more favorable pharmacological profile coupled with the expected increase in metabolic stability make dicarbavasopressin antagonists more viable potential therapeutic candidates than their disulfide congeners.

Registry No. 2, 113084-42-7; 3, 113084-43-8; 4, 90332-82-4; 5, 114359-16-9; 6, 110500-78-2; 7, 114923-99-8; 8, 94497-37-7; 9, 114820-55-2; AVP, 113-79-1; LVP, 50-57-7; benzyl cyclohexanediacetic acid, 113009-25-9; BOC-L-glutamic acid α-benzyl ester, 30924-93-7; adenylate cyclase, 9012-42-4.

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Articles

Dimeric 1,4-Dihydropyridines as Calcium Channel Antagonists

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A series of 1,*n*-alkanediylbis(1,4-dihydropyridines) (*n* = 2, 4, 6, 8, 10, 12) bridged at C_3 of 2,6-dimethyl-3-carboxy-5-carbethoxy-4-(3-nitrophenyl)-1,4-dihydropyridine were synthesized and evaluated in a radioligand binding assay, [^{3}H]nitrendipine in intestinal smooth muscle, as Ca^{2+} channel ligands. Binding activity was comparable to that of nitrendipine itself but independent of chain length, suggesting the lack of a major binding contribution by the second 1,4-dihydropyridine group. Analogues lacking the second 1,4-dihydropyridine nucleus or possessing an inactive function (4-nitrophenyl) were no less active, confirming that this series of ligands likely does not bridge adjacent 1,4-dihydropyridine receptors of the Ca^{2+} channel.

The Ca^{2+} channel antagonist nifedipine has proved to be of value in a number of cardiovascular diseases, including angina and hypertension.[1,2] Nifedipine and other 1,4-dihydropyridines, both antagonist and activator, have proven to be valuable molecular probes for the delineation of the structural requirements of the 1,4-dihydropyridine receptor component of the Ca^{2+} channel.[3-5] Additionally, these agents have proved of value in protocols designed to isolate and reconstitute the 1,4-dihydropyridine-sensitive Ca^{2+} channel.[6,7] However, much remains to be learned of the relationship between the 1,4-dihydropyridine binding site and the functional machinery of the Ca^{2+} channel,[5,8] and nothing is known of the topographic relationship between 1,4-dihydropyridine binding sites. This relationship assumes increased importance because of recent reports that activator and antagonist 1,4-dihydropyridines may occupy discrete binding sites.[9,10]

Polyvalent ligands have been of value in probing the interbinding site distances at pharmacological receptors. Early examples include the bis(onium) neuromuscular- and ganglion-blocking agents.[11] More recently, dimeric enkephalins have been used to probe the different distributions of μ- and δ-opiate receptors,[12] and dimeric analogues of gonadotropin releasing hormone have been used to study the effects of receptor microaggregation on ligand-induced activity.[13] Successful bridging of two adjacent receptors by a divalent ligand may enhance affinity by a minimum of twofold and a maximum corresponding to the square of the affinity constant of the appropriate monovalent ligand. The lowest enhancement of activity may be difficult to detect.

As an initial effort to probe the distribution of 1,4-dihydropyridine binding sites associated with voltage-dependent Ca^{2+} channels, we have synthesized and evaluated a series of 1,*n*-alkanediylbis(1,4-dihydropyridines) in which two 1,4-dihydropyridine molecules are linked through C_3 ester substituents.

