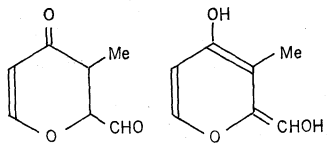
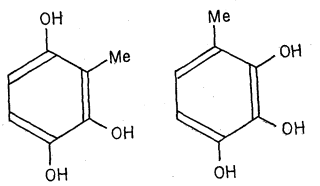
THE STRUCTURE OF THE ANTIFUNGAL ANTIBIOTIC VERSICOLIN

Sir:

The established metabolites of *Aspergillus versicolor* include numerous xanthones and anthraquinones, and a substituted imide[1]. Recently DHAR and BOSE[2] reported the isolation of a new antifungal antibiotic, named versicolin, from the culture filtrate of a strain of *A. versicolor*. Low yielding cultures of *A. versicolor* could be regenerated by mutagenic treatment[3]. Versicolin was notable because of its high specific activity against several human dermatophytes, particularly *Trichophyton rubrum* which causes 90% of the skin infections in eastern India[2,4]. It was essentially inactive against gram-positive and gram-negative bacteria, yeasts and a variety of other fungi[2,4]. Versicolin was subsequently assigned[5] a dihydro-γ-pyrone structure (I), which was considered to exist primarily as the enolic tautomer (II) in order to explain both spectroscopic data and functional group reactions. Re-interpretation of the published evidence[2,4,5] suggests that versicolin is 2,3,6-trihydroxytoluene (III), and this conclusion has been confirmed by synthesis.





From published data[2,4,5], versicolin is an optically inactive compound $C_7H_8O_3$, m.p. 125~126°C, very susceptible to oxidation either by air or with FEHLING's or TOLLENS' reagents, and containing unsaturation together with an enolic or phenolic hydroxyl group. Surprisingly in view of the proposed enolic ether forms (I and II) versicolin was stable towards acids, although unstable towards alkalis as expected. Despite a positive reaction with 2,4-dinitrophenylhydrazine, neither the infrared spectrum [ν_{max}^{Nujol} 3345 (OH), 1640 and 1600 cm^{-1} (C=C)] nor the proton magnetic resonance spectrum showed the presence of the aldehyde function expected from structure (I), and they were interpreted[5] in terms of the di-enolic tautomer (II). The p.m.r. spectrum[5,6] showed resonances at δ 2.08 (3H, s, $CH_3\overset{1}{C}=$), δ 3.1 (1H, bs), δ 7.3 (2H, m), and an AB quartet (J=8 Hz) at δ 6.18 and 6.52. However, the components of the AB system are too similar in chemical shift for the structure (II), in which the olefinic protons are in quite different environments[7]. Furthermore, there seems no reason why such a dienol (II) should be the preferred tautomeric form.

This evidence is in good agreement with a 1,2,3,4-tetrasubstituted benzene structure, (III) or (IV). Both III and IV are known; the former has m.p. 121.5°C, with a triacetate m.p. 91.5°C[8], whilst the latter has m.p. 142~144°C[9] (142°C[10], 140~141°C[11]). Versicolin has m.p. 125~126°C[2,4,5], and with zinc and acetic acid affords a compound m.p. 95°C, described as a diacetate $C_{11}H_{18}O_5$, ν_{max} 1740 and 1176 cm^{-1}, but for which no analytical data was presented[5]. Accordingly, 2,3,6-trihydroxytoluene (III) was synthesised by FLAIG, SALFELD and BAUME's method[8] from 2-methyl-resorcinol. The product, m.p. 123~125°C, had an infrared spectrum (in Nujol) identical with that published[4] for versicolin. The only significant differences between the p.m.r. spectrum of this product, which showed absorption[6] at δ 2.08 (3H, s), an AB quartet at δ 6.10 and 6.44 (J=8 Hz), and two resonances exchangeable with deuterium oxide at δ 6.87 (1H, bs) and δ 7.46 (2H, m), and that of versicolin occur in the chemical shifts of the exchangeable protons, which are concentration and temperature dependent[12]. The mass spectrum of synthetic III showed the intense ions at *m/e* 140 (M^+), 139 (M^+-H), 111 (M^+-CHO), 94, 65, and 39 described[5] for versicolin, in addition to the expected[13] ions at *m/e* 123 (M^+-OH), 122 (M^+-H_2O), and 121 (139^+-H_2O).

Versicolin is clearly 2, 3, 6-trihydroxytoluene (III). As described for versicolin[2,4], the quinol (III) gives a positive reaction with 2,4-dinitrophenylhydrazine, probably as a result of oxidation to a quinone under the reaction conditions. Such facile oxidation probably also explains the differences in the published ultraviolet spectra attributed to versicolin, which was originally[2] reported as having λ_{max}^{EtOH} 288 nm ($E_{1cm}^{1\%}$ 280), but which was subsequently[4,5] altered to λ_{max}^{EtOH} 222, 256, 390, and 520 nm (log ε 4.4, 3.9, 2.6 and 2.2 respectively). 2,3,6-Trihydroxytoluene (III) exhibits λ_{max}^{EtOH} 287 nm (log ε 3.44) immediately on dissolving in ethanol, but this maximum diminishes rapidly, particularly in the presence of traces of alkali, with the simultaneous development of maxima at 252 and 392 nm. An isobestic point at 276 nm relates successive spectra, and as expected the final spectrum resembles that of 2-hydroxy-3-methyl-*p*-benzoquinone[8], λ_{max}^{EtOH} 255 and 396 nm (log ε 4.17 and 3.16 respectively). In acidic solution 2,3,6-trihydroxytoluene is somewhat more stable, and initially shows $\lambda_{max}^{0.1N\ HCl}$ 284 nm (log ε 3.45) similar to that recorded[5] for versicolin, $\lambda_{max}^{0.1N\ HCl}$ 285 nm (log ε 3.37).

In contrast to the previous formulation (II), 2,3,6-trihydroxytoluene (III) is readily accessible by synthesis should versicolin prove of value in antifungal therapy. In view of the ease of oxidation of versicolin, its biological activity may in fact be due to the corresponding *p*-quinone rather than to the quinol itself. In *A. versicolor* versicolin probably arises biosynthetically by secondary decarboxylation and oxidation of a polyketide related to 6-methylsalicylic acid[14].

Acknowledgements

The author thanks Mr. C. ARANDJELOVIC for p.m.r. spectra and Mr. K. GOGGIN for mass spectra.

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(Received July 9, 1971)

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