the pore in the fungal spore wall were developed, such information could be used in the design of antifungal agents other than the 8-quinolinols and derivatives.

Experimental Section

Melting points were taken in a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer Model 221 spectrophotometer.

Preparation of Substituted Bis(8-quinolinolato)copper(II) Derivatives. To a solution of 0.02 mol of ligand in MeOH or MeOH–DMF mixture was added 0.01 mol of $Cu(OAc)_2 \cdot H_2O$ dissolved in MeOH, and the mixture was stirred for 1 hr. The product was removed by filtration, washed with H_2O followed by Me_2CO, and dried at 60° overnight. The materials were usually pure enough for analysis.

Acknowledgments. This work was supported in part by U. S. Public Health Service Grant No. AI-05808. Thanks are due to P. Pechukas, Columbia University, for helpful discussion; M. LaCorte, 1967 summer assistant at Boyce Thompson Institute, for assistance in calculating the data of Table III; A. T. Grefig for obtaining the infrared spectra; and P. K. Godfrey and J. Baricko for carrying out the antifungal testing.

Supplementary Material Available. Infrared spectra will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-74-824.

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Antiviral Agents. Chemical Modifications of a Disulfide Antibiotic, Acetylaranotin†

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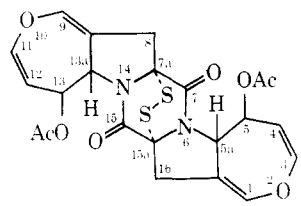
The reactivity of the cyclic disulfide linkage in acetylaranotin (1) was investigated. Novel insertion reactions with elemental sulfur and hydrogen cyanide gave tetrasulfide 8 and dithiocarbamate **16**. Cleavages with methanethiol and dimethyl disulfide gave dithiol **5** and bis(methyl disulfide) **7**. Because of lability of the disulfide linkage of acetylaranotin toward acids and bases, acetoxy groups were removed by an indirect method to give diol **26**. When atmospheric oxygen was present in a basic medium a sulfur disproportionation reaction gave tetrasulfide diol **29**. Diol **26** was oxidized to diketone **30** and acylated to give di- and monocarbethoxy derivatives **31** and **32**. In mice, tetrasulfide 8 and trisulfide 10 gave protection equivalent to that of acetylaranotin against a lethal respiratory virus, Coxsackie A-21. Carbethoxy derivatives **31** and **32** were less active. Marginal activities of trithiocarbonate **18** and monosulfide **22** give the first indication that an S–S linkage may not be an absolute requirement for antiviral activity in this family of compounds. In an enzyme inhibition assay against an RNA polymerase system from Coxsackie A-21 virus, several compounds were more than 1000 times more inhibitory toward the viral polymerase than they were toward the RNA polymerase of the uninfected host cells. An improved color test for the detection of thiols is described.

Earlier reports from these laboratories and from workers at Eli Lilly and Co. have described the isolation,[1b,2a] characterization,[1b,2] and antiviral activities[3,4] of acetylaranotin (AAS_2, 1). The structural elucidation of 1 was completed by X-ray crystallography,[5] revealing the indicated absolute configuration. The same central epidithiopiperazinedione grouping also is found in gliotoxin (2), sporidesmin (3),[6a] oryzachlorin,[7] the chaetocins,[8a] verticillin,[8b] the melinocidins,[8c] and chetomin.[8d] Although these fungal metabolites are generally more toxic to mammals than acetylaranotin, gliotoxin has shown some prophylactic antiviral activity *in vivo*[9] as well as *in vitro*.[9-12]

The relatively few chemical modifications of gliotoxin (2) and sporidesmin (3) have led predominantly to sulfur-free or nonbridged structures which lacked significant biological activity.[6a] This report describes an investigation of the reactivity of the disulfide linkage of AAS_2, aimed primarily at the preparation of sulfur-retaining and ring-bridged derivatives. As a working hypothesis, such derivatives were considered to be of special interest when they retained a potentiality for reaction with enzymatic thiol groups, *i.e.*, Enz-SH + RSSR′ → Enz-SSR.‡ As a practical matter, the range of synthetic procedures applicable to AAS_2 derivatives was found to be fairly limited due to the

†For a preliminary communication of this work, see ref 1a.

‡Susequent biochemical evidence inicates that acetylaranotin does indeed inhibit a viral RNA polymerase by reacting with enzymatic sulfhydryl groups.[6b]



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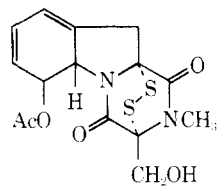

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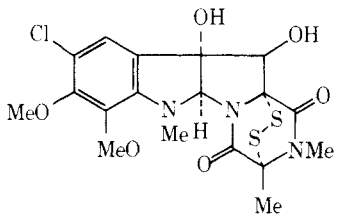
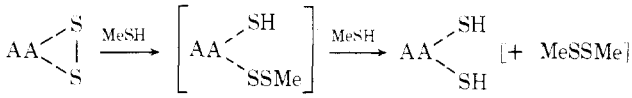


3

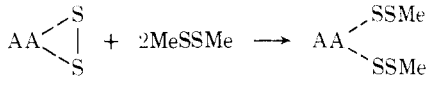
lability of the divinyl ether, acetoxy, and disulfide groupings.

Reduction of the disulfide linkage in AAS_2 (1) proceeded satisfactorily with sodium borohydride to give dithiol **5**. It was found that this dithiol was obtained in better yield (92%) and with greater simplicity when an excess of methanethiol in unheated pyridine was used as the reducing agent. Reaction presumably proceeds *via* a mixed disulfide **4** which then reacts with more methanethiolate ion to give **5**. This reduction procedure proved to be very useful for processing certain fermentation batches of AAS_2 that contained such large amounts of the related $AA(SMe)_2$[2a,1b] (**6**) that the AAS_2 could not be purified by recrystallization. These mixtures were reduced and the dithiol was removed by filtration. Oxidation of the dithiol back to disulfide **1** proceeded very satisfactorily with either 1,2-diiodoethane[13] or 2,3-dichloro-5,6-dicyanoquinone.[14]



1 4 5

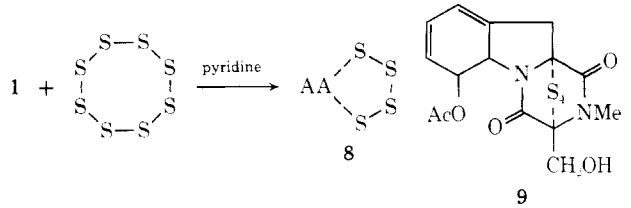
Under the same conditions, reaction of disulfide **1** with excess dimethyl disulfide gave bis(methyl disulfide) **7** in



1 7

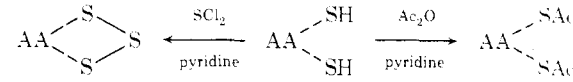
64% yield. Catalysis[15] by an added thiol was not required, though a little methanethiol could have been present in the dimethyl disulfide. Elemental sulfur was readily inserted into the disulfide linkage of **1** without requiring addition of dithiol **5** or any other thiol catalyst.[16] With a large excess of sulfur only 2 equiv reacted, forming tetrasulfide **8** in 81% yield. A tetrasulfide **9** from gliotoxin (**2**) was prepared similarly.

