Angiotensin-Converting Enzyme Inhibitors: Importance of the Amide Carbonyl of Mercaptoacyl Amino Acids for Hydrogen Bonding to the Enzyme[1]

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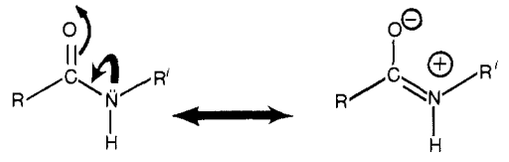
A series of mercaptoacyl amino acids and related compounds was synthesized and evaluated for inhibition of angiotensin-converting enzyme (ACE) in order to determine the nature and importance of the putative interaction between ACE and the amide moiety of inhibitors such as captopril (3-mercapto-2-methylpropanoyl-L-proline). It was concluded that the interaction involves a hydrogen bond from a donor site on ACE to the oxygen of the amide carbonyl. Compounds in which the amide moiety is replaced by other groups (ester, ketone, sulfonamide) capable of accepting a hydrogen bond are effective inhibitors, but compounds in which only the geometrical features of the amide are retained are ineffective inhibitors. The presence of an NH group is not necessary for effective inhibition. The activity of a series of mercaptoacyl cycloalkyl carboxylic acids parallels the activity of the isosteric series of mercaptoacyl imino acids.

In our recently proposed model for the active site of angiotensin-converting enzyme (ACE),[2,3] one of the key interactions between substrates (**1**) and inhibitors (**2** and **3**) and the enzyme was postulated to involve the C-terminal amide bond of substrates and inhibitors. This was assumed to involve a hydrogen bond between the oxygen of the amide carbonyl and a proton donor provided by the enzyme, as depicted in Figure 1.

In view of the electronic and geometric features of the amide bond, represented by classical resonance structures **4** and **5**, this interaction might result from a combination

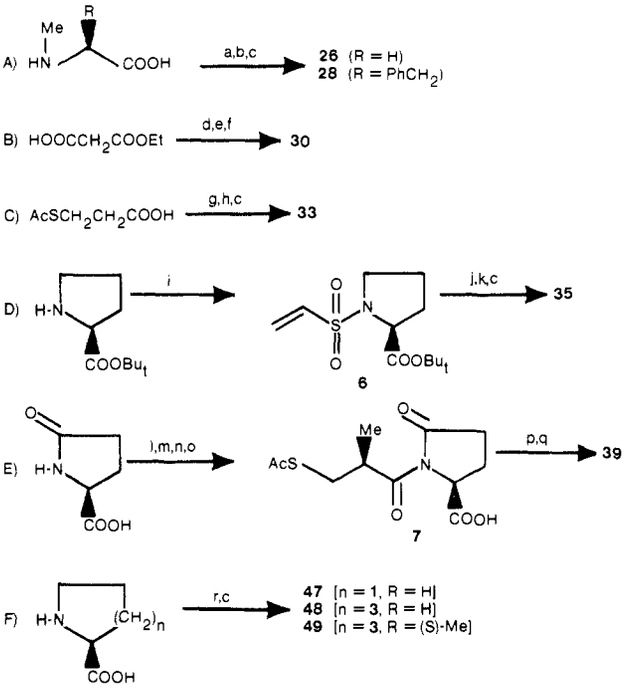


4 **5**

of effects. One must consider not only the hydrogen-bonding capabilities of the oxygen acceptor and the nitrogen donor but also the planarity and partial double-bond character of the carbon–nitrogen bond. The analogues in Tables I and II were synthesized and tested for ACE inhibitor activity in order to assess the relative importance of such factors in the interaction of the C-terminal amide of substrates and inhibitors with ACE.

Chemistry. Mercaptoacyl amino acids **24**, **27**, **36**, and **40**, as well as acid **25**, have been reported previously.[2] Various techniques were utilized in the synthesis of the amides and esters listed in Tables I and II. The methyl amides **26** and **28** were prepared by Schotten–Baumann acylations (Scheme I, A) and subsequent introduction of the thiol function. Amide **30** and ester **33** were obtained via sequences involving *N,N'*-dicyclohexylcarbodiimide-1-hydroxybenzotriazole[4] (Scheme I, B) and *N,N'*-carbonyldiimidazole[5] (Scheme I, C) mediated coupling reactions, respectively. Reaction of proline *tert*-butyl ester with 2-chloroethylsulfonyl chloride afforded vinylsulfonylproline *tert*-butyl ester **6** (Scheme I, D), which on conjugate addition of thiolacetic acid and deprotection afforded sulfonamide **35**. The base-labile analogue **39** was prepared by aprotic acylation[6a] of L-pyroglutamic acid with

Scheme I. Miscellaneous N- and O-Acylation Routes[a]



[a] Reagents: a, $BrCH_2CH_2COCl$; b, $PhCOS^-K^+$; c, aqueous NH_3; d, DCC-HOBt; e, $HSCH_2CH_2NH_2$; f, aqueous NaOH; g, CDI; H, L-(-)-3-phenyllactic acid; i, $ClCH_2CH_2SO_2Cl$; j, MeCOSH; k, TFA-anisole; l, BSTFA-propylene oxide-molecular sieves; m, $AcSCH_2CH(CH_3)COCl$; n, DCHA salt; o, 10% aqueous $KHSO_4$; p, $Hg(OCOCF_3)_2$; q, H_2S; r, $AcSCH_2CH(R)COCl$.

racemic 3-(acetylthio)-2-methylpropanoyl chloride, separation of the desired, *S,S* diastereomer[6b] **7** (Scheme I, E) via salt formation, and nonhydrolytic thiol unmasking using mercuric trifluoroacetate.[7] Amides **47–49** were prepared by Schotten–Baumann acylations, followed by thiol deprotection (Scheme I, F).

Amino acid **37** was prepared by alkylation of proline *tert*-butyl ester with 1,3-dibromopropane, bromide displacement with sodium thioacetate, and complete deprotection of the resulting intermediate **8** (Scheme II, A).

(1) Presented in part at the ACS/CJC Chemical Congress, Honolulu, HI, Apr 1–6, 1979, and in part at the ASBC/AAI Annual Meeting, Atlanta, GA, June 4–8, 1978.
(2) M. A. Ondetti, B. Rubin, and D. W. Cushman, *Science*, **196**, 441 (1977).
(3) D. W. Cushman, H. S. Cheung, E. F. Sabo, and M. A. Ondetti, *Biochemistry*, **16**, 5484 (1977).
(4) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(5) H. A. Staab and A. Mannschreck, *Chem. Ber.*, **95**, 1284 (1962).
(6) (a) H. R. Kricheldorf, *Liebigs Ann. Chem.*, **763**, 17 (1972). (b) X-Ray analysis of the structures of captopril (**40**) and **39**, as well as of other analogues of captopril, confirm the assignment of the *S,S* stereochemistry to the more levorotatory diastereomer: J. Z. Gougoutas, B. Toeplitz, and M. Malley, manuscript in preparation.
(7) O. Nishimura, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.* **26**, 1576 (1978).

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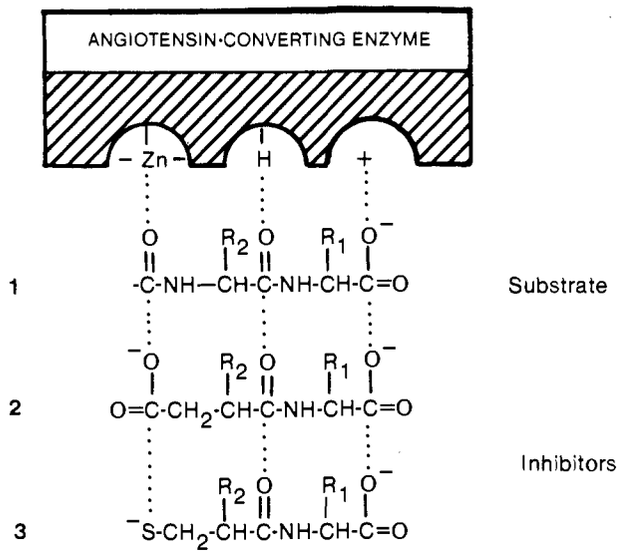
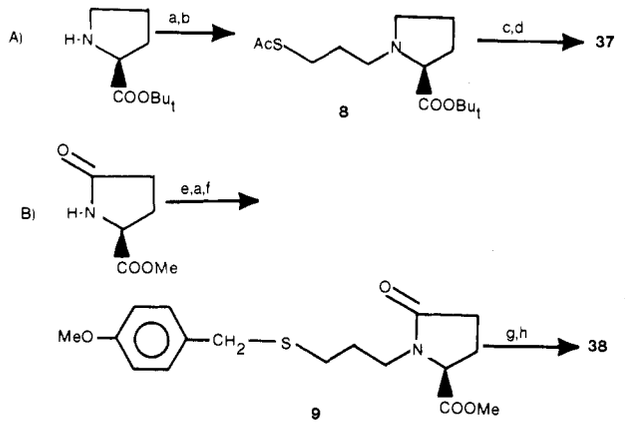


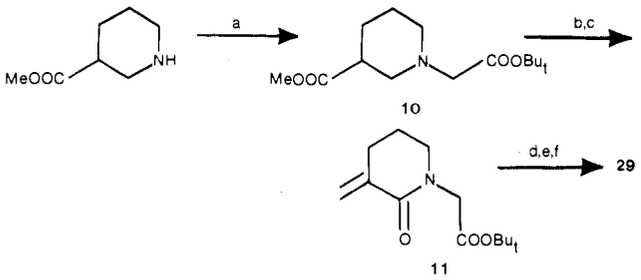
Figure 1. Model for the active site of ACE.

