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STEROLS AND TRITERPENE FROM *Trichosanthes kirilowii*

Bui Hoang Vinh[1,2], Pham Thi Trang Tho[1], Bui Huu Tai[1], Hoang Le Tuan Anh[1], Trinh Nam Trung[2], Hoang Viet Dung[2], Ninh Khac Ban[1], Chau Van Minh[1], Phan Van Kiem[1], Nguyen Xuan Nhiem[1*]

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

[2]*Vietnam Military Medical University*

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Abstract

By various chromatographic methods, two sterols and one triterpene, 22*E*,24*Z*-stigmasta-7,22,24(28)-triene-3*β*-ol, ergosta-6,22-diene-3*β*,5*α*,8*α*-triol, and 3*β*-hydroxymultiflora-8-ene-17-oic acid were isolated from the roots of *Trichosanthes kirilowii* MAXIM. Their structures were elucidated by spectroscopic methods and in comparison with the reported data. To our best knowledge, compound **1** was isolated from the nature for the first time. Compounds **2** and **3** were reported from *Trichosanthes* genus for the first time.

Keywords. *Trichosanthes kirilowii*, Cucurbitaceae, triterpene.

1. INTRODUCTION

Trichosanthes kirilowii MAXIM belongs to the cucumber family (Cucurbitaceae) and is distributed in Vietnam, China, Korea, and Japan. Its roots have been used in Vietnam traditional medicine as an anti-inflammatory agent, a cough and sore throat medicines [1]. Previous phytochemical investigations of *T. kirilowii* have yielded lignans [2], flavones [3], and triterpenes [4-6]. In addition, some of these compounds showed cytotoxic [2, 7, 8], antitumor [9], anti-inflammatory [5], and antibacterial activities [10]. As part of our ongoing chemical investigation from the roots of *T. kirilowii*, three triterpenes were isolated. We report herein the isolation and structural elucidation of these compounds.

2. MATERIAL AND METHODS

2.1. Animal materials

The roots of *T. kirilowii* were collected in Hoabinh, Vietnam in September, 2013, and identified by Dr. Ninh Khac Ban, Institute of Marine Biochemistry, VAST, Vietnam. A voucher specimen (TK01) was deposited at the Herbarium of Institute of Marine Biochemistry, VAST, Vietnam.

2.2. General experimental procedures

Chemical shifts are reported in parts per million from TMS. 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, HMBC, and COSY experiments, were carried out using 5-mm probe tubes at temperature of 22.2 ^{o}C in CD_3OD solutions, with TMS as the internal standard. Melting points were recorded in Kofler micro-hostage. 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 roots of *T. kirilowii* (2.0 kg) were extracted with MeOH three times using sonicator for 15 h to yield 90.0 g of a dark solid MeOH extract, which was then suspended in water and successively partitioned with $CHCl_3$ to obtain $CHCl_3$ (TK1, 40.0 g), and water (TK2, 50.0 g) residues after removing solvent in *vacuo*. The TK1 layer was chromatographed on a silica gel column and eluted with *n*-hexane−acetone gradient (40:1→1:1, v/v) to obtain five fractions, TK1A (9.0 g), TK1B (10.0 g),

TK1C (5.0 g), TK1D (5.5 g), and TK1E (5.0 g). The TK1B fraction was chromatographed on a silica gel column eluting with *n*-hexane−ethyl acetate (4:1, v/v) to give three smaller fractions, TK1B1−TK1B3. The TK1B2 fraction was chromatographed on an RP-18 column eluting with acetone−water (4:1, v/v) to yield compounds **1** (5.0 mg) and **2** (5.0 mg). The TK1C fraction was chromatographed on a silica gel column eluting with dichloromethane−ethyl acetate (15:1, v/v) to obtain four smaller fractions, TK1C1-TK1C4. The TK1C3 fraction was chromatographed an RP-18 column eluting with MeOH−water (5:1, v/v) to yield compound **3** (7.0 mg).

22E,24Z-Stigmasta-7,22,24(28)-triene-3β-ol (1): White amorphous powder, $C_{29}H_{46}O$, MW = 410, m.p. 135-137^{o}C, ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$), see table 1.

Ergosta-6,22-diene-3β,5α,8α-triol (2): White amorphous powder, $C_{28}H_{46}O_3$, MW = 430, m.p. 163-165^{o}C, ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$), see table 1.

3β-Hydroxymultiflora-8-ene-17-oic acid (3): White amorphous powder, $C_{30}H_{48}O_3$, MW = 456, m.p. 211-212^{o}C, ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$ and CD_3OD), see table 1.

