2	0.90 (s)	11.5
15	4.61 (2H,dd, J = 14, 18)	66.2	4.38 (br.d, J = 12)	72.9
			4.14 (br.d, J = 11.5)	
1′		170.4	4.37 (d, J = 8)	103.9
2′	2.04	29.2	3.30 m	71.6
3′		170.5	3.24 m	77. 8
4′	2.04	29.2	3.18 m	75.2
5′			4.75	78.2
6′			3.85 (dd, J = 1.8, 2)	62.7
			3.67 (dd, J = 11.8, 5.2)	

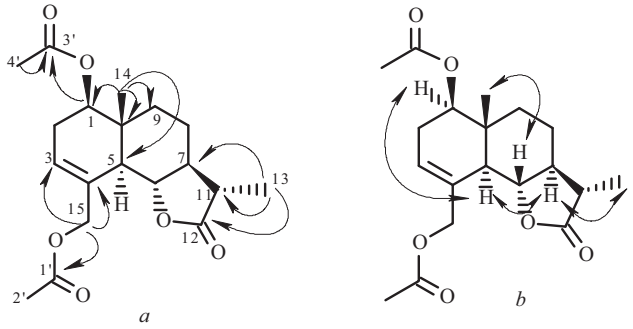

Fig. 1. Key HMBC (H→C) correlations of compound **1** (*a*) and key ROESY (H→H) correlations of compound **1** (*b*).

Consequently, all the signals of protons and carbons of **1** were assigned unambiguously, and its structure was established as 1*β*,15-diacetoxy-5,7*α*,6,11*β*(*H*)-eudesm-3,4-en-6,12-olide.

Compound **2** was isolated as a yellow gum, and its molecular formula was determined to be $C_{21}H_{32}O_9$ by HR-ESI-MS (*m/z* found 451.1941 $[M + Na]^+$, calcd 451.1939).The ^{1}H and ^{13}C NMR and the subsequent 2D NMR (HMBC, HSQC) (Fig. 2a) experiments suggested that **2** was a sesquiterpene lactone glycoside [20]. Typical signals for *β*-D-glucopyranoside were readily recognized from the NMR spectra, which was further confirmed as D-glucose after acid hydrolysis of **2**. The remaining signals indicated that the aglycone was a sesquiterpene lactone and was similar to the known eudesmanolide (1*β*, 6*α*)-1,6,14-trihydroxyeudesm-3-en-12-oic acid *γ*-lactone [17]. The HMBC correlations of H-15 at δ 4.38 to C-1′ at δ 103.9 and another H-1′ (δ_H 4.37) to C-15 (δ_C 72.9) connected the glucose group to C-15. Meanwhile the doublet signal for H-1′ (δ_H 4.37, d, J =7.9 Hz) and the resonance for C-1′ (δ_C 103.9) indicated the *β*-configuration of the glucoside in **2**.

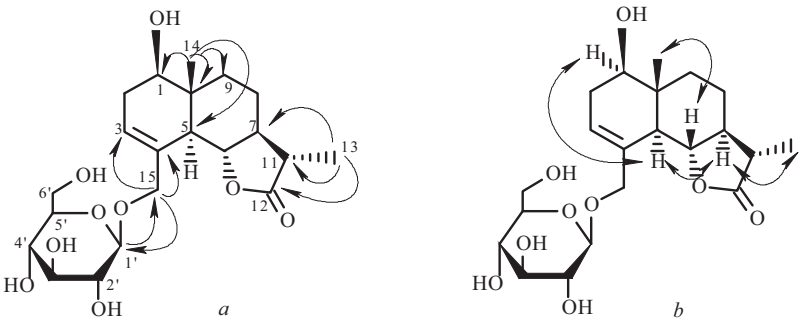

Fig. 2. Key HMBC (H→C) correlations of compound **2** (*a*) and key ROESY (H→H) correlations of compound **2** (*b*).

Finally, the stereochemistry was established by ROESY experiments (Fig. 2b). The clear cross-peaks between H-6β and H-14β and H-5 and H-1, H-7, H-9α indicated that C-14 had the β-configuration, while H-1, H-5, and H-7 were α-oriented. Therefore, the structure of **2** was established as 1β-hydroxy-3,4-en-15-*O*-β-glucopyranosyl-5,7α,6,11β(*H*)-eudesm-6,12-olide.

The known compounds **3**–**12** were identified by comparison of their MS and ^{1}H and ^{13}C NMR spectral data with those in the literature [10–17].

It is worth pointing out that the occurrence of sesquiterpene lactones is characteristic of *Sonchus* genus [8]. This study further confirm this conclusion.

The reason that the new compounds had weak or no activity against *Streptococcus mutans* ATCC 25175 may be because they have no antibacterial group.

