The Photoelimination of *N*-Nitroso-*N*-acetyl-α-amino Acids; a New Synthesis of 1,2,4-Oxadiazoles

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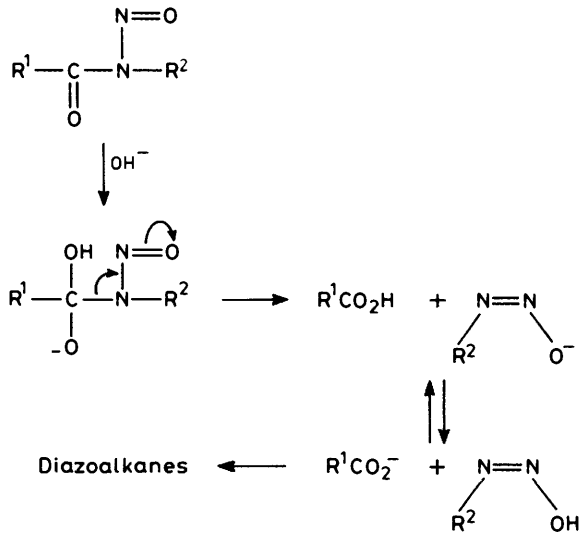
The excitation of *N*-nitroso-*N*-acetyl-α-amino acids, nitrosopeptide model compounds, under neutral and weakly basic conditions, caused the homolysis of the N–N bond followed by decarboxylation to give hyponitrous acid (HNO) and *N*-acetylimines which were susceptible to nucleophilic addition. While weak bases caused the carboxylate group to assist intramolecular rearrangement to a small extent, they functioned primarily to provide nucleophilic NO^-, which initiated nucleophilic attack leading to the *C*-nitroso derivatives. These *C*-nitroso derivatives spontaneously cyclized to 1,2,4-oxadiazoles much more rapidly than tautomerism to the corresponding oximes; the latter oximes failed to cyclize under the basic conditions.

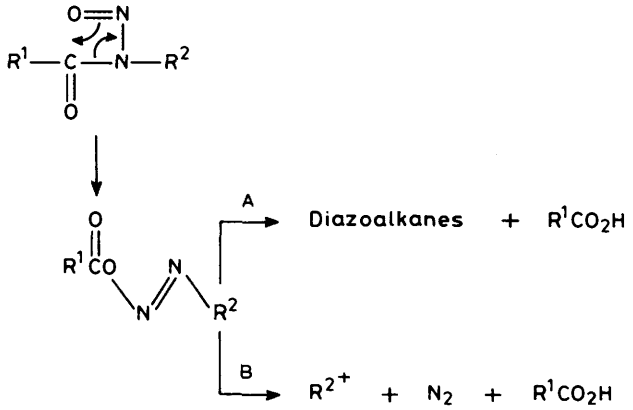
Nitrosamines (R^1R^2N–NO) have been well established to be animal carcinogens and have been shown to be widely distributed in the human environment.[1,2] The less known *nitrosamides* (RR'CON–NO) have recently been established to be even more powerful proximate carcinogens.[3] The oncogenic toxicity of nitrosamides is to be expected since it has been proposed that nitrosamines undergo α-hydroxylation to generate nitrosamides (or the equivalents) which are the immediate precursors of carcinogens.[4,5] In spite of this relationship, nitrosamides are not as well understood as nitrosamines, primarily owing to their instability and difficulty in manipulation under ambient conditions.[6] In contrast, the nitrosamines are extraordinarily stable owing to the extensive delocalization of the *N*-nitroso moiety. In view of the fact that living systems contain amide linkages as a vital chemical constituent, the chemistry of nitrosamides and, more importantly, nitrosopeptides needs to receive its due attention. We have therefore investigated some fundamental reactions of nitrosamides derived from peptide model compounds.

Nitrosamides rearrange readily by mild thermolysis and by base treatment.[7–10] The rearrangement induced by strong bases, such as alkali ethoxide, has been shown to be initiated by the attack of bases either at the carbonyl group or at the nitroso group[11] as in Scheme 1. It is also suggested that weaker bases, such as carboxylate anion or imidazole,[12,13] exclusively attack the carbonyl group to give the *cis*-diazotate–diazoic acid pair which establishes an equilibrium in protic solvent. These further decompose to the corresponding diazoalkanes which serve as alkylating agents.[7,8]

The thermal rearrangement of nitrosamides in various solvents[7,8] leads to unstable *trans*-diazo esters which decompose further to either diazoalkanes or carbonium ions depending on the nature of R^2 (Scheme 2). The net result of *N*-nitrosamide thermolysis in aprotic neutral solvents is very similar to that of deamination of the corresponding primary amines by diazotization.[7,8] In both reactions, diazonium ions and diazoalkanes coexist in an equilibrium state, the relative importance of which is determined by the nature of R^2 and the conditions of the reactions. It should be noted that carbonium ions and/or diazoalkanes have been suggested to be the immediate precursors[4,5] of carcinogenesis induced by nitrosamines. This research concerns with the preparation and the photochemical behaviour of nitrosamides derived from *N*-acetyl-α-amino acids, *i.e.*, peptide models. The photochemistry of nitrosamides have been investigated by our group extensively in recent years.[6,14–18]



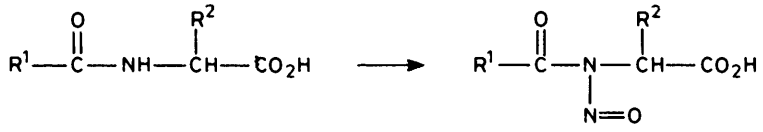
Scheme. 1.



