

**Figure 3.** Time course of depletion of heart norepinephrine by MBA. Male Wistar rats were administered one dose (0.3 mmol/kg) of MBA at various times before sacrifice. All animals were sacrificed within 45 min of 11:00 a.m. Results are represented as per cent control  $\pm$  the standard error of the mean.

Removal of the solvent and crystallization from EtOH gave **5a**: mp 240–241°; nmr ( $D_2O$ )  $\delta$  4.25 (q, 2 H,  $J = 7.0$  Hz) and 1.25 (t, 3 H,  $J = 7.0$  Hz, ethyl ester protons). Anal. ( $C_{13}H_{19}N_3Cl_2O_2$ ) C, H, N, Cl.

**Tyrosine Hydroxylase Inhibition Studies.** The materials and methods used in tyrosine hydroxylase purification and assay were the same as previously reported.<sup>14,15</sup> Tyrosine hydroxylase kinetics were determined by the method of Lineweaver-Burk<sup>16</sup> with substrate concentrations varying from  $6 \times 10^{-6}$  to  $10^{-4}$  M and by the method of Dixon<sup>17</sup> with substrate concentrations set at  $5 \times 10^{-5}$  and at  $1 \times 10^{-5}$  M. DMPH<sub>4</sub> concentration was constant in the inhibition studies at  $10^{-3}$  M but was later varied between  $1 \times 10^{-2}$  and  $1 \times 10^{-5}$  M.

**Biogenic Amine Studies.** Male Wistar rats (90–120 g) were sacrificed with a guillotine and a glass and Teflon homogenizer was used in all homogenization procedures.

Biogenic amines were determined by the modification of the procedures of Chang, *et al.*<sup>18</sup> (norepinephrine and dopamine), and of Maickel, *et al.*<sup>19</sup> (serotonin), as described by Johnson, *et al.*<sup>2</sup> The control values obtained for biogenic amines were, in  $\mu g/g \pm S.E.$ ,  $0.57 \pm 0.02$  for heart norepinephrine,  $0.46 \pm 0.01$  for brain norepinephrine,  $0.50 \pm 0.01$  for brain serotonin, and  $0.45 \pm 0.02$  for brain dopamine.

## References

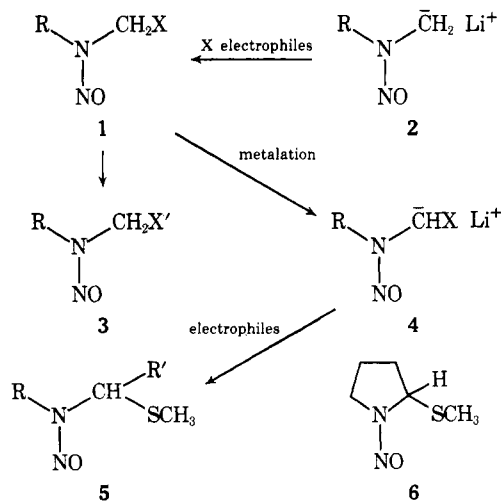
- J. D. Milkowski, F. M. Miller, E. M. Johnson, Jr., and N. Zenker, *J. Med. Chem.*, **13**, 740 (1970).
- E. M. Johnson, Jr., N. Zenker, and J. Wright, *Biochem. Pharmacol.*, **21**, 1777 (1972).
- S. Imashuku, E. A. LaBrosse, E. M. Johnson, Jr., V. H. Morgenroth, III, and N. Zenker, *Biochem. Med.*, **5**, 22 (1971).
- K. E. Moore and J. A. Dominic, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **30**, 859 (1971).
- C. C. Porter and D. C. Titus, *J. Pharmacol. Exp. Ther.*, **139**, 77 (1973).
- N. Zenker, *J. Med. Chem.*, **9**, 826 (1966).
- J. Wright, J. W. King, V. H. Morgenroth, III, and N. Zenker, *Pharmacol. Future Man, Proc. Int. Congr. Pharmacol.*, 5th, 1972, 256 (1973).
- B. K. Koe, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **30**, 866 (1971).
- M. Ikeda, L. A. Fahien, and S. Udenfriend, *J. Biol. Chem.*, **241**, 4452 (1966).
- T. Nagatsu, M. Levitt, and S. Udenfriend, *J. Biol. Chem.*, **239**, 2910 (1964).
- R. J. Taylor, Jr., and L. Ellenbogen, *Life Sci.*, **6**, 1463 (1967).
- V. H. Morgenroth, III, and N. Zenker, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **32**, 769 (1973).
- T. N. Ghosh, B. Bhattacharya, and S. Datta, *J. Indian Chem. Soc.*, **34**, 417 (1957).
- B. N. Lutsky and N. Zenker, *J. Med. Chem.*, **11**, 1241 (1968).
- Y. H. Caplan, N. Zenker, D. Blake, and E. M. Johnson, Jr., *J. Med. Chem.*, **14**, 405 (1971).
- H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934).
- M. Dixon, *Biochem. J.*, **55**, 170 (1953).
- C. C. Chang, *Neuropharmacology*, **3**, 643 (1964).
- R. P. Maickel, R. H. Cox, J. Saillant, and F. P. Miller, *Neuropharmacology*, **7**, 275 (1968).

