

ISOLATION OF FLAVONOLS FROM THE STEMS OF MALAYSIAN
Uncaria cordata var. *ferruginea* (BLUME) RIDSD.

(Pemisahan Flavonol dari batang *Uncaria cordata* var. *ferruginea* (Blume) Ridsd. Malaysia)

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Abstract

Continuing our interest in the genus, a phytochemical investigation on *Uncaria cordata* var. *ferruginea* collected from Hutan Pasir Raja, Malaysia has been carried out. Two flavonols known as quercetin and kaempferol were isolated from the methanolic stem extract of this plant along with other phenolic compounds and terpenes. The two flavonols are reported for the first time from this species. The structure of the compounds were elucidated on the basis of spectroscopic data, mostly 1D-NMR, 2D-NMR as well as comparison with literature.

Keywords: flavonoid, flavonol, kaempferol, quercetin, *Uncaria cordata*

Abstrak

Dalam meneruskan minat kami terhadap genus ini, satu kajian fitokimia ke atas *Uncaria cordata* var. *ferruginea* yang diambil dari Hutan Pasir Raja, Malaysia telah dijalankan. Dua flavonol dikenali sebagai quercetin dan kaempferol telah diasingkan daripada ekstrak metanol bahagian batang tumbuhan ini bersama sebatian fenolik lain dan terpena. Kedua-dua flavonol ini dilaporkan buat pertama kali daripada spesies ini. Struktur sebatian telah dielusidasi berdasarkan data spektroskopi, terutamanya 1D-NMR, 2D-NMR dan juga perbandingan dengan kajian literatur.

Kata kunci: flavonoid, flavonol, kaempferol, quercetin, *Uncaria cordata*

Introduction

The genus *Uncaria* (Rubiaceae) is well known for centuries among medicinal practitioners due to its medicinal properties and ability in healing various diseases such as diarrhea, dysentery, fevers, diabetes and others. Many studies have been carried out scientifically for its pharmacological activities on *Uncaria* species [1]. For example, *Uncaria tomentosa* which is widely explored has been proven to have anticancer, anti-inflammatory and other biological properties. A biological study on five species of Malaysian *Uncaria* including *Uncaria longiflora* var. *pteropoda*, *Uncaria acida*, *Uncaria lucida*, *Uncaria callophylla* and *Uncaria cordata* have indicated that they are rich sources of antioxidants and potential anti-diabetic agents which may be used in the prevention and control of type II diabetes [2].

Uncaria is amongst the genera known for its alkaloid content. Previously, we have reported the isolation of two new heteroyohimbine-type oxindole alkaloids, namely rauniticine-*allo*-oxindole B and rauniticinic-*allo* acid B along with five of their stereoisomers including four pentacyclic oxindole alkaloids, isopteropodine, pteropodine, uncarine F and isopteropodic acid from Malaysian *Uncaria longiflora* var. *pteropoda* [3 – 5]. In contrast, the non-alkaloid constituents have not been widely reported from *Uncaria* genus. Lately, the isolation of flavonoids and other chemical constituents present in *Uncaria* have gained more interest. The flavonoids reported from the genus are mostly isolated from *Uncaria gambir* particularly flavane derivatives including flavan-3-ols and their dimers [6]. Recently, we have reported the isolation of a novel flavonoid, (-)-2*R*, 3*R*-3,5,4'-trihydroxyflavan-[6,7:5'',6'']-2''-pyranone named uncariechin, along with (-)-*epiafzelechin* and (-)-*epicatechin* from the methanol extract of the leaves of *Uncaria longiflora* var. *pteropoda* [7]. Therefore, continuing our interest on the phytochemicals from *Uncaria*, the isolation of two flavonols, quercetin (**1**) and kaempferol (**2**) from the stems of Malaysian *Uncaria cordata* var. *ferruginea* was reported in this study.

