Anal. Calcd. for $C_{17}H_{20}N_2O_5$ (332.25): C, 61.43; H, 6.07; N, 8.43. Found: C, 61.69; H, 6.30; N, 8.49.

α-Methylamino-β-(3-indole)-propionic Acid (IX).—To a solution of 0.40 g. of potassium hydroxide in 2 ml. of water was added 332 mg. (0.001 mole) of the dimethyl ester VIII and the mixture maintained at reflux temperature for a period of 50 hours. The cold solution was acidified with hydrochloric acid and clarified by filtration. The filtrate was neutralized with sodium acetate and the solution was concentrated to dryness under diminished pressure. One milliliter of water was added, and after 15 hours at 0°, the crystalline deposit was collected by filtration and was recrystallized from 1 ml. of water; yield 165 mg. (75%), m.p. 231–233°.[28]

Anal. Calcd. for $C_{12}H_{14}N_2O_2$ (218.25): C, 66.03; H, 6.47; N, 12.84. Found: C, 64.66; H, 6.49; N, 12.47.

The infrared spectrum was identical with that displayed by a specimen of naturally occurring L-abrine.

The hydantion was prepared with potassium cyanate and was crystallized from water; m.p. 213–214°.[28b]

The picrate was prepared in aqueous solution and was recrystallized from a mixture of ethyl acetate and petroleum ether; m.p. 173–175°.[29]

α-Acetylmethylamino-β-phenylpropionic Acid.—A solution of 450 mg. (0.002 mole) of the sodium derivative VII, 253 mg. (0.002 mole) of benzyl chloride and 10 mg. of sodium iodide in 3 ml. of absolute methanol was maintained at reflux temperature for a period of 5 hours. The methanol was distilled under reduced pressure and the residue was treated with a solution of 500 mg. of sodium carbonate in 5 ml. of water. After 15 hours at reflux temperature, the solution was clarified by filtration and was acidified with hydrochloric acid; yield 170 mg. (34%), m.p. 146–147°.[30]

(28) Each investigator who has reported experience with this substance has observed a different melting point: (a) W. G. Gordon and R. W. Jackson, *J. Biol. Chem.*, **110**, 154 (1935), 297°; (b) E. J. Miller and W. Robson, *J. Chem. Soc.*, 1910 (1938), 245°; (c) F. F. Blicke and P. E. Norris, THIS JOURNAL, **76**, 3213 (1954), 272–275°. The compound is considerably more soluble in water and is more difficult to recrystallize and to obtain in anhydrous state than is the naturally occurring L-enantiomorph which melts in the neighborhood of 295°, N. Ghatak and R. Kaul, *J. Indian Chem. Soc.*, **9**, 383 (1932); T. Hoshino, *Ann.*, **520**, 31 (1935).

(29) This picrate generally deposits from aqueous solution as yellow-orange needles which melt initially at *ca.* 110–120°. If temperature elevation is continued, resolidification occurs with final melting at 173–175°.

(30) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **99**, 143 (1932).

Anal. Calcd. for $C_{12}H_{15}NO_3$ (221.25): C, 65.14; H, 6.83; N, 6.33. Found: C, 65.29; H, 6.87; N, 6.25.

N-Methyl-DL-phenylalanine.—A solution of 90 mg. (0.0004 mole) of α-acetylmethylamino-β-phenylpropionic acid, derived from the above experiment, in 2 ml. of 6 *N* hydrochloric acid solution was maintained at reflux temperature for a period of 15 hours. The solution was concentrated under reduced pressure, the residue was dissolved in a small quantity of water and was neutralized with sodium acetate. The crystalline deposit was collected by filtration and was recrystallized from water; yield 65 mg. (90%), m.p. 252–254°.[31]

Anal. Calcd. for $C_{10}H_{13}NO_2$ (179.12): C, 67.04; H, 7.31; N, 7.82. Found: C, 66.89; H, 7.10; N, 7.84.

