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FURTHER SESQUITERPENE LACTONES AND FLAVONES FROM *Centaurea bruguierana*

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SUMMARY

A further investigation of the aerial parts of *Centaurea bruguierana* (DC.) Hand.-Mazz. afforded four sesquiterpene lactones namely, cnicin, 11,13-dehydromelitensin, 8α-(5-hydroxy)-angeloyloxy-11,13-dehydromelitensin, 8α-(3',4'-dihydroxyethyl)-acryloxy-11,13-dehydromelitensin and five flavonoids; eupatilin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, jaceosidin, pectolinarigenin and cirsilineol. The structures were determined by spectral methods especially by high field NMR spectroscopy.

ÖZET

Centaurea bruguierana (DC.) Hand.-Mazz. bitkisinin toprak üstü kısımlarından dört seskiterpen lakton; 11,13-dehydromelitensin, cnicin, 8α-(5-hidroksi)-angeloyiloksi-11,13-dehidromelitensin, 8α-(3',4'-dihidroksietil)-akriloksi-11,13-dehidromelitensin ve beş flavonoid bileşik; eupatilin, 5-hidroksi-6,7,3',4'-tetrametoksiflavon, jaceosidin, pectolinarigenin ve cirsilineol elde edilmiştir. Bileşiklerin yapıları spektroskopik yöntemler ile açıklanmıştır.

Key words: *Centaurea bruguierana;* Compositae; sesquiterpene lactones; flavones.

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INTRODUCTION

In course of our research of the genus *Centaurea,* we have examined *Centaurea bruguierana* (DC.) Hand.-Mazz. *(Syn. C. phllocephala* Boiss.) a Saudi Arabian plant, which was distributed in Najid province of Saudi Arabia.

The plant has been investigated previously by one of us, F.M. Harraz, and the presence of a germacranolide; cnicin, an eudesmanolide; 11,13-dehydromelitensin 8α-acetate and three flavonoids; circilineol, cirsimaritin and hispudilin 7-O-rutinoside were reported (1). In the present paper, we report the isolation and characterization of four sesquiterpene lactones **(1-4)** and five flavones**(5-9)** from the aerial parts of *C. bruguierana.* The sesquiterpene lactone, cnicin **(1)** and the flavon, cirsilineol **(9)** were previously isolated from many *Centaurea* species (1-3) while the others 8α-(3',4'-dihydroxyethyl)-acryloxy-11,13-dehydromelitensin **(2)** (4,5), 8α-(5-hydroxy)-angeloyl-11,13-dehydromelitensin **(3)** (6), 11,13-dehydromelitensin **(4)** (7) and flavones, eupatilin **(5)** (8), 5-hydroxy-6,7,3',4'-tetramethoxyflavone **(6)** (9), jaceosidin **(7)** (10), pectolinarigenin **(8)** (11) were reported for the first time from *Centaurea bruguierana.*

RESULTS AND DISCUSSION

Compound 1 was cnicin, a common sesquiterpene lactone of *Centaurea* species, it was identified by comparison of its spectral data with those reported in the literature (2,3).

Compound 2 was obtained as glassy gum. The IR spectrum showed the characteristic doublets of α,β-unsaturated-γ-lactone carbonyl at 1770 cm^{-1}, an ester carbonyl at 1725 and 1270 cm^{-1}, hydroxyl(s) at 3450 cm^{-1} and unsaturations at δ 1630, 980, 860 cm^{-1}. Extensive ^{1}H NMR spin decoupling experiments (Table 1), ^{1}H NMR (Table 2) and ^{13}C NMR spectra were evident for the structure of compound **2.** Starting irradiation of the typical doublets of exocyclic methylene group at δ 6.16 (d, *J*=3 Hz, H-13) and 5.56 (d, *J*=3.5 Hz, H-13') led to the unambiguous sequences H_7-H_9 and H_7-H_5. The signals at δ 5.77 (dd, *J*= 10.5 and 17 Hz, H-1), δ 5.07 (dd, *J*=1 and 10.5 Hz, H-2a) and δ 5.03 (dd, *J*=1 and 17 Hz, H-2b) were easily assigned as a vinyl group and the broadened singlets at δ 5.42 and 4.96 indicated the presence of a terminal methylene group in the molecule. Further spin decoupling experiments permitted identification of the ester function, irradiation of the signal at δ 4.64 (dd, *J*=4 and 7 Hz, H-3')

