Biologically Oriented Organic Sulfur Chemistry. 8. Structure–Activity Relationships of Penicillamine Analogs and Derivatives[1]

B. J. SWEETMAN, M. M. VESTLING, S. T. TICARIC, P. L. KELLY, L. FIELD,*

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37203

P. MERRYMAN, AND I. A. JAFFE

Department of Medicine, New York Medical College, New York, New York 10029

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D-Penicillamine (**1**) is of interest for the treatment of rheumatoid arthritis, but it causes many side effects. Evalnation of substances structurally related to **1** was made for effectiveness in reducing the tensile strength of rat skin *in vivo*, a parameter of **1** significant to collagen biochemistry, if not to rheumatoid arthritis. The moieties SH, NH_2, and CO_2H all appeared to be necessary for reduction of tensile strength, for activity was lacking whenever any one of them was omitted or was blocked by substitution. Variations of the skeleton are possible, since α-amino-β-mercaptocyclohexaneacetic acid·HCl (**13**) is at least as active as **1**. Latentiation of **1** and related compds led to one product (**17**), contg an *S*-(*o*-carboxyphenylthio) moiety, which had activity comparable to **1**. Slight activity was seen for the Me ester of **1** and for α-mercaptopropionylglycine. Indications are that **1** may owe its effect in rheumatoid arthritis to something other than to dissociation of the rheumatoid factor, to function as an antiinflammatory or immunosuppressive agent, or to strengthening of a lysosomal membrane.

D-Penicillamine (**1**) is of interest in treating a wide variety of illnesses, *e.g.*, Wilson's Disease,[2] cystinuria,[3] idiopathic pulmonary fibrosis,[4] rheumatoid arthritis,[5] and scleroderma.[6] Significant clinical improvement

$(CH_3)_2C(SH)CH(NH_2)CO_2H$
1

in a number of patients having rheumatoid arthritis has been attributed to prolonged oral administration of **1**,[5] but the clinical usefulness of **1** is limited by many side effects, among which renal damage is particularly troublesome.[7]

The mechanism by which **1** acts in rheumatoid arthritis is not clear. Chemically, **1** is known to chelate heavy metals[8] and to effect thiol–disulfide interchange to some extent;[9] indeed, its chelation capability is important, for example, in the treatment of Pb poisoning.[10] However, neither chelation nor thiol–disulfide interchange seems likely to explain the effect of **1** in rheumatoid arthritis.[11] On the other hand, **1** forms thiazolidines with aldehydes or ketones,[12] and this characteristic has been suggested as a possible explanation for the effect of **1** in solubilizing collagen, a principal protein constituent of connective tissue, and in markedly reducing the tensile strength of skin in rats.[13] Whether or not these effects of **1** on collagen and skin are relevant to diseases of connective tissue is uncertain, but at the time we began our investigation, **1** was the only compound known to have such effects *in vivo*.

This paper reports the results of several biological tests carried out on **1** and on a series of analogs and derivatives of **1** in a search for other substances of potential interest in diseases of connective tissue. For synthesis of compounds related to **1** that would be more active medicinally and/or less toxic, 3 approaches suggested themselves: (**1**) determination of the functional groups and skeletal features of **1** that are required for action on connective tissue; clarification of this point hopefully may lead also to a clearer view of the mechanism of action of **1** in diseases of connective tissue; (**2**) investigation of latentiating moieties[14] for **1** in the hope of obtaining pharmacologically more useful compounds from **1**; (3) coupling of molecules embodying the best structural parameters with the most promising latentiating moieties.

Biological Results.—Rheumatoid arthritis is one of the rheumatic diseases that include diseases of connective tissue.[15] Although it may be coincidental that **1** has a favorable influence in rheumatoid arthritis and that it also reduces the tensile strength of rat skin *in vivo*[13] and *in vitro*,[16] the most attractive means (at least in the present state of knowledge) for studying the structural features of **1** likely to be important in arthritis seemed to be to follow the effect of structural variations of **1** on the capacity for reducing the tensile strength of rat skin *in vivo*. In view of widespread interest in the biochemistry of collagen, such results

(1) (a) Paper 7, L. Field, W. S. Hanley, I. McVeigh, and Z. Evans, *J. Med. Chem.*, **14**, 202 (1971). (b) This investigation was supported by Public Health Service Research Grant AM11685 from the National Institute of Arthritis and Metabolic Diseases to Vanderbilt University and by Contract No. DA-49-193-MD-2445 from the U. S. Army Medical Research and Development Command and Public Health Service Research Grant AM11586 from the National Institute of Arthritis and Metabolic Diseases to New York Medical College.

(2) (a) J. M. Walshe, *Lancet*, **i**, 188 (1960); (b) I. Sternlieb and I. H. Scheinberg, *J. Amer. Med. Ass.*, **189**, 748 (1964).