EXPERIMENTAL

General Experimental Procedures. Melting points were measured on an XT-4 micro-melting point apparatus, uncorrected. NMR spectra were recorded on a Bruker ACF-500/300 NMR instrument (^{1}H 500/300 MHz, ^{13}C 125/75 MHz) with TMS as internal standard. Mass spectra were obtained on an MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and a Micro Q-TOF MS (HR-ESI-MS) spectrometer, respectively. GC/MS was done on a Varian CP-3800 gas chromatograph equipped with a Saturn 2200 mass detector, with a CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm). All solvents used were of analytical grade (Tianjin Chemical Plant). Silica gel (100–200 mesh, 200–300 mesh; Qingdao Marine Chemical Co., Ltd.), D-101 porous resin (Chemical Factory of Tianjin University), Sephadex LH-20 (25–100 mm; Pharmacia), and RP-C_{18} (20–45 mm; Fuji Silysia Chemical Co., Ltd.) were used for column chromatography. Thin-layer chromatography and preparative TLC were performed on silica gel GF254 (Qingdao Marine Chemical Co., Ltd.). L-Cysteine methyl ester hydrochloride, trimethylchlorosilane, and standard D-glucose were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai).

Plant Material. The whole plant of *S. arvensis* L. was collected in February 2007 in Shaoyang, Hunan Province, P. R. China and identified by Professor Minjian Qin (China Pharmaceutical University). A voucher specimen (No. 20070201) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation. The whole plant of *S. arvensis* (9.4 kg) was pulverized and extracted with refluxing (85% ethanol three times, 3 h each time). The EtOH extract was concentrated under reduced pressure (1345 g) and was then suspended in hot water and extracted with petroleum ether and ethyl acetate successively. The residue (190 g) was subjected to D-101 porous resin eluted with gradient EtOH–H_2O (0%, 25%, 50%, 75%, 95%) to afford five fractions (Fr. A–E). Fraction B (11 g) was chromatographed on a column of silica gel eluted successively with a gradient of $CHCl_3$–MeOH (10:1 to 0:1) to give five subfractions. Fraction B1 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (0:1 to 1:1) to give four subfractions. Fraction B1c was further purified by Sephadex LH-20 (MeOH) to obtain **2** (6 mg). Fraction C (33 g) was chromatographed on a column of silica gel eluted successively with a gradient of $CHCl_3$–MeOH (10:1 to 0:1) to give six subfractions. Fraction C2 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (1:2 to 2:1) to give four subfractions. Fraction C2a was further purified by Sephadex LH-20 (MeOH) to obtain **3** (7 mg). Fraction C3 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (1:2 to 2:1) to give four subfractions. Fraction C3a was further purified by Sephadex LH-20 (MeOH) to obtain **4** (10 mg) and **5** (10 mg). Fraction D (21 g) was chromatographed on a column of silica gel eluted successively with a gradient of $CHCl_3$–MeOH

(1:0 to 0:1) to give five subfractions. Fraction D3 was chromatographed on a column of silica gel eluted successively with a gradient of PE–EtOAc to give four subfractions. Fraction D3b was further purified by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to give **6** (6 mg) and **7** (7 mg). Fraction D3c was further separated by preparative TLC with petroleum ether–acetone (1:1) to yield **11** (12 mg, R_f 0.51) and **12** (3 mg, R_f 0.42). Fraction D3d was further purified on a column of silica gel to give **10** (200 mg). Fraction E (18 g) was chromatographed on a column of silica gel eluted successively with a gradient of PE–EtOAc (1:0 to 0:1) to give five subfractions. Fraction E2 was further chromatographed on a column of silica gel eluted successively with a gradient of PE–$CHCl_3$ (5:1 to 1:1) to give three subfractions. Fraction E2b was further separated by preparative TLC with PE–EtOAc (5:1) to give **1** (5 mg, R_f 0.56). Fraction E3 was further chromatographed on a column of silica gel eluted successively with a gradient of PE–$CHCl_3$ (3:1 to 0:1) to give **8** (40 mg) and **9** (50 mg).

Hydrolysis and GC Chromatography. Compound **2** (3 mg) dissolved in MeOH (4 mL) was treated separately with 5% H_2SO_4 (4 mL) at 90°C for 2 h. After adding H_2O (4 mL), we evaporated the reaction mixture to 8 mL under vacuum to remove MeOH and then kept it at 60°C for 15 min. The residue was dried using a rotary evaporator. The gas-liquid chromatography experiment was done using the published method [20]. The absolute configuration of the monosaccharide were confirmed to be that of D-glucose by comparison of the retention times of monosaccharide derivatives with those of standard samples of D-glucose (14.01 min).

MIC Determinations. The MIC values of isolates **1, 2** against selected oral bacteria were determined using liquid cultures in 96-well culture plates according to the modified method described by Shapiro et al. [21].

1β,15-Diacetoxy-5,7α,6,11β(*H*)-eudesm-3,4-en-6,12-olide (1). Yellow oil. ^{1}H and ^{13}C NMR, see Table 1. ESI-MS (pos.) *m/z* 368 $[M + NH_4]^+$; HR-ESI-MS (pos.) *m/z* found 373.1625 $[M + Na]^+$ (calcd for $C_{19}H_{26}O_6$, 373.1622).

1β-Hydroxy-3,4-en-15-*O*-β-glucopyranosyl-5,7α,6,11β(*H*)-eudesman-6,12-olide (2). Yellow gum. ^{1}H and ^{13}C NMR, see Table 1. ESI-MS *m/z*: (pos.) 446 $[M + NH_4]^+$, (pos.) 429 $[M + H]^+$, (neg.) 473 $[M + Cl]^-$, (neg.) 427 $[M – H]^-$; HR-ESI-MS (pos.) *m/z* found 451.1941 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_9$, 451.1939).

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