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Gypsophin: A novel α-glucosidase inhibitory cyclic peptide from the roots of *Gypsophila oldhamiana*

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Abstract—An unusual new cyclic peptide with a pyrrolidine-2,5-dione unit, gypsophin (**1**), was isolated from *Gypsophila oldhamiana.* Its structure was elucidated by the spectroscopic evidences. The stereochemistry was determined by application of the Marfey's method and the single-crystal X-ray diffraction. Compound **1** exhibited inhibitory activity against α-glucosidase with IC_{50} of 305 μM.
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Cyclic peptides are characteristic components of Caryophyllaceae plants, which can be used as a marker of secondary metabolites for Caryophyllaceae plants. However, up to now no cyclic peptides have been found in plants of the genus *Gypsophila*.[1] *Gypsophila oldhamiana* (Miq.) (Caryophyllaceae) is a small perennial herb widely distributed in the north regions of China, its roots have been used as a substitute for the traditional Chinese medicine Yin-Chai-Hu (roots of *Stellria dichotoma* var. Lanceolata Bge) to treat fever, consumptive disease, and infantile malnutrition syndrome.[2] In previous chemical study, saponins, sterols, and glycosides[3–5] have been reported as the main constituents of the roots from *G. oldhamiana*. Continuing the search for biologically active compounds of this plant, we have isolated an unusual cyclic peptide containing a pyrrolidine-2,5-dione unit (Fig. 1), named gypsophin (**1**). In this paper, we describe the structure elucidation of gypsophin (**1**) and its inhibitory effect on α-glucosidase.

The roots of *G. oldhamiana* were collected in Jinan, Shandong Province, People's Republic of China, and identified by Prof. Yun-Yao Li of the Department of Pharmacognosy, Shandong University. A voucher specimen (No. 041022) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University. The dried roots (30 kg) were extracted with aq. 70% EtOH under reflux. After removal of the solvent in vacuum, the residue was suspended in H_2O and partitioned between *n*-BuOH and water. The *n*-BuOH-soluble portion (724 g) was subjected to a silica gel column ($CHCl_3$/MeOH, 10:1 → 1:1), in which a fraction eluted with $CHCl_3$/MeOH (5:1) was further separated by RP-C18 column (MeOH/H_2O, 7:3 → 8:2) and purified by Sephadex LH-20 to afford **1** (1.5 g, 0.005% yield).

Gypsophin (**1**), obtained as amorphous powder, showed the pseudomolecular peak at *m/z* 772 $[M+H]^+$, and the molecular formula $C_{41}H_{53}N_7O_8$, was established by HR ESI MS ($[M+H]^+$, *m/z* 772.4041, calcd 772.4028), corresponding to 19 degrees of unsaturation. The absorptions between 3100 and 3500 cm^{-1}, between 1600 and 1730 cm^{-1} and 1524 cm^{-1} in the IR spectrum suggested the presence of amide, carbonyl, and aromatic functions, respectively.[6] Standard amino acid analysis of hydrolysate of **1** showed the presence of each 1 mol glycine (Gly), leucine (Leu), isoleucine (Ile), proline (Pro), aspartic acid (Asp), and 2 mol phenylalanine (Phe), except for Gly, all of which were proved to be L-amino acids by Marfey's method.[7] The ^1H NMR spectrum of **1** in DMSO-d_6 showed four methyl groups (δ 0.78, 0.79, 0.91, 0.99) and five NH group protons (δ 9.56, 8.82, 8.32, 7.42, 7.40), and seven proton resonance (δ 3.88–4.75), which were indicative of α-protons of amino acid residues. The presence of twelve olefins and eight carbonyl carbons was indicated by the ^{13}C NMR spectra. Assignment of ^1H NMR signals (Table 1) to specific protons in individual residues was obtained by TOCSY

