Tetrahedron. Vol. 32, pp. 1353 to 1356 Pergamon Press 1976. Printed in Great Britain

PENTAKETIDE METABOLITES OF *VERTICILLIUM DAHLIAE*

IDENTIFICATION OF (+)-SCYTALONE AS A NATURAL PRECURSOR TO MELANIN

A. A. BELL,* R. D. STIPANOVIC and J. E. PUHALLA
Agricultural Research Service, U.S. Department of Agriculture, National Cotton Pathology Research Laboratory, P.O. Drawer JF, College Station, TX 77840, U.S.A.

(*Received in USA* 22 *September* 1975; *Received in UK for publication* 16 *January* 1976)

Abstract—Culture filtrates of the brown *brm*-1 mutant of *Verticillium dahliae* Kleb. restored normal black pigment (melanin) synthesis when added to plate cultures of 10 albino mutants. The active metabolite was isolated and identified as (+)-scytalone [3,4-dihydro-3,6,8-trihydroxy-1(2H)naphthalenone] by physical characteristics (m.p., UV, IR, NMR, MS) and chemical conversions. Related metabolites, flaviolin and *cis*-4-hydroxyscytalone, were also isolated and identified. Flaviolin and its derivatives contribute to the reddish brown color of the *brm*-1 mutant. Scytalone may be a general intermediate of melanin biosynthesis in fungi.

The synthesis of dark pigments (melanins) in animals generally involves the action of tyrosinase on tyrosine.[1,2] Intermediates in this reaction are DOPA (3,4-dihydroxyphenylalanine), cyclic DOPA (5,6 - dihydroxy - 2,3 - dihydroindole - 2 - carboxylic acid), dopachrome (2 - carboxy - 2,3 - dihydroindole - 5,6 - quinone, or red pigment), 5,6-dihydroxyindole, and indole-5,6-quinone. The last compound or a mixture of the intermediates apparently condenses to form the melanin polymer.[2]

Some fungal melanins have also been classified as "indole" melanins,[3,4] while others appear to be synthesized from catechol[5,6] or 1,8-dihydroxynaphthalene.[7,8] Evidence for the structure of fungal melanins mostly is indirect and frequently based on products formed by alkaline fusion of a melanin residue remaining after extensive solvent extraction and prolonged acid hydrolysis.

Evidence for indole involvement in fungal melanin synthesis includes: isolation from fungi of a highly active tyrosinase, tyrosine, and sometimes DOPA; a positive reaction of fusion products with Ehrlich's indole reagent; and the presence of nitrogen in "purified" melanins. In no case, however, has the % N in fungal melanins equalled that found in melanins of known indole structure. Ellis and Griffiths[3] concluded that melanin of *Verticillium dahliae*, as well as several other fungi, was indolic in nature. They obtained a positive reaction of alkali fusion products with Ehrlich's reagent for indoles and based their conclusions primarily on this evidence.

Catechol melanins yield catechol during pyrolysis in N_2 and yield salicylic acid and protocatechuic acid during alkali fusion.[5] The same products were obtained from the natural melanin of the fungus *Ustilago maydis*, indicating that its melanin was formed from catechol.[5] Catechol also reversed near-UV inhibition of microsclerotia formation and melanin synthesis in *Verticillium dahliae*.[6,9] There was little or no N in *Verticillium* melanin, and a yellow pigment with a UV spectrum similar to benzoquinone was formed in the culture during melanogenesis.[10] Thus, Gafoor and Heale[6] concluded that catechol is the substrate for melanin in *V. dahliae*.

Free 1,8-dihydroxynaphthalene has not been isolated from a fungus; however, Allport and Bu'Lock[7,8] isolated its dimer 4,4',5,5'-tetrahydroxybinaphthyl and its dimethoxyether from the black sporophores of the fungus *Daldinia concentrica*. They further showed that the binaphthyl or 1,8-dihydroxynaphthalene, could be oxidized into a black polymer (or melanin). Aspergilline, a black, high molecular weight substance from spores of *Aspergillus niger* contains a perylenequinone that may be formed from 1,8-dihydroxynaphthalene.[11]

The fungus *Verticillium dahliae* accumulates dense black melanin granules in the outer wall and surrounding matrix of its microsclerotial cells but has hyaline hyphae and conidia.[12,13] In our laboratory we[14,15] recently isolated a series of mutants from this fungus that allows direct identification of the biochemical intermediates of fungal melanin and determination of its probable chemical structure. Culture filtrates or extracts of a brown microsclerotia mutant (*brm*-1) of *V. dahliae* serve as a substrate for melanin synthesis in an albino microsclerotia mutant (*alm*-1) of *V. dahliae* or in chlamydospores of an albino mutant of *Thielaviopsis basicola*.[14] *Brm*-1 produces several metabolites, but only one is the melanin substrate.[16] Melanin formed from the *brm*-1 metabolite by *alm*-1 microsclerotia was chemically[17] and ultrastructurally[18] indistinguishable from the normal melanin of the fungus.

