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AN INITIAL STUDY ON CHEMICAL CONSTITUENTS OF *BISCHOFIA JAVANICA*

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ABSTRACT

From the methanol extract of *Bischofia javanica* Blume leaves, five compounds including 5'-*β*-D-glucopyranosyloxyjasmonic acid methyl ester (**1**), 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (**2**), hexyl-*O*-*β*-D-glucopyranoside (**3**), friedelan-3-one (**4**), and gallic acid (**5**) were isolated. Their structures were elucidated by NMR spectra as well as in comparison with previously reported data. This is the first report on the isolation of **1**, **2**, and **3** from *Bischofia javanica*.

Keyword: Bischofia javanica, jasmonic acid derivative, phenylethanoid.

1. INTRODUCTION

Bischofia javanica Blume species, belonging to the family Euphorbiaceae, is found widely in Vietnam, India, China, Indonesia, and Philippine [1]. In folk medicinal remedies, *B. javanica* was used for treatment of various diseases such as cancer, inflammation, tuberculosis, diarrhea, sore throat, burns and different allergic conditions. The barks, leaves, roots, and fruits of this plant are used to treat diphtheria, pharyngitis, tonsillitis, different skin diseases, and nervous disorders [2]. Antileukemic activity of the leaves extract of *B. javanica* was evaluated on human leukemic cell lines by Lingadurai *et al*. The methanol extract of this plant showed significant cytotoxicity against HL-60 cell line with an IC_{50} value as low as 3.5 µg/ml. In addition, methanol extract *B. javanica* (10 µg/ml) was demonstrated to induce apoptosis of HL-60 cancer cells which was strongly supported for the ethno-medicinal use of *B. javanica* leaves in the treatment of cancer [3]. Betulinic acid and its derivatives from chloroform extract of the bark of *B. javanica* were found to be catalytic inhibitors of Topo II activities with IC_{50} values ranging from 0.38 to 58 µM [4]. Besides, methanol extract of *B. javanica* leaves was reported to have antioxidant, antiinflammatory and antinociceptive activities [5, 6]. However, chemical compositions from *B. javanica* have not been extensively investigated to date. Several triterpenoids and phenolics such as betulinic acid, ursonic acid, β-amyrine, chrysoeriol, quercetin have been isolated from the leaves of *B. javanica* [4, 7, 8].

To clarify the active components, the methanol extract of leaves of *B. javanica* was subjected to chemical study. Herein, we report the isolation and structural elucidation of five

compounds, 5'-*β*-D-glucopyranosyloxyjasmonic acid methyl ester (**1**), 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (**2**), hexyl-*O*-*β*-D-glucopyranoside (**3**), friedelan-3-one (**4**), and gallic acid (**5**) from the methanol extract of *B. javanica*.

2. EXPERIMENTAL

2.1. Plant material

The leaves of *Bischofia javanica* Blume were collected at Melinh, Vinhphuc province, Vietnam in June, 2012. Its scientific name was identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (BJ1-2012) is deposited at the Faculty of Basic Science, University of Transport and Communications.

