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Pentacyclic Triterpenoids from *Mallotus apelta*

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A new triterpene (**1**) and six known pentacyclic terpenoids (**2**-**7**) were isolated from the methanol extract of the dried leaves from *Mallotus apelta*. Based on the spectral and chemical evidence, their structures were determined to be 3α-hydroxyhop-22(29)-ene (**1**), hennadiol (**2**), friedelin (**3**), friedelanol (**4**), epifriedelanol (**5**), taraxerone (**6**), and epitaraxerol (**7**).

Key words: Euphorbiaceae, *Mallotus apelta*, Triterpene, Malloapelta A

INTRODUCTION

Mallotus apelta Muell. -Arg, (Euphorbiaceae) is widely distributed in the northern areas of Vietnam, and has been used in traditional Vietnamese medicine for the treatments of chronic hepatitis, white blood and enteritis. (Chi, 1997; Loi, 2001). Many antibiotic triterpenoids, steroids, diterpenoids, alkaloids, coumarinolignoids and benzopyran derivatives have been isolated from its roots (An *et al.,* 2002; Cheng *et al.,* 1998, 1999a, 1999b, 2000), of which, malloapeltine has been reported to have significant anti-HIV activity (Cheng *et al.,* 1998).

As a part of an ongoing research program on bioactive compounds from Vietnamese medicinal plants, this paper reports the isolation and structural determination of a new triterpene and six known pentacyclic triterpenoids. The structures of these compounds were determined to be 3α-hydroxyhop-22(29)-ene (**1**), hennadiol (**2**), friedelin (**3**), friedelanol (**4**), epifriedelanol (**5**), taraxerone (**6**), and epitaraxerol (**7**), based on the spectral and chemical evidence.

MATERIALS AND METHODS

General experimental procedures

The melting points were determined using a Kofler micro-hotstage. The IR spectra were obtained on a Hitachi 270-30 type spectrometer, using KBr disks. The optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. The FAB-MS and HR-FAB-MS were obtained using a JEOL JMS-DX 300 spectrometer. The ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker DRX300 spectrometer, and those of the ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) on a Bruker AM600 FT-NMR spectrometer, with TMS as an internal standard. Column chromatography (CC) was performed on a silica gel column (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck).

Plant material

The leaves from *M. apelta* were collected at Tamdao Mountain, Vinhphuc province, Vietnam during December 2002, and identified by Prof. Vu Van Chuyen, Hanoi University of Pharmacy, Vietnam. A voucher specimen (INPC 2847) was deposited in the herbarium of the Institute of Natural Product Chemistry, VAST, Vietnam.

Extraction and isolation

The dried and powdered leaves from *M. apelta* (4.5 kg) were extracted three times with hot MeOH to obtain a methanol extract (200 g), which was suspended in water and sequentially extracted using *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The combined hexane and chloroform fraction (32 g) was subjected to chromatography on a silica gel column, using $CHCl_3$-MeOH (from 100:0 to 0:100) as an eluent, yielding five fractions (Fr. A-E). Fraction A (7 g) was subjected to chromatography on a silica gel column, using hexane-acetone (100:1) as an eluent, to yield compounds **3** (32 mg) and **6** (136 mg). Fraction B

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(15 g) was subjected to chromatography on a silica gel column, using *n*-hexane-acetone (10:1) as the eluent, to yield compounds **1** (64 mg) and **2** (6.0 mg). Fraction C (5 g) was subjected to chromatography on a silica gel column, using *n*-hexane-acetone (8:1) as the eluent, to give compounds **4** (77 mg), **5** (17 mg), and **7** (370 mg) as white crystals.

3α-Hydroxyhop-22(29)-ene (1)

White crystals; mp 223-224°C; $[\alpha]_D^{25}$-2.5° (c 1.0, $CHCl_3$); positive FAB-MS *m/z*: 449.38 $[M+Na]^+$; HR-FAB-MS *m/z*: 449.3760 $[M+Na]^+$ (Calcd. for $C_{30}H_{50}ONa$: 449.3759); ^{13}C-NMR ($CDCl_3$, 150 MHz) and ^1H-NMR ($CDCl_3$, 600 MHz): see Table I.

