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Formation of Aggregates of *N*-Acyl-L-amino Acids in Methanol Solutions. An Origin of CD

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CD, UV, and FT-IR measurements were carried out on methanol solutions of *N*-dodecanoyl-L-glutamic acid and of *N*-dodecanoyl-L-valine over a concentration range of 5×10^{-3}—1×10^{-1} M. A large positive CD band at 212 nm and a small negative one at 240 nm were observed. The positive CD decreased along with a simultaneous increase in the negative CD upon heating. Weak absorption due to a carboxylic carbonyl was found in the wavelength region where the CD bands were observed. The IR measurements reveal that carboxylic acid dimers are formed predominantly over the concentration range studied, and that an amide group does not take part in the formation of a hydrogen bond.

The two CD bands are explained in terms of the presence of two rotational conformers about the C_1–C_2 bond of the carboxylic group. This conclusion differs from the assignment of the positive CD band previously reported, which was ascribed to hydrogen bonding between the C*–COOH carbonyl and NH groups.

N-Acylamino acids are compounds of current interest, since they possess two hydrogen-bonding groups, CONH and COOH, and an asymmetric carbon which make it possible to form a chiral aggregate by hydrogen bonding. Sakamoto and Hatano[1)] reported a positive CD band at around 212 nm in methanol, 1-propanol, and 1-octanol solutions of *N*-acylamino acids, such as *N*-dodecanoyl-L-glutamic acid (DGA), *N*-dodecanoyl-L-valine (DV), *N*-acetyl-L-glutamic acid, and *N*-acetyl-L-valine, over a concentration range of 10^{-4}—10^{-1} M ($1\ \mathrm{M}=1\ \mathrm{mol\,dm^{-3}}$). They ascribed these CD bands to the formation of chiral intermolecular hydrogen bonds between the carbonyl of C*–COOH and the NH of the amide group. Their report attracted our attention, because we have been studying the formation of hydrogen bonds between amino acid derivatives in mixtures of water and alcohol.

We recognized, however, the following two uncertain points concerning their conclusions. The first is that "The CD band should be inherent to the solution system, since acylamino acids and alcohol, *per se*, have no absorption band at this wavelength." This sentence tells us that they observed the CD over a wavelength region where there is no light absorption by samples. This contradicts the definition of CD: that is, the difference in the absorbance between right- and left-circularly polarized light. We, therefore, considered that the CD signal might be due to differential light scattering of circularly polarized light by aggregates.[2)] However, as mentioned later, light scattering is not likely to occur in a methanol solution of DGA. This suggests the necessity of carrying out careful UV measurements of the solution.

The second point concerns their assignment of the CD band. They correlated the CD band with the formation of an intermolecular hydrogen bond on the basis of their observation of a decrease in the intensity of CD upon heating, or upon the addition of urea. There have been, however, many reports concerning positive CD bands at about 210 nm and negative ones at about 240 nm for both aqueous and ethanol solutions of α-substituted S-type carboxylic acids,[3—11)] though they did not refer to the negative CD band at all. The conclusion obtained so far can be summarized as follows: 1) Both the positive and negative CD bands are assigned to the n–π* transition of the carboxylic carbonyl, and are interpreted as indicating the presence of two rotational isomers.[3—11)] 2) The CD bands are not related to the formation of a hydrogen bond.[6,8,11)] Thus, the conclusions presented by Sakamoto and Hatano are different from these.

Apart from the assignment of the positive CD band, we wonder whether or not a hydrogen-bonding interaction would take place at such a low concentration, such as 10^{-4} M, in a hydrogen-bonding solvent like methanol. If it does occur, which of the four kinds of hydrogen bonds (Fig. 1) is formed predominantly?

In order to solve these problems, UV, CD, and FT-IR measurements were carried out on methanol solutions of DGA and DV in this work.