Materials and Methods

General

Column chromatography packed with Sephadex LH-20 was carried out and flavonoids were detected on thin layer chromatography (TLC) stained with aluminium chloride (AlCl₃) reagent. A positive result was indicated by the observation of yellow spots visualized under UV light at 365 nm. Mass spectra were measured on an Agilent 1100 Series Technologies HPLC-TOF LC/MS, where the analysis for compound **1** was run in the positive mode, while compound **2** was analysed in the negative mode. The ultraviolet (UV) spectra were obtained in methanol on a Shimadzu UV-Vis 160i. The infrared (IR) data was recorded on a Perkin Elmer model FT-IR spectrometer as KBr disks. ¹H and ¹³C-NMR data for compound **1** was obtained in acetone-*d*₆ on Bruker 600 Ultrashield NMR spectrometer measured at 600 and 150 MHz, respectively while ¹H-NMR and ¹³C-NMR data for compound **2** was obtained in MeOD on Bruker 500 Ultrashield NMR spectrometer measured at 500 and 125 MHz, respectively.

Plant material

Stems of *Uncaria cordata* var. *ferruginea* was collected from Hutan Pasir Raja, Terengganu in December 2011 and was identified by Dr. Shamsul Khamis (Universiti Putra Malaysia). The voucher specimens were deposited at the Herbarium of Institute of Bioscience, Universiti Putra Malaysia.

Extraction and isolation of compounds

Stems of Malaysian *Uncaria cordata* var. *ferruginea* were cut into small pieces, air-dried and ground into fine powder. The finely ground stems were then weighed and soaked in methanol for 72 hours at room temperature. The solvent was evaporated off under reduced pressure by using a rotary evaporator. Then the extract was dissolved with methanol and water (1:1) before being subjected to liquid-liquid partitioning using chloroform and ethyl acetate. The ethyl acetate fraction (9.8 g) was further fractionated using a column packed with Sephadex LH-20 employing CHCl₃: MeOH (9:1) as the solvent system to give four sub-fractions. Based on their TLC profiles, flavonoids were detected in sub-fractions f2 and f4 by using aluminium chloride reagent. Repeated column chromatography of these two sub-fractions using Sephadex LH-20 successfully yielded compound **1** and compound **2**.

Results and Discussion

Compound **1** was isolated as a yellow amorphous solid with melting point 316 °C. The mass spectrum of this compound showed a molecular ion [M+H]⁺ peak at *m/z* 303 which corresponded to the molecular formula C₁₅H₁₀O₇. Its UV absorptions maxima at 295, 338 and 385 nm were found to be in agreement with a flavonol-type compound [7]. The IR spectrum displayed absorption bands at 3411, 1665 and 1615 cm⁻¹ indicating the presence of functional groups of OH, C=O and C=C aromatic, respectively. The ¹H NMR spectrum of compound **1** showed the characteristic signals of flavonoid-type compound with ABX system. In A-ring system, two doublet peaks of *meta*-coupled aromatic protons present at δ 6.28 (1H, d, *J* = 2.4 Hz) and at δ 6.54 (1H, d, *J* = 2.4 Hz) were assigned as H-6 and H-8, respectively. The rest of proton signals belonging to the ring-B system appeared at δ 7.01 (1H, d, *J* = 8.4 Hz, H-5'), 7.72 (1H, dd, *J* = 8.4, 2.4 Hz, H-6') and 7.85 (1H, d, *J* = 2.4 Hz, H-2'). Its COSY spectrum showed the H-H correlation indicating H-5' and H-6' to be neighbouring protons. Its ¹³C NMR spectrum showed the presence of 15 carbon signals which were typical of a flavonol skeleton. The most downfield signal at δ 175.67 was attributed

to carbonyl C-4 due to electron withdrawing oxygen and anisotropic effects. In addition, five oxyaryl carbons observed at δ 135.85, 144.97, 147.51, 161.45 and 164.16 were attributed to C-3, C-3', C-4', C-5, and C-7. Four quaternary carbons at δ 103.23, 122.86, 146.05 and 156.90 were assigned as C-10, C-1', C-2 and C-9, respectively. The rest of the signals at δ 93.57, 98.29, 114.87, 115.34 and 120.56 were subsequently determined as aromatic methine carbons C-8, C-6, C-2', C-5' and C-6', respectively which are confirmed by HMQC correlations. All carbon assignments were further confirmed by HMBC and NOESY correlations. In short, the characterization of compound **1** is given below.