Chemistry

Ethyl acetoacetate and a substituted benzaldehyde were

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(1) Fleckenstein, A. *Calcium Antagonism in Heart and Smooth Muscle. Experimental Facts and Therapeutic Prospects.* Wiley-Interscience: New York, 1984.
(2) *Cardiovascular Effects of Dihydropyridine-type Calcium Antagonists and Agonists. Bayer Symposium IX*; Fleckenstein, A., van Breemen, C., Gross, R., Hoffmeister, F., Eds.; Springer-Verlag: New York, 1985.
(3) Loev, B.; Goodman, M. M.; Snader, K. M.; Tedeschi, R.; Macko, E. *J. Med. Chem.* **1974**, *17*, 956.
(4) Mannhold, R.; Rodenkirchen, R.; Bayer, R. *Prog. Pharmacol.* **1982**, *5*, 25.
(5) Su, C. M.; Yousif, F. B.; Triggle, D. J.; Janis, R. A. In *Cardiovascular Effects of Dihydropyridine-type Calcium Antagonists and Agonists*; Fleckenstein, A., van Breemen, C., Gross, R., Hoffmeister, F., Eds.; Springer-Verlag: New York, 1985.
(6) Curtis, B. M.; Catterall, W. A. *Biochemistry* **1984**, *23*, 2113.
(7) Borsotto, M.; Barhanin, J.; Fosset, M.; Lazdunski, M. *J. Biol. Chem.* **1985**, *260*, 14255.
(8) Triggle, D. J.; Janis, R. A. *Annu. Rev. Pharmcol. Toxicol.* **1987**, *27*, 347.
(9) Dubé, G. P.; Baik, Y. H.; Vaghy, P. L.; Schwartz, A. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 1295.
(10) Kokobun, S.; Prod'hom, B.; Becker, C.; Porzig, H.; Reuter, H. *Mol. Pharmacol.* **1986**, *30*, 571.
(11) Barlow, R. B.; Ing, H. R. *Br. J. Pharmacol.* **1948**, *3*, 298.
(12) Costa, T.; Wuster, M.; Herz, A.; Shimohigashi, Y.; Chen, H.-C.; Rodbard, D. *Biochem. Pharmacol.* **1985**, *34*, 25.
(13) Smith, W. A.; Conn, P. M. *Endocrinology* **1984**, *114*, 553.

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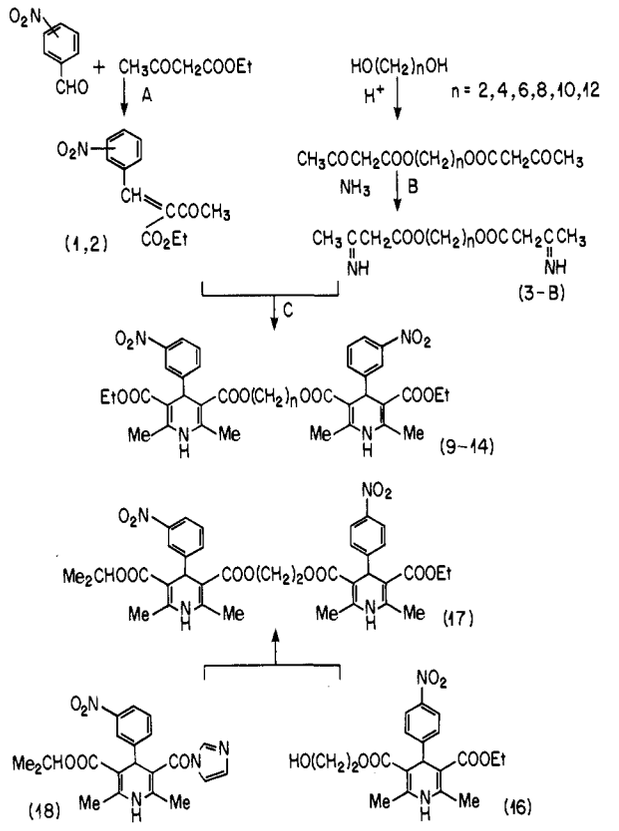
Scheme I

