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Sesquiterpene lactones in *Blainvillea rhomboidea*

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Abstract

The investigation of an extract of the aerial parts of *Blainvillea rhomboidae* afforded, in addition to known compounds, six further melampolides, two germacrolides and four guaianolides. HPLC analysis revealed that the compounds, except for one ethoxylated derivative, are natural constituents. They are sequestered by capitate glandular trichomes of the plant surface. The taxonomic relevance of the results is briefly discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Blainvillea*; Heliantheae; Asteraceae; Sesquiterpene lactones; Chemotaxonomy

1. Introduction

Blainvillea is a genus with approximately 10 species of pantropical distribution. Its delineation is not very clear and no monograph has been published since the first description of the genus by Cassini (1823). Formerly placed in the Ecliptinae (Stuessy, 1977; Robinson, 1981) the genus is now seen to have its closest relatives in the *Wedelia* group of the Verbesininae (Karis & Ryding, 1994). Four species of *Blainvillea* have been chemically investigated before and revealed to be a rich source of sesquiterpene lactones of different skeletal types (Bohlmann, Ziesche, King & Robinson, 1981; Singh, Sharma, Joshi, Jakupovic & Bohlmann, 1985; Rojatkar et al., 1986; Singh, Bhala, Jain & Jakupovic, 1988; Kijjoa, Bastos, Gedris & Herz, 1993; Sawaikar, Rojatkar & Nagasampagi, 1997; Singh, Jain & Krishna, 1998).

2. Results and discussion

The ethanolic extract of the aerial parts of *Blainvillea rhomboidea* afforded the known melampolides 8β-angeloyloxy-9β-hydroxy-14-oxo-acanthospermolide (**1**) and 8β-angeloyloxy-14-oxo-acanthospermolide (**2**), previously reported from *Grazielia intermedia* (Eupatorieae, Asteraceae) (Bohlmann, Zdero, King & Robinson, 1981). In addition, the melampolides **3**–**8**, the germacrolides **9**–**11** and the guaianolides **12**–**15** were identified.

^{1}H NMR spectra of compound **3** (Table 1) indicated structural similarity with the acanthospermolide skeleton. NOE spectra revealed close proximity between H-1 and H-14 thus confirming *cis*-configuration of the C-1/C-10 double bond. While the total number of protons in compounds **3** and **1** was identical and most of the shift values were similar, the signals of H-8 and H-9 in compound **3** were significantly shifted downfield. This situation suggested that the compounds were isomers with interchanged substituents at carbons 8 and 9. The β-position of the angelate side chain at C-9 and the hydroxyl at C-8 was deduced by clear nOe from H-7. Compound **3**, therefore, was named 8β-hydroxy-

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Table 1
^{1}H NMR spectral data of compounds **3**–**6** (300 MHz, $CDCl_3$)

H	3	4	5	6
1	6.60 ddd (5.8, 12)	6.81 dd (4.5, 9.1)	6.60 ddd	6.63 ddd
2a	2.80 dq (3, 12)	2.83 m	2.54 m	2.58 m
2b	2.64 dq (5, 12)	2.64 m	2.30 m	2.32 dq
3a	2.53 ddd	2.70 m	2.41 m (5.8, 13.4)	2.42 ddd
3b	2.01 ddd	2.05 m	2.11 m (13.4)	2.18 m
5	5.40 bd (1.4, 10)	5.48 bd (10.3)	5.07 bd (10)[a]	5.09 bd[a]
6	4.88 dd (9.4, 10)	5.13 dd (10, 10.3)	5.11 dd (8.3, 10)[a]	5.13 dd[a]
7	3.15, dq (3, 3.3, 9.4)	3.05 dq (3, 3.3, 10)	2.49 m	2.52 m
8	4.14 bd (9)	4.61 bs	6.42 ddd (1.8, 7.5)	6.46 ddd
9a	4.61 bd (9)	5.75 bs	2.82 dd (7.5, 13.2)	2.83 dd
9b			1.95 dd (1.5, 13.2)	1.98 dd
13a	6.28 d (3.3)	6.29 d (3.3)	6.22 d (3.5)	6.24 d (3.3)
13b	5.81 d (3)	5.57 d (3)	5.58 d (3.1)	5.61 d (3)[a]
14	9.40 d (1.9)	9.47 d (1.5)	9.45 d (1.5)	9.47 d
15	1.68 d (1.4)[b]	1.68 d (1.4)	1.94 s	1.72 bs
2′				5.64 m[a]
3′	6.10 qq	6.14 qq	6.79 qq	
4′	1.99 dq[b]	1.95 dq	1.80 dq	2.00 bs[b]
5′	1.72 dq[b]	1.84 dq	1.81 s	1.98 bs[b]

[a] Signals overlapped.
[b] Three proton intensity.

