First Natural Urease Inhibitor from *Euphorbia decipiens*

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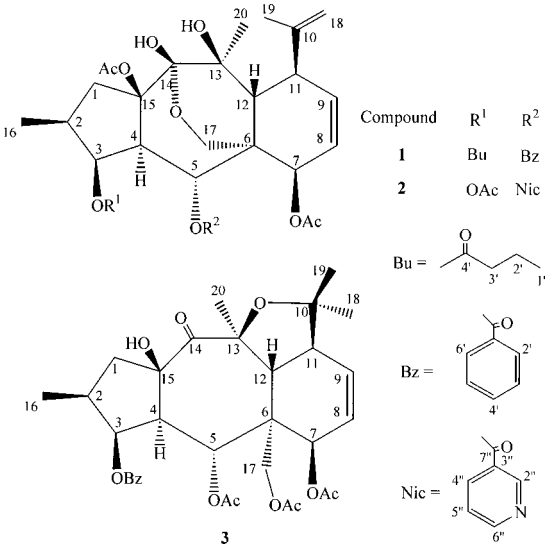
Three new diterpene esters with a myrsinol-type skeleton have been isolated from *Euphorbia decipiens* BOISS. & BUHSE. The structure elucidation of the isolated compounds was based primarily on two-dimensional (2D)-NMR techniques including correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) experiments. Compounds 1 and 3 are active against prolyl endopeptidase and compound 2 showed inhibitory activity against urease enzyme.

Key words *Euphorbia decipiens*; Euphorbiaceae; diterpene ester; myrsinol-type diterpene; urease; prolyl endopeptidase

In the course of our continuing research on biologically active compounds from *Euphorbia decipiens*, a series of studies on the isolation and structure elucidation of secondary metabolites from this plant have been performed.[1—6] Continuing the previous studies, in this paper we report on the isolation and structure of three new diterpenes from the whole plant of *Euphorbia decipiens*. Compounds **1** and **3** showed 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.[7] Compound **2** showed inhibitory activity against the activity of urease. The enzyme urease has been implicated in a variety of pathological conditions, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation.[8]



Results and Discussion

Compound **1** was assigned the molecular formula $C_{35}H_{44}O_{11}$ on the basis of high-resolution electron impact mass spectrometery (HR-EI-MS). Its IR spectra showed a characteristic absorption for carbonyl groups at 1740 cm^{-1} and those at 1600, 1570 and 710 cm^{-1} for a benzene ring. A sharp absorption at 3460 cm^{-1} indicated a non-hydrogen-bonded hydroxyl group in the molecule.

In the EI-MS spectrum the ions at *m*/*z* 580, 520, 460 and 400 indicated the presence of acetate groups which were eliminated from the molecular ion at *m*/*z*=640 in the form of acetic acid. The peak at *m*/*z*=105 $(C_6H_5CO)^+$, 121 $(C_6H_5COO)^+$ and at *m*/*z* 71 (C_3H_7CO) indicated the presence of a benzoate and butanoate ester group respectively in the molecule.

The ^{1}H-NMR of **1** in $CDCl_3$ (Table 1) showed two singlets for acetate methyl groups at δ 2.18 and 2.07. There were three signals due to protons geminal to ester groups which were observed as a doublet at δ 5.68 (d, *J*=10.7 Hz, H-5), a doublet at δ 4.52 (*J*=6.0 Hz, H-7) and a triplet at δ 5.55 (t, *J*=3.4 Hz, H-3). The spectrum also showed three methyl signals in the molecule, comprised of a secondary methyl at δ 0.88 (d, *J*=7.4 Hz, 3×H-16), one olefinic methyl at δ 1.91 (s, H_3-19) and one tertiary methyl at δ 1.34 (s, H_3-20); these signals seems to be geminal to an oxygen bearing group.

