New Sesquiterpene Dilactones from *Mikania micrantha*

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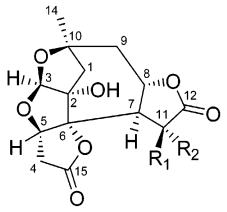
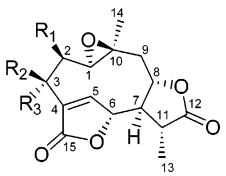
Four new sesquiterpene dilactones, mikamicranolide (**1**), 11β,13-dihydromikamicranolide (**2**), 3α-hydroxy-11β,13-dihydrodeoxymikanolide (**3**), and 2β,3β-dihydroxy-11β,13-dihydrodeoxymikanolide (**4**), were isolated from the whole plants of *Mikania micrantha*. Their structures were elucidated on the basis of spectroscopic analysis. Compounds **1** and **2** possess an unusual rearranged 12,8-germacranolide sesquiterpene skeleton.

Mikania micrantha, a fast-growing perennial creeping vine native to Central and South America, is one of the worst exotic weeds in Southeast Asia and South Asia. It has been present in South China since the 1980s and has caused a significant adverse impact on tree crops in its established area.[1] This weed damages or kills other plants by cutting out light and smothering them. It also competes for water and nutrients, but more importantly, the plant releases allelochemicals that inhibit the growth of other plants.[2,3] Previous chemical investigations have reported the occurrence of germacranolide sesquiterpenes from this plant.[4−6] During our ongoing allelochemical research on invasive plants in South China, we have reinvestigated this weed and have isolated four new and four known sesquiterpene dilactones. In this paper, we describe the isolation and the structure elucidation of these new compounds (**1**−**4**).



1 $R_1, R_2 = CH_2$
2 $R_1 = H, R_2 = CH_3$



3 $R_1 = R_2 = H, R_3 = OH$
4 $R_1 = R_2 = OH, R_3 = H$

The EtOH extract of the powdered dry whole plants of *M. micrantha* was fractionated sequentially with petroleum ether, $CHCl_3$, and EtOAc. The $CHCl_3$-soluble fraction was subjected to repeated column chromatography over silica gel. Two new compounds, named mikamicranolide (**1**) and 11β,13-dihydromikamicranolide (**2**), were isolated along with four known sesquiterpenes, dihydromikanolide, deoxymikanolide, 11β,13-dihydrodeoxymikanolide, and dihydroscadenolide, which were identified by comparison of their spectral data with literature values.[6−8] The EtOAc fraction was separated by repeated column chromatography over silica gel and Sephadex LH-20 to yield further two new sesquiterpene dilactones, 3α-hydroxy-11β,13-dihydrodeoxymikanolide (**3**) and 2β,3β-dihydroxy-11β,13-dihydrodeoxymikanolide (**4**).

Mikamicranolide (**1**) was found to have the molecular formula $C_{15}H_{16}O_7$ by high-resolution time-of-flight (TOF) mass spectrometry, giving a $[M + Na]^+$ peak at *m/z* 331.0804. The IR spectrum showed hydroxyl (3479 cm^{-1}), olefinic bond (1658 cm^{-1}), and carbonyl (1797−1761 cm^{-1}) absorptions. The ^1H NMR spectrum of **1** exhibited 15 nonexchangeable protons, including a tertiary methyl (δ 1.39), two olefinic protons (δ 6.38 and 5.86), an acetal proton (δ 6.09), and two oxygenated methine protons (δ 5.32 and 4.85). The ^{13}C NMR spectrum of **1** gave signals for two ester carbonyl carbons (δ 174.9 and 169.1), an olefinic quaternary carbon (δ 136.9), an olefinic methylene (δ 123.3), an acetal methine (δ 115.8), three oxygenated quaternary carbons (δ 90.2, 88.4, and 84.4), two oxygenated methines (δ 81.4 and 75.9), three aliphatic methylenes (δ 45.2, 43.6, and 38.5), an aliphatic methine (δ 46.6), and a methyl carbon (δ 30.9). Interpretation of the $^1H-^1H$ COSY spectrum of **1** suggested the presence of two partial structures, A and B (Figure 1). The proton signals for segment A at δ 2.45 (1H, dd, J = 12.8, 5.2 Hz), 1.79 (1H, dd, J = 12.8, 12.0 Hz), 4.85 (1H, ddd, J = 10.8, 10.8, 4.4 Hz), 3.87 (1H, ddd, J = 10.4, 2.4, 2.4 Hz), 6.38 (1H, br s), and 5.86 (1H, br s) were assignable to H-9β, H-9α, H-8, H-7, H-13a, and H-13b, respectively, characteristic of 11(13)-germacren-12,8-olides.[7] The further connectivity of the above partial structures was deduced from the HMBC spectrum (Figure 1). The HMBC correlations from the acetal methine proton (H-3) to the three oxygenated quaternary carbons at δ 84.4 (C-10), 88.4 (C-2), and 90.2 (C-6), the methine carbon at δ 81.4 (C-5) of segment B, and the methylene carbon at δ 45.2 (C-1), and from the methine proton (δ 5.32, H-5) of segment B to the acetal carbon at δ 115.8 (C-3), C-2 and C-6, as well as from the methylene protons (δ 2.80 and 2.54, H_2-1) to C-10, C-2, C-6, and C-3, indicated the connectivity of C-3 via two individual oxygen bridges to both C-5 and C-10, and C-2 to C-6. The correlations from both H-5 and the methylene protons (δ 3.16, H_2-4) of segment B to the carbonyl carbon at δ 174.9 (C-15) and C-6 suggested the presence of a five-membered lactone ring moiety. Further, the above HMBC correlations also implied that a hydroxyl group was attached to C-2. On the other hand, the oxygenated methine proton (H-8) of segment A showed

