Studies on the Constituents of *Mikania hirsutissima* (Compositae)

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Two novel norhumulene-type sesquiterpenes, named mikaniahumulene I (1) and II (2) were isolated along with nine known compounds, seven kaurenic acid-type diterpenes (3—9), a coumarin (10) and a flavone (11), from the aerial parts of *Mikania hirsutissima* DC (Compositae). The structures of new norhumulenes were determined by spectroscopic means. The cytotoxic activities of isolated compounds against leukemia cells (L 1210) were investigated; among the isolated compounds, 1, 5, 8, and 11 showed relatively strong cytotoxicity.

Key words *Mikania hirsutissima*; Compositae; norhumulene-type sesquiterpene; cytotoxic activity

Mikania hirsutissima is a medicinal plant which has been used as a folk medicine for treatment of diarrhea, rheumatism and gout in Brazil.[1)] In the course of our search for biologically active substances from South American medicinal plants, we found that the alcohol extracts of the aerial part of *M. hirsutissima* exhibited a cytotoxic activity (IC_{50}=25 μg/ml) against leukemia cells (L 1210). In this paper, we describe the isolation and structural elucidation of two new norhumulene-type sesquiterpenes, called mikaniahumulene I (**1**) and II (**2**) along with nine known compounds, seven kaurenic acid-type diterpenes (**3**—**9**),[2a—g)] a coumarin (**10**, herniarin)[3)] and a flavone (**11**, chrysin)[4)] from *M. hirsutissima*. (Fig. 1)

The aerial part of *M. hirsutissima* was extracted with ethanol under ultrasonication. The ethanol extracts were concentrated and then chromatographed on an HP-20 resin (Nippon Rensui[@]) column eluted successively with 40% MeOH, 70% MeOH, MeOH and acetone. The growth inhibition of each fraction against leukemia cells was tested and the MeOH fraction was found to be the most active. Thus, this fraction was further purified by silica gel column chromatography followed by HPLC to give mikaniahumulene I (**1**) and II (**2**) in addition to the nine known compounds.

Structures of Mikaniahumulene I (1) and II (2) Mikaniahumulene I (**1**) was obtained as colorless crystals, mp 136—139 °C, $[\alpha]_D$ −5.0° (c=0.8, MeOH). Its HR-MS spectrum showed a molecular ion peak at *m/z* 222.1618 $(M)^+$ corresponding to the molecular formula $C_{14}H_{22}O_2$. The ^{1}H-NMR and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra of **1** suggested the presence of *trans*-double bond [δ 5.97 (1H, d, J=16.9 Hz, H-2), δ 6.71 (1H, d, J=16.9 Hz, H-3)], three tertiary methyl groups [δ 1.08 (6H, s, H-12, H-14) and δ 1.10 (3H, s, H-13)] and two methylene sequences of C-5—C-7 and C-10—C-11 (see Table 1). The ^{13}C-NMR and ^{1}H–^{13}C COSY spectra of **1** revealed the presence of a carbonyl group (δ 202.3), the epoxide ring (δ 61.9 and 64.4) and two quaternary carbons (δ 37.7) including the epoxy carbon (δ 61.9). Thus, two of three tertiary methyl groups should be attached to the quaternary carbon at δ 37.7 to compose the geminal dimethyl group and the other one should be on the epoxy carbon at δ 61.9. To investigate the connectivities of these functional groups, we measured the heteronuclear multiple bond correlation (HMBC) spectrum of **1**. As shown in Fig. 2, the carbonyl carbon signal at δ 202.3 showed long-range correlations with the protons at δ 2.35 (H-10), δ 2.47 and 3.06 (H-11) and δ 6.71 (H-3) which exhibit the cross peaks due to long-range coupling with the carbons at δ 22.6 and 29.5 (C-12 and C-13), δ 37.7 (C-4) and δ 45.6 (C-5). Furthermore, the following ^{13}C–^{1}H long-range correlations were observed between the epoxy carbon (C-9) at δ 64.4 and H-7, H-10, H-11, H-14; the quaternary epoxy carbon (C-8) at δ 61.9 and H-6, H-7, H-10, suggesting that the plane structure of **1** is defined as shown in Fig. 2.

