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Cyclonatsudamine A, a new vasodilator cyclic peptide from *Citrus natsudaidai*

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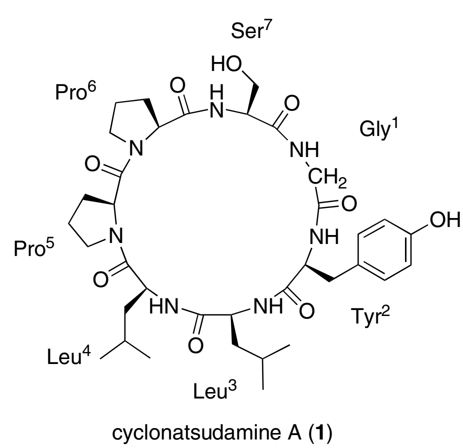
Abstract—A new cyclic heptapeptide, cyclonatsudamine A (**1**), *cyclo* (-Gly-Tyr-Leu-Leu-Pro-Pro-Ser-), has been isolated from the peels of *Citrus natsudaidai* and the structure was elucidated by 2D NMR analysis and chemical degradation. Cyclonatsudamine A (**1**) relaxed norepinephrine-induced contractions of rat aorta, which may be mediated through the increased release of NO from endothelial cells.

The vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation. Several endothelium-dependent vasodilators, such as bradykinin, acetylcholine, and histamine, have been reported to elevate Ca^{2+} levels in endothelial cells and activate NO release, leading to vasorelaxation.[1] On the other hand, contractile response in smooth muscle is caused by an influx of Ca^{2+} through voltage-dependent Ca^{2+}-channels (VDC) and/or receptor-operated Ca^{2+}-channels (ROC).[2] The endothelium-independent vasodilators, such as nicardipine, niphedipine, dirtiazem, and verapamil, have been reported to inhibit VDC and led to an decrease in the intracellular Ca^{2+} concentration in smooth muscle, leading to vasorelaxation.[2]

Recently, we have reported that cyclic peptides such as cyclosquamosin B from *Annona squamosa*,[3] dichotomin J from *Stellaria dichotoma* var. *lanceolata*,[4] and cycloleonuripeptide F from *Leonurus heterophyllus*[5] showed vasorelaxant activities. During our search for bioactive compounds targeting aortic smooth muscle from medicinal plants, we found that the extract from the peels of *Citrus natsudaidai* (Rutaceae) showed a vasorelaxant effect on rat aorta. Our efforts at identifying new vasodilators resulted in the isolation of a new cyclic heptapeptide, cyclonatsudamine A (**1**). This paper describes the isolation, structure elucidation, and conformational analysis of cyclonatsudamine A (**1**) by spectroscopic data and chemical means as well as its vasodilator effect on rat aorta.



cyclonatsudamine A (**1**)

Keywords: Cyclic heptapeptide; Cyclonatsudamine A; *Citrus natsudaidai*; Vasodilator.
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The peels of *C. natsudaidai* were extracted with MeOH, and the MeOH extract was in turn partitioned with $CHCl_3$ and H_2O. Chromatographic purification of the $CHCl_3$ soluble fraction showing orange spots by TLC Dragendorff reagent resulted in the isolation of a new cyclic peptide, cyclonatsudamine A (**1**, 0.008% yield), together with a known cyclic hexapeptide, *cyclo* (-Gly-Leu-Val-Leu-Pro-Ser-) (**2**).[6]

Table 1. ^{1}H (800 MHz) and ^{13}C (201 MHz) NMR data for cyclonatsudamine A in pyridine-d_5

