[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

Synthesis and Fungistatic Activity of Some 5-(1-Methyl-2-thioalkylethylidene)-rhodanines

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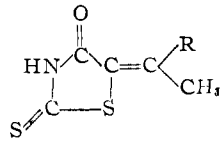
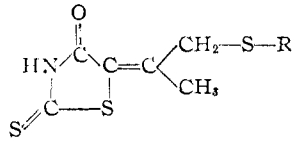
Nine 5-(1-methyl-2-thioalkylethylidene)-rhodanines (II) have been synthesized by condensation of rhodanine with alkyl acetonyl sulfides. Of the sulfides required for this work, seven are here reported for the first time. The fungistatic action of the new rhodanine compounds II has been compared with that of the 5-(1-alkylethylidene)-rhodanines (I).

In some earlier communications we have described the condensation of rhodanine with methyl ketones[1] and the mildew-proofing activity[2,3] of the resulting 5-(1-alkylethylidene)-rhodanines (I). As



I



II

may be seen in Fig. 1, these condensation products I possess considerable fungistatic activity toward *Aspergillus niger*, a non-cellulolytic fungus, the activity reaching a maximum where R is C_2H_5 or C_3H_7.[4] In order to determine the effect produced by the introduction of a third sulfur atom into the molecule we have now synthesized some 5-(1-methyl-2-thioalkylethylidene)-rhodanines (II),[5] The necessary alkyl acetonyl sulfides were prepared by allowing an alcohol solution of the proper sodium mercaptide to react with chloroacetone.[6,7] Our results in this step are summarized in Table I.

TABLE I

ALKYL ACETONYL SULFIDES CH_3COCH_2SR

R	B.p., °C.	Press., mm.	$n^{25}D$	Yield, %	Formula	Analyses,[a] % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
CH_3	152.5–153	(At.)	1.4713	54	C_4H_8OS	46.13	46.20	7.74	7.88
C_2H_5	168–172[b]	(At.)	....	54					
C_3H_7	101–101.5	48	1.4681	60	$C_7H_{15}ON_3S$[c]				
$(CH_3)_2CH$	92–92.5	48	1.4627	69	$C_7H_{15}ON_3S$[d]	44.42	44.34	7.98	7.94
C_4H_9	115–119[e]	50		56					
$(CH_3)_2CH—CH_2$	109–109.5	48	1.4621	60	$C_7H_{14}OS$	57.48	57.35	9.65	9.43
$(CH_3)_3C$	103.5–104	48	1.4632	73	$C_7H_{14}OS$	57.48	57.44	9.65	9.56
C_5H_{11}	133–133.5	48	1.4647	75	$C_8H_{16}OS$	59.94	59.99	10.06	9.73
C_6H_{13}	147.5–148.5	48	1.4646	77	$C_9H_{18}OS$	62.02	62.34	10.41	10.47

[a] Analyses by Clark Microanalytical Laboratories, Urbana, Illinois. [b] Lit. (ref. 6) 170–172°. [c] Semicarbazone, white needles from dilute alcohol, m.p. 153.5–154°; *Anal.* Calcd.: S, 22.20. Found: S, 22.33 (Analysis by the Micro-Tech Laboratories). [d] Semicarbazone, fine white needles from alcohol–water, m.p. 155–155.5°. [e] Lit. (ref. 7) 116° (50 mm.).

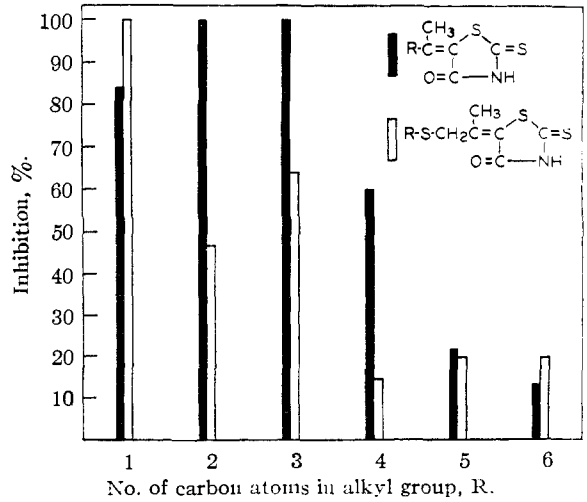


Fig 1.—Fungistatic action of 5-ethylidenerhodanine derivatives against *Aspergillus niger* at 250 p.p.m.

