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SESQUITERPENE LACTONES IN A POPULATION OF *BALSAMITA MAJOR* CULTIVATED IN BULGARIA

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Key Word Index—*Balsamita major*, Asteraceae; sesquiterpene lactones.

Abstract—Seven C-9β-hydroxylated or esterified germacranolides were isolated from the flowers of a *Balsamita major* population cultivated in Bulgaria: the known 9β-propionyloxy- and 9β-isobutyryloxy-costunolides, 1α,10β-epoxyhaageanolide, 9β-hydroxyartemorin, and the new esters the propionyl-, isobutyryl- and 2-methylbutyryl-1α,10β-epoxyhaageanolides. These lactones differ from the presently known eudesmanolides found in a *B. major* population cultivated in Poland, probably as result of the existence of chemotypes, frequently observed in the tribe Anthemideae.

INTRODUCTION

As part of our investigations on the sesquiterpene lactones in Bulgarian species of the Anthemideae [1, 2], we report here our findings on *Balsamita major* Desf†. This plant of Asian origin is widely grown in Europe and Asia. It is a large and up to 1 5 m high perennial plant with yellow flowers. Both flowers and leaves contain essential oils with carvone, thujone and β-cubebene as main components [4] Decoctions of leaves and flowers are used medicinally and against insects [5, 6]; this latter activity is due to the presence of pyrethrin I in the essential oil [4]. So far, only three selinane-type lactones, erivanin [7], isoerivanin and dehydroisoerevanin [8] have been reported for *B major*; they co-occur in a population from Poland.

RESULTS AND DISCUSSIONS

The material used was flowers of plants cultivated in the Botanical Garden of the Bulgarian Academy of Sciences, located near Sofia. The extraction procedure is described in the Experimental. Preparative chromatography on silica eventually yielded seven substances, six crystalline and one amorphous. All exhibited a strong IR band at 1750–1770 cm^{-1} for a γ-methylene lactone grouping proved further by ^{1}H NMR. Structure elucidation was carried out by means of ^{1}H NMR, EIMS and CIMS spectra compared with literature data.

All isolated compounds displayed several 250 MHz ^{1}H NMR signals with almost identical chemical shifts and multiplicity (Table 1), indicating their close structural relationships. Irradiation of the one-proton double doublet, located at δ4.25–4.61 ($J = J_1 = 10$ Hz) and common for all compounds (**1–7**), assigned to H-6 by its coupling with one vinylic, H-5, and one *trans* axial, H-7, protons. The two one-proton doublets assigned for H-13 and H-13′ (Table 1) confirmed the compounds as γ-methylene lactones. Irradiation of H-7 and H-9 demonstrated also the presence of two C-8 protons.

Table 1. ^{1}H NMR spectra of sesquiterpene lactones **1–7** (250 MHz, $CDCl_3$, TMS as reference; chemical shifts in δ)

H	**1, 2**	**3–6**	**7**
1	5 19 *dd*	2 85 *dd*	4 20 *br d*
2α,β	2 32 *m*	1 46–2 29 *m*	1.97–2.26 *m*
3α,β	2.32 *m*	1 46–2 29 *m*	1.97–2 26 *m*
5	4.69 *d*	5 20*, 5 24 *br d*	5 20 *br d*
6	4 57 *dd*	4 61 *dd*	4.25 *dd*
7	2 75 *m*	2 66*, 2.75† *dd*	2 76 *m*
8α	2 05 *m*	2 25 *br d*	2.77 *m*
8β		2 76 *ddd*	1.91 *ddd*
9	5 22 *dd*	3 21*, 4.39† *dd*	3 98 *br d*
13	6.31 *d*	6 30 *d*	6.22 *d*
13′	5 56 *d*	5.58*, 5 53 *d*	5 53 *d*
14	1 46 *br s*	1 15*, 1 25 *s*	5 38 *br s*
15	1 74 *br s*	1 85 *s*	1.59 *br s*

MeCH$_2$COO (in **1** and **4**): δ 1 17 (3H, *dd*, $J = J_1 = 7$), 2.40 (2H, *m*); (Me)$_2$CHCOO (in **2** and **5**) δ 1 19 and 1 20 (2Me, two *d*, $J = 6.9$), 2.61 (H-2, *m*)

MeCH$_2$CH(Me)COO (in **6**) δ 1 18 (3H, *d*, $J = 7$ 1), 0.97 (3H, *dd*, $J = J_1 = 7$), 2 40 (H, *m*)

*For compound **3**

†for compounds **4–6**

The δ values of **1**, **2**, **4–6** are on average within ±0 05 ppm The methylene envelope (δ1.40–2.80) of all compounds was partially resolved by decoupling.

