Cyclic Peptides, Acyclic Diterpene Glycosides and Other Compounds from *Lycium chinense* MILL.[1,2]

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The chemical structures of four cyclic peptides, lyciumins A—D (1—4), three acyclic diterpene glycosides, lyciumosides I—III (5—7) and other three compounds, a tryptophan derivative glycoside (8), a monoterpene glycoside (9) and a steroidal glycoside (10) isolated from *Lycium chinense*, have been elucidated by a combination of chemical, ^{1}H- and ^{13}C-NMR, and mass spectrometric studies. Lyciumins are interesting because of their monocyclic octapeptides containing a novel C–N linkage between tryprophan N_1 and glycine C_α.

Keywords Lycii Radicis Cortex; *Lycium chinense*; Solanaceae; lyciumin; cyclic peptide; acyclic diterpene glycoside

An oriental crude drug, Lycii Radicis Cortex, the root bark of *Lycium chinense* MILL. has been used as an antifebrile, a tonic and an antihypertensive agent.[3] With regard to the constituents of this crude drug and the fresh plant itself, the less polar of these have previously been extensively studied[4]; however, the more polar have not been sufficiently examined. As far as the water-soluble constituents in the above crude drug and the fresh plant are concerned, only betaine, vitamin C and rutin obtained from roots and leaves of *L. chinense* were known.[3] Our present study has concentrated on the water-soluble constituents of Lycii Radicis Cortex, and the fresh roots, stems and leaves of *L. chinense*. We have isolated four new cyclic peptides, lyciumins A—D (**1—4**),[2] and three acyclic diterpene glycosides, lyciumosides I—III (**5—7**), together with three other compounds, a tryptophan derivative glycoside (**8**), a monoterpene glycoside (**9**) and a furostanol glycoside (**10**) from various parts of the plants as listed in Table I. This paper deals with their structure characterization.

Cyclic Peptides Lyciumin A (**1**), obtained as a white amorphous powder ($[\alpha]_D$ +10.1°), gave a negative reaction to ninhydrin reagent and a pseudo-molecular ion peak at *m/z* 872 $[M-H]^-$ in the negative fast atom bombardment mass spectrometer (FAB-MS). Its ultraviolet (UV) spectrum showed absorption maxima at 273, 281 and 291 nm suggesting the presence of an indole skeleton. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table VI) of **1** displayed the signals due to nine carbonyl and fourteen aromatic carbons, which were made up of nine tertiary and five quaternary carbons. From the above evidence, **1** was assumed to be a peptide. On acid hydrolysis of **1**, amino acid components were determined as L-glutamic acid (Glu), L-serine (Ser), glycine (Gly), proline (Pro), L-valine (Val) and L-tyrosine (Tyr) (1 : 1 : 1 : 1 : 1 : 1) according to amino acid analysis and the method described by Mimura *et al.*[5] Compound **1** underwent the dinitrophenyl (DNP) reaction to yield a single DNP derivative, while, on benzylation, **1** gave two-moles of benzyl derivative. The above evidence suggested the presence of Tyr and Ser in **1**. Partial acid hydrolysis of **1** yielded four degradation products and the subsequent acid hydrolysis of these led to the identification of amino acids and determination of amino acid sequences as listed in Table II.

α-Chymotrypsin hydrolysis of **1** provided two degradation products, and acid hydrolysis of each of these afforded amino acids, as listed in Table III. Two decomposition products were analyzed by amino acid sequencer and found to be X_1–Val–Gly–Ser–X_2 and pyroGlu–Pro–Tyr.

TABLE I. Distribution of Lyciumins A—D (**1—4**), Lyciumosides I—III (**5—7**) and **8** in Crude Drug and Various Parts of Fresh Plants

		1	2	3	4	5	6	7	8
Crude drug	Lycii Radicis Cortex	○	○	○	○				○
Fresh plant	Root-bark	○	○	○	○				
	Stems	○	○			○			
	Leaves					○	○	○	

TABLE II. Products of Partially Acid Hydrolysis of **1**

Products-1	Pro–Tyr
2	pyroGlu–Pro–Tyr
3	Pro–Tyr–X_1–Val–Gly–Ser–X_2
4	Tyr–X_1–Val–Gly–Ser–X_2

X_1, X_2 = unidentified compounds.

TABLE III. Products of α-Chymotrypsin Hydrolysis of **1**, **2**, **3** and **4**

1	X_1–Val–Gly–Ser–X_2,	pyroGlu–Pro–Tyr
2	X_1–Val–Gly–Ser–X_2,	pyroGlu–Pro–Trp
3	X_1–Val–Phe–Ser–X_2,	pyroGlu–Pro–Tyr
4	X_1–Val–Gly–Ile–X_2,	pyroGlu–Pro–Tyr

X_1, X_2 = unidentified compounds.

TABLE IV. Products of Proline-Specific Endopeptidase Hydrolysis of **1**, **2**, **3** and **4**

1	pyroGlu–Pro,	Tyr–X_1–Val–Gly–Ser–X_2
2	pyroGlu–Pro,	X_1–Val–Gly–Ser–X_2
3	pyroGlu–Pro,	Tyr–X_1–Val–Phe–Ser–X_2
4	pyroGlu–Pro,	Tyr–X_1–Val–Gly–Ile–X_2

X_1, X_2 = unidentified compounds.

Next, proline-specific endopeptidase hydrolysis of **1** gave two products, and acid hydrolysis of these products provided amino acids as listed in Table IV. Thus, the proline has the L configuration. These degradation products were analyzed by amino acid sequencer to show the presence of Tyr–X_1–Val–Gly–Ser–X_2 and pyroGlu–Pro.

