

## Sesquiterpene Lactones and other Chemical Constituents of *Mikania hoehnei* R.

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O estudo fitoquímico de *Mikania hoehnei* conduziu ao isolamento e à identificação de acetato de lupeol, estigmasterol,  $\beta$ -sitosterol, campesterol, 3-O- $\beta$ -D-glicopiranosil estigmasterol, 3-O- $\beta$ -D-glicopiranosil sitosterol, 2,6-dimetoxibenzoato de benzila, luteolina, caempferol e duas lactonas sesquiterpênicas diidrocostuslactona e 8 $\beta$ -hidroxizaluzanina D. Estas substâncias foram identificadas com base na análise dos espectros de IV, EM e RMN de  $^1\text{H}$  e  $^{13}\text{C}$  e os dados foram comparados com os descritos na literatura.

Phytochemical study of *Mikania hoehnei* yielded lupeyl acetate, stigmasterol,  $\beta$ -sitosterol, campesterol,  $\beta$ -sitosteryl glucopyranoside, stigmasteryl glucopyranoside, benzil 2,6-dimethoxybenzoate, luteolin, kaempferol and two sesquiterpene lactones: dehydrocostuslactone and 8 $\beta$ -hydroxyzaluzanin D. IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR and MS spectroscopic analyses and comparisons with previously reported data were used for the identification of these compounds.

**Keywords:** *Mikania hoehnei*, Asteraceae, guaianolides, flavonoids

### Introduction

*Mikania hoehnei* B. Robinson, first described by B. Robinson in 1934, is an endemic vine found in Brazil from Rio de Janeiro to Santa Catarina.<sup>1,2</sup> Only one report has appeared dealing with the terpenoids of *M. hoehnei*,<sup>3</sup> in which the presence of lupeol and stigmasterol was indicated. We selected the entire plant for phytochemical investigation as part of our studies on members of the Eupatorieae.<sup>4-6</sup>

The genus *Mikania* has undergone taxonomic studies, where morphologic information was considered<sup>1,7-9</sup> and an evaluation of the existence of correlations between the terpenoid chemistry and the phylogeny of those species was done.<sup>10,11</sup> *Mikania hoehnei* B. Robinson belongs to the subtribe Mikaniinae, section Mikania (L.) Willd. (Holmes, private communication) and phytochemical investigation of the whole plant led to the isolation of two sesquiterpene lactones. Their structures were proposed on the basis of spectroscopic data and comparisons of the attributed signals with previously reported data.<sup>12-17</sup>

Although the occurrence of sesquiterpene lactones is common in species of *Mikania*, guaianolides have been

found in only 3 of the 46 species studied so far, e.g. in *M. vitifolia*,<sup>18</sup> *M. haenkeana*<sup>19</sup> and *M. mendocina*.<sup>20</sup> There also seems to be a remarkable difference between the compounds identified in *M. hoehnei* and those reported for other *Mikania* species.

### Experimental

#### General

The IR spectra were obtained on NaCl film in a Perkin Elmer model 1420 spectrophotometer.  $^1\text{H}$  NMR (300 MHz) and  $^{13}\text{C}$  NMR (75 MHz) spectra were recorded on a Bruker DPX 300 in  $\text{CDCl}_3$  with TMS as internal standard. EIMS was obtained at 70 eV on HP 5988-A. 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 hoehnei* B. Robinson 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 04309) was deposited

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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. hoehnei* plants (2.8 kg) were exhaustively extracted at room temperature with hexane, ethyl acetate and ethanol in successive phases. Evaporation of solvents under reduced pressure furnished 64.0 g, 25.0 g and 35.0 g respectively, of crude extracts.

The bulk of the hexane extract (60.0 g) was chromatographed over silica gel under vacuum (VLC) and eluted with hexane, gradually increasing the polarity with ethyl acetate and then methanol. Twelve fractions were collected. Fraction 2 (60.0 mg) was submitted to prep. TLC (silica gel), eluting with hexane-ethyl acetate 9:1 (v/v), to afford 16.0 mg of lupeyl acetate. CC of fraction 10 (600.0 mg) on silica gel (hexane, ethyl acetate and methanol in mixtures of increasing the polarity) followed by precipitation (methanol) of subfr. 10.13 (45.0 mg) yielded 5.0 mg of a mixture of  $\beta$ -sitosterol, campesterol and stigmaterol and 5.3 mg of a mixture of  $\beta$ -sitosteryl glucopyranoside and stigmasteryl glucopyranoside.

The crude ethyl acetate and ethanol extracts were separately suspended in MeOH-H<sub>2</sub>O 19:1 (v/v). The solutions were partitioned first with hexane and then with dichloromethane. After evaporation at reduced pressure, the dichloromethane fractions were chromatographed separately on Si gel 60 (CC) with hexane and gradually increasing polarity, with ethyl acetate and then methanol. All fractions monitored by TLC.