9β-angeloyloxy-14-oxo-acanthospermolide. A very similar case of reciprocal substitution was recently described for the 2-methylbutyrate derivatives of hydroxy-14-oxo-acanthospermolide in *B. acmella* (Singh et al., 1985).

In MS investigations compound **4** showed the same molecular weight of M^+ 360 ($C_{20}H_{24}O_6$) and the same fragmentation pattern as **3**. Besides the general similarity in the ^{1}H NMR spectrum, differences in the chemical shift values and coupling constants of H-8 (δ 4.61) and H-9 (δ 5.75) as well as the presence of W-coupling between H-14 and H-9 suggested an inverse stereochemistry at C-9 with the oxygen-ester in the α-position.

Table 2
^{1}H NMR spectral data of compounds **7**–**11** (300 MHz, $CDCl_3$)

H	7	8	9	10	11
1	6.75 dd (7, 10)	6.67 ddd (1, 7, 10)	5.38 dd (4.6, 11)	5.38 dd (3, 12.5)	5.90 dd (4, 10)
2a	2.68 m	2.62 m		2.58 ddd (4, 15)	3.50 m
2b	2.35 m[a]	2.35 m	[2.15–2.45; 4 H]	2.43 m	2.32 m[a]
3a	2.40 m[a]	2.43 m (12.6)		2.50 m	2.50 m[a]
3b	2.07 dd (9, 14)	2.13 m (12.6)		2.25 dd (5, 12)	2.40 m[a]
5	4.88 bd (10.3)	5.58 bd (10.4)	4.77 bd (10.2)	4.85 bd (10.3)	5.03 bd (9.9)
6	5.13 dd (10.3)	4.69 bd (10.4)	5.04 dd (9, 10.2)	5.04 dd (9, 10)	5.16 dd (9, 9.9)
7	2.42 m[a]		3.02 ddd (3.1, 3.5, 9)	3.02 ddd (3.1, 3.5, 9)	2.93 ddd (3, 3.4; 9)
8	5.09 bd (8)	2.99 d (16.4)	6.09 d (2)	6.15 bs	5.82 bd (7)
9a	3.56 dd (2.2, 8)	3.37 d (16.2)	4.49 d (2)	4.47 d (1.5)	3.60 dd (7, 14)
9b					2.28 bd (14)
13a	6.33 d (3.5)	1.90 d (1.3)[b]	6.38 d (3.5)	6.42 d (3.5)	6.35 d (3.4)
13b	5.64 d (3.1)		5.78 d (3.1)	5.93 d (3.1)	5.69 d (3)
14	9.45 d (2.2)	9.41 s	4.56 d (12), 4.40 d (12)	4.68 d (13), 4.51 dd (1.5, 13)	
15	1.89 d (1.1)[b]	1.97 d (2)	1.63 d (1.3)	1.84 d (1)	1.88 s
2′	3.40 dq (9, 7.1), 3.25 dq				
3′	1.19 t (7.1)[b]		6.19 qq	6.18 qq	6.81 qq
4′			2.00 dq[b]	1.93 dq	1.72 dq
5′			1.91 dq[b]	1.83 dq	1.70 dq
OAc				2.07 s[b]	

[a] Signals overlapped.
[b] Three proton intensity.

