



Highly oxygenated guaianolides from *Anthemis cretica* subsp. *cretica*

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Abstract

Two new guaianolides, i.e. anthemolide B and 8-*O*-angeloyl-9-*O*-acetylanthemolide B, in addition to 12 known closely related guaianolides were identified in the aerial parts of the flowering plant *Anthemis cretica* subsp. *cretica*. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Anthemis cretica* subsp. *cretica*; Asteraceae; Aerial parts; Sesquiterpene lactones; Guaianolides; Anthemolide B; 8-*O*-Angeloyl-9-*O*-acetylanthemolide B

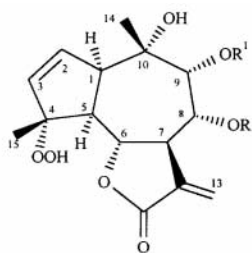
1. Introduction

Genus *Anthemis* L. (family Asteraceae, tribe Anthemidae), comprising ca 130 species, is a floral element of the Mediterranean (Gajic, 1975; Tutin, Heywood, Burges, Moore, Valentine, Walters et al., 1976; Heywood & Humphries, 1978), but some species are also found in southwest Asia and South Africa (Heywood & Humphries, 1978). In Serbia nine species are known (Gajic, 1975). Among the sesquiterpene lactones, isolated from this genus, only three types, i.e. germacranolides (and *seco*-germacranolides) (Bohlmann & Zdero, 1975; Benesova, Herout, & Sorm, 1964; Benesova, Samek, Herout, & Sorm, 1970; Holub & Samek, 1977; Samek, Holub, Grabarczyk, Drozd, & Herout, 1977; Mayer & Ruecker, 1987; El-Alfy, Shehata, Koheil, & El-Dahmy, 1989; Ruecker, Mayer, & Lee, 1989; Abou El-Ela, Jakupovic, Bohlmann, Ahmed, Seif El-Din, Khafagi et al., 1990; Sarg, El-Dahmy, & Salem, 1990; Bruno, Diaz, & Herz, 1991) eudesmanolides (De Pascual Teresa, Caballero, Anaya, Caballero, & Gonzalez, 1986; De Pascual

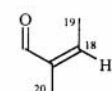
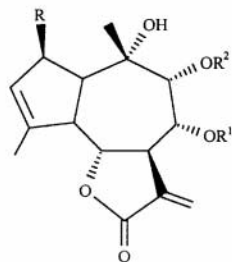
Teresa, Anaya, Caballero, & Caballero, 1988) and guaianolides (Holub, Budešinsky, Samek, Drozd, Grabarczyk, Ulubelen et al., 1982; El-Alfy et al., 1989; Ruecker, Mayer, & Lee, 1989; Di Benedetto, Menichini, Gacs-Baitz, & Delle Monache, 1991; Bruno, Bondi, Vassalo, Gedris, & Hertz, 1997; Bulatovic, Vajs, Macura, Juranic, & Milosavljevic, 1997) have been reported so far. The exception was an allergenic lactone with unusual structure (anthecotulolide), a constituent of *A. cotula* (Bohlmann, Zdero, & Grenz, 1969; Baruah, Bohlmann, & King, 1985).

Continuing our phytochemical examinations of the wild-growing Yugoslavian *Anthemis* species and our search for new compounds of pharmacologic interest, we now report the investigation of the aerial parts of *Anthemis cretica* L. subsp. *cretica* (syn. *A. orientalis* subsp. *montana* Hayek, *A. montana* L.) (Gajic, 1975; Tutin et al., 1976) originating from the northern part of Sar-planina (Sara mountain, positioned between Serbia and Macedonia). This species, usually occurs on high, grassy and rocky terrain of the south Balkan Peninsula (Gajic, 1975) and has not been studied previously. Studies of the aerial parts of *A. carpatica* collected from a different location on the same mountain revealed twelve new highly oxygenated guaianolides,

