Studies on the Metabolites of *Verticillium* sp. Structures of Verticillins A, B, and C [1a]

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Three new antibiotics, verticillins A (Ia), B (XVIII), and C have been isolated from *Verticillium* sp. Stereostructures of verticillins A and B, which are derivatives of bi-(3,11a-epidithio-1,4-dioxopyrazino[1',2':1,5]pyrrolo-[2,3-*b*]indol-10b-yl), have been elucidated by chemical and physicochemical methods. Verticillin C is thought to be an epitrithio-analogue of verticillin B.

A SPECIES of *Verticillium* (strain TM-759), an imperfect fungus isolated from a basidiocarp of *Coltricia cinnamomea* (*Polystictus cinnamomeus*), produces the antibiotics, verticillins A[1] (Ia), B (XVIII), and C. Antimicrobial activity has been found against Gram-positive bacteria and mycobacteria but not against Gram-negative bacteria and fungi. The cytotoxicity effects (ED_{50}) of verticillins A and B against HeLa cells were both 0·2 γ ml^{-1}.

Verticillin A (Ia), $C_{30}H_{28}N_6O_6S_4$, $[\alpha]_D$ +703·7°, shows $\nu_{max.}$ (Nujol) 3420 and 3335 (OH and NH), and 1703, 1694, and 1675 cm^{-1} (acid amide) and $\lambda_{max.}$ (dioxan) 306 nm (ε 5960). Its n.m.r. spectrum contains signals at τ 8·22 (s, Me), 7·15 (s, NMe), 4·25 (1H, s, >C*H*·OH), and 3·80 (1H, s, N·C*H*·N). It shows an ion at *m/e* 64 due to the loss of S_2 (*cf.* ref. 2) in its mass spectrum, and four Cotton effects, at 236 ([θ] +331,400), 272 (−25,000), 307 (+57,500), and 375 nm (−2750). The presence of acid amide i.r. bands in conjunction with the positive Cotton effect at 236 nm indicates the presence of a dioxopiperazine system.[3,4] The Cotton effect at 275 nm is assigned to the disulphide chromophore,[5] the presence of which is supported by the mass spectrum. These data suggest that verticillin A has the same epidithiodioxopiperazine system as gliotoxin[6] (II) and sporidesmin[7] (III) *etc.*[4,8,9] Furthermore, verticillin A gave bi-indol-3-yl[10] (IV) in *ca.* 50% yield on being heated under reflux with 5% potassium hydroxide in dioxan–water for 2·5 h. This result, the n.m.r. data, and the empirical formula indicate that verticillin A must have a symmetrical dimeric structure.

Treatment of verticillin A (Ia) with Raney nickel or with sodium borohydride gave complex mixtures of products, as did treatment with triphenylphosphine. However, reduction with aluminium amalgam[11] in dioxan at room temperature gave a tetradethio-derivative (V), which when heated under reflux with 5% potassium hydroxide for 3·5 h gave bi-indol-3-yl (IV), glycine (VI), and *N*-methylalanine (VII). However, treatment of compound (V) with 3% potassium carbonate in methanol at room temperature overnight gave the bi-indolyl (IV) and a dioxopiperazine derivative (VIII) (which afforded a 2,4-dinitrophenylhydrazone) in quantitative yields. When compound (VIII) was reduced with sodium borohydride, it gave two stereoisomeric alcohols, (IX) and (X), which were both hydrolysed with 6N-hydrochloric acid to give serine (XI) and *N*-methylalanine (VII). Moreover, since the n.m.r. spectra of these alcohols (IX) and (X) both showed the presence of a methyl group (doublet), an *N*-methyl group, and a CH_2·OH system, their structures must be as illustrated. These results indicate that the dioxopiperazine derivative (VIII) is an aldehyde, and that the tetradethio-derivative derived from (Ia) can consequently be represented by formula (V). The isolation of glycine (VI) under the more severe hydrolysis conditions is explained by decarbonylation of compound (VIII) with alkali before hydrolysis.

Verticillin A gave only a monoacetate (Ib) when treated with acetic anhydride–pyridine at room temperature. On reduction with aluminium amalgam this acetate (Ib) gave two tetradethio-derivatives, (XII) (M^+ 556) and (XIII) (M^+ 554). The former (XII) was hydrolysed with 4% potassium carbonate in methanol for 7·5 h at room temperature to give compound (XIV), M^+ 386, and the aldehyde (VIII). However when heated under reflux with methanolic 5% potassium hydroxide for 3 h, the tetradethio-derivative (XII) gave compound (XIV), glycine (VI), and *N*-methylalanine (VII) [*cf.* hydrolysis of (V)]. The tetradethio-derivative (XIII) was also hydrolysed by 4% potassium carbonate (in methanol at 60° for 5·5 h) to give an amorphous powder (XV), M^+ 384, and the aldehyde (VIII). Hydrogenation of compound (XV) over 10% palladised

[1] (*a*) Preliminary communication, H. Minato, M. Matsumoto, and T. Katayama, *Chem. Comm.*, 1971, 44; (*b*) K. Katagiri, K. Sato, S. Hayakawa, T. Matsushima, and H. Minato, *J. Antibiotics*, 1970, **23**, 420.

[2] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry,' Holden-Day, San Francisco, 1964, vol. 2, p. 249.

[3] H. Herrmann, R. Hodges, and A. Taylor, *J Chem. Soc.*, 1964, 4315; D. Balasubramanian and D. B. Wetlasfer, *J. Amer. Chem. Soc.*, 1966, **88**, 3449.

