830. *Sporidesmins. Part V.*[1] *The Stereochemistry of the Bridged Dioxopiperazine Ring in Sporidesmins and in Gliotoxin.*

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The optical rotatory dispersion (o.r.d.) and circular dichroism curves of gliotoxin, sporidesmin, and some of their derivatives have been determined. No obvious relationship was observed between the o.r.d. curves, though dehydrogliotoxin and anhydrosporidesmin-B exhibited single, negative Cotton effects. By contrast, the circular dichroism curves of sporidesmin, sporidesmin-B, gliotoxin, and dehydrogliotoxin were similar and it can be concluded that they have the same absolute configuration. Alkaline treatment of anhydrosporidesmin-B gave the pyrroloindole (VI) from which DL-*N*-methylalanine was isolated. A mechanism for the elimination of sulphur from anhydrosporidesmin-B under alkaline conditions is proposed.

The formula (I; R = OH) for sporidesmin was deduced by *X*-ray crystallographic [2] and chemical [3] methods but the absolute stereochemistry of the sporidesmins or of gliotoxin (II *) [4] is still unknown. A chemical approach to this problem requires the transformation of sporidesmin into an entity lacking all the asymmetric centres except those bearing the disulphide bridge. Accordingly attempts were made to dehydrate sporidesmin-B (I; R = H). Treatment of sporidesmin-B with boron trifluoride gave a stable complex from which sporidesmin-B was recovered after decomposition with water. On heating with acetic acid, sporidesmin-B acetate [5] gave the pyrroloindole (III) [6] but with boron trifluoride in ether a product, $C_{18}H_{18}ClN_3O_4S_2$, was isolated which had an absorption band at 321 mμ. Other physical properties, given in the Experimental section, and the chemical properties discussed below were consistent with its formulation as the indole (IV).

The disulphide-bridged dioxopiperazine ring in anhydrosporidesmin-B (IV) and in dehydrogliotoxin (V) is structurally associated in a similar manner with an aromatic system, *i.e.* an indole and a benzene ring. The o.r.d. curves of (IV) and (V) are given in Fig. 1. The troughs of the negative Cotton effects observed occur at the same frequencies as those of L-tryptophan and L-cystine, respectively. The o.r.d. curves of sporidesmin, sporidesmin-B, and gliotoxin are given in Fig. 2. No obvious relationship between these curves is apparent. However the circular dichroism curves of sporidesmin, sporidesmin-B, gliotoxin, and dehydrogliotoxin (Figs. 3 and 4) are closely similar, suggesting that the asymmetric centres bearing the disulphide group have the same absolute configuration in both the sporidesmins and in gliotoxin. The band at 321 mμ in the spectrum of anhydrosporidesmin-B corresponds to the minimum $[\theta]_{320-330}$ −19,200, and to zero rotation in the o.r.d. curve.

An alternative approach to the stereochemistry of the bridged dioxopiperazine system lay in the possibility of stereospecific replacement of the sulphur atoms with hydrogen. At pH 12 the optical rotation of solutions of anhydrosporidesmin-B (IV) decreased to zero in 4 hr. at 20°, and the ultraviolet spectrum of the solution was identical with that of the pyrroloindole (VI). The latter was isolated from the reaction mixture and had $[\alpha]_D$ 0°. Acid hydrolysis of the total products of this reaction gave methylamine and the expected

* It is hoped to present further experimental evidence for this formula in a future publication with Dr. G. Lowe.

[1] Part IV, preceding Paper.
[2] Fridrichsons and Mathieson, *Tetrahedron Letters*, 1962, 1265.
[3] Hodges, Ronaldson, Taylor, and White, *Chem. and Ind.*, 1963, 42.
[4] Bell, Johnson, Wildi, and Woodward, *J. Amer. Chem. Soc.*, 1958, **80**, 1001.
[5] Ronaldson, Taylor, White, and Abraham, *J.*, 1963, 3172.
[6] Hodges, Ronaldson, Shannon, Taylor, and White, *J.*, 1964, 26.