Scheme II. N-Alkylation Routes[a]



[a] Reagents: a, $Br(CH_2)_3Br$; b, $MeCOS^-Na^+$; c, TFA-anisole; d, aqueous NH_3; e, NaH, f, *p*-$MeOC_6H_4CH_2SH$-Na NaOMe; g, aqueous NaOH; h, CF_3SO_3H-TFA.

Scheme III. Synthesis of Amide **29** *via* α-Methylene Lactam Rearrangement[a]

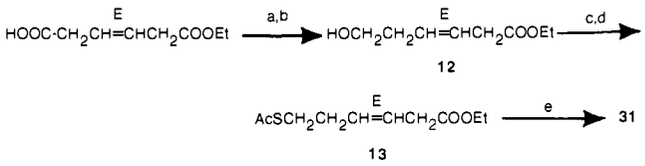


[a] Reagents: a, $ClCH_2COO$-*t*-Bu-K_2CO_3; b, NaOH; Ac_2O; d, MeCOSH; e, TFA-anisole; f, aqueous NH_3.

Amide **38** was similarly prepared (Scheme II, B) from methyl L-pyroglutamate via a sequence involving protected intermediate **9**. In this case, the *p*-methoxybenzyl group was removed with trifluoromethanesulfonic acid.[8]

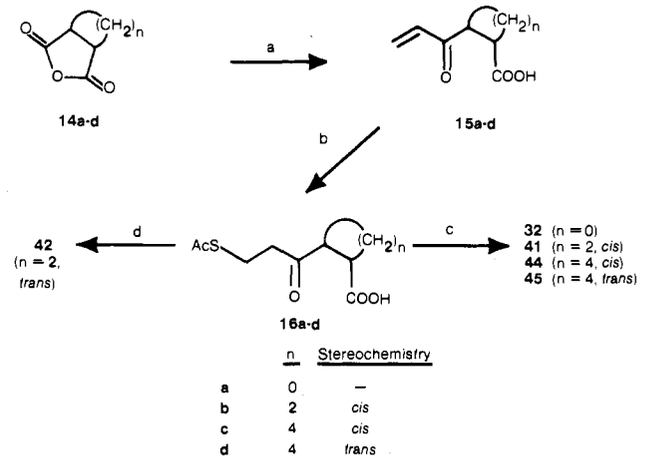
Analogue **29** was prepared as depicted in Scheme III from methyl nipecotate. The corresponding *N*-alkyl derivative **10** was converted via α-methylene lactam rearrangement[9] to lactam **11**, the key intermediate in the synthesis of **29**.

Scheme IV. Synthesis of Olefin **31**[a]



[a] Reagents: a, *p*-nitrophenol-DCC; b, $NaBH_4$-glyme; c, TsCl-Et_3N-CH_2Cl_2; d, MeCOSH-Et_3N; e, 2 N HCl.

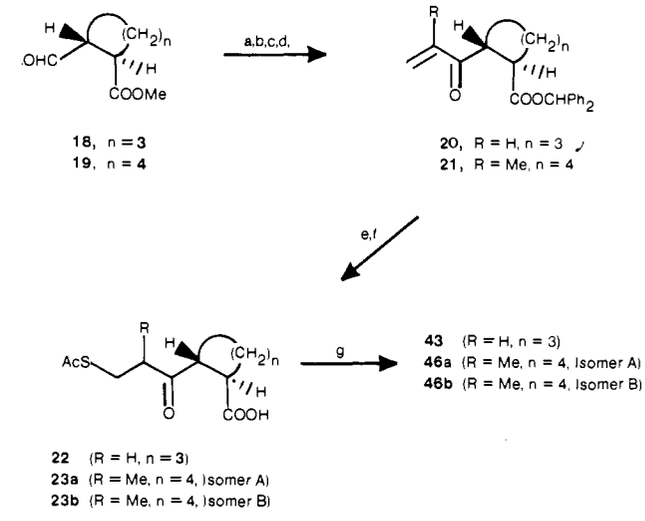
Scheme V. Friedel-Crafts Route to Ketones[a]



	n	Stereochemistry
a	0	–
b	2	*cis*
c	4	*cis*
d	4	*trans*

[a] Reagents: a, $H_2C{=}CH_2$-$AlCl_3$-$ClCH_2CH_2Cl$; b, MeCOSH; c, aqueous NH_3; d, NaOMe-MeOH.

Scheme VI. Grignard Route to Ketones[a]



[a] Reagents: a, $H_2C{=}C(R)$-MgBr; b, NaOH; c, Ph_2CN_2; d, oxidation; e, MeCOSH; f, TFA-anisole; g, aqueous NH_3.

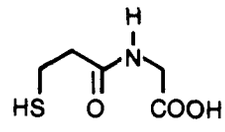
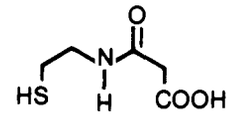
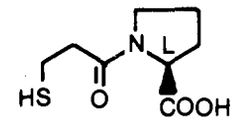
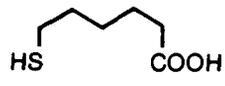
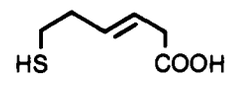
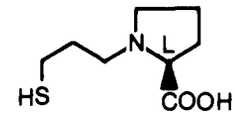
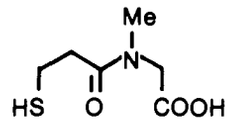
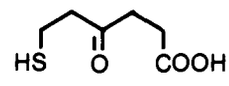
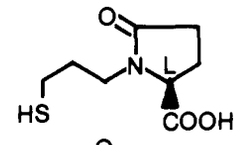
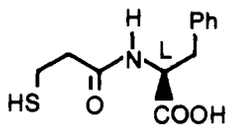
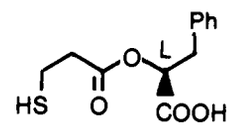
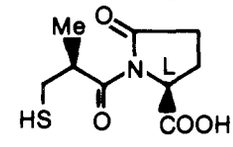
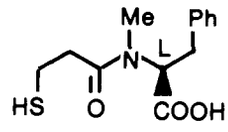
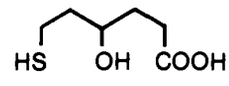
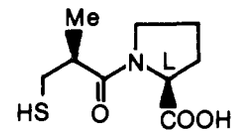
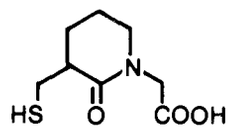
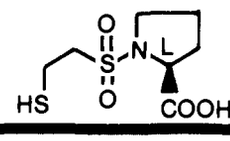
Olefinic acid **31** was prepared from 5-(ethoxycarbonyl)-3-pentenoic acid (Scheme IV). Reduction of the *p*-nitrophenyl ester with $NaBH_4$ yielded alcohol **12**, which was converted via **13** to analogue **31**.

The ketones listed in Tables I and II were prepared by the methods depicted in Schemes V and VI. All but one of the ketones were prepared by Michael addition of thiolacetic acid to enones **15a–d** (Scheme V), **20** and **21**

(8) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, 107 (1974).

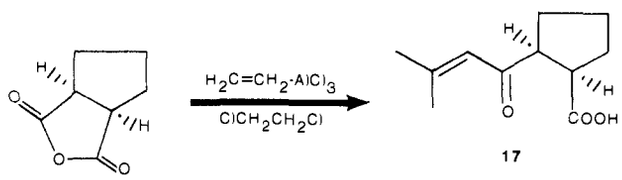
(9) (a) D. Lee, C. J. Morrow, and H. Rapoport, *J. Org. Chem.*, **39**, 893 (1974). (b) D. L. Lee and H. Rapoport, *J. Org. Chem.*, **40**, 3491 (1975).

Table I. Inhibition of Angiotensin-Converting Enzyme by Mercaptoacyl Amino Acids and Analogues

no.	structure	I_{50}[a] (μM)	no.	structure	I_{50}[a] (μM)	no.	structure	I_{50}[a] (μM)
24		2.8	**30**		580	**36**		0.25
25		1100	**31**		720	**37**		240
26		3.0	**32**		11	**38**		640
27		0.85	**33**		0.70	**39**		0.009
28		31	**34**[b]		1200	**40**		0.02
29[b]		10	**35**		11			

[a]The I_{50} values were determined by the procedure of D. W. Cushman and H. S. Cheung, *Biochem. Pharmacol.*, 20, 1637 (1971). [b]Racemate.

(Scheme VI), followed by hydrolytic removal of the *S*-acetyl group (Scheme V), with prior removal of the carboxyl-protecting group when necessary (Scheme VI). Ketone **42** was prepared from **16b** by carrying out the hydrolytic removal of the *S*-acetyl group under conditions which epimerized the ketone-bearing side chain. In the case of enone **21**, conjugate addition of the thiolacetic acid produced a pair of diastereomers, which were separated by fractional crystallization after removal of the carboxyl protecting group, yielding acids **23a** and **23b**. The precursor enones **15a–d** (Scheme V) were conveniently obtained by Friedel–Crafts acylation of ethylene with anhydrides **14a–d**. In the case of *cis*-anhydrides, **14b** and **14c**, no isomerization was observed. Unexpectedly, however, *cis*-cyclopentane-1,2-dicarboxylic anhydride did not afford the expected enone but gave a rearrangement product **17**,



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resulting from incorporation of 2 mol of ethylene. The formation of such isobutenyl ketones in Friedel–Crafts acylations of ethylene has been reported previously.[10,11] Consequently, an alternative route to ketone **43** proceeding through enone **20** (Scheme IV) was devised. This same route was employed in the synthesis of enone **21** as depicted.

