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Spermine alkaloids from *Albizia adinocephala* with activity against *Plasmodium falciparum* plasmepsin II

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Abstract

Crude MeOH extracts from the stem bark and leaves of a Panamanian specimen of *Albizia adinocephala* (Leguminosae) were found to inhibit the malarial enzyme plasmepsin II. Bioassay guided fractionation led to the isolation of two new bioactive spermine alkaloids, budmunchiamines L4 and L5. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Albizia adinocephala*; Leguminosae; Stem bark; Leaves; Spermine alkaloid; Budmunchiamine; Plasmepsin II; Malaria

1. Introduction

Nearly 40% of the world's population live in areas at risk from malaria, with approximately 300–500 million new infections each year. It is estimated that malaria infections result in 0.5–2.5 million deaths each year, with the majority of victims being children under the age of five. The recent emergence of (multi)-drug resistant malaria parasites and insecticide resistant mosquitoes has triggered an alarming increase in the incidence and spread of the disease. No effective vaccine is available and few new anti-malarial drugs are undergoing clinical trials. Therefore, there is an urgent need for new therapies based on novel mechanisms of action (Berry, 2000; Olliaro and Yuthavong, 1999).

Plasmepsins are aspartyl proteases that appear to be indispensable for parasite survival and have a profound effect on parasite multiplication in vitro (Semenov et al., 1998). Plasmepsin II is one particular enzyme that has been well characterised, with sufficient quantities of expressed enzyme available for assay development and high throughput screening. These reasons made plasmepsin II an attractive molecular target for our natural product extract library.

MeOH extracts from the stem bark and leaves of *Albizia adinocephala* (J.D.Sm.) Britt. & Rose ex Record (Leguminosae) collected from Panama were found to inhibit plasmepsin II. Bioassay guided fractionation of the stem bark extract led to the isolation of two new macrocyclic spermine alkaloids, budmunchiamines L4 (**1**) and L5 (**2**). The leaves also contained budmunchiamine L5 (**2**). Their structure elucidation and bioactivity against plasmepsin II are outlined below.

2. Results and discussion

Budmunchiamine L4 (**1**) was isolated as a colourless oil that gave an $[M+H]^+$ ion peak of m/z 439.4357 [Δmmu 1.9] in the (+)-ESIMS consistent with the molecular formula $C_{26}H_{54}N_4O$ and corresponding to two double bond equivalents. Analysis of the NMR data for **1** showed resonances consistent with an amide moiety [^{13}C: δ 173.5 ppm; ^{1}H: δ 8.61 (*s*)], an observation further supported by characteristic IR absorptions for an amide at 3400 and 1640 cm^{-1}. Further analysis of the ^{1}H NMR data for **1** showed a triplet at δ 0.87 and a broad singlet at δ 1.28, characteristic of a straight chain alkane. With no sp or sp^2 carbons present in the NMR data it was evident that **1** was a monocyclic macrocycle. Also evident in the NMR data for **1** were resonances

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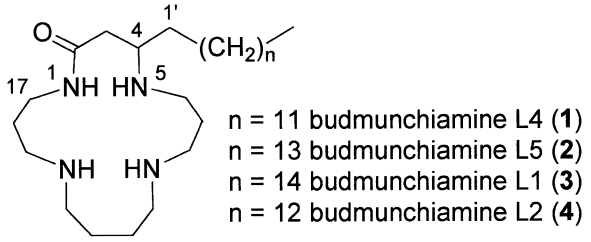