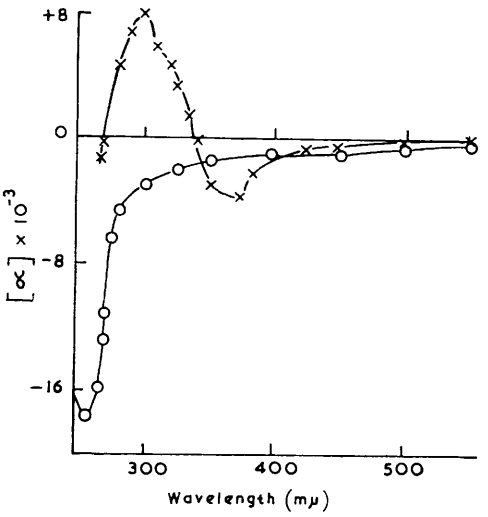


FIG. 1. Optical rotatory dispersion curves of anhydrosporidesmin-B ×—×—×, and dehydrogliotoxin ○—○—○.

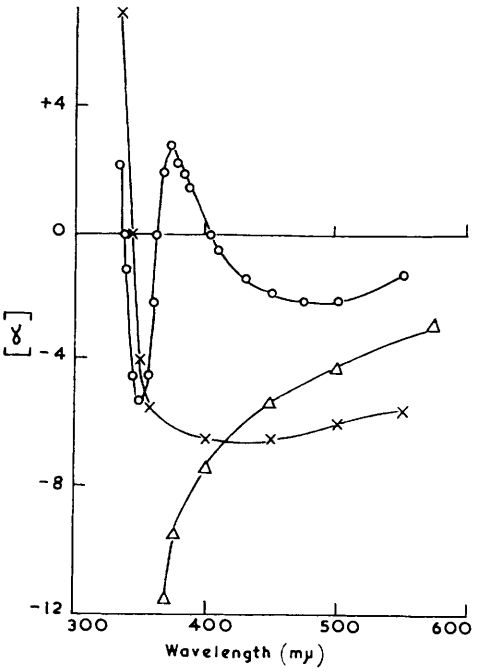


FIG. 2. Optical rotatory dispersion curves of sporidesmin ○—○—○ ($\times 10^{-1}$), sporidesmin-B ×—×—× ($\times 10^{-1}$), and gliotoxin △—△—△ ($\times 10^{-2}$).

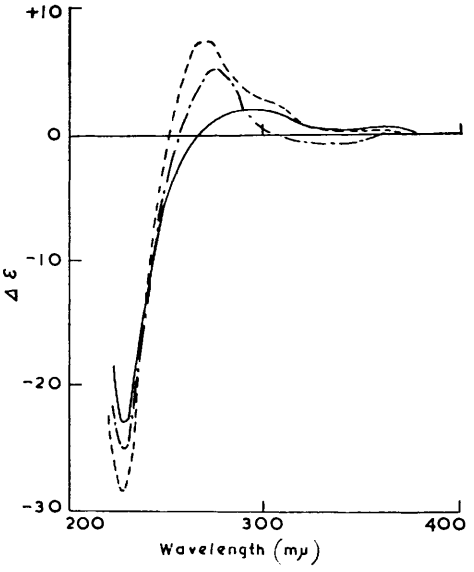


FIG. 3. Circular dichroism curves of sporidesmin ——, sporidesmin-B —●—●—, and gliotoxin – – – –.