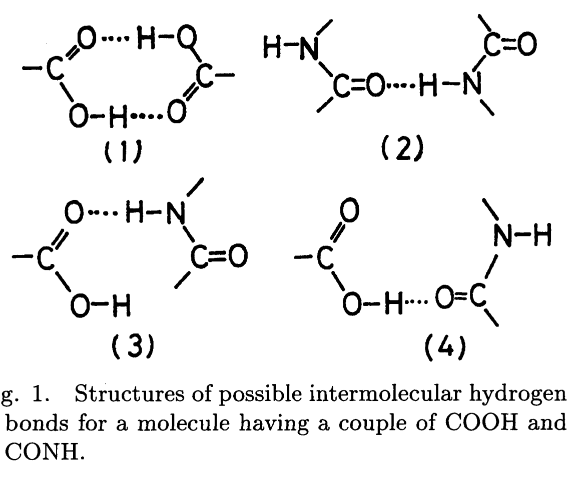

Fig. 1. Structures of possible intermolecular hydrogen bonds for a molecule having a couple of COOH and CONH.

Experimental

Materials. DGA and DV were synthesized according to a method reported by Takehara, et al.[12] The amino acids were converted to their sodium salts and then returned to acids after acylation. Complete acidification was confirmed by measuring the FAB (fast atom bombardment) mass spectra of the products. The mass spectra were acquired with a JEOL DX-303 mass spectrometer and processed with a JEOL DA-500 data system. Glycerol, or *m*-nitrobenzyl alcohol, were used as a liquid matrix. *N*-Ethylacetamide (Kanto Chemicals) and (*S*)-(+)-2-methylbutanoic acid (Sigma) were used as they were. Spectrograde methanol (Dohjin) was used as a solvent in all of the measurements.

Measurements. CD spectra were measured using a JASCO J-500A spectropolarimeter and 1 mm cells. A JASCO UVDEC-610 spectrophotometer was used to obtain the UV spectra. The use of 1 mm cells was essential for measurements due to the absorption by methanol in the wavelength region below 210 nm. IR spectra were obtained using a JASCO FT/IR-8000 spectrometer and a 0.1 mm cell at 4 cm^{-1} resolution using a liquid-nitrogen cooled mercury–cadmium–telluride detector. Measurements using a 0.5 mm cell were possible only over the wavenumber region above 1600 cm^{-1} due to methanol absorption.

Results and Discussion

Measurements of UV Spectra. The CD spectra of DGA and of DV are shown in Figs. 2 and 3, respectively. The spectra are almost the same as those reported by Sakamoto and Hatano,[1] except that the molar ellipticity that we obtained was larger than that reported. DGA has a large positive CD band with a peak at 212 nm and a small negative one with a peak at 243 nm. DV also has a negative CD band at 241 nm.

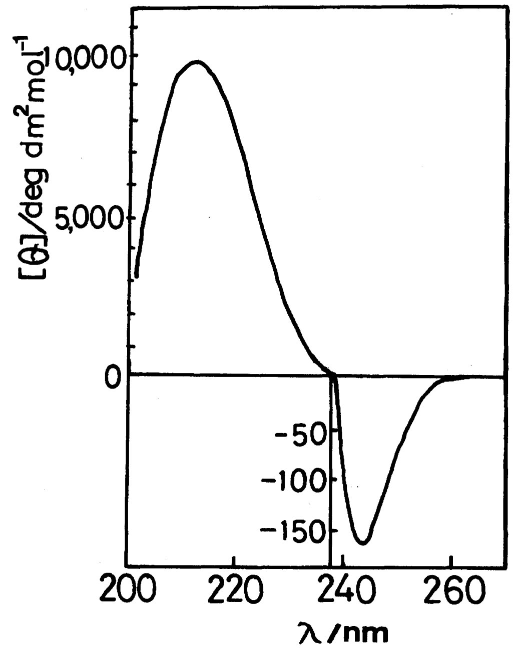

Fig. 2. CD spectrum of *N*-dodecanoyl-L-glutamic acid at 3.03×10^{-3} M.

The UV absorption spectra of DGA and DV in methanol are shown in Fig. 4. DGA and DV have a large asymmetric absorption band with a peak at 197 nm. The peak of the positive CD band does not correspond to that of the UV absorption band in both compounds, as can be seen from Figs. 2, 3, and 4. What should be noted here is that the solutions show weak absorption over the wavelength region of the positive and negative CD bands.

