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Short Report

# Kaurene Diterpenes and Other Chemical Constituents from *Mikania stipulacea* (M. Vahl) Willd.

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O estudo fitoquímico de *Mikania stipulacea* conduziu ao isolamento e à identificação de lupeol, α-amirina, estigmasterol, β-sitosterol, campesterol, 3-O-β-D-glicopiranosil estigmasterol, 3-O-β-D-glicopiranosil sitosterol, da cumarina isoescopoletina e dos ácidos vanílico, cinamoilgrandiflórico e *ent*-caurenóico, juntamente com dois diterpenos novos, os ácidos *ent*-9α-hidroxi-15β-*E*-cinamoiloxi-16-cauren-19-óico e *ent*-9α-hidroxi-15β-*Z*-cinamoiloxi-16-cauren-19-óico. Estas substâncias foram identificadas com base na análise dos espectros de IV, EM e RMN de $^{1}H$ e $^{13}C$.

Phytochemical study of *Mikania stipulacea* yielded lupeol, α-amyrin, stigmasterol, β-sitosterol, campesterol, β-sitosteryl glucopyranoside, stigmasteryl glucopyranoside, the coumarin isoscopoletin, and vanillic, cinnamoylgrandifloric and *ent*-kaurenoic acids, besides two new diterpene acids: *ent*-9α-hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic and *ent*-9α-hydroxy-15β-*Z*-cinnamoyloxy-16-kauren-19-oic. IR, $^{1}H$ and $^{13}C$ NMR and MS spectroscopic analyses were used for the identification of these compounds.

**Keywords:** *Mikania stipulacea*, Asteraceae, diterpenes, *ent*-kaurenes

## Introduction

The tribe Eupatorieae forms the major part of the Asteraceae family and comprises nearly 2,400 species[1, 2]. *Mikania* Willd is the only genus in the sub-tribe Mikaniinae of Eupatorieae. These species are native to Central and South America, where they are known as "guaco" and used to treat fever, rheumatism, flu and respiratory tract diseases[3, 4]. As might be expected for such a large and diverse genus, its chemistry is also diverse[5]. Less than 10% of the more than 430 known *Mikania* species have been studied. Sesquiterpene lactones are found in nearly two thirds of these species[5]. In the other species whose chemistry has been investigated, diterpenes, mainly of the *ent*-kaurene type, predominate[6].

Continuing our work on this genus[7, 8] we report herein the isolation of two new kaurene-type diterpenes from *M. stipulacea*. Their structures were proposed on the basis of spectroscopic data and comparisons of the attributed signals with previously published data on related compounds.

## Experimental

### *General*

The IR spectra were obtained on KBr pellets in a Perkin Elmer model 1420 spectrophotometer. $^{1}H$ NMR (300 MHz) and $^{13}C$ NMR (75 MHz) spectra were recorded on a Bruker DPX 300 in $CDCl_3$ with TMS as internal standard. EIMS was obtained at 70 eV on HP 5988-A. Optical rotations were measured with a Polartronic HH8. Prep. TLC was carried out on Si gel PF-254 (Merck), CC on Si gel 60 (0.063 a 0.200) (Merck) and VLC on Si gel 60 H (0.005 – 0.045) (Merck).

### *Plant material*

*Mikania stipulacea* (M. Vahl) Willd was collected in Restinga de Maricá, Rio de Janeiro, RJ, Brazil, in July, 1996, and identified by Professor Dr. Janie G. Silva (Instituto de Biologia da Universidade Federal Fluminense - Rio de Janeiro). A voucher specimen (SPFR 04123) was deposited in the herbarium of the Department of Biology, FFCLRP/USP and was used for the authentication of the species.

### *Extraction and fractionation*

Dried and powdered whole *M. stipulacea* plants (1.7 kg) were exhaustively extracted at room temperature with hexane, ethyl acetate and ethanol. Evaporation of the solvents under reduced pressure furnished 46.0 g, 23.0 g and 225.0 g respectively, of crude extracts. The bulk of each extract (45.0 g hexane, 22.0 g ethyl acetate, 60.0 g ethanol) was chromatographed separately over silica gel under vacuum

(VLC) and eluted with hexane, gradually increasing the polarity with ethyl acetate and then methanol.

From the crude hexane extract, 14 fractions were collected after VLC.