## Synthesis of $\alpha$ -Heterosubstituted Nitrosamines. Novel Test Substances for Cancer and Mutagenesis Research?†

Dieter Seebach\* and Dieter Enders

Institut für Organische Chemie der Universität,  
63 Gießen, West Germany. Received March 4, 1974

Because of their environmental occurrence, high activity, and often application-independent, organospecific effects, nitrosamines have gained broad application and worldwide interest for practical and mechanistic studies in cancer and mutagenesis research; the alkylation hypothesis of carcinogenesis requires that they are hydroxylated enzymatically to give, for instance, **1** ( $X = OH$ ) which is a potential precursor of alkylating diazonium ions and/or diazo compounds.<sup>1–4</sup> In order to further test this theory, new methods of preparation of  $\alpha$ -heteronitrosamines were desirable. Hitherto, only ethers of type **1** ( $X = OR$ ) were readily available.<sup>†5–9</sup> We describe here a general route to and first examples of the preparation of  $\alpha$ -sulfur-,  $\alpha$ -selenium-,  $\alpha$ -silicon-, and  $\alpha$ -tin-substituted nitrosamines **1**, **3**, **5**, and **6** (see Table I). The method rests upon the availability of lithiated nitrosamines such as **2**<sup>10–12</sup> whose reactions with the heteroelectrophiles dimethyl and diphenyl disulfide, diphenyl diselenide, trimethylchlorosilane, and trimethylchlorotin lead to the derivatives **1**. These were amenable to further structural modifications either by transformation of  $X$  in **1** into  $X'$  in **3** (*cf.*  $SCH_3 \rightarrow SOCH_3$ ) or by metalation of **1** to give **4** which was derivatized to higher heteronitrosamines **5**. Cyclic compounds such as **6** were also available by our route. The yields were good to excellent; data of the products prepared are listed in Table I. The new compounds can be stored for months in a refrigerator (0°). The low-molecu-



† Financial support by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Studienstiftung des Deutschen Volkes is gratefully acknowledged.

† Most of the numerous examples known and easiest to prepare are cyclic derivatives. One  $\alpha$ -acyloxy derivative<sup>7</sup> is also known.

**Table I. Yields and Some Physical Data of  $\alpha$ -Heterosubstituted Nitrosamines**

$$\begin{array}{c}
 \text{R}' \\
 | \\
 \text{R}-\text{N}-\text{CHX(X')} \\
 | \\
 \text{NO}
 \end{array}$$