With just 1 equiv of sulfur, tlc data indicated that disulfide **1** formed a mixture of trisulfide **10** and tetrasulfide



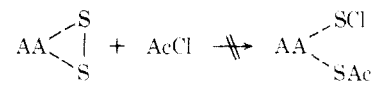
8 9

8.§ Trisulfide **10** was formed much more cleanly from the condensation of sulfur dichloride with dithiol **5**. Spectral data showed that the trisulfide was not merely a fortuitous mixture of the disulfide and the tetrasulfide, **1** and **8**. Each compound showed uniquely characteristic peaks. Thus, for the di-, tri-, and tetrasulfides the amide carbonyl peaks appeared at 5.85, 5.89, and 5.91 μ, respectively. Each of their nmr spectra showed a unique, singlet acetate peak at δ 2.00, 2.11, and 2.17 ppm, respectively.



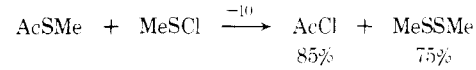
10 5 11

With acetic anhydride dithiol **5** gave the *S,S*-diacetyl derivative **11**. It was thought that a reaction of disulfide **1** with acetyl chloride might give a sulfenyl chloride derivative **12** which could be a useful intermediate for further

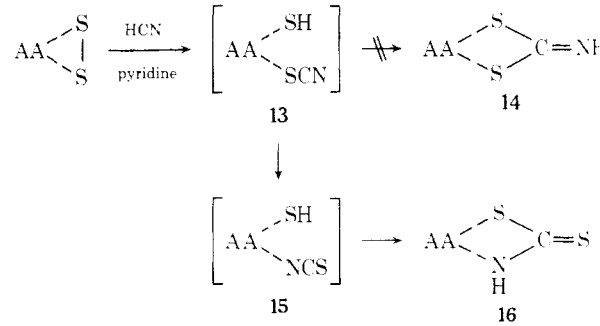


1 12

transformations or for a spontaneous intramolecular cyclization involving addition of the sulfenyl chloride moiety to an olefinic linkage. Actually, the disulfide was unchanged after being dissolved for 2 hr at 25° in undiluted acetyl chloride. In this system it may be that the equilibrium lies far to the left, since Douglass[18] has reported a facile reaction of the reverse type, between methyl thiolacetate and methanesulfenyl chloride.



Disulfide **1** and an excess of hydrogen cyanide gave a 1:1 adduct with an ir spectrum (5.82, 5.73, 3.12 μ) lacking the peak near 4.7 μ expected for thiocyanate **13**. Moreover, the nmr spectrum showed *two* acetate peaks (at δ 1.97 and 2.00, with NH at δ 8.80 ppm), thus indicating a molecular dissymmetry which would be unexpected in iminodithiocarbonate **14**, a possible cyclization product from **13**.



13 14 15 16

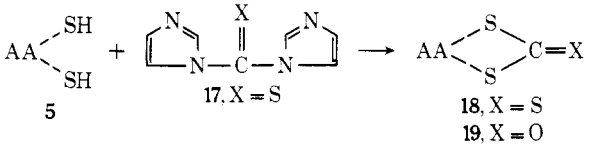
This adduct is believed to be dithiocarbamate **16**, a cyclization product which would be expected to form after a preliminary rearrangement[19] of thiocyanate **13** to isothiocyanate **15**. In contrast to this condensation of **1** with hydrogen cyanide, acyclic disulfides attached to fully substi-

§Rahman, *et al.*,[17a] subsequently reported the isolation of a naturally occurring trisulfide derivative (sporidesmin E) of sporidesmin (**3**) and the conversion of the disulfide to the trisulfide by treatment with a mixture of phosphorus pentasulfide and sulfur. This group later achieved the same conversion with dihydrogen disulfide, which converted dehydrogliotoxin into a mixture of the tri- and tetrasulfides.[17b]

tuted carbon atoms have been reported to be unreactive toward cyanide ion.[20]

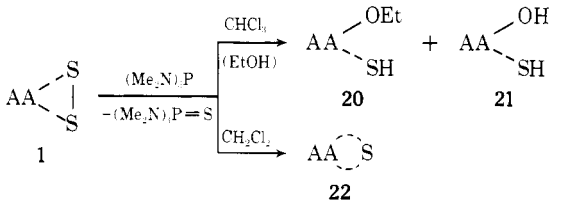
Further unusual reactivity of the disulfide linkage in 1 and other epidithiodiketopiperazines[6] is shown in their very helpful color test responses to silver nitrate and iodine–azide sprays[6] on thin-layer chromatograms, responses not shown by simple, acyclic disulfides. This heightened reactivity is in accord with X-ray crystallographic results[5a] showing a considerable angular deformation in the disulfide bridge of 1, where the system C–S–S–C defines a dihedral angle of only 15–18°. This contrasts with a preferred angle near 90° in linear disulfides.[21]

Dithiol 5 was allowed to react with thiocarbonyl diimidazole[22] in pyridine to give trithiocarbonate 18 in 70% yield. An analogous reaction with carbonyl diimidazole formed dithiocarbonate 19.

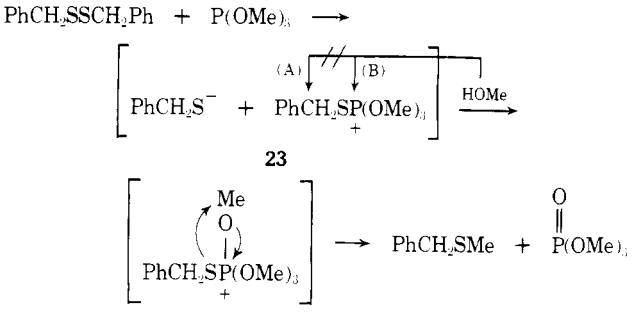


Partial desulfurization[23] of disulfide 1 with hexamethylphosphorous triamide in $CHCl_3$ yielded ethoxythiol 20 rather than the intended cyclic monosulfide 22. The ethoxy group is presumably derived from the 0.75% of EtOH included in the commercial $CHCl_3$ as a stabilizer. The possibility that this product was actually an EtOH solvate of 22 was excluded by an improved color test (described at the beginning of the Experimental Section) which strongly indicated the presence of a thiol group.

Additionally, nmr data for 20 also indicated the presence of a thiol group (δ 3.50 ppm), an ethoxyl group (methyl triplet centered at δ 1.25), and a molecular dissymmetry not to be expected in a mere EtOH solvate of monosulfide 22 (*i.e., two* acetate peaks were shown at 2.05 and 2.10 ppm). Similar evidence indicated that a minor coproduct obtained with 20 was hydroxythiol 21.



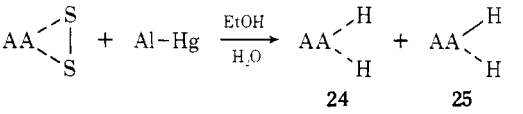
The above affixation of an ethoxy group is in interesting contrast to results found by Davidson.[24] He found that the reaction of dibenzyl disulfide with trimethyl phosphite in MeOH solution gave products indicating that MeOH attacked a phosphonium salt intermediate 23 at a P atom (reaction mode B) rather than at a C atom (reaction mode A).



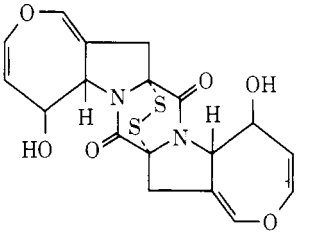
Considering stereochemical implications, if the present reaction with disulfide 1 proceeds *via* a phosphonium ion intermediate analogous to 23, then a subsequent S_N2 attack of EtOH at a carbon atom, in analogy with reaction mode A, would proceed with the inversion of configuration represented in ethoxythiol 20.

In an alcohol-free solvent (CH_2Cl_2) the synthesis of cyclic monosulfide 22 was successful, indicating that at least in this case there was an overall retention of configuration (or inversions at both of the carbon atoms involved, in which case the product is actually AA(S)#).

