# Verticillin A, a New Antibiotic from *Verticillium sp.*

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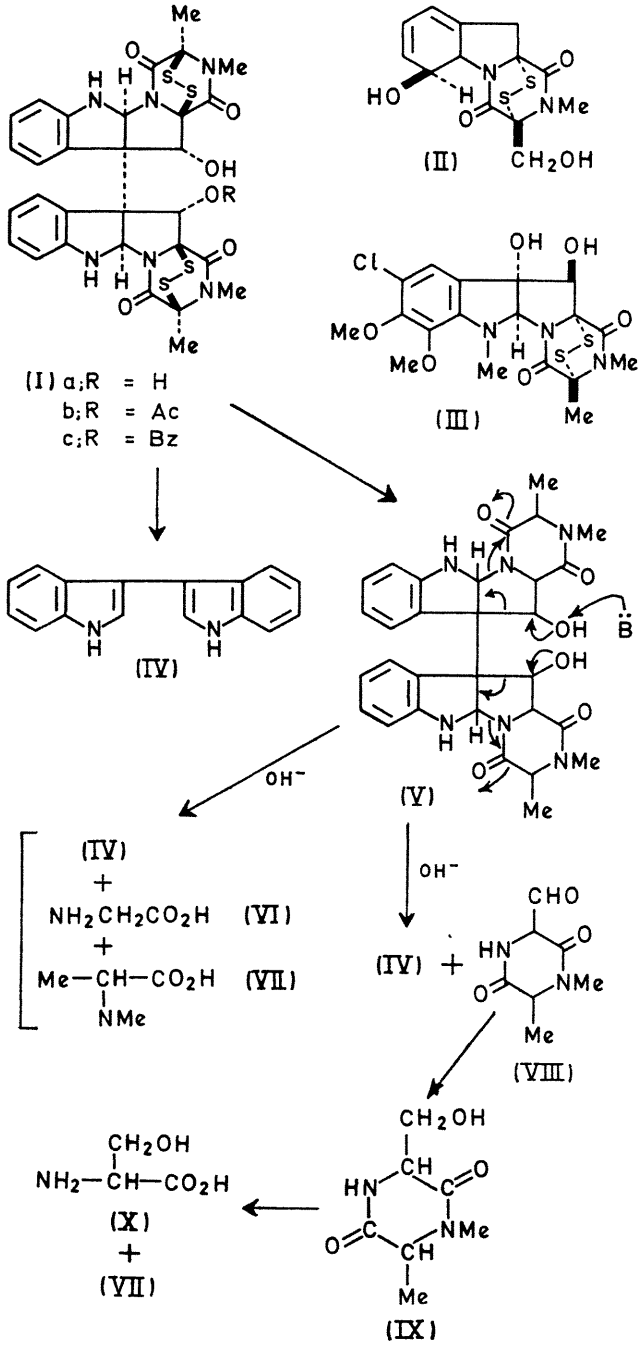
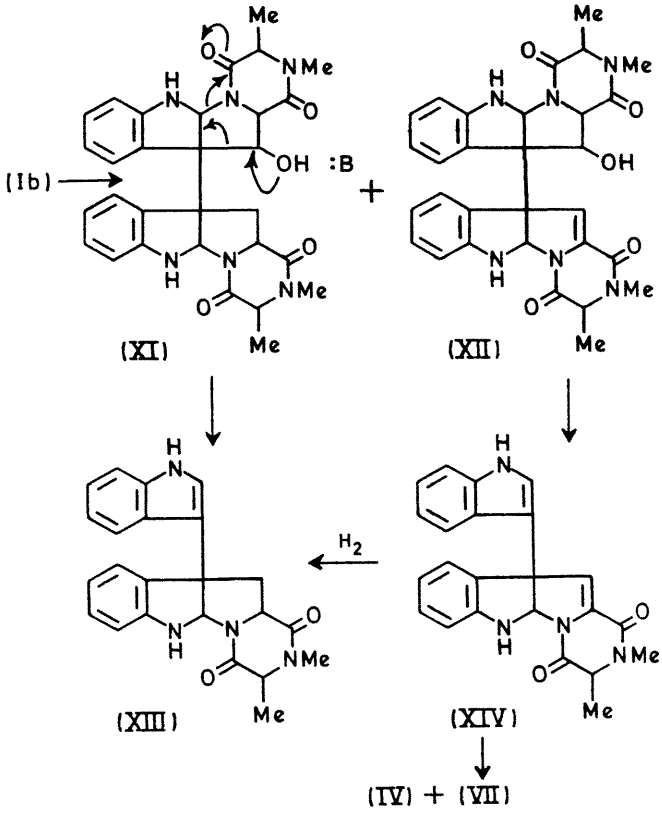
*Summary* The stereostructure of a new antibiotic, verticillin A has been elucidated by chemical and physicochemical methods.

