Chemical Studies on Carcinogenic Nitrosamines. 1. Hydrolysis of α-Acetoxynitrosamines

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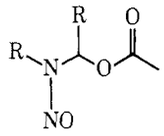
α-Hydroxynitrosamines have been implicated as activated carcinogens. Protected derivatives of these species were found to undergo two mutually exclusive reactions on treatment with simple nucleophiles. Attack at the α carbon resulted in hydrolysis or methanolysis, while attack at the carbonyl carbon by *n*-propylamine, for example, yielded *N*-*n*-propylacetamide and the unstable α-hydroxynitrosamine. The hydrolysis and methanolysis appear to occur via an S_N1 reaction, involving a resonance-stabilized benzylnitrosimminium ion. The deacylation by primary amines was found to be strongly activated by the nitrosamino group, probably via an inductive effect.

Nitrosamines are believed to be activated in vivo to a proximate carcinogen. One possibility for this process that has been frequently discussed is α hydroxylation, following which, decomposition could provide several powerfully electrophilic species capable of alkylating nucleic acids (Scheme I). α-Acetoxynitrosamines (1) are currently receiving considerable attention since on hydrolysis they may give rise to the elusive α-hydroxynitrosamines. Indeed, it has been conclusively demonstrated that such derivatives (1) are both carcinogenic



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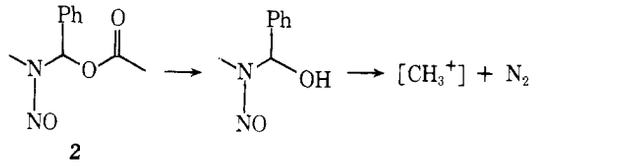
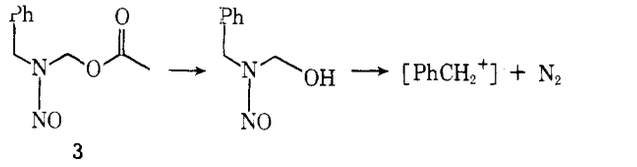
and capable of directly causing bacterial mutations without prior enzymatic activation, an observation that has been attributed to their prior hydrolysis to the α-hydroxynitrosamines.[1-3] Recently[1b] we demonstrated that the two structural isomers of α-acetoxymethylbenzylnitrosamine possessed markedly different mutagenic activity, i.e., **2** was a powerful bacterial mutagen, whereas **3** was essentially inactive, the expected hydrolysis schemes for these compounds being shown in eq 1 and 2.[4]



2

+ PhCHO + OH^- (1)

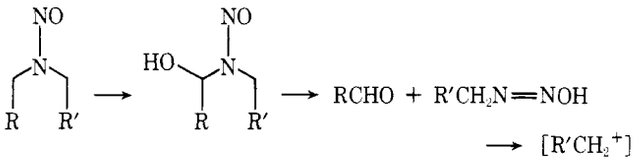


3

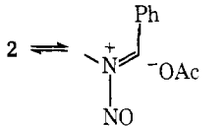
+ CH_2O + OH^- (2)

In experiments to effect mild hydrolysis of the ester functions of these two compounds, they were both treated in a buffered solution with hog liver esterase. Both compounds hydrolyzed readily, but **2** reacted three times more rapidly. The relative lability of **2** was much more apparent in the absence of enzyme. While **3** was stable in aqueous neutral solution, **2** displayed a half-life of only 19 min in water. Rapid hydrolysis of **2** was also apparent in pH 8 buffer, while **3** hydrolyzed some 32-fold more slowly. In weakly acidic solutions

Scheme I



(pH 3), **2** again hydrolyzed extremely rapidly, while **3** was stable. We attribute the striking reactivity of **2** to its ability to form a highly resonance-stabilized benzylnitrosimminium ion, **4** (eq 3). This type of S_N1 process, via imminium ion,[12] **4**,



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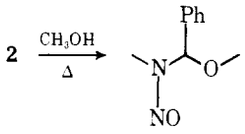
involving fission at the alkyl–oxygen bond, is unlikely to be operative for **3**, as the resultant cation would lack benzylic stabilization. It is instead more likely that the slow hydrolysis of **3** in pH 8 buffer involves the more conventional acyl–oxygen fission.

