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Flavonoids and other compounds from *Vitex limonifolia*

Nguyen Thi Kim Thoa[2], Ninh Khac Ban[1], Do Thi Trang[1], Tran My Linh[1], Vu Huong Giang[1], Nguyen Xuan Nhiem[1], Phan Van Kiem[2*]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Viet Nam*

[2]*Faculty of Basic Science, Hanoi University of Mining and Geology, Viet Nam*

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Abstract

Using combined chromatographic methods, three flavonoids, 5,4′-dihydroxy-3,7-dimethoxyflavone (**1**), vitecetin (**2**), 5,4′-dihydroxy-7,3′-dimethoxyflavone (**3**), a lignan, verrucosin (**4**), and a γ-pyrone glycoside, maltol *O*-β-D-glucopyranoside (**5**) were isolated from the methanol extract of the leaves of *Vitex limonifolia*. Their structures were identified on the basis of spectroscopic evidence and comparison with those reported in the literature. Compounds **1** and **3-5** were reported from *Vitex* genus for the first time.

Keywords. *Vitex limonifolia*, flavonoid, lignan, γ-pyrone glycoside.

1. INTRODUCTION

The genus *Vitex* is one of the largest genus in the *Verbenaceae* family with approximately 250 species.[1] The plants are mostly shrubs or trees, and mainly found in the tropical areas with a few in subtropical regions.[1] Traditionally, some of its species are being used for rheumatic pains, sprains, anti-fungal, and anti-cancer activities.[2] Phytochemical study of the *Vitex* genus revealed the presence of flavonoids, terpenoids, ecdysteroids, and iridoid glycosides, etc…[2] This paper reported the isolation and structure elucidation of three flavonoids, one lignan, and one γ-pyrone glycoside from the methanol extract of the leaves of *Vitex limonifolia* (figure 1).

2. MATERIALS AND METHODS

2.1. Plant materials

The leaves of *Vitex limonifolia* Wall. ex C.B.Clarke were collected in Bach Ma National Park, Thua Thien Hue, Vietnam in September, 2015, and identified by one of the authors, Prof. Dr. Ninh Khac Ban. A voucher specimen was deposited at the Herbarium Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. The NMR spectra were recorded using a Bruker DRX 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz). Column chromatography was performed using silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd.), and thin layer chromatography (TLC) was performed using a precoated 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 leaves of *V. limonifolia* (4.2 kg) were extracted with hot MeOH three times (3×5 L) under reflux for 12 h to yield 350 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with CH_2Cl_2 and EtOAc to obtain the CH_2Cl_2 (VIL1, 130.0 g), EtOAc (VIL2, 27.0 g), and H_2O (VIL3, 190.0 g) extracts after removal of the solvents *in vacuo.*

The VIL1 fraction was chromatographed on a silica gel column eluting with a gradient of *n*-hexane:acetone (100:0 → 0:1) to give six fractions, VIL1A–VIL1F. VIL1B was chromatographed on an RP-18 column eluting with MeOH:water (5:1, v/v) to give two fractions, VIL1B1 and VIL1B2. VIL1B1 was chromatographed on a silica gel column eluting with *n*-hexane:EtOAc (1.5:1, v/v) to yield compound **4** (4.1 mg). VIL1B2 was chromatographed on a silica gel column eluting with *n*-hexane:EtOAc (1.7:1, v/v) to yield compounds **1** (4.4 mg) and **3** (12.5 mg). The VIL1C was chromatographed on an

RP-18 column eluting with MeOH:water (5:1, v/v) to give three fractions, VIL1C1-VIL1C3. VIL1C

