Vietnam Journal of Science and Technology ***56*** *(6) (2018) 681-687*

DOI: 10.15625/2525-2518/56/6/12584

STEROLS AND FLAVONE FROM THE LEAVES OF *VERNONIA AMYGDALINA* GROWING IN THUA THIEN HUE

Hoang Le Tuan Anh[1, 2, *], Le Thi Lien[1, 2], Pham Viet Cuong[1], Masayoshi Arai[3], Tran Phuong Ha[1], Ton That Huu Dat[1], Le Canh Viet Cuong[1]

[1]*Mientrung Institute for Scientific Research, Vietnam Academy of Science and Technology (VAST), 321 Huynh Thuc Khang, Hue city, Thua Thien Hue, Viet Nam*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[3]*Research Center for Drug Discovery, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka 565-0871, Japan*

[*]Email: *hoangletuananh@hotmail.com*

Received: 22 May 2018; Accepted for publication: 4 September 2018

ABSTRACT

By using various chromatographic methods, two sterols and one flavone, (22*R*,23*S*,24*R*,28*S*)-28-methoxy-7,8,9,11-tetradehydro-3*β*,16*α*,21,24-tetrahydroxy-21,23 :22,28-diepoxy-5α-stigmastane (**1**), (23*S*,24*R*,28*S*)-3*β*,22*α*-dihydroxy-7,8,9,11-tetradehy dro-24,28-epoxy-5*α*-stigmastane-21,23-carbolactone (**2**)**,** and luteolin (**3**) were isolated from the methanol extract of the leaves of *Vernonia amygdalina*. Their structures were determined using 1D, 2D-NMR and ESI-MS analysis as well as by comparison with the reported data. Compounds **1** and **2** were reported from nature for the first time.

Keywords: Vernonia amygdalina, sterol, luteolin.

Classification numbers: 1.1.1; 1.1.6

1. INTRODUCTION

Vernonia amygdalina Delile is a shrub or small tree that is mainly grown in tropical areas of Africa [1]. In Africa, it can be used as a traditional treatment for diabetes, emesis, nausea, dermatitis, arthristis, ascariasis, stomached, anaemia, jaundice, pneumonia, fever, tonsillitis and anti-inflammatory [2-4]. The studies of the chemical components have shown that *V. amygdalina* contains steroids, terpenoids, saponins, polyphenolics, alkaloids, cardiac glycosides, anthraquinone and coumarins [5-9]. Biological activities of extracts and isolated compounds from *V. amygdalina* have been reported, such as antidiabetic, antibacterial, antifungal, antiparasite, antiviral, anticancer, anti-obesity, antioxidant, antihypertensive, and liver protective activity [1, 10-12]. Recently, *V. amygdalina* was introduced and grown in Thua Thien Hue as a

potential remedy for the management of diabetes mellitus. In this paper, we report the isolation and structural elucidation of two sterols and one flavone from the leaves of *V. amygdalina*.

2. MATERIAL AND METHODS

2.1. Plant Materials

The leaves of *Vernonia amygdalina* Delile were collected in Phong Dien, Thua Thien Hue, Vietnam, in August 2017, and were identified by MSc. Nguyen Quynh Nga, National Institute of Medicinal Materials. A voucher specimen (MISR-2017-14) was deposited at Mientrung Institute for Scientific Research, Vietnam Academy of Science and Technology, Viet Nam.

