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Sesquiterpene Lactones from Flowers of *Picris hieracioides* L

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Synopsis. The isolation and structural elucidation of four guaianolides from the antimicrobial fractions of the flowers of *Picris hieracioides* L. (Compositae) are reported. Two of them are new compounds.

The flowers of *Picris hieracioides* L. var. *japonica* Regel (Compositae) are bitter and these cause a contact allergy for human skin. Schönbeck reported that the antibiotic activity of flowers of many higher plants is stronger than that of the other parts, leaves, roots and branches.[1] In the course of our investigation of antimicrobial compounds from flowers of higher plants,[2] we found that the extract of the flowers of *P. hieracioides* L. var. *japonica* Regel showed antimicrobial activity by the paper-disk method against *Bacillus subtilis.* Meanwhile, Nishimura et al. reported the isolation of some sesquiterpene lactone glycosides from whole plants of *P. hieracioides* L. var. *japonica* Regel.[3] We report here the isolation of two new sesquiterpene lactones, hieracin I and II with the known guaianolides, jacquilenin[4] and 8-deoxylactucin[5] from the antimicrobial fractions of methanol extract of the flowers of *P. hieracioides* L. var. *japonica* Regel.

The methanol extract of flowers (3.0 kg) of *P. hieracioides* L. var. *japonica* Regel. was concentrated in vacuo and the residue was partitioned with ethyl acetate and water. The antimicrobial activity [paper disk method against *B. subtilis* (IFO 3734)] was found in the ethyl acetate fraction. The residue of the ethyl acetate phase was chromatographed on silica gel with hexane-ethyl acetate. The active fractions were purified by HPLC to give an antimicrobial compound (**A**, 30 mg) and other compounds (**B**, 150 mg; **C**, 5.2 mg; and **D**, 0.5 mg).

8-Deoxylactucin (1). The spectroscopic properties (High resolution MS IR, ^{1}H NMR, and ^{13}C NMR) suggested that the compound **A** is a sesquiterpene lactone. The presumed structure from the ^{1}H-^{1}H COSY[6] and the J-resolved 2D[7] agreed with 8-deoxylactucin (**1**). The reported ^{1}H NMR data[5,8] of 8-deoxylactucin **1** corresponded with our data of the compound **A**.

Jacquilenin (2). The spectral data showed that compound **B** is very similar to 8-deoxylactucin (**1**) although one of the double bonds is saturated. Finally the structure of **B** was identified to be jacquilenin (**2**) by comparing the ^{1}H NMR (^{1}H-^{1}H COSY and NOESY[9]) and the ^{13}C NMR data with those of jacquilenin (**2**).[5,10]

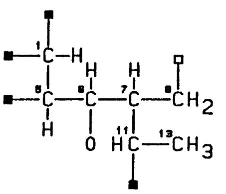
Two new guaianolides, which were named hieracin I (**3**) and hieracin II (**4**), were isolated from a more polar fraction than 8-deoxylactucin (**1**).

Hieracin I (3). Compound **C** has a molecular formula $C_{15}H_{20}O_5$ (MS: *m/z* 280.1339, Calcd M, 280.1331). The IR [3450 (br), 1765, 1690, 1620 cm^{-1}] and ^{1}H NMR spectra suggested **C** should be a guaianolide similar to **1** and **2**. The MS (M^+-H_2O at *m/z* 262.1149, Calcd 262.1204), IR, ^{1}H NMR, and ^{13}C NMR spectra [δ 59.08 (d) and 71.90 (s)] proved that **C** has a tertiary hydroxyl group instead of a double bond (C1—C10) of jacquilenin **2**. The partial structure (**P**) was clearly revealed by the ^{1}H-^{1}H COSY spectrum of **C**. These observations led to the structure **3** for compound **C** which was named hieracin I. The chemical shift of H-13 (δ 1.24, d, J=7.0 Hz) and the coupling constant ($J_{7\alpha\text{-}11\beta}$=12.5

