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FUNGAL METABOLITES—III[1]

QUINONES FROM *FUSARIUM SOLANI* D_2 PURPLE AND STRUCTURE OF (+)-SOLANIOL[2]

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Abstract—The known quinone pigments javanicin (I), bostrycoidin (II) and fusarubin (III), and a new compound, (+)-solaniol, have been isolated from cultures of *Fusarium solani* D_2 purple. The quinonoid structure IV has been assigned to (+)-solaniol on the basis of physical methods, and proven by synthesis of (±)-solaniol.

WEISS and NORD[3] have reported the presence of several pigments in cultures of *Fusarium solani* D_2 purple. One of these was isolated and identified as javanicin (Fig. 1, I), but the remainder were not investigated. Further work on this group of pigments has now led to the characterization of three additional naphthoquinone derivatives. Javanicin crystallized from the n-hexane extract of the mycelium, as reported by Weiss and Nord.[3] Aside from lipids and javanicin, the n-hexane soluble fraction also contained small amounts of the three other pigments found in the culture filtrate. The culture filtrate afforded bostrycoidin[1] (Fig. 1, II), fusarubin[4] (Fig. 1, III), and a new compound for which the name solaniol is suggested. Javanicin was also present in the culture filtrate but was not isolated.

Since the total amount of (+)-solaniol, m.p. 190–194° dec, isolated was only 89 mg, no attempt was made to determine its structure by degradative experiments or preparation of derivatives. The structure of solaniol was established solely by physical methods and proven by synthesis.

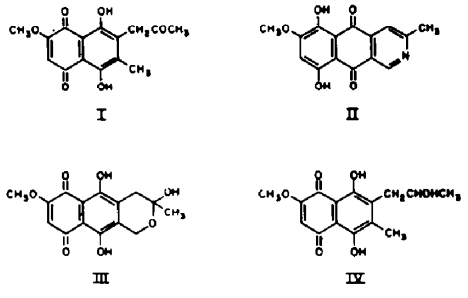

FIG. 1 Structure of quinone pigments found in cultures of *Fusarium solani* D_2 purple.

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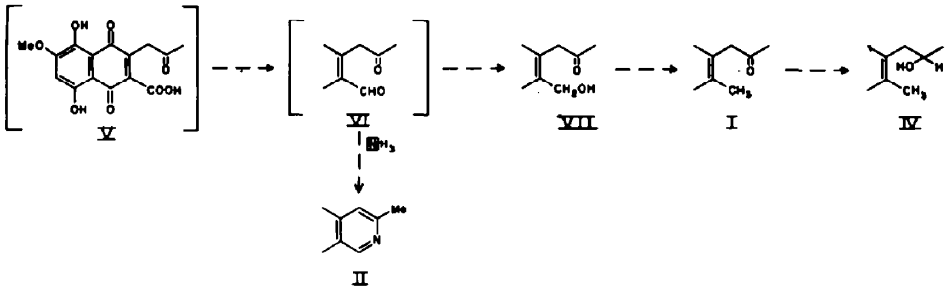

FIG. 2 Possible biogenetic relationship of pigments in *F. solani* D_2 purple.

(+)-Solaniol was easily decolorized by sodium dithionite and the reduced product was readily oxidized by air, a behavior indicating that the metabolite is a quinone. The UV-visible spectra of (+)-solaniol, fusarubin and javanicin were almost superposable, suggesting that (+)-solaniol is a substituted naphthazarin differing from fusarubin and javanicin only in the kind of substituents.

(+)-Solaniol has a mol wt of 292 and an elemental composition of $C_{15}H_{16}O_6$ as shown by mass spectrometry. Partial mass spectral data are listed in Table 1. Elemental

TABLE 1. DATA FOR SELECTED IONS IN MASS SPECTRUM OF (+)-SOLANIOL

m/e	$\%\Sigma_{28}$	Measured mass[a]	Calculated mass[b]	Error (ppm)	Elemental composition[b]
292	3·8	292·0948	292·0947	0·3	$C_{15}H_{16}O_6$
248	9·8[c]	248·0687	248·0685	0·8	$C_{13}H_{12}O_5$
45	3·2	45·03402	45·03404	0·4	C_2H_5O

[a] By peak-matching method.