Finally, the stereostructure of **1** was investigated by difference nuclear Overhauser effect (NOE) experiments. Irradiation at δ 3.06 (H-11) produced significant enhancement of the proton signals at δ 2.87 (H-9) and 6.71 (H-3), and on irradiation at δ 6.71 and 1.08 (H-14), NOEs were observed on the H-9, H-11, and on the H-6α and H-10α, respectively. Thus, the relative stereostructure of **1** was confirmed as shown in Fig. 2.

Mikaniahumulene II (**2**) was obtained as an oily material, $[\alpha]_D$ −7.2° (c=1.0, MeOH). Its HR-MS spectrum showed the molecular ion peak at *m/z* 238.1567 $(M)^+$ corresponding to the molecular formula $C_{14}H_{22}O_3$. A detailed comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **1** indicated that **2** must have the same framework as that of **1** and the signifi-

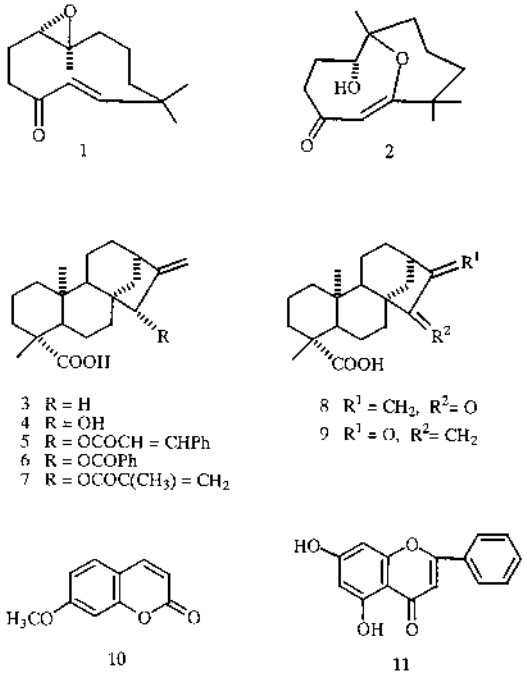

Fig. 1

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Table 1. ^{1}H- and ^{13}C-NMR Data for Mikaniahumulene I (**1**) and II (**2**)

	^{1}H-NMR (400 MHz, δ, $CDCl_3$)		^{13}C-NMR (100 MHz, δ, $CDCl_3$)	
Position	1	2	1	2
1			202.3	202.7
2	5.97 d (16.9)	5.81 s	128.7	119.1
3	6.71 d (16.9)		159.5	176.7
4			37.7	40.5
5α	1.29 m	1.23 dd (2.6, 12.3)	45.6	43.9
5β	1.88 m	1.68 m		
6α	1.19 m	1.61 m	17.9	18.9
6β	1.67 m	1.65 m		
7α	0.92 dd (10.3, 13.9)	1.39 dd (13.0, 14.3)	42.3	41.1
7β	2.24 dd (9.5, 13.9)	2.10 dd (7.3, 14.3)		
8			61.9	82.2
9	2.87 dd (3.7, 11.0)	3.76 dd (4.4, 12.1)	64.4	73.8
10α	1.44 dd (2.9, 12.5)	1.80—2.00 m	27.1	29.4
10β	2.35 m			
11α	2.47 m	2.36 m	34.1	39.4
11β	3.06 dd (2.9, 12.5)	3.36 m		
12	1.10 s	1.10 s	22.6	27.7
13	1.08 s	1.19 s	29.5	26.5
14	1.08 s	1.35 s	16.2	18.4

The numbers in parentheses are *J* values in Hz.

