Three Cyclooctapeptides and One Glycoside from *Microtoena prainiana*

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Three new cyclic octapeptides, microtoenins A−C (**1**−**3**), and a new glycoside, 3‴-*O*-methylcrenatoside (**4**), along with several known compounds, were isolated from the ethanolic extract of the stems of *Microtoena prainiana*. Their structures were determined by spectral and chemical evidence. At a concentration of 0.01 mg/mL, 3‴-*O*-methylcrenatoside (**4**), crenatoside (**5**), and isocrenatoside (**6**) inhibited angiotensin converting enzyme (ACE) activity by more than 30%.

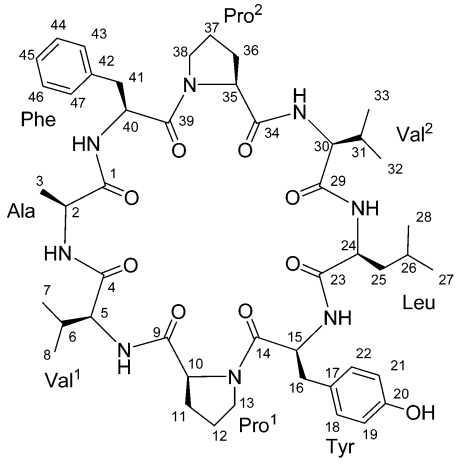
The genus *Microtoena* comprises about 21 species, distributed from Southeast Asia to South China. *M. insuavis* (Hance) Prain is used as a folk remedy for treatment of enteritis and diarrhea. *M. prainiana* Diels (Labiatae) is a plant widely distributed in Sichuan, Yunnan, and Guizhou Provinces, China.[1] Chemical research on this genus has not been reported. In our program searching for compounds to reduce high blood pressure, it was found that the EtOH extract of *M. prainiana* exhibited ACE inhibitory activity. The EtOH extract was successively triturated with petroleum ether, $CHCl_3$, EtOAc, and n-BuOH. The EtOAc fraction was found to be the highest in activity.

Repeated chromatography of the inactive $CHCl_3$ fraction provided cyclic peptides, microtoenins A−C (**1**−**3**), and indole-3-aldehyde,[2] scopoletin,[3] ethyl caffeate,[4] 2-anilino-1,4-naphthoquinone,[5] (4*E*)-2-anilino-4-(phenylimino)naphthalene-1(4*H*)-one,[6] apigenin,[7] luteolin,[7] betulinic acid,[8] pachypodol,[9] and kumatakenin.[10,11] Bioassay-guided isolation of the EtOAc fraction led to active compounds 3‴-*O*-methylcrenatoside (**4**), crenatoside (**5**),[12] and isocrenatoside (**6**),[13] as well as two inactive compounds, apigenin 7-(6″-*p*-coumaroyl)glucoside[14] and hesperidin.[15] At a concentration of 0.01 mg/mL, compounds **4**−**6** inhibited ACE activity by more than 30%. Compounds **1**−**4** were new. The structures of all the compounds were determined on the basis of spectral and chemical methods.

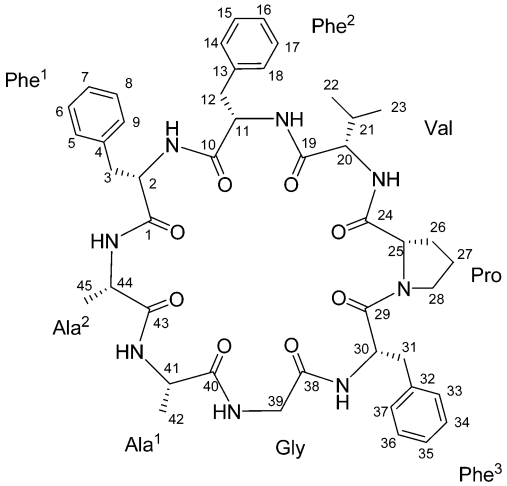
Results and Discussion

Compound **1** was negative to ninhydrin but positive after it was hydrolyzed with 6 N HCl(aq). The molecular formula was assigned as $C_{47}H_{66}N_8O_9$ on the basis of the ion peak at *m*/*z* 887.5036 ($[M + H]^+$) in the HRESIMS spectrum. The IR absorptions at 3435 and 1635 cm^{-1} were consistent with amide. The ^{13}C NMR spectrum showed eight amide carbonyl signals in the range δ 171.2−174.0 and eight methine signals in the range δ 48.7−62.1. The ^1H NMR spectrum showed six signals between δ 7.58 and 10.87 for amide N−H. The above evidence suggested that compound **1** was a cyclopeptide.

Standard amino acid analysis of the hydrolysate revealed the presence of 1 equiv of alanine (Ala), tyrosine (Tyr), leucine (Leu), and phenylalanine (Phe) and 2 equiv of valine (Val) and proline (Pro), which was consistent with the conclusion from $^1H-^1H$ COSY, TOCSY, HSQC, and HMBC experiments (Figure S1, Supporting Information).



