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Insecticidal piperidine alkaloid from *Microcos paniculata* stem bark

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Abstract

The stem bark of *Microcos paniculata* contained a new alkaloid, *N*-Methyl-6β-(deca-l′,3′,5′-trienyl)-3β-methoxy-2β-methylpiperidine, which showed good insecticidal activity against *Aedes aegypti* second instar larvae.

Keywords: *Microcos paniculata*; Tiliaceae; Stem bark; Alkaloids; *N*-methyl-6β-(deca-l′,3′,5′-trienyl)-3β-methoxy-2β-methylpiperidine; Insecticidal; *Aedes*

1. Introduction

Microcos paniculata L. (Tiliaceae) is a shrub that is abundant in secondary forests and also grown as hedges (Dassanayake and Fosberg, 1991). The traditional use of its leaves in the control of headlice prompted our study on its insecticidal activity. The only reported work on the genus is the isolation of a piperidine alkaloid, *N*-methyl-6α-(deca-l′,3′,5′-trienyl)-3β-methoxy-2α-hydroxymethyl-piperidine (micropine) from *M. philippinensis* (Aguinaldo and Reed, 1990).

We now report the isolation of an insecticidal alkaloid *N*-methyl-6β-(deca-l′,3′,5′-trienyl)-3β-methoxy-2β-methylpiperidine (**1**) from *M. paniculata* stem bark.

2. Results and discussion

Both the dichloromethane and methanol extracts of *M. paniculata* stem bark showed moribund/toxic and growth-inhibitory effects on the second instar larvae of the mosquito *Aedes aegypti*. Moribund larvae appeared to be dead, but made occasional zig-zag movements when exposed to a light source. They did not develop into the next instar and their survival time depended on the concentration of extract in the medium. Larvae in media containing the extract in low concentrations (< 20 ppm) remained moribund for up to five or six days and then died. The bodies of affected larvae became lean, long and transparent, suggesting that sub-lethal concentrations of the extract have effects on growth and development.

The MC_{50} (concentration at which 50% of larvae were moribund) of the dichloromethane and methanol extracts of stem bark against the second instar were found to be 17.5 and 16.0 ppm and the LC_{50} of the extracts at 24 HAT (hours after treatment) to be 47.5 and 47.0 ppm, respectively, indicating that both extracts showed comparable activity. The constituent responsible for the larvicidal activity was identified by bioassay-guided fractionation of a mixture of the extracts. The combined extract was subjected to repeated vacuum liquid, flash and preparative thin

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layer chromatography with fractions at each stage being monitored for mosquito larvicidal activity. Fractionation yielded the active compound (**1**) with an MC_{50} of 1.0 ppm and LC_{50} of 2.1 ppm at 24 h against *A. aegypti* second instar larvae. The MC_{50} and LC_{50} of compound (**1**) at 24, 48 and 72 h indicated that very few of the moribund larvae survived, most dying about 72 h after treatment.

Compound (**1**) absorbed in UV with a λ_{max}. 268 nm and in IR with ν_{max}, 3015 and 1640 cm^{-1}, indicating that it was unsaturated. Its mass spectrum showed a molecular ion at m/z 277 suggesting that it contained nitrogen and was probably an alkaloid. Accurate mass determination by HRCIMS of the M + 1 peak at m/z 278 gave the molecular formula $C_{18}H_{31}NO$.

The ^{1}H-NMR spectrum showed the presence of four methyl signals, namely, a triplet at δ 0.89, a doublet at δ 1.21 and two singlets at δ 2.15 and 3.33. These respectively suggested the presence of CH_3CH_2, CH_3CH, OCH_3 and NCH_3 groups. Six protons appeared in the δ 5.70 – 6.15 region in ^{1}H-NMR, and the ^{13}C-DEPT spectra showed six CH carbons at δ 130.6, 130.7, 131.1, 132.5, 135.6 and 137.8 indicated that three double bonds were present and that the compound was monocyclic.

