Highly Oxygenated Guaianolides from *Anthemis carpatica*

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Twelve new guaianolides, (**1**−**12**), six of them (**2**−**5**, **9** and **12**) containing a hydroperoxy function, were isolated from the aerial parts of the flowering plant *Anthemis carpatica*. Their structures were elucidated by spectroscopic methods, including 2D NMR experiments.

The genus *Anthemis* L. (family Asteraceae, tribe Anthemidae) comprises about 130 species that commonly occur in the Mediterranean, although some also can be found in southwest Asia and South Africa.[1] In Serbia nine species are known.[2] Three main classes of compounds of chemosystematic interest, such as polyacetylenes, flavonoids, and sesquiterpene lactones, typical for Anthemidae, have been detected in the genus *Anthemis*.[3,4] Among the sesquiterpene lactones, only three types, that is, germacranolides (and *seco*-germacranolides),[5−15] eudesmanolides,[16,17] and guaianolides,[5,14,18,19] have been isolated from the genus. The exception is an allergenic lactone with unusual structure (anthecotuloide), the constituent of *A. cotula*.[20,21] Continuing our chemotaxonomic examinations of the Yugoslavian flora belonging to Asteraceae and our search for new compounds of pharmacological interest, we now report the investigation of the aerial parts of *Anthemis carpatica* Willd, a species usually occurring at elevated, shady and grassy, sandy terrains of the Balkan Peninsula, eastern Alps, and east Carpathian Mountains.[2] A flavonoid quercetin[22] (leaves) and polyacetylenes[4] (roots) are the only secondary metabolites reported for *A. carpatica*.

Results and Discussion

Repeated Si gel column chromatography of the extract, obtained using the usual extraction procedure for isolation of sesquiterpene lactones,[23] combined with preparative TLC of some fractions, yielded sesquiterpene lactones **1**−**12**. The application of 2D NMR methods, such as double quantum filtered (DQF) COSY, TOCSY, phase-sensitive (PS) NOESY, HMQC, and HMBC, performed on compounds **1** and **5**−**11** enabled their complete ^{1}H- and partial ^{13}C-NMR assignments. The ^{1}H-NMR spectra of the remaining lactones were assigned by characteristic chemical shifts and couplings and by comparison with spectra of the above analogues. The spectral data of all compounds were in accordance with the same type of (guaiadien-12,6α-olide) skeleton, oxygenated at C-9 and C-10, that is, 10α-OH and 9α-OAc (or OH). The majority of isolated lactones (with exception of **1** and **10**) also contained 8α-(acyloxy) (or hydroxy) functionality. Lactones **2**−**5**, **9**, and **12** exhibited a hydroperoxy function, giving rise to a lowfield ^{1}H-NMR OOH signal [δ ca. 13.3−13.6, 10.26, and 8.60 in C_5D_5N, $(CD_3)_2CO$, and $CDCl_3$, respectively]. Because of a low solubility in nonpolar solvents, the NMR spectra of the hydroperoxy lactones were run in C_5D_5N, which caused downfield shifts of most ^{1}H resonances in comparison to those in nonaromatic solvents (compare ^{1}H-NMR data of **11**, measured in C_5D_5N and $CDCl_3$, Table 4). The presence of an OOH group in these lactones was corroborated by intensive $[M + H-O]^+$ and $[M + H-H_2O_2]^+$ fragments in DCIMS, typical for organic hydroperoxides,[24] and a specific peroxide (red) colored TLC test with *N*,*N*-dimethyl-*p*-phenyllenediammonium dichloride.[25] The similar frequency of a lactone carbonyl band ($\leq$ 1750 cm^{-1}) in all compounds, typical for α,β-unsaturated γ-lactones, together with two characteristic doublets (or double doublets in some cases) in the olefinic region (δ > 5.5 in all solvents, Tables 1−4) of their ^{1}H-NMR spectra,[26] assigned to exomethylene protons (H-13 and H-13′), revealed the exocyclic 11,13-double bond. According to the position of the remaining double bond, all isolated lactones could be divided into three groups: (i) Δ^2, (ii) Δ^3, and (iii) Δ^4.

Assignment of a 2-double bond in lactones **1**−**5** was based on the occurrence of a characteristic lowfield AB portion of an ABX system (δ ca. 6.2−6.5 in C_5D_5N), assigned to vinyl protons H-2 and H-3. In addition to mutual (vicinal) coupling, typical for adjacent olefinic protons of a cyclopentene ring ($J_{2,3}$ = 5.8−6.0 Hz), H-2 and H-3 coupled to H-1 (δ ca. 4.2−4.4, ddd, C_5D_5N) via three and four bonds, respectively. An additional common feature of the lactones from this group was a pair of three-proton singlets assigned, according to chemical shifts (δ 1.45−1.52 and δ 1.71−1.73 in C_5D_5N) to methyls (H-14 and H-15, respectively) bonded to oxygenated carbons. Lactones **2**−**5** also exhibited a lactonic double doublet (H-6) at δ 4.83−4.99 (in C_5D_5N) distinctive for 5αH,6βH,7αH-guaianolides. In lactone **1**, the chemical shift of H-6 (in $CDCl_3$) was observed at δ 4.33 (Table 1).

Lactone **1** ($C_{17}H_{22}O_6$), named anthemolide A, containing one acetoxy group, was the only member of Δ^2-series without 8-functionality. A consecutive elimination of two molecules of H_2O from the $[M + H]^+$ ion in DCIMS clearly indicated the presence of two hydroxyls (bonded to C-4 and C-10) in **1**. The NOEs H-15/H-6 and H-14/H-6 (observed in PS NOESY) were in agreement with the 4β,10β-dimethyl (4α,10α-dihydroxy) arrangement in **1**. A chemical shift of H-5 (δ 2.73, in $CDCl_3$) similar to

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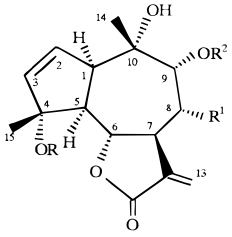
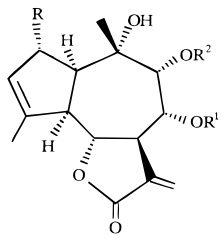
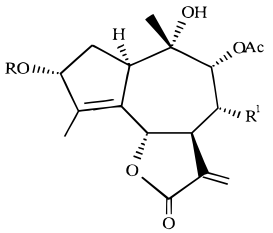


	R	R^1	R^2
1	H	H	Ac
2	OH	OAc	H
3	OH	OH	Ac
4	OH	OAc	Ac
5	OH	OiBut	Ac



	R	R^1	R^2
6	H	H	Ac
7	H	Ac	H
8	H	Ac	Ac
9	OOH	iBut	Ac



	R	R^1
10	H	H
11	H	OAc
12	OH	OAc

that in the related 4α,10α-dihydroxy-Δ^2-guaianolides (i.e. δ 2.73 and 2.67),[27] was also consistent with this proposal. The occurrence of a three-proton acetoxy singlet in the ^{1}H- ($CDCl_3$) NMR spectrum of **1** (δ 2.19) and a one-proton dd (δ 5.12) typical for a (pseudo)-equatorial proton α-positioned to an acetoxy group, coupled (according to DQF COSY) to an adjacent methylene (δ 2.40 and 1.70, H-8α and H-8β, respectively), indicated C-9 as the location of the acetoxy group. The differentiation of the signals of H-8α and H-8β was based on their NOEs, such as H-8α/H-13′ and H-8β/H-6. The magnitudes of scalar couplings $J_{9,8\alpha}$ and $J_{9,8\beta}$, together with a NOE H-9/H-14, revealed 9β−H (9α-OAc) arrangement. A NOE H-1/H-5 was in accordance with 1α,5α−H geometry.

