The Structure and Biosynthesis of Hinnuliquinone, A Pigment from *Nodulisporium hinnuleum*

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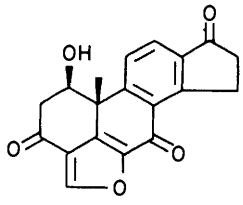
The pigment, hinnuliquinone, isolated from the fungus *Nodulisporium hinnuleum*, has been shown to be 2,5-bis[2-(1,1-dimethylprop-2-enyl)-1*H*-indol-3-yl]-3,6-dihydroxycyclohexa-2,5-diene-1,4-dione. It is biosynthesized from tryptophan and mevalonic acid.

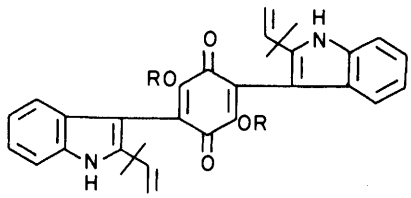
The fungus, *Nodulisporium hinnuleum* (ACC 3199), produces the steroidal antibiotic, demethoxyviridin (1),[1] when grown on a surface culture on a Raulin-Thom medium. In the course of biosynthetic studies [2] on this metabolite, we have isolated a new fungal pigment, hinnuliquinone (2), the structure of which has formed the subject of a preliminary communication.[3] In this paper we present the full evidence for its structure and some further evidence for its biosynthesis. The initial vegetative mycelial growth of the fungus is white but after *ca.* 10 days growth on surface culture sporulation occurs and it becomes black. Extraction of the mycelium with acetone or chloroform after 20 days growth afforded demethoxyviridin (1), often directly, whilst chromatography of the mother liquors gave the dark-red pigment, hinnuliquinone (2), $C_{32}H_{30}N_2O_4$, M^+ 506. The i.r. and u.v. spectra [$\nu_{max.}$ 3 410, 3 330, and 1 640 cm^{-1}, $\lambda_{max.}$ 280 (log ε 4.44), 288 (4.24), and 506 nm (2.98)] established the hydroxyquinonoid nature of the pigment. On reaction with acetic anhydride in pyridine, the pigment gave a diacetate (3), $C_{36}H_{34}N_2O_6$, whilst treatment with methanolic diazomethane gave a dimethyl ether (4), $C_{34}H_{34}N_2O_4$. These compounds retained the NH absorption ($\nu_{max.}$ 3 380 cm^{-1}) in their i.r. spectra. The dimethyl ether (4) underwent reductive acetylation with zinc and acetic acid to give a leuco-derivative (5), $C_{38}H_{40}N_2O_6$, M^+ 620 [$\lambda_{max.}$ 280 (log ε 3.32) and 288 nm (3.29)]. Addition of alkali to an ethanolic solution of the pigment caused the appearance of a strong band in the u.v. at 322 nm (log ε 4.31) typical of a 2,5-dihydroxy-*p*-benzoquinone.[4] In addition to aromatic signals (δ 7.03—7.42), the ^1H n.m.r. spectrum (determined in $CDCl_3$) of the pigment and its derivatives contained singlet signals (δ 1.49) assigned to four CMe groups and an ABX system typical of two vinyl groups (δ 5.02, 5.24, and 6.13, *J* 10 and 17 Hz). Although we were unable to observe the ^{13}C signals of the quinonoid moiety, the ^{13}C n.m.r. spectrum contained resonances which could be assigned to an isopentenyl system (δ 26.9, 39.2, 112.2, and 145.5 p.p.m.) and an indole [δ 128.5 (C-2), 99.4 (C-3), 128.4 (C-4), 119.3, 120.3, and 121.9 (C-5—C-7), 111.9 (C-8), and 134.8 (C-9)]. Typical of many quinones, the mass spectrum contained a strong $M + 2$ peak (508, 20%) and it showed an ($M - 69$) fragment corresponding to the loss of an isopentenyl unit.

Oxidation of the pigment with alkaline hydrogen peroxide gave 2-(1,1-dimethylprop-2-enyl)indole (6) identified by its ^1H n.m.r. spectrum.[5] The presence of a free β-indole position in (6) was revealed by a ^1H n.m.r. signal at δ 6.33 and indicated the position of attachment of the quinone ring. The unstable C-3 carboxylic acid (7) was also isolated from the oxidation. Indole-3-carboxylic acids are known to undergo ready decarboxylation.[6] This evidence leads to the structure (2) for hinnuliquinone.

