Stereochemistry of Piperazine-2,5-dione Formation by Self-condensation of DL-Amino Acid Esters

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'Racemic' piperazine-2,5-diones (diketopiperazines, dkps) have been synthesized by the self-condensation of DL-amino acid esters without solvent. It was found that 'racemic' dkps consisted of *cis*- and *trans*-isomers, and that *cis*-dkp was preferentially formed in the early stage of the self-condensation, the *cis*:*trans* ratios gradually decreasing with increasing reaction time. These results may be attributed to the difference in the rates of cyclization of two kinds of diastereoisomeric dipeptide esters, intermediates in the formation of dkps from DL-amino acid esters. It was further confirmed that the pre-*cis*-dipeptide ester, which formed *cis*-dkp, cyclized faster than the pre-*trans*-dipeptide ester in methanol. Differences in steric hindrance in the cyclization reaction of pre-*cis*- and pre-*trans*-isomers may be an important factor in the stereochemistry of self-condensation.

It is well known that piperazine-2,5-dione (diketopiperazine, dkp) is formed by the self-condensation of amino acid esters,[1] and this method has been applied to the synthesis of dipeptides by partial hydrolysis of the dkps prepared from amino acid esters.[2,3] It is also known that dkps are formed readily from dipeptide derivatives, including esters[1,4] and amides.[5,6] A kinetic study of dipeptide ester cyclization in aqueous solution has also been reported.[7] However, the mechanism of dkp formation from amino acid esters has not been well defined. Such reactions apparently proceed *via* the preliminary condensation of two molecules of amino acid ester, followed by an intramolecular cyclization of the intermediate dipeptide ester, to give the corresponding dkp.[8] The intermediate dipeptide ester, however, has not been detected.

Generally, the dkps produced from DL-amino acid esters, which have been called 'racemic' dkps, have been assumed to contain two kinds of diastereoisomeric dkps, the *trans*- and *cis*-isomers. A thermodynamic study of the *cis–trans* isomerization of dkps has been carried out,[9] but the diastereoisomeric ratio of 'racemic' dkps formed by self-condensation of DL-amino acid esters has never been clarified. In the present study, the course of the formation of dkps and dipeptide esters *via* the self-condensation of DL-amino acid esters has been studied, and a possible reaction pathway for dkp formation is reported.

Results and Discussion

The substrates used for this study were DL-Ala-OMe, DL-Ala-OEt, DL-Ala-OPr^i, DL-Val-OMe, and DL-Leu-OMe. Figures 1 and 2 give plots of time *vs.* ratio of *cis*:*trans* dkps yields for the self-condensation of DL-Ala-OEt at 80 °C. The *cis*-isomer is the major product in the beginning of the reaction, the *cis*:*trans* ratio gradually decreasing with increasing reaction time. All the substrates used in this study show similar diastereoisomer ratios to that of DL-Ala-OEt.

Table 1 shows the reaction time, total yields, and *cis*:*trans* ratios of dkps, which were formed at 80 °C. Only in the case of DL-Ala-OMe was the *trans*-isomer formed in excess over the *cis*-isomer after one day of reaction. Amino acid esters with bulkier ester moieties or alkyl groups gave a slower rate of condensation reactions. These results suggests that a bulkier ester moiety is a less effective leaving group, and that the bulkier alkyl group could produce steric hindrance during the reaction.

It is important to note that higher *cis*:*trans* ratios were observed in reactions using amino acid esters with bulkier alkyl groups. For example, *cis*:*trans* ratios of diastereoisomeric dkps at similar chemical yields increased in the following order: DL-Ala-OMe (yield; 2.4%, *cis*:*trans*; 1.8); DL-Leu-OMe (3.2%, 2.7); and DL-Val-OMe (2.8%, 3.3), as shown in Table 1. Judging from these results, it may be assumed that the α-alkyl group of the amino acid ester plays an important role in determining the diastereoisomeric ratio of dkps in the cyclization reaction.

