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# Sterols from stems of *Momordica cochinchinesis* (Lour.) Spreng

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**Abstract**

Three known sterols, polypodine B (**1**), (22*E*,24*R*)-24-methylcholesta-2,22-diene-3*β*,5*α*,6*β*-triol (**2**) and chondrillasterol (**3**) were isolated from the stems of *Momordica cochinchinesis* (Lour.) Spreng. Their chemical structures were successfully determined using NMR and ESI-MS analysis as well as in comparison with the reported data. All compounds were reported from *Momordica* genus for the first time.

**Keywords.** *Momordica cochinchinesis*, Cucurbitaceae, steroid.

## 1. INTRODUCTION

*Momordica* genus belongs to Cucurbitaceae family, including 60 species. In Vietnam, there are three species, comprising of *M. charantia*, *M. cochinchinensis*, and *M. grosvenori*. *M. cochinchinensis* is a dioecious species with yellow flowers, green globe fruits turn orange and red when ripened [1]. The main ingredient of *M. cochinchinensis* oil is *β*-carotene, which is used to treat diseases related to vitamin A as slow growth in children, dry eyes, night blindness, tired and anorexic people. Additionally, *M. cochinchinensis* oil is used in the treatment of the wound and burns. The seeds are used to treat abscess, mumps, swelling breast, engorgement, hemorrhoids and pile. The roots are bitter, cool thus used for preventing low-temperature effects, diuretics [1]. The chemical studies of *M. cochinchinensis* indicated the presence of triterpenoids [2], carotenoids [3]. These compounds showed the anti-inflammatory [2], stomach ulcers, and wound healing activities [4]. In this paper, we report the isolation, structural elucidation of one ecdysterone and two sterols from the stems of *M. cochinchinensis*.

## 2. MATERIAL AND METHODS

### 2.1. Plant materials

The stems of *M. cochinchinensis* were collected in Quoc Oai, Hanoi, Vietnam in September 2013 and identified by Prof. Dr. Ninh Khac Ban, Institute of Ecology and Biological Resources, VAST. A voucher specimen (MC1309) was deposited at the Herbarium of the Institute of Marine Biochemistry, VAST.

### 2.2. General experimental procedures

*Thin layer chromatography* (TLC): Performed on the pre-coated silica-gel DC-Alufolien 60 F254 (Merck 1.05715), RP18 F254s plates (Merck). Compounds were detected by ultraviolet light at 254 nm and 365 nm and visualized by spraying with aqueous 10 % $H_2SO_4$ and heating for 5 minutes.

*Column chromatography* (CC): performed on silica gel (0.040÷0.063 mm, Merck) or RP-18 resins (30÷50 μm, Fujisilisa Chemical Ltd.).

All NMR spectra were recorded on a Bruker AM-500 at Institute of Chemistry, VAST.

The ESI-MS were obtained from an Agilent 1100 series at Institute of Marine Biochemistry, VAST.

### 2.3. Extraction and isolation

The dried powder of stems of *M. cochinchinesis* was extracted 3 times with methanol at 50 °C. The extract was filtered through filter paper, then solvent was removed under reduced pressure to yield 40 g of a dark solid extract. The extract was suspended in water and partitioned with dichloromethane giving dichloromethane (9.0 g) and water extracts (30.0 g).

The dichloromethane extract was chromatographed on a silica gel column eluting with *n*-hexane:acetone (10:1→1:1, v/v) to give 5 subfractions, C1-C5.

