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CHEMISTRY OF N-NITROSAMINES DERIVED FROM α-AMINO ACIDS

by

Joël Sebastien Polo

Ingénieur Chimiste, (1973)

Ecole Supérieure de Chimie Industrielle

de Lyon (France)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Chemistry

SIMON FRASER UNIVERSITY

November 1979

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"Chemistry of N-Nitrosamines Derived from α-Amino Acids"

Author:

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Joël Sebastien POLO

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November 21st 1979.

(date)

APPROVAL

Name: Joel Sebastien Polo

Degree: Doctor of Philosophy

Title of Thesis: Chemistry of N-Nitrosamines Derived from α-Amino Acids

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ABSTRACT

Chemistry of N-Nitrosamines Derived from α-Amino Acids

In relation to their carcinogenic activity, a series of nitroso derivatives of N-alkyl and N-acyl α-amino acids was synthesized and their physical properties as well as their thermal, basic and photolytic decompositions were investigated.

Studies by ^{13}C and ^{15}N nmr spectroscopy revealed the configuration of the nitroso derivatives in which the Z-isomer was shown to be stabilized by an intramolecular hydrogen bonding. Nitrososarcosine was the first nitrosamine to exhibit the E-Z isomerism in ^{15}N nmr spectroscopy.

N-alkyl-nitrosamino acids underwent efficient oxidative photodecarboxylation, followed by addition of nitroxyl (HNO) to give N-alkyl-α'-amidoximes. The reaction was shown to involve an imine intermediate.

The nitroso derivatives of N-acyl-α-amino acids also decarboxylated under u.v. irradiation to generate an N-acyl-imine, a synthetically useful intermediate, which underwent facile nucleophilic 1,4-addition. When the nucleophile was

the conjugate base of nitroxyl, C-nitroso adducts were obtained and rearranged intramolecularly to give derivatives of 1,2,4-oxadiazole.

Thermolysis of N-nitroso-N-acetyl-D,L-phenylalanine (1) in methanol gave 2-methoxy-3-phenyl-propanoic acid (2) and its methyl ester, which were assumed to arise from diazoalkane and/or 1,2,3-oxadiazol-5-one (3) intermediates. In benzene, thermolysis of 1 gave α-acetoxy carboxylic acid, a product resulting from the normal thermal decomposition of nitrosamides, and phenylethylacetate arising from a diazoalkane intermediate. Some spectroscopic evidence for the latter is also presented.

Under basic conditions, 1 underwent rapid decomposition, the mechanism of which depended upon the amount and the strength of the base added. For several strong bases, the presence of more than one equivalent generated a diazocarboxylate anion. The characterization of this anion was attempted by uv spectroscopy and by chemical tranformations. In the presence of one or less than one mole equivalent of a strong base (or in the presence of an excess of a weak base), an intramolecularly catalyzed nucleophilic deamination was observed. In methanol, α-methoxy acid 2 was obtained and in non polar solvents products derived from oxadiazolone 3 were observed.

v

to Odile

" - Les hommes ont oublié cette vérité, dit le renard. Mais tu ne dois pas l'oublier. Tu deviens responsable pour toujours de ce que tu as apprivoisé. Tu es responsable de ta rose ...

- Je suis responsable de ma rose... répéta le petit prince, afin de se souvenir. "

- "Le Petit Prince"
Antoine de Saint-Exupéry

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Y.L. Chow, for providing me with challenging opportunities and professional supervision for the period of this research. To my friend and mentor, Dr. K.S. Pillay, I extend a very special thanks for our many stimulating discussions. I would like to offer my deep appreciation for the interest and support derived from members of Prof. Chow's group. For an unflagging and valuable friendship, I owe much to Dr. K. Hanaya. A venture of this kind would not have been possible without the unstinting support of the faculty and staff of the Department of Chemistry, Simon Fraser University.

A special thanks to Ms Leslie Vincent, who endured the typing of the manuscript. While, for financial support, I am grateful to the Department of Chemistry and the Natural Sciences and Engineering Research Council of Canada.

Finally, to my parents, who have encouraged me in so many ways, a very special thank you.

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CHAPTER I

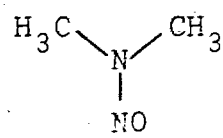
INTRODUCTION

I-1 Nitrosamines and Cancer

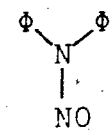
Nitrosamines have been known for more than a hundred years (1), but it was only in 1956 that Magee and Barnes (2) discovered their tumorigenicity. Since then, over 150 nitrosamines have been tested for carcinogenic activity and of these, more than 75% have been found to be carcinogenic towards animals (3).

Nitrosamines are formed by the interaction of nitrous acid with secondary or tertiary amines (4). Recently, traces of nitrosamines have been found in environmental samples (5) and have been shown to occur in the mammalian stomach (6). Secondary amines are known to occur in various foods such as vegetables, fish, cheese, mushrooms, fruits, wine and beer. Furthermore, nitrites are found in the environment either naturally or from reduction of nitrates by microorganisms (7), or are added to food as preservatives. The latter fact explains the detection of N-nitrosodimethylamine I-1 and N-nitrosopyrrolidine I-2 in cured meat, fish and fried bacon at levels of up to 200 µg/kg (8). Nitrosation by nitrous acid is known to be pH dependent and is optimal at pH 1-3 (4) which is the prevailing condition in the mammalian stomach. Simultaneous injection of morpholine and

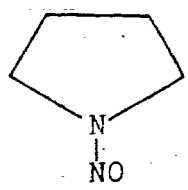
sodium nitrite resulted in the induction of tumors in the liver of rats similar to that induced by direct injection of nitrosomorpholine I-3 (9). The in vivo formation of nitrosamine was also demonstrated when N-nitrosodiphenylamine I-4 was isolated from stomach contents of human subjects after intragastrical injection of sodium nitrate and diphenylamine (10).



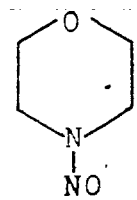
I-1



I-4



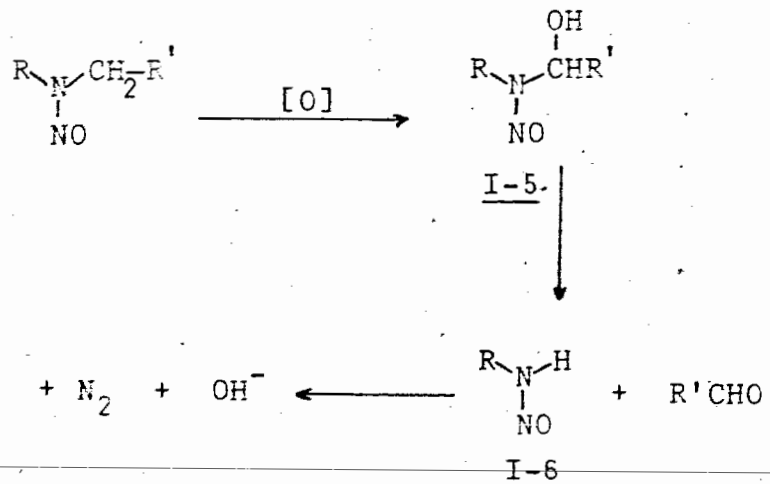
I-2



I-3

The mechanism of the carcinogenic action of nitrosamines has been suggested by Magee (11) to involve alkylation at the nitrogen-7 of guanidine in nucleic acids, by alkylating agents generated in vivo. Indeed, Magee (11) demonstrated the in vivo and in vitro nucleic acid alkylation by means of labelled nitrosamines. Nitroso compounds are usually classified into two groups based on their biological activity: i) those that act directly and ii) those that require metabolic or chemical activation to be

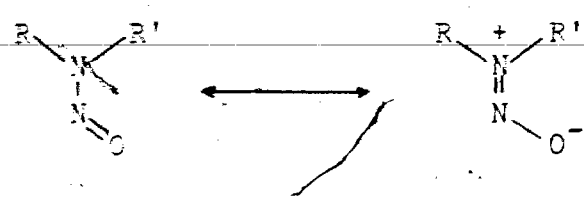
effective. The former group includes nitroso derivatives of amides and related compounds such as ureas and urethans. They are much less stable than nitrosamines and are known to decompose at alkaline pH to give diazoalkanes (12-15) which are potent alkylating agents (16). The second group includes aliphatic and aromatic nitrosamines and their derivatives. As a rule, they show organ specificity to an exceptional degree. Presently it is believed that the α-carbon of a typical dialkylnitrosamine is oxidized to yield the corresponding α-hydroxy compound I-5 which undergoes heterolysis of the C-N bond to give the unstable monoalkylnitrosamine I-6. The latter collapses to the alkylating carbenium ion R^+ (17).



I-2 N-Nitrosamines

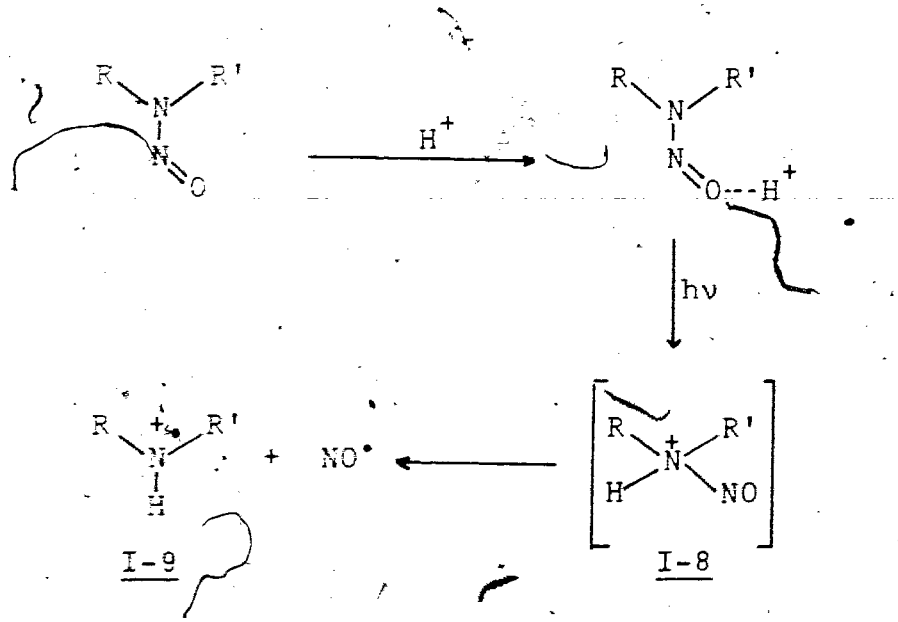
N-nitrosamines are prepared by nitrosation of secondary N-alkylamines with nitrites, nitric oxide, nitrogen trioxide, nitrogen tetroxide, nitrosyl chloride, or nitrosyl tetrafluoroborate (4). Under nitrosating conditions, primary alkylamines undergo the well known diazotization reaction (16) and tertiary amines α-cleavage with formation of secondary nitrosamines (4).

Nitrosamines are extremely stable compounds which exhibit ir absorptions at ν_{max}: 1320 (N=O), 1080 (N-N stretch.) and 660 (N-N=O deform.) cm^{-1} and uv absorptions at λ_{max} (ε): 250 nm (8000) and 340 nm (100). The ground state electronic configuration of nitrosamines has been assessed by SCF calculations (18) to possess a 48% contribution of polar resonance form I-7. The planar geometry of the nitrosamino group has also been demonstrated by X-ray diffraction studies (19) and the resulting energy barrier for rotation around the N-N bond was evaluated to be 24 kcal/mole (20).

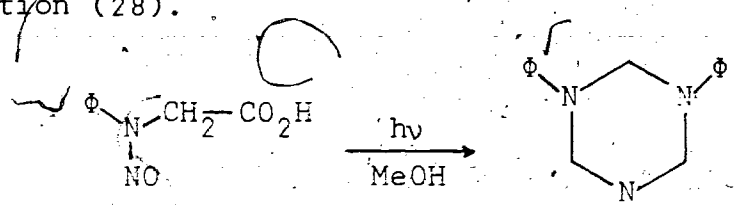


I-7

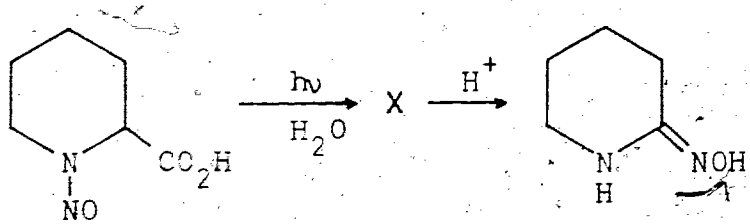
Nitrosamines are stable towards uv irradiation under neutral or basic conditions (21-23). However, in the presence of a dilute acid, they readily undergo N-N homolysis (24). It is believed that in the ground state a proton complexes with the oxygen of the nitroso group (25). In the excited state the proton is transferred to the amino nitrogen to give nitrosammonium ion I-8 which then undergoes homolysis to give aminium radical I-9 and nitric oxide. This aminium radical can subsequently add to olefins (26), abstract hydrogen atoms (27) or undergo β-elimination (28).



The requirement of an acid for nitrosamines to undergo photolysis, prompted Chow (28) to investigate the photoreactions of nitroso derivatives such as N-nitroso-N-phenylglycine I-10 and N-nitrosopipecolinic acid II-1-h* which bear an internal carboxylic function. Both compounds underwent light induced decarboxylation without addition of an external acid. The photolysis of nitroso derivative I-10 in methanol resulted in the formation of triazine I-11 and that of nitroso II-1-h in water gave a hygroscopic intermediate which, on treatment with an acid, isomerized to 2-piperidonoxime II-16-h. However neither nitrosonipecotinic acid I-13-a itself nor N-nitrosopiperidine I-13 and N-methyl-N-nitrosoaniline I-12 in the presence of one equivalent of acetic acid showed any change under prolonged irradiation (28).



I-10 I-11



II-1-h II-16-h

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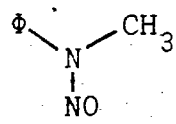
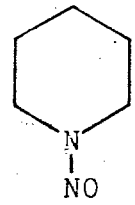
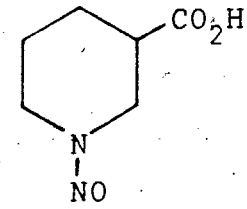
* Some chemicals introduced in this chapter are numbered II-x in order to keep a systematic notation in chapter II.



I-12



I-13

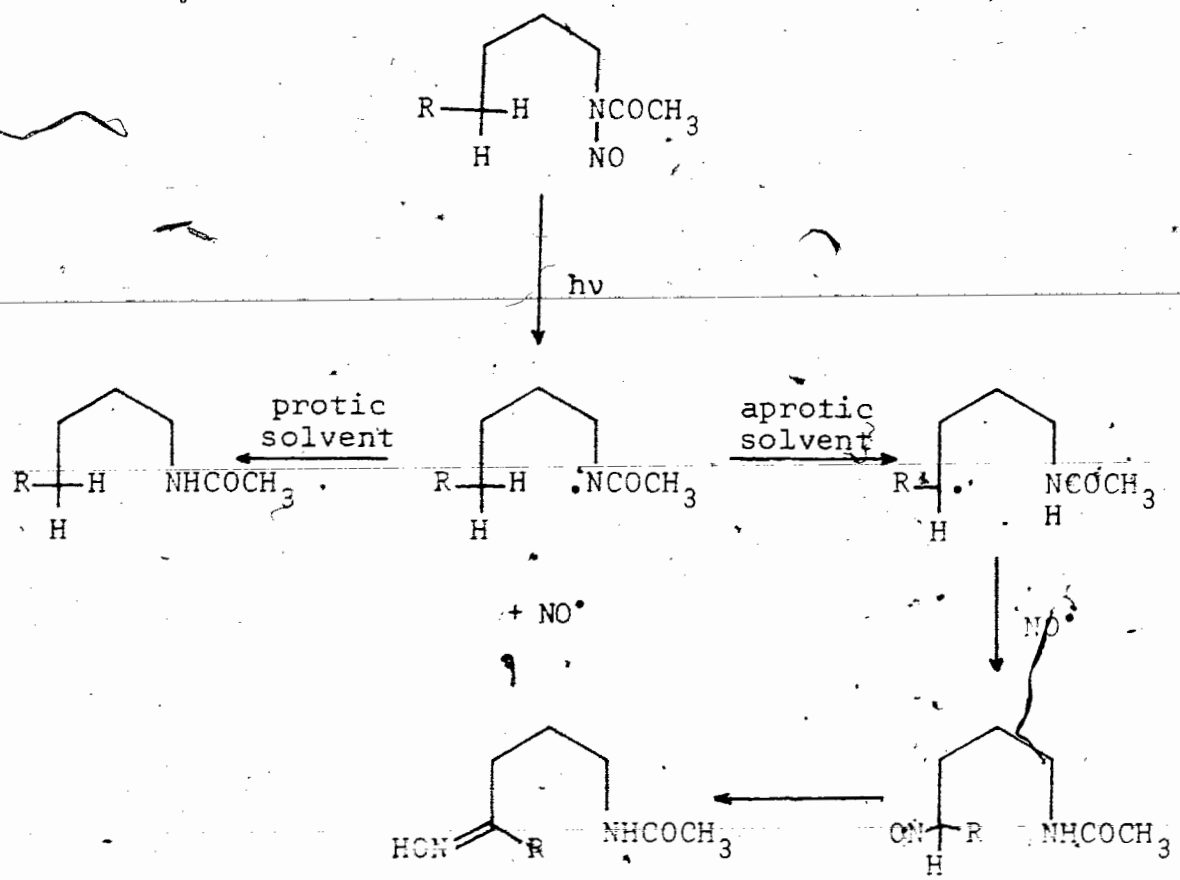


I-13-a

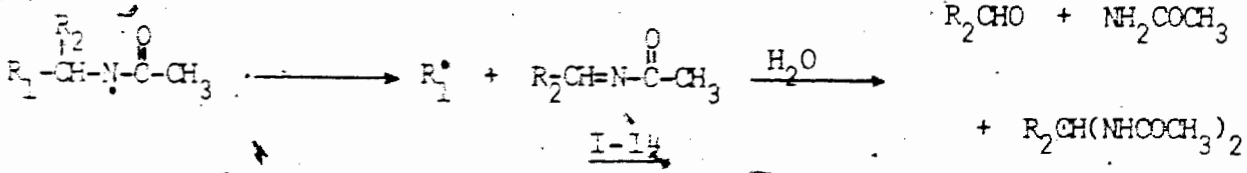
I-3 N-Nitrosamides

N-nitrosamides are prepared by nitrosation of N-alkylamides with sodium nitrite, nitrous anhydride, nitrosyl chloride, dinitrogen tetroxide (29) or nitrosyl tetrafluoroborate (30). In general, nitrosamides are unstable and their instability increases from tertiary to primary carbinamines (31). They exhibit ir absorptions at ν_{max}: 1755-1715 cm^{-1} (C=O) and at 1535-1515 cm^{-1} and uv absorptions at λ_{max} (ε) : 242-246 (4000-6000), 406-415 (100) and 425-435 (10) nm.

Photolysis of nitrosamides generates amido radicals which primarily undergo hydrogen atom abstraction either intramolecularly or intermolecularly from the solvent (32-38). The intramolecular hydrogen atom abstraction occurs via a six-membered transition state to give δ-oximino amides as shown in the following scheme.



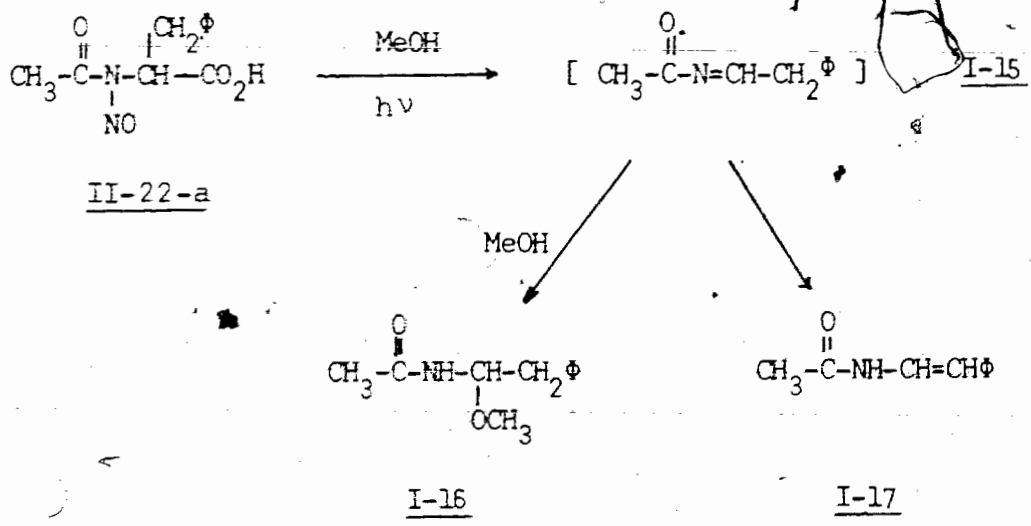
In the absence of an abstractable hydrogen, amido radicals undergo β-scission of a C-H or C-C bond to generate alkylidene-acetamide I-14 which then undergo hydrolysis (32).



R_1 = H, Alkyl

R_2 = H, Alkyl

Intermediates such as I-14 have also been proposed to be involved in the light induced oxidative decarboxylation of nitrosamides derived from α-amino acids (28). Thus, photolysis of N-nitroso-N-acetyl-DL-phenylalanine II-22-a generated N-acylimine I-15 which then reacted with the solvent, methanol, to give N-acetyl-1-phenyl-2-methoxyethylamine I-16 or rearranged to N-acetyl-β-styrylamine I-17.



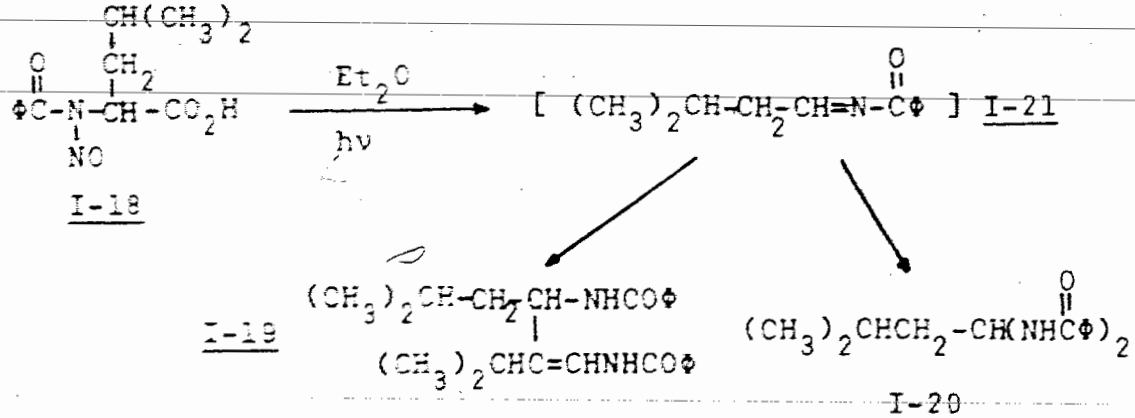
II-22-a

I-15

I-16

I-17

Furthermore, photolysis of N-nitroso-N-benzoyl-DL-leucine I-18 in ether resulted in the formation of compounds I-19 and I-20 via the imine intermediate I-21 (28).



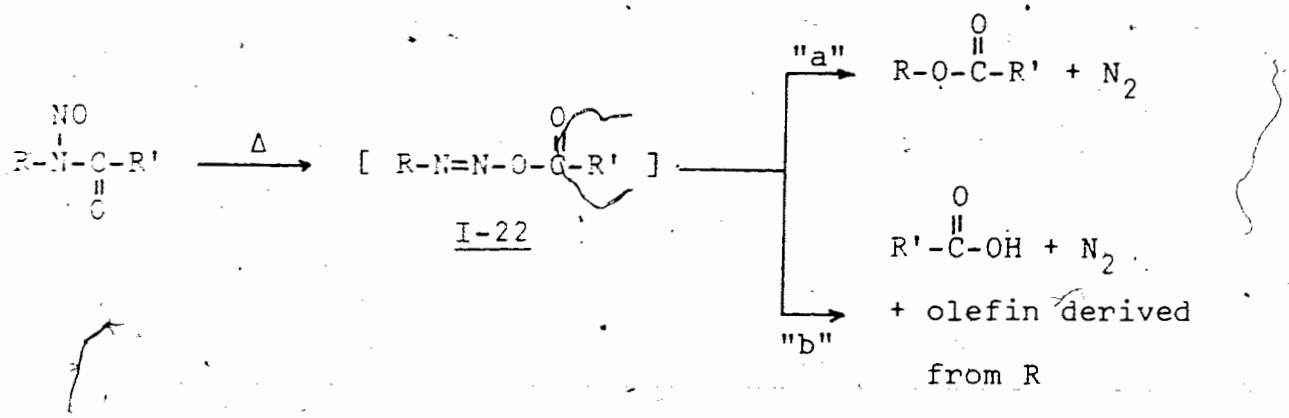
I-18

I-21

I-19

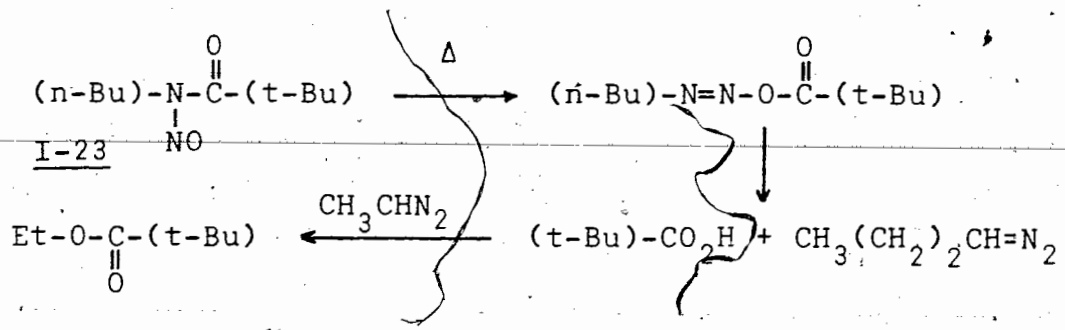
I-20

N-nitrosamides, in contrast to nitrosamines, are only stable at or below room temperature. It is now well established (9) that the rate determining step of the thermolysis of nitrosamides is the initial formation of the unstable diazoester I-22. The latter undergoes further decomposition, the pathways of which depend upon the nature of the substituent R.

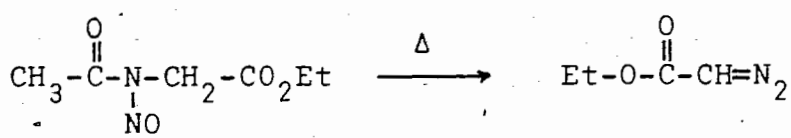


Thus, aryldiazoesters decompose to give biaryls and carboxylic acids via the intermediacy of either benzyne (40) or aryl radicals (41). On the other hand, alkyl diazoesters yield predominently carboxylic esters (pathway "a")in the case of primary alkyl groups (36) and carboxylic acids and olefins (pathway "b") in the case of secondary and tertiary alkyl groups (42). The existence of a diazoalkane intermediate in the decomposition of primary alkyldiazoesters was confirmed by intercepting the carboxylic acid molecule with externally added diazoalkane. Thus, decomposition of N-(n-butyl)-N-nitrosotrimethylacetamide I-23 inthe presence of an excess of diazoethane resulted in the formation of the

ethyl ester and diazobutane (42). Furthermore, thermolysis of



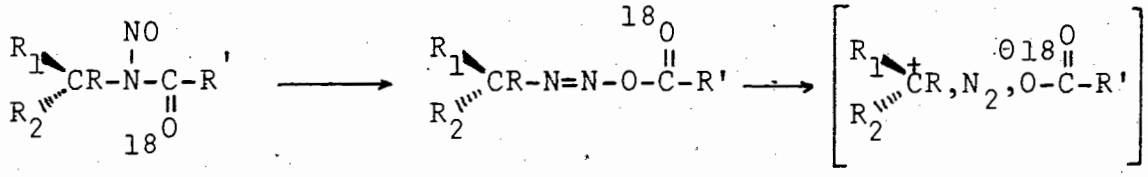
ethyl-N-acetyl-N-nitrosoglycine I-24 was reported to give the stable ethyldiazoacetate I-25 (39).



I-24 I-25

In non polar solvents, optically active secondary diazoesters rearrange intramolecularly with predominence of retention of configuration (42). In polar solvents, secondary and tertiary diazoesters decompose into an intimate ion pair which collapses in the solvent cage, to give the observed esters (42). In the case of optically active and ^{18}O labelled substrates, secondary alkyl diazoesters lead to esters showing some label scrambling and partial racemization (42) whereas tertiary alkyl diazoesters

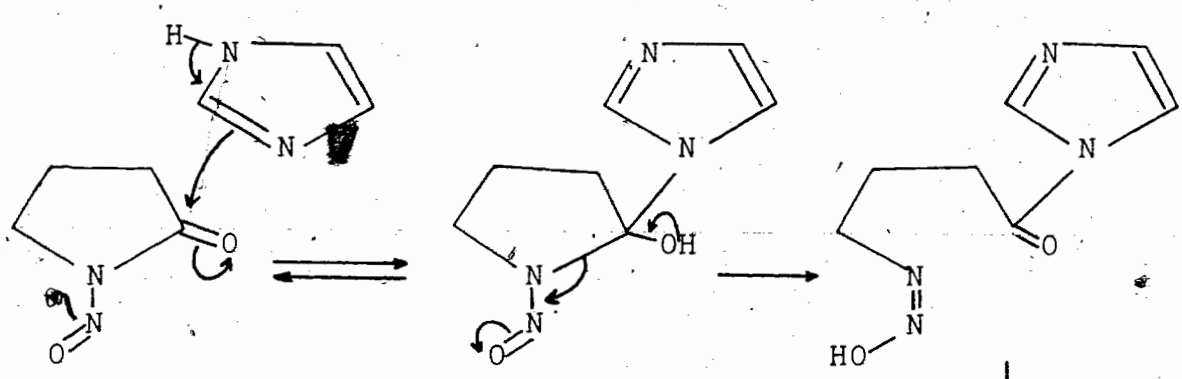
decompose with retention of configuration and only a small amount of label scrambling (42). It is postulated that the decomposition of diazoester I-26 involves the formation of ion-pair I-27 which induces label scrambling and racemization. Any inversion is intramolecular and a tertiary carbenium ion, with its greater size, would have a slower rate of rotation.



I-26 I-27

The rearrangement induced by strong bases such as alkali alkoxides, has been shown to be initiated by the attack of the base at the carbonyl or at the nitroso group (43). The nature of the solvent has a marked effect on the site of the attack; aprotic solvents favour attack at the nitroso nitrogen whereas protic solvents favour attack at the carbonyl carbon. The resulting cis-diazotate is stable in aprotic solvents (44) but hydrolyses readily in the presence of water to give diazotic acid which decomposes into a diazoalkane derivative or an alkyl carbenium ion (44).

Recently, deamination of nitrosamides has also been observed in weakly acidic (45) as well as weakly basic (46) media. In both cases, the decomposition of N-nitrosopyrrolidone I-28 was markedly catalysed by bases such as $MeCO_2^-$, HCO_2^- , pyridine, imidazole and n-butylamine. At pH= 7-9, the deamination reaction involves an addition elimination pathway as shown in the following scheme. The preference for nucleophilic rather than general base-catalysed hydrolysis is related to the enhanced leaving properties of the N-nitrosamino fragment.



I-28

I-4 Research Outline

Since the chemistry of nitrosamines, and in particular that of nitroso derivatives of amino acids remains largely unknown, our understanding of the mechanism of their carcinogenicity is hampered. Nitroso derivatives of amino acids are potent

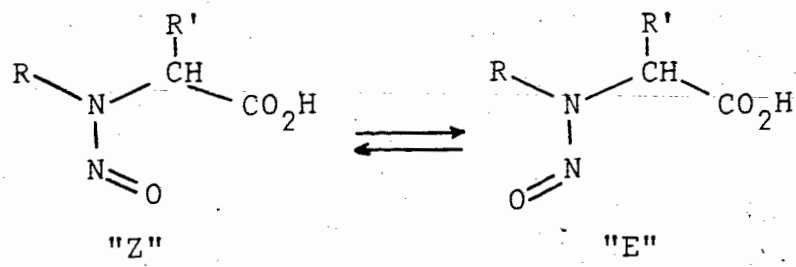
carcinogens (6) and are known to occur in the gastro-intestinal tract of humans, either by direct nitrosation of naturally occurring amino acids (6) or by metabolic oxidation of nitrosamines (17). It was, therefore interesting to investigate further the chemistry of these compounds in order to acquire a clear understanding of their carcinogenic behavior.

The present work involves the synthesis of a series of nitroso derivatives of N-alkyl and N-acyl α-amino acids and the investigation of their photochemical, thermal and base induced decomposition.

CHAPTER II

RESULTS

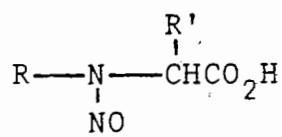
In the following chapters, the reported percentage yields are based on one mole of starting material and are obtained from gas chromatographic or nmr spectroscopic analyses unless specified as isolated percentages. The neutral and acidic fractions always refer to the material obtained from an acid-base extraction of the crude product. The Z and E-isomers of nitrosamino acids refer to the configuration shown below.



II-1 Preparation and Properties of N-Alkyl-N-Nitroso-α-Amino Acids

The nitroso derivatives of N-alkyl-α-amino acids II-1 were prepared by nitrosation of the parent amino acids with sodium nitrite or nitrosyl tetrafluoroborate; the α-amino acids were either obtained commercially or synthesized via the straight-

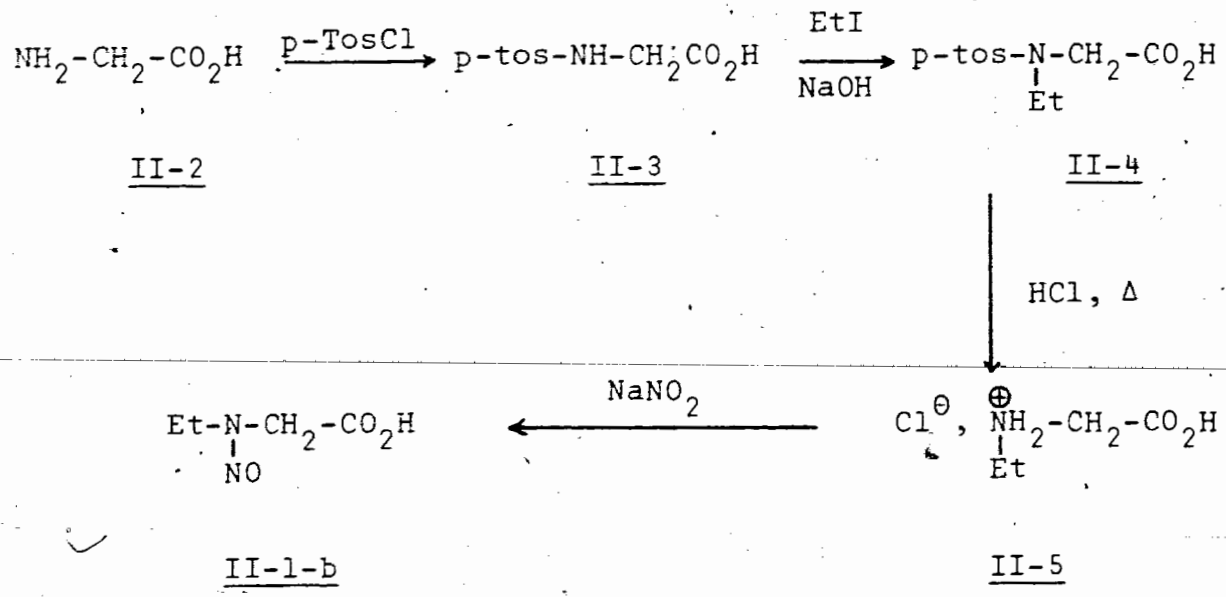
forward routes depicted in schemes 2-1 and 2-2. All known compounds were purified by recrystallization and gave similar melting points to those reported in the literature except for N-isopropyl derivative II-1-c which showed a discrepancy of 60°C (47). Our sample of II-1-c was confirmed by the pertinent ir, nmr, uv and ms data. The new nitrosamines II-1-d, II-1-e and II-1-f gave satisfactory elemental analyses.



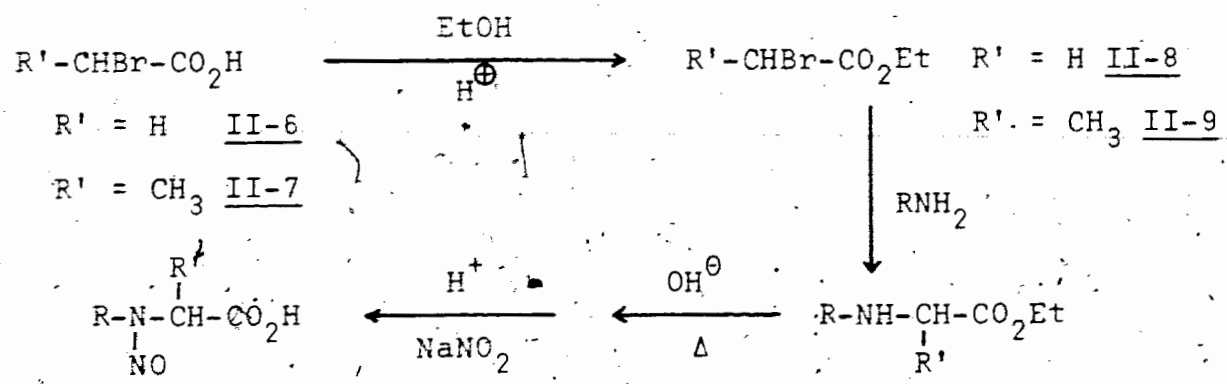
II-1

$R = CH_3$	$R' = H$	a
$R = C_2H_5$	$R' = H$	b
$R = C_3H_7$	$R' = H$	c
$R = (CH_2)_3\phi$	$R' = H$	d
$R = iC_3H_7$	$R' = CH_3$	e
$R = tC_4H_9$	$R' = CH_3$	f
$R,R' = (CH_2)_3$		g
$R,R' = (CH_2)_4$		h

All nitroso derivatives II-1 show two uv absorption bands at about 350 nm (ε ~100) and 240 nm (ε ~10,000) attributable to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. Their ir spectra exhibited the characteristic carbonyl stretching frequency of carboxylic acids at ~1730 cm^{-1} as well as that of the nitroso group at ~1450 cm^{-1}.



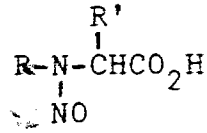
Scheme 2-1: Synthesis of N-ethyl-N-nitrosoglycine (II-1-b)



II-1-c	R = iC_3H_7	R' = H	II-10
II-1-d	R = $(CH_2)_3\phi$	R' = H	II-11
II-1-e	R = iC_3H_7	R' = CH_3	II-12
II-1-f	R = tC_4H_9	R' = CH_3	II-13

Scheme 2-2: Synthesis of Nitrosamino Acids II-1-c, II-1-d, II-1-e and II-1-f

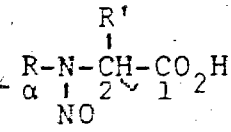
Table 2-1. ^{1}H nmr Data of N-Nitroso-N-Alkyl-α-Amino Acids



Compound	- CH -		R		Solvent
	Z	E	Z	E	
II-1-a[e]	5.72	5.02	6.10	6.87	$CDCl_3$
II-1-b[e]	5.60	4.99	5.69 q (7 Hz)	6.27 q (7 Hz)	D_2O
			8.59 t (7 Hz)	8.9 q (7 Hz)	
II-1-c	5.82	-	5.21 sp (7 Hz)	-	DMSO-d_6
			8.61 d (8 Hz)		
II-1-d[f]	5.70	5.00	5.72 t (7 Hz)	6.38 m	Acetone-d_6
			7.3; 7.9 m	7.45; 7.9 m	
			2.79 m	2.79 m	
II-1-e[a]	5.55	-	5.16 sp (7 Hz)		DMSO-d_6
			8.38 d (7 Hz)		
II-1-f[b]	5.5	-	8.39		Pyridine
II-1-g[c)e]	5.25	4.5	5.65-6.3	5.65-6.3	Pyridine
II-1-h[d)e]	4.25	3.95	5.2 (Heq)	4.9 (Heq)	Pyridine
			5.9 (Hax)	6.95 (Hax)	

a) R' : 8.75 d (7 Hz) b) R' : 8.35 d (6.5 Hz) c) R' : 7.9 m, taken from Ref. 48 d) R' : 7.5 m; 8.5 m, taken from Ref. 48 e) the isomeric ratio at equilibrium was 1:1 f) at equilibrium ~15% of E-isomer was present

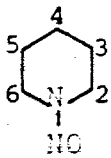
Table 2-2. ^{13}C nmr Data of N-Nitroso-N-Alkyl-α-Amino Acids



Compound	C_1		C_2		C_α (R)		Others
	Z	E	Z	E	Z	E	
II-1-a[a)]	169.8	172.2	48.6	55.8	41.2	34.4	
II-1-b[b)]	167.7	170.5	48.2	53.2	46.5	40.2	13.9 (Z), 11.0 (E)
II-1-c[a)]	167.2	-	44.6	-	55.1	-	20.9
II-1-d[c)]	166.6	-	45.7	-	52.4	-	29.9, 30.7, 141.4, 128.4, 125.9
II-1-e[d)]	169.6	-	52.6	-	55.0	-	21.5, 21.2, 13.4 (R')
II-1-f[d)]	170.2	-	52.6	-	61.3	-	28.1, 13.1 (R')

a) Taken in $CDCl_3$ b) Taken in DMSO-d_6 c) Taken in Acetone-d_6
d) Taken in $CDCl_3$ + Pyridine

Table 2-3. ^{13}C nmr Shifts of Derivatives of Nitrosopiperidine and Nitrosopyrrolidine

Compound		C_2	C_3	C_4	C_5	C_6	$C(CO_2H)$
 a)		39.0	25.5	24.7	27.2	50.8	
 b)		39.2	32.0	42.2	33.6	50.2	
II-1-h c)	Z	50.3	25.0	21.0	26.1	48.4	169.6
	E	60.9	27.5	21.0	23.6	38.0	171.6
 c)e)	Z	40.1	40.0	26.7	24.5	49.6	173.6
	E	51.0	41.3	26.7	22.9	39.0	
 c)f)		38.1	27.0	39.9	28.7	48.8	175.0
II-1-g d)	E	62.3	27.3	22.4	45.6		173.2
	Z	58.4	20.5	28.3	49.5		171.1

a) Ref. 24 (neat liquid) b) Ref. 25 $CDCl_3$ c) In DMSO-d_6 d) In $CDCl_3$ + Pyridine e) This compound was synthesized by catalytic hydrogenation (PtO_2) of nicotinic acid followed by nitrosation. f) This compound was obtained by nitrosation of isonipecotic acid.

Figure 2-1. Correlation Diagram for ^{13}C nmr Chemical Shifts of Five and Six Membered Nitrosamines

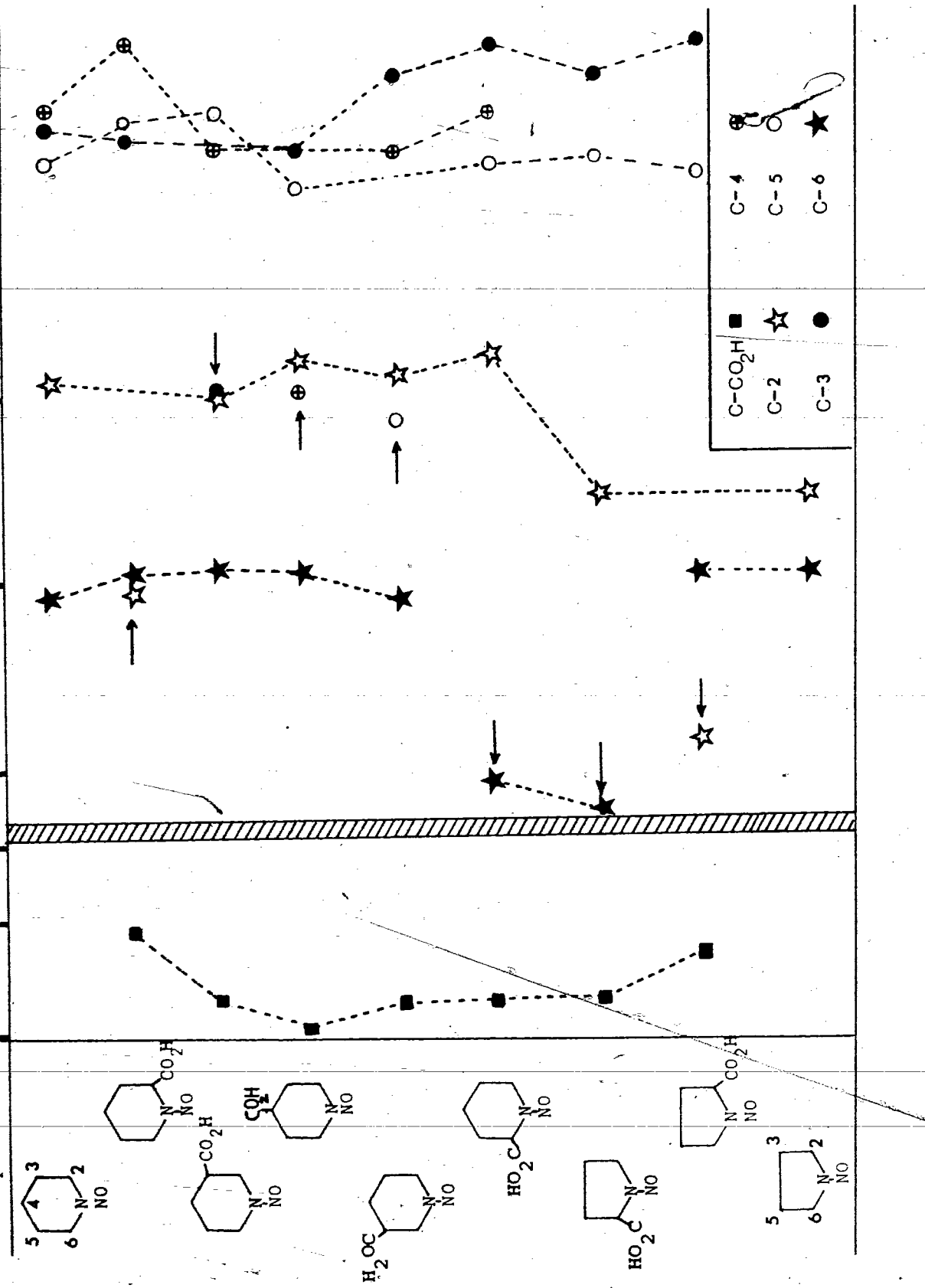


Table 2-4. Relative Intensities of ms Fragments of Some Nitrosoamino-Acids

Compound	M^+	$[M-OH]^+$	$[M-NO]^+$	$[M-CO_2H]^+$	$[M-NO-CO_2H]^+$	$[M-HNO-CO_2H]^+$	Others
II-1-a[a]	88	0.2	22	100	91.5	98	m/e = 43 (100)
II-1-b[a]	51	<0.1	2.4	81	96	100	m/e = 54 (93)
II-1-c[b]	18	<0.1	18	10	15	37	m/e = 91 (100)
II-1-c[c]	<0.1	22	16	0.3	4	25	m/e = 70 (100)
II-1-c[a]	20	<0.1	6	8	38	33	m/e = 41 (91)
II-1-f[a]	37	0.3	1	100	92	62	m/e = 54 (93)
II-1-h[a]	15	<0.1	6	51	100	22	

a) run at 90°C; b) run at 30°C; c) run at 60°C

Table 2.5. uv Data of Nitrosamino Acids λ_{max} (ε)

Compound	$n \rightarrow \pi^*$	$\pi \rightarrow \pi^*$	Solvent
II-1-a [a]	340 (86)	233 (6200)	H_2O
	352 (93)	233 (6300)	EtOH
	358 (105)	233 (5800)	CH_2Cl_2
II-1-b	350 (89)	235 (6100)	MeOH
II-1-c	348 (90)	233 (5900)	MeOH
II-1-d	348 (92)	231(5900)	MeOH
II-1-e	350 (89)	233 (6000)	MeOH
II-1-f	351 (95)	234 (5200)	MeOH
II-1-g [a]	343 (91)	240 (7600)	H_2O
	354 (100)	235 (6700)	EtOH
	361 (100)	237 (6600)	CH_2Cl_2
II-1-h [a]	345 (91)	240 (7300)	H_2O
	355 (93)	240 (6900)	EtOH
	361 (112)	238 (6900)	CH_2CH_2

a) taken from Ref. 42

^{1}H nmr: Nitrosamino acids are known to preferentially crystallize in the E or Z-conformation and to isomerize slowly in solution (48,49). Determination of the crystalline conformation is generally accomplished by ^{1}H nmr spectroscopy (48): the nmr spectrum of a freshly prepared solution of a nitrosamino acid exhibits the signals due to the conformation at the solid state and when isomerization occurs, a new set of signals, corresponding to the other isomer, appears and increases slowly to reach its maximum intensity at equilibrium. The relative ratio of the two isomers at equilibrium can be measured from the ratio of areas of corresponding signals. Assignment of each set of signals is then achieved by extending to nitrosamino acids the well established anisotropic effect of the nitrosamino group on the α-protons (48,50), i.e., α-protons which are syn to the nitroso group are shifted upfield with respect to the anti α-protons. The same method was used to determine the conformation of the new compounds reported in Table 2-1.

