Racemization in Peptide Synthesis. Mechanism-specific Models

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SEVERAL model systems were suggested[1-4] for the study of racemization. In these systems a peptide with an optically active *C*-terminal L-amino-acid, or an optically active L-acylamino-acid is coupled to an ester of a second amino-acid or peptide. The products of the coupling are examined: the D-amino-acid-containing peptide is separated by fractional crystallization[1,2] or counter-current distribution,[3] or by vapour-phase chromatography;[4] the amount of the peptide with a D-amino-acid serves as a measure of racemization occurring in the coupling. These model systems or model peptides are extremely useful in the evaluation of coupling methods and in the selection of optimal conditions for the peptide bond-forming step. On the other hand, such model peptides do not permit conclusions on the influence of different protecting groups or different amino-acid side-chains.†

Stouffer, Jarvis, and du Vigneaud[5] observed that *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester is readily racemized in the presence of triethylamine. The base-catalyzed racemization of active esters of protected amino-acids was, therefore, suggested[6] for the study of racemization.[7] This approach does not necessitate the separation of reaction products; it can be executed simply by the observation of the change with time in the values of rotation. Already the preliminary investigations[6] pointed to differences in the rate of racemization of different amino-acids and the influence of protecting groups also was indicated. It is very likely that these differences are due to the fact that in the racemization of protected and activated amino-acids more than one mechanism can be operative.[8] The most common and best studied mechanism involves an oxazolinone (azlactone) intermediate. The formation of such an intermediate is greatly reduced in benzyloxycarbonylamino-acids or more generally in amino-acid derivatives bearing a urethane-type amino-protecting group. Such a double protection, which prevents both undesired acylation and racemization, does not hinder the loss of optical purity by a second mechanism: the base-catalyzed reversible β-elimination of good leaving groups.

† An additional shortcoming of model peptides (refs. 1–4) is that the D-amino-acid-containing product has to be isolated. This can be avoided if the acetyl-(or benzoyl)-L-isoleucine is coupled, *e.g.*, to glycine ethyl ester. The amount of D-alloisoleucine in the crude product is a measure of racemization and can be easily determined by quantitative amino-acid analysis. This approach is currently used in this laboratory; its details will be published later.

This is the cause of racemization of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester.‡ Finally, the abstraction by tertiary base of a proton from the α-carbon atom explains the increased tendency for racemization in the derivatives of the aromatic amino-acids[6] where delocalization of the negative charge can stabilize the resulting carbanion. In active esters of phenylglycine the aromatic ring is directly attached to the asymmetric (α) carbon atom, which is not only benzylic but also further influenced by the negative inductive effect of an active ester grouping. Therefore, the rapid racemization of benzyloxycarbonyl-L-phenylglycine *p*-nitrophenyl ester§ is not quite unexpected. No oxazolinone intermediate should be assumed in this process since a a urethane-type amino-protecting group is present. The extremely rapid racemization of phthalylphenylglycine *p*-nitrophenyl ester also supports¶ this argument.

Because of the existence of these diverse mechanisms, it is unlikely that any single model peptide could allow the drawing of conclusions which would be valid for the entire gamut of intermediates in peptide synthesis. It is more probable that an analysis through several models, each racemized by a distinct mechanism, should lead to a better understanding and handling of the racemization problem.

We report the application of three active esters, benzoyl-L-leucine *p*-nitrophenyl ester,[9] *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester,[10] and benzyloxycarbonyl-L-phenylglycine *p*-nitrophenyl ester. Each of these esters represents one of the mechanisms discussed above. They were used for a study of the influence of the nature of the tertiary base which causes their racemization. The significance of tertiary bases in racemization was recently pointed out by Anderson and his associates,[11] but it was felt that a series of measurements on mechanism-specific models could yield additional useful information. Rotation measurements were performed at 24°, with a Carey 60 recording spectropolarimeter, at $\lambda = 590$ mμ, with manually-operated slit (0·11 mm.); a cell of 1 cm. path length was used.

