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Antitumor Agents. 31.[1] Helenalin *sym*-Dimethylethylenediamine Reaction Products and Related Derivatives

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Several novel cyclopentenone bearing lactams and related derivatives have been synthesized as potential alkylating antitumor agents. The synthesis of these compounds involved the reaction of helenalin with *sym*-dimethylethylenediamine. These lactams were initially formed by a Michael addition of the amine to the α-methylene grouping of the γ-lactone ring, followed by a nucleophilic ring closure by the attack of the second amine group on the γ-lactone carbonyl. Reaction of helenalin (**1**) with dimethylamine gave, in addition to the single Michael reaction adduct (**2**) of the γ-lactone ring, a new double Michael addition product (**9**). The regeneration of **1** from **2** could be effected in 50% yield by silica gel column chromatography. In vitro assay for the cytotoxicity of these compounds against the growth of tissue culture cells originating from human epidermoid carcinoma of the larynx (H.Ep.-2) showed decreased significant activity due to the loss of the α-methylene-γ-lactone alkylating moiety. Cytotoxicity and in vivo antitumor activity in Walker 256 carcinosarcoma screen were enhanced with the introduction of a cinnamate ester group to the parent molecule. Preliminary in vivo tumor assay also indicated that compounds possessing a cyclopentenone and a C-6 hydroxyl group in either a bicyclic ring system or a tricyclic ring system with a saturated α-methylene grouping of the γ-lactone ring were active against Walker 256 carcinosarcoma growth in rats and marginally active against P-388 lymphocytic leukemia in mice.

Helenalin *sym*-dimethylethylenediamine reaction products were synthesized from helenalin so that the structure–antitumor relationships[2] would be expanded and those results are now reported. The first objective was to determine if the tricyclic ring system of helenalin itself contributes significantly to antitumor or cytotoxic activity. These *sym*-dimethylethylenediamine products were obtained by cleaving the γ-lactone ring of helenalin. The β-unsubstituted cyclopentenone ring was retained since it has been shown that this system contributed significantly to in vitro cytotoxicity (H.Ep.-2)[3,4] and in vivo antitumor activity (Walker 256 carcinosarcoma in rats).[5] The second objective was to generate a C-8 hydroxyl group in addition to the C-6 hydroxyl function for the subsequent introduction of diester moieties, such as cinnamoyl groups, for possible enhancement of the in vivo antitumor activity. The cinnamate ester of helenalin was found to be more cytotoxic than helenalin.[6]

Chemistry. Sesquiterpene lactones containing an α-methylene-γ-lactone grouping are known to form readily the monoadducts with secondary amines via Michael type reaction. For example, the α-methylene moiety of helenalin (**1**) forms an adduct (**2**) with dimethylamine.[3] With this in mind, it was thought that the γ-lactone ring of helenalin might be cleaved and thus generate the desired C-8 free hydroxyl group if an intramolecular nucleophilic attack of the γ-lactone carbonyl could be accomplished by a second amine group which is available in the initial amine adduct. Among the several secondary diamines examined, it was found that reaction of helenalin with *sym*-dimethylethylenediamine gave rise to the expected novel lactam **3** which was stable. Compound **3** was formed by an initial Michael addition of *sym*-dimethylethylenediamine to the α-methylene grouping of **1**, followed by a subsequent nucleophilic ring closure by attack of the second amine group on the γ-lactone carbonyl. The lactam **3**, $C_{19}H_{30}O_4N_2$, mp 209 °C, showed IR bands ($CHCl_3$) at 3540, 3250 (OH), 1700 (cyclopentenone), and 1630 cm^{-1} (amide C=O), and the absence of the lactonic carbonyl absorption. The NMR spectrum ($CDCl_3$) of the lactam was in accord with the assigned structure **3**, i.e., it indicated the presence of two *N*-methyl groups [δ 2.33 (3 H, s, CH_2N-*Me*) and 3.05 (3 H, s, CON-*Me*)], two *C*-methyl groups [δ 1.16 (3 H, d, J = 6 Hz, Me-10) and 1.57 (3 H, s, Me-5)], and a cyclopentenone ring system [δ 6.09 (1 H, dd, J = 3.0 and 6.0 Hz, H-3) and 7.81 (1 H, dd, J = 2.0 and 6.0 Hz, H-2)].

