H, s, CH_3C=CH), 1.28 (3 H, t); ^{19}F NMR (CCl_4) 118 ppm upfield from $CFCl_3$ [d, J_{HF} = 30 Hz, (4Z)-fluoro]. Anal. ($C_{22}H_{26}ClFO_3$) C, H, Cl, F.

Ethyl (*E,Z,E,E*)-3,7-Dimethyl-4-fluoro-9-(2-chloro-4-methoxy-3,6-dimethylphenyl)-2,4,6,8-nonatetraenoate (10c). The compound was prepared in 47% yield using a procedure similar to that described for **10b**: yellow crystals; mp 115–117 °C (CH_2Cl_2–petroleum ether); IR (KBr) 1716 cm^{-1} ($COOC_2H_5$); UV max (EtOH) 208 nm (ε 24 500), 365 (45 400); ^1H NMR ($CDCl_3$) δ 6.76 [d, J = 16 Hz, (E)-ArCH=CH], 6.55 (d, J = 12 Hz, CH_3C=CH), 6.47 (d, J = 16 Hz, ArCH=CH), 6.38 (dd, J_{HH} = 12 Hz, J_{HF} = 36 Hz, CH=CF), 6.21 (s, CH=$COOC_2H_5$), 4.18 (2 H, q), 3.80 (3 H, s), 2.37, 2.25 (2 s, 2 aromatic CH_3's), 2.33 [s, (2E)-CH_3C=CH$COOC_2H_5$], 2.08 (s, CH_3C=CH), 1.29 (3 H, t); ^{19}F NMR ($CDCl_3$) δ 118.5 ppm upfield from $CFCl_3$ (d, J_{FH} = 36 Hz). Anal. ($C_{22}H_{26}ClFO_3$) C, H, Cl, F.

Ethyl (*E,Z,E,E*)-3,7-Dimethyl-4-fluoro-9-(2,6-dichloro-3-methyl-4-methoxyphenyl)-2,4,6,8-nonatetraenoate (10d). This compound was prepared in 55% yield using a procedure similar to that described for **10a**: yellow crystals; mp 127–132 °C (CH_2Cl_2–hexane); UV max (EtOH) 206 nm (ε 32 100), 364 (48 000); MS (m/e) 412 (M^+), ^1H NMR (CCl_4) δ 6.80 (1 H, d, J = 16 Hz), 6.75 (1 H, s), 6.60 (1 H, d, J = 16 Hz), 6.51 (d, J = 12 Hz, CH_3C=CH), 6.23 (dd, J_{HH} = 12 Hz, J_{HF} = 30 Hz, CH=CF), 6.14 (1 H, s), 4.11 (2 H, q), 3.22 (3 H, s), 2.31 (s, CH_3C=CH$COOC_2H_5$), 2.22 (3 H, s), 2.06 (3 H, s, CH_3C=CH), 1.28 (3 H, t). Anal. ($C_{21}H_{23}Cl_2FO_3$) C, H, Cl, F.

Ethyl (*E,Z,E,E*)-3,7-Dimethyl-4-fluoro-9-(3-chloro-2,4,6-trimethylphenyl)-2,4,6,8-nonatetraenoate (10e). This compound was prepared in 52% yield using a procedure similar to that described for **10b**: yellow crystals; mp 100–104 °C (hexane); IR (KBr) 1719 ($COOC_2H_5$), 1620, 1190 cm^{-1}; UV max (EtOH) 355 nm (ε 49 200); MS (m/e) 376 (M^+); ^1H NMR (CCl_4) δ 6.83 (1 H, s), 6.66–6.0 (5 olefinic protons), 4.11 (2 H, q), 2.26, 2.29 (9 H, br s), 2.20 (3 H, s), 2.04 (3 H, s), 1.28 (3 H, t). Anal. ($C_{22}H_{26}ClFO_2$) C, H, Cl, F.

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Notes

Synthesis and Angiotensin-Converting Enzyme Inhibitory Activity of 3-(Mercaptomethyl)-2-oxo-1-pyrrolidineacetic Acids and 3-(Mercaptomethyl)-2-oxo-1-piperidineacetic Acids

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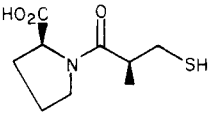
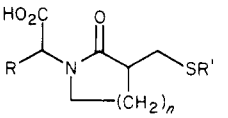
A number of γ- and δ-lactam derivatives were synthesized and their in vitro angiotensin-converting enzyme (ACE) inhibitory activities were compared. The structures of these compounds were designed to include many of the important features of captopril. The synthesis involved the preparation of a variety of novel 3-methylene-2-pyrrolidinones (**3–5** and **16**) and 3-methylene-2-piperidinones (**3a–5a**, **10–12**, and **17**). The key intermediate 3-methylenelactams **3** and **3a** were obtained from 3-(hydroxymethyl)lactams **2** and **2a** by a direct dehydration with dicyclohexylcarbodiimide using cuprous iodide as a catalyst. Introduction of the sulfhydryl group was accomplished by a Michael addition to these α,β-unsaturated lactams. The compound with the highest in vitro activity was 3-(mercaptomethyl)-2-oxo-1-piperidineacetic acid (**7a**). The activity of the **7a** both in vitro and in vivo (dog) was shown to be less than that of captopril by a factor of about 100.

In the last few years several papers have been published describing antihypertensive agents of the type that work by inhibiting the angiotensin-converting enzyme (ACE).[1-5] The most exciting compound thus far has been the clinically proven, orally effective agent captopril (SQ 14 225; I). In the reports on structural variations of I, it was



I



II, R = H, CH_3, *n*-Pr, Ph; R' = H, Ac; n = 1, 2

usually found that the ACE inhibitory activity was best when the important functional groups were retained.