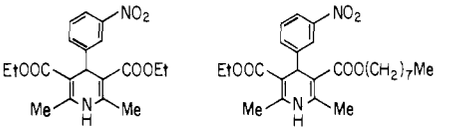


condensed in a Knoevenagel reaction to give the ethyl benzylideneacetoacetate (**1** and **2**) as described in General Method A (see Scheme I). The bis(β-keto esters) (**3–8**) and subsequent bis(aminocrotonates) were made by a transesterification reaction between the appropriate 1,*n*-alkanediol and methyl acetoacetate[14] followed by reaction with ammonia as described in General Method B (see Scheme I). Other β-keto esters were synthesized by reaction of the appropriate alcohol with diketene. The 1,*n*-alkanediylbis(aminocrotonate) and the Knoevenagel product were condensed in a Hantzsch synthesis[15] to obtain the desired 1,*n*-alkanediylbis(1,4-dihydropyridine) as described in General Method C (see Scheme I). An unsymmetrically substituted 1,*n*-alkanediylbis(1,4-dihydropyridine) (**17**) was prepared by condensation of the imidazole (**18**) and ω-(hydroxyalkyl)-1,4-dihydropyridine (**16**).

Results and Discussion

The series of symmetrically substituted bis(1,4-dihydropyridines) and several related compounds were evaluated for their Ca^{2+} channel antagonistic activity in the [^{3}H]nitrendipine binding assay by using a microsomal preparation of guinea pig ileal longitudinal smooth muscle.[16] The validity and limitations of this assay have been discussed.[17] The data are summarized in Table I. The underlying premise of this approach was that if bridging to adjacent 1,4-dihydropyridine receptors occurred, then a defined peak of activity would be expected according to interreceptor distance.

However, in the symmetrical bis(1,4-dihydropyridines) where the bridging carbon chain length varied from 2 to 12, activity varied over only 1 order of magnitude and was essentially independent of chain length from $n = 4$ to 12. This suggests that the second 1,4-dihydropyridine nucleus has not bridged to an adjacent binding site. The dimeric compounds **9–14** were examined as a mixture of D,L racemate and meso forms and **17** as the threo and erythro diastereomers. Since, 1,4-dihydropyridine sites have defined stereochemical requirements, it is very probable that these forms interact with different affinities and may not be recognized as equally effective "bridging agents". This represents a potential limitation to our approach. However, the relative activities of compounds **17**, **21**, and **22** lend support to the conclusion that the dimeric compounds do not bridge adjacent receptors. Compound **17** is an unsymmetrical bis(1,4-dihydropyridine) bearing one group with a 4-nitrophenyl substituent. This latter substitution, or indeed any 4-substitution in the phenyl ring, is known to greatly reduce activity in simple 1,4-dihydropyridines related to nifedipine.[3,4,16] The activity of **17**, approximately 20-fold higher than its symmetrical analogue **9** and approximately as active as the longer chain analogues (Table I) suggests that the second 1,4-dihydropyridine residue (bearing the 4-nitrophenyl substituent) does not contribute to specific binding at a discrete 1,4-dihydropyridine receptor. The high activity of compound **21** and **22**, which



21 **22**

are monomeric analogues of **9** and **12**, possessing the bridging carbon chain only, also suggests that the second 1,4-dihydropyridine group is noncontributing. Compound **22**, bearing the C_8 ester substituent, is the most active member of the series, being some 15 times more potent than nitrendipine (2,6-dimethyl-3-carbomethoxy-5-carbethoxy-4-(3-nitrophenyl)-1,4-dihydropyridine) in the same preparation. This is consistent with previous conclusions that 1,4-dihydropyridines with nonidentical ester functions show enhanced activity, presumably reflecting the asymmetric disposition of binding sites at the 1,4-dihydropyridine receptor.[18]

The high activities of both the monomeric and dimeric 1,4-dihydropyridines suggests, consistent with previous proposals,[5,19] that there exists adjacent to the specific

