PERGAMON

Phytochemistry 54 (2000) 625–633

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Further sesquiterpene lactones from *Anthemis carpatica*

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Received 16 December 1999; received in revised form 27 April 2000

Abstract

A new germacranolide, (*E*)-1α,10β-epoxy-3β-acetoxy-6α-hydroxygermacra-4,11(13)-dien-12,8α-olide, together with nine new highly oxygenated guaiadien-12,6α-olides of anthemolide, and cumambrin type were identified in the repeated examination of the aerial parts of the flowering *Anthemis carpatica*. In addition, six known guaianolides belonging to the same groups, also isolated previously from *A. carpatica*, along with two guaianolides, 2β-hydroxyepiligustrin and cumambrin B, not found before in this species, were isolated this time.

Keywords: *Anthemis carpatica*; Asteraceae; Sesquiterpene lactones; Guaidienolides; Anthemolides; 9α-Oxygenated cumambrins; (*E*)-1α,10β-Epoxy-3β-acetoxy-6α-hydroxygermacra-4,11(13)-dien-12,8α-olide; 3β-Acetoxydeacetyltulirinol

1. Introduction

In our previous phytochemical study of the aerial parts of *Anthemis carpatica* and *A. cretica* subsp. *cretica* (Bulatović et al., 1997; Bulatović, 1998; Vajs et al., 1999), several highly oxygenated guaiadien-12,6α-olides (anthemolides A–E and 9α-oxygenated cumambrins) have been isolated. All of them exhibited 11(13)-double bond, 9α-OH (or OAcyl) and 10α-OH functionalities. The remaining double bond was positioned in a five-membered ring at 2-, 3- or 4-position. The majority of these lactones also contained 8α-hydroxyl (or OAcyl) group, and many of them a hydroperoxy function in the five-membered ring. Some of the guaia-3,11(13)-dienolides found in *A. cretica* subsp. *cretica* were also isolated previously from *A. hydruntina* (di Benedetto et al., 1991) and recently from *A. aetnensis* (Bruno et al., 1997).

Due to a possible phytochemical and pharmacological importance of such highly oxygenated natural products, as demonstrated in numerous examples reviewed by Casteel (1992, 1999), we have repeated the investigation of the aerial parts of *A. carpatica* Willd. (collected at the same locality as before, see Section 3).

2. Results and discussion

Using the same extraction procedure as before (Bohlmann et al., 1984), in combination with silica gel CC and preparative TLC, 17 guaianolides (**1–17**) and a germacranolide **18** have been isolated. Five of them, i.e. anthemolide A (**1**), 8-*O*-isobutyryl-9-*O*-acetylanthemolide B (**5**), 9α-acetoxycumambrin A (**9**), 2α-hydroperoxy-8-*O*-isobutyryl-9α-acetoxycumambrin B (**11**) and anthemolide C (**12**) were also isolated in our first attempt from *A. carpatica* (Bulatović et al., 1997; Bulatović, 1998). Lactone **5**, together with anthemolide B (**4**), was reported as the constituent of *A. cretica* subsp. *cretica* as well (Bulatović, 1998; Vajs et al.

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00160-6

1999). Among the known guaianolides isolated for the first time from *A. carpatica* were cumambrin-B (**7**) (Yoshioka et al., 1973) and 2β-hydroxyepiligustrin (**8**) (Todorova and Krasteva, 1996).

The ^{1}H-NMR spectra of new guaianolides, assigned by comparison to those of the known closely related compounds (**1, 2, 8**) and in some cases using 2D-NMR methods, such as double quantum filtered (DQF) COSY, phase sensitive (PS) NOESY, direct and long-range C,H-HETCOR (HSQC and HMBC, respectively), are listed in Tables 1–5.

All hydroperoxy lactones (**3–6, 11, 17**) exhibited a positive specific peroxide (red) colored TLC test with *N*,*N*-dimethyl-*p*-phenyllenediammonium dichloride (Knappe and Peteri, 1962), and a low-field ^{1}H-NMR signal typical of the OOH group (Tables 1 and 4).

According to the ^{1}H-NMR data (Table 1), lactone **2** ($C_{21}H_{28}O_8$) contained acetate and isobutyrate ester groups. A part of its ^{1}H-NMR spectrum comprising signals of protons from the ester side chains, seven-membered lactone rings (i.e. H-6, H-7, H-8, H-9, H-13, H-13′, H-14) were almost identical to that of 4α-hydroperoxy-Δ^2-lactone (**5**), containing one more oxygen, whose structure and stereochemistry was identified previously by extensive 2D-NMR study (Bulatović et al., 1997; Milosavljević et al., 1998) (see Table 1). This was in accordance with identical 8α-isobutyryloxy-9α-acetoxy-10α-hydroxy arrangement in **2**. The major differences between the ^{1}H-NMR spectra of **2** and **5** were those concerning protons from the five-membered ring, especially H-5, exhibiting upfield shift in **2** ($\Delta\delta$ 0.26 ppm) in comparison to the same proton in **5**. This, according to the literature data (Zdero et al., 1987), indicated 4α-OH substitution in **2** (instead of 4α-OOH in **5**).

The ^{1}H-NMR data of **3** ($C_{17}H_{22}O_7$) were similar to those of **1**, the only Δ^2-guaianolide lacking 8-oxygen functionality, also isolated previously from *A. carpatica* (Bulatović et al., 1997). The major difference between the spectra of these compounds was a paramagnetic shift of H-5 ($\Delta\delta$ 0.20 ppm) in **3** in comparison to that in **1** (containing 4α-hydroxyl group), indicating 4α-positioned OOH group in **3** (Zdero et al., 1987), same as in all other Δ^2-hydroperoxy guaianolides originating from the same source. At the same time, the similarity of chemical shifts and couplings of the remaining groups in **1** and **3** were in agreement with the same (usual) relative stereochemistry in these molecules.