^{13}C nmr: The same observation was made in ^{13}C nmr spectra of dialkylnitrosamines: the carbon atom α to the nitrosamino group resonates at higher field when it is syn to the nitroso group than when it is anti (41,52,53,54). As ^{13}C nmr spectra of nitrosamino acids have never been reported, it was interesting to study the anisotropic property of the nitrosamino group on this class of derivatives. Two independent methods of

spectral assignment were applied to the spectrum of N-nitroso-sarcosine (II-1-a). The first technique depended on the known Z-configuration of II-1-a in the solid state (48) and its slow isomerization in solution to a 1:1 ratio of E-Z isomers in the equilibrium state. As shown in Figure 2-2, the zero-hour spectrum (spectrum a) exhibited the lines due to the Z-isomer: after 45 minutes, a new set of lines emerged (spectrum b) as the population of the E-isomer increased; in 3 hours the equilibrium was reached showing two sets of signals with equal intensities (spectrum c). The second method was the selective decoupling technique reported by Wilson (54): two spectra were recorded with different decoupler offset values; the crossover points, when the corresponding shifts are connected, correspond to the carbon shifts on the x-axis and to the proton shifts along the y-axis, as shown on Figure 2-3. The well established ^{1}H nmr chemical shifts of E and Z-isomers of II-1-a (48) enabled us to correlate the proton with the carbon shifts and to assign the ^{13}C nmr signals to their corresponding carbon in each isomer. For example, the methyl protons of the E-isomer (τ = 6.87) resonating at higher field than that of the Z-isomer (τ = 6.10) must correspond to the high field carbon; the methyl carbon of the Z-isomer therefore must be assigned to the down field signal. The second method confirmed the assignments obtained from the first one (Table 2-2). The assignments of the other open chain derivatives

Figure 2-2. Isomerization of Z-Nitroso-sarcosine in CD_3OD at 35°C followed by ^{13}C nmr.

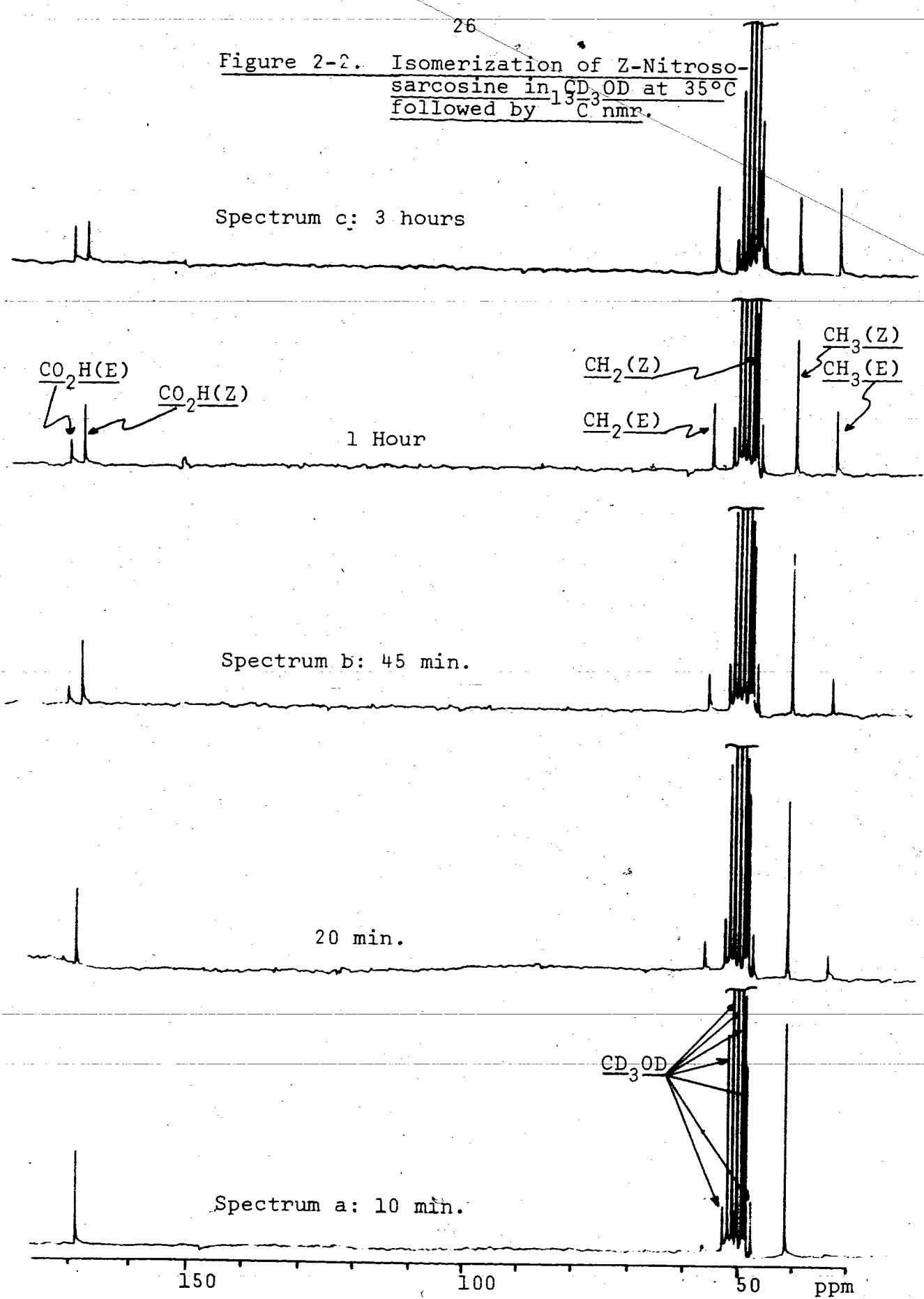
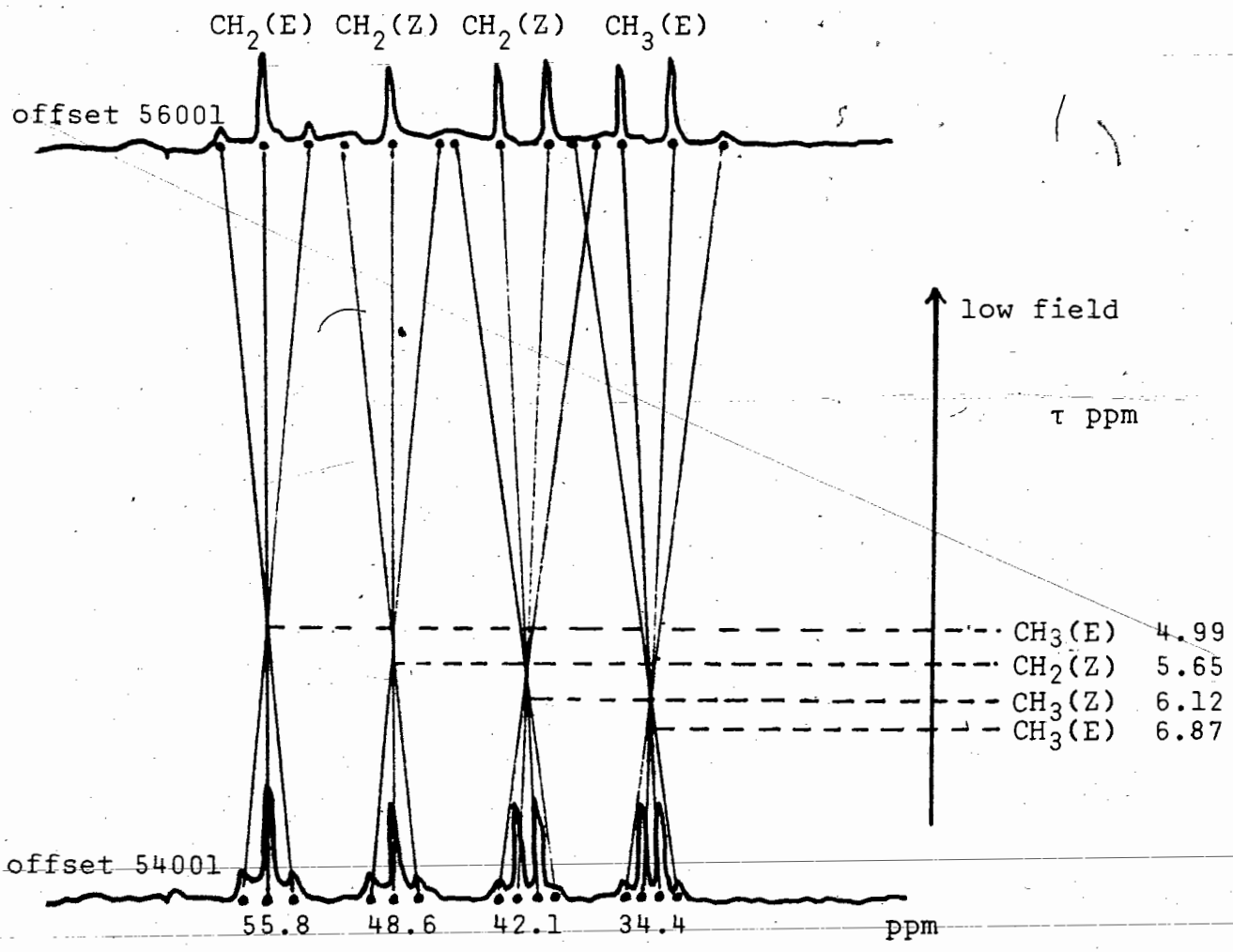


Figure 2-3. Spectral Assignment of the ^{13}C nmr Spectrum of Nitrososarcosine, II-1-a, by Selective Decoupling Experiments.



(Table 2-2) were made by utilizing off resonance decoupling (ord) splitting patterns and by comparing the chemical shifts of some carbons with the corresponding ones in II-1-a. The spectral assignments of the cyclic derivatives (Table 2-3) were made by using ord data and the correlation diagram of Figure 2-1. The diagram was constructed by comparing the chemical shifts of the different carbons of nitroso piperidine (52-54) and nitrosopyrrolidine (54) with those of the corresponding carbons in the nitrosamino acids. All carbons bearing the carboxyl group are indicated by an arrow and consistently show a shift of 10-14 ppm downfield from the corresponding carbon in the parent nitrosopiperidine or nitrosopyrrolidine.

Similarly to the α-carbons in dialkylnitrosamines, the corresponding carbons in nitrosamino acids experience the effect of the nitroso group. Thus, a carbon α to the nitrosamino group resonates at a higher field when it is syn to the nitroso group than when it is anti. The large difference in shieldings of the syn and anti carbons of nitrosamines has been attributed to a steric compression effect (53) rather than to an electric field effect (51,52). Although recent theoretical calculations on ketoximes (56) did not completely explain the experimental values, similar steric effects are currently accepted as the cause of related differential shieldings in amides (57,58) and oximes (56-60). The syn-anti

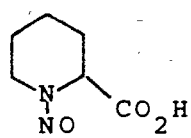
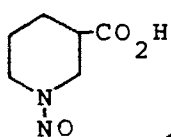
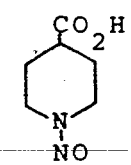
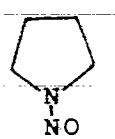
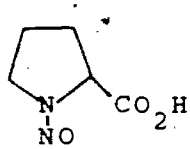
differential shielding of the corresponding α-carbon in E and Z-isomers (Δ^{α}_{SA}) is defined as follows:

$$\Delta^{\alpha}_{SA} = \delta_{\underline{syn}} \text{ (of one isomer)} - \delta_{\underline{anti}} \text{ (of the other)}$$

Thus, nitrososarcosine (II-1-a) displays two Δ^{α}_{SA} values: one for the methylene carbon [Δ^{α}_{SA} (CH_2) = 7.2 ppm] and one for the methyl carbon [Δ^{α}_{SA} (CH_3) = 6.8 ppm]. Open-chain derivatives show an averaged Δ^{α}_{SA} ~6.5 ppm (see Table 2-6) substantially smaller than that observed in the six-membered ring nitrosamino acids (Δ^{α}_{SA} ~10.6 ppm) but larger than that of five-membered ring derivatives (Δ^{α}_{SA} ~3.9 ppm). This trend was also observed in dialkylnitrosamines, where alicyclic derivatives exhibited an averaged Δ^{α}_{SA} of 8.2 ppm (51,54), six-membered ring derivatives showed a Δ^{α}_{SA} average of 11.2 ppm (52-54) and five-membered ring compounds an average of 4.6 ppm (51,54). Similar observations have also been made for oximes (60) for which Δ^{α}_{SA} was shown to correlate satisfactorily with the dihedral angle formed by the C=N and the α C-H bonds. The differential shielding of nitrosamino groups has been proposed to simply be another example of a γ-effect (53).

The β-carbons relative to the nitrosamino group also experience the anisotropic effects of the nitroso moiety but to a lesser extent than the α-carbons (see Δ^{β}_{SA} in Table 2-6).

Table 2-6. Syn-Anti Differential ^{13}C Shieldings for α and β-Carbons in Nitrosamine Derivatives

Compound	Δ^{α}_{SA}	Δ^{β}_{SA}	
		C-alkyl	C-CO_2H
$CH_3N(NO)CH_3$	7.8[b] 7.9[a]	--	--
$CH_3N(NO)CH_2CH_3$	7.5 (CH_3)[b] 8.9 (CH_2)[b]	3.1[d]	
$CH_3N(NO)CH_2CO_2H$	6.8 (CH_3) 7.2 (CH_2)	--	2.4
$CH_3CH_2N(NO)CH_2CH_3$	8.6[a)b)]	3.0[a]	--
$CH_3CH_2N(NO)CH_2CO_2H$	6.3 (C_2H_5) 5.0 (CH_2)	2.9	2.8
	11.8[c] 11.0[f] 11.2[b]	1.7[c] 3.5[e]	--
	10.6 (C_2) 10.4 (C_6)	2.5 (C_3) 2.5 (C_5)	2.0
	10.9 (C_2) 10.8 (C_6)	1.3 (C_3) 1.6 (C_5)	0
	10.7	1.7	
	4.6[a)b)]	3.5[a]	
	3.9 (C_2) 3.9 (C_5)	1.9 (C_3) 1.0 (C_4)	2.1

a) Ref. 51; b) Ref. 54; c) Ref. 50; d) Ref. 61; f) Ref. 53
e) calculated for the methyl group (60)

The carboxylic carbons of the derivatives of α-amino acids also occupying the β-position, exhibit a relatively large Δ^{β}_{SA} (2.0 ÷ 2.8 ppm). However, due to complex electrostatic interactions, it is difficult to attribute the Δ^{β}_{SA} of carboxylic carbons to a steric compression rather than to an electric field effect.

^{15}N nmr: The ^{15}N chemical shifts of both nitrogen atoms of II-1-a were measured as shown in Table 2-7. The chemical shift of the amino-nitrogen could be recorded at the natural abundance level, whereas only a ^{15}N enriched sample could give a signal for the nitroso-nitrogen. The chemical shifts recorded were of the same order of magnitude as those reported for nitrosamines (54,63,64). Two lines of equal intensity was observed for each nitrogen due to the E and Z-isomers*. The syn-anti differential shielding of the amino-nitrogen was at least three times smaller than that of the nitroso-nitrogen. The assignment reported in Table 2-7 was made possible by i) the recent study of Gouesnard and Martin (54), who showed that, during protonation of nitrosamines, the ^{15}N chemical shift of the amino group moves downfield and that of the nitroso group upfield as the proton concentration increases and ii) by assuming that the Z-isomer is stabilized by an intramolecular hydrogen bond which is not likely to exist in the E-isomer (vide infra).

*The sample contained an equimolar isomeric mixture

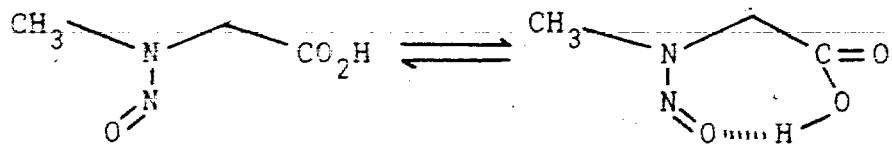
Table 2-7. ^{15}N Chemical Shifts of N-Nitrososarcosine (II-1-a)

Scale	$>\underline{N}-{}^{15}N\Theta$		$>N-{}^{15}\underline{N}O$	
	Z	E	Z	E
frequency scale ($+NMe_4$)[a]	+198	+196	492	499
σ scale (NO_2Me)	+136	+138	-158	-165

a) Calculated from $Me_4N^{\oplus}$ = 334 ppm upfield from $MeNO_2$

The ^{15}N chemical shifts for the E-Z isomerism in nitroso derivatives of N-alkylamines have never been reported. For example, Gouesnard and Martin (54) have measured the ^{15}N nmr spectra of a series of asymmetrically substituted dialkylnitrosamines and reported one line for each nitroso and amino nitrogen atoms. It was concluded that the steric compression effect experienced in ^{13}C nmr does not directly affect the ^{15}N-chemical shifts of the nitrosamino group. As E-Z isomers are configurational isomers one would have expected two sets of two lines for the Z and E-isomers as observed in ^{13}C nmr. Our results show that there is a marked difference in the

^{15}N chemical shifts of not only the nitroso but also the amino nitrogen atoms in II-1-a. Since it has been shown that the chemical shifts of the amino and nitroso nitrogens of nitrosamines undergo drastic variations upon protonation of the nitroso group (54), the difference in ^{15}N shifts observed in II-1-a may arise from hydrogen-bonding as shown below. The relatively large Δ^{β}_{SA} experienced by the carboxylic carbon in ^{13}C nmr may also be accounted for by the same effect. Although hydrogen-bonding through a seven-membered ring is thermodynamically less favoured than that through a six-membered ring (65), it has been shown (66) that the oxygen atom is the most basic atom and that protonation of oxygen may be expected to be the dominant mechanism (66).

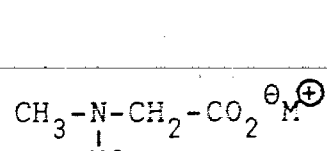


E Z

II-2 Preparation and Properties of Nitrososarcosine Salts

A series of salts derived from II-1 were prepared and their properties studied. The sodium and potassium salts (II-14-b and II-14-c) were prepared by the reaction of the free acid II-1 with NaOH or KOH in MeOH. The lithium salt (II-14-a)

was obtained from the reaction of II-1 with LiOH in water and the dicyclohexylamine salt (II-14-d) according to the reported procedure (67). The purity of the new salts (II-14-a, II-14-b and II-14-c) was ascertained by elemental analysis.



	M = Li	II-14-a
	M = Na	II-14-b
	M = K	II-14-c
II-14	M = $NH_2(C_6H_{11})_2$	II-14-d

The ir spectrum of each derivative exhibited the two characteristic bands for carboxylate groups at 1640-1600 cm^{-1} and ~1340 cm^{-1} and for the N=O stretching absorption at ca. 1440 cm^{-1}. The ^1H and ^{13}C nmr spectra of all derivatives showed two sets of lines which were attributed to the two isomeric forms E and Z. The ^1H chemical shifts of the methyl group of both isomers and that of the methylene group of the Z-isomer were identical to those of the free acid within experimental error, whereas the methylene protons of the E-isomer consistently exhibited a 0.2 ppm upfield shift. The ^{13}C chemical shifts for the same isomer (E or Z) of all salts did not vary significantly. As expected, the methyl carbons, being screened by the nitrosamino group, did not experience the deshielding effect due to the ionization of the carboxylic group. However, the methylene carbons showed approximately a 2.7 ppm downfield shift in comparison to the corresponding carboxylic acid similar to that observed in the α

Table 2-8. Spectral Characteristics of Nitrososarcosine Salts

Compound	% Solid State	^{13}C nmr a)			^{1}H nmr b)		uv c)	ir e)	pH d) (pKa)
		CH_3	CH_2	CO_2H	CH_3	CH_2	λ_{max} (ε) nm	cm^{-1}	in H_2O
II-1-a E	0	34.65	56.23	172.90	6.10	5.72	341 (84)	1730	
II-1-a Z	100	41.55	49.37	170.45	6.87	5.02		1440	
II-14-a E	5	35.01	58.88	175.64	6.23	5.83	338 (88)	1620 1350	5.66
II-14-a Z	95	41.97	52.12	173.35	6.77	5.25		1440	(10.3)
II-14-b E	95	35.00	58.81	175.52	6.17	5.78	337 (91)	1600 1340	5.10
II-14-b Z	5	41.97	52.11	173.20	6.85	5.20		1430	(9.2)
II-14-c E	43	34.86	58.76	175.55	6.17	5.78	336 (84)	1600 1350	4.49
II-14-c Z	57	41.86	52.01	173.21	6.85	5.20		1440	(8.0)
II-14-d E	90	34.80	58.7	175.1	6.15	5.78	337.5 (69)	1640 1340	7.85
II-14-d Z	10	41.50	51.7	172.7	6.75	5.21		1450	

a) taken in D_2O with internal TMS capillary b) taken in D_2O, reported in τ values from TMS c) taken in H_2O d) 0.1 Molar aqueous solutions at 25°C. e) in nujol

carbon of aliphatic carboxylates (68). The carboxylate carbon also showed a downfield shift of approximately 2.8 ppm, which is slightly smaller than the 4.7 ppm shift observed by the aliphatic carboxylic acids upon ionization (68).

The uv spectra of these compounds all exhibited a weak absorption (ε ~90) in the 340 nm region corresponding to the $n\rightarrow\pi^*$ transition. The metallic salts exhibited an increasing bathochromic shift of the $n\rightarrow\pi^*$ band as the atomic number of the metal decreased.

The isomeric composition of the crystalline salts was determined by taking ^1H nmr spectra immediately after dissolution in water. The potassium salt II-14-c consisted of a mixture of the Z and E isomers in a 1:0.75 ratio, whereas the sodium and lithium salts (II-14-b and II-14-a) were found to contain over 95% of E and Z isomers, respectively. However, all derivatives slowly isomerized in water at room temperature to give a 1:1 ratio of the two isomers*. The lithium and sodium salts II-14-a and II-14-b isomerized with approximately the same rate (see Fig. 2-4) but much slower than the free acid II-1-a. For example, complete isomerization of Z-nitroso-

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* This observation is contradictory to Lijinsky's report (48) which claims an excess of the E-isomer for the sodium salt equilibrium.

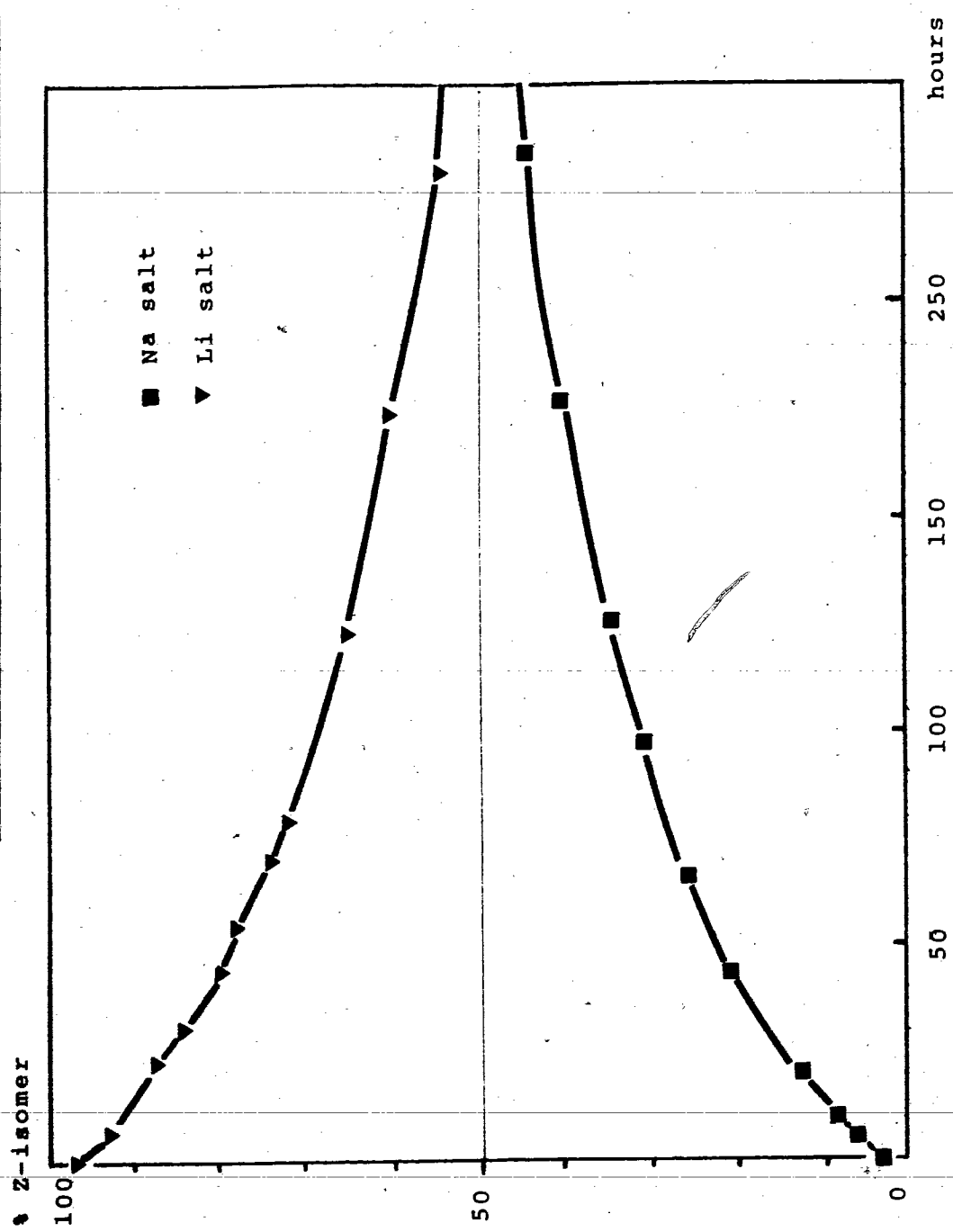


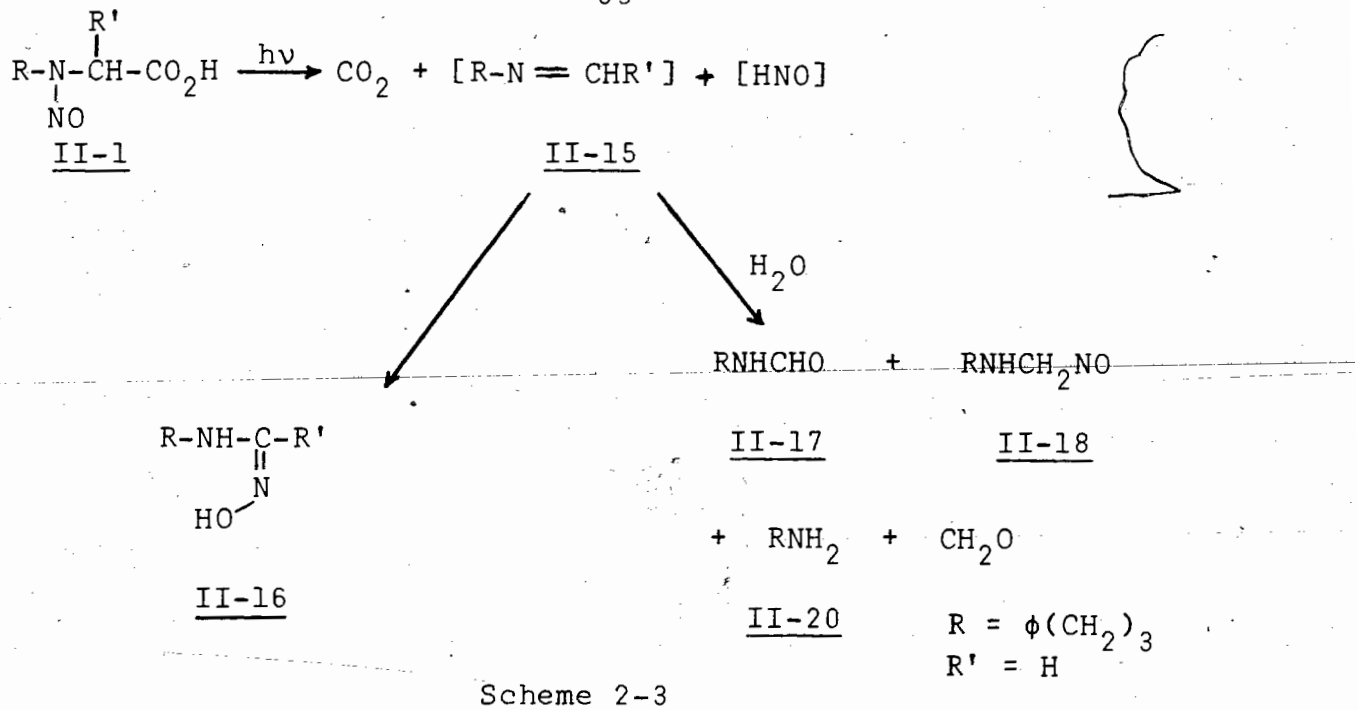
Figure 2-4. Isomerization of Lithium and Sodium Salts of Nitrososarcosine in Water at Room Temperature.

sarcosine in water took about 3 hours at room temperature, whereas only 2% of the crystalline isomer of a metallic salt was isomerized during the same period. The crystalline form of the dicyclohexylamine salt II-14-d was shown to contain 10% of Z-isomer and to isomerize in water at room temperature to a mixture containing 15% of Z-isomer.

The pH of a 0.1 M solution of each salt in water was measured and is reported in Table 2-8. As expected, the pH of the dicyclohexylamine salt was slightly basic (pH = 7.85). However, surprisingly the pH of the solutions of the metallic salts were found slightly acidic, with the pH value decreasing as the atomic number of the metal increased.

II-3 Photolysis of N-Alkyl-N-Nitroso-α-Amino Acids

The photolysis of nitroso derivatives of acyclic N-alkyl-α-amino acids in various solvents were investigated. The photoreactions carried out at room temperature and under nitrogen showed a zero order decrease of the 350 nm band and a rapid evolution of CO_2. In general, the photolysate was evaporated to give high yields of N-alkylformamidoxime II-16 (see Table 2-9) and in some cases (II-1-b and II-1-d) a trace of the parent α-amino acid was also detected.



Scheme 2-3

Table 2-9. Percentage Yields of Amidoximes: Photoproducts of N-Alkyl-N-Nitroso-α-Amino Acids

Starting Nitrosaminoacid II-1		Yield of Amidoxime II-16	Solvent
a	R = CH_3 R' = H	72	MeOH
b	R = C_2H_5 R' = H	69	MeOH
c	R = iso-C_3H_7 R' = H	76	MeOH
d	R = $(CH_2)_3\phi$ R' = H	82	MeOH
g	R,R' = $(CH_2)_3$	68 a) 78 b)	ether H_2O, HCl
h	R,R' = $(CH_2)_4$	76 a) 83 a) 48 a)b)	ether H_2O, AcOH c) H_2O, HCl

a) isolated as hydrochloride salt b) a small amount of a hygroscopic material was observed c) the photolysate was boiled with HCl

The molecular formulae of amidoximes were ascertained by elemental analysis and by high resolution mass spectroscopy (hrms). These compounds exhibited characteristic absorption bands at ca. 1680 cm^{-1} for the C=N group, at ca. 900 cm^{-1} for the N-O stretching and at 3250-2750 cm^{-1} for the hydroxyl group. The ^{1}H nmr spectra of the formamidoximes II-16-a to II-16-d each exhibited a singlet at τ 2.9-3.4 (see Table 2-10) for the vinylic proton, arising from a single isomer of the E-Z aldoximes. As the chemical shift of the vinylic proton was comparable to that of the trans vinylic proton of Z-aldoximes at τ 3.2 (69,70) the Z-configuration was assigned to the formamidoximes. The presence of a strong hydrogen bond stabilizing the Z-configuration was confirmed by the significant shift of the hydroxyl stretching towards the lower frequency region (3250-2750 cm^{-1}) compared to the free hydroxyl stretching frequency of oximes (3650-3500 cm^{-1}) (71,72).

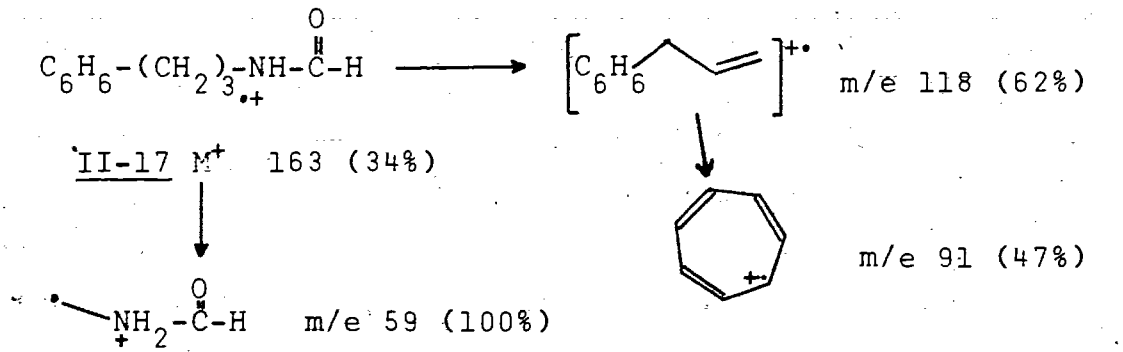
The photolysis of a suspension of N-nitroso-N-(3-phenyl) propylglycine (II-1-d) in water, under nitrogen at room temperature showed the emergence of new uv absorption bands at ν_{max} = 328, 505, 540 and 720 nm which increased steadily to a maximum and then decreased slowly on further irradiation (see Figure 2-5). During the photolysis, a blue colour typical of C-nitroso derivatives (73) was also observed and the change in its intensity paralleled that of the new uv bands.

Table 2-10. ^1H nmr and ir Data of Amidoximes II-16

Compound II-16	^1H nmr (τ)		ir (cm^{-1})			
	R	R'	C=N	N-O	NH	OH
a[1]	7.12	3.00	1680	920	3400	3100-2900
b[2]	6.89, 8.83	3.3	1680	940	3360	2800
c	6.48, 8.8	2.93	1675	900	3380	3250
d[2]	2.8, 6.94 7.34, 8.20	3.4	1680	900	3350	2750
g	7.52, 7.9	6.6	1648	940	3380	3060
h[2]	7.73, 8.2	6.8	1670	940	3380	3100

1) taken in D_2O 2) taken in $CDCl_3$

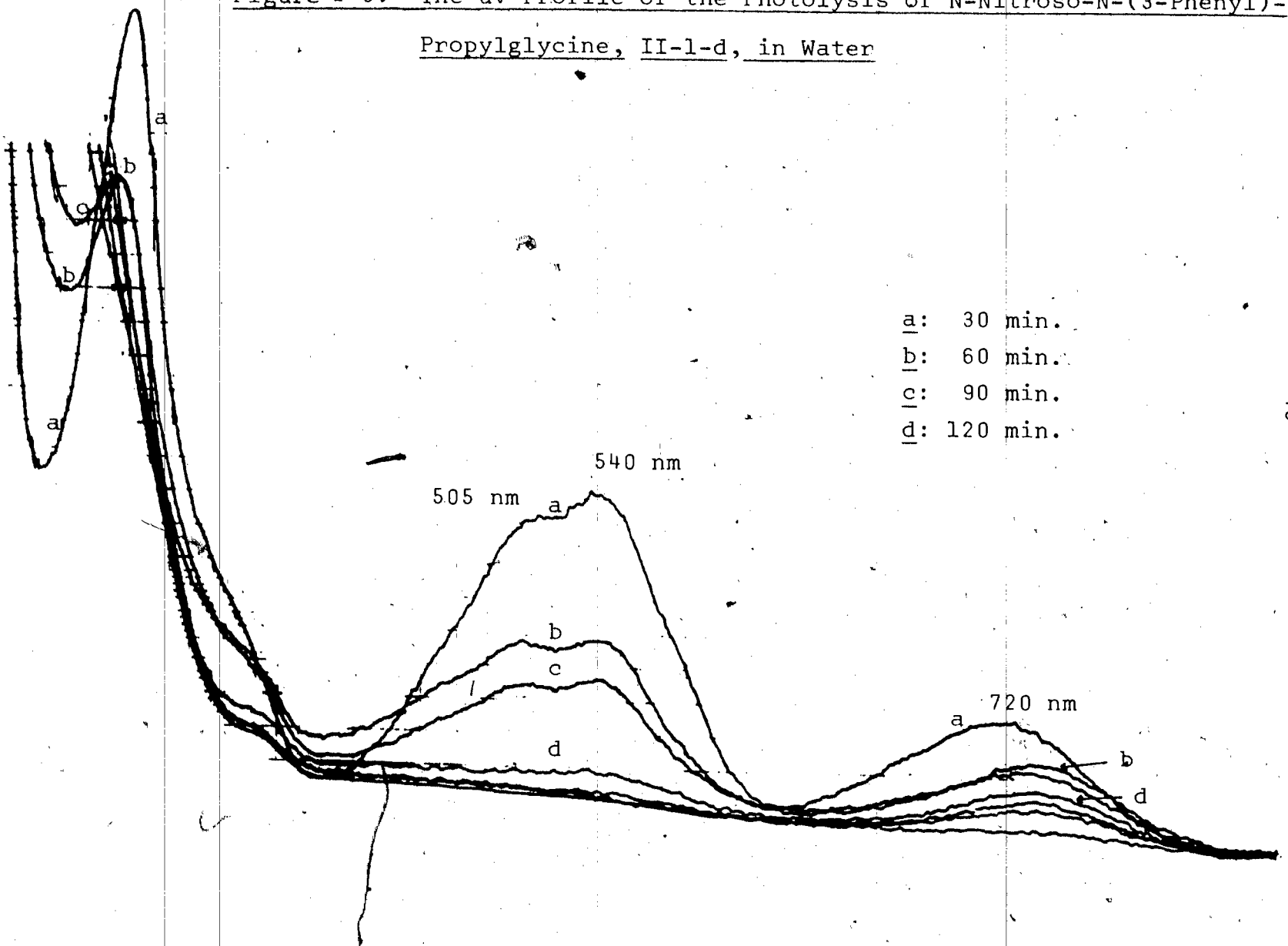
After completion of the photolysis, the solvent was distilled to give a small amount of formaldehyde which was detected as its 2,4-dinitrophenylhydrazone derivative. The fraction obtained from extraction of the concentrated photolysate afforded N-(3-phenyl)-propylformamidoxime (II-16-d) (5%) and a residue which exhibited a strong ir absorption at 1700 cm^{-1} (NHC=O). This fraction was shown by gc-ms to be a mixture of three major and two minor components. The mass spectrum of one of the major compounds (23%) showed an intense molecular ion peak at m/e 163 and a spectral pattern compatible with fragmentation of N-formyl-3-phenyl-propylamine (II-17) (Scheme 2-4). The assignment was further confirmed by gc mixed injection with an authentic sample.* The other two major



Scheme 2-4

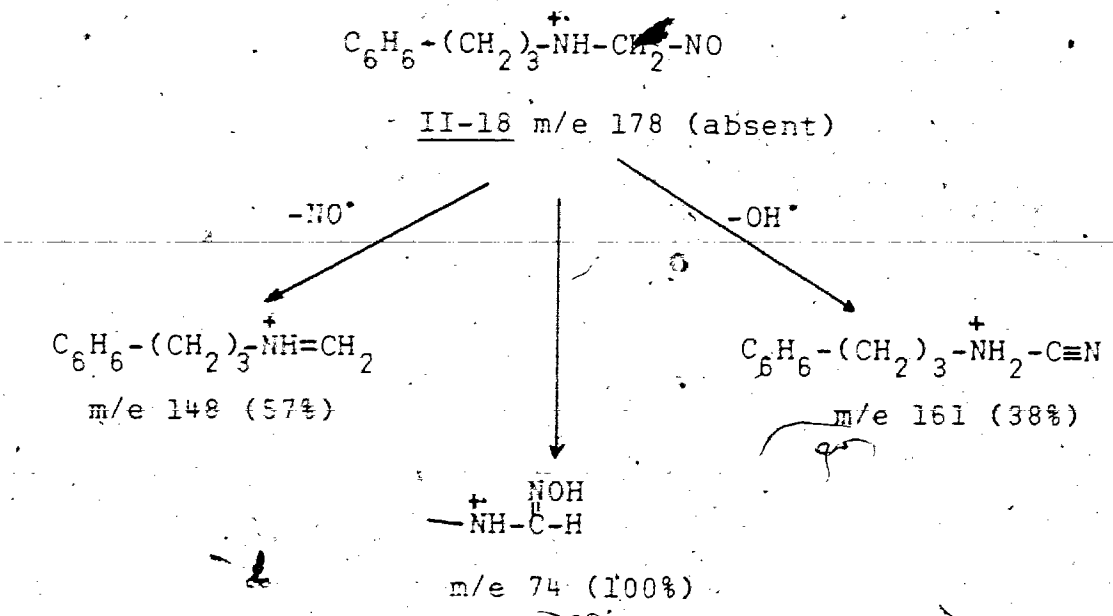
* An authentic sample of II-17 was synthesized by chloral formylation of 3-phenylpropylamine (II-20).

Figure 2-5. The uv Profile of the Photolysis of N-Nitroso-N-(3-Phenyl)-Propylglycine, II-1-d, in Water

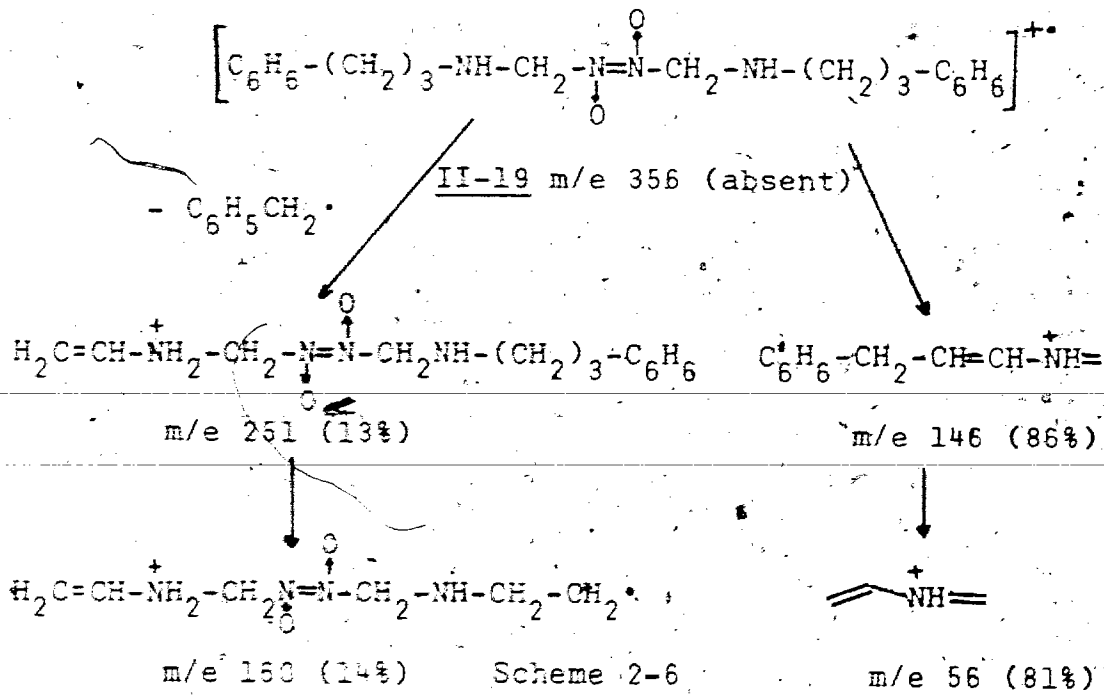


peaks (10% total) were barely separated on gc, but gave quite different mass spectra. The structure of N-(3-phenyl)-propylamino-1-nitrosomethane (II-18) was assigned to the less polar component on the basis of its ms pattern showing a peak at m/e 148 (57%) for the loss of NO (Scheme 2-5). The presence of high molecular weight fragments (m/e = 251) in the mass spectrum of the second peak suggested that II-18 existed as a dimer in the solid state (Scheme 2-6). It is very likely that dimer II-19 partially dissociated in the column to give the monomer II-18. Basic extraction of the mother liquor of the photolysate gave 3-phenylpropylamine II-20 (12%) and acidic extraction gave unreacted starting material II-1-d (8%). The structure of compounds II-20 and II-1-d was confirmed by comparison of their spectral data with those of authentic samples.

Photolysis of cyclic nitrosamino acids II-1-g and II-1-h also showed a zero-order decrease of the 350 nm band as well as a rapid CO_2 evolution. Irradiation of nitrosopipecolinic acid (II-1-h) in ether gave a hygroscopic precipitate which exhibited ir absorptions similar to those of 2-piperidonoxime (II-16-h). Work up of the photolysate gave II-16-h (76%) and another small amount of the same hygroscopic material. When the photolysate resulting from a photoreaction carried out in water and acetic acid was boiled with HCl, the hydrochloride of amidoxime II-16-h (37%) was isolated. Neutralization



Scheme 2-5



Scheme 2-6

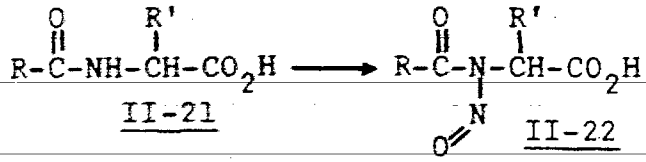
of the mother liquor gave amidoxime II-16-h (46%) but none of the amorphous solid could be detected. On the other hand, photolysis of II-1-h in water and dilute HCl, gave, besides II-1-h hydrochloride (48%), the same amorphous solid. The latter gave II-16-h hydrochloride (6%) upon treatment with concentrated HCl. It is believed that the amorphous solid which exhibits similar ir absorptions to II-16-h is the E-isomer of II-16-h and that it is converted to the Z-isomer upon HCl treatment or attempted recrystallization. The structure of both amidoxime II-16-h and its HCl salt were ascertained by spectral comparison with authentic samples (74).