J(Hz) compounds **1** and **2**, 1,2α = 10.8, 1,2β = 2.5, 5,6β = 6β,7α = 10; 7α,8β = 9 5, 7α,13 = 3 5, **3** 1β,2β = 2 5; 1β,2α = 5 6; 6β,7α = 7α,8β = 8β,9α = 10, 7α,13 = 3 5, **4–6**, 1β,2β = 2, 1β,2α = 11 1, 5,6β = 6β,7α = 7α,8β = 10 1; 8β,9α = 10.7, 7α,13 = 3 5, **7**, 1α,2β = 5 6, 6β,7α = 7α,8β = 8β,9α = 10, 7α,13 = 3 5

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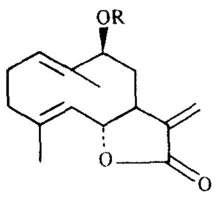
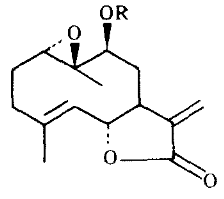
[†] Synonyms: *Chrysanthemum balsamita* (L) Baillon, non L, *Pyretrum majus* (Desf) Tzvelev [3]



1 R = COEt

2 R = COCHMe$_2$

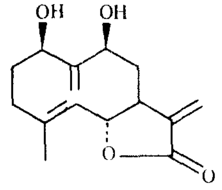


3 R = H

4 R = COEt

5 R = COCHMe$_2$

6 R = COCHMeEt



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In addition to these common features, the ^{1}H NMR spectra of (**1**–**7**) contained signals characteristic for different substituents The spectra of **1** and **2** displayed (a) a signal for one strongly deshielded carbinolic proton, H-9, coupled with the two H-8, (b) two singlets for two olefinic methyls, (c) signal for one olefinic proton, H-1, coupled with one axial and one equatorial protons at C-2 The negative D_2O exchange experiments, the loss of 74 *m/z* (C_2H_5COOH) or of 88 *m/z* (C_3H_7COOH) from $[M]^+$ of **1**, (*m/z* 304, $C_{18}H_{24}O_4$) or of **2** (*m/z* 318, $C_{19}H_{24}O_4$) respectively, and the deshielded H-9 carbinolic proton, indicated **1** and **2** as C-9 esters While **1** was clearly a C-9 propionate, the ^{1}H NMR signals for an isopropylic group indicated **2** as a C-9 isobutyrate These data coincided well with those of the literature [8, 9] and identified (**1**) as the known 9β-propionyloxycostunolide, isolated from *Inula royaleana* [9], and **2** as the 9β-isobutyryloxycostunolide, found in *Inula helenium* [10]

The structures of **3**–**6** were elucidated in a similar way Instead of the characteristic ^{1}H NMR signal of the vinylic proton H-1 in **1** and **2** (Table 1), the compounds **3**–**6** showed a double doublet at δ 2 85 ± 0 05 for one proton coupled with a methylene group The C-10 carbon bore a less deshielded tertiary methyl than that in **1** and **2** (δ 1 20 ± 0.05). The $[M]^+$ of (**3**) corresponded to $C_{15}H_{20}O_4$, but D_2O exchanged only one hydroxylic proton Hence, one oxygen in (**3**), and also in (**4**–**6**), formed a C-1 (10)- epoxide ring. In the ^{1}H NMR spectra of **4**–**6**, the carbinolic proton at the hydroxyl bearing C-9 displayed one deshielded double doublet at δ 4 39 ± 0 05/δ 3 21 in **3** Their spectra also showed signals for protons in propionyl-, isobutyryl- and 2-methylbutyryl-acyls, forming the relevant C-9 esters. From these data, (**3**) is identified as the known 1α,10β-epoxyhaageanolide, found in *Inula heterolepis* [9], while **4**–**6** appear to be the new C-9 esters of **3**, i e the 9β-propionyloxy-, 9β-isobutyryloxy- and 9β-(2-methyl)-butyryloxy-, 1α,10β-epoxyhaageanolide respectively The chemical shifts and the coupling constants of the protons in **4**–**6** coincided well with those of the parent compound (**3**) and showed that they shared a common stereochemistry