The overall appearance of the ^{1}H-NMR spectrum of lactone **6** ($C_{22}H_{28}O_9$) was rather close to the spectra of 8,9-diesters of anthemolide B (e.g. **5**) (Bulatović et al., 1997; Vajs et al., 1999). The nature of the ester side chains in **6** was evident from the ^{1}H-NMR spectrum containing signals typical of the acetate and tiglate (Joseph-Nathan et al., 1984) (Table 1, footnote a). The couplings of H-8 and H-9 (measured in C_5D_5N), together with NOEs of these protons, such as H-6/H-8 and H-6/H-9, observed in PS NOESY, fully accorded with the 8α,9α-diacyloxy pattern, same as that in all guaianolides isolated from this (Bulatović et al., 1997) and the related species (Vajs et al., 1999; di Benedetto et al., 1991; Bruno et al., 1997) so far. The NOEs between H-13′ and the tigloyl protons (H-3′ and H-5′)

Table 1
^{1}H 300 MHz ($CDCl_3$) NMR data of compounds **2**, **3**, **5**, and **6** (δ, multiplicity, *J*, Hz)

H	**2**[a]	**3**[b]	**5**[ac]	**6**[a]
1	3.62 *br d* (10.4)	3.63 *ddd* (10.2, 2.6, 2.2)	3.62	3.64 *ddd* (10.0, 2.6, 2.0)
2	5.86 *s*	6.00 *dd* (5.9, 2.6)	6.03	6.04 *dd* (5.8, 2.6)
3	5.86 *s*	5.91 *dd* (5.9, 2.2)	5.92	5.93 *dd* (5.8, 2.0)
5	2.74 *dd* (11.8, 10.4)	2.93 *dd* (11.4, 10.2)	3.00	3.02 *dd* (11.7, 10.0)
6	4.40 *dd* (11.8, 9.2)	4.31 *dd* (11.4, 9.4)	4.37	4.40 *dd* (11.7, 9.6)
7	3.49 *dddd* (10.8, 9.2, 3.6, 3.4)	3.06 *ddddd* (11.9, 9.4, 3.2, 3.4, 2.4)	3.50	3.52 *m*
8α		2.31 *ddd* (15.2, 4.2, 2.4)		
8β	5.27 *dd* (10.8, 2.6)	1.73 *ddd* (15.2, 11.9, 2.4)	5.24	5.35[d]
9	5.33 *d* (2.6)	5.08 *dd* (4.2, 2.4)	5.34	5.35[d]
13′	5.88 *d* (3.6)	5.52 *d* (3.2)	5.86	5.78 *d* (2.8)
13	6.36 *d* (3.4)	6.23 *d* (3.4)	6.35	6.30 *d* (3.0)
14	1.25 *s*	1.09 *s*	1.26	1.27 *s*
15	1.49 *s*	1.39 *s*	1.46	1.47 *s*
OAc	2.21 *s*	2.15 *s*	2.22	2.20 *s*
OOH		8.02 *s*[e]	7.60	7.63 *s*

[a] Ester (C-8) side chains: in **2** and **5**: 2.56 *sep* (7.0) 1H; 1.20 *d* (7.0) 6H (isobutyrate); in **6**: 6.88 *br q* (7.2) 1H, H-3′; 1.85 *br d* (7.2) 3H, H-4′; 1.84 *br s* 3H, H-5′ (tiglate).
[b] Measured in $CDCl_3/CD_3OD$, ca. 10:1.
[c] Multiplicity as in **6**.
[d] Overlapping signals; resolved in C_5D_5N: δ 5.89 *dd* (10.8, 2.6), H-8 and δ 6.05 *d* (2.6), H-9.
[e] Measured in $(CD_3)_2CO$.

Table 2
^{1}H 300 MHz ($CDCl_3$) NMR data of compound **10** at room temperature and at −51°C (δ, multiplicity, *J*, Hz)[a]; ratio of conformers, **10A/10B**, 1:2.0

H	Room temperature	−51°C	
	10 [9]	**10A [9A]**	**10B [9B]**
1	2.83[b] [2.85]	ca. 3.1[c] [ca. 3.0]	ca. 2.7[c] [2.68]
2α, 2β	2.35[b] [2.35]	2.4–2.65 [c] [2.4–2.6]	2.2–2.3[c] [2.2–2.3]
3	5.52[c] *br s* [5.55]	5.55 *br s* [5.50]	5.55 *br s* [5.50]
5	2.83[b] [2.85]	ca. 3.15[c] [ca. 3.09]	ca. 2.82[c] [ca. 2.75]
6	4.20 *t* (9.6) [4.18]	4.36 *t* (9.7) [4.29]	4.23 *t* (10.0) [4.15]
7	3.71[b] [3.61]	3.54 *dddd* (10.9, 9.7, 3.2, 2.6) [3.44]	3.85 *dddd* (10.0, 8.8, 3.0, 3.5) [3.81]
8	5.50[b] [5.40]	5.22 *dd* (10.9, 2.5) [5.15]	5.64 *dd* (8.8, 5.0) [5.56]
9	5.32 *d* (4.2) [5.30]	5.33 *d* (2.5) [5.28]	5.33 *d* (5.0) [5.27]
13′	5.50[b] [5.60]	5.92 *d* (2.6) [5.87]	5.42 *d* (3.0) [5.42]
13	6.20 *d* (2.7) [6.33]	6.37 *d* (3.2) [6.34]	6.23 *d* (3.5) [6.20]
14	1.21 *s* [1.19]	1.26[c] [1.20]	1.21[c] [1.17]
15	1.89 *br s* [1.86]	1.83 *br s* [1.79]	1.92 *br s* [1.88]
OAc	2.08 *br s* [2.08, 2.13]	2.25 *s* [2.25, 2.09][d]	2.07 *s* [2.24, 2.04][c]
OiBut	2.65 *sep* (7.2) 1H; 1.24 *d* (7.2) 3H; 1.23 *d* (7.2) 3H	2.57[c] 1H; ca. 1.20[c] *d* (7.0) 6H	2.75[c] 1H; 1.29[c] *d* (7.0) 3H; 1.27[c] *d* (7.0) 3H

[a] Assigned by means of DQF COSY and PS NOESY and by analogy to the NMR data of **9** measured at the room temperature, and conformers **9A** and **9B** measured at −57°C, in square brackets (Bulatović et al.,1997).
[b] Very broad unresolved signals due to a conformational change at a medium rate.
[c] Overlapping signals.
[d] The assignment to the conformers was tentative.

indicated 8α-tigloyloxy-9α-acetoxy pattern. The very similar shifts of H-5 in **5** and **6** (Table 1) indicated the presence of a 4α-OOH substituent. The relative configuration at C-4, i.e. 4α-OOH, 4β-methyl, as well as of the rest of the molecule (same is in the esters of anthemolide B), was corroborated by the following NOEs: H-15/H-6, H-15/H-9, H-15/H-8. H-14/H-9, H-14/H-8, H-1/H-5 and H-5/H-7.