In a preliminary report we[14] erroneously identified the melanin substrate from *brm*-1 as cyclic DOPA. This conclusion was primarily based on: misinterpretation of the MS peak at *m/e* 195 (10%) as the parent ion, positive reactions with several indole reagents, formation of a red chelate with Fe^{3+}, and coaccumulation of a red quinone (thought to be dopachrome) in a culture medium. The NMR also showed protons similar to those expected for cyclic DOPA.

Our investigation of the *brm*-1 metabolites, reported here, shows that the natural melanin substrate produced by *brm*-1 is (+)-scytalone (**1**). The related metabolites, flaviolin (**2**) and *cis*-4-hydroxyscytalone (**3**) were also isolated and identified from *brm*-1 cultures.

Compound **1** began accumulating in culture media at 4 to 5 days after inoculation with *brm*-1 conidia and reached maximum concentrations in 2–4 weeks. Addition of 10^{-4} to 10^{-5} M catechol to the liquid medium greatly increased production of (+)-scytalone (**1**). This effect was apparently due to a similarly enhanced production of microsc-

lerotia. These appear to be the only fungal cells responsible for synthesizing scytalone (1) as well as melanin.[16] In typical experiments, 2–3 g of pure scytalone (1) were obtained from 10 liter of 4-week-old cultures.

Compound 1 was recrystallized from ether as cream-colored prisms with m.p. 164–168.5°. High resolution mass measurement indicated a molecular formula of $C_{10}H_{10}O_4$ (Found: 194.058537 (89%); calculated: $C_{10}H_{10}O_4$ 194.057890, $C_9H_7NO_4$ 194.045330). The peak at *m/e* 195 (10%) was due to incorporation of ^{13}C into the molecule (Found: 195.059956; calculated for $C_9C^{13}H_{10}O_4$: 195.061240, for $C_9H_8NO_4$ [cyclic DOPA]: 195.053150). The absence of nitrogen in 1 was further confirmed by elemental analysis that showed less than 0.1% N. Compound 1 readily loses H_2O (*m/e* 176, 85%) in the mass spectrometer to give the radical ion 6,8-dihydroxy-1(4H)naphthalenone. The base peak (*m/e* 150) results from a reverse Diels–Alder reaction in which $HOCH{=}CH_2$ is lost. This is a typical fragmentation reaction for 1-tetralones.[19]

The IR spectrum of 1 indicated the presence of a ketone (1635 cm^{-1}) conjugated with a β-enol. The enol was substantiated by an NMR peak at δ 12.45 that exchanged with D_2O. This proton apparently accounts for the slight acidity of 1.

The NMR spectrum [$(CD_3)_2CO$] indicated two aromatic protons. The proton at δ 6.04 was a doublet, and the proton at δ 6.15 was a doublet of doublets. The coupling constant (2.5 c/s) between the aromatic protons indicated their *meta*-orientation. The peak at δ 6.15 is assigned to the C_5 proton since spin decoupling confirmed that it was coupled to the multiplet at δ 2.6–3.2 (C_4).

The protons at C_4 are a conformationally mobile benzylic system. In either chair conformation, one of the C_4 protons is approximately at a 90° angle to the C_5 aromatic proton and therefore shows maximum coupling (J = −0.4 c/s†) with it. The other C_4 proton is positioned at a very small angle with respect to the C_5 aromatic proton and shows little coupling.

Spin decoupling also showed that the methine proton at δ 4.20 is coupled to the protons at δ 2.6–3.2. The chemical shift of the proton at δ 4.20 indicates that it is bonded to a carbon atom to which an electron withdrawing group, such as an alcohol, is attached.