A two-proton double triplet at δ 2.09 (J = 7.1 and 7.0 Hz) was shown by COSY experiments to be coupled to the olefinic signal at δ 5.75. These experiments also showed that the methyl triplet at δ 0.89 was correlated with a multiplet at $\sim\delta$ 1.34 which was in turn correlated with the signal at $\sim\delta$ 1.36. The signal at δ 2.09 was found to be coupled not only to the olefinic signal at δ 5.75 but also to this signal at $\sim\delta$ 1.36. The olefinic signals were correlated with each other, suggesting that they were part of a long-chain substituent consisting of a butanyl group attached to three conjugated double bonds. A similar side chain was reported to be present in micropine (**2**),which was isolated from *M. philippinensis* (Aguinaldo and Reed, 1990). The ^{1}H- and ^{13}C-NMR side chain signals of alkaloids (**1** and **2**) were sufficiently similar to suggest that they were the same.

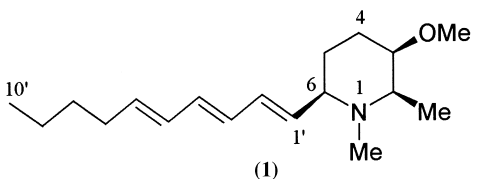
The molecular formula of $C_{18}H_{31}NO$ suggested that alkaloid (**1**) may contain a piperidine ring as in micropine. The piperidine nitrogen must be methylated and the ring must carry an OCH_3 substituent as methyl singlets were observed at δ 2.15 and 3.33 in the ^{1}H NMR spectrum of the alkaloid. Molecular formula considerations suggested that the remaining carbon should be a methyl substituent on the piperidine ring. The proposed structure then agrees with the molecular formula and only the positions of CH_3, OCH_3 and the side chain in the piperidine ring need to be assigned.

HMQC showed that the downfield ^{13}C-NMR signals due to saturated carbon atoms at δ 79.6, 57.3, 68.8 and 62.6 were correlated with the 1 Hz NMR signals at δ 3.14, 3.33, 2.47 and 2.10, respectively, and the carbons at δ 40.9 and 18.4 with the methyl singlet at δ 2.15 and the methyl doublet at δ 1.21. The signal at δ 79.6 must be due to the carbon carrying the OMe group, those at δ 68.8 and 62.6 due to the ring carbon atoms attached to nitrogen and those at δ 57.3, 40.9 and 18.4, respectively, due to OMe, *N*Me and the ring Me carbons. The ^{13}C-NMR signal at δ 26.6 showed HMQC correlations with part of the 1 Hz NMR multiplet at δ 1.30 – 1.45 and the doublet at δ 2.05, while that at δ 28.3 with part of the same multiplet and the multiplet at δ 1.81. These two carbon signals must be due to the remaining methylene carbon atoms of the ring.

HMBC showed that the ^{1}H-NMR signal at δ 6.06 – 6.15 and that at δ 1.81 were correlated with the signal at δ 68.2, indicating that the side chain appeared on a carbon atom attached to nitrogen and to the methylene carbon showing the ^{13}C-NMR signal at δ 28.3. Since the signal at δ 1.81 was also correlated with the carbon atoms showing ^{13}C-NMR signals at δ 26.6 and 79.6, this methylene group was attached either to the second methylene group or the carbon carrying the methoxy substituent. The COSY spectrum suggested that the former was the case.

The COSY spectrum also showed correlations between the methyl signal at δ 1.21 and the multiplet at δ 2.10, which in turn was correlated with the broad singlet at δ 3.14, indicating that the carbon atoms with the methyl and methoxy substituents were next to each other. Since the multiplet at δ 2.10 is due to the protons of a carbon attached to nitrogen, the CH_3 and OCH_3 groups are therefore, respectively, at C-2 and C-3 and the side chain at C-6 of the piperidine ring, with C-4 and C-5 remaining unsubstituted. The HMQC, HMBC and COSY spectra are in agreement with the proposed structure.

