Steric Effects in the Nitrosation of Piperidines

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SUMMARY

The rates of reaction with nitrous acid of four methyl-substituted piperidines to form the corresponding N-nitrosopiperidines were compared with that of piperidine. The relative rates for piperidine, 2-methyl-, 2,6-dimethyl-, and 2,2,6,6-tetramethyl-piperidine were approximately 100:20:10:1, showing considerable steric hindrance to nitrosation by the α methyl groups. The rate of formation of nitrosopiperidine from the tertiary amine N-methylpiperidine was about 10,000 times slower than that from piperidine.

INTRODUCTION

The possible formation in vivo of carcinogenic nitrosamines by interaction of amines with nitrous acid in the stomach merits consideration as a cause of human cancer (4). It has been accepted that the extent of the nitrosation reaction depends on the nature of the amine (7). Many hundreds of amines would qualify as progenitors of nitrosamines in man (3), among them some drugs that are amines substituted on the carbon atoms neighboring the nitrogen atom. There have been few studies of the kinetics of nitrosation of secondary amines (Refs. 1, 5, and 8; for review, see Ref. 6) and none of tertiary amines or of sterically hindered secondary amines. Widely different rates of nitrosation of such amines would have a strong bearing on the significance of formation of the nitrosamines in the genesis of cancer.

As models for these studies we chose 4 readily available methyl piperidines, and we compared the rates of formation of the corresponding nitrosamines with that of nitrosopiperidine from piperidine under comparable conditions.

MATERIALS AND METHODS

Reagents. Piperidine, 2-methylpiperidine, 2,6-dimethylpiperidine, 2,2,6,6-tetramethylpiperidine, and N-methylpiperidine were obtained from Aldrich Chemical Company, Milwaukee, Wis. They were purified by distillation, and purity was 99% or greater.

Kinetics. The amine (1 mmole) was placed in a 5-ml volumetric flask. For the secondary amines, equimolar concentrations of nitrite and amine were found to result in straight-line kinetic plots. An aliquot of sodium nitrite was added to give a ratio of amine to nitrite of 1:1. A constant volume (250 μl) of glacial acetic acid in each reaction mixture provided a pH of 4.1 to 4.2. The solutions were diluted with water to 5 ml, stoppered, mixed, and immersed immediately in thermostatically controlled water baths. All of the mixtures were investigated at 27°; mixtures containing 2,2,6,6-tetramethyl- and N-methylpiperidine were also run at 57°.

At intervals, the flasks were temporarily removed from the bath while 500-μl aliquots were withdrawn. The aliquots were added to 10-ml vials, each of which contained a magnetic stirring bar, excess calcium oxide (400 to 500 mg), and 5.00 ml of a carbon disulfide solution of n-tridecane or n-tetradecane (99 +%, 1 μl/5.00 ml). The vials were capped and stirred vigorously for 10 to 15 min. Decantation from the sludge (calcium oxide, hydroxide, acetate, and dithiocarbamates) provided a clear carbon disulfide solution containing only the internal standard alkane and the nitrosamine produced in the original mixture from the time of mixing to the time the aliquot was added to the calcium oxide.

The N-methylpiperidine mixtures also contained the original tertiary amine since, unlike secondary amines, tertiary amines cannot react with carbon disulfide in the presence of calcium hydroxide to form insoluble dithiocarbamate salts. For these cases, the carbon disulfide solutions were decanted into a 2nd vial containing a magnetic stirring bar and 200 to 300 mg of potassium bisulfate. The vials were capped and stirred for several min. Decantation again provided a clear carbon disulfide solution containing only the nitrosamine and the calibrating alkane.

Measurement of the nitrosamines present in the carbon disulfide solutions was performed by injecting several ml of each solution directly into a gas chromatographic column of 15% polyphenyl ether on Chromosorb W, AW-DMCS, 60 to 80 mesh (2-ft x ¼-inch glass, helium 60 ml/min, flame-ionization detector). The oven was operated isothermally, but temperatures ranging from 150–170° were used in different experiments.

The relative retention times of the nitrosamines are given in Table 1. The areas under the nitrosamine and alkane peaks were compared by means of response ratios derived...
Table 1
Retention times of nitrosopiperidines on a gas chromatographic column of 15% polyphenyl ether on Chromosorb W

The retention times of nitrosopiperidine and methyl-substituted nitrosopiperidines are compared with those of saturated alkanes used as standards for calibration.

<table>
<thead>
<tr>
<th>Compound</th>
<th>150°</th>
<th>160°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetradecane</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Pentadecane</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Hexadecane</td>
<td>5.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Nitrosopiperidine</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Nitroso-2-methylpiperidine</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Nitroso-2,6-dimethylpiperidine</td>
<td>4.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Relative rate constants for nitrosation of piperidines

The pseudo 4th-order rate constants for the reaction of piperidine and 4 methyl-substituted piperidines were calculated from the kinetic plots in Charts 1 to 3.

