Peptide Racemization Mechanism. A Kinetic Isotope Effect as a Means of Distinguishing Enolization from Oxazolone Formation

Sir:

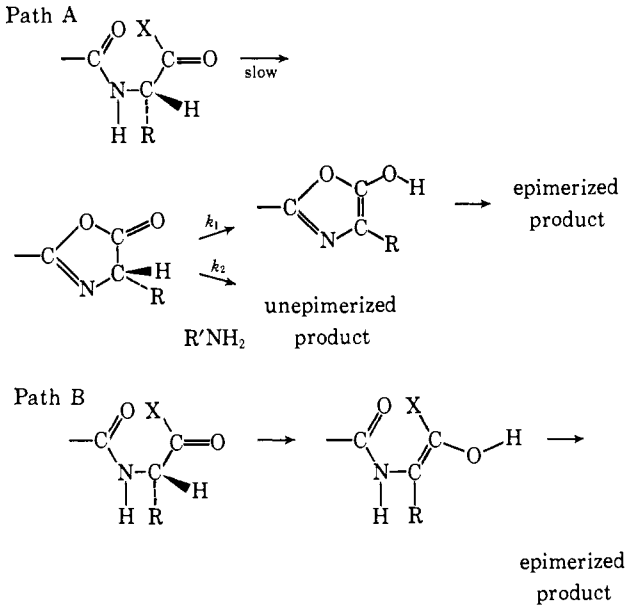
Of the two mechanisms of α epimerization open to carboxyl-activated peptide derivatives, the oxazolone mechanism, A, has been convincingly shown[1] to account for most of the racemization observed when phenyl esters of peptide and acylamino acids are treated with strong bases. A distinction between A and B for more routine peptide coupling conditions which involve weakly basic peptide amines and which yield only traces of racemate may be of considerable importance since it may permit a rational suppression of racemization.[2]

We wish to describe a method for assessing the kinetic isotope effect observed for the racemization of α-deuterated benzoyl-L-leucine (**1**) and benzyloxycarbonylglycyl-L-phenylalanine (**2**), which occurs during coupling with ethyl glycinate. Provided oxazolone formation is rate determining and $k_1 \gg k_2$, mechanism A must show an isotope effect of unity,[3] while B is expected to show a substantial isotope effect. Provided these conditions can be justified, a method for measuring k_H/k_D is a method for distinguishing A from B.

Table I. Kinetic Isotope Effects for Peptide Racemization

Coupling method[a]	Conditions	Young, 1 → 3		Anderson, 2 → 4	
		% rac[b]	k_H/k_D[c]	% rac[b]	k_H/k_D[c]
(1) 3-Acyloxy-2-hydroxy-*N*-ethylbenzamides	(i) Ester, 1 hr, 22°, DMF, 1.0 equiv of tetramethylguanidine; then GlyOEt·HCl, 12 hr	32	1.0		
	(ii) 1.0 equiv of GlyOEt, DMF, 12 hr 22°	1.0	1.0	0.06	1.3
	(iii) 2°	0.3	1.0	0.012	1.0
(2) 2-Acyloxy-*N*-ethylbenzamides	1.0 equiv of Et_3N, 1.0 equiv of Et_3NHBF_4, 22°, 15 min, DMF; then GlyOEt·HCl, 24 hr	19	1.0		
(3) Mixed anhydride	1.0 equiv of *i*-BuOCOCl, 1.0 equiv of *N*-methylmorpholine, THF, 30 sec, −14°; then GlyOEt	3.0	1.0	0.013	1.5
(4) Acyl azide	(i) Et_2O, 3°, GlyOEt	0.17	1.0	0.03	2.6
	(ii) Et_2O, 3°, Et_3N, 15 min; then GlyOEt	1.4	1.1	1.6	2.9
	(iii) DMF, 3°, GlyOEt			0.48	2.1
	(iv) DMF, 3°, Et_3N, 15 min; then GlyOEt			50.3	1.3

[a] Detailed reaction conditions may be found in ref 4 and D. S. Kemp, Z. Bernstein, and J. Rebek, Jr., *J. Amer. Chem. Soc.*, **92**, 4756 (1970). [b] Calculated for the α-H compounds. [c] Estimated errors are ±0.2.



