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Biflavones and megastigmane glycosides from the leaves of *Antidesma bunius*

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Abstract

Two biflavones, podocarpusflavone A (**1**) and amentoflavone (**2**) and two megastigmane glycosides, byzantionoside B (**3**) and (6*S*,9*R*)-roseoside (**4**) were isolated from the methanol extract of the leaves of *Antidesma bunius*. Their structures were determined by spectroscopic methods and in comparison with the published data.

Keywords. *Antidesma bunius*, Euphorbiaceae, biflavone, megastigmane.

1. INTRODUCTION

Antidesma bunius (L.) Spreng belongs to Euphorbiaceae family and widely distributes throughout Vietnam and China. The fruits of *A. bunius* are edible and have been used to prepare drink supplement or healthy foods. In traditional medicine, *A. bunius* was used for the treatment of inflammation and infection diseases [1]. Phytochemical analysis of this plant indicated the presence of flavonoids, phenolics and organic acids [2]. In addition, this plant exhibited antioxidant [3] and antimicrobial activities [4]. Herein, we report the isolation and structure elucidation of two biflavones and two megastigmane glycosides from the methanol extract of *A. bunius* leaves.

2. MATERIAL AND METHODS

2.1. Plant material

The leaves of *Antidesma bunius* (L.) Spreng were collected in Daklak province, Vietnam, in March 2013, and identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature. A voucher specimen (AB1303) was deposited at Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). NMR measurements, including ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC experiments, were carried out using 5-mm probe tubes at temperature of 22.2^{o}C. Optical rotations were determined on a Jasco DIP-1000 polarimeter. Column chromatography was performed using a silica-gel (Kieselgel 60,70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (30-50 µm, Fujisilisa Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried powder leaves of *A. bunius* (2.0 kg) were sonicated in methanol (MeOH) three times to yield 110.0 g of a dark solid extract, which was then suspended in water and successively partitioned with CH_2Cl_2 and ethyl acetate (EtOAc) to give CH_2Cl_2 (AD1, 17.0 g), EtOAc (AD2, 20.0 g), and water layers (AD3, 73.0 g) after removing solvent *in vacuo*. The EtOAc layer (AD2, 20.0 g) was chromatographed on a RP-18 column eluting with MeOH/water (3/1, v/v) to give three fractions, AD2A-AD2C. The AD2B fraction was applied to a silica gel column eluting with CH_2Cl_2/MeOH/water (5/1/0.1, v/v/v) to yield compounds **2** (30.0 mg) and **1** (40.0 mg). The water layer (AD3, 73.0 g) was subjected to a Diaion HP-20 column eluting with water to remove sugar, then increase concentration of methanol in water (25, 50, 75, and 100 %) to obtain 4 fractions, AD3A-AD3D. The AD3B fraction

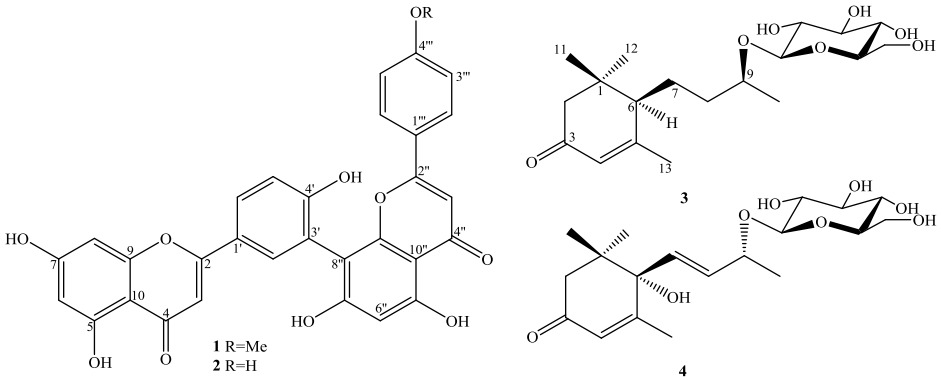

Figure 1: Chemical structures of compounds **1**-**4**

was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (8/1, v/v) to give three fractions, AD3B1-AD3B3. The AD3B1 fraction was further purified by a RP-18 column eluting with MeOH/water (1/2, v/v) to yield compounds **3** (20.0 mg) and **4** (15.0 mg).

Podocarpusflavone A (1): Yellow powder, $C_{31}H_{20}O_{10}$, ESI-MS *m/z* 551 $[M-H]^-$, ^{1}H- and ^{13}C-NMR ($DMSO-d_6$), see table 1.

