BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 1138—1141 (1970)

# The Optical Rotatory Dispersion and Circular Dichroism of Aromatic Amino Acids[*1,2]

Naokazu SAKOTA, Kozo OKITA and Yasuhiko MATSUI

*Department of Industrial Chemistry, Faculty of Engineering, Ehime University, Bunkyo-cho, Matsuyama*

(Received August 8, 1969)

The CD and ORD of D-phenylglycine and L-phenylalanine were measured at various pH's. The superficial small pH dependence of the CD maximum around 220 mμ and the anomalous large $[\theta]$ value at the CD maximum in both aromatic amino acids suggest an interaction between the $^1L_a$ transition of the benzene ring and the $n\to\pi^*$ transition of the carboxyl group. The ORD and CD of L-α-amino-β-benzalpropionic acid, L-α-amino-β-benzylpropionic acid, and L-2-amino-4-phenyl-butanol-1 were also measured, and the screening effect of the methylene groups which exist between a benzene ring and an asymmetric carbon atom was discussed.

Studies of the optical rotatory dispersion (ORD) and circular dichroism (CD) of optically active aromatic compounds have revealed that in some compounds, $^1L_a$ and $^1L_b$ transitions of the benzene ring in Platt's notation[1] are optically active.[2,3] It has also been reported that the $^1L_b$ transition of the benzene ring is optically active in such aromatic amino acids as tyrosine,[3] tryptophan,[3] and phenylalanine.[4] However the absorption maximum corresponding to the $^1L_a$ transition is situated so close to that of the $n\to\pi^*$ transition of the carboxyl group that it is not obvious whether the $^1L_a$ transition of aromatic amino acid is optically active or not.

Sakota (one of the present authors) has previously shown[5] that the CD curve of an aromatic amino acid is closely related to that of its acryloyl derivative, although a new CD maximum around 230 mμ appears in the acryloyl derivatives of aliphatic amino acids. These facts suggest that a Cotton effect in 220 mμ regions of aromatic amino acids corresponds to the phenyl chromophore rather than to the carboxyl chromophore. Furthermore, in comparing the ORD curves of phenylalaninol and tyrosinol with those of the corresponding amino acids, Rosenberg[6] has suggested that the $^1L_a$ transition of the benzene ring is optically active.

In the present experiment, the present authors have measured the ORD and CD curves of D-

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[*1] Studies of Optical Rotatory Dispersion, Part V. Part IV: N. Sakota, T. Tanigaki, K. Okita and N. Koine, *Nippon Kagaku Zasshi*, **90**, 593 (1969).

[*2] Presented at the 22nd Annual Meeting of the Chemical Society of Japan, Tokyo, 1969.

1) J. R. Platt, *J. Chem. Phys.*, **16**, 832 (1948).

2) L. Verbit and Y. Inoue, *J. Amer. Chem. Soc.*, **89**, 5717 (1967).

3) E. Iizuka and J. T. Yang, *Biochem.*, **3**, 1519 (1964).

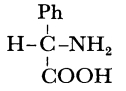
4) A. Moscowitz, A. Rosenberg and A. E. Hansen, *J. Amer. Chem. Soc.*, **87**, 1813 (1965).

5) N. Sakota, *Nippon Kagaku Zasshi*, **89**, 425 (1968).

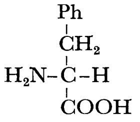
6) A. Rosenberg, *J. Biol. Chem.*, **241**, 5119 (1966).

phenylglycine and L-phenylalanine at various pH's, and also, the ORD and CD curves of three more optically active aromatic amino acids synthesized. The results gave direct support to the above-mentioned suggestions, indicating the screening effect of the methylene groups which exist between an asymmetric carbon atom and a benzene ring in aromatic amino acids.

