phosphate and of $M/10$ sodium monobasic phosphate solutions) and finally with 10% sulfuric acid. Each individual acid extract was worked up according to the general procedure and analyzed for quiniine and quinidine.

The method offered no significant improvement over the usual method of isolation.

Summary

The mechanism of the partial racemization of quinine to a mixture of quinine, epiquinine, quinidine and epiquinidine involves two steps: the alkoxide catalyzed oxidation of quinine to quininone by a ketone followed by the alkoxide catalyzed reduction of quininone to the stereoisomeric mixture by a primary or secondary alcohol. The dependence of the proportion of isomers in the reduction mixture on the nature of the alkoxide ion employed was investigated. It was found that sodium isopropoxide converted quininone to quinidine in good yield while sodium pentylate-3 reduced quininone predominantly to quinine. There was discovered the disproportionation of quinine to quininone in potassium *t*-alkoxides and the oxidation of the epibases to quininone by sodium *t*-butoxide and fluorenone.

A new and probably general method for the racemization or epimerization of secondary alcohols was developed: quinine was partially racemized in toluene by treating with sodium *t*-butoxide and an oxidation–reduction system such as fluorenone–fluorenol.

NEW YORK 27, N. Y. RECEIVED OCTOBER 24, 1946

[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Alkyl Thiolsulfinates

BY LAVERNE D. SMALL, JOHN HAYS BAILEY AND CHESTER J. CAVALLITO

The antibacterial agent isolated from *Allium sativum* has been assigned the probable structure, $CH_2{=}CH{-}CH_2{-}\underset{\underset{O}{|}}{S}{-}S{-}CH_2{-}CH{=}CH_2$.[1]

This compound, allyl 2-propene-1-thiolsulfinate, represented the only known example of a derivative of the hypothetical thiolhyposulfurous acid, $H{-}\underset{\underset{O}{|}}{S}{-}S{-}H$.[2]

There is now described the preparation and properties of a series of synthetic alkyl thiolsulfinates. This new class of compounds of which the antibacterial agent of garlic represents the prototype, has the feature of possessing marked antimicrobial activity. This property is believed to be associated with the marked reactivity of the $-\underset{\underset{O}{|}}{S}{-}S-$ group toward biologically essential sulfhydryl groups.[1,3]

The following method for the preparation of thiolsulfinates proved to be unsatisfactory

$$R{-}MgCl + SOCl_2 \longrightarrow R{-}SO{-}Cl + MgCl_2$$
$$R{-}SO{-}Cl + R'{-}S{-}M \longrightarrow R{-}SO{-}S{-}R' + MCl$$

where M is H or Na. By using ethyl as R and R′ in a variety of solvents at several concentrations and temperatures, yields were obtained of less than 0.3% thiolsulfinate as shown by antibacterial assay.

The alkyl thiolsulfinates were satisfactorily prepared by oxidation of the corresponding disulfides with an organic per-acid in an appropriate solvent. A number of per-acids could be used, including peracetic, perbenzoic, perfuroic, perphthalic and percamphoric; perlauric acid was not as satisfactory. Perbenzoic or peracetic acid was the oxidizing agent of choice in the presently described work.

The solvent for the oxidation was not critical although chloroform was preferred. Ethanol, ether, acetone, acetaldehyde, acetic acid, acetonitrile, or carbon tetrachloride also could be used.

An equimolecular ratio of organic per-acid to disulfide gave the best yields of thiolsulfinate (20% to 65%), the yields decreasing with a deficiency or an excess of per-acid. The oxidation of normal disulfides from methyl through amyl proceeded readily, thirty minutes reaction time at 25° being sufficient for maximum yield. Secondary disulfides proceeded with more difficulty, isopropyl disulfide underwent oxidation more slowly and gave poorer yields than the normal compound. In the oxidation of the secondary isomer, peracetic was better than perbenzoic acid, and ultraviolet light irradiation improved the yield with perbenzoic acid. No measurable yield of thiolsulfinate could be obtained by the presently described procedures, from oxidation of di-tertiary disulfides such as *t*-butyl and *t*-hexyl disulfide. Since normal alkyl disulfides yielded a monoxide and the tertiary did not, it was of interest to extend the series to a mixed type such as *t*-butyl ethyl disulfide. The latter yielded a thiolsulfinate which was assigned the structure $C_2H_5{-}\underset{\underset{O}{|}}{S}{-}S{-}C(CH_3)_3$ in light of the hindrance to oxidation of the sulfur demonstrated by the tertiary group.

(1) Cavallito, Buck and Suter, THIS JOURNAL, **66**, 1950 (1944).