1



2

The fragments Ala-Val[1]-Pro[1]-Tyr and Leu-Val[2]-Pro[2]-Phe in compound **1** were elucidated by the following HMBC correlations: 2-NH and H-2 (Ala)/C-4 (Val[1]), 5-NH (Val[1])/C-9 (Pro[1]), H-13 (Pro[1])/C-14 (Tyr), and 24-NH (Leu)/C-29 (Val[2]), 30-NH and H-30 (Val[2])/C-34 (Pro[2]), H-38 (Pro[2])/C-39 (Phe). In the ESIMS/MS experiments (Figure S2, Supporting Information), compound **1** showed fragment ions at *m*/*z* 218 and 472 due to the fragments of Phe-Ala and Val[1]-Pro[1]-Tyr-Leu, respectively. Therefore, the structure of compound **1** was determined to be cyclo-(Ala-Val[1]-Pro[1]-Tyr-Leu-Val[2]-Pro[2]-Phe). The proposed structure was further supported by the following NOE correlations: 15-

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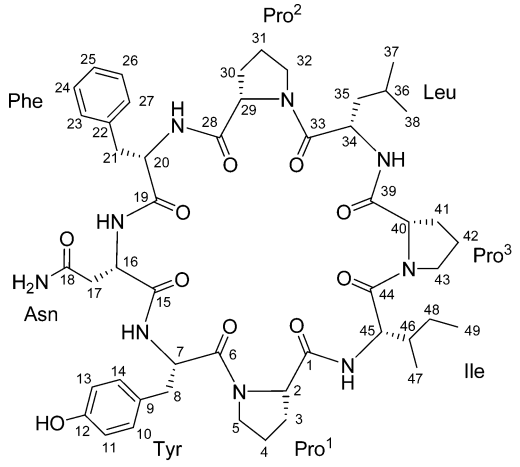
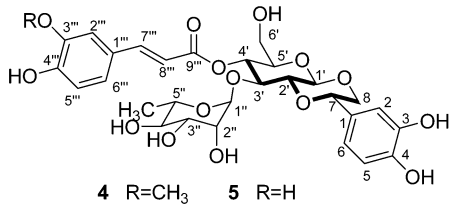
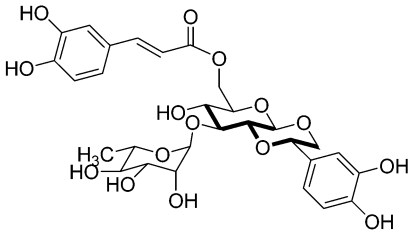


3



4 R=CH_3 **5** R=H



6

NH (Tyr)/H-24 (Leu) and 40-NH (Phe)/H-2 (Ala) in the NOESY experiments.

The absolute configuration of the residues in **1** was characterized by acid hydrolysis and analysis of the hydrolysate by Marfey's method.[16] The Marfey's derivatives of DL- and L-amino acids of all these amino acids were prepared as standards and compared with those of the hydrolysate by co-injecting on HPLC. All amino acids of compound **1** were found to have the L configuration.

Compound **2** was negative to ninhydrin but positive after it was hydrolyzed with 6 N HCl(aq). Its molecular formula was assigned as $C_{45}H_{56}N_8O_8$ according to the ion peak at *m*/*z* 837.4295 ([M + H]$^+$) in the HRESIMS spectrum. IR absorptions at 3429 and 1656 cm^{-1} suggested the presence of amide moieties. Eight signals in the range δ 169.4−174.6 for amide carbonyl groups, seven signals in the range δ 49.3−61.4 for methines, and a methylene signal at δ 44.0 were recognized in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed seven amide protons between δ 7.45 and 10.17. Thus, compound **2** was also a cyclopeptide.

Standard amino acid analysis of the hydrolysate prepared from **2** with 6 N HCl(aq) revealed the presence of 1 equiv of Val, Pro, and glycine (Gly), 2 equiv of Ala, and 3 equiv of Phe, which was in accordance with the results from TOCSY, HSQC, and HMBC experiments (Figure S3, Supporting Information).

The sequence of amino acids in **2** was established as cyclo-(Phe^1-Phe^2-Val-Pro-Phe^3-Gly-Ala^1-Ala^2) by the following HMBC correlations: H-2 (Phe^1)/C-10 (Phe^2), H-11 (Phe^2)/C-19 (Val), H-20 (Val)/C-24 (Pro), H-28 b (Pro)/C-29 (Phe^3), H-30 (Phe^3)/C-38 (Gly), H-39 (Gly)/C-40 (Ala^1), 41-NH (Ala^1)/C-43 (Ala^2). In addition, the information provided by NOESY experiments (Figure S3, Supporting Information) and ESIMS/MS data (Figure S4, Supporting Information) confirmed the proposed structure. The configuration of all of the amino acids in **2** was determined to be L by analysis of its hydrolysate with Marfey's method.

Compound **3** was negative to ninhydrin but positive after it was hydrolyzed with 6 N HCl(aq). The ion peak at *m*/*z* 964.4916 ([M + Na]$^+$) in the HRESIMS provided the molecular formula $C_{49}H_{67}N_9O_{10}$. IR absorptions at 3435 and 1645 cm^{-1}, nine ^{13}C NMR signals in the range δ 171.5−175.1 for carbonyls, eight ^{13}C NMR signals for methines in the range δ 51.0−63.1, and ^{1}H NMR signals for seven amide protons between δ 7.19 and 10.10 suggested that **3** was also a cyclopeptide.

Standard amino acid analysis of the hydrolysates of **3** indicated the presence of 1 equiv of Tyr, aspartic acid (Asp), Phe, Leu, and isoleucine (Ile) and 3 equiv of Pro. In the course of the analysis of ^{1}H−^{1}H COSY, TOCSY, HSQC, and HMBC data (Figure S5, Supporting Information), it became apparent that **3** incorporated an asparagine (Asn) residue instead of an Asp in view of the molecular formula and the lack of an IR band assignable to a carboxyl group.