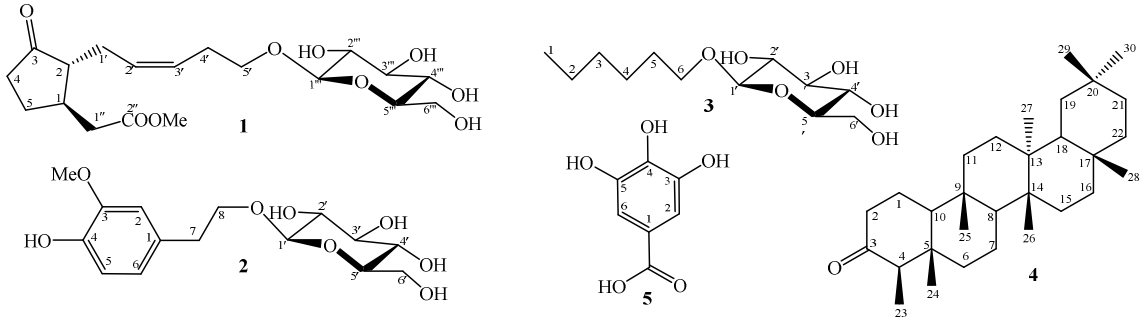

Figure 1. Chemical structures of **1** – **5.**

2.2. General experimental procedures

The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on Agilent 400-MR-NMR spectrometer and TMS was used as an internal standard. Column chromatography was performed using silica gel (Kieselgel 60, 70 - 230 mesh and 230 – 400 mesh, Merck, Whitehouse Station, NJ) or RP-18 resins (30 – 50 μm, Fuji silysia Chemical Ltd.). Thin layer chromatography (TLC) was carried out using pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried and powdered of *B. javanica* leaves (2.0 kg) were extracted with methanol at 40^{o}C for three times (10.0 L each). The organic layer was filtered and removed under *vacuo* to obtain 100.0 g of crude extract. This extract was suspended in distilled water (2.0 L) and successively partitioned with dichloromethane, ethyl acetate to give dichloromethane (BJC, 30.0 g), ethyl acetate (BJE, 12.0 g) extracts, and water soluble part. The BJC extract (30.0 g) was chromatographed on a silica gel column, eluting with a gradient elution of dichloromethane – methanol (50/1, 20/1, 5/1, 1/1, 0/1; v/v) to yield 5 fractions (BJC1 - BJC5). Fraction BJC2 (4.0 g) was chromatographed on a silica gel column, eluting with dichloromethane – acetone (10/1; v/v) to obtain 2 smaller fractions, named BJC2A and BJC2B. Compound **4** (10.0 mg) was obtained from fraction BJC2A using a silica gel column and eluted with dichloromethane – methanol (12/1; v/v). Compound **5** (7.0 mg) was obtained from fraction BJC2B using a RP-18 column and eluted with acetone/water (2/1; v/v).

Fraction BJC4 (3.0 g) was chromatographed on a RP-18 column, eluting with methanol/water (2/1; v/v) to obtain 3 sub-fractions, named BJC4A – BJC4C. Fraction BCJ4A was chromatographed on a RP-18 column, eluting with methanol/water/formic acid (2.5/1/0.01, v/v/v) to yield compound **3** (10.0 mg). Fraction BJC4C was chromatographed on a silica gel column eluting with dichloromethane/methanol (6/1; v/v) and further chromatographed on a RP-18 column eluting with acetone/water/formic acid (1/1/0.01; v/v/v) to obtain compounds **1** (6.0 mg) and **2** (8.0 mg).

5'-*β*-D-Glucopyranosyloxyjasmonic acid methyl ester (1): white powder. MF: $C_{19}H_{30}O_9$ (M = 402). ESI-MS: *m/z* 425 $[M+Na]^+$. ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD); see Table 1.

2-(4-Hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (2): amorphous powder. MF: $C_{15}H_{22}O_8$ (M = 330). ESI-MS: *m/z* 331 $[M+H]^+$. ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD); see Table 1.

Hexyl-*O*-*β*-D-glucopyranoside (3): white amorphous powder. MF: $C_{12}H_{24}O_6$ (M = 264). ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm): 4.22 (1H, d, *J* = 7.6 Hz, H-1′), 3,88 (1H, dd, *J* = 6.8, 15.2 Hz, H_a-1), 3.84 (1H, d, *J* = 12.0 Hz, H_a-6′), 3.64 (1H, dd, *J* = 4.8; 12.0 Hz, H_b-6′), 3.51 (1H, dd, *J* = 7.2; 15.2 Hz, H_b-1), 3.32* (1H, H-3′), 3.30* (1H, H-5′), 3.24* (1H, H-4′), 3.14 (1H, t, *J* = 7.6 Hz, H-2′), 1.59 (2H, m, *J* = 6.8 Hz, H-2), 1.35* (2H, H-3), 1.29* (2H, H-5), 1.28* (2H, H-4) and 0.89 (3H, t, *J* = 6.8 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ_C: 104.4 (C-1′), 78.1 (C-3′), 77.9 (C-5′), 75.1 (C-2′), 71.6 (C-4′), 70.9 (C-1), 62.7 (C-6′), 32.9 (C-4), 30.8 (C-2), 26.8 (C-3), 23.7 (C-5) and 14.4 (C-6). *: overlapped signals.