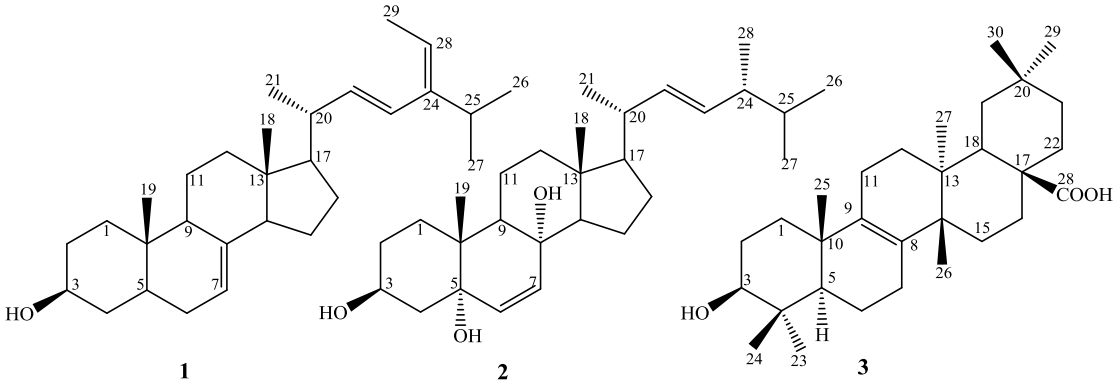

Figure 1: Chemical structures of compounds **1-3** from *Trichosanthes kirilowii*

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR of **1** showed protons of two tertiary methyl groups at δ_H 0.80 and 0.58, four secondary methyl groups at δ_H 1.72 (d, *J* = 7.0 Hz), 1.08 (d, *J* = 6.5 Hz), 1.03 (d, *J* = 6.5 Hz), and 1.02 (d, *J* = 6.5 Hz), four olefinic protons at δ_H 6.17 (d, *J* = 16.0 Hz), 5.51 (dd, *J* = 9.0, 16.0 Hz), 5.31 (q, *J* = 7.0 Hz), and 5.16 (dd, *J* = 2.5, 5.0 Hz), and one hydroxyl methine proton at 3.59 (m), suggested the presence of a sterol skeleton. The ^{13}C-NMR and DEPT spectra of **1** showed the signals of 29 carbons, including four quaternary at δ_C 34.25, 43.36, 139.48, and 143.35, eleven methine at δ_C 30.28, 40.29, 41.27, 49.48, 55.11, 56.01, 71.07, 117.58, 117.99, 123.50, and 136.41, eight methylene at δ_C 21.58, 22.97, 28.22, 29.66, 31.51, 37.17, 38.02, and 39.51, and six methyl carbons at δ_C 12.17, 13.05, 13.31, 20.96, 22.23, and 22.63. Analytical ^{1}H- and ^{13}C-NMR data of **1** indicated that the structure of **1** is similar to these of stigmasta-7,22,24(28)-triene-3-ol [11]. The position of the hydroxyl group at C-3 was confirmed by HMBC correlations between H-1 (δ_H 1.11 and 1.85)/H-2 (δ_H 1.40 and 1.81)/H-4 (δ_H 1.30 and 1.73)/H-5 (δ_H 1.40) and C-3 (δ_C 71.07). In addition, HMBC correlations between H-7 (δ_H 5.16) and C-9 (δ_C 49.48)/C-14 (δ_C 55.11), H-21 (δ_H 1.08) and C-17 (δ_C 56.01)/C-20 (δ_C 41.27)/C-22 (δ_C 136.41); H-23 (δ_H 6.17) and C-20 (δ_C 41.27)/C-22 (δ_C 136.41)/C-24 (δ_C 143.35)/C-25 (δ_C 30.28)/C-28 (δ_C 117.99); H-29 (δ_H 1.72) and C-24 (δ_C 143.35)/C-28 (δ_C 117.99) proved the positions of three double bonds at C-7/C-8, C-22/C-23, and C-24/C-28. Based on the above evidence, the structure of **1** was elucidated to be stigmasta-7,22,24(28)-triene-3-ol. Compound **1** was reported as its acetate ester form [11]. To our best knowledge, compound **1** was isolated as the first time from nature.

Compound **2** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **2** showed protons of two tertiary methyl groups at δ_H 0.82 (s, H-18) and 0.88 (s, H-19); four secondary methyl groups at δ_H 0.82 (d, *J* = 6.5 Hz), 0.83 (d, *J* = 6.5 Hz), 0.89 (d, *J* = 6.5 Hz) and 1.00 (d, *J* = 6.5 Hz), four olefinic protons at δ_H 5.14 (dd, *J* = 8.0, 15.5 Hz), 5.22 (dd, *J* = 7.5, 15.5 Hz), 6.24 (d, *J* = 8.5 Hz) and 6.50 (d, *J* = 8.5 Hz), one hydroxyl methine proton at δ_H 3.95 (m), suggested that the structure of **2** belonged to an ergostane skeleton. The ^{13}C-NMR and DEPT spectra of compound **2** showed the signals of 28 carbons, including four non-protonated carbons at δ_C 36.95, 44.55, 79.43, and 82.15, eleven methine at δ_C 33.06,