Table 3
^{1}H NMR spectral data of compounds **12**–**15** (300 MHz, $CDCl_3$)

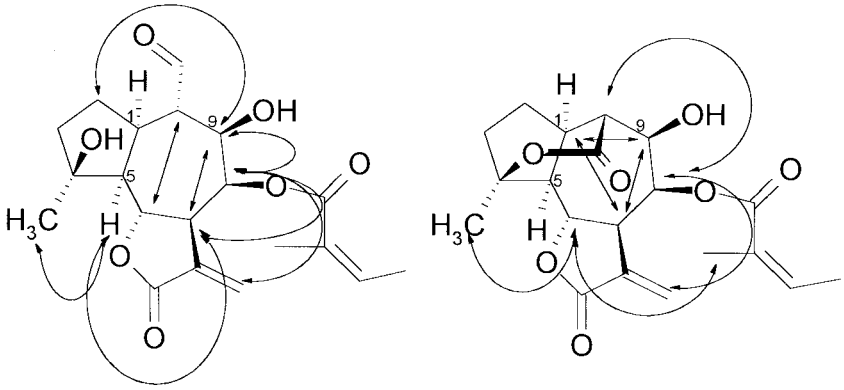
H	12	13	14	15
1	2.60 m	2.60 m	2.65 t (5.6)	2.69t (5.6)
2a				
2b	[1.7-2.3; 4H][a]	[1.7-2.3; 4H]	[1.7-2.3; 4H]	[1.7-2.3; 4H]
3a				
3b				
5	2.17 t (11)	2.20 t	2.38 t (5.6)	2.29 t (5.6)
6	4.92 dd (11, 10)	5.02 dd	4.80 dd (5.6, 10)	4.68 dd (5.6, 10)
7	3.05 ddt (1, 3, 10)	2.95 ddt	3.25 dddd (1, 2.6, 3.3, 10)	2.90 t (10, 12)
8	5.90 dd (2.3)	4.50 dd	6.08 dd (1, 4.7)	5.71 bd (4.3)
9	4.25 dd (2.3, 10.4)	5.30 dd	4.05 dt (4.7, 10)	4.00 dt (4.3, 10)
10	3.00 dt (4.4, 10.4)	3.32 dt	2.89 bd (4.7)	2.87 bd (4.3)
11				2.45 dt (3, 12)
13a	6.36 d (3.3)	6.42 d	6.36 d (3.3)	3.81 dd (2.7, 10)
13b	5.70 d (2.7)	5.65 d	5.84 d (2.6)	3.70 dd (3.4, 10))
14	9.57 d (4.5)	9.41 d		
15	1.57 s[b]	1.57 s	1.59 s	1.53 s
3′	6.18 qq	6.20 qq	6.13 qq	6.21 qq
4′	1.98 dq[b]	1.99 dq	1.96 dq	2.02 dq
5′	1.89 dq[b]	1.86 dq	1.85 dq	1.91 dq
O-Me				3.40 s[b]
9-OH			4.28 d (10)	4.21 d (10)

[a] Signals overlapped.
[b] Three proton intensity.

The ^{1}H NMR spectra of compounds **5** and **6** were almost identical with that of the melampolide **2**, except for the signals of the side chain. The typical shift values (δ 6.79, 1H, qq; δ 1.81, 3H, s, δ 1.80, 3H, dq) indicated a tiglate ester in compound **5**. The 11,13-dihydroderivative of this constituent was previously reported to occur in *B. acmella* (Singh et al., 1985). Compound **6**, according to the signals of the side chain protons (δ 5.64, 1H, m; δ 2.00, 3H, bs, δ 1.98, 3H, bs) and the mass fragmentation patterns (see Section 3), was identified as 8β-senecioyloxy-14-oxo-acanthospermolide.

The ^{1}H NMR spectra of compounds **7** and **8** (Table 2) revealed two additional 14-oxo-melampolides. However, in contrast to the previous compounds the typical signals for the side chain esters were missing in both constituents. The aldehyde proton H-14 showed W-type coupling with a signal at δ 3.56 dd in **7** and δ 3.37 dd in **8**, respectively, thus indicating the position of H-9 in both molecules. Due to the downfield shift C-9 had to be oxygenated. The position of H-8 (δ 5.09 in compound **7** and δ 2.99 in **8**) followed from coupling with H-9. The aliphatic region of the spectrum of **7** showed the typical pattern of an ethoxygroup. Its position at C-8 explained the shift value of H-8 in comparison with the other melampolides. The similarity of **7** to compounds **1** and **3** in the large coupling of H-8 with H-9 as well as the lack of coupling with H-7 led us to the stereochemical β-assignment of both oxygen functions at C-8 and C-9, respectively. Compound **8**, in contrast to **7**, showed an upfield shift of H-8 as would be expected for an epoxide. The exocyclic methylene protones and H-7 were lacking. Instead, an additional methyl group appeared at δ 1.90 with a small long range coupling (1.3 Hz) to H-6. This was indicative of a double bond between C-7 and C-11. The stereochemistry of the epoxide could not be resolved due to the lack of H-7. However, NOE-experiments indicated an equiplanar relationship for H-9, H-8 and H-13 and calculations using PC-MODEL lend support for the α-position of the respective protons. Mass spectroscopic data with M^+ 260 for $C_{15}H_{16}O_4$ were in agreement with the proposed structure.