Self-condensation reactions of L-amino acid esters were

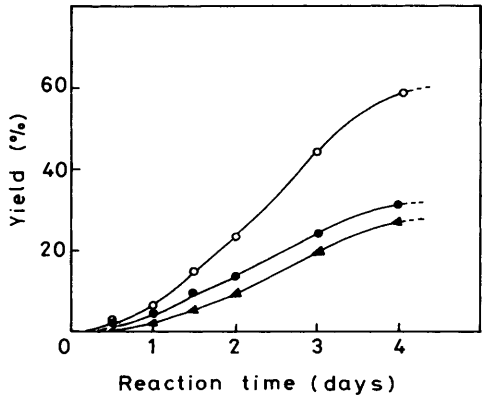


Figure 1. Yields of dkps from DL-Ala-OEt at 80 °C: (○—○), total dkps; (●—●) *cis* dkp; (▲—▲), *trans* dkp

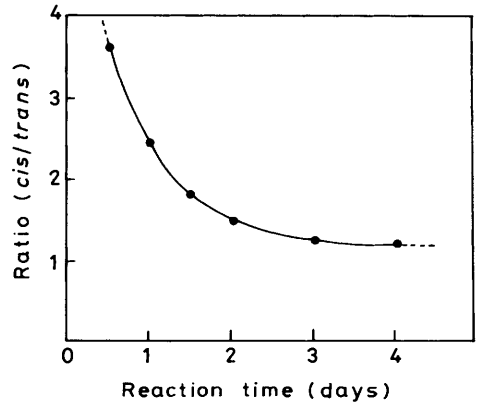


Figure 2. The *cis*/*trans* ratio of dkps from DL-Ala-OEt at 80 °C

Table 1. Formation of dkps from DL-amino acid ester at 80 °C

DL-Ala-OMe	(1)	0.25	0.5	1	2	
	(2)	2.4	10.0	40.3	64.0	
	(3)	1.8	1.3	0.98	0.89	
DL-Ala-OEt	(1)	1	1.5	2	3	4
	(2)	6.5	15.0	22.7	44.1	58.6
	(3)	2.4	1.8	1.5	1.2	1.2
DL-Ala-OPr^i	(1)	3	5	7	10	14
	(2)	0.59	2.4	4.1	9.5	12.8
	(3)	2.6	2.5	2.3	2.0	1.8
DL-Val-OMe	(1)	4	6	8	11	14
	(2)	0.7	1.6	2.8	3.2	8.9
	(3)	4.5	3.4	3.3	3.0	2.3
DL-Leu-OMe	(1)	1	2	3	4	5
	(2)	0.4	3.2	11.1	25.5	29.3
	(3)	5.3	2.7	1.8	1.3	1.3

(1) Reaction time (days) (2) Yield (%) (3) *cis*:*trans* ratio

Table 2. *trans*:(*cis* + *trans*) Ratios of dkps formed from L-amino acid esters at 80 °C

	L-Ala-OMe	L-Ala-OEt	L-Ala-OPr^i	L-Val-OMe	L-Leu-OMe
(1)	2	4	14	14	5
(2)	35.1	35.8	15.9	5.1	31.5
(3)	5.3	11.0	5.0	0.0	2.5

(1) Reaction time (days) (2) Yield (%) (3) *trans*:(*cis* + *trans*) × 100 (%)

