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CHEMICAL CONSTITUENTS OF ETHYL ACETATE EXTRACT OF THE LIVERWORT MARCHANTIA POLYMORPHA GROWING IN VIETNAM

Nghiên cứu thành phần hóa học của cao chiết Ethyl Acetate từ loài địa tiễn Marchantia Polymorpha thu hái ở Việt Nam

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ABSTRACT

Liverwort *Marchantia polymorpha* has various pharmaceutical properties in Asian countries. However, chemical data of the Vietnamese liverwort are scarce. This paper was conducted to investigate the phytochemical data of Vietnamese *M. polymorpha*. The ethyl acetate extract of *M. polymorpha* was chemically investigated using multiple chromatographic method. NMR spectroscopic method was exploited to conclude the chemical structures of compounds. They were elucidated as (-)-thujopsenone (**1**), 6*β*-hydroxystigmast-4-en-3-one (**2**), 12-oleanene-3,11-dione (**3**), and cerevesterol (**4**). This is the first time compounds 6*β*-hydroxystigmast-4-en-3-one (**2**), 12-oleanene-3,11-dione (**3**), and cerevesterol (**4**) found in the genus *Marchantia*.

Keywords: *Marchantia polymorpha, thujopsenone, sesquiterpene, stigmastane.*

TÓM TẮT

Ở Việt Nam, loài địa tiễn *Marchantia polymorpha* sinh trưởng phổ biến ở các vùng khí hậu lanh. Loài này được biết đến là một bài thuốc quý trong y học cổ truyền ở nhiều nước. Tuy nhiên, có rất ít nghiên cứu về thành phần hoá học của loài địa tiễn đặc hữu của Việt Nam. Bài báo này mô tả thành phần hoá học của loài địa tiễn *M. polymorpha* sinh trưởng ở tỉnh Lâm Đồng. Sử dụng nhiều phương pháp sắc ký khác nhau, cao ethyl acetate của loài địa tiễn *M. polymorpha* được thực hiện phân tích hóa học, xác định sự có mặt của bốn hợp chất. Các phương pháp hoá lý hiện đại được thực hiện để xác định cấu trúc của các hợp chất. Các hợp chất được xác định là (-)-thujopsenone (**1**), 6*β*-hydroxystigmast-4-en-3-one (**2**), 12-oleanene-3,11-dione (**3**), và cerevesterol (**4**). Các hợp chất 6*β*-hydroxystigmast-4-en-3-one (**2**), stigmast-4-en-3-one (**3**), và cerevesterol (**4**) lần đầu tiên được tìm thấy trong chi *Marchantia*.

Từ khoá: *Marchantia polymorpha, thujopsenone, sesquiterpene, stigmastane.*

1. Introduction

Marchantia polymorpha L. (family Marchantiacea) is a popular liverwort growing in mountainous regions in Asia [1]. This medicinal source is native to Vietnam. It is thought to be a precious source in traditional medicines in India, China, and Europe [1,2]. This liverwort has been traditionally used to treat several diseases such as inflammation and liver disease, bites by insect, and snake, treatments of wounds and boils [1]. Over 50 compounds were detected in this liverwort [3-10]. In Vietnam, to date, 11 known compounds were reported in the native *M. polymorpha* [11-12]. Isolated compounds were divided

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into three main catergories: bibenzyls, sesquiterpenoids, and phenolics. The crude extracts of *M. polymorpha* showed antioxidant, antibacterial, inhibition of tyrosinase and alpha-glucosidase, and cytotoxicity against several cancer cell lines [13-18]. The ethyl acetate extract of *M. polymorpha* was chemically investigated by using multiple chromatographic methods. NMR spectroscopic method was exploited to conclude the chemical structures of compounds. Four compounds were identified (Figure 1). They were elucidated as (-)-thujopsenone (**1**), 6*β*-hydroxystigmast-4-en-3-one (**2**), 12-oleanene-3,11-dione (**3**), and cerevesterol (**4**).

