was prepared by the addition of 28 ml of 2.5 *N* ethanolic HCl to the base dissolved in ether. The salt was recrystallized from ethanol, mp 184–186°.

Anal. Calcd for $C_{11}H_{13}ClF_3N$: C, 52.5; H, 5.2; Cl, 14.1; F, 22.7; N, 5.6. Found: C, 52.6; H, 5.3; Cl, 14.6; F, 22.3; N, 5.6.

***m*-Methyl-N-propylbenzylamine (VI)** was prepared by procedure B from α-bromo-*m*-xylene and *n*-propylamine in 68% yield, bp 120–122° (10–12 mm). The hydrochloride was recrystallized from acetone, mp 176–178°.

Anal. Calcd for $C_{11}H_{18}ClN$: C, 66.2; H, 9.1; Cl, 17.8; N, 7.0. Found: C, 66.0; H, 9.3; Cl, 17.7; N, 6.9.

N-Propyl-*m*-(trifluoromethyl)benzylamine (III) was prepared by the above procedure from *m*-(trifluoromethyl)benzyl chloride and *n*-propylamine in 86% yield, bp 110–112° (20 mm). The hydrochloride was recrystallized from ethanol, mp 211–212°.

Anal. Calcd for $C_{11}H_{15}ClF_3N$: C, 52.1; H, 6.0; Cl, 22.5; F, 14.0; N, 5.5. Found: C, 52.5; H, 6.1; Cl, 22.0; F, 14.1; N, 5.3.

Procedure C. Reduction of N-Cyclopropyl-α,α,α-trifluoro-*m*-toluamide with Diborane.—A solution of 2.3 g (0.01 mole) of N-cyclopropyl-α,α,α-trifluoro-*m*-toluamide in 10 ml of THF was added dropwise under nitrogen to a cooled solution of 1 *M* borane in THF (20 ml, 0.02 mole). The reaction mixture was heated at reflux temperature for 2 hr. The mixture was cooled to room temperature, 5 ml (0.025 mole) of 5 *N* HCl was added, and the reaction mixture was heated on the steam bath to distil off the THF. The mixture was diluted with 10 ml of water, cooled, made alkaline with 3 g of NaOH pellets, and extracted with ether. Concentration of the ether solution yielded an oil which produced only one spot on thin layer chromatography. The yield of N-cyclopropyl-*m*-(trifluoromethyl)benzylamine was 2 g (93%). The hydrochloride was prepared by the addition of ethanolic HCl to the amine dissolved in ether, mp 185–186°.

Acknowledgment.—We wish to thank Mr. L. Brancone and co-workers for the microanalyses, Mr. C. Pidacks and staff for the chromatography, and Mr. W. Fulmor and associates for the spectral data. In particular, we gratefully acknowledge the assistance given by Mr. G. O. Morton in his interpretation of the nmr spectra of the reaction products.

Phenazines, Phenoxazinones, and Dioxopiperazines from *Streptomyces thioluteus*[1]

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Streptomyces thioluteus is known to produce thiolutin, aureothricin,[2] aureothin,[3] 1,6-phenazinediol (**1**),[4] 1,6-phenazinediol 5-oxide, and 1,6-phenazinediol 5,10-dioxide (iodinin).[5] In our study of this organism we have encountered all of these metabolites as well as several new ones described here.

Using previously described methods[6] the chloroform extracts of washed cells and filtered beer from *S. thioluteus* fermentations were fractionated to give pure compounds which were then compared with authentic samples. Thus identified was 1-phenazinol (**2**),[7] 2-amino-3H-phenoxazin-3-one (**6**),[8] 6-methoxy-1-phenazinol (**3**), and 1,6-dimethoxyphenazine (**4**).[9] Although **4** is a known compound and **3** has been made recently by the reduction of myxin,[10] neither had been found before in nature.

