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Germacranolides from *Mikania guaco*☆

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Dedicated to Professor Dr. H. Rimpler, Institut für Pharmazeutische Biologie, Universität Freiburg on the occasion of his 65th birthday.

Abstract

Fourteen novel sesquiterpene lactones of the germacranolide type have been isolated from the aerial parts of *Mikania guaco*: six costunolide, two melampolide and six germacra-4-*trans*,10(14),11(13)-trien-12,6α-olide derivatives. Except for one compound all the others possess a carbonyl function at C-9. Eight were obtained in the form of four isomer pairs which were difficult to separate. Structure elucidation was based on mass and 1D and 2D NMR measurements. Low energy conformations were obtained by quantum mechanical calculations. Pyrrolizidine alkaloids could not be detected. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Mikania guaco*; Asteraceae; Structure elucidation; Sesquiterpene lactones; Germacranolides

1. Introduction

Mikania guaco Humb. et Bonpl. (Asteraceae, tribe Eupatorieae) is a herbaceous creeper and climbs over shrubs and trees. The plant has a longstanding reputation as an anti-inflammatory remedy in the traditional medicine of Central America (Morton, 1981). Some time ago, four sesquiterpene lactones (Sls) of the eudesmanolide type were isolated, but pharmacological studies have not been undertaken (Castro et al., 1986). The search for Sls with potential anti-inflammatory activity has prompted us to reinvestigate the aerial parts of *M. guaco*. The present communication reports the isolation and structure elucidation of 14 new sesquiterpene lactones of the germacranolide type.

2. Results and discussion

The lipophilic extract of the aerial parts of *M. guaco* was separated using common CC in addition to high and low pressure as well as thin-layer chromatography (see Experimental). Bioguided fractionation using the agar plate diffusion assay for antibacterial activity as well as the electrophoretic mobility shift assay with the transcription factor NF-κB as molecular target for anti-inflammatory activity (Lyß et al., 1997) led to detection of 14 Sls (**1**–**14**) (Fig. 1), eight of which were obtained in the form of four isomer pairs differing only in the acyl moiety.

Compound **1** was obtained in a mixture with **2** as a white amorphous powder. TLC analysis using two different solvent mixtures revealed one spot which gave a brownish colour after spraying with anisaldehyde-sulfuric acid reagent. The mixture could not be resolved completely by HPLC analysis, but both compounds could eventually be separated with heavy losses. This was shown with compound **2** which was obtained in an amount of 1.5 mg. Therefore structure elucidation was carried out on the mixture.

The molecular mass of 420 was deduced from the mass spectrum (CI, isobutane, ESI). Moreover, fragment ions indicated the occurrence of an *O*-acetyl and an *O*-C_5-acyl moiety (see Experimental). The molecular formula $C_{22}H_{28}NaO_8$ was calculated by high resolution molecular mass measurement on the basis of the ion at *m*/*z* 443.

The ^{13}C NMR spectrum displayed 28 carbon signals with different intensities, 22 of higher and six signals of lower intensity. The 22 signals were assigned by DEPT-135 to the resonances of seven quaternary, six CH, six

☆ Part V in the series Phytochemical and biological studies of Costa Rican Asteraceae.

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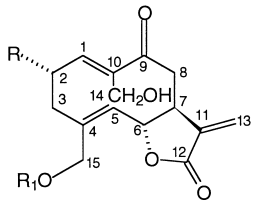
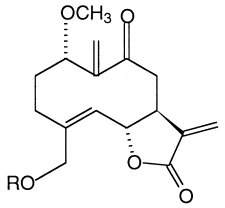
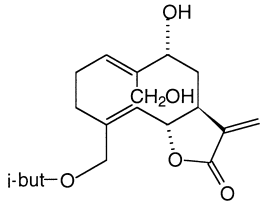


1 R: OAc; R_1: i-val
2 R: OAc; R_1: 2-mebut
3 R: H; R_1: i-but
4 R: H; R_1: i-val
5 R: H; R_1: 2-mebut



9 R: i-but
13 R: i-val
14 R: 2-mebut



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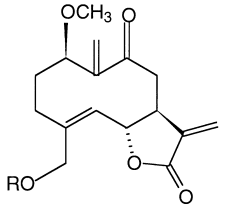
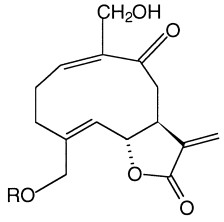


10: R: i-but
11: R: i-val
12: R: 2-mebut



7 R: i-val
8 R: 2-mebut

Fig. 1. Structures of the isolated sesquiterpene lactones.

CH_2, and three CH_3 carbon atoms. Five signals could be readily assigned to an isovaleryl and two to an acetoxyl moiety (see Table 1) (Budesinsky and Saman, 1995). The remaining 15 carbons indicated the occurrence of a sesquiterpene skeleton. The chemical shifts of five signals with lower intensity agreed with those of 2-methylbutyric acid (Budesinsky and Saman, 1995). Except for one carbon signal at δ 61.9 no further one with lower intensity could be detected. It follows that the main compound (**1**) differed from the minor (**2**) only in the C_5-acyl moiety and that both compounds possessed the same skeleton.

The presence of an α-methylene-γ-lactone moiety was evident from ^{13}C NMR signals at δ 120.8, 137.1 and 168.5. Consequently, in the ^1H NMR spectrum two characteristic one-proton doublets appeared downfield at δ 6.31 (H-13a) and 5.60 (H-13b), both being coupled to H-7 (δ 2.80). The coupling constants between H-6 and H-7 ($J_{6,7}=9.5$ Hz) together with the allylic coupling between H-7 and H-13 ($J_{7,13}=3.0$ Hz) indicated *trans* attachment of the α-methylene-γ-lactone ring (Samek, 1970), which was bound to a germacradienolide system. Two double bonds at Δ^4 and $\Delta^{1,10}$ could be deduced from two carbon signals (δ_C 134.6 and 133.1) typical for methine groups and from 2D COSY studies (Marco et al., 1997). The cyclodecadiene ring exhibits an oxygen function at C-2. Its presence was corroborated by the carbon signal at δ 70.1 and its location at C-2 followed from ^1H–^1H COSY, GHSQCR and GHMQCR analysis. The large coupling $J_{1,2}=10.4$ Hz agreed with an α-configuration (Bohlmann et al., 1982a; Vasquez et al., 1990). This value was similar to that reported for tamaulipin A angelate, for which the molecular structure was established by X-ray diffraction analysis (Vasquez et al., 1990). An α H-2 would have shown a smaller coupling constant ($J_{1,2}=6$ Hz) (Marco et al., 1997). The hydroxy group was esterified by acetic acid as proved by correlations between the ester carbonyl of the acetoxy group and H-2 in the GHMQCR spectrum (Table 3).

The 2D COSY spectrum only showed geminal couplings of the protons at C-8 and vicinal couplings of H-8 to H-7. Consequently, C-9 must be quarternary and resonated at δ 203.0. This was unambiguously confirmed by a GHMQCR experiment which exhibited correlations between the signal at δ_C 203 (C-9) and H-8α and β, H-7 and H-1 (see Table 3). The neighbouring oxygen function induced a downfield shift for C-8 to δ 40.5 (Vasquez et al., 1990) compared with an unsubstituted germacranolide, e.g. tamaulipin A angelate (δ 27.9). Although, the signal of C-1 should also be significantly shifted downfield, because of the conjugation between the carbonyl group and the double bond at $\Delta^{1,10}$. However, the resonance for C-1 at δ 134.6 showed only a slight downfield shift of about 8 ppm in comparison to a 1,10 double bond without any oxygen substitution (Vasquez et al., 1990). This could be explained by a keto group which is not coplanar with the 1,10-double bond, but most probably lies below the plane as already assumed earlier in the case of a costunolide derivative with a C-9 keto group where the same effects were observed (Bohlmann et al., 1981), in agreement with earlier studies which showed that the influence of a carbonyl group on neighbouring atoms strongly depends on its torsion angle (Montecalvo and St. Jacques, 1975). In **1** and **2,** the typical upfield signals of C-14 and C-15 methyl groups were replaced by two characteristic signals of oxygen bearing methylene groups in the ^1H and ^{13}C NMR spectrum. Assignment was achieved by GHSQCR and GHMQCR spectra. The magnitude of the ^{13}C NMR shifts suggested that they were bound to trans double bonds, respectively (Herz and Kumar, 1981; Lange and Lee, 1986; Pearce et al., 1986). The resonance at δ_C 59.1 was assigned to C-14, because of long range couplings of the respective doublet at δ_H 4.43 to C-1, 9 and 10 in the GHMQCR spectrum (Table 3) and the allylic coupling observed between H-1 and H-14a in the 2D COSY. Furthermore, C-14 contains a hydroxyl since in the 2D COSY spectrum both H-14 protons were coupled with the broad signal of the OH group. The remaining two one proton

Table 1
^{13}C NMR-spetral data of compounds **1**–**8** (75 MHz, $CDCl_3$)

