

Isopteropodic Acid from Malaysian *Uncaria longiflora* var. *pteropoda*

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Abstract: We have recently reported on the antioxidant and antimicrobial properties of the stems and leaves extract of Malaysian *Uncaria longiflora* variety *pteropoda*. Continuing our interest in the genus, we have carried out a phytochemical study on the plant with the aim of isolating its bioactive alkaloids. Acid-base extraction of the crude methanolic extract of the plant followed by radial chromatography and repeated preparative thin layer chromatography techniques afforded isopteropodic acid along with three other pentacyclic oxindole alkaloids, pteropodine, isopteropodine and uncarine F. Structural elucidation of the alkaloids was accomplished by spectroscopic methods including 1D-NMR, 2D-NMR, UV, IR and MS as well as comparison with literature. This is the first paper reporting the isolation of isopteropodic acid from this species.

Key words: Isopteropodic acid • Uncarine F • Pentacyclic oxindole alkaloids • *Uncaria longiflora* • NMR

INTRODUCTION

The *Uncaria* genus has been instrumental in the discovery of medicinal natural products since this species have been extensively studied since early 1990s. One of the earlier phytochemical reports was published in 1928 and revealed the isolation of rhynchophylline from *U. rhynchophylla* [1]. The most widely explored *Uncaria* species is the Peruvian *U. tomentosa* (cat's claw) which has yielded diverse bioactive compounds. Today, over 150 compounds have been isolated and identified from this genus and the most recognized compound is the alkaloid mitraphylline which has been identified in 20 out of the 34 species [2].

In Malaysia, more than 10 species of *Uncaria* is available and *U. longiflora* (Poir.) Merr. is one of the representatives [3]. This species of *Uncaria* is a complex species comprising of at least three major entities, namely *U. pteropoda* Miq., *U. longiflora* (Poir.) Merr. *sensu stricto* and *U. havilandiana* S. Moore [4]. It represents a rather well studied group from viewpoint of its alkaloidal content reported to be present in the flowers, shoots, leaves, barks, stems or hooks and roots [1, 5-7]. In 1991, Kam and colleagues [7] had reported the alkaloidal distribution and the isolation of new indole alkaloids in a number of Malaysian *Uncaria* species including *Uncaria longiflora* var. *pteropoda*. They reported on the isolation

of two alkaloids, isopteropodine and pteropodine from the leaves extract of *U. longiflora* var. *pteropoda* (Miq.) which was in accordance with previous reports done by Yeoh *et al.*, in 1966 [5].

In our previous work [8], we have found that the methanolic extract of the leaves and stems of Malaysian *U. longiflora* var. *pteropoda* possess strong antioxidant and antimicrobial activities. Thus, a reinvestigation of the plant is expected to yield more bioactive compounds such as alkaloids which could be good candidates for drugs and therapeutically important compounds.

MATERIALS AND METHODS

General Procedures: TLC and PTLC were performed using pre-coated aluminium-backed supported silica gel 60 F₂₅₄ (0.2 mm thickness) and glass supported silica gel 60 F₂₅₄ (0.5 and 1.0 mm thickness), respectively. Column chromatography was carried out using silica gel 60, 70-230 mesh ASTM (Merk 7734) whereas radial chromatography was done using glass plates with Merck's silica gel Kieselgel 60 PF₂₅₄ Merk Art 7749. Spots and bands for compounds on TLC, PTLC and radial plates were detected using UV light (254 and 365 nm). Mass spectra were measured on Agilent Technologies 6890N GC equipped with Agilent Technologies 5973 inert mass selective detector. The ultraviolet (UV) spectra were obtained in

methanol on Shimadzu UV-Vis 160i. The infrared (IR) data was recorded on a Perkin Elmer FT-IR spectrometer as KBr disc. Optical rotations were measured on a JASCO P1020 digital polarimeter. Melting points were determined using X-4 melting-point apparatus with microscope JM628 digital thermometer. The ^1H and ^{13}C -NMR were analyzed in chloroform-*D* on Bruker 300 Ultrashield NMR spectrometer measured at 300 and 75 MHz, respectively.

