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Three New Diterpenoids from *Euphorbia decipiens*

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Three new diterpene esters with a tricyclic myrsinol-type skeleton have been isolated from *Euphorbia decipiens* Boiss. & Buhse. The structure elucidation of the isolated compounds was based primarily on 1D and 2D-NMR analysis, including COSY, HMQC, HMBC and NOESY correlations. The compound **1** showed inhibitory activity against prolyl endopeptidase and compound **2** showed analgesic activity.

Key words: *Euphorbia decipiens* Boiss. & Buhse, Euphorbiaceae, diterpene esters, tricyclic diterpenes, myrsinol-type diterpenes, prolyl endopeptidase

Euphorbia decipiens grows wild in different parts of Iran at high altitude [1]. Some of the plants belonging to genus *Euphorbia* are used in folk medicine, for instance *E. kansui* is considered as a herbal remedy for edema, ascites and cancer in China and investigation of this plant showed two antileukemic diterpene esters with an ingenane carbon skeleton [2]. The macrocyclic and polycyclic diterpenes isolated from different species of *Euphorbia* plants with ingenane, tigliane and daphnane skeletons have skin-irritant, tumour-promoting and anti-tumour activities [3–5]. Some esters of myrsinol isolated from *E. myrsinites* showed anti-HIV-1 reverse transcriptase (RT) inhibition [6]. We report the isolation and structure elucidation of three new esters of a tricyclic diterpene (**1**–**3)** with a myrsinane nucleus. The compound **1** showed inhibitory activity against prolyl endopeptidase. Prolyl endopeptidase (PEP, EC 3.4.21.26) is the only serine protease, which is known to cleave a peptide substrate in the C-terminal side of a proline residue. Prolyl endopeptidase plays an important role in the metabolism of peptide hormones and neuropeptides and recognized to be involved in learning and memory [7].

RESULTS AND DISCUSSION

Compound **1** was exhibited a molecular ion at *m/z* 654.2698 in the HREIMS, indicating its molecular formula as $C_{35}H_{42}O_{12}$. Its IR spectra showed characteristic peak for carbonyl groups at 1730 $cm^{-1}$and at 1580, 1600 and 710 cm^{-1} for a benzene ring. A

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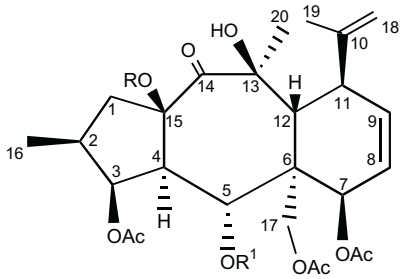
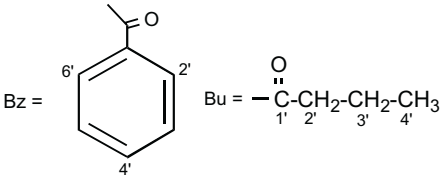


Compound	R	R^1
1	Ac	Bz
2	H	Bz
3	H	Bu



sharp peak at 3480 cm^{-1} indicated a non-hydrogen-bonded hydroxyl group in the molecule. In EIMS spectrum the ions at *m/z* = 594, 534, 474 and 414 indicated the presence of acetate groups, which were eliminated from the molecular ion at *m/z* = 654 in the form of acetic acid. The base peak at *m/z* = 105 $(C_6H_5CO)^+$, and others at 121 $(C_6H_5COO)^+$ and 533 $(M^+ - 121)$ indicated the presence of a benzoate ester group in the molecule.

Table 1. ^{1}H-NMR data (in $CDCl_3$) for compound **1, 2** and **3**.