C	1[bd]	2[bd]	3[b]	4	5	6[c]	7[e]	8[e]
1	134.6 CH	134.6 CH	141.0	141.0	141.0	127.6	136.7 CH	136.7 CH
2	70.1 CH	70.1 CH	26.8	26.8	26.8	26.0	23.8 CH_2	23.8 CH_2
3	41.1 CH_2	41.1 CH_2	35.3	35.4	35.4	35.2	34.0 CH_2	34.0 CH_2
4	139.4 C	139.4 C	140.0	139.9	139.9	139.7	138.4 C	138.5 C
5	133.1 CH	133.0 CH	130.8	130.9	130.9	131.4	128.7 CH	128.6 CH
6	78.6 CH	78.6 CH	78.9	78.9	78.9	79.8	78.4 CH	78.4 CH
7	48.3 CH	48.3 CH	48.8	48.8	48.8	41.4	47.8 CH	47.8 CH
8	40.5 CH_2	40.5 CH_2	40.1	40.2	40.2	35.7	42.5 CH_2	42.5 CH_2
9	203.0 C	203.0 C	203.6	203.6	203.6	69.3	202.6 C	202.6 C
10	146.5 C	146.5 C	–[f]	146.0	146.0	141.4	143.5 C	143.5 C
11	137.1 C	137.1 C	137.6	137.7	137.7	137.9	137.7 C	137.7 C
12	168.5 C	168.5 C	168.8	168.8	168.8	170.0	169.1 C	169.1 C
13	120.8 CH_2	120.8 CH_2	120.4	120.4	120.4	119.8	119.9 CH_2	119.9 CH_2
14	59.1 CH_2	59.1 CH_2	58.4	58.5	58.5	59.2	66.1 CH_2	66.1 CH_2
15	61.8 CH_2	61.9 CH_2	62.0	61.8	61.3	61.7	60.5 CH_2	60.5 CH_2
1′	170.4 C	170.4 C						
2′	20.9 CH_3	20.9 CH_3						
1″	172.5 C	176.2 C	176.8	172.7	176.2	176.9	172.6 C	176.4 C
2″	43.1 CH_2	41.0 CH	34.0	43.3	41.0	34.0	43.3 CH_2	41.0 CH
3″	25.6 CH	26.7 CH_2	19.0a	25.7	26.7	18.9	25.7 CH	26.7 CH_2
4″	22.4[a] CH_3	11.6 CH_3	18.9a	22.4	11.7	18.9	22.4 CH_3	11.6 CH_3
5″	22.3[a] CH_3	16.5 CH_3		22.4	16.6		22.4 CH_3	16.5 CH_3

[a] Assignment interchangeable.
[b] Assignments determined by GHSQCR and GHMQCR correlations.
[c] Assignments determined by GHSQCR correlations.
[d] Multiplicities from DEPT spectrum.
[e] Multiplicities from APT spectrum.
[f] not detectable.

Table 2
^{13}C NMR spectral data of compounds **9**–**14** (75 MHz, $CDCl_3$)[a]

C	9	10	11	12	13	14
1	77.9 CH	76.7 CH	76.6 CH	76.6 CH	77.9 CH	77.9 CH
2	36.0 CH_2	33.9 CH_2	33.9 CH_2	33.9 CH_2	36.1 CH_2	29.8 CH_2
3	29.7 CH_2	32.5 CH_2	32.4 CH_2	32.4 CH_2	29.8 CH_2	29.8 CH_2
4	143.2 C	144.8 C	144.6 C	144.6 C	142.7 C	142.7 C
5	127.3 CH	126.2 CH	126.4 CH	126.4 CH	127.5 CH	127.5 CH
6	77.7 CH	78.7 CH	78.6 CH	78.6 CH	77.7 CH	77.7 CH
7	46.9 CH	50.2 CH	50.2 CH	50.2 CH	46.9 CH	46.9 CH
8	39.8 CH_2	35.6 CH_2	35.6 CH_2	35.6 CH_2	39.8 CH_2	39.8 CH_2
9	199.7 C	201.9 C	201.9 C	201.9 C	199.7 C	199.7 C
10	151.1 C	152.4 C	152.4 C	152.4 C	151.1 C	151.1 C
11	138.1 C	138.0 C	138.0 C	138.0 C	138.1 C	138.1 C
12	168.9 C	169.1 C	169.1 C	169.1 C	168.9 C	168.9 C
13	119.6 CH_2	118.6 CH_2	118.6 CH_2	118.6 CH_2	119.6 CH_2	119.6 CH_2
14	123.4 CH_2	123.4 CH_2	123.5 CH_2	123.5 CH_2	123.5 CH_2	123.5 CH_2
15	63.4 CH_2	61.7 CH_2	61.4 CH_2	61.4 CH_2	63.3 CH_2	63.3 CH_2
OCH_3	56.0 CH_3	55.5 CH_3	55.7 CH_3	55.7 CH_3	56.0 CH_3	56.0 CH_3
1′	176.6 C	176.4 C	172.6 C	176.0 C	172.5 C	176.2 C
2′	33.9 CH	33.9 CH	43.2 CH_2	41.0 CH	43.1 CH_2	40.9 CH
3′	18.9[b] CH_3	18.9[b] CH_3	25.6 CH	26.6 CH_2	25.6 CH	26.6 CH_2
4′	18.8[b] CH_3	18.8[b] CH_3	22.4 CH_3	11.6 CH_3	24.6 CH_3	11.6 CH_3
5′			22.4 CH_3	16.4 CH_3	22.4 CH_3	16.4 CH_3

[a] Assignments determined by GHSQCR and GHMQCR correlations, multiplicities by DEPT analysis.
[b] Assignment interchangeable.

Table 3
^{1}H NMR data as well as GHMQCR and NOESY correlations of compounds **1** and **2** (300 MHz, $CDCl_3$)

H	**1**	**2**					**1**	**2**	**A**[e]	**B**[e]
	δ (ppm)		mult	GHMQCR, corr. with C[d]	NOESY, corr. with H[d]	H	J (Hz)			
1	5.54	5.54	*d*	2, 9, 10, 14	3α, 5, 7	1/2β	10.4	10.4	10.7	10.2
2β	5.40	5.40	*m*	1, 1′, 3, 10	14a, 15a	2β/3α	10.4	10.4	10.4	6.2
3α	2.29[a]	2.29[a]	*m*[b]	2, 4, 5, 15	1, 5	2β/3β	5.3	5.3	5.8	9.3
3β	2.84	2.84	*m*[b]	2, 4, 5, 15	15a	5/6	9.5	9.5	9.8	9.4
5	5.08	5.08	*d*	3, 6, 7, 15	1, 3α, 7	6/7	9.5	9.5	8.6	9.6
6	4.80	4.80	*t*[c]	3, 4, 5	8β, 14b	7/8α			1.0	10.3
7	2.80	2.80	*m*[b]	6, 9, 10, 11	3α, 5	7/8β	11.4	11.4	10.7	2.8
8α	2.77[a]	2.77[a]	*m*[b]	6, 7, 9, 10, 11		7/13a	3.0	3.0		
8β	2.98	2.98	*dd*	6, 7, 9	6, 14b	7/13b	3.0	3.0		
13a	6.31	6.31	*d*	7, 12		8α/8β	12.5	12.5		
13b	5.60	5.60	*d*	7, 12		14a/14b	13.9	13.9		
14a	4.43	4.43	*d (br)*	1, 9, 10	2, 3″ (**2**)	14b/OH	5.6	5.6		
14b	4.10	4.10	*dd*		2, 6, 8β	15a/15b	13.7	13.7		
15a	4.74	4.74	*d*	1″, 3, 4, 5	3β					
15b	4.65	4.65	*d*	1″, 3, 4, 5	3β	3″/4″	6.6			
OH at C-14	2.50	2.50	*m*			3″/5″	6.6			
2′	2.08	2.08	*s*	1′, 2		2″/5″		7.0		
2″	2.28[a]		*d*	1″, 3″, 4″, 5″	4″, 5″	3″/4″		7.4		
3″	2.14		*m*	2″, 4″, 5″						
4″	0.98		*d*	2″, 3″	2″					
5″	0.98		*d*	2″, 3″	2″					
2″		2.46	*m*	1″, 3″						
3″		1.72	*m*	1″, 4″						
3″		1.52	*m*	1″, 4″						
4″		0.93	*t*	2″, 3″						
5″		1.20	*d*	1″, 2″, 3″						

[a] Assignment determined by GHSQCR correlations.
[b] Multiplicity not determined (signal overlap).
[c] Pseudo triplet.
[d] Valid for **1** and **2.**
[e] Calculated coupling constants for conformation A and B of **1** and **2**.

doublets at δ_H 4.65 and 4.74, exhibiting a geminal coupling, were assigned to H-15a and H-15b and the carbon signal at δ_C 61.82 to C-15. The latter assignment as well as the esterification of the C-15 hydroxy group by isovaleric and 2-methylbutyric acid was confirmed by the GHMQCR spectrum (correlations of H-15a and b with C-3, 4 and 5 and the ester carbonyl of both C_5-acyloxy groups). The two substances were neither heliangolides (1,10-*trans*, 4-*cis*; $J_{6,7}$ and $J_{7,13} = 2$ Hz) nor melampolides (1,10-*cis*, 4-*trans*) but *trans*-1(10),*trans*-4-germacradienolides, as indicated by the magnitude of the coupling constants $J_{7,13}$ (> 3 Hz), $J_{6,7}$ (9.5 Hz) and by the chemical shifts for C-14 and C-15 (about δ 60) as well as by the NOESY spectrum. Assuming that H-7 is normally α-orientated, as shown by X-ray analysis (Witt and Watkins, 1978; Picher et al., 1984), the interactions between H-7, H-5 and H-1 in a NOESY experiment confirmed α-orientation, respectively. Hence, the ten-membered ring is in the $^{15}D_5$, $_1D^{14}$-conformation (= UU) for which crossed, trans double bonds and a double chair conformation was proved by X-ray diffraction (Witt and Watkins, 1978; Picher et al., 1984; Watson and Kashyap, 1986; Vasquez et al., 1990). Moreover, the relative stereochemistry at C-2 and C-6 was confirmed by the NOESY spectrum. The NOE between H-2 and H-14 as well as H-14 and H-6 showed the α-configuration of the C-2 acetoxy group and the *trans*-fused lactone ring.