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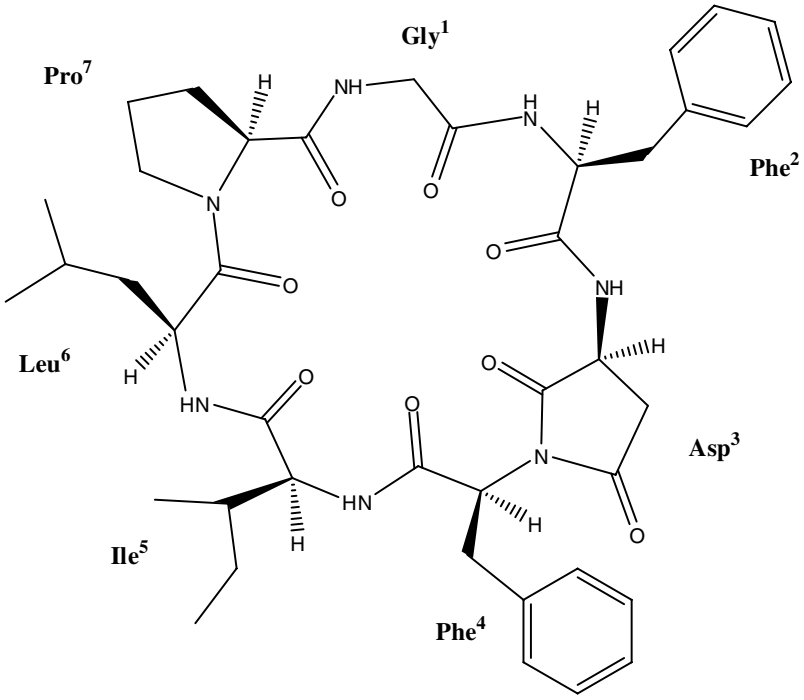

Figure 1. The structure of gypsophin (**1**).

Table 1. The ^{1}H and ^{13}C NMR assignments for compound **1** (in DMSO-d_6)[a]

	Position	δ_C	δ_H		Position	δ_C	δ_H
Gly[1]	NH		8.82 (t, 6.2)		*m*-CH	128.1	7.20–7.27 (m)
	α-CH_2	42.6	3.88 (m), 3.47 (m)		*p*-CH	126.3	7.16 (m)
	CO	168.7			CO	167.1	
Phe[2]	NH		8.32 (d, 9.1)	**Ile**[5]	NH		7.40 (d, 10.3)
	α-CH	53.0	4.75 (dd, 10.6, 4.7)		α-CH	58.8	4.33 (m)
	β-CH_2	37.6	2.57 (dd, 13.5, 4.7)		β-CH	37.6	1.70 (m)
			2.84 (dd, 13.5, 10.6)		γ-CH	24.4	1.72 (m)
	i-C	137.5			γ-CH_3	15.3	0.91 (3H, d, 6.8)
	o-CH	128.5	7.07–7.11(m)		δ-CH_3	10.6	0.78 (3H, t, 7.2)
	m-CH	128.4	7.07–7.11(m)		CO	169.2	
	p-CH	126.4	7.20–7.27 (m)	**Leu**[6]	NH		7.42 (d, 7.3)
	CO	170.5			α-CH	48.9	4.71 (m)
Asp[3]	NH		9.56 (d, 6.9)		β-CH_2	42.2	1.58 (m), 1.45 (m)
	α-CH	48.4	3.93 (m)		γ-CH	24.0	1.80 (m)
	β-CH_2	34.7	3.00 (dd, 18.3, 7.0)		δ-CH_3	23.5	0.79 (3H, d, 6.8)
			1.93 (dd, 18.3, 4.5)		δ-CH'_3	21.6	0.99 (3H, d, 6.4)
	γ-CO	174.3			CO	170.0	
	CO	173.4		**Pro**[7]	α-CH	61.2	4.17 (t, 8.8)
Phe[4]	α-CH	54.4	4.77 (dd, 10.6, 4.7)		β-CH_2	28.2	2.09 (m), 1.90 (m)
	β-CH_2	32.7	3.12 (dd, 14.3, 10.6)		γ-CH_2	25.3	2.05 (m)
			3.51 (dd, 14.3, 4.7)		δ-CH_2	46.8	3.76 (br t, 8.1)
	i-C	137.0					3.42 (m)
	o-CH	128.1	7.20–7.27 (m)		CO	171.9	

[a] TMS was used as internal standard, *J* in Hz. The ^{1}H and ^{13}C NMR data were measured at 500 MHz and 125 MHz, respectively.

