

Structures of Cochlioquinones A and B, New Metabolites of *Cochliobolus miyabeanus*: Chemical and X-Ray Crystallographic Determination

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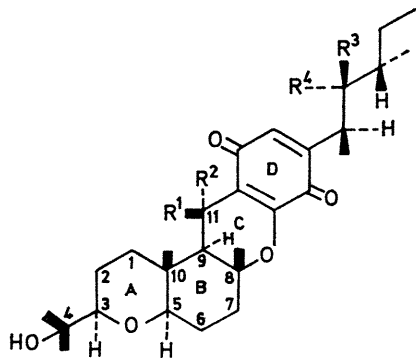
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Summary The structures of cochlioquinones A and B, new metabolites isolated from a strain of *Cochliobolus miyabeanus*, have been determined from chemical, spectroscopic, and crystallographic evidence, the main feature being a *p*-benzoquinone ring linked to a sesquiterpene moiety and to a C₇ side-chain.

Cochliobolus miyabeanus, a parasitic mould of rice, has been found to produce ophiobolins.¹ After growing a particular strain† of the same organism under suitable conditions² we isolated a mixture of yellow pigments, localized both in the mycelium and in the medium; they can be extracted with *n*-hexane and separated by fractional crystallization

followed by chromatography. Two main components were obtained; cochlioquinone A (I),[‡] C₃₀H₄₄O₈, m.p. 130—132° from *n*-hexane, *R_F* 0.47 on silica gel t.l.c., solvent chloroform–ethyl acetate (7:3), and cochlioquinone B (II), C₂₈H₄₀O₆, m.p. 168—169° from diethyl ether, *R_F* 0.56 in the same system.

The *p*-quinonoid nature of these compounds was evident on conventional chemical examination and on the basis of their u.v. spectra [λ_{\max} 398 nm in methanol for (I) and λ_{\max} 401 nm in methanol for (II)]. We report the chemical evidence which led us to assign (apart from stereochemistry) structures (I) to cochlioquinone A and (II) to cochlioquinone B, respectively. The presence in (I) of three additional rings was demonstrated from the absence of further unsaturation, beside the quinone moiety (chemical and n.m.r. evidence), and from the clarification of the chemical nature of the remaining six oxygens. In fact two of them belong to an acetoxy-group [present in the n.m.r. spectrum of (I) and absent in that of (IVa)]; one is a secondary allylic OH [characteristic downfield shift of the corresponding doublet in (III) and (V)]; one is a tertiary OH [formation of the corresponding acyl derivative, *e.g.* (V), with the more reactive 3-nitrobenzoyl chloride from (IV) and trichloroacetyl isocyanate³ from compound (IX)]; two are etheral oxygens (chemical inertness, i.r. and n.m.r. considerations). The structure of the open side-chain of (I) was demonstrated by the isolation of two degradation products; oxidation of (I) both with alkaline KMnO₄ and H₂O₂ followed by methylation yielded respectively C₂H₅·CH(CH₃)·CH(OAc)·CH(CH₃)·CO₂CH₃ and C₂H₅·CH(CH₃)·CH(OAc)·CH(CH₃)·C(CO₂CH₃):CH·CO₂CH₃ (structures secured by high-resolution mass spectrometry, n.m.r., and n.m.d.r.).

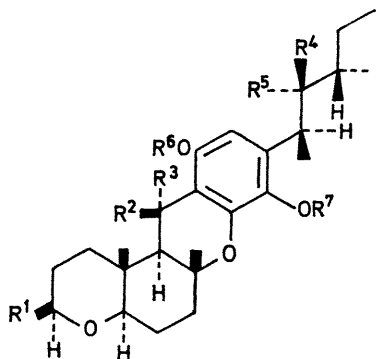


- (I) R¹ = R³ = H; R² = OH; R⁴ = OAc
 (II) R¹ = R² = H; R³R⁴ = =O
 (III) R¹ = R³ = H; R² = R⁴ = OAc
 (VI) R¹R² = =O; R³ = H; R⁴ = OAc

† *Helminthosporium oryzae* Breda de Haan 3.179 of Mycotheca Universitatis Catholicae Sacri Cordis.

‡ Elemental analysis and/or mass-spectrometric measurements of all the reported compounds were in agreement with the structures shown.

The presence of only one quinonic H, *ortho* to the above side-chain, was confirmed by n.m.r. spectrum of (II). The nature and the mutual relationships of substituents on the cyclized C₁₅ side-chain were based on n.m.r., n.m.d.r., and tickling in various solvents and/or at various temperatures of compounds (III) to (XIII) prepared by standard techniques. Carbonyl i.r. frequencies (1685–1695 cm⁻¹ in CCl₄) of ketones (VI), (VIII), and (X), demonstrated that ring c was six-membered. Accordingly, one of the phenolic functions in (IV) is rather hindered and this property has