The conformation of Sl **1** and **2** based on extensive NMR studies was in accordance with the mostly common one found for *trans*,*trans*-germacradiene-6,12-olides in solution (Watson and Kashyap, 1986). To find out whether further conformations may exist we calculated possible low-energy conformations of compound **1**. Two conformations, showing an up–up arrangement (UU-conformation) of the two hydroxymethyl groups, but differing in the orientation of the C-9 carbonyl group, were obtained (see Fig. 2, **1**/**2**: A and B). The theoretical values of the coupling constants were deduced from these molecular models (see Table 3). As expected, only the conformation, in which the carbonyl group showed antiparallel orientation to the 1,10 double bond, was in good agreement with the NMR data. Thus, in this case only one conformation was present, as it was previously shown for 14-acetoxy-8α-hydroxycostunolide

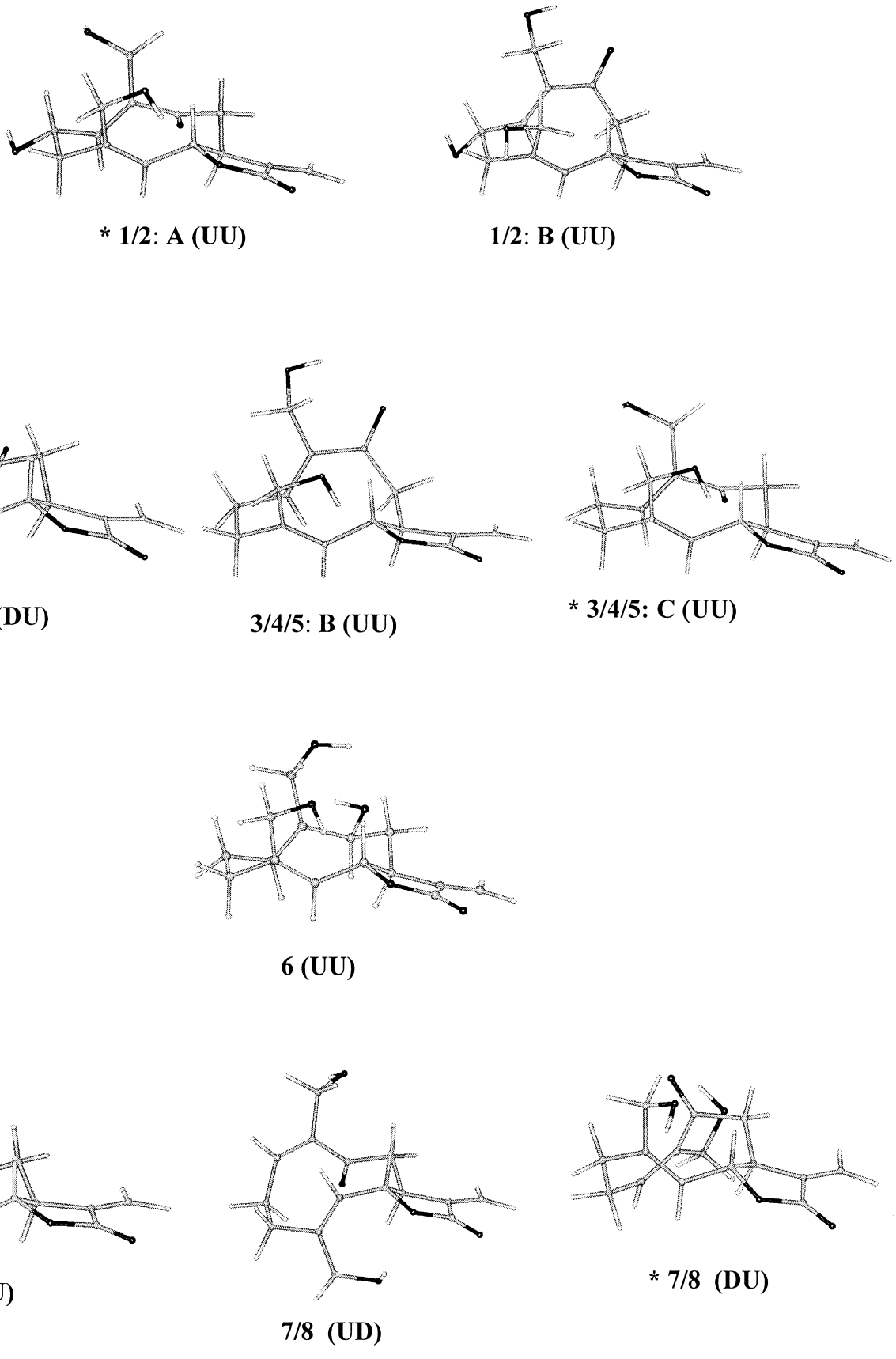


Fig. 2. Low energy conformations of the isolated sesquiterpene lactones **1**–**10** without acyl side chains as found by quantum mechanical calculations (*mainly occurring conformations considering the NMR data) (*continued on next page*).

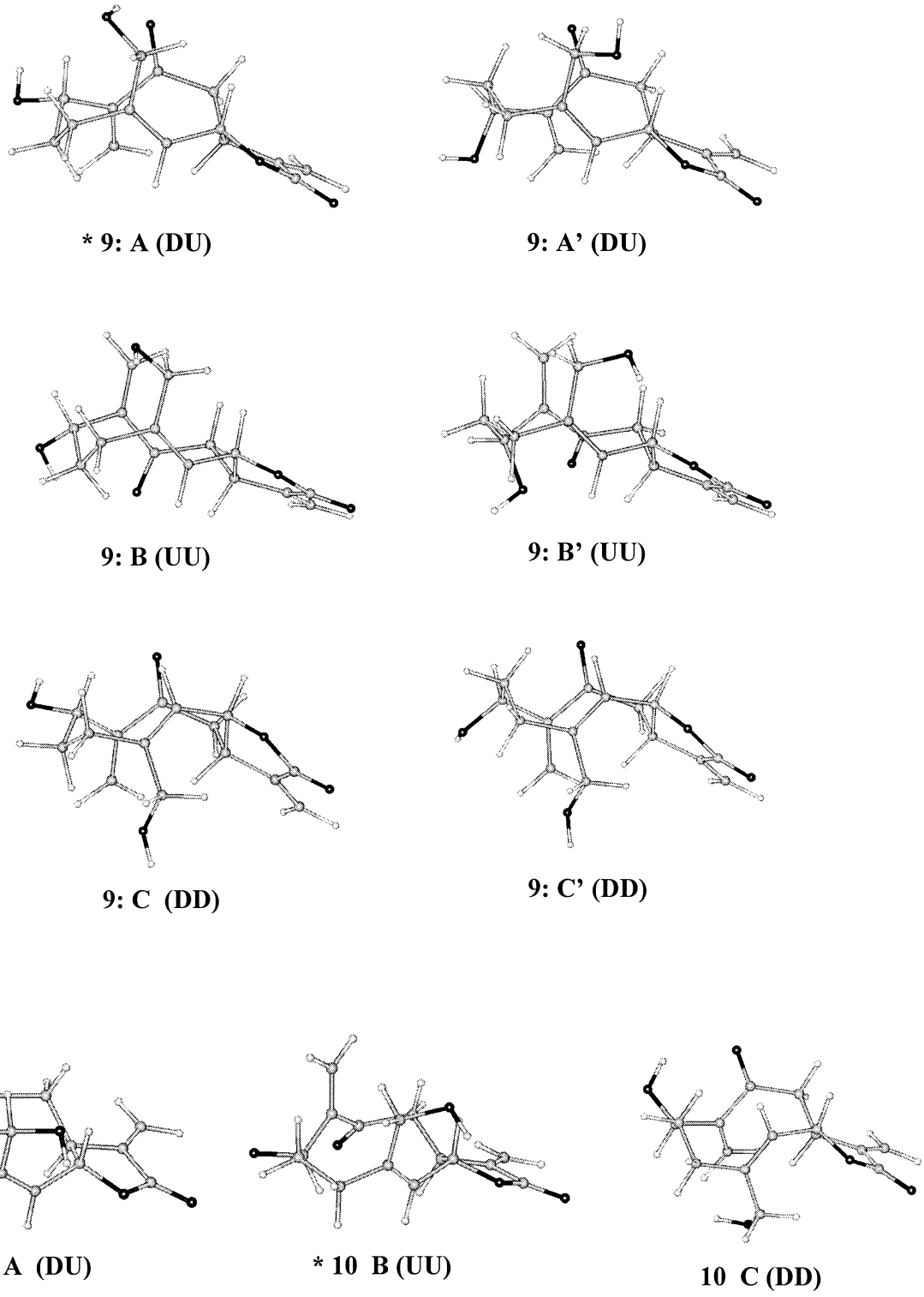


Fig. 2 (*continued*).

