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o-NITROBENZOYL GROUP AS A NEW AMINO PROTECTING GROUP FOR PEPTIDE SYNTHESIS AND THE SYNTHESIS OF SOME ANTHRANILYL PEPTIDES

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Abstract—N (*o*-nitrobenzoyl)amino acids can be coupled with other amino acids using DCC and the resulting product on hydrogenation gives peptides, containing the anthranilyl group as $-NH_2$ end group. N (anthranilyl)amino acids or peptides can also be obtained by reaction of isatoic anhydride on amino acids or peptides. The anthranilyl end group is easily cleaved by metal (Cu^{+2}) catalysed hydrolysis to give α-amino acid peptides and the insoluble copper(II) anthranilate.

Metal ion sensitive protecting groups can offer an unique choice for the synthesis of peptides. Corey and Dawson employed the 8-quinoloxycarbonyl group[1] for protecting the amino group in peptide synthesis and recently the α-picolinyl group as an amino-protecting group, removable by metal ion catalysed hydrolysis, was reported.[2] In the present communication, we report the use of the *o*-nitrobenzoyl group for the protection of the amino group in peptide synthesis.

Certain naturally occurring peptides-like compounds are known to contain *p*-aminobenzoic acid residues e.g.; the folic acid-vitamins and *p*-aminohippuric acid. On the other hand N-anthranilyl-(*o*-aminobenzoyl) residues are not of common occurrence, though 2-glutamyl-3-pyruyulanthranilate has been suggested as an intermediate in the biosynthetic pathway for anthranilate.[3]

Carboxylamides or esters of anthranilic acid are easily hydrolysed, particularly in presence of metal ion and such a reaction has been used for the precipitation of metal anthranilate from a homogenous solution.[4] The reaction must involve anthranilyl-amino and carboxyamide groups in complexation of metal ion, resulting in the weakening of the amide bond. The usual benzoylation method using acid chloride cannot be used for the synthesis of anthranilyl derivatives, because anthranilic acid chloride is readily cyclised to dianthranilide.[5] However, isatoic anhydride provides a suitable route for the synthesis of anthranilic acidamide derivatives. The other approach for the synthesis of N (*o*-aminobenzoyl)amino acids is *via* preparation of N (*o*-nitrobenzoyl)amino acid followed by hydrogenation to the corresponding amino compound. The N (*o*-aminobenzoyl)amino acids can be considered as dipeptides of anthranilic acid with another α-amino acid, having anthranilic acid as the amino end group. When a di- or tripeptide is reacted with isatoic anhydride, the anthranilyl group is added as the new amino end group. In such peptides, the terminal anthranilyl group is easily cleaved by metal ion catalysed hydrolysis producing an insoluble metal anthranilate and the original peptides or amino acids. The normal peptide bonds are not cleaved under these mild conditions. Hence, it is possible to prepare N (*o*-nitrobenzoyl)amino acids and condense them with other amino acids by the standard methods to produce N (*o*-nitrobenzoyl)peptides.

Finally the N (*o*-nitrobenzoyl)peptides are converted into the corresponding N (*o*-aminobenzoyl)-peptides by catalytic hydrogenation and the N (*o*-aminobenzoyl) group is selectively cleaved by metal catalysed hydrolysis to give α-amino acid peptides. Although N (anthranilyl)amino acids or peptides are prepared by the isatoic anhydride method in one step, for α-amino acid peptide synthesis, it is necessary to proceed *via* N (*o*-nitrobenzoyl)amino acids and reduce it only after it has been coupled to another amino acid. The method has some analogy to the method employing N (*o*-nitrophenyloxyacetyl)[6] group, the removal of which is carried out by its hydrogenation to the corresponding amino compound followed by cyclisation to a lactam.

