Sporidesmins. Part VIII.[1] Isolation and Structure of Sporidesmin-D and Sporidesmin-F

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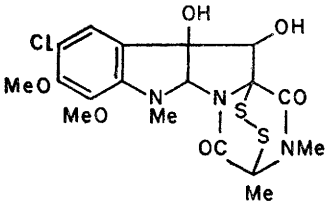
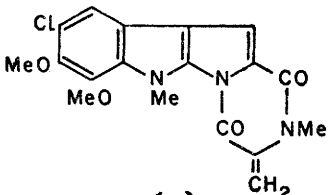
Chromatography of crude extracts of cultures of *Pithomyces chartarum* on silicic acid has been shown to separate the biologically active sporidesmins from a group of inactive metabolites. One of these inactive compounds has been purified and shown to be the dimethyl thioether analogue of sporidesmin, named spordesmin-D. Evidence is presented for the structure of another inactive metabolite, sporidesmin-F, which has not been obtained in crystalline form. Sporidesmin has been converted into sporidesmin-D by treatment with sodium borohydride and methyl iodide.

It has been reported [2] that chromatography on silicic acid is a convenient means of purification of sporidesmin (I). Further examination of crude extracts [2] (containing about 40% sporidesmin) on this adsorbent has led to the isolation of a number of new sporidesmin-like metabolites



(I)



(II)

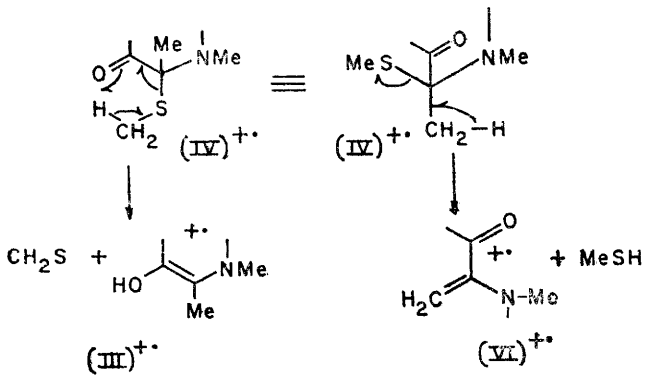
of *Pithomyces chartarum*. One of these, named sporidesmin-D, was eluted after the sporidesmin-containing fractions when the extract was chromatographed on silicic acid in the solvent, benzene–ether–acetic acid (44 : 6 : 1). Elemental analysis of sporidesmin-D suggested the empirical formula $C_{20}H_{26}ClN_3O_6S_2$ and the presence of solvent of crystallization (diethyl ether or ethanol). Signals were observed in the ^1H n.m.r. spectrum that could be assigned to such solvents. When the metabolite was introduced directly into the mass spectrometer ion source, partial sublimation at about 100° removed the solvent of crystallization, which was identified by its spectrum. The solvents thus identified (ethanol, ether) were those from which sporidesmin had been obtained in unsolvated form.[2] After sublimation of the solvent the total ion current decreased to zero. Further heating of the sample to about 163° enabled us to obtain the mass spectrum of the metabolite; the precise mass of the molecular ion corresponded to that required for the empirical formula.

The n.m.r. signals at τ 6·05, and 6·95 were not observed after deuteriation of sporidesmin-D, which also formed a diacetate ($\nu_{max.}$ 1765 and 1750 cm.$^{-1}$, τ 8·15 and 8·37), thus demonstrating the presence of two hydroxy-groups. In the ethanolate, the proton with signal at τ 6·95 (CHO*H*) was coupled to the proton with signal at τ 5·35 (C*H*OH, *J* 3 Hz), but such coupling was not observed in the non-solvated molecule. This result is in accord with our inability to obtain solvates of esters of

[1] Part VII, R. Hodges, J. S. Shannon, and A. Taylor, *J. Chem. Soc.* (*C*), 1966, 1823.

