A new flavone from Malaysia Borneo *Marsdenia tinctoria*

Nur Atiqah Mohd Nasuha and Yeun-Mun Choo

Faculty of Science, Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia

**ABSTRACT**

*Marsdenia tinctoria* is an indigo producing plant commonly found in Borneo, Malaysia. In this present study, one new flavone kapitone (1) and three known compounds, that is 3,2′-dihydroxyflavone (2), 1-methylcyclobutene (3) and dimethyl isatoate (4) were isolated from the Malaysia Borneo *M. tinctoria* R. Br. (Apocynaceae). These compounds were isolated and characterised using extensive chromatographic and spectroscopic methods.

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**1. Introduction**

*Marsdenia tinctoria* R. Br. (Apocynaceae) is an indigo plant, which is known as ‘Akar Tarum’ or ‘Rengat’ in local Malaysia language. *M. tinctoria* is a perennial climber that is widely distributed in India to South China and Malaysia. It is utilised as traditional medicine to treat rheumatic ailments and hepatomegaly (Gao et al. 2009). In addition to its therapeutic properties, the plant is also cultivated to produce the blue indigo dye (Fujisawa & Nakashizuka 2012). Previous reports on *Marsdenia* sp. from India, China and Bangladesh yielded steroidal compounds (Chowdhury et al. 1994; Gao et al. 2009; Gupta et al. 2009, 2011). In the present report, the Malaysia Borneo *M. tinctoria* was collected from Kapit, Sarawak, Malaysia, for chemical constituents investigation. The plant was cultivated by the local Iban community in their farm as part of their heritage culture to produce blue dye for the traditional *Pua Kumbu* textiles (Fujisawa & Nakashizuka 2012).
2. Results and discussion

The *M. tinctoria* from Kapit, Sarawak, Malaysia, yielded one new flavone, kapitone (1) and three known compounds, that is 3,2'-dihydroxyflavone (2), 1-methylcyclobutene (3) and dimethyl isatoate (4) through extensive chromatographic purifications of the *M. tinctoria* extracts. The structures of the compounds 1–4 were characterised using NMR, HRESIMS, IR and UV spectroscopy (Figure 1), and the NMR data are available in the supplementary document.

2.1. Kapitone (1)

Kapitone (1) is 2,3-bis(2-hydroxyphenyl)-4H-chromen-4-one and a new flavone isolated as purplish amorphous solid from *M. tinctoria*. The HRESIMS showed *m/z* 313.1343 [M – OH]⁺ and 312.1311 [M – H₂O]⁺ consistent with molecular formula C₂₁H₁₄O₄ and 15° of unsaturation, suggesting a highly conjugated structure. The observation of neutral losses in the HRESIMS of OH and H₂O species suggested the presence of hydroxyl group. The presence of the hydroxyl group is also consistent with the observation of IR band at 3274 cm⁻¹. The IR spectrum also indicated the presence of carbonyl (1687 cm⁻¹) group. The ¹H NMR spectrum displayed twelve resolved aromatic proton signals and a hydroxyl signal (δ_H 11.78). Of the 12 aromatic protons, six were observed as broad doublet (brd) or doublet of doublet (dd) at δ_H 9.27, 8.70, 8.46, 7.87, 7.79, and 7.13, and the other six were observed as broad triplet (brt) or triplet of doublet (td) at δ_H 7.82, 7.55, 7.56, 7.51, 7.43 and 7.07. The observed spin systems of these aromatic protons are typical of the 1,2-disubstituted aromatic ring system and suggested the presence of three 1,2-disubstituted aromatic ring moiety in the structure which was further confirmed by the COSY experiment. The COSY spectrum indicated of three similar partial structure –CH=CH–CH = CH–, corresponding to C(5)-C(6)-C(7)-C(8), C(3')-C(4')-C(5')-C(6') and C(3'')-C(4'')-C(5'')-C(6''), and hence confirming the presence of three...
1,2-disubstituted aromatic rings (rings A, C and D, respectively). The $^{13}$C NMR showed the presence of twelve $sp^2$ methine and nine $sp^2$ quaternary carbon signals. The observation of a total of 21 $sp^2$-type carbon signals and the appearance of the compound 1 as purple amorphous solid suggested the presence of a highly conjugated structure in agreement with the MS results. The carbon signals at $\delta_C$ 187.7 indicated the presence of carbonyl carbon, while the $\delta_C$ 159.5 (C(2)), 151.4 (C(8a)) and 151.2 (C(2')) suggested that these quaternary $sp^2$ carbon are located adjacent to an oxygen atom. The HMBC spectrum (Figure S1) showed $J^b$ correlations from the carbonyl C(4) to H(5), C(4a) to H(6) and H(8), and C(8a) to H(5) and H(7) thereby connecting the rings A and B together and suggested the presence of a chromenone moiety. The structure of ring C is confirmed by the observation of HMBC $J^b$ correlations from C(2) to H(6’), C(1’) to H(3’) and H(5’), and C(2’) to H(4’) and H(6’) indicating the presence of 2-hydroxylphenyl substitution at C(2) of the chromenone moiety. And lastly, the $J^3$ HMBC correlations from C(3) to H(6''), C(1'') to H(3'') and H(5''), and C(2'') to H(4'') and H(6'') connect the of 2-hydroxyphenyl (ring D) to the C(3) of the chromenone moiety and confirmed the structure of kapitone (1) as 2,3-bis(2-hydroxyphenyl)-4H-chromen-4-one.