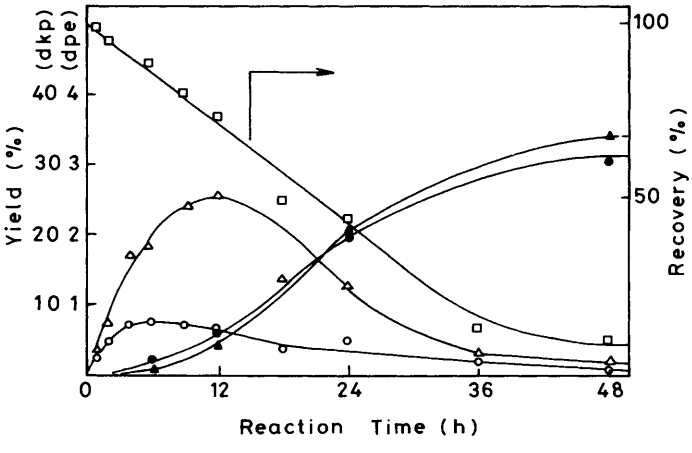
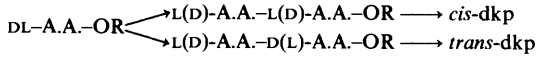


Figure 3. Formation of dkps and dipeptide esters (dpes) from DL-Ala-OMe at 80 °C: (□—□), substrate; (●—●), *cis* dkp; (▲—▲), *trans* dkp; (○—○), pre-*cis* dpe; (△—△), pre-*trans* dpe

carried out to estimate the extent of racemization (Table 2). It was found that the racemization could have occurred in some cases to a maximum extent of about 10%. Little racemization took place in the beginning of the reaction. Practically no racemization was observed in the case of L-Val-OMe. These results suggest that racemization during the reaction has little influence on the *cis*:*trans* ratios of dkps produced by self-condensation of DL-amino acid esters.

Condensation reactions were carried out at 80 °C and at 111 °C. Although rates of reactions at 111 °C were faster than at 80 °C, the decrease in the *cis*:*trans* ratios of dkps were similar at both temperatures. More racemization took place at higher temperatures. No racemization was observed, however, in the case of L-Val-OMe, even at 111 °C.

From these results, it can be inferred that the diastereoisomeric dkps were produced from corresponding diastereoisomeric dipeptide esters which were formed from DL-amino acid esters (Scheme). *cis*-Dkp is produced from L(D)-A.A.–L(D)-A.A.–OR (the amino acid residue is abbreviated as A.A.), which we call the 'pre-*cis*' dipeptide ester, and *trans*-dkp is produced from L(D)-A.A.–D(L)-A.A.–OR, which we call the 'pre-*trans*' dipeptide ester.



Scheme.

Considering the Scheme, factors controlling the ratio of dkps could be: (1) differing rates of formation between diastereoisomeric dipeptide esters; (2) differing rates of cyclization between diastereoisomeric dipeptide esters; and (3) thermodynamic equilibrium between dkps.

Diastereoisomeric dkps are known to isomerize into other diastereoisomers under alkaline conditions.[10] It is reported that the rate of racemization of amino acids is low under dry conditions.[11] Factor (3) may be excluded because of the low racemization value in the control experiment (Table 2). However, if heating was continued for a longer time, factor (3) might have some influence on the ratio of diastereoisomeric dkps.

In order to verify whether the controlling factors are (1) and (2), analyses of the resulting diastereoisomeric dipeptide esters in the reaction mixtures were carried out. The dipeptide esters could not be analysed by high performance liquid chromatography (h.p.l.c.), with which dkp analyses were carried out. Therefore, the diastereoisomeric dipeptide esters were converted into *N*-trifluoroacetyl-(TFA-)dipeptide esters and these were analysed by g.l.c. The time courses of the yields of dipeptide esters and dkps, which were produced from DL-Ala-OMe at 80 °C, and the decay curve of the starting material are shown in Figure 3. It is apparent that the pre-*trans* isomer is produced more readily than is the pre-*cis* isomer. Similar time courses were observed in the case of DL-Ala-OPr^i at 80 °C (Figure 4). In the case of DL-Ala-OMe, the rate of cyclization of the dipeptide ester is higher than the rate of formation of the dipeptide ester from the amino acid ester. Therefore, formation of the dipeptide ester should be a rate-determining step in the reaction sequence.

