(bp 30-60°) followed by recrystallization from CH_2Cl_2 yielded analytically pure 2i, mp 108-111.5°. *Anal.* ($C_{19}H_{18}Cl_2O_5S$) C, H, Cl, S.

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Biologically Oriented Organic Sulfur Chemistry. 12. Further Principles of Structure–Activity Relationships for Penicillamine Analogs and Derivatives[1,†]

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Further congeners of penicillamine (1) were studied for relation of structural factors to reduction of the skin tensile strength (sts) of rats, *in vivo*. The results are relevant to collagen biochemistry and perhaps to the mechanism by which 1 acts in rheumatoid arthritis. Previous conclusions as to effects on sts were confirmed and extended, *viz.*, the apparent necessity of the functions SH and CO_2H (inactivity of the disulfide and of amides of 1); the feasibility of skeletal variation (activity of a cyclopentyl variant, 10), but within limitations (both branching carbons at the β position seem essential); and the feasibility of latentiating 1 [activity of the 2,2-dimethylthiazolidine 13 and of the zinc(II) chelate 16]. The three compounds 10, 13, and 16 thus are added to the very few known to have the effect of 1 in reducing sts. Relevant chemical features that emerged were these: rapid solvolysis of an α-amino β-thiolactone salt 14, relative to the amide 18, which points to a neighboring-group effect of $-NH_2$ on $-C(O)-$; conversion of 14 to a polymer of 1, *via* its conjugate base [with further indication of a neighboring-group effect of $-NH_2$ on $-C(O)-$]; and the first isolation of the much studied (in solution) zinc(II) chelate of 1 (16).

Penicillamine (1) has shown favorable effects on laboratory parameters and clinical aspects of rheumatoid arthritis.[2] When fed to rats, it also reduces skin tensile strength (sts) and solubilizes collagen.[3,4] While there is no evidence to suggest that the effect of 1 on collagen (a principal protein of connective tissue) and its efficacy in rheumatoid arthritis are related, these studies were undertaken in order to shed light on this question.[5] Moreover, in several experimental models currently employed in studying antiarthritic drugs, 1 was without marked activity.[5] Because of the consistent effect of 1 on sts, on the other hand, the sts model was selected for the screening of analogs.[5] In any event, whether or not there ultimately proves to be a relation to rheumatoid arthritis, sts is interesting and important *per se* in relation to collagen biosynthesis.

A previous paper reported studies of structural features of 1 necessary for reduction of sts and of the possible latentiation of 1 as a means of increasing the activity in sts effects and/or decreasing the toxicity;[5] the rationale for the present studies also was discussed.[5] This paper reports a continuation of that study.

Biological Results (Table I). Functional group variations may be considered first. Previous variations suggested that the CO_2H, NH_2, and SH moieties of 1 all were necessary for reduction of sts.[5] The importance of the SH group now has been confirmed by inactivity of the disulfide 2 of D-penicillamine. The amides 3 and 4, carboxyl-blocked analogs of DL-1, also were inactive, confirming the apparent necessity of the free carboxyl group. Inactivity of 5, which we thought might solubilize collagen by forming a thiazolidine (*cf.* ref 5), also may stem from lack of CO_2H; of course, inactivity also may be a consequence merely of too gross a structural disparity to 1.

For assessment of the effects of structure on activity, one must bear in mind that modifications may affect drug stability, absorption from the gastrointestinal (GI) tract, and subsequent distribution as profoundly as activity at the actual site of action. Since most compounds were administered orally, in common with general practice, our definition of activity necessarily encompasses such variations. However, the amide 4 was injected intraperitoneally because of limited availability, our first use of a parenteral route (dosage, 48 mg in 0.5 ml of 9% saline per day to each rat for 14 days; in preliminary testing for adverse reactions at this level, three rats remained healthy and gained weight during 3 days).

The carbon skeleton proved earlier to be very sensitive to alteration, since 6 was inactive.[5] The results of Table I for DL-cysteine hydrochloride (7; inactive) and both *threo*- and *erythro*-β-methylcysteine (8a and 8b; inactive) confirm the importance of β-disubstitution. Nevertheless, earlier activity for the cyclohexyl analog 9 shows that skeletal modifications *are* possible,[5] and the activity of

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Table I. Rat Skin Tensile Strength (sts) after Administration of Congeners of **1**[a]

