Base-Catalyzed Decomposition of *N*-Nitrosobis(2-oxopropyl)amine

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N-Nitrosobis(2-oxopropyl)amine, a potent carcinogen for the pancreas in Syrian hamsters, undergoes a facile, base-catalyzed, intramolecular aldol condensation to yield *N*-nitroso-5-hydroxy-5-methyl-3-piperidone. This cyclic nitrosamine then decomposes to yield 3-hydroxy-5-methylpyridine.

Introduction

During the course of our biochemical investigations of *N*-nitrosobis(2-oxopropyl)amine (BOP), a potent carcinogen for the pancreas in Syrian hamsters (*1*), we noticed that stock solutions of the nitrosamine in neutral buffers gradually turned yellow over about a 12-h period. Previously we have investigated the base-catalyzed deacylation of *N*-nitroso(2-oxopropyl)propylamine (NOPPA) to yield *N*-nitrosomethylpropylamine (NMPA) (*2*). We were unable, however, to detect any significant formation of NMPA over a 16-h period at pH values lower than 12.0. We concluded that nonenzymatic conversion of NOPPA to NMPA does not take place to a significant extent under physiological conditions. The decomposition of BOP clearly proceeded at neutral pH. Furthermore, if the reaction was similar to the base-catalyzed decomposition of NOPPA, the product would be *N*-nitrosomethyl(2-oxopropyl)amine, a product that has been detected in vivo following administration of BOP (*3*) and a potent pancreatic carcinogen in its own right (*4*, *5*). In view of their possible biochemical importance, we here outline the products of decomposition of BOP in aqueous solution.

Experimental Section

BOP was purchased from Ash Stevens Inc. (Detroit, MI). ^{1}H NMR spectra were obtained with a Nicolet 360-MHz spectrometer at the University of Toronto Biomedical Research NMR Centre. GC–MS was performed with a Hewlett-Packard (Avondale, PA) 5985B instrument at the Best Institute, University of Toronto. The column packing was OV-1 (4 ft), initial temperature 80 °C, and the gradient 6 deg/min. HPLC was performed with a 30 cm × 3.9 mm C18 μBondapak column (Waters Associates, Millford, MA); detection was by a Hewlett-Packard model HP-1040A diode array spectrophotometer.

Results and Discussion

In order to characterize the products of the reaction(s) of BOP in aqueous solution, we constructed a continuous flow reactor, using the flow cell of an HPLC diode array detector. A buffer solution (1 L; 20 mM phosphate at pH 11 or 8 or 20 mM borate at pH 10 or 9) was continuously pumped at 5 mL/min through the cell. After equilibration, 1 mL of 100 mM BOP in 1 mM phosphate, pH 7, was added, and spectra were recorded every 75 s by measuring the absorption of the solution at 4-nm intervals from 190 to 450 nm. Figure 1a shows the reaction profile at pH 11. There was an initial rapid increase in absorbance at 345 nm which subsequently decreased slowly with the appearance of a second stable peak at 298 nm. Figure 1b shows the complete UV spectra at 27 and 900 min of the two products at pH 11.

The rate of formation of the first product was proportional to the hydroxide ion concentration and independent of buffer concentration; the pH-independent rate constant was 3×10^{-5} M^{-1} min^{-1}. In order to isolate a sample for characterization, 500 mL of 100 μM BOP in 20 mM sodium phosphate, pH 12, was neutralized with 1 M sodium dihydrogen phosphate after 15 min. The solution was extracted with dichloromethane and the solvent removed in vacuo. The residue was applied to a C18 μBondapak HPLC column and eluted with 1 mM phosphate, pH 7.0. Figure 2 shows that only one peak at 4.3 min, in addition to unreacted BOP at 6.3 min, was observed under these conditions. The UV spectrum of the isolated component was identical with that shown in Figure 1a when the pH was adjusted to 11. The product was extracted into dichloromethane, dried, and dissolved in chloroform-*d* for examination by NMR.

The 360-MHz ^{1}H NMR spectrum (Figure 3a) showed two strong signals at δ 1.52 and 1.48 and a large number of smaller signals between δ 2.5 and 5.1. An expansion of this latter region is shown in Figure 3b. Several spin decoupling and nuclear Overhauser effect (NOE) experiments were then performed to aid the assignment of signals (data provided as supplementary material).