The ^{1}H NMR spectra of **9** and **10** closely resembled each other, indicated the structure of a germacrolide with an angelate side chain and showed similarity to the ovatifolin derivatives previously reported from *Grazielia intermedia* (Bohlmann, Zdero, et al., 1981). In both compounds, H-9 was shifted downfield due to the presence of an hydroxyl. The stereochemistry at the centers C-8 and C-9 was concluded from the coupling patterns. Compound **9** showed a molecular weight of M^+ 362 for $C_{20}H_{26}O_6$ which was in agreement with the proposed structure of 8β-angeloyloxy-9β,14-dihydroxy-1(10)-4(5)-*trans*,*trans*-germacradienolide. In compound **10** the hydroxyl of C-14 was acetylated as was indicated by an additional methyl signal at δ 2.07



12 14

Fig. 1. Nuclear Overhauser effects of compound **12** and **14** observed in a TROESY-experiment.

and the chemical shift of H-14a,b. A similar compound with a methylbutyrate side chain was previously reported from *B. acmella* (Singh et al., 1985).

A third germacrolide, **11**, showed unusual shifts for H-2a (δ 3.50 ppm) and H-9a (δ 3.60 ppm). This was indicative of a deshielding effect by the proximity of a carboxyl group as previously described for grazielia acid (Bohlmann, Zdero, et al., 1981). Fragmentation in mass spectroscopic measurements confirmed the presence of a carboxyl group and showed a molecular weight of M^+ 360 for $C_{20}H_{24}O_6$ The compound was identified as the tiglate analog of grazielia acid.

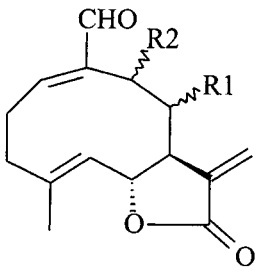
The ^{1}H NMR and COSY spectra of compounds **12** and **13** (Table 3) revealed the characteristcs of 11,13-unsaturated 7,6-lactonized guaianolides. The position of H-5 (δ 2.17; 2.2 ppm) was established through its vicinal coupling with H-6. The singlet signal for the methyl function of H-15 indicated a quarternary carbon at C-4. The lack of an additional olefinic proton and the downfield shift of the methyl group (δ 1.57 ppm) strengthened the assumption of a hydroxyl function at C-4. The position of H-10 could be deduced from coupling with H-9. Both protons, H-5 and H-10, showed an additional vicinal coupling which led to the assignment of H-1. Compound **12** and **13** showed the typical signal for an aldehyde function (δ 9.57 ppm in **12** and 9.41 ppm in **13**) attached to C-10, and the spectra were nearly identical except for the signals of H-8 and H-9, both of which showed chemical shifts due to a hydroxyl and an angelate ester substituent, respectively. Coupling to H-7 indicated that in **12** the ester function was attached to C-8, whereas it was attached to C-9 in compound **13**. The stereochemistry (Fig. 1) was established through coupling information (Table 4) and was confirmed by TROESY spectra.

^{1}H NMR data together with COSY, TOCSY and MS measurements led to the structure of compound **14** which was based on the same guaianolide skeleton as the previous two compounds. Clear NOE-effects (Fig.1) made a precise determination of the stereochemistry possible. The unusual coupling of H-9 with a hydroxyl group was confirmed by D_2O-exchange, after which a doublet at δ 4.28 ppm disappeared and the signals at δ 4.05 ppm (dt, H-9) collapsed to a triplet. All proton bearing carbons were confirmed through an HSQC-experiment (for data see Section 3). The unusual 4,14-lactone moiety was furthermore confirmed by calculations using PC-MODEL (MMX forcefield). The results (Table 4) of the calculated versus the measured coupling constants were in good agreement with the proposed stereochemistry. Compound **15** differed from **14** only in an additional CH_2OMe function and the lack of the exocyclic methylene protones. This indicated a methanol adduct to C-13 which was confirmed by a coupling path between H-7, H-11, H13a,b and a clear nOe between the O-Me function and H-13a,b. The stereochemistry at C-11 was deduced from an additional nOe between H-11 and H-7. Unfortunately, HMBC-experiments were not possible due to the small amount of sample.