Compd	Structure	No. of rats	Av sts, g/cm		Av wt gain of rats, % of original wt	
			Control	Test	Control	Test
1	D-$(CH_3)_2C(SH)CH(NH_2)CO_2H$	3	14.0[b]	7.5[b]	*b*	*b*
2	D-$[SC(CH_3)_2CH(NH_2)CO_2H]_2$	3	11.5	11.0	71	60
3	DL-$(CH_3)_2C(SH)CH(NH_3^+)C(O)NHPh$-*p*-$ClBr^-$	3	10.5	11.0[c]	71	56
4	DL-$(CH_3)_2C(SH)CH(NH_3^+)C(O)NEt_2Br^-$ [d]	2	7.5	8.5	68	101
5	o-$HSC_6H_4NH_3^+Cl^-$	4	7.5	7.0	68	28
7	DL-$HSCH_2CH(NH_3^+)CO_2HCl^-$	4	9.1	8.4	50	56
8a	*threo*-$CH_3CH(SH)CH(NH_3^+)CO_2HCl^-$	3	9.1	9.4	50	38
8b	*erythro*-$CH_3CH(SH)CH(NH_2)CO_2H$	4	7.5	7.2	68	88
10	DL-c-$(CH_2)_4C(SH)CH(NH_3^+)CO_2HCl^-$	4	9.1	5.4	50	14
13	DL-$(CH_3)_2\overline{CSC(CH_3)_2NH_2^+}CHCO_2HCl^-$	3	8.9	4.6	73	42[e]
14	DL-$(CH_3)_2\overline{CSC(O)}CHNH_3^+Br^-$	3	9.1	8.3	50	49
15	Polypeptide polymer of DL-**1**[f]	2	7.5	9.3	68	44
16	Zinc chelate of **1**[g]	4	8.9	5.0	73	60

[a] For details of testing, see ref 5 and 9; variations from an sts average of *ca.* 10–20% are not unusual (ref 5). Reference 5 discusses the likelihood with animals showing poor weight gain that their sts may have been reduced less than if the gains had been normal, but not more. [b] Given for comparison; see ref 5 for details. Body weights after 14 days were 88% of those for control rats that received no drug (ref 5). [c] Two rats died on days 7 and 10. [d] Compound **4** was administered by injection (see text). [e] One sick rat is included in this calculation. [f] See text for structure. Given as the Na salt at 0.6% of the diet (*vs.* 0.25% for **1**) because of the higher molecular weight; lack of enough **15** precluded testing more than two rats. [g] See text for structure.

the cyclopentyl analog **10** now confirms this conclusion.

$CH_3(C_2H_5)C(SH)CH(NH_2)CO_2H$ DL-c-$(CH_2)_5C(SH)CH(NH_3Cl)CO_2H$

6 9 (c represents cyclo)

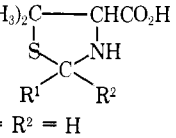
DL-c-$(CH_2)_4C(SH)CH(NH_3Cl)CO_2H$

10 (c represents cyclo)

Latentiation affords another potential means of improving pharmacological activity.[6] The activity of **11** was the first indication that it was indeed feasible to latentiate **1**.[5] Latentiated variants of **1**, such as **11** and **13**, of course may merely be converted rapidly to **1** *in vivo*; whether they release **1** slowly *in vivo* at preferred sites and confer useful properties is a question best deferred until the most attractive latentiating possibilities can be selected from more candidates than are yet available.

D-o-$HO_2CC_6H_4SSC(CH_3)_2CH(NH_2)CO_2H$

11



12, $R^1 = R^2 = H$

13, $R^1 = R^2 = CH_3$ (HCl salt)

When an earlier effort was made to latentiate **1** by forming the thiazolidine **12** with formaldehyde, the product was inactive, evidently because **12** is too stable to release **1** *in vivo*.[5] On the other hand, the 2,2-dimethylthiazolidine **13** is fully as active as **1**. This comparison suggests that thiazolidines properly substituted at position 2 will be promising latentiated forms of **1** with a very useful range of latentiating capabilities adjustable between the extremes seen for **12** and **13**.

The thiolactone **14**, an internally latentiated form of **1**, was inactive, possibly owing to high reactivity toward interfering nucleophiles *in vivo (vide infra)*.

A polypeptide-like polymer of **1** (**15**) prepared from **14** was inactive, as had been the amides **3** and **4**. Presumably amides are too poorly hydrolyzed *in vivo* to be promising for latentiating compounds like **1**.

Since **1** is a good chelating agent,[7,8] **1** might owe its action in sts and/or arthritis to an effect on trace metal ions. Chelation of copper seems unlikely to be the cause of reduction of sts.[9] The zinc(II) chelate **16** of **1** became of interest because of a report that administration of D-**1** to patients with Wilson's disease and cystinuria resulted in a more positive zinc balance.[10a] Excretion of zinc in the urine was increased, but increased absorption of zinc from the GI tract more than offset this loss, so that a positive balance resulted.[10a] This finding suggested that **1**, by chelation with zinc, might alter the transport of zinc to a binding site that could play a role in collagen biosynthesis and in arthritis. Indications that zinc accumulates in inflamed joints lends further interest to factors that affect its distribution.[10b]

Table I shows that the zinc(II) chelate **16** has at least the same activity as **1** itself. It may become desirable later to determine whether this activity results simply from rapid dissociation of **16** to give **1**, by comparing separate effects of **1**, **16**, and zinc(II) ion. For the present, however, three conclusions are justified: (1) that reasonable stability of **16** in water, indicated by previous studies,[7] is now confirmed by notable differences in aqueous solutions of **1** and **16** during titration, in circular dichroism (CD), and in uv spectra; (2) that the activity of **1** at least is not *negated* by zinc(II) ion, as it is by some latentiating groups;[5] and (3) that zinc(II) ion (and perhaps other metal ions) is among reasonable candidates for latentiating **1**, from which a final choice can be made when a variety of latentiating functions has been developed.

