Terpenes from *Mikania* aff. *jeffreyi* (Asteraceae)

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Ten terpenes, three steroids and one flavone have been isolated from the leaves of *Mikania* aff. *jeffreyi*, Asteraceae. Among them there are two new kaurane and one new bis-norlabdane diterpene derivatives. The kaur-16-en-18-oic acid, 4-epi-kaurenoic acid, is predominant in the plant leaves. The structures were elucidated by spectroscopic analysis.

Key words: Mikania aff. *jeffreyi*, Diterpenoids, Kauran-18-oic Acid Derivatives

Introduction

The region of Chapada Diamantina, Bahia, Brazil presents a large diversity of endemic and unknown species [1]. *Mikania* of the family Asteraceae, tribe Eupatorieae, is one of the most prominent genera. The literature shows that diterpenes are largely found in plants of the genus *Mikania*, and kaurane diterpenoids are predominant among them [2]. If these diterpenoids are present in the plant very little or no quantity of other diterpenes is found [3]. Some of the conspicuous constituents of those plants are kauran-19-oic acid derivatives. As part of our continuous interest in the chemistry of *Mikania* species [4] we have analyzed the leaves of the new and endemic climbing herb plant *Mikania* aff. *jeffreyi*. The phytochemical analysis afforded six diterpenes (**1**, **3**, **6**, **8**, **10**, **14**), one bis-norditerpene (**7**), two sesquiterpenes (**5**, **11**), one triterpene (**2**), three steroids (**4**, **12**, **13**), and one flavone (**9**). Compounds **3**, **7** and **8** are described for the first time.

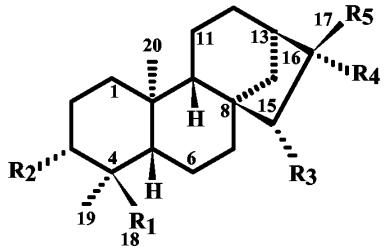
Results and Discussion

The hexane extract of the leaves of *Mikania* aff. *jeffreyi* afforded *ent*-kaur-16-en-18-oic acid (**1**), lupeol (**2**), *ent*-16(β) kauran-16-ol (**6**), sitosterol (**12**), stigmasterol (**13**), and a small quantity of *ent*-labda-8(17),12(*E*),14-trien-18-oic acid, ozic acid (**14**).

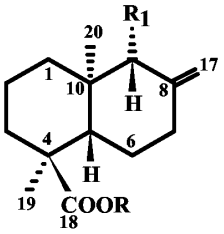
The dichloromethane extract of the leaves of the plant afforded five kaurane diterpenes (**1**, **3**, **6**, **8**, **10**) and a small quantity of the bis-norlabdane diterpene (**7**) besides lupeol (**2**), stigmastenone (**4**), spathulenol (**5**), 3',4'-methylenedioxy-7-methoxyflavone (**9**), and oplopanone (**11**, Fig. 1).

The structures of the known diterpenes **1**, **6** and **14** have been identified by comparison with literature data [5 – 7] respectively. Compound **1** has been obtained as its methyl ester **1a** and it is the conspicuous compound of the leaves. The negative specific rotation ($[\alpha]_D^{25} = -92^\circ$, $CHCl_3$) of **1**, reported here for the first time, indicated that it belongs to the *ent* series.

Compounds **3**, **7** and **8** are new. Compound **3** has also been obtained as its dimethyl ester **3a**. The ^{13}C NMR spectrum of **3a** presented 22 signals assigned by means of DEPT 135° and 90° spectra to four methyls, nine methylenes, four methines and hence four quaternary carbon atoms. The presence of two methyl esters groups becomes evident by the chemical shifts of two methoxyl and two carboxyl groups. No double bond carbon signal was found (Table 1). The ^{1}H NMR spectrum showed four singlets at $\delta = 3.65$, 3.67, 1.02 and 1.15 corresponding to two methoxyl and two methyl groups (Table 2). A multiplet at $\delta = 2.46$, assigned to H-13 of a kaurane, suggested **3** to be a kaurandioic acid. The HREIMS exhibited, as molecular ion, a peak at $m/z = 362.2730$. That information, together with the NMR data, indicated the molecular formula of **3a** as $C_{22}H_{34}O_4$ that agrees with the proposed structure of a kaurandioic acid. Comparison of the ^{13}C NMR spectrum of **3a** with that of **1a** located the ester groups at C-18 and C-17. This spectrum also suggested that the carbomethoxyl group at C-16 should be in β position by the protection made by this group at C-14. The ^{13}C-14 chemical shift is $\delta = 37.9$ instead of $\delta = 41.3$, expected for α substitution. The stereochemistry of C-16 was confirmed by