To further investigate the behavior of **2** and **3** toward nucleophilic substitution, each compound was treated with dry methanol at reflux for about 24 h (eq 4). The methyl ether **5**



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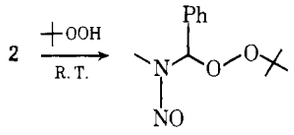

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was cleanly produced from **2** in quantitative yield (79% isolated); no reaction occurred at 25 °C with or without *p*-toluenesulfonic acid catalysis. When isomer **3** was refluxed with dry methanol, however, only the starting material was recovered. Again it appears that **2** yields the cation **4**, which is trapped by methanol, while **3** cannot likewise react. Additionally, **2** was treated with *tert*-butyl hydroperoxide for 2 weeks at room temperature (eq 5). The α-peroxynitrosamine **6** formed in nearly quantitative yield. If a sufficiently mild

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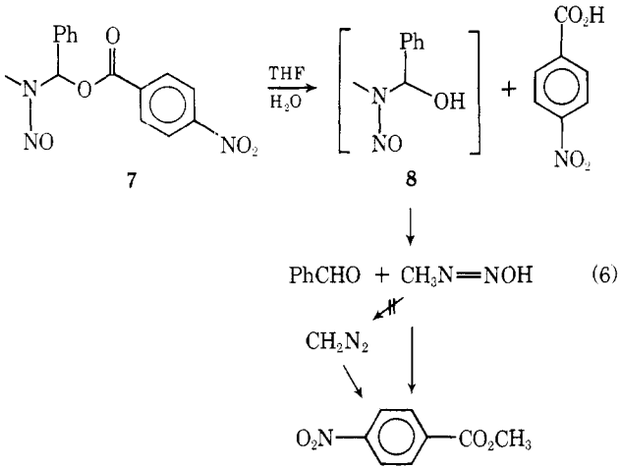
reductive method can be found, this compound could prove to be a precursor of the α-hydroxynitrosamine **8**. Exchange of the acyloxy function was found to occur when **2** was refluxed in methylene chloride solution with an excess of chloroacetic acid. The identity of methyl(α-chloroacetoxybenzyl)nitrosamine was confirmed by comparison with an authentic sample prepared in standard fashion.

In an effort to trap ion **4** with water, we hydrolyzed the nitrosamine **7** in THF containing 1 equiv of H_2O at reflux. Under these conditions, methyl *p*-nitrobenzoate was isolated in 75% crude yield. It seems likely that initial replacement of *p*-nitrobenzoate by hydroxyl is followed by collapse of the α-hydroxynitrosamine to methyl diazotate, which directly alkylates the *p*-nitrobenzoic acid (eq 6). In addition, replacement of the water in the hydrolysis experiment with deuterium oxide gave methyl *p*-nitrobenzoate, containing no deuterium, thereby eliminating diazomethane as a possible alkylating intermediate.[5]



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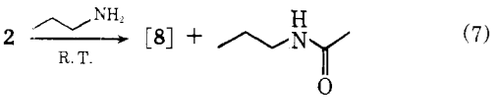
In sharp contrast to the above behavior, when nitrosamine **2** was exposed to neat *n*-propylamine at room temperature for 5 min, a smooth exothermic reaction occurred (eq 7). *N*-*n*-propylacetamide was isolated in quantitative yield and a mixture of benzaldehyde and *N*-benzylidene-*n*-propylamine was also isolated. These products clearly result from nucleophilic attack at the carbonyl carbon, followed by spontaneous decomposition of the α-hydroxynitrosamine **8**, vide supra. The resulting benzaldehyde is incompletely converted to the imine under the reaction conditions.



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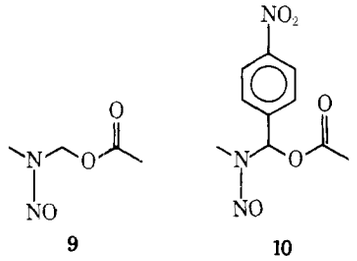
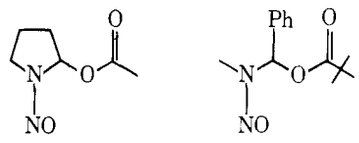
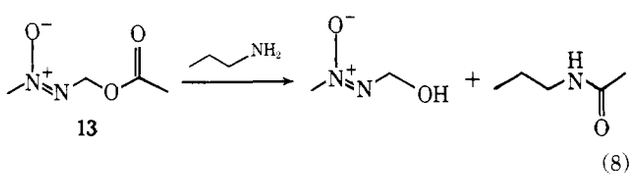
The generality of this exothermic reaction of α-acetoxynitrosamines with *n*-propylamine was demonstrated. Thus, α-acetoxydimethylnitrosamine (**9**), prepared according to Keefer,[2a] reacted as rapidly with *n*-propylamine as did **2** to give a 68% isolated yield of the amide. Nitrosamine **3** gave an exothermic reaction on treatment with the amine at 25 °C, resulting in an inseparable mixture of the amide and an unknown aromatic impurity. Nitrosamine **10** reacted readily at 0 °C in like fashion. The cyclic α-acetoxynitrosamine **11** similarly gave a 77% isolated yield of *n*-propylacetamide.





The reaction with *n*-propylamine is subject to steric effects. The α-pivalate **12** reacted exclusively at the carbonyl carbon to give cleanly a 1:1 mixture of *n*-propylpivalamide and *N*-benzylidene-*n*-propylamine. However, the reaction required 40 h at reflux (48 °C) to reach completion. Though a steric effect is not unexpected, its magnitude is remarkable.