[b] J. H. Beynon and A. E. Williams, *Mass and Abundance Tables for Use in Mass Spectrometry*. Elsevier Publishing Company, Amsterdam (1963).

[c] Most intense peak of spectrum.

analyses of (+)-solaniol led to an erroneous elemental composition as was the case for spinochrome D,[5] another substituted naphthazarin. Repeated elemental analyses of (+)-solaniol which may have led to the correct values were not carried out because of the limited quantity of sample available.

The mol wt of (+)-solaniol is 2 a.m.u. larger than that of javanicin and the two compounds differ in elemental composition by two hydrogens. A comparison of the IR spectra of (+)-solaniol and javanicin suggests that the ketone CO in javanicin is reduced to an OH in (+)-solaniol, accounting for the difference in elemental composition between the two compounds. Thus, javanicin shows no OH absorption above 3100 cm^{-1} because of intramolecular hydrogen-bond formation between the phenolic OH's and the quinone CO's; ketone CO absorption is at 1717 cm^{-1} and intramolecularly hydrogen-bonded quinone CO's absorb at 1602 cm^{-1}. By contrast

(+)-solaniol shows a broad OH absorption band with maxima at 3350 and 3280 cm^{-1} and a single absorption band in the CO region at 1602 cm^{-1}. The presence of an asymmetric centre at the carbinol group would account for the optical activity of (+)-solaniol which is unique among the pigments of *F. solani* D_2 purple in having this property.

The tentative conclusion that the ketone CO in javanicin is reduced to a secondary OH in (+)-solaniol is supported by the fact that (+)-solaniol has three active hydrogens whereas javanicin has only two, as shown by exchange of the active hydrogens with deuterium followed by mass spectrometric analysis. The mass spectrum of (+)-solaniol provides evidence for the presence of a 2-hydroxypropane side chain in the molecule. Cleavage of the C_α—C_β bond (C_α being next to the ring) with retention of the positive charge at the C atom bearing the O atom accounts for the abundant peak observed at *m/e* 45. On the other hand, loss of the same two C atoms via a McLafferty rearrangement with charge retention on the ring would lead to *m/e* 248 which is the most abundant ion in the spectrum. The elemental composition of the ions at *m/e* 45 and *m/e* 248 is in agreement with the fragmentation pathways suggested above.

In support of the proposed structure IV for (+)-solaniol the PMR spectrum shows the presence of a quinonoid ring proton at τ 3·85 (1H, s),* MeO protons at τ 6·08 (3H, s), intramolecularly hydrogen-bonded phenolic protons at τ −3·28 and −2·97 (1H each, both s's), benzylic protons at τ 7·67 (3H, s) and at τ 7·08 (2H, d with spacing of 6 Hz), a methine proton at τ 5·80 (1H, m), a OH proton at τ 6·08 (1H, s) and aliphatic Me protons at τ 8·67 (3H, d with spacing of 6 Hz). After shaking a deuteriochloroform solution of (+)-solaniol with deuterium oxide, the relative intensity of resonance signals at τ −3·28, −2·97 and 6·08 decreased with respect to other signals in the spectrum, thus indicating the presence of three readily exchangeable protons.

Structure IV deduced for (+)-solaniol on the basis of physical evidence was proved to be correct by synthesis of (±)-solaniol. Javanicin was reduced with sodium borohydride and the resulting hydroquinone oxidized in air. (±)-Solaniol, m.p 186–189° dec, was isolated in 25% yield from the crude reaction mixture by preparative TLC. The synthetic product and the material isolated from *F. solani* D_2 purple were identical except for slight differences in m.p. and solubility both of which may be due to the racemic nature of the synthetic material.

