[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MICHIGAN]

Studies on the Base-catalyzed Acylation of Acylamino Acids. I. Stoichiometry

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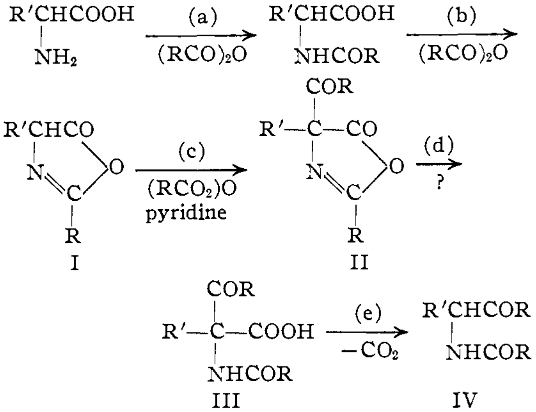
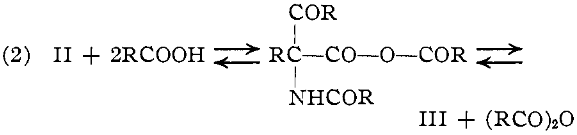
The base-catalyzed conversion of α-amino acids to α-acylamino ketones by acid anhydrides or acyl halides[1] has been the subject of several recent communications.[2,3,4,5] The most probable mechanism for this reaction[2] involves azlactonization of the acylamino acid first formed, base-catalyzed acylation at the reactive 4-position, followed by cleavage to a β-keto acid which decarboxylates (Eqn. 1). The first two steps correspond to known reactions under similar conditions.[6] The reactivity toward bases of the hydrogen in the 4-position is likewise well-established,[6] though few 4-acyloxazolones have been prepared by direct acylation of an oxazolone.[5,7] However, the penultimate step—the cleavage of the 4-acyloxazolone to a β-keto acid—is hypothetical, and its initiation is difficult to formulate.

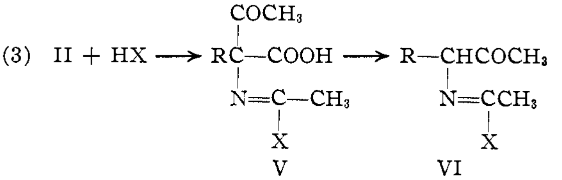
(1)



We had felt, as had others,[8,9] that the carboxylic acid produced in the first three steps as a by-product might cleave the azlactone in a manner reminiscent of similar cleavages by water, alcohols and amines.[6,7] The irreversible decarboxylation of the β-keto acid would complete the reaction and shift the equilibria to the right. Azlactones behave in many respects like acid anhydrides, and the reversible formation of anhydrides from acids by the action of other anhydrides is well-established. Equation (2) is plausible with carboxylic acids, but it is difficult to extend it to the halogen acids produced when acid halides are used instead of anhydrides,[1,2] especially since the hydrogen halide has been neutralized by pyridine. Though an imido halide (V) could be written, and its decarboxylation to VI and hydrolysis to IV in the isolation procedure[2] are reasonable, the preferred mode of reaction of HA reagents with oxazolones gives compounds of the different type VII.[7] Perhaps a different mechanism operates with acid halides, especially in view of the poor yields usually obtained with acid halides.[2]

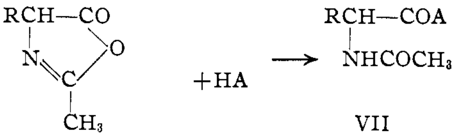


$$\text{(2)}\quad \text{II} + 2\text{RCOOH} \rightleftarrows \text{R}\overset{\text{COR}}{\underset{\text{NHCOR}}{\text{C}}}\text{—CO—O—COR} \rightleftarrows \text{III} + (\text{RCO})_2\text{O}$$



$$\text{(3)}\quad \text{II} + \text{HX} \longrightarrow \underset{\text{V}}{\text{R}\overset{\text{COCH}_3}{\underset{\substack{\text{N=C—CH}_3\\ |\\ \text{X}}}{\text{C}}}\text{—COOH}} \longrightarrow \underset{\text{VI}}{\text{R—}\underset{\substack{\text{N=CCH}_3\\ |\\ \text{X}}}{\text{C}}\text{HCOCH}_3}$$

(4)



We have studied the stoichiometry of the overall reaction Eqn. (1) to learn whether Eqn. (2) accurately represented the cleavage in step (d). Reactions (a), (b) and (c) each consume one mole of anhydride. One of these would be regenerated according to Eqn. (2), so that the net reaction (Eqn. (5)) should require two moles of anhydride per mole of amino acid. The use of considerable excesses of acid anhydride and pyridine has been customary. We have employed

$$\text{R'CH(NH}_2\text{)COOH} + 2(\text{RCO})_2\text{O} \longrightarrow \text{R'CH(NHCOR)COR} + \text{CO}_2 + 2\text{RCOOH} \quad (5)$$

limited quantities of acetic anhydride, using α-aminophenylacetic acid as a typical amino acid,[10] obtaining the results in Table I.

TABLE I

The percentages shown are the means of several determinations, differing by not more than three absolute per cent.

Moles Ac_2O/mole amino acid	CO_2, %
2	63
2.5	82
3	98

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[1] Dakin and West, *J. Biol. Chem.*, **78**, 91 (1928). The reaction is sometimes designated as the "Dakin-West reaction."

[2] Cleland and Niemann, THIS JOURNAL, **71**, 842 (1949).

[3] Searles and Cvejanovich, THIS JOURNAL, **72**, 3200 (1950).

