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Sesquiterpene lactones in *Viguiera eriophora* and *Viguiera puruana* (Heliantheae; Asteraceae)

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Abstract

Extracts of the aerial parts of *Viguiera eriophora* ssp. *eriophora* and *Viguiera puruana* afforded, in addition to known compounds, six new heliangolides and a germacrolide, whose structures were determined by spectral analysis. HPLC analysis and LC NMR experiments revealed the natural occurrence of the compounds in glandular trichomes. The taxonomic relevance of the results is briefly discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Viguiera eriophora* ssp. *eriophora*; *Viguiera puruana*; Helianthinae; Asteraceae; Sesquiterpene lactones; Chemotaxonomy

1. Introduction

In continuation of our chemotaxonomic studies on terpenoids of species of the subtribe Helianthinae, Asteraceae (Spring and Buschmann, 1996), we have analyzed leaf extracts of *Viguiera eriophora* (Greenmann) ssp. *eriophora* Panero & Schilling and *Viguiera puruana* Paray. Both taxa belong to the section Maculatae (Panero and Schilling, 1988) of the genus *Viguiera*, a newly erected section which was formerly treated as a series within the sect. Viguiera (sect. Chloracra S.F. Blake) of the subgenus *Viguiera* (*Calanticaria* S.F. Blake) (Blake, 1918). The section Maculatae, in the sense of Panero and Schilling (1988) consists of 13 species which are part of the endemic Mexican flora. Six taxa of the section have been chemically investigated for terpenoids in the past. These studies revealed the presence of various heliangolides in *V. eriophora* Greenm. (Delgado et al., 1982), *V. quinqueradiata* (Cav.) A. Gray (Delgado et al., 1984a) and *V. sphaerocephala* (DC.) Hemsl. (Ortega et al., 1980), while *V. insignis* Miranda (Delgado et al., 1983), *V. maculata* Blake (Delgado et al., 1984b) and *V. oaxacana* (Delgado et al., 1984c) were reported to produce no sesquiterpene lactones.

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2. Results and discussion

The extract of the aerial parts of *V. eriophora* ssp. *eriophora* afforded the heliangolide 17, 18-dehydroviguiepinin (**1**) as major constituent (0.02% of the dry wt.) and minor amounts (0.003 and 0.002% of the dry wt. respectively) of erioflorin (**7**) and acetylerioflorin (**8**) (Fig. 1). They were identified by comparison of ^{1}H NMR spectra with those reported earlier from the same taxon (Delgado et al., 1982). Among the minor components, LC-^{1}H NMR measurements established the presence of budlein A (**3**) (De Vivar et al., 1976), calaxin (**4**) (Bohlmann et al., 1981a) and leptocarpin (**9**) (Martinez et al., 1979) as additional known compounds.

The fraction dominated by compound **1** in HPLC separation contained two minor constituents, **2** and **5**, which were separated by repeated HPLC with 30% CH_3CN. The ^{1}H NMR spectrum of compound **2** (Table 1) indicated close structural similarity with that of **1** except for the lack of the exocyclic methylene signals. Instead of the olefinic protons, two doublets of doublets at δ 3.66 (J=4.6, 10.4 Hz) and δ 3.64 (J=4.6, 10.4 Hz) occurred and showed coupling with a proton at δ 3.00 (dt; J=4.6, 7.0 Hz). In combination with a singlet at δ 3.38 (3H), these were consistent with the presence of an 11(13)-dihydro-13-methoxy substitution on the lactone ring. Besides the expected differences of the chemical shifts of **2** measured in $CDCl_3$ and CH_3CN/D_2O,