The nature of the carbonyl activation by an α-nitrosamino functionality was probed by replacing the nitrosamino group by other functional groups possessing the same inductive effect. Thus, methylazoxymethanol acetate (**13**), which is isomeric with **9**, was treated with *n*-propylamine (eq 8) under the usual conditions. The reaction was complete within 35 min at 25 °C without any noticeable exotherm, and the resulting methylazoxymethanol and *n*-propylacetamide could not be separated by preparative TLC. Methoxymethyl acetate (**14**) was similarly exposed to *n*-propylamine (eq 9). In this experiment an exothermic reaction occurred to produce a quantitative yield of *n*-propylacetamide and hexahydro-1,3,5-tripropyl-*s*-triazine (**15**). Apparently, the liberated formaldehyde is converted to the imine, which spontaneously trimerizes under the reaction conditions. As an unactivated control 2-phenylethyl acetate (**16**) was treated with the amine under the usual conditions (eq 10). No reaction occurred and the ester was recovered unchanged. These experiments imply that the observed carbonyl activation by the α-nitrosamino group is purely an inductive effect.



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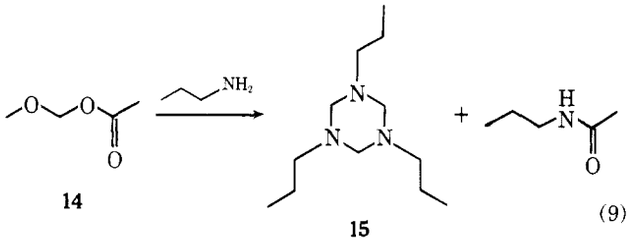

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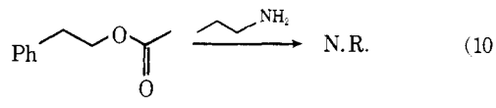

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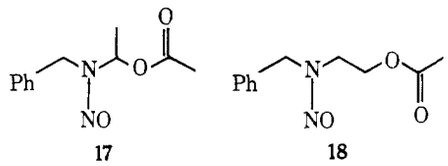
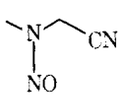
The importance of the α position with regard to this carbonyl activation was studied by comparing the reactivity of benzyl(α-acetoxyethyl)nitrosamine (**17**) and benzyl(β-acetoxyethyl)nitrosamine (**18**). The α-acetoxy isomer **17** gave the usual exothermic reaction with *n*-propylamine, resulting in the isolation of a 78% yield of *n*-propylacetamide, while under identical conditions the β-acetoxy isomer **18** gave no exotherm and was only about 50% complete (NMR). In the latter experiment, a 46% isolated yield of the liberated alcohol was



realized and 12% of the unreacted starting material was recovered. The difference in reactivity between **17** and **18** is reminiscent of the hydrolysis of α- and β-cyanonitrosamines recently reported by Harrington and co-workers.[6] They observed a rate enhancement in the hydrolysis of the cyano group which was due to interaction with the nitrosamino function. Furthermore, they found that the rate of hydrolysis



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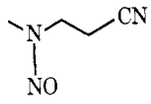

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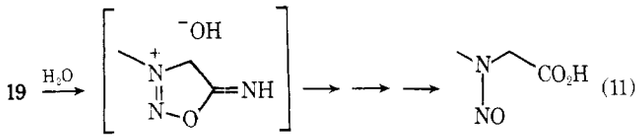
was 500 times faster for the α-isomer **19** than for the β-isomer **20.** They invoked a five-membered cyclic intermediate in the α case (eq 11) and a six-membered cyclic intermediate in the



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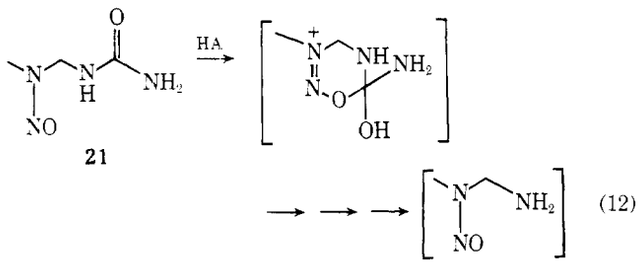
β case. This type of cyclic intermediate (eq 12) was also suggested by Michejda and co-workers in the hydrolysis of α-ureidonitrosamines (**21**).[7]



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(12)