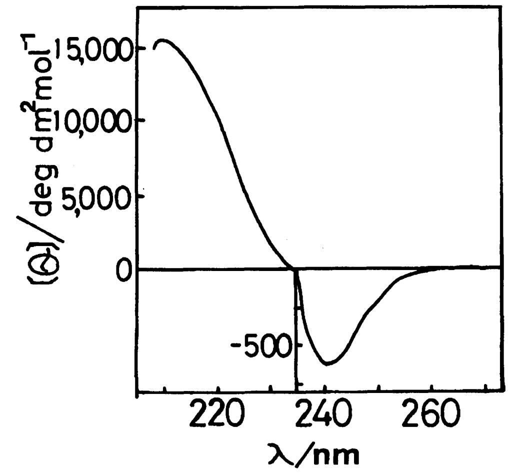

Fig. 3. CD spectrum of *N*-dodecanoyl-L-valine at 5.69×10^{-3} M.

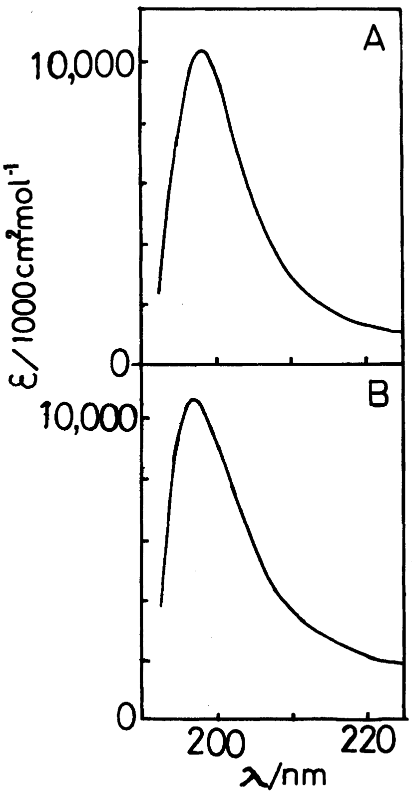

Fig. 4. UV spectra of (A) *N*-dodecanoyl-L-glutamic acid at 2.42×10^{-3} M, and of (B) *N*-dodecanoyl-L-valine at 4.47×10^{-4} M, in methanol solutions.

In order to assign the observed absorption and CD bands, the UV absorption spectra of the following two compounds were measured: *N*-ethylacetamide (EAA) that possesses only the carbonyl of CONH, and (*S*)-(+)-2-methylbutanoic acid ((*S*)-MBA) that has only the carbonyl of C*–COOH. The UV spectra of these two compounds are shown in Fig. 5. From a comparison between Figs. 4 and 5, the UV absorption band of DGA and of DV at 197 nm can be assigned to the amide carbonyl. Furthermore, we recognize that solutions of DGA and of DV have an absorption band due to the carboxylic carbonyl over the wavelength region where the CD bands of DGA and of DV are observed, although we can neither recognize it clearly as a peak nor a shoulder.

So far, we have investigated various problems concerning CD measurements resulting from systematic errors and artifacts which are inevitable in a modern CD spectrometer.[2,13—16] If chiral aggregates are formed in solution, there may be an apparent CD signal due to the differential scattering of circularly polarized light by them.[2] The result of light-scattering measurements indicates that the differential scattering by DGA does not likely occur. Together with the finding of the UV absorption band, the result concerning light scattering led us to the conclusion that the observed CD spectra are not apparent CD signals, but true ones.

Measurements of IR Spectra. FT-IR measurements were carried out on methanol solutions of DGA and of DV over a concentration range of 5×10^{-3}—1×10^{-1} M; almost the same spectra were obtained for the two compounds. Here, we analyzed only the IR spectra of DV, since an analysis of the IR spectra becomes much too complicated for DGA, due to the presence of two carboxylic groups.

In Fig. 6, the IR spectrum of DV at 1.0×10^{-1} M, the highest concentration studied, is shown over the wavenumber region of 1800—1500 cm^{-1}. A couple of bands having peaks at 1734 and 1718 cm^{-1} were assigned to free and hydrogen-bonding carboxylic carbonyls, respectively, showing that a considerable number of the carboxylic carbonyls take part in hydrogen bonding. The band at 1657 cm^{-1} can be assigned to the amide I band, while the band at 1552 cm^{-1} to the amide II band. No appreciable change was observed in the shape of the IR spectra along with a decrease in the concentration, except for a very small decrease in the intensity of the band for the hydrogen-bonding carboxylic carbonyl.

