Sporidesmins. Part XII.[1] Isolation and Structure of Sporidesmin G, a Naturally-occurring 3,6-Epitetrathiopiperazine-2,5-dione

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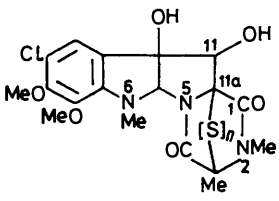
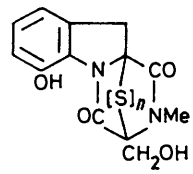
When the tail fractions from chromatograms of sporidesmin E (I; n = 3) were rechromatographed, a new crystalline metabolite of *Pithomyces chartarum* was isolated with molecular formula $C_{18}H_{20}ClN_3O_6S_4$. The new compound, named, sporidesmin G (I; n = 4), gave sporidesmin (I; n = 2) on irradiation or treatment with triphenylphosphine, and sporidesmin D (V) after reduction and methylation. It was shown that the model compound, 1,4-dimethyl-3,6-epidithiopiperazine-2,5-dione, gave the cyclic tetrasulphide on treatment with dihydrogen disulphide. Similarly, sporidesmin and sporidesmin E when treated with dihydrogen disulphide gave the new metabolite.

THE presence of metabolites of *Pithomyces chartarum* whose elemental analysis suggested S : Cl ratios >3 has been reported.[2] These compounds were found in the tail fractions of chromatograms of sporidesmin E (I; n = 3). It was easily possible to separate sporidesmin E from another sulphur-rich component by t.l.c. using benzene–ether–acetic acid (70 : 30 : 1), or light petroleum–t-butyl alcohol (25 : 1), or benzene–ethyl acetate (5 : 1),



(I)



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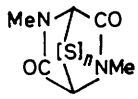

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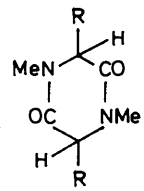

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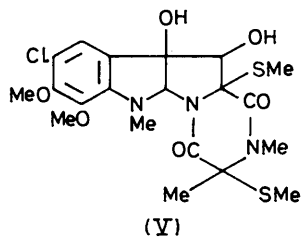

(V)

as developing solvent. However, the metabolite was only obtained in quantity by chromatography on thick-layer plates with benzene–ethyl acetate (5 : 1), followed by elution of the sulphur-rich fractions and rechromatography by use of repeated elutions with light petroleum–t-butyl alcohol (25 : 1). The sulphur-rich fraction eluted from this chromatogram was finally purified by preparative t.l.c. with chloroform as the developing solvent.

The yield of the new metabolite, named sporidesmin G, was low; about 0·25% of the yield of sporidesmin was isolated, or 0·25 ng ml^{-1} based on the original culture of *P. chartarum*. The metabolite was obtained as plates of its solvate with ether and analysis showed S, 20·9%. Its u.v. spectrum was similar to those reported for other sporidesmins[3,4] but its c.d. spectrum was different; negative molecular elipticities were observed at 253 and 276 nm, as compared to those of sporidesmin, $[\theta]_{min.}$ 233 nm,[5] and sporidesmin *E*, $[\theta]_{min.}$ 263 nm.[2] Similar changes have been reported in the dehydrogliotoxin series when the c.d. of epidithio-, epitrithio-, and epitetrathio-derivatives were determined.[6] The i.r. spectrum of the new metabolite was also similar to that of other sporidesmins; the main absorptions distinguishing it are given in the Experimental section. It was biologically active and inhibited the growth of HeLa cells[5] at 2 ng ml^{-1}, thus being slightly less active than sporidesmin (1 ng ml^{-1}) and considerably less toxic than sporidesmin E (100 pg ml^{-1}). Thus the toxicity of sporidesmin G may be due, in part, to traces of sporidesmin E not detected by other analyses. No ions containing sulphur, apart from S_8, S_7 *etc.*, were found in the mass spectrum of sporidesmin G; the ratio of abundance of the ions at m/e 411 and 409 was that of the natural abundance of ^{37}Cl and ^{35}Cl. Further work on the structure and analysis of the new metabolite was prevented by shortage of material.