From the dichloromethane fraction (10.0 g) of crude ethyl acetate extract, eleven fractions were collected. Fraction 3 (45.0 mg) after prep. TLC (silica gel) eluting with dichloromethane, afforded 1.5 mg of benzil 2,6-dimethoxybenzoate. CC of fraction 6 (1.04 g) on silica gel (hexane, ethyl acetate and methanol in a mixture of increasing polarity), afforded 13 subfractions. Subfr. 6.4 yielded 100.0 mg of 8 $\beta$ -hydroxyzaluzanin D (**1**).

From the dichloromethane extract (2.0 g) of crude EtOH extract, twelve fractions were collected. Fraction 6 (93.0 mg) was submitted to prep. TLC (silica gel). Elution with hexane-dichloromethane 3:2 afforded 5.0 mg of dehydrocostuslactone.

The hydroalcoholic extract (2.0 g) from the crude ethanol extract, soluble in methanol, was chromatographed on a Sephadex LH - 20 column using methanol as eluent, and twelve fractions were collected. Fraction 4 yielded 15.0 mg of luteolin and fraction 7 yielded 4.0 mg of kaempferol.

8 $\beta$ -hydroxyzaluzanin D (**1**). Colorless gum; C<sub>17</sub>H<sub>20</sub>O<sub>5</sub>; IR  $\nu_{\max}$  cm<sup>-1</sup> (NaCl film): 3494 (OH alcohol), 1750 ( $\gamma$ -

lactone), 1730 (acetyl group), 1664 and 1641 (c=c double bonds); EI-MS, *m/z* (relative intensity in %): 262 [M<sup>+</sup> - ketene] (14), 91 (24), 43 [CH<sub>3</sub>CO]<sup>+</sup> (100); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>), see Table 1. <sup>13</sup>C NMR, DEPT 135<sup>o</sup> and HMQC (75 MHz, CDCl<sub>3</sub>): 45.0 (d, C-1), 36.5 (t, C-2), 74.5 (d, C-3), 147.7 (s, C-4), 50.4 (d, C-5), 77.6 (d, C-6), 50.1 (d, C-7), 65.7 (d, C-8), 41.8 (t, C-9), 142.8 (s, C-10), 136.0 (s, C-11), 170.8 (s, C-12), 121.6 (t, C-13), 117.4 (t, C-14), 114.2 (t, C-15), 169.4 (s, C-1'), 21.2 (q, C-2').

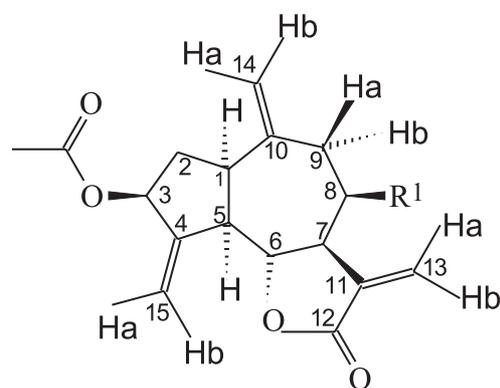
## Results and Discussion

The chromatographic fractionation of hexane, ethyl acetate and ethanol extracts yielded stigmaterol,  $\beta$ -sitosterol and campesterol, lupeyl acetate,<sup>21</sup>  $\beta$ -sitosteryl and stigmasteryl glucopyranosides,<sup>22</sup> benzil 2,6-dimethoxybenzoate,<sup>23</sup> luteolin,<sup>24</sup> kaempferol<sup>24</sup> and dehydrocostuslactone.<sup>13,14</sup> The structures were established by comparison of their spectroscopic properties (mainly IR, <sup>1</sup>H and <sup>13</sup>C NMR) with those reported in the literature and in some cases by direct comparison with authentic samples.

Structure of the guaianolide **1** was deduced from the IR, MS, <sup>13</sup>C and <sup>1</sup>H NMR spectra data and the stereochemistry was defined from the coupling constants' value *J* and nOe difference correlations. This lactone has been previously reported as a synthetic compound obtained in the controlled acetylation of the natural guaianolide Integrifolin (8-epi-desacylcynaropicrin).<sup>12</sup> The IR spectrum displayed bands at 1750 cm<sup>-1</sup> ( $\gamma$ -lactone), at 1730 cm<sup>-1</sup> (acetyl group), at 3494 cm<sup>-1</sup> (hydroxyl group) and weak bands at 1664, 1641 cm<sup>-1</sup> (c=c double bonds).

Inspection of the <sup>1</sup>H NMR spectrum of compound **1** indicated several signals very close to those observed in Zaluzanin D<sup>15</sup>. The position and the stereochemistry ( $\beta$ -orientation) of hydroxyl group at C-8 were determined by nOe correlation (Figure 1). nOe's were observed between the H-8 ( $\delta$  4.37) and H-13a ( $\delta$  5.62), H-7 ( $\delta$  2.98) and H-9b ( $\delta$  2.61). In the same way, correlation of the H-3 ( $\delta$  5.50) with H-2b ( $\delta$  2.45) and H-15a ( $\delta$  5.32) in the nOe spectrum confirmed the  $\alpha$ -orientation of H-3 which was attached to the carbon atom which is linked to the acetate group, as previously defined as Zaluzanin D (compound **2**).

