Secondary Metabolites from *Centaurea deusta* with Antimicrobial Activity

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The aerial parts of *Centaurea deusta* Ten. afforded in addition to several known compounds, mainly sesquiterpene lactones, one new eudesmanolide and one new elemane derivative. Structures of the new compounds were elucidated by spectroscopic methods. The *in vitro* antifungal and antibacterial activities of the isolated compounds was tested, using the micro-dilution method. All compounds tested showed high antifungal activity.

Introduction

Continuing our research on the chemical constituents of Greek *Centaurea* sp. (Skaltsa *et al.*, 1999; 2000a; 2000b), we report here the results of the investigation of secondary metabolites from *C. deusta* Ten. [= *C. alba* subsp. *deusta* (Ten.) Nyman], a species, belonging to section *Phalolepis* (Mabberlay, 1997). Isolated were the flavonoids cirsimaritin and salvigenin (Mabry *et al.*, 1970), the nor-isoprenoid 3*S*,5*R*-loliolide (**11**) (Hodges and Porte, 1964), two elemanolides (**1**, **2**), two related elemanes (**3**, **4**), four germacranolides (**5**–**8**) and two eudesmanolides (**9**–**10**). Compounds **4** and **10** are new naturally occurring sesquiterpene lactones. The *in vitro* antifungal and antibacterial activity of the isolated compounds were tested, using the micro-dilution method (Hanel and Raether, 1988; Daouk *et al.*, 1995).

Results and Discussion

The crude extracts of the aerial parts of *C. deusta* afforded the dehydromelitensine derivatives, 8α-(3,4-dihydroxy-2-methylene-butanoyloxy) (**1**) (Bruno *et al.*, 1995), 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy) (**2**) (Bruno *et al.*, 1994), the elemanes methyl 8α-(3,4-dihydroxy-2-methylene-butanoyloxy)-6α, 15-dihydroxy-elema-1,3, 11(13)-trien-12-oate (**3**) (Cardona *et al.*, 1997) and methyl 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-6α, 15-dihydroxyelema-1, 3, 11(13)-trien-12-oate (**4**), the germacranolides cnicin (**5**) (Rustaiyan *et al.*, 1982), 4′-acetylcnicin (**6**) (Jakupovic *et al.*, 1986), 3′-acetylcnicin (**7**) (Polo, 1994) and 8α-(4-acetyl-2-hydroxymethyl-buten-2-oyloxy)-salonitenolide (**8**) (Huneck *et al.*, 1986) and the eudesmanolides 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-4-*epi*-sonchu-carpolide (**9**) (Skaltsa *et al.*, 2000b) and 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-sonchucarpolide (**10**). Compounds **4** and **10** are new natural products.

The MS of **4** exhibited a molecular peak at *m/z* 452.2067, which agreed with the molecular formula $C_{23}H_{32}O_9$ and its IR spectrum afforded absorptions characteristic of hydroxyl and ester groups. The signals in the ^{1}H NMR spectrum (Table I) at δ 5.64 *dd*, 4.93 *d*, 4.89 *d*, 5.35 *s* and 4.98 *s* were assigned to H-1, H-2a, H-2b, H-3a and H-3b, respectively, of an elemane framework. The methyl ester chain on C-7 was evident from the two singlets for H-13a and H-13b at δ 6.27 and 5.72, respectively, and the methyl singlet at δ 3.76. A typical doublet at δ 2.13 (J = 10.0 Hz) for H-5, the signal at δ 4.18 (*t*, J = 10.6 Hz) for H-6 and the low field double triplet at δ 5.43 corresponding to H-8 indicated a *trans*-disposition of H-5/H-6, H-6/H-7 and H-7/H-8 and so the oxygenated functions