α-N-Methyl-DL-histidine Dihydrochloride.—To a solution of 450 mg. (0.002 mole) of the sodium derivative VII in 5 ml. of methanol containing 46 mg. (0.002 mole) of sodium was added at 0° 306 mg. (0.002 mole) of 4(5)-chloromethylimidazole hydrochloride.[32] After 1 hour at 0° the methanol was distilled under diminished pressure, 2 ml. of 6 *N* hydrochloric acid was added and the solution was maintained at reflux temperature for a period of 15 hours. The hydrochloric acid was distilled under reduced pressure, the residue was dissolved in water, was neutralized with sodium acetate and was treated at 90° with 900 mg. of picric acid. The precipitate which weighed 1.2 g. and which melted at 121–127° was recrystallized from water; yield 820 mg. (65%), m.p. 121–131°.[33] The picrate was treated with benzene and dilute hydrochloric acid and the acid extract was washed with several successive quantities of benzene. The hydrochloric acid solution was concentrated under diminished pressure to yield 180 mg. of the crystalline dihydrochloride which was recrystallized from a small quantity of dilute hydrochloric acid; m.p. 130–135°.[33]

Anal. Calcd. for $C_7H_{11}N_3O_2 \cdot 2HCl \cdot {}^1/_2H_2O$ (251.10): C, 33.48; H, 5.62; N, 16.74. Found: C, 33.40; H, 5.66; N, 17.09.

Acknowledgment.—These studies were assisted by a grant from the National Science Foundation.

(31) E. Friedman and S. Gutmann, *Biochem. Z.*, **27**, 491 (1910).

(32) F. L. Pyman, *J. Chem. Soc.*, 668 (1911); J. R. Totter and W. J. Darby, "Organic Syntheses," Coll. Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 460.

(33) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, 734 (1921). V. Deulofeu and A. E. A. Mitta, *J. Org. Chem.*, **14**, 915 (1949).

BOSTON 15, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

Some 3-Substituted Rhodanines

BY FRANCES C. BROWN, CHARLES K. BRADSHER, ELIZABETH C. MORGAN, MARVIN TETENBAUM AND PELHAM WILDER, JR.

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Forty-four 3-phenyl-, 3-benzyl- and related rhodanine derivatives have been synthesized from the corresponding amines and tested for fungitoxic and bacteriotoxic activity. In the 3-phenylrhodanine series, the fungitoxic activity toward *A. niger* is noteworthy, and the bacteriotoxic activity negligible, while the reverse is true of the 3-benzylrhodanines.

In view of the mildew-preventing activity exhibited by some 5-substituted rhodanine derivatives,[1] it seemed advisable to investigate the effect of variations in other portions of the rhodanine molecule on toxicity toward fungi and bacteria. Rhodanine[2] (I) and its derivatives retaining hydrogen attached to the nitrogen atom have been patented as fungicides while the compounds containing a hydrocarbon residue attached to the nitrogen atom[3] were patented as pesticides, with mention being made of their usefulness as fungicides. The improvement in insecticidal activity of 3-methylrhodanine over rhodanine is evident from the data in the Ciba patent, but the relation between the structure of the hydrocarbon residue and the activity of the molecule as a whole was not reported.

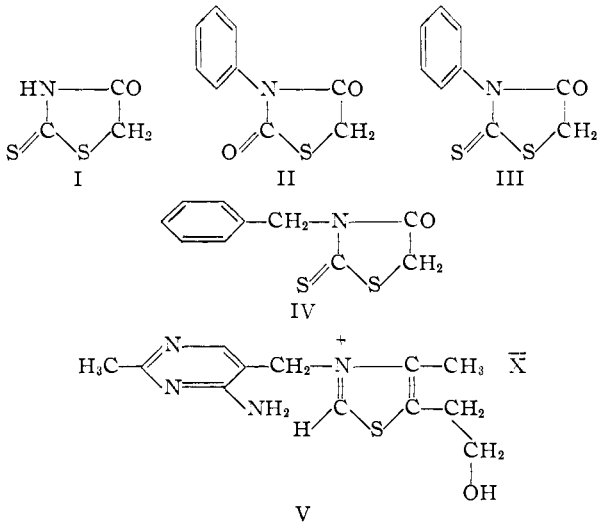
The fungicidal activity of 3-phenyl-2,4-thiazoli-

(1) F. C. Brown and C. K. Bradsher, *Nature*, **168**, 171 (1951), F. C. Brown, C. K. Bradsher and E. N. Lawton, *Ind. Eng. Chem.*, **45**, 1027 (1953); F. C. Brown, C. K. Bradsher and S. M. Bond, *ibid.*, **45**, 1030 (1953); F. C. Brown, C. K. Bradsher, S. M. Bond and R. J. Grantham, *ibid.*, **46**, 1508 (1954); C. K. Bradsher, F. C. Brown and R. J. Grantham, THIS JOURNAL, **76**, 114 (1954).

