722. *The Reaction of* N-*Maleoylamino-acids with Benzylamine.*

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N-Maleoyl-amino-acids and -dipeptides, prepared by shaking an aqueous solution of the amino-acid (dipeptide) with maleic anhydride in benzene, were treated with benzylamine. Only in the case of DL-α-maleoylaminobutyric acid could the pure (*N*-benzyl-β-aspartyl) peptide be obtained; it was hydrogenolysed to the free dipeptide. In all other instances complex mixtures were produced which contained, *inter al.*, the β-aspartyl peptides.

One of the most direct preparations of β-aspartyl peptides consists in the condensation of maleic anhydride with amino-acid esters to yield *N*-maleoyl derivatives which react with benzylamine to form (*N*-benzyl-β-aspartyl) dipeptide esters; these are then hydrolysed and catalytically hydrogenolysed to the free β-aspartyl peptides.[1] This route, however, does not permit the isolation of (*N*-benzyl-β-aspartyl) dipeptides which are soluble in water and, therefore, not precipitated after hydrolysis of the esters.

The condensation of maleic anhydride with free amino-acids and peptides, and the reaction of the *N*-maleoylamino-acids with benzylamine, were investigated, with a view to shortening the above route, to avoid the use of amino-acid esters, and to prepare the (*N*-benzyl-β-aspartyl) dipeptides not obtained by hydrolysis of their esters.

N-Maleoylamino-acids can be prepared either by shaking an aqueous solution of the amino-acid with maleic anhydride dissolved in benzene,[2] or by reaction of the amino-acid with maleic anhydride in acetic acid.[3] The first method is especially applicable to water-soluble amino-acids whose maleoyl derivatives are much less soluble in that solvent, thus

[1] Liwschitz and Zilkha, *J. Amer. Chem. Soc.*, 1955, **77**, 1265.
[2] Irsay, Liwschitz, and Zilkha, *Bull. Res. Council Israel*, 1957, **6***A*, 272.
[3] King, Clark-Lewis, Wade, and Swindin, *J.*, 1957, 873.

promoting their precipitation almost from the start, and yields are good for comparatively short reaction times. Not only amino-acids but also dipeptides react in this way, as exemplified in the preparation of the *N*-maleoyl derivatives of glycylglycine, glycyl-DL-alanine, and DL-alanyl-DL-phenylalanine.

In contrast to the smooth addition of benzylamine to the double bond of *N*-maleoyl-amino-acid esters, this reaction did not proceed as expected with *N*-maleoylamino-acids. It was studied under various conditions, and it seemed best to perform it in aqueous solution with an excess of benzylamine at reflux temperature for 3—4 hours. After that, unchanged benzylamine was extracted with ether, and, on acidification with hydrochloric acid, substances were obtained which did not contain double bonds (as shown by negative potassium permanganate tests) or free amino-groups (negative ninhydrin reaction). This seemed to indicate that these compounds were the expected (*N*-benzyl-aspartyl) dipeptides. However, only in the case of α-maleoylaminobutyric acid, could a product be isolated whose analysis conformed to the *N*-benzylaspartyl dipeptide, *i.e.*, (*N*-benzyl-β-aspartyl)-α-aminobutyric acid. Hydrogenolysis of this compound in ethanol yielded (β-aspartyl)-α-aminobutyric acid. Chromatography gave the bluish spot characteristic of β-aspartyl dipeptides [4] and the same R_F value as the (β-aspartyl)-α-aminobutyric acid synthesised previously.[5] Its higher melting point (225°) indicates that the substance which belonged to the "mixed" (LD, DL) type was not accompanied by the "pure" (LL, DD) racemic diastereoisomers.

In one experiment with *N*-maleoylglycine, *NN'*-dibenzylasparagine was obtained which could only have arisen by a transamidation reaction with benzylamine. Chromatography of the residue from another experiment after hydrogenolysis gave a complex pattern of spots in which the following substances could be identified: (β-aspartyl)glycine (easily recognised by its peculiar ninhydrin colour which is altogether different from the purple colour of the α-isomer [4]), aspartic acid, glycine, and N^{α}-benzylasparagine. An additional spot was due to alanylglycine. Besides, there appeared a red spot of very low R_F value, which did not change in colour after several weeks, and which has not yet been identified.

