TRITERPENOIDS AND PHENOLIC COMPOUNDS FROM *EUPHORBIA TITHYMALOIDES* L. (EUPHORBIACEAE)

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Abstract

Friedelan-3β-ol (**1**), β-sitosterol (**2**), oryzanol-C (**3**), 2,4,6-trimethoxyacetophenone (**4**), 3,4,3'-tri-*O*-methylellagic acid (**5**), scoparone (**6**), hopenone B (**7**), and tetradecane-1,2-diol (**8**) were isolated from the leaves and stems of *Euphorbia tithymaloides* L. (Euphrobiaceae) collected in northern Vietnam. Their structures were determined by spectroscopic analysis.

Keywords: *Euphorbia tithymaloides*, *Pedilanthus tithymaloides*, Euphorbiaceae, triterpenoid, phenolic compound.

1. INTRODUCTION

Euphorbia is the largest genus in the spurge (Euphorbiaceae) family and an interesting genus for natural product chemists owing to its biological diversity, large distribution, diverse chemistry, and specific anticancer and multidrug-resistant activities of its constituents [1]. *Euphorbia tithymaloides* L. (syn. *Pedilanthus tithymaloides* (L.) Poit.) is a perennial succulent spurge. The shrub is native to tropical and North America and Central America. The Vietnamese species (local name: *Thuoc dau*) is usually grown as medicinal and ornamental plant [2]. Its fresh leaves are used to treat cuts and wounds. A few phytochemical investigations on *P. tithymaloides* reported the isolation of long-chain alcohols, sterols, terpenes [3, 4], and flavonoids [5], including a noticeable report on antiplasmodial and antimycobacterial poly-*O*-acylated jatrophane diterpenes from the white latex [3].

2. EXPERIMENTAL

2.1. General

ESI-MS spectra were measured on a LC-MS Agilent 6310 system. ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra were recorded on a Bruker Avance 500 NMR spectrometer. Diaion HP-20 (Mitsubishi, Japan) and silica gel Merck (Darmstadt, Germany) were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on precoated silica gel Merck 60 F_{254} plate.

2.2. Plant Materials

The fresh leaves and stems of *E. tithymaloides* were collected in Hanoi, Vietnam two times; the first collection (14 kg) in April 2009 and the second collection (15 kg) in June 2010. A voucher specimen (HCTN 409) has been deposited in the Laboratory of Chemistry of Natural Products, VNU University of Science, Vietnam National University, Hanoi.

2.3. Extraction and Isolation of 1-8

The samples were air-dried and then oven-dried at 45-50 ^{o}C. The materials for the extraction were 1.3 kg of the dried stems and 334.7 g of the dried leaves from the first collection and 1 kg of the dried stems and 3.5 kg of the fresh stems from the second collection. The general extraction procedure involved maceration of the samples with MeOH at room temperature, followed by fractionation of the resultant MeOH extract into *n*-hexane-, CH_2Cl_2-, and EtOAc-soluble fractions by liquid-liquid extraction between water and the organic solvents in the order of the increasing polarities. The *n*-hexane-soluble fractions from the leaves (10 g) and stems (14 g) of the first collection were separated by repeated silica gel column chromatography using gradient *n*-hexane-acetone as the mobile phase and then further recrystallization gave **1** (18.6 mg), **2** (100 mg), and **3** (30 mg). Similar TLC patterns were observed for the *n*-hexane- and CH_2Cl_2-soluble fractions from the fresh stems (3.4 g and 11 g, respectively) as well as the dried stems (5.2 g and 6 g, respectively); they were combined accordingly and subjected to

repeated silica gel column chromatography using gradient *n*-hexane-EtOAc to give **4** (36.1 mg), **5** (5 mg), **6** (76.5 mg), and **7** (2 mg). The water phase from the extraction of the dried stems was concentrated and chromatographed on Diaion HP-20 using MeOH-H_2O 20 %, 40 %, and 60 %. The fraction eluted with MeOH-H_2O 60% was chromatographed on silica gel eluting with gradient CH_2Cl_2-MeOH 19:1, 9:1, and 6:1 to give **8** (10 mg).