(2) E. Alvord, (to Grasselli Chemical Co.), U. S. Patent 1,962,109 (June 5, 1934).

(3) CIBA, A. G. Swiss Patent 242,300 (Oct. 1, 1946).

denedione[4] (II) and some of its derivatives has also been patented. A study of 3-phenylrhodanine (III) and its derivatives offered the possibility of varying the nature and position of the substituent attached to the benzene ring. The same possibility is present with 3-benzylrhodanine (IV) in which the phenyl group and the rhodanine nucleus are separated by a methylene bridge. This structure reminds one of thiamine (V), in which a methylene bridge separates a pyrimidine ring from a thiazole ring.

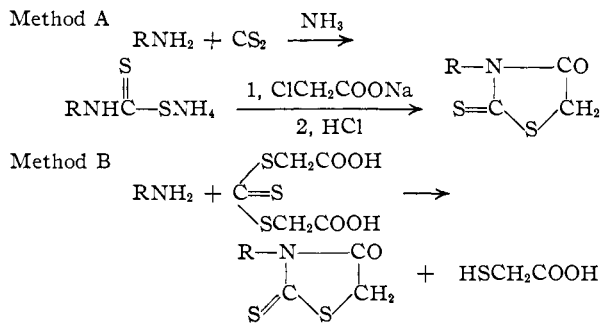


I II III IV V

Recently van der Kerk and his co-workers[5] have reported fungicidal tests on 3-phenylrhodanine and five of its *para*-substituted derivatives. Their results agree in some points with our findings.

The present communication describes the preparation and test for bactericidal and fungicidal activity of forty-four compounds belonging to the 3-phenyl- and 3-benzylrhodanine series. The compounds were prepared from primary aromatic or benzylamines which were commercially available or were synthesized for the purpose. The substituents on the benzene ring were chosen to give as wide a variation as possible with respect to such factors as size, electronegativity and chemical reactivity.

Two general methods of synthesis, both of which start with the primary amine and were developed by Holmberg,[6] were used. With method A, a more



detailed procedure was obtained by combining the directions for the preparation of the dithiocarbamate salt[7] with those for the latter steps in the preparation of rhodanine.[8] With method B,[9] the amine, or its hydrochloride with sufficient base for neutralization, was refluxed with dicarboxymethyltrithiocarbonate. Ring closure was effected during the same operation.

The two series of derivatives of III and IV were tested for their ability to inhibit the growth of *A. niger*, *B. subtilis* and *E. coli*. A serial dilution method was used and the results are recorded in Table I.

The two series present some interesting contrasts. Twelve of the twenty-six 3-phenylrhodanine derivatives inhibit completely the growth of *A. niger* at 250 p.p.m. and two of these derivatives, 3-(*p*-methoxyphenyl)-rhodanine and 3-(*p*-acetophenyl)-rhodanine, are equally effective at a tenfold greater dilution, *i.e.*, 25 p.p.m. The derivatives of 3-benzylrhodanine have little inhibitory power for the growth of *A. niger*, and although twelve of the eighteen compounds showed some activity at 250 p.p.m., none gave complete inhibition at 200 p.p.m. With the 3-phenylrhodanines the introduction of any substituent except an *ortho* methyl group increases the effectiveness, while the introduction of any substituent into 3-benzylrhodanine decreases markedly its moderate fungicidal activity toward *A. niger*.

In the phenyl group of the 3-phenylrhodanine series, all three orientations of substituents relative to the rhodanine ring have certain derivatives which at a concentration of 250 p.p.m. are effective in the inhibition of growth of *A. niger*. With chloro, bromo, methyl and carboxyl substituents, data are presented which indicate that with the halogen derivatives, *para* substitution is the most effective, while with the other two, *meta* substitution produces greater inhibition.

