Sporidesmins. Part 17.[1] Isolation of Sporidesmin H and Sporidesmin J

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Two new sporidesmins have been isolated from the polar constituents of culture extracts of *Pithomyces chartarum*. One of these, named sporidesmin H, is possibly a 3-cholorindoline (1) and the other, sporidesmin J, is shown to be de-N^6-methylsporidesmin (2).

DURING fractionation of extracts of cultures of *P. chartarum* described in earlier reports in this series of papers [2-4] it was noticed that polar fractions were biologically active,[5] and catalysed the conversion of azide to nitrogen by iodine.[6] The preparation of a quantity of sporidesmin for use as a relay in the synthetic studies of Kishi *et al.*[7] gave an opportunity to examine these polar fractions in greater detail. When crude extracts [2] were eluted from silica gel with a gradient of ethyl acetate in benzene, fractions were obtained, in increasing polarity, containing: sporidesmin B,[2] sporidesmin,[2] sporidesmins E[3] and G,[4] sporidesmin D,[8] and finally mixtures of depsipeptides. These peptides were largely insoluble in ether, and the ether-soluble, biologically active [5] materials were shown to contain at least two new epipolythiodioxopiperazines [9] by preparative layer chromatography (p.l.c.). The most polar of these, named sporidesmin J, was produced by the fungus in *ca.* 1% of the quantity of spirodesmin.[5] It was crystalline and its elemental analysis and mass spectrum indicated a molecular formula $C_{17}H_{18}ClN_3O_6S_2$. It was, therefore, a demethylsporidesmin. The mass spectrum of sporidesmin J had an abundant ion $C_{10}H_{10}ClNO_3^+$, but the ion $C_{11}H_{12}ClNO_3^+$ present in all other sporidesmin mass spectra [10] was absent. Hence the absent methyl group was one normally located in the indoline moiety. The ^1H n.m.r. spectrum showed that the N-CH_3 signal at $\delta_H \simeq 3.0$, assigned to the indoline nitrogen methyl [11] in the spectrum of sporidesmin, was absent and a new exchangeable proton (δ_H 5.15) coupled to the bridgehead proton (δ_H 5.67) was present. Thus sporidesmin J is de-N^6-methylsporidesmin, a conclusion confirmed by its conversion into diacetylsporidesmin [2] upon acetylation and methylation.

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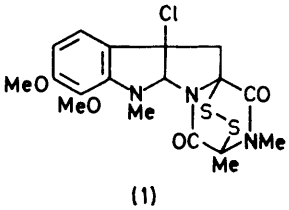
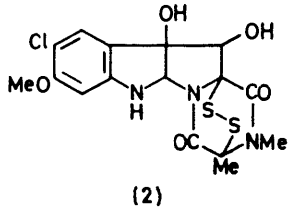
[11] J. W. Ronaldson, *Austral. J. Chem.*, 1975, **28**, 2043; 1976, **29**, 2307.

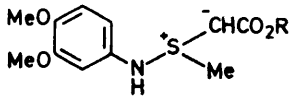
A second component from the p.l.c. separation was not obtained pure. The best samples of this material, named sporidesmin H, inhibited the growth of He La cells [5] at 3×10^{-9} g/ml. Elemental analysis of the amorphous solid gave sulphur : chlorine ratios of 2 : 1, but did not correspond to a theoretical empirical formula, possibly because of the instability of the metabolite. In the mass spectrum of sporidesmin H, ions at *m/e* 443 and 441 were observed. These ions were of low abundance but their relative abundances were those calculated for ions of the elemental compositions: $C_{18}H_{20}{}^{35}ClN_3O_4S_2$ and $C_{18}H_{20}{}^{37}ClN_3O_4S_2$. An abundant ion $C_{18}H_{17}N_3O_4{}^+$ was observed in this spectrum. The formation of such an ion from $C_{18}H_{20}ClN_3O_4S_2$ requires the loss of the elements H_3ClS_2. The loss of the elements H_2S_2 from the molecular ions of epidithiodioxopiperazines is well documented [10] but the loss of HCl has not been recorded in the sporidesmin series. As in the mass spectra of all epidithiodioxopiperazines, that of sporidesmin H contained abundant ions corresponding to $S_8{}^+$, $S_7{}^+$, and $S_6{}^+$, *etc.* No absorption bands were found in the 3 600—3 200 cm^{-1} region of the i.r. spectrum of sporidesmin H and no exchangeable protons (with D_2O) were observed in its ^1H n.m.r. spectrum. In the latter spectrum signals assigned to a C-Me group, 2 NMe groups, 2 OMe groups, a bridgehead proton, and a methylene group similar to that found in sporidesmin B [2] were seen. The remaining two protons were unique in the sporidesmin series. Their chemical shifts (δ_H 6.98 and 6.43) and coupling constant (J = 8.5 Hz) showed them to be *ortho* hydrogens on the aromatic ring. There are, therefore, three possibilities for the orientation of these protons in sporidesmin H. We were unable to degrade the metabolite to the corresponding isatin, and thus establish this orientation. However, 6,7-dimethoxy- and 4,7-dimethoxy-3-methylthio-1*H*-indol-2-ones were synthesised in *ca.* 50% yield by Gassman's [12] method. In addition cyclisation of 4-aminoveratrole gave not only 5,6-dimethoxy-3-methylthio-1*H*-indol-2-one (50%) but