Photolysis of nitrosoproline (II-1-g) in ether gave 2-pyrrolidonoxime (II-16-g) (78%) and a hygroscopic precipitate which turned to a resin upon exposure to air. In water containing diluted HCl, photolysis of II-1-g gave the hydrochloride of II-16-g (68%) and the same hygroscopic material (24%). The ^{1}H nmr of the latter exhibited the same signals as that of II-16-g namely two triplets at τ 6.33, 7.17 and a multiplet at τ 7.77. It is believed that, in analogy with the photolysis of II-1-h, the hygroscopic material corresponds to the E-isomer of the amidoxime II-16-g. The structures of amidoxime II-16-g was confirmed by spectral comparison with an authentic sample, and that of its salt by spectral and elemental analyses.

The photolysis of the sodium salt of nitrososarcosine (II-14-b) in methanol, also showed a zero-order decrease of the 350 nm band. During the photoreaction a new absorption at λ_{max} 305 nm appeared, probably due to a C-nitroso dimer and disappeared upon prolonged irradiation. The crude product was shown from ^{1}H nmr to be a complex mixture of products from which formamidoxime II-16-a (10%) was isolated.

II-4 Preparation and Properties of N-Nitroso-N-Acyl-α-Amino Acids

Acylated amino acids II-21 were nitrosated with dinitrogen tetraoxide (method B) or nitroxyl tetrafluoroborate (method C) to give the corresponding nitrosamides II-22. Due to the instability of the nitrosamido group (13,14) the nitrosamides were not purified further and were stored in a dry container at -20°C. These nitrosamides were yellow oils except II-22-a which was a low melting solid. The relevant spectral data are reported in Table 2-11.



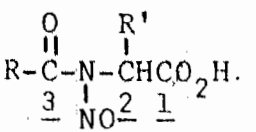
II-21 → II-22

a) R = CH_3 R' = $CH_2\phi$ c) R = CH_3 R' = $CH_2CH(CH_3)_2$

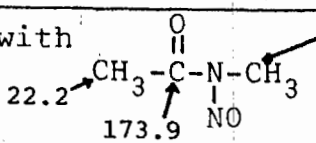
b) R = ϕ R' = $CH_2\phi$ d) R = CH_3 R' = CH_2OCOCH_3

Scheme 2-7

Table 2-11. Spectral Data of N-Acyl-N-Nitroso-α-Amino Acids

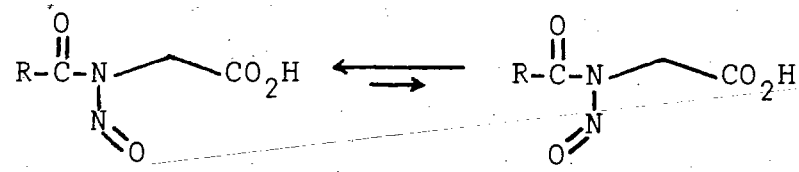


	ir cm^{-1}	^1H nmr τ/TMS			^{13}C nmr δ/TMS					uv (ε) λ_{max} nm [c]
		$C_2\underline{H}$	R	R'	C_1	C_2	C_3	R	R'	
II-22-a	1715	4.40	7.40	2.7	172.6	52.0	173.5[a]	22.1	33.3	405 (41)
	1490			6.56					126.9, 128.3	423 (40)
				6.95					128.6, 135.4	
II-22-b	1720	4.20	2.70	2.7	171.6	52.4	171.9	127.6	32.8	
				6.47	or			129.9	126.8, 128.3	
				6.74	171.9		171.6	131.9	128.5, 135.4	
								132.2		
II-22-c	1725	4.67	7.18	7.9-8.17	172.8	49.7	173.7	22.4[b]	21.4, 22.5[b]	405 (~50)
				9.18					25.0, 36.3	420 (~45)
II-22-d	1740	4.52	7.28	5.41	170.3	49.4	173.4	22.2	20.3	405 (~65)
	1380			5.88					59.9	481 (~50)
	1220			8.12					168.7	

a) this signal was assigned to C_3 by comparison with 

b) these two chemical shifts could be exchanged c) in methanol

Nitrosamides, analogous to nitrosamines, may also exhibit the Z-E isomerism. However, dipole-dipole repulsion between the carbonyl and the nitroso groups is expected to favour the E-configuration provided the R group is not so bulky as to offset the effect by a non-bonded interaction. This has been confirmed by Chow (33) who studied the conformational equilibrium of nitrosamides by nmr spectroscopy. Thus, the nmr spectra of nitrosamides derived from primary carbinylamines exhibited only one signal for the syn-N-methylene protons. The ^{1}H and ^{13}C nmr spectra of nitroso derivatives of N-acyl-α-amino acids showed only one set of lines due to one isomer. In order to determine the nature of this isomer, the ^{13}C nmr spectra of N-acetyl derivatives II-22-a, II-22-c and II-22-d were compared to that of N-methyl-N-nitrosoacetamide (see Table 2-11) for which the nitroso group is known to be anti to the carbonyl groups (33). The ^{13}C chemical shifts of the carbonyl and methyl carbons of the N-acetyl groups were all identical, within experimental errors. This led us to believe that nitrosamides derived from amino acids also occupy the E-configuration.



"E" "Z"

Nitrosamides derived from amino acids exhibit a uv absorption centered at λ_{max} ~400 nm corresponding to the $n \rightarrow \pi^*$ transition of the nitrosamido group (see Table 2-11). However, when the spectrum was taken immediately after addition of an excess amount of a strong base, this absorption was replaced by a set of two absorptions at λ_{max}=335 and 410 nm. The rate of disappearance of the former absorption and the appearance of the latter were shown to be dependent upon the ratio of the concentration of the base to that of the nitroso derivative. Fig. 2-6 and Fig. 2-7 show the uv spectra of methanolic solutions of II-22-a containing different concentrations of KOH. The concentrations and molar ratios are listed in Table 2-12. For those samples containing less than one mole equivalent of potassium hydroxide (samples 1-5 and 11-13), a partial decrease of uv absorption at λ_{max} = 400 nm was observed. Samples of equimolar composition (samples 6 and 14) showed the appearance of a new absorption at λ_{max} = 335 nm and for those containing an excess of potassium hydroxide (samples 7-10 and 15-16) the 400 nm absorptions were replaced by two new absorptions at λ_{max}=335 and 410 nm. A residual nitrosamide

Table 2-12. Experimental Conditions for the Decompositions Shown in Fig. 2-6 and 2.7

	Sample #	[II-22-a] X 10^3 mole/l	[OH^-] X 10^3 mole/l	$\frac{[OH^-]}{[II\text{-}22\text{-}a]}$
Fig. 2-6	1	1.0	0	0
	2	0.9	0.1	0.11
	3	0.8	0.2	0.25
	4	0.7	0.3	0.43
	5	0.6	0.4	0.67
	6	0.5	0.5	1.00
	7	0.4	0.6	1.50
	8	0.3	0.7	2.33
	9	0.2	0.8	4.00
	10	0.1	0.9	9.00
Fig. 2-7	11	12.0	0	0
	12	12.0	2.4	0.2
	13	12.0	6.0	0.5
	14	12.0	12.0	1.0
	15	12.0	24.0	2.0
	16	12.0	60.0	5.0

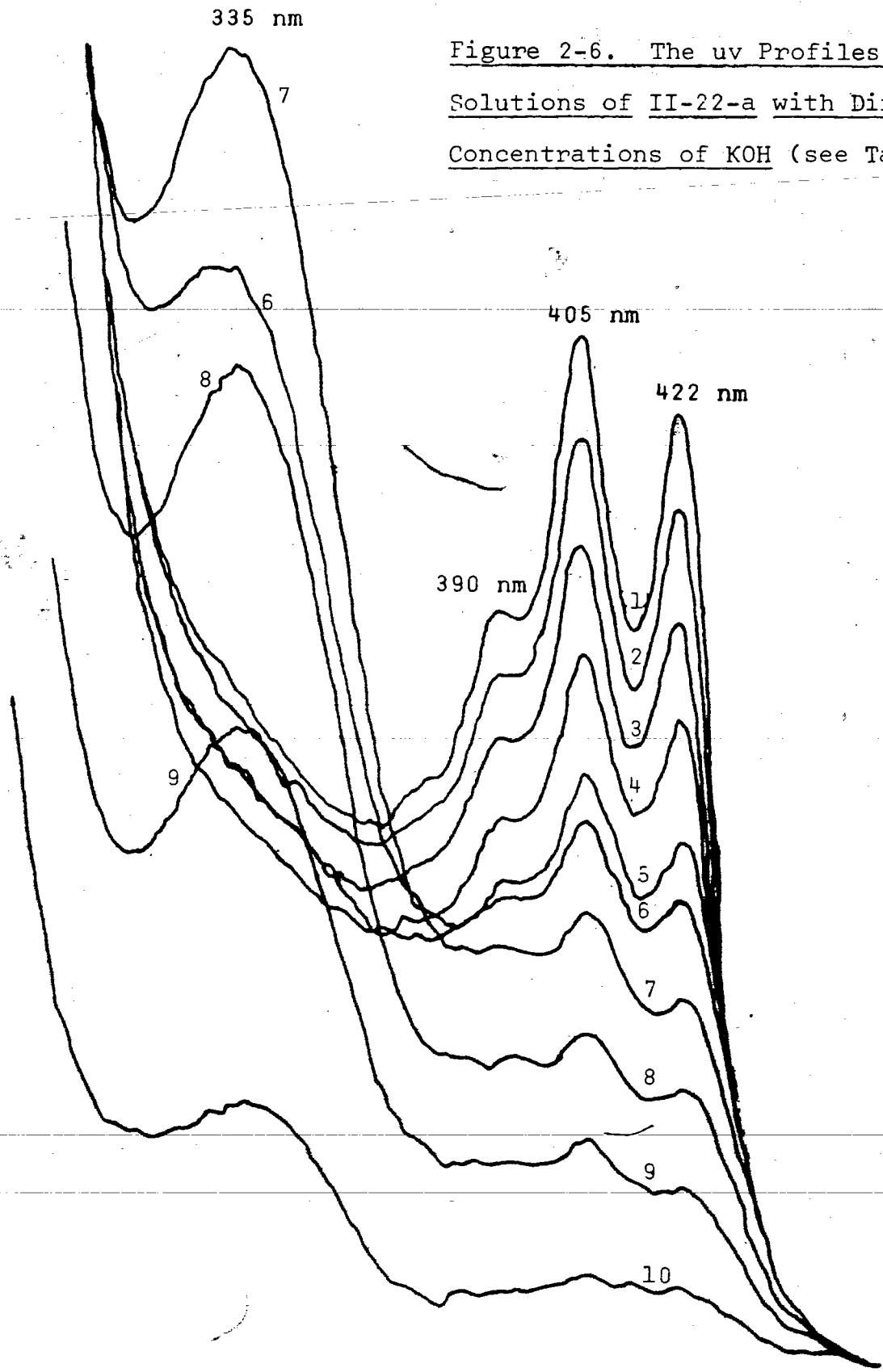


Figure 2-6. The uv Profiles of Methanolic Solutions of II-22-a with Different Concentrations of KOH (see Table 2-12).

Figure 2-7. The uv Profiles of Methanolic Solutions of II-22-a with Different Equivalents of KOH (see Table 2-12)

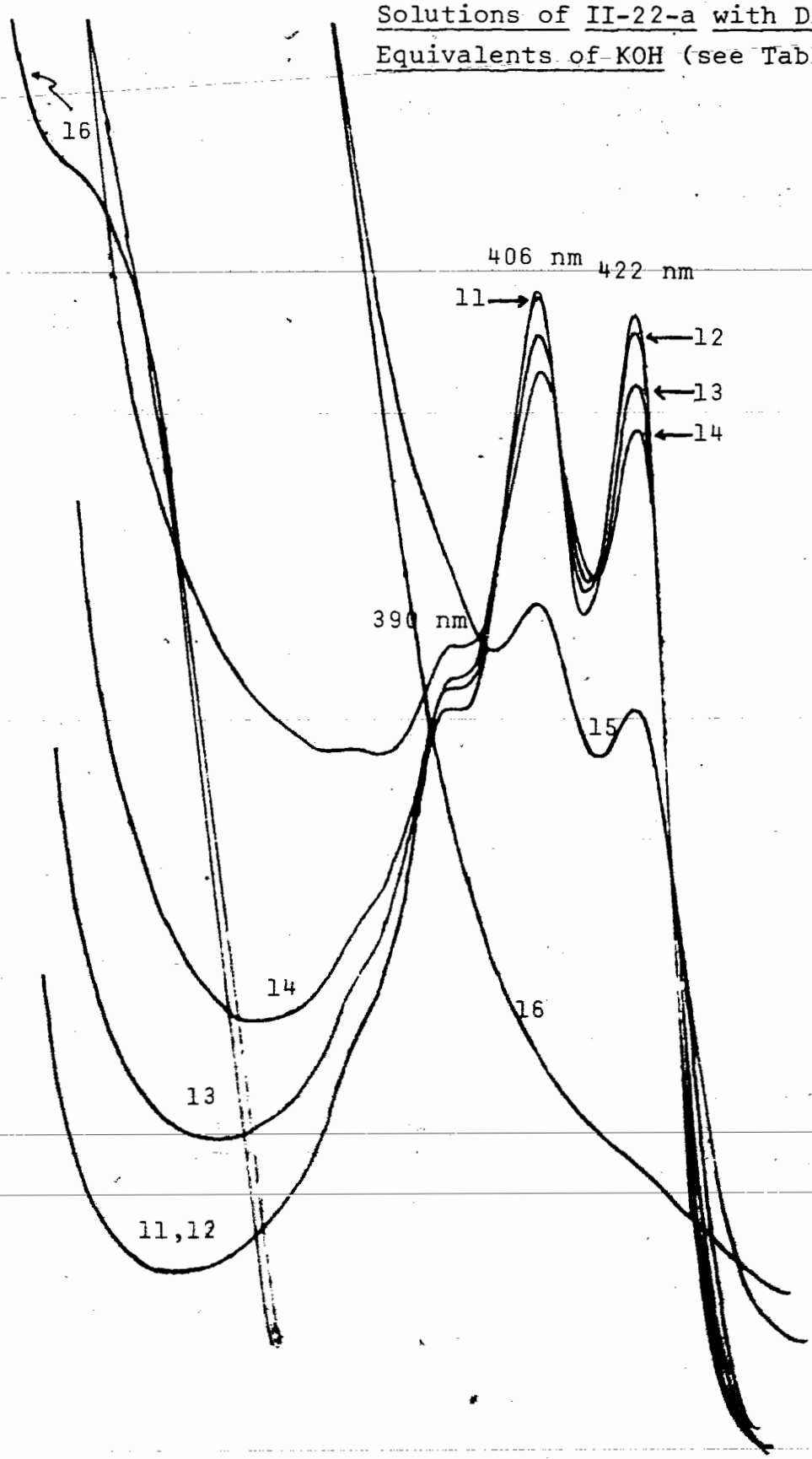
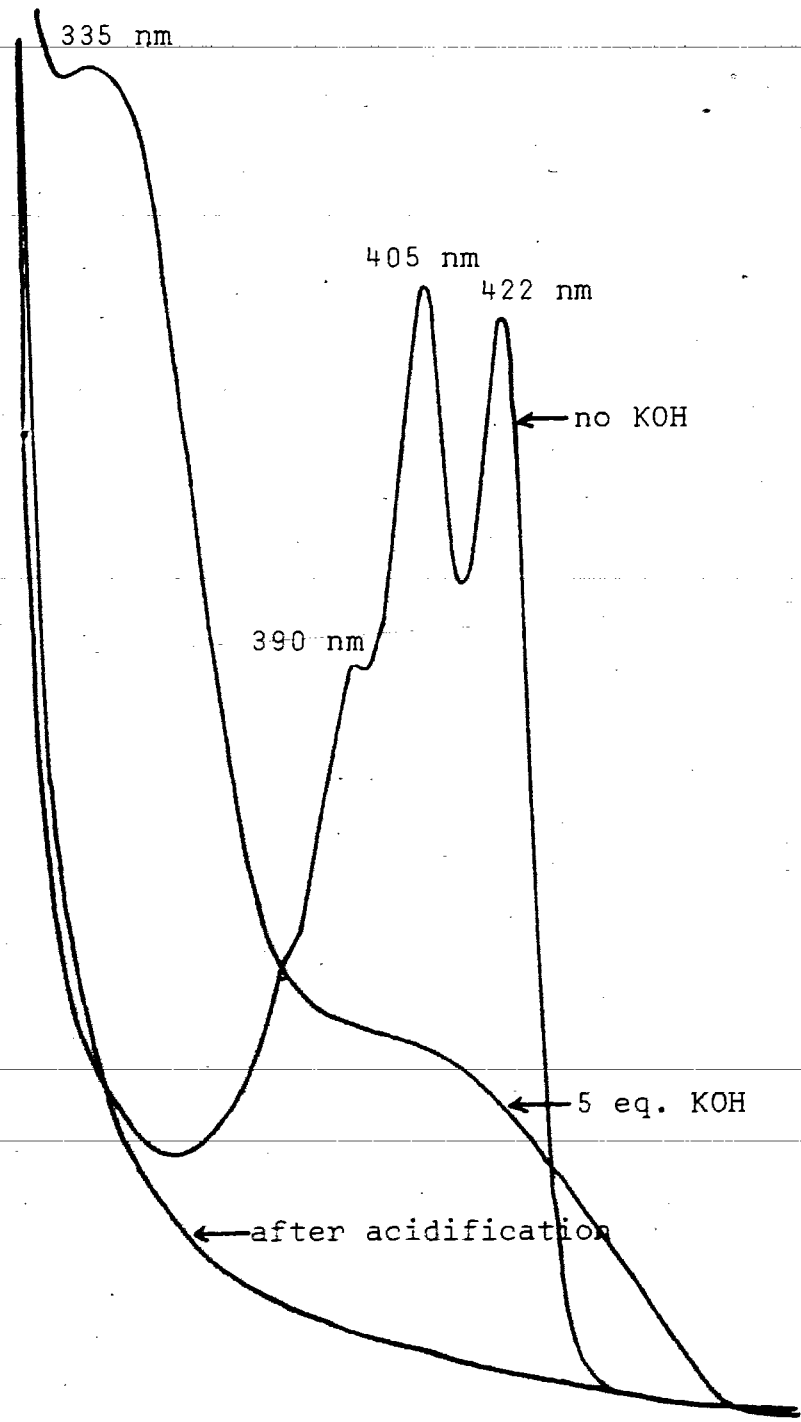


Figure 2-8. Decomposition of II-22-a in the Presence of 5 Mole Equivalents of KOH at Room Temperature.



absorption (λ_{max} = 400 nm) was still visible when two equivalents of KOH were added (sample 15) but the transformation was practically instantaneous in the presence of 5 equivalents of base as shown in Fig. 2-8. In all cases, the absorption at 410 nm was much weaker than that centered at 335 nm. However, the uv spectrum of a benzene solution of nitroso II-22-a did not show any change after addition of up to 6 mole equivalents of triethylamine.*

II-5 Decomposition of Nitrosamido Acids Under Basic Conditions

II-5-1 Kinetic Study

Although nitrosamides are known to be thermally labile compounds, a methanol or benzene solution of nitrosoamide II-22-a could be kept at room temperature, in the dark for a period of hours, and nearly indefinitely at 0°C, without any decomposition However, in benzene and in the presence of 1,2,3 or 5 mole equivalents of triethylamine, II-2-a decomposed slowly at room temperature, as indicated by the decrease of the nitrosamide absorption at 400 nm (see Fig. 2-9). The disappearance of the absorption followed first order kinetics. In all cases, the

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* The same absorption at λ_{max} 330 nm was once observed after addition of triethylamine to an aged sample of II-22-a, in benzene

spectra showed a residual absorption at ~400 nm the absorbance of which never exceeded 20% of that of the zero hour curve. The rate constants of each reaction were calculated by plotting the logarithm of the absorbance at 400 nm as a function of time. The results of Table 2-13 show that an increasing amount of triethylamine increases the rates of the decomposition only slightly but that the rate constant of the reaction drastically increases when the temperature is raised.

Table 2-13. Rate Constants for the Decomposition of II-22-a in Benzene in the Presence of Triethylamine and KOH

mole equivalents of base	T °C	k (X $10^5 s^{-1}$)	τ (hour)
1 $(Et)_3N$	22	2.5	7.8
2	22	3.2	6.1
5	22	3.6	5.3
3	30	8.3	2.3
1	80	22.8	0.8
1 KOH	22°	9.7	1.9
	40°C	82.2	0.2
0	80	23.0	0.8

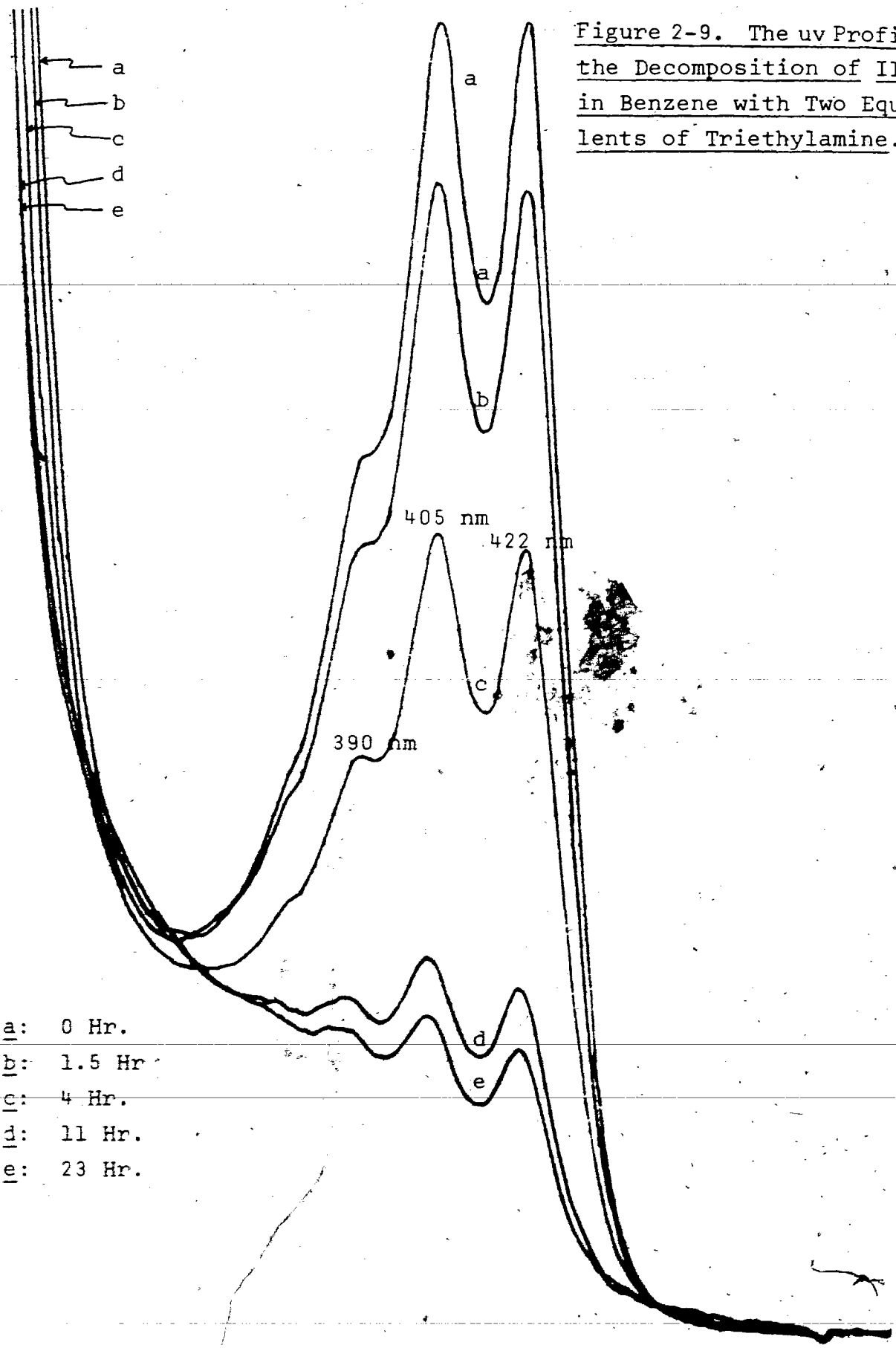
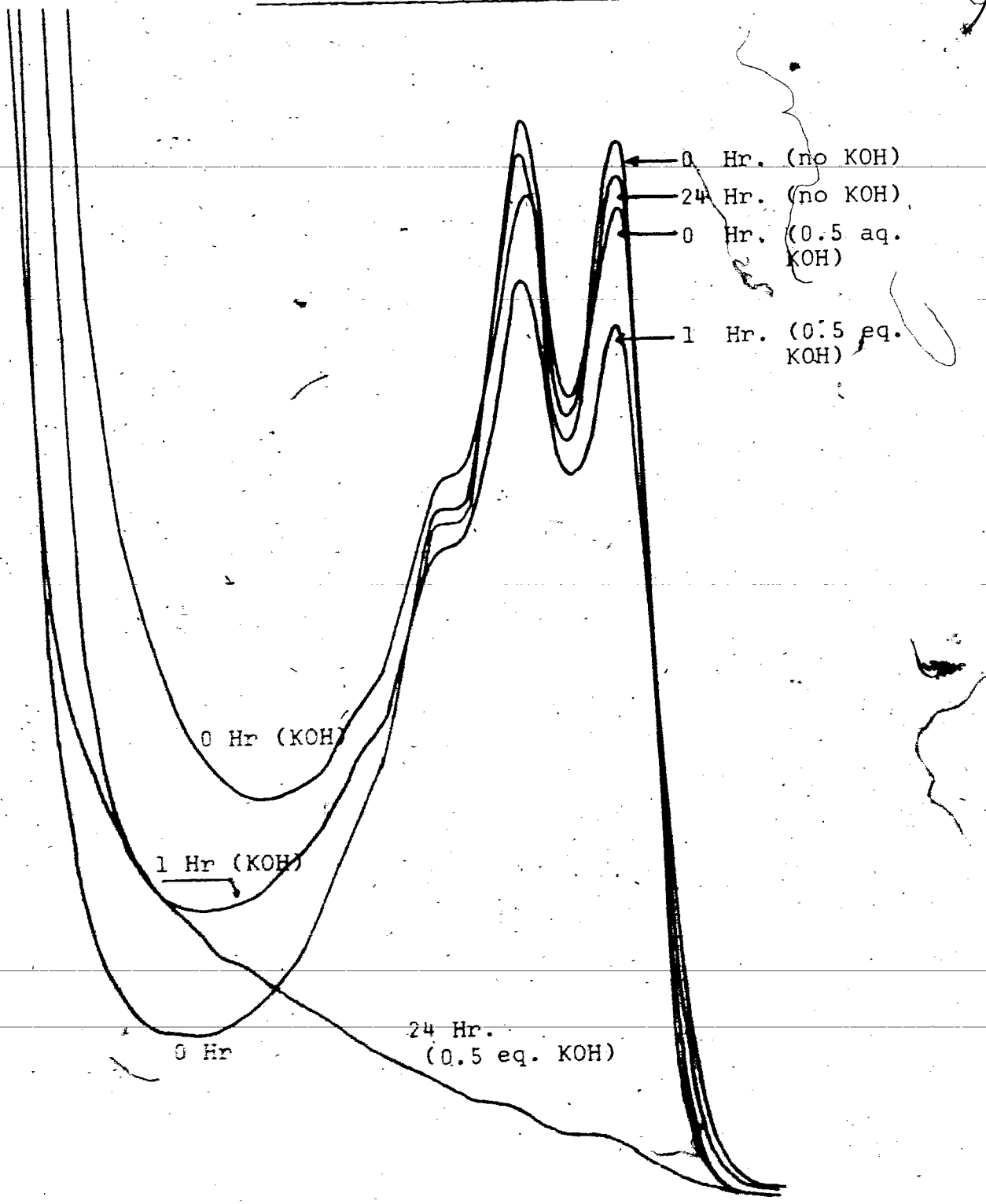


Figure 2-9. The uv Profile of the Decomposition of II-22-a in Benzene with Two Equivalents of Triethylamine.

a: 0 Hr.
b: 1.5 Hr
c: 4 Hr.
d: 11 Hr.
e: 23 Hr.

Figure 2-10. Decomposition of II-22-a in the Presence of 0.5 Mole Equivalent of KOH in Methanol at Room Temperature.



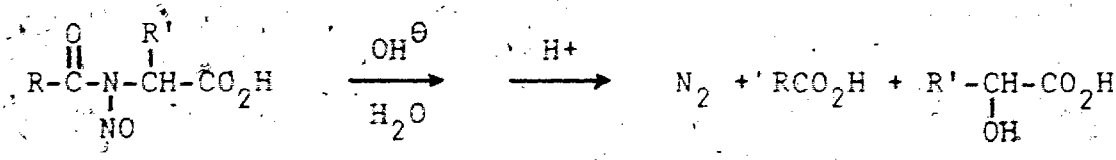
In methanol and in the presence of up to one equivalent of potassium hydroxide nitrosamide II-22-a also underwent slow decomposition. The decomposition of solutions containing 0.2, 0.5 and 1 mole equivalent of potassium hydroxide was monitored by following the decrease of the 400 nm absorption. U.v. profiles similar to that shown on Fig. 2-10 (0.5 equivalent of KOH) were obtained for samples containing 0.2 and 1 equivalent of base. The decomposition was faster as the concentration of KOH was increased. A control sample containing no KOH, kept under the same conditions (at room temperature in the dark), hardly showed any decomposition after one day. This demonstrated that the decomposition was indeed markedly accelerated under basic conditions. The effects of temperature were shown by the complete decomposition of a sample containing one equivalent of potassium hydroxide after one hour at 40°C. In contrast, the same sample decomposed to the extent of 30% at room temperature and none at all at 0°C (Fig. 2-12). The rate constants of the reaction were calculated and are reported in Table 2-13.

The decomposition of II-22-a in the presence of 2 and more equivalents of KOH was nearly instantaneous and the nitrosamide absorption at 400 nm was replaced by a set of two new absorptions at 330 and 410 nm. The species showing these absorptions was quite stable since the new absorptions persisted for few

days. However, they disappeared instantaneously upon acidification of the solution (Fig. 2-8).

II-5-2 Product Analysis of the Basic Decomposition of Nitrosamido Acids

In protic solvents with an excess of a strong base: When a suspension of nitrosamide II-22 in water at 0°C, was treated with an excess of potassium hydroxide, a strong evolution of gas indicated a rapid decomposition. The reaction was over in few minutes and upon acidification more gas was evolved. The nmr spectra of the acidic fractions exhibited the characteristic signals of hydroxy acids II-23 as only product.



II-22 II-23

$R = CH_3$	$R' = CH_2\phi$	a	(82%)
$R = \phi$	$R' = CH_2\phi$	b	(99%)
$R = CH_3$	$R' = CH_2CH(CH_3)_2$	c	(65%)

Scheme 2-8

2-Hydroxy-3-phenylpropanoic acid (II-23-a) was isolated as its methyl ester* II-24, whose structure was confirmed by spectral and elemental analyses. Acetic acid generated from the reaction of nitrosamide II-22-a was detected in the nmr spectrum of the crude product, showing a singlet at τ 8.0, but was not isolated. The decomposition of II-22-b gave benzoic acid which was isolated as such and as its methyl ester. 2-Hydroxy-4-methylvaleric acid (II-23-c) was purified by column chromatography and had identical spectral properties to those of an authentic sample (75).

The gas evolved during addition of nitrosamide II-22-a to a methanolic potassium hydroxide solution at 0°C, was measured. The total volume represented the decomposition of approximately 39% of the starting nitrosamide II-22-a.

In order to differentiate the products generated under basic conditions from those formed upon acidification of the crude reaction product, the following experiment was carried out. A methanolic solution of nitrosamide II-22-a was allowed to decompose at room temperature with an excess of sodium methoxide. Methanol was evaporated and replaced by water.

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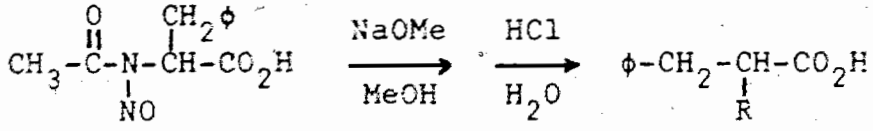
* All methyl esters were prepared by diazomethane treatment of the corresponding acids.

Upon acidification with hydrochloric acid, more gas was evolved and usual workup gave a mixture of three acids. 2-Methoxy-3-phenylpropanoic acid (II-25) (48%) and 2-hydroxy-3-phenylpropanoic acid (II-23-a) (14%) were identified as their methyl esters by gc peak matching with authentic samples. 2-Chloro-3-phenylpropanoic acid (II-26) (7%) was tentatively assigned on basic of the gc-ms fragmentation pattern of its methyl ester showing a molecular ion peak at m/e 198-200 in a 3:1 ratio.



II-22-a

R = OCH_3 II-25

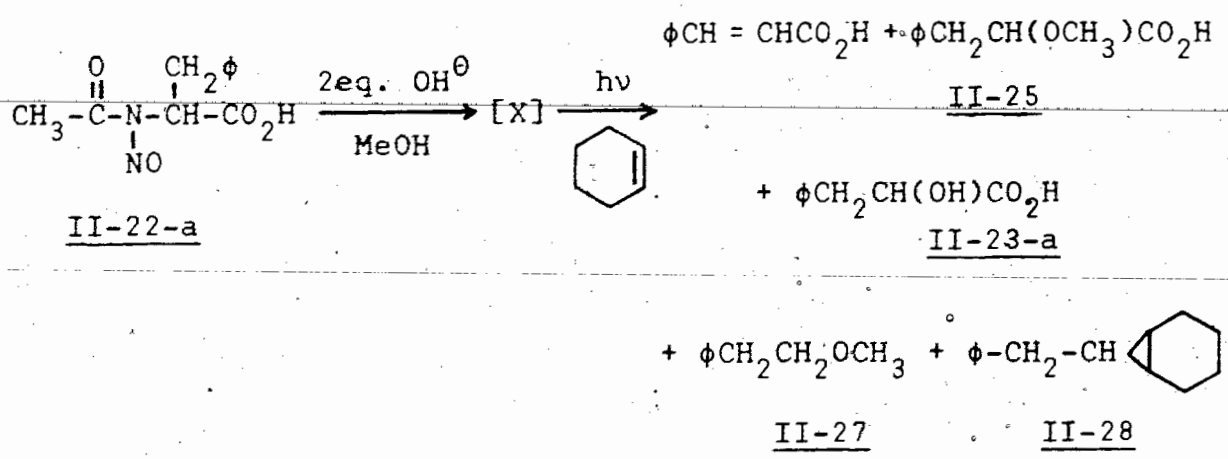
R = OH II-23-a

R = Cl II-26

Scheme 2-9

Several attempts were made to trap species "X" responsible for the 330 amd 410 nm uv absorptions. On the assumption that this intermediate was a diazoalkane derivative, photolysis of "X" in the presence of cyclohexene was investigated. The second approach involved the alkylation of the carboxylate group of "X" with an alkylating agent such as phenacylbromide.

A solution containing species "X" was generated as usual from the reaction of nitrosamido acid II-22-a with two equivalents of potassium hydroxide in methanol. Within two hours



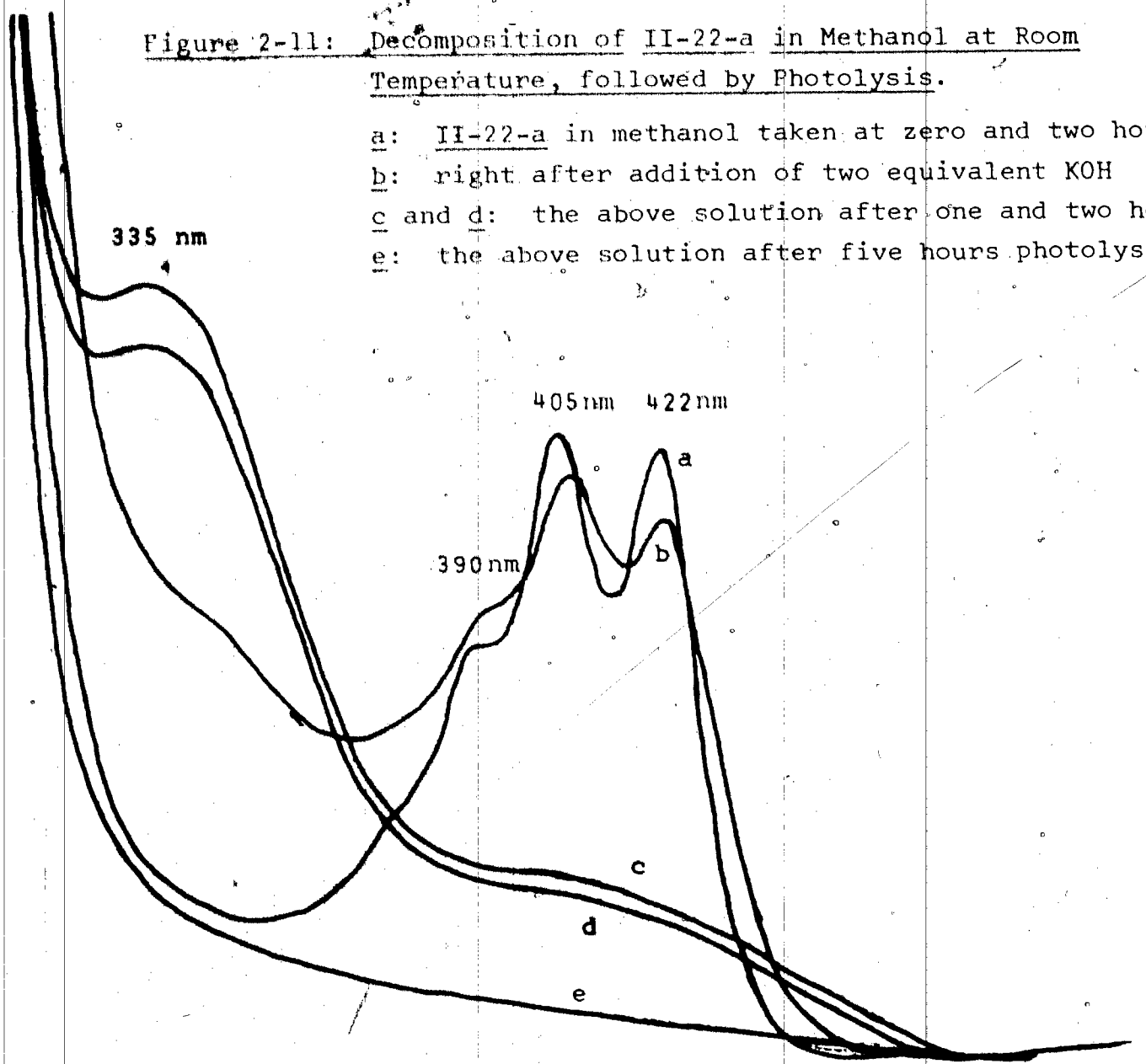
II-22-a

Scheme 2-10

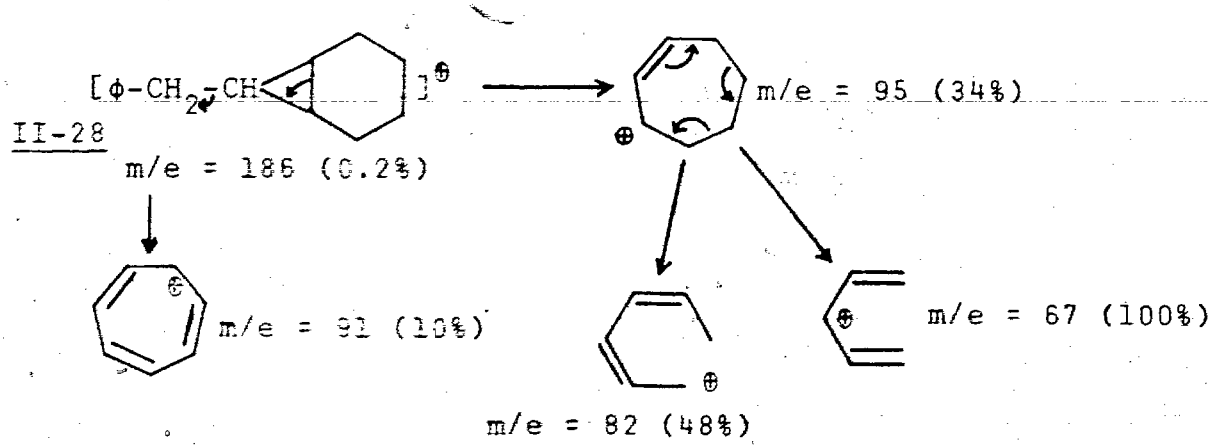
this solution showed no nitrosamide absorption at 400 nm but absorptions at 330 and 410 nm at their maximum (see Fig. 2-11). The solution, after addition of cyclohexene, was irradiated at 0°C in a pyrex apparatus for 5 hours until the absorption bands at 330 nm and 410 nm had completely disappeared (see Fig. 2-11). The acidic fraction was shown to contain in addition to two minor unidentified components, cinnamic acid (12%), hydroxy acid II-23-a (18%) and methoxy acid II-25 (64%). The compounds were identified as their methyl esters by gc peak matching with authentic samples. The yields based on II-22-a, were estimated from peak area measurements. The neutral fraction contained 1-cyclohexenol, 1-methoxy-2-phenylethane II-27 (~1%), 7-benzyl-

Figure 2-11: Decomposition of II-22-a in Methanol at Room Temperature, followed by Photolysis.

a: II-22-a in methanol taken at zero and two hours.
b: right after addition of two equivalent KOH
c and d: the above solution after one and two hours.
e: the above solution after five hours photolysis.



bicyclo[4.1.0]heptane II-28 (~1%) and one minor unidentified compound. Ether II-27 was identified by gc peak matching with an authentic sample. Addition product II-28 was identified by gc-ms and showed a molecular ion peak at m/e 186 and base peak at m/e 67 characteristic of bicyclo[4.1.0]heptanes (75b).



Scheme 2/11

Treatment of a solution containing species "X" with an excess of phenacylbromide in refluxing methanol caused rapid disappearance of the absorptions at 330 and 410 nm. The acidic fraction was shown to contain methoxy acid II-25 (36%) and a trace of hydroxy acid II-23-a by examination of the nmr spectrum. The neutral fraction exhibited ir absorptions of phenacylbromide at 1675, 1280 and 1190 cm^{-1} and a weak absorption at 2080 cm^{-1} which may be attributed to

phenacyl 3-phenyl-2-diazopropanoate (II-29).** The carbonyl stretching of the ester group of II-29 could not be confirmed since the crude product contained relatively large amount of phenacylbromide. Attempted chromatography of the mixture on basic alumina failed to give the pure ester II-29.

In methanol with one equivalent of strong base:

a) Treatment of nitrosamido acid II-22-a with one equivalent of sodium methoxide in methanolic solution for 72 hours at 5°C followed by evaporation of the solvent, gave methoxy acid II-25 as shown by the nmr signal at τ 6.62 ($-OCH_3$). Acidic extraction of the crude product followed by esterification with diazomethane yielded the corresponding ester II-37 in 90% overall yield.*

b) Treatment of II-22-a with one equivalent of potassium hydroxide at 40°C gave a first order decrease of the $n\rightarrow\pi^*$ band. ($k = 82.2 \times 10^{-5}$ sec^{-1}) which was at least three times as fast as that at 22°C (see Fig. 2-12). Esterification of an aliquot of the concentrated reaction mixture followed by gc analysis gave methoxy ester II-37 as a major product and a trace of hydroxy ester II-24 (~3%). Acidic work up of the rest of the crude product gave methoxy acid II-25 (95%) identified as its methyl ester II-37.

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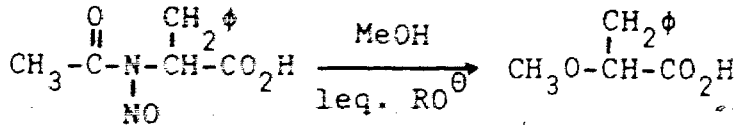
* Ester II-37 gave satisfactory spectral and elemental analyses.