Friedelan-3-one (4): white amorphous powder. MF: $C_{30}H_{50}O$ (M = 426). ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 1.16 (s, 3H), 1.02 (s, 1H), 0.98 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.83 (d, *J* = 6.5 Hz, 3H), 0.84 (s, 3H) and 0.70 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (ppm): 213.4 (C-3), 59.4 (C-10), 58.2 (C-4), 53.1 (C-8), 42.7 (C-18), 42.1 (C-5), 41.5 (C-2), 41.2 (C-6), 39.7 (C-13), 39.2 (C-22), 38.2 (C-14), 37.4 (C-9), 35.9 (C-11), 35.6 (C-16), 35.3 (C-19), 35.0 (C-29), 32.7 (C-15), 32.4 (C-21), 32.0 (C-30), 31.7 (C-28), 30.5 (C-12), 29.9 (C-17), 28.1 (C-20), 22.3 (C-1), 20.2 (C-27), 18.6 (C-26), 18.2 (C-7), 17.9 (C-25), 14.6 (C-24), and 6.83 (C-23).

Gallic acid (5): yellow amorphous powder. MF: $C_7H_6O_5$ (M = 170). ^{1}H-NMR (400 MHz, CD_3OD) δ_H: 7.03 (2H, s, H-2, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ_C: 171.08 (COOH), 146.28 (C-3, C-5), 139.21 (C-4), 122.86 (C-1) and 110.33 (C-2, C-6).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white powder. The ^{1}H-NMR showed the presence of two olefin protons at δ_H 5.38 (1H, m) and 5.50 (1H, m); an anomeric proton at δ_H 4.25 (1H, d, *J* = 8.4 Hz) assigned for a sugar moiety; and a methoxy group at δ_H 3.66 (3H, s). The ^{13}C-NMR and DEPT of **1** showed 19 carbon signals, including two carbonyl groups (δ_C 221.6 and 174.5); two olefin carbons (δ_C 129.0 and 128.9); an oxymethylene carbon (δ_C 70.0); two methine carbons and three methylene carbons from δ_C 26.3 to 55.0; and five oxymethine and an oxymethylene of a *β*-glucopyranoside (δ_C 104.3, 75.1, 78.1, 71.6, 77.9 and 62.7). The one bond proton-carbon signals were assigned on the basis of HSQC correlations (*Table 1*). The COSY correlations between H-1 (δ_H 2.38) and H-2 (δ_H 1.97); between H-1′ (δ_H 2.40) and H-2′ (δ_H 5.38); between H-2′ (δ_H 5.38) and H-3′ (δ_H 5.50); between H-3′ (δ_H 5.50) and H-4′ (δ_H 2.42); between H-4′ (δ_H 2.42) and H-5′ (δ_H 3.54 and 3.88) confirmed the constitutional fragments (*Figure 2*). The HMBC correlations from H-5′ (δ_H 3.54 and 3.88) to carbons C-3′ (δ_C 129.0), C-4′ (δ_C 29.0), C-1‴ (δ_C