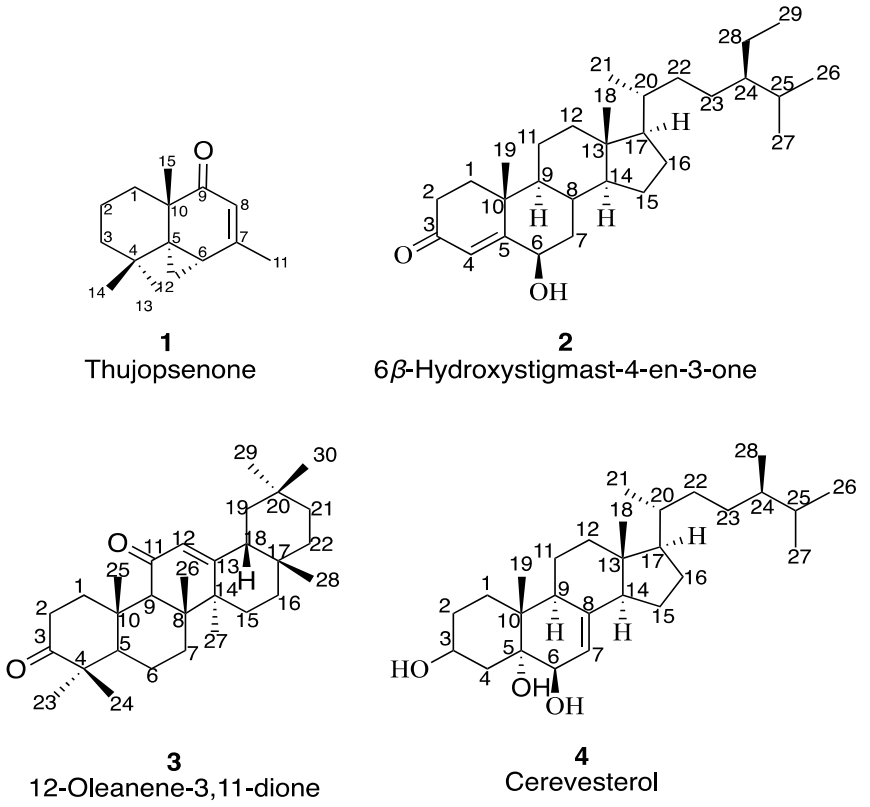


Figure 1. Chemical structures of isolated compounds **1-4**

2. Experimental

2.1. General experimental procedures

Bruker Avance III and MicrOTOF–Q mass spectrometers were chosen for NMR and HRESIMS spectra. NMR (^{1}H, 500 MHz and ^{13}C, 125 MHz) spectra were recorded using deuterated solvents: $CDCl_3$, DMSO-d_6, CD_3OD. Normal phase and reverse phase silica gel (Merck) were used for TLC (thin-layer chromatography) method. Silica gel 60 (Himedia) was used for CC (column chromatography) method. Yeast *Saccharomyces cerevisiae* *α*-glucosidase enzyme (Sigma-Aldrich) and the positive control acarbose (Sigma-Aldrich) were purchased for alpha-glucosidase inhibitory assay.

2.2 Plant Material

Liverwort *M. polymorpha* L was gathered in *Pinus* trees growing in Lam Dong Province (March 2023). The scientific name (registration No. US-LVUE02) was identified by the botanist namely Dr. Tram Vo Khanh Trinh

(University of Sciences, Ho Chi Minh City, Vietnam).

2.3 Extraction and Isolation of Compounds

4.5 kg of dried, ground *M. polymorpha* powder was extracted with ethyl acetate (3 x 10 L, each 8 hours) at room condition. The extracted solution was collected, filtered, and evaporated to provide 169.32 g of a crude ethyl acetate extract. This extract was chromatographed [normal phase silica gel column, isocratic mode, mobile phase as *n*-hexane: ethyl acetate (1:1, v/v)], providing six fractions **EA1-EA6**. Fraction EA3 was the most bioactive fraction based on inhibition against alpha-glucosidase, thus, selected for next chemical analysis. The isolation of **1-4** was shown in Figure 2.

(-)-Thujopsenone (1)

Colorless oil. ^{1}H NMR and ^{13}C NMR spectra were recorded in acetone-d_6. The data were shown in Table 1. These spectroscopic values were compatible with those reported by Masuo and co-workers [6].