and HMQC experiments to show the complete spin systems of the amino acid residues. The corresponding carbons (Table 1) were determined on the basis of the HMQC and HMBC experiments. The carbonyl signal at 173.4 was assigned as amide carbonyl carbon of aspartic acid residue, which was illuminated by HMBC cross-peaks for this carbon with both Hβ and NH (δ 9.56) of aspartic acid residue. Accordingly, another carbonyl signal at δ 174.3 was assigned as γ-carbon of aspartic acid residue by the HMBC correlations (Fig. 2). The sequence of the amino acid residues in gypsophin (**1**) was determined to be *cyclo*-(**Gly**[1]-**Phe**[2]-**Asp**[3]-**Phe**[4]-**Ile**[5]-**Leu**[6]-**Pro**[7]) by detailed analysis of HMBC correlations of Hα and NH to carbonyl carbon in each residue as shown in Figure 2. Considering the 17 degrees of unsaturation of the identified amino acid res-

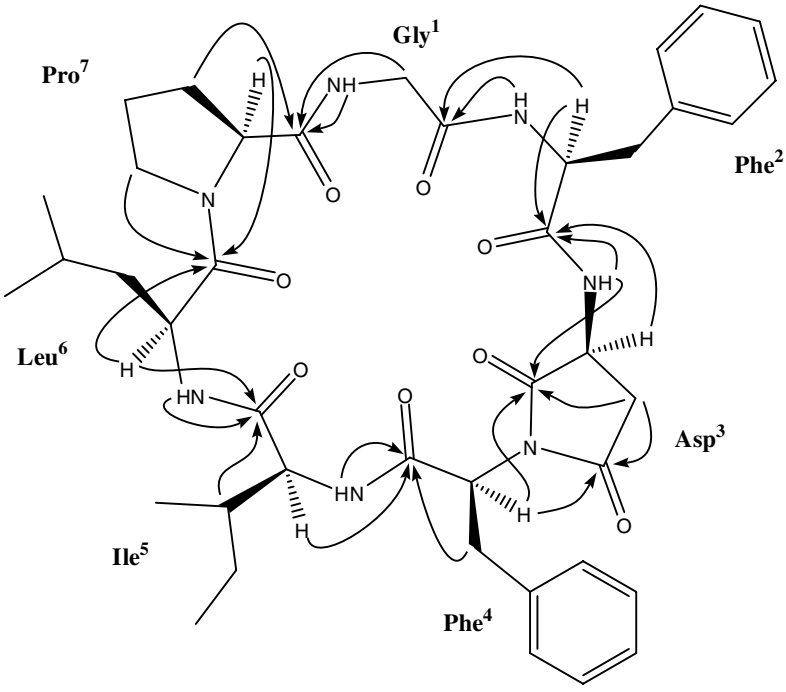

Figure 2. Selected HMBC correlations (from H to C) of **1**.

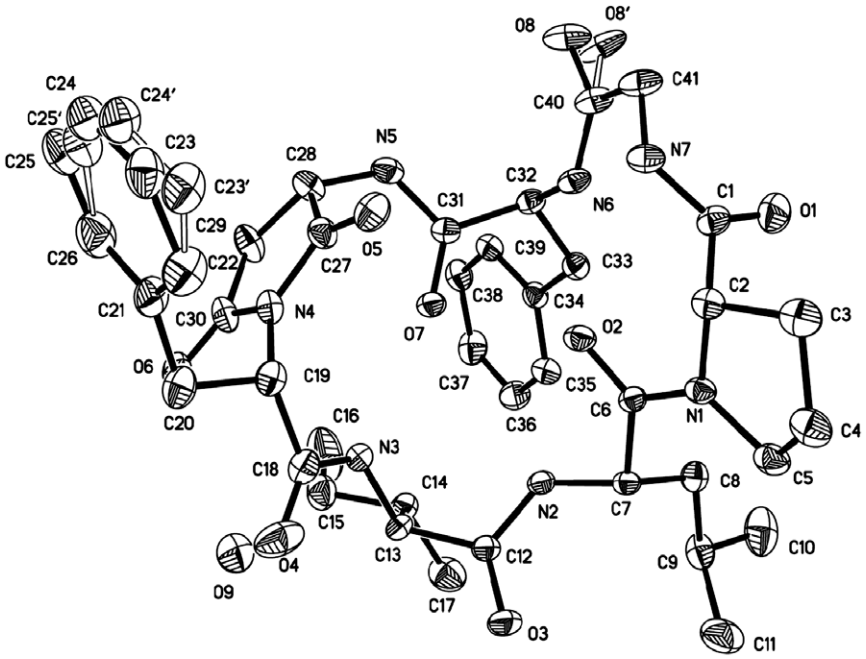

Figure 3. X-ray diffraction structure of **1**, with the numbering of the atom.

idues besides one of the cycle, an extra degree of unsaturation strongly indicated that another cyclic moiety is involved in the structure of **1**. The HMBC correlations of Hα (δ 4.77) of **Phe**[4] to two carbonyl carbons (δ 173.4, 174.3) of **Asp**[3] revealed that an additional five-number ring was formed by dehydrating between **Asp**[3] and **Phe**.[4] Thus, the structure of **1** was deduced to be an unusual cyclic peptide containing a pyrrolidine-2,5-dione unit as shown in Figure 1.

