Optically Active Cyclic Dipeptide Carrying 9-Anthryl Groups

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Optically active 3-(9-anthryl)alanine was synthesized for the first time and its cyclic dipeptide, cyclo(D-9-anthrylalanine)$_2$, was prepared. Conformation of the cyclic dipeptide in solution was investigated by means of ^{1}H NMR, absorption, circular dichroism (CD), and fluorescence spectroscopy. The coupling parameter $J_{NH\text{-}C^{\alpha}H}$, observed in the NMR spectra indicated a planar configuration for the 2,5-piperazinedione ring of the dipeptide. The cyclic dipeptide showed a moderately large exciton couplet in CD spectrum and a small splitting in the ^1B_b absorption band, but no excimer emission in fluorescence spectrum. These spectroscopic data suggest an unfolded-unfolded conformation for the side chains of the cyclic dipeptide.

Bichromophoric compounds in which two aromatic groups are linked to a ring system have been investigated.[1-3] Study on electronic interactions between two rigidly-fixed chromophores may provide basic information on photochemical processes in chromophoric assemblies, such as polymers, bilayers, and monolayers. In the previous paper, syntheses and conformational analyses of two cyclic dipeptides carrying 1- and 2-naphthyl groups, cyclo(L-1- and 2-naphthylalanine)$_2$ [c(1- and 2-napAla)$_2$] have been described.[4] The 2,5-piperazinedione ring of the two cyclic dipeptides was found to be nearly planar and the folded-unfolded asymmetric side-chain conformation was most populated. In this article, the same line of study was extended to a cyclic dipeptide carrying 9-anthryl group, cyclo(D-9-anthrylalanine)$_2$ [c(D-antAla)$_2$]. Its conformation in solution was studied by means of ^{1}H NMR, absorption, circular dichroism (CD), and fluorescence spectroscopy. The optically active 9-anthrylalanine was synthesized for the first time in this study. The determination of the absolute configuration of the amino acid has been described elsewhere.[5]

Experimental

Materials. D-3-(9-Anthryl)alanine and Cyclo(D-9-anthrylalanine)$_2$: Optically active 9-anthrylalanine was synthesized for the first time in this study. The cyclic dipeptide was prepared by a similar mannar as in the case of c(1- and 2-napAla)$_2$. The elimination of *N*-benzyloxycarbonyl group from a linear dipeptide was conducted by a catalytic hydrogenation using ammonium formate as a hydrogen donor.[6]

Ac-DL-9-antAla-OH[7]: Sodium (2.5 g) was dissolved in dry ethanol (200 ml) and diethyl acetamidomalonate (23.23 g) was added to the solution. 9-(Chloromethyl)anthracene (20 g) was then added and the mixture was refluxed for 4 h. After cooling to room temperature, sodium chloride was filtered off and the filtrate was evaporated. Diisopropyl ether (100 ml) was added to the residue and the mixture was cooled with ice to precipitate crude crystals of diethyl α-(9-anthrylmethyl)acetamidomalonate (25 g). The latter were dissolved in ethanol (200 ml) and 2M NaOH (100 ml, 1M=1 mmol dm^{-3}) was added. The mixture was heated to ca. 60 °C for 30 min and neutralized with concd HCl to pH= 3. Ethanol was evaporated and water was added to precipitate Ac-DL-antAla-OH. The curde material was redissolved in ethanol (100 ml) under heating and 2M NaOH (50 ml) was added. Crystalline precipitate was collected and treated with 1M HCl to obtain a free acid form of Ac-DL-antAla-OH, yield 15.9 g. Mp 265—267 °C. Found: C, 74.34; H, 5.28; N, 4.35%. Calcd for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.57; N, 4.56%.

