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Chemical Constituents of *Centaurea cuneifolia*

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The whole plant material fo *Centaurea cuneifolia* Sm. afforded three sesquiterpene lactones; cnicin **(1)**, dehydromelitensin **(2)** and dehydromelitensin-8-[(2′ − α-hydroxy-β-hydroxyethyl)-acryloyl)] **(3)**, the lignan; (-)-arctigenin **(4)**, flavonoids; salvigenin **(5)**, eupatilin **(6)**, jaceosidin **(7)**, acacetin **(8)**, kaemferol **(9)**, 5,7,4′-trihydroxy-3-methylether **(10)**, 5-hydroxy-3′,′,6,7-tetramethoxyflavone **(11)** and 5-hydroxy-3′,4′,7,8-tetramet hoxyflavone **(12)**, a triterpene; α-amyrin and a sterol; β-sitosterol. The structures of the isolates were determined by IR, UV, ^{1}H and ^{13}C NMR and MS spectroscopy.

Key words: *Centaurea cuneifolia*; Compositae; sesquiterpene lactones; elemanolide; lignan; flavonoids; triterpenes; steroids.

Introduction

The largest genus *Centaurea* of the family Compositae is represented by about 170 species in Turkey and has been the subject of many chemical investigations which have led to the isolation f various types of compounds such as sesquiterpene lactones[1−2], acetylenes[3,4], flavonoids[5−8] and lignans.[9−11]

In our previous study[5] on *Centaurea cuneifolia* Sm., we reported the presence of six methoxylated flavonoids namely scutellarein-6,7,4′-trimethylether (salvigenin) **(5)**, 6-hydroxyluteolin-6,3′,4′-trimethylether (eupatilin) **(6)**, 6-hydroxyluteolin-6,3′-dimethylether (jaceocidin) **(7)**, apigenin-4′-methylether (acacetin) **(8)**, scutellarein-4′,7-dimethylether **(14)**, and a triterpene, oleanolic acid.

In our continuing study on the chemical constituents of *Centaurea cuneifolia*, we report here the isolation and identification of three sesquiterpene lactones; cnicin **(11)**[12], dehydromelitensin[13] **(2)**, and dehydromelitensin-8-[2′ − α-hydroxy-β-hydroxyethyl)-acryloyl] **(3)**[7], a lignan (-)-arctigenin **(11)**[9,10] and four more flavonoids 3,5,7,4′-tetrahydroxyflavone (kaemferol) **(9)**, 5,7,4′-trihydroxy-3-methylether **(10)**, 5-hydroxy-3′,4′,6,7-tetramethoxyflavone **(11)** and 5-hydroxy-3′,4′,7,8-tetramethoxyflavone **(12)** as well as previously isolated flavonoids **(5-8)** by our group.The plants also yielded the well known triterpene, α-amyrin, and the steroid, β-sitosterol. This is the first report on the presence of the sesquiterpene lactones and the lignan in *Centaurea cuneifolia*.

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Experimental

Instrumentation: ^{1}H NMR (200 MHz) and ^{13}C NMR (50.32 MHz) were recorded on a Bruker AC; MS spectra were recorded on a VG Zabspec.; IR spectra were recorded on a Perkin Elmer 963; and UV spectra were recorded on a Varian Techtron 635 instrument for flavonoids.

Plant Material: *Centaurea cuneifolia* Sm. was collected from Kıyıköy (Tekirdağ) in June 1996 and identified by Prof. Dr. Ali Çırpıcı (Marmara University). A voucher specimen was deposited in the Herbarium of the Atatürk Faculty of Education, Marmara University (MARA 5634).