Condensation of the alkyl acetonyl sulfides with rhodanine (Table II) was carried out in an alcoholic solution of ammonium hydroxide in the presence of ammonium chloride.[1,8] It can be seen from Table II that the new series II contains only one derivative (R = CH_3) which at 250 p.p.m. is 100% effective in inhibiting the growth of *A. niger*, and from Fig. 1 that there is a general decline in activity with increasing size of the R group.[9] It is interesting to note that if one regards the sulfide group as equivalent to a methylene that a maximum in activity occurs at the same place in both series, where the side chain (R in I) is three atoms in length. The failure of the new series II to show enhanced activity lends further support to the ob-

(1) F. C. Brown, C. K. Bradsher, S. G. McCallum and M. Potter, *J. Org. Chem.*, **15**, 174 (1950).

(2) F. C. Brown and C. K. Bradsher, *Nature*, **168**, 171 (1951).

(3) F. C. Brown, C. K. Bradsher and E. N. Lawton, *Ind. Eng. Chem.*, **45**, 1027 (1953).

(4) Even tests at higher dilution show very little difference between the two compounds (concentration, parts per million, causes inhibition %): C_2H_4 100, 100; 50, 88; 25, 59; 10, 0. C_3H_7 100, 100; 50, 90; 20, 48; 10, 20.

(5) F. J. Bandelin and J. V. Tuschhoff, THIS JOURNAL, **74**, 4271 (1952), have recently reported on a group of germicides in which the thioether linkage appears to act like the methylene group of a hydrocarbon chain.

(6) W. Autenrieth, *Ber.*, **24**, 159 (1891).

(7) G. H. Morey (to Commercial Solvents Corporation), U. S. Patent 2,363,462 (November 21, 1944).

(8) We are indebted to Mrs. Marny Potter for carrying out some of these condensation reactions.

(9) The tests for fungistatic activity were carried out by Mrs. Marny Potter and Mrs. Rita S. Kardon.

TABLE II

5-(1-METHYL-2-THIOALKYLETHYLIDENE)-RHODANINES (II)

R	M.p.,[a] °C.	Yield,[b] %	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Inhibition, % *A. niger* at 250 p.p.m.
CH_3	110–110.5	48	$C_7H_9ONS_3$	38.33	38.73	4.13	4.22	100[g]
C_2H_5	127–128	31	$C_8H_{11}ONS_3$	41.17	41.45	4.75	5.07	47
C_3H_7	90.5–91.5	31[c]	$C_9H_{13}ONS_3$	43.69	44.01	5.30	5.47	64
$(CH_3)_2CH$	112–112.5	21	$C_9H_{13}ONS_3$	43.69	44.03	5.30	5.38	49
C_4H_9	106.5–107.5	44[d]	$C_{10}H_{15}ONS_3$	45.94	46.03	5.78	5.42	15
$(CH_3)_2CH—CH_2$	112–112.5	21	$C_{10}H_{15}ONS_3$	45.94	46.12	5.78	5.56	14
$(CH_3)_3C$	127.5–128	30	$C_{10}H_{15}ONS_3$	45.94	46.23	5.78	6.10	39
C_5H_{11}	91–91.5[e]	14	$C_{11}H_{17}ONS_3$	47.96	47.99	6.22	6.02	20
C_6H_{13}	91.5–92	76[f]	$C_{12}H_{19}ONS_3$	49.79	49.52	6.62	6.68	20

[a] Except as noted all compounds crystallized from cyclohexane or benzene–cyclohexane as yellow needles. [b] Except as noted yields are for material melting within at least five degrees of the analytical sample. [c] For crude product, m.p. 79–81°. [d] Crude product m.p. 93–95°. Yellow flakes. [f] M.p. 83–85°. [g] P.p.m. causing % inhibition 250, 100; 100, 100; 50, 64; 25, 28.

servation of Davies and Sexton[10] that sulfur *per se* makes no contribution to fungistic activity.