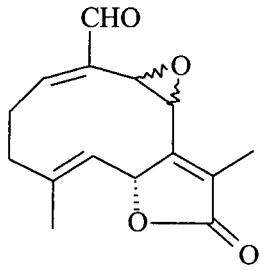
Microsampling of glandular trichomes (Spring, 1991) from the leaf surface of *B. rhomboidea* was performed in order to check the natural occurrence and the localization of the compounds within the plant. All

Table 4
Comparison of measured versus calculated coupling constants of relevant protons of compound **12** and **14**

	Protons								
	6-7	7-8	8-9	9-10	10-1	1-5	1-2a	1-2b	5-6
Compound **12**									
J (Hz) calculated	10.8	1.2	2.5	11	12.4	10.8	9	8	10.7
J (Hz) found	10	1	2.3	10.4	10.9	11	–	–	11
Compound **14**									
J (Hz) calculated	10.9	1	3.5	2.6	0.5	4.5	1	6.9	5.6
J (Hz) found	10.5	1	4.7	4.7	-	5.6	-	5.6	5.6



	R1	R2
1	8ß-O-ang	9ß-OH
2	8ß-O-ang	H
3	8ß-OH	9ß-O-ang
4	8ß-OH	9α-O-ang
5	8ß-O-tig	H
6	8ß-O-sen	H
7	8ß-O-Et	9α-OH



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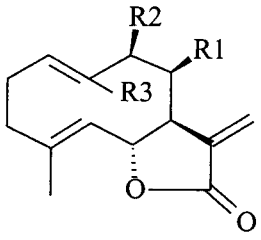
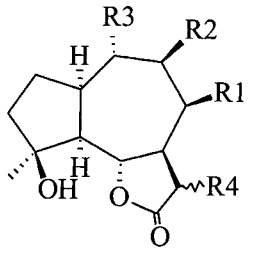
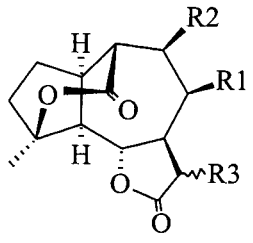


	R1	R2	R3
9	O-ang	OH	CH_2OH
10	O-ang	OH	CH_2OAc
11	O-tig	H	COOH



	R1	R2	R3	R4
12	O-ang	OH	CHO	$=CH_2$
13	OH	O-ang	CHO	$=CH_2$



	R1	R2	R3
14	O-ang	OH	$=CH_2$
15	O-ang	OH	ß-CH_2OMe

Table 5
HPLC retention times of **1**–**8** in 50% MeOH (RRT_1) and in 30% CH_3CN (RRT_2) relative to dimethylphenol

	1	2	3	4	5	6	7	8
RRT_1	1.78	4.10	1.16	2.57	3.70	2.24	0.45	0.42
RRT_2	3.85	7.70	1.20	3.42	6.72	4.12	0.49	0.65

compounds reported here, except for **7**, were detected in HPLC analysis of trichome extracts according to their characteristic migration (see Section 3 for relative retention times in two independent solvent systems; 50% MeOH, 30% CH_3CN) and their UV-absorbance at double wavelength detection (A_{225}:A_{265}). Their relative peak proportion in glandular trichome extracts according to their absorbance at UV A_{225} was 25% (**1**), 4% (**2**), 9% (**3**), 1% (**4**), 2% (**5**), 2% (**6**), 6% (**8**), 5% (**9**), 34% (**10**), 1% (**11**), 5% (**12**), 3% (**13**), 2% (**14**) and 1% (**15**). The absence of **7** in trichome extracts which were prepared and analyzed in avoidance of EtOH indicates that the compound is a preparation artifact putatively deriving from a 8,9-epoxidized precursor after reaction with the extraction solvent. Compound **15** was coeluting with **14**, thus prohibiting to prove its natural existence in glandular trichomes via HPLC analysis. However, the proportion between the peak at the relevant position did not change between preparations in MeOH and CH_3CN, respectively, as should be expected if **15** would be considered to be an artifact. Moreover, according to our experience with sesquiterpene lactones the spontaneous reaction of the exocyclic methylene group with MeOH is unlikely and otherwise should be expected to occur in many other compounds, too.

