

THE ISOLATED COMPOUNDS FROM THE TWIGS OF *MORUS ALBA* L. IN DONG THAP

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ABSTRACT

From the ethyl acetate extract of the twigs of *Morus alba* L. (Moraceae) growing in Dong Thap, Vietnam, a pentacyclic triterpenoid, β -amyirin- β -D-glucopyranoside (**1**), a Diels – Alder type adduct, mulberrofuran G (**2**), a benzofuran, moracin M (**3**) and a prenylated flavonoid, kuwanon G (**4**) were isolated through repeated silica gel column chromatography. Their structures were identified by physicochemical and spectrometric methods.

Keywords: β -amyirin- β -D-glucopyranoside, mulberrofuran G, moracin M, kuwanon G, *Morus alba*.

1. INTRODUCTION

The genus *Morus* (Moraceae) comprises about a dozen of species which are widely distributed in India, China, Japan, North Africa, Arabia, South Europe, etc. [1]. Five species were found in Vietnam [2]. White mulberry (*Morus alba* L.) is widely cultivated in many Asian countries where the leaves are used as the best feed for silkworms because they contain many nutritional components [3]. Almost all parts of this plant is used in folk medicine in Vietnam as well as in China, Korea and Japan [4]. In recent studies many potentially active compounds have been isolated from *Morus alba* L. including flavonoids [5], benzofurans and stilbenoid [6], lignans [7], alkaloids and amino acids [8], coumarins [9], and triterpenoids [10]. In this study, we report the isolation of four compounds from twigs of *Morus alba* in Dong Thap. Their spectroscopic data were compared with previously reported ones to determine their chemical structures as: a triterpenoid, β -amyirin- β -D-glucopyranoside (**1**), a Diels – Alder type adduct, mulberrofuran G (**2**), a benzofuran, moracin M (**3**) and a prenylated flavonoid, kuwanon G (**4**). Although there are many reports on the chemical composition of root bark and leaf of *Morus alba* L., those on the twigs are few. This is the first study on the chemical composition of white mulberry twigs from Dong Thap, Vietnam.

2. MATERIALS AND METHODS

2.1. Plant material

The twigs of *Morus alba* were harvested in June 2015 in Sa Dec, Dong Thap, Vietnam. We collected the twigs of adult plants in the morning.

2.2. Instruments

Melting points were determined on a Yanaco micro melting point apparatus and were uncorrected. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh; E. Merck). Silica gel 60F₂₅₄ precoated plates (Merck) were used for TLC, developed with a CHCl₃–CH₃OH (6:1; 4:1), visualized under UV light λ 254 nm. ¹H-NMR and ¹³C-NMR spectra were recorded on a Varian Unity INOVA-400 spectrometer (USA) using tetramethylsilane (TMS) as internal standard. EI-MS data were collected on a Quattro II spectrometer.

2.3. Extraction and isolation

The twigs of *Morus alba* (6 kg) were dried and powdered and soaked with methanol (7 L x 3 times) at room temperature. The extract was concentrated to obtain crude methanol residue (315 g) which was resuspended in water (1 L) and partitioned successively in hexane, ethyl acetate, to afford hexane (42 g), ethyl acetate (89 g), and water soluble fractions.

The ethyl acetate fraction (89 g) was chromatographed over a silica gel column (0.6 kg, 120 × 10 cm) by gradient elution with hexane and increasing concentrations of acetone (25:1; 20:1; 15:1; 10:1; 7:1; 4:1; 2:1; 1:1, each 2 L) to afford eight fractions (fraction 1 - 8). The fraction 4 (2 g) was further subjected to silica gel CC (50 × 2 cm) eluted with hexane:acetone (10:1) to yield β -amyrin-glucopyranoside (**1**) (15 mg). Silica gel CC (100 g, 60 × 3 cm) of fraction 6 (18 g) with gradient elution of hexane:acetone (7:1; 4:1; 2:1, each 1 L) gave mulberrofuran G (**2**) (10 mg) and moracin M (**3**) (9 mg). Purification of fraction 7 (15 g) by CC on silica gel (100 g, 60 × 3 cm) and elution with hexane:acetone (2:1) afforded kuwanon G (**4**) (58 mg).