Two other phenazines, not identical with any known naturally occurring phenazines, were isolated in amounts too small for complete identification. Their intense yellow-orange color with sodium hydrosulfite solution showed that both had a carbonyl containing substituent in conjugation with the ring system.[11] The ester resembled, but was not identical with, the methyl ester of phenazine-1-carboxylic acid (**5**).[12] The phenol had an ultraviolet and visible absorption spectrum identical with that of griseolutic acid (1-hydroxymethyl-4-methoxyphenazine-6-carboxylic acid).[13] The isolation of such a variety of substituted phenazines from one organism is unusual and suggests that all types of naturally occurring phenazines are biosynthesized from a common intermediate.[14]

S. thioluteus also produced a phenoxazinone which resembled **6** in color tests and spectra but was more polar. The mass spectrum molecular weight of 256 and important fragmentation products of $M - H_2O$ and $M - CH_2OH$ suggested the previously unknown structure, **7**, 2-ethanolamino-3H-phenoxazin-3-one. This was synthesized in two steps[15] from 2-hydroxy-3H-phenoxazin-3-one[16] and found to be identical with the natural product.

When the cell-extract residue was treated with hexane, then ethanol, the insoluble portion contained 3,6-dibenzylidene-2,5-dioxopiperazines. The major one, compound **8**, recognized by its absorption at 338 mμ and basic hydrolysis to benzaldehyde, was identical with an authentic sample.[17] The minor one had an nmr band at δ 3.61 suggesting a methoxyl group. Therefore glycine anhydride, benzaldehyde, and anisaldehyde were condensed[18] to give a mixture of **8**, **9**, and the dianisylidenedioxopiperazine. After separation, the previously unknown **9**, 3-anisylidene-6-benzylidene-2,5-dioxopiperazine, was identical with the natural product.

Phenazines **3** and **4** showed some weak antibacterial

(1) The U. S. Public Health Service Grant AI 06230-03 supported this investigation.

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(10) O. E. Edwards and D. C. Gillespie, *Tetrahedron Letters*, No. 40, 4867 (1966); M. Weigele and W. Leimgruber, *ibid.*, No. 8, 715 (1967). The melting points and spectra of the 10-oxide of **3**, reported as a degradation product of myxin, were identical with those of our N-oxide of **3** prepared enzymatically with sonicated *Brevibacterium iodinum* cells (for details of method see reference in footnote 5).

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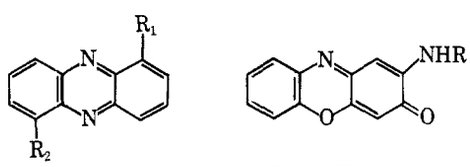
TABLE I

PRODUCTION, ISOLATION, AND PROPERTIES OF METABOLITES FROM *S. thioluteus*

Seed flasks: medium, days incubated	Production: medium, days incubated	Portion extracted	Product isolated,	Yield	Method of isolation from $CHCl_3$ extract	Properties, comparison with authentic sample
YD, 5	PGB + $CaCO_3$, 6	Beer	**2**	0.25[b]	Cc	Mp 155–157°,[a] pc solvents A, B, and C, tlc solvent D
YD, 5	Pab, 7	Whole broth				Mp 190–192°,[a] pc solvents A, B, and C, tlc solvent D
	SBM/J, 3	Beer	**3**[c]		Cc	
	PGB + $CaCO_3$, 6	Beer		7.0[b]		
YD, 5	Pab, 7	Whole broth	**4**		Cc	Mp 249–253°,[a] pc solvents A, B, and C, tlc solvent D
	PGB + $CaCO_3$, 6	Beer				
YD, 5	Pab, 7	Whole broth	**6**		Tlc solvent D	Pc solvents F, B, and E (Whatman No. 1), tlc solvent D
M − 5 + $CaCO_3$, 5	SBM/J, 3	Whole broth	**7**	0.4[b]	Extract with aqueous acid; neutralize acid solution, then extract with $CHCl_3$. Tlc solvent D	Mp 204–207°, pc solvents E, F, and 70% acetone (Whatman No. 1): λ_{max}^{EtOH} 438, 422, 238 mμ and $\lambda_{max}^{EtOH-acid}$ 445, 238 mμ. No $FeCl_3$ or ester test
M − 5, 5	SBM/J, 7	Cells	**8**	25.0[b]	Cc of hexane and EtOH, insoluble portion	Mp 303–305°[a]
			9[d]	4.0[b]		Mp 270–273°,[a] $\lambda_{max}^{CHCl_3}$ 350 mμ, $\lambda_{max}^{dilute\ base}$ 398 mμ; both tlc solvent I, pc solvents A and B
YD, 5	PGB + $CaCO_3$, 6	Beer	Phenazine ester		Neutral portion of hexane soluble part of late cc fractions	Mp 120–124°, mixture melting point with **5** methyl ester depressed 10–15°: λ_{max} 250, 362 mμ, OD = 9.05, 1.78; positive ester test
			Phenazine phenol 0.5[b]		Base soluble portion of a late cc fraction	Mp 245°; positive $FeCl_3$ test; pc solvents A, B, and C: $\lambda_{max}^{CHCl_3}$ 270, 330, 339 (shoulder), 348 (shoulder), 357, 365, 373, 450 mμ