The spectral data for 1, along with its ability to chelate Fe^{3+}, shows that it is scytalone [3,4-dihydro-3,6,8-trihydroxy-1(2H)naphthalenone (1)]. Scytalone (1) was previously isolated from culture filtrates of the fungi *Scytalidium*[20] and *Phialophora lagerbergii*.[21] Spectral data similar to ours were obtained; however, scytalone isolated from these fungi showed no optical rotation, while our preparation from *V. dahliae* was (+)-scytalone $[\alpha]_D^{25}$ = +32° (*c*; 0.25; 95% EtOH).

Identification of compound 1 as scytalone was further confirmed by chemical conversions to known compounds. In 50% KOH under nitrogen or 6 N HCl at 98°C for 30 min, 1 was dehydrated to 1,3,8-trihydroxynaphthalene (4). The structure of 4 was confirmed by its melting point and by conversion to the known 1,3,8-trimethoxynaphthalene.[22]

When 1 was stirred in 95% EtOH containing 0.1 N KOH in air, an orange quinone was produced. This quinone was identified as 2-hydroxyjuglone (5) by comparison of its NMR, UV-visible, and MS spectra with those of synthetically prepared 2-hydroxyjuglone (5).[23] 1,3,8-Trihydroxynaphthalene (4) which rapidly oxidized to 5 in dilute base, apparently is an intermediate in the reaction.

Flaviolin (2)[24] was produced chemically from compound 1 by oxidation with Jones reagent.[25] This quinone (2) is a known autooxidation product of 1,3,6,8-tetrahydroxynaphthalene (6)[26] that should be formed from scytalone (1) by oxidation with the reagent.

The *brm*-1 mutant consistently produced a red pigment (2) that coaccumulated with scytalone (1). High resolution mass measurements indicated the formula $C_{10}H_6O_5$ (Found: 206.020919; calcd.: 206.021500). Comparison of the acidic and basic UV spectra of this compound with those of flaviolin (2)[20] showed they were identical. Flaviolin (2) was also produced biologically from scytalone (1) by incubating mM 1 and mM NADP with *brm*-1 microsclerotia overnight.

A third metabolite of *brm*-1 was identified as 4-hydroxyscytalone (3). The MS indicated a molecular formula of $C_{10}H_{10}O_5$ (M^+, *m/e* 210, 64%). Compound 3 appeared identical to scytalone (1) at the C_1, C_3 and C_5–C_8 carbons. Both 1 and 3 readily lost H_2O and $CH_2{=}CHOH$ in the MS. Both compounds also underwent acidic or basic dehydration to phenols. The NMR of 1 or 3 showed two *meta*-arranged aromatic protons. Identical structure of 1 and 3 at the C_1, C_8 positions was further indicated by: similar red chelates of 1 or 3 with Fe^{3+}, similar IR absorption peaks indicating ketones conjugated with β-enols (1635 cm^{-1} for 1; 1622 for 3) and similar NMR peaks indicating the strongly chelated protons of the C_8-hydroxyl (δ 12.96 for 1; δ 12.89 for 3). Thus, the additional oxygen of 3 compared to 1 is at C_2 or C_4.

Several observations indicate that a hydroxyl is added to the C_4 position of 3. The MS of 3 had a peak at *m/e* 166 (25%) from loss of $CH_2{=}CHOH$ by a reverse Diels–Alder reaction. This requires a CH_2 group at C_2. Compound 3, in dilute base and air, dehydrated and oxidized to flaviolin and a second red quinone. A phenol and transient violet quinone (probably 5,7 - dihydroxynaphtho - 1,4 - quinone) were formed during the reaction. The conversion of 5,7 - dihydroxynaphtho - 1,4 - quinone to a mixture of flaviolin and 2,6,8-trihydroxynaphtho-1,4-quinone is expected, because juglone has been shown to oxidize to a mixture of 2- and 3-hydroxyjuglone under similar mild oxidative conditions.[22] Flaviolin (2) would not have been obtained as a product of alkaline oxidation if the hydroxyl had been in the C_2 position. Final confirmation of the structure of 4-hydroxyscytalone (3) was obtained, when flaviolin (2) was fed to 7-day-old cultures of the *brm*-1 mutant, and 4-hydroxyscytalone (3) was produced as the only major product.