Alcohol **34** was prepared by $NaBH_4$ reduction of intermediate **16a**.

Results and Discussion

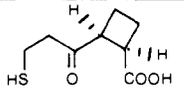
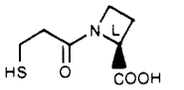
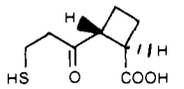
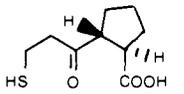
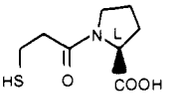
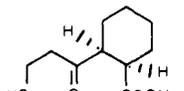
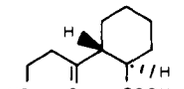
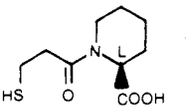
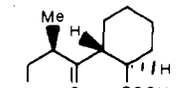
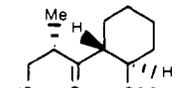
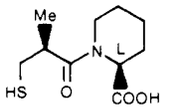
Compounds **24–49** were evaluated for in vitro inhibition of rabbit lung ACE using hippurylhistidylleucine as substrate (Tables I and II).[3] Comparison of the activities of the simple prototype mercaptoacyl amino acid, mercaptopropanoylglycine (**24**, Table I), and mercaptohexanoic acid (**25**) clearly indicates that the amide bond plays a critical role in binding of **24** to ACE. With regard to the nature of the interaction, comparison of the activities of acyclic secondary amides (e.g., **24**) with their tertiary *N*-methyl derivatives (**26**) appears to rule out the possibility that the interaction involves the secondary amide nitrogen in the role of hydrogen donor. Firmer support for this contention is provided by the potent inhibition exhibited by derivatives of cyclic α-imino acids, such as **36** and **40**.

Similar correlations indicate that the conformational restriction and planarity imposed by the amide does not per se play a major role in the binding of inhibitors to ACE. Analogue **30** maintains the conformational restriction im-

(10) T. Matsumoto, K. Hata, and T. Nishida, *J. Org. Chem.*, **23**, 106 (1958).

(11) H. T. Taylor, *J. Chem. Soc.*, 3922 (1958).

Table II. Inhibition of Angiotensin-Converting Enzyme by Mercaptoketo Acids and Their Amide Analogues

no.	ketone[a]	I_{50}[b] (μM)	no.	amide	I_{50}[b] (μM)
41		34	47		1.7
42		37			
43		2.4	36		0.25
44		1.4			
45		1.7	48		0.46
46a[c]		0.74			
46b[c]		3.7	49		0.13

[a] Ketones 41-46 are racemic. [b] See Table I, footnote *a*. [c] Configurational assignments were made on the basis of observed inhibitory potencies.

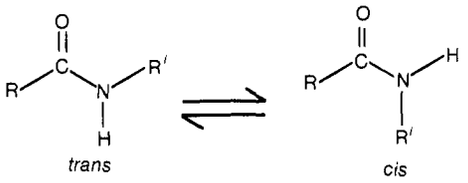
posed by the planar amide moiety of **24** but is 200 times less active. Assuming that the zinc–sulfhydryl and carboxyl interactions are equivalent in the binding of **24** and **30** to ACE, hydrogen-bond acceptor interaction by the amide carbonyl oxygen in **30** is precluded by the interchange of the carbonyl group and the secondary nitrogen. The poor activity of analogue **30**, therefore, probably reflects the negligible role played by the geometric components of planarity and restriction of the amide in its interaction with the enzyme. Further evidence in support of this argument is the comparable 250-fold decrease in activity observed on isosteric replacement of the amide[12] in **24**, presumably in the trans configuration, by a *trans*-olefin (**31**). Although the possibility that the cis conformer of amide **24** binds preferentially to the enzyme cannot be rigorously excluded, it appears to be discounted by the equivalent activities exhibited by the conformationally frozen trans monocyclic analogue **29**[13] and acyclic amide **24**.

A more revealing correlation, which permits a more precise distinction among these factors, is provided by ketone **32** and ester **33**, which possess activities comparable to their amide counterparts **24** and **27**, respectively. Neither of these amide surrogates possesses a secondary nitrogen capable of acting as a donor in a hydrogen bond and neither maintains the conformational restriction of the amide. The structural feature common to the ketone, ester, and amide groups is, of course, the planar carbonyl group, which can function as an acceptor in a hydrogen bond.

The importance of this hydrogen-bonding interaction between inhibitors and ACE is reinforced by inspection of the series of cyclic α-imino acid derivatives **36–40**. Amino acid **37**, which cannot function as a hydrogen bond acceptor, is 1000-times less active than amide **36**. This comparison must be qualified, however, since the fundamental differences in electronic nature (zwitterionic vs. neutral) and geometry (tetrahedral vs. planar) between **37** and **36** cannot be overlooked. Pyroglutamic acid derivative **38**, a regioisomer of **36**, provides a more valid comparison, and its poor activity must be due to its inability to hydrogen bond with the enzyme. The possibility that the 5-oxo group of **38** might interfere with binding to the enzyme, thereby invalidating this comparison, is readily dismissed by the equivalent potencies exhibited by **39** and **40**.

Two other functional groups were evaluated for their ability to act as acceptors in hydrogen bonds with ACE. Both the alcohol **34** and the sulfonamide **35** were found to be considerably less active than corresponding carbonyl analogues. This result may be attributable to a change in hydrogen-bond acceptor characteristics or to the change from trigonal to tetrahedral geometry.

A final consideration regarding the olefinic nature of the amide bond concerns the existence of cis and trans forms.



In the case of *N*-acyl α-amino acid derivatives (i.e., secondary amides), the trans form is energetically favored. *N*-Acyl α-imino acid derivatives (i.e., tertiary amides), however, often contain substantial amounts of the cis conformer, which is observed spectroscopically in solution. Since some of the most potent ACE inhibitors fall into the latter class (e.g., **36**, Table III), it was of interest to attempt to correlate observed inhibitory potencies with relative cis–trans populations as determined by ^{13}C NMR spectroscopy. Comparison of inhibitors **24** and **27** (all trans) with **26** and **28** (cis–trans mixtures), respectively, however, clearly demonstrates that no such correlation exists. The possibility that the enzyme might preferentially bind either the cis or trans form of a given inhibitor cannot be addressed by such a spectroscopic study.

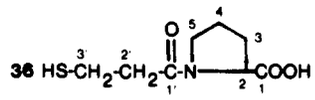
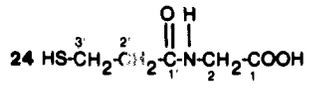
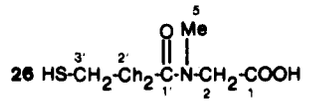
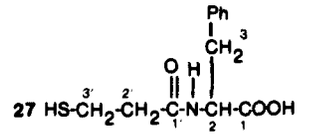
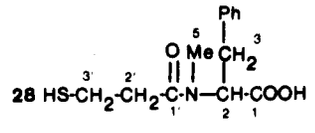
In view of the potent inhibitory activity of the simple keto acid **32**, it was of interest to explore structure–activity relationships in a series of analogues.[14] The cyclopentane- and cyclohexanecarboxylic acid derivatives **43–45** (Table II) were found to be more potent inhibitors than acyclic ketone **32**, an observation which parallels the trend in the amide series. Further enhancement in potency is observed on substitution α to the carbonyl group with an alkyl group of appropriate configuration, i.e., **46a** vs. **46b**. In this instance, the relative configurational assignments for the α-methyl group are based on the observed biological ac-

(12) The isoteric replacement of amide bonds in enkephalins by *trans*-olefins has recently been reported: M. M. Hann and P. G. Sammes, *J. Chem. Soc., Chem. Commun.*, 234 (1980); M. J. Cox, D. W. Heaton, and J. Horbury, *ibid.*, 799 (1980).

(13) This compound has recently been described by S. Klutchko, M. L. Hoefle, R. D. Smith, A. D. Essenburg, R. B. Parker, V. L. Nemeth, M. J. Ryan, D. H. Dugan, and H. R. Kaplan, *J. Med. Chem.*, **24**, 104 (1981).

(14) The racemic carboxylic ketone analogue of amide **40** has recently been described by A. Sugie and J. Katsube, *Chem. Pharm. Bull.*, **27**, 1708 (1979).

Table III. ^{13}C NMR Chemical Shifts[a] of Selected Derivatives[b,c]

			acyl Chain			amino Acid						relative peak heights for C_2	I_{50}
no.	structure	pH[b]	3'	2'	1'	5	4	3	2	1	aromatic	*cis/trans*	(μM)
36		7.2	38.6	20.3 20.2	173.6 172.9	48.7(t) 47.8(c)	24.0(t) 23.4(c)	32.2(c) 30.6(t)	63.6(c) 62.5(t)	178.0		48/52	0.25
24		7.1	40.2	20.8	175.0				44.2	177.4		0/100	2.8
26		7.1	37.5	20.4	175.1 174.7	37.3(t) 35.9(c)			59.4(c) 52.8(t)	177.2 176.8		50/50	3.0
27		7.6	40.5	20.8	174.1			38.6	57.0	178.0	138.8 129.4 130.1 127.6	0/100	0.85
28		7.2	37.8 37.1	20.2	174.6 175.1	33.3(t) 31.1(c)		36.4(c) 35.8(t)	65.8(c) 61.8(t)	178.2 177.2	139.5 129.9 129.7 129.4 127.6 127.3	46/54	31

[a] In parts per million relative to $SiMe_4$. [b] See Experimental Section. [c] Chemical-shift assignments, as well as *cis* (c) and *trans* (t) designations, were made according to C. M. Deber *et al. J. Am. Chem. Soc.*, **96**, 5009 (1974); and D. E. Dorman and F. A. Bovey, *J. Org. Chem.*, 38, 2379 (1973).

tivities and on structure–activity relationships established in the mercaptoacyl amino acid series. Considering the racemic nature of these ketones, their activities closely approach those of the corresponding amides **36**, **48**, and **49**. The somewhat better activity observed with the amides may reflect the fact that ketones mimic but do not exactly reproduce the rigidity and planarity of the amide. In this context it is interesting to note that comparison of **41** and **42**, as well as **44** and **45**, indicates that the stereochemical relationship between vicinal substituents has little effect on activity.