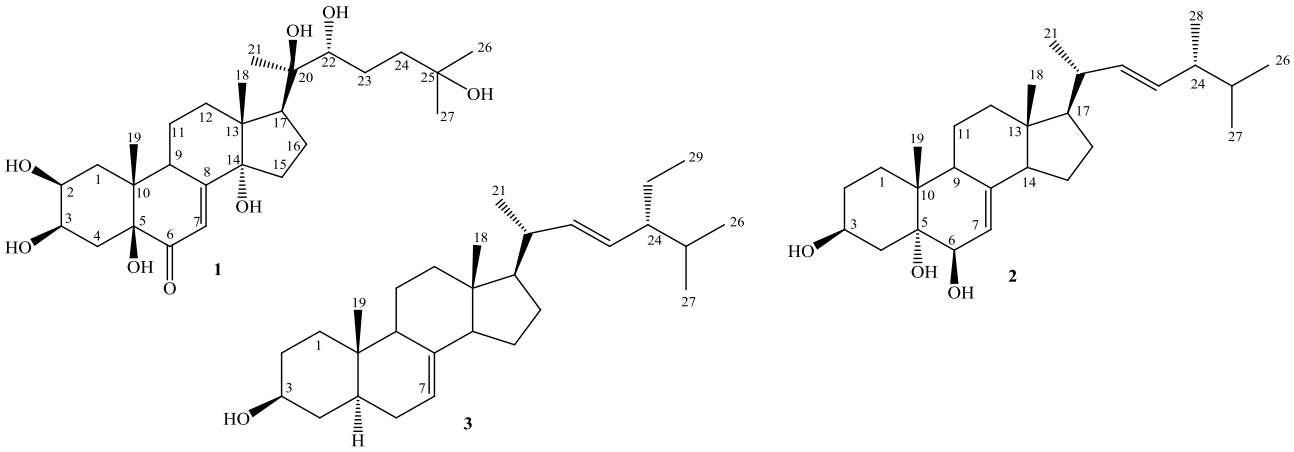

*Figure 1:* Chemical structures of compounds **1**-**3**

Fraction C2 was chromatographed on an RP-18 column eluting with acetone–water (6:1, v/v) to give two smaller fractions, C2.1 and C2.2. Compound **3** (5.0 mg) was yielded from C2.2 fraction using a silica gel column eluting with *n*-hexane:acetone (2:1, v/v). Fraction C3 (1.2 g) was chromatographed on an RP-18 column eluting with acetone - water (2:1, v/v) to give four smaller fractions, C3.1-C3.4. Fraction C3.2 was chromatographed on a Sephadex LH-20 column eluting with dichloromethane:methanol (1:3, v/v) to yield compound **2** (10.0 mg). Fraction C4 (1.5 g) was continued to fractionated on a silica gel column eluting with dichloromethane:methanol:water (5:1:0.1, v/v/v) to give two fractions, C4.1 and C4.2. Fraction C4.1 (600 mg) was chromatographed on a RP-18 column eluting with acetone:methanol:water (4:4:1 v/v/v) to yield compound **1** (15.0 mg).

**Polypodine B** (**1**): White amorphous powder; ESI-MS *m/z* 495 $[M-H]^-$; $C_{27}H_{44}O_8$; $^1$H-NMR (500 MHz, $CD_3OD$) and $^{13}$C-NMR (125 MHz, $CD_3OD$), see Table 1.

**(22*E*,24*R*)-24-Methylcholesta-2,22-diene-3β,5*α*,6β-triol** (**2**): White amorphous powder; ESI-MS *m/z* 429 $[M-H]^-$; $C_{28}H_{46}O_3$; $^1$H-NMR (500 MHz, DMSO-$d_6$) and $^{13}$C-NMR (125 MHz, DMSO-$d_6$), see table 1.

**Chondrillasterol** (**3**)**:** White amorphous powder; ESI-MS *m/z* 411 $[M-H]^-$; $C_{29}H_{48}O$; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta_H$: 3.59 (m, H-3), 5.16 (m, H-7), 0.55 (s, H-18), 0.80 (s, H-19), 1.03 (d, *J* = 6.5 Hz, H-21), 5.03 (dd, *J* = 8.5, 16.0 Hz, H-22), 5.19 (dd, *J* = 8.5, 16.0 Hz, H-23), 0.79 (d, *J* = 6.5 Hz, H-26), 0.85 (d, *J* = 6.5 Hz, H-27), 0.81 (t, *J* = 7.5 Hz, H-29); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta_C$: 38.0 (C-1), 31.5 (C-2), 71.1 (C-3), 37.2 (C-4), 40.3 (C-5), 29.7 (C-6), 117.5 (C-7), 139.6 (C-8), 49.5 (C-9), 34.2 (C-10), 21.6 (C-11), 39.5 (C-12), 43.3 (C-13), 55.1 (C-14), 23.0 (C-15), 28.4 (C-16), 55.9 (C-17), 12.1 (C-18), 13.1 (C-19), 40.8 (C-20), 20.9 (C-21), 138.1 (C-22), 129.5 (C-23), 51.2 (C-24), 31.8 (C-25), 18.8 (C-26), 21.4 (C-27), 25.4 (C-28), 12.4 (C-29).