Similar treatment of helenalin acetate (**4**) with *sym*-dimethylethylenediamine led to the lactam acetate **5**. Esterification of **5** with cinnamoyl chloride yielded the

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Table I. Cytotoxicity and Physical Constants of Helenalin *sym*-Dimethylethylenediamine Reaction Products and Related Compounds

Compd	Formula	Analyses[a]	Mp, °C	Recrystn solvent	ED_{50},[b] μg/mL (H.Ep.-2)
1	$C_{15}H_{18}O_4$		170-172[c]	c-C_6H_{12}	0.08
2	$C_{17}H_{25}O_4N$		214 dec[c]	$CHCl_3$-EtOH	0.60
3	$C_{19}H_{30}O_4N_2$	C, H, N	209	Me_2CO-Et_2O	1.87
4	$C_{17}H_{20}O_5$		181[d]	CH_2Cl_2	0.29
5	$C_{21}H_{32}O_5N_2$	C, H, N	208	Me_2CO	4.96
6	$C_{30}H_{38}O_6N_2$	*e*	Oil	*j*	0.84
7	$C_{28}H_{36}O_5N_2$	*f*	Oil	*j*	2.89
8	$C_{37}H_{42}O_6N_2$	*g*	Oil	*j*	0.75
9	$C_{19}H_{32}N_2O_4$	C, H, N	205-206 dec	$CHCl_3$-Et_2O	0.85
10	$C_{17}H_{29}O_4N$	C, H, N	154-155	CH_2Cl_2-Et_2O	3.69
11	$C_{17}H_{27}O_4N$		161-162.5[c]	CH_2Cl_2-Et_2O	6.04
12	$C_{15}H_{20}O_4$		223-226[c,h]	CH_3CN	0.81
13	$C_{24}H_{24}O_5$		*i*		0.04

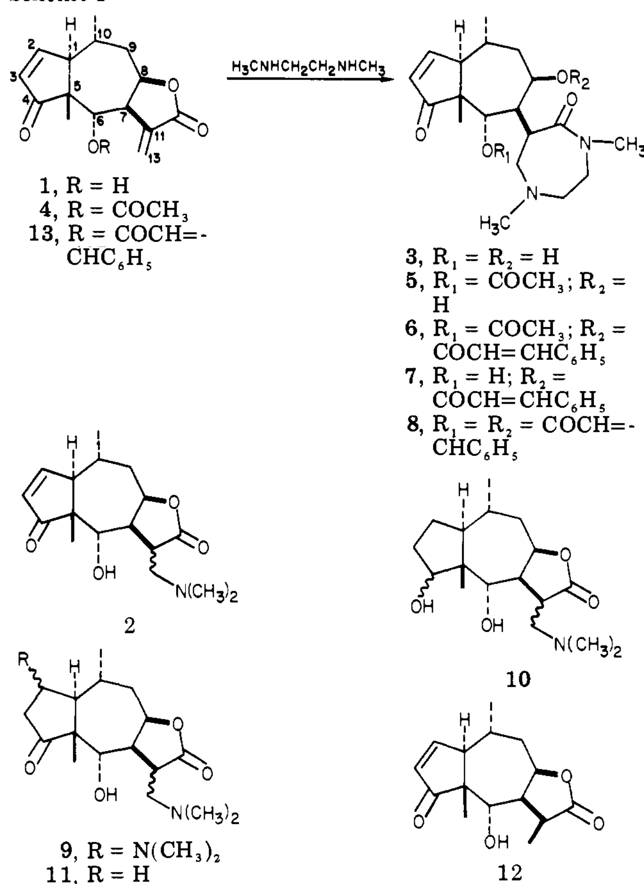
[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. [b] The values of ED_{50} are used for expressing the potency of cytotoxicity which is the calculated effective dose that inhibits the net cell growth to 50% of control growth. An ED_{50} of ≤4 μg/mL is required for significant cytotoxicity. [c] See ref 7. [d] See ref 8. [e] *m/e* 522.2738 ($C_{30}H_{38}O_6N_2$). [f] *m/e* 480.2621 ($C_{28}H_{36}O_5N_2$). [g] *m/e* 610.3037 ($C_{37}H_{42}O_6N_2$). [h] See ref 9. [i] See ref 6. [j] An examination of this compound on TLC in various solvent systems showed the presence of only a single spot. Spectral data of this compound were also in accord with the assigned structure.

corresponding acetyl cinnamate 6. Esterification of 3 with the same reagent gave rise to a mixture of mono- (7) and dicinnamate (8) which was separated by preparative thin-layer chromatography. For the structure–activity correlation purposes, reaction of helenalin with dimethylamine was carried out. It gave, in addition to the previously reported dimethylamine adduct 2,[3] a new double Michael addition product, compound 9, which could be converted into compound 2 by recrystallization from ethanol–chloroform via a β-elimination of the 2-dimethylamino group of the cyclopentanone. It should also be noted that the regeneration of the α-methylene-γ-lactone ring bearing helenalin (1) from its corresponding dimethylamino derivative 2 could be effected in 50% yield by silica gel column chromatography. Compound 2 was also reduced catalytically to the diol 10 for a better insight of the cytotoxic effect of the cyclopentanone ring of compound 11 (Scheme I).