Table 1: The NMR data for compounds **1-3** and reference compounds

		1			**2**			**3**	
C	δ_C [#]	δ_C [a,c]	δ_H [a,d] (mult., *J* = Hz)	δ_C [$]	δ_C [a,c]	δ_H [a,d] (mult., *J* = Hz)	δ_C [@]	δ_C [b,c]	δ_H [b,d] (mult., *J* = Hz)
1	36.8	37.17	1.11 (m)/1.85 (m)	34.7	34.67	1.70 (m)/1.96 (m)	35.1	34.49	0.89 (m)/2.13 (m)
2	**27.5**	**31.51**	1.40 (m)/1.81 (m)	39.3	39.32	1.24 (m)/1.97 (m)	28.0	27.70	1.86 (m)/2.10 (m)
3	**73.5**	**71.07**	3.59 (m)	66.5	66.48	3.95 (m)	78.0	78.97	3.19 (dd, 5.0, 11.0)
4	**33.8**	**38.02**	1.30 (m)/1.73 (m)	36.9	36.90	1.80 (m)/2.11 (m)	39.4	37.33	-
5	40.0	40.29	1.40 (m)	82.2	82.15	-	50.9	50.66	1.04*
6	29.5	29.66	1.28 (m)/1.78 (m)	135.4	135.40	6.50 (d, 8.5)	19.6	19.31	1.39 (m)/1.69 (m)
7	117.4	117.58	5.16 (dd, 2.5, 5.0)	130.7	130.74	6.24 (d, 8.5)	35.5	35.16	1.13 (m)/1.77 (m)
8	139.4	139.48	-	79.4	79.43	-	134.1	134.07	-
9	49.3	49.48	1.67*	51.7	51.67	1.57*	134.6	134.11	-
10	34.2	34.25	-	37.0	36.95	-	40.6	40.33	-
11	21.5	21.58	1.50 (m)/1.69 (m)	20.6	20.62	1.41(m)/1.60 (m)	21.1	20.76	1.91*
12	39.4	39.51	1.27 (m)/2.02 (m)	30.1	30.95	1.53 (m)/1.84 (m)	28.7	27.63	1.64 (m)
13	43.4	43.36	-	44.6	44.55	-	37.7	38.88	-
14	55.0	55.11	1.84 (m)	56.2	56.17	1.23 (m)	42.1	41.95	-
15	22.9	22.97	1.42 (m)/1.51 (m)	23.4	23.39	1.23 (m)/1.51 (m)	25.4	25.19	1.32 (m)/1.52 (m)
16	28.2	28.22	1.32 (m)/1.85 (m)	28.6	28.65	1.75 (m)	30.5	29.92	1.37 (m)/2.18 (m)
17	56.0	56.01	1.34 (m)	33.1	33.06	2.34 (m)	37.8	37.64	-
18	12.2	12.17	0.58 (s)	12.9	12.87	0.82 (s)	45.1	44.83	1.50 (m)
19	13.0	13.05	0.80 (s)	18.2	18.17	0.88 (s)	37.3	37.15	1.34 (m)/1.68 (m)
20	41.3	41.27	2.16 (m)	42.8	42.76	1.86 (m)	31.3	31.26	-
21	21.0	20.96	1.08 (d, 6.5)	17.6	17.56	0.89 (d, 6.5)	30.7	30.39	1.27 (m)/1.64 (m)
22	136.4	136.41	5.51 (dd, 9.0, 16.0)	132.3	132.29	5.22 (dd, 7.5, 15.5)	31.1	30.73	1.64 (m)/2.39 (d, 15.5)
23	123.5	123.50	6.17 (d, 16.0)	135.2	135.19	5.14 (dd, 8.0, 15.5)	28.6	28.02	0.98 (s)
24	143.3	143.35	-	39.7	39.74	2.02 (m)	22.4	22.18	0.96 (s)
25	30.2	30.28	2.54 (m)	51.1	51.06	1.50 (m)	20.1	19.93	0.95 (s)
26	22.6	22.63	1.02 (d, 6.5)	19.9	19.95	0.83 (d, 6.5)	31.4	31.04	1.03 (s)
27	22.2	22.23	1.03 (d, 6.5)	19.6	19.64	0.82 (d, 6.5)	16.5	15.65	0.79 (s)
28	118.0	117.99	5.31 (q, 7.0)	20.9	20.87	1.00 (d, 6.5)	181.3	182.07	-
29	13.3	13.31	1.72 (d, 7.0)				18.0	17.51	0.89 (s)
30							33.3	33.94	1.21 (s)

[a]recorded in $CDCl_3$,[b]recorded in $CDCl_3$ and CD_3OD [c]125 MHz, [d]500MHz, [#]δ_C of 22E,24Z-stigmasta-7,22,24(28)-triene-3β-yl acetate [11], , [$]$\delta_C$ of ergosta-6,22-diene-3β,5α,8α-triol [12] [@]δ_C of 3β-hydroxymultiflora-8-ene-17-oic acid [13].