(2) Hinsberg, (*Ber.*, **41**, 2838 (1908)) postulated that a thiolsulfinate was formed by the oxidation of bis-(*p*-acetaminophenyl) disulfide with nitric acid; however later (*ibid.*, **42**, 1278 (1909)) he indicated that the product was an equimolecular mixture of disulfide and disulfoxide. Toennies (THIS JOURNAL, **56**, 2198 (1934)) and Toennies and Lavine (*J. Biol. Chem.*, **113**, 571 (1936)) believed that cystine, upon oxidation to the disulfoxide, went through the monoxide intermediate (thiolsulfinate) which could not be isolated.

(3) Cavallito, *J. Biol. Chem.*, **164**, 29 (1946).

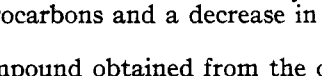
Oxidation of ethyl diselenide under the conditions described with perbenzoic acid did not yield products with antibacterial activity.

Temperature of oxidation is not critical, the reaction of disulfide with perbenzoic acid proceeding from at least −20 to 50°, above which there is usually considerable decomposition of thiolsulfinate.

The alkyl thiolsulfinates are mobile liquids at room temperature. They are soluble in most organic solvents such as alcohols, ketones, ethers, halogenated hydrocarbons and aromatic hydrocarbons, and with increase in size of the carbon chains, show an increase in solubility in the petroleum hydrocarbons and a decrease in water solubility.

The compound obtained from the oxidation of allyl disulfide with perbenzoic acid was found to be identical with the natural product from garlic. This serves to substantiate the thiolsulfinate structure for this antibiotic since the oxidation is carried out on preformed disulfide. This reasonably eliminates the —S—O—S— structure for these compounds, particularly since oxidation of the mercaptans directly with two moles of peracid yielded some thiolsulfinate, but in much poorer yield than from the disulfide.

Whereas allyl 2-propene-1-thiolsulfinate is very unstable in the pure state, the saturated thiolsulfinates are relatively stable at temperatures up to 40 to 50° and may be kept at room temperature for several weeks without measurable deterioration. The *n*-butyl and *n*-amyl derivatives appear to be somewhat less stable than the lower homologs, although all preparations keep well at 0 to 5°. The particular instability of the allyl compound appears to be associated with the double bond structure whereas the antibacterial activity is derived from the —S—S— grouping. The saturated thiolsulfinates prepared are all distillable at reduced or very low pressures. Thiolsulfinates are unstable toward alkalies, yielding the disulfide and sulfur dioxide. Dilute acids have no action, although iodine is liberated from hydriodic acid. Cysteine reacts readily with thiolsulfinates to yield almost quantitatively, crystalline compounds, having the formula



Experimental

Preparation of Disulfides.—The methyl, *n*-propyl, *n*-butyl and *n*-amyl disulfides were Eastman Kodak Co. preparations further purified by fractional distillation until their molar refractivities were in fairly good agreement with calculated values. The di-tertiary disulfides were obtained from the Phillips Petroleum Company. The isopropyl disulfide was prepared by iodine oxidation of isopropyl mercaptan. The *t*-butyl ethyl disulfide was obtained in 63% yield by oxidation with iodine of an equimolecular mixture of ethyl and *t*-butyl mercaptans in ethanol, followed by fractional distillation of the oxidation mixture; b. p. 58–61° at 11 mm. pressure.

Anal. Calcd. for $C_6H_{14}S_2$: C, 48.04; H, 9.39. Found: C, 48.22; H, 9.45.

Allyl disulfide (b. p. 58–59° at 5 mm. pressure) was prepared in 70% yield by iodine oxidation of freshly distilled allyl mercaptan in cold pyridine–ethanol solution and, more easily, in 62% yield from allyl chloride through the Bunte salt.[4,5]

Oxidation of Disulfides.—Into a three-liter, three-neck flask fitted with a stirrer and dropping funnel was added 0.1 mole of the disulfide in approximately one liter of chloroform. To this was added slowly (about thirty minutes) with rapid stirring and cooling with ice-water, 0.1 mole of perbenzoic acid[6] in 200 cc. of chloroform. The reaction mixture was allowed to stand from thirty minutes to one hour at room temperature, after which it was shaken once with 0.1 mole of 5% aqueous sodium bicarbonate solution and then with sufficient 2% aqueous sodium bicarbonate solution to remove acids. The chloroform solution was shaken finally with 100 cc. of water. Each aqueous wash solution was extracted with 50 to 100 cc. of chloroform which was then added to the main chloroform solution.