Hennadiol (2)

White crystals; mp 235-237°C; $[\alpha]_D^{25}$-10° (c 1.00, $CHCl_3$); positive FAB-MS *m/z*: 465 $[M+Na]^+$; ^1H-NMR ($CDCl_3$, 600 MHz) δ: 4.95 (1H, br s, Ha-29), 4.96 (1H, br s, Hb-29), 4.15 (2H, br s, H-30), 3.18 (1H, dd, J = 10.0, 6.5 Hz, H-3), 2.38 (1H, m, H-19), 1.05 (3H, s, H-26), 0.97 (3H, s, H-23), 0.96 (3H, s, H-27), 0.85 (3H, s, H-25), 0.79 (3H, s, H-28), 0.76 (3H, s, H-24); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 39.1 (t, C-1), 27.8 (t, C-2), 79.4 (d, C-3), 39.3 (s, C-4), 55.7 (d, C-

Table I. ^1H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR spectral data (150 MHz, $CDCl_3$) of **1** and the reference triterpenes (Rowan *et al.*, 1992)[a]

No.	3β-Methoxyhop-22(29)-ene	Hop-22(29)-ene (21αH)	Hop-22(29) ene (21βH)	δ_C of **1**	δ_H of **1**
1	38.9	40.4	40.4	33.2	1.25-1.43 (2H, m)
2	22.4	18.6	18.7	25.4	1.55-1.95 (2H, m)
3	88.9	42.2	42.1	76.3	3.39 (1H, d, $W_{1/2}$ = 7.0)
4	39.0	33.3	33.3	37.2	-
5	55.9	56.2	56.1	50.1	1.35 (1H, m)
6	18.5	18.8	18.7	18.3	1.38 (2H, m)
7	33.9	33.4	33.3	33.2	1.25 (2H, m)
8	42.0	42.3	42.1	41.9	-
9	50.6	50.5	50.4	49.5	1.35 (1H, m)
10	37.4	37.5	37.5	37.5	-
11	21.3	21.0	20.9	20.9	1.33-1.56 (2H, m)
12	24.2	24.1	24.0	23.9	1.40-1.50 (2H, m)
13	49.7	48.8	49.5	48.9	1.20 (1H, m)
14	42.3	42.0	41.9	42.1	-
15	33.6	32.7	33.6	33.6	1.25-1.39 (2H, m)
16	21.9	21.0	21.6	21.6	1.48-1.64 (2H, m)
17	55.1	54.0	54.9	54.9	1.39 (1H, m)
18	45.0	44.3	44.8	44.7	-
19	42.1	40.3	41.9	41.9	1.03-1.61 (2H, m)
20	27.6	27.4	27.4	27.4	1.86 (2H, m)
21	46.7	48.0	46.5	46.5	2.70 (1H, m)
22	148.8	148.3	148.7	148.7	-
23	28.3	33.5	33.4	28.6	0.94 (3H, s)
24	16.3	21.7	21.6	22.5	0.84 (3H, s)
25	16.3	16.0	15.9	15.7	0.84 (3H, s)
26	16.7	16.7	16.7	16.6	0.94 (3H, s)
27	16.7	16.9	16.7	16.8	0.95 (3H, s)
28	16.0	15.1	16.1	16.1	0.73 (3H, s)
29	110.3	109.5	110.1	110.1	4.79 (2H, br s)
30	25.2	19.7	25.0	25.3	1.77 (3H, s)
OMe	57.7				

[a]Chemical shift (d) in ppm. All assignments were assigned on the basis of DEPT (distortionless enhanced by polarization transfer), ^1H-^1H COSY (^1H-^1H chemical shift correlation spectroscopy), HMQC (heteronuclear multiple quantum coherence), and HMBC (heteronuclear multiple bonds correlation) spectra.

5), 18.7 (t, C-6), 34.7 (t, C-7), 41.3 (s, C-8), 50.8 (d, C-9), 37.6 (s, C-10), 21.4 (t, C-11), 25.1 (t, C-12), 38.4 (d, C-13), 43.2 (s, C-14), 27.8 (t, C-15), 35.9 (t, C-16), 43.4 (s, C-17), 49.3 (d, C-18), 44.2 (d, C-19), 155.2 (s, C-20), 32.2 (t, C-21), 40.3 (t, C-22), 28.4 (q, C-23), 15.8 (q, C-24), 16.4 (q, C-25), 16.5 (q, C-26), 14.9 (q, C-27), 18.1 (q, C-28), 107.3 (t, C-29), 65.4 (t, C-30).