H	**1**	**2**	**3**
1α	3.14 (dd, *J* = 9.1, 14.2 Hz)	3.15 (dd, *J* = 9.2, 14.2 Hz)	3.16 (dd, *J* = 9.0, 14.5 Hz)
1β	1.61 (m)	1.59 (m)	1.58 (m)
2	2.09 (m)	2.08 (m)	2.09 (m)
3	5.29 (t, *J* = 3.4 Hz)	5.29 (t, *J* = 3.4 Hz)	5.30 (t, *J* = 3.5 Hz)
4	2.35 (dd, *J* = 3.5, 11.6 Hz)	2.35 (dd, *J* = 3.5, 11.5 Hz)	2.36 (dd, *J* = 3.4, 11.5 Hz)
5	6.37 (d, *J* = 11.6 Hz)	6.38 (d, *J* = 11.5 Hz)	6.32 (d, *J* = 11.3 Hz)
7	4.86 (m)	4.87 (m)	4.87 (m)
8	5.95 (ddd, *J* = 2.0, 6.4, 9.5 Hz)	5.95 (ddd, *J* = 2.0, 6.5, 9.5 Hz)	5.96 (ddd, *J* = 2.0, 6.4, 9.5 Hz)
9	5.72 (dd, *J* = 4.5, 9.5 Hz)	5.72 (dd, *J* = 4.5, 9.5 Hz)	5.73 (dd, *J* = 4.5, 9.5 Hz)
11	3.27 (dd, *J* = 2.0, 8.2 Hz)	3.27 (dd, *J* = 2.0, 7.2 Hz)	3.26 (dd, *J* = 2.0, 5.1 Hz)
12	4.07 (d, *J* = 8.0 Hz)	4.07 (d, *J* = 8.0 Hz)	4.08 (d, *J* = 8.0 Hz)

Table 1 (continuation)

16	0.89 (d, *J* = 6.7 Hz)	0.88 (d, *J* = 6.7 Hz)	0.87 (d, *J* = 6.5 Hz)
17	3.95 (d, *J* = 12.0 Hz)	3.96 (d, *J* = 12.0 Hz)	3.97 (d, *J* = 12.0 Hz)
17′	4.33 (d, *J* = 12.0 Hz)	4.33 (d, *J* = 12.0 Hz)	4.34 (d, *J* = 12.0 Hz)
18	4.86 m [2H]	4.87 m [2H]	4.87 m [2H]
19	1.75 (s)	1.76 (s)	1.77 (s)
20	1.66 (s)	1.66 (s)	1.67 (s)
Acetyl			
	2.10 (s)	2.11 (s)	2.11 (s)
	2.05 (s)	2.06 (s)	2.07 (s)
	1.93 (s)	1.94 (s)	1.94 (s)
	1.72 (s)	–	–
Butanoyl			
2′			2.12 (m)
3′			1.51 (m)
4′			0.90 (t, *J* = 7.4 Hz)
Benzoyl			
2′, 6′	7.87 (dd, *J* = 1.5, 7.3 Hz)	7.85 (dd, *J* = 1.4, 8.4 Hz)	
3′, 5′	7.35 (br. t, *J* = 7.8 Hz)	7.35 (br. t, *J* = 7.4 Hz)	
4′	7.44 (t.t, *J* = 2.0, 8.0 Hz)	7.48 (t.t, *J* = 2.0, 9.0 Hz)	