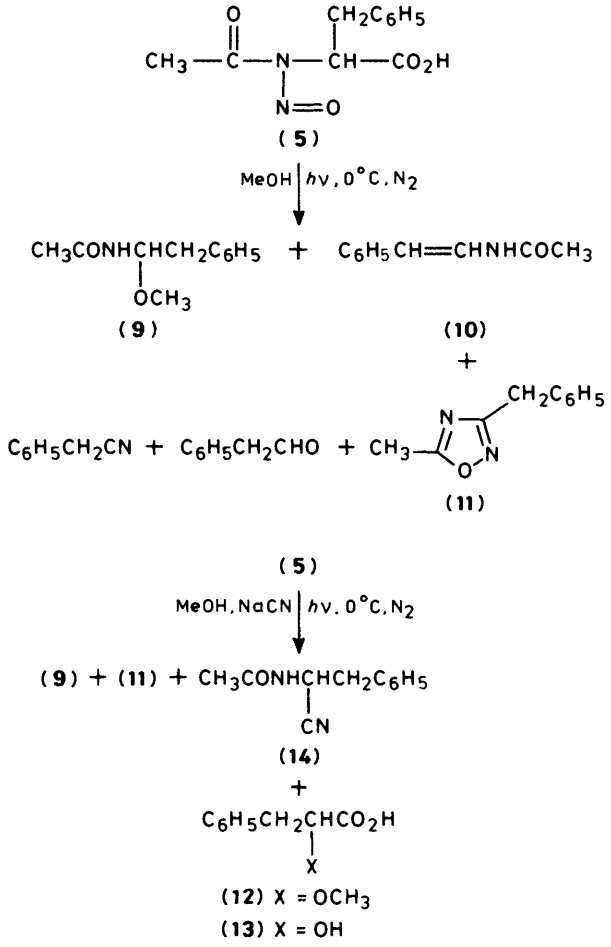
Scheme 2. Path A, R^1 = primary group; path B, R^2 = secondary or tertiary

Results

Acylated α-amino acids (1)—(4) were nitrosated with dinitrogen tetraoxide (N_2O_4) or nitrosonium tetrafluoroborate[19,20]



(1) $R^1 = CH_3$, $R^2 = C_6H_5CH_2$ (5)
(2) $R^1 = CH_3$, $R^2 = (CH_3)_2CHCH_2$ (6)
(3) $R^1 = CH_3$, $R^2 = CH_3COOCH_2$ (7)
(4) $R^1 = C_6H_5$, $R^2 = C_6H_5CH_2$ (8)



(NOBF$_4$) to give yellow nitrosamido acids (**5**)—(**8**). These nitrosamides possess characteristic i.r., u.v., ^{1}H and ^{13}C n.m.r. spectra[6,14] as described in the Experimental section. In contrast to nitrosamines that show *Z*–*E* isomerism and give two sets of ^{1}H and ^{13}C n.m.r. signals,[21,22] nitrosamides in general, and, also, (**5**)—(**8**), give one set of n.m.r. signals for each nitrosamide. This indicated that nitrosamides preferentially possess the *E*-conformation owing to dipole–dipole repulsion of the C=O and N=O groups provided R^2 is not too bulky to offset the effects.[23] Similarity of the n.m.r. chemical shifts of (**5**)—(**8**) with those of simple nitrosamides lead us to assign the *E*-conformation to these nitrosamido acids (**5**)—(**8**).

Compounds (**5**)—(**8**) also showed low-intensity u.v. absorptions at 390, 405, and 420 nm, characteristic of nitrosamides, arising from the $n \rightarrow \pi^*$ transition of the nitroso group.[23] They were too unstable to withstand further purification, but could be kept indefinitely at −10 °C. All

Table. Photolysis of (**5**) in the presence of triethylamine

In CH_3OH[a]		In CH_3CN[b]	
[(**5**)]/[Et_3N]	(**11**) (%)	[(**5**)]/[Et_3N]	(**11**) (%)
1:0	*ca.* 1	1:0.6	26
1:1	17	1:1.3	42
1:2	52	1:2.5	63
1:5	68	1:4.9	68

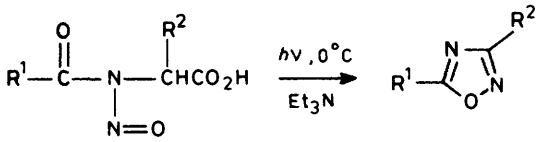
[a] The concentration of (**5**) was 0.01M. [b] The concentrations of (**5**) were 0.7—1.4mM.

photolysis was carried out at 0 °C under nitrogen. Irradiation of *N*-nitroso-*N*-acetyl-DL-phenylalanine (**5**) in methanol gave *N*-acetyl-2-phenyl-1-methoxyethylamine (**9**) (45%) and *N*-acetyl-*trans*-β-styrylamine (**10**) (33%) after chromatography. By g.c.–m.s. analysis, the crude photolystate was shown to contain methoxyamide (**9**) (35%), styrylamine (**10**) (39%) and a few percent each of phenylacetaldehyde, phenylacetonitrile, and 3-benzyl-5-methyl-1,2,4-oxadiazole (**11**). On a g.c. column, (**9**) was shown to decompose to give (**10**), which explained the reversed percentage yield upon g.c. analysis. However, (**9**) and (**10**) were stable to photolysis under comparable conditions.

In the presence of bases, nitrosamido acid (**5**) was shown to undergo ready carboxylate group-assisted rearrangement to give diazoalkane intermediates which reacted with solvent to give[24] hydroxy acid (**12**) or methoxy acid (**13**). Such rearrangement was relatively slow in the presence of weak bases such as sodium cyanide and amines. Photolysis of (**5**) in the presence of sodium cyanide in methanol afforded methoxy amide (**9**) (7%), *N*-acetyl-2-phenyl-1-cyanoethylamine (**14**) (19%), and a much increased yield of oxadiazole (46%): (**12**) and (**13**) derived from base-catalysed rearrangement[24] were also obtained in 24% yield. Similar photolysis of (**5**) in methanol in the presence of sodium carbonate or in tetrahydrofuran in the presence of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) did not give better yields of oxadiazole (**11**); in fact, more complex mixtures were obtained in the latter case. Nor did photolysis of the dicyclohexylamine salt of (**5**) in methanol give any better yields of oxadiazole (**11**). In the presence of these weak bases, carboxylic acids (**12**) and (**13**) were always obtained.

