experiments performed on the fraction A, using as a reference a sample of **V**, prepared as above.

Axamide-4 (**VI**). The occurrence of isonitriles in marine sponges, has generally been accompanied by the corresponding isothiocyanates and formamides, and this was considered to be evidence of the strict biogenetic relationship between these three derivatives. Unfortunately our attempts to isolate **VI** were abortive, probably due to the very small quantities of Axamide-4 present in the sponge. However, co-GLC experiments, performed on the fraction C, revealed the presence of a product with the same retention time of a synthetic sample of Axamide-4 [M^+ 247, m.p. 81–84°, $[\alpha]_D$ +63,3, ν_{max} 3380 to 3170, 1683, 1655 cm^{-1}; δ 0.97 (3H, s), 1.67 (3H, s), 1.73 (3H, s), 4.56 (2H, m)], obtained by treatment of **IV** with acetic acid in anhydrous ether, in the previously described experimental conditions[3].

Diketopiperazines containing L-proline from Streptomyces lavendulae and their stereochemistry in solution

A. KUBO[1–3], K. TAKAHASHI[4] and T. ARAI[5]

Laboratory of Organic Chemistry, Meiji College of Pharmacy, Setagaya (Tokyo, 154, Japan), Division of Chemotherapy, Chiba Cancer Center Research Institute, Nitona (Chiba, 280, Japan), and Department of Antibiotics, Research Institute for Chemobiodynamics, Chiba University, Izumi (Narashino, 275, Japan), 10 June 1976

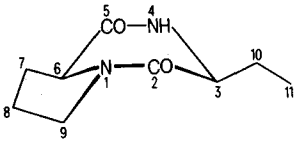
Summary. L-Valyl-L-proline (**I**), L-leucyl-L-proline (**II**), L-phenylalanyl-L-proline (**III**) and L-isoleucyl-L-proline (**IV**) anhydrides were isolated from the cultures of *Streptomyces lavendulae* No. 314 and, on the basis of ^{13}C-NMR and CD spectra, their stereochemistry in solution is described.

In the previous communication[6], we have described the isolation of novel antitumor antibiotics, Chlorocarcin A, B and C from the culture filtrate of *Streptomyces lavendulae* No. 314. We report here the isolation of several diketopiperazines[7] containing L-proline (**I-IV**) as companion substances, and we will discuss their stereochemistry in solution on the basis of ^{13}C-NMR and CD spectra.

The companion substances (**I-IV**) were isolated from the crude basic components by column chromatography over silica gel along with Chlorocarcins having potent antitumor activity. However, four crystalline compounds (**I-IV**) did not exhibit inhibitory activity against experimental L1210 lymphocytic leukemia. These optically active substances (**I-IV**) were shown to have the molecular formula: I, $C_{10}H_{16}O_2N_2$ (M^+, 196) m.p. 186.5–187.5 °C; II, $C_{11}H_{18}O_2N_2$ (M^+, 210) m.p. 159–161 °C; **III**, $C_{14}H_{16}O_2N_2$ (M^+, 244) m.p. 134–134.5 °C; IV, $C_{11}H_{18}O_2N_2$ (M^+, 210) m.p. 124–127 °C, $[\alpha]_D^{20}$ −167.2 °C (c, 0.67, $CHCl_3$) and were identified as L-valyl-L-proline (**I**)[8–12], L-leucyl-L-proline (**II**)[8–11,13–17], L-phenylalanyl-L-proline (**III**)[8,11] and L-isoleucyl-L-proline (**IV**) anhydrides respectively. Among them, **IV** was a novel diketopiperazine and its structure was elucidated by the examination of mass[18] and ^{1}H-NMR spectra and the direct comparison of the synthetic material prepared from L-proline and L-isoleucine. This is, to the best of our knowledge, the first time **IV** has been isolated from *Streptomyces* sp. or indeed from any natural source.