Extraction and Fractionation

Air-dried and powdered whole plant material of *Centaurea cuneifolia* Sm. (950 g) was extracted with 1:1:1 petrol ether-ether-ethanol at room temperature. After filtration the extract was evaporated *vacuo* to a small volume. This extracts was treated with MeOH and kept in a refrigerator for 2 hours to remove the long chain saturated hydrocarbons. After elimination of the precipitate by filtration and evaporation of MeOH *in vacuo*, the residue (35 g) was coarsely prefractionated on a silica gel column eluting with petrol ether, a gradient of petrol ether-Et_2O up to 100% Et_2O, followed by MeOH up to 100%. The similar fractions were combined and further chromatographed on small columns when necessary.

Isolation of the Compounds

The relevant fractions were further separated and cleaned by repeated preparative TLC using CH_2Cl_2:EtOH (9:1; 8:2) mixtures as developing solvents to yield cnicin (**1**, 520 mg), dehydromelitensin (**2**, 15 mg dehydromelitensin-8-[(2′ − α-hydroxy-β-hydroxyethyl)-acryloyl] (**3**, 12 mg), and (-)-arctigenin (**4**, 20 mg). Flavonoids were obtained from fractions 31, 35, 41 and 42, using an LH-20 sephadex column and purified by prep. TLC. The yields were as follows: **5** (9 mg), **6** (11 mg), **7** (7 mg), **8** (11 mg), **9** (14 mg), **10** (7 mg), **11** (7 mg), **12** (15 mg).

Results and Discussion

From the fractions (38-40), compounds **1-3** were obtained. Their structures were identified by spectral methods as cnicin **(1)**, dehydromelitensin **(2)** and dehydromelitensin-8-[(2′ − α-hydroxy-β-hydroxyethyl)-acryloy]) **(3)**.

The IR spectrum of **1** showed hydrogen bonded OH group (s) at 3400 cm^{-1}, an intense carbonyl absorption band of an α,β-unsaturated-γ-lactone at 1760 cm^{-1}, an ester carbonyl at 1710 cm^{-1} and double bonds at 1660 and 1625 cm^{-1}. The ^{1}H NMR spectrum of **1** (Table 1) exhibited a charcteristic doublet of an exocyclic methylene group conjugated with a γ-lactone at δ 6.22 (J=3.5 Hz, H-13) and 5.84 (J=3.0 Hz, H-13′), two doublets at δ 4.30 (J=14 Hz, H-15) and 4.06 (J=14 Hz, H-15′) of an oxymethylene group (O-CH_2), a doublet of doublets at δ 5.11 (J=2.5 5, 11 H-8) and a doublet at δ 4.97 (J=10 Hz, H-5). The spectrum also showed the signals of an α-hydroxy-β-hydroxyyethylacryloxy group as an ester function with a doublet of doublets at δ 4.55 (J=3.5 6.0 Hz, H-3′), two proton singlets at δ 6.40 (H-5′a) and 6.11 (H-5′b), and a pair of doublet of dublets at δ 3.75 (J=3.5 11 Hz, H-4′a) and 3.55 (J=6, 11 Hz, H-4′b.) All of the civinal protons were assigned by extensive spin-decoupling experiments. The signal of H-7 at δ 3.30 (dddd, J=3.0, 3.5, 9, 11 Hz) was selected as a convenient point of reference. Starting with H-7 and then H-1 (δ 5.14, m) the sequences H-6 to H-8 and, H_2-13 to H_2-2 to H_2-3 were clearly deduced. ^{13}C

Table 1 ^{1}H NMR Data of Compounds **1-4** (200 MHz, $CDCI_3$ TMS)