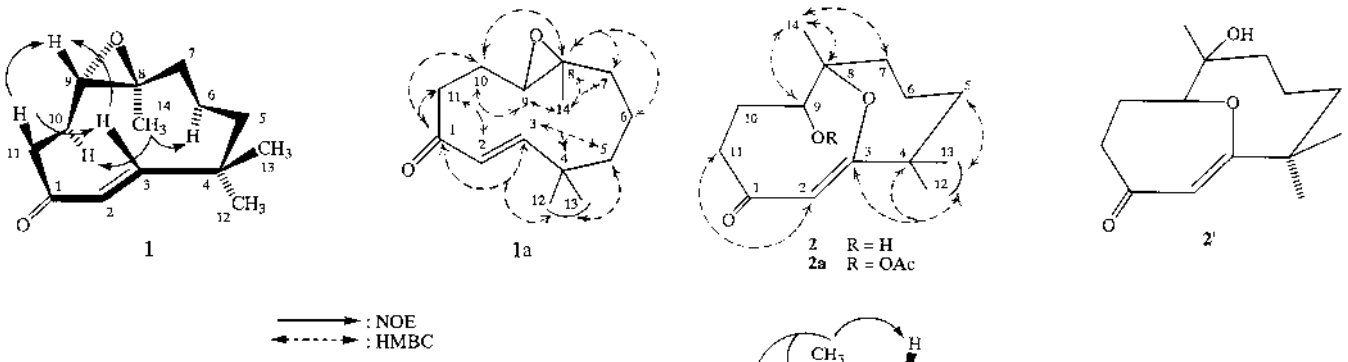

Fig. 2

cant downfield shifts of the carbon signals due to C-3 (δ 176.7), C-8 (δ 82.2) and C-9 (δ 73.8) suggested the ring opening of the epoxide ring including C-8 and C-9 and the connection of oxygen functions to these carbon atoms. Considering of these spectral data and the degree of unsaturation, **2** should be an oxygen-bridged bicyclic norhumulene-type sesquiterpene represented by the structures **2** or **2′** in Fig. 3. To discriminate between the structures **2** and **2′**, we tried acetylation of mikaniahumulene II. Treatment of **2** with acetic anhydride–pyridine gave a monoacetate (**2a**) which exhibited the proton signal due to H-9 (δ 5.05) at 1.29 ppm lower field as compared with that (δ 3.76) of **2**, thereby suggesting the presence of a hydroxyl group at C-9. Thus, the plane structure of mikaniahumulene II was represented by the structure **2** which was also supported by the long range correlations observed in the HMBC spectrum (Fig. 3).

The relative stereostructure of **2** was confirmed as shown in Fig. 3 from the results of difference NOE experiments on **2**. The structures of compounds (**3**—**11**) were identified by comparison of their spectral data with those described in the literatures.[2a—g] The cytotoxic activities of isolated compounds against leukemia cells were investigated according to ref. 5. Among the isolated compounds (**1**, **5**, **8**, and **11**) showed relatively strong cytotoxicity while the major component (**3**) of this plant exhibited weak cytotoxicity.

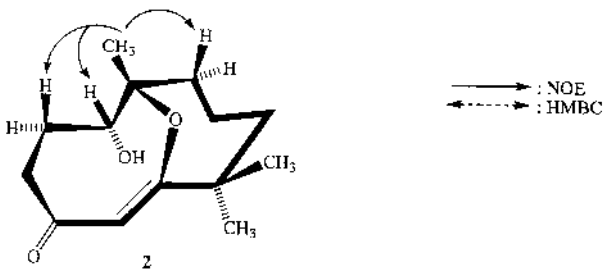

Fig. 3

Table 2. Growth Inhibitory Effects of the Compounds Isolated from *M. hirsutissima* againt Leukemia Cells (L 1210)

Compounds	IC_{50} (μg/ml)	Compounde	IC_{50} (μg/ml)
1	10	**7**	40
2	>50	**8**	3
3	40	**9**	40
4	20	**10**	>50
5	3	**11**	5
6	>50		

Experimental

Plant material was purchased from LABORATORIO FARMAERVAS

LTDA. in Sao Paulo, Brazil and deposited in the Laboratory of Medicinal Chemistry, Nihon University. The ^{1}H- and ^{13}C-NMR were measured on a JEOL GSX-400 spectrometer in $CDCl_3$ containing tetramethylsilane (TMS) as internal standard. The MS spectra were recorded on a Hitachi RMU 6M instrument. Optical rotation was measured on a JASCO DIP-370 polarimeter.