Elucidation of the structure and relative stereochemistry of the hydroperoxy lactones **2**−**4**, the esters of anthemolide B, was mostly based on the close similarity of their ^{1}H- (C_5D_5N) NMR data (Tables 1 and 2) to those of **5** ($C_{21}H_{28}O_9$), the member of the group extensively studied by means of 2D NMR methods (Figure 1). The position of the hydroperoxy group in **5**, and thus in **2**−**4**, was determined by HMBC. In the ^{13}C-NMR spectrum of lactone **5**, a nonprotonated carbon resonating at δ 96.4 was unambiguously assigned, according to the characteristic chemical shift,[28] as a hydroperoxidation site, and identified as C-4 on the basis of (heteronuclear) correlations via two and three bonds, respectively (Figure 1b). HMBC correlations of protons of the remaining methyl, H-14 (δ 1.52) were in accordance with the proposed structure for this lactone. The ^{1}H-NMR data of **2**−**5** were in agreement with the same number and disposition of oxygen functions in these molecules. The major difference was in the acylation pattern at C-8 and C-9. Occurrence of a three-proton singlet of an acetate methyl (δ 2.11) and two groups of signals typical for an isobutyrate moiety, such as a one-proton septet and a pair of three-proton doublets (see Table 1), indicated the nature of ester residues in **5**. Dipolar couplings of CH and CH_3 of isubutyrate moiety to the exomethylene vinyl proton H-13′, together with other NOEs observed in the PS NOESY (Figure 1a), indicated an 8α-position of the isobutyryloxy group and a 9α-position of the acetoxy group in this lactone.

Two singlets of acetate methyls in the ^{1}H-NMR spectrum of **4** ($C_{19}H_{24}O_9$), together with the signals of H-8 and H-9, similar to those in **5** (Table 1), indicated an 8α,9α-diacetoxy structure for **4**. The proposed 4α-OOH configuraton in **4** (same as in **5**) was also confirmed by the chemical shift of H-5 (δ 3.02) measured in $CDCl_3$ (see footnote *d*, Table 1), similar to that of H-5 in structurally related 4α-hydroperoxy-Δ^2-guaianolides.[27] In the isomeric lactones **2** and **3** ($C_{17}H_{22}O_8$), the occurrence of only one singlet of the acetate methyl in the ^{1}H-NMR spectra suggested monoacetate structures. The coupling pattern of H-8 and H-9 in these lactones, similar to that in **4** and **5**, was in agreement with the same 8α,9α-disposition of the acyloxy/hydroxy functions. A diamagnetic shift of H-9 in **2** and H-8 in **3**, in comparison to the chemical shifts of the corresponding

Table 1. ^{1}H- (300 MHz) NMR Data of Compounds **1**−**5** (δ, mult., *J* in Hz)

H	**1**[a] ($CDCl_3$)	**2** (C_5D_5N)	**3** (C_5D_5N)	**4** (C_5D_5N)	5[a] (C_5D_5N)
1	3.62, ddd, 10.4, 2.5, 2.0	4.37, ddd, 10.3, 2.5, 2.1	4.22, ddd, 10.4, 2.4, 2.2	4.18, ddd, 10.3, 2.6, 2.1	4.17, ddd, 10.4, ca. 2.5, 2.0
2	5.93, dd, 5.8, 2.5	6.49, dd, 5.9, 2.5	6.48, dd, 6.0, 2.4	6.41, dd, 5.9, 2.6	6.41, dd, 5.9, ca. 2.5[b]
3	5.87, dd, 5.8, 2.0	6.25, dd, 5.9, 2.1	6.31, dd, 6.0, 2.2	6.27, dd, 5.9, 2.1	6.26, dd, 5.9, 2.0
5	2.73, dd, 11.3, 10.4	3.49, dd, 11.8, 10.3	3.60, dd, 11.8, 10.4[b]	3.60, dd, 11.9, 10.3[d]	3.60, dd, 11.8, 10.4
6	4.33, dd, 11.3, 9.5	4.90, dd, 11.8, 9.3	4.83, dd, 11.8, 9.6	4.99, dd, 11.9, 9.5	4.99, dd, 11.8, 9.4
7	3.00, m	4.04 dddd, 10.8, 9.3, 3.5, 2.2	3.60, m[b]	3.73, dddd, 11.1, 9.5, 3.0, 2.4	3.74, dddd, 11.0, 9.4, ca. 3.5, ca. 3
8α	2.40, m				
8β	1.70, m	5.66, dd, 10.8, 2.1	4.55, dd, 10.6, 3.0	5.72, dd, 11.1, 2.6	5.71, dd, 11.0, 2.6
9	5.12, dd, 4.0, 2.4	4.52, br s	5.96, d, 3.0,	6.01, d, 2.6	5.98, d, 2.6
13′	5.55, d, 3.1	5.97, dd, 2.2, ca. 1	6.54, dd, 3.4, 1.8	5.94, dd, 2.4, <1	5.94, dd, ca. 3, <1
13	6.31, d, 3.4	6.38, dd, 3.5, ca. 1	6.74, dd, 3.2, 1.8	6.38, dd, 3.0, <1	6.41, d, ca. 3.5[b]
14	1.14, s	1.47, s	1.45, s	1.52, s	1.52, s
15	1.49, s	1.71, s	1.73, s	1.72, s	1.72, s
OAc	2.19, s	2.00, s	2.03, s	2.10, s; 2.13, s	2.11, s
O-*i*-But					2.65, sep, 7.0 1.25, 1.18 2 × d, 7.0
OOH		13.31, br s	10.26, s[c]	13.45, br s	13.30, br s

[a] Assigned by means of DQF COSY and PS NOESY. [b] Overlapped signals. [c] Measured in $(CD_3)_2CO$. [d] In $CDCl_3$ (not shown) the chemical shift of H-5 was δ 3.02.