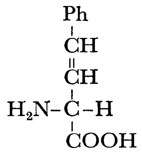
Table 1


$\begin{array}{c}\text{Ph}\\ |\\ \text{H-C-NH}_2\\ |\\ \text{COOH}\end{array}$

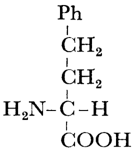
D-Phenylglycine (I)


$\begin{array}{c}\text{Ph}\\ |\\ \text{CH}_2\\ |\\ \text{H}_2\text{N-C-H}\\ |\\ \text{COOH}\end{array}$

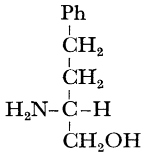
L-Phenylalanine (II)


$\begin{array}{c}\text{Ph}\\ |\\ \text{CH}\\ \|\\ \text{CH}\\ |\\ \text{H}_2\text{N-C-H}\\ |\\ \text{COOH}\end{array}$

L-α-Amino-β-benzalpropionic acid (III)


$\begin{array}{c}\text{Ph}\\ |\\ \text{CH}_2\\ |\\ \text{CH}_2\\ |\\ \text{H}_2\text{N-C-H}\\ |\\ \text{COOH}\end{array}$

L-α-Amino-β-benzylpropionic acid (IV)


$\begin{array}{c}\text{Ph}\\ |\\ \text{CH}_2\\ |\\ \text{CH}_2\\ |\\ \text{H}_2\text{N-C-H}\\ |\\ \text{CH}_2\text{OH}\end{array}$

L-2-Amino-4-phenylbutanol-1 (V)

## Experimental

**Materials.** Chromatographically- and optically-pure D-phenylglycine (I) and L-phenylalanine (II) were purchased from the Yoneyama Chemical Industry Co., Ltd.

**Preparations.** *L-α-Amino-β-benzalpropionic Acid (III).* DL-α-Amino-β-benzalpropionic acid (11 g, 0.0615 mol), prepared from cinnamaldehyde and sodium cyanide by the method of Ref. 7, was dissolved in 32 m*l* of 2N NaOH (0.062 mol). To this solution 6.4 g (0.062 mol) of acetic anhydride and 32 m*l* (0.062 mol) of 2N NaOH were added at 0°C over a period of about 1 hr. Then, the mixture was stirred for one more hour, after which the pH of the solution was adjusted with 6N HCl to 2.0 and the acetyl-DL-α-amino-β-benzalpropionic acid thus precipitated was filtered out in order to dry it. The yield was 11 g (81%), mp 133—134°C. Acetyl-DL-α-amino-β-benzalpropoinic acid (11 g, 0.05 mol) was dissolved in 25 m*l* of 2N LiOH and adjusted to pH 7.5, the clear upper solution of a suspension of 2.5 g of Biodiastase (purchased from the Amano Pharmaceutical Co., Ltd.) in 10 m*l* of water was then added. After the mixture had stood at 37°C for 5 hr, the crystalline precipitate (III) was filtered off and recrystallized from methanol-water. Yield 3 g (75%), $[\alpha]_D^{20}$ −20° (*c* 1, 0.1N HCl).

Found: C, 67.50; H, 6.10%. Calcd for $C_{10}H_{11}O_2N$: C, 67.77; H, 6.45%.

*L-α-Amino-β-benzylpropionic Acid (IV).* L-α-Amino-β-benzalpropionic acid (2.5 g, 0.014 mol) was dissolved in 10% acetic acid, 200 mg of 10% palladium charcoal (purchased from the Kawaken Fine Chemical Co., Ltd.) was added, and the mixture was hydrogenated at room temperature for 5 hr. Then, the catalyst was filtered off and the filtrate was concentrated *in vacuo.* IV was precipitated by the addition of methanol and filtered off. Yield 2.3 g (95%), $[\alpha]_D^{20}$ −18° (*c* 1, 0.1N HCl). Ref.[8] $[\alpha]_D^{20}$ −17.7° (*c* 1, 0.1N HCl).

*L-2-Amino-4-phenylbutanol-1 (V).* L-α-Amino-α-benzylpropionic acid (2 g, 0.012 mol) was added to a solution of 1.2 g (0.032 mol) of $LiAlH_4$ in 30 m*l* of ether over a 20-min period. Stirring was continued for 30 more min, and then water was added to decompose the unreacted $LiAlH_4$. The precipitate was filtered off and washed with ether. The ether solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo.* The crystalline material was filtered off and recrystallized from ethyl acetate. Yield 0.4 g (24.4%), mp 95—96°C, $[\alpha]_D^{20}$ −12° (*c* 1, MeOH).