β -amyrin- β -D-glucopyranoside (**1**): colourless crystal, R_f : 0.6 (CHCl₃: CH₃OH, 4:1), m. p. 213–215°C. ¹H-NMR (400 MHz, C₆D₅N) δ_H 5.35 (1H, *t*, J = 2.8 Hz, H-12), 5.06 (1H, *d*, J = 6.0 Hz, H-6'), 4.57 (1H, *br d*, J = 8.0 Hz, H-1'), 4.47 (1H, *dd*, J = 4.0, 9.6 Hz, H-2'), 4.29 (2H, *m*, H-3', H-4'), 4.06 (1H, *t*, J = 6.4; 12.8, H-6'), 3.99 (1H, *m*, H-5'), 3.94 (1H, *m*, H-3), 2.73 (1H, *m*, H-18), 0.99 (3H, *s*, H-29), 0.98 (3H, *s*, H-23), 0.94 (3H, *s*, H-28), 0.91 (3H, *s*, H-27), 0.89 (3H, *s*, H-25), 0.87 (3H, *s*, H-26), 0.85 (3H, *s*, H-30), 0.66 (3H, *s*, H-24). ¹³C-NMR (100 MHz, C₆D₅N) δ_C 141.4 (C-13), 122.6 (C-12), 103.0 (C-1'), 79.2 (C-3), 79.0 (C-5'), 78.6 (C-3'), 75.8 (C-2'), 72.2 (C-4'), 63.4 (C-6'), 57.2 (C-5), 56.7 (C-9), 50.9 (C-18), 46.5 (C-19), 43.0 (C-14), 40.5 (C-8), 39.8 (C-4), 38.0 (C-1), 37.4 (C-22), 36.9 (C-10), 34.8 (C-21), 32.7 (C-29), 32.6 (C-7), 30.8 (C-17), 30.0 (C-20), 29.0 (C-15), 29.0 (C-23), 26.9 (C-28), 24.9 (C-2), 24.0 (C-16), 21.8 (C-27), 20.4 (C-30), 19.9 (C-11), 19.7 (C-6), 19.5 (C-26), 12.6 (C-24), 12.4 (C-25). EI-MS m/z 588 [M]⁺ (molecular formula, C₃₆H₆₀O₆).

Mulberrofuran G (**2**): white amorphous powder, R_f : 0.55 (CHCl₃: CH₃OH, 6:1), m.p. 180–182°C. ¹H-NMR (400 MHz, CD₃OD) δ_H 7.40 (1H, *d*, J = 6.8 Hz, H-4), 7.19 (1H, *d*, J = 6.8 Hz, H-14''), 7.16 (1H, *d*, J = 6.8 Hz, H-20''), 6.99 (1H, *br s*, H-3), 6.96 (2H, *br s*, H-2', H-7), 6.88

(1H, *d*, $J = 0.8$ Hz, H-6'), 6.80 (1H, *dd*, $J = 1.6$; 6.8 Hz, H-5), 6.52 (1H, *dd*, $J = 2.0$, 6.8 Hz, H-13''), 6.47 (1H, *br d*, $J = 3.6$ Hz, H-2''), 6.41 (1H, *d*, $J = 2.0$ Hz, H-17''), 6.38 (1H, *d*, $J = 2.0$ Hz, H-11''), 6.20 (1H, *dd*, $J = 1.6$, 6.8 Hz, H-19''), 3.42 (1H, *m*, H-3''), 3.36 (1H, *m*, H-4''), 3.02 (1H, *ddd*, $J = 4.4$, 9.2, 13.6 Hz, H-5''), 2.73 (1H, *dd*, $J = 4.4$, 13.6 Hz, H-6''), 2.09 (1H, *dd*, $J = 3.2$, 12.8 Hz, H-6''), 1.86 (3H, *s*, H-7''). $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) δ_{C} 160.1 (C-10''), 158.4 (C-18''), 157.9 (C-12''), 157.8 (C-6), 157.3 (C-5'), 156.8 (C-2), 155.8 (C-3'), 154.9 (C-16''), 153.6 (C-7a), 133.9 (C-1''), 131.5 (C-1'), 130.6 (C-14''), 128.0 (C-20''), 123.4 (C-2''), 123.2 (C-3a), 122.0 (C-4), 118.3 (C-4'), 117.4 (C-15''), 114.0 (C-9''), 113.3 (C-5), 110.0 (C-19''), 107.0 (C-13''), 105.6 (C-6'), 105.1 (C-2'), 104.5 (C-17''), 104.2 (C-11''), 103.2 (C-8''), 102.1 (C-3), 98.5 (C-7), 37.7 (C-3''), 36.8 (C-5''), 35.4 (C-4''), 28.9 (C-6''), 23.9 (C-7''). EI-MS m/z 562 $[\text{M}]^+$ (molecular formula, $\text{C}_{34}\text{H}_{26}\text{O}_8$).