In the presence of *ca.* 2.5 equiv. triethylamine, similar photolysis of (**5**) in either methanol or acetonitrile gave >65% of oxadiazole (**11**). Indeed, in acetonitrile, the neutral fraction gave (**11**) as the only product. But, from the photolysis in methanol (**9**), (**10**), phenylacetaldehyde, and phenylacetonitrile were detected as minor components. In a series of experiments, when the ratio of triethylamine to (**5**) was varied in the range 0—5, oxadiazole increased from *ca.* 1% to 68% reaching *ca.* 50% at 2 equiv. triethylamine (Table). This conclusively proved the necessity of having bases present to generate oxadiazole (**11**).

N-Nitroso-*N*-acetyl-DL-leucine (**6**) was irradiated in acetonitrile in the presence of 2 mol. equiv. triethylamine to give 3-



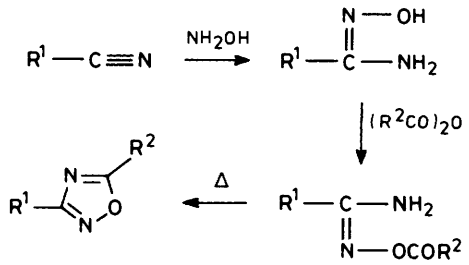
(6) $R^1 = CH_3$, $R^2 = (CH_3)_2CHCH_2$ (15)
(7) $R^1 = CH_3$, $R^2 = CH_3COOCH_2$ (16)
(8) $R^1 = C_6H_5$, $R^2 = C_6H_5CH_2$ (17)

isobutyl-5-methyl-1,2,4-oxadiazole (**15**) in 63% yield in addition to the base-catalysed rearrangement products corresponding to (**12**) and (**13**). Photolysis of *N*-nitroso-*NO*-diacetyl-DL-serine (**7**) in acetonitrile and *N*-nitroso-*N*-benzoyl-DL-phenylalanine (**8**) in methanol in the presence of triethylamine, however, gave poor yields of the oxadiazoles (**16**) (12%) and (**17**) (5%). For the latter photolysis, the rearrangement catalysed by base occurred rapidly on addition of triethylamine and before irradiation as shown by drastic decreases of the nitrosamide u.v. absorption in the 400 nm range. This led to the formation of a large quantity (> 55%) of methoxy acid (**12**) and hydroxy acid (**13**). The poor yields in the former reaction arose from solubility of the products in water and, also, from the volatility of (**16**).

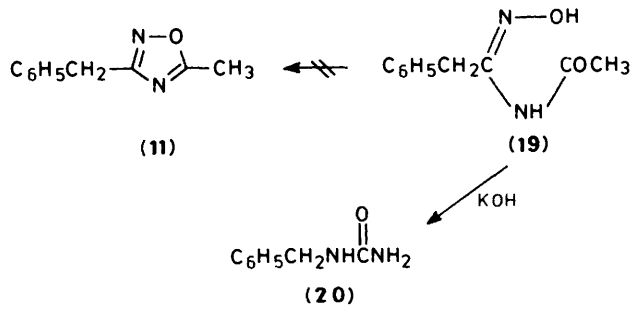
The presence of a methoxy and a cyano group in (**9**) and (**14**), respectively, at C-1 was readily shown by the n.m.r. signals at δ 3.33 (s, 3 H) and 5.4 (m, 1 H) and i.r. absorptions at 1 070 cm^{-1} for the former, and by δ 5.08 (dt, 1 H) and 2 240 cm^{-1} for the latter. Styrylamine (**10**) exhibited a well defined AB quartet at δ 6.21 and 7.75 (*J* 15 Hz) arising from *trans*-olefinic protons; the u.v. absorption at 287 nm clearly indicated the presence of a *trans*-styryl group. The methoxy and hydroxy acids (**12**) and (**13**) and analogous compound were reported previously.[24]

While the physical data of oxadiazoles (**11**) and (**15**)—(**17**) showed some similarities, such as an i.r. absorption at 1 590 cm^{-1}, and ^{13}C n.m.r. signals at δ *ca.* 175 and 169 p.p.m., these only indicate the presence of the oxadiazole nucleus as a common entity. As two positional isomers could be formed in each photorearrangement, (**11**) and its corresponding isomer, 5-benzyl-3-methyl-1,2,4-oxadiazole (**18**), were synthesized by unambigious preparation sequence as shown below. The acetylation of amidoximes gave the *O*-acetates, but not the amides, as clearly indicated by the i.r. absorptions at 1 740 cm^{-1}. Synthesis using phenylacetonitrile and acetic anhydride gave an oxadiazole identical with (**11**). The alternative oxadiazole obtained from acetonitrile and phenylacetic anhydride gave (**18**) which exhibited ^{13}C n.m.r. signals for C-3 (δ 116.6 p.p.m.) as a quartet (*J* 6.8 Hz) and C-5 (δ 176.8 p.p.m.) as a triplet (*J* 7.5 Hz); these patterns arose from long-range coupling with the β-hydrogens of the CH_3 and CH_2 groups, respectively.

It was envisaged that oxadiazole (**11**) could be formed from the intermediate, *N*-acetylphenylacetamidoxime (**19**), by cyclization during photolysis of nitrosamide (**5**). Amidoxime (**19**) was prepared as a 1 : 1 mixture of the *E* and *Z* isomers,[25] as shown by two equal singlets at δ 4.53 and 4.43 for the benzylic protons. The i.r. absorptions at 1 700 and 1 670 cm^{-1} confirmed the C=O and C=N bonds in the *N*-acetylated amidoxime group. The treatment of (**19**) either with triethylamine in acetonitrile or with potassium hydroxide in methanol, however, did not give any oxadiazole (**11**). Under the latter conditions, benzylurea (**20**) was obtained in good yield (59%). The reaction is similar to the Beckmann rearrangement in its overall pattern. It is noteworthy that this occurs under strongly basic conditions.