The NMR spectrum allowed the first product to be unequivocally identified as *N*-nitroso-5-hydroxy-5-methyl-3-piperidone. This cyclic *N*-nitrosamine exists principally as two diastereomers in which the nitroso group is either *E* or *Z* with respect to the carbonyl group (Figure 4). NOE experiments allowed protons to be assigned to the two isomers, while spin decoupling experiments aided the assignments of individual protons in each diastereomer. NOE experiments also suggested that the major conformer contains an equatorial methyl group and an axial hydroxyl group. This assignment is consistent with the greater steric demand of the methyl group, and the possibility that the *N*-nitroso group may hydrogen bond with an axial hydroxyl group. The assignments of the signals, listed in Table I, were also based on previous work by Chow and Colon (*6*), Fraser and Ng (*7*), and Kokkinakis et al. (*8*) for other cyclic *N*-nitrosamines. The small signals at δ 4.45 and 3.67 are probably due to the minor conformer.

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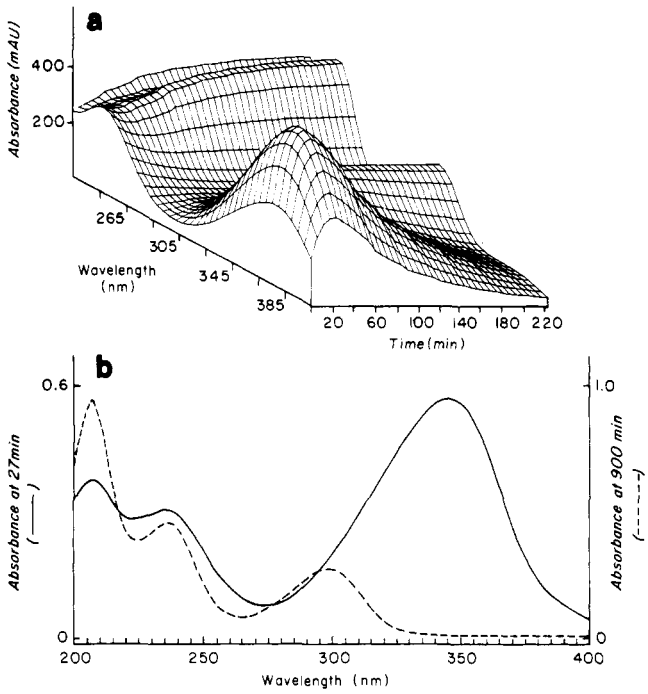

Figure 1. (a) Change in UV absorbance during decomposition of BOP at pH 11. (b) UV spectra at 27 (—) and 900 min (- - -) of the first and second products, respectively, of decomposition of BOP and pH 11.

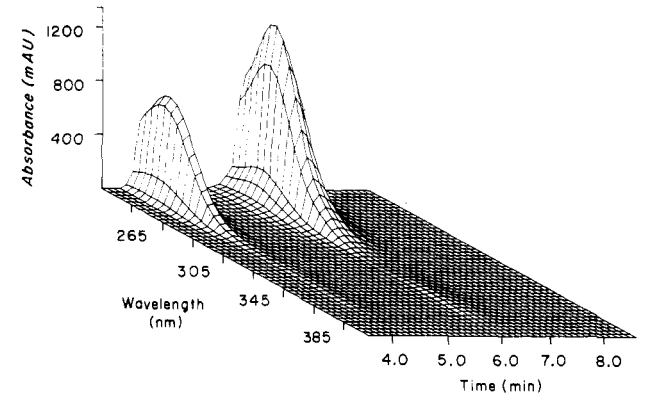

Figure 2. HPLC reaction mixture 15 min after adding BOP to phosphate buffer, pH 12.

Table I. ^{1}H NMR Data of *N*-Nitroso-5-hydroxy-5-methyl-3-piperidone[a]

proton(s)[b]	δ	proton(s)[b]	δ
5e	1.48	5z	1.52
2ea	4.89, 4.84	2za	3.98, 3.93
2ee	5.10, 5.05	2ze	4.72, 4.77[c]
4ea	2.54, 2.50	4za	2.60, 2.65
4ee	2.70, 2.75[c]	4ze	2.80, 2.75[c]
6ea	3.81, 3.77	6za	4.28, 4.19
6ee	3.97, 3.93	6ze	4.78, 4.74[c]

Coupling Constants (Hz)

J(2ea,2ee) = 18.4	J(2za,2ze) = 15.8
J(4ea,4ee) = 16.2	J(4za,4ze) = 16.9
J(6ea,6ee) = 15.6	J(6za,6ze) = 14.0
J(2ea,6ea) ≃ 0.5	J(2za,6za) ≃ 0.5
J(4ee,6ee) ≃ 2.5	J(4ze,6ze) ≃ 3.0

[a] Values were determined from the 360-MHz spectrum in $CDCl_3$ at 25 °C. [b] Protons are numbered as shown in Figure 4. [c] Values are approximate due to overlapping signals.