	R_1	R_2	R_3	R_4	R_5
1	COOH	H	H	Δ16	
1a	COOMe	H	H	Δ16	
3	COOH	H	H	COOH	H
3a	COOMe	H	H	COOMe	H
8	Me	OCOMe	H	OH	Me
10	COOH	H		Δ15	COH



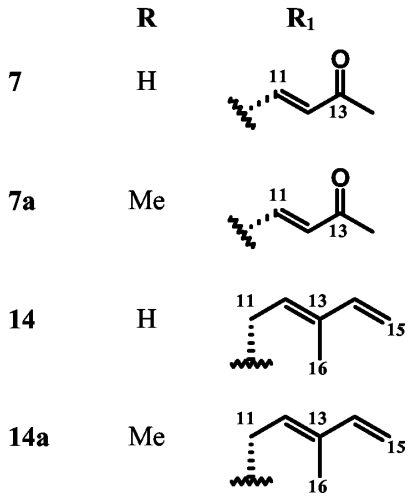


Fig. 1. Structures of the diterpenes **1**, **3**, **7**, **8**, **10** and **16**.

the chemical shifts of H-13 and H-16 which exhibit different resonance signals for the two stereoisomers. For 16α-COOMe the chemical shifts of H-13 and H-16 are $\delta = 2.50$ (m) and $\delta = 2.82$ (ddd; 6.0; 6.0 and 12.0 Hz), respectively, and for 16β-COOMe, H-13 and H-16 are $\delta = 2.44$ (m) and $\delta = 2.62$ (dd; 6.8 and 8.7 Hz), respectively [8, 9]. The ^{1}H NMR spectrum signals for **3a** agree with those of the 16β-COOMe stereochemistry (Table 2) and confirms the structure of 16β-kauran-17,18-dioic acid dimethyl ester.

Table 1. ^{13}C NMR data (75 MHz, δ, $CDCl_3$) of compounds **1a**, **3a**, **8**, **10** and **12a**.

C	**1a**	**3a**	**8**	**10**
1	39.9 t	39.4 t	38.2 t	39.4 t
2	17.9 t[a]	18.1 t	23.5 t	17.6 t
3	36.7 t	36.7 t	80.9 d	36.9 t
4	47.4 s	47.7 s	37.6 s	47.4 s
5	50.4 d	50.3 d	55.0 d	49.7 d
6	23.1 t	23.6 t	19.9 t	21.7 t
7	40.5 t	40.2 t	41.7 t	37.5 t
8	44.2 s	45.1 s	45.0 s	50.9 s
9	55.9 d	55.9 d	56.4 d	46.8 d
10	38.6 s	38.5 s	39.8 s	38.9 s
11	17.7 t[a]	17.6 t	18.0 t	18.1 t
12	33.1 t	31.1 t	26.7 t	25.1 t
13	43.8 d	41.1 d	48.7 d	37.9 d
14	39.5 t	37.9 t	37.4 t	42.9 t
15	48.9 t	44.6 t	57.6 t	161.7 d
16	155.6 s	45.2 d	79.2 s	148.5 s
17	102.9 t	177.7 s	24.3 q	189.4 d
18	179.4 s	179.3 s	28.1 q	184.3 s
19	17.8 q	17.6 q	16.4 q	17.8 q
20	16.3 q	16.3 q	17.7 q	16.0 q
OCH_3	51.8 q	51.7 q	–	–
$OCOCH_3$	–	–	170.9 s	–
$OCOCH_3$	–	–	21.2 q	–

[a] Assignments may be interchanged in the same column.

