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Sesquiterpene lactones from *Achillea collina* J. Becker ex Reichenb

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Abstract

The flower heads of *Achillea collina* afforded 31 individual sesquiterpene lactones, among which nine guaianolides (**1**, **16**, **20**, **21**, **23**, **27**–**30**), a germacranolide (**18**) and a dimeric guaianolide (**31**) were found to be new natural products. The lactones **2**–**4**, **12**, **14**, **15**, **17**, **19** and **25** were isolated for the first time from the investigated species. Their structures were established by spectral methods.

Keywords: *Achillea collina*; Asteraceae; Sesquiterpene lactones; Guaianolides

1. Introduction

Achillea collina J. Becker ex Reichenb belonging to the *A. millefolium* group (Richardson, 1976) is widely used in folk medicine for the preparation of herbal teas with antiphlogistic activity (Wichtl, 2002). Phytochemical studies of the species have shown the presence of proazulene sesquiterpene lactones (Bahn-Nhu et al., 1979; Verzár-Petri et al., 1980; Kastner et al., 1991a), 3-oxa-guaianolides (Kastner et al., 1991c), highly oxygenated guaianolides (Glasl et al., 2001), matricarin and matricin derivatives (Glasl et al., 1994; Kubelka et al., 1999). It should be noted that all the studied so far taxa of *A. collina* are rich in guaianolides, but one for which are reported germacranolides and eudesmanolides only (Mustakerova et al., 2002). Continuing our study on the chemical constituents of Bulgarian *Achillea* species we now report the sesquiterpene lactone profile of *A. collina*.

2. Results and discussion

The flower heads of *A. collina* were extracted and worked-up as described in Section 3 part to give 8α-acetoxy-11-*epi*-tannunolide C (**1**), 8α-acetoxytannunolide B (**2**) (Todorova et al., 2006), 8α-acetoxy-6-*epi*-tannunolide B (**3**) (Todorova et al., 2006), dehydroleucodin (**4**) (Bohlmann and Zdero, 1972), 8α-angeloxyartabsin (**5**) (Verzár-Petri et al., 1980; Schroeder et al., 1994), 8α-tigloxyartabsin (**6**) (Kastner et al., 1991a; Schroeder et al., 1994), achillicin (**7**) (Verzár-Petri et al., 1980; Schroeder et al., 1994), 8α-angeloxy-3-oxa-artabsin (**8**) (Kastner et al., 1991c; Schroeder et al., 1994), 8α-tigloxy-3-oxa-artabsin (**9**) (Kastner et al., 1991c; Schroeder et al., 1994), 3-oxa-achillicin (**10**) (Kastner et al., 1991c; Schroeder et al., 1994), matricarin (**11**) (Ahmed et al., 2003), 8-desacetyl-8α-angeloyl-4-*epi*-matricin (**12**) (Todorova et al., 2006), 8-desacetyl-8α-tigloyl-4-*epi*-matricin (**13**) (Kastner et al., 1991b), matricin (**14**) (Flaskamp et al., 1982), 4-*epi*-matricin (**15**) (Appendino and Gariboldi, 1982), 5α-hydroxymatricarin (**16**), 3β-hydroxy-11αH, 13-dihydrocostunolide (**17**) (Glasl et al., 2002), 8α-acetoxy-3β-hydroxy-11(αH),13-dihydrocostunolide (**18**), 3β-hydroxy-13-acetoxygermacra-1(10)*E*,4*E*, 7(11)-trien-12,6α-olide (**19**) (Zdero and Bohlmann, 1990), 8α-isobutyryloxy-11(βH),