No.	Electrophile	R	R'	X (X')	Mp or bp (Torr). <sup>a</sup> °C	Yield, %	Formula	Analyses <sup>b</sup>
<b>1a</b>	CH <sub>3</sub> SSCH <sub>3</sub>	CH <sub>3</sub>	H	SCH <sub>3</sub>	50 (0.7)	85	C <sub>3</sub> H <sub>8</sub> N <sub>2</sub> OS	C, H, N
<b>1b</b>	C <sub>6</sub> H <sub>5</sub> SSC <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	H	SC <sub>6</sub> H <sub>5</sub>	120 (0.03)	83	C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> OS	C, H, N
<b>1c</b>	C <sub>6</sub> H <sub>5</sub> SeSeC <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	H	SeC <sub>6</sub> H <sub>5</sub>	100 (0.02)	67	C <sub>8</sub> H <sub>10</sub> N <sub>2</sub> OSe	C, H, N
<b>1d</b>	(CH <sub>3</sub> ) <sub>3</sub> SiCl	(CH <sub>3</sub> ) <sub>3</sub> C	H	Si(CH <sub>3</sub> ) <sub>3</sub>	46–47	80	C <sub>8</sub> H <sub>20</sub> N <sub>2</sub> OSi	C, H, N
<b>1e</b>	(CH <sub>3</sub> ) <sub>3</sub> SnCl	(CH <sub>3</sub> ) <sub>3</sub> C	H	Sn(CH <sub>3</sub> ) <sub>3</sub>	47–48 dec	>95	C <sub>8</sub> H <sub>20</sub> N <sub>2</sub> OSn	C, H, N
<b>1f</b>	CH <sub>3</sub> SSCH <sub>3</sub>	(CH <sub>3</sub> ) <sub>3</sub> C	H	SCH <sub>3</sub>	39–41	90	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> OS	C, H, N, S
<b>3</b>		(CH <sub>3</sub> ) <sub>3</sub> C	H	SO(CH <sub>3</sub> ) <sup>d</sup>	110 (0.06)	100	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S	H, N; C <sup>c</sup>
<b>5a</b>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Br	(CH <sub>3</sub> ) <sub>3</sub> C	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	SCH <sub>3</sub>	71.5–72.5	95	C <sub>13</sub> H <sub>20</sub> N <sub>2</sub> OS	C, H, N, S
<b>5b</b>	C <sub>6</sub> H <sub>5</sub> CHO	(CH <sub>3</sub> ) <sub>3</sub> C	C <sub>6</sub> H <sub>5</sub> CH(OH)	SCH <sub>3</sub>	98–99	90	C <sub>13</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N, S
<b>5c</b>	Cyclohexenone	(CH <sub>3</sub> ) <sub>3</sub> C	1-Hydroxy- 2-cyclo- hexenyl	SCH <sub>3</sub>	110–111	80	C <sub>15</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
<b>5d</b>	CH <sub>3</sub> SSCH <sub>3</sub>	(CH <sub>3</sub> ) <sub>3</sub> C	SCH <sub>3</sub>	SCH <sub>3</sub>	96.5–97 dec	85	C <sub>7</sub> H <sub>16</sub> N <sub>2</sub> OS <sub>2</sub>	C, H, N
<b>6</b>	CH <sub>3</sub> SSCH <sub>3</sub>	–(CH <sub>2</sub> ) <sub>3</sub> –		SCH <sub>3</sub>	80 (0.1)	75	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> OS	C, H, N

<sup>a</sup> Bath temperature during molecular distillation. <sup>b</sup> Analyses of indicated elements were within  $\pm 0.4\%$  of the theoretical values. <sup>c</sup> C: calcd, 40.42; found, 39.76. <sup>d</sup> By oxidation of **1f** with  $\text{KIO}_4$ .

lar-weight derivatives **1a,f**, **3**, **5d**, and **6** had an appreciable solubility in water (work-up must be carried out with saturated brine) or were even hygroscopic (*cf.* **3**) as indicated in the Experimental Section.

We are currently investigating reactions of nitrosamine anions with oxygen- and halogen-introducing electrophiles. Although animal tests have not been performed with the new nitrosamines as yet, utmost care is advisable in their preparation and handling.

