

## Metabolic Products of *Stemphylium radicinum*. Part IV.<sup>1</sup> Minor Products

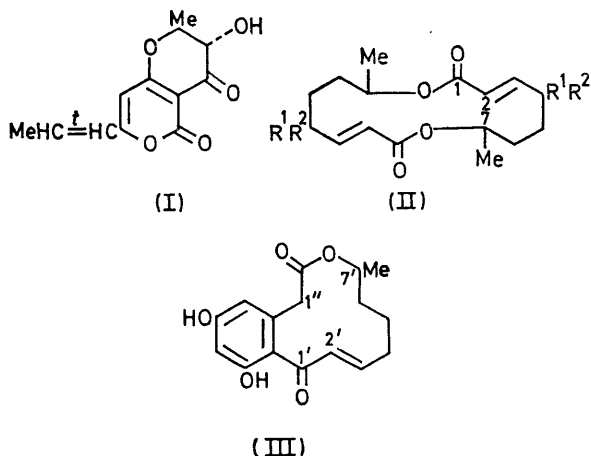
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Pyrenophorol, dehydrocurvularin, and a new  $C_9H_{12}O_3$  compound, shown to be 2,3-dihydro-2-hydroxy-2,4-dimethyl-5-*trans*-propenylfuran-3-one, have been isolated in low yield from culture filtrates of the phytopathogenic fungus *Stemphylium radicinum*, C.M.I. 105654, in addition to radicinin and pyrenophorin which were obtained previously. The pyrenophorin was sometimes accompanied by an isomeric compound, possibly an artifact derived from it by an intramolecular Michael condensation.

RADICININ<sup>2</sup> (I) and pyrenophorin<sup>3</sup> (II;  $R^1R^2 = O$ ) have previously been isolated<sup>2,4</sup> from culture filtrates of *Stemphylium radicinum*. In connection with studies<sup>1</sup> on the biosynthesis of radicinin, a search was made for minor metabolic products which might prove to be intermediates in the biosynthetic pathway. No such intermediates were detected, but a number of products were isolated in yields of 1–5 mg./l.

Fractional crystallisation of the crude product obtained by extraction of culture filtrates of *S. radicinum*, C.M.I. 105654, with chloroform gave radicinin. Column chromatography of the residual gum then yielded, in order of increasing polarity, pyrenophorin, an unstable  $C_9H_{12}O_3$  ketone, m.p. 99°, radicinin, pyrenophorol<sup>5</sup> (II;  $R^1R^2 = H, OH$ ), and dehydrocurvularin<sup>6</sup> (III). Pyrenophorol has previously been isolated from *Byssoschlamys nivea*,<sup>5</sup> and dehydrocurvularin from *Curvularia lunata*<sup>7</sup> and *Alternaria cucumerina*.<sup>8</sup>

In the n.m.r. spectrum of dehydrocurvularin, examination of the AB part ( $\tau$  3.4) of the ABX<sub>2</sub> system at  $\tau$  3.4 and 7.65 by double irradiation gave the value of  $J_{2,3'}$  as 16 Hz, consistent with the *trans*-configuration assigned<sup>6</sup> from consideration of the i.r. spectrum.



The mother liquors of the pyrenophorin fraction sometimes contained an optically active isomeric keto-lactone,  $C_{16}H_{20}O_6$ , readily separated from pyrenophorin

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<sup>1</sup> Part III, J. F. Grove, *J. Chem. Soc. (C)*, 1970, 1860.

<sup>2</sup> J. F. Grove, *J. Chem. Soc.*, 1964, 3234.

<sup>3</sup> S. Nozoe, K. Hirai, K. Tsuda, K. Ishibashi, M. Shirasaka, and J. F. Grove, *Tetrahedron Letters*, 1965, 4675.

by fractional crystallisation and sublimation *in vacuo*. It resembled pyrenophorin in having two keto-groups, which were reduced by sodium borohydride, and two lactone rings, as shown by titration. The lactone rings both terminated in  $-CO\cdot O\cdot CHMe\cdot CH_2-$  groups as judged by the n.m.r. spectrum, which showed two three-proton doublets ( $J$  6.5 Hz) at  $\tau$  8.85 and 8.60, coupled to two one-proton multiplets at  $\tau$  5.05 and 5.55, respectively. The keto-lactone differed from pyrenophorin in the absence of ethylenic double bonds and the absence of a two-fold axis of symmetry, as revealed by i.r. and n.m.r. spectroscopy. It was easily separated from pyrenophorin on rechromatography and its presence in the pyrenophorin fraction suggests therefore that it is an artifact formed from pyrenophorin during work-up. Of the possible structures resulting from intramolecular double Michael condensation of pyrenophorin, the symmetrical structure (IV) is excluded. Some evidence for the unsymmetrical structure (V), derived by sequential Michael addition, was obtained from the mass spectrum, which showed abundant fragment ions at  $m/e$  208 and 180, consistent with the stepwise elimination of ring c.