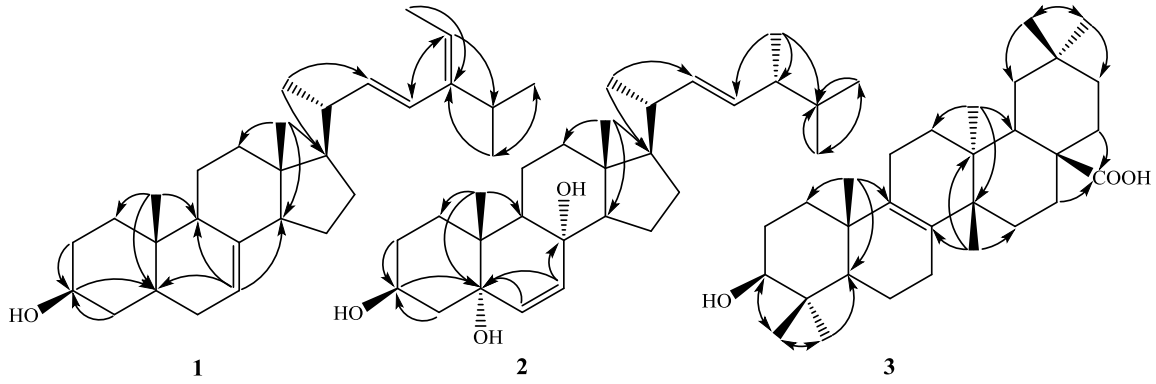

Figure 2: The important HMBC correlations of compounds **1-3**

39.74, 42.76, 51.06, 51.67, 56.17, 66.48, 130.74, 132.29, 135.19, and 135.40, seven methylene at δ_C 20.62, 23.39, 28.65, 30.95, 34.67, 36.90, 39.32, and six methyl carbons at δ_C 12.87, 17.56, 18.17, 19.64, 19.95, and 20.87. The position of the hydroxyl group at C-5 was confirmed by HMBC correlations between H-19 (δ_H 0.88) and C-1 (δ_C 34.67)/C-5 (δ_C 82.15)/C-9 (δ_C 51.67)/C-10 (δ_C 36.95). The HMBC correlations from H-7 (δ_H 6.24) to C-8 (δ_C 79.43)/C-9 (δ_C 51.67)/C-14 (δ_C 56.17) suggested the double bond at C-6/C-7 and hydroxyl group at C-8. The remaining of double bond at C-23/C-24 was proved by HMBC correlations between H-21 (δ_H 0.89) and C-17 (δ_C 33.06)/C-20 (δ_C 42.76)/C-22 (δ_C 132.29). Consequently, compound **2** was elucidated to be ergosta-6,22-diene-3β,5α,8α-triol [12].

The ^{1}H-NMR of compound **3** exhibited one hydroxyl methine proton at δ_H 3.19 (dd, J = 5.0, 11.0 Hz) and seven tertiary methyl groups at δ_H 0.79, 0.89, 0.95, 0.96, 0.98, 1.03, and 1.21, (each 3H, singlet). The ^{13}C-NMR and DEPT spectra of compound **3** showed the presence of 30 carbons including one carboxylic, eight quaternary, three methine, eleven methylene, seven methyl carbons. The ^{1}H- and ^{13}C-NMR data of **3** was similar to those of 3*β*-hydroxymultiflora-8-ene-17-oic acid. The position of hydroxyl group at C-3 was confirmed by the HMBC correlations between H-23 (δ_H 0.98)/H-24 (δ_H 0.96) and C-3 (δ_C 78.97)/C-4 (δ_C 37.33)/C-5 (δ_C 50.66). The *β* configuration of this hydroxyl group was based on the large coupling constant between H-2 and H-3, J = 11.0 Hz. The double bond at C-8/C-9 was proved by the HMBC correlations from H-25 (δ_H 0.95) to C-9 (δ_C 134.11) and from H-26 (δ_H 1.03) to C-8 (δ_C 134.07). Thus, compound **3** was defined to be 3*β*-hydroxymultiflora-8-ene-17-oic acid. This compound was isolated from *Momordica charantia* [13] but first time from the *Trichosanthes* genus.

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Corresponding author: **Nguyen Xuan Nhiem**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: nxnhiem@yahoo.com.