ORGANIC LETTERS

2004
Vol. 6, No. 23
4139−4142

Novel Sesterterpenes from *Leucosceptrum canum* of Nepalese Origin

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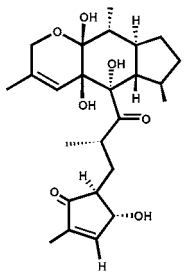
Received June 4, 2004 *(Revised Manuscript Received September 7, 2004)*

ABSTRACT



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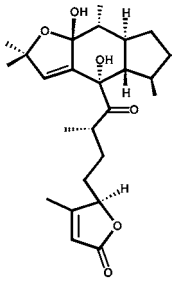

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Two novel sesterterpenoids, leucosesterterpenone (1) and leucosesterlactone (2), with novel skeleta were isolated from the hexane extract of the medicinal plant, *Leucosceptrum canum*. Their structures were established by the analysis of NMR data and the single-crystal X-ray diffraction of compound 1. Compounds 1 and 2 were found to exhibit activity against prolylendopeptidase (PEP).

Leucosceptrum canum Sm. (Lamiaceae), a small tree, locally known as Bhusure in Nepal, is distributed in the temperate Himalayans regions, Myanmar, and China.[1,2] The plant is used as an insecticidal agent in remote areas of Nepal. Our previous study on this plant has yielded a novel sesterterpene, leucosceptrine (**3**).[3] In this paper, we report the isolation of two more novel sesterterpenes, leucosesterterpenone (**1**) and leucosesterlactone (**2**). A known primary precursor *trans*-phytol was also obtained from this plant.[4,5]

Leucosesterterpenone (**1**) was obtained as colorless crystals. Its molecular formula was established by (−ve) HRFAB MS to be $C_{25}H_{36}O_7$ with the $[M - H]^-$ ion appearing at *m*/*z* 447. The HREI MS spectrum of **1** showed an ion peak at

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(1) Hooker, J. D. *Flora of British India*; Bishen Singh Mahendra Pal Singh Publishers: Dehradun, 1983; Vol. IV, p 699.

(2) Flora of Kathmandu Valley. In *Bull. Department Med. Plant, Nepal, No. 11*; His Majesty's Government of Nepal, Ministry of Forests and Soil Conservation, Department of Medicinal Plants: Kathmandu, Nepal, 1986; p 561.

(3) Choudhary, M. I.; Ranjit, R.; Atta-ur-Rahman; Shrestha, T. M.; Yasin, A.; Parvez, M. *J. Org. Chem.* **2004**, *69*, 2906.

(4) **Plant Material.** The aerial parts of *L. canum* Sm. were collected from Godawari, Kathmandu, Nepal on November 26, 2000, at an altitude of 1550 m. The plant was identified by senior scientific officer, Mrs. Tirth Maiya Shrestha, and a voucher specimen (T037) was deposited at The National Herbarium and Research Laboratory, Department of Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Kathmandu, Nepal.

(5) **Extraction and Isolation.** Aerial parts of *L. canum* were cut into small pieces and dried in the shade. The air-dried and powdered plant material (1.75 kg) was soaked in hexane (10 L) at room temperature for 2 days, filtered and evaporated under reduced pressure. This process was repeated three times to yield 34.03 g of hexane extract. The plant material was subsequently soaked in dichloromethane, ethyl acetate, and methanol. The resulting organic extracts were filtered and concentrated under reduced