The vicinal olefinic protons showing signals at δ 6.35 (br t, *J*=8.9 Hz, H-9) and at δ 6.28 (br t, *J*=7.9 Hz, H-8) are separated by a methine proton at δ 3.18 (d, *J*=6.4 Hz, H-11) from the terminal olefinic protons at δ 4.80 (br s) and 4.55 (br s) in an isopropenyl group, H-18. The downfield chemical shift of H-11 can be considered a consequence of its location between the two double bonds.[11] The long range coupling between H-17 at δ 3.77 (dd, *J*=1.0, 11.2 Hz, H-17a), 4.09 (d, *J*=11.2 Hz, H-17b) and H-5 at δ 5.68 (d, *J*=10.7 Hz), upfield shift of H-12 at δ 2.89 (br s) and H-1 at δ 2.39 (dd, *J*=10.8, 14.7 Hz), are due to the formation of a six membered hemiacetal ring in **1**. Hα-1 (δ 2.39) and H-12 (δ 2.89) of compound **1** appear upfield compared to the corresponding signals of decipinone and myrsinane diterpenoids,[1—3] as a result of the lack of the keto group at C-14.[9] The C(14)=O and OH of the C-17 of the myrsinane skeleton form a hemiacetal function in **1**, exhibiting C-14 at δ 98.4 (s) (Table 2).[9] The connectivity between H-17/C-14 in HMBC spectrum (Table 1) further confirmed the hemiacetal function in **1**.

The broad band and distortionless enhancement by polarization transfer (BB and DEPT) decoupled ^{13}C-NMR of **1** showed 33 signals due to 35 carbons including six CH_3, five CH_2, twelve CH and ten quaternary carbons, out of which seven were oxygen bearing (two tertiary alcohol, one tertiary ester and four ester carbonyls). The ^{1}H–^{1}H- and ^{1}H–^{13}C connectivities were supported by the correlation spectroscopy (^{1}H–^{1}H-COSY) and heteronuclear multiple quantum coher-

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ence (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 heteronuclear multiple bond correlation (HMBC), as in the case of benzoyl carbonyl at *ca*. δ 165.0 with H-5, butanol carbonyl at *ca.* δ 171 with H-3 and for acetate group δ at *ca.* 170 with H-7 (Table 1). The stereochemistry of **1** was determined by comparison of the ^{1}H-NMR coupling constants of **1** with those recorded for myrsinol esters[1—6] with similar structure as well as by nuclear Overhauser enhancement spectroscopy (NOESY) spectra. The coupling constant of H-3 (t, J=3.4 Hz) and H-4 (dd, J=5.2, 10.6 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 (10.6 Hz) between H-4 and H-5 showed the *trans* relationship between them. The NOESY cross peaks between δ 5.55 (H-3)/δ 2.72 (H-4), δ 5.68 (H-5)/δ 2.89 (H-12), established that H-5, H-12 must be located on one face of the molecule. In NOESY spectra cross peaks between 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 on one face of the molecule and H-12 and H-18 in the other.

The ^{1}H and ^{13}C-NMR data are very similar to those reported for decipinone B,[4] except that one acetate group at C-3 is replaced by butanoyl group. The signals at δ 2.11 (m, H-2′), 1.51 (m, H-3′), 0.90 (t, J=7.4 Hz, H-4′) the ^{1}H-NMR spectra and signals at δ 171.4 (s, C-1′), 36.7 (t, C-2′), 18.3 (t, C-3′) and 13.7 (q, C-4′) in ^{13}C-NMR are compatible with the proposed structure.[3]

