by column chromatography of **2** (10 mg) in $CHCl_3$–Me_2CO (1:1) on silica gel (1 × 10 cm) or by stirring of **2** (10 mg) with silica gel (1.5 g) in Me_2CO at room temperature overnight.

Catalytic Hydrogenation of Compound 2. Compound 10. Compound **2** (350 mg, 1.14 mmol) in EtOAc (10 mL) was hydrogenated in the presence of prereduced PtO_2 (100 mg) at room temperature and atmospheric pressure. The uptake of H_2 ceased after 1 h. After removal of the catalyst, the filtrate was concentrated in vacuo and the residue was crystallized from Et_2O to furnish colorless needles of **10** in quantitative yield: mp 154–155 °C; IR (Nujol) 3490 (OH) and 1760 cm^{-1} (γ-lactone CO); NMR ($CDCl_3$) 0.99 (3 H, d, J = 6 Hz, CH_3-10), 1.02 (3 H, s, CH_3-5), and 3.35 [6 H, s, $N(CH_3)_2$].

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Mercaptoimidazolylpropionic Acid Hydrobromide. Inhibition of Tadpole Collagenase and Related Properties

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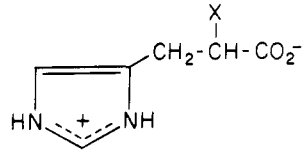
A mercapto analogue of histidine (**1**), (*RS*)-2-mercapto-3-(5-imidazolyl)propionic acid (**2**), was prepared by treatment of (*RS*)-2-bromo-3-(5-imidazolyl)propionic acid with trithiocarbonate. Decomposition of the resulting intermediate with hydrochloric acid followed by Sephadex G-15 chromatography permitted isolation of **2** as a hydrobromide complex having unusual stability and properties as evidenced by IR and ^1H NMR data. The potency of this complex in inhibiting tissue (*Rana catesbiana*) collagenase was estimated by radial diffusion assay. The amount of **2** required to produce 50% inhibition was 3.8 ± 1.5 mM compared to 8.7 ± 2.5 mM for cysteine. Preliminary tests of oxygen susceptibility, mutagenicity, and toxicity suggest that this substance may warrant study as a therapeutic agent for control of collagenase-linked corneal ulcerations.

Excess collagenase has been found in several pathological states including corneal ulceration,[1] tumor invasion,[2] and rheumatoid arthritis.[3] Previous studies from this[4] and other laboratories[5] have shown that histidine (**1**) is an inhibitor of bacterial collagenase. Related studies have



1, X = NH_2
2, X = SH
3, X = Br
4, X = Cl

shown that cysteine and certain other sulfhydryl compounds are also inhibitors of both bacterial and tissue collagenase.[6,7] The possibility that a synergistic effect on the inhibition of tissue collagenase might be realized by incorporation of both a sulfhydryl and an imidazole group into the same molecule[7] prompted us to prepare and test (*RS*)-2-mercapto-3-(5-imidazolyl)propionic acid (**2**).[8]

In this communication we describe the preparation and characterization of the hydrobromide of **2** and its inhibition of tadpole collagenase. In addition to its inhibitory behavior, this compound proved to have some unexpected chemical properties which may be of interest to a variety of other investigators studying mercapto compounds and their biological behavior.

Chemistry. A. Synthesis. (*RS*)-2-Bromo-3-(5-imidazolyl)propionic acid (**3**)[9] was treated with trithiocarbonate.[10] The resulting thioester was decomposed with HCl and the product was purified by gel filtration (Sephadex G-15) in aqueous acetic acid. The major component contained both an imidazole ring and an SH group. The same product was obtained when HCl was substituted for acetic acid in gel filtration. The product (mol wt 172) emerges from the column after the salt. Study of interactions of small molecules with cross-linked dextrans[11] has revealed that adsorption is enhanced when the excluding effect of charge is overcome.[12] The conclusion (below) that **2** chromatographs as a hydrobromide complex in which bromide ion is not free to exchange is consistent with these effects. In contrast, the chloro

