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# Studies on optically Active Amino Acids. XVII.[1] Studies on α-Alkyl-α-amino Acids. XIII.[2] Absolute Configuration and Circular Dichroism in Some α-Methyl-α-amino Acids

Shun-ichi Yamada, Kazuo Achiwa, Shiro Terashima, Hatsuhiko Mizuno, Norio Takamura[3a] and Maurice Legrand[3b]

*Faculty of Pharmaceutical Sciences, University of Tokyo[3a] and Centre de Recherches Roussel-Uclaf.[3b]*

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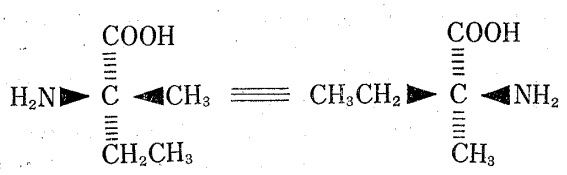
The CD curves of α-methyl-α-amino acids of known absolute configurations such as (*S*)(+)-isovaline, (*S*)(+)-α-methylaspartic acid, (*S*)(+)-α-methylserine, (*S*)(+)-α-methylphenylglycine and (*S*)(−)-α-methylphenylalanine were measured in acidic, neutral and alkaline medium between 185—250 mμ, and the signs of CD maxima were discussed from the stereochemical properties of α-methyl-α-amino acids.

In our previous papers the absolute configuration of the representative α-methyl-α-amino acids such as (+)-isovaline,[4] (+)-α-methylaspartic acid,[5] (−)-α-methyl DOPA,[5a,6] (−)-α-methylphenylalanine,[7] (+)-α-methyphenylglycine,[8] (+)-α-methylserine,[9] and others[4–9] has been elucidated to be (*S*)-series[4b,10] by their chemical correlations with glyceraldehyde. Different from naturally occurring α-amino acids, the absolute configuration of α-methyl-α-amino acids can be assigned to be both an L and a D series depending upon the consideration as to which is the main skeleton of the amino acids as shown in Chart 1, that is, it can be readily seen that optically active α-methyl-α-amino acids have optical properties of both D and L-series. Therefore, the investigation of relationships between the absolute configurations of α-methyl-α-amino acids and their optical properties seems to be very attractive and important from the point of the assignment of absolute configuration by optical methods.




L-α-methylbutyrine D-α-ethylalanine
(*S*)(+)-isovaline

Chart 1

In this paper, we present the results obtained from circular dichroism (CD) measurements in acidic, neutral and basic mediums on a series of above mentioned α-methyl-α-amino acids whose absolute configurations were determined in our laboratory.

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3) Location: *a*) *Hongo, Tokyo*; *b*) *Romainville Seine France* (*Maurice Legrand*).
4) *a*) S. Yamada and K. Achiwa, *Chem. Pharm. Bull.* (Tokyo), **12**, 1525 (1964); *b*) K. Achiwa and S. Yamada, *ibid.*, **14**, 537 (1966).
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10) For a full discussion of the (*R*) and (*S*) notation for defining the absolute configuration, see R.S. Cahn, C.K. Ingold and V. Prelog, *Experientia*, **12**, 81 (1956).

The use of anomalous ORD measurements has not hitherto been applied to the assignment of absolute configuration of α-amino acids since only uncharacteristic plain dispersion curves were observed in the range extending to 240 mμ[11] from 600 mμ. Recently however, a new instrument for the measurement of ORD curves has become available which permits the measurement down to nearly 200 mμ where a Cotton effect by the $n \rightarrow \pi^*$ transition of carboxyl group (–COOH) or carboxylate anion ($-COO^-$) was observed and all L-amino acids derived from protein were found to show positive Cotton effect curves at about 210—220 mμ.[12]

On the other hand, one[13] of the present authors has measured CD curves on a series of α-amino acids down to 185 mμ, and in the case of L-amino acids which have no other chromophores (*i.e.* imidazole, indole and phenyl) than carboxyl group or carboxylate anion, positive CD maximum was always observed at 208—209 mμ in an acidic solution, 204 mμ (200—207 mμ) in a neutral solution and near 214 mμ (202—217 mμ) in a basic solution.