H	**1***	**2**	**3**	**H**	**4**
1	5.14 m	5.78 dd (11, 17)	5.78 dd (11, 17)	2	6.64 d (2
2a	2.64 ddd	5.04 dd (1, 11)	5.06 dd (1, 11)	2′	6.46 d (2
2b	2.37 ddd	4.98 dd (1, 17)	5.01 dd (1, 17)	5	6.82 d (9
3a	2.41 ddd (4, 11, 14)	5.40 s	5.42 brs	5′	6.75 d (9
3b	2.08 ddd (5.5, 11, 14)	4.94 s	4.98 brs	6	6.53 dd (
5	4.97 d (10)	2.52 d (11.5)	2.58 d (11.5)	6′	6.53 dd (
6	5.25 dd (9, 10)	4.14 t (11)	4.23 t (11.5)	7	2.70m
7	3.30 dddd (3, 3.5, 9, 11)	2.64 dddd (3.5, 3, 11.5, 11)	7′	2.45 m	
8	5.11 ddd (2.5, 5, 11)	4.10 ddd (4, 2.5, 11)	5.28 dt (4, 11, 11)	8	2.89 dd (
9a	2.62 dd (5, 12.5)	1.87 dd (4, 13)	2.04 dd (4, 13)	8′	2.92 dd (
9b	2.29 dd (2.5, 12.5)	1.61 m	1.69 dd (11, 13)	9_a	4.15 d (6
13a	6.22 d (3.5)	6.18 d (3)	6.14 d (3.5)	9_b	3.92 d (7
13b	5.84 d (3)	5.98 d (3)	5.54 d (3)	OCH_3	3.81 s×2
14	1.58 s	1.10 s	1.19 s		3.85 s
15a	4.06 d (14)	4.09 d (13)	4.11 d (13.5)		
15b	4.30 d (14)	3.97 d (13)	4.00 d (13.5)		
3′	4.55 dd (3.5, 6)		4.63 dd (4, 7)		
4′a	3.75 dd (3.5, 11)		3.84 dd (4, 11.5)		
4′b	3.55 dd (6, 11)		3.60 dd (6, 11.5)		
5′a	6.40 brs		6.38 brs		
5′b	6.11 brs		6.06 brs		

* in CD_3OD

^{a,b} assignments interchangeable

NMR (APT) spectrum of **1**, which is given for the first time in this report, shows 20 carbons consisting of 1 primary (CH_3), 7 secondary (CH_2), 6 tertiary (CH) and 6 quaternary carbon atoms supporting the proposed structure (Fig. 1). The molecular formula of **1** was determined to be $C_{20}H_{26}O_7$ by EI-mass spectrometry with a molecular ion peak at *m/z* 378 $[M]^+$ and, prominent peaks at *m/z* 265 and 114 suggesting the loss of an α-hydroxy-β-hydroxyethylacryloxy moiety from the molecular ion. All of the spectral data were in good agreement with those reported for cnicin, a germacranolide, previously isolated from *Cnicus benedictus*.[14]

Table 2 ^{13}C NMR Spectral data of Compounds **1,3-4**

C	**1***	**3**	**C**	**4**
1	129.7 d	145.0 d	1	130.7 s
2	26.9 t	113.2 t	1′	129.5 s
3	35.1 t	114.8 t	2	114.2 d
4	142.2 s	143.2 s	2′	111.6 d
5	130.8 d	50.6 d	3	149.1 s
6	78.6 d	77.9 d	3′	147.9 s
7	54.0 d	53.0 d	4	146.7 s
8	71.9 d	71.1 d	4′	144.6 s
9	48.2 t	44.9 t	5	111.8 d
10	133.2 s	41.9 s	5′	111.3 d
11	137.4 s	139.1 s	6	122.1 d
12	166.5 s	170.0 s	6′	120.6 d
13	125.3 t	120.2 t	7	38.2 t^a
14	17.1 q	18.3 q	7′	34.5 t^a
15	66.6 t	67.4 t	8	46.6 d
1′	171.3 s	165.1 s	8′	40.9 d
2′	145.5 s	136.7 s	9	71.3 t
3′	74.4 d	71.2 d	9′	178.8 s
4′	60.8 t	64.8 t	OCH_3	55.9 q
5′	127.3 t	127.5 t		