Friedelin (3)
White crystals; mp 265-267°C; $[\alpha]_D^{25}$-24.8° (c 1.0, $CHCl_3$); positive FAB-MS m/z: 427.29 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.36 (m, H-2), 2.24 (q, *J* = 6.4 Hz, H-4), 0.87 (d, *J* = 6.4 Hz, H-23), 0.71 (s, H-24), 0.85 (s, H-25), 0.99 (s, H-26), 1.03 (s, H-27), 1.16 (s, H-28), 0.94 (s, H-29), 0.99 (s, H-30); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 22.6 (t, C-1), 41.9 (t, C-2), 213.4 (s, C-3), 58.6 (d, C-4), 42.5 (s, C-5), 41.7 (t, C-6), 18.6 (t, C-7), 53.5 (d, C-8), 37.8 (s, C-9), 59.9 (d, C-10), 36.0 (t, C-11), 30.9 (t, C-12), 40.1 (s, C-13), 38.7 (s, C-14), 32.8 (t, C-15), 36.4 (t, C-16), 30.4 (s, C-17), 43.2 (d, C-18), 35.7 (t, C-19), 28.5 (s, C-20), 33.1 (t, C-21), 39.6 (t, C-22), 7.1 (q, C-23), 15.0 (q, C-24), 18.3 (q, C-25), 20.6 (q, C-26), 19.0 (q, C-27), 32.4 (q, C-28), 35.4 (q, C-29), 32.1 (q, C-30).

Friedelanol (4)
White crystals; mp 293-294°C; $[\alpha]_D^{25}$+16.6° (c 1.0, $CHCl_3$); positive FAB-MS *m/z*: 451.39 $[M+Na]^+$, positive HR-FAB-MS *m/z*: 451.3916 (Calcd. for $C_{30}H_{52}ONa$: 451.3916); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.34 (1H, ddd, *J* = 10.8, 10.8, 4.6 Hz, H-3), 1.54 (1H, dd, *J* = 6.5, 2.6 Hz, H-18), 0.89 (3H, d, *J* = 6.6 Hz, H-23), 0.77 (s, H-24), 0.81 (s, H-25), 1.01 (s, H-26), 0.98 (s, H-27), 0.99 (s, H-28), 0.94 (s, H-29), 1.17 (s, H-30); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 19.6 (t, C-1), 36.7 (t, C-2), 72.2 (d, C-3), 53.2 (d, C-4), 37.4 (s, C-5), 41.4 (t, C-6), 17.8 (t, C-7), 53.0 (d, C-8), 38.7 (s, C-9), 60.1 (d, C-10), 35.3 (t, C-11), 30.6 (t, C-12), 39.7 (s, C-13), 38.3 (s, C-14), 32.8 (t, C-15), 36.1 (t, C-16), 30.1 (s, C-17), 42.9 (d, C-18), 36.1 (t, C-19), 28.1 (s, C-20), 32.4 (t, C-21), 39.3 (t, C-22), 9.9 (q, C-23), 14.6 (q, C-24), 18.1 (q, C-25), 20.1 (q, C-26), 18.6 (q, C-27), 31.8 (q, C-28), 32.1 (q, C-29), 35.1 (q, C-30).

Epifriedelanol (5)
White crystals; mp 280-283°C; $[\alpha]_D^{25}$+22° (c 1.0, $CHCl_3$); positive FAB-MS *m/z*: 429 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 3.73 (br d, H-3), 0.91 (d, *J* = 7.0 Hz, H-23), 0.93 (s, H-24), 0.83 (s, H-25), 0.98 (s, H-26), 0.95 (s, H-27), 0.97 (s, H-28), 0.92 (s, H-29), 1.14 (s, H-30); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 16.2 (t, C-1), 35.7 (t, C-2), 73.1 (d, C-3), 49.6 (d, C-4), 37.5 (s, C-5), 42.1 (t, C-6), 17.9 (t, C-7), 53.6 (d, C-8), 38.2 (s, C-9), 61.8 (d, C-10), 35.6 (t, C-11), 31.0 (t, C-12), 38.7 (s, C-13), 40.0 (s, C-14), 33.2 (t, C-15), 36.5 (t, C-16), 30.0 (s, C-17), 43.2 (d, C-18), 35.9 (t, C-19), 28.7 (s, C-20), 32.7 (t, C-21), 39.7 (t, C-22), 12.0 (q, C-23), 16.8 (q, C-24), 18.6 (q, C-25), 19.0 (q, C-26), 20.5 (q, C-27), 32.5 (q, C-28), 35.4 (q, C-29), 32.2 (q, C-30).

