REPORT

Haplophytin B from *Maclurodendron porteri*

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Abstract: An alkaloid from *Maclurodendron porteri* has been isolated and characterized. Extraction process was conducted by acid-base extraction method followed by column chromatography. The structure was established by nuclear magnetic resonance spectroscopy and mass spectrometry. The compound was identified as haplophytin B which occurs commonly in the Rutaceae family. However, this is the first time this alkaloid was isolated and reported from the species. The compound showed no inhibition against *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli* and no cytotoxic activity against H199 and A549 cell lines.

Keywords: *Maclurodendron porteri*, Rutaceae, furanoquinoline alkaloid, haplophytin B.

INTRODUCTION

Maclurodendron porteri (Hook.f) T.G. Hartley belongs to the Rutaceae family. *M. porteri* is locally known as *merlimau* that can reach 20 m tall and grows throughout lowland to lower montane forest (Hanum *et al*., 2001). Previous phytochemical study by Bowen and Osborne (1998) resulted in the isolation of two furanoquinoline alkaloids, skimmianine and kokusaginin from the leaves of *M. porteri*. The present study described the isolation of a furanoquinoline alkaloid, haplophytin B from *M. porteri*. Haplophytin B (syn. haploperin, evoxin) is an isomer of haplotin (Ali *et al*., 2008) which was previously isolated from *Haplophyllum acutifolium* (Ali *et al*., 2001; Eastwood *et al*., 1954; Waffo *et al*., 2007). Previous research reported that haplophytin B showed weak antioxidant activity (Kiplimo *et al*., 2011) and haplophytin A induced apoptosis effect on HL-60 cells (Won *et al*., 2010). Investigation of antimicrobial and cytotoxic activities of haplophytin B revealed no activity against selected Gram-positive and Gram-negative microbes as well as against H1299 and A549 cell lines. This paper reports isolation of haplophytin B from *M. porteri* for the first time.

MATERIALS AND METHODS

General Procedures

The melting point was measured on a Buchi B545 melting point apparatus and uncorrected. The ^{13}C-NMR and ^{1}H-NMR were recorded on a Bruker 400 MHz in CD_3OD. Chemical shifts are reported in ppm and d scale and the coupling constants are given in Hz. The ultraviolet (UV) spectrum was recorded using a Secomam Uvi light XT2 spectrophotometer in methanol. The infrared (IR) spectrum was obtained from a Perkin Elmer infrared spectrophotometer. Silica gel (70-230 and 230-400 mesh, Merck) was used for column chromatography.

Plant materials

Maclurodendron porteri was collected from Pandan River waterfalls, Kuantan in April 2011. The plant was identified by Dr. Shamsul Khamis of Universiti Putra Malaysia. The voucher specimen (MT-11-07) was deposited at Herbarium of Faculty of Pharmacy, International Islamic University Malaysia.

Cell Lines and Microorganisms

H1299 and A549 cells were courtesy from Prof. Masa-Aki Ikeda of Tokyo Medical and Dental University. Two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus anthracis* ATCC 14578) and two Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218) were used for antibacterial test.

Cytotoxicity Assay

Cytotoxicity assay against H1299 and A549 cell lines were conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test according to Mosmann (1983). The monolayer cells were cultured in DMEM supplemented with 10% (v/v) foetal bovine serum and antibiotic (1% penicillin-streptomycin) in a 96-well plate at a density of 2 x 10^4 cells/mL. After reaching

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confluence ($2x10^5$ cell/mL), then the cells were treated with the samples. Compound 1 was dissolved in DMSO (1 mg/mL). The cells were treated in triplicates with serial dilution of sample (0.01-1 mg/mL) for 24 hr. Doxorubicin was used as a commercialized anticancer standard. To reduce the interference of residue of supernatant, the monolayer cell was washed two times with phosphate buffer saline (PBS), the 20mL of MTT stock solution (5 mg/mL) was added and the plates were further incubated overnight at 37°C. DMSO (100mL) was added to each well to dissolve the purple formazan crystal. After 1 h, the solubilized formazan was measured at 570 and 630 nm (reference) with a UV microplate reader. The half reduction in cell number relative to control or IC_{50} was established by extrapolation from linear regression of experimental data.