10.1021/ol040040y CCC: $27.50
Published on Web 10/16/2004

m/*z* 430 ($C_{25}H_{34}O_6$), representing the loss of H_2O from the M^+. The IR absorption at 3456 cm^{-1} indicated the presence of hydroxyl groups.[6] Analysis of the ^1H NMR, ^{13}C NMR, and HMQC data[7] revealed the presence of seven quaternary carbons, nine methine, four methylene, and five methyl groups. The downfield ^{13}C NMR signals at δ 73.2, 96.9, and 82.6 assigned to C-4, C-5, and C-12, respectively, indicated the presence of hydroxyl groups. A downfield carbon signal at δ 96.9 appeared due to C-5 with a hemiacetal functionality. The signal for an oxymethylene carbon at δ_C 64.6 (δ_H 4.14 and 3.94, d, $J_{1\alpha,1\beta}$ = 16.5 Hz, H_2-1) for C-1, olefinic carbons at δ_C 121.5 (δ_H 5.01) and 137.2 for C-2 and C-3, respectively, and a methyl carbon at δ_C 17.5 (δ_H 1.63) appeared due to the carbons of an unsaturated pyran ring A. The carbon signal at δ 223.6 and the IR absorption at 1705 cm^{-1} indicated the presence of a ketonic group. Out of five methyl carbons, the secondary methyl groups resonating at δ_C 10.9, 21.7, and 16.0 correlated with the ^1H NMR doublets at δ 1.01 ($J_{22,6}$ = 6.7 Hz, H-22), 0.78 ($J_{23,10}$ = 6.1 Hz, H-23), and 1.18 ($J_{24,14}$ = 6.8 Hz, H-24), respectively. The remaining two tertiary methyl carbons appeared at δ 17.5 (C-21) and 10.1 (C-25) directly correlating with δ_H 1.63 and 1.77, respectively, in the HMQC spectrum. Most of the chemical shifts of leucosesterterpenone (**1**) resembled those of leucosceptrine (**3**)[3] except for some shifts due to the presence of an α,*β*-unsaturated five-membered ketonic ring instead of an α,*β*-unsaturated five-membered lactone ring. The main difference between leucosesterterpenone (**1**) and leucosceptrine (**3**)[3] is the length of the carbon chain from four (C-13 to C-16) to three (C-13 to C-15), since C-16 was involved in the formation of a five-membered ring. The downfield proton resonated at δ 7.11, the downfield carbon signals at δ 142.4 and 154.8, and a ketonic carbon signal at δ_C 206.7, indicating a double bond in conjugation with a carbonyl group. This was also supported by the mass fragment at *m*/*z* 111 that resulted from the cleavage of the C-15/C-16 bond. COSY 45° interactions between δ_H 7.11 and δ_H 4.53 and δ_H 4.53 and δ_H 2.39, as well as HMBC correlations between δ_H 1.77 (H-25) and δ_C 206.7 (C-17) and δ_H 4.53 (H-20) with δ_C 142.4 (C-18) and 154.8 (C-19) (Figure 1) further supported the presence of a five-membered α,*β*-unsaturated carbonyl-containing ring with a hydroxyl group at C-20 (δ_C 75.7).[8]

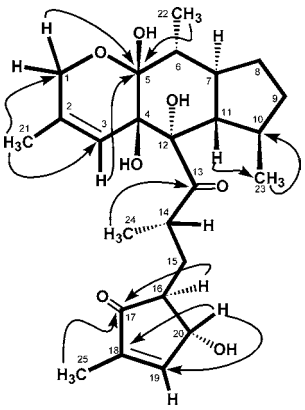


Figure 1. Key HMBC correlations in compound **1**.

Since compound **1** was obtained as colorless prismatic crystals from chloroform solution, an X-ray diffraction analysis was carried out.[9] The result supported the gross structure of compound **1**, and allowed for the assignment of the relative configurations for all chiral centers (Figure 2).

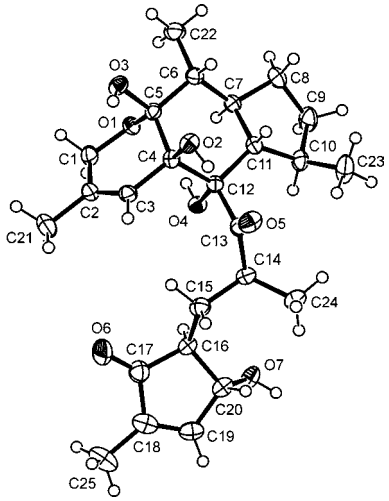


Figure 2. Computer-generated ORTEP diagram of the final X-ray model of compound **1**.

A biogenetic pathway for compound **1** is proposed in Scheme 1. Like leucosceptrine (**3**),[3] **1** may also originate from geranylfarnesyl pyrophosphate (GFPP). A series of enzymatic reduction, oxidation, and cyclization reactions can lead to a novel skeleton for compound **1**, via intermediates [A] and [B].

