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TERPENOIDS FROM *ADIANTUM EMARGINATUM*

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SUMMARY

*From the petroleum ether fraction of the methanolic extract of Adiantum emarginatum Bory, three triterpenoids including tetrahymanol (**1**), isoadiantol B (**2**), hydroxyadiantone (**3**), and a diterpenoid 8,13-epoxy-14-labden-19-oic acid (**4**) have been isolated. Their structures were deduced from the spectral evidence and on the basis of X-ray diffraction studies.*

I - INTRODUCTION

Adiantum emarginatum Bory (*A. capilus-veneris* L.) belonging to family Adiantaceae is a common fern and wildly distributed in Vietnam. The dried whole plant has been used in traditional medicine for various diseases such as inflammation, kidney stone, and diabetes mellitus [1, 2]. The genus *Adiantum* contains numbers of interesting components in which triterpenoid presented as the major class of the genus [3 - 5].

Diabetes mellitus is a disorder characterized by hyperglycemia and long term complication such as retinopathy, cataract, atherosclerosis, impaired wound healing, Alzheimer's, rheumatoid, and arthritis. Increased glycation of proteins and accumulation of advanced glycation endproducts (AGEPs) have been implicated in the pathogenesis of diabetic complications. Glycation and AGEP formation are also accompanied by formation of free radicals via autoxidation of glucose and glycated proteins. Compounds with combined antiglycation and antioxidant properties may offer therapeutic potential [6].

In the course of chemical studies on medicinal plants, four compounds have been isolated from the petroleum fraction of *A. emarginatum* with anti-glycation guided fractionation.

II - EXPERIMENT

1. Plant material

The whole plant of *A. emarginatum* Bory (ron den) was collected in Tam Dao mountain, Vinh Phuc province, Vietnam and was identified by Dr Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. The voucher of specimen was deposited at Institute of Natural Products Chemistry (INPC), Vietnamese Academy of Science and Technology (VAST).

2. General experimental procedures

The ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker NMR

spectrometer. Chemical shifts are referenced to δ using tetramethylsilan (TMS) as an internal standard. The EI-MS spectrum was obtained using an IMS-DA 500 mass spectrometer. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 - 0.063 mm, Merck). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates.

3. Extraction and Isolation

Dried aerial parts of *A. emarginatum* were extracted three times with MeOH. The MeOH extract was suspended in water and partitioned in turn with petroleum ether, ethyl acetate, and butanol to obtained petroleum ether, ethyl acetate and butanol fractions. All the fractions were screened for antioxidant and antiglycation activities. The petroleum ether fraction showed the most promising activity then selected for further investigation on bioactive chemical components. The petroleum ether fraction was chromatographed on silica gel eluting with petroleum ether and acetone gradient (from 20:1 to 1:1) to give three fractions P1, P2, and P3. P1 fraction was chromatographed on silica gel using petroleum ether - ethyl acetate (10:1) as eluent to afford compounds **1** and **2**. Repeated flash column chromatography on silica gel of P2 fraction using dichloromethane - acetone obtained compound **3**. Compound **4** was recrystallized from P3 using petroleum ether - acetone system.

Tetrahymanol (1): Colorless needles; EI-MS *m/z*: 428.3 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.73 (3H, s, H-24), 0.77 (3H, s, H-28), 0.79 (2H, s, H-25), 0.79 (3H, s, H-28), 0.82 (3H, s, H-30), 0.93 (3H, s, H-26), 0.94 (3H, s, H-27), 0.95 (3H, s, H-23), and 3.14 (1H, dd, *J* = 5.3, 1.6 Hz, H-3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 38.7 (C-1), 27.4 (C-2), 79.1 (C-3), 38.8 (C-4), 55.2 (C-5), 18.7 (C-6), 33.1 (C-7), 41.9 (C-8), 50.2 (C-9), 37.1 (C-10), 21.1 (C-11), 21.3 (C-12), 50.3 (C-13), 41.7 (C-14), 33.1 (C-15), 18.7 (C-16), 56.2 (C-17), 37.3 (C-18), 40.3 (C-19), 18.7 (C-20), 42.1 (C-21), 33.2 (C-22), 28.0 (C-23), 15.4 (C-24), 15.9 (C-25), 16.5 (C-26), 16.5 (C-27), 15.9 (C-28), 21.5 (C-29), and 33.4 (C-30).