(Bohlmann et al., 1985). To the best of our knowledge, compounds **1** and **2** are here described for the first time. These costunolide derivatives can be described as 2α-acetoxy-14-hydroxy-15-isovaleryloxy-9-oxo-costunolide (**1**) and 2α-acetoxy-14-hydroxy-15-(2-methylbutyryloxy)-9-oxo-costunolide (**2**), respectively. The skeleton without the acyl moieties could be named miguanin, so Sl **1** is 2α-acetoxy-15-isovaleryl-miguanin and **2** 2α-acetoxy-15-(2-methylbutyryl)-miguanin.

Compound **3** gave a grey-brownish colour with anisaldehyde-sulfuric acid reagent and possessed a molecular mass of 348 (ESI–MS). Its ^{13}C NMR (see Table 1)

revealed the presence of 18 carbon atoms, four of which could be readily identified as belonging to an isobutyryloxy substituent (Budesinsky and Saman, 1995). The remaining carbon signals resembled those obtained from the skeleton of Sl **1** and **2**, except for C-1, C-2 and C-3 and to a lesser extent for C-5 and C-14. The signal of the quarternary C-10 could not be detected. The respective protons bound to these carbon atoms differed in their chemical shifts from those found for compounds **1** and **2** in the ^{1}H NMR spectrum, too. The other signals as well as the experimental coupling constants were identical. Thus, the occurrence of a germacrolide, the same as in **1** and **2**, was confirmed (see Table 4). Here, the C-15 hydroxy group was esterified by isobutyric acid indicated by the correlation of the ester carbonyl and both protons at C-15 in the GHMQCR spectrum. The differences in the chemical shifts could be explained when an unsubstituted C-2 was assumed. The lack of an oxygen function induced a pronounced upfield shift of C-2 and, to a lesser degree, of C-3 and a downfield shift of C-1. The signal for H-1 appeared as doublets of doublets and was shifted ca. 0.2 ppm downfield compared to **1**. Assignment of the stereochemistry followed from the NOESY spectrum by which the occurrence of a [$^{15}D_5$, $_1D^{14}$] was confirmed. Molecular models were created and theoretical values of the coupling constants calculated. From the three possibilities (A: DU, B: UU and C: UU) (see Fig. 2) only conformation C was in full agreement with the NOESY experiment and the coupling constants (see Table 4). Notations refer to the orientation of the methyl groups attached to C-10 and C-4, respectively. Thus, compound **3** is 14-hydroxy-15-isobutyryloxy-9-oxo-costunolide or 15-isobutyryl-miguanin, which has been found for the first time.

Compound **4** was isolated in a mixture with **5** as a colourless resin and showed the same behaviour in TLC analysis as the mixture of **1** and **2**. The occurrence of a pair of isomers was confirmed by the ESI and GC/MS mass spectra with a molecular mass of 362. ^{1}H and ^{13}C NMR data revealed the same skeleton as in compound **3** (see Tables 1 and 4), but differences in the acyl moiety. Thus, Sl **4** was 15-isovaleryl-miguanin and **5** 15-(2-

Table 4
^{1}H NMR data of compounds **3**–**5** as well as GHMQCR and NOESY correlations of **3** (300 MHz, $CDCl_3$)

H	**3**				**4**	**5**			**3**	**4**	**5**	**A**[e]	**B**[e]	**C**[e]
	δ (ppm)	mult	GHMQCR, corr. with C	NOESY, corr. with H	δ (ppm)		mult	H	J (Hz)					
1	5.74	*dd*	9	5, 8β	5.73	5.73	*dd br*	1/2α[d]	5.9	6.0	6.0	11.2	4.8	4.4
2a	2.54[a]	*m*[b]	3, 4		2.54	2.54	*m*[b]	1/2β[d]	11.0	11.0	11.0	4.3	10.6	11.2
2b	2.22[a]	*m*[b]	1, 3		2.16–2.22	2.16–2.22	*m*	2α/3α				11.7	9.4	5.4
3a	2.70[a]	*m*[b]	2, 4, 5, 15		2.70	2.70	*m*[b]	2α/3β				2.2	0.4	1.4
3b	2.20[a]	*m*[b]	2, 4, 5, 15		2.16–2.22	2.16–2.22	*m*	2β/3α				4.4	8.9	12.5
5	4.92	*d*	3, 7, 15	1, 7	4.92	4.92	*d*	2β/3β				11.6	9.6	5.4
6	4.83	*t*[c]	4, 5, 8	8β, 15b	4.81	4.81	*t*[c]	5/6	10.0	10.0	10.0	11.4	9.6	10.2
7	2.84	*m*[b]		5	2.84	2.84	*m*	6/7	10.0	10.0	10.0	9.1	9.5	8.8
8α	2.75	*dd*	6, 7, 9, 11		2.75	2.75	*dd*	7/8α	1.7	1.7	1.7	2.6	10.3	1.0
8β	3.07	*dd*	6, 7, 9	1, 6, 14b	3.06	3.06	*dd*	7/8β	10.3	10.3	10.3	6.1	3.0	10.1
13a	6.30	*d*	7, 11, 12		6.30	6.30	*d*	7/13a	3.4	3.3	3.3			
13b	5.61	*d*	7, 12		5.61	5.61	*d*	7/13b	3.2	3.3	3.3			
14a	4.28	*d*	1, 9		4.28	4.28	*d*	8α/8β	12.5	12.5	12.5			
14b	4.16	*d*	1, 9	8β	4.15	4.15	*d*	14a/14b	13.1	13.6	13.6			
15a	4.73	*d*	1′, 3, 4, 5	6	4.71	4.71	*d*	15a/15b	13.7	13.6	13.6			
15b	4.64	*dd*	1′, 3, 4, 5		4.65	4.65	*d*							
OH at	1.84	*s br*			1.80	1.80	*m*	2′/3′	7.1					
C-14								2′/4′	7.1					
2′	2.61	*sept*	1′, 3′, 4′		2.24		*m*[b]							
3′	1.21	*d*	1′, 2′		2.13		*m*[b]	3′/4′		6.6				
4′	1.21	*d*	1′, 2′		0.98		*d*	3′/5′		6.6				
5′					0.98		*d*							
								2′/5′			7.1			
2′						2.44	*m*	3′/4′			7.3			
3′a						1.70	*m*							
3′b						1.50	*m*							
4′						0.92	*t*							
5′						1.18	*d*							

[a] Assignment determined by GHSQCR correlations.
[b] Multiplicity not determined (signal overlap).
[c] Pseudo triplet.
[d] 1/2a and 1/2b for **3**, **4** and **5.**
[e] Calculated coupling constants for different conformations (A, B and C) of **3**–**5**.

methyl-butyryl)-miguanin. For these Sls the UU-conformation, as for compound **3**, can be assumed, too.

Compound **6** was obtained as a colourless resin which gave a violet colour after spraying with anisaldehyde-sulfuric acid. A molecular mass of 350 was deduced from the ESI and CI (isobutane) mass spectra. ^{13}C NMR data were close to those of Sls **3**–**5** except for the signals of C-7, C-8, C-9 and C-10. For compound **6** the signal of the keto carbonyl at C-9 (δ_C 203) was replaced by a signal at δ_C 69.26, which corresponds to an oxygenated methine group. In agreement with this the signals of C-1, C-7, C-8 and C-10 were shifted upfield. The ^1H NMR and 2D COSY spectra were in full agreement with these observations, displaying among others the double doublet at δ 4.78 of H-9 which coupled with the two protons at C-8. The stereochemistry of the protons at C-3 and C-8 was deduced by the NOESY spectrum and that of H-9 from the coupling constants. In fact, coupling constants of $J_{8\alpha,9} = 5.6$ Hz and $J_{8\beta,9} = < 1$ Hz agreed with an α-orientated C-9 hydroxy group (Rustaiyan et al., 1986; Bruno et al., 1991), whereas a β-orientation would give values of $J_{8\alpha,9} =$ about 2 Hz and $J_{8\beta,9} =$ about 10 Hz (Bohlmann et al., 1982b; Rustaiyan et al., 1986). The $^{15}D_5$, $_1D^{14}$-conformation was confirmed by the NOESY spectrum. NOEs involving H-7 and H-5 as well as H-5 and H-1 indicated that these substituents were below the plane, the NOE between H-6β and H-15a showed an above position. Quantum mechanical calculations proposed only one low energy form (see Fig. 2). The theoretical values of the coupling constants calculated from this molecular model were in good agreement with the experimental ones (see Table 5). Slight differences were only obtained for $J_{1,2\alpha}$ and $J_{1,2\beta}$. Again Sl **6**, which is 9α,14-dihydroxy-15-isobutyryloxy-costunolide, has not been found in nature up to now.