The correlation of this carbonyl activation with the IR stretching frequency of the ester C=O group was examined. The α-acetoxynitrosamines **2, 3, 9,** and **17** have C=O stretching bands at 1750 cm^{-1} and all react exothermically with *n*-propylamine at room temperature. Nitrosamine **10,** with an unusually high carbonyl stretch at 1765 cm^{-1}, also reacted readily. The cyclic α-acetoxynitrosamine **11** and the α-pivaloxynitrosamine **12** have C=O stretches at 1740 cm^{-1}. The former reacted exothermically under the usual conditions; the latter was very sluggish, but this can be attributed to steric effects. The β-acetoxynitrosamine **18** has a C=O stretch at 1740 cm^{-1} and reacted slowly under conditions where the α-isomer **17** reacted exothermically and completely. Methylazoxymethanol acetate (**13**) has a C=O stretch at 1750 cm^{-1} and reacted as rapidly as the α-acetoxynitrosamines. The control compound, 2-phenylethyl acetate (**16**) has a "normal" C=O stretch at 1735 cm^{-1} and failed to react under the usual conditions. Methoxymethyl acetate (**14**) has a C=O stretch at 1745 cm^{-1} and reacted exothermically with *n*-propylamine as did the α-acetoxynitrosamines. From these data we may conclude that acetate esters having an α-nitrosamino group, α-azoxy group, or α-methoxy group (1745–1750 cm^{-1}) are sufficiently activated to react exothermically and rapidly with *n*-propylamine, while those having a β-nitrosamino group (1740 cm^{-1}) react slowly. Normal esters having C=O stretching at 1735 cm^{-1} or lower do not react under these conditions. Since the inductively similar, but structurally dissimilar, nitrosamino, azoxy, and methoxy groups activate equally well, it appears that the carbonyl activation which we have observed with the nitrosamino group is purely an inductive effect and does not involve a cyclic intermediate.

Experimental Section

General. Solvents and commercially available starting materials were reagent grade and were used as received unless specified otherwise. Thin-layer chromatograms were run on Analtech analytical silica gel plates. Preparative plates were prepared from EM Laboratories plate silica gel. Melting points were taken with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 700 instrument, and NMR spectra were recorded with a Varian T-60 or a Hitachi Perkin-Elmer R22 spectrometer using tetramethylsilane as an internal standard (δ 0). The high-resolution mass spectra were obtained through the courtesy of Dr. Catherine E. Costello at M.I.T. The microanalyses were performed by Robertson Laboratory, Florham Park, N.J.

Hydrolysis of 2 and 3. Compounds **2** and **3** (5 μmol) were dissolved in 1.0 mL of buffer (20 mM trimethylamine hydrochloride, pH 8.0) at room temperature, and 5 units of esterase was added. [EC 3.1.1.1. hog liver esterase (Sigma): 1 unit hydrolyzes 1 μmol of butyrate per min at pH 8.0 and 25 °C]. Similar solutions of **2** and **3** were prepared, without the addition of enzyme, in distilled water, in buffer, and in 0.001 N HCl (pH 3.0). The disappearance of **2** and **3** with time, and the concomitant appearance of the products benzaldehyde and benzyl alcohol, respectively, was monitored by HPLC with UV detection at 254 nm. Chromatography was performed on a reverse-phase μC_{18}-Bondapak column (Waters Associates, Milford, Mass.) using a mobile phase of MeOH–H_2O (50:50), delivered at 1.5 mL/min. The decreasing size of the absorption peaks due to **2** and **3** from successive aliquots allowed determination of the amount of each compound remaining at a given time under each reaction condition. In all cases, hydrolyses were first order with respect to **2** and **3.** Linear regression analysis of the data gave the following results:

	Half-lives $\left(\text{times at which } \frac{[\text{compound}]}{[\text{compound}]_0} = \frac{1}{2}\right)$			
	In water	In acid, pH 3.0	In buffer, pH 8.0	In buffer, pH 8.0, with enzyme
2	19 min	17 min	26 min	16 min
3	stable	stable	16 h	1 h

Methyl(α-acetoxybenzyl)nitrosamine (2) was prepared as previously described.[1b] Anal. Calcd for $C_{10}H_{12}N_2O_3$: C, 57.69; H, 5.81; N, 13.45. Found: C, 58.06; H, 5.81; N, 12.98.

Benzyl(acetoxymethyl)nitrosamine (3) was prepared as previously described.[1b] Anal. Calcd for $C_{10}H_{12}N_2O_3$: C, 57.69; H, 5.81; N, 13.45. Found: C, 57.80; H, 6.01; N, 12.76.

Treatment of 2 with Methanol; Methyl(α-methoxybenzyl)-nitrosamine (5). Compound **2** (1.0 mmol, 0.208 g) was dissolved in dry methanol (4 mL) and allowed to stand at 25 °C. After 1 h, TLC analysis indicated that no reaction had occurred; however, refluxing for 16 h resulted in a complete reaction. The solvent was removed in vacuo, giving 0.224 g of yellow liquid which was purified by preparative TLC on silica gel (hexane–ethyl acetate, 2:1). This gave 0.142 g (79%) of pure methyl(α-methoxybenzyl)nitrosamine (**5**), which was a very pale-yellow oil. By NMR integration, the compound exists almost exclusively as the *E* isomer: IR (film) 2950 (w), 1470 (s), 1350 (s), 1210 (s), and 1110 (s) cm^{-1}; NMR ($CDCl_3$) δ 2.77 (s, 3 H), 3.47 (s, 3 H), 6.65 (s, 1 H), and 7.29 (s, 5 H). The high-resolution mass spectrum did not show a molecular ion. A satisfactory peak was obtained for the loss of CH_3O. Anal. Calcd for $C_8H_9N_2O$: 149.07148 ($M^+ - CH_3O$). Found: 149.07250.