The *vicinal*-diol in 4-hydroxyscytalone (3) from *V. dahliae* apparently is *cis*. Compound 3 was isolated from *Pyricularia oryzae* as a *trans*-substituted diol.[27] The smaller coupling constant (J = 2.5 c/s) between the protons on C_3 and C_4 of 3 from *V. Dahliae* compared to that reported between the same protons in the *trans* isomer (J = 8.0 c/s)[27] indicates an axial-equatorial interaction[28] in the former, and thus the *cis*-arrangement of 3 from *V. dahliae*. This is further supported by the lower m.p. (98–108°) of 3 from *V. dahliae* compared to that (166°) of *trans*-3. The isomeric difference of the compounds is further indicated by the NMR spectra of the C_2 protons. The thermodynamically more stable *trans* conformation has both hydroxyl groups equatorial, and exists predominantly in this conformation. Thus, the protons on C_2 appear

†The J value was assigned a negative value, because the protons were benzylic; see pp. 316–331 of Ref. 28.

as two distinct peaks at 2.70 and 3.06 δ. In the *cis*-diol (3), the thermodynamic stability of the two chair conformers is similar and they rapidly interconvert. Therefore, both protons of C_2 appear at an average chemical shift of 2.86 δ.

When (+)-scytalone was added to plate cultures of albinos of *V. dahliae* or *T. basicola*, visible melanization occurred within 5 min and intense melanization occurred after 1 hr. Scytalone isolated by other workers was not reported to possess biological activity, but it probably was not tested as a substrate for melanin synthesis.[20,21]

Flaviolin (2) or *cis*-4-hydroxyscytalone were not substrates for melanin synthesis in the albino fungi. Thus, these compounds are not intermediates in melanin biosynthesis but probably are formed from the accumulated scytalone (1). Flaviolin (2) was made from scytalone (1) by either chemical or biological oxidation of 1, and 4-hydroxyscytalone (3) was the only major product obtained from 2 by biological reduction. Thus, the genetic lesion in *brm*-1 appears to affect an enzyme that converts scytalone (1) to the next compound in the biosynthetic pathway to melanin.

A natural role of scytalone (1) in melanin synthesis is indicated by several observations. While wild-type isolates of *V. dahliae* did not accumulate 1 in cultures, trace amounts could be recovered from cultures undergoing active melanin synthesis. None of 10 different albinos of *V. dahliae* produced even traces of 1, but each readily converted (+)-scytalone (1) to melanin. Thus, albinism is associated with genetic blocks in scytalone biosynthesis. Recently, we (unpublished) found that the albino microsclerotial mutant *alm*-9 converts (+)-scytalone to a naphthalendiol. This compound, which appeared to be 1,8-dihydroxynaphthalene, was enzymatically or auto-oxidatively converted to a black pigment (melanin).

The black pigment of *V. dahliae* apparently is an allomelanin[2] derived by a pentaketide biosynthetic scheme. Synthesis of (+)-scytalone and flaviolin from radioactive acetate has been demonstrated in another fungus.[21]. The position of the oxygens in 3 also are consistent with a pentaketide origin. The melanin probably consists of 1,8-dihydroxynaphthalene subunits arranged in a polymer similar to that proposed by Allport and Bu'Lock.[7,8] The same or similar allomelanin probably occurs in many other fungi, since pentaketides occur extensively among fungi containing black or brown pigments.[29,30]

†Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

EXPERIMENTAL

M.ps were determined on a Kofler-hostage† apparatus. Mass spectra were measured with a Varian† CH-7 by direct probe insertion. NMR spectra were recorded on a JEOL-MH-100 with TMS as an internal standard. Hydroxyl protons were determined by rapid exchange with D_2O. UV-visible spectra were determined in 95% EtOH, 95% EtOH plus 0.03 M NaOH (EtONa) or 95% EtOH plus 0.015 M HCl (EtOH–HCl) with a Beckman† ACTA-MVI and are reported as λ_{max} (ε).

Extraction and isolation of **1, 2** *and* **3**. The mutants derived from *Verticillium dahliae* strain T9[16] were grown at 24° in the dark on potato-carrot-dextrose-agar[31] or on still cultures containing Brandt's liquid sucrose nitrate medium[32] amended to contain 3×10^{-5} M catechol.