Table I. Inhibition by 1,4-Dihydropyridines of [^{3}H]Nitrendipine Binding in Intestinal Smooth Muscle

compd	chain length	IC_{50}, M (95% CL)	n^a
	Symmetrical Bis(1,4-dihydropyridines)		
9	2	3.9×10^{-10} (1.5–10.2)	7
10	4	2.2×10^{-11} (0.7–7.2)	6
11	6	4.5×10^{-11} (1.8–11.3)	7
12	8	3.0×10^{-11} (1.2–7.4)	6
13	10	3.3×10^{-11} (1.2–8.9)	7
14	12	5.0×10^{-11} (1.9–13.3)	9
	Unsymmetrical Bis(1,4-dihydropyridine)		
17	2	1.7×10^{-11} (0.8–3.6)	7
	Alkyl Ester 1,4-Dihydropyridines		
21	2	4.6×10^{-11} (1.5–13.8)	5
22	8	1.0×10^{-11} (0.4–2.30)	7
nitrendipine		1.5×10^{-10} (0.6–3.2)	5

[a] Number of separate observations.

(14) Bader, A.; Cummings, L.; Vogel, H. *J. Am. Chem. Soc.* **1951**, *73*, 4195.
(15) Stout, D. M.; Meyers, A. I. *Chem. Rev.* **1982**, *82*, 223.
(16) Bolger, G. T.; Gengo, P.; Klockowski, R.; Luchowski, E.; Siegel, H.; Janis, R. A.; Triggle, A. M.; Triggle, D. J. *J. Pharmacol. Expt. Ther.* **1983**, *225*, 291.
(17) Triggle, D. J.; Janis, R. A. *Drug Dev. Res.* **1984**, *4*, 257.
(18) Towart, R.; Wehinger, E.; Meyer, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *317*, 183.

1,4-dihydropyridine binding site a hydrophobic area with which nonpolar substituents, but not a second 1,4-dihydropyridine ring, interact to maintain and enhance actvity.

Although it can be argued that separation of the 1,4-dihydropyridine functions by longer polymethylene chains might have permitted bridging of adjacent binding sites, recent evidence suggests that the major protein, mass 170K, associated with the voltage-dependent Ca^{2+} channel possesses only one binding site for the 1,4-dihydropyridine category of Ca^{2+} channel ligand.[20] However, cooperative binding of 1,4-dihydropyridine ligands has been described in cardiac myocytes,[10] suggesting the possibility of interactions between or associations of binding sites that may be amenable to ligand bridging. Resolution of the uncertainties of the stoichiometries of 1,4-dihydropyridine binding is important to the analysis of the actions of potential bivalent ligands.

Experimental Section

Melting points were determined with a Meltemp apparatus (Laboratory Devices) and are reported uncorrected. Infrared spectra were obtained with a Nicolet FT-IR spectrophotometer. NMR spectra were determined with a Varian T60A or a Varian EM90 spectrometer and are reported as δ (ppm) values with $CDCl_3$ as a solvent and TMS as the internal standard unless otherwise stated. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical values. Compound **18** was the generous gift of Dr. Schwenner, Bayer A. G. Wuppertal.

General Method A. A solution of 3- or 4-nitrobenzaldehyde (0.03 mol) and ethyl acetoacetate (0.03 mol) in 50 mL of benzene, 0.2 mL of piperidine, and 0.1 mL of acetic acid was refluxed with stirring for 3 h, and water was removed with a Dean–Stark apparatus. The reaction mixture was then diluted with 250 mL of diethyl ether and washed with 200 mL of 5% HCl (two times), 200 mL of 5% $NaHCO_3$ (two times), 200 mL of 5% acetic acid, and 200 mL of H_2O (two times). The organic layer was dried over magnesium sulfate, the volume was then reduced in vacuo, and the resultant oil was recrystallized from ethyl acetate to obtain the desired product. Ethyl (3-nitrobenzylidene)acetoacetate (**1**) was obtained in 47% yield: mp 110–111 °C; IR (KBr) 1742, 1724, 1524 cm^{-1}; NMR (DMSO-d_6) δ 1.3 (t, 3 H, J = 8 Hz), 2.4 (s, 3 H), 4.3 (q, 2 H, J = 7 Hz), 7.3–8.3 (m, 5 H).