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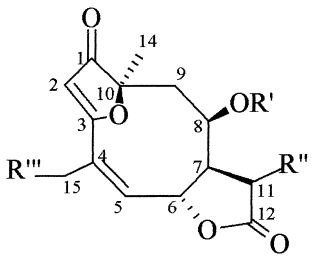
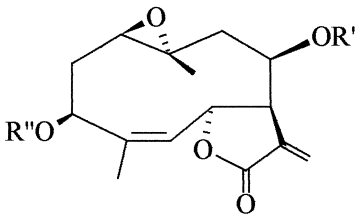
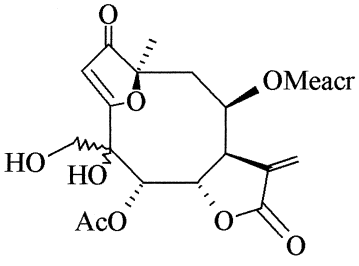


	R'	R''	R'''
1	Meacr	$=CH_2$	OH
2	Meacr	α-CH_2-OMe	OH
3	Ang	$=CH_2$	OH
4	Meacr	$=CH_2$	H



	R'	R''
7	Meacr	H
8	Meacr	Ac
9	Ang	H
10	Ang	Ac
11	Ang	Ethyl
12	Tig	H
13	Epang	H
14	Epang	Ac
15	Epang	iBut
16	Epang	Mebut
17	Epang	iVal
18	Sar	Ac



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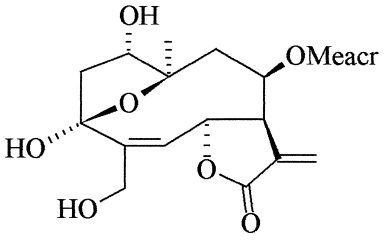

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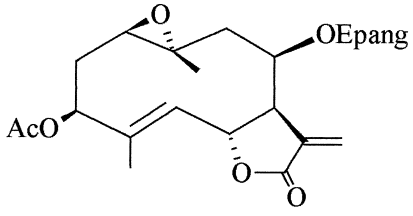

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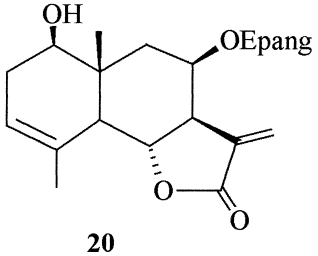

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Fig. 1. Compounds isolated from *V. eriophora* ssp. *eriophora* (**1**–**9**) and from *V. puruana* (**7**, **9**–**20**). The absolute configuartion of the discussed compounds was not determined and the chemical formulae are given in analogy to known compounds.

Table 1
^{1}H NMR spectral data of compounds **2**, **5** and **6** from *V. eriophora* (500 MHz, $CDCl_3$)

H	**2**	**2** (CH_3CN/D_2O)	**5**	**6**
1				4.04 *dd* (4.4, 8.1[a])
2	5.68 *bs*	5.83 *bs*	5.78 *s*	2.58 *dd* (4.4, 14.2)
				2.43 *d* (14.2)
5	6.19 *dt* (1.6,3.6)	6.13 *dt* (1.5, 3.6)	5.27 *s*	5.91 (3.9) *d*
6	5.11 *m*	5.19 *ddd* (1.9, 3.6, 7.0)	4.82 *d* (5)	5.39 *t* (3.9)
7	3.30 *ddd* (5,7,12.8)	3.24 *ddd* (1.9, 6.8, 7.0)	4.04 *m* (1, 2.5, 2.5, 5)	4.23 *m*
8	5.13 *m*	5.11 *ddd* (1.9, 3.6, 4.5)	5.08 *ddd* (1, 3.6, 4.5)	5.66 *dd* (4.9, 10)
9a	2.46 *dd* (5.1, 15.2)	2.43 *dd* (4.5, 15.1)	2.65 *dd* (4.5, 15.9)	2.03 *dd* (4.9, 14.4)
9b	2.30 *dd* (3, 15.2)	2.44 *dd* (3.6, 15.1)	2.31 *dd* (3.6, 15.9)	1.85 *dd* (10, 14.4)
11	3.00 *dt* (4.6, 7)	2.98 *ddd* (3.5, 5, 6.8)		
13a	3.64 *dd* (4.6, 10.4)	3.86 *dd* (5, 10)	6.34 *d* (2.5)	6.29 *d* (2.6)
13b	3.66 *dd* (4.6, 10.4)	3.62 *dd* (3.5, 10)	5.71 *d* (2.5)	5.64 *d* (2.1)
14	1.48 *s*	1.48 *s*	1.48 *s*	1.55 *s*
15a	4.42 *d* (13.5)	4.29 *ddd* (1.5, 2, 14.3)	4.23 *m* (12.2)	4.24 *dd* (5, 13)
15b	4.42 *d* (13.5)	4.27 *ddd* (1.5, 2, 14.3)	4.17 *m* (12.2)	4.14 *dd* (5, 13)
3′a	6.07 *dq* (1.1, 1.4)	5.97 *s*	6.01 *s*	5.95 *dq* (1.1, 1.2)
3′b	5.68 *t*-like (1.3, 1.4)	5.71 *s*	5.60 *bs*	5.53 *t*-like (1.1, 1.2)
4′	1.90 *dd* (1.1, 1.3)	1.84 *s*	1.87 *s*	1.83 *dd* (1.2, 1.2)
O-Me	3.38 *s*	3.32 *s*		
O-Ac			2.11 *s*	

