**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 $\delta$ 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 $\lambda_{max}$ ($\epsilon$) 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 ($CDCl_3$) $\delta$ 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 $\lambda_{max}$ ($\epsilon$) 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-$d_6$) $\delta$ 1.70 (s, 9 H, $Me_3C$), 2.25 (s, 6 H, $SCH_3$), 5.3 (s, 1 H, CH); uv $\lambda_{max}$ ($\epsilon$) 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 $\delta$ 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 $\lambda_{max}$ ($\epsilon$) 236 (6600), 360 (83).

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## Synthesis and Pharmacology of Homoarginine Bradykinin† Analogs


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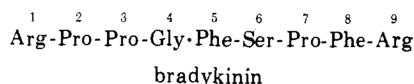
Of the numerous analogs of bradykinin which have been synthesized, few have displayed any antibradykinin activity.[1,2] The importance of Arg[1] and Arg[9] residues has been well documented in that deletion of either of the latter residues or substitution of another residue at these positions results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



bradykinin

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 positions. 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.

†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 |

[a]Peptides 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 |

[a]Each analog was tested on rat fundus strip, rat colon, and rat duodenum.

**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 Harg[1]-Brad and Harg[9]-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($NO_2$) by the method of Hayakawa[11] 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.

subsequently *tert*-butyloxycarbonylated by the method of Schwyzer.[12] Arg($NO_2$) was purchased from Schwarz Bioresearch and *tert*-butyloxycarbonylated.[12] All other Boc-protected amino acid derivatives were obtained from Miles Research Products Division. All *tert*-butyloxycarbonylamino acids were checked for purity by tlc before use. Paper chromatograms were run on Whatman No. 1 paper in the following solvent systems: A, *n*-BuOH-HOAc-$H_2O$ (4:1:1); B, *i*-PrOH-$H_2O$ (2:1); C, *n*-BuOH-HOAc-pyridine-$H_2O$ (15:3:10:12). Chromatograms were developed by ninhydrin, $Cl_2$-starch-iodide,[13] and Sakaguchi reagents. DMF, $Et_3N$, and dioxane were distilled prior to use. All other solvents were reagent grade and were used as received.

**Synthesis of Peptides.** Peptides were synthesized by the solid-phase procedure essentially as described by Stewart and Young.[14] After coupling of the last amino acid on the resin, peptides were cleaved from the resin using liquid HF and subsequently purified by ion-exchange chromatography on IRC-50.[15] Purity of peptides was ascertained by paper chromatography in systems A, B, and C.

**Pharmacology.** The three peptides were tested for bradykinin-like activity using the cascade technique of Vane.[16] Assays were performed on three smooth muscle tissues (rat fundus strip, rat colon, and rat duodenum) which were superfused with oxygenated Krebs-Henselheit solution at 37°. The three peptides were tested for antagonism of bradykinin activity also using the cascade technique. In each experiment, a subthreshold dose of each peptide was added to a known reference dose of bradykinin.

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## Quinazolines and 1,4-Benzodiazepines. 69.[1] 1-Vinyl-1,4-benzodiazepin-2-ones and 1-Vinylquinazolin-2(1*H*)-ones

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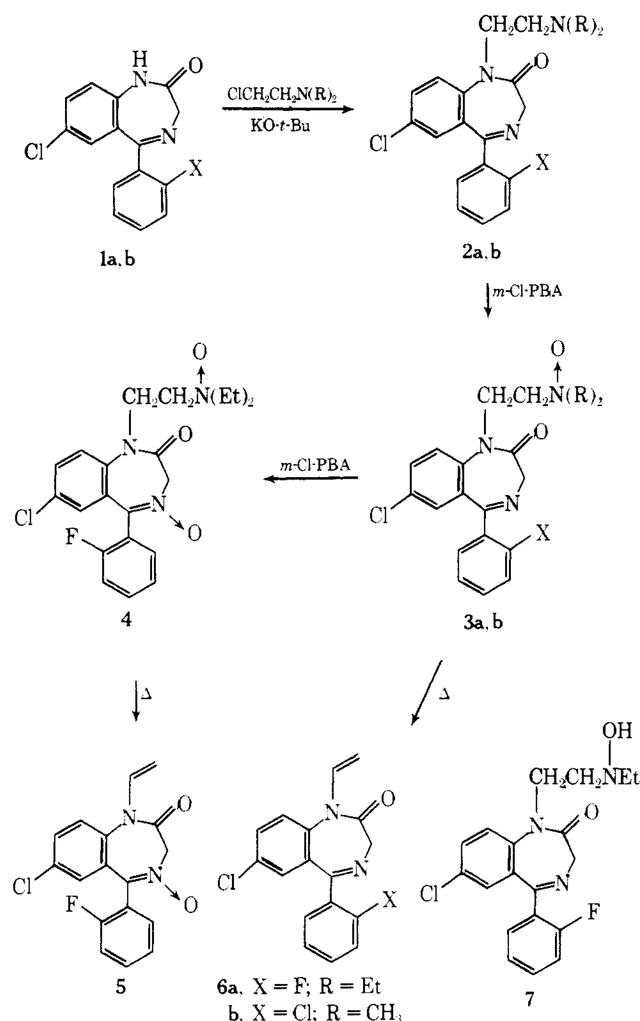
We have found that *N*-vinylamides are easily accessible from dialkylaminoethylamido *N*-oxides by a Cope elimination[2] and we have utilized this reaction in the preparation of the title compounds.

Oxidation of the benzodiazepine derivatives **2** (Scheme I) with 1 equiv of *m*-chloroperbenzoic acid occurred quite selectively at the more basic nitrogen in the side chain and led to the *N*-oxides **3**. Treatment of **3a** with an additional equivalent of peracid yielded in a much slower reaction the di-*N*-oxide **4**. These amine oxides were very water soluble and had to be extracted from the aqueous phase with a mixture of ethanol and methylene chloride after saturation with sodium chloride. Both **3a** and **3b** crystallized with incorporation of water and were analyzed as hydrates. Because of the thermal lability of these *N*-oxides, high-temperature drying had to be avoided.

Scheme I




Thermolysis of **3a** in boiling toluene gave a mixture of the 1-vinylbenzodiazepine **6a** and the hydroxylamine **7**. Due to the considerable differences in polarity, these compounds were readily separated by chromatography on silica gel. A similar mixture of vinyl derivative and hydroxylamine was obtained by thermolysis of the di-*N*-oxide **4**. In this case only the vinylbenzodiazepine **5** which crystallized from the mixture was isolated. Treatment of the oxide of the dimethylaminoethyl derivative **3b** under the same reaction conditions gave exclusively the vinyl compound **6b**. The benzodiazepine **2b** was prepared in an analogous manner to that used for the synthesis of **2a**[3] by the alkylation of the 1-potassium salt of **1b**[4] with 2-dimethylaminoethyl chloride in dimethylformamide.

Alkylation of the quinazolinone **8**[5] (Scheme II) under comparable conditions led to a mixture of both the N-alkylated and the known[6] O-alkylated derivatives, compounds