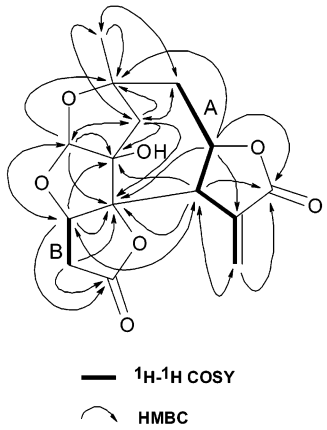


Figure 1. $^1H-^1H$ and major long-range $^{13}C-^1H$ correlations of **1**.

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Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a]

position	**1**[b]	**2**[b]	**3**[c]	**4**[c]
1β	2.54 d (13.6)	2.51 d (13.6)	2.78 dd (11.2, 1.6)	2.99 d (10.0)
1α	2.80 d (13.2)	2.83 d (13.2)		
2β			2.35 ddd (12.0, 6.0, 1.6)	
2α			1.66 ddd (12.0, 11.2, 10.4)	3.61 dd (10.0, 4.0)
3	6.09 s	6.09 s	4.90 dd (10.4, 6.0)	4.75 dd (4.0, 1.6)
4	3.16 d (7.6)	3.25 dd (12.0, 9.2)		
		3.17 dd (12.0, 6.4)		
5	5.32 t (7.6)	5.66 dd (9.2, 6.4)	7.88 br s	7.99 br s
6			5.32 br s	5.39 br s
7	3.87 ddd (10.4, 2.4, 2.4)	3.04 t (10.8)	2.60 br dd (12.4, 10.0)	2.65 br dd (12.8, 10.0)
8	4.85 ddd (10.8, 10.8, 4.4)	4.85 ddd (10.0, 10.0, 5.2)	4.55 ddd (10.0, 10.0, 4.4)	4.62 ddd (10.0, 10.0, 6.0)
9β	2.45 dd (12.8, 5.2)	2.44 dd (12.8, 5.2)	1.96 dd (14.4, 4.4)	2.01 m
9α	1.79 dd (12.8, 12.0)	1.72 dd (12.8, 10.0)	2.04 m	2.04 m
11		3.16 m	2.91 m	2.98 m
13a	6.38 br s	1.41 d (6.8)	1.34 d (7.2)	1.35 d (7.2)
13b	5.86 br s			
14	1.39 s	1.38 s	1.19 s	1.20 s

[a] Chemical shifts (δ) in ppm; coupling constants (parentheses) given in Hz. [b] In C_5D_5N. [c] In acetone-d_6.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

position	**1**[b]	**2**[b]	**3**[c]	**4**[c]
1	45.2	45.1	60.4	62.2
2	88.4	88.2	32.9	68.8
3	115.8	115.7	66.5	68.9
4	38.5	38.4	134.9	135.1
5	81.4	80.7	148.7	148.9
6	90.2	91.1	80.3	81.3
7	46.6	50.3	54.4	54.7
8	75.9	75.7	78.4	78.1
9	43.6	44.1	43.6	44.0
10	84.4	83.9	57.5	58.0
11	136.9	39.3	40.6	40.5
12	169.1	177.7	176.3	176.3
13	123.3	16.2	13.5	13.5
14	30.9	31.0	20.7	21.3
15	174.9	174.6	171.2	171.4

[a] Chemical shifts (δ) in ppm. [b] In C_5D_5N. [c] In acetone-d_6.