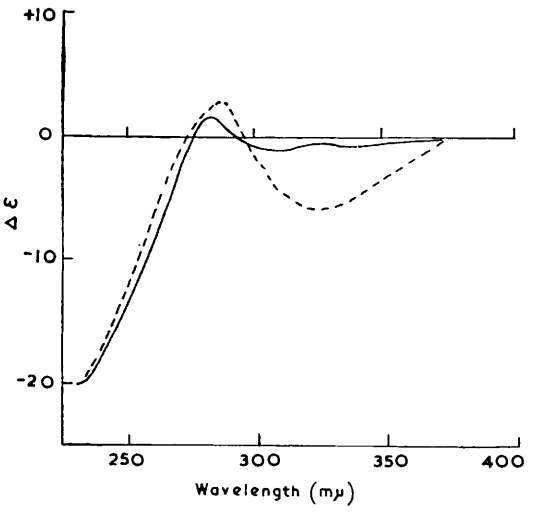
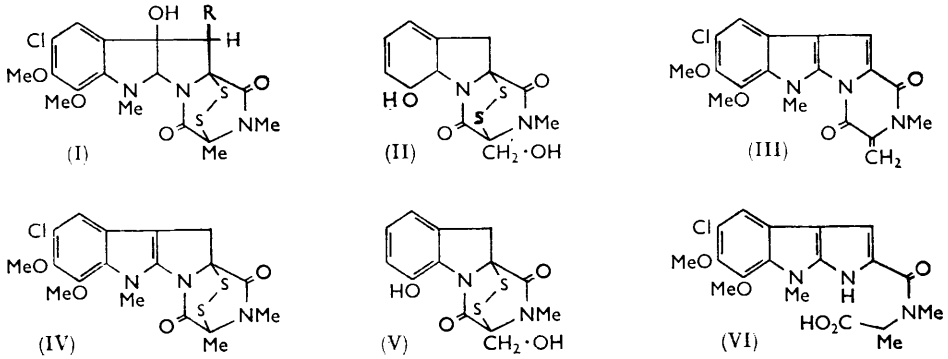
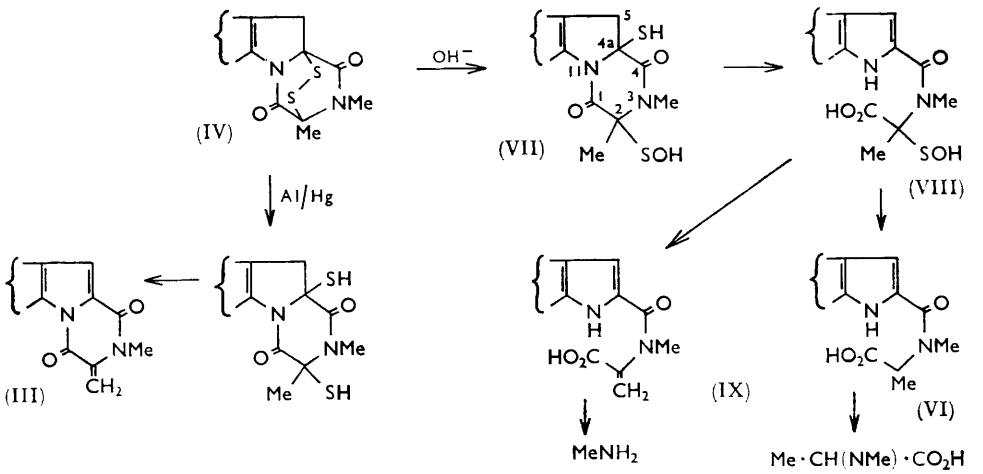


FIG. 4. Circular dichroism curves of dehydrogliotoxin —— and anhydrosporidesmin-B – – – –.

N-methylalanine.[6] Since the pyrroloindole (III) was obtained after reduction of anhydrosporidesmin-B with aluminium amalgam, when presumably a dithiol was the intermediate, it appears that elimination of hydrogen sulphide from the system occurs easily. We suggest that the disulphide reacts with hydroxyl ion producing the 4a-mercapto-2-sulphenic acid



(VII) isomer exclusively. Elimination of the thiol with simultaneous opening of the dioxopiperazine ring gives (VIII), with pyrroloindole absorption in the ultraviolet, whilst disproportionation of the 2-sulphenic acid group gives the pyrroloindoles (VI) and (IX), as illustrated. These reactions receive support from a comparative study of sporidesmin and sporidesmin-B; only in the case of the former are ketonic products formed.