In summary, we conclude that the binding of mercaptoacyl amino acid inhibitors to ACE involves a hydrogen bond between a donor site on the enzyme and the oxygen of the amide carbonyl. Other carbonyl-containing groups, such as esters and ketones, as well as sulfonamides, can substitute for the amide with diminished efficiency but an alcohol cannot. Groups which retain only the geometrical properties of the amide, such as olefins and inverted amides, are clearly inferior substitutes. We suggest that this hydrogen-bonding interaction is equally important in the binding of substrates to ACE. These findings further strengthen our active-site model for ACE and enhance its utility as a heuristic tool for the design of novel inhibitors.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on Varian Associates Models T-60 and XL-100-15 spectrometers in the indicated solvents. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as internal standard, and apparent splittings are given in all cases. ^{13}C NMR studies for measuring comparative cis–trans populations (Table III) were obtained on a Varian Associates XL-100-15 spectrometer at 38 °C. Solutions (0.25 M) for these studies were prepared in 5% $NaHCO_3$–D_2O with dioxane (δ 67.4) as internal standard, and the observed pH (not corrected for deuterium isotope effect) was recorded. All other ^{13}C NMR spectra were measured in deuteriochloroform on a JEOL FX 60-Q Fourier transform spectrometer and are referenced to the central solvent resonance at δ 77 from tetramethylsilane. All ^{13}C NMR shifts are reported relative to tetramethylsilane. Infrared spectra were determined on Perkin-Elmer Models 621 and 21 recording spectrometers. Mass spectra were obtained on an AEI-MS-902 mass spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 10-cm microcell.

Reactions were run at room temperature under argon or nitrogen unless otherwise noted. Standard workup procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were washed in the indicated order using the following aqueous solutions: bicarbonate (saturated $NaHCO_3$), bisulfate (5% $KHSO_4$), hydroxide (3% NaOH), and acid (1 N HCl). Extracts were dried over $MgSO_4$ or Na_2SO_4, filtered, and evaporated in vacuo. Dicyclohexylamine (DCHA) and cyclohexylamine (CHA) salts were prepared in the indicated solvents by addition of a slight excess of amine to the acid. Free acids were liberated from DCHA salts by partition between 5% $KHSO_4$ and EtOAc and standard workup.

Chromatographic separations employed Baker silica gel using the indicated eluants. Analytical and preparative (PLC) thin-layer

chromatography were carried out using E. Merck F-254 silica gel plates.

Scheme I. A. *N*-(3-Mercapto-1-oxopropyl)-*N*-methylglycine (26). 3-Bromopropionyl chloride (8.5 g, 50 mmol) was added to a solution of sarcosine (4.45 g, 50 mmol) in 75 mL of 1.33 N NaOH at 0 °C. The pH was about 7. The ice bath was removed. After 3.5 h at room temperature, a suspension of thiobenzoic acid (7.5 g, 35 mmol) and K_2CO_3 (4.8 g, 35 mmol) in 50 mL of water was added, and the mixture was stirred overnight. The clear solution was acidified and worked up (EtOAc), yielding 14.6 g. Crystallization from Et_2O afforded 7.9 g (56%) of *N*-[3-(benzoylthio)-1-oxopropyl]-*N*-methylglycine, mp 139–140 °C.

Concentrated NH_4OH (4 mL) was added to a suspension of the above acid (2.81 g, 10 mmol) in 5 mL of water, and a clear solution resulted. After 2 h the reaction mixture was diluted with water, filtered, washed with ethyl acetate, acidified, and worked up to yield 1.65 g of residue.

A DCHA salt was prepared (EtOAc): yield 2.73 g; mp 157–158 °C. The free acid was obtained as an oil: TLC R_f 0.65 ($CHCl_3$–MeOH-38% aqueous HOAc, 3:2:1). Anal. ($C_6H_{11}NO_3S \cdot C_{12}H_{19}N$) C, H, N, S.

***N*-(3-Mercapto-1-oxopropyl)-*N*-methyl-L-phenylalanine (28).** This compound was prepared from *N*-methyl-L-phenylalanine by the procedure utilized for the synthesis of **26** and was obtained as an oil: TLC R_f 0.35 (benzene–HOAc, 3:1). Anal. ($C_{13}H_{17}NO_3S$) C, H, N, S.

B. 3-[(2-Mercaptoethyl)amino]-3-oxopropanoic Acid (30). 1-Hydroxybenzotriazole (1.35 g, 10 mmol) was added to a solution of 2-aminoethanethiol hydrochloride (1.14 g, 10 mol) and Et_3N (1.4 mL, 10 mmol) in 30 mL of $CHCl_3$ at 0 °C. *N,N'*-Dicyclohexylcarbodiimide (2.06 g, 10 mmol) and monoethyl malonate (1.32 g, 10 mmol) in 5 mL of $CHCl_3$ were added. After 15 min, the bath was removed, and the mixture was stirred overnight. The mixture was filtered, and the filtrate was concentrated and worked up (EtOAc, bicarbonate, bisulfate). The resulting ethyl ester (1.28 g) was stirred for 4 h with 30 mL of 0.85 N NaOH and 19.2 mL of MeOH. Ag-50W-X2 (H^+ form) resin was added until the reaction mixture was acidic, the suspension was applied to a 60-mL column of the same resin, and the product was eluted with water, yielding 1.0 g. Crystallization from EtOAc–hexane yielded 0.69 g (64%): mp 52–54 °C; TLC R_f 0.62 ($CHCl_3$–MeOH–38% HOAc, 3:2:1). Anal. ($C_5H_9NO_3S$) C, H, N, S.

C. L-β-(3-Mercapto-1-oxopropoxy)benzenepropanoic Acid (33). 3-(Acetylthio)propionic acid (1.48 g, 10 mmol) and *N,N'*-carbonyldiimidazole (1.62 g, 10 mmol) were stirred in 10 mL of THF for 20 min. L-(−)-3-phenyllactic acid (1.66 g, 10 mmol) and Et_3N (1.4 mL, 10 mmol) in 7.5 mL of THF were added, and the mixture was stirred overnight. The THF was removed in vacuo, and the residue was worked up (EtOAc, acid) and chromatographed (12% AcOH/benzene) to give L-β-[3-(acetylthio)-1-oxopropoxy]benzenepropanoic acid: yield 1.7 g (57%); TLC R_f 0.42 (benzene–acetic acid, 7:1). The thioester (1.5 g, 5.1 mmol) was stirred in 7.5 mL of water and 7.5 mL of concentrated NH_3 for 15 min. The mixture was chilled, acidified, and worked up (EtOAc), and the residue was chromatographed (7% AcOH/benzene) to give **33**: 0.36 g (28%); TLC R_f 0.25 (benzene–HOAc, 14:1), $[\alpha]^{25}_D$ −14.6° (c 0.13, MeOH). From a small aliquot a cyclohexylamine salt was prepared, mp 99–100 °C. Anal. ($C_{12}H_{14}O_4S \cdot C_{12}H_{23}N$) C, H, N, S.

D. 1-[(2-Mercaptoethyl)sulfonyl]-L-proline (35). 2-Chloroethanesulfonyl chloride (8.2 g, 0.05 mol) in 100 mL of CH_2Cl_2 was added over 20 min to a solution of proline *tert*-butyl ester (6.9 g, 0.04 mmol) and Et_3N (14 mL, 0.1 mol) in 200 mL of CH_2Cl_2 at 0 °C. After stirring for 2 h, the mixture was worked up (CH_2Cl_2, bisulfate, bicarbonate). The semisolid residue was chromatographed (50% EtOAc–hexane) and crystallized from Et_2O–hexane to give vinylsulfonylproline *tert*-butyl ester **6**: yield 7.1 g (68%); mp 84–87 °C; TLC R_f 0.57 (EtOAc).

The above ester (5.0 g, 0.019 mol) and thiolacetic acid (1.43 mL, 0.02 mol) were mixed in 100 mL of Et_2O and allowed to stand overnight. The mixture was worked up (Et_2O, bisulfate, bicarbonate) to yield a yellow oil. NMR showed a small amount of starting material still present, so the procedure was repeated using half of the above quantities of Et_3N and thiolacetic acid. Workup as before afforded a crude product free of starting material, which was filtered through a short silica gel column and crystallized from Et_2O–hexane to give 1-[[2-(acetylthio)ethyl]-sulfonyl]-L-proline *tert*-butyl ester: yield 2.9 (45%); mp 46–50 °C.

The above *tert*-butyl ester (2.9 g, 0.0086 mol) was dissolved in 15 mL of anisole and 45 mL of trifluoroacetic acid and let stand for 1 h. The mixture was evaporated in vacuo to a gummy residue, which was taken up in EtOAc and treated with a large volume of hexane. The supernatant was decanted, and the procedure was repeated. The resulting semisolid was crystallized from EtOAc–hexane to give 1-[[2-(acetylthio)ethyl]sulfonyl]-L-proline: yield 1.9 g (78%); mp 75–77 °C; TLC R_f 0.22 (benzene–HOAc, 7:1).

