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Isoquinoline Alkaloids and Antimalarial Properties of *Popowia Perakensis* Extract

(Alkaloid Isokuinolina dan Sifat-sifat Antimalarial Ekstrak Popowia Perakensis)

Saripah Salbiah Syed Abd. Aziz[1], Mat Ropi Mukhtar[2], A. Hamid A. Hadi[2], Noor Rain Abdullah[3] , and Khalijah Awang[2]

[1] Department of Chemistry, Faculty of Science, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia.
[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[3] Herbal Medicine Research Centre, Institute for Medical Research, 50588 Jalan Pahang, Kuala Lumpur, Malaysia.

Abstract

Further investigation on the alkaloidal composition on the bark of the Malaysian *Popowia perakensis* King collected from Mersing, Johore afforded four isoquinoline alkaloids, i.e atherospermidine **1**, (-)-anonaine **2**, (-)-norstephalagine **3** and (-)-asimilobine **4**. These compounds were elucidated and identified *via* spectroscopic methods mainly ^{1}H-NMR, ^{13}C-NMR, IR, UV, GCMS and by comparison with literature data. The crude alkaloid extract was screened for antimalarial activity towards *Plasmodium falciparum in vitro* using the lactate dehyrogenase (LDH) assay to give IC_{50} value of 6.85 μg/ml.

Keywords: Isoquinoline, *Popowia perakensis*, alkaloid, antimalarial activity, *Plasmodium falciparum*.

Abstrak

Kajian lanjut ke atas kandungan alkaloid terhadap kulit pokok *Popowia perakensis* King berasal dari Malaysia yang diambil dari Mersing, Johor telah menghasilkan empat alkaloid isokuinolina iaitu aterospermidina **1**, (-)-anonaina **2**, (-)-norstephalagina **3** dan (-)-asimilobina **4**. Sebatian-sebatian ini telah ditentukan dan dikenalpasti melalui kaedah spektroskopi iaitu ^{1}H-NMR, ^{13}C-NMR, IR, UV, GCMS dan perbandingan dengan data kajian terdahulu. Ekstrak mentah telah disaring terhadap aktiviti antimalaria ke atas *Plasmodium falciparum in vitro* menggunakan laktat dehidrogenas (LDH) dan memberikan bacaan IC_{50} 6.85 μg/ml.

Kata kunci: Isokuinolina, *Popowia perakensis*, alkaloid, aktiviti antimalarial, *Plasmodium falciparum*.

Introduction

Popowia (family Annonaceae) is a genus of small trees or shrubs widely distributed in Tropical Africa, Madagascar, South India, Burma, Thailand, Indo-China, Malaysia and Australia (Sinclair, 1955; Ng, 1989). Among other *Popowia* species, *P. pisocarpa* has undergone detailed chemical studies. Previous investigation on *P. pisocarpa* an Indonesian species afforded bisbenzylisoquinolines alkaloids (Jossang et al. 1986). Meanwhile the study on alkaloids of *P.* cf. *cynocarpa* from New Guinea has revealed the presence of aporphine and bisbenzylisoquinoline alkaloids (John et al. 1970).

In previous studies we have reported the isolation of two oxoaporphine alkaloids; liriodenine and lanuginosine, together with a bisbenzylisoquinoline; (-)-*O*-methyldauricine from the bark of Malaysian *Popowia perakensis* (Saripah Salbiah et al. 2006). As part of a continuing investigation on the alkaloids content of this species, we now report the isolation and structural elucidation of four isoquinoline alkaloids from the bark of *Popowia perakensis* namely atherospermidine **1**, (-)-anonaine **2**, (-)-norstephalagine **3** and (-)-asimilobine **4** as well as bioassay data of the crude extract.

Experimental

General methods

The solvents used in this work were hexane, petroleum ether (40-60^{o}C), dichloromethane, chloroform, acetone and methanol. All solvents are from AR grade except those that are used for bulk extractions (distilled). Other chemicals were hydrochloric acid, ammonium solution and anhydrous sodium sulphate. Silica gel 60 (70-230 and 230-400 mesh) was used for column chromatography. Thin Layer Chromatography (TLC) was carried out on precoated silica Kieselgel 60, F_{254} plates. TLC spots were visualized under ultraviolet light (254 and 365 nm) and by spraying with Dragendorff's reagent. The Mayer's reagent (potassium mercuric iodide) was used to test the presence of alkaloids in the sample. A positive test for alkaloids was indicated by the production of a turbid solution or a white precipitate.