** See structure of II-29 in Scheme 3-11.

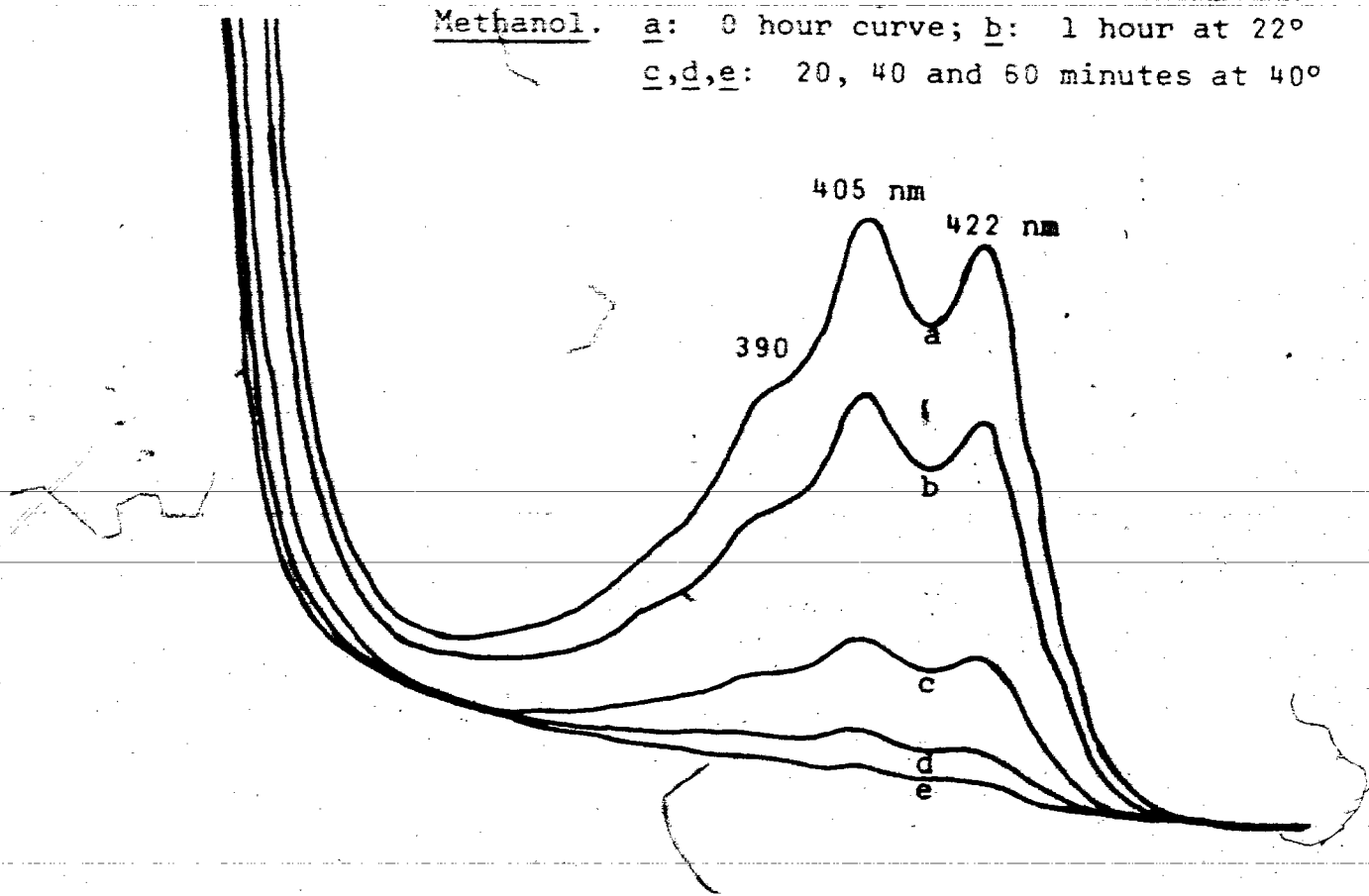


II-22-a II-25

R = H, CH_3

Scheme 2-12

Figure 2-12. Decomposition of Nitrosamide II-22-a in the Presence of 1 mole Equivalent of KOH in Methanol. a: 0 hour curve; b: 1 hour at 22° c,d,e: 20, 40 and 60 minutes at 40°



In benzene with triethylamine: When a benzene solution of nitroso derivative II-22-a was treated with 1, 3 or 5 mole equivalents of triethylamine at room temperature, it underwent slow decomposition, as shown by the gradual decrease of the 400 nm band (see Fig. 2-9). In all cases, a residual absorption centered at 400 nm and representing approximately 20% of the zero hour curve was observed. The crude products obtained after evaporation of the solvent showed identical ir spectra, featuring broad absorptions at 3500 and 2500 cm^{-1} for carboxylic acids, a medium absorption at 2080 cm^{-1} characteristic of a diazo linkage, and strong bands at 1740 and 1250 cm^{-1} for an ester group. The fraction obtained after basic (10% Na_2CO_3) extraction of the crude product was shown to contain unreacted nitrosamido acid II-22-a (ca. 20%)*, phenylethylacetate (II-31) (16%) and 2'-phenylethyl-3-phenyl-2-diazopropanoate (II-30) (17%)** by examination of its ir and nmr spectra. The starting material was characterized by a singlet at τ 7.5 for the methyl group and a double doublet at τ 4.4 (J = 6,10 Hz) for the methine proton, in the nmr spectrum. Phenylethylacetate showed a singlet at τ 8.0 for the methyl protons and a triplet at τ 5.72, whereas

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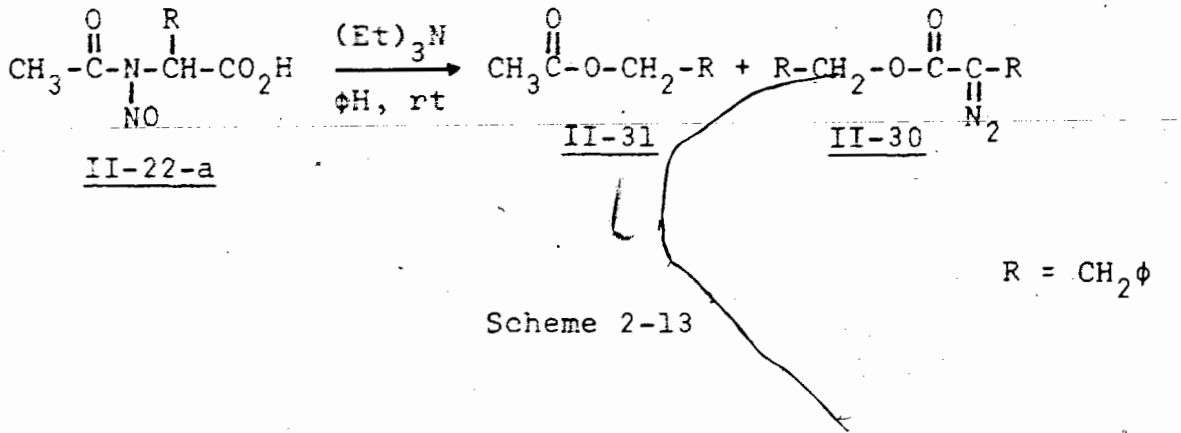
* Dissociation of the acid group in II-22-a requires a stronger pH.

** The yield of II-30 is based on 1 mole of II-22-a to give a maximum of 0.5 mole of II-30.

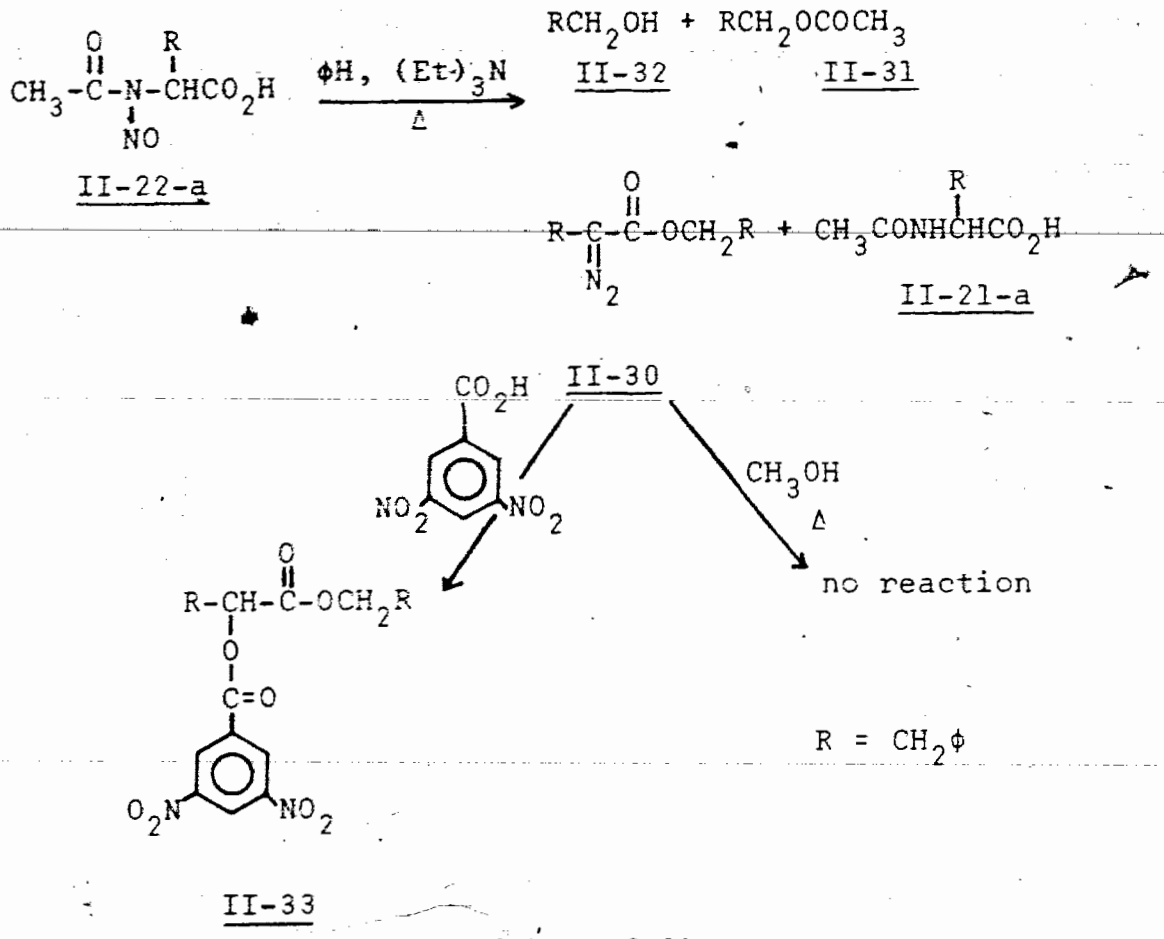
diazo ester II-30 was characterized by a triplet at τ 5.7 and a singlet at τ 6.5 for the methylene group alpha to the diazo linkage. The yields were calculated from nmr integration and were identical within experimental errors in all three experiments.



Scheme 2-13

When a benzene solution of nitrosamide II-22-a containing one equivalent of triethylamine was refluxed, no residual absorption at 400 nm was observed. The rate of disappearance of the nitrosamido absorption at 400 nm was about twice as fast as in the thermolysis of II-22-a in refluxing benzene without triethylamine. The neutral fraction obtained after basic extraction showed the same ir characteristics as that obtained in the room temperature reaction; e.g., strong absorptions at 2080 and 1690 cm^{-1} for diazo ester II-30 (76) and strong absorptions at 1745 and 1240 cm^{-1} for ester II-31. Chromatography of this fraction over basic alumina afforded 2-phenylethylacetate (II-31) (3%), diazo ester II-30 (17%) and phenylethyl alcohol (II-32) (7%). The acidic fraction was mainly parent amide

II-21-a (2%) as indicated by its ir spectrum.



Scheme 2-14

Ester II-31 was obtained by elution with hexane and identified by comparison of its spectral data with those of an authentic sample. Diazo ester II-30, eluted with a mixture of 10% benzene in hexane, was a bright yellow oil which showed to be pure by tlc and hplc analyses but gave poor elemental analysis. The diazo ester function was characterized by the strong ir absorptions at 2080 and 1690 cm^{-1} (76) and a weak uv absorption

at λ_{max} 406 nm (ε ~35). The proton nmr spectrum exhibited two triplets at τ 5.66 and 7.08 (J = 6 Hz) which collapsed into a singlet upon irradiation of one of the other signal. The singlet at τ 6.44 was attributed to the methylene protons alpha to the diazo group by comparison of the chemical shift with that of a similar type of diazo ester derivative (76). Unfortunately, due to its long T_1 (77) the diazo carbon could not be observed by ^{13}C nmr spectroscopy, the other carbons showed chemical shifts and ord splitting patterns consistent with the structure. Furthermore, the 3,5-dinitrobenzoate derivative of diazo ester II-30 (II-33) was prepared in 27% yield based on II-30. The structure of derivative II-33 was confirmed by elemental analysis and ir, nmr and high resolution mass spectroscopies. The last compound phenethyl alcohol II-32 was obtained by elution with benzene containing 25% of hexane. Its structure was determined by comparison of its ir and nmr spectra with those of an authentic sample. It is believed that phenethyl alcohol was formed by the decomposition of an intermediate during the separation, since the ir spectrum of the neutral fraction did not exhibit any absorption in the 3600 - 3300 cm^{-1} region.

In a similar experiment, the crude neutral fraction containing diazo ester II-30 was refluxed in methanol for one hour. The ir spectrum of the resulting crude product showed no

modifications in comparison to that of the starting mixture, indicating that no reaction had occurred.

When nitrosamide II-22-a was allowed to decompose in refluxing methanol, in the presence of one equivalent of triethyl amine, only methoxy acid II-25 (93%) was obtained.

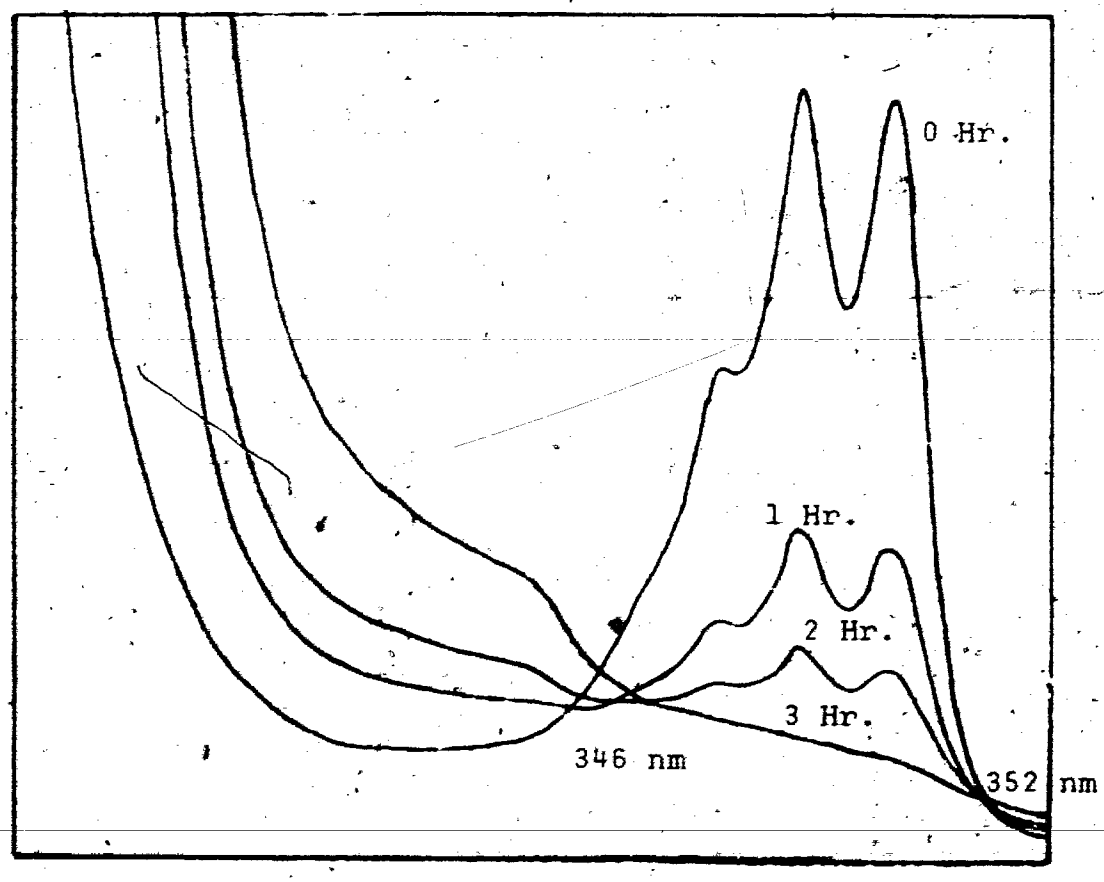
II-6 Decomposition of N-Nitrosamido Acids under Thermal Conditions

II-6-1 Thermal decomposition of N-Nitroso-N-acetyl-D,L-Phenylalanine (II-22-a) in Benzene

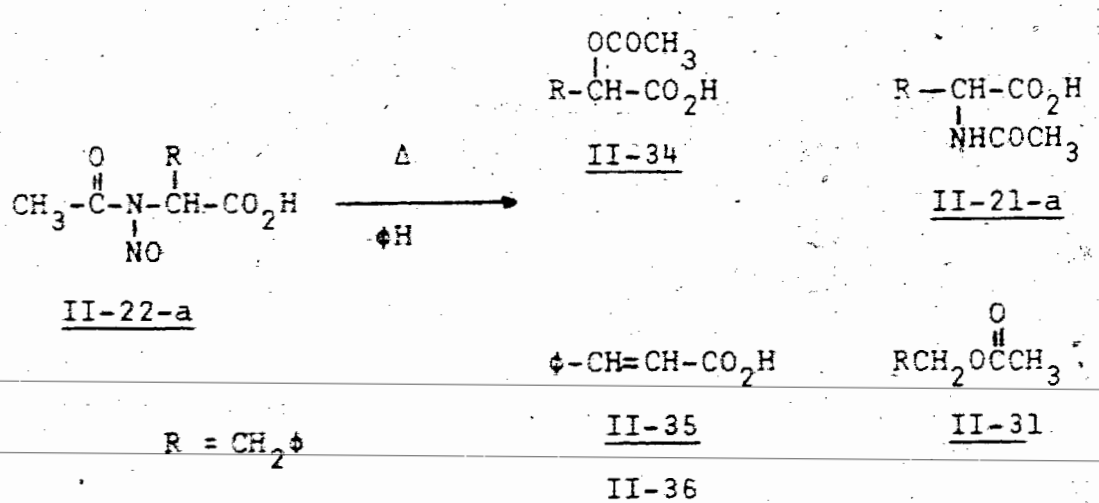
When nitrosamido acid II-22-a was refluxed in benzene in the dark under inert atmosphere, it underwent rapid decomposition as shown by the first order decrease ($k = 2.3 \times 10^{-4} sec^{-1}$) of the 400 nm absorption. The uv profile of the reaction (Fig. 2-13), exhibited two isoabsorptive points at λ = 352 and 446 nm, indicating the presence of one intermediate.

The crude product showed ir absorptions at 2080 cm^{-1} characteristic of a diazo group and at 2500 and 1730 cm^{-1} for a carboxylic acid group. The former band along with a multiplet at τ 6.3 in the nmr spectrum disappeared when the sample was

Figure 2-13. Thermolysis of II-22-a in Refluxing Benzene



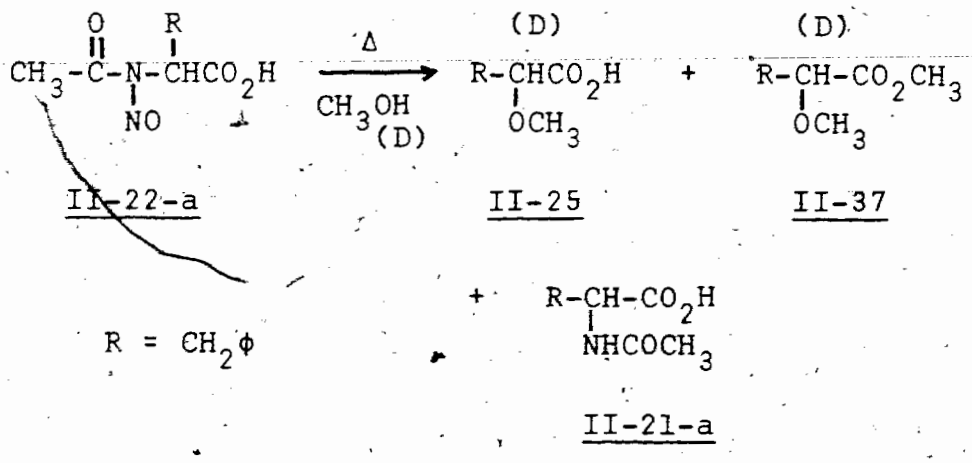
stored at room temperature overnight. The "aged" crude mixture was analyzed by gc-ms of its methyl ester to give 3-phenyl-2-acetoxypropanoic acid (II-34) (43%), N-acetyl-phenylalanine (II-21-a) (21%), trans-cinnamic acid (II-35) (4%), 2-phenylethyl acetate (II-31) (<1%) along with a compound (<1%) whose methyl ester had a fragmentation pattern and a retention time very similar to that of methyl cinnamate. This compound was assigned to cis-cinnamic acid (II-36). All compounds except II-36 were identified on the basis of the fragmentation pattern of their methyl esters and upon mixed injection with authentic samples. The methyl esters of II-21-a and II-35 were obtained via diazomethane esterification of the parent acids and that of II-34 by acetylation of the corresponding alcohol II-24.



Scheme 2-18

II-6-2 Thermal Decomposition of N-Nitroso-N-Acetyl-D,L-Phenylalamine, II-22-a, in Methanol

The thermolysis of nitrosamido acid II-22-a was carried out in refluxing dry methanol under nitrogen in the dark. The decomposition of II-22-a was monitored by following the first order decrease of the 400 nm absorption ($k = 2.2 \times 10^{-4} sec^{-1}$). Basic extraction of the crude mixture gave methyl methoxy ester II-37 (20%) and extraction of the acidified mother liquor gave methoxy acid II-25 (58%) and the parent amide II-21-a (~10%).



Scheme 2-16

Methoxy ester II-37 was identified on the basis of its ir spectrum and gc retention time in comparison to those of an

authentic sample. The methoxy acid II-25 and the parent amide II-21-a were identified as their methyl esters by gc matching with authentic samples.

When the same reaction was carried out at 40°C, II-22-a decomposed by a first order kinetics with a rate constant of ($k = 1.7 \times 10^{-5} sec^{-1}$). A similar work up gave methoxy acid II-25 (60%), methoxy ester II-37 (10%) and the parent amide II-21-a (7%).

Thermolysis of nitrosamido acid II-22-a in refluxing O-deuterated methanol also showed a first order decrease of the 400 nm absorption ($k = 1.5 \times 10^{-4} sec^{-1}$) and gave methoxy acid II-25 (45%), methoxy ester II-37 (45%) and a trace of parent amide II-21-a. The total amount of deuterium incorporation at the α-position of the carbonyl group in both methoxy acid II-37 and methoxy ester II-25 was approximated to 36% by comparison of the integration of the methine protons at τ 6.0 and that of the methyl protons at τ 6.3 in the nmr of the crude product. A more accurate value was obtained from gc-ms analysis using the $[M^+-49]$ and $[M^+-32]$ ions of ester II-37. The deuterium content of the ester present in the crude product resulting from the thermal reaction in MeOD, was 40%.* Esterification of the crude

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* It was shown by nmr that starting nitrosamide II-22-a did not incorporate deuterium in methanol-d at room temperature under neutral conditions for 24 hours.

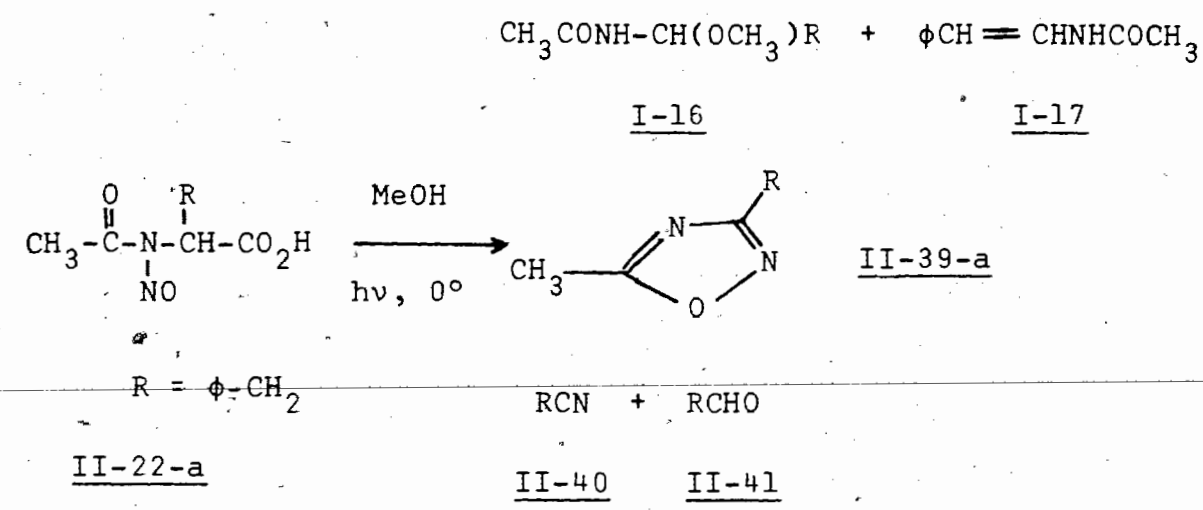
with diazomethane, followed by gc-ms analysis showed the same amount of deuterium incorporation. This indicates that the extent of deuterium incorporation in both methoxy acid II-25 and ester II-37 is identical.

II-7 Photolysis of N-Acyl-N-Nitroso-α-Amino Acids

II-7-1 Photolysis of N-Nitroso-N-Acetyl-D,L-Phenylalanine II-22-a in Methanol

Photolysis of nitrosamido acid II-22-a in methanol, under nitrogen and at 0°C was reinvestigated. Previous results (28) had given N-acetyl-1-phenyl-2-methoxyethylamine I-16 (45%) and N-acetyl-β-styrylamine I-17 (23%). The photolysis was run under the same conditions and the photolysate was directly analyzed by gc-ms to give phenylacetaldehyde (II-41) (3%), phenylacetonitrile (II-40) (3%), 3-benzyl-5-methyl-1,2,4-oxadiazole (II-39-a) (2%), methoxy amide I-16 (35%) and styrylamine derivative I-17 (39%) accompanied by two minor unidentified components.

All compounds were identified on the basis of their ms fragmentation pattern and by gc peak matching with authentic



Scheme 2-18

samples.* The yields were estimated from relative peak areas measurements. Methoxy amide I-16 was shown to decompose partially in the gc column to give styrylamine I-17. This explained the discrepancy between the yields reported on this work from those previously reported.

II-7-2 Photolysis of N-Nitroso-N-Acetyl-D,L-Phenylalanine II-22-a Under Basic Conditions

In methanol and in the presence of sodium cyanide: The presence of a shoulder at λ_{max} = 340 nm in the uv spectrum of

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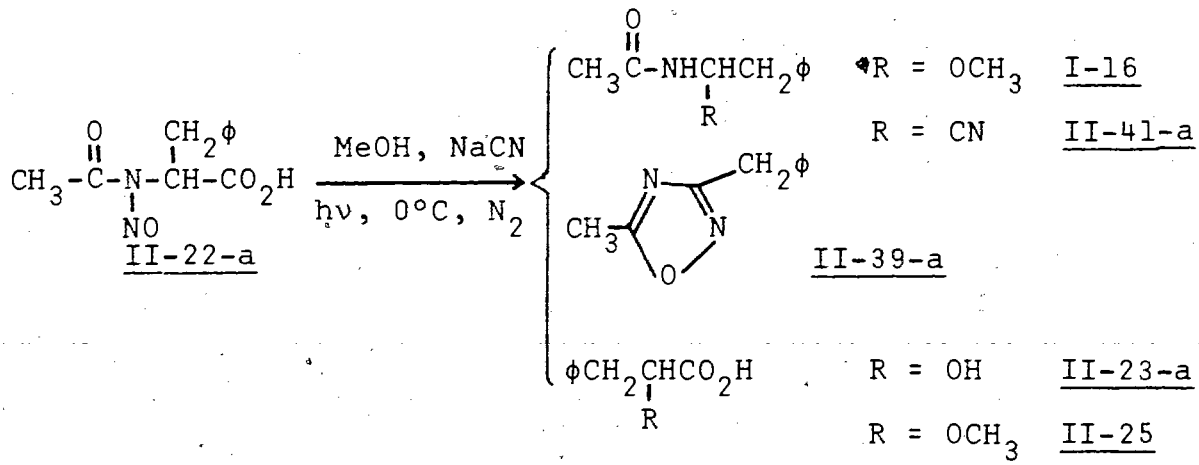
* Authentic samples of I-16 and II-39-a are described further in the text and an authentic sample of I-17 was kindly provided by Dr. Y.L. Chow.

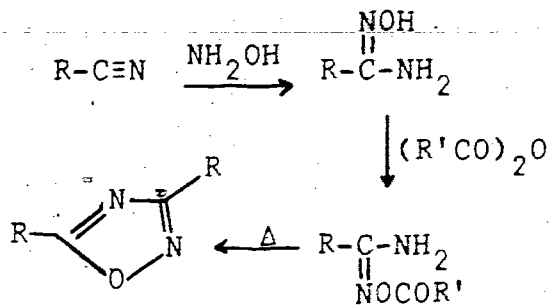
a solution of nitrosamide II-22-a in a saturated methanolic solution of sodium cyanide indicated ca. 10% decomposition of II-22-a prior to the irradiation. The photolysis of this solution at 0°C under nitrogen, resulted in the formation of non-photolytic basic decomposition products (24%), methoxy acid II-25 and hydroxy acid II-23-a and photo products (72%), methoxy amide I-16, oxadiazole II-39-a and N-acetyl-2-phenyl-1-cyanoethylamine (II-41-a). Chromatography of the neutral fraction



Scheme 2-18

on basic alumina gave oxadiazole II-39-a (46%), methoxy amide I-16 (7%) and nitrile (II-41-a) (19%). Gc analysis of the crude neutral fraction also showed the presence of two unidentified minor components. The molecular formula of oxadiazole II-39-a was ascertained by elemental analysis and hrms. The presence of the 1,2,4-oxadiazole ring was confirmed by a strong characteristic absorption at 1590 cm^{-1} in the ir spectrum (79), a

deshielded methyl group resonating at τ 7.52 in nmr and an uv absorption at λ_{max} = 226 nm (ε = 290). Ultimately, the structure of II-39-a was confirmed by the unequivocal synthesis of the two positional isomers of benzyl-methyl-1,2,4-oxadiazole i.e., II-39-a and 5-benzyl-3-methyl-1,2,4-oxadiazole (II-39-e) (Scheme 2-19).



R = CH_3 II-42

R = $CH_2\phi$ II-43

R = CH_3, R' = $CH_2\phi$ II-44

R = $CH_2\phi$, R' = CH_3 II-45

II-39-e R = CH_3, R' = $CH_2\phi$

II-39-a R = $CH_2\phi$, R' = CH_3

Scheme 2-19

The spectral data of II-39-a, II-39-e and those of the photoadduct are summarized in Table 2-14. The C-5 carbon atom, being alpha to an oxygen and a nitrogen atom is expected to resonate at a lower field than C-3 which is alpha to two nitrogen atoms (80). The splitting patterns for C-3 and C-5 of II-39-e were obtained by off acquisition decoupled ^{13}C nmr and showed a triplet and a quartet, respectively. Compound II-39-a was found to be stable towards thermolysis (200°C),

Table 2-14. ^{13}C and ^{1}H nmr Data for the Two Isomers of Benzyl-Methyl-1,2,4-Oxadiazole

		C_3	C_5	CH_3	CH_2	ϕ			
^{13}C nmr[a]	II-39-e[c]	166.6(q)[d]	176.8(t)[e]	10.3(q)[f]	31.6(t)[g]	132.9	126.3	127.9	128.2
	II-39-a	168.8	176.0	11.5	31.5	135.1	126.4	128.0	128.3
	photoproduct	168.6	175.8	11.7	31.6	134.8	126.3	127.9	128.2
^{1}H nmr[b]	II-39-e			7.65	5.92		2.70		
	II-39-a			7.63	6.03		2.77		
	photoproduct			7.52	5.98		2.68		

a) taken in $CDCl_3$, in δ from TMS; b) taken in $CDCl_3$ in τ from TMS; c) off-acquisition decoupled spectrum

d) J = 6.8 Hz; e) J = 7.5 Hz; f) J = 131.4 Hz; g) J = 130.2 Hz

basic treatment (sodium hydroxide-methanol) and uv irradiation (254 nm). In all three experiments over 90% of the starting material was recovered.

The structure of methoxy amide I-16 was confirmed by spectroscopic and elemental analyses. The molecular formula of nitrile II-41-a was ascertained by elemental analysis and hrms. The ir spectrum exhibited absorptions at 2240 cm^{-1} (C≡N) and at 1660 and 1540 cm^{-1} (NHCO). The ^1H nmr spectrum showed a singlet a τ 8.03 for the methyl and an ABX pattern for the phenethyl groups.

The ir spectrum of the acidic fraction exhibited the characteristic absorptions for a carboxylic acid group at 1710, 2500 and 3300 cm^{-1}. A singlet at τ 6.62 in the nmr spectrum indicated the presence of a methoxy derivative. The mixture was esterified with isopropanol and the resulting esters were separated by preparative tlc to give isopropyl 2-methoxy-3-phenylpropanoate (II-46) (16%) and isopropyl 2-hydroxy-3-phenylpropanoate (II-47) (8%). The structure of methoxy ester II-46 was confirmed by strong ir absorptions at 1740 and 1100 cm^{-1} for the ester group and by appropriate nmr signals for a methoxy (τ = 6.67) and an isopropyl group. The ir spectrum of hydroxy ester II-47 showed strong absorptions at 3500 cm^{-1} (OH) and at 1740 and 1100 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-O$). The corresponding nmr

spectrum exhibited an ABX pattern for the substituted phenethyl group and the expected splitting pattern for the isopropyl group. The molecular formulae of both II-46 and II-47 were further ascertained by elemental analysis and hrms.

In methanol and in the presence of sodium carbonate: Nitrosamido acid II-22-a in a methanol solution saturated with sodium carbonate decomposed partially as indicated by the appearance of new uv absorption at λ_{max} ~350 nm. Photolysis of this solution at 0°C, resulted in a rapid disappearance of the nitrosamido absorption at 400 nm. The usual work up of the photolysate gave a neutral fraction containing oxadiazole II-39-a (12.5%) and methoxy amide I-16 (46%) as shown by the nmr signals at τ 5.98(s) and τ 7.5(s) for compound II-39-a and at τ 4.6 and 7.1(d) for I-16. The yields were estimated from the integration of the two methyl signals.

In tetrahydrofuran in the presence of 1,5-diazabicyclo [5.4.0]undec-5-ene (DBU): Nitrosamido acid II-22-a decomposed partially when dissolved in a tetrahydrofuran solution containing DBU, as shown by the new 350 nm absorption band. Photolysis of this solution at 0°C, resulted in the complete disappearance of the nitrosamide absorption at 400 nm. Usual work up of the photolysate gave an acidic fraction which was shown by gc-ms to contain benzoic acid (6%), phenylacetic

acid (11%), oxadiazole II-39-a (22%) and hydroxy acid II-23-a (23%). The acids were analyzed as their methyl esters and all compounds were identified on basis of their ms fragmentation patterns and upon mixed injection with authentic samples. The yields were estimated from the relative areas of each peak.

In methanol in the presence of triethylamine: The photolysis of a methanolic solution of nitrosamido acid II-22-a containing slightly over two mole equivalents of triethylamine at 0°C, gave oxadiazole II-39-a (64%) and methoxy acid II-25 (9%). The two products were identified by tlc and gc peak matching with authentic samples and the yields were estimated by measuring the areas of the corresponding signals in the nmr spectrum of the crude mixture.

The variation of the percentage yield of oxadiazole II-39-a as a function of the amount of triethylamine was investigated. The photolysates resulting from the irradiation of nitrosamide II-22-a in methanolic solutions containing 1, 2 and 5 mole equivalents of triethylamine were analyzed by gc-ms to give oxadiazole II-39-a, and other products derived from N-acylimine intermediate II-54 (vide infra); e.g., styrylamine II-38, methoxy amide I-16, aldehyde II-38 and nitrile II-40. The percentage yields were estimated from peak area measurement

and that of oxadiazole II-39-a was calculated relative to dibenzofuran used as internal standard. The percentage yield of oxadiazole II-39-a was found to increase steadily as the amount of trimethylamine increased, to reach a maximum of ~70% for approximately two mole equivalents of base (Fig. 2-14).

Photolysis of methanolic solution of N-nitroso-N-acetyl-D,L-phenylalanine dicyclohexylamine salt (II-48)* at 0°C gave aldehyde II-41 (20%), oxadiazole II-39-a (15%), methoxy amide I-16 (31%) and styrylamine II-17 (26%) (from gc analysis).

In acetonitrile with triethylamine: Photolysis of II-22-a in acetonitrile containing two mole equivalents of triethylamine gave oxadiazole II-39-a (63%, after distillation), as only product. The structure was confirmed by comparison of the spectral data with those of an authentic sample (vide supra).

In order to maximize the yield of formation of oxadiazole II-39-a , the photolysis of nitrosamide II-22-a was carried out in acetonitrile containing differing triethylamine-nitrosamide molar ratios. The yields of II-39-a were calculated as pre-

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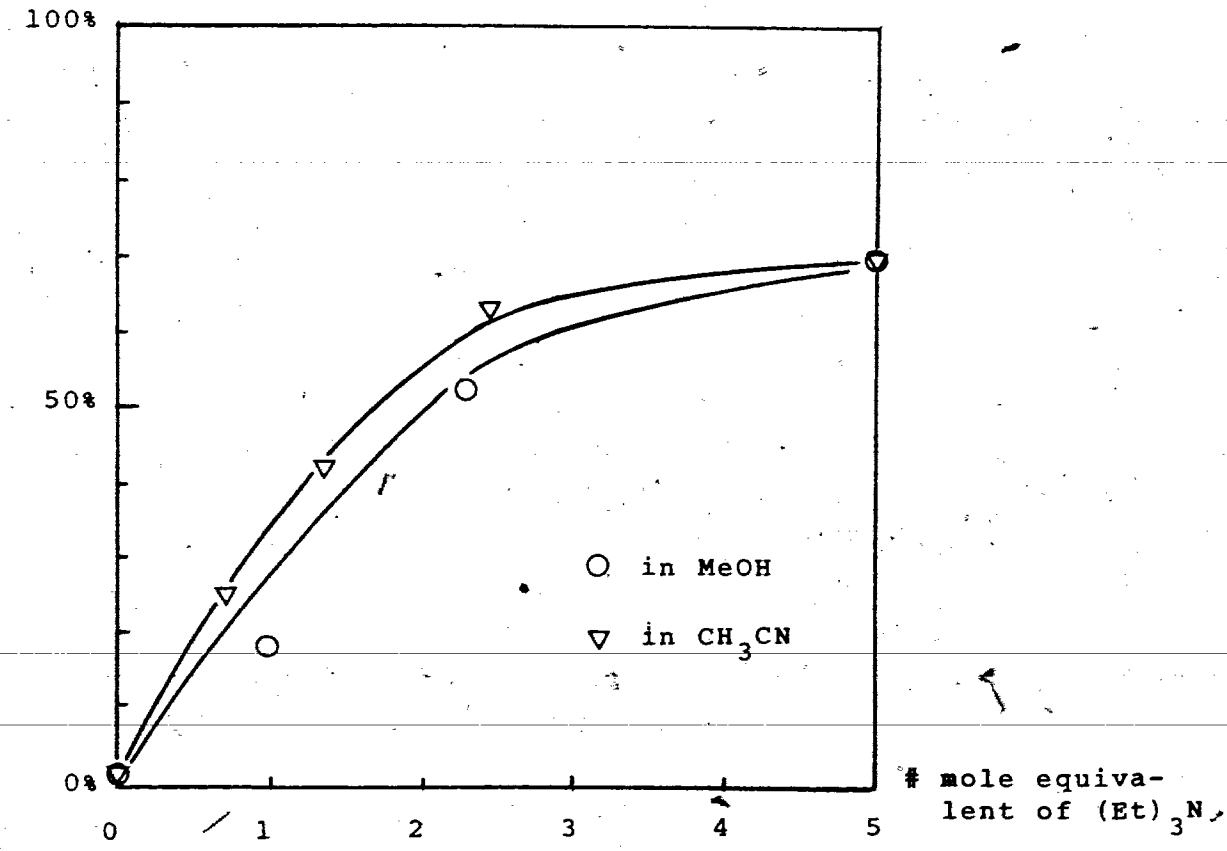
* II-48 was prepared by addition of dicyclohexylamine to a slight excess of II-22-a in ether.

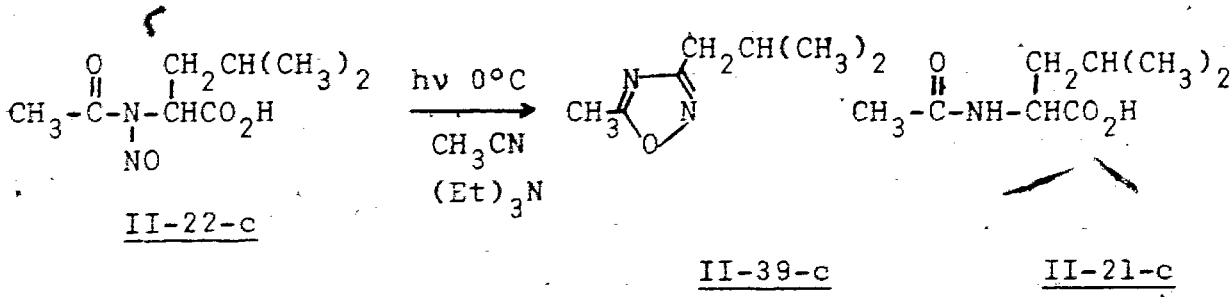
viously described and are plotted as a function of the number of mole equivalents of triethylamine (Fig. 2-14). No oxadiazole was detected in the absence of triethylamine but appeared and increased steadily when the amount of base increased to reach a maximum of ~70% when two or more equivalents of base were present.

Figure 2-14. % Yield of 3-Benzyl-5-Methyl-1,2-4, Oxadiazole as a Function of the Number of Triethylamine Equivalents



II-7-3 Photolysis of N-Nitroso-N-Acetyl-D,L-Leucine II-22-c

Photolysis of nitrosamide II-22-c, in acetonitrile containing two molar equivalents of triethylamine, at 0°C, gave 3-isobutyl-5-methyl-1,2,4-oxadiazole II-39-c (68%) along with the parent amido acid II-21-c (7%) and ca 2% of an unidentified compound.



II-22-c II-39-c II-21-c

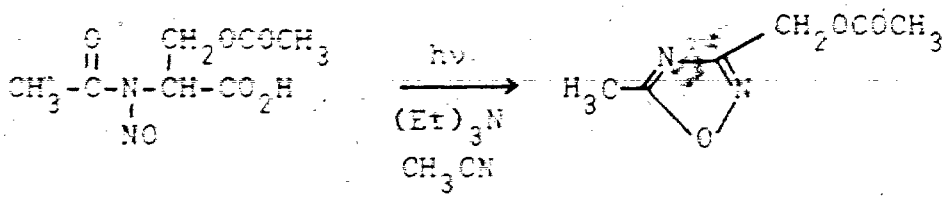
Scheme 2-20

The parent acid II-21-c was identified as its methyl ester by mixed injection with an authentic sample in gc. The oxadiazole II-39-c was isolated by preparative gc and was found to be quite volatile similarly to alkyl substituted 1,2,4-oxadiazoles (81). Analytically pure sample could not be obtained, but its structure was determined by spectroscopic analysis. The 1,2,4-oxadiazole ring was confirmed by the strong ir absorption at 1590 cm^{-1} and the two low field singlets at δ 175.8 (C-5) and 169.7 ppm (C-3) in the ^{13}C nmr spectrum. The ^{1}H nmr spec-

trum showed a deshielded singlet at τ 7.44 for the methyl group and the expected splitting pattern for the isobutyl group.

II-7-4 Photolysis of N-Nitroso-N,O-Diacetyl-D,L-Serine II-22-d

A sample of nitrosamide II-22-d containing ca. 15% of parent amido acid II-21-d (as estimated from integration in nmr spectrum) was photolysed in acetonitrile at 0°C in the presence of over two equivalents of trimethylamine to give 3-acetoxymethyl-5-methyl-1,2,4-oxadiazole (II-39-d) (12%) as the only isolable product.



II-22-d II-39-d

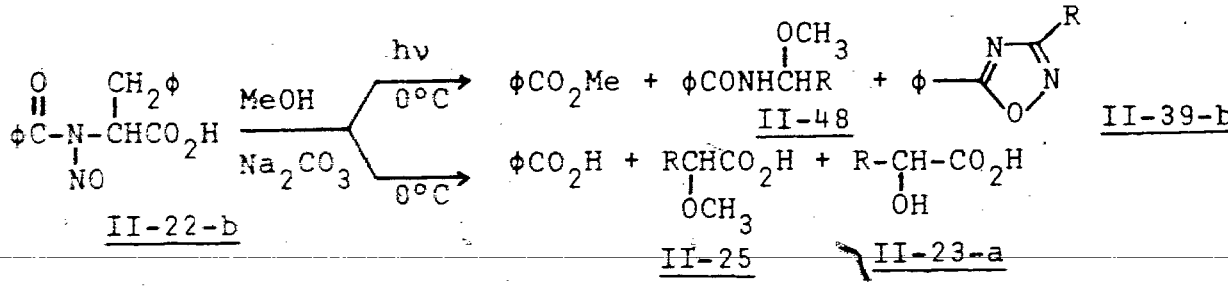
Scheme 2-21

The presence of the oxadiazole ring in II-39-d was confirmed by its characteristic ir absorption at 1590 cm^{-1} and the two low field signals at δ 175.3 (C-5) and 168.6 ppm (C-3) in the ^{13}C nmr spectrum. The acetoxymethyl group was characterized by strong absorptions at 1750 and 1220 cm^{-1}, by singlets at

τ 7.85 (CH_3) and 4.82 (CH_2) in the proton nmr spectrum and by a low field signal at δ 165.0 ppm (C=O) in the ^{13}C nmr spectrum. The 5-methyl group gave a singlet at τ 7.3 in proton nmr.

II-7-5 Photolysis of N-Benzoyl-N-Nitroso-D,L-Phenylalanine II-22-b

The addition of the nitroso derivative II-22-b to a saturated solution of sodium carbonate in methanol, resulted in a fair amount of decomposition of the nitrosamide, as evidenced by the strong uv absorption band at λ_{max} = 350 nm. The photolysis of this solution gave a small amount of the expected 3-benzyl-5-phenyl-1,2,4-oxadiazole (II-39-b) (5%) and N-benzoyl-1-phenyl-2-methoxyethlyamine II-48 (5%) along with traces of methyl benzoate (4%). The acidic fraction was shown to contain benzoic acid (45%), methoxy acid II-25 (47%) and hydroxy acid II-23-a (8%).



R = $CH_2\phi$

Scheme 2-22

The molecular formula of oxadiazole II-39-b was confirmed by hrms. Due to an extensive conjugation the ir absorption characteristic of oxadiazole ring was slightly shifted towards the low frequency region (1560 cm^{-1} as compared to 1590 cm^{-1} for non-conjugated 1,2,4-oxadiazoles). The ^{13}C nmr spectrum exhibited two low field signals at 156.9 and 152.4 ppm attributed to C_5 and C_3 of the ring, respectively. Furthermore, the melting point observed was identical to that previously reported (82).

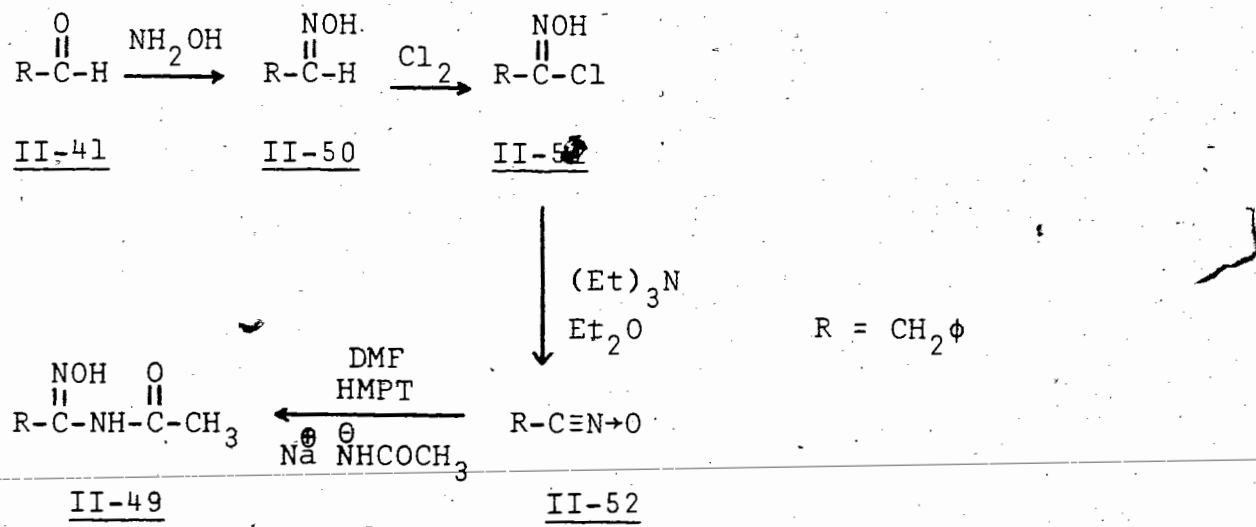
Elemental analysis and hrms gave the molecular formula of methoxy adduct II-48. Its ir spectrum showed typical absorption bands for secondary amides at 1640 and 1530 cm^{-1}. The methoxy group was characterized by a singlet at τ 6.6 in the ^{1}H nmr spectrum.

Benzoic acid was isolated by sublimation and methoxy acid II-25 and hydroxy acid II-23-a were analyzed by gc as their methyl esters.

II-7-6 Attempt at Elucidation of the Mechanism for Oxadiazole Formation

Synthesis and basic treatment of N-acetyl-phenylacetamidoxime II-49: A first attempt to synthesize amidoxime II-49 by N-acetylation of the corresponding oxime II-43 with acetic ethylcarbonic anhydride ($EtOCOOCOCH_3$) as described in the literature (83) failed and gave O-acetylation product.