Resolution of Ac-DL-antAla-OH: The racemic acetylamino acid (14.8 g) was dissolved in ethanol (200 ml) under heating and *l*-ephedrine (9.5 g) was added. After standing overnight at room temperature the mixture was cooled with ice to precipitate Ac-D-antAla-OH *l*-ephedrine complex (the absolute configuration has been determined as described below). The complex was recrystallized twice from ethanol and redissolved in ethanol under heating. Concd HCl (10 ml) and water (300 ml) were added to the ethanol solution and the mixture was cooled with ice to precipitate Ac-D-antAla-OH, yield 3.3 g. Mp 269—270 °C. (Found: C, 74.04; H, 5.57; N, 4.47%). CD: $[\theta]_{258(max)}=1.12\times10^5$ (ethanol, $c=1.95\times10^{-4}$ M). Evaporation of the filtrate gave an L-rich Ac-antAla-OH *l*-ephedrine complex, from which the acetylamino acid containing more L-enantiomer was recovered.

Ac-D-antAla-OMe: Ac-D-antAla-OH was dissolved in methanol saturated with HCl and the solution was kept stirring at room temperature for 3 h. Evaporation gave crystals which was recrystallized from methanol/diisopropyl ether mixture. Mp 208—209 °C. Found: C, 74.53; H, 5.94; N, 4.41%. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36%.

D-antAla-OH·HCl: Ac-D-antAla-OH (3.3 g) was suspended in concd HCl (150 ml)/acetic acid (50 ml) mixture and refluxed for 4 h. After a further addition of water, the mixture was cooled with ice to precipitate crystalline D-antAla HCl, 2.84 g. Mp 244 °C. $[\alpha]_D=-43°$ (ethanol, $c=0.058$ g dl^{-1}, 21 °C).

D-antAla-OMe: D-AntAla·HCl (150 mg) was suspended in 1M HCl/methanol mixture and the suspension was refluxed for 1 h. The solvent was evaporated and the residual crystal was recrystallized from methanol/ether mixture to give D-antAla-OMe·HCl, yield 157 mg. Mp 229C°. The hydrochloride (50 mg) was treated with 3% $NaHCO_3$ and the free methyl ester was extracted with ethyl acetate, yield 30 mg. Mp 76 °C.

Absolute Configuration and Optical Purity of the 9-Anthryl-alanine Resolved by Using *l*-Ephedrine: The absolute configuration of the optically active amino acid was determined on the basis of the "Dnp-aromatic rule".[8] The *N*-dinitrobenzoyl derivative of the optically active antAla

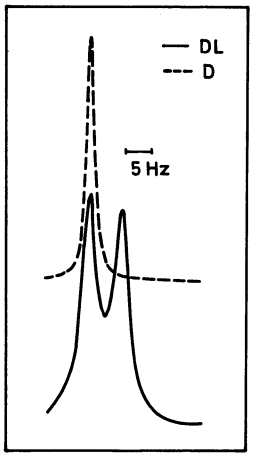

Fig. 1. Proton NMR spectra of D- and DL-antAla-OMe in the presence of 0.6 molar tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium-(III) in deuteriochloroform. (——): DL-isomer, (------): D-isomer.

showed a positive Cotton effect around 400 nm, which is indicative of D-configuration. The detail has been reported elsewhere.[5]

^{1}H NMR spectra of D- and DL-antAla-OMe were measured in $CDCl_3$ in the presence of 0.6 equivalent molar tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium(III). Figure 1 shows the NMR spectra in the O-CH_3 proton region. The racemic compound showed two peaks whereas the D-isomer showed only one peak under the same conditions. The optical purity of the D-isomer is better than ca. 99%. It is interesting to note that the O-CH_3 signal of D-isomers of 1- and 2-naphthylalanine and 1-pyrenylalanine has been observed at lower field than that of the corresponding L-isomers. The present result in Fig. 1 conforms to this rule.

Z-D-antAla-OH: D-AntAla-OH·HCl (2.62 g) was dissolved in 1M NaOH (18 ml), and benzyloxycarbonyl chloride (2.0 ml) and 1M NaOH (9 ml) were added dropwise simultaneously at O°C under vigorous stirring. Precipitate appeared after one day. The mixture was acidified with HCl to pH=3 and the crystal precipitated was collected, washed with water and hexane, and dried. The curde crystal was recrystallized from ethyl acetate, yield 2.91 g. Mp 215—218°C.

