[Chem. Pharm. Bull.
23(11)2474—2477(1975)]
UDC 547.466.1.057.02 : 547.86.3.02 : 542.98

Studies on Lactams. VI.*,[1] Stereochemistry of L-Prolyl-L-valine Anhydride

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(Received March 13, 1975)

L-Prolyl-L-valine anhydride (I) was obtained from the cultured broth of *Streptomyces* No. K-73 as a by-product of an antibiotic No. 13—1. The structural elucidation of I were done by the determination of infrared, nuclear magnetic resonance (NMR), ^{13}C-NMR, and mass spectra. Conformation of 2,5-diketopiperazine group compounds was discussed from the NMR spectra.

Recently, L-prolyl-L-valine anhydride (I) was obtained from *Rosellinia necatrix* BERLESE,[3] and *Aspergillus ochraceus* and *Oospora destructor*.[4] The present work reports the first isolation of I from *Streptomyces* sp., and we discuss the stereochemistry of I from the studies of nuclear magnetic resonance (NMR) spectra.

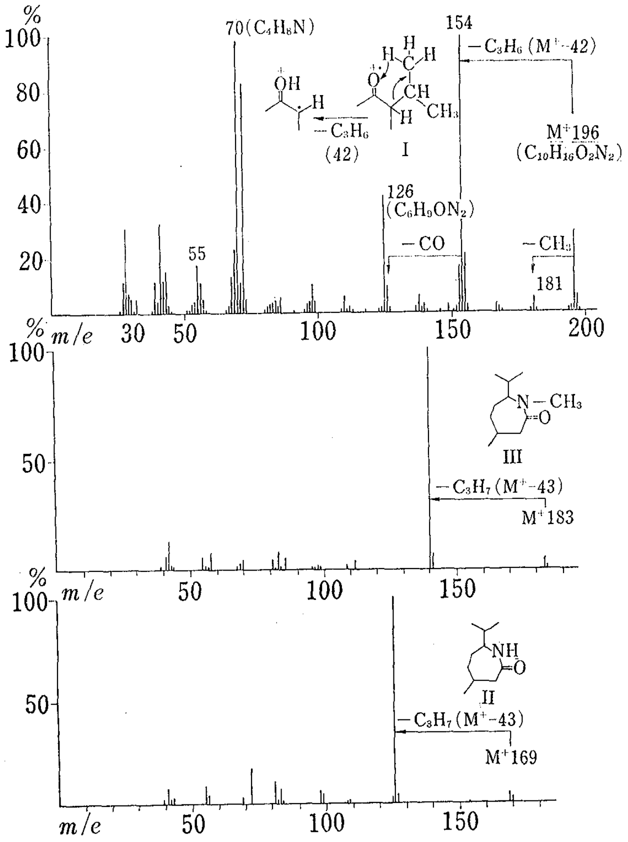

Fig. 1. Mass Spectra of I, II, and III

Streptomyces No. K-73 was cultured for 7—14 days at about 25° in the starch-peptone medium. L-Prolyl-L-valine anhydride (I) was obtained from the filtrate broth by chloroform extraction.

The spectral data for I agreed with its formulation; $\nu_{\max}^{\text{KBr}}$ 3230 (NH), 1660 (CONH) cm^{-1}; Mass Spectrum *m/e*: 196 (M^+). NMR spectra suggested the presence of an NH group at 7.00 ppm ($CDCl_3$) or 8.63 ppm (C_5D_5N). The NMR spectra also showed the presence of isopropyl group from the decoupling and by triple resonance methods, and the presence of $CH_2CH_2CH_2CH<$ group was confirmed by an application of homonuclear internuclear double-resonance(INDOR) technique.[5]

Mass spectrum of I (Fig. 1) showed an intense peak at *m/e*: 154 (M$-$42), which was attributed to the McLafferty rearrangement product from molecular ion, in contrast to intense M$-$43 peaks in the spectra of ($-$)-menthone lactam (II) and N-methyl-($-$)-menthone

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* Dedicated to the memory of Prof. Eiji Ochiai.

[1] Part V: H. Ogura, K. Takeda, and H. Takahashi, *Chem. Pharm. Bull.* (Tokyo), 23, 000) 1975).

[2] Location: *Shirokane, Minato-ku, Tokyo, 108 Japan.*

[3] Y.-S. Chen, *Bull. Agr. Chem. Soc. Japan*, **24**, 372 (1960).

[4] Y. Kodaira, *Agr. Biol. Chem.* (Tokyo), **25**, 261 (1961).

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lactam[6] (III) (Fig. 1). This evidence clearly suggested that the position of the isopropyl group was at C-3.

From proton noise decoupled and off-resonance ^{13}C-NMR spectra (Table I), the structure of I was further confirmed. Signals appearing in the lowest region at 170.75_2 and 165.26_8 were assigned to 2-C and 5-C carbonyl carbon.[7] Other signals were reasonably assigned as shown in Table I.