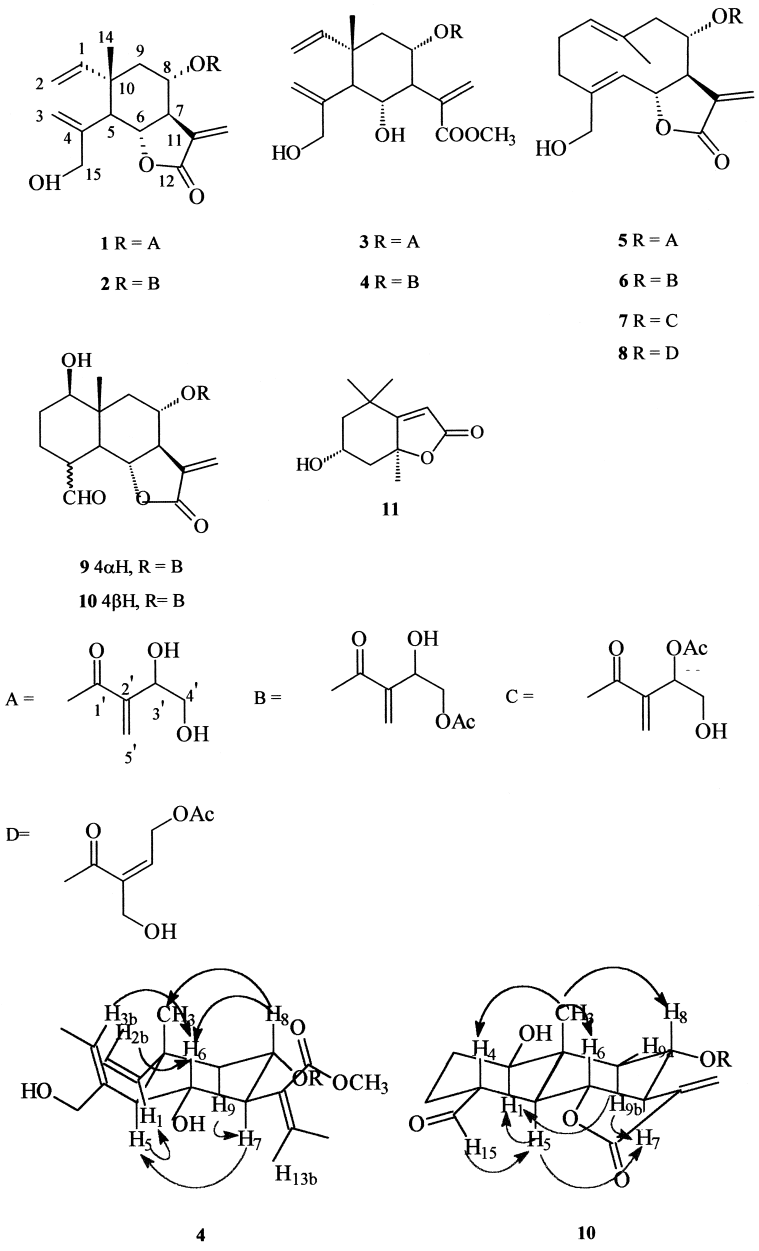

Fig. 1. Structures of the isolated compounds. 8α-(3,4-dihydroxy-2-methylenebutanoyloxy)dehydromelitensine (**1**), 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)dehydromelitensine (**2**), methyl 8α-(3,4-di-hydroxy-2-methylene-butanoyloxy)-6α,15-dihydroxyelema-1,3,11(13)-trien-12-oate (**3**), methyl 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-6α,15-dihydroxy-elema-1,3,11(13)-trien-12-oate (**4**), cnicin (**5**), 4′-acetylcnicin (**6**), 3′-acetylcnicin (**7**), 8α-(4-acetyl-2-hydroxymethyl-buten-2-oyloxy)-salonitenolide (**8**), 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-4-*epi*-sonchucarpolide (**9**), 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-sonchucarpolide (**10**), 3*S*, 5*R*-loliolide (**11**).