Table 3
^{13}C 75 MHz ($CDCl_3$) NMR chemical shifts[a] of conformers **10A** and **10B** at −51°C

C	**10A [9A]**[b]	**10B [9B]**[b]
1	42.0[c] [42.4]	53.4 [54.1]
2	32.7 [33.7]	33.3 [33.7]
3	127.4 [127.7]	125.5 [125.6]
4	139.0	142.9
5	52.4 [52.9]	53.7 [54.1]
6	80.2 [80.4]	79.7 [80.0]
7	41.8[c] [42.3]	45.9 [46.2]
8	71.2 [71.5]	71.2 [71.9]
9	77.9 [78.4]	69.7 [70.4]
10	73.7	76.6
11	134.8	137.1
12	169.9	169.7
13	125.5 [125.6]	121.8 [121.9]
14	21.7[c] [21.7]	27.6 [27.9]
15	16.5 [16.7]	18.0 [18.3]
OAc	171.0; 21.3[c]	169.5; 19.3
OiBut	176.0; 33.7; 18.7; 18.5	176.5; 33.9; 20.6; 19.0

[a] Assigned by means of HSQC and HMBC.
[b] Chemical shifts of conformers **9A** and **9B** (δ_C), measured in HSQC at −57°C (Bulatović et al., 1997), in square brackets.
[c] The assignments can be interchanged.

The ^{1}H- and ^{13}C-NMR spectra of guaianolide **10** ($C_{21}H_{28}O_7$), belonging to cumambrin (Δ^3) group, were very similar to those of co-occurring 8α,9α-diacetoxy analogue **9**, also isolated previously from *A. carpatica* (Bulatović et al., 1997) (see Tables 2 and 3). The only structural difference between these lactones was in one ester residue. As it was proved by the ^{1}H- and ^{13}C-NMR data, in combination with the low temperature HMBC (vide infra), lactone **10** contained 8α-isobutyroloxy-9α-acetoxy moiety. As in **9** (Bulatović et al., 1997; Milosavljević et al., 1998), a conformational exchange occurring between two forms (**10A** and **10B**, Fig. 1) at the room temperature at a medium rate (on the NMR time scale) caused considerable broadening of the most of the ^{1}H resonances of **10**. At a low temperature (−51°C, $CDCl_3$), most of the ^{1}H- and ^{13}C-NMR signals split into pairs of sharp, well-resolved resonances. Within each pair, the ^{1}H signals were connected by positive cross-peaks in PS NOESY, which is typical of the conformers mutually exchanging at a slow rate (**10A** ⇄ **10B**, Fig. 1). The ^{1}H- and ^{13}C-NMR data of **10A** and **10B** measured in the low temperature spectra are listed in Tables 2 and 3. The similarity of ^{1}H- and ^{13}C-NMR data of **9A** and **9B** to those of **10A** and **10B**, respectively, indicated the same conformational types in these lactones; whereas in lactone **9**, under the similar experimental conditions (−57°C, $CDCl_3$), **9B** was slightly predominant in the conformational equilibrium (**9A**/**9B** ca. 1:1.2) (Bulatović et al. 1997); the

Table 4
^{1}H 200 MHz ($CDCl_3$) NMR data of lactones **13**–**17** (δ, multiplicity[a], *J*, Hz)

H	**13**[b]	**14**[c]	**15**[c]	**16**[b,c]	**17**[c]
1	3.6–3.8[d]	3.73 *m*	ca. 3.74[d]	ca. 3.75[d]	3.72[d]
2α	ca. 1.9 *m*	1.91 *ddd* (14.8, 6.9, 2.4)	1.91	1.87	ca. 2.22[d]
2β	ca. 2.3 *m*	ca. 2.4 *m*	ca. 2.3	ca. 2.3	ca. 2.1[d]
3	4.58 *br d* (ca. 6.5)	4.62 *br d* (7.6)	4.62	4.57	4.89[d]
6	4.80 *br d* (10.8)	4.81 *dq* (10.8, 1.6)	4.81	4.86	4.84[d]
7	3.6–3.8[d]	3.65 *dddd* (10.8, 10.0, 3.6, 2.6)	3.69[d]	3.70[d]	3.68[d]
8	5.15 *dd* (10.4, 2.4)	5.25 *br d* (10.0)	5.24	5.32	5.24
9	3.80 *d* (2.4)	5.27 *br s*	5.29	5.28	5.30
13′	5.89 *dd* (3.2, 0.8)	5.87 *br d* (2.6)	5.90	5.80	5.91
13	6.32 *d* (3.4)	6.33 *d* (3.6)	6.34	6.28	6.35
14	1.00 *s*	1.10 *s*	1.10	1.08	1.12
15	1.95 *br s*	2.00 *t* (1.6)	1.99	1.98	2.01
OAc	2.20 *s*	2.20 *s*	2.20	2.19	2.20[d]
OOH					7.79 *br s*

[a] Multiplicity in **15**–**17** same as in **13** and **14**.
[b] Measured in $CDCl_3/CD_3OD$ (ca. 10:1).
[c] Ester (C-8) side chains: in **14**: δ 2.36 *q* (7.6) 2H; 1.17 *t* (7.6) 3H (propionate); in **15** and **17**: δ 2.57 *qui* (7.2) 1H; 1.20, 1.19 2 × *d* (7.2) 2 × 3H (isobutyrate); in **16**: δ 6.86 *qq* (7.4, 1.6) 1H, H-3′; 1.85 *br d* (7.4) 3H, H-4′; 1.84 *br s* 3H, H-5′ (tiglate).
[d] Overlapped signals.

equilibrium population of **10B** was twice as that of **10A** (**10A**/**10B** ca. 1:2.0). This was obviously due to the larger 8α-OiBut group in **10** (in comparison to 8α-OAc in **9**), whose attachment to C-8 was evident from a three-bond C,H-correlation (in **10B**) between H-8 (δ 5.64) and isobutyrate carbonyl (δ 176.5) observed in a low-temperature HMBC of **10**. An additional proof for 8α-OiBut, 9α-OAc pattern in **10** was obtained from three-bond correlations between the acetate carbonyl and H-9 (δ_C 171.0/δ_H 5.33 and δ_C 169.5/δ_H 5.33 in **10A** and **10B**, respectively), detected in the same HMBC spectrum. A more detailed conformational analysis of **9** and **10**, involving low-temperature NMR measurements in different solvents (CD_2Cl_2, CD_3OD, $(CD_3)_2CO$ and toluene-d_8), as well as (MM+) calculations of the internal energies of the possible conformations is in progress.