Moracin M (**3**): brown crystal, R_f : 0.52 (CHCl_3 : CH_3OH , 4:1), m.p. 260 - 263°C. $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ_{H} 7.36 (1H, *d*, $J = 8.0$ Hz, H-4), 6.92 (1H, *d*, $J = 0.8$ Hz, H-3), 6.91 (1H, *d*, $J = 2.4$ Hz, H-7), 6.77 (1H, *d*, $J = 2.4$ Hz, H-6'), 6.76 (1H, *d*, $J = 2.4$ Hz, H-2'), 6.73 (1H, *dd*, $J = 2.4$, 8.0 Hz, H-5), 6.25 (1H, *t*, $J = 2.4$ Hz, H-4'). $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) δ_{C} 160.0 (C-3', C-5'), 157.3 (C-7a), 156.9 (C-6), 156.1 (C-2), 133.8 (C-1'), 123.0 (C-3a), 122.0 (C-4), 113.2 (C-5), 103.9 (C-2', C-6'), 103.5 (C-4'), 102.2 (C-3), 98.5 (C-7). EI-MS m/z 562 $[\text{M}]^+$ (molecular formula, $\text{C}_{34}\text{H}_{26}\text{O}_8$).

Kuwanon G (**4**): amorphous powder, R_f : 0.55 (CHCl_3 : CH_3OH , 4:1), m.p. 214 - 218°C. $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ_{H} 7.35 (1H, *d*, $J = 8.4$ Hz, H-27 or H-6'), 7.15 (1H, *d*, $J = 8.4$ Hz, H-27 or H-6'), 6.75 (1H, *d*, $J = 8.0$ Hz, H-33), 6.51 (1H, *br s*, H-5'), 6.48 (1H, *d*, $J = 7.6$ Hz, H-3'), 6.15 (1H, *br s*, H-30), 6.09 (1H, *dd*, $J = 2.4$, 8.0 Hz, H-32), 6.0 (2H, *br s*, H-6, H-24), 5.91 (1H, *br d*, $J = 8.8$ Hz, H-26), 5.18 (2H, *m*, H-10, H-15), 4.60 (1H, *br s*, H-20), 4.35 (1H, *br d*, $J = 9.6$ Hz, H-14), 3.68 (1H, *br s*, H-19), 3.18 (2H, *br s*, H-9), 1.97 (2H, *br d*, $J = 14.0$ Hz, H-18), 1.64 (3H, *s*, H-12), 1.49 (3H, *br s*, H-17), 1.46 (3H, *s*, H-13). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ_{C} 210.2 (C-21), 183.9 (C-4), 165.9 (C-23), 165.6 (C-7, C-25), 162.5 (C-2, C-8a, C-4'), 161.7 (C-29, C-2'), 161.0 (C-31), 157.7 (C-5), 134.4 (C-16, C-33), 132.7 (C-11, C-27, C-6'), 124.6 (C-15), 123.0 (C-10, C-28), 121.6 (C-3), 115.9 (C-22), 113.8 (C-1'), 108.6 (C-8, C-26), 108.2 (C-32), 107.9 (C-5'), 105.7 (C-4a), 103.7 (C-24), 103.6 (C-3'), 102.9 (C-30), 98.5 (C-6), 47.8 (C-20), 39.1 (C-18), 25.9 (C-12, C-17), 24.7 (C-9), 23.1 (C-14, C-19), 17.7 (C-13). EI-MS m/z 692 $[\text{M}]^+$ (molecular formula, $\text{C}_{40}\text{H}_{36}\text{O}_{11}$).