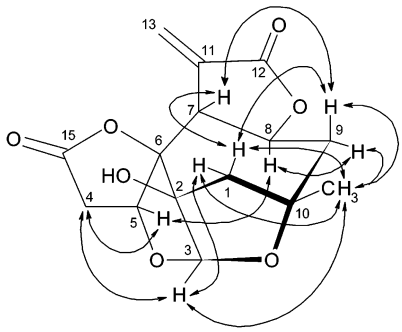

Figure 2. Selected NOESY correlations for **1**.

correlations to C-10, C-6, the quaternary olefinic carbon at δ 136.9 (C-11), and the carbonyl carbon at δ 169.1 (C-12). The methine proton (H-7) of segment A showed correlations to C-2, C-5, C-6, and C-12. The methylene protons (H_2-9) of segment A showed correlations to C-10, C-1, and the methyl carbon at δ 30.9 (C-14). Furthermore, the methyl protons (δ 1.39, H_3-14) were correlated to C-10, C-1, and the methylene carbon at δ 43.6 (C-9).

On the basis of the spectral evidence described above, the planar structure of **1** was deduced as shown in Figure 1. The assignments of the ^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2.

The relative stereochemistry of **1** was determined by a NOESY experiment (Figure 2). In this spectrum, the strong cross-peaks between H-7/H-9α, H-7/H-1α, and H-1α/H-9α indicated that the seven-membered ring formed by C-1, C-2, C-6, C-7, C-8, C-9, and C-10 was in the chair ($^{2,6}C_9$) form, with C-1, C-7, C-8, and C-10 held in the same plane, while C-2 and C-6 were oriented up and C-9 down the plane. The observation of the NOE interactions between H-3/H-1β, H-3/H_3-14, H-3/H_2-4, H-5/H-8, and H-8/H-9β and the absence of the interactions between H-3/H-5, H-7/H-8, and H-8/H-9α indicated the α-orientation of H-3 and β-orientations of H-5 and H-8. Therefore, the complete structure of **1** was determined as shown.

11β,13-Dihydromikamicranolide (**2**) was determined to have a molecular formula of $C_{15}H_{18}O_7$ by the combined analysis of its HRTOFMS, APCIMS, EIMS, ^{13}C NMR, and DEPT data. The IR spectrum showed hydroxyl (3477 cm^{-1}) and carbonyl (1795−1780 cm^{-1}) absorptions. The ^{1}H NMR spectrum of **2** (Table 1) was very similar to that of **1** except that the signals for the olefinic methylene protons (H_2-13) in **1** were absent in **2**. Instead, a doublet at δ 1.41 (J = 6.8 Hz) for protons of a secondary methyl group and a multiplet at δ 3.16 for an additional methine proton were present. Analysis of the spectroscopic evidence suggested that compound **2** is the 11,13-dihydride of **1**. This was supported by the ^{13}C NMR data of **2** (Table 2), which were assigned by comparison with those of **1**, and detailed analyses of the ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC spectra. The large coupling constant between H-7 and H-11 ($J_{7,11}$ = 10.8 Hz)[8] and the observation of a NOE interaction between H_3-13 and H-7 in the NOESY spectrum indicated the β-orientation of H-11. Thus, **2** has the structure depicted.

Compounds **1** and **2** are interesting because they possess an unusual rearranged 12,8-germacranolide sesquiterpene skeleton. These are the first two examples to be assigned with this skeleton and could be considered 3,4-seco-germacranolides with an additional bond between C-2 and C-6.