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). ESI-MS spectra were recorded on Agilent 1260 Series Single Quadrupole LC/MS Systems. Optical rotations, Jasco P-2000 digital polarimeter. Plant sample was extracted on a JP. Selecta 300867 sonicator. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150 µm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a 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

Air-dried leaves of *V. amygdalina* (1.2 kg) were extracted with 100 % methanol (5L × 3 times) under sonication at 50 ^{o}C for 4 h to yield a dark solid extract (200 g). This extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane and ethyl acetate to give corresponding *n*-hexane (VAH, 52 g), dichloromethane (VAD, 42 g), ethyl acetate (VAE, 31 g) and water layer (VAW, 75 g). The VAE fraction (31 g) was subsequently chromatographed on a silica gel column eluting with dichloromethane/methanol (gradient from 100/1 – 0/1, v/v) to give five fractions (VAE1-VAE5). The VAE1 (1.2 g) fraction was further separated on a silica gel column eluting with dichloromethane/methanol/water (25/1/0.05, v/v/v) to give two smaller fractions (VAE1.1 and VAE1.2). Compound **1** (VA4, 5 mg) was yielded from VAE1.1 (0.3 g) fraction by purify on a RP-18 column eluting with methanol/water (4/1, v/v). The VAE1.2 (0.2 g) fraction was further separated on a Sephadex LH-20 column eluting with methanol/water (1/1, v/v) to afford compound **3** (VA6, 15 mg). The VAE2 (0.9 g) fraction was continuously chromatographed on a RP-18 column eluting with methanol/water (3/1, v/v) to yield compound **2** (VA11, 7 mg).

(22*R*,23*S*,24*R*,28*S*)-28-methoxy-7,8,9,11-tetradehydro-3*β*,16*α*,21,24-tetrahydroxy - 21,23:22,28-diepoxy-5α-stigmastane (**1**): white powder; mp: 208-209^{o}C; $[\alpha]^{25}_{D}$ = + 78,1 (c = 0.12, MeOH); molecular formula $C_{30}H_{46}O_7$; ESI-MS*: m/z* 553.4 [M + Cl]$^{-}$. ^{1}H- and ^{13}C-NMR data, see Table 1.

(23*S*,24*R*,28*S*)-3*β*,22*α*-dihydroxy-7,8,9,11-tetradehydro-24,28-epoxy-5*α*-stigma stane-21,23-carbolactone (**2**): white powder; mp: 208-210 ^{o}C; $[\alpha]^{25}_{D}$ = + 57,0 (c = 0.14, MeOH); molecular formula $C_{29}H_{42}O_5$. ^{1}H- and ^{13}C-NMR data, see Table 1.

Luteolin (**3**): Yellow powder; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 6.65 (1H, s, H-3), 6.19 (1H, s, H-6), 6.44 (1H, s, H-8), 7.42 (1H, s, H-2′), 6.89 (1H, d, *J* = 7.5 Hz, H-5′), 7.40 (1H,

d, J = 7.5 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 163.9 (C-2), 102.8 (C-3), 181.6 (C-4), 161.4 (C-5), 98.8 (C-6), 164.1 (C-7), 93.8 (C-8), 157.3 (C-9), 103.6 (C-10), 121.5 (C-1′), 113.3 (C-2′), 145.7 (C-3′), 149.7 (C-4′), 116.0 (C-5′), 118.9 (C-6′).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder and its molecular formula was determined as $C_{30}H_{46}O_7$ by the ESI-MS at *m/z* 553.4 $[M + Cl]^-$ with a combination of ^{1}H- and ^{13}C-NMR data (Table 1). The ^{1}H-NMR spectrum of **1** showed five methyl group signals at δ_H 0.61 (3H, s, H-18), 0.93 (3H, s, H-19), 0.95 (6H, d, J = 6.5 Hz, H-26, H-27), and 1.45 (3H, s, H-29); two olefinic proton signals at δ_H 5.44 (1H, d, J = 4.5 Hz, H-7) and 5.57 (1H, d, J = 5.5 Hz, H-11); one distinctive H-3 proton signal at δ_H 3.54 (1H, m, H-3), and one methoxy group signal at δ_H 3.22 (3H, s, OCH_3). The ^{13}C-NMR and HSQC spectra (Table 1) of **1** revealed 30 carbon signals, of which 29 were assigned to steroidal skeleton and the remaining signal belonged to methoxy group. Furthermore, the ^{13}C NMR spectrum of steroid moiety contained characteristic signals corresponding to two tri-substituted double bonds [δ_C 145.1 (C-9), 136.5 (C-8), 122.1 (C-7), and 119.4 (C-11)], two dioxygenated carbons [δ_C 114.1 (C-28), 100.0 (C-21)], five oxygenated carbons [δ_C 91.9 (C-23), 83.1 (C-24), 81.6 (C-22), 77.3 (C-16), and 71.4 (C-3)]. The above-mentioned data suggested that **1** was a $\Delta^{7,9(11)}$ stigmastane-type [2, 13]. Two oxymethine signals at 3.53 (1H, m) and 4.34 (1H, t, J = 7.0) suggested the presence of a β-hydroxy group at C-3 and a α-hydroxy group at C-16 by comparison with those literature [14]. Two methyl signals at δ_H 0.95 (H-26)/ δ_C 17.4 (C-26); δ_H 0.95 (H-27)/ δ_C 18.1 (C-27), and a methine signal at δ_H 2.06 (H-25)/ δ_C 33.1 (C-25) showed the presence of an isopropyl moiety.