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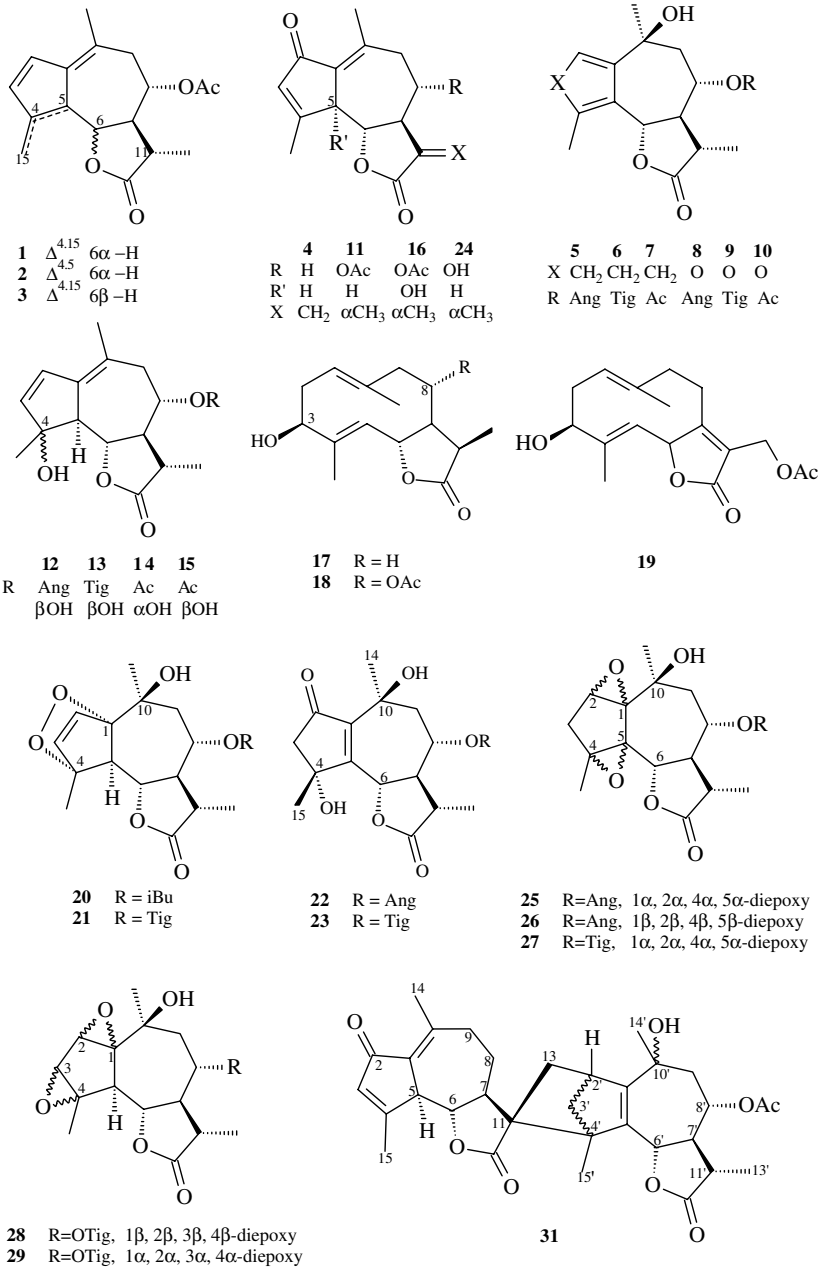
13-dihydro-10-*epi*-tanaparthin-α-peroxide (**20**), 8α-tigloyloxy-11(βH),13-dihydro-10-*epi*-tanaparthin-α-peroxide (**21**), 4α,10β-dihydroxy-8α-angeloxy-2-oxo-6βH,7α H, 11βH-1(5)-guaien-12,6α-olide (**22**) (Glasl et al., 2001), 4α,10β-dihydroxy-8α-tigloyloxy-2-oxo-6βH,7αH,11β H-1(5)-guaien-12,6α-olide (**23**), desacetylmatricarin (**24**) oxy-10β-hydroxy-6βH,7αH,11βH-12,6α-guaianolide (**29**), 1α,2α,3α,4α-diepoxy-8α-angeloyloxy-10β-hydroxy-6βH, 7αH,11βH-12,6α-guaianolide (**30**), and achicollinolide (**31**). From aforementioned products, compounds **1**, **16**, **18**, **20**, **21**, **23**, **27**–**31** have not been reported in the bibliography.



(Ahmed et al., 2003), 1α,2α,4α,5α-diepoxy-8α-angeloxy-10β-hydroxy-6βH,7αH,11βH-12,6α-guaianolide (**25**) (Todorova et al., 2006) and 1β,2β,4β,5β-diepoxy-8α-angeloxy-10β-hydroxy-6βH,7αH,11β H-12,6α-guaianolide (**26**) (Glasl et al., 2001), 1α,2α,4α,5α-diepoxy-8α-tigloyloxy-10β-hydroxy-6βH,7αH,11β H-12,6α-guaianolide (**27**), 1β,2β, 3β,4β-diepoxy-8α-tigloyloxy-10β-hydroxy-6βH,7αH,11βH-12,6α-guaianolide (**28**), 1α,2α,3α,4α-diepoxy-8α-tigloyl-

The ^{1}H NMR spectrum of **1** (Table 1) indicated the presence of an unsaturated guaianolide with a 2,3-double bond (a pair of doublets at δ 6.38 and 6.56 ppm), an exocyclic methylene (broad singlets at δ 5.17 and 5.24 ppm) and a tetrasubstituted double bond (δ 1.93 ppm, C-14 methyl group). The ^{1}H NMR data were found to be similar to those reported for tannunolide C (Barrero et al., 1990) with exception of the stereochemistry at C-11 and additional signals

Table 1
^{1}H (250 MHz) NMR data of lactones **1**, **16**, **20**, **21**, **23** and **27**–**30**