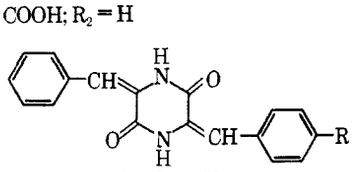
[a] Mixture melting point with an authentic sample was undepressed. [b] Approximate yield given in mg per l. of whole broth. [c] *Anal.* Calcd for $C_{13}H_{10}O_2N_2$: C, 69.0; H, 4.5; N, 12.4; mol wt, 226. Found: C, 68.6; H, 4.6; N, 12.6; mol wt (Rast), 238. [d] *Anal.* Calcd for $C_{19}H_{16}O_3N_2$: C, 71.2; H, 5.0; N, 8.7. Found: C, 71.8; H, 5.3; N, 8.3.

activity; dioxopiperazines **8** and **9** were inactive toward all microorganisms used.



1, $R_1 = R_2 = OH$
2, $R_1 = OH$; $R_2 = H$
3, $R_1 = OCH_3$; $R_2 = OH$
4, $R_1 = R_2 = OCH_3$
5, $R_1 = COOH$; $R_2 = H$

6, R = H
7, $R = -CH_2CH_2OH$



8, R = −H
9, $R = -OCH_3$

Experimental Section[19]

Fermentations.—*S. thioluteus* is maintained on YD slants, transferred every 10–14 days, incubated at 28°, and not refrigerated for storage. For production, 5–7 day old slants were used to inoculate seed flasks (50 ml of media/250-ml erlenmeyer flask). The whole broth from these was inoculated at 5% into production media (100 ml/250-ml flask). Details and results are shown in Table I.

Syntheses. A. 6-Methoxy-1-phenazinol (3).—Synthetic **4**[9] (100 mg) was refluxed 3 hr with 7 ml of glacial acetic acid and 7 ml of 48% hydrobromic acid. The reaction mixture was diluted with water, adjusted to pH 5.2, and extracted with chloroform. The chloroform solution was extracted with dilute base; the alkaline solution was acidified and extracted with chloroform. Column chromatography of the final chloroform solution separated **1** and **3**. Compound **3** had a yield of 14 mg, mp 190–193°: λ_{max}^{EtOH} 271 mμ (ε 67300), 371 (2780), 438 (1880).

B. 2-Ethanolamino-3H-phenoxazin-3-one (7).—A mixture of 15 mg of 2-hydroxy-3H-phenoxazin-3-one,[16] 3 ml of dry benzene, and 0.1 ml of thionyl chloride was refluxed for 0.5 hr. The mixture was then taken to dryness, fresh dry benzene was added, and the solution taken to dryness again, twice. The black residue was only partly soluble in benzene; 0.1 ml of ethanolamine was added and after shaking overnight this mixture was extracted with chloroform. The chloroform solution was extracted with aqueous acid; the acid solution was neutralized and extracted with chloroform. Tlc showed only one spot. After column chromatography, eluting with 1% ethanol in chloroform, synthetic **7** melted at 203–205° and had the same crystal form after microsublimation on the hot stage as natural **7**.

