

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) δ 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.^{14,15} Tyrosine hydroxylase kinetics were determined by the method of Lineweaver-Burk¹⁶ with substrate concentrations varying from 6×10^{-6} to 10^{-4} M and by the method of Dixon¹⁷ with substrate concentrations set at 5×10^{-5} and at 1×10^{-5} M. DMPH₄ concentration was constant in the inhibition studies at 10^{-3} M but was later varied between 1×10^{-2} and 1×10^{-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.*¹⁸ (norepinephrine and dopamine), and of Maickel, *et al.*¹⁹ (serotonin), as described by Johnson, *et al.*² The control values obtained for biogenic amines were, in $\mu\text{g/g} \pm \text{S.E.}$, 0.57 ± 0.02 for heart norepinephrine, 0.46 ± 0.01 for brain norepinephrine, 0.50 ± 0.01 for brain serotonin, and 0.45 ± 0.02 for brain dopamine.

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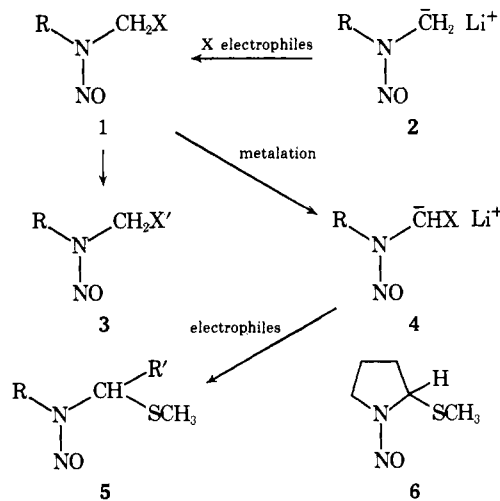
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Synthesis of α -Heterosubstituted Nitrosamines. Novel Test Substances for Cancer and Mutagenesis Research?†

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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 carcinogenicity requires that they are hydroxylated enzymatically to give, for instance, **1** ($X = \text{OH}$) which is a potential precursor of alkylating diazonium ions and/or diazo compounds.^{1–4} In order to further test this theory, new methods of preparation of α -heteronitrosamines were desirable. Hitherto, only ethers of type **1** ($X = \text{OR}$) were readily available.^{†,5–9} We describe here a general route to and first examples of the preparation of α -sulfur-, α -selenium-, α -silicon-, and α -tin-substituted nitrosamines **1**, **3**, **5**, and **6** (see Table I). The method rests upon the availability of lithiated nitrosamines such as **2**^{10–12} 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.* $\text{SCH}_3 \rightarrow \text{SOCH}_3$) 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-



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‡ Most of the numerous examples known and easiest to prepare are cyclic derivatives. One α -acyloxy derivative⁷ is also known.