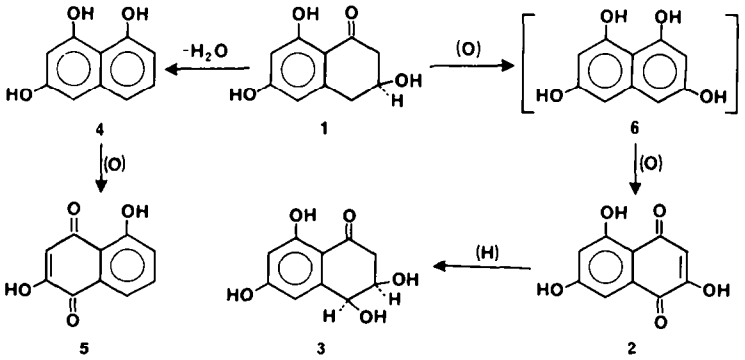
Agar cultures were chopped and pentaketides were extracted with acetone. Acetone was removed *in vacuo* to leave an aqueous phase. Liquid cultures were centrifuged and the supernatant was filtered. Either aqueous preparation was adjusted to pH 5 with H_3PO_4, saturated with NaCl, and extracted with ethyl acetate. The organic solvent was evaporated, and the concentrate was used for chromatography.

The crude pentaketides were dissolved in ethyl ether and applied to a 2 × 20 cm column of alternate 2 cm layers of silica gel and polyamide. The column was first eluted with ethyl ether to remove 1 and 3, and subsequently with methanol to remove 2. TLC plates (silica gel GF_{254}) were developed with ethyl ether : naphtha solvent (3 : 1) or chloroform : methanol (9 : 1).

Identification of **1**. Concentrated column eluates gave crude crystals of 1 upon the addition of hexane. Recrystallization from ethyl ether gave 1 as off-white crystals, m.p. 164.5–168.5°. CD (95% EtOH): θ_{307} +4269°, θ_{218} −33,325°. MS (probe 80°): *m/e* (%) 194 (M⁺; 89), 177 (12), 176 (M–H_2O; 85), 151 (17), 150 (M–CH_2=CHOH; 100), 148 (18), 134 (10), 122 (M–CH_2=CHOH, CO; 26), 121 (10), 69 (35), 65 (10). NMR [$(CD_3)_2CO$]: 2.6–3.2 (4H, m), 4.20 (1H, m), 6.04 (1H, d, J = 2.5 c/s) 6.15 (1H, dd, J = 2.5 and −0.4 c/s), 12.45 (1H, s, exchanged with D_2O) δ. λ_{max}^{EtOH} (ε) 218 (sh), 222 (9200), 232 (6100), 239 (sh), 285 (9900), 317 (sh) nm; λ_{max}^{EtONa} (ε) 254 (4300), 335 (20,600) nm. ν_{max} (KBr) 1635, 1592, 1275, 1175 cm⁻¹.

Identification of **2**. Flaviolin (2) was purified by TLC and occurred as the top orange-red band. It was prepared synthetically by dissolving scytalone in acetone, cooling to 0°, and adding Jones reagent[25] dropwise. Crystallization from ethyl ether gave 2 as a red powder. NMR [$(CD_3)_2CO$]: 6.15 (1H, s, slow exchange with D_2O), 6.68 (1H, d), 7.15 (1H, d), 12.65 (1H, s, exchanged with D_2O) δ. MS (probe = 90°): (Found: M⁺ 206.020919, 100%; $C_{10}H_6O_3$ requires: 206.021500), *m/e* (%) 178 (33, M–CO), 150 (23, M–2CO), 137 (49), 136 (13, M–$C_3H_2O_2$), 109 (13), 108 (14), 69 (37). $\lambda_{max}^{EtOH-HCl}$ (ε) 213 (21,500), 264 (15,000), 309 (7300), 399 (2100), 450 (2400) nm; λ_{max}^{EtONa} (ε) 284.5 (23,800), 363 (6000), 554 (2200) nm. ν_{max} (KBr) 1592, 1385, 1240, 1175 cm⁻¹. Variation of the pH of a flaviolin solution produced the same color changes noted previously.[33]