[2] J. W. Ronaldson, A. Taylor, E. P. White, and R. J. Abraham, *J. Chem. Soc.*, 1963, 3172.

sporidesmins, or of sporidesmin-B, which lacks a secondary hydroxy-group.[2] Sporidesmin-D diacetate was smoothly converted into anhydrodesthiosporidesmin [3] (II) with boron trifluoride–ether complex. Thus the same carbon–nitrogen nucleus was present in sporidesmin as in sporidesmin-D. Sporidesmin-D was biologically inactive, suggesting that the disulphide bridge present in sporidesmin (I) was modified in the new metabolite.[4] The nature of this modification was evident from the ^{1}H n.m.r. spectrum of sporidesmin-D, which had all the signals found in the spectrum of sporidesmin and in addition, two signals, each equivalent to three protons, at τ 7·60 and 7·68. It was therefore assumed, in agreement with the analytical data, that sporidesmin-D was the dimethyl thioether analogue of sporidesmin, *i.e.* (IV). Support for this assumption was obtained from the mass spectrum of sporidesmin-D. Ions were observed at *m/e* 457, 455, 411, and 409 corresponding to the loss of two methylthio-groups. The peak observed at *m/e* 457 could be resolved into signals for the two ionic species $C_{19}H_{22}[^{37}Cl]N_3O_6S$ and $C_{19}H_{24}[^{35}Cl]N_3O_6S$. The peak at *m/e* 409 could also be similarly resolved and the metastable peak *m** *ca.* 368



showed that the ion reaction $C_{19}H_{22}[^{35}Cl]N_3O_6S \longrightarrow C_{18}H_{20}[^{35}Cl]N_3O_6 + CH_2S$ occurred. These facts indicate that the methylthio-groups are lost by two different ion reactions: $(IV^+) \longrightarrow (III^+) + CH_2S$, and $(IV^+) \longrightarrow (VI^+) + MeSH$, possibly by the mechanisms indicated. It has been shown recently [5] that aranotin (VII) also occurs with its dimethyl thioether analogue, and the reported chemical shifts of the methylthio-signals of the latter are similar to those of sporidesmin-D.

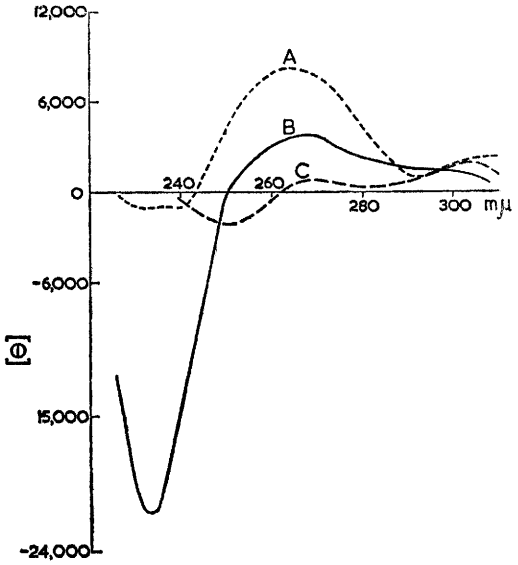
The indole tetrasulphide (VIII) is converted into its dimethyl thioether when treated with lithium aluminium hydride in the presence of methyl iodide.[6] In our hands, sporidesmin, its monoacetate, and its diacetate were converted in high yield into sporidesmin-D and its mono- and di-acetates, respectively, when treated with methyl iodide and sodium borohydride in pyridine. Hence the formula (IV) is proposed for sporidesmin-D.

* The c.d. curve for sporidesmin shown in an earlier publication [7] was incorrectly drawn.

[3] R. Hodges, J. W. Ronaldson, J. S. Shannon, A. Taylor, and E. P. White, *J. Chem. Soc.*, 1964, 26.

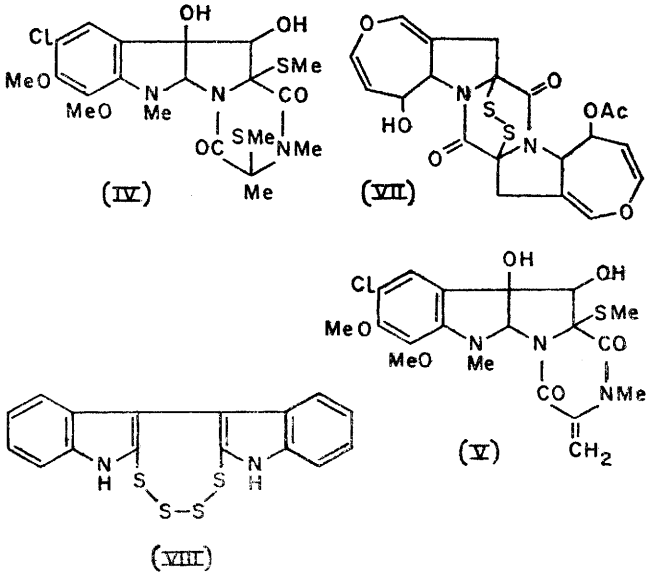
[4] D. Brewer, D. E. Hannah, and A. Taylor, *Canad. J. Microbiol.*, 1966, **12**, 1187.