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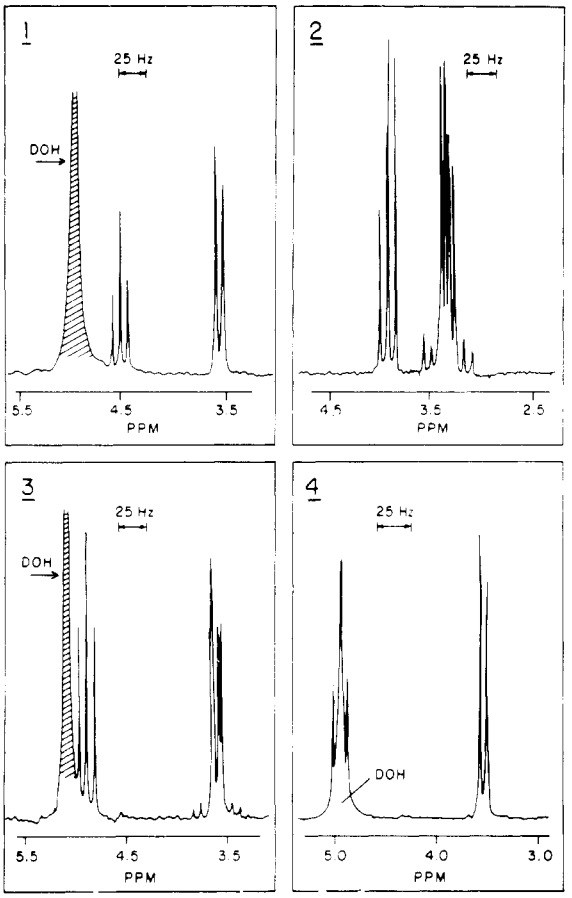

Figure 1. ^{1}H NMR splitting patterns of protons of C-2 and C-3 of compounds 1, 2 (as hydrobromide), 3 (as monohydrate), and 4. In each case ca. 80 mg of compound was dissolved in 1.0 mL of DCl acidified D_2O (pD ~0.4). Ppm are relative to DSS. Ring proton resonances in each case were similar to that of histidine.

analogue 4 (mol wt 175.5) coelutes with salt. TLC of column fractions revealed that the Ellman-positive material was essentially homogeneous. A trace contaminant, probably the disulfide of 2, may have been produced during TLC sample application. The elemental compositions[13] of samples isolated from both acetic acid or HCl gel filtration were consistent with the hydrobromide of 2. Clearly, the conjugate acid of 2 binds bromide with remarkable affinity since only a stoichiometric amount of this ion is released during synthesis of 2. The presence of bromide was verified by TLC using appropriate standards.

The imidazolylmethyl carbonium ion (*m/e* 81, base peak) and QM^+ of 173 in the CI mass spectrum confirm the skeletal structure of 2. In separate studies we have shown that 2 may be aminoethylated to give (*RS*)-2-(*S*-cysteaminyl)-3-(5-imidazolyl)propionic acid[14] or Gly ψ His.[15] The utility of aminoethylation in the synthesis of (*S*)-2-(*S*-cysteaminyl)-4-methylpentanoic acid,[16] or Gly ψ Leu,[15] also has been reported by this laboratory.[16]

B. Nature of the Noncovalent Complex. Elemental analyses and sulfhydryl titers of 1.0 SH group for fw 253 demonstrated that 2 was isolated as the hydrobromide. The moderate IR absorption at 2445 cm^{-1} compared with weak absorption at 2550–2600 cm^{-1} (more typically observed for SH groups) implies that the SH group is hydrogen bonded intramolecularly.[17,18] Compounds 1, 3, and 4 do not show absorption in this region.

Although the resonance of imidazole protons of 1–4 is unremarkable, the acylic protons exhibit distinctive coupling patterns (Figure 1) ranging from the deceptively simple,[19] nearly AX_2 pattern for the amino and chloro derivatives 1 and 4 to the ABC pattern for the mercapto and bromo derivatives 2 and 3. Interpretations of spectra of diastereotopic groups require appropriate caution.[20] In the present instance a wealth of ^{1}H NMR spectra of appropriate model compounds, including histidine and cysteine, facilitates the interpretation.[21] The proton spectra observed in acidic solution for both histidine and cysteine approach the doublet and triplet pattern of an AX_2 system.[22] Clearly, neither the electronegativity nor the anisotropy of the mercapto group is sufficient to promote the intrinsic nonequivalence of the methylene protons to an ABC resonance pattern. Similarly, the size of neither a mercapto group nor a bromine atom is likely to affect rotomer populations in compounds 2 and 3[23] relative to 1 and 4.