	Position	δ_H (int.; mult.; J (Hz))	δ_C
Gly[1]			
	α	3.93 (1H, dd, 3.6, 17.6)	42.7
		4.38 (1H, dd, 6.0, 17.6)	
	C=O		171.3
	NH	8.27 (1H, br s)	
Tyr[2]			
	α	4.74 (1H, ddd, 5.8, 7.2, 7.8)	58.4
	β	3.22 (1H, dd, 7.2, 14.0)	36.8
		3.34 (1H, dd, 7.8, 14.0)	
	γ		127.4
	δ	7.23 (2H, d, 8.2)	130.8
	ε	7.07 (2H, d, 8.2)	116.2
	ζ		157.9
	C=O		172.6
	NH	9.58 (1H, d, 5.8)	
Leu[3]			
	α	4.63 (1H, m)	54.1
	β	2.02 (1H, m)	39.6
		2.10 (1H, m)	
	γ	1.62 (1H, m)	24.8
	δ	0.79 (3H, d, 6.4)	23.4
		0.85 (3H, d, 6.9)	21.0
	C=O		172.6
	NH	9.34 (1H, d, 7.3)	
Leu[4]			
	α	5.17 (1H, ddd, 7.8, 7.8, 7.8)	49.9
	β	1.77 (1H, m)	41.7
		2.02 (1H, m)	
	γ	1.92 (1H, m)	24.9
	δ	0.89 (3H, d, 6.9)	23.1
		0.93 (3H, d, 6.9)	22.7
	C=O		170.4
	NH	7.87 (1H, d, 7.8)	
Pro[5]			
	α	4.95 (1H, dd, 4.6, 8.2)	59.9
	β	1.84 (1H, m)	28.7
		2.11 (1H, m)	
	γ	1.77 (1H, m)	25.2
		2.02 (1H, m)	
	δ	3.82 (2H, m)	47.8
	C=O		171.2
Pro[6]			
	α	4.83 (1H, d, 8.7)	61.7
	β	2.04 (1H, m)	32.0
		2.57 (1H, dd, 5.9, 11.9)	
	γ	1.60 (1H, m)	22.3
		1.79 (1H, m)	
	δ	3.49 (1H, dd, 9.1, 10.1)	47.1
		3.55 (1H, m)	
	C=O		172.7
Ser[7]			
	α	5.22 (1H, m)	58.8
	β	4.47 (1H, dd, 4.2, 10.8)	62.5
		4.57 (1H, dd, 7.4, 10.8)	
	C=O		170.9
	NH	9.06 (1H, d, 7.8)	

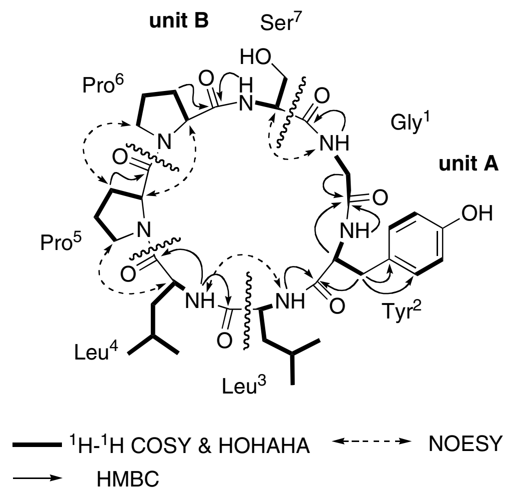

Figure 1. Selected 2D NMR correlations of cyclonatsudamine A (**1**) in pyridine-d_5.

Cyclonatsudamine A (**1**), colorless solid, $[\alpha]_D^{20}$ −93 (*c* 0.3, MeOH), showed molecular formula, $C_{36}H_{53}N_7O_9$, which was determined by HRESIMS [*m*/*z* 728.3950, $(M+H)^+$, Δ −3.3 mμ], indicating 14 degrees of unsaturation in the molecule. The IR absorption bands were characteristic of amino (3309 cm^{-1}) and amide carbonyl (1653 cm^{-1}) groups. Amino acid analysis of **1**

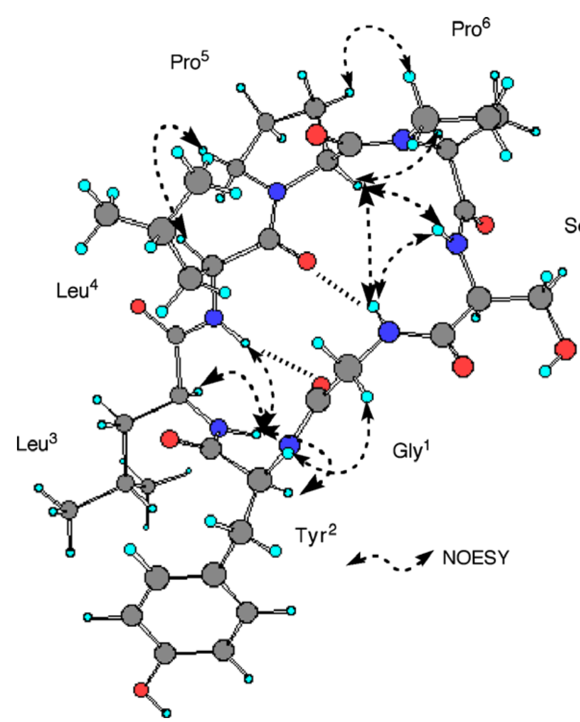

Figure 2. Stable conformation with selected NOESY correlations of cyclonatsudamine A (**1**).

