Sporidesmins. Part X.[1] Synthesis of Polysulphides by Reaction of Dihydrogen Disulphide with Disulphides and Thiols

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The structure of sporidesmin E suggests that it is biosynthesised from sporidesmin by a sulphurising enzyme analogous to a peroxidase. Accordingly, the reaction of dihydrogen disulphide with sporidesmin, dehydrogliotoxin, and other disulphides, has been studied. Polysulphides were obtained in all cases, but the reaction of dibenzyl disulphide with dihydrogen disulphide was slow. By contrast phenylmethanethiol reacted smoothly with the reagent to give di-, tri-, tetra-, and penta-sulphides, and this oxidation appears to be general. The epitrithiadioxopiperazines were light-sensitive and were converted photolytically into equimolecular mixtures of di-, and tetra-sulphides. By use of ^{35}S-labelled polysulphides it was shown that the photolysed sulphur was that inserted by the dihydrogen disulphide.

THE isolation of sporidesmin E (I; $n = 3$) and its conversion into sporidesmin (I; $n = 2$) [1] and sporidesmin D (II) [2] established a stereochemical relationship between these metabolites of *Pithomyces chartarum*. It is known that disulphides on treatment with hydrogen peroxide provide sulphoxides of the type $\cdot S \cdot S(\rightarrow O)$.[3] Analogously it might be expected that disulphides would react with dihydrogen disulphide [equation (1)] to give

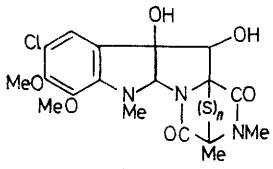
$$-S-S- + H_2S_2 \longrightarrow H_2S + -S-S(\rightarrow S)- \rightleftharpoons -S-S-S- \quad (1)$$

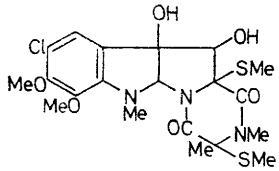
trisulphides, though this reaction has not apparently been reported. However the equilibrium between the linear and the branched trisulphide is soundly based.[4,5]

In the reaction between sporidesmin and dihydrogen disulphide, hydrogen sulphide, sporidesmin E, and possibly other polysulphides were obtained. In the case of dehydrogliotoxin (III; $n = 2$) the reaction was similar and the trisulphide (III; $n = 3$) and tetrasulphide (III; $n = 4$) were isolated. This trisulphide (III; $n = 3$) had physical properties similar to those of

[1] Part IX, R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1665.
[2] W. D. Jamieson, R. Rahman, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1564.
[3] B. Milligan and J. M. Swan, *J. Chem. Soc.*, 1965, 2901.
[4] T. Wieland and H. Schwahn, *Chem. Ber.*, 1956, **89**, 421.
[5] D. Barnard, T. H. Houseman, M. Porter, and B. K. Tidd, *Chem. Comm.*, 1969, 371.

sporidesmin E; its n.m.r. spectrum was complex and fragmentation in the mass spectrometer occurred with the sequential loss of one, two, and three sulphur atoms.


(I)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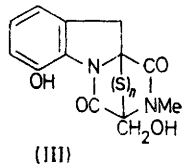
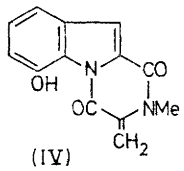
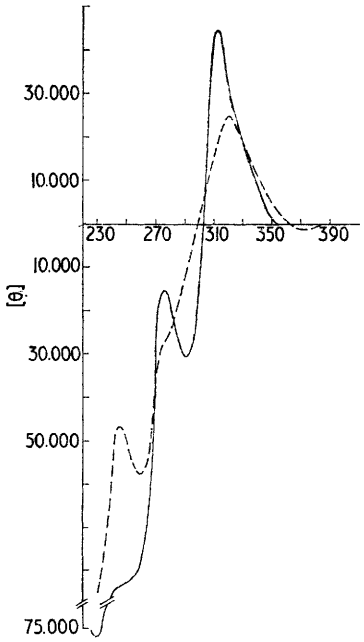

(III)


(IV)

Its c.d. curve (Figure) was closely similar to that reported [1] for sporidesmin E, showing a negative ellipticity at 260 mμ. Since the absolute configurations of the asymmetric centres of the dioxopiperazine rings in