Spectroscopic methods

The ultraviolet absorption spectra were taken using a UV-VIS NIR Scanning Spectrophotometer (model Shimadzu UV-310 IPC) with methanol as a solvent. The infrared spectra were measured on a Perkin Elmer system 2000 FTIR spectrometer with chloroform as a solvent. The optical rotations were determined on Jasco (Japan) P1010 with tungsten lamp. EIMS spectra were recorded on Shimadzu GC-MS-QP2000A Mass Spectrometer 70 eV. The NMR (^{1}H, ^{13}C and 2D) spectra were obtained in deuterated chloroform on a JEOL JNM-FX100 (400 MHz).

Plant material

The bark of *Popowia perakensis* King (Annonaceae), was collected at Mersing, Johore in October 2000. The plant was identified and collected by the Phytochemical group of Chemistry Department, University of Malaya, Kuala Lumpur. A voucher specimen (KL 4962) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and isolation of the alkaloids

Dried, grounded bark of *Popowia perakensis* King (2.2 kg) was first defatted with hexane for 3 days at room temperature and then hexane extract was filtered. After being dried under room temperature for 24 hours the barks were rinsed with 10% of ammonia solution and left to soak overnight; this was to aggregate the nitrogen-containing compounds in the plant. The bark was then re-extracted with dichloromethane solvent using soxhlet extractor (17 hours). The dichloromethane extract was concentrated to about 500 mL by using the rotary evaporator.

The dichloromethane extract was re-extracted with 5 % hydrochloric acid. This procedure was repeated three times and the hydrochloric acid portion was kept and washed with CH_2Cl_2. Later the aqueous solution was basified with 10 % ammonia solution to pH 11 and re-extracted with CH_2Cl_2 until a negative Mayer test was obtained. The CH_2Cl_2 extracts were dried with Na_2SO_4 and evaporated to dryness to furnish 8.1 g (0.37 %) of crude alkaloid.

The crude alkaloid extract (5.0 g) were chromatographed on a column silica gel for TLC using the solvent CH_2Cl_2 in CH_3OH gradient mixtures. A total of 213 fractions were collected and combined on the basis of TLC profiles. Fractions 67-68 (4.5 mg, 99 CH_2Cl_2: 1 CH_3OH), contained a single compound that was identified as atherospermidine **1** (Bick et al. 1956; Bick & Douglas, 1966). UV λ max (CH_3OH): 249, 280, 315, 430. IR ν max (cm^{-1}, liquid film): 1660, 750. EIMS m/z: 305, 290, 262, 206, 176, 175, 149. ^{1}H NMR ($CDCl_3$) ppm: δ 8.57 (1H, *dd*, *J*=8.0, J'=1.0Hz, H-11), δ 8.53 (1H, *dd*, *J*=8.0, *J*'=1.0 Hz, H-8), δ 7.40- 7.70 (2H, *m*, H-9 or 10), δ 8.13 (H-4, *J*=5.3 Hz), δ 8.91 (H-5, *J*=5.3 Hz), δ 6.25 (2H, *s*, OCH_2O) and δ 4.22 (3H, *s*, C3-OCH_3).

Fractions 127-130 were combined (60 mg) and applied to silica gel using CH_2Cl_2 - CH_3OH (98:2, gradient) as eluting solvent to afford (-)-anonaine **2** (12.7 mg) (Chang et al.2000; Urzua et al. 1982; Nieto et al. 1976). $[\alpha]_D^{26}$: -20.8^o (*c*=6.07 x 10^{-4}, CH_3OH). UV λ max (CH_3OH): 234, 272, 312. IR ν max (cm^{-1}, liquid film): 1041 (C-O strecthing), 965 (-OCH_2O-). EIMS m/z: 265, 264, 236, 206, 178. ^{1}H NMR ($CDCl_3$) ppm: δ 7.23-7.26 (3H, *m*, H-8,9,10), δ 8.06 (1H, d, *J*=7.6 Hz, H-11), δ 6.57 (1H, *s*, H-3), 5.95 and δ 6.10 (2H, *d*, *J*= 1.4 Hz, OCH_2O).