Fraction 3 was precipited from MeOH, giving 1.0 g of solid. CC of solid (3A) on silica gel with hexane and gradually increasing the polarity with ethyl acetate and then methanol, yielded 22 subfractions: Subfr. 3A.6 yielded 79.0 mg of crystals which were recrystallized from hexane–ethyl acetate (98:2) and gave a mixture of lupeol and α-amyrin (69.0 mg); Subfr. 3A.9 (99.0 mg) was also recrystallized from hexane–ethyl acetate (98:2) to provide a mixture of β-sitosterol, stigmasterol and campesterol (49.2 mg); Subfr. 3A.12 (43.0 mg) was purified by prep. TLC (hexane–ethyl acetate 85:15) affording 10.0 mg of cinnamoylgrandifloric acid. Methanol-soluble fr. 3B (600.0 mg) was purified by CC on silical gel with hexane and increasing amounts of ethyl acetate and methanol, affording 16 subfractions: *ent*-16-Kauren-19-oic acid (5.0 mg) and a mixture of β-sitosterol, stigmasterol and campesterol (3.0 mg) were obtained from subfr. 3B.5 (100.0 mg) after prep. HPLC (Si-60 column, using Hexane-iso-propyl alcohol 98:2).

Fraction 4 (2.0 g) was purified by consecutive CCs (silica gel) to give subfr. 4.10.8 (30.0 mg), which, when submitted to prep. HPLC (Si-60 column, using Hexane-iso-propyl alcohol 97:3), yielded a mixture of compounds **1** and **2** (5 mg).

Fraction 5 (1.3 g) was separated by CC (silica gel) into 16 subfractions: Subfr. 5.8 (50.0 mg) eluting with hexane-ethyl acetate 1:1 after prep. TLC (silica gel) yielded compound **1** (3.0 mg).

Finally, fraction 10 (500.0 mg) after CC on silica gel (hexane-ethyl acetate and methanol in mixtures of increasing polarity) and precipitation in ethyl acetate of subfr. 10.6 led to the isolation of a mixture of β-sitosteryl glucopyranoside and stigmasteryl glucopyranoside (4.0 mg).

From the crude ethyl acetate extract, 15 fractions were collected after VLC.

Fractions 4 and 5 were pooled (3.44 g) and chromatographed over a silica gel column (CC) eluted with hexane, gradually increasing the polarity with ethyl acetate and then methanol. Subfrs. 4/5.12 (100.0 mg) and 4/5.17 (84.0 mg) were submitted to prep. TLC (silica gel), eluting with hexane-ethyl acetate 1:1, to afford compound **1** (17.0 mg) and isoscopoletin (1.0 mg), respectively.

CC of fraction 6 (500.0 mg) on silica gel (hexane, ethyl acetate and methanol in a mixture of increasing the polarity) followed by precipitation (methanol) of subfr. 6.15 (41.0 mg) yielded a vanillic acid (3.0 mg).

Fractions 8, 9, 10, 11 and 12 after precipitation in ethyl acetate yielded a mixture of β-sitosteryl glucopyranoside and stigmasteryl glucopyranoside (1.3 g).

From the crude ethanol extract, 15 fractions were collected after VLC.

Fractions 3, 4 and 5 were combined (351.0 mg) and dissolved in acetonitrile. The soluble part (subfr. 3/4/5-C, 281.0 mg) was submitted to prep. HPLC (Si-60 column, using hexane-iso-propyl alcohol 97:3), yielding a compound **1** (2.0 mg).

Frs. 6 and 7 showed similarity on TLC and were pooled (50.0 mg) and submitted to prep. TLC (silica gel), yielding isoscopoletin (3.0 mg).

*Ent-9α-hydroxy-15β-E-cinnamoyloxy-16-kauren-19-oic acid* (**1**): colourless crystals (not sufficient to obtain a sharp mp but homogeneous by TLC in several solvent systems).$[\alpha]_D^{25}$ - 0.14º (*c* $1.508\times10^{-3}$, $CHCl_3$); IR: ($\nu_{max}/cm^{-1}$) 3600 - 2600 (OH acid), 3500 (OH), 1690, 1720 (C=O), 1630 (C=C) (KBr); EIMS, *m/z* (relative intensity in %): 298 [(M-148) – $H_2O$]$^+$ (19), 161 [(M-148) – $H_2O$ - 137]$^+$ (46), 131 [M-333]$^+$ (100), 148 [M-316]$^+$ (60), 103 [131-CO]$^+$ (55). $^1$H NMR (300 MHz, $CDCl_3$), see Table 1. $^{13}$C NMR (PND, DEPT 135º and HMQC for correlating directly bonded $^1$H and $^{13}$C nuclei (75 MHz, $CDCl_3$), see Table 2.