6β-Hydroxystigmast-4-en-3-one (2)

Colorless oil. ^{1}H NMR and ^{13}C NMR spectra were recorded in acetone-d_6. The data were shown in Table 2. These spectroscopic values were compatible with those reported by Grecca and co-workers [19].

12-Oleanene-3,11-dione (3)

Colorless oil. ^{1}H NMR and ^{13}C NMR spectra were recorded in acetone-d_6. The data were shown in Table 3. These spectroscopic values were compatible with those reported by He-jiao and co-workers [20].

Cerevesterol (4)

White amorphous powder. ^{1}H NMR and ^{13}C NMR spectra were recorded in acetone-d_6. The data were shown in Table 4. These spectroscopic values were compatible with those reported by Haque and co-workers [21].

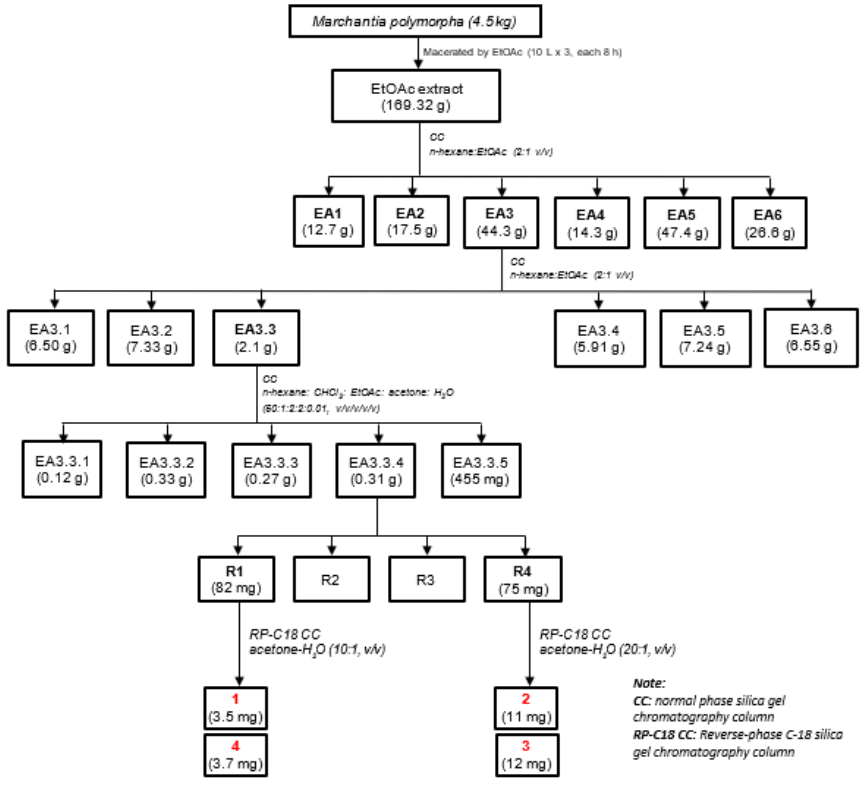


Figure 2: Isolation procedure of compounds **1-4**

3. Results and discussion

Compound **1**, colorless oil was isolated from the **EA3.3** fraction. The ^{1}H NMR spectrum showed an olefinic proton (δ_H 5.44, d, J = 1.0, H-8), and four methyl groups [δ_H 2.09, 1.40, 1.15, 0.70]. The JMOD spectrum exhibited a ketone carbonyl carbon (δ_C 201.5), one olefinic methine carbon (δ_C 118.3), four methylene carbons (δ_C 33.7, 24.8, 19.1 and 18.4), one sp3 methine (δ_C 24.8) and four quaternary carbons (δ_C 164.3, 47.0, 40.6, and 35.1). These data confirmed that **1** was a thujopsane sesquiterpene. HMBC correlations fully supported the structural elucidation of **1** (Figure 3). The HMBCs of H_3-14 (δ_H 1.25) and H_3-13 (δ_H 0.07) to C-4 (δ_C 40.6) and C-5 (δ_C 35.1), of H-15 (δ_H 1.40) to C-9 (δ_C 201.5), C-10 (δ_C 47.0), and C-5 (δ_C 35.1) defined the structure of the A-ring (Figure 2). Both methyl H_3-11 (δ_H 2.09) and H_2-12 (δ_H 0.88) gave HMBCs to C-6 (δ_C 24.8), while H-6 gave HMBCs to C-11 and C-12, confirming the position of H-12. Compound **1** had a consistent NMR data to thujopsenone [6], thus compound **1** was assigned to be thujopsenone.

