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Ecdysteroids from leaves of *Vitex trifolia*

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Abstract

Four known ecdysteroids, ecdysone (**1**), 20-hydroxyecdysone (**2**), 20-hydroxyecdysone 2,3-monoacetonide (**3**), and turkesterone (**4**) were isolated from leaves of *Vitex trifolia*. The structure of these compounds was elucidated by means of 1D- and 2D-NMR spectra and was compared with those reported in literature. Compound **3** was reported from *Vitex* genus for the first time; compounds **1**, **2**, and **4** from *V. trifolia* for the first time.

Keywords. *Vitex trifolia, ecdysteroids*.

1. INTRODUCTION

Vitex trifolia L, belonging to *Verbanesceae* family, is a tropical shrub widely distributed in Pacific-Asian countries, such as India, Sri Lanka, China, Philippines, Indonesia, North Australia, New Caledonia, etc.[1] The leaves of *V. trifolia* are used in traditional medicine for the treatment of rheumatic pain, inflamamation, analgesic, anticonvulsant, and sedative.[2] Ecdysteroids were discovered as insect molting hormones, displayed the important physiological effects on insects and play defensive role. Ecdysteroids also exert beneficial pharmacological properties such as decreasing the blood cholesterol and glucose level in experimental animals, anticancer, and wound healing activities.[3] This paper reported the isolation and structure elucidation of fourecdysteroids from the leaves of *V. trifolia* (Fig. 1).

2. MATERIAL AND METHODS

2.1. Plant materials

The leaves of *Vitex trifolia* L. were collected in Bachma National Park, Thua Thien Hue, Viet Nam 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). ESI-MS spectra were recorded on an Agilent 1100 spectrometer. HR-ESI-MS spectra were recorded on an Agilent 6550 iFunnel Q-TOF LC/MS system. 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. trifolia* (2.2 kg) were extracted with hot MeOH three times (3 × 4 L) using sonicator for 3 h to yield 130 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with CH_2Cl_2, EtOAc to obtain the CH_2Cl_2 (VIT1, 51.0 g), EtOAc (VIT2, 27.0 g), and H_2O (VIT3, 52.0 g) extracts after removal of the solvents *in vacuo*.

VIT2 was chromatographed on a silica gel column eluting with a gradient solvent of *n*-hexane:acetone (100:0 → 0:1) to give four fractions, VIT2A-VIT2D. VIT2D was chromatographed on a silica gel column eluting with CH_2Cl_2:MeOH (10:1, v/v) to give three sub-fractions, VIT2D1-VIT2D3. VIT2D1 was chromatographed on an RP-18 column