The ^{1}H-NMR spectra of **14** ($C_{20}H_{26}O_8$), **15** ($C_{21}H_{28}O_8$), **16** ($C_{22}H_{28}O_8$) and **17** ($C_{21}H_{28}O_9$), closely resembling each other (Table 4), were typical of the 8α,9α-diacyloxy-Δ^4-guaianolides, whose structure determination, involving the application of various 2D-

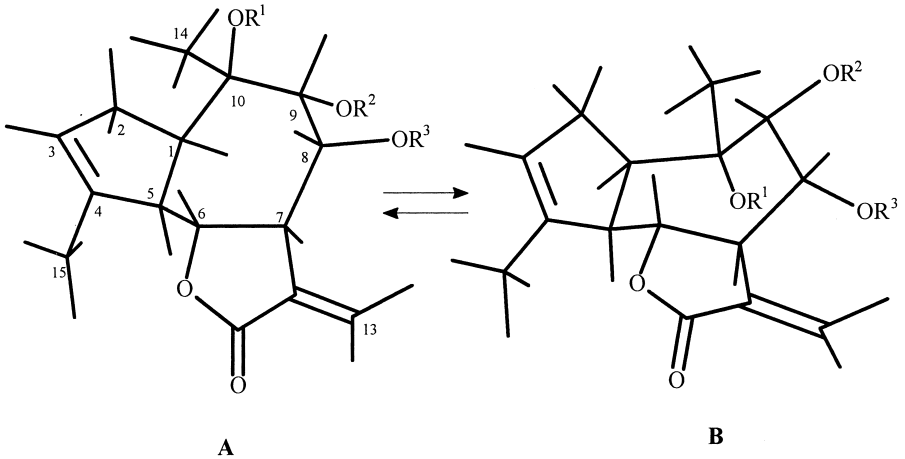
Table 5
^{1}H 300 MHz NMR data[a] of lactones **18**–**20** (δ, multiplicity, *J*, Hz)

H	**18** ($CDCl_3$)	**18** (C_6D_6)	**19** ($CDCl_3$)	**20** $(CD_3)_2CO$
1	2.90 *dd* (9.4, 5.6)[b]	2.30–2.40[b]	4.33 *dd* (10.6, 6.2)	5.45 *dd* (11.3, 5.6)
2α	1.9–2.1[b]	ca. 1.62[b]	ca. 2.15 *m*[b]	2.28 *ddd* (11.5, 11.5, 7.2)
2β	2.33 *ddd* (14.5, 6.6, 5.6)	1.96 *ddd* (14.2, 6.6, 5.8)	ca. 2.05 *m*[b]	ca. 2.05 *m*[b]
3	5.36 *br t* (7.6)[c]	5.16 *br t* (7.5)[c]	4.92 *dd* (7.5, 10.4)	4.95 dd (7.2, 10.6)
5	5.51 *br d* (9.3)[c]	5.00 *br d* (9.2)[c]	5.30 *br d* (10.4)	5.24 *br d* (10.6)
6	4.35 *ddd* (9.7, 9.7, 2.9)[d]	ca. 3.60 *m*[b]	4.53 *dd* (9.1, 10.4)	5.67 *dd* (9.8, 10.6)
7	2.95 *m*[b]	2.30–2.40[b]	2.83 *dddd* (9.0, 9.1, 3.4, 3.0)	3.23 *dddd* (9.8, 8.8, 3.5, 3.0)
8	4.06 *br m*	ca. 3.54 *m*[b]	4.48 *br t* (9.0)[c]	4.88 *dd* (10.2, 8.8)
9	2.0–2.10[b]	1.88 *dd* (13.8, 3.4) (9α); 1.47 *dd* (13.8, 11.6) (9β)	5.34 *br d* (10.0)	5.56 *br d* (10.2)
13′	6.20 *dd* (2.3, ≤1)	5.90 *dd* (2.2, 1.4)	6.22 *dd* (3.0, 0.8)	5.80 *dd* (3.0, 0.5)
13	6.34 *dd* (2.6, ≤1)	6.42 *dd* (2.6, 1.4)	6.32 *dd* (3.4, 0.8)	6.10 *dd* (3.5, 0.5)
14	1.26 *s*	0.84 *s*	1.86 *d* (1.4)	1.87 *d* (1.6)
15	1.85 *d* (ca. 1)	1.24 *br s*	1.78 *d* (1.2)	1.99 *d* (1.4)[b]
OAc	2.11 *s*	1.64 *s*	2.06 *s*	2.10 *s*; 2.01 *s*[b]; 2.00 *s*[b]
OH	ca. 2.0[b]	1.11 *d* (3.6)		

[a] Assigned by means of COSY and NOESY.
[b] Overlapping signals.
[c] The splittings of these signals did not correspond to the exact vicinal couplings due to additional broadening caused by small (homo) allylic couplings.
[d] Shifts to δ ca. 5.35 upon acetylation of **18**.

NMR techniques on 8α,9α-diacetoxy analogues, i.e. anthemolide D (3α-hydroxy) and anthemolide E (3α-hydroperoxy), was reported previously (Bulatović et al., 1997). Monoacetate **13** ($C_{17}H_{22}O_7$), also belonging to the Δ^4-group, according to the chemical shifts and couplings of H-8 and H-9 (δ 5.15 and 3.80, respectively, Table 4), exhibited 8α-acetoxy,9α-hydroxy pattern. The common feature of **14–17** was the acetoxy group (δ 2.20), whereas the nature of the remaining (larger) ester residue was evident from the characteristic ^{1}H-NMR signals (see Table 4, footnote c). The correlations in NOESY between H-13′ and the protons of these ester side chains, such as CH_2 (δ 2.36) from propionate (**14**), $CH(CH_3)_2$ (δ 2.57, 1.20 and 1.19) from isobutyrates (**15–17**), and H-3′ (δ 6.86) from tiglate (**16**), clearly indicated that in all cases the larger OAcyl group was in 8α-position, whereas the OAc group was in 9α-position. The NOEs, such as H-14/H-6, H-14/H-8 and H-14/H-9, were also in agreement with the stereochemical proposal concerning these lactones. 3α-Position of the hydroperoxy group in **17**, same as in anthemolide E (Bulatović et al., 1997), was evident from the downfield shift of H-3 (Δδ ca. 0.3) and H-2α (Δδ ca. 0.3) in comparison to the chemical shifts of the same proton in **13–16**, as well as the NOE between H-3 and H-2β.