Fractions 141-144 (65 mg) were chromatographed further on a silica gel column using step gradient elution with CH_2Cl_2- CH_3OH to offer (-)- norstephalagine **3** (9.2 mg, 98 CH_2Cl_2: 2 CH_3OH) (Achenbach et al. 1982; Hocquemiller et al. 1981). $[\alpha]_D^{26}$: -35.8^o (*c*=5.23 x 10^{-4}, MeOH). UV λ max (CH_3OH): 241, 278. IR ν max (cm^{-1}, liquid film):

1050 and 940. EIMS m/z: 295, 294, 266, 236, 180,165. ^{1}H NMR ($CDCl_3$) ppm: δ 7.16-7.31 (3H, *m*, H-8,9,10), δ 8.02 (1H, d, *J*=7.8 Hz, H-11), 5.92 and δ 6.07 (2H, *d*, *J*= 1.5 Hz, OCH_2O) and δ 4.02 (3H, *s*, C3-OCH_3).

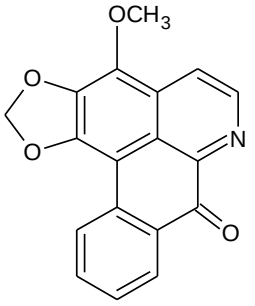
Fractions 145-148 (72 mg) were combined and separated using the same conditions as above to afford (-)-asimilobine **4** (15.5 mg, 98 CH_2Cl_2: 2 CH_3OH) (John et al., 1970). $[\alpha]_D^{26}$:-27.9^o (*c*=4.05 x 10^{-4}, CH_3OH). UV λ max (CH_3OH): 222, 273, 308. IR ν max ($cm^{-1,}$ liquid film): 3435 (OH). EIMS m/z: 267, 266, 252, 238, 236, 29. ^{1}H NMR ($CDCl_3$) ppm: δ 7.22 (3H, *m*, H-8,9,10), δ 8.35 (1H, *d*, *J*=7.6 Hz, H-11), δ 6.62 (1H, *s*, H-3) and δ 3.63 (3H, *s*, C1-OCH_3).

Screening for antimalarial activity

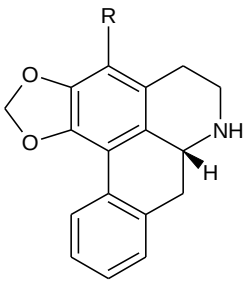
As there are no records of *Popowia* species being used traditionally in the treatment of fever or malaria (Burkill, 1935), this work was undertaken to investigate the possibility of anti-malarial properties of *Popowia perakensis* (KL 4962). The *in vitro* testing of the antimalarial assay was carried out on the dichloromethane alkaloidal crude extract of the bark. The test was done by measuring the lactate dehydrogenase (LDH) activity of the parasite according to the methods described previously (Makler & Hinrichs, 1993; Makler et al. 1993).

Results and Discussion

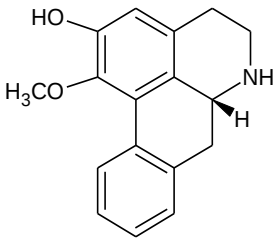
Four alkaloids were obtained by repeated column chromatography from the alkaloidal crude extract of the bark of *Popowia perakensis*. On the basis of spectral data, they were identified as atherospermidine **1**, (-)-anonaine **2**, (-)-norstephalagine **3** and (-)-asimilobine **4**.



1



2 R=H
3 R= OCH_3



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Compound **1** was isolated as a bright yellow solid. The mass spectrum showed a molecular ion peak at m/z 305, which suggested the molecular formula of $C_{18}H_{11}NO_4$. The UV spectrum showed maxima absorptions at 249, 280, 315 and

430 nm indicating the existence of a highly unsaturated chromophore. The IR spectrum exhibited a strong peak at 1660 cm^{-1} attributed a highly conjugated ketone function. The ^1H NMR spectrum showed a methoxyl singlet at δ 4.22 and another singlet at δ 6.25 indicative of methylenedioxy group of an oxoaporphine at C-1 and C-2. Four aromatic protons resonated at δ 8.57 (1H, *dd*, *J*=8.0, *J'*=1.0 Hz, H-11), 8.53 (1H, *dd*, *J*=8.0, *J'*=1.0 Hz, H-8) and 7.40- 7.70 (2H, *m*, H-9 or 10). H-11 has the highest chemical shift due to the hydrogen bonding with the methylenedioxy group and the deshielding effect of the facing ring A. The absence of the proton singlet at ~ δ 7.20 further supported that C-3 was substituted with a methoxy group. Furthermore, two sets of doublets with coupling constants of 5.3 Hz were observed at δ 8.13 and 8.91 attributable to H-4 and H-5, respectively. Comparing their data with those of literature identified the known compound **1** as atherospermidine.