A reductive desulfurization of AAS_2 with aluminum amalgam was adapted from a procedure used with gliotoxin.[26] A sulfur-free product 24 was obtained along with a smaller amount of a second, very similar product which is probably an isomer 25 of 24.



Modification of the Dihydrooxepin Moieties. The diol 26 corresponding to cleavage of the acetoxy groups of acetylaranotin was wanted not only for antiviral testing, but also as a likely intermediate for the preparation of other derivatives. However, a variety of methods for ester cleavage under mild conditions was unsuccessful when applied to acetylaranotin. There was either extensive degradation or no reaction. This is at least partly understandable in view of the known lability of disulfide compounds to acids and bases[27] and the especially high lability of the sterically strained disulfide system in acetylaranotin. A satisfactory synthesis of diol 26 was eventually accomplished by an indirect route, starting with dithiol 5. The acetate groups were removed smoothly in methanolic ammonia at room temperature, facilitated by the high solubility of the dithiol in this system. A crystalline solid believed to be the ammonium salt of the corresponding diol dithiol 27 separated during reaction. This apparently labile mercaptide salt was not isolated but was conveniently oxidized to the desired diol disulfide 26 in an overall yield of 63% by adding ethylene iodide[28] to the methanolysis mixture.



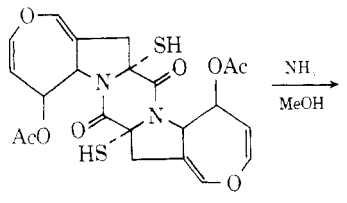
The methanolysis reaction appeared to be adversely sensitive to atmospheric oxygen. In one run where all of the air was not flushed out of the reaction system with gaseous ammonia, the product was not diol disulfide 26, but the corresponding diol tetrasulfide 29. The additional

#Safe and Taylor[25] independently desulfurized the disulfides sporidesmin (3) and dehydrogliotoxin, using triphenylphosphine. The resulting monosulfides gave circular dichroism curves which were roughly the inverse of those from the disulfides. They concluded from these curves that in each case *both* of the attached carbon atoms had been inverted. X-Ray crystallography has shown that in the epidithiopiperazinedione portion, chaetocin has an absolute configuration (*S*) that is just the reverse of those of AAS_2, sporidesmin, and gliotoxin (*R*), and the circular dichroism curve of chaetocin is likewise essentially reversed from those of these other three antibiotics.[8a] Thus, Safe and Taylor's conclusion appears to be supported.

Table I. Inhibition of Fungi and Bacteria *in Vitro*

Organism	Drug concn (μg/ml) for inhibition: Diol 26	Acetylaranotin
Candida albicans E 83	2.5	10
Cryptococcus neoformans E 138	1	10
Microsporum canis ATCC 10214	0.5	10
Microsporum gypseum ATCC 14683	1	10
Phialophthora jeanselmi E 16	10	>250
Trichophyton tonsurans NIH 662	1	10
Trychophyton mentagrophytes E 11	1	25
Trichophyton rubrum E 97	1	10
Mycobacterium smegmatis ATCC 606	5	2.5
Staphylococcus aureus Rose ATCC 14154	25	100
Streptococcus pyogenes C 203	10	50
Proteus vulgaris ATCC 9484	50	>250

two atoms of sulfur must have come from a disproportionation involving a desulfurizing degradation of part of the starting material.



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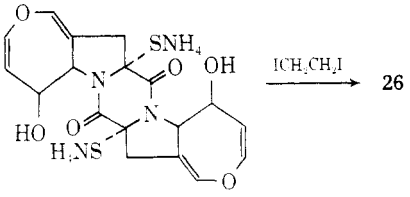
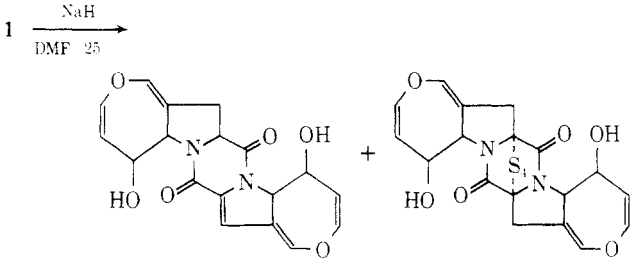


27

In another disproportionation reaction a sulfur-free product **28** with an additional double bond was indeed isolated, along with the same diol tetrasulfide **29.** These

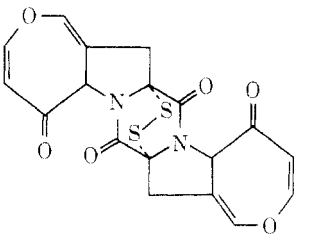


28 29

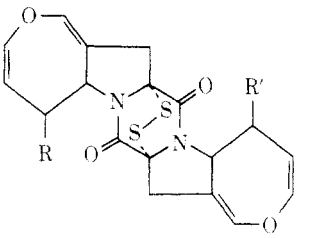
products resulted from an attempted base-catalyzed elimination of the elements of acetic acid from acetylaranotin, using a suspension of sodium hydride in dry, unheated DMF.

Disproportionation without deacetylation gave tetrasulfide **8** when acetylaranotin in DMF was subjected to hydrogenation at 85° over a rhodium sulfide catalyst under conditions reported to convert benzene to cyclohexane.[29]

A chromium trioxide–pyridine complex[30] was effective for oxidation of diol disulfide **26** to the corresponding diketone **30.** Esterification of diol **26** was surprisingly sluggish. Unreacted diol was recovered after treatment for 7 days with a large excess of benzoyl chloride in pyridine at room temperature. Under comparable conditions even *tert*-butyl alcohol is reported to be benzoylated in 80% yield after standing overnight.[31] Neither *p*-toluenesulfonyl chloride nor methanesulfonyl chloride reacted with diol **26** in pyridine or *sym*-collidine at room temperature. Ethyl chlorocarbonate was more reactive. It gave 31% of the desired dicarbethoxy derivative **31** after 24 hr, though 10% of monoesterified product **32** was also obtained.



30



31, R = R' = OCOOEt
32, R = OCOOEt; R' = OH

Biological Testing Results.** The trisulfide **10** and tetrasulfide **8** derived from acetylaranotin had activities comparable with acetylaranotin[3] in protecting mice when injected subcutaneously at the maximum tolerated dose after intraperitoneal infection with a lethal (*ca.* LD_{95}) inoculum of Coxsackie A-21 virus. Diketone **30** and dicarbethoxy derivative **31** were less active. Trithiocarbonate **18,** ethoxythiol **20,** and monosulfide **23** were marginally active, indicating that the presence of an S–S bond may not be an absolute requirement for antiviral activity in this series. The other compounds did not show significant activity in mice, although, like acetylaranotin, diol **26** did protect HeLa cells in tissue culture against the cytopathic effect of Coxsackie A-21 virus. Tetrasulfide **8** and dithiol **5** were similar to AAS_2 in their inhibition of several rhinovirus strains in tissue culture. None of the present compounds showed activity against influenza or herpes simplex viruses in tissue culture.

Diol **26** was more toxic in mice than acetylaranotin. The minimum lethal subcutaneous doses were 7.5 and 48 mg/kg, respectively. The growth of a number of fungi and bacteria was also inhibited by lower concentrations of diol **26** than acetylaranotin (Table I). Toxicities and antifungal activities similar to those of diol **26** have been reported for gliotoxin and for two new epidithia- and epitrithiapiperazinediones characterized by DeVault and Rosenbrook.[32] Tetrasulfide **8** did not inhibit any of the fungi or bacteria of Table I at concentrations up to 50 μg/ml.