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

[5] C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Amer. Chem. Soc.*, 1962, **84**, 4552; M. Carmack and L. A. Neubert, *ibid.*, 1967, **89**, 7134; H. Ziffer, U. Weiss, and E. Charney, *Tetrahedron*, 1967, **23**, 3881.

[6] M. R. Bell, J. R. Johnson, B. S. Wildi, and R. B. Woodward, *J. Amer. Chem. Soc.*, 1958, **80**, 1001; A. F. Beecham, J. Fridrichsons, and A. McL. Mathieson, *Tetrahedron Letters*, 1966, 3131 and references therein.

[7] R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1665 and references therein.

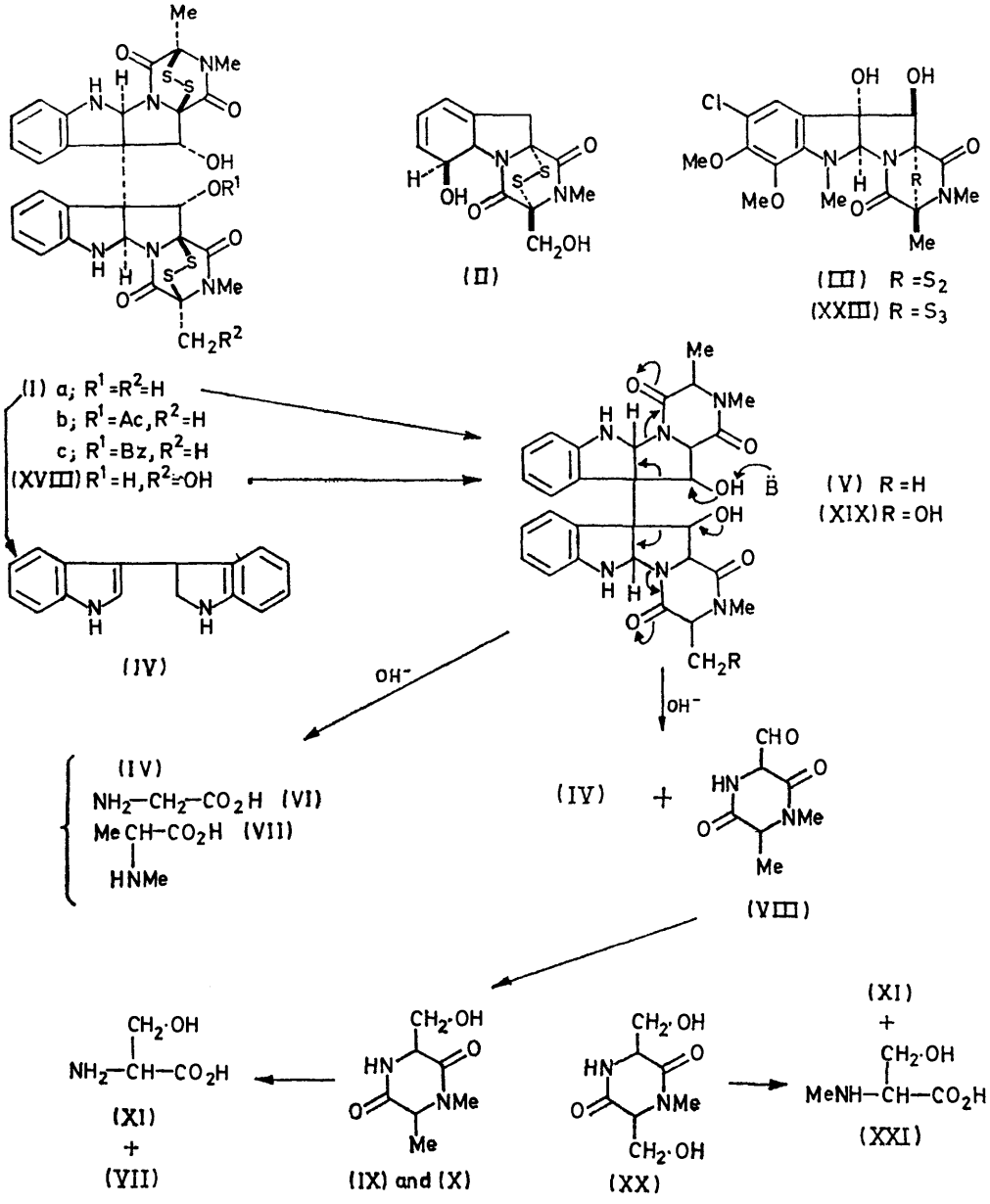
[8] N. Neuss, R. Nagarajan, B. B. Malloy, and L. Huckstep, *Tetrahedron Letters*, 1968, 4467; R. Nagarajan, N. Neuss, and M. M. Marsh, *J. Amer. Chem. Soc.*, 1968, **90**, 6518; D. B. Cosulich, N. R. Nelson, J. H. van den Hende, *ibid.*, p. 6519.

[9] D. Hauser, H. P. Weber, and H. D. Sigg, *Helv. Chim. Acta*, 1970, **53**, 1061.

[10] S. Gabriel, W. Gerhard, and R. Wolter, *Ber.*, 1923, **56**, 1033.

[11] J. Dutcher, J. R. Johnson, and W. F. Bruce, *J. Amer. Chem. Soc.*, 1945, **67**, 1736.

charcoal in ethanol gave compound (XIV); this agrees with the illustrated structure (XV) or that of an isomer having an exocyclic methylene group. When compound (XV) was heated at 160° for 12 h in 10% potassium hydroxide–ethylene glycol, it gave bi-indolyl (IV) and *N*-methylalanine (VII). These results confirm structures (XV) and (XIV), and, consequently, (XIII) and (XII).