TABLE I. ^{13}C-NMR Chemical Shifts of I in $CDCl_3$

δ^{TMS} ppm	16.16_2	18.92_9	22.42_4	28.53_9	45.13_9	58.82_6	60.62_2	165.26_8	170.75_2
(off-resonance)	(q)	(q)	(t)	(d)(t)	(t)	(d)	(d)	(s)	(s)
Number of carbon	11	12	8	7, 10	9	3	6	5	2

TABLE II. Chemical Shifts (ppm) and Coupling Constants (Hz) of I

	C_5D_5N (coupling constants)		$CDCl_3$	C_5D_5N-$CDCl_3$ (1:1)
3-H	4.09	$(J_{3,10}=2.5)$	3.97	3.97
NH	8.63		7.00	—
6-H	4.17	$(J_{6,7e}=1;\ J_{6,7a}=7.8)$	4.10	4.08
7-H*e*	2.28	$(J_{7e,6}=1;\ J_{7e,8a}=10.5)$ $(J_{7e,8e}=7.2)^{a)}$	2.34	
7-H*a*	2.16	$(J_{7a,6}=7.8;\ J_{7a,7e}=-12.0;\ J_{7a,8a}=8.0)$ $(J_{7a,8e}=7.2)^{a)}$		
8-H*e*	1.6—1.8	$(J_{8e,9a}=7.0;\ J_{8e,9e}=7.0)$	2.3—1.8	
8-H*a*	1.5—1.7	$(J_{8a,9e}=7.0;\ J_{8a,9a}=4.0)$		
9-H*e*	3.46	$(J_{9a,9e}=-12.0;\ J_{9e,8e}=7.0;\ J_{9e,8a}=7.0)$	3.56	3.42
9-H*a*	3.65	$(J_{9a,8a}=4.0)$	3.68	3.60
10-H	2.77	$(J_{10,11}\text{-}CH_3=6.0;\ J_{10,12}\text{-}CH_3=6.0;\ J_{10,3}=2.5)$	2.61	2.73
11-CH_3	1.15	$(J=6.0)$	0.94	1.03
12-CH_3	1.22	$(J=6.0)$	1.12	1.14

a) Coupling constants obtained by INDOR technique.

Conformation of L-prolyl-L-valine anhydride (I) in solution is discussed on the basis of the NMR spectra. Lin and Webb[8] reported the conformation of diketopiperazine ring assumed a nearly planar (A) conformation or a flagpole boat (B) conformation from the X-ray analysis of L-seryl-L-tyrosine anhydride and glycyl-L-tyrosine anhydride monohydrate, respectively, with the torsional angle in 5°, 6°, and 4°, 7°, respectively. On the other hand, racemic 3,4-dehydroprolylproline anhydride (IV) showed the bowsprit boat (C) conformation from the X-ray analysis.[9] Almost identical results were reported with L-prolyl-L-leucine anhydride[10] and L-prolyl-L-proline anhydride.[11]

L-Prolyl-L-valine anhydride (I) assumes the bowsprit boat conformation (D) from the NMR studies. Conformation of pyrrolidine ring was confirmed, as shown in Fig. 2, by means of chemical shift and coupling constant in NMR spectra (Table II) comparing to those of pyrrolidine derivatives.[12]

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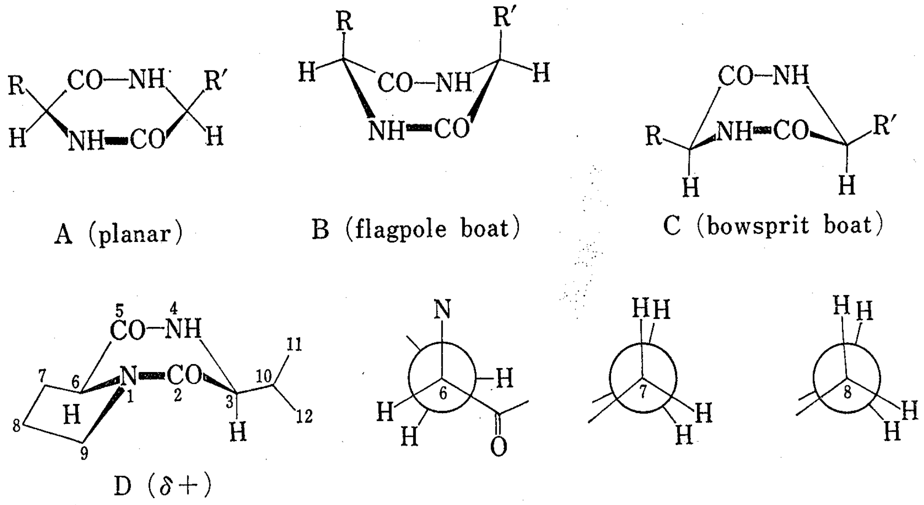