The presence of four quinone pigments in *F. solani* D_2 purple raises the question of their biogenetic relationship. Fig. 2 shows a possible biosynthetic pathway for the four compounds and is an extension of the pathway in *F. javanicum* proposed by Gatenbeck and Bentley.[6] The acid V or its biological equivalent derived by the acetate–malonate pathway may undergo stepwise reduction yielding in succession the aldehyde VI, fusarubin (VII) (drawn as the hydroxyketone rather than the hemiketal III), javanicin (I) and (+)-solaniol (IV). The aldehyde VI or its biological equivalent could lead to bostrycoidin (II) as suggested earlier.[1] The acid V and aldehyde VI have not so far been isolated as natural products.

EXPERIMENTAL

M.ps were taken on a Kofler micro hot stage. UV-visible spectra were measured in p-dioxane using a Beckman DK-2 recording spectrophotometer. IR spectra were determined on KBr discs with a Perkin–

* s = singlet; d = doublet; m = multiplet, etc.

Elmer model 237 spectrophotometer. PMR spectra were taken in $CDCl_3$ on a Varian A60 spectrometer using TMS as internal standard. Mass spectra were determined with a CEC 21-110B mass spectrometer equipped with a direct sample introduction system, and operated at a source temp of 150°, an ionizing potential of 70 ev and an ionizing current of 100 μa. Optical rotation was measured on a Rudolph Automatic Recording Spectropolarimeter. Analytical TLC was carried out on silica gel G layers (SGG) with $CHCl_3$–MeOH–AcOH (94:1:5) as developer or on polyamide layers (PA) with MeOH as developer. Preparative TLC was carried out on silica gel G plates (20 cm × 20 cm × 2 mm thickness) using 3 passes of 1% AcOH in $CHCl_3$ as developer. Microanalyses were performed by Pascher Mikroanalytisches Laboratorium, Bonn, Germany. The source of authentic samples of javanicin, fusarubin and bostrycoidin has been given.[1,7]

Isolation of quinone pigments

The isolate of *F. solani* D_2 purple used in this work and culture conditions were the same as those used by Weiss and Nord.[3] The yields given below are based on 20 l. of culture medium.

(a) *Javanicin* (I). The culture was filtered and the mycelium washed with water, freeze-dried (yield 160 g) and extracted (Soxhlet, 24 hr) with n-hexane (3·5 l.). The red crystalline material which separated out during extraction was filtered (yield 2·3 g) and was shown by TLC to be javanicin with a trace of fusarubin. Two recrystallizations from EtOH afforded 1·6 g of javanicin as red needles with metallic lustre, m.p. 206–210° dec. Identity with an authentic sample was established by TLC (SGG) and comparison of IR spectra. The n-hexane soluble fraction of the extracts contained mostly lipids but was also shown by TLC on SGG to contain small amounts of compounds I to IV (inclusive).

(b) *Bostrycoidin* (II). The culture filtrate (pH 6·95) was extracted with ether (4 × 5 l.). The combined ether extracts were concentrated to 6 l., and back-extracted with 1% $NaHCO_3$aq (2 × 2 l.) and water (2 l.). The aqueous extracts were discarded and the ether phase extracted with 2N HCl (4 × 1 l.). The aqueous phase was cooled to 0° and 50% NaOHaq (416 ml) at 0° was added. The pigment was extracted from the aqueous phase with ether (3 × 1 l.). The ether extracts were dried over Na_2SO_4, concentrated to 20 ml and allowed to crystallize at room temp overnight: yield 106 mg. The product was recrystallized once from $CHCl_3$–n-hexane and twice from ether (Soxhlet) to yield 60 mg of dark brown needles m.p. 241–243° dec. The identity of this compound was established by direct comparison (m.p., mixed m.p., TLC on SGG and PA, UV-visible and IR spectra) with authentic bostrycoidin. TLC on SGG of the ether extract of a freshly prepared culture filtrate showed that the pigments bostrycoidin, fusarubin, javanicin and (+)-solaniol were present. The possibility that bostrycoidin was inadvertently synthesized during the isolation procedure was thus eliminated.