(3) (a) J. C. Crawhall, E. F. Scowen, and R. W. E. Watts, *Brit. Med. J.*, **1**, 588 (1963); (b) H. Boström and P. O. Wester, *Acta Med. Scand.*, **181**, 475 (1967).

(4) *Cf.* R. I. Henkin, H. R. Keiser, I. A. Jaffe, I. Sternlieb, and I. H. Scheinberg, *Lancet*, **ii**, 1268 (1967).

(5) I. A. Jaffe, *Postgrad. Med. J., Oct. Suppl.*, **44**, 34 (1968).

(6) E. D. Harris, Jr., and A. Sjoerdsma, *Lancet*, **ii**, 996 (1966).

(7) *Cf.* I. A. Jaffe, G. Treser, Y. Suzuki, and T. Ehrenreich, *Ann. Intern. Med.*, **69**, 549 (1968).

(8) M. L. Sharma and L. D. Tuck, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, MEDI 59.

(9) L. Eldjarn and L. Hambraeus, *Scand. J. Clin. Lab. Invest.*, **16**, 153 (1964).

(10) W. G. Levine in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Ed., 3rd ed, Macmillan Co., New York, N. Y., 1965, p 939.

(11) I. A. Jaffe, *Arthritis Rheum.*, **8**, 1064 (1965).

(12) A. H. Cook and I. M. Heilbron in "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, N. J., 1949, p 921.

(13) (a) M. E. Nimni, *J. Biol. Chem.*, **243**, 1457 (1968); (b) K. Deshmukh and M. E. Nimni, *ibid.*, **244**, 1787 (1969).

(14) (a) N. J. Harper, *Fortschr. Arzneimittelforsch.*, **4**, 221 (1962); (b) L. Field, B. J. Sweetman, and M. Bellas, *J. Med. Chem.*, **12**, 624 (1969).

(15) J. L. Hollander in "Arthritis and Allied Conditions," 7th ed, J. L. Hollander, Ed., Lea and Febiger, Philadelphia, Pa., 1966, p 23.

(16) R. D. Harkness and M. E. Nimni, *Acta Physiol. Acad. Sci. Hung.*, **33**, 325 (1968).

should be of value quite apart from arthritis. The procedure used in evaluating skin tensile strength was based on techniques of Nimni,[17a,b] as described earlier.[17c] It involved mixing the compound to be tested so that it would provide the molar equiv of 0.25% by wt of **1** in the diet of weanling male rats of the Holtzman strain. After **14** days of feeding *ad libitum*, control and test animals were sacrificed, and the tensile strength of dorsal skin strips was measured. Earlier results[17c] suggest that variations of about ±10–20% from an average value are not unusual. Based on previous observations, an increase in soluble collagen can be assumed to accompany diminished skin tensile strength.[13,17c]

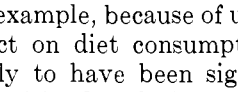
A problem with this method of testing might seem to lie in variation of amounts of test compounds actually ingested, for example, because of unpalatability or of an anorexic effect on diet consumption. Such an effect seems unlikely to have been significant however, except perhaps with **12** and **13**, because with compounds of Table I (and II), except **12** and **13**, body weights after the **14**-day period ranged from ~86 to 108% of those of control rats that received no drug (~88% with **1**) and averaged ~93% of control weights. No animals died, and the health of all rats seemed comparable after **14** days (including controls, but not rats that received **12** and **13**). Rats that received **12** and **13** had 77% and 60%, respectively, of the control weights; with **12** and **13** therefore, weight loss may reflect reduced food consumption, reduced consumption of **12** or **13**, and *less* reduction of skin tension than would have ensued from consumption of the usual molar amounts of test compounds. On the other hand, large reductions in the range of interest to us can scarcely be other than genuine results (albeit minimum ones), since our experience is that partial starvation has no effect on skin tension.

From a chemical viewpoint, two problems with this method are that rather large amounts of compound are required for adequate tests (~6 g) and that liquids cannot readily be used because of the possibility of heterogeneity in the diet prepared. As described later, however, efforts to find other parameters of **1** that might correlate with the effect of **1** in rheumatoid arthritis have been unavailing so far.