The optical rotatory properties of the *N*-methylalanine obtained from anhydrosporidesmin-B were investigated using D- and L-amino-acid oxidases.[7] No reaction occurred with L-amino-acid oxidase; with D-amino-acid oxidase the amino-acid was partly oxidised. Synthetic *N*-methylalanine[6] was resistant to oxidation with L-amino-acid oxidase though oxidation did occur with D-amino-acid oxidase. Similar results have been obtained using proline[8] as substrate for these enzymes. We conclude that the disproportionation of the sulphenic acid group in (VIII) is attended by racemisation of the asymmetric centre at position 2.

[7] Bonetti and Dent, *Biochem. J.*, 1954, **57**, 77.
[8] Purikh, Greenstein, Winitz, and Birnbaum, *J. Amer. Chem. Soc.*, 1958, **80**, 953.

Experimental

Infrared and ultraviolet spectra were determined on Perkin-Elmer 137 instruments. Radio-frequency spectra were measured in $CDCl_3$ on a Varian instrument. Subscripts refer to wavelength in mμ.

Sporidesmin.—$[\theta]_{356-366}$ +1035, $[\theta]_{295-305}$ +6270, $[\theta]_{230-235}$ −69,000, [*c* 1·17 g./l. (EtOH), 420—320 mμ; 0·117 g./l., 320—270 mμ; 0·0117 g./l., 270—220 mμ] $[M]_{589}$ −61°, $[M]_{487}$ −122°, $[M]_{375}$ +150°, $[M]_{350}$ −294° (*c* 0·13, dioxan).

Sporidesmin-B.—$[\theta]_{360}$ +740, $[\theta]_{295-300}$ +8100, $[\theta]_{268}$ +23,400, $[\theta]_{230-235}$ −94,000, [*c* 0·79 g./l., (EtOH) 400—320 mμ; 0·079 g./l., 320—270 mμ; 0·0079 g./l., 270—230 mμ], $[M]_{589}$ −78°, $[M]_{400}$ +257°, $[M]_{355}$ +156°, $[M]_{340}$ +740°, (*c* 0·78, EtOH).

Gliotoxin.—The crude metabolite was repeatedly recrystallised from methanol until the specific optical rotation did not increase on further recrystallisation, $[\theta]_{325-340}$ −1425, $[\theta]_{274}$ +17,300, $[\theta]_{230-235}$ −84,000, [*c* 0·94 g./l., (EtOH), 380—320 mμ; 0·094 g./l., 320—260 mμ; 0·0094 g./l., 260—220 mμ], $[M]_{589}$ −936°, $[M]_{355}$ −3970°, (*c* 0·0675, dioxan).

Dehydrogliotoxin.—Gliotoxin (14 mg.), tetrachloro-1,2-benzoquinone (10 mg.), and benzene (2 ml.) were heated under reflux for 2 hr. The red solution was evaporated and the residue triturated with methanol (0·2 ml.). The crystalline *solid* (7 mg.) was collected and recrystallised from methanol as colourless needles (3·5 mg.), m. p. 182—183° (Found: C, 48·0; H, 3·65; N, 8·7; S, 19·5. $C_{13}H_{12}N_2O_4S_2$ requires C, 48·2; H, 3·7; N, 8·65; S, 19·8%), $\lambda_{max.}$ (EtOH) 214, 272, 300 mμ (log ε 4·34, 3·73, 3·67), $\nu_{max.}$ (KBr) 1660, 1600 cm.$^{-1}$, $[\theta]_{340}$ −1880, $[\theta]_{330}$ −1250, $[\theta]_{300}$ −2840, $[\theta]_{280-285}$ +5600, $[\theta]_{230}$ *ca.* −66,000, [*c* 0·53 g./l., (EtOH), 400—360 mμ; 0·053 g./l., 360—250 mμ; 0·008 g./l., 250—230 mμ], $[M]_{589}$ −1310°, (*c* 0·23, $CHCl_3$), $[M]_{255}$ −57,400° (*c* 0·0015, dioxan).