Antimicrobial Assay

The agar disc diffusion technique was used to determine the antimicrobial activity by following a published method (Qaralleh *et al*., 2009) for the determination of antimicrobial activity. An inoculum with concentration of 10^6-10^7 CFU/mL was spread on Mueller-Hinton agar plates. By using a forceps, the sterile filter papers (6 mm diameter) containing compound **1** (20/mg), standard antibiotics (30/mg of chloramphenicol) and the negative control (DMSO) were put on the surface of inoculated agar plate. The plates were incubated for 24 h at 37°C. The sample was tested in triplicate and the diameter zone of inhibition was measured in millimeters.

Extraction and Isolation

The dried and powdered leaves of *M. porteri* (1.18/kg) was extracted with MeOH (5/L, three times for 3 days each) at room temperature. The extract was evaporated to give 200/mL gummy extract and then mixed with 5% tartaric acid (1/L). The acidified solution was left overnight and the non alkaloidal materials were separated. The solution was extracted with ethyl acetate to give alkaloid extract (crude B). The crude B alkaloid (0.5/g) was purified via column chromatography (230-400 mesh, 50g) and eluted with hexane: EtOAc (2:8); EtOAc; EtOAc: MeOH (9:1, 8:2→1:9) sequentially to afford 39 tubes (5/mL). Fractions 33-39 were combined and recrystalized with hot MeOH to give compound 1 (148 mg).

RESULTS

***Haplophytin B* (Fig. 1)**

White powder

MP: 140-141ºC.

$[\alpha]_D$: +78.1 (*c* 0.032, MeOH).

Rf: 0.4 (EtOAc).

IR (KBr): 3467, 2978, 1626, 1511, 1093, 1064, 725 cm^{-1}.

UV/Vis λ_{max} (MeOH) nm : 231, 249, 319.

^1H NMR (400 MHz, CD_3OD): 8.04 (1H, d, *J*=9.4 Hz, H-5), 7.78 (1H, d, *J*=2.8 Hz, H-2), 7.39 (1H, d, *J*=9.4 Hz, H-6), 7.34 (1H, d, *J*=2.8, H-3), 4.51 (1H, s, H1'-b, overlapped partially), 4.50 (3H, s, OCH_3-4, overlapped partially), 4.17 (1H, dd, *J*= 9.8, 8.2, H1'-a), 4.03 (3H, s, OCH_3-8), 3.85 (1H, dd, *J*=8.0, 2.8, H-2'), 1.32 (3H, s, H-4'), 1.26 (3H, s, H-5')

^{13}C NMR (100 MHz, CD_3OD): 164.6 (C, C-9a), 157.8 (C, C-4), 151.9 (C, C-7), 143.8 (CH, C-2), 141.5 (C, C-8a), 140.6 (C, C-8), 118.2 (CH, C-5), 114.6 (C, C-4a),113.3 (CH, C-6), 104.9 (CH, C-3), 102.0 (C, C-3a), 76.4 (CH, C-2'), 71.4 (C, C-3'), 71.1 (CH_2, C-1'), 60.5 (CH_3, OCH_3-4), 58.7 (CH_3, OCH_3-8), 25.5 (CH_3, C-4'), 23.5 (CH_3, C-5').

