Pergamon

Tetrahedron, Vol. 52, No. 2, pp. 395-404, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)00857-8

A90720A, A Serine Protease Inhibitor Isolated From A Terrestrial Blue-Green Alga *Microchaete loktakensis*

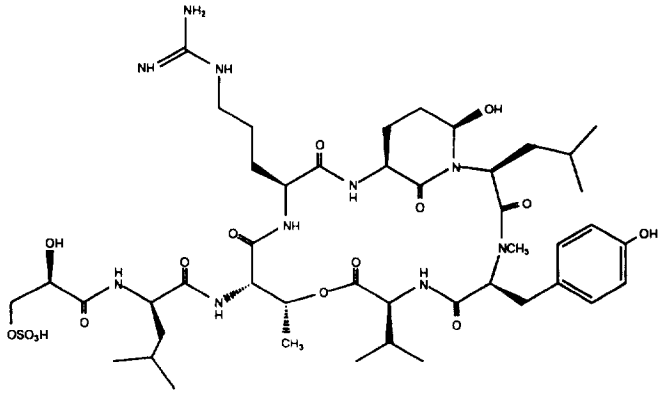
Rosanne Bonjouklian*, Tim A. Smitka, Ann H. Hunt, John L. Occolowitz, Thomas J. Perun Jr., Lawrence Doolin, Stephanie Stevenson, Lisa Knauss, Ranmali Wijayaratne, Stanley Szewczyk, Gregory M. L. Patterson#

Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN 46285

#Department of Chemistry, University of Hawaii, Honolulu, HI 96822

Abstract: The isolation and structure elucidation of A90720A,**1**, a cyclic depsipeptide from the terrestrial blue-green alga *Microchaete loktakensis* is described. **1** was isolated on the basis of its thrombin inhibitory activity and its structure was determined using spectral methods and amino acid analysis. The absolute configurations of the constituent amino acids were found to be as follows: the nucleus contains L-threonine, L-arginine, L-3-amino-6-hydroxy-2-piperidone (Ahp, i.e., glutamic acid-γ-carboxaldehyde), L-leucine, L-N-methyl-tyrosine and L-valine. The side chain off the L-threonine amino group contains D-leucine, followed by a 3-O-sulfated R-glycerate moiety.

Cardiovascular diseases such as stroke and coronary artery occlusion continue to be major causes of morbidity and mortality, and it is likely that improved pharmacological control of blood clot formation will remain a major goal. Heparin or aspirin are frequently used to lengthen clotting time but are not without side effects, especially for chronic use. The process of blood coagulation is triggered by a complex proteolytic cascade that leads to the formation of fibrin. Thrombin is a serine protease that cleaves a peptide fragment from fibrinogen which then leads to the generation of fibrin, a major component of blood clots. In a search for novel thrombin inhibitors we have screened a large number of extracts from microbiological sources, including blue-green algae, in a high volume colorimetric bioassay using bovine thrombin and a synthetic peptide substrate.[1] Studies on the bioactive metabolites produced by this class of photosynthetic microbes has led to a wide variety of unique natural products with therapeutic potential. In this paper we describe the isolation and structure elucidation of A90720A (**1**), a potent inhibitor of thrombin, trypsin and plasmin, obtained from the extract of the blue-green alga *Microchaete loktakensis*. A90720A is a new cyclic depsipeptide which contains a sulfated glyceric acid moiety in the sidechain and is structurally related to previously described blue-green algal-derived cyclic peptides such as the aeruginopeptins, cyanopeptolins, microcystilide A, and the micropeptins, all obtained from *Microcystis* species[2a-e], as well as dolastatin 13 from the blue-green algal-grazing sea hare *Dolabella auricularia*. [3] No useful antibacterial, antifungal or cytotoxic activities of **1** have yet been detected. However, it was found to be a fairly non-specific serine protease inhibitor active against bovine thrombin (IC_{50} = 270 ng/ml), bovine trypsin (IC_{50} = 10 ng/ml) and human plasmin (IC_{50} = 30 ng/ml). Of the previously mentioned cyclic peptides, only the micropeptins are reported to be inhibitors of trypsin and plasmin.