It is thus concluded that verticillin A has the formula (Ia).

Since, as already mentioned, the c.d. data for verticillin A (Ia) are antipodal to those of gliotoxin [6] (*II*), sporidesmin [7] (III), and aranotin homologues,[4,8] which have the *R*-configuration at the two asymmetric centres in the dioxopiperazine ring, compound (Ia) should have the *S*-configuration. The Sandoz group [9] has elucidated the structure of chaetocin (XVI) by chemical and *X*-ray methods. This compound is an isomer of verticillin A and its c.d. data are in good agreement with those of verticillin A. The stereostructure of verticillin A can therefore be represented by formula (XVII).

It remained only to assign the configuration of the hydroxy-groups. The i.r. spectrum of verticillin A (Ia) shows the presence of two intramolecularly hydrogen-bonded hydroxy-groups (3425 cm^{-1}), two bonded carbonyl groups (acid amide) (1669 cm^{-1}), and two non-bonded carbonyl groups (1691 cm^{-1}). The monoacetate (Ib) shows one bonded hydroxy-group (3425 cm^{-1}), one bonded carbonyl group (1671 cm^{-1}), and three non-bonded carbonyl groups (1693 cm^{-1}). These results indicate the presence of hydrogen bonding to the

oxygen atom of the acid amide group (see Table). A molecular model shows distances of *ca.* 1·4 and *ca.* 3·5 Å between the hydrogen atom of an α-hydroxy-group * and the carbonyl oxygen atom and between the hydrogen and sulphur atoms, respectively, whereas corresponding distances of *ca.* 3·4 and 1·9 Å are observed for the hydrogen atom of a β-hydroxy-group. Therefore, it is assumed that the hydroxy-group is α-oriented.

Moreover, verticillin A monobenzoate (Ic) shows a Cotton effect at 223 nm ([θ] +50,000) due to the benzoate group. Application of the benzoate sector rule [12] confirms the α-configuration of the hydroxy-group. From these results, the absolute configuration of verticillin A can be represented as in formula (XVII).

* The α-configuration of the hydroxy-group is that shown in formulae (Ia) and (XVII).

Verticillin B (XVIII), $C_{30}H_{28}N_6O_7S_4$, $[\alpha]_D$ +704·7°, $\lambda_{max.}$ 306 nm (ε 5600), showed an i.r. spectrum similar to that of verticillin A. However, its n.m.r. spectrum indicated that it had not a symmetrical dimeric structure, showing signals for one methyl group at τ 8·25, two *N*-methyl groups at 7·17 and 6·74, and one C*H*$_2$·OH system at 5·48. When verticillin B was acetylated with acetic anhydride–pyridine at room temperature, it afforded a diacetate. Moreover its c.d. data were in good agreement with those of verticillin A. It was therefore considered that verticillin B had the structure (XVIII).

When heated with 2% potassium carbonate in pyridine–water at 50° for 2 h, verticillin B gave bi-

[12] N. Harada, Mo. Ohashi, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1968, **90**, 7349.

indol-3-yl (IV) in good yield. When verticillin B was reduced with aluminium amalgam under the same conditions as for verticillin A (Ia), it gave a tetradethio-derivative (XIX). This compound was hydrolysed

Intramolecular hydrogen-bonds of verticillin A (Ia) and its monoacetate (Ib) as shown by i.r. spectra

	ν_{OH}				$\nu_{C=O}$			
	ν_{OH}/cm^{-1}	ε	$\Delta\nu_{\frac{1}{2}}$/cm^{-1}	A * $\times 10^{-4}$	$\nu_{C=O}$/cm^{-1}	ε	$\Delta\nu_{\frac{1}{2}}$/cm^{-1}	A * $\times 10^{-4}$
(Ia)	3425	192·9	95·2	6·64	1669	1246·4	22·1	9·96
					1691	1220·7	21·0	9·27
(Ib)	3425	162·6	68·0	4·00	1671	832·7	20·5	6·11
					1693	2352·2	23·5	19·65

(Ia), c 0·3835 $\times 10^{-3}$ mol l^{-1} in $CHCl_3$; 5 mm cell. (Ib), c 0·3690 $\times 10^{-3}$ mol l^{-1} in $CHCl_3$; 5 mm cell.

* D. A. Ramsay, *J. Amer. Chem. Soc.*, 1952, **74**, 72.

with 5% potassium carbonate in methanol overnight at room temperature. The reaction mixture was diluted with water and extracted with chloroform to give bi-indolyl (IV). To the aqueous layer was added sodium borohydride and the mixture was left for 4 h at room temperature to give the stereoisomeric alcohols (IX) and (X), and a diol (XX), m.p. 210—213°. Compound (XX) gave serine (XI) and *N*-methylserine (XXI) on hydrolysis with 6N-hydrochloric acid. These results lead to the conclusion that verticillin B has structure (XVIII). Moreover, its c.d. data indicate that the absolute stereo-structure can be represented by formula (XXII).

It is relevant that the Sandoz group [13] has recently isolated 11α,11′α-dihydroxychaetocin (XXIV) from the fungus *Verticillium tenerum*.