## 3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The $^1$H-NMR spectrum of **1** showed the signals of five methyl groups at 0.92 (3H, s), 0.94 (3H, s), 1.21 (3H, s), and 1.22 (6H, s), one olefinic proton at $\delta_H$ 5.88 (1H, t, *J* = 7.5 Hz), and three oxymethine protons at $\delta_H$ 3.33 (m), 3.97 (m), and 4.00 (br d, *J* = 3.0 Hz). The $^{13}$C-NMR and DEPT spectra of **1** exhibited the signals of 27 carbons, including one carbonyl carbon at $\delta_C$ 202.4; seven non-protonated carbons at $\delta_C$ 45.4, 48.5, 71.3, 77.9, 80.3, 84.1, and 167.5; six methines at $\delta_C$ 39.0, 50.4, 68.4, 70.2, 78.4, and 120.6; eight methylenes at $\delta_C$ 21.5, 22.5, 27.4, 31.7, 32.6, 34.2, 36.2, and 42.4; five methyl carbons at $\delta_C$ 16.9, 18.0, 21.0, 29.0, and 29.7. Analysis the NMR data of compound **1** indicated that structure of **1** was ecdysteroid skeleton and very similar to those of polypodine B [5]. The position of functional groups was assigned by HMBC correlations as well as compared with those of reference compounds. The HMBC correlations from H-2 ($\delta_H$ 3.97) to C-1 ($\delta_C$ 34.2)/C-3 ($\delta_C$ 70.2)/C-4 ($\delta_C$ 36.2)/C-10 ($\delta_C$ 45.4); from H-3 ($\delta_H$ 4.01) to C-1 ($\delta_C$ 34.2)/C-2 ($\delta_C$ 68.4)/C-4 ($\delta_C$ 36.2)/C-5 ($\delta_C$ 80.3); from H-19 ($\delta_H$ 0.94) to C-1 ($\delta_C$ 34.2)/C-5 ($\delta_C$ 80.3)/C-9 ($\delta_C$ 39.0)/C-10 ($\delta_C$ 45.4) suggested hydroxyl groups at C-2, C-3, and C-5. The HMBC correlations between H-7 ($\delta_H$ 5.88) and C-5 ($\delta_C$ 80.3)/C-6 ($\delta_C$ 202.4)/C-8 ($\delta_C$ 167.5)/C-9 ($\delta_C$ 39.0)/C-

14 ($\delta_C$ 84.1) suggested the position of carbonyl group at C-6 and the double bond at C-7/C-8. The HMBC correlations from H-18 ($\delta_H$ 0.92) to C-12 ($\delta_C$ 32.6)/C-13 ($\delta_C$ 48.5)/C-14 ($\delta_C$ 84.1)/C-17 ($\delta_C$ 50.4) suggested the hydroxyl groups at C-14. Three hydroxyl groups at C-20, C-22, and C-25 were also