C. 3-Anisylidene-6-benzylidene-2,5-dioxopiperazine (9).—A mixture of 575 mg of glycine anhydride, 1.65 g of anhydrous sodium acetate, 0.55 ml of acetic anhydride, 0.75 ml of benzaldehyde, and 0.75 ml of anisaldehyde was heated for 8 hr at 120–130°. After numerous washings with water and ethanol the pale yellow solid remaining was separated by column and thin layer chromatography. Three products (**8**, mp 303–305°; **9**, mp 275–277°; and the dimethoxy analog, mp 325°) were obtained: λ_{max}^{EtOH} 368 mμ (saturated solution); $\lambda_{max}^{CHCl_3}$ 360 (ε 10,320);

(19) For details of experimental methods see ref 6. Analyses were by G. Robertson, Florham Park, N. J. Nmr data were obtained on a Varian A-60 spectrophotometer using tetramethylsilane as an internal standard. Submerged fermentations were carried out on a rotary action shaker Model V, New Brunswick Scientific Co., New Brunswick, N. J., at 215 rpm and 28°. All paper (pc) and thin layer chromatographic (tlc) comparisons were with authentic samples run on the same paper or plate; the R_f values given are approximate. All column chromatography (cc) was carried out with chloroform solutions and chloroform eluent unless otherwise noted. Media: YD, PGB, and SBM/J, see ref 6; Pablum, see N. N. Gerber and H. A. Lechevalier, *Appl. Microbiol.*, **13**, 935 (1965); M − 5, 5 g of Wilson's peptone No. 851F, (The Wilson Co., Chicago, Ill.), 5 g of BYF No. 50X (A fraction of autolyzed brewers yeast sold by Amber Laboratories, Inc., Milwaukee, Wis.), 10 g of Cerelose, 20 g of Brer Rabbit Green Label Molasses, tap water to 1 l., pH adjusted to 8.5 before autoclaving. When calcium carbonate was added it was always 100 mg per flask.

$\lambda_{max}^{dilute\ NaOH}$ 393; λ_{max}^{Nujol} 3.22, 6.04, 6.30, 7.78, 8.08, 8.58–8.80, 9.85, 10.7, 11.35, 12.18, 12.95, 13.6–13.9 μ.

Antimicrobial Assay.—These were carried out as previously described[8a] using a representative selection of bacteria, fungi, and actinomycetes. Compounds **8** and **9** were inactive at 50 μg/ml. The minimum inhibitory concentrations of **4** and **3** in μg/ml are given in parentheses after the microorganisms; a dash means inactive at 50 μg/ml, an asterisk indicates static activity: *Trichophyton mentagrophytes* 171 (25*) (25*), *Sarcina lutea* 14 (–) (20), *Corynebacterium fimi* 22 (–) (20), *Mycobacterium smegmatis* 607 (25*) (50), *M. rhodochrous* 271 (5*) (50), *Norcardia asteroides* 3409 (5*) (40), *N. coeliaca* 3520 (20*) (50*), *Actinoplanes* sp. W13 (25*) (50), *Microellobosporia cinerea* 3855 (–) (50*).

Acknowledgments.—The author wishes to thank Mrs. M. P. Lechevalier for invaluable guidance in fermentation procedures and for testing the pure substances against microorganisms. The excellent technical assistance of Mrs. Eva M. Fekete is acknowledged. The author is grateful to Dr. Y. Okami for an authentic sample of griseolutic acid, to Dr. R. Brown for compound **8**, to Dr. W. D. Celmer for aureothricin, and, especially, to Dr. B. C. Das and Dr. E. Lederer for the mass spectrum of compound **7**.

Synthesis and Selected Reactions of 3-Methyl-2,5-dihydrothiophene 1-Oxide[1]

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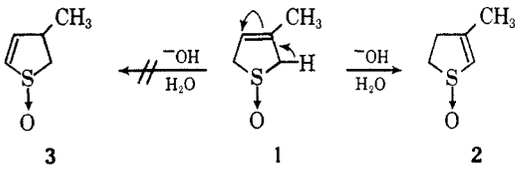
As an extension of the studies with the dihydrothiophene 1-oxides,[3] we have prepared the previously unknown 3-methyl-2,5-dihydrothiophene 1-oxide (**1**) and have investigated some selected reactions of this compound.