Table 1: Spin-decoupling experiments on Compound 2 ($CDCl_3$)

Irradiated H	Observed H	Multiplicities changes
6.16 (H-13)	2.97 (H-7)	dddd ----- ddd (J= 3.5, 11, 11 Hz)
2.97 (H-7)	4.24 (H-6) 5.29 (H-8)	t--------d (J= 11 Hz) ddd-----dd (J= 4,10 Hz)
4.24 (H-6)	2.58 (H-5)	d-------s
5.29 (H-8)	2.05 (H-9b) 1.69 (H-9a)	dd-----d (J= 14 Hz) dd-----d (J= 14 Hz)
5.77 (H-1)	5.07 (H-2a) 5.03 (H-2b)	dd-----br s dd-----br s
4.64 (H-3')	3.85 (H-4'a) 3.60 (H-4'b)	dd-----d (J= 11 Hz) dd-----d (J= 11 Hz)

collapsed the two double doublets at δ 3.82 (*J*=4 and 11 Hz, H-4'a) and 3.60 (*J*= 7 and 11 Hz, H-3'b) to doublets and sharpened the vinylic singlets at δ 6.39 (H-5'a) and 6.08 (H-5'b) indicating that the nature of the ester function as (3',4'-dihydroxyethyl)-acryloic acid. The lower chemical shift of H-8 (ddd, 5.29) indicated that the ester function is at C-8. ^{13}C NMR (APT) spectrum ($CDCl_3$) of compound **2** showed 20 carbon singlets, 1xCH_3, 7xCH_2, 6xCH and 6xC. Multiplicities were assigned by DEPT spectra. 145.53 (C-1), 113.20 (C-2), 115.15 (C-3), 143.58 (C-4), 50.46 (C-5), 78.63 (C-6), 52.31 (C-7), 69.69 (C-8), 44.97 (C-9), 41.88 (C-10), 136.60 (C-2'), 169.51 (C-12), 120.163 (C-13), 18.60 (C-14), 67.35 (C-15), 165.14 (C-1'), 139.10 (C-11), 71.17 (C-3'), 65.76 (C-4'), 127.63 (C-5'). In the view of spectral data **2** was identified as 8α-(3',4'-dihydroxyethyl)-acryloxy-11,13-dehydromelitensin. All spectral data of **2** were in good agreement with those reported by Bruno and Herz (4).

Compound 3 has the same structural skeleton with that of compound **2,** it only differs on the nature of ester function. The ^1H NMR spectrum indicated the presence of an angeloyloxy group as ester function at C-8 showing the signals of a methine quartet at δ 6.45, a methyl doublet at δ 2.07 and a methylene singlet adjacent to a hydroxyl group at δ 4.27. Spin decoupling experiments clearly showed the relations between these protons. Irradiation of H-3' turned the doublet at δ 2.08 (H-4') to a br singlet conversly, the former turned to a br singlet. On the basis of all spectral data compound **3** was identified as 8α-(5-hydroxy)-angeloyl-11,13-dehydromelitensin.

Table 2: ^{1}H NMR spectra of Compounds **2-8**, 200 MHz, $CDCl_3$

H	**2**	**3**	**4***
1	5.78 dd	5.77 dd	5.79 dd
2a	5.07 dd	5.08 dd	5.03 dd
2b	5.03 dd	5.04 dd	4.99 dd
3a	5.42 br s	5.44 br s	5.39 br s
3b	4.98 br s	4.97 br s	4.90 br s
5	2.59 d	2.59 d	2.49 d
6	4.23 t	4.25 t	4.16 t
7	2.97 ttt	2.98 ttt	2.65 ttt
8	5.29 ddd	5.36 ddd	3.60 ?
9a	2.03 dd	2.12 dd	1.83 dd
9b	1.69 dd	1.67 dd	1.62 dd
13	6.16 d	6.18 d	6.16 d
13'	5.56 d	5.69 d	6.03 d
14	1.19 s	1.18 s	1.19 s
15a	4.11 d	4.11 d	4.04 d
15b	3.99 d	4.00 d	3.95 d
3'	4.64 dd	6.47 q	–
4'a	3.85 dd	2.08 d	–
4'b	3.60 dd	–	–
5'a	6.39 br s	4.29 br s	–
5'b	6.08 br s	–	–

