Received 20 March 2008; Accepted 7 August 2008

Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
18 (Supl.): 683-685, Dez. 2008

Artigo

Preliminary studies on the volatile constitution of *Mikania* species

Anair A. Reis,[1] Tatiana L. Ferraz,[1] Dirceu Martins,[1] Frederico G. Cruz,[1] Maria Lenise da S. Guedes,[2] Nidia F. Roque*,[1]

[1]Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Campus Universitário de Ondina, 40170-290 Salvador-BA, Brazil,
[2]Instituto de Biologia, Universidade Federal da Bahia, Campus Universitário de Ondina, 40170-290 Salvador-BA, Brazil

RESUMO: "Estudos preliminares dos constituintes voláteis de espécies de *Mikania*". Óleos voláteis obtidos das partes aéreas de plantas de três espécies de *Mikania* foram analisados por CG-EM e RMN. Quarenta e seis terpenos, entre monoterpenos, sesquiterpenos e diterpenos, foram identificados. A análise classificou *Mikania hookeriana* como produtora de diterpenos, de acordo com a maioria das espécies de *Mikania* que ocorrem no Brasil.

Unitermos: *Mikania hookeriana, Mikania hagei, Mikania jeffrey,* Asteraceae, terpenos voláteis, diterpenos kaurano.

ABSTRACT: Volatile oils obtained from the aerial parts of three *Mikania* species plants were analyzed by GC-MS and NMR. Forty-six terpenes among monoterpenes, sesquiterpenes and diterpenes were identified by this methodology. The analysis classified *Mikania hookriana* as diterpene producer as the majority *Mikania* species occurring in Brazil.

Keywords: *Mikania hookeriana, Mikania hagei, Mikania jeffrey,* Asteraceae, volatile diterpenes, kaurane diterpenoids.

INTRODUCTION

We have been studding *Mikania* plants, from Asteraceae family, for twenty years (Knudsen et al., 1986; Nunez et al., 2004). This genus is the largest of the Eupatorieae tribe, with more than 450 species. Brazil is the country where the genus is best represented with more than 150 species occurring all over the country. Many new endemic *Mikania* species were discovered in Chapada Diamantina, Bahia, Brazil (Hind, 1993).

As terpenes are de major constituents isolated from plants of this genus and essential oils compounds have large applications in pharmaceutics and cosmetics industries (Silva-Santos, et al., 2004;), the analysis of the essential oils of same *Mikania* species was accomplished. In this paper we describe for the first time the composition of the volatile oil from the aerial part plants of three *Mikania* species, *M. jeffreyi*, *M. hagei* and *M. hookeriana* collected in Chapada Diamantina, Bahia. 15-oxy-kaurenoic acid derivatives were found in the resin of *M. hookeriana* (Reis et al., 2003). These compounds have anti-bacterial properties (Davino et al., 1989) and are also present in the active fractions of *M. glomerata*, used in Brazil, for treatment of respiratory diseases (Moura et al., 2002). *M. hagei* and *M. jeffreyi* have not been studied from the chemical point of view.

MATERIAL AND METHODS

The aerial parts of *M. hookeriana* DC and *M. hagei* R. King/H. Robinson were collected in Chapada Diamantina in November 1999 and *M. jeffreyi* J. D. Hind in October 2000 in the same region. Voucher specimens were deposited at the herbarium Alexandre Leal Costa (HALC) of the Instituto de Biologia, UFBA, under the numbers 045688, 045687 and 048657, respectively.

The stems and leaves of *M. hookeriana* were separated and the fresh and ground material submitted to hydrodistillation in a Clevenger-type apparatus for 2 hours. The stem oil yield was 0.20 v/w % and the leave oil yield was 0.15 v/w %. The aerial parts of *M. hagei* and *M. jeffreyi* were submitted to the same procedure without separation of the aerial plant parts. The yields of the extractions were 0.10% v/w for *M. hagei* and 0.70% v/w for *M. jeffreyi*. The crude oils obtained were analyzed by a methodology that combines ^{13}C NMR spectra, CG-MS and Kovats Index. NMR spectra were recorded at 300 MHz for ^{1}H and 75 for ^{13}C on a Varian Gemini 300 using $CDCl_3$ as solvent and internal standard. GC-MS analyses were performed using a gas chromatograph HP 6890 interfaced with a HP 5873 Mass Selective Detector (ionization voltage 70 eV) equipped with capillary HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm), using He as the carrier gas

* E-mail: nifroque@ufba.br, Tel +55-71-32636812, Fax +55-71-32374117

Table 1. Volatile constituents from *Mikania* species.