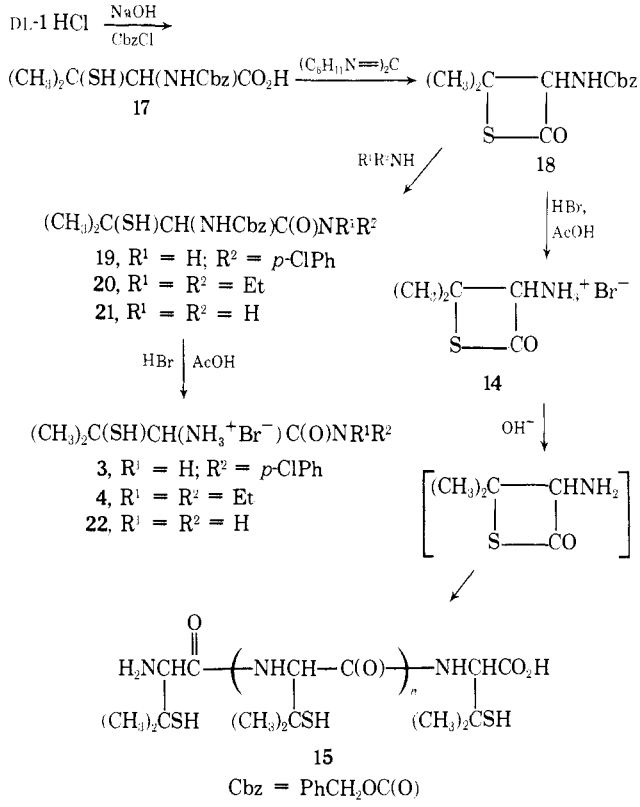
All but one of the compounds in Table I have asymmetric carbon atoms. Since DL-**1** and D-**1** have much the same effect in solubilizing collagen, however,[11] stereoisomeric aspects of these compounds are not being dwelt upon for the present. D-**1** reduced the skin tensile strength of rats to 54% of that for controls.[5] For the three comparably active compounds of Table I, the reductions were: **10**, 59%; **13**, 52%; and **16**, 56%.

Chemistry. The preparation of the amino β-thiolactone salt **14** followed a procedure of Sheehan and Pollak, in which *N*-carbobenzoxy-DL-penicillamine (**17**) was allowed to react with dicyclohexylcarbodiimide to form **18**, which then was decarbobenzoxylated with HBr to **14** (Scheme I).[12,‡] Utilizing D-**1**, we were able to prepare the carbobenzoxy derivative D-**17** but could not convert D-**17** to the thiolactone D-**18**, evidently owing to sparing solubility of D-**17** in suitable solvents. However, when DL-**1** was used as specified,‡ DL-**17** gave DL-**18** (84%). The DL-**18** is conveniently identifiable by the characteristic ir absorption of

‡ J. C. Sheehan and K. Pollak, unpublished results.

the ring carbonyl at 1755–1760 cm^{-1}.[12] Subsequent reactions related to Scheme I were based on DL-1 and should be so understood.

Scheme I

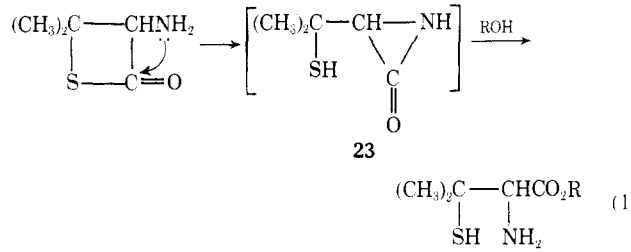


As Scheme I shows, amides 3 and 4 were prepared by decarbobenzoxylating the Cbz-blocked amides 19 and 20 with HBr in acetic acid, a method based on one of Sheehan and Pollak for the corresponding anilide.‡ The hydrochloride of the unsubstituted amide 22 has been reported previously.[13] However, although the *N*-carbobenzoxyamide 21 could be obtained in 30% yield, the hydrobromide 22 was hygroscopic and unsuitable for testing (*cf.* ref 5 regarding liquids). During exchange of the HBr of 22 with *p*-toluenesulfonic acid, HBr was distilled in methanol; a nonhygroscopic powder presumed to be the tosylate was isolated, but satisfactory analyses could not be obtained. The amide salt 3 was not hygroscopic, but the *N,N*-diethylamide 4 led to considerable problems with hygroscopicity and could be recrystallized only in small amounts. We conclude that conversion of the thiolactone 18 to amides is a generally useful reaction, the preparation of unsubstituted and mono- and disubstituted amides all testifying to the generality of the method, but that problems may ensue from the properties of the hydrobromide salts.

Decarbobenzoxylation of 18, as reported,[12,‡] gave the salt 14. Both the amide 18 and the salt 14 are intriguing biologically (as latentiated forms of 1) and chemically (as small-ring sulfur-containing heterocycles and as starting materials for congeners of 1). Study of the stability of 18 and 14 under various conditions therefore became desirable.

