[Contribution from the Department of Chemistry, Duke University]

Synthesis and Antimicrobial Activity of Some 5-(1-Methylalkylidene)-2,4-thiazolidinediones[1]

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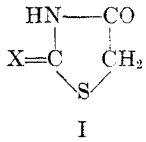
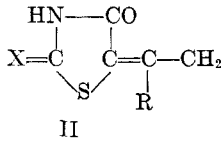
The condensation of methyl ketones with 2,4-thiazolidinedione to yield 5-(1-methylalkylidene)-2,4-thiazolidinedione (IIB) has been accomplished for the first time. Although towards *A. niger* and *B. subtilis* certain of these condensation products show antimicrobial activity comparable with that of the most potent of the corresponding rhodanine derivatives (IIA), maximum activity is found only with longer R groups on the alkylidene side chain.

Many derivatives of rhodanine (IA) have shown fungitoxic or bacteriotoxic activity.[2] Among these are two series, 5-(1-methylalkylidene)rhodanines (IIA) and 5-(1-methyl-2-alkylthioethylidene)rhodanines (III) which have been prepared and tested for activity against *Aspergillus niger*.[3] With this organism the former series shows a peak of activity when R is ethyl or *n*-propyl, while maximum activity in the latter series occurs when R is methyl. It was concluded that the sulfide group in the alkylidene chain could replace the methylene group with little change in activity.



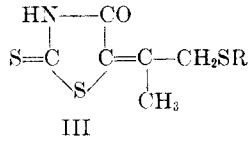
I

A: X = S;
B: X = O



II

A: X = S;
B: X = O



III

The type of linkage of sulfur to carbon may affect the microbiological activity of the molecule. In addition to the sulfur in the thiazolidine ring, rhodanine contains a thione group which may tautomerize to a sulfhydryl group. Replacement of the thione group by a carbonyl, as in 2,4-thiazolidinedione (IB) gives a molecule in which less tendency for tautomerization is present.

Previous work on the related dithiocarbamates leaves some doubt as to the importance of the thione group. Thus, van der Kerk and Klöpping[4] found that the substitution of $\rangle C{=}O$ for $\rangle C{=}S$ in the dithiocarbamates gave compounds which showed greatly decreased activity towards *A. niger*, while Davies and Sexton[5] found little difference in activity towards *Pen. digitatum* between $C_6H_5NH{-}\overset{\overset{S}{\|}}{C}{-}SCH_3$ and $C_6H_5NH\overset{\overset{O}{\|}}{C}{-}SCH_3$. The change in test organism may account for the different results, but a further study of the question seems desirable. Consequently, a series of 5-(1-methylalkylidene)-2,4-thiazolidinediones (IIB) was synthesized for comparison of the antimicrobial properties of series members with those of the corresponding derivatives of rhodanine.

Two compounds of the desired series (IIB) have been made by desulfurization of the corresponding rhodanine, using either lead acetate[6] or chloroacetic acid.[7] They are the compounds in which R is methyl melting at 166°[6] or 160–162°[7] and R is ethyl, melting at 141–145°.[7] The logical method of preparation would be the direct condensation of 2,4-thiazolidinedione and the ketone in the presence of the proper condensing agent, by a method analogous to that used for rhodanine derivatives,[3a] but this method has been reported as unsuccessful when it is applied to the reaction between simple aliphatic ketones and 2,4-thiazolidinedione.[7]

In preliminary studies of the preparation of members of this series, a 15% yield of 5-isopropylidene-2,4-thiazolidinedione[8] and a smaller yield of 5-*sec*-butylidene-2,4-thiazolidinedione were obtained by the condensation of 2,4-thiazolidinedione and the appropriate ketone in alcoholic solution in the presence of ammonia and ammonium chloride. An attempted reaction of these compounds in the presence of zinc chloride[9] gave 2,4-thiazolidinedione as the only recoverable product.

(1) Presented before the Medicinal Chemistry Division of the American Chemical Society, Dallas, Texas, April 9, 1956.