It is reported that in acid solution *cyclo*-L-alanyl-L-alanine-(*cis*-dkp) hydrolyses 2.5 times faster than does the *trans* isomer;[12] this is also true of alkaline conditions.[13] However, in the present study, the reactions were carried out without solvent. The amount of methanol found was thus too small to

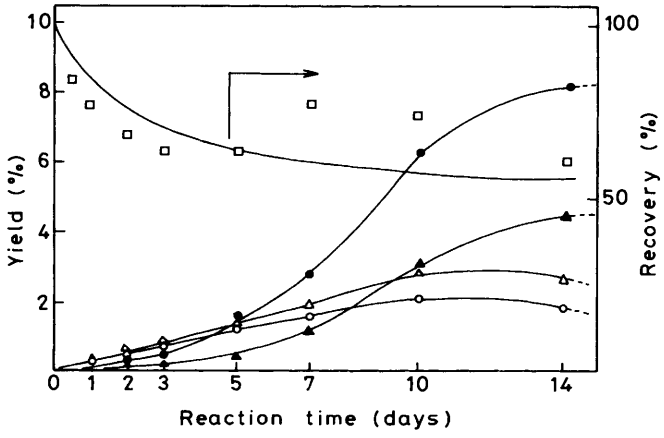


Figure 4. Formation of dkps and dipeptide esters (dpes) from DL-Ala-OPr^i at 80 °C: (□—□), substrate; (●—●), *cis* dkp; (▲—▲), *trans* dkp; (○—○), pre-*cis* dpe; (△—△), pre-*trans* dpe

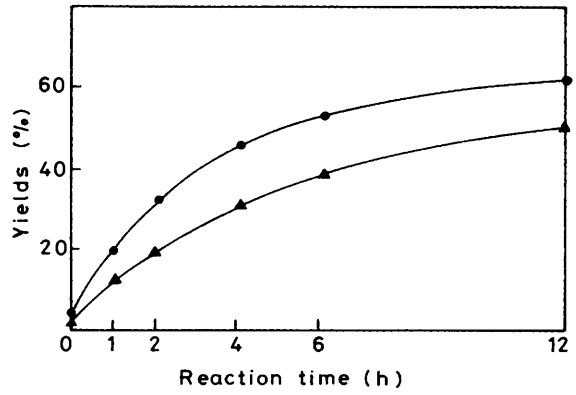


Figure 5. Formation of dkps from L-Ala-L-Ala-OMe and L-Ala-D-Ala-OMe in methanol at 65 °C: (●—●), *cis* dkp; (▲—▲), *trans* dkp

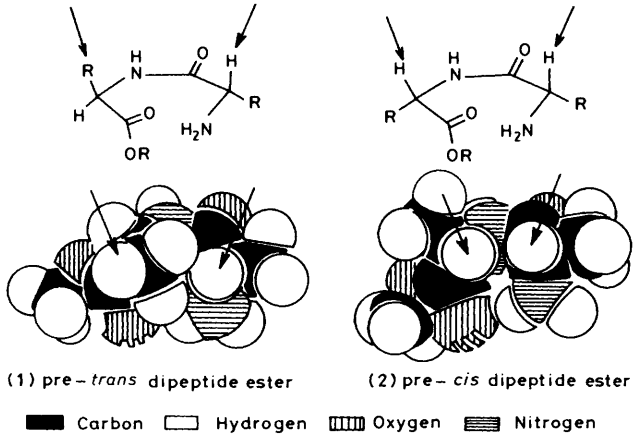


Figure 6. Steric hindrance in the cyclization reaction of the dipeptide ester. CPK models are obtained by assuming the following factors: (1) the peptide bond is planar; (2) the *N*-terminal amine attacks *C*-terminal ester moiety; and (3) the pre-*cis* isomer cannot take up a conformation where two alkyl groups attached to α-carbons are neighbours

cause methanolysis of the dkp, and so very little dipeptide ester could be detected after prolonged reaction time (Figure 3).