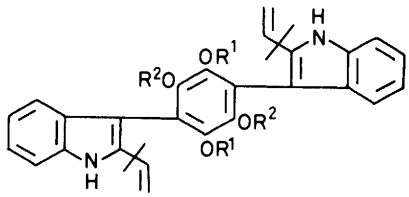
A plausible biogenesis of hinnuliquinone (2) involves transamination of tryptophan to form indolylpyruvic acid (8) or its equivalent, and self-condensation of two moles of the latter. This was supported in initial biosynthetic experiments



(1)



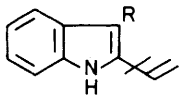
(2) R = H
(3) R = Ac
(4) R = Me



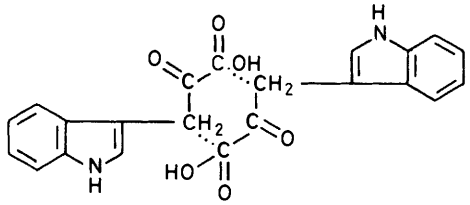
(5) R^1 = Ac, R^2 = Me

by the incorporation of [U-^{3}H]tryptophan (0.8%) and [2-^{14}C]mevalonate (0.2%) into the metabolite by *N. hinnuleum*. However the incorporation of [U-^{3}H]tryptophan did not define the origin of the *p*-benzoquinone ring. [1-^{14}C]Tryptophan was incorporated to a comparable extent (1.9%) and thus the C_3 side chain was also involved in the final metabolite. The possibility that free indol-3-ylpyruvic acid (8) was involved in the biosynthesis was examined by converting a sample of [3-^{14}C]tryptophan through a biosynthetically based transamination reaction [7] into the pyruvic acid. However the incorporation (0.35%) was disappointingly low and would not exclude a reversal of the transamination sequence and incorporation *via* another derivative of tryptophan. Attempts to mimic the self-condensation reaction in the laboratory were not successful.

Hinnuliquinone (2) is a member of a small group of prenylated bis(indolyl)-2,5-dihydroxybenzoquinone fungal pigments which also include asteriquinone [8] and cochliodinol.[9] A larger group of fungal benzoquinones are dimers of C_6—C_3 precursors.[10]



(6) R = H
(7) R = CO_2H



(8)

Experimental

Light petroleum refers to the fraction with b.p. 60—80 °C.

Isolation of Hinnuliquinone (2).—*Nodulisporium hinnuleum* (ACC 3199) [1] was grown on a surface culture (5 l) in Roux bottles (100-ml medium each) for 20 days on a Raulin-Thom medium. The mycelium was filtered, dried and extracted with acetone in a Soxhlet apparatus. The solvent was evaporated and the residue (20 g) triturated with light petroleum (3 × 100 ml) to remove lipid material. The remaining insoluble black solid (*ca.* 15 g) was taken up in hot acetone and treated with charcoal. The acetone was concentrated to afford demethoxyviridin (*ca.* 2 g). The mother liquors were concentrated and adsorbed onto silica (Merck 7736) and chromatographed. Elution with ethyl acetate–light petroleum (1 : 1) gave 2,5-*bis*[2-(1,1-*dimethylprop*-2-*enyl*)-1H-*indol*-3-*yl*]-3,6-*dihydroxycyclohexa*-2,5-*diene*-1,5-*dione* (hinnuliquinone) (2) (1.5 g) which crystallized from chloroform as red needles, m.p. 243 °C (Found: C, 75.65; H, 6.3; N, 5.5. $C_{32}H_{30}O_4N_2$ requires C, 75.9; H, 6.0; N, 5.5%), $\nu_{max.}$ 3 410, 3 300, and 640 cm^{-1}; $\lambda_{max.}$ (EtOH), 280 (log ε 4.44), 288 (4.24), and 506 nm (2.98); $\lambda_{max.}$ (EtOH–NaOH) 280 (log ε 4.27), 288 (4.29), 322 (4.31), and 516 nm (3.17); δ ($CDCl_3$; 90 MHz) 1.49 (12 H, d, *J* 2 Hz, CMe), 5.07 and 5.24 (4 H, m, C=CH_2), 6.13 (2 H, ddd, *J* 2, 10.8 and 17 Hz, CH=C), 7.03—7.42 (8 H, m, ArH), and 8.3 (2 H, br s, NH); *m/z* 508 (20%) (*M* + 2), 507 (16), 506 (45, M^+), 437 (30), 369 (100), 208 (13), 196 (11), 182 (20), 181 (24), 180 (26), 170 (22), 168 (17), 167 (22), 158 (18), 156 (10), 130 (11), and 69 (43). The *methyl ether* (4), prepared in methanol with ethereal diazomethane, crystallized from methanol as dark red plates, m.p. 284 °C (Found: C, 76.4; H, 6.5; N, 5.1. $C_{34}H_{34}N_2O_4$ requires C, 76.4; H, 6.4; N, 5.2%); $\nu_{max.}$ 3 380, 1 655, and 1 608 cm^{-1}; δ ($CDCl_3$) 1.50 (12 H, br s, CMe), 3.68 (6 H, s, OMe), 5.06 and 5.23 (4 H, m, C=CH_2), 6.14 (2 H, dd, *J* 11 and 17 Hz, CH=C), and 6.95—7.58 (10 H, m, ArH and NH); $\lambda_{max.}$ 280 (log ε 4.51), 287 (4.49), and 488 nm (3.01); *m/z* 536 (*M* + 2, 50%), 535 (30), 534 (90), 465 (20), and 396 (100). The *acetate*, prepared with acetic anhydride in pyridine, crystallized from acetone as purple rods, m.p. 240 °C (Found: C, 73.4; H, 5.9; N, 4.7. $C_{36}H_{34}N_2O_6$ requires C, 73.2; H, 5.8; N, 4.7%); $\nu_{max.}$ 1 775 and 1 670 cm^{-1}; $\lambda_{max.}$ 268 (log ε 4.4), 275 (4.39), 286 (4.33), and 536 nm (3.26); δ 1.40 (12 H, s, CMe), 1.75 (6 H, s, OAc), 4.96 and 5.25 (4 H, m, C=CH_2), 6.15 (2 H, dd, *J* 11 and 17 Hz, CH=C), 6.8—7.5 (8 H, m, ArH), and 8.0 (2 H, m, NH).