at C-6 and C-8 should be α-oriented. From a pair of doublets at δ 4.07 and 3.93 a hydroxymethyl group as substituent at C-4 was also evident. The identity of a 4-acetoxy-3-hydroxy-2-methylenebutanoyloxy ester side chain was deduced from the chemical shifts of its protons. This side chain sustains independently to the central skeleton as it is shown by the lack of signals in the NOESY spectrum. In addition, from the NOE experiments further information was obtained (Fig. 1). The chair conformation of the cyclohexane ring derives from the NOE signals between H-8, H-6 and 10-CH_3 and H-5 with H-1, H-7 and one of the two protons of the 15-hydroxylmethyl-group (H-15b). The α-/β-orientation of the two vinyl groups gave rise to the NOE signals of H-2b with the 14-CH_3

and H-3b with H-6. We have assigned to compound **4** the structure of the new methyl 8α-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-6α, 15-dihydroxyelema-1, 3, 11(13)-trien-12-oate.

The MS of **10** showed a molecular peak at *m/z* 436.1715 $[M]^+$ which agreed with the molecular formula $C_{22}H_{28}O_9$. Its ^1H and ^{13}C NMR spectra showed typical signals that suggested an eudesmane framework. The analysis of the NMR spectra with the aid of $^1H-^1H$ COSY, HMQC and HMBC (Table I) showed that **10** has an eudesmanolide nucleus with an 8α-acyl side chain with identical functionalisation and stereochemistry to compound **9** (Skaltsa *et al.*, 2000b) except for C-4. Due to the different orientation of the aldehyde group the following differences are observed: H-5 is shielded at δ 1.87 (vs δ 1.94 in compound **9**) giving a triplet with a coupling constant of 11.2 Hz, showing that this proton has a *trans*-diaxial disposition with H-4 and H-6. This suggests a change in the configuration of C-4: H-4 is axial, 15-CHO is equatorial and appears as a doublet slightly shielded at δ 9.69 (vs **9**, where appears as singlet at δ 9.90), due to the coupling with H-4, which is also shielded. The conformation of the decaline skeleton is confirmed by the NOESY spectrum. In the ^1H NMR spectrum a sharp singlet at δ 2.05 (3H) suggested the presence of an acetate group. For the acetyl group we assigned the position C-4′ in the basis of the low field shift of the signals of the H-4′a (δ 4.29) and H-4′b (δ 4.19) in **10**. A similar low field shift is observed for those protons in 4′-acetylcnicin (**6**) (Jakupovic *et al.*, 1986) vs cnicin (**5**) (Barrero *et al.*, 1988; Rustaiyan *et al.*, 1982). Consequently, compound **10** is the new 8α-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-sonchucarpolide.

Table I. ^1H NMR (400 MHz, $CDCl_3$, δ values) data of **4** and **10** and ^{13}C NMR (50.3 MHz, $CDCl_3$) data of **10**.

Position	**4**, δ_H, *J* (Hz)	**10**, δ_H, *J* (Hz)	**10**, δ_C
1	5.64 *dd* (10.8, 17.2)	3.42 *dd* (4.6, 11.2)	76.3
2a	4.93 *d* (10.8)	1.84 *m*	27.6
2b	4.89 *d* (17.2)	1.62 *m*	
3a	5.35 *br s*	1.98 *m*	22.6
3b	4.98 *br s*	1.53 *m*	
4	–	2.49 *tt* (4.2, 11.2)	42.6
5	2.13 *d* (10.0)	1.87 *t* (11.2)	48.2
6	4.18 *t* (10.6)	3.96 *t* (11.2)	78.6
7	2.73 *t* (10.8)	2.88 *tt* (3.0, 11.3)	52.7
8	5.43 *dt* (4.4, 11.0)	5.27 *dt* (4.6, 10.3)	69.8
9a	1.87 *dd* (4.4, 12.4)	2.56 *dd* (4.2, 13.0)	47.5
9b	1.57 *t* (12.4)	1.29 *dd* (11.2, 14.1)	
10	–	–	40.8
11	–	–	136.0
12	–	–	169.2
13a	6.27 s	6.12 *d* (2.9)	120.4
13b	5.72 *s*	5.54 *d* (2.9)	
14	1.15 *s*	1.00 *s*	14.1
15a	4.04 *d* (12.8)	9.69 *d* (4.1) (CHO)	201.8
15b	3.93 *d* (12.8)		
1′	–	–	164.6
2′	–	–	138.3
3′	4.63 *dd* (3.6, 6.6)	4.70 *dd* (3.7, 6.8)	69.7
4′a	4.18 *dd* (3.7, 11.8)	4.29 *dd* (3.7, 11.5)	67.1
4′b	4.07 *dd* (6.9, 11.9)	4.19 *dd* (6.8, 11.2)	
5′a	6.27 *br s*	6.35 *br s*	127.6
5′b	5.90 *br s*	6.03 *br s*	
CH_3O	3.76 *s*	–	
–OAc	2.04 *s*	2.05 *s*	
–OOC**C**H_3			20.8
–OO**C**CH_3			171.4