Isolation of Thiolsulfinates.—**A.**—The chloroform from the above solution was removed by distillation under reduced pressure. To the residue was added about 25 cc. of Skellysolve B and the mixture was extracted in a separatory funnel with water in 200-cc. portions. Each water extract was filtered through a wet filter paper and the paper and remaining oil was added to the Skellysolve layer and shaken with the next 200 cc. of water. Completion of extraction was tested for by adding a drop of 10% aqueous sodium hydroxide solution to a few cc. of the clear water extracts. Development of a turbidity within a few seconds indicated the presence of sufficient thiolsulfinate to warrant further extraction of the oily material. The clear aqueous extracts of the thiolsulfinate were combined and extracted with four 25 to 50 cc. portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate after which the solvent was distilled off under reduced pressure leaving behind the thiolsulfinate. This method depends upon the relative water insolubility of disulfides as compared with the more water soluble thiolsulfinates. The *n*- and isopropyl and allyl derivatives were particularly suited for this type of isolation.

B.—The chloroform solution was dried over anhydrous sodium sulfate, filtered and distilled under reduced pressure by means of a water pump to remove the solvent. The residue was then distilled with the aid of a mechanical vacuum pump, a two-stage fractionating oil-diffusion pump also being used for attaining the lower pressures. The disulfides were distilled off at lower temperatures or higher pressures than the corresponding thiolsulfinates. The distillation temperature for most of the compounds was maintained within the range of 25 to 50°, the pressures being adjusted to obtain the desired distillation rate of approximately 2 to 4 cc. per hour.

Antibacterial and Antifungal Tests.—The antibacterial tests with most of the organisms were carried out as described in a previous publication.[7] The media employed were, beef extract broth for all of the aerobic bacteria excepting the pneumococci and *Streptococcus hemolyticus* C203 which were grown in veal infusion broth containing 10% horse serum and 0.1% dextrose; the fungi were grown in a medium composed of 4% glycerol and 1% peptone; the Long's medium was used for the acid-fast organism. All thiolsulfinates were first dissolved in water and secondary dilutions were made with the growth media. The bacterial inoculum was 1 cc. of a 1:1000 dilution of a twenty-four hour broth culture of the test organism, except with *B. tuberculosis ranae* for which a 1:100 dilution was used. Incubation was for fifteen hours at 37° for the bacteria and forty-eight hours

(4) Twiss, *J. Chem. Soc.*, **105**, 36 (1914).
(5) Westlake and Dougherty, THIS JOURNAL, **63**, 658 (1941).
(6) "Organic Syntheses," Coll. Vol. I, 2nd ed., p. 431.
(7) Cavallito and Bailey, THIS JOURNAL, **68**, 489 (1946).

for the acid-fast organism. Complete absence of growth was taken as the bacteriostatic level. Samples from all tubes showing bacteriostasis were diluted 1:10 in sterile broth for observation of bactericidal action.

Antifungal tests were run in a medium composed of 1% bacto-tryptone and 4% glycerol. Incubation was for fourteen days at 25°. The inoculum consisted of 1 cc. of a suspension of spores (*A. niger* and *P. notatum*) or finely divided mold mat in a concentration of 12.5 mg. per cc. Since the tests were run in 25-cc. volume, the final concentration of the inoculum in the tests was 0.5 mg. per cc.

Discussion

A few observations may be made relative to the chemical structure and antibiotic activity of the thiolsulfinates. In general, it requires about the same quantities of the lower molecular weight thiolsulfinates (2, 4 and 6 carbons) to inhibit gram-positive as compared with gram-negative bacteria, but as the carbon chain length increases, activity against gram-negative organisms de-