The ORD curves of some α-methyl-α-amino acids, *i.e.* isovaline[14] α-methyl DOPA[15] α-methylserine[16] and α-ethylserine[16] have been measured down to 300 mμ from higher wave

Table I. Circular Dichroism Data for (*S*)-α-Methyl-α-amino Acids in Various Mediums

| Medium | 3N HCl $\lambda_{max}$ (mμ) | $\Delta\varepsilon$ | $H_2O$ $\lambda_{max}$ (mμ) | $\Delta\varepsilon$ | 1N NaOH $\lambda_{max}$ (mμ) | $\Delta\varepsilon$ |
|---|---|---|---|---|---|---|
| (*S*)-Isovaline | 212 | +0.16 | 200 | +0.14 | 223.2 | +0.14 |
| (*S*)-α-Methylserine | 212 | +0.13 | 184 | +0.22 | | |
| (*S*)-α-Methylaspartic Acid | 200 | −0.11 | 201 | +0.45 | 222 | −0.10 |
| | | | 241 | −0.004 | | |
| (*S*)-α-Methylphenylglycine | | | 193[a] | −6.30 | 212.5 | +3.15 |
| | 218 | +6.64 | 202 | +6.23 | | |
| | 248 | −0.048 | 248 | −0.605 | 247.5 | −0.01 |
| | 254 | −0.13 | 254 | −0.091 | 255 | −0.021 |
| | 264 | −0.18 | 261.5 | −0.13 | 261 | −0.041 |
| | 267 | −0.15 | 267 | −0.12 | 268 | −0.037 |
| (*S*)-α-Methylphenylalanine[b] | 195[a] | −0.59 | 195[a] | −0.20 | | |
| | 215 | +0.41 | 217 | +0.18 | 217 | +2.05 |
| | 225 | −0.065 | 222 | −0.082 | | |
| | | | 243 | −0.010 | | |
| | 248 | −0.015 | 248 | −0.015 | | |
| | | | 252 | −0.015 | | |
| | 255 | −0.019 | 255 | +0.003 | | |
| | 258 | +0.003 | 258.5 | +0.007 | | |
| | 262 | −0.020 | 262 | −0.014 | | |
| | 267.5 | −0.015 | 265 | +0.006 | | |

*a*) These values show the $\Delta\varepsilon$ at the lowest wave lengths measured.
*b*) pH 2.7 in HCl and pH 7.4 in $H_2O$

11) *a*) C. Djerassi, "Optical Rotatory Dispersion, Application to Organic Chemistry", McGraw-Hill, New York, 1960, Chapter 12; *b*) J. Stem, Y.S.R. Krisha-Prasad and J.A. Schellman, *Tetrahedron*, **13**, 176 (1961).
12) *a*) J.P. Jennings and W. Klyne, *Biochem. J.*, **1963**, 86; *b*) I.P. Dirkx and F.L.J. Sixma, *Rec. Trav. Chim.*, **83**, 522 (1964); *c*) W. Gaflield, *Chem. Ind.* (London), **1964**, 1460; *d*) J.P. Jennings, W. Klyne and P.M. Scopes, *J. Chem. Soc.*, **1965**, 294; *e*) D.W. Urry and H. Eyring, *J. Am. Chem. Soc.*, **86**, 4574 (1964); *f*) E. Iizuka and J.T. Yang, *ibid.*, **86**, 1519 (1964); *g*) A. Kjaer, W. Klyne, P.M. Scopes and D.R. Sparrow, *Acta. Chem. Scand.*, **18**, 2412 (1964); *h*) J.C. Craig and S.K. Roy, *Tetrahedron*, **21**, 391 (1965).
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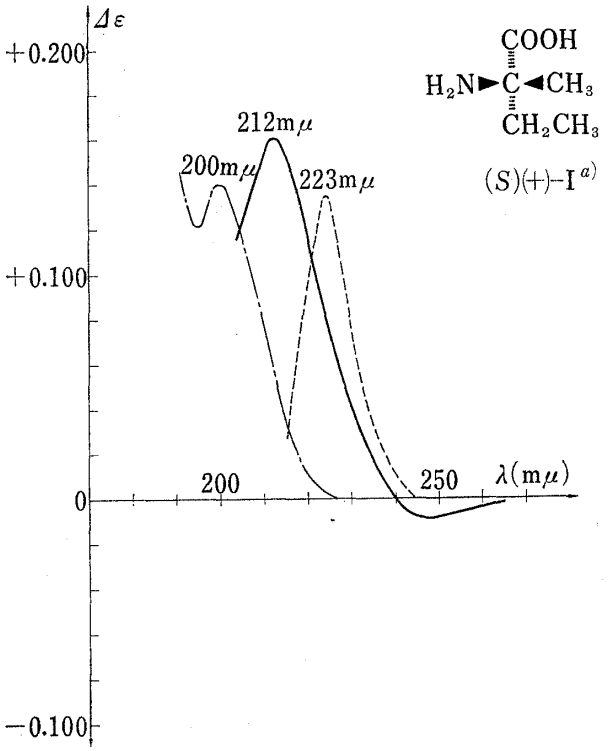