Found: C, 72.60; H, 9.21%. Calcd for $C_{10}H_{15}ON$: C, 72.65; H, 9.28%.

**Measurements.** The optical rotatory dispersion and circular dichroism were measured at room temperature with a Jasco-model ORD/UV-5 optical rotatory dispersion recorder with a CD attachment. The cells used were 1 mm long except for III (0.1 mm). Samples of I and II were dissolved in distilled water (about 0.1—0.5 mg/m*l*), and the pH was adjusted with aqueous sodium hydroxide or hydrochloric acid.

## Results and Discussion

The CD curves of D-phenylglycine (I) and L-phenylalanine (II) are shown in Fig. 1 and Fig. 2 respectively. The pH-dependent CD measure-

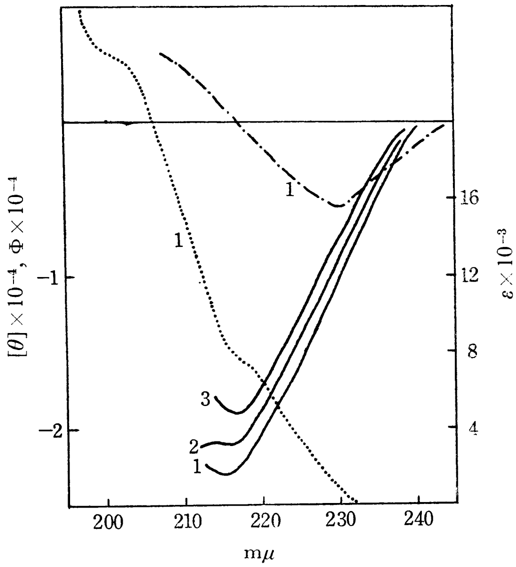


Fig. 1. CD (———), ORD (—•—) and UV (·········) spectra of D-phenylglycine (I).
(1: pH 2.45, 2: pH 6.40, 3: pH 9.75)

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7) S. Pinner and A. Spilker, *Ber.*, **22**, 689 (1989).

8) P. E. Gagnon and B. Nolin, *Can. J. Res.*, **B27**, 742 (1949).

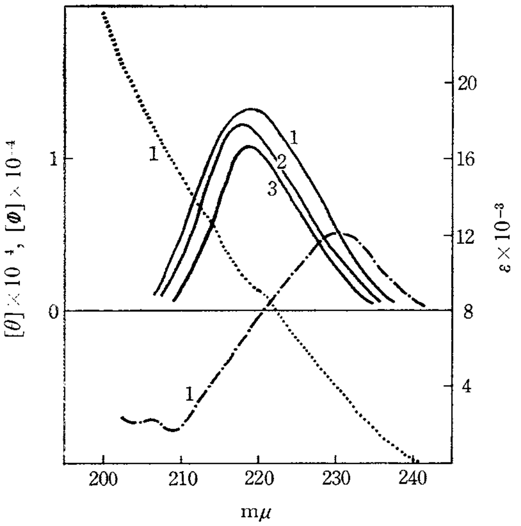


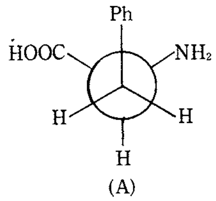
Fig. 2. CD (———), ORD (—·—) and UV (·········) spectra of L-phenylalanine (II).
(1: pH 2.45, 2: pH 6.40, 3: pH 9.81)

ments had little influence on the wavelength of the CD maximum in the 220 mμ region, though in the case of aliphatic amino acids dramatic changes have been reported.[9] These facts suggest that the CD maximum in the 220 mμ region for I and II is attributable to the aromatic chromophore rather than to the carboxyl chromophore. However, the 220 mμ CD maximum should be partly ascribed to the carboxyl $n\rightarrow\pi^*$ transition, because the superficial small pH dependence of the 220 mμ CD maximum observed in these two aromatic amino acids is presumably associated with the anomalous large contribution of the $^1L_a$ transition of the aromatic chromophore.