Z-(D-antAla)$_2$-OMe: Z-D-antAla-OH (50 mg), D-antAla-OMe·HCl (39.5 mg), and triethylamine (17.4 l) were dissolved in DMF (2 ml), and water-soluble carbodiimide (26.4 mg) and 1-hydroxybenzotriazole hydrate (18.6 mg) were added under cooling with ice. The mixture was stirred under cooling for 4 h and at room temperature for 2 d. Precipitate, which appeared by the addition of 1M HCl (50 ml), was collected and washed with water, 3% $NaHCO_3$, and water. The crude solid was recrystallized from tetrahydrofuran/ethyl acetate mixture, yield 50 mg. Mp 238—240°C. Found: C, 78.02; H, 5.43; N, 4.53%. Calcd for $C_{43}H_{36}N_2O_5$: C, 78.16; H, 5.49; N, 4.24%.

Cyclo(D-antAla)$_2$: Z-(D-antAla)$_2$-OMe (40 mg) was dissolved in DMF (30 ml) and 10% palladium/activated carbon (100 mg) was added. Ammonium formate (40 mg) dissolved in methanol (5 ml) was added to the mixture under stirring at room temperature. The progress of the reaction was followed by TLC [Z-(D-antAla)$_2$-OMe, R_f=0.85, c(D-antAla)$_2$, R_f=0.4; ethyl acetate]. After 3 d, the catalyst was removed by filtration and the solvent was evaporated. The residual solid was reprecipitated from tetrahydrofuran (solvent)/methanol (nonsolvent), yield 10 mg, mp>300°C. TLC showed a single spot (chloroform, ethyl acetate). No impurity was found in IR, ^{1}H NMR (Fig. 2), UV (Fig. 3), and fluorescence (Fig. 5) spectra. IR (KBr disc): 1668 (amide C=O), 1438 (amide C-N) cm^{-1}.

Measurements. 90 MHz ^{1}H NMR spectra were recorded on a JEOL FX90Q instrument in DMF-d_7. Since the NH signal overlapped with the aromatic signals, the coupling parameter $J_{NH-C^\alpha H}$, was determined from the $C^\alpha H$ signals under irradiating $C^\beta H$ protons.

Circular dichroism was measured on a JASCO J-20 spectropolarimeter with quartz cells of 0.05 and 1.0 cm path lengths at the dipeptide concentration of 1.0×10^{-4} mol dm^{-3}. Fluorescence spectra were measured on a Hitachi MPF-4 instrument in a cuvette equipped with a stopcock. The sample solution was purged with nitrogen gas before each measurement.

Results and Discussion

Synthesis of Optically Active 9-Anthrylalanine. Synthesis of racemic 9-anthrylalanine has been reported[7] but its optical resolution has never been reported so far. Since optically active aromatic amino acids carrying large aromatic ring are important intermediate to prepare novel aromatic polypeptides in which aromatic groups are arranged regularly along a helical main chain, i.e., one-dimensional aromatic crystals, their preparation deserves special attention.

Optical resolution of antAla was attempted by a stereoselective deacylation of Ac-antAla with acylase at first, but the enzymic reaction did not occur at all. This contrasts the cases of 1- and 2-naphthylalanine, the acetyl derivatives of which were deacylated stereoselectively with acylase.[9,10] Enzymic deacylation of Ac-DL-pyrAla was also possible, although the yield was very low. The bulkiness of the 9-anthryl group may be the reason for the failure of the enzymic reaction.