Compounds	KI	Mha	Mho		Mje
		S+L	S	L	S+L
α-Thujene	930	-	-	-	0.18
α-Pinene	937	1.02	23.34	15.79	17.35
Camphene	953	-	-	-	0.09
β-Thujene	976	0.15	0.87	1.09	1.22
β-Pinene	980	0.30	3.93	8.07	25.64
β-Myrcene	991	-	-	-	3.37
α-Phellandrene	1006	-	-	-	1.97
3-Carene	1012	-	-	-	0.32
α-Terpinene	1019	-	-	-	0.24
p-Cymene	1027	-	0.62	0.20	7.77
Limonene	1031	0.40	0.35	0.38	30.83
(E)-β-Ocimene	1051	-	-	-	0.32
γ-Terpinene	1062	-	-	-	0.38
Terpinolene	1090	-	-	-	0.22
Terpineol-4	1178	-	-	0.16	0.67
α-Terpineol	1191	-	-		0.32
Myrtenal	1195	-	-	0.42	-
δ-Elemene	1339	-	-	0.24	-
Cyclosativene	1366	-	-	-	0.10
α-Copaene	1375	-	0.17	1.31	0.96
β-Bourbonene	1384	0.58	-	0.34	-
β-Cubebene	1389	-	0.21	0.23	-
β-Elemene	1392	4.29	-	-	-
Cyperene	1396	-	-	-	0.05
Caryophyllene	1417	2.07	1.76	1.60	0.29
α-Humulene	1452	1.50	0.77	0.77	0.09
Alloaromadendrene	1459	-	-	0.45	-
γ-Muurolene	1476	0.51	-	0.45	0.08
Germacrene D	1480	4.02	11.14	5.19	3.62
β-Selinene	1485	45.78	-	0.29	-
α-Selinene	1493	10.28	-	-	-
α-Muurolene	1498	-	-	0.45	0.09
Germacrene A	1503	8.11	-	-	-
α-Farnesene	1509	-	-	-	0.08
γ-Cadinene	1514	0.43	-	1.44	0.12
δ-Cadinene	1523	-	0.25	1.69	0.43
Elemol	1549	0.33	-	0.69	-
Germacrene B	1555		-	0.41	0.17
Spathulenol	1576	0.43	2.95	1.99	0.31
Cariophyllene Oxide	1580	0.51	1.64	1.35	-
τ-Muurolol	1642	1.99	-	6.35	-
α-Cadinol	1654	3.44	-	3.30	-
Kaur-16-ene	2026	-	1.18	0.24	-
Kaur-16-en-18-al	2244	-	6.32	0.38	-
Kaur-16-en-18-ol	2334	-	1.36	0.11	-
Kaur-16-en-18-oic Acid	2385	-	0.66	0.30	-
Total		86.14	55.52	56.37	97.28
Monoterpenes %		1.87	29.11	26.11	90.89
Sesquiterpenes %		84.27	16.89	29.23	6.39
Diterpenes %			9.52	1.03	

Mha - *M. hagei*; Mho - *M. hookeriana*; Mje - *M. jeffreyi;* S - stems; L - leaves.

(1.0 mL min^{-1}). The oven temperature was programmed from 60° to 240 °C at 3°/ min and isothermal at 240 °C for 10 min. The injector and detector temperatures were 240° and 300 °C, respectively. The RIs were calculated by the Kovats algorithm (Kovats, 1958) with C_9-C_{25} hydrocarbons as standard. The library used for MS comparison was NIST 1998.

RESULTS AND DISCUSSION

Forty-six terpenes were identified in the three oils analyzed. The percentages of mono-, sesqui- and diterpenes identified in each oil are showed in Table 1. As in other *Mikania*, the chemical composition of the oils showed from one to three major compounds. Therefore, after obtaining the oils, ^{13}C NMR spectra (BBD and DEPT) were run, in order to confirm the identification made by CG-MS analysis and Kovats index (KI) of the principal oil constituents.

