The recent thermodynamic examination of the cholinergic receptors by Belleau[35] gives considerable testimony to the conceptual trap that may develop when the most common drug functionality is used as a basis for depicting drug–receptor molecularity. His suggestion is that many years of study, concerned with simple quaternary salts, has given an inaccurate visualization of the cholinergic receptor, and that it is only possible to generate a precise visualization when potent, structurally pertinent compounds are studied.

The diminished activity of compounds from the *para* series, in relation to corresponding structures from the *meta* series, poses a difficulty in rationalizing these observed facts with current concepts of α- and β-adrenergic activations.[36–38]

From the *meta* series, compounds **41** and **45** possess the small amine moiety in conjunction with a pseudocatechol system, which satisfy, structurally and biologically, α-receptor agonist concepts. Likewise, **49** with a bulkier nitrogen substituent and the pseudocatechol ring system, meets the rather vague, residual definitions for β-receptor agonists.

In contrast, the corresponding compounds from the *para* series, **76–78**, have analogous substitutions on the nitrogen atom, but differ only by encompassing an isomeric pseudo-catechol system. Since we have no evidence, either *in vitro* or *in vivo*, that compounds of the *para* series are altered, destroyed, or transported differently from those of the *meta* series, we can only conclude that they are "bound" differently.

This constrains us to the conclusion that, when present, the catechol structure, or its equivalent, plays a role energetically superior to the amino function. It must then follow, that nonphenolic or monophenolic phenethanolamines can not chemically relate to the same total receptor functionalities as the catecholamines or their equivalents reported here. Hence, great care must be exercised when such partial structures are substrates for catecholamine-receptor theories.

These suggestions cannot be construed to mean that the amine portion of a catecholamine is unimportant. For significant sympathomimetic action, the literature gives considerable silent testimony to the fact that the amine must bear at least one hydrogen atom, other than the one arising from any potential protonation process. In this work, the only tertiary amine reported is **75**, in which the amino nitrogen is part of a piperidine ring. It is somewhat discomforting to rationalize away the relative inactivity of this compound on the basis of rather vague steric effects.

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(36) B. Belleau in "Adrenergic Mechanisms," J. R. Vane, G. E. W. Wolstenholme, and M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1960, pp 223–244.

(37) E. J. Ariëns and A. M. Simonis, *J. Pharm. Pharmacol.*, **16**, 137 (1964).

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Studies on Latent Derivatives of Aminoethanethiols as Potentially Selective Cytoprotectants. VI. Synthesis of N-(2-Mercaptoethyl)carbamoylamino Acids[1]

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The N-(2-mercaptoethyl)carbamoyl derivatives of a series of amino acids were synthesized by reaction of 2-thiazolidinone with the sodium salts of β-alanine, glycine, L-leucine, L-alanine, L-methionine, and L-aspartic acid. Attempts to prepare the acids by hydrolysis of the corresponding ethyl esters, obtained by condensation of cysteamine with the amino acid ester isocyanates, were unsuccessful and led only to formation of hydantoins. When administered to tumor-bearing rats, the glycine and alanine derivatives released small but significant levels of cysteamine in three tissues. With four others studied, the derivatives of phenylalanine, methionine, aspartic acid, and β-alanine, little or no cysteamine was found in any of the 15 tissues assayed.

The effectiveness of either radiation or alkylating agents in cancer chemotherapy might be increased significantly by delivery of cytoprotectants as 2-mercaptoethylamine[2] selectively to normal tissues most susceptible to damage, particularly intestinal epithelium and bone marrow. Larger doses of nitrogen mustards or radiation than are normally safely tolerated might then be administered.

One possible manner of such selective delivery would be by administration of 2-mercaptoethylamine as a "latent" chemical derivative from which it could be released enzymatically or otherwise at intracellular sites in the critical tissues. This paper describes the synthesis and properties of a series of N-(2-mercaptoethyl)carbamoylamino acids as possible derivatives of cysteamine of this type.