A90720A (**1**)

Isolation and Structure Elucidation. The terrestrial blue-green alga *Microchaete loktakensis* was collected from a soil sample acquired near Townsville, Queensland, Australia, and the pure strain was isolated from a subculture grown on solid media as previously described.[4] Taxonomic studies revealed the filamentous and heterocystous nature of this species, which is quite different from the unicellular morphology of various *Microcystis* species recently found to produce the cyclic depsipeptides mentioned above. Freeze-dried biomass was generated by liquid cultivation of the microorganism in glass carboys exposed to fluorescent lighting under controlled conditions, followed by filtration and lyophilization. Extraction with EtOH/H_2O (30:70) was followed by a series of reversed-phase chromatography steps to give the bioactive material, A90720A (**1**, 0.05% dry weight).

Analysis of proton and carbon NMR spectra of **1** (Table 1), including COSY, ROESY, and C/H correlation spectra, indicated that A90720A was peptide-like and this finding was confirmed by initial results from amino acid analysis: one Arg, one Val, one Thr, and two Leu. The NMR substructures included one Arg, one Val, one Thr, one complete Leu and one Leu lacking an amide proton resonance (Leu'), one collection of resonances corresponding to the ring and α/β protons of a Tyr, one methyl singlet attached to nitrogen, and the following two spin systems: (**2**) -$OCH_2CH(OH)$- and (**3**) -$NHCHCH_2CH_2CHOH$. The unusual chemical shift of the Thr β-CH resonance (5.48 ppm in d_6-DMSO) is diagnostic for acylation of the threonine hydroxyl, suggesting the presence of a lactone.

The molecular formula of **1** was established as $C_{45}H_{72}N_{10}O_{16}SNa$ by HRFABMS [*m/z* 1063.4720 $(M + Na)^+$, Δ +2.6 mmu]. Other ions observed included m/z 1041 $(M+H)^+$, 983 $(M+Na-SO_3)^+$, and 961 $(M+H-SO_3)^+$. The accurate mass of m/z 983 was 983.5153, which agreed with the calculated mass of 983.5178 and confirmed the presence of a sulfate functionality in the molecule.

Treatment of **1** with ammonia without subsequent purification resulted in a preparation which gave a FABMS containing an ion at m/z 979 $(M+H-SO_3+H_2O)^+$, corresponding to ring-opened, desulfated A90720A. The CID spectrum of m/z 979 yielded a series of ions which suggested the following partial sequence: - **X** - Leu - NMeTyr - Val-OH, where **X** = $C_5H_7NO_2$. Partial hydrolysis of **1** with 6N aqueous HCl for two days at a nominal 40°C gave a mixture of compounds; accurate mass measurements for ions observed in the FABMS of the hydrolysis mixture allowed the peptides listed in Table 2 to be identified.

Table 1. ^{13}C and ^{1}H NMR Chemical shifts in CD_3CN/CD_3OH and DMSO-d_6, and selected inter-residue NOE's.