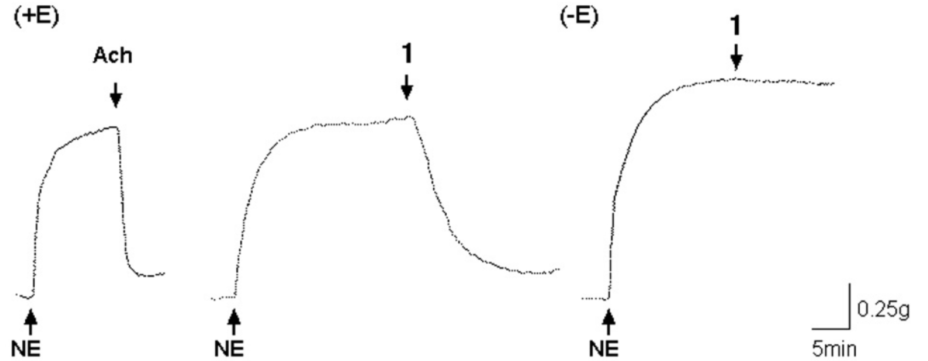

Figure 3. Typical recording of the relaxation effect of cyclonatsudamine A (**1**, 10^{-4} M) on aortic rings precontracted with 3×10^{-7} M norepinephrine (NE) with endothelium (+E) and without endothelium (−E).

showed it to consist of Leu × 2, Pro × 2, Gly, Ser, and Tyr, all of which proved to be L-amino acids by Marfey's derivatization, followed by HPLC analysis.[7] The UV absorption (ε 1400) at 278 nm of **1** also supported the Tyr residue. In the NMR spectra, five amide proton signals and seven amide carbonyl signals corresponding to the above seven amino acids were observed. Because the two proline-containing heptapeptide structure with one Tyr residue satisfies the 13 degrees of unsaturation, the remaining unsaturation is explained by a cyclic structure. Complete assignments for the ^{1}H and ^{13}C NMR signals in pyridine-d_5 were accomplished using a combination of 2D NMR experiments in an 800 MHz NMR machine, such as ^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC spectra (Table 1).

The sequence of the seven amino acids was elucidated by detailed analysis of HMBC correlations as well as NOESY correlations as shown in Figure 1. Partial units A (Gly-Tyr-Leu) and B (Pro-Ser) were elucidated by HMBC correlations for each Hα and the next NH to the amide carbonyl carbon. Connection between units A and B was assigned by NOESY correlation between Gly^1-NH and Ser^7-Hα. Connection of Pro^5-Pro^6 sequence and its *cis* geometry between Pro^5 and Pro^6 could be deduced by the combination of the strong NOE correlation between Hα in Pro^5 and Hα in Pro^6, the ^{13}C chemical shifts (δ_C 31.9 and 22.2) of β and γ positions in Pro^6 residue,[8] and the occurrence of a doublet signal of Hα in Pro^6.[9] The remaining Leu^4 residue and cyclic peptide nature were analyzed by the NOESY correlation between Leu^3-NH and Leu^4-NH, and between Leu^4-Hα and Pro^5-Hδ and revealed the whole sequence of cyclonatsudamine A (**1**) to be *cyclo* (-Gly-Tyr-Leu-Leu-Pro-Pro-Ser-) (Fig. 1).

Conformation of cyclic peptides has been intensively studied, because their biological activities are known to be closely related to their conformational states. We have reported the conformations of a series of cyclic heptapeptides such as yunnanin A[10] and pseudostellarin D[11] in order to clarify the relationship between their conformations and their biological activities.