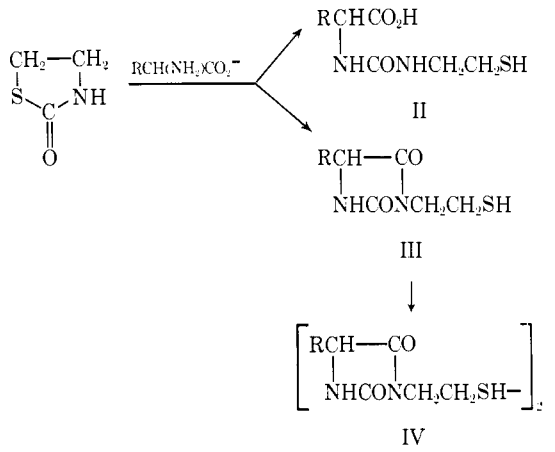
The mercaptoethylcarbamoyl derivative (II) of β-alanine, glycine, L-leucine, L-phenylalanine, L-glutamic acid, L-alanine, L-methionine, and L-aspartic acid were ultimately prepared by an entirely new direct route which consisted of heating a mixture of 2-thiazolidinone[3]

(1) Supported by a Cancer Chemotherapy National Service Center research contract (PH43-62-170) from the National Cancer Institute, National Institutes of Health, Bethesda, Md. Previous paper in this series, K. Pollack and O. M. Friedman, *J. Med. Chem.*, **9**, 622 (1966).

(2) For a discussion and other references, see S. F. Contractor, *Biochem. Pharmacol.*, **12**, 821 (1963).

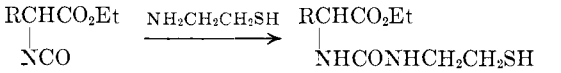
(3) J. C. Michels and G. Gever, *J. Am. Chem. Soc.*, **78**, 5349 (1956).

and the sodium salts of the appropriate amino acids to 130–170°. Leucyl hydantoin mercaptan (III) precipitated in moderate yields from the aqueous extracts of the reaction mixtures. In all other cases only very small amounts of the corresponding hydantoin mercaptans were obtained. Upon acidification and elimination of H_2O, followed by extractions of the residues with alcohol and $CHCl_3$, the filtrates gave the desired products (II), which were identified by their infrared spectra, chemical properties, and elemental analyses. In some cases, conversion to the cyclohexylamine salts was necessary to obtain analytically pure samples.



Leucyl hydantoin mercaptan (III) gave, upon oxidation with 3% hydrogen peroxide, the corresponding high-melting hydantoin disulfide IV, identified by the infrared spectra and analytical data.

Earlier, the esters I of the mercaptoethylcarbamoyl derivatives of a series of eight amino acids were prepared by reaction of the corresponding isocyanates[4] with 2-mercaptoethylamine. The esters I were isolated in good yields as oils difficult to purify. Attempts to hydrolyze these esters (I) to the corresponding acids II were unsuccessful. The only identifiable products isolated under mild alkaline conditions were the hydantoin disulfides IV of leucine and methionine, and the hydantoin mercaptan III of leucine (identified as the disulfide) under acid conditions.



Biological Studies.—In our initial screen for biological activity these compounds were administered to Sprague–Dawley rats bearing the Walker 256 tumor. After 10 min the treated animals and appropriate controls were sacrificed and 15 tissues, including intestine, bone marrow, and tumor, were assayed for cysteamine.[5] The methodology is described in detail in a previous paper[6] in this series.