Taraxerone (6)
White crystals; mp 240-243°C; $[\alpha]_D^{25}$+9.6° (c 1.0, $CHCl_3$); positive FAB-MS *m/z*: 447.3 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 5.58 (dd, *J* = 8.1, 3.3 Hz, H-15), 2.57 (m, H-2a). 2.31 (m, H-2b), 1.12 (3H, s, CH_3), 1.06 (s, 3×CH_3), 0.97 (s, CH_3), 0.93 (s, 2×CH_3), 0.83 (s, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 38.7 (t, C-1), 34.5 (t, C-2), 217.7 (s, C-3), 47.9 (s, C-4), 56.1 (d, C-5), 20.3 (t, C-6), 35.5 (t, C-7), 39.2 (s, C-8), 49.1 (d, C-9), 36.1 (s, C-10), 17.8 (t, C-11), 38.1 (t, C-12), 38.1 (s, C-13), 158.0 (s, C-14), 117.5 (d, C-15), 37.0 (t, C-16), 37.9 (s, C-17), 49.2 (d, C-18), 41.0 (t, C-19), 29.1 (s, C-20), 33.9 (t, C-21), 33.4 (t, C-22), 26.5 (q, C-23), 21.7 (q, C-24), 15.1 (q, C-25), 30.2 (q, C-26), 25.9 (q, C-27), 30.3 (q, C-28), 33.7 (q, C-29), 21.8 (q, C-30).

Epitaraxerol (7)
White crystals; mp 270-272°C; $[\alpha]_D^{25}$-9.7° (c 1.0, $CHCl_3$); positive FAB-MS *m/z*: 449.3 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 5.54 (dd, *J* = 8.0, 3.0 Hz, H-15), 3.41 (t, *J* = 2.7 Hz, H-3), 1.11 (s, CH_3), 0.96 (s, 3×CH_3), 0.93 (s, 2×CH_3), 0.88 (s, CH_3), 0.84 (s, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 38.1 (t, C-1), 25.4 (t, C-2), 76.6 (d, C-3), 38.4 (s, C-4), 49.1 (d, C-5), 19.1 (t, C-6), 34.1 (t, C-7), 39.5 (s, C-8), 49.3 (d, C-9), 37.9 (s, C-10), 17.7 (t, C-11), 35.9 (t, C-12), 37.9 (s, C-13), 158.1 (s, C-14), 117.0 (d, C-15), 37.0 (t, C-16), 37.9 (s, C-17), 49.4 (d, C-18), 41.6 (t, C-19), 29.1 (s, C-20), 33.5 (t, C-21), 33.2 (t, C-22), 28.5 (q, C-23), 22.5 (q, C-24), 15.6 (q, C-25), 30.2 (q, C-26), 26.4 (q, C-27), 30.1 (q, C-28), 33.7 (q, C-29), 21.6 (q, C-30).

RESULTS AND DISCUSSION

Compound **1** was formed as white crystals. Its molecular formula was determined by HR-FAB-MS to be $C_{30}H_{50}O$ (*m/z*: 449.3760 $[M+Na]^+$; Calcd. for $C_{30}H_{50}ONa$: 449.3759). The IR spectrum of compound **1** exhibited an absorption maximum at 3400 cm^{-1}, which was assigned to hydroxy stretching. The ^{1}H-NMR spectrum of compound **1** showed six tertiary methyls at δ 0.73-0.95, one isopropenyl at δ 1.77 (3H, s) and 4.79 (2H, br s), and one C-3 proton at δ 3.39 (1H, d, $W_{1/2}$ = 7.0 Hz). The ^{13}C-NMR spectrum of compound **1** revealed 30 carbons, including 7 methyl, 11 methylene, 6 methine groups, and 6 quaternary carbons. The H-C assignments were determined from the HMQC spectrum. The structure of compound **1** was determined by comparison with the data from hop-22(29)-ene and 3β-methoxyhop-22(29)-ene, and from tracing the connectivities shown in the HMBC spectrum (Table I). This com-

