The Synthesis of 2-Carboxy-6-nitrobenzimidazole 1-Oxides by Intramolecular Oxidation of α-(2,4-Dinitrophenylamino)-αβ-unsaturated Acyl Derivatives

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In basic solutions of α-(2,4-dinitrophenylamino)-αβ-unsaturated acyl derivatives, a 2-nitro-group intramolecularly oxidizes the β-position of the unsaturated acyl chain. Retroaldol cleavage of the oxygenated β-carbon occurs with ring closure of the scission product yielding an aldehyde and a 2-carboxy-6-nitrobenzimidazole 1-oxide (65—75%). This reaction occurs for the methyl ester of α-(2,4-dinitrophenylamino)acrylic acid and its β-substituted anologues, α-(2,4-dinitrophenylamino)crotonate and α-(2,4-dinitrophenylamino)cinnamate, in that respective order of reactivity, as well as the propyl amide of α-(2,4-dinitrophenylamino)acrylic acid. These reactions occur in protic and aprotic solvents with a large number of bases, and a tentative concerted mechanism is proposed. Reaction of *N*-2,4-dinitrophenylglycine methyl ester with base gives a moderate yield (50—60%) of 2-carboxy-6-nitrobenzimidazole 1-oxide as above. The *N*-2,4-dinitrophenyl methyl esters of serine, threonine, and β-phenylserine undergo retroaldol scission in base to the corresponding aldehyde and *N*-2,4-dinitrophenylglycine methyl ester, which quickly undergoes this same ring closure. This reaction occurs with sodium carbonate in methanol but not in dimethylformamide, and in contrast to the intramolecular oxidation is analogous to known base-catalyzed heterocyclic ring closure condensations of active methylene compounds with aromatic nitro-groups. The ring closures of *N*-2,4-dinitrophenylamino-acid derivatives are suggested as reactions possibly useful in *N*-terminal amino-acid determination of peptides or glycoproteins.

RECENT Reports from this laboratory [1,2] stated that base-catalysed elimination in 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-L-serine methyl ester yields rapidly in the cold the monosaccharide and methyl α-(2,4-dinitrophenylamino)acrylate (IX) which underwent ring closure in base to yield on work-up 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide (XV).[2] We here present evidence to show that besides (XV) the reaction of (IX) also produces an equimolar quantity of formaldehyde, which was isolated and characterised. No previous report of such a reaction of α-(*o*-nitrophenylamino)-αβ-unsaturated esters has been made. We have, therefore, extended the reaction to the β-methyl (XI) and β-phenyl (XII) analogues of (IX) as well as its propyl amide (X). Of interest in determining parameters for the reaction was knowledge about its general base catalysis and preferential solvent media. We show that the ring closures of (IX), (X), (XI), and (XII) are effected under very mild conditions with a variety of bases in many solvents. Although several benzimidazole ring closures are known,[3,4] the benzimidazole 1-oxides have a unique tautomeric structure and have received particular

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[1] J. R. Vercellotti and A. E. Luetzow, *J. Org. Chem.*, 1966, **31**, 825.

[2] A. E. Luetzow, N. E. Hoffman, and J. R. Vercellotti, *Chem. Comm.*, 1966, 301.

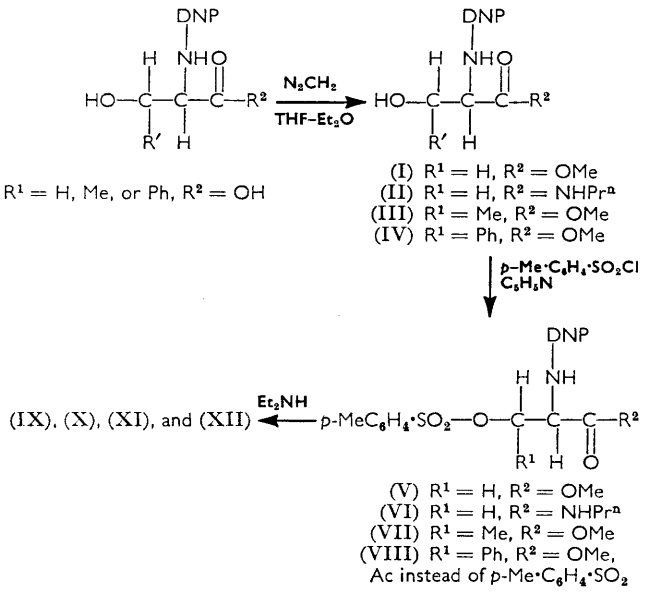
[3] K. Hofmann, " The Chemistry of Heterocyclic Compounds," ed. A. Weissberger, " Imidazole and Its Derivatives," Part I, Interscience, New York, 1953, p. 247.

[4] E. S. Schipper and A. R. Day, " Heterocyclic Compounds," ed. R. C. Elderfield, Wiley, New York, 1957, vol. 5, p. 194.

attention.[5-10] We therefore report here the synthesis of precursors as well as substrates for the reaction and develop a rationale for the proposed mechanism.

SCHEME 1

Synthesis of substrates



The respective amino-acid starting materials (Scheme 1) were first treated with 1-fluoro-2,4-dinitrobenzene to make the *N*-2,4-dinitrophenyl (DNP) derivatives.[11] t.l.c. prevents addition of excess diazomethane, which was found to be responsible for a red contaminant. Physical data for these compounds are in Table 1.

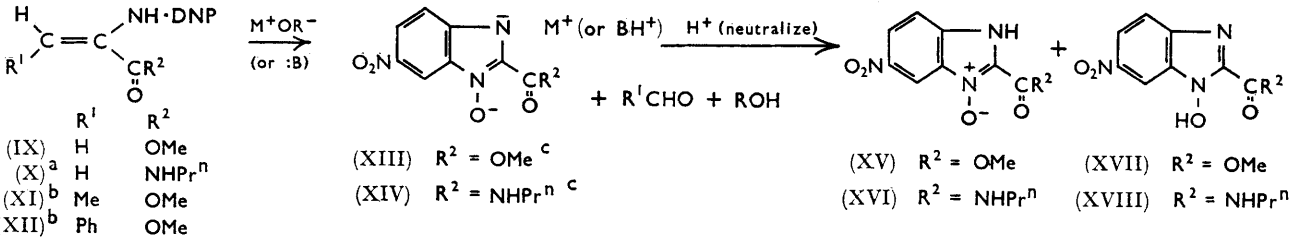
In order to examine the effect of an amide linkage on the ring closure reaction, the propyl amide of L-serine was synthesised from the methyl ester by a modification of Ginsberg's procedure.[14] The DNP-group was then added to the propyl amide to give (II).