H	**5**	**6**	**7**	**8**
2'	7.34 d	7.35 d	7.33 d	7.82 d br
6'	7.53 dd	7.54 dd	7.47 dd	
3'				6.94 d br
5'	6.98 d	6.98 d	7.04 d	
3	5.59 s	5.98 s	6.55 s	6.54 s
8	6.63 s	6.62 s	6.60 s	6.59 s
12-OH	13.07 s	12.76 s	13.10 s	12.77 s
OCH_3	3.96 s	3.94 s	4.00 s	3.92 s
	3.97 s	3.97 s	4.03 s	3.97 s
	4.05 s	3.98 s	–	–
	–	4.01 s	–	–

J (Hz) = **2**:2a, 1= 10; 2b, 1= 17; 2a, 2b= 1; 5, 6=11; 6, 7= 11; 7, 8= 10; 9a, 8= 11; 9b, 8= 4; 9a, 9b= 14; 3', 4'a= 4; 3', 4'b= 7; 4'a, 4'b= 11; **3:** 3', 4'= 7; **5-8:** 2', 6'= 2.5; 3', 5'= 2.5; 5', 6'= 8.

Compound 4 exhibited frequencies corresponding to an α, β-unsaturated-γ-lactone carbonyl at 1735 cm^{-1}, unsaturations at 1662, 990 cm^{-1} and hydroxyl(s) at 3440 cm^{-1} in its IR spectrum. The ^{1}H NMR spectrum (Table 2) showed exocyclic methylene group protons at δ 6.16 (d, J=3 Hz, H-13) and 6.03 (d, J= 3.5 Hz, H-13'). The paramagnetic shift of H-13' clearly indicated that compound **4** contains an α-oriented OH group at C-8. This is very characteristic for the 11,13-dehydro sesquiterpene lactones carrying and α-oriented free hydroxy group on C-8. The spectrum also displayed the other proton resonances of the skeleton, while it did not show any signal for an ester group.

Based on all spectral data and comparison them with those reported in the literature, compound **4** was identified as 11,13-dehydromelitensin.

UV (Table 3) and ^{1}H NMR (Table 2) spectral data of compounds **5-9** revealed that they all are 6-methoxylated flavonoids. **9** was cirsilineol which was also isolated in the previous work (1). Compounds **5, 6** and **8** were purple under UV light (366 nm) and the color did not change after exposure NH_3 vapours and spraying NA reagent(Diphenyl boric acid-β-aminoethylester), suggesting that they have no free hydroxyl groups either at C-3' or C-4'. The compounds, **5, 6** and **8** were found identical with eupatilin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone and pectolinarigenin, respectively. Compound **7** was purple under UV light

Table 3: UV spectral data of compounds **5-8,** MeOH λ (max.) nm.

	5	**6**	**7**	**8**
MeOH	340 272	339 273	342 275	334 274
NaOMe	360 313 277	339 275 268	402 331 275	385 312 272
$AlCl_3$	349 277	348 280	346 275	341 275
$AlCl_3$ + HCl	353 285	348 280	355 282	348 280
NaOAc	338 279	339 275	342 275	334 272
NaOAc + H_3BO_3	339 278	338 275	342 275	334 272

and turned to yellow color by both exposure NH_3 vapour and spraying NA reagent suggesting a free hydroxyl group at C-4'. From spectral data **7** was identified as jaceosidin (see Table 2 and 3).