Amentoflavone (2): Yellow amorphous powder, $C_{30}H_{18}O_{10}$, ESI-MS *m/z* 537 $[M-H]^-$, ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

Byzantionoside B (3): White powder, $C_{19}H_{32}O_7$, $[\alpha]_D^{25}$: +50.0 (*c* = 0.1, MeOH), ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

(6*S*,9*R*)-roseoside (4): White powder, $C_{19}H_{30}O_8$, $[\alpha]_D^{25}$: +65.0 (*c* = 0.1, MeOH), ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow amorphous powder. The molecular formula of **1** was determined to be $C_{31}H_{20}O_{10}$, by the combination of ESI-MS ion at *m/z* 551 $[M-H]^-$ and ^{13}C-NMR data. The ^{1}H-NMR spectrum of compound **1** showed the following signals: three aromatic protons of the ABX system in B ring at δ_H 7.15 (1H, d, *J* = 8.0 Hz), 7.99 (1H, d, 1.5 Hz), and 8.01 (1H, dd, *J* = 1.5, 8.0 Hz); four aromatic protons of *para*-substituted aromatic ring at δ_H 6.92 (2H, d, *J* = 9.0 Hz), and 7.67 (2H, d, *J* = 9.0 Hz); three singlet protons at δ_H 6.41 (1H, s), 6.82 (1H, s), 6.88 (1H, s); two *meta*-protons of aromatic ring at δ_H 6.18 (1H, d, *J* = 2.0 Hz), and 6.45 (1H, d, *J* = 2.0 Hz). The ^{13}C-NMR and DEPT spectra of compound **1** showed the presence of two carbonyl at δ_C 181.74 and 182.15, sixteen non-protonated at δ_C 2 × 103.72, 104.01, 121.01, 119.97, 122.98, 154.54, 157.37, 159.54, 160.54, 161.45, 162.00, 162.20, 163.22, 163.79, and 164.11, twelve methine carbons at δ_C 94.03, 98.70, 98.83, 103.02, 103.24, 2×114.48, 116.18, 127.84, 2×127.98, 132.76, and one methoxy group at δ_C 55.50, assigned to a biflavone. The ^{1}H- and ^{13}C-NMR spectra of **1** were identical to those of podocarpusflavone A [5]. The position of methoxy group at C-4' was confirmed by the HMBC correlation from methoxy (δ_H 3.75) to C-4' (δ_C 162.20). The HMBC correlations from H-6 (δ_H 6.18)/H-8 (δ_H 6.45) to C-7 (δ_C 164.11); from H-6 (δ_H 6.18) to C-5 (δ_C 161.45)/C-7 (δ_C 164.11); from H-2′ (δ_H 7.99)/H-5′ (δ_H 7.15)/H-6′ (δ_H 8.01) to C-4′ (δ_C 159.54) suggested the positions of hydroxyl groups at C-5, C-7, and C-4′ of flavone unit I. The HMBC correlations between H-6″ (δ_H 6.41) and C-5″ (δ_C 160.54)/C-7″ (δ_C 162.00)/C-8″ (δ_C 104.01)/C-10″ (δ_C 103.72), H-2' (δ_H 7.67)/H-3’ (δ_H 6.92) and C-4’ (δ_C 162.20) suggested that the hydroxyl groups were at C-5″, C-7″, and C-4' of flavone unit II. In addition, the HMBC cross peaks from H-2′ (δ_H 7.99)/H-5′ (δ_H 7.15) to C-3′ (δ_C 119.97) and H-2′ (δ_H 7.99)/H-6″ (δ_H 6.41) to C-8″ (δ_C 104.01) indicated the linkages between the two flavone units at C-3′ and C-8″. Consequently, the structure of **1** was determined to be podocarpusflavone A [5]. This compound was reported from the genus *Antidesma* for the first time.

The molecular formula of **2** was determined to be $C_{30}H_{18}O_{10}$, by ESI-MS ion at *m/z* 537 $[M-H]^-$ and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **2** showed the following signals: three aromatic protons of the ABX system in aromatic ring at δ_H 7.09 (1H, d, *J* = 8.5 Hz), 7.83 (1H, dd, *J* = 1.5, 8.5 Hz), and 7.96 (1H, d, *J* = 1.5 Hz), four aromatic protons of *para*-substituted aromatic ring at δ_H 6.73 (2H, d, *J* = 8.5 Hz), and 7.49 (2H, d, *J* = 8.5 Hz), five aromatic protons at δ_H 6.16 (1H, s), 6.36 (1H, s), 6.43 (1H, s), 6.57 (1H, s), and 6.58 (1H, s). The ^{13}C-NMR and DEPT spectra revealed the signals of 30 carbons, including two carbonyl at δ_C 183.59 and 184.02, sixteen non-protonated at δ_C 105.15, 105.28, 105.37, 121.44, 123.13 × 2, 156.34, 159.21, 160.78, 162.39, 162.44, 163.09, 163.26, 165.78 × 2, and 165.92, twelve methine carbons at δ_C 95.19, 99.89, 100.15,