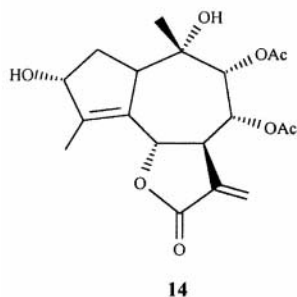
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	R	R ¹
1:	H	H
2:	Ac	H
3:	Ac	Ac
4:	i-But	Ac
5:	Ang =	Ac

	R	R ¹	R ²
6:	H	H	Ac
7:	H	Ac	H
8:	OAc	i-But	Ac
9:	OAc	H	Ac
10:	OAc	Ac	Ac
11:	OAc	H	i-But
12:	OAc	H	i-Val
13:	OAc	H	2-MeBut



six of them containing a hydroperoxy function (Bulatovic et al., 1997).

2. Results and discussion

The usual extraction procedure for isolation of sesquiterpene lactones (Bohlmann, Zdero, King, & Robinson, 1984), was as previously (Bulatovic et al., 1997), followed by silica gel CC, combined (in some cases) with preparative TLC, afforded sesquiterpene lactones **1–14**. According to spectral data all of these compounds possessed the same type of (10 α -hydroxyguaia-1,2,6-trien-12,6 α -olide) skeleton, oxygenated at 8 α - and 9 α -positions. All lactones exhibited a 11(13)-double bond, as assigned from IR (≤ 1750 cm⁻¹, typical for α,β -unsaturated lactone) and ¹H NMR ($\delta > 5.5$, 2 \times d or dd, H-13 and H-13') data. The remaining double bond was positioned in the five-membered ring, i.e. Δ^2 (**1–5**), Δ^3 (**6–13**) or Δ^4 (**14**). This was similar to the situation observed in compounds isolated from *A. carpatica*, which also contained the same three types of guaia-1,2,6-trienolides. Moreover, 8-*O*-acetylanthemolide B

(**2**), 8,9-di-*O*-acetylanthemolide B (**3**), 8-*O*-isobutyryl-9-*O*-acetylanthemolide B (**4**), a mixture of 9 α -acetylcumambrin-B (**6**) + 9 α -hydroxycumambrin-A (**7**) and anthemolide D (**14**), were identified in *A. carpatica*, as well (Bulatovic et al., 1997). Hitherto, in addition to *A. carpatica* (Bulatovic et al., 1997), only two species of the genus were reported to contain the hydroperoxy lactones: *A. nobilis* (Roman Camomile) whose ethanol extract of the blossoms yielded 1 β -hydroperoxyisobibin (a germacranolide) (Mayer & Ruecker, 1987; Ruecker et al., 1989) and 4 α -hydroperoxyromanolide (a guaianolide structurally close to **2–5**), and *A. aetnensis* (Bruno, Bondi, Vassalo, Gedris, & Herz, 1997) whose aerial parts contained, in addition to several guaia-3,11(13)-dienolides (vide infra), a germacranolide with 1 β -hydroperoxy function.

Among the Δ^3 -lactones (**8–13**), bearing the 2 β -acetoxy function, hydruntinolide B (**10**) was reported previously as a constituent of *A. hydruntina* (species endemic to southern Italy) (Di Benedetto et al., 1991). This lactone, together with the related guaianolides with the hydruntinolide substitution pattern, i.e. **8**, **9** and a mixture **11** + **12** + **13** (also observed in our

extract) have been isolated recently from the related *A. aetnensis* (an endemic species growing in volcanic debris on the upper slopes of Mount Etna) (Bruno et al., 1997).