The ^{1}H-NMR (in $CDCl_3$ and C_6D_6) spectra (see Table 5) of lactone **18** ($C_{17}H_{22}O_6$) were substantially different from those of the co-occurring guaianolides (if not otherwise stated, the NMR data quoted in further text are referring to those measured in $CDCl_3$). Lactone **18** contained a hydroxyl (3446 cm^{-1}) and an acetoxy group (δ 2.11). The COSY experiments (both in $CDCl_3$ and C_6D_6) enabled spectral assignment and identification of gross structure of **18**. Starting the COSY analysis from the unambiguously identified low-field signals of the α-methylene-γ-lactone moiety (δ 6.20 and 6.43, H-13′ and H-13, respectively), the assignment of the multiplet at δ 2.95 (partially overlapped with a *dd* at δ 2.90) as H-7, according to its allylic couplings to H-13 and H-13′, was straightforward. Proton H-7 exhibited further COSY correlations to the vicinal protons giving resonances at δ 4.35 (H-6) and 4.06 (H-8). The assignment of the carbinol proton H-6 was based on its coupling to the vinyl proton H-5 (δ 5.51) and also to the OH group ($J_{OH,\,6} = 2.9$ Hz) whose signal, masked with other resonances, was located in COSY and PS NOESY (exhibiting the positive cross-peak in the latter due to a chemical exchange with water) at δ ca. 2.00. The narrow doublet of OH was clearly visible in C_6D_6 at δ 1.11. A downfield shift of H-6 to δ ca. 5.35 upon acetylation of **18**, corroborated the assignment of H-6 as the carbinol proton. The narrow methyl doublet at δ 1.85 ($J_{5,\,15}$ ca. 1 Hz), exhibiting allylic coupling to H-5, was assigned as H-15. The remaining proton coupled to H-7, i.e. a broad unresolved multiplet (δ 4.06), originating from the lactonic proton (H-8), coupled to a methylene group (H-9) whose unresolved signals were detected in the region of ca. δ 2.0–2.1. The acetoxy group could be associated with a low-field, somewhat broadened, triplet at δ 5.36 (H-3), characteristic of an allylic proton geminal to the OAc group and coupled to an aliphatic methylene, the latter exhibiting two groups of mutually coupled signals, *ddd* at δ 2.33 (H-2β) and the multiplet overlapped with other signals at δ ca. 1.9–2.1 (H-2α).



A B

	R^1	R^2	R^3
9 :	H	Ac	Ac
10 :	H	Ac	*i*-But

Fig. 1. Conformational equilibrium in **9** and **10**.

This CH_2 group was also connected by cross-peaks in COSY to one-proton double doublet at δ 2.90 (H-1) which could be assigned, according to the chemical shift, to a proton on a 1,10-epoxide ring. Finally, a three-proton singlet (δ 1.26), characteristic of the methyl geminal to an oxygen, could be attached to the epoxide ring. These data accorded with a biogenetically plausible germacran-12,8-olide skeleton **18**. The proposed stereochemistry of **18** was mostly based on H,H-couplings and NOESY data. From the pairs of spatially close protons (exhibiting NOE), which could be divided into two groups (occupying α- or β-side of the 10-membered ring), like H-2α/H-14, H-3/H-5, H-5/H-7, H-5/H-14, H-7/H-14 (α-side) and H-1/H-8, H-1/H-15 and H-6/H-15 (β-side), followed the 1α,10β-epoxy, 3α-acetoxy, 6α-hydroxy, 7α-H and 8β-H configurations. The absence of NOE between H-5 and H-15 indicated (4*E*)-configuration. The observed vicinal couplings, such as $J_{1,\,2\alpha} = 9.4$ Hz, $J_{2\beta,\,3} = 6.6$ Hz, $J_{2\alpha,\,3} \geq 7.6$ Hz, $J_{5,\,6} = J_{6,\,7} = 9.7$ Hz (Table 5), are in agreement with this stereochemical proposal.

(Un)fortunately, during the extensive, long-lasting 2D-NMR (HSQC and HMBC) measurements in $CDCl_3$, obviously containing traces of acidic impurities, lactone **18** was transformed into a single product **19**. According to the ^{1}H-NMR data (see Table 5), the product **19**, with same molecular formula as **18** contained, instead of 1,10-epoxide ring, an additional double bond whose proton (δ 5.34 *br d*), coupled (according to COSY) to H-8 with a large coupling constant ($J_{8,\,9} = 10.4$ Hz) and thus assigned as H-9. The remaining spectral changes associated with the structural transformation, such as the simplification of the signal of H-8, a downfield shift of H-1 to δ 4.33 and H-14 to δ 1.86, the latter exhibiting a small (allylic) coupling to H-9 ($J_{9,\,14} = 1.4$ Hz) and a disappearance of the signal of aliphatic (C-9) methylene, indicated acid-catalyzed opening of the 1,10-epoxide ring in **18**, followed by a stereospecific elimination of a proton from C-9, leading to the 1-hydroxy-Δ^9-moiety (Fig. 2). The structure of **19** was also compatible with the long-range C,H-correlations observed in HMBC (Fig. 2).

As far as the geometry of **19** is concerned, the observed NOESY data and couplings could be rationalized in terms of the chair–boat conformation (Fig. 3), similar to that observed in tulirinol (**21**) and its acetate (**22**) (Doskotch et al., 1980), germacrolides differing from **19** by the lack of 3-oxygen functionality.The lack of NOE between H-5 and H-15 (as in **18**) and the occurrence of NOE between H-9 and H-14 indicated (5*E*,9*Z*)-configurations. The remaining NOEs (Fig. 3), together with H,H-couplings (see Table 5), were in agreement with the structure of (5*E*,9*Z*)-1α,6α-dihydroxy-3β-acetoxygermacra-5,9,11(13)-trien-12,8α-olide (alternative name: 3β-acetoxydeacetyltulirinol). An additional proof for the structure (and the relative stereochemistry) of **19**, and thus (indirectly) of **18**, was obtained from the similarity of ^{1}H-NMR: data [in $(CD_3)_2CO$, see Table 5] of triacetate **20**, obtained by the standard acetylation procedure (Ac_2O/pyridine), to those of **22**, also characterized by X-ray crystallography and CD spectra (Doskotch et al., 1980). Finally, the NOESY data of **20** were almost identical to those of **19** (Fig. 2).

