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FORMATION AND REACTIONS OF AMINO ACIDS AND PEPTIDE OXAZOLONES

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The most common route to racemization during peptide coupling reactions involves oxazolone formation. Little work has been reported on the relative ease of formation of oxazolones under various conditions. Kemp and Chien[1] explored the nature of the dependence of the rate of oxazolone formation on the base and showed that the amide involved in the cyclization can have a dual nucleophilicity, depending on the relative concentrations of neutral amide and amide anion present in the media. Young and coworkers[2] found that the yields of L-peptide in the dicyclohexylcarbodiimide coupling of benzoyl, acetyl, and formyl-leucine with glycine ethyl ester in dichloromethane were 54%, 70% and 94%, respectively. These values must be related to the relative ease of formation of the oxazolone intermediate. Determan[3] examined amino acid derivatives bearing acyl and alkoxycarbonyl blocking groups by IR and NMR spectroscopy. He attributes the ease of oxazolone formation in the former case to the higher nucleophilicity of the amide oxygen atom.

During a peptide coupling reaction, three factors may be considered in relation to racemization via the oxazolone route: a) the ease of oxazolone formation; b) the rate of oxazolone racemization; c) the rate of ring-opening of oxazolone. We studied these factors for benzoyl-L-phenylalanine, which racemizes via the amino acid oxazolone [2-phenyl-L-4-benzyloxazolone] and benzyloxycarbonylaminoisobutyryl-L-phenylalanine, which racemizes via the peptide oxazolone [2-(1'-benzyloxycarbonylamino-1'-methyl)-ethyl-4-benzyloxazolone.

Amino acid oxazolone formation appears to be a far more facile process than peptide oxazolone formation. Treatment of benzoyl-L-phenylalanine (1.09 g.;7.3 mmole) in 11 ml. of a 1:1 acetic anhydride-dioxane mixture at

20° gives the maximum negative polarimetric reading in 75 minutes, corresponding to the formation of L-oxazolone. Similar results for the formation of amino acid oxazolones have been obtained for acetyl-L-phenylalanine[4] and benzoyl-L-leucine[5]. Reaction of benzyloxycarbonylaminoisobutyryl-L-phenylalanine, under the same conditions, gives a maximum negative reading after 14 hours.

On treatment with basic reagents, oxazolones racemize by abstraction of a proton from the acidic asymmetric center. This reaction can be followed by polarimetry. Alternatively, attack on the carbonyl function leads to a ring-opened product which can be followed by the disappearance of the carbonyl absorption in the IR spectrum. Previous investigations in our laboratory have determined the second order rate constants for the racemization of 2-phenyl-L-4-benzyloxazolone[6] and for the racemization and ring-opening of the peptide oxazolone, 2-(1'-benzyloxycarbonylamino-1'-methyl)-ethyl-4-benzyloxazolone[7] by DL-phenylalanine methyl ester in dioxane at 25°. We determined the second order rate constant for 2-phenyl-L-4-benzyloxazolone from the slope found by plotting three pseudo-first order rate constants against concentration of DL-phenylalanine methyl ester. A summary of these second-order rate constants is given in Table 1.

Under our reactions conditions, the peptide oxazolone racemizes 11-12 times faster than it ring-opens. For the amino acid oxazolone, racemization proceeds almost 200 times faster than ring-opening. The amino acid oxazolone racemizes 5 times faster than the peptide oxazolone because of the additional conjugation possible in the former case between the aromatic ring and the carbon-nitrogen double bond. Ring-opening for the peptide oxazolone is 3 to 4 times slower than for the amino acid oxazolone.

These results may serve to explain why the Young model system[8] is a more stringent test for racemization than Anderson's method[9]. The former involves benzoyl-L-leucine which racemizes via an amino acid oxazolone while the latter utilizes benzyloxycarbonylglycyl-L-phenylalanine which racemizes via a peptide oxazolone. Our experiments indicate that an amino acid oxazolone can form more readily, racemize more quickly and ring-open more slowly

than a peptide oxazolone.

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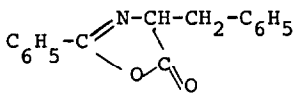
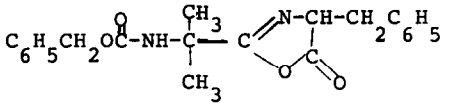
Table 1. Second order rate constants for racemization and ring opening of oxazolones in dioxane at 25°.

	Amino acid oxazolone	Peptide oxazolone
		
k_{ro} (ℓ/mole-min)	0.019	0.065
k_{rac} (ℓ/mole-min)	3.69	0.750

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