(11) $R^1 = C_6H_5CH_2$, $R^2 = CH_3$
(18) $R^1 = CH_3$, $R^2 = C_6H_5CH_2$



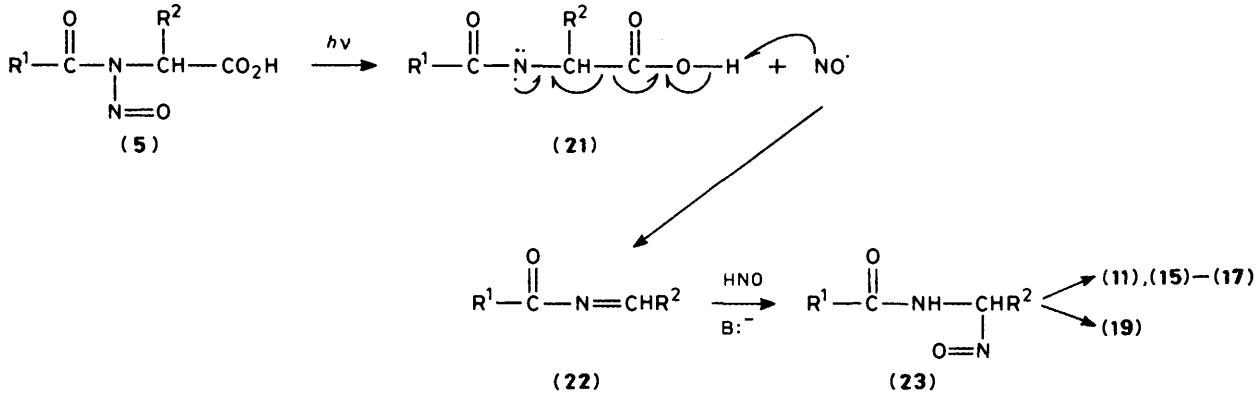
(11) (19) (20)

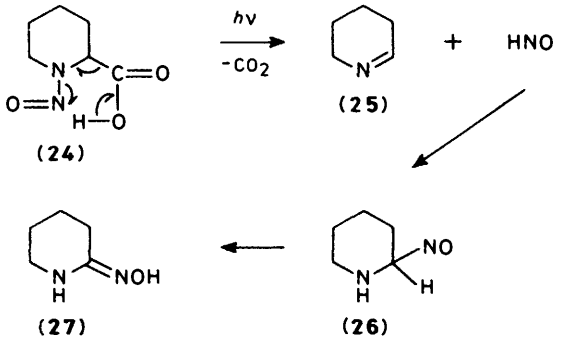
Discussion

The products arising from photoexcitation of (**5**)—(**8**) have one common feature in that the original carboxy group of the amino acids has been eliminated. This feature contrasts with the products arising from base-catalysed rearrangement in which the carboxy group survives the ground-state intramolecular reaction,[24] and can be readily recognized in products (**12**) and (**13**). By analogy with the well established reaction patterns of excited-state nitrosamines,[14,23] the primary photochemical reaction of these nitrosamido acids (**5**)—(**8**) must be the homolysis of the N–N bond to form the amidyl radicals (**21**) and nitric oxide. This is followed by decarboxylation to give[26] *N*-acylimine (**22**) and hyponitrous acid HNO as necessitated by the observed product pattern, although the mechanistic detail of the decarboxylation step remains to be elaborated.

All the photolysis products can be explained by reactions involving *N*-acylimine (**22**) with reasonable mechanisms. Methoxyamide (**9**) and cyanoamide (**14**) are obviously formed by nucleophilic attack of methanol and cyanide ion on (**22**), and phenylacetaldehyde by that of water followed by the elimination of an acetamide. Since *N*-acylimine (**22**) possesses the two dipoles of the functional groups C=O and C=N connected in series, it is very susceptible to nucleophilic attack. Styrylamine (**10**) can arise from a 1,3- or 1,5-prototropy from the benzylic C–H bond to the NH or OH bond, although it can also be formed by elimination of (**9**) as demonstrated. As such reactions are generally slow, the yields of (**10**) are low. In principle, the formation of the C≡N group from *N*-acylimine (**22**) requires further oxidation steps; such a step may be represented by the elimination of CH_3COH species, or may involve oxidation by nitrogen oxides (NO_2 or NO_3); examples of such oxidative steps have been published.[16,17]

The photolytic decarboxylation of (**5**)—(**8**) resembles that observed in photolysis of *N*-alkyl-*N*-nitrosamines,[27] *e.g.* *N*-nitrosopiperidine-1-carboxylic acid (**24**). Decarboxylation from (**24**) is followed by the addition of HNO to the imine bond C=N of (**25**) to form a *C*-nitroso compound (**26**) which tautomerizes rapidly to amidoxime (**27**). In the photoreaction of nitrosamido acids (**5**)—(**8**), the reaction pattern is dichotomous in that addition also occurs but very inefficiently unless weak bases are present, and because the *C*-nitroso compound (**23**)





rapidly cyclizes to oxadiazoles (**11**) and (**15**)—(**17**), leaving no chance for tautomerization to oximes such as (**19**). The observation that acylamidoxime (**19**) does not cyclize to oxadiazole (**11**) indirectly support our proposal that *C*-nitroso compound (**23**) directly cyclizes to (**11**). Whatever mechanism is involved in this cyclization, the corresponding oxime (**19**) is not nucleophilic enough, even in the presence of KOH, to lead to ring formation.

Why is nucleophilic attack of HNO to the C=N bond in (**25**) facile while that to the C=N bond of (**22**) is sluggish? The function of weak bases is essentially to prompt the dissociation of HNO to generate the conjugate base NO^- (pK_a 4.7—4.8)[28] which is the nucleophilic species attacking (**22**). Imines, such as (**25**), have[29] pK_a 5—7 and can act as a weak base in the photolysis of (**24**) to catalyse a similar addition. However, the C=N–C=O moiety in (**22**) is not basic enough, probably pK_a *ca.* 20, to promote HNO dissociation and requires the assistance of added base. Unfortunately, bases also dissociate the CO_2H group of (**5**)—(**8**) to generate the carboxylate group which attack nitrosamido group intramolecularly to initiate the rearrangement.[24] In view of the facile photolysis in neutral conditions, though giving only (**9**) and (**10**) as the major products, it is believed that weak bases have no effect in assisting the primary step of the photolytic decarboxylation as shown by the sequence (**5**) → (**21**) → (**24**).