The amide 18 proved to be surprisingly stable in protic solvents. Based on lack of change of the absorbance at 1755 cm^{-1}, 18 in *t*-BuOH, MeOH, or THF–17% H_2O underwent no significant loss in 5–7 days at ~25° or in 1–3 days under reflux. The salt 14 is stable for months at ambient conditions (by ir). In marked contrast to the amide 18, however, ~50% of 14 reacts during 16–21 hr in H_2O (ir) and in D_2O (nmr) or during 53 hr (nmr) in MeOH (saturated solutions, 25°); 100% of 14 was lost after ~3 days in H_2O (ir) and in D_2O (nmr) or during ~12 days (nmr) in MeOH. Nmr gave more satisfactory results than ir in these assessments.

The rapid solvolysis of 14 relative to 18 might be explained in terms of acid catalysis by HBr present from dissociation of 14. This explanation seems improbable, however, because when acid catalysis was simulated with the amide 18 in MeOH (by adding 1 equiv of ethylamine hydrochloride), the nmr spectrum did not change significantly in two days, thus indicating insignificant ring cleavage. The rapid solvolysis of 14, on the other hand, seems understandable in terms of a neighboring-group assistance of $-NH_2$ (which should be present to some extent from dissociation of the salt), as suggested in eq 1. The feasibility of the aziridinone 23 (α-lactam) as an intermediate in eq 1 is supported by the observation and isolation of such structures.[14] Two other points also support the intermediate 23. (1) When the per cent of 14 remaining (nmr) was plotted as a function of time for 0.1 and 0.75 *M* solutions of 14 in D_2O, the curves were superimposable. Hence, a concentration-dependent intermolecular catalysis by $-NH_2$ seems unlikely (the range of concentrations was dictated by saturation at the upper limit and by marginal nmr signals at the lower). (2) When 14 was solvolyzed in D_2O containing 1–2 molar equiv of HBr, nmr showed that 87% loss required 72 hr. The contrast of this result with that for 14 alone under conditions identical in all other respects (100% loss in 66 hr) suggests that the proposed neighboring-group effect of $-NH_2$, present as the conjugate base, diminishes with increasing concentration of acid, as one would expect.



Neutralization of 14 in H_2O or DMF results in the formation of a polymer of variable molecular and equivalent weights (15, 30–55% yield). These variable results may arise from anomalies in measurements, as well as from variations in the reactions themselves. For example, since heating was not employed for drying (in order to obviate further chemical changes), the presence of a little trapped solvent could have resulted in low molecular weights. Inadvertent titration of SH moieties may have led to low results in the determination of equivalent weights. Polymer 15 prepared from 14 in H_2O appeared to have n = 2–7 and that obtained in DMF to have n = 2–30. These values were based on extremes found by osmometric molecular-weight determination, nmr integration ratio of methoxyl to *gem*-dimethyl of methylated 15, and acidimetric titration of carboxyl end groups. Loss of fine structure in the ir spectrum also supported formulation of 15 as a polymer.

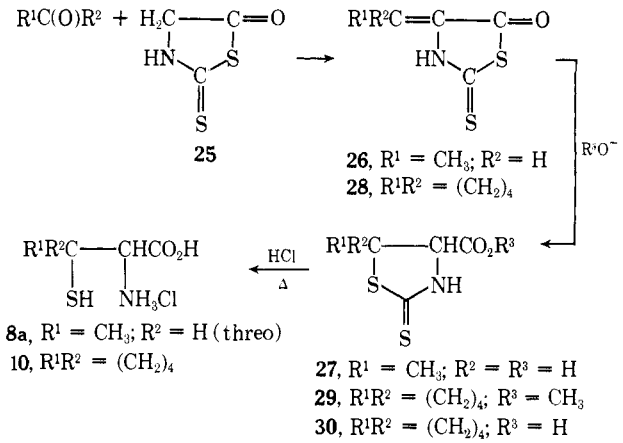
It is noteworthy that reaction in water under conditions of high dilution led only to 1 (tlc) and that no polymer was found. This result suggests that unless the concentration of the α-amino β-thiolactone is sufficiently high for intermolecular condensation, a neighboring-group ($-NH_2$) assisted solvolysis dominates, which leads to 1.

Attempts to form a dimer (the diketopiperazine) of 1 by heating the methyl ester of D-1 (24) were unsuccessful. The methyl ester 24 was surprisingly stable. It was unchanged after 24 hr neat, 96 hr in $CDCl_3$, or 7 days in re-

fluxing benzene. When heated neat, **24** was stable at 65°, but it decomposed in a synthetically unattractive manner to several products at 75° (tlc).