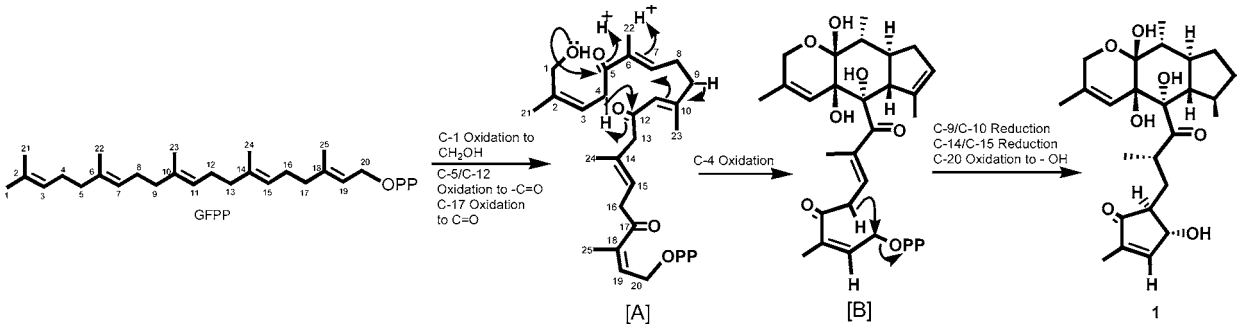
pressure. Some fatty acids were removed from the hexane extract through precipitation with acetone. The filtrate was again concentrated under reduced pressure to afford a semidried extract (26.89 g). This hexane extract was repeatedly chromatographed on a silica gel column using various polarities of solvents, starting from hexane, and proceeding with hexane−chloroform, chloroform−methanol, and finally methanol to obtain subfractions LCH 207-C and LCH 207-D. Fraction LCH 207-D obtained on elution with chloroform−methanol (95:5) was recrystallized in hexane−chloroform (40:60) with a few drops of methanol through slow evaporation. The colorless crystals obtained were washed with diethyl ether, which afforded the pure compound **1** (20.35 mg). Similarly, repeated column chromatography of the fraction LCH 207-C afforded white crystals of **2** (100 mg), eluting with pure chloroform. The purity of the crystals was checked by TLC (visualized at 254 and 366 nm) and by spraying with ceric ammonium sulfate spraying reagent.

(6) Pavia, D. L.; Lampman, G. M.; Kriz, G. S. *Introduction to Spectroscopy*, 2nd ed.; Harcourt Brace College Publishers: Fort Worth, TX, 1996.

(7) Atta-ur-Rahman; Choudhary, M. I. *Solving Problems by NMR Spectroscopy*; Academic Press: San Diego, 1996.

(8) **Compound 1:** colorless block crystals, mp 154−157 °C (dec.), R_f = 0.30 (2% MeOH/$CHCl_3$), $[\alpha]^{25}_D$ 240° (*c* = 0.04, $CHCl_3$), UV (MeOH) λ_{max} nm (log ϵ) 390 (2.78), 341 (2.76), 194 (5.14); λ_{min} nm (log ϵ) 368 (2.61), 339 (2.64); IR ($CHCl_3$) ν_{max} 3456 (OH), 2935 and 2868 (CH), and 1705 (C=O) cm^{-1}; ^1H ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data see Table 1; FAB MS $M^+ - 1$ *m*/*z* 447; HREI MS *m*/*z* 430.2012 (calcd for $C_{25}H_{36}O_7$, *m*/*z* 430.1924), EI MS *m*/*z* (rel intensity %) 430 (33), 319 (10), 292 (25), 249 (60), 234 (50), 203 (35), 139 (50), 111 (46), 110 (73), 83 (100).

Scheme 1. Biogenesis of Leucosesterterpenone (**1**)