Table I. Yields and Some Physical Data of α -Heterosubstituted Nitrosamines

No.	Electrophile	R	R'	X (X')	Mp or bp (Torr). ^a °C	Yield, %	Formula	Analyses ^b
1a	CH ₃ SSCH ₃	CH ₃	H	SCH ₃	50 (0.7)	85	C ₃ H ₈ N ₂ OS	C, H, N
1b	C ₆ H ₅ SSC ₆ H ₅	CH ₃	H	SC ₆ H ₅	120 (0.03)	83	C ₈ H ₁₀ N ₂ OS	C, H, N
1c	C ₆ H ₅ SeSeC ₆ H ₅	CH ₃	H	SeC ₆ H ₅	100 (0.02)	67	C ₈ H ₁₀ N ₂ OSe	C, H, N
1d	(CH ₃) ₃ SiCl	(CH ₃) ₃ C	H	Si(CH ₃) ₃	46–47	80	C ₈ H ₂₀ N ₂ OSi	C, H, N
1e	(CH ₃) ₃ SnCl	(CH ₃) ₃ C	H	Sn(CH ₃) ₃	47–48 dec	>95	C ₈ H ₂₀ N ₂ OSn	C, H, N
1f	CH ₃ SSCH ₃	(CH ₃) ₃ C	H	SCH ₃	39–41	90	C ₈ H ₁₄ N ₂ OS	C, H, N, S
3		(CH ₃) ₃ C	H	SO(CH ₃) ^d	110 (0.06)	100	C ₆ H ₁₄ N ₂ O ₂ S	H, N; C ^c
5a	C ₆ H ₅ CH ₂ Br	(CH ₃) ₃ C	C ₆ H ₅ CH ₂	SCH ₃	71.5–72.5	95	C ₁₃ H ₂₀ N ₂ OS	C, H, N, S
5b	C ₆ H ₅ CHO	(CH ₃) ₃ C	C ₆ H ₅ CH(OH)	SCH ₃	98–99	90	C ₁₃ H ₂₀ N ₂ O ₂ S	C, H, N, S
5c	Cyclohexenone	(CH ₃) ₃ C	1-Hydroxy- 2-cyclo- hexenyl	SCH ₃	110–111	80	C ₁₂ H ₂₂ N ₂ O ₂ S	C, H, N
5d	CH ₃ SSCH ₃	(CH ₃) ₃ C	SCH ₃	SCH ₃	96.5–97 dec	85	C ₇ H ₁₆ N ₂ OS ₂	C, H, N
6	CH ₃ SSCH ₃	–(CH ₂) ₃ –		SCH ₃	80 (0.1)	75	C ₅ H ₁₀ N ₂ OS	C, H, N

^a Bath temperature during molecular distillation. ^b Analyses of indicated elements were within $\pm 0.4\%$ of the theoretical values. ^c C: calcd, 40.42; found, 39.76. ^d By oxidation of 1f with KIO₄.

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 (CCl₄), Me₄Si 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 (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° and the mixture was treated 10 min later with the electrophiles. After 3 hr the cooling bath was removed and working up with CH₂Cl₂ 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 δ 1.98, 2.03 (s, 3 H, SCH₃, *Z*, *E*), 3.03, 3.80 (s, 3 H, NCH₃, *E*, *Z*), 4.54, 5.15 (s, 2 H, CH₂, *Z*, *E*); *E*:*Z* = 7.5; uv λ_{\max} (ϵ) 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 δ 2.95, 3.67 (s, 3 H, CH₃, *E*, *Z*), 4.75, 5.40 (s, 2 H, CH₂, *Z*, *E*), 7.23 (m, 5 H, C₆H₅); *E*:*Z* = 5.7; uv λ_{\max} (ϵ) 213 (7700), 240 (6700), 358 (620).

***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 C₆H₆) of the excess diselenide as described in the previous procedure, 1.54 g (67%) of a yellow oil: nmr δ 2.91, 3.63 (s, 3 H, CH₃, *E*, *Z*), 4.76, 5.52 (s, 2 H, *J* (⁷⁷SeCH₂-*E*) = 19.5 Hz, *J* (⁷⁷SeCH₂-*Z*) = 16 Hz, CH₂, *Z*, *E*), 7.23, 7.45 (m, 5 H, C₆H₅); *E*:*Z* = 3.0; uv λ_{\max} (ϵ) 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 Me₃SiCl, 7.5 g (80%) of essentially pure product was isolated after work-up with CH₂Cl₂-saturated NaCl solution. Crystallization (pentane, -40°) furnished colorless crystals: nmr (CDCl₃) δ 0.04 (w), 0.13 (s, 9 H, Me₃Si), 1.50 (s, 9 H, Me₃C), 3.00 (s, 2 H, CH₂).

***N*-Nitrosotrimethylstannylmethyl-*tert*-butylamine (1e).** *N*-Nitrosomethyl-*tert*-butylamine (1.16 g, 10 mmol) and 2 g (10 mmol) of Me₃SnCl 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 (Et₂O-pentane, -30°) gives colorless crystals: nmr δ 0.05 (s, 9 H, *J* (¹¹⁷-¹¹⁹SnCH) = \sim 53 Hz, Me₃Sn), 1.54 (s, 9 H, Me₃C), 2.90 (s, 2 H, *J* (¹¹⁷-¹¹⁹SnCH₂) = 38.5 Hz, not resolved, CH₂); uv λ_{\max} (ϵ) 228 (7950), 349 (64).