Table I. Half-Time of Sulfhydryl Group Loss of the Hydrobromide of 2 by Oxidation as Compared with *N*-Acetylcysteine and Cysteine

Compd tested	Concn, mM[a]	O_2 flow, mL/min[b]	Half-time, h[c]
N-Acetylcysteine	0.28	200	12.8
	0.27	350	10.9
Hydrobromide of 2	0.25	214	12.7
	0.14	400	8.7
Cysteine	0.24	214	6.4
	0.16	360	5.3

[a] The test sample was 35 mL, 0.1 M in phosphate buffer (pH 7.4) which was oxygen saturated. [b] Oxygen gas saturated with water vapor was bubbled through the solution at 20 °C by means of a gas dispersion tube. [c] Aliquots (200 μL) were removed and sulfhydryl group titers were determined by the quantitative Ellman determination.[31]

The remaining factor likely to account for the more complex spectra is interaction with a second molecule: HBr in the case of 2 and H_2O in the case of 3. Indeed, 3 is isolated as a stable monohydrate[9] while evidence for the complex of 2 with HBr is discussed above. In contrast, acids 1 and 4 are readily isolated free of other ligands. Accordingly, the ABC spectra of 2 and 3 may reasonably be interpreted as rotomer preference resulting from complex formation. Previous interpretations of ABC patterns of histidine derivatives at high pH have been attributed to rotomer preferences arising from intramolecular electrostatic interactions between imidazole rings and carboxylate anions.[23] Under the acidic conditions described in the present study, this electrostatic restraint is not operative and emphasizes the likelihood of conformational restraint due to complex formation.

Thus, the trends observed in the 90-MHz proton spectra constitute further clues to the unusual nature of the hydrobromide of mercaptoimidazolylpropionic acid. The physical data suggest that the stability of the hydrobromide of 2 may be due to cooperative effects that include hydrogen bonding of the sulfhydryl group and an imidazolium–bromide interaction.

C. Sensitivity to Oxygen. Acetylcysteine has been used in the treatment of corneal ulceration in preference to cysteine because of greater resistance of acetylcysteine to oxidation.[1] It was, therefore, of interest to obtain an indication of the relative ease of oxidation of compound 2 as compared to cysteine and acetylcysteine. The data in Table I indicate that the new inhibitor resembles acetylcysteine more closely than cysteine in its resistance to oxidation.

Biological Activity. A. Collagenase Inhibition. As indicated above, both histidine and cysteine have been reported to be inhibitors of bacterial and tissue collage-

Table II. Summary of I_{50} Values of Mercaptoimidazolylpropionic Acid (2) and Related Compounds for Inhibition of Tadpole Collagenase

Compd	N^a	I_{50} ± SEM, mM
(*RS*)-2-Mercapto-3-(5-imidazolyl)-propionic acid hydrobromide (2)	3	3.8 ± 1.5
L-Histidine (1)	3	⩾100
(*RS*)-2-Bromo-3-(5-imidazolyl)-propionic acid (3)	2	No inhibn
L-Cysteine	4	8.7 ± 2.5
N-Acetyl-L-cysteine	2	⩾100

[a] *N* is the number of determinations.

nase.[4,7] The inhibitory effect of these substances on the bacterial enzyme appears to be linked to an interaction with zinc; a similar mechanism may be operative with tissue collagenase.[24] The purpose of this study was to determine if a combination of structural features of the two inhibitors would result in a compound of synergistically enhanced potency.[7] Accordingly, the amino group of histidine was replaced with an SH group to produce **2**.

The inhibitory effect of **2** on tadpole collagenase was estimated using the recently described radial diffusion assay.[7] A comparison of the concentration of this new inhibitor required for 50% inhibition (I_{50}) of tissue collagenase to that required for some known inhibitors is shown in Table II. The results show that, neglecting the effect of the common carboxyl group, the compounds with two additional coordinating groups are the most inhibitory of the series. Thus, cysteine and histidine are more effective than *N*-acetylcysteine or bromoimidazolylpropionic acid, respectively. Substitution of the amino group of histidine with an SH group enhances its inhibitory character to the level of cysteine, but a strong synergistic effect was not realized by this combination. The possible modulating effect of the presence of a stoichiometric amount of bromide on the inhibitory behavior of **2** may warrant further study.

