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CHEMICAL CONSTITUENTS FROM SEEDS OF *Lactuca sativa*[a]

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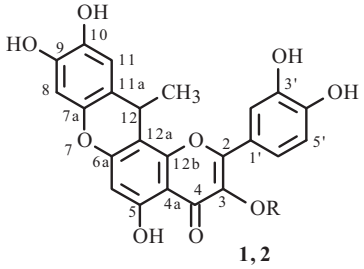
*A new flavonol glycoside with a rare structure type, lactucasativoside A (**1**), was isolated from the seeds of* Lactuca sativa*, together with three known compounds, japonicin A (**2**), isoquercitrin (**3**), and caffeic acid (**4**). Their structures were established on the basis of extensive spectroscopic analysis. Compound **2** was obtained from the genus* Lactuca *for the first time.*

Keywords: *Lactuca sativa* L., Compositae, flavonoids, lactucasativoside A.

Lactuca sativa L. (Compositae), a plant well known as lettuce, is a kind of favorite vegetable and is widely cultivated in China. The seeds of this plant are traditionally used as a folk medicine in China and Iran to relieve hyperpyrexia, inflammation, anuria, neurasthenia, gastrodynia, and osteodynia [1–3]. Phytochemical analysis showed the presence of triterpenoids, saponins, and phenols in the lettuce seeds [3].

In our recent research, the anti-inflammatory and analgesic activities of lettuce seeds were evaluated from mouse ear edema resulting from xylene and mouse writhing symptom caused by acetic acid. The results indicated that the 50% EtOH extract possessed the best anti-inflammatory activity, and the 95% EtOH extract showed the best analgesic activity. In order to reveal the active components, the chemical constituents of the seeds were isolated via extraction with 95% and 50% EtOH, purified by column chromatography and pre-HPLC, and identified on the basis of spectral data of MS and NMR. A new flavonol glycoside with a rare structure type, lactucasativoside A (**1**), together with three known compounds, japonicin A (**2**), isoquercitrin (**3**), and caffeic acid (**4**), was isolated from the seeds of *Lactuca sativa*.

Compound **1**, brown powder, had the molecular formula $C_{29}H_{26}O_{14}$, determined by analysis of its (–) HR-ESI-MS (*m/z* 597.1238 $[M - H]^-$, calcd for $C_{29}H_{25}O_{14}$ 597.1244), indicating the presence of 17 degrees of unsaturation. The ^1H and ^{13}C NMR data (Table 1) of **1** revealed structural features similar to those of japonicin A (**2**) [4], except for the presence of a sugar moiety. In addition, the chemical shift for C-2 was 9.8 ppm downfield (δ_C 159.0) and C-3 was 1.9 ppm upfield (δ_C 136.1). The glycosylation shifts indicated that the sugar unit was linked to C-3 of the aglycone, which was confirmed by the HMBC correlation of H-1″ (δ_H 5.35) to C-3 (δ_C 136.1). The remaining fragment connection was completely confirmed by HMBC correlations (Fig. 1).



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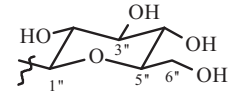

2: R = H

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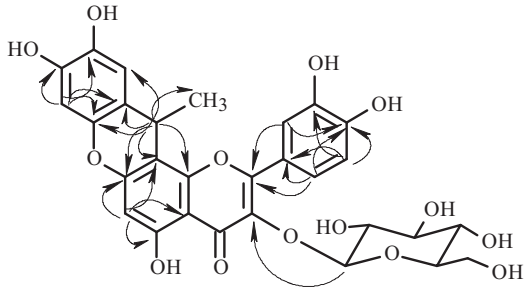
TABLE 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data of **1** and **2** (CD_3OD, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	–	159.0	–	149.2
3	–	136.1	–	138.0
4	–	179.7	–	177.7
4a	–	108.2	–	107.1
5	–	160.7	–	160.3
6	6.39 (1H, s)	99.9	6.40 (1H, s)	99.3
6a	–	158.4	–	158.1
7a	–	144.3	–	144.6
8	6.53 (1H, s)	104.4	6.57 (1H, s)	104.6
9	–	146.1	–	146.2
10	–	143.5	–	143.6
11	6.75 (1H, s)	115.1	6.80 (1H, s)	115.3
11a	–	117.1	–	116.0
12	4.35 (1H, q, J = 7.2)	28.7	4.43 (1H, q, J = 7.2)	28.9
12a	–	106.4	–	106.4
12b	–	154.7	–	154.5
1′	–	123.1	–	124.3
2′	7.81 (1H, d, J = 1.8)	117.4	7.86 (1H, d, J = 1.8)	117.4
3′	–	146.1	–	146.6
4′	–	150.2	–	148.5
5′	6.93 (1H, d, J = 8.4)	116.2	6.97 (1H, d, J = 8.4)	116.6
6′	7.72 (1H, dd, J = 8.4, 1.8)	123.3	7.76 (1H, dd, J = 8.4, 1.8)	121.9
12-Me	1.44 (3H, d, J = 7.2)	26.3	1.51 (3H, d, J = 7.2)	26.3
1″	5.35 (1H, d, J = 7.8)	104.1		
2″	3.52 (1H, t, J = 7.8)	75.7		
3″	3.45 (1H, t, J = 7.8)	78.1		
4″	3.37 (1H, t, J = 7.8)	71.3		
5″	3.25 (1H, m)	78.5		
6″	3.73 (1H, dd, J = 12.0, 2.4, H-6″a)	62.5		
	3.59 (1H, dd, J = 12.0, 5.4, H-6″b)			