The c.d. curves for the sporidesmin-D isolated from cultures of *Pithomyces chartarum* and for that prepared from sporidesmin were identical. Thus the reaction proceeds with retention of configuration. In the Figure, the c.d. curve of sporidesmin is compared with that of



C.d. curves of A, sporidesmin-F; B, sporidesmin; and C, sporidesmin-D

sporidesmin-D;* it is clear that the large negative ellipticity at *ca.* 235 mμ shown by sporidesmin is not observed in the case of sporidesmin-D. However, this Cotton effect cannot be assigned unequivocally to the



asymmetric disulphide chromophore, because aranotin (VII) and its dimethyl thioether derivative exhibit similar negative ellipticites at about 230 mμ. Apart

[5] R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. DeLong, M. M. Marsh, and N. Neuss, *J. Amer. Chem. Soc.*, 1968, **90**, 2980.

[6] W. Carpenter, M. S. Grant, and H. R. Snyder, *J. Amer. Chem. Soc.*, 1960, **82**, 2739.

from the band at 230 mμ the dichroic dispersions of sporidesmin and sporidesmin-D are similar, hence it is likely that their absolute configurations are the same.

The mother liquors from the sporidesmin-D recrystallizations were combined and rechromatographed on silicic acid in light petroleum–t-butyl alcohol (17 : 3). Sporidesmin-D was obtained from the first fractions, but later fractions contained a new metabolite, named sporidesmin-F. This material, which has not been crystallized, had a u.v. spectrum similar to that of sporidesmin. In the mass spectrometer a molecular ion was obtained, the precise mass of which corresponded to the formula $C_{19}H_{22}[^{35}Cl]N_3O_6S$. The ions observed at *m/e* 411 and 409, possibly produced by loss of CH_2S by the mechanism given before, indicated the presence of a single methylthio-group. The chemical shifts and intensities of most of the signals in the ^1H n.m.r. spectrum were the same as those in the spectrum of sporidesmin-D. However only one three-proton signal (τ 7·69) was seen, and the doublets at τ 4·02 and 4·92 (*J* 2 Hz), absent in the spectrum of sporidesmin-D, strongly suggested the presence of a methylene substituent on a dioxopiperazine ring.[7,8] Like sporidesmin-D, sporidesmin-F showed no large negative Cotton effect at 235 mμ and a positive dichroism at 265 mμ. The latter molecular ellipticity was, however, ten times greater than that observed in the cases of sporidesmin and sporidesmin-D. All of these data are consistent with formula (V) for sporidesmin-F.

Unlike the sporidesmins and gliotoxins,[9] sporidesmins-D and -F showed little or no biological activity.[10] They were inefficient catalysts of the decomposition of azide with iodine[11] and reacted very slowly with neutral aqueous silver nitrate (giving purple spots on thin-layer chromatograms).

If the structure proposed for sporidesmin-F is substantiated, we consider it to be of biosynthetic significance. It has been shown[8] that 3-methylenedioxopiperazines are produced by *Penicillium terlikowskii* and the reasons for considering these as intermediates in the biosynthesis of gliotoxin have been discussed.[8] The structure of sporidesmin-F suggests another step in the biosynthesis of the epidithiadioxopiperazine system, and in addition that there is more than one mechanism for the reaction of the sulphur donor with the receptor olefin.