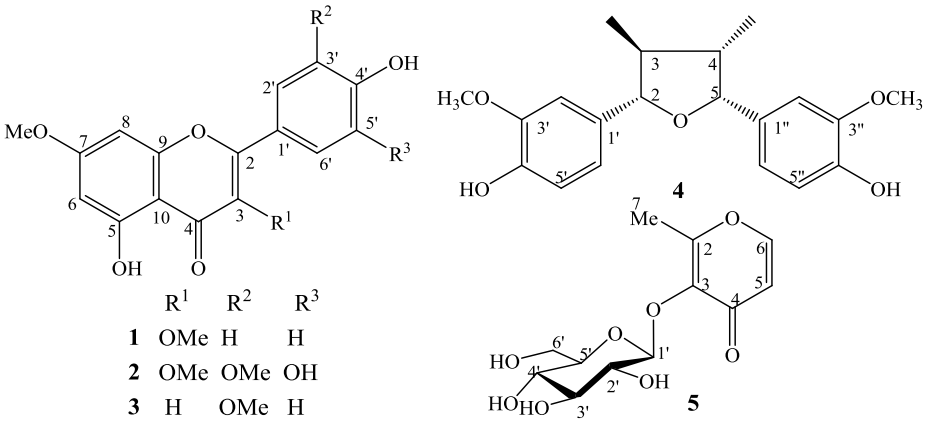

Figure 1: The chemical structures of compounds **1**-**5**

and was chromatographed on a Sephadex LH-20 column and eluting with MeOH:water (1:1, v/v) to give compound **2** (5.0 mg). The VIL3 was chromatographed on a Diaion HP-20P column eluting with H_2O containing increasing concentrations of MeOH (25, 50, 75, and 100 %) to obtain four sub-fractions, VIL3A-VIL3D. VIL3A was chromatographed on a silica gel column eluting with gradient of CH_2Cl_2:MeOH (100:0 → 0:1, v/v) to give seven fractions, VIL3A1-VIL3A7. VIL3A5 was chromatographed on an RP-18 column eluting with MeOH:water (5:1, v/v) to give three smaller fractions, VIL3A5A-VIL3A5C. Compound **5** (9.2 mg) was obtained from VIL3A5A on a silica gel column, using EtOAc:MeOH:water (3.5:1:0.15, v/v/v).

5,4′-Dihydroxy-3,7-dimethoxyflavone (**1**): yellowish powder; $C_{17}H_{14}O_6$; HR-ESI-MS *m/z*: 313.0711 $[M-H]^-$ (Calcd. for $[C_{17}H_{13}O_6]^-$, 313.0718); ^{1}H- and ^{13}C-NMR (DMSO-d_6), see table 1.

Vitecetin (2): yellowish powder; $C_{18}H_{16}O_8$; HR-ESI-MS *m/z*: 359.0752 $[M-H]^-$ (Calcd. for $[C_{18}H_{15}O_8]^-$, 359.0772); ^{1}H- and ^{13}C-NMR (DMSO-d_6), see table 1.

5,4′-Dihydroxy-7,3′-dimethoxyflavone (**3**): yellowish powder; $C_{17}H_{14}O_6$; HR-ESI-MS *m/z*: 313.0712 $[M-H]^-$ (Calcd. for $[C_{17}H_{13}O_6]^-$, 313.0718); ^{1}H- and ^{13}C-NMR (DMSO-d_6), see table 1.

Verrucosin (**4**): colorless oil; $[\alpha]_D^{25}$: +12.0 (*c* 0.1, $CHCl_3$); $C_{20}H_{24}O_5$; HR-ESI-MS *m/z*: 313.0712 $[M-H]^-$ (Calcd. for $[C_{20}H_{23}O_5]^-$, 343.1551); ^{1}H-NMR ($CDCl_3$) δ_H 5.11 (d, *J* = 8.5 Hz, H-2), 2.24 (m, H-3), 1.77 (m, H-4), 4.39 (d, *J* = 9.5 Hz, H-5), 7.04 (d, *J* = 1.5 Hz, H-2′), 6.92 (d, *J* = 8.0 Hz, H-5′), 6.99 (dd, *J* = 1.5, 8.0 Hz, H-6′), 6.85 (d, *J* = 1.5 Hz, H-2″), 6.88 (d, *J* = 8.0 Hz, H-5″), 6.82 (dd, *J* = 1.5, 8.0 Hz, H-6″), 3.86 (s, 3′-OMe), 3.91 (s, 3″-OMe), 1.05 (d, *J* = 7.0 Hz, 3-Me), and 0.66 (d, *J* = 7.0 Hz, 4-Me); ^{13}C-NMR ($CDCl_3$) δ_C 87.4 (C-2), 47.8 (C-3), 46.0 (C-4), 83.2 (C-5), 133.2 (C-1′), 109.4 (C-2′), 146.5 (C-3′), 145.2 (C-4′), 114.2 (C-5′), 119.3 (C-6′), 132.8 (C-1″), 109.8 (C-2″), 146.2 (C-3″), 144.6 (C-4″), 113.9 (C-5″), 119.9 (C-6″), 55.9 (3′-OMe), 55.9 (3″-OMe), 14.97 (3-Me), and 15.01 (4-Me).