Treatment of 2 with Methanol and *p*-Toluenesulfonic Acid. Compound **2** (0.50 mmol, 0.104 g) was dissolved in dry methanol (2 mL) at 25 °C and one crystal of *p*-toluenesulfonic acid was added. The acid dissolved and the pale-yellow solution was allowed to stand for 22 h without any detectable reaction by TLC.

Treatment of 3 with Methanol. Compound **3** (0.60 mmol, 0.125 g) was dissolved in dry methanol (5 mL) and refluxed for 24 h without any detectable reaction by TLC or NMR. The starting material was quantitatively recovered.

Methyl(α-*tert*-butylperoxybenzyl)nitrosamine (6). Compound **2** (2 mmol, 0.4164 g) was stirred in *tert*-butyl hydroperoxide (8 mL) under N_2 for 2 weeks. A crude yellow liquid of high purity was obtained by simple removal of the hydroperoxide in vacuo. It was rinsed with several portions of CH_2Cl_2 and dried in vacuo. Filtration through a short column of silica with hexane–ether (9:1) removed a small amount of baseline impurity to give, after drying, 0.451 g (95%) of **6.** By NMR integration, the compound exists as a mixture of *E*:*Z* isomers (approximately 98:2, respectively): IR (film) 1470 (s) cm^{-1}; NMR ($CDCl_3$) δ 1.33 (s, *E*-$C(CH_3)_3$), 2.84 (s, *E*-CH_3), 3.68 (s, *Z*-CH_3), 2.84 (s, *E*-CH_3), 3.68 (s, *Z*-CH_3), 7.30 (s, *E*-CH), and 7.37 (s, *E*-C_6H_5).

Anal. Calcd for $C_{12}H_{18}N_2O_3$: C, 60.49; H, 7.61; N, 11.76. Found: C, 60.58; H, 7.93; N, 11.32.

Methyl(α-chloroacetoxybenzyl)nitrosamine. This compound was prepared according to Wiessler.[8] Passage through a short column

of silica gel with benzene elution gave a yellow liquid which crystallized on standing (86%). By NMR integration the compound exists as a mixture of *E*:*Z* isomers (approximately 95:5, respectively). The analytical sample was recrystallized from hexane: mp 58–60 °C; IR ($CHCl_3$) 1765 cm^{-1}; NMR ($CDCl_3$) δ 2.84 (s, *E*-CH_3), 3.59 (s, *Z*-CH_3), 4.18 (s, *Z*-CH_2), 4.26 (s, *E*-CH_2), 7.47 (s, *E*-C_6H_5), 8.41 (s, *E*-CH).

Anal. Calcd for $C_{10}H_{11}ClN_2O_3$: C, 49.50; H, 4.57; N, 11.57; Cl, 14.61. Found: C, 49.43; H, 4.52; N, 11.78; Cl, 14.60.

Treatment of 2 with Chloroacetic Acid. Compound **2** (1.0 mmol, 0.208 g) and chloroacetic acid (10 mmol, 0.945 g) were dissolved in methylene chloride (1 mL). The resulting solution was allowed to stand at 25 °C for 3.5 h without any detectable reaction by TLC. After refluxing for 19 h the reaction solution was worked up by pouring into a separatory funnel containing methylene chloride (20 mL) and saturated sodium bicarbonate (30 mL). The organic layer was separated, washed with water, and dried over sodium sulfate. The solvent was removed in vacuo, yielding 0.153 g of yellow liquid which by NMR was a 3:2 mixture of chloroacetoxynitrosamine and benzaldehyde (probably formed on workup). A trace of starting acetoxy compound was also present. Since the mixture could not be separated on an analytical TLC plate, no purification was attempted.

Methyl(α-*p*-nitrobenzoyloxybenzyl)nitrosamine (7). This compound was prepared in 55% crude yield according to Wiessler.[8] By NMR integration, the compound exists as a mixture of *E*:*Z* isomers (approximately 96:4, respectively). Recrystallization from benzene–hexane gave a 49% yield of **7**: mp 115–116 °C (lit.[8] mp 115–116 °C); NMR ($CDCl_3$) δ 3.02 (s, *E*-CH_3), 3.73 (s, *Z*-CH_3), 8.37 (s, $(ABq)_2$, *E*-C_6H_4), and 8.62 (s, *E*-CH).

Hydrolysis of Methyl(α-*p*-nitrobenzoyloxybenzyl)nitrosamine (7). Compound **7** (0.50 mmol, 0.157 g) and water (0.50 mmol, 0.009 g) were dissolved in distilled THF (0.5 mL) and allowed to stand for 45 min at 25 °C without any detectable reaction by TLC. Refluxing for 23 h followed by removal of the solvent in vacuo gave 0.068 g (75%) of pale-yellow solid which by NMR was found to be pure methyl *p*-nitrobenzoate. Purification by preparative TLC on silica with hexane–ethyl acetate (9:1) afforded 0.036 g of pale-yellow crystals, mp 93–94 °C (lit.[9] mp 95–96 °C). The mixture melting point with an authentic sample of methyl *p*-nitrobenzoate was 93–94 °C.