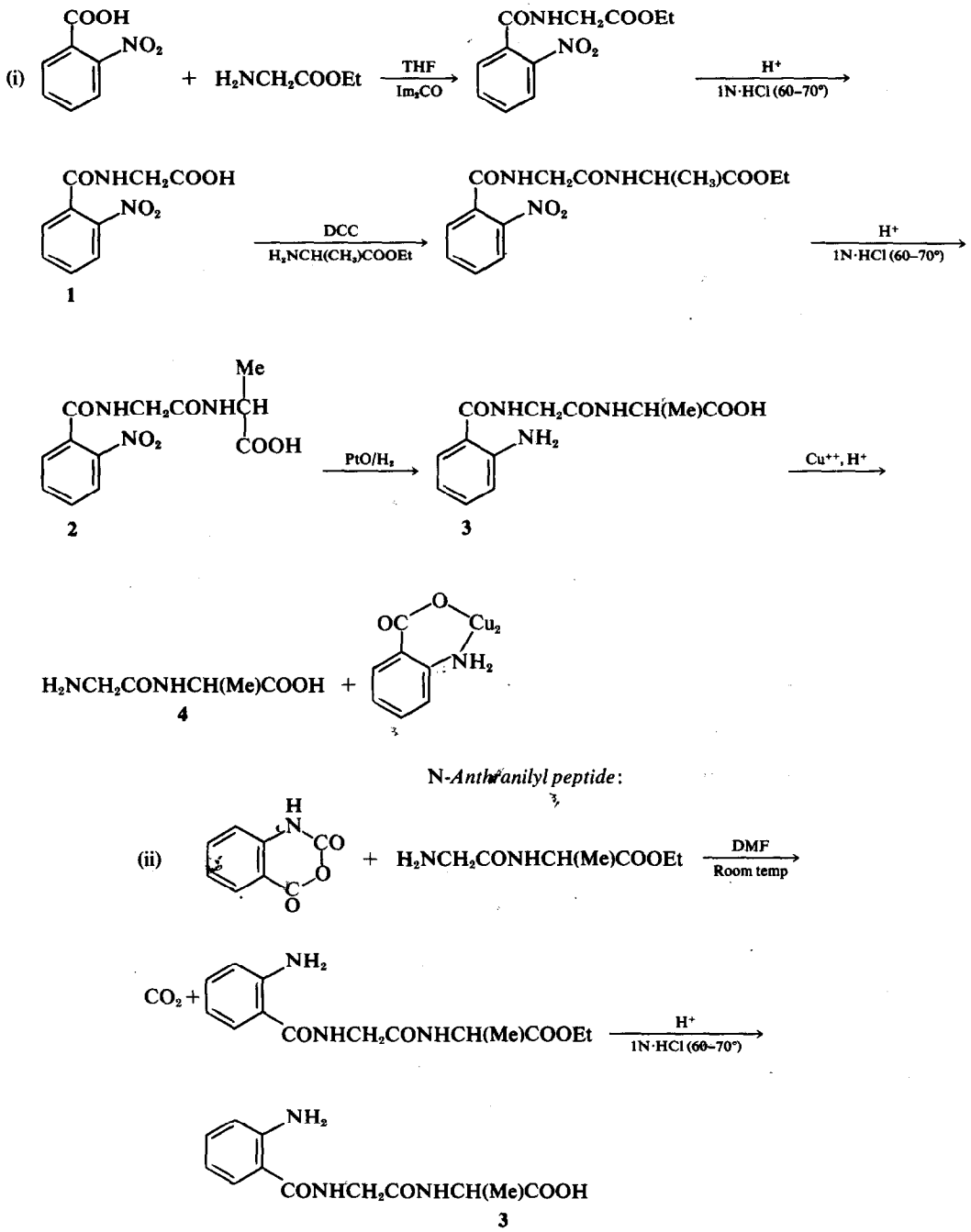
The course of the reactions expected is as given over.

EXPERIMENTAL

As an example, the synthesis of a dipeptide glycylalanine is described, using the N (*o*-nitrobenzoyl) group as an amino protecting group.

N (*o-nitrobenzoyl)glycine* (1). To a soln of *o*-nitrobenzoic acid (1·67 g, 0·01 mole) in dry THF (10 ml), N,N'-carbonyldiimidazole (1·62 g, 0·01 mole)[7,8] was added. The

α-amino acid peptide



N-Anthranilyl peptide:

soln was stirred for 1 hr or such time at room temp till the evolution of CO_2 ceased. At this stage glycine ethyl ester hydrochloride (1·03 g, 0·01 mole) was added to the mixture.