Instead of the C-10 methyl in compounds **1**–**6**, compound **7**, according to its ^{1}H NMR, contained one C-10 exomethylene group Two secondary hydroxyls in **7** were established by D_2O exchange and by the two broad doublets at δ 3 98 and δ 4.20 of two carbinolic protons The $[M+1]^+$ signal in CIMS, corresponding to $[C_{15}H_{20}O_4+H]^+$, split off two molecules of water and one of carbon monoxide This, and the ^{1}H NMR data in Table 1 identified **7** as the known 9β-hydroxyartemorin, isolated earlier from *Inula heterolepis* and considered as a possible artefact of **3**, both having been found together in the same *Inula* species [9]

As usual, the intensive signals with higher *m/z* in the CIMS (probe with isobutane) were more informative for the type of substituents in compounds **1**–**7** than the EIMS The CI spectra displayed also some peculiarities, for example as: (a) the esters with isobutyric, (**2**, **5**), acids formed $[M]^+$ (100%) ions, (b) those with propionic (**1** and **4**), and with 2-methylbutyric (**6**) acids, as well as the alcohols (**3** and **7**) displayed $[M+1]^+$ [100%, 37% for (**7**)] ions, (c) certain differences in the intensity of the signals, produced by splitting of the acyl and/or water from $[M]^+$ or $[M+1]^+$ were also observed (Experimental) This could help in the identification and structure elucidation of similarly substituted lactones

As a contribution to the phytochemistry of *Balsamita major* Desf, one other finding of our work is that the Bulgarian population contained only C-9β-hydroxylated germacranolides and their esters (7 compounds in total) In the chrysanthemum complex, only a few *trans*, *trans*-1(10),4(5)-germacradienolides have been found so far, and none with a C-9 hydroxyl [11] The lactones, known until now for *Balsamita major* Desf, are only of the eudesmane type [1, 2] and probably are specific for the Polish population Hence, this plant appears to occur in nature as more than one chemotype

EXPERIMENTAL

A voucher specimen of the plant has been deposited at the herbarium of the Institute of Botany, Bulgarian Academy of Sciences (c o Dr L Evstatieva), Sofia Mps uncorr IR in KBr pellets or in discs [comp (**7**)] ^{1}H NMR (Table 1) were recorded at WM 250 MHz in $CDCl_3$ and TMS as ref MS by direct inlet, EI at 70 eV, CI with *iso*-butane at 200 eV The air-dried and coarsely ground flowers (300 g) were extracted with 3 × 500 ml $CHCl_3$, evapd to dryness *in vacuo*, the residue stirred with 250 ml of 50% aq EtOH and defatted with petrol Precipitation of the aq EtOH part with $Pb(OAc)_2$ in EtOH, filtration, removal of the EtOH from the filtrate *in vacuo* and re-extraction of the H_2O residue with $CHCl_3$ yielded the crude lactone fraction (15 2 g) This material (4 0 g) was sepd on a prep silica column by elution with $CHCl_3$–Et_2O mixtures The lactone fractions (IR monitoring) were further purified by prep TLC and recrystallization from Et_2O

9β-Propionyloxy costunolide (**1**) Colourless crystals, mp 143–145° (colourless oil acc [8]), ν_{max}^{KBr} cm^{-1} 1760, 1720, 1660, 960, MS *m/z*, (rel int , %) EI 304 $[M]^+$ (5), $C_{18}H_{24}O_4$, 248 (3), 230 $[M-C_2H_5COOH]^+$, (39), 57 $[C_2H_5CO]^+$ (100), CI – 305

$[M+H]^+$ (100), 231 $[M+H-C_2H_5COOH]^+$ (70), 213, $[231-H_2O]^+$ (8).