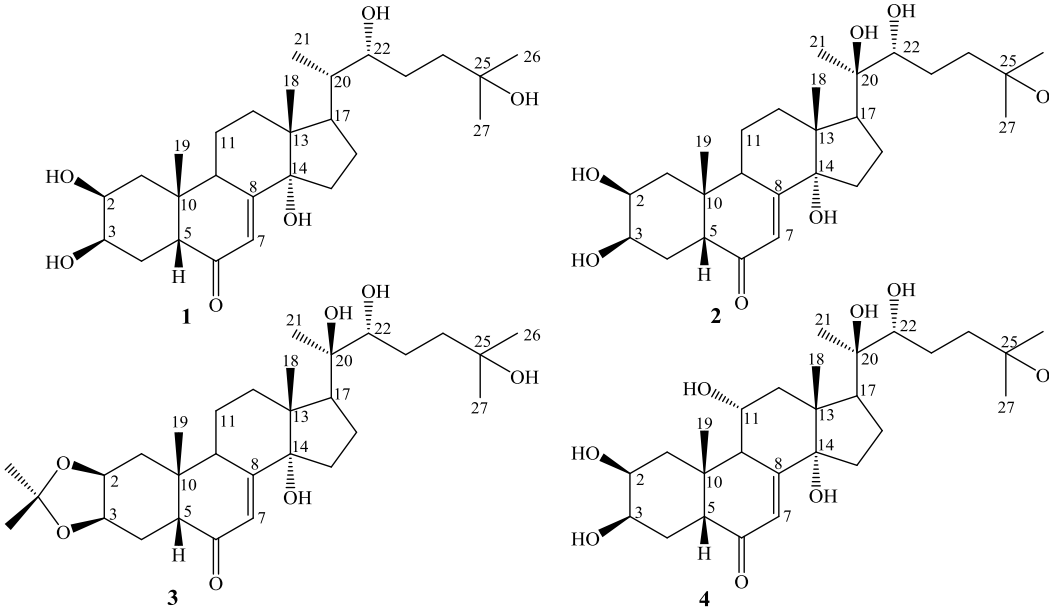

Figure 1: The chemical structures of compounds **1**-**4**

eluting with MeOH:water (1:1.5, v/v) to give four smaller fractions, VIT2D1A-VIT2D1D. VIT2D1A was chromatographed on a Sephadex LH-20 column eluting with MeOH:water (1:1, v/v) to give compound **1** (9.0 mg). VIT2D1C was chromatographed on a silica gel column eluting with CH_2Cl_2:acetone:water (1.5:1:0.05, v/v/v) to yield compound **2** (10.0 mg). VIT2D2 was chromatographed on an RP-18 column eluting with MeOH:water (1:1.5, v/v) to give two smaller fractions, VIT2D2A and VIT2D2B. VIT2D2B was chromatographed on a silica gel column eluting with CH_2Cl_2:acetone:water (1.5:1:0.05, v/v/v) to yield compound **3** (8.0 mg).

VIT3 was chromatographed on a Diaion HP-20P column, using H_2O to remove sugar and then eluting with the increasing MeOH in water (25, 50, 75, and 100 %) to obtain four sub-fractions, VIT3A-VIT3D. VIT3B was chromatographed on an RP-18 column eluting with MeOH:water (1:1.5, v/v) to give three smaller fractions, VIT3B1-VIT3B3. Compound **4** (12.0 mg) was obtained from VIT3B2 fraction using an RP-18 column (acetone:water, 1:3.7, v/v).

Ecdysone (1): white amorphous powder; $[\alpha]_D^{25}$: +70.0 (*c* 0.1, MeOH); ESIMS *m/z* 465 $[M+H]^+$, CTPT $C_{27}H_{44}O_6$; MW: 464; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

20-Hydroxyecdysone (2): white amorphous powder; $[\alpha]_D^{25}$: +52.0 (*c* 0.1, MeOH); ES-IMS *m/z* 481 $[M+H]^+$, CTPT $C_{27}H_{44}O_7$; MW: 480; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

20-Hydroxyecdysone 2,3-monoacetonide (3): white amorphous powder; $[\alpha]_D^{25}$: +50.0 (*c* 0.1, MeOH); ES-IMS *m/z* 521 $[M+H]^+$, CTPT $C_{30}H_{48}O_7$; MW: 520; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

Turkesterone (4): white amorphous powder; $[\alpha]_D^{25}$: +85.0 (*c* 0.1, MeOH); $C_{27}H_{44}O_8$; MW: 496; HR-ESI-MS *m/z*: 495.2950 $[M-H]^-$ (Calcd. for $[C_{27}H_{43}O_8]^-$, 495.2963); ^{1}H-NMR (CD_3OD) δ_H 4.03 (dt, *J* = 4.0, 12.0 Hz, H-2), 3.98 (br d, *J* = 2.0 Hz, H-3), 2.36 (dd, *J* = 4.0, 13.0 Hz, H-5), 5.82 (d, *J* = 2.5 Hz, H-7), 3.17 (dd, *J* = 2.0, 8.5 Hz, H-9), 4.13 (m, H-11), 0.90 (s, H-18), 1.08 (s, H-19), 1.23 (s, H-21), 3.37 (m, H-22), 1.22 (s, H-24), and 1.22 (s, H-27); ^{13}C-NMR (CD_3OD) δ_C39.1 (C-1), 68.9 (C-2), 68.6 (C-3), 33.3 (C-4), 52.8 (C-5), 206.7 (C-6), 122.7 (C-7), 165.7 (C-8), 42.9 (C-9), 39.9 (C-10), 69.5 (C-11), 43.8 (C-12), 49.0 (C-13), 84.9 (C-14), 31.9 (C-15), 21.5 (C-16), 50.3 (C-17), 18.9 (C-18), 24.6 (C-19), 77.8 (C-20), 21.0 (C-21), 78.4 (C-22), 27.3 (C-23), 42.4 (C-24), 71.3 (C-25), 29.0 (C-26), and 29.7 (C-27).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The ^{1}H-NMR spectrum of **1** showed the signals of one olefinic proton at δ_H 5.84 (d, *J* = 2.0 Hz), three oxymethine protons at δ_H 3.97 (br s), 3.86 (dt, *J* = 3.5, 12.0 Hz), and 3.61 (br d, *J* = 9.5 Hz), five methyl groups at δ_H 1.22 (s), 1.21 (s), 0.99 (s), 0.97 (d, *J* = 7.0 Hz), and 0.76 (s) (table 1). The ^{13}C-NMR and DEPT spectra of **1** displayed the signals of 27 carbons, including one carbonyl at δ_C 206.5; two olefinic carbons at δ_C 167.6 and 122.0; four quaternaries at δ_C 85.1, 71.4, 48.1, and 39.3; seven methines at δ_C 75.3, 68.7, 68.5, 51.8, 48.8, 43.4, and