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

| C | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| | $\delta_C$ [$] | $\delta_C$ [a] | $\delta_H$ [a] mult. (*J*, Hz) | $\delta_C$ [#] | $\delta_C$ [b] | $\delta_H$ [b] mult. (*J*, Hz) |
| 1 | 34.9 | 34.2 | 1.70 (m) | 33.6 | 31.2 | 1.24 (m)/1.61 (m) |
| 2 | 68.1 | 68.4 | 3.97 (m) | 33.9 | 32.5 | 1.30 (m) |
| 3 | 69.9 | 70.2 | 4.01 (br d, 3.0) | 67.6 | 66.0 | 3.77 (m) |
| 4 | 36.1 | 36.2 | 1.78 (m)/2.10 (dd, 3.0, 15.0) | 41.9 | 40.2 | 1.50 (m)/1.89 (m) |
| 5 | 79.9 | 80.3 | 2.41 (m) | 76.2 | 74.5 | - |
| 6 | 201.0 | 202.4 | - | 74.3 | 72.1 | 3.39 (m) |
| 7 | 119.9 | 120.6 | 5.88 (d, 2.5) | 120.5 | 119.4 | 5.08 (t, 2.5) |
| 8 | 167.0 | 167.5 | - | 141.6 | 139.7 | - |
| 9 | 38.4 | 39.0 | 3.20 (t, 8.5) | 43.8 | 42.3 | 1.93 (m) |
| 10 | 44.8 | 45.4 | - | 38.1 | 36.7 | - |
| 11 | 22.1 | 22.5 | 1.78 (m)/1.83(m) | 22.5 | 21.3 | 1.42 (m) |
| 12 | 32.2 | 32.6 | 1.91(m)/2.16 (m) | 40.0 | 39.0 | 1.14 (m)/1.88 (m) |
| 13 | 48.2 | 48.5 | - | 43.8 | 43.0 | - |
| 14 | 84.1 | 84.1 | - | 55.3 | 54.2 | 1.80 (m) |
| 15 | 31.8 | 31.7 | 1.61 (t, 10.0)/1.98 (m) | 23.5 | 22.6 | 1.37 (m)/1.50 (m) |
| 16 | 21.4 | 21.5 | 2.00 (m)/1.75 (m) | 28.5 | 27.7 | 1.24 (m)/1.57 (m) |
| 17 | 50.1 | 50.4 | 2.41 (t, 9.0) | 56.12 | 55.3 | 1.26 (m) |
| 18 | 18.0 | 18.0 | 0.92 (s) | 12.6 | 12.1 | 0.54 (s) |
| 19 | 17.3 | 16.9 | 0.94 (s) | 18.8 | 17.7 | 0.90 (s) |
| 20 | 76.9 | 77.9 | - | 40.8 | 40.1 | 2.01 (m) |
| 21 | 21.7 | 21.0 | 1.22 (s) | 21.5 | 21.0 | 0.99 (d, 6.5) |
| 22 | 77.7 | 78.4 | 3.33 (m) | 136.2 | 135.4 | 5.19 (dd, 8.0, 16.0) |
| 23 | 27.6 | 27.4 | 1.78 (m)/1.31 (m) | 132.2 | 131.4 | 5.25 (dd, 7.0, 16.0) |
| 24 | 42.3 | 42.4 | 1.82 (m)/1.46(m) | 43.2 | 42.0 | 1.85 (m) |
| 25 | 69.7 | 71.3 | - | 33.4 | 32.5 | 1.47 (m) |
| 26 | 30.1 | 29.0 | 1.21 (s) | 20.2 | 19.7 | 0.83 (d, 7.0) |
| 27 | 30.2 | 29.7 | 1.22 (s) | 19.9 | 19.5 | 0.81 (d, 7.0) |
| 28 | 24.2 | 24.3 | 0.88 (s) | 17.9 | 17.3 | 0.89 (d, 6.5) |
| 29 | 28.0 | 28.0 | 0.97 (s) | | | |
| 30 | 22.1 | 22.1 | 0.87 (s) | | | |

[a)] Recorded in $CD_3OD$; [b)]DMSO-$d_6$; [$]$\delta_C$ of polypodine B in pyridine-$d_5$ [5];
[#]$\delta_C$ of (22E,24R)-24-methylcholesta-2,22-diene-3β,5α,6β-triol [6].

confirmed by HMBC correlations between H-21 ($\delta_H$ 1.22) and C-17 ($\delta_C$ 50.4)/C-20 ($\delta_C$ 77.9)/C-22 ($\delta_C$ 78.4); between H-26 ($\delta_H$ 1.21)/H-27 ($\delta_H$ 1.22) and C-25 ($\delta_C$ 71.3). The NMR data of **1** were in good agreement with those of polypodine B [5]. Thus, the structure of **1** was elucidated as polypodine B.