H	**1** ($CDCl_3$)	**16** ($CDCl_3$)	**20** ($CDCl_3$)	**21** ($CDCl_3$)	**23** (acetone-d_6)	**27** (acetone-d_6)	**28** ($CDCl_3$)	**29** ($CDCl_3$)	**30** ($CDCl_3$)
2	6.38 *d*	–	6.65 *d*	6.67 *d*	–	3.78 *d*	3.62 *d*	3.63 *brs*	3.65 *brs*
3	6.56 *d*	6.21 *brs*	6.29 *d*	6.30 *d*	2.56 *d*	1.65 *dd*	3.38 *d*	3.29 *brs*	3.29 *brs*
					2.62 *d*	1.97 *d*			
5	3.45 *d*	–	2.38 *d*	2.42 *d*	–	–	2.20 *d*	2.42 *d*	2.43 *d*
6	3.67 *t*	3.96 *d*	3.80 *t*	3.83 *t*	5.78 *d*	5.56 *d*	4.31 *t*	4.50 *t*	4.50 *t*
7	2.55 *ddd*	3.10 *ddd*	2.30 *m*	2.30 *m*	2.72 *ddd*	2.43 *ddd*	2.18 *ddd*	2.40 *m*	2.38 *ddd*
8	4.76 *dt*	4.85 *dt*	5.22 *ddd*	5.29 *ddd*	5.58 *ddd*	5.57 *ddd*	5.18 *ddd*	5.40 *ddd*	5.40 *ddd*
9	2.25 *dd*	3.23 *dd*	2.28 *dd*	2.25 *dd*	2.22 *dd*	2.05 *dd*	2.24 *dd*	2.12 *dd*	2.18 *dd*
9′	2.15 *dd*	2.25 *dd*	2.03 *dd*	2.06 *dd*	2.10 *dd*	1.90 *dd*	2.05 *dd*	2.01 *dd*	2.00 *dd*
11	2.48 *dq*	2.50 *dq*	2.35 *dq*	2.35 *dq*	2.80 *dq*	2.70 *dq*	2.65 *dq*	2.55 *dq*	2.60 *dq*
13	1.33 *d*	1.35 *d*	1.20 *d*	1.19 *d*	1.22 *d*	1.22 *d*	1.30 *d*	1.28 *d*	1.29 *d*
14	1.93 *brs*	2.45 *s*	1.61 *s*	1.63 *s*	1.63 *s*	1.14 *s*	1.42 *s*	1.26 *s*	1.26 *s*
15	5.17 *brs*	2.28 *s*	1.70 *s*	1.71 *s*	1.72 *s*	1.51 *s*	1.52 *s*	1.55 *s*	1.55 *s*
15′	5.24 *brs*								
R	2.10 *s*	2.12 *s*	2.58 *qq*	6.89 *m*	6.94 *m*	6.96 *m*	6.89 *m*	6.89 *m*	6.18 *qq*
			1.20 *d*	1.84 *d*	1.85 *d*	1.85 *d*	1.84 *d*	1.84 *d*	2.02 *dq*
			1.21 *d*	1.86 *brs*	1.87 *brs*	1.87 *brs*	1.86 *brs*	1.86 *brs*	1.88 *dq*
					4.58 *brs* (OH)	4.85 *brs* (OH)			
					3.95 *brs* (OH)				

J (Hz): **1**: 2,3 = 5.5; 5,6 = 6,7 = 7,8 = 8,9 = 10.4;8,9′ = 2.2; 7,11 = 12.0, 9,9′ = 12.5; 11,13 = 6.8; **16**: 6,7 = 7,8 = 7,11 = 8,9 = 10.9; 8,9′ = 2.0; 9,9′ = 13.3; 11,13 = 7.0; **20** and **21**: 2,3 = 5.5; 5,6 = 6,7 = 7,8 = 7,11 = 10.3; 8,9 = 5.5;8,9′ = 2.0; 9,9′ = 14.5; 11,13 = 6.8; **23**: 3,3′ = 18.9; 6,7 = 7,8 = 8,9′ = 10.6; 7,11 = 12.0; 8,9 = 2.0; 9,9′ = 14.8; 11,13 = 6.5; **27**: 2,3 = 3.0; 3,3′ = 18.0; 6,7 = 7,8 = 8,9′ = 7,11 = 10.3; 8,9 = 4.5; 9,9′ = 14.8; 11,13 = 6.5; **28**: 2,3 = 1.1; 5,6 = 6,7 = 10.6; 7,11 = 11.3; 7,8 = 8,9′ = 9.5; 8,9 = 2.9; 9,9′ = 14.5; 11,13 = 6.8; **29** and **30**: 5,6 = 6,7 = 7,11 = 11.7; 7,8 = 11.0; 8,9′ = 4.3; 8,9 = 7.2; 9,9′ = 15.0, 11,13 = 6.8; OTig: 3″,4″ = 7.0; 3″,5″ = 4″,5″ = 1.2; OiBu: 2″,3″ = 2″,4″ = 7.0; OAng: 3″,4″ = 7.0; 3″,5″ = 4″,5″ = 1.2.

for an acetoxy group at C-8. The *trans*-disposition of H-6, H-7 and H-8 followed from the large vicinal coupling constants ($J_{6,7} = J_{7,8} = 10.4$ Hz) and was confirmed by the observed strong NOE between H-6 and H-8. The α-orientation of the methyl group at C-11 clearly followed from the value of the vicinal coupling $J_{7,11}$ (12.0 Hz) as well as from NOE interactions H-6/H-11 and H-8/H-11 in NOESY spectrum. Thus, lactone **1** was identified as 8α-acetoxy-11-*epi*-tannunolide C.