Fig. 1. CD Curves of (*S*)(+)-Isovaline

——: in 3N HCl —·—: in $H_2O$
- - - -: in 1N NaOH
*a*) (*R*)-Isomer was used for measurements.

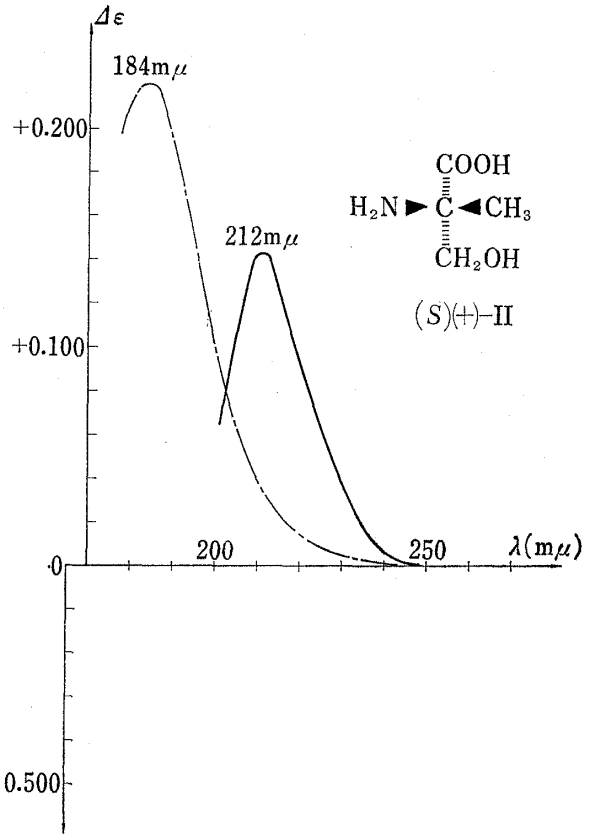


Fig. 2. CD Curves of (*S*)(+)-α-Methylserine

——: in 3N HCl
—·—: in $H_2O$

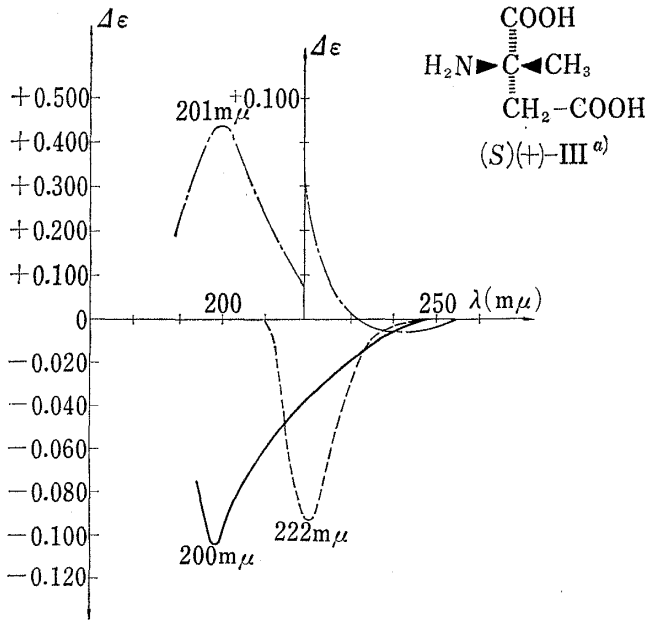


Fig. 3. CD Curves of (*S*)(+)-α-Methylaspartic Acid

——: in 3N HCl —·—: in $H_2O$ - - - -: in 1N NaOH
*a*) (*R*)-Isomer was used for measurements.

lengths by several researchers. However, in these cases it was impossible to observe a Cotton effect caused by a $n\rightarrow\pi^*$ transition of the carboxyl group or the carboxylate anion.

We have therefore measured CD curves of a series of the following α-methyl-α-amino acids; (*S*)(+)-isovaline (I), (*S*)(+)-α-methylserine (II), (*S*)(+)-α-methylaspartic acid (III), (*S*)(+)-α-methylphenylglycine (IV), and (*S*)(−)-α-methylphenylalanine (V) in solution in 3N hydrochloric acid, in water and in 1N sodium hydroxide down to the vicinity of 180 mμ to reach the region of the $n\rightarrow\pi^*$ transition of the carboxyl group or the carboxylate ion and the results are presented in Table 1 and Fig. 1—5 for respective amino acids.