Hydrolysis of 7 with D_2O. Compound **7** (0.50 mmol, 0.157 g) and deuterium oxide (0.50 mmol, 0.010 g) were dissolved in distilled THF (1.5 mL), and the solution was refluxed for 30 h, after which time most of the solvent had evaporated. The reaction was found to be incomplete by TLC, so an additional (0.50 mmol) portion of D_2O and more THF (1.5 mL) were added, and refluxing was continued for an additional 26 h after which time the reaction was still incomplete. Removal of the solvent in vacuo gave 0.130 g of pale-yellow solid, which by TLC contained benzaldehyde, methyl *p*-nitrobenzoate, and starting material. Preparative TLC on silica with hexane–ethyl acetate (9:1) gave 0.038 g (42%) of pure methyl *p*-nitrobenzoate, mp 93–94 °C. An NMR spectrum of this material was identical with that from the hydrolysis experiment with H_2O; no 1:1:1 triplet characteristic of $-CH_2D$ was observed.[10]

Control for the Hydrolysis of 7. Methanol (1.0 mmol, 0.040 mL) and *p*-nitrobenzoic acid (1.0 mmol, 0.167 g) were dissolved in distilled THF (1 mL). The resulting solution was refluxed for 21 h. The solvent was removed in vacuo, giving 0.151 g of recovered *p*-nitrobenzoic acid, mp 238–240 °C. The starting *p*-nitrobenzoic acid melted at 239–240 °C.

Treatment of 2 with *n*-Propylamine. Compound **2** (3.0 mmol, 0.634 g) was dissolved in *n*-propylamine (Eastman) (5 mL) and allowed to stand for 5 min at ambient temperature. TLC analysis at this time revealed that the reaction was complete. The excess amine was removed in vacuo, giving a 0.791 g of yellow liquid which was found to give two major spots on TLC. Preparative TLC as above gave 0.194 g of pale-yellow liquid (44%) in the upper band, which was identified as a 2:1 mixture of *N*-benzylidene-*n*-propylamine and benzaldehyde (NMR). The lower band gave 0.308 g (100%) of *n*-propylacetamide, which was identified by comparison of the IR and NMR spectra to those from an authentic sample.

Methyl(acetoxymethyl)nitrosamine (9). This compound was prepared in 9% yield as described by Keefer.[2a] The NMR spectrum of the crude product was identical with that reported by Keefer and revealed no impurities.

Treatment of Methyl(acetoxymethyl)nitrosamine (9) with *n*-Propylamine. Compound **9** (1.0 mmol, 0.132 g) was dissolved in *n*-propylamine (1 mL). TLC analysis after 3 min revealed that the reaction was complete. After 20 min, the excess amine was removed in vacuo, yielding 0.170 g of yellow oil. Preparative TLC on silica using chloroform–methanol (10:1) gave 0.069 g (68%) of pure *n*-propylacetamide, which was identified by comparison of the IR and NMR spectra with those from an authentic sample.

Treatment of Benzyl(acetoxymethyl)nitrosamine (3) with *n*-Propylamine. Compound **3** (3.0 mmol, 0.624 g) was dissolved in *n*-propylamine (2 mL). The usual exothermic reaction occurred and after 18 min the excess amine was removed in vacuo. This afforded 0.818 g of yellow liquid which by NMR contained *n*-propylacetamide and an unknown aromatic impurity, which was not benzyl-*n*-propylamine or benzyl alcohol. Filtration of the crude product through a short column of silica, followed by preparative TLC, gave a mixture of *n*-propylacetamide and the aromatic impurity. The usual solvent systems failed to separate these two compounds.

Methyl(α-acetoxy-*p*-nitrobenzyl)nitrosamine (10) was prepared on a 50-mmol scale by the method of Wiessler.[8] Compound **10** was purified by washing with saturated sodium bisulfite, followed by column chromatography [silica gel H, EM reagent; benzene–ether (19:1)] in 11% yield as light-yellow crystals. By NMR integration the compound exists as a mixture of *E*:*Z* isomers (approximately 95:5, respectively). The analytical sample was recrystallized from hexane–chloroform: mp 108–109 °C; IR ($CHCl_3$) 1765 (s) and 1480 (s) cm^{-1}; NMR ($CDCl_3$) δ 2.29 (s, *Z*-CH_3), 2.36 (s, *E*-CH_3), 2.89 (s, *E*-CH_3), 3.64 (s, *Z*-CH_3), 8.06 ($(ABq)_2$, J_{AB} = 9 Hz, $\Delta\nu_{AB}$ = 0.583 ppm, *E*-C_6H_4), and 8.46 (s, *E*-CH).