There has been considerable interest in the stereochemistry of diketopiperazine ring through ^{1}H-NMR (in solution)[19–22] and X-ray crystallographic (in the solid state)[18,23–25] studies. As to proline derivatives, glycyl-L-proline[18] and L-leucyl-L-proline (**II**)[24] anhydrides were recently shown to adopt the bowsprit boat conformation by X-ray analysis, and L-valyl-L-proline anhydride (**I**)[12] assumed the same conformation in solution by NMR studies. Then we further examined the ^{13}C-NMR and CD

^{13}C-NMR chemical shifts (δ^{TMS}) of diketopiperazines (**I–III**) in $CDCl_3$[a]



bowsprit boat

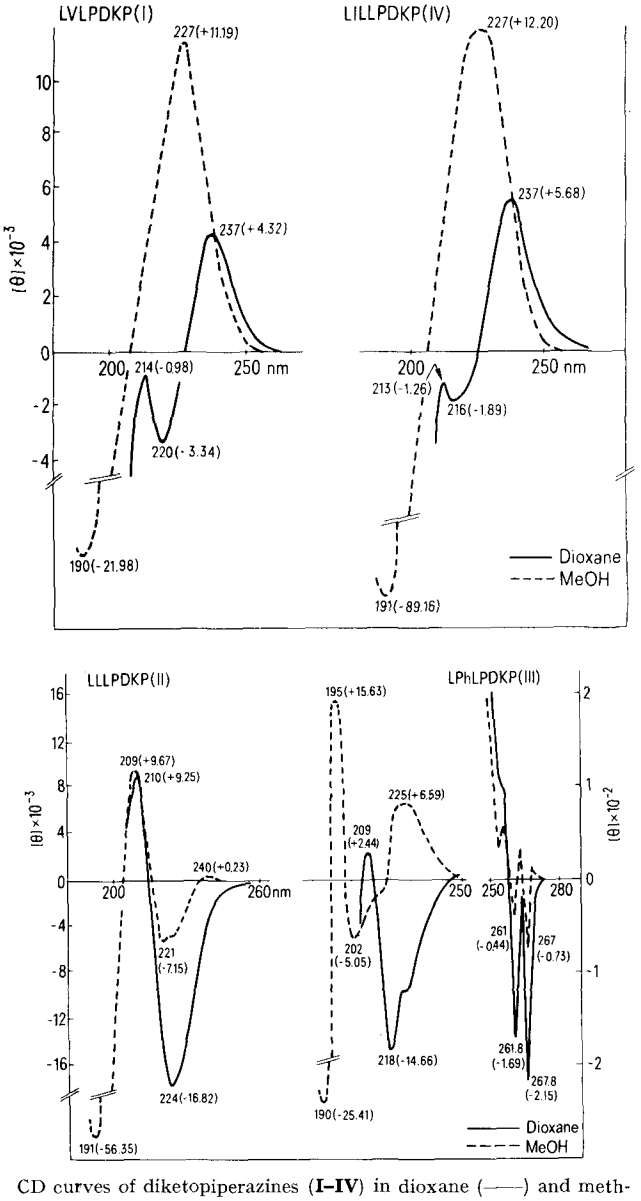
DKP	Number of carbon (off resonance)									
	2 (s)	3 (d)	5 (s)	6 (d)	7 (t)	8 (t)	9 (t)	10	11	$\underline{C}H_3$ (q)
I	170.41	58.78	165.08	60.47	28.50	22.27	45.01	28.50 (d)		16.03,19.02
II	170.54	53.58	166.38	59.04	28.11	23.31	45.53	38.64 (t)	24.61 (d)	21.36,22.79
III	169.43	56.31	165.07	59.04	28.30	22.46	45.33	36.82 (t)		
Leucine[26]		55.5						45.2 (t)	25.2 (d)	22.3,23.5

[a] ^{1}H-Noise decoupled and off resonance ^{13}C-NMR spectra were taken with Jeol FX-60 FT-NMR spectrometer operating at 15.0 MHz.

spectra of these diketopiperazines (**I-IV**), hitherto little investigated class of compounds. From proton noise decoupled and off-resonance ^{13}C-NMR spectra, it was suggested that **I**, **II** and **III** adopt the bowsprit boat conformation, and the leucyl side chain is fully extended by comparison with ^{13}C-NMR data of leucine[26] (see table). Consequently it was proved that the stereochemistry of **II** in solution was identical with that found crystallographically[24].