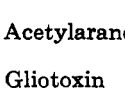
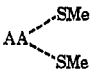
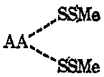
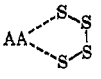
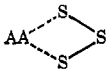
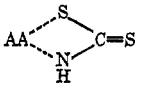
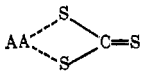
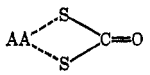
The inhibitory activities of acetylaranotin and its derivatives *in vitro* against an RNA polymerase from Coxsackie A-21 are listed in Table II and compared with the drug concentrations required to inhibit the RNA polymerase of the HeLa host cells. It is seen that compounds **26, 28, 29,** and **32** exerted a highly selective inhibition of the viral enzyme.

Experimental Section

Melting points are uncorrected. Solids were pressed with KBr for ir spectral determinations on a Perkin-Elmer Model 21 spectrophotometer; nmr data (Me_4Si) were obtained for all compounds with a Varian Model A-60 spectrophotometer and were compatible with the assigned structures. Evaporations were conducted under reduced pressure with a water aspirator. Tlc utilized phosphor-doped silica gel; plates were developed most satisfactorily with $CHCl_3$–Me_2CO, 9:1 by volume. Spots were revealed with a uv lamp (254 nm) and, in the case of all of the sulfur-containing products, by spraying with 2% aqueous $AgNO_3$. The spots

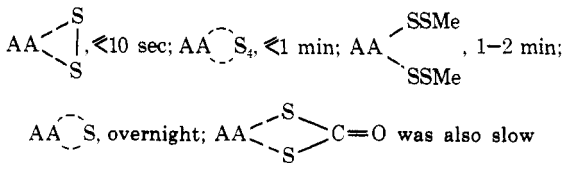
**The antiviral activities of acetylaranotin and the antiviral testing procedures used in these laboratories are described in ref 3.

Table II. Inhibition of Viral RNA Polymerase Activity *in Vitro*[a]

No.	Compound	Drug concn (μg/ml) for 50% inhibn		"Therapeutic index" (C_1/C_2)
		Cellular (C_1)	Viral (C_2)	
1	Acetylaranotin 	75	0.1	750
2	Gliotoxin	0.6	0.003	200
5		>160	5.5	>29
6		100	1	100
7		20	0.07	290
8		115	0.2	600
9	Tetrasulfide from gliotoxin	0.9	0.002	450
10		30	0.8	38
11		40	0.09	440
16		>25	3	>8
18		>100	18	>5
19		111	0.5	220
20		82	5.8	14
21		164	121	1
22		14.4	3.8	4
24		80	30	3
25		60	20	3
26	Diol dilsulfide	0.9	0.007	1,300
28	Desulfurized[b] diol	3	0.00009	33,000
29	Diol tetrasulfide	3	0.0001	30,000
30	Diketone disulfide	0.8	0.06	13
31	Dicarbethoxy disulfide	50	0.08	620
32	Monocarbethoxy disulfide	15	0.0002	75,000

[a]Viral RNA polymerase activity in HeLa cells infected with Coxsackie A-21 virus was measured by the uptake of ^{14}C-labeled uridine into material insoluble in trichloroacetic acid while host cell RNA synthesis was inhibited by actinomycin C. Cellular RNA polymerase activity was similarly measured in uninfected cells without actinomycin C. Details of this assay are described in ref 3c. [b]Analyses indicated that this sample of **28** retained 0.74% of sulfur, indicating the presence of a small amount of a sulfur-containing contaminant.

from the $AgNO_3$ spray aged from yellow tan to dark brown; the rate of their first appearance at 25° correlated with structure, *i.e.*



Preparative thick-layer chromatography was done on hard-surfaced, 20 × 20 × 0.2 cm plates of phosphor-doped silica gel ("F-254," from E. Merck, AG) distributed by the Brinkmann Instrument Co., applying the solute as a $CHCl_3$ solution and usually developing the chromatogram with $CHCl_3$–Me_2CO (9:1). Product bands were detected by uv, scraped off, and extracted with $CHCl_3$–EtOH (3:1), and the extracts were evaporated. The residues were separated from small amounts of siliceous material by dissolving them in CH_2Cl_2 and then filtering. Many of the products were then crystallized from CH_2Cl_2–EtOH, boiling out most of the CH_2Cl_2 (to bp >70°).

Improved Color Test for Thiols. Although the iodine–azide test[33] is relatively specific for thiols, it also gives a positive re-

sponse to such activated disulfides as diacyl disulfides, sporidesmin, gliotoxin,[6] and AAS_2.[4] However, we find that neither AAS_2 nor gliotoxin gives a color response with another thiol reagent, 5,5′-dithiobis(2-nitrobenzoic acid), thus allowing derived thiols to be readily distinguished from them. Although the latter reagent[34] is normally used in an aqueous solution, we find that its sensitivity is remarkably enhanced in DMF solution. Thus a 0.01 *M* solution of this reagent (0.01 ml) in DMF gave an immediate, strong salmon-orange color when combined with *ca.* 0.01 mg of thiols **5** or **21**, or with traces of *p*-thiocresol or benzyl, *n*-butyl, or dodecyl mercaptan. The reagent solution was still effective after 2 years at 25° in a brown bottle.

5,5aβ,13,13aβ-Tetrahydro-5β,13β-diacetoxy-8*H*,16*H*-7a,15a-epidithio-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]-pyrazine-7,15-dione (1). Certain fermentation batches of AAS_2 (**1**) have contained so much $AA(SMe)_2$ (**6**) that a satisfactory separation was accomplished only *via* reduction of AAS_2 to give $AA(SH)_2$ (**5**). Reoxidations of dithiol **5** to **1** are described below. A more detailed procedure for use of 1,2-diiodoethane in an analogous oxidation is described in the preparation of diol **26**.

Procedure A. To a solution of 5.1 mg (0.01 mmol) of $AA(SH)_2$ (**5**) in 0.1 ml of MeOH saturated with NH_3 was added 0.05 ml of MeOH containing 3.1 mg (0.011 mmol) of ICH_2CH_2I.[13] A white solid separated immediately and some gas was evolved (presumably $CH_2{=}CH_2$). After 5 min at 25° the MeOH and NH_3 were removed by evaporation and the solid was washed by centrifugation with MeOH, with ether, and with H_2O: yield, 5.4 mg; tlc and ir showed no difference from AAS_2.

Procedure B. To a solution of 5.0 mg (0.022 mmol) of 2,3-dichloro-5,6-dicyanoquinone[14] in 0.5 ml of dioxane was added 10.1 mg (0.02 mmol) of $AA(SH)_2$ (**5**). Stirring was continued for 15 hr even though all of the dithiol had dissolved after 5 min. Dioxane was removed by evaporation, the residue was dissolved in 0.5 ml of $CHCl_3$, and the solution was extracted with 3 × 0.1 ml of 5% aqueous $NaHCO_3$. Evaporation of the dried ($MgSO_4$) $CHCl_3$ solution left 9.8 mg (97%) of an ivory-colored solid with ir and tlc identical with those of AAS_2.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7a,-15a-dimercapto-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,-2′-*d*]pyrazine-7,15-dione (5). Method A. A solution of 1.000 g of AAS_2 (**1**) in 20 ml of dry pyridine was saturated with gaseous CH_3SH. After 3 hr at about 25° the resulting crystals were collected and washed with ether: 0.847 g; mp 252–254° dec; ir 5.92, 5.78 μ. Fermentation batches of AAS_2 containing a preponderance of the bis(methylthio) analog **6** may also serve as starting material (see preceding experiment). *Anal.* ($C_{22}H_{22}N_2O_8S_2$) C, H, N, S.