Reductive Acetylation of the Methyl Ether (4).—The dimethyl ether (4) (25 mg) in acetic anhydride (10 ml) was treated with an excess of zinc dust and sodium acetate and heated under reflux for 1½ h under nitrogen. Water was added and the mixture was filtered through Celite and extracted with ethyl acetate. The solvent was evaporated and the residue crystallized from ethanol to afford the *leuco-compound* (5) as needles, m.p. 310 °C (Found: C, 72.5; H, 6.7; N, 4.4. $C_{38}H_{40}N_2O_6$ requires C, 72.7; H, 6.2; N, 4.5%); $\nu_{max.}$ 3 460 and 1 730 cm^{-1}; $\lambda_{max.}$ 280 (log ε 3.32) and 288 nm (3.29); δ [$(CD_3)_2CO$] 1.46 (12 H, s, CMe), 1.70 (6 H, s, OAc), 3.23 (6 H, OMe), 4.99 (2 H, dd, *J* 1.8 and 10.8 Hz), 5.05 (2 H, dd, *J* 1.8 and 18 Hz), 6.20 (2 H, dd, *J* 10.8 and 18 Hz), and 6.85—7.5 (10 H, m, ArH and NH); *m/z* 620 (100%), 578 (17), 536 (80), 467 (10), 399 (25), 310 (8), 69 (35), and 43 (55).

Oxidative Cleavage of Compound (2).—Hinnuliquinone (200 mg) was suspended in 0.1M-sodium hydroxide (40 ml) and treated with 30% hydrogen peroxide (20 ml) at room temperature for 5 h. The solution was acidified with dil. hydrochloric acid, and the product was recovered in ethyl acetate as a red gum (120 mg). Preparative t.l.c. in ethyl acetate–light petroleum (1 : 1) gave 2-(1,1-dimethylprop-2-enyl)indole (6) [5] as a yellow oil (30 mg) which went red on standing in air, δ ($CDCl_3$) 1.48 (6 H, s, C–Me), 5.03 and 5.19 (2 H, m, C=CH_2), 6.08 (1 H, dd, *J* 10 and 18 Hz), 6.33 (1 H, d, *J* 2 Hz), 7.03—7.7 (4 H, m, ArH), and 7.9 (1 H, br m, NH); $\nu_{max.}$ 3 420 cm^{-1} (NH). The lower band gave 2-(1,1-dimethylprop-2-enyl)indole-3-carboxylic acid (20 mg) (7) as unstable needles, $\nu_{max.}$ 3 440, 3 200—2 800br, and 1 665 cm^{-1}; $\lambda_{max.}$ 286 (log ε 3.07) and 278 nm (3.06).

Biosynthetic Experiments.—(*a*) DL-Tryptophan (^{3}H-generally labelled) (3.51 × 10^8 d.p.m.) in aqueous ethanol (10 ml) was distributed between 49 8-day old cultures (100 ml each) of *Nodulisporium hinnuleum*. The fermentation was harvested after a further 7 days and the hinnuliquinone (300 mg) was isolated as described previously. It was converted into the leuco-compound (5) as above. This showed 6 930 d.p.m. mg^{-1} corresponding to an incorporation of 0.8%.

(*b*) [2-^{14}C]Mevalonic acid lactone (0.1 mC) in ethanol (5 ml) was distributed between 15 cultures as above and the hinnuliquinone (100 mg) isolated and converted into the leuco-compound (5) which showed 4 220 d.p.m. mg^{-1} corresponding to an incorporation of 0.19%.

(*c*) [3-^{14}C]-DL-Tryptophan (25 μC) was diluted with tryptophan (500 mg) and converted into indol-3-ylpyruvic acid (44 mg, 2.78 × 10^6 d.p.m.) following the literature procedure.[7] This was dissolved in ethanol (1 ml) and distributed equally between five cultures (100 ml each) of *Nodulisporium hinnuleum* 10 days after inoculation. After a further 8 days the hinnuliquinone (52 mg) was isolated and converted into the leuco-compound (5). The latter possessed 189 d.p.m. mg^{-1} corresponding to an incorporation of 0.35%.

Acknowledgements

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