Verticillin C, $[\alpha]_D$ +765°, $C_{30}H_{28}N_6O_7S_5$, has one more sulphur atom in the molecule than verticillin B. We therefore consider that one dioxopiperazine ring has a trisulphide bridge rather than a disulphide bridge. Reduction of verticillin C with aluminium amalgam gave products showing the same behaviour as those from verticillin B on t.l.c. Its pentadethio-derivative was hydrolysed with 5% potassium carbonate in methanol to give bi-indolyl (IV) and a water-soluble product. The latter was reduced with sodium borohydride to give three alcohols, (IX), (X), and (XX). Therefore, verticillins C and B (XVIII) have the same structure save for the sulphide bridge.

Taylor *et al.* have reported [14] that sporidesmin E (XXIII) is converted into sporidesmin (III) by treatment with triphenylphosphine and that the latter can be sulphurated with sulphur and phosphorus pentasulphide to regenerate the former. Attempted transformation of verticillin C into verticillin B (XVIII) was, however, unsuccessful under the same conditions.

† Fermentation was performed by Dr. S. Hayakawa and Mr. K. Ishii in this laboratory.

‡ Elemental analyses were carried out for solvent-free samples, after quantitative analysis for crystallisation solvents by use of an apparatus [15] for C and H determination combined with differential thermal analysis. In the mass spectrum, the parent peak (M^+) was not observed under any conditions, but the molecular weight was shown to be 674 by vapour pressure osmometry.

EXPERIMENTAL

Unless otherwise stated, n.m.r. spectra were taken for solutions in deuteriopyridine with a Varian A60 spectrometer. M.p.s were measured with a Kofler hot-stage apparatus and are corrected.

Isolation of Verticillins A (Ia), *B* (XVIII), *and C.*—*Verticillium* sp. strain TM-759 on an agar slant was incubated in a shaken 500 ml Sakaguchi flask containing 100 ml of a medium containing (in g l^{-1}) glucose (30), peptone (20), and sodium chloride (5). The pH of the medium was adjusted to 6·8 prior to sterilisation. The inoculated flask was incubated at 27° for 24 h with shaking to produce a primary seed culture. Two samples of seed culture broth (5 ml) were transferred each into a 2 l Erlenmyer flask containing the same medium (500 ml) and incubated on a rotary shaker (180 rev. min^{-1}) at 27° for 3 days. The secondary seed culture broth thus obtained was transferred to a 30 l jar fermenter containing the same medium (20 l) and Nissan Uniol D-2000 (2 ml) as a defoamer. Fermentation was carried out at 27—28° for 2 days with an air flow rate of 20 l min^{-1} at a pressure of 0·5—0·7 kg cm^{-2} and with an agitator speed of 250 rev. min^{-1}.†

The mycelium thus obtained (12 jar fermenters; wet weight 14 kg) was collected by centrifugal separation and extracted with acetone (20 l) and then ethyl acetate (2 × 15 l) with stirring at room temperature. The extracts were combined and evaporated *in vacuo* at 50—60°. The residue was dissolved in ethyl acetate; the solution was washed with 5% sodium carbonate and water, dried (Na_2SO_4), and evaporated *in vacuo*. The residue (25·8 g) was added to ether (200 ml) and stirred at room temperature for 30 min. The ether-insoluble product (13·7 g) was dissolved in benzene–chloroform (1 : 1; 1300 ml), chromatographed on silica gel (700 g), and eluted successively with benzene–chloroform (1 : 1), chloroform, and chloroform–methanol (9 : 1). Elution with benzene–chloroform (1 : 1) gave verticillin A (Ia) (5·0 g); elution with chloroform–methanol (9 : 1) gave verticillin B (XVIII) (2·53 g) and then verticillin C (0·13 g).

Verticillin A ‡ (Ia) {*bi*-(3,11a-*epithio*-1,2,3,4,5a,10b,11,11a-*octahydro*-11-*hydroxy*-2,3-*dimethyl*-1,4-*dioxo*-6H-*pyrazino*-[1′,2′:1,5]*pyrrolo*[2,3-*b*]*indol*-10b-*yl*)} was obtained as pale yellow plates, $C_{30}H_{28}N_6O_6S_4$,$CHCl_3$, m.p. 199—213° (decomp.) (from chloroform) (Found: C, 51·55; H, 4·0; N, 11·9; O, 13·5; S, 18·2. $C_{30}H_{28}N_6O_6S_4$ requires C, 51·7; H, 4·05; N, 12·05; O, 13·8; S, 18·4%), as pale yellow needles, $C_{30}H_{28}N_6O_6S_4$,0·67C_5H_5N, m.p. 202—217° (decomp.) (from pyridine) (Found: C, 52·1; H, 3·8; N, 12·15; O, 13·4; S, 17·85%), or as a pale yellow amorphous powder, $C_{30}H_{28}N_6O_6S_4$, m.p. 203—214° (decomp.) (from tetrahydrofuran) (Found: C, 52·3; H, 4·2; N, 11·7; O, 13·7; S, 17·95%), $[\alpha]_D^{26}$ +703·7° (±17·9°) (*c* 0·422 in dioxan), $\lambda_{max.}$ (dioxan) 306 nm (ε 5960), $\nu_{max.}$ (Nujol) 3420, 3335, 1703, 1694, 1675, 1608, 1594, 1350, 1300, 1246, 1202, 1141, 1092, 1064, 982, 753, and 745 cm^{-1}.

Verticillin B (XVIII) (the mono-3-hydroxymethyl analogue of verticillin A) was obtained as pale yellow prisms, m.p. 230—233° (decomp.) (from chloroform), $[\alpha]_D^{21}$ +704·7° (±22·4°) (*c* 0·493 in dioxan), $\lambda_{max.}$ (dioxan) 306 nm (ε 5600), $\nu_{max.}$ ($CHCl_3$) 3400, 1677, 1605, 1352, 1302, 1140, 1091, 1063,

[13] D. Hauser, H. R. Loosli, and P. Niklaus, *Helv. Chim. Acta*, 1972, **55**, 2182.