Friedelan-3β-ol (**1**): White needles, m.p. 281-282 ^oC. ^{1}H-NMR ($CDCl_3$, δ, ppm, *J*/Hz): 0.86 (3H, s, CH_3-25), 0.94 (3H, d, *J* = 7.5 Hz, CH_3-23), 0.95 (3H, s, CH_3-24), 0.97 (3H, s, CH_3-30), 0.99 (6H, s, CH_3-26, CH_3-27), 1.01 (3H, s, CH_3-29), 1.17 (3H, s, CH_3-28), 3.73 (1H, s, H-3). ^{13}C-NMR/DEPT ($CDCl_3$): 11.6 (q, C-23), 15.8 (t, C-7), 16.4 (q, C-24), 17.6 (t, C-1), 18.3 (q, C-25), 18.6 (q, C-27), 20.1 (q, C-26), 28.2 (s, C-20), 30.1 (s, C-17), 30.7 (t, C-12), 31.8 (q, C-30), 32.1 (q, C-28), 32.4 (t, C-15), 32.9 (t, C-21), 35.0 (q, C-29), 35.2 (t, C-2), 35.4 (t, C-19), 35.6 (t, C-11), 36.1 (t, C-16), 37.1 (s, C-5), 37.9 (s, C-9), 38.4 (s, C-14), 39.3 (t, C-22), 39.7 (s, C-13), 41.8 (t, C-6), 42.9 (d, C-18), 49.2 (d, C-4), 53.2 (d, C-8), 61.4 (d, C-10), 72.8 (d, C-3).

β-Sitosterol (2): White needles, m.p. 134-136 ^oC. IR (KBr): ν_{max} cm^{-1} 3427, 1637, 1461, 1379, 1056.

Oryzanol-C (3): White needles, m.p. 198-199 ^oC. ESI-MS (positive mode): *m/z* 639.6 ($[M+Na]^+$, $C_{41}H_{60}O_4Na$). ^{1}H-NMR ($CDCl_3$, δ, ppm, *J*/Hz): 0.18 (1H, d, *J* = 3.9 Hz, H-19a), 0.43 (1H, d, *J* = 3.9 Hz, H-19b), 0.89 (3H, s, CH_3-28), 0.89 (3H, d, *J* = 6.4 Hz, CH_3-21), 0.91 (3H, s, CH_3-30), 0.92 (3H, s, CH_3-29), 0.98 (3H, s, CH_3-18), 1.03 (3H, d, *J* = 6.8 Hz, CH_3-26), 1.04 (3H, d, *J* = 6.8 Hz, CH_3-27), 2.24 (1H, septet, *J* = 6.8 Hz, H-25), 3.93 (3H, s, CH_3O-3′), 4.64 (1H, dd, *J* = 10.5 Hz, 4.5 Hz, H-3), 4.65 (1H, br s, H-31a), 4.72 (1H, br s, H-31b), 6.3 (1H, d, *J* = 15.5 Hz, H-α), 6.91 (1H, d, *J* = 8.5 Hz, H-5′), 7.04 (1H, d, *J* = 1.5 Hz, H-2′), 7.07 (1H, dd, *J* = 8.5 Hz, 1.5 Hz, H-6′), 7.61 (1H, d, *J* = 15.5 Hz, H-β).

2,4,6-Trimethoxyacetophenone (**4**): White amorphous powder. ^{1}H-NMR ($CDCl_3$, δ, ppm, *J*/Hz): 2.46 (3H, s, CH_3CO), 3.79 (6H, s, CH_3O-2, CH_3O-6), 3.83 (3H, s, CH_3O-4), 6.1 (2H, s, H-3, H-5).

3,4,3'-Tri-*O*-methylellagic acid (**5**): White amorphous powder. ^{1}H-NMR ($CDCl_3$ + CD_3OD, δ, ppm, *J*/Hz): 4.05 (3H, s), 4.22 (3H, s), 4.26 (3H, s) (CH_3O-3, CH_3O-3', CH_3O-4), 7.68 (1H, s), 7.69 (1H, s) (H-5, H-5').