Compound **2**, obtained as a colorless oil, displayed the IR

Table 1. ^{1}H-NMR and HMBC Data of Compounds **1**—**3**

Position	**1**		**2**		**3**	
	H[a]	HMBC[b]	H[a]	HMBC[b]	H[a]	HMBC[b]
1α	2.39 dd (10.8, 14.4)	2, 3, 4, 14, 15	2.41 dd (10.7, 14.6)	2, 3, 4, 15	2.82 dd (9.9, 14.8)	2, 3, 4, 15
1β	2.47 d (13.8)	1, 15	2.51 br d (14.4)	1, 15	1.72 dd (9.5, 15.0)	1, 15
2	2.71 m		2.73 m		2.10 m	
3	5.55 t (3.4)	1, 15, C-1′	5.51 t (6.2)	1, 15, O<u>C</u>OCH$_3$	5.61 t (4.1 Hz)	1, 15, O<u>C</u>OPh
4	2.72 dd (5.2, 10.6)	5, 6, 14	2.71 dd (5.0, 10.4)	5, 6, 3	3.01 dd (4.1, 11.0)	5, 6, 14
5	5.68 d (10.7)	4, 6, 7, 17, O<u>C</u>OPh	5.53 d (8.5)	4, 6, 7, 17, O<u>C</u>ONic	6.35 d (11.0)	4, 6, 7, O<u>C</u>OCH$_3$
7	4.52 d (6.0)	5, 6, 8, 9, O<u>C</u>OCH$_3$	4.46 d (6.1)	5, 6, 8, 9, O<u>C</u>OCH$_3$	5.17 d (5.3)	5, 6, 8, 9, O<u>C</u>OCH$_3$
8	6.28 br t (7.9)	6, 7, 9, 11	6.24 br t (6.4)	6, 7, 9, 11	6.00 ddd (2.8, 5.2, 8.1)	6, 7, 9, 11
9	6.35 br t (8.9)	7, 11, 12	6.38 br t (8.3)	7, 11, 12	5.82 dd (2.0, 9.8)	7, 11, 12
11	3.18 d (6.4)	6, 8, 9, 10, 12, 13, 18, 19	3.17 br d (6.8)	6, 8, 9, 10, 12, 13, 18, 19	2.85 br d (12.6)	6, 8, 9, 10,12, 13, 18, 19
12	2.89 br s	5, 6, 10, 11, 13, 17	2.91 br s	5, 6, 10, 11, 13, 17	3.13 d (12.7)	5, 6, 10, 11, 13, 17
16	0.88 d (7.4)	1, 2, 3	0.88 d (7.0)	1, 2, 3	0.67 d (7.9)	1, 2, 3
17a	3.77 dd (1.0, 11.2)	5, 6, 7, 12, 14	3.68 dd (1.2, 11.2)	5, 6, 7, 12, 14	4.20 d (12.0)	5, 6, 7, 12, O<u>C</u>OCH$_3$
17b	4.09 d (11.2)	5, 6, 7	3.92 d (11.3)	5, 6, 7	4.59 d (12.0)	5, 6, 7, O<u>C</u>OCH$_3$
18a	4.80 s	9, 10, 11, 19	4.82 br s	9, 10, 11, 19	1.42 s	9, 10, 11, 19
18b	4.55 s		4.54 br s		—	
19	1.91 s	10, 11, 18	1.95 s	10, 11, 18	1.03 s	10, 11, 18
20	1.34 s	12, 13, 14	1.31 s	12, 13, 14	1.52 s	12, 13, 14
OCO<u>C</u>H$_3$						
	2.18 s		2.08 s		2.15 s	
	2.07 s		2.02 s		1.90 s	
	—		1.63 s		1.68 s	
	—		—		—	
Benzoyl						
2′, 6′	7.86 br d (7.2)				7.80 dd (1.0, 8.0)	
3′, 5′	7.38 br t (7.8)				7.37 br t (7.9)	
4′	7.52 br t (3.3)				7.51 m	
Butanoyl						
2′	2.11 m					
3′	1.51 m					
4′	0.90 t (7.0)					
Nictinoyl:						
2″			9.28 br s			
4″			8.36 br d (6.5)			
5″			7.42 dd (4.8, 7.7)			
6″			8.76 br d (3.8)			

a) Recorded in $CDCl_3$. *b*) Correlated carbons.

absorptions at 3450, 1730 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 HR-EI-MS as $C_{32}H_{39}NO_{11}$, *m/z* 613.2532. In the EI-MS the ion peak at *m/z* 595 $[M-H_2O]^+$, 553 $[M-HOAc]^+$ and 493 $[M-2\times HOAc]^+$ and the base peak *m/z* 106 $(C_5H_4NCO)^+$ indicated the presence of hydroxyl, acetate, and nicotinoate functionality in **2**.

The ^{1}H- and ^{13}C-NMR spectra of **2** (Tables 1, 2) were similar to those recorded for **1**, except that it was shown that the benzoyl moiety was substituted by a nicotinate moiety at δ 9.28 (br s, H-2″), 8.36 (br d, J=6.5 Hz, H-4″), 7.42 (dd, J=4.8, 7.7 Hz, H-5″), and 8.76 (br d, J=3.8 Hz, H-6″). The BB and DEPT decoupled ^{13}C-NMR spectra showed five signals at δ 147.8 (s, C-2″), 127.4 (d, C-3″), 140.0 (d, C-4″), 124.9 (d, C-5 ″), 150.3 (d, C-6″) in contrast with a nicotinate moiety. Another difference was that butanoyl group was replaced by acetate group at C-3. In the EI-MS, presence of the ions at *m/z* 106 $[C_6H_4ON]^+$, 124 $[C_6H_5O_2N+1]^+$, and lack of peak for butanoyl group at *m/z* 71 $(C_3H_7CO)^+$ in EI-MS further confirmed the structure. The locations of ester in the groups were established from the HMBC spectrum. The HMBC (Table 1) between δ_C 165.0 to δ_H 5.53 (H-5) indicated that the nicotinate moiety was at C-5 (Table 1). And the HMBC between the carbonyl signal at *ca.* δ_C 170 to δ_H 5.51 (H-3) and 4.46 (H-7) indicated that acetate groups were at C-3 and C-7. The relative stereochemistry of **2** was confirmed by NOESY correlations and coupling constants, which were similar to compound **1**.