Maltol *O-β*-D-glucopyranoside (**5**): White amorphous powder; $[\alpha]_D^{25}$: –15.0 (*c* 0.1, MeOH); $C_{12}H_{16}O_8$, MW: 288; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellowish powder and the molecular formula was determined to be $C_{17}H_{14}O_6$ on the basic of HR-ESI-MS ion at *m/z* 313.0711 $[M-H]^-$ (Calcd. for $[C_{17}H_{13}O_6]^-$, 313.0718); The ^{1}H-NMR spectrum of **1** showed the signals of six aromatic protons at δ_H 6.36 (1H, s), 6.73 (1H, s), 6.95 (2H, d, *J* = 8.5 Hz), and 7.97 (2H, d, *J* = 8.5 Hz), two methoxy groups at δ_H 3.86 (3H, s) and 3.80 (3H, s), suggested the presence of a flavone. The ^{13}C-NMR and DEPT spectra showed the the signals of 17 carbons, including nine non-protonated carbons at δ_C 105.2, 120.5, 137.8, 155.9, 156.3, 160.3, 160.9, 165.1, and 178.1; six methines at δ_C 92.3, 98.0, 115.7×2, and 130.2×2; two methoxy carbons at δ_C 56.1 and 59.1 This also confirmed the presence of the flavonol structure with two methoxy groups. The hydroxyl group at C-4 of B ring was confirmed by HMBC correlations between H-2′/H-6

(δ_H 7.97) and C-2 (δ_C 155.9)/C-4′ (δ_C 160.3) (Figure 2). The HMBC correlations between H-6 (δ_H 6.36)/H-8 (δ_H 6.73) and C-7 (δ_C 165.1); methoxy proton (δ_H 3.80) and C-3 (δ_C 137.8); and between methoxy proton and C-7 (δ_C 165.1) suggested the positions of two methoxy groups at C-3 and C-7. All NMR assignments of **1** were confirmed by detailed analyses of HSQC and HMBC spectra (table 1),

Table 1: The ^{1}H- and ^{13}C-NMR data for compounds **1-3** and **5**

C	1			2			3			C	5		
	$\delta_C^{\#}$	$\delta_C^{a,c}$	$\delta_H^{a,d}$ (*J* = *Hz*)	$\delta_C^{¥}$	$\delta_C^{a,c}$	$\delta_H^{a,d}$ (*J* = *Hz*)	$\delta_C^{\$}$	$\delta_C^{a,c}$	$\delta_H^{a,d}$ (*J* = *Hz*)		$\delta_C^{@}$	$\delta_C^{b,c}$	$\delta_H^{b,d}$ (*J* = *Hz*)
2	156.0	155.9	-	155.9	156.0	-	161.0	161.1	-	2	164.7	164.6	-
3	137.9	137.8	-	138.2	138.0	-	103.2	103.3	6.93 (s)	3	143.7	143.6	-
4	178.1	178.1	-	178.1	177.9	-	181.1	181.9	-	4	177.7	177.2	-
5	160.9	160.9	-	161.0	161.0	-	163.8	164.0	-	5	117.4	117.3	6.47 (d, 5.5)
6	97.8	98.0	6.36 (s)	97.9	97.8	6.37 (s)	97.7	97.9	6.35 (s)	6	157.3	157.6	8.03 (d, 5.5)
7	165.2	165.1	-	165.2	165.1	-	164.9	165.1	-	7	15.9	15.8	2.49 (s)
8	92.4	92.3	6.73 (s)	92.4	92.3	6.75 (s)	92.4	92.7	6.76 (s)	1′	105.5	105.5	4.85 (d, 8.0)
9	156.4	156.3	-	156.3	156.2	-	157.0	157.2	-	2′	75.4	75.4	3.41 (t, 8.0)
10	105.3	105.2	-	104.1	105.1	-	104.5	104.7	-	3′	78.1	78.0	3.42 (m)
1′	120.6	120.5	-	119.6	119.1	-	121.3	121.4	-	4′	71.1	71.1	3.37 (dd, 8.0, 8.5)
2′	130.3	130.2	7.97 (d, 8.5)	105.2	104.3	7.26 (d, 2.0)	110.4	110.2	7.57 (s)	5′	78.0	78.6	3.27 (m)
3′	115.8	115.7	6.95 (d, 8.5)	148.2	148.1	-	147.9	148.0	-	6′	62.5	62.5	3.69 (dd, 5.5, 12.0) 3.85 (dd, 2.0, 12.0)
4′	160.3	160.3	-	137.9	138.5	-	150.7	150.9	-				
5′	115.8	115.7	6.95 (d, 8.5)	145.6	145.8	-	115.7	115.8	6.94 (d, 7.5)				
6′	130.3	130.2	7.97 (d, 8.5)	109.8	109.6	7.32 (d, 2.0)	120.2	120.5	7.58 (d, 7.5)				
3-OMe	59.8	59.7	3.80 (s)	59.8	59.6	3.81 (s)							
7-OMe	56.1	56.1	3.86 (s)	56.2	56.1	3.87 (s)	55.7	56.0	3.90 (s)				
3′-OMe				56.1	56.1	3.85 (s)	55.9	56.0	3.86 (s)				