<table>
<thead>
<tr>
<th>Amine</th>
<th>Temperature</th>
<th>[NO2]: [amine]</th>
<th>Pseudo 4th-order rate constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperidine</td>
<td>27°</td>
<td>1:1</td>
<td>19.2</td>
</tr>
<tr>
<td>2-Methylpiperidine</td>
<td>27°</td>
<td>1:1</td>
<td>3.1</td>
</tr>
<tr>
<td>2,6-Dimethylpiperidine</td>
<td>27°</td>
<td>1:1</td>
<td>1.2</td>
</tr>
<tr>
<td>2,2,6,6-Tetramethylpiperidine</td>
<td>27°</td>
<td>1:1</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>57°</td>
<td>1:1</td>
<td>13</td>
</tr>
<tr>
<td>N-Methylpiperidine</td>
<td>57°</td>
<td>1:1</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:1</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4:1</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8:1</td>
<td>0.57</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

We used 0.2 M amine + 0.2 M sodium nitrite in acetic acid at pH 4.1 and 27°, and the relative rates of reaction of the secondary amines are shown graphically in Chart 1. The rate of formation of nitrosamine from the piperidine falls when one or more methyl groups is introduced α to the nitrogen atom. The relative rates, as calculated from the pseudo 4th-order rate constants for piperidine, 2-methylpiperidine, 2,6-dimethylpiperidine, and 2,2,6,6-tetramethylpiperidine, were approximately 100:20:10:1 (Table 2). There appeared to be considerable steric hindrance to nitrosation of the amine by the presence of even 1 methyl group adjacent to the nitrogen atom, and this hindrance from appropriate blank runs. These nitrosamine values were used to calculate the amine concentrations for use in the kinetic calculations. The validity of this method was demonstrated by following to completion several reactions in which the final nitrosamine concentration was equal to the initial amine concentration, thus showing that no concurrent side reactions took place.

The pH's of the reaction mixtures were measured and found to remain constant within 0.03 unit over 7 days, the period required for some of the experiments.

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Increased with the number of methyl groups. There was no large difference in the pK of the secondary amines that could be responsible for these differences in nitrosation rates (e.g., the pK of piperidine is 11.12; the pK of tetramethylpiperidine is 11.07).

The formation of N-nitrosopiperidine from N-methylpiperidine under the same conditions (at 27°) was so slow as to be unmeasurable. For comparison of the reaction with nitrous acid of this compound with that of piperidine, a similar study was carried out at a higher temperature. The formation of N-nitrosopiperidine was compared with the formation of N-nitroso-2,2,6,6-tetramethylpiperidine from the secondary amine at 57°. At this temperature, the reactions of both compounds were measurable, and the relative rates of formation of nitrosamine are shown in Chart 2. It appears that the reaction rate of N-methylpiperidine is more than 2 orders of magnitude slower than that of 2,2,6,6-tetramethylpiperidine and therefore is at least 10,000 times slower than that of piperidine itself.

As shown in Chart 3, there is a considerable increase in the reaction rate of N-methylpiperidine with an increasing ratio of nitrite to amine, the rate constant increasing somewhat less than linearly with the nitrite ion concentration. There is also an increase in the rate of nitrosation of the secondary amines at higher nitrite concentrations, but we found that increasing the ratio of nitrite to amine above 1:1 resulted in nonlinear pseudo 4th-order kinetic plots. This indicates that different mechanisms are involved in the nitrosation of secondary and tertiary amines.

Only limited conclusions can be drawn about the relevance of the results reported here to the problem of formation of carcinogenic nitrosamines in the stomach from ingested amines and nitrite. The largest risk seems to be from unhindered, relatively weakly basic secondary amines, since the N-nitroso derivatives of these are known to be strong carcinogens. The nitrosation of sterically hindered secondary amines is likely to be less important, since the rate of reaction is slower and the N-nitroso compounds formed might be less carcinogenic than the unsubstituted nitrosamines (2). Tertiary amines, although reacting much more slowly than secondary amines, do give rise to nitrosamines that are strong carcinogens. The increased rate of reaction with higher ratios of nitrite to amine is obviously important. The relative rates of reaction might be of less biological significance than it seems, since the residence time of amines and nitrite is measured in hours in the stomach or in days in stored food. This could be sufficient time for the formation of significant quantities of carcinogenic nitrosamines, even from amines, especially tertiary amines, which react relatively slowly.

ACKNOWLEDGMENTS

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REFERENCES

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