In the solution of a nonpolar solvent, such as carbon tetrachloride, both the amide I band at 1657 cm^{-1} and the amide II band at 1552 cm^{-1} can be assigned to an amide group that forms hydrogen bonding. However, it is necessary to confirm whether the above mentioned assignment is reasonable for a solution of polar solvents, like methanol. Thus, in order to assign the amide I and amide II bands observed in a methanol solution of DV to either a free or hydrogen-bonding amide group, the IR spectra of EAA in methanol were measured over the concentration range from 5×10^{-2} to 4 M. Figure 6 shows the IR spectrum of EAA at 1.0×10^{-1} M, together with that of DV. Two additional bands appeared at 1636 and 1560 cm^{-1}, other than the bands at 1660 and 1552 cm^{-1}, which are similar to those of DV. The ratio between the absorbance at 1636 cm^{-1} and that at 1660

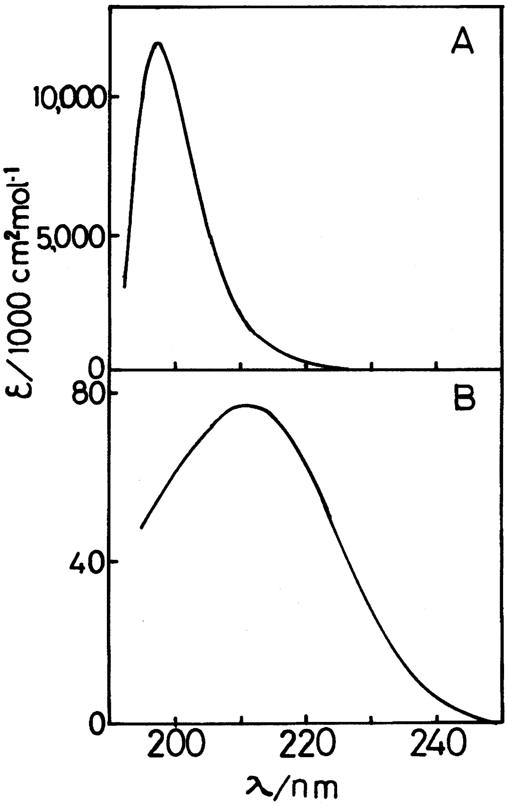

Fig. 5. UV spectra of (A) *N*-ethylacetamide at 5.40×10^{-4} M, and of (B) (*S*)-(+)-2-methylbutanoic acid at 1.57×10^{-2} M, in methanol solutions.

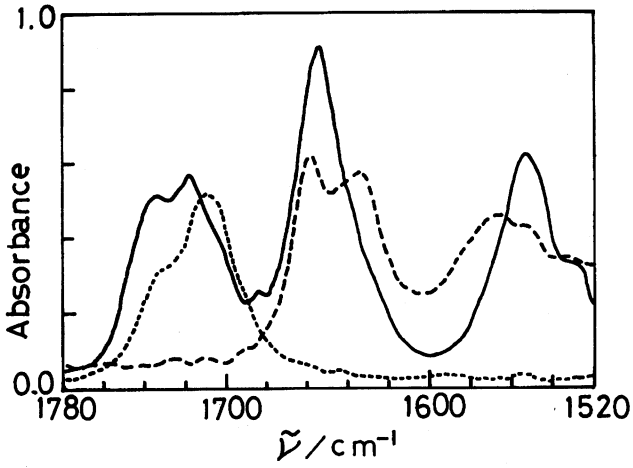

Fig. 6. IR spectra of (—) *N*-dodecanoyl-L-valine at 1.0×10^{-1} M, of (- - -) *N*-ethylacetamide at 1.0×10^{-1} M, and of (····) (*S*)-(+)-2-methylbutanoic acid at 1.0×10^{-1} M, in methanol solutions.

cm^{-1} became greater with an increase in the concentration. The same correlation was observed between the bands at 1560 and 1552 cm^{-1}. Thus, one can attribute the bands at 1660 and 1552 cm^{-1} to the free amide I and amide II bands, respectively. Further, the bands at 1636 and 1560 cm^{-1} can be assigned to the amide I and amide II bands of intermolecularly hydrogen-bonding amide groups, respectively.

From a comparison of the spectrum of DV with that of EAA, the amide I and the II band of DV can be designated to a free amide carbonyl and to a free NH, respectively. This indicates that both the amide carbonyl and the NH group of DV in methanol solution do not take part in hydrogen bonding over the concentration range studied. Thus, one can conclude that the types of hydrogen bonds illustrated as (2), (3), and (4) in Fig. 1 are not formed in the solution.