[a)]recorded in DMSO-d_6, [b)] CD_3OD, [c)]125 MHz, [d)]500 MHz, [#]δ_C of 5,4′-dihydroxy-3,7-dimethoxyfavone[3], [¥]δ_C of vitecetin[4], [$]$\delta_C$ of 5,4′-dihydroxy-7,3′-dimethoxyflavone[5], [@]δ_C of maltol *O*-*β*-D-glucopyranoside[6].

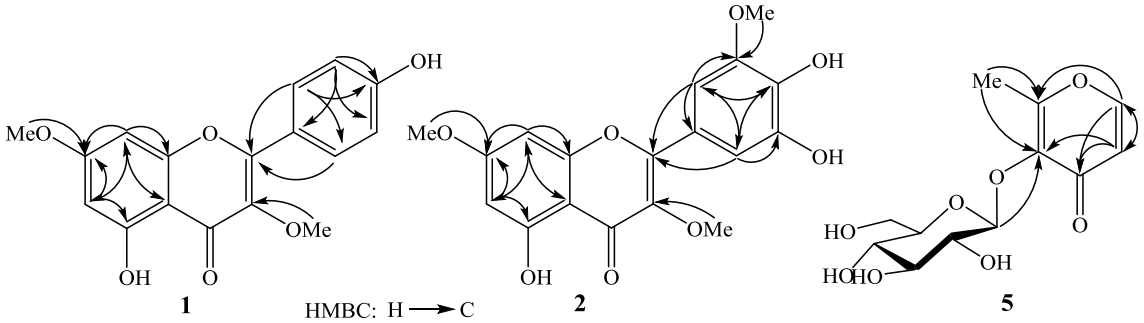

Figure 2: The key HMBC correlations of compounds **1**, **2**, and **5**

which are in good agreement with those reported in the literature.[3] Thus, compound **1** was identified as 5,4′-dihydroxy-3,7-dimethoxyflavone.

Compound **2** was also obtained as a yellowish powder and the molecular formula was determined to be $C_{18}H_{16}O_8$ by HR-ESI-MS ion at *m/z* 359.0752 $[M-H]^-$ (Calcd. for $[C_{18}H_{15}O_8]^-$, 359.0718). The ^{1}H-NMR spectrum of **2** showed the signals of four aromatic protons at δ_H 6.37 (s), 6.75 (s), 7.26 (d, *J* = 2.0 Hz), and 7.32 (d, *J* = 2.0 Hz), three methoxy groups at δ_H 3.81, 3.85, and 3.87 (each 3H, s). The ^{13}C-NMR and DEPT spectra showed the the signals of 18 carbons, including eleven non-protonated carbons at δ_C 105.1, 119.1, 138.0, 138.5, 145.8, 148.1, 156.0, 156.2, 161.0, 165.1, and 177.9; four methines at δ_C 92.3, 97.8, 104.3, and 109.6; three methoxy carbons at δ_C 56.1×2 and 59.6. The ^{1}H- and ^{13}C-NMR data of **2** were similar to those of vitecetin

.[4] All NMR assignments of **2** were confirmed by detailed analyses of HSQC and HMBC spectra. The HMBC correlation between H-6 (δ_H 6.37)/H-8 (δ_H 6.75) and C-7 (δ_C 165.1); methoxy proton (δ_H 3.81) and C-3 (δ_C 138.0); and between methoxy proton (δ_H 3.87) and C-7 (δ_C 165.1) suggested the positions of two methoxy groups at C-3 and C-7. The two hydroxyl groups at C-4′ and C-5′ and methoxy group at C-3′ of B ring were proved by HMBC correlations between H-2′ (δ_H 7.26) and C-2 (δ_C 156.0)/C-1′ (δ_C 119.1)/C-3′ (δ_C 148.1)/C-6′ (δ_C 109.6); H-6′ (δ_H 7.32) and C-2 (δ_C 156.0)/C-1′ (δ_C 119.1)/C-2′ (δ_C 104.3)/C-4′ (δ_C 138.5)/C-5′ (δ_C 145.8); and between methoxy proton (δ_H 3.85) and C-3′ (δ_C 148.1). Thus, compound **2** was identified as vitecetin and this compound was already reported from *Vitex peduncularis*.[4]