Table 2. ^{1}H NMR data [300 MHz, δ, m, J (Hz), $CDCl_3$,] of compounds **3a**, **8** and **10**.

H	**3a**	**8**	**10**
3-αax		4,64 dd (11.6, 4.7)[b]	
13	2.46 m	[a]	3.05 m
15			6.59 s
16	2.61 dd (like brt) (7.5, 7.6)		
17		1.36 s	9.73 s
18		1.05 s	
19	1.15 s	0.84 s	1.16 s
20	1.02 s	0.84 s	1.10 s
$-OCH_3$	3.65 s, 3.67 s		
$-OCOCH_3$		2.05 s	

[a] Data not observed; [b] in C_6D_6 solution.

The ^{13}C NMR spectrum of **8** showed 22 signals, and two of them ($\delta = 79.2$ and 80.9) are assigned to oxygenated carbons. The spectrum did not show any signals of double bond carbons. The peaks at $\delta =$ 170.9 (s) and 21.1 (q) suggested an acetoxyl group being present in the molecule. The LREIMS showed a $[M]^+$ at $m/z = 348$ and other peaks at $m/z = 330$ $[M\text{-}H_2O]^+$, at $m/z = 288$ $[M\text{-}CH_3\text{-}COOH]^+$ and at $m/z = 270$ $[M\text{-}H_2O\text{-}CH_3\text{-}COOH]^+$. This spectrum together with the ^{13}C NMR spectroscopic data suggested the structure of an hydroxyacetoxykaurane diterpene with a molecular formula $C_{22}H_{36}O_3$ which agrees with the elemental analysis. The ^{1}H NMR spectrum of **8** (Table 2) pointed out signals for five methyl groups

Table 3. ^{13}C NMR and ^{1}H NMR data of compounds **7a**[a] and **14a** [75 MHz, 300 MHz]; [$CDCl_3$, δ, J (Hz)].

	7a[a]					**14a**	
Position	δ^{13}C[a]	HMBC		δ^{1}H[a]		δ^{13}C	δ^{1}H
		$^{2}J_{CH}$	$^{3}J_{CH}$	H_{eq}	H_{ax}		
1	39.6 t		H_3-20	1.39 m	1.14 m	38.1 t	
2	18.1 t			1.54 m[b]	1.54 m[b]	18.3 t	
3	37.0 t		H_3-19	1.74 m	1.60 m	36.9 t	
4	47.5 s			–	–	47.6 s	–
5	48.9 d		H_3-19, H_3-20	1.97 dd (12.5, 2.5)		49.7 d	
6	25.4 t			1.54 m[b]	c	26.7 t	
7	36.1 t		H-17	2.4 ddd (14, 4.5, 2)	2.15 dt (14, 4.5)	37.5 t	
8	147.8 s			–	–	147.8 s	–
9	60.6 d		H-12, H-17, H-17', H_3-20	2.57 brd (10.9)		56.9 d	
10	38.7 s	H_3-20		–	–	38.8 s	–
11	145.6 d			6.86 dd (16, 10.5)		22.9 t	
12	133.8 d		H-14	6.11 d (16)		133.6 d	5.41 t (6.3)
13	198.0 s	H-14		–		133.4 s	–
14	27.4 q			2.28 s		141.5 d	6.33 dd (17.5, 10.7)
15	–	–	–	–		109.8 t	4.88 d (10.4), 5.04 d (17.6)
16	–	–	–	–		11.8 q	1.75 s
17	109.2 t			4.80 d (1.5), 4.43 d (1.5)		107.9 t	4.82 d (1.4), 4.47 brs
18	178.9 s		H_3-19, OCH_3	–		179.2 s	–
19	16.8 q			1.19 s		16.6 q	1.16 s
20	15.4 q			0.92 s		14.6 q	0.75 s
OCH_3	52.0 q			3.68 s		51.8 q	3.65 s

[a] The ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) data were obtained from HMQC and HMBC; [b] overlapped signal; [c] data not observed.

and a double doublet at $\delta = 4.64$ (11.6 and 4.7 Hz) assigned to H-3 in agreement with the proposed structure. The structure of **8** was determined as *ent*-16β-3β-acetoxykauran-16-ol by comparing the NMR data of **8** with those of 3-acetoxy pentacyclic triterpenes [10] and **6**. The absolute stereochemistry was suggested by the negative specific rotation, $[\alpha]_D^{25} = -23^\circ$, when compared with other kaurane diterpenes.