Plant Material: Stems of *Uncaria longiflora* var. pteropoda were collected from Hutan Simpan Bangi, Selangor, Malaysia. The voucher specimen (HTBP 1336) was deposited at herbarium of Taman Botani Putrajaya, Malaysia.

Extraction and Isolation: The stems of plant (0.85 kg) were cut into small pieces, air-dried and ground into fine powder. The finely ground plant materials were weighed and extracted exhaustively with methanol at room temperature for 72 hours. The solvent were evaporated off under reduced pressure and the weight of the extract was recorded. The weighed crude extract (25 g) was acidified with 5% HCl. Filtration to remove non-alkaloidal material followed by basification with 37% NH_4OH release the alkaloids which were taken into CHCl_3 to give 2.25 g crude alkaloid fraction. The crude alkaloid fraction was then dissolved in methanol and subjected to radial chromatography (4 mm thickness silica gel plate) with DCM:EtOAc followed by EtOAc:MeOH with a gradual increase of solvent polarity to give several sub-fractions. Repeated preparative thin layer chromatography using CHCl_3 :MeOH on the targeted sub-fractions successfully yielded isopteropodic acid (15 mg) along with pteropodine (20 mg), isopteropodine (50 mg) and uncarine F (8 mg).

Isopteropodic Acid (1): Whitish opaque needles (15 mg), mp 268 – 271°. $[\alpha]_D$ -65.8 (EtOH; c 0.012); MS m/z : 354, $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_4$; UV (MeOH) λ_{max} nm: 242, 219; IR (KBr) ν_{max} cm^{-1} : 3523 (OH), 3217 (NH), 3114 (C-H aromatic), 1698 (C=O acid), 1674 (C=O amide), 1636 (C=C olefinic), 1471 (C=C aromatic), 1195 (C-O cyclic ether); ^1H NMR (CDCl_3 , 300 MHz) δ ppm: 9.23 (1H, br s, N-H), 7.51 (1H, s, H-17), 7.29 (1H, d, J = 7.5 Hz, H-9), 7.21 (1H, ddd, J = 7.5, 1.2 Hz, H-11), 7.04 (1H, ddd, J = 7.5, 1.2 Hz, H-10), 6.94 (1H, d, J = 7.5 Hz, H-12), 4.38 (1H, q, J = 6 Hz, H-19), 3.36 – 3.22 (2H, m, H-21 β , H-5 β), 2.66 – 2.39 (5H, m, H-15, H-21 α , H-6 β , H-5 α , H-3), 2.01 (1H, m, H-6 α), 1.67 – 1.62 (2H, br m, H-14 α , H-20), 1.44 (3H, d, J = 6 Hz, CH_3), 0.88 (1H, m, H-14 β); ^{13}C NMR (CDCl_3 , 75MHz) δ ppm: 181.98 (N-C=O), 171.51

(O-C=O), 156.53 (C-17), 140.33 (C-13), 133.70 (C-8), 127.74 (C-11), 124.44 (C-9), 122.60 (C-10), 110.05 (C-16), 109.38 (C-12), 72.44 (C-19), 71.26 (C-3), 57.10 (C-7), 54.10 (C-5), 53.50 (C-21), 37.83 (C-20), 34.63 (C-6), 30.37 (C-15), 30.04 (C-14), 18.63 (CH_3).