Compounds **7** and **8**, which were present as a mixture, had the same molecular mass ($M_r = 362$, ESI–MS) and similar spectroscopic data as Sls **4**–**5**. Again, **7** and **8**

Table 5
^1H NMR data and NOESY correlations of compounds **6**–**8** (300 MHz, $CDCl_3$)

H	**6** δ (ppm)	mult	NOESY, corr. with H	**7** δ (ppm)	**8** δ (ppm)	mult	NOESY, corr. with H[d]	H	**6**	**6**[f]	**7** *J* (Hz)	**8**	**7**/**8**[g]
1	5.45	*t(br)*	5	5.83	5.83	*t(br)*	14b	1/2α[e]	6.8	4.3	8.8	8.8	3.2
2α	2.33[ab]	*m*[c]		2.24	2.24	*m*[c]		1/2β[e]	9.3	11.2	8.8	8.8	4.6
2β	2.33[ab]	*m*[c]		2.74	2.74	*m*	15b	2α/3α		5.8			2.8
3α	2.18[a]	*m*[c]	5, 8α	1.75	1.75	*m*[c]	5	2α/3β		1.2			13.2
3β	2.55[a]	*m*[c]		2.57	2.57	*ddd*		2β/3α		12.2			4.2
5	4.99	*d*	1, 3α, 7	5.09	5.09	*d*	3α, 7	2β/3β		5.8			2.5
6	4.62	*dd*	8β, 15b	4.73	4.73	*dd*	8β	5/6	10.3	10.4	10.4	10.4	11.3
7	3.15	*m*	5	3.25	3.25	*m*	5, 14a	6/7	8.8	8.5	9.8	9.8	9.8
8α	2.42	*ddd*	3α	3.36	3.36	*dd*		7/8α	1.5	1.0	3.7	3.7	1.6
8β	2.07	*ddd*	6	2.29	2.29	*dd*	6	7/8β	9.3	9.5	12.2	12.2	11.8
9	4.78	*dd*						7/13a	3.7		3.2	3.2	
13a	6.27	*d*		6.26	6.26	*d*		7/13b	3.2		3.2	3.2	
13b	5.54	*d*		5.49	5.49	*d*		8α/8β	14.2		14.2	14.2	
14a	4.09	*d*		4.50	4.50	*dd*	7	8α/9	5.6	5.8			
14b	3.89	*d*		4.24	4.24	*d*	1	8β/9	< 1	1.2			
15a	4.59	*d*		4.65	4.65	*d*		14a/14b	12.0		12.5	12.5	
15b	4.54	*d*	6	4.36	4.36	*d*	2β	14a/OH			6.8	6.8	
OH at C-14				1.63	1.63	*m*		15a/15b	12.7		13.2	13.2	
2′	2.59	*sept*		2.20		*m*		2′/3′	7.1				
3′	1.20	*d*		2.08		*m*		2′/4′	7.1				
4′	1.20	*d*		0.95		*d*		3′/4′			6.6		
5′				0.95		*d*		3′/5′			6.6		
2′					2.38	*m*		2′/5′				7.1	
3′a					1.68	*m*		3′/4′				7.4	
3′b					1.48	*m*							
4′					0.90	*t*							
5′					1.14	*d*							

[a] Assignment determined by GHSQCR correlations.
[b] 2a and 2b.
[c] Multiplicity not determined (signal overlap).
[d] Valid for **4** and **5**.
[e] 1/2a and 1/2b for **6**.
[f] Calculated coupling constants from the model structure of **6**.
[g] Calculated coupling constants for the DU conformation of compound **7** and **8**.

should be isomers because only one pseudomolecular ion at m/z 363 appeared besides other signals from Na and K adducts. The ^{13}C and ^{1}H NMR data revealed the presence of a mixture of two components in different proportion, one with an isovaleryloxy (**7**) and the other with a 2-methylbutyryloxy residue (**8**) (see Tables 1 and 5). Differences in the chemical shifts of C-1, C-2, C-10 and C-14 suggested a germacranolide, but of a subtype different from Sls **1**–**6**. This was confirmed by the chemical shift of C-14 which was shifted downfield from ca 58 to 66.1 ppm and was characteristic for a vinylic hydroxymethyl group located on a 1,10-*cis* double bond (Asada et al., 1984; Lange and Lee, 1986). In the ^{1}H NMR spectrum, the H-1 signal appears as a broad triplet (Asada et al., 1984; Ming et al., 1989) while in germacrolides the corresponding signal exists as a double doublet. Additionally, the chemical shift suggests again a *cis* relationship with the hydroxymethyl group. That **7** and **8** were 1,10-*cis*,4-trans-germacran-12,6-olides with a *trans*-fused γ-lactone ring (melampolides) was also confirmed by the coupling constants $J_{1,2\alpha}$ and $J_{1,2\beta} = 8.8$ Hz, $J_{5,6} = 10.4$ Hz as well as $J_{7,13a}$ and $J_{7,13b} = 3.2$ Hz (Samek, 1970; Asada et al., 1984; Ming et al., 1989). The assignment of the relative stereochemistry of the protons at C-2, C-3 and C-8 was achieved by a NOESY spectrum. Quantum mechanical calculations afforded three low energy conformers (see Fig. 2) which differed mainly in the orientation of the substituents at the double bonds: UU, UD and DU. NOEs including H-1/H-14a, H-5/H-14a and H-7/H-14a showed a *cis*-configuration of the 1,10-double bond as well as orientation of both substituents below the plane. No NOE could be observed between H-5 and H-15, but between H-5 and H-7. Therefore a 4,5-*trans* double bond with an up arrangement of C-15 was present. Due to these observations only the DU-conformation which is in agreement with the X-ray structure of melampolide is possible (Ming et al., 1989) In contrast, Bohlmann et al. (1984) postulated for 8α-(2′,3′-epoxy-2′-methylbutyryloxy)-9-oxo-germacra-4*E*,1(10)-*Z*-dien-6β,12-olide a UD conformation. Theoretical values of the coupling constants calculated from the DU conformer agreed only partly with the experimental ones. These differences in the coupling constants $J_{1,2\alpha}$ and $J_{1,2\beta}$ may be explained by a different behaviour of this part of the molecule in solution than in the vacuum used for calculation. Altogether, compound **7** could be identified as 14-hydroxy-15-isovaleryloxy-9-oxo-melampolide and **8** as 14-hydroxy-15-(2-methylbutyryloxy)-9-oxo-melampolide. Both melampolides are described for the first time.

Compound **9** was separated from **10** by preparative TLC. Silica gel plates were developed several times in diethylether and afforded a colourless resin which gave a grey-brownish colour after spraying with anisaldehyde-sulfuric acid. The molecular mass of 362 was deduced from the mass spectra (CI, isobutane and ESI) and agreed with the molecular formula $C_{20}H_{26}O_6$. Fragment ions at m/z 275 [M + H–$C_4H_8O_2^+$] and 257 [M + H–H_2O–$C_4H_8O_2$] in the CI mass spectrum indicated the occurrence of a C_4-acyl acid residue which was identified as isobutyric acid according to the signals in the ^{1}H and ^{13}C NMR spectra (see Tables 2 and 6). The remaining chemical shifts in the ^{1}H and ^{13}C NMR spectra (^{1}H-^{1}H COSY, GHMQCR, GHSQCR and DEPT) revealed that compound **9** partly possessed the same structure elements found in Sl **3**, e.g. a 6,7-*trans*-α-methylene-γ-lactone ring, a carbonyl group at C-9, a Δ^4 double bond and a hydroxymethyl group at C-15 esterified with isobutyric acid (see Tables 2 and 6). However, the corresponding signals for C-1 and C-14 at about δ_C 140 and δ_C 60, respectively were replaced by signals at 77.9 and 123.4. The first signal corresponds to an oxygen substituted methine carbon, which correlated in the GHSQCR spectrum with a broad doublet that was assigned to H-1 in the COSY experiment. Additionally, a signal appearing at δ_C 56.0 corresponds to a methoxy group, which must be located at C-1 since only one –CH–O– group was observed. The signal at δ_C 123.4 correlated with two downfield one-proton singulets in the GHSQCR spectrum at δ_H 6.08 and 5.92 and well agreed with an exocylic methylene group attached to C-10. This fact was confirmed by long range couplings between H-14a and H-14b and H-1 and correlations of the exocyclic protons to C-1, C-9 and C-10 in the GHMQCR spectrum. Due to the presence of the carbonyl function at C-9 the C-1 signal was shifted downfield about 10 ppm compared with values reported in the literature for unsubstituted compounds (Segal et al., 1983; Sanz et al., 1989; Marco et al., 1991). Since the coupling constants $J_{1,2\alpha}$ and $J_{1,2\beta}$ show values of similar magnitude (Geissman, 1970; Segal et al., 1983; Sanz et al., 1989; Marco et al., 1991), the relative stereochemistry at C-1 was determined from the NOESY spectrum. NOEs between H-7α and H-14 as well as H-14 and the methoxy group showed that H-1 was β. Moreover, *trans*-configuration of the Δ^4 double bond as well as the position of C-15 above the plane were confirmed by an NOE between H-5 and H-7α. Calculation of low energy conformations afforded the three conformers DU, UU and DD, which existed in two forms, respectively (see Fig. 2). These subtypes differed in the position of C-2, which is either above or below the plane. Similar conformations have already been reported from the structural related germacranolide lucentolide (Marco et al., 1997). Here, the DU conformer predominated, as it was also reported for 8β-acetoxy-1β-peroxycostunolide (Schmeda-Hirschmann et al., 1985). NOESY experiments as well as the comparison of the experimental with the calculated coupling constants from the force field molecular models provided evidence for the occurrence of the DU conformer A with C-2 facing down (see Fig. 2 and Table 6). Thus, compound **9** is 1α-methoxy-15-isobutyryloxy-9-oxo-germacra-4-*trans*-10 (14),11(13)-trien-12,6α-olide.