3α-Hydroxy-11β,13-dihydrodeoxymikanolide (**3**) was established as having a molecular formula of $C_{15}H_{18}O_6$ by HRTOFMS, APCIMS, EIMS, and the NMR spectra (^{1}H, DEPT, and ^{13}C). Its ^{1}H and ^{13}C NMR spectra indicated two methyl groups, two methylenes, and seven methines, with four of them oxygenated and one olefinic, as well as four quaternary carbons, of which two were ester carbonyls, one olefinic and another bonded to oxygen. Finally, ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC experiments furnished the assignments of all ^{1}H and ^{13}C NMR signals (Tables 1 and 2) and indicated that the structure of **3** is similar to 11β,13-dihydrodeoxymikanolide except that **3** has a hydroxyl group at C-3. The α-orientation of the hydroxyl group at C-3 was indicated by the ^{1}H NMR coupling pattern of H-3 (dd, $J_{3,2\alpha}$ = 10.4 Hz, $J_{3,2\beta}$ = 6.0 Hz) and the NOESY spectrum (Figure 3), which showed the presence of the NOE interactions between H-3/H-1, H-3/H-5, and H-3/H-2β and the absence of an interaction between H-3/H-2α.

2β,3β-Dihydroxy-11β,13-dihydrodeoxymikanolide (**4**) was assigned a molecular formula of $C_{15}H_{18}O_7$, which was also derived from HRTOFMS, APCIMS, EIMS, and the NMR

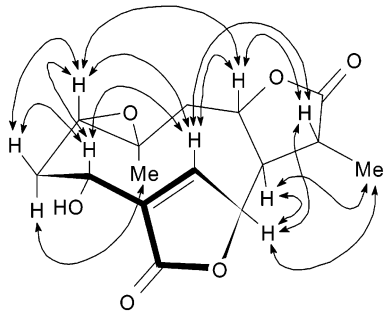

Figure 3. Selected NOESY correlations for **3**.

spectra (^{1}H, DEPT, and ^{13}C). Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), for which all assignments were based on ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC experiments, indicated a structure similar to **3** except for an additional hydroxyl group attached to C-2 in **4**. The β-orientations of both hydroxyl groups at C-2 and C-3 were evidently indicated by the ^{1}H NMR coupling constants, $J_{1,2} = 10.0$ Hz and $J_{2,3} = 4.0$ Hz,[9] as well as the NOESY spectrum, which exhibited NOE correlations between H-2/H-3 and H-2/H_3-14.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yangimoto Seisakusho micro-hot stage melting apparatus and were uncorrected. Optical rotations were obtained on a Perkin-Elmer 343 spectropolarimeter with MeOH as solvent. The UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 25 UV−vis spectrophotometer. The IR spectra were measured in KBr on a WQF-410 FT-IR spectrophotometer. The ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were recorded on a Bruker DRX-400 instrument using TMS as an internal reference. HRTOFMS data were obtained on an API QSTAR mass spectrometer in positive-ion mode for **1** and negative ion mode for **2**−**4**. APCIMS were recorded on an API 2000 LC/MS/MS system in negative-ion mode. EIMS were collected on a Micromass Platform EI 200 GC/MS instrument at 70 eV by direct inlet. For column chromatography, silica gel 60 (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China) and Sephadex LH-20 were used. TLC was performed on precoated plates (Kieselgel 60GF_{254}, Merck) with detection effected by spraying with H_2SO_4 (10%) in EtOH followed by heating.

Plant Material. The whole plants (the aerial parts and the roots) of *M. micrantha* were collected from Dongguan, Guangdong, People's Republic of China, in July 2002. An authenticated voucher specimen (No. CHL020701) was deposited at the herbarium of South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, People's Republic of China.

Extraction and Isolation. The powdered dry whole plants of *M. micrantha* (3.2 kg) were extracted with 95% EtOH three times at room temperature. The EtOH extract, after concentration in vacuo, was suspended in H_2O, and the aqueous suspension was sequentially extracted three times each with petroleum ether, $CHCl_3$, and EtOAc. The combined $CHCl_3$ solution, upon evaporation, yielded a deep brown syrup (44.5 g). This syrup was subjected to silica gel column chromatography, eluted with $CHCl_3$−MeOH mixtures of increasing polarities (100:0 to 50:3), to obtain six fractions (I−VI). Fraction III, obtained on elution with $CHCl_3$−MeOH (99:1), was rechromatographed on a silica gel column eluted with benzene−EtOAc (9:1) followed by crystallization from acetone to give compound **2** (36 mg, 0.00113% yield), dihydromikanolide[6,7] (120 mg, 0.00375% yield), deoxymikanolide[6,7] (210 mg, 0.00656% yield), and a subfraction III-1. Subfraction III-1 was further separated by silica gel column chromatography eluted with benzene−EtOAc (2:1) to afford **1** (18 mg, 0.00056% yield), 11β,13-dihydrodeoxymikanolide[8] (15 mg, 0.00047% yield), and dihydroscadenolide[6,7] (38 mg, 0.00118% yield). The combined EtOAc solution, after concentration, afforded a light brown syrup. This syrup was divided into eight fractions (VII−XIV) by silica gel column chromatography eluted with $CHCl_3$−MeOH mixtures of increasing polarities (19:1 to 5:1). Fraction X, obtained on elution with $CHCl_3$−MeOH (18:2), was further separated by silica gel chromatography eluted with $CHCl_3$−MeOH (20:1), followed by purification on a Sephadex LH-20 column with MeOH as eluent, to afford **3** (14 mg, 0.00044% yield) and **4** (18 mg, 0.00056% yield).

