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ACID HYDROLYSIS OF DIASTEREOMERIC DIKETOPIPERAZINES

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Diketopiperazines (DKP) have in recent years been of interest as models in conformational studies. Investigations have been based on NMR (1,2), ORD and CD (3), and other methods (4,5). Kinetic studies concerning alkaline and acid hydrolysis of DKP have also been reported (6,7).

This communication presents a study of the hydrolysis of diastereomeric, LL (cis) and LD(trans), forms of both leucine and alanine DKP in 0.5 M hydrochloric acid. Significant differences in rates of hydrolysis were found.

The DKP were synthesized from the corresponding carbobenzoxydipeptide methyl esters. These were dissolved in methanol and hydrogenated with palladium on charcoal as catalyst. As no acid was present, the liberated amino group was not protonated. After the hydrogenation was completed (30-60 min), the catalyst was filtered off and the methanol was evaporated under reduced pressure. The resulting dipeptide methyl ester was again dissolved in a small amount of hot methanol which was kept boiling for a short time. On cooling, the corresponding DKP crystallized out, and was filtered off and washed with small amounts of cold methanol. The purity of the products was high; the crystals of the two diastereomers of alanine DKP were used in X-ray crystallographic structure determination without further purification (8). This convenient way of synthesizing DKP provides a supplement to methods recently reported (9,10).

The analytical procedure was based on the ninhydrin reaction; the DKP with no free amino group does not react with ninhydrin, while the product of the hydrolysis, the dipeptide, forms Ruhemann's purple wich was measured spectrophotometrically at 570 mμ.

As further hydrolysis of the dipeptide is much slower than the ring opening (7), see below, the consecutive step could be neglected in the rate constant calculations.

The experiments were performed in sealed tubes which contained loose innertubes. Prior to the start of the reaction the outer tube contained 1.00 ml DKP solution, pipetted from a stock solution, and the inner tube contained 1.00 ml M HCl. The ampules were fastened vertically in a rack which was immersed in a thermostated oilbath. After temperature equilibration (10 min) the two solutions were mixed by turning the rack upside down. The concentrations in the reaction mixture were then 0.00011 - 0.00013 M DKP and 0.50 M HCl. At appropriate time intervals estimated to cover the range of 20-70% hydrolysis, a pair of ampules were removed and cooled in a dry-ice ethanol bath.

Each run involved 14 samples (7 parallels) of each of the LL and LD forms, 4 blank samples and 10 (5 parallels) standards of different concentrations of dipeptides. All the standards were removed from the thermostat together with the last sample.

As a check on possible hydrolysis of the dipeptides, two samples with the most concentrated standard of dipeptide were prepared in the same way as the other samples, but they were not heated in the oil bath.

The ampules were opened and 1.00 ml of the contents were pipetted out and treated with ninhydrin according to the Moore and Stein procedure (11). The colour yield was determined quantitatively on a Beckman DU spectrophotometer at 570 mu.

The heated and non-heated standards with the same concentration of dipeptide gave the same colour yield with ninhydrin. This indicated that the hydrolysis of the dipeptides under these conditions was negligible.

From a plot based on the dipeptide standards, the extinction coefficient of the dipeptides was found, and the amount of dipeptide present in each of the test samples was calculated. Least square plots gave the following pseudo first order rate constants and activation parameters:

	Alanine DKP		Leucine DKP	
	LL	LD	LL	LD
Temp, ^{o}C	$k \times 10^3$, min^{-1}	$k \times 10^3$, min^{-1}	$k \times 10^3$, min^{-1}	$k \times 10^3$, min^{-1}
60	5.8	2.7		
70	13.3	6.8		
80	28.7	15.7	18.3	5.0
90			45.0	11.8
100			91.0	27.0
E_a, kcal	20.0 ± 0.4	20.6 ± 0.4	21.0 ± 0.5	22.1 ± 0.5
$\Delta H^{\ddagger}$, kcal	19.0 ± 0.4	19.9 ± 0.4	20.0 ± 0.5	21.4 ± 0.5
$\Delta S^{\ddagger}$, E.U.	-12.3 ± 1.0	-10.9 ± 1.0	-9.4 ± 1.2	-8.7 ± 1.2

The rate constants were estimated to be good to 2%.

It is seen that LL Ala DKP was hydrolysed 1.6 times faster than LL Leu DKP, while LD Ala DKP was hydrolysed 3.2 times faster than LD Leu DKP at 80°C. Both polar and steric effects may play a role in determining the ratios.

For the diastereomeric pairs:

$$k_{\text{LL Ala DKP}} / k_{\text{LD Ala DKP}} \approx 2.0$$

$$k_{\text{LL Leu DKP}} / k_{\text{LD Leu DKP}} \approx 3.5$$

These differences may probably be due to steric effects, as in the LL (cis) form one of the sides of the DKP ring is only shielded by two hydrogen atoms. The two substituents on the same side of the ring may also give a higher strain in the ring.

The activation parameters may indicate that the LD isomers have slightly higher activation energies. These differences are, however, not significant. For a more

thorough discussion of this matter more experimental data for diastereomeric pairs of DKP of other amino acids are needed. This is under investigation.

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