The methodology adopted allowed a more precise identification of the main compound (45.78%) from the stems and leaves of *M. hagei*, as β-selinene. The comparison of the mass spectra with those of NIST 98 library suggested that the compound could be eremophilene or β-selinene with the same confidence (97%). The KI for both compounds is the same 1486 (Joulain & König, 1998) so, as the major signals present in the ^{13}C NMR spectra equal those of β-selinene, the identity of the compound could be established. The ^{13}C NMR spectrum also confirmed the presence of α-selinene in the oil.

The major compound (23.34 %) of the volatile oil from the stems of *M. hookeriana* was identified as α-pinene followed by germacrene D (11.14 %) and kaurenal (6.32 %). The oil from the leaves has the same two major constituents.

The three major compounds of *M jeffreyi* are limonene, α and β-pinene. Sesquiterpenes are less representative in this species.

In all oils, the presence of the monoterpenes α and β-pinene, limonene, β-thujene and the sesquiterpenes cariophyllene and germacrene D was observed. Diterpenes, detected by the retention time and mass fragmentation pattern, are present as traces in all species. Only in *M. hookeriana* we could identify kaurane diterpenes and they are even representatives (9.52%). The low percentage of identified compounds in the oils from *M. hookeriana* is due to the presence of diterpenes. That compounds are not identified by the CG-MS analysis.

The study of the $CHCl_3$/MEOH extract from aerial parts of *M. hookeriana* was made and 15-oxy-kauranoic acid derivatives were present as major compounds (Reis et al., 2003). This suggests that volatile oil analysis could be used to indicate the presence of fixed kaurane or other diterpenes in plants. Thus volatile chemical composition of *Mikania* species could direct the search for plants rich in the bioactive kaurenoic acids derivatives, as shown by this analysis in *Mikania hookeriana*.

Herz (1998) described the occurrence of a dichotomy between diterpenes and sesquiterpenes lactones in the constitution of *Mikania* species. Furthermore, reports on *Mikania* chemical constitution suggested that when kaurane diterpenes are present in the plant, the other kind of diterpenoids, whether they are present, is found in very small amounts (Nunez et al., 2004). As showed in this report, the volatile compounds analysis could detect the presence of diterpenes in the oil. In that way *M. hookeriana* could be classified as diterpene producer as the majority *Mikania* species occurring in Brazil.

ACKNOWLEDGEMENTS

This work was supported by CNPq and FINEP.

REFERENCES

Davino SC, Giesbrecht AM, Roque NF 1989. Antimicrobial activity of kaurenoic acid derivatives substituted on C-15. *Braz J Med Biol Res 22*: 1127-1129.

Herz W 1998. Terpenoid chemistry of *Mikania* species. *J Indian Chem Soc 75*: 559-564.

Hind JN 1993. Notes on the compositae of Bahia. *Kew Bull 48*: 245-246.

Joulain D, König W 1998. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons* E.B.-Verlag: Hamburg.

Knudsen FS, Vilegas W, Oliveira F, Roque NF 1986. New pimaradiene diterpenes from *Mikania triangularis*. *Phytochemistry 25*: 1240-1242

Kovats E 1958. Gas-chromatographische charakterisierung organischer verbindungen. Teil 1. Retentionsindices aliphatischer halogenide, alkohole, aldehyde und ketone. *Helv Chim Acta* 1915-1932.

Moura RS, Costa SS, Jansen JM, Silva CA, Lopes CS, Bernardo-Filho M, Silva VN, Criddle DN, Portela BN, Rubenich LMS, Araujo RG, Carvalho LCRM 2002. Bronchodilator activity of *Mikania glomerata* Sprengel on human bronchi and guinea-pig trachea. *J Pharm Pharmacol 54*: 249-256.

Nunez CV, Amendula MC, Lago JHG, Roque NF 2004. Diterpene acids from *Mikania* sp. nov *Biochem Sist Ecol 32*: 233-237.

Reis AA, Mendes CC, Roque NF 2003. Terpenes from *Mikania hookeriana*. *Biochem Syst Ecol 31*: 1061-1062.

Silva-Santos A, Antunes MAS, Bizzo HR, D'Avila CA, Souza-Santos LC 2004. The application of essential oils and terpenics/terpenoids compounds in the fields of pharmaceutic and cosmetic through the knowledge registered in patents. *Rev Bras Farmacogn 14*: 48-50.