Ethyl (4-nitrobenzylidene)acetoacetate (**2**) was obtained in 30.6% yield: mp 188–189 °C; IR (KBr) 1720, 1700, 1523 cm^{-1}; NMR (DMSO-d_6) δ 1.3 (t, 3 H, J = 8 Hz), 2.4 (s, 3 H), 4.3 (q, 2 H, J = 7 Hz), 7.4–8.3 (m, 5 H).

General Method B. To a solution of the appropriate diol (1 equiv) being stirred at 100 °C was added excess methyl acetoacetate (5–10 equiv). The reaction mixture was allowed to stir at 100 °C for 18 h. After the mixture had cooled to room temperature, the excess methyl acetoacetate was removed by vacuum distillation (bp (10 mmHg) 55 °C) to yield a crude white oily solid. The crude product was dissolved in 50 mL of benzene and warmed on a steam bath, and ammonia was bubbled through for 4 h. The benzene was then removed in vacuo to give a green oil that was crystallized from methanol to yield white crystals of the 1,n-alkanediyl bis(aminocrotonates).

Compounds **3–8** were prepared by this general technique. 1,2-Ethanediyl bis(aminocrotonate) (**3**, n = 2) in 64% yield with mp 148–149 °C; 1,4-butanediyl bis(aminocrotonate) (**4**, n = 4) in 27% yield, mp 132–133 °C; 1,6-hexanediyl bis(aminocrotonate) (**5**, n = 6) in 48% yield, mp 115–116 °C; 1,8-octanedily bis(aminocrotonate) (**6**, n = 8) in 81% yield, mp 96–97 °C; 1,10-decanediyl bis(aminocrotonate) (**7**, n = 10) in 73% yield, mp 102–105 °C; 1,12-dodecanediyl bis(aminocrotonate) (**8**, n = 12) in 39% yield, mp 89–90 °C.

(19) Triggle, D. J.; Janis, R. A. *J. Cardiovasc. Pharmacol.* **1984**, *6*, S949.

(20) Galizzi, J.-P.; Borsotto, M.; Barhanin, J.; Fosset, M.; Lazdunski, M. *J. Biol. Chem.* **1986**, *261*, 1393.

Table II. Physical and Analytical Data for 1,n-Alkanediylbis(1,4-dihydropyridines)

compd	chain length	mp, °C	yield, %	formula	anal.[a]
9	2	204–205	33	$C_{36}H_{38}N_4O_{12}$	CHN
10	4	157–159	37	$C_{38}H_{42}N_4O_{12}$	CHN
11	6	105–107	87	$C_{40}H_{46}N_4O_{12}$	CHN
12	8	181–183	91	$C_{42}H_{50}N_4O_{12}$	CHN
13	10	106–110	20	$C_{44}H_{54}N_4O_{12}$	CHN
14	12	118–123	23	$C_{46}H_{58}N_4O_{12}$	CHN

[a] All compounds were analyzed for C, H, and N to within ±0.4% of the theoretical value.

General Method C. To a round-bottom flask equipped with a Dean–Stark trap was added 1 equiv of the appropriate 1,n-alkanediyl bis(aminocrotonate) (**3–8**) dissolved in benzene. Then an excess (>2:1 equiv) of **1** was added. The reaction mixture was refluxed with stirring for 3 days. Following in vacuo removal of benzene, the yellow oil was chromatographed on silica gel with ethyl acetate–$CHCl_3$ (1:1) as the eluent and crystallized from ethyl acetate–hexane to afford the product as a yellow solid (Table II).