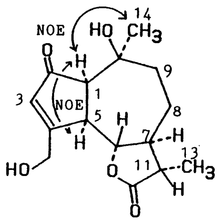
Table 1. NMR Data of 8-Deoxylactucin (**1**) and Jacqilenin (**2**)

C	8-Deoxylactucin (1)		Jacquilenin (2)	
	^{13}C NMR	^{1}H NMR[a)]	^{13}C NMR	^{1}H NMR
1	131.0		131.1	
2	195.2		195.1	
3	133.2	6.44 (s)	133.2	6.43 (s)
4	171.7		171.6	
5	49.8	3.69 (d, 10.3)	49.5	3.59 (d, 10)
6	83.9	3.61 (dd, 10.3, 10.3)	83.7	3.61 (dd, 10, 10)
7	52.7	2.90 (dddd, 13, 10.3, 3.1, 3.1)	56.1	1.98 (m)
8	24.2	α 2.21 (ddd, 13, 5.7, 2.5)	25.8	α 1.99 (m)
		β 1.45 (dddd, 13, 13, 13, 2.6)		β 1.39 (m)
9	37.3	α 2.51 (ddd, 13, 13, 2.5)	37.7	α 2.40 (m)
		β 2.40 (ddd, 13, 5.7, 2.6)		β 2.42 (m)
10	153.3		153.5	
11	138.4		41.3	2.28 (dq, 12.4, 7.0)
12	172.0		177.2	
13	119.3	a 6.18 (d, 3.1)	12.2	1.25 (d, 7.0)
		b 5.49 (d, 3.1)		
14	21.9	2.45 (s)	21.8	2.43 (s)
15	62.4	a 4.89 (d, 17.8)	62.5	a 4.85 (d, 17.7)
		b 4.54 (d, 17.8)		b 4.48 (d, 17.7)

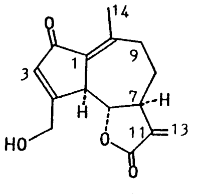
a) The coupling constants were estimated from the *J*-resolved 2D spectrum.



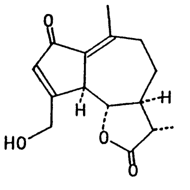
Partial Structure (P)



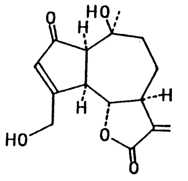
hieracin I (3)



8-deoxylactucin (1)



jacquilenin (2)



hieracin II (4)

Table 2. NMR Data of Hieracin I (3) and II (4)

	Hieracin I (3)		Hieracin II (4)
C	^{13}C (DEPT)[11]	^{1}H[a]	^{1}H
1	59.2 (CH)	2.72 (d, 6.8)	2.74 (d, 6.6)
2	207.0		
3	129.8 (CH)	6.33 (s)	6.35 (s)
4	180.4		
5	50.7 (CH)	3.25 (dd, 9.6, 6.8)	3.35 (dd, 9.9, 6.6)
6	83.3 (CH)	4.34 (dd, 9.6, 9.6)	4.40 (dd, 9.9, 9.9)
7	52.7 (CH)	1.78 (m)	2.01 (m)
8	23.8 (CH_2)	a 1.95 (m) b 1.60 (m)	
9	41.0 (CH_2)		
10	71.9		
11	41.7 (CH)	2.30 (dq, 12.5, 7.0)	
12	177.9		
13	12.7 (CH_3)	1.24 (d, 7.0)	a 6.23 (d, 3.7) b 5.55 (d, 3.7)
14	32.3 (CH_3)	1.63 (s)	1.50 (s)
15	63.1 (CH_2)	a 4.83 (d, 17.8) b 4.51 (d, 17.8)	a 4.87 (d, 17.8) b 4.55 (d, 17.8)

a) The coupling constants were estimated from the J-resolved 2D spectrum.