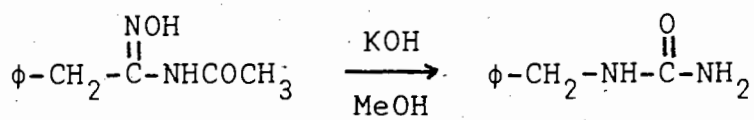
On the other hand, acetamidoxime II-49 was synthesized in 34% yield via addition of sodium acetamide to nitrile oxide II-52 (see Scheme 2-23) in a similar manner to the Behn's addition of thiols to the same nitrile oxide (84).



II-41 II-50 II-51 II-52 II-49

Scheme 2-23

The elemental analysis and high resolution mass spectroscopy ascertained the molecular formula of II-49 to be $C_{10}H_{12}N_2O_2$. The ir spectrum exhibited strong absorptions at 1700 and 1670 cm^{-1} which were attributed to the C=O and C=N bond stretchings, respectively. The nmr spectrum showed two singlets of equal intensity at τ 5.47 and 5.57 for the benzylic protons, indicating a mixture of E and Z-oximes in a 1:1 ratio. The mother liquor was shown by tlc not to contain any oxadiazole II-39-a. Furthermore, no reaction was observed when acetamidoxime II-49 was treated with an excess of triethylamine in acetonitrile. However, treatment of II-49 with KOH in methanol at room temperature gave N-benzylurea II-53 (59%), a Beckman rearrangement product. The structure of II-53 was assigned on the basis of its ms and mp as compared to those of an authentic sample (75-b).

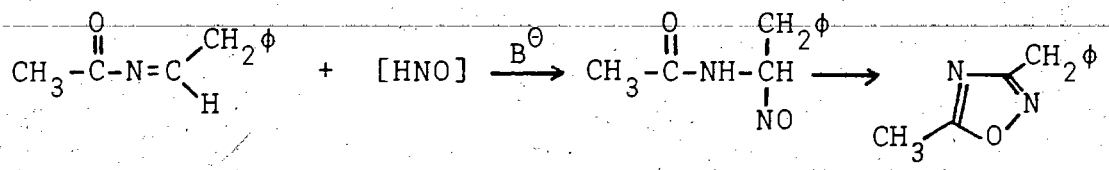


II-49 II-53

Scheme 2-24

Attempt to trap the N-acylimine II-54 with nitroxyl: In order to demonstrate that oxadiazole II-39-a obtained from the

photolysis of nitrosamido acid, II-22-a, is a rearrangement product of C-nitroso intermediate II-55 resulting from the addition of nitroxyl to the N-acylimine II-54, the reaction between the latter and nitroxyl was attempted.



II-54 II-55 II-39-a

Scheme 2-25

N-acetylimine II-54 was generated in situ by DBU dehydrohalogenation of N-acetyl-N-chloro-2-phenylethylamine II-56 (Scheme 2-26). The latter was obtained in 65% overall yield by N-acetylation of phenethylamine followed by N-chlorination (85).

Nitroxyl (or hyponitrous acid) is known to be a decomposition product of the basic hydrolysis of Piloty's salt (N-hydroxy benzenesulfonamide) (86). It is also known to be an unstable species having a lifetime of 0.1 sec. (12) under the conditions of flash photolysis. It is therefore important, for our purposes that both HNO and N-acylimine II-56 are continuously and simultaneously generated in the reaction mixture. In order to

determine the optimum conditions for the nitroxyl generation, the rates of hydrolysis of Piloty's salt in methanol were measured as a function of the base concentration. The profile of the decomposition of Piloty's salt in various sodium hydroxide-methanol solutions was traced by uv spectroscopy as shown in Fig. 2-15, and the rate constants calculated by plotting the usual first order kinetics graph (Fig. 2-16). In the presence of one equivalent of NaOH (spectrum a) the decomposition was slow, and almost instantaneous in the presence of 100 equivalents of NaOH (spectrum e). In the presence of 5 equivalents of base (spectrum b) two isosbestic points at λ = 275 and 306 nm were observed indicating the presence of a minimum of two species in the reaction mixture, namely the starting Piloty's salt (λ_{max} = 252, 259, 265 and 272 nm) and the resulting benzenesulfonate (λ_{max} = 270 nm). Spectra c and d were recorded for a mixture of Piloty's salt with 10 and 20 equivalents of NaOH, respectively. Spectrum c also exhibits two isosbestic points at ~275 and 298 nm whereas the two isosbestic points of spectrum d are more difficult to assign but can definitively be located between 283 and 288 nm. The rate constants of the disappearance of Piloty's salt are listed in Table 2-15.

Figure 2-15. Decomposition of Piloty's Salt in Methanol in the Presence of NaOH

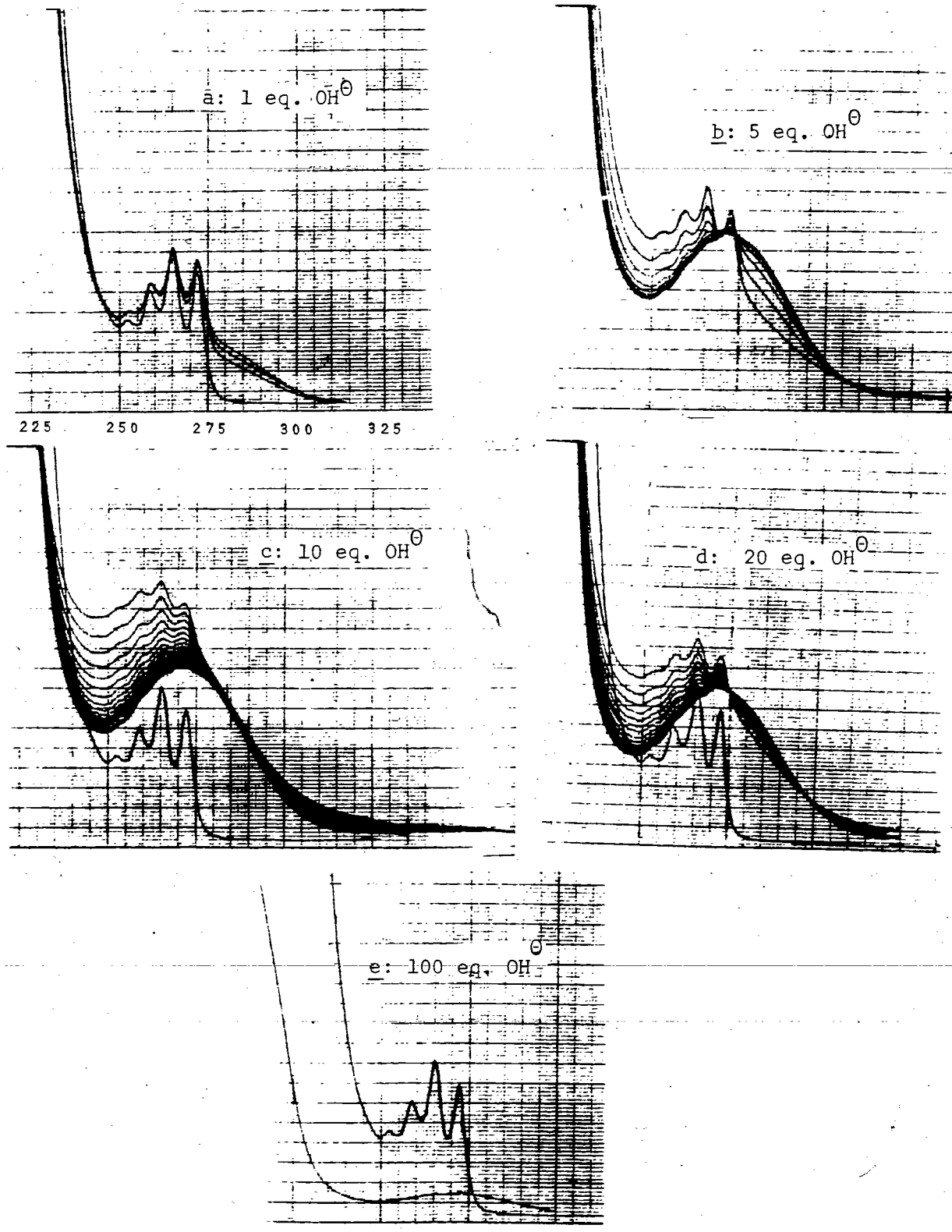


Figure 2-16. 1st Order Kinetics Plot of the Decomposition of Piloty's Salt in Methanol in the Presence of NaOH

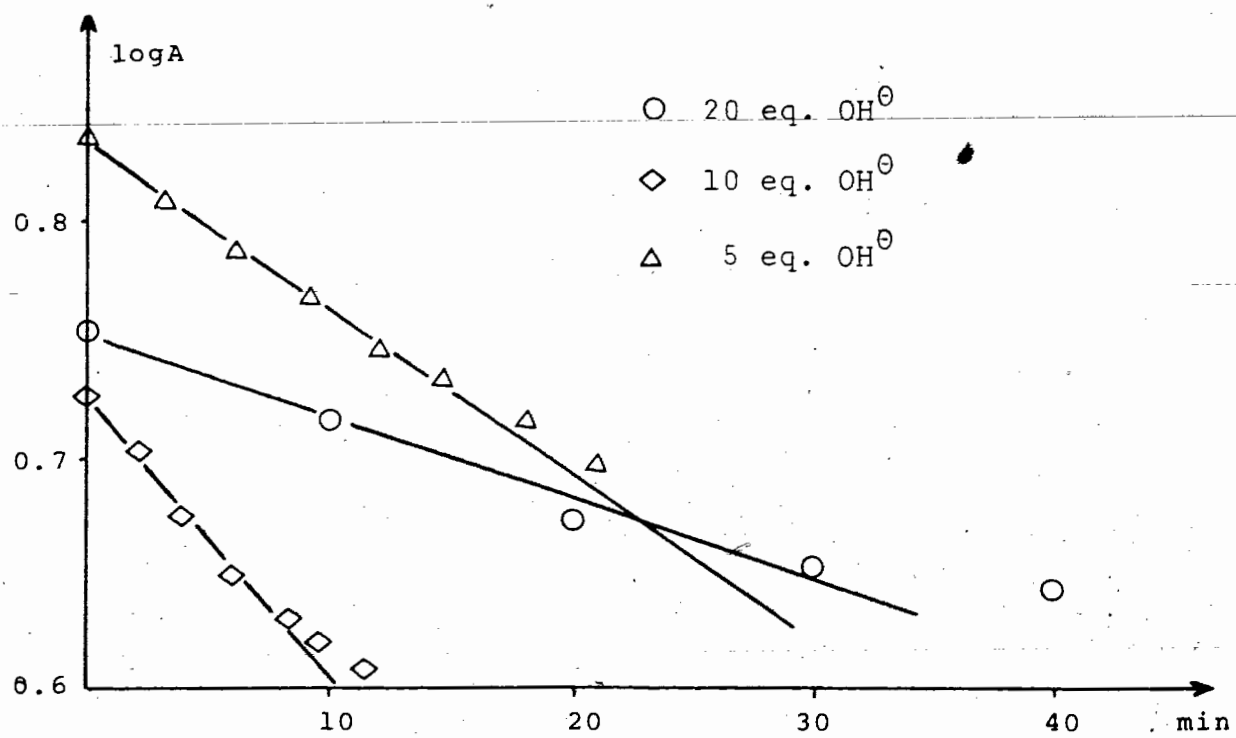
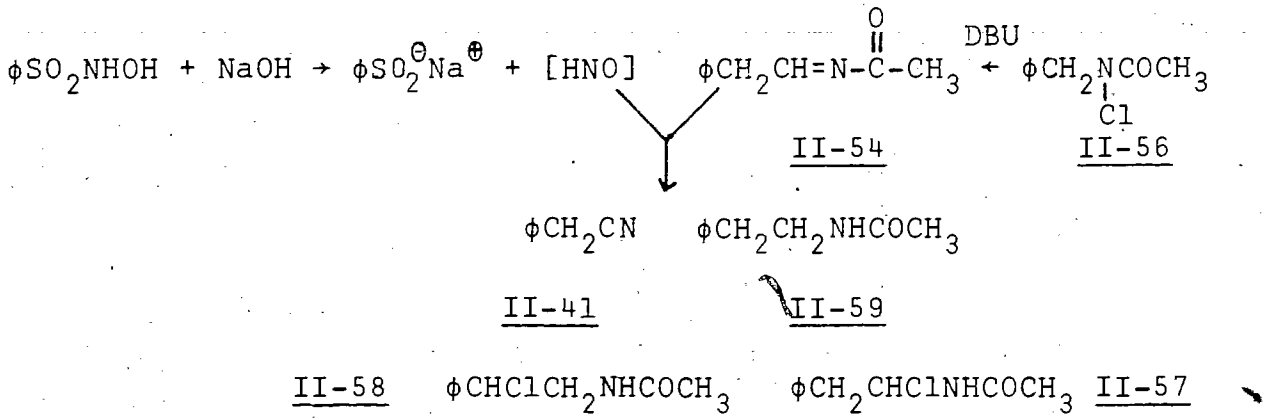


Table 2-15. Rate Constants for the Decomposition of Piloty's Salt as a Function of the Number of NaOH Equivalents

eq. NaOH	1	5	10	20	100
$k(X\ 10^{6}\ sec^{-1})$	slow	2	4.4	5.3	fast

N-acylimine II-54 was continuously generated by slowly mixing a solution of N-chloramide II-56 with a solution of DBU. The reaction was very rapid as demonstrated by the instantaneous formation of a heavy precipitate. The resulting imine II-54 was added to a methanol solution of Piloty's salt to which 10 mole equivalents of sodium hydroxide had just been added. The ir and nmr spectra of the crude product did not exhibit the characteristic absorptions for oxadiazole II-39-a. The neutral fraction was shown to be a mixture of phenylacetonitrile II-41 (38%) and N-acetylphenylethylamine II-59 (35%) accompanied by N-acetyl-(1-chloro-2-phenyl)-ethylamine II-57 (5%) and N-acetyl-(2-chloro-2-phenyl)-ethylamine II-58 (5%).



Scheme 2-26

The first two components were identified on the basis of their ms fragmentation patterns which were obtained by gc-ms analysis

and on gc peak matching with authentic samples. The two minor components were assigned from their ms fragmentation patterns. Both compounds exhibited a M^+ peak at 197-199 in a 3:1 ratio characteristic of the presence of a chlorine atom. Chloro derivative II-57 exhibited a strong signal at m/e = 162 (91%) for the loss of chlorine atom, whereas this fragment was absent in the mass spectrum of chloro compound II-58. The gc-ms trace showed no evidence of formation of either oxadiazole II-39-a nor methoxy adduct I-16.

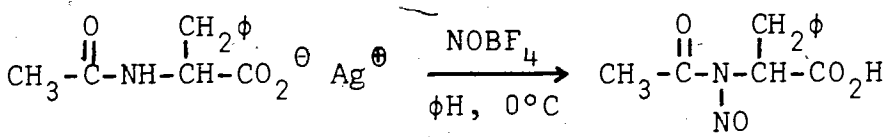
In another reaction, the direct slow addition of N-chloramide II-56 to a methanolic solution of Piloty's salt and 10 mole equivalents of NaOH gave a neutral fraction whose ir and nmr spectra did not exhibit the characteristic signals for oxadiazole II-39-a. Chromatography of this fraction on silica gel gave an unidentified compound (20%) and parent amide II-59 (55%) and no trace of oxadiazole II-39-a.

II-8 Nitrosation of N-Acetyl-D,L-Phenylalanine Silver Salt II-60 via Nitrosyl Tetrafluoroborate

Silver salt II-60 was prepared in 71% yield from the carboxylate anion of amido acid II-21-a and silver nitrate. Silver salt II-60 turned black on exposure to light and its ir spectrum showed absorption frequencies at 3380 (NH), 1610 ($CO_2^{\ominus}$) and 700

(C_6H_6) cm^{-1}.

Reaction of silver salt II-60 with nitrosyl tetrafluoroborate in benzene at 0°C gave nitrosamido acid II-22-a (17%) as shown by the typical uv bands of a nitrosamido group at λ_{max} 418, 400 and 390 nm. The yield was calculated from the absorbance of these absorptions.



II-60 II-22-a

Scheme 2-27

CHAPTER III

DISCUSSION

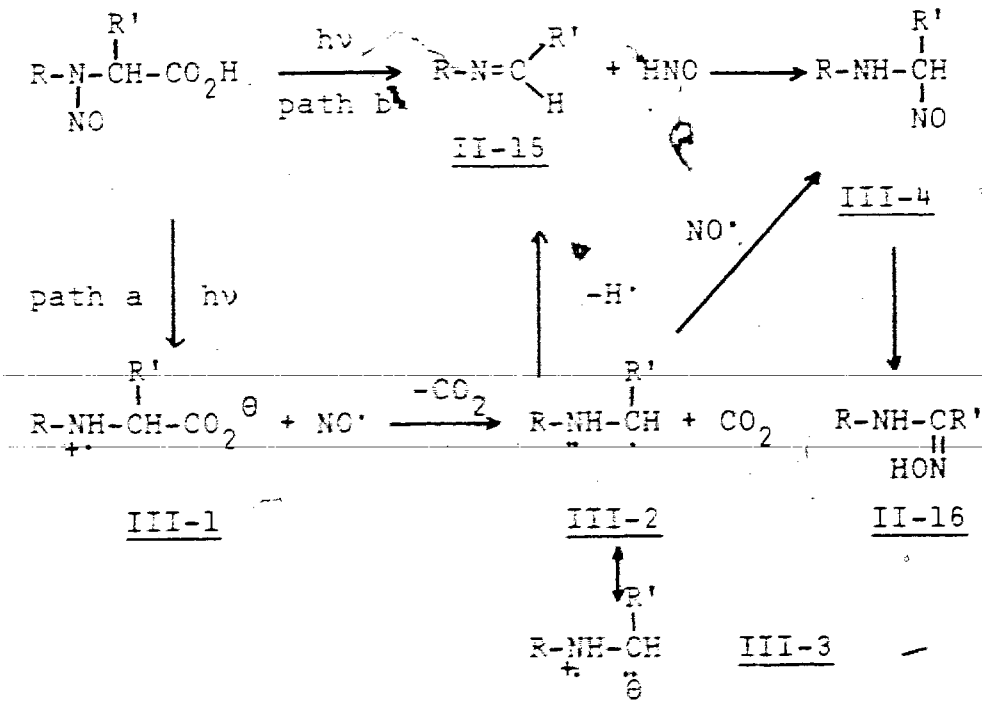
III-1 Photodecarboxylation of N-Nitroso-N-Alkyl-α-Amino Acids

Irradiation of the $n \rightarrow \pi^*$ band of dialkyl nitrosamines under neutral conditions does not induce cleavage of the nitrosamine group (22). However, the nitrosamine-acid complex, formed in the presence of a dilute acid, does undergo N-N bond homolysis on irradiation, and results in the generation of aminium radical (24). The concentration and strength of the acid play an important role in this reaction. Thus, complete protonation of the nitroso oxygen suppresses the photodissociation (25), whereas acetic acid is not sufficiently strong to induce it (28).

In contrast, nitroso derivatives of N-alkyl-α-amino acids underwent efficient photodecomposition without addition of an external acid. The facile photolysis can be attributed to i) the increase in acidity of the carboxylic acid in nitrosamino acids (pka of II-1-a = 3.2) (42) as compared to acetic acid (pka - 4.75) and/or ii) the possibility of an intramolecular proton-associated complex. These views are further supported by the inertness of N-nitrosonipecotinic acid II-1-e

towards uv irradiation. The carboxylic function in I-13-a is in the β-position relative to the nitroso group, and therefore expected to be just as acidic as acetic acid. Also, the preferred conformation would not allow the acid and the nitroso groups to form a proton-associated complex.

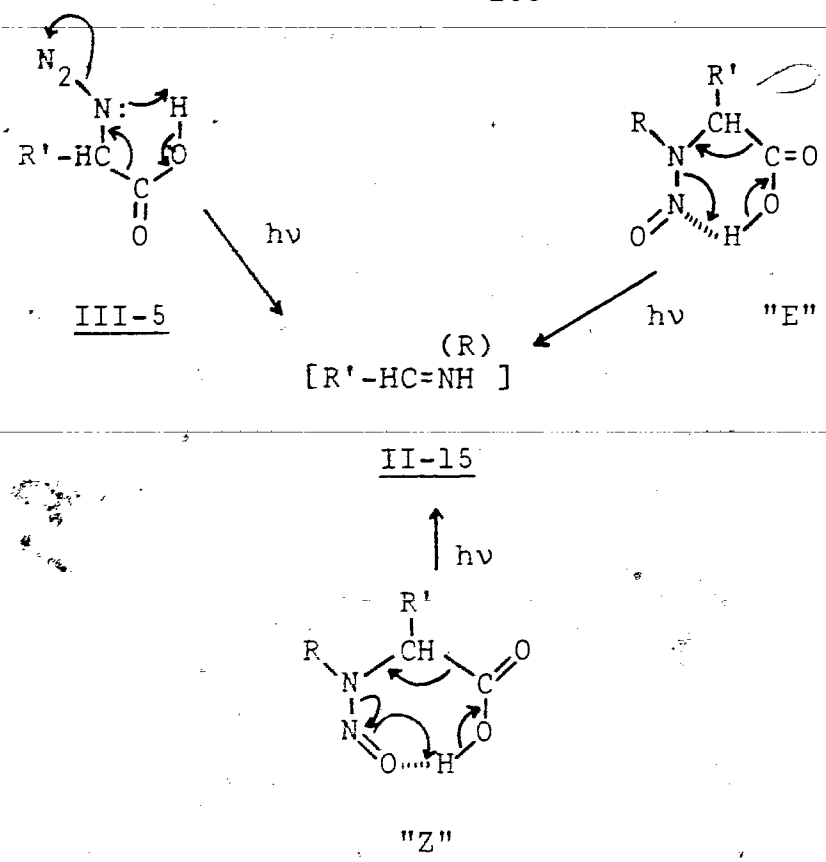
The formation of trimer I-11 in the photolysis of N-nitroso-N-phenylglycine I-10 indicates the intermediacy of imine II-15 (28) which was also proposed as a precursor for 2-piperidonoxime (II-16-h) in the photolysis of N-nitrosopipecolinic acid II-1-h (28). Accordingly, photolysis of nitrosamino acid II-1-d in water gave 3-phenylpropylamine (II-20) and formaldehyde resulting from the hydrolysis of the imine intermediate. The formation of this intermediate can simply



Scheme 3-1

be explained by a stepwise mechanism (path a, Scheme 3-1) via intermediacy of aminium radical III-1 followed by decarboxylation and loss of a hydrogen atom. Decarboxylation from aminium radicals such as III-1 has been proposed previously in photosensitized decarboxylation of N-(O-chlorophenyl)-glycine (90). The intermediacy of α-amino alkyl radicals such as III-2 has been suggested by Schollkopf and Ludwig (91) for the Stevens rearrangement of quaternary ammonium salts with base. Esr studies of such radicals (92) have shown a significant degree of delocalization of spin density from carbon to the adjacent nitrogen atom, and can justifiably be considered as nitrogen-centered radicals (form III-3). Direct recombination of III-2 with the stable nitric oxide radical is therefore not likely although not completely ruled out.

Alternatively, a concerted elimination of nitroxyl and CO_2 (pathway b, Scheme 3-1) cannot be ruled out. A similar concerted mechanism has been proposed in the photodecomposition of α-azido-carboxylic acids III-5 (93, 94) in which alkylimines were also found to be intermediates. Should such a mechanism be prevailing, one would expect the E-isomer, out of the two isomers in equilibrium under the photolytic conditions (95), to be the photolabile species. This assumption is based upon the following reasoning: in the $n\rightarrow\pi^*$ excited state, the polarity of the nitroso group is reversed and, hence, the



Scheme 3-2

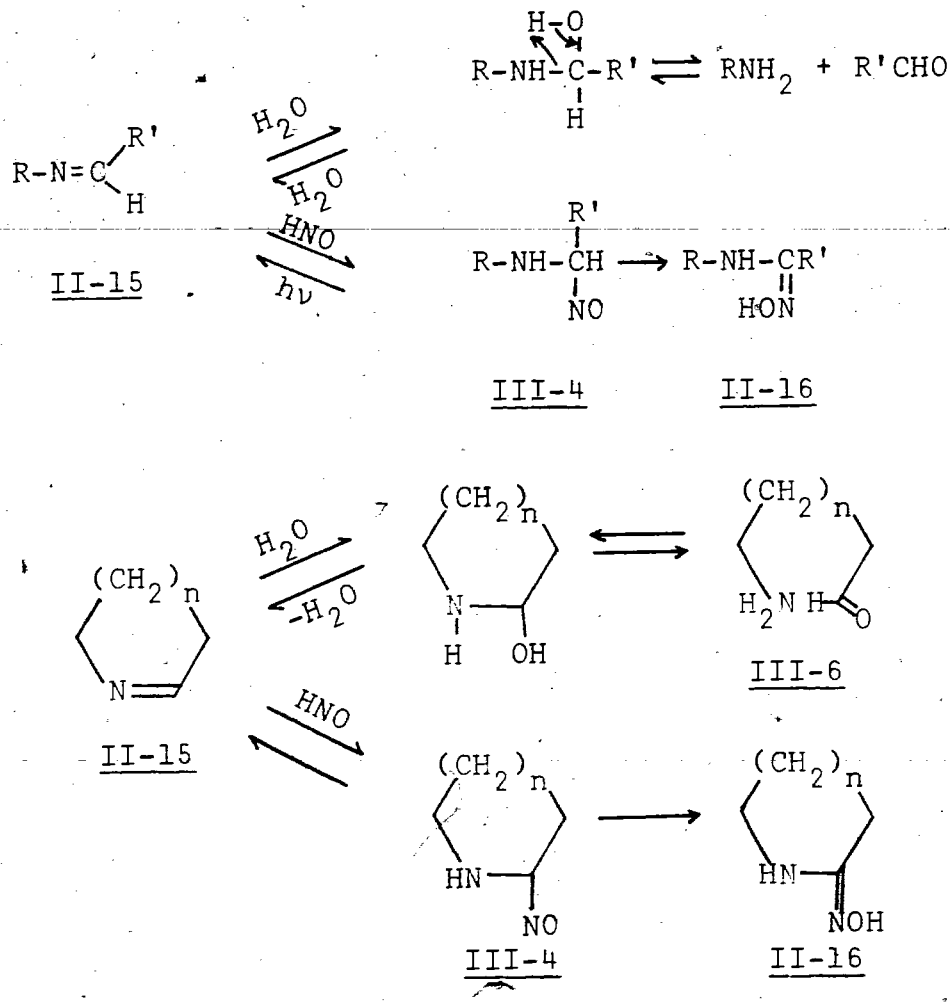
proton of the carboxylic group is expected to coordinate with the nitrogen atom of the nitroso group to give a six-membered transition state for elimination. Furthermore, this will result in the elimination of nitroxyl as [HNO] (88,89) rather than [NOH] in the case of the Z-isomer (Scheme 3-2).

The product amidoxime obviously results from the tautomerization of the C-nitroso derivative III-4 formed by the addition of nitroxyl to imine II-15. Primary and secondary

nitroso-alkanes are known to tautomerize very rapidly in the presence of an acid or a base or in polar solvents (96). It is, therefore, not surprising that C-nitroso compounds could not be detected during the photolysis of the free acid II-1-a in methanol, whereas a typical uv absorption at ~305 nm for C-nitroso dimers could be observed in the photolysis of the sodium salt of II-14-a in the same solvent. In general, tautomerization of C-nitroso derivatives leads to a mixture of Z and E oximes (97). In contrast, only Z-amidoximes were detected in the present work. Owing to an intramolecular hydrogen bonding, the Z-isomer is the thermodynamically most stable configuration. It is likely that, initially, both E and Z-isomers are formed and that the former isomerizes slowly to the more stable Z-isomer.

When the photolysis was carried out in water, a priori contradictory results were obtained. In the case of acyclic nitrosamino acid II-1-d, both amidoxime II-16-d and products arising from the hydrolysis of intermediate imine were observed. The hydrolysis products, however, could not be detected from the photoreaction of cyclic analogues. This may be due to the facile recyclization of δ and γ-amino aldehydes III-6 to give back the parent cyclic imine. The competing nitroxyl addition to the imine leads to C-nitroso derivative III-4 which is readily and irreversibly tautomerized

to the corresponding oximes (Scheme 3-3).



Scheme 3-3

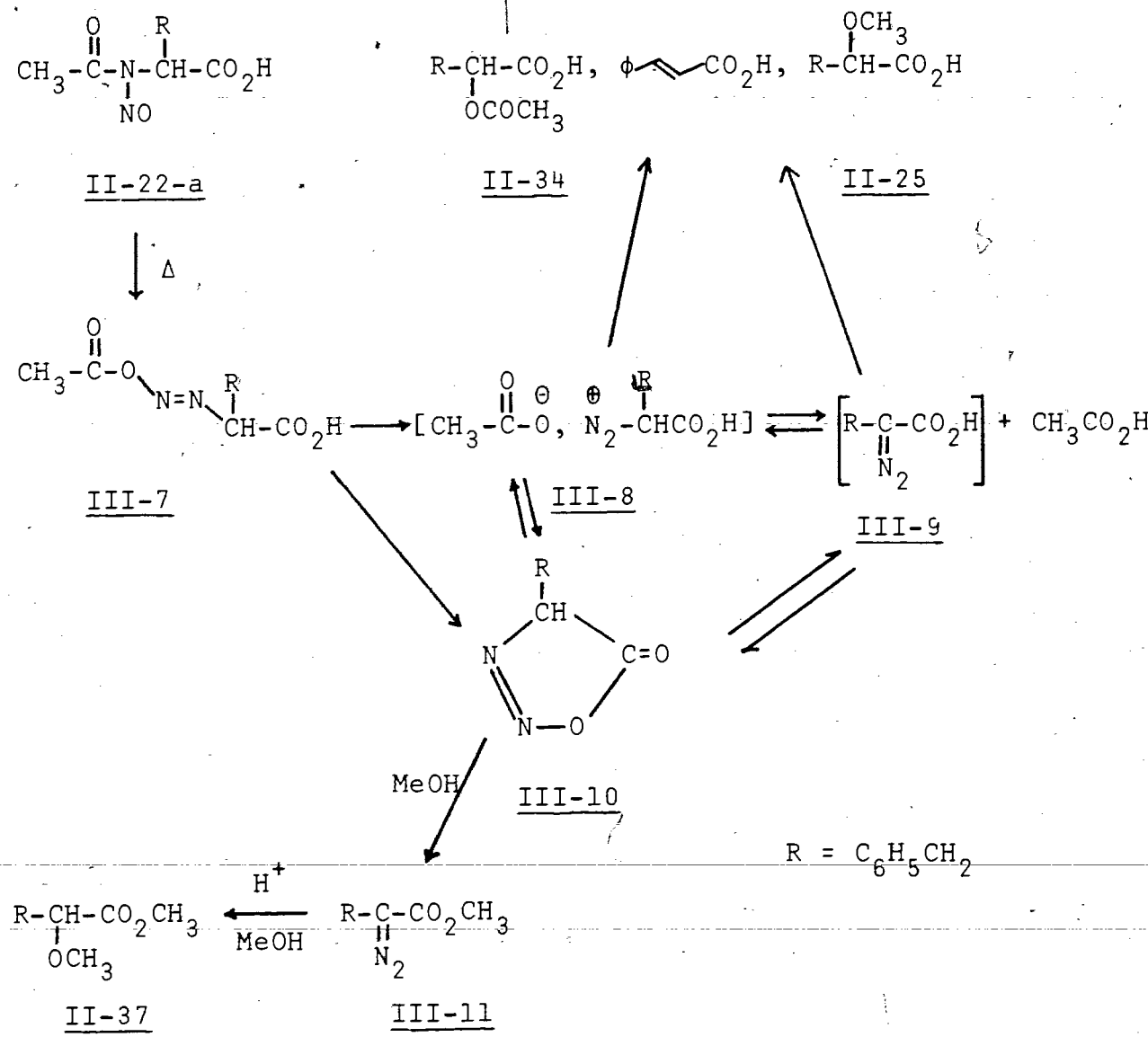
III-2 Decomposition of N-Nitroso-N-Acyl-α-Amino Acids Under Thermal or Basic Conditions

III-2-1 Thermolysis of N-Nitroso-N-Acyl-α-Amino Acids

The mechanism of the thermal decomposition of nitrosamides has been thoroughly investigated (12-15) and it is now widely accepted that the first step of the reaction involves the rearrangement of the nitrosamido group into a trans-diazoester derivative. In aprotic solvents, the latter decomposes readily to yield carboxylic esters as major products whereas in protic solvents, the formation of carboxylic acids and olefins prevail. It is shown that in aprotic solvents diazonium ions and diazoalkanes coexist in the equilibrium state and that their relative importance is determined by the nature of the N-alkyl substituents and the conditions of the reaction.

Methoxy acid II-25 and methoxy ester II-37, obtained from the thermolysis of nitrosamido acid II-22-a in methanol, can be best accounted for by the intermediacy of diazoacetate III-7 or diazo acid III-9. Analogues of such intermediates have been shown to be present in the thermolysis of alkyl-nitrosamides (15). Since generation of a carbenium ion center next to a carboxylic group is not favorable, diazonium ion III-8 is more likely to decompose into diazo-

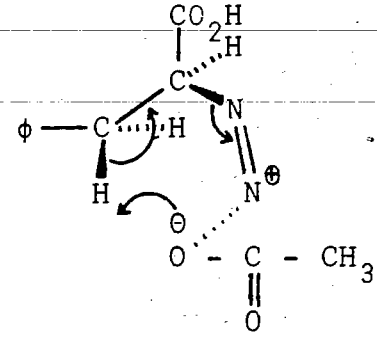
acid III-9 rather than into a carbenium ion. In methanol, diazoacid III-9 decomposes readily to give methoxy acid II-25. Incorporation of deuterium in II-25 from the solvent further supports the existence of a diazo intermediate. However, the formation of II-25 directly from III-8 is also likely since the extent of deuterium incorporation was only 40%.



Scheme 3-4

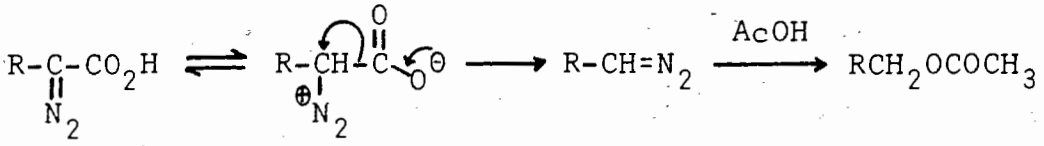
The isolation of methyl α-methoxy-ester II-37 is surprising since the formation of the ester linkage cannot be satisfactorily explained by intermediates such a diazoacid III-9 or diazoacetate III-7. It is believed that 1,2,3-oxadiazol-5-one III-10, formed either from diazoester III-7 by an intramolecular SN2 reaction, from diazoacid III-9 by prototropy, or from III-8 reacts with methanol to give diazo-ester III-11 which leads to methyl α-methoxy-ester II-37. The incomplete deuterium incorporation from the solvent in II-37 supports the involvement of a diazo intermediate but not necessarily exclusively.

The products, acetoxy acid II-34, cis and trans cinnamic acids, can be rationalized by a nucleophilic attack of acetate anion on diazonium ion III-8 and by elimination reaction from III-8, respectively. Formation of both cis and trans cinnamic acids suggests that elimination occurs by a "not completely concerted mechanism", as stated by Bieron and Dinan (14). Intermediacy of an α-carboxyl carbene cannot be completely ruled out. However, the presence of an absorption at 2080 cm^{-1} in



"not completely concerted elimination"

the ir spectrum of the crude thermolysis product, and the formation of phenylethyl acetate (II-31) suggest a more complex reaction pathway. It is tempting to assign the 2080 cm^{-1} band to diazoacid III-9 which can serve as a precursor to acetoxy acid II-34 or alternatively can undergo decarboxylation as shown in Scheme 3-5, to give diazoalkane III-13 which then leads to phenylethyl acetate II-31.



III-9 III-12 III-13 II-31

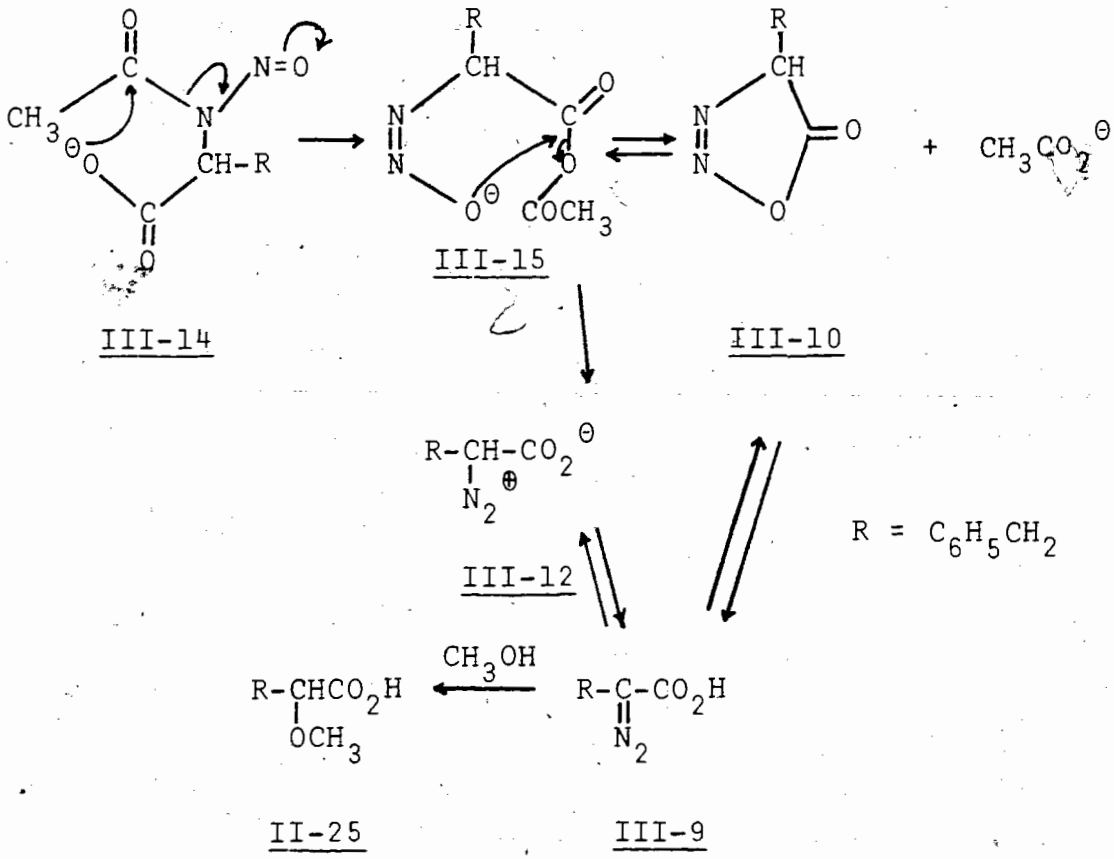
Scheme 3-5

III-2-2 Decomposition of N-Acyl-α-Amino Acids Under Basic Conditions

Results from the present work show that nitrosamido acids undergo deamination under basic conditions and that the mechanism of the reaction depends upon the number of mole equivalent of base used as well as its strength.

In the presence of one mole equivalent of potassium hydroxide or sodium methoxide in methanol nitrosamide II-22-a must exist as its conjugate base III-14 which undergoes facile

deamination. In analogy to the intermolecular nucleophilic catalyzed deamination of nitrosamides by weak bases such as acetates (45), the reaction may proceed via an intramolecular displacement as shown in Scheme 3-6. Formation of diazotate III-15 can be interpreted in terms of an addition-elimination pathway involving intramolecular nucleophilic attack by the acetate anion at the carbonyl carbon atom. Such an intra-



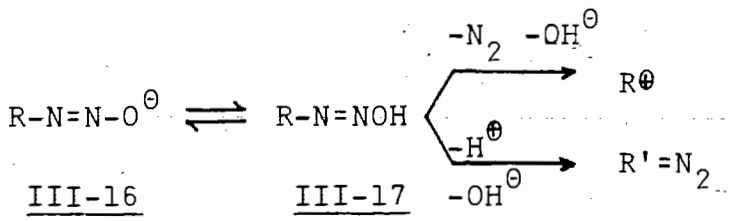
Scheme 3-6

molecular catalysis was shown to operate in the hydrolysis of phtalamic acid (78) for which an anhydride intermediate was also proposed. In comparison, the enhanced electron defficiency of the carbonyl of the nitrosamido group in III-14 and the better leaving properties of the nitrosamino group make this pathway even more feasible. The participation of the carboxylate anion in the decomposition of the nitrosamido group is confirmed by the kinetic study of the deamination of II-22-a in benzene, at room temperature with one, two or five mole equivalents of triethylamine. The rate constant of the disappearance of nitrosamide II-22-a varied only slightly due to the equilibrium dissociation. Since, as shown by Challis (46), the rate constant of the deamination reaction of nitrosamides varies rapidly with the concentration of the catalyst, direct participation of triethylamine in the nucleophilic attack at the carbonyl group is ruled out. These results are in accordance with a rate-determining intramolecular displacement by the carboxylate anion.

Temperature was found to play an important role in this reaction; the rate of disappearance of nitrosamide increased rapidly with an increase in temperature (see Table 2-13). The competitive thermal process which could occur at elevated

temperature is not believed to be important since at 40°C, the rate of the basic decomposition reaction was at approximately ten times as fast as that of the thermolysis. Furthermore, the product pattern of the decomposition of II-22-a with triethylamine in benzene at room temperature was identical to that at 80°C but completely different from that obtained in the thermolysis reaction.

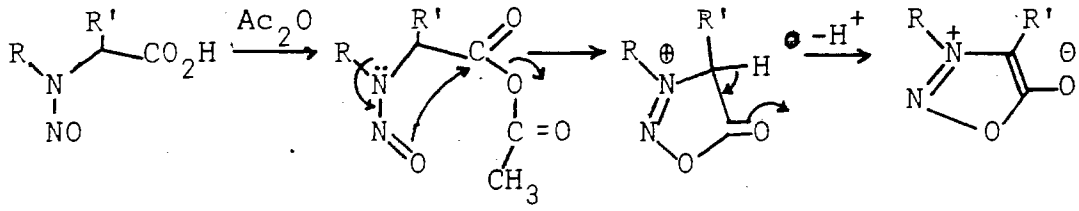
In protic solvents, diazotate III-16 is known to be in equilibrium with its protonated form, diazotic acid III-17 (38). The latter dissociates rapidly into carbenium ion or diazo derivatives (38). Although the partition of diazotic



Scheme 3-7

acid III-17 is a low activation energy process (87), the formation of carbenium ion from diazotate III-15 may require a prohibitively high activation energy because of the presence of the electron withdrawing carbonyl group. Similar conclusions can be drawn for the diazoalkane pathway since diazoalkane formation is first order in base and is usually observed in

highly basic media (~3M aqueous hydroxide) (98). A likely alternative pathway for the decomposition of diazotate III-15 is an intramolecular nucelophilic displacement of the acetate anion similar to that observed in the formation of sydnones III-18 from anhydride III-19 (99). The Z-configuration of the diazotate group, which is usually observed in the decomposition of nitrosamides by alkoxides (44), provides diazotate III-15 with the geometrical requirements for the facile cyclization.

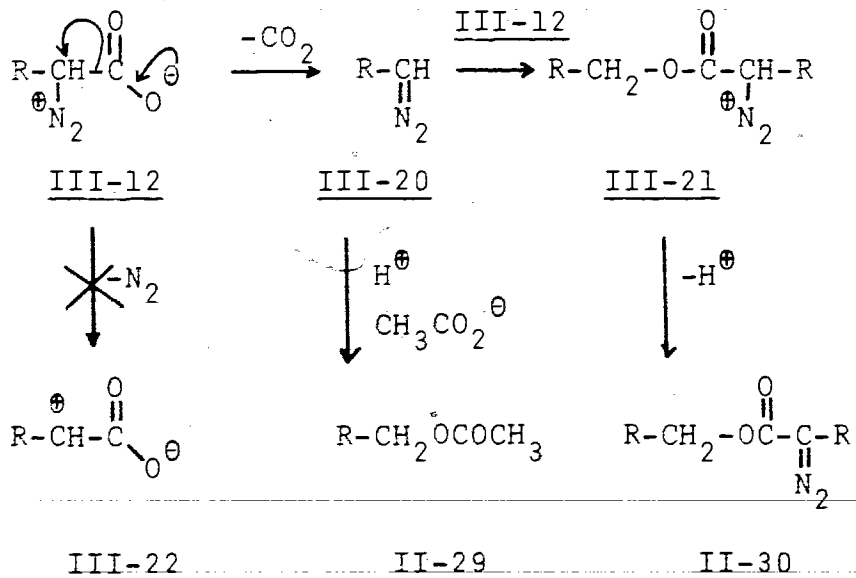


III-19 III-18

Scheme 3-8

The resulting oxadiazolone III-10 is a tautomer of diazo acid III-9. In methanol the latter decomposes readily to give methoxy acid II-25. In aprotic solvents such as benzene, diazo ester II-30 is the major product and its formation can be explained via the decomposition of diazonium acetate III-12, a tautomeric structure of diazo acid III-9. Alkyl diazonium ions are commonly accepted as reactive intermediates in numerous

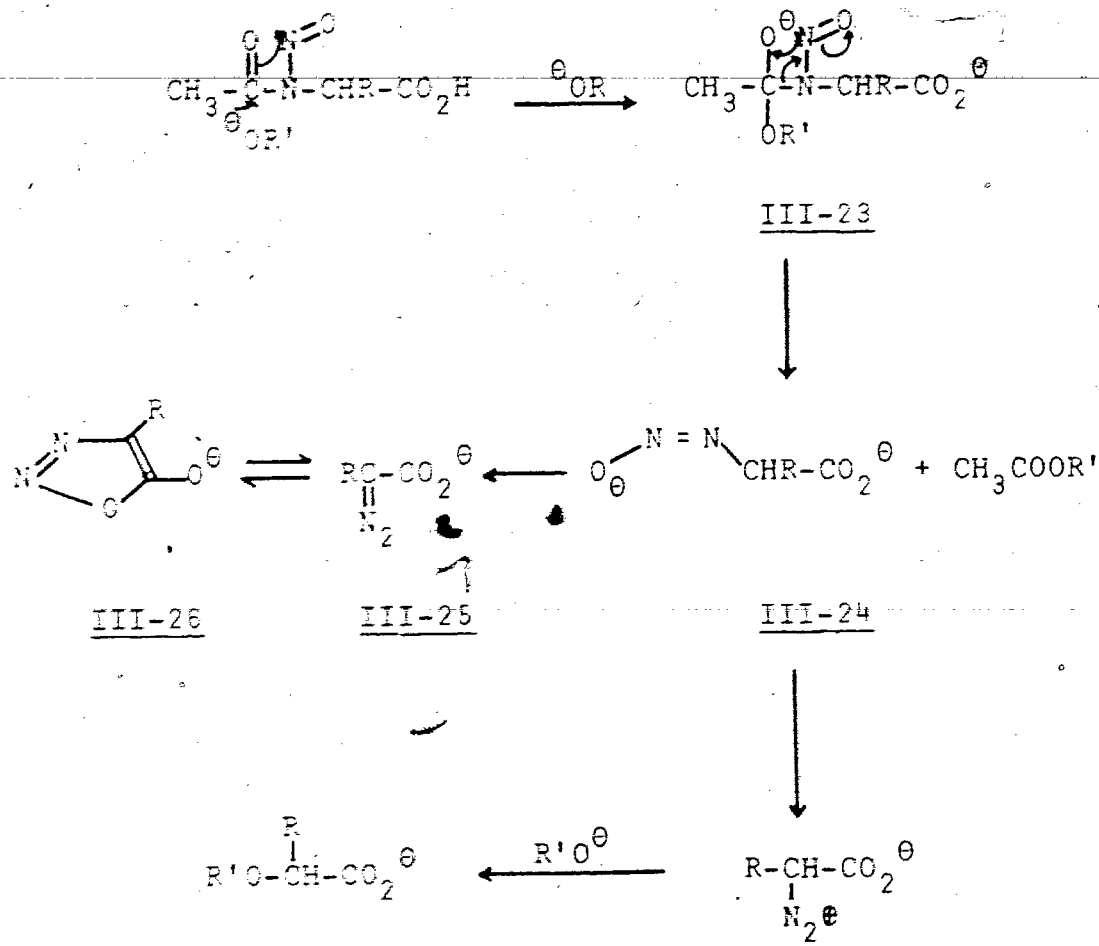
reactions such as diazotization of alkylamines (100). The general fate of these ions is the generation of carbenium ions via the rate determining loss of nitrogen (101). However, elimination of nitrogen from III-12 to give the corresponding carbenium ion is not likely to occur because of the destabilizing effect of the carboxylate anion. On the other hand, it is probable that diazonium acetate III-12 undergoes decarboxylation to give diazoalkane III-20. Esterification of III-12 by diazoalkane III-20 followed by proton elimination results in the stable diazoester II-30. Similar deprotonation has already been observed in the diazotization of ethyl aminoacetate (102) and in the thermolysis of ethyl N-acetyl-N-nitrosoglycinate (58) to give ethyl diazoacetate. Alternatively, diazoalkane



Scheme 3-9

III-20 may react with acetic acid to give phenylethyl acetate II-29.