It has been shown[6] that thiodehydrogliotoxin (II; n = 3), reacts readily with dihydrogen disulphide to give the epitetrathiopiperazinedione (II; n = 4). Recently Trown[7] reported the preparation of the epidithiopiperazinedione (III; n = 2) and we have repeated this work and shown that this disulphide also reacts with dihydrogen disulphide to give the tetrasulphide (III; n = 4) in 80—90% yield. The dibromide (IV; R = Br) and the dithiol (IV; R = SH) also provide the epitetrathio-compound (III; n = 4) in high yield when treated with dihydrogen disulphide or dihydrogen polysulphide.

[1] Part XI, S. Safe and A. Taylor, *J. Chem. Soc.* (*C*), 1971, 1189.
[2] R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1665.
[3] J. W. Ronaldson, A. Taylor, E. P. White, and R. J. Abraham, *J. Chem. Soc.*, 1963, 3172.
[4] W. D. Jamieson, R. Rahman, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1564.
[5] H. Herrmann, R. Hodges, and A. Taylor, *J. Chem. Soc.*, 1964, 4315.
[6] S. Safe and A. Taylor, *J. Chem. Soc.* (*C*), 1970, 432.
[7] P. W. Trown, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 402.

Since the dithiol (IV; R = SH) was obtained in almost quantitative yield by reduction of the tetrasulphide (III; $n = 4$) with sodium borohydride, the series of reactions (IV; R = Br) ⟶ (III; $n = 4$) ⟶ (IV; R = SH) ⟶ (III; $n = 2$) is the method of choice for the preparation of the latter compound. Similar conclusions have been reported recently by other workers.[8]

This work indicated that the reaction of dihydrogen disulphide with epidi- and epitri-thiopiperazinediones was probably general. It was therefore applied to sporidesmin E and the product obtained in *ca.* 40% yield had the same optical rotation, i.r. and u.v. spectra as sporidesmin G. Its R_F values in several solvents were also identical with those of the metabolite. The semi-synthetic product gave an analysis agreeing with the formula $C_{18}H_{20}ClN_3O_6S_4,C_4H_{10}O$, and its mode of preparation strongly suggested that its structure was (I; $n = 4$). Confirmation of this structure was obtained by conversion of the semisynthetic product into sporidesmin and dethiosporidesmin [1] (I; $n = 1$) photochemically [6] and by the action of triphenylphosphine.[1] It was also converted in high yield into sporidesmin D [4] (V) by reduction with sodium borohydride and methylation of the resulting dithiol.

Sporidesmin G gave a diacetate whose elemental analysis did not agree with that required by the formula $C_{22}H_{24}ClN_3O_8S_4$, probably because of its instability. However, ions at *m/e* 623 and 621 were observed in its mass spectrum in accord with this molecular formula, and like the fragmentation of other epitetrathiopiperazinediones [(II; $n = 4$); (III; $n = 4$)] ions were observed corresponding to the loss of 1, 2, 3, and 4 sulphur atoms.

The n.m.r. spectrum of sporidesmin G was closely similar to that of sporidesmin; no duplication of signals, as seen in spectra of sporidesmin E and its derivatives, was observed. The n.m.r. spectrum could not be measured at low temperatures because of the low solubility of the tetrasulphide in commonly available deuteriated solvents. At −20 °C in toluene the absorption bands in the spectrum of sporidesmin G were significantly broader and it was concluded that the free energy of conversion of the various possible conformers of the epitetrathio-group was low. The epitetrathiopiperazinedione (III; $n = 4$) also had very low solubility and a study of conformational equilibrium in this series of compounds awaits the synthesis of a suitably soluble example.

EXPERIMENTAL

U.v. spectra were measured on a Cary 14 spectrophotometer, i.r. spectra on a Perkin-Elmer 521 instrument, and n.m.r. spectra on a Varian A60A machine using tetramethylsilane as internal standard. Mass spectra were obtained by use of a Consolidated Electrodynamics Corporation 21-110B mass spectrometer with photoplate and electrical detection. C.d. measurements were made on a Cary 60 instrument. Silica gel (Merck) was used for thick- (100 × 20 cm plates, 0·8 mm thick) and thin-layer (20 × 20 cm plates, 0·3 mm thick) chromatography; bands were detected by their fluorescence when irradiated by short-wave u.v. light, and by spraying the plates with aqueous 5% silver nitrate solution. The identity of compounds from different sources was based on their i.r., u.v., and n.m.r. spectra.