TABLE I

THIOLSULFINATES—PHYSICAL AND ANALYTICAL DATA

Formula	Distillation condn.[a] Temp., °C.	Press., mm.	$n^{25}D$	d^{25}_4	Mol. ref. Calcd.	Mol. ref. Found	Approx. water soly., %	Yield, %[b]	Analyses, %[c] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
$CH_3S(O)SCH_3$[d]	64[e]	0.5	1.5481	1.222	29.76	28.64	∞	20[f]	21.80	22.06	5.49	5.78
$C_2H_5S(O)SC_2H_5$	67	.5	1.5244	1.104	38.99	38.33	11	45	34.75	35.06	7.29	7.46
n-$C_3H_7S(O)SC_3H_7$-*n*	25–35	.01	1.5098	1.041	48.23	47.76	2	45	43.33	43.50	8.49	8.43
i-$C_3H_7S(O)SC_3H_7$-*i*	25–30	.10	1.5090	1.057	48.23	46.98	2.5	7–30[g]	43.33	43.13	8.49	8.59
$C_2H_5S(O)SC_4H_9$-*t*	25–35	.10	1.5092	1.043	48.23	47.61	3	60	43.33	43.63	8.49	8.63
$C_3H_5S(O)SC_3H_5$	Unstable		1.5600	1.109	47.30	47.33	2.5	63	44.44	44.82	6.17	6.24
n-$C_4H_9S(O)SC_4H_9$-*n*[h]	20–30	10^{-5}	1.5041	0.992	57.47	57.98	0.1	54	49.45	49.44	9.34	9.43
n-$C_5H_{11}S(O)SC_5H_{11}$-*n*	45	$\lesssim 10^{-5}$	1.4990	0.988	66.70	66.07	0.015	56	54.00	54.03	9.97	9.79

[a] Variations in temperature or pressure affect rate of distillation. [b] Yields from perbenzoic acid oxidations unless otherwise stated. [c] Sulfur determinations by the oxygen-bomb method (30 atmospheres oxygen pressure) of the thiolsulfinates were found, by the analytical staff, to be erratic and usually low; this difficulty was not evident with the disulfides. [d] Freezing point approximately −10°. [e] The methyl and ethyl derivatives are more stable to distillation. [f] Low yield may result from losses of product during aqueous washing of the oxidation mixture in chloroform. [g] Lower yields with perbenzoic acid, higher with peracetic. Separation by distillation from by-products difficult, several fractions of lower carbon content obtained. [h] Freezing point approximately −40°.

TABLE II

ANTIBACTERIAL AND ANTIFUNGAL DATA MINIMUM CONCENTRATION OF THIOLSULFINATE REQUIRED FOR COMPLETE GROWTH STASIS,[a] EXPRESSED AS MILLIMOLES PER CC. × 10^4

Organism	Methyl	Ethyl	*n*-Propyl	*i*-Propyl	*t*-Butyl ethyl	*n*-Butyl	*n*-Amyl	Allyl
Staphylococcus aureus 209	3	1.5	2	3	6	0.5	0.1	0.6
Staphylococcus aureus SA10	4.5	0.6	1.5	3	6	.5	.1	.6
Staphylococcus albus 151	>6	1.5	3	3	6	1.5	.1	1.5
Sarcina lutea	1.5	0.6	0.3	1.2	3	0.05	.03	0.2
Streptococcus fecalis 10C1	>6	1.5	4	6	9	3	.3	2
Streptococcus hemolyticus 68C	4.5	1.5	1.5	3	6	0.5	.1	0.6
Streptococcus hemolyticus C203	1.5	1.5	3	6	6	.6	.7	1
Bacillus subtilis C4	3	1.5	1	3	6	.3	.03	0.5
Bacillus subtilis S8	0.6	0.6	1	6	3	.5	.07	.3
Bacillus cereus 15	1.5	6	1.5	3	6	.5	.07	.3
Bacillus coli ATC4157	3	1.5	3	6	6	>6	>1	1.5
Bacillus typhosus	1.5	1.5	3	6	9	>6	>1.3[b]	1
B. dysenteriae Hiss	1.5	0.6	2	6	9	4.5	[a]	0.3
B. dysenteriae Flexner ATC9380	1.5	1.5	2	6	6	6	[a]	.3
Bacillus paratyphosus A ATC9150	3	1.5	3	9	12	>6	[a]	1
B. typhi murium	3	1.5	3	12	>12	>6	[a]	1
Diplococcus pneumoniae I	6	1.5	5	12	>12	3	[a]	2
Diplococcus pneumoniae III	>6	1.5	6	>12	>12	3	[a]	..
Bacillus welchii	3	4.5	1.5			1.5	[a]	1
Bacillus histolyticus	1.5	1.5	1.5			1.5	[a]	0.6
Bacillus tuberculosis ranae ATC110	1	0.01	0.1	0.1	0.6	0.03	0.007	.5
Aspergillus niger	2	0.07	.6	3	3	1.2	.7	.6
Penicillium notatum	>8	.4	..	..	..	1.2	..	.9
Trycophyton gypseum	2	.7	.3	0.6	1	1.2	.07	.06
Microsporon audouini	0.8	.07	> .03	.3	0.3	0.06	.04	.06

[a] Bactericidal values range from concentrations equal to, to approximately ten times the bacteriostatic levels. The higher molecular weight compounds, in general, require higher ratios for bactericidal levels. The activity against the fungi is essentially fungicidal. [b] Indicates >1.3; end-point not obtained as result of low solubility of the compound in aqueous media.

creases while that against gram-positive bacteria increases. Branching results in lowered activity, the order of activity being *n*-propyl > isopropyl > *t*-butyl ethyl derivatives.