B. Mutagenicity. An indirect mutagenicity assay using repair-deficient strains of *Bacillus subtilis*[25] was performed to determine the possible activity of compound **2**. The tester strains were exposed to the mercapto compound (0.10 M) and chloroacetaldehyde (0.10 M), a known mutagen.[26,27] In all tests chloroacetaldehyde showed its characteristic growth inhibition,[26] whereas compound **2** did not inhibit any of the repair-deficient strains. These results suggest that mercaptoimidazolylpropionic acid is not mutagenic in microbial assays.

C. Acute Toxicity. Toxicity was estimated in 12-h starved Swiss Wistar male mice (weight range: 16–25 g). Solutions of the hydrobromide of **2** were adjusted to pH 6.9 prior to ip injection of 0.2 mL. Five mice were injected with normal saline as controls. The following dose levels were also given to each of five mice: 189, 418, 885, and 1980 mg/kg. Three days after injection weight gains of 22, 18, 23, and 18%, respectively, were observed for the test groups while a 20% weight gain was observed for the saline control group. All the mice remained in good health for the 7-day period of observation after injection.

Conclusions

Several properties of mercaptoimidazolylpropionic acid suggest that this collagenase inhibitor may warrant further study of its therapeutic potential. The hydrobromide is freely soluble in water. It compares favorably with cysteine in its ability to inhibit tadpole collagenase while being similar to acetylcysteine in resistance to oxidation. In addition, preliminary tests of mutagenicity and toxicity show no untoward effects. This favorable combination of properties suggests that mercaptoimidazolylpropionic acid may have potential for treatment of collagenase-linked corneal ulcerations.

Experimental Section

Melting points were determined on a Fischer-Johns apparatus and are uncorrected. IR data were recorded on a Perkin-Elmer 257 spectrophotometer. NMR spectra were obtained in the Fourier mode on a 90-MHz Bruker WH-90 DS spectrometer equipped with a Nicolet 1180 computer. Mass spectra were obtained with a Finnigan 3300-6110 computerized GLC–mass spectrometer. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. TLC cellulose-coated glass plates (Celplate, without fluorescent indicator) and silica gel plates (silica gel 60 F-254) were obtained from Brinkman Instruments, Westbury, N.Y. The developing solvent was butanol–acetic acid–water [12:3:5 (v/v)] unless otherwise indicated. Imidazole- and sulfhydryl-containing substances were located by Pauly[28] and Ellman[29] reagent stains, respectively. Quantitative determinations of imidazoles and sulfhydryl groups for column chromatography were performed with diazotized sulfanilic acid[30] and 5,5′-dithiobis(2-nitrobenzoic acid),[31] respectively.

Collagenase. Tadpole collagenase was prepared from tail fin and back skin by culture as described by Nagai et al.[32] and was purified by precipitation with ammonium sulfate and by chromatography on Sephadex G-200.[33] Pooled fractions containing collagenase activity were concentrated 20-fold by ultrafiltration using an Amicon UM2 filter at 4 °C.

Assay of Collagenase. Inhibition of tadpole collagenase was determined using a radial diffusion assay described by Yankeelov et al.[7] The substrate matrix consists of a Tris-buffered (pH 7.6) 1% Sea Plaque agarose support containing lathyritic rat skin collagen (0.1%). Assay dishes are prepared by adding the agarose–collagen suspension (6 mL, 37 °C) to 50 × 12 mm Petri dishes. Dishes are allowed to set at 20 °C for 2 h. Resulting gels are stored at 4 °C for at least 40 h during which time fibrillar collagen depolymerizes to give a clear matrix. Wells (3.0 mm) are punched (four to a dish) and 15 μL of test solution containing collagenase or collagenase and inhibitor is added. The plates are incubated for 41 h at 28 °C. Subsequent incubation at 37 °C for 2 h produces sharp zones of lysis. Activities are a function of zone diameter and are expressed as radial diffusion units.[7]

(*RS*)-2-Bromo-3-(5-imidazolyl)propionic Acid (3). This compound was either prepared as the monohydrate as previously described[9] or obtained from Pierce Chemical Co., Rockford, Ill. (*RS*)-2-Chloro-3-(5-imidazolyl)propionic acid (**4**) was prepared as previously described.[9]