A SPECIES of *Verticillium* (strain TM-759), an imperfect fungus isolated from a basidiocarp of *Coltricia cinnamomea* (*Polystictus cinnamomeus*), produced a new antibiotic, verticillin A (Ia),[1] the acetate of which was used for biological evaluation. Antimicrobial activity was found against Gram-positive bacteria and mycobacteria but not against Gram-negative bacteria and fungi The cytotoxicity effect ($ED_{50}$) against HeLa cells was 0·2 $\gamma$/ml.




Verticillin A (Ia), $C_{30}H_{28}O_6N_6S_4$,† $[\alpha]_D$ +703·7°, $\nu_{max}$ (Nujol) 3420 and 3335 (OH and NH), 1703, 1694, and 1675 (acid amide) $cm^{-1}$, $\lambda_{max}$ (dioxan) 306 nm ($\epsilon$ 5960), showed an ion at *m/e* 64 due to the loss of $S_2$ [2] in the mass spectrum and four Cotton effects, at 236 nm ([$\theta$] + 331,400), 272 (−25,000), 307 (+57,500), and 375 (−2750) in the c.d. The presence of acid amide bands in the i.r. in conjunction with the positive Cotton effect at 236 nm indicates the presence of a dioxopiperazine moiety[3,4] in verticillin A. The Cotton effect at 272, 307, and 375 nm are assigned to the disulphide chromophore,[5] the presence of which is supported by the mass spectrum. These data suggest that verticillin

† Verticillin A was obtained as pale yellow plates, $C_{30}H_{28}O_6N_6S_4.CHCl_3$, m.p. 199—213° (decomp.) (from chloroform), as pale yellow needles, $C_{30}H_{28}O_6N_6S_4.\frac{2}{3}C_5H_5N$, m.p. 202—217° (decomp.) (from pyridine), or as a pale yellow amorphous powder, $C_{30}H_{28}O_6N_6S_4$, m.p. 203—214° (decomp.) (from tetrahydrofuran). Elemental analyses for solvent-free samples were obtained, after quantitative analyses of solvated crystals. Analyses were confirmed by differential thermal analysis; the molecular weight, 674, was measured by the vapour pressure–osmometry method.

A has the same disulphide-bridged dioxopiperazine system as gliotoxin[6] (II) or sporidesmin[7] (III).

The n.m.r. spectrum,‡ of verticillin A has $\tau$ 8·22 (s,Me), 7·15(s, *N*-Me), 4·25 (1H, s, >C*H*OH), and 3·80 (1H, s, N–C*H*-N). Moreover, on being heated under reflux with 5% potassium hydroxide in dioxan–water for 2·5 h verticillin A gave 3,3′-bi-indolyl[8] (IV) in *ca.* 50% yield. These results and the empirical formula, show that verticillin A must have a symmetrical dimeric structure.