It has been stated[5] that an unsaturated and/or negative substituent in the para position is most beneficial to the fungicidal activity of the 3-phenylrhodanines. While a large number of effective compounds reported in this paper belong to the electron-attracting group, one of the two most effective compounds, 3-(*p*-methoxyphenyl)-rhodanine has a substituent of opposite polarity. Also it appears that the introduction of a substituent into positions other than *para* can give compounds which are more effective than the parent substance, specifically, with chlorine and bromine in the *ortho* and *meta* positions. Further, with methyl and carboxyl substituents the meta compound seems to possess greater activity than the *para* isomers.

While the 3-phenylrhodanine derivatives are superior to the 3-benzyl analogs in activity toward *A. niger*, the picture is reversed with respect to bactericidal activity toward *B. subtilis* and *E. coli*.

(4) N. K. Sundholm and J. B. Skaptason (to United States Rubber Co.), U. S. Patent 2,510,725 (June 6, 1950).

(5) G. J. M. van der Kerk, H. C. van Os, G. de Vries and A. K. Sijpesteijn, *Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent*, **18**, 402 (1953).

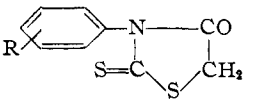
(6) B. Holmberg, *J. prakt. Chem.*, **81**, 451 (1910).

(7) F. B. Dains, R. Q. Brewster and C. P. Olander, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 447.

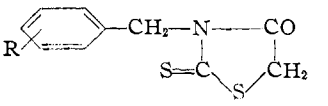
(8) C. E. Redemann, R. N. Icke and G. A. Alles, ref. 7, Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 763.

(9) L. G. S. Brooker, G. H. Keyes, R. H. Sprague, R. H. Van Dyke, E. Van Lare, G. Van Zandt and F. L. White, THIS JOURNAL, **73**, 5326 (1951).

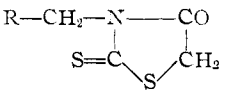
TABLE I

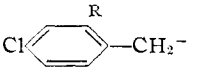


R	*A. niger* % inhibition, at 250 p.p.m.	Lowest concn. giving 100% inhibition, p.p.m. *A. niger*	*B. subtilis*	*E. coli.*
H[a]	18		>250	>250
2-CH_3[b]	14		>250	>250
3-CH_3[c]	100	250	250	>250
4-CH_3[b]	27		>250	>250
4-OCH_3[a]	100	25	200	250
4-F	41		>250	>250
2-Cl	100	200	>250	>250
3-Cl	100	250	>250	>250
4-Cl[d]	100	50	250	200
2-Br	100	200	>250	>250
3-Br	100	250	250	>250
4-Br[a]	100	50	>250	>250
4-I	61		>250	>250
3-COOH	100	100	100	200
4-COOH[e]	73		100	200
4-$COCH_3$	100	25	>250	100
3-CF_3	40		>250	>250
4-SO_2NH_2	40		>250	>250
2,5-diCH_3	18		>250	>250
2,6-diCH_3	21		>250	>250
2,5-diCl	100	250	>250	>250
2-CH_3-3-Cl	53		>250	>250
2-CH_3-4-Cl	27		>250	>250
2-CH_3-4-Br	82		>250	>250
2-Br-4-CH_3	100	200	>250	>250
2-OH-5-NO_2	62		100	200



R	*A. niger* % inhibition at 250 p.p.m.	Lowest concn. giving 100% inhibition, p.p.m. *B. subtilis*	*E. coli.*
H[c]	100[f]	100	25
2-CH_3	66	100	200
3-CH_3	58	50	100
4-CH_3	41	25	250
4-OCH_3	100[g]	100	>250
4-F	50	25	5
2-Cl	41	50	100
4-Cl	52	2.5	10
4-Br	84	10	50
4-I	26	25	>250
2,4-diCl	66	10	>250
3,4-diCl	57	2.5	100
4-NO_2	100[h]	25	>250
2-SCH_3	20	2.5	>250



R	*A. niger* % inhibition at 250 p.p.m.	Lowest concn. giving 100% inhibition, p.p.m. *B. subtilis*	*E. coli.*
Cl- 	34	10	>250
	59	200	200
	33	200	100
Cl- 	42	200	25

[a] Ref. 6. [b] R. Andreasch and A. Zipser, *Monatsh.*, **26**, 1191 (1905). [c] R. Andreasch, *ibid.*, **29**, 399 (1908). [d] F. Brown, C. K. Bradsher, S. M. Bond and R. J. Grantham, *Ind. Eng. Chem.*, **46**, 1508 (1954). [e] Ref. 9. [f] Inhibition at 200 p.p.m., 100%; at 100 p.p.m., 59%; at 50 p.p.m., 36%. [g] Inhibition at 200 p.p.m., 53%. [h] Inhibition at 200 p.p.m., 40%; at 100 p.p.m., 43%.