[a] Coupling with 1-OH.

respectively, several differences in coupling constants (Table 1) were observed. These are explainable by different conformations of **2** under aprotic ($CDCl_3$) or protic (CH_3CN/D_2O) conditions. MS data (APCI) showed $m/z = 393$ $[M+H]^+$ indicative of $C_{20}H_{24}O_8$, thus confirming the proposed substitution. The relative stereochemistry at C-11 was deduced from an NOE observed between H-13b and H-7. Since **2** could be detected in HPLC analysis of the extracts from glandular trichomes which were micromechanically sampled from the leaf surface and were not brought into contact with methanol, we assume **2** to be a natural plant product. We found a similar case of unusual 13-*O*-methyl substitution just recently in *Blainvillea rhomboidea* (Spring et al., 1999).

Compound **5** gave ^{1}H NMR signals compatible with the presence of a 1-keto-2,3-unsaturated furanoheliangolide skeleton with a methacrylate side chain. Significant shifts of the signals of H-6 (δ 4.82 *d*, approximately 0.5 ppm upfield) and H-7 (δ 4.04 *dddd*, approximately 0.2 ppm downfield) together with an additional acetyl signal at δ 2.11 (*s*, 3H) indicated major structural differences at C-4 and C-5 when compared with **1**. Similar chemical shifts and the lack of coupling between H-6 and H-5 were previously observed for a 5-*O*-acetyl derivative of **1** obtained by Delgado et al. (1982). However, compound **5** differed from the latter derivative in the substituents of C-4. A pair of signals at δ 4.23 and δ 4.17 showed geminal coupling and coupling with an OH-signal at δ 2.95 (disappeared upon addition of D_2O), thus indicating the presence of a CH_2OH-function. C-4 had to be quaternary, because of the lack of additional vicinal couplings of H-15a,b. The proposed structure with a tertiary hydroxyl function at C-4 was confirmed by MS investigations (APCI) which showed $m/z = 437$ $[M+H]^+$, representing a molecular weight of 436 ($C_{21}H_{24}O_{10}$). Additionally in a related compound, isocentratherin (Manchand et al., 1983), the stereochemistry at C-5 was assigned by X-ray analysis. Comparison of calculated and observed coupling constants (PCMODEL, MMX forcefield; Table 2) in **5** and isocentratherin led us to the assignment of an α-oriented acetyl function at C-5. This further implies that the structure given by Delgado et al. (1982) has to be revised. NOE-measurements unfortunately could not give reliable hints for the stereochemistry at C-4. Only an NOE between 15-H and 5-H was observable, leaving the question of α- or β-orientation open.

