# The Mechanism of Racemisation during the Coupling of Acylpeptides

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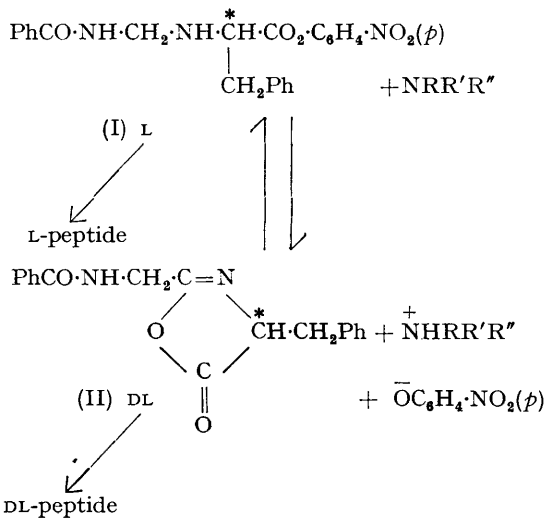
IT is now well established that the coupling of acylpeptides with amino-compounds is attended by the risk of racemisation, except when the acid azide route is used;[1] to this exception may now

[1] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1963, 881; F. Weygand, A. Prox, L. Schmidhammer, and W. König, *Angew. Chem. Internat. Edn.*, 1963, **2**, 183.

be added the use of 1-piperidyl esters, which also couple without racemisation in standard tests.[2] There is however still debate as to whether this base-catalysed racemisation proceeds through the intermediate formation, racemisation, and coupling of the oxazolone, formed as in the Scheme, or whether direct exchange of the hydrogen at the dissymmetric centre of the activated acylpeptide is responsible.[3] We now have strong evidence for the mechanism set out in the Scheme.

We have shown earlier[4] that the base-catalysed racemisation of benzoyl-L-leucine *p*-nitrophenyl ester proceeds chiefly if not exclusively through the oxazolone, but this is not a typical case and we have therefore examined the racemisation of acyldipeptide *p*-nitrophenyl esters. When a solution of the ester (I) in dichloromethane was treated with one molar proportion of triethylamine the optical rotation fell by 50% in 50 minutes at room temperature—far more rapidly than with phthaloyl-L-phenylalanine *p*-nitrophenyl ester (*ca.* 5% in the same time) in which the hydrogen at the dissymmetric centre should be more acidic, and this suggests that for the acyldipeptide ester oxazolone formation (not feasible for the phthaloyl-amino-ester) provides a favourable route. Surprisingly, no oxazolone-carbonyl infrared absorption developed in the solution; nevertheless, we have shown that in the presence of base equilibrium is indeed established between esters such as (I) and the corresponding oxazolones such as (II): when an equimolar mixture of benzyloxycarbonyl-glycyl-L-phenylalanine *p*-nitrophenyl ester and the oxazolone (II) in dichloromethane was treated with triethylamine, the ester (I) was recovered (after destruction of the benzyloxycarbonyl compound by means of hydrogen bromide in acetic acid). No such exchange occurred between benzyloxy-carbonyl-L-phenylalanine *p*-nitrophenyl ester (which does not form an oxazolone) and the oxazolone (II) under similar conditions. We conclude that the reversible reactions given in the Scheme do take place, and that the absence of oxazolone-carbonyl infrared absorption is due to the low concentration of oxazolone at equilibrium. This is confirmed by the change in infrared absorption which occurs when equimolar amounts of *p*-nitrophenol and triethylamine are added to a solution of the oxazolone, the oxazolone-carbonyl peak being replaced by that of the ester.

It does not necessarily follow that racemisation proceeds by this route, but we believe that the following experiment provides evidence that it does. To a solution of the ester (I) in dichloromethane was added one molar proportion of triethylamine and 10 molar proportions of the oxazolone derived from benzyloxycarbonylglycyl-L-phenylalanine. This large excess of oxazolone intercepts the *p*-nitrophenoxide anion and, acting as a "scavenger," largely prevents the formation of benzoylglycyl-DL-phenylalanine *p*-nitrophenyl ester by the reverse reaction. When the optical rotation of the solution had fallen to half its initial value, the mixed esters were recovered and treated with hydrogen bromide in acetic acid; the benzoyl-glycylphenylalanine *p*-nitrophenyl ester so obtained was scarcely changed in rotation, and no racemate could be found. Control experiments showed that 10% of racemate can be detected under these conditions. If racemisation occurred directly by ionisation of the hydrogen at the dissymmetric centre of the ester (I) then racemate corresponding in amount to the fall in optical rotation of the solution would be present and should certainly be detected. We conclude therefore that the racemisation proceeds, in this typical case also, chiefly if not exclusively through the oxazolone.




(*Received, August 4th*, 1965; *Com.* 487.)

[2] S. M. Beaumont, B. O. Handford, J. H. Jones, and G. T. Young, *Chem. Comm.*, 1965, 53; B. C. Handford, J. H. Jones, G. T. Young, and T. F. N. Johnson, *J. Chem. Soc.*, in the press.

[3] For a review, see I. Antonovics, A. L. Heard, M. W. Williams, and G. T. Young, "Proc. 6th European Peptide Symposium, Athens, 1963," ed. L. Zervas, Pergamon Press, in the press.

[4] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1964, 3701.