All eighteen of the compounds studied in the 3-benzylrhodanine series completely inhibited the growth of *B. subtilis* at 200 p.p.m. Ten of these compounds allowed no growth of this organism at 25 p.p.m. and three, 3-(*p*-chlorobenzyl)-rhodanine, 3-(3,4-dichlorobenzyl)-rhodanine and 3-(*o*-methylmercaptobenzyl)-rhodanine, were equally effective at 2.5 p.p.m. This effectiveness is in marked contrast to the relative inactivity of the 3-phenylrhodanine derivatives.

Many of the 3-benzylrhodanine derivatives inhibit the growth of *E. coli*, an organism generally considered more resistant than *B. subtilis*. In this connection specific mention should be made of the activity of 4-fluorobenzylrhodanine, which at a concentration of 5 p.p.m. completely inhibits the growth of *E. coli*. With increasing atomic weight of the halogen in the *p*-halogen-benzylrhodanine, the inhibition of the growth of *E. coli* becomes less. Aside from *p*-fluorobenzylrhodanine the following compounds inhibit the growth of *E. coli* at lower concentrations than are effective for *B. subtilis:* 3-benzylrhodanine, 3-(2-thenyl)-rhodanine, 3-(5-chloro-2-thenyl)-rhodanine, 3-(*p*-acetophenyl)-rhodanine and 3-(*p*-chlorophenyl)-rhodanine.

Experimental

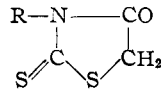
Two typical procedures for making the 3-substituted rhodanines are described. Ethyl alcohol and ligroin were the most useful solvents for recrystallization. Details of yields, melting points and analyses are described in Table II. No effort was made to obtain the optimum conditions for the maximum yield with individual compounds, as the purpose was the preparation of pure compounds in sufficient quantity for the microbiological tests.

Method A.—To 30 ml. of concentrated ammonia in an ice-salt bath was added 18.9 g. (0.24 mole) of carbon bisulfide. Twenty-eight grams (0.2 mole) of *p*-chlorobenzylamine was added dropwise over a period of 15 minutes and stirring continued for ten minutes. The dithiocarbamate precipitated and was allowed to stand overnight. It was filtered, washed with cold ether and dried by suction. The sodium chloroacetate solution was prepared by mixing cooled solutions of 19 g. (0.2 mole) of chloroacetic acid in 21 ml. of water and 7 g. of sodium hydroxide in 20 ml. of water and adding solid, anhydrous sodium carbonate until the solution was basic. The sodium chloroacetate was stirred, cooled to 5–10° and the dithiocarbamate added during ten minutes. Stirring was continued while the flask was allowed to warm to room temperature. The solid was added to a warm solution of 66 ml. of concentrated hydrochloric acid and 26 ml. of water and the mixture heated to 85–90° for 15 minutes. A yellow oil formed, was extracted twice with hot water to remove *p*-chlorobenzylamine hydrochloride, and on cooling solidified, yielding 62% of 3-(*p*-chlorobenzyl)-rhodanine.

Method B.—To 11.5 g. (0.05 mole of di-(carboxymethyl)-trithiocarbonate in 200 ml. of water was added 5.5 g.