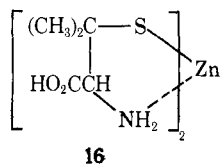
Preparation of **8a** and **10** differed little from reported procedures (Scheme II), although syntheses were less easy than anticipated and warrant brief mention. Compound **26** was obtained from the condensation of **25** with acetaldehyde using piperidine in ethyl acetate (52%).[15] The conversions **25** → **26** → **27** → **8a** afforded an overall yield of 11% without complication.[16] The erythro isomer **8b** also was prepared. Both diastereoisomers **8a** and **8b** have been reported from the reaction of 4-ethylidene-2-phenyl-5-oxazolone with phenylmethanethiol under alkaline conditions followed by hydrolysis, separation of the diastereoisomers, debenzoylation, and debenzylation of the product.[17] The preparation and separation presented no difficulty, except for initial crystallization of some of the products, and afforded **8b** in 2% yield overall from the oxazolone. There still appears to be some question as to whether **8b** is in fact the erythro diastereoisomer.[16] Infrared spectra of **8a** and **8b** seemed consistent with diastereoisomerism, however, and since both 8a and **8b** were inactive in sts tests we did not pursue this matter.

Scheme II



The preparation of **28** and its rearrangement to **29** proceeded without difficulty. However, several efforts to hydrolyze **29** with tin and HCl led only to trace amounts of **10**, although Cook and Pollock reported a 40% yield;[18] direct hydrolysis (concentrated HCl) of **29** to **10** in a sealed tube also was unsuccessful (*cf.* ref 19). Fortunately, conversion of **29** to **10** finally could be effected by first hydrolyzing **29** to **30** with 5 *N* HCl (44% yield); **30**, with concentrated HCl under vigorous conditions, then gave **10** (55%).

Heavy metal chelates of **1** have been of interest in relation to treatment of Wilson's disease[20,21] and of lead poisoning.[8] The biologically intriguing zinc(II) chelate exists in solution primarily as two ligand molecules per metal ion, with S and N as primary bonding sites (minor contribution by CO_2H),[7a] evidently as tentatively depicted in structure **16**.[7a,b,d] Heretofore, the chelate of zinc(II) with



16

1 had been studied only in solution, with no attempts to isolate it.[7] However, chelate **16** could be isolated as a solid by a method we later found to be somewhat similar to one of Akihama and Toyoshima,[22] in which the zinc(II) chelate of guanidine was isolated by combining correct molar proportions of reactants and removing the solvent. We

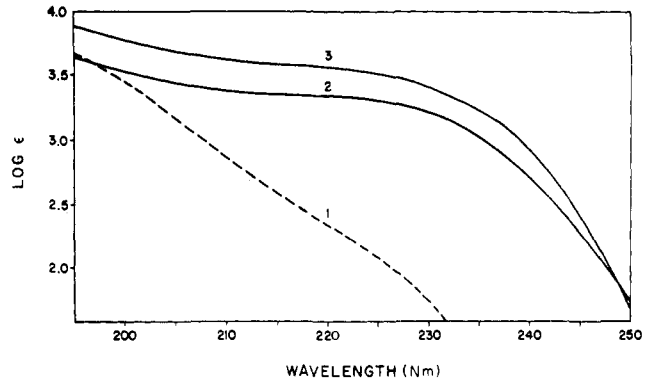


Figure 1. Ultraviolet spectra in aqueous solution: curve 1, 2:1 mixture of D-penicillamine and $ZnCl_2$; curve 2, **16a**; curve 3, **16b**.

dissolved D-1 and zinc(II) acetate dihydrate in 2:1 ratio in H_2O and lyophilized the product repetitively, the residue being redissolved after each lyophilization (eq 2). After attainment of constant weight, the weight of residue was 101% of that expected for loss of acetic acid (and H_2O) according to eq 2; when the anhydrous product **16a** was allowed to stand under ambient conditions, an increase in weight was observed, consistent with formation of a dihydrate. To confirm the structure of **16a**, anhydrous zinc(II) propionate was substituted in eq 2. The weight of the residue was 100% of expectation (eq 2); the product **16b** was identical in all respects with **16a** and was clearly different from D-1 [decomposition points, titration, uv spectra (Figure 1), ir and nmr spectra, inner orbital photoelectron spectroscopy (ESCA), CD, and simple neutralization equivalents (±3%, phenolphthalein indicator)].

$$2(CH_3)_2C(SH)CH(NH_2)CO_2H + Zn[OC(O)R]_2 \longrightarrow \mathbf{16} + 2RCO_2H \quad (2)$$

16a, R = CH_3
16b, R = CH_3CH_2

Titration curves in H_2O of **16** and D-1 show marked differences which suggest that **16** is indeed a chelate (*cf.* ref 7b). Slight changes of slope for **1** are consistent with the zwitterionic structure expected. In contrast, the curves of both **16a** and **16b** show pronounced inflections at the equivalence point, suggesting that the acidic protons are no longer bound in a zwitterionic structure and that the N atom is bound to zinc rather than being involved in dipolar ion formation with the carboxyl moiety.

Further evidence that **16** is a chelate is provided by the uv spectrum (Figure 1). A mixture of D-1 with zinc chloride should not undergo loss, in solution, of the strong acid HCl and should be much like **16a** and **16b** if the latter were *not* chelates. The uv spectra (Figure 1), however, show the chelate (curves 2 and 3) to be markedly different from the mixture of D-1 and zinc chloride (curve 1).