Fig. 2

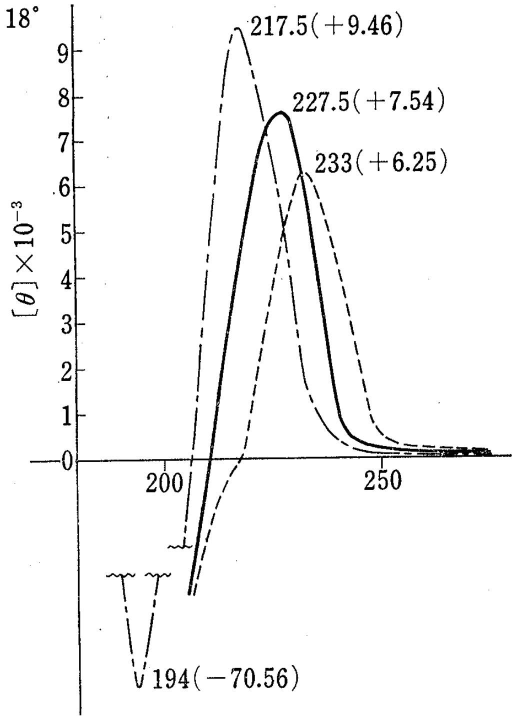

Fig. 3. CD Curves of I in Various Solvents

——: in 5%HCl
——: in MeOH
——: in dioxane

Circular dichroism (CD) curves of I in various solvents shown in Fig. 3, and the positive Cotton effect observed at around 220—234 nm can be attributed to the *n*-*π** transition, because the bands are blue-shifted in more polar solvents. This observation was also reported by Urry,[13] and the molecular rotational strength was discussed comparing with pyrrolid-2-one.

In simple 3,6-disubstituted diketopiperazines, $R^1=CH_3$, $R^2=H$; $R^1=R^2=CH_3$; $R^1=R^2=CH_2\text{-}COOH$; $R^1=R^2=(CH_2)_3NH_2$; and $R^1=R^2=$ isopropyl showed a positive *n*-*π** Cotton effect at around 220—230 nm.[13] On the other hand, glycyl-L-serine anhydride (X) showed a negative *n*-*π** Cotton effect in various solvents (Table III).

Further elucidation of the sign of the *n*-*π** Cotton effect relating to the stereochemistry of 2,5-diketopiperazine and the lactam rule[6,14] is under investigation in our laboratories.

Experimental

Temperatures are uncorrected. NMR spectra were measured at 100 MHz with a JMS-PS 100 PFT-100 spectrometer, and Me_4Si was used as an internal reference. CD curves were measured with a Japan Spectroscopic Model J-20 recording polarimeter. Mass spectra were measured with JEOL-OlS spectrometer by a direct inlet system at 75 eV.

3-Isopropyl-2,5-dioxo-1,4-diazabicyclo[4.3.0]nonane (L-Prolyl-L-valine Anhydride) (I)——The culture of *Streptomyces* No. K-73 used in this work was furnished by Kayaku Antibiotics Research Laboratory.[15] For isolation, the broth was carried out by extraction of I with $CHCl_3$ at pH 5.0—5.5, the obtained crude material was crystallized and recrystallized from BuOH to colorless needles, mp 189.5° (reported 191—193°,[3])

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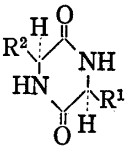
[13] D.W. Urry, *Ann. Rev. Phys. Chem.*, **1968**, 477.

[14] H. Ogura, H. Takayanagi, and K. Furuhata, *Chemistry Letters*, (Tokyo), 1973, 387.

[15] Y. Koyama, S. Chihara, I. Haneda, K. Hasuda, S. Takano, and Y. Koide, Japanese Patent 49—007689 (1974).

TABLE III. CD Data of 2,5-Diketopiperazines[8]



No.	R^1	R^2	[θ] nm H_2O	F_3CCH_2OH	MeCN	Trimethylphosphate	Dioxane
V	Me	H	231 (1000)				
VI	Me	Me	237 (200)	237 (300)	237 (2750)	238 (2000)	
VII	$CHMe_2$	$CHMe_2$	221 (16000)	219 (17500)	223 (14300)	223 (11800)	223 (12000)
VIII	CH_2COOH	CH_2COOH	218 (4000) (pH 1.8)				
IX	$(CH_2)_3NH_2$	$(CH_2)_3NH_2$	225 (2000) (pH 11.7)				
	$(CH_2)_3NH_3^+$	$(CH_2)_3NH_3^+$	222 (2000) (pH 5)				
X	CH_2OH	H	210 (−35000)	210 (−28000)	221 (−25000)	222 (−28000)	

188—189°[4]). $[\alpha]_D^{20}$ −150° (c 1.0, MeOH), −146° (c 1.0, $CHCl_3$). $[\theta]_{227.5}^{20}$ +7540 (MeOH). *Anal.* Calcd. for $C_{10}H_{16}O_2N_2$: C, 61.20; H, 8.22; N, 14.27. Found: C, 61.06; H, 8.30; N, 14.36. Mass Spectrum (M^+) *m/e*: Calcd. for $C_{10}H_{16}O_2N_2$: 196.1212. Found: 196.1220. IR ν_{max}^{KBr} cm^{-1}: 3230 (NH), 1660 (CONH).