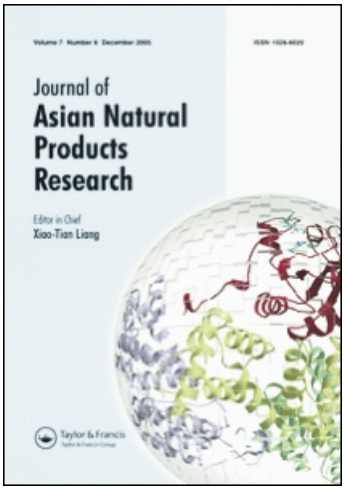

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Shi-Xiu Feng[a]; Li-Dong Lin[a]; Han-Hong Xu[b]; Xiao-Yi Wei[a]
[a] South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou, China [b] Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agriculture University, Guangzhou, China

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Two new piperidine alkaloids from the leaves of *Microcos paniculata*

Shi-Xiu Feng[a], Li-Dong Lin[a]*, Han-Hong Xu[b] and Xiao-Yi Wei[a]

[a]*South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou, China;* [b]*Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agriculture University, Guangzhou, China*

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Two new piperidine alkaloids, microcosamines A (**1**) and B (**2**), were isolated from the leaves of *Microcos paniculata*. Their structures were elucidated by spectroscopic analysis. Both new compounds showed significant larvicidal activity against *Culex quinquefasciatus*.

Keywords: piperidine alkaloids; *Microcos paniculata*; larvicidal activity; microcosamine A; microcosamine B

1. Introduction

Microcos paniculata L. (Tiliaceae) is a shrub native to South China, South Asia, and Southeast Asia. Its leaves are a folk medicine, used as herbal tea for the treatment of cold, enteritis, and skin rash in South China [1]. We previously investigated the leaves of this plant and isolated triterpenoids and flavonoids as the predominant constituents [2]. The occurrence of insecticidal piperidine alkaloids in its stem bark was also reported [3]. In continuing our study on the bioactive constituents of this Chinese herb, we reinvestigated the leaves of this plant by enlargement of the amount of plant material. The reinvestigation led to the isolation of two new piperidine alkaloids, microcosamines A (**1**) and B (**2**). This paper deals with the isolation, structural elucidation, and insecticidal activity of the new compounds.

2. Results and discussion

The leaves of *M. paniculata* were extracted with acidic water. The resulting aqueous solution was basified and then extracted with $CHCl_3$. The $CHCl_3$-soluble fraction was subjected to repeated column chromatography over silica gel, ODS, and Sephadex LH-20 to yield compounds **1** and **2** (Figure 1).

Microcosamine A (**1**) was obtained as colorless needles. Its molecular formula was determined as $C_{16}H_{27}NO$ from the ESI-MS ions at *m/z* 499 $[2\ M + H]^+$ and 250 $[M + H]^+$, as well as HR-ESI-MS which gave a pseudomolecular ion at *m/z* 250.2162 $[M + H]^+$. The IR spectrum exhibited bands for OH (3399 cm^{-1}), NH (3268 cm^{-1}), and unsaturated groups (3010 and 1635 cm^{-1}). The UV absorption maximum at 270 nm indicated the presence of a conjugated triene system [4]. The presence of a deca-1*E*,3*E*,5*E*-trienyl group was readily recognized from the proton signals for six methine groups in the region of δ 5.57–6.15, a 2H quartet at δ 2.07 (q, $J = 7.2$ Hz, H-7′), and a 3H triplet at δ 0.86 (t, $J = 6.8$ Hz, CH_3-10′) in the ^1H NMR spectrum (Table 1), as well as the carbon resonances for six olefinic methine carbons in the region of δ 129.0–135.6; three methylene carbons at δ 32.5 (C-7′), 31.4 (C-8′), and 22.2 (C-9′); and a methyl carbon at δ 13.9 (C-10′)

*Corresponding author. Email: linld@scbg.ac.cn

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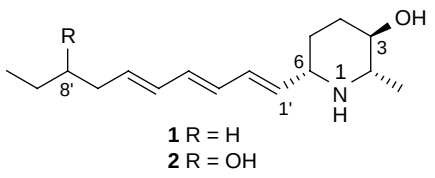

Figure 1. Structures of compounds **1** and **2**.