The sequence of the amino acid residues was elucidated by the following HMBC correlations: H-2 (Pro^1)/C-6 (Tyr), H-7 (Tyr)/C-15 (Asn), H-16 (Asn)/C-19 (Phe), 20-NH (Phe)/C-28 (Pro^2), H-29 (Pro^2)/C-33 (Leu), H-34 (Leu)/C-39 (Pro^3), H-40 (Pro^3)/C-44 (Ile). Consequently, the structure of **3** was determined as cyclo-(Pro^1-Tyr-Asn-Phe-Pro^2-Leu-Pro^3-Ile). The ESIMS/MS data (Figure S6, Supporting Information) and NOESY experiments supported the conclusion. The amino acids in **3** were determined to have an L configuration by Marfey's method.

Compound **4** was isolated as an amorphous powder. HRESIMS gave an ion peak at *m*/*z* 659.1949 [M + Na]$^+$, indicating a molecular formula of $C_{30}H_{36}O_{15}$. Alkaline treatment of **4** followed by acid hydrolysis gave glucose and rhamnose. The ^{1}H NMR signals at δ 7.27 (1H, d, J = 1.8 Hz, H-2‴), 7.09 (1H, dd, J = 8.4, 1.8 Hz, H-6‴), 6.79 (1H, d, J = 8.4 Hz, H-5‴), and 7.56, 6.39 (each 1H, d, J = 15.6 Hz, H-7‴, H-8‴) revealed the presence of an *E*-caffeoyl (feruloyl) moiety. The protons at δ 6.73 (1H, d, J = 1.8 Hz, H-2), 6.69 (1H, d, J = 7.8 Hz, H-5), 6.61 (1H, dd, J = 7.8, 1.8 Hz, H-6) and 4.56 (1H, dd, J = 10.8, 2.4 Hz, H-7), 3.94 (1H, dd, J = 11.4, 2.4 Hz, H-8a), 3.50 (1H, dd, J = 11.4, 10.8 Hz, H-8b) were assigned to a 3,4-dihydroxyphenylethanol moiety. The above assignments were confirmed by the ^{13}C NMR spectrum. The above information revealed a close structural similarity to crenatoside (**5**),[2] with the exception of an extra methoxy group at δ 3.80 (3H, s). This methoxy group could be located at C-3‴ by a NOESY cross signal between OCH_3/H-2‴ and HMBC correlation between OCH_3 (δ 3.80) and C-3‴ (Figure S7, Supporting Information). Therefore, **4** incorporated a feruloyl moiety. The ^{1}H NMR signals at δ 4.54 (1H, d, J = 7.8 Hz) and 4.97 (1H, d, J = 1.8 Hz) were respectively assigned to the anomeric protons of β-glucopyranosyl and α-rhamnopyranosyl moieties. The linkage of the sugars and the *E*-feruloyl moiety was determined by the following HMBC correlations (Figure S7, Supporting Information): H-4′ (δ 4.90) and C-9‴ (δ 166.1), H-1″ (δ 4.97), and C-3′ (δ 74.9). The relative configuration of C-7 was determined by the NOESY correlations (Figure S7, Supporting Information) of H-1′ with H-8b and of H-7 with H-2′ and H-8a. In view of the similar optical rotation of **4** and crenatoside (**5**),[12] compound **4** was thus assigned as 1,2-*O*-[2*S*-(3,4-dihydroxyphenyl)-1,2-

Table 1. ACE Inhibitory Activity of Compounds **4**, **5**, and **6** from *Microtoena prainiana*

sample	inhibition (%)		
	1.0 mg/mL	0.1 mg/mL	0.01 mg/mL
4	99.8	67.5	32.5
5	99.7	75.5	34.6
6	99.3	71.4	35.2
captopril (positive control)			97.7

ethanediyl]-3-*O*-α-L-rhamnopyranosyl-4-*O*-feruloyl-β-D-glucopyranoside (3‴-*O*-methylcrenatoside).

At a concentration of 0.01 mg/mL, compounds **4**−**6** inhibited the ACE activity by 32.5%, 34.6%, and 35.2%, respectively (Table 1). Some cytotoxic and anti-HIV cyclopeptides have been reported;[17−19] thus, cyclopeptides **1**−**3** were screened in vitro for cytotoxicity with human breast cancer (Bre04), human lung cancer (Lu04), and human neuroma (N04) cell lines, and they exhibited no activity (GI_{50} > 100 μg/mL). The antiviral test of compounds **1**−**6** was measured on Vero cell lines infected with Herpes simplex virus type 2 (HSV-2, strain 333); they were inactive at 250 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were determined on an XRC-1 melting point apparatus (Scientific Instruments Factory, Sichuan University). Optical rotations were measured on a Perkin-Elmer model 341 polarimeter. UV spectra and IR spectra were carried out on a Perkin-Elmer Lambda 35 UV/vis spectrometer and a Perkin-Elmer Spectrum One FT-IR spectrometer. ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, TOCSY, HSQC, HMBC, and NOESY spectra were performed on a Bruker Avance 600 spectrometer. Chemical shift values are in ppm (δ) with TMS as internal standard. Electrospray ionization mass spectra (ESIMS) were acquired with a Finnigan LCQ^{DECA} mass spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained on an API Q-STAR PULSAR *i* mass spectrometer. High-performance liquid chromatography (HPLC) was performed using a Perkin-Elmer series 200 pump equipped with a Perkin-Elmer series 200 UV/vis detector. Amino acid analysis was carried out on a Hitachi L-8800 amino acid analyzer. N_α-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide (FDAA, Marfey's reagent), hippuric acid-histidine-leucine (HHL), and *O*-phthaldialdehyde were purchased from Sigma-Aldrich, Inc., Germany. Silica gel (200−300 mesh) was obtained from Qingdao Ocean Chemical Company, China. MCI was purchased from Mitsubishi Company, Japan. Polyamide was acquired from Shanghai Chemical Reagent Factory, China. C-18 silica gel (RP-18 silica 60, 40−63 μm) was obtained from Merck & Co., Inc., Germany.