3. RESULTS AND DISCUSSION

Compound 1 was obtained as colourless crystals. The mass spectrum (EI-MS) exhibited a molecular ion peak at m/z 588 $[\text{M}]^+$, corresponding to the molecular formula $\text{C}_{36}\text{H}_{60}\text{O}_6$. In the $^1\text{H-NMR}$ spectrum of **1**, a broad doublet signal at δ 4.57 ($J = 8.0$ Hz) was ascribed to H-1' anomeric proton while protons from hexose moiety appeared at 5.06 (1H, *d*, $J = 6.0$ Hz) and 4.06 (1H, *t*, $J = 6.4$; 12.8 Hz) were methylene protons of H-6', 4.47 (1H, *dd*, $J = 4.0$, 9.6 Hz, H-2'), 4.29 (2H, *m*, H-3', H-4'), and 3.99 (1H, *m*, H-5'). A downfield triplet centered at δ 5.35 ($J = 2.8$ Hz) was assigned to H-12 vinylic proton, multiplet signals at δ 3.94 and 2.73 were assigned to H-3, H-18, respectively. Eight singlet signals of methyl protons were found at δ 0.99 (H-29), 0.98 (H-23), 0.94 (H-28), 0.91 (H-27), 0.89 (H-25), 0.87 (H-26), 0.85 (H-30), 0.66 (H-24), all located on the saturated carbons. The $^{13}\text{C-NMR}$ spectrum of **1** displayed signals for thirty six carbons which consist of thirty carbons of pentacyclic triterpenic skeleton and six carbons of

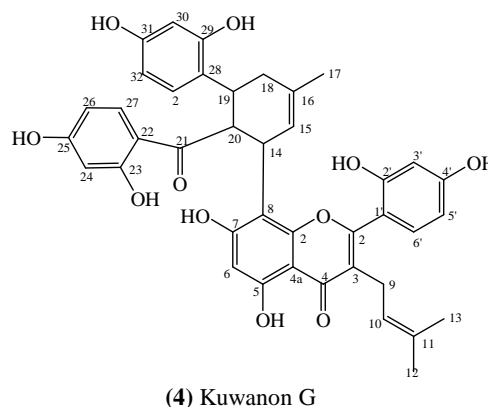
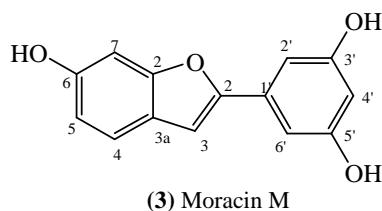
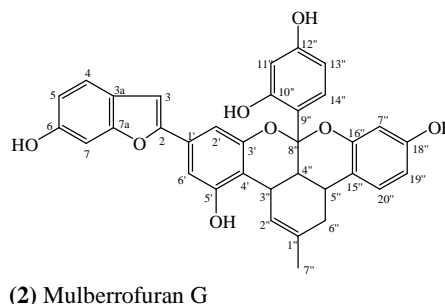
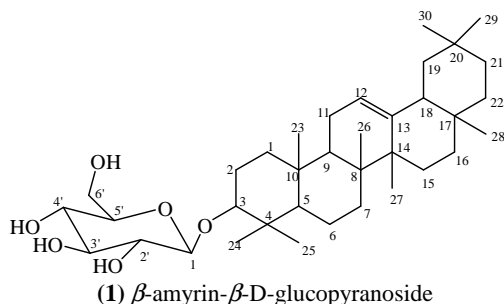
glucosidic sugar. The signals appeared at δ 141.4 and 122.6 are assigned for vinylic carbons (C-13 and C-12), at δ 103.0 for anomeric carbon (C-1') and δ 79.2 for carbinol carbon (C-3). The methyl carbons showed up at δ 29.0 (C-23), 12.6 (C-24), 12.4 (C-25), 19.5 (C-26), 21.8 (C-27), 26.9 (C-28), 32.7 (C-29), 20.4 (C-30). Sugar carbons appeared at δ 75.8 (C-2'), 78.6 (C-3'), 72.2 (C-4'), 79.0 (C-5'), 63.4 (C-6'). The assignments of the carbon chemical shift of **1** were made by comparison with data from literature [11]. Comparison of these spectral data with data from literature [11] allowed us to identify **1** as β -amyrin- β -D-glucopyranoside.