Leucosesterlactone (**2**)[5] was obtained as white crystals and showed the M^+ at *m*/*z* 432.2444 (HREI MS), in agreement with the formula $C_{25}H_{36}O_6$. The IR absorptions indicated the presence of hydroxyl (3437 cm^{-1}) and ketonic (1739 cm^{-1}) groups. A detailed study of ^1H and ^{13}C NMR data (Table 1) indicated the presence of six methyls (C-1, C-21, C-22, C-23, C-24, and C-25) and four methylenes (C-8, C-9, C-15, and C-16). Three secondary methyl groups resonated at δ_C 13.2, 20.6, and 15.3, correlating with ^1H NMR doublets at δ 0.81 ($J_{22,6}$ = 6.7 Hz, H-22), 1.12 ($J_{23,10}$ = 6.4 Hz, H-23), and 1.17 ($J_{24,14}$ = 6.5 Hz, H-24), respectively. The remaining three tertiary methyl groups resonated at δ_C 28.2, 21.0, and 13.9, directly correlating with δ_H 1.81 (d, $J_{21,3}$ = 1.2 Hz, H-21), 2.03 (d, $J_{3,1}$ = 1.2 Hz, H-1), and 2.02 (dd, $J_{25,18}$ = 0.6 Hz, $J_{25,17}$ = 0.4 Hz), respectively. Signals for the carbons of two trisubstituted double bonds (C-3, C-4, and C-18, C-19), six methine carbons (C-6, C-7, C-10, C-11, C-14, and C-17), and five quaternary carbons, including two carbonyl carbons, appeared in the ^{13}C NMR spectrum (Table 1). The IR and ^{13}C NMR spectra indicated the presence of an α,β-unsaturated five-membered lactone ring (ν_{max} 1739 cm^{-1}, δ_C

Table 1. NMR Data of Leucosesterterpenone (**1**) and Leucosesterlactone (**2**) in $CDCl_3$

	1		**2**	
position	δ_H (*J* in Hz)[a]	δ_C[b]	δ_H (*J* in Hz)[a]	δ_C[b]
1	3.94, 4.14 (d, 16.5)	64.6	2.03 (d, 1.2)	21.0
2		137.2		69.2
3	5.01 (d, 1.3)	121.5	5.99 (brs)	123.4
4		73.2		157.9
5		96.9		94.0
6	1.92 (m)	41.9	1.95 (m)	45.1
7	1.55 (m)	43.3	1.87 (m)	47.8
8	1.25, 1.91 (m)	33.2	1.31, 1.81(m)	30.2
9	2.32, 1.41 (m)	32.7	1.35, 2.01 (m)	32.3
10	1.95 (m)	31.0	2.35 (m)	33.2
11	1.85 (m)	54.6	2.12 (m)	61.5
12		82.6		87.1
13		223.6		211.0
14	3.55 (m)	40.7	2.51 (m)	38.6
15	1.18, 1.80 (m)	27.8	1.25,1.91 (m)	30.0
16	2.39 (m)	53.6	1.19 (m)	28.3
17		206.7	4.60 (brd, 6.7)	86.9
18		142.4		172.3
19	7.11 (d, 1.7)	154.8	5.75 (m)	117.1
20	4.53 (d, 1.6)	75.7		175.8
21	1.63 (s)	17.5	1.81 (d, 1.2)	28.2
22	1.01 (d, 6.7)	10.9	0.81 (d, 6.7)	13.2
23	0.78 (d, 6.1)	21.7	1.12 (d, 6.4)	20.6
24	1.18 (d, 6.8)	16.0	1.17 (d, 6.5)	15.3
25	1.77 (s)	10.1	2.02 (dd, 0.8, 0.6)	13.9
OH	3.94 (s)		3.91 (s)	
OH	4.93 (s)			

[a] ^1H NMR in 300 MHz. [b] ^{13}C NMR at 100 and 125 MHz for compounds **1** and **2**, respectively.