(1) D. W. Cushman, H. S. Cheung, E. F. Sabo, and M. A. Ondetti, *Biochemistry*, **16**, 5484 (1977).
(2) D. W. Cushman, H. S. Cheung, E. F. Sabo, and M. A. Ondetti, *Prog. Cardiovasc. Dis.* **21**(3), 176 (1978).
(3) I. Mita, J. Iwao, M. Oya, T. Chiba, and T. Iso, *Chem. Pharm. Bull.*, **26**(4), 1333–1335 (1978).
(4) A. Sugie and J. Katsube, *Chem. Pharm. Bull.*, **27**(7), 1708–1709 (1979).
(5) D. Cushman and H. Cheung, *Biochem. Pharmacol.*, **20**, 1637–1648 (1971).

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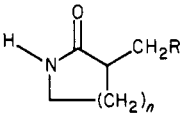
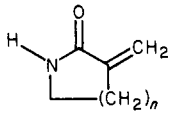
Table I. 3-Substituted 2-Piperidinones and 2-Pyrrolidinones[a]



no.	R	n	% yield	mp, °C	recrystn solvent	formula	anal.
2	OH	1	48	100-101	*i*-PrOH	$C_5H_9NO_2$	C, H, N
2a[b]	OH	2	66	81-83[c]	*i*-PrOH/hexane	$C_6H_{11}NO_2$	C, H, N
8	$SCOCH_3$	2	48	124-126	EtOAc	$C_8H_{13}NO_2S$	C, H, N, S
9	SH	2	61	83-85	EtOAc/hexane	$C_6H_{11}NOS$	H, N; C[d]

[a] All compounds exhibited IR and ^1H NMR spectra consistent with the assigned structures. [b] Starting material for 2a, ethyl 2-oxo-3-piperidinecarboxylate, was commercially available. [c] Reported mp 79-80 °C.[6] Literature procedure for the preparation of 2a was modified to give higher yields; see Experimental Section. [d] C: calcd, 49.62; found 50.15.

Table II. 3-Methylene-2-pyrrolidinone and 3-Methylene-2-piperidinone[a]



no.	n	% yield	mp, °C	recrystn solvent	formula	anal.
3	1	56	106-108	EtOAc	C_5H_7NO	C, H, N
3a	2	67	55-57	*b*	C_6H_9NO	C, H, N

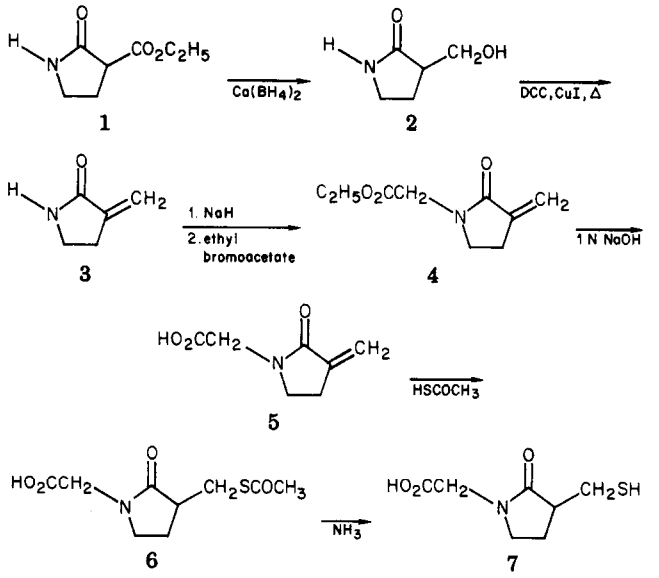
[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] Compound was distilled, bp 110 °C (1.0 mmHg).

Because of the proposed receptor-site mechanism of action,[1] the relative order of these groups, as well as stereochemistry, was considered significant in drug design.

We studied a series of γ- and δ-lactams (II) for ACE inhibiting activity. These compounds also contain some of the important features of I, in that the mercaptan and the carboxylic acid functions are separated by the same number of atoms. Compounds of type II do not, however, possess a proline moiety. Instead, they contain a lactam ring which retains the tertiary amide feature and incorporates the methyl group that is present in I. Structure–activity relationship studies were directed mainly to variations of the R group and the lactam ring size.

Chemistry. The synthesis of lactams of formula II was envisioned via Michael addition of a suitable sulfhydryl moiety to an appropriately substituted α,β-unsaturated lactam. To study the feasibility of this approach, ethanethioic acid was successfully added to 3-methylene-2-piperidinone (3a) to give the *S*-acetyl derivative 8 (Scheme II). Cleavage of the *S*-acetyl group with ammonia generated the free mercaptan 9.

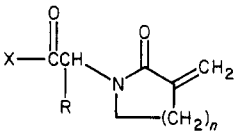
Scheme I



Subsequently, various *N*-(carboxyalkyl)-3-methylenelactams (5, 5a, 10, 11 and 12) (Table III) were prepared and reacted with ethanethioic acid to give the desired compounds (6, 6a, 7, 7a, 13, 14, and 15) (Table IV). Scheme I shows the full synthetic route to these target compounds as exemplified in the 2-pyrrolidinone series.