Experimental Section§

Materials. D- and DL-penicillamine (1), D-penicillamine disulfide (2), DL-cysteine hydrochloride (7), 2,2,5,5-tetramethyl-4-

§ Melting points, determined in capillary tubes using a Thomas-Hoover stirred-liquid apparatus or a Mel-Temp block, are corrected. Ir spectra were obtained using a Beckman Model IR10 spectrophotometer with thin films of liquids and KBr pellets of solids; bands reported were of at least medium intensity. Nmr spectra were obtained using a Varian Model A-60 spectrometer with TMS as an internal (and occasionally external) standard, uv spectra with a Cary Model 14 spectrophotometer, and circular dichroism (CD) spectra with a Cary Model 60 recording spectropolarimeter. Molecular weights (Mecrolab Model 301 vapor-pressure osmometer), equivalent weights except for **16a** and **16b** (titration of samples dissolved in THF with 0.01 *N* base to a phenolphthalein end point), and elemental analyses were determined by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results for these elements were within ±0.4% of the theoretical values.

thiazolidinecarboxylic acid hydrochloride (13), and all other compounds not described were used as purchased. D-1 methyl ester·HCl was prepared as reported earlier;[5] it was converted to the free base 24 by neutralization (NaOH) and extraction into $CHCl_3$. To prepare 5, HCl gas was bubbled (5–10 min) into a solution of 2-aminobenzenethiol (25 g, 200 mmol) in Et_2O (50 ml). The precipitate which formed immediately was separated (27.4 g, 85%): mp 208–210°. Recrystallization (H_2O) gave 5 with mp 211–212° dec (lit.[23] 210–211° dec).

N-Carbobenzoxy-DL-penicillamine Thiolactone (18) and DL-Penicillamine Thiolactone Hydrobromide (14). These were prepared essentially according to Sheehan and Pollak.[12,‡] Equivalent amounts of carbobenzoxy chloride (benzyl chloroformate) in dioxane and of 4 *N* aqueous NaOH were added concurrently (15 min) to DL-1·HCl in 2 molar proportions of 4 *N* NaOH at 0°. After 2 hr, acidification and extraction gave 17, a bicarbonate solution of which was washed (Et_2O) and acidified: yield 74%. In CH_2Cl_2, this 17 was treated (10 min) with 1 equiv of dicyclohexylcarbodiimide; after 24 hr, filtration and evaporation gave oil. An Et_2O solution was carefully treated with oxalic acid until bubbling ceased, the urea was removed, and a concentrate taken up in $CHCl_3$ for removal of oxalic acid: yield of 18, 84%; mp 90–95° (lit.[12] 93–95°). The 18 in 17 ml/g of glacial AcOH saturated with HBr, after 0.5 hr, was treated with dry Et_2O to incipient turbidity and chilled. Washing (Et_2O) and drying of the precipitate gave 14 (83%), mp 189–190° (lit.[12] 189–190°).

DL-Penicillamine-*p*-chloroanilide Hydrobromide (3) and -*N*,*N*-diethylamide Hydrobromide (4). In a modification of a procedure for the anilide,‡ crude 18 (49 mmol) in dry CH_2Cl_2 (20 ml) was added to *p*-chloroaniline (49 mmol) in dry CH_2Cl_2 (20 ml). The solution became cloudy in ~15 min, but stirring was continued for 24 hr. The precipitate of white 19 (11.6 g, 61%) was collected by filtration and washed with CH_2Cl_2. All of this crude 19 was stirred in glacial AcOH (150 ml), previously saturated with HBr, for 1 hr ($CaCl_2$ drying tube). Dry Et_2O (~300 ml) then was added, and the solution was kept at 0° for 3 days. The precipitate was collected, washed well with dry Et_2O, and dried over concentrated H_2SO_4 and NaOH pellets to give 3 (7.9 g, 79% yield) as white powder: mp 149–151° (frothing); ir (KBr) 3220–2790, 1680, 1595, 1540, 1490, 1400, 820 cm^{-1}. *Anal.* ($C_{11}H_{16}BrClN_2OS$) C, H, Cl.

The diethylamide 4 was made similarly in 81% yield. Recrystallization (amounts of ~1–2 g proved best) from EtOH–Et_2O gave 4 (40% yield): mp 153–154°; ir (KBr) 3500–2600, 1650, 1470, 1380, 1370, 1275, and 1135 cm^{-1}. *Anal.* ($C_9H_{21}BrN_2OS$) C, H, Br, N, S.

Amide of N-Carbobenzoxy-DL-penicillamine (21). An excess of ammonia (~25 ml) was condensed (cold finger, Dry Ice–Me_2CO) into a stirred solution of crude 18 (1.00 g, 3.8 mmol). The solution was stirred at *ca.* −33° (boiling point of NH_3) for ~4 hr and then was allowed to come to ~25°. The solution was evaporated; the oil that remained, when washed with 2 *N* HCl, H_2O, 5% $NaHCO_3$, and again with H_2O, solidified: 0.32 g (30% of 21). Recrystallization from Et_2O–petroleum ether gave 0.21 g (20%) of light green 21: mp 112.5–113°; ir (KBr) 3390, 3290, 1710, 1670, 1520, 1405, 1320, 1230, 1040, and 720 cm^{-1}. *Anal.* ($C_{13}H_{18}N_2O_3S$) H, N; C: calcd, 55.26; found, 55.69. Attempted conversion of 21 to 22 with glacial AcOH saturated with HBr gave a product too hygroscopic for characterization.