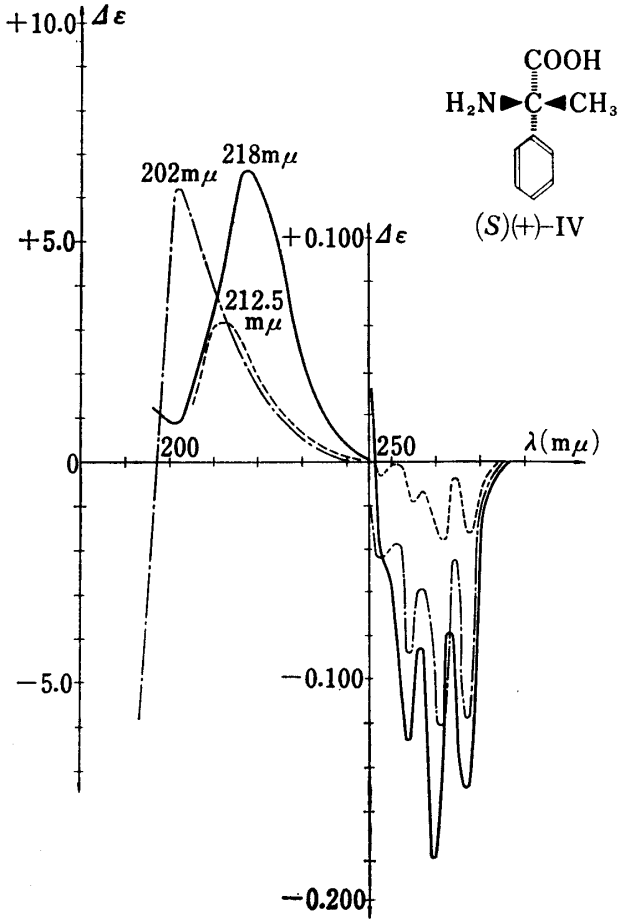


Fig. 4. CD Curves of (*S*)(+)-α-Methylphenylglycine

——: in 3N HCl ——: in $H_2O$
- - - -: in 1N NaOH

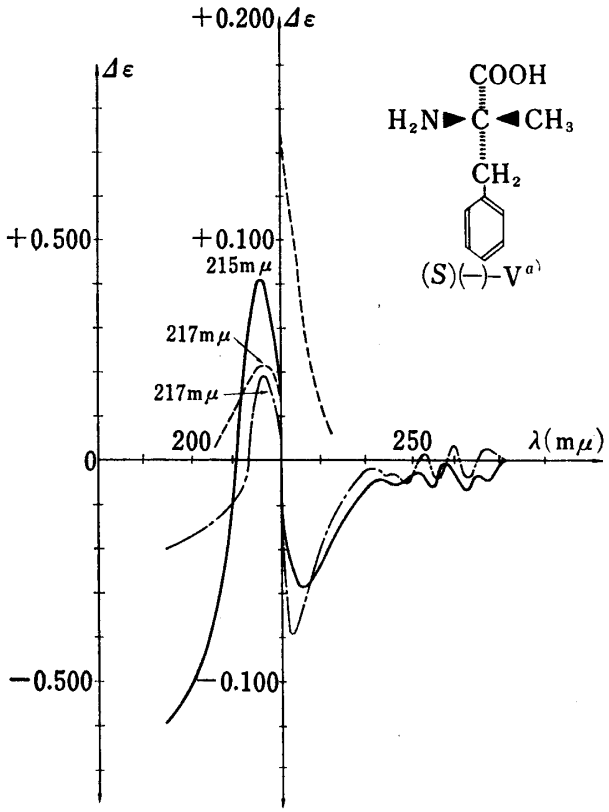


Fig. 5. CD Curves of (*S*)(+)-α-Methylphenylalanine

——: in HCl (pH 2.7) ——: in $H_2O$ (pH 7.4)
- - - -: in 1N NaOH
*a*) (*R*)-Isomer (HCl) was used for measurements.

It was observed that (*S*)(+)-I always shows a positive CD maximum in aqueous acidic, neutral and alkaline solutions (Fig. 1) in the range of 200—223 mμ (Fig. 1). This result is the same as for the L-amino acids which always showed a positive Cotton effect in aqueous solution in the pH range from 1 to 13.[13] Similar results in which the sign of the CD curve is constantly positive in acidic, neutral and alkaline solutions were observed in the case of (*S*)(+)-IV and (*S*)(−)-V (Fig. 4 and 5). However, for (*S*)(+)-III of the same configuration, (*S*)(+)-III in neutral solution exhibit a positive sign, whereas (*S*)(+)-III in acidic and alkaline solutions reverses the sign of CD maxima and display a negative sign. The sign varies according to the acidity and basicity of the medium. (Fig. 3). Thus, it is to be noted that the assignment of the absolute configuration of α-methyl-α-amino acids by the sign of CD maximum between about 200 mμ and 220 mμ is found to be impossible since the sign of CD maximum is very sensitive to the acidity of the medium.

It is thought that the location of CD maximum in acidic solution is due to the $n\rightarrow\pi^*$ transition of carboxyl group and in neutral and alkaline solutions due to that of carboxylate ion (Chart 2). However, the wave lengths of $\lambda_{max}$ and the amplitudes ($\Delta\varepsilon$) described in Table I provide no special information. Attempts at a theoretical interpretation of these results seem premature, but the explanation might be given as follows.

As shown in Chart 3, α-methyl-α-amino acid can be divided into two parts by means of a plane A in which there exist N–$C_2$ and $C_1$–$C_2$ bondings, hence two substituents $R_1$ and $R_2$ are situated symmetrically against plane A. A carboxylate anion can be located in the plane