The DNP-αβ-unsaturated amino-acid acyl derivatives were synthesised by introducing a leaving group at the 3-position of the hydroxy-amino-acids. In the case of the DNP-serine methyl ester and propyl amide, as well as the DNP-threonine methyl ester, the leaving group introduced was the toluene-*p*-sulphonyl group (V), (VI), and (VII). The DNP-β-phenylserine methyl ester proved resistant to toluene-*p*-sulphonation, perhaps because of steric hindrance. Instead, the 3-acetoxy-DNP-β-phenylserine methyl ester (VIII) was made. To effect elimination of leaving groups and form the αβ-unsaturated compounds from (V), (VII), and (VIII), the mildly basic conditions suggested by Photaki[15] were employed. The propyl amide of α-(2,4-dinitrophenylamino)acrylic acid was not isolable in the above preparation. For this amide the rate of elimination of toluene-*p*-sulphonate was slower than the rate of base-catalysed ring closure. Ring closure of the propyl amide was, therefore, effected *in situ* beginning with the *O*-toluene-*p*-sulphonate (VI).

Elemental analyses, melting points, and other physical characteristics of the DNP-amino-acid acyl derivatives

SCHEME 2

Reactants and products for the 2-carboxy-6-nitrobenzimidazole 2-oxide ring closure



[a] The *in situ* reaction of *N*-2,4-dinitrophenyl-*O*-toluene *p*-sulphonyl-L serine propyl amide first produces (X), which then goes on to form (XIV). [b] Possible *cis–trans*-isomer mixture. [c] See Scheme 3 for mesomers of this salt.

The methyl esters of these were synthesised by using diazomethane[12] in tetrahydrofuran–ether. Although Fletcher and his co-workers[13] reported the esterification of *N*-DNP-amino-acids, impurities were formed in their reaction mixture which demanded separation of the product by column chromatography. The methylation procedure described here is superior to that of Fletcher[13] because careful monitoring of the reaction by t.l.c. prevents addition of excess diazomethane, which was found to be responsible for a red contaminant. Physical data for these compounds are in Table 1.

(I)—(IV) and (XIX) as well as their β-hydroxy-esters (V)—(VIII) used in the elimination reactions are listed in Table 1. Compounds (I)—(IX), (XI), (XII), and (XIX) were characterised by satisfactory i.r. and n.m.r. spectra, bearing analogies to known compounds in their absorptions.[1,2] The crotonate (XI) and cinnamate (XII)

[5] (*a*) J. D. Loudon and G. Tennant, *Quart. Rev.*, 1964, **18**, 389; (*b*) p. 397; (*c*) p. 404.
[6] G. W. Stacy, T. E. Wollner, and T. R. Oakes, *J. Heterocyclic Chem.*, 1966, **3**, 51.
[7] G. W. Stacy, B. V. Ettling, and A. J. Papa, *J. Org. Chem.*, 1964, **29**, 1537.
[8] D. W. Russell, *Chem. Comm.*, 1965, 498, and earlier references cited.
[9] R. J. Pollitt, *Chem. Comm.*, 1965, 262.
[10] S. Takahashi and H. Kano, *Chem. and Pharm. Bull.* (*Japan*), 1964, **12**, 282, and earlier references cited.
[11] F. Sanger, *Biochem. J.*, 1946, **39**, 507.
[12] T. J. de Boer and H. J. Backer, *Rec. Trav. chim.*, 1954, **73**, 229.
[13] C. M. Fletcher, A. G. Lowther, and W. S. Reith, *Biochem. J.*, 1954, **56**, 106.
[14] S. Ginsberg and I. B. Wilson, *J. Amer. Chem. Soc.*, 1964, **86**, 4716.
[15] I. Photaki, *J. Amer. Chem. Soc.*, 1963, **85**, 1123; 1965, **87**, 3489.

TABLE 1

Analytical data for compounds synthesised by general methods [a]

Compound	Formula	Requires (%)				Found (%)			Yield (%) and crystal form	M. p.	Recryst. solvent	R_F value [a] and solvent
		M	C	H	N	C	H	N				
(I) *N*-2,4-Dinitrophenyl-DL-serine methyl ester [b]	$C_{10}H_{11}N_3O_7$	285	42·1	3·9	14·7	42·6	3·9	14·7	69 yellow needles	99°	EtOH–H_2O	0·80 D
(II) *N*-2,4-Dinitrophenyl-L-serine propylamide [c]	$C_{12}H_{16}N_4O_6$	312	46·0	5·2	17·9	45·7	5·6	18·2	60 yellow needles	155—156	EtOH–H_2O	0·25 B
(III) *N*-2,4-Dinitrophenyl-DL-threonine methyl ester	$C_{11}H_{13}N_3O_7$	299	44·2	4·4	14·1	44·2	4·5	14·2	77 yellow plates	128—129	C_6H_6	0·54 B
(IV) *N*-2,4-Dinitrophenyl-DL-β-phenylserine methyl ester	$C_{16}H_{15}N_3O_7$	361	53·2	4·2	11·6	53·4	4·1	11·9	93 yellow threads	134—135	95% EtOH	0·77 B
(V) *N*-2,4-Dinitrophenyl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester	$C_{17}H_{17}N_3O_9S$	439	46·5	3·9	9·6	46·4	3·9	10	98 yellow prisms	151—152	Acetone–water	0·75 B
(VI) *N*-2,4-Dinitrophenyl-*O*-toluene-*p*-sulphonyl-L-serine propylamide [c]	$C_{19}H_{22}N_4O_8S$	466	48·9	4·8	12·0 S 7·1	49·0	4·9	11·8 S 6·9	80 orange needles	164—165	Acetone–water	0·54 B
(VII) *N*-2,4-Dinitrophenyl-*O*-toluene-*p*-sulphonyl-DL-threonine methyl ester	$C_{18}H_{19}N_3O_9S$	453	47·7	4·2	9·3 S 7·1	47·6	4·0	9·1 S 7·3	71 yellow rods	127—128	EtOH	0·67 B
(VIII) *N*-2,4-Dinitrophenyl-*O*-acetyl-DL-β-phenylserine methyl ester [f]	$C_{18}H_{17}N_3O_8$	403	53·6	4·3	10·42	53·6	4·1	10·6	91 yellow needles	112—113	EtOH	0·48 C
(XIX) *N*-2,4-Dinitrophenylglycine methyl ester [d]										118—119 [d]	EtOH	0·83 B