Since the substances isolated after the reaction of the *N*-maleoyl derivatives with benzylamine did not give positive ninhydrin reactions, the appearance of the component amino-acids on chromatograms must be due to fission of the peptide bonds during the hydrogenolysis in acetic acid solution. This had already been observed in the preparation of (α-D-aspartyl)glycine.[6] However, even when ethanol was used as a solvent in the hydrogenolysis similar mixtures were obtained. The appearance of alanylglycine, identified on paper chromatograms in two different solvent systems, can only be explained in terms of a decarboxylation of (α-aspartyl)glycine which was formed together with its β-isomer, but being apparently more susceptible to decomposition its presence could not be established.

In order to verify whether the *N*-maleoyl derivatives were liable to hydrolyze in the aqueous medium in which the condensation with benzylamine was performed, thereby lowering the yield of the *N*-benzylaspartyl dipeptides, each of the substances prepared was boiled in aqueous solution for 10 minutes and the mixture was chromatographed together with the component amino-acid or peptide. In turned out that part of it had indeed undergone hydrolysis. On the other hand, the *N*-benzyl derivatives, including (*N*-benzyl-β-aspartyl)-α-aminobutyric acid and the compounds which proved to be mixtures, when treated likewise proved stable and no ninhydrin-positive substances could be detected by chromatography.

The reaction product of *N*-maleoyl-β-alanine with benzylamine yielded, after hydrogenolysis, spots on chromatograms among which β-aspartyl-β-alanine, aspartic acid, β-alanine, and N^{α}-benzylasparagine were identified.

[4] LeQuesne and Young, *J.*, 1952, 24.
[5] Liwschitz and Zilkha, *J.*, 1957, 4394.
[6] Liwschitz, Nemes, and Levi, unpublished work.

Experimental

Melting points were determined in a Fisher–Johns apparatus.

Preparation of N-*Maleoyl-amino-acids and -peptides.*—The amino-acid (0·2 mole), dissolved in the minimum amount of water, was shaken with a solution of maleic anhydride (0·2 mole) in benzene for several hours. In the case of the peptides, 0·004 mole was used, and reaction times are therefore much shorter. The precipitates, which started to form almost from the start, were filtered off, washed with water and ethanol, and dried. They were essentially pure and were recrystallised only for analysis. Details are given in the Table.

N-Maleoyl-[a]	Reaction time (min.)	Yield (%)	M. p.	Formula	Carbon Found	Carbon Calc.	Hydrogen Found	Hydrogen Calc.	Nitrogen Found	Nitrogen Calc.
glycine[b]	180	78	188°							
β-alanine	240	79	163	$C_7H_9NO_5$	45·0	44·9	4·8	4·8	7·4	7·5
DL-α-aminobutyric acid	210	70	150	$C_8H_{11}NO_5$	48·2	47·7	5·5	5·5	6·4	7·0
DL-valine[c]	180	90	197	$C_9H_{13}NO_5,0{\cdot}5H_2O$	47·9	48·1	5·7	6·2	6·0	6·2
glycylglycine	10	95	206	$C_6H_{10}N_2O_6$	41·5	41·6	4·7	4·4	12·1	12·2
glycyl-DL-alanine	10	95	147	$C_9H_{12}N_2O_6$	44·2	44·2	5·2	4·9	11·9	11·5
DL-alanyl-DL-phenylalanine...	10	90	174	$C_{16}H_{18}N_2O_6$	57·0	57·3	6·0	5·4	8·1	8·4

[a] Recrystallised from water unless indicated otherwise. [b] This substance had already been prepared by Werbin and Spoerri,[7] who reported m. p. of 189—190°. [c] Recrystallised from ethanol. The anhydrous compound, m. p. 166—167°, has been described by Fox and Minard.[8]

(N-*Benzyl*-β-DL-*aspartyl*)-DL-α-*aminobutyric Acid.*—DL-α-Maleoylaminobutyric acid (7 g. was dissolved in water (20 ml.), and benzylamine (15 ml.) was added. The mixture was heated under reflux for 3 hr. After cooling, aqueous sodium hydroxide solution was added and the solution was extracted with ether (4 × 20 ml.). On acidification of the aqueous layer to pH 4 with hydrochloric acid, the *N*-benzyl dipeptide was precipitated (5·3 g., 50%), m. p. 188° (from water) (Found: C, 58·2; H, 6·5; N, 9·2. $C_{15}H_{20}N_2O_5$ requires C, 58·3; H, 6·5; N, 9·1%).

