Isolation of Three New Isochroman-3-one Metabolites from *Oidiodendron rhodogenum* Robak

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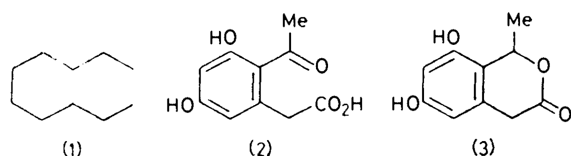
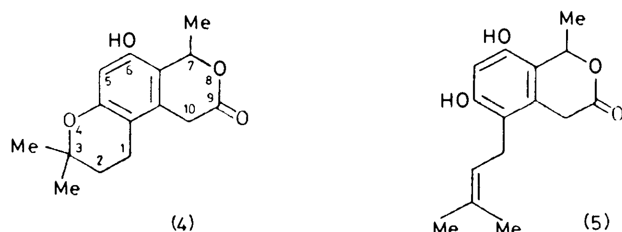
The fungus *Oidiodendron rhodogenum* Robak produces curvulinic acid, fuscin, and three new isochroman-3-one metabolites for which the structures (3)—(5) are proposed. A second strain grown under the same conditions produces fuscin and dihydrofuscin.

AMONG fungal metabolites the polyketides are a large group of biosynthetically related compounds which have been classified [1] according to length and probable folding of the acetate/malonate derived chain. Of the various modes of cyclisation of pentaketides, few examples of the type (1) have been isolated. In this paper the isolation of three fungal metabolites which structurally belong to this class is reported.

RESULTS AND DISCUSSION

A strain of *Oidiodendron rhodogenum* Robak, CMI 235 256, grown in still culture, produces curvulinic acid [2] (2) and three new metabolites, (3)—(5). Of these the major metabolite is $C_{10}H_{10}O_4$ to which structure (3) is assigned. The ^1H n.m.r. spectrum of (3) shows



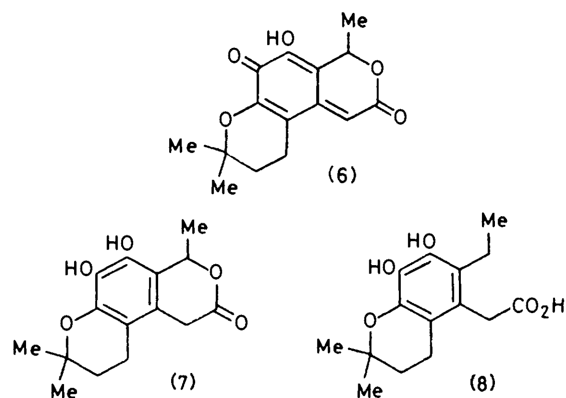


similarities to that of curvulinic acid. Thus *meta*-related aromatic protons and methylenes consistent with the grouping $Ar-CH_2-CO_2-$ are common to both. The methylene of (3), however, appears as an AB pattern. The acetyl-methyl signal of curvulinic acid is absent in (3). Instead, a methyl doublet appears at δ 1.48, coupled to a methine at δ 5.59. Together with an i.r. absorption at 1 735 cm^{-1}, these facts are consistent with structure (3). This is confirmed by sodium borohydride reduction of curvulinic acid which gives the metabolite, identical in all respects except optical activity.

The other two metabolites both have the formula $C_{15}H_{18}O_4$ and from their ^1H n.m.r. spectra are related to (3). The more abundant, crystalline metabolite shows two methyl singlets at δ 1.28 and 1.32 and an A_2B_2 pattern at δ 1.81 and 2.55, suggesting the grouping $Me_2\overset{\overset{O}{|}}{C}-CH_2CH_2Ar$ arrangement. This data is consistent with structure (4) and long-range coupling between the lower-field signal of the A_2B_2 pattern and the $ArCH_2CO_2$ methylene confirms the aromatic substitution position. This is further supported by the splitting pattern following off-resonance decoupling of the ^{13}C n.m.r. spectrum of (4). The aromatic carbon bearing a proton appears as a doublet, but shows none of the longer-range coupling expected if the dihydropyran ring were fused at C-6 and not at C-10a.