Method B. To a solution of 0.0152 g (0.4 mmol) of $NaBH_4$ in 2 ml of absolute EtOH was added 5 ml of CH_2Cl_2 and then 0.202 g (0.4 mmol) of AAS_2. There was an immediate, brief, brisk effervescence and a slight evolution of heat. After 5 min at about 25°, the resulting clear solution was chilled, 10 ml of ice-cold H_2O was added, and the pH brought to 6 by bubbling in a stream of CO_2. The CH_2Cl_2 layer was separated and the aqueous suspension extracted (with 5 × 5 ml of CH_2Cl_2) until no more solid remained. The CH_2Cl_2 extracts were washed with 2 × 5 ml of H_2O, combined, dried ($MgSO_4$), and evaporated, leaving 0.191 g of white solid. Crystallization from $CHCl_3$, cooling finally at 0°, gave 0.110 g of colorless crystals: mp 252–254° dec; ir ≡ product of method A. *Anal.* C, H.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7b,15a-bis(methylthio)-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,-2-*a*:1′,2′-*d*]pyrazine-7,15-dione (6). A partially purified fermentation product appeared by tlc to be roughly a 3:7 mixture of AAS_2 and $AA(SMe)_2$ (**6**). A solution of 6.94 g of this mixture in a minimum volume (52 ml) of dry pyridine was filtered from 73 mg of a solid which tlc and ir indicated to be mostly AAS_2. The filtrate was saturated with MeSH, nullifying the exotherm with a pan of cool H_2O. After 22 hr the solid which had separated was collected and washed with pyridine and with ether to give 2.13 g of ivory-colored solid. By tlc and ir it was indistinguishable from dithiol **5**, but it was later found also to contain about 3% of **6**.

The pyridine mother liquor (but not the washes) was diluted with 120 ml of H_2O. (Stench!) After 15 min the solution was decanted from a little brown oil. Dilution with 100 ml of water at each of four 10-min intervals precipitated a solid which was collected and washed with water: 3.50 g; pale tan leaflets, mp 243–245°. Recrystallization of 0.50 g of these leaflets, adding 10 ml of absolute EtOH to a solution in 1 ml of CH_2Cl_2 and then boiling down until the boiling point reached 78°, returned 0.49 g of tan leaflets, mp 246–248°. Their ir spectrum was indistinguishable from that of authentic[2a] **6**.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7b,15-bis(methyldithio)-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,-2′-*d*]pyrazine-7,15-dione (7). A solution of 0.202 g of AAS_2 and 1.0 ml of MeSSMe in 5 ml of dry pyridine was allowed to stand for 6 hr and then was evaporated almost to dryness at <45°. The residual thick syrup, agitated with 3 ml of EtOH, gave 0.188 g of solid, mp 121–124°. Recrystallization from CH_2Cl_2–EtOH returned 0.153 g (64%) of colorless needles: mp 128–131° (gel); $[\alpha]^{25}D$ −322 ± 0.59° ($CHCl_3$, *c* 0.338). *Anal.* ($C_{24}H_{26}N_2O_8S_4$) C, H, N, S.

5,5aβ,13,13aβ-Tetrahydro-5β,13β-diacetoxy-8*H*,16*H*-7a,15a-epitetrathio-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]-pyrazine-7,15-dione (8). A solution of 504 mg (1.0 mmol) of AAS_2 (**1**) and 128 mg (4.0 mmol) of S in 20 ml of dry pyridine was allowed to stand for 1.5 hr and then was evaporated to dryness at <40°. The residual solid was washed with CS_2 and then crystallized from CH_2Cl_2–EtOH to give 477 mg of ivory-colored crystals which gradually sintered and darkened from 205°: ir, nmr, mass spectral, and tlc results confirmed identity and homogeneity. *Anal.* ($C_{22}H_{20}N_2O_8S_4$) C, H, N, S.

8,12-Dihydro-8β-hydroxy-5β-(hydroxymethyl)-14-methyl-5,-12a(7aβ*H*)-(iminomethano)[1,2,3,4]tetrathiazocino[6,5-*a*]indole-6(5*H*),13-dione (9). A solution of 0.104 g (0.32 mmol) of gliotoxin **2** and 0.051 g (1.6 mmol) of sulfur in 4 ml of dry pyridine was allowed to stand at 25° for 1 hr and then was evaporated essentially to dryness at <30° (15 mm). The residual solid was washed with CS_2: 0.124 g; mp 167–170°. Recrystallization from CH_2Cl_2–hexane without heating gave 0.091 g (73%) of ivory-colored rosettes: mp 194–196° dec; ir 5.97, 6.08 μ; uv (MeOH) 212 nm (ε 13,400) and 272 (sh, 5000); $[\alpha]^{25}D$ −453 ± 1.5° ($CHCl_3$, *c* 0.322). *Anal.* ($C_{13}H_{14}N_2O_4S_4$) C, H, N, S.

5,5aβ,13,13aβ-Tetrahydro-5β,13β-diacetoxy-8*H*,16*H*-7a,15a-epitrithio-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]-pyrazine-7,15-dione (10). A suspension of 0.253 g (0.5 mmol) of dithiol **5** in 10 ml of CH_2Cl_2 containing 0.791 g (1.0 mmol) of dry pyridine was swirled during the dropwise addition of a solution of 0.515 g (0.5 mmol) of SCl_2 (freshly distilled from 0.5% by volume of diethyl phosphite and stabilized with 0.008 mol % of diethyl phosphite[35]) in 5 ml of CH_2Cl_2. The solid dissolved immediately. The reaction solution was kept at about 25° for 15 hr, washed three times with H_2O, dried ($MgSO_4$), and concentrated. It deposited 0.119 g of ivory-colored crystals. From a $CDCl_3$ solution an nmr signal ascribable to the OAc groups appeared as a sharp singlet at δ 2.11 ppm. *Anal.* ($C_{22}H_{20}N_2O_8S_3$) C, H, N, S.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7b,-15a-di(thioacetoxy)-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-7,15-dione (11). A suspension of 0.253 g of dithiol **5** in 2 ml of dry pyridine containing 0.2 ml of acetic anhydride was stirred for 14 hr. A trace of undissolved solid was removed by filtration and the filtrate was evaporated to drynss, finally at 0.1 mm. Recrystallization of the residue from benzene–hexane gave 0.220 g of colorless crystals: mp 140–144°; ir 5.74, 5.85, 6.01 μ (sh); nmr δ 1.95 (OAc, 6 H) and 2.35 (SAc, 6 H); $[\alpha]^{25}D$ −277 ± 0.38° ($CHCl_3$, *c* 0.525). *Anal.* ($C_{26}H_{26}N_2O_{10}S_2{\cdot}C_6H_6$) C, H, N, S.