Plant Material. The stems of *M. prainiana* were collected from Tianquan County, Sichuan Province, China, in October 2002 and identified by Professor Fa-Ding Fu at Chengdu Institute of Biology, the Chinese Academy of Sciences (CAS). A voucher specimen (no. TQ-6) is deposited in the Herbarium of Chengdu Institute of Biology, CAS.

Extraction and Isolation. The dried and powdered stems of *M. prainiana* (22 kg) were soaked with 95% EtOH (3 × 160 L) at room temperature. After concentrating in vacuo, 1500 g of residue was obtained. The residue was suspended in H_2O (5 L) and then triturated successively with petroleum ether (60−90 °C) (5 × 5 L), $CHCl_3$ (5 × 3 L), EtOAc (5 × 5 L), and n-BuOH (5 × 5 L) to afford corresponding fractions. Each fraction was examined for ACE inhibitory activity, and the EtOAc fraction showed the strongest activity.

The EtOAc fraction (302 g) was chromatographed over a silica gel column (2500 g, $CHCl_3$−MeOH−H_2O, 100:1:0.02, 50:1:0.02, 20:1:0.02, 10:1:0.02, 5:1:0.02, 0:1:0.02, each 5000 mL, Ø × *l* = 12 × 55 cm) to afford four subfractions. 3‴-*O*-Methylcrenatoside (**4**) (304 mg, 0.0014% dry weight) and compound **6** (30 mg, 0.00014%) were isolated from fraction 1 (30 g) using a silica gel column (350 g, $CHCl_3$−MeOH, 10:1, 3000 mL, 7.5 × 14 cm) and were each purified by recrystallization from acetone. Fraction 2 (30 g) was chromatographed over silica gel (200 g, EtOAc−MeOH, 20:1, 2000 mL, 6.5 × 15 cm) to afford **5** (800 mg, 0.0036%). Apigenin 7-(6″-*p*-coumaroyl)glucoside (5 mg, 0.00002%) was obtained from fraction 3 (300 mg) using a silica gel column (15 g, $CHCl_3$−MeOH−H_2O, 15:1:0.02, 350 mL, 2.5 × 20 cm). Fraction 4 (3 g) was chromatographed over a polyamide column (70 g, acetone−H_2O, 1:5, 1000 mL, 5 × 10 cm) to afford hesperidin (3 mg, 0.000014%).

The $CHCl_3$ fraction (100 g) was chromatographed over MCI gel (500 g) using MeOH−H_2O (7:3, 8:2, 9:1) to afford fraction 5. Fraction 5 (38 g) was chromatographed over silica gel, resulting in nine subfractions (fractions 6−14). Indole-3-aldehyde (20 mg, 0.00009%) and scopoletin (15 mg, 0.00007%) were obtained by recrystallization of fraction 6 (100 mg) and fraction 7 (80 mg) from acetone, respectively. Ethyl caffeate (30 mg, 0.00014%) was isolated from fraction 8 (400 mg). Fraction 9 (240 mg) was separated over a silica gel column, yielding 2-anilino-1,4-naphthoquinone (3 mg, 0.00001%) and (4*E*)-2-anilino-4-(phenylimino)naphthalene-1(4*H*)-one (2 mg, 0.000009%). The separation of fraction 10 (150 mg) using a C_{18} silica gel column (30 g, MeOH−H_2O, 1:1) afforded apigenin (38 mg, 0.00017%) and luteolin (15 mg, 0.00007%). Fraction 11 (2 g) was chromatographed over silica gel to give betulinic acid (27 mg, 0.00012%). Microtoenin A (**1**) (58 mg, 0.00026%) and microtoenin B (**2**) (30 mg, 0.00014%) were isolated from fraction 12 (2.4 g) using a silica gel column [120 g, petroleum ether (60−90 °C)−EtOAc−MeOH, 5:1:0.2, 2000 mL, 5.6 × 10 cm] and were further purified by recrystallization from acetone. Fraction 13 (35 mg) was chromatographed over silica gel to afford pachypodol (8 mg, 0.00004%) and kumatakenin (3 mg, 0.00001%). Microtoenin C (**3**) (58 mg, 0.00026%) was isolated from fraction 14 (2 g) over a silica gel column [60 g, petroleum ether (60−90 °C)−acetone, 1:1, 2000 mL, 5 × 6.5 cm] and was further purified by recrystallization from acetone.