From the above evidence, the terminal Glu should be of the pyro-glutamine type, and X_1 and X_2 were identified as

TABLE V. ^{1}H-NMR Data for **1**, **2**, **3** and **4** (DMSO-d_6)

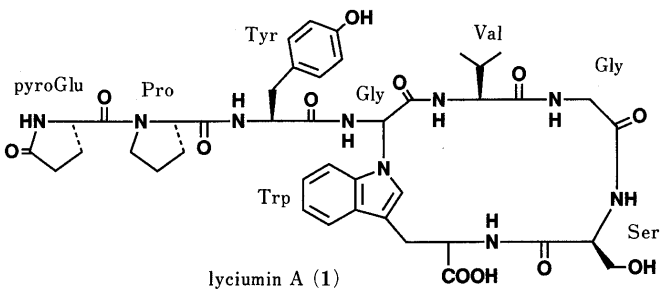
	NH	α	β	γ	δ	Ar
Lyciumin A (**1**)						
pyroGlu		4.35 (m)	2.29 (m) 1.90 (m)	2.10 (2H, m)		
Pro		4.35 (m)	2.16 (m) 1.75 (m)	1.82 (m) 1.68 (m)	3.60 (m) 3.35 (m)	
Tyr	7.97 (br d, 7)	4.33 (t, 7)	2.63 (2H, d, 7)			7.71 (s, OH), 6.63 (2H, d, 8, H-2, 6), 6.38 (2H, d, 8, H-3, 5)
Gly^4	9.37 (d, 8)	6.67 (d, 8)				
Val	7.92 (br d, 7)	3.99 (t, 7)	2.03 (m)	0.87 (3H, d, 7) 0.82 (3H, d, 7)		
Gly^6	8.68 (t, 6)	4.08 (dd, 6, 15) 3.23 (dd, 6, 15)				
Ser	7.72 (br d, 7)	4.11 (dd, 7, 11)	3.59 (m) 3.49 (m)			
Trp	7.83 (d, 7)	4.40 (t, 7)	3.30 (m) 3.01 (m)			7.54 (d, 8, H-4), 7.38 (d, 8, H-7), 7.14 (t, 8, H-6), 7.04 (t, 8, H-5), 6.91 (s, H-2)
Lyciumin B (**2**)						
pyroGlu		4.36 (m)	2.21 (m) 1.83 (m)	2.08 (2H, m)		
Pro		4.36 (m)	2.10 (m) 1.95 (m)	1.72 (2H, m)	3.64 (dd, 5, 11) 3.11 (dd, 9, 11)	
Trp^3	7.86 (d, 6) 10.66 (s)	4.32 (m)	3.26 (m) 2.93 (br s)			7.56 (d, 8, H-4), 7.28 (d, 8, H-7), 7.08 (t, 8, H-6), 7.01 (t, 8, H-5), 6.87 (s, H-2)
Gly^4	9.35 (d, 8)	6.67 (d, 8)				
Val	7.89 (br d, 7)	4.01 (t, 7)	2.08 (m)	0.87 (3H, d, 7) 0.82 (3H, d, 7)		
Gly^6	8.55 (br s)	4.08 (dd, 6, 15) 3.25 (m)				
Ser	7.69 (br d, 7)	4.18 (dd, 6, 12)	3.55 (m) 3.30 (m)			
Trp	7.69 (br d, 7)	4.52 (m)	3.26 (m) 2.93 (br s)			7.55 (s, H-2), 7.37 (d, 8, H-4), 7.26 (d, 8, H-7), 7.00 (t, 8, H-6), 6.86 (t, 8, H-5)
Lyciumin C (**3**)						
pyroGlu		4.36 (m)	2.24 (m) 1.79 (m)	2.09 (2H, m)		
Pro		4.36 (m)	1.96 (m) 1.79 (m)	1.79 (2H, m)	3.73 (t, 7) 3.41 (m)	
Tyr	7.99 (d, 6)	4.36 (m)	2.74 (m)			7.54 (s, OH), 6.68 (2H, d, 8, H-2, 6), 6.39 (2H, d, 8, H-3, 5)
Gly^4	9.15 (d, 8)	6.63 (d, 8)				
Val	7.90 (m)	4.31 (m)	1.86 (m)	0.78 (3H, d, 7) 0.63 (3H, d, 7)		
Phe	8.30 (d, 7)	4.36 (m)	3.06 (m) 3.01 (m)			7.22 (5H, m)
Ser	7.85 (d, 7)	4.16 (m)	3.65 (2H, m)			
Trp	7.75 (d, 7)	4.42 (m)	3.09 (m) 3.01 (m)			7.51 (d, 8, H-4), 7.35 (d, 8, H-7), 7.17 (t, 8, H-6), 7.11 (t, 8, H-5), 7.09 (s, H-2)
Lyciumin D (**4**)						
pyroGlu		4.36 (m)	2.31 (m) 1.85 (m)	2.10 (2H, m)		
Pro		4.36 (m)	1.97 (m) 1.80 (m)	1.83 (2H, m)	3.58 (m) 3.41 (m)	
Tyr	7.85 (d, 5)	4.33 (m)	2.64 (2H, m)			7.53 (s, OH), 6.52 (2H, d, 8, H-2, 6), 6.35 (2H, d, 8, H-3, 5)
Gly^4	9.15 (d, 9)	6.67 (d, 9)				
Val	7.70 (d, 8)	4.04 (m)	2.10 (m)	0.86 (3H, d, 6) 0.84 (3H, d, 6)		
Gly^6	8.30 (t, 5)	4.01 (m) 3.29 (m)				
Ile	7.47 (d, 7)	4.07 (m)	1.77 (m)	1.49 (m) 1.14 (m) 0.83 (3H, d, 8)	0.82 (3H, t, 8)	
Trp	7.56 (d, 6)	4.38 (m)	3.20 (m) 3.00 (m)			7.54 (d, 8, H-4), 7.39 (d, 8, H-7), 7.11 (t, 8, H-6), 7.01 (t, 8, H-5), 6.84 (s, H-2)

tryptophan (Trp) analog. Consequently, an amino acid disposition of **1** was shown to be pyroGlu–Pro–Tyr–X_1–Val–Gly–Ser–X_2. Next, the proton signals from the proton (^{1}H)-NMR spectrum in dimethylsulfoxide-d_6 (DMSO-d_6) of each of the amino acids from **1** were assigned by ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{1}H homonuclear Hartmann–Hahn spectroscopy (HOHAHA) and ^{1}H–^{13}C COSY spectra as listed in the Table V.