Scoparone (**6**): White amorphous powder. ^{1}H-NMR ($CDCl_3$, δ, ppm, *J*/Hz): 3.93 (3H, s, CH_3O-7), 3.96 (3H, s, CH_3O-6), 6.29 (1H, d, *J* = 9.5 Hz, H-3), 6.84 (1H, s, H-8), 6.86 (1H, s, H-5), 7.62 (1H, d, *J* = 9.5 Hz, H-4).

Hopenone B (7): White amorphous powder. ^{1}H-NMR ($CDCl_3$, δ, ppm, *J*/Hz): 0.73 (3H, s, CH_3-28), 0.93 (3H, s, CH_3-25), 0.95 (3H, s, CH_3-27), 1.01 (1H, s, CH_3-26), 1.03 (3H, s, CH_3-23), 1.07 (3H, s, CH_3-24), 1.75 (3H, s, CH_3-30), 2.4 (1H, ddd, *J* = 15.5 Hz, 8.0 Hz, 7.5 Hz, H-2a), 2.5 (1H, ddd, *J* = 15.5 Hz, 7.5 Hz, 4.0 Hz, H-2b), 2.69 (1H, br q, *J* = 6.5 Hz, H-21), 4.78 (2H, br s, 2H-29). ^{13}C-NMR/DEPT ($CDCl_3$): 15.7 (q, C-25), 16.2 (q, C-28), 16.4 (q, C-26), 16.6 (q, C-27), 19.8 (t, C-6), 21.1 (q, C-24), 21.59 (t, C-11), 21.6 (t, C-16), 23.9 (t, C-12), 24.9 (q, C-30), 26.6 (q, C-23), 27.4 (t, C-20), 32.7 (t, C-7), 33.7 (t, C-15), 34.2 (t, C-2), 36.9 (s, C-10), 39.6 (t, C-1), 41.7 (s, C-8), 41.9 (t, C-19), 42.2 (s, C-14), 44.8 (s, C-18), 46.5 (d, C-21), 47.4 (s, C-4), 49.6 (d, C-13), 49.7 (d, C-9), 54.9 (2 × d, C-5, C-17), 110.2 (t, C-29), 148.6 (s, C-22), 218.1 (s, C-3).

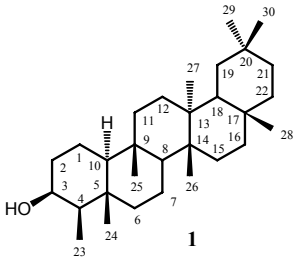
Tetradecane-1,2-diol (8): White amorphous powder. ESI-MS (positive mode): *m/z* 222 ($[M+Na–CH_2OH]^+$, $C_{14}H_{30}O_2Na–CH_2OH$). ^{1}H-NMR (CD_3OD, δ, ppm, *J*/Hz): 0.92 (3H, t, *J* = 7.0 Hz, CH_3-14), 1.31 (20H, br s, CH_2-4 → CH_2-13), 1.54 (2H, m, CH_2-3), 3.79 (1H, m, H-2), 3.9 (1H, dd, *J* = 10.5 Hz, 6.0 Hz, H-1a), 3.96 (1H, dd, *J* = 10.5 Hz, 4.5 Hz, H-1b).

3. RESULTS AND DISCUSSION

The leaves and stems of *E. tithymaloides* were extracted with MeOH and the extracts were partitioned between water and organic solvents. The *n*-hexane- and CH_2Cl_2-soluble fractions were chromatographed repeatedly on silica gel to yield seven compounds. The following triterpenoids and phenolic compounds were isolated for the first time from *E. tithymaloides*, friedelan-3β-ol (**1**) [3, 6], oryzanol-C (**3**) [7], 2,4,6-trimethoxyacetophenone (**4**) [8], and hopenone B (**7**) [9]. β-Sitosterol (**2**), 3,4,3'-tri-*O*-methylellagic acid (**5**) [10], and scoparone (**6**) [11] isolated from the *n*-hexane- and CH_2Cl_2-soluble fraction of the leaves and stems; and tetradecane-1,2-diol (**8**) [12] from the water phase of the dried stems are the known compounds from *E. (Pedilanthus) tithymaloides* [3, 4, 13]. The structures of compounds **1-8** were determined by comparing their MS and NMR spectroscopic data with those reported in the literature [6-11].