Stabilities of Thiolactones 14 and 18. The resistance of thiolactones 14 and 18 to solvolysis was determined by the general procedure below, the per cent decomposition being approximated either by nmr [disappearance in 14 of the methyl singlet (δ ~1.8–1.9) and appearance of a doublet (δ ~1.4–1.5), and (except in MeOH) loss of the methine peak at δ ~5.2 and appearance of a peak at δ ~4] or by ir (disappearance in 14 and 18 of the very characteristic‡ ν_{CO} at 1755 cm^{-1}).

Compound 14 or 18 was dissolved in H_2O, D_2O, MeOH, or *t*-BuOH, and the resulting solution was stirred during times and under conditions outlined in the discussion. The solution was monitored by either nmr or ir, and the amount of 14 or 18 remaining was calculated as follows: nmr (integral of methyl *or* methine protons of 14 or 18 at time t × 100)/(integral of all methyl or methine protons); ir (area of ν_{CO} at 1755 cm^{-1} at time t × 100)/(area of ν_{CO} at 1755 cm^{-1} at t = 0), with a particular solution always being monitored in the same AgCl cell; where changes were slight, ν_{1755} was compared by inspection with $\nu_{CO\text{-}O}$ ~1700 cm^{-1}.

Preparation of the Polymer of Penicillamine (15). Method A. In H_2O. NaOH (0.09 g, 2.3 mmol) in 0.5 ml of H_2O was added to a solution of 14 (0.5 g, 2.3 mmol) in 8 ml of H_2O. Precipitate appeared immediately, but the mixture was stirred for ~6 hr. The precipitate was collected and washed several times with H_2O. Drying over P_2O_5 under vacuum to constant weight yielded light tan powder (15, 0.17 g, 55%), mol wt 338. After a further drying period of 4 weeks for complete removal of H_2O, the molecular weight was found to be ~1200 and to be unchanged by drying for 4 weeks more: mp ~230° dec; ir (KBr) 3320, 2980, 2960, 2550, 1650, 1500, 1395, 1375, 1215, and 1140 cm^{-1}. Methylation (CH_2N_2) of this 15 in DMSO-d_6 yielded material having an nmr spectrum that gave a ratio of methoxyl to *gem*-dimethyl corresponding to a mol wt ~1200.

Method B. In DMF. A solution of KOH (0.13 g, 2.3 mmol) in EtOH (1 ml) was added to 14 (0.50 g, 2.3 mmol) in 8 ml of *N*,*N*-dimethylformamide. The solution was stirred for 24 hr; some precipitate formed. H_2O was added until precipitation was complete, and the precipitate was washed with H_2O until washings gave no precipitate with $AgNO_3$–HNO_3. Drying for 2 weeks under vacuum over P_2O_5 gave light tan granular solid (15, 0.09 g, 30%), having a mol wt of 730§ but an equiv wt of 4200.§ Repetition with 2.13 g (10 mmol) of 14 gave 0.44 g (33%) of 15. Drying for 4 weeks gave 15 having a mol wt of 477 and an equiv wt of 812.§

β-Methylcysteine (8). Compound 25[5] (washed free of HCl and stored over $CaCl_2$ to prevent decomposition) with AcH gave 26 (52%, mp 193–193.5°),[15] which with KOH–MeOH gave 27 (73%, mp 179–181°).[16] Concentrated HCl (sealed tube) converted 27 to the threo form of 8a [30%, mp 182.5–184° dec (lit.[16] 184–185°)].[16]

4-Ethylidene-2-phenyl-5-oxazolone (54%, mp 92–94°)[24] and $PhCH_2SH$ gave, after hydrolysis, benzoyl-DL-α-amino-β-benzylthio-*n*-butyric acid (63%, mp 138–164°).[17] The B isomer (allo or erythro; *cf.* ref 16) gave the erythro form of 8b (3%);[17] mp 201–203° dec (lit.[17] 203–204° dec).

DL-2-(1-Mercaptocyclopentyl)glycine Hydrochloride (10). Cyclopentanone (100 ml), 25 (*vide supra*, 25 g), and $ZnCl_2$ (25 g) in EtOAc (150 ml) gave brown 28 (*cf.* ref 18). Recrystallization (EtOAc) gave 28 (25.1 g, 67%), mp 243° dec. The 28 (10.0 g) with Na (5.0 g) in MeOH, after acidification, gave 29 (10.3 g, 89%, mp 83–94°), which was recrystallized from Me_2CO–H_2O: 5.5 g (47%, *cf.* ref 18); mp 130–132° (lit.[18] 133°). Compound 29 (1.0 g, 4.3 mmol) was heated under reflux for 2.5 hr in 5 *N* HCl (20 ml), and the resulting solution was decanted from tar and kept at 0° until 30 separated (0.41 g, 44%): mp 166.5–168°. Recrystallization from H_2O gave 30, mp 167–168° (lit.[18] 168°). Hydrolysis of 30 (5.0 g, 23 mmol) with concentrated HCl (~50 ml) in a sealed tube at ~120° for 21 hr gave 10 (4.4 g, 91%), mp 190–192°. Reprecipitation from EtOH with Et_2O afforded 10 (2.68 g, 55%), mp 196–197°. Two additional reprecipitations gave analytically pure 10, mp 198–199° (frothing) [lit.[18] 199–200° (frothing)]. The overall yield of 10 from 25 was ~8%; ir (KBr) 3390, 3200–2500, 1730, 1570, 1480, 1430, 1380, 1340, 1200, 1100, 950, 850, 630 cm^{-1}. *Anal.* ($C_7H_{14}ClNO_2S$) C, H, Cl, N, S.