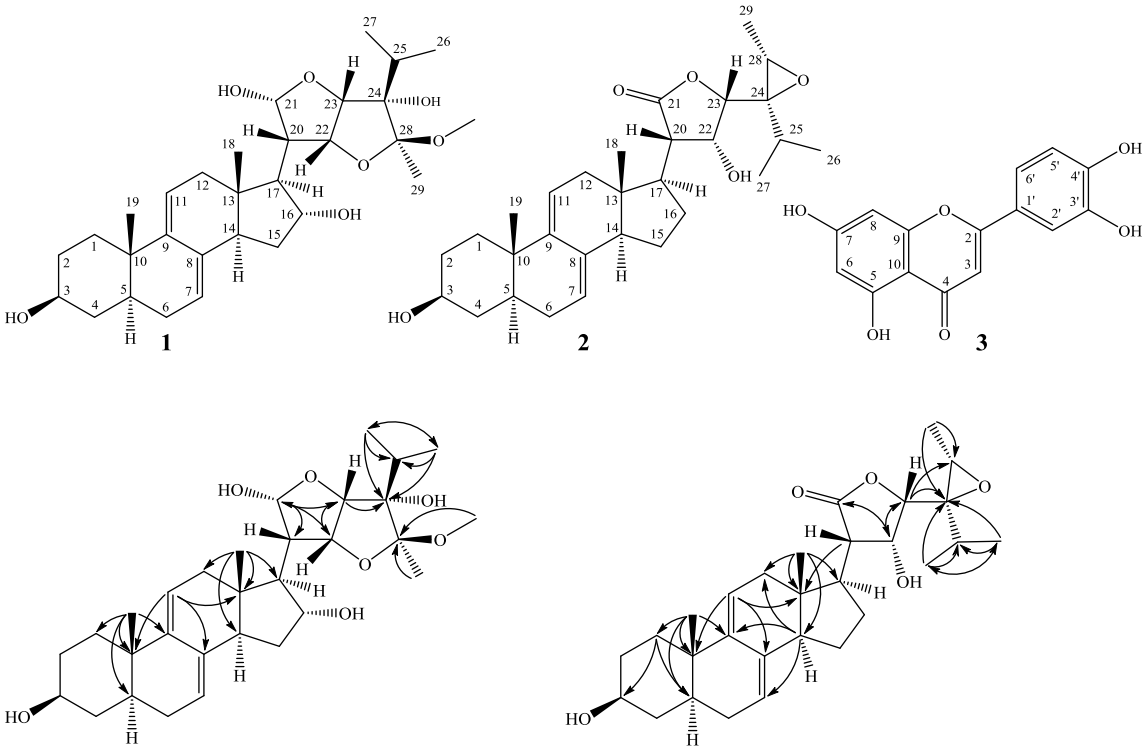

Figure 1. Chemical structures of compounds **1**-**3** and key HMBC correlations of compounds **1**-**2**.