Compund **1** was isolated from the *n*-hexane-soluble fraction as white needles, m.p. 281-282 ^oC. The ^{1}H-NMR ($CDCl_3$) spectrum of **1** implied the presence of seven singlet methyl groups [δ_H 0.86 (3H, s), 0.95 (3H, s), 0.97 (3H, s), 0.99 (6H, s), 1.01 (3H, s), and 1.17 (3H, s)], a secondary methyl group [δ_H 0.94 (3H, d, *J* = 7.5 Hz)], and an oxymethine [δ_H

3.73 (1H, s)]. The ^{13}C-NMR ($CDCl_3$) and DEPT spectra of **1** showed thirty carbon-13 signals including eight methyl groups (8q) at δ_C 11.6, 16.4, 18.3, 18.6, 20.1, 31.8, 32.1, and 35.0; eleven methylenes (11t) at δ_C 15.8, 17.6, 30.7, 32.4, 32.9, 35.2, 35.4, 35.6, 36.1, 39.3, and 41.8; five methines (5d) at δ_C 42.9, 49.2, 53.2, 61.4, and 72.8; and six quaternary carbons (7s) at δ_C 28.2, 30.1, 37.1, 37.9, 38.4, and 39.7. The ^{1}H-NMR and ^{13}C-NMR spectra determined the structure of **1** to be friedelan-3β-ol [3, 6].



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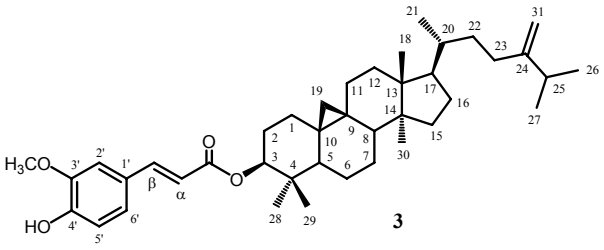

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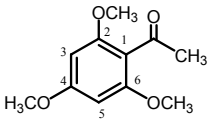

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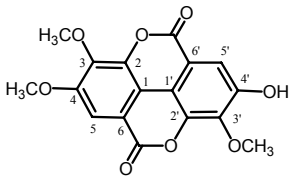

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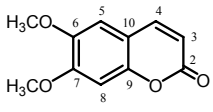

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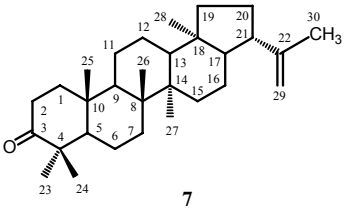

