



Isoflavanone glycoside from the leaf of *Mangifera indica* L.

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ABSTRACT

A new isoflavonoid glycoside, *malaysianone B* has been isolated from the methanol extract of the leaf of *Mangifera indica* by various chromatography techniques. Its structure was established on the basis of spectroscopic evidence and comparison with the published data.

Keywords: Anacardiaceae, *Mangifera indica*, mango, isoflavanone, *malaysianone B*.

INTRODUCTION

Mangifera indica L. (Mango), which belongs to the family Anacardiaceae, order Rutales, is a popular and economically important tropical fruit throughout the world, due to its excellent eating quality and nutritional composition [1]. In many Asian countries, all part of the plant including the fruit, flower, leaf and stem of mango has been used for diverse medicinal purposes [2]. Mango contains various classes of polyphenols, carotenoids and ascorbic acid demonstrating different health-promoting properties, mainly from their antioxidant activities [3]. The first compounds defined as major polyphenolics present in mango are gallic acid and gallotannins[4], while the other polyphenols, such as mangiferin, quercetin, kaempferol, *p*-hydroxybenzoic acid, *m*-coumaric acid, *p*-coumaric acid and ferulic acid, were also identified [5-9]. The aqueous extract of the stem bark of *Mangifera indica* L. (Anacardiaceae Family) was reported to contain anti-inflammatory activity, inhibit ear-oedema induced by the four irritants, inhibit the induction of prostaglandin E (PGE) and leukotriene-B4 (LTB4) released by macrophages [10]. In this paper, we report the isolation and structure elucidation of a new isoflavonoid glycoside derivative from the methanol extract of *M. indica* leaf.

EXPERIMENTAL SECTION

General experimental procedures

The following instruments were used: IR spectra was measured with a Perkin Elmer Spectrum One FTIR spectrometer, the ¹H and ¹³C NMR spectra were recorded with a Bruker Advance [300 MHz (¹H) and 75 MHz (¹³C)], and HRESI-MS were obtained with a JEOL AccuTOF-T100LP mass spectrometer. The following adsorbents were used for purification: vacuum liquid chromatography (Si-gel 60, Merck catalog number: 1.07749) and column chromatography (Si-gel 60, Merck catalog number: 1.09385), and for analysis (TLC, aluminum based, Merck,

Kieselgel 60 F₂₅₄ 0.25 mm). Solvents used in this research are analytical grade and industrial grades that were distilled before used.

Plant material

The leaves of *M. indica* were collected from Felda Seriting Hilir, Bahau, Negeri Sembilan, Malaysia. The plant was identified by a botanist from Universiti Putra Malaysia.

Extraction and isolation

The air-dried leaves (1.0 kg) was ground and extracted successfully with petroleum ether and MeOH at room temperature. The resulting of MeOH extract was filtered and evaporated under reduced pressure to give a dark green residue (28.4 g). Part of the MeOH extract (20 g) was subjected to vacuum liquid chromatography using a stepwise gradient solvent system of *n*-Hexane-CHCl₃(20% CHCl₃ - 100% CHCl₃ and washed with 100% MeOH) to give 10 fractions (A₁-A₁₀). The fraction eight (5.3 g) was refractionated with vacuum liquid chromatography (eluted with mixtures of CHCl₃/MeOH 80:20) to give 13 fractions (A₈₍₁₎-A₈₍₁₃₎). Purification of fraction A₈₍₁₀₎(77.6 mg) with preparative thin layer chromatography (silica gel GF₂₅₄, 20x20, 1 mm plate) using solvent system CHCl₃-MeOH(8:2), gave compound **1**(22 mg).

Compound **1**: yellow reddish crystal. IR (KBr) ν_{\max} cm⁻¹: 3435, 2930,1625,1512,1449, 1384, 1317, 1277, 1166, 1084, 1036, and 613. ¹H and ¹³C NMR spectral data: see Table 1.HRESI-MS *m/z*: [M]⁺ 448.1365 (Calc. for C₂₂H₂₄O₁₀, 448.1371).

RESULTS AND DISCUSSION

The dried powder of *M. indicaleaf* was extracted successively with petroleum ether and methanol at room temperature. The methanol extract was subjected to silica gel column chromatography and preparative-TLC to give a new compound **1**.