(β-DL-*Aspartyl*)-DL-α-*aminobutyric Acid.*—(*N*-Benzyl-β-DL-aspartyl)-DL-α-aminobutyric acid (4 g.) was suspended in ethanol (100 ml.), and the catalyst (0·3 g. of palladium chloride–Norite 3 : 10) added. Hydrogenolysis was carried out in a Parr low-pressure apparatus at 70—80° for 10 hr. Most of the substance adhered to the catalyst and was freed by dissolution in hot water from which it crystallised (1·4 g., 52%) on cooling after filtration, m. p. 225° [Found: N(total), 12·4; N(Van Slyke), 6·3. Calc. for $C_8H_{14}N_2O_5$: N(total), 12·8; N(Van Slyke), 6·4%]. The substance was chromatographed in 80% phenol and in butan-1-ol–acetic acid–water (4 : 1 : 1). In both cases a single bluish spot resulted which had the same R_F value as a sample of (β-DL-aspartyl)-DL-α-aminobutyric acid prepared by a different route,[5] chromatographed simultaneously.

Reaction between N-*Maleoylglycine and Benzylamine.*—(*a*) *N*-Maleoylglycine (17·3 g.) in water (60 ml.) was heated with benzylamine (11 g.) under reflux for 4 hr. After cooling, the mixture was made alkaline and extracted several times with ether. After acidification to pH 4 with hydrochloric acid it was set aside for 2 days. The white precipitate which had formed was *NN'*-dibenzylasparagine, m. p. 214° (from water) (Found: C, 69·0; H, 6·4; N, 9·5. Calc. for $C_{18}H_{20}N_2O_3$: C, 69·2; H, 6·5; N, 9·0%).

(*b*) *N*-Maleoylglycine (14·7 g.) in water (25 ml.) and benzylamine (36 g.) were heated under reflux for 3 hr. The cooled mixture, after addition of 20% sodium hydroxide solution (20 ml.), was extracted with ether and acidified with trifluoroacetic acid. The substance which was precipitated was filtered off and dried (17 g.), m. p. 187—189° (from water). It did not decolorise potassium permanganate solution and gave a negative ninhydrin test. Analyses did not conform to the expected (*N*-benzyl-β-aspartyl)glycine or to any single substance which could have been formed in the reaction. In order to identify its components it was hydrogenolysed (1·3 g.) in glacial acetic acid (100 ml.) with the usual catalyst (0·3 g.) for 8 hr. at 70—80°. After the catalyst had been filtered off, the solvent was evaporated *in vacuo* and the residue chromatographed in the two solvent systems mentioned above. The following substances could be

[7] Werbin and Spoerri, *J. Amer. Chem. Soc.*, 1947, **69**, 1681.
[8] Fox and Minard, *J. Amer. Chem. Soc.*, 1952, **74**, 2085.

identified by simultaneous chromatography with authentic samples: (β-aspartyl)glycine, aspartic acid, glycine, alanylglycine, and N^{α}-benzylasparagine.

Reaction between N-*Maleoylglycylglycine and Benzylamine.*—*N*-Maleoylglycylglycine (1·6 g.) in water (3 ml.) was heated with benzylamine (4 g.) under reflux for 3 hr. After cooling the reaction mixture was extracted with ether, and adjusted to pH 3 with hydrochloric acid. On storage overnight in an ice-box, *N*-benzyl-DL-aspartic acid was deposited; after recrystallisation (from water) it (1 g.) had m. p. 204° (Found: C, 59·5; H, 6·0; N, 6·3. Calc. for $C_{11}H_{13}NO_4$: C, 59·1; H, 5·8; N, 6·3%).

Reaction between N-*Maleoyl-β-alanine and Benzylamine.*—*N*-Maleoyl-β-alanine (18·7 g.) in water (15 ml.) was heated with benzylamine (21·4 g.) under reflux for 3 hr. After cooling, excess of benzylamine was extracted with ether, and the solution was acidified to pH 4 with hydrochloric acid. A crystalline precipitate formed overnight (ice-box). This had m. p. 185—190°. It gave a negative ninhydrin reaction and did not decolorise potassium permanganate solution. Since analyses did not reveal its identity it was hydrogenolysed in ethanol for 8 hr. After the catalyst had been filtered off, the solvent was removed *in vacuo*, and chromatography of the residue, as above, revealed β-aspartyl-β-alanine, aspartic acid, β-alanine, and N^{α}-benzylasparagine.

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