

## SESQUITERPENE LACTONES AND FLAVONES FROM *CENTAUREA CINERARIA* SUBSP. *UMBROSA*

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**Key Word Index**—*Centaurea cineraria* ssp. *umbrosa*; Cynareae; Compositae; germacradienolides; elemadienolides; flavones.

**Abstract**—Extraction of the aerial parts of *Centaurea cineraria* ssp. *umbrosa* afforded the germacradienolides cnicin and cnicin 4'-O-acetate, a new elemadienolide and the flavones salvigenin, 5-hydroxy-3',4',6,7-tetramethoxyflavone, eupatilin and jaceosidin.

Representatives of the large genus *Centaurea* typically furnish functionalized germacradienolides and guaianolides. In the present report we describe our work on *Centaurea cineraria* L. ssp. *umbrosa* (Lacaita) Pign. (syn. *C. ucrae* Lacaita ssp. *ucrae*), one of the three subspecies of *C. cineraria* endemic to the western part of Sicily [1]. Isolated from the aerial parts were cnicin (**1a**), the major sesquiterpene lactone constituent and a common constituent of *Centaurea* species, cnicin 4'-O-acetate (**1b**) recently found in *Centaurea calcitrapa* [2], the new elemadienolide **2** which is formally a Cope rearrangement product of **1a**, and the four flavones salvigenin (**3**), 5-hydroxy-3',4',6,7-tetramethoxyflavone (**4**), eupatilin (**5**) and jaceosidin (**6**).

The structure and stereochemistry of **2**, a gum, were evident from the extensive NMR decoupling experiments. <sup>1</sup>H and <sup>13</sup>C NMR spectra are given in Tables 1 and 2 respectively. Three hydroxyl groups (two primary and one secondary) were present as shown by the <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> with addition of D<sub>2</sub>O and by reaction with trichloromethyl isocyanate. Irradiation at the frequencies of the typical doublets of the exocyclic methylene group identified H-7 as a multiplet at δ2.97. Further irradiation at the latter frequency collapsed the H-6 triplet at δ4.23 and the H-8 *td* at δ5.30 to a doublet and *dd*, respectively. Irradiation at the frequency of H-6 permitted identification of H-5, a doublet at δ2.59, and sharpened two broadened singlets at δ5.42 and 4.98 which could therefore be ascribed to H-3a,b. Irradiation at the frequencies of H-3a,b sharpened two germinally coupled broadened doublets at δ4.11 and δ3.99 (H-15a,b). Irradiation at the frequency of H-8 collapsed two mutually coupled *dds* at δ2.03 and 1.69 (H-9β and H-9α) to doublets.

Three signals at δ5.70, 5.07 and 5.03 were typical of a vinyl group; they were better resolved in CDCl<sub>3</sub>-C<sub>6</sub>D<sub>6</sub> in which H-1 was a *dd* at δ5.52, H-2a a *dd* at δ4.86 and H-2b a *dd* at δ4.78. The nature of the ester side chain, suggested by the loss of a unit of *m/z* 114 in the mass spectrum was

established by sequential decoupling, beginning with irradiation of H-3' which collapsed the two *dds* at δ3.83

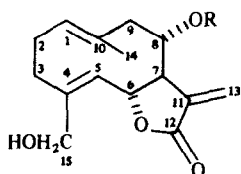
Table 1. <sup>1</sup>H NMR spectra of compounds **1b** and **2** (270 MHz)

H	<b>1b</b> *	<b>2</b> †
1	5.01 <i>br dd</i> (10,5)	5.78 <i>dd</i> (17,11)
2a	2.24 <i>m</i>	5.07 <i>dd</i> (11,1)
2b	2.20 <i>m</i>	5.03 <i>dd</i> (17,1)
3a	2.57 <i>m</i>	5.42 <i>br s</i>
3b	1.94 <i>m</i>	4.98 <i>br s</i>
5	4.84 <i>d</i> (9)	2.59 <i>d</i> (10)
6	5.12 <i>t</i> (9)	4.23 <i>t</i> (10)
7	3.10 <i>tt</i> (9,3)	2.97 <i>tt</i> (10,3)
8	5.14 <i>td</i> (9,2)	5.30 <i>td</i> (10,4)
9a	2.48 <i>m</i>	2.03 <i>dd</i> (13,4)
9b	2.40 <i>m</i>	1.69 <i>dd</i> (13,10)
13a	6.28 <i>d</i> (3)	6.13 <i>d</i> (3)
13b	5.75 <i>d</i> (3)	5.53 <i>d</i> (3)
14‡	1.51 <i>brs</i>	1.19 <i>s</i>
15a	4.32 <i>brd</i> (13)	4.11 <i>br d</i> (13)
15b	4.10 <i>brd</i> (13)	3.99 <i>br d</i> (13)
3'	4.70 <i>dd</i> (7,4)	4.62 <i>dd</i> (7,4)
4'a	4.27 <i>dd</i> (11,4)	3.83 <i>dd</i> (11,4)
4'b	4.19 <i>dd</i> (11,7)	3.59 <i>dd</i> (11,7)
5'a	6.38 <i>br s</i>	6.39 <i>br s</i>
5'b	6.08 <i>br s</i>	6.06 <i>br s</i>
15-OH		1.83 <i>br s</i>
3'-OH		3.08 <i>br s</i>
4'-OH		2.36 <i>br s</i>
OAc‡	2.08 <i>s</i>	