Pteropodine (2): Whitish amorphous solid (20 mg), mp 206 – 210°. $[\alpha]_D$ -123.4 (EtOH; c 0.011); MS m/z : 368, $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$; UV (MeOH) λ_{max} nm: 240, 204; IR (KBr) ν_{max} cm^{-1} : 3206 (NH), 3108 (C-H aromatic), 1721 (C=O ester), 1686 (C=O amide), 1629 (C=C olefinic), 1471 (C=C aromatic), 1086 (C-O cyclic ether); ^1H NMR (CDCl_3 , 300 MHz) δ ppm: 8.66 (1H, br s, N-H), 7.49 (1H, s, H-17), 7.22 (1H, d, J = 7.5 Hz, H-9), 7.19 (1H, ddd, J = 7.5, 1.2 Hz, H-11), 7.07 (1H, ddd, J = 7.5, 1.2 Hz, H-10), 6.83 (1H, d, J = 7.5 Hz, H-12), 4.57 (1H, q, J = 6 Hz, H-19), 3.63 (3H, s, OCH_3), 3.37 – 3.19 (2H, m, H-21 β , H-5 β), 2.50 – 2.31 (5H, m, H-15, H-21 α , H-6 β , H-5 α , H-3), 2.03 (1H, m, H-6 α), 1.74 (1H, m, H-14 α), 1.61 (1H, br m, H-20), 1.51 (1H, m, H-14 β), 1.43 (3H, d, J = 6 Hz, CH_3); ^{13}C NMR (CDCl_3 , 75MHz) δ ppm: 181.0 (N-C=O), 167.77 (O-C=O), 155.29 (C-17), 140.48 (C-13), 133.41 (C-8), 127.98 (C-11), 123.27 (C-9), 122.74 (C-10), 109.25 (C-12), 109.22 (C-16), 74.42 (C-3), 72.23 (C-19), 56.08 (C-7), 55.17 (C-5), 53.63 (C-21), 50.93 (OCH_3), 37.88 (C-20), 34.59 (C-6), 30.98 (C-15), 29.59 (C-14), 19.0 (CH_3).

Isopteropodine (3): Colourless needles (50 mg), mp 216 – 220°. $[\alpha]_D$ -101.5 (EtOH; c 0.02); MS m/z : 368, $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$; UV (MeOH) λ_{max} nm: 249; IR (KBr) ν_{max} cm^{-1} : 3206 (NH), 3110 (C-H aromatic), 1718 (C=O ester), 1698 (C=O amide), 1632 (C=C olefinic), 1470 (C=C aromatic), 1082 (C-O cyclic ether); ^1H NMR (CDCl_3 , 300 MHz) δ ppm: 7.83 (1H, br s, N-H), 7.43 (1H, s, H-17), 7.29 (1H, d, J = 7.5 Hz, H-9), 7.21 (1H, ddd, J = 7.5, 1.2 Hz, H-11), 7.04 (1H, ddd, J = 7.5, 1.2 Hz, H-10), 6.88 (1H, d, J = 7.5 Hz, H-12), 4.36 (1H, q, J = 6 Hz, H-19), 3.62 (3H, s, OCH_3), 3.33 – 3.21 (2H, m, H-21 β , H-5 β), 2.60 – 2.34 (5H, m, H-15, H-21 α , H-6 β , H-5 α , H-3), 2.01 (1H, m, H-6 α), 1.67 – 1.69 (2H, m, H-14 α , H-20), 1.43 (3H, d, J = 6 Hz, CH_3), 0.88 (1H, m, H-14 β); ^{13}C NMR (CDCl_3 , 75MHz) δ ppm: 180.88 (N-C=O), 167.64 (O-C=O), 155.00 (C-17), 140.04 (C-13), 133.75 (C-8), 127.69 (C-11), 124.64 (C-9), 122.58 (C-10), 109.85 (C-16), 109.46 (C-12), 72.16 (C-19), 71.34 (C-3), 56.86 (C-7), 54.15 (C-5), 53.53 (C-21), 50.99 (OCH_3), 37.90 (C-20), 34.86 (C-6), 30.49 (C-15), 30.19 (C-14), 18.65 (CH_3).