Table 6
^{1}H NMR data as well as GHMQCR and NOESY correlations of compounds **9** and **10** (300 MHz, * 400 MHz)

H	**9** δ * (ppm)	mult	GHMQCR, corr. with C	NOESY, corr. with H	**10** δ * (ppm)	mult	GHMQCR, corr. with C	NOESY, corr. with H	H	**9**	A[d]	A′[d] J (Hz)	**10**	B[e]
1	4.03	*d(br)*			4.23	*dd*		3α	1β/2α	8	9	5.4		
2α	2.03[a]	*m*[b]		5	2.15[a]	*m*[b]		5	1β/2β		1.2	1.5		
2β	2.14[a]	*m*[b]			1.78	*m*		14	1α/2α				2	1.2
3α	2.51[a]	*m*[b]		5	2.12[a]	*m*[b]		1	1α/2β				10	10.5
3β	2.10[a]	*m*[b]			2.50	*m*[b]			2α/3α		3.6	0.4		3.6
5	5.31	*d*	3, 7, 15	2α, 3α, 7	5.19	*d*	3, 7, 15	2α, 7	2α/3β		13.5	7.9		3.0
6	4.69	*m*			4.44	*t*[c]		15a	2β/3α		2.9	12.2		13.5
7	3.30	*m*		5, 14b	3.15	*m*		5	2β/3β		3.5	0.5		3.6
8α	3.50	*dd*	6, 7, 9	14b	2.67	*dd*	6, 7, 9, 11		5/6	10	11.6	11.3	10	11.5
8β	2.38	*dd*	6, 7, 9, 10, 11		2.90	*t(br)*	6, 7, 9	14, 15b	6/7		8.9	9.6	10	9.4
13a	6.26	*d*	7, 11, 12		6.22	*d*	7, 11, 12		7/8α	4	2.8	1.8	1.5	1.0
13b	5.50	*d*	7, 11, 12		5.50	*d*	7, 11, 12		7/8β	12	12.4	12	12	9.6
14a	6.08	*s*	1, 9, 10	OCH_3	5.99	*d*	1, 9, 10	2β, 8β, OCH_3	7/13a	3			3	
14b	5.92	*s*	1, 9, 10	7, 8α	5.99	*d*	1, 9, 10	2β, 8β, OCH_3	7/13b	3			3	
15a	4.54	*d(br)*	1′, 4		4.60	*dd*	1′, 3, 4, 5	6	8α/8β	14			12	
15b	4.38	*d(br)*	1′, 4		4.46	*d*	1′, 3, 4, 5	8β	14a/14b				1.5	
OCH_3	3.17			14a	3.18	*s*	1	14	15a/15b	13			13	
2′	2.56	*sept*	1′, 3′, 4′		2.55	*sept*	1′, 3′, 4′		2′/3′	7			7	
3′	1.17	*d*	1′, 2′		1.16	*d*	1′, 2′		2′/4′	7			7	
4′	1.17	*d*	1′, 2′		1.16	*d*	1′, 2′							

[a] Assignment determined by GHSQCR correlations.
[b] Multiplicity not determined (signal overlap).
[c] Pseudo triplet.
[d] Calculated coupling constants from model structures of **9.**
[e] Calculated coupling constants from model structure of **10**.

Compound **10** had MS and NMR features similar to those of **9** suggesting the occurrence of isomers. Both Sls differed in the stereochemistry of the methoxy group at C-1 and in the conformation of the skeleton. The most conspicuous difference was observed in the multiplicity of the signal for two H-14, which resonated at δ_H 5.99 and appeared as a doublet. Furthermore, in the ^{13}C NMR spectrum chemical shifts for C-2, C-8 and C-15 were shifted upfield, that of C-3 and C-7 downfield of 2–3 ppm. The NOESY experiment confirmed β-orientation of the methoxy group and of the exocyclic methylene group at C-10 as well as the orientation of C-14 and C-15 above the plane by interactions between H-2β, H-8β, H-14, H-15 and the methoxy group. Therefore, only the UU conformation (B) which was obtained by quantum mechanical calculation agreed with the NMR data, but not the low energy forms of a DU (A) and DD (C) conformation (see Fig. 2). Theoretical values of the coupling constants were also in good agreement with the experimental ones (see Table 6). Thus, compound **10** possessed the same conformation as gallicin, which lacks the carbonyl function at C-9 and is substituted by a hydroxy instead of a methoxy group (González et al., 1979). Compound **10** is 1β-methoxy-15-isobutyryloxy-9-oxogermacra-4-*trans*,10(14),11(13)-trien-12,6α-olide, a new epimer of Sl **9**.

Both epimers can be easily distinguished by the different behaviour of the methylene protons at C-14. The following explanation may be applicable: In the DU conformation A of compound **9** the distance between the oxygen at C-9 and one proton at C-14 is 3.88 Å, and 2.64 Å between the oxygen at C-9 and the other proton at C-14; on the other hand in compound **10** with the β-methoxy group, these values were 3.71 and 3.3 Å, respectively. The distances were calculated using HyperChem™. The influence of the two electronegative oxygen atoms on both protons is much more regular for compound **10** than for **9** and hence both protons at C-14 might be chemically equivalent and resonated as one signal.

Compounds **11** and **12** were obtained and identified as a mixture for the reasons mentioned above. The mass spectra (CI, isobutane, negative mode and ESI) showed one molecular ion peak at *m*/*z* 376 and one pseudomolecular ion peak at *m*/*z* 377. ^{1}H and ^{13}C NMR data agreed well with those of compound **10** (see Tables 2 and 7), with the isobutyryloxy moiety replaced by isovaleryloxy in **11** and 2-methylbutyryloxy in **12**. Comparison of the experimental and theoretical coupling constants confirmed the same conformation (UU, B) as found for Sl **10**. **11** and **12** have been isolated for the first time.

An additional mixture of Sls contained compounds **11** and **12** and a further pair of isomers. The mass spectra [EI, CI (NH_3)] from which a molecular weight of 376 was determined did not give any hint about the complexity of the mixture. However, ^{1}H and ^{13}C NMR spectra revealed the occurrence of two isomeric pairs. Besides the signals

Table 7
^{1}H NMR data of compounds **11**–**14** ($CDCl_3$, 300 MHz)

H	11 δ (ppm)	12 δ (ppm)		13 δ (ppm)	14 δ (ppm)	mult	H	11 J (Hz)	12 J (Hz)	13 J (Hz)	14 J (Hz)
1	4.24	4.24	*dd*	3.99	3.99	*d(br)*	1β/2α			8	8
2α	2.15[a,b]	2.15[a,b]	*m*[c]	2.03[a,d]	2.03[a,d]	*m*[c]	1β/2β				
2β	1.80[a,b]	1.80[a,b]	*m*[c]	2.15[a,d]	2.15[a,d]	*m*[c]	1α/2α	2.5	2.5		
3α	2.12[a,b]	2.12[a,b]	*m*[c]	2.48[a,d]	2.48[a,d]	*m*[c]	1α/2β	10	10		
3β	2.50[a,b]	2.50[a,b]	*m*[c]	2.10[a,d]	2.10[a,d]	*m*[c]	5/6	10.5	10.5	10	10
5	5.20	5.20	*d*	5.28	5.28	*d*	6/7	10.5	10.5		
6	4.46	4.46	*t*[e]	4.68[a]	4.68[a]	*m*	7/8α	2	2	4	4
7	3.15	3.15	*m*	3.28[a]	3.28[a]	*m*	7/8β	12	12		
8α	2.67[b]	2.67[b]	*dd*	3.47[b]	3.47[d]	*dd*	7/13a	3	3	3	3
8β	2.90[b]	2.90[b]	*t(br)*	2.36[a,d]	2.36[a,d]	*m*[c]	7/13b	3	3	3	3
13a	6.23	6.23	*d*	6.21	6.21	*d*	8α/8β	12	12	14	14
13b	5.51	5.51	*d*	5.47	5.47	*d*	15a/15b	13	13		
14a	6.00	6.00	*d*	6.04	6.04	*s*	3′/4′	6.6	7.4	6.6	7.4
14b	6.00	6.00	*d*	5.90	5.90	*s*	3′/5′	6.6		6.6	
15a	4.62	4.62	*dd*	4.54[a]	4.54[a]	*d*	2′/5′		7.1		7
15b	4.45	4.45	*d*	4.34[a]	4.34[a]	*d*					
OCH_3 at C-9	3.19	3.19	*s*	3.15	3.15	*s*					
2′	2.20		*m*[c]	2.18[a]		*m*[c]					
3′	2.10		*m*[c]	2.06[a]		*m*[c]					
4′	0.93		*d*	0.93[a]		*d*					
5′	0.93		*d*	0.93[a]		*d*					
2′		2.40	*m*[c]		2.38[a]	*m*[c]					
3a′		1.65	*m*[c]		1.64[a]	*m*[c]					
3b′		1.45	*m*[c]		1.43[a]	*m*[c]					
4′		0.89	*t*		0.87[a]	*m*[c]					
5′		1.15	*d*		1.10	*d*					

[a] Assignment determined by GHSQCR correlations.
[b] Assignment according to **10**.
[c] Multiplicity not determined (signal overlap).
[d] Assignment according to **9**.
[e] Pseudo triplet.

for **11** and **12**, those for two further Sls differing in the acyl moiety, but possessing both the skeleton of compound **9**, were detected (see Tables 2 and 7). Compound **13** was the isovaleryloxy and **14** the 2-methylbutyryloxy derivative of **9**. The same conformation (DU), as determined for **9** can be assumed.