The above results suggest that the hydrogen bond formed by the carboxylic carbonyl of DV is of type (1) shown in Fig. 1: two hydrogen bonds in a ring structure by a couple of carboxylic groups. If this is true, the absorption bands of DV at 1734 cm^{-1} and at 1718 cm^{-1} should be due to the free carboxylic carbonyl and the hydrogen-bonding one, which belongs to the ring structure, respectively. In order to confirm whether or not these assignments are reasonable, we measured the IR spectra of (*S*)-MBA in methanol solutions. Figure 6 shows the IR spectrum of (*S*)-MBA in methanol at 1.0×10^{-1} M. A couple of bands at 1726 and 1709 cm^{-1} can be assigned to a free carboxylic carbonyl and the hydrogen-bonding one belonging to the ring structure. These two bands of (*S*)-MBA are almost in the same wavenumber region where the carboxylic carbonyl bands of DV are observed. The shift by 17 cm^{-1} in the spectrum of (*S*)-MBA is almost equal to that obtained for DV. These results lead us to conclude that the type of hydrogen bond (1) shown in Fig. 1 is formed in methanol solutions of DV. Our results also indicate that carboxylic acid dimers are formed predominantly from 5×10^{-3} M, the lowest concentration studied, up to 1×10^{-1} M, in spite of the presence of an amide group as another hydrogen-bonding group.

Changes in the CD Spectra with Temperature. One more unsolved problem is whether or not the CD bands observed are correlated to the formation of the carboxylic acid dimer, as revealed by IR measurements. The CD measurements were carried out for the esters of many carboxylic acids; the spectra were found to be the same as those obtained for the carboxylic acids.[1,3,5,7,9,10] Korver and van Gorkom reported the CD spectrum of methyl butanoate that can form neither intermolecular nor intramolecular hydrogen bonds.[8] All of these results indicate that the observed CD has no relation with the formation of carboxylic acid dimer.

Listowsky et al.[5] presented for the first time the temperature-dependence of the CD for α-hydroxy carboxylic acids, which show an increase in the intensity of the negative CD with a concomitant decrease in that of the positive CD upon raising the temperature. They explained the data in terms of a change in equilibrium between two rotational isomers which are assumed to be responsible for each band. The positive and negative CD bands have therefore been interpreted as indicating the presence of conformational equilibrium at around the C_1–C_2 bond of carboxylic acids.[6—11] We measured the CD spectra of DGA at 1.0×10^{-2} M while increasing the temperature from 20 to 60°C. Figure 7 shows the change in the extrema of the positive and negative CD bands with temperature. The positive maximum decreases with a temperature increase, whereas the negative minimum increases. The same result was also obtained for DV. We think that our results can also be explained on the basis of the presence of two conformers and a shift of the equilibrium between the two.

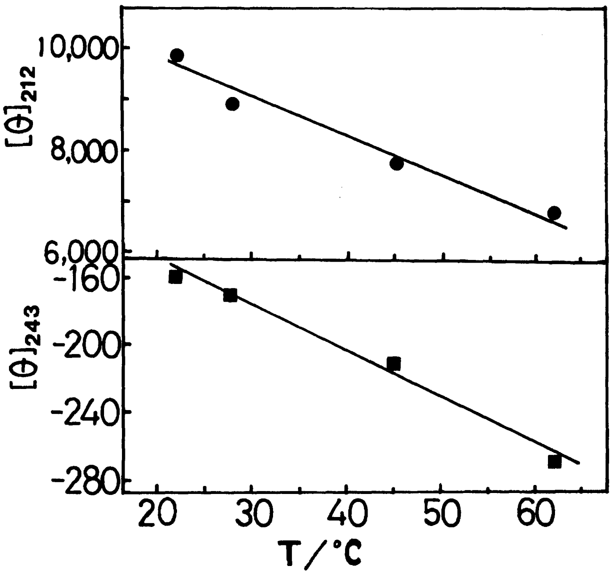

Fig. 7. Effect of the temperature on the molar ellipticity of a positive CD maximum at 212 nm and of a negative CD minimum at 243 nm for *N*-dodecanoyl-L-glutamic acid at 1.0×10^{-2} M in methanol solution.

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