Multiplicities were assigned by DEPT Spectra

* in CD_3OD

^{a} interchangeable in the same column

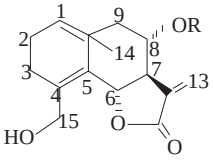
The CI-mass spectrum of **2** gave a molecular ion peak at *m/z* 265 $[M+H]^+$ suggesting the molecular formula $C_{15}H_{20}O_4$. The ^{1}H NMR spectrum was very similar to that of melitensin[8], an elamandienolide type sesquiterpene lactone. The difference between the two compounds was the presence of an α-methylene-γ-lactone ring in compound **2**. This was very clear from the typical doublets of C-13 protons at δ 6.1 (d, *J*=3 Hz, H-13) and 5.98 (d, *J*=3 Hz, H-13′) in ^{1}H NMR spectrum and moreover, the chemical shifts of H-13 and H-13′ was also evidence for the presence of a free α-OH group at C-8.[15] All of the spectral data (Table 1) of **2** were idential with those of dehydromelitensin which was first determined in *Centaure pullata*.[13]

From the ^{1}H and ^{13}C NMR spectra (Table 1), compound **3** was found as dehydromelitensin esterified with and α-hydroxy-β-hyroxyethylacryloxy moiety on C-8. All of the spectral data were identical with those of elemandienolide which was previously isolated from *Centaurea cineraria* subsp. *umbrosa* by Bruno, M. et *al.*[7] Hence **3** was identified as dehydromelitensin-8-[(2′ − α-hydroxy-β-hydroxyethyl)-acryloyl].

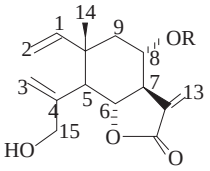
The IR and NMR spectral properties of compound **4**; $[\alpha]_4$-21.97° (EtOH; c 0.91), suggested an

aromatic compound. Its El-mass spectrum showed a molecular ion peak at m/z 372 suggesting the molecular formula, $C_{21}H_{24}O_6$. ^{1}H NMR spectrum (Table 2) showed methoxy group singlets and six aromatic protons as well as the presence of a lignan-olide skeleton in compound **4**. The latter was also seen in the IR spectrum with a strong absorption peak at 1748 cm^{-1}. ^{13}C NMR (APT) spectrum (Table 2) revealed 1 primary (CH_3), 3 recondary (CH_2), 8 tertiary (CH) and 7 quaternary carbon atoms. The spectral data were identical with those of (-)-arctigenin, a lignan, previously isolated from *Wikstroemia indica*.[9]

The flavonoids **(5-12)** isolated in this study were the common flavonoids of the family Compositae previously isolated from several *Centaurea* species. They were identified by a combination of UV and ^{1}H NMR spectra and comparing their spectral properties reported in the literature as well as TLC comparison with authentic samples.

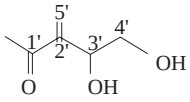


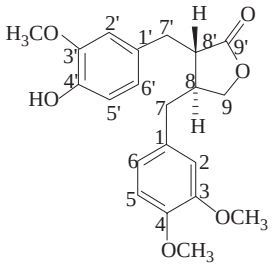
1 R = A



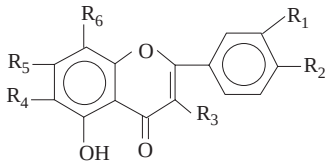
2 R = H
3 R = A

A =





4



	R_1	R_2	R_3	R_4	R_5	R_6
5	H	OMe	H	OMe	OMe	H
6	OMe	OMe	H	OMe	OH	H
7	OMe	OH	H	OMe	OH	H
8	H	OMe	H	H	OH	H
9	H	OH	OH	H	OH	H
10	H	OH	OMe	H	OH	H
11	OMe	OMe	H	OMe	OMe	H
12	OMe	OMe	H	H	OMe	OMe
13	H	OMe	H	H	OMe	H
14	H	OMe	H	OMe	OMe	H

Scheme 1.

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