5,5aβ,13,13aβ-Tetrahydro-5β,13β-diacetoxy-18-thio-8*H*,16*H*-15a,7a-(iminomethanothio)-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-7,15,18-trione (16). A solution of 0.504 g of AAS_2 in 10 ml of dry pyridine at 0° was combined with 0.1 ml of ice-cold, dry HCN,[36] warmed to 25°, allowed to stand for 1 hr, and then evaporated almost to dryness at 40° (15 mm). The residual slush was washed with 3 ml of EtOH, leaving 0.423 g of solid. Recrystallization from pyridine–EtOH, finally at 0°, gave 0.280 g of tan crystals which gave only a single spot on tlc, R_f 0.27. The crystals were stirred in hexane for 15 min and washed with hexane, leaving 0.221 g of tan crystals which sintered from 200°: ir 5.73, 5.78 (sh), 5.82, 5.90 (sh), 6.02 μ. *Anal.* ($C_{23}H_{21}N_3O_8S_2$) C, H, N, S.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7a,-15a-dimercapto-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-7,15-dione Cyclic 7,15-Thiocarbonate (18). A solution of 0.089 g (0.5 mmol) of 1,1′-thiocarbonyldiimidazole[22] in 5 ml of pyridine was stirred under N_2 with 0.253 g (0.5 mmol) of dithiol **5** until (15 min) all of the solid had dissolved. After 18 hr at 25°, the solution was evaporated to dryness at <25°, finally at 0.05 mm. Crystallization of the residue from CH_2Cl_2–EtOH gave 0.192 g (70%) of golden yellow crystals which sintered from 210°: ir 5.74, 5.85, 6.05 μ. The mass spectrum showed a major peak at *m/e* 440 (M − CS_3). *Anal.* ($C_{23}H_{20}N_2O_8S_3$) C, H, N; S: calcd, 17.6; found, 17.1.

In an earlier run using CH_2Cl_2 rather than pyridine as the sol-

vent, the reaction was incomplete; an imidazole-containing, uncyclized intermediate ($C_{26}H_{24}N_4O_8S_3$) was a coproduct. Reaction of dithiol 5 with thiophosgene in pyridine gave AAS_2 rather than 18; in cold, aqueous Na_2CO_3 the yield of 18 was 6%.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7a,15a-dimercapto-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione Cyclic 7a,15a-Carbonate (19). To a stirred suspension of 0.202 g (0.4 mmol) of dithiol 5 in 3 ml of CH_2Cl_2 was added dropwise during 2 min a solution of 0.065 g (0.4 mmol) of 1,1′-carbonyldiimidazole in 0.5 ml of dry THF. The solid did not dissolve then or after addition of 5 ml of pyridine. The mixture was stirred under N_2 at 25° for 2 hr and under reflux for 3 hr. The next morning the solid was colleced and washed with CH_2Cl_2: 0.141 g (66%); mp 222–223° dec; ir 5.75, 5.83, 5.88 (sh), 6.13, 6.01 μ (sh). *Anal.* ($C_{23}H_{20}N_2O_9S_2$) C, H, N, S.

15aξ-Ethoxy-5,5aβ,7a,8,13,13aβ,15a,16-octahydro-5β,13β-diacetoxy-7aα-mercapto-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione (20) and 5,5aβ,7a,8,13,13a,15a,-16-Octahydro-5β,13β-diacetoxy-15aβ-hydroxy-7aα-mercapto-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione (21). A solution of 252 mg (0.5 mmol) of AAS_2 and 90 mg (0.55 mmol) of hexamethylphosphorous triamide in 5 ml of $CHCl_3$ was kept at 25° under a N_2 atmosphere for 16 hr, when tlc indicated no remaining AAS_2. Evaporation of the $CHCl_3$ and washing of the residue with petroleum ether left 0.252 g of residual solid. (The evaporated washes left 0.088 g of an oily solid with ir the same as that of authentic $(Me_2N)_3P{=}S$.) Preparative thick-layer chromatography of 252 mg of residue gave three main bands, R_f's 0.18 +, 0.51 +++, and 0.65 ++++. Crystallization of the solute from the middle band from CH_2Cl_2–EtOH gave mostly dense, tan, hexagonal plates covered with a much smaller amount of nondense clusters of colorless rods. The rods (plus a greater amount of plates) were decanted separately: 19.7 mg. The remaining plates (76.6 mg, mp 223–225°, only slightly contaminated with rods) were crystallized from CH_2Cl_2–EtOH, affording 64.8 mg of straw-colored rods (!): mp 223–225° dec; tlc showed only one spot, R_f 0.53. The above color test for thiols was strongly positive: ir 3.93 μ (–SH); *m/e* 518 (M); nmr data are in the Discussion section. *Anal.* [$C_{24}H_{26}N_2O_9S$ (518.55)] C, H, N, S.

The solute (18.7 mg) from the band at R_f 0.18 crystallized from CH_2Cl_2–EtOH as dense yellow granules covered with a few white crystals. The white crystals were selectively removed by decantation. After the remaining yellow granules were picked free of white crystals, the granules amounted to 7.2 mg, sintering from 200 to >300°: ir 3.92 μ (–SH). The color test for thiols was strongly positive. As with the preceding thiol **20**, the nmr spectrum indicated –SH (δ 3.16 ppm) and a lack of symmetry, *i.e.*, showing *two* acetate peaks at δ 2.04 and 2.10 ppm, though no –OEt group was indicated. *Anal.* ($C_{22}H_{22}N_2O_9S{\cdot}H_2O$) C, H, N.

5,5aβ,13,15aβ-Tetrahydro-5β,13β-diacetoxy-8H,16H-7a,15a-epithio-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione (22). The preceding reaction was repeated except that the hexamethylphosphorous triamide was dissolved in CH_2Cl_2 (not $CHCl_3$) and then added to an ice-cold solution of the 252 mg of AAS_2 in CH_2Cl_2, generating a transient, salmon-pink color. After 15 hr at 25° under N_2, the mixture was processed as before. The fastest of the bands (R_f 0.66) from each of six, thick-layer chromatographic plates, run concurrently, gave a total of 76 mg of product. Crystallization from CH_2Cl_2–EtOH gave a little yellow gum plus ivory-colored crystals which were detached and collected separately: 21 mg; mp 185–190°. The above color test for a thiol was negative and no infrared peak was present near 4.0 μ: ir 5.77, 5.89, and 6.02 μ (*vs.* 5.74, 5.85, and 6.02 μ for AAS_2). Unlike thiol **20**, the pattern of nmr peaks indicated a symmetry similar to that of AAS_2, *i.e.*, with only a singlet –OAc peak at δ 2.15 ppm; tlc gave just one spot, R_f 0.68. *Anal.* ($C_{22}H_{20}N_2O_8S$) C, H, N, S.

5,5aβ,7a,8,13,13aβ,15a,16-Octahydro-5β,13β-diacetoxy-7H,-15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione (24) and an Isomer (25?). A suspension of 0.250 g of AAS_2 and 0.50 g of small snips of Al amalgam[37] in 125 ml of absolute EtOH under N_2 was magnetically stirred for 70 hr, adding additional 0.50-g portions of amalgam at 5, 22, and 24 hr and adding 1.00 g of amalgam and 0.50 ml of H_2O at 30 hr. H_2O (15 ml) was added to convert $Al(OEt)_3$ to $Al(OH)_3$. Most of the unreacted amalgam was removed by filtration of the gelatinous mixture through an unpapered Hirsch filter funnel. THF (100 ml) was added to the filtrate and the gelatinous solid was removed by centriguation, washing with 2 × 200 ml of a 1:1 mixture of absolute EtOH and THF. The supernatant solutions were evaporated and the thin layer of residue was extracted by swirling with 2 × 10 ml of THF. The extracts were evaporated and a solution of the residual gum (0.294 g) in $CHCl_3$ was subjected to thick-layer chromatography on silica gel *vs.* $CHCl_3$–EtOAc (1:1).