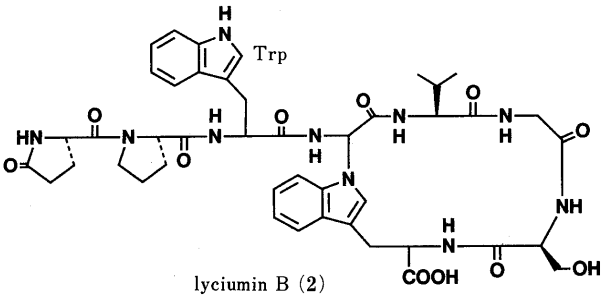
However, signals at δ6.67 (d, J=8 Hz) and 9.37 (d, J=8 Hz, NH) coupled to each other could not be interpreted. Moreover, the indole NH could not be found. Subsequently, rotating frame nuclear Overhauser effect spectroscopy (ROESY) and differential nuclear Overhauser effect (NOE) spectra revealed the sequence of amino acids in **1**, *e.g.* NOE was observed between an amide proton (NH) and a proton attached to the α-carbon of the neighboring amino acid. Particularly, it should be noted that a proton signal at δ9.37 possessed NOEs with those of H-2 (δ6.91) of the Trp indole ring, the NH (δ7.92) of Val and the proton (δ4.33) of C_α of Tyr. Moreover, an unidentified proton signal at δ6.67 coupled with the above mentioned NH at δ9.37, also had NOEs with those of the NH of Val and the H-2 and H-7 (δ7.38) of Trp. Therefore, it was suggested that **1** contained an internal bonding between the indole N_1 of the Trp (X_2) and the CH (δ6.67) of the other amino acid component (X_1). In particular, an unidentified –N–CH– sequence was revealed, belonging to part of an additional Gly. Thus all proton signals could be unambiguously assigned and the molecular formula derived from an $[M-H]^-$ peak matched to this structure. The proline residue within the native sequence allows the possibility of *cis*/*trans* isomerization about the amide linkage resulting in two configuration isomers. Consequently, the structure of **1** could be represented as (glycyl^4-C_α, tryptophan^8-indole N_1)-cyclo-L-pyroglutaminyl-L-prolyl-L-tyrosyl-glycyl-L-valyl-

TABLE VI. ^{13}C-NMR Data for **1**, **2**, **3** and **4** (DMSO-d_6)

	α	β	γ	δ	Ar								
					1	2	3	4	5	6	7	8	9
Lyciumin A (**1**)													
pyroGlu	54.6	23.9	27.3										
Pro	59.4	29.0	24.2	46.1									
	(58.5)	(31.7)	(21.6)	(46.8)									
Tyr	53.8	36.3			126.5	129.8	114.9	155.6	114.9	129.8			
Gly^4	61.4												
Val	56.3	29.0	19.2										
			18.5										
Gly^6	43.5												
Ser	55.4	62.0											
Trp	53.8	28.3				123.8	113.2	119.1	121.3	119.1	109.4	135.9	128.4
CO and COOH	166.5, 169.2×2, 170.6, 171.3×2, 171.5, 175.7 (176.9), 177.3												
Lyciumin B (**2**)													
pyroGlu	55.0	24.8	27.8										
Pro	61.4	30.1	25.5	47.7									
	(61.3)	(32.9)	(22.8)	(48.2)									
Trp	55.5	28.1				124.3	109.4	120.2	122.5	119.0	110.3	136.9	127.8
	55.5	28.1				124.5	114.1	120.8	123.2	119.8	112.6	137.1	129.2
Gly^4	62.1												
Val	60.6	29.8	20.0×2										
Gly^6	44.1												
Ser	56.5	62.3											
CO and COOH	168.3, 170.6, 171.2, 172.3, 173.6, 173.8, 173.9, 177.0, 180.9												
Lyciumin C (**3**)													
pyroGlu	59.2	26.2	29.1										
Pro	63.1	31.2	26.8										
Tyr	57.0	37.9			128.1	131.0	117.1	157.5	117.1	131.0			
Gly^4	64.7												
Val	62.2	31.0	20.2	20.7									
Phe	57.0	38.7			139.2	131.8	130.3	130.5	130.3	131.8			
Ser	58.5	62.2											
Trp	58.0	31.0				125.4	115.6	121.0	123.9	121.6	110.9	138.8	128.1
CO and COOH	170.7, 172.2, 174.0×2, 174.7, 174.9, 175.1, 175.2, 182.6												
Lyciumin D (**4**)													
pyroGlu	55.2	23.8	29.0										
Pro	59.4	28.9	24.4	46.2									
	(58.5)	(32.0)	(21.8)	(46.8)									
Tyr	53.7	36.4			125.9	129.7	115.0	156.2	115.0	129.7			
Gly^4	60.8												
Val	59.0	29.0	18.4	19.2									
Gly^6	43.0												
Ile	56.9	36.6	24.6	11.0									
			15.0										
Trp	53.7	28.7				123.0	113.4	118.6	121.4	119.0	109.6	136.1	128.0
CO and COOH	166.2, 168.2, 169.9, 170.5, 170.7, 171.2, 171.4, 171.5, 177.3												



lyciumin A (1)



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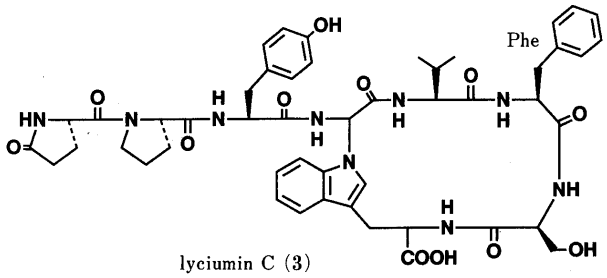
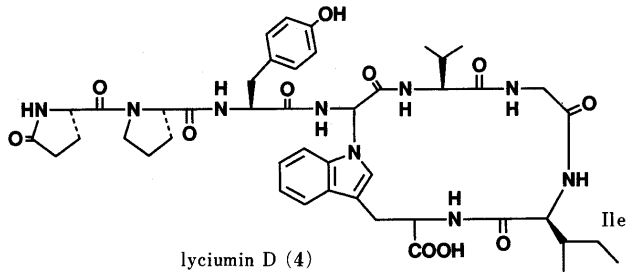


lyciumin C (3)



lyciumin D (4)

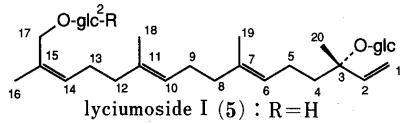
glycyl-L-seryl-tryptophan.