Uncarine F (4): Whitish amorphous solid (8 mg), mp 120 – 124°. $[\alpha]_D$ +51.3 (EtOH; c 0.008); MS m/z : 368, $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$; UV (MeOH) λ_{max} nm: 237, 205; IR (KBr) ν_{max} cm^{-1} : 3448 (NH), 3144 (C-H aromatic), 1706 (C=O ester),

1706 (C=O amide), 1620 (C=C olefinic), 1472 (C=C aromatic), 1083 (C-O cyclic ether); ^1H NMR (CDCl_3 , 300 MHz) δ ppm: 7.62 (1H, br s, N-H), 7.43 (1H, s, H-17), 7.39 (1H, d, $J=7.5$ Hz, H-9), 7.20 (1H, ddd, $J=7.5, 1.2$ Hz, H-11), 7.04 (1H, ddd, $J=7.5, 1.2$ Hz, H-10), 6.86 (1H, d, $J=7.5$ Hz, H-12), 4.20 (1H, q, $J=6$ Hz, H-19), 3.65 (3H, s, OCH_3), 3.29 (1H, m, H-5 α), 3.01 (5H, m, H-21 α), 2.72 (1H, m, H-15), 2.53–2.32 (3H, m, H-3, H-6 α , H-5 β), 2.22–2.11 (2H, m, H-14 β , H-21 β), 2.06–1.84 (2H, m, H-6 β , H-20), 1.23 (3H, d, $J=6$ Hz, CH_3), 1.05 (1H, m, H-14 α); ^{13}C NMR (CDCl_3 , 75MHz) δ ppm: 181.57 (N-C=O), 167.43 (O-C=O), 153.86 (C-17), 140.50 (C-13), 133.60 (C-8), 127.60 (C-11), 124.96 (C-9), 122.10 (C-10), 109.62 (C-12), 104.96 (C-16), 74.70 (C-19), 67.37 (C-3), 56.56 (C-7), 53.93 (C-5), 53.54 (C-21), 50.93 (OCH_3), 36.83 (C-20), 34.79 (C-6), 26.94 (C-14), 24.99 (C-15), 18.62 (CH_3).

RESULTS AND DISCUSSION

Extensive chromatographic techniques on the stems extract of *Uncaria longiflora* var. pteropoda afforded isopteropodic acid (1) along with three other pentacyclic oxindole alkaloids namely pteropodine (2), isopteropodine (3) and uncarine F (4). The structures of the compounds are shown in Figure 1.

Alkaloid 1 was isolated as whitish opaque needles. The mass spectrum showed a molecular ion peak at m/z 354 corresponding to the molecular formula of $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_4$. The UV region showed absorption at 219 nm and 242 nm suggesting that a hydroxyl substituent may be present. The IR spectrum revealed the presence of non-hydrogen-bonded OH (sharp peak) at 3523 cm^{-1} . Typical IR characteristics of heteroyohimbine-type oxindole alkaloids were observed at 3217 cm^{-1} (NH), 3114 cm^{-1} (C-H aromatic), 1698 cm^{-1} (C=O acid), 1674 cm^{-1} (C=O amide), 1636 cm^{-1} (C=C olefinic), 1471 cm^{-1} (C=C aromatic) and 1195 cm^{-1} (C-O cyclic ether) suggesting that alkaloid 1 belongs to pentacyclic oxindole alkaloid. In the ^1H NMR spectrum, four aromatic proton signals resonating at δ 7.29, δ 7.21, δ 7.04 and δ 6.94 indicate that the aromatic ring is unsubstituted in the oxindole moiety. A singlet at δ 7.51 belonging to an olefinic proton H-17 and a broad singlet at δ 9.23 attributed to a proton attached with nitrogen were also observed. A quartet at δ 4.38 ($J=6$ Hz) and a three-proton doublet signal at δ 1.44 ($J=6$ Hz) were also present corresponding to H-19 and H-18, respectively. The ^{13}C NMR spectrum further confirmed the existence of 20 carbons including a methyl group, an acid carbonyl and oxindole carbonyl signals at δ 18.63, δ 171.51 and δ 181.98 respectively.