The key intermediate 3-methylenelactams 3 and 3a were prepared in good yields by direct dehydration of 3-(hydroxymethyl)lactams 2 and 2a with dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts of cuprous iodide in a nonprotic solvent such as toluene or

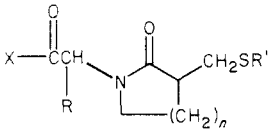
Table III. 3-Methylene-2-oxo-1-pyrrolidine- and -piperidineacetic Acids, Esters, and Amides[a]



no.	R	n	X	% yield	mp, °C	recrystn solvent	formula	anal.
4a	H	2	OC_2H_5	68	*b*	*c*	$C_{10}H_{15}NO_3$	H, N; C[d]
5	H	1	OH	62[e]	140-142	EtOAc	$C_7H_9NO_3$	C, H, N
5a	H	2	OH	84[f]	147-148	EtOAc	$C_8H_{11}NO_3$	C, H, N
10	CH_3	2	OH	38[g]	152-154	EtOAc	$C_9H_{13}NO_3$	C, H, N
11	1-Pr	2	OH	11[g]	123-125	EtOAc	$C_{11}H_{17}NO_3$	C, H, N
12	Ph	2	OH	41[g]	171-173	EtOAc	$C_{14}H_{15}NO_3$	C, H, N
16	H	1	NH_2	48	174-176	i-PrOH/hexane	$C_7H_{10}N_2O_2$	C, H, N
17	H	2	NH_2	60	167-169	MeOH	$C_8H_{12}N_2O_2$	C, H, N

[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] Compound was an oil which polymerized on heating above 50 °C. [c] Purified by silica gel chromatography. [d] C: calcd, 60.89; found, 60.25. [e] Percent yield based on 3 as the starting material. [f] Percent yield based on 4a. [g] Percent yield based on 3a.

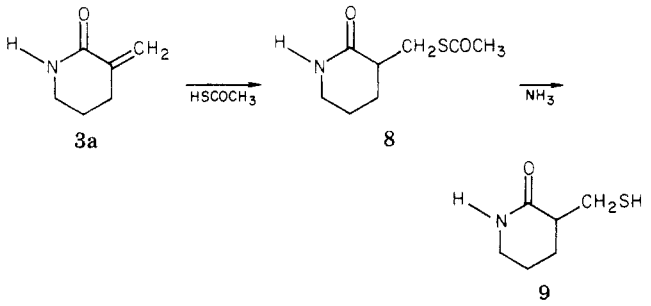
Table IV. Angiotensin-Converting Enzyme Inhibitory Activity and Physical Data on 3-(Mercaptomethyl)-2-oxo-1-pyrrolidine- and -piperidineacetic Acids and Derivatives[a]



compd	R	R'	*n*	X	% yield	mp, °C	recrystn solvent	formula	anal.	IC_{50}, M
6	H	$COCH_3$	1	OH	58[b]	138-140	EtOAc	$C_9H_{13}NO_4S \cdot C_{12}H_{23}N$	C, H, N	3.3×10^{-5}
6a	H	$COCH_3$	2	OH	88[b]	151-152	MeCN	$C_{10}H_{15}NO_4S \cdot C_{12}H_{23}N$	C, H, N	1.0×10^{-4}
7	H	H	1	OH	71[b]	142-144	MeCN	$C_7H_{11}NO_3S \cdot C_{12}H_{23}N$	C, H, N	3.7×10^{-6}
7a	H	H	2	OH	71[b]	138-140	MeCN	$C_8H_{13}NO_3S \cdot C_{12}H_{23}N$	C, H, N	1.0×10^{-6}
13	CH_3	$COCH_3$	2	OH	79[c]	85-90[d]	Et_2O/hexane	$C_{11}H_{17}NO_4S$	C, H, N	3.5×10^{-5}
14	1-Pr	$COCH_3$	2	OH	58[b]	120-122[d]	*e*	$C_{13}H_{21}NO_4S \cdot C_{12}H_{23}N$	C, H, N	3.0×10^{-5}
15	Ph	$COCH_3$	2	OH	41[b]	116-120[d]	*e*	$C_{16}H_{19}NO_4S \cdot C_{12}H_{23}N$	C, H, N	1.9×10^{-5}
18	H	$COCH_3$	2	NH_2	74	150-152	MeOH	$C_{10}H_{16}N_2O_3S$	C, H, N	N[f]
19	H	H	2	NH_2	100	138-140	EtOH	$C_8H_{14}N_2O_2S$	C, H, N	N
Captopril										1.2×10^{-8}

[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] Compound isolated as the dicyclohexylamine salt. [c] Compound isolated as a free acid. [d] Mixture of diastereomers. [e] Not recrystallized. [f] N = not active at 1.0×10^{-4}.

Scheme II



chlorobenzene at reflux. The structures of **3** and **3a** were confirmed by NMR, which showed in each case only an exocyclic double bond. Thus, in the case of **3**, the unequivalent vinyl protons appeared at 5.35 and 6.00 ppm and with **3a**, at 5.30 and 6.18 ppm. The starting alcohols **2** and **2a** were obtained in fair to good yields using calcium borohydride (sodium borohydride and calcium chloride) reduction of the corresponding esters **1** and ethyl 2-oxo-3-piperidinecarboxylate.

Carbethoxyalkylation of the anions of **3** and **3a** with ethyl bromoacetate gave **4** and **4a**. These oily esters were unstable products which gradually polymerized on standing at room temperature or heating. They were purified by silica gel chromatography or hydrolzyed directly with 1 N sodium hydroxide to the crystalline free acids **5** and **5a**. Carbethoxyalkylation with substituted α-haloacetic acid esters led to the racemic 3-methylene-2-oxo-1-piperidineacetic acids **10–12** (Table III).

Acetylthio derivatives **6**, **6a**, and **13–15** (Table IV) were synthesized by addition of ethanethioic acid to the corresponding unsaturated acids. Compounds **6** and **6a** were obtained as racemates. Compounds **13–15** were obtained as mixtures of two diastereomeric pairs of enantiomers, which were present in approximately equal ratios as indicated by TLC and NMR. Cleavage of the *S*-acetyl derivatives **6** and **6a** using ammoniacal methanol yielded the free mercaptans **7** and **7a**, which were characterized as cystalline dicyclohexylamine salts. Similar treatment of **13–15** gave intractable gums, and conversion to amine salts failed to yield any crystalline materials. The lactam 1-acetamides **16–19** were prepared to determine the effect of amide formation on activity.