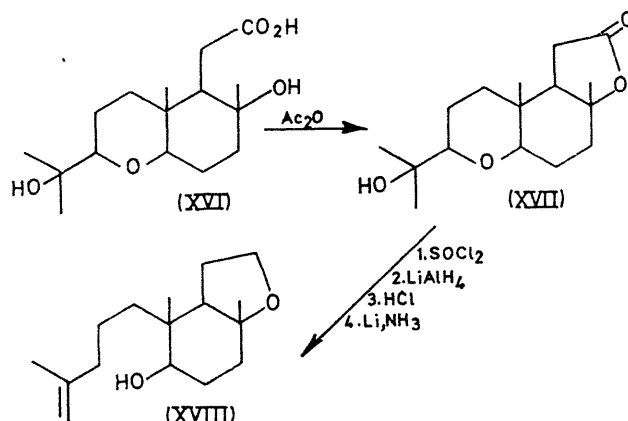


- (IV) R¹ = CMe₂OH; R² = R⁴ = R⁶ = R⁷ = H; R³ = OH
R⁵ = OAc
(IVa) R¹ = CMe₂OH; R² = R⁴ = R⁶ = R⁷ = H; R³ = R⁵
= OH
(V) R¹ = CMe₂·O·CO(3-NO₂)C₆H₄; R² = R⁴ = H; R³ = O·CO·
(3-NO₂)C₆H₄; R⁵ = OAc; R⁶ = R⁷ = CO(3-NO₂)C₆H₄
(VII) R¹ = CMe₂OH; R² = R⁴ = H; R³ = OH; R⁵ = OAc;
R⁶ = Me; R⁷ = Ac
(VIII) R¹ = CMe₂OH; R², R³ = O; R⁴ = H; R⁵ = OAc;
R⁶ = Me; R⁷ = Ac
(IX) R¹ = CMe₂OH; R² = R⁴ = H; R³ = OH; R⁵ = OAc;
R⁶ = R⁷ = Me
(X) R¹ = CMe₂OH; R², R³ = O; R⁴ = H; R⁵ = OAc;
R⁶ = R⁷ = Me
(XI) R¹ = CMe₂OH; R² = R⁴ = H; R³ = Δ⁹⁽¹¹⁾; R⁵ = OAc;
R⁶ = R⁷ = Me
(XIa) R¹ = CMe₂OH; R² = R⁴ = H; R³ = Δ⁹⁽¹¹⁾; R⁵ = OH;
R⁶ = R⁷ = Me
(XII) R¹ = CMe₂CH₂; R² = R⁴ = H; R³ = Δ⁹⁽¹¹⁾; R⁵ = OAc;
R⁶ = R⁷ = Me
(XIII) R¹ = Ac; R² = R⁴ = H; R³ = Δ⁹⁽¹¹⁾; R⁵ = OAc;
R⁶ = R⁷ = Me
(XIV) R¹ = CMe₂OH; R² = R⁴ = H; R³ = OH; R⁵ = OAc;
R⁶ = Me; R⁷ = *p*-iodobenzoyl
(XV) R¹ = CMe₂OH; R² = R⁴ = H; R³ = OH; R⁵ = OAc;
R⁶ = Me; R⁷ = *p*-iodobenzenesulphonyl

been exploited for preparing compounds (XIV) and (XV), this last being the more suitable for X-ray analysis. Moreover, (I) was transformed into (II) through appropriate reactions involving hydrogenolysis of the allylic OH, elimination of the acetyl group, and oxidation of the resulting secondary alcohol to a ketone. Alkaline H₂O₂ oxidation of (II) yielded mainly (XVI) which was transformed into the γ -lactone (XVII) (i.r. 1770 cm⁻¹ in CS₂). This product was converted into (XVIII), the length of the side-chain of which was demonstrated by the appearance of an ion at *m/e* 82 in its mass spectrum. The sesquiterpene origin of this moiety was confirmed by biosynthetic experiments. Structures (I) and (II), apart from the stereochemistry and *p*-relationship of the two side-chains, are therefore the correct structures for cochlioquinones A and B. The mutual relationship of the two side-chains was assigned by analogy

with helicobasidin,⁴ tauranin,⁵ and siccanin and the siccano-chromenes.⁶

In order to solve the remaining ambiguities and to determine the stereochemistry, compound (XV) was used as the most suitable heavy-atom derivative for the X-ray analysis of (I). Crystals of (XV) are rhombohedral, *a* = 41.181, *c* = 6.683 Å. Space group R3, Z = 9. Crystal data were determined, and intensity measurements made, on a Siemens AED diffractometer (Mo-K α radiation, λ taken as 0.7107 Å).



The co-ordinates of the iodine atom were determined from a three-dimensional Patterson function, sharpened to point-atoms at rest. The positions of a further thirty atoms were then deduced from a three-fold superposition minimum function, based on the iodine-iodine vectors. The remaining atoms were subsequently found in series of Fourier syntheses, and the whole structure refined by block-diagonal least-squares to a current set of atomic co-ordinates and isotropic thermal parameters corresponding to an *R* value of 0.08. These co-ordinates define bond lengths which deviate by a maximum of 0.14 Å (in two cases) and by an average of 0.04 Å from acceptable values for formula (XV). Additional crystallographic evidence was taken into account in order to establish structure (XV) unambiguously. The full stereochemistry of the molecule can be seen in the general projection of the structure given in the Figure, which shows the relative configuration of the

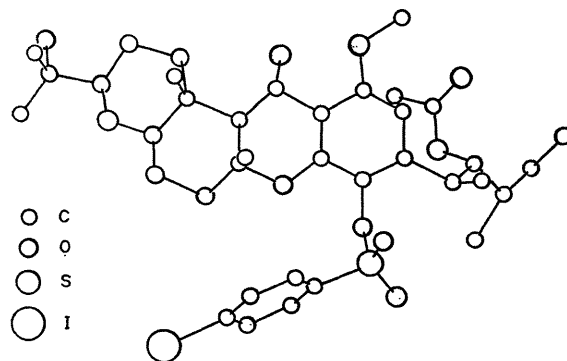


FIGURE. General projection of the structure of compound (XV).

asymmetric carbon atoms and many other configurational and conformational details

In the crystal, the molecules form a continuous spiral around the three-fold axes, composed of three separate hydrogen-bonded chains about each such axis

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