The known 6-methoxyflavone eupafolin was isolated and identified by MS and comparative TLC analysis with the authentic compound (Liu and Mabry, 1981). β-Amyrin, β-amyrin acetate as well as stigmasterol and β-sitosterol were identified by comparative GC/MS analysis, too.

All Sls presented here possess a germacranolide skeleton. The eudesmanolides which were previously found in the aerial parts of *M. guaco* from a different location in Costa Rica could not be detected. This difference may be explained by the occurrence of geographical variability which has also been found in the case of *M. micrantha* (Cuenca et al., 1988). Germacranolides of the germacrolide and melampolide type are characteristic Sls of the genus *Mikania*. However, whereas more often Sls with a 7,8 attached lactone ring have been found in other *Mikania* species, only germacradiene-6,12-olides were detected in *M. guaco* investigated here. These Sls were isolated by bio-guided fractionation using the transcription factor NF-κB as molecular target. This protein is a pivotal mediator in the immune system and regulates the transcription of various inflammatory cytokines (Lyß et al., 1997). All Sls were studied in more detail for their antiinflammatory activity which will be reported in a subsequent paper.

The genus *Mikania* belongs to the tribe Eupatorieae from which most species with pyrrolizidine alkaloids are known, like in the tribe Senecioneae (Asteraceae) (Mattocks, 1968,1986). Unsaturated representatives of these secondary metabolites are toxic. They possess especially hepatotoxic properties. Therefore, it is essential to clarify if plants used in traditional medicine are free of this type of alkaloids. Saturated and unsaturated pyrrolizidine alkaloids can be distinguished by the reagent of Dann and Mattocks (detectable limit 0.5–1.0 μg) after TLC analysis (Mattocks, 1968; Röder and Neuberger, 1988). Here we proved that no type of pyrrolidizidine alkaloids could be detected in the methanolic extract of

the aerial parts from *M. guaco* under these conditions. The Dragendorff reagent, an unspecific reagent for alkaloids, also gave a negative result. From this point of view preparations from *M. guaco* are quite safe. However, it has to be kept in mind that due to the presence of Sls, external use of *M. guaco* preparations can induce contact dermatitis (Hausen and Vieluf, 1997).

3. Experimental

3.1. General experimental procedures

Optical rotation was measured with Perkin-Elmer 241 polarimeter at 27°C. Mass spectra were recorded in the direct inlet mode using chemical ionization with NH_3 or isobutane as reactant gas (CI-MS) on a Finnigan MAT 8200 (130 eV) or Finnigan MAT 44S, EI–MS on a Finnigan MAT 44 (70 eV), ESI–MS on a Finnigan TSQ 7000; GC/MS on a Finnigan GC 9610/MS 4500, 70 eV using an OV-1-DF capillary column (25 m×0.25 mm I.D., 0.25 μM film-thickness), carrier He at 24 ml/min (total flow), split 1:24, column flow 1.0 ml/min, gradient: 120–270°C, rate: 10°C/min, GC/MS-CI with isobutane as reactant gas on a Varian 3700/MAT 44S, 170 eV, DB-1 CB (5 m×0.32 mm×0.25 μm) carrier He, split 1:20, gradient: 50–300°C, rate 20°C/min; GC analyses were carried out with a Varian 3700 equipped with a split injector and FID, using an OV-1-DF capillary column (25 m×0.25 mm I.D., 0.25 μM film-thickness), carrier nitrogen at 25 ml/min, split 1:42.7, column flow 0.59 ml/min, temperatures: injector and detector 290°C, gradient as above, retention time (RT) of the standard santonin: 10.92 min; NMR spectra were recorded with a Varian UNITY 300 (300/75 MHz), compounds **9** and **10** partly with a Bruker Arx 400 (400 MHz). All NMR spectra were taken at room temperature with TMS as internal standard.

TLC for the detection of Sls was performed using silica 60 plates (Merck) with CH_2Cl_2–EtOAc–MeOH (8:1:1) (system I) and toluene–EtOAc (1:2) (system II) as solvents and anisaldehyde–H_2SO_4 as visualizing agent, heating at 120°C. TLC for the detection of pyrrolizidine alkaloids was carried out using silica 60 plates with CH_2Cl_2–MeOH–NH_3 (25%) (85:14:1) as solvent and the reagent of Dann and Mattocks (Röder and Neuberger, 1988). Here plates were subsequently sprayed with 30% H_2O_2, acetanhydride-benzene (80–100°C)-toluene (1:4:5) and finally with *p*-dimethylaminobenzaldehyde (2.00 g), 37% HCl (54.0 ml), EtOH (90%) (ad 100.0 ml). The plates were heated at 120°C for 30 min after each spraying. Monocrotaline was used for comparison. Low pressure chromatography was carried out with a pump from Latek (model S1990) combined with a UV detector and with columns from Latek LC-1/2-23 and Kronlab Classic CL 15/450 and 20/450.

3.2. Calculation of the conformations for Sls **1**, **3**, **6**, **7**, **9**, **10** and the coupling constants

We generated low-energy conformations of the Sls using the conformational search option of ChemPlus® (v. 2.0), which is operated under the Molecular Modelling package Hyperchem® (v. 5.1 Professional). Energy minimizations were performed with Hyperchem's semi-empirical quantum mechanical method AM1 using the Polak–Ribiere minimization algorithm.

Starting structures were created with Hyperchem and initially minimized to an RMS gradient < 0.01 kcal mol^{-1} Å^{-1}. All rotatable cyclic bonds were included as variable torsions and allowed to be changed simultaneously. The search was performed applying a usage directed search method and standard settings for duplication tests. A search run was terminated after energy minimization of 2500 unique starting geometries. Acyl side chains of the respective Sls were not included in the conformational search. The resulting structures were energy minimized to an RMS gradient as above. Coupling constants were calculated with PCMODEL™ using the MOPAC form of the conformations created with HyperChem™.

3.3. Plant material

Aerial parts of *M. guaco* were collected near Rio Corinto, Costa Rica, in March 1995 and identified by L. Poveda, Professor of Botany, Universidad National, Costa Rica. Voucher specimens (no. FM 1022) are deposited at the Herbarium of Inventory Program, INBio, Costa Rica.

3.4. Extraction and isolation

Dried, powdered aerial parts of *M. guaco* (1147 g) were exhaustively extracted with *n*-hexane–Et_2O–MeOH (1:1:1) at room temp. The crude extract (48.5 g) was treated with MeOH at −20°C for 48 h. After filtering and evaporation of the solvent a viscous residue (29.8 g) was obtained. The MeOH soluble part (in four portions) was separated by CC on Sephadex LH-20 with MeOH as solvent. Nine fractions were obtained. They were studied for their antibacterial activity in the agar plate diffusion test (Burkhardt, 1992) using *Bacillus subtilis* and *Staphylococcus aureus* as well as for their antiinflammatory activity in the NF-κB EMSA (Lyß et al., 1997). Fractions 4–6 showed inhibitory activity in both assays. Further isolation of the Sls followed from fractions 5 and 6. During the isolation procedure studies on the antibacterial activity were repeated from time to time. CC of fraction 5 on Sephadex LH-20 with cyclohexane–CH_2Cl_2-MeOH (7:4:1) gave 10 subfractions (5.1–5.10).

Subfr. 5.2 (3.14 g) was submitted to CC on silica gel with CH_2Cl_2–EtOAc mixtures of increasing polarities (9:1–4:6) and afforded 15 fractions (5.2.1.–5.2.15). CC

of 5.2.14 (440 mg) on silica gel 60 using CH_2Cl_2–EtOAc–MeOH (8:1:1) yielded eight fractions. Further separation of subfr. 5.2.14.4 (166 mg) on silica gel 60 with *n*-hexane–EtOAc mixtures (100–50%, rate 5%) afforded 10 fractions. Subfr. 5.2.14.4.6. gave 21.8 mg of a mixture of **1** and **2**, subfr. 5.2.14.4.10 a mixture of **4** and **5** (6.8 mg). A further subfr. 5.2.14.4.7 (12.3 mg), containing compounds **1** and **2**, was separated by HPLC on a RP-18 column (Hypersil ODS, 5 μm, 12.5×4.6 cm) with a water-MeOH gradient (flow: 3.2 ml/min, MeOH–H_2O, 45–60%, within 45 min) and 1.5 mg of compound **2**, nearly pure, was obtained.

Subfr. 5.7 (88 mg) was chromatographed on RP-18 (Eurosil Bioselect, 20–45 μm) by low pressure chromatography with a H_2O–MeOH mixture (58:42) and subsequently on a RP-18 column (Hypersil ODS, see above) by HPLC with H_2O–MeOH (65:35). Thus, compound **3** (6 mg) and **6** (5 mg) were isolated.