Monte Carlo conformational search was conducted by using the Monte Carlo (MC/MM) search. After the conformational search, each of the resulting conformations was subjected to the energy-minimization calculation using AMBER94 force field and one of the minimum-energy conformers is shown in Figure 2. The results showed that the molecule had two β-turns incorporating a classical β-bulge motif with a *cis* amide bond. There is a weak intramolecular hydrogen bond between Gly^1–NH and Pro^5–CO of a type $5 \rightarrow 1$ found in cycloleonuripeptide D[12] and [Phe^4, Val^6]antamanide.[13] The detailed conformation around this $5 \rightarrow 1$ hydrogen bond is shown in Figure 2 and the conformational angles around three related residues, Pro-Pro-Ser, are almost the same as those in cycloleonuripeptide D.[12] These conformational characteristics of cyclonatsudamine A (**1**) may be favorable and common features for heptapeptides consisting of all L amino acids such as in evolidine,[13] hymenamide,[14] and phakellistatin,[15] which may be related to the biological activity.

After achieving a maximal response to thoracic aortic rings with endothelium by NE (3×10^{-7} M), cyclonatsudamine A (**1**) showed vasorelaxant action at 10^{-4} M (Fig. 3), whereas the known cyclic peptide, **2**, did not.[16] The vasorelaxant activity of cyclonatsudamine A (**1**) was observed in a concentration-dependent manner (10^{-4} M, 80% relaxation; 3×10^{-7} M, 46% relaxation) and did not cause vascular relaxation in endothelium-denuded aortic tissues. Treatment with N^G-monomethyl-L-arginine (L-NAME, 10^{-4} M), an inhibitor of nitric oxide (NO) synthase, also inhibited cyclonatsudamine A-induced vasorelaxation. The vasodilator effect of **1** may be mediated through the increased release of NO from endothelial cells.

Acknowledgments

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References and notes

1. Muller, J. M.; Davis, M. J.; Kuo, L.; Chilian, W. M. *Am. J. Physiol.* **1999**, *276*, 1706; Muller, B.; Kleschyov, A. V.; Gyorgy, K.; Stoclet, J. C. *Physiol. Res.* **2000**, *49*, 19.
2. Karaki, H.; Ozaki, H.; Hori, M.; Mitsui-Saito, M.; Amano, K.; Harada, K.; Miyamoto, S.; Nakazawa, H.; Won, K. J.; Sato, K. *Pharmacol. Rev.* **1997**, *49*, 157.
3. Morita, H.; Iizuka, T.; Choo, C. Y.; Chan, K. L.; Takeya, K.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4609.
4. Morita, H.; Iizuka, T.; Choo, C. Y.; Chan, K. L.; Itokawa, H.; Takeya, K. *J. Nat. Prod.* **2005**, *68*, 1686.
5. Morita, H.; Iizuka, T.; Gonda, A.; Itokawa, H.; Takeya, K. *J. Nat. Prod.* **2006**, *69*, 839.
6. Matsumoto, T.; Tashiro, N.; Nishimura, K.; Takeya, K. *Heterocycles* **2002**, *56*, 477.
7. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
8. Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379.
9. Kopple, K. D.; Schumper, T. J.; Go, A. *J. Am. Chem. Soc.* **1974**, *96*, 2597.
10. Morita, H.; Kayashita, T.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1997**, *53*, 1607.
11. Morita, H.; Kayashita, T.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1995**, *51*, 12539.
12. Morita, H.; Gonda, A.; Takeya, K.; Itokawa, H.; Iitaka, Y. *Tetrahedron* **1997**, *53*, 1617.
13. Eggleston, D. S.; Baures, P. W.; Peishoff, C. E.; Kopple, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 4410.
14. Kobayashi, J.; Tsuda, M.; Nakamura, T.; Mikami, Y.; Shigemori, H. *Tetrahedron* **1993**, *49*, 2391; Tsuda, M.; Shigemori, H.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **1993**, *49*, 6785; Kobayashi, J.; Nakamura, T.; Tsuda, M. *Tetrahedron* **1996**, *52*, 6355.
15. Pettit, G. R.; Xu, J.; Cichacz, Z. A.; Williams, M. D.; Boyd, M. R.; Cerny, R. L. *BioMed. Chem. Lett.* **1994**, *4*, 2091.
16. Nagai, M.; Noguchi, M.; Iizuka, T.; Otani, K.; Kamata, K. *Biol. Pharm. Bull.* **1996**, *19*, 228.