The HRMS (EI) of compound **16** revealed a peak at *m*/*z* 320.1261 $[M]^+$, corresponding to a molecular formula $C_{17}H_{20}O_6$. The signals for two vinylic methyls (δ 2.45 and 2.28 ppm) as well as for an olefinic proton (δ 6.21 ppm) in the ^{1}H NMR spectrum (Table 1) of **16** indicated the presence of a guaianolide closely related to matricarin (**11**) (Ahmed et al., 2003). As the H-6 signal was a doublet, a hydroxy group at C-5 was very likely. Its α-orientation followed from deshieldings of H-7 (δ 3.10 ppm) and H-9 (δ 3.23 ppm). ^{13}C NMR data (Table 2) were also in agreement with proposed structure of 5α-hydroxymatricarin (**16**).

The ^{1}H NMR data of **18** (Table 3) revealed a germacranolide structure very similar to that of lactone **17**. However, an additional signal at high-frequency (δ 5.08 ppm) as well as a singlet at δ 2.08 ppm indicated the presence of an acetoxy group. Its location at C-8 followed from ^{1}H–^{1}H COSY experiments, while the relative stereochemistry was assigned on the basis of the values of the vicinal coupling constants and observed NOE between H-6 and H-8 in the NOESY spectrum. ^{13}C NMR data (Table 3) also confirmed the proposed structure. Thus, germacranolide **18** was identified as 8α-acetoxy-3β-hydroxy-11α,13-dihydro- costunolide.

Table 2
^{13}C (100 MHz) NMR data of lactones **16**, **21**, **28**–**30** in $CDCl_3$ and **23** in acetone-d_6

C[a]	**16**	**21**	**23**	**28**	**29**	**30**
1	135.1	99.0	143.8	71.8	72.0	71.7
2	192.8	134.1	206.5	56.1	56.6	56.7
3	135.9	136.1	53.6	57.5	58.3	58.3
4	169.8	94.3	76.6	67.5	75.1	70.5
5	78.4	69.6	164.8	44.3	49.7	49.9
6	82.8	75.5	76.6	77.2	74.7	78.9
7	50.7	51.7	53.9	57.9	53.8	53.8
8	71.2	71.8	73.8	68.5	71.2	70.5
9	42.3	42.5	42.6	50.1	42.9	43.4
10	148.1	71.2	71.7	78.2	78.8	78.2
11	40.5	42.1	41.9	41.2	41.3	41.4
12	176.6	176.9	177.3	172.2	177.2	177.5
13	15.0	14.2	14.9	14.8	15.2	15.5
14	15.5	23.6	29.7	29.7	29.3	29.3
15	21.9	13.7	27.6	19.8	20.4	20.3
R	170.3	166.6	166.7	166.7	166.8	168.1
	21.1	138.9	139.3	138.2	139.1	141.0
		128.1	129.9	128.2	128.1	126.7
		14.6	16.3	14.5	14.6	20.8
		12.0	12.6	12.1	12.0	15.9

[a] Assigned by DEPT, HMQC and HMBC.

The glaucolide **19** was found for the first time in *Achillea* species. ^{13}C NMR data of **19** are given in Table 3 as they have not been reported previously.

The ^{1}H NMR data of the lactones **20** and **21** (Table 1) resembled those of the known 8α-acyloxy-11βH,13-dihydrotanaparthin-α-peroxides (Jakupovic et al., 1986). However, the shieldings of H-5 and H-7 and the deshieldings of H-2 and H-9 required β-orientation of the C-10-hydroxy

Table 3
^{1}H (250 MHz) of **18** and ^{13}C (62.8 MHz) NMR data of **18** and **19** in $CDCl_3$

H/C	**18**		**19**
	δ_H, *m* (*J*, Hz)	δ_C[b]	δ_C[b]
1	4.95 *brd* (12.0)	128.0	126.3
2	2.50 *m*[a]	34.9	34.9
2′	2.12 *m*		
3	4.23 *dd* (5.7, 10.6)	70.8	74.4
4		141.6	142.0
5	4.67 *brd* (9.8)	123.6	120.5
6	4.93 *t* (9.8)	77.8	80.8
7	2.55 *m*[a]	52.9	170.2
8	5.08 *dt* (3.0, 10.7)	76.0	26.0
9	2.20 *dd* (3.0, 12.7)	47.6	40.3
9′	2.50 *m*[a]		
10		134.2	135.8
11	2.83 *qu* (7.4)	39.7	124.9
12		178.5	170.6
13	1.18 *d* (7.4)	10.7	55.3
14	1.48 *s*	16.7	16.0
15	1.75 *brs*	12.0	11.2
$OCOCH_3$		169.8	172.5
$OCOCH_3$	2.08 *s*	21.1	20.8