Position		CD_3CN/CD_3OH ^{13}C	CD_3CN/CD_3OH ^{1}H	DMSO-d_6 ^{1}H	NOE's
Glc	CO	173.1			
	2	72.0	4.34	4.10	Leu NH
	2-OH			5.96	
	3,3'	70.5	4.00, 4.26	3.71, 4.03	
Leu	CO	174.9			
	NH		7.79	7.73	Glc 2
	α	52.7	4.62	4.69	Thr NH
	β,β'	41.9	1.65, 1.70	1.60	
	γ	25.6	1.71	1.50	
	δ,δ'	21.9,23.1	0.95, 0.98	0.90, 0.91	
Thr	CO	171.4			
	NH		7.95	8.55	Leu α
	α	56.6	4.75	4.59	Arg NH
	β	73.1	5.52	5.48	Arg NH, Ahp NH
	γ	18.7	1.33	1.19	
Arg	CO	172.8			
	NH		8.00	8.52	Thr α, Thr β
	α	54.8(br)	4.26	4.30	Ahp NH
	β,β'	27.9(br)	2.09	1.43, 2.04	
	γ,γ'	26.2	1.53, 1.62	1.43	
	δ,δ'	41.5	3.13	3.07	
	NH		7.11	7.51	
	$HN=CNH_2$	157.8			
Ahp	CO	170.7			
	NH		7.61	7.35	Thr β; Arg α
	α	50.6	4.52	4.38	
	β,β'	22.1	1.82, 2.60	1.72, 2.50	
	γ,γ'	30.8	1.83	1.71	
	δ	75.2	5.02	4.89	Leu' β'
	δ-OH		5.60	5.99	N-CH_3, Val NH, Val γ
Leu'	CO	173.1			
	α	49.7	4.72	4.61	MeTyr 2/6, MeTyr α
	β,β'	39.1	0.65, 1.60	0.41, 1.52	β': Ahp δ
	γ	24.9	0.99	0.98	
	δ,δ'	22.6, 18.7	0.54, 0.71	0.49, 0.69	δ':MeTyr 2/6, MeTyr 3/5
NMeTyr	CO	171.8			
	N-CH_3	31.7	2.76	2.71	Ahp δ-OH , Val NH, Val γ,γ'
	α	62.8	4.98	4.92	Val NH
	β,β'	33.8	2.76, 3.30	2.69, 3.10	
	H1	128.9			
	H2/H6	131.1	6.97	6.89	Leu' α, Leu' δ'
	H3/H5	116.4	6.66	6.63	Leu' δ'
	4	156.9			
	4-OH			9.22	
Val	CO	174.8			
	NH		7.90	7.46	MeTyr α , N-CH_3 , Ahp δ–OH
	α	59.1	4.35	4.81	
	β	31.2	2.07	2.09	
	γ,γ'	19.5, 24.0	0.86, 0.922	0.74, 0.86	both: N-CH_3 γ: Ahp δ–OH

These fragments as a group contained sequence information complementary to that derived from NMR spectra, but they did not distinguish between Leu and Leu', and they did not help locate the sulfate group.

Table 2. Peptides from Partial Hydrolysis of **1** with 6N HCl.

Fragment	Mass	Δ(mmu)[a]
$C_3H_5O_3$-Leu	220.1178	0.7
Thr-Arg	276.1664	0.8
NMeTyr-Val	295.1661	0.3
Leu-Thr-Val	332.2218	3.3
Leu-Thr-Arg	389.2544	1.8
Val-NMeTyr-Leu	408.2512	1.3
Val-Thr(Leu)-Arg	488.3193	0.4
Leu-Thr-Val-NMeTyr	509.2954	5.4
x-Leu-NMeTyr-Val	521.2962	1.3

[a]Δ = (exact mass calc'd - observed)

Sequential information from NMR for the A90720A substructures was provided by the ROESY spectrum and by long-range C/H correlations (Figure 1). Four consecutive NH/α ROESY correlations provided the linkage (**4**): - **2** - Leu - Thr - Arg - **3** -. A similar NH/α correlation linked the Val and NMeTyr, and the N-methyl protons had a long-range correlation to the Leu' carbonyl carbon. A ROESY crosspeak between the α resonances of NMeTyr and Leu' required that the peptide bond between these groups be cis, as in **5**. Additional C/H long-range correlations connected the Leu' α proton to its own CO and to a final amide carbonyl, and this carbon in turn had long-range correlations from both the methine protons of **3**. The Val carbonyl carbon had correlations from its own α proton and from the Thr β hydrogen. The two fragments **4** and **5** contained all of the eight carbonyls indicated by the ^{13}C NMR spectrum, suggesting that the lactone carbonyl which acylates the Thr hydroxyl must be the Val carbonyl, in agreement with the FABMS finding of a C-terminal Val after mild basic hydrolysis of A90720A. The Thr-Val ester survived treatment with 6N HCl in three of the fragments listed in Table 2.