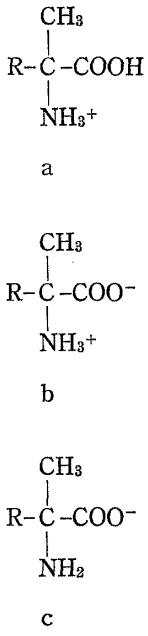


Chart 2

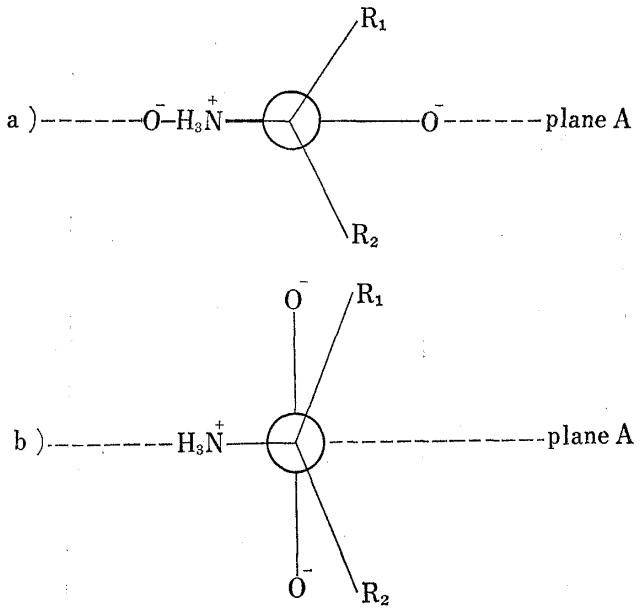


Chart 3

(Chart 3-b) perpendicular to plane A or in a plane A (Chart 3-a). When an $n\rightarrow\pi^*$ transition of carboxylate anion takes place in a plane perpendicular to a plane A (Chart 3-b) or in a plane A (Chart 3-a), it is assumed that the Amplitude ($\Delta\varepsilon$) and its sign of $\alpha$-methyl-$\alpha$-amino acids in CD measurements depend only upon the difference of the perturbating contributions from two substituents at the asymmetric center. Hence, the $\Delta\varepsilon$ value of $\alpha$-methyl-$\alpha$-amino acids is expected to be estimated as the sum of the $\Delta\varepsilon$ value of the two constituents at $\alpha$-position, that is, the $\Delta\varepsilon$ value of (*S*)-isovaline (I) is considered to be the sum of $\Delta\varepsilon$ values of L-butyrine and D-alanine whose $\Delta\varepsilon$ values were already reported by Legrand.[13] The expected $\Delta\varepsilon$ of (*S*)-isovaline in neutral solution is +0.07 which was calculated from the results that $\Delta\varepsilon$ value of L-butyrine and D-alanine at pH 7.0 are +0.75 ($\lambda_{max}$ 204 m$\mu$) and −0.68 ($\lambda_{max}$ 204 m$\mu$) respectively. This calculated value is in agreement with the experimental value +0.14 ($\lambda_{max}$ 200 m$\mu$). In the case of the carboxyl group which is present in acidic solution, it is clear that the symmetry of the carboxyl group is less than that of the carboxylate anion, but roughly speaking, the same explanation, as above, might be allowed in the cases of carboxyl groups (in an acidic solution). For example,, the experimental value $\Delta\varepsilon$ of (*S*)(+)-isovaline in an acidic solution is +0.16, the calculated value is +0.15 which was derived from the results of the $\Delta\varepsilon$ value of L-butyrine and D-alanine which was reported to be +1.19 ($\lambda_{max}$ 209 m$\mu$)

Table II. The Comparison of the Experimental and Calculated $\Delta\varepsilon$ of (*S*)-$\alpha$-Methyl-$\alpha$-amino Acids in Various Mediums

| (*S*)-series | Acidic medium ($\Delta\varepsilon$) Experimental (3N HCl) | Acidic medium ($\Delta\varepsilon$) Calculated pH 1.0 | Neutral medium Experimental $H_2O$ | Neutral medium Calculated pH 7.0 | Alkaline medium Experimental 1N NaOH | Alkaline medium Calculated pH 13.0 |
|---|---|---|---|---|---|---|
| Isovaline | +0.16 (212 m$\mu$)[a] | +0.15 (208—209 m$\mu$)[b] | +0.14 (200 m$\mu$) | +0.07 (204 m$\mu$) | +0.14 (223 m$\mu$) | +0.09 (214—216 m$\mu$) |
| $\alpha$-Methylserine | +0.13 (212 m$\mu$) | +0.22 (208 m$\mu$) | +0.22 (184 m$\mu$) | +0.10 (204 m$\mu$) | | |
| $\alpha$-Methylaspartic acid | −0.11 (200 m$\mu$) | −0.23 (208 m$\mu$) | +0.45 (201 m$\mu$) | +0.42 (202—204 m$\mu$) | −0.10 (222 m$\mu$) | +1.62 (204—214 m$\mu$) |
| $\alpha$-Methylphenylalanine | +0.41 (215 m$\mu$) | +3.46 (208—218 m$\mu$) | +0.18 (217 m$\mu$) | +2.32 (204—218 m$\mu$) | +2.05 (217 m$\mu$) | +2.47 (214—216 m$\mu$) |