The ^{1}H NMR spectrum of **10** displayed signals corresponding to an aldehyde at $\delta = 9.73$, three methyl groups, one vinyl hydrogen and a multiplet at $\delta = 3.05$ (Table 2). The ^{13}C NMR spectroscopic data suggested the structure of a 18-kaurenoic acid with an α,β-unsaturated aldehyde group (Table 1). Comparison of these data with those of *ent*-kaur-15-en-17-al [11] established the structure of **10** as *ent*-kaur-15-en-17-al-18-oic acid. Compound **10** was previously isolated from *Mikania banisteriae* [12], but its ^{13}C NMR data were not previously published.

Compound **7** was also obtained from the leaves of *Mikania* aff. *jeffreyi*, after methylation, as its methyl ester **7a**. The ^{1}H NMR spectrum of **7a** showed four signals assigned to three hydrogen atoms, each one at $\delta = 0.92$, 1.19, 2.28 and 3.68 corresponding to three methyl and one methoxyl groups, and one methyl is located in α position to a carbonyl group. Two double bond $\delta = 4.80$ (d; 1.6 Hz), 4.44 (d; 1.6 Hz), 6.11 (d; 15.7 Hz) and 6.86 (dd; 15.7 and 10.3 Hz) and a CH resonance, represented by a doublet at $\delta = 2.57$ (10.3 Hz) (Table 3) were also observed. These data suggested the presence of one exocyclic double bond and another α,β-unsaturated one neighboring a CH group. The ^{13}C NMR spectrum of **7a** disclosed the presence of 19 carbons in the molecule whose chemical shifts are compatible with those of a bisnor-diterpen-18-oic methyl ester derivative. ^{13}C NMR together with DEPT 135° and 90° spectra showed the presence of four CH_3, six CH_2, four CH, and five quaternary carbon atoms in the molecule, and two of them are assigned to one carbonyl and one carboxyl ester carbon (Table 3). The two highest peaks in the LREIMS occur at $m/z = 289$ and 245 suggesting $[M\text{-}CH_3]^+$ and $[M\text{-}COOCH_3]^+$ fragments, and the molecular formula $C_{19}H_{28}O_3$ is confirmed by the HREIMS. Comparison of the NMR data of **7a** with those of bisnor-15,16-(11*E*)-labda-13-oxy-8(17),11-diene, isolated from *Alpinia formosana* [13], and **1a** characterized **7a** as bisnor-15,16-(11*E*)-labda-8(17),11-dien-13-oxy-18-oic acid methyl ester. The bidimensional HMQC and ^{1}H-^{1}H COSY spectra agree with the structure. Compound **7a** is probably a natural oxidation product of **14**.

Comparison of the NMR data of compounds **2**, **5**, **9** and **11** with those of the literature [14–17] identified their structures. Compounds **4**, **12** and

13 were identified by comparison with authentic samples.

The chemical composition of the leaves of *Mikania* aff. *jeffreyi* showed as principal constituents 18-kaurenoic acid derivatives which are not very common in the nature. The occurrence of kaurenes in the plant are in agreement with the composition of other plants of the *Mikania* genus. Compounds **11** and **9** were not previously described from *Mikania* species.