Microtoenin A (1): white amorphous powder; mp 280−282 °C; $[\alpha]_D^{20}$ −104.8° (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.31) nm; IR (KBr) ν_{max} 3435, 2927, 1635, 1517, 1454 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz) δ 5.34 (1 H, m, H-2), 1.49 (3H, d, *J* = 6.0 Hz, H-3), 8.94 (1H, d, *J* = 8.4 Hz, 2-NH), 4.69 (1H, dd, *J* = 8.4, 6.0 Hz, H-5), 2.51 (1H, m, H-6), 1.01 (9H, d, *J* = 6.6 Hz, H-7, H-8, H-33), 7.58 (1H, d, *J* = 8.4 Hz, 5-NH), 4.30 (1H, d, *J* = 7.8 Hz, H-10), 2.26 (1H, m, H-11a), 1.04 (1H, overlapped, H-11b), 1.50 (4H, m, H-12, H-37), 3.72 (1H, m, H-13a), 3.55 (2H, m, H-13b, H-38b), 4.95 (1H, overlapped, H-15), 3.20 (2H, m, H-16a, H-41a), 2.81 (1H, m, H-16b), 7.00 (2H, d, *J* = 8.4 Hz, H-18, H-22), 7.09 (2H, d, *J* = 8.4 Hz, H-19, H-21), 11.55 (1H, brs, 20-OH), 10.87 (1H, brs, 15-NH), 5.38 (1H, m, H-24), 2.00 (1H, m, H-25a), 1.79 (2H, m, H-25b, H-26), 0.85 (3H, d, *J* = 6.0 Hz, H-27), 0.91 (3H, d, *J* = 6.0 Hz, H-28), 8.62 (1H, d, *J* = 8.4 Hz, 24-NH), 4.74 (1H, t, *J* = 8.4 Hz, H-30), 2.45 (1H, m, H-31), 1.04 (3H, d, *J* = 6.6 Hz, H-32), 8.11 (1H, d, *J* = 9.0 Hz, 30-NH), 4.00 (1H, d, *J* = 7.8 Hz, H-35), 2.21 (1H, m, H-36a), 0.73 (1H, m, H-36b), 3.65 (1H, m, H-38a), 4.86 (1H, m, H-40), 2.95 (1H, m, H-41b), 7.04 (2H, d, *J* = 7.2 Hz, H-43, H-47), 7.25 (2H, dd, *J* = 7.3, 7.2 Hz, H-44, H-46), 7.20 (1H, overlapped, H-45), 10.83 (1H, brs, 40-NH); ^{13}C NMR (C_5D_5N, 150 MHz) δ 174.0 (C, C-1), 48.7 (CH, C-2), 17.8 (CH_3, C-3), 171.2 (C, C-4), 61.3 (CH, C-5), 31.1 (CH, C-6), 19.8 (CH_3, C-7), 17.7 (CH_3, C-8), 171.7 (C, C-9), 62.1 (CH, C-10), 30.5 (CH_2, C-11), 21.9 (CH_2, C-12), 46.4 (CH_2, C-13), 172.2 (C, C-14), 53.8 (CH, C-15), 37.6 (CH_2, C-16), 126.2 (C, C-17), 130.9 (CH, C-18, C-22), 116.6 (CH, C-19, C-21), 158.2 (C, C-20), 173.5 (C, C-23), 51.5 (CH, C-24), 42.6 (CH_2, C-25), 25.2 (CH, C-26), 22.3 (CH_3, C-27), 23.0 (CH_3, C-28), 171.5 (C, C-29), 62.0 (CH, C-30), 31.3 (CH, C-31), 19.9 (CH_3, C-32), 18.5 (CH_3, C-33), 171.3 (C, C-34), 61.9 (CH, C-35), 30.5 (CH_2, C-36), 21.8 (CH_2, C-37), 46.3 (CH_2, C-38), 171.6 (C, C-39), 54.1 (CH, C-40), 38.3 (CH_2, C41), 136.1 (C, C-42), 129.7 (CH, C-43, C-47), 129.1 (CH, C-44, C-46), 127.6 (CH, C-45); ESIMS *m/z* 909 ([M + Na]$^+$, 100), 887 ([M + H]$^+$, 15), 885 ([M − H]$^-$, 100); ESIMS/MS on [M + H]$^+$ *m/z* 887 (8), 869 (10), 859 (45), 740 (15), 691 (100), 669 (14), 620 (18), 578 (13), 528

(11), 415 (11), 310 (3); ESIMS/MS/MS on $[M + H - Phe]^+$ *m/z* 740 (20), 712 (100), 472 (10), 359 (11), 267 (20), 218 (17), 196 (90), 147 (18); HRESIMS *m/z* 887.5036 ($[M + H]^+$, $C_{47}H_{67}N_8O_9$ requires 887.5031).