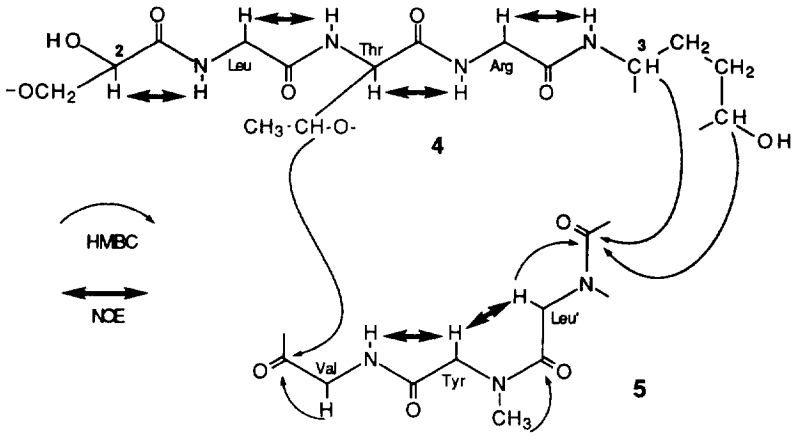
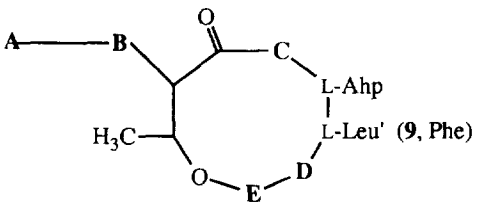
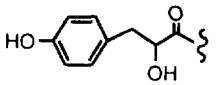


Figure .1 A90720A substructures.

The Leu' nitrogen and the amide carbonyl attached to it combine with **3** to form a hemiacetal, 3-amino-6-hydroxy-piperidone (Ahp, i.e., glutamate-δ-carboxaldehyde), linking the Leu' and Arg residues; the Ahp unit was first reported in an analogous cyclodepsipeptide with a somewhat different amino acid composition, dolastatin 13 (**9**) [3] (Figure 2). The cyclic peptide produced by combining fragments **4** and **5** contained all of the elements of A90720A except for the sulfate group. The proton 1D and COSY NMR spectra showed OH resonances for the NMeTyr OH, the hydroxyl on **3**, and the secondary hydroxyl on **2**; no coupling was observed between the CH_2 protons of **2** and a hydroxyl proton, leaving this as the sulfate site and completing the structure of **1**. Both micropeptin 90[2e] and oscillapeptin[5] contain analogous sulfate groups.

Figure 2. Structures of A90720A (**1**), Microcystolide A (**6**), Micropeptin A (**7**), Cyanopeptolin A (**8**) and Dolastatin 13 (**9**)



Compound	A	B	C	D	E
A90720A (**1**)	$Na^+ {}^-SO_3CH_2CH(OH)CO$-	D-Leu	L-Arg	L-NMeTyr	L-Val
Microcystolide A (**6**)		L-Gln	L-Tyr	L-NMeTyr	L-Ile
Micropeptin A (**7**)	$CH_3(CH_2)_6CO$-	L-Glu	L-Lys	L-NMeTyr	L-Val
Cyanopeptolin A (**8**)	$CH_3(CH_2)_4CO$-	L-Asp	L-Arg	L-NMePhe	L-Val
Dolastatin 13 (**9**)	$HOCH_2CH(OCH_3)CO$-	Val	cis-Abu*	N-MePhe	Val

* cis-Abu = α,β-dehydro-cis-butanoic acid (see ref. 3)