2-Hydroxyethyl Acetoacetate (15). Freshly distilled diketene (3.13 mL, 0.04 mol) was added dropwise to a stirring 60 °C solution of ethylene glycol (4.5 mL, 0.08 mol) and 0.2 mL of triethylamine in 25 mL of toluene. During the addition the temperature of reaction mixture was never allowed to exceed 90 °C. After the addition was complete, the reaction mixture was heated at 110 °C for 3 h. The toluene was removed in vacuo, and the brown residue was then chromatographed on silica gel with ethyl acetate as the eluent. The appropriate fractions were pooled and evaporated to afford **15** as a yellow oil in a yield of 4.1 g (70.2%): NMR δ 2.3 (s, 3 H), 3.2 (br s, 1 H), 3.5 (s, 3 H), 3.8 (t, 3 H, J = 4 Hz), 4.2 (t, 3 H, J = 4 Hz); IR ($CHCl_3$) 3469, 1724 cm^{-1}.

2-Hydroxyethyl 2,6-Dimethyl-3-carbethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (16). A solution of **15** (0.6 g, 4.0 mmol) and **2** (1.0 g, 4.0 mmol) in 50 mL of ethanol was saturated with ammonia at 25 °C for 3 h. The reaction mixture was then refluxed for 18 h. Following in vacuo removal of the ethanol, the yellow residue was chromatographed on silica gel with ethyl acetate–hexanes (2:1) as the eluent. The orange oil that was obtained could not be crystallized and was used in the subsequent step without further purification: NMR δ 1.1 (t, 3 H, J = 7 Hz), 2.3 (s, 6 H), 3.2 (br, 1 H), 3.6–3.9 (m, 2 H), 4.0–4.4 (m, 2 H), 5.1 (s, 1 H), 7.0 (br s, 1 H), 7.3–8.2 (m, 4 H); IR ($CHCl_3$) 3437, 1697, 1350 cm^{-1}.

1,2-Ethanediyl 2,6-Dimethyl-5-carbethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine-3-carboxylate 2,6-Dimethyl-5-(isopropoxycarbonyl)-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (17). To a solution of **16** (1.03 g, 3.0 mmol) and sodium hydride (0.6 g, 3.0 mmol) in 20 mL of dry THF was added **18** (1.1 g, 2.6 mmol), dissolved in 20 mL of dry THF, dropwise under N_2 at 25 °C. After the addition was complete, the reaction mixture was heated to reflux and stirred for 24 h. The product was purified by column chromatography on silica gel with ethyl acetate–hexanes (1:1) as the eluent and crystallized from ethyl acetate to yield 1.09 g of **17**: mp 204–208 °C; NMR (DMSO d_6–TMS) δ 1.1 (d, 6 H, J = 6 Hz), 1.3 (t, 3 H, J = 7 Hz), 2.2 (s, 3 H), 2.25 (s, 9 H), 2.4–2.6 (m, 1 H), 3.9–4.4 (t, 4 H, J = 7 Hz), 4.7–5.1 (m, 4 H), 7.2–8.1 (m, 8 H); IR (KBr) 3361, 1693 cm^{-1}. Anal. ($C_{37}H_{40}N_4O_{12}$) C, H, N.

Octyl Acetoacetate (19). Compound **19** was synthesized by the same procedure described above for **15** with freshly distilled diketene (12.5 mL, 0.16 mol) and n-octanol (23.6 mL, 0.15 mol). Following column chromatography on silica gel with ethyl acetate as the eluent, **19** was obtained as a yellow oil in a yield of 2.3 g (62.2%): NMR δ 0.9–1.9 (m, 15 H), 2.3 (s, 3 H), 3.4 (s, 2 H), 4.1 (t, 2 H, J = 7 Hz).

Octyl Aminocrotonate (20). This compound was obtained from **19** (20 g, 0.09 mol) as an oil in essentially quantitative yield by saturating a solution in 25 mL of ethanol at 25 °C with ammonia for 2 h and then removing the ethanol in vacuo.