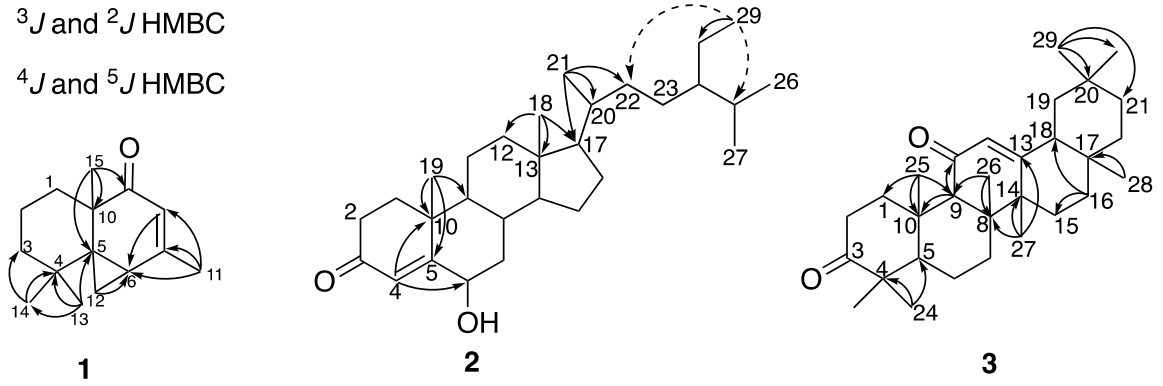


Figure 3. Selected HMBC correlations of **1-3**

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **(-)-thujopsenone**

No	1		(-)-Thujopsenone		
	δ_H[a]	δ_C[b]	δ_H[c]	δ_H[d]	δ_C[e]
1		24.8			23.8
2		19.1			21.2
3		33.7			32.5
4		40.6			39.9
5		35.1			34.1
6	0.89 (s)	24.8			24.7
7		164.3			160.0
8	5.44 (s)	118.3	5.38 (s, *br*)	5.63 (s, *br*)	118.1
9		201.5			198.8
10		47.0			45.5

No	1		(-)-Thujopsenone		
	δ_H[a]	δ_C[b]	δ_H[c]	δ_H[d]	δ_C[e]
11	2.09 (d, 1.0)	38.2	2.03 (d, 1.5)	1.70 (d, 1.5)	36.8
12	0.88 (s)	18.4			18.2
13	0.70 (s)	29.4	0.68 (s)	0.44 (s)	29.0
14	1.15 (s)	27.4	1.15 (s)	1.00 (s)	27.0
15	1.40 (s)	24.2	1.38 (s)	1.58 (s)	23.8

[a]500 MHz, multiplicity with *J* values in Hz), [b]125 MHz
[c]90 MHz, $CDCl_3$ multiplicity with *J* values in Hz
[d]90 MHz, CD_6D_6 multiplicity with *J* values in Hz
[e]22.5 MHz, $CDCl_3$