[14] R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1665.

[15] T. Ieki and K. Daikatsu, 36th Symposium of Organic Micro-analysis, Tokyo, May 23—25, 1969.

973, and 856 cm^{-1}, τ 8·25 (s, Me), 7·17 (s, NMe), 6·74 (s, NMe), 5·48 (s, CH_2·OH), 4·23 (2H, s, >CH·OH), and 3·78 (2H, s, N·CH·N), c.d. (in dioxan) 237 ([θ] +350,000), 274 (−28,000), 306 (+61,900), and 370 nm (−3070) (Found: C, 47·15; H, 3·85; Cl, 7·15; N, 10·45; O, 14·5; S, 16·3. $C_{30}H_{28}N_6O_7S_4$,0·5$CHCl_3$ requires C, 47·4; H, 3·7; Cl, 6·9; N, 10·9; O, 14·5; S, 16·6%).

Verticillin C was obtained as a pale yellow amorphous powder, m.p. 230—235° (decomp.) (from methanol–water), $[\alpha]_D^{21}$ +765·0° (±23·0°) (*c* 0·506 in dioxan), $\lambda_{max.}$ (dioxan) 303 nm (ε 5500), $\nu_{max.}$ ($CHCl_3$) 3420, 1673, 1605, 1362, 1305, 1141, 1093, 1064, 973, and 861 cm^{-1}, τ 8·25 (s, Me), 7·20 (s, NMe), 6·57 (s, NMe), 5·40 (s, CH_2·OH), 4·28 (2H, s, >CH·OH), and 3·78 (2H, s, N·CH·N), c.d. (in dioxan) 237·5 ([θ] +278,000), 277 (−25,700), 311 (+63,100), and 377 nm (−1380) (Found: C, 46·15; H, 3·85; N, 10·45; O, 17·9; S, 21·0. Calc. for $C_{30}H_{28}N_6O_7S_5$,2H_2O: C, 46·15; H, 4·15; N, 10·75; O, 18·45; S, 20·55%).

Acetylation of Verticillin A (Ia).—Verticillin A (Ia) (500 mg) was added to acetic anhydride (1 ml) and dry pyridine (5 ml) and left overnight at room temperature to give 11-O-*acetyl verticillin A* (Ib) (520 mg), obtained as a yellow amorphous powder, m.p. 220—243° (decomp.) (from chloroform–methanol), $\nu_{max.}$ ($CHCl_3$) 3408, 1767, 1692, 1609, 1596, 1338, 1145, 1088, 1050, and 858 cm^{-1}, τ ($CDCl_3$) 8·13 (s, 2Me), 7·58 (s, OAc), 7·10 (s, NMe), 7·03 (s, NMe), 4·98 (1H, s), and 4·85 (3H, s) (Found: C, 51·9; H, 3·9; N, 11·1; O, 14·8; S, 17·05. $C_{32}H_{30}N_6O_7S_4$ requires C, 52·0; H, 4·1; N, 11·4; O, 15·15; S, 17·35%).

Treatment of Verticillin A (Ia) *with 5% Potassium Hydroxide.*—Verticillin A (639 mg) was dissolved in a solution of potassium hydroxide (600 mg) in dioxan (10 ml) and water (2 ml) and heated under reflux for 2·5 h. The solution was evaporated, and the residue was dissolved in water (10 ml) and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a crystalline residue (247 mg), which was recrystallised from ethyl acetate to give bi-indol-3-yl [10] (IV) (112 mg), m.p. 285—287°. The water layer was acidified with acetic acid and extracted with ethyl acetate to give sulphur (30·8 mg). The residual water layer was treated with Amberlite IRA-400 and then Amberlite IR-120 resin to give an amino-acid mixture. This was separated into glycine (12·4 mg) and alanine (12·7 mg) by preparative t.l.c. on MN-cellulose powder (solvent phenol–water, 3 : 1).

Reduction of Verticillin A (Ia) *with Aluminium Amalgam.*—A solution of verticillin A (500 mg) in dioxan (45 ml) was added dropwise to aluminium amalgam [11] [prepared by treatment of aluminium (5 g) with N-sodium hydroxide and 0·5% mercuric chloride] in dioxan (50 ml) with stirring in an ice-bath. The mixture was stirred for 4 h at room temperature, then filtered, and the filtrate was evaporated. The residue (420 mg) was separated into *tetradethioverticillin A* (V), an amorphous powder (224 mg), m.p. 254—256° (decomp.) (from ethyl acetate), R_F 0·21, $\lambda_{max.}$ (EtOH) 243 (ε 11,200) and 303 nm (4600), $\nu_{max.}$ ($CHCl_3$) 3284, 1661, 1611, 1099, 1059, and 876 cm^{-1} (Found: C, 62·4; H, 5·7; N, 14·6; O, 16·8%; M^+, 572. $C_{30}H_{32}N_6O_6$ requires C, 62·9; H, 5·65; N, 14·7; O, 16·75%; *M*, 572·6); its *stereoisomer*, an amorphous powder (43 mg), R_F 0·32, $\lambda_{max.}$ (EtOH) 243 (ε 11,800) and 302 nm (5100), $\nu_{max.}$ ($CHCl_3$) 3414, 1667, 1607, and 1094 cm^{-1}, M^+ 572; and another amorphous powder (103 mg), R_F 0·25, by preparative t.l.c. on silica gel (solvent chloroform–methanol, 10 : 1).