1

Fig. 1. Key HMBC correlations of compound **1**.

Acid hydrolysis of **1** afforded *β*-D-glucose as confirmed by comparison with an authentic sample and established by the J value (J = 7.8 Hz) of the anomeric proton (H-1″). Thus, compound **1** was readily established as 3,3′,4′,5,9,10-hexahydroxy-12-methylchroman[2,3-h]flavone 3-*O*-*β*-D-glucopyranoside, named lactucasativoside A (**1**).

Compounds **2**–**4** were identied as japonicin A (**2**) [4], isoquercitrin (**3**) [5], and caffeic acid (**4**) [5], respectively, by comparing their spectroscopic data with those reported in the literature and were isolated from the seeds of *Lactuca sativa* for the first time.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a Yanaco MP-S3 apparatus (uncorrected). Optical rotation was measured on a Rudolph Autopol IV polarimeter. UV spectroscopy was carried out on a Shimadzu UV-2550 spectrophotometer. IR spectrum was obtained using a Bio-Rad FTS 165 spectrophotometer. NMR spectra were recorded on a Varian VNMRS 600 spectrometer. HR-ESI-MS data were acquired using a Q STAR Elite mass spectrometer (Applied Biosystems). D101 macroporous resin (Cangzhou Baoen Chemical Co., Ltd, China), silica gel (Qingdao Haiyang Chemical Co., Ltd, China), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden) were used for chromatography.

Plant Material. The seeds of *Lactuca sativa* L. were collected from the Miquan Vegetable Planting Base in Xinjiang, China in 2008. The seeds were authenticated by chief researcher Y. F. Zhang at the Xinjiang Institute of Materia Medica. A voucher specimen was deposited in the Herbarium of the Xinjiang Institute of Materia Medica.

Extraction and Isolation. The dried seeds (10 kg) were extracted with 95% EtOH and 50% EtOH (2 × 100 L) successively to give a crude extract (1108 g) after evaporation *in vacuo*. The extract was suspended in water and extracted with petroleum ether. The aqueous layer (614 g) was condensed and chromatographed over a macroporous absorbent resin column with gradient elution by water, 30% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH successively. The 50% EtOH fraction (15 g) was chromatographed over a silica gel column, eluted with $CHCl_3$–CH_3OH (50:1–2:8) in a gradient manner to give six fractions. Fraction 3 was subjected to Sephadex LH-20 (eluted with MeOH) to give 13 subfractions. Fraction 3–9 was purified by prep-HPLC using CH_3OH–0.2% HCOOH (45:55) as eluent to afford **1** (10 mg). Fraction 3–12 was purified through Sephadex LH-20 (MeOH) to give **2** (3 mg). Fraction 3–8 was purified by Sephadex LH-20 (MeOH) to give **3** (15 mg). The 30% EtOH fraction (55 g) was subjected to silica gel column chromatography with gradient elution by $CHCl_3$–CH_3OH (50:1–2:8) to give nine fractions. Fraction 7 was chromatographed over Sephadex LH-20 gel (MeOH) to yield **4** (5 mg).

Lactucasativoside A (1). $C_{29}H_{26}O_{14}$, brown powder (MeOH), mp > 270°C. $[\alpha]_D^{20}$ –10.00° (*c* 0.01, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 227 (3.98), 250 (3.74), 270 (3.76), 274 (3.84), 355 (3.66). IR (KBr, ν, cm^{-1}): 3368, 1656, 1597, 1518, 1488, 1456, 1383, 1351, 1261, 1180, 1071. (–) HR-ESI-MS *m*/*z* 597.1238 $[M - H]^-$ (calcd for $C_{29}H_{25}O_{14}$, 597.1244). ^1H and ^{13}C NMR (Table 1).

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