Pale yellow amorphous solid, wt: 3.2 mg; MS $m/z = 303$ $[M+H]^+$, $C_{15}H_{10}O_7$; UV (MeOH) λ_{max} nm: 295, 338, 385; IR (KBr) ν_{max} cm^{-1} : 3411 (free OH), 1665 (C=O), 1615 (C=C aromatic); 1H NMR (Acetone- d_6 , 600 MHz) δ ppm : 12.19 (1H, br s, OH), 7.85 (1H, d, $J = 2.0$ Hz, H-2'), 7.72 (1H, dd, $J = 8.5, 2.0$ Hz, H-6'), 7.01 (1H, d, $J = 8.5$ Hz, H-5'), 6.54 (1H, d, 2.0 Hz, H-8), 6.28 (1H, d, 2.0 Hz, H-6); ^{13}C NMR (Acetone- d_6 , 150 MHz) δ ppm : 175.67 (C=O), 164.16 (C-7), 161.45 (C-5), 156.90 (C-9), 147.51 (C-4'), 146.05 (C-2), 144.97 (C-3'), 135.85 (C-3), 122.86 (C-1'), 120.56 (C-6'), 115.34 (C-5'), 114.87 (C-2'), 103.23 (C-10), 98.29 (C-6), 93.57 (C-8). Thus, based on the 1D and 2D NMR spectral data, as well as comparison with the literature [8], compound **1** was identified as quercetin.

Compound **2** was isolated as yellow amorphous powder with melting point 277°C. Its mass spectrum showed a molecular ion $[M-H]^-$ peak at m/z 285 which corresponds to the molecular formula $C_{15}H_{10}O_6$. Its UV absorptions at 310 and 380 nm were indicative of a flavonol-type compound. The IR spectrum displayed absorption bands at 3324, 1660 and 1614 cm^{-1} which were due to the functional groups of OH, C=O, and C=C aromatic, respectively. The 1H NMR spectrum of this compound showed proton signals of a typical flavonol-type compound with ABX system (6-8 ppm). For the A-ring protons, two doublet peaks observed at δ 6.18 (1H, d, $J = 2$ Hz) and at δ 6.39 (1H, d, $J = 2$ Hz) were assigned as H-6 and H-8, respectively. Both of these protons were *meta*-coupled as indicated by their coupling constant value. In the B-ring system, proton signals appeared as a pair of doublet of doublets at δ 6.90 (2H, dd, $J = 7, 2$ Hz, H-3', H-5') and at δ 8.09 (2H, dd, $J = 7, 2$ Hz, H-2', H-6'). These protons were *ortho*-coupled based on their J values and were adjacent to each other as was evident from the COSY spectrum. Its ^{13}C NMR spectrum exhibited 13 carbons signal attributed to 15 carbons with the peak at δ 129.27 representing equivalent carbons C-2' and C-6' and the peak at δ 114.89 representing equivalent carbons C-3' and C-5'. A very downfield signal at δ 175.95 was attributed to carbonyl C-4 due to electron withdrawing oxygen and anisotropic effect. In addition, four oxyaryl carbons observed at δ 135.72, 159.13, 161.10 and 164.17 were attributed to C-3, C-4', C-5 and C-7, respectively. Four quaternary carbons at δ 103.14, 122.33, 146.62 and 156.84 were then assigned as C-10, C-1', C-2 and C-9, respectively. The rest of the carbon signals observed at δ 93.07 (C-8), 97.86 (C-6), 114.89 (C-3', C-5'), and 129.27 (C-2', C-6') were methine carbons. The carbon assignments were confirmed by HMQC and HMBC data. The characterization of compound **2** is given below.