Anal. Calcd for $C_{10}H_{11}N_3O_5$: C, 47.43; H, 4.38; N, 16.60. Found: C, 47.43; H, 4.41; N, 16.80.

Treatment of 10 with *n*-Propylamine. Compound **10** (0.5 mmol, 0.127 g) was added at 0 °C to *n*-propylamine (3 mL). Analytical TLC (eluent either ether or benzene) of an aliquot (taken immediately after mixing) showed the complete disappearance of starting material. Concentration in vacuo gave a clean 1:1 mixture of *n*-propylacetamide and *N*-(*p*-nitrobenzylidene)-*n*-propylamine as determined by NMR.

α-Acetoxynitrosopyrrolidine (11) was prepared as previously described.[1a]

Treatment of α-Acetoxynitrosopyrrolidine (11) with *n*-Propylamine. Pure **11** (0.221 mmol, 0.035 g) was dissolved in *n*-propylamine (0.5 mL), resulting in an immediate exotherm. After 2 min, TLC showed that the reaction was complete. After 14 min, the excess amine was removed in vacuo to give 0.061 g of yellow liquid which by NMR was essentially pure *n*-propylacetamide. Preparative TLC on silica with hexane–ethyl acetate (2:1) afforded 0.017 g (77%) of pale-yellow oil whose IR spectrum was identical with that from an authentic sample of amide.

Methyl(α-pivaloyloxybenzyl)nitrosamine (12) was prepared on a 10-mmol scale by the method of Wiessler.[8] Compound **12** was purified by column chromatography (silica gel H; EM reagent; benzene–ether, 19:1) in 29% yield as a light-yellow liquid. By NMR intergration, the compound exists as a mixture of *E*:*Z* isomers (approximately 97:3, respectively): IR (film) 1740 (s) and 1475 (s) cm^{-1}; NMR ($CDCl_3$) δ 1.34 (s, *E*-$C(CH_3)_3$), 2.86 (s, *E*-CH_3), 3.60 (s, *Z*-CH_3), 7.48 (s, *E*-C_6H_5), and 8.36 (*E*-CH).

Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.53; H, 7.20; N, 11.09.

Treatment of 12 with *n*-Propylamine. Compound **12** (0.571 mmol, 0.143 g) was dissolved in *n*-propylamine (3 mL). Analytical TLC after stirring for 1 h at ambient temperature indicated no reaction; 40 h at reflux was necessary to effect complete reaction. The reaction was conveniently followed by analytical TLC [benzene–ether (9:1)]. Concentration in vacuo gave a clean 1:1 mixture of *N*-(*n*-propyl)pivalamide and *N*-benzylidene-*n*-propylamine as determined by NMR.

Treatment of Methylazoxymethanol Acetate (13) with *n*-Propylamine. Compound **13** (0.50 mmol, 0.0661 g) was dissolved in *n*-propylamine (1 mL) as usual. After 35 min at 25 °C (no exotherm or color change), the excess amine was removed in vacuo to give 0.113 g of pale-yellow liquid. NMR analysis revealed that the crude product was a 1:1 mixture of *n*-propylacetamide and methylazoxymethanol.[11] The reaction was complete. Preparative TLC on silica ($CHCl_3$–CH_3OH, 8:1) failed to separate the two products.

Treatment of Methoxymethyl Acetate with *n*-Propylamine. To a 10-mL flask were added methoxymethyl acetate (Eastman) (0.312 g, 3.0 mmol) and *n*-propylamine (3 mL). The resulting colorless solution became warm to the touch after 2 min. After standing at ambient temperature for 30 min, the excess amine was removed in vacuo, giving 0.506 g (98%) of yellow liquid, which contained no ester by IR and appeared to be a mixture of *n*-propylacetamide and hexahydro-1,3,5-tripropyl-*s*-triazine (3:1) by NMR. A Sadtler NMR spectrum (8949) for the triazine was superimposable on the unknown signals in the mixture NMR. Attempted separation of the two products on silica using hexane–ethyl acetate (1:1) gave 0.337 g (theoretical 0.303 g) of pale-yellow liquid from the band with

the same R_f as n-propylacetamide. An NMR spectrum revealed that the amide still contained a small amount of triazine.

Treatment of 2-Phenylethyl Acetate (16) with n-Propylamine. To a 10-mL flask were added 2-phenylethyl acetate (0.279 g, 1.7 mmol) and anhydrous n-propylamine (2 mL). The resulting colorless solution, from which no heat was evolved, was allowed to stand at room temperature for 30 min before the amine was removed in vacuo. This gave 0.272 g (99%) of pale-yellow liquid, which by NMR was pure unchanged starting material.