Kinetic studies (46) have shown that the catalytic rate constant for the decomposition of nitrosamides by $OH^{\ominus}$ is 5.6 X 10^6 times greater than that by $AcO^{\ominus}$. Therefore, in the presence of an excess of potassium hydroxide or sodium methoxide the intramolecular attack by the acetate anion is not likely. In methanol or in water, the initial attack of the base is more likely to occur at the carbonyl rather than at the nitroso nitrogen (43) to give III-23 which rearranges readily into diazotate III-24. In analogy to alkyl diazotates which generate diazo derivatives in strongly basic media (44), diazotate III-24 decomposes to give diazo carboxylate III-25. Diazo derivatives such as III-25 have been known to exist in solution (103) as well as in the solid state (104). These derivatives are stable in alkaline solution but decompose rapidly with evolution of nitrogen on neutralization (105). The uv spectra of such derivatives have not been reported. However, it is believed that the two absorptions at 300 and 410 nm exhibited by species X are due to diazo carboxylate III-25. The 410 nm absorption is probably due to the $n \rightarrow \pi^*$ transition of the diazo linkage whereas that at 330 nm may likely be due to a small contribution of oxadiazole III-26 in equilibrium with III-25. Characterization of the structure of diazocarboxylate



III-23

III-26 III-25 III-24

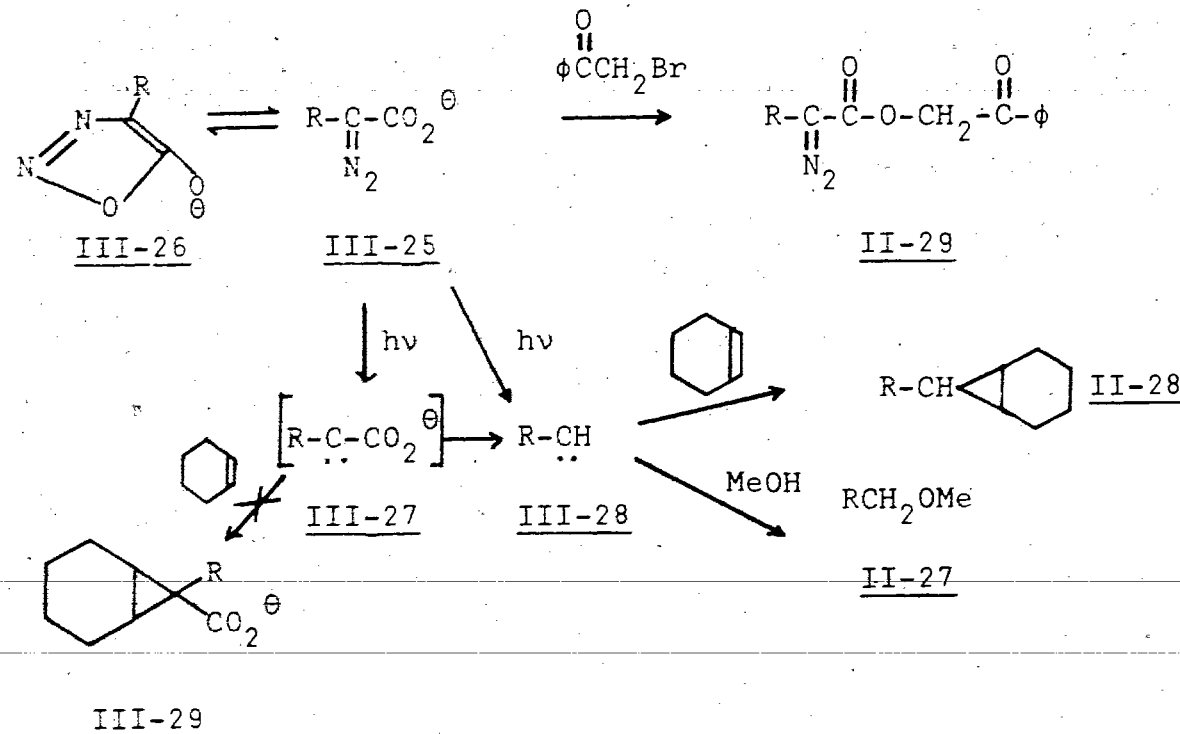
III-12

R' = H, CH_3

R = $C_6H_5CH_2$

Scheme 3-10

III-25 was attempted by alkylation with phenacylbromide. The 2080 cm^{-1} ir absorption appearing in the crude product could be attributed to the stretching frequency of the diazo linkage of diazoester II-29 However, isolation of II-29 could not be achieved. In analogy to diazoalkanes, photolysis of diazoboxylate III-25 was expected to generate carboxylate carbene III-27. However, photolysis of diazocarboxylate III-25 in the presence of cyclohexene did not give the expected addition product III-29. The formation of ether II-27 and bicyclo adduct II-28 indicated that a decarboxylation has occurred at a certain stage to generate phenylethylcarbene III-28 which is trapped by methanol and cyclohexene, respectively.

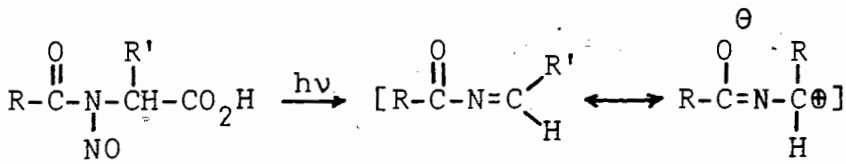


Scheme 3-11

The gas evolution occurring upon addition of an excess of base to aqueous or methanolic solutions of nitrosamide acid II-22 indicated partial decomposition. The volume of nitrogen evolved at room temperature indicated that nitrosamide acid II-22-a deaminated to an extent of approximately 40%. Furthermore, decomposition of II-22-a in methanol with sodium methoxide, followed by careful removal of methanol and acidic work up gave 48% of methoxy acid II-25. These results are indicative of a dual pathway for the decomposition of diazotate III-24 formed from II-22-a by the action of a base. The most likely alternative to the diazoacetate III-25 pathway is the formation of diazonium ion III-12 followed by nucleophilic displacement by hydroxyl or methoxide anions.

III-3 Photolysis of N-Acyl-N-Nitroso-α-Amino Acids

Irradiation of the $n \rightarrow \pi^*$ transition of nitroso derivatives of N-acyl-α-amino acids results in an efficient decarboxylation. The photoreaction has been proposed (28) to involve the corresponding imine intermediate (III-31) as in the case of N-alkyl-nitrosamino acids. Since the C=N bond of N-acyl-imine III-31 is conjugated with the π system of the carbonyl, one would expect this intermediate to be very susceptible to nucleophilic attack. Accordingly, products arising from the



II-22 III-31 III-31-a

R = alkyl, aryl

Scheme 3-12

nucleophilic addition of methanol, enamide, amide or cyanide were observed in both polar and non-polar solvents. Although nucleophilic attack of $NO^{\ominus}$ on acyl-imine III-31 is believed to be occurring*, particularly under basic conditions, neither the corresponding C-nitroso nor its tautomeric oxime could be detected. The absence of products derived from the nitroxyl addition to the N-acylimine under neutral conditions is at first surprising since N-alkylimines II-15 did undergo addition followed by tautomerization to give the corresponding amidoxime as the major product. The pKa of the conjugate acids of N-alkylimine cannot be measured due to their instability but are expected to be at least as high, if not higher, as those of diphenylketimines (pKa = 5-7) (106). Since nitroxyl has been proposed to be of a similar acid strength as acetic acid (pKa = 4.75) (107), addition of nitroxyl to N-alkylimine is believed to occur via the iminium ion. On the other hand, the basicity of the nitrogen of N-acylimine is greatly reduced due to the presence of the electron withdrawing carbonyl group and

* A radical mechanism is ruled out because of the pH dependence of the reaction.

hence is not basic enough to induce the dissociation of HNO. Thus, the products are derived from the addition of methanol which is present in larger concentration than nitroxyl. However, in the presence of an external base such as triethylamine, the nucleophilic species $NO^{\ominus}$ is formed which readily attacks the N-acylimine to give the corresponding C-nitroso intermediate.

The formation of N-acylimine III-31 can be visualized via a radical mechanism* as shown in Scheme 3-13. As in the case of dialkylnitrosamides, it is likely that photolysis of nitrosamido acids generates amido radical III-32 which decarboxylates readily to give III-33. Further proton elimination from III-33 gives N-acylimine III-31. Although amido radicals are known to readily undergo inter (33) as well as intramolecular (32) hydrogen atom abstraction, the parent amide II-21 was observed only as a minor product. Decarboxylation of III-32 must, therefore, occur at a much faster rate than that of hydrogen atom abstraction.

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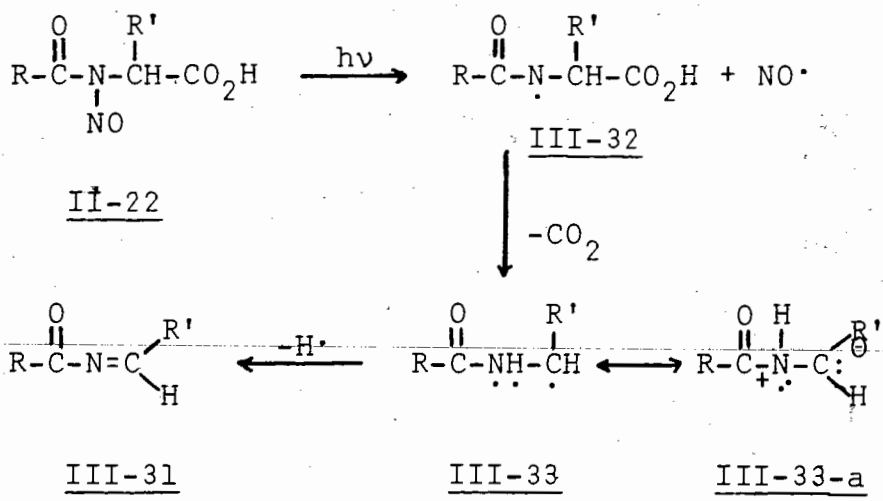
* A concerted elimination of HNO and CO_2 similarly to N-alkylnitrosamino acids cannot, however, be completely ruled out.



II-22

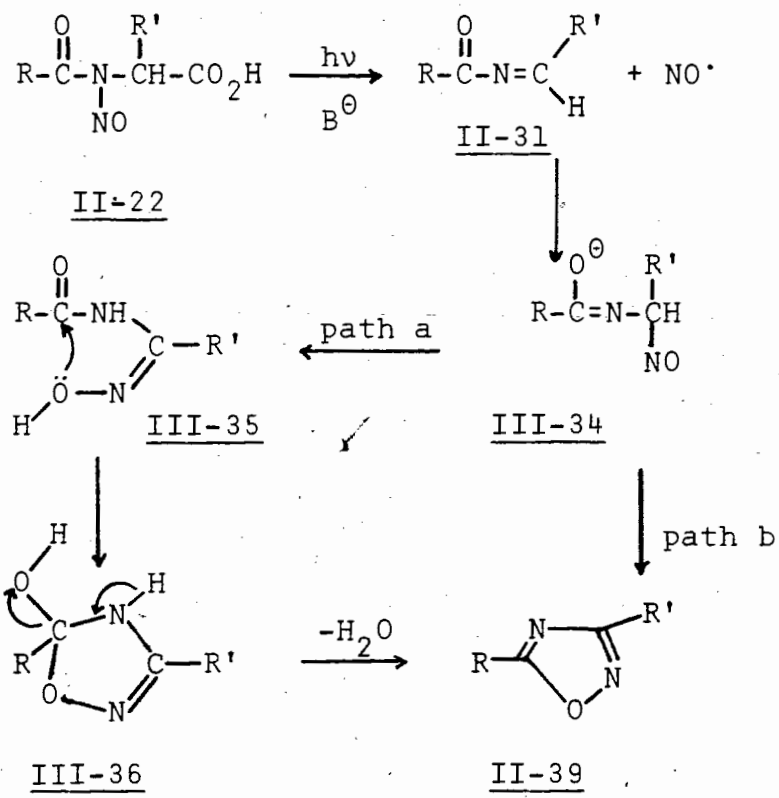
Scheme 3-13

Under mildly basic conditions, the photodecomposition of nitrosamido acids II-22 is much faster than the thermal decomposition and results in the formation of 1,2,4-oxadiazole II-39 in 12-70% yield. The variation of the yield of oxadiazole II-39-a with the concentration of the base is in agreement with the alleged role of the externally added base.

It appears that the optimum condition for the formation of oxadiazole II-39 requires at least two mole equivalents of base (see Fig. 2-14). Under these conditions the acid-base equilibrium is shifted towards the formation of $NO^{\ominus}$ anion.

The formation of oxadiazole II-39 can be satisfactorily explained by the intramolecular cyclization of oxime III-35, as shown in path a of Scheme 3-14. Such mechanism has been

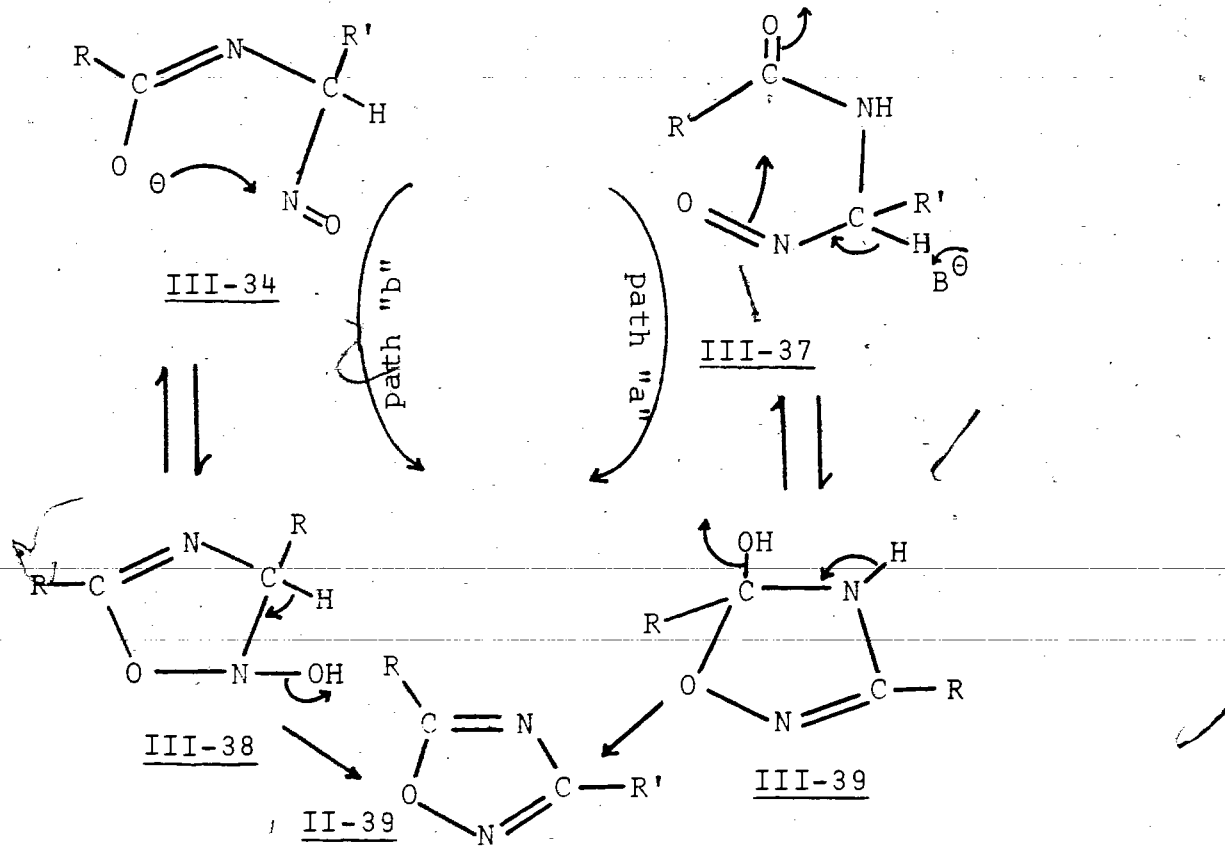
proposed in the synthesis of carbethoxyoxadiazole by nitrosation of an acylaminomalonic ester (108). The postulated N-acylamidoxime III-35 has however never been isolated. In order to confirm this mechanistic route, a mixture of Z and E-isomers of N-acylamidoxime II-49 was synthesized by another route. In the presence of a base, one would expect the Z-isomer to undergo cyclization to give the corresponding oxadiazole. However, the oxime failed to give any trace of oxadiazole II-39-a under



Scheme 3-14

various basic conditions, thus ruling the above mechanism out.

Since amidoximes III-35 were neither isolated nor detected in the photoreactions of nitrosamido acids II-22 under basic conditions, it is very likely that C-nitroso III-34 undergoes intramolecular cyclization (path b in Scheme 3-14) at a much faster rate than that of tautomerization to the corresponding oxime. The intramolecular cyclization of III-34 can occur either by the initial attack of the carboxamide oxygen on the nitroso nitrogen atom (path b) or by the attack of the nitroso oxygen on the carbonyl group (path a) as shown in Scheme 3-15. In view of the total lack of oxime III-35 in the product mix-



Scheme 3-15

ture, it may be assumed that the intramolecular attack as in path b occurs from anion III-34 as soon as it is formed. Similar nucleophilic attack on the nitroso nitrogen atom has been observed previously (109 - 115). Furthermore, hydroxylamine derivatives such as III-40 formed in the aldol-type condensation of aromatic nitroso compounds with active methylene groups (Erlich-Sachs reaction) readily lose a molecule of water to form anils III-41 (116). Similar dehydration from III-38 to give the stable oxadiazole would be expected to be facile.

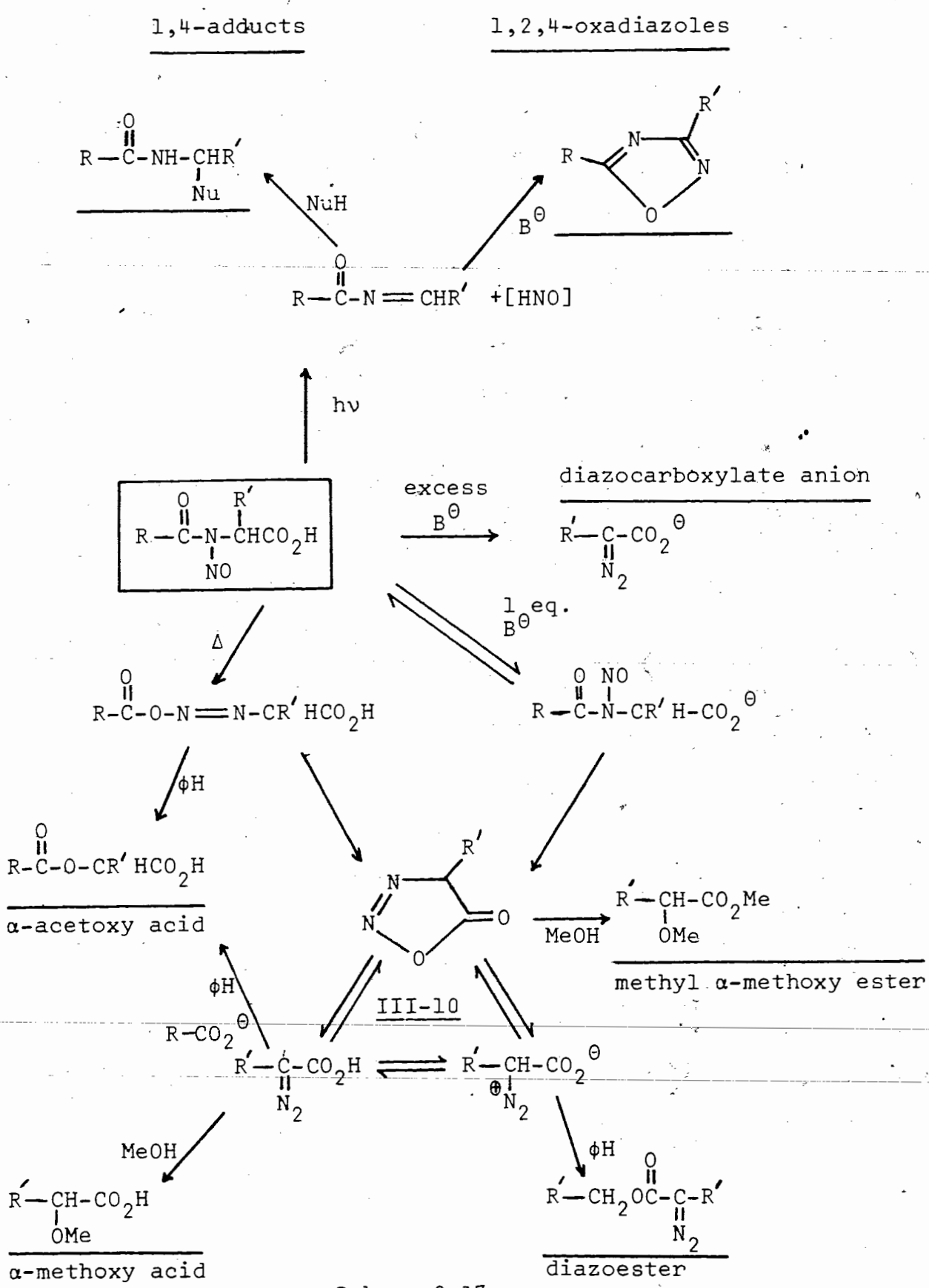
$$ArNO + CH_2XY \longrightarrow \underset{\text{III-40}}{ArN(OH)CHXY} \xrightarrow{-H_2O} \underset{\text{III-41}}{ArN{=}CXY}$$

Scheme 3-16

III-4 Conclusion

The synthesis of α-amidoximes is of particular interest since some of them are known to possess antibacterial activity (117). Primary α-amidoximes are readily prepared by the reaction of hydroxylamine with nitriles (107). However, N-alkylated α-amidoximes are not easily accessible. The photooxidative decarboxylation of N-alkyl-n-nitroso-α-amino acids described in this thesis provides an efficient and simple route to this class of compounds.

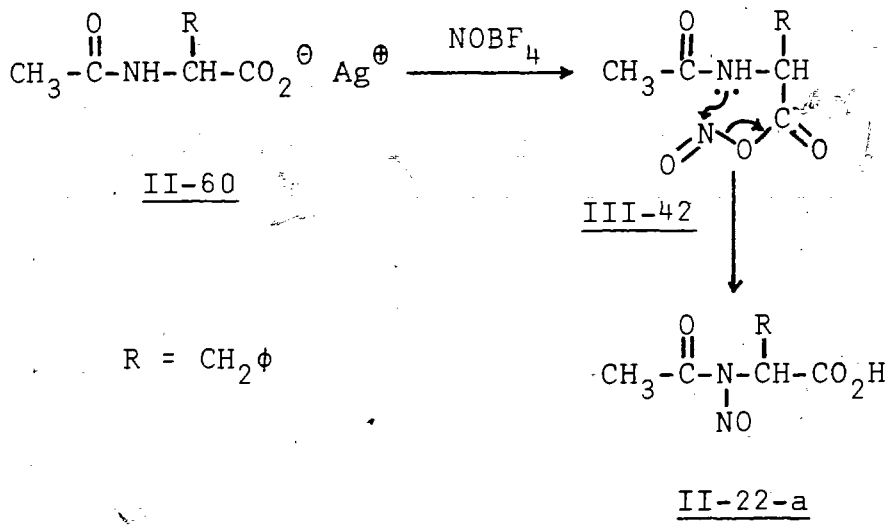
The chemistry of nitrosamines derived from N-acetylated α-amino acids, investigated in the present work (Scheme 3-17), shows that these compounds are very versatile. Their photolysis in neutral conditions is one of the best method to generate the synthetically useful (118) N-acylimine intermediate. Under basic conditions, their photolysis provides an efficient approach to the 1,2,4-oxadiazole skeleton. Some derivatives of 1,2-4-oxadiazole have been found to possess biological activities (119). Under thermal conditions, nitrosamido acids may have generated the elusive 1,2,3-oxadiazol-5-one (III-10) via esters of diazotic acid. In the presence of weak bases or of one or less than one equivalent of strong bases, intramolecularly catalyzed deamination occurs, which may have also generated III-10 as intermediate. Finally in the presence of


1,4-adducts
1,2,4-oxadiazoles
NuH
B⊖
+[HNO]
hν
excess B⊖
diazocarboxylate anion
1 eq. B⊖
Δ
ϕH
α-acetoxy acid
MeOH
methyl α-methoxy ester
III-10
R-CO2⊖
α-methoxy acid
diazoester

Scheme 3-17

an excess of strong bases the stable diazocarboxylate anion III-25 is generated.

Results in §II-8 show that nitrosation of the amido group of an α-amido acid can be achieved via an acyl nitrite intermediate (III-42) which is usually obtained by the reaction of a carboxylate anion with a source of NO^+ (120). It is believed that specific in vivo nitrosation of the terminal amido group of peptides can occur through a five-membered transition state as shown in Scheme 3-18. The nitrosating species $NO^{\oplus}$ may arise



Scheme 3-18

from sodium nitrite or from dialkylnitrosamines by transnitrosation (131). In view of the results obtained in the present work, it is possible to visualize an in vivo degradation of

peptides *via* the intramolecularly catalyzed deamination of the terminal peptidic linkage, leading to diazoalkane derivatives, which are known to be a likely cause of the carcinogenic activity of nitrosamines.

CHAPTER IV

EXPERIMENTAL

IV-1 General Techniques

Unless otherwise indicated the following general conditions prevail. Infrared (ir) spectra were measured on a Perkin-Elmer model 457 as liquid film or nujol mull. The absorption bands (cm^{-1}) are designated as s, m, w or b for strong, medium, weak, or broad respectively. Ultraviolet (uv) spectra were recorded on a Unicam SP 800 or a Cary 17 spectrophotometer. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian A 56/60 or a Varian XL-100 spectrophotometer equipped with a Nicolet 1080 computer using deuterochloroform as solvent and TMS as internal standard. The chemical shift for the proton nmr spectra are reported in τ values, coupling constants (J) and half-height widths (W1/2) in hertz (Hz). The splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), qi (quintet), sp (septet), m (multiplet), b (broad) and the number of protons relative to each signal is indicated as a multiple of H. The D_2O exchangeable proton is indicated by D_2O exch. . In the ^{13}C nmr spectra, the chemical shifts are reported in δ values relative to TMS and the splitting patterns resulting from off resonance decoupling (ord) are indicated in

parenthesis. The decoupling experiments were done on the same XL-100 spectrometer. The experiments were performed by Mr. A. Brooke, Ms. E. Cheah or by the author. The mass spectra (ms) and the gas chromatography mass spectra (gc-ms) were obtained by Mr. G. Owen on a Hitachi-Perkin-Elmer RMU-7 mass spectrometer coupled with a System Industries data acquisition system/150. High resolution mass spectra (hrms) were performed at the University of British Columbia mass spectrometric services.

The gas chromatographic (gc) analyses were performed on a Varian 1400 chromatograph equipped with a flame ionization detector. Preparative gc runs were executed on a Varian 1700 equipped with a thermal conductivity detector. The retention times (rt) are reported in minutes (min). The thin layer chromatographic (tlc) analyses were performed on silica gel impregnated with uv indicator or on alumina and were then developped with iodine. Separations by column chromatography were performed using neutral or basic alumina (Brockman activity I, Fisher Scientific Co, 80-200 mesh) or silica gel (Baker analysed, 60-200 mesh).

Melting points were measured on a Fisher-Johns hot stage and were not corrected. Elemental analyses were performed by Mr. M.K. Yang with a Perkin-Elmer 240 microanalyser.

IV-2 Chemicals

The solvents were reagent grade and distilled prior to use. Benzene (distilled from H_2SO_4) was stored over sodium ribbon, acetonitrile (distilled over P_2O_5) and methanol (distilled from magnesium) were kept over molecular sieve 3A. The following amino acids: sarcosine (Fluka), D,L-phenylalanine (BDH), D,L-serine (BDH) and D,L-leucine (BDH) were reagent grade and used without further purification. Triethylamine was distilled (bp: 87°C) and stored over potassium hydroxide pellets. The nitrogen gas used was scrubbed with Fieser's solution, followed by concentrated H_2SO_4 and potassium hydroxide pellets.

IV-3 General Procedure for Photolysis

The photolyses were carried out in a previously described (32) photovessel using a pyrex cold finger. The condensor was fitted with a calcium chloride tube or a mercury trap. The reactants were dissolved in the appropriate solvent and the resulting solution was introduced in the photocell. The solution was magnetically stirred while a stream of dry nitrogen was bubbled through the gas inlet for 10 to 15 min. before the start of the irradiation. When required, the solution was cooled by immersing the photocell in an external ice bath. The solution was then irradiated by placing a Hanovia 654 A36 (200 w) medium pressure mercury lamp into the lamp well. The reaction was monitored by recording the uv spectrum of diluted aliquots of

the photolysate taken at regular intervals. The mixture was photolysed until the $n \rightarrow \pi^*$ absorption of the N-nitroso group (ca. 350nm for nitrosamines and ca. 400nm for nitrosamides) had completely disappeared. The zero hour aliquot was kept under the same conditions but in the dark and its uv spectrum, recorded after completion of the reaction showed no appreciable change. This assured that no dark reaction had taken place. The solvent was removed under vacuum at ca. 10°C using a rotatory evaporator. The residue was examined by tlc and ir and nmr spectroscopy and the different components were separated by means of usual extractions and chromatographic techniques.

IV-4 General Methods of Nitrosation

All N-alkyl-N-nitroso-amino acids were recrystallized before use, but the N-acyl-N-nitroso-amino acids were used as obtained without any further purification.

IV-4-1 Method A: Sodium Nitrite ($NaNO_2$) Nitrosation

An amino acid (0.01 mole) was dissolved in water (50 ml) containing concentrated HCl (2 ml, 0.06 mole). A solution of $NaNO_2$ (1g, 0.015 mole) in water (20 ml) was slowly added to the ice cold and stirred solution of amino acid. After completion of the addition, the mixture was further stirred for 2 hours at 0°C. In the reactions where the nitroso derivative precipated, filtration, washing with cold water and drying over P_2O_5 gave

the crude nitroso compound. When no precipitate was obtained the reaction mixture was extracted with ethyl acetate and in some cases continuously extracted with ether or ethyl acetate. The extracts were dried over $MgSO_4$ and evaporated to give the solid nitroso-amino acid.

IV-4-2 Method B: Dinitrogen Tetroxide (N_2O_4) Nitrosation

The method described by White (29) was modified as below. N_2O_4 (MCB) was bubbled through ice cold dry CH_2Cl_2 (50 ml) until the solution gained 1g (0.01 mole) in weight. The resulting yellow solution was cooled to -78°C with a dry ice-acetone bath and fused sodium acetate (800mg, 0.01 mole) was added at once. The colour of the solution turned steel grey. The amino acid derivative (0.01 mole) was added in several portions. When the addition was complete, the solution was allowed to come to ice temperature while stirred in the dark. Stirring was continued for another 2 hours. The reaction mixture was washed with dilute Na_2CO_3 (1%) several times until the pH of the washings reached 5-6. The organic phase was washed with water and dried over $MgSO_4$. Evaporation of the solvent afforded the crude nitroso derivative.

IV-4-3 Method C: Nitrosyl Tetrafluoroborate Nitrosation (30)

An amino acid (0.01 mole) was added in several portions to a cooled (ice-salt bath) and stirred suspension of $NOBF_4$ (D.F.

Goldsmith Cie.) (1.57 g, 0.014 mole) in dry acetonitrile (10 ml) After completion of the addition stirring was continued for an additional hour and the mixture was evaporated to dryness. The residual solids were extracted several times with ethyl acetate or ether. The combined extracts were washed with a saturated solution of NaCl, dried over $MgSO_4$ and evaporated to yield the crude nitroso derivative.

IV-5 Preparation of N-Alkyl-N-Nitroso- α-Amino Acids

IV-5-1 N-Nitrososarcosine, II-1-a

Nitrosation of sarcosine (5g, 0.06 mole) was carried out as described in methods A or C to give II-1-a (6.1g, 85%): 69-70°C, reported 66-67°C (42) ir: 1730 (s) and 1440 (s) cm^{-1}; ^1H and ^{13}C nmr data, see tables 2-1 and 2-2 ; ms and uv data, see tables 2-4 and 2-5 . When the ^1H nmr spectrum was recorded immediately after dissolution of the sample, the ratio of the two sets of singlets at τ 5.72, 6.10 and 5.02, 6.87 was approximately 9:1, and gradually changed to become 1:1 after staying at room temperature and in the dark for one day. The same observation was made in ^{13}C nmr experiments: only one set on signals (at 167.2, 44.9 and 38.6 ppm) appeared when the spectrum was recorded shortly after dissolution, and a new set (at 169.5 53.7 and 31.4 ppm) gradually increased to reach the same intensity as that of the first one ater 3 hours in the probe (30-40°C). Two ORD spectra with different decoupler offset values were recorded and the $J^{13}C$-H were measured (see table 2-1). The ^{15}N natural abundance spectrum was measured in the fourier mode with a Varian XL-100 at 10.135 MHz. The spectrum was taken

as a degassed neat oil, in a 12 mm tube at the ambient probe temperature (35- 40°C). External ^{1}H lock and noise decoupling were used. In order to obtain a signal-to-noise ratio greater than three, 4785 60° pulses were accumulated with a 10 sec repetition rate. Calibration was achieved by means of an acidified ammonium chloride sample (1M $^{15}NH_4Cl$ in 2M HCl). The chemical shifts obtained are reported in the frequency as well as in the shielding constant scale in table 2-7.

^{15}N enriched nitrososarcosine (150 mg, 63%) was prepared by nitrosation (method A) of sarcosine (180 mg, 0.002 mole) with 50% ^{15}N enriched $NaNO_2$ (Isomet, Palisades Park, N.J, USA) : ^{15}N nmr (CH_3OH) δ ppm from $^{+}NH_4Cl$: 508.4 (s) and 514.8 (s). The two singlets showed the same intensity. The spectrum was recrded one day after preparation of the sample. A number of 256 scans was accumulated with a delay time of 30 sec between pulses. External ^{1}H lock was used and the spectrum was recorded without noise decoupling.

Table 4-1 $J^{13}C$-H of Nitrososarcosine at different decoupler offset values

decoupler offset Hz	$J^{13}CH_2$		$J^{13}CH_3$	
	C_Z	C_E	C_Z	C_E
56001	65	70	70	75
54001	35	40	30	25

IV-5-2 N-Nitrososarcosine Lithium Salt, II-14-a

An equivalent amount of LiOH (10 ml, 0.47 N in H_2O) was added to a solution of II-1-a (650 mg, 5.5 mmole) in H_2O (5 ml). The resulting solution was concentrated under vacuum to about 5 ml and acetone was added until the solution became turbid. Upon cooling, white crystals appeared and after filtration and drying over P_2O_5 gave II-14-a (388 mg, 57%); mp 250°C; ir ν_{max}: 1630(s), 1440(m,b), 1340(m), 1290(m), and 700(m) cm^{-1}; ^1H nmr (D_2O) τ: 5.25 (s), 5.78(s), 6.23(s), and 6.77(s); uv λ_{max} (ε): 436 (84) nm. Anal. calcd. for $C_3H_5N_2O_3Li$: C 29.05, H 4.06, N 22.59; found: C 30.07, H 4.31, N 22.19

When taken immediately after dissolution, the intensity of the set of singlets at τ 5.78 and 6.23 was approximately 24 times as large as that of the singlets at τ 5.25 and 6.77. However the latter increased slowly to finally reach the same intensity as the previous one.

The pH of a solution of II-14-a in water (0.1 M) was measured to be 5.66

IV-5-3 N-Nitrososarcosine Sodium Salt, II-14-b

An equivalent amount of NaOH (18.3 ml, 0.68 N in MeOH) was added to a solution of II-1-a (1.46 g, 12.4 mmole) in methanol (20 ml). The solution was evaporated under vacuum to about 10 ml and was cooled in the fridge to give white crystals. The crystals were filtrated and washed with acetone and ether and were dried over P_2O_5 to afford II-14-b as white needles (1.2 g, 70%); mp 250°C; ir ν_{max}: 1600 (s), 1440 (m), 1380 (m), 1340 (m), 1290 (m), 980 (m) 770 (m), and 690 (m) cm^{-1}; ^1H nmr (D_2O) τ: 5.2 (s), 5.78 (s), 6.17 (s) and 6.85 (s); uv λ_{max} (ε): 437 (91) nm. Anal. calcd. for $C_3H_5N_2O_3Na$: C 25.72, H 3.60, N 20.00; found: C 25.78, H 3.58, N 20.03.

When the nmr spectra was taken immediately after dissolution, the intensity of the set of singlet at τ5.2 and 6.85 was approximately 25 times as large as that of the singlets at τ5.78 and 6.17. The latter increased slowly to finally reach the same intensity as that of the former set.

The pH of a solution of II-14-b in water (0.1 M) was measured at 25 °C to be 5.10.

IV-5-4 N-Nitrososarcosine Potassium Salt, II-14-c

A molar equivalent of KOH (5.4 ml, 1.24 N in MeOH) was added to a solution of II-1-a (804 mg, 6.8 mmole) in MeOH (10 ml). The solution was concentrated under vacuum to about 5 ml and acetone was added dropwise until the solution became turbid. The crystals deposited upon cooling at 0 °C were filtrated and dried over P_2O_5 to give II-14-c (570 mg, 54%) as very hygroscopic white needles; ir ν_{max}: 1600 (s), 1290 (s), 1190 (m), 1050 (m), 975 (m), 770 (m) , and 685 (m) cm^{-1}; ^1H nmr (D_2O) τ: 5.20 (s), 5.78 (s), 6.17 (s), and 6.85 (s); uv λ_{max} (ε): 438 (88) nm. Anal. calcd. for $C_3H_5N_2O_3K$: C 23.07, H 3.23, N 17.93; found: C 23.02, H 3.21, N 18.12.

Immediately after dissolution, the nmr singlets at τ5.78 and 6.85 and those at τ5.20 and 6.17 had their intensity in a ratio of 57:43 and slowly changed to a 1:1 ratio.

The pH of a solution of II-14-c in water (0.1 M) at 25 °C was measured to be 4.49.

IV-5-5 N-Nitrososarcosine Dicyclohexylamine Salt, II-14-d

Dicyclohexylamine (Aldrich, 3.4 ml, 0.018 mole) was slowly added to a solution of II-1-a (2.1g, 0.018 mole) in EtOH (10ml) at room temperature. The salt II-14-d (3.1g, 69%) was obtained as pale yellow needles; mp 168-170°C (lit. (67) mp 175-176°C); ir ν_{max}: 1640 (s), 1450 (m), and 760 (m) cm^{-1}; ^{1}H nmr (D_2O) τ: 5.21 (s), 5.78 (s), 6.15 (s), 6.75 (s), 6.75 (s), 6.7 (bs), and 8.5 (m); ^{13}C nmr (D_2O) δ: 143.2 (s), 55.3 (t), 48.3 (t), 38.0(q) 31.4 (q), 49.9 (d), 26.8 (t), 22.6 (t), and 22.0 (t); uv λ_{max}(ε): 437.5 nm (69).

The integration of the singlets at τ5.21 and 6.75 and at τ 5.78 and 6.15 immediatly after dissolution showed a E:Z ratio of 9:1 and changed to 8:1 after one week.

The pH of a solution of II-14-d in water (0.1M) at 25°C was measured to be 7.85.

IV-5-6 N-Ethyl-N-Nitrosoglycine, II-1-b

Tosylation of glycine (MCB, 22.5g, 0.3 mole) (123) gave p-tosylglycine (41.6g, 61%); mp 147-148°C (lit.(123) mp 149-150°C) ir ν_{max}: 3280 (s), 1720 (s), 1160 (s) ,and 820 (s) cm^{-1}; ^{1}H nmr (pyridine) τ: 5.73 (s,2H),and 7.8 (s,3H).

A solution of ethyliodide (MCB, 15g, 0.096 mole) and II-3 (20.6g, 0.09 mole) in 3N NaOH was heated with shaking in a sealed tube at 70°C for 4 hrs. After acidification (pH 2) a solid precipitated out and was recrystallised from water to give

N-ethyl-N-p-tosylglycine II-4 (13.5g,61%) as white needles; mp 142-144°C; ir ν_{max}: 1730 (s), 1255 (s),and 700 (s) cm^{-1}; ^1H nmr τ:5.63 (s,2H), 6.45 (q,J=5 Hz,2H), 7.76 (s,3H), and 8.83 (t, J=7 Hz,3H).

A solution of tosylate II-4 (13.5g,0.05 mole) in concentrated HCl (60 ml) was heated in a sealid tube at 100°C for 24 hrs. The solution was evaporated to dryness under vacuum and the resulting residue was taken up in ethanol (150 ml). After filtration and concentration of the ethanolic solution to ca. 50 ml, crystallisation was induced by adding ether. After cooling, N-ethylglycine p-toluenesulfonic acid salt IV-1 (9.8g,71%) was filtered and dried; mp 180-185°C; ir ν_{max}: 1720 (s), 1200 (s), and 690 (s) cm^{-1}; ^1H nmr (D_2O) τ: 1.69 (A of A_2B_2, 2H), 2.28 (B of A_2B_2, J=9 Hz, 2H), 6.08 (s, 2H), 6.84 (q, J= 7Hz, 2H), 7.63 (s, 3H), and 8.70 (t, J=7Hz, 3H).

Salt IV-1 (9.8g, 0.036 mole) dissolved in concentrated HCl (50 ml) was cooled to 0°C overnight to give white crystals of N-ethylglycine hydrochloride II-5 (4.70g,95%); mp 160-166°C; ir ν_{max}: 1750 (s), 1420 (s), 1180 (s), and 790 (s) cm^{-1}; ^1H nmr τ: 6.04 (s, 2H), 6.80 (q, J=7.5 Hz, 2H), and 8.68 (t, J=7.5 Hz,3H).

The hydrochloride II-5 (4.7g;0.032 mole) was nitrosated by method A to yield II-1-b (2.3g,54%) as yellow crystals; mp 83-85°C; ir ν_{max}: 3100-2600 (b,m), 1730 (s), 1460 (s), 1370 (s), 1305 (s), 1210 (s), 1110 (s),and 700 (s) cm^{-1}; ^1H nmr and ^{13}C nmr data are found in tables 2-1 and 2-2 : ms and uv data are in Table 2-5.

IV-5-7 N-Nitroso-N-Isopropylglycine, II-1-c

Bromoacetic acid II-6 was obtained in 27% yield by bromination of acetic acid (123);ir ν_{max}: 1730 (s) and 730 (s) cm^{-1}; ^1H nmr τ:-1.87 (s, D_2O exch.) and 5.98 (s).

Bromo derivative II-6 was esterified (47) to yield ethylbromoacetate II-8 (80%); bp 154-155°C/760 Torr; ir ν_{max}: 1740 (s), 1230 (s), and 1030 (s) cm^{-1}; ^1H nmr τ: 5.73 (q, J=7Hz, 2H), 6.15 (s, 2H), and 8.70 (t, J=7Hz, 3H).

Bromoacetate II-8 was treated with isopropylamine (47) to give ethyl N-isopropylglycinate II-10 (84%); ir ν_{max}: 3340(m), 1740 (s), 1200 (s), and 1030 (m) cm^{-1}; ^1H nmr τ: 5.8 (q,J=7Hz, 2H), 6.6 (s, 2H), 7.18 (sp, J=7Hz, 1H), 8.25 (s, D_2O exch., 1 H), 8.73 (t, J=7Hz, 3H), and 8.92 (d, J=7Hz, 6H).

Glycinate II-10 (12g, 0.082 mole) in aqeous NaOH solution was heated for 1 hr to give a solution of N-isopropylglycine. The solution was nitrosated by method A to yield II-1-c (4.7g, 38%); mp (from water) 137-138°C (lit. (47) mp 76-78°C); ir ν_{max}: 2600(b,s), 1730 (s), 1360 (s), 1240 (s), 1070 (m), 890 (m), 690 (m), and 650 (s) cm^{-1}; ^1H and ^{13}C nmr data, see tables 2-1 and 2-2 ; ms and uv data, see tables 2-4 and 2-5. No other signal appeared in the ^1H nmr spectrum when the sample was left in solution at room temperature for two weeks.

IV-5-8 N-Nitroso-N-(3-Phenylpropyl)-Glycine, II-1-d

A solution of ethylbromoacetate II-8 (16.6g, 0.1 mole) and 3-phenylpropylamine (BDH, 27g, 0.2 mole) in benzene (80 ml) was refluxed for 5 hours. Filtration of the precipitate and evaporation of the solvent gave a crude oil (17.9g) which upon distillation (bp 191°C/29 Torr) gave pure N-(3-phenylpropyl)-glycine ethyl ester II-11 (14.3g, 64%); n_D^{26} 1.4980; ir ν_{max}: 3320 (m), 3060 (w), 3020 (m), 1730 (s), 1200 (s), 1020 (s), 740 (m) and 700 (s) cm^{-1}; ^1H nmr τ: 2.84 (m, 5H), 5.88 (q, J=7Hz, 2H), 6.68 (s,2H), 7.4 (m, 4H), 8.06 (s,D_2O exch., 1H), 8.2 (m, 1H), and 8.78 (t, J=7Hz, 3H).

Ester II-11 (15g, 0.07 mole) was saponified with sodium hydroxide (5g in 50 ml H_2O) and after acidification to pH 2, yielded N-(3-phenylpropyl)-glycine hydrochloride IV-2 (15.3g, 98%); ir ν_{max}: 3500 (b,m), 2400 (b,m), 1740 (s), 1580 (m), 1410 (m), 740 (m) and 690 (s)cm^{-1}.

Hydrochloride IV-2 (4.6g, 0.02 mole) was nitrosated by method A to yield II-1-d (3.1g, 70%); mp 106-108°C (from water); ir ν_{max}: 3100-2840 (b,m), 2700 (m), 2600 (m), 1730 (s), 1450 (m), 1310 (s), 1285 (s), 1210 (s), 740 (m) and 700 (s) cm^{-1}; ^1H and ^{13}C nmr data, see tables 2-1 and 2-2 ; ms and uv data, see tables 2-4 and 2-5 . Anal. calcd. for $C_{11}H_{14}N_2O_3$: C 59.45, H 6.35, N 12.60; found: C 59.43, H 6.47, N 12.86.