Hz) indicated that the methyl group at C-11 is α-oriented.[10] The stereochemistry at C-1, C-5, C-6, C-7 and C-10 was clarified by comparing the coupling constants ($J_{5\alpha\text{-}6\beta}$=9.6 and $J_{6\beta\text{-}7\alpha}$=9.6 Hz) with those of jacquilenin (**2**) ($J_{5\alpha\text{-}6\beta}$=10 Hz and $J_{6\beta\text{-}7\alpha}$=10 Hz) and the NOESY spectrum of hieracin I (**3**). The NOE were observed between H-14 and H-1α and between H-1α and H-5α. Whereas no NOE were observed between H-5α and H-6β, between H-6β and H-7α, and between H-7α and H-11β (Fig. 1).

Hieracin II (4). The spectral deta: MS (Found: m/z 278.1144; 260.1082, Calcd for $C_{15}H_{18}O_5$: M, 278.1152; M−H_2O, 260.1047), IR, and ^{1}H NMR [δ 6.23 (1H, d, J=3.7 Hz) and 5.55 1H, d, J=3.7 Hz)] suggested that compound **D** has an *exo*-methylene group (C11-C13) instead of a methyl group at C-11 of hieracin I (**3**). The novel compound **D** was named hieracin II (**4**).

8-Doxylactucin (**1**) showed moderate activity against *Bacillus subtilis* whereas jacquilenin (**2**) showed no activity against *B. subtilis*. Clearly the antimicrobial activity of **1** should depends on the α-methylene γ-lactone moiety.

Experimental

NMR spectra were measured with a JEOL GX-270 spectrometer in $CDCl_3$ solution containing tetramethylsilane as internal standard. IR spectra were measured on Jasco IR-810 spectrometer. Mass spectra were recorded by JEOL JMS-D300. HPLC was performed by JASCO BIP-1 HPLC system with UV detector on a silica-gel column (Lichrosorb Si-60 10 μm, 7.5×500 mm), an Asahipak-310 column, or an ODS column (INERTSIL 10 μm, 7.5×300 mm). Thin-layer chromatography was carried out on Kieselgel GF_{254} (Merck) in 0.25 mm thickness. Wakogel C-200 (Wako Pure Chemical Industries) was used for column chromatography.

Isolation of Sesquiterpene Lactones. The flowers (3.0 kg) of *Picris hieracioides* were collected in Yamanashi prefecture, Japan in September 1985 and extracted with methanol. The extract was concentrated in vacuo and the residue was partitioned with ethyl acetate and water. The antimicrobial activity was tested by paper-disk method against *Bacillus subtilis* (IFO 3734) for each fraction. The activity was found in the ethyl acetate phase. The residue of the ethyl acetate phase was chromatographed on silica gel with hexane–ethyl acetate. The repeated purification by HPLC on a silica-gel column (Lichrosorb Si-60, 10 μm, 7.53×500 mm) eluted with hexane–ethyl acetate (3:5) and ethyl acetate, and on an ASAHIPAK-310 column eluted with 40%-methanol and on an ODS column (INERTSIL 10 μm, 7.53×300 mm) eluted with 35%-methanol to give 8-deoxylactucin (**1**, 30 mg), jacquilenin (**2**, 150 mg), hieracin I (**3**, 5.2 mg), and hieracin II (**4**, 0.5 mg).

8-Deoxylactucin (1). MS: Found: m/z 260.1033, Calcd for $C_{15}H_{16}O_4$: M, 260.1048; IR (film) 3420, 1770, 1690, 1620, 1250, 1130, and 990 cm^{-1}.

Jacquilenin (2). Mp 147—152 °C; MS: Found: m/z 262.1194, Calcd for $C_{15}H_{18}O_4$: M, 262.1204; IR (KBr) 3350, 1750, 1660, 1260, and 995 cm^{-1}.

Hieracin I (3). MS: Found: m/z 280.1339; 262.1149, Calcd for $C_{15}H_{20}O_5$: M, 280.1311; M^+−H_2O, 262.1204; IR (neat) 3450, 1765, 1690, 1620, and 985 cm^{-1}.

Hieracin II (4). MS: Found: m/z 278.1144; 260.1082, Calcd for $C_{15}H_{18}O_5$: M, 278.1152; M^+−H_2O, 260.1047; IR 3400, 1760, 1680, 1620, and 985 cm^{-1}.

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