Biological Results. The in vitro angiotensin-converting enzyme (ACE) inhibitory activities of a group of γ- and δ-lactams (Table IV) were determined. The most active compounds in this series, **7** and **7a**, were those possessing the free mercaptan and carboxylic acid functions. The IC_{50} values of these racemic structures were 3.7×10^{-6} and 1.0×10^{-6} M, respectively, compared to 1.2×10^{-8} M for captopril. The size of the lactam ring appears to have some effect on activity; thus, the six-membered lactam **7a** was about 4 times as active as the five-membered lactam **7**.

In studying the effects of varying the R group of type II compounds in the δ-lactam series ($n = 2$), it was necessary to compare the activities of the *S*-acetyl derivatives because the free mercaptans were not obtained in satisfactory crystalline form. In addition, the interpretation of the biological data were R was other than hydrogen (compounds **13–15**) was complicated by the fact that these compounds were a mixture of diastereomers. Based on the work in the captopril series,[1] where it was shown that activity of the stereoisomer with the *S,S* configuration was significantly greater than that of the other isomers, it was presumed that the observed biological activity was attributable to one of the four stereoisomers present. Testing results showed that the compound where R was hydrogen (**6a**) was least active, the compounds where R was alkyl (methyl and 1-propyl, **13** and **14**) were more active than **6a**, and the compound where R was phenyl (**15**) was most active.

Since captopril and its *S*-acetyl derivative ($IC_{50} = 1.2 \times 10^{-8}$ and 4.4×10^{-7}, respectively) were appreciably more active than the corresponding compounds in this series and since the oral activity of **7a** was not promising, synthetic work was not pursued further. Neither the amides **18** and **19** nor the intermediate 3-methylene derivatives in Table III were active.

Compound **7a** was the most potent compound and it was chosen for secondary evaluation against various agonists in isolated guinea pig ileum strips and against angiotensin I in conscious dogs.

Compound **7a** and captopril were evaluated for their effects on the contractile responses of guinea pig ileum to angiotensin I (AI; 2×10^{-8} M), angiotensin II (AII; 2×10^{-8} M), bradykinin (BK; 10^{-8} M), and carbachol (10^{-7} M). The IC_{50} for compound **7a** (dicyclohexylamine salt) was 2.2×10^{-5} M and 7×10^{-8} for captopril, whereas the BK PC_{50} for **7a** was 1.4×10^{-6} M and 1×10^{-8} M for captopril. Compound **7a** in concentrations of 3×10^{-5} M produced 20% reductions in the contractile responses to AII and carbachol. This was attributed to the dicyclohexylamine

portion of the salt, which alone at comparable concentrations produced similar reductions. Captopril had no effect on AII or carbachol at concentrations of 3×10^{-5} M.

Compound **7a** was evaluated for oral and iv ACE inhibitory activity in a conscious normotensive dog. Compound **7a** (as the dicyclohexylamine salt), 30 mg/kg iv, inhibited the pressor response to AI 67% and the response returned to 75% of the control after 24 min, whereas 30 mg/kg po (via gelatin capsule) produced only a brief 20% inhibition. Compound **7a** (as the free acid), 16 mg/kg iv, inhibited the pressor response to AI 77%, and the response returned to 75% of the control response after 24 min. In contrast, both oral and iv captopril demonstrated significantly greater activity than **7a**. Captopril, 0.3 mg/kg po, inhibited the AI response by an average ($N = 4$) of 75%, and the response returned after 225 min. Captopril, 0.3 mg/kg iv ($N = 1$), inhibited the pressor response to AI by 67%, and the response returned after 55 min.

Compound **7a** was also evaluated in a barbital sodium (300 mg/kg iv) anesthetized, artifically ventilated, bilaterally vagotomized mongrel dog against AI (0.3 μg/kg iv) and BK (1 μg/kg iv) in iv doses of 1.0 to 30.0 mg. The doses of **7a** which produced a 50% reduction in the AI response were 10–30 mg/kg compared to 0.03–0.1 mg/kg for captopril. The doses of **7a** that produced a 50% increase in the recovery of blood pressure following bradykinin were 10–30 mg/kg compared to approximately 0.01 mg/kg for captopril.

Conclusion

The γ- and δ-lactams (II) were active as angiotensin-converting enzyme inhibitors. This activity was apparently due to the presence and arrangement of the same functional groups that are also necessary for the good activity of captopril. Lactams of type II were generally less active than captopril, by a factor of about 100, indicating that activity drops considerably when a cyclic amino acid, such as proline, is not part of the molecule. It has been previously reported that amides of aliphatic amino acids, such as glycine, are also far less potent that I.[1] Although the tertiary amide feature of captopril was retained in compounds of type II, this was not enough to generate the high level of ACE inhibitory activity that is necessary in a useful drug.

Experimental Section

Melting points were determined with the Thomas-Hoover capillary melting point apparatus which was calibrated against known standards. IR spectra were run on a Digilab FTS 14 spectrometer. NMR spectra were run on a Varian EM 390 spectrometer using Me_4Si as an internal standard. Satisfactory elemental analyses (±0.4% of calculated values) were obtained for all compounds except where noted otherwise. Mass spectra were determined on a Finnigan 1015 spectrometer.

General Procedure A. Preparation of 3-Methylenelactam 1-Acetic Acids (and Esters). A solution of 0.28 mol of **3** or **3a** in 600 mL of toluene was cooled to 10 °C. With stirring, under nitrogen, a quantity of 0.28 mol of 50% sodium hydride–mineral oil was added over 5 min, allowing the temperature to reach 20 °C. After 10 min at 20 °C, the mixture was cooled to 15 °C and 0.28 mol of an appropriate α-bromo ester was added over 10 min. After stirring the mixture for 2 h at room temperature, the toluene was distilled off at reduced pressure. Ether (500 mL) was added to the residue. The solids were filtered and washed with ether, and the filtrate was concentrated to give a crude product containing mineral oil. This ester was used directly in the next hydrolysis step or, as in the case of **4a**, purification was accomplished by silica gel column chromatography.