The ^{1}H NMR data of **6** closely resembled those of niveusin-type sesquiterpene lactones (Ohno and Mabry, 1980) with α-orientation of the C-1 hydroxyl according to the shift values of H-1, H-6 and H-9b as discussed by Herz and Kumar (1981). The side chain of compound **6** showed the typical pattern of methacrylate. This was confirmed by MS data which gave M^+ 380 for $C_{19}H_{24}O_8$ and a major fragment peak of 69 (C_4H_5O) for the side chain. According to our knowledge, this side chain derivative of the 1,3,15-hydroxylated furanoheliangolide skeleton has not yet been reported from Asteraceae.

HPLC analysis of the extract of *V. puruana* revealed the presence of numerous constituents over a wide range of polarity. Among them, the lack of 1-keto-2,3-

Table 2
Calculated and observed coupling constants (Hz) of **5** and isocentratherin (Manchand et al., 1983)

Position	**5** obsd.	**5**α-OAc calcd.	**5**β-OAc calcd.	Isocentratherin obsd.	Isocentratherin calcd.
5–6	0	1.3	9.9	0	1
6–7	5	5.7	5.2	5	4.5
7–8	1	1.7	1.5	0	1.5
8–9a	4.5	4.8	5.0	12	11
8–9b	3.6	2.1	1.9	0	2

unsaturated furanoheliangolides became obvious from the absence of compounds with a second UV-maximum at 260–280 nm at double wavelength detection (A225:265 nm). Instead, ^{1}H NMR measurements of the purified compounds indicated the presence of several 1,10-epoxidized heliangolides with varying substituents at C-8 and C-3 position. The known compounds eriofloriin (**7**) (Delgado et al., 1982), leptocarpin (**9**) (Martinez et al., 1979), acetylleptocarpin (**10**) (Delgado et al., 1984a), heliangin (**12**) (Morimoto et al., 1966), four 8β-(2′*R*, 3′*R*)-epoxyangeloyloxy-heliangolide derivatives (**13**–**15**, **17**) (Gao et al., 1989; Schuster et al., 1992), and the eudesmanolide 8β-2,3-epoxyangeloyloxy-balchanin (**20**) (Bohlmann et al., 1981b) were identified by comparing ^{1}H NMR and MS data with those given in the literature.

In addition, three so far undescribed derivatives (**11**, **16**, **18**) were identified. Their basic spectral data were identical with those of the aforementioned 1,10-epoxidized heliangolides. Compound **11** differed from leptocarpin (**9**) in the occurrence of an additional ethyl function (δ 3.73, q, 2 H, and δ 1.25, t, 3 H; $J=7$) in the ^{1}H NMR spectrum (Table 3). This indicated the presence of an ethoxy instead of a hydroxy group at C-3 and was in agreement with the molecular ion of M^+ 390 ($C_{22}H_{30}O_6$). The ^{1}H NMR spectrum of compound **16** was most similar to that of **13**, but the C-3 hydroxyl was esterified with α-methylbutyric acid. The ^{1}H NMR data of compound **18** indicated the presence of a sarracinate ester at C-8 and an acetyl residue at C-3.

Finally, the ^{1}H NMR spectrum of compound **19**, though showing the same substituents at C-3 and C-8 as

Table 3
^{1}H NMR spectral data of compounds **11**, **16**, **18**, **19** from *V. puruana* (500 MHz, $CDCl_3$)