Table 2. ^{13}C-NMR Assignment for Compounds **1**—**3** (δ ppm)[a]

Position	**1**[b]	**2**[b]	**3**[b]	DEPT
1	38.3[b]	38.4	46.1	CH_2
2	33.1	33.3	35.7	CH
3	73.1	71.8	79.1	CH
4	54.4	54.7	49.6	CH
5	72.9	72.1	68.9	CH
6	45.7	45.6	47.1	C
7	66.4	66.0	68.1	CH
8	127.3	127.0	126.3	CH
9	137.3	137.3	128.7	CH
10	147.3	147.5	79.1	C
11	40.7	38.4	47.4	CH
12	40.8	40.5	41.7	CH
13	77.4	77.3	83.6	C
14	98.4	97.1	205.4	C
15	98.6	98.5	85.3	C
16	16.1	16.3	14.3	CH_3
17	66.4	65.9	61.2	CH_2
18	112.2	110.5	—	CH_2
18			29.6	CH_3
19	22.3	22.5	24.6	CH_3
20	24.4	22.1	24.8	CH_3
$OCO\underline{CH_3}$				
	22.1	22.0	21.2	CH_3
	20.8	21.0	20.7	CH_3
	—	20.8	20.5	CH_3
$O\underline{C}OCH_3$				
	170.2	174.3	170.1	CO
	170.1	170.1	170.0	CO
	—	170.1	169.7	CO
Benzoyl				
1′	130.8		129.4	C
2′, 6′	129.6		129.5	CH
3′, 5′	128.3		128.3	CH
4′	133.2		133.2	CH
7′	165.1		165.0	CO
Butanoyl				
1′	171.4			CO
2′	36.7			CH_2
3′	18.3			CH_2
4′	13.7			CH_3
Nicitinoyl				
2″		147.8		CH
3″		127.4		CH
4″		140.0		CH
5″		124.9		CH
6″		150.3		CH
CO		165.0		CO

a) Assignment made by a combination of DEPT HMQC NMR data. *b*) Obtained in $CDCl_3$.

Compound **3** exhibited a molecular ion at *m/z* 612.2548 in HR-EI-MS, indicating its molecular formula as $C_{33}H_{40}O_{11}$. In the EI-MS, the ions at *m/z* 584 ($[M-CO]^+$), 524 ($[M-CO-AcOH]^+$), 464 ($[M-CO-2AcOH]^+$), 122 ($PhCO_2H^+$), and 105 ($PhCO^+$) indicated the presence of carbonyl, acetate, and benzoate functionalities in the molecule which were supported by IR absorptions at 3490 (OH), 1740, 1705 (RCO_2R', $RCOR'$), and 1620 (C=C) cm^{-1}.

The ^{1}H- and ^{13}C-NMR data showed ($CDCl_3$, Table 1 and Table 2) a close resemblance in most features to those of **1**. However, two main differences were apparent in the spectrum of compound **1**: (i) One tertiary methyl signal at δ 1.42 (s, H-18) in **3** replaced the olefinic protons of **1** appearing at δ 4.80 br s and 4.55 br s, H-18. (ii) The lack of the olefinic signal in ^{13}C-NMR of C-18 (δ 112.2), C-10 (δ 147.3) of **1** and the addition of the signals at δ_C 79.1 (C-10), a methyl group resonating at δ_C 29.6 (C-18) in **3**. These differences pointed to a rearranged structure and contain a tetrahydrofuran moiety due to the ether bridge between C-10 and C-13. Moreover, the signals at δ_C 79.1 (s) and δ_C 83.6 (s) assigned as C-10 and C-13, respectively reflect their presence in an ether linkage between C-10 and C-13 involving sp^3 carbons. The presence of a geminal dimethyl substituted tetrahydrofuran moiety was also indicated in the HMBC spectrum (Fig. 1, Table 1) by correlations of H-18, H-19 with C-10 and C-11. The extension of the sequence (ether bridge between C-10 and C-13) was achieved because H-11 correlated with C-10, C-12, C-13, H-12 with C-10 and C-11 and C-13 and H-20 with C-12 and C-13.