Base Hydrolysis of the Above Ester. The crude ester was dissolved in 700 mL of ethanol. Sodium hydroxide (800 mL, 1 N) was added and the mixture was maintained at reflux for 15 min. The ethanol was distilled off at reduced pressure, and the separated mineral oil was extracted away with 300 mL of ether. The aqueous phase was treated with 200 mL of 4 N hydrochloric acid and concentrated to dryness in vacuo, removing the last amounts of water by adding ethanol and concentrating. The residue was triturated with 500 mL of warm methylene chloride and filtered, and the filter cake was washed with 100 mL of methylene chloride. The filtrate was concentrated to give a tacky product. This material was triturated with 100 mL of ether and filtered to give a solid product. Recrystallization from ethyl acetate gave pure white crystals of the 3-methylenelactam 1-acetic acid derivatives.

General Procedure B. Michael Addition of Ethanethioic Acid to α,β-Unsaturated Lactams. A solution of 0.05 mol of the appropriate α,β-unsaturated lactam in 30 mL of ethanethioic acid was warmed at 50 °C for 5 min. Toluene (75 mL) was added and the volatiles were removed at reduced pressure. The last traces of ethanethioic acid were removed by similar addition and removal of toluene. The solid products **8**, **13**, and **18** were purified by recrystallization, and the noncrystalline products **6**, **6a**, **14**, and **15** were converted to the crystalline dicyclohexylamine salts by addition of an equivalent amount of dicyclohexylamine to an ethyl acetate solution of the free acids.

General Procedure C. Preparation of Free Mercaptans via Ammonia Cleavage of *S*-Acetyl Derivatives. A methanol (200 mL) solution of 0.023 mol of 3-[(acetylthio)methyl]lactams (in the case of the acid lactams **6** and **6a**, the dicyclohexylamine salts were first converted to the free acids by ethyl acetate partition with a 5% potassium bisulfate solution of the dicyclohexylamine salts) was saturated with ammonia gas at 10 °C. The solution was allowed to stand at room temperature for 2 h, warmed on the steam bath for 5 min to drive off the excess ammonia, and concentrated to remove the solvent and to give the crude product, which in the case of **9** was a crystalline solid. In the case of the acid derivatives **7** and **7a**, the resulting noncrystalline ammonium salts were converted to the free acids by dissolution in 20 mL of water and filtration through a cation-exchange resin (AG-50W-X2, Bio-Rad Labs). The resin was washed with another 30 mL of water, and the filtrate was concentrated to dryness in vacuo, removing the last traces of water by ethanol addition and removal. Crystalline dicyclohexylamine salts were prepared by treating a solution of these free acids in ethyl acetate with an equivalent amount of dicyclohexylamine.

3-(Hydroxymethyl)-2-pyrrolidinone (2). A solution of 70.3 g (0.45 mol) of ethyl 2-oxo-3-pyrrolidinecarboxylate,[6] 49.6 g (0.45 mol) of calcium chloride, and 800 mL of methanol was cooled to 0 °C and treated with one portion of 16.9 g (0.45 mol) of sodium borohydride, keeping the temperature at 0–5 °C with cooling for 2 h. The mixture was allowed to warm to room temperature overnight. The solids were filtered and washed with 50 mL of methanol. The methanol filtrate was concentrated to ca. 300 mL volume and ca. 2 L of ether was added to precipitate a gummy material. After decantation, the gum was triturated with 200 mL of ether and decanted. Water (500 mL) was added to the gum, and the separated solid was filtered and washed with 200 mL of water. The aqueous filtrates were saturated with potassium carbonate and extracted with three portions of 600 mL of methylene chloride. The combined extracts were dried over potassium carbonate, filtered, and concentrated. Trituration with ether gave 21.0 g (48%) of product, mp 98–100 °C. Recrystallization from 2-propanol gave pure product: mp 100–101 °C; IR ($CHCl_3$) 3340 (NH), 1700 cm^{-1} (lactam C=O); NMR (Me_2SO-d_6) δ 1.8–2.4 (m, 3 H, H-3 and H-4), 3.15 (t, 2 H, H-5), 3.55 (t, 2 H, CH_2OH), 4.58 (t, 1 H, CH_2OH), 7.5 (br s, 1 H, NH). Anal. ($C_5H_9NO_2$) C, H, N.

3-(Hydroxymethyl)-2-piperidinone (2a). This compound was prepared from ethyl 2-oxo-3-piperidinecarboxylate[7] by the same procedure used to prepare **2**. Recrystallization from 2-propanol–hexane gave pure product in 66% yield: mp 81–83 °C (lit.[8] mp 79–80.5 °C); MS, m/e (relative intensity) 129 (M^+, 25),

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112 (25), 99 (100), 98 (70), 84 (35).

3-Methylene-2-pyrrolidinone (3). A quantity of 2.0 g of cuprous iodide was added to a stirred solution of 18.7 g (0.16 mol) of **2**, 100 mL of chlorobenzene, and 37.1 g (0.018 mol) of dicyclohexylcarbodiimide (DCC) at 125 °C. The mixture was heated at reflux for 10 min and cooled. Water (200 mL) was added and the mixture was stirred for 1 h. Ether (500 mL) was added and the entire mixture was filtered. The aqueous phase was separated. The organic phase was extracted with 200 mL of water, and this was combined with the first aqueous phase. The solution was charcoaled, filtered, saturated with potassium carbonate, and extracted with two 500-mL portions of methylene chloride. The organic extract was dried (K_2CO_3), filtered, and concentrated to give 8.6 g (56%) of **3**, mp 89–93 °C. Recrystallization from ethyl acetate gave pure product: mp 106–108 °C; IR (KBr pellet) 3200 (NH), 1700 (lactam C=O), 1670 cm^{-1} (C=C); NMR ($CDCl_3$) δ 2.9 (m, 2 H, H-4), 3.48 (t, 2 H, H-5), 5.35 (m, 1 H, C=CH_2), 6.00 (m, 1 H, C=CH_2), 7.8 (br s, 1 H, NH); MS, *m/e* (relative intensity) 97 (M^+, 100), 68 (20); TLC (silica gel/EtOAc), one spot, R_f 0.25. Anal. (C_5H_7NO) C, H, N.