Table 2. ^{1}H- (300 MHz) ($CDCl_3$) NMR Data of Compounds **6**,[a] **7**,[a] **8**, and **9** (δ, mult., J in Hz)

H	6	7	8	9
1	ca. 2.60, m	3.02, dt, 8.9, 3.2	2.85[b,c]	3.05, br d, ca. 8.4
2α,2β	2.2−2.3, m	2.4−2.6, m	2.35[b]	5.28, br s (2b)
3	5.48, sex, 1.7	ca. 5.5[d]	5.55, br s	5.60, br s
5	2.75, br t, 9.5	2.95, br t, ca. 10	2.85[b,c]	3.31, br dd, 10.7, ca. 8.4
6	4.11, dd, 10.6, 9.7	4.21, dd, 10.2, 9.4	4.18, t, ca. 10	4.23, dd, 10.7, 10.1
7	ca. 3.5[b]	3.57, m	3.61[b]	3.47, dddd, 11.2, 10.1, 3.3, 2.8
8	4.09, dd, 8.6, 4.5	5.22, dd, 10.9, 2.8	5.40[b]	5.22, dd, 11.2, 2.3
9	5.14, d, 4.5	3.89, d, 2.8	5.30, d, 4.2	5.38, d, 2.3
13′	5.99[b]	5.72, br d, 2.7	5.60[b]	5.82, d, 2.8
13	6.21, d, 3.5	6.27, dd, 3.5, ca. 0.5	6.33, d, 3.6	6.32, d, 3.3
14	1.23, s	1.13, s	1.19, s	1.27, s
15	1.88, q, ca. 1.8	1.80, br s	1.86, br s	1.97, br s
OAc	2.18, s	2.19, s	2.08, s[b], 2.13, s	2.23, s
O-*i*-But				2.56, sep, 7.0 1.20, d, 7.0
OOH				8.6, br s

[a] The ^{1}H-NMR data assigned by means of DQF COSY, PS NOESY, HMQC, and HMBC of mixture containig 85% of **6** and 15% of **7**. [b] Very broad unresolved signals due to a conformational exchange at a medium rate. [c] Overlapped signals. [d] Overlapped with the signal of H-3 in **6**.

Table 3. Low Temperature (−57 °C, $CDCl_3$) ^{1}H- (300 MHz) Data and ^{13}C-NMR Chemical Shifts (δ_C)[a] of Conformers **8A** and **8B** (1:1.16, respectively)

	conformer **8A**		conformer **8B**	
H/C	δ_H, mult., J in Hz	δ_C	δ_H, mult., J in Hz	δ_C
1	ca. 3.0[b]	42.4	ca. 2.68	54.1
2	2.4−2.6, m	33.7	2.2−2.3	33.7
3	5.50, br s	125.6[c]	5.50, br s	127.7[c]
5	ca. 3.09[b]	52.9	ca. 2.75[b]	54.1
6	4.29, t, 9.8	80.4	4.15, t, 9.9	80.0
7	3.44, dddd, 11.1, 9.8, 2.9, 3.3	42.3	3.81, dddd, 9.9, 9.1, 3.5, 3.2	46.2
8	5.15, dd, 11.1, 2.6	71.5	5.56, dd, 9.1, 5.1	71.9
9	5.28, d, 2.6	70.4[c]	5.27, d, 5.1	78.4[c]
13′	5.87, d, 2.9		5.42, d, 3.2	
13	6.34, d, 3.3	125.6	6.20, d, 3.5	121.9
14	1.20, s	21.7	1.17 s,	27.9
15	1.79, br s	16.7	1.88, br s	18.3
OAc	2.25, 2.09, 2 × s[c]	21.5[c]	2.24, 2.04, 2 × s[c]	ca. 21[c]

[a] Detected in HMQC. [b] Overlapped with other signals. [c] The assignment to the conformers tentative.

signals in **4** and **5**, together with the resonances of H-8 in **2** and H-9 in **3** at almost the same chemical shifts as in **4** and **5** (see Table 1), could be rationalized in terms of 8α-acetoxy-9α-hydroxy and 8α-hydroxy-9α-acetoxy patterns in **2** and **3**, respectively. The 8α-hydroxy substitution in **3** was also confirmed by the observed (well-known) paramagnetic shift and the increase of geminal coupling of H-13′and H-13 upon transformation of 8α-OAcyl to 8α-OH.[26] Whereas in 8α-acyloxy lactones (**2**, **4**, and **5**) the resonances of H-13′ and H-13 were detected in the regions of δ 5.94−5.97 and δ 6.38−6.41, respectively, in compound **3** these signals occurred at lower field, with a concomitant increase of geminal coupling to 1.8 Hz.

The ^{1}H- ($CDCl_3$) NMR data (Table 2) of lactones **6**−**9** indicated the same basic (cumambrin) type of structure, i.e., 8α-hydroxy (or acyloxy)-10α-hydroxy-1α,5α−H-guaia-3,11(13)-dien-12,6α-olide,[18,29−32] containing an additional 9α-OAc (or OH) functionality and, in the case of **9**, also a 3α-hydroperoxy group. Recognition of the Δ^3-pattern in these lactones was based on the occurrence of a one-proton olefinic signal, H-3 (δ 5.48−5.60, br s or sextet, $^4J_{3,15}$ ca. 1.8 Hz), weakly coupled to an allylic methyl, H-15 (δ 1.80−1.97, br s or q). The HMQC and HMBC data, revealing the carbon resonances at δ ca. 124.0−127.0 and 141.8−150.9 (C-3 and C-4, respectively),[18,31,32] confirmed the presence of a Δ^3-double bond.

In the ^{1}H-NMR spectrum of diacetoxy lactone **8** ($C_{19}H_{24}O_7$) in $CDCl_3$ (and also in C_6D_6) at room temperature, almost all resonances were rather broadened, due to a conformational exchange at intermediate rate (on the NMR time scale). Although most of the information regarding multiplicities was lost, the correlations observed in DQF COSY and PS NOESY (Figure 2) enabled spectral assignment and identification of **8** as 9α-acetoxycumambrin A.

The proposed structure of lactone **8** and the existence of conformational equilibrium was also confirmed by a low-temperature NMR ($CDCl_3$) study. At −57 °C, most of the broad multiplets (observed at room temperature) split into pairs of sharp, well-resolved signals (with a ratio of ca. 1:1.16 within each pair), connected by positive cross peaks in PS NOESY, thus indicating the existence of two conformers (**8A** and **8B**), with the latter slightly predominating. The ^{1}H- and ^{13}C-NMR data of **8A** and **8B**, obtained by first-order analysis with the aid of DQF COSY and HMQC (Table 3), were in accordance with different conformations of the seven-membered ring in these conformers.

