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

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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), ¹³C-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.

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; $\nu_{\text{max}}^{\text{KBr}}$ 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 (CDCl₃) or 8.63 ppm (C₅D₅N). The NMR spectra also showed the presence of isopropyl group from the decoupling and by triple resonance methods, and the presence of CH₂CH₂CH₂CH< 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

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

* Dedicated to the memory of Prof. Eiji Ochiai.

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lactam⁶⁾ (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 ¹³C-NMR spectra (Table I), the structure of I was further confirmed. Signals appearing in the lowest region at 170.75₂ and 165.26₈ were assigned to 2-C and 5-C carbonyl carbon.⁷⁾ Other signals were reasonably assigned as shown in Table I.

TABLE I. ¹³C-NMR Chemical Shifts of I in CDCl₃

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 ₅ D ₅ N (coupling constants)		CDCl ₃	C ₅ D ₅ N-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-He	2.28	(J _{7e,6} =1; J _{7e,8a} =10.5) (J _{7e,8e} =7.2) ^{a)}	2.34	
7-Ha	2.16	(J _{7a,6} =7.8; J _{7a,7e} =-12.0; J _{7a,8a} =8.0) (J _{7a,8e} =7.2) ^{a)}		
8-He	1.6—1.8	(J _{8e,9a} =7.0; J _{8e,9e} =7.0)	2.3—1.8	
8-Ha	1.5—1.7	(J _{8a,9e} =7.0; J _{8a,9a} =4.0)		
9-He	3.46	(J _{9a,9e} =-12.0; J _{9e,8e} =7.0; J _{9e,8a} =7.0)	3.56	3.42
9-Ha	3.65	(J _{9a,8a} =4.0)	3.68	3.60
10-H	2.77	(J _{10,11} -CH ₃ =6.0; J _{10,12} -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 Webb⁸⁾ 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 anhydride¹⁰⁾ 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

Model J-20 recording polarimeter. Mass spectra were measured with JEOL-OIS 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⁾

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 $n\text{-}\pi^*$ 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, $\text{R}^1=\text{CH}_3$, $\text{R}^2=\text{H}$; $\text{R}^1=\text{R}^2=\text{CH}_3$; $\text{R}^1=\text{R}^2=\text{CH}_2\text{-COOH}$; $\text{R}^1=\text{R}^2=(\text{CH}_2)_3\text{NH}_2$; and $\text{R}^1=\text{R}^2=\text{isopropyl}$ showed a positive $n\text{-}\pi^*$ Cotton effect at around 220–230 nm.¹³⁾ On the other hand, glycyl-L-serine anhydride (X) showed a negative $n\text{-}\pi^*$ Cotton effect in various solvents (Table III).

Further elucidation of the sign of the $n\text{-}\pi^*$ 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

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TABLE III. CD Data of 2,5-Diketopiperazines⁸⁾

No.	R ¹	R ²	[θ] nm				
			H ₂ O	F ₃ CCH ₂ OH	MeCN	Trimethyl-phosphate	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 ₂ COOH	CH ₂ COOH	218 (4000) (pH 1.8)				
IX	(CH ₂) ₃ NH ₂	(CH ₂) ₃ NH ₂	225 (2000) (pH 11.7)				
	(CH ₂) ₃ NH ₃ ⁺	(CH ₂) ₃ NH ₃ ⁺	222 (2000) (pH 5)				
			210 (-35000)	210 (-28000)	221 (-25000)	222 (-28000)	
X	CH ₂ OH	H					

188—189°⁴⁾. $[\alpha]_D^{20} -150^\circ$ (*c* 1.0, MeOH), -146° (*c* 1.0, CHCl₃). $[\theta]_{227.5}^{20} +7540$ (MeOH). *Anal.* Calcd. for C₁₀H₁₆O₂N₂: 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 C₁₀H₁₆O₂N₂: 196.1212. Found: 196.1220. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3230 (NH), 1660 (CONH).