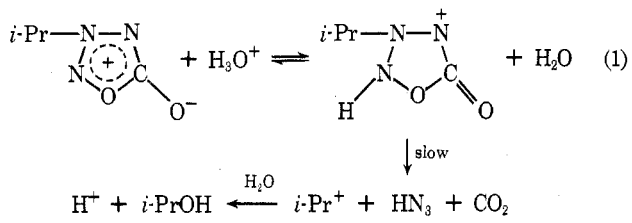

drolysis in the presence of hydrochloric acid were found to be isopropyl chloride and hydrazoic acid.

Experimental Section

The isopropyloxatriazole **2b** prepared by the method of Boyer and Canter[13] had bp 63 °C (0.5 mm) [lit.[13] bp 60–61.5 °C (0.45–0.50 mm)]. Kinetics were followed spectrophotometrically at 255 nm in a Unicam Model S.P. 800 spectrometer using a constant temperature cell thermostated to ±0.1 °C.

Registry No.—**2b**, 7724-83-6; $HClO_4$, 7601-90-3; H_2SO_4, 7664-93-9; HCl, 7647-01-0.

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Reactions of Carbamylimidazoles with Nucleophiles—an Example of an Intramolecular Acyl Transfer Reaction

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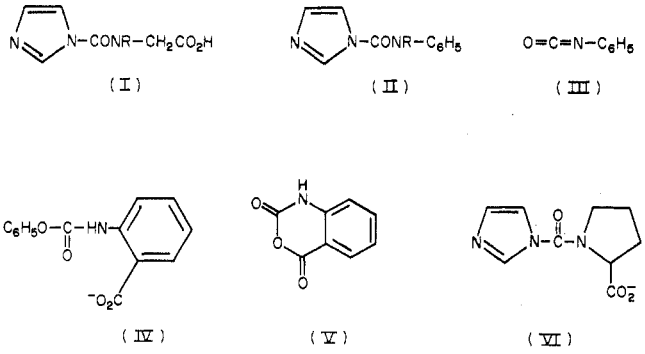
Received April 6, 1976

Glycine in an aqueous imidazole buffer has been shown[1] to react with carbonyldiimidazole to yield initially *N*-[imidazolyl-(1)-carbonyl]glycine (I), R = H. This intermediate



slowly polymerizes to yield oligoglycines. The suggested route for this polymerization is via a *N*-carboxyanhydride. The present report presents evidence that the cyclization of I proceeds via an addition–elimination, intramolecular acyl transfer reaction[2] involving a carbamylimidazole.[3,4] In general 1-substituted carbamylimidazoles react with nucleophiles via

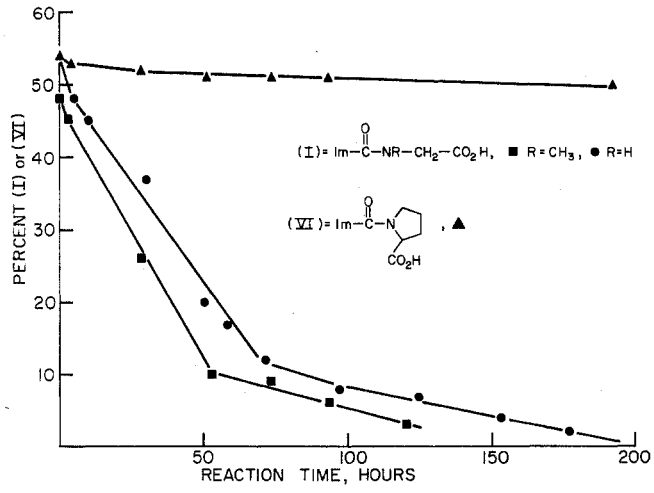


Figure 1. Plot of survival of I and VI as a function of time.

an intermolecular elimination–addition mechanism. Staab and Benz,[5] for example, showed that II, R = H, reacts with amines, while II, R = CH_3, does not. They concluded from this that II, R = H, reacts via its isocyanate (III). Kinetic evidence has been presented[3,4] to show that the hydrolysis of II, R = H, also proceeds via an intermolecular elimination–addition acyl transfer mechanism involving an isocyanate. In this case, II, R = CH_3, hydrolyzes much slower than II, R = H. *N*-Aryl carbamates, like the carbamylimidazoles, undergo hydrolysis[6] via the elimination–addition route. However, phenyl *N*-(*o*-carboxyphenyl)carbamate (IV) follows an addition–elimination route[7] via isatoic anhydride (V). This reaction is analogous to the one which we have discovered.