Diethyl 2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (21). A solution of ethyl aminocrotonate (1 g, 8.0 mmol) and **1** (2.04 g, 8.0 mmol) in 50 mL of ethanol was heated to reflux with stirring for 48 h. Following in

vacuo removal of ethanol, the yellow oil was crystallized from ethyl acetate to afford **21** as yellow crystals in a yield of 2.57 g (91.5%): mp 161–163 °C; NMR δ 1.3 (t, 6 H, J = 4 Hz), 2.4 (s, 6 H), 4.1 (q, 4 H, J = 4 Hz), 5.1 (s, 1 H), 6.1 (br s, 1 H), 7.2–8.1 (m, 4 H); IR (KBr) 3346, 1645 cm^{-1}. Anal. ($C_{19}H_{22}N_2O_6$) C, H, N.

Octyl 2,6-Dimethyl-3-carbethoxy-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate (22). A solution of 1 (5 g, 0.02 mol) and **20** (4.0 g, 0.02 mol) in 50 mL of ethanol was heated to reflux with stirring for 24 h. The ethanol was removed in vacuo, and the product was purified by column chromatography on silica gel with hexanes–ethyl acetate (5:1) as the eluent. Crystallization from ethyl acetate–hexanes afforded **22** as a yellow waxy solid in a yield of 1.77 g (19.3%): mp 70–72 °C; NMR δ 0.9–1.6 (m, 18 H), 2.4 (s, 6 H), 3.8–4.3 (m, 4 H), 5.1 (s, 1 H), 5.8 (br s, 1 H), 7.2–8.1 (m, 4 H); IR (KBr) 3330, 1695 cm^{-1}. Anal. ($C_{25}H_{34}N_2O_6$) C, H, N.

Pharmacology. Inhibition of [^{3}H]Nitrendipine Binding. The ability of the compounds to block voltage-dependent Ca^{2+} channels in smooth muscle was evaluated by inhibition of specific [^{3}H]nitrendipine binding as previously described by Bolger and co-workers.[16] Briefly, terminal ileum from male guinea pigs was removed and placed in ungassed physiologic saline at 37 °C of the following composition (mM): NaCl, 137; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.36; $NaHCO_3$, 11.9; dextrose 5.55. The longitudinal muscle layer was removed, and the isolated strips were placed in ice cold 50 mM Tris·HCl buffer, pH 7.2, at 4 °C, minced, and homogenized in 10–20 volumes/wet weight tissue in ice-cold Tris buffer. The homogenate was then centrifuged at 4 °C at 11000g for 20 min, and the supernatant was centrifuged at 10000g for 10 min and then recentrifuged at 45000g for 45 min. The pellet from the 45000g spin was used for the binding studies. Protein concentration determination was by the method of Bradford.[21]

Membrane protein (20–80 μg) was incubated in a total volume of 5 mL of 50 mM Tris·HCl (pH 7.2) for 60 min at 25 °C with 0.1 nM [^{3}H]nitrendipine (2,6-dimethyl-3-ethoxy-5-[^{3}H]methoxy-4-(3-nitrophenyl)-1,4-dihydropyridinedicarboxylate, specific activity 80 Ci/mmol) (New England Nuclear, Boston, MA), and varying concentrations of specific compounds (where indicated). Equilibrium binding is reached in this period.[14] Nonspecific binding is defined in duplicate tubes by the addition of unlabeled 10^{-7} M nitrendipine. Binding experiments were performed in subdued light. Analyses of data employed standard programs[22] implemented on an IBM computer.