EXPERIMENTAL

Physical constants of new compounds were measured, and are presented as in earlier papers of this series. Mass spectra were obtained by direct introduction into the source of a Consolidated Electrodynamics Corporation 110-B instrument. Ions were detected electrically and their precise masses were measured by reference to ions of known mass in the spectrum of perfluorokerosene. Calibration of the instrument showed that the accuracy of mass measurement was within 5 p.p.m. C.d. measurements were obtained with a Cary 60 instrument. Silicic acid used for t.l.c. was Mallinckrodt TLC-7G, and that for preparative column chromatography was Mallinckrodt silicar CC-4 (100—200 mesh). Full details of chromatographic separations can be obtained from ref. 12. Sulphur compounds were detected on t.l.c. plates by spraying with 5% silver nitrate. Identities were established by comparison of i.r. and ^1H n.m.r. spectra.

Isolation of Sporidesmin-D and Sporidesmin-F.—The crude residue (3·25 g.) obtained from the isopropyl ether extract described by Ronaldson and his co-workers,[2] in benzene–diethyl ether–acetic acid (44 : 6 : 1) was adsorbed on silicic acid (180 g.; 25 × 4·5 cm.) saturated with the same solvent. The column was eluted with this solvent, fractions (17 × 200 ml.) were collected, each was examined by t.l.c., and like fractions were combined. Fractions 3 and 4 gave sporidesmin (0·9 g.);[2] fractions 6—11 (0·58 g.) gave colourless needles (0·26 g.) from ether. Recrystallization from ether gave *sporidesmin-D etherate*, m.p. 110—120°, $\nu_{max.}$ (KBr) 3450, 3330, 1680, 1665, and 1605 cm.$^{-1}$ (Found: C, 51·55; H, 6·65; N, 6·9; O, 19·85. $C_{20}H_{26}ClN_3O_6S_2,C_4H_{10}O$ requires C, 51·6; H, 7·1; N, 6·5; O, 19·7%), τ ($CDCl_3$) 2·90 (1H), 4·70 (1H), 5·33 (1H), 6·12 (4H), 6·19 (3H), 6·50 [4H, q, *J* 7 Hz (Et_2O)], 6·62 (3H), 6·92 (3H), 7·60 (3H), 7·68 (3H), 8·13 (3H), 8·79 [6H, t, *J* 7 Hz, (Et_2O)]. This ether solvate was recrystallized from ethanol, whence *sporidesmin-D ethanolate*, m.p. 105—107°, separated (Found: C, 48·0; H, 5·9; Cl, 6·7; N, 7·6; O, 20·4; S, 11·7. $C_{20}H_{26}ClN_3O_6S_2,C_2H_5OH$ requires C, 48·1; H, 5·8; Cl, 6·5; N, 7·7; O, 20·4; S, 11·6%), $\lambda_{max.}$ (MeOH) 216, 252, and 300 mμ (log ε 4·45, 4·00, and 3·28), $[\alpha]_D^{23}$ 58° (*c* 0·11 in $CHCl_3$), $[\theta]_{303}$ 2025°, $[\theta]_{268}$ 715°, $[\theta]_{252}$ 950° (*c* 0·00042 in MeOH), *m/e* 503·0931 ($C_{20}H_{26}[^{35}Cl]N_3O_6S_2$ requires 503·0951), 457·1058 ($C_{19}H_{24}[^{35}Cl]N_3O_6S$ requires 457·1074), 457·0926 ($C_{19}H_{22}[^{37}Cl]N_3O_6S$ requires 457·0888), 409·1040 ($C_{18}H_{20}[^{35}Cl]N_3O_6$ requires 409·1041, *m* ca.* 368), 409·0841 ($C_{18}H_{18}[^{37}Cl]N_3O_6$ requires 409·0855), and 241 and 226 (*m* ca.* 212), τ ($CDCl_3$) 2·90 (1H), 4·70 (1H), 5·35 (1H), 6·95 (1H, *J* 3 Hz), 6·05 (1H), 6·13 (3H), 6·19 (3H), 6·29 [2H, q, *J* 7 Hz, (EtOH)], 6·63 (3H), 6·93 (3H), 7·60 (3H), 7·68 (3H), 8·13 (3H), 8·43 (1H), 8·79 [3H, t, *J* 7 Hz, (EtOH)]. The mother liquors from sporidesmin-D were evaporated and the residue (0·874 g.) in t-butyl alcohol (5 ml.) was added to t-butyl alcohol–light petroleum (3 : 17; 5 ml.) and the resulting solution was run on to silicic acid (180 g.; 25 × 4·5 cm.). The column was developed with t-butyl alcohol–light petroleum (3 : 17), and fractions (200 ml.) were collected. Sporidesmin-D was obtained from fraction 5. Fractions 11 and 12 were combined and evaporated; the residue (16 mg.) in ethyl acetate treated with light petroleum (b.p. 60—80°) gave *sporidesmin-F* as an amorphous solid, m.p. 65—75°, *m/e* 457, 455·0911 ($C_{19}H_{22}[^{35}Cl]N_3O_6S$ requires 455·0917), 411, 409, 407 (*m* ca.* 363·5), 241, 226 (*m* ca.* 212), and 168, $[\theta]_{310}$ 2275°, $[\theta]_{264}$ 8300°, $[\theta]_{240}$ −1500° (*c* 0·00006 in MeOH), $\lambda_{max.}$ (MeOH) 216, 250, and 298 mμ (log ε 4·46, 4·14, and 3·30), $\nu_{max.}$ (KBr) 3430, 1690, 1615, and 1605 cm.$^{-1}$, τ ($CDCl_3$) 2·91 (1H), 4·00, 4·04, 4·90, and 4·94 (2H, *J* 2 Hz), 4·64 (1H), 5·37 (1H), 6·12 (3H), 6·18 (3H), 6·60 (3H), 6·76 (3H), and 7·69 (3H).