H	**11**	**16**	**18**	**19**
1	2.84 *m*[a]	2.90 *m*[a] (4.4)	2.87 *m*[a]	2.74 *dd* (2.3, 11)
2a	2.47 *dt* (5, 14.8)	2.60 *dt* (4.4, 15.7)	2.58 *dt* (4.7, 15)	2.48 *ddd* (2.3, 5.6, 12.8)
2b	1.75 *m*[a]	1.75 *m*	1.76 *m*	1.61 *m* (5, 11, 12.8)
3a	4.51 *bs*	5.29 *m*[a]	5.30 *m*	5.10 *dd* (5, 5.6)
5	5.40 *d* (10.9)	5.32 *m*[a]	5.28 *bd* (10.5)	5.50 *bd* (10)
6	6.65 *dd* (1,10.9)	6.07 *dd* (2.1, 11)	6.16 *dd* (2, 10.5)	5.19 *t* (9.5)
7	2.88 *bs*[a]	2.92 *m*	2.92 *m*[a]	2.93 *m*
8	5.22 *bs*	5.30 *m*[a]	5.20 *bs*	5.76 *bd* (6)
9a	2.82 *dd*[a] (4.3, 11)	2.74 *dd* (4.5, 15.2)	2.84 *m*[a]	2.79 *dd* (6, 15.4)
9b	1.34 *dd* (1, 11)	1.39 *dd* (2.5, 15.2)	1.38 *m*[a]	1.33 *bd* (< 1, 15.4)
13a	6.39 *d* (1.5)	6.42 *d* (2.2)	6.41 *d* (2)	6.37 *d* (3.4)
13b	5.78 *d* (1.2)	5.81 *d* (2)	5.82 *d* (2)	5.62 *d* (3.2)
14	1.48 *s*	1.52 *s*	1.48 *s*	1.24 *s*
15	1.83 *bs*	1.93 *d* (1.4)	1.93 *bs*	1.90 *d* (1)
3′	6.12 *qq* (1.5, 7.3)	3.07 *q* (5.5)	6.45 *q* (7)	3.08 *q* (5.4)
4′	1.99 *dq* (1.5, 7.3)	1.32 *d* (5.5)	2.09 *d* (7)	1.27 *d* (5.4)
5′	1.87 *dq* (1.5)	1.50 *s*	4.26 *dd* (5[b], 13)	1.53 *s*
			4.20 *dd* (5[b], 13)	
2″		2.47 *q* (7, 7.5)		
3″		1.73 *m*[a]		
		1.55 *m*[a]		
4″		0.95 *t* (7)		
5″		1.24 *d* (7.5)		
OEt	3.73 *q* (7)			
	1.25 *t* (7)			
OAc			2.15 *s*	2.13 *s*

[a] Signals overlapped.
[b] Coupling with 5′-OH.

compound **14**, showed significant differences especially with respect to the chemical shift of H-6. Coupling constants of $H_{7,13a/b}$ ($J > 3$ Hz) indicated a germacrolide for **19** rather than a heliangolide skeleton. Spectral data (including MS with M^+ 420 for $C_{22}H_{28}O_8$) were in agreement with the proposed structure. The desacetyl derivative of **19** has previously been isolated from *V. microphylla* (Torres-Valencia et al., 1999).

All compounds of *V. puruana* with an 8β-epoxyangelate side chain (except **20**) were found in both diastereomeric forms, 2′*R*,3′*R* and 2′*S*, 3′*S*, with an approximate ratio of 3:1. HPLC allowed separation of the diastereomers of **13**–**17** and **19**, but they were insufficient to establish the absolute stereochemistry by X-ray or chemical transformation as recently suggested (Torres-Valencia et al., 1999). However, comparison of the slightly different chemical shifts between the 2′*R*,3′*R* and 2′*S*,3′*S* diastereomers with the results published for compound **13** from *V. laciniata* (Gao et al., 1989) led us to the assumption, that *V. puruana* produces about three times more of the 2′*R*,3′*R* diastereomer than of the alternative form.