Carbonyldiimidazole (0.4 M) was added to 0.1 M [α-^{14}C]-glycine, [α-^{14}C]sarcosine, and [α-^{14}C]proline (specific activity in each case, 0.05 mCi/mmol) in imidazole buffer (0.5 M) at pH 7.0 and 0 °C. The carbamylimidazole intermediates I, R = H, I, R = CH_3, and VI were obtained in 84, 48, and 98% yield, respectively, from the three amino acids. The intermediates were identified by their electrophoretic behavior in systems II and III (unit negative charge[1,4,8]) and by their positive reaction with a sulfanilamide reagent.[1,9] As shown in Figure 1, the lifetime of I, R = CH_3, is strikingly similar to that of I, R = H, when the two are formed in approximately the same initial yield. By contrast, VI is extremely long lived.

To determine the rate of appearance of the sarcosine peptides, the origins of the system II electrophoreses papers were cut out, eluted with deionized water, and rerun in system I. The sarcosine peptides, which have almost the same mobility value as sarcosylglycine (see below), appeared at a rate similar to that found for glycine (see Figure 2). To further confirm this the previous experiment was modified as follows. Unlabeled amino acids were used to generate the initial intermediates, I and VI. Immediately after the dissolution of the carbonyldiimidazole at 0 °C, pH 7.0, [α-^{14}C]glycine (0.2 M glycine, 0.5 M imidazole, pH 6.85, specific activity 0.05 mCi/mmol) was added to each of the reaction mixtures in twofold molar excess. The appearance of aminoacylglycine with time is shown in Figure 3. Again, sarcosine behaves like glycine whereas proline is much less reactive. Cochromatography in solvent system IV and coelectrophoresis in solvent system I with authentic samples of sarcosylglycine and prolyglycine were used to establish the nature of the peptides generated in the above experiments.

There are three reasonable mechanisms for the formation of a *N*-carboxyanhydride from VII, R = H. By analogy with the behavior[7] of compound IV the addition–elimination route (2) involving direct closure of VII to the *N*-carboxyanhydride

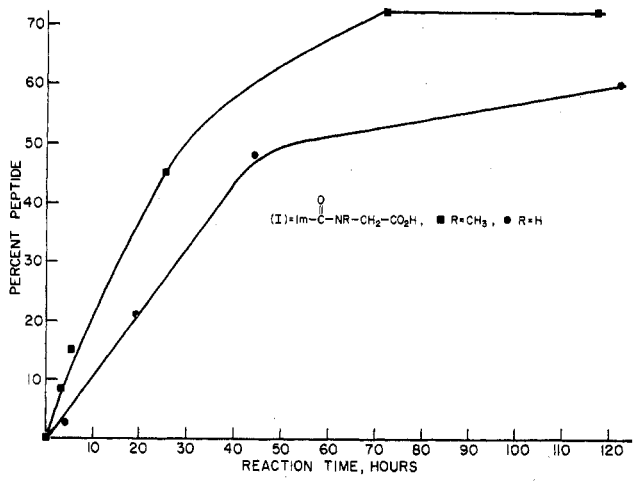
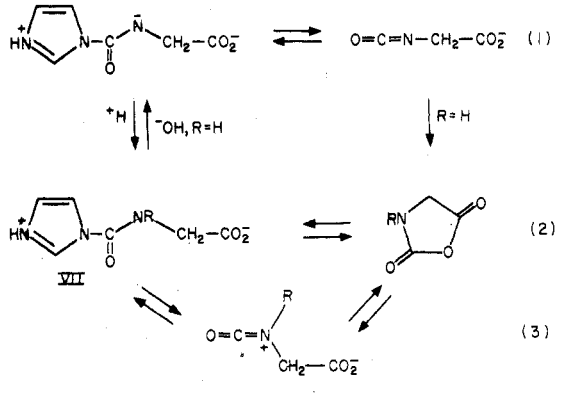


Figure 2. Plot of appearance of peptide as a function of time in the reaction of glycine and the reaction of sarcosine with carbonyldiimidazole in imidazole buffer.