TABLE II

PREPARATION OF 3-SUBSTITUTED RHODANINES



R	Prepn. method	M.p., °C.	Yield, %	Formula	Calcd. C, %	Calcd. H, %	Found C, %	Found H, %
4-Fluorophenyl	B	166–167.5	66	$C_9H_6FNOS_2$	47.56	2.66	47.69	2.72
2-Chlorophenyl	A	116.5–117.5		$C_9H_6ClNOS_2$	44.35	2.48	44.49	2.58
3-Chlorophenyl	A	160.5–161	62	$C_9H_6ClNOS_2$	44.35	2.48	44.68	2.39
2-Bromophenyl	A	136–137		$C_9H_6BrNOS_2$	37.51	2.10	37.70	2.08
3-Bromophenyl	A	178–179	21[a]	$C_9H_6BrNOS_2$	37.51	2.10	37.94	2.11
4-Iodophenyl	A	163 dec.	8[a]	$C_9H_6INOS_2$	32.25	1.80	32.41	1.77
3-Carboxyphenyl	A	269–270 dec.	11[a]	$C_{10}H_7NO_3S_2$	47.42	2.79	47.41	2.53
4-Acetophenyl	A	147–148	4[b]	$C_{11}H_9NO_2S_2$	52.57	3.61	52.78	3.34
3-Trifluoromethylphenyl	B	176–177	41	$C_{10}H_6F_3NOS_2$	43.31	2.18	43.62	2.29
4-Sulfonamidophenyl	A	>240 dec.	2[a]	$C_9H_8N_2O_3S_3$	37.49	2.80	37.79	2.79
2,5-Dimethylphenyl	A	135–136	11[c]	$C_{11}H_{11}NOS_2$	55.67	4.67	55.69	4.62
2,6-Dimethylphenyl	A	161–163	12[a]	$C_{11}H_{11}NOS_2$	55.67	4.67	55.98	4.82
2,5-Dichlorophenyl	A	152–154		$C_9H_5Cl_2NOS_2$	38.86	1.81	39.11	1.63
2-Methyl-3-chlorophenyl	A	121–122		$C_{10}H_8ClNOS_2$	46.60	3.13	46.70	3.07
2-Methyl-4-chlorophenyl	A	145–146		$C_{10}H_8ClNOS_2$	46.60	3.13	46.28	2.64
2-Methyl-4-bromophenyl	A	135–136		$C_{10}H_8BrNOS_2$	39.74	2.67	39.79	2.42
2-Bromo-4-methylphenyl	A	118–119	18[a]	$C_{10}H_8BrNOS_2$	39.74	2.67	39.78	2.62
2-Hydroxy-5-nitrophenyl	A	225–226 dec.	20[d]	$C_9H_6N_2O_4S_2$	39.99	2.24	40.37	1.79
2-Methylbenzyl	A	122–123	81	$C_{11}H_{11}NOS_2$	55.67	4.67	56.10	4.89
3-Methylbenzyl	A	63–64	8[e]	$C_{11}H_{11}NOS_2$	55.67	4.67	55.90	4.75
4-Methylbenzyl	A	74–75	22	$C_{11}H_{11}NOS_2$[f]	55.67	4.67	56.01	4.88
4-Methoxybenzyl	A	99.5	20[e]	$C_{11}H_{11}NO_2S_2$	52.15	4.38	52.10	4.46
4-Fluorobenzyl	A	64.5–65	31[e]	$C_{10}H_8FNOS_2$[g]	49.77	3.34	50.19	3.34
2-Chlorobenzyl	A	87.5–88	13[e]	$C_{10}H_8ClNOS_2$	46.60	3.13	46.90	2.96
4-Chlorobenzyl	A	88–89	62	$C_{10}H_8ClNOS_2$[h]	46.60	3.13	47.00	3.35
4-Bromobenzyl	A	96–97	17[e]	$C_{10}H_8BrNOS_2$	39.74	2.67	39.57	2.64
4-Iodobenzyl	A	122–123	33	$C_{10}H_8INOS_2$	34.39	2.31	34.69	2.16
2,4-Dichlorobenzyl	A	103–104	17[e]	$C_{10}H_7Cl_2NOS_2$	41.10	2.42	41.20	2.48
3,4-Dichlorobenzyl	A	106	48[e]	$C_{10}H_7Cl_2NOS_2$	41.10	2.42	41.28	2.52
4-Nitrobenzyl	B	145	11[e]	$C_{10}H_8N_2O_3S_2$	44.76	3.01	44.79	3.09
2-Methylmercaptobenzyl	A	119–121	8[e]	$C_{11}H_{11}NOS_3$	49.04	4.12	49.20	4.34
2-(4-Chlorophenyl)-ethyl	A	108–109	11[e]	$C_{11}H_{10}ClNOS_2$	48.61	3.71	48.74	3.83
2-Furfuryl	A	73–74	11	$C_8H_7NO_2S_2$	45.05	3.31	45.37	3.63
2-Thenyl	A	94.5–95	76	$C_8H_7NOS_3$	41.90	3.08	41.98	3.28
5-Chloro-2-thenyl	A	92.5–93.5	83	$C_8H_6ClNOS_3$	36.43	2.29	36.69	2.26