Acetylation of Sporidesmin-D.—Sporidesmin-D etherate

[7] H. Herrmann, R. Hodges, and A. Taylor, *J. Chem. Soc.*, 1964, 4315.
[8] M. S. Ali, J. S. Shannon, and A. Taylor, *J. Chem. Soc.* (*C*), 1968, 2044.
[9] A. F. Beecham, J. Fridrichsons, and A. McL. Mathieson, *Tetrahedron Letters*, 1966, 3131.
[10] D. Brewer, R. Rahman, and A. Taylor, unpublished work.
[11] D. Brewer and A. Taylor, *Canad. J. Microbiol.*, 1967, **13**, 1577.
[12] R. Rahman, Ph.D. Thesis, Dalhousie University, 1969.

(0·25 g.) in pyridine (0·8 ml.) was treated with acetic anhydride (0·2 ml.), kept for 72 hr. at 2°, and evaporated at 20°/11 mm. The residual gum was treated with iced water (20 ml.) and the mixture was extracted with ether (3 × 25 ml.). The extract was evaporated (20°/11 mm.), dissolved in benzene–ether–acetic acid (44 : 6 : 1; 5 ml.), and adsorbed on silicic acid (42 g.; 18 × 2 cm.); the column was eluted with the same solvent. The first 150 ml. of eluate were evaporated, and the residue gave *sporidesmin-D diacetate*, m.p. 202—204° (from ethanol), $[\alpha]_D^{20}$ 6° (*c* 0·1 in $CHCl_3$) (Found: C, 49·7; H, 5·3; Cl, 6·2; N, 6·8; O, 21·5; S, 10·7. $C_{24}H_{30}ClN_3O_8S_2$ requires C, 49·0; H, 5·1; Cl, 6·0; N, 7·2; O, 21·8; S, 10·9%), $\nu_{max.}$ (KBr) 1765, 1750, 1693, 1660, 1603, 1468, 1415, 1375, 1350, 1315, 1305, 1265, 1230, 1105, 1050, 1010, 985, 955, 940, 900, 890, 835, 790, and 780 cm.$^{-1}$, $\lambda_{max.}$ (MeOH) 218, 253, and 307 mμ (log ε 4·50, 4·04, and 3·30), τ ($CDCl_3$) 3·22 (1H), 3·87 (1H), 4·20 (1H), 6·12 (3H), 6·19 (3H), 6·59 (3H), 6·94 (3H), 7·55 (3H), 7·70 (3H), 7·90 (3H), 8·15 (3H), and 8·37 (3H). The succeeding eluate from the column (65 ml.) was evaporated, and the residue (63 mg.) gave *sporidesmin-D monoacetate* (43 mg.), m.p. 185—187° (from aqueous ethanol) (Found: C, 48·5; H, 5·2; Cl, 6·4; N, 7·8; O, 20·8; S, 11·4. $C_{22}H_{28}ClN_3O_7S_2$ requires C, 48·4; H, 5·1; Cl, 6·5; N, 7·7; O, 20·5; S, 11·7%), $\lambda_{max.}$ (MeOH) 217, 252, and 302 mμ (log ε 4·47, 4·07, and 3·34), $\nu_{max.}$ (KBr) 3460, 3020, 3000, 1765, 1685, 1670, and 1605 cm.$^{-1}$, τ ($CDCl_3$) 3·05 (1H), 4·09 (1H), 4·65 (1H), 5·92 (1H), 6·13 (3H), 6·18 (3H), 6·62 (3H), 6·92 (3H), 7·55 (3H), 7·69 (3H), 8·10 (3H), and 8·44 (3H).