Penicillamine has 3 functional groups (SH, NH_2, CO_2H) on its C skeleton, along with **1** optically active center. In order to learn which functional group or groups were essential for reduction of skin tensile strength (and hopefully promising therefore for use with arthritis), the various possibilities for the 3 functional groups were systematically tested, first singly and then in combinations of two, using **2**–**8** (Table I). Because of the difficulty mentioned in testing liquids, salts **2**, **3**, **5**, **7**, and **8** were used instead of the parent compounds. Hydroxythiol **4** was chosen instead of 2-methyl-2-butanethiol because **4** seemed likely to be less volatile and less offensive in odor. Unfortunately, **4** was the first liquid tried, and satisfactory testing of it could not be achieved. Hence, **5** was used instead to determine whether a water-soluble thiol would be active. Only **6** in this series of compounds has an optically active center; the D isomer of **6** was tested rather than the L because the counterpart L-penicillamine is more toxic, owing to its greater anti-B_6 effect.[11]

TABLE I

RAT SKIN TENSILE STRENGTHS IN THE DETERMINATION OF THE ESSENTIAL FUNCTIONAL GROUPS OF **1**[a]

Compd	Structure	Average skin tensile strength, g/cm: Control	Test
1	D-$(CH_3)_2C(SH)CH(NH_2)CO_2H$	14.0[a]	7.5[a]
2	$(CH_3)_2CHCH_2CO_2Na$	9.5	9.7
3	$(CH_3)_2CHCH_2NH_3Cl$	7.7	9.4
4	$(CH_3)_2C(SH)CH_2CH_2OH$	*b*	*b*
5	$(C_2H_5)_2C(SH)CO_2Na$	5.8	7.6
6	D-$(CH_3)_2CHCH(NH_2)CO_2H$	9.5	9.0
7	$(CH_3)_2C(SH)CH_2CO_2K$	6.3	6.3
8	$(CH_3)_2C(SH)CH_2NH_3Cl$	8.2	9.3
9	D-$(CH_3)_2C(SH)CH(NH_2)CO_2CH_3 \cdot HCl$	10.6	8.8
10	DL-$(CH_3)_2C(SCH_3)CH(NH_2)CO_2H$	6.4	6.7
11	DL-$(CH_3)_2C(SCH_3)CH(NHAc)CO_2H$	6.4	6.1
12		7.4[c]	8.5[c]

[a] For details of testing, see ref 17c. Usually, 3 tests were done with each rat and 3–4 rats were used with each drug. [b] Test not done because **4** has too low a melting point; see text. [c] Preliminary report published with chemical details but not biological ones [B. J. Sweetman, M. Bellas, and L. Field, *J. Med. Chem.*, **12**, 888 (1969)].

Results of the tests are shown in Table I. Penicillamine (**1**) reduced the skin tensile strength to ~54% of the control value.[17c] For all of the other compounds, the search was for activity comparable with that of **1**. Compounds **2**, **3**, and **5**–**8** caused no reduction in skin tensile strength. All the functional groups of **1** thus seem necessary for activity, although this is not intended necessarily to imply that all 3 functional groups are involved in whatever biochemical interactions occur in the tissues. Nimni, *et al.*, similarly found that oral administration of **6** to rats did not cause accumulation in skin of neutral salt-soluble collagen.[18] Interestingly, **8** and **9**, but not **3**, appear to react with elastin (as well as collagen) *in vitro*.[19]

It seemed desirable to test the conclusion that all the functional groups of **1** were necessary for activity by blocking each of them in turn with substituents. This approach must be viewed with caution, of course, since a blocking group might be lost *in vivo*. Furthermore, a blocking group might inhibit activity solely for steric reasons, rather than because a necessary functional group was made unavailable. Compounds **9**–**12**, shown in Table I, were tested in rats with the results that are given in the Table. The ester **9** was used as a "carboxyl-blocked" analog of **1**, and the *S*-methylpenicillamine **10** as a "thiol-blocked" analog. Compounds **11** and **12** are blocked at both the amine and the thiol functions. The ester **9** and thiazolidine **12** were prepared from D-penicillamine, and **10** and **11** from commercially available DL-*N*-acetylpenicillamine. DL-*N*-

(17) (a) M. E. Nimni and L. A. Bavetta, *Science*, **150**, 905 (1965); (b) *Cf.* M. E. Nimni, *Biochim. Biophys. Acta*, **111**, 576 (1965); (c) I. A. Jaffe, P. Merryman, and D. Jacobus, *Science*, **161**, 1016 (1968).

(18) M. E. Nimni, K. Deshmukh, N. Gerth, and L. A. Bavetta, *Biochem. Pharmacol.*, **18**, 707 (1969).

(19) (a) Private communication from R. Jaffe and C. Franzblau, Boston University School of Medicine, Boston, Mass. (b) It is worth noting that **1** has been reported to alter connective tissue markedly by preventing the normal cross-linkage of elastin (another important protein in connective tissue), as well as of collagen; S. R. Pinnell, G. R. Martin, and E. J. Miller, *Science*, **161**, 475 (1968).