*Ent-9α-hydroxy-15β-Z-cinnamoyloxy-16-kauren-19-oic acid* (**2**), unseparated from **1**: colourless gum; $^1$H NMR (300 MHz, $CDCl_3$), see Table 1. $^{13}$C NMR (PND, DEPT 135º and HMQC for correlating directly bonded $^1$H and $^{13}$C nuclei (75 MHz, $CDCl_3$), see Table 2.

## Results and Discussion

Whole *M. stipulacea* plants were extracted with hexane, ethyl acetate and ethanol. The chromatographic fractionation of these extracts yielded stigmasterol, β-sitosterol and campesterol, cinnamoylgrandifloric[9], *ent*-kaurenoic acid[10, 11], vanillic acid[12], isoscopoletin[13], lupeol[14], α-amyrin[14], β-sitosteryl glucopyranoside and stigmasteryl glucopyranoside[15]. The above compounds were individually identified by spectroscopic analyses and comparisons with reported data.

The *ent*-kaurene diterpene **1**, obtained as a pure solid exhibited a $^1$HNMR spectrum with typical signals of the ester *E*-cinnamate, $\delta$ 7.69 d and 6.46d (AB system, $J_{AB}$ 16 Hz). The H-15 exhibited an even higher paramagnetic shift when compared with the H-15 chemical shift of *ent*-kaurenoic acid (**3**) ($\delta$ 2.09) and of cinnamoylgrandifloric acid (**4**) ($\delta$ 5.41), (Table 1). The stereochemistry at C-9 and C-15 was deduced from the chemical shift of H-15 which could be explained only by a deshielding effect of a β-oriented oxygen function in C-9.

**Table 1.** $^1$H NMR data ($\delta$) for **1** and **2** in $CDCl_3$ at 300 MHz compared with **3** ($CDCl_3$)[10] and **4** ($CDCl_3$)[9].

| H | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| 2' | 6.46 d (16 Hz) | 5.96 d (12.8 Hz) | - | 6.47 d (16 Hz) |
| 3' | 7.69 d (16 Hz) | 6.96 d (12.8 Hz) | - | 7.69 d (16 Hz) |
| 5', 9' | 7.32-7.63 m | 7.32-7.63 m | - | 7.53 m |
| 6', 7', 8' | 7.32-7.63 m | 7.32-7.63 m | - | 7.38 m |
| 13 | 2.82 br s | 2.74 br s | 2.62 br s | 2.82 br s |
| 15 | 6.08 br s | 6.08 br s | 2.09 br s | 5.41 br s |
| 17 | 5.16 br s | 5.16 br s | 4.79 br s | 5.07 br s |
| 17' | 5.19 br s | 5.19 br s | 4.79 br s | 5.20 br s |
| Me-18 | 1.23 s6 | 1.23 s | 1.24 s | 1.21 s |
| Me-20 | 1.11 s6 | 1.07 s | 0.95 s | 0.99 s |

**Table 2.** $^{13}$C NMR data ($\delta$) for **1** and **2** in $CDCl_3$ at 75 MHz compared with **4** ($CDCl_3$)[9] , **5** ($C_5D_5N$)[17] and **6** ($C_5D_5N$)[18].

| *C* | 1 | 2 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| 1 | 37.9 t | 37.9 t | 40.7 t | 36.8 t | 38.7 t |
| 2 | 18.9 t | 18.9 t | 18.5 t | 19.9 t | 19.8 t |
| 3 | 37.9 t | 37.9 t | 37.8 t | 38.6 t | 36.4 t |
| 4 | 44.5 s | 44.5 s | 43.8 s | 44.0 s | 43.9 s |
| 5 | 49.8 d | 49.8 d | 56.7 d | 49.8 d | 57.2 d |
| 6 | 20.8 t | 20.8 t | 20.9 t | 22.7 t | 22.0 t |
| 7 | 33.8 t* | 33.8 t** | 34.9 t | 40.8 t | 41.2 t |
| 8 | 53.1 s | 53.1 s | 47.7 s | 49.8 s | 48.4 s |
| 9 | 76.8 s | 77.2 s | 53.1 d | 76.9 s | 54.0 d |
| 10 | 43.8 s | 43.8 s | 40.0 s | 44.5 s | 40.2 s |
| 11 | 29.2 t | 29.2 t | 19.1 t | 29.5 t | 18.7 t |
| 12 | 32.1 t | 32.1 t | 32.7 t | 32.9 t | 33.1 t |
| 13 | 41.2 d | 41.2 d | 42.7 d | 42.9 d | 42.9 d |
| 14 | 37.4 t* | 37.4 t** | 37.4 t | 34.6 t | 36.7 t |
| 15 | 79.2 d | 79.2 d | 83.2 d | 44.4 t | 82.7 d |
| 16 | 154.8 s | 154.8 s | 155.4 s | 156.3 s | 161.3 s |
| 17 | 110.4 t | 110.4 t | 110.1 t | 102.9 t | 117.8 t |
| 18 | 28.9 q | 28.9 q | 28.9 q | 29.6 q | 29.3 q |
| 19 | 183.0 s | 183.0 s | 184.2 s | 180.7 s | 180.3 s |
| 20 | 17.4 q | 17.4 q | 15.94 q | 18.0 q | 16.3 q |
| 1' | 167.1 s | 167.1 s | 166.9 s | | |
| 2' | 118.4 d | 120.0 d | 118.6 d | | |
| 3' | 144.9 d | 143.5 d | 144.7 d | | |
| 4' | 134.5 s | 134.5 d | 134.6 s | | |
| 5' | 128.1 d | 128.9 d | 128.8 d | | |
| 6' | 128.9 d | 127.9 d | 128.1 d | | |
| 7' | 130.3 d | 129.9 d | 130.1 d | | |
| 8' | 128.9 d | 127.9 d | 128.1 d | | |
| 9' | 128.1 d | 128.9 d | 128.8 d | | |