[a] See introduction to Experimental section as well as individual general procedures. [b] See ref. 1. [c] Amidation of L-serine methyl ester, ref. 14, followed by *N*-2,4-dinitrophenylation; $[\alpha]_D^{25}$ +118° ± 10° (*c* 0·27 in EtOH). [d] M. p. in accord with ref. 13. N.m.r. and i.r. data also confirmatory of structure. $[\alpha]_D^{25}$ +49·2° (*c* 1·14 in EtOAc). [f] C_5H_5N–Ac_2O acetylation, 1·5 hr. at room temp.

TABLE 2

Effect of reaction conditions on ring closure of methyl α-(2,4-dinitrophenylamino)acrylate (IX) to 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide (XV)

Expt.	Substrate (mmoles)	Base	Mequiv. of base	Solvent	Vol. (ml.)	Time (hr.)	Product yield (%)
a	0·37	$NaHCO_3$	1·2	MeOH	10	7	79
b	0·37	Na_2CO_3	2·4	DMSO	10	2	72
c	0·37	Pr^nNH_2	26	MeOH	10	22	45 [a]
d	0·37	Pr^nNH_2	1·3	MeOH	10	16	36 [a]
e	0·56	NaOAc	0·28	DMSO	10	16	53
f	0·56	NaOAc	0·56	DMSO	10	16	70
g	0·20	NaOAc	0·02	DMSO	5	21	32 total 10·4 Na^+ salt 21·6 H^+ form
h	0·15	None		MeOH	20	7 days	0
i	0·15	Na_2CO_3	0·05	MeOH	20	6 days	29
j	1·12	Et_2NH	10·0	EtOAc	20	20	17
k	0·54	Na_2CO_3	3·3	DMF	2	½	72
l	0·21	Et_3N	3·6	EtOAc	10	2 days	21
m	0·21	Et_3N	3·6	MeOH	10	2 days	42
n	0·21	Et_3N	3·6	DMF	10	2 days	56
o	0·52	Na_2CO_3	3·7	MeOH	4	10 min. at 60°	70
p	0·41	NaOMe	5 drops 4 N	MeOH	2	20 min.	68

[a] Isolated from this reaction mixture as the 2-propylcarbamoyl-6-nitrobenzimidazole-1-oxide.

TABLE 3

Ring closure of α-2,4-dinitrophenylamino-αβ-unsaturated acyl derivatives to 2-carboxy-6-nitrobenzimidazole 1-oxides [a]

Compound		Substrate (mmoles)	Base	Base (mequiv.)	Solvent	Vol. (ml.)	Time	Product yield (%)
(VI)	*N*-2,4-Dinitrophenyl-*O*-toluene-*p*-sulphonyl-L-serine propylamide [b]	0·99	NaOH	2·9	MeOH	20	1 hr. (10 min. at 70°)	74
(VI)	*N*-2,4-Dinitrophenyl-*O*-toluene-*p*-sulphonyl-L-serine propylamide [b]	1·07	Et_2NH	9·7	EtOAc	20	4 hr.	28
(XI)	Methyl α-(2,4-dinitrophenylamino)crotonate [c]	0·36	Na_2CO_3	2·4	DMF	10	22 hr. (1 hr. at 70°)	69
(XI)	Methyl α-(2,4-dinitrophenylamino)crotonate [c]	0·08	NaOMe	0·8	MeOH	10	2 hr. at 50°	79
(XII)	Methyl α-(2,4-dinitrophenylamino)cinnamate [c]	0·39	Na_2CO_3	2·4	DMF	10	22 hr. (2 hr. at 70°)	71

[a] Reactions at room temperature unless otherwise noted. Work-up as in Experimental section for synthesis of 2-carboxy-6-nitrobenzimidazole-1-oxides. [b] *In situ* generation of *N*-propyl-α-(2,4-dinitrophenylamino)acrylamide. Product is 2-propylcarbamoyl-6-nitrobenzimidazole-1-oxide (XVI). [c] Product is 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide (XV).

could possess *cis–trans*-isomers. No knowledge of the preferable geometrical isomers of (XI) and (XII) is available and we cannot assign absolute structures.