The ^{1}H- and ^{13}C-NMR data of **3** were very similar to those recorded for cheiradone, a compound isolated from *Euphorbia cheiradenia*,[9] but there were some differences for cheiradone. The slightly upfield shift of H-5 at δ 6.35 (t, J=11.0 Hz) and the downfield shift of H-3 at δ 5.61 (d, J=4.1 Hz) showed that the position of the benzoate group changed from C-5 to C-3 in **3**. Migration of benzoate moiety

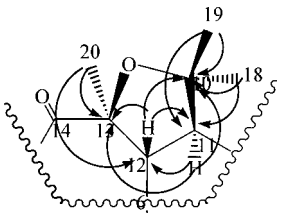

Fig. 1. Some Important HMBC Crrelations for **3**

Table 3. *In Vitro* Quantitative Urease Inhibition Activity of Compound **2**

Compound	*IC_{50} (μM)±S.E.M.	Thiourea (positive control) for urease
2	81.39±0.0023	21±0.011 μM

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

Table 4. *In Vitro* Quantitative Inhibition of Prolyl Endopeptidase by Compounds **1** and **3**

Compound	*IC_{50} (μM)	Bacitracin (positive control) for PEP
1	3.195	129.26 μM
3	10.52	—

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

from C-5 to C-3 also confirmed through HMBC *i.e.* a relatively upfield carbonyl signal at δ 165.0 and its connectivity with H-3 (δ 5.61) in the HMBC spectrum. The relative positions of the other ester groups were also determined from the HMBC data (Table 1), with cross-peaks observed between the H-5, H-7, and H-17 signals and the carbonyl signal of the acetates at *ca.* δ 170. The stereochemistry of **3** was determined by comparison of the ^{1}H-NMR coupling constants of **3** with those recorded for other myrsinol esters[1—6,9] as well as from the NOESY spectrum. The coupling constant of H-3 (t, J=4.1 Hz) and H-4 (dd, J=4.1, 11.0 Hz) indicated that H-2 to H-4 must lie on one face of the molecule with the same dihedral angles between H-2/H-3 and H-3/H-4. The J value (11.0 Hz) between H-4 and H-5 showed their *trans* relationship and the coupling constant of H-12 (d, J=12.7 Hz) indicated a *trans* relationship between H-12 and H-11. The cross peaks between H-3/H-4, H-17/H-7, H-11; and H-18/H-20, in the NOESY spectrum, indicated that these protons all lay on one face of the molecule. A cross-peak between H-5/H-12, and H-19 together with the observed coupling constant (J=11.0 Hz) between H-4 and H-5, confirmed the configuration of **3**.

Studies on the enzyme inhibition have led to the discoveries of drugs. Urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs . Although certain synthetic classes of compounds like hydroxamic acids, imidazoles and phosphazenes have shown potential Urease inhibition,[8] unfortunately, no natural product with this activity has been discovered so far. So there is a need to search for inhibitors of urease from natural resources. Compound **2** is the first naturally occurring urease inhibitor with IC_{50} of 81.4±0.0023 (Table 3).

Low molecular weight inhibitors of Prolyl endopeptidase (PEP) have been reported in the literature but the majority of these are synthetic.[13] Most of the natural PEP inhibitors isolated have been of microbial origin while PEP inhibitors from plants have rarely been investigated.[10] The compounds **1** and **3** have shown IC_{50} of 3.2 and 10.5 μM, respectively, with the positive control of Bacitracin (Table 4).

Experimental

General 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 Kandovan mountain, 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 this department.

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 extractive (51 g) was then extracted with chloroform. The chloroform extractive (44 g) was subjected to CC over a silica gel column (880 g) using hexane with a gradient of $CHCl_3$ up to 100% and followed by methanol. Twenty fractions were collected. Fraction no. 13 of the first column which contained compounds **1**—**3** was loaded on a silica gel column chromatograph using a system of hexane : EtOAc (50 : 50) to purify compounds **1** [(8.1 mg, *Rf* 0.63; $CHCl_3$–acetone (93 : 7)], **2** [(83.0 mg, *Rf* 0.65; $CHCl_3$–acetone (93 : 7)] and **3** [(10.2 mg, *Rf* 0.68; $CHCl_3$–acetone (95 : 5)].