Acetylpenicillamine, the "amine-blocked" analog, previously has been found inactive in reducing rat skin tensile strength,[16] and, unlike **1**, it is also inactive in reducing the rheumatoid factor in humans.[20] The thiazolidine **12** may be considered to be a doubly blocked form of **1** because it resists hydrolysis even by 1 *N* HCl or 1 *N* NaOH.[21]

The blocked compounds, **9–12**, with the possible exception of ester **9**, caused no significant reduction in tensile strengths. The ester **9** was somewhat effective in this *in vivo* test, but the result seems of marginal significance (tensile strength for **9** ~83% of the control value *vs.* ~54% for **1**); perhaps **9** is converted partially to **1** *in vivo*. Although as mentioned, **9** gave the same reaction with elastin (and collagen) as **1**, **10–12** did not react with either elastin or collagen, *in vitro*.[19a] *In vivo*, the blocked compounds seem to substantiate the view that all the functional groups of **1** are essential for reduction of skin tensile strength in rats.

The next step in assessing the requirements for a compound capable of reducing tensile strength was to examine skeletal and other structural variants of **1**. The compounds chosen for this purpose were **13–16**. The structures and test results are given in Table II.

TABLE II

RAT SKIN TENSILE STRENGTHS WITH SKELETAL VARIANTS OF **1** AND WITH LATENTIATED FORMS OF **1**[a]

Compd	Structure	Average skin tensile strength, g/cm Control	Test
1	D-$(CH_3)_2C(SH)CH(NH_2)CO_2H$	14.0[a]	7.5[a]
13	DL-*c*-$(CH_2)_5C(SH)CH(NH_2)CO_2H \cdot HCl$[e]	5.8	2.6
14	"DL"-$(C_2H_5)(CH_3)C(SH)CH(NH_2)CO_2H$[b]	6.4	6.0
15	DL-$CH_3CH(SH)C(O)NHCH_2CO_2H$	14.7	10.6
16	$HSCH_2CH_4NHCH_2CO_2H \cdot HCl$	6.4	6.3
17	D-*o*-$HO_2CC_6H_4SSC(CH_3)_2CH(NH_2)CO_2H$	5.4[c]	2.9[c]
		7.5[c]	4.4[c]
18	DL-*o*-$HO_2CC_6H_4SSC(CH_3)_2CH(NHAc)CO_2H$	14.7[c]	13.3[c]
19	$Cl_3CCH(OH)S(CH_2)_2NH_3Cl$	7.2[d]	6.8[d]
20	DL-*c*-$(CH_2)_5C(S)NHC(=S)CHCO_2C_2H_5$	6.4	6.3

[a] For details of testing, see footnote *a*, Table I. [b] "DL" is the manufacturer's label designation for product with mp 161–163° dec. [c] Preliminary report published with chem details but not biol ones [L. Field and P. M. Giles, Jr., *J. Org. Chem.*, **36**, 309 (1971)]. Two separate tests with **17** were done. [d] Preliminary report published with chem details but not biol ones.[14b] [e] *c* represents cyclo.

Although **13–15** have optically active centers, racemic **13–15** seemed likely to suffice for preliminary evaluation. This assumption was borne out by the notable activity of **13** (tensile strength ~45% of the control value, in comparison with ~54% for **1**). Compd **13** thus seems to be somewhat superior to **1** itself; we plan to attempt resolution of racemic **13** or of similar compds in future work. In the light of the result with **13**, the inactivity found with **14** is surprising, but it confirms an earlier report.[18] Even if one assumes the D configuration at C-2 of **14** to be as important as it is with **1**, **14** should be 50% D configuration at C-2 regardless of the configuration at C-3. Perhaps the configuration at C-3, of the commercial product tested, is such as to influence the D configuration at C-2 adversely. α-Mercaptopropionylglycine (**15**) was chosen because, like **1**, it has been used in cystinuria therapy[22] and because it contains the 3 functional groups of **1**, albeit in modified form. The result with **15** seems marginally significant (the tensile strength with **15** was ~72% of the control *vs.* ~54% with **1**). Compound **16** also contains the 3 functional groups of **1**, although it contains a primary SH moiety (which at least with 2-aminoethanethiol does not effect a decrease in the rheumatoid factor in humans);[20] **16** was inactive.

The second approach mentioned above to the synthesis of compds related to **1**, which might be more active and/or less toxic, was to investigate latentiating groups for **1**. Consequently, several thiols in combination with potential latentiating groups, **17–20** (Table II), were evaluated for their effects on skin tensile strength. The results are shown in Table II. Previously, the *o*-carboxyphenylthio moiety, *o*-$HO_2CC_6H_4S$, demonstrated promise as a latentiating group for 2-aminoethanethiol in the form of *o*-(2-protoaminoethyldithio)benzoate (**21**), an active antiradiation agent.[23]