The acrylate (IX) hydrolysed on warming with acid to 2,4-dinitroaniline (isolated crystalline and identified by i.r. and n.m.r. spectra) and pyruvic acid (qualitative t.l.c. identification). Such ready protonation of the α-amino-acrylate's β-position and imine hydrolysis confirmed the enamine nature of (IX) previously suggested.[16,17]

The unsaturated amino-acid derivatives which were converted into 2-carboxy-6-nitrobenzimidazole 1-oxides are shown in Scheme 2. The reactions occur very quickly simply by shaking the substrates at room temperature in the presence of a small quantity of base. Elevation of temperature decreases the reaction time. The product is isolated as a highly coloured salt of the 2-carboxy-6-nitrobenzimidazole 1-oxide, which can be neutralised with acid to the cream coloured protonated form of the heterocycle, *e.g.*, (XV). Yields for these reactions under many conditions of solvent, temperature, and base are shown in Tables 2 and 3. In general the yields are good (65—75% isolable product under favourable reaction conditions). A pseudokinetic study by t.l.c. estimation of disappearance of reactants and formation of products posits this order of reactivity under identical conditions (Table 4):

Methyl DNP-α-aminoacrylate > -crotonate > -cinnamate

TABLE 4

Reaction times of α-(2,4-dinitrophenylamino)-esters under similar reaction conditions [a]

Compound		Time of reaction (min.)
(IX)	Methyl α-(2,4-dinitrophenylamino)acrylate	5
(XI)	Methyl α-(2,4-dinitrophenylamino)crotonate	35
(XII)	Methyl α-(2,4-dinitrophenylamino)cinnamate	85

[a] MeOH solution (15 ml.); 0·1 g. $NaHCO_3$; 0·1 mmole substrate; wrist action shaker at room temperature; samples withdrawn at 5 min. intervals; t.l.c., Solvent A, to disappearance of starting materials.

In the development of a mechanism for these reactions certain results were pertinent. Upon elemental analysis and equivalent weight determination of the product in the conversion of (IX) into (XIII),[2] loss of the elements of formaldehyde was recognised, and the characteristic odour of formaldehyde was noticed coming from the reaction mixture in several solvents. Subsequent attempts to distil the formaldehyde from methanol met with difficulty. The aprotic solvent dimethylformamide permitted distillation of formaldehyde in good yield, which was determined as its crystalline dimedone derivative. With methanol as solvent formaldehyde could be determined by the chromotropic acid method.[18,19] The assay indicated an 89% yield of the equimolar quantity of formaldehyde expected from this reaction. In extending the reaction to the β-methyl derivative (XI) the expected aldehyde from the scission is acetaldehyde. Difficulty was experienced in collecting a suitable quantity of acetaldehyde for characterisation, but acetaldehyde could be qualitatively detected by g.l.c. The basic conditions used in this reaction were probably responsible for aldol condensation of the acetaldehyde as fast as it formed. The β-phenyl-DNP-α-aminoacrylate (XII) also underwent aldehyde scission and ring closure to give benzaldehyde, which was identified in good yield as its semicarbazone.

In general, the above reactions of (IX), (XI), and (XII) only occur with a base present. From considerable study of the reaction and careful t.l.c. examination of solutions, we are confident that the transformation (IX) ⟶ (XIII) does not occur in neutral or acidic media or under ordinary conditions of visible light. This is shown in Table 2, where a number of ring closure conditions for the simplest unsaturated compound (IX) are listed. When (IX) was left in uncatalysed methanol solution for a week at room temperature or exposed to daylight no change was discernible (Table 2, h). A variety of bases in solvents with a range of polarities effected the ring closure of (IX); those bearing an anionic charge (Table 2, a,b,e—g,i,k,o,p) promoted greater

[16] G. Riley, J. Turnbull, and W. Wilson, *J. Chem. Soc.*, 1957, 1373.
[17] F. Micheel and W. Busse, *Chem. Ber.*, 1958, **91**, 985.
[18] D. A. MacFadyen, *J. Biol. Chem.*, 1945, **158**, 107.
[19] C. E. Bricker and H. R. Johnson, *Ind. Eng. Chem. Analyt.*, 1945, **17**, 400.

reactivities than the amines (*e.g.*, lower yields in Table 2, c,d,j,l—n). When propylamine is used as the base (Table 2, c,d) on the methyl ester (IX), amidation also takes place and the ring closure product is the 2-propylcarbamoyl-benzimidazole (XIV). With diethylamine in an aprotic solvent (Table 2, j), the 2-methoxycarbonyl-benzimidazole (XIII) is recovered with little indication of amidation. The protonated amine cation undergoes exchange with the benzimidazole anion during the reaction as a partial product the protonated benzimidazole mixed with the salt. A similar proton exchange from the protonated base occurs when less than stoicheiometric amounts of base are employed (Table 2, g). The reaction in Table 2, g represents *ca.* 180% yield in terms of base present, indicative of base catalysis by the benzimidazole anion. The basicities of benzimidazoles have been studied in some detail,[20] and the proton exchange results of Table 2, g are in accord with equilibrium pK_a values reported for other benzimidazoles. Although yields were good for the ring closure of the β-methyl (XI) and β-phenyl (XII) unsaturated analogues (Table 4), these reactions were slower under identical conditions than for the unsubstituted, less sterically hindered acrylate (IX).

Although the ring closures of (IX), (XI), and (XII) are achieved in nearly equal yield both in aprotic and protic solvents, the polarity of the solvent enhances reactivity even with a weaker base. For the substrate (IX) this is demonstrated in Table 2, l—n. Here identical quantities of substrate and base (Et_3N) are used in the solvents ethyl acetate, methanol, and dimethylformamide to give yields of 21, 42, and 56%, respectively. It is suggested that the polarity of the solvent might stabilise a charged transition state in the reaction. The solvation effect of polar solvents is indicated by the deep red solution which the reaction mixture forms even with weak bases (amines, carbonates, acetate) in dimethylformamide or dimethyl sulphoxide as solvent.