[a] Yield after two recrystallizations. [b] Yield after three recrystallizations. [c] Yield after five recrystallizations. [d] Yield after four recrystallizations. [e] Yield after one recrystallization. [f] *Anal.* Calcd.: S, 27.02. Found: S, 26.82. [g] *Anal.* Calcd.: N, 5.81. Found: N, 5.57. [h] *Anal.* Calcd.: Cl, 13.76. Found: Cl, 13.80.

of *p*-fluoroaniline, and the mixture refluxed for 1.5 hours. After ten minutes of refluxing, the originally clear solutions became turbid, and precipitation continued during the reflux period. The precipitate was filtered, washed with cold water and gave 66% yield of the 3-(*p*-fluorophenyl)-rhodanine.

Preparation of Amines

With one exception, *p*-fluoroaniline[10] (b.p. 70–71°, at 12 mm.) (lit. 98–99° at 33 mm.) which was synthesized by the catalytic reduction of *p*-fluoronitrobenzene, the substituted anilines were commercially available. Benzylamine, five of its derivatives, containing the *o*- and *p*-chloro, 2,4- and 3,4-dichloro-, *p*-methoxy substituents, and furfurylamine were available, but the remaining benzylamines and related compounds were synthesized.

***o*-Methylbenzylamine** was prepared by the $LiAlH_4$ reduction of *o*-tolunitrile using the method of Nystrom and Brown[11] but the hydrolysis was accomplished by the procedure of Amundsen and Nelson.[12] The reduction was completed by refluxing the ether solution from four to six hours. The properties of other amines obtained by this method are described in Table III. *o*-Methylthiobenzylamine was made by stirring the corresponding amide (0.12 mole) with $LiAlH_4$ (0.15 mole) in ether solution at room temperature for 20 hours.[13] The amide was made from thiosalicylic acid by the procedure of McClelland and Warren.[14] To show that the methylmercapto group would not be affected by the long contact with $LiAlH_4$, thioanisole (0.2 mole) was stirred with $LiAlH_4$ (0.25 mole) in ether for 20 hours and recovered quantitatively. The benzylamines react readily with atmospheric CO_2; consequently, picrates of the amines were analyzed.

A modified Delépine reaction[15] was successful in the preparation of ***p*-nitrobenzylamine hydrochloride** and ***p*-iodobenzylamine**. In the latter synthesis it is important to filter the quaternary salt, wash it thoroughly with cold alcohol to remove the unreacted benzyl halide and resuspend the salt in alcohol before saturation with hydrogen chloride gas. The *p*-iodobenzylamine hydrochloride was purified by dissolving it in alcohol and adding ether to the alcoholic solution. It melted at 240° dec. (lit. 240°)[16] and was obtained

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(16) C. L. Jackson and C. F. Mabry, *Am. Chem. J.*, **2**, 257 (1880).

TABLE III

Amine	Yield, %	B.p., °C.	B.p., °C. (lit.)	M.p., °C.	Picrate M.p., °C. (lit.)	Formulas	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
m-Methylbenzyl	48	201	201–202[a] (755 mm.)	190	198 dec.[b]					
p-Methylbenzyl	62	200.5	198–200[c] (680 mm.)	205–215 dec.	205 dec.[c]					
p-Fluorobenzyl	60	183		203		$C_{13}H_{11}FN_4O_7$	44.07	44.42	3.13	3.15
o-Methylmercaptobenzyl	38	145 (4 mm.)		203–204		$C_{14}H_{14}N_4O_7S$	43.98	44.17	3.69	3.67
β-(*p*-Chlorophenyl)-ethyl	23	90 (5 mm.)	114–116[d] (15 mm.)	210		$C_{14}H_{13}ClN_4O_7$	43.70	43.78	3.41	3.51

