The Hydrolysis of Certain *N*-Benzoylamino-acids

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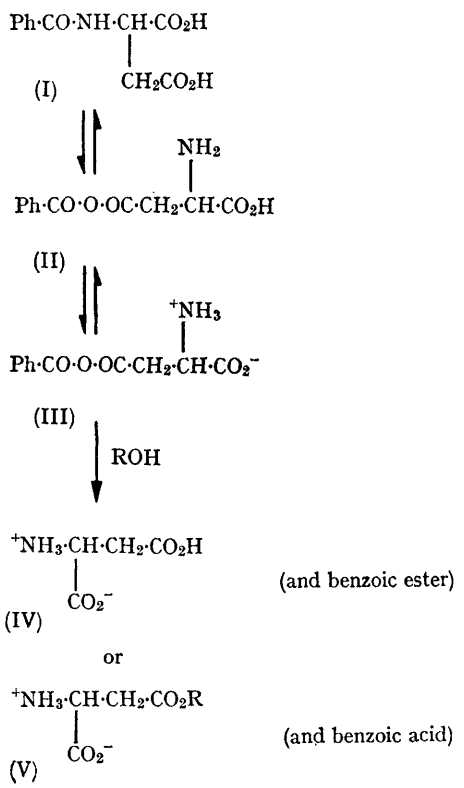
OUR observation of the unexpectedly rapid hydrolysis of *N*-benzoylaspartic acid in water at 100°, led us to investigate the behaviour of a number of *N*-benzoylamino-acids in water, and dilute acid, at 100°. This report concerns the hydrolysis of the *N*-benzoyl derivatives of glutamic acid and aspartic acid under the above conditions in dilute solution.

N-Benzoylglutamic acid in water at 100° gave no significant amounts of glutamic acid in 24 hours, but was quickly converted into pyrrolid-2-one-5-carboxylic acid and benzoic acid. However, in dilute hydrochloric acid it was hydrolysed to glutamic acid, though pyrrolid-2-one-5-carboxylic acid was shown to build up early in the reaction and then later to diminish and disappear. These results suggest that *N*-benzoylglutamic acid rearranges in solution at 100° to give benzoic acid and pyrrolid-2-one-5-carboxylic acid which, though stable in water, is hydrolysed in dilute acid media to give glutamic acid.

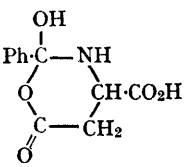
In water at 100° *N*-benzoylaspartic acid is readily hydrolysed to aspartic acid and benzoic acid. This reaction cannot proceed through a lactam intermediate because azetidin-2-one-4-carboxylic acid[1] is stable under these conditions. Further information was obtained concerning the course of these reactions by boiling *N*-benzoylaspartic acid under reflux in anhydrous methanol and also in anhydrous Methyl Cellosolve when the products (in addition to benzoic acid and benzoic ester) were free aspartic acid and the β-ester in proportions of roughly 1 : 2. The sequence of steps (I → IV or V) provides a route for the solvolysis of *N*-benzoylaspartic acid consistent with the available evidence. *N*-Benzoylaspartic acid (I) undergoes N → O migration, giving the mixed β-aspartyl–benzoic anhydride (II) which is prevented from reverting to (I) by formation of the zwitterion (III). This zwitterion mixed benzoic anhydride may then be attacked by solvent in two ways to give either benzoic ester and

aspartic acid (IV) or benzoic acid and the β-aspartic ester (V). Conversion of (II) into the zwitterion (III) must be important in suppressing the back reaction to regenerate *N*-benzoylaspartic acid(I),

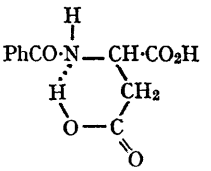


since *N*-benzoyl-β-alanine (in which the α-carboxyl group is absent) is quite resistant to hydrolysis under these mild conditions. We have at present no evidence to show how (I) is converted into (II), though this may occur *via* (VI) or by the rearrangement of the hydrogen-bonded structure (VII).

This mechanism has two interesting features. It is readily adaptable to explain the behaviour of *N*-benzoylglutamic acid because the γ-glutamyl–benzoic anhydride similar to (II) would be readily attacked by the α-amino-group to give pyrrolid-2-one-5-carboxylic acid. It may also be applied to help explain the selective release of aspartic acid from peptides and proteins. If both α- and β-carboxyl groups of an *N*-acylaspartic acid must be free to permit hydrolytic release of the aspartic acid (as this theory would imply) then aspartic acid should be released from a peptide chain by first splitting the chain to the carboxyl-side of the



(VI)



(VII)

aspartic acid residue. This may occur by the transient formation of the *N*-acylaspartic anhydride, as suggested by Blackburn and Lee.[2] Degradation of proteins under these conditions usually requires 8—16 hours to release all their aspartic acid,[2,3] whereas *N*-benzoylaspartic acid has a half-life of 115 minutes in water at 100°. This indicates that the initial chain-splitting to the carboxyl-side of the aspartic residue should be the slow rate-determining step. Once this has occurred it should be followed by the much more rapid release of *C*-terminal aspartic acid from the peptide fragment. This would explain the scarcity of peptides bearing *N*-terminal or *C*-terminal aspartic acid among fragments obtained by the degradation of tryptic peptides of tobacco mosaic virus,[4] and wool and bovine plasma albumin.[2]

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[1] Prepared according to E. A. Talley, T. J. Fitzpatrick, and W. L. Porter, *J. Amer. Chem. Soc.*, 1956, **78**, 5836.
[2] S. Blackburn and G. R. Lee, *Biochem. J.*, 1954, **58**, 227.
[3] S. M. Partridge and H. F. Davis, *Nature*, 1950, **165**, 62.
[4] C. M. Tsung and H. Fraenkel-Conrat, *Biochemistry*, 1965, **4**, 793.