Table 1: The ^{1}H- and ^{13}C-NMR data for compounds **1-4** and reference compounds

Pos.	**1**			**2**		**Pos.**	**3**			**4**		
	δ_C[@]	δ_C[a]	δ_H[a] (mult., *J*, Hz)	δ_C[b]	δ_H[b] (mult., *J*, Hz)		δ_C[#]	δ_C[a]	δ_H[a] (mult., *J*, Hz)	δ_C[$]	δ_C[a]	δ_H[a] (mult., *J*, Hz)
						Aglycone						
2	163.8	163.79	-	165.92	-	1	37.2	37.31	-	42.2	42.42	-
3	103.0	103.02	6.82 (s)	103.45	6.57 (s)	2	48.0	48.07	1.97 (d, 17.5)	50.5	50.71	2.54 (d, 17.5)
									2.49 (d, 17.5)			2.17 (d, 17.5)
4	181.7	181.74	-	184.02	-	3	202.3	204.42	-	201.3	201.20	-
5	161.4	161.45	-	163.09	-	4	125.3	125.37	5.82 (s)	127.1	127.18	5.89 (d, 1.5)
6	98.8	98.83	6.18 (d, 2.0)	100.15	6.16 (d, 1.5)	5	170.0	170.13	-	167.2	167.24	-
7	164.1	164.11	-	165.78	-	6	52.3	52.38	2.02 (m)	79.9	80.00	-
8	94.0	94.03	6.45 (d, 2.0)	95.19	6.43 (d, 1.5)	7	26.7	26.82	1.53 (m)/1.98 (m)	134.9	135.29	5.88*
9	157.3	157.37	-	159.21	-	8	37.7	37.80	1.66 (m)	131.4	131.56	5.88*
10	103.7	103.72	-	105.15	-	9	75.0	75.48	3.91 (m)	77.0	77.28	4.44 (m)
1′	121.0	121.01	-	121.44	-	10	19.8	19.87	1.21 (d, 6.0)	20.8	21.18	1.31 (d, 6.5)
2′	131.3	131.38	7.99 (d, 1.5)	132.76	7.96 (d, 1.5)	11	27.5	27.53	1.11 (s)	19.2	19.54	1.03 (s)
3′	120.0	119.97	-	123.13	-	12	29.0	29.08	1.03 (s)	23.0	23.42	1.02 (s)
4′	159.6	159.54	-	160.78	-	13	25.0	24.97	2.07 (s)	24.4	24.68	1.94 (d, 1.5)
5′	116.2	116.18	7.15 (d, 8.0)	117.27	7.09 (d, 8.5)	9-*O*Glc						
6'	127.8	127.84	8.01 (dd, 1.5, 8.0)	128.96	7.83 (dd, 1.5, 8.5)	1′	102.0	102.11	4.35 (d, 8.0)	102.5	102.75	4.36 (d, 8.0)
2″	163.2	163.22	-	165.78	-	2′	75.4	75.14	3.16 (dd, 8.0, 9.0)	75.1	75.26	3.18 (dd, 8.0, 9.0)
3″	103.2	103.24	6.88 (s)	104.06	6.58 (s)	3′	78.0	78.15		77.9	78.13	
4″	182.1	182.15	-	183.59	-	4′	71.7	71.83		71.3	71.68	
5″	160.5	160.54	-	162.44	-	5′	77.7	77.89		77.8	78.04	
6″	98.7	98.70	6.41 (s)	99.89	6.36 (s)	6'	62.8	62.91	3.66 (dd, 5.5, 11.5)	62.3	62.2.85	3.64 (dd, 5.5, 11.5)
									3.87 (dd, 2.0, 11.5)			3.87 (dd, 2.0, 11.5)
7″	161.0	162.00	-	163.26	-							
8″	104.1	104.01	-	105.28	-							
9″	154.5	154.54	-	156.34	-							
10″	103.6	103.72	-	105.37	-							
1′	123.0	122.98	-	123.13	-							
2′, 6′	128.0	127.98	7.67 (d, 8.5)	129.31	7.49 (d, 8.5)							
3′, 5′	114.5	114.48	6.92 (d, 8.5)	116.87	6.73 (d, 8.5)							
4′	162.2	162.20	-	162.39	-							
4′-OCH_3	55.5	55.50	3.75 (s)									

[a)]Recorded in CD_3OD, [b)]recorded in DMSO-d_6,*overlapped signals, [@]δ_C of podocarpusflavone A in CD_3OD [5], [#]δ_C of byzantionoside B in CD_3OD [7], [$]$\delta_C$ of (6S,9R)-roseoside in CD_3OD [8].