104.3); from H-2′ (δ_H 5.38) to carbons C-4′ (δ_C 29.0), C-2 (δ_C 55.0); from H-3′ (δ_H 5.50) to carbon C-1′ (δ_C 39.5) confirmed the position of double bond at C-2′/C-3′ and the position of glucose moiety at C-5′. Besides, the HMBC correlations from methoxy group (δ_H 3.66) and H-2′ (δ_H 2.38) to carbonyl carbon C-1′ (δ_C 174.5) were observed confirming the linkage of methyl ester at C-2′. Based on the above evidence, chemical structure of **1** was established as 5'-*β*-D-glucopyranosyloxyjasmonic acid methyl ester [9] and shown in *Figure 1*. This compound was previously isolated from leaves of *Thyus vulgaris* [10], *Phyllanthus urinaria* [11] and possessed a weak cytotoxic activity against CHO (Chinese hamster ovary) and J774 (Murine macrophage) cells [11].

Table 1. ^{1}H- and ^{13}C-NMR data for **1**-**2** and reference compounds.

	1				**2**		
C	$^{d}\delta_C$ [9]	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (*J*, Hz)	C	$^{a}\delta_C$ [12]	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (*J*, Hz)
1	39.2	39.2	2.38 (m)	1	131.8	131.5	-
2	55.0	55.0	1.97 (dt, 5.0; 10.1)	2	114.0	113.6	6.80 (s)
3	221.6	221.6	-	3	148.9	148.8	-
4	38.6	38.6	2.39*	4	146.0	145.8	-
			2.12 (m)				
5	28.1	28.1	2.20 (m)	5	116.2	116.0	6.63*
			1.50 (m)				
1′	26.4	26.3	2.40*	6	122.5	122.4	6.63*
2′	128.9	128.9	5.38 (m)	7	36.8	36.7	2.79 (t, 6.8)
3′	129.0	129.0	5.50 (m)	8	72.0	72.0	4.00 (m)
							3.66 (m)
4′	29.0	29.0	2.42*	1′	104.4	104.3	4.24 (d, 7.5)
5′	70.2	70.2	3.88*	2′	75.2	75.1	3.13 (dd, 7.5, 9.0)
			3.54 (dd, 7.2, 10.0)				
1″	174.5	174.5	-	3′	78.2	78.1	3.31 (t, 9.0)
2″	39.5	39.5	2.71 (dd, 3.6, 14.6)	4′	71.8	71.6	3.22 (t, 9.0)
			2.40*				
COOMe	52.1	52.1	3.66 (s)	5′	78.0	77.9	3.21 (m)
1‴	104.4	104.3	4.25 (d, 7.5)	6′	62.9	62.7	3.81 (dd, 3.0, 12.0)
							3.62 (dd, 5.0, 12.0)
2‴	75.1	75.1	3.15 (dd, 7.5, 9.0)	OMe	56.6	56.3	3.78 (s)
3‴	78.1	78.1	3.32 (t, 9.0)				
4‴	71.6	71.6	3.30 (t, 9.0)				
5‴	77.9	77.9	3.29 (m)				
6‴	62.8	62.7	3.84 (dd, 3.0, 12.0)				
			3.64 (dd, 5.0, 12.0)				

*Measured in [a] CD_3OD [b]100 MHz, [c]400 MHz. *overlapped signals. $\delta_C^{\#}$ 5'-β-D-glucopyranosyloxyjasmonic acid methyl ester* [9], *$\delta_C^{\$}$ 2-(4-hydroxy-3-methoxyphenyl)ethyl-O-β-D-glucopyranoside* [12].