Table 1: The ^{1}H- and ^{13}C-NMR data for compounds **1-3** in CD_3OD

C		**1**			**2**			**3**	
	δ_C[#]	δ_C	δ_H (mult., *J* = Hz)	δ_C[¥]	δ_C	δ_H (mult., *J* = Hz)	δ_C[$]	δ_C	δ_H (mult., *J* = Hz)
1	37.5	37.4	1.43 (m)/1.80 (m)	38.0	37.3	1.45 (m)/1.81 (m)	38.7	38.8	1.22 (m)/2.00 (m)
2	68.7	68.7	3.86 (dt, 3.5, 12.0)	68.3	68.6	3.87 (dt, 4.0, 12,0)	73.5	73.5	4.28 (m)
3	68.5	68.5	3.97 (br s)	68.2	68.4	3.98 (br s)	73.2	73.2	4.31 (m)
4	32.8	32.9	1.73 (m)	32.5	32.7	1.73 (m)	27.7	27.7	1.30 (m)/2.00 (m)
5	51.8	51.8	2.40 (dd, 4.5, 12.5)	51.4	51.7	2.40 (dd, 5.0, 13.0)	52.5	52.5	2.26 (t, 8.5)
6	206.4	206.5	-	203.5	206.5	-	205.6	205.7	-
7	122.0	122.0	5.84 (d, 2.0)	121.7	122.1	5.84 (d, 2.5)	121.8	121.8	5.81 (d, 2.0)
8	167.5	167.6	-	166.1	168.0	-	167.2	167.2	-
9	35.9	35.3	3.17 (t, 8.0)	34.6	35.0	3.17 (t, 8.5)	35.7	35.7	2.96 (t, 8.0)
10	39.2	39.3	-	38.8	39.2	-	38.9	38.9	-
11	21.6	21.6	1.71 (m)/1.82 (m)	21.2	21.5	1.73 (m)/1.82 (m)	21.6	21.6	1.77 (m)/2.01 (m)
12	32.1	32.1	1.60 (m)	32.1	32.4	1.90 (m)	32.5	32.5	1.89 (m)
			2.10 (m)			2.14 (m)			2.14 (m)
13	48.2	48.1	-	48.2	48.6	-	49.0	49.5	-
14	85.1	85.1	-	84.4	85.2	-	85.2	85.2	-
15	32.1	32.1	1.79 (m)	31.8	31.7	1.63 (m)	31.7	31.7	1.61 (m)
			1.96 (m)			1.98 (m)			1.99 (m)
16	27.0	27.0	1.52 (m)/1.98 (m)	21.6	21.5	1.75 (m)/2.00 (m)	21.5	21.5	1.72 (m)/2.00 (m)
17	48.8	48.8	2.10 (m)	50.2	50.4	2.39 (m)	50.5	50.5	2.41 (t, 9.0)
18	16.2	16.2	0.76 (s)	17.9	18.0	0.91 (s)	18.0	18.0	0.90 (s)
19	24.4	24.5	0.99 (s)	24.5	24.4	0.99 (s)	24.0	23.9	0.99 (s)
20	43.3	43.4	1.78 (d, 2.5)	77.0	77.9	-	77.9	77.9	-
21	13.3	13.3	0.97 (d, 7.0)	21.7	21.1	1.22 (s)	21.0	21.1	1.22 (s)
22	75.3	75.3	3.61 (br d, 9.5)	77.7	78.3	3.36*	78.4	78.4	3.33*
23	25.5	25.4	1.34 (m)/1.55 (m)	27.5	27.3	1.31 (m)/1.70 (m)	27.3	27.4	1.30 (m)/1.67 (m)
24	42.2	42.3	1.42 (m)/1.80 (m)	42.6	42.3	1.45 (m)/1.81 (m)	42.9	42.4	1.46 (m)/1.81 (m)
25	71.4	71.4	-	69.8	71.3	-	71.3	71.3	-
26	29.3	29.1	1.21 (s)	30.1	29.0	1.21 (s)	28.9	29.0	1.21 (s)
27	29.5	29.6	1.22 (s)	30.1	29.7	1.23 (s)	29.7	29.7	1.22 (s)
2,3-ACN							109.5	109.5	-
ACN-Me							28.8	28.8	1.49 (s)
ACN-Me							26.6	26.6	1.34 (s)