Experimental

Alkyl Acetonyl Sulfides.[8,9]—These were prepared by the following general procedure: To a solution prepared by dissolving 7.7 g. of sodium in 200 ml. of ethanol, one-third mole of mercaptan was added. The mixture was stirred and chilled in an ice-bath while 33.3 g. of chloroacetone was added dropwise over a period of 20 minutes with stirring (precipitation of sodium chloride). The mixture was refluxed for two hours with vigorous stirring. The salt was removed by filtration. Fractionation yielded the mercaptan as a yellow-greenish or greenish liquid.

Fungistatic Testing.—These tests measured the inhibition in the rate of radial growth of *Aspergillus niger* produced when the culture medium contained a known concentration, usually 250 parts per million (p.p.m.), of the rhodanine derivative. The procedure was essentially that of Leonard and Blackford.[11]

(10) W. H. Davies and W. A. Sexton, *Biochem. J.*, **40**, 331 (1946).

(11) J. M. Leonard and V. L. Blackford, *J. Bact.*, **57**, 339 (1949).

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[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Isolation and Identification of a Naturally Occurring Analog of Methionine[1a]

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A heat-labile compound more effective in preventing the toxicity of sulfanilamide than molar equivalents of methionine for *Escherichia coli* has been isolated from cabbage juice and identified as a 3-amino-3-carboxypropyldimethylsulfonium salt, $(CH_3)_2S^+—CH_2—CH_2—CH(NH_2)—COOH$. The enhanced activity of this methionine analog and its wide distribution in plants suggest an important role in the storage or transfer of methyl groups.

During the course of a survey of the effects of natural materials on the reversal of sulfonamide toxicity for *E. coli* in the basal medium employed for the assay of vitamin B_{12},[2] cabbage juice was observed to exert an effect on aseptic addition which was markedly decreased when the sample was heated prior to assay or autoclaved in the assay medium. This loss in activity was far in excess of that to be expected from destruction of methionine, *p*-aminobenzoic acid or vitamin B_{12} which support growth under the conditions employed.

On the basis of measurements by bioautograph techniques,[3] the loss in activity appeared to be due to the destruction of a single compound which differed in R_f from all compounds known to be active in the assay as shown in Fig. 1. An effect of the active principle in reversing the toxicity of sulfanilamide for *E. coli* was noted only under conditions of limiting methionine biosynthesis. The ability of the compound to replace only methionine among the various reversing agents for sulfonamide inhibitions in *E. coli* suggested that the active principle was probably closely related biochemically if not structurally to methionine. The same zone which gave biological activity on paper chromatograms gave a positive ninhydrin reaction (yellow turning to purple on standing or after prolonged heating), a weak positive test for sulfur with the iodine–azide reagent,[4] and a negative nitroprusside test for

(1) (a) Preliminary reports covering portions of this work have been presented: W. Shive, R. A. McRorie, G. L. Sutherland, M. S. Lewis, M. Rupp, J. L. Reger and F. Armstrong, 121st Meeting, American Chemical Society, Milwaukee, Wis., March, 1952; W. Shive, *International Review of Vitamin Research*, **23**, 329 (1952); (b) Eli Lilly and Co. Research Assistant; (c) Eli Lilly and Co. Postdoctorate Research Fellow, 1949–1951; (d) Eli Lilly and Co. Postdoctorate Research Fellow, 1948–1949.

(2) W. Shive, *Ann. N. Y. Acad. Sci.*, **52**, 1212 (1950); W. Shive, E. R. Alexander and M. S. Lewis, in preparation.

(3) W. A. Winsten and E. Eigen, *Proc. Soc. Exp. Biol. Med.*, **67**, 513 (1948).

(4) E. Chargaff, C. Levine and C. Green, *J. Biol. Chem.*, **175**, 67 (1948).