Microtoenin B (2): white amorphous powder; mp 288−290 °C; $[\alpha]_D^{20}$ −68.3° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (4.61) nm; IR (KBr) ν_{max} 3429, 2927, 1656, 1534, 1453 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz) δ 5.43 (1H, m, H-2), 3.23 (1H, m, H-3a), 3.38 (1H, m, H-3b), 7.45(3H, d, J = 7.2 Hz, H-5, H-9, 20-NH), 7.28 (4H, m, H-6, H-8, H-14, H-18), 7.15 (3H, m, H-7, H-15, H-17), 7.64 (1H, d, J = 7.2 Hz, 2-NH), 4.64 (1H, m, H-11), 3.20 (1H, m, H-12a), 3.32 (1H, m, H-12b), 7.37 (3H, m, H-16, H-34, H-36), 7.93 (1H, brs, 11-NH), 4.08 (1H, t, J = 9.6 Hz, H-20), 1.96 (1H, m, H-21), 0.88 (3H, d, J = 6.6 Hz, H-22), 0.78 (3H, d, J = 6.6 Hz, H-23), 3.78 (1H, d, J = 7.8 Hz, H-25), 2.04 (1H, q, J = 6.0 Hz, H-26a), 0.98 (1H, m, H-26b), 1.52 (1H, m, H-27a), 1.43 (1H, m, H-27b), 3.64 (1H, m, H-28a), 3.53 (1H, t, J = 10.2 Hz, H-28b), 4.68 (1H, m, H-30), 3.27 (1H, t, J = 12.6 Hz, H-31a), 3.16 (1H, m, H-31b), 7.30 (2H, d, J = 8.4 Hz, H-33, H-37), 7.25 (1H, m, H-35), 10.17 (1H, brs, 30-NH), 4.61 (1H, m, H-39a), 3.85 (1H, dd, J = 16.8, 5.4 Hz, H-39b), 8.57 (1H, brs, 39-NH), 5.02 (1H, m, H-41), 1.72 (3H, d, J = 7.2 Hz, H-42), 8.54 (1H, d, J = 8.4 Hz, 41-NH), 4.60 (1H, m, H-44), 1.49 (3H, d, J = 7.2 Hz, H-45), 9.03 (1H, d, J = 3.6 Hz, 44-NH); ^{13}C NMR (C_5D_5N, 150 MHz) δ 171.7 (C, C-1), 53.6 (CH, C-2), 39.2 (CH_2, C-3), 137.2 (C, C-4), 130.7 (CH, C-5, C-9), 128.3 (CH, C-6, C-8), 126.7 (CH, C-7), 171.3 (C, C-10), 58.4 (CH, C-11), 37.4 (CH_2, C-12), 136.7 (C, C-13), 129.7 (CH, C-14, C-18), 129.03 (CH, C-15, C-17), 127.3 (CH, C-16), 173.6 (C, C-19), 58.6 (CH, C-20), 27.0 (CH, C-21), 20.6 (CH_3, C-22), 18.7 (CH_3, C-23), 172.0 (C, C-24), 61.4 (CH, C-25), 30.9 (CH_2, C-26), 21.9 (CH_2, C-27), 46.5 (CH_2, C-28), 174.6 (C, C-29), 53.7 (CH, C-30), 38.2 (CH_2, C-31), 136.5 (C, C-32), 129.8 (CH, C-33, C-37), 129.09 (CH, C-34, C-36), 127.6 (CH, C-35), 169.4 (C, C-38), 44.0 (CH_2, C-39), 173.7 (C, C-40), 49.3 (CH, C-41), 18.0 (CH_3, C-42), 173.1 (C, C-43), 52.1 (CH, C-44), 17.0 (CH_3, C-45); ESIMS *m/z* 859 ($[M + Na]^+$, 100), 835 ($[M - H]^-$, 100); ESIMS/MS on $[M - H]^-$ *m/z* 835(3), 817 (20), 791 (7), 710 (5), 693 (15), 660 (3), 634 (17), 606 (11), 558 (100), 540 (2), 492 (6), 345 (5), 288 (2); ESIMS/MS/MS on $[M - 3H - Phe - Gly - Ala]^-$ *m/z* 558 (2), 540 (60), 513 (10), 470 (10), 430 (62), 362 (52), 345 (100), 327 (15), 302 (5), 274 (7), 215 (11), 200 (11), 195 (3); HRESIMS *m/z* 837.4295 ($[M + H]^+$, $C_{45}H_{57}N_8O_8$ requires 837.4299).

Microtoenin C (3): white amorphous powder; mp 256−258 °C; $[\alpha]_D^{20}$ −93.8° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.36) nm; IR (KBr) ν_{max} 3435, 2961, 1645, 1517, 1452 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz) δ 3.87 (1H, m, H-2), 1.74 (2H, m, H-3a, H-42a), 2.33 (2H, m, H-3b, H-36), 1.91 (2H, m, H-4a, H-48b), 1.84 (1H, m, H-4b), 3.41−3.52 (7H, m, H-5a, H-8b, H-17b, H-21a, H-32a, H-43), 3.79 (1H, t, J = 8.4 Hz, H-5b), 4.63 (1H, m, H-7), 4.05 (1H, t, J = 6.6 Hz, H-8a), 7.28 (2H, d, J = 7.8 Hz, H-10, H-14), 7.11−7.19 (5H, m, H-11, H-13, H-24, H-25, H-26), 11.43 (1H, s, 12-OH), 7.19 (1H, overlapped, 7-NH), 5.15 (1H, m, H-16), 3.90 (1H, m, H-17a), 8.57, 7.90 (each 1H, s, 18-NH_2), 10.10 (1H, s, 16-NH), 5.12 (1H, m, H-20), 3.59 (1H, d, J = 12.6 Hz, H-21b), 7.34 (2H, d, J = 7.2 Hz, H-23, H-27), 8.63 (1H, d, J = 8.4 Hz, 20-NH), 4.32 (1H, d, J = 7.8 Hz, H-29), 2.41 (1H, q, J = 6.0 Hz, H-30a), 1.64 (2H, m, H-30b, H-42b), 1.35 (1H, m, H-31a), 0.76 (1H, m, H-31b), 3.34 (1H, t, J = 10.2 Hz, H-32b), 5.10 (1H, m, H-34), 2.04 (1H, m, H-35a), 1.79 (1H, m, H-35b), 0.79 (3H, d, J = 6.0 Hz, H-37), 1.11 (3H, d, J = 6.0 Hz, H-38), 8.04 (1H, brs, 34-NH), 4.77 (1H, t, J = 7.8 Hz, H-40), 2.28 (1H, m, H-41a), 2.13 (1H, m, H-41b), 4.48 (1H, brs, H-45), 2.60 (1H, brs, H-46), 1.07 (3H, d, J = 6.0 Hz, H-47), 1.52 (1H, m, H-48a), 0.84 (3H, t, J = 7.2 Hz, H-49), 8.91 (1H, brs, 45-NH); ^{13}C NMR (C_5D_5N, 150 MHz) δ 174.3 (C, C-1), 61.0 (CH, C-2), 29.5 (CH_2, C-3), 27.2 (CH_2, C-4), 49.1 (CH_2, C-5), 175.1 (C, C-6), 63.1 (CH, C-7), 36.1 (CH_2, C-8), 139.6 (C, C-9), 132.5 (CH, C-10, C-14), 117.3 (CH, C-11, C-13), 158.3 (C, C-12), 171.8 (C, C-15), 54.6 (CH, C-16), 38.1 (CH_2, C-17), 171.51 (C, C-18), 171.58 (C, C-19), 59.7 (CH, C-20), 39.9 (CH_2, C-21), 130.8 (C, C-22), 130.6 (CH, C-23, C-27), 129.8 (CH, C-24, C-26), 128.0 (CH, C-25), 171.54 (C, C-28), 62.4 (CH, C-29), 32.0 (CH_2, C-30), 22.8 (CH_2, C-31), 48.0 (CH_2, C-32), 171.65 (C, C-33), 51.0 (CH, C-34), 46.8 (CH_2, C-35), 26.3 (CH, C-36), 25.2 (CH_3, C-37), 22.5 (CH_3, C-38), 172.79 (C, C-39), 60.1 (CH, C-40), 28.5 (CH_2, C-41), 26.2 (CH_2, C-42), 48.6 (CH_2, C-43), 172.71 (C, C-44), 61.3 (CH, C-45), 35.3 (CH, C-46), 16.9 (CH_3, C-47), 26.2 (CH_2, C-48), 11.2 (CH_3, C-49); ESIMS *m/z* 964 ($[M + Na]^+$, 100), 940 ($[M - H]^-$, 100); ESIMS/MS on $[M - H]^-$ *m/z* 940 (2), 922 (32), 843 (20), 730 (7), 664 (100), 646 (40), 619 (5), 517 (25), 422 (32); ESIMS/MS/MS on $[M - Tyr - Asn]^-$ *m/z* 664 (2), 646 (100), 619 (15), 517 (20), 454 (5), 420 (3), 357 (3), 260 (3), 209 (5); HRESIMS *m/z* 964.4916 ($[M + Na]^+$, $C_{49}H_{67}N_9O_{10}Na$ requires 964.4908).