Optical resolution by diastereomeric complex formation of Ac-DL-antAla-OH with 18 different chiral amino compounds was attempted. Only three of them, i.e., *l*-ephedrine, quinine, and *l*-cinchonidine formed ethanol-insoluble diastereomeric complex. These amines complexed preferentially with the D-enantiomer of Ac-antAla-OH. Among the three, the complex with *l*-ephedrine was most easily purified by recrystallization. The absolute configuration of antAla which forms insoluble complex with *l*-ephedrine was found to be a D-isomer according to the "Dnp-aromatic rule" as described above.[5]

Conformation of Cyclo(D-9-anthrylalanine)$_2$. ^{1}H NMR Spectra: Proton NMR spectrum of c(D-antAla)$_2$ in DMF-d_7 is shown in Fig. 2. The coupling parameter $J_{NH-C^\alpha H}$ was measured under irradiating $C^\beta H$ was protons and is shown in Table 1. The coupling parameter is related to the dihedral angle $\theta_{NH-C^\alpha H}$, which is approximately equal to ϕ+60° (for D-iso-

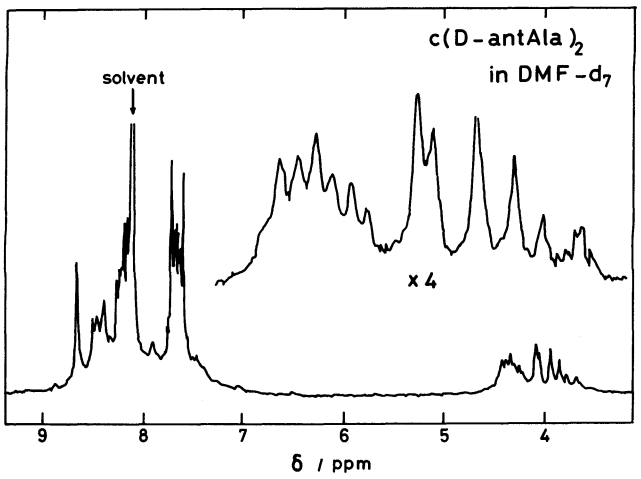

Fig. 2. Proton NMR spectrum of c(D-antAla)$_2$ in *N,N*-dimethylformamide-d_7. Concentration=4 mg/0.4 ml.

Table 1. The Coupling Parameter $J_{NH-C^{\alpha}H}$, and the Dihedral Angles $\theta_{NH-C^{\alpha}H}$, ϕ, and β for Cyclo(D-9-anthhrylalanine)$_2$ in *N,N*-Dimethylformamide-d_7

$J_{NH-C^{\alpha}H}$ (Hz)[a]	$\theta_{NH-C^{\alpha}H}$[b]	ϕ[c]	β[d]
2.6±0.2	±115°, ±57° (+57°)	−3°	3°

a) Estimated from the peak separation of $C^{\alpha}H$ signals under irradiating the $C^{\beta}H$ protons. b) θ was calculated according to the Bystrov equation: $J_{NH-C^{\alpha}H}=9.4\cos^2\theta-1.1\cos\theta+0.4$. c) $\phi=\theta-60°$. d) A supplement of the dihedral angle between two amide planes calculated assuming standard structural parameters for peptide bonds.

mer) where ϕ is the rotational angle of N-C^{α} bond defined according to the IUPAC-IUB nomenclature committee.[11] The dihedral angle and the rotational angle calculated according to the Bystrov equation[12] are also listed in Table 1. The Bystrov equation afforded four solutions for θ, but only one of the four, which is given in the parenthesis, corresponds to a reasonable skeletal conformation of the 2,5-piperazinedione ring. That is, the 2,5-piperazinedione ring is usually assumed to involve two planar amide bonds, and the dihedral angle between the two amide planes (β) may vary between ±45°. The θ value in the parenthesis corresponds to β=3°, whereas the other three solutions of the Bystrov equation gave β values outside the possible range. The determined β value indicates a planar conformation of the 2,5-piperazinedione ring of c(D-antAla)$_2$.