The different outcome of the epoxide cleavage in **18**, in comparison to the well-established biogenetic route leading to eudesmanolides, involving acid-catalyzed transannular cyclization in (*E*)-1β,10α-epoxygermacra-4-en-12,6α- and 12,8α-olides (Iriuchijima and Tamura, 1967; Sutherland, 1974; Rodrigues et al., 1978) may also be regarded as an indication of the different configuration (i.e. 1α,10β-) of the epoxide function in **18**. The characteristic example is also diacetate of 1β,10α-epoxychamissonin (**23**), the lactone with the similar

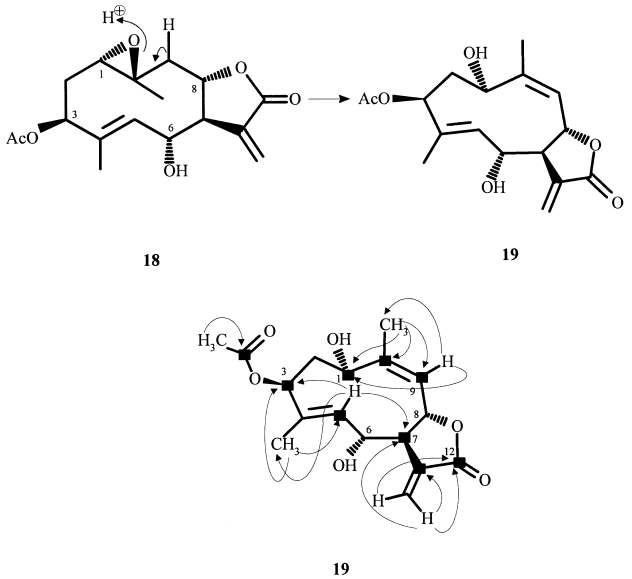


Fig. 2. Acid-catalyzed opening of the epoxode ring in **18** and long-range C,H-correlations observed in HMBC of **19**.

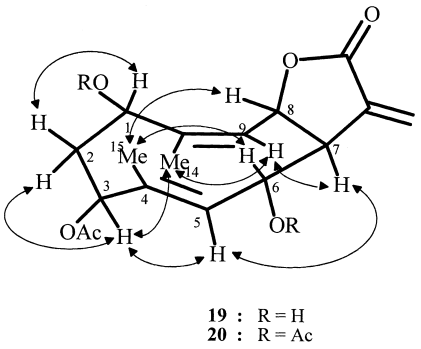
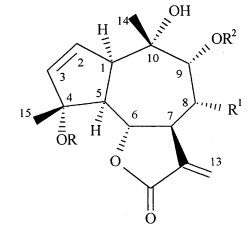
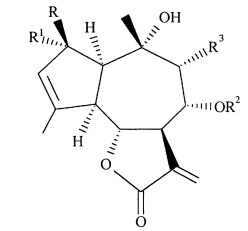


Fig. 3. Significant NOEs observed in PS NOESY of **19** and **20**.

gross structure as **18**, but the different stereochemistry which upon treatment with *p*-TsOH/Ac_2O was smoothly converted to eudesmanochamissonin tetraacetate (Geissman et al., 1973).

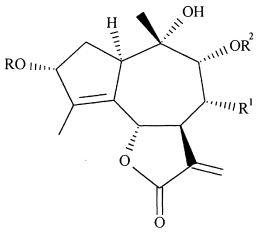


	R	R^1	R^2
1 :	H	H	Ac
2 :	H	O-*i*-But	Ac
3 :	OH	H	Ac
4 :	OH	OH	H
5 :	OH	O-*i*-But	Ac
6 :	OH	OTig*	Ac

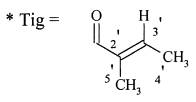


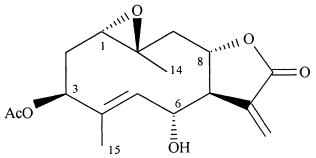
	R	R^1	R^2	R^3
7 :	H	H	H	H
8[a] :	OH	H	H	H
9 :	H	H	Ac	OAc
10 :	H	H	*i*-But	OAc
11 :	H	OOH	*i*-But	OAc

a $\Delta^{10(14)}$



	R	R^1	R^2
12 :	H	H	Ac
13 :	H	OAc	H
14 :	H	OProp	Ac
15 :	H	O-*i*-But	Ac
16 :	H	OTig*	Ac
17 :	OH	O-*i*-But	Ac





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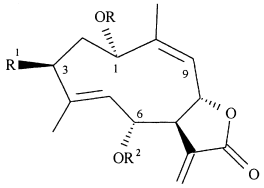
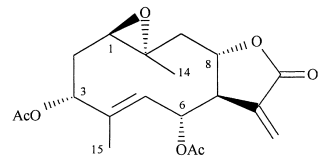


	R	R^1	R^2
19 :	H	OAc	H
20 :	Ac	OAc	Ac
21 :	H	H	Ac
22 :	Ac	H	Ac



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3. Experimental

3.1. General

CC: silica gel 60 (Merck), 0.063–0.200 mm; TLC: Kieselgel 60 GF_{254}, layer thickness 0.25 and 1 mm; IR: transparent dry films; ^{1}H-NMR: various deuterated solvents (see Tables 1–5), at 200 and 300 MHz relative to TMS at ($\delta = 0.00$); 2D-NMR spectra measured as usual (Milosavljević et al., 1998); DCIMS double focusing (BE geometry); ESIMS double focusing (EB geometry) + electro spray interface.

3.2. Plant material

The plant material was collected during the flowering season (July 1996) at the north part of Šara Mountain (location Lavlja Vrata, altitude of ca. 1900 m). Voucher specimen (No. 210796AC) was deposited in the herbarium of The Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade.

3.3. Extraction procedure

A crude extract (68.8 g) of air-dried aerial parts (2115 g) was obtained by extraction with freshly distilled solvents: Et_2O (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 $CHCl_3$ (400 ml) soluble portion of the whole crude extract was added to 300 g of silica gel and dried at a room temperature overnight. 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 Et_2O.