Of the six compounds studied to date the glycine derivative (II, R = H) and the L-alanine derivative (II, R = CH_3) gave evidence of significant release of cysteamine in three tissues; and interestingly both derivatives produced small but significant concentration of cysteamine (0.3 and 0.5 mμmole/mg of tissue, respectively) in intestine and none in tumor. With the derivatives of the four other amino acids, L-phenylalanine (II, R = $C_6H_5CH_2$), L-methionine (II, R = $CH_3SCH_2CH_2$), L-aspartic acid (II, R = $HOOCCH_2$), and β-alanine, little or no cysteamine was found in the tissues of normal Sprague–Dawley rats to which they were administered.[7]

It is of interest that neither of the two active compounds nor the six others were hydrolyzed to cysteamine when tested with rat kidney homogenate[7] with demonstrable acylase activity.[8] Presumably the tissues in which cysteamine was found—intestine, stomach, spleen, and thymus—contain an enzyme(s) other than acylase capable of hydrolyzing the particular β-mercaptoethylcarbamylamino acid.

Experimental Section[9]

N-(2-Mercaptoethyl)carbamoylamino Acid Ethyl Esters (I).—The esters I of L-leucine, L-methionine, L-phenylalanine, L-aspartic acid, L-glutamic acid, β-alanine, DL-β-aminobutyric acid, and β-aminoisobutyric acid were obtained as impure oils. They were characterized by infrared spectra ($CHCl_3$) which showed typical ester and ureido >C=O and SH (weak) at 5.75, 5.98, and 3.9 μ, respectively; sulfhydryl content determined by titration with I_2 in ethanol corresponded with theory; and oxidation with 3% H_2O_2 to the disulfide gave a product lacking the 3.9-μ SH peak but with an otherwise identical infrared spectrum. We were unable to purify these compounds satisfactorily. The preparation of **N-(2-mercaptoethyl)carbamoyl-L-phenylalanine ethyl ester** is described as a representative example. 2-Mercaptoethylamine hydrochloride (Aldrich) (2.27 g, 0.02 mole) and 1.68 g (0.02 mole) of $NaHCO_3$ were dissolved in H_2O (10 ml), and 4.4 g (0.02 mole) of L-phenylalanine ethyl ester isocyanate, prepared by the procedure of Goldschmidt and Wick,[4] in 10 ml of acetone was added slowly. Vigorous evolution of CO_2 resulted. The H_2O–acetone solvent was adjusted so that complete solution resulted. The mixture was left standing for 20 min, evaporated on the vacuum pump until all the acetone was removed, and extracted with two 30-ml portions of $CHCl_3$. The $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated, leaving 5.8 g (90% yield) of ester as a yellow oil. The infrared spectrum ($CHCl_3$) includes bands at 3.90 (w), 5.75 (s), and 5.98 (s) μ.

Anal. Calcd for $C_{14}H_{20}N_2O_3S$: C, 56.7; H, 6.7; N, 9.4. Found: C, 55.2; H, 6.6; N, 9.2.

The **disulfide** was obtained by leaving a solution of 1 g of the mercaptan I to stand in 25 ml of 3% H_2O_2 and 20 ml of ethanol at room temperature overnight, evaporating the ethanol under vacuum, followed by extracting ($CHCl_3$) and evaporating the dried $CHCl_3$ extracts. The infrared spectrum ($CHCl_3$) was identical with I, except for the loss of SH absorption at 3.9 μ.

Base Hydrolysis of N-(2-Mercaptoethyl)carbamoyl-L-methionine Ethyl Ester.—The methionine derivative I (R = $CH_3SCH_2CH_2$) (1.1 g) was allowed to stir for 15 hr at room temperature in a solution of 15 ml of 1 *N* KOH and 20 ml of methanol. Colorless crystals slowly precipitated from the solution which increased in yield on removal of the methanol. They were filtered and found to be insoluble in dilute or concentrated HCl and soluble only in 20% KOH at 100°. The infrared spectrum (KBr) lacked the carbonyl, carbamoyl, and mercaptan bands characteristic of I, but had two distinct new carbonyl bands at 5.62 and 5.83 μ characteristic of imides and hydantoins.[10] The filtrate was acidified with Amberlite[11] cation-exchange resin to pH 3 and evaporated to dryness under vacuum. The colorless oil resulting could not be recrystallized or identified. The crystalline solid identified as the hydantoin disulfide IV (R = $CH_3SCH_2CH_2$), mp 118–120°, was obtained in 32% yield.