Alkaloid **2** was obtained as a brownish amorphous form, $[\alpha]_D^{26}$ -20.8° (*c*=6.07 x 10^{-4}, CH_3OH). The mass spectrum revealed a molecular ion peak at m/z 265, which correlated to a molecular formula of $C_{17}H_{15}NO_2$. The peak at m/z 264 was indicative of an aporphine nature. Furthermore, a peak at m/z 236 $[M-29]^+$ was due to the loss of a methylene imine resulting from retro Diels-Alder cleavage. Its UV spectrum showed maxima absorptions at 234, 272 and 312 nm, thus suggesting a 1,2-disubstituted aporphine skeleton. The IR spectrum exhibited an absorption band at 1041 cm^{-1} which was characteristic of the C-O stretching vibrations of methoxyl or methylenedioxy group. A peak at 965 cm^{-1} was also observed which was assignable to a methylenedioxy group. The ^1H NMR data showed a proton singlet at δ 6.57 which was assigned for the proton at C-3. H-11 appeared as a doublet (*J*=7.6 Hz) at δ 8.06. A multiplet corresponding to the three protons (H-8, H-9 and H-10) at δ 7.23-7.26 showed that ring D was not substituted. In addition, signals due to methylenedioxy protons were observed as a *dd* at δ 5.95 and 6.10 (*J*= 1.4 Hz). Finally, alkaloid **2** was identified as anonaine.

Alkaloid **3**, $[\alpha]_D^{26}$ -35.8° (*c*=5.23 x 10^{-4}, CH_3OH) was isolated as brownish oil. The mass spectrum was most informative showing the molecular ion peak at m/z 295, thus giving a possible molecular formula of $C_{18}H_{17}NO_3$. The UV spectrum exhibited maxima at 241 and 278 nm typical of a 1,2-disubstituted aporphine. The infra-red spectrum indicated two peaks at 1050 and 940 cm^{-1}. The former was assigned to the C-O stretching vibrations of methoxyl or methylenedioxy while the latter was consistent with the characteristic of the methylenedioxy group. The ^1H NMR spectrum of **3** is similar to those of **1** except for the signal of H-3 was replaced by the methoxyl group at δ 4.02. Hence, alkaloid **3** can be deduced to be norstephalagine.

Alkaloid **4** was isolated as a brown powder, $[\alpha]_D^{26}$ -27.9° (*c*=4.05 x 10^{-4}, CH_3OH). Alkaloid **4** showed the characteristic of UV maxima of an isoquinoline chromophore at 222, 273 and 308 nm. The EI mass spectrum exhibited a molecular ion peak at m/z 267 corresponding to the molecular formula of $C_{17}H_{17}NO_2$ and the base peak at m/z 266 was indicative of an aporphine skeleton. The presence of an intense peak at 238 $[M-29]^+$, due to the loss of methylene

imine, indicated that alkaloid **4** is an *N*-unsubstituted (NH) aporphine. The IR spectrum showed absorption at 3435 cm^{-1} indicating the presence of the hydroxyl group. The ^1H NMR spectrum displayed signals for four aromatic protons. A singlet at δ 6.62 was assigned to a proton attached to C-3. H-11 appeared downfield compared to compound **2** and **3** (*d*, *J*=7.6 Hz) at δ 8.35 which was due to the deshielding effect of the facing aromatic ring A. Compound **2**, **3** and **4** contain unsubstituted ring D based on a multiplet signal corresponding to three aromatic protons (H-8, 9 and 10). There was also signal for the methoxyl group at C-1. Based on this data alkaloid **4** was deduced as asimilobine.

As for bioassay investigation, the crude alkaloid extract of bark of *Popowia perakensis* showed better antimalarial activity towards the resistant strain Gombak A (IC_{50} of 6.85 μg/ml). Therefore, it was capable of inhibiting the growth of cultured *Plasmodium falcifarum* strain, Gombak A.

Conclusion

All four isoquinoline alkaloids isolated are known and were identical by comparison of their spectral data with those previously published. To the best of our knowledge, this is the first report on the isolation of atherospermidine **1**, (-)-anonaine **2** and (-)-norstephalagine **3** from the *Popowia* species. However, (-)-asimilobine **4** have been isolated from *Popowia pisocarpa* (Indonesia) and *Popowia* cf. *cynocarpa* Laut & K. Schum (New Guinea), respectively. On the other hand, the crude alkaloid extract showed potential antimalarial properties against *in vitro* culture of chloroquine resistant Gombak A of *Plasmodium falcifarum* with its IC_{50} value of 6.85 μg/ml.

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