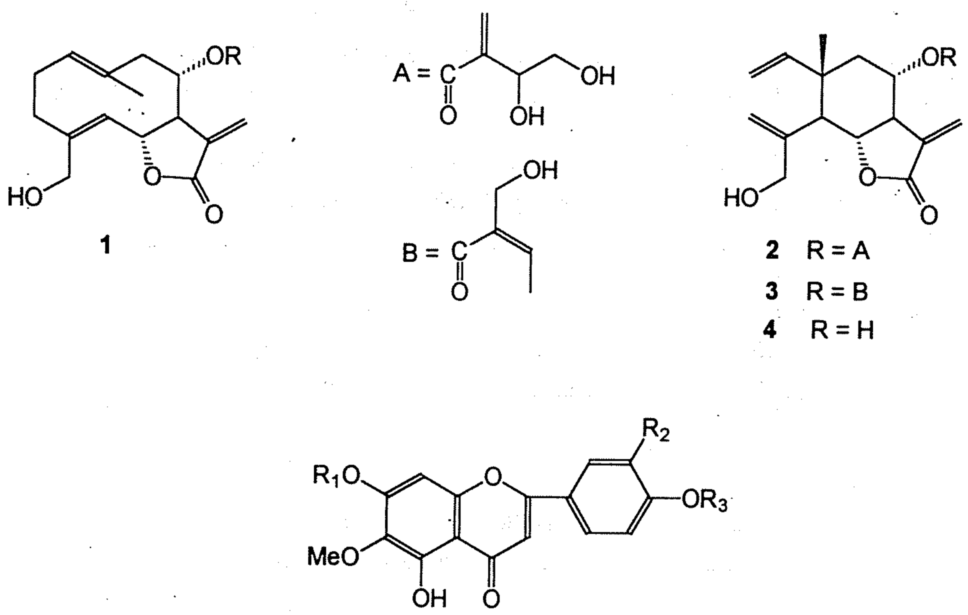
EXPERIMENTAL

General Experimental Procedures- Spectra were recorded with the following instruments: ^1H NMR and ^{13}C NMR on a Bruker AC 200L; IR on a Perkin-Elmer 983; UV Varian Techtron 635.

Plant Material- *Centaurea bruguierana* was collected from Saudi Arabia in May 1992. A voucher specimen is deposited in the Department of Veterinary Medicine, Qassim, Burideah, Saudi Arabia.

Extraction and Isolation- Air-dried aerial parts were extracted with EtOH at room temp. and evaporated in *vacuo*. The crude extract was successively extracted with petrol and $CHCl_3$. Chloroform-soluble part was fractionated on a silica gel column and eluted with toluen-$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$-EtOAc (9:1) and EtOAc up to 100%. All fractions were monitored on tlc, similar ones were combined and each fraction was subjected to a small column and further prep.tlc to obtain pure compounds. This procedure was repeated two or three times when necssary.

Compounds **5-9** and **1-4** were isolated from $CHCl_3$-EtOAc (1:1) and EtOAc fractions, respectively. Compounds **1-4** were obtained as follows; **1** (15 mg), **2** (13 mg), **3** (7 mg), **4** (10 mg). Compounds **5-9** were separetly subjected to sephadex LH-20 columns (2x20 cm) and eluted with MeOH to give pure samples. Yields were; **5** (11 mg), **6** (7 mg), **7** (3 mg), **8** (5 mg), **9**(4 mg).



1

2 R = A

3 R = B

4 R = H

	5	**6**	**7**	**8**	**9**
R_1	H	Me	H	H	Me
R_2	OMe	OMe	OMe	H	OMe
R_3	Me	Me	H	Me	H

Figure 1

REFERENCES

1. Harraz, F.M., Kassem, F.F. and El-Shaer, N.S. *Alex. J. Parm. Sci.* **8,** 219-222 (1994).
2. Jakupovic, J., Jia, Y., Pathak, V.P., Bohlmann, F. and King, R.M. *Planta Medica* **52,** 399 (1986).
3. Barrero, A.F., Sanchez, J.I., Rodrigez, I. and Sonia, C. *An. Quim, Ser.* **C. 84,** 344 (1988).
4. Bruno, M. and Herz, W. *Phytochemistry* **27,** 1873-1875 (1988).
5. Bruno, M., Fazio, C., Passananti, S., Paternostro, M.P., Diaz, J.G. and Herz, W. *Phytochemistry* **35,** 1371-1372 (1994).
6. Tsankova, E. and Ognyanov, I. *Planta Medica* 465-466 (1985).
7. Gonzalez, A.G., Bermejo, J., Cabrera, I. and Massanet, G.M. *An. Quim.* **70,** 74 (1974).
8. Kupchan, S.M., Sigel, C.W., Hemingway, R.J., Knox, J.R., Udayamurthy, M.S. *Tetrahedron* **25,** 1603-1615 (1969).
9. Dobberstein, R.H., Tin-Wa, M., Fong, H.H.S., Crane, F.A., Farnsworth, N.R. *J. Parm. Sci.* **66,** 600-602 (1977).
10. Breton Funes, J.L., Marrero, B.G. and Gonzalez, A.G. *An. Quim.* **64,** 187-192 (1968).
11. Herz, W. and Sudarsanam, V. *Phytochemistry* **9,** 895-896 (1970).