ESI-MS: m/z 347 $[M]^+$, 288, 244, 227 $[M\text{-side chain}]^+$ (100%) (calc. for $C_{18}H_{21}NO_6$, 347.1363)

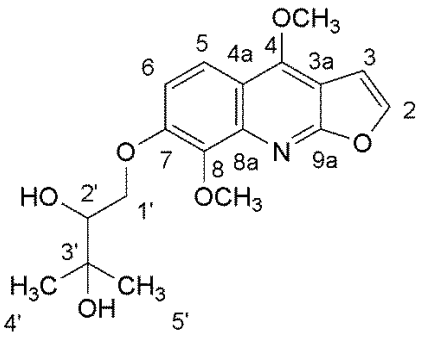

Fig. 1: Compound **1** (haplophytin B)

DISCUSSION

The lemon-like smell of fresh leaves of *M. porteri* was dried, powdered and extracted with MeOH. The MeOH extract was concentrated in *vacuo* and subjected to 5 % tartaric acid solution for a total of 1/L. The mixture was settled down overnight and decanted to remove acid insoluble materials. Subsequent fractionation with extraction process yielded crude alkaloid B (0.5/g). The crude alkaloid B was subjected to silica gel (230-400 mesh) column chromatography (50/g) and eluted with mixture of hexane: EtOAc (2:8); EtOAc; EtOAc: MeOH (9:1, 8:2→1:9) to afford 39 fractions. The eluents collected were recrystalized with hot MeOH to give compound **1** (148 mg).

Compound **1** (fig. 1) was obtained as a white powder which gave positive reaction with Dragendorff reagent. It also showed positive response to UV light (366 nm) indicating the presence of conjugated system in the molecule which was supported by its UV spectrum showing the bands at 249 and 319 nm. The presence of NH/OH group was indicated by a broad band at 3467 cm^{-1}. C=N stretching and vinyl stretching were observed at 1093 cm^{-1} and 1626 cm^{-1}, respectively. The molecular mass was found at m/z 347 $[M]^+$ The lost of

dihydroxyprenyloxy side chain was shown by the peak at m/z 227. The formula was identified as $C_{18}H_{21}NO_6$ which consist of nine degrees of unsaturation.

Proton NMR spectrum of **1** showed two singlet signals resonated at d 1.26 and 1.32 due to two methyl groups. The signals for two methoxyl groups appeared at d 4.03 and 4.50. The methylene group appeared as two separate signals at d 4.17 (dd, *J*= 9.8, 8.2, H1'-a) and d 4.51 (overlapping, H1'-b, signal pattern overlapped with a methoxyl) which were confirmed by HMQC spectrum. The methylene proton was correlated with a methine proton at d 3.85 (dd, *J*=8.0 and 2.8, H-2) which was clearly observed in COSY spectrum. The spectrum also showed a clear separated *ortho* signals downfield specifically for aromatic signals at d 8.04 (d, *J*= 9.4, H-5) and 7.39 (d, *J*= 9.4, H-6). The protons of disubstituted furan ring showed doublets at d 7.78 (*J*=2.8, H-2) and d 7.34 (*J*=2.8, H-3). The carbon NMR of 1 exhibited 18 carbons signals which consisted of four methyls, one methylene, five methines and eight quarternary carbon atoms. The HMBC spectrum indicated a cross peak for C-8 and C-4 with two methoxyl group at d 4.03 and d 4.50 allowing their assignments. The presence of dihydroxyprenyloxy was observed from carbon signals at 71.1, 76.4 and 71.4 indicated for C-1' C2' and C-3' respectively. The carbon of methylene at d 71.1 was observed as a cross peak to the methylene proton at d 4.17 and 4.51. Based on the spectral data, it was concluded that compound **1** was haplophytin B, which was previously reported from another Rutaceae family, *Haplophyllum acutifolium* (Ali *et al*., 2001).

Bioactivity investigation of compound **1** was carried out against four strains of microbes (*Staphylococus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*) and two cell lines (H1299 and A549). However, compound **1** gave no activity for the entire tests mentioned.

ACKNOWLEDGEMENT

This research was partially funded by Endowment Grant (International Islamic University Malaysia).

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