$^-O_2CC_6H_4SSCH_2CH_2NH_3^-$

21

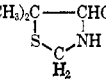
In Table II, the entry is striking for **17**, where the *o*-carboxyphenylthio moiety has been used to latentiate **1**.[24] Compd **17** had essentially the same effect on skin tension as **1** (with **17** the value was 54–59% of the control value, *vs.* 54% with **1**). The equiv action of **17** and **1** hints that the *o*-carboxyphenylthio moiety may be lost quite readily *in vivo*. The inactivity of **18**, the latentiated counterpart of *N*-acetylpenicillamine,[24] shows that the activity of **17** is not produced by the latentiating group itself in the form of *o*-mercaptobenzoic acid or a metabolic product from it. Latentiated forms of 2-aminoethanethiol (**19**) and of the active thiol **13** (**20**) were inactive. Efforts to latentiate **1** itself with chloral and related compounds led to synthetic problems that still are unresolved.[14b] Structure **20** probably bears much the same relation to the active thiol **13** that the thiazolidine **12** bears to **1** in being too stable to provide the active thiol *in vivo*. The slight activity mentioned for the ester of **1** (**9**) indicates that esters of **1** may deserve further attention as a means for latentiating **1**.

Various experiments done either in connection with the foregoing work or in the hope of finding parameters for testing penicillamine other than by skin tensile tests gave a certain amount of useful negative evidence on the manner by which **1** may act in rheumatoid arthritis. D-Penicillamine (**1**), besides effecting a reduction in rat skin tensile strength, has been found to dissociate the rheumatoid factor *in vitro*.[25] Jaffe previously found that quite a variety of compds with a free SH group effected an *in vitro* dissociation.[25] The results with the penicillamine analogs and derivatives shown in Table III substantiate the view that thiols induce the dissociation, but that other substances do not; the sulfide **19** no doubt functions as a latentiated thiol (*cf.* ref 14b). The fact that virtually all thiols that

(20) I. A. Jaffe and P. Merryman, *Ann. Rheum. Dis.*, **27**, 14 (1968).
(21) Ref 12, p 926.
(22) (a) J. S. King, Jr., *Proc. Soc. Exp. Biol. Med.*, **129**, 927 (1968); (b) J. Thomas, A. Lemonnier, R. Lelue, C. Charpentier, L. Levillain, C.-T. Thuong, A. Balan, and P. Aboulker, *J. Urol. Nephrol.*, **74**, 977 (1968).
(23) (a) R. R. Crenshaw and L. Field, *J. Org. Chem.*, **30**, 175 (1965); (b) L. Field and P. M. Giles, Jr., *J. Med. Chem.*, **13**, 317 (1970).
(24) L. Field and P. M. Giles, Jr., *J. Org. Chem.*, **36**, 309 (1971).
(25) I. A. Jaffe, *J. Lab. Clin. Med.*, **60**, 409 (1962).

TABLE III

EFFECTS OF COMPOUNDS ON THE *in Vitro* DISSOCIATION OF THE RHEUMATOID FACTOR

Compd	Structure	Activity[a]
1	D-$(CH_3)_2C(SH)CH(NH_2)CO_2H$	++[a]
2	$(CH_3)_2CHCH_2CO_2Na$	0
3	$(CH_3)_2CHCH_2NH_3Cl$	0
6	D-$(CH_3)_2CHCH(NH_2)CO_2H$	0
8	$(CH_3)_2C(SH)CH_2NH_3Cl$	+++
12		0[b]
14	"DL"-$(C_2H_5)(CH_3)C(SH)CH(NH_2)CO_2H$	+++
19	$Cl_3CCH(OH)S(CH_2)_2NH_3Cl$	+++[c]
	$HS(CH_2)_2NH_2$	++++[a]
	$HSCH_2CH(NH_2)CO_2H$	++++[a]

[a] For details, see ref 25. The activity is based on quant precipitin curves obtd after incubation of the test compd at 0.1 *M* with 0.2 ml of std test serum (1:10) for 30 min at 37°. [b] Footnote *c*, Table I. [c] Footnote *d*, Table II.

have been tried dissociated the rheumatoid factor (even though no thiol but **1** has been known to affect skin tensile strength), taken with the high concentrations needed in the *in vitro* test relative to those for *in vivo* effects, buttress the earlier conclusion that the dissociation of the rheumatoid factor by **1** probably is unrelated to the mechanism of action of **1** on rheumatoid arthritis.[11]

N-Acetylpenicillamine was found to reduce the severity of induced dermal inflammation in rabbits when it was administered in repeated doses, which led to the suggestion that SH compounds may be of use in treatment of rheumatoid arthritis by direct effect on the inflammatory process.[26] However, when **1** was given sc against carrageenin-induced edema in the hind paw of 12 rats, an assay used for antiinflammatory drugs,[27] it led only to 30% inhibition at 2 doses of 50 mg/kg each, which is at the borderline of significant activity (there was no activity at 100 mg/kg in erythema block).[28] Thus since **1** seemed to have little antiinflammatory action in this assay, the possibility that **1** acts primarily as an antiinflammatory agent in rheumatoid arthritis is unattractive to us (*cf.* also ref **11**). The assay seemed to be of dubious value in suggesting synthetic guidelines for compds related to **1**, and no further compds were tested.