From Table II, it can be seen that **8** possessed the highest antifungal potential, while **3** is of lower activity. All compounds tested show greater antifungal potential than miconazole, a commercial fungicide, which was used as a control, except against *Cladosporium cladosporioides*. These results are in agreement with our previously reported results on sesquiterpene lactones (Skaltsa *et al.*, 2000b). Concerning the antibacterial potential, only **5** is active against all bacterial strains tested, as expected (Vanhaelen-Fastre and Vanhaelen, 1976).

The main compounds of *C. deusta* Ten. are cnicin and 4′-acetylcnicin. When its chemical profile is compared to previously studied *Centaurea* sp. belonging to the same section, it appears that their main constituents are germacranolides: *C. alba* (Geppert *et al.*, 1983; Fernández *et al.*, 1995); *C. alba* ssp. *caliacrae* (Geppert *et al.*, 1983).

Additionally, the chemistry of this taxon is characterized by the absence of guaianolides, common metabolites of other *Centaurea* species (Connolly and Hill, 1991; Fraga, 1992; 1993; 1994; 1995; 1996; 1997; 1998; 1999; 2000).

Experimental

General procedures

NMR: 400, 200 MHz (^1H) and 50.3 MHz (^{13}C). Chemical shifts are reported in δ (ppm) values. MS: Fisons VG Autospec GC 8000 (CI, CH_4); Hewlett-Packard mod. 5988A; (EI, 70 eV). FT-IR Spectrometer: Perkin-Elmer Paragon 500. Polar-

Table II. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations, nmol/ml.

Fungi	**2** MIC (MFC)	**3** MIC (MFC)	**8** MIC (MFC)	**10** MIC (MFC)	**11*** MIC (MFC)	Miconazole MIC (MFC)	Bifonazole MIC (MFC)
A. niger	2.5 (2.5)	1.2 (1.2)	0.6 (0.6)	0.6 (0.6)	7.5 (7.5)	3.0 (8.0)	0.3 (0.3)
A. ochraceus	2.5 (2.5)	1.2 (1.2)	0.6 (0.6)	0.6 (0.6)	7.5 (7.5)	3.0 (8.0)	0.3 (0.3)
A. versicolor	2.5 (2.5)	0.6 (1.2)	0.6 (1.2)	0.3 (0.6)	7.5 (7.5)	4.0 (8.0)	0.3 (0.3)
A. flavus	2.5 (2.5)	1.2 (1.2)	0.3 (0.6)	0.6 (1.2)	7.5 (7.5)	1.0 (8.0)	0.3 (0.3)
P. ochrochloron	5.0 (5.0)	1.2 (2.4)	0.6 (1.2)	1.2 (2.4)	10.0 (12.0)	2.0 (10.0)	0.45 (0.45)
P. funiculosum	1.25 (1.25)	1.2 (2.4)	0.6 (1.2)	1.2 (2.4)	10.0 (12.0)	4.0 (10.0)	0.45 (0.45)
T. viride	5.0 (7.5)	1.2 (2.4)	1.2 (2.4)	1.2 (1.2)	10.0 (15.0)	4.0 (4.0)	0.45 (0.45)
C. cladosporioides	1.25 (1.25)	0.3 (0.3)	0.15 (0.3)	0.15 (0.15)	3.75 (3.75)	0.06 (0.06)	0.2 (0.3)
A. alternata	1.25 (1.25)	0.075 (0.075)	<0.075 (0.075)	0.15 (0.15)	3.75 (3.75)	1.0 (1.0)	0.3 (0.3)