(1)



(2)



(3)

also the product from the alternative mode of rearrangement of the intermediate (3), *i.e.* 4,5-dimethoxy-3-methylthio-1*H*-indol-2-one (<1%). Thus compounds having the three possible orientations of methoxy-groups in sporidesmin H were available. The n.m.r. spectra of these compounds permitted assignment of the signals of their aromatic protons (see Table), and especially that

Chemical shifts of aromatic protons in ^1H n.m.r. spectra of dimethoxy-3-methylthio-1*H*-indol-2-ones and in sporidesmin H

Methoxy-substituents	δ_{H_4}	δ_{H_5}	δ_{H_6}	δ_{H_7}
4,5			6.82	6.58
5,6	6.98			6.62
4,7		6.32	6.80	
6,7	7.05	6.60		
Sporidesmin H	6.98	6.43		
Sporidesmin J	7.14			

of the proton at position 4 which was 0.2 p.p.m. downfield of the others. It follows that sporidesmin H also has a proton at position 4 (10) and that the orientation of the methoxy-groups is probably the same as in all other sporidesmins. 6,7-Dimethoxy-3-methylthio-1*H*-indol-2-one differed from the other 1*H*-indol-2-ones because it did not have an absorption band at 310 nm—a characteristic shared by sporidesmin H. All these data and biogenetic considerations [1,13] are consistent with structure (1) for sporidesmin H.

EXPERIMENTAL

U.v. spectra were recorded on a Cary 14 instrument, and i.r. spectra on a Perkin-Elmer 237 spectrometer. Mass spectra were obtained by direct introduction of the sample into the source of a Dupont 21-110B mass spectrometer. Precise mass measurements were obtained by the peak matching method by comparison to an ion in the spectrum of perfluorokerosene. ^1H N.m.r. spectra were recorded using C^2HCl_3 as a solvent, on Varian A-60-A and HA-100 instruments. All chemical shifts are reported in p.p.m. downfield from the signal of tetramethylsilane; the letter e in the n.m.r. data indicates that the proton was exchangeable. Silica gel (Merck), layers 1-mm thick supported by glass plates 1 m × 20 cm, was used for p.l.c.

Isolation of Sporidesmin H and Sporidesmin J.—The lipid-free methanol extract [2] (18 g, *ca.* 6 g mixed sporidesmins) was dissolved in benzene (200 ml) and applied to a silica gel (2 kg) column (100 mesh, diam. 7 cm). The column was developed with benzene–ethyl acetate (4 : 1), the ethyl acetate concentration being increased by 1% as each 2 l of eluant ran through the column. The first 37.6 l passed through the column contained no sporidesmins, the next 2.5 l contained mostly sporidesmin B contaminated with sporidesmin, and the following 1.6 l gave sporidesmin (4 g). Further elution with 2.4 l of the solvent gave a mixture of sporidesmin and sporidesmin E. The next 5.6 l contained traces of a sporidesmin G and sporidesmin D. The following 3 l, on evaporation, gave a gum (2.5 g) which was triturated with ether (5 × 20 ml) and the ethereal solutions combined, concentrated, and applied to a preparative layer plate, which was developed with chloroform–acetic acid (49 : 1). The band of lowest R_F

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[13] D. R. Morris and L. P. Hager, *J. Biol. Chem.*, 1966, **241**, 1763.