Just as the NMR spectrum of **2**, the NMR spectrum of guaianolide **1** exhibited two characteristic doublets at  $\delta$  6.42 and 5.62 (*J* 3.8 and 3.2 Hz respectively) corresponding to hydrogens of H - 13a and H - 13b of an exocyclic methylene group conjugated with a  $\gamma$ -lactone. The exocyclic methylene groups attached to C-10 and C-4 were characterized by two singlets at  $\delta$  4.99 (H-14a), 5.10 (H-14b) and a pair of triplets (*J* 2.0 Hz) at  $\delta$  5.32 (H-15a) and 5.52 (H-15b), respectively.



1: R<sup>1</sup> = OH

2: R<sup>1</sup> = H

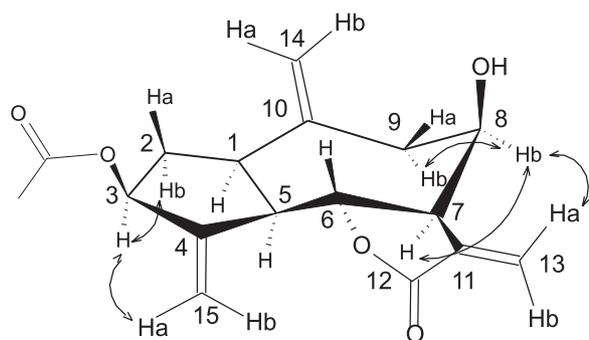


Figure 1. nOe correlations of compound 1.

The stereochemistry *cis* of the ring junction at C-1 and C-5 was established by the coupling constant ( $J$  8.3 Hz) between H-1 and H-5. In the case of 1 $\beta$ , 5 $\alpha$ -*trans*-guaianolide, a value of 10.0 Hz or greater is expected according to that reported in the literature.<sup>25,26</sup> The lactone ring junction was confirmed to be *trans* (6 $\beta$  H, 7 $\alpha$  H) by the large coupling constant ( $J \sim 3.0$  Hz) of the H-13- $\alpha$ -methylene protons this is due to H-6/H-7 ( $J$  8.9 Hz) coupling constant.<sup>16</sup>

All other signals were in agreement with the proposed structure of the guaianolide **1** (Table 1). Heteronuclear multiple quantum correlation <sup>1</sup>H – <sup>13</sup>C (HMQC) allowed us to assign unambiguously the signals of all carbons.

Besides the importance chemosystematic,<sup>27</sup> sesquiterpene lactone possesses a wide spectrum of biological activity.<sup>28</sup> Zaluzanin C, Zaluzanin D and its derivatives

Table 1. <sup>1</sup>H NMR data ( $\delta$ ) for **1** at 300 MHz

H	1
1	2.95 td (8.3, 8.0 Hz) <sup>a</sup>
2a	1.86 ddd (14.0, 8.0, 6.3 Hz)
2b	2.45 ddd (14.0, 8.0, 6.3 Hz)
3	5.50 br t (6.3 Hz)
5	2.82 dd (10.0, 8.3 Hz)*
6	4.47 dd (10.0, 8.9 Hz)
7	2.98 br d (8.9 Hz)
8	4.37 td (5.6, 2.7 Hz)
9a	2.43 dd (12.0, 5.6 Hz)
9b	2.61 dd (12.0, 5.6 Hz)
13a	5.62 d (3.2 Hz)
13b	6.42 d (3.2 Hz)
14a	4.99 s
14b	5.10 s
15a	5.32 t (2.0 Hz)
15b	5.52 t (2.0 Hz)
2'	2.10 s

<sup>a</sup> Signals attributed according spectrum in C<sub>5</sub>D<sub>5</sub>N.

have shown several biological activities, such as activity against the P 388 lymphocytic leukemia *in vitro*,<sup>29,30</sup> antifungal activity,<sup>31,32</sup> inhibitory activity on nitric oxide production and nuclear factor KB,<sup>33</sup> inhibitory activity on ethanol absorption.<sup>34</sup>

On the basis of the above data, we conclude that *M. hoehnei* belongs to the Brazilian *Mikania* species groups, which produce sesquiterpene lactones. As other authors have also reported,<sup>4,11</sup> we conclude after analysis of all this information that it is too early to establish correlations between the terpene chemistry and the morphology of this huge genus.

## Acknowledgements

We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial aid and a fellowship to J. S. C., Professor Dr. Janie Garcia da Silva for plant identification and Professor Walter C. Holmes (Department of Biology, Baylor University, Waco, Texas) for information that he includes an *M. hoehnei* in the *Mikania* section. This study was supported by grants from CAPES, CNPq and FAPESP.

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Received: May 15, 2002

Published on the web: July 29, 2003

**FAPESP helped in meeting the publication costs of this article.**