The second $C_{15}H_{18}O_4$ metabolite is non-crystalline with ^1H n.m.r. signals attributable to the $Me_2C{=}CHCH_2$-Ar group. Structure (5) is assigned to it, and this has been confirmed by acid-catalysed ring-closure to give (4).

A second strain of *Oidiodendron rhodogenum* Robak, numbered ACC 6689 in our collection, produces no detectable quantities of the above metabolites when grown under the same conditions. The related metabolites fuscin (6) and dihydrofuscin [3] (7) have been



isolated, however. A small quantity of fuscin is also produced by CMI 235 256. Reduction of fuscin by sodium dithionite or by catalytic hydrogenation yields dihydrofuscin, but as a crystalline modification. The identity of the semi-synthetic material has been established by the superimposability of its solution i.r. spectrum with that of the natural metabolite. Prolonged catalytic hydrogenation of fuscin gives the tetrahydro-compound (8).

In contrast to fuscin and dihydrofuscin, (3) and (4) showed no antibacterial activity against *Staphylococcus aureus*.

EXPERIMENTAL

I.r. spectra were determined for Nujol mulls except where indicated otherwise. ^1H N.m.r. spectra were determined at 100 MHz and ^{13}C n.m.r. at 22.5 MHz, both with tetramethylsilane as internal standard. Letters following ^{13}C n.m.r. chemical shifts refer to pattern following off-resonance decoupling. Silica gel for chromatography was Hopkins and Williams MFC or Merck Kieselgel 60. T.l.c. was performed on Merck GF silica gel eluting with one of the systems chloroform–methanol–acetic acid (90 : 5 : 5) (system A); chloroform–acetone–acetic acid (95 : 4 : 1) (system B); or ethyl acetate–toluene–acetic acid (50 : 50 : 1) (system C). Preparative t.l.c. was carried out on layers 1 mm thick. Light petroleum had b.p. 60—80 °C.

Isolation of Metabolites.—*Oidiodendron rhodogenum* Robak (CMI 235 256) was grown as surface culture for 21 days in glass Thompson bottles each containing 350 ml of Czapek–Dox medium. The culture filtrate (27 l) was extracted with ethyl acetate (1 × 9 l, 1 × 5 l) at pH 2.0. The extract was dried (Na_2SO_4) and concentrated to a small volume producing crystals of *curvulinic acid* (7.8 g) whose i.r. spectrum was identical with that of an authentic sample. The mother-liquors were concentrated to an oil (40 g) which was chromatographed on silica gel (1.5 kg). Elution with increasing concentrations of chloroform in toluene, and then ethyl acetate in chloroform, gave three fractions, A—C.

Fraction A (5.9 g) containing mainly material of R_F 0.76 (system A) was crystallised from chloroform–light petroleum to give slightly yellow crystals (4.1 g) which were purified by preparative t.l.c. (system A) providing *5-hydroxy-*1,8,9-10-*tetrahydro-*4,8,8-*trimethyl-*2H,4H-*benzo*[1,2-b:4,3-c′]*dipyran-2-one* (4), m.p. 182 °C (acetone–light petroleum) (Found: C, 68.7; H, 7.0%; M^+, 262.116 8. $C_{15}H_{18}O_4$ requires C, 68.7; H, 6.9%: M, 262.120 5); $\nu_{max.}$ 3 375, 1 720, and 1 620 cm^{-1}; δ_H ($CDCl_3$) 1.28 (3 H, s), 1.32 (3 H, s), 1.57 (3 H, d, J 6 Hz), 1.81 (2 H, t, J 6 Hz), 2.55 (2 H, m), 3.52 and 3.72 (2 H, AB q, J 20 Hz), 5.86 (1 H, q, J 6 Hz), 6.26 (1 H, s), and 6.32 (1 H, s, disappears on addition of D_2O); δ_C ($CDCl_3$ + [$^2H_6$]DMSO) 18.75 (t), 21.67 (q), 25.99 (q), 26.97 (q), 31.08 (t), 32.54 (t), 73.35 (s), 74.26 (d), 102.32 (d), 108.43 (s), 114.10 (s), 128.21 (s), 151.24 (s), 154.05 (s), and 170.10 (s).