3‴-*O*-Methylcrenatoside (4): white amorphous powder; mp 224−226 °C; $[\alpha]_D^{20}$ − 53.2° (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ϵ) 328 (4.18), 290 (3.96), 231 (4.03), 219 (4.07), 205 (4.31) nm; IR (KBr) ν_{max} 3403, 2957, 1719, 1638, 1603, 1517, 1448, 1302, 1274, 1243, 1174, 1122, 1064, 1042, 1018 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 6.73 (1H, d, J = 1.8 Hz, H-2), 8.90 (1H, s, 3-OH), 8.89 (1H, s, 4-OH), 6.69 (1H, d, J = 7.8 Hz, H-5), 6.61 (1H, dd, J = 7.8, 1.8 Hz, H-6), 4.56 (1H, dd, J = 10.8, 2.4 Hz, H-7), 3.94 (1H, dd, J = 11.4, 2.4 Hz, H-8a), 3.50 (1H, dd, J = 11.4, 10.8 Hz, H-8b), 4.54 (1H, d, J = 7.8 Hz, H-1′), 3.38 (1H, m, H-2′), 4.03 (1H, t, J = 9.6 Hz, H-3′), 4.90 (1H, t, J = 9.6 Hz, H-4′), 3.70 (1H, t, J = 7.8 Hz, H-5′), 3.43 (2H, m, H-6′a, H-5″), 3.40 (1H, m, H-6′b), 4.86 (1H, t, J = 6.0 Hz, 6′-OH), 4.97 (1H, d, J = 1.8 Hz, H-1″), 3.53 (1H, brs, H-2″), 3.24 (1H, m, H-3″), 3.09 (1H, t, J = 9.6 Hz, H-4″), 1.02 (3H, d, J = 6.0 Hz, H-6″), 4.53 (2H, overlapped, 2″-OH, 4″-OH), 4.45 (1H, d, J = 5.4 Hz, 3″-OH), 7.27 (1H, d, J = 1.8 Hz, H-2‴), 9.61 (1H, s, 4‴-OH), 6.79 (1H, d, J = 8.4 Hz, H-5‴), 7.09 (1H, dd, J = 8.4, 1.8 Hz, H-6‴), 7.56 (1H, d, J = 15.6 Hz, H-7‴), 6.39 (1H, d, J = 15.6 Hz, H-8‴), 3.80 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 128.6 (C, C-1), 114.2 (CH, C-2), 145.8 (C, C-3), 145.7 (C, C-4), 115.9 (CH, C-5), 117.8 (CH, C-6), 76.7 (CH, C-7), 71.6 (CH_2, C-8), 97.5 (CH, C-1′), 81.0 (CH, C-2′), 74.9 (CH, C-3′), 69.3 (CH, C-4′), 76.8 (CH, C-5′), 61.0 (CH_2, C-6′), 100.9 (CH, C-1″), 71.0 (CH, C-2″), 70.9 (C-3″), 72.0 (CH, C-4″), 69.4 (CH, C-5″), 18.5 (CH_3, C-6″), 126.2 (C, C-1‴), 111.8 (CH, C-2‴), 148.5 (C, C-3‴), 150.1 (C, C-4‴), 116.1 (CH, C-5‴), 123.8 (CH, C-6‴), 146.5 (CH, C-7‴), 114.5 (CH, C-8‴), 166.1 (C, C-9‴), 56.3 (CH_3, OCH_3); ESIMS *m/z* 659 ($[M + Na]^+$, 85), 675 ($[M + K]^+$, 10), 1295 ($[2M + Na]^+$, 100), 635 ($[M - H]^-$, 100), 671 ($[M + Cl^-]^-$, 20), 1271 ($[2M - H]^-$, 25); HRESIMS *m/z* 659.1949 ($[M + Na]^+$, $C_{30}H_{36}O_{15}Na$ requires 659.1951).