parison suggested that compound **1** was 3α-hydroxyhop-22(29)-ene. The C-21 and C-30 resonances of compound **1** (δ 46.5 and 25.3) were similar to those of hop-22(29)-ene (21βH) (δ 46.5 and 25.0), but different from those of hop-22(29)-ene (21αH) (δ 48.0 and 19.7, respectively) (Rowan *et al.*, 1992). There was an upfield shift consistent with a pseudo-axial configuration of the C-21 isopropenyl group. Moreover, the B, C, D and E rings of compound **1** matched those of hop-22(29)-ene, with 21βH (hopene-B) (Rowan *et al.*, 1992; Shiojima *et al.*, 1990), with the exception of the A ring (Table I). ^{13}C-^{1}H long-range correlations between the H-23 (δ 0.94)/H-24 (δ 0.84) protons and the C-3 carbon (δ 76.3), between the H-3 proton (δ 3.39) and C-1 (δ 33.2)/C-4 (δ 37.2)/C-5 (δ 50.1) carbons were observed in the HMBC spectrum. This confirmed that the hydroxy group was attached to C-3. Furthermore, an NOE correlation between the methyl protons, CH_3-24 (δ_H 0.84) and the H-3 proton (d_H 3.39) was observed in the NOESY spectrum. In addition, the chemical shifts at C-3 [δ_C 76.3/δ_H 3.39 (1H, d, $W_{1/2}$ = 7.0)] indicated that the stereochemistry at C-3 must be a 3α-hydroxy position (Betancor *et al.*, 1980). Based on the above data, compound **1** was determined to be 3α-hydroxyhop-22(29)-ene, which was named malloapelta A.

Compounds **2-7** were identified as hennadiol (Betancor *et al.*, 1980), friedelin (Ageta *et al.*, 1995), friedelanol (Samaraweera *et al.*, 1983), epifriedelanol (Kundu *et al.*, 2000), taraxerone (Sakurai *et al.*, 1987) and epitaraxerol (Rahman *et al.*, 1997), respectively, by a comparison with the ^{1}H-, ^{13}C-NMR and MS data reported in the literature. The ^{1}H- and ^{13}C-NMR data of friedelanol (**4**) were assigned by analysis of the ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. To the best of our knowledge, this is the first report of the ^{13}C-NMR data of friedelanol.

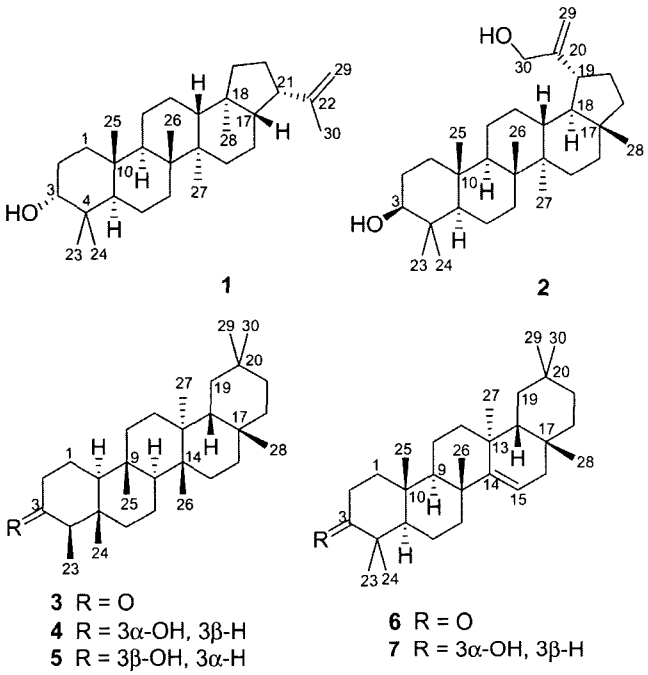

Fig. 1. Structures of Compounds **1-7**

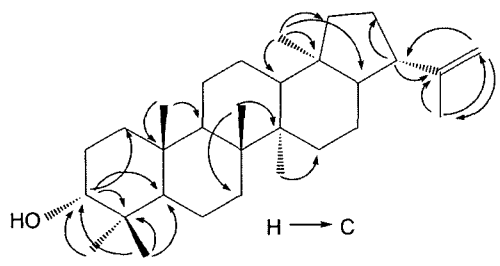

Fig. 2. Selected ^{1}H-^{13}C long-range correlations in the HMBC spectrum of **1**

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