3-Methylene-2-piperidinone (3a). This compound was prepared from **2a** by a procedure similar to that used to prepare **3**, except that boiling toluene was used as the solvent. The product was purified by distillation (yield 67%), bp 110 °C (1 mmHg). This material crystallized: mp 55–57 °C; IR (KBr pellet) 3240 (NH), 1673 (lactam C=O), 1628 cm^{-1} (C=C); MS, *m/e* (relative intensity) 111 (M^+, 100), 82 (44), 67 (11); NMR ($CDCl_3$) δ 1.90 (m, 2 H, H-5), 2.60 (m, 2 H, H-4), 3.40 (m, 2 H, H-6), 5.30 (m, 1 H, C=CH_2), 6.18 (m, 1 H, C=CH_2), 7.0 (br s, 1 H, NH). Anal. (C_6H_9NO) C, H, N.

3-Methylene-2-oxo-1-pyrrolidineacetic Acid (5). This compound was prepared by general procedure A from compound **3** and ethyl bromoacetate without isolation of the intermediate ester in 62% yield. Recrystallization from ethyl acetate gave pure product: mp 140–142 °C; IR (KBr pellet) 1740 (carboxy C=O), 1670 (C=C), 1630 cm^{-1} (lactam C=O); MS, *m/e* (relative intensity) 155 (M^+, 25), 111 (50), 110 (100), 83 (14); NMR (Me_2SO-d_6) δ 2.7 (m, 2 H, H-4), 3.42 (t, 2 H, H-5), 4.0 (s, 2 H, NCH_2COOH), 5.32 (m, 1 H, C=CH_2), 5.75 (m, 1 H, C=CH_2), 12.8 (br s, 1 H, COOH). Anal. ($C_7H_9NO_3$) C, H, N.

3-Methylene-2-oxo-1-piperidineacetic Acid Ethyl Ester (4a) and 3-Methylene-2-oxo-1-piperidineacetic Acid (5a). These compounds were prepared using general procedure A. The ester **4a** was purified by silica gel column chromatography using ethyl acetate as an eluant to give a 68% yield of viscous oil: TLC (silica gel/EtOAc), one spot, R_f 0.6; IR (film) 1745 (ester C=O), 1660 (C=C), 1618 cm^{-1} (lactam C=O); MS, *m/e* (relative intensity) 197 (M^+, 27), 152 (8), 124 (100), 110 (19), 96 (12), 81 (14), 67 (16); NMR ($CDCl_3$) δ 1.30 (t, 3 H, OCH_2CH_3), 1.95 (m, 2 H, H-5), 2.65 (t, 2 H, H-4), 3.50 (t, 2 H, H-6), 4.20 (m, 4 H, OCH_2CH$_3$ and NCH_2COOH), 5.32 (m, 1 H, C=CH_2), 6.25 (m, 1 H, C=CH_2). Anal. ($C_{10}H_{15}NO_3$) H, N; C: calcd, 60.89; found, 60.25.

The purified ester **4a** was hydrolyzed to the carboxylic acid **5a** according to general procedure A in 84% yield: mp 147–148 °C (from ethyl acetate); IR (KBr pellet) 1740 (carboxy C=O), 1655 (C=C), 1595 cm^{-1} (lactam C=O); MS, *m/e* (relative intensity) 169 (M^+, 28), 125 (57), 124 (100), 110 (11), 96 (12), 81 (10), 67 (10); NMR ($CDCl_3$) δ 1.8–2.0 (m, 2 H, H-5), 2.55 (t, 2 H, H-4), 3.45 (t, 2 H, H-6), 4.15 (s, 2 H, NCH_2COOH), 5.30 (m, 1 H, C=CH_2), 6.18 (m, 1 H, C=CH_2), 10.5 (s, 1 H, COOH). Anal. ($C_8H_{11}NO_3$) C, H, N.

3-[(Acetylthio)methyl]-2-oxo-1-pyrrolidineacetic Acid *N*-Cyclohexylcyclohexanamine Salt (6). This compound was prepared by general procedure B from **5**: yield, melting point, etc. are shown in Table IV. Anal. ($C_9H_{13}NO_4S$·$C_{12}H_{23}N$) C, H, N.

3-[(Acetylthio)methyl]-2-oxo-1-piperidineacetic Acid *N*-Cyclohexylcyclohexanamine Salt (6a). This compound was prepared by general procedure B from **5a**: yield, melting point, etc. are shown in Table IV; IR (KBr pellet) 1700 (*S*-acetyl C=O), 1650 (lactam C=O), 1630 cm^{-1} (carboxy anion C=O); NMR ($CDCl_3$) δ 1.0–2.1 (m, 24 H, dicyclohexane CH_2, H-4 and H-5), 2.25 (s, 3 H, SCOCH_3), 2.45 (m, 1 H, H-3), 2.7–3.4 (m, 6 H, CHN, CH_2S, H-6), 3.85 (q, 2 H, NCH_2COOH), 8.2 (br s, 2 H, NH and COOH). Anal. ($C_{10}H_{15}NO_4S$·$C_{12}H_{23}N$) C, H, N.