(2) (a) F. C. Brown and C. K. Bradsher, *Nature*, **168**, 171 (1951), (b) F. C. Brown, C. K. Bradsher, E. C. Morgan, M. Tetenbaum, and P. Wilder, *J. Am. Chem. Soc.*, **78**, 384 (1956).

(3) (a) F. C. Brown, C. K. Bradsher, S. G. McCallum, and M. Potter, *J. Org. Chem.*, **15**, 174 (1950); (b) C. K. Bradsher, F. C. Brown, and R. J. Grantham, *J. Am. Chem. Soc.*, **76**, 114 (1954).

(4) H. L. Klöpping and G. J. M. van der Kerk, *Rec. trav. chim.*, **70**, 917 (1951).

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

(6) C. C. J. Culvenor, W. Davies, J. A. Maclaren, P. F. Nelson, and W. E. Savige, *J. Chem. Soc.*, 2573 (1949).

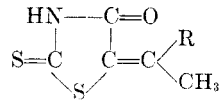
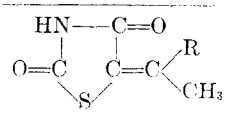
(7) W. J. Croxall, C-P. Lo, and E. Y. Shropshire, *J. Am. Chem. Soc.*, **75**, 5419 (1953).

(8) Observation of Mr. Philip Evans.

(9) E. B. Knott, *J. Chem. Soc.*, 1490 (1954).

TABLE I

COMPARISON OF ANTIMICROBIAL ACTIVITY OF SUBSTITUTED RHODANINES AND THIAZOLIDINEDIONES

R	 *A. Niger*, % inhibition 250 ppm.	50 ppm.	*B. Subtilis* Lowest conc'n in ppm. with 100% inhibition	 *A. Niger*, % inhibition 250 ppm.	50 ppm.	*B. Subtilis* Lowest conc'n ppm. with 100% inhibition
Methyl	84	55	>250	57	−2	>250
Ethyl	100	88 100	200	82	12	>250
Propyl	100	90 100	200	100	32 20	>250
Butyl	60	50	50	100	43 64	>250
Isobutyl	79	59 87	100	100	61 42	250
Amyl	21	22	>250	100	42 100	100
Hexyl	14	3	>250	49	4 44	>250
3-Butenyl	—	—	—	100	40 46	>250

It seemed probable that displacement of the equilibrium by the removal of water might give better yields. This was accomplished by running the reaction in dry benzene with catalytic amounts of piperidinium acetate and removing the water with a Dean and Stark water separator.[10] The yield of 5-isopropylidene-2,4-thiazolidinedione, melting at 163–166° was 87%. Under similar conditions, the reaction gave smaller yields with methyl *n*-alkyl ketones, methyl isobutyl ketone, and 5-hexene-2-one, but failed with methyl isopropyl ketone, diethyl ketone, acetophenone, and *m*-nitroacetophenone.

The effectiveness of the two series of compounds in preventing the growth of *A. niger* and of *B. subtilis* is reported in Table I. Neither series of compounds inhibitied the growth of *E. coli* at a concentration of 250 parts per million.

With *A. niger*, both series have certain compounds which completely inhibit the growth at 100 ppm. However, with the compounds containing a thione group the peak of activity occurs when R contains a smaller number of carbon atoms. Thus in the rhodanine derivatives the most effective compounds are those in which the alkyl group has two or three carbon atoms while in the thiazolidinedione series the best compounds contain four or five carbon atoms in the R group. Likewise in the thiazolidinedione series, the peak of activity towards *B. subtilis* comes with a larger alkyl group than in the rhodanine series.

Three of the thiazolidinedione derivatives reported in Table I afford a comparison of the effect of branching the carbon chain, or of unsaturation in the normal alkyl group, on the inhibition of the growth of *A. niger*. Measurements of growth of the organism in the presence of 50 ppm. of the 5-(1-methylalkylidene)-2,4-thiazolidinedione, (R = butyl, isobutyl, or 3-butenyl) indicate that the differences in the percent inhibition are within the limits of experimental accuracy. The butyl derivative inhibits the growth of *B. subtilis* at 100 ppm. while with its isomer or unsaturated analog, a concentration of 200 ppm. is necessary for complete inhibition of growth. It seems evident that the increase or decrease in the number of carbon atoms of the R group in the compounds on either side of the peak of activity is a more important factor in determining the degree of activity of the molecule than is the branching of the carbon chain or the presence of unsaturation.