Sporidesmin-B–Boron Trifluoride Complex.—Sporidesmin-B (10 mg.) in ether (2 ml.) was treated with boron trifluoride etherate (0·1 ml.). The crystalline complex ($\nu_{max.}$ 1720, 1670, 1650 cm.$^{-1}$) was shaken with water (1 ml.) and ether (2 ml.). The ethereal solution gave sporidesmin-B, m. p. 180°, on evaporation.

Anhydrosporidesmin-B.—Sporidesmin-B acetate (540 mg.) in ether (10 ml.) was allowed to react with boron trifluoride etherate (1 ml.) for 16 hr. at 20°. The products were adsorbed from benzene on silica gel, eluted with benzene–ether (99 : 1), and recrystallised from ethanol, giving yellow plates (170 mg.) of *anhydrosporidesmin-B*, m. p. 185—187° (decomp.) (Found: C, 49·2; H, 3·95; Cl, 8·9; N, 9·4; O, 15·1; S, 14·65. $C_{18}H_{18}ClN_3O_4S_2$ requires C, 49·15; H, 4·15; Cl, 8·05; N, 9·55; O, 14·6; S, 14·6%), $\lambda_{max.}$ (MeOH) 232, 321 mμ (log ε 4·42, 4·08), $[\theta]_{320-330}$ −19,200, $[\theta]_{285}$ +9600, $[\theta]_{230}$ *ca.* −66,000, [*c* 0·014 g./l., (EtOH), 400—260 mμ; 0·002 g./l., 260—230 mμ], $[M]_{589}$ −1760° (*c* 0·2, $CHCl_3$), $[M]_{362}$ −15,800° (*c* 0·018, dioxan), $[M]_{300}$ +35,400° (*c* 0·0022, dioxan), $\nu_{max.}$ ($CHCl_3$) 1715, 1710, 1575, 1480 cm.$^{-1}$ (no OH- or NH-stretching bands), τ 2·86 (intensity 1), 5·60 (intensity 3), 5·93 (intensity 3), 6·00 (intensity 3), 6·64 (intensity 1), 6·73 (intensity 3), 6·91 (intensity 1), 7·83 (intensity 3).

Action of Alkali on Anhydrosporidesmin-B.—(*a*) Sodium hydroxide solution (15%; 0·5 ml.) was added to anhydrosporidesmin-B (17·8 mg.) in ethanol (50 ml.). The mixture was acidified immediately with acetic acid, concentrated to 5 ml., diluted with water, and extracted with chloroform. The acidic product provided crystals (5·8 mg.) of *N*-(5-chloro-6,7-dimethoxy-8-methylpyrrolo[2,3-*b*]indol-2-ylcarbonyl)-*N*-methylalanine (VI).

(*b*) Anhydrosporidesmin-B (2·82 mg.) was dissolved in chloroform (0·5 ml.) and the solution made up to 1 ml. with methanol. Sodium hydroxide solution (2N; 5 μl.) was added and the molecular rotation of the solution changed as follows: 15 min. −1140°; 40 min. −879°; 150 min. −285°; 270 min. −88°. The solution [$\lambda_{max.}$ 236, 283, 330 mμ (log ε 4·47, 4·31, 4·37)] was evaporated, the residue treated with concentrated hydrochloric acid (0·2 ml.), and the mixture heated at 110° in a sealed tube for 24 hr. The mixture was evaporated, the residue digested with phosphate buffer (0·05M, pH 7·25; 150 μl.), the digest centrifuged, and the supernatant solution (= hydrolysate) collected. L-Amino-acid oxidase (Sigma, from *Crotalus adamanteus* venom, 25 mg.) was dissolved in phosphate buffer (0·05M, pH 7·25; 2 ml.) and D-amino-acid oxidase (from pig kidney,[9] 50 mg.) was dissolved in phosphate buffer (0·05M, pH 8·2; 2 ml.). D-Isoleucine (25 mg., containing about 10% of the L-isomer) was dissolved in phosphate buffer (0·05M, pH 8·2; 2 ml.) and L-isoleucine (25 mg.) in phosphate buffer (0·05M, pH 7·25; 2 ml.). Four reaction mixtures were then prepared as listed, together with a control containing no enzyme. Dilutions were made with phosphate buffer (0·05M) at the pH values given in the