Lyciumins B (**2**), C (**3**) and D (**4**), showed $[M-H]^-$ ions at *m/z* 895, 962 and 898, respectively, in the negative FAB-MS. Acid hydrolysis of **2**, **3** and **4** indicated that each consisted of 1 mol of L-Glu, Gly, Pro, L-Ser and L-Val, of L-Glu, L-Phe, Pro, L-Ser, L-Tyr and L-Val, and of L-Glu, Gly, L-Ile, Pro, L-Tyr and L-Val, respectively. This evidence showed that Tyr^3 was substituted by Trp in **1** in **2**, Phe for Gly^6 in **1** in **3** and Ile for Ser^7 in **1** in **4**. α-Chymotrypsin hydrolysis and proline-specific endopeptidase hydrolysis of **2**, **3** and **4** gave a number of degradation products, and then acid hydrolysis of each of these products afforded amino acids, thus proline should possess an L-type configuration. Each of the degradative products was analyzed by amino acid sequencer to give the results listed in Tables III and IV. The ^1H- and ^{13}C-NMR of each peptide forming **2**, **3** and **4** could be assigned two dimensional (2D) COSY spectra as listed in Tables V and VI. The structures of **2**, **3** and **4** were found to be as shown in the formulae.

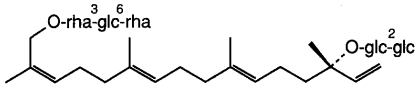
Acyclic Diterpene Glycosides Lyciumoside I (**5**), $[\alpha]_D$ −21.0°, showed a peak due to $[M-H]^-$ at *m/z* 629, together with a fragment ion at *m/z* 467 $[M-\text{hexose}-H]^-$, under negative FAB-MS. Acid hydrolysis of **5** gave glucose but no aglycone. The ^1H-NMR spectrum of **5** revealed the presence of four methyl groups [δ 1.38, 1.59×2 and 1.77 (each s)], a mono-substituted double bond [δ 5.21 (1H, d, $J=10$ Hz, H-1), 5.23 (1H, d, $J=18$ Hz, H-1) and 5.93 (1H, dd, $J=10$, 18 Hz, H-2)], three olefinic protons [δ 5.11 (2H, t, $J=7$ Hz, H-6, H-10) and 5.38 (1H, t, $J=7$ Hz, H-14)] adjacent to the methylene group, six methylene groups [δ 1.61 (2H, m, H_2-4), 1.97—2.16 (10H, m)], an oxygenated methylene group [δ 4.19, 4.30 (each 1H, d, $J=11$ Hz, H_2-17)], and two anomeric signals [δ 4.22, 4.35 (each 1H, d, $J=8$ Hz)]. This signal pattern was similar to that of capsianoside II (**11**) isolated from *Capsicum* spp.[6] A comparative study of the ^{13}C-NMR spectra of **5** with that of **11** revealed that both aglycone moiety signals were identical, thus the absolute configuration at C-3 was deduced to be 3*S*.[6] Moreover, it suggested the presence of two terminal β-glucopyranosyl residues, linked to C-3 and C-17 positions of the aglycone. The structure of **5** was determined as shown in the formula.

Lyciumoside II (**6**), $[\alpha]_D$ −19.6°, showed a peak due to $[M-H]^-$ at *m/z* 791, together with fragment ions at *m/z* 629 $[M-\text{hexose}-H]^-$ and 467 $[629-\text{hexose}-H]^-$ under negative FAB-MS, indicating that **6** has one more hexosyl moiety than **5**. Compound **6** on acid hydrolysis gave glucose, but no aglycone. The ^1H-NMR spectrum of **6** revealed the presence of a similar aglycone pattern to that of **5** and three anomeric signals [δ 4.34, 4.36 and 4.63 (each 1H, d, $J=8$ Hz)]. In comparing the ^{13}C-NMR spectrum of **6** with that of **5**, the signal attributable to the C-2 of the C-17-*O*-glucosyl moiety was shifted to δ 82.0. Consequently, lyciumoside II was assigned the structure shown in the formula.

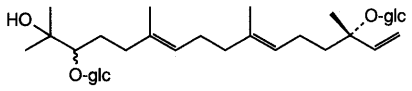
Lyciumoside III (**7**), $[\alpha]_D$ −31.1°, exhibited peaks due to $[M-H]^-$ and $[M-\text{hexose}-H]^-$ at *m/z* 647 and 485 under negative FAB-MS. The ^1H-NMR spectrum showed two anomeric proton signals at δ 4.34 (1H, d, $J=8$ Hz) and 4.43 (1H, d, $J=7$ Hz) whose pattern was analogous to that of **5**. However, **7** showed signals due to a hydroxy methine signal at δ 3.37 (1H, m), two olefinic protons at δ 5.12, 5.20 (each 1H, t, $J=7$ Hz) and five methyl groups, unlike those of **5**. The ^{13}C-NMR spectral data, except for the signals due to two oxygenated carbons at δ 90.5 (d) and 75.0 (s), two methyl groups at δ 24.1 and 26.6 and an anomeric carbon of a β-glucopyranosyl moiety at δ 106.6, were superimposable on those of **5**, suggesting **7** to be an acyclic diterpene glycoside. In the ^1H–^1H COSY spectrum of **7**, correlations in a sequence of H_2-12 [δ 2.26 (m)], H_2-13 [δ 1.41 (m), 1.52 (m)] and H-14 [δ 3.37 (m)] were observed. The ^1H–^{13}C long-range COSY spectrum (10 Hz) of **7** exhibited cross peaks between H_3-18 [δ 1.59 (s)] and C-12 (δ 37.0), between H-14 and glucose C-1 (δ 106.6), and between H_3-16 [δ 1.17 (s)] and C-14 (δ 90.5). Therefore, lyciumoside III was assigned the structure shown in the formula of **7**.