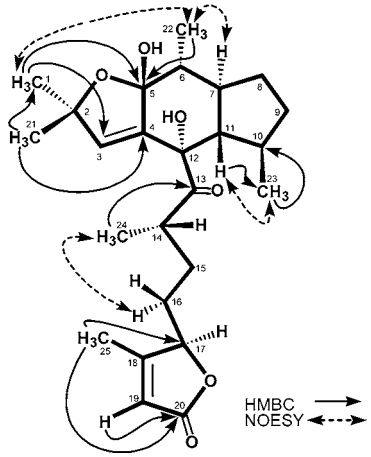
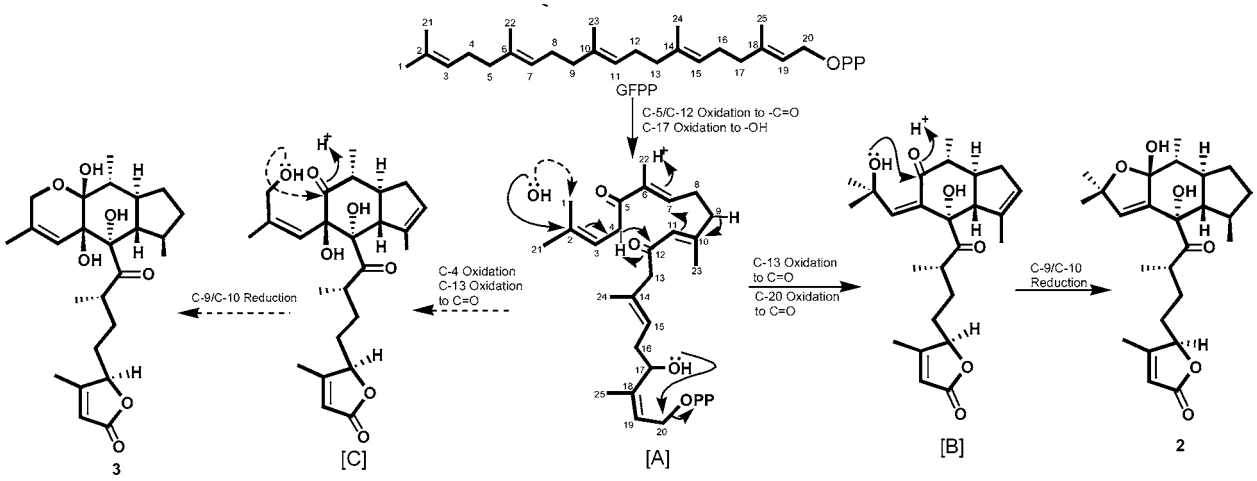


Figure 3. Key HMBC and NOESY correlations in compound **2**.

(9) **X-ray Diffraction Studies on Compound 1:** $C_{25}H_{36}O_7 \cdot 0.10\ H_2O$, M = 448.34, tetragonal system, space group $P4_32_12$, a = 11.3790(10) Å, b = 11.3790(10) Å, c = 36.967(7) Å, V = 4786.5(11) Å^3, $F(000)$ = 1944, Z = 8, d = 1.250 Mg/m^3. A crystal of dimensions 0.20 × 0.20 × 0.18 mm was used for measurement on a Nonius kappa CCD diffractometer with graphite monochromated radiations. The total number of independent reflections measured was 3217 [R(int) = 0.055]. The crystal structure was solved by direct methods (Altomare, A.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A. *J. App. Cryst.* **1993**, *26*, 343) using Fourier techniques (Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; de Gelder, R.; Israel, R.; Smiths, J. M. M. *The DIRDIF-94 Program System, Technical Report of the Crystallography Laboratory*; University of Nijmegen: Nijmegen, The Netherlands, 1994). The structure was refined by full matrix least-squares calculations on F2 with the aid of the program *SHELXL97* (Sheldrick, G. M. *SHELXL97*; University of Göttingen: Göttingen, Germany, 1997). The final indices R_1 = 0.040 and wR_2 = 0.087.

Scheme 2. Biogenesis of Leucosesterlactone (**2**)



175.8, 172.3, and 117.1), which was further supported by the mass fragment at *m*/*z* 97, and a proton multiplet at δ_H 5.75.[10] Most of the chemical shifts in compound **2** closely resembled those of leucosceptrine (**3**).[3] The main difference between leucosesterlactone (**2**) and leucosceptrine (**3**)[3] is the contraction of the six-membered hemiacetal ring into a five-membered hemiacetal ring in compound **2**. The absence of an oxymethylene carbon and the presence of an oxymethine carbon (δ_C 69.2, C-2), along with an additional methyl signal (C-1), indicated the presence of a dihydrofuran ring. Two methyls, C-21 (δ_C 28.2) and C-1 (δ_C 21.0), were found to be attached to the same carbon C-2 in the HMBC spectrum (Figure 3). This was also supported by mass fragments at *m*/*z* 251, 181, 112, and 320.

The relative stereochemistry and the conformation of compound **2** were established by a NOESY experiment (Figure 3). In the NOESY spectrum, the correlations between H_3-1/H_3-22, H_3-22/H-7, H_3-24/Ha-16, and Ha-16/H-17 indicated that H_3-1, H-7, and H-17 were in the α-configuration. Similarly, the H_3-23/H-11 correlation indicated that H-11 was in the β-configuration.