Experimental Section

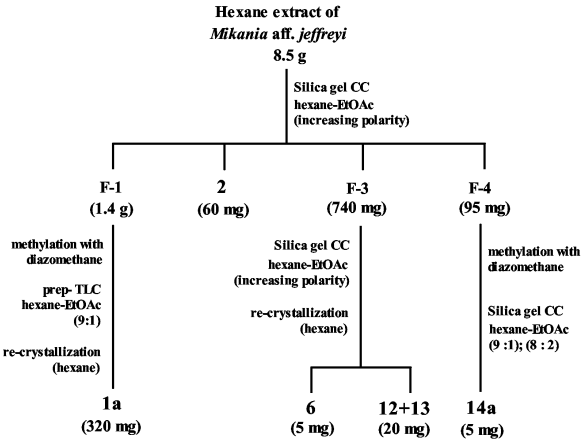
Column chromatography was carried out using silica gel (70 – 230 mesh, Merck). For TLC, glass-supported silica gel F250 plates and for prep-TLC GF254, both from Merck, were used. Sephadex LH-20 (Sigma) was used for exclusion chromatography. Optical rotations were measured in a digital polarimeter JASCO DIP-370. NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C in a Varian Gemini 300 using $CDCl_3$ as solvent and internal standard. All the LREIMS spectra were carried out in an GCMS Hewlett Packard 6890-chromatograph equipped with a HP-5MS column (30×0.25 nm, film thickness 0.25 μm) and coupled with an EIMS, 70 eV, HP-5973. Helium was used as carrier gas at a flow rate of 1 ml/sec. The temperature programming was from 60 °C rising 290 °C at 10°/min, then isothermal at 290 °C for 20 min. The injector and (FID) detector temperatures were 240 °C and 300 °C, respectively. HREIMS were performed using a VG Auto Spec-Fisions Instrument using an electron ionization of 70 eV. Elemental analyses was obtained in a Perkin-Elmer Elemental Analyzer, model 2400 CHN.

Plant material

The aerial parts of the plant were collected in November 1997 in Morro do Pai Incio, Palmeiras, Chapada Diamantina, Bahia, Brazil. The plant was initially identified as belonging to the *Mikania* genus by the botanist Maria Lenise da S. Guedes from the Instituto de Biologia, Universidade Federal da Bahia (UFBA). A voucher specimen, number ALCB 031763, was deposited at the Herbarium Alexandre Leal Costa, Instituto de Biologia, UFBA. After being analyzed by four botanists (see acknowledgements) the plant is considered to be a new *Mikania* species, named initially *Mikania* aff. *jeffreyi* D. J. N. Hind. Dr. Roberto L. Esteves from Universidade Federal do Rio de Janeiro, Brazil, has been working with the plant material in order to classify the species.