New lactones **1** (C₁₅H₂₀O₇) and **5** (C₂₂H₂₈O₉), exhibiting a positive specific peroxide (red) colored TLC test with *N,N*-dimethyl-*p*-phenylenediammonium dichloride (Knappe & Peteri, 1962), contained a hydroperoxy function. The presence of the OOH group in **5** was also confirmed by abundant [M + H–O]⁺ and [M + H–H₂O₂]⁺ fragments in DCI-MS, typical of organic hydroperoxides (Schwarz & Schiebel, 1983) and a low-field (OOH) NMR signal (Table 1). Although guaianolide **5** was eluted as an inseparable (ca 1:1) mixture with **4**, the comparison of the NMR spectrum of this mixture to that of pure compound **4** enabled assignment of the majority of the ¹H NMR signals (Table 1) essential for structure determination. Lactones **1** and **5** were identified as anthemolide B and 8-*O*-angeloyl-9-*O*-acetyl-anthemolide B, respectively, by similarity of their ¹H NMR data to those of the co-occurring lactones **2–4** (Bulatovic et al., 1997). Chemical shifts and coupling pattern of H-8 and H-9 in **1** and **5** (Table 1) indicated 8 α ,9 α -dihydroxy and 8 α ,9 α -diacyloxy patterns, respectively. The 8 α -hydroxy substitution in **1** was corroborated by the observed (well known) paramagnetic shift and the increase of geminal coupling *J*_{13,13'} upon transformation 8 α -OAcyl to 8 α -OH (Yoshioka, Mabry, Irwin, Geissman, & Samek, 1971). Whereas in the corresponding 8 α -acyloxy lactones the resonance of H-13' was detected at $\delta < 5.7$ (*J*_{13,13'} < 0.9 Hz), in compound **1** the signal of H-13' was at lower field, i.e. δ 6.28, with concomitant increase of the geminal coupling to 1.5 Hz. The nature of the ester side chains in **5** was evident from the ¹H NMR signals characteristic of acetate and angelate (Table 1). A NOE between H-13' and H-20 of the angelate residue observed in NOESY (of the mixture **4** + **5**) clearly indicated the 8 α -angeloyloxy-9 α -acetoxy pattern in **5**. This was supported by a downfield shift of H-8 ($\Delta\delta$ ca 0.12 ppm) and an upfield shift of H-13' ($\Delta\delta$ ca –0.13 ppm) in **5** on comparison to the chemical shifts of the same protons in **4** (Table 1). A 4 α -configuration of the OOH group in **1** (same as in **2–4**) was assigned on the basis of the similarity of chemical shift of H-5 (δ 2.86), to that of the same proton in the structurally related 4 α -hydroperoxy- Δ^2 -guaianolides (Zdero, Bohlmann, & Mueller, 1987). In the ¹H NMR spectra of **4** + **5**, measured in various solvents, such as (CD₃)₂CO (Table 1), C₅D₅N and CDCl₃ + CD₃OD (1:1), the signals of H-5 from **4** and **5** were almost superimposed, thus indicating the same relative configuration at C-4, that is 4 α -OOH. Chemical shifts (in all three NMR solvents used) of protons sensitive to the configuration at C-10, such as H-14, H-6 and H-7 (Sosa et al., 1989) in **5** were similar to those in **4**

Table 1
¹H 300 MHz NMR data of compounds **1**, **4** and **5** (δ , mult., *J*, Hz)