With the occurrence of melampolides, germacrolides and guaianolides in *B. rhomboidea*, this species produces a greater variety of sesquiterpene lactone skeletons than previously reported from any other member of this genus. Acanthospermolide type melampolides appear to be most characteristic for this pantropical genus. They were reported from *B. acmella* (Singh et al., 1985), *B. dichotoma* (Bohlmann, Ziesche, et al., 1981) and *B. gayana* (Kijjoa et al., 1993), but lacked in three independant investigations of *B. latifolia* (Rojatkar et al., 1986; Singh et al., 1988; Sawaikar et al., 1997). Guaianolides were only reported from an Indian population of *B. latifolia* (Singh et al., 1988, 1998).

Table 6
HPLC retention times of **9**–**15** in 50% MeOH (RRT_1) and in 30% CH_3CN (RRT_2) relative to dimethylphenol

	9>	10	11	12	13	14	15
RRT_1	1.0	2.0	0.99	0.38	0.39	0.91	0.91
RRT_2	0.80	2.37	1.28	0.44	0.45	1.10	1.08

3. Experimental

3.1. Plant material

B. rhomboidea Cass. was collected near Cajuru, State of São Paulo, Brazil, in March 1993. Species determination was carried out by Professor Hermógenes de Freitas Leitão Filho, Instituto de Biologia, UNICAMP, SP, Brazil. A voucher specimen (SPFR 04501) is deposited at the herbarium of the Dept. de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, USP.

3.2. Extraction and structure elucidation

Dried and pulverized aerial parts of *B. rhomboidea* (7.5 kg) were exhaustively extracted with EtOH affording 250 g of crude extract after vacuum evaporation of the solvent. The residual extract was dissolved in MeOH:H_2O (9:1) and partitioned consecutively with hexane and CH_2Cl_2. After evaporation of the solvent in vacuo the CH_2Cl_2-soluble part (48 g) was redissolved and chromatographed over silicagel D. The final purification of sesquiterpene lactone-containing fractions was performed by HPLC (Hypersil ODS, 5 μm; 4 × 250 mm; 50% MeOH or 30% alternatively used as solvents; UV detection simultaneously at 225 and 265 nm; dimethylphenol as int. standard). Screening for sesquiterpene lactones in extracts of glandular trichomes gained via microsampling from the leaf surface was carried out in the usual manner (Spring, 1991) using the same HPLC parameters. The retention times of **1**–**15** relative to dimethylphenol in 50% MeOH (RRT_1) and in 30% CH_3CN (RRT_2) are presented in Tables 5 and 6.

3.2.1. 8β-Hydroxy-9β-angeloyloxy-14-oxo-acanthospermolide (*3*)

$C_{20}H_{24}O_6$, EIMS 70 *m/z* (rel. intensity): 360 $[M]^+$ (not observed), 260 $[M\text{-angelate}]^+$ (4), 242 $[260\text{-}H_2O]^+$ (3), 83 $[C_5H_7O]^+$ (100).

3.2.2. 8β-Hydroxy-9α-angeloyloxy-14-oxo-acanthospermolide (*4*)

$C_{20}H_{24}O_6$, APCI + Q1MS; grad. 10–100% CH_3CN in 40 min: 361 $[M+H]^+$, 343 $[361\text{-}H_2O]$, 261 $[361\text{-angelate}]^+$, 243 $[261\text{-}H_2O]^+$, 83 $[C_5H_7O]^+$.

3.2.3. 8β-Tigloyloxy-14-oxo-acanthospermolide (*5*)

$C_{20}H_{24}O_6$, EIMS 70 *m/z* (rel. intensity): 344 $[M]^+$ (1), 244 $[M\text{-tiglate}]^+$ (10), 83 $[C_5H_7O]^+$ (100).