The above ester (0.64 g, 0.0023 mol) was dissolved in 5 mL water and 5 mL of concentrated NH_4OH and stirred for 1 h. The solution was acidified and worked up (EtOAc) to yield an oily residue, which was chromatographed (10% acetic acid–benzene), and the main fraction was crystallized ($CHCl_3$–hexane) to give **35**: yield 0.44 g (81%); TLC R_f 0.40 (benzene–HOAc, 7:1), mp 99–101 °C; $[\alpha]^{26}_D$ −64.3° (c 1, DMF). Anal. ($C_7H_{13}O_4NS_2$) C, H, N, S.

E. 1-(3-Mercapto-2-methyl-1-oxopropyl)-5-oxo-L-proline (39). Bis(trimethylsilyl)trifluoroacetamide (20.4 mL, 77 mmol) was added to a suspension of L-pyroglutamic acid (4.5 g, 35 mmol) in 35 mL of propylene oxide and 210 mL of CH_3CN. The mixture was stirred for 15 min, then DL-3-(acetylthio)-2-methylpropanoyl chloride (5.7 mL, 36.8 mmol) was added, and the mixture was stirred overnight. The mixture was cooled to 0 °C, acidified slowly with 25 mL of 1 N HCl, and concentrated in vacuo, and the residue was worked up (EtOAc) to yield 16.8 g of oil. The DCHA salt was prepared (Et_2O) and recrystallized from MeOH–Et_2O to give **7**, 1-[3-(acetylthio)-2-methyl-1-oxopropyl)-5-oxo-L-proline, DCHA salt: yield 5.75 g (36%); mp 197–199 °C; $[\alpha]_D$ −70.5° (c 1, MeOH). Anal. ($C_{11}H_{15}NO_6S \cdot C_{12}H_{23}N$) C, H, N, S. The mother liquor was concentrated to yield the diastereomeric (*R,S*) DCHA salt: mp 168–169 °C; $[\alpha]_D$ +6.2° (c 1, CH_3OH). Anal. ($C_{11}H_{15}NO_6S \cdot C_{12}H_{23}N$) C, H, N, S.

The free acid was liberated from the higher-melting DCHA salt in 91% yield (3.9 g, 14.1 mmol), dissolved in 150 mL of MeCN and 1.14 mL (28.2 mmol) of MeOH, treated with mercuric trifluoroacetate (6.61 g, 15.2 mmol), and stirred for 3.5 h. The resulting suspension was treated with H_2S at 0 °C for 3 min, purged with nitrogen, filtered through Celite, and concentrated in vacuo to 3.4 g of crude semisolid. Trituration with warm EtOAc–hexanes yielded 2.67 g, mp 101–108 °C. This material was worked up (EtOAc, bisulfate) to yield a crystalline solid, which was triturated with hexanes to afford 2.49 g (70%) of **39**: mp 104–106.5 °C; $[\alpha]^{25}_D$ −108.8° (c 1, MeOH). Anal. ($C_9H_{13}NO_4S$) C, H, N, S.

F. L-1-(3-Mercapto-1-oxopropyl)-2-azetidinecarboxylic Acid (47). 3-(Acetylthio)propionyl chloride (6.7 g, 40 mmol) was added to a suspension of L-azetidine-2-carboxylic acid (4.04 g, 40 mmol) and *N*-methylmorpholine in dimethylacetamide (150 mL). The temperature rose to 32 °C, and the reaction mixture was heated on a steam bath for 3 h; the resulting clear solution was allowed to stand overnight. The crystals that precipitated were filtered. The dimethylacetamide was removed in vacuo, and the residue was worked up (EtOAc, acid wash) to yield 5.3 g (57%) of L-1-[3-(acetylthio)-1-oxopropyl]-2-azetidinecarboxylic acid, which did not crystallize.

Concentrated NH_4OH (12 mL) was precooled to 10 °C and added to the above acid (4.9 g, 19 mmol), and the resulting clear solution was stirred at room temperature under nitrogen for 3 h. The reaction mixture was then acidified and worked up (EtOAc) to give 2.3 g (64%) of **47** as a viscous residue, which did not crystallize: TLC R_f 0.23 (benzene–HOAc, 7:2); $[\alpha]^{25}_D$ −10.1° (c 1, EtOH); ^1H NMR ($CDCl_3$) δ 0.75 (m, 1 H, SH), 1.30–2.30 (m, 6 H, $HSCH_2CH_2CO$ and $CH_2CHCOOH$), 3.25 (m, 2 H, CH_2N), 4.02 (m, 1 H, NCHCOOH), 8.65 (s, 1 H, COOH). Anal. ($C_7H_{11}NO_3S \cdot 0.5H_2O$) C, H, N.

L-1-(3-Mercapto-1-oxopropyl)-2-piperidinecarboxylic Acid (48). This compound was prepared from L-pipecolic acid by the procedure utilized for the synthesis of **47** and was obtained as an oil which failed to crystallize: TLC R_f 0.80 (benzene–HOAc, 7:1); $[\alpha]^{25}_D$ −51.5° (c 1, EtOH). Anal. ($C_9H_{15}NO_3S$), C, H, N.

(1*S*,2*S*)-1-(3-Mercapto-2-methyl-1-oxopropyl)-2-piperidinecarboxylic Acid (49). This compound was prepared

by procedure utilized for the synthesis of **47**, utilizing (*S*)-3-(acetylthio)-2-methylpropanoyl chloride and L-pipecolic acid, and it was obtained as an oil which failed to crystallize: TLC R_f 0.57 (benzene–HOAc, 7:1); $[\alpha]^{25}_D$ −210° (c 1, EtOH). Anal. ($C_{10}H_{17}NO_3S \cdot 0.25H_2O$), C, H, N.

Scheme II. A. 1-(3-Mercaptopropyl)-L-proline (37). Proline *tert*-butyl ester (6.8 g, 0.04 mmol) and 1,3-dibromopropane (12.2 mL, 0.12 mol) were mixed and heated on the steam bath for 3 h. The mixture was poured into saturated $NaHCO_3$ solution and worked up (Et_2O), and the residue was distilled. The main fraction, bp 110–114 °C (0.5 mm), amounted to 7.0 g (59%) of 1-(3-bromopropyl)-L-proline *tert*-butyl ester.

Sodium hydride (2.0 g of 50% dispersion, 0.042 mol) was washed free of oil and suspended in 50 mL of THF under nitrogen. Thiolacetic acid (3.5 mL, 0.048 mol) in 10 mL of THF was added over 30 min. When gas evolution had ceased, the above bromopropylproline ester (6.2 g, 0.021 mol) in 10 mL of THF was added over 10 min, and the mixture was stirred overnight. The resulting suspension was worked up (Et_2O, bicarbonate), yielding a yellow oil. Chromatography (0–30% EtOAc–hexane) afforded 1-[3-(acetylthio)propyl]-L-proline *tert*-butyl ester (**8**): yield 5.1 g (84%); TLC R_f 0.43 (EtOAc).

The above *tert*-butyl ester (2.5 g, 0.087 mol) was dissolved in 10 mL of anisole and 25 mL of trifluoroacetic acid and let stand for 1 h. The mixture was then evaporated in vacuo to a brown oil, which was triturated repeatedly with Et_2O–hexane. The residue was dissolved in 20 mL of water and 5 mL of concentrated NH_3 under nitrogen and stirred for 1 h. The resulting solution was concentrated to 10 mL in vacuo and applied to a 200-mL column of AG-50W resin. The column was eluted with water until the eluant had pH >6 and then with pH 6.5 pyridine–acetic acid buffer. The nitroprusside-positive fractions were pooled and lyophilized to give a dark brown gum (0.7 g), which was chromatographed (10% AcOH–40% MeOH–CH_2Cl_2). The main fraction was taken up in water, treated with charcoal, Millipore-filtered, and lyophilized, yielding 0.4 g (25%) of **37** as a hygroscopic oil: TLC R_f 0.39 ($CHCl_3$–MeOH–HOAc, 5:4:1). Anal. ($C_8H_{15}O_2NS \cdot 0.5H_2O$) C, H, N; S: calcd 16.17; found, 15.33. Since acceptable analysis could not be obtained due to the extremely hygroscopic nature of **37**, homogeneity was confirmed by paper electrophoresis (Camag, 50 V/cm): single spot, pH 1.9, −3.5 cm, 30 min; single spot, pH 6.5, 0 cm, 40 min. NMR (D_2O) δ 1.6–2.3 (br, 6 H, proline 3-CH_2, 4-CH_2, $HSCH_2CH_2$), 2.55 (t, J = 8 Hz, 2 H, $HSCH_2$); 3.30 (t, (J = 9 Hz, 2 H, CH_2N], 3.9 (m, 2 H, proline 5-CH_2), 4.3 (m, 1 H, proline 2-CH).

B. L-3-(Mercaptopropyl)-5-oxo-2-pyrrolidinecarboxylic Acid (38). L-Pyroglutamic acid (25 g, 0.19 mol), MeOH (80 mL), benzene (50 mL), and concentrated H_2SO_4 (25 g) were combined and heated at reflux for 18 h. The reaction mixture was cooled in an ice bath and treated with 10% NaOH to pH 6. The mixture was concentrated in vacuo, then diluted with CH_2Cl_2, and filtered through Celite. The filtrate was dried (Na_2SO_4) and concentrated in vacuo. The residue was distilled to yield 20 g (73%) of methyl L-pyroglutamate bp 120 °C (0.002 mm).