Chiralities of the standard amino acids in **1** were determined by C18 reverse phase HPLC analysis of chiral OPA-NAC (o-phthal-dialdehyde-N-Ac-L-cysteine)[6] derivatives: L-Arg, L-Val, L-Thr, L-Leu, D-Leu. The presence of L-N-methyltyrosine and, again, D- and L-Leu was confirmed by similar analysis of DNPA (Marfey reagent, 2,4-dinitro-phenyl-L-alaninamide) derivatives.[7] During the course of this work several similar structures have been reported, as previously mentioned, including microcystilide A [2c] (**6**), micropeptin [2d] A (**7**), cyanopeptolin A [2b] (**8**), and the aeruginopeptins [2a]. See Figure 2. Unlike **1**, however, all of these depsipeptides contain exclusively L-amino acids, both in the lactone ring and in the tail. The chirality of the Ahp unit has not been described except on the basis of inferences from NMR characteristics and its derivation

from L-glutamic acid, in the case of **8** [2b]. The compounds **6-8** all contain a blocked L-Leu between Ahp and an N-methyl Tyr or Phe, as does **1**, and the virtual identity of chemical shifts for the Leu' protons in the four compounds (see Figure 2 and Table 3) suggests that the orientation of the Leu' sidechain with respect to the adjacent aromatic ring is identical in the four materials. Thus, based on NMR data alone, the L-Leu of **1** would be part of the depsipeptide ring, and the D-Leu and glyceric acid make up the tail;this conclusion is confirmed by the crystallographic results mentioned below. In addition, the data of Table 3 suggest that the stereochemistry of the Ahp residues in 6, 7, and 8 must be the same as that of A90720A, which is shown to be L-Ahp in the trypsin-A90720A cocrystal.[8] The observation of two small values of $J_{\gamma\delta}$ and one large and one small $J_{\alpha\beta}$ for the Ahp ring of **1**, as also described for **8**, is consistent with a twisted chair conformation for the six-membered ring, having the α proton and the δ-OH group in axial positions. Thus, in solution, the hexadepsipeptide ring of A90720A appears to be a rather rigid, constrained structure, as indicated by a large number of interresidue NOEs; many of these are shown in Figure 3 and are shared with the related compounds.

Table 3. ^{1}H NMR of A90720A (**1**) and Related Compounds in DMSO-d_6: Comparison of Leu' and Ahp Chemical Shifts.

Compound : Position		1	6[a]	7[b]	8[c]
Ahp	NH	7.35	7.33	7.31	7.31
	α	4.38	4.37	4.36	4.38
	β	1.72	1.73	1.71	1.70
	β'	2.50	2.5	2.53	2.53
	γ	1.71	1.7	1.71	1.70
	γ'	1.71	1.73	1.71	1.70
	δ	4.89	4.87	4.89	4.87
	δ-OH	5.99	5.98	6.02	6.03
Leu'	α	4.61	4.58	4.58	4.56
	β	0.41	0.39	0.42	0.27
	β'	1.52	1.55	1.54	1.55
	γ	0.98	1.00	0.96	0.95
	δ	0.49	0.49	0.48	0.42
	δ'	0.69	0.69	0.68	0.66

a. Microcystilide A, ref 2c, b. Micropeptin A, ref 2d, c. Cyanopeptolin A, ref 2b

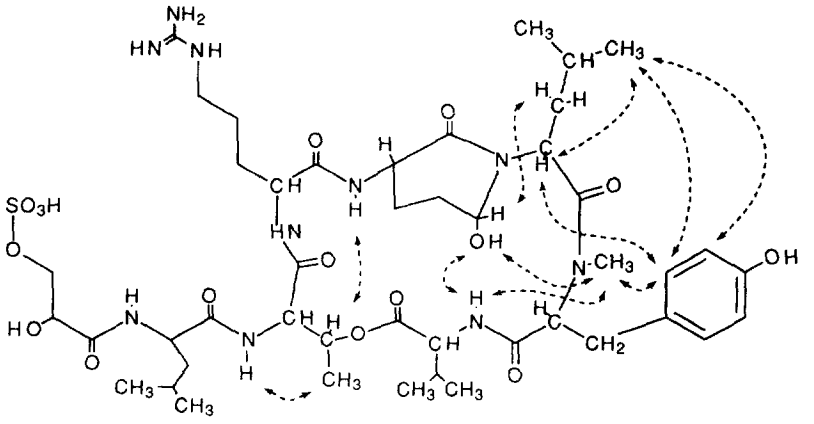


Figure 3. A90720A (1). Arrows show selected structural NOEs.