Table 1: ¹H NMR spectroscopy data of compound **1**.

No	δ_{H} (mul., <i>J</i> in Hz)	δ_{C}	HMBC (¹ H \leftrightarrow ¹³ C)
2	3.65 (<i>m</i>) 3.91 (<i>m</i>)	61.1 -	
3	3.40 (<i>m</i>)	55.9 -	
4	-	197.0 H-6, H-2'/H-6'	
5a	-	104.1 H-3	
5	-	161.8 H-6	
6	6.12 (<i>s</i>)	91.0 -	
7	-	159.4 H-6, H-1''	
8	-	104.1 H-6, H-1'', H-2''	
8a	-	161.8 H-2, H-3, H-1''	
1'	-	131.8 H-2'/H-6'	
2'/6'	7.67 (<i>d</i> , <i>J</i> =8.4)	131.7 H-3'/H-5'	
3'/5'	6.80 (<i>d</i> , <i>J</i> =8.4)	114.3 H-2'/H-6'	
4'	-	162.0 H-2'/H-6', H-3'/H-5', OCH ₃	
1''	4.90 (<i>m</i>)	74.9 H-2'', H-3'', H-4'', H-5''	
2''	3.44 (<i>m</i>)	70.1 H-1'', H-3'', 5''	
3''	3.46 (<i>m</i>)	78.4 H-2'', H-4'', H-5''	
4''	3.86 (<i>m</i>)	71.9 H-1'', H-3'', H-5'', H-6''	
5''	3.40 (<i>m</i>)	81.1 H-1'', H-2'', H-3'', H-4'', H-6''	
6''	3.72 (<i>dd</i> , 4.8; 9.0) 3.61 (<i>dd</i> , 4.8; 9.0)	63.0 H-4'', H-5''	
OCH ₃	3.81 (<i>s</i>)	54.8 -	

Measured in methanol-*d*₆ at 300MHz(¹H) and 75 MHz (¹³C).

Compound **1** was isolated as a yellow reddish crystal. A molecular formula of C₂₂H₂₄O₁₀ was deduced for **1** from its [M]⁺ ion at *m/z* 448.1365 in the HRESI-MS, a formula characteristic for a flavanoneglucoside. The IR spectrum indicated the presence of hydroxyl (3435 cm⁻¹), carbonyl (1625cm⁻¹) and aromatic rings (1512, 1449 cm⁻¹). The ¹³C NMR and DEPT-135 spectrum of **1**(Table 1) showed 19 carbon signals representing 24 carbons and are distributed into six aromatic carbons (four oxyaryl, three quarternary, and five methine) at δ_{C} 91.0-165.7, nine aliphatic carbons (five methine, two methylene and one methyl) at δ_{C} 54.8-81.1, and a carbonyl carbon at δ_{C} 197.0. The ¹H NMR and COSY spectra of **1** (Table 1) showed ABX aliphatic signal the characteristic of isoflavanone signal for H-2_a at

δ_{H} 3.65 (1H, *m*), H-2_b at δ_{H} 3.91 (1H, *m*) and H-3 at δ_{H} 3.40 (1H, *m*). A singlet at δ_{H} 6.12 is due to an aromatic proton suggesting that three substituents were linked to A-ring. The presence of AA'BB' aromatic system could be observed at δ_{H} 7.67 (2H, *d*, $J = 8.4$ Hz, H-2'/H-6') and 6.80 (2H, *d*, $J = 8.4$ Hz, H-3'/H-5'). In addition, a singlet at δ_{H} 3.81 due to a methoxy group was also observed. The ^1H and ^{13}C NMR spectra (Table 1) revealed an anomeric signals at δ_{H} 4.90 (1H, *m*) and δ_{C} 74.9 together with six carbon signals in the region of sugars indicating **1** to be an isoflavanone C-glycoside and not O-glycoside since the anomeric carbon of this glycoside is more deshielded. These features are characteristic of a dihydrobiochanin A [5,7-dihydroxy-3-(4-methoxyphenyl)chroman-4-one] derivative [11-12] with addition of a glycosidic substituent at ring A. The 2D-NMR (HMQC, COSY, and HMBC) measurements, therefore, were used to determine the exact position of the unit structure of H-2_a and H-2_b.