in the ^{13}C NMR and DEPT spectra (Table 1) [3,4]. After excluding the signals due to the decatrienyl group, the remaining resonances in the ^{1}H NMR, ^{13}C NMR, and DEPT spectra showed the presence of a methyl group [δ 1.17 (d, $J = 6.4$ Hz, CH_3-2); δ 19.0, CH_3-2], two methylene groups [δ 2.04 (m, H_{eq}-4), 1.71 (m, H_{eq}-5), 1.24–1.37 (overlapping, H_{ax}-4 and H_{ax}-5); δ 33.9 (C-4), 32.0 (C-5)], and three methine groups with one oxygenated [δ 3.13 (m, H-3); δ 73.6 (C-3)] and two bearing nitrogens [δ 3.16 (m, H-6), 2.50 (dq, $J = 10.0$, 6.4 Hz, H-2); δ 58.6 (C-6), 58.4 (C-2)]. This finding in combination with analysis of $^{1}H-^{1}H$ and $^{13}C-^{1}H$ COSY spectra indicated the presence of a 6-substituted 2-methyl-3-piperidinol ring [5]. The attachment of the trienyl group to C-6 was derived from the $^{1}H-^{1}H$ COSY and ^{1}H-NMR spectra, which showed that H-1′ was coupled to H-6 with a coupling constant of 7.2 Hz. The large proton coupling constant ($J = 10.0$ Hz) between H-2 and H-3 in the ^{1}H NMR spectrum indicated that H-2 and H-3 are in axial configuration. The strong NOE interaction between H-2 and H-6 observed in the NOESY spectrum indicated that H-2 and H-6 were located on the same side. This evidence indicated that Me-2, OH-3, and the trienyl group at C-6 were all in equatorial orientation, which confidently established the relative stereochemistry of the piperidinol ring [5]. Thus, the structure of compound **1** was determined as 6α-(deca-1*E*,3*E*,5*E*-trienyl)-3β-hydroxy-2α-methylpiperidine.

Microcosamine B (**2**) was obtained as white amorphous powder. Its formula was determined as $C_{16}H_{27}NO_2$ by HR-ESI-MS in conjunction with the ^{1}H NMR, ^{13}C NMR, and DEPT spectral data (Table 1). The ^{1}H NMR, ^{13}C NMR, and DEPT spectra of **2** were similar to those of **1**, except that the signals at δ 3.54 and δ 72.4 for an additional oxygenated H—C group were present while

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** in $CDCl_3$.

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2	2.50 (dq, $J = 10.0$, 6.4)	58.4 (d)	2.50 (dq, $J = 10.0$, 6.8)	58.3 (d)
3	3.13 (m)	73.6 (d)	3.12 (m)	73.2 (d)
4	1.24–1.37 (m, H_{ax}) 2.04 (m, H_{eq})	33.9 (t)	1.33 (m, H_{ax}) 2.00 (m, H_{eq})	33.8 (t)
5	1.24–1.37 (m, H_{ax}) 1.71 (m, H_{eq})	32.0 (t)	1.33 (m, H_{ax}) 1.69 (m, H_{eq})	31.8 (t)
6	3.16 (m)	58.6 (d)	3.16 (m)	58.5 (d)
1′	5.57 (dd, $J = 15.2$, 7.2)	135.2 (d)	5.58 (dd, $J = 15.2$, 7.2)	135.7 (d)
2′	6.13 (m)	130.1 (d)	6.13 (m)	130.1 (d)
3′	6.14 (m)	130.4 (d)	6.02 (m)	130.8 (d)
4′	6.15 (m)	132.9 (d)	6.12 (m)	132.2 (d)
5′	6.04 (m)	129.9 (d)	6.08 (m)	133.1 (d)
6′	5.67 (dt, $J = 15.2$, 7.2)	135.6 (d)	5.68 (dt, $J = 15.2$, 7.4)	130.6 (d)
7′	2.07 (q, $J = 7.2$)	32.5 (t)	2.15 (m, H_a) 2.27 (m, H_b)	40.3 (t)
8′	1.36 (m)	31.4 (t)	3.54 (tt, $J = 7.4$, 5.0)	72.4 (d)
9′	1.34 (m)	22.2 (t)	1.44 (m)	29.5 (t)
10′	0.86 (t, $J = 6.8$)	13.9 (q)	0.90 (t, $J = 7.2$)	9.9 (q)
Me–C(2)	1.17 (d, $J = 6.4$)	19.0 (q)	1.15 (d, $J = 6.0$)	18.9 (q)

Chemical shifts (δ) in ppm; coupling constants (parentheses) given in Hz.

the resonances for one of the CH_2 groups in **1** were absent in **2**. Thus, the difference in the molecular formula between **1** and **2** was due to the presence of an extra hydroxyl substituent in **2**. The position of this hydroxyl group was assigned at H-8′ by the $^1H-^1H$ COSY spectrum, in which the signal at δ 3.54 (tt, $J = 7.4$, 5.0, H-8′) was coupled to the 2H multiplet at δ 1.44 (H-9′), which was in turn correlated with the 3H triplet at δ 0.90 (t, $J = 7.2$ Hz, CH_3-9′) for the terminal methyl group. Therefore, compound **2** was deduced as 6α-(8-hydroxy-deca-1*E*,3*E*,5*E*-trienyl)-3β-hydroxy-2α-methylpiperidine.