The possibility has been suggested that the effectiveness of certain drugs in arthritis, *e.g.*, chloroquine and cortisone, may be associated with the capacity of the compd to stabilize lysosomal membranes.[29] To determine whether such a property might be a factor in the action of **1** in the treatment of rheumatoid arthritis, Touster and Stahl examined this possibility.[30] Although liver lysosomes had to be used, the presence of **1** at 10^{-3} *M*, if anything, appeared to enhance release of acid hydrolases of the lysosomes rather than to diminish it.[32] In any event, effects of **1** on liver lysosomes seem to afford no promise as a means of estimating effects of compds like **1** in rheumatoid arthritis.

Immunosuppressive drugs have become of considerable interest in research on arthritis.[33] However, **1** also seems unlikely to exert its effect by any very simple mechanism of immune suppression, since only 250 mg/kg of **1** in 10 doses before and after sensitization (but not 125 or 62.5 mg/kg) suppressed the antibody in the sheep-erythrocyte-antibody mouse assay (5 mice at each dose level; **1** given ip).[28,34] This is a low order of activity, considerably less than for azathioprine or cortisone. Furthermore, confirming earlier evidence that **1** did not influence the immune response of rabbits injected with formalin-killed *E. coli* organisms,[11] tests of **1** against adjuvant arthritis[35] also were essentially negative.[28,36]

In a continuing study of potential antiradiation drugs, **17** and **18** were tested but were inactive. The ALD_{50} (mg/kg), the drug dose (mg/kg), and the per cent survival after 30 days, respectively, were for **17**, 400, 100, and 0%, and for **18**, 900, 600, and 0%.[37]

Chemistry.—Preparation of the samples used for biological testing usually went smoothly by known methods. Only 3 syntheses warrant discussion.

First, 3-mercapto-3-methylbutanoic acid (**22**) had

$(CH_3)_2C(SH)CH_2CO_2H$
22

been prepared previously starting with β,β-dimethylacrylic acid and H_2S, but only in 15% yield; the unchanged acrylic acid was the major substance isolated.[38] Hence an alternate route to the acid **22** was devised which started with β,β-dimethylacrylic acid and phenylmethanethiol. A second step, cleavage of the benzyl moiety with Na–NH_3, was needed to produce the mercapto acid **22**. However, the overall yield for this 2-step sequence was 76%. LAH reduced the acid **22** to the carbinol **4**.

Second, the preparation of the Me ester of penicillamine·HCl (**9**) has been reported numerous times.[39] The most useful preparation seems to be that of Sheehan and Tishler[39c] from **1** and dimethyl sulfite; in our hands, this gave the ester in 83% yield.

Lastly, alkylation of **1** with MeI in the presence of NaOMe in several trials did not give D-*S*-methylpenicillamine cleanly, even though alkylation of an SH group attached to a primary C in an aminothiol should

(26) K. R. Bailey and A. L. Sheffner, *Biochem. Pharmacol.*, **16**, 1175 (1967).

(27) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962); responses to standard agents are reported.

(28) We thank Dr. W. B. Lacefield of Eli Lilly & Co., Indianapolis, Ind., for arranging for these tests and for their interpretation.

(29) (a) *Cf.* H. J. Sanders, *Chem. Eng. News*, **46**, July 29, 64 (1968); (b) A. J. Anderson, *Biochem. J.*, **113**, 457 (1969).

(30) O. Touster and P. D. Stahl, Department of Molecular Biology, Vanderbilt University, Nashville, Tenn., private communication. Liver lysosomes, prepd by the method of DeDuve,[31] were incubated in 0.25 *M* sucrose at pH 5.0. The release of several marker enzymes from the particles at 37° was not inhibited by the presence of **1** at 10^{-5}, 10^{-4}, or 10^{-3} *M* concns.

(31) C. DeDuve, B. C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans, *Biochem. J.*, **60**, 604 (1955).

(32) *Cf.* also ref 29b.

(33) H. J. Sanders, *Chem. Eng. News*, **46**, 64 (Aug 12, 1968).

(34) H. C. Nathan, S. Bieber, G. B. Elion, and G. H. Hitchings, *Proc. Soc. Exp. Biol. Med.*, **107**, 796 (1961).

(35) B. B. Newbould, *Brit. J. Pharmacol.*, **21**, 127 (1963).

(36) Edema inhibition by 25 mg/kg/day of **1**, 31.8–39.5%. X-ray scores on a scale of 0–5 (max bone damage) were: phenylbutazone (50 mg/kg) 1.2–2.0; **1**, 2.0–4.0; control, 2.8–4.5.

(37) For leading references to earlier work and to details of testing, see ref 23b. We thank Drs. D. P. Jacobus, T. R. Sweeney, E. A. Steck, D. L. Klayman, and Miss Marie Grenan of the Walter Reed Army Institute of Research for these results.