(detected by u.v. absorption and aqueous $AgNO_3$ [9]) was eluted from the silica gel (EtOAc), the eluate evaporated, and the residue, recrystallised from ether gave *sporidesmin J* (2) (21 mg), m.p. 168—169 °C (Found: C, 44.6; H, 4.2; Cl, 7.8; N, 8.7; S, 13.3. $C_{17}H_{18}ClN_3O_6S_2$ requires C, 44.4; H, 3.95; Cl, 7.7; N, 9.1; S, 13.9%), $[\alpha]_D^{18}$ +43° (*c*, 0.25, $CHCl_3$), *m/e* 459.032 5 ($C_{17}H_{18}ClN_3O_6S_2$ requires 459.032 6), 397, 395, 256, 229, 227.034 5 ($C_{10}H_{10}{}^{35}ClNO_3$ requires 227.034 9), δ 7.14 (H), 5.67 (H, J = 4 Hz), 5.17 (H, e, J = 1.5 Hz), 5.05 (H, e), 4.74 (H, J = 1.5 Hz), 3.88 (3 H), 3.85 (3 H), 3.06 (3 H), 2.03 (3 H), and 1.74 (H, e). The band ($AgNO_3^+$) of greatest R_F was eluted similarly from the silica gel, the ethyl acetate eluate concentrated, and the solution (1 ml) treated with isopropyl ether (5 ml). *Sporidesmin H* (1) separated from diethyl ether–light petroleum (b.p. 40—60 °C) as a colourless amorphous solid (34 mg), m.p. 150—152 °C, *m/e* 443, 441, 339.120 7 ($C_{18}H_{17}N_3O_4$ requires 339.121 9), 256, and 176; $\lambda_{max.}$ (MeOH) 216, 252, and 290 nm (log ε 4.37, 4.05, and 3.81); $\nu_{max.}$ (KBr) 1 700 cm^{-1}; δ 6.98 (H, J = 8 Hz), 6.43 (H, J = 8 Hz), 5.38 (H), 3.83 (3 H), 3.75 (3 H), 3.30 (3 H), 3.27 (H, J = 16 Hz), 3.03 (3 H), 2.70 (H, J = 16 Hz), and 2.02 (3 H).

Sporidesmin Diacetate.—Sporidesmin J (7 mg) in pyridine (0.1 ml) was treated with acetic anhydride (0.05 ml) and the solution kept at 4 °C for 3 days. The volatile components of the mixture were evaporated at 20 °C and the residue [$\nu_{max.}$ (KBr) 1 740 cm^{-1}; *m/e* 481, 479, 361, 359, 228] treated with dimethyl sulphate (0.1 ml) at 95 °C for 2 min. The reaction mixture was applied to a p.l.c. plate (20 cm × 20 cm) and sporidesmin diacetate (3 mg), m.p. 185—187 °C, isolated in the usual way.

4,5-Dimethoxy- and 5,6-Dimethoxy-3-methylthio-1H-indol-2-ones.—Chlorine (2 ml) was diluted at −70 °C with dichloromethane (100 ml) and the solution treated with a solution (20 ml) of ethyl methylthioacetate (5.9 g) in dichloromethane during a period of 1.5 h. The resulting colourless solution was stirred at −70 °C while a solution (50 ml) of 4-aminoveratrole (13.5 g) in dichloromethane was added during 0.75 h. After a further 2 h at −70 °C, triethylamine (10 ml) was added and the mixture was stirred for 1 h at −70 °C when the reaction mixture was allowed to warm to room temperature. The dichloromethane was evaporated at water pump pressure, ether (150 ml) was added to the residue, and the mixture was treated with dilute hydrochloric acid (2N; 20 ml) and then stirred overnight at room temperature. The precipitate (A) was washed with water and ether, and the ether phase separated from the aqueous phase which was extracted (× 2) with ether. The combined ethereal extracts were washed with water, dried (Na_2SO_4), filtered, and evaporated. The residue was taken up in hot ethyl acetate (10 ml), kept at 4 °C for 18 h, and the crystals that separated (B) collected. The mother liquors were evaporated, the residue taken up in toluene (10 ml), and the solution carefully applied to the top of a silica gel column (Merck, silica gel for thin layer chromatography (254); 25 × 4 cm, packed as a slurry in toluene). The column was developed with toluene (50 ml) and then toluene–ethyl acetate (1 : 1) until a pale yellow band was eluted (8 l) when fractions (11 ml) were collected. T.l.c. of the fractions showed the presence of 1*H*-indol-2-ones (by heating the plates in air, or with u.v. radiation) in fractions 15—20 and 31—37 (C). Fractions 15—20 were combined, evaporated, and the residue (100 mg) recrystallised from toluene (5 ml). *4,5-Dimethoxy-3-methylthio-1H-indol-2-one* separated from ethanol as colourless needles, m.p. 146—147 °C (90 mg; 0.8%) (Found: C, 54.0; H, 5.7; S, 13.1. $C_{11}H_{13}NO_3S{\cdot}0.25H_2O$ requires C, 54.2; H, 5.5; S, 13.1%), $\lambda_{max.}$ (MeOH) 258 and 310 nm (log ε 3.89 and 3.40), $\nu_{max.}$ (KBr) 1 715, 1 630, 1 490, 1 255, 1 060, and 790 cm^{-1}; *m/e* 239, 224, 192 (*m* ca.* 155, $239^+ \longrightarrow 192^+ + 47$), 177 (*m* ca.* 163, $192^+ \longrightarrow 177^+ + 15$); δ 8.43 (H, e), 6.82 (H, J = 8 Hz), 6.58 (H, J = 8 Hz), 4.36 (H), 4.00 (3 H), 3.83 (3 H), and 2.07 (3 H). The crystalline precipitates (A), (B), and (C) were combined (5.2 g, 49%) and recrystallised from toluene (100 ml) as needles (m.p. 156 °C) and rhombs (m.p. 162 °C) having identical i.r. spectra. *5,6-Dimethoxy-3-methylthio-1H-indol-2-ones* separated from ethanol as colourless dihedral rhombs, m.p. 162 °C (Found: C, 55.2; H, 5.6; N, 5.8; O, 19.9; S, 13.4. $C_{11}H_{13}NO_3S$ requires C, 55.2; H, 5.4; N, 5.9; O, 20.1; S, 13.4%), $\lambda_{max.}$ (MeOH) 220, 270, and 300 nm (log ε 4.43, 3.83, and 3.56); $\nu_{max.}$ (KBr) 1 730, 1 635, 1 510, 1 205, 1 120, and 825 cm^{-1}; *m/e* 239, 192, and 177; δ 9.30 (H, e) 6.98 (H), 6.62 (H), 4.27 (H), 3.88 (6 H), and 1.97 (3 H).

