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# The Formation of Lactams of *N*-(2-Amino)benzoylamino Acids

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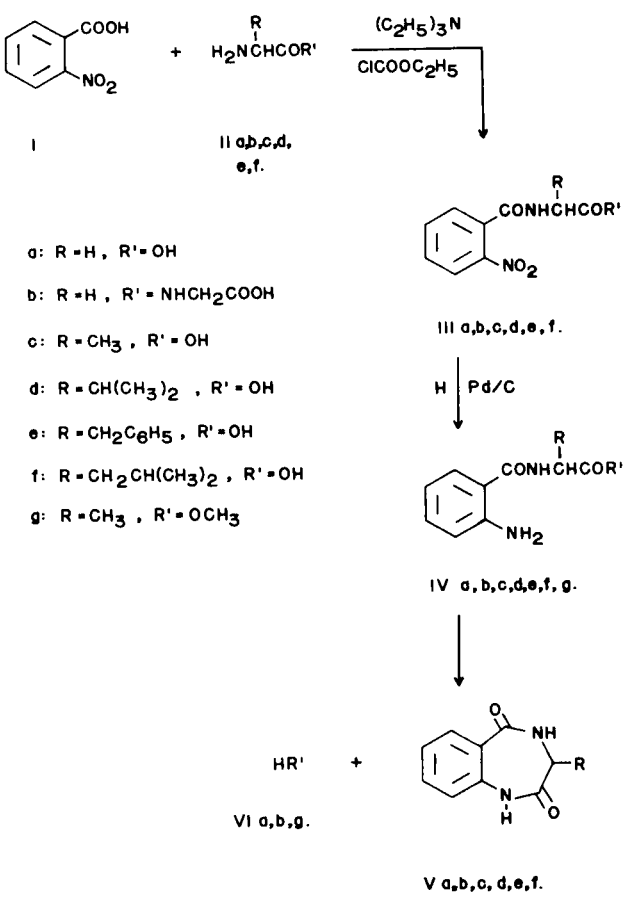
*N*-(2-Nitro)benzoylamino acids were prepared by 2-nitrobenzoylation of amino acids via the mixed ethylcarbonic anhydride procedure. They were reduced catalytically to *N*-(2-amino)benzoylamino acids which underwent cyclization to the corresponding lactams under a variety of conditions. The use of this reaction sequence for stepwise degradation of peptides seems possible.

In 1952 Holley and coworkers reported (1) that 2-aminophenoxyacetyl- as well as 2-amino-*N*-phenylglycyl derivatives undergo cleavage and cyclization in hot water to the corresponding lactams. In compounds such as 2-aminophenoxyacetyl- or 2-amino-*N*-phenylglycyl-peptides these groups can be used as *N*-protective groups and their cleavage and cyclization releases the free peptide. This method has also been developed into a stepwise degradation of peptides by repeated addition of the protective group and subsequent cleavage with concurrent lactam formation. 2-Amino-*N*-phenylglycine itself, lactamizes so readily that the free acid has never been obtained (2). Similar seven membered lactams were, however, less well known and their easy formation was not self-evident. Only recently has a patent been published (3) which describes the formation of 2-aminobenzylmercaptoacetic acid and its lactamization in boiling xylene. The following work describes the synthesis of *N*-(2-amino)benzoylamino acids and their derivatives and their relatively easy lactamization under a variety of conditions.

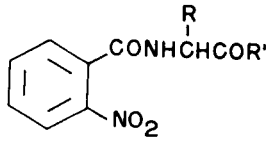
The 2-nitrobenzoylation of amino acids was carried out via a mixed ethylcarbonic anhydride (4) with results, summarized in Table I. The yields of products are good and compare favorably with similar acylations of free amino acids by this procedure. The low yield in the case of glycylglycine (IIIb) is probably due to its preparation - prior to use - by partial hydrolysis of 2,5-diketopiperazine (glycine anhydride). It was used *in situ*, *i.e.*, in a very dilute aqueous alkaline solution. *N*-(2-nitro)benzoyl-DL-phenylalanine (IIIe) and *N*-(2-nitro)benzoyl-DL-leucine (IIIf) could not be obtained in crystalline form and were characterized as anilides. The 2-nitrobenzoylamino acids exhibit the specific infrared absorptions of the nitro group and of the amide bond. Their catalytic reductions yield the corresponding *N*-(2-amino)benzoylamino acids (IV) (Table II). *N*-(2-Amino)benzoyl-DL-valine (IVd) and *N*-(2-amino)-benzoyl-DL-phenylalanine (IVe) were characterized as derivatives since they could not be obtained in crystalline form. The infrared spectra of these compounds exhibit the specific absorptions of the primary amino group.