Fraction B was triturated with acetone to give orange crystals of *fuscin*, identified by comparison (i.r., t.l.c.) with an authentic sample. The mother-liquors were chromatographed by preparative t.l.c. (system A) and a band R_F 0.49 was eluted to give *6,8-dihydroxy-5-(3-methylbut-2-enyl)-1-methylisochroman-3-one* (5) (0.29 g) as an oil (Found: M^+, 262.120 4. $C_{15}H_{18}O_4$ requires M, 262.120 5); $\nu_{max.}$ (MeCN) 3 380, 1 745, and 1 615 cm^{-1}; δ_H ($CDCl_3$) 1.54 (3 H, d, J = 6 Hz), 1.66 (3 H, s), 1.76 (3 H, s), 3.25 (2 H, br d, J 6 Hz), 3.54 and 3.76 (2 H, AB q, J 18 Hz), 5.04 (1 H, br t, J 6 Hz), 5.83 (1 H, q, J 6 Hz), 6.42 (1 H, s), and 7.47 and 8.15 (each 1 H, br s, disappears on addition of D_2O).

Fraction C, containing mainly material of R_F 0.31 (system A) was triturated with chloroform to yield *6,8-dihydroxy-1-methylisochroman-3-one* (3) (5g) as white crystals, m.p. 184 °C (decomp.) (ethyl acetate–light petroleum) (Found: C, 61.9; H, 5.2%; M^+, 194.057 9. $C_{10}H_{10}O_4$ requires C, 61.9; H, 5.2%; M, 194.057 9); $[\alpha]_D^{22}$ = −3.85° (c 4.0, MeOH); $\nu_{max.}$ 3 290, 1 685, 1 620, and 1 530 cm^{-1}; $\nu_{max.}$ (MeCN) 3 350, 1 735, and 1 620 cm^{-1}; δ_H ([$^2H_6$]-DMSO) 1.48 (3 H, d, J 6 Hz), 3.46 and 3.85 (2 H, AB q, J 18 Hz), 5.59 (1 H, q, J 6 Hz), 6.10 and 6.14 (2 H, AB q, J 2 Hz), and 9.56 (2 H, br, disappears on addition of D_2O); δ_C ([$^2H_6$]DMSO) 21.34 (q), 34.34 (t), 74.00 (d), 100.97 (d), 104.98 (d), 112.95 (s), 131.80 (s), 153.36 (s), 158.02 (s), and 170.43 (s).

A second strain of *Oidiodendron rhodogenum* Robak, numbered ACC 6689 in our collection, was grown as a surface culture for 21 days in 60 glass Thompson bottles each containing 350 ml of Czapek–Dox medium. The culture filtrate (20 l) was extracted at its natural pH of 4.5 with ethyl acetate (1 × 6 l, 1 × 4 l). The extract was dried (Na_2SO_4) and concentrated to a small volume producing red crystals of *fuscin* (2.3 g), m.p. 230 °C (ethanol) (lit.,[3] 230 °C); δ_H (CF_3CO_2H) 1.42 (3 H, s), 1.44 (3 H, s), 1.70 (3 H, d, J 6 Hz), 1.98 and 2.78 (4 H, A_2B_2 pattern, J 6 Hz), 5.98 (1 H, q, J 6 Hz), and 6.72 (1 H, s). The mother-liquors were concentrated to dryness and the residue boiled with methylene chloride (2 × 100 ml). Each time the methylene chloride was decanted and the combined solutions concentrated. The resulting residue was absorbed on silica gel and added to a column of silica gel (250 g) made up in chloroform. Elution with chloroform (800 ml) gave a fraction which was allowed to crystallise from chloroform–methanol. Close examination revealed a mixture of red and yellow crystals which were separated by hand picking. The yellow crystals were recrystallised twice from chloroform and methanol to give *dihydrofuscin*, m.p. 206—208 °C (lit.,[3] 206 °C) (Found: M^+, 278.116 2. $C_{15}H_{18}O_5$ requires M, 278.115 4); $\nu_{max.}$ 3 360, 1 710, 1 690 (sh), and 1 630 cm^{-1}; δ_H ([$^2H_6$]DMSO) 1.25 (3 H, s), 1.27 (3 H, s), 1.43 (3 H, d, J 6 Hz), 1.72 (2 H, t, J 6 Hz), 2.4—2.6 (2 H, m), 3.48 and 3.58 (2 H, AB q, J 20 Hz), 5.61 (1 H, q, J 6 Hz), and 8.15 and 8.58 (each 1 H, s, disappears on addition of D_2O).