Isolation of Compounds (1—11) The aerial part of dried *M. hirsutissima* (1.9 kg) was extracted with ethanol (2 l×3) under ultrasonication. The alcoholic extract was concentrated under reduced pressure to give 91.8 g oily material which was then chromatographed on a HP-20 resin (Nippon Rensui®) column eluted successively with stepwise gradients of MeOH–H_2O [2 : 3 (2 l), 7 : 3 (2 l)], MeOH (3 l) and then acetone (2 l). The cytotoxicity of each fraction against leukemia cells was tested and the MeOH fraction was found to be most active. This fraction (47.6 g) was then chromatographed on a silica gel column eluted successively with stepwise gradients of hexane–EtOAc [10 : 1 (3.6 l) (fr. 1), 5 : 1 (3 l) (fr. 2), 1 : 1 (6 l)], EtOAc (1.0 l) (fr. 3) and then MeOH (2.0 l) (fr. 4). Concentration of fr. 1 left a solid material which was recrystallized from hexane–EtOAc to give **3** (5.6 g). Fr. 2 (4.7 g) was purified by high performance liquid chromatography (HPLC) to give **4** (43 mg, HPLC: Shodex SIL-5E, 10×250 mm, hexane–EtOAc=2 : 1; flow rate, 3.0 ml/min; retention time, 10.0 min), **5** (112 mg, HPLC: Shodex SIL-5E, 10×250 mm, hexane–EtOAc=5 : 1; flow rate, 3.0 ml/min; retention time, 11.2 min), **6** (15 mg; retention time, 32.0 min), **7** (6.0 mg; retention time, 22.5 min) (HPLC for **6** and **7**; Shodex C18-5E, 10×250 mm, 85% MeOH; flow rate, 3.0 ml/min), **8** (10 mg; retention time, 13.6 min) (HPLC: Shodex C18-5E, 10×250 mm, 90% MeOH; flow rate, 3.0 ml/min), **9** (40 mg; retention time, 54.0 min), **11** (33 mg; retention time, 20.0 min) (HPLC for 9 and 11: Shodex C18–5E, 10×250 mm, 70% MeOH; flow rate, 2.5 ml/min), **10** (150 mg; retention time, 19.4 min) (HPLC: Senshu Pak Silica-4251-N, 10×250 mm, hexane–EtOAc=5 : 1; flow rate, 4.0 ml/min) and **1** (28 mg; retention time, 18.0 min) (HPLC: Senshu Pak Silica–4251–N, 10×250 mm, hexane–EtOAc=3 : 1; flow rate, 3.0 ml/min). Fr. 3 was chromatographed on a silica gel column eluted successively with hexane : EtOAc=7 : 1, 4 : 1, 2 : 1, 1 : 1, and then EtOAc. The hexane : EtOAc=1 : 1 and EtOAc fractions were combined and then concentrated under reduced pressure to leave an oily material which was purified by HPLC (Kaseisorb LC 60-5, 10×250 mm; flow rate, 3.0 ml/min) to give **2** (33 mg; retention time, 16.0 min).

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Refereces and Note

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2) *a*) **3** (*ent*-kaur-16-en-19-oic acid): Monte. F. J. Q., Dantas E. M. G., Braz F. R., 27, 3209—3212 (1988); *b*) **4** (*ent*-kaur-15α-hydroxy-16-en-19-oic acid): Hutchinson M., Lewer P., MacMillan J., *J. Chem. Soc*., *Perkin Trans*. 1, 1984, 2363—2366; *c*) **5** (*ent*-kaur-15α-cinnamoyloxy-16-en-19-oic acid): Vichnewski W., Filo F. L., Murai H., Herz W., *Phytochemistry*, **16**, 2028—2029 (1977); *d*) **6** (*ent*-kaur-15α-benzoyloxy-16-en-19-oic acid): Bohlmann F., Natu A. A., Mahanta P. K., *ibid*., **17**, 483—485 (1978). *e*) **7** (*ent*-kaur-15α-methacryloxy-16-en-19-oic acid): Bohlmann F., Zdero C., Robinson H., and King R. M., *ibid*., **20**, 522—523 (1981); *f*) **8** (*ent*-kaur-15-oxo-16-en-19-oic acd): Monte. F. J. Q., Dantas E. M. G., Braz F. R., *ibid*., **27**, 3209—3212 (1988); *g*) **9** (*ent*-kaur-16-oxo-18-oic acid): Hanson J. R., Silverns M., Piozzi F., Savona G., *J. Chem. Soc*., *Perkin Trans*. 1, **1976**, 114—117.
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