[#]δ_C of ecdysone[4], [¥]δ_C of 20-hydroxyecdysone[5], [$]$\delta_C$ of 20-hydroxyecdysone-2,3-monoacetonide[6], [Ψ]δ_C of turkesterone[7], *overlapped signals.

35.3; eight methylenes at δ_C 42.3, 37.4, 32.9, 32.1×2, 27.0, and 25.4, and 21.6; and five methyl carbons at δ_C 29.6, 29.1, 24.5, 16.2, and 13.3. The ^{1}H-NMR, ^{13}C-NMR and MS data of **1** were found in good agreement ecdysone.[4]. The HMBC correlations between H-5 (δ_H 2.40) and C-4 (δ_C 32.9)/C-6 (δ_C 206.5)/C-7 (δ_C 122.0); H-7 (δ_H 5.84) and C-5 (δ_C 51.8)/C-9 (δ_C 35.3)/C-14 (δ_C 85.1) indicated the position of oxo group at C-6 and the double bond at C-7/C-8. The HMBC correlation between H-1 (δ_H 1.43 and 1.80) and C-2 (δ_C 68.7)/C-3 (δ_C 68.5) and between H-3 (δ_H 3.97) and C-5 (δ_C 51.8) suggested two hydroxyl groups at C-2 and C-3. The HMBC correlations between H-18 (δ_H 0.76) and C-12 (δ_C 32.1)/C-13 (δ_C 48.1)/C-14 ((δ_C 85.1)/C-17 (δ_C 48.8); between H-22 (δ_H 3.61) and C-21 (δ_C 13.3)/C-23 (δ_C 25.4)/C-24 (δ_C 42.3); and between H-26 (δ_H 1.21)/H-27 (δ_H 1.22) and C-24 (δ_C 42.3)/C-25 (δ_C 71.4) indicated the position of three hydroxyl groups at C-14, C-22, and C-25. Thus, compound **1** was identified as ecdysone, a compound was previously reported to be isolated from *V. megapotamica*.[8]

The ESI-MS of **2** exhibited an ion peak at *m/z* 481 $[M+H]^+$, corresponding to the molecular formula

of $C_{27}H_{44}O_7$. The ^{1}H-NMR spectrum of **2** showed the signals of one olefinic proton at δ_H 5.84 (d, J = 2.5 Hz), three oxymethine protons at δ_H 3.98 (br s), 3.87 (dt, J = 4.0, 12.0 Hz), and 3.36 (overlapped signals with NMR solvent), five methyl groups at δ_H 1.23 (s), 1.22 (s), 1.21 (s), 0.99 (s), and 0.91 (s). The ^{13}C-NMR and DEPT spectra showed signals of 27 carbons, including one carbonyl, two olefinic carbons, five quaternaries, six methines, eight methylenes, and five methyl carbons. By comparing the NMR data, the structure of **2** can be suggested to be similar with that of **1**. The difference between them is an additional hydroxyl group at C-20 of compound **2**, which has been confirmed by the HMBC correlations from H-21 (δ_H 1.22) to C-17 (δ_C 50.4)/C-20 (δ_C 77.9)/C-22 (δ_C 78.3). Moreover, the HMBC correlations between H-5 (δ_H 2.40) and C-6 (δ_C 206.5)/C-7 (δ_C 122.1); H-7 (δ_H 5.84) and C-5 (δ_C 51.7)/C-9 (δ_C 35.0)/C-14 (δ_C 85.2) indicated the position of oxo group at C-6 and the double bond at C-7/C-8. All NMR assignments of **2** were confirmed by detailed analyses of HSQC and HMBC spectra, which are in good agreement with those reported in literature.[5] Thus, compound **2** was identified as 20-hydroxyecdysone. Previously, this compound was isolated from *V. canescens*[9] and *V. leptobotrys*.[10]

