135. *The Preparation of Benziminazoles from α-Benzamido-acids and Peptides.*

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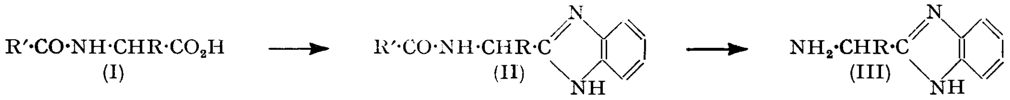
Exploratory studies have been made on the identification of the *C*-terminal amino-acid residues of peptides as benziminazole derivatives.

HUGHES and LIONS [1] reported that fusion of hippuric acid (I; R = H, R′ = Ph) with *o*-phenylenediamine at 160° gave in good yield 2-benzamidomethylbenziminazole (II; R = H, R′ = Ph), which could be hydrolysed to 2-aminomethylbenziminazole (III; R = H). However, Lettré, Fritsch, and Porath [2] obtained α-benzamido β-2-benziminazolylpropionic acid in only 5·5% yield from *N*-benzoylaspartic acid, the α-carboxyl group of this compound being unreactive under these conditions. In order to assess the feasibility of using the reaction sequence (I) → (II) → (III) to convert the *C*-terminal amino-acid residues of peptides into their benziminazole derivatives, we have studied the reactions of hippuric acid, *N*-benzoylalanine (I; R = Me, R′ = Ph), and *N*-benzoylphenylalanine (I; R = CH_2Ph, R′ = Ph). The compounds in the order named required increasingly drastic conditions to force condensation with *o*-phenylenediamine (see Experimental). The reactions are accelerated by acid catalysts, but these are undesirable for general use because of the acid-lability of some peptide linkages.[3]



The hydrochlorides of the aminoalkylbenziminazoles (III; R = H, Me, and CH_2Ph), derived from glycine, alanine, and phenylalanine, respectively, were obtained by hydrolysis of benzamido-compounds and were readily distinguished by paper chromatography.

Benzoylglycylglycine (I; R = H, R′ = $PhCO{\cdot}NH{\cdot}CH_2$) on fusion with *o*-phenylenediamine afforded the benziminazole (II; R = H, R′ = $PhCO{\cdot}NH{\cdot}CH_2$). On hydrolysis this gave glycine and 2-aminomethylbenziminazole, detected by paper chromatography, in

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[1] Hughes and Lions, *J. Proc. Roy. Soc., N.S.W.*, 1938, **71**, 209.

[2] Lettré, Fritsch, and Porath, *Chem. Ber.*, 1951, **84**, 719.

[3] Christensen, *Compt. rend. Trav. Lab. Carlsberg*, 1953, **28**, 265.

approximately equimolar amounts. The same products were obtained, in the expected relative amounts, from glycylglycine (I; R = H, R' = $NH_2{\cdot}CH_2$) and from diglycylglycine (I; R = H, R' = $NH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2$); the intermediate benziminazoles (II; R = H, R' = $NH_2{\cdot}CH_2$) and (II; R = H, R' = $NH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2$) were very soluble in water, and were not isolated analytically pure. The behaviour of peptides contrasts with that of α-amino-acids, which do not form benziminazoles when fused with *o*-phenylenediamine.[1]

EXPERIMENTAL

Preparation of Benzamidoalkylbenziminazoles.—(a) *Without catalyst.* The benzamido-acid (1 mole) was heated with *o*-phenylenediamine (2 moles) in a sealed tube under the conditions tabulated below. These conditions represent the optimal discovered; lower temperatures or shorter reaction times resulted in lower yields.

Starting material	Temp.	Reaction Time (hr.)	Product	Yield (%)	M. p.	Formula	Analysis	C (%)	H (%)
N-Benzoylglycine	118°	10	2-Benzamidomethylbenziminazole	77	228° [a, c]	$C_{15}H_{13}ON_3,H_2O$	Found :	66·5	5·5
							Calc. :	66·8	5·5
N-Benzoylalanine	137	10	2-1'-*Benzamidoethylbenziminazole*	53	254° [b]	$C_{16}H_{15}ON_3$	Found :	71·8	5·6
							Reqd. :	72·4	5·6
N-Benzoylphenylalanine	250	3·5	2-α-*Benzamidophenethylbenziminazole*	65	237° [a]	$C_{22}H_{19}ON_3$	Found :	77·0	5·4
							Reqd. :	77·4	5·6

[a] Crystallized from aqueous ethanol. [b] Crystallized from ethyl acetate. [c] Reported m. p. 230°.[1]

(b) *With acid as catalyst.* The benzamido-acid (1 mole), *o*-phenylenediamine (2 moles), concentrated hydrochloric acid (2 moles), and 75% phosphoric acid (2 moles) were heated in a sealed tube for 10 hr. At 100°, the yield of 2-benzamidomethylbenziminazole was 73%, that of 2-1'-benzamidoethylbenziminazole, 91%. At 110°, the yield of 2-benzamidomethylbenziminazole was 94%.