We have recently described[4] a microassay for racemates which produces samples whose radioisotopic content is proportional to the amount of D enantiomer formed in a peptide coupling step. By substituting for singly labeled starting material a mixture of α deuterio peptide acid labeled with ^{14}C and protio acid labeled with ^{3}H and by comparing the initial $^{3}H/^{14}C$ ratio with that of the final sample, we have extended this microassay to permit estimation of k_H/k_D for racemization processes which contribute as little as 0.001% to the overall product composition.[5,6] Results

(1) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 3701 (1964); I. Antonovics and G. T. Young, *ibid.*, 595 (1967); M. Goodman and L. Levine, *J. Amer. Chem. Soc.*, **86**, 2918 (1964); W. J. McGahren and M. Goodman, *Tetrahedron*, **23**, 2017 (1967).

(2) For discussions of this problem, see: G. T. Young, *Proc. 8th Eur. Peptide Symp., Noordwijk, 1966*, 55 (1967); D. S. Kemp and S. W. Chien, *J. Amer. Chem. Soc.*, **89**, 2743, 2745 (1967); G. T. Young and J. H. Jones, *J. Chem. Soc.*, 436 (1968).

(3) If k_2 is of the same magnitude as k_1, an appreciable isotope effect for oxazolone racemization will result in a corresponding nonunity isotope effect for the overall path A. Secondary isotope effects are expected to lie within the margin of error of the assay.

(4) D. S. Kemp, S. W. Wang, G. Busby III, and G. Hugel, *J. Amer. Chem. Soc.*, **92**, 1043 (1970).

(5) Resolved [7-^{14}C]Bz-L-[2-^{2}H]LeuOH (>97% α-^{2}H by nmr) was prepared by repeated equilibration of 2-phenyl-4-isobutyl[2-^{14}C]oxazol-5-one in DOAc, cleavage with D_2O, and resolution with cinchonine; it was mixed with Bz-L-[4- or -5-^{3}H]LeuOH and freed of labeled DL by dilution.[4] Resolved Z-[1-^{14}C]Gly-L-[2-^{2}H]PheOH was prepared by analogous equilibration and cleavage of 2-phenyl-4-benzyloxazol-5-one (>97% α-^{2}H by nmr), acid hydrolysis, coupling of the DL-Phe with Z-[1-^{14}C]GlyOH,[2,6] and resolution of the product with (−)-α-phenyl-

for the synthesis of the Young peptide, ethyl benzoylleucylglycinate (**3**), and the Anderson peptide, ethyl benzyloxycarbonylglycylphenylalanylglycinate (**4**), are given in Table I.

The most striking result of Table I is the isotope effect of unity observed for all benzoylleucine couplings. Since methyl benzoyl-L-leucinate is observed to racemize in methanol containing potassium methoxide with an isotope effect of 3.8, one can conclude that appreciable isotope effects should be observed for the simple enolization mechanism; the oxazolone route, A, therefore appears to account for the major part of racemization observed for benzoylleucine couplings. It should be noted that two of the cases of Table I appeared as likely candidates for simple enolization: method 1i involves intermediacy of a catechol monoester anion, for which racemization *via* an internal proton transfer may now be excluded; method 2 involves conditions (tertiary amine, high conjugate acid concentration) for which the normal specific base catalysis mechanism is suppressed.[2b] We now attribute the racemization observed with method 2 to general base catalyzed oxazolone formation.

Isotope effects observed for the Anderson couplings are perhaps within experimental error of unity for methods 1ii and 1iii,[7] 3, and 4iv, but reflect substantial isotopic selectivity for most of the azide couplings. Before the oxazolone route can be excluded for these cases, it must be established that for the Anderson oxazolone, 2-benzyloxycarbonylaminomethyl-4-benzyloxazol-5-one (**5**), under the reaction conditions 4i–iii, k_1 in fact greatly exceeds k_2.

In accord with the study of Goodman and McGahren[8] L-**5**[9] was found to react with ethyl glycinate under condition 4i to yield tripeptide which was only 54% racemic, and an isotope effect of *ca.* 3.5 was observed; under condition 4iii the corresponding numbers were 80% and *ca.* 5–7. On the other hand, complete racemization (k_H/k_D = 1.0) was observed with **5** under conditions 4ii or 4iv. The isotope effect in case 4ii is therefore inexplicable by the oxazolone mechanism, and in the presence of triethylamine the acyl azide of **2** in ether must racemize largely by the direct enolization mechanism. The evidence does not permit clear-cut assignment of mechanism for the three remaining azide cases, but they seem best interpreted as involving contributions from both mechanisms.