The ^{1}H-NMR spectrum of **3** showed the following proton signals: three aromatic protons with ABX system of B ring at δ_H 7.57 (1H, s), 7.58 (1H, d, *J* = 7.5 Hz), and 6.94 (1H, d, *J* = 7.5 Hz), two *meta* aromatic protons of A ring at 6.35 (1H, s) and 6.93 (1H, s), one proton of C ring at δ_H 6.76 (1H, s), and two methoxy groups at δ_H 3.86 (3H, s) and 3.90 (3H, s). The ^{13}C-NMR and DEPT spectra of **3** showed signals of 17 carbons, including nine non-protonated carbons, six methines and two methoxy carbons. The NMR data of **3** were found to be similar with those of 5,4′-dihydroxy-7,3′-dimethoxyflavone.[5] Thus, **3** was determined to be 5,4′-dihydroxy-7,3′-dimethoxyflavone.

The molecular formula of **4** was determined $C_{20}H_{24}O_5$ by HR-ESI-MS ion at *m/z* 313.0712 $[M-H]^-$ (Calcd. for $[C_{20}H_{23}O_5]^-$, 343.1551). The ^{1}H-NMR spectrum of **4** showed the protons signals of six aromatic protons at δ_H 7.04 (d, *J* = 1.5 Hz, H-2′), 6.92 (d, *J* = 8.0 Hz, H-5′), 6.99 (dd, *J* = 1.5, 8.0 Hz, H-6′), 6.85 (d, *J* = 1.5 Hz, H-2″), 6.88 (d, *J* = 8.0 Hz, H-5″), and 6.82 (dd, *J* = 1.5, 8.0 Hz, H-6″), and two oxygenated methine protons at δ_H 5.11 (1H, d, *J* = 8.5 Hz) and 4.39 (1H, d, *J* = 9.5 Hz), and two methoxy groups at δ_H 3.86 and 3.92 (each 3H, s). The ^{13}C-NMR and DEPT spectra of **4** showed the signals of 20 carbons, including six quaternary carbons at δ_C 132.8, 133.2, 144.6, 145.2, 146.2, and 146.5; ten methines at δ_C 46.0, 47.8, 83.2, 87.4, 109.4, 109.8, 113.9, 114.2, 119.3, 119.9, and four methyl carbons at δ_C 15.4.97, 15.01, and 55.9 × 2. The NMR data of **4** is in good agreement with those reported in the literature.[7] Thus, compound **4** was determined to be verrucosin.

The ^{1}H-NMR spectrum of **5** showed the signals of two olefinic protons at δ_H 6.47 (1H, d, *J* = 5.5 Hz) and 8.03 (1H, d, *J* = 5.5 Hz), one anomeric proton at δ_H 4.85 (d, *J* = 8.0 Hz), and one methyl group at δ_H 2.49 (3H, s). The ^{13}C-NMR and DEPT spectra of **5** displayed the signals of 12 carbons, including one cacbonyl, one methylene, seven methines, and two quaternary carbons. Of which, six carbons were assigned to *γ*-pyrone moiety with a methyl group and six carbons to a sugar unit. The HMBC correlations between H-5 (δ_H 6.47) and C-3 (δ_C 143.6)/C-4 (δ_C 177.2); H-6 (δ_H 8.03) and C-4 (δ_C 177.2)/C-2 (δ_C 164.6); between methyl protons (δ_H 2.49) and C-2 (δ_C 164.6)/C-3 (δ_C 143.6)/C-1′ (δ_C 105.5) indicated *γ*-pyrone aglycone with the methyl group at C-2. The ^{13}C-NMR of sugar at δ_C 105.5, 75.4, 78.1, 71.1, 78.0, and 62.5 and coupling constant of H-1′ and H-2′, *J* = 8.0 Hz suggested the presence of *O-β*-D-glucopyranosyl moiety. In addition, the HMBC correlation between H-1′ (δ_H 4.85) and C-3 (δ_C 143.6) indicated the position of sugar moiety at C-3. Based on the above envidence and literature[6], compound **5** determined to be maltol *O-β*-D-glucopyranoside.

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Corresponding author: **Phan Van Kiem**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam
E-mail: phankiem@yahoo.com.