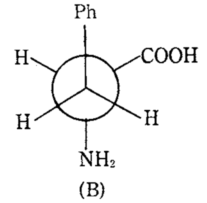
In fact, the molecular ellipticities $[\theta]$ at the 220 mμ CD maximum of I and II are about 20000—23000 and 11000—13000 respectively, though the aliphatic amino acids show the value of 3000—5000,[9] and the aromatic compounds without a carboxyl group, about 3000.[6] Therefore, it may be deduced that the 220 mμ CD maximum in aromatic amino acids should not be ascribed to the mere sum of the contributions of an aromatic chromophore and a carboxyl chromophore, but, rather, to the results of the interaction of the two chromophores.

Lucas[10] measured the UV spectra of the compounds with the general formulae of $C_6H_5(CH_2)_n$-$CH{:}CH_2$ and $C_6H_5(CH_2)_nCH(COOH)CH_2COOH$; he suggested the presence of the interaction of the two chromophores in the case of $n=1$. As in the case of I, there exists an aromatic chromophore interacted with a carboxyl group through an asymmetric carbon atom, it can be proved that I should show a $[\theta]$ value larger than the sum of the contributions of the two chromophores.

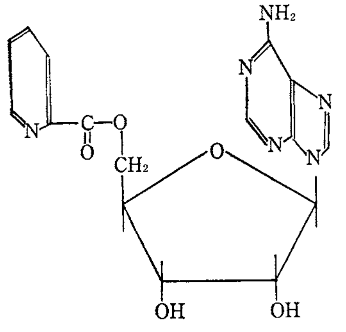
On the other hand, II contains a methylene group and an asymmetric carbon atom between carboxyl and phenyl chromophores, so it is difficult to explain the relatively large $[\theta]$ value at 220 mμ by Lucas's theory. Recently, Cavanaugh[11] mentioned that in light of the NMR measurements, the conformer, (A) or (B), in which the phenyl group



(A)



(B)



(C)

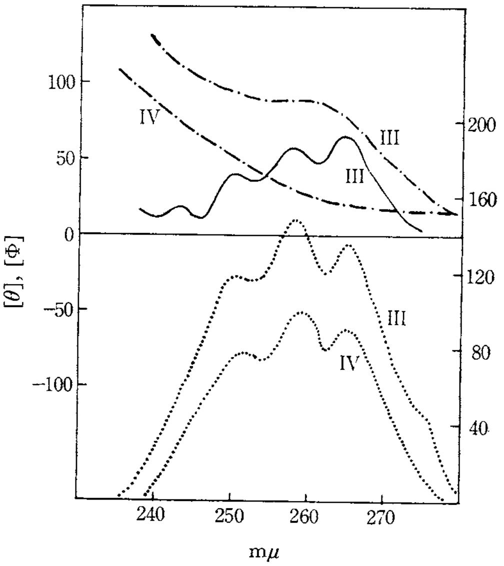


Fig. 3. CD (———), ORD (—·—) and UV (·········) spectra of L-α-amino-β-benzalpropionic acid (III) and L-α-amino-β-benzylpropionic acid (IV) (in 0.1N HCl).

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[9] M. Legland, *Bull. Soc. Chim. Fr.*, **1965**, 679.
[10] R. Lucas, *ibid.*, **1932**, 51, 289, 965.
[11] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, **90**, 4533 (1968).

is gauche to the carboxyl group is considerably predominant in the phenylalanine anion and in the dipolar ion. Urry[12] has also concluded that the large molecular ellipticity for adenosine-5′-mononicotinate (C) is caused by the reciprocal relation between the adenine and the nicotinic acid residue. In phenylalanine, it may be suggested that the benzene-ring plane can run nearly parallel to the carboxyl group, therefore, the $[\theta]$ value at 220 m$\mu$ becomes larger by means of interaction between the aromatic $^1L_a$ transition and the carboxyl $n \rightarrow \pi^*$ transition.