During the course of the structure elucidation process, we were able to take advantage of the fairly potent antitrypsin activity of **1** by attempting a bovine trypsin-A90720A cocrystallization experiment. While this successful effort has been published [8], it is useful here to note that the conformations of the cyclic peptide in solution as determined by NMR and in the crystalline enzyme-bound state appear very similar. The nucleus in the bound state has an elliptical shape stabilized by two transannular hydrogen bonds involving the Ahp residue, one between the Ahp NH and the Val carbonyl and the second between the Ahp δ hydroxyl and the Val NH. Although we did not explicitly search for evidence of hydrogen bonds in the A90720A NMR studies (such as by temperature or solvent variation) and have yet to perform a rigorous evaluation of coupling constants for dihedral angle determination, the presence of the same two transannular bonds in solution is suggested by inter-residue NOEs shown in Figure 3, one between the Ahp NH and the Thr β CH, adjacent to the lactone, and one between the Ahp δ OH and the Val NH. Similarly, the Leu' and Nmethyl tyrosine side chains are connected by a number of NOE's in Figure 3, and these two sidechains occupy the trypsin hydrophobic pocket in the cocrystal.

Finally, dolastatin 13 (**9**) contains an O-methylated glyceric acid unit in its side chain but its absolute configuration has not been previously reported. We were eventually able to *chemically* solve the chirality of the glycerate moiety of **1** by using a coupled column HPLC procedure where Cu (II) - **L**-proline eluent of the acid hydrolysate of **1**, followed by Fe(III)-glycerate detection led to the identification of the **R** glycerate enantiomer.[9] This result was confirmed by the trypsin-A90720A cocrystal structure.

Thus, in summary, physical and chemical evidence points to the presence of all **L** amino acids in the ring of **1**, and the presence of **D** leucine and **R** glycerate in the sidechain. In comparison, the *Microcystis*-derived cyclic peptides[2a-e] possess only **L** amino acids.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 500 spectrometer at 298°K; solvents used were DMSO-d_6, CD_3OD or CD_3OH, and CD_3CN plus a few drops of either CD_3OD or CD_3OH to bring the A90720A fully into solution. Standard vendor software and pulse sequences were used for various two-dimensional NMR experiments including COSY, TOCSY, ROESY (150 msec mixing time), and direct and long-range (70 msec evolution time) carbon/proton correlations. Mass spectral analyses were conducted in FAB mode on a VG-ZAB-2SE mass spectrometer using bombardment with Cs ions having a net energy of 20 keV. Samples were dispersed in either glycerol/oxalic acid or a 5:1 mixture of dithiothreitol/dithioerythreitol ("magic bullet"). Accurate mass determinations were performed at a resolution of 10,000 (10% valley) using external mass standards.

Culture Conditions. An epilithic form of *Microchaete loktakensis* Bruhl & Biswas (Nostacales, Microchaetaceae), designated strain number IC-39-2, was isolated from an algal sample collected from the grounds of the Australian Institute of Marine Science (Townsville, Queensland) in 1990. Cultures were grown in BG-11 liquid medium in 45 L glass bottles using the procedure described previously. After 30 days, the alga was harvested by filtration and freeze-dried. Yields of lyophilized alga averaged 0.4 g/L.