Circular Dichroism: CD and absorption spectra of c(D-antAla)$_2$ and Ac-D-antAla-OMe are shown in Fig. 3. The CD spectrum of the cyclic dipeptide shows a distinct exciton couplet at the ^1B_b absorption band. The molar ellipticities for one molar cyclic dipeptide are: $[\theta]_{248.5}=-3.1\times10^5$, $[\theta]_{262.5}=-5.3\times10^5$ deg cm^2 dmol^{-1}. According to the exciton coupling rule,[13]

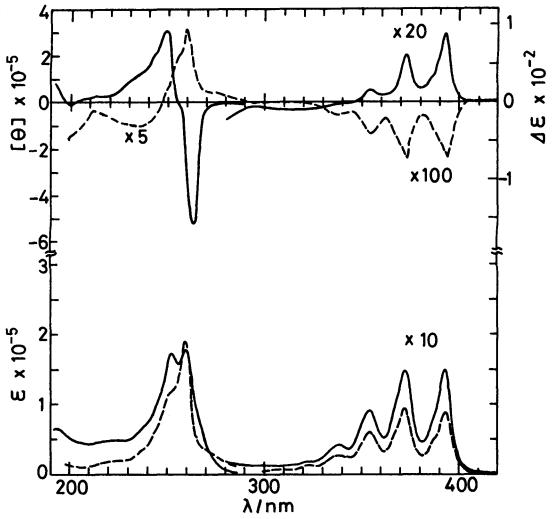

Fig. 3. Circular dichroism and absorption spectra of c(D-antAla)$_2$ (——) and Ac-D-antAla-OMe (- - - -) in trimethyl phosphate. [antAla]=1.0×10^{-4} mol dm^{-3} for c(D-antAla)$_2$ and 1.9×10^{-4} mol dm^{-3} for Ac-D-antAla-OMe, room temperature. [θ] and ε are calculated with respect to two anthryl groups for c(D-antAla)$_2$ and one anthryl group for Ac-D-antAla-OMe.

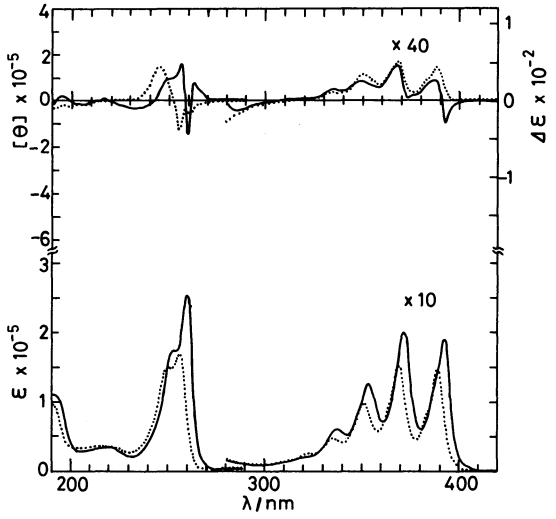

Fig. 4. Circular dichroism of Z-(D-antAla)$_2$ in trimethyl phosphate (—) and in 2,2,2-trifluoroethanol (·····). [antAla]=1.0×10^{-4} mol dm^{-3}, room temperature. [θ] and ε are calculated with respect to two anthryl groups.

the sign of the exciton couplet indicates that the transition dipole moments of the ^1B_b absorption which are parallel to the long axis of the anthryl group, are in a left-handed helical arrangement in c(D-antAla)$_2$.