H	1 (CDCl ₃ + CD ₃ OD, 1:1)	4 ((CD ₃) ₂ CO)	5 ^a ((CD ₃) ₂ CO)
1	3.68 ddd (10.3, 2.6, 2.0)	3.72 ddd (10.2, 2.1, 2.5)	$\geq 3.75^b$
2	6.03 dd (5.9, 2.6)	5.96 dd (5.9, 2.5)	ca 5.95 ^b
3	5.89 dd (5.9, 2.0)	5.87 dd (5.9, 2.1)	ca 5.85 ^b
5	2.86 dd (11.7, 10.3)	2.97 dd (11.9, 10.2)	2.99 dd (11.8, 10.4)
6	4.35 dd (11.7, 9.6)	4.77 dd (11.9, 9.3)	4.80 dd (11.8, 9.4)
7	3.30 m	3.57 m	$\geq 3.60^b$
8	3.90 dd (10.3, 2.7)	5.35 ^c	5.47 dd (11.0, 2.7)
9	3.74 d (2.7)	5.35 ^c	5.38 d (2.7)
13'	6.28 dd (3.2, 1.5)	5.85 dd (ca 3, <1)	5.72 dd (3.0, ≤ 0.8)
13	6.40 dd (2.9, 1.5)	6.15 dd (3.4, 0.9)	6.11 dd (3.4, ≤ 0.8)
14	1.07 s	1.26 s	1.29 s
15	1.41 s	1.41 s	1.43 s
OAc		2.15 s	2.15 s
OBu		2.59, 1H sep (7.0); 1.15, 6H d (7.0)	
OAng			1.87 qui 3H (ca 1.5), H-20; 2.00 dq 3H (7.3, ca 1.5), H-19; 7.25 1H qq (7.3, ca 1.5), H-18.
OOH	d	10.29 s	10.30 s

^aMeasured in the spectrum of ca 1:1 mixture of **4** + **5**.

^bPartially overlapped with the signal of the same proton from **4**.

^cOverlapped signals; resolved in C₅D₅N: d 5.71, dd, 11.0, 2.6 (H-8) and d 5.98, d, 2.6 (H-9) (Bulatovic et al., 1997).

^dNot visible due to exchange with deuterium from CD₃OD.

(Table 1). This agreed with the same relative configuration at C-10 (i.e. 10α -hydroxy, 10β -methyl). The same type of evidence, i.e. the similarity of the chemical shifts of the above groups in **1** to those in the closely related 10α -hydroxyguaianolides (Zdero et al., 1987; Bulatovic et al., 1997) led to the analogous configuration at C-10. The NOESY correlations in **5**, such as H-14/H-6, H-14/H-8, H-14/H-9 and H-15/H-6, H-1/H-5 and H-1/H-7 were in agreement with the above stereochemical proposal.

3. Experimental

3.1. General

CC: silica gel 60 (Merck), 0.063–0.200 mm; TLC: Kieselgel 60 GF₂₅₄, layer thickness 0.25 mm; IR: transparent dry films; ¹H NMR: various deuterated solvents (see Table 1), at 300 MHz relative to TMS at $\delta = 0.00$; NOESY measured as usual (Macura & Ernst, 1980) with mixing time of 1 s, repetition rate 1.5 s, 128 scans per evolution domain point and digitalization of 512 data points for both domains (acq. time 57 ms); DCI-MS double focusing (BE geometry); ESI-MS double focusing (EB geometry) + electro spray interface; Elemental analyses: a standard (Pregl) combustion method.

3.2. Plant material

The plant material was collected during the flowering season (July 1994) at location Osljak (Sara mountain, altitude of ca 2200 m). Voucher specimen (No. 220794AM) was deposited in the herbarium of The Institute for Medicinal Plant Research 'Dr. Josif Pancic', Belgrade.

3.3. Extraction procedure

A crude extract (19.5 g) of air-dried aerial parts (850 g) was obtained by extraction with freshly distilled solvents: Et₂O (peroxides free)–petrol–MeOH (1:1:1) at room temperature (24 h), followed by treatment with MeOH to remove long chain saturated hydrocarbons, using the usual procedure (Bohlmann et al., 1984).

3.4. Isolation procedure

A solution of the crude extract (19.5 g) in CHCl₃ (150 ml) was added to 100 g of silica gel, and dried on a rotavap. The adsorbed mixture was applied to a silica gel column and the elution was started with petrol. The polarity of the solvent was gradually increased by addition of Et₂O.

Fraction A (616 mg) eluted with petrol–Et₂O (1:1), after the repeated silica gel CC (CH₂Cl₂–MeOH, 95:5), followed by CC (C₆H₆–Et₂O–MeOH, 7:2:1) and crystallization (CHCl₃ + MeOH) afforded the 1:1 mixture (22 mg) of lactones **4** and **5**.