The lactamization of these compounds could be effected by solution and reflux in water (Method A), by reflux in boiling xylene (Method B) or boiling ethanediol (Method C), or by application of heat to the substance without any solvent (Method D). The synthetic results are summarized in Table III. Water could be used whenever the starting material was crystalline and did dissolve in hot water. In one instance *N*-(2-amino)benzoyl-DL-alanine (IVc) a product of hydrolysis - anthranilic acid - was iso-

TABLE I

*N*-(2-Nitro)benzoylamino Acids



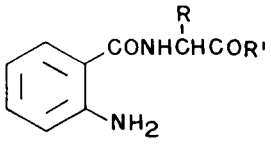

III a,b,c,d,e,f.

| Compound | Yield % | M.p. | Recryst. Solv. | | Analysis Calcd. | Found |
|---|---|---|---|---|---|---|
| IIIa | 69.5 | 193-194° | Water | C: | 48.2 | 48.0 |
| R=H : R'=OH | | | | H: | 3.5 | 3.7 |
| | | | | N: | 12.5 | 12.3 |
| IIIb | 28.4 | 200-201° | Water | C: | 47.0 | 47.0 |
| R=H : R'=$NHCH_2COOH$ | | | | H: | 3.9 | 4.0 |
| | | | | N: | 15.0 | 14.4 |
| IIIc | 67 | 122-124° | Water | C: | 50.4 | 50.6 |
| R=$CH_3$ : R'=OH | | | | H: | 4.2 | 4.1 |
| IIId | 66 | 171° | AcOEt | C: | 54.1 | 53.9 |
| R=$CH(CH_3)_2$ : R'=OH | | | | H: | 5.3 | 5.3 |
| | | | | N: | 10.5 | 10.2 |
| IIIe (crude) | 87.5 | | | | | |
| R=$CH_2C_6H_5$ : R'=OH | | | | | | |
| (characterized as anilide) | | 214° | EtOH | C: | 67.9 | 67.6 |
| | | | | H: | 4.9 | 4.9 |
| | | | | N: | 10.8 | 10.6 |
| IIIf (crude) | 71.5 | | | | | |
| R=$CH_2CH(CH_3)_2$ : R'=OH | | | | | | |
| (characterized as anilide) | | 192° | Isopropanol | C: | 64.3 | 64.6 |
| | | | | H: | 5.9 | 6.2 |
| | | | | N: | 11.8 | 11.7 |

lated in addition to the lactam (Vc, Table III). The cyclic structure of the products is substantiated by the elementary analysis; by the chemical evidence of the loss of the free amino and carboxyl groups and by the infrared spectra which fail to show these two functional groups but exhibit instead specific absorptions for secondary amides in cyclic structures (5). The fact that methyl *N*-(2-amino)benzoyl-DL-alaninate (IVg) as well as *N*-(2-amino)benzoylglycylglycine (IVb) cyclize to products, identical with those obtained from *N*-(2-amino)benzoyl-DL-alanine (IVc) and *N*-(2-amino)benzoylglycine (IVa) respectively, represents additional proof for the structure of the products. In the case of *N*-(2-amino)benzoylglycylglycine (IVb) a cyclic compound containing both glycine residues could have been formed. The reaction proceeds however with cleavage of the glycylglycine bond and consequent release of the free terminal glycine unit and cyclization of the residue. It is obvious that the released terminal glycine can again be subjected to *N*-(2-amino)-benzoylation. In this way a method for sequential release of amino acids from a peptide chain is at hand. For this purpose the yields in the 2-nitrobenzoylation reaction have to be increased by using amino acid (or peptide) derivatives instead of free acids. The reaction is then carried out in an anhydrous medium. Also the residual peptide must be recovered as completely as possible.