(4) S. Goldschmidt and M. Wick, *Ann.*, **575**, 217 (1952).

(5) K. A. Herrington, K. Pointer, A. Meister, and O. M. Friedman, *Cancer Res.*, **27**, 130 (1967).

(6) K. A. Herrington, C. J. Small, A. Meister, and O. M. Friedman, *ibid.*, **27**, 148 (1967).

(7) K. A. Herrington and O. M. Friedman, unpublished results.

(8) S. C. J. Fu and A. M. Birnbaum, *J. Am. Chem. Soc.*, **75**, 918 (1953).

(9) Analyses by Dr. S. Nagy, Belmont, Mass., and Dr. K. Fitz, Needham, Mass. Infrared spectra were obtained in a Perkin-Elmer Infracord.

(10) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, p 221.

(11) Amberlite IR-120 (Mallinckrodt), a strongly acidic, sulfonated polystyrene cation-exchange resin.

TABLE I
N-(2-MERCAPTOETHYL)CARBAMOYLAMINO ACIDS (II)

RCHCOOH
|
NHCONHCH$_2$CH$_2$SH

R	% yield	Mp, °C	Ir spectra (max), μ	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
H		121–123	5.9, 6.15, 6.35	$C_5H_{10}N_2O_3S$			15.7	18.0			15.6 15.5	18.2 18.3
CH_2CH_2COOH	82	124–125	5.9, 6.15, 6.35	$C_6H_{11}N_2O_3S$	37.5	6.2	14.6	16.6	37.6	6.1	14.5	16.8
L-CH_2CH_2COOH	30	112–115	5.9, 6.25, 7.2	$C_8H_{14}N_2O_5S$	38.4	5.6	11.9	13.5	38.2	5.7	11.7	13.1
L-$CH_2CH(CH_3)_2$	20	139–141	6.5, 7.2	$C_9H_{18}N_2O_3S \cdot C_6H_{11}NH_2$	54.05	9.3	12.6	9.7	54.2	9.0	12.3	9.5
L-$CH_2C_6H_5$	22	137–140	6.15, 6.5, 6.9, 7.15	$C_{12}H_{16}N_2O_3S \cdot C_6H_{11}NH_2$	59.0	7.9	11.4	8.7	59.2	8.0	11.5	9.0
L-CH_3	41	95.5–98.5	6.15, 6.4, 6.9, 7.1	$C_6H_{12}N_2O_3S$	37.5	6.25			37.3 37.4	6.4 6.3		
L-$CH_2CH_2SCH_3$	15.6	89–92	6.5, 7.25	$C_8H_{16}N_2O_3S$	47.9	8.3			47.9	8.4		
L-CH_2COOH	2.3	124–128	6.45, 6.9, 7.2	$C_7H_{12}N_2O_5S \cdot 2C_6H_{11}NH_2$	52.5	8.9			52.7 52.4	9.2 9.2		

Anal. Calcd for $C_{16}H_{26}N_4O_4S_4$: C, 41.2; H, 5.6; N, 12.0; S, 27.5. Found: C, 41.2; H, 5.7; N, 12.1; S, 27.6.

1,2-[1-(4-Isobutylhydantoin)]ethyl disulfide (IV) was obtained from N-(2-mercaptoethyl)carbamoyl L-leucine ethyl ester I [R = $(CH_3)_2CHCH_2$] in 24% yield in an analogous manner, mp 172–175°.

Anal. Calcd for $C_{18}H_{30}N_4O_4S_2$: C, 50.2; H, 7.0; N, 13.0; S, 14.9. Found: C, 50.0; H, 6.9; N, 12.8; S, 14.7.