Pale yellow amorphous solid, wt: 1.3 mg; MS $m/z = 285$ $[M-H]^-$, $C_{15}H_{10}O_6$; UV (MeOH) λ_{max} nm: 310, 380; IR (KBr) ν_{max} cm^{-1} : 3324 (free OH), 1660 (C=O) 1614 (C=C aromatic); 1H NMR (MeOD, 500 MHz) δ ppm : 8.09 (2H, dd, $J = 8.9, 2.0$ Hz, H-2', H-6'), 6.90 (2H, dd, $J = 8.9, 2.0$ Hz, H-3', H-5'), 6.39 (1H, d, $J = 2.0$ Hz, H-8), 6.18 (1H, d, $J = 2.0$ Hz, H-6); ^{13}C NMR (MeOD, 150 MHz) δ ppm : 175.95 (C=O), 164.17 (C-7), 161.10 (C-5), 159.13 (C-4'), 156.84 (C-9), 146.62 (C-2), 135.72 (C-3), 129.27 (C-2', C-6'), 122.33 (C-1'), 114.89 (C-3', C-5'), 103.14 (C-10), 97.86 (C-6), 93.07 (C-8). On the basis of spectral data and comparison with literature [9], compound **2** was identified as 3,4',5,7-tetrahydroxyflavone or kaempferol.

Table 1 shows the ^{13}C NMR data compounds **1** and **2** along with literature values. Figure 1 shows the structure of the flavonoids.

Table 1. ^{13}C NMR data for compounds 1 and 2

Compound 1			Compound 2		
Position	δ_{C} (ppm)	δ_{C} (ppm) in MeOD ^a	Position	δ_{C} (ppm)	δ_{C} (ppm) in DMSO ^b
2	146.05	147.8	2	146.62	147.3
3	135.85	137.3	3	135.72	136.0
4	175.67	177.4	4	175.95	176.4
5	161.45	162.4	5	161.10	161.2
6	98.25	99.4	6	97.86	98.7
7	164.16	165.6	7	164.17	164.4
8	93.57	94.5	8	93.07	94.0
9	156.90	158.3	9	156.84	156.7
10	103.23	104.5	10	103.14	103.5
1'	122.86	124.0	1'	122.33	122.1
2'	114.87	116.1	2'	129.27	130.0
3'	144.97	146.2	3'	114.89	115.9
4'	147.51	148.7	4'	159.13	159.7
5'	115.34	116.3	5'	114.89	115.9
6'	120.56	121.5	6'	129.27	130.0

^a[8], ^b[9]

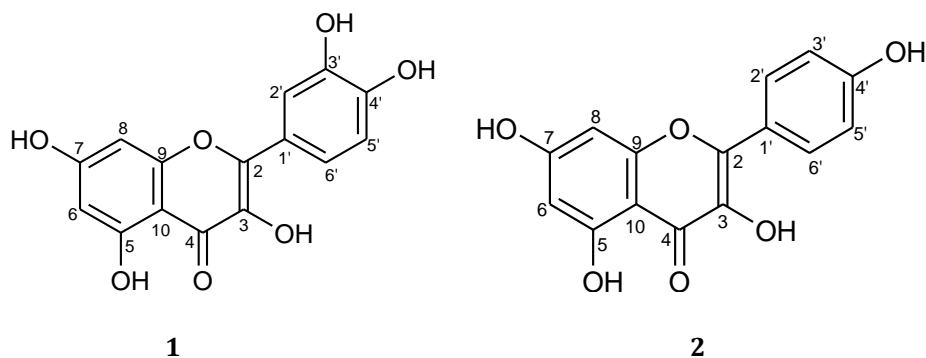


Figure 1. Structure of flavonols isolated from the stems of Malaysian *Uncaria cordata* var. *ferruginea*: (1) Quercetin, (2) Kaempferol

Conclusion

Phytochemical study of the methanolic stem extract of Malaysian *Uncaria cordata* var. *ferruginea* has led to the isolation of two flavonols, quercetin and kaempferol along with other phenolic compounds and terpenes. The structure of the compounds was elucidated on the basis of spectroscopic data. The presence of other non-alkaloidal constituents should be further explored in order to justify their chemotaxonomic significance in the *Uncaria* genus.

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