Nitrosamido acids (**5**)—(**8**) can be regarded as nitroso-peptide model compounds with a carboxy terminal. The ready decarboxylation by photoexcitation and ensuing reactions are complex and can be important in understanding nitroso-induced toxicity in biological transformations.

Experimental

I.r. spectra were taken with a Perkin-Elmer model 457 instrument as liquid films or Nujol mulls. U.v. spectra were recorded on a Unicam SP 800 or Cary 17 spectrophotometer. N.m.r. spectra were recorded on a Varian A56/60 or a Varian XL-100 spectrophotometer equipped with a Nicolet 1080 computer using tetramethylsilane as internal standard. M.p.s were measured on a Fisher–Johns hot stage and were not corrected. G.c. was performed on a Varian 1400 chromatograph (f.i.d.) and g.c.–m.s. analysis on a Hitachi–Perkin-Elmer RMU-7 instrument coupled with a System Industry Data Acquisition System-150 computer. Elemental analyses were performed by Mr. M. K. Yang with a Perkin-Elmer 240 microanalyser.

Preparation of N-*Nitroso*-N-*acetyl*-α-*amino Acids*.—*N*-Acetyl-α-amino acids (**1**)—(**4**) were treated with N_2O_4–sodium acetate (anhydrous) in CH_2Cl_2 at −70 °C or $NOBF_4$ in acetonitrile at 0 °C. The residue was extracted with ether; the ether solution was washed with 1% aqueous $NaHCO_3$ and water. Evaporation of ether gave the nitrosamido acids (**5**)—(**8**). These products were used directly. Compound (**5**) was isolated as yellow solid (75—90%), m.p. 75—79 °C (decomp.); $\nu_{max.}$ 1 715(s), 1 490(s), 1 300(s), 1 120(s), 940(s), and 700 cm^{-1}; δ_H 9.12 (s, D_2O exch.), 7.3 (m, 5 H), 5.60 (*J* 10.5 and 6 Hz, 1 H), 3.44 (J_{AB} 14.5 Hz, 1 H), 6.95 (1 H), and 7.40 (s, 3 H); δ_C 22.1 (q), 33.3 (t), 52.0 (d), 126.9, 128.3, 135.4, 172.6, and 173.5 p.p.m.

To a solution of (**5**) (200 mg, 0.8 mmol) in dry ether (10 ml) was added a solution of dicyclohexylamine (162 mg, 0.9 mmol) in ether (10 ml). Upon cooling the resulting solution to −20 °C, yellow crystals appeared. Filtration, washing with a cold mixture of ether–ligroin (1:1), and drying gave the dicyclohexylamine salt (260 mg, 73%), m.p. 101—102 °C (decomp. with gas evolution); $\nu_{max.}$ 3 020(w), 3 040(w), 1 725(m), 1 630(s), 1 500(m), 1 450(m), 1 370(s), 1 115(m), 940(s), and 700(w) cm^{-1}; $\lambda_{max.}$(MeOH) 424 (ε 227), 405 (233), and 390 nm (173) (Found: C, 66.3; H, 8.8; N, 9.5. Calc. for $C_{23}H_{35}N_3O_4$: C, 66.2; H, 8.45; N, 10.1%). Upon dissolving the salt in [$^2H_6$]DMSO, bubbles were evolved, indicating decomposition. The resulting ^1H n.m.r. spectrum showed signals at δ 7.25(s), 3.97(s), and 1.95(s).

Compound (**8**) was obtained as a yellow resinous oil (83%), $\nu_{max.}$ 1 720(s) and 1 360(s) cm^{-1}; δ_H 9.05 (br s, D_2O exch.), 7.30 (m, 10 H), 5.80 (*J* 6 and 10 Hz, 1 H), 3.53 and 3.26 (*J* 14 Hz, 2 H); δ_C 32.8(t), 52.4(d), 126.8, 128.3, 128.5, 127.6, 129.9, 131.9, 132.2, 135.4, 171.6, and 171.9 p.p.m.

Compound (**6**) was isolated as a yellow oil, $\nu_{max.}$ 1 725(s) and 1 380(s) cm^{-1}; δ_H 9.93 (br s, D_2O exch.), 5.33 (*J* 9 and 5.5 Hz, 1 H), 2.82 (s, 3 H), 2.1—1.8 (br m, 3 H), 0.82 (d, *J* 5 Hz, 6 H); δ_C 21.4(q), 22.4(q), 22.5(q), 25.0(d), 36.3(t), 49.7(d), 172.8, and 173.7 p.p.m.

Compound (**7**) was isolated as a yellow oil (95%), $\nu_{max.}$ 1 740-(s), 1 380(s), and 1 220(s), cm^{-1}; δ_H 9.1 (br s, D_2O exch.), 5.48 (*J* 9 and 4 Hz, 1 H), 4.59 and 4.12 (J_{AB} 12 Hz, 2 H), 2.72 (s, 3 H), and 1.88 (s, 3 H); δ_C 20.3(q), 22.2(q), 49.4(d), 59.9(t), 168.7(s), 170.3(s), and 173.4(s) p.p.m.

Photolysis of Nitrosamido Acids.—The photolysis of (**5**)—(**8**) follows the general procedure described previously,[14-18] using a 200 W Hanovia medium-pressure Hg lamp (654A36) with a filter solution of sodium hydrogen phthalate–sodium nitrite circulating around and an ice-bath to hold the temperature at 0 °C. The reaction was traced by following the disappearance of the absorption at 420, 405, and 390 nm. In the presence of bases, the solution was cooled to −20 °C before nitrosamides were added. The initial solution generally showed a weak absorption at 340 nm indicating that base-catalysed rearrangement had occurred to a small extent. Photolysates were evaporated and diluted with water. The aqueous solution was made basic with Na_2CO_3 solution and was extracted with ether to afford a neutral fraction. The aqueous phase was made acidic and was extracted with ether to afford acid [*e.g.* (**12**) and (**13**)] derived from the rearrangement. This fraction was treated with diazomethane and the known methyl esters[20] were analysed by g.c.