As far as has been determined, thiolsulfinates react only with the sulfhydryl groups in amino acids and, conceivably, of protein matter as well and it has been postulated that they act by blocking essential protein —SH groups. The more or less non-specificity of action of the lower members may be related to their high diffusibility and low selective adsorption on protein surfaces. Increasing molecular size would be expected to decrease ease of diffusibility and decrease the number of opportunities for reaction with —SH groups screened by other protein structures. However, as the longer carbon chain molecules become more lipid soluble and less water soluble, an increase in the lipid content of cells (as in the acid-fast organisms) or even more probable, by having present —SH enzymes of a lipoprotein character, one should expect a selective concentration of these thiolsulfinates in the vicinity of the vulnerable —SH groups. To explain the trend in activity, this could mean that the more sensitive organisms contain more lipoprotein type of —SH enzymes than do less sensitive organisms; that the more sensitive organisms contain more total cellular lipids; that the essential —SH groups in sensitive organisms are more superficially located in the enzyme or that a combination of these factors is involved.

Acknowledgment.—We are indebted to M. E. Auerbach and staff for the analytical data, to W. F. Warner for cup-plate assays, and wish to thank Dr. C. M. Suter and Dr. J. S. Buck for encouragement and suggestions offered during the course of this investigation.

Summary

Eight synthetic alkyl thiolsulfinates have been prepared by controlled oxidation of the corresponding disulfides and isolated in the pure state. These compounds represent a new class of sulfur esters modelled after the natural antibiotic from *Allium sativum* and the structure of the latter is defined.

All thiolsulfinates prepared demonstrate antibacterial and antifungal action, with the higher members showing increasing specificity of action. This new class of antimicrobial agents appears to act by binding sulfhydryl groups essential for cell metabolism.

RENSSELAER, NEW YORK RECEIVED FEBRUARY 28, 1947

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1118]

The Electron Diffraction Investigation of Isomeric Lewisites[1]

BY JERRY DONOHUE,[2] GEORGE HUMPHREY AND VERNER SCHOMAKER

This investigation of the two isomers of β-chlorovinyldichloroarsine, Lewisite I (b.p. 190° at 760 mm.) and Lewisite II (b.p. 150.2° at 760 mm.), was undertaken in order to determine which is the *cis* form and which is the *trans* form. MacDowell and Emblem[3] found the dipole moments of Lewisite I and Lewisite II to be 2.21×10^{-18} e.s.u. and 2.61×10^{-18} e.s.u., respectively,[4] and on this basis assigned the *trans* structure to Lewisite I and the *cis* structure to Lewisite II. Confirmation of this assignment seemed desirable because of the small difference between the observed moments and because of the uncertainty which we believe would necessarily arise from the presence of two polar groups ($\rangle AsCl_2$ and $Cl\rangle C{=}C\langle$ or $\rangle C{=}C\langle Cl$) joined by a single bond with more or less unpredictable freedom of internal rotation in the neighborhood of an unknown preferred orientation. In addition, it was hoped that information could be obtained concerning the influence of the carbon–carbon double bond on the arsenic–carbon single bond distance and of the organic group on the arsenic–chlorine distance.

Experimental

The sample of the higher boiling isomer I was obtained from Dr. C. E. Redemann of the University of Chicago, and that of isomer II was provided by Edgewood Arsenal through the courtesy of Gen. W. C. Kabrich and Col. M. F. Peake. The constants given by Redemann for the sample of isomer I are: b.p., 72–73° at 10 mm.; d^{25} 1.8799; and $n^{25}D$ 1.6068; in fairly close agreement with the accepted values. According to Edgewood Arsenal the sample of isomer II had a refractive index corresponding to a composition of 4% of isomer I and 96% of isomer II, while chemical analysis showed 4.68% of isomer I,

(1) The work herein reported was done under Contract OEMsr-753 between the National Defense Research Committee and the California Institute of Technology.

(2) Predoctoral Fellow of the National Research Council.

(3) C. A. MacDowell and H. G. Emblem, British Report (from Sutton Oaks).

(4) These values are not consistent with the value 1.77×10^{-18} e. s. u. found by C. T. Zahn and H. Mohler, *Helv. Chim. Acta*, **21**, 1292 (1938), for a preparation of β-chlorovinyldichloroarsine which, on the basis of this value, they concluded was a mixture of the *cis* and *trans* isomers.