Isoadiantol B (2): Colorless needles; EI-MS *m/z*: 414.4 $[M]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.66 (3H, s, H-28), 0.77 (3H, s, H-24), 0.79 (3H, s, H-25), 0.83 (3H, s, H-23), 0.93 (3H, s, H-26), 0.95 (3H, s, H-27), 1.13 (3H, d, *J* = 6.2 Hz, H-29), and 3.71 (1H, m, H-3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 40.3 (C-1), 18.7 (C-2), 42.1 (C-3), 33.3 (C-4), 56.1 (C-5), 18.7 (C-6), 33.3 (C-7), 41.9 (C-8), 50.5 (C-9), 37.4 (C-10), 20.9 (C-11), 22.8 (C-12), 48.5 (C-13), 42.0 (C-14), 32.7 (C-15), 23.9 (C-16), 55.0 (C-17), 45.1 (C-18), 39.7 (C-19), 24.3 (C-20), 47.5 (C-21), 72.8 (C-22), 33.4 (C-23), 21.6 (C-24), 15.9 (C-25), 16.8 (C-26), 16.8 (C-27), 15.2 (C-28), and 21.7 (C-29).

Hydroxyadiantone (3): Colorless crystals; mp. 270-275ºC; EI-MS *m/z*: 428.4 $[M]^+$; IR (KBr) ν_{max} cm^{-1}: 3430, 1082, 1695 (OH), and 1715 (C=O).

8,13-Epoxy-14-labden-19-oic acid (4): Colorless needles; positive FAB-MS *m/z*: 321 $[M+H]^+$, 303 $[M-H_2O+H]^+$, negative FAB-MS *m/z*: 319 $[M-H]^-$, 275 $[M-C_2H_5O]^-$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.70 (3H, s, H-20 1.29), (3H, s, H-17), 1.22 (3H, s, H-18), 1.25 (3H, s, H-18), 1.26 (1H, brs, H-5), 1.18 (1H, brs, H-9), 4.88 (2H, dddd, *J* = 1.45, 10.70, 17.35 Hz, H-15), and 5.81 (1H, dd, *J* = 10.70, 17.35 Hz, H-14); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 39.3 (C-1), 19.0 (C-2), 37.9 (C-3), 43.7 (C-4), 55.0 (C-5), 21.4 (C-6), 43.2 (C-7), 74.7 (C-8), 56.8 (C-9), 37.7 (C-10), 15.5 (C-11), 35.6 (C-12), 73.3 (C-13), 147.9 (C-14), 110.3 (C-15), 28.9 (C-16), 25.0 (C-17), 28.7 (C-18), 182.3 (C-19), and 13.1 (C-20).

X-Ray data of tetrahymanol (1): A needle-shaped colorless crystal of compound **1** with dimensions 0.44 x 0.21 x 0.13 mm was selected for X-Ray diffraction studies. $C_{30}H_{52}O$ $0.2H_2O$ (the water molecule is only 15.5% occupied in the unit cell): M_τ 431.09; momoclinic; *a* = 25.719 (3) Å, *b* = 13.5581 (13) □, *c* = 30.804 (4) Å, *V* = 10251(2) $Å^3$, space group = *P*2(1)2(1)2(1)2(1), *Z* = 16, D_{calc} = 1.117 mg/m^3. *F*(000) 3862, Mo Kα (0.7107 Å). Intensity data of compound A were collected on a Bruker Smart APEX II, CCD 1-K area-

detector diffractometer. Data reductions were performed using SAINT. The structure was solved by direct methods and refined by full-matrix least squares on F^2 using the SHELXTL-PC package. The intensity data within the θ range 0.69 - 25.00□ were collected at 372(2) K. A total of 53843 reflections were recorded, of which 18002 reflections were observed on the basis of $I > 2$ (I). The final R and R_w were 0.0775 and 0.1798, respectively. The figure was plotted with the aid of PLUTO.