(1) N-*Nitroso*-N-*acetyl*-DL-*phenylalanine* (**5**). (a) In methanol. The neutral fraction (0.87 g) from photolysis of (**5**) (1.0 g) in methanol (100 ml) was chromatographed on alumina go give three fractions which were recrystallized from cyclohexane to afford styrylamine (**10**) (220 mg), m.p. 99—101 °C; $\lambda_{max.}$(EtOH) 287 (ε 21 200) and 221 nm (13 800); $\nu_{max.}$ 3 290, 1 640, and 1 575 cm^{-1}; δ_H 6.21 (d, *J* 15 Hz, 1 H), 7.75 (dd, *J* 10 and 15 Hz, 1 H), 10.0 (br d, *J* 10 Hz, 1 H, D_2O exch.), 2.05 (s, 3 H), and 7.3 (br s, 5 H) (Found: C, 74.3; H, 7.0; N, 8.7. Calc. for $C_{10}H_{11}NO$: C, 74.5; H, 6.9; N, 8.7%).

Further elution gave several fractions which were recrystallized from cyclohexane to give methoxyamide (**9**) (370 mg), m.p. 95—96 °C; $\nu_{max.}$ 1 660(s), 1 530(s), 1 070(s), and 700(s) cm^{-1}; δ 8.22 (d, *J* 10 Hz), 7.27 (s, 5 H), 5.4 (m, 1 H), 3.33 (s, 3 H), 2.93 (d, *J* 5.5 Hz, 2 H), and 1.92 (s, 3 H); *m/e* 162 (19%), 134 (13), 116 (71), 102 (83), 91 (62), 74 (75), 60 (100), 46 (54), and 43 (54) (Found: C, 68.5; H, 7.9; N, 7.3. Calc. for $C_{11}H_{15}NO_2$: C, 68.4; H, 7.8; N, 7.25%).

The crude product showed g.c. peaks (10% SE 30; 150—240 °C at 10° min^{-1}) corresponding to phenylacetaldehyde (2 min; 3%), phenylacetonitrile (2.75 min; 3%), oxadiazole (**11**) (6 min; 2%), methoxyamide (**9**) (13 min; 35%), and strylamine (**10**) (8 min; 39%). When pure (**9**) was injected into the chromatograph at 240 °C, peaks for both (**9**) and (**10**) were observed.

(b) In methanol–NaCN. Irradiation of (**5**) (2.36 g) and NaCN (1 g) in methanol (220 ml) afforded the neutral (1.2 g) and acidic (550 mg) fraction. The neutral fraction showed g.c. peaks at 5 (6%; phenylacetaldehyde), 6.5 (6%; phenylacetonitrile), 11.5 [57%; (**11**)], 12.7 [9%; (**9**)], and 15.9 min [23%; (**14**)]. This (870 mg) was chromatographed on basic alumina using chloroform as eluant. Fractions (440 mg) were rechromatographed and distilled (20 °C at 1 mmHg) to give (**11**) as an oil, $\nu_{max.}$ 1 590(s), 1 385(s), 1 270(s), 735(s), and 700(s) cm^{-1}; δ_H 7.32 (s, 5 H), 4.02 (s, 2 H), and 2.48 (s, 3 H); δ_C 175.8(s), 168.6(s), 134.8(d), 128.2, 129.9, 126.3, 31.6(t), and 11.7 p.p.m.(q); *m/e* 174 (M^+, 82%), 132 (100), 131 (66), 91 (44), 77 (43), and 43 (43) (Found: C, 69.0; H, 5.9; N, 16.3. Calc. for $C_{10}H_{10}N_2O$: C, 68.95; H, 5.8; N, 16.1%).

A following fraction (54 mg) was recrystallized from cyclohexane to give (**9**) as crystals, m.p. 95—96 °C.

Further elution with chloroform containing 1% of methanol gave a yellowish oil (175 mg) which crystallized on standing. Recrystallization from ether gave (**14**), m.p. 105—106 °C (decomp.); $\nu_{max.}$ 3 290(m), 2 240(w), 1 660(s), 1 540(m), 1 460(m), 1 330(m), and 710(m) cm^{-1}; δ_H 7.27 (s, 5 H), 6.5 (br s), 5.08 (dt, *J* 8 and 6 Hz, 1 H), 3.07 (d, *J* 6 Hz, 2 H), and 1.97 (s, 3 H); *m/e* 188.0945 (M^+, 39%; calc. for $C_{11}H_{12}N_2O$: 188.09 49), 129 (91), 102 (24), 91 (100), 60 (30), and 43 (42) (Found: C, 70.5; H, 6.5; N, 14.9. Calc. for $C_{11}H_{12}N_2O$: C, 70.2; H, 6.4; N, 14.9%).

(c) In methanol–Na_2CO_3. A solution of (**5**) (1.3 g, 5.5 mmol) and Na_2CO_3 (2 g) in methanol (130 ml) was irradiated for 2 h and was worked up to give a neutral fraction (615 mg). The percentage of (**9**) and (**11**) was estimated from ^1H n.m.r. signals at δ 3.3(s) for (**9**) and 2.5(s) for (**11**) and from their g.c. peaks to be 46 and 12%, respectively.

(d) In tetrahydrofuran–DBU. The neutral fraction obtained from irradiation of (**5**) (236 mg, 1 mmol) and DBU (152 mg, 1 mmol) showed the peaks of (**11**) (22%) and many other minor products.

(e) In methanol–Et_3N. A solution of (**5**) (970 mg, 4.1 mmol) and triethylamine (980 mg, 9.7 mmol) in methanol was irradiated for 2 h to give a neutral fraction (470 mg) which was shown to contain (**9**) (9%) and (**11**) (64%) by analysis of the g.c. and ^1H n.m.r. spectra.

(f) In acetonitrile–Et_3N. A solution of (**5**) (235 mg, 1 mmol) and triethylamine (202 mg, 2 mmol) in dry acetonitrile (120 ml) was irradiated for 1.5 h to give a neutral fraction (220 mg) which showed the ^1H n.m.r. spectra and g.c. peak of (**11**).