Isolation of Sporidesmin G.—The gum (3 g) from the methanol phase of the crude extract of *P. chartarum* [3,9] was subjected to t.l.c. with light petroleum (b.p. 30—60°)–t-butyl alcohol (10 : 1). Sporidesmin (0·41 g) was recovered from the more polar, u.v.-fluorescent band. The u.v.-fluorescent band of greatest R_F value was rechromatographed with benzene–ethyl acetate (5 : 1). The band of greatest R_F value gave sporidesmin E (0·14 g); the more polar band was rechromatographed (multiple development) with light petroleum–t-butyl alcohol (25 : 1) until the sporidesmin G band was completely separated from the other u.v.-fluorescent bands. The sporidesmin G was rechromatographed with chloroform. *Sporidesmin G* (9-*chloro*-3,11a-*epitetrathio*-2,3,5a,6,10b,11-*hexahydro*-10b,11-*dihydroxy*-7,8-*dimethoxy*-2,3,6-*trimethyl*-11a*H*-*pyrazino*-[1′,2′:1,5]*pyrrolo*[2,3-b]*indole*-1,4-*dione*) had m.p. 148—153° (from ether at 4°) (Found: Cl, 7·4; S, 20·9. $C_{18}H_{20}ClN_3O_6S_4,C_4H_{10}O$ requires Cl, 5·8; S, 20·9%), $[\alpha]_D^{20}$ −217° (*c* 0·023 in $CHCl_3$), $\lambda_{max.}$ (MeOH) 216, 250, and 298 nm (log ε 4·65, 4·06, and 3·47), $\nu_{max.}$ ($CHCl_3$) 3570, 3520, 1690, and 1660, (KBr) 1210, 825, 725, 585, 510, 505, 480, 410, and 395 cm^{-1}.

Preparation of Sporidesmin G from Sporidesmin and Sporidesmin E.—Sporidesmin [3] (0·25 g) and hydrogen polysulphide [6] (0·5 ml) were mixed and kept for 1 h at 22°. The mixture was extracted with chloroform; the extract was concentrated and chromatographed with benzene–ethyl acetate (7 : 3). The band of greatest R_F was that of sporidesmin and sporidesmin E (0·18 g); this was eluted and treated again with hydrogen polysulphide (0·5 ml). Chromatography as before gave two bands, that of greatest R_F containing sporidesmin and sporidesmin E. The latter was eluted and the whole procedure was repeated. Material from the bands of lowest R_F from the three chromatograms was combined and recrystallised from ether, giving sporidesmin G (125 mg), m.p. 148° (Found: C, 43·8; H, 4·85; Cl, 6·15; N, 6·85; S, 20·75. Calc. for $C_{18}H_{20}ClN_3O_6S_4,C_4H_{10}O$: C, 44·0; H, 4·9; Cl, 5·8; N, 6·9; S, 20·9%), $[\alpha]_D^{22}$ −218° (*c* 0·2 in $CHCl_3$), $[\theta]_{253}$ −6850, $[\theta]_{276}$ −3920, $[\theta]_{302}$ 4900, $[\theta]_{313}$ 3400 (*c* 0·0012 in dioxan), τ ($CDCl_3$) 2·82 (1H), 4·90 (1H), 5·35 (1H), 6·10 (3H), 6·16 (3H), 6·60 (3H), 6·93 (3H), and 8·03 (3H). Sporidesmin G (0·04 g), pyridine (1 ml), and acetic anhydride (0·3 ml) were mixed and kept at 20° for 5 days. The mixture was poured into water (40 ml) and extracted with chloroform (3 × 20 ml); the extract was evaporated and the residue chromatographed with benzene–ethyl acetate (4 : 1). The band of greatest R_F was eluted and the gum obtained had *m/e* 623, 621; 591, 589; 559, 557; 527, 525; 495, 493; 435, 433; 393, 391; and 375, 373.

Conversion of Sporidesmin G into Sporidesmin D (V).—Sporidesmin G (25 mg) was dissolved in pyridine (0·5 ml) and methyl iodide (2 ml) was added. Sufficient methanol was then added for complete dissolution, then sodium borohydride (80 mg) was added during 1 h. The mixture was stirred for 4 h, poured into water (40 ml), and extracted with chloroform (3 × 20 ml). After concentration, the

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[9] J. Done, P. H. Mortimer, A. Taylor, and D. W. Russell, *J. Gen. Microbiol.*, 1961, **26**, 207.

residue was chromatographed with benzene–ethyl acetate (2 : 1). Sporidesmin D was recovered from the main band as plates (from ethanol), m.p. 106—107°.