Benzyl(α-acetoxyethyl)nitrosamine (17). This compound was prepared using the method developed by Keefer[2a] by substituting acetaldehyde for paraformaldehyde. The crude black product obtained as described by Keefer was filtered through silica with benzene to give 13.7 g (31%) of yellow liquid which by TLC and NMR contained benzyl acetate (major) and **17** (minor). Distillation at 0.7 mm and 50–54 °C gave 8.7 g (29%) of pure benzyl acetate. The residue, which was predominantly **17** (5.0 g, 11%), was purified by preparative TLC on silica using hexane–ethyl acetate (9:1). From 0.539 g of crude **17**, 0.303 g of pure material was obtained. By NMR integration, the compound exists almost exclusively as the *E* isomer: IR (film) 3000 (m), 1750 (s), 1480 (s), 1380 (s), 1220 (s), and 1070 (s) cm^{-1}; NMR ($CDCl_3$) δ 1.82 (d, J = 6 Hz, *E*-CH_3), 2.02 (s, *E*-CH_3), 4.85 (ABq, J_{AB} = 15 Hz, $\Delta\nu_{AB}$ = 0.342 ppm, *E*-CH_2), and 6.9–7.5 (m, 6 H).

Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.60; mol wt, 222.10044. Found: C, 59.84; H, 6.44; N, 12.33; mol wt, 222.10127.

Treatment of 17 with n-Propylamine. Compound **17** (1.36 mmol, 0.303 g) was treated with n-propylamine (2 mL) as described above for 30 min. The usual exothermic reaction occurred to give after workup 0.428 g of yellow-orange oil, which was found by NMR to contain n-propylacetamide and an unknown aromatic impurity. Preparative TLC with hexane–ethyl acetate (2:1) gave from the area just above the baseline 0.107 g (78%) of yellow liquid which was pure amide by NMR.

***N*-Nitroso-*N*-benzylethanolamine.** *N*-Benzylethanolamine (Aldrich) (3.0 mmol, 0.453 g) and distilled triethylamine (3.3 mmol, 0.458 mL) were dissolved in methylene chloride (3 mL), and the resulting solution was cooled in an ice bath while flushing with N_2. After 10 min nitrosyl chloride (1.44 M, 3.3 mmol) in methylene chloride was added via syringe and the bath was removed. The mixture was allowed to come to 25 °C over a 30-min period before adding excess dry ether. The mixture was filtered and the filtrate concentrated to dryness in vacuo to give 0.536 g (99%) of yellow liquid which was quite pure by TLC. Filtration of a benzene solution through a small column of silica (15 g) removed the high running impurities. The alcohol was eluted with benzene–ether (4:1) to give 0.378 g (70%) of pale-yellow liquid which was pure product by NMR. By NMR integration, the compound exists as a mixture of *E*:*Z* isomers (approximately 39:61, respectively): IR (film) 3400 (broad), 2950 (w), 1460 (m), and 1145 (s) cm^{-1}; NMR ($CDCl_3$) δ 3.40 (broad s, 1 H), 3.62 (s, *Z*-A_2B_2), 4.02 (m, *E*-A_2B_2), 4.85 (s, *E*-CH_2), 5.32 (s, *Z*-CH_2), and 6.9–7.4 (m, 5 H).

Anal. Calcd for $C_9H_{12}N_2O_2$: mol wt, 180.08987. Found: mol wt, 180.08918.

Benzyl(β-acetoxyethyl)nitrosamine (18). *N*-Nitroso-*N*-benzylethanolamine (0.833 mmol, 0.150 g) was dissolved in acetic anhydride (1 mL) and a trace of concentrated H_2SO_4 was added from a capillary tube. The yellow solution was allowed to stand overnight at 25 °C, after which time the acetylation was found to be complete by TLC. Removal of the excess acetic anhydride and acetic acid in vacuo gave 0.157 g (85%) of yellow liquid which was pure (**18**) by NMR. By NMR integration, the compound exists as a mixture of *E*:*Z* isomers (approximately 51:49, respectively): IR (film) 2975 (m), 1740 (s), 1460 (m), and 1240 (s) cm^{-1}; NMR ($CDCl_3$) δ 2.05 (s, *Z*-CH_3), 2.07 (s, *E*-CH_3), 3.90 (m, *Z*-A_2B_2), 4.38 (s, *E*-A_2B_2), 4.90 (s, *E*-CH_2), 5.38 (s, *Z*-CH_2), and 7.0–7.5 (m, 5 H).

Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.60; mol wt, 222.10044. Found: C, 58.98; H, 6.41; N, 12.26; mol wt, 222.10156.

Treatment of 18 with n-Propylamine. Compound **18** (0.707 mmol, 0.157 g) was dissolved in n-propylamine (1 mL) and allowed to stand at 25 °C for 30 min before removing the excess amine in vacuo. This gave 0.345 g of yellow liquid which by NMR was a mixture of n-propylacetamide, **18**, and *N*-nitroso-*N*-benzylethanolamine. Preparative TLC on silica using benzene–ether (4:1) gave 0.019 g (12%) of starting ester (NMR) and 0.058 g (46%) of *N*-nitroso-*N*-benzylethanolamine (NMR). The amide was not isolated.

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