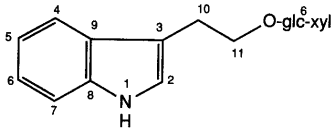
lyciumoside I (5) : R=H
lyciumoside II(6) : R=glc



capsianoside II(11)



lyciumoside III(7)



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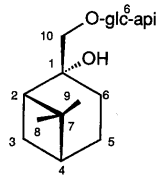

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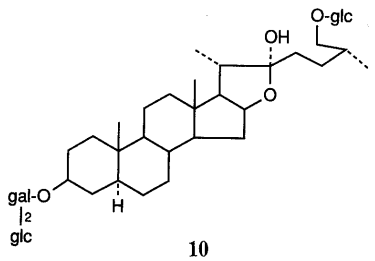

10

Other Compounds Compound **8**, $[\alpha]_D$ −32.7°, showed a peak due to $[M-H]^-$ at *m/z* 454 and fragment peaks at *m/z* 322 [M−pentose−H]$^-$ and 159 [322−hexose−H]$^-$ under negative FAB-MS. Compound **8**, on acid hydrolysis, liberated glucose and xylose. The ^{1}H-NMR spectrum of **8** displayed evidence of five aromatic signals [δ 7.55 (1H, d, J=8 Hz, H-4), 7.32 (1H, d, J=8 Hz, H-7), 7.13 (1H, s, H-2), 7.07 (1H, t, J=8 Hz, H-6) and 7.00 (1H, t, J=8 Hz, H-5)], and an imino signal [δ 10.60 (1H, s)], which could be assigned to the tryptophan indol moiety, and two anomeric proton signals [δ 4.34 (1H, d, J=7 Hz) and 4.32 (1H, d, J=8 Hz)], indicating that **8** is a tryptophan glycoside analog. The ^{13}C-NMR spectrum of **8** showed a β-xylopyranosyl-(1→6)-β-glucopyranosyl signal. Moreover, aromatic signals attributable to tryptophan C-2—C-9, together with an oxygenated methylene signal [δ 71.5] and a methylene signal [δ 26.8] were also observed. The structure of **8** was as shown in the formula.

Compound **9**, $[\alpha]_D$ −49.4°, showed a peak due to $[M-H]^-$ at *m/z* 463 and fragment peaks at *m/z* 331 [M−pentose−H]$^-$ and 169 [331−hexose]$^-$ under negative FAB-MS. Acid hydrolysis of **9** liberated glucose and apiose. The ^{1}H-NMR spectrum of **9** exhibited signals arising from two anomeric protons at δ 4.23 (1H, d, J=8 Hz) and 5.02 (1H, d, J=2 Hz), two methyl groups at δ 0.93 (s) and 1.01 (s) and a hydroxymethyl group at δ 3.46 (1H, d, J=10 Hz) and 4.13 (1H, d, J=10 Hz). The ^{13}C-NMR spectrum of **9** exhibited signals due to twenty-one carbons, including signals arising from a terminal β-apiofuranosyl moiety and a C_6-*O*-substituted β-glucopyranosyl moiety, a quaternary oxygenated carbon (δ 82.4), an oxygenated methylene group (δ 73.6), three methylene groups (δ 24.3, 24.7 and 35.6), two methyl groups (δ 22.2 and 26.1), two methine carbons (δ 49.1 and 51.4) and one quaternary carbon (δ 45.0), indicating that the aglycone moiety of **9** was a monoterpene derivative. The ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra suggested it to be a pinane derivative because of the presence of a –CH–CH_2–CH–CH_2–CH_2– residue. In the ^{1}H–^{13}C long-range COSY spectrum (10 Hz) of **9**, signal correlations were observed between an anomeric proton of apiosyl (δ 5.02) and a glucosyl C-6 carbon signal (δ 68.8), between the signal of H-2 (δ 1.70) and a C-1 carbon signal (δ 82.4), between signals of H_3-8 and H_3-9 [δ 0.93 (3H, s) and 1.01 (3H, s)] and a C-7 carbon signal (δ 45.0), indicating that the aglycone of **9** was 1,10-dihydroxypinane. The relative configuration of the aglycone was determined as that shown in the formula based on the fact that irradiation of the signal of the H_3-9 (δ 0.93) caused enhancement of the signal due to H_2-10 (δ 4.13 and 3.46), H-6 (δ 1.62) and H-2 (δ 1.70), and irradiation of the signal of H_3-8 (δ 1.01) caused enhancement of the H-2 and H-3β [δ 2.09 (br d, J=10 Hz)] in the NOE experiments in **9**. Consequently, the structure of **9** was determined as shown.

Compound **10** was deduced to be a furostanol glycoside on the basis of the positive color developed with Ehrlich's reagent.[7] Enzymatic hydrolysis of **10** with β-glucosidase gave glucose and a glycoside (**10a**). On acid hydrolysis, **10a** gave tigogenin, as the aglycone, galactose and glucose. The ^{13}C-NMR spectrum of **10a** indicated the presence of a terminal β-glucopyranosyl moiety and a C_2-*O* substituted β-galactopyranosyl moiety. The structure of **10a** was found to be tigogenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside. Therefore, **10** was assigned the structure, proto-**10a**, shown in the formula.

Cyclic peptides, lyciumins A (**1**) and B (**2**) were shown to have inhibitory activity on angiotensin-converting enzyme. These peptides were also found in the root of *L. barbarm* L. and have been detected by HPLC.

Acyclic diterpene glycosides, lyciumoside I—III (**5**—**7**) occur rarely in nature.