The protons of the 2-methyl group showed NOESY correlations with 2-H, 3-H and the OCH_3 protons, but not with the 6-H. The 2-, 3- and 6-protons were also correlated in the NOESY spectrum indicating that they were *cis* to each other. All three substituents on the piperidine ring must also therefore be *cis* to each other and the mosquito larvicidal constituent of *M. paniculata* stem bark should therefore be *N*-methyl-6β-(deca-1′,3′,5′-trienyl)-3β-methoxy-2β-methylpiperidine (**1**) or the identical *N*-methyl-6α-(deca-1′,3′,5′-trienyl)-3α-methoxy-2α-methylpiperidine. The stereochemistry of piperidine (**1**) thus differs from that of the previously isolated piperidine from *Microcos* species, micropine at 3-position.



(**1**)

3. Experimental

3.1. General

UV spectra were recorded in EtOH solution and IR for KBr discs; NMR spectra were determined in $CDCl_3$ at 500 MHz with $SiMe_4$ as internal standard; optical rotations $CHCl_3$, 22°; Chromatographic separations were achieved using silica gel $PF_{254+366}$ (PLC) and Merck 9385 Kieselgel 60 for vacuum liquid (VLC), medium pressure (MPLC) and flash (FC) chromatography.

3.2. Plant material

Microcos paniculata stem bark was collected from Rajakadaluwa in the Chilaw district of Sri Lanka and identified by Dr. Magdon Jayasuriya of the National Herbarium, Royal Botanic Gardens, Peradeniya where a voucher specimen has been deposited.

3.3. Screening

Extracts, fractions and compounds were screened for activity against the second instar larvae of *Aedes aegypti*. A piece of filter paper with mosquito eggs was placed in a beaker of water kept at 27°C for 24 h. After 48 h, the second instar larvae was available for testing.

Initially, solutions of concentration 500 ppm for plant extracts, 50 ppm for fractions and 20 ppm for pure compounds were prepared by dissolving the appropriate weight in acetone or water (~1 ml). Polyethyleneglycol in isopropanol (1:1, 24 μl/4 mg) was added and the solution made up to 200 ml with water. Ten second instar larvae were introduced into 25 ml of this solution in a beaker for each test and each concentration was replicated four times. Similar solutions with acetone or water and polyethyleneglycol solution were used as controls. Active extracts, fractions and compounds were tested at lower concentrations using serial dilutions of the above solutions. The number of larvae moribund or dead was recorded at 24 h intervals until death or pupation. Larvae which did not show zig-zag movement when probed were considered as moribund and moribund larvae which did not show movements when exposed to a tungsten bulb (60 W) were considered dead. The median 50% moribund (MC_{50}) and lethal (LC_{50}) concentrations were determined using probit analysis (MSTAT package).

3.4. Extraction

The dried, ground stem bark (3.75 kg) was successively extracted with CH_2Cl_2 and MeOH at ambient temperature for two 24 h periods each. Concentration of CH_2Cl_2 and MeOH solutions gave 31.0 and 75.0 g of solids, respectively.

3.5. Bioassay of extracts

The CH_2Cl_2 and MeOH extracts, respectively, showed MC_{50} of 17.5 and 16.0 ppm and LC_{50} of 47.5 and 47.0 ppm 24 HAT. The MC_{50} of both extracts remained almost unchanged (±1 ppm) when measured at 24 h intervals for 120 HAT. The LC_{50} of the CH_2Cl_2 extract decreased to 40.2, 34.5 and 21.5 ppm at 48, 72 and 120 HAT, while that of the MeOH extract decreased similarly to 32.9, 20.5 and 17.1 ppm. Since both extracts showed similar activity, the extracts were combined and subjected to bioassay-guided fractionation in order to isolate the active compound.