C.d. curves for tetrasulphide (III; $n = 4$) (———) and trisulphide (III; $n = 3$) (· · · · · ·). Wavelengths are in mμ

gliotoxin and in sporidesmin [6,7] are the same, dihydrogen disulphide must react with dehydrogliotoxin and sporidesmin with retention of configuration. Thus the reaction does not involve fission or formation of C–S bonds, the donated sulphur atom being added to the S–S link as indicated in equation (1). The trisulphide (III; $n = 3$) was converted into the tetrasulphide (III; $n = 4$) when treated with dihydrogen disulphide but (see later) we were unable to obtain evidence for the presence of penta- or higher sulphides. The tetrasulphide may be regarded as analogous to S_8, and the absence of higher sulphides is surprising in view of the known stability of S_{12}.[8] The physical properties of the tetrasulphide were in accord with the structure (III; $n = 4$) postulated. Its n.m.r. spectrum indicated the presence of three aromatic protons, an *N*-methyl group, and two methylene groups. A molecular ion was observed at *m/e* 388, which fragmented with sequential loss of one, two, three, and four sulphur atoms. In the c.d. curve (Figure) a negative Cotton effect is observed at 290 mμ; thus as the chain of sulphur atoms is lengthened the negative Cotton effect shifts bathochromically by *ca.* 30 mμ per sulphur atom and its ellipticity decreases. However in the case of this tetrasulphide (III; $n = 4$) negative Cotton effects are also observed at 260 and 230 mμ. Like the trisulphide (III; $n = 3$) and dehydrogliotoxin, the tetrasulphide (III; $n = 4$) was converted in high yield into the phenol (IV) by heating in pyridine at 100°. Further evidence for the close relationship between the compounds (III; $n = 2$, 3, or 4) was that, in agreement with previous work,[9] the trisulphide (III; $n = 3$) on irradiation at 310 mμ was converted into an equimolecular mixture of dehydrogliotoxin (III; $n = 2$) and the tetrasulphide (III; $n = 4$). Under similar conditions sporidesmin E gave sporidesmin, and diphenyl disulphide was obtained from diphenyl trisulphide (see later). When the trisulphide (III; $n = 3$) (0·4 μc/mmole), synthesised from $H_2{}^{35}S_2$, was irradiated, the dehydrogliotoxin produced was only weakly radioactive (0·0015 μc/mmole), but the tetrasulphide (III; $n = 4$, 0·8 μc/mmole) was twice as radioactive as the starting material. These results show that the trisulphide behaves as a sulphurating reagent [equation (2)].

$$R_2S_3 + R_2S_3 \xrightarrow{h\nu} R_2S_4 + R_2S_2 \qquad (2)$$

Such a suggestion is in accord with similar well-charted reactions of alkyl trisulphides.[9,10]

This ready reaction of dihydrogen disulphide with sporidesmin and dehydrogliotoxin led us to examine briefly its reaction with diphenyl disulphide and dibenzyl disulphide. In the former case the reaction was similar to that in the case of sporidesmin, except that it was shown by reversed phase partition chromatography that penta- and possibly hexa-sulphides were formed in addition to diphenyl tri- and tetra-sulphides. In contrast, little or no reaction was observed when dibenzyl disulphide or cystine was treated with the reagent. Thus, although the reaction of dihydrogen disulphide with disulphides may be regarded as another example of the well known thermal equilibrium–' dis-

[6] H. Herrmann, R. Hodges, and A. Taylor, *J. Chem. Soc.*, 1964, 4315.
[7] A. F. Beecham, J. Fridrichsons, and A. McL. Mathieson, *Tetrahedron Letters*, 1966, 3131.
[8] M. Schmidt, G. Knippschild, and E. Wilhelm, *Chem. Ber.*, 1968, **101**, 381.
[9] S. F. Birch, T. V. Cullum, and R. A. Dean, *J. Inst. Petroleum*, 1953, **39**, 206.
[10] E. N. Gur'yanova, Ya. Syrkin, and L. S. Kuzina, *Doklady Akad. Nauk S.S.S.R.*, 1952, **86**, 107; T. L. Pickering, K. J. Saunders, and A. V. Tobolsky, *J. Amer. Chem. Soc.*, 1967, **89**, 2364.

proportionation' of mixtures of polysulphides,[10] it differs in one respect: its rate is greatly dependent on the structure of the organic residues.

Such discrimination was not observed when thiols were treated with dihydrogen disulphide. Benzenethiol and phenylmethanethiol readily gave mixtures of polysulphides, and the n.m.r. spectra of the latter[11] showed that the ratio of di-, tri-, tetra-, and penta-sulphides was 2 : 10 : 7 : 4.