The results of this study indicate that in the series represented by II, the thione group may be replaced by the carbonyl group with little change in activity towards *A. niger*, provided the number of methylene groups in the alkylidene group is increased.

EXPERIMENTAL

5-ISOPROPYLIDENE-2,4-THIAZOLIDINEDIONE

(*a*). *Ammonium chloride method.* To a mixture of 17.5 g. (0.15 mole) of 2,4-thiazolidinedione and 11.3 ml. (0.15 mole) of acetone in 10 ml. of concentrated ammonia and 80 ml. of ethyl alcohol was added a solution of 10 g. of ammonium chloride in 20 ml. of hot water. A white precipitate formed immediately. After refluxing the solution for three hours, the mixture was cooled, and the precipitate was filtered. Addition of cold water to the solution gave a second crop. The precipitates were combined, yielding 3.6 g., melting over a range of 146–154°. Two recrystallizations from alcohol raised the melting point to 166–167°.

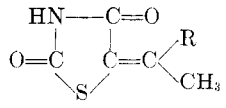
Anal. Calc'd for $C_6H_7NO_2S$: C, 45.84; H, 4.49. Found: C, 46.23; H, 4.40.

(*b*) *By displacement of the equilibrium.* A mixture of 20 g. (0.17 mole) of 2,4-thiazolidinedione, and 31 ml. (0.45 mole) of acetone in 200 ml. of dry benzene containing 0.5

(10) A. C. Cope, C. M. Hoffman, C. Wyckoff, and E. Hardenbergh, *J. Am. Chem. Soc.*, **63**, 3452 (1941).

TABLE II

5-(1-METHYLALKYLIDENE)-2,4-THIAZOLIDINEDIONES



R	Time of refluxing (hrs.)	M.P., °C.	Yield, %	Formula	Analyses Calc'd C	Calc'd H	Found C	Found H
Methyl	22	165–166.5	87	$C_6H_7NO_2S$	45.84	4.49	46.23	4.40
Ethyl	164	145–146.5	19	$C_7H_9NO_2S$	49.08	5.30	49.16	5.39
Propyl	120	136–137	23	$C_8H_{11}NO_2S$	51.87	5.99	52.18	6.20
Butyl	41	120–121.5	36[a]	$C_9H_{13}NO_2S$	54.24	6.57	54.27	6.40
Isobutyl	90	101–102	16	$C_9H_{13}NO_2S$	54.24	6.57	54.04	6.74
Amyl	52	108–109	43[b]	$C_{10}H_{15}NO_2S$	56.31	7.09	56.49	6.98
Hexyl	96	91–92	27	$C_{11}H_{17}NO_2S$	58.12	7.54	57.97	7.14
3-Butenyl	72	116–116.5	65	$C_9H_{11}NO_2S$	54.80	5.62	54.84	5.65

[a] After one recrystallization. [b] After two recrystallizations.

ml. of glacial acetic acid and 1.0 ml. of piperidine was refluxed in a flask to which a Dean and Stark water separator was attached. The refluxing was continued for 22 hours. The benzene was removed by evaporation, giving 24.7 g. (87%) of product melting at 163–166°. Recrystallization from ethyl alcohol yielded the compound with a melting point of 165–166°.

Data for the preparation of individual compounds in the series are collected in Table II. In several cases it was advantageous, after removal of the bulk of the solvent, to add the mixture to ice-water or to wash the precipitate repeatedly with dilute ethyl alcohol.

The method of testing for activity towards *A. niger*, a modification of that used by Leonard and Blackford,[11] and for *B. subtilis*, has been reported previously.[2b,12]

Acknowledgment. This work was supported by research grants from the Duke University Research Council and from the National Microbiological Institute of the National Institutes of Health, Public Health Service [Grant E-695(C)].

DURHAM, NORTH CAROLINA

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

(12) We are indebted to Mrs. Dorcas Clarke and Mrs. Marilena Ferguson for carrying out the tests for antimicrobial activity.