Isolation. Lyophilized *M. loktakensis* (38 g) was extracted with EtOH/H_20 (3:7) for 24 hr, then filtered through diatomaceous earth and concentrated. The extract (8.98 g) was dissolved in H_20 and chromatographed on a 2.5 cm i.d. X 45 cm steel column of HP-20SS (Diaion) using a 120 min linear gradient of 0% to 100% MeOH at a flow rate of 8 mL/min. Fractions containing A90720A (**1**), as determined by bioassay, were combined and concentrated to yield a residue (48.5 mg). Further purification over a Zorbax Phenyl column (2.5 cm i.d. X 25 cm, DuPont) using a 95 min linear gradient of CH_3CN: 0.2% HOAc buffer (adjusted to pH 5.1 with NaOH) afforded a single component. **1** elutes at 5.3 min using an analytical Zorbax Phenyl column (4.1 mm i.d. X 25 cm, 31:69 CH_3CN/0.2% HOAc, pH 5.1 with NaOH) at a wavelength of 215 nm.) This material was desalted by passage of an aqueous solution of **1** over an HP20 (Diaion) column and the pure factor was eluted with MeOH to afford 18 mg (0.05% dry weight) of A90720A (**1**).

A90720A (**1**): Colorless solid; $[\alpha]_D$ -32.5° (c 0.004, MeOH); UV(MeOH) λ_{max} 278 (ε 20670) and 213 (ε 138100) nm; IR (KBr) υ_{max} 3374, 2962, 1739, 1664, 1518, 1446, 1207 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); FABMS *m/z* 1063 $(M+Na)^+$, 1041 $(M+H)^+$, 983 $(M+Na-SO_3)^+$ and 961 $(M+H-SO_3)^+$; HRFABMS *m/z* 1063.4720 $(M+Na)^+$; calcd for $C_{45}H_{72}N_{10}O_{16}SNa$, 1063.4746).

Hydrolyses of 1 for Mass Spectral Analysis. Acidic: 2 mg of **1** was dissolved in 0.5 ml of 6 N HCl and heated at 40°C for two days. Basic: **1** (0.5 mg) was dissolved in 50 µl methanol, 1ml H_2O, and 50 µl conc. NH_4OH and heated at 40°C for 6 hr. Then 50 µl conc. NH_4OH was added and the temp elevated to 45°C. At 7.5 hrs HPLC showed no intact A90720A remaining, and the solution was lyophilized. See Table 2.

Determination of Absolute Configuration of Amino Acids by HPLC. A90720A (10 mg) was dissolved in 8 ml of 6 N HCl in a sealed tube, heated at 110 - 120°C for 24 hr, and then lyophilized. Two derivatization methods were followed: **OPA Method:** Literature methods were followed. Analysis by HPLC: Waters NovaPak phenyl column (**I,** 8 mm i.d. x 100 mm) with a 10 min linear gradient at 2 ml/min (10:90 to 25:75 CH_3CN/0.2% HOAc buffer, pH to 4.3 with 5 N NaOH) or a Zorbax C 18 column (**II,** 4.6 mm x 25 cm) with a 10 min linear gradient at 1.5 ml/min (10:90 to 31:69 CH_3CN/0.2% HOAc buffer, pH to 5.7) was used. Detection (337 nm). In this manner the following chiral retention times were determined as

compared to authentic standards: (Column I): L-arginine (5.5 min), L-threonine (4.6 min), L-valine (9.9 min), (Column II): L-leucine (3.3 min), and D-leucine (3.9 min). **Marfey Method:** Literature methods were used. Analysis by HPLC: Waters Novapak C18 column (8mm x 10 cm) with isocratic 25:75 CH_3CN/0.1% HOAc at 2 ml/min and detection at 340 nm. In this manner the hydrolysate-derived tyrosine derivative eluted at 8.24 min which matched the L-N-Me-tyrosine standard. Similarly, D and L leucine derivatives were found with retention times of 9.59 and 4.87 min, respectively, using 37:63 CH_3CN/0.1% HOAc.