Conversion of Sporidesmin G into Sporidesmin.— Sporidesmin G (125 mg) was dissolved in ether and the solution was kept at 22° for 24 h in the presence of fluorescent lighting. The solution was concentrated and sporidesmin G (95 mg) was recovered after chromatography [benzene–ethyl acetate (7 : 3)]. The combined sporidesmin and sporidesmin E band was separated by chromatography with light petroleum–t-butyl alcohol (9 : 1).

Conversion of Sporidesmin G to Dethiosporidesmin (I; $n = 1$).—Sporidesmin G (25 mg) was dissolved in chloroform (3 ml) and triphenylphosphine (25 mg) was added. The mixture was kept at 20° for 20 min, then concentrated and chromatographed [benzene–ethyl acetate (2 : 1)]. Dethiosporidesmin (15 mg), m.p. 72—74°, was recovered from the band of lowest R_F. When triphenylphosphine (12 mg) was used, sporidesmin E (I; $n = 3$) [2] and sporidesmin were obtained.

3,6-*Dibromo*-1,4-*dimethylpiperazine*-2,5-*dione*.— 1,4-Dimethylpiperazine-2,5-dione (K and K Laboratories) (2·84 g) in *o*-dichlorobenzene (40 ml) was treated for 1 h with a solution (40 ml) of bromine (6·4 g) in *o*-dichlorobenzene. The mixture was heated to 100° and kept at this temperature until no further hydrogen bromide was evolved. The cooled mixture was diluted with light petroleum (b.p. 30—60°; 1 l) and the solution was kept at 4° for 12 h. The dibromide (6 g), m.p. 140—143°,[9] separated from diethyl ether–chloroform as needles.

1,4-*Dimethyl*-3,6-*epitetrathiopiperazine*-2,5-*dione*.— (*a*) The above dibromide (0·35 g) and hydrogen polysulphide (1·5 g) were heated at 90° for 4 h. The cold mixture was digested several times with carbon disulphide and the final residue (0·17 g) was recrystallised from methanol. The *tetrasulphide* (III; $n = 4$) (needles) had m.p. 195—200° (from n-butyl alcohol) (Found: C, 27·1; H, 2·9; N, 10·5; S, 47·8. $C_6H_8N_2O_2S_4$ requires C, 26·9; H, 3·0; N, 10·45; S, 47·7%), $\lambda_{max.}$ (MeOH) 280 nm (log ε 3·28), $\nu_{max.}$ ($CHCl_3$) 1690, (KBr) 1410, 1305, 1255, 1210, 1030, 770, 745, 685, 560, 505, 405, and 395 cm^{-1}.

(*b*) 3,6-Dimercapto-1,4-dimethylpiperazine-2,5-dione (10 mg) and hydrogen polysulphide (0·5 g) were heated together at 90° for 2 h. The mixture was worked-up as described above to give the epitetrathiopiperazine (10 mg) (III; $n = 4$), m.p. 195—200°.

(*c*) The same product was obtained (10 mg) when the disulphide (III; $n = 2$) (10 mg) was treated under the same conditions with hydrogen polysulphide (0·5 g).

3,6-*Dimercapto*-1,4-*dimethylpiperazine*-2,5-*dione*.— The epitetrasulphide (III; $n = 4$) (0·17 g) was suspended in methanol (3 ml) and the stirred mixture was treated with sodium borohydride (0·1 g) during 1·7 h under an atmosphere of nitrogen. The mixture was stirred for 2 h, diluted with water, acidified with diluted hydrochloric acid, and extracted with chloroform (2 × 20 ml). The dried (Na_2SO_4) extract was evaporated and the residue gave the dithiol (IV; R = SH), needles (0·12 g), m.p. 110—113° (from ethanol) (Found: C, 35·2; H, 4·8; N, 13·6; S, 30·4. Calc. for $C_6H_{10}N_2O_2S_2$: C, 35·0; H, 4·85; N, 13·6; S, 31·0%).

We thank Dr. P. W. Trown for details of the preparation of 3,6-dibromo-1,4-dimethylpiperazine-2,5-dione prior to publication.

[1/1477 *Received, August 16th,* 1971]