Hydrolysis of the Tetradethio-derivative (V) *with 5% Potassium Hydroxide.*—Compound (V) (30 mg) in 5% potassium hydroxide was heated under reflux for 3·5 h; the mixture was then extracted with ethyl acetate. The ethyl acetate layer gave bi-indol-3-yl (IV) (11 mg). The aqueous layer was filtered through a column of Dowex-1 resin and eluted with 2N-hydrochloric acid to liberate amino-acids. The hydrochloric acid solution was evaporated *in vacuo* and the residue was dissolved in saturated sodium hydrogen carbonate solution (0·5 ml). To this solution was added a solution of 2,4-dinitrofluorobenzene (40 mg) in 95% ethanol (1 ml). This mixture was stirred for 2 h at room temperature and extracted with chloroform to remove an excess of the reagent. The aqueous layer was acidified with 2N-hydrochloric acid and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated. The residue (11·4 mg) was separated into *N*-2,4-dinitrophenyl-*N*-methylalanine (5·1 mg) and *N*-2,4-dinitrophenylglycine (3·4 mg) by preparative t.l.c. on silica gel (solvent chloroform–methanol–acetic acid, 95 : 5 : 1).

Hydrolysis of the Tetradethio-derivative (V) *with 3% Potassium Carbonate in Methanol.*—A solution of compound (V) (109 mg) in 3% potassium carbonate in methanol (5 ml) was left overnight at room temperature under nitrogen during which time bi-indol-3-yl (IV) (24·6 mg) was deposited as needles. The solution was filtered, diluted with water (5 ml), and extracted with chloroform to give more bi-indolyl (IV) (19 mg). Half of the aqueous layer was acidified with 2N-sulphuric acid and treated with Brady's reagent (1 ml) overnight at room temperature. The resulting *4,5-dimethyl-3,6-dioxopiperazine-2-carbaldehyde* (VIII) *2,4-dinitrophenylhydrazone* (6·5 mg) crystallised from methanol as yellowish orange needles, m.p. 220—230° (decomp.) (Found: C, 44·9; H, 3·7; N, 23·85. $C_{13}H_{14}N_6O_6$ requires C, 44·55; H, 4·05; N, 24·0%). The other half of the aqueous layer was reduced with sodium borohydride (30 mg) for 6 h at room temperature. The mixture was acidified with 2N-hydrochloric acid, filtered through a column of Dowex 50 W × 8 resin, and eluted with water. The eluate was evaporated *in vacuo* and the residue (28·6 mg) was separated into *3-hydroxymethyl-1,6-dimethylpiperazine-2,5-dione* (IX), prisms (from ethanol), m.p. 217—220° (10 mg), R_F 0·17, $\nu_{max.}$ (KBr) 3337, 3163, 1692, 1649, 1342, 1066, and 839 cm^{-1}, τ (D_2O) 8·43 (CH_3, d, *J* 7·0 Hz), 7·03 (NCH_3, s), and 6·0 (CH_2·OH, m) (Found: C, 48·95; H, 6·9; N, 16·25. $C_7H_{12}N_2O_3$ requires C, 48·85; H, 7·05; N, 16·25%), and its oily *isomer* (X) (7 mg), M^+ 172, R_F 0·24, τ (D_2O) 8·48 (d, CH_3, *J* 6·8 Hz), 7·01 (s, NCH_3), 6·0 (CH_2·OH, m), by preparative t.l.c. (solvent chloroform–methanol, 10 : 2).

Hydrolysis of the Alcohol (IX) *with 6N-Hydrochloric Acid.*—A solution of the alcohol (IX) (5 mg) in 6N-hydrochloric acid (1 ml) was heated at 120° (bath temp.) for 7 h and evaporated *in vacuo* to leave a mixture of amino-acids, which was shown to consist of *N*-methylalanine (VII) and serine (XI) by t.l.c. and paper chromatography [solvents phenol–water (3 : 1) and 99% ethanol–conc. NH_4OH (7 : 3)] of amino-acids and their 2,4-dinitrophenyl derivatives.

Hydrolysis of the Isomeric Alcohol (X) *with 6N-Hydrochloric Acid.*—Compound (X) (3 mg) was hydrolysed under the same conditions and gave *N*-methylalanine (VII) and serine (XI).

Reduction of 11-O-*Acetylverticillin A* (Ib) *with Aluminium Amalgam.*—A solution of the acetate (Ib) (1·16 g) in dioxan

(20 ml) was added dropwise to aluminium amalgam [from aluminium (10 g)] in dioxan (50 ml) with stirring in an ice-bath. The mixture was stirred for 3 h at room temperature and filtered, and the residue was washed with chloroform–methanol. The filtrate and washings were combined and evaporated; the residue (788 mg) was separated into the *tetradethio-derivative* (XII), an amorphous powder (from benzene), m.p. 240° (decomp.) (520 mg), R_F 0·41, M^+ 556, $\lambda_{max.}$ (EtOH) 243 (ε 12,700) and 302 nm (5200), $\nu_{max.}$ ($CHCl_3$) 3374, 1660, 1608, 1065, and 860 cm^{-1} (Found: C, 64·85; H, 5·5; N, 14·75; O, 14·15. $C_{30}H_{32}N_6O_5$ requires C, 64·75; H, 5·8; N, 15·1; O, 14·35%) and another *tetradethio-derivative* (XIII), an amorphous powder (from chloroform–methanol), m.p. 280 (decomp.) (210 mg), R_F 0·57, M^+ 554, $\lambda_{max.}$ (EtOH) 240 (ε 26,600) and 297 nm (6400), $\nu_{max.}$ ($CHCl_3$) 3392, 1658, 1606, 1094, and 864 cm^{-1} (Found: C, 64·9; H, 4·9; N, 15·1; O, 14·4. $C_{30}H_{30}N_6O_5$ requires C, 64·95; H, 5·45; N, 15·15; O, 14·45%) by preparative t.l.c. on silica gel (solvent chloroform–methanol, 10 : 1).