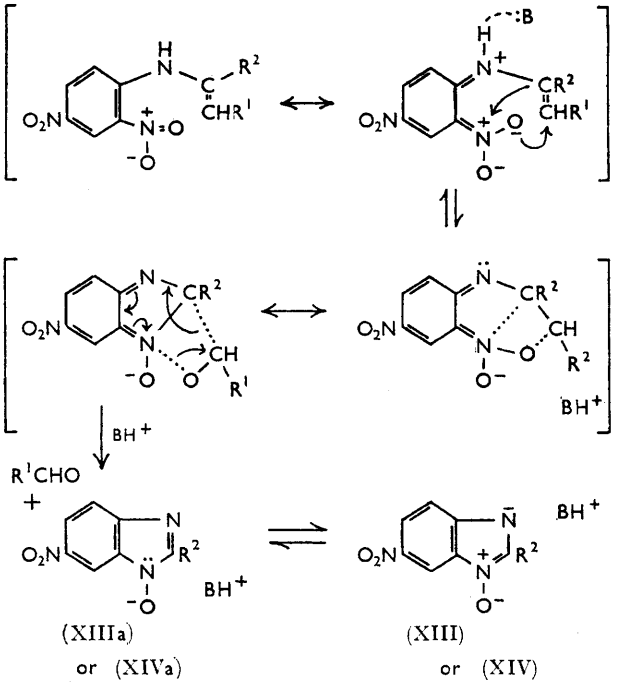
Considerable attention was devoted to detecting possible intermediates of the ring closure. No intermediates were apparent on thorough t.l.c. examination of reaction mixtures. In particular no evidence was found for an intramolecular nitronate ester[21] or a nitroso-derivative.[22] Such a nitroso-compound might be expected from a nitro-oxygen transfer to the terminal vinyl group of (IX), (XI), or (XII). Ring closure to both the 2-methoxycarbonyl- and 2-n-propylcarbamoyl-benzimidazoles proceeded with the substrate carboxy-derivative intact in every solvent (amidation of the ester was apparent with propylamine, as pointed out above).

During ring closure in an alcoholic solvent, the addition of alcohol across the double bond of the unsaturated acid was discernible only in the case of methyl DNP-α-aminocrotonate (XI) in methanolic methoxide. Thus, methyl α-(2,4-dinitrophenylamino)-β-methoxybutyrate was isolated from quenched reaction mixtures (acid neutralisation), purified by t.l.c., and characterised by n.m.r. spectroscopy, elemental analysis, and other physical data. This alcohol addition product undergoes subsequent elimination of alkoxide so that oxidation, retroaldol scission, and benzimidazole ring closure eventually proceed to completion.

Because no intermediates were discernible indicative of a stepwise mechanism, a concerted mechanism is tentatively suggested (Scheme 3). Attack of the *o*-nitro-oxygen at the β-position of the unsaturated acid is accompanied by bridging of the α-carbon of the acyl chain to the nitrogen of the nitro-group. At the same time base removes an imino-proton. The resulting four-centred transition state then collapses into the ring closure product and the aldehyde. The resulting benzimidazole exists in the reaction mixture as an anion. Thus, a stoicheiometric amount of base is consumed in the reaction. Negative charge distribution on the benzimidazole ring is shown through the mesomers (XIII) and (XIIIa) or their respective protonated forms (XV) and (XVII). This tautomerism has been discussed.[6,7,10] Although this concerted mechanism is electronically

SCHEME 3

Suggested concerted mechanism for the ring closure and intramolecular oxidation



R^1 = H, Me, or Ph
R^2 = CO_2Me (XIII) and (XIIIa) or $CO{\cdot}NHPr^n$ (XIV) and (XIVa)

[20] D. R. Rabiger and M. M. Joullie, *J. Org. Chem.*, 1964, **29**, 476.

[21] N. Kornblum and R. A. Brown, *J. Amer. Chem. Soc.*, 1965, **87**, 1742.

[22] S. J. Etheredge, *Tetrahedron Letters*, 1965, 4527.

balanced, the possibility of a radical anion as the reactive intermediate is under further investigation.[23,24]

An intramolecular transfer of oxygen from a nitro-group to an unsaturated carbon–carbon bond has not been reported previously as occurring in the presence of base. Several examples of nitro-oxidation of a double bond which occurred in acid are known and have been reviewed.[5c] This retroaldol scission bears analogy to the work of Snell and his co-workers [25—28] whoused nitrophenyl Schiff base groups to introduce electron delocalisation similar to that of the pyridoxal Schiff bases in the reverse aldol cleavage of β-hydroxy-amino-acids. Thus, 4-nitrosalicylaldehyde and other aromatic aldehydes could be substituted for pyridoxal in the cleavage.[27] However, a specific pyridoxal-like effect of the *N*-2,4-dinitrophenyl group on β-hydroxyamino-acid ester or amide retroaldol cleavage in base has not been reported before.

In addition to the electron withdrawing effect of the dinitrophenyl group, it must be emphasised that in this concerted mechanism simultaneous charge shift from the α-carbon of the acyl derivative to the nitro-nitrogen coincides with the approach of the nitro-oxygen. Although the nitro-group very often acts as an electrophilic recipient for active methylene group anions,[5a] it is here the nitro-oxygen itself which generates the electron density on the α-carbon. The principal driving force for the ring closure would then be aromatisation to the heterocyclic moiety.

While synthesising and handling DNP-β-phenylserine methyl ester (IV) in the presence of base, we noted a strong odour of benzaldehyde. A synthetic route to β-phenylserine is through aldol condensation of benzaldehyde and glycine ester Schiff bases.[29,30] The products of reaction of (IV) in sodium carbonate and methanol were benzaldehyde (isolated as its semicarbazone), DNP-glycine methyl ester (XIX), and 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide (XIII). The benzimidazole (XIII) was thought to result here from an intramolecular attack of the active α-methylene group of the DNP-glycine methyl ester (XIX) on the *ortho*-nitro group.[5] In confirmation the ring closure to (XIII) was performed on authentic (XIX) in methanolic sodium carbonate and found to proceed quite well, though more slowly than those of (IX), (XI), and (XII) (cf. Tables 4 and 5). The serine and threonine analogues (I) and (III) of (XIX) were also run under the conditions above (Table 5) and found to produce the same benzimidazole salt (XIII) as (IV) and (XIX). Although no glycine was isolable in the reactions of (I) and (III), the reactions of (I), (III), and (IV) are all assumed to undergo the same retroaldol scission and ring closure of the DNP-glycine ester.