[a] Overlapped signals.
[b] Assigned by DEPT, HSQC and HMBC.

group. The configuration at C-10 as well as at the other chiral centers was confirmed by the observed NOEs in NOESY spectra. Thus, NOE interactions between H-5 and H-7, H-5 and H-14, and H-7 and H-14 indicated that all these protons were orientated to the α-face of the molecule. On the other hand, the observed NOEs H-2/H-9, H-2/H-6, H-3/H-6, H-6/H-8, H-6/H-11 and H-8/H-11 indicated their syn-β-disposition and showed that C-2/C-3 double bond was situated above the plane of the molecule. Hence, the lactones **20** and **21** were identified as 8α-isobutyroyloxy-11βH,13-dihydro-10-*epi*-tanaparthin-α-per oxide and 8α-tigloyloxy-11βH,13-dihydro-10-*epi*-tanaparthin-α-peroxide, respectively.

The HRMS (ESI) analysis of **23** revealed the adduct ion at *m*/*z* 442.1829 $[M+CH_3CN+Na]^+$ compatible with a sesquiterpene lactone with a molecular formula $C_{20}H_{26}O_7$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) showed 2-oxo-4α,10β-dihydroxy-guaianolide structure very similar to that of lactone **22** (Glasl et al., 2001), but the angeloxy group at C-8 was replaced with a tigloxy moiety. The relative stereochemistry of **23** was confirmed by the observed NOE interactions between H-15/H6, H-6/H-8, H-6/OH (C-10) indicating their syn-β-orientation.

The HRMS (EI) of **27** revealed a molecular ion at *m*/*z* 378.1678 $[M]^+$ corresponding to a molecular formula $C_{20}H_{26}O_7$. ^{1}H NMR data (Table 1) indicated 1α,2α,4α,5α-diepoxyguaianolide structure very similar to that of lactone **25**, recently found in *A. asplenifolia* of Bulgarian origin (Todorova et al., 2006). However, the presence of a tiglate at C-8 instead an angelate residue followed from the typical NMR signals of the ester group. The relative stereochemistry of **27** was deduced from NOESY spectrum where NOE interactions H-6/OH (C-10), H-6/H-8 and H-6/H-15 indicated their syn-β-orientation.

The ^{1}H NMR data (Table 1) of **28** and **29** showed 1,2,3,4-diepoxyguaianolide structures related to those of the known 8α-acyloxy derivatives of 11βH,13-dihydroartecanin and 11βH,13-dihydrocanin, respectively (Jakupovic et al., 1988). However, the strong diamagnetic shifts of the H-5 and H-7 signals and paramagnetic shifts of the H-2, H-6, H-8 and H-14 signals as well as the lack of NOE correlation between H-6 and H-14 showed that **28** and **29** differed from the reported analogues (Jakupovic et al., 1988) in the configuration at C-10. It should be noted that NOE interaction between H-5 and H-15 required β-orientation of the oxirane rings in **28**, while NOE between H-6 and H-15 in addition to H-15/H-5 directed to α-disposition of the epoxides in **29**. These observed NOEs can be assumed as characteristic NMR features for differentiation of artecanin and canin derivatives. ^{13}C NMR data (Table 2) were also in agreement with the proposed structures. Thus, lactones **28** and **29** were identified as 8α-tigloyloxy-11βH,13-dihydro-10-*epi*-artecanin and 8α-tigloyloxy-11βH,13-dihydro-10-*epi*-canin, respectively.

The NMR data (Tables 1 and 2) of lactone **30** were almost identical with those of **29**, except for the signals of the ester group at C-8. The tigloyl side chain was replaced with angeloyl as deduced from characteristic NMR signals.