The complete structure and stereochemistry of **1** were established unequivocally by single-crystal X-ray analysis. Colorless crystals of gypsophin in the form of cubes were obtained by slow evaporation from MeOH–H_2O (7:3) solution at room temperature. The X-ray structure[8] of **1** is represented in Figure 3. The configuration of the chiral centers in the crystals was as shown in Figure 3. Except for glycine, the relative configurations of α-carbons (C2, 7, 13, 19, 28 and C32) in the other six

amino acid residues were all established to be S. Combining this result with the configuration of the hydrolysates of compound **1** established by Marfey's analysis permits assignment of the absolute configuration of compound **1** as shown in Figure 1.

α-Glucosidases are located in the brush-border surface membrane of intestinal cells and are the key enzymes of carbohydrate digestion. In fact, α-glucosidases not only control oligosaccharide metabolism, but also process protein glycosylation, which is involved in a wide range of biological processes, including promotion of protein folding in the endoplasmic reticulum and stabilization of cell-surface glycoproteins. Thus, glycosidase inhibitors arouse great interest as potential therapeutic agents such as anti-diabetics, anti-obesities, anti-viral, anti-cancer agents, and therapeutic agents for some genetic disorders.[9–11] Since some cyclic peptides have been reported to possess potent biological activities of anti-virus,[12] anti-tumor[13], and inhibition of protein synthesis,[14] the compound **1** was tested for α-glucosidase inhibitory activities using the same enzyme inhibition assay as previously described,[15] and it showed moderate α-glucosidase inhibitory activity with IC_{50} = 305 μM. Under the same test condition, the positive control (Arcarbose) exhibited inhibitory activity against α-glucosidase with IC_{50} = 388 μM.

Acknowledgment

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6. Compound **1:** white amorphous powder; mp 264–266 °C; $[\alpha]_D^{25}$ −55.3 (*c* 0.2, MeOH); IR (KBr) ν_{max}: 3427, 3314, 1722, 1680, 1657, 1624, 1524, 1458, 1383 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1; ESI MS *m/z*:772 $[M+H]^+$; HR ESI MS *m/z*:772.4041 $[M+H]^+$(calcd 772.4028 for $C_{41}H_{54}N_7O_8$).
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8. Colorless cube crystals of gypsophin (**1**), crystallized from MeOH–H_2O (7:3), belong to the monoclinic space group *P*2 (1). Crystal data: $C_{41}H_{53}N_7O_8$ $(H_2O)_{0.5}$, *M* = 780.91, *a* = 9.830 (2) Å, *b* = 22.617 (5) Å, *c* = 10.286 (2) Å, *β* = 115.373 (3)°, *V* = 2066.2 (7) $Å^3$, *Z* = 4, *d* = 1.255 Mg/m^3, Mo Kα radiation, linear absorption coefficient $\mu = 0.089\ mm^{-1}$. The sample selected for investigation had dimensions of 0.50 × 0.48 × 0.40 mm. The data were collected on a Bruker SMART-1000 CCD diffractometer with graphite monochromated Mo Kα radiation (*λ* = 0.71073 Å) at room temperature, maximum 2*θ* value of 50.02° was set. A total of 10,894 reflections were collected to give 3731 independent reflections (R_{int} = 0.0229), of which 3009 were observed. The structure was solved using directed methods with SHELXTL software package and refined by full-matrix least-squares techniques. The non-hydrogen atoms were assigned anisotropic displacement parameters in the refinement. The hydrogen atoms were treated using a riding model. The structure was refined on F^2 using SHELX-97. The final *R* and ωR_2 indices on F^2 were 0.0372 and 0.00882 ($\omega = 1/[\sigma^2(F_o^2) + (0.0541P)^2 + 0.2147P]$, where $P = (F_o^2 + 2F_c^2)/3$). The crystal used for the diffraction study shows no decomposition during data collection. The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 615443.
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