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

C	1		2	
	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)
1	35.3	1.34 (m) 1.98(m)	36.0	1.35 (m) 2.00 (m)
2	32.4	1.50 (m) 1.87 (m)	32.3	1.50 (m) 1.86 (m)
3	71.4	3.54 (m)	71.5	3.53 (m)
4	38.5	1.31(m) 1.72 (brd 12.0)	38.8	1.31 (m) 1.71 (m)
5	40.6	1.45(m)	40.7	1.43 (m)
6	31.0	1.92(m)	31.0	1.30 (m) 1.93 (m)
7	122.1	5.45 (d, 5.0)	121.3	5.44 (brs)
8	136.7	-	137.6	-
9	145.1	-	144.9	-
10	37.0	-	37.0	-
11	119.4	5.56 (d, 6.5)	120.5	5.58 (d, 6.5)
12	42.5	2.04 (m) 2.72 (dd, 4.0, 10.5)	42.8	2.13 (m) 2.82 (m)
13	44.3	-	43.3	-
14	49.7	2.58 (m)	51.4	2.86 (dd, 4.0, 10.5)
15	36.0	1.37 (dd, 3.0, 13.5) 2.00 (dd, 3.0, 13.5)	24.2	1.52 (m) 1.84 (m)
16	77.3	4.34 (t, 7.0)	28.5	1.52 (m) 1.80 (m)
17	56.3	2.07 (dd, 2.5, 6.5)	46.3	2.00 (m)
18	14.5	0.61 (s)	12.6	0.61 (s)
19	19.8	0.93 (s)	19.9	0.94 (s)
20	48.8	2.23 (t, 5.0)	52.8	2.28 (m)
21	100.0	5.46 (brs)	177.8	-
22	81.6	4.57 (t, 5.5)	73.8	4.42 (dd, 2.5, 4.0)
23	91.9	4.58 (brs)	80.5	4.77 (d, 2.5)
24	83.1	-	65.2	-
25	33.1	2.06 (m)	31.0	1.83 (m)
26	17.4	0.95 (d, 6.5)	18.4	1.20 (d, 7.0)
27	18.1	0.95 (d, 6.5)	18.6	1.15 (d, 7.0)
28	114.1	-	57.6	2.29 (s)
29	17.4	1.45 (s)	13.1	1.37 (d, 5.5)
28-OCH_3	48.5	3.22 (s)		

[a] Recorded in CD_3OD, [b] 125 MHz, [c] 500 MHz.

The complete assignment of all protons and carbons of **1** was conducted by analysis of the HMBC spectrum. The HMBC correlations (Figure 1) between H-11 (δ_H 5.56) and C-8 (δ_C 136.7)/ C-10 (δ_C 37.0)/ C-13 (δ_C 44.3) as well as between H-19 (δ_H 0.93) and C-1 (δ_C 35.3)/ C-5

(δ_C 40.6)/ C-9 (δ_C 145.1)/ C-10 (δ_C 37.0) suggested the presence of a $\Delta^{7,9(11)}$ diene. For the side chain, the HMBC correlations from H-21 (δ_H 5.46) to C-20 (δ_C 48.8)/C-22 (δ_C 81.6)/C-23 (δ_C 91.9); from H-22 (δ_H 4.57) to C-21 (δ_C 100.0)/C-23 (δ_C 91.9); from H-23 (δ_H 4.58) to C-21 (δ_C 100.0)/ C-24 (δ_C 83.1) indicated the presence of two furan rings, which were fused at C-22 and C-23. The isopropyl group was determined to be attached to C-24 by the HMBC correlations from H-25 (δ_H 2.06) and H-26/H-27 (δ_H 0.95) to C-24 (δ_C 83.1).