Urease Assay and Inhibition Reaction mixtures comprising 25 μl of enzyme (jack bean urease) solution and 55 μl of buffers containing 100 mM urea were incubated with 5 μl of test compounds (1 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[11] Briefly, 45 μl of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, U.S.A.). All reactions were performed in triplicate in a final volume of 200 μl. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, U.S.A.). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4 \cdot 3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula $100-(OD_{testwell}/OD_{control})\times 100$. Thiourea was used as the standard inhibitor of urease.

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. The 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.*[12] 100 mM Tris(hydroxyl methyl)-aminomethane-HCl buffer containing 1 mM EDTA, pH 7.0, 247 μl, 15 μl PEP (0.02 unit/300 μl) and test sample in 8 μl of MeOH, were mixed in a 96-well microplate and preincubated for 10 min at 30 °C. The reaction was initiated by adding 30 μl of 2 mM 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 a 96-well microplate reader (Molecular Devices, Spectramax 340 U.S.A.). The IC_{50} values were performed in triplicate.

Compound **1**: Colorless oil, ^{1}H: ^{13}C-NMR (in $CDCl_3$) data see Tables 1 and 2. IR ν_{max} ($CHCl_3$): 3460, 3000—2900, 1740, 1600,1570, 1450, 1240, 1020, 710, 600 cm^{-1}. UV (MeOH) λ_{max}: 270.0, 227.2, 199.4 nm. EI-MS *m/z* (rel. int.): 640 $[M]^+$ (1), 580 $[M-HOAc]^+$ (1), 520 $[M-2\times HOAc]^+$ (1), 460 $[M-3\times HOAc]^+$ (1), 400 $[M-4\times HOAc]^+$, 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 $[C_6H_5COO]^+$ (12), 105 $[C_6H_5CO]^+$ (100), 85 (65), 83 (73); 71 $[C_3H_7CO]^+$ (85). CI-MS (CH_4) *m/z*: 641 $[M+1]^+$ (6), 581 (25), 521 (50), 355 (12),123 (58), 105 (100), 71 (81); 41 (34). HR-EI-MS: *m/z* 640.2872 (Calcd for $C_{35}H_{42}O_{12}$, 640.2883). $[\alpha]_D^{23}$ −3.28° (c=0.12, $CHCl_3$).

Compound **2**: Colorless oil, ^{1}H: ^{13}C-NMR (in $CDCl_3$) data see Tables 1 and 2. IR ν_{max} ($CHCl_3$): 3450, 2930, 2850, 1730, 1640, 1600, 1580, 1450, 1240, 1110, 1020, 750, 600 cm^{-1}. UV (MeOH) λ_{max}: 270, 199.1 nm. EI-MS *m/z* (rel. int.): 595 $[M-H_2O]^+$ (1), 553 $[M-HOAc]^+$ (1), 493 $[M-2\times HOAc]^+$ (2), 432 (4), 430 (14), 387 (8), 370 (22), 124 $[C_6H_5NO_2]^+$, 106 $[C_5H_4NCO]^+$ (100). CI-MS (CH_4) *m/z*: 613 $[M]^+$ (13), 553 (10), 493 (40), 431 (45), 371 (43), 311 (35), 123 (23), 106 (100), 61 (42). HR-EI-MS: *m/z* 613.2532 (Calcd for $C_{35}H_{42}O_{12}$, 613.2522). $[\alpha]_D^{23}$ −6.63° (c=1.66, $CHCl_3$).

Compound **3**: Colorless oil, ^{1}H: ^{13}C-NMR (in $CDCl_3$) data see Tables 1

and 2. IR ($CHCl_3$) ν_{max} 3490, 2960, 2940, 1740, 1705, 1620, 1600, 1550, 1250, 1100, 1020, 710, 600 cm^{-1}. UV (MeOH) λ_{max} 229, 202 nm. EI-MS *m/z* 584 $[M-CO]^+$ (34), 524 $[M-CO-AcOH]^+$ (12), 464 $[M-CO-2\times HOAc]^+$ (17), 122.5 ($PhCO_2H^+$), 105 ($PhCO^+$) (100), 464 (17), 293 (73), 233 (93), 191 (73), 105 (100). CI-MS (CH_4) *m/z* 613 $[M+1]^+$, 553 $[M+1-HOAc]^+$, 493 $[M+1-2\times HOAc]^+$, 415, 285. HR-EI-MS *m/z* 612.2548 (Calcd for $C_{33}H_{40}O_{11}$, 612.2570). $[\alpha]_D^{23}$ −9.68° (*c*=0.14, $CHCl_3$).

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