## Experimental Section

Melting points (uncorrected) were determined according to Tottoli on a Büchi melting point apparatus. Nmr spectra were recorded on a Jeol Minimar 100 spectrometer ( $\text{CCl}_4$ ),  $\text{Me}_4\text{Si}$  standard. The ratios of conformational isomers present in the nmr samples may not reflect thermodynamic ratios since the spectra were taken immediately after purification. Ir spectra were taken with a Perkin-Elmer 225 spectrophotometer; they were in agreement with the structures given. Uv spectra ( $\text{MeOH}$ ) were taken with a Leitz-Unicam 800 spectrometer.

**Metalation of Nitrosamines.** Nitrosamines (10 mmol) were added with stirring to a solution of lithium diisopropylamide (10.5 mmol) in anhydrous THF (25 ml) (from 1.5 ml of diisopropylamine and 6.5 ml of *n*-BuLi; 1.6 *M* in *n*-hexane) at  $-78^{\circ}$  and the mixture was treated 10 min later with the electrophiles. After 3 hr the cooling bath was removed and working up with  $\text{CH}_2\text{Cl}_2$  yielded the crude products.

**N-Nitrosomethylthiodimethylamine (1a).** *N*-Nitrosodimethylamine (2.22 ml, 30 mmol) and 13.3 ml (150 mmol) of dimethyl disulfide gave 3.05 g (85%) of a yellow oil after distillation of the crude product: nmr  $\delta$  1.98, 2.03 (s, 3 H, SCH<sub>3</sub>, *Z*, *E*), 3.03, 3.80 (s, 3 H, NCH<sub>3</sub>, *E*, *Z*), 4.54, 5.15 (s, 2 H, CH<sub>2</sub>, *Z*, *E*); *E*:*Z* = 7.5; uv  $\lambda_{\text{max}}$  ( $\epsilon$ ) 235 (7000), 356 (100).

**N-Nitrosophenylthiodimethylamine (1b).** From 0.74 ml (10 mmol) of *N*-nitrosodimethylamine and 10.9 g (50 mmol) of diphenyl disulfide, a crude product was obtained which was put on a silica gel column with pentane. Elution with this solvent removed excess disulfide while ether eluted the product: 1.52 g (83%) of a yellow oil; nmr  $\delta$  2.95, 3.67 (s, 3 H, CH<sub>3</sub>, *E*, *Z*), 4.75, 5.40 (s, 2 H, CH<sub>2</sub>, *Z*, *E*), 7.23 (m, 5 H, C<sub>6</sub>H<sub>5</sub>); *E*:*Z* = 5.7; uv  $\lambda_{\text{max}}$  ( $\epsilon$ ) 213 (7700), 240 (6700), 358 (62).

**N-Nitrosophenylselenodimethylamine (1c).** N-Nitrosodimethylamine (0.74 ml, 10 mmol) and 8.7 g (28 mmol) of diphenyl

diselenide gave, after similar separation (elution with  $C_6H_6$ ) of the excess diselenide as described in the previous procedure, 1.54 g (67%) of a yellow oil: nmr  $\delta$  2.91, 3.63 (s, 3 H,  $CH_3$ , E, Z), 4.76, 5.52 (s, 2 H,  $J$  ( $^{77}SeCH_2E$ ) = 19.5 Hz,  $J$  ( $^{77}SeCH_2Z$ ) = 16 Hz,  $CH_2$ , E, Z), 7.23, 7.45 (m, 5 H,  $C_6H_5$ );  $E:Z$  = 3.0; uv  $\lambda_{max}$  ( $\epsilon$ ) 218 (9200), 244 (9350), 358 (134).

**N-Nitrosotrimethylsilylmethyl-*tert*-butylamine (1d).** From 5.8 g (50 mmol) of *N*-nitrosomethyl-*tert*-butylamine and 25.6 ml (200 mmol) of freshly distilled  $\text{Me}_3\text{SiCl}$ , 7.5 g (80%) of essentially pure product was isolated after work-up with  $\text{CH}_2\text{Cl}_2$ -saturated NaCl solution. Crystallization (pentane,  $-40^\circ$ ) furnished colorless crystals: nmr (CDCl<sub>3</sub>)  $\delta$  0.04 (w), 0.13 (s, 9 H,  $\text{Me}_3\text{Si}$ ), 1.50 (s, 9 H,  $\text{Me}_3\text{C}$ ), 3.00 (s, 2 H,  $\text{CH}_2$ ).