CD curves of these diketopiperazines (**I-IV**) are shown in the figure: **I** and **IV** showed a positive n $\rightarrow \pi^*$ Cotton effect[27] at 237 nm ([θ] +4320) and 237 nm ([θ] +5680). On the other hand, **II** and **III** showed a negative n $\rightarrow \pi^*$ Cotton effect at 224 nm ([θ] −16820) and 218 nm ([θ] −14650) respectively. Since the presence of the proline ring may enhance the stability of the bowsprit boat form[25], it should be pointed out that this striking difference in the sign of the n $\rightarrow \pi^*$ Cotton effect was influenced by the conformation of the side chain in accord with ^{1}H- and ^{13}C-NMR data. In conclusion, the above results clearly indicate that the sign of the n $\rightarrow \pi^*$ Cotton effect depends on the presence of the methyl group at C-10 carbon of the side chain on these rigid diketopiperazine ring.

Investigations on this point are continuing in our laboratory.



CD curves of diketopiperazines (**I-IV**) in dioxane (——) and methanol (----).

[1] Laboratory of Organic Chemistry, Meiji College of Pharmacy, Setagaya, Tokyo, 154, Japan.

[2] Acknowledgments. The authors would like to express their sincere thanks to Prof. H. Ogura, Kitasato University, for kind supply of the authentic L-valyl-L-proline anhydride and to Mr. A. Takakuwa, JASCO, for measuring the CD curves with J-40 automatic recording spectropolarimeter.

[3] To whom correspondences should be addressed.
[4] Division of Chemotherapy, Chiba Cancer Center Research Institute, Nitona, Chiba, 280, Japan.
[5] Department of Antibiotics, Research Institute for Chemobiodynamics, Chiba University, Izumi, Narashino, 275, Japan.

[6] T. Arai, K. Yazawa, Y. Mikami, A. Kubo and K. Takahashi, J. Antibiot., Tokyo *29*, 398 (1976).
[7] P. G. Sammes, in *Fortschr. Chem. Organ. Naturstoffe*, vol. 32 (Springer-Verlag, Wien 1975), p. 51–118.
[8] Y.-S. Chen, Bull. Agr. Chem. Soc. Japan *24*, 372 (1960).
[9] Y. Kodaira, Agr. Biol. Chem., Tokyo *25*, 261 (1961).
[10] G. Luedemann, W. Charney, A. Woyclesjes, E. Pettersen, W. D. Peckham, M. J. Gentles, H. Marshall and H. L. Herzog, J. Org. Chem. *26*, 4128 (1961).
[11] S. Tamura, A. Suzuki, Y. Aoki and N. Otake, Agr. Biol. Chem., Tokyo *28*, 650 (1964).
[12] H. Ogura, K. Furuhata and K. Furuhata, Chem. Pharm. Bull., Tokyo *23*, 2474 (1975).
[13] J. L. Johnson, W. G. Jackson and T. E. Eble, J. Am. Chem. Soc. *73*, 2947 (1951).
[14] N. Inagaki, Chem. Pharm. Bull., Tokyo *10*, 152 (1962).
[15] T. Kosuge and H. Kamiya, Chem. Pharm. Bull., Tokyo *10*, 154 (1962).
[16] L. A. Mitscher, M. P. Kunstmann, J. H. Martin, W. W. Andres, R. H. Evans, Jr. K. J. Sax and E. L. Patterson, Experientia *23*, 796 (1967).
[17] K. Takahashi, M. Tadenuma, K. Kitamoto and S. Sato, Agr. Biol. Chem., Tokyo *38*, 927 (1974).
[18] G. R. Pettit, R. B. von Dreele, G. Bolliger, P. M. Traxler and P. Brown, Experientia *29*, 521 (1973).
[19] K. D. Kopple and D. H. Marr, J. Am. Chem. Soc. *89*, 6193 (1967).
[20] K. D. Kopple and M. Ohnishi, J. Am. Chem. Soc. *91*, 962 (1969).
[21] Z., K. D. Kopple, J. Org. Chem. *35*, 253 (1970).
[22] Z., K. D. Kopple and C. A. Bush, Tetrahedron Lett. *1972*, 483.
[23] E. Sletten, J. Am. Chem. Soc. *92*, 172 (1970).
[24] I. L. Karle, J. Am. Chem. Soc. *94*, 81 (1972).
[25] C.-F. Lin and L. E. Webb, J. Am. Chem. Soc. *95*, 6803 (1973).
[26] L. F. Johnson and W. C. Jankowski, in *Carbon-13 NMR Spectra* (John Wiley & Sons, Inc., New York 1972), Spectrum No. 203.
[27] N. J. Greenfield and G. D. Fasman. J. Am. Chem. Soc. *92*, 177 (1970).