The position of methyl and methoxy groups at C-28 was confirmed by the HMBC correlations from H-29 (δ_H 1.45) and 28-OCH_3 (δ_H 3.22) to C-28 (δ_C 114.1). The NMR data of **1** compared with those of aglycone of vernonioside B2 [14] were relevant to conclude that compound **1** is (22*R*,23*S*,24*R*,28*S*)-28-methoxy-7,8,9,11-tetradehydro-3*β*,16*α*,21,24-tetrahydroxy-21,23:22,28-diepoxy-5α-stigmastane. This compound was formed when vernonioside B_2 was hydrolyzed with *β*-glucosidase [14]. To the best of our knowledge, compound **1** was isolated from nature for the first time (Figure 1).

Compound **2** was obtained as a white powder. The ^{1}H-NMR spectrum of **2** observed five methyl group signals at δ_H 0.61 (3H, s, H-18), 0.94 (3H, s, H-19), 1.20 (3H, d, *J* = 7.0 Hz, H-26), 1.15 (3H, d, *J* = 7.0 Hz, H-27), and 1.37 (3H, d, *J* = 5.5 Hz, H-29); two proton olefinic signals at δ_H 5.44 (1H, brs, H-7), and 5.58 (1H, d, *J* = 6.5 Hz, H-11), and the distinctive H-3 proton signal at δ_H 3.53 (1H, m, H-3). The ^{13}C-NMR and HSQC spectra of **2** showed 29 carbon signals (Table 1) including five methyl signals, seven methylene carbons, 11 methine carbons and six non-protonate carbons. The ^{1}H and ^{13}C NMR spectroscopic data of **2** were similar to those of **1**. The significant difference between **1** and **2** was observed at the side chain. The HMBC correlations from H-22 (δ_H 4.42) to C-21 (δ_C 177.8)/C-23 (δ_C 80.5); from H-23 (δ_H 4.77) to C-21 (δ_C 177.8) suggested the presence of a γ-lactone ring in the molecule. Meanwhile, the signals of two oxygenated carbons at δ_C 57.6 (C-28), 65.2 (C-24) implied the presence of epoxy group. In addition, the HMBC correlations of H-25 (δ_H 1.83)/H-26 (δ_H 1.20) to C-24 (δ_C 65.2)/C-25 (δ_C 31.0), of H-29 (δ_H 1.37) to C-24/C-28 (δ_C 57.6) led to the assignment of isopropyl, methyl groups at C-24, C-28, respectively. These evidences allowed us to construct the 2,3-epoxy-4-methylpentyl sub-structure. The connection between this sub-structure and γ-lactone ring via C-23/C-24 linkage was confirmed by the HMBC correlations of H-23 to C-24/C-25/C-28. Base on above evidence and comparison with the reported data [15], compound **2** was determined as an aglycone of vernonioside B_1: (23*S*,24*R*,28*S*)-3*β*,22*α*-dihydroxy-7,8,9,11-tetradehydro-24,28-epoxy-5*α*-stigmastane-21,23-carbolactone. This compound was also isolated from nature for the first time (Figure 1). Compound **3** was obtained as a yellow powder. The NMR spectra of **3** indicated that the structure of **3** is to be a flavone and its data were similar to those of luteolin [16]. Thus, compound **3** was identified as luteolin.

4. CONCLUSIONS

From the methanol extract of the leaves of *Vernonia amygdalina*, two sterols, 22*R*,23*S*,24*R*,28*S*)-28-methoxy-7,8,9,11-tetradehydro-3*β*,16*α*,21,24-tetrahydroxy-21,23:22,28-diepoxy-5α-stigmastane (**1**), (23*S*,24*R*,28*S*)-3*β*,22*α*-dihydroxy -7,8,9,11-tetradehydro-24,28-epoxy-5*α*-stigmastane-21,23-carbolactone (**2**), and one flavone, luteolin (**3**), were isolated and identified. Compounds **1** and **2** have been isolated from nature for the first time.

Acknowledgement. This research was supported by Vietnam Academy of Science and Technology under grant number VAST.NĐP.03/17-18.

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