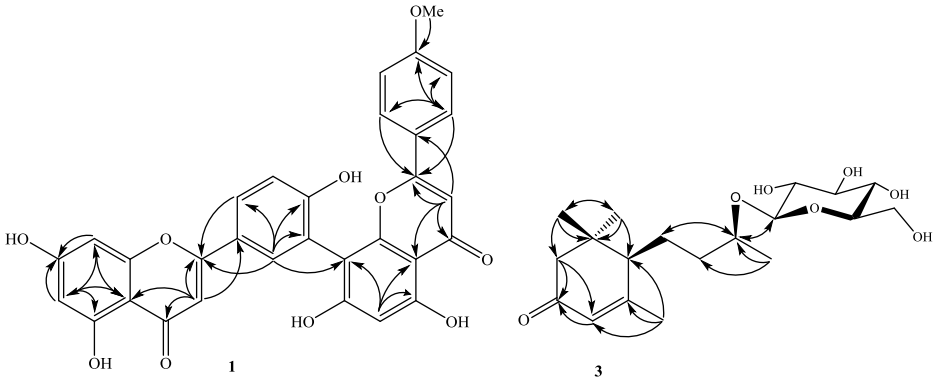

Figure 2: The important HMBC correlations of compounds **1** and **3**

103.45, 104.06, 116.87 × 2, 117.27, 128.96, 129.31 × 2, 132.76, indicating of the presence of two flavone units. The ^{1}H- and ^{13}C-NMR spectra of **2** were almost similar to those of **1** except for disappearance of methoxy group at C-4. The positions of the remaining functional groups were based on the HSQC and HMBC spectra. Thus, the structure of compound **2** was elucidated to be amentoflavone, it was previously isolated from *A. laciniatum* [6].

The ^{1}H-NMR spectrum of **3** showed the signals of one olefinic proton at δ_H 5.82 (1H, s), one secondary methyl group at 1.21 (3H, d, J = 6.0 Hz), three tertiary methyl groups at δ_H 1.03 (3H, s), 1.11 (3H, s), and 2.07 (3H, s), assigned to a megastigmane aglycone; one anomeric proton at δ_H 4.35 (1H, d, J = 8.0 Hz) assigned to one sugar moiety. The ^{13}C-NMR and DEPT spectra of compound **3** displayed the signals of 19 carbons, including one carbonyl at δ_C 204.42, two non-protonated at δ_C 37.1 and 170.13; eight methine at δ_C 52.38, 71.83, 75.14, 75.48, 77. 89, 78.15, 102.11, and 125.37; four methylene at δ_C 26.82, 37.80, 48.07, and 62.91; four methyl carbons at δ_C 19.87, 24.97, 27.53 and 29.08. Analysis of ^{1}H- and ^{13}C-NMR data indicated that structure of **3** was identical to byzantionoside B [7]. The ^{13}C-NMR data of sugar moiety (δ_C 102.11, 78.15, 77.89, 75.14, 71.83, and 62.91) and coupling constant of glc H-1′ and glc H-2′, J = 8.0 Hz proved the presence of β-D-glucopyranosyl moiety in **3**. The position sugar unit at C-9 of aglycone was confirmed by HMBC correlations between glc H-1′ (δ_H 4.31) and C-9 (δ_C 75.48). The HMBC correlations from H-2 (δ_H 1.97 and 2.49)/H-4 (δ_H 5.82) to C-3 (δ_C 204.42); from H-4 (δ_H 5.82) to C-2 (δ_C 48.07)/C-3 (δ_C 204.42)/C-5 (δ_C 170.13) confirmed the ketone group and the double bond at C-3 and C-4/C-5. Thus, the structure of **3** was elucidated to be byzantionoside B [7].

The signals of 19 carbons were observed in the ^{1}H-, ^{13}C-NMR and DEPT spectra of **4** including one carbonyl, three non-protonated, nine methines, two methylene, and four methyl carbons. The NMR data of **4** were almost similar to those of **3** except for an addition hydroxyl group at C-6 and double bond at C-7/C-8. Furthermore, NMR data of **4** were identical to those of (6*S*,9*R*)-roseoside [8]. Thus, the structure of **4** was determined as (6*S*,9*R*)-roseoside [8].

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