Fraction A (1.2 g) eluted with petrol–Et_2O (2:3), after the repeated silica gel CC (CH_2Cl_2–MeOH, 98:2), followed by preparative TLC (CH_2Cl_2–MeOH, 98:2, two developments), afforded lactone **9** (4.3 mg).

Fraction B (1.9 g) eluted (after A) with petrol–Et_2O (2:3) yielded by silica gel CC (toluene–Et_2O–MeOH, 7:2:1) fractions I–III. Fraction I, after preparative TLC (toluene–Et_2O–MeOH, 7:2:1, two developments), yielded lactone **10** (55.5 mg) and fraction Ia. Lactones **5** (2.3 mg) and **15** (3.3 mg) were isolated from Ia by preparative TLC (CCl_4–Et_2O–MeOH, 5:4:1, two developments). Fraction II, purified by repeated CC ($CHCl_3$–MeOH, 10:1), followed by preparative TLC (EtOAc–petrol–MeOH, 7:2.5:0.5, four developments), afforded lactones **16** (1.1 mg) and **17** (1.4 mg). Lactone **2** (1.1 mg) was isolated from fraction III by CC (CH_2Cl_2–MeOH, 95:5), followed by preparative TLC (petrol–EtOAc, 1:4, three developments).

Fraction C (1.0 g) eluted (after B), after CC (CH_2Cl_2–MeOH, 98:2), followed by preparative TLC (CCl_4–Et_2O–MeOH, 5:4.5:0.5, three developments), yielded lactones **6** (2.3 mg) and **11** (1.1 mg).

Fraction D (1.1 g) eluted with petrol–Et_2O (3:7)

afforded, after repeated silica gel CC (C_6H_6–Et_2O–MeOH, 7:2:1), lactone **3** (3.2 mg) and fractions I–III. Fraction I, after CC (CH_2Cl_2–MeOH, 95:5), yielded lactone **18** (3.6 mg). Lactone **14** (1.6 mg) was obtained from fraction II by two preparative TLCs [(CH_2Cl_2–MeOH, 9:1, two developments) and (EtOAc–petrol–MeOH, 7:2.5:0.5, two developments)]. Lactones **1** (1.6 mg) and **12** (2.1 mg) were isolated from fraction III by preparative TLC (toluene–Me_2CO, 3:2, two developments).

Lactone **7** (3.0 mg) was isolated from fraction E (0.7 g) eluted with petrol–Et_2O (3:7), by repeated CC (CH_2Cl_2–MeOH, 95:5), followed by preparative TLC (CH_2Cl_2–MeOH, 95:5, two developments).

Fraction F (1.1 g) eluted with Et_2O, upon repeated column chromatography (CH_2Cl_2–MeOH, 9:1), followed by preparative TLC (CH_2Cl_2–MeOH, 9:1, two developments) gave lactone **8** (5.0 mg).

Lactones **4** (2.1 mg) and **13** (3.2 mg) were isolated from fraction G (0.6 g) eluted with Et_2O (after F), by repeated CC (CH_2Cl_2–MeOH, 9:1), followed by preparative TLC.

3.5. 8α-Isobutyryloxyanthemolide A (*2*)

Colorless gum; $[\alpha]_D^{25}$ −13.6° (CH_2Cl_2; *c* 0.11); IR ν_{max} cm^{-1}: 3446 (OH), 1740 (C═O, α,β-unsat. γ-lactone, C═O, OAc, OiBut) , ca. 1652 (C═C); ^{1}H-NMR: see Table 1; DCIMS (isobutane probe), 150 eV, *m/z* (rel. int.): 409 $[M + H]^+$ (97), 391 $[M + H - 18]^+$ (100), 373 $[M + H - 2 \times 18]^+$ (73).

3.6. 8-Deoxy-9-O-acetylanthemolide B (*3*)

Amorphous solid; $[\alpha]_D^{25}$ −54° (MeOH; *c* 0.32); IR ν_{max} cm^{-1}: 3376 (OH, OOH), 1752 (C═O, α,β-unsat. γ-lactone), 1740 (C═O, OAc), ca. 1650 (C═C); ^{1}H-NMR: see Table 1; ESIMS (in MeOH–H_2O, 1:1 +1% NH_4OAc) *m/z* (rel. int.): 356 $[M + NH_4]^+$ (100).

3.7. 8-O-Tigloyl-9-O-acetylanthemolide B (*6*)

Colorless gum; IR ν_{max} cm^{-1}: 3424 (OH, OOH), 1747 (C═O, α,β-unsat. γ-lactone, OAc), ca. 1730 sh (C═O, tiglate), 1650 (C═C); ^{1}H-NMR: see Table 1; DCIMS: isobutane probe, 150 eV, *m/z* (rel. int.): 437 $[M + H]^+$ (45), 421 $[M + H - 16]^+$ (58), 419 $[M + H - 18]^+$ (82), 403 $[M + H - 34]^+$ (100), 385 $[M + H - 34 - 18]^+$, 377 $[M + H - 60]^+$ (14).

3.8. 8-O-Isobutyryl-9α-acetoxycumambrin B (*10*)

Amorphous solid; $[\alpha]_D^{25}$ +21° ($CHCl_3$; *c* 0.16) ; IR ν_{max} cm^{-1}: 3484 (OH), 1742 (C═O, α,β-unsat. γ-lactone, esters), 1662 (C═C); ^{1}H- and ^{13}C-NMR: see Tables 2 and 3, respectively; DCIMS isobutane probe, 150 eV, *m/z* (rel. int.): 393 $[M + H]^+$ (100), 375 $[M + H - 18]^+$ (25), 333 $[M + H - 60]^+$ (4), 305 $[M + H - 88]^+$ (21), 287 $[M + H - 18\text{-}88]^+$ (10), 245 $[M + H - 60\text{-}88]^+$ (18), 227 $[M + H - 18 - 60 - 88]^+$ (42).

3.9. 9-O-Deacetylanthemolide D (*13*)

Pale yellow oil; IR ν_{max} cm^{-1}: 3475 (OH), 1742 (C═O, α, β-unsat. γ-lactone, OAc), 1666 (C═C); ^{1}H-NMR: see Table 4; DCIMS isobutane probe, 150 eV, *m/z* (rel. int.): 339 $[M + H]^+$ (43), 321 $[M + H - 18]^+$ (48), 303 $[M + H - 2 \times 18]^+$ (30), 279 $[M + H - 60]^+$ (100), 261 $[M + H - 18 - 60]^+$ (82).