2.2. 3,2’-Dihydroxyflavone (2), 1-methylcyclobutene (3) and dimethyl isatoate (4)

3,2’-Dihydroxyflavone (2) is a known flavone which is previously obtained through synthesis (Savi et al. 2010). Compound 3 is 1-methylcyclobutene which is previously reported from Abies delavayi (Yang et al. 2014). Dimethyl isatoate (4) is also another known compound which is previously obtained through synthesis (Moriyama et al. 2012). This is the first isolation report for compounds 2 and 4 to the best of our knowledge. The NMR spectrum and spectroscopic data of compounds 2–4 are available in the supplementary document.

3. Experimental section

3.1. General experimental procedures

NMR spectra data were obtained from 600 MHz Bruker AVANCE III (Bruker, Fallanden, Switzerland) NMR spectrometers with chemical shifts expressed in ppm and TMS as an internal standard in CDCl$_3$. HRESIMS data were obtained from the Agilent 6530 Q-TOF (Agilent Technologies, Santa Clara, CA, USA) mass-spectrometer equipped with the Agilent 1200 series Rapid Resolution LC system. The UV data were recorded using Agilent Cary 60 UV–vis Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) using quartz cell. IR was carried out on the Perkin-Elmer RX1 FTIR (Perkin-Elmer, Waltham, MA, USA) using NaCl cell.

3.2. Plant material and extraction

M. tinctoria was collected from Kapit, Sarawak, Malaysia. A voucher of the specimen is available at the Herbarium of Sarawak Biodiversity Centre, Kuching, Sarawak, Malaysia (Research Permit No. SBC-RA-0089-CYM). 0.5 kg of wet whole plant was extracted with H$_2$O (1 L) at 100 °C for 30 min and left for overnight in room temperature. The aqueous extract was then freeze-dried to give 2.05 g of dried solid. The dried solid was further subjected to sequential partitioning by CHCl$_3$ and MeOH to give CHCl$_3$ extract (270 mg) and MeOH extract (615 mg).
3.3. Isolation

The CHCl₃ extract was subjected to purification by column chromatography using silica gel 60 (0.040–0.063; Merck, Darmstadt, Germany) and solvent system of chloroform (100%) which resulted in fractions A1, B1 and C1. The centrifugal chromatography (Kieselgel 60 with gypsum silica gel; Merck, Darmstadt, Germany) was extensively used for the subsequent isolation and purification of pure compounds. Purification of fraction A1 using centrifugal chromatography with solvent system hexane:chloroform (6:4) afforded compound 3. Purification of fraction B1 using centrifugal chromatography with solvent system hexane:chloroform (6:4) afforded compound 2. Purification of fraction C1 using centrifugal chromatography with solvent system hexane:chloroform (7:3) afforded compound 1. The MeOH extract was subjected to purification by column chromatography using silica gel 60 (0.040–0.063; Merck, Darmstadt, Germany) and solvent system of chloroform (100%) which resulted in fractions A2, B2 and C2. Purification of fraction B2 using centrifugal chromatography with solvent system hexane:chloroform (6:4) afforded compound 4. The yields of the compounds were as follows: 1 (1.0 mg, 0.0002% yield), 2 (1.5 mg, 0.0003% yield), 3 (6.8 mg, 0.0014% yield) and 4 (7.9 mg, 0.0016% yield).