Experimental

The optical rotations were measured with a JASCO DIP 360 digital polarimeter. The UV spectra were recorded using a Hitachi U-3200 type spectrometer. The MS were measured using JEOL JMS-DX 303HF and JMS-HX110 instruments (ion source, Xe atom beam; accelerating voltage, 3 kV; matrix, MeOH/glycerin or MeOH/*m*-nitrobenzyl alcohol (*m*-NBA). The NMR spectra were recorded using a JEOL JNM-GX-400 and Bruker AM-500 spectrometers; chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Applied Biosystems Inc. gas phase sequencer was used for the determination of amino acid sequences. The purity of peptides was checked by HPLC [Toso Co., Ltd., column, TSK gel 80 TM (ODS, 4.6×250 mm, 5 μm); solvent CH_3CN–0.05% trifluoroacetic acid (TFA) water=5%—95%, gradient elution]. Gas-liquid chromatography (GLC) was carried out on

a Shimadzu gas chromatograph, model GC-3BF. Column chromatography was carried out on MCI-gel CHP-20P (75—150 μm, Mitsubishi Chemical Industries Co., Ltd.), Kieselgel 60 (230—400 mesh, Merck) and Bondapak C_{18} (Waters Associates, Inc.). TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck) using a $CHCl_3$–MeOH–H_2O system as the developing solvent for the free compounds and detection was achieved by spraying plates with 20% H_2SO_4 reagent, followed by heating.

Isolation Crude drug, Lycii Radicis Cortex (2 kg) was extracted with MeOH and the solution evaporated under reduced pressure to afford a residue (164 g), which was shaken with benzene and water. After removal of the aqueous phase, 155 g of residue was obtained. This was subjected to column chromatography on MCI gel CHP-20P (eluted stepwise with $H_2O \rightarrow 40\% \rightarrow 60\% \rightarrow 80\% \rightarrow 100\%$ MeOH), silica gel (eluted with $CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) and Bondapak C_{18} (eluted with 30—50% MeOH, gradient elution) to yield lyciumins A (**1**, 516 mg), B (**2**, 91 mg), C (**3**, 5 mg) and D (**4**, 16 mg), compounds **8** (5 mg), **9** (15 mg) and **10** (20 mg). *Lycium chinense* MILL.: In a similar fashion, fresh root-bark (178 g) collected in Kumamoto was extracted with MeOH (4.5 g) and separated to afford lyciumins A (**1**, 10 mg), B (**2**, 19 mg), C (**3**, 7 mg) and D (**4**, 2 mg). The fresh stems (1.1 kg) were extracted with MeOH (89.9 g) and separated as in the case of the crude drug to afford lyciumins A (**1**, 6 mg) and B (**2**, 30 mg) and lyciumoside I (**5**, 52 mg). The fresh leaves (2.5 kg) were extracted with MeOH (89 g) and separated as in the case of the crude drug to afford lyciumosides I (**5**, 174 mg), II (**6**, 56 mg) and III (**7**, 19 mg).

Lyciumin A (**1**): A white powder, $[\alpha]_D^{21}$ +10.1° (c=0.54, DMSO). UV (MeOH) nm (ε): 273 (5200), 281 (sh, 5000) and 291 (sh, 3200). Positive FAB-MS m/z: 874.3728 (Calcd 874.3735: $C_{45}H_{52}N_9O_{12}$, $[M+H]^+$). Negative FAB-MS m/z: 872 $[M-H]^-$, 760 $[M-H-Glu]^-$, 648 [m/z 760−Pro]$^-$, 500 [m/z 648−Tyr]$^-$.

Lyciumin B (**2**): A white powder, $[\alpha]_D^{20}$ −3.5° (c=0.74, DMSO). Positive FAB-MS m/z: 897.3900 (Calcd 897.3895: $C_{44}H_{53}N_{10}O_{11}$, $[M+H]^+$). Negative FAB-MS m/z: 895 $[M-H]^-$.

Lyciumin C (**3**): A white powder, $[\alpha]_D^{24}$ −11.9° (c=0.97, DMSO). Positive FAB-MS m/z: 964.4211 (Calcd 964.4205: $C_{49}H_{58}N_9O_{12}$, $[M+H]^+$). Negative FAB-MS m/z: 962 $[M-H]^-$.

Lyciumin D (**4**): A white powder, $[\alpha]_D^{25}$ −8.4° (c=0.45, DMSO). Positive FAB-MS m/z: 900.4249 (Calcd 900.4256: $C_{45}H_{58}N_9O_{11}$, $[M+H]^+$). Negative FAB-MS m/z: 898 $[M-H]^-$, 786 $[M-H-Glu]^-$, 674 [m/z 786−Pro]$^-$, 526 [m/z 674−Tyr]$^-$.

Amino Acid Analysis of 1, 2, 3 and 4 Each sample (1 mg) was dissolved in 50 μl of 6 M HCl, allowed to stand in an oven at 150 °C for 3 h, and then dried. The residue was subsequently treated with 30 μl of 0.1 M sodium borate and 20 μl of *o*-phthalaldehyde/*N*-acetyl-L-cysteine reagent.[5] The resulting mixture was allowed to stand for 10 min at room temperature and then an aliquot of the solution was directly injected into an HPLC chromatographic system. Column TSK-80TM (4.6 × 150 mm, 5 μm) at 40 °C; mobile phase, (A) 50 mM sodium acetate, (B) MeOH; gradient, 0—11 min 0% B, 11—36 min 0—10% B, 36—46 min 10—25% B, 46—86 min 25—45% B, step-wise gradient; flow rate, 1 ml/min. L-Glu 8.1 min, L-Ser 20.0, Gly 39.1, L-Tyr 47.9, L-Val 51.6, L-Phe 58.7, L-Ile 66.2, L-Lys 67.4.

DNP-lation of 1 To a mixture of **1** (3 mg), $NaHCO_3$ (3 mg) in H_2O (1 ml) and 1-fluoro-2,4-dinitrobenzene (FDNB, 15 μl) were added. The reaction mixture was shaken for 3 h and extracted with ether. The water layer was then acidified with HCl to pH 1, after extraction with ether. The organic layer was concentrated and chromatographed on silica gel ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) to afford a mono-DNP (3 mg) derivative. Negative FAB-MS m/z: 1061 $[M]^-$, 1038, 872.

Benzylation of 1 and 2 Each sample (3 mg) and Cs_2CO_3 (100 μl, 13 mg/ml H_2O) were dissolved in MeOH–H_2O (10 : 1, 1 ml) and then evaporated. The dried residue was dissolved in dimethylformamide (DMF) (0.5 ml), benzyl bromide (10 μl) added and the solution left to stand for 6 h. The reaction mixture was diluted with EtOAc and washed with water. The EtOAc layer was concentrated and the residue was purified on silica gel ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) to give the benzyl ether (2.5 mg). **1**-Benzylate: negative FAB-MS m/z: 1052 $[M-H]^-$, 962. **2**-Benzylate: negative FAB-MS m/z: 985 $[M-H]^-$, 895.