3.10. 8α-Propionyloxyanthemolide C (*14*)

Amorphous solid; IR ν_{max} cm^{-1}: 3424 (OH), 1739 (C═O, α,β-unsat. γ-lactone, ester), 1651 (C═C); ^{1}H-NMR: see Table 4; DCIMS (NH_3 probe), 150 eV, *m/z* (rel. int.): 412 $[M + NH_4]^+$ (5), 394 $[M + NH_4 - 18]^+$ (55), 377 $[M + H - 18]^+$ (25), 359 $[M + H - 2 \times 18]^+$ (63), 334 $[M + NH_4 - 18 - 60]^+$, 303 $[M + H - 18 - 74]^+$ (22), 285 $[M + H - 2 \times 18 - 74]^+$ (100).

3.11. 8α-Isobutyryloxyanthemolide C (*15*)

Colorless gum; $[\alpha]_D^{25}$ +51° (MeOH; *c* 0.14) ; IR ν_{max} cm^{-1}: 3396 (OH), 1741 (C═O, α,β-unsat. γ-lactone, esters), 1655 (C═C); ^{1}H-NMR: see Table 4; DCIMS isobutane probe, 150 eV, *m/z* (rel. int.): 409 $[M + H]^+$ (12), 391 $[M + H - 18]^+$ (100), 373 $[M + H - 2 \times 18]^+$ (42), 349 $[M + H - 60]^+$ (10), 321 $[M + H - 88]^+$ (53), 303 $[M + H - 18 - 88]^+$ (53), 285 $[M + H - 2 \times 18 - 88]^+$ (21).

3.12. 8α-Tigloyloxyanthemolide C (*16*)

Colorless gum; $[\alpha]_D^{25}$ +57°(MeOH; *c* 0.11); IR ν_{max} cm^{-1}: 3418 (OH), 1753 (C═O, α,β-unsat. γ-lactone, OAc), 1725 (C═O, OTig) 1650 (C═C); ^{1}H-NMR: see Table 4; DCIMS (isobutane probe), 150 eV, *m/z* (rel. int.): 421 $[M + H]^+$ (9), 403 $[M + H - 18]^+$ (100), 385 $[M + H - 2 \times 18]^+$ (32), 361 $[M + H - 60]^+$ (5), 321 $[M + H - 100]^+$ (92), 303 $[M + H - 18 - 100]^+$ (53), 285 $[M + H - 2 \times 18 - 100]^+$ (18).

3.13. Anthemolide F (*17*)

Amorphous solid; $[\alpha]_D^{25}$ +97° (MeOH; *c* 0.33); IR ν_{max} cm^{-1}: 3456 (OH, OOH), 1740 (C═O, α,β-unsat. γ-lactone, ester), 1652 (C═C); ^{1}H-NMR: see Table 4; DCIMS isobutane probe, 150 eV, *m/z* (rel. int.): 425 $[M + H]^+$ (6), 409 $[M + H - 16]^+$ (22), 408 [M +

H − 17]$^+$ (42), 407 [M + H − 18]$^+$ (100), 391 [M + H − 34]$^+$ (37), 373 [M + H − 34 − 18]$^+$ (27).

3.14. (*E*)-1α,10β-Epoxy-3β-acetoxy-6α-hydroxygermacra-4,11(13)-dien-12,8α-olide (**18**)

Amorphous solid; $[\alpha]_D^{25}$ +54° ($CHCl_3$; *c* 0.35); IR ν_{max} cm^{-1}: 3446 (OH), 1741 (C═O, α,β-unsat. γ-lactone, OAc), 1658 (C═C); ^{1}H- and ^{13}C-NMR: see Table 5; DCIMS isobutane probe, 150 eV, *m*/*z* (rel. int.): 323 [M + H]$^+$ (100), 305 [M + H − 18]+ (2), 281 [M + H − 42]$^+$ (7), 263 [M + H − 60]$^+$ (12), 245 [M + H − 18 − 60]$^+$ (4).

3.15. (4*E*,9*Z*)-1α,6α-Dihydroxy-3β-acetoxygermacra-4,9,11(13)-trien-12,8α-olide (3β-acetoxydeacetyltulirinol) (**19**)

Amorphous solid; IR ν_{max} cm^{-1}: 3391 (OH), 1746 (C═O, α,β-unsat. γ-lactone, OAc), 1659 (C═C); ^{1}H-NMR: see Table 5; ^{13}C-NMR ($CDCl_3$, detected in HSQC and HMBC), δ: 66.2 (C-1), 33.8 (C-2), 76.5 (C-3), 132.0 (C-5), 71.0 (C-6), 52.5 (C-7), 74.1 (C-8), 127.5 (C-9), 141.5 (C-10), 137.0 (C-11), 170.0 (C-12), 124.5 (C-13), 18.0 (C-14), 12.0 (C-15), OAc (22.0, 170.0); DCIMS (isobutane probe), 150 eV, *m*/*z* (rel. int.): 323 [M + H]$^+$ (100), 305 [M + H − 18]$^+$ (55), 263 [M + H − 60]$^+$ (25), 245 [M + H − 18 − 60]$^+$ (77).

3.16. (4*E*,9*Z*)-1α,6α,3β-Triacetoxygermacra-4,9,11(13)-trien-12,8α-olide (3β-acetoxytulirinol acetate) (**20**)

Amorphous solid; $[\alpha]_D^{25}$ +43° (CH_2Cl_2; *c* 0.06); IR ν_{max} cm^{-1}: 1740 sh,1736 (C═O, α,β-unsat. γ-lactone, OAc), 1375, 1236, 1027; ^{1}H-NMR: see Table 5; DCIMS isobutane probe, 150 eV, *m*/*z* (rel. int.): 407 [M + H]$^+$ (61), 347 [M + H − 60]$^+$ (100), 287 [M + H − 2 × 60]$^+$ (17).

3.17. Lactones **1**, **4**, **5**, **7**, **8**, **9**, **11** and **12**

The identification of the known lactones was based on a comparison of their spectral data to the published ones, i.e. **1**, **5**, **9**, **11** and **12** (Bulatović et al, 1997), **4** (Vajs et al., 1999), **7** (Yoshioka et al., 1973) and **8** (Todorova et al., 1996).

Acknowledgements

The authors from Yugoslavia are grateful to the Ministry for Science and Technology, Republic of Serbia for financial support.

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