After standing for 3 hr, the precipitated imidazole hydrochloride was filtered off and THF was removed at reduced pressure. The product was dried and on recrystallisation with EtOH gave N (*o*-nitrobenzoyl)glycine ester which on hydrolysis (1 N HCl, 1 hr, 60–70°) gave N (*o*-nitrobenzoyl)glycine (3·95 g, 90%, mp 185–188°). (Found: C = 43·2, H = 3·3; N = 11·8. $C_6H_4NO_2$-$CONHCH_2COOH$ requires: C = 43·7, H = 3·5, N = 12·5%).

N (*o-nitrobenzoyl*)*glycylalanine* (2). To the soln of 1 in EtOAc, was added alanine ethyl ester (1·17 g, 0·01 mole) and dicyclohexylcarbodiimide[9] (2·06 g, 0·01 mole) in EtOAc soln (20 ml). The mixture was stirred at 0° for

0·5 hr, and then it was allowed to stand at room temp for 1 hr. The precipitated N,N′-dicyclohexylurea was filtered off. The filtrate was evaporated to dryness and the residue consisting of crude N (*o*-nitrobenzoyl)glycylalanine ester separated. This on hydrolysis (1·0 N HCl, 1 hr, 70–80°) gave N (*o*-nitrobenzoyl)glycylalanine yield 85%, mp 238° (dec). (Found: C = 48·7, H = 4·1, N = 13·6. $C_6H_4NO_2$-$CONHCH_2CONHCH(CH_3)COOH$ requires: C = 49·1, H = 4·4, N = 14·2%).

Hydrogenation of N (*o-nitrobenzoyl*)*glycylalanine* (2). The product 2 was subjected to catalytic hydrogenation and the nitro group was reduced to an amino group.

A soln of 0·50 millimole of (2) and 45 mg (0·53 millimole) $NaHCO_3$ in 3 ml water, to which 29 mg PtO_2 was added, was hydrogenated in a microhydrogenation apparatus.[6] After the theoretical amount of H_2 has been absorbed (30 to 40 min), the hydrogenation was stopped. The mixture was filtered, the catalyst was washed with 1 ml water, and the Na salts were neutralized by the addition of 0·53 ml 1 N HCl. The mixture was cooled and filtered and 3 so formed was washed with a little cold water and dried *in vacuo*, yield 85%, mp 250–255° (dec). (Found: C = 54·2, H = 5·3, N = 15·1. $C_6H_4NH_2CONHCH_2CONHCH$-$(CH_3)COOH$ requires: C = 54·3, H = 5·6, N = 15·8%).

Hydrolysis of N (*o-aminobenzoyl*)*glycylalanine* (3). a sample of the dipeptide glycyl-L-alanine, mp 235–236° (dec) $[\alpha]_D^{20} - 50°$[10] prepared by coupling benzoyloxycarbonyl derivative of glycine with L-alanine ester, using dicyclohexylcarbodiimide as coupling reagent.

Synthesis of N (*o-aminobenzoyl*)*glycylalanine* (3) *from glycylalanine and isatoic anhydride*. To a soln of isatoic anhydride (1·63 g, 0·01 mole) in DMF (20 ml), glycylalanine ester hydrochloride (2·01 g, 0·01 mole) was added. The soln was stirred for 10 min at room temp, when the evolution of CO_2 ceased.

After standing for 0·5 hr, the excess of DMF was removed at reduced pressure. The product was dried and on recrystallisation with EtOH gave N (anthranilyl)glycylalanine ester hydrochloride. This on hydrolysis gave N (anthranilyl)glycylalanine, m.p. 251° (dec), yield 75%. (Found: C = 54·3, H = 5·2, N = 15·1. $C_6H_4NH_2CONH$-$CH_2CONHCH(CH_3)COOH$ requires: C = 54·5, H = 5·6, N = 15·8%). This product was identical with the N (*o*-aminobenzoyl)glycylalanine prepared by using the N (*o*-nitrobenzoyl) group as N-protecting group. Glycylalanine could be recovered from this product by copper(II) catalysed hydrolysis as described. Other peptides synthesised using *o*-nitrobenzoyl group as N-protecting group and certain anthranilyl peptides synthesised using isatoic anhydride are given in Table 1.