Thus with the benzamido group (or its anion) the oxazolone pathway appears to be the major contributor to racemization. With a less nucleophilic peptide amide the competition is more delicately balanced, and in at least one azide coupling direct enolization is competitive.

ethylamine; the sample was mixed with Z-Gly-L-[*3*-^3H]PheOH and diluted with unlabeled DL.[4]

(6) Though not strictly valid for all mechanistic cases, calculations of Table I assume that $k_H/k_D = ([^3H_{prod}]/[^{14}C_{prod}])\,([^{14}C_{sm}]/[^3H_{sm}])$.

(7) The value of 1.5 for the anhydride case is significantly larger than unity and may reflect either a contribution from path B or a finding of $k_2 \sim k_1$ for THF.

(8) M. Goodman and W. J. McGahren, *Tetrahedron*, 23, 2031 (1967).

(9) L-**5** was prepared in *ca.* 10% yield by reaction of **2** in MeCN with the ketoketenimine obtained from Woodward's reagent K and triethylamine.[10] By-products were removed by extraction with water. Optical purity of **5** was established by a cleavage with hydrazine[8] at 0° in methanolic THF which yielded hydrazide of **2**, >95% L, assessed by isotopic dilution.

(10) R. B. Woodward and D. J. Woodman, *J. Org. Chem.*, 34, 2742 (1969).

(11) A. P. Sloan Fellow, 1968–1970; financial support from National Institutes of Health Grant GM 13453 is gratefully acknowledged. Address correspondence to this author.

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A Radical Intermediate in the Photolysis of *o*-Phthalaldehyde at 77°K

Sir:

Following our report,[1] two papers[2,3] discussed the mechanism of the photochemical isomerization of *o*-phthalaldehyde (**1**) to phthalide (**3**), and both proposed an intermediate hydroxyketene (**2**). We now wish to report that the mechanism of the photochemical reaction is strongly temperature dependent and that we have secured evidence for a free-radical intermediate in the photolysis of **1** at low temperature.

When a sample of **1** (carbonyl at 1670 cm^{-1}), neat or as a mull in Nujol, was irradiated at 77°K,[4] a minute ir

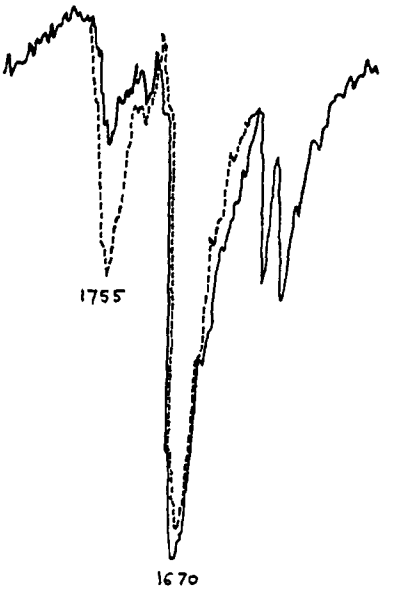

Figure 1. Infrared spectra of **1** (neat): after 5-min irradiation at 77°K with a 450-W Hanovia lamp (solid line), and after warm-up of that sample to room temperature in the dark (dotted line). Considerable scale expansion was required for seeing the 2060-cm^{-1} peak in the first spectrum.

absorption at 2060 cm^{-1} appeared almost immediately, and its intensity remained constant while a phthalide-like carbonyl peak at 1755 cm^{-1} slowly increased with the irradiation time.[5] The 2060 peak was stable at

(1) J. Kagan, *Tetrahedron Lett.*, 6097 (1966).

(2) S. P. Pappas and J. E. Blackwell, Jr., *ibid.*, 3337 (1968).

(3) K. F. Cohen, J. T. Pinhey, and R. J. Smith, *ibid.*, 4729 (1968).

(4) We used a variable-temperature cell (No. VLT-2, Beckman) which was placed in a Rayonet reactor at 253.7 nm, or in front of a 450-W Hanovia lamp housed in a water-cooled quartz well with a Vycor filter.

(5) Very significant conversion took place upon prolonged irradiation at 77°K, especially with the Hanovia lamp.