Identification of **3**. Compound 3 was purified by TLC from the solns left from crystallization of 1. Both 1 and 3 appeared as quenched spots under 254 nm and yellow fluorescent spots under 365 nm UV light. However, 3 occurred at a distinctly lower R_f than 1. Crystallization from ethyl ether gave 3 as off-white



crystals, m.p. 98–108° with resolidification to give two types of crystals—m.p. 158–164° and 179–181°. $[\alpha]_D^{23} = -56°$ (95% EtOH; $c = 0.25$). MS (probe = 125°): *m/e* (%) 210 (M^+; 64), 192 (M–H_2O, 16), 166 (M–CH_2=CHOH; 25), 138 (M–CH_2=CHOH, CO; 64), 137 (M–CH_2=CHOH, CHO; 100), 131 (10), 130 (10), 81 (12). NMR [$(CD_3)_2$]CO]: 2.86 (2H, d, J = 4.8 c/s), 4.37 (1H, m), 4.86 (1H, broad d, J = 2.5 c/s), 6.73 (1H, dd, J = 2.0 and 1.0 c/s), 6.29 (1H, d, J = 2.0 c/s), 12.89 (1H, s, exchanged with D_2O), other hydroxyls appeared as a broad band 3.07–7.0 δ. λ_{max}^{EtOH} (ε) 217 (13,700), 234 (8300), 284 (13,100), 312 (6500) nm; λ_{max}^{EtONa} (ε) 256 (5800), 337 (28,500) nm. ν_{max} (KBr): 1622 cm^{-1}.

Dehydration of **1** *and* **4**. (+)-Scytalone (**1**) was dissolved in 50% KOH under N_2 and heated at 95° for 30 min. The mixture was poured over ice, adjusted to pH 5.0, and extracted with ethyl ether. Alternatively, **1** was dissolved in conc. HCl that was immediately diluted to 6N. This mixture was heated under N_2 in a sealed tube for 30 min at 95°, poured over ice, and extracted with ethyl ether. TLC of either ether extract gave 1,3,8 trihydroxynaphthalene (**4**) as the predominant quenching band under UV (254 nm).

From ethyl ether, **4** was recovered as light yellow crystals, m.p. 193–203° (Lit. 205–207°).[30] MS (probe 18°): *m/e* (%) 176 (77), 149 (29), 134 (100), 106 (41), 105 (18), 91 (10), 78 (31), 77 (18), 71 (13), 57 (25). λ_{max}^{EtOH} (ε) 230 (43,700), 292 (4900), 304 (5300), 329 (3700), 340 (3900) nm; λ_{max}^{EtONa} (ε) 337.5 (7900) nm.

The structure of **4** was confirmed by conversion to 1,3,8-trimethoxynaphthalene. 12 mg of **4** was dissolved in acetone (15 ml); and dimethyl sulfate (0.5 ml) and anhyd K_2CO_3 (500 mg) were added to the soln. The soln was refluxed for 16 hr. Water (50 ml) was added, and the solution extracted with ethyl ether (2 × 50 ml). The organic layer was washed with 2N NaOH (50 ml), water (4 × 50 ml), and saturated brine (50 ml); then it was dried over anhyd Na_2SO_4. The solvent was evaporated and the product purified by TLC (silica gel 254, benzene). The lower of two bands proved to be the trimethoxy ether of **4**, m.p. 76–79° from hexane (Lit. 81°).[21] MS (probe 18°): *m/e* (%) 218 (100), 175 (14), 145 (16), 96 (25), 95 (38), 66 (11). λ_{max}^{EtOH} 230 (47,100), 285 (5300), 294 (5400), 319 (3200), 331 (3400) nm.

Oxidation of **1** *to* **5**. (+)-Scytalone (**1**) was dissolved in 95% EtOH containing 0.1 N KOH and stirred at room temp. for 1 hr. Then, two vol. saturated brine was added, pH was adjusted to 3, and 1 vol. of ethyl acetate was used to extract the aqueous phase. Purification by TLC gave 2-hydroxyjuglone (**5**) as a prominant yellow-orange band. Crystallization from benzene gave **5** as an orange powder. NMR [$(CD_3)_2$CO]: 4.34 (1H, bs, exchange with D_2O), 6.27 (1H, s, slow exchange with D_2O), 7.25–7.45 (1H, m), 7.60–7.81 (2H, m), 12.70 (1H, s, exchange with D_2O) δ. MS (probe 75°): *m/e* (%) 190 (M^+, 100), 162 (M–CO, 45), 134 (M–2CO, 32), 121 (86), 120 (17), 105 (14), 93 (24), 92 (26). $\lambda_{max}^{CHCl_3}$ (ε) 284 (12,100), 429 (3500) nm, $\lambda_{max}^{CH_3OH\text{-}HCl}$ (ε) 282.5 (6900), 411 (2300) nm, $\lambda_{max}^{CH_3ONa}$ 261 (14,100), 386 (1600), 470 (2100) nm.