The ^{1}H-NMR of **1** in $CDCl_3$, (Table 1) exhibited three oxymethine protons at δ 5.29 (t, *J* = 3.4 Hz, H-3), 6.37 (d, *J* = 11.6 Hz, H-5), 4.86 (m, H-7), one oxymethylene as a couple of doublets at δ 3.95 (d, *J* = 12.0 Hz, H-17) and 4.33 (d, *J* = 12.0 Hz, H-17′). The signals at δ 7.87 (dd, *J* = 1.5, 7.3 Hz, H-2′, H-6′), 7.44 (tt, *J* = 2.0, 8.0 Hz, H-4′) and 7.35 (br t, *J* = 7.8 Hz, H-3′, H-5′) represented a benzoyl group. In addition to four methyl signals related to four acetyl groups at 2.10 (s), 2.05 (s), 1.93 (s) and 1.72 (s), one secondary and two tertiary methyls at δ 0.89 (d, *J* = 6.7 Hz), 1.75 (s) and 1.66 (s) were recorded for H-16, H-19, and H-20, respectively. The vicinal olefinic protons showing signals at δ 5.72 (dd, *J* = 4.5, 9.5 Hz, H-9) and at δ 5.95 (ddd, *J* = 2.0, 6.4, 9.5 Hz, H-8) are separated by a methine proton at δ 3.27 (dd, *J* = 2.0, 8.2 Hz, H-11) from the terminal olefinic protons at δ 4.86 (m) in an isopropenyl group, H-18. The ^{13}C-NMR (BB and DEPT) of **1** showed 33 signals due to 35 carbons including seven CH_3, three CH_2, twelve CH and eleven quaternary carbons, out of which eight were oxygen-bearing (one tertiary alcohol, one tertiary ester, one ketonic and five ester carbonyls). The ^{1}H-^{1}H- and ^{1}H-^{13}C connectivities were supported by the ^{1}H-^{1}H-COSY and HMQC spectra. The location of acetate and benzoate groups were also deduced by observing the cross peaks between corresponding protons and carbonyl carbon of ester groups in HMBC, which was in the case of benzoyl carbonyl at *ca*. δ 165.0 with H-5 and for other acetate groups δ at *ca*. 170 and H-3, H-7 and H-17 (Table 3). The stereochemistry of **1** was determined by comparison of the ^{1}H-NMR coupling constants of **1** with those recorded for myrsinol esters [8–12] with similar structure as well as by NOESY spectra. The coupling constant of H-3 (t, *J* = 3.4 Hz)

and H-4 (dd, J = 3.5, 11.6 Hz) indicated that H-2 to H-4 must lie on one face of the molecule with the same dihedral angle between H-2/H-3 and H-3/H-4. The J value (11.6 Hz) between H-4 and H-5 showed the *trans* relationship between them. The coupling constant of H-12 (d, J = 7.9 Hz) indicated the *trans* relationship between H-12 and H-11. The NOESY cross peaks between H-3/H-4, H-5/H-12, established that H-5, H-12 must be located on one face of the molecule. In NOESY spectra cross peaks between (δ 4.86) H-7, H-18 with H-12 and H-11 were also detected, through which we concluded that H-7 and H-11 must be in one face of the molecule and H-12 and H-18 in another. The ^{1}H and ^{13}C-NMR data are very similar to those reported for decipinone [10]. The upfield shift for C [13] and down field shift for C [15] (δ 82.0 (s) and 88.1 (s), resp. are the main difference to the ^{13}C-NMR data of decipinone. The above data confirm the position of OH and AcO at C [13] and C [15], respectively [11].

Table 2. ^{13}C-NMR data (in $CDCl_3$ BB and DEPT) for compound **1** and **2**[a].

C	**1**	**2**	DEPT
1	45.5	44.9	CH_2
2	37.3	37.5	CH
3	79.2	79.2	CH
4	53.1	53.2	CH
5	69.7	70.5	CH
6	48.7	48.7	C
7	67.7	67.7	CH
8	122.0	122.1	CH
9	136.0	135.8	CH
10	146.0	146.9	C
11	45.7	45.9	CH
12	40.9	41.0	CH
13	82.0	84.0	C
14	205.5	205.2	C
15	88.1	85.9	C
16	14.2	14.2	CH_3
17	62.4	62.2	CH_2
18	113.1	113.2	CH_2
19	20.4	20.0	CH_3
20	23.3	23.4	CH_3
OAc	170.7 (21.0)	171.7 (21.0)	C, (CH_3)
	170.3 (20.9)	170.3 (20.8)[b]	C, (CH_3)

Table 2 (continuation)

	169.9 (20.8)[b]	169.9 (20.7)[b]	C, (CH_3)
	169.8 (20.7)[b]	–	–
Benzoyl			
1′	129.7	129.8	C
2′, 6′	129.5	129.6	CH
3′, 5′	128.2	128.2	CH
4′	133.0	133.0	CH
7′	165.0	165.1	C

[a]All the ^{1}H and ^{13}C connectivities were assigned by HMQC experiments.
[b]The assignment may be interchanged.