Exhaustive study of conditions for this reaction of (XIX) has not been made. However, the ring closure of (XIX) does not occur in the aprotic solvent, dimethylformamide. In the protic solvent, methanol, with base, we suggest a mechanism analogous to that suggested for the ring closures of *o*-nitrobenzyl-malonic ester [31] or -acetoacetic ester.[32] A similar reaction has been used to synthesise 2-phenylbenzimidazole 1-oxide from 1-benzyl-2-nitrobenzene,[6,7] and to synthesise phenanthridine

SCHEME 4

Retroaldol cleavage of DNP-β-hydroxy amino-acid esters and ring closure of the DNP-glycine methyl ester scission product

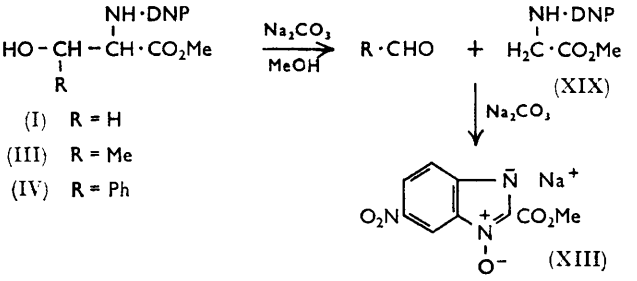


TABLE 5

Ring closure of DNP-glycine methyl esters (XIX) to (XV).[a] Availability of (XIX) for ring closure after retroaldol cleavage of (I), (III), and (IV)

Compound		Substrate (mmoles)	Base (mequiv.)	Reaction time	Product yield (%)
(I)	*N*-2,4-Dinitrophenyl-DL-serine methyl ester	0·70	3·8	1 day	54
(III)	*N*-2,4-Dinitrophenyl-DL-threonine methyl ester	0·66	3·8	3 days	52
(IV)	*N*-2,4-Dinitrophenyl-DL-β-phenylserine methyl ester	2·5	21·6	1·16 hr.	39
(XIX)	*N*-2,4-Dinitrophenylglycine methyl ester	0·75	4·3	1 day	63

[a] Reactions at room temperature with t.l.c. monitoring to completion (solvent A); MeOH (*ca.* 20 ml.) as reaction solvent; Na_2CO_3 as base.

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N-oxides by ring closure of favourably oriented 2′-(active benzylic methylene)-2-nitrobiphenyls.[33] Loudon and Tennant's discussion [5b] of the role of the active methylene group in these reactions and initial reduction of the nitro-group, presumably by the reaction medium, is pertinent to the present work, as is the fact that methanol can reduce an aromatic nitro-group.[34]

The ready reductive ring closure of DNP-amino acids to 3,4-dihydro-2-hydroxy-7-nitroquinoxalines [35] has been utilised for *N*-terminal amino-acid identification in the progressive degradation of polypeptides. We suggest the use of the present 6-nitrobenzimidazole 1-oxide ring formation for quantitative identification of DNP-αβ-unsaturated amino-acid amides. These unsaturated peptides are often the elimination products of serine or threonine phosphates or other esters [15,16] and of glycosides to the β-hydroxy-group in glycoprotein substances.[36] The characteristic u.v. absorption shifts of (XIII), (XV), or (XVII) ($\lambda_{max.}$ 253 and 301 mμ) away from the parent unsaturated 2,4-dinitrophenyl derivative (IX) ($\lambda_{max.}$ 346 mμ) can serve as a method of identification. The conversion of the DNP-derivatives of glycine, serine, and threonine into their methyl esters, followed by ring closure to 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide, may also find a useful function in peptide analysis.

EXPERIMENTAL

Thin-layer chromatography was carried out on silica gel G (Merck), activated at 110° for 1 hr. using the ascending technique. Solvents: A, benzene; B, ethylacetate–ether, 1 : 1; C, benzene–ethyl acetate–ether, 2 : 1 : 1; D, butanol–acetic acid–water, 3 : 1 : 1; detection of zones visually or with an ultraviolet lamp. Nuclear magnetic resonance spectra were measured on a Varian A 60 spectrometer. Proper n.m.r. integrals for all proton ratios were observed and correlated to previously reported values for analogous compounds (see discussion in refs. 1 and 2).

N-2,4-*Dinitrophenyl Amino-acids*.—These were prepared in quantitative yield by the method of Sanger,[11] as detailed earlier.[1] The crude products were nearly homogeneous by t.l.c. except for small amounts of inorganic salts. They were not further purified before esterification to the methyl ester.

General Procedure for Methylation of N-2,4-*Dinitrophenyl Amino-acids*.— Crude *N*-2,4-dinitrophenyl-DL-amino-acid (0·071 mole) was dissolved in tetrahydrofuran–ether (1 : 1; 400 ml.). The solution was filtered through sintered glass to remove traces of inorganic salts, cooled in an ice-bath, and treated with small portions of diazomethane [12] in ether. After each addition (the reaction is almost instantaneous) the reaction was checked for completeness by t.l.c. (solvent B). Immediately after completion (disappearance of slower migrating yellow zone) the reaction mixture was evaporated at room temperature to a syrup which was crystallised from an appropriate solvent (Table 1).

General Procedure for the O-*Toluene*-p-*sulphonates of* α-*Dinitrophenylamino*-β-*hydroxy-acyl Derivatives*.— The DNP-β-hydroxy-amino-acid ester or amide (0·02 mole) was treated with toluene-*p*-sulphonyl chloride (0·06 mole) in anhydrous pyridine (160 ml.) with stirring at −16 to +25° until completion of reaction was indicated by t.l.c. (see Table 1 for solvents and R_F values) (*ca* 20 hr.). *N*-2,4-Dinitrophenyl-DL-serine methyl ester (I) was run at −16° to reduce the rate of toluene-*p*-sulphonate elimination from the product (V), which occurs appreciably at higher temperatures. The β-methyl and β-phenyl analogues formed more stable toluene-*p*-sulphonates and their reactions could be run at room temperature. Sometimes part of the product separated from the reaction mixture as a solid and was collected by filtration. The supernatant was then added to an ice–water slurry (400 ml.) containing 100 ml. of hydrochloric acid (12M) from which additional amounts of product were isolated as a powder by filtration. The crude product was recrystallised from an appropriate solvent (Table 1).