[9] Greenstein and Winitz, "Chemistry of the Amino Acids," Wiley, New York, 1961, p. 1788.

Table. Each of the 5 reaction mixtures was placed in a tube of capacity 3 ml. The tubes were evacuated, filled with oxygen, and incubated horizontally at 37° for 18 hr. The resulting solutions were chromatographed as described previously.[6] The chromatograms developed with

Tube No.	Hydrolysate	L-Oxidase	D-Oxidase	L-Isoleucine	D-Isoleucine	Buffer
1	10 μl.	—	5 μl.	—	5 μl.	40 μl., pH 8·2
2	10 μl.	—	5 μl.	5 μl.	—	40 μl., pH 8·2
3	10 μl.	5 μl.	—	—	5 μl.	40 μl., pH 7·25
4	10 μl.	5 μl.	—	5 μl.	—	40 μl., pH 7·25

the acidic solvent were sprayed with a freshly prepared solution of sodium naphtho-1,2-quinone-4-sulphonate (0·2 g.) in sodium carbonate solution (5%; 100 ml.). Blue spots having the R_F value of methylamine were obtained in addition to pink spots having the R_F value of *N*-methylalanine. The chromatograms developed with the basic solvent were sprayed with ninhydrin and the amino-acids estimated as described by Russell.[10] The optical densities at 570 mμ of the amino-acid spots from each reaction mixture and the control (tube 5) are tabulated. Synthetic

Tube No.	1	2	3	4	5
N-Methylalanine	0·05	0·038	0·088	0·095	0·090
Isoleucine	0·09	0·210	0·215	0·02	0·200

N-methylalanine [6] (10 mg.) in phosphate buffer (0·05M, pH 7·25; 1 ml.) was treated as described above for the hydrolysate of (VI). The optical densities of the resulting amino-acid spots are tabulated.

Tube No.	1	2	3	4	5
N-Methylalanine	0·190	0·180	0·320	0·280	0·300
Isoleucine	0·08	0·215	0·220	0·040	0·230

Action of Alkali on Sporidesmin and Sporidesmin-B.—Sporidesmin benzene solvate (46·1 mg.) in ethanol (6 ml.) was treated with sodium hydroxide solution (0·01N; 25 ml.). The molecular rotation of the solution changed as follows: 5 min. −317°; 30 min. −45°; 100 min. +450°; 195 min. +587°; 16 hr. +630°. Two mols. of alkali were consumed and the solution, when treated with an excess of 2,4-dinitrophenylhydrazine in ethanolic hydrochloric acid, gave a brown precipitate (8 mg.), m. p. 236—238°, $\nu_{max.}$ (KBr) 1675, 1330, 1045, 925, 910 cm.$^{-1}$. When sporidesmin-B was similarly treated, the change in molecular rotation was +720°, two equivalents of alkali were consumed but no precipitate was obtained when the solution was treated with 2,4-dinitrophenylhydrazine.

Reduction of Anhydrosporidesmin-B.—Anhydrosporidesmin-B (12 mg.), aluminium amalgam (50 mg), and methanol (3 ml.) were heated under reflux for 10 min. and the product adsorbed from benzene on silica gel. Elution with benzene–ether (99 : 1) gave unchanged anhydrosporidesmin-B (8·1 mg.). Further elution with benzene–ether (19 : 1) gave 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 (III), (2·2 mg.).

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[10] Russell, *J. Chromatog.*, 1960, **4**, 251.