\* CDCl<sub>3</sub>-MeOD(3:2).

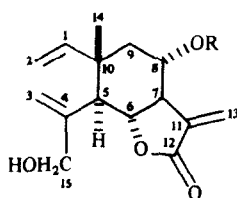
† CDCl<sub>3</sub>.

‡ Intensity three protons.

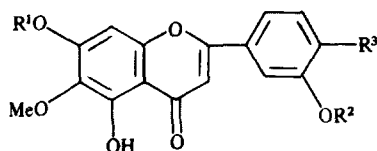
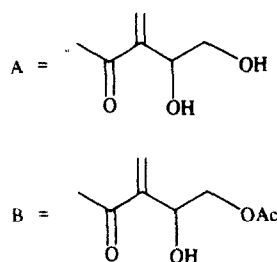


**1a** R = A

**1b** R = B



**2** R = A



**3** R<sup>1</sup>, R<sup>2</sup> = Me, R<sup>3</sup> = H

**4** R<sup>1</sup>, R<sup>2</sup> = Me, R<sup>3</sup> = OMe

**5** R<sup>1</sup>, R<sup>2</sup> = Me, R<sup>3</sup> = OMe

**6** R<sup>1</sup>, R<sup>2</sup> = H, R<sup>3</sup> = OMe

and 3.56 (H-4'a,b) and sharpened two vinylic signals at  $\delta$ 6.39 and 6.06.

The coupling constants involving H-5, H-6, H-7 and H-8, as well as the negative Cotton effect ( $[\theta]_{259} - 1030$ ), established the relative and absolute stereochemistry in the formula.

Table 2. <sup>13</sup>C NMR spectrum of compound **2** (67.89 MHz, CDCl<sub>3</sub>)<sup>†</sup>

C		C	
1	145.6 <i>d</i>	11	139.0 <i>s</i> *
2	113.1 <i>t</i>	12	169.1 <i>s</i>
3	115.1 <i>t</i>	13	120.1 <i>t</i>
4	143.6 <i>s</i> *	14	18.4 <i>q</i>
5	50.6 <i>d</i>	15	67.3 <i>t</i>
6	78.7 <i>d</i>	1'	165.2 <i>s</i>
7	52.4 <i>d</i>	2'	136.7 <i>s</i>
8	69.7 <i>d</i>	3'	71.2 <i>d</i>
9	45.0 <i>t</i>	4'	64.8 <i>t</i>
10	41.9 <i>s</i>	5'	127.5 <i>t</i>

<sup>†</sup> All multiplets were identified by single frequency decoupling.

\*These assignments may be interchanged.

## EXPERIMENTAL

Above-ground parts (580 g) of *C. cineraria* L. ssp. *umbrosa* (Lacaita) Pign., collected near Capo Zafferano 40 km west of Palermo, Sicily, in Aug. 1986 (voucher specimen as deposit in the herbarium of the botanical garden at Palermo) were extracted with CHCl<sub>3</sub>. Work-up in the usual fashion gave 9 g of crude gum which was adsorbed on 20 g of silica gel (Merck No. 7734, deactivated with 15% H<sub>2</sub>O) and subjected to CC over 400 g of the same adsorbent, 250 ml fractions being collected as follows: 1–4 (petrol), 5–10 (petrol–EtOAc, 4:1), 11–14 (petrol–EtOAc, 3:2) 15–20 (petrol–EtOAc, 2:3), 21–24 (petrol–EtOAc, 1:4), 25–28 (EtOAc), 29–32 (EtOAc–MeOH, 9:1).