Table 3. HMBC[a] data for **1** and **2**.

1	**2**
H_{α}-1: C-2, C-3, C-4, C-14, C-15	H_{α}-1: C-2, C-3, C-4, C-15
H_{β}-1: C-1, C-15	H_{β}-1: C-1, C-15
H-3: C-1, C-15, O*C*OCH_3	H-3: C-1, C-15, O*C*OCH_3
H-4: C-5, C-6, C-14	H-4: C-5, C-6, C-3
H-5: C-4, C-6, C-7, C-17, O*C*OPh	H-5: C-4, C-6, C-7, C-17, O*C*OPh
H-7: C-5, C-6, C-8, C-9, O*C*OCH_3	H-7: C-5, C-6, C-8, C-9, O*C*OCH_3
H-8: C-6, C-7, C-9, C-11	H-8: C-6, C-7, C-9, C-11
H-9: C-7, C-11, C-12	H-9: C-7, C-11, C-12
H-11: C-6, C-8, C-9, C-10, C-12, C-13, C-18, C-19	H-11: C-6, C-8, C-9, C-10, C-12, C-13, C-18, C-19
H-12: C-5, C-6, C-10, C-11, C-13, C-17	H-12: C-5, C-6, C-10, C-11, C-13, C-17
H-16: C-1, C-2, C-3	H-16: C-1, C-2, C-3
H-17: C-5, C-6, C-7, C-12, O*C*OCH_3	H-17: C-5, C-6, C-7, C-12, O*C*OCH_3
H-17′: C-5, C-6, C-7, O*C*OCH_3	H-17′: C-5, C-6, C-7, O*C*OCH_3
H-18: C-9, C-10, C-11, C-19	H-18: C-9, C-10, C-11, C-19
H-19: C-9, C-10, C-11, C-18	H-19: C-10, C-11, C-18
H-20: C-12, C-13, C-14	H-20: C-12, C-13

[a]Protons correlating with carbon resonance.

Compound **2**, obtained as a colorless oil, displayed the IR absorptions at 3460, 1740 and 1640 cm^{-1} indicating the presence of hydroxy, carbonyl, and unsaturation in the molecule, respectively. Its molecular formula was assigned on the basis of CIMS as $C_{33}H_{40}O_{11}$, *m/z* 612 $[M]^{+}$. In the EIMS the ion at *m/z* 594 $[M\text{-}H_2O]^{+}$, 522 $[M\text{-}HOAc]^{+}$ and 492 $[M\text{-}2x\ HOAc]^{+}$ and the base peak *m/z* 105 $(C_6H_5CO)^{+}$ indicated the presence of hydroxyl, acetate, and benzoate functionality in **2**. The ^{1}H and ^{13}C-NMR spectra of **2** are very similar to those of **1**, except that **2** has one less acetate moiety. The upfield shift of C-15 to δ 85.9 and lack of one acetate peak in ^{1}H and

^{13}C-NMR is compatible with the proposed structure [11]. The position of benzoate and ester groups were established by the observed HMBC cross peaks [(H-(3), H-(7), H-17, H-17′)/carbonyl signals (at δ *ca*. 169–170)], and relatively upfield carbonyl signal at δ 165.1 and its connectivity with H-5 (δ 6.38) in HMBC plot confirm position of the benzoate moiety at C-5.

Compound **3**, a minor constituent, was identified as the butanoyl ester of compound **2**. All the signals in the ^{1}H-NMR spectra of **3** were similar to those recorded for **2** except the benzoyl moiety, which was substituted by butanoyl signals at δ 2.12 (m, H-2′), 1.51 (m, H-3′), 0.90 (t, J = 7.4 Hz, H-4′). The presence of butanoyl group also confirmed through base peak at *m/z* 71 $(C_3H_7CO)^+$ instead of benzoyl moiety in this compound.