In order to isolate a sample of the second reaction product, 500 mL of 200 μM BOP in 20 mM sodium phosphate, pH 12, was allowed to stand at room temperature for 18 h. After neutralization, the reaction mixture

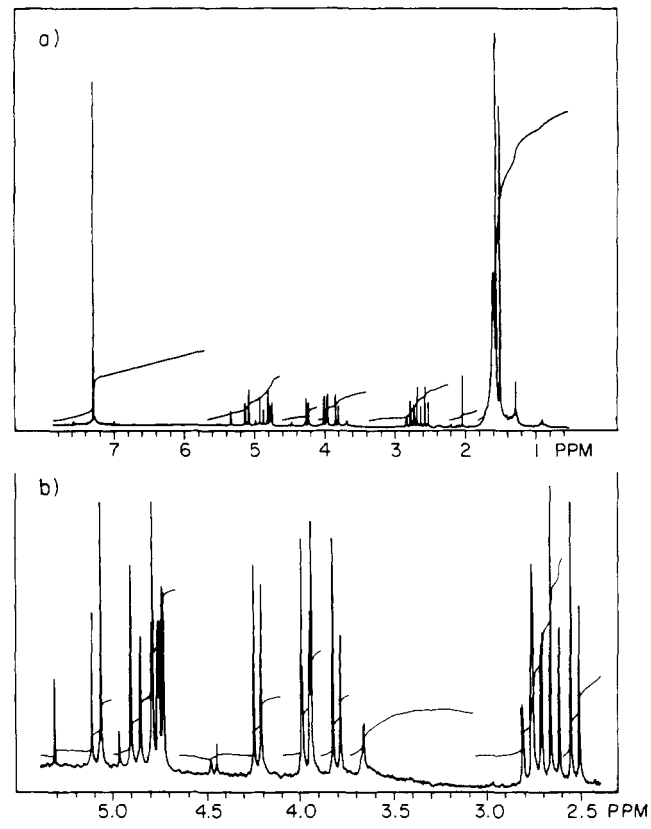

Figure 3. (a) 360-MHz ^{1}H NMR spectrum of *N*-nitroso-5-hydroxy-5-methyl-3-piperidone in $CDCl_3$. (b) Expanded portion of NMR spectrum from 2.4 to 5.3 ppm.

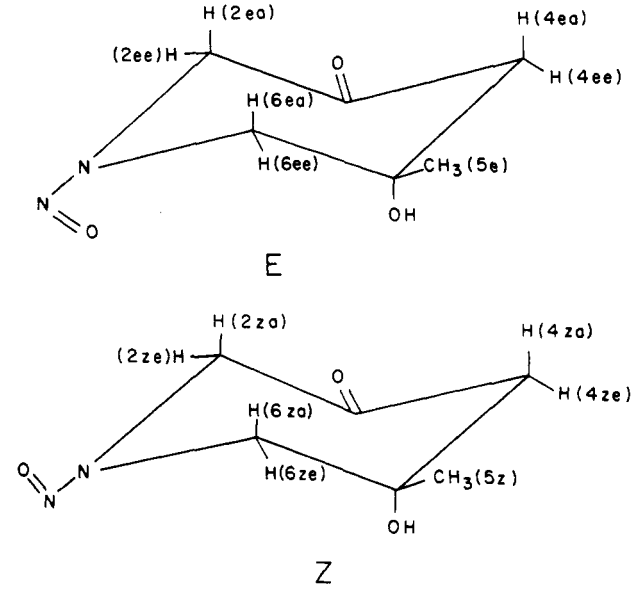

Figure 4. Structures of the two diastereomers of *N*-nitroso-5-hydroxy-5-methyl-3-piperidone.

was extracted with ethyl acetate. The solvent was removed in vacuo, and the sample was applied to a C18 μBondapak HPLC column and eluted with 1 mM phosphate, pH 7.0, containing 20% acetonitrile. Under these conditions, the product eluted at 10.1 min, BOP at 6.0 min, and *N*-nitroso-5-hydroxy-5-methyl-3-piperidone at 3.9 min. The component eluting at 10.1 min was extracted into ethyl acetate, dried, and dissolved in deuteriated chloroform.