***N*-Nitrosomethylthiomethyl-*tert*-butylamine (1f).** *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°): nmr δ 1.62 (s, 9 H, Me₃C), 2.18 (s, 3 H, CH₃), 4.50 (s, 2 H, CH₂); uv λ_{\max} (ϵ) 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 KIO₄ 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 CHCl₃, to the isolation of 0.9 g (100%) of a bright yellow oil (hygroscopic): nmr δ 1.64 (s, 9 H, Me₃C), 2.60 (s, 3 H, CH₃), 4.24, 4.89 (d, 2 H, *J*_{gem} = 12.5 Hz, CH₂); uv λ_{\max} (ϵ) 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 C₆H₅CH₂Br we obtained 1.3 g (96%) of a beige solid material which was dissolved in a small amount of CCl₄. Precipitation with pentane gave 0.95 g (75%) of colorless crystals: nmr (C₆D₆) δ 1.10 (s, 9 H, Me₃C), 1.70 (s, 3 H, SCH₃), 3.35, 3.84 (ABM system, CH₂ and CH), 7.04 (m, 5 H, C₆H₅); uv λ_{\max} (ϵ) 214 (7300), 239 (4400), 367 (43).

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

phenylethane (5b). 1f (2.43 g, 15 mmol) and 1.52 ml (15 mmol) of C_6H_5CHO yielded 3.6 g (90%) of a mixture of diastereomers which was recrystallized twice from $CHCl_3$ - Et_2O -pentane to give colorless crystals of the predominant diastereomer: nmr δ 1.16 (s, 9 H, Me_3C), 2.22 (s, 3 H, SCH_3), 3.44 (br s, 1 H, OH), 3.72 (d, 1 H, CH), 5.15 (d, 1 H, CH), 7.10 (m, 5 H, C_6H_5); uv λ_{max} (ϵ) 211 (8300), 241 (4200), 367 (38).

N-Nitroso-1-methylthio-1-(1-hydroxy-2-cyclohexenyl)-methyl-*tert*-butylamine (5c). 1f (0.81 g, 5 mmol) and 0.49 ml (5 mmol) of 2-cyclohexen-1-one produced 1.0 g (80%) of a mixture of diastereomers formed by 1,2 addition. An analytical sample was prepared by recrystallization from EtOH in the form of colorless crystals: nmr ($CDCl_3$) δ 1.15-2.2 (m, 6 H), 1.65 (s, 9 H, Me_3C), 2.24 (s, 3 H, SCH_3), 4.18 (s, 1 H, CH), 4.44 (s, 1 H, OH), 5.88 (m, 2 H); uv λ_{max} (ϵ) 244 (3640), 371 (34).

N-Nitroso-1,1-bis(methylthio)methyl-*tert*-butylamine (5d). 1f (2.43 g, 15 mmol) and 6.63 ml (75 mmol) of dimethyl disulfide gave 2.8 g (85% spectroscopic) of a yellow solid. Recrystallization (Et_2O) afforded yellow crystals (2.34 g, 75%): nmr ($DMSO-d_6$) δ 1.70 (s, 9 H, Me_3C), 2.25 (s, 6 H, SCH_3), 5.3 (s, 1 H, CH); uv λ_{max} (ϵ) 238 (5150), 376 (42).

1-Nitroso-2-methylthiopyrrolidine (6). *N*-Nitrosopyrrolidine (2.74 ml, 30 mmol) and 13.2 ml (150 mmol) of dimethyl disulfide gave, after column chromatography (silica gel, Et_2O), 3.3 g (75%) of a yellow oil: nmr δ 2.1 (m, 4 H), 2.2 (s, 3 H, SCH_3), 2.38, 3.56 (m, 2 H), 5.70 (m, 1 H); uv λ_{max} (ϵ) 236 (6600), 360 (83).