9β-Isobutyryloxy costunolide (**2**). Colourless crystals, 152–154°; ν_{max}^{KBr} cm^{-1} 1760, 1720, 1660, 1160, 1140, 960; MS· *m/z*, (%), EI 318 $[M]^+$ (6), $C_{19}H_{26}O_4$, 230 $[M-C_3H_7COOH]^+$ (30); CI· 318 $[M]^+$ (100), 231 $[M-C_3H_7COO]^+$ (60), 213 $[231-H_2O]^+$ (3).

1α,10β-Epoxyhaageanolide (**3**). Colourless crystals, mp 136–139° (a gum acc. [9]), ν_{max}^{KBr} cm^{-1} 3400, 1760, 1660, 1260, 1140, 950, 750, MS· *m/z*, (%), EI 246 $[M-H_2O]^+$ (1), 228 $[M-2H_2O]^+$ (2), 149 (52), 81 (100); CI; 265 $[M+H]^+$ (100), 247 $[M+H-H_2O]^+$ (25), 229 $[M+H-2H_2O]^+$ (10), 219 $[247-CO]^+$ (3), 201 $[229-CO]^+$ (1)

*9β-Propionyloxy-*1α,*10β-epoxyhaageanolide* (**4**). Colourless crystals, mp 180–183°; ν_{max}^{KBr} cm^{-1} 1750, 1720, 1660, 1270, 1180, 1140, 970; MS. *m/z* (%), EI 320 $[M]^+$ (1) $C_{18}H_{24}O_5$, 292 $[M-CO]^+$ (0.5), 264 $[M-56]^+$ (1 5), 246 $[M-C_2H_5COOH]^+$ (2), 228 $[246-H_2O]^+$ (1 5), 57 $[C_2H_5CO]^+$ (100), CI. 321 $[M+H]^+$ (100), 303 $[M+H-H_2O]^+$ (10), 247 $[M+H-C_2H_5COOH]^+$ (40), 229 $[M+H-H_2O-C_2H_5COOH]^+$ (30)

*9β-Isobutyryloxy-*1α,*10β-epoxyhaageanolide* (**5**) Colourless crystals, mp 190–192° ν_{max}^{KBr} cm^{-1}. 1760, 1720, 1670, 1280, 1220, 1150, 960; MS, *m/z*, (%), EI 334 $[M]^+$ (2.5) $C_{19}H_{26}O_5$, 306 $[M-CO]^+$ (0.5), 264 (3), 246 $[M-C_3H_7COOH]^+$ (6); CI· 334 $[M]^+$ (100), 316 $[M-H_2O]^+$ (45), 247 $[M-C_3H_7COO]^+$ (50), 229 $[M-H_2O-C_3H_7COO]^+$ (38).

9β-(2-*Methyl*)*-butyryloxy-*1,10*-epoxyhaageanolide* (**6**). Colourless crystals, mp 127–129°, ν_{max}^{KBr} cm^{-1}· 1760, 1720, 1660, 1280, 1210, 1145, 960, MS: *m/z*, (%), EI: 348 $[M]^+$ (2 5), $C_{20}H_{28}O_5$, 320 $[M-CO]^+$ (1), 291 $[M-C_4H_9]^+$ (1 5), 264 $[M-84]^+$ (2), 246 $[M-C_4H_9COOH]^+$ (1 5), 85 (100), CI 349 $[M+H]^+$ (100), 331 $[M+1-H_2O]^+$ (3), 247 $[M+H-C_4H_9COO]^+$ (18), 229 $[M+H-H_2O-C_4H_9COO]^+$ (10).

9β-Hydroxyartemorin (**7**). Oil as one TLC spot, partially crystallized after time. ν_{max}^{layer} cm^{-1} 1760, 1670, 965, 262; MS: *m/z*, (%), EI. 246 $[M-H_2O]^+$ (15) $C_{15}H_{18}O_4$, 228 $[M-2H_2O]^+$ (18), 203 (22), 83 (90), 43 (100); CI: 265 $[M+H]^+$ (37), 247 $[M+1-H_2O]^+$ (15), 185 (8), 169 (15), 151 (27), 73 (100).

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