Biological Results. Compounds prepared in this study were first assayed for their cytotoxicity against the growth of tissue culture cells originating from human epidermoid carcinoma of the larynx (H.Ep.-2) according to a rapid microtiter method previously described.[12] A comparison of the ED_{50} values for the cytotoxicity of the compounds listed in Table I disclosed that the loss of the α-methylene-γ-lactone alkylating moiety[13] via γ-lactone ring cleavage as in compounds 3 and 5–8 results in decreased cytotoxicity (compare compound 3 with 1, 5 and 6 with 4, and 7 and 8 with 13). Cytoxicity is enhanced significantly when a cinnamate ester group is added to the parent molecule as observed previously[6] (compare compound 8 with 3, 6 with 5, and 8 with 7). The cyclopentenone bearing tricyclic compounds 2 and 12 are only two- to threefold more cytotoxic than the bicyclic lactam 3. The significant level of cytotoxicity shown by the double Michael addition product 9 may be attributable to the regeneration of the cyclopentenone alkylating center[5] as in 2 in the tumor cell (compare compound 9 with 2) since removal of this dimethylamino substituent at the 2 position leads to compound 11 which is only marginally active. Compound 10 shows approximately the same marginal cytotoxicity as 11.

Compounds prepared in this study were also evaluated for their in vivo antitumor activity against the Walker 256 carcinosarcoma in Sprague–Dawley male rats (~100 g) and

Scheme I



the P-388 lymphocytic leukemia in male DBA/2 mice (~20 g) according to standard NCI protocols.[10] As shown in Table II, compounds 1, 3, 8, 9, 12, and 13 exhibited significant activity in Walker 256 carcinosarcoma in rats at a low dose (2.5 mg/kg). The dicinnamate 8 is more active than the diol 3 in this Walker 256 system. Compounds 1–3, 7, 12, and 13 were marginally active in the P-388 assay. These data would indicate that a cyclopentenone and a C-6 free hydroxyl group in a bicyclic ring system (such as in compounds 3 and 7) or in a tricyclic ring system in which the α-methylene grouping of the γ-lactone ring is saturated (such as in compounds 2 and 12) are

Table II. Effects of Helenalin *sym*-Dimethylethylenediamine Reaction Products and Related Derivatives on Inhibition of Tumor Growth

		P-388 lymphocytic leukemia		Walker 256 ascites	
Compd	N^d	Av days survived	T/C[a]	Av days survived	T/C[a]
1	6	13.4/10.6	127 (16.8)[e]	26.33/8.33	316 (2.5)
2	6	13.0/10.0	130 (25)		
3	6	12.0/9.5	126 (25)	21.25/12.50	170 (2.5)
4	6	8.0/10.2	78 (25)		
5	6	10.2/10.2	100 (25		
6	6	10.4/10.2	102 (25)		
7	6	12.8/10.4	123 (10)		
8	6	10.4/10.4	100 (10)	36.0/12.5	288 (2.5)
9	6	10.6/10.4	102 (10)	21.5/15.8	136 (2.5)
12	6	11.9/8.7	138 (48)	15.0/7.25	207 (2.5)
13	6	12.2/9.9	123 (4)	27.0/17.2	157 (2.5)
Melphalan[b]	6	16.3/9.68	168 (25)	23.0/7.25	317 (2.5)
5-Fluorouracil[c]	6	19.7/10.6	186 (25)		

[a] A compound is active if it exhibits a T/C (mg/kg) ⩾125%.[10] [b] Wellcome Research Laboratories, Research Triangle Park, N.C. [c] Calbiochem, La Jolla, Calif. [d] N is the number of animals per group. [e] See ref 11 for T/C values at different dose levels. Toxicity day is 4 days after the day of the first injection; the number in parentheses represents the dose required for no toxicity to be demonstrated by day 4, i.e., all six animals survived day 4.

responsible for significant in vivo activity in the Walker 256 and the P-388 screens. Further investigation of the structure–activity relationships of cyclopentenone bearing sesquiterpene lactones and related compounds is in progress.