Compound 2 was isolated as a white amorphous powder with a molecular formula of $C_{34}H_{26}O_8$ as determined by the EI-MS peak at m/z 562 $[M]^+$. 1H -NMR spectrum of **2** displayed three group of signals of aromatic protons being characteristic for ABX spin systems denoted by the resonances at δ 7.40 (1H, *d*, $J = 6.8$ Hz, H-4), 6.80 (1H, *dd*, $J = 1.6; 6.8$ Hz, H-5), 6.96 (1H, *br s*, H-7); 6.38 (1H, *d*, $J = 2.0$ Hz, H-11''), 6.52 (1H, *dd*, $J = 2.0, 6.8$ Hz, H-13''), 7.19 (1H, *d*, $J = 6.8$ Hz, H-14''); 6.41 (1H, *d*, $J = 2.0$ Hz, H-17''), 6.20 (1H, *dd*, $J = 1.6, 6.8$ Hz, H-19''), 7.16 (1H, *d*, $J = 6.8$ Hz, H-20''). Two singlet peaks at δ 6.99 and 6.96 were successively assigned to H-3 and H-2', a doublet signal at δ 6.88 ($J = 0.8$ Hz) was assigned to H-6' indicating three aromatic protons. In addition, signal of vinylic proton at δ 6.47 was assigned to H-2''. Three signals of methine at δ 3.42 (1H, *m*), 3.36 (1H, *m*) and 3.02 (1H, *ddd*, $J = 4.4, 9.2, 13.6$ Hz) were predicted for H-3'', H-4'', H-5''. Two doublet signals at δ 2.73 (1H, *dd*, $J = 4.4, 13.6$ Hz) and 2.09 (1H, *dd*, $J = 3.2, 12.8$ Hz) were assigned to methylene protons of H-6'', singlet signal at δ 1.86 to methyl protons, H-7''. The ^{13}C -NMR spectrum displayed signals for thirty four carbons. Nine aromatic carbons displayed downfield signals at δ 160.1 (C-10''), 158.4 (C-18''), 157.9 (C-12''), 157.8 (C-6), 157.3 (C-5'), 156.8 (C-2), 155.8 (C-3'), 154.9 (C-16''), 153.6 (C-7a) because they were linked to oxygen atom. Eighteen remaining aromatic carbon signals were found at δ 131.5 (C-1'), 130.6 (C-14''), 128.0 (C-20''), 123.2 (C-3a), 122.0 (C-4), 118.3 (C-4'), 117.4 (C-15''), 114.0 (C-9''), 113.3 (C-5), 110.0 (C-19''), 107.0 (C-13''), 105.6 (C-6'), 105.1 (C-2'), 104.5 (C-17''), 104.2 (C-11''), 102.1 (C-3), 98.5 (C-7). Other assignments were made to two vinylic carbon signals at δ 133.9 (C-1'') and 123.4 (C-2''), three methine carbons signals at 37.7 (C-3''), 35.4 (C-4'), 36.8 (C-5''), a quaternary carbon at δ 103.2 (C-8''), a methylene carbon at δ 28.9 (C-6''), and a methyl carbon at δ 23.9 (C-7''). Comparison of the spectral data of **2** with literature [12] identified **2** as the Diels–Alder type adduct, mulberrofuran G.