X-Ray data of hydroxyadiantone (3): A plate-shaped colorless crystal of compound **3** with dimensions 0.71 x 0.30 x 0.07 mm was selected for X-Ray diffraction studies. $C_{29}H_{48}O_2$: M_τ 857.35 (two plate was occupied in the unit cell); triclinic; a = 6.5662 (8) Å, b = 7.2458 (9) □, c = 28.278 (3) Å, V = 1199.0(2) Å^3, space group = $P2(1)$, Z = 1, D_{calc} = 1.187 mg/m^3. $F(000)$ 476, Mo Kα (0.7107 Å). Intensity data of compound A were collected on a Bruker Smart APEX II, CCD 1-K area-detector diffractometer. Data reductions were performed using SAINT. The structure was solved by direct methods and refined by full-matrix least squares on F^2 using the SHELXTL-PC package. The intensity data within the θ range 0.72 - 25.00□ were collected at 373(2) K. A total of 12768 reflections were recorded, of which 8395 reflections were observed on the basis of $I > 2$ (I). The final R and R_w were 0.0641 and 0.1592, respectively. The figure was plotted with the aid of PLUTO.

III - RESULTS AND DISCUSSION

Compound **1** was obtained as colorless needles from the bioactive petroleum ether fraction of *A. emarginatum*. The molecular formula of **1** was suggested as $C_{30}H_{52}O$ by EI-MS spectrum at m/z 428.3 (M)$^+$. The ^{1}H-NMR and ^{13}C-NMR spectra showed signals of 30 carbons including 8 methyl, 11 methylene, 5 methine, and 6 quaternary carbons. The ^{13}C-NMR chemical shifts of **1** were very similar to those of the known compound tetrahymanyl acetate except for the absence of an acetoxy group at C-3. Instead, the presence of a hydroxyl group at C-3 (δ_H 3.14/ δ_C 79.1) was obtained in the NMR spectra. Finally, the structure and the relative configuration of the gammacerane triterpenoid **1** were deduced on the basis of X-Ray diffraction studies. The hydroxyl group at C-3 was determined as beta-oriented (Fig. 2). Thus, **1** was identified as tetrahymanol. Interestingly, this is the first study on X-Ray diffraction of this compound [7].

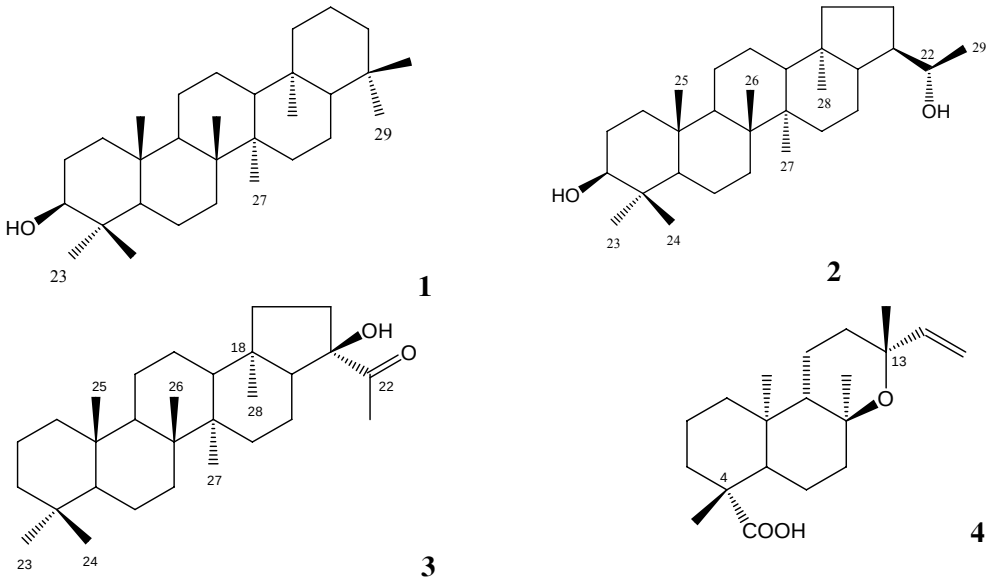
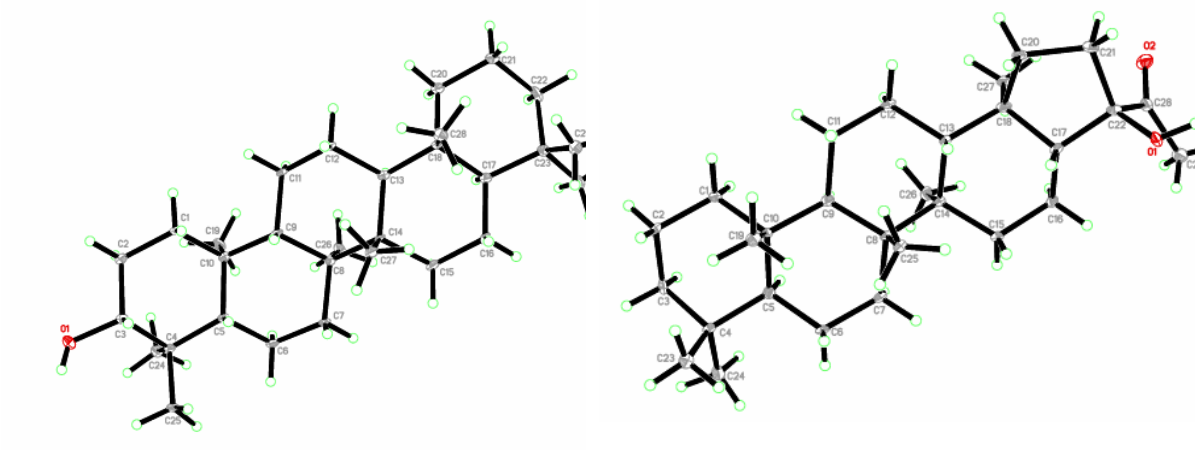