consistent with five amino methylenes [^{13}C: δ 46.8, 47.5, 48.0, 48.3, 49.4; ^{1}H: δ 2.60–3.04 (10H, *m*)] and an amino methine [^{13}C: δ 56.5; ^{1}H: δ 3.09 (1H, *m*)], an amide methylene [^{13}C: δ 37.9; ^{1}H: δ 3.35 (1H, *m*), 3.43 (1H, *m*)] and a keto methylene group [^{13}C: δ 39.0; ^{1}H: δ 2.52 (1H, *dd*, J = 15.5, 7.8 Hz), 2.72 (1H, *br d*, J = 15.5 Hz)]. These data suggested that **1** was a spermine type alkaloid. Comparison of the NMR data for **1** with the known spermine alkaloids budmunchiamines L1 (**3**) and L2 (**4**) (Misra et al., 1995) indicated that only difference between **1**, **3** and **4** was the length of the saturated alkane side chain. Hence the structure of **1** is as shown. Although bundmunchiamine L4 (**1**) has been previously reported as a synthetic intermediate in the preparation of budmunchiamine C (Popaj and Hesse, 2001), this is the first report of **1** as a natural product.

Budmunchiamine L5 (**2**) was isolated as a colourless oil that gave a molecular formula in the (+)-ESIMS ($C_{28}H_{58}N_4O$) 28 amu higher than that for **1**. Comparison of the NMR data for **2** with **1** indicated that both compounds were almost identical. This suggested the only difference between **2** and **1** was **2** had a saturated alkane side chain that was two methylene units longer than **1**.

Spermine alkaloids have been found to possess a variety of biological activities (Guggisberg and Hesse, 1983, 1998). Alkaloids related to **1** and **2**, which contain a spermine unit cyclized to an 3-amino substituted fatty chain, have been previously isolated from *Pithecolobium saman* (syn. *Samania saman*) (Wiesner et al., 1952, 1968a, b) and specimens of *Albizia* sp. collected from both India (Pezzuto et al., 1991; Mar et al., 1991; Pezzuto et al., 1992; Misra et al., 1995) and Kenya (Rukunga and Waterman, 1996a, b). *A. gummifera* is used in traditional Kenyan medicine as a treatment for many aliments, including malaria (Rukunga and Waterman, 1996a, b). Budmunchiamines L4 (**1**) and L5 (**2**) have mild activity against plasmepsin II, with IC_{50} values of 14 and 15 μM, respectively.

3. Experimental

3.1. General

Optical rotations were recorded on a Jasco DIP-1000 Digital Polarimeter. IR spectra were recorded on a Perkin Elmer BioRad FT-IR spectrophotometer. HPLC was performed on Waters systems complete with Waters Millenium^{32} software, Waters 996 PDA detector, Waters 600 gradient controller and pump, Waters 717plus autosampler and Waters Fraction Collector II fraction collector. One- and two-dimensional NMR experiments were obtained on a Bruker Avance DRX 500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C and spectra referenced to residual protons in the deuterated solvent. High resolution mass spectral data were recorded on a Perspective Biosystems Mariner Biospectrometrey TOF mass spectrometer.

3.2. Plant material

The stem bark and leaves of *A. adinocephala* (J.D. Sm.) Britt. & Rose ex Record were collected from I. Amador, Panama in May 1995. A voucher specimen (F2260) was lodged with the Universidad De Panama Herbarium.

3.3. Extraction and isolation

Ground and dried stem bark (23 g) was extracted with MeOH (5 l) and concentrated in vacuo. The crude extract (1.1 g) was partitioned using modified Kupchan conditions (Pettit et al., 1985). The activity was localised in the MeOH/H_2O fraction (730 mg) which was subjected to reversed phase preparative HPLC (16 ml/min, gradient elution from 42 to 90% CH_3CN in H_2O with 0.1% formic acid over 12 min; 5 μm Merck Hunter C18 100 mm×21.2 mm column) to generate one active fraction (173 mg). Further separation with Sephadex LH-20 gel using 100% MeOH as the eluant and then reversed phase semi-preprative HPLC (4 ml/min, gradient elution from 30 to 80% CH_3CN in H_2O over 15 min; 5 μm Thermoquest C18 150 mm×10 mm column) yielded budmunchiamines L4 (**1**) (21 mg) and L5 (**2**) (5.7 mg). A similar bioactivity guided isolation from the leaves led to the isolation of budmunchiamine L5 (**2**).