Methyl α-(2,4-*Dinitrophenylamino*)*acrylate* (IX).—Employing Photaki's [15] elimination conditions a solution of (V) (7·5 g., 0·0172 mole) in ethyl acetate (450 ml.) was treated at room temperature with diethylamine (1·2 ml., 0·021 mole) with stirring. After 1 hr. the mixture was cooled in an ice-bath and the crystallised diethylammonium toluene-*p*-sulphonate was removed by filtration. The supernatant was evaporated to a syrup which was crystallised from hot methanol, yielding (IX) (3·5 g., 75%) as orange needles, m. p. 92°,* $\lambda_{max.}$ (MeOH) 346 mμ (ε 1·93 × 10⁴).

Methyl α-(2,4-*Dinitrophenylamino*)*crotonate* (XI).— To methanol (50 ml.) was added the threonine methyl ester (III) (1·06 g., 2·34 mmoles) and anhydrous sodium carbonate (1·0 g.). The suspension was shaken with warming (45°) for *ca*. 15 min., the sodium carbonate removed by filtration, and the supernatant evaporated to near dryness. The remaining solid was triturated with benzene (two 25 ml. portions), and the benzene solution filtered and evaporated to a syrup which crystallised from hot ethanol, yielding *methyl* α-(2,4-*dinitrophenylamino*)*crotonate* (XI) (0·60 g., 90%) as yellow plates. Recrystallisation from ethanol–water gave an analytical sample, m. p. 96—97°, $R_{F(B)}$ 0·71 (Found: C, 47·2; H, 4·0; N, 14·8. $C_{11}H_{11}N_3O_6$ requires C, 47·0; H, 3·9; N, 15·0%), $\lambda_{max.}$ (KBr) 2·98 (NH), 5·78 (CO_2Me), 6·16 (Ar), 6·30 (Ar), 6·58 (NO_2), 7·42 (NO_2), 7·82 (CO_2Me), 8·72, 10·78, 11·92, 13·40, and 14·03 μ, $\lambda_{max.}$ (MeOH) 341 mμ (ε 1·61 × 10⁴).

Methyl α-(2,4-*Dinitrophenylamino*)*cinnamate* (XII).—A solution of (VIII) (1·0 g., 2·48 mmoles) in ethyl acetate (25 ml.) was heated with diethylamine (1 ml., 10 mmoles) at 60° for 2 hr. on a water-bath. Reduction of the volume of the solution to half, cooling, and filtering gave the *product* (XII) (0·80 g.) as fine threads. Additional product (0·07 g.) was obtained by evaporating the supernatant to dryness, triturating the solid with ethanol (5 ml.)–water (15 ml.), and filtering. Total yield of (XII) was 0·87 g. (100%). An analytical sample was obtained from hot ethanol, m. p. 178—179°, $R_{F(A)}$ 0·22, $R_{F(B)}$ 0·55 (Found: C, 56·0; H, 4·1; N, 12·5. $C_{16}H_{13}N_3O_6$ requires C, 56·0; H, 3·8; N, 12·2%), $\lambda_{max.}$ (KBr) 2·98 (NH), 5·81 (CO_2M),

* Ref. 1 gives m. p. 67—70°. This value for a better dried sample supercedes the earlier value.

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6·18 (Ar), 6·28 (Ar), 6·59 and 6·69 (NO_2), 6·99 and 7·45 (NO_2), 7·62, 7·82, 8·28, 9·01, 10·90, 12·78, 13·40, and 14·40 μ, $\lambda_{max.}$ (MeOH) 264 and 348 mμ (ε 2·5 × 10^4 and 1·65 × 10^4).

General Procedure for the Cyclisation of the N-2,4-*Dinitrophenyl-αβ-unsaturated Amino-acids to* 6-*Nitrobenzimidazole* 1-*Oxide Derivatives*.—The unsaturated amino-acid acyl derivative and base were shaken in a solvent at room temperature, except when noted (Tables 2 and 3). The completeness of the reaction was estimated by t.l.c. (solvent A). The reaction mixture was evaporated to a solid below 60° under vacuum. Where the base was an amine, an oil rather than a solid resulted. The residue was triturated with benzene (15 ml., two portions) with warming. If an oil had been present, it solidified upon trituration. The solid was removed by filtration through a sintered glass funnel. The benzene filtrate contained traces of side products and starting material if still present. The solid 2-carboxy-6-nitrobenzimidazole 1-oxide salt (XIII) or (XIV) was dissolved from the filter with water (5 ml.) followed by acetone (3 ml.). Where the base was an amine 2 drops of a saturated sodium carbonate solution were added to the water to dissolve any 2-carboxy-6-nitrobenzimidazole 1-oxide present in the protonated form (XV)—(XVIII). Hydrochloric acid (6N) was added dropwise with stirring to the orange solution until the colour disappeared and a white solid of 2-carboxy-6-nitrobenzimidazole 1-oxide (XV), (XVII) precipitated out. Yields are in Tables 2 and 3.

2-*Propylcarbamoyl*-6-*nitrobenzimidazole* 1-*Oxide*. (XVI) *from* N-2,4-*Dinitrophenyl*-O-*toluene*-p-*sulphonyl*-L-*serine Propyl Amide* (VI).—To a solution of the amide (VI) (0·446 g. 0·95 mmole) in methanol (20 ml.) at 50° was added dropwise with stirring a solution of sodium hydroxide (0·118 g., 2·9 mmoles) in methanol (1 ml.). After allowing the reaction mixture to cool to room temperature (30 min.), work-up as described for 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide yielded the *product* (XVI) as yellow threads (0·187 g., 74%). An analytical sample was prepared by crystallising from hot methanol, m. p. 197°, $R_{F(B)}$ 0·00, $R_{F(D)}$ 0·77 (Found: C, 50·2; H, 4·7; N, 20·9. $C_{11}H_{12}N_4O_4$ requires C, 50·0; H, 4·6; N, 21·2%), $\lambda_{max.}$ (KBr) 3·25b, 6·08 (amide I), 6·20 (Ar), 6·63 (NO_2, amide II), 7·45 (NO_2), 11·82, 12·53, and 13·42 μ.