*3.2.13. 8β-Senecioyloxy-14-oxo-acanthospermolide (**6**)*

$C_{20}H_{24}O_5$, APCI + Q1MS; grad. 10–100% CH_3CN in 40 min: 345 $[M+H]^+$, 245 [M-senecioate $+H]^+$, 83 $[C_5H_7O]^+$.

3.2.5. 8β-Ethoxy-9α -hydroxy-14-oxo-acanthospermolide (7)

$C_{17}H_{22}O_5$, APCI + Q1MS; grad. 10–100% CH_3CN in 40 min: 307 $[M+H]^+$, 289 $[307\text{-}H_2O]^+$, 261 $[307\text{-}C_2H_6O]^+$, 243 $[261\text{-}H_2O]^+$.

*3.2.6. 8,9-Epoxy-14-oxo-1(10)Z,4(5)E,7(11)-germacratrien-6,12-olide (**8**)*

$C_{15}H_{16}O_4$, EIMS 70 *m/z* (rel. intensity): 260 $[M]^+$ (10), 242 $[M\text{-}H_2O]^+$ (8).

*3.2.7. 8β-Angeloyloxy-9β,14-dihydroxy-1(10),4(5)-trans,trans-germacradienolide (**9**)*

$C_{20}H_{26}O_6$, EIMS 70 *m/z* (rel. intensity): 362 $[M]^+$ (1), 344 $[M\text{-}H_2O]^+$ (8), 262 [M-angelate]$^+$ (7), 83 $[C_5H_7O]^+$ (100).

*3.2.8. 8β-Angeloyloxy-9β-hydroxy-ovatifolin (**10**)*

$C_{22}H_{28}O_6$, EIMS 70 *m/z* (rel. intensity): 388 $[M]^+$ (<1), 260 $[M–H_2O–angelate]^+$ (3), 83 $[C_5H_7O]^+$ (100).

*3.2.9. 8β-Tigloyloxy-grazielia acid (**11**)*

$C_{20}H_{24}O_6$, APCI + Q1MS; grad. 10–100% CH_3CN in 40 min: 361 $[M+H]^+$, 343 $[361\text{-}H_2O]^+$, 261 [361-tiglate]$^+$, 243 $[261\text{-}H_2O]^+$, 215 $[261\text{-}HCOOH]^+$, 83 $[C_5H_7O]^+$.

*3.2.10. 8β-Angeloyloxy-14-oxo-4β,9β-dihydroxy-guaia-11(13)-en-6,12-olide (**12**)*

$C_{20}H_{26}O_7$, ESI + Q1MS: 379 $[M+H]^+$.

*3.2.11. 9β-Angeloyloxy-14-oxo,4β,8β-dihydroxy-guaia-11(13)-en-6,12-olide (**13**)*

$C_{20}H_{26}O_7$, ESI + Q1MS: 379 $[M+H]^+$.

*3.2.12. 8β-Angeloyloxy-9β-hydroxy-guaia-11(13)-en-6(12), 4(14)-diolide (**14**)*

$C_{20}H_{24}O_7$, EIMS 70 *m/z* (rel. intensity): 376 $[M]^+$ (5),: 358 $[M\text{-}H_2O]^+$ (13), 276 [M-angelate]$^+$ (8) 83 $[C_5H_7O]^+$ (100). ^{13}C values according to GHSQC in $CDCl_3$: δ 37.9 ppm (C-1), δ 30.3 ppm (C-2), δ 37.6 ppm (C-3), δ 50.1 ppm (C-5), δ 73.1 ppm (C-6), δ 44.7 ppm (C-7), δ 67.7 ppm (C-8), δ 73.7 ppm (C-9), δ 50.9 ppm (C-10), δ 123.2 ppm (C-13), δ 21.8 ppm (C-15).

*3.2.13. 8β-Angeloyloxy-9β-hydroxy-guaia-13-methoxy-6(12), 4(14)-diolide (**15**)*

$C_{21}H_{28}O_8$, EIMS 70 *m/z* (rel. intensity): 408 $[M]^+$ (3),: 390 $[M\text{-}H_2O]^+$ (11), 290 [M-angelate]$^+$ (6) 83 $[C_5H_7O]^+$ (100).

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