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Compound **3** was isolated from the *n*-hexane-soluble fraction as white needles, m.p. 198-199 ^{o}C. The positive-mode ESI-MS of **3** gave a quasi-molecular ion peak at *m/z* 639.6 ([M+Na]$^+$), indicating a molecular formula $C_{41}H_{60}O_4$. The ^{1}H-NMR ($CDCl_3$) spectrum of **3** identified a triterpenoid of 24-methylenecycloartane series. The signals for the cyclopropane methylene at δ_H 0.18 (1H, d, J = 3.9 Hz) and 0.43 (1H, d, J = 3.9 Hz); four tertiary methyl groups at δ_H 0.89 (3H, s), 0.92 (6H, s), and 0.98 (3H, s); a secondary methyl group at δ_H 0.89 (3H, d, J = 6.3 Hz); and an isopropyl group at δ_H 1.03 (3H, d, J = 6.8 Hz) and 1.04 (3H, d, J = 6.8 Hz) were observed. In the downfield region signals for the double bond methylene at δ_H 4.65 (1H, s) and 4.72 (1H, s) and an oxymethine at δ_H 4.64 (1H, dd, J = 10.5 Hz, 4.5 Hz) were observed. A feruloyl group [δ_H 6.91 (1H, d, J = 8.5 Hz), 7.04 (1H, d, J = 1.5 Hz), and 7.07 (1H, dd, J = 8.5 Hz, 1.5 Hz); 6.3 (1H, d, J = 15.5 Hz) and 7.61 (1H, d, J = 15.5 Hz); and 3.93 (3H, s)] was bonded to C-3 causing downfiled shift of H-3 (δ_H 4.64) in comparison of that of 24-methylenecycloartanol ($\delta_{H\text{-}3}$ 3.28 ($CDCl_3$); $\Delta\delta_H$ +1.36 ppm) [4]. This feruloyl group was determined to be β-oriented on the basis of the proton-proton coupling constant between H-2 $_{ax}$ and H-3$_{ax}$ (J = 10,5 Hz). On the basis of the NMR analysis, **3** was determined to be 24-methylenecycloartanyl ferulate (oryzanol-C) [5].

Compund **4** was isolated from the *n*-hexane-soluble fraction as a white amorphous powder. Inspection of the ^{1}H-NMR spectrum of **4** exhibited the presence of an acetyl group at δ_H 2.46 (3H, s), three methoxy groups at δ_H 3.79 (6H, s) and 3.83 (3H, s), and a two-proton singlet of an substituted benzene at δ_H 6.10 (2H, s). The ^{1}H-NMR spectroscopic data indicated that **4** possessed an acetophenone-type structure and its substitution pattern was either 2,4,6-trimethoxy [8] or 3,4,5-trimethoxy [14]. The ^{1}H-NMR data of **4** were superimposed on those of 2,4,6-trimethoxy-acetophenone.

Compound **7** was isolated from the *n*-hexane-soluble fraction as a white amorphous powder. In the ^{1}H-NMR spectrum of **7**, six methyl groups resonated at δ_H 0.73 (3H, s), 0.93 (3H, s), 0.95 (3H, s), 1.01 (3H, s), 1.03 (3H, s), and 1.07 (3H, s), and an isopropenyl group at δ_H 1.75 (3H, s) and 4.78 (2H, s br). Thirty ^{13}C-NMR signals including seven methyl groups, ten methylenes, five methines, five carbons, a carbonyl ketone [δ_C 218.1 (s, C-3)], and a 1,1-disubstituted double bond [δ_C 110.2 (t, C-29) and 148.6 (s, C-22)] suggested a triterpenoid structure of **7**. The presence of the isopropylene implied the lup-22(29)-ene or hop-22(29)-ene structure of **7**. By comparing the ^{13}C-NMR spectroscopic data of **7** with those of literature values the structural similarity of **7** and 2-oxohopan-22-ol (rings A, B,

and C) and hop-22(29)-en (rings D and E) was seen [15]. On the basis of the NMR analysis **7** was determined to be 3-oxo-hop-22(29)-en; this structure occurs in nature in the form of two C-21 epimers: moretenone (21α-H) [16, 17] and hopenone B (21β-H) [9]. The stereochemistry at C-21 greatly affected the chemical shifts of C-21 and C-30: δ_C 47.9 (C-21) and 19.7 (C-30) of mortenone and 46.5 (C-21) and 25.0 (C-30) of hop-22(29)-ene [15]. The latter values agreed well with those of **7**. Therefore, the structure of **7** was determined to be hopenone B. Hopenone B is a rare naturally occuring compound which was isolated from *Euphorbia cyparissias* in 1969 and structurally elucidated with insufficient ^{1}H-NMR data [9].

4. CONCLUSION

The study isolated eight compounds from the leaves and stems of *E. tithymaloides*. Of the compounds isolated triterpenoids and phenolic compounds are of special interest. Friedelane, cycloartane, and hopane triterpenoids may be biogenetically related in this plant.

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