Compound **2**, colorless oil was isolated from the **EA3.3** fraction. The ^{1}H NMR spectrum showed an olefinic proton (δ_H 5.67, H-4), a proton with the hydroxyl group as a narrow signal (δ_H 4.29, m, H-6), together with three doublet signals as three methyl groups [δ_H 0.96 (3H, d, J = 3.0), 0.82 (3H, *d*, J = 3.5), and 0.81 (3H, d, J = 2.5)], a methyl triplet (δ_H 0.85, J = 3.5, 4.5), and two methyl singlets [δ_H 1.39, and 0.78]. The JMOD and HSQC spectra exhibited a ketone carbonyl carbon (δ_C 200.0), one olefinic methine carbon (δ_C 126.4), one oxygenated tertiary carbon (δ_C 73.2), six methyl carbons (δ_C 19.7, 19.4, 19.2, 19.0, 12.7, 12.0), ten methylene carbons (δ_C 40.7, 38.1, 37.1, 34.8, 34.5, 28.9, 26.1, 25.0, 23.4, and 21.7), seven sp3 methine carbons (δ_C 56.9, 56.7, 54.5, 45.5, 36.4, 30.4, and 29.2), three quaternary carbons (δ_C 169.0, 43.1, and 38.6). All mentioned data indicated that **2** was a stigmasterol. HMBCs supported the chemical structure of **2** (Figure 2). At first, the HMBCs of H-4 (δ_H 5.67) and H_3-19 (δ_H 1.39) to C-10 (δ_C 38.6), besides, H_3-19 gave a HMBC to C-5 (δ_C 169.0) and C-19 (δ_C 54.5), supporting the position of H-19. Both H_3-18 (δ_H 0.78) and H_3-21 (δ_H 0.95) gave a HMBC to C-17 (δ_C 56.7). In addition, both H_3-21 and H_3-29 (δ_H 0.85) gave HMBCs to C-22 (δ_C 34.5), indicating their locations. Moreover, H_3-29 gave HMBCs to C-25 (δ_C 29.2) and C-28 (δ_C 23.4), defined the structure of the alkyl-group (Figure 3). Compound **2** had a consistent NMR data to 6*β*-hydroxystigmast-4-en-3-one [19], confirming its structure.

Compound **3**, a white powder was isolated from the **EA3.3** fraction. The ^{1}H NMR spectrum showed a singlet olefinic proton (δ_H 5.55, H-12), eight singlet signals as eight methyl groups [δ_H 1.43, 1.25, 1.19, 1.08, 1.06, 0.92, 0.88, and 0.86], two tertiary protons [δ_H 2.56, H-9 and 2.17, H-18]. The JMOD and HSQC spectra exhibited two ketone carbonyl carbons (δ_C 215.0 and 206.2), one olefinic methine carbon (δ_C 128.6), eight methyl carbons (δ_C 33.5, 29.1, 27.0, 23.9, 23.8, 21.6, 19.5, and 16.1), nine methylene carbons (δ_C 46.0, 40.0, 36.2, 34.7, 34.6, 32.7, 27.3, 26.8, and 19.5), and seven quaternary carbons (δ_C 171.2, 48.4, 46.0, 42.8, 37.4, 33.3, and 31.0). These finding implied that **3** was an olean-12-ene-11-ketone skeleton (Shirota et al., 1996). HMBCs supported the chemical structure of **3** (Figure 3). Initially,

the HMBCs of H-9 (δ_H 2.56) and H-25 (δ_H 1.25) to C-10 (δ_C 37.3), and H-9 also gave a HMBC to C-11 (δ_C 206.2). Both methyl H_3-26 (δ_H 1.43) and H_3-27 (δ_H 1.19) gave HMBCs to C-8 (δ_C 46.0), while H-27 gave HMBCs to C-13 and C-14, supporting the position of H-27. In addition, the methyl H_3-29 (δ_H 0.92) gave a HMBC to C-20 (δ_C 31.0), C-21 (δ_C 34.7), and C-30 (δ_C 33.5), supporting the position of H-29. Compound **3** had a consistent NMR data to 12-oleanene-3,11-dione [20] (Table 3), indicating compound **3** was assigned to be 12-oleanene-3,11-dione.

Table 2. ^{1}H and ^{13}C NMR spectral data of **2**, **4**, **6*β*-hydroxystigmast-4-en-3-one**, and **cerevisterol**