Amino Acid Analysis of 1−3. Microtoenins A (**1**) (5.4 mg), B (**2**) (5.7 mg), and C (**3**) (5.4 mg) were treated separately with 6 N HCl (10.8, 11.4, and 10.8 mL, respectively) at 110 °C for 30 h in a sealed tube, after which the reaction mixtures were concentrated in vacuo to dryness and redissolved in 0.02 N HCl (2.7, 2.9, and 2.7 mL, respectively) and then subjected to amino acid analysis.

Acid Hydrolysis of Cyclopeptides. 1−3 (each 0.5 mg) were separately treated with 6 N HCl (each 1 mL) at 110 °C for 30 h. The reaction mixture was concentrated in vacuo to dryness. These acid hydrolysates were then subjected to Marfey's derivatization.[16]

Marfey's Derivatization and Analysis. To each acid hydrolysate was added 50 μL of 1 M sodium bicarbonate and 1 mL of 0.1% FDAA in acetone. The solution was stirred at 40 °C for 60 min. Then the mixture was neutralized with 25 μL of 2 N HCl, and the derivatized sample was concentrated in vacuo to dryness and redissolved in H_2O (each 1 mL). As reference DL- and L-amino acids were derivatized in the same manner. HPLC analysis involved elution of 20 μL of the derivatized solution through a C_{18} column (Lichrospher 100, RP-18e, 5 μm, 4 × 250 mm, Merck KGaA. Germany) with a flow rate of 1 mL/min in 60 min linear gradient of CH_3CN in H_2O (0%−60%) containing 0.05% TFA and detection at 340 nm.

Retention times (t_R, min) of Marfey's derivatives of authentic amino acids: 32.31 (L-Asp), 32.96 (D-Asp), 34.34 (Gly), 36.14 (L-Ala), 37.69 (D-Ala), 36.78 (L-Pro), 37.80 (D-Pro), 39.33 (L-Tyr), 40.35 (D-Tyr), 41.84 (L-Val), 45.23 (D-Val), 45.13 (L-Ile), 48.68 (D-Ile), 46.09 (L-Leu), 49.71 (D-Leu), 46.32 (L-Phe), 48.59 (D-Phe).

Retention times (t_R, min) of the Marfey's derivatives of the acid hydrolysate of microtoenin A (1): 36.50 (L-Ala), 37.08 (L-Pro), 39.77 (L-Tyr), 42.23 (L-Val), 46.48 (L-Leu), 46.73 (L-Phe).

Retention times (t_R, min) of the Marfey's derivatives of the acid hydrolysate of microtoenin B (2): 35.08 (Gly), 36.71 (L-Ala), 37.20 (L-Pro), 42.19 (L-Val), 46.52 (L-Phe).

Retention times (t_R, min) of the Marfey's derivatives of the acid hydrolysate of microtoenin C (3): 32.03 (L-Asp), 36.88 (L-Pro), 39.33 (L-Tyr), 45.30 (L-Ile), 46.03 (L-Leu), 46.36 (L-Phe).

Alkaline Treatment of Compound 4 followed by Acid Hydrolysis. Compound **4** (10 mg) in 2% NaOH solution was kept overnight under N_2 at room temperature. The reaction mixture was acidified with diluted HCl(aq) and extracted with Et_2O. The aqueous layer was extracted with n-BuOH. The n-BuOH layer was evaporated to dryness. The residue was dissolved in 1% H_2SO_4 solution and heated on a H_2O bath (95 °C) for 1 h and then cooled. The mixture was extracted with Et_2O. The aqueous layer was neutralized with $Ba(OH)_2$(aq), and the precipitate was filtered off. The filtrate was evaporated to dryness. Glucose and rhamnose were detected in the residue by TLC comparison with authentic samples.

Angiotensin I Converting Enzyme Assay. ACE activity was determined by the method of Carmel.[20] Briefly, 10 μL of ACE extract (containing 10 μg of total protein) was incubated with 10 μL of samples of various concentration for 30 min at 37 °C. Then 120 μL of HHL was added and incubated for 15 min at 37 °C. Afterward, 40 μL of 1 M NaOH and 10 μL of 20 g/L *O*-phthaldialdehyde were added, and the reaction was terminated after 10 min by the addition of 20 μL of 3 M HCl(aq). The fluorescence intensity of the product His-Leu was measured at 405 nm (excitation) and 535 nm (emission) with a fluorescence spectrophotometer (Wallac Victor2). For each assay, the blank control, negative control, and positive control were prepared. If the letters *N*, *S*, and *B* respectively represent the measured fluorescence intensity values of the reacting systems of negative control, detected sample, and blank control, then the ACE inhibitory rate (%) = 100% × ($N - S$)/($N - B$). At least three separate determinations were conducted for each sample. In this assay protocol, the positive control (captopril) completely inhibited ACE activity at the concentration of 40 nM with an IC_{50} of 20.3 nM. These data are in accordance with those reported,[21] indicating the reliability of this assay protocol.

In Vitro Cytotoxicity Assay. Cytotoxic assays were performed by the sulforhodamine B (SRB) method.[22] The cell lines used were human breast cancer (Bre04), human lung cancer (Lu04), and human neuroma (N04).

Antiherpetic Activity Assay. Inhibition of Herpes simplex virus type 2 (HSV-2) replication was evaluated in the Vero cell line using assays of cytopathic effect (CPE).[23] Acyclovir (ACV) was used as positive drug control.

Supporting Information Available: Figures S1−S7, selected 2D NMR, and mass fragmentation data for compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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