Detection of Formaldehyde Liberated during the Cyclisation of Methyl α-(2,4-*Dinitrophenylamino*)*acrylate* (IX) *to* 2-*Methoxycarbonyl*-5-*nitrobenzimidazole* 1-*Oxide* (XIII).—(a) *Dimedone derivative*. A solution of the acrylate (IX) (0·1455 g., 0·542 mmole) and sodium carbonate (0·1725 g., 1·63 mmoles) in dimethylformamide (2 ml.) was shaken for 15 min., after which the solution smelled strongly of formaldehyde. Air was then bubbled through the solution for 10 min., which carried formaldehyde through a glass tube to a methanol (3 ml.) trap cooled in a Dry-Ice bath. The formaldehyde present in the methanol was characterised as the dimedone derivative by a standard method.[37] Two recrystallisations of the derivative from 95% ethanol gave m. p. and mixed m. p. 191° (lit.,[37] 191°), i.r. spectrum identical with that of authentic material.

The dimethylformamide solution was diluted with acetone–water (1 : 1; 10 ml.). Addition of hydrochloric acid (5 drops; 6N) and cooling gave 2-methoxycarbonyl-6-nitrobenzimidazole 1-oxide (0·092 g., 71·5%) as fine cream coloured needles.

(b) *Chromotropic acid*. A suspension of sodium carbonate (0·0353 g., 0·336 mmole) in methanol (10 ml.) containing the acrylate (IX) (0·0267 g., 0·1 mmole) was shaken for 1 hr. with warming (50°). The solution was diluted with sulphuric acid (15 ml.; 0·5M) and sodium arsenite solution (26 g. in 1000 ml.) (25 ml.) added. 2-Methoxycarbonyl-6-nitrobenzimidazole 1-oxide (XV) (0·0146 g., 62%) was removed by filtration and the filtrate diluted with water to 200 ml. Samples (1 ml.) of this filtrate, analysed for formaldehyde by the chromatropic acid method of MacFadyen,[18] gave a formaldehyde concentration of 1·36 g./ml., corresponding to an 89% yield of formaldehyde.

Detection of Acetaldehyde Liberated during the Cyclisation of Methyl α-(2,4-*Dinitrophenylamino*)*crotonate* (XI) *to* (XV). A solution of the crotonate (XI) (0·028 g., 0·1 mmole and sodium carbonate (0·05 g., 0·5 mmole) in dimethylformamide (1 ml.) was shaken for 10 min., when the solution smelled of acetaldehyde. Peaks with retention times of 9·5 min. were obtained when a 10-μl. sample of the reaction mixture or a solution (10 μl.) of acetaldehyde (0·05 ml.) in dimethylformamide (10 ml.) were injected into a gas chromatograph. Neither the solvent alone nor any other component of the mixture gave a peak with this retention time. The Barber-Colman model 5000 gas chromatograph equipped with a hydrogen flame ionisation detector was used. A 182·88 cm. by 3·5 mm. inside diameter glass U-tube packed with 100—120 mesh Porapak Q was employed with nitrogen as the carrier gas.

Detection of Benzaldehyde Liberated during the Cyclisation of the Cinnamate (XII).—A solution of the cinnamate (XII) (0·130 g., 0·388 mmole) and sodium carbonate (0·10 g., 1·4 mmoles) in dimethylformamide (10 ml.) was shaken for 22 hr. at room temperature and then warmed at 70° for an additional 2 hr. to complete the reaction (t.l.c.). The reaction mixture had the odour of benzaldehyde. The dimethylformamide and benzaldehyde in the solution were distilled off by means of a vacuum pump (2 mm.) at 50°. To the distillate was added semicarbazide hydrochloride (0·2 g.) and sodium acetate (0·2 g.). The solution was heated for a *ca.* 2 min. at 70° and then allowed to stand for 30 min. The solvent was distilled by vacuum pump and the remaining solid was crystallised from 50% methanol yielding benzaldehyde semicarbazone (0·041 g., 65%), m. p. and mixed m. p. 212—213° (from 95% ethanol), identical (i.r. spectrum) with an authentic sample.

Methyl α-(2,4-*Dinitrophenylamino*)-β-*methoxybutyrate*.—The crotonate (XI) (0·1 g.) in methanol (10 ml.) was treated with sodium methoxide (1 drop; 0·47 g. sodium dissolved in 30 ml. of methanol) at the start of reaction and a further quantity (0·1 ml.) after 0·5 hr. After 0·75 hr. the solution was neutral to phenolphthalein and t.l.c. revealed three zones, $R_{F(A)}$ 0·48 (starting material), 0·27 (butyrate), and 0·00 (XIII), in nearly equal concentrations. The mixture was cooled, 1 drop of glacial acetic acid added, and the residue evaporated to dryness. After trituration with benzene and filtering through a sintered glass funnel, the remaining yellow solid was treated with methanol–water 1 : 1 (15 ml.), 2 drops of 5% hydrochloric acid were added, and white crystals of the benzimidazole (XV) were collected (0·02 g.). The remaining solution was evaporated to a small volume and the crotonate (XI) was separated from zone $R_{F(A)}$ 0·27 (butyrate) by preparative t.l.c. (solvent A). Elution of the excised zone with acetone gave the *product* (0·126 g.), m. p. 97—98° (from ethanol–water), $R_{F(B)}$ 0·76,

[37] N. D. Cheronis and J. B. Entrikin, "Identification of Organic Compounds," Interscience, New York, 1963, p. 263.

$R_{F(C)}$ 0·32, $\lambda_{max.}$ (KBr) 2·98, 3·40, 5·70, 6·18, 6·38, 6·57, 7·00, 7·46, 7·80, 8·60, 8·75, 9·10, 10·80, 11·92, 13·40, and 13·80 μ (Found: C, 46·1; H, 4·7. $C_{12}H_{15}N_3O_7$ requires C, 46·0; H, 4·8%).

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