A band at R_f 0.46 gave 0.055 g of a straw-colored glass which was sublimed at 175° (3 × 10^{-5} mm) to give 0.026 g of canary-yellow crystals, mp 185–190°. After the crystals had remained for 2 hr in the open air they melted at 120°, resolidified, and remelted at 188–190°; ir 5.77, 6.01, 8.13, μ (lit.[1b] mp 201–202°; ir 5.78, 6.02, 8.12 μ); *m/e* 442 (M). The nmr spectrum was very similar to that of AAS_2 except for a new quartet (2 protons) centered at δ 4.37, assignable to the new protons adjacent to the amide carbonyl groups (lit.[1b] quartet at δ 4.36). *Anal.* ($C_{22}H_{22}N_2O_8$) C, H, N.

A second band at R_f 0.27 similarly gave an extract which was sublimed at 190° (10^{-5} mm), affording 0.0076 g of a canary-yellow solid: mp 100–110° (gel); ir and nmr were very similar to those of **24** (ir 5.76, 5.99, 8.13 μ) except that nmr showed an additional peak at δ 4.89 ppm. *Anal.* ($C_{22}H_{22}N_2O_8$) N, H; C: calcd, 59.7; found, 58.9.

5,5aβ,13,13aβ-Tetrahydro-5β,13β-dihydroxy-8H,16H-7a,15a-epidithio-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]-pyrazine-7,15-dione (26). A solution of 1.047 g (2.00 mmol) of $AA(SH)_2$ (**5**) in 2 ml of MeOH saturated with NH_3 was filtered from 0.035 g of an insoluble contaminant [$AA(SMe)_2$ (**6**)], washing with three 0.5-ml portions of saturated NH_3–MeOH. The filtrates were collected in a 15-ml centrifuge tube and then resaturated with NH_3. The centrifuge tube was sealed with a rubber membrane cap which was then pierced with two hypodermic needles. By means of these needles, ammonia was flushed through the head space of the reaction vessel for 5 min. This stream of NH_3 evaporated about 15% of the reaction solution and presumbly removed essentially all of the atmospheric oxygen. After 15.5 hr at 25° the reaction mixture was an apparently solid mass of crystals. A solution–suspension of 0.068 g (2.16 mmol) of 1,2-diiodoethane (freshly recrystallized from CH_2Cl_2–EtOH) in 10 ml of MeOH was added in two portions, agitating the reaction mixture vigorously as the appearance of the solid changed and a substantial amount of gas bubbled out. The pale yellow solid was collected and washed sparingly with MeOH. A solution of this solid (0.607 g) in 30 ml of CH_2Cl_2 was combined with 3.3 ml of acetone and then chromatographed through a 7-mm column containing 1 g of silica gel which had previously been partly deactivated by equilibration overnight with atmospheric moisture. Another 25 ml of CH_2Cl_2–acetone (9:1) was passed through the column, the combined eluates were concentrated to a thick slurry, 20 ml of absolute EtOH was added, and boiling was continued until the boiling point reached 70°. Crystallization gave 0.530 g (63%) of ivory-colored needles: mp 223–225° dec; ir 2.99 (–OH), 5.99 μ (–NHC=O); nmr ($CDCl_3$) δ 5.83 (2HOH). *Anal.* ($C_{18}H_{16}N_2O_6S_2$) C, H, N, S.

5,5aβ,7a,8,13,13aβ-Hexahydro-5β,13β-dihydroxy-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione (28) and 5,5aβ,13,13aβ-Tetrahydro-5β,13β-dihydroxy-8H,16H-7a,15a-epitetrathio-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]pyrazine-7,15-dione (29). A suspension of 3.5 mmol of NaH (from 154 mg of a 54.7% emulsion of NaH in mineral oil, washed free of oil by decantation with petroleum ether) and 252 mg of acetylaranotin in 10 ml of dry DMF was stirred under N_2 for 4 hr and then acidified with 0.9 ml of HOAc. The suspension was stirred until an aliquot applied to moistened indicator paper no longer showed any flecks of alkalinity from unreacted NaH particles. The mixture was evaporated to dryness and the residue agitated with 20 ml of $CHCl_3$ and 10 ml of H_2O. The $CHCl_3$ solution was washed with water, dried ($MgSO_4$), and then subjected to thick-layer chromatography. A band at R_f 0.14 gave 24 mg of pale yellow crystals or 10 mg of **28** after recrystallization from CH_2Cl_2–EtOH, boiling out the CH_2Cl_2: mp 205–228° dec; ir 6.01, 6.08 (sh), and 5.90 μ; uv (MeOH) 224 nm (ε 14,800), 253 (8200), 327 (12,300); *m/e* 356 (M). *Anal.* ($C_{18}H_{16}N_2O_6$, 356.3) H, N; C: calcd, 60.7; found, 59.6; S: found, 0.7.

A chromatographic band at R_f 0.34 gave 37 mg of yellow crystals or 27 mg of **29** after recrystallization as above: mp 231–233° dec; ir 6.02, 6.08 (sh), 6.20, 5.92 (sh), 2.94 μ (–OH); uv (MeOH) 220 nm (ε 20,400); *m/e* 356 (M − S_4), 32, 16 (S_2 and S, very strong). *Anal.* [$C_{18}H_{16}N_2O_6S_4$ (484.6)] C, H, N; S: calcd, 26.5; found, 26.0. Infrared and nmr spectra and tlc comparison indicated identity with **29** prepared in the following experiment.

5,5aβ,13,13aβ-Tetrahydro-5β,13β-dihydroxy-8H,16H-7a,15a-epitetrathio-7H,15H-bisoxepino[3′,4′:4,5]pyrrolo[1,2-a:1′,2′-d]-pyrazine-7,15-dione (29). Deacetylation of 1.013 g of $AA(SH)_2$ (**5**) was done just as in the synthesis of diol disulfide **26** except that air was flushed out of the reaction vessel in a less stringent man-

ner, merely running a stream of gaseous ammonia down into the open centrifuge tube and then closing it tightly with a cork. After the oxidation with ICH_2CH_2I there separated only 0.024 mg of a strongly orange solid, which was removed by filtration and discarded. The filtrate was evaporated to dryness at <25°. The residue was washed with water, causing it to solidify. An infrared spectrum of this solid showed a peak at 5.76 μ which was about half as strong as in the starting diacetate, indicating an incomplete deacetylation. The dried solid was washed with CH_2Cl_2 and the concentrated washes were subjected to thick-layer chromatography. The strongest of the resulting bands (R_f 0.3) gave 0.200 g of light yellow crystals. Two recrystallizations from CH_2Cl_2–EtOH gave 0.101 g of cream-colored needles, mp 230–231° dec, plus 0.019 g, mp 228–230°, from the partially evaporated mother liquor. *Anal.* ($C_{18}H_{16}N_2O_6S_4$) C, H, N, S. Infrared, nmr, and tlc data showed this material to be identical with the coproduct described in the preceding experiment.

