Racemisation in Peptide Synthesis

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Summary The second-order rate constants for the racemisation of 4-isobutyl-2-phenyl-L-oxazolin-5-one by triethylamine and di-isopropylethylamine in chloroform at 25° were 1400 l mol^{-1} min^{-1} and 113 l mol^{-1} min^{-1}, respectively.

RACEMISATION during peptide synthesis proceeds most commonly *via* the oxazolin-5-one route. This involves formation of the oxazolin-5-one ring, racemisation of the oxazolin-5-one, and finally opening of the oxazolin-5-one ring. This route competes with the direct formation of the peptide bond. The significance of these two competing routes in determining the optical purity of the products has been pointed out by several authors.[1]

I report an investigation of the rate of racemisation of 4-isobutyl-2-phenyl-L-oxazolin-5-one; the rates obtained are compared with the rates of racemisation of benzoyl-L-leucine *p*-nitrophenyl ester found by Bodanszky and Bodanszky.[2]

The 4-isobutyl-2-phenyl-L-oxazolin-5-one was synthesized by the action of dicyclohexylcarbodi-imide on benzoyl-L-leucine.[3] The racemisation of the oxazolin-5-one by triethylamine and di-isopropylethylamine in chloroform was studied at 25° with a Perkin-Elmer 141 Polarimeter. Pseudo-first-order rate constants were obtained from plots of the logarithm of the observed rotation against time. These pseudo-first-order constants were independent of the oxazolin-5-one concentration, but increased with increasing amine concentration. A plot of the pseudo-first-order constants against amine concentration gave a straight line through the origin, the slope was equal to the second-order racemisation constants: triethylamine: 1400 l mol^{-1} min^{-1}; di-isopropylethylamine : 113 l mol^{-1} min^{-1}. The constant for di-isopropylethylamine was estimated to be good to $\pm 2\%$, whereas the experimental error for the triethylamine runs was approximately 3 times higher on account of the rapid reaction.

Bodanszky and Bodanszky studied under similar conditions, *i.e.* chloroform as solvent and 24°, the racemisation of the benzoyl-L-leucine *p*-nitrophenyl ester. (The effect of the difference in reaction temperatures of 1° is neglected in the discussion.) With 0·05 M-substrate and 0·1 M-

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tertiary amine, they obtained half-lives of 30 min with triethylamine and 41 min with di-isopropylethylamine.

Calculated from the observed second-order constants given above, a concentration of 0·1 M-tertiary amine would give half-lives of 0·005 min with triethylamine and 0·06 min with di-isopropylethylamine. This implies that the much slower rates which were observed by Bodanszky and Bodanszky were in fact the rates of *formation* of the oxazolin-5-one, and not the rates of *racemisation* of the oxazolin-5-one.

Bodanszky and Bodanszky observed only a minor difference between triethylamine and di-isopropylethylamine in the racemisation of benzoyl-L-leucine *p*-nitrophenyl ester, while the racemisation of benzyloxycarbonyl-L-phenylglycine *p*-nitrophenyl ester, expected to proceed by direct proton abstraction, with triethylamine was 35 times faster than with di-isopropylethylamine.

The racemisation of the present oxazolin-5-one with triethylamine was 12 times faster than with di-isopropylethylamine. The di-isopropylethylamine has much more pronounced steric shielding around the nitrogen atom than has triethylamine, and significant differences in rates should be expected regardless of the structure of the substrate. The lack of significant differences in the rates of racemisation of benzoyl-L-leucine *p*-nitrophenyl ester with triethylamine and di-isopropylethylamine can be explained by the suggestion put forward by Kemp and Chien[4] that the formation of an oxazolin-5-one from an activated acylamino-acid consists of a rapid equilibrium between the starting material and the amide anion, followed by a rate-determining attack of the amide oxygen on the activated carboxyl carbon.

The observed rate constants together with the data obtained by Bodanszky and Bodanszky show that the formation of an oxazolin-5-one from an activated acylamino-acid was 6000 times slower than the racemisation of the oxazolin-5-one when triethylamine catalysed the reactions, and 700 times slower when di-isopropylethylamine was the catalyst.

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