A detailed kinetic analysis cannot be carried out because this reaction is not a homogeneous system, and the resulting dkps crystallized out. Although we met with great difficulty in determining the rate constants, the rates of decay and of dipeptide ester formation were determined for DL-Ala-OMe. Based on Figure 3, the decay of the starting material is considered to be a zero order reaction. The value of the decay constant is about 2.0×10^{-2} mmol h^{-1}. The rates of dipeptide ester formation were estimated by extrapolation to the starting point of the reaction, using the rate constants in the early stage of the reaction. The values for pre-*trans* and pre-*cis* formation are both about 2.0×10^{-3} mmol h^{-1}. Thus there should be no difference between the rates of formation of the two diastereoisomers of the dipeptide ester. Therefore, factor (2) must have influenced the ratio of dkp diastereoisomers. Further detailed kinetic studies are required on the formation of dipeptide esters from DL-amino acid esters in homogeneous systems.

Subsequently, cyclization reactions of two diastereoisomeric alanyl-alanine methyl esters in methanol were carried out. This reaction is known to give optically pure dkp.[14,15] Figure 5 shows that the pre-*cis* isomer cyclized faster than the pre-*trans* isomer. This result is supported by the fact that more pre-*trans* than pre-*cis* isomer was found on g.l.c. analysis (Figure 3). This result may be attributed to the difference in steric hindrance in the cyclization reaction of pre-*cis* and pre-*trans* isomers. In the case of the pre-*trans* isomer, steric hindrance exists between the alkyl group and the α-hydrogen attached to the α-carbon of the other amino acid residue. This result suggests that amino acid esters with bulkier alkyl groups show a lower rate of cyclization of the pre-*trans* isomer. Examination of the appropriate CPK model shows that the alkyl group attached to the α-carbon of the amino acid ester plays an important role in determining the diastereoisomeric ratio of dkps (Figure 6).

In conclusion: (1) 'racemic' dkps, produced by self-condensation of DL-amino acid esters, consist of *cis*- and *trans*-isomers; (2) the ratio of diastereoisomeric dkps varies with reaction time; (3) *cis*-dkp is preferentially formed in the beginning of the reaction and the *cis*:*trans* ratios gradually decrease with increasing reaction time; (4) these results may be attributed to a difference in the rates of cyclization of two kinds of intermediate dipeptide esters which are formed from DL-amino acid esters, and not to the thermodynamic equilibrium between the diastereoisomeric dkps; and (5) a difference in steric requirements for the cyclization reaction of pre-*cis*- and pre-*trans*-dipeptide esters may be important in the formation of dkps.

Experimental

These experimental results were obtained from a single measurement for each substrate and temperature.

Reaction Procedure.—Amino acid ester hydrochlorides or toluene-*p*-sulphonates were synthesized from amino acids by Fischer's method.[16] Amino acid ester hydrochloride or toluene-*p*-sulphonate, diethyl ether, and an equimolar aqueous solution of 6M-sodium hydroxide were stirred vigorously, and free amino acid ester was extracted with ether. The extract was dried (K_2CO_3) and evaporated under reduced pressure to give the free amino acid ester; this was immediately placed in several sealed tubes (8 mm i.d. × *ca.* 15 cm) and allowed to react in a benzene bath (80 °C) or toluene bath (111 °C). Each sealed tube contained *ca.* 1 mmol of amino acid ester. Sample tubes were analysed at intervals of time.

Cyclization Reaction of Dipeptide Ester in Methanol.—Benzyloxycarbonyl-(Z)-L-Ala-L-Ala-OMe (617 mg, 2 mmol) in methanol (20 ml) containing an equimolar amount of 12M-hydrochloric acid was hydrogenated over 5% palladium-on-charcoal (100 mg) under hydrogen (1 atm) for 12 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The resulting oily product was dissolved in methanol (*ca.* 10 ml), an equimolar amount of triethylamine was added, and the mixture was diluted to 20 ml with methanol. 2 ml Aliquots of the solution were immediately placed in sealed tubes (8 mm i.d. × *ca.* 10 cm) and these were allowed to react in a methanol bath (65 °C). Sample tubes were analysed at intervals of time. The cyclization reaction of L-Ala-D-Ala-OMe was carried out in a similar manner.