8*H*,16*H*-7a,15a-Epidithio-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-5(5aβ*H*),7,13(13aβ*H*),15-tetrone (30). A stored sample of the chromic oxide–pyridine reagent of Collins, Hess, and Frank[30] was found to be only 49% soluble in CH_2Cl_2 (at 3 ml/0.1 g) so was considered to be 49% "real." A solution of 0.252 g (0.6 mmol) of diol **26** in 5 ml of CH_2Cl_2 was stirred for 20 min at 30° with 3.78 g (7.2 mmol) of the above reagent. Solids were collected and washed with CH_2Cl_2. The filtrates were diluted with 50 ml of CH_2Cl_2, washed with water, dried ($MgSO_4$), and evaporated, finally at 0.05 mm, removing all odor of pyridine. The residue was crystallized from $CHCl_3$, dissolved in $CHCl_3$–acetone (9:1), filtered, and then "chromatographed" on 0.2 g of silica gel which had been preequilibrated overnight with the atmosphere, eluting with 5 ml of the same solvent mixture. The residue from evaporation of the eluate (at <25°) was crystallized from CH_2Cl_2–EtOH to give 0.0445 g of needles which darkened from 170° and sintered from *ca.* 217 to 230°; ir 5.85 μ (-C=CC=O); uv (CH_3CN) 254 nm (ϵ 9100). *Anal.* ($C_{18}H_{12}N_2O_6S_2 \cdot H_2O$) C, H, N, S. Earlier attempts to oxidize diol **26** to diketone **30** with Ac_2O–DMSO,[38] dicyclohexyl carbodiimide–DMSO,[39] or MnO_2[40] were not successful. Diketone **30** appeared to be especially labile. On SiO_2 tlc plates the product spot was initially colorless under ordinary light, but after irradiation under a uv lamp (254 nm) for 5 sec and then for 1 min the spot became, respectively, first yellow, then a dark tan color, when seen under ordinary light. Unirradiated tlc spots sprayed with 1% silver nitrate solution turned black immediately rather than giving the usual yellow or tan color. The above analyses also fit an unhydrated, monosulfoxide (SS=O) structure. However, a sulfoxide structure was contraindicated by the nmr spectrum, which did not show the doubling of peaks that results when the symmetrical acetylaranotin is converted to an unsymmetrical derivative.

5,5aβ,13,13aβ-Tetrahydro-13β-carbethoxy-5β-hydroxy-8*H*,16*H*-7a,15a-epidithio-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-7,15-dione (32) and 5,5aβ,13,13aβ-Tetrahydro-5β,13β-dicarbethoxy-8*H*,16*H*-7a,15a-epidithio-7*H*,15*H*-bisoxepino[3′,4′:4,5]pyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-7,15-dione (31). A solution of 250 mg (0.594 mmol) of diol disulfide **26** in 4 ml of dry pyridine was agitated at 0–5° during the dropwise addition of 500 mg (0.44 ml, 4.60 mmol) of ethyl chloroformate. A gum separated immediately. After 16 hr at 25° the mixture was evaporated to dryness and the residue was washed with water. A solution of the resulting solid in 6 ml of $CHCl_3$ was processed by thick-layer chromatography. A band at R_f 0.56 gave 35.5 mg of solid. Crystallization from CH_2Cl_2–EtOH gave 29 mg of canary-yellow crystals of **32**: mp 218–220° dec; ir 5.74, 5.85 (sh), 5.90 (sh), 5.98 μ; nmr δ 1.30 (3 H, CH_3), with a doubling of many other peak patterns giving evidence of molecular assymetry, in contrast to **32**. *Anal.* ($C_{21}H_{20}N_2O_8S_2$) C, H, N, S.

A band at R_f 0.70 gave 125 mg of solid product or 104 mg of colorless crystals of **31** after crystallization from CH_2Cl_2–EtOH: mp 229–230° dec; ir 5.73, 5.85, 5.91 (sh), 6.09 μ; $[\alpha]^{25}D$ −492 ± 0.53° ($CHCl_3$, *c* 0.376); nmr δ 1.30 (6 H, CH_3). *Anal.* ($C_{24}H_{24}N_2O_{10}S_2$) C, H, N, S.

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Structure and Antischistosomal Activity in the Nitrofurylvinyl and the Niridazole Series. Noninterchangeability of the Nitroheterocyclic Rings†

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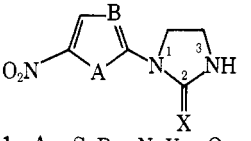
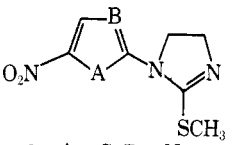
Good antischistosomal activity is shown by nitrofurylvinyl derivatives such as amides of 3-(5-nitro-2-furyl)acrylic acid (**4b** and **5b**), as well as by nitrothiazole derivatives such as niridazole (**1a**). The effects of interchanging the nitroheterocyclic groupings have been studied by the synthesis and biological comparison of five pairs of exact analogs. Replacement of nitrofuran by nitrothiazole in the nitrofurylacrylic acid amides (**4b** and **5b**) gave **4a** and **5a** and resulted in complete loss of antischistosomal activity. Substitution of nitrothiazole by nitrofuran in niridazole (**1a**) and two new active analogs **2a** and **3a** gave the exact analogs **1b**, **2b**, and **3b**, respectively, with essentially complete loss of activity. These findings are surprising in view of the close similarity of biochemical and morphological effects produced by compounds of the nitrofurylvinyl and of the niridazole series. Comparisons of partition coefficients and of nitro group oxidation potentials suggest that these factors alone cannot explain all the data, and it is suggested that subtle structural differences as well as differences in metabolism are also involved.

In previous studies[2,3] we have determined structural features which appear to be essential for antischistosomal activity of 5-nitro-2-furyl derivatives. These features comprise a nitrofuran linked *via* an olefinic bond to a terminal nitrogen substituent of low basicity. However, a nitrothiazole derivative (niridazole, **1a**) is also prominent among the relatively few nitroheterocyclic derivatives which show good schistosomicidal activity. We have already pointed out[2] that nitrofuran derivatives, such as *trans*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole (**6**, SQ 18,506) and amides of 3-(5-nitro-2-furyl)acrylic acid (*e.g.*, **4b** and **5b**), as well as the nitrothiazole **1a** exhibit the same time course and pattern of biochemical and morphological changes in schistosomes, suggesting a common mode of action. Furthermore, in spite of the dissimilar side chains borne by the nitroheterocyclic rings, comparison of models revealed striking similarities. Specifically, superimposition of the nitroheterocyclic moieties of **6** or **4b** *vs.* **1a** resulted in reasonable overlap not only of the respective terminal side-chain nitrogen substituents but also of the vinyl group of **6** or **4b** with the N_1–C_2 bond of **1a**.[4] These conclusions rested on assumptions about the preferred conformations of the compounds under discussion. These assumptions have recently been supported by X-ray crystallographic studies‡,§ with **1a** and **6**.

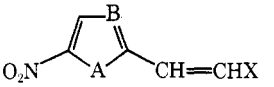
The question now arose as to the possible interchangeability of the nitroheterocyclic groupings in the nitrofurylvinyl and niridazole series. It is already known that in the nitrofurylvinyl series replacement of nitrofuran by nitrophenyl results in complete loss of activity,[4] while replacement by nitrothienyl has an adverse effect.[5] Furthermore, in the niridazole series, replacement of nitrothiazole by



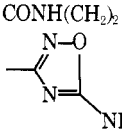
1a, A = S; B = N; X = O
b, A = O; B = CH; X = O
2a, A = S; B = N; X = S
b, A = O; B = CH; X = S



3a, A = S; B = N
b, A = O; B = CH



4a, A = S; B = N; X = $CONHCH(CH_3)_2$
b, A = O; B = CH; X = $CONHCH(CH_3)_2$
5a, A = S; B = N; X = $CONH(CH_2)_2$·c-NC_5H_{10}·HCl
b, A = O; B = CH, X = $CONH(CH_2)_2$·c-NC_5H_{10}·HCl



6, A = O; B = CH; X =

nitrophenyl and nitropyridyl similarly results in complete loss of activity.# However, no data have hitherto been available for direct comparison of the biologically effective nitrofuran and nitrothiazole groupings. Consequently, the

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‡ R. T. Puckett and B. Biffar, Ciba-Geigy Corp., unpublished data.

§ L. Amzel, Johns Hopkins University, personal communication.

= D. W. Henry and E. Bueding, unpublished data.