6,7-Dimethoxy-3-methylthio-1H-indol-2-one.— 2,3-Dimethoxyaniline hydrochloride [14] (8.3 g) was suspended in dichloromethane (100 ml) and the stirred suspension treated at 0 °C with a solution (30 ml) of sodium hydroxide (2 g) in water. The dichloromethane solution was separated, washed with water, dried (Na_2SO_4), and added at −70 °C to a solution of ethyl methylsulphenylacetate chloride, prepared from chlorine (1 ml) and ethyl methylthioacetate (2.95 g) as described in the previous paragraph. After 1 h at −65 °C triethylamine (5 ml) was added to the mixture which was then stirred for 0.5 h at −60 °C. It was then allowed to warm to room temperature when the dichloromethane was evaporated off and the residue mixed with ether (50 ml) and dilute hydrochloric acid (2N; 10 ml). The mixture was stirred for 18 h when the precipitate (A) (2.5 g) was collected. The ethereal phase was separated from the filtrate and the raffinate extracted with ether. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue (2 g) was combined with the precipitate (A), taken up in hot toluene (50 ml), and the yellow needles (2.79 g, 48%), m.p. 173—176°, that separated on cooling, were collected. *6,7-Dimethoxy-3-methylthio-1H-indol-2-one* separated from ethanol as colourless needles, m.p. 177—178 °C (Found: C, 55.1; H, 5.7; N, 5.9; S, 13.4%), *m/e* 239, 192, 177, 149, and 134; $\lambda_{max.}$ (MeOH) 225, 256sh, and 310sh nm (log ε 4.01, 3.38, and 2.36); $\nu_{max.}$ (KBr) 1 715, 1 640, 1 510, 1 460, 1 080, and 715 cm^{-1}; δ 8.37 (H, e), 7.05 (H, J = 8 Hz), 6.60 (H, J = 8 Hz), 4.26 (H), 3.89 (6 H), and 2.08 (3 H).

4,7-Dimethoxy-3-methylthio-1H-indol-2-one.—This 1*H*-indole-2-one was prepared in the same way as the 6,7-dimethoxy-isomer starting from chlorine (1.65 ml), ethyl methylthioacetate (4.8 g), 2,5-dimethoxyaniline (11 g), and triethylamine (8.2 ml). After the reaction mixture had warmed to room temperature it was washed with water (2 × 100 ml), evaporated, and the residue treated with ether (100 ml) and dilute hydrochloric acid (2N; 16 ml). The crude 1*H*-indol-2-one (6.1 g, 65%, m.p. 168—172 °C) recrystallised from toluene as orange prisms and colourless needles both melting at 172—173 °C. The mixed crystals (0.24 g) were stirred with ethanol (30 ml) and alumina (Woelm, basic, 1 g) for 30 min, and the mixture heated to boiling and filtered hot. *4,7-Dimethoxy-3-methylthio-1H-indol-2-one* separated from the filtrate as colourless needles

[14] R. Hodges and A. Taylor, *J. Chem. Soc.*, 1964, 4310.

(0.2 g), m.p. 177—179 °C (Found: C, 55.1; H, 5.5; N, 5.9; O, 20.2; S, 13.4%), *m/e* 239, 192, 177, 150, 149, and 134; $\lambda_{max.}$ (MeOH) 251sh and 312 nm (log ε 3.46 and 3.71), $\nu_{max.}$ 1 700, 1 510, 1 260, 1 080, and 780 cm^{-1}; δ 7.93 (H, e), 6.80 (H, J = 9 Hz), 6.32 (H, J = 9 Hz), 4.32 (H), 3.85 (3 H), 3.82 (3 H), and 2.11 (3 H).

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