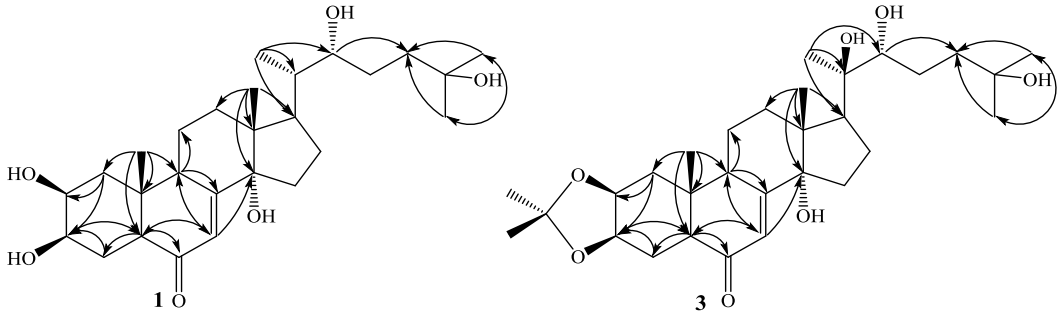

Figure 2: The key HMBC correlations of compounds **1** and **3**

The structure of **3** was identified from the NMR and MS data and from their comparison with the corresponding data of 20-hydroxyecdysone (**2**). The addition of the acetonide group was confirmed by presence of two methyl groups at δ_H 1.49 (s) and 1.34 (s), and the downfield shifts of protons H-2 (δ_H 4.31) and H-3 (δ_H 4.28) in comparison with those of **2** [H-2 (δ_H 3.98) and H-3 (δ_H 3.87)]. In addition, the ^{13}C-NMR spectrum of **3** had a set of acetonide carbon signals at δ_C 109.5, 28.8 and 26.6 accompanied by significant downfield shifts of C-2 (δ_C 73.2) and C-3 (δ_C 73.5) in comparison with those of **2** [C-2 (δ_C 68.6) and C-3 (δ_C 68.4)]. Furthermore, the HMBC correlations from H-2 (δ_H 4.31)/H-3 (δ_H 4.28) to C-2,3-ACN (δ_C 109.5); from methyl groups (δ_H 1.49)/(δ_H 1.34) to 2,3-ACN (δ_C 109.5) also suggested the position of acetonide group at C-2/C-3. All NMR assignments of **3** were confirmed by detailed analyses of HSQC and HMBC spectra, which are in good agreement with those reported in literature.[6] Therefore, **3** was identified as 20-hydroxyecdysone 2,3-monoacetonide.

The molecular formula of **4** was determined to be $C_{27}H_{44}O_8$ on the basic of HR-ESI-MS ion at *m/z* 495.2950 $[M-H]^-$ (Calcd. for $[C_{27}H_{43}O_8]^-$, 495.2963). The ^{1}H-NMR spectrum of **4** showed the signals one olefinic proton at δ_H 5.82 (d, J = 2.5 Hz), four hydroxyl methines at δ_H 4.13 (m), 4.03 (dt, J = 4.0, 12.0 Hz), 3.98 (br d, J = 2.0 Hz), and 3.37 (m), five tertiary methyl groups at δ_H 1.24, 1.23, 1.22, 1.08, 0.90 (each 3H, s). The ^{13}C-NMR and DEPT spectra of **4** displayed the signals of 27 carbons, including one carbonyl at δ_C 206.7; six non-protonated carbons at δ_C 165.7, 84.9, 77.8, 71.3, 49.0, and 39.9; eight methines at δ_C 122.7, 78.4, 68.9, 68.6, 52.8, 42.9, 69.5, and 50.3; seven methylenes at δ_C 43.8, 42.4, 39.1, 33.3, 31.9, 27.3, and 21.5; and five methyl carbons at δ_C 29.7, 29.0, 24.6, 21.0, and 18.9. The ^{1}H- and ^{13}C-NMR data of **4** were very similar to those of turkesterone.[7] From the above evidence, compound **4** was identified as turkesterone, a compound was also isolated from *V. scabra*.[5].

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