TABLE II

*N*-(2-Amino)benzoyl Acids



IV a,b,c,d,e,f.

| Compound | Yield % | M.p. | Recryst. Solv. | | Analysis Calcd. | Found |
|---|---|---|---|---|---|---|
| IVa | 86.5 | 143° | Acetonitrile | C: | 55.7 | 55.5 |
| R=H : R'=OH | | | | H: | 5.2 | 5.2 |
| | | | | N: | 14.4 | 14.7 |
| IVb | 73.8 | 218° | Water | C: | 52.5 | 52.5 |
| R=H : R'=$NHCH_2COOH$ | | | | H: | 5.2 | 5.5 |
| | | | | N: | 16.2 | 16.8 |
| IVc | 89 | 122° | Nitromethane | C: | 57.7 | 57.9 |
| R=$CH_3$ : R'=OH | | | | H: | 5.8 | 5.8 |
| | | | | N: | 13.5 | 13.1 |
| IVd (crude) | 98 | | | | | |
| R=$CH(CH_3)_2$ : R'=OH (characterized as *N*-tosyl derivative) | | 196° | Ethanol-Water | C: | 58.5 | 58.5 |
| | | | | H: | 5.6 | 5.5 |
| | | | | N: | 7.2 | 6.9 |
| IVe (crude) | 96 | | | | | |
| R=$CH_2C_6H_5$ : R'=OH (characterized as methyl ester hydrochloride) | | 155° | Methanol-Ether | C: | 61.1 | 61.3 |
| | | | | H: | 5.7 | 5.9 |
| | | | | N: | 8.4 | 8.2 |
| IVf (crude) | 96 | | | | | |
| R=$CH_2CH(CH_3)_2$ : R'=OH | | | | | | |

## EXPERIMENTAL (6)

Formation of *N*-(2-Nitro)benzoylamino Acids (Table I).
*N*-(2-Nitro)benzoylglycine (IIIa).

To a solution of 33.4 g. (0.2 mole) of 2-nitrobenzoic acid and 20.2 g. (0.2 mole) of triethylamine in 300 ml. of dry tetrahydrofuran, cooled in an ice-salt bath to -15°, was added 21.6 g. (0.2 mole) of ethyl chloroformate. The mixture was stirred with exclusion of moisture for 30 minutes. A solution of 15 g. (0.2 mole) of glycine in 100 ml. of 2 *N* sodium hydroxide was then added during 20 minutes. The solution was stirred continuously and slowly brought to room temperature overnight. Tetrahydrofuran was then distilled off and the residual aqueous solution was cooled, brought to *p*H 9 by addition of 2 *N* sodium hydroxide, extracted several times with ether, and then freed from ether by slight evacuation. Acidification with concentrated hydrochloric acid in the cold precipitated an oil which turned solid after trituration. It was filtered, dried, and washed with ether to eliminate residual 2-nitrobenzoic acid; 31.1 g. of product was obtained, $\nu$ max (KBr), 1530, 1375, 860, 720 (C-$NO_2$) (7), 1650 $cm^{-1}$ (sec. amide) (5).

*N*-(2-Nitro)benzoylglycylglycine (IIIb).

A suspension of 11.4 g. (0.1 mole) of 2,5-diketopiperazine (glycine anhydride) in 100 ml. of 0.1 *N* sodium hydroxide was stirred at room temperature until completely dissolved and hydrolysed to glycylglycine. This solution was added to a cold (ice-salt bath) solution of 0.1 mole of 2-nitrobenzoic-ethyl-carbonic anhydride in 250 ml. of tetrahydrofuran, prepared as described for *N*-(2-nitro)benzoylglycine. The resultant mixture was worked up as described above to yield 8 g. of pure product, $\nu$ max (KBr), 1530, 1360, 860, 700 (C-$NO_2$), 1650 $cm^{-1}$ (sec. amide).

Reduction of *N*-(2-Nitro)benzoylamino Acids (Table II).
*N*-(2-Amino)benzoylglycine (IVa).

A solution of 3.8 g. (0.0169 mole) of *N*-(2-nitro)benzoylglycine in 100 ml. of ethanol, together with 0.3 g. of palladium-charcoal (10%), was subjected to catalytic hydrogenation. After the theoretical quantity of hydrogen had been absorbed, the catalyst was filtered off and the solution concentrated *in vacuo*; 3.2 g. of solid product remained, $\nu$ max (Nujol), 3390, 3300, 3250 ($NH_2$), 1645 (sec. amide), 1715, 930 (COOH), 1600, 1580, 1175, 760 $cm^{-1}$ (2-subst. phenyl).

*N*-(2-Amino)benzoyl-DL-alanine (IVc).

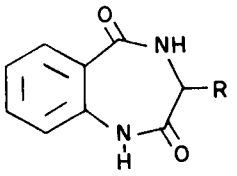
This compound showed the following infrared spectra; $\nu$ max (Nujol), 3445, 3330, 3270 ($NH_2$), 1625 (sec. amide), 1730, 900 (COOH), 1580, 1180, 755 $cm^{-1}$ (2-subst. phenyl).