Partial Acid Hydrolysis of 1, 2, 3 and 4 Each peptide sample (20 nmol) was acid hydrolyzed with 6 N HCl gas at 100 °C for 30 min and then dried. The residue was separated by HPLC [column, TSK gel 80 TM (4.6 × 250 mm, 5 μm); solvent, 5%—60% CH_3CN–0.05% TFA water (gradient): detector, UV 210 nm] to afford peptide fragments listed in Table II.

α-Chymotorypsin Hydrolysis of 1, 2, 3 and 4 A mixture of the appropriate specimen (10 nmol), α-chymotrypsin (10 μl, 150 μg/ml 0.005 N HCl) and 20 mM $NaHCO_3$ was incubated at 37 °C overnight. The reaction mixture was then separated by HPLC to afford the peptide fragments in Table III.

Proline-Specific Endopeptidase Hydrolysis of 1, 2, 3 and 4 A mixture of each sample (15 nmol), proline-specific endopeptidase (0.5 unit/5 ml H_2O) and 0.1 M phosphoric acid (30 μl) was incubated at 40 °C for 5 h. The reaction mixture was then separated by HPLC to afford the peptide fragments listed in Table IV.

Lyciumoside I (**5**): A white powder, $[\alpha]_D^{27}$ −21.0° (c=0.50, MeOH). Positive FAB-MS m/z: 653.3506 (Calcd 653.3513: $C_{32}H_{54}NaO_{12}$, $[M+Na]^+$). Negative FAB-MS m/z: 629 $[M-H]^-$, 467 $[M-H-glc]^-$. ^{1}H-NMR (CD_3OD) δ: 1.38, 1.59 × 2, 1.77 (each s, H_3-20, 19, 18, 16), 1.61 (2H, m, H_2-4), 1.97—2.16 (10H, m, H_2-5, 8, 9, 12, 13), 4.19, 4.34 (each 1H, d, J=11 Hz, H_2-17), 5.11 (2H, t, J=7 Hz, H-6, 10), 5.21 (1H, d, J=10 Hz, H-1), 5.23 (1H, d, J=18 Hz, H-1), 5.38 (1H, t, J=7 Hz, H-14), 5.93 (1H, dd, J=10, 18 Hz, H-2), 3.16—3.39 (m, sugar), 3.64, 3.70 (each 1H, dd, J=5, 12 Hz, glc H-6), 3.80, 3.85 (each 1H, dd, J=2, 12 Hz, glc H-6), 4.22, 4.35 (each 1H, d, J=8 Hz, glc H-1). ^{13}C-NMR (CD_3OD) δ: 116.0, 144.6, 81.6, 42.8, 23.7, 125.9, 136.0, 40.9, 27.4, 125.9, 135.6, 41.1, 27.7, 131.3, 132.6, 22.1, 67.9, 16.3, 16.4, 23.4 (C-1—20), 99.6, 75.1, 78.2, 71.6, 77.6, 62.7 (C-3-*O*-glc C-1—6), 102.4, 75.2, 78.3, 71.8, 77.9, 62.9 (C-17-*O*-glc C-1—6).

Lyciumoside II (**6**): A white powder, $[\alpha]_D^{26}$ −19.6° (c=0.49, MeOH). Positive FAB-MS m/z: 815.4041 (Calcd 815.4041: $C_{38}H_{64}NaO_{17}$, $[M+Na]^+$). Negative FAB-MS m/z: 791 $[M-H]^-$, 629 $[M-H-glc]^-$, 467 [m/z 629−glc]$^-$. ^{1}H-NMR (CD_3OD) δ: 1.37, 1.59 × 2, 1.79 (each s, H_3-20, 19, 18, 16), 1.60 (2H, m, H_2-4), 1.98—2.15 (10H, m, H_2-5, 8, 9, 12, 13), 4.26, 4.29 (each 1H, d, J=12 Hz, H_2-17), 5.11 (2H, m, H-6, 10), 5.21 (1H, d, J=10 Hz, H-1), 5.22 (1H, d, J=18 Hz, H-1), 5.37 (1H, br t, J=7 Hz, H-14), 5.93 (1H, dd, J=10, 18 Hz, H-2), 3.17—3.87 (m, sugar), 4.34, 4.36, 4.63 (each 1H, d, J=8 Hz, glc H-1). ^{13}C-NMR (CD_3OD) δ: 116.1, 144.6, 81.6, 42.8, 23.7, 125.9, 136.0, 40.9, 27.4, 125.9, 135.6, 41.1, 27.7, 131.2, 132.6, 22.1, 68.4, 16.3, 16.4, 23.4 (C-1—20), 99.6, 75.2, 78.3, 71.4, 77.6, 62.9 (C-3-*O*-glc 1—6), 101.2, 82.0, 77.9, 71.6, 77.6, 62.7 (C-17-*O*-inner glc 1—6), 104.8, 76.0, 78.3, 71.8, 77.8, 62.9 (C-17-*O*-terminal glc 1—6).