A biogenetic pathway for compound **2** is presented in Scheme 2. It might be derived from enzymatic reduction, oxidation, and cyclization via intermediates [A] and [B] as in compound **3**.[3]

Compounds **1**−**3** are members of a new class of sesterterpenes named leucosesterterpenes. The characteristic features of this class include a tricyclic skeleton with a cyclic ether and a 10-carbon side chain.

Both compounds **1** and **2** exhibited activity against the enzyme prolylendopeptidase (PEP). PEP catalyzes the degradation of proline-containing neuropeptides such as vasopressin, substance P, and thyrotropin-releasing hormones that are involved in the processes of learning and memory.[11]

Compounds **1** and **2** showed moderate inhibitory activity against the PEP, i.e., IC_{50} = 322.21 ± 5.23 and 296.91 ± 3.28 μM, respectively, against the enzyme.[12]

Acknowledgment. Ms. Rosa Ranjit gratefully acknowledges the financial support of the Third World Organization for Women in Science (TWOWS), Trieste, Italy, for providing a Ph.D. scholarship to study at the H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan. We are also grateful to the Third World Academy of Sciences (TWAS), Trieste, Italy, for a fellowship to Mr. Krishna P. Devkota under the TWAS Associateship Scheme. A specific inhibitor of PEP, *N*-benzyloxycarbony-pro-prolinal, was kindly provided by Dr. Hideaki Shimizu, Central Institute for Microbiological Research, Tokyo, Japan.

Supporting Information Available: Tables of X-ray data and computer-generated diagram of the final X-ray model of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) **Compound 2:** white needle-shaped crystals, mp 155−160 °C (dec.), R_f = 0.32 (1% MeOH/$CHCl_3$), $[\alpha]^{25}_D$ 80° (*c* = 0.04, $CHCl_3$), UV (MeOH) λ_{max} nm (log ϵ) 389 (2.77), 343 (2.85), 243 (3.99), 206 (4.21); λ_{min} nm (log ϵ) 376 (2.63), 339 (2.76), 235 (3.97), 197 (4.12); IR ($CHCl_3$) ν_{max} 3437 (OH), 2956 and 2872 (CH) and 1739 (C=O) cm^{-1}; ^1H ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data see Table 1; FAB MS (−ve) $M^+ - 1$ *m*/*z* 431; HREI MS *m*/*z* 432.2445 (calcd for $C_{25}H_{36}O_6$, *m*/*z* 432.2444), EI MS *m*/*z* (rel intensity %) 432 (7), 349 (22), 320 (10), 292 (15), 251 (4), 233 (5), 196 (9), 181 (15), 153 (39), 112 (17), 109 (20), 97 (30), 83 (100).

(11) Kobayashi, W.; Miyase, T.; Sano, M.; Umehara, K.; Warashina, T.; Noguchi, H. *Biol. Pharm. Bull.* **2002**, *25*, 1049.

(12) **PEP Inhibitory Activity of Compound 1.** The PEP inhibition activity was assayed using the modified method of Yoshimoto et al. (Yoshimoto, T.; Walter, R.; Tsuru, D. *J. Biol. Chem.* **1980**, *255*, 4786). Tris (hydroxymethyl)-aminomethane−HCl buffer (100 mM), containing 247 μL of 1 mM EDTA, pH 7.0, 15 μL of PEP (0.02 unit/300 μL), and test sample in 8 μL of MeOH was mixed in 96-well microplate and preincubated for 10 min at 30 °C. The reaction was initiated by adding 30 μL of 2 mM *N*-benzyloxycarbonyl-Gly-Pro-*p*NA (in 40% 1,4-dioxane) as the substrate. The amount of *p*-nitroaniline released was determined spectrophotometrically as indicated by the increase in absorption at 410 nm on a 96-well microplate reader at 30 °C. The IC_{50} values were the average of at least three determinations. Bacitracin was used as a standard inhibitor in this assay (IC_{50} = 129.26 ± 3.28 μM: Fan, W.; Tezuka, Y.; Ni, K. M.; Kadota, S. *Chem. Pharm. Bull.* **2001**, *49*, 396).