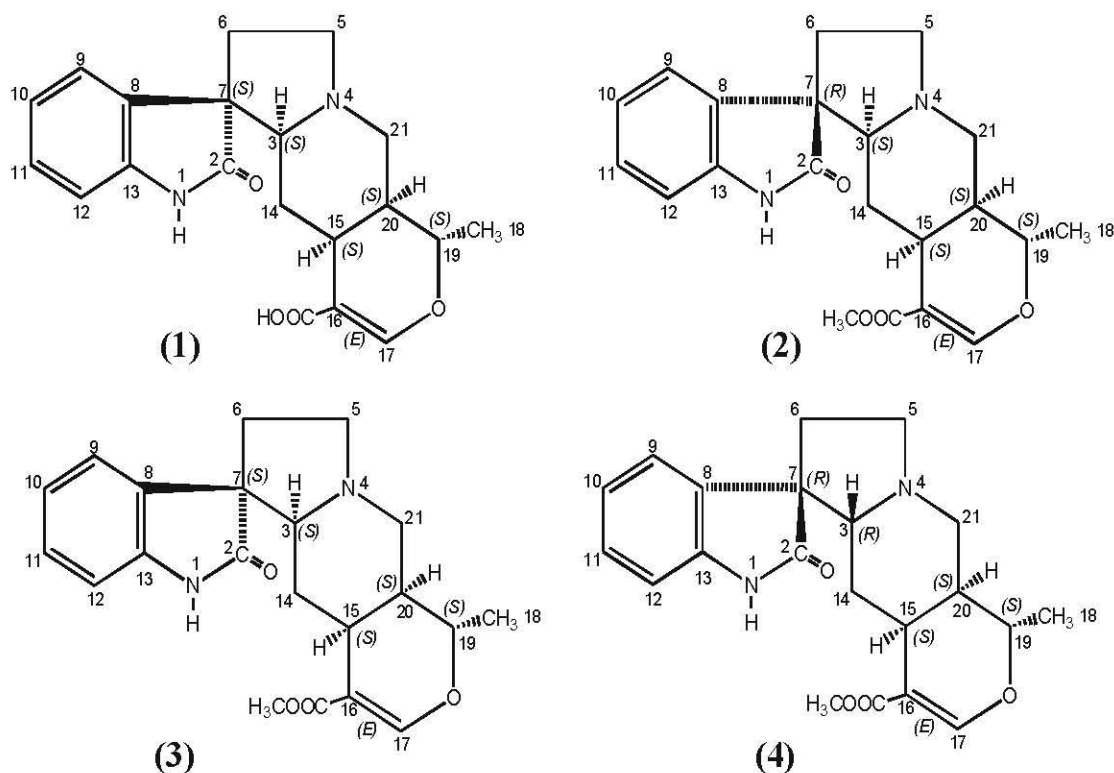


Fig. 1: Structures of the isolated compounds

Furthermore, the presence of the carboxylic acid moiety at C-16 was confirmed by the fragment ion at m/z 337 and a base peak at m/z 209 in the mass spectrum. Based on these spectral data as well as comparison with literature [9], alkaloid 1 is identified as isopteropodic acid, a 16-carboxy derivative of isopteropodine which belongs to the *allo*-group of heteroyohimbine-type oxindole alkaloids [10].

Pteropodine (2), isopteropodine (3) and uncarine F (4) were identified based on their spectral data as well as comparison with the literatures value [5, 11]. This is the first paper reporting the isolation of isopteropodic acid (1) from *Uncaria longiflora* var. *pteropoda*. It was previously reported as a constituent of *U. sinensis* [9]. Uncarine F has been previously isolated from a few other *Uncaria* species including *U. longiflora* var. *longiflora*, *U. bernaysii*, *U. lanosa*, *U. orientalis* and *U. sinensis* [2, 6].

CONCLUSION

In this study, extensive chromatographic techniques on *U. longiflora* var. *pteropoda* stems extract have successively yielded isopteropodic acid along with three other pentacyclic oxindole alkaloids identified as pteropodine, isopteropodine and uncarine F. Their structures were determined on the basis of spectroscopic evidence including 1D (^1H , ^{13}C) and 2D-NMR (COSY, HMQC) as well UV, IR and MS data and comparison with literature [5, 9, 11].

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