(c) *Fusarubin* (III). The ether phase remaining after extraction with 2N HCl was extracted with 1N Na_2CO_3 (4 × 1 l.). The ether phase was discarded, and the aqueous extracts were acidified to pH 7 with conc HCl and extracted with ether (3 × 1 l.). The ether extracts were dried over Na_2SO_4 and concentrated to 20 ml. The tarry ppt was crystallized and the remaining ether evaporated to dryness. The residue was refluxed with 50 ml $CHCl_3$ and the suspension filtered. The insoluble residue was recrystallized from $CHCl_3$ (Soxhlet) affording 98 mg of fusarubin identical with authentic material by TLC on SGG and IR spectroscopy.

(d) (+)-*Solaniol* (IV). The $CHCl_3$ soluble fraction described in section c was percolated through a column of silicic acid (18 mm diam × 100 mm). The column was washed with $CHCl_3$ until the eluate was nearly colorless. The colored eluate was evaporated to dryness and the residue refluxed in 40 ml of AcOH for 5 min converting any fusarubin present into anhydrofusarubin to avoid interference from fusarubin in the next isolation step. The AcOH-solution was evaporated to dryness and the residue separated into 3 components by preparative TLC on 4 plates. The 3 components in decreasing order of mobility were anhydrofusarubin, javanicin and solaniol. The material in the solaniol zone was eluted with 1% AcOH in acetone, and recrystallized once from $CHCl_3$–n-hexane and twice from ether (Soxhlet) to yield 89 mg of solaniol, dark red needles, m.p. 190–194° dec, $[\alpha]_{589}^{25}$ +122° (*c*, 0·053 in MeOH), single spot by TLC on SGG and PA. A methanolic soln of solaniol gave a dark green color with $FeCl_3$aq and a violet color with a methanolic lead acetate soln. (Found: C, 62·57, 62·34; H, 5·55, 5·69. $C_{15}H_{16}O_6$ requires: C, 61·64; H, 5·52%); λ_{max} (log ε) 536 (shoulder, 3·72), 500 (3·91), 472 (shoulder, 3·84), 304 (3·97), 227 mμ (4·53).

Synthesis of (±)-solaniol

A suspension of javanicin (500 mg) in MeOH (500 ml) was stirred vigorously while three 71 mg portions of $NaBH_4$ were added at 20 min intervals. Stirring was continued for 1·5 hr after completing the addition of $NaBH_4$ to ensure complete air oxidation of the hydroquinone. The dark red soln was diluted with

water (2 l.) made slightly acidic with HCl aq and extracted with ether (2 × 1 l.). The ether extracts were dried over Na_2SO_4 and evaporated to dryness affording 400 mg of residue estimated from its absorbance at 500 mμ to contain 240 mg of (±)-solaniol. The residue was purified by preparative TLC on 3 plates. The pigment in the major red zone was eluted with 1% AcOH in acetone, and recrystallized from ether (Soxhlet) affording 122 mg (25% yield) of (±)-solaniol as fine red needles, m.p. 186–189° dec. (Found: C, 61·70; H, 5·43; O, 32·69; OMe, 10·41. $C_{15}H_{16}O_6$ requires: C, 61·64; H, 5·52; O, 32·85; OMe, 10·62%).

The m.p. of (±)-solaniol was undepressed on admixture with (+)-solaniol. Synthetic solaniol was identical by TLC on PA and SGG, and in IR, UV-visible, mass and PMR spectra to the naturally occurring solaniol. The former was about 3 times more soluble in ether and $CHCl_3$ than the latter.

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