Mikamicranolide (1): colorless needles, mp 246−250 °C; $[\alpha]^{24}_D$ −64.0° (*c* 0.0625, pyridine); UV (MeOH) λ_{max} (log ϵ) 211 (3.97) nm; IR (KBr) ν_{max} 3479 (OH), 1797 (C=O), 1761 (C=O), 1658 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N), see Tables 1 and 2; EIMS *m/z* 290 [M − H_2O]$^+$ (41), 262 [M − H_2O − CO]$^+$ (8), 244 [M − H_2O − CO − H_2O]$^+$ (21), 233 (14), 216 (18), 206 (19), 191 (19), 179 (27), 151 (36), 150 (72), 136 (47), 122 (100); HRTOFMS *m/z* 331.0804 [M + Na]$^+$ (calcd for $C_{15}H_{16}O_7Na$, 331.0793).

11β,13-Dihydromikamicranolide (2): colorless needles, mp 264 °C (dec); $[\alpha]^{24}_D$ +13.6° (*c* 0.1175, acetone); IR (KBr) ν_{max} 3477 (OH), 1795 (C=O), 1780 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N), see Tables 1 and 2; EIMS *m/z* 310 [M]$^+$ (4), 292 [M − H_2O]$^+$ (12), 264 [M − H_2O − CO]$^+$ (9), 246 [M − H_2O − CO − H_2O]$^+$ (12), 222 (19), 218 (11), 208 (8), 191 (17), 179 (27), 152 (34), 124 (50), 73 (100); APCIMS *m/z* 309 [M − H]$^-$; HRTOFMS *m/z* 309.0985 [M − H]$^-$ (calcd for $C_{15}H_{17}O_7$, 309.0974).

3α-Hydroxy-11β,13-dihydrodeoxymikanolide (3): colorless needles, mp 203−208 °C; $[\alpha]^{24}_D$ +113.2° (*c* 0.1175, acetone); UV (MeOH) λ_{max} (log ϵ) 208 (4.11) nm; IR (KBr) ν_{max} 3431 (OH), 1774 (C=O), 1745 (C=O), 1649 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6), see Tables 1 and 2; EIMS *m/z* 279 [M − CH_3]$^+$ (4), 251 [M − CH_3 − CO]$^+$ (9), 233 [M − CH_3 − CO − H_2O]$^+$ (2), 192 (4), 177 (2), 152 (6), 136 (9), 112 (100), 99 (30); APCIMS *m/z* 293 [M − H]$^-$; HRTOFMS *m/z* 293.1037 [M − H]$^-$ (calcd for $C_{15}H_{17}O_6$, 293.1025).

2β,3β-Dihydroxy-11β,13-dihydrodeoymikanolide (4): colorless prisms, mp 137−140 °C; $[\alpha]^{24}_D$ +82.4° (*c* 0.1263, acetone); UV (MeOH) λ_{max} (log ϵ) 215 (4.10) nm; IR (KBr) ν_{max} 3415 (OH), 1751 (C=O), 1616 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6), see Tables 1 and 2; EIMS *m/z* 311 [M + H]$^+$ (2), 292 [M − H_2O]$^+$ (2), 251 [M − CH_3 − CO_2]$^+$ (11), 233 [M − CH_3 − CO_2 − H_2O]$^+$ (4), 164 (16), 149 (18), 136 (68), 121 (65), 112 (100), 99 (66); APCIMS *m/z* 309 [M − H]$^-$; HRTOFMS *m/z* 309.0962 [M − H]$^-$ (calcd for $C_{15}H_{17}O_7$, 309.0974).

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