Frs 4,5 were rechromatographed over silica gel (petrol–Et<sub>2</sub>O, 1:1) to give 8 mg of salvigenin **3** identified by its physical and spectroscopic data [4]. Frs 8–10 dissolved in MeOH upon refrigeration (–20°) deposited 60 mg of 5-hydroxy-3',4',6,7-tetramethoxyflavone (**4**) identified by its physical and spectroscopic data [5]. Frs 11, 12 were rechromatographed over silica gel (CHCl<sub>3</sub>) to give a mixture of eupatilin **5** [6, 7] and jaceosidin **6** [8, 9]. The two compounds were separated by radial chromatography (CHCl<sub>3</sub>–MeOH 49:1) to give 5 mg of **5** and 5 mg of **6** whose physical and spectroscopic properties were in agreement with those reported in the literature.

Frs 15–17 were purified by CC (CHCl<sub>3</sub>). Using repeated radial chromatography (CHCl<sub>3</sub>–MeOH, 49:1) it was possible to isolate 35 mg of **1b** [2] whose <sup>1</sup>H NMR spectrum is listed in Table 1.

Rechromatography of fr. 18–19 by CC (petrol–EtOAc, 3:7) and further purification by radial chromatography (petrol–EtOAc) gave 45 mg of **2** as a gum, IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>–1</sup>: 3640, 3610, 1775, 1715, 985; CD curve (MeOH)  $[\theta]_{259} - 1030$ ,  $[\theta]_{292} 0$ ,  $[\theta]_{225} + 8910$ , MS (CI) *m/z* (rel. int.): 379 [M+H] (1) 265 (20) 247 (29), 229 (21) 115 (110); <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra are given in Tables 1 and 2.

Frs 20, 21 were dissolved in  $\text{CHCl}_3$  and refrigeration ( $-20^\circ$ ) resulted in crystallization of 2.5 g of cnicin (**1a**) [10] whose  $^1\text{H}$  NMR spectrum compared with that of an authentic sample.

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## TERPENES FROM THE RED ALGA *SPHAEROCOCCUS CORONOPIFOLIUS* OF THE NORTH ADRIATIC SEA\*

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**Key Word Index**—*Sphaerococcus coronopifolius*; Rhodophyta; sesquiterpene; diterpenes.

**Abstract**—From the red alga *Sphaerococcus coronopifolius* collected in the north Adriatic Sea, four terpenes were isolated, viz. the sesquiterpene alloaromadendrene, the diterpenes bromosphaerol, sphaerococcenol **A** and the new compound sphaeroxetane. The structure of the new diterpenoid was proposed on the basis of its spectral data, including 2D-NMR spectroscopy, in comparison with known related compounds. All  $^{13}\text{C}$  chemical shifts of known compounds are assigned.

## INTRODUCTION

Continuing our studies on the seaweeds of the north Adriatic Sea, we have investigated the red alga *Sphaerococcus coronopifolius* collected near Plomin, Yugoslavia. From the same alga, collected in Spain, Fenical *et al.* [2] described the isolation of sphaerococcenol **A** (**1**), while Fattorusso *et al.* [3] reported the isolation of bromosphaerol (**2**) as the main diterpenoid together with a series of minor diterpenoids [4–6] isolated from the same alga collected in Sicily (Tyrrhenian Sea). We wish to describe herein the isolation from the alga, of a new bromoditerpenoid, named sphaeroxetane (**3**), of two previously reported related diterpenoids, sphaerococcenol **A** [2] and bromosphaerol [3], and of the sesquiterpene alloaromadendrene [7] (**4**).

## RESULTS AND DISCUSSION

The chloroform-soluble material from chloroform-methanol extracts of dried *S. Coronopifolius* was chromatographed on silica gel, using increasing concentrations of diethyl ether in petrol as the eluent. Three bromoditerpenes and one sesquiterpene were isolated. In order of decreasing polarity, these were bromosphaerol (**2**) (0.04% dry wt), sphaerococcenol **A** (**1**) (0.01%), sphaeroxetane (**3**) (0.008%) and alloaromadendrene (**4**) (0.01%). The spectra of **1** and **2** were in excellent agreement with published data [2,3]. Using 2D-NMR spectroscopy, proton-proton correlation (COSY) and 2D-heteronuclear correlation we were able to assign all the  $^{13}\text{C}$  chemical shifts, not completely assigned in the literature, for the two known diterpenoids (see Table 1).

The spectral data, including 2D-NMR spectroscopy, of **4** were in agreement with the gross structure of an aromadendrene skeleton. The stereochemistry was deduced by NOESY with the aid of a Dreiding model. The

\*Part 3 in the series 'Chemical studies of north Adriatic seaweeds'. For part 2 see ref. [1].