(*RS*)-2-Mercapto-3-(5-imidazolyl)propionic Acid (2) Hydrobromide. The monohydrate of **3** (3.52 g, 14.8 mmol) was dissolved in 30 mL of water. Sodium thiocarbonate (70 mL, 40% solution) was added. After standing for 90 h at room temperature, 6 N HCl was slowly added with stirring until the solution was distinctly acidic. The mixture was filtered and the filtrate was evaporated to dryness (40 °C). The residue was dissolved in water and chromatographed on a 3.0 × 104 cm column of Sephadex G-15 using 0.1 M acetic acid as eluent. The fractions containing Ellman-positive material were combined, concentrated to 25 mL, and rechromatographed under the same conditions. The viscous oil solidified completely after storage at −20 °C for 1 month. The yield of microcrystalline white solid, mp 117–118 °C, was 0.72 g (18.5%). Anal. ($C_6H_8N_2O_2S$·HBr) C, H, N, S. Titration with Ellman's reagent revealed 1.03 sulfhydryl groups per fw 253. Alternatively, the hydrobromide of **2** may be isolated by gel filtration using 0.01 M HCl as eluent and then recrystallizing from 0.01 M HCl–aqueous acetone (mp 118–119 °C, 1.02 SH groups per formula weight).

TLC on cellulose plates showed essentially one component (R_f 0.51) which stained with both Pauly and Ellman reagents. A trace contaminant (R_f 0.28) was Pauly positive and Ellman negative. The amount of the contaminant could be increased by prior air oxidation of the sample and decreased by treatment with dithiotreitol. On silica gel the R_f value of the hydrobromide of **2** is 0.11 compared to 0.18 for **3**: IR (KBr) 3350–3600, 2445, 1710, 1620 cm^{-1}; NMR δ 3.3 (2 H), 3.9 (1 H), 7.43 (1 H), 8.70 (1 H); MS *m/e* 172 (M^+), 81 (M − 91), 173 (QM^+).

The halogen present was identified by TLC in the following manner. Silica gel plates were freed of interfering material by a full-length sham chromatographic development in 1-propanol–concentrated ammonium hydroxide [25:11 (v/v)]. The plates were air-dried prior to application of the test samples. Standards of NH_4Br (10 mg/mL) and NH_4Cl (10 mg/mL) were compared to the hydrobromide of **2** (20 mg/mL) using the propanol–ammonia development. Plates were dried with a stream of warm air and then placed in an oven at 120 °C for 5 min. The plates were sprayed with silver nitrate (1% in 95% acetone–water), dried, and placed under an ultraviolet (254 nm) lamp for 10 min. The halides appeared as dark spots against a white background. R_f values: NH_4Br, 0.54; NH_4Cl, 0.50; hydrobromide of **2**, 0.54. The Pauly-positive component of **2** hydrobromide was located at R_f 0.39.

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Nucleoside 5′-Monophosphate Analogues. Synthesis of 5′-Sulfamino-5′-deoxynucleosides

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The synthesis of two new nucleotide analogues is described. 5′-Sulfamino-5′-deoxyadenosine (**1**) was prepared by reaction of 5′-amino-5′-deoxyadenosine with $(CH_3)_3N{\cdot}SO_3$, and 5′-sulfamino-5′-deoxythymidine (**2**) was prepared from 5′-amino-5′-deoxythymidine by a similar reaction. The 5′-sulfamino nucleosides are shown to be quite stable to hydrolysis in acidic or basic aqueous solution. Tests show that these compounds do not inhibit the growth of *Escherichia coli* or L1210 cells at concentrations $<10^{-4}$ M. At 10^{-4} M compound **2** was found to give 70% inhibition of the replication of herpes simplex virus (type 1) with no effect on host cell growth (CV-1 monkey line).

In recent years there has been a great deal of interest in the preparation of analogues of nucleoside 5′-monophosphates. Much of this interest is a result of the knowledge that natural nucleosides require phosphorylation at their 5′ position before they can be utilized for many metabolic functions. Similarly, many therapeutically useful nucleoside analogues, such as arabinosylcytosine and 5-fluoro-2′-deoxyuridine, must be phosphorylated in vivo before they exhibit biological activity.[1] The analogues of nucleoside 5′-monophosphates not only provide potential new drugs but also they are important tools in the study of enzyme mechanisms and biochemical pathways.[2]

We wish to report the synthesis of the first two compounds (**1** and **2**) of a new class of nucleoside 5′-monophosphate analogues, the 5′-sulfamino-5′-deoxynucleosides. These analogues are of special interest for several reasons. The sulfamino group is very stable in aqueous solution and is not expected to cleave easily under physiological con-

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