(g) The dicyclohexylamine salt of (**5**) in methanol. The salt (125 mg, 0.3 mmol) in methanol (100 ml) was irradiated for 15 min to give a neutral fraction (45 mg) which showed the following g.c. peaks: phenylacetaldehyde (1.1 min; 20%), (**11**) (3.5 min; 15%), (**10**) (4.6 min; 16%), and (**9**) (5.0 min; 31%).

(2) N-*Acetyl*-N-*nitroso*-DD-*leucine* (**6**). A solution of (**6**) (2.33 g, 11 mmol) and triethylamine (2.2 g, 22 mmol) in acetonitrile (230 ml) was irradiated for 1.25 h. The solvent was distilled through a Widmer column and the residue was worked up to give a neutral fraction which contains triethylamine (1.0 min), unknown compound (3 min; 5%) and (**15**) [5 min; 68% estimated from (**11**) as internal standard]. Preparative g.c. gave (**15**) as a volatile oil, $\nu_{max.}$ 1 620(m) and 790(m) cm^{-1}; δ_H 2.58 (d, *J* 7 Hz, 2 H), 2.56 (s, 3 H), 2.12 (nonet, *J* 7 Hz, 1 H), and 0.99 (d, *J* 6 Hz, 6 H); δ_C 175.8(s), 169.7(s), 34.5(t), 26.8(d), 22.1(q), and 12.0(q) p.p.m.; *m/e* 140 (M^+, 1.7%), 125 (12), 98 (100), 83 (31), 56 (53), and 43 (97); upon irradiation of the doublet at δ_H, the nonet at δ 2.12 collapsed into a triplet (*J* 7 Hz).

(3) N-*Nitroso*-NO-*diacetyl*-DL-*serine* (**7**). A solution of (**7**) (360 mg, 80% pure) and triethylamine (370 mg) in acetonitrile (90 ml) was irradiated for 1 h and worked up as above to give a neutral fraction which gave an oil (25 mg, 12%). Distillation at room temperature and 0.1 mmHg gave an oil, (**16**) (20 mg), $\nu_{max.}$ 3 500(w br), 1 750(s), 1 590(s), 1 370(m), 1 220(s), and 1 040(s) cm^{-1}; δ_H 5.18 (s, 2 H), 2.60 (s, 3 H), and 2.15 (s, 3 H); δ_C 175.3, 168.6, 165.0, 56.7(t), 20.6(q), and 13.6(q) p.p.m.; *m/e* 156 (M^+, 3%), 114 (22.7), 113 (10), 104 (11.8), 102 (12), 86 (11), 85 (22), 84 (11), and 43 (100).

(4) N-*Benzoyl*-N-*nitroso*-DL-*phenylalanine* (**8**). A solution containing (**8**) (1.3 g, 4.3 mmol) in methanol (230 ml) was cooled to 0 °C under nitrogen. Sodium carbonate (2 g) was added in small portions whereupon an intense absorption at $\lambda_{max.}$ 350 nm was observed along with that of the nitrosamido group at the 400 nm region. The solution was irradiated for 10 h, until no change in the u.v. absorption could be detected. The usual work-up afforded neutral (270 mg) and acidic (736 mg) fractions.

The neutral fraction was chromatographed over silica gel (20 g) using a mixture of ether–light petroleum (1:1) as eluant. The first fraction (82 mg) consisted of several products which were separated by preparative t.l.c. [silica gel; ether–light petroleum (1:4); eluted twice]: the fastest moving spot was extracted with ether to yield a yellow oil (21 mg, 4%) identified as methyl benzoate, identical i.r. and n.m.r. spectra with those of an authentic sample. The second spot was extracted with ether to yield a solid (42 mg, 5%), which after recrystallization from ethanol gave (**17**) as needles, m.p. 81—82 °C (lit.,[30] 88 °C); $\nu_{max.}$ 3 060(w), 3 030(w), 1 620(w), 1 560(m), 1 450(s), 1 370(3), 730(m), 715(s), 695(m), and 650(m) cm^{-1}; δ_H 8.1 (m, 2 H), 7.3 (m, 8 H), and 4.18 (s, 2 H); δ_C 156.9, 152.4, 132.5, 128.9, 128.5, 128.0, 126.9, and 32.31 p.p.m.; *m/e* 236.0947 (M^+, 69%, calc. for $C_{15}H_{12}N_2O$: 236.0950), 207 (20), 131 (14), 116 (15), 105 (100) 103 (42), 91 (33), and 77 (55).

The second fraction (65 mg) from column chromatography solidified upon evaporation of the solvent. Recrystallization from cyclohexane gave *N*-benzoyl-1-phenyl-2-methoxyethylamine (58 mg, 5%) as crystals, m.p. 123—125 °C, $\nu_{max.}$ 3 320(m), 1 640(s), 1 530(s), 1 280(m), 1 100(m), 1 060(m), 700(s), and 690(s) cm^{-1}; δ_H 7.7 (m, 2 H), 7.5 (m, 3 H), 7.26 (s, 5 H), 6.26 (br d, *J* 6 Hz, 1 H), 5.65 (br m, 1 H), 3.36 (s, 3 H), and 3.02 (d, *J* 6 Hz, 2 H); *m/e* 255 (M^+, 0.1%), 223.1036 (25; calc. for $C_{15}H_{14}NO$, 223.0997), 178 (57), 164 (58), 162 (34), 105.0342 (100, calc. for C_7H_5O: 105.0344), 91 (37), and 77 (61) (Found: C, 75.0; H, 6.8; N, 5.4. Calc. for $C_{16}H_{17}NO_2$: C, 75.3; H, 6.7; N, 5.5%). The third fraction (50 mg) was shown by t.l.c. to contain the same methoxy adduct. The acidic fraction was shown to contain (**12**) (87%) by g.c. analysis of the methyl ester.