The isomeric lactones **6** and **7** ($C_{17}H_{22}O_6$) were eluted as an inseparable mixture containing, according to ^{1}H NMR, ca. 85% of **6** and 15% of **7**. The assignment of the ^{1}H-NMR signal of both compounds (Table 2) was accomplished using DQF COSY of the mixture. Chemical shifts and couplings of H-8 and H-9 for **6** were typical for the 8α-hydroxy-9α-acetoxy arrangement (same as in **3**), leading to the structure of 9α-acetoxycumambrin B. In lactone **7** the NMR data of H-8 and H-9 were in agreement with that of 9α-hydroxycumambrin A (containing 8α-acetoxy-9α-hydroxy moiety, the same as in **2**). The proposed structures of **6** and **7** were confirmed by acetylation (Ac_2O/Py) of **6** + **7** to the co-occurring 8α,9α-diacetoxy lactone **8**. Lactone **6**, as did **8**, gave broad ^{1}H-NMR lines (although not to such an extent), also indicating conformational equilibrium at a medium rate. Lactone **7** gave well-resolved, narrow ^{1}H-NMR resonances and exhibited ^{1}H- and ^{13}C-NMR signals similar to those of conformer **8A** (Table 3), which could be explained by the existence of only one conformation in **7** (at room temperature). The NOEs H-14/H-9, H-14/H-8, and H-14/H-6, and the absence of NOE between

Table 4. ^{1}H- (300 MHz) NMR Data of Compounds **10**,[a] **11**,[a] and **12** (δ, mult., J in Hz)

H	**10** ($CDCl_3$)	**11** ($CDCl_3$)	**11**(C_5D_5N)	**12** (C_5D_5N)
1	3.78, m	3.74, m	4.14, m	4.22, m
2α	1.93, ddd, 13.9, 7.9, 2.1	1.91, ddd, 14.8, 7.9, 2.3	2.40, ddd, 14.2, 8.0, 2.4	2.81, ddd, 14.8, 7.5, 1.5
2β	2.30, ddd, 13.9, 7.5, 6.4	ca. 2.3, m	2.71, ddd, 14.2, 7.4, 6.2	2.57, ddd, 14.8, 7.5, 7.0
3	ca. 4.6[b]	4.63, br d, 7.0	4.93, br d, ca. 7	5.12[c]
6	4.65[b]	4.82, dq, 10.8, ca. 1.7	5.24, br d, 11.2	5.21, br d, 10.8[c]
7	3.2, br t, ca. 10	3.65, dddd, 10.8, 10.2, 3.3, 2.9	3.69, dddd, 11.2, 10.4, <3, 3.6	3.83, dddd, 10.8, 10.7, 3.4, 3.0
8α	2.42, ddd, 16.4, 4.0, ca. 2			
8β	1.71, ddd, 16.4, 11.6, 2.5	5.22, br d, 10.2	5.63, dd, 10.4, 2.6	5.63, dd, 10.7, 2.5
9	5.07, dd, 4.1, 2.5	5.31, br s	5.93, d, 2.6	5.92, d, 2.5
13′	5.49, d, 3.1	5.88, d, 2.9	5.99, d, <3	5.98, d, 3.0
13	6.27, d, 3.4	6.35, d, 3.3	6.43, d, 3.6	6.41, d, 3.4
14	1.00, s	1.10, s	1.34, s	1.32, s
15	1.99, t, 1.7	2.0, t, 1.7	2.27, br s	2.20, br s
OAc	2.18 s	2.10, 2.22, 2 × s	2.02, 2.16, 2 × s	2.00, 2.13, 2 × s
OOH				13.6, br s

[a] Assigned by DQF COSY and PS NOESY. [b] Overlapped signals; in C_6D_6: H-3, δ 4.20, br d, 8.2 Hz, and H-6, δ 3.69, br d, 11.2 Hz. [c] Partially overlapped with signal of H_2O from C_5D_5N.

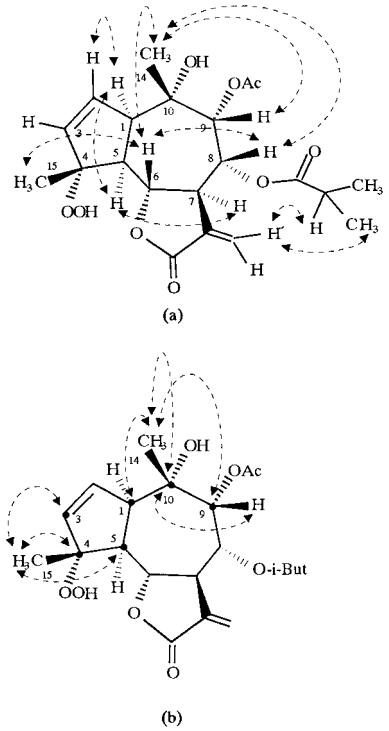


Figure 1. Significant correlations observed in (a) PS NOESY and (b) HMBC of **5**.

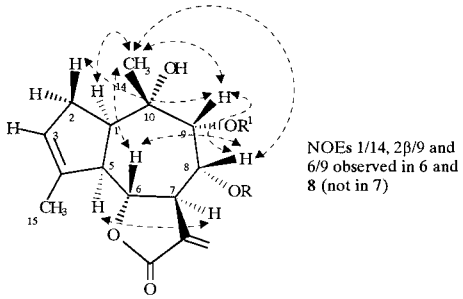


Figure 2. Significant NOEs in PS NOESY of **6** (R = H, R^1 = Ac), **7** (R = Ac, R^1 = H) and **8** (R = R^1 = Ac).

H-9 and H-6 (detected in **8** and **6**, at room temperature, Figure 2) in **7**, as well as the chemical shift of C-14 (δ 21.7 and 21.0 in **8A** and **7**, respectively) typical for a (pseudo)axial methyl, were in agreement with a distorted C_s chair conformation of seven-membered rings with the plane of symmetry passing through C-8 and midway through the C-1 and C-5 bond, similar to that in 10-*epi*-8-deoxycumambrin measured in the solid state by X-ray crystallography.[33] The similarity of the ^{1}H- and ^{13}C-NMR data of compound **6** (Table 2) to those of conformer **8B** (Table 3) indicated the predominance of this conformation in **6**. The chemical shift of C-14 in **6** and **8B**, i.e. δ 27.8 and 27.9, respectively, typical for a (pseudo)equatorial methyl, and the NOEs H-9/H-6 and H-9/H-2β in **8** and **6** could be explained by a twisted chair C_2 conformation of seven-membered ring, in **8B** (and in the major conformer of **6**) similar to that observed previously by X-ray measurement of some crystalline guaianolides, such as cumambrin A[31] and euparotin bromoacetate.[34] A more detailed conformational analysis of **7** and **8**, evaluation of geometry of the conformers (e.g., **8A** and **8B**), and determination of the corresponding kinetic parameters are in progress.

Lactone **9**, with the same molecular formula ($C_{21}H_{28}O_9$) as **5**, was the only member of the Δ^3-group containing a hydroperoxy function. The constitution and stereochemistry of the part comprising the seven-membered and lactone rings in **9** was analogous to that in **5**. This proposal was based on the almost identical interproton coupling patterns (Table 2) and PS NOESY data (i.e., H-1/H-5, H-5/H-7, H-6/H-14, H-8/H-14, and *i*-Bu/H-13′) of **9** to those in **5** (see Figure 1a). The same substitution type (i.e., 8α-isobutyryloxy-9α-acetoxy) was observed previously in the closely related guaianolide, hydruntinolide B, isolated from *Anthemis hydruntina*.[18] The position of the hydroperoxy group in **9** was assigned to C-2 on the basis of its characteristic chemical shift (δ ca. 91.7) and a direct (HMQC) correlation to H-2 (δ 5.28, br s). A cross peak between H-2 and H-14 observed in the PS NOESY of **9**, together with a negligible vicinal H-1/H-2 scalar coupling, differing from the previously observed $J_{1a,2a}$ of 5.5−5.6 Hz in the structurally similar 1α−H,2β-OAc-Δ^3-guaianolides, hydruntinolides A−C,[18] was in accordance with 2β−H (and 2α-hydroperoxy) position.