Analyses.—The samples were analysed by h.p.l.c. (Jasco TRI ROTAR-V) for dkps, and by g.l.c. (Hitachi 163) for TFA-dipeptide esters. The column used for h.p.l.c. was Finepak C_{18} [Jasco, 4.6 mm i.d. × 25 cm; eluant: H_2O–MeOH (97:3 for Ala dkp; 65:35 for Val dkp; 53:47 for Leu dkp)] and a u.v. detector (210 nm) was used. The column used for g.l.c. was FFAP, which was packed in a stainless steel column (3 mm i.d. × 3 m) and a FID detector was used. The detector and injection ports were maintained at 300 °C. The oven was programmed to rise from 80 to 220 °C at 5 °C min^{-1}. The nitrogen carrier flow rate was 25 ml min^{-1}.

For dkp analysis, the heated sample was removed from the

reaction bath, and immediately acidified with a few ml of 10% (v/v) glacial acetic acid–methanol solution to prevent further self-condensation of the amino acid ester. It was also necessary that the reaction mixture was completely dissolved in methanol to prepare an analytical sample. Quantitative analyses were carried out by comparison with authentic samples, which were synthesized from dipeptide esters by refluxing in methanol.[15]

For the analysis of dipeptide esters, the reaction mixture was mixed immediately with trifluoroacetic anhydride (TFAA, 2 ml) and dichloromethane (10 ml), and the solution was kept for at least 6 h at room temperature. Under these conditions, no racemization of the dipeptide ester was observed. After evaporation under reduced pressure, *N*-trifluoroacetyl-1-naphthylamine (TFA-1-NA) (0.5 ml) in methanol (0.5 mM) was added as an internal standard. Quantitative analyses were carried out by means of calibration curves. However, it was considered possible that diastereoisomeric dipeptide esters had different reactivities in the TFA derivatization reaction. Thus, a mixture of diastereoisomeric dipeptide esters, which were prepared by catalytic hydrogenation of Z-L(L)-Ala-L(D)-Ala-OMe, was tested. The results of TFA derivatization showed no difference in the reactivities of the two diastereoisomeric dipeptide esters. Therefore, the diastereoisomeric ratio of dipeptide esters was correctly analysed by TFA derivatization.

Materials.—All m.p.s are uncorrected. Optical rotations at the sodium D line were measured with a Jasco DIP-181 polarimeter.

TFA-L-Ala-L-Ala-OMe. This material was synthesized from Z-L-Ala-L-Ala-OMe by a modification of the method of Tomida *et al.*:[17] yield 71%, m.p. 138—141 °C, $[\alpha]_D^{25}$ −85.6 (*c* 0.5 in methanol) (Found: C, 39.95; H, 4.85; N, 10.3. $C_9H_{13}F_3N_2O_4$ requires C, 40.00; H, 4.84; N, 10.36%).

Z-L-Ala-L-Ala-OPr^i. This material was synthesized by a modification of the method of Weygand *et al.*:[18] yield 72%, m.p. 114—115 °C, $[\alpha]_D^{25}$ −51.2 (*c* 0.5 in methanol) (Found: C, 60.8; H, 7.2; N, 8.35. $C_{17}H_{24}N_2O_5$ requires C, 60.69; H, 7.19; N, 8.32%).

TFA-L-Ala-L-Ala-OPr^i. This material was synthesized from Z-L-Ala-L-Ala-OPr^i in a similar manner as for TFA-L-Ala-L-AlaOMe: yield 63%, m.p. 141—143 °C, $[\alpha]_D^{25}$ −82.2 (*c* 0.5 in methanol) (Found: C, 44.55; H, 5.75; N, 9.65. $C_{11}F_3H_{17}N_2O_4$ requires C, 44.29; H, 5.74; N, 9.39%).

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