3-(Mercaptomethyl)-2-oxo-1-pyrrolidineacetic Acid *N*-Cyclohexylcyclohexanamine Salt (7). This compound was prepared by general procedure C from **6**: yield, melting point, etc. are shown in Table IV; IR (KBr pellet) 1680 (lactam C=O), 1640 cm^{-1} (carboxy anion C=O). Anal. ($C_7H_{11}NO_3S$·$C_{12}H_{23}N$) C, H, N.

3-(Mercaptomethyl)-2-oxo-1-piperidineacetic Acid *N*-Cyclohexylcyclohexanamine Salt (7a). This compound was prepared by general procedure C from **6a**: yield, melting point, etc. are shown in Table IV. Anal. ($C_8H_{13}NO_3S$·$C_{12}H_{23}N$) C, H, N.

Ethanethioic Acid *S*-[(2-Oxo-3-piperidinyl)methyl] Ester (8). This compound was prepared by general procedure B from **3a**: yield, melting point, etc. are shown in Table I; IR (KBr pellet) 3300 (NH), 1695 (*S*-acetyl C=O), 1675 cm^{-1} (lactam C=O), NMR ($CDCl_3$) δ 1.2–2.0 (m, 4 H, H-4, H-5), 2.2 (s, 3 H, SCOCH_3), 2.5 (m, 1 H, H-3), 3.0–3.4 (m, 4 H, CH_2S, H-6), 6.5 (br s, 1 H, NH). Anal. ($C_8H_{13}NO_2S$) C, H, N, S.

3-(Mercaptomethyl)-2-piperidinone (9). This compound was prepared by general procedure C from **8**: yield, melting point, etc. are shown in Table I; IR (KBr pellet) 2540 (SH), 1675 cm^{-1} (lactam C=O). Anal. ($C_6H_{11}NOS$) H, N; C: calcd, 49.62; found, 50.15.

α-Methyl-3-methylene-2-oxo-1-piperidineacetic Acid (10). This compound was prepared by general procedure A from **3a** and ethyl 2-bromopropanoate: yield, melting point, etc. are shown in Table III. Anal. ($C_9H_{13}NO_3$) C, H, N.

3-Methylene-2-oxo-α-propyl-1-piperidineacetic Acid (11). This compound was prepared by general procedure A from **3a** and ethyl 2-bromopentanoate: yield, melting point, etc. are shown in Table III. Anal. ($C_{11}H_{17}NO_3$) C, H, N.

3-Methylene-2-oxo-α-phenyl-1-piperidineacetic Acid (12). This compound was prepared by general procedure A from **3a** and ethyl α-bromobenzeneacetate: yield, melting point, etc. are shown in Table III. Anal. ($C_{14}H_{15}NO_3$) C, H, N.

3-[(Acetylthio)methyl]-α-methyl-2-oxo-1-piperidineacetic Acid (13). This compound was prepared by general procedure B from **10**. It was isolated as a crystalline free acid, mp 85–90 °C (ether–hexane). This material had a good elemental analysis (C, H, N) but was an inseparable mixture of diastereomers as indicated by TLC, which showed two overlapping spots at 0.40–0.60 and 0.45–0.65 (silica gel/20% MeOH–$CHCl_3$). Anal. ($C_{11}H_{17}NO_4S$) C, H, N.

3-[(Acetylthio)methyl]-2-oxo-α-propyl-1-piperidineacetic Acid (14). This compound was prepared by general procedure B from **11**: yield, melting point, etc. are shown in Table IV. Anal. ($C_{13}H_{21}NO_4S$·$C_{12}H_{23}N$) C, H, N.

3-[(Acetylthio)methyl]-2-oxo-α-phenyl-1-piperidineacetic Acid (15). This compound was prepared by general procedure B from **12**: yield, melting point, etc. are shown in Table IV. Anal. ($C_{16}H_{19}NO_4S$·$C_{12}H_{23}N$) C, H, N.

3-Methylene-2-oxo-1-pyrrolidineacetamide (16). A quantity of 2.38 g (0.013 mol) of crude ester **4** was stirred with 50 mL of concentrated ammonium hydroxide at room temperature overnight. The solution was concentrated to dryness at reduced pressure. The residue was heated with 75 mL of methanol and filtered to remove some insoluble material. Concentration gave 0.95 g (48%) of white crystals, mp 174–176 °C (polymerizes). Recrystallization from 2-propanol–hexane did not change the melting point: IR (KBr pellet) 3310 and 3160 (NH_2), 1705 (lactam C=O), 1675 (C=C), 1650 cm^{-1} (amide C=O); MS, *m/e* (relative intensity) 154 (M^+, 18), 137 (33), 110 (100), 96 (14), 83 (14); NMR (Me_2SO-d_6) δ 2.6 (m, 2 H, H-4), 3.3 (t, 2 H, H-5), 3.78 (s, 2 H, NCH_2CONH$_2$), 5.2 (m, 1 H, C=CH_2), 5.6 (m, 1 H, C=CH_2), 7.0 (br s, 1 H, CONH$_2$), 7.3 (br s, 1 H, CONH$_2$). Anal. ($C_7H_{10}N_2O_2$) C, H, N.

3-Methylene-2-oxo-1-piperidineacetamide (17). This compound was prepared from the ester **4a** by the same procedure used to prepare **16**: yield, melting point, etc. are shown in Table III; IR (KBr pellet) 3330 and 3175 (NH_2), 1705 (lactam C=O), 1655 (C=C), 1610 cm^{-1} (amide C=O). Anal. ($C_8H_{12}N_2O_2$) C, H, N.