The occurrence of a doublet for H-6 (δ ca. 4.7−4.8) with a fine structure caused by a small homoallylic coupling to H-15 (δ ca. 2.0) in the NMR ($CDCl_3$) spectra of lactones **10** ($C_{17}H_{22}O_6$) and **11** ($C_{19}H_{24}O_8$), named anthemolides C and D, respectively, indicated the 4-double bond in these molecules. In the NMR (C_5D_5N) spectra of lactones **12** ($C_{19}H_{24}O_9$, anthemolide E) and **11**, both signals were shifted downfield (Table 4). The presence of a 4-double bond was also confirmed by the absence of a signal due to H-5 in the ^{1}H-NMR spectra of the members of this group, and the detection of

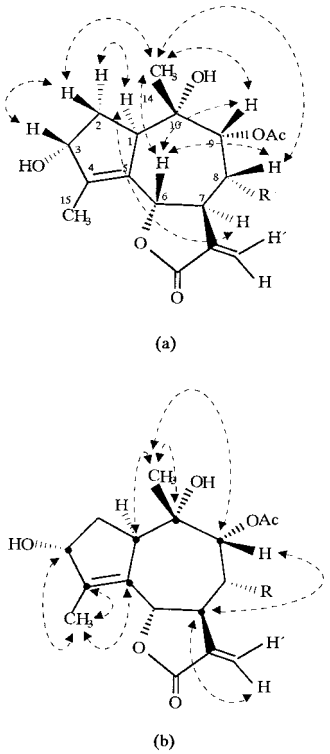


Figure 3. Significant correlations observed in (a) PS NOESY and (b) HMBC of **10** (R = H) and **11** (R = OAc).

signals of two nonprotonated olefinic carbons in the HMBC of **10** and **11** (Figure 3b) at δ ca. 145.0 and 132.0 (in $CDCl_3$), assigned, according to characteristic chemical shifts,[35] to C-4 and C-5, respectively. Lactones **11** and **12** displayed rather similar ^{1}H-(C_5D_5N) NMR spectra, containing two three-proton acetoxy singlets and a pair of characteristic downfield signals of protons adjacent to the acetoxy groups, assigned as H-8β and H-9β by the similarity of their NMR data to those of the same protons in 8α,9α-diacyloxy lactones **4** and **5** (see Table 1). The proposed 8α,9α-diacetoxylation pattern was also verified by DQF COSY and PS NOESY of **11** (Figure 3). The only structural difference between **11** and **12** was in the oxygen function attached to C-3, that is, OH and OOH, respectively. Placement of the OH group at the 3-position in **11** was based on a one-proton signal in its ^{1}H- ($CDCl_3$) NMR spectrum typical for a carbinol proton of an allylic secondary alcohol (δ 4.63, br d, J = 7.0 Hz) coupled (according to DQF COSY) to protons of an adjacent methylene (resonating in the region of δ 1.9−2.3) assigned as H-2α and H-2β. The 3β−H configuration (and 3α−OH) was deduced from the cross peaks in PS NOESY (Figure 3a) of this proton to H-2β and H-14 and corroborated by the observed vicinal couplings ($J_{2\alpha,3}$ = 2.3 Hz and $J_{2\beta,3}$ = 7.0 Hz). In the ^{1}H- (C_5D_5N) NMR spectrum of hydroperoxy lactone **12** the signals attached to the cyclopentene rings (i.e., H-3 and H-2) showed rather similar couplings, but different chemical shifts. The signal of H-3 in **12** in comparison to the chemical shift of H-3 in **11**, exhibited a downfield shift ($\Delta\delta$ = 0.19 ppm), indicating C-3 as the position of the hydroperoxy group. The 3α-configuration of the OOH group followed from the observed downfield shift of H-2α ($\Delta\delta$ = 0.41 ppm) in **12** in comparison to the chemical shift of the same proton in the 3α-hydroxy lactone **11**. Downfield shifts, after substitution of OH with OOH, of the *syn* protons at the adjacent carbon were observed previously.[27]

Contrary to **11** and **12**, lactone **10** was not oxygenated at C-8. A pair of one-proton triple doublets was assigned, according to characteristic chemical shifts and couplings (see Table 4) and 2D correlations (Figure 3), to the 8-methylene group. A proton (δ 5.07), adjacent to an acetoxy group, coupled to this methylene and dipolarly coupled to H-6 and H-14 (Figure 3a), was identified as 9β−H. The remaining scalar and dipolar couplings, as well as HMBC correlations observed for this part of the molecule (see Figure 3) fitted to the 10β-methyl-9α-acetoxy-pattern. The constitution and stereochemistry of the five-membered ring (i.e., 3α-hydroxy-1α-H) was based on the chemical shifts and couplings of H-3 and H-2 (almost the same as in **11**, see Table 4) and dipolar coupling H-3/H-2β (observed in PS NOESY, see Figure 3a).

Similar Δ^2-, Δ^3-, and Δ^4-guaianolides (without 9-functionality) have been isolated previously from some *Eriocephalus* species (tribe Anthemidae).[27] As far as the genus *Anthemis* is concerned, the only species yielding hydroperoxy lactones was *A. nobilis* (Roman camomile) with two lactones, such as 1β-hydroperoxyisonobilin (a germacranolide)[13,14] and 4α-hydroperoxyromanolide, a guaianolide closely related to our Δ^2-lactones (**2**−**5**),[14] isolated from the EtOH extracts of the blossoms.

Experimental Section

General Experimental Procedures. The spectra were recorded with the following instruments: IR, Perkin-Elmer FT-IR Spectrometer 1725 X; ^{1}H and ^{13}C (1D and 2D) NMR, Bruker AMX 300; DCIMS and HRCIMS (150 eV, isobutane), Finnigan MAT mass spectrometer 8230, double focusing (BE geometry); ESIMS (a sample + ammonium acetate, dissolved in MeOH−H_2O, 1:1), Finnigan MAT 900, double focusing (EB geometry) equipped with a Finnigan MAT electrospray interface; optical rotations, Perkin-Elmer 141 MC polarimeter. Elemental analyses were performed using the standard combustion (Pregl) method.

Plant Material. Plant material was collected during the flowering season (July 1994) at the north part of Sara Mountain, situated between Serbia and Macedonia (location Lavlja vrata, altitude of ca. 1900 m). Voucher specimen (no. 210794AC) was deposited in the herbarium of The Institute for Medicinal Plant Research "Dr. Josif Pancic", Belgrade.