*a*) figures in the parenthesis of experimental column show the wave lengths of CD maximum of $\alpha$-methyl-$\alpha$-amino acids
*b*) figures in the parenthesis of calculated column show the wave lengths of CD maxima of component D and L-amino acids

and −1.04 ($\lambda_{max}$ 208 m$\mu$).[13] As shown in Table 2, besides (*S*)(+)-isovaline the experimental signs of the CD maxima of (*S*)-II, (*S*)-III and (*S*)-V in various media were found to be in accordance with the calculated ones; with one exception the case of (*S*)-α-methylaspartic acid in alkaline solution. The sign of CD maxima of α-methyl-α-amino acids is much more complicated than the case of α-amino acids, but this may be well explained by considering these acids as the above.

## Experimental

CD curves were measured with the Roussel-Joan Dichrograph CD 185. The measurements were performed in 1 cm cell in the range 600—240 $cm^{-1}$ and in 0.01 cm between 240—190 $cm^{-1}$ wave number.

All the optically active α-methyl-α-amino acids such as (*R*)(−)-isovaline, (*S*)(+)-α-methylserine, (*R*)(−)-α-methylaspartic acid, (*S*)(+)-α-methylphenylglycine and (*R*)(+)-α-methylphenylalanine hydrochloride, were prepared in our laboratory according to the respective references.[4-9]

The properties of each amino acid and the conditions for CD measurements are as follows. The $\Delta\varepsilon$ value at every wave length is shown in Table 1 and Figs. 1—5.

**(*R*)(−)-Isovaline**[4,5]——$[\alpha]_D^{21}$ −11.9 ($c$=0.786, $H_2O$), mp >250° Concentrations for CD measurements, 5.70 mg in 5 ml of 3N HCl, 5.70 mg in 5 ml of $H_2O$, and 5.04 mg in 5 ml of 1N NaOH.

**(*R*)(−)-α-Methylaspartic Acid**[5,7]——$[\alpha]_D^{17}$ −52.9° ($c$=0.680, $H_2O$), mp 256—257° (decomp.). Concentrations for CD measurements, 5.97 mg in 5 ml of 3N HCl, 6.33 mg in 5 ml of $H_2O$, and 6.09 mg in 5 ml of 1N NaOH.

**(*S*)(+)-α-Methylserine**[9]——$[\alpha]_D^{19}$ +4.7° ($c$=0.888, $H_2O$), mp 264° (decomp.). Concentrations for CD measurements, 4.52 mg in 5 ml of 3N HCl, 5.35 mg in 5 ml of $H_2O$.

**(*S*)(+)-α-Methylphenylglycine**[8]——$[\alpha]_D^{25}$ +84.3° ($c$=0.976, 1N HCl), mp>250°. Concentrations for CD measurements, 4.89 mg in 5 ml of 3N HCl, 5.1 mg in 5 ml of $H_2O$, and 5.17 mg in 5 ml of 1N NaOH.

**(*R*)(+)-α-Methylphenylalanine Hydrochloride Monohydrate**[7]——$[\alpha]_D^{15}$ +6.6° ($c$=1.034, $H_2O$), mp 210—213° (decomp.). Concentration for CD measurements, 6.62 mg in 5 ml of $H_2O$ adding HCl to pH 2.7, 6.56 mg in 5 ml of $H_2O$ whose pH value was adjusted to 7.4 with NaOH solution, and 5.33 mg in 5 ml of 1N NaOH.