The EIMS of achicollinolide (**31**) exhibited a very weak molecular ion at *m*/*z* 550 $[M]^+$ corresponding to a molecular formula $C_{32}H_{38}O_8$ and significant peaks at *m*/*z* 306 ($C_{17}H_{22}O_5$) and *m*/*z* 244 ($C_{15}H_{16}O_3$) due to a spontaneous retro-Diels–Alder fragmentation of **31**. The dimeric guaianolide structure was further established by counting the number of resonances in ^{1}H and ^{13}C NMR spectra (Table 4) and then ascertaining the nature of the linkage by 2D NMR techniques (COSY, HMQC, HMBC and NOESY). The presence of dehydroleucodin (**4**) as one of the monomers could be easily deduced from the ^{1}H and ^{13}C NMR data (Bohlmann and Zdero, 1972). The second lactone of the dimeric molecule was assigned to be a 12,6-*trans*-guaianolide carrying a hydroxy group at C-10, an α-acetoxy group at C-8 and a tetrasubstituted C-1/C-5 double bond. The heteronuclear correlation between C-11 and H-15′ in the HMBC spectrum and homonuclear correlations H-2′/H-13a and H-2′/H-13b in the COSY spectrum provided strong evidence for the connection of two monomers via new carbon–carbon bonds formed between C-13/C-2′ and C-11/C-4′ as shown in Fig. 1. The observed clear NOE interactions between H-7/H-15′, H-6/H-13a and H-6/H-13b allowed the assignment of the configuration at C-11. Unfortunately, correlations seen in the NOESY spectrum of **31** were ambiguous in defining the stereochemistry at C-2′, C-4′ and C-10′. Achicollinolide (**31**) is the first representative of 2,4-linked dimeric guaianolides, formed apparently from Diels–Alder reaction of achillicin (**7**) as a dienophile with dehydroleucodin (**4**) as a diene.

The reported investigations on sesquiterpene lactones in the species of *A. millefolium* group allowed their division

Table 4
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of lactone **31** in $CDCl_3$

Position	δ_H, *m* (*J*, Hz)	δ_C [b]	Position	δ_H, *m* (*J*, Hz)	δ_C [b]
1	–	131.8	1′	–	150.9
2	–	196.4	2′	3.03 *brs*	42.4
3	6.17 *brs*	135.8	3′	1.45 *brd* (8.5) 1.47 *brd* (8.5)	53.4
4	–	170.5	4′	–	56.1
5	3.46 *d* (10.4)	52.4	5′	–	144.3
6	3.58 *t* (10.4)	81.6	6′	5.56 *d* (10.6)	79.6
7	2.55 *m*[a]	53.9	7′	2.28 *m*[a]	53.9
8	2.30 *m*[a] 2.45 *m*[a]	24.3	8′	5.35 *dt* (4.9, 4.9, 10.1)	72.8
9	2.30 *m*[a] 2.50 *m*[a]	36.7	9′	1.93 *dd* (4.9, 14.6) 2.25 *dd* (4.9, 14.6)	45.7
10	–	152.8	10′	–	71.2
11	–	60.16	11′	2.50 *m*[a]	42.5
12	–	179.0	12′	–	178.0
13	1.50 *m* 2.13 *dd* (3.5, 12.5)	34.7	13′	1.27 *d* (6.8)	14.8
14	2.42 *brs*	20.9	14′	1.48 *s*	28.6
15	2.25 *brs*	19.9	15′	1.49 *s*	17.1
OAc			OAc	2.09 *brs*	21.2 169.8

[a] Overlapped signals.
[b] Assigned by DEPT, HMQC and HMBC.

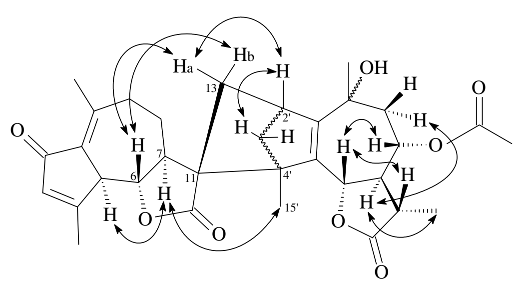


Fig. 1. Correlations seen in the NOESY spectrum of **31**.

into two subgroups based on the presence/absence of azulenogenic guaianolides. The proazulene containing subgroup includes *A. asplenifolia, A. roseoalba, A. ceretanica* and *A. collina* with common components artabsin and matricin derivatives. 3-Oxa-artabsin esters have also been detected in all the proazulene containing species (Kubelka et al., 1999; Rauchensteiner et al., 2002). The proazulene lactone pattern of each species differs in the proportion of the respective acyl ester moieties (Kubelka et al., 1999). Thus, the co-occurrence of acetyl, angeloyl and tigloyl esters (**5**–**10** and **12–15**) in the investigated sample corresponded to the literature data for *A. collina*. In addition, several structural types of nonazulenogenic lactones were isolated from the studied taxon, which have not been reported in *A. collina* previously but are components of some of the above discussed proazulene containing species. Thus, chemical analogues of matricarin have been identified in *A. roseoalba* (Kastner et al., 1991c) and *A. ceretanica* (Glasl et al., 1997) while germacranolides and glaucolides have been found only in *A. asplenifolia* so far (Todorova et al., 2006). Furthermore, compounds **22**, **23** and **25–27**, precursors of which are regarded to be guaia-1,4-dienolides **5–7** (Beauhaire and Fourrey, 1982) were isolated too. In addition, the lactones **20, 21** and **28–30** which are suggested to originate from the respective guaia-1,3-dienolides (Bohlmann and Zdero, 1982) were also identified. It should be noted, that these hypothetical precursors have not been found in the studied sample. The literature survey revealed that 1,4-endoperoxy- and 1,2,3,4-diepoxy-guaianolides are distributed in many species of Asteraceae family, while only one guaia-1,3-dienolide (isoachifolidien) has been isolated so far (Rücker et al., 1992). This fact probably due to the higher reactivity of guaia-1,3-dienolides in comparison with their isomers guaia-1,4-dienolides.