Figure 3 shows the ORD, CD, and UV spectra in the 260 m$\mu$ region for L-$\alpha$-amino-$\beta$-benzalpropionic acid (III) and L-$\alpha$-amino-$\beta$-benzylpropionic acid (IV) in 0.1 N HCl. III reveals a multiple-humped positive CD maximum corresponding to the 250—265 m$\mu$ absorption maximum, and a weak Cotton effect in the ORD curve, these characteristics show that the $^1L_b$ transition of III is optically active. Although IV also shows the multiple-humped absorption band of the $^1L_b$ transition in 250—265 m$\mu$, neither the CD maximum nor the anomalous dispersion curve are observed. The results imply that the $^1L_b$ transition is not optically active in the case of IV. From these facts, it is evident that the $^1L_b$ transition of the compounds with the $C_6H_5(CH_2)_nCH(NH_2)COOH$ formula is optically active in $n=0$ or $n=1$, but inactive in $n=2$.

The ORD, CD and UV spectra for III and IV in the 220 m$\mu$ region are shown in Fig. 4. The ORD and CD curves of III shows the CD maximum and an anomalous dispersion, these characteristic may be explained in a manner similar to that used for I. Although IV has a CD maximum at 207 m$\mu$, it is not obvious that this CD maximum is to be attributed to either the $^1L_a$ transition of the aromatic chromophore or to the $n \rightarrow \pi^*$ transition of the carboxyl chromophore. The CD, ORD and UV spectra of L-2-amino-4-phenylbutanol-1 (V), which was synthesized from IV by the reduction of the carboxyl group, were measured in 0.1 N HCl (Fig. 5).

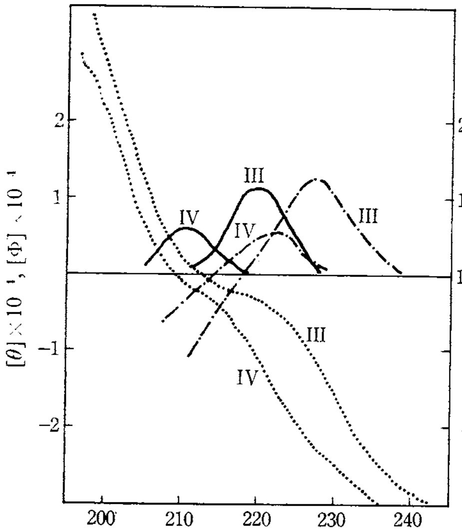

Fig. 4. CD (———), ORD (—·—) and UV (·········) spectra of L-$\alpha$-amino-$\beta$-benzalpropionic acid (III) and L-$\alpha$-amino-$\beta$-benzylpropionic acid (IV).

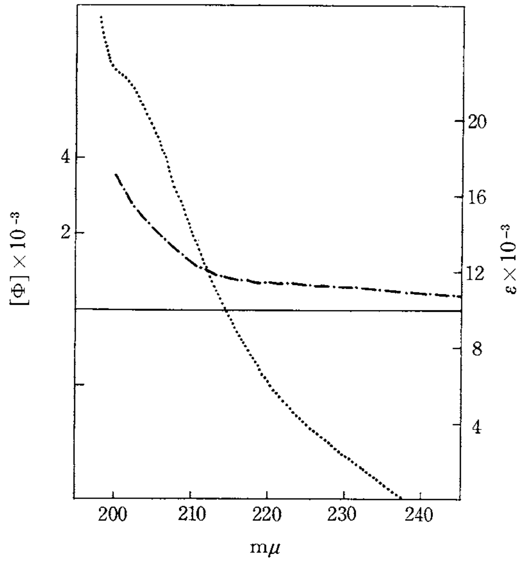

Fig. 5. ORD (—·—) and UV (·········) spectra of L-2-amino-4-phenylbutanol-1 (V) (in 0.1 N HCl).

V had a large shoulder of $^1L_a$ transition in the 200—205 m$\mu$ region of the UV absorption spectrum, but no anomalous dispersion was observed. Therefore, it is evident that the $^1L_a$ transition of V is not optically active; this implies that the 207 m$\mu$ CD maximum of IV is to be attributed not to the $^1L_a$ transition of the benzene ring but to the carboxyl $n \rightarrow \pi^*$ transition.

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[12] D. W. Miles and D. W. Urry, *J. Phys. Chem.*, **71**, 4448 (1967).