Bis(3-mercapto-D-valinato)zinc(II) (16). A solution of D-1 (14.9 g, 100 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (10.98 g, 50 mmol) in a minimum of deionized H_2O was lyophilized (3–6 hr). The weight of the residue was noted, and the solid then was redissolved and the lyophilizing process repeated. The entire process was repeated 10–13 times until the weight was essentially constant. The material then was kept under reduced pressure until there was no further weight loss. The weight of the residue was 18.20 g (101%); mp progressive darkening from *ca.* 192°.

Chelate 16b was prepared similarly from D-1 and Zn[O-C(O)CH_2CH_3]$_2$ and was identical in all respects with 16a: equiv wt calcd 180, found (aqueous NaOH, phenolphthalein) 186 (16a), 186 (16b); ir (KBr) 3410, 3100, 2960, 1615, 1390 cm^{-1}; nmr (D_2O, external TMS) δ 1.4 (s), 3.7 (s); (DMSO-d_6, external TMS) 1.2–1.3 (d), 3.2 (s); uv, Figure 1; CD, minima at 231 nm (1 has a minimum at 225 nm and also a maximum at 210 nm). ESCA showed the same N (1s), S (2p), and Zn (3d) bonding energies and the same broad lines for both 16a and 16b; the N (1s) and S (2p) photopeaks of D-1 were different from those of 16a and 16b, principally in being sharper. *Anal.* (after drying, as a precaution, at ~60° for 24 hr; prior to this drying, the analyses reflected an indication of hydration) ($C_{10}H_{20}N_2O_4S_2Zn$) C, H, N, S, Zn.

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Metabolism of 5-(*p*-Hydroxyanilino)-1,2,3,4-thiatriazole† in Rats

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Plasma level, excretion, and metabolite identification studies were performed after oral administration of tritiated 5-(*p*-hydroxyanilino)-1,2,3,4-thiatriazole to male Sprague–Dawley rats. At a dose of 250 mg/kg, an average of 74.8 and 19.8% of the administered radioactivity was excreted in the urines and feces, respectively; at a dose of 1 g/kg, the values averaged 44.1 (urines) and 49.7% (feces). The major metabolic pathway for the disposition of the title compound in these rats was ethereal sulfate conjugation. The glucuronide conjugate and products resulting from fragmentation of the thiatriazole moiety were found in smaller amounts.

The compound, 5-(*p*-hydroxyanilino)-1,2-3,4-thiatriazole (1), has been under investigation at Abbott Laboratories as a possible antihypertensive agent. In order to obtain some idea of its metabolism in animal systems, studies using tritium-labeled drug were performed in rats. These include studies on blood levels of radioactivity, excretion, and metabolite identification.

Results and Discussion

Results of the blood level studies appear in Table I and Figure 1. It appears that more than one molecular species are present in the plasma. If the initial decline can be attributed to the parent drug, the half-life of this portion is 4.5–5.5 hr. The rise in plasma levels of radioactivity at about 6 hr may be due to the formation of metabolite(s) which have different volumes of distribution from that of 1.

If rats are sacrificed at 4 hr after an oral dose (250 mg/kg) and the tissues are analyzed for drug radioactivity, a large quantity of radioactivity (60%) was found in the gastrointestinal tract.‡ This may indicate either (1) a slow absorption or (2) biliary excretion of drug. The results obtained from bile-duct cannulated rats (Table II) seem to favor the idea of slow absorption of drug, since apparently no great amount of drug is routed *via* the bile. It also ap-

Table I. Blood Levels of Radioactivity after Administration of Abbott-31699-^{3}H to Male Sprague–Dawley Rats[a]

Time after administration, hr	Plasma levels of radioactivity, μg of drug/ml of plasma	
	Rat α	Rat β
0.25	12.8	17.0
0.50	21.5	26.9
1.0	56.6	24.7
2.0	23.3	20.5
3.0	21.3	17.6
4.0	19.4	16.4
5.0	15.3	19.7
6.0	15.6	19.9
7.5	16.3	20.2
12	12.0	17.4
24	5.88	11.3
30	5.03	10.1
48	3.71	7.92

[a] Dose = 250 mg/kg orally in tragacanth suspension.

† Abbott-31699.

‡ G. J. Ikeda, unpublished observations, Abbott Laboratories, North Chicago, Ill.