Lyciumoside III (**7**): A white powder, $[\alpha]_D^{26}$ −31.1° (c=0.48, MeOH). Positive FAB-MS m/z: 671.3617 (Calcd 671.3619: $C_{32}H_{56}NaO_{13}$, $[M+Na]^+$). Negative FAB-MS m/z: 647 $[M-H]^-$, 485 $[M-H-glc]^-$. ^{1}H-NMR (CD_3OD) δ: 1.13, 1.17 (each 3H, s, H_3-17, 16), 1.38, 1.59 × 2 (each s, H_3-20, 19, 18), 1.41, 1.52 (each 1H, m, H_2-13), 1.63 (2H, m, H_2-4), 1.98—2.27 (8H, m, H_2-5, 8, 9, 12), 2.26 (2H, m, H_2-12), 3.37 (1H, m, H-14), 5.12 (1H, t, J=7 Hz, H-6), 5.20 (1H, m, H-10), 5.20 (1H, d, J=11 Hz, H-1), 5.23 (1H, d, J=18 Hz, H-1), 5.93 (1H, dd, J=11, 18 Hz, H-2), 3.15—3.87 (m, sugar), 4.34 (1H, d, J=8 Hz, glc H-1), 4.43 (1H, d, J=7 Hz, glc H-1). ^{13}C-NMR (CD_3OD) δ: 116.1, 144.6, 81.6, 42.9, 23.8, 126.2, 136.1, 41.0, 27.6, 126.0, 136.1, 37.0, 30.8, 90.5, 75.0, 24.1, 26.6, 16.2, 16.4, 23.4 (C-1—20), 99.7, 75.3, 78.4, 71.5, 77.7, 62.8 (C-3-*O*-glc C-1—6), 106.6, 76.1, 78.6, 71.8, 78.0, 63.0 (C-14-*O*-glc C-1—6).

Compound **8**: A pale yellow powder, $[\alpha]_D^{20}$ −32.7° (c=0.40, MeOH). Positive FAB-MS m/z: 478.1685 (Calcd 478.1689: $C_{21}H_{29}NaO_{10}$, $[M+Na]^+$). Negative FAB-MS m/z: 454 $[M-H]^-$, 322 $[M-H-xyl]^-$, 159 [m/z 322−glc−H]$^-$. ^{1}H-NMR (CD_3OD) δ: 3.05—4.20 (m, sugar, H_2-10, H_2-11), 4.32 (1H, d, J=7 Hz, xyl H-1), 4.34 (1H, d, J=8 Hz, glc H-1), 7.00 (1H, t, J=7 Hz, H-5), 7.07 (1H, t, J=8 Hz, H-6), 7.13 (1H, s, H-2), 7.32 (1H, d, J=8 Hz, H-7), 7.55 (1H, d, J=8 Hz, H-4). ^{13}C-NMR (CD_3OD) δ: 123.7, 112.6, 119.3, 119.6, 122.2, 112.2, 128.9, 138.0, 26.8, 71.5 (C-1—11), 105.5, 74.8, 78.0, 71.5, 77.0, 69.8 (glc C-1—6), 104.5, 75.1, 77.7, 71.2, 66.9 (xyl C-1—5).

Compound **9**: A white powder, $[\alpha]_D^{19}$ −46.4° (c=0.78, MeOH). Positive FAB-MS m/z: 487.2153 (Calcd 487.2155: $C_{21}H_{36}NaO_{11}$, $[M+Na]^+$). Negative FAB-MS m/z: 463 $[M-H]^-$, 331 $[M-H-api]^-$, 169 [m/z 331−glc]$^-$. ^{1}H-NMR (CD_3OD) δ: 0.93, 1.01 (each 3H, s, H_3-9, 8), 1.08 (1H, d, J=10 Hz, H-3), 1.30 (1H, m, H-6), 1.39—1.45 (2H, m, H_2-5), 1.62 (1H, m, H-6), 1.70 (1H, br s, H-2), 2.09 (1H, d, J=10 Hz, H-3β), 2.15 (1H, br s, H-4), 3.18—3.90 (m, sugar), 3.46, 4.13 (each 1H, d, J=10 Hz, H_2-10), 3.76, 3.96 (each 1H, d, J=10 Hz, api H_2-4), 4.23 (1H, d, J=8 Hz, glc H-1), 5.02 (1H, d, J=2 Hz, api H-1). ^{13}C-NMR (CD_3OD) δ: 82.4, 51.4, 35.6, 49.1, 24.3, 24.7, 45.0, 26.1, 22.2, 73.6 (C-1—10), 105.2, 75.4, 78.1, 71.9, 78.0, 68.8 (glc C-1—6), 111.2, 77.2, 80.7, 75.2, 65.9 (api C-1—5).

Compound **10**: A white powder, Ehrlich reagent (+).

A solution of **10** (50 mg) and β-glucosidase (25 mg) in water (30 ml)

was incubated at 37 °C overnight. The reaction mixture was purified by silica gel chromatography with $CHCl_3$–MeOH–H_2O (8:2:0.5) to give **10a** (30 mg). **10a**: A white powder, positive FAB-MS *m/z*: 763.4246 (Calcd 763.4245: $C_{39}H_{64}NaO_{13}$, $[M+Na]^+$). ^{1}H-NMR (C_5D_5N) δ: 0.91 (1H, br d, J=4 Hz, H-9), 0.83, 0.98 (each 3H, s, H_3-18, 19), 1.08, 1.16 (each 3H, d, J=7 Hz, H_3-21, 27), 4.93, 5.30 (each 1H, d, J=8 Hz, gal H-1, glc H-1). ^{13}C-NMR (C_5D_5N) δ: 30.9, 26.2, 76.9, 27.0, 36.9, 26.4, 26.5, 35.5, 40.3, 35.2, 21.2, 40.3, 40.9, 56.5, 32.1, 81.4, 63.0, 16.3, 24.0, 42.5, 14.9, 109.7, 26.8, 26.8, 27.5, 65.1, 16.6 (C-1—27), 102.5, 81.8, 75.2, 69.9, 76.6, 62.2 (gal C-1—6), 106.0, 75.5, 78.0, 71.7, 78.4, 62.8 (glc C-1—6).

Acid Hydrolysis of 5—10 Each solution of **5—10** (2 mg) in 1 N HCl (1 ml) was heated at 80 °C in a sealed tube for 3 h. The reaction mixture was identified by TLC (solvent, $CHCl_3$: MeOH : acetone : H_2O = 3:3:3:1); *Rf* 0.40 (glucose), 0.35 (galactose), 0.53 (xylose), 0.55 (apiose) and (solvent; benzene : EtOAc = 3:1); *Rf* 0.52 (tigogenin), and also by GLC [2% OV-17 on Chromosorb W (60—80 mesh), N_2 flow rate 1 kg/cm^2, 3 mm × 1 m glass column, column temperature 150 °C]; t_R (min): 21.2, 25.0 (trimethylsilyl (TMS)-galactose)), t_R 25.0, 37.9 (TMS-glucose).

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