Hydrolysis of the Tetradethio-derivative (XII) *with* 4% *Potassium Carbonate in Methanol.*—A solution of compound (XII) (170 mg) in 4% potassium carbonate in methanol (10 ml) was left for 7·5 h at room temperature under nitrogen. It was then evaporated, and the residue was dissolved in water (5 ml) and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated; the residue (104 mg) crystallised from chloroform to give *compound* (XIV) as prisms (80 mg), m.p. 252—254°, M^+ 386, $\lambda_{max.}$ (MeOH) 220 (ε 36,800), 240sh (9200), 282·5 (6800), and 290 nm (6900), $\nu_{max.}$ ($CHCl_3$) 3454, 1666, 1608, and 1066 cm^{-1}, τ ($CDCl_3$) 8·60 (d, Me, *J* 7·0 Hz), 7·05 (s, NMe), 6·92 (2H, m), 6·10 (1H, m), 5·90 (1H, m), 4·11 (1H, s), 3·50—2·20 (10H), and 1·88 (1H, m), c.d. (in MeOH) 217 ([θ] −82,500), 249 (+28,800), 275 (+6700), 287sh (+12,100), and 298 nm (+20,900) (Found: C, 71·4; H, 5·75; N, 14·5. $C_{23}H_{22}N_4O_2$ requires C, 71·5; H, 5·75; N, 14·5%. Found: $CHCl_3$, 23·4. $C_{23}H_{22}N_4O_2,CHCl_3$ requires $CHCl_3$, 23·6%).

One-third of the aqueous layer was acidified with 2N-sulphuric acid and treated with Brady's reagent to give the 2,4-dinitrophenylhydrazone (23·8 mg) of (VIII). The other two-thirds of the aqueous layer was reduced with sodium borohydride to give a mixture of compounds (IX) (18 mg) and (X) (14 mg).

Hydrolysis of the Tetradethio-derivative (XII) *with* 5% *Potassium Hydroxide in Methanol.*—A solution of compound (XII) (16 mg) in 5% potassium hydroxide in methanol (1·2 ml) was heated under reflux for 3 h and extracted with chloroform. The chloroform layer gave compound (XIV) (4·2 mg). The aqueous layer was acidified with 2N-hydrochloric acid and extracted with chloroform. The aqueous layer was filtered through a column of Dowex-1 resin to give a mixture of amino-acids, which was treated with 2,4-dinitrofluorobenzene as in the case of (V) to give *N*-2,4-dinitrophenyl-*N*-methylalanine (1·7 mg) and *N*-2,4-dinitrophenylglycine (1·1 mg).

Hydrolysis of the Tetradethio-derivative (XIII) *with* 4% *Potassium Carbonate in Methanol.*—A solution of compound (XIII) (72 mg) in 4% potassium carbonate in methanol (4 ml) was heated at 60° for 5·5 h under nitrogen, then evaporated. The residue was dissolved in water (5 ml) and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving an amorphous residue (60 mg), which was purified by t.l.c. to give *compound* (XV) as an amorphous powder (31 mg), R_F 0·30 (benzene–methanol, 10 : 1), M^+ 384, $\lambda_{max.}$ (EtOH) 220 (ε 53,000), 237sh (28,000), 260 (16,000), and 290 nm (10,000), $\nu_{max.}$ ($CHCl_3$) 3450, 1678, 1646, and 1610 cm^{-1}, τ ($CDCl_3$) 8·46 (d, Me, *J* 7·0 Hz), 7·0 (NMe), 5·95 (1H), 4·65 (1H), and 3·81 (1H) (Found: C, 71·7; H, 5·15; N, 14·35. $C_{23}H_{20}N_4O_2$ requires C, 71·85; H, 5·25; N, 14·6%). The aqueous layer was reduced with sodium borohydride (10 mg) for 4 h at room temperature. The mixture was acidified with 2N-hydrochloric acid, filtered through a column of Dowex 50 W × 8 resin, and eluted with water. The eluate was evaporated *in vacuo* to leave a mixture of compounds (IX) (5·2 mg) and (X) (3·7 mg).

Catalytic Hydrogenation of Compound (XV).—A solution of compound (XV) (5·3 mg) in 95% ethanol (5 ml) was hydrogenated over 10% palladised charcoal (5 mg) for 21 h at room temperature. The mixture was filtered, and the filtrate was evaporated; the residue (5 mg) crystallised from methanol to give compound (XIV).