* The nos. on top denote the compounds shown in Fig. 1. All the components were tested in triplicate and MICs and MFCs were presented as mean values.

imeter: Perkin-Elmer 341. The optical rotation values were determined at 25 °C at 589 nm in $CHCl_3$ (Uvasol).

Chromatography

Vacuum liquid chromatography (VLC): silica gel (Merck; 43–63 μm), CC: silica gel (SDS; 40–63 μm), gradient elution with the solvents mixtures indicated in each case; HPLC: CE 1100 Liquid Chromatography Pump Techsil 10-C18 (250 × 10 mm).

Absorbents for TLC: Merck RP 18 F_{254}; Merck silica gel 60 F_{254}; solvents for TLC: mixt. of CH_2Cl_2 with MeOH; mixt. of cyclohexane with EtOAc. Detection on TLC-plates: UV-light, spray reagent (anisaldehyde-H_2SO_4 on silica gel).

Plant material

Aerial parts of *Centaurea deusta* were collected on Mount Pelion in June 1997 and authenticated by Dr. Th. Constantinidis (Institute of Systematic Botany, Agricultural University of Athens). A voucher specimen is deposited in the Herbarium of the Institute of Systematic Botany, University of Patras under the number Skaltsa and Lazari 107 (UPA).

Bioassays

For the bioassays nine fungi were used: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus flavus* (ATCC 9643), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839), *Trichoderma viride* (IAM 5061), *Cladosporium cladosporioides* (ATCC 13276) and *Alternaria alternata* (DSM 2006).

The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological research "Sinisa Stankovic", Belgrade, Yugoslavia.

The micromycetes were maintained on malt agar (MA) and the cultures were stored at +4 °C and subcultured once a month (Booth, 1971).

To investigate the antifungal activity of the compounds the modified microdilution technique was used (Hanel and Raether, 1988; Daouk *et al.*, 1995). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (vol/vol). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The inocula were stored at +4 °C for further use. Dilutions of the inocula

were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs), which inhibited fungal growth, were performed by a serial dilution technique using 96-well microtitre plates. Extracts of compounds investigated were dissolved in malt medium broth with fungal inoculum to achieve concentrations of 0.03–4 μg/ml. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MIC. The minimum fungicidal concentrations (MFCs), were determined by serial subcultivation of 2 μl into microtitre plates containing 100 μl of broth per well and further incubation for 72 h at 28 °C. The lowest concentration which killed the 99.5% of the original inoculum, spores and mycelium of the fungi, was defined as the MFC. Commercial fungicides, miconazole and bifonazole, were used as control (0.03–5 μg/ml). The antimicrobial activity of the compounds against the Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* BBL 12084, the Gram-negative bacteria *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853) was evaluated using the microdilution technique. Streptomycin (solution of 1 mg/ml in H_2O, kindly provided by Alkon Lab. Hellas) was used as standard form the antibacterial activity.

Extraction and chromatography

The fresh plant material (0.86 kg) was finely ground and extracted at room temperature with cyclohexane–Et_2O–MeOH (1:1:1 v/v). The extract was washed with brine, the aq. layer re-extracted with EtOAc, and the organic layer dried with Na_2SO_4 and concentrated under reduced pressure. The residue 22 g was prefractionated by VLC (Coll and Bowden, 1986) on silica gel, using cyclohexane–EtOAc–Me_2CO mixtures of increasing polarity as eluents to give several fractions. Frs C (hexane–EtOAc, 1:4), D (EtOAc), E (EtOAc–acetone, 9:1) and F (EtOAc–acetone, 4:1 v/v) were subjected to further chromatographic separations as described below.