2.1. Chemotaxonomic aspects

Comparison of the sesquiterpene lactone pattern of *V. puruana* with other members of its section on a compound to compound basis could lead to the impression that section Maculatae is a phytochemically very diverse group. Thus *V. puruana* and *V. eriophora* in the current study share only two out of 20 compounds. However, looking at the basic skeletal types the impression is completely different and suggests a high infrasectional homogeneity. All members of the section that so far furnished sesquiterpene lactones contained 1,10-epoxyheliangolides (Ortega et al., 1980; Delgado et al., 1982, 1984a). In contrast, 1-keto-2,3-unsaturated furanoheliangolides which are very dominating in many other sections of *Vigueira* (for ref. see De Vivar and Delgado, 1985; Spring and Buschmann, 1996) were less abundant in the section Maculatae, and germacrolides may even be regarded as rare. While sharing the basic steps of sesquiterpene lactone biosynthesis, a high degree of interspecific diversity in the section Maculatae results from modifications at specific positions such as C-8 and C-3. Thus a methacrylate side chain at C-8 as well as an acetylation of the hydroxyl group at C-3, each occurs in three of the four taxa. The presence of a second side chain with four or five carbons in C-3 position is characteristic of *V. puruana* and, within the Helianthinae, has so far only been reported for *V. laciniata* (Gao et al., 1989) and *Tithonia rotundifolia* (Schuster et al., 1992). The cooccurrence of 1,10 epoxyheliangolides and germacrolides in *V. puruana* was found in *V. sphaerocephala* (Ortega et al., 1980) and was previously also reported from *Helianthus tuberosus* (Spring, 1991a) and *Tithonia rotundifolia* (De Vivar et al., 1982).

The abundance in sesquiterpene lactones of *V. puruana* and *V. eriophora* is in contrast to the reported absence of such compounds in three other taxa of the section Maculatae, namely *V. insignis*, *V. maculata* and *V. oaxacana* (Delgado et al., 1983, 1984b, c). This is most likely due to the lack of glandular trichomes (the source of sesquiterpene lactones in Helianthinae) on leaves and stems of the previously extracted plant material. Since by far most such plants produce sesquiterpene lactones in glandular trichomes located at flowering parts (especially anther appendages), it would be of interest to reinvestigate these species. *V. maculata* Blake, however, according to Panero and Schilling (1988) is a synonym for *V. eriophora* ssp. *eriophora* and may lead to similar results as reported here for this taxon.

3. Experimental

3.1. Extraction of plant material

V. eriophora (Greenman) ssp. *eriophora* Panero & Schilling was collected near Huajuapan, Oaxaca, Mexico, in September 1985 by J.L. Panero (voucher specimens deposited at TENN and MEXU; Panero & Panero 4). *V. puruana* Paray derived from an area near Jungapeo, Michoacán, Mexico, and was collected in November 1986 by J.L. Panero et al. (voucher at TENN). Air-dried leaves (30 g each) of both taxa were extracted with CH_2Cl_2 and were treated as usually. After drying the extract, the residue was redissolved in CH_3CN, diluted with water and centrifuged in order to remove insoluble parts. The clear supernatant was applied to HPLC (Hypersil ODS, 5 μm; 4 × 250 mm; 30% CH_3CN, 1.3 ml/min; UV detection simultaneously at 225 and 265 nm; 2,5-dimethylphenol as int. standard). Alternatively, MeOH (50%, 1 ml/min) was used for HPLC purification of fractions on the same type of column. The occurrence of the isolated compounds as natural products of the plant was checked by comparison of HPLC analysis of the purified compounds and extracts from trichomes of the leaf surface as described previously (Spring, 1991b).

Several impure fractions of small sample amounts, in order to prevent further loss of material through subfractionation, were applied to LC-^{1}H NMR and LC-MS measurements as described earlier (Spring et al., 1997). Both types of spectroscopic measurements were carried out under identical HPLC conditions in order to allow direct correlation of the obtained data. LC-^{1}H NMR spectra were measured on a Varian Unity *Inova*; 500 MHz, UV-detector type 9050 equipped with a NMR flow cell of 60 μl detectable volume. LC-MS experiments were performed on a Finnigan TSQ 700 under atmospheric pressure chemical ionization (APCI).