Compound **2** was obtained as a white amorphous powder. The $^{1}$H-NMR spectrum of **2** showed the signals of two tertiary methyl groups at $\delta_H$ 0.54 (3H, s) and 0.90 (3H, s), four secondary methyl groups at $\delta_H$ 0.81 (3H, d, *J* = 7.0 Hz), 0.83 (3H, d, *J* = 7.0 Hz), 0.89 (3H, d, *J* = 6.5 Hz), and 0.99 (3H, d, *J* = 6.5 Hz). The olefin proton signals at $\delta_H$ 5.19 (1H, dd, *J* = 8.0, 16.0 Hz) and 5.25 (1H, dd, *J* = 7.0, 16.0 Hz) suggested the presence of a double bond in *E* configuration. The $^{13}$C-NMR and DEPT spectra of **2** exhibited the signals of 28 carbons, including four non-protonated carbons at $\delta_C$ 139.7, 74.5, 43.0 and 36.7; eleven methines at $\delta_C$ 135.4, 131.4, 119.4, 72.1, 66.0, 55.3, 54.2, 42.3, 42.0, 40.1, 32.5; seven methylenes at $\delta_C$ 40.2, 39.0, 32.5, 31.2, 27.7, 22.6, and 21.3; six methyl carbons at $\delta_C$ 21.0, 19.7, 19.5, 17.7, 17.3 and 12.1. The above spectral data analysis suggested the structure of compound **2** as a sterol. The NMR data of **2** were in good agreement with those of 3*β*, 5*α*, 6*β*-trihydroxysterol reported in the literature [6]. In addition, the HMBC correlations between H-19 ($\delta_H$ 0.90) and C-1 ($\delta_C$ 31.2)/C-5 ($\delta_C$ 74.5)/C-9 ($\delta_C$ 42.3)/C-10 ($\delta_C$ 36.7); between H-7 ($\delta_H$ 5.08) and C-6 ($\delta_C$ 72.1)/C-8 ($\delta_C$ 139.7)/C-9 ($\delta_C$ 42.3)/C-14 ($\delta_C$ 54.2) suggested the position of two hydroxyl groups at C-5, C-6 and double bond at the C-7/C-8. The double bond at C-22/C-23 was confirmed by HMBC correlations from H-21 ($\delta_H$ 0.99) to C-17 ($\delta_C$ 55.3)/C-20 ($\delta_C$ 40.1)/C-22 ($\delta_C$ 135.4); from H-28 ($\delta_H$ 0.89) to C-23 ($\delta_C$ 131.4)/C-24 ($\delta_C$ 42.0)/C-25 ($\delta_C$ 32.5). From the above evidence and comparison with the reported data [6], compound **2** was determined as (22*E*,24*R*)-24-methylcholesta-2,22-diene-3*β*,5*α*,6*β*-triol. .

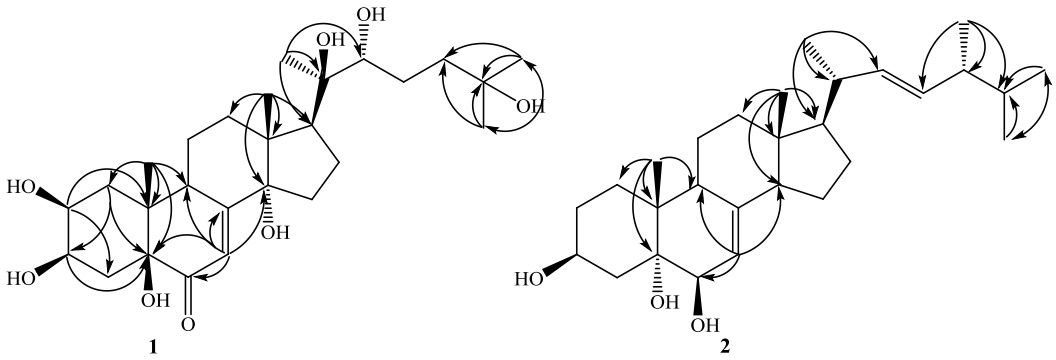


*Figure 2:* The key HMBC correlations of compounds **1** and **2**

The molecular formula of **3** was suggested as $C_{29}H_{48}O$ (M = 412) by the ESI-MS ion peak at *m/z* 411 $[M-H]^-$ and NMR data. The $^{1}$H-NMR spectrum of **3** showed signals for 6 methyl groups at $\delta_H$ 0.55 (3H, s), 0.79 (3H, d, *J* = 6.5 Hz), 0.80 (3H, s), 0.81 (3H, t, *J* = 7.5 Hz), 0.85 (3H, d, *J* = 6.5 Hz) and 1.03 (3H, d, *J* = 6.5 Hz). Besides, the presence of two olefin proton signals at $\delta_H$ 5.19 (1H, dd, *J* = 8.5, 16.0 Hz) and 5.03 (1H, dd, *J* = 8.5, 16.0 Hz) indicated the *E* configuration for this double bond. The $^{13}$C-NMR and DEPT spectra of **3** displayed signals of 29 carbons, including three quaternary carbons at $\delta_C$ 139.6, 43.3, 34.2; eleven methines at $\delta_C$ 138.1, 129.5, 117.5, 71.1, 55.9, 55.1, 51.2, 49.5, 40.8, 40.3 and 31.8; nine methylenesat $\delta_C$ 39.5, 38.0, 37.2, 31.5, 29.7, 28.4, 25.4, 23.0, and 21.6; six methyl carbons at $\delta_C$ 21.4, 20.9, 18.8, 13.1, 12.4, and 12.1. From the above evidence and comparison with the reported data [7], compound **3** was determined as chondrillasterol. This is the first time compounds **1-3** were isolated from genus *Momordica*.

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