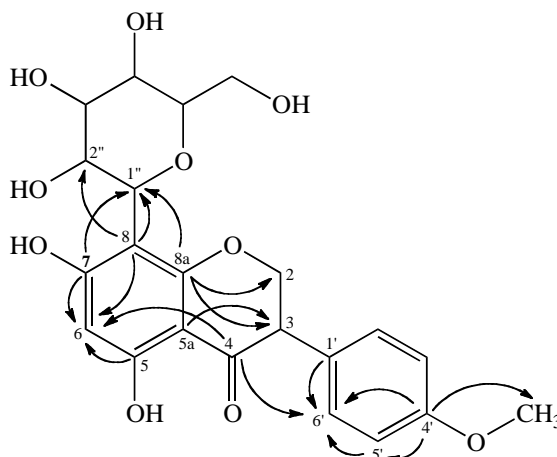


Figure 1: Key HMBC correlations ($^1\text{H} \leftrightarrow ^{13}\text{C}$) of compound **1**

The heteronuclear multiple bond correlation spectroscopy (HMBC) spectrum (Table 1, Fig. 1) showed cross peaks between proton at δ_{H} 7.67 (H-2'/H-6') and 6.80 (H-3'/H-5') with carbon at δ_{C} 162.0 (C-4') and between proton at δ_{H} 3.81 (CH₃O) with carbon C-4', respectively, thus determine the position of the methoxy group to be at C-4'. Furthermore, the HMBC spectra (see Fig. 1) also showed long-range correlation between anomeric proton signal at δ_{H} 4.90 with the ^{13}C signals at δ_{C} 159.4 (C-7), δ_{C} 104.1 (C-8) and δ_{C} 161.8 (C-8a), establishing the location of the glucosidic ring at carbon C-8 of A-ring. The correlation between δ_{H} 7.67 (H-2'/H-6') with carbonyl carbon at δ_{C} 197.0 further confirms the attachment of B-ring to C-3 suggesting the isoflavanone moiety. The other correlations are in agreement with the proposed structure. From the above evidence, the structure of **1** was concluded to be a new compound assigned as malaysianone B, adihydrobiochanin A-glycoside derivative.

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REFERENCES

- [1] Youngmok K., Angela J. Lounds-Singleton, Stephen T. T. *Food Chemistry*, **2009**, 115, 989–993.
- [2] Burkill, I.H. A dictionary of economic products of the Malay Peninsular. Ministry of Agriculture and Co-operative, Malaysia, Vol 1&2, **1966**, 112, 1424-1431.
- [3] Talcott, S. T., Moore, J. P., Lounds-Singleton, A. J., Percival, S. S. *Journal of Food Science*, **2005**, 70, 337–341.
- [4] Saleh, N. A. M., El-Ansari, M. A. I. *Planta Medica*, **1975**, 28, 124–130.
- [5] Kitanov, G.M., Assenov, I. *Pharmazie bulgaria*, **1988**, C13(12), 879-880.
- [6] Schieber, A., Ullrich, W., Carle, R. *Innovative Food Science and Emerging Technologies*, **2000**, 1, 161–166.
- [7] Sanchez, G. M., Re, L., Giuliani, A., Nunez, A., Davison, G. P., Leon, O. S. *Pharmacological Research*, **2000**, 42, 565–573.
- [8] Schieber, A., Berardini, N., Carle, R. *Journal of Agricultural and Food Chemistry*, **2003**, 51, 5006–5011.
- [9] Rodriguez, J., Pierro, D. D., Gioia, M., Monaco, S., Delgado, R., Coletta, M. *Biochimica et Biophysica Acta*, **2006**, 1760(9), 1333–1342.

- [10] Garrido, G., Gonzalez, D., Lemus, Y., Garcia, D., Lodeiro, L., Quintero, G. *Pharmacological Research*, **2004**, 50, 143–149.
- [11] Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K., Itokawa, H. *Chemistry and Pharmaceutical Bulletin*, **1992**, 40, 2970–2974.
- [12] Heinonen, S., Wahala, K., Adlercreutz, H. *Journal of Agricultural and Food Chemistry*, **2004**, 52, 6802–6809.