In the evaluation of insecticidal activity, compounds **1** and **2** exhibited potency against the larvae of *Culex quinquefasciatus* with LC_{50} values of 5.2 and 17.0 μg/ml, respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanagimoto Seisakusho MD-S2 micro-melting point apparatus and are uncorrected. Optical rotations were obtained on a PerkinElmer 341 polarimeter with MeOH as solvent. UV spectra were recorded in MeOH on a PerkinElmer Lambda 25 UV–vis spectrophotometer. IR spectra were measured in KBr on a WQF-410 FT-IR spectrophotometer. ^1H (400 MHz), ^{13}C (100 MHz), and 2D-NMR spectra were recorded on a Bruker DRX-400 instrument using TMS as an internal standard. The chemical shifts are given in δ (ppm) and coupling constants in Hz. ESI-MS were collected on MDS Sciex API 2000 LC/GC/MS instrument in positive ion mode. HR-ESI-MS data were obtained on an API QSTAR mass spectrometer in positive ion mode. TLC was performed on precoated plates (Kieselgel $60GF_{254}$; Merck, White House Station, NJ, USA) with detection effected by spraying with Dragendorff reagent. For column chromatography, silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd, Qingdao, China) and Sephadex LH-20 were used. Solvents used were of analytical grade and purchased from Guangzhou Chemical Company, Guangzhou, China.

3.2 Plant material

The leaves of *M. paniculata* L. were purchased from Guangzhou Qingping Professional Market for Traditional Chinese Medicine, Guangdong, China, in August 2006, and identified by Prof. Fuwu Xing, South China Botanical Garden, Chinese Academy of Sciences, China. An authenticated voucher specimen (no. 677869) has been deposited at the Laboratory of Phytochemistry, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China.

3.3 Extraction and isolation

The powdered, air-dried leaves of *M. paniculata* (19.5 kg) were extracted with 2% HCl, twice each for 24 h at room temperature. The filtrate was alkalized to pH 10 with aqueous NH_3 and then extracted with $CHCl_3$. The $CHCl_3$ layer, after evaporation, yielded a light brown solid (5.5 g). This solid was subjected to silica gel column chromatography, and eluted with $CHCl_3$–MeOH mixtures of increasing polarities (10:0–8:2) to obtain fractions 1–6. Fraction 2 (0.8 g), obtained on elution with $CHCl_3$–MeOH (9:1), was rechromatographed on an RP-18 column eluted with MeOH–H_2O (6:4), followed by purification on a Sephadex LH-20 column with MeOH as eluent, to afford compound **1** (45 mg, 0.0024% yield). Fraction 4 (0.5 g), obtained on elution with $CHCl_3$–MeOH (9:1), was rechromatographed on an RP-18 column eluted with MeOH–H_2O (6:4), followed by purification on a Sephadex LH-20 column with MeOH as eluent, to afford compound **2** (30 mg, 0.0016% yield).

3.3.1 Microcosamine A (*1*)

Colorless needles (MeOH), m.p. 108–110°C (MeOH). $[\alpha]_D^{20} + 4$ (*c* 1.0, MeOH). UV (MeOH) λ_{max} (nm) (log ε): 270 (3.94). IR

(KBr) ν_{max} (cm^{-1}): 3399, 3268, 3010, 2960, 1718, 1635, 1457. For ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1. ESI-MS *m/z*: 499 [2 M + H]$^+$, 250 [M + H]$^+$. HR-ESI-MS *m/z*: 250.2162 [M + H]$^+$ (calcd for $C_{16}H_{28}NO$, 250.2165).

3.3.2 *Microcosamine B (**2**)*

White amorphous power, m.p. 95–98°C. $[\alpha]_D^{20}$ + 5 (*c* 1.1, MeOH). UV (MeOH) λ_{max} (nm) (log ε): 270 (4.15), 279 (4.19). IR (KBr) ν_{max} (cm^{-1}): 3382, 3268, 3014, 2964, 1724, 1635, 1455. For ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1. ESI-MS *m/z*: 266 [M + H]$^+$. HR-ESI-MS *m/z*: 266.2114 [M + H]$^+$ (calcd for $C_{16}H_{28}NO_2$, 266.2115).

3.4 *Larvicidal assay*

A laboratory colony of *C. quinquefasciatus* was used for the larvicidal activity. The larvae were reared in dechlorinated water, fed with yeast tablets, and maintained at 26 ± 1°C and 75% RH, under 12L:12D photoperiod cycles. Larvicidal activity against *C. quinquefasciatus* was assessed by the previously described method [6] with minor modification. Briefly, 40 larvae of early fourth instar were released in a 100 ml glass beaker containing 35 ml of dechlorinated water and 0.5 ml of the desired concentrations of compounds in acetone. Triplicates for each concentration were run at a time. The control solution was set up with 0.5 ml of acetone in 35 ml of dechlorinated water. Mortality counts were made using Abbott's formula after 24 h of the treatment, and the LC_{50}, regression equation and the 95% confidence limit of upper confidence limit and lower confidence limit, and Chi-square values were calculated using probit analysis. The average mortality of three replications at each concentration and control was calculated, and LC_{50}, which was defined as the concentration causing 50% mortality, was determined. The LC_{50} values for compounds **1** and **2** were determined to be 5.2 and 17.0 μg/ml, respectively. Chi-square values were significant at $P < 0.05$ level.

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