Determination of Absolute Configuration of Glycerate by HPLC: Achiral/Chiral Column Switching Procedure: The resolution of glyceric acid from other amino acid constituents of the hydrolyzate was achieved on a Dionex ICE-AS6 (25 cm x 9.0 mm i.d.) ion exchange column using a mobile phase of 0.2 mM H_2SO_4 (1 ml/min, 205 nm). The eluted glyceric acid was collected in a 100 µl loop of a six-port valve and switched on to a Zorbax Rx-C8 (25 cm x 4.6 mm i.d.) column using an eluent containing Cu(II)-L-proline and 0.05% $HClO_4$ adjusted to pH 5.5 with NaOH. The column was cooled to 5° C and the mobile phase flow rate was 0.7 ml/min. The glyceric acid from the column effluent was then detected by the method of Lunder and Messori.[7] To the effluent, 2.5 mM $FeCl_3$ diluted in 0.5% $HClO_4$ was added through a mixing T (flow rate = 0.7 ml/min) using a third pump. The resulting solution was mixed thoroughly and its absorbance measured at 420 nm.

Acknowledgement. We thank Dan Baker, Billy Allen, Pat Baker and Ron Sparks for bulk production and purification support. We are also grateful to Bob Ellis for amino acid analysis, and Denny Berry, Marci Kellam, Trelia Craft, Donetta Gifford-Moore and Gerry Smith for biological support. Work at the University of Hawaii was supported by NSF grant # CHE-9024748.

References and Notes

1. See Shuman, R. T.; Rothenberger, R. B.; Campbell, C. S.; Smith, G. F.; Gifford-Moore, D. S.; Gesellchen, P. D. *J. Med. Chem.* **1993**, *36*, 314-319, and references cited therein.

2. a) Aeruginopeptins: Harada, K.; Mayumi, T.; Shimada, T.; Suzuki, M.; Kondo, F.; Watanabe, M. *Tet. Lett.* **1993**, *34*, 6091-6094. b) Cyanopeptolins: Martin, C.; Oberer, L.; Ino, T.; Konig, W. A. ;Busch, M.; Weckesser, J. *J. Antibiotics*, **1993**, *46*, 1550-1556. c) Microcystilide A: Tsukamoto, S.; Painuly,P.; Young,K. A.; Yang, X.; Shimizu, Y. *J. Am. Chem. Soc.*, **1993**, *115*, 11046-11047. d) Micropeptins: Okino,T.; Murakami, M.; Haraguchi, R.; Munekata, H.; Matsuda, H.; Yamaguchi, K. *Tet. Lett.*, **1993**, *34*, 8131-8134. e) Micropeptin 90: Ishida, K.; Murakami, M.; Matsuda, H.; Yamaguchi, K. *Tet. Lett.*, **1995**, *36*, 3535-3538.

3. Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Cerny, R. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.*, **1989**, *111*, 5015-5017.

4. See references cited in Smitka, T. A.; Bonjouklian, R.; Doolin, L.; Jones, N. D.; Deeter, J. B.; Yoshida, W.; Prinsep, M. R.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.*, **1992**, *57*, 857-861.

5. Shin, H. J.; Murakami, M.; Matsuda, H.; Ishida, K.; Yamaguchi, K. *Tet. Lett.*, **1995**, *36*, 5235-5238.

6. Aswad, D. W. *Analytical Biochem.*, **1984**, *137*, 405-409.

7. Adamson, J. R.; Hoang, T.; Crivici, A.; Lajoie, G. A. *Analytical Biochem.*, **1992**, *202(1)*, 210-214.

8. Lee, A.Y.; Smitka, T.A.; Bonjouklian, R.; Clardy, J. ,*Chemistry and Biology* , **1994**, *1*, 113-118.

9. Lunder, T. L.; Messori, F., *Chromatographia*, **1979**, *12*, 716. Our report of this application to A90720A is in preparation for submission to *J. Chromatography*.

(*Received in USA* 13 *November* 1994; *accepted* 5 *October* 1995)