Synthesis of Oxadiazoles (**11**) *and* (**18**).—(1) The reaction of hydroxylamine with phenylacetonitrile[31] gave the amidine oxime $C_6H_5CH_2C(NH_2)$=NOH (95%), m.p. 57—59 °C, $\nu_{max.}$ 3 500—3 100(br s), 1 650(s), 1 460(s), and 760(s) cm^{-1}; δ_H 8.3 (br s, D_2O exch.), 7.3 (s, 5 H), 4.5 (br s, D_2O exch.), and 3.49 (s, 2 H). This compound was acetylated with acetic anhydride to yield the corresponding *O*-acetate (89%) as a solid, m.p. 121—123 °C; $\nu_{max.}$ 3 400(s), 3 320(s), 1 740(s), 1 630(s), 1 230(s), 900-(s), and 750(s) cm^{-1}; δ_H 7.3 (s, 5 H), 4.8 (br s, D_2O exch.), 3.55 (s, 2 H), and 2.13 (s, 3 H). The *O*-acetate was heated in water to give (**11**) (61%) as an oil, $\nu_{max.}$ 1 590(s), 1 500(m), 1 450(m), 1 430-(m), 1 380(m), 1 360(m), 1 270(m), 740(s), and 700(s) cm^{-1}.

(2) The reaction of acetonitrile with hydroxylamine gave acetamidoxime, $CH_3C(NH_2)$=NOH (9%), m.p. 125—128 °C (lit.,[32] 133.5 °C), $\nu_{max.}$ 3 500(s), 1 650(s), 1 040(m), and 890(s) cm^{-1}. Treatment with phenylacetic anhydride gave the *O*-acetate (19%) as crystals, m.p. 86—91 °C, $\nu_{max.}$ 3 420(m), 3 300(m), 1 740(s), 1 600(s), 1 220(s), and 720(s) cm^{-1}; δ_H 7.37 (s, 5 H), 5.0 (br s, D_2O exch.), 3.72 (s, 2 H), and 1.85 (s, 3 H).

Steam distillation of the crude *O*-acetate gave (**18**) (78%) as a yellow oil, $\nu_{max.}$ 1 580(s), 1 500(m), 1 460(m), 1 430(m), 1 400(s), 1 340(s), 740(s), and 700(s) cm^{-1}; δ_H 7.30 (s, 5 H), 4.08 (s, 2 H), and 2.35 (s, 3 H); δ_C 10.3(q), 31.6(t), 126.3, 127.8, 127.9, 132.9, 166.6(s), and 176.8(s); *m/e* 174 (46%), 117 (59), 104 (100), 91 (64), 90 (34), 77 (15), 65 (29), and 39 (22). When an off-acquisition decoupled ^{13}C spectrum was recorded, the lines at δ 166.6 and 176.8 p.p.m. were respectively split into a quartet (*J* 6.8 Hz) and a triplet (*J* 7.5 Hz). Both (**11**) and (**18**) were recovered when heated (200 °C) for 12 h or treated with 1M-NaOH in methanol, or photolysed (254 nm) for 6 h.

Synthesis of N-*Acetylphenylacetamidoxime* (**19**).—The chloroxime $C_6H_5CH_2CCl$=NOH was prepared by chlorination of phenylacetaldehyde oxime according to the method described by Behn, m.p. 85—88 °C (lit.,[31] 89—91 °C); $\nu_{max.}$ 3 200(s), 1 600(m), 1 080(m), and 990(s) cm^{-1}; δ_H 9.0 (br s, D_2O exch.), 7.30 (s, 5 H), and 3.79 (s, 2 H).

A solution of triethylamine (90 mg, 0.9 mmol) in ether (5 ml) was added to a solution of the chloroxime (130 mg, 0.8 mmol) in ether (20 ml). The precipitate was filtered off and the filtrate was added dropwise to a suspension of sodium acetamide (600 mg, 7 mmol) in DMF (25 ml) containing a few drops of hexamethylphosphoric triamide. This solution developed a brown colour. The mixture was stirred at room temperature overnight. Water (25 ml) was added to the mixture and the resulting solution was first extracted with ether (4 × 30 ml) and then continuously with ether for 12 h. The combined extracts were thoroughly washed with water (9 × 10 ml), dried, and evaporated to give *N*-acetylphenylacetamidoxime (**19**) (50 mg, 33%) as a solid which was purified by sublimination at 60 °C and 0.1 mmHg, m.p. 130—130.5 °C, $\nu_{max.}$ 3 320(m), 1 700(s), 1 670(s), 1 550(m), 1 460(m), 1 270(m), 1 260(m), 1 230(m), and 720(m) cm^{-1}; δ_H 8.9 (br s, D_2O exch.), 7.3 (s, 5 H), 4.53 (s, 1 H), 4.43 (s, 1 H), and 2.1 (s, 3 H); *m/e* 192.0901 (M^+, 49%; calc. for $C_{10}H_{12}N_2O_2$: 192.0903), 107 (16), 106 (100), 91 (37.5), 77 (15.5), and 60 (62.3) (Found: C, 62.7; H, 6.5; N, 14.4. Calc. for $C_{10}H_{12}N_2O_2$: C, 62.5; H, 6.3; N, 14.6%).

Treatments of (**19**) *with Base.*—Triethylamine (25 mg, 0.25 mmol) in acetonitrile (2 ml) was added to a solution of the acetamidoxime (**19**) (18 mg, 0.09 mmol) in acetonitrile (3 ml). The mixture was stirred in the dark at room temperature overnight. The resulting solution was analysed by t.l.c. to show the presence of unchanged acetamidoxime (17 mg, 94%).

A solution of (**19**) (17 mg, 0.09 mmol) in methanol (2 ml) was treated with a solution of potassium hydroxide (*ca.* 10 mg) in methanol (3 ml). The resulting mixture was stirred at room temperature for 18 h. The reaction was followed by t.l.c. to give no spot corresponding to (**11**). The solvent was evaporated off to give a solid residue which was dissolved in CH_2Cl_2 (25 ml). The resulting solution was washed with water (10 ml), dried ($MgSO_4$), and evaporated to yield (**20**) (8 mg, 59%) as solid, m.p. 139—142 °C (lit.,[32] 147—148 °C); $\delta_H(D_2O)$ 7.3(s), 4.36(s), and 4.3(s); *m/e* 150 (M^+, 80%), 106 (100), 91 (50), 79 (25), and 77 (25).

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