Low molecular weight inhibitors of PEP have been reported in literature but majority of these are synthetic substrate mimetic with very few natural products. Most of natural inhibitors have been isolated as PEP inhibitor from microbial origin but PEP inhibitors have been rarely investigated from plant material [13]. The compound **1** has shown IC_{50} of 139±7.4 μM (Table 4) with the positive control of PEP (Z-Pro-prolinal), which is as moderate as previously reported natural inhibitors in activity [14,15].

Table 4. *In vitro* quantitative inhibition of prolyl endopeptidase by compound **1**.

Compound	*IC_{50} (μM)	Z-Pro-prolinal (positive control) for PEP
1	139.2 ± 7.4	1.27 ± 0.001 nM

*IC_{50} values are the ± standard mean (SEM) error of three assays.

EXPERIMENTAL

General experimental methods: Column chromatography (CC): silica gel, 70–230 mesh. Flash chromatography (FC): silica gel 230–400 mesh. TLC: pre-coated silica gel G-25-UV_{254} plates: detection at 254 nm, and by ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR Spectra: Hitachi-UV-3200 and Jasco-320-A spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR, COSY, HMQC and HMBC Spectra: Bruker spectrometers operating at 500 and 400 MHz; chemical shifts δ in ppm and coupling constants in Hz. EI-, CI MS: JMS-HX-110 with a data system.

Plant material. The plant *Euphorbia decipiens* Boiss. & Buhse (Euphorbiaceae) was collected at the mountain Kandovan, north of Karaj, Iran, in 1998, and identified by Mr. Bahram Zehzad (plant taxonomist) at the Department of Biological Sciences, Shahid Beheshti University, Eveen, Tehran. A voucher specimen (no. 98112) has been deposited at the herbarium of the Biology Department of Shahid Beheshti University, Eveen, Tehran.

Extraction and purification. The air-dried ground plant (4 kg) was exhaustively extracted with acetone at room temperature. The extract was evaporated and the residue (62 g) defatted by extraction with hexane. The defatted extract (51 g) was extracted with chloroform. The chloroform extract (44 g) was subjected to CC over a silica gel column (880 g) using hexane with gradient of $CHCl_3$ up to 100% and followed by methanol. Twenty fractions were collected. Fraction no. 11 of the first column was loaded on $AgNO_3$ impregnated silica gel (flash silica 230–400 mesh) and eluted with pure $CHCl_3$. The fraction no. 16 thus obtained was again loaded on preparative plates using system of hexane:EtOAc (55:45) to purify

compounds **1** [(31.5 mg, Rf 0.64; $CHCl_3$-acetone (93:7)], **2** [(23.5 mg, Rf 0.67; $CHCl_3$-acetone (93:7)] and **3** [(3.6 mg, Rf 0.69; $CHCl_3$-acetone (95:5)].

3,7,15,17-*O*-tetraacetyl-5-*O*-benzoyl-13-hydroxymyrsinol (1): Colorless oil (31.5 mg): $C_{35}H_{42}O_{12}$; $[\alpha]_D^{23}$ –18.09 (c = 0.63, $CHCl_3$); UV (MeOH) λ_{max}: 271.7, 229.2, 202.4 nm; IR ν_{max} ($CHCl_3$): 3480, 2960, 2930, 1730, 1600,1580, 1450, 1240, 1020, 710, 600 cm^{-1}; HREIMS: *m/z* 654.2698 (calcd. for $C_{35}H_{42}O_{12}$, 654.2676); EIMS *m/z* (rel. int.): 654 $[M]^+$ (1), 594 (1), 536 (1), 534 (1), 474 (1), 414 (1), 384 (12), 324 (12), 282 (26), 264 (57), 251 (24), 239 (27), 237 (27), 207 (38), 184 (10), 152 (13), 158 (71), 175 (48), 156 (56), 131 (52), 125 (47), 121 (12), 105 (100), 85 (65), 83 (73); 77 (23) CIMS (CH_4) *m/z*: 655 $[M+1]^+$ (6), 595 (25), 535 (50), 493 (8), 475 (12), 413 (14), 355 (12), 123 (58), 105 (16), 61 (100); 41 (34); For ^1H-^{13}C NMR (in $CDCl_3$) data see Table 1 and 2.