Fig. 1: Structures of **1 — 4**



1 **3**

Fig. 2: X-ray structures of **1** and **3** showing the atom-numbering scheme

Compound **2** was recrystallized from petroleum ether - acetone. The NMR spectra of **2** displayed the resonances due to the signals of a tertiary carbon bearing a hydroxyl group (δ_H 3.71/δ_C 72.8), seven methyl, eleven methylene, five olefinic methine and five quaternary carbons. The NMR data of **2** were coincident to those of isoadiantone B. The results were confirmed by the exhibition of the ion peak at *m/z* 414.4 $[M]^+$ (corresponding to the molecular formula of $C_{29}H_{50}O$) in the EI-MS spectrum of **2**. From the above evidence, **2** was determined to be isoadiantone B, which previously isolated from *A. monochlamys*. However, this is the first report of **2** from *Adiantum emarginatum* [8].

Compound **3** was obtained as colorless crystals. The infrared absorption (IR) spectrum indicated the presences of a hydroxyl group at 3430 cm^{-1} and a ketone group at 1715 cm^{-1}. The ^{1}H-NMR spectrum displayed the signals of six methyl groups and a carbonyl methyl group at δ 2.23. The structure and absolute configuration of **3** was confirmed by the X-Ray diffraction studies. Accordingly, the hydroxyl group at C-21 was determined as β-oriented. Thus, **3** was identified as hydroxyadiantone, which was also isolated from the leaves of *A. monochlamys*. However, this is the first report of **3** from *Adiantum emarginatum* [9, 10].

The molecular formula of **4** was suggested as $C_{20}H_{30}O_3$ by positive FAB-MS at *m/z* 321 $[M+H]^+$, 303 $[M-H_2O+H]^+$, negative FAB-MS *m/z*: 319 $[M-H]^-$. The ^{1}H-NMR spectrum of **4** showed singlet signals of four methyl groups at δ 0.70, 1.22, 1.29, 1.25, and 1.26, a double bond at δ_H 4.88 (2H, dddd, *J* = 1.45, 10.70, 17.35 Hz) and 5.81 (1H, dd, *J* = 10.70, 17.35 Hz). The ^{13}C-NMR spectrum of **4** exhibited signals of 20 carbons, including 3 methine, 8 methylene, 4 methyl, 2 quaternary, 2 tertiary carbons bearing an oxygen atom, and a carboxylic group at δ_C 182.0, suggesting a diterpene compound. The double bond moiety was evident at δ_C 110.3 (C-15)/147.9 (C-14), two tertiary carbons bearing an oxygen atom were assigned at δ_C 73.3 (C-13) and 74.7 (C-8). The 19-oic acid position was determined by comparisons of chemical shifts of **4** to those of published data [11]. All the NMR and MS data led to the suggestion of the structure of **4** as shown in Fig. 1, its NMR data found to match well with those of 8,13-epoxy-14-labden-19-oic acid isolated from *Leyssera gnaphaloides* [12]. This is the first report of **4** from *Adiantum* species.

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