When the nmr sample was left in the dark at room temperature, a new set of signals appeared at τ5.00 (s), 6.38 (m), 7.45 (m) and 8.20 (m), whose intensities reached the equilibrium state after two days (ca.15% of those of the initial set of signals).

IV-5-9 N-Nitroso-N-Isopropyl-D,L-Alanine, II-1-e

A solution of α-bromopropanoic acid (Eastman Kodak) in ethanol was refluxed to give ethyl- α -bromopropanoate II-9 ; ir ν_{max}: 1740 (s), 1225 (s) and 1160 (s) cm^{-1}; ^1H nmr τ: 5.63 (q, J=7Hz, 1H), 5.79 (q, J=7Hz, 2H), 8.22 (d, J=7Hz, 3H) and 8.73 (t, J=7Hz, 3H).

Ester II-9 was treated with isopropylamine (47) to give N-isopropyl-D,L-alanine ethyl ester II-12 (85%); ir ν_{max}: 3300 (w), 1730 (s), and 1180 (s) cm^{-1}; ^1H nmr τ: 5.82 (q, J=7Hz, 1H), 6.58 (q, J=7Hz, 2H), 7.22 (sp, J=6Hz, 1H), 8.3 (s, D_2O exch.), 8.70 (d, J=7Hz, 2H), 8.71 (t, J=7Hz, 3H) and 8.94 (d, J=6Hz, 6H).

A solution of ester II-12 in aqueous sodium hydroxide was refluxed for one hr and after acidification to pH 2 was nitrosated directly by method A, to give II-1-e (31%); ir ν_{max}: 2500-3100 (b,m), 1740 (s), 1380 (s), 1220 (m) and 1180 (m) cm^{-1}; ^1H and ^{13}C nmr data, see tables 2-1 and 2-2 ; ms and uv data, see tables 2-4 and 2-5 ; Anal. calc. for $C_6H_{12}N_2O_3$: C 44.99, H 7.55, N 17.49; found: C 45.06, H 7.59, N 17.61.

IV-5-10 N-Nitroso-N-t-Butyl-D,L-Alanine, II-1-f

N-t-butyl-D,L-alanine ethyl ester II-12 was obtained in 40% yield from bromide II-9 and t-butylamine as described by Greco and al (47); ir ν_{max}: 1740 (s), 1370 (s) and 1180 (s) cm^{-1}; ^1H nmr τ: 5.81 (q, J=7Hz, 2H), 6.58 (q, J=7Hz, 1H), 8.3 (bs, D_2O exch.), 8.73 (t, J=7Hz, 3H), 8.75 (d, J=7Hz, 1H) and 8.95 (s, 9H).

The ester II-12 was refluxed in aqueous sodium hydroxide solution and after acidification, was nitrosated by method A to give white crystals (from water) of II-1-f (32%); ir ν_{max}: 3100-2400 (b,m), 1740 (s), 138 (s), 1290 (s) and 720 (d,m) cm^{-1}; ^1H and ^{13}C nmr data, see tables 2-1 and 2-2 ; uv and ms data, see tables 2-4 and 2-5 ;

IV-5-11 N-Nitrosoproline, II-1-g

Compound II-1-g (3.6g, 58%) was obtained by nitrosation of L-proline (MCB, 5g, 0.04 mole) by method A; mp 104-106°C (lit. (121) 109-110°C); ir ν_{max}: 2500 (b,m), 1720 (s), 1370 (s), 1200 (m), 720 (s) and 680 (s) cm^{-1}; ^1H and ^{13}C nmr data, see tables 2-1, 2-3 ms and uv data, see tables 2-4 and 2-5 ;

IV-5-12 N-Nitrosopipecolinic Acid, II-1-h

L-Pipecolinic acid (MCB, 10.5g, 0.81 mole) was nitrosated according to method A to give II-1-h (10.7g, 81%); mp 93-95°C (lit. (121) mp 91-93°C); ir ν_{max}: 3200-2500 (m,b), 1720 (s), 1465 (s), 1405 (s), 1330 (s), 1248 (s), 1190 (s), 1015 (s), 930 (s), and 740 (s) cm^{-1}; ^{1}H and ^{13}C nmr data, see tables 2-1 and 2-3 ; ms and uv data, see tables 2-4 and 2-5 ;

IV-6 Photolysis of N-Nitroso-N-Alkyl-α-Amino Acids

IV-6-1 Photolysis of N-Nitrososarcosine, II-1-a

A solution of II-1-a (1.2g, 0.01 mole) in methanol (150ml), was irradiated for 75 min. until the absorption at 350 nm completely disappearred. The solvent was removed under reduced pressure to yield a yellowish solid (670mg); mp 105-115°C; ir ν_{max}: 3400 (m), 1680 (m), 1320 (m), 1280 (m), 1020 (s), 900 (m) and 750 (m) cm^{-1}; ^{1}H nmr (D_2O) τ: 3.00 (s, 1H), 7.12 (s, 3H). Recrystallization from benzene yielded N-methyl-formamidoxime II-16-a(572mg, 72%) as white plates; mp 134-135°C; ir ν_{max}: 3400 (m), 3100-2900 (b,m), 1680 (m), 1320 (m), 1290 (m), 920 (m), 900 (m) and 750 (m) cm^{-1}; ^{1}H nmr (D_2O) τ: 3.00 (s,1H), and 7.12 (s,3H); ms m/e (%): 74.0486 (M^+, 69, calc. for $C_2H_6N_2O$: 74.0480), 57 (57), 42 (54), 30 (100) and 28 (84). Subblimation at room temperature and at 1.5 Torr yielded an anlytical sample; Anal. calc. for $C_2H_6N_2O$: C 32.43, H 8.16, N 37.81; found: C 32.58,

H 8.21, N 37.59.

IV-6-2 Photolysis of N-Ethyl-N-Nitrosoglycine, II-1-b

The residue (910mg) obtained from the photolysis of II-1-b (1.06g, 0.008 mole, 90min.) in methanol (120ml) at room temperature showed virtually the same ir and nmr spectra as those of N-ethyl-formamidoxime II-16-b . The residue was treated with hot benzene to give some insoluble material (56mg) which gave a positive ninhydrin test and showed comparable ir and nmr spectra with those of N-ethylglycine. Upon cooling, the benzene solution yielded formamidoxime II-16-b (449 mg, 69%) as white crystals; mp 81-83°C; ir ν_{max}: 3360 (m), 3200 (m,b), 2800 (b,s), 1680 (s), 1490 (m), 1260 (m), 1160 (m), 940 (s), 890 (s), 750 (m) and 730 (m) cm^{-1}; ^1H nmr τ: 3.3 (s, 1H), 6.89 (q, J=7Hz, 2H),7.1 (s, D_2O exch.), and 8.83 (t, J=7Hz, 3H); ms m/e (%): 88.0639 (M^+, 93, calcd. for $C_3H_8N_2O$: 88.0630), 73 (67), 71 (42), 55 (58), 44 (100), 43 (62), 42 (38) and 30 (67); Anal. calcd.for $C_3H_8N_2O$: C 40.90, H 9.15, N 31.79; found: C 40.97, H 9.03, N 31.52.

IV-6-3 Photolysis of N-Nitroso-N-Isopropylglycine, II-1-c

The crude solid (655 mg) obtained from the photolysis of a solution of II-1-c (1.02 g, 0.007 mole, 45 min.) in methanol (100 ml) was almost pure II-16-c as shown by its ir and nmr spectra; mp 40-44°C. The solid was subblimed under 0.1 Torr, at room temperature, to give N-isopropyl-formamidoxime II-16-b,

(540 mg, 76%); mp 46-48°C; ir ν_{max}: 3380 (m), 3250 (b,m), 1675 (s), 1380 (d,m), 1340 (m), 1270 (m), 900 (m), and 855 (m) cm^{-1}; ^1H nmr (D_2O) τ: 2.93 (s, 1H), 6.48 (sp, J=7Hz, 1H) and 8.8 (d, J=7Hz, 6H); ms m/e (%): 102.0799 (M^+, 36, calcd. for $C_4H_{10}N_2O$: 102.0793), 87 (47.1), 71 (15.5), 69 (33), 60 (32), 58 (23), 44 (100), 43 (94), 42 (69), 41 (66) and 39 (25). Anal. calcd. for $C_4H_{10}N_2O$: C 47.04, H 9.87, N 27.43; found: C 46.77, H 9.66, N 27,25.

IV-6-4 Photolysis of N-Nitroso-N-(3-Phenylpropyl)glycine, II-1-d

In methanol: A methanolic solution (230 ml) of II-1-d (1.57 g, 0.007 mole) was photolysed under nitrogen, at room temperature for 1.5 hours until the absorption at λ=350 nm had completely disappeared. A slightly pink solid (1.46 g) was obtained after evaporation of the solvent, which showed ir and nmr spectra similar to those of amidoxime II-16-d. This residue showed two spots on tlc (silica gel, 10% MeOH in $CHCl_3$) at Rf: 0.45 and 0.05 (positive to ninhydrin test). Recrystallization from cyclohexane gave N-(3-phenylpropyl)-formamidoxime II-16-d (1.02 g, 82%) as white plates; mp 100-101°C; ir ν_{max}: 3350 (m), 3050 (m), 2750 (b,m), 1680 (s), 1490 (m), 1450 (m), 1330 (m), 1240 (m), 900 (s), 770 (s), 740 (s) and 690 (s) cm^{-1}; ^1H nmr τ: 2.1 (bs, D_2O exch., 1H), 2.84 (m, 5H), 3.40 (bd, J≃6Hz, changes to a sharp singlet after D_2O addition, 1H), 5.0 (bs, D_2O exch., 1H), 6.94 (bt, J≃7Hz, changes to a sharp

triplet, J=8Hz, after D_2O addition, 1H), 7.34 (t, J=8Hz, 2H), and 8.20 (qi, J=8Hz, 2H); ^{13}C nmr δ: 145.5 (d), 142.0 (s), 128.4 (d), 125.9 (d), 44.5 (t), 33.7 (t) and 32.8 (t); ms m/e (%): 178.1125 (M^+, 18, calcd. for $C_{10}H_{14}N_2O$: 178.1125), 146 (100), 117 (60), 91 (71), 73 (60) and 57 (58); Anal. calcd. for $C_{10}H_{14}N_2O$: C 67.39, H 7.92, N 15.72; found: C 67.48, H 7.96, N 15.80.

In water: The nitroso derivative II-1-d (820 mg, 3.7 mmoles) was suspended in water (230 ml) and was photolysed at room temperature, under nitrogen, for 4 hours. During the progress of the photolysis, the solution developped a violet-blue colour having λ_{max} at 328, 505, 540 and 725 nm, which reached its maximum intensities after 30 min. of irradiation and faded away gradually to give a clear light brown solution at the end of the photolysis. A small amount of starting material sticking to the photocell wall, just above the water level remained unreacted. The solution was concentrated under atmospheric pressure to about 40 ml, with the distillation receiver kept in a dry-ice acetone bath. The distillate was treated with an ethanolic 2,4-dinitrophenylhydrazine solution to give an orange precipitate; mp 164-166°C (from ethanol-water), mmp with an authentic sample of 2,4-dnph derivative of formaldehyde 165°C. The concentrated photolysate (pH= 6-7) was extracted with CH_2Cl_2 (3×20 ml) to give after drying ($MgSO_4$) and evaporation a brown semi solid (346 mg); ir ν_{max}: 3360 (m), 2800 (b,m), 1680 (s), 1600 (m), 1500 (m), 1460 (m), 1370 (m), 910 (m), 750 (s) and 700 (s) cm^{-1}; ^1H nmr τ: 1.9 (bs), 2.75 (s), 5.85 (bm, wl/2= 20Hz), 7.4 (bm, wl/2= 15Hz) and 8.15

(bm, wl/2=25Hz). This crude mixture was treated with hot cyclohexane ot afford II-16-d (30 mg, 5%) as light brown plates; mp 91-94°C; mixed mp with authentic II-16-d 97-99°C. The insoluble part was taken up in CH_2Cl_2 (20 ml) and washed several times with diluted HCl solution (0.5N). The organic phase was further washed with water, dried ($MgSO_4$) and evaporated to yield a brown oil (280 mg); ir ν_{max}: 3200 (b,m), 1700 (s), 1600 (m), 1500 (m), 1450 (s), 1350 (s), 1130 (m), 750 (m) and 700 (s) cm^{-1}. This oil was analysed by gc-ms (10% SE-30, 150°C to 250°C at 10°C/min) to give two major components as well as two minor unknown compounds; the compounds are described in order of increasing retention time (rt) and the yields indicated are estimated from gc peak areas measurement; rt 2.5 min., II-18, 10%; rt 2.7 min., unknown, 5%; rt 3.6 min., unknown, 5%; rt 6.1 min., II-17, 23%; The peak at rt 2.5 min. was shown to be composed of two products by examining the mass spectra of the beginning and the end of the peak; the beginning of the peak showed the following ms m/e (%): 161 (38), 148 (57), 120 (37), 118 (17), 117 (19), 105 (50), 91 (60), 74 (100), and 42 (30); the end of the peak showed the following ms m/e (%): 251 (13), 160 (14), 147 (29), 146 (86), 117 (40), 91 (100), 77 (13) and 56 (81). The peak at rt 6.1 min. was identified as N-formyl-3-phenylpropylamine II-17 and showed the following ms m/e (%): 163 (M^+, 34), 118 (62), 117 (52), 105 (18), 91 (47), 77(15) and 59 (100). The aqueous photolysate was acidified to pH=2-3 and extracted with ether (2×30 ml) to give after drying ($MgSO_4$) and evaporation unreacted II-1-d (65 mg,8%); mp 103-105°C; mixed mp with authentic II-1-d 104-106°C.

The water phase was then basified with 10% NaOH solution to pH=10 and extracted with ether (3×20 ml) to give 3-phenylpropylamine II-20 (62 mg, 12%) as a yellow oil which showed identical ir spectrum to that of an authentic sample of II-20.

Preparation of N-formyl-3-phenylpropylamine, II-17; Formylation of 3-phenylpropylamine II-20 was performed with chloral in $CHCl_3$ according to Blicke's procedure (124) and gave formamide II-17 (70%) which was purified by distillation (bp 140°C/1 Torr); ir ν_{max} 3280 (m), 1655 (s), 1520 (m), 1490 (m), 1450 (m), 1380 (m), 740 (m) and 690 (s) cm^{-1}; ^1H nmr τ:1.86 (bs, wl/2=3Hz, 1H), 2.78 (s, 5H), 3.7 (bs), 6.8 (m,2H), 7.4 (m, 2H) and 8.2 (m, 2H); ms m/e (%): 163 (M^+, 7), 146 (100), 117 (31), 91 (87), 65 (18) and 59 (25).

IV-6-5 Photolysis of N-Nitrosoproline, II-1-g

In ether: When an ether solution (200 ml) of II-1-g (535 mg, 3.7 mmoles) was photolysed at room temperature and under nitrogen, it gradually turned cloudy and an amorphous resin deposited on the wall of the photocell. After 75 min. the absorptions at 381 and 368 nm had completely disappeared. Concentration of the ether solution gave 2-pyrrolidonoxime II-16-g (185 mg, 50%) as white crystals; mp 150-152°C (from water); mixed mp with an authentic sample 156-158°C; ir ν_{max}: 3340 (m), 3060 (b), 2700 (bm), 1675 (s), 1308 (s), 1282 (s), 1075 (s) and 940 (s) cm^{-1}. The mother liquor was evaporated to give a resin which was taken up in hot

benzene to give on cooling, another crop of II-16-g (65 mg, 18%). The filtrate was evaporated to yield an amorphous solid which turned to a sticky resin on exposure ot air. This solid was not soluble in acetone.

In water and in the presence of hydrochloric acid; The same nitroso derivative II-1-g (1.5 g, 0.01 mole) was photolysed in water (200 ml) containing HCl (7 ml, 2.8N), at room temperature and under nitrogen . After 4 hours of irradiation, the absorption band at 350 nm had completely disappeared and the photolysis was continued for another 2 hours. The photolysate was filtrated through a Dowex 3 (50 ml) column and the resulting filtrate (pH=5-6) was evaporated to dryness to give a yellowish oil; ir ν_{max}: 3400-2200 (s), 1300 (s), 1065 (s), 990 (s), and 935 (s) cm^{-1}. The residue was treated with hot isopropyl alcohol (25 ml) to leave a very hygroscopic residue (350 mg); ^1H nmr τ: 6.33 (t), 7.17 (t), and 7.77 (m). Cooling of the filtrate gave 2-pyrrolidonoxime hydrochoride (600 mg, 42%), as white crystals; mp 197-200°C (from isopropyl alcohol); ir ν_{max}: 2500-3200 (b), 1690 (s), 1555 (w), 1310 (m), 1060 (s), 990 (s) cm^{-1}; ^1H nmr (D_2O) τ: 6.27 (t, J=7Hz, 2H), 7.1 (t, J=7Hz, 2H), and 7.73 (m, 2H). Anal. calcd. for $C_4H_8N_2O.NCl$: C 35.17, H 6.64, N 20.51; found: C 35.58, H 6.85, N 20.07. Further evaporation of the mother liquor gave another crop of II-16-g hydrochloride (520mg, 36%).

IV-6-6 Photolysis of N-Nitrosopipecolinic acid, II-1-h

In ether: An ether solution (200 ml) containing II-1-h (600 mg, 3.8 mmoles) was photolysed at room temperature, under nitrogen for two hours until over 90% of the absorption at 350 nm had disappeared. The fluffy precipitate formed during the photolysis was filtered and turned to a resin upon exposure to air; ir ν_{max}: 3400-2500 (b,m), 1600-1650 (s) and 1130 (s) cm^{-1}. The ether solution was concentrated under vacuum and after cooling gave 2-piperidonoxime (II-16-h) (300 mg, 76%) as white crystals; mp 114-116°C (from benzene-petroleum ether); mixed mp with an authentic sample 114-116°C; ir and nmr spectra were identical to those of the authentic sample (125). The mother liquor was evaporated to dryness to give an amorphous solid with a strong smell and whose ir spectrum was similar to that of II-16-h.

In water and in the presence of hydrochloric acid: A solution of II-1-h (1.82g, 11.5 mmoles) in water (200 ml) containing HCl (2.84N, 4.2 ml) was photolysed for nine hours. The water phase was evaporated to dryness to give a yellowish residue which was taken up in 100% ethanol. Addition of benzene gave II-16-h hydrochloride (640 mg, 37%) as a white precipitate; mp 210-215°C; ir ν_{max}: 3150 (m), 1665 (s) and 1510 (s) cm^{-1}, superimposable with that of an authentic sample of II-16-h hydrochloride (125). Further addition of petroleum ether gave an amorphous solid whose ir spectrum was similar to that of II-16-h hydrochloride. Treatment of the amorphous solid with

1N HCl and extraction with CH_2Cl_2 gave II-16-h hydrochloride (104 mg, 6%). On standing, the mother liquor gave another crop of II-16-h hydrochloride (180 mg, 11%).

In another experiment, a solution of II-1-h (1.57g, 10 mmoles) in water (250 ml) and acetic acid (1.3g, 20 mmoles) was photolysed for four hours. Hydrochloric acid (2N, 5ml) was added to the photolysate and the resulting solution was boiled for five min. Usual work up of the solution gave II-16-h hydrochloride (560 mg, 37%) but no amorphour material. The mother liquor was dissolved in water, neutralized with Na_2CO_3 and extracted with ether to give II-16-h (530 mg, 46%).

IV-6-7 Photolysis of N-Nitrososarcosine Sodium Salt, II-14-b

A solution of II-14-b (1.27 g, 9 mmoles) in methanol (230 ml) was photolysed at room temperature and under nitrogen for 3.5 hours. Immediately after the start of the irradiation, a new absorption maximum at 305 nm appeared and decreased to finally disappear with that at 345 nm. The solvent was evaporated at atmospheric pressure to yield a residue which was dissolved in water (10 ml, pH = 13). The resulting solution was continuously extracted with ether during 36 hours to afford after drying (Na_2SO_4) and evaporation formamidoxime II-16-a (70 mg, 10%);

ir ν_{max}: 3400 (b), 1660 (s), 1380 (m), 1320 (m), 1280 (m), 1160 (m), 1020 (m), 890 (m), 760 (m) and 740 (m); ^{1}H nmr τ: 1.75 (b,s), 3.32 (s), 4.5 (b,s) and 7.1 (s). The mother liquor was evaporated to dryness to give a yellowish residue (970 mg); ir 3500 (b), 2500 (b), 1720 (m), 1660 (b,s), 1400 (s) and 1220 (s) cm^{-1}; ^{1}H nmr τ: 1.52 (s), 2.0 (d, J=4Hz), 6.09 (s), 6.11 (s), 6.18 (s), 6.95 (s), 7.1 (s), 7.15 (s) and 7.5 (b,s). Attempts to separate the components of this mixture failed

IV-7 Preparations of N-Nitroso-N-Acyl-α-Amino Acids

IV-7-1 N-Nitroso-N-Acetyl-D,L-Phenylalanine, II-22-a

D,L-Phenylalanine was treated with acetic anhydride in aqueous sodium hydroxide to give N-acetyl-D,L-phenylalanine II-21-a (85 to 100%); mp 151-153°C; ir ν_{max}: 3390 (s), 1700 (s), 1550 (s), 750 (s) and 700 (s) cm^{-1}.

N-Acetyl derivative II-21-a was nitrosated either with N_2O_4 (method B) or with $NOBF_4$ (method C) to yield II-22-a (75 to 95%) as a yellow solid ; mp 75-79°C (decomposed) (lit. (28) mp 65-70°C); ir ν_{max}: 1715 (s), 1490 (s), 1300 (s), 1120 (s), 940 (s) and 700 cm^{-1}; ^{1}H nmr τ: 0.88 (s, D_2O exch.), 2.7 (m,5H), 4.40 (X of ABX, J= 10.5 and 6 Hz, 1H), 6.56 (A of ABX, J_{AB}= 14.5 Hz, 1H), 6.95 (B of ABX, 1H) and 7.40 (s,3H); ^{13}C nmr δ: 22.1 (q), 33.3 (t), 52.0 (d), 126.9, 128.3, 135.4, 172.6, and 173.5. The ^{1}H nmr spectrum of II-22-a taken in methanol-d did not show any exchan-

geable proton other that the acid proton.

IV-7-2 N-Nitroso-N-Acetyl-D,L-Phenylalanine Dicyclohexylamine salt II-48

To a solution of II-22-a (200 mg, 0.8 mmole) in dry ether (10 ml) was added a solution of dicyclohexylamine (162 mg,0.9mmole) in ether (10ml). Upon cooling the resulting solution at -20°C, yellow crystals appeared and after filtration, washing with a cold mixture of ether-ligroin 1:1 and drying gave pure II-48 (260 mg, 73%); mp 101-102°C (decomposed with gas evolution); ir ν_{max}: 3020 (w), 3040 (w), 1725 (s), 1630 (s), 1500 (m), 1450 (m), 1370 (s), 1115 (m), 940 (s) and 700 (w) cm^{-1}; uv $\lambda_{max}(\varepsilon)$ (MeOH): 424 (227), 405 (233) and 390 (173). Anal. calcd. for $C_{23}H_{35}N_3O_4$: C 66.16, H 8.45, N 10.06; found C 66.26, H 8.83, N 9.54. Upon dissolving the salt in DMSO-d_6, bubbles were evolved, indicating decomposition of the compound. The resulting ^1H nmr showed the following signals τ 2.75 (s), 6.03(s), 8.05 (s), 8.01-8.9 (m).

IV-7-3 N-Nitroso-N-Benzoyl-D,L-Phenylalanine II-22-b

N-Benzoyl-D,L-phenylalanine (125) was nitrosated according to method C to give II-22-b (83%) as a yellow resinous oil; ir ν_{max}: 1720 (s) and 1360 (s) cm^{-1}; ^1H nmr τ: 0.95 (b s, D_2O exch.) 2.70 (m, 10H), 4.20 (X of ABX, J= 6 and 10 Hz, 1H), 6.47 and 6.74 (AB of ABX, J= 14 Hz, 2H); ^{13}C nmr δ: 32.8 (t), 52.4 (d), 126.8 , 128.3, 128.5, 135.4, 127.6, 129.9, 131.9, 132.2, 171.6,

and 171.9.

IV-7-4 N-Nitroso-N-Acetyl-D,L-Leucine II-22-c

D,L-Leucine was acetylated with acetic anhydride to yield N-acetyl-D,L-leucine II-21-c (70%); mp 155-157°C; ir ν_{max}: 3420 (s), 1700 (s), 1620 (s), 1560 (s) and 1245 (s) cm^{-1}. II-21-c was nitrosated with $NOBF_4$ (method C) to give II-22-c (100%) as a yellow oil; ir ν_{max}: 1725 (s) and 138 (s) cm^{-1}; ^1H nmr τ: 0.07 (bs, D_2O exch.), 4.67 (X of ABX, J= 9 and 5.5 Hz, 1H), 7.18 (s, 3H), 7.9-8.17 (bm, 3 H), 9.18 (d, J= 5 Hz, 6 H); ^{13}C nmr δ : 21.4 (q), 22.4 (q), 22.5 (q), 25.0 (d), 36.3 (t), 49.7 (d) 172.8 and 173.7

IV-7-5 N-Nitroso-N-diAcetyl-D,L-Serine II-22-d

D,L-Serine was diacetylated according to the method described by Narita (126) to yield N,O-diacetyl-D,L-serine II-21-d (13%); mp 130-133°C (lit. (126) mp 136-137°C); ir ν_{max}: 1730 (s), 1600 (s), 1550 (s), 1250 (s), 1240 (s) and 3350 (s) cm^{-1}; ^1H nmr (D_2O) τ: 7.92 (s, 3 H), 7.90 (s, 3 H), 5.5 (d, J= 4 Hz, 2H) and 5.2 (t, J= 4 Hz, 1 H).

Diacetyl II-21-d was nitrosated with $NOBF_4$ (method C) to give II-22-d (95%) as a yellow oil; ir ν_{max}: 1740 (s), 1380 (s) and 1220 (s) cm^{-1}; ^1H nmr τ: 0.9 (bs, D_2O exch.), 4.52 (X of ABX, J=9 and 4 Hz, 1 H), 5.41 and 5.88 (AB of ABX, J_{AB}= 12 Hz, 2 H), 7.28 (s,

3 H) and 8.12 (s,3 H); ^{13}C nmr : 20.3 (q), 22.2 (q), 49.4 (d), 59.9 (t), 168.7 (s), 170.3 (s) and 173.4 (s).

IV-8 Decomposition of N-Nitrosamides Under Basic Conditions

IV-8-1 Kinetic Study of the Base Decomposition of II-22-a

A stock solution of II-22-a in MeOH (S_0, 0.119 N) was freshly prepared and kept in the dark. Two stock solutions of KOH in water (S_1, 0.121N and S_2, 121 N) were prepared and titrated with oxalic acid. Six solutions of II-22-a in methanol with 0, 0.2, 0.5, 1, 2 and 5 mole equivalents of base were prepared according to the following procedure: the required volume of S_1 or S_2 was diluted with ~5 ml MeOH; 1 mp of S_0 was added to the resulting solution and the total volume was adjusted to 10 ml in a volumetric flask. The whole procedure was carried out at room temperature and the samples were stored in the dark. Uv spectra of the samples were taken approximately 1 min., 1 hr. and 1 day after preparation (see Fig. 2-7).

IV-8-2 Decomposition of Nitroso-Amido Acids II-22 in Water With an Excess of Potassium Hydroxide

a) N-Acetyl-N-Nitroso-D,L-Phenylalanine II-22-a

An aqueous solution (10 ml) of potassium hydroxide (300 mg,

5 mmoles) was added dropwise to a suspension of II-22-a (236 mg, 1 mmole) in water (10 ml) at 0°C in the dark. A gas evolution was observed and the solution turned from light to dark yellow. The resulting mixture was stimed for 0.5 hr. at 0°C and then was acidified with 1N HCl to pH ≈ 2-3 (more gas was evolved during acification). Extraction with ether (3 X 3 ml), drying ($MgSO_4$) and evaporation gave a yellow oil (162 mg): ^1H nmr τ 2.6 (bs, D_2O exch.), 2.78(s), 5.58 (X of ABX, J = 7 and 5.5 Hz), 6.83 and 7.11 (AB of ABX, J_{AB} = 14 Hz) and 8.0(s). The oil was dissolved in dry ether (10 ml) and treated with an ethanol solution of diazomethane until no bubbles were evolved. Evaporation of the solvent gave hydroxy ester II-23-a (148 mg, 82%) as a yellow oil; ir ν_{max}: 3500(b), 1735(s), 1270(bs), 1220(s), 750(s) and 700(s) cm^{-1}; ^1H nmr τ: 2.78(s,5H), 5.38 (Z of ABX, J = 5 and 6.5 Hz,1H), 6.27(s,3H), 6.92 and 7.1 (AB of ABX, J = 14 Hbz,2H) and 7.2 (brs, D_2O exch.); ms m/e (%): 180.0790 (M^+,15,calcd. for $C_{10}H_{12}O_3$ 180.0786), 162(73), 131(36), 121(47), 103(47), 91(100) and 77(30). An analytical sample was obtained by distillation (room temperature under 0.1 mm Hg). Anal. Calcd. for $C_{10}H_2O_3$: C 66.65, H 6.71; Found: C 66.73, H 6.74.

b) N-Benzoyl-N-Nitroso-D,L-Phenylalanine II-22-b

Compound II-22-b (200 mg, 0.67 mmole), in water (20 ml)

was treated, at 0°C in the dark, with an aqueous potassium hydroxide solution (1N) until the pH of the solution reached 10-11. Same work up as described above gave a yellow solid (180 mg). A small amount of this residue was treated with diazomethane in ether and the recovered oil was analyzed by gc (3% SE 30, 120°C) to give the following peaks: rt 0.75 min, 80% methylbenzoate ; rt 1.75 min, 99% (II-24). Both peaks were identified upon mixed infection with authentic samples. the initial solid residue was sublimed (RT/0.1 Torr) and afforded benzoic acid as white crystals; mp 121-122°C; no depression when mixed with an authentic sample.

c) N-Acetyl-N-Nitroso-D,L-Leucine, II-22-c

An aqueous potassium hydroxide solution (6N) was added to a suspension of the nitroso derivative II-22-c (0.195 g, 0.96 mmole) in water (15 ml) at 0°C in the dark, until pH = 11. A strong gas evolution was observed. The solution was stirred for 0.5 hr. and acidified with HCl solution to pH = 3. The resulting solution was extracted with ether (3 X 50 ml) and the extracts were combined, washed with water, dried over $MgSO_4$ and evaporated to give a yellow oil (134 mg): ir ν_{max}: 3500-2600(bs), 1720(s), 1270(s), 1230(s), 1140(s) and 1080(s) cm^{-1}; ^1H nmr δ: 1.8(bs, D_2O exch.), 5.67(dd, J = 6 and 7 Hz), 7.9(s), 8.3(m) and 9.05(d, J = 6 Hz). The oil was passed through a silicic acid column (1g) using CH_2Cl_2 as eluant. This

afforded leucid acid II-23-c (82 mg, 65%) as a white solid. Sublimination (RT/0.3 Torr) followed by recrystallization from ether-petroleum ether gave white crystals; mp 53-56°C; lit. (130) ; 77°C; ir ($CHCl_3$) ν_{max}: 3550(bm), 3200(b), 1720(s), 1470(m), 1370(m), 1270(m), 1145(m) and 1090(m) cm^{-1}; ^1H nmr τ: 1.9(brs, D_2O exch.), 5.68(X of ABX, J = 6 and 7 Hz, 1H), 8.3(m, 3H), and 9.03(d, J = 6 Hz, 6H); ^{13}C nmr δ: 21.3(q), 23.1(q), 24.4(d), 43.1(t), 68.9(d) and 180.0(s); ms m/e (%): 132 (M^+ absent), 81(87), 69(100) and 43(92).

d) Measurement of the Gas Evolved from the Reaction of II-22-a with Potassium Hydroxide

A solution of II-22-a (0.66 g, 2.8 mmoles) in methanol (60 ml) was added dropwise to a stirred solution of methanol (20 ml) containing potassium hydroxide (0.87 g, 22 mmoles). The reaction vessel was immersed in an ice bath, and was connected to a burette filled with water. During addition bubbles were evolved. When the addition was complete the mixture was stirred for another 30 minutes until no change in the gas colume could be detected. The total gas volume was measured: 24.1 cm^3 (~1 mmole, ~38%).

IV-8-3 Decomposition of N-Acetyl-N-Nitroso-D,L-Phenylalanine in Methanol with an Excess of Sodium Methoxide

Sodium methoxide (~1 g Na, 10 ml CH_3OH) was added to a solution of II-22-a (823 mg, 3.5 mmoles) in methanol (100 ml) and the resulting solution was stirred in the dark at room temperature for three days. Upon addition of KOH, the colour of the solution turned darker and the uv pattern characteristic of the nitrosamido group was replaced by a stronger absorption at λ = 340 nm which shifted slowly to higher frequency and decreased to reach a minimum at 358 nm after 24 hours and was steady during the next 40 hours.

The methanol was evaporated to dryness to have an oily residue which was treated with 6N HCl (10 ml). During acidification bubbles were evolved and the colour of the solution turned lighter. Extraction with ether (3 X 30 ml), washing with water and drying ($MgSO_4$) gave after evaporation a yellow oil (520 mg): ir ν_{max} 3500-2800(bm), 2500(bm), 1720(s), 1500(m), 1450(m), 1200(bs), 1100(bs), and 700(s); ^1H nmr τ: 1.66(bs, D_2O exch.), 2.78(s), 5.6(m), 6.0(dd, J = 7 and 6 Hz), 6.67(s), 6.95(m) and 8.0(s). An aliquot of this oil was esterified with diazomethane and analyzed by gc-ms (3% Silar 10c, 180°C iso): 1.6 min., 48%, (II-37); 1.8 min., 7% (methyl ester of II-26); 4.0 min., 14% (II-24). Compounds II-37 and II-24

were identified by gc peak matching with authentic samples. The ms of the third peak showed fragments at m/e: 198(22%) and 200(7%).

IV-8-4 Attempts to Trap Species "X"

a) Photolysis of Species "X" in the Presence of Cyclohexene

To an ice cold solution of II-22-a (0.59 g, 2.5 mmoles) in methanol (110 ml), a methanolic potassium hydroxide solution (4.2 ml, 1.24N, 5.2 mmoles) was added dropwise vigorous nitrogen. The solution turned to dark yellow, and the uv absorption spectrum was taken regularly thereafter. The original absorption at λ_{max} = 400 nm and 420 nm gradually decreased to be replaced by two new absorptions at λ_{max} = 410 nm and 330 nm. After two hours cyclohexane (2.05 g, 0.025 mole) was added to the mixture and the resulting solution was irradiated at 0°C under nitrogen. After five hours of irradiation both absorptions at 330 nm and 410 nm had disappeared. The solvent was then evaporated under reduced pressure at 15°C to leave a yellowish residue (0.71 g). The residue was dissolved in water (10 ml) (pH ≃ 10) and extracted with ether (3 X 3 ml). The ether extracts were combined, dried over $MgSO_4$ and evaporated to yield the neutral fraction (20 mg) as a yellow oil. This oil

was analyzed by gc-ms (10% SE 30, 100 to 220°C at 8°C/min.) to give the following components: 1.7 min., 2-cyclohexenol; 3.7 min., 1%, (II-27); 11 min., <1%, [unknown, m/e (%) 178(10), 135(21), 97(100), 84(30), 81(39), 79*44), 68(33), 67(70), 55(33), 54(32.3) and 41(59) and 11.5 min., 1%, [II-28, m/e (%) 186(M^+,.2), 178(13), 149(12), 97(96), 95(34), 94(21), 91(10), 82(48), 81(31), 79(30), 77(13), 68(36), 67(100), 55(28), 54(41), 53(22), 41(57) and 39(25)]. The first two peaks were identified by gc peak matching with authentic samples and comparison of ms with those already reported (75).

The water phase was carefully acidified with diluted HCl solution to pH ~6. The solution because turbid and some gas bibbles were evolved. This solution was extracted with other (3 X 30 ml) to give a yellow oil (133 mg); ir ν_{max}: 3300-2800 (bs), 2400(bm), 1710(bs), 1630(m), 1500(m), 1455(m), 1230(bs), 1120(s) and 700(s) cm^{-1}; ^1H nmr τ: 0.1(bs, D_2O exch.), 2.7(m), 3.96(s), 4.17(s), 6.10(dd, J = 5 and 7 Hz), 6.67(s) and 6.95(m). A small aliquot of this fraction was esterified with diazomethane to yield an oil; ir λ_{max}: 3500(bw), 1730(s), 1200(s), 1170(s), 1120(s) and 700(s) cm^{-1}. This mixture was analyzed on gc (3% Silar 10 C, 160°C to 220°C at 10°C/min.) to give, in addition to two minor peaks, the following major components: 3.4 min., 9.8% (methyl cinnamate); 3.9 min., 20.3% (II-24) and 6 min., 1.3% (II-37). All these compounds were identified

by gc peak matching with authentic samples. The water phase was further acidified to pH 2 and extracted with ether (3 X 30 ml) to yield a yellow oil (278 mg) which showed the same spectral characteristics as the oil described above. Gc-ms analysis of the esterified oil under the same conditions gave the following composition: 3.4, 2.5% (methyl cinnamate); 3.9, 43.8% II-24 and 6 min., 17% (II-37).

b) Alkylation of Species "X" with Phenacylbromide

A solution of methanol (50 ml) containing species "X" prepared as usual from II-22-a (229 mg, 0.97 mmole) and KOH (1.9 mmoles) (in 15 min.) was refluxed for 50 minutes in the presence of phenacylbromide (1.9 mmoles). Ether extraction (3 X 30 ml) at pH 10 of the concentrated reaction mixture gave the neutral fraction (370 mg); ir ν_{max}: 2080(w), 1750(s), 1675(s), 1280(s), 1190(s) and 750(s) cm^{-1}; ^1H nmr τ: 2.1(m), 2.5(m), 2.67(s), 4.6(s), 5.57(s), 5.82(dd, J = 5, 7 Hz), 6.2(s), 6.5(s), 6.53(s) and 6.72(s). Column chromatography on basic alumina gave the following fractions. Elution with benzene containing 50% of hexane gave an oil (17 mg); ir ν_{max}: 2080(w), 1700(s), 1680(s), 1280(s), 1210(s) and 680(s) cm^{-1}. Elution with benzene containing 20% of hexane gave crystals (3 mg); mp 160-1°C; ms (m/e) 318, 316, 223, 105, 103, 77. Further elution with ethylacetate only gave a residual oil (13 mg).

Acidification (pH = 2) of the mother liquor followed by ether extraction (3 X 30 ml) gave II-25 (63 mg, 36%).

IV-8-5 Decomposition of N-Acetyl-N-Nitroso-D,L-Phenylanine, II-22-a, with One Equivalent of Strong Base

a) Sodium Methoxide

A solution of sodium methoxide in methanol (6.6 ml, 0.97 ml, 0.54 mmole) was added to a solution of II-22-a (138 mg, 0.58 mmole) in dry methanol (10 ml). The resulting mixture was stored in the dark at 5°C for 3 days. The solvent was evaporated under vacuum at room temperature to yield a yellow oil (145 mg); ^{1}H nmr τ: 1.2(bs), 2.8(s), 6.1(m,wl/2 = 13 Hz), 6.62(s), 6.83(s), 6.9(br m,wl/2 ~13 Hz) and 8.02(s). This oil was taken up in CH_2Cl_2 (10 ml) and washed with diluted HCl solution (2 X 10 ml, 0.1N HCl) followed by water (2 X 5 ml) to give after drying and evaporation methoxy acid II-25 (95 mg, 90%) as a slightly yellow oil; ^{1}H nmr τ: 0.3(bs, D_2O exch.), 2.76(s,5H), 6.0(X of ABX, J = 5 and 8 Hz, 1H), 6.62(s,3H) and 6.88 and 7.02 (AB of ABX, J = 13 Hz, 2H). The latter oil was dissolved in dry ether and treated with an ethanol solution of diazomethane to yield methoxy ester II-37 (103 mg, 90%) as a yellowish oil; ir ν_{max}: 1750(s), 1200(s), 1120(s), and 700(s) cm^{-1}; ^{1}H nmr τ: 2.78(s,5H), 6.02(X of ABX, J = 6 and

7 Hz, 1H), 6.31(x,3H), 6.67(s,3H), 6.88 and 7.05 (AB of ABX, J = 14 Hz, 2H); ^{13}C nmr δ: 172.0, 136.6, 128.9, 127.9, 128.2, 81.4(d), 58.0(q), 51.5(q) and 38.9(t); ms m/e (%): 194.0940 (M^+,24, Calcd. for $C_{11}H_{14}O_3$: 194.0943), 163(17), 162(83), 135(100), 131(37), 117(27), 105(35), 103(69), 91(84) and 77(33). An analytical sample was obtained on distillation at room temperature under 0.1 Torr. Anal. calcd. for $C_{11}H_{14}O_3$: C 68.02, H 7.27. Found: C 68.30, H 7.35.

b) Potassium Hydroxide

A solution of methanolic potassium hydroxide ~~(10 ml,~~ 6.35 X 10^{-2} M) containing II-22-a (150 mg, 0.64 mmole) was heated at 40°C in a dark flask under nitrogen. As seen on Fig. 2-12, more than 90% of the absorption at 400 nm had disappeared after 1 hour. A small aliquot of the original reaction mixture, when kept at 0°C for the same time period, did not show any decrease whereas another sample showed a 30% decrease when kept at room temperature for 1 hour.

The solvent was evaporated under vacuum to yield a yellow oil (122 mg); ir ν_{max}: 3450(bm), 2500(b), 1710(bs), 1600(bs), 1600(bs), 1275(bs), 1110(bs) and 700(s) cm^{-1}. A small aliquot (15 mg) of this residual oil was esterified with diazomethane to give an oil: ir ν_{max} 3400(bs), 1745(m), 1600(s), 1410(s), 1200(m),

1110(s) and 700(s) cm^{-1}. Gc analysis (3% Silar 10 C, 160°C to 220°C at 10°C/min.) showed this oil to contain essentially methoxy ester II-37 with a trace of II-24. Both peaks were identified by mixed injection with authentic samples. The rest of the crude oil (107 mg) was dissolved in water (10 ml, pH = 6-7) and the resulting solution was extracted with CH_2Cl_2 to give the neutral extract (68 mg) as a yellow oil. The water phase was reextracted at pH = 3-4 with methylene chloride to yield the acidic extract (25 mg) as an oil. Both oils showed identical ir spectra and gc analysis of their methyl esters (diazomethane) gave a single peak matched with authentic II-37 (3% Silar 10 C, 160°C to 220°C at 10°/min.). The total yield of II-25 (95%) is taking into account the samples for uv measurement.

IV-8-6 Decomposition of N-Acetyl-N-Nitroso-D,L-Phenylalanine II-22-a, with Triethylamine

a) In Benzene, at Room Temperature

Three benzene solutions of II-22-a (2.3, 2.45 and 1.22 10^{-3} N) containing respectively 1, 2 and 5 equivalents of triethylamine were prepared and were monitored by uv spectroscopy. The first spectrum was taken immediately after preparation of the sample and the others at regular time intervals

while the solutions were kept at room temperature under nitrogen in the dark (see Fig. 2-9). The rates of decomposition were calculated and are reported in Table 2-13.

Three quantitative experiments were conducted at room temperature using the conditions in Table 4-2

Table 4-2. Experimental Conditions for Decomposition of II-22-a in Benzene with $(Et)_3N$

Run #	II-22-a		$(Et)_3N$		II-22-a/$(Et)_3N$
	mg	mmole	mg	mmole	(mole)
1	180	0.76	77	0.76	1.0
2	205	0.87	263	2.16	3.0
3	184	0.78	395	4.0	5.1

Uv monitoring of Reaction 1 showed a 68% decrease of the nitroxamido absorption after 24 hours and another 4% in the following 60 hours. The benzene was removed under vacuum to yield a yellow oil: ir 2500(bm), 2080(m), 1730(s), 1600(m), 1380(s), 1240(s), 1180(s), 940(m), 750(m), 700(m) and [redacted](s) cm^{-1}. The oil was dissolved in methylene chloride (25 ml) and the resulting solution was washed with 10% sodium carbonate (10 ml) and water subsequently (2 X 10 ml). The organic phase was dried ($MgSO_4$) and evaporated to yield the neutral fractions

as a yellow oil (102 mg); ir ν_{max}: 2080(m), 1740(s), 1690(m), 1620(m), 1500(m), 1460(m), 1380(s), 1240(s), 1180(s), 940(m), 750(s) and 700(s) cm^{-1}; ^1H nmr τ: 0.8(bs), 1.8(m), 4.5(dd, J = 5.5 and 9 Hz), 5.72(unresolved t, 5-6 Hz), 6.45(s), 7.0(m), 7.5(s), 8.0(s) and 8.75(m). Integration of the signals at τ 4.5, 5.72, 6.45 and 7.5 gave the following yields: II-22-a (20%); II-31 (16%) and II-30 (17%). The aqueous phase was acidified to pH = 3-4 and extracted with methylene chloride (2 X 30 ml) to give an oily residue (3 mg).

Reactions 2 and 3 were run and worked up in a similar manner to Reaction 1. The ir and nmr spectra of the neutral fractions were identical to those in Reaction 1 and the corresponding yields were the same, within the experimental errors.

b) In Benzene at Reflux

A solution of dry benzene (30 ml) containing II-22-a (1.09 g, 4.62 mmoles) and triethylamine (466 mg, 4.61 mmole) was refluxed in the dark, under nitrogen, for 1.25 min., when the 400 nm absorption had totally disappeared. The resulting yellow solution was washed with 10% sodium carbonate solution (10 ml) and water (2 X 10 ml), dried over $MgSO_4$ and evaporated to yield a yellow oil (526 mg); ir ν_{max}: 2080(s), 1745(s), 1690(s),

1375(m), 1240(m), 1180(s), 1110(m), 940(m), 750(m), 700(s) and 680(m) cm^{-1}; ^1H nmr τ: 2.75(bs, wl/2 = 4 Hz), 5.6(m), 6.4(m), 6.9(m), 7.48(s), 7.97(s) and 8.75(t, J = 7 Hz) the intensity of the band at 2080 cm^{-1} represented ~77% of that at 1745. The water phase was made acidic by addition of diluted HCl solution (0.5N) and extracted with CH_2Cl_2 (2 X 15 ml). The combined extracts were dried ($MgSO_4$) and evaporated to yield the acidic fraction (22 mg) as a yellow oil; ir ν_{max}: 3500-2900(m,b), 2580(m), 1720(s), 1500(m), 1450(m), 1180(m), 1085(m) and 700(s) cm^{-1}.

The basic fraction was chromatographed over basic alumina (40 g). On elution with hexane 2-phenylethylacetate II-31 (20 mg, 3%) was obtained; ir ν_{max}: 1740(s), 1240(s) and 700(s) cm^{-1}; ^1H nmr τ: 2.78(s,5H), 5.72(t, 5 = 7H, 2H), 7.08(t, J = 7H, 2H) and 7.98 (s,3H). Elution with a mixture containing 10% of benzene in hexane afforded diazoester II-30 (71 mg, 17%) as a bright yellow oil; ir 2080(s), 1690(s), 1500(m), 1460(m), 1390(s), 1330(b,m), 1180(m), 1110(s), 740(m) and 700(s) cm^{-1}; ^1H nmr τ: 2.84(m,10H), 5.66(t,J = 6 Hz, 2H), 6.44(s,2H) and 7.08(t,J = 6 Hz, 2H); ^{13}C nmr δ: 167.8, 137.5, 136.9, 128.8, 128.6, 126.9, 128.2, 126.3, 65.2(t), 35.1(t) and 28.9(t); λ_{max} (ε) ($CHCl_3$): 406 nm (~35); upon irradiation of the triplet at τ 5.66, the triplet at τ 7.08 collapsed into a singlet and vice versa. This fraction gave one spot on tlc as well as one peak on hplc analysis. Further elution with hexane containing

75% of benzene afforded 2-phenylethyl alcohol II-32 (40 mg, 7%); ir λ_{max}: 3350(bs), 1500(m), 1460(m), 1040(s), 750(s), and 700(s) cm^{-1}; ^1H nmr τ: 2.78(s,5H), 6.18(t, J = 6.5 Hz, 2H), 7.17(t,J = 6.5 Hz, 2H) and 8.25(bs, D_2O exch.).