Extraction Procedure. A crude extract (35.8 g) of air-dried aerial parts (1060 g) was obtained by extraction with Et_2O−petroleum−MeOH (1:1:1) at room temperature (24 h), followed by treatment with MeOH to remove long-chain saturated hydrocarbons, using the usual procedure.[23]

Isolation Procedure. A solution of the crude extract (35.5 g) in $CHCl_3$ (200 mL) was added to 130 g of Si gel 60 (Merck), 0.063−0.200 mm, and dried on a rotavap. The adsorbed mixture was applied to a Si gel column, and the elution was started with petroleum. The polarity of the solvent was gradually increased by addition of Et_2O.

Fraction A (690 mg) eluted with petroleum-Et_2O (7:3), after repeated Si gel column chromatography (C_6H_6−Et_2O−MeOH, 7:2:1) and crystallization (EtOAc) afforded lactones **5** and **9** (10 and 7 mg, respectively).

Lactones **1** (2 mg) and **10** (2 mg) were isolated from fraction B (350 mg), eluted with petroleum−Et_2O (1:1), by repeated Si gel column chromatography (C_6H_6−Et_2O−MeOH, 7:2:1), followed by preparative TLC (Kieselgel 60 GF_{254}, layer thickness 0.25 mm, CH_2Cl_2−MeOH, 19:1, two developments).

Fraction C eluted with Et_2O (1.1 g) yielded, by Si gel column chromatography (C_6H_6−Et_2O−MeOH, 7:2:1) fractions I (51 mg) and II (610 mg). Lactones **4** (2.5 mg) and **11** (13 mg) were isolated from fraction I by repeated column chromatography (same conditions as above). Fraction II, purified by repeated column chromatography (same conditions as above), followed by preparative TLC (C_6H_6−Et_2O−MeOH, 7:2:1, three developments) afforded lactone **8** (5 mg) and a fraction (with lower R_f value) that, after two preparative TLCs, [(i.e., C_6H_6−EtOAc, 1:1 two developments) and then C_6H_6−Et_2O−MeOH, 7:2:1 (three developments)] yielded lactone **12** (2.4 mg).

Fraction D (1.9 g) eluted with Et_2O and yielded, upon repeated column chromatography (C_6H_6−Et_2O−MeOH, 7:2:1), lactone **2** (8.5 mg) and a fraction (with higher R_f value) whose less polar part (taken as emulsion in *n*-hexane) was subjected to further purification. A portion (ca. 25 mg, after evaporation of *n*-hexane) afforded lactone **3** (3.4 mg) by LC (Lichroprep Si 60, Si gel, 40−63 mm, at 2−6 bar, petroleum−EtOAc, 2:8). The remaining portion of this fraction was purified by three preparative TLCs (two developments in each case), carried out in the following order: (i) C_6H_6−Me_2CO, 1:1, (ii) C_6H_6−Et_2O−MeOH, 7:2:1, and (iii) CH_2Cl_2−MeOH, 19:1, to yield a mixture (1.5 mg) of lactones **6** and **7** in the ratio of 8.5:1.5, respectively.

Anthemolide A (1): colorless gum; IR (film) ν_{max} 3401 (OH), 1750 sh (C=O, α,β-unsaturated γ-lactone, 1739 (C=O, acetate), 1653 (C=C),1373, 1260, 1090, 1043 cm^{-1}; ^1H NMR (see Table 1); HRCIMS *m/z* $[M + H]^+$ 323.1499 (calcd for $C_{17}H_{23}O_6$ 323.1495); DCIMS *m/z* $[M + H]^+$ 323 (14), $[M + H - 18]^+$ 305 (68), $[M + H - 2 \times 18]^+$ 287 (100), $[M + H - 18-60]^+$ 245 (3), $[M + H - 2 \times 18-60]^+$ 227 (29).

8-*O*-Acetylanthemolide B (2): white solid; mp 111 °C; $[\alpha]^{25}_D$ +16° (*c* 0.06, Me_2CO); anal. calcd for $C_{17}H_{22}O_8$, C 57.62, H 6.26; found C 57.51, H 6.35; IR (dry film) ν_{max} 3474, 3295 (OH, OOH), 3057 (C=C−H), 1752 (C=O, α,β-unsaturated γ-lactone), 1720 (C=O, acetate), 1654 (C=C), 1373, 1272, 1256, 1237, 1195, 1166, 1038 cm^{-1}; ^1H NMR (see Table 1); DCIMS *m/z* $[M + H]^+$ 355 (60), $[M + H - 16]^+$ 339 (74), $[M + H - 18]^+$ 337 (100), $[M + H - 34]^+$ 321 (97), $[M + H - 2 \times 18]^+$ 319 (19.5), $[M + H - 34-18]^+$ 303 (15), 169 (64).

9-*O*-Acetylanthemolide B (3): colorless gum; IR (film) ν_{max} 3418 (OH, OOH), 1745 (C=O, α,β-unsaturated γ-lactone + acetate), 1657 (C=C), 1371, 1237, 1153, 1052; ^1H NMR (see Table 1); HRCIMS *m/z* $[M + H]^+$ 355.1385 (calcd for $C_{17}H_{23}O_8$ 355.1393); ESIMS *m/z* $[M + NH_4]^+$ 372 (100).

8,9-di-*O*-Acetylanthemolide B (4): white solid; mp 96 °C; $[\alpha]^{25}_D$ −8° (*c* 0.22, MeOH); IR (dry film) ν_{max} 3416 (OH, OOH), 1748 (C=O, α,β-unsaturated γ-lactone + acetate), 1658 (C=C), 1371, 1244, 1158, 1098, 1058 cm^{-1}; ^1H NMR (see Table 1); HRCIMS *m/z* $[M + H]^+$ 397.1507 (calcd for $C_{19}H_{25}O_9$ 397.1498); DCIMS *m/z* $[M + H]^+$ 397 (11), $[M + H - 16]^+$ (21), $[M + H - 18]^+$ 379 (14), $[M + H - 34]^+$ 363 (26), $[M + H - 34-18]^+$ 345 (27), 295 (30), 257 (42), 211 (72.5), 199 (100).

8-*O*-Isobutyryl-9-*O*-acetylanthemolide B (5): colorless crystals (from EtOAc); mp 168 °C; $[\alpha]^{25}_D$ −42° (*c* 0.12, MeOH); *anal.* C 59.30%, H 6.71%, calcd for $C_{21}H_{28}O_9$, C 59.42%, H 6.65%; IR (dry film) ν_{max} 3428 (OH, OOH), 1732 (C=O, α,β-unsaturated γ-lactone + ester), ∼1640 (C=C), 1385, 1261, 1096, 1026 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (C_5D_5N, 75.4 MHz) δ 51.18 (C-1), 133.51 (C-2), 137.80 (C-3), 96.18 (C-4), 47.71 (C-5), 78.46 (C-6), 42.86 (C-7), 72.65 (C-8), 79.22 (C-9), 73.62 (C-10), 137.03 (C-11), 169.70 (C-12), 22.95 (C-14), 20.33 (C-15) 175.83, 34.22, 18.90, 18.71 (*i*-Bu), 170.75, 20.92 (OAc); DCIMS *m/z* $[M + H]^+$ 425 (27), $[M + H - 16]^+$ 409 (18), $[M + H - 18]^+$ 407 (47), $[M + H - 34]^+$ 391 (100), $[M + H - 34-18]^+$ 373 (83), $[M + H - 18-34-60]^+$, 313 (12), 285 (19), 242 (20.5).