Acknowledgements—The authors are grateful to Drs. Ronald Grigsby, who performed the high-resolution MS measurements, B. W. Bycroft, who provided a sample of flaviolin, and Anthony Waiss, Jr., who measured the optical rotations. The excellent technical assistance of Mrs. Mildred E. Hummel, Mr. Gilbert Tribble and Mrs. Linda Hall is appreciated.

REFERENCES

[1]R. A. Nicolaus, M. Piattelli and E. Fattorusso, *Tetrahedron* **20**, 1163 (1964).
[2]G. A. Swan, *Fortschritte d. Chem. org. Naturst.* **31**, 521 (1973).
[3]D. H. Ellis and D. A. Griffiths, *Can. J. Microbiol.* **20**, 1379 (1974).
[4]A. T. Bull, *Arch. Biochem. Biophys.* **137**, 345 (1970).
[5]M. Piattelli, E. Fattorusso, R. A. Nicolaus and S. Magno, *Tetrahedron* **21**, 3229 (1965).
[6]A. Gafoor and J. B. Heale, *Microbios* **3**, 131 (1971).
[7]D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.* 4090 (1958).
[8]D. C. Allport and J. B. Bu'Lock, *Ibid.* 654 (1960).
[9]W. H. Brandt, *BioScience* **15**, 669 (1965).
[10]A. Gafoor and J. B. Heale, *Microbios* **3**, 87 (1971).
[11]M. Barbetta, G. Casnati and A. Ricca, *Rend. 1st. Lombardo Sci Lett. A* **101**, 75 (1967).
[12]M. F. Brown and T. D. Wyllie, *Phytopathology* **60**, 538 (1970).
[13]D. A. Griffiths, *Kleb. Arch. Mikrobiol.* **74**, 207 (1970).
[14]A. A. Bell and J. E. Puhalla, *Proc. Amer. Phytopatholog. Soc.* **1**, 53 (1975).
[15]J. E. Puhalla, *Proc. Beltwide Cotton Prod. Res. Conf.* p. 26. Jan. 6–9, New Orleans, La. (1975).
[16]A. A. Bell, J. E. Puhalla, R. D. Stipanovic and W. J. Tolmsoff, *Can. J. Microbiol.* **22**, in press (1976).
[17]W. J. Tolmsoff, A. A. Bell and M. H. Wheeler, *Proc. Beltwide Cotton Prod. Res. Conf.* p. 27. Jan. 6–9, New Orleans, La. (1975).
[18]M. H. Wheeler, W. J. Tolmsoff and A. A. Bell, *Proc. Beltwide Cotton Prod. Res. Conf.* p. 27. Jan. 6–9, New Orleans, La. (1975).
[19]J. H. Bowie, *Austral. J. Chem.* **19**, 1619 (1966).
[20]J. A. Findlay and D. Kwan, *Can. J. Chem.* **51**, 1617 (1973).
[21]D. C. Aldridge, A. B. Davies, M. R. Jackson and W. B. Turner, *J. Chem. Soc.* Perkin I, 1540 (1974).
[22]H. Stetter and H. Heidel, *Chem. Ber.* **99**, 2172 (1966).
[23]H. Singh, T. L. Folk and P. J. Scheuer, *Tetrahedron* **25**, 5301 (1969).
[24]B. W. Bycroft and J. C. Roberts, *J. Chem. Soc.* 2063 (1962).
[25]K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *Ibid.* 39 (1946).
[26]P. M. Baker and B. W. Bycroft, *Chem. Comm.* 71 (1968).
[27]S. Iwasaki, H. Muro, K. Sasaki, S. Nozoe and S. Okuda, *Tetrahedron Letters* 3537 (1973).
[28]L. M. Jackman and S. Sternhell, *Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* (2 Edition). p. 288. Pergamon Press, Oxford (1969).
[29]J. D. Bu'Lock, *The Biosynthesis of Natural Products*, Chap. 2. McGraw-Hill, London (1965).
[30]W. B. Turner, *Fungal Metabolites*, Chap. 5. Academic Press, New York (1971).
[31]J. E. Puhalla, *Phytopathology* **63**, 1488 (1973).
[32]W. H. Brandt, *Can. J. Bot.* **42**, 1017 (1964).
[33]B. D. Astill and J. C. Roberts, *J. Chem. Soc.* 3302 (1953).