Reduction of Fuscin.—(*a*) *By sodium dithionite.* Fuscin was reduced with sodium dithionite according to the procedure of Michael.[3] The crude product crystallised from chloroform–methanol to give *dihydrofuscin*, m.p. 205—206 °C; $\nu_{max.}$ 3 450, 3 260, 1 710, and 1 620 cm^{-1}. The chloroform solution i.r. was identical to that of dihydrofuscin.

(*b*) *By catalytic hydrogenation of fuscin.* A solution of fuscin (100 mg) in glacial acetic acid (20 ml) was shaken in an atmosphere of hydrogen in the presence of platinum oxide catalyst. After 1 equiv. hydrogen had been consumed the catalyst was removed by filtration and the filtrate evaporated *in vacuo*. The resulting residue was crystallised from chloroform–methanol to give *dihydrofuscin*, identical to the above dithionite product. This experiment was repeated except that the reaction mixture was allowed to consume 2 equiv. of hydrogen. Crystallisation of the crude product from chloroform–light petroleum gave *(7,8-dihydroxy-2,2-dimethyl-6-ethylchroman-5-yl)acetic acid*, m.p. 142—142.5 °C (Found: C, 64.0; H, 7.1%. $C_{15}H_{20}O_5$ requires C, 64.27; H, 7.19%); $\nu_{max.}$ 3 520, 3 390, 2 500—3 100 (br), 1 705, and 1 615 cm^{-1}; δ_H ([$^2H_6$]DMSO) 0.96 (3 H, t, J 6 Hz), 1.24 (6 H, s), 1.7 (2 H, t, J 6 Hz), 2.6—2.4 (4 H, m), and 3.42 (2 H, s).

Reduction of Curvulinic Acid.—To a solution of curvulinic acid (1.0 g) dissolved in methanol (75 ml) was added portionwise and with stirring, sodium borohydride (1.0 g). The reaction mixture was stirred for 2 h following the addition, acidified to pH 2.0 with hydrochloric acid, and diluted with water (100 ml). The methanol was evaporated *in vacuo* and the resulting aqueous solution extracted with ethyl acetate (3 × 50 ml). The ethyl acetate solution was dried

(Na_2SO_4) and concentrated to an oil, which was chromatographed according to the method of Still *et al.*,[4] eluting with ethyl acetate–toluene (1 : 1). Combination and evaporation of appropriate fractions followed by crystallisation of the residue from ethyl acetate–toluene (1 : 1) gave white crystals of racemic 6,8-*dihydroxy*-1-*methylisochroman*-3-*one*, m.p. 180—181 °C (decomp.) (0.55 g). The product was identical by solution i.r. and t.l.c. (systems A, B, and C) comparison with an authentic sample.

Conversion of (5) *to* (4).—The isochroman-3-one (5) (0.35 g) was dissolved in glacial acetic acid (17 ml) and water (7 ml) and concentrated hydrochloric acid (7 ml) added. After 2 h water (100 ml) was added and the mixture extracted with ether (3 × 25 ml). The ether solution was dried (Na_2SO_4) and evaporated to a gum (0.315 g) which was chromatographed according to the method of Still *et al.*,[4] eluting with ethyl acetate–toluene (1 : 9). Combination and evaporation of appropriate fractions and crystallisation of the residue from acetone–hexane (1 : 3) gave (4), m.p. 181—181.5 °C (0.179 g). The product was identical by t.l.c. (systems A, B, and C) and i.r. comparison with an authentic sample.

The author thanks Mr. D. Greatbanks and Mr. R. Pickford for helpful discussion of the ^{13}C n.m.r. spectra.

[0/600 *Received*, 23*rd April*, 1980]

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