A mixture of 9α-Acetoxycumambrin B (6) and 9α-Hydroxycumambrin A (7) (8.5:1.5): white solid; IR (dry film) ν_{max} 3457 (OH), 3041 (=C−H), 1747 (C=O, α,β-unsaturated γ-lactone + acetate), 1661 (C=C), 1371, 1273, 1240, 1147, 1111, 1077, 1037; HRCIMS *m/z* $[M + H]^+$ 323.1492 (calcd for $C_{17}H_{23}O_6$ 323.1495); DCIMS *m/z* $[M + H]^+$ 323 (39), $[M + H - 18]^+$ 305 (53.5), $[M + H - 60]^+$ 263 (51), $[M + H - 18-60]^+$ 245 (100), $[M + H - 60-2 \times 18]^+$ 227 (76), 197 (32), 181 (22); ^1H NMR (see Table 2); ^{13}C NMR ($CDCl_3$, detected via HMQC and HMBC correlations), lactone **6** δ 56.4 (C-1), 34.3 (C-2), ca. 125 (C-3), 143.1 (C-4), 55.5 (C-5), 80.3 (C-6), ca. 50 (C-7), 73.5 (C-8), 71.8 (C-9), 169.8 (C-12), 122.9 (C-13), 27.8 (C-14), 19.2 (C-15), 21.0, 169.8 (OAc); lactone **7** δ ca. 43 (C-1), ca. 33 (C-2), ca. 127 (C-3), ca. 54 (C-5), ca. 81 (C-6), ca. 42 (C-7), ca. 73 (C-8), ca. 78 (C-9), 169.8 (C-12) ca.125 (C-13), 21.0 (C-14), 17.5 (C-15), ca. 21, 169.8 (OAc).

9α-Acetoxycumambrin A (8): white solid; mp 122 °C; $[\alpha]^{25}_D$ +43° (*c* 0.07, Me_2CO); *anal.* C 62.51%, H 6.72%, calcd for $C_{19}H_{24}O_7$, C 62.62%, H 6.64%; IR (dry film) ν_{max} 3477 (OH), 1748 (C=O, α,β-unsaturated γ-lactone + OAc), 1661 (C=C), 1371, 1249, 1149, 1096, 1063, 1039 cm^{-1}; ^1H NMR (see Tables 2 and 3); ^{13}C NMR ($CDCl_3$, detected via HMQC and HMBC correlations, see also Table 3) δ 53.7 (C-1), 33.2 (C-2), 126.2 (C-3), 141.8 (C-4), 53.7 (C-5), ca. 79.8 (C-6), ca. 45 (C-7), ca. 71.7 (C-8 and C-9) 77.1 (C-10), 169.8, or 169.0 (C-12), ca. 122 (C-13), ca. 25 (C-14), 17.3 (C-15), 20.8, 20.5, 169.8, and/or 169.0, (OAc), DCIMS *m/z* $[M + H]^+$ 365 (100), $[M + H - 18]^+$ 347 (27), $[M + H - 60]^+$ 305 (31), 227 (26).

2a-Hydroperoxy-8-*O*-isobutyryl-9α-acetoxycumambrin B (9): white solid; mp 135 °C; $[\alpha]^{25}_D$ −39° (*c* 0.18, MeOH); *anal.* C 59.32%, H 6.70%, calcd for $C_{21}H_{28}O_9$, C 59.42%, H 6.65%; IR (dry film) ν_{max} 3450 (OH, OOH), 1745 (C=O, α,β-unsaturated γ-lactone + ester), 1661 (C=C), 1370, 1274, 1232, 1148, 1100, 1061, 1019 cm^{-1}; ^1H NMR (see Table 3); ^{13}C NMR ($CDCl_3$, detected via HMQC and HMBC correlations) δ ca. 49 (C-1), 91.7 (C-2), 124.6 (C-3), 150.9 (C-4), 52.5 (C-5), ca. 80 (C-6), ca. 43 (C-7), ca. 72 (C-8), ca. 79 (C-9), 170.3 (C-12), 124.6 (C-13), 22.7 (C-14), 17.5 (C-15), 18.7, 34.5, ca. 175 (*i*-But), 21.0, 170.3 (OAc); DCIMS *m/z* $[M + H]^+$ 425 (35), $[M + H - 16]^+$ 409 (96), $[M + H - 18]^+$ 407 (100), $[M + H - 34]^+$ 391 (58), $[M + H - 34-18]^+$ 373 (60), $[M + H - 60]^+$ 365 (9).

Anthemolide C (10): colorless gum; IR (film) ν_{max} 3441 (OH), 1752 (C=O, α,β-unsaturated lactone), 1731 (C=O, acetate), 1375, 1246, 1150, 1113, 1085, 1028 cm^{-1}; ^1H NMR (see Table 4); ^{13}C NMR ($CDCl_3$, detected via HMQC and HMBC correlations) δ ca. 49 (C-1) ca. 35 (C-2), ca. 80 (C-3), 144.4 (C-4), 132.5 (C-5), ca. 80 (C-6), ca. 40 (C-7), ca. 30 (C-8), ca. 77.5 (C-9), ca. 75.5 (C-10), ca. 120 (C-13), ca. 21 (C-14), 14.5 (C-15), ca. 22, 170.3 (OAc); HRCIMS *m/z* $[M + H]^+$ 323.1485 (calcd for $C_{17}H_{23}O_6$ 323.1495); DCIMS *m/z* $[M + H]^+$ 323 (7.5), $[M + H - 18]^+$ 305 (100), $[M + H - 2 \times 18]^+$ 287 (31), $[M + H - 60]^+$ 263 (5), $[M + H - 60-18]^+$ 245 (8.5), $[M + H - 60-2 \times 18]^+$ 227 (24).

Anthemolide D (11): white solid; mp 127 °C; $[\alpha]^{25}_D$ +23° (*c* 0.15, Me_2CO); *anal.* C 59.87%, H 6.46%, calcd for $C_{19}H_{24}O_8$, C 59.99%, H 6.36%; IR (dry film) ν_{max} 3452 (OH), 1746 (C=O, α,β-unsaturated γ-lactone + OAc), 1657 (C=C), 1385, 1260, 1152, 1044 cm^{-1}; ^1H NMR (see Table 4); ^{13}C NMR ($CDCl_3$, detected via HMQC and HMBC correlations) δ 48.2 (C-1), ca. 35 (C-2), 80.2 (C-3), 145.0 (C-4), 131.6 (C-5), 77.9 (C-6), ca. 41 (C-7), 71.5 (C-8), 77.9 (C-9), 73.9 (C-10), ca. 170 (C-12), 124.6 (C-13), 20.4 (C-14), 13.6 (C-15), 20.4, ca. 170, 170.4 (OAc); DCIMS *m/z* $[M + H]^+$ 381 (14), $[M + H - 18]^+$ 363 (100), $[M + H - 2 \times 18]^+$ 345 (61), $[M + H - 60]^+$ 321 (55.5), $[M + H - 60-18]^+$ 303 (29), $[M + H - 60-2 \times 18]^+$ 285 (18.5).