3. Experimental

3.1. General

NMR spectra: Bruker DRX-250 (^{1}H 250 MHz/^{13}C 62.8 MHz) and Bruker DRX-400 (^{1}H 400 MHz/^{13}C 100 MHz), deuterated solvents, TMS as internal standard; Mass spectra (EI and HRMS): VG Autospec and Micromass LCT, respectively; IR spectra (film): Specord IR-75; column chromatography (CC): Silica gel 60 (Merck) and Sephadex LH-20 (Sigma); medium pressure chromatography (MPCC): LiChroprep®Si 60 40–63 μm (Merck) and LiChroprep®RP-18, 40–63 μm (Merck); analytical and preparative thin layer chromatography (TLC): Silica gel 60 GF_{254} (Merck); spot detection: UV light at 254 nm, spraying with sulfuric acid, modified acetic acid–phosphoric acid reagent (Stahl, 1967).

3.2. Plant material

From wild-growing population of *A. collina* of Vitosha mountain (ca. 950 m altitude) 30 individuals were collected and planted in the experimental field of the Institute of Botany, BAS (ca. 500 m altitude) near Sofia in 2001. The taxonomy identification of the plant material was based on morphological and caryological data. The flower heads of cultivated plants were collected in July 2004. Voucher specimen (SOM CO 989) was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

3.3. Extraction, isolation and identification

Fresh flower heads (1 kg) were extracted exhaustively with $CHCl_3$ at room temperature. After evaporation of the solvent the residue (10 g) was defatted by precipitation with MeOH. The filtrate was concentrated under vacuum and the resulting gum (4.1 g) was separated into 6 fractions

by CC on Sephadex LH-20 using MeOH as eluent. Fraction 2 (2.5 g) containing sesquiterpene lactones (IR control) was subjected on Silica gel CC using $CHCl_3/Me_2CO$ mixtures with increasing polarity to give subfractions F-2/1–F-2/8. Repeated CC ($CHCl_3/Me_2CO$, 20:1) of F-2/1 (0.5 g) followed where necessary by prep. TLC (hexane/Et_2O, 1:1, 2×) afforded compounds **1** (4.8 mg), mixture of **2** and **3** (6.0 mg) and **4** (2.0 mg). MPCC (LiChroprep®Si 60, $CHCl_3/Me_2CO$, 20:1 to Me_2CO) of F-2/3 (0.39 g) followed where necessary by prep. TLC (hexane/Et_2O, 1:2, 3×) allowed the isolation of **5** (1.2 mg), **6** (1.1 mg), **7** (8.2 mg), **8** (1.0 mg), **9** (0.8 mg), **10** (2.9 mg), **11** (3.1 mg), **12** (0.9 mg), **13** (0.7 mg), **14** (2.8 mg) and **15** (6.5 mg). MPCC (LiChroprep®RP-18, MeOH/H_2O, 6:4) of F-2/4 (0.15 g) followed by prep. TLC (hexane/Et_2O, 1:2, 3×) gave **16** (0.8 mg), **17** (1.8 mg), **18** (4.5 mg), **19** (3.1 mg), **20** (1.6 mg) and **21** (5.4 mg). Repeated CC ($CHCl_3/Me_2CO$, 9:1) of F-2/5 (0.16 g) followed where necessary by prep. TLC (hexane/Et_2O, 1:3, 2×) afforded **17** (8.1 mg), **19** (7.8 mg), **22** (1.2 mg), **23** (1.1 mg), **24** (28 mg), mixture of **25** and **26** (1.5 mg), **27** (1.2 mg), **28** (1.5 mg), **29** (6.7 mg) and **30** (1.1 mg). Lactone **31** (2.1 mg) was isolated from F-2/6 (0.3 g) by MPCC (LiChroprep®RP-18, MeOH/H_2O, 7:3) and prep. TLC (hexane/Et_2O, 1:5, 2×).

Known compounds were identified by comparison of their spectral data with those reported in the literature. It has to be noticed that the isolated amounts do not represent the real concentration of the compounds in the plant.

*3.3.1. 8α-Acetoxy-11-epi-tannunolide C (**1**)*

Colorless oil; IR (film) ν_{max} 3400, 1760, 1715, 1645, 1630 cm^{-1}; HRMS (ESI) *m/z*: 352.1193 $[M+CH_3CN+Na]^+$ (calcd. for $C_{19}H_{23}NO_4Na$, 352.1163); ^1H NMR data: see Table 1.