HPLC retention times of compounds from *V. eriophora* in 50% MeOH (RRT_1) and in 30% CH_3CN (RRT_2) relative to 2,5-dimethylphenol (retention time ca 13 min in both solvents): **1**, 0.36/041; **2**, 0.37/0.43; **3**, 0.54/0.60; **4**, 0.98/1.04; **5**, 0.38/0.45; **6**, 0.33/0.34; **7**, 0.47/0.63; **8**, 0.64/0.94; **9**, 0.69/0.96 (RRT_1/RRT_2).

HPLC retention times of compounds from *V. puruana* in 50% MeOH (RRT_1) and in 30% CH_3CN (RRT_2) relative to 2,5-dimethylphenol (retention time ca. 13 min in both solvents): **7**, 0.47/0.63; **9**, 0.69/0.96; **10**, 1.60/3.12; **11**, 0.71/0.85; **12**, 0.62/0.84; **13**, 0.34/0.43; **14**, 0.54/1.04; **15**, 1.24/2.71; **16**, 2.32/4.71; **17**, 2.82/5.65; **18**, 0.47/0.60; **19**, 0.70/1.25; **20**, 1.62/1.52 (RRT_1 / RRT_2).

*3.1.1. 1-Keto-3,10-epoxy-11α-methoxymethyl-8β-O-methacryloyl-15-hydroxy-2,4-germacradiene, 6α,12-olide (**2**)*

$C_{20}H_{24}O_8$, APCI +; grad. 40–60% MeOH in 20 min: 393 $[M+H]^+$, 375 $[M- H_2O]^+$, 306 [M — methacrylate]$^+$, 288 [375 — methacrylate]$^+$ 69 $[C_4H_5O]^+$.

*3.1.2. 1-Keto-3,10-epoxy-8β-O-methacryloyl-4,15-dihydroxy-5-acetoxy-2,11-germacradiene, 6α,12-olide (**5**)*

$C_{21}H_{24}O_{10}$, APCI +; grad. 40–60% MeOH in 20 min: 437 $[M+H]^+$, 69 $[C_4H_5O]^+$.

*3.1.3. 1α,3α,15-Trihydroxy-3,10-epoxy-8β-O-methacryloyl-4,11-germacradiene, 6α, 12-olide (**6**)*

$C_{19}H_{24}O_8$, ESI^-: 379 $[M-H]^-$.

*3.1.4. 3β-Ethoxy-leptocarpin (**11**)*

$C_{22}H_{30}O_6$, APCI +: 391 $[M+H]^+$, 363 [391 — C_2H_4]$^+$, 345 [391 — C_2H_5O]$^+$, 263 [363 — angelate]$^+$, 83 $[C_5H_7O]^+$.

*3.1.5. 3β-(2′-Methylbutanoyloxy)-8β-epoxyangeloyloxy-leptocarpin (**16**)*

$C_{25}H_{34}O_8$, APCI+: 463 $[M+H]^+$, 361 [463 — methylbutyrate]$^+$, 347 [463 — epoxyangelate]$^+$, 245 [463 — methylbutyrate–epoxyangelate]$^+$, 99 $[C_5H_7O_2]^+$, 85 $[C_5H_9O]^+$.

*3.1.6. 3β-O-Acetyl-8β-sarracinoyloxy-leptocarpin (**18**)*

$C_{22}H_{28}O_8$, APCI +: 421 $[M+H]^+$, 305 [421 — sarracinate]$^+$, 99 $[C_5H_7O_2]^+$.

*3.1.7. 3β-O-Acetyl-8β-epoxyangeloyloxy-tithifolin (**19**)*

$C_{22}H_{28}O_8$, APCI +: 421 $[M+H]^+$, 379 [421 — acetate]$^+$, 263 [379 — epoxyangelate]$^+$, 99 $[C_5H_7O_2]^+$.

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