Sulfoxide **1** was obtained by the hydrogen peroxide oxidation of the corresponding sulfide which had been prepared by a known method[4] and purified using the aqueous sulfuric acid technique developed in our laboratory.[3] The structure of **1** was supported by elemental analysis, spectral data, and by chemical conversion to the well-characterized sulfone.[5]

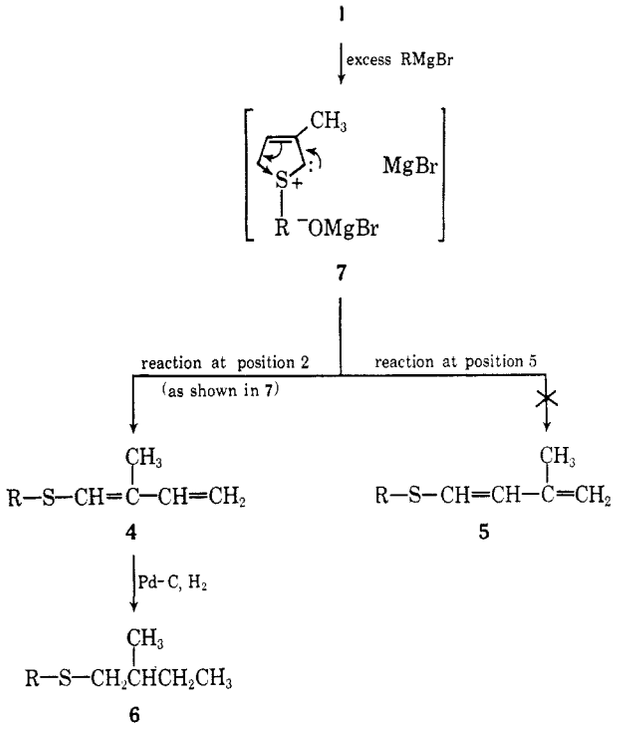
The behavior of **1** under alkaline conditions was studied to determine if isomerization of the olefinic bond would occur as was observed with the corresponding sulfone.[6] On treatment with 0.5 *N* sodium hydroxide, **1** underwent partial isomerization to give 3-methyl-4,5-dihydrothiophene 1-oxide (**2**). Sulfoxide **2** was not isolated; however, it was detected in-



3 **1** **2**

directly in two ways. First, half of a chloroform extract of the aqueous reaction mixture was treated with 30% hydrogen peroxide and the sulfones corresponding to **1** and **2** were identified by gas–liquid partition chromatography (glpc). The other half of the organic extract was treated with 48% hydriodic acid, and the sulfides corresponding to **1** and **2** were identified by glpc analysis. Results using this technique indicated that after 96 hr or longer, **1** and **2** were present in the original reaction mixture in a ratio of 1.8:1. No evidence for formation of the isomeric 3-methyl-2,3-dihydrothiophene 1-oxide (**3**) was obtained.

The behavior of **1** in the presence of Grignard reagents was especially interesting since reaction at the nonequivalent α-carbon atoms could yield the two isomeric butadienyl sulfides **4** and **5** *via* ring cleavage. When **1** was treated with excess phenyl- and *n*-propylmagnesium bromide, 1-phenylthio- and 1-*n*-propyl-



4a and **6a**, R = C_6H_5
4b and **6b**, R = *n*-C_3H_7

thio-2-methyl-1,3-butadiene (**4a** and **4b**) were obtained in 70 and 49% yield, respectively. These dienyl sulfides readily polymerized on standing at room temperature but were stable for extended periods when stored in the dark at 0°. No by-products were detected in either reaction.

The general dienyl sulfide structure of these compounds was supported by analytical data and by ultraviolet absorption at 288 mμ (ϵ 21,700) for **4a** and at 281 (17,300) for **4b**. Strong absorption at 6.19 and 6.31 μ in the infrared spectrum of **4a** and at 6.18 in the

(1) Presented at the Southeastern Regional Meeting of the American Chemical Society, Louisville, Ky., Oct 1966.

(2) (a) Abstracted in part from the Ph.D. dissertation of D. W. Kreh, Virginia Polytechnic Institute, 1966; (b) to whom any correspondence should be addressed: Building 231, Tennessee Eastman Co., Kingsport, Tenn., 37662; (c) George Mason College, Fairfax, Va. 22030.

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