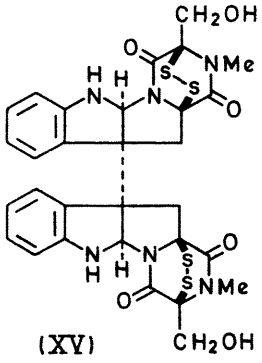
When verticillin A (Ia) was reduced with aluminium amalgam,[9] it gave a dethio-derivative (V), $M^+$ 572, which was hydrolysed with 5% potassium hydroxide under reflux for 3·5 h to give 3,3′-bi-indolyl (IV), glycine (VI), and *N*-methylalanine (VII). On the other hand, treatment of (V) with the same alkali at room temperature, gave two components, (IV) and a dioxopiperazine derivative (VIII), 2,4-dinitrophenylhydrazone, m.p. 220—230°), in quantitative yield. Compound (VIII) was reduced with sodium borohydride to give an alcohol (IX), m.p. 217—220°, which was hydrolysed with 6N-HCl to give serine (X) and *N*-methylalanine (VII).

Moreover, on aluminium amalgam reduction, verticillin A acetate§ (Ib), $C_{32}H_{30}O_7N_6S_4$, m.p. 220—243° (decomp.) gave two dethio-derivatives, (XI), $M^+$ 556 and (XII), $M^+$ 554, which were hydrolysed with 5% potassium carbonate at room temperature to give (XIII), m.p. 252—254° and (XIV), respectively, together with (VIII). When compound (XIV), which was converted into (XIII) by hydrogenation, was heated at 160° for 12 h in 5% potassium hydroxide–ethylene glycol, it gave (IV) and *N*-methylalanine (VII). From these results, it is concluded that verticillin A has the formula (Ia).

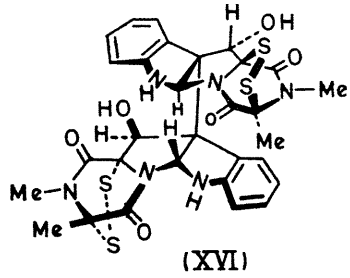
As c.d. data of (Ia) were antipodal to those of gliotoxin[4] (II), sporidesmin[6] (III), and aranotin,[10] which had the *R*-configuration on the two asymmetric centres in the dioxopiperazine ring, (Ia) should have the *S*-configuration.

The structural elucidation of chaetocin (XV) by chemical and *X*-ray methods has been reported[11]. Since 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 should be represented by the formula (XVI).

It remained only to assign the configuration of the hydroxy group. In the i.r. study, (Ia) shows a hydrogen-bonded H–O stretching frequency at 3425 $cm^{-1}$ due to two bonded hydroxy groups ($A \times 10^{-4}$, 6·64), a hydrogen-bonded carbonyl band at 1669 $cm^{-1}$ ($A \times 10^{-4}$, 9·96), and



(XV)


(XVI)

another carbonyl band at 1691 $cm^{-1}$ ($A \times 10^{-4}$, 9·27), whereas the monoacetate (Ib) shows one bonded hydroxy group at 3425 $cm^{-1}$ ($A \times 10^{-4}$, 4·00) one bonded carbonyl group at 1671 $cm^{-1}$ ($A \times 10^{-4}$, 6·11) and three other carbonyl groups at 1693 $cm^{-1}$ ($A \times 10^{-4}$, 19·65). These results indicate the presence of hydrogen bonding to the oxygen of the carbonyl group. Moreover, verticillin A monobenzoate (Ic) shows a Cotton effect at 223 nm ($[\theta]$ +50,000) due to the benzoate group. The configuration of the hydroxy groups is, therefore, thought to be $\alpha$ as shown in the formula (I) by application of the benzoate sector rule.[12] From these results, the absolute configuration of verticillin A can be represented by the formula (XVI).

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‡ For the solution in $C_5D_5N$–$D_2O$.
§ Verticillin A gave only a monoacetate (Ib) with $Ac_2O$–$C_5H_5N$ at room temperature.

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