*,** Assignments may be interchanged

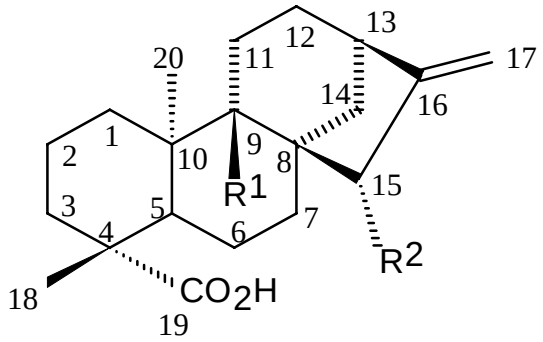
A comparison of the $^{13}$C NMR spectral data of **1** and **4** showed that the hydroxyl group at C-9 in **1** produced a deshielding effect on the signals of C-8, C-10 and C-11 and a shielding effect on the signals of C-1, C-5, C-7 and C-12 (γ-gauche effect), thus confirming the position and the stereochemistry proposed earlier for C-9 (Table 2). In accordance with this conclusion, the $^{13}$C NMR data of **1** were similar to those for *ent*-9α-hydroxy-16-kauren-19-oic acid (**5**). Some differences were due to the additional O-cinnamoyl group at C-15, which caused a strong high frequency shift of this carbon. In addition, direct comparison of **1** with **4** and grandifloric acid (**6**), in which the C-9 (without the hydroxyl group) appears respectively at $\delta$ 53.1 and 54.0, confirmed the kaurene skeleton with an O-cinnamoyl group at C-15 and an OH group at C-9.

The axial *trans* position of the C-19 carboxyl group in relation to H-5 was deduced from the chemicals shifts of C-5, C-18, C-19 and C-20[16].

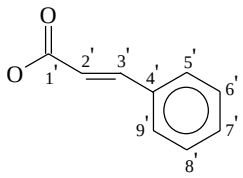
The *ent* series of compound **1** was established by measurement of the optical rotation. The structure was confirmed by MS and IR data.

The diterpene **2** was obtained mixed with compound **1**. The presence of the *E* and *Z*-cinnamate esters in the diterpenes was suggested by the observation of their typical signals, respectively, at $\delta$ 7.69 d and 6.46d (AB system, $J_{AB}$ 16 Hz) and $\delta$ 6.96 d and 5.96 d (AB system, $J_{AB}$ 12.8 Hz) in their $^1$HNMR spectrum. **1** and **2** were 2',3' - *E*/*Z* - isomers which could be identified by their different chemical shifts and couplings.

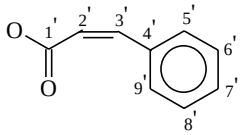
On the basis of the present data, we conclude that *M. stipulacea* belongs to a fairly large group of Brazilian *Mikania* species which produce *ent*-kauranes rather than sesquiterpene lactones[5, 7].




1: $R^1$ = OH $R^2$ =

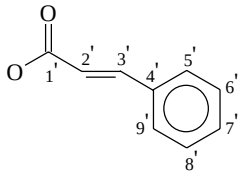


2: $R^1$ = OH $R^2$ =



3: $R^1$ = H $R^2$ = H-2

4: $R^1$ = H $R^2$ =



5: $R^1$ = OH $R^2$ = H-2

6: $R^1$ = H $R^2$ = OH

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