Hydrolysis of Compound (XV) *with Potassium Hydroxide.*—Compound (XV) (42 mg) was dissolved in a solution of potassium hydroxide (110 mg) in ethylene glycol (1 ml) and water (0·05 ml). The solution was heated at 160° for 12 h under nitrogen, diluted with water, and extracted with chloroform to give bi-indolyl (IV) (6·5 mg). To the aqueous layer was added sodium borohydride (20 mg.) The mixture was left for 2 h at room temperature, then acidified with 2N-hydrochloric acid and extracted with chloroform. The aqueous layer was neutralised with 2N-sodium carbonate and added to a solution of 2,4-dinitrofluorobenzene (20 mg) in 99% ethanol (4 ml) and 2N-sodium carbonate (0·2 ml). The resulting solution was stirred for 2 h at room temperature and extracted with chloroform to remove an excess of the reagent. The aqueous layer was acidified with 2N-hydrochloric acid and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated. The residue (15·1 mg) was purified by t.l.c. to give *N*-2,4-dinitrophenyl-*N*-methylalanine (5·9 mg).

Acetylation of Verticillin B (XVIII).—Verticillin B (XVIII) (537 mg) was added to acetic anhydride (1 ml) and dry pyridine (4 ml) and left overnight at room temperature to give *di*-O-*acetylverticillin B* (620 mg), obtained as an amorphous powder (from methanol), m.p. 200° (decomp.), $\nu_{max.}$ ($CHCl_3$) 3422, 1769, 1757, 1698, 1608, 1596, and 1049 cm^{-1}, τ ($CDCl_3$) 8·13 (s, Me), 7·90 (s, OAc), 7·58 (s, OAc), 7·10 (s, NMe), and 7·03 (s, NMe) (Found: C, 49·1; H, 4·0; N, 9·75; S, 15·05. $C_{34}H_{32}N_6O_9S_4,2H_2O$ requires C, 49·0; H, 4·35; N, 10·1; S, 15·4%).

Treatment of Verticillin B (XVIII) *with* 2% *Potassium Carbonate.*—Verticillin B (XVIII) (20 mg) was dissolved in a solution of potassium carbonate (60 mg) in pyridine (1 ml) and water (2 ml), heated at 50° for 2 h, and left overnight at room temperature. The solution was evaporated *in vacuo* and extracted with chloroform–acetone to give bi-indolyl (IV) (6 mg).

Reduction of Verticillin B (XVIII) *with Aluminium Amalgam.*—A solution of compound (XVIII) (200 mg) in dioxan (10 ml) was reduced with aluminium amalgam [from aluminium (2 g)] under the same conditions as in the case of (Ia). A tetradethio-derivative (XIX) was obtained as an amorphous powder (166 mg) and hydrolysed with 5% potassium carbonate in methanol without purification.

Hydrolysis of the Tetradethio-derivative (XIX) *with* 5% *Potassium Carbonate in Methanol.*—A solution of crude

compound (XIX) (144 mg) in 5% potassium carbonate in methanol (7 ml) was left overnight at room temperature under nitrogen, during which time bi-indolyl (IV) was deposited as needles (25·4 mg). The filtrate was extracted with chloroform to give more bi-indolyl (IV) (13·9 mg). To the aqueous layer was added sodium borohydride (50 mg) and the mixture was left for 4 h at room temperature, acidified with 2N-hydrochloric acid, filtered through a column of Dowex 50 W × 8 resin, and eluted with water. The eluate was evaporated *in vacuo*; the residue (60 mg) was separated into the alcohols (X) (12·1 mg), R_F 0·29, and (IX) (11·6 mg), R_F 0·21, and *3,6-bis-hydroxymethyl-1-methylpiperazine-2,5-dione* (XX), prisms, m.p. 210—213° (from methanol) (18·5 mg), R_F 0·10 (Found: C, 44·4; H, 6·4; N, 15·0. $C_7H_{12}N_2O_4$ requires C, 44·7; H, 6·45; N, 14·9%), by preparative t.l.c. (solvent chloroform–methanol, 10 : 2).

Hydrolysis of the Diol (XX) *with* 6N-*Hydrochloric Acid.*—A solution of the diol (XX) (3 mg) in 6N-hydrochloric acid (1 ml) was heated at 110° for 20 h and evaporated *in vacuo* to leave a mixture of amino-acids, which was shown to consist of serine (XI) and *N*-methylserine (XXI) by comparison with authentic samples [t.l.c. (solvent EtOH–conc. NH_4OH, 7 : 3) and paper chromatography (solvents phenol–water, 7 : 3, n-butanol–acetic–acid–water, 4 : 1 : 5)].

Reduction of Verticillin C with Aluminium Amalgam and Hydrolysis of Its Pentadethio-derivative.—A solution of verticillin C (30 mg) in dioxan (1 ml) was reduced with aluminium amalgam [from aluminium (0·3 g)] under the same conditions as in the case of (Ia). A pentadethio-derivative was obtained as an amorphous powder (22 mg), which showed the same i.r. and t.l.c. properties as compound (XIX). This crude product was hydrolysed with 5% potassium carbonate in methanol. The mixture was evaporated, and the residue was dissolved in water and extracted with chloroform to give bi-indolyl (IV) (5·5 mg). To the aqueous layer was added sodium borohydride (7 mg); the mixture was left for 4 h at room temperature, acidified with 2N-hydrochloric acid, filtered through a column of Dowex 50 W × 8 resin, and eluted with water. The eluate was evaporated *in vacuo* and the residue (15 mg) was separated into the alcohols (IX) (2·3 mg), (X) (2·7 mg), and (XX) (4·0 mg) by preparative t.l.c.

We thank Dr. Y. Matsui and Mr. M. Takasuka for determining the hydrogen-bonding properties (i.r. spectra), and Dr. K. Kuriyama for c.d. measurements. We also thank Mr. T. Ieki and Mr. K. Daikatsu for elemental analyses.

[3/377 *Received*, 19*th February*, 1973]