A solution of diazoester II-30 (10 mg, 0.04 mmole) in ether (5 ml) containing 3,5-dinitrobenzoic acid (25 mg, 0.12 mmole) was stirred at room temperature, in the dark, overnight. After evaporation of the solvate, the resulting solid residue was dissolved in ether (30 ml) and washed with 10% sodium carbonate solution (10 ml) followed by water (2 X 10 ml). The organic phase was dried over $MgSO_4$ and evaporated to yield a solid residue (5 mg, 27%). Recrystallization from methylene chloride-hexane gave II-33 as white plates; mp 125-126°C; ir (CH_2Cl_2) ν_{max}: 1745(s), 1630(m), 1550(s), 1350(s), 1280(s), and 1170(s) cm^{-1}; ^1H nmr τ: 0.86(t,J = 3 Hz, 1H), 1.02(d,J = 3 Hz, 2H), 2.8(m,10H), 4.55(X of ABX, J = 6 and 8 Hz, 1H), 6.66 and 6.80 (AB of ABX, J_{AB} = 13 Hz, 2H) and 7.08(t, J = 7 Hz, 2H); ms M/e (%): 464.1210 (M^+, 0.04, Calcd. for $C_{24}H_{20}N_2O_8$: 464.1220), 195(7), 149(4), 131(4), 105(15), 104(100) and 91(5). Anal. Calcd for $C_{24}H_{20}N_2O_8$: C 62.07, H 4.34, N 6.03. Found: C 62.11, H 4.35, N 5.95.

In another experiment, II-22-a (145 mg, 0.61 mmole) was refluxed for 1 hour in benzene (20 ml) in the presence of two

mole equivalents of $(Et)_3N$ (124 mg, 1.23 mmoles). After the usual work up, a yellow oil (106 mg) was obtained whose ir spectrum contained the diazo absorption at 2080 cm^{-1}. The intensity of this absorption was ~80% that of the carbonyl at 1740 cm^{-1}. When this oil was refluxed in methanol, no change in the ir spectrum of the product could be observed.

c) In Methanol with Triethylamine

A solution of II-22-a (136 mg, 0.58 mmole) in dry methanol (20 ml) and in the presence of 1 molar equivalent of Et_3N (59 mg, 0.58 mmole) was refluxed in the dark under nitrogen. After 30 minutes the uv absorption at λ = 400 mm completely disappeared. Evaporation of the solvent yielded a yellow oil which did not exhibit any ir absorption in the 2000 cm^{-1} region. This oil was taken up in methylene chloride (25 ml), washed with 1N HCl solution (10 ml) and water (2 X 10 ml) and after drying ($MgSO_4$) and evaporation, gave methoxy acid II-25 (97 mg, 93%) identified by its nmr and ir spectra.

IV-9 Thermolysis of N-Acetyl-N-Nitroso-D,L-Phenylamine II-22-a

IV-9-1 Thermolysis in Benzene

A solution of II-22-a (251 mg, 1.06 mmole) in dry benzene (20 ml) was refluxed in the dark under nitrogen for 2.5 hours

when over 90% of the absorption at 400 nm had disappeared (see Fig. 2-13). The spectra also disclosed two isoabsorptive points at λ_{max} = 352 and 446 nm. The solvent was removed at room temperature, under vacuum to yield a yellow oil (150 mg); ir (benzene) ν_{max}: 2500(bm), 2080(m), 1730(b,s), 1630(s), 1370(s), 1200(b,s), 1020(b,s), 840(s) and 690(b,s) cm^{-1}; ^1H nmr τ: 0.7(bs), 2.75(m), 4.6(m), 5.68(bt,J = 7 Hz), 6.3(m), 6.8(m), 7.95(s) and 8.0(m). The intensity of the 2080 cm^{-1} band represented ~40% of that at 1730 cm^{-1}. When this crude oil was left at room temperature for one day, the band at 2080 cm^{-1} and the multiplet at τ 6.3 completely disappeared.

A small amount of the crude product (63 mg) was esterified with diazomethane to yield a yellow oil (54 mg); ir ν_{max}: 1740(s), 1280(m), 1220(m), 1170(s), 1050(m) and 700(s) cm^{-1}. This oil was analyzed by gc-ms (10% SE 30, 130 to 240°C at 8°C/min.) to give the following compounds described in increasing retention time order: 5.4 min., 1% (II-31); 6 min., 1% [unknown, ms m/e (%): 163(38), 131(100), 103(68.2) and 77(40)]; 7.5 min., 4% (methyl cinnamate); 9.5 min., 43% (methyl ester of II-34); 12.1 min., 21% (methyl ester of II-21-a); 17.5 min., 5% [unknown, ms m/e (%): 162(63), 131(100), 103(48), 91(90) and 77(23)]. The identified compounds were characterized by gc peak matching with authentic samples which were either commercially obtainable or synthesized that of methyl ester of II-34 was obtained by acetylation of II-24 with

acetyl chloride and pyridine (123); ir ν_{max}: 1750(s), 1230(s), and 700 cm^{-1}; ^1H nmr τ: 2.72(s,5H), 4.81(X of ABX, J = 6 and 8 Hz, 1H), 6.28(s,3H), 6.78 and 6.91 (AB of ABX, J_{AB} = 14 Hz, 2H) and 7.95(s,3H).

IV-9-2 Thermolysis in Methanol

a) In CH_3OH

A solution of II-22-a (226 mg, 0.96 mmole) in dry methanol (30 ml) was refluxed in the dark, under nitrogen for 3.5 hours when over 90% of the absorption at 400 nm disappeared the methanol was evaporated to yield the crude product as a yellow oil (155 mg); ir ν_{max}: 3400(b), 2600(b), 1720(s), 1630(m), 1200(m), 1100(m) and 1020(m); ^1H nmr τ: -0.1(bs,D_2O exch.), 2.78(s), 6.0(X of ABX, J = 6 and 7 Hz), 6.32(s), 6.77(s), 6.9(m), 7.95(s) and-8.05(s). The signals at 6.32, 6.77 and 8.05 were in a 21:64:1 ratio. Part of this residual oil (113 mg) was taken up in 10% sodium carbonate (10 ml) and was extracted with ether (3 X 15 ml). The work up of the ether extracts to give II-34 as yellow oil (27 mg, 18%); ir ν_{max}: 1740(s), 1450(m), 1440(m), 1200(s), 1110(s), and 100(s) cm^{-1}; ^1H nmr τ: 2.72(s, 5H), 5.98(X of ABX, J = 6 and 7 Hz, 1H), 6.25(s,3H), 6.61(s,3H) and 6.92 and 7.07 (AB of ABX, J = 17 Hz, 2H). This product gave one single peak on gc analysis (10% SE 30, 150-200°C, 8°C/min.) which had the same retention time as authentic II-37.

The other part of the crude oil was esterified with diazomethane and analyzed on gc (10% SE 30, 150-200°C at 8°C/min.) to give two peaks: 4.5 min., 95% (II-37) and 9 min., 5% (methyl ester of II-21-a). Both products were identified upon mixed injection with authentic samples.

In an other experiment a solution of II-22-a (130 mg), 0.55 mmole) in dry methanol (20 ml) was heated in the dark, under nitrogen, at 40°C for 5 days when the uv spectrum showed that over 83% of II-22-a had disappeared. Evaporation of the solvent gave a pale yellow oil (79 mg) whose nmr showed the characteristic signals for the methoxy ester II-37 and the methoxy acid II-25 in a 1:6 ratio, as evaluated from peak area measurements.

b) In CH_3OD

A solution of II-22-a (118 mg, 0.5 mmole) in methanol (MSD, 20 ml) was reflexed in the dark, under nitrogen for 6 hours, until the 400 nm absorption had completely disappeared. Evaporation of the solvent yielded a yellow oil (85 mg). The ^1H nmr spectrum of this oil exhibited two singlets at τ 6.3 and 6.68 in a 1:2 ratio, indicating the presence of methoxy acid II-25 and methoxy ester II-37 in a 1:1 ratio; the integration of the methine proton at τ 6.0 represented 45% of that

of the singlet at τ 6.3, indicating a total deuterium incorportation of 36%.

This oil was analyzed on gc-ms (10% SE 30, 150.2, 40°C at 10°C/min.) and the percentage of deuterium incorporation in the methoxy ester was calculated using the intensities of the characteristic fragments at m/e = 162 and 135. An authentic non-deuterated sample was run prior to every measurement, under the same conditions, in order to obtain the intensities of the M+1, M and M-1 molecular ion peaks of the two fragments considered. The same analysis was done on an esterified (diazomethane) sample of the crude oil and the total amount of deuterium incorporation was measured. The amount of deuterium incorporation in the methoxy acid was calculated by difference. The collected data and the respective amounts of deuterium exchange are reported in Table 4-3.

Table 4-3. Deuterium Incorporation During Thermolysis of II-22-a in Methanol-d

Fragment	% m/e authentic	% m/e before esterification	% D	% m/e after esterification	% D
163	11.4	73.4		79.3	
162	100.0	100.0	37	100.0	39
161	5.6	5.8		5.8	
136	16.5	83.8		77.7	
135	100.0	100.0	43.4	100.0	41
134	6.5	4.0		9.9	
		average % D	40		40

IV-10 Photolysis of N-Acyl-N-Nitroso-α-Amino Acids, II-22

II-10-1 N-Acetyl-N-Nitroso-Phenylalanine II-22-a

a) In Methanol in the Presence of Sodium Cyanide

A solution of II-22-a (2.36 g, 0.01 mole) and sodium cyanide (1 g) in methanol (220 ml) was cooled to 0°C and bubbled with nitrogen. The uv spectrum of a diluted sample of this solution showed absorptions at λ_{max} = 420, 402 and 375 nm for the nitrosamide group and a large shoulder at λ_{max} = 340 nm with a much higher absorbance. The solution was photolysed for 3 hours until the absorption disappeared. The darker photolysate was concentrated under vacuum at room temperature to about 10 ml and diluted with water (20 ml). The resulting solution (pH = 10) was extracted with ether (3 X 50 ml) which after drying (over $MgSO_4$) and evaporation, yielded the neutral fraction as a brownish oil (1.2 g). The water phase was acidified with 6N HCl to pH = 2 and extracted with ether (3 X 50 ml). The extracts were combined, washed with water (20 ml), dried over $MgSO_4$ and evaporated to yield the acidic fractions as a dark yellow oil (550 mg).

The neutral fraction exhibited ir absorptions at ν_{max}: 1660(s), 1590(s), 1520(s), 1370(s), 740(s), 700(s) and 680(s) cm^{-1} and its nmr spectrum showed a multiplet (wl/2 = 6 Hz) at

τ 2.67 and singlets at τ 5.95, 6.67, 7.50 and 8.07. Gc-ms analysis (10% SE 30, 150°C to 240°C at 10°C/min.) gave the following peaks described in the order of increasing retention times: 5 min., 6%, (unknown); 6.5 min., 6%, (unknown); 11.5 min., 57%, [II-39-a, m/e (%): 174(67), 132(100), 131(66), 104(43), 103(45), 91(43), 77(35) and 43(52)]; 12.7 min., 9%, [I-16, m/e (%): 161(34), 119(91), 118(49), 102(49), 91(45), 60(100) and 43(64)]; 15.9 min., 23%, [II-41-a m/e(%): 188(33), 161(10), 130(33), 129(97), 91(100) and 43(65)].

Column chromatography (basic alumina, 50 g) of a portion (870 mg) of the basic fraction afforded the following fraction. Elution with chloroform gave a colourless oil (71 mg) which showed one single compound on the analysis (silica gel, pet. ether-ether 50:50) with Rf = 0.54, contaminated with a more polar compound at Rf = 0.3. Distillation at 20°C/0.5 mm Hg gave II-39-a as colourless oil: ir ν_{max}: 1590(s), 1385(s), 1270(s), 735(s) and 700(s) cm^{-1}; ^{1}H nmr τ: 2.68(s,5H), 5.98 (s,2H), and 7.52(s,3H); ^{13}C nmr δ: 175.8(s), 168.6(s), 134.8(d), 128.2, 129.9, 126.3, 31.6(t) and 11.7(q); ms m/e (%) 174(M^{+},82), 132(100), 131(66), 91(44), 77(43) and 43(43). Anal. calcd. for $C_{10}H_{10}N_2O$: C 68.95, H 5.79, N 16.08; found: C 68.97, H 5.87, N 16.29.

Further elution with chloroform gave an oil (420 mg) whose

tlc analysis showed to be a mixture of oxadiazole II-39-a and a component of lower Rf value. This fraction was rechromatographed on basic alumina (30 g) to give the following compounds: compound II-39-a (270 mg) was obtained on elution with a mixture of pet. ether-ether (50:50). On elution with 25% pet. ether in ether a colourless oil (54 mg) was obtained, which crystallized on standing. Recrystallization from cyclohexane gave white crystals of I-16; mp 95-96°C; ir ν_{max}: 1660(s), 1530(s), 1070(s) and 700(s) cm^{-1}; ^1H nmr τ: 178 (d,J = 10 Hz), 2.73(s,5H), 4.6(m,1H), 6.67(s,3H), 7.07(d, J = 5.5 Hz, 2H) and 8.08(s,3H); ms m/e (%): 162(19), 134(13), 116(71), 102(83), 91(62), 74(75), 60(100), 46(54) and 43(54). Anal. calcd. for $C_{11}H_{15}NO_2$: C 68.37, H 7.82, N 7.25; found: C 68.54, H 7.92, N 7.29.

Elution of the original column with chloroform containing 1% of methanol gave a yellowish oil (175 mg) which crystallized on standing. Recrystallization from ether gave II-41-a; mp 105-106°C (dec); ir ν_{max}: 3290(m), 2240(w), 1660(s), 1540(m), 1460(m), 1330(m) and 710(m) cm^{-1}; ^1H nmr τ: 2.73(s,5H), 3.5 (bs), 4.92(dt, J = 8 and 6 Hz, 1H), 6.93(d, J = 6 Hz, 2H) and 8.03(s,3H); ms m/e (%): 188.0945 (M^+,39, calcd. for $C_{11}H_{12}N_2O$: 188.0949), 129(91), 102(24), 91(100), 60(30) and 43(42). Anal. calcd. for $C_{11}H_{12}N_2O$: C 70.19, H 6.43, N 14.88; found, C 70.46 H 6.52, N 14.89.

Further elution gave an unknown oil (45 mg) which showed ir absorptions at ν_{max}: 3280(s), 1650(s), 1530(s), 1290(s), 1030(m), 750(s) and 680(s) cm^{-1} and whose ^1H nmr spectrum exhibited the following signals at τ: 2.7(m,2I/w = 2 Hz), 5.36(t,5 = 6.5 Hz), 6.88(d,J = 7 Hz) and 8.0(s).

The acidic fraction was dissolved in benzene (50 ml) and refluxed with isopropyl alcohol (10 ml) and p-troluenesulfonic acid (50 mg) until no water was formed (~2 hours). The solvent was evaporated to yield a yellow oil (440 mg); ir ν_{max}: 3500(b, m), 1740(s), 1275(m,b), 1200(m), 1100(s) and 700(s); ^1H nmr τ: 2.75(s), 4.95(sp, J = 6 Hz), 5.61(dd, J = 5.5 and 7 Hz), 6.1(dd, 6 and 7 Hz), 6.66(s), 7.0(m), 8.8(d,J - 7 Hz) and 8.88(d, J = 7 Hz). After repeated (preparative) chromatography (silica and mixtures of ether-pet. ether) moving component was obtained as an oil (13 mg); ir ν_{max}: 1740(s), 1375(m), 1270(m), 1180(m), 1100(m) and 700(m); ^1H nmr τ: 2.75(s), 4.9(m), 5.5(m), 6.4(s), 6.6(m) and 8.7(m). The second compound identified as II-46 was obtained as a colourless oil (90 mg, 16%); ir ν_{max}: 1740(s), 1450(m), 1370(m), 1270(m), 1190(s), 1100(s), 740(m) and 700(s); ^1H nmr τ: 2.80(s,5H), 4.97(sp,J = 6 Hz, 1H), 6.07(t,J = 6.5 Hz, 1H), 6.67(s,3H), 7.0(d,J = 6.5 Hz, 2H), 8.78(d,J = 6 Hz, 3H) and 8.85(d,J = 6 Hz, 3H); ^{13}C nmr δ: 171.0(s), 136.6, 129.0, 127.8, 126.2, 81.5(d), 68.1(d), 57.8(q), 38.9(t), 21.6(q) and 21.5(q); ms m/e (%): 222.1242 (M^+, 0.3, calcd. for $C_{13}H_{18}O_3$:

222.1255), 190(84), 135(100), 103(53), 91(56), 71(44). Distillation under reduced pressure (70°C/2 Torr) gave an analytical sample: Anal. calcd. for $C_{13}H_{18}O_3$: C 70.24, H 8.16; found: C 70.40, H 8.14.

The third component II-47 (41 mg, 8%) was also isolated as a colourless oil; ir ν_{max}: 3500(b,m), 1740(s), 1500(m), 1470(m), 1460(m), 1380(m), 1270(m), 1210(m), 1100(s), 750(m), and 700(s) cm^{-1}; ^1H nmr τ: 2.77(s,5H), 4.93(sp, J = 6 Hz, 1H), 5.65 (X of ABX, J = 5.5 and 8 Hz, 1H), 7.01 and 7.11 (AB of ABX, J = 16 Hz, 2H), 7.1 (bs, D_2O exch.) and 9.75(d, J = 6 Hz, 6H); ^{13}C nmr δ: 176.1(s), 138.9, 132.0, 130.7, 129.2, 73.8(d), 72.1(d), 43.1(t) and 24.4(q); ms m/e (%) 208.1094 (M^+, 3, calcd. for $C_{12}H_{16}O_3$ 208.1100), 191(17), 190(83), 148(57), 147(30), 145(25), 121(93), 104(35), 103(72), 91(100), 77(37) and 43(60). Anal. calcd. for $C_{12}H_{16}O_3$: C 69.21, H 7.74; found: C 69.03, H 7.67.

b) In Methanol and Sodium Carbonate

A solution of II-22-a (1.3 g, 5.5 mmole) in methanol (130 ml) containing sodium carbonate (2 g) showed the characteristic uv absorptions of the nitrosamido group at λ = 420 and 400 nm with a shoulder at λ = 350 nm. After 2 hours of irradiation at 0°C, this solution was concentrated under vacuum to about 10 ml. Addition of water (15 ml) and ether extraction of the

resulting mixture (pH = 10) yielded after drying and evaporation the neutral fraction (615 mg) as a yellow oil; ^{1}H nmr τ: 2.70(m, wl/2 6 Hz), 4.6(m), 5.98(s), 6.7(s), 7.1(d,J = 6.0 Hz), 7.5(s) and 8.03(s); the peak areas of the methyl signals at τ 6.7 for I-16 and 7.5 for II-39-a were in a ratio 3.7:1. The estimated yields for oxadiazole II-39-a were 12.5% and for methoxy derivative I-16, 46%.

c) In THF in the Presence of DBU

A solution of II-22-a (236 mg, 1 mmole) and one mole equivalent of DBU (152 mg, 1 mmole) in dry THF (120 ml) showed an absorption at λ_{max} 400 nm for the nitrosamido group as well as an absorption at λ_{max} 350 nm; the absorbance of the latter being approximately twice as large as that of the former. The mixture was irradiated at 0°C under nitrogen for 90 min. and the solvent was evaporated under vacuum to give a yellow oil (457 mg) whose complex nmr spectrum showed singlets at τ 5.98 and 7.5. Water (10 ml) was added and the resulting mixture was acidified with HCl to pH = 2. Extraction with ether (2 X 30 ml) afforded a yellow oil (120 mg) which was esterified with diazomethane. The residue obtained after evaporation was analyzed by gc-ms (3% Silar 10 C, 90 to 180°C at 10°/min.) to give the following peaks: 2.3 min., 6% methyl benzoate; 3.0 min., 11% methyl phenethylacetate; 3.5 min., 29%, butyrolactone; 5.5 min.,

22% (II-39-a); 6.5 min., 23% (II-24). All compounds were identified by peak matching with authentic samples and from their ms fragmentation patterns.

d) In MeOH in the Presence of $(Et)_3N$

A solution of II-22-a (970 mg, 4.1 mmoles) and triethylamine (980 mg, 9.7 mmoles) in methanol (120 ml) was irradiated at 0°C under ni-rogen for 1.75 hours when the absorption at λ_{max} 400 nm disappeared completely. The solvent was evaporated at room temperature, under vacuum, and the residue dissolved in ether (50 ml). The resulting solution was subsequently washed with 10% sodium carbonate solution, 0.1N HCl solution and water, to give a yellowish oil (467 mg). The nmr spectrum of this oil exhibited the characteristic signals for II-39-a (64%) and I-16 (9%), the methyl groups of which being in a 6.7:1 ratio, respectively. Both products were identified by tlc and gc peak matching with authentic samples.

In another experiment a stock solution (So) containing II-22-a (944 mg, 4 mmoles) in methanol (100 ml) and one (S_1) containing triethylamine (1.01 g, 0.01 mole) in methanol (100 ml) were prepared. Four different solutions were made up from those stock solutions in the proportions indicated in Table 4-4. Each of them was photolysed at 0°C under nitrogen and under identical conditions. The resulting photolysates were added

with dibenzofuran as the internal standard and was injected into gc for direct analysis.

The photolysate of the experiment without triethylamine was submitted by gc-ms analysis (10% Se 30, 120 to 220°C at 8°C/min.), to give the following peaks: 2 min., 3% (II-41); 2.75 min., 3% (II-40); 3.5 min., 3% (unknown); 6 min., 3%, (II-39-a); 8 min., 35% (I-17); 8.6 min., 8% (unknown); 13 min., 26% (I-16); 13.5 min., 13% (I-16). All compounds were identified upon mixed injection with authentic samples. Upon injection of pure I-16 two peaks appeared, whose intensities ratio varied with the temperature. The first peak at 13 min. had a ms similar to that of I-17 and the ms of the second peak at 13.5 min. contained m/e = 178 corresponding to $[M^+ - OCH_3]$. Obviously I-16 decomposed in the column to give I-17 and I-16.

Table 4-4. Experimental Conditions and Results of Photolysis of II-22-a in MeOH with $(Et)_3N$

Run #	[II-22-a] M	[Et_3N] M	Ratio base/II-22-a	Total Vol. ml	Time of Photolysis (min.)	% Yield II-6
1	0.01	0.01	0	100	20	-1
2	0.01	0.01	1	100	20	17
3	0.01	0.02	2	100	20	52
4	0.01	0.05	5	100	20	68

e) In CH_3CN in the Presence of $(Et)_3N$

Trimethylamine (202 mg, 2 mmoles) was added to a cold solution of II-22-a (235 mg, 1 mmole) in dry acetonitrile (120 ml). The resulting solution was irradiated under nitrogen at 0°C for 1.5 hours, the peak at λ_{max} = 400 nm disappeared completely. The solvent was evaporated under vacuum to yield a yellow oil (220 mg) which was dissolved in ether (20 ml) and washed with diluted HCl solution (2 X 10 ml 0.1N HCl) followed by water. The ether phase was dried ($MgSO_4$) and evaporated to yield a yellowish oil (150 mg) which was distilled at room temperature under 0.5 Torr to give II-39-a (109 mg, 63%) as a slightly yellow oil which gave one single compound on tlc and gc analysis.

In another experiment four solutions containing different molar ratio of triethylamine to II-22-a in acetonitrile (as indicated in Table 4-5) were photolysed under nitrogen and at 0°C for 30 minutes where the nitrosamido absorption had disappeared. Each photolysate was evaporated to approximately 10 ml and adjusted to 10 ml exactly in a volumetric flask. Samples for gc analysis were made up from 1 ml of the latter solution and 1 ml of a dibenzofuran solution in acetonitrile (0.59 mN). The analyses were conducted on a 3% SE 30 column and at 120°C and the yields are reported in Table 4-5.

Table 4-4. Experimental Conditions and Results of Photolysis of II-22-a in CH_3CN with $(Et)_3N$

# mole II-22-a X 10^3 (mg)	# mole $(Et)_3N$ X 10^3 (mg)		# II-39-a
1.39 (238)	0.86 (87)	0.62	26
0.82 (193)	1.08 (104)	1.31	42
0.89 (210)	2.18 (221)	2.45	63
0.71 (167)	3.47 (350)	4.9	68

f) Photolysis of N-Acetyl-N-Nitroso-D,L-Phenylanine Dicyclohexylamine Salt II-48 in MeOH

A solution of II-48 (125 mg, 0.3 mmole) in methanol (100 ml) was irradiated under nitrogen at 0°C for 15 min., until the absorption at λ_{max} = 405 and 423 nm had completely disappeared. The solvent was evaporated under vacuum at room temperature to yield a yellow residual oil, which was dissolved in ether (30 ml), washed with diluted HCl solution (2 X 10 ml, 0.1N HCl) and with water (10 ml). After drying (Na_2SO_4) and evaporation, a yellow semi-solid (45 mg) was obtained; ir ν_{max}: 3280(bs), 1650(s), 1370(m), 1280(m), 1120(m), 1070(m) and 700(s) cm^{-1}. Gc-ms analysis (10% SE 30, 140°C to 220°C at 1°/min.) gave the

following peaks: 1.1 min., 20% (II-41); 3.5 min., 15% (II-39-a); 4.0 min., 6% (dicyclohexylamine); 4.6 min., 16% (I-17), 5.0 min., 31% (I-16); 7.5 min., 10% [unknown m/e (%), 161(33.7), 119(100), 118(55.3), 91(32.4), 43(71.4)]. The first five components were identified on basis of their ms and upon mixed injection with authentic samples.

The acidic water phase gave crystals (10 mg) of dicyclohexylamine hydrochloride: ir 2850-2600 (multiple bands), 2520(m), 2420(m), 1460(m) cm^{-1}; the spectrum was superimposable with that of an authentic sample. The mother liquor was further extracted with ether to give no appreciable amount of material.

IV-10-2 N-Acetyl-N-Nitroso-D,L-Leucine II-22-c

An ice cold solution of II-22-c (2.33 g, 0.011 mole) and triethylamine (2.2 g, 0.022 mole) in acetonitrile (235 ml) was kept under nitrogen and irradiated for 1.25 hours when the absorption at 400 nm completely disappeared. The solvent was distilled under atmospheric pressure, using a Widmer spinal column. The volume of the residue was made 10 ml using a volumetric flask and the resulting solution was analyzed by gc-ms (10% SE 30, 100°C iso). The chromatographs showed the presence of three peaks: 1.0 min., (triethylamine); 3.0 min., 5% [unknown, ms

m/e (%) 102(100, 57(34.7), 56(44.7), 44(42.2) and 42(57.7)]; 5 min., 68%, (II-39-c). The first peak was identified on the basis of mixed injection with an authentic sample. The third peak was isolated by preparative gc to give a volatile yellow oil; ir ν_{max}: 1620(m), 790(m) cm^{-1}; ^{1}H nmr τ: 7.42(d, J = 7 Hz, 2H), 7.44(s,3H), 7.88(mo, J = 7 Hz, 1H) and 9.01 (d, J = 6 Hz, 6H); ^{13}C nmr δ: 175.8(s), 169.7(s), 34.5(t), 26.8(d), 22.1(q) and 12.0(q); ms m/e (%): 140(M^+,1.7), 125(12), 98(100), 83(31), 56(53) and 43(87); upon irradiation of the doublet at τ 9.01 in the ^{1}H nmr spectrum, the nonet at τ 7.88 collapsed into a triplet (J = 7 Hz).

A small sample of the residual oil was treated with diazomethane and the resulting mixture was analyzed by gc-ms (10% SE 30, 100° to 240°C at 8°C/min.). In addition to the previously described peaks, a new peak [9.6 min., m/e (%) 187(M^+, 0.6), 131(10.7), 128(61.5), 86(100) and 43(28.2)] was observed and identified as the methylester of II-21-c by peak matching with an authentic sample. The yield of oxadiazole II-39-c was measured by means of oxadiazole II-39-a as internal standard.

IV-10-3 Photolysis of N,O-Diacetyl-N-Nitroso-D,L-Leucine II-22-d

A solution containing triethylamine (370 mg, 3.3 mmoles)

and II-22-d (360 mg, approximately 80% purity, 1.3 mmole) in acetonitrile (90 ml) was irradiated under nitrogen at 0°C for 1 hour. The solvent was evaporated under vacuum to yield a yellow oil which was dissolved in methylene chloride (40 ml) and washed with diluted HCl solution (0.5N, 2 X 20 ml) followed by water. After drying ($MgSO_4$), evaporation of the solvent gave II-39-d (25 mg, 12%) as a slightly yellow oil. Distrillation at room temperature under 0.1 Torr gave a nearly colourless oil (20 mg); ir ν_{max}: 3500(wb), 1750(s), 1590(s), 1370(m), 1220(s), and 1040(s); ^1H nmr τ: 4.82(s,2H), 7.40(3,3H), 7.85 (s,3H); ^{13}C nmr δ: 175.3, 168.6, 165.0, 56.7(t), 20.6(q) and 13.6(q); ms m/e (%) 156(M^+,.3), 114(22.7), 113(10), 10.4(11.8), 102(12), 86(11), 85(22), 84(11) and 43(100). Anal. calcd. for $C_6H_8N_2O_3$: C 46.15, H 4.16, N 17.94; found: C 47.03, H 5.66, N 17.48. The continuous extraction of the water phase gave only triethylamine HCl salt as identified by its ir spectrum.

IV-10-4 N-Benzoyl-N-Nitroso-D,L-Phenylalanine II-22-b

A solution containing II-22-b (1.3 g, 4.3 mmoles), 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 λ_{max} 350 nm was observed along with that of the nitrosamido group at 400 nm. The solution was irradiated for 10 hours, until no change in the uv absorption

could be detected. The solvent was evaporated at room temperature to yield a semi-solid to which water (10 ml) was added to give a yellow solution (pH = 10). This solution was extracted with ether (4 X 30 ml), the extracts were combined, dried ($MgSO_4$) and evaporated to yield the neutral fraction (270 mg) as a yellow oil. The water phase was acidified to pH = 2 with 1N HCl and extracted with ether (4 X 30 ml). The extracts were combined, dried ($MgSO_4$) and evaporated to yield the acidic fraction (736 mg) as a yellow oil.

The neutral fraction was chromatographed over Silica gel (20 g) using a mixture of ether-petroleum ether (1:1) as eluant. The first fraction (82 mg) consisted of several products which were separated by preparative tlc (silica gel, ether-pet. ether 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 ir and nmr spectra and tlc mobility as 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 II-39-b as white needles: mp 81-82°C; lit. (82) mp 88°C; ir ν_{max}: 3060(w), 3030(w), 1620(w), 1560(m), 1450(s), 1370(3), 730(m), 715(s), 695(m) and 650(m) cm^{-1}; ^1H nmr τ: 1.9(m,2H), 2.6(m,8H) and 5.82(s,2H); ^{13}C nmr δ: 156.9, 152.4, 132.5, 128.9, 128.5, 128.0, 126.9, 32.31; ms m/e (%), 236.0947(M^+,69, calcd. for $C_{15}H_{12}N_2O$: 236.0950), 207(20),

131(14), 116(15), 105(100), 103(42), 91(33) and 77(55). Another unidentified product (3 mg) was obtained from extraction of the slowest moving spot; ir ν_{max}: 3400(bm), 1740(s), 1460(s), 1280(s), 970(s), 760(s) and 700(s) cm^{-1}.

The second fraction (65 mg) of the column chromtography, solidified upon evaporation of the solvent. Recrystallization from cyclohexane gave II-48 (58 mg, 5%) as white crystals; mp 123-125°C; ir ν_{max}: 3320(m), 1640(s), 1530(s), 1280(m), 1100(m), 1060(m), 700(s) and 690(s) cm^{-1}; ^1H nmr τ: 2.3(m,2H), 2.5(m, 3H), 2.74(s,5H), 3.74(bd, J = 6Hz,1H), 4.35(bm,wl/2 = 8 Hz, 1H), 6.64(s,3H) and 6.98(dJ = 6 Hz, 2H); ms m/e (%): 255(M^+,0.1), 224.1026(25, calcd. for $C_{15}H_{14}NO$, 223.0997), 178(57), 164(58), 162(34), 105.0342 (100, calcd. for C_7H_5O: 105.0344), 91(37) and 77(61). Anal. calcd. for $C_{16}H_{17}NO_2$: C 75.27, H 6.71, N 5.49; found: C 75.00, H 6.83, N 5.37. The third fraction (50 mg) was shown by tlc to contain some of the methoxy adduct II-48.

The yellow acidic fraction was sublimed at room temperature and under 0.1 Torr to yield white crystals of benzoic acid (60 mg); mp 114-115°C; mixed mp with an authentic sample 119-120°C. The yellow residue was treated with ether (~5 ml) to give II-21-b (150 mg) (mp and mixed mp 119-120°C) and a yellow filtrate. The latter after treatment with an etheral

solution of diazomethane gave a yellow oil (540 mg) which was analyzed by gc (3% silar 10 C, 170°C). This gave the following peaks: 0.9 min., 10% (methyl benzoate); 1 min., 2% (unknown); 1.6 min., 1% (unknown); 1.9 min., 71% (II-37) and 3.6 min., 16% (II-25). All compounds were identified by mixed injection with authentic samples.

IV-11 Synthesis and Properties of Benzyl-Methyl-1,2,4-Oxadiazoles

IV-11-1 Synthesis of 3-Benzyl-5-Methyl-1,2,4-Oxadiazole II-39-a

The reaction of hydroxylamine with phenylacetonitrile (BOH) (83) gave II-43 (95%); mp 57-59°C; ir ν_{max}: 3500-3100(b, s), 1650(s), 1460(s) and 760(s) cm^{-1}; ^1H nmr τ: 1.7(bs, D_2O exch.), 2.72(s,5H), 5.5(bs, D_2O exch.) and 6.51(s,2H).

II-43 was acetylated with acetic anhydride (123) to yield II-45 (89%) as a white solid; mp 121-123°C; ir ν_{max}: 3440(s), 3320(s), 1740(s), 1630(s), 1230(s), 900(s) and 750(s) cm^{-1}; ^1H nmr τ: 2.70(s,5H), 5.2(bs, D_2O exch.), 6.45(s,2H), and 7.87(s,3H).

Heating II-45 in water gave II-39-a (61%) as a colourless oil; ir ν_{max}: 1590(s), 1500(m), 1450(m), 1430(m), 1380(m), 1360(m), 1270(m), 740(s) and 700(s) cm^{-1}; ^1H nmr τ: 2.77(s,

5HO, 6.03(s,2H) and 6.63(s,3H); ^{13}C nmr δ: 11.5(q), 31.5(t), 126.4, 128.0, 128.3, 135.1, 168.8(s) and 176.0(s); ms m/e (%): 174(M^+,81), 133(41), 132(100), 131(65), 105(31), 104(42), 103(49), 91(42), 88(31), 86(69), 84(77), 77(32) and 43(36).

IV-11-2 Synthesis of 3-Methyl-5-Benzyl-1,2,4-Oxadiazole II-39-e

The reaction of acetonitrile with hydroxylamine (127) gave II-42 (9%); mp 125-128°C; lit. (82) mp 133.5; ir ν_{max}: 3500(s), 1650(s), 1040(m) and 890(s) cm^{-1}.

Acetylation of II-42 with phenylacetic anhydride (128) gave II-44 (19%) as white crystals; mp 86-91°C; ir ν_{max}: 3420(m), 3300(m), 1740(s), 1600(s), 1220(s) and 720(s) cm^{-1}; ^1H nmr τ: 2.73(s,5H), 5.0(bs, D_2O exch.), 6.28(s,2H), and 8.15(s,3H).

Steam distillation of II-44 gave II-39-e (78%) as a slightly yellow oil; ir ν_{max}: 1580(s), 1500(m), 1460(m), 1430(m), 1400(s), 1340(s), 740(s) and 700(s) cm^{-1}; ^1H nmr τ: 2.70(s, 5H), 5.92(s,2H) and 7.65(s,3H); ^{13}C nmr δ: 10.3(q), 31.6(t), 126.3, 127.8, 127.9, 132.9, 166.6(s) and 176.8(s); ms m/e (%): 174(46), 117(59), 104(100), 91(64), 90(34), 77(15), 65(29), and 39(22). When an off acquisition decoupled C spectrum was recorded the lines at 166.6 and 176.8 were respectively split into a quartet and a triplet.

IV-11-3 Reaction of 3-Benzyl-5-Methyl-1,2,4-Oxadiazole II-39-a

a) Basic Treatment

A solution of oxadiazole II-39-a (200 mg, 1.2 mmole) and NaOH (1 g, 0.025 mole) in methanol (20 ml) was stirred overnight at room temperature to give unreacted II-39-a (188 mg, 94%).

b) Thermal Treatment

Oxadiazole II-39-a (340 mg, 1.4 mmole) was heated in a sealed tube, at 200°C for 12 hours to give the unreacted starting material (320 mg, 94%) as indicated by its ir and nmr spectra.

c) Uv Irradiation

A solution of acetonitrile (200 ml) containing oxadiazole II-39-a (300 mg, 1.3 mole) was irradiated at room temperature in a quartz vessel, with a 60 W low pressure mercury lamp for 6 hours to give unreacted II-39-a (305 mg, 100%).

IV-12 Attempts to Elucidate the Mechanism of Oxadiazole Formation

IV-12-1 Synthesis of N-Acetyl-Phenylacetamidoxime II-49

Chloroxime II-51 was prepared by chlorination of oxime II-50 (128) according to the method described by Behn (84): mp 85-88°C; lit. (84) mp 89-91; ir ν_{max}: 3200(s), 1660(m), 1080(m), 990(s); ^{1}H nmr τ: 1.0(bs, D_2O exch.), 2.70(s,5H), and 6.21(s,2H).

A solution of triethylamine (90 mg, 0.9 mmole) in ether (5 ml) was added to a solution of the chloroxime II-51 (130 mg, 0.8 mmole) in ether (20 ml). The precipitate formed was filtered and the filtrate was added dropwise to a suspension of sodium acetamide (600 mg, 7 mmole)(129) in DMF (25 ml) containing few drops of HMPT. A brown colour appeared. The mixture was stirred at room temperature overnight. Water (25 ml) was added to the reaction mixture and the resulting solution was first extracted with ether (4 X 30 ml) and then continuously with ether for 12 hours. The combined extracts were throughly washed with water (9 X 10 ml), dried and evaporated to give N-acetylphenylacetamidoxime II-49 (50 mg, 33%) as a white solid which was purified by sublimination at 60°C under 0.1 Torr; mp 130-130.5°C; ir ν_{max}: 3320(m), 1700(s), 1670(s), 1550(m), 1460(m), 1270(m), 1260(m), 1230(m) and 720(m) cm^{-1}; ^{1}H nmr τ: (bs, D_2O exch.), 1.1(bs, D_2O exch.), 2.7(s,5H),

5.47(s,1H), 5.57(s,1H) and 7.9(s,3H); ms m/e (%): 192.0901 (M^+, calcd. for $C_{10}H_{12}N_2O_2$ 192.0903, 49), 107(16), 106(100), 91(37.5), 77(15.5) and 60(62.3). Anal. calcd. for $C_{10}H_{12}N_2O_2$, C 62.49, H 6.29, N 14.57; found: C 62.66, H 6.46, N 14.39.

IV-12-2 Basic Treatment of N-Acetyl-Phenylacetamidoxime II-49

a) $(Et)_3N$ in CH_3CN

Triethylamine (25 mg, 0.25 mmole) in acetonitrile (2 ml) was added to a solution of the acetamidoxime II-49 (18 mg, 0.09 mmole) in acetonitrile (3 ml). The mixture was stirred in the dark, at room temperature overnight. The resulting solution was analyzed by tlc to give unreacted acetamidoxime II-49 (17 mg, 94%).

b) KOH in MeOH

A solution of II-49 (17 mg, 0.09 mmole) in methanol (2 ml) was treated with a solution of potassium hydroxide (~10 mg) in methanol (3 ml). The resulting mixture was stirred at room temperature for 18 hours. The reaction was followed by tlc but no formation of II-39-a could be detected. The solvent was evaporated 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 II-53 (8 mg, 59%) as a white solid, mp 139-142°C; lit. (130) mp 147-148°C; ^1H nmr (D_2O) τ: 2.7(s), 5.64(s), 5.7(s); ms m/e (%): 150(M^+,80), 106(100), 91(50), 79(25), 77(25).

IV-12-3 Attempts at Trapping II-34 with Nitroxyl

a) Preparation of N-Hydroxybenzenesulfonamide

Benzenesulfonyl chloride (10 g, 0.057 mole) was treated with hydroxylamine in the presence of NaOH according to the original method of Piloty (86) to give Piloty's salt (6 g, 61%); mp 123-124°C; lit. (88) ml 126°C; ir ν_{max}: 3420(s), 3260(s), 2920(s), 2860(s), 1330(s), 750(s) and 690(s) cm^{-1}; uv (MeOH) λ_{max}: 252(550), 259(725), 265(975) and 272(850).

b) Synthesis of N-Chloro-N-Acetyl-2-Phenylethylamine II-56

Phenethylamine (BDH) was acetylated with acetic anhydride to give the corresponding amide IV-3 (80%); mp 45-49°C; ir ν_{max}: 3300(s), 1650(s), 1550(s), 1450(s), 750(s) and 700(s) cm^{-1}; ^1H nmr τ: 2.78(s,5H), 3.6(bs, D_2O exch.), 6.8(m,wl/2 = 24 Hz,2H), 7.2(m,wl/2 = 19 Hz,2H) and 8.07(s,3H).

Treatment of amide IV-3 (5.3 g, 0.032 mole) with bleach solution (60 ml, commercial Javex) in ether at 0°C and in the dark (85) yielded II-56 (5.3 g, 83%) as a yellow oil which, when stored at -20°C, gave crystals which melted at room temperature; tlc (silica gel/ether) gave one single spot (Rf = .85) and no trace of the starting material; ir ν_{max}: 1675(s), 1380(s), 750(s) and 700(s) cm^{-1}.

c) Kinetic Study of Piloty's Salt Decomposition

Five solutions of Piloty's salt in methanol containing 1, 5, 10, 20 and 100 mole equivalents of base (NaOH), respectively were prepared. The uv spectrum of each solution was recorded immediately (approximately 1 min.) after preparation, and at regular time intervals thereafter. The spectra are shown in Fig. 2-15, and the rates of decomposition were calculated and reported in Table 2-15.

d) Trapping Reaction of N-Acylimine, II-54, with HNO

A solution of II-56 (240 mg, 1.2 mmole) ether (10 ml) and a solution of DBU (330 mg, 2.2 mmole) in ether (10 ml) were slowly mixed into a dropping funnel. A heavy precipitate appeared immediately and the solution turned brown. The resulting mixture was simultaneously added through a sandglass

wool filter to a stirred solution of Piloty's Salt (854 mg, 5 mmoles) in methanol (40 ml) to which NaOH (2 g in 5 ml H_2O) had just been added. The whole addition process took about 15 minutes. The resulting mixture was stirred for 15 more mins. and the methanol was evaporated to yield a residue which was diluted with water (20 ml). The resulting solution was extracted with ether (4 X 30 ml) to give after drying ($MgSO_4$) and evaporation a yellow oil (300 mg) whose nmr spectrum did not exhibit the characteristic signals of oxadiazole II-39-a. The oil was dissolved in methylene chloride (20 ml), washed with diluted HCl solution (0.5N, 20 ml), and water (20 ml) to give after drying ($MgSO_4$) and evaporation a yellow oil (150 mg); ir ν_{max}: 3300(m), 2225(w), 1650(s), 1550(s), and 700(s) cm^{-1}; ^1H nmr τ: 2.7(s), 2.8(s), 3.7(bs), 6.31(s), 6.58(m,wl/2 = 14 Hz), 7.15(m,wl/2 = 14 Hz) and 8.12(s). Gc-ms analysis (10% SE 30, 120 to 200°C at 10°C/min.) gave the following components: 1.9 min., 38% (II-41); 3.6 min. 35%, (II-59); 5.6 min., 5% (II-58) [m/e (%); 199(3.3), 197(10.3), 140(34), 138(100), 72(25), 43(20)] and 8 min., 5% (II-57) [199(3.2), 197(9.4), 162(91), 140(32), 138(100), 125(27), 72(92), 43(47)]. Both peaks at 1.0 and 3.6 min. were identified by gc peak matching with authentic samples and on basis of their ms. The gc-ms trace contained one of the m/e = 174 and 193 fragments typical for compounds II-39-a and I-16.

In another experiment, an ether solution (10 ml) containing N-chloramide II-56 (220 mg, 1.1 mmole) was added dropwise to a methanol solution (50 ml) of Piloty's salt (867 mg, 5 mmoles) to which NaOH (2 g, ~50 mmoles) had just been added. The resulting mixture was stirred in the dark for 30 min. after completion of the addition until the KI paper test was negative. The solvent was evaporated to give a residue which was taken up in water (10 ml) and extracted with ether (3 X 50 ml). Drying ($MgSO_4$) and evaporation of the extract gave a yellow oil (391 mg); ir ν_{max}: 3300(b), 1700(s), 1660(s) and 1360(s) cm^{-1}; ^1H nmr τ: 2.7(s), 5.8(b,s), 6.5(m), 7.3(s), 7.75(s), 8.0(s) and 8.2(s). Chromatography of this oil over silica gel (10 g) gave a first fraction (150 mg, 37%) by elution with pentane-ether (8:2); ir ν_{max}: 3400(b), 1700(s), 1370(s) and 1180(m); ^1H nmr τ: 7.3(s), 7.75(s) and 8.2(s). Elution with ether gave II-59 (96 mg, 50%); ir identical to authentic sample. Further elution with methanol did not give any substantial material.

IV-13 Nitrosation of N-Acyl-D,L-Phenylalanine via Acyl Nitrite

IV-13-1 Preparation of N-Acetyl-D,L-Phenylalamine Silver Salt II-60

A solution of II-21-a (439 mg, 2.72 mmoles) in water (20 ml) was neutralized with a 0.1N KOH solution to the turning

point of phenyl phtalein. Silver nitrate (357 mg, 2.7 mmoles) was added at ounce to the resulting solution in the dark, and the heavy precipitate was filtered after the solution had been cooled at 5°C for overnight. The white cake obtained was dried over P_2O_5 under vacuum to give II-60 (607 mg, 71%) as a white powder; ir ν_{max}: 3380(m), 1610(s), 1560(s), 1520(m), 1410(s) and 700(s) cm^{-1}. Silver salt II-60 was kept at room temperature in the dark.

IV-13-2 Nitrosation of N-Acetyl-D,L-Phenylalanine Silver Salt, II-60, with $NOBF_4$

Silver salt II-60 (362 mg, 1.5 mmoles) was added at once to a suspension of $NOBF_4$ (133 mg, 1.2 mmoles) in dry benzene (30 ml) at 0°C in the dark. The resulting suspension was stirred for 4 hours at 0°C. The reaction was monitored by uv measurements of filtered aliquots. After 4 hours uv absorption bands at λ_{max} 418, 400 and 390 characteristic of the nitrosamido group in II-22-a reached their maximum intensities. The yield in II-22-a (17%) was evaluated from the uv band absorbance.

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