The ready reaction of sporidesmin with dihydrogen disulphide suggests that sporidesmin E might be similarly biosynthesised. Such a biosynthetic reaction would, by chemical analogy, be like that of a peroxidase. Like sporidesmin E[12] the compounds (III; $n = 3$ or 4) were biologically active, with the trisulphide having the greatest activity. Studies of the mode of action of these compounds should be facilitated by this preparation of isotopically marked species.

EXPERIMENTAL

Spectroscopic measurements were obtained as described in previous papers in this series. Glass plates (20 × 100 cm.) and silica gel (Merck, 0·8 mm. thick) were used for preparative t.l.c. Reversed phase partition chromatography was run on cellulose plates (Eastman) impregnated with paraffin oil, with methanol–water (9 : 1) as developing solvent. Radioactivity was measured with a Nuclear-Chicago scintillation counter; samples (1 mg.) were dissolved in toluene (15 ml.) containing diphenyloxazole (75 mg.).

Dihydrogen Disulphide.—Dihydrogen polysulphide was prepared from sodium sulphide hydrate (453 g.), sulphur (87 g.), and water (400 ml.).[13] The sulphide (90 g.) was cracked,[14] and the disulphide (20 g.) (Found: H_2S, 51·2. H_2S_2 requires H_2S 51·2%) was collected.

Sporidesmin E.—A mixture of sporidesmin benzene solvate[15] (125 mg.), carbon disulphide (5 ml.), and dihydrogen disulphide (0·4 ml.) was kept for 40 min. at 22°. The reaction was exothermic and hydrogen sulphide was evolved. The mixture was treated with acetone (4 ml.), then evaporated, and the residue was digested with chloroform. Preparative t.l.c.[1] of the concentrated chloroform solution gave sporidesmin E etherate (82 mg.) [Found: S, 16·2%; *M*, 582 (cryoscopic). Calc. for $C_{18}H_{20}ClN_3O_6S_3,C_4H_{10}O$: S, 16·6%; *M*, 579); its i.r., n.m.r., and mass spectra were identical with those of the natural product.[1]

Reaction of Dehydrogliotoxin with Dihydrogen Disulphide.—Dehydrogliotoxin (III; $n = 2$) (100 mg.),[16] carbon disulphide (5 ml.), and dihydrogen disulphide (0·2 ml.) were heated under reflux for 1 hr. Acetone (5 ml.) was added, the mixture was evaporated, and the residue was triturated with tetrahydrofuran. The solution was subjected to preparative t.l.c. in benzene–ethyl acetate (8 : 1). The band of greatest R_F gave dehydrogliotoxin[16] (8 mg.); the band of intermediate R_F gave 1,2,3,4,10,10a-*hexahydro*-3-*hydroxymethylene*-6-*hydroxy*-2-*methyl*-1,4-*dioxo*-3,10a-*epitrithiopyrazino*[1,2-a]*indole* as plates (20 mg.) [from ether–tetrahydrofuran (9 : 1)], m.p. 172—174° (Found: C, 44·3; H, 3·7; N, 8·2; S, 27·5. $C_{13}H_{12}N_2O_4S_3$ requires C, 43·8; H, 3·4; N, 7·9; S, 27·0%), $\nu_{max.}$ ($CHCl_3$) 3575, 1695, 1655, and 1610 cm.$^{-1}$, $[\theta]_{320}$ 25,400, $[\theta]_{260}$ −58,100 (*c* 0·00014 in dioxan), *m/e* 356, 324, 292, 260 (256, S_8), 242, and 159. The band of lowest R_F (72 mg.), eluted with ether, gave the *tetrasulphide* (III; $n = 4$) as colourless needles, m.p. 90—93° [from carbon tetrachloride–chloroform (10 : 1)] (Found: C, 31·3; H, 2·6; N, 5·0; S, 23·7. $C_{13}H_{12}N_2O_4S_4,CCl_4$ requires C, 31·0; H, 2·6; N, 5·2; S, 23·6%), $\nu_{max.}$ ($CHCl_3$) 3595, 1675, 1650, and 1610 cm.$^{-1}$, $[\theta]_{313}$ 44,000, $[\theta]_{290}$ −30,400, $[\theta]_{260}$ −81,000, $[\theta]_{230}$ −176,000 (*c* 0·00008 in dioxan), *m/e* 388, 356, 292, 260, (256, S_8), 242, and 159. A reaction on the same scale with the crude dihydrogen polysulphide gave the epitrithio-compound (III; $n = 3$) (20 mg.) and the epitetrathio-compound (III; $n = 4$) (70 mg.).