Table 1.

S. No.	N-Anthranilyl-peptide[a]	Yield	MP (°C)	Elemental (%) composition Calc. (Found) C	H	N	α-Aminoacid peptide[b]	Yield (%)	MP (°C)	Elemental (%) composition Calc. (Found) C	H	N
1.	Anthranilyl-glyalanine	75	251 (dec)	54·5 (54·2)	5·6 (5·3)	15·8 (15·2)	Glyalanine	80	245 (dec)	25·4 (24·8)	4·2 (3·8)	19·1 (18·7)
2.	Anthranilyl-glyvaline	70	260 (dec)	57·3 (57·1)	6·4 (6·2)	14·3 (13·9)	Glyvaline	75	265 (dec)	48·2 (47·9)	8·04 (7·8)	16·0 (15·5)
3.	Anthranilylgly-val-tyrosine	60	275 (dec)	60·5 (59·8)	6·5 (6·2)	12·2 (11·7)	Glyval-tyrosine	65	270 (dec)	59·9 (59·5)	6·5 (6·3)	13·5 (12·9)
4.	Anthranilylgly-val-tyr-proline	55	280 (dec)	59·8 (59·3)	6·6 (6·3)	12·6 (12·2)	Glyval-tyr-proline	60	285 (dec)	58·05 (57·8)	6·9 (6·6)	13·7 (13·2)

[a]Prepared via N (*o*-nitrobenzoyl) protecting group, as well as by reaction of isatoic anhydride on α-amino acid peptide (b).

[b]The products of metal catalysed hydrolysis of N (anthranilyl) peptides (a).

The removal of the protecting group was finally achieved by reacting 3 with Cu(II) ions, when the N (*o*-aminobenzoyl) group was cleaved in the form of its copper(II) complex.

To a suspension of (3) (1·375 gm, 0·005 mole) in 25 ml water, cupric acetate (0·6725 g, 0·003 mole) and 2 ml dil HCl was added, and the mixture was allowed to stand at room temp with occasional shaking for 30 min. The mixture was then concentrated to half its volume under reduced pressure and filtered. From the filtrate the excess of Cu(II) ions were removed by passing H_2S through it. This was followed by filtration and on evaporating the filtrate under vacuum gave crude (4) 80%, mp 245° (dec). (Found: C = 24·8, H = 3·8, N = 18·7. H_2NCH_2CONH-$CH(CH_3)COOH$ requires: C = 25·4, H = 4·2, N = 19·1%).

The sample of glycylalanine so prepared was identified by TLC and paper chromatography by comparing it with

Racemization. This problem has not been encountered to any appreciable extent in the using *o*-nitrobenzoyl group. This is to be expected because of the low temperatures and the less polar solvents employed.

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REFERENCES

[1]E. G. Corey and R. L. Dawson, *J. Am. Chem. Soc.* **84**, 4899 (1962).

[2]A. K. Koul, N. K. Mathur, N. S. Ramegowda, B. Prashad and J. M. Bachhawat, *Synthetic Communications*, In press.

[3]V. F. Lingens, W. Luck and W. Geobel, *Z. Naturforsch.* Pt. b, **18**, 851 (1963).

[4]H. Singh, D. S. Mittal and N. K. Mathur, *Z. Anal. Chem.* **254**, 361 (1971).
[5]E. H. Rodd, *Chemistry of Carbon Compounds* Volume III A, pp. 579–580. Elsevier, New York (1954).
[6]R. W. Holley and A. D. Holley, *J. Am. Chem. Soc.* **74**, 3069 (1952).
[7]R. Paul and G. W. Anderson, *Ibid.* **82**, 4596 (1960).
[8]F. H. Carpenter and D. J. Gish, *Ibid.* **74**, 3818 (1952).
[9]M. Bodanzsky and V. du Vigneaud; *Ibid.* **81**, 5688 (1955).
[10]E. H. Rodd, *Chemistry of Carbon Compounds* Vol I, Part B, P831. Elsevier, New York (1952).