Ethanethioic Acid *S*-[[1-(2-Amino-2-oxoethyl)-2-oxo-3-piperidinyl]methyl] Ester (18). This compound was prepared

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by general procedure B from 17: yield, melting point, etc. are shown in Table IV; IR (KBr pellet) 3380 and 3200 (NH_2), 1705 (S-acetyl C=O), 1670 (lactam C=O), 1635 cm^{-1} (amide C=O). Anal. ($C_{10}H_{16}N_2O_3S$) C, H, N.

3-(Mercaptomethyl)-2-oxo-1-piperidineacetamide (19). This compound was prepared by general procedure C from 18: yield, melting point, etc. are shown in Table IV; IR (KBr pellet) 3360 and 3180 (NH_2), 2540 (SH), 1665 (lactam C=O), 1630 cm^{-1} (amide C=O). Anal. ($C_8H_{14}N_2O_2S$) C, H, N.

Pharmacology Methods. Assay for in Vitro Angiotensin-Converting Enzyme Inhibitory Activity. ACE inhibitory activity was determined by means of a commercial kit for the radioassay of ACE activity (Ventrex Laboratories; Portland, Maine). In this assay, ACE inhibitory activity was determined by using unpurified guinea pig serum ACE in the presence and absence of the test compound. ACE from guinea pig serum and the test compounds were preincubated for 10 min before the addition of the labeled substrate [^{3}H]hippurylglycylglycine. After a 60-min incubation at 37 °C, the reaction was stopped by the addition of 0.1 N hydrochloric acid. ACE cleaves the hippuryl–glycyl bond to form the dipeptide glycylglycine and [^{3}H]-hippuric acid. The [^{3}H]hippuric acid was then extracted with ethyl acetate, and the ACE of a given sample was calculated as the amount of [^{3}H]hippuric acid generated. Activity is reported as the IC_{50}, which is the approximate molar concentration of test compound causing a 50% inhibition of the control converting-enzyme activity.

Guinea Pig Ileum Assay. Compounds were evaluated for their effects on contractile responses of guinea pig ileum to angiotensin I (AI; 2×10^{-8} M), angiotensin II (AII; 2×10^{-8} M), bradykinin (BK; 10^{-8} M), and carbachol (10^{-7} M). In this test, 3- to 5-cm segments of ileum from female guinea pigs were mounted in 50-mL baths containing aerated Tyrode's solution with a resting force of 0.2–0.5 g at 37 °C. The contractile responses were recorded via force-displacement transducers (FT .03C; Grass Instrument, Quincy, Mass.) and a strip chart recorder (Model 260; Gould-Brush, Cleveland, Ohio). Test drugs were added to the bath 2 min prior to the agonist. In a given experiment, four tissues were exposed to only one agonist and one antagonist. Activity was expressed as the approximate molar concentration producing 50% inhibition (IC_{50}) of AI, AII, or carbachol or a 50% potentiation (PC_{50}) of the bradykinin response.

Oral and Intravenous ACE Inhibitory Activity in a Concious Normotensive Dog. In these experiments, inhibition of the blood-pressure response to reproducible submaximal iv doses of AI was used as an index of ACE inhibition. Dogs were prepared for blood-pressure monitoring and iv drug injection by implantation of a silastic catheter in the left femoral artery and vein, respectively. The arterial catheter was connected to a pressure transducer (P23AA; Statham; Hato Rey, Puerto Rico), and blood pressure and heart rate were recorded on a strip chart recorder (Type RM dynograph, Beckman Instruments, Schiller Park, Ill). All experiments were performed in a single dog over a period of 6 days.

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Synthesis and Antiviral Activity of 1-(2-Deoxy-β-D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone

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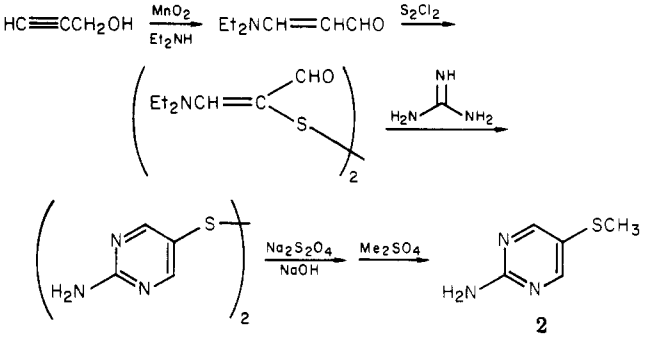
1-(2-Deoxy-β-D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone (**1b**) was synthesized via modification of the silyl method. **1b** inhibits the Herpes simplex virus type 1 (98%) and type 2 (97%) at a concentration which is nontoxic to human HeLa cells. The compound shows 50 times greater binding affinity (lower K_i) to the virus-specific thymidine kinase than to the thymidine kinase of uninfected HeLa cells.

A variety of 5-substituted 2′-deoxyuridine analogues have shown antiherpes activity. These include compounds where the 5-substituent is halogen, alkyl, vinyl, ethynyl, hydroxymethyl, or thiocyanato.[1] 5-(Methylmercapto)-2′-deoxyuridine, previously synthesized in this laboratory,[2] was also shown to be an inhibitor of herpes simplex type 1 virus (HSV-1).[3] We are currently studying 5-substituted 2-pyrimidinone nucleoside analogues. 2-Pyrimidinones may be considered to be uracil analogues lacking the N-3 hydrogen and the C-4 oxygen. Several nucleoside derivatives of 2-pyrimidinones have shown biological activity. 5-Fluoro-2-pyrimidinone deoxyriboside inhibited DNA synthesis in *Escherichia coli*, whereas the corresponding 2-pyrimidinone deoxyriboside was inactive.[4] 2-Pyrimidinone β-D-ribofuranoside was reported to inhibit the growth of *E. coli*[5] and vaccinia virus.[6] 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2-pyrimidinone 5′-phosphate and the corresponding 5-methyl-2-pyrimidinone nucleotide

Scheme I



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