Experimental Section

Chemistry. Unless otherwise specified, melting points were determined on a Thomas-Hoover melting point apparatus and were corrected. NMR spectra were measured with a Jeolco C-60 HL spectrometer (Me_4Si) and chemical shifts reported in δ (ppm) units: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and the J values in hertz. Silica gel for preparative TLC refers to Merck silica gel GF-254; and silica gel for the TLC refers to Merck silica gel G developed with chloroform–acetone (1:1) and visualized by spraying with sulfuric acid and heating. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga.

Reaction of Helenalin with *sym*-Dimethylethylenediamine. Compound 3. To a solution of helenalin (1) (200 mg, 0.76 mmol) in absolute EtOH (5 mL) was added *sym*-dimethylethylenediamine (49 mg, 0.57 mmol). The mixture was stirred at room temperature for 24 h. After evaporation of the solvent in vacuo, the brown residue was purified by preparative TLC followed by recrystallization from Me_2CO–Et_2O (1:1) to yield the lactam 3 as colorless prisms (95 mg, 36%): mp 209 °C. The relevant features of the IR and NMR data are noted and described in the Chemistry section.

Reaction of Helenalin Acetate with *sym*-Dimethylethylenediamine. Compound 5. A solution of helenalin acetate (4) (150 mg, 0.56 mmol) in absolute EtOH (3 mL) was treated with *sym*-dimethylethylenediamine (150 mg, 1.70 mmol) at room temperature for 50 h. The residue was purified by preparative TLC ($CHCl_3$–Me_2CO, 1:2) and recrystallized from Me_2CO to afford 5 as colorless prisms (120 mg, 62%): mp 208 °C; IR 3240 (OH), 1739 (OAc), 1715 (cyclopentenone CO), and 1634 cm^{-1} (lactam C=O); NMR ($CDCl_3$) 1.17 (3 H, d, J = 6.0 Hz, CH_3-10), 1.62 (3 H, s, CH_3-5), 1.93 (3 H, s, OAc), 2.34 (3 H, s, NCH_3), 3.06 (3 H, s, lactam NCH_3), 4.11 (1 H, m, H-8), 5.41 (1 H, d, J = 8.0 Hz, H-6), 6.05 (1 H, dd, J_A = 3.0 Hz, J_B = 6.0 Hz, H-3), 7.21 (1 H, OH), and 7.71 (1 H, dd, J_A = 2.0 Hz, J_B = 6.0 Hz, H-2).

Esterification of Compound 5 with Cinnamoyl Chloride. Compound 6. To a solution of compound 5 (50 mg, 0.13 mmol) in anhydrous benzene (2 mL) was added pyridine (1 mL) and cinnamoyl chloride (250 mg, 1.51 mmol). The solution was allowed to stand at room temperature for 20 h and then evaporated in vacuo. The product was extracted with Et_2O and the Et_2O extract was washed with 5% $NaHCO_3$ and H_2O, dried (anhydrous Na_2SO_4), and evaporated. The yellowish oil was purified by preparative TLC to furnish pure 6 as colorless oil (65 mg, 97%): IR ($CHCl_3$) 1742 (OAc), 1715 (cinnamate CO and cyclopentenone CO), 1645 (lactam CO), and 1635 cm^{-1} (shoulder, C=C); NMR ($CDCl_3$) 1.35 (3 H, d, J = 6.0 Hz, CH_3-10), 1.37 (3 H, s, CH_3-5), 2.00 (3 H, s, OAc), 2.43 (3 H, s, NCH_3), 2.98 (3 H, s, lactam NCH_3), 5.55 (1 H, s, H-6), 5.66 (1 H, m, H-8), 6.13 (1 H, dd, J_A = 6.0 Hz, J_B = 3.0 Hz, H-3), 7.78 (1 H, dd, J_A = 2.0 Hz, J_B = 6.0 Hz, H-2), 7.55 (5 H, aromatic protons), 6.33 (1 H, d, J = 17.0 Hz, COC*H*=CHPh), and 7.82 (1 H, d, J = 17.0 Hz, COCH=C*H*Ph).