The above ester (18.0 g, 0.126 mmol) and sodium hydride (3.22 g, 0.134 mol; oil-free) were stirred for 5 h in 240 mL of DMF, treated with 1,3-dibromopropane (62 mL, excess) in 120 mL DMF, and heated at 50 °C for 18 h. The DMF and excess 1,3-dibromopropane were removed in vacuo. The residue was worked up (benzene, bicarbonate) and chromatographed (hexane, benzene, 10% EtOAc–CH_2Cl_2) to give methyl L-3-(bromopropyl)-5-oxo-2-pyrrolidinecarboxylate: yield 11 g (33%); TLC R_f 0.3 (EtOAc).

A solution of *p*-methoxy-α-toluenethiol (8.98 g, 0.058 mol), NaOMe (3.14 g, 0.058 mol), and the above halide (10.25 g, 0.039 mol) in MeOH (100 mL) was stirred for 18 h, and the mixture was concentrated and worked up (Et_2O). The residue was chromatographed (15% EtOAc–hexane) to give methyl L-*N*-[β-[(*p*-methoxybenzyl)thio]propyl]-5-oxo-2-pyrrolidinecarboxylate (**9**): yield 8.3 g (63%); TLC R_f 0.5 (EtOAc).

The above ester (3.65 g, 0.011 mol) and 1 N NaOH (11 mL, 0.011 mol) were stirred in MeOH (50 mL) for 18 h. The MeOH was removed in vacuo. The residue was partitioned between water and Et_2O. The aqueous layer was acidified and saturated with NaCl and worked up (EtOAc) to L-*N*-[3-[(*p*-methoxybenzyl)-thio]propyl]-5-oxo-2-pyrrolidinecarboxylic acid: yield 3.5 g (100%); TLC R_f 0.1 (benzene–HOAc, 7:1).

A solution of the above acid (3.5 g, 0.011 mol), trifluoromethanesulfonic acid (1 mL), and anisole (10 mL) in trifluoroacetic acid (40 mL) was stirred for 1 h. The reaction mixture was concentrated and worked up (EtOAc). The residue was treated with 150 mL of 25% NH_4OH (previously purged with nitrogen) and stirred for 1 h. The reaction mixture was chilled in an ice bath, acidified, and worked up (Et_2O). The residue was chromatographed (2% MeOH–2% AcOH–$CHCl_3$) and crystallized from benzene to give **38**: yield 0.9 g (36%); mp 80–82 °C; TLC R_f 0.15 (12% AcOH–benzene). Anal. ($C_8H_{13}NO_3S$) C, H, N. S.

Scheme III. Methyl *N*-[(*tert*-Butyloxycarbonyl)-methyl]nipecotate (10). A slurry of K_2CO_3 (14.5 g, 0.105 mol6) in *tert*-butyl chloroacetate (12.7 g, 0.084 mol) and methyl nipecotate (12.0 g, 0.084 mol) was stirred vigorously at 50 °C for 3.5 h. The resultant thick paste was worked up (CH_2Cl_2, carbonate), and the residue was distilled in vacuo to yield 15 g (70%) of **10**, bp 95–100 °C (0.001 mm), used without further purification.

α-Methylene Lactam (11). A solution of **10** (23 g, 0.089 mol), NaOH (3.68 g, 0.092 mol), and water (50 mL) in MeOH (900 mL) was stirred for 3 days, and the mixture was concentrated. The resultant clear glass was refluxed in acetic anhydride (400 mL) for 2 h. The mixture was concentrated, and the residue was worked up to yield 8 g of an oily residue. The oil was dissolved in hexane–EtOAc (7:3) and filtered through silica gel (200 mL). The solvent was removed, and the resultant solid was recrystallized from hexane to yield 12 g (60%) of **11**, mp 84–86 °C. Anal. ($C_{12}H_{19}NO_3$) C, H, N.

3-(Mercaptomethyl)-2-oxo-1-piperidineacetic Acid (29). A solution of α-methylene lactam **11** (12 g, 0.053 mol) in thiolacetic acid (12 mL) was stirred for 3 days. The mixture was concentrated and the resultant oil was chromatographed (20% EtOAc–hexane) to give *tert*-butyl 3-[(acetylthio)methyl]-2-oxo-1-piperidineacetate as an oil: yield 12 g (75%); TLC R_f 0.65 (EtOAc).

A solution of the above ester (12 g, 0.04 mol) and anisole (40 mL) in trifluoroacetic acid (120 mL) was stirred for 1 h. The reaction mixture was concentrated, and the residue was converted to a DCHA salt (EtOAc). The purified salt (mp 150–152 °C) (lit.[13] mp 151–152 °C) was decomposed to give 3-[(acetylthio)-methyl]-2-oxo-1-piperidineacetic acid an oil: yield 6.1 g (62%).

The above thiol ester (6.06 g, 0.025 mol) was treated with 25% ammonium hydroxide (120 mL) and stirred for 1 h. The reaction mixture was then chilled in an ice bath, acidified, and worked up (EtOAc). The resultant oil was converted to a DCHA salt (EtOAc): yield 7 g (73%); mp 135–137 °C (lit.[13] mp 138–140 °C). For testing, the salt was converted to the free acid **29**: TLC R_f 0.15 (benzene–HOAc, 7:1). Anal. ($C_8H_{13}NO_3S$) C, H, N, S.

Scheme IV. (*E*)-6-Hydroxy-3-hexenoate (12). A mixture of ethyl 3-hexenedioate[15] (20.0 g, 116 mmol) and recrystallized *p*-nitrophenol (17.2 g, 124 mmol) in 240 mL of EtOAc was treated with *N*,*N*′-dicyclohexylcarbodiimide (25.6 g, 124 mmol) at 0 °C. The mixture was stirred for 1 h and then overnight at room temperature. The mixture was filtered, the filtrate was evaporated, and the residue was triturated with isopropyl ether to give ethyl *p*-nitrophenyl-3-hexenedioate: mp 78–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, J = 7 Hz, 3 H, CH_3), 3.10–3.60 (m, 4 H, $CH_2CH{=}CHCH_2$), 4.25 (q, J = 7 Hz, 2 H, OCH_2CH_3), 5.80–6.05 (m, 2 H, CH=CH), 7.20–8.50 (A_2B_2 m, 4 H, aromatic). Anal. ($C_{14}H_{15}NO_6$) C, H, N.

Sodium borohydride (4.7 g, 125 mmol) was suspended in 65 mL of dry 1,2-dimethoxyethane. This was warmed in a water bath at 38 °C, and a solution of the above *p*-nitrophenyl ester (7.32 g, 25 mmol) in 35 mL of dimethoxyethane was added dropwise in 8 min. The temperature rose to 46 °C, and 20 min later the temperature had dropped to 38 °C. After cooling in an ice bath the mixture was acidified with 6 N HCl, keeping the temperature below 30 °C. Some water was added and, after stirring for a few minutes, the product was extracted into EtOAc. The wet EtOAc extracts were taken to dryness in vacuo. The residue was worked up (EtOAc, hydroxide) to give 1.65 g (42%) of oily ester **12**: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3 H, CH_3), 2.20 (s, 1 H, OH), 2.10–2.54 and 2.97–3.20 (m, 2 H each, $CH_2CH{=}CHCH_2$), 3.68 (t, J = 6 Hz, 2 H, CH_2OH), 4.18 (q, J = 7 Hz, 2 H, OCH_2CH_3), 5.54–5.80 (m, 2 H, CH=CH); used without further purification.

(15) V. Vukou and R. J. Crawford, *Can. J. Chem.*, **53**, 1367 (1975).

Ethyl (*E*)-6-(Acetylthio)-3-hexenoate (13). To a stirred solution of ester **12** (3.16 g, 20 mmol) and Et_3N (2.9 mL, 2.1 g, 21 mmol) in CH_2Cl_2 (10 mL) was added a solution of *p*-TsCl (4.0 g, 21 mmol) in CH_2Cl_2 (10 mL) dropwise over 10 min. The resulting mixture was stored overnight at 5 °C and worked up (EtOAc, $KHSO_4$) to give crude tosylate as an oil: yield 6.1 g (98%); ^1H NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 3 H, CH_3), 2.48 (s, 3 H, aromatic CH_3), 2.20–2.63 and 2.93–3.23 (m, 2 H each, CH_2CH=$CHCH_2$), 4.07 (t, J = 6 Hz, 2 H, CH_2OTs), 4.17 (q, J = 7 Hz, 2 H, OCH_2CH_3), 5.48–5.73 (m, 2 H, CH=CH), 7.43–7.93 (A_2B_2 m, 4 H, aromatic); used without further purification.

Thiolacetic acid (10.7 mL, 11.4 g, 150 mmol) was added to a solution of the above tosylate (9.1 g, 29.2 mmol) and Et_3N (18.9 mL, 13.6 g, 135 mmol) in EtOAc (75 mL), and the mixture was stored overnight. Workup ($NaHCO_3$) and distillation of the crude product afforded ester **13**: yield 4.86 g (76%); bp 112–114 °C (0.2 mm); ^1H NMR ($CDCl_3$) δ 1.28 (t, J = 7, 3 H, CH_3), 2.73 (s, 3 H, $COCH_3$), 2.26–2.36 and 2.92–3.13 (m, 2 H each, CH_2CH=$CHCH_2$), 4.20 (q, J = 7 Hz, 2 H, OCH_2CH_3), 5.48–5.68 (m, 2 H, CH=CH).