Anhydrodesthiosporidesmin (II).—Sporidesmin-D diacetate (0·117 g.) in ether (8 ml.) was treated with boron trifluoride–ether complex (1·5 ml.) and the mixture was kept at room temperature for 8 hr. The solution was decanted from the solid that separated, the crystals (25 mg.), m.p. 268°, were washed with ether (3 × 10 ml.), and the washings and decanted solution were combined, and evaporated. The residue was adsorbed from benzene on silicic acid (17 × 2 cm.). The column was washed with benzene (700 ml.) and the yellow band eluted with the next 1200 ml. of eluate was collected, giving 7-chloro-1,2,4,10-tetrahydro-8,9-dimethoxy-3,10-dimethyl-2-methylene-1,4-dioxo-3*H*-pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indole (II),[3] m.p. 268° (from chloroform–ether) (total yield was 32 mg., 45%).

Sporidesmin Monoacetate.—Sporidesmin diacetate [2] (0·148 g.) in formic acid (90%, 8 ml.) was treated with methanesulphonic acid (2 ml.) and the solution was kept at 20° for 2 hr. Water was added, the mixture was extracted with ether, the ethereal solution was evaporated, and the residue was adsorbed on a preparative t.l.c. plate (20 × 20 cm.). The plate was developed with chloroform–acetic acid (19 : 1), and three zones were obtained; that of intermediate R_F (10 mg.) in chloroform deposited *sporidesmin monoacetate* as colourless needles, m.p. 200—204°, *m/e* 517, 515; 485, 483; 453, 451; 393, 391 (*m* ca.* 339); 378, 376; 375, 373; 281, 279; and 241, 226, $\lambda_{max.}$ (MeOH) 217, 252, and 300 mμ (log ε 4·44, 4·01, and 3·42), $\nu_{max.}$ (KBr) 3350, 1760, 1715, and 1675 cm.$^{-1}$, τ ($CDCl_3$) 3·05 (1H), 4·12 (1H), 4·65 (1H), 6·14 (3H), 6·20 (3H), 6·67 (3H), 6·99 (4H), 7·95 (3H), and 8·37 (3H).

Conversion of Sporidesmin into Sporidesmin-D.—Sporidesmin benzene solvate [2] (0·27 g.) in pyridine (1 ml.) was treated with methyl iodide (5 ml.), and the yellow precipitate was dissolved by adding methanol (1 ml.). The solution was treated with a solution of sodium borohydride (75 mg.) in methanol (4 ml.) and then with methyl iodide (1 ml.). The mixture was kept for 3 hr. at 20°, then evaporated (40°/22 mm.); the residual gum was treated with water (20 ml.) and the mixture was extracted with ether (3 × 25 ml.). The extract was evaporated, and the residue (0·249 g.) was chromatographed as described for the isolation of sporidesmin-D but with the solvent benzene–ether–acetic acid (88 : 12 : 1). The fractions containing sporidesmin-D (t.l.c.) were collected and evaporated, and the residues were recrystallized from ether, giving sporidesmin-D etherate, m.p. 110—120° (67%).

Sporidesmin-D Monoacetate.—Sporidesmin monoacetate (4·5 mg.) was treated in pyridine (0·2 ml.) with sodium borohydride (4 mg.) in methanol and methyl iodide as described for sporidesmin. Sporidesmin-D monoacetate was isolated by preparative t.l.c. as described in its preparation from sporidesmin-D.

Sporidesmin-D Diacetate.—Sporidesmin diacetate (60 mg.) in pyridine (0·5 ml.) was treated with sodium borohydride (38 mg.) and methyl iodide (0·5 ml.) as described for sporidesmin, giving sporidesmin-D diacetate (30 mg.), m.p. 202—204°.

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