Compound **2** was obtained as amorphous powder. The ^{1}H-NMR showed three protons of a 1,3,4-trisubstituted aromatic ring at δ_H 6.80 (s, H-2), 6.63 (overlapped, H-5 and H-6); an anomeric proton at δ_H 4.24 (1H, d, *J* = 7.6 Hz); and a methoxy group at δ_H 3.78 (3H, s). The ^{13}C-NMR and DEPT showed six carbon signals of a 1,3,4-trisubstituted aromatic ring at δ_C 148.8 (C-3), 145.8 (C-3), 131.5 (C-1), 122.4 (C-6), 116.0 (C-5) and 113.6 (C-2); six carbons of a

β-glucose at δ_C 104.3 (C-1′), 75.1 (C-2′), 78.1 (C-3′), 71.6 (C-4′), 77.9 (C-5′) and 62.7 (C-6′). Besides, the signals of a oxymethylen carbon at δ_C 72.0 (C-8); a methylene carbon at δ_C 36.7 (C-7); and a methoxy group at δ_C 56,3 were observed. The NMR data of **2** were similar to those of 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside [12]. The chemical structure of **2** was assigned with the aid of HSQC (Table 1) and HMBC (Figure 2) spectra. The HMBC correlation from anomeric proton H-1′ (δ_H 4.24) to carbon C-8 (δ_C 72.0) confirmed the position of *β*-glucose moiety at C-8. The position of methoxy group at C-3 was confirmed by the HMBC correlations from methylene proton H-7 (δ_H 2.79) to carbons C-2 (δ_C 113.6)/C-6 (δ_C 122.4), from H-6 (δ_H 6.62) to carbon C-4 (δ_C 145.8) and from methoxy signal (δ_H 3.78) to carbon C-3 (δ_C 148.8). Based on the above evidence, chemical structure of **2** was established as 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside and shown in Figure 1. Compound **2** were previously isolated from various plant such as *Tetrastigma hemsleyanum* [13], *Nanophyton erinaceum* [14] and *Laurus nobilis* [12]. It was reported to have antioxidant and hepatoprotective effects [15].

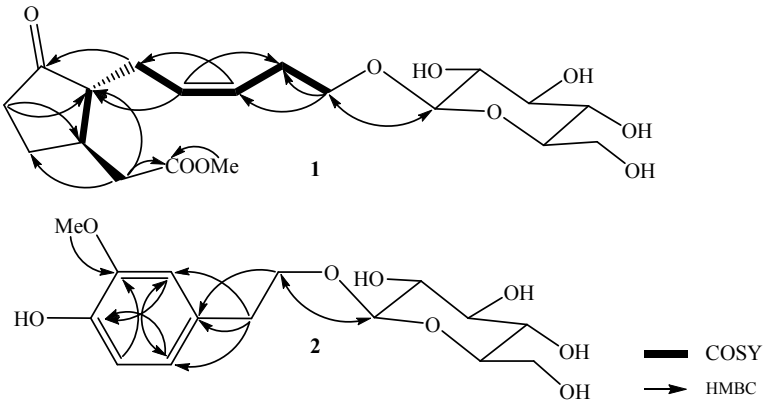

Figure 2. The key COSY and HMBC correlations of **1** and **2.**

Compounds **3**, **4**, and **5** were identified as hexyl-*O*-*β*-D-glucopyranoside [16], friedelan-3-one [17], and gallic acid [18] based on their spectral evidence, which were in agreement with those of the reported data in the literature, respectively. Among isolated compounds, the compounds **1**, **2** and **3** were firstly isolated from *B. javanica* leaves. Meanwhile, compounds **4** and **5** have been previously isolated from this plant, showing broad range of biological activities [7, 8]. Gallic acid (**5**) is known as the active principle responsible for the regeneration of β-cells and normalizing all the biochemical parameters related to the patho-biochemistry of diabetes mellitus and hence it could be used as a potent antidiabetic agent [18].

4. CONCLUSIONS

Five compounds 5'-*β*-D-glucopyranosyloxyjasmonic acid methyl ester (**1**), 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (**2**), hexyl-*O*-*β*-D-glucopyranoside (**3**), friedelan-3-one (**4**), and gallic acid (**5**) were isolated from dichloromethane soluble fraction of *B. javanica* leaves. Compounds **1**, **2** and **3** were isolated from *B. javanica* for the first time.

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