No	2		6*β*-hydroxystigmast-4-en-3-one		4		Cerevisterol	
	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]	δ_H [e]	δ_C [f]
1		37.1		32.9		32.9		32.9
2		34.8		30.9		30.9		30.9
3		200.0	4.06 (*m*)	67.7	4.06 (*m*)	67.7	4.06 (*m*)	67.7
4	5.67 (s)	126.4		39.5		39.5		39.5
5		169.0		75.9		75.9		75.9
6	4.29 (m)	73.2	3.62 (*m*)	73.7	3.62 (*m*)	73.7	3.60 (*m*)	73.7
7		38.1	5.34 (*d*, 5.4)	117.5	5.34 (*d*, 5.4)	117.5	5.33 (*d*, 5.4)	117.5
8		30.4		144.0		144.0		144.0
9		54.5		43.5		43.5		43.5
10		38.6		37.2		37.2		37.1
11		21.7		22.1		22.1		22.0
12		40.7		39.2		39.2		39.2
13		43.1		43.8		43.8		43.8
14		56.9		54.8		54.8		54.8
15		25.0		22.9		22.9		22.9
16		28.9		27.9		27.9		27.9
17		56.7		56.0		56.0		56.0
18	0.78 (s)	12.0	0.60 (s)	12.3	0.60 (s)	12.3	0.58 (s)	12.3
19	1.39 (s)	19.4	1.08 (s)	18.8	1.08 (s)	18.8	1.07 (s)	18.8
20		36.4		40.4		40.4		40.4
21	0.96 (d, 3.0)	19.0	1.02 (d, 6.6)	19.6	1.02 (d, 6.6)	19.6	1.00 (d, 6.6)	19.6
22		34.5	5.16 (dd,	135.4	5.16 (dd, 7.2,	135.4	5.16 (dd, 7.2,	135.4

No.	3		12-Oleanene-3,11-dione	
	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[d]
19	1.62 m, 1.11 m	45.5	1.64 m, 1.10 m	46.0
20		31.3		31.0
21	1.36 m, 1.22 m	34.7	1.37 m, 1.18 m	34.7
22	1.45 m, 1.32 m	36.7	1.45 m, 1.31 m	36.2
23	1.06 (s)	21.6	1.07 (s)	21.6
24	1.08 (s)	26.7	1.10 (s)	27.0
25	1.25 (s)	15.9	1.27 (s)	16.1
26	1.19 (s)	18.9	1.18 (s)	19.5
27	1.43 (s)	23.6	1.37 (s)	23.9
28	0.86 (s)	29.0	0.85 (s)	29.1
29	0.92 (s)	23.3	0.91 (s)	23.8
30	0.88 (s)	33.8	0.89 (s)	33.5

[a]500 MHz, multiplicity with *J* values in Hz, [b]125 MHz
[c]500 MHz, $CDCl_3$ multiplicity with *J* values in Hz
[d]125 MHz, $CDCl_3$

Compound **4**, colorless oil was isolated from the **EA3.3** fraction. The ^{1}H NMR spectrum showed an olefinic proton (δ_H 5.34, d, *J* = 5.4, H-7), two protons attached to a double bonds [(δ_H 5.21, dd, *J* = 7.2, 15, H-23), and (δ_H 5.16, dd, *J* = 7.2, 15, H-22)], two protons with the hydroxyl group [(4.06, m, H-3), and (3.62, m, H-6)], together with four doublet signals as four methyl groups [δ_H 1.02 (3H, d, *J* = 6.6), 0.91 (3H, d, *J* = 7.2), 0.83 (3H, d, *J* = 6.6), and 0.81 (3H, d, *J* = 6.6)], and two singlet methyl signals [δ_H 1.08 (3H, s), and 0.60 (3H, s)]. The JMOD and HSQC spectra exhibited three olefinic methine carbon (δ_C 135.4, 133.2, and 117.5), two oxygenated tertiary carbon (δ_C 73.7, and 67.7), six methyl carbons (δ_C 21.1, 19.9, 19.6, 18.8, 17.6, and 12.3), seven methylene carbons (δ_C 39.5, 39.2, 32.9, 30.9, 27.9, 22.9, and 22.1), six sp3 methine (δ_C 56.0, 54.8, 43.5, 42.8, 40.4, and 33.1) and four quaternary carbons (δ_C 144.0, 75.9, 43.8, and 37.2). The above spectroscopic data demonstrated that **4** could be a steroid. Compound **4** had a consistent NMR data to cerevisterol reported in the literature [21] (Table 2), indicating **4** was cerevisterol.

4. Conclusions

From the liverwort *M. polymorpha* growing in Lam Dong Province, four compounds were isolated and elcucidated, including (-)-thujopsenone **(1)**, 6*β*-hydroxystigmast-4-en-3-one **(2)**, 12-oleanene-3,11-dione (**3**), and cerevisterol (**4**). This is the first time that isolated compounds 6*β*-hydroxystigmast-4-en-3-one **(2)**, 12-oleanene-3,11-dione (**3**), and cerevisterol (**4**) have been reported in the genus *Marchantia*.

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