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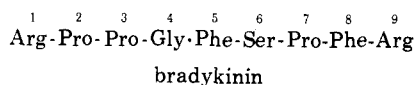
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Synthesis and Pharmacology of Homoarginine Bradykinin† Analogs

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Of the numerous analogs of bradykinin which have been synthesized, few have displayed any antibradykinin activity.^{1,2} The importance of Arg¹ and Arg⁹ residues has been well documented in that deletion of either of the latter residues or substitution of another residue at these positions results in a significant loss of biological activity.³⁻⁹



The purpose of these studies was to determine the effect of extending the aliphatic chain which connects the guanyl group with the main chain in positions 1 and 9 on the biological activity, by substitution of Harg at these posi-

†All amino acids were of the L configuration. Abbreviations used: Boc = *tert*-butyloxycarbonyl; Harg = homarginine; Brad = bradykinin.

Table I. Synthetic Harg-Brad Analogs

Compound	Amino acid composition ^a					
	Arg	Harg	Pro	Phe	Ser	Gly
Harg ¹ -Brad	1.07	0.97	2.81	1.86	1.07	1.00
Harg ⁹ -Brad	0.98	1.05	2.80	2.14	0.93	1.00
Harg ^{1,9} -Brad		1.87	2.88	2.04	0.92	1.00

^aPeptides were hydrolyzed and analyzed for amino acid content as described in the text.

Table II. Relative Potency of Brad Analogs

Compound	Activity on smooth muscle ^a
Arg ^{1,9} -Brad	1
Harg ¹ -Brad	0.06
Harg ⁹ -Brad	0.05
Harg ^{1,9} -Brad	0.02-0.03

^aEach analog was tested on rat fundus strip, rat colon, and rat duodenum.

tions. Thus, the synthesis of the three peptides shown in Table I was undertaken. At the time that this work was being completed, the synthesis of these three peptides was published by Arold and Stibenz,¹⁰ who used conventional methods of peptide synthesis to obtain these compounds. However, no biological data on these peptides were reported.

Biological Results. The three peptides prepared were tested for bradykinin-like activity on rat fundus strip, rat colon, and rat duodenum. All three peptides displayed bradykinin-like activity on each test tissue. The relative potencies of the analogs with respect to bradykinin are given in Table II. Interestingly, both the Harg¹-Brad and Harg⁹-Brad retained a significant amount of activity. Although these values are still relatively low as contrasted with bradykinin, they are significant when compared with the relatively lower values which have been obtained by substitutions of other amino acids at the 1 and 9 positions.³⁻⁹ These data support the current thought that the basic guanyl groups at positions 1 and 9 are necessary for bradykinin-like activity. Since in effect, the chain length of the molecule is being somewhat increased by substitution of Harg, these results show that activity is largely but not totally dependent on the size of the molecule. This is evidenced by the fact that Harg^{1,9}-Brad exhibited approximately one-half the activity of the other two peptides, showing that the activity rapidly diminishes as chain length is increased.

The three peptides were also tested in a second series of experiments for antagonism of bradykinin activity using the cascade technique. None of the peptides were found to antagonize the bradykinin response.

In conclusion, the study of additional analogs of bradykinin which embody subtle changes in structure could lead to a better understanding of the hormone-receptor interaction, which in turn would aid in the design of an effective antimetabolite of bradykinin.

Experimental Section‡

Homoarginine was purchased from Cyclo Chemical Corp. and was converted to Harg(NO₂) by the method of Hayakawa¹¹ and

‡Peptides for amino acid analysis were hydrolyzed in evacuated vessels in 6 N HCl for 24 hr at 110°. Amino acid analyses were performed on a Technicon 5.5-hr amino acid analyzer by the Marschall Division Analytical Services Department of Miles Laboratories.