(38) O. Süs, *Justus Liebigs Ann. Chem.*, **559**, 92 (1948).

(39) The following references illustrate three of the methods used: (a) Merck & Co., Inc., British Patent 622,298 (1949); *Chem. Abstr.*, **43**, 7039 (1949); (b) F. C. Copp and S. Wilkinson, British Patent 585,250 (1947); *Chem. Abstr.*, **41**, 4175 (1947); (c) J. C. Sheehan and M. Tishler, U. S. Patent 2,491,523 (1949); *Chem. Abstr.*, **44**, 3034 (1950).

lead to a clean S-substituted product.[40] As an alternative to the direct preparation of D-*S*-methylpenicillamine from **1**, we prepared DL-*S*-methylpenicillamine (**10**) in 2 steps from commercially available DL-*N*-acetylpenicillamine by modifying a reported method.[41]

Experimental Section[42]

Materials.—**D-Penicillamine** (**1**) was donated by the Merck Sharp and Dohme Research Laboratories, West Point, Pa., or was purchased from Aldrich Chemical Co. Aldrich Chemical Co. supplied **DL-*N*-acetylpenicillamine, DL-β-mercaptoisoleucine (14),** and **cysteamine-*N*-acetic acid·HCl (16).** Pierce Chemical Co. supplied D-valine (**6**), and Santen Pharmaceutical Co. (*via* Calbiochem Co.) supplied α-mercaptopropionylglycine (**15**). All of the foregoing were used as supplied.

Sodium isovalerate (**2**) was prepared by neutralizing isovaleric acid with an equiv amt of 30% aq NaOH, evapg, and drying *in vacuo* at 50° for 24 hr. *i*-BuNH$_3$Cl (**3**) was obtained by dissolving *i*-BuNH$_2$ in ice-cold MeOH, satg with dry HCl, removing solvent, recrysig **3** from Et$_2$O–MeOH, and drying *in vacuo*: needles, mp 175–178°, lit.[43] mp 177–178°.

Sodium 2-mercapto-2-ethylbutanoate (**5**) was prepd by treating 2-mercapto-2-ethylbutanoic acid[44] (0.059 mole) in abs EtOH with NaOEt (0.059 mole), removing the solvent, and washing the solid with anhyd Et$_2$O, mp 225–240° dec.

2-Mercapto-2-methylpropylammonium chloride (**8**) was prepd by a reported method, but with AlHg instead of LAH;[45] hygroscopic colorless plates, mp 231–233° (sealed tube), lit. mp 238–238.5°,[46] 220–222°;[45] the ir of (**8**) was identical with that of an authentic sample.[46] For the prepn of **α-amino-β-mercaptocyclohexaneacetic acid·HCl** (**13**) and **2-thio-4-carbethoxy-5,5-pentamethylenethiazolidine** (**20**), glycinamide·HCl (452 mmoles, Aldrich Chemical Co.) was converted using CS$_2$ (452 mmoles), K$_2$CO$_3$ (452 mmoles), and KOH (452 mmoles to neutralize HCl) in H$_2$O (240 ml), then (after 24 hr) concd HCl (300 ml), to "2-mercaptothiazol-5-one;"[47] the latter was condensed with cyclohexanone using morpholine to give "2-thio-4-cyclohexylidene-5-thiazolidone;"[48] treatment of the latter with NaOEt, then cold HCl, gave **20**, mp 145–149° (lit.[48] mp 136–138°);[48] hydrol of **20** with concd HCl in a sealed tube at 140° for 24 hr gave **13** in an overall yield from glycinamide·HCl of 7%, mp 221–223° dec (lit.[48] mp 223–224° dec). Compds **12**,[49] **17**,[24] **18**,[24] and **19**[24b] were obtd as reported previously; we thank P. M. Giles, Jr., for the prepn of **17** and **18**.

3-Mercapto-3-methylbutanol (**4**).—3-Mercapto-3-methylbutanoic acid (*vide infra*; 50 g, 0.373 mole) was added to LAH (28.4 g, 0.749 mole) in Et$_2$O (600 ml) during 2 hr. The mixt was heated under reflux for 20 hr, before H$_2$O (28 ml), NaOH soln (15%, 28 ml), and H$_2$O (84 ml) were added successively; the mixt then was heated under reflux until excess hydride decompd. Residue was sepd by filtration and washed with Et$_2$O (4 × 200 ml). The Et$_2$O soln was dried (MgSO$_4$) and Et$_2$O was removed; yield of **4** as a pale yellow oil, 42.7 g. This **4** (35 g) was distd to give colorless **4** (25.8 g, 70% yield): bp 87–89° (20 mm); n^{25}D 1.4780; ir and nmr spectra as expected. *Anal.* (C$_5$H$_{12}$OS) C, H, S.