Acid Hydrolysis of N-(2-Mercaptoethyl)carbamoyl-L-leucine Ethyl Ester.—The ester (1 g) was dissolved in 10 ml of glacial acetic acid and 10 ml of concentrated HCl, and the mixture was heated to 80° for 30 min. The solvents were evaporated to dryness under vacuum. A colorless oil resulted which slowly crystallized to a solid, mp 41–43°. The infrared spectrum showed the hydantoin structure with bands at 5.62 and 5.85 μ, as well as an SH band at 3.9 μ. The compound was dissolved in 10 ml of 3% H_2O_2. Colorless crystals soon appeared which were filtered and dried, mp 180–183°, identified as 1,2-[1-(4-isobutylhydantoin)]ethyl disulfide by infrared spectra and mixture melting point. The product of acid hydrolysis was therefore the hydantoin mercaptan III [R = $(CH_3)_2CHCH_2$].

N-(2-Mercaptoethyl)carbamoylamino Acids.—The preparation of the glycine derivative is described as a representative example. The other seven compounds for which data are given in Table I were prepared by essentially the same procedure.

N-(2-Mercaptoethyl)carbamoylglycine (II, R = H).—Glycine (0.75 g, 0.01 mole) and 1.03 g (0.01 mole) of 2-thiazolidinone, prepared by the procedure of Michels and Gever,[3] were dissolved in 2 ml of 1 *N* NaOH (0.02 mole). The solvent was removed under vacuum, and the residue was heated to 140° for 35 min, dissolved in 10 ml of water, and acidified to pH 3 with Amberlite[11] cation-exchange resin. On gradual removal of solvent, crystals deposited. A total of 1.55 g was collected in four crops, mp 121–123°. The infrared spectrum (KBr) included bands at 2.95–3.45 (m, broad), 5.9 (s), 6.15 (s), 6.35 (s), 7.2 (m), 7.8 (m), 8.2 (s), 9.4 (w), 10.8 (w) μ.

Anal. Calcd for $C_5H_{10}N_2O_3S$: N, 15.7; S, 18.0. Found: N, 15.6, 15.5; S, 18.2, 18.32.

1,2-[1-(4-Isobutylhydantoin)]ethyl Mercaptan [III, R = $(CH_3)_2CHCH_2$].—L-Leucine (5.26 g) was heated with 8.26 g of 2-thiazolidinone and the reaction mixture was extracted with water in a fashion as described above. The separated hydantoin was collected on a filter, washed with water, and air dried to yield 1.13 g (13.1%) of mercaptan hydantoin, mp 87–89.5°. The crude hydantoin was dissolved in $CHCl_3$ and treated with charcoal. Evaporation gave a white crystalline material which was collected with ether to yield the pure product. The infrared spectrum ($CHCl_3$) included bands at 5.65 (w), 5.85 (s), 6.9 (m), 7.05 (m), 7.5 (w), 8.35 (w) μ.

Anal. Calcd for $C_9H_{16}N_2O_2S$: C, 50.0; H, 7.4. Found: C, 50.32; H, 7.32.

1,2-[1-(4-Isobutylhydantoin)]ethyl Disulfide [IV, R = $(CH_3)_2CHCH_2$].—Mercaptan hydantoin (0.5 g) was dissolved in 10 ml of methanol and added to a stirred solution of an excess of 3% H_2O_2. The mixture was stirred overnight at room temperature, the solvents were removed under reduced pressure, and the residue was extracted three times ($CHCl_3$). The combined extracts were dried (Na_2SO_4) and the solvents were removed to give a white solid (0.35 g, 70%), mp 169–172°. The infrared spectrum ($CHCl_3$) included bands at 3.5 (w), 4.3 (w), 5.65 (w), 5.85 (s), 6.9 (m), 7.05 (m), 7.5 (w), 8.35 (w) μ.

Anal. Calcd for $C_{18}H_{30}N_4O_4S_2$: C, 50.2; H, 7.0. Found: C, 49.4; H, 7.1.

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