Column chromatography of fr C (CH_2Cl_2–MeOH, 10:0 to 8:2) followed by further CC and several HPLC (MeOH–H_2O, 1:1) allowed the isolation of **2** (29.5 mg), **6** (386.0 mg), **7** (11.4 mg), **9** (9.5 mg) and **11** (7.8 mg). VLC of fr D (CH_2Cl_2–MeOH, 10:0 to 8:2) followed by further CC and several HPLC (MeOH–H_2O, 4:3, 1:1) allowed the isolation of **1** (29.5 mg), **2** (14.0 mg), **4** (3.6 mg), **5** (727.5 mg), a mixture of **5** and **9** (8.0 mg), a mixture of **5** and **1** (72.2 mg), **6** (129.1 mg), **7** (3.9 mg), **8** (3.3 mg) and **10** (7.2 mg). CC of fr E (CH_2Cl_2–MeOH, 10:0 to 7:3) followed by further CC and several HPLC (MeOH–H_2O,1:1) allowed the isolation of **5** (283.7 mg), **6** (4.6 mg) and **10** (6.0 mg). CC of fr F (CH_2Cl_2–MeOH, 10:0 to 7:3) followed by further CC and several HPLC (MeOH–H_2O, 1:1) allowed the isolation of **3** (3.8 mg) and **5** (39.2 mg).

Methyl 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-6α,15-dihydroxyelema-1,3,11(13)-trien-12-oate (**4**)

Oil; $[\alpha]_D^{20}$ +11.58 ($CHCl_3$, *c* 0.10); IR ν_{max}^{KBr} cm^{-1}: 3600–3300 (OH), 1773, 1764 (C=O, γ-lactone, ester), 1719, 1712 (C=O, acetate). CIMS *m/z* 452.2067 $[M]^+$ (19) ($C_{23}H_{32}O_9$ requires 452.2046), 420 $[M-MeOH]^+$ (5), 374 $[M-AcOH-H_2O]^+$ (8), 360 $[M-AcOH-MeOH]^+$ (6), 342 $[360-H_2O]^+$ (5), 241 (100). ^1H NMR spectral data: see Table I.

8α-(3-hydroxy-4-acetoxy-2-methylenebutanoyloxy)-sonchucarpolide (**10**)

Oil; $[\alpha]_D^{20}$ +8.59 ($CHCl_3$, c 0.09); IR ν_{max}^{KBr} cm^{-1}: 3600–3300 (OH), 1773, 1764 (C=O, γ-lactone, ester), 1719, 1712 (C=O, acetate, aldehyde). CIMS *m/z* 436.1715 $[M]^+$ (100) ($C_{22}H_{28}O_9$ requires 436.1733), 420 $[M+H-H_2O]^+$ (30), 430 $[M.CO]^+$ (14), 376 $[M-AcOH]^+$ (80), 363 $[M-Ac-H_2CO]^+$ (64), 279 $[M-\mathbf{B}]^+$ (15), 262 $[M-\mathbf{B}OH]^+$ (52). ^1H NMR and ^{13}C NMR spectral data: see Table I.

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Barrero A. F., Sanchez J. F., Rodriguez I. and Soria Sanz C. (1988), Germacranolides of *Centaurea malacitana*. Anales de Química, Serie C, **84**, 344–347.

Booth C. (1971), Fungal Culture Media. In: Methods in Microbiology, 4th ed. (J. R. Norris and D. W. Ribbons, eds). Academic Press, London, New York, pp. 49–94.

Bruno M., Fazio C., Passanati S., Paternostro M. P., Diaz J. G. and Herz W. (1994), Sesquiterpene lactones from *Centaurea sphaerocephala* ssp. *sphaerocephala*. Phytochemistry **35**, 1371–1372.

Bruno M., Fazio C., Paternostro M. P., Diaz J. G. and Herz W. (1995), Sesquiterpene lactones and other constituents of *Centaurea napifolia*. Planta Med. **61**, 374–375.