Subfr. 5.2.14.5 (152 mg) was submitted to low pressure chromatography on RP-18 (Eurosil Bioselect, 20–45 μm) with a H_2O–MeOH gradient (40–65%) which afforded 5.5 mg of a mixture of **7** and **8**.

CC of subfr. 5.2.6 (543 mg) on Sephadex LH-20 with cyclohexane–CH_2Cl_2–MeOH (7:4:1) yielded five fractions. Subfr. 5.2.6.4 (150 mg) was separated by low pressure chromatography on RP-18 (Eurosil Bioselect, 20–45 μm) and 60% aqueous MeOH as eluent. Prep. TLC, by which silica gel G plates were developed four times with Et_2O, was carried out with subfr. 5.2.6.4.3 (50.6 mg) yielding 8.3 mg of **9** and 6.0 mg of **10**. Both compounds were slightly impure. Subfr. 5.2.6.4.10 afforded 31 mg of a mixture of **11**, **12**, **13** and **14**, which gave a broad peak in HPLC. Further separation attempts were unsuccessful.

Subfr. 5.2.6.3 (122 mg) was rechromatographed on Sephadex LH-20 with cyclohexane–CH_2Cl_2–MeOH (7:4:1). One of the subfr. (80 mg) was submitted to CC on silica gel 60 using mixtures of *n*-hexane–EtOAc (100–50%, rate 5%). One fraction (2.2 mg) yielded **11** and **12** in a mixture.

Fraction 6 (4.65 g) was subjected to CC on Sephadex LH-20 with cyclohexane–CH_2Cl_2–MeOH (7:4:1) and gave 18 subfractions (6.1–6.18). Low pressure chromatography of subfr. 6.11 (417 mg) on RP-18 (Eurosil Bioselect, 20–45 μm) using 50% aqueous MeOH afforded an additional amount of **3** (8.2 mg).

The flavone eupafolin was obtained from fraction 8 (410 mg). Identification of the triterpenes occurred in subfraction 6.4, while that of the sterols in subfr. 5.2.5.

3.5. 2α-Acetoxy-15-isovaleryl-miguanin (*1*) and 2α-acetoxy-15-(2-methylbutyryl)-miguanin (*2*)

RT: 15.67 min (**1**), 15.80 min (**2**); R_f: 0.64 (I) and 0.46 (II) (**1** and **2**); optical rotation: dextrorotatory (**2**), no value given, because of the small amount; UV (online): $\lambda_{max} = 209.9$ nm (**1** and **2**); CI–MS (isobutane) *m/z* 421 $[m+H]^+$ (11), 415 (9), 391 $[M+H–CH_2O]^+$ (6), 361 $[M+H–CH_3COOH]^+$ (5), 331 $[M+H–CH_2O–CH_3COOH]^+$ (100), 319 $[M+H–C_5\text{-acid}]^+$ (8), 289 $[M+H–CH_2O–C_5\text{-acid}]^+$ (4), 258 (8), 229 $[M+H–CH_2O–CH_3COOH\text{-}C_5\text{-acid}]^+$ (23), 185 (6), 169 (10), 103 (6); ESI–MS *m/z* 475 $[M+Na+CH_3OH]^+$ (44), 443 $[M+Na]^+$ (100), 415 $[M+Na+CH_3OH–CH_3COOH]^+$, 413 $[M+Na–CH_2O]^+$ (32), 385 $[M+Na+CH_3OH–CH_2O–CH_3COOH]^+$ (2), 383 $[M+Na–CH_3COOH]^+$ (3), 353 $[M+Na–CH_2O–CH_3COOH]^+$ (28).

3.6. 15-Isobutyryl-miguanin (*3*)

Amorphous, optical rotation: dextrorotatory, value not given, because of the small amount; UV (online): $\lambda_{max} = 219.3$ nm; RT: 12.16 min; R_f: 0.57 (I) and 0.34 (II); ESI–MS *m/z* 387 $[M+K]^+$ (100), 371 $[M+Na]^+$ (37), 366 $[M+H_2O]^+$ (22), 349 $[M+H]^+$ (3).

3.7. 15-Isovaleryl-miguanin (*4*) and 15-(2-methylbutyryl)-miguanin (*5*)

RT: 12.59 min (**4**) and 12.59 (**5**); R_f: 0.57 (I) and 0.36 (II) (**4** and **5**); GC/CI–MS (isobutane): *m/z* 363 $[M+H]^+$ (56), 349 (9), 333 $[M+H–CH_2O]^+$ (10), 261 $[M+H–C_5\text{-acid}]^+$ (100), 243 (10), 231 $[M+H–CH_2O–C_5\text{-acid}]^+$ (18), 103 (16), 85 (30); ESI–MS *m/z* 401 $[M+K]^+$ (100), 385 $[M+Na]^+$ (23), 380 $[M+H_2O]^+$ (18), 363 $[M+H]^+$ (2).

3.8. 9α,14-Dihydroxy-15-isobutyryloxy-costunolide (*6*)

RT: 12.17 min; R_f: 0.39 (I) and 0.12 (II); optical rotation: dextrorotatory, values omitted, because of the small amount; UV (online): $\lambda_{max} = 205.2$ nm; CI–MS (isobutane) *m/z* 351 $[M+H]^+$ (3), 349 (16), 333 $[M+H–H_2O]^+$ (59), 263 $[M+H–C_4H_8O_2]^+$ (38), 245 $[M+H–H_2O–C_4H_8O_2]^+$ (50), 233 (22), 89 (100), 71 (19); ESI–MS *m/z* 389 $[M+K]^+$ (100), 373 $[M+Na]^+$ (23), 368 $[M+H_2O]^+$ (4).

3.9. 14-Hydroxy-15-isovaleryloxy-9-oxo-melampolide (*7*) and 14-hydroxy-15-(2-methylbutyryloxy)-9-oxo-melampolide (*8*)

RT: 15.82 (**7** and **8**); R_f: 0.52 (I) and 0.26 (II); UV (online) $\lambda_{max} = 200.6$ nm (**7** and **8**); ESI–MS *m/z* 401 $[M+K]^+$ (100), 385 $[M+Na]^+$ (45), 363 $[M+H]^+$ (2).

3.10. 1α-Methoxy-15-isobutyryloxy-9-oxo-germacra-4-trans,10(14),11(13)-trien-12,6α-olide (*9*)

RT: 15.42 min, R_f: 0.73 (I) and 0.47 (II); α_D 0 ($CHCl_3$); CI–MS (isobutane) 363 $[M+H]^+$ (41), 345 $[M+H–H_2O]^+$ (3), 277 (26), 275 $[M+H\text{-}i\text{-butyric acid}]^+$ (32), 257 $[M+H\text{-}H_2O\text{-}i\text{-butyric acid}]^+$ (9), 245 (69), 231 (15), 215 (14), 199 (19), 89 (100), 71 (27); ESI–MS 401

$[M+K]^+$ (11), 385 $[M+Na]^+$ (100), 363 $[M+H]^+$ (2), 243 (4).

3.11. 1β-Methoxy-15-isobutyryloxy-9-oxo-germacra-4-trans,10(14),11(13)-trien-12,6α-olide (**10**)

RT: 15.59 min; R_f: 0.67 (I) and 0.49 (II); α_D 0 ($CHCl_3$); CI–MS (isobutane) 363 $[M+H]^+$ (14), 277 (65), 275 (16), 245 (59), 231(15), 201 (20), 103 (19), 89 (100), 71 (28); ESI–MS 401 $[M+K]^+$ (26), 385 $[M+Na]^+$ (100), 363 $[M+H]^+$ (9), 243 (32).

3.12. 1β-Methoxy-15-isovaleryloxy-9-oxo-germacra-4-trans,10(14),11(13)-trien-12,6α-olide (**11**) and 1β-methoxy-15-(2-methylbutyryloxy)-9-oxo-germacra-4-trans,10-(14),11(13)-trien-12,6α-olide (**12**)

RT: 16.86 min; R_f: 0.70 (I) and 0.51 (II) (**11** and **12**); CI-MS (isobutane, negative ions) 376 $[M]^-$ (14), 274 (10), 252 (100), 101 (72); ESI–MS 415 $[M+K]^+$ (47), 399 $[M+Na]^+$ (100), 377 $[M+H]^+$ (6), 243 (14).

3.13. 1α-Methoxy-15-isovaleryloxy-9-oxo-germacra-4-trans,10(14),11(13)-trien-12,6α-olide (**13**) and 1α-methoxy-15-(2-methylbutyryloxy)-9-oxo-germacra-4-trans,10-(14),11(13)-trien-12,6α-olide (**14**)

RT: 16.71; R_f: 0.77 (I) and 0.49 (II) (**13** and **14**); EI–MS 376 $[M]^+$ (2), 345 (22), 317 (26), 274 (2), 261 (2), 242 (2), 231 (1), 215 (4), 185 (2), 169 (3), 161 (4), 137 (6), 133 (4), 105 (10), 91 (9), 85 (51), 81 (13), 57 (100), 53 (29), 41 (35); CI–MS (NH_3) 394 $[M+NH_4]^+$ (100), 377 $[M+H]^+$ (5).

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