Anthemolide E (12): colorless gum; IR (film) ν_{max} 3428 (OH, OOH), 1748 (C=O, α,β-unsaturated γ-lactone + OAc), 1658 (C=C), 1384, 1260, 1152, 1052 cm^{-1}; ^1H NMR (see Table 4); HRCIMS *m/z* $[M + H]^+$ 397.1488 (calcd for $C_{19}H_{25}O_9$ 397.1498); DCIMS *m/z* $[M + H]^+$ 397 (16), $[M + H - 16]^+$ 381 (15), $[M + H - 18]^+$ 379 (100), $[M + H - 34]^+$ 363 (29), $[M + H - 2 \times 18]^+$ 361 (16), $[M + H - 34-18]^+$ 345 (18), $[M + H - 60-18]^+$ 319 (18), $[M + H - 60-34]^+$ 303 (9.5), $[M + H - 60-34-18]^+$ 285 (14.5).

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References and Notes

(1) Heywood, V. H.; Humphries, C. J. In *Biology and Chemistry of the Compositae*; Heywood, V. M., Harborne, J. B., Turner, B. L., Eds.; Academic: New York, 1978; pp 852−898.
(2) Gajic, M. In *Flore de la Republique Socialiste de Serbie*; Josifovic, M., Ed.; Academie Serbe des Sciences et des Arts: Beograd, 1975; Vol. 7, pp 83−90.
(3) Greger, H. In *Biology and Chemistry of the Compositae*; Heywood, V. M., Harborne, J. B., Turner, B. L., Eds. Academic: New York, 1978; pp 899−941.
(4) Christensen, L. P. *Phytochemistry* **1992**, *31*, 7−49, and references therein.
(5) El-Alfy, T. S.; Shehata, A. H.; Koheil, M. A.; El-Dahmy, S. I. *Fitoterapia* **1989**, *60*, 556−558.
(6) Abou El-Ela, M.; Jakupovic, J.; Bohlmann, F.; Ahmed, A. A.; Seif El-Din, A.; Khafagi, S.; Sabri, N.; El-Ghazouly, M. *Phytochemistry* **1990**, *29*, 2704−2706.
(7) Bruno, M.; Diaz, J. G.; Herz, W. *Phytochemistry* **1991**, *30*, 3458−3460.
(8) Bohlmann, F.; Zdero, C. *Chem. Ber.* **1975**, *108*, 1902−1910.
(9) Benesova, V.; Herout, V.; Sorm, F. *Collect. Czech. Chem. Commun.* **1964**, *29*, 3096−3100.
(10) Benesova, V; Samek, Z.; Herout, V.; Sorm, F. *Tetrahedron Lett.* **1970**, 5017−5020.
(11) Holub, M.; Samek, Z. *Collect. Czech. Chem. Commun.* **1977**, *42*, 1053−1064.
(12) Samek, Z.; Holub, M.; Grabarczyk, H.; Drozdz, B.; Herout, V. *Collect. Czech. Chem. Commun.* **1977**, *42*, 1065−1068.
(13) Mayer, R.; Ruecker, G. *Arch. Pharm. (Weinheim)* **1987**, *320*, 318−322.
(14) Ruecker, G.; Mayer, R.; Lee, K. R. *Arch. Pharm. (Weinheim)* **1989**, 821−826.
(15) Sarg, T.; El-Dahmy, S.; Salem, S. *Sci. Pharm.* **1990**, *58*, 33−35.
(16) De Pascual Teresa, J.; Caballero, E.; Anaya, J.; Caballero, C.; Gonzalez, M. S. *Phytochemistry* **1986**, *25*, 1365−1369.
(17) De Pascual Teresa, J.; Anaya, J.; Caballero, E.; Caballero, M. C. *Phytochemistry* **1988**, *27*, 855−860.
(18) Di Benedetto, R.; Menichini, F.; Gacs-Baitz, E.; Delle Monache, F. *Phytochemistry* **1991**, *30*, 3657−3659.
(19) Holub, M.; Budesinsky, M.; Samek, Z.; Drozdz, B.; Grabarczyk, H.; Ulubelen, A.; Oksuz, S.; Rychlewska, U. *Collect. Czech. Chem. Commun.* **1982**, *47*, 670−675.
(20) Baruah, R. N.; Bohlmann, F.; King, R. M. *Planta Med.* **1985**, *6*, 531−532.
(21) Bohlmann, F.; Zdero, C.; Grenz, M. *Terahedron Lett.* **1969**, 2417−2418.
(22) Greger, H. *Naturwissenschaften* **1969**, *56*, 467−468.
(23) Bohlmann, F.; Zdero, C.; King, H. R.; Robinson, E. H. *Phytochemistry* **1984**, *23*, 1979−1988.
(24) Schwarz, H.; Schiebel, H.-M. In *The Chemistry of Peroxides*; Patai S., Ed.; John Wiley & Sons: Chichester, 1983; Chapter 4, pp 113−115.
(25) Knappe, E.; Peteri, D. *Z. Anal. Chem.* **1962**, *190*, 386−389.
(26) Yoshioka, H.; Mabry, T. J.; Irwin, M. A.; Geissman, T. A.; Samek, Z. *Tetrahedron* **1971**, *27*, 3317−332.
(27) Zdero, C.; Bohlmann, F.; Mueller, M. *Phytochemistry* **1987**, *26*, 2763−2775.
(28) Budesinsky, M.; Saman, D. In *Annual Reports on NMR Spectroscopy*; Academic: New York, 1995; Vol. 30, p 367, and references therein.
(29) Irwin, M. A.; Geissman, T. A. *Phytochemistry* **1969**, *8*, 305−311.
(30) Geissman, T. A.; Griffin, S.; Waddell, T. G. *Phytochemistry* **1969**, *8*, 145−150.
(31) Davies-Coleman, M. T.; English, R. B.; Rivet, D. E. A. *Phytochemistry* **1992**, *31*, 2165−2167.
(32) Haruna, M.; Kato, M.; Ito, K.; Nikai, T.; Sugihara, H.; Murata, H. *Phytochemistry* **1981**, *20*, 2583−2584.
(33) Sosa, V. E.; Oberti, J. C.; Gil, R. R.; Ruveda, E. A.; Goedeken, V. L.; Gutierrez, A. B.; Herz, W. *Phytochemistry* **1989**, *28*, 1925−1929.
(34) McPhail, A. T.; Sim, G. A. *Tetrahedron* **1973**, *29*, 1751−1758.
(35) Budesinsky, M.; Saman, D. In *Annual Reports on NMR Spectroscopy*; Academic: New York, 1995; Vol. 30, pp 394−395, and references therein.

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