*3.3.2. 5α-Hydroxymatricarin (**16**)*

Colorless oil; IR (film) ν_{max} 3450, 1760, 1745, 1680, 1615 cm^{-1}; HRMS (EI) *m/z*: 320.1261 $[M]^+$ (calcd. for $C_{17}H_{20}O_6$, 320.1259); ^1H NMR data: see Table 1; ^{13}C NMR data: see Table 2.

*3.3.3. 8α-Acetoxy-3β-hydroxy-11(αH),13-dihydrocostunolide (**18**)*

Colorless oil; IR (film) ν_{max} 3420, 1760, 1730, 1460 cm^{-1}; HRMS (EI) *m/z*: 308.1625 $[M]^+$ (calcd. for $C_{17}H_{24}O_5$, 308.1624); ^1H and ^{13}C NMR data: see Table 3.

*3.3.4. 8α-Isobutyryloxy-11(βH),13-dihydro-10-epi-tanaparthin-α-peroxide (**20**)*

Colorless oil; IR (film) ν_{max} 3570, 1750, 1715, 1650 cm^{-1}; HRMS (EI) *m/z*: 366.1679 $[M]^+$ (calcd. for $C_{19}H_{26}O_7$, 366.1679); ^1H NMR data: see Table 1.

*3.3.5. 8α-Tigloyloxy-11(βH),13-dihydro-10-epi-tanaparthin-α-peroxide (**21**)*

Colorless oil; IR (film) ν_{max} 3550, 1760, 1720, 1640 cm^{-1}; HRMS (ESI) *m/z*: 401.1583 $[M+Na]^+$ (calcd. for $C_{20}H_{26}O_7Na$, 401.1576); ^1H NMR data: see Table 1, ^{13}C NMR data: see Table 2.

*3.3.6. 4α,10β-Dihydroxy-8α-tigloyloxy-2-oxo-6βH,7αH,11βH-1(5)-guaien-12,6α-olide (**23**)*

Colorless oil; IR (film) ν_{max} 3400, 1780, 1720, 1700, 1640 cm^{-1}; HRMS (ESI) *m/z*: 442.1829 $[M+CH_3CN+Na]^+$ (calcd. for $C_{22}H_{29}NO_7Na$, 442.1842); ^1H NMR data: see Table 1, ^{13}C NMR data: see Table 2.

*3.3.7. 1α,2α,4α,5α-Diepoxy-8α-tigloyloxy-10β-hydroxy-6βH,7αH,11βH-12,6α-guaianolide (**27**)*

Colorless oil; IR (film) ν_{max} 3450, 1770, 1715, 1650 cm^{-1}; HRMS (EI) *m/z*: 378.1678 $[M]^+$ (calcd. for $C_{20}H_{26}O_7$ 378.1679); ^1H NMR data: see Table 1.

*3.3.8. 1β,2β,3β,4β-Diepoxy-8α-tigloyloxy-10β-hydroxy-6βH,7αH,11β-12,6α-guaianolide (**28**)*

Colorless oil; IR (film) ν_{max} 3400, 1780, 1720, 1630 cm^{-1}; HRMS (ESI) *m/z*: 401.1591 $[M+Na]^+$ (calcd. for $C_{20}H_{26}O_7Na$, 401.1576); ^1H NMR data: see Table 1, ^{13}C NMR data: see Table 2.

*3.3.9. 1α,2α,3α,4α-Diepoxy-8α-tigloyloxy-10β-hydroxy-6βH,7αH,11βH-12,6α-guaianolide (**29**)*

Colorless oil; IR (film) ν_{max} 3550, 1780, 1720, 1630 cm^{-1}; HRMS (ESI) *m/z*: 422.1836 $[M+CH_3CN+Na]^+$ (calcd. for $C_{22}H_{29}NO_7Na$, 442.1842); ^1H NMR data: see Table 1, ^{13}C NMR data: see Table 2.

*3.3.10. 1α,2α,3α,4α -Diepoxy-8α-angeloyloxy-10β-hydroxy-6β H,7αH,11βH-12,6α-guaianolide (**30**)*

Colorless oil; IR (film) ν_{max} 3500, 1770, 1720, 1640 cm^{-1}; HRMS (ESI) *m/z*: 422.1854 $[M+CH_3CN+Na]^+$ (calcd. for $C_{22}H_{29}NO_7Na$, 442.1842); ^1H NMR data: see Table 1, ^{13}C NMR data: see Table 2.

*3.3.11. Achicollinolide (**31**)*

Colorless oil; IR (film) ν_{max} 3400, 1770, 1715, 1650 cm^{-1}; EIMS (70 eV) *m/z* (rel. int.): 550 $[M]^+$ (<1), 306 $[C_{17}H_{22}O_5]^+$ (71), 288 (22), 246 $[306-CH_3COOH]^+$ (56), 244 $[C_{15}H_{16}O_3]^+$ (98), 228 (100), 213 (84), 203 (76), 185 (82), 173 (78), 159 (72), 145 (74), 107 (71), 69 (82), 55 (84); ^1H and ^{13}C NMR data: see Table 4.

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