(*E*)-6-Mercapto-3-hexenoic Acid (31). Ester **13** (2.5 g, 12 mmol) and 2 N HCl (25 mL) were refluxed under nitrogen for 2 h. Workup (EtOAc, NaCl) afforded crude **31** as an oil (1.4 g), which was chromatographed (MeOH–$CHCl_3$, 1:9) to afford acid **31**: yield 0.59 g (35%); TLC R_f 0.50 (MeOH–$CHCl_3$), 1:9); ^{13}C NMR ($CDCl_3$) δ 24.1 ($HSCH_2CH_2$), 36.7 ($HOOCCH_2$), 37.7 ($HSCH_2$), 123.4 and 132.4 (CH=CH), 177.7 (COOH). A CHA salt (EtOAc) had mp 93–95 °C. Anal. ($C_6H_{10}O_2S \cdot C_6H_{13}N$) C, H, S, N.

Schemes V and VI. Enones 15a–d (Scheme V) and 17. *trans*-2-(1-Oxo-2-propenyl)cyclohexanecarboxylic Acid (15d). To a vigorously stirred suspension of anhydrous $AlCl_3$ (66.7 g, 0.5 mol) in 1 L of 1,2-dichloroethane was added *trans*-1,2-cyclohexanecarboxylic anhydride (**36d**; 38.5 g, 0.25 mol). Ethylene was then bubbled through the resulting solution for 4.5 h. The solution was poured into 900 mL of 5% HCl and worked up (Et_2O). The residue was heated on a steam bath with 300 mL of 10% aqueous K_2CO_3 for 15 min, the cooled mixture was extracted with several portions of Et_2O, and the aqueous layer was acidified with dilute HCl. Workup (Et_2O) afforded a colorless crystalline solid: yield 31.6 g (70%). Crystallization (Et_2O–hexane) afforded the analytical sample, mp 101–103 °C, after drying in vacuo over P_2O_5: NMR ($CDCl_3$) δ 1.00–2.30 (m, 8 H, $(CH_2)_4$), 2.70 (m, $J_{a,a}$ = 12.5 Hz, $J_{a,a}$ = 10.5 Hz, $J_{a,e}$ = 3.5 Hz, 1 H, CHCO), 5.66–6.54 (ABC pattern, 3 H, CH=CH_2), 11.64 (s, 1 H, COOH). Anal. ($C_{10}H_{14}O_3$) C, H.

The following enones were prepared by the method described above.

4-Oxo-5-hexenoic acid (15a): 22% yield; bp 102–107 °C (0.5 mm); mp 47–51 °C; ^1H NMR ($CDCl_3$) δ 2.50–3.20 [4 H, m $(CH_2)_2$], 5.75–6.42 (3 H, m, -CH=CH_2), 11.42 (s, 1 H, COO*H*); ^{13}C NMR ($CDCl_3$) δ 26.7 and 33.4 (CH_2CH_2COOH), 127.2 and 135.8 (H_2C=CH), 173.5 (COOH), 197.8 (CO). Anal. ($C_6H_8O_3$) C, H.

***cis*-2-(1-Oxo-2-propenyl)cyclobutanecarboxylic acid (15b):** 61% yield; mp 71–75 °C (Et_2O–hexane); ^1H NMR ($CDCl_3$) δ 2.08–2.40 (m, 4 H, $(CH_2)_2$), 3.44 (m, 1 H, CH_aCOOH), 3.76 (m, 1 H, CH_bCO), 5.70–6.46 (m, 3 H, CH=CH_2), 11.44 (s, 1 H, COOH); decoupling by irradiation at 230 Hz eliminated all proton couplings except 8.5 Hz between H_a and H_b. Anal. ($C_8H_{10}O_3$) C, H.

***cis*-3-(1-Oxo-2-propenyl)cyclohexanecarboxylic acid (15c):** 79% yield; colorless foam. Due to the instability of this material, it was used without further purification. Recrystallization of a small sample (Et_2O–hexane) afforded crystals: mp 90–93 °C; ^1H NMR ($CDCl_3$) δ 1.30–2.40 [m, 8 H, $(CH_2)_4$], 2.83 (m, 1 H, *CH*COOH), 3.23 (m, 1 H, *CH*CO), 5.67–6.93 (m, 3 H, CH=CH_2), 10.93 (s, 1 H, COOH). Anal. ($C_{10}H_{14}O_3$) C, H.

***cis*-2-(3-Methyl-1-oxo-2-propenyl)cyclopentanecarboxylic acid (17)** was obtained from *cis*-1,2-cyclopentanedicarboxylic anhydride[16] in 14% yield after chromatography: mp 85–88 °C (hexane); TLC R_f 0.08 ($CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.60–2.30 [m, 6 H, $(CH_2)_3$], 1.90 (d, J = 0.5 Hz, 3 H, CH_3 cis to ketone), 2.12 (d, J = 0.5 Hz, 3 H, CH_3 trans to ketone), 2.95 (q, J = 7 Hz, 1 H, *CH*COOH), 3.22 (q, J = 7 Hz, 1 H, CHCO); mass spectrum, m/e 196 (M^+). Anal. ($C_{11}H_{16}O_3$) C, H.

Enones 20 and 21 (Scheme VI). Methyl *trans*-2-Formylcyclopentanecarboxylate (18). Silver nitrate (33 g, 194 mmol) in 55 mL of H_2O was added over 10 min to a stirred refluxing solution of methyl *trans*-2-(dibromethyl)-1-cyclopentanecarboxylate[17] (25 g, 83.3 mmol) in 140 mL of THF. After 30 min, the mixture was cooled, saturated with NaCl, neutralized with saturated $NaHCO_3$ solution, and filtered through Celite. The filtrate was worked up (Et_2O), yielding 7.85 g of an amber oil, which was distilled: yield 4.1 g (32%); bp 60–72 °C (0.3–0.4 mm); ^1H NMR ($CDCl_3$) δ 1.50–2.40 [m, 6 H, $(CH_2)_3$], 3.0–3.21 (m, 2 H, OHCC*HCH*COOMe), 3.75 (s, 3 H, OMe), 9.80 (s, 1 H, *CH*O); used without further purification.

Diphenylmethyl *trans*-2-(1-Oxo-2-propenyl)cyclopentanecarboxylate (20). Vinylmagnesium bromide in THF [from vinyl bromide (2.5 g, 23 mmol) and Mg (0.39 g, 16 mmol)] was added dropwise over 30 min to aldehyde **39a** (2.5 g, 16 mmol) in 20 mL of THF at 0–5 °C, and the mixture was stirred for 15 min. Saturated aqueous NH_4Cl solution (20 mL) was then added dropwise, and the mixture was worked up, yielding 2.3 g of yellow oil: TLC R_f 0.53 (1% MeOH–$CHCl_3$). This material was refluxed for 1.5 h in 18 mL 1 N NaOH. After cooling, the solution was washed with EtOAc, acidified with cold 10% $KHSO_4$, and worked up (EtOAc), yielding 2.0 g of oil: TLC R_f 0.16 (1% MeOH–$CHCl_3$). This material was dissolved in 50 mL of EtOAc and treated with diphenyldiazomethane (2.2 g, 11.3 mmol) in several portions over 30 min, then stirred overnight, and worked up (EtOAc, bicarbonate), yielding 3.65 g of reddish viscous oil, which was chromatographed ($CHCl_3$) to yield 1.35 g (35%) of oil: TLC R_f 0.10 ($CHCl_3$). The oil was stirred overnight with activated MnO_2 (13 g) in 60 mL of CH_2Cl_2. The mixture was filtered, the filtrate was evaporated, and the residue was chromatographed (benzene) to give **20** as a yellow oil: yield 0.7 g (32%); TLC R_f 0.44 ($CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.50–2.17 [m, 6 H, $(CH_2)_3$], 3.07–3.70 (m, 2 H, CHCO, *CH*COOCHPh$_2$), 7.28 (s, 10 H, Ph); used without further purification.

Diphenylmethyl *trans*-2-(1-Oxo-2-methyl-2-propenyl)-cyclohexanecarboxylate *trans*-2-(Methoxycarbonyl)cyclohexanecarboxylic acid was converted to methyl *trans*-2-formylcyclohexanecarboxylate (**19**) by diborane reduction, followed by a modified Collins oxidation. Enone **21** was prepared from aldehyde **19** as described for enone **20**, utilizing the Grignard reagent derived from 2-bromopropene and using the modified Collins oxidation procedure instead of MnO_2. Enone **21** was obtained as a crystalline solid: mp 92–94 °C (hexane); ^1H NMR ($CDCl_3$) δ 1.20–2.20 [m, 8 H, $(CH_2)_4$], 1.78 (d, J = 0.5 Hz, 3 H, CH_3), 2.96 (m, $J_{a,a}$ = 11.0 Hz, $J_{a,a}$ = 11.0 Hz, $J_{a,e}$ = 4.0 Hz, 1 H, CHCO), 3.35 (m, $J_{a,a}$ = 11.0 Hz, $J_{a,a}$ = 11.0 Hz, $J_{a,e}$ = 4.0 Hz, 1 H, *CH*COOCHPh$_2$), 5.66 and 5.96 (br s, 2 H, C=CH_2), 6.76 (s, 1 H, *CH*Ph$_2$), 7.24 (s, 10 H, Ph). Anal. ($C_{24}H_{26}O_3$) C, H.

Thiolacetic Acid Adducts 16a–d (Scheme V) and 22 and 23a,b (Scheme VI). 6-(Acetylthio)-4-oxohexanoic Acid (16a). Thiolacetic acid (4.9 g, 65 mmol) was added dropwise to **15a** (6.8 g, 58 mmol) in 30 mL of CH_2Cl_2, and the mixture was stirred for 45 min and filtered. The filtrate was concentrated to yield 9.9 g (84%) of crystalline solid, which was triturated with petroleum ether and crystallized (benzene) to give **16a**: yield 6.2 g (52%); mp 72–74 °C. Anal. ($C_8H_{12}O_4S$) C, H, S.