Convenient derivatives were: 2-*benzamidomethylbenziminazole picrate*, m. p. 248° (Found: C, 52·6; H, 3·6. $C_{21}H_{16}O_8N_6$ requires C, 52·5; H, 3·3%); 2-1'-*benzamidoethylbenziminazole picrate*, m. p. 199° (Found: C, 53·0; H, 3·9. $C_{22}H_{18}O_8N_6$ requires C, 53·4; H, 3·6%); and 2-α-*benzamidophenethylbenziminazole picrate*, m. p. 221° (Found: C, 58·8; H, 4·0. $C_{28}H_{22}O_8N_6$ requires C, 58·8; H, 3·8%).

Chromatographic Identification of the 2-Aminoalkylbenziminazoles.—The 2-benzamidoalkylbenziminazoles were hydrolysed with boiling 12N-hydrochloric acid (10 parts) for 5 hr. Benzoic acid was removed with ether; the aqueous solutions on evaporation to dryness afforded the hydrochlorides of the 2-aminoalkylbenziminazole. 2-Aminomethylbenziminazole dihydrochloride, recrystallised from ethanol, melted at 267° (reported m. p. 263° [1]). The hydrochlorides of 2-1'-aminoethylbenziminazole, m. p. 85°, and of 2-α-aminophenethylbenziminazole were very deliquescent and soluble in water, and were not investigated further except by chromatography on Whatman No. 1 filter paper. The benziminazoles were located as golden-yellow zones by spraying the chromatogram with an ethanolic solution of ninhydrin and heating. R_F values for the hydrochlorides are shown below.

Solvent system	(III; R = H)	(III; R = Me)	(III; R = CH_2Ph)
Butanol saturated with 2% aqueous hydrochloric acid	0·18	0·59	0·73
Pyridine–butanol–ethanol–water 12 : 66 : 4 : 8 (by vol.)	0·54	0·70	0·94
Pyridine–ethanol–water 12 : 80 : 8 (by vol.)	0·55	0·84	1·0

2-*Hippuramidomethylbenziminazole* (II; R = H, R' = $PhCO{\cdot}NH{\cdot}CH_2$).—Benzoylglycylglycine (1 g.) and *o*-phenylenediamine (0·9 g.) were heated in a sealed tube at 137° for 10 hr. Crystallization of the melt from ethyl acetate gave white needles of 2-*hippuramidomethylbenziminazole* (0·8 g.), m. p. 186° (Found: C, 66·2; H, 5·1. $C_{17}H_{16}O_2N_4$ requires C, 66·2; H, 5·2%). The *picrate* melted at 176° (Found: C, 52·4; H, 4·0. $C_{23}H_{19}O_9N_7$ requires C, 51·6; H, 3·6%). The benziminazole when hydrolysed with 12N-hydrochloric acid yielded glycine and 2-aminomethylbenziminazole in approximately equimolar amounts, as shown by paper chromatography.

Reactions of Glycylglycine and of Diglycylglycine with o-*Phenylenediamine.*—The di- and tripeptide (1 mole) were fused with *o*-phenylenediamine (2 moles) at 137° for 10 hr. No piperazine-2 : 5-dione (R_F 0·19) was detected in the melts by paper chromatography, propanol–water 1 : 1 (by vol.) being used as the developing agent, and the chlorine–starch–iodide method[4] for locating piperazine-2 : 5-dione. The melts were hydrolysed with hydrochloric acid, and glycine and 2-aminomethylbenziminazole identified by paper chromatography.

We are grateful to Professor W. Cocker for constant support and encouragement, and to the Medical Research Council of Ireland for a research grant (to R. C.).

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[*Received, October 3rd*, 1955.]

[4] Smith and Rydon, *Nature*, 1952, **169**, 922.