3.3.1. Budmunchiamine L4 (*1*)

Colourless oil. $[\alpha]_D = -13^\circ$ (*c* 0.65, MeOH); IR (film) ν_{max} 3400, 2920, 2850, 2780, 1640 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$); δ 0.87 (3H, *t*, J = 6.8 Hz, H-13′), 1.25 (24H, *m*, H-1′ to H-12′), 1.50–2.00 (8H, *m*, H_2-7, H_2-11, H_2-12, H_2-16), 2.52 (1H, *dd*, J = 15.5, 7.8 Hz, H-3$_a$), 2.72 (1H, *br d*, J = 15.5 Hz, H-3$_b$), 2.60–3.04 (10H, *m*, H_2-6, H_2-8, H_2-10, H_2-13, H_2-15), 3.09 (1H, *m*, H-4), 3.35 (1H, *m*, H-17$_a$), 3.43 (1H, *m*, H-17$_b$), 8.61 (1H, *s*, 1-NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1 (*q*, C-13′), 22.7 (t, C-12′), 24.5, 25.1, 25.2, 25.9, 27.0, 29.3 to 29.7, (13 t, C-7, C-11, C-12, C-16, C-2′ to C-10′), 31.8, 31.9 (2 t, C-1′, C-11′), 36.9 (*t*, C-17), 38.0 (*t*, C-3), 45.8 (*t*, C-6), 46.5, 47.0, 47.3, 48.4, (4 *t*, C-8, C-10, C-13, C-15), 55.5 (*d*, C-4), 172.5 (*s*, C-2); HRESIMS $(M+H)^+$ m/z 439.4357 (calculated for $C_{26}H_{55}N_4O$ 439.4376).

3.3.2. Budmunchiamine L5 (2)

Colourless oil. $[\alpha]_D = -20^\circ$ (*c* 0.21, MeOH); IR (film) ν_{max} 3410, 3020, 2930, 5860, 1660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, *t*, $J = 6.7$ Hz, H-15′), 1.28 (28H, *m* H-1′ to H-14′), 1.44–2.10 (8H, *m*, H_2-7, H_2-11, H_2-12, H_2-16), 2.45–3.60 (10H, *m*, H_2-6, H_2-8, H_2-10, H_2-13, H_2-15), 3.09 (1H, *m*, H-4), 3.43 (4H, m, H_2-3, H_2-17), 8.03 (1H, *s*, 1-NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1 (*q*, C-15′), 22.7 (*t*, C-14′), 24.4, 24.5, 25.5, 25.7, 26.2, 29.3 to 29.7, (15 *t*, C-7, C-11, C-12, C-16, C-2′ to C-12′), 31.9 (2 *t*, C-1′, C-13′), 38.1 (*t*, C-3), 38.4 (*t*, C-17), 41.1, 43.8, 48.8, 48.9, 49.1, (5 *t*, C-6, C-8, C-10, C-13, C-15), 56.5 (*d*, C-4), 162.7 (*s*, C-2); HRESIMS $(M + H)^+$ *m*/*z* 467.4587 (calculated for $C_{28}H_{59}N_4O$ 467.4689).

3.4. Plasmepsin II assay

The activity of plasmepsin II was measured as the cleavage of FRET substrate (Dabcyl-gERJFLSFPD-Edans, where *g* is γ-aminobutyric acid and *J* is L-norleucine). Proplasmepsin II was autoactivated by incubation in 0.1 M citrate pH 4.5, 0.1% Tween-20 and 50 mM DTT at room temperature for 40 min. The assay was initiated by adding activated plasmepsin II to a reaction mixture containing 50 mM sodium acetate at pH 4.7, 0.1% Tween-20, 10 μM substrate peptide and inhibitors at various concentrations. After a 60 min incubation at room temperature, substrate peptide cleavage was measure in a Tecan Ultra plate reader using 360 nm excitation and 535 nm emission fluorescence filters. Pepstatin A was used as a standard inhibitor.

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