seems the most probable route to the *N*-carboxyanhydride. The elimination–addition route (1) via the anionic zwitterion[4]



seems unlikely since glycine and sarcosine exhibit such similar behavior with respect to the disappearance of VII and to the appearance of peptide. Route 3, via the neutral zwitterionic isocyanate, seems unlikely on intuitive grounds and in light of the observation of Hegarty et al.[4] that the methyl analogue II, R = CH_3, hydrolyzes much slower than II, R = H. Again the similar behavior of glycine and sarcosine makes this route unlikely. The behavior of proline reflects the ring strain of its *N*-carboxyanhydride relative to that of most other amino acid *N*-carboxyanhydrides.[10]

This appears to be the first reported instance of an intramolecular acyl transfer reaction involving a carbamyl imidazole, and suggests that, despite Hegarty's work,[3,4] a careful study of the reaction of nucleophiles with carbamyl imidazoles at pH 7.0 is worthwhile.

Experimental Section

Sarcosine (98%, mp 208 °C) was purchased from Aldrich and purified by recrystallization from 3–4% aqueous ethanol. Glycine and proline were purchased from Calbiochem, *N,N'*-carbonyldiimidazole and imidazole from Sigma, and sarcosylglycine and prolylglycine from Vega-Fox-Biochemicals. Radioactive [α-^{14}C]glycine and [α-^{14}C]-proline were purchased from Schwarz. Radioactive [α-^{14}C]sarcosine was purchased from California Bionuclear Corp.

Paper electrophoresis was done on Whatman 3MM paper, using varsol as coolant, or using a Savant flat plate electrophoresis system. The buffers were I, 0.05 M formic acid adjusted to pH 2.7 with concentrated ammonium hydroxide; II, 0.03 M potassium phosphate, pH 7.1; III, 0.2 M lithium hydroxide adjusted to pH 4.5 with glacial acetic acid.

Paper chromatography was done in solvent system IV, isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:2).

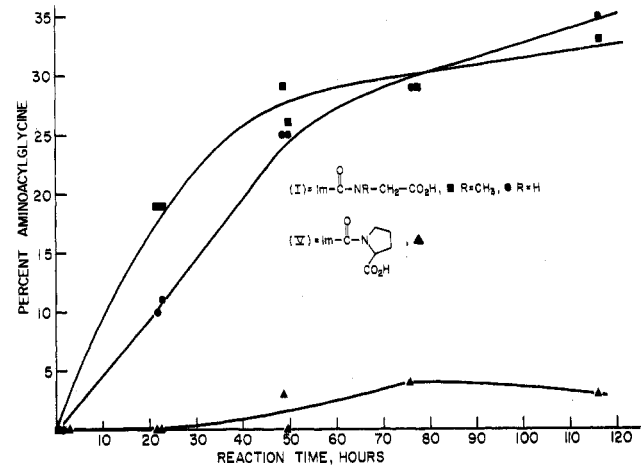


Figure 3. Plot of appearance of aminoacylglycine as a function of time in the reaction of glycine with I and VI.

Electrophoretograms of radioactive samples were treated as described earlier.

Acknowledgment. This work was supported by NSF Grant MPS 73-08792.

Registry No.—I (R = CH_3), 59643-40-2; I (R = H), 59643-41-3; VI, 59643-42-4; carbonyldiimidazole, 530-62-1; glycine, 56-40-6; sarcosine, 107-97-1; proline, 147-85-3.

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The Structures of Staphigine and Staphirine. Two Novel Bisditerpene Alkaloids from *Delphinium staphisagria*

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Received March 12, 1976

We wish to report the structures of staphigine (1) and staphirine (2), two new bisditerpene alkaloids isolated from the mother liquors of *Delphinium staphisagria*. ^{13}C and ^{1}H NMR spectroscopy played a major role in the determination of these structures. These alkaloids are unusual in containing a lactam moiety in addition to many of the uncommon features of the staphisine skeleton (3).[1]

The mother liquors accumulated during the isolation of delphinine from the seeds of *D. staphisagria* were found to contain a relatively large amorphous fraction of alkaloids.[2] From these mother liquors, we have recently isolated three new bisditerpene alkaloids, staphidine (4), staphinine (5), and