Circular dichroic and absorption spectra of a linear dipeptide, Z-(D-antAla)$_2$-OMe in trimethyl phosphate (TMP) and in 2,2,2-trifluoroethanol (TFE) are shown in Fig. 4. A small and complex CD pattern is observed in TMP, whereas a small exction couplet at ^1B_b band in TFE. Two anthryl groups of the linear dipeptide may take a variety of orientations and the CD spectrum

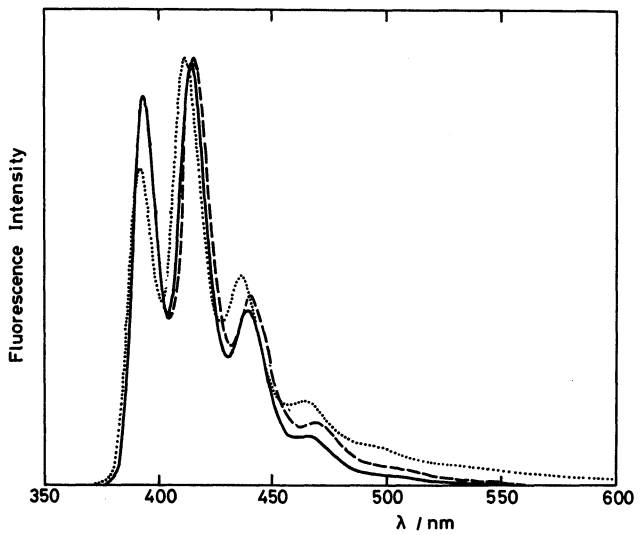

Fig. 5. Fluorescence spectra of c(D-antAla)$_2$ in trimethyl phosphate (—), and Z-(D-antAla)$_2$-OMe in trimethyl phosphate (-----) and in 2,2,2-trifluoroethanol (·····). [antAla]=1.0×10^{-5} mol dm^{-3}, room temperature.

should represent an average of these conformations. Rizzo and Jäckle[14] reported that linear dipeptides of aromatic amino acids (phenylalanine, histidine, tyrosine, and tryptophan) take extended conformations in aprotic solvents and folded conformations in protic solvents. This rule was also applicable to linear dipeptides of 1- and 2-naphthylalanines.[4] Hence the difference in CD spectrum in TMP (aprotic solvent) and TFE (protic solvent) may be interpreted by the different average conformations of Z-(D-antAla)$_2$-OMe in the two solvents.

Fluorescence Spectra: Figure 5 shows fluorescence spectra of c(D-antAla)$_2$ in TMP, and Z-(D-antAla)$_2$-OMe in TMP and in TFE. The cyclic dipeptide exhibits no excimer emission, indicating that the two anthryl groups are separated from each other by more than ca. 4 Å. The linear dipeptide shows no excimer in TMP but shows a small amount of excimer emission in TFE. The difference is consistent with the different conformations of the linear dipeptide in protic (TFE) and aprotic (TMP) solvent as described above. In the protic solvent the linear dipeptide takes folded compact conformations which favors excimer formation, whereas in the aprotic solvent the dipeptide takes extended conformations in which two anthryl groups are well separated from each other.

Conclusion on the Conformation of c(D-9-antAla)$_2$. Cyclo(D-antAla)$_2$ has a planar 2,5-piperazinedione ring in solution. It shows a moderately large exciton couplet in CD and a small exciton splitting in absorption spectrum, but no excimer emission in fluorescence spectrum. These spectroscopic properties suggest either folded-unfolded or unfolded-unfolded conformation of side chains. However, a CPK model analysis and a preliminary result of empirical conformational energy calculation[15] suggest that the folded (g^+) conformation is not allowed for the side chain of c(D-antAla)$_2$ due to a steric repulsion between the anthryl group and the 2,5-piperazinedione ring. Hence the side-chain conformation of c(D-antAla)$_2$ is concluded as unfolded-unfolded conformation. This conclusion is in contrast with the case of c(1- and 2-napAla)s, where folded-unfolded asymmetrical conformation was proposed.[4] In the latter two cases, the interaction between the 2,5-piperazinedione ring and the aromatic ring may not be so severe as to eliminate the folded conformation. In the unfolded-unfolded conformation of c(D-antAla)$_2$, the two anthryl groups are separated by about 10 Å. This interchromophore distance is evidently too far to form excimer in the excited state, but may be close enough to induce an exciton couplet in CD spectrum at the ^1B_b absorption band, which has very strong transition dipole moment.

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