Cyclization of *N*-(2-Amino)benzoylamino Acids (Table III).
*N*-(2-Amino)benzoylglycine Lactam (Va) (Method A).

A suspension of 0.9 g. (0.00463 mole) of *N*-(2-amino)benzoylglycine (IVa) in 20 ml. of water at *p*H 4 was refluxed for 25 minutes. The solid dissolved and upon cooling a precipitate appeared which was filtered and air dried; 0.5 g. of product was obtained, $\nu$ max (Nujol), 3175, 3070 (sec. amide in cyclic lactams) (5), 1680, 1655 (sec. amide), 1660, 1580, 1175, 760 $cm^{-1}$ (2-subst. phenyl).

TABLE III

Lactams of *N*-(2-Amino)benzoylamino Acids



V a,c,d,e,f.

| Lactams | Reactants | Method | Yield % | M.p. | Recryst. Solv. | | Analysis Calcd. | Found |
|---|---|---|---|---|---|---|---|---|
| Va | IVa | A | 54.5 | 318-322° (a) | Water | C: | 61.4 | 61.3 |
| R=H : | R'=OH | D | 69.5 | " | " | H: | 4.5 | 4.7 |
| | | C | 69 | " | " | N: | 15.9 | 16.0 |
| | IVb | A | 59 | " | " | | | |
| | R'=NHCH$_2$COOH | | | | | | | |
| Vc | IVc | A | 32 | 316° | DMF-Water | C: | 63.1 | 63.1 |
| R=CH$_3$ : | R'=OH | B | 58 | " | " | H: | 5.3 | 5.4 |
| | | | | | | N: | 14.7 | 14.6 |
| | IVg | B | 30 | " | " | | | |
| | R'=OCH$_3$ | | | | | | | |
| Ve | IVe | D | 44 | 275-278° | " | C: | 72.2 | 72.2 |
| R=CH$_2$C$_6$H$_5$ : | R'=OH | | | | | H: | 5.3 | 5.1 |
| | | | | | | N: | 10.5 | 10.6 |
| Vd | IVd | D | 64.5 | 266° | " | C: | 66.0 | 66.0 |
| R=CH(CH$_3$)$_2$ : | R'=OH | | | | | H: | 6.4 | 6.5 |
| | | | | | | N: | 12.9 | 12.9 |
| Vf | IVf | D | 55 | 252° | " | C: | 67.3 | 67.2 |
| R=CH$_2$CH(CH$_3$)$_2$ : | R'=OH | | | | | H: | 6.9 | 7.2 |
| | | | | | | N: | 12.1 | 11.8 |

(a) The melting points vary somewhat and depend on the mode of heating. Those reported were taken on an instrument preheated to approximately 10° of the melting point.

*N*-(2-Amino)benzoyl-DL-alanine Lactam (Vc) (Method B).

A suspension of 0.6 g. (0.00384 mole) of *N*-(2-amino)benzoyl-DL-alanine (IVc) in 50 ml. xylene was subjected to slow distillation. The material dissolved in hot xylene and the solution was concentrated to 10 ml. Upon cooling a precipitate appeared which was filtered and air dried; 0.32 g. of product was obtained, $\nu$ max (Nujol), 3145, 3070 (sec. amide in cyclic lactams), 1690, 1660 (sec. amide), 1580, 1185, 755 (2-subst. phenyl).

*N*-(2-Amino)benzoylglycine Lactam (Va) (Method C).

A suspension of 1 g. (0.00515 mole) of *N*-(2-amino)benzoylglycine (IVa) in 10 ml. of ethanediol was refluxed for 90 minutes. During that time complete solution was effected and a red color developed. After cooling the resultant precipitate was filtered, washed with ethanol and air dried.

*N*-(2-Amino)benzoyl-DL-valine Lactam (Vd) (Method D).

One gram (0.00424 mole) of crude *N*-(2-amino)benzoyl-DL-valine was heated in a test tube, equipped with a thermometer, to a temperature of 140-190° for a period of 3-5 minutes. Water vapour was evolved and the liquid solidified upon cooling. It was heated with a little water, filtered and air dried, 0.6 g. of product was obtained.

Acknowledgment.

The authors are indebted to Mrs. M. Goldstein and Dr. Ch. Feilchenfeld and their collaborators for the microanalyses and the infrared spectra respectively. Support of this work by a research grant from the Research and Development Authority of the Hebrew University is gratefully acknowledged.

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(6) All melting points were taken on a Fisher-Johns apparatus and are uncorrected.
(7) Ref. 5, p. 50.

Received April 18, 1966 Jerusalem, Israel