Cardona M. L., García B., Muñoz M. C., Navarro F. I. and Pedro J. R. (1997), New sesquiterpenes and other constituents from *Centaurea paui*. Liebigs Ann./ Recueil **3**, 527–532.

Coll J. C. and Bowden B. F. (1986), The application of vacuum liquid chromatography to the separation of terpene mixtures. J. Nat. Prod. **49**, 943–946.

Connolly J. D. and Hill R. A. (1991), Dictionary of Terpenoids, Vol. **1**, Chapman and Hall, London.

Daouk R. K., Dagher S. M. and Sattout E. J. (1995), Antifungal activity of the Essential oil of *Origanum syriacum* L. J. Food Prot. **58**, 1147–1149.

Fernández I., Pedro J. R. and Polo E. (1995) Sesquiterpene lactones from *Centaurea* alba and *C. conifera*. Phytochemistry **38**, 655–657.

Fraga B. M., (1992; 1993; 1994; 1995; 1996; 1997; 1998; 1999; 2000), Natural Sesquiterpenoids. Nat. Prod. Rep. **9**, 217–241 and 557–580; **10**, 397–419; **11**, 533–554; **12**, 303–320; **13**, 307–326; **14**, 145–162; **15**, 73–92; **16**, 21–38; **16**, 711–730; **17**, 483–504.

Geppert B., Drozdz B., Kielczewski M. and Holub M. (1983), Sesquiterpene lactones. XXIII. Isolation of sesquiterpene lactones from *Centaurea* L. species Acta Soc. Bot. Pol. **52**, 23–34.

Hanel H. and Raether W. (1988), A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. Mycoses **31**, 148–154.

Hodges R. and Porte A. L. (1964), The structure of loliolide. Tetrahedron **20**, 1463–1467.

Huneck S., Jakupovic J. and Schuster A. (1986), Further compounds from *Centaurea stoebe*. Planta Med. **5**, 398–399.

Jakupovic J., Jia Y., Pathack V. P., Bohlmann F. and King R. M. (1986), Bisabolene derivatives and sequiterpene lactones from *Centaurea* species. Planta Med. **5**, 399–401.

Mabberlay D. J., (1997). The Plant Book, 2nd edition. Cambridge University Press, Cambridge, U.K. p. 440.

Mabry T. J., Markham K. R. and Thomas M. B. (1970), The Systematic Identification of Flavonoids. Springer, New York.

Polo-Toribio E. (1994), Sesquiterpene lactones from *Centaurea alba* and *C. conifera*. Tesis de licenciatura, Valencia (Burjassot).

Rustaiyan A., Niknejad A. and Aynechi Y. (1982), Chemical constituents of *Centaurea brugueriana*. Planta Med. **44**, 185–187.

Skaltsa H., Lazari D., Georgiadou E., Kakavas S. and Constantinidis Th. (1999), Sesquiterpene lactones from *Centaurea* species: *C. thessala* subsp. *drakiensis* and *C. attica* subsp. *attica*. Planta Med. **65**, 393.

Skaltsa H., Lazari D., Garcia B., Pedro J., Sokovic M. and Constantinidis Th. (2000a), Sesquiterpene lactones from *Centaurea achaia*, a greek endemic species. Antifungal activity. Z. Naturforsch. **55c**, 534–539.

Skaltsa H., Lazari D., Panagouleas C., Georgiadou E., Garcia B. and Sokovic M. (2000b), Sesquiterpene lactones from *Centaurea thessala* Hausskn. ssp. *drakiensis* (Freyn & Sint.) Georg. and *Centaurea attica* Nym. ssp. *attica*. Antifungal activity. Phytochemistry **55**, 903–908.

Vanhaelen-Fastre R. and Vanhaelen M. (1976), Antibiotic and cytotoxic activity of cnicin and its hydrolysis products. Chemical structure-biological activity relation. Planta Med. **29**, 179–189.