Compound 3 was obtained as brown crystals. The molecular formula of **3** was established as $C_{34}H_{26}O_8$ by EI-MS m/z 562 $[M]^+$. The 1H -NMR spectrum of **3** indicated aromatic proton signals at δ 7.36, 6.73 and 6.91 (H-4, H-5, H-7), 1,3,5-distributed benzene signals at δ 6.76, 6.25, and 6.77 (H-2', H-4', and H-6') and one proton of furan nucleus signal at 6.91 (H-3). The ^{13}C -NMR spectrum revealed the presence of fourteen aromatic carbons consisting of five carbons linked to oxygen showing signals downfield at δ 160.0 (C-3', C-5'), 157.3 (C-7a), 156.9 (C-6), 156.1 (C-2), and nine remaining carbons showing signals at δ 133.8 (C-1'), 123.0 (C-3a), 122.0 (C-4), 113.2 (C-5), 103.9 (C-2', C-6'), 103.5 (C-4'), 102.2 (C-3), 98.5 (C-7). By comparing NMR data of **3** to those of a benzofuran [13], its structure was determined to be moracin M.

Compound 4 was isolated as amorphous powder with a molecular formula of $C_{40}H_{36}O_{11}$ determined by the EI-MS peak at m/z 692 $[M]^+$. 1H -NMR spectrum of **4** displayed the presence of three characteristic ABX spin systems as follows: H-24, H-26, H-27 corresponding to the resonances at δ 6.0 (*br s*), 5.91 (*br d*, $J = 8.8$ Hz), 7.35 or 7.15 (*d*, $J = 8.4$ Hz); H-30, H-32, H-33 with signals at δ 6.15 (*br s*), 6.09 (*dd*, $J = 2.4, 8.0$ Hz), and 6.75 (*d*, $J = 8.0$ Hz, H-33), and H-3', H-5', H-6' successively denoted by the resonances at δ 6.48 (*d*, $J = 7.6$ Hz), 6.51 (*br s*), and 7.35 or 7.15 (*d*, $J = 8.4$ Hz). Another aromatic proton at δ 6.0 (*br s*) was assigned to H-6.

Moreover, protons in a cyclohexene moiety were found as vinylic proton at δ 5.18 (H-15), three methine protons at δ 4.35 (H-14), 3.68 (H-19), 4.60 (H-20), and two methylene protons at δ 1.97 (H-18). Nine protons were assigned to a prenyl group showing signals at δ 3.18 (*br s*, H-9), 5.18 (*m*, H-10), 1.64 (*s*, H-12), 1.46 (*s*, H-13). The ^{13}C -NMR spectrum displayed forty carbon signals. Two ketonic carbon signals appeared at δ 210.2 (C-21) and 183.9 (C-4), six vinylic carbons at δ 162.5 (C-2), 134.4 (C-16), 132.7 (11), 124.6 (C-15), 123.0 (C-10), 121.6 (C-3). Eight signals of aromatic carbons at δ 165.9 (C-23), 165.6 (C-7, C-25), 162.5 (C-8a, C-4'), 161.7 (C-29, C-2'), 161.0 (C-31), 157.7 (C-5) appeared toward lower field than fifteen remaining aromatic carbon signals at δ 134.4 (C-33), 132.7 (C-27, C-6'), 123.0 (C-28), 115.9 (C-22), 113.8 (C-1'), 108.6 (C-8, C-26), 108.2 (C-32), 107.9 (C-5'), 105.7 (C-4a), 103.7 (C-24), 103.6 (C-3'), 102.9 (C-30), 98.5 (C-6) because they were combined with oxygen. Three methine carbons and one methylene carbon of a cyclohexene moiety appeared at δ 47.8 (C-20), 23.1 (C-19), 23.1 (C-14) and 39.1 (C-18). Signals were found for one methylene carbon of the prenyl moiety at δ 24.7 (C-9) and three methyl carbons at 25.9 (C-17), 25.9 (C-12), 17.7 (C-13). These data suggested that compound **4** is a prenylated flavonoid [14], its structure was found to be identical with kuwanon G.



4. CONCLUDING REMARKS

Four compounds (**1-4**) were isolated from the ethyl acetate fraction of the twigs of *Morus alba* L. growing in Dong Thap, Vietnam by column chromatography on silica gel. Compounds **1-4** were identified as β -amyrin- β -D-glucopyranoside (**1**), mulberrofuran G (**2**), moracin M (**3**) and kuwanon G (**4**) by spectroscopic methods and by comparing their data with literature values. These compounds have been found in root barks and leaves of *Morus alba* L., it is the first time they were isolated from twigs of this plant.

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