**N-Nitrosodimethylstannylmethyl-*tert*-butylamine (1e).** *N*-Nitrosomethyl-*tert*-butylamine (1.16 g, 10 mmol) and 2 g (10 mmol) of Me<sub>3</sub>SnCl afforded 2.8 g (100%) of crystalline **1e** which, on contact with air, was decomposed to give starting nitrosamine and a high-melting, unidentified tin derivative. Recrystallization (Et<sub>2</sub>O-pentane, -30°) gives colorless crystals: nmr  $\delta$  0.05 (s, 9 H,  $J$  (<sup>117</sup>-<sup>119</sup>SnCH) = ~53 Hz, Me<sub>3</sub>Sn), 1.54 (s, 9 H, Me<sub>3</sub>C), 2.90 (s, 2 H,  $J$  (<sup>117</sup>-<sup>119</sup>SnCH<sub>2</sub>) = 38.5 Hz, not resolved, CH<sub>2</sub>); uv  $\lambda_{\text{max}}$  ( $\epsilon$ ) 228 (7950), 349 (64).

**N-Nitrosomethylthiomethyl-*tert*-butylamine (If).** *N*-Nitrosomethyl-*tert*-butylamine (2.15 g, 18.5 mmol) and 8.85 ml (100 mmol) of dimethyl disulfide gave 2.7 g (90%) of beige crystals (pentane,  $-30^{\circ}$ ): nmr  $\delta$  1.62 (s, 9 H, Me<sub>3</sub>C), 2.18 (s, 3 H, CH<sub>3</sub>), 4.50 (s, 2 H, CH<sub>2</sub>);  $\nu$   $\lambda_{\max}$  ( $\epsilon$ ) 237 (5200), 365 (62).

***N*-Nitroso-*N*-*tert*-butylaminodimethyl Sulfoxide (3).** Addition, within 30 min, of a warm aqueous solution of 2.3 g (10 mmol) of  $\text{KIO}_4$  to a solution of 0.82 g (5.05 mmol) of **1f** in 40 ml of MeOH led, after stirring for 2 hr at room temperature, filtering, evaporating the filtrate, and extracting the residue several times with  $\text{CHCl}_3$ , to the isolation of 0.9 g (100%) of a bright yellow oil (hygroscopic): nmr  $\delta$  1.64 (s, 9 H,  $\text{Me}_3\text{C}$ ), 2.60 (s, 3 H,  $\text{CH}_3$ ), 4.24, 4.89 (d, 2 H,  $J_{\text{gem}} = 12.5$  Hz,  $\text{CH}_2$ ); uv  $\lambda_{\text{max}}$  ( $\epsilon$ ) 225 (6600), 252 (7000), 369 (72).

**1-*N*-Nitroso-*N*-*tert*-butylamino-1-methylthio-2-phenylethane (5a).** From 0.85 g (5.25 mmol) of **1f** and 0.6 ml (5 mmol) of  $\text{C}_6\text{H}_5\text{CH}_2\text{Br}$  we obtained 1.3 g (96%) of a beige solid material which was dissolved in a small amount of  $\text{CCl}_4$ . Precipitation with pentane gave 0.95 g (75%) of colorless crystals: nmr ( $\text{C}_6\text{D}_6$ )  $\delta$  1.10 (s, 9 H,  $\text{Me}_3\text{C}$ ), 1.70 (s, 3 H,  $\text{SCH}_3$ ), 3.35, 3.84 (ABM system,  $\text{CH}_2$  and  $\text{CH}$ ), 7.04 (m, 5 H,  $\text{C}_6\text{H}_5$ ); uv  $\lambda_{\text{max}}$  ( $\epsilon$ ) 214 (7300), 239 (4400), 367 (43).

**2-N-Nitroso-N-tert-butylamino-2-methylthio-1-hydroxy-1-**