Extraction and isolation of the compounds

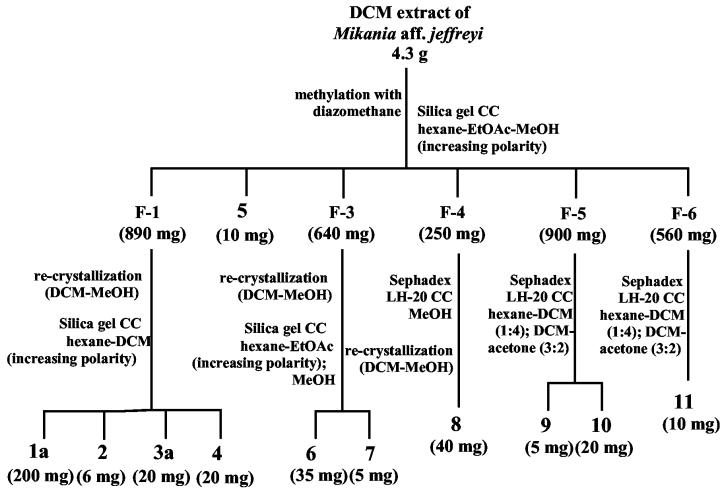
The ground air-dried leaves (1080 g), were separated from the stems and extracted with hexane and then with methanol at room temperature. The crude methanol extract (29 g) was submitted to a separation between hexane and MeOH:H_2O (5%) to give a hexane and an alcoholic extract (23.5 g). The alcoholic extract was separated again between DCM and MeOH: H_2O (40%) to give 5.1 g of the DCM extract. The hexane extracts were combined (8.5 g) and submitted to chromatography over silica gel eluting with mixtures of hexane and ethyl acetate of increasing polarity. The fractions were then purified (Scheme 1) to give **1a**: *ent*-kaur-16-en-18-oic acid methyl ester (320 mg); **2**: lupeol (60 mg); **6**: 16(β)-kauran-16-ol (5 mg); **12**+**13**: sitosterol and stigmasterol (20 mg); **14a**, *ent*-12 (*E*),labda-8(17),12,14-trien-18-oic acid methyl ester (5 mg). The DCM extract was dissolved in hot methanol and upon cooling, a solid grease material was formed. After filtration and evaporation of the solvent, the DCM extract (4.3 g) was submitted to diazomethane methylation. The methylated extract was submitted to chromatography over silica-gel eluting with mixtures of hexane, ethyl acetate and methanol of increasing polarity (Scheme 2) to give **1a** to **11** in the order of elution: **1a**: *ent*-kaur-16-en-18-oic acid methyl ester (200 mg); **2**: lupeol (6 mg); **3a**: *ent*-16β-kauren-17,18-dioic acid dimethyl ester (20 mg); **4**: stigmastenone (20 mg); **5**: spathulenol (10 mg); **6**: 16(β)-kauran-16-ol (35 mg); **7**: *ent*-11(*E*) bisnor-15,16-labda-8(17),11-dien-13-oxy-18-oic acid methyl ester (5 mg); **8**: *ent*-16(β)-3(β)-acetoxykauran-16-ol (40 mg); **9**: 7-metoxy-3':4'-methylendioxyflavone (5 mg); **10**: *ent*-kaur-15-en-17-al-18-oic acid (20 mg); **11**: oplopanone (10 mg) (Scheme 2).



Scheme 1. Fractionation of the hexane extract.

*Ent-16β-kauran-17,18-dioic acid dimethyl ester (***3a***)*

Colorless amorphous solid. – $[\alpha]_D^{25} = -16,9°$ (0.65 mg/ml $CHCl_3$). – ^{1}H and ^{13}C NMR (Tables 2 and 1). – HREIMS $m/z = 362.2730$ ($C_{22}H_{34}O_4$) calcd. 362.2748. – MS (EI, 70 eV): m/z(%) = 362(7) [M^+], 347(2), 331(16), 330(51), 303(55), 302(74), 287(12), 271(10), 243(29), 123(100), 121(65), 109(68), 91(87), 81(75), 55(68), 41(41).



Scheme 2. Fractionation of the DCM extract.

*Ent-11(E) bisnor-15,16-labda-8(17),11-dien-13-oxa-18-oic acid methyl ester (***7a***)*

Colorless oil. – $[\alpha]_D^{25} = -25.6^\circ$ (0.4 mg/ml, $CHCl_3$). – ^{1}H and ^{13}C NMR (Table 3) – HREIMS $m/z = 304.2015$ ($C_{19}H_{28}O_3$) calcd. 304.2031. – MS (EI, 70 eV): $m/z(\%) =$ 304(8) [M^+], 289(5), 261(4), 245(7), 244(5), 201(8), 187(6), 161(14), 159(7), 137(6), 124(15), 122(14), 121(100), 109(25), 81(23), 55(7).

*Ent-16(β)-3(β)-acetoxykauran-16-ol (***8***)*

Colorless crystals, m.p. 205 – 206.5 °C (hexane). – $[\alpha]_D^{25} = -23.2^\circ$ (0.7 mg/ml, $CHCl_3$). – ^{1}H and ^{13}C NMR (Tables 2 and 1). – MS (EI, 70 eV): $m/z(\%) = 348(1)$ [M^+], 300(21), 315(4), 288(12), 270(24), 255(38), 245(25), 227(37), 215(39), 187(54), 136(56), 121(64), 105(47), 94(57), 69(34), 43(100). – $C_{22}H_{36}O_3$: calcd. C 75.80, H 10.42; found C 75.74, H 10.48.

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