[a] Ref. 19. [b] C. W. Shoppee, *J. Chem. Soc.*, 696 (1932). [c] K. G. Lewis, *ibid.*, 2250 (1950). [d] D. H. Hey and J. M. Williams, *ibid.*, 1527 (1951).

in 30% yield. On neutralization with sodium hydroxide, extraction with ether, and vacuum distillation, the free amine boiled at 113–115° (4 mm.), solidified to a white solid, and gave a picrate melting at 237° dec. (lit. 231° dec.[17]). This method was found to be superior to that recommended by Shoppee.[17]

The Gabriel synthesis for the preparation of *p*-bromobenzylamine[17] was modified in the last step by the use of hydrazine.[18] N-(*p*-Bromobenzyl)-phthalimide was converted to the *p*-bromobenzylamine, which distilled at 100° at 5 mm. (lit. 102° at 12 mm.[19]) in 72% yield.

2-Thenylamine (b.p. 56–58°(3 mm.)) and **5-chloro-2-thenylamine** (b.p. 76–79°(3 mm.)) were made by aminomethylation of thiophene and of 2-chlorothiophene.[20]

The method of Leonard and Blackford[21] was used with tests on the inhibition of growth of *A. niger* (ATCC 215–4247). Concentration of the candidate fungicide was measured in parts per million (p.p.m.). If no growth occurred at 250 p.p.m., tests were run at lower concentrations. Controls, which were run parallel with each test, measured the growth of the organism in the absence of a potential inhibitor and in separate plates in the presence of 25 and of 50 p.p.m. of the fungicide 2,2′-dihydroxy-5,5′-dichlorodiphenyl (G-4).

Tests against the gram-positive bacteria, *B. subtilis*, 9945, and the gram-negative bacteria *E. coli*, 4157, were run in a Difco Bacto-Agar medium containing 250 or less p.p.m. of the candidate bacteriocide. Complete inhibition of growth of the bacteria 72 hours after streaking the culture was taken as the criterion of inhibition. If no growth occurred at concentrations of 250 p.p.m., measurements were made at greater dilutions.

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DURHAM, N. C.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Erythromycin. VI. Degradation Studies[1]

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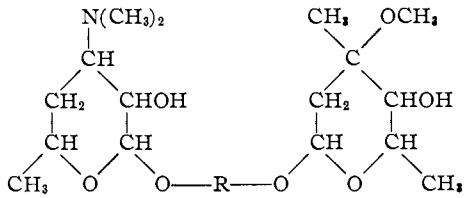
Erythromycin has been shown to be a bisglycoside of a twenty-one carbon polyhydroxy ketolactone. By reduction and hydrolysis of erythromycin, the aglycone, dihydroerythronolide, has been isolated and characterized. Other degradation products of erythromycin have been obtained and studied.

In the first paper[2] of this series, erythromycin was shown to be a glycoside containing the dimethylaminodeoxy sugar desosamine and the methoxydeoxy sugar cladinose.[3] The partial structure I was suggested.

The work reported in this paper is concerned with the aglycone portion [R(OH)$_2$] of I and evidence is presented that this is a twenty-one carbon polyhydroxy ketolactone, for which we propose the name erythronolide.

The presence of the free hydroxyl group vicinal to the dimethylamino group in the desosamine moiety of I has been inferred previously from the



I, R = $C_{21}H_{36}O_6$[4]

reduced pK'_a values of the monoacyl derivatives of I.[2,5] Direct evidence for this free hydroxyl group has been obtained now as a consequence of the isolation of methylamine as a product of the

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(2) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, THIS JOURNAL, **76**, 3121 (1954).
(3) The structure of cladinose shown here has been reported recently; P. F. Wiley and O. Weaver, THIS JOURNAL, **77**, 3422 (1955).
(4) The composition of erythromycin was given in the first paper of this series (ref. 2) as $C_{37}H_{67-69}NO_{13}$ and the composition of R as $C_{21}H_{36-38}O_6$. On the basis of analytical data and chemical information presented in this paper, the correct empirical formula of erythromycin is $C_{37}H_{67}NO_{13}$ and R is $C_{21}H_{36}O_6$.
(5) H. W. Murphy, "Antibiotics Annual," 1953–1954, Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 500–513.