Fraction B (274 mg), eluted with petrol–Et₂O (3:7), yielded by silica gel CC (C₆H₆–Et₂O–MeOH, 7:2:1) fractions I and II. Fraction I, by preparative TLC (CH₂Cl₂–MeOH, 97:3, two developments) afforded lactone **10** (3 mg). Lactone **4** (2 mg) was isolated from fraction II by preparative TLC (same conditions as above).

Fraction C (422 mg) eluted with Et₂O yielded, by silica gel CC (C₆H₆–Et₂O–MeOH, 7:2:1) lactones **3** (18 mg) and **14** (8.5 mg).

Fraction D (424 mg), eluted with Et₂O (after C) yielded, upon crystallization (MeOH) lactone **2** (4 mg). The rest of the fraction was subjected to CC (CH₂Cl₂–MeOH, 95:5) to yield lactone **9** (3.5 mg) and a fraction which, after preparative TLC (CHCl₃–MeOH, 10:1, two developments) afforded two mixtures of the closely related lactones. One of them (7 mg), as in the previously studied *A. carpatica* (Bulatovic et al., 1997), contained **6** + **7** (in the ratio of ca 7.5:2.5, respectively). The second mixture (2 mg), similarly to *A. aetnensis* (Bruno et al., 1997), consisted of three Δ^3 -lactones, i.e. **12** (the major constituent), **13** and **14** (in the ratio of ca 3:1:1, respectively).

Fraction E (162 mg) eluted with Et₂O (after D), upon two CCs (C₆H₆–Et₂O–MeOH, 7:2:1 and CH₂Cl₂–MeOH, 95:5) yielded lactone **8** (11.5 mg).

Lactone **1** (5 mg) was obtained from fraction F (23.5 mg), eluted with Et₂O–MeOH (95:5), upon repeated CC (CH₂Cl₂–MeOH, 95:5), followed by preparative TLC (CH₂Cl₂–MeOH, 9:1, two developments).

Anthemolide B (**1**): colorless solid (Found: C, 57.5; H, 6.5. C₁₅H₂₀O₇ requires: C 57.7; H 6.5%); IR ν_{\max} cm^{−1}: 3376 (OH, OOH), 1751 (C=O, α,β -unsat. γ -lactone), 1654 (C=C), 1374, 1274, 1025; ¹H NMR (see Table 1); ESI-MS (in MeOH–AcOH, 100:1) *m/z* (rel. int): 335.0 [M + Na]⁺ (100), 351.1 [M + K]⁺ (33.5).

8-*O*-isobutyryl-9-*O*-acetylanthemolide B (**4**): for the ¹H [(CD₃)₂CO] NMR data see Table 1; all other spectroscopic data as reported (Bulatovic et al., 1997).

8-*O*-Angeloyl-9-*O*-acetylanthemolide B (**5**) + **4** (1:1): colorless solid; IR ν_{\max} cm^{−1}: 3424 (OH, OOH), 1746 (C=O, α,β -unsat. γ -lactone, OAc), 1658 (C=C), 1370, 1234, 1147, 1060; ¹H NMR (see Table 1); DCI-MS (isobutane probe), 150 eV, *m/z* (rel. int): 437 [M + H]⁺ (44), 421 [M + H-16]⁺ (84.5), 419 [M + H-18]⁺ (21.5), 403 [M + H-34]⁺ (100), 385 [M + H-34-18]⁺ (50), 325 [M + H-18-34-60]⁺ (32), ions of the co-occurring **4** (Bulatovic et al., 1997) also observed.

The remaining lactones (**2**, **3**, **6**, **7**) (Bulatovic et al., 1997), (**8**, **9**, **11**, **12**, **13**) (Bruno et al., 1997) and (**10**) (Di Benedetto et al., 1991) were identified by comparison of spectroscopic data to those reported.

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