Our investigations confirmed the known effect of solvents on racemization which was comparatively slow in tetrahydrofuran, faster in ethyl acetate, even more in chloroform, and very pronounced in dimethylformamide. These are the solvents commonly used in peptide synthesis. This solvent effect is independent of the mechanism since it was observed in the same sense on all the three active esters. Similarly, the rate of racemization was found in all three systems to be directly proportional to the concentration of the tertiary base. On the other hand, it was interesting to observe how differently the three active esters behave toward a series of tertiary amines (Table). The effect of steric hindrance is quite obvious in the case of racemization by β-elimination or by abstraction of a proton from the α-carbon of the ester, but even the strongly hindered *NN*-di-isopropylethylamine leads to rapid racemization of azlactone intermediates. Only extreme hindrance, such as in tribenzylamine, inhibits the

TABLE

Effect of tertiary bases on the rate of racemization

Amines	$t_{\frac{1}{2}}$ (min.)[a] A	B	C
Triethylamine	30	57	23
Tri-n-propylamine	48	128	27
Tri-n-butylamine	46	93	32
Tri-n-pentylamine	31	92	37
Tri-(3-methylbutyl)amine ..	35	59	35
Tri-n-octylamine	26[b]	86	24
Tri-n-dodecylamine	70	100	30
Tribenzylamine[c]	stable	stable	stable
NN-Diisopropylethylamine[d] ..	41	stable	800
NN-Methyldiethylamine ..	34	35	15
N-Methylpiperidine	46	42	20
N-Ethylpiperidine	41	62	45

[a] $t_{\frac{1}{2}}$ is the time (min.) required for the loss of half of the original value of rotation. A = benzoyl-L-leucine *p*-nitrophenyl ester; B = *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester; C = benzyloxycarbonyl-L-phenylglycine *p*-nitrophenyl ester. 0·05M-Solutions of active esters in chloroform were used. The amines were present in 0·1M-concentration with active esters A and C and 0·4M with ester B. [b] 140 min. in ethyl acetate. [c] In dimethylformamide B is stable, A and C are racemized extremely slowly. [d] In dimethylformamide $t_{\frac{1}{2}}$ is 230 min. for B and 9 min. for C.

‡ The formation of *N*-benzyloxycarbonyl-*S*-benzyl-DL-cysteine thiobenzyl ester from *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester in the presence of triethylamine at 100° (M. Bodanszky, unpublished) is good support for the assumption of racemization by β-elimination.

§ The ready racemization of t-butyloxycarbonyl-L-phenylglycine *p*-nitrophenyl ester was observed in co-operation with Dr. Miguel A. Ondetti of the Squibb Institute for Medical Research, New Brunswick, New Jersey. Benzyloxycarbonyl-L-phenylglycine *p*-nitrophenyl ester was prepared according to the general procedure described in *Biochem. Prep.*, 1962, **9**, 110.

¶ Phthalylphenylglycine was prepared from optically pure L-phenylglycine with *N*-ethoxycarbonylphthalimide [G. H. L. Nefkens, G. I. Tesser, and F. J. F. Nivard, *Rec. Trav. Chim.*, 1960, **79**, 688] and the acid was esterified with nitrophenol. (*cf. Biochem. Prep.*, 1962, **9**, 110). The protected amino-acid active ester [m.p. 160—162°, $[\alpha]_D^{24}$ − 22° (*c* 4, tetrahydrofuran)] is of undetermined optical purity. A 50% drop in the value of its rotation in tetrahydrofuran containing 1% triethylamine occurs in less than 1 min.

racemization of an oxazolinone. That differences in the amine are less significant in this mechanism can be well understood after inspection of the molecular model of an azlactone: the α-carbon (in position 4 of the oxazolinone) is sufficiently exposed and can be approached by the amine even if the free electron-pair of the latter is not too easily available.

In the case of the azlactone mechanism satisfactory protection can be provided by the use of urethane-type amino-protecting groups. Inhibition of racemization by application of sterically hindered amines might gain importance where derivatives of *S*-benzylcysteine, *O*-benzylserine, or of aromatic amino-acids are involved.

This work was supported by a grant from the National Institute of Health.

(*Received, May 1st,* 1967; *Com.* 416.)

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