Potassium 3-Mercapto-3-methylbutanoate (**7**).—A mixt of phenylmethanethiol (124 g, 1.0 mole), β,β-dimethylacrylic acid (100 g, 1.0 mole), and piperidine (180 ml) was heated under reflux for 24 hr.[50] The mixt was acidified with dil HCl and extd with Et$_2$O (2 × 200 ml). The Et$_2$O layer then was extd with 10% Na$_2$CO$_3$ soln (3 × 200 ml). The carbonate mixt was acidified with concd HCl and extd with Et$_2$O. The ext was dried (MgSO$_4$). Filtration and removal of Et$_2$O gave pale yellow **3-benzylmercapto-3-methylbutanoic acid** (174 g, 78% yield). Distn gave acid with bp 167–169° (1.6 mm); lit.[38] bp, 195° (12 mm); ir and nmr spectra as expected. *Anal.* (C$_{12}$H$_{16}$O$_2$S) C, H, S.

3-Mercapto-3-methylbutanoic acid was prepd from undistd 3-benzylmercapto-3-methylbutanoic acid (170 g, 0.76 mole) in liq NH$_3$ (500 ml) by slow addn of Na (42 g, 1.83 g-atoms). The NH$_3$ was removed under an N$_2$ stream during a 24-hr period. The residue was acidified with dil HCl and extd with Et$_2$O (2 × 300 ml). The Et$_2$O soln was extd with Na$_2$CO$_3$ soln (10%, 4 × 200 ml). The carbonate ext was acidified with concd HCl and extd with Et$_2$O. After the Et$_2$O ext had been dried (MgSO$_4$), removal of Et$_2$O left pale yellow 3-mercapto-3-methylbutanoic acid (99 g, 97% yield) which partially crystd in long prisms on standing. Distn of a sample from another prepn gave a colorless solid, bp 119–120° (12 mm), mp 36–37° [lit.[38] bp 118–120° (12 mm), mp 38°]. **Potassium 3-mercapto-3-methylbutanoate** (**7**) was then prepd by neutralizing 3-mercapto-3-methylbutanoic acid with aq KOH and removing the H$_2$O. The salt was dried overnight *in vacuo* to give a white powder, mp 230–234° dec.

D-Penicillamine Methyl Ester·HCl (**9**).—Essentially as reported,[39c] a mixt of **1** (20 g, 0.134 mole), dimethyl sulfite (Eastman Organic Chemicals; 30.5 g, 0.276 mole), HCl gas (139 g), and anhyd MeOH (400 ml) was heated under reflux for 20 hr; C$_6$H$_6$ (100 ml) then was added, and solvents were removed under reduced pressure at about 70°. The residue of crude **9** was slurried with hot *tert*-BuOH, and solid **9** was separated by filtration. The filtrate upon cooling deposited more **9** (identical ir spectrum), which was combined. The **9** then was recrystd from *tert*-BuOH to give 22.2 g (83% yield), with mp 186–188° dec; lit.[39c] mp 184° dec; ir and nmr spectra as expected.

DL-*N*-Acetyl-*S*-methylpenicillamine (**11**).—In a modification of a reported procedure,[41] DL-*N*-acetylpenicillamine (35 g, 0.18 mole) was dissolved in anhyd MeOH (300 ml) to which Na (8.6 g, 0.37 g-atom) had been added. MeI (28 g, 0.20 mole) mixed with anhyd MeOH (20 ml) then was added in 1 portion. The mixt was stirred at ~25° overnight. The solvent was removed, and the residue was dissolved in H$_2$O. After filtration, the soln was adjusted to pH 1.5 with 6 *M* HCl. A copious amt of white solid appeared. The mixt was extd with 7 l. of EtOAc in numerous portions. The resulting soln was dried (MgSO$_4$), the EtOAc was removed, and the residue was recrystd twice from H$_2$O to give 29.4 g (80% yield) of **11**, mp 203–205° dec; lit.[41] mp 196–197°. The **11** thus prepd was converted to **DL-*S*-methylpenicillamine** (**10**) by the procedure of Marshall, *et al.*,[41] mp 250° dec; lit.[51] mp 250° dec.

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(42) Melting points, detd in capillary tubes using a Hershberg stirred-liquid apparatus or a Mel-Temp block, are cor; boiling points are uncor. Where analyses are indicated only by symbols of the elements, results of analyses for these elements were within ±0.4% of the theoretical values (Galbraith Analytical Laboratories, Knoxville, Tenn.). Ir spectra were obtained using a Beckman Model IR10 spectrophotometer with thin films of liquids and KBr pellets of solids; bands reported were of a least medium intensity. Nmr spectra were obtained using a Varian Model A-60 spectrometer (TMS as internal standard) (with **9** in D$_2$O, HDO was set at δ 4.61 as a standard). Solvents were evapd under reduced pressure using a rotary evaporator.

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