Conversion of the Epitrisulphide (III; n = 3) *into its Tetrasulphide Derivative.*—The epitrisulphide (III; $n = 3$) (4 mg.), carbon disulphide (1 ml.), and dihydrogen disulphide (0·03 ml.) were heated under reflux for 1 hr. The mixture was treated as before and the epitetrathiodioxopiperazine (III; $n = 4$) (2 mg.) was isolated.

Reaction of Diphenyl Disulphide with Dihydrogen Disulphide.—Diphenyl disulphide (45 mg.), carbon disulphide (1 ml.), and dihydrogen disulphide (0·5 ml.) were heated under reflux for 1 hr. in the dark. Acetone (5 ml.) was added and the mixture was kept in the dark for 1 hr. and evaporated; the residue was digested with ether. Preparative t.l.c. of the solution (Woelm neutral alumina) in light petroleum (b.p. 30—60°) at 0° in the absence of light gave diphenyl disulphide (31 mg.) (from the band of highest R_F) and diphenyl polysulphide (15 mg.) (from the band of slightly lower R_F). Repetition of the reaction with the recovered diphenyl disulphide gave diphenyl polysulphide (10 mg.). Reversed phase partition chromatography of this polysulphide mixture as before, showed the presence of at least four components.

Oxidation of Benzenethiol with Dihydrogen Polysulphide.—Benzenethiol (45 mg.), carbon disulphide (1 ml.), and dihydrogen polysulphide (0·5 ml.) were heated under reflux for 10 min. in the dark. The mixture was treated as in the reaction with diphenyl disulphide, and the latter compound (16 mg.) and diphenyl polysulphide (28 mg.) were obtained.

Oxidation of Phenylmethanethiol with Dihydrogen Polysulphide.—Phenylmethanethiol (150 mg.), carbon disulphide (3 ml.), and dihydrogen polysulphide (0·3 ml.) were heated under reflux for 16 hr. Acetone (5 ml.) was added, the mixture was evaporated, and the residue was chromatographed on silica gel. The polysulphide band (100 mg.) was eluted and rechromatographed by reversed phase partition chromatography as before.

Photolysis of the Epitrithiodioxopiperazine (III; n = 3).—The trisulphide (III; $n = 3$) (75 mg.) in tetrahydrofuran (10 ml.) was photolysed for 2 min. in a Srinivasan reactor ($\lambda_{max.}$ 310 mμ). The solution was evaporated and the products were separated by chromatography as before. Dehydrogliotoxin (III; $n = 2$) (12 mg.)[16] was isolated

[11] J. R. Van Wazer and D. Grant, *J. Amer. Chem. Soc.*, 1964, **86**, 1450.
[12] D. Brewer, R. Rahman, S. Safe, and A. Taylor, *Chem. Comm.*, 1968, 1571.
[13] K. H. Butler and O. Maass, *J. Amer. Chem. Soc.*, 1930, **52**, 2184.
[14] F. Fehér, W. Laue, and G. Winkhaus, *Z. anorg. Chem.*, 1956, **288**, 113.
[15] J. W. Ronaldson, A. Taylor, E. P. White, and R. J. Abraham, *J. Chem. Soc.*, 1963, 3172.
[16] G. Lowe, A. Taylor, and L. C. Vining, *J. Chem. Soc.* (*C*), 1966, 1799.

from the band of highest R_F and unchanged starting material (39 mg.) from the intermediate band. The tetrasulphide (III; $n = 4$, 12 mg.) was obtained from the most polar band.

Photolysis of Diphenyl Polysulphide.—Diphenyl polysulphide (20 mg.) in diethyl ether (5 ml.) was illuminated with artificial fluorescent light for 18 hr. Chromatography as before gave diphenyl disulphide (8 mg.), m.p. 60—61°.

1,2,3,4-*Tetrahydro*-6-*hydroxy*-2-*methyl*-3-*methylene*-1,4-*dioxopyrazino*[1,2-a]*indole*.—(*a*) The trisulphide (III; $n = 3$) (30 mg.) in pyridine (0·5 ml.) was heated at 100° for 30 min. The mixture was diluted with water (15 ml.), and then extracted with chloroform. The extract was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated, and the residue gave the phenol (IV),[16] m.p. 218—219° (from ether) (22 mg.), with physical properties identical to those of the product synthesised from 7-methoxyindole-2-carboxylic acid.

(*b*) The tetrasulphide (III; $n = 4$) (20 mg.) under the same conditions gave the phenol (IV) (14 mg.), m.p. 218°.

(*c*) Dehydrogliotoxin (III; $n = 2$) (20 mg.) treated similarly gave the phenol (IV) (16 mg.).

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