The 360-MHz ^{1}H NMR spectrum and the electron impact mass spectrum of this product are shown in Figure 5. The NMR spectrum indicates the presence of a methyl group and three aromatic protons. Identification of the

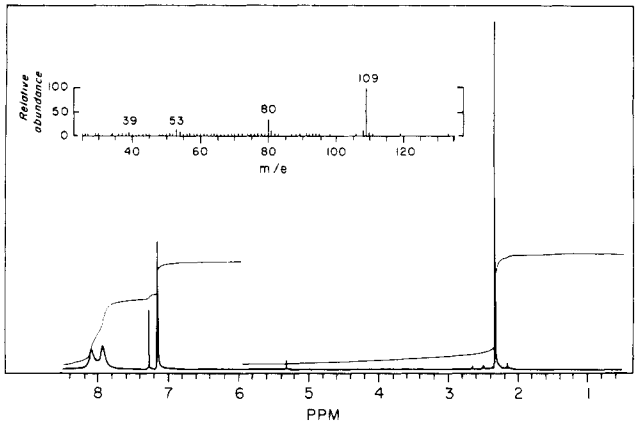

Figure 5. 360-MHz ^1H NMR spectrum in $CDCl_3$ and electron impact mass spectrum (insert) of 3-hydroxy-5-methylpyridine.

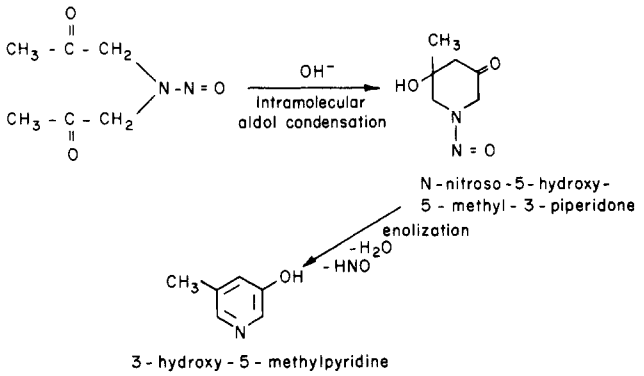

Figure 6. Reaction pathway for decomposition of BOP in aqueous solution.

product as 3-hydroxy-5-methylpyridine was confirmed by the molecular ion at m/e 109 and the fragment at m/e 80, probably 3-methylpyrrole.

Isolation of pure *N*-nitroso-5-hydroxy-5-methyl-3-piperidone followed by an examination of its decomposition by HPLC confirmed that 3-hydroxy-5-methylpyridine is a direct product of the decomposition of the cyclic *N*-nitrosamine. We did not carry out an extensive kinetic study of the formation of the pyridine derivative, but its formation was independent of pH over the range of 4–10, and it was independent of the presence of oxygen in solution.

The results indicate that the aqueous decomposition of BOP does not involve a simple deacylation as we observed for NOPPA but rather a facile, base-catalyzed, intramolecular aldol condensation to yield *N*-nitroso-5-hydroxy-5-methyl-3-piperidone (Figure 6). In an unusual reaction, this cyclic nitrosamine then loses water and HNO to yield 3-hydroxy-5-methylpyridine in a reaction for which the driving force is clearly aromatization. The species HNO has previously been proposed as an intermediate in the reaction of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide with thiols (*9*). It remains to be determined whether *N*-nitroso-5-hydroxy-5-methyl-3-piperidone and/or 3-hydroxy-5-methylpyridine are formed in experimental animals following BOP administration and whether they are involved in any way in carcinogenesis by BOP.

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Registry No. BOP, 60599-38-4; *N*-nitroso-5-hydroxy-5-methyl-3-piperidone, 111582-21-9; 3-hydroxy-5-methylpyridine, 42732-49-0.

Supplementary Material Available: Details of spin decoupling and nuclear Overhauser effect experiments in the 360-MHz ^1H NMR spectrum of *N*-nitroso-5-hydroxy-5-methyl-3-piperidone (1 page). Ordering information is given on any current masthead page.

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