By utilizing the above procedure, the following adducts were prepared.

***cis*-2-[3-(Acetylthio)-1-oxopropyl]cyclobutanecarboxylic acid (16b):** mp 50–54 °C (diisopropyl ether). Anal. ($C_{10}H_{14}O_4S$) C, H, S.

***cis*-2-[3-(Acetylthio)-1-oxopropyl]cyclohexanecarboxylic acid (16c):** mp 79–81 °C (diisopropyl ether). Anal. ($C_{12}H_{18}O_4S$) C, H, S.

***trans*-2-[3-(Acetylthio)-1-oxopropyl]cyclohexanecarboxylic acid (16d):** mp 65–68 °C (diisospropyl ether). Anal. ($C_{12}H_{18}O_4S$) C, H, S.

***trans*-2-[3-(Acetylthio)-2-methyl-1-oxopropyl]cyclohexanecarboxylic Acid [23a (Isomer A) and 23b (Isomer B)].** Enone **21** (4.0 g, 11 mmol) and thiolacetic acid (4.5 mL) were stirred for 2 h in 40 mL of CH_2Cl_2 and the mixture was concen-

(16) S. F. Birch, R. A. Dean, N. J. Hunter, and E. V. Whitehead, *J. Org. Chem.*, **20**, 1178 (1955).

(17) W. Boland and L. Jaenicke, *Chem. Ber.*, **110**, 1823 (1977).

trated. The residue was chromatographed (benzene; 50% $CHCl_3$–benzene) to give an oil: yield 4.7 g (97%); TLC R_f 0.27 (50% $CHCl_3$–benzene). A mixture of this oil (4.7 g, 10.7 mmol) and anisole (21.6 g, 200 mmol) was cooled to 0 °C, and trifluoroacetic acid (60 mL) was added dropwise over 60 min. After 60 min, the mixture was concentrated, and the residue was dissolved in ether and extracted with saturated $NaHCO_3$. The aqueous layer was washed with Et_2O, acidified, and worked up (Et_2O) to give 3.0 g of oil. The ^1H NMR of this material shows two sharp peaks for the *S*-acetylmethyl groups, indicating a mixture of isomers. Fractional crystallization (isopropyl ether–hexane) gave isomer A (**23a**): yield 700 mg (24%); mp 103–108 °C. Anal. ($C_{13}H_{20}O_4S$) C, H, S. The mother liquors were taken to dryness to give 1.1 g of oil, very much enriched in isomer B, as judged by ^1H NMR. This crude isomer B was used without further purification.

***trans*-2-[3-(Acetylthio)-1-oxopropyl]cyclopentanecarboxylic Acid (41a).** Using the procedure described above for **23a,b**, enone **20** was converted to acid **22**, obtained as an oil, which was used without further purification in the synthesis of **43**: TLC R_f 0.53 ($CHCl_3$–MeOH, 9:1).

Ketone Analogues 32 (Table I) and 41–46 (Table II). ***trans*-2-(3-Mercapto-2-methyl-1-oxopropyl)cyclohexanecarboxylic Acid (Isomer A, 46a).** Acid **23a** (isomer A; 680 mg, 2.5 mmol) was stirred with 1.5 mL of concentrated NH_4OH and 1.5 mL of water for 30 min, and the mixture was cooled, acidified, and worked up (EtOAc). The residue was chromatographed (2% MeOH–$CHCl_3$) and recrystallized (isopropyl ether) to give acid **46a**: yield 230 mg (40%); mp 87–90 °C; TLC R_f 0.43 (10% MeOH–$CHCl_3$). Anal. ($C_{11}H_{18}O_3S$) C, H, S.

By the method described above, the following ketone analogues were prepared.

***trans*-2-(3-Mercapto-2-methyl-1-oxopropyl)cyclohexanecarboxylic Acid (isomer B, 46b)**: mp 91–93 °C (diisopropyl ether), 22% yield from crude **23b** (isomer B) described above. Anal. ($C_{11}H_{18}O_3S$) C, H, S.

***trans*-2-(3-Mercapto-1-oxopropyl)cyclohexanecarboxylic acid (45)**: mp 71–74 °C (diisopropyl ether–hexane); 64% yield from **16d**. Anal. ($C_{10}H_{16}O_3S$) C, H, S.

***cis*-2-(3-Mercapto-1-oxopropyl)cyclohexanecarboxylic acid (44)**: mp 82.5–84.5 °C (diisopropyl ether); 72% yield from **16c**. Anal. ($C_{10}H_{16}O_3S$) C, H, S.

***cis*-2-(3-Mercapto-1-oxopropyl)cyclobutanecarboxylic acid (41)**: 66% yield as an oil from **16b**; TLC R_f 0.43 ($CHCl_3$–MeOH, 9:1); dicyclohexylamine salt, mp 129–132 °C (EtOAc). Anal. ($C_8H_{13}O_3S \cdot C_{12}H_{23}N$), C, H, N, S.

6-Mercapto-4-oxohexanoic acid (32): mp 40–42 °C (Et_2O–hexane); 58% yield from **16a**. Anal. ($C_6H_{10}O_3S$) C, H, S.

***trans*-2-(3-Mercapto-1-oxopropyl)cyclopentanecarboxylic acid (43)**: 45% yield as an oil from **22**; TLC R_f 0.45 ($CHCl_3$–MeOH, 9:1); dicyclohexylamine salt, mp 126–128 °C (diisopropyl ether). Anal. ($C_9H_{14}O_3S \cdot C_{12}H_{23}N$) C, H, N, S.

***trans*-2-(3-Mercapto-1-oxopropyl)cyclobutanecarboxylic acid (42)**. Acid **16b** (460 mg, 2 mmol) and NaOMe (324 mg, 6 mmol) were stirred for 2 h at 0 °C in 10 mL of MeOH, and the mixture was acidified, concentrated, and worked up (EtOAc). The residue was purified on a preparative TLC plate (silica gel, 10% MeOH–$CHCl_3$, R_f 0.55) and converted to a DCHA salt (ether): yield 250 mg (34%); mp 140–144 °C. Anal. ($C_8H_{12}O_3S \cdot C_{12}H_{23}N$) C, H, N, S.

4-Hydroxy-6-mercaptohexanoic Acid (34). Sodium borohydride (11.4 g, 300 mmol) was added portionwise over 3 h to a suspension of **16a** (12.24 g, 60 mmol) in 120 mL of H_2O at 0 °C. The mixture was stirred for 60 min, acidified by the slow addition of 2 N HCl, and let stand overnight. Workup (EtOAc) gave an oil, which was mainly the lactone derivative of hydroxy acid **32** as judged by IR. The oil was dissolved in benzene, a small amount of *p*-toluenesulfonic acid was added, and the mixture was heated under reflux in a nitrogen atmosphere and with a water trap for 1.5 h. After the mixture was cooled, a small amount of insoluble material was removed by filtration, and the solvent was removed in vacuo, leaving an oil, which was chromatographed ($CHCl_3$) to give 7.4 g (84%) of colorless oil. Distillation of a 2-g sample gave 1.2 g (51%) of 4,5-dihydro-5-(2-mercaptoethyl)-2(3*H*)-furanone, bp 118–120 °C (0.6 mm). The above lactone (2.0 g, 13.7 mmol) in 20 mL of 1 N NaOH was heated under reflux for 2 h, and the mixture was cooled and then acidified with dilute acetic acid. Workup afforded an oil (1.8 g, 80%), TLC R_f 0.10 (5% MeOH–$CHCl_3$), which was converted to the DCHA salt (EtOAc): yield 1.7 g; mp 123–126 °C. Anal. ($C_{18}H_{35}O_3NS$) C, H, N, S.

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Catechol *O*-Methyltransferase. 10. 5-Substituted 3-Hydroxy-4-methoxybenzoic Acids (Isovanillic Acids) and 5-Substituted 3-Hydroxy-4-methoxybenzaldehydes (Isovanillins) as Potential Inhibitors

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A series of 5-substituted 3-hydroxy-4-methoxybenzoic acids (isovanillic acids) and -benzaldehydes (isovanillins) have been synthesized and evaluated as inhibitors of rat liver catechol *O*-methyltransferase. The compounds exhibited either noncompetitive or competitive patterns of inhibition when 3,4-dihydroxybenzoic acid was the variable substrate. The benzaldehydes were significantly more potent inhibitors than the corresponding benzoic acids, and electron-withdrawing substituents in the 5 position greatly enhanced their inhibitory activity.

The extraneuronal inactivation of catecholamines and the detoxification of many xenobiotic catechols are dependent upon the enzyme catechol *O*-methyltransferase (COMT, EC 2.1.1.6). COMT is a soluble, magnesium-requiring enzyme which catalyzes the transfer of a methyl group from *S*-adenosylmethionine (SAM) to a catechol substrate, resulting in the formation of the meta- and para-O-methylated products.[1]

Inhibition of COMT as a means of controlling endogenous catecholamine metabolism or preventing the inactivation of exogenously administered catechols has been the subject of considerable research interest.[2] Many of the COMT inhibitors identified to date have potent inhibitory effects in vitro, but in general they are less effective in vivo. Their reduced effectiveness in vivo appears to result from a number of factors, including poor bioa-

(1) R. T. Borchardt, "Enzymatic Basis of Detoxication", W. B. Jakoby, Ed., Vol. II, Wiley, New York, 1980, p 43.

(2) H. C. Guldberg and C. A. Marsden, *Pharmacol. Rev.*, 27, 135 (1975).

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