3.6. Bioassay guided fractionation

The extract (85 g) was fractionated by VLC on silica gel (275 g) using hexane and then hexane–CH_2Cl_2 (95:5) through CH_2Cl_2 to CH_2Cl_2–MeOH (9:1) (1 l each) as eluants to give 11 fractions. Larvicidal screening showed that the ninth and tenth most polar fractions [CH_2Cl_2–MeOH (19:1 to 9:1)], respectively, caused 35% and 5% mortality at 20 ppm 48 HAT. At this concentration, both fractions caused 100% moribundity 24 HAT and no recovery was seen 48 HAT. VLC was repeated twice and the combined active fraction (5.0 g) subjected to MPLC on silica gel (50 g) using CH_2Cl_2 and CH_2Cl_2–MeOH mixtures (99.5:0.5 to 9:1). The eluates were combined after TLC analysis into 11 fractions which were screened again. Fractions 5–7 [CH_2Cl_2–MeOH (98:2 to 19:1)] showed moribundity of 90–100% 24 HAT and 100% at 48 to 120 h and 100% mortality after 7 days.

FC of the combined fractions on silica gel (12 g) with EtOAc–MeOH (96:4) gave seven fractions. Fractions 6 and 7 showed 100% mortality 24 HAT at 10 ppm and were combined (107 mg). PLC with EtOAc–MeOH (9:1) twice gave three separable bands. Of these, the least polar [8.5 mg, R_f 0.135 with EtOAc–MeOH (9:1)] was larvicidal with MC_{50} of 1.03, 1.21 and 1.21 ppm and LC_{50} of 2.13, 1.15 and 1.00 ppm, respectively, at 24, 48 and 72 HAT. *N*-methyl-6β-(deca-1′,3′,5′-trienyl)-3β-methoxy-2β-methyl-piperidine (**1**), recrystallised from CH_2Cl_2, mp 52–53°C $[\alpha]_D^{22}$ + 29.2°; HRCIMS 278.2481 ($M^+ + H$); Calc. for $C_{18}H_{32}NO$: 278.2484; UV: λ_{max}(nm) 268; IR. ν_{max} (cm^{-1}): 3015 and 1640; ^{1}H-NMR: δ 0.89 (3H, *t*, J = 7.0 Hz, H-10′), 1.21 (*d*, J = 7.1 Hz, 2eq-CH_3), 1.25–1.45 (6H, *m*, H-4ax, H-5ax, H-8′ and H-9′), 1.81 (1H, *ddd*, J = 9.7, 3.2 and 3.2 Hz, H-5eq), 2.05 (1H, *m*, H-4eq), 2.09 (2H, *dt*, J = 7.1 and 7.0 Hz, H-7′), 2.10 (1H, *dq*, J = 9.1 and 7.1 Hz, H-2ax), 2.15 (3H, *s*, *N*-CH_3), 2.47 (1H, *ddd*, J = 11.4, 8.4 and 3.2 Hz, H-6ax), 3.14 (1H, *ddd*,

J = 10.7, 9.1 and 4.8 Hz, H-3ax), 3.33 (3H, s, O-CH_3), 5.70 (1H, *dt*, J = 15.5 and 7.2 Hz, H-1), 5.75 (1H, *dd*, J = 15.8 and 8.4 Hz, H-6′), 6.10–6.15 (4H, *m*, H-2′–5′); ^{13}C-NMR: δ 14.3 (C-10′), 18.4 (CH_3), 22.6 (C-9′), 26.6 (C-4), 28.3 (C-5), 31.8 (C-7′), 32.9 (C-8′), 40.9 (*N*-CH_3), 57.3 (O-CH_3), 62.6 (C-2), 68.8 (C-6), 79.6 (C-3), 130.6, 130.7, 131.1 and 132.5 (C-2′–5′), 135.6 (C-6′) and 137.8 (C-1′); MS m/z (rel. int., %), 277 [M^+] (82), 262 (50), 248 (16), 246 (24), 234 (44), 220 (42), 206 (50), 162 (44), 105 (76), 91 (94), 79 (54), 62 (46), 58 (70), 55 (56), 44 (86) and 41 (100).

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