3,7,17-*O*-triacetyl-5-*O*-benzoyl-13, 15-dihydroxymyrsinol (2): Colorless oil (23.5 mg): $C_{33}H_{40}O_{11}$; $[\alpha]_D^{23}$ – 7.89 (c = 0.19, $CHCl_3$); UV (MeOH) λ_{max}: 274.6, 226.6, 201.2 nm; IR ν_{max} ($CHCl_3$): 3460, 2930, 2850, 1740, 1640, 1600, 1450, 1240, 1110, 1020, 750, 710, 600 cm^{-1}; EIMS *m/z* (rel. int.): 594 $[M\text{-}H_2O]^+$ (1), 552 $[M\text{-}HOAc]^+$ (1), 492 (2), 432 (4), 430 (14), 387 (8), 370 (22), 105 (100); CIMS (CH_4) *m/z*: 612 $[M]^+$ (13), 552 (100), 492 (40), 431 (45), 371 (43), 311 (35), 123 (23), 105 (15), 61 (422); For ^1H-^{13}C NMR (in $CDCl_3$) data see Table 1.

3,7,17-*O*-triacetyl-5-*O*-butanoyl-13, 15-dihydroxymyrsinol (3): Colorless oil (3.6 mg): $C_{30}H_{42}O_{11}$; $[\alpha]_D^{23}$ – 26.38 (c = 0.072, $CHCl_3$); UV (MeOH) λ_{max}: 202.0 nm; IR ν_{max} ($CHCl_3$): 3610, 2930, 2850, 1740, 1450, 1260, 1150, 1020, 720, 610 cm^{-1}; EIMS *m/z* (rel. int.): 578 $[M]^+$ (1), 560 (1, $[M\text{-}H_2O]^+$), 518 (1, $[M\text{-}AcOH]^+$) 458 (1, $[M\text{-}2x\ AcOH]^+$), 384 (3), 282 (13), 264 (27), 239 (11), 228 (8), 185 (18), 175 (22) 158 (40), 156 (37), 132 (20) 131 (40), 125 (46), 71 (100), 69 (17) 60 (23); CIMS (CH_4) *m/z*: 579 $[M+1]^+$, 561, 519, 473, 459, 441, 413, 371,353, 293, 311, 89, 61; For ^1H-NMR (in $CDCl_3$) data see Table 1.

Enzyme inhibition assay. Chemicals. Prolyl endopeptidase (*Flavobacterium meningosepticum* origin) was purchased from Seikagaku Corporation (Tokyo, Japan) and *N*-benzyloxycarbonyl-Gly-Pro-*p*NA was procured from BACHEM Fine Chemicals Co. Specific inhibitor of PEP, *N*-benzyloxycarbonyl-pro-prolinal, was kindly donated by Dr. Hideaki Shimizu, Yakult Central Institute For Microbiological Research, Tokyo, Japan.

PEP inhibition assay: The PEP inhibition activity was assayed by a modification of the method of Yoshimoto *et al*. [16] 100 mM Tris (hydroxymethyl)-aminomethane-HCl buffer containing 1 mM EDTA, pH 7.0, 247 µL, PEP (0.02 unit/ 300 μL) 15 µL and test sample in 8 µL MeOH, were mixed in 96-well microplate and preincubated for 10 minutes at 30°C. The reaction was initiated by adding 30 µL of 2 *m*M of *N*-benzyloxycarbonyl-Gly-Pro-*p*NA (in 40% 1,4-dioxane) as the substrate. The amount of released *p*-nitroaniline was determined spectrophotometrically, as increase in absorption at 410 nm, with 96-wells microplate reader (Molecular Devices, Spectramax 340 USA). The IC_{50} values were the average of at least three determinations performed in triplicate.

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