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Registry No. 1, 39562-16-8; **2**, 15802-69-4; **3**, 113567-97-8; **4**, 113567-98-9; **5**, 113567-99-0; **6**, 113568-00-6; **7**, 113568-01-7; **8**, 113568-02-8; (±)-**9**, 113568-03-9; **9**, 113568-12-0; (±)-**10**, 113568-04-0; **10**, 113568-13-1; (±)-**11**, 113568-05-1; **11**, 113568-14-2; (±)-**12**, 113568-06-2; **12**, 113568-15-3; (±)-**13**, 113568-07-3; **13**, 113568-16-4; (±)-**14**, 113568-08-4; **14**, 113568-17-5; **15**, 33736-01-5; **16**, 113568-09-5; **17** (isomer 1), 113568-10-8; **17** (isomer 2), 113584-19-3; **18**, 113568-11-9; **19**, 16436-00-3; **20**, 27618-18-4; **21**, 21829-28-7; **22**, 88284-24-6; $H_3CCOCH_2CO_2Et$, 141-97-9; $H_3CCOCH_2CO_2Me$, 105-45-3; $HO(CH_2)_2OH$, 107-21-1; $HO(CH_2)_4OH$, 110-63-4; $HO(CH_2)_6OH$, 629-11-8; $HO(CH_2)_8OH$, 629-41-4; $HO(CH_2)_{10}OH$, 112-47-0; $HO(CH_2)_{12}OH$, 5675-51-4; $H_3C(CH_2)_7OH$, 111-87-5; diketene, 674-82-8; ethyl aminocrotonate, 7318-00-5; 3-nitrobenzaldehyde, 99-61-6; 4-nitrobenzaldehyde, 555-16-8.

(21) Bradford, M. *Anal. Biochem.* **1976**, *72*, 248.
(22) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations*; Springer-Verlag: New York, 1981.

Decomposition of *N*-(2-Chloroethyl)-*N*-nitrosocarbamoyl Amino Acid Amides

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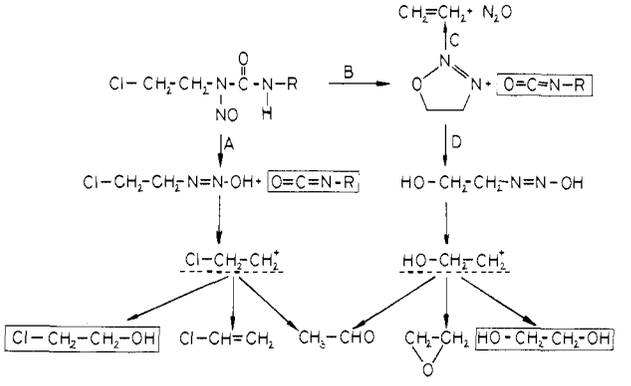
The chemical decomposition of *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl (Q(NO)) prolinamide and valinamide were studied under physiological conditions. The volatile products were identified with GC. Q(NO)-Pro-NH_2 gave twice the amount of ethylene glycol and only one-fifth of the 2-chloroethanol produced by Q(NO)-Val-NH_2 or BCNU, pointing to different pathways of their decomposition. The carbamoylating activity was also investigated in the presence of cyclohexylamine, and it was found to lead mainly to intramolecular carbamoylation with the formation of hydantoin derivatives.

The (2-chloroethyl)nitrosoureas (CENU-s) are highly active antitumor agents, and several of them are in clinical use as well. These compounds decompose rapidly under physiological conditions, producing alkylating and carbamoylating moieties (for a review, see ref 1).

The chemistry of BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] was perhaps the most thoroughly examined.[2-4] Brundrett has shown that BCNU decomposes at pH 7.4 through 2-chloroethanediazohydroxide and yields 2-chloroethanol and acetaldehyde as major products (Scheme I, path A), while at pH 5 it decomposes through 4,5-dihydro-1,2,3-oxadiazole with ethylene glycol and acetaldehyde as major products (Scheme I, paths B, D).[5] Previously, Colvin et al. observed that dMCNU (1-(2-chloroethyl)-3,3-dimethyl-1-nitrosourea), lacking in vitro antitumor activity, has a much higher half-life in aqueous solution at pH 7.4 than the other CENU-s, and its decomposition does not yield chloroethanol, but mainly acetaldehyde.[3] These differences were thought to be due to

Scheme I. Decomposition of (2-Chloroethyl)nitrosoureas in Aqueous Solution



the chemical structure of the molecule having a tertiary nitrogen atom in the N3 position of the urea, while other

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(1) Weinkam, R. J.; Lin, H.-S. *Adv. Pharmacol. Chemother.* **1982**, *19*, 1.

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