[4] Wiley and Borum, Abst. Sept. 1949 Am. Chem. Soc. Meeting, p. 42M.

[5] Attenburrow, Elliot and Penny, *J. Chem. Soc.*, 310 (1948).

[6] Carter, "Azlactones," in Adams, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946.

[7] Cornforth, "Oxazolones," in Clark and Robinson, Editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1948.

[8] Scott Searles, private communication.

[9] George H. Cleland, private communication.

[10] The reaction with this amino acid gives quantitative yields of a solid product.[1]

The table shows clearly that the reaction is not complete unless three moles of acetic anhydride are used per mole of amino acid. Where the reaction is incomplete, the yield of carbon dioxide corresponds closely to the fraction of the required three moles employed.

Our results provide additional support for the mechanism outlined in Eqn. (1). Eqn. (1) indicates the requirement of three moles of anhydride, provided that the cleavage (Step (d)) does not proceed according to Eqn. (2). In other mechanisms which have been proposed,[1,4,11] only two moles are required.

We are currently engaged in a study of the cleavage of oxazolones of types I and II by carboxylic acids and halogen acids. It is expected that the results of this investigation will enable us to formulate a more satisfactory representation of step (d). Acid cleavage of some type is indicated by the results of Searles and Cvejanovich,[3,8] who have shown with a preformed oxazolone (type I) that the initial rate of carbon dioxide evolution drops to zero very rapidly if the acetic anhydride contains no acetic acid; the evolution is resumed upon addition of acetic acid.

Sarcosine (N-methylglycine), which cannot form an azlactone, does not undergo this reaction at 100°.[1] Wiley[4] has shown that the normal product, N-methylacetamidoacetone, can be obtained from acetylsarcosine at 140°. The longer contact time and the higher temperature required when an azlactone cannot be formed indicate that a different, slower mechanism operates in this case. Glycine also reacts at 140°. The azlactone derived from glycine has two active hydrogens in the 4-position, both of which appear to be replaceable.[12] Cleland and Niemann[2] have proposed an explanation for the different behavior of glycine. By arrangement with Professor Wiley, we are studying the stoichiometric requirements of sarcosine to obtain further evidence concerning the apparent duality of mechanism.

A consideration of other possible mechanisms led us to ask whether the carbon dioxide actually was liberated from the amino acid, as is generally assumed, or whether it might come from the acid anhydride. By using C^{14} carboxyl-labeled alanine, we confirmed this assumption. None of the radioactivity from samples of tagged alanine was found in the acetamidobutanone produced from them by the Dakin–West reaction. Most of the original activity appeared in the carbon dioxide evolved.

Experimental

Stoichiometric Studies.—Samples of α-aminophenylacetic acid[13] weighing 0.1–0.2 g. were mixed with weighed amounts of acetic anhydride (Merck Reagent, refractionated) and pyridine (Mallinckrodt Reagent, dried with barium oxide). After sweeping the system with dry, carbon dioxide-free air, the reaction mixture was heated on the steam-bath. The carbon dioxide was entrained by a slow stream of air, bubbled through sulfuric acid, and absorbed in ascarite. A barium hydroxide solution following the ascarite tube provided assurance of complete absorption. A three-way stopcock could be turned to divert the gas stream through a second weighed ascarite tube, permitting removal of the first for periodic weighing. The tubes were weighed alternately until they had reached constant weight. Heating and sweeping were continued for an additional hour to ensure complete reaction. Several determinations for each ratio were made, and the deviations from the mean never exceeded 3% absolute.

α-Phenyl-α-acetamidoacetone was isolated in several runs as a check on the method. The weight of crude material, m. p. 101–103° (uncor.) (lit.[1] 103–104°), was 5–7% less than that calculated from the carbon dioxide evolved.

Tracer Experiments.—Radioactive (C^{14}) alanine[14] (0.2 mg.) was diluted with purified ordinary alanine (10 g.) by dissolving the mixture in water and evaporating to dryness. A sample (0.2 g.) of this diluted material was mixed with 2.5 ml. of acetic anhydride and 1.5 ml. of pyridine. While heating on a steam-bath, a slow stream of dry, carbon dioxide-free air was drawn through the mixture by means of a water aspirator. After a sulfuric acid wash, the carbon dioxide was precipitated as barium carbonate by 1 *N* barium hydroxide. In two experiments, the yield of barium carbonate was 67.5 and 58.3%.

The radioactivity was measured in a "flow betameter,"[15] using a stream of "Q-gas."[15] The barium carbonate had 92 ± 4% of the activity of the alanine. The discrepancy may result from incomplete preliminary sweeping or a slight leak in one of the connections which permitted air to enter. The acetamidobutanone[12] exhibited no activity detectable above the background count.

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Summary

1. The pyridine-catalyzed conversion of α-aminophenylacetic acid to α-phenyl-α-acetaminoacetone requires three moles of acetic anhydride for complete reaction. With ratios less than three to one, the yield of carbon dioxide is proportionately lower. These results are consistent with the azlactone mechanism, but they cast doubt upon the mode of cleavage by acetic acid previously considered.

2. The assumption that the amino acid furnishes the carbon dioxide liberated has been verified with radioactive alanine.

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[11] Levene and Steiger, *J. Biol. Chem.*, **79**, 95 (1928).

[12] Wiley and Borum, THIS JOURNAL, **70**, 2005 (1948).

[13] Steiger, *Org. Syn.*, **22**, 23 (1942).

[14] Tracerlab, Inc., Boston, Mass.

[15] Nuclear Instrument and Chemical Co., Chicago, Illinois.