Esterification of Compounds 3 with Cinnamoyl Chloride. Compounds 7 and 8. A mixture of compound 3 (100 mg, 0.29 mmol) and cinnamoyl chloride (500 mg, 3.01 mmol) in dry benzene (2 mL) and pyridine (1 mL) was allowed to stand at room temperature for 20 h. The reaction mixture was worked up in an analogous manner as described for the conversion of 5 to 6. The oily residue was chromatographed on Al_2O_3 (Brockmann, Stufe II, 30 × 1.5 cm) using benzene as eluent. The benzene eluates were further purified by preparative TLC ($CHCl_3$–Me_2CO, 2:1) to yield two pure compounds, 7 (70 mg, 51%) and 8 (70 mg, 40%).

Compound 7 (colorless oil): IR ($CHCl_3$) 3200–3400 (OH), 1712 (cinnamate CO and cyclopentenone CO), 1645 (lactam CO), and 1589 cm^{-1} (C=C); NMR ($CDCl_3$) 1.15 (3 H, d, J = 6.0 Hz, CH_3-10), 1.40 (3 H, s, CH_3-5), 2.22 (3 H, s, NCH_3), 2.97 (3 H, s, lactam NCH_3), 4.09 (1 H, d, J = 4.5 Hz, H-6), 5.91 (1 H, m, H-8), 6.12 (1 H, dd, J_A = 6.0 Hz, J_B = 3.0 Hz, H-3), 7.75 (1 H, dd, J_A = 6.0 Hz, J_B = 2.0 Hz, H-2), 7.35–7.60 (5 H, m, aromatic protons), 6.91 (1 H, d, J = 16.0 Hz, COC*H*=CHPh), and 7.77 (1 *H*, d, J = 16.0 Hz, COCH=C*H*Ph).

Compound 8 (colorless oil): IR ($CHCl_3$) 1718 (cinnamate CO and cyclopentenone CO), 1647 (lactam CO), and 1589 cm^{-1} (C=C); NMR ($CDCl_3$) 1.35 (3 H, d, J = 6.0 Hz, CH_3-10), 1.36 (3 H, s, CH_3-5), 2.43 (3 H, s, NCH_3), 2.97 (3 H, s, lactam NCH_3), 5.66 (1 H, br s, H-6), 5.72 (1 H, m, H-8), 6.07 (1 H, dd, J_A = 6.0 Hz, J_B = 3.0 Hz, H-3), 7.70 (1 H, dd, J_A = 6.0 Hz, J_B = 2.0 Hz, H-2), 7.30–7.60 (10 H, m, aromatic protons), 6.28 (1 H, d, J = 16.0 Hz), 6.32 (1 H, d, J = 16.0 Hz), 7.65 (1 H, d, J = 16.0 Hz), and 7.67 (1 H, d, J = 16.0 Hz) (two cinnamates CH=CH).

Reaction of Helenalin with Dimethylamine. Compounds 2 and 9. To an ice-cold solution of helenalin (1, 500 mg, 1.90 mmol) in absolute EtOH (10 mL) was passed dry dimethylamine for 5 min. The resulting mixture was stored overnight in the refrigerator. The solid formed was filtered and washed with cold EtOH (2 mL). Fractional recrystallization from $CHCl_3$–Et_2O gave a bis adduct, compound 9: mp 205–206 °C dec; IR (Nujol) 3420 (OH), 2782 (NCH_3), 1770 (γ-lactone CO), and 1725 cm^{-1} (cyclopentanone CO); NMR ($CDCl_3$) 1.12 (3 H, s, CH_3-5), 1.35 (3 H, d, J = 6.0 Hz, CH_3-10), 2.23 [6 H, 2, $N(CH_3)_2$], and 2.30 [6 H, s, $N(CH_3)_2$].

The mother liquor after the removal of 9 was further concentrated and the residue was recrystallized from $CHCl_3$–EtOH to yield the monoadduct, compound 2: mp 214 °C dec. The IR and NMR spectra of 2 are identical with those previously reported.[3]

Conversion of Helenalin Dimethylamine Adduct (2) to Helenalin (1). This could be readily done in 50% yield either

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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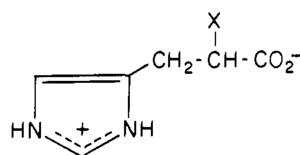
A mercapto analogue of histidine (**1**), (*RS*)-2-mercapto-3-(5-imidazolyl)propionic acid (**2**), was prepared by treatment of (*RS*)-2-bromo-3-(5-imidazolyl)propionic acid with trithiocarbonate. Decomposition of the resulting intermediate with hydrochloric acid followed by Sephadex G-15 chromatography permitted isolation of **2** as a hydrobromide complex having unusual stability and properties as evidenced by IR and ^{1}H 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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