3.4. Kapitone (1)

Purplish amorphous solid; UV (EtOH) λmax (log ε) 570 (3.19), 280 (3.39), 240 (3.58), 230 (3.57), 220 (3.58) nm; IR (NaCl) νmax 3274, 2927, 2855, 1687, 1545, 1462, 1322 cm⁻¹; 1H NMR (CDCl₃, 600 MHz) δ 11.78 (1H, br s, 2'-OH), 9.27 (1H, br d, J = 8 Hz, H-6''), 8.70 (1H, br d, J = 8 Hz, H-3''), 8.46 (1H, dd, J = 8 and 1 Hz, H-6'), 7.87 (1H, br d, J = 8 Hz, H-3'), 7.82 (1H, td, J = 8 and 1 Hz, H-4'), 7.79 (1H, br d, J = 8 Hz, H-5), 7.56 (1H, br t, J = 8 Hz, H-7), 7.55 (1H, br t, J = 8 Hz, H-5'), 7.51 (1H, td, J = 8 and 1 Hz, H-4''), 7.43 (1H, td, J = 8 and 1 Hz, H-5''), 7.13 (1H, br d, J = 8 Hz, H-8), 7.07 (1H, br t, J = 8 Hz, H-6). 13C NMR (CDCl₃, 150 MHz) δ 187.7 (C, C-4), 159.5 (C, C-2), 151.4 (C, C-8a), 151.2 (C, C-2'), 138.0 (C, C-2''), 137.0 (CH, C-7), 134.7 (CH, C-4'), 129.9 (CH, C-4''), 127.6 (CH, C-6'), 127.6 (CH, C-3'), 127.5 (CH, C-5'), 126.8 (CH, C-5''), 125.8 (CH, C-5), 125.7 (CH, C-6''), 123.7 (C, C-1'), 122.3 (CH, C-6), 121.0 (C, C-4a), 120.7 (C, C-1'), 116.8 (CH, C-3''), 112.5 (CH, C-8), 107.4 (C, C-3); HMBC: 2 J C-6'' to H-5''; 3 J C-2 to H-6''; C-3 to H-6''; C-5 to H-5; C-4a to H-6 and H-8; C-5 to H-7; C-6 to H-8; C-7 to H-5; C-8 to H-6; C-8a to H-5 and H-7; C-1' to H-3' and H-5'; C-2' to H-4' and H-6'; C-3' to H-5'; C-4' to H-6'; C-5' to H-3'; C-6' to H-4'; C-1'' to H-3'' and H-5''; C-2'' to H-4'' and H-6''; C-3'' to H-5''; C-4'' to H-6''; C-5'' to H-3''; C-6'' to H-4''. HRESIMS m/z 313.1343 [M - OH]⁺ (calcd for C₂₁H₁₄O₄–OH, 312.0865) and 312.1311 [M – OH]⁺ (calcd for C₂₁H₁₄O₄–H₂O, 312.0786).

4. Conclusion

In this present study, we have successfully isolated and characterised a new flavone kapitone (1) which possessed the 2,3-bis(2-hydroxyphenyl)-4H-chromen-4-one structure and three known compounds, that is 3,2'-dihydroxyflavone (2), 1-methylcyclobutene (3) and dimethyl isatoate (4) from Malaysia Borneo M. tinctoria. This is the first isolation from natural product report for compounds 2 and 4 to the best of our knowledge. The compounds were not subjected to further bioassays characterisation due to the minute amount of samples isolated in this study.
Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S17.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Yeun-Mun Choo http://orcid.org/0000-0001-9891-5898

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