The  $C_9H_{12}O_3$  ketone contained an hydroxy-group ( $\nu_{max}$  3340  $cm^{-1}$ ; broad singlet at  $\tau$  5.15). The remaining eleven protons were contained in two  $R_3CMe$  groups (singlets at  $\tau$  8.32 and 8.50) and a *trans*-propenyl substituent ( $A_3MX$  system at  $\tau$  8.05, 3.70, and 3.15;  $J_{MX}$  15.5 Hz). Although it was acetylated under mild conditions, the hydroxy-group must therefore be tertiary: it was not enolic since the parent ketone was neutral and gave no colour with iron(III) chloride. The carbonyl group ( $\nu_{max}$  1680  $cm^{-1}$ ) must be part of a conjugated dienone system, since the dihydro-compound obtained by palladium-charcoal reduction showed n.m.r. signals consistent with the presence of a *n*-propyl substituent while retaining i.r. absorption corresponding to a fully substituted enone group ( $\nu_{max}$  1690 and 1610  $cm^{-1}$ ). The u.v. absorption of this derivative ( $\lambda_{max}$  281 nm.) and of the parent dienone ( $\lambda_{max}$  312 nm.) indicated that the third oxygen atom was present in an ether linkage attached to the chromophore. These spectroscopic

<sup>4</sup> D. D. Clarke and F. F. Nord, *Arch. Biochem. Biophys.*, 1955, 59, 269.

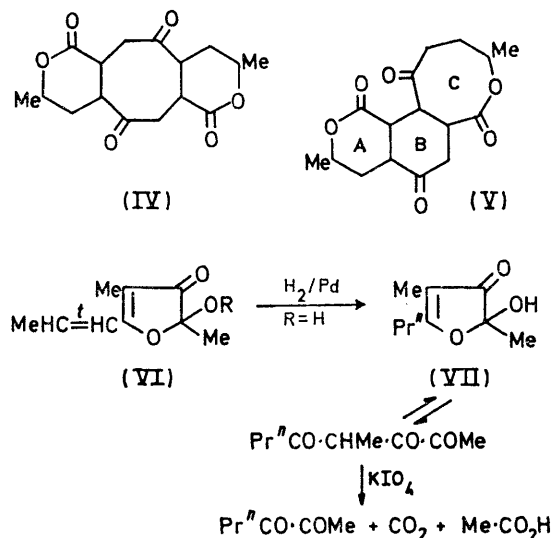
<sup>5</sup> Z. Kis, P. Furger, and H. P. Sigg, *Experientia*, 1969, 25, 123.

<sup>6</sup> H. D. Munro, O. C. Musgrave, and R. Templeton, *J. Chem. Soc. (C)*, 1967, 947.

<sup>7</sup> O. C. Musgrave, *J. Chem. Soc.*, 1956, 4301.

<sup>8</sup> A. N. Starratt and G. A. White, *Phytochem.*, 1968, 7, 1883.

observations are consistent only with the furan-3-one structures (VI; R = H) and (VII) for the  $C_9H_{12}O_3$  ketone and its dihydro-derivative respectively. The i.r. spectrum of the furanone (VI; R = H) in carbon



tetrachloride showed intermolecular hydrogen bonding between the OH and C=O groups ( $\nu_{\text{max}}$  3340 and 1690  $\text{cm}^{-1}$ ) in addition to intramolecular hydrogen bonding; but in more dilute solution only the latter was seen ( $\nu_{\text{max}}$  3580 and 1714  $\text{cm}^{-1}$ ). The i.r. spectrum of the acetyl derivative ( $\nu_{\text{max}}$  1765, 1718, and 1648  $\text{cm}^{-1}$ ) was also consistent with the unsaturated ketol acetate structure (VI; R = Ac).

Oxidation of the dihydro-compound (VII) with periodate in *N*-hydrochloric acid required vigorous conditions, giving carbon dioxide (1 mol.) and hexane-2,3-dione, isolated as the bisdinitrophenylhydrazone, instead of the expected hexan-3-one. Hexan-3-one was not oxidised to the 2,3-dione under these conditions, but oxidation of the activated methylene group of  $\beta$ -diketones and  $\beta$ -keto-acids by periodate is well known<sup>9,10</sup> and intermediates of both types may be present. In a model reaction under the same conditions, ethyl  $\alpha$ -ethylacetoacetate gave, after hydrolysis, carbon dioxide and pentane-2,3-dione. The normal fission product, pentan-2-one, was obtained when the periodate was omitted.

#### EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus and are corrected. Unless otherwise stated, i.r. spectra were determined for mulls in Nujol, u.v. spectra and optical rotations for solutions in ethanol, and n.m.r. spectra (at 100 MHz) for solutions in deuteriochloroform with tetramethylsilane as internal standard. Light petroleum had b.p. 60–80°.

*Isolation of the Minor Metabolic Products.*—*S. Radicinum*, C.M.I. 105654, was cultured and harvested as previously

<sup>9</sup> C. F. Huebner, S. R. Ames, and E. C. Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621.

described.<sup>1</sup> Extraction of the culture filtrate (30 l. batches) with chloroform gave a crude solid product (15 g.). A portion (5 g.) was fractionally crystallised from acetone, giving radicinin (3.5 g.) and a brown oil (1.5 g.) which was chromatographed in benzene (20 ml.) on acid alumina [Woelm, grade II (45 g.)] in u.v. light. After elution of lipids (520 mg.; u.v. end absorption only) with benzene (300 ml.), elution with benzene-methanol (composition of mixture in parentheses) gave a series of fractions with specific u.v. absorption: (i) 100 ml. (200:1), 106 mg. solid,  $\lambda_{\text{max}}$  220 nm.; (ii) 100 ml. (100:1), 162 mg., yellow oil,  $\lambda_{\text{max}}$  310 nm.; (iii) 150 ml. (100:1), 110 mg., semi-solid,  $\lambda_{\text{max}}$  310 nm.; (iv) 50 ml. (50:1), 13 mg., solid,  $\lambda_{\text{max}}$  340 nm., which crystallised from acetone in needles of radicinin; (v) 50 ml. (50:1), 35 mg., oil,  $\lambda_{\text{max}}$  300 nm.; (vi) 50 ml. (50:1), 82 mg., semi-solid,  $\lambda_{\text{max}}$  210 nm.; (vii) 100 ml. (50:1), 59 mg., oil,  $\lambda_{\text{max}}$  310 nm.; (viii) 100 ml. (20:1), 36 mg., oil,  $\lambda_{\text{max}}$  340 nm.

Fractions (ii), (v), and (vii) were intractable. Fractional crystallisation of fraction (i) from ethanol yielded needles (53 mg.), m.p. 162–168°, of crude pyrenophorin, and, from some batches of culture filtrate, a solid residue which crystallised from benzene in prisms. It was freed (t.l.c.) from pyrenophorin by sublimation at 160°/10<sup>-1</sup> mm. followed by recrystallisation from benzene, giving prisms, m.p. 252°,  $[\alpha]_{\text{D}}^{20} -33^\circ$  (*c* 0.1), of a *keto-lactone* [Found: C, 62.45; H, 6.6%; *M* (mass spectrum), 308; equiv. (lactone titration) 160.  $C_{16}H_{20}O_6$  requires C, 62.3; H, 6.5%; *M*, 308],  $\nu_{\text{max}}$  (OH absent) 1748, 1728, 1720, and 1700  $\text{cm}^{-1}$ ,  $\nu_{\text{max}}$  ( $\text{CCl}_4$ ) 1750, 1725, 1715, and 1700  $\text{cm}^{-1}$ , u.v. end-absorption only,  $\tau$  5.05 (1H, m), 5.55 (1H, m), 6.5–8.5 (12H), 8.60 (3H, d), and 8.85 (3H, d). It gave no colour with iron(III) chloride. The yellow precipitate formed with 2,4-dinitrophenylhydrazine was intractable.

Fraction (vi) crystallised from ethyl acetate in prisms (15 mg.), m.p. 132–135°. Recrystallisation from ethyl acetate-light petroleum afforded prisms, m.p. 135°,  $[\alpha]_{\text{D}}^{20} -3^\circ$ ,  $[\alpha]_{546}^{20} -6^\circ$ ,  $[\alpha]_{436}^{20} -26^\circ$  (*c* 0.375), of pyrenophorol (II;  $R^1R^2 = \text{H,OH}$ ) (lit.,<sup>5</sup> m.p. 135°,  $[\alpha]_{\text{D}}^{20} -3^\circ$ ) [Found: C, 61.3; H, 7.8%; *M* (mass spectrum), 312. Calc. for  $C_{16}H_{24}O_6$ : C, 61.5; H, 7.75%; *M*, 312],  $\nu_{\text{max}}$  3290, 1725, and 1655  $\text{cm}^{-1}$ , u.v. end-absorption only,  $\tau$  3.1 (dd, *J* 16 and 5.5 Hz, H-3), 4.0 (dd, *J* 16 and 1.5 Hz, H-2), 4.9 (m, H-7), 5.75 (m, H-4), 7.7 (s, OH), 8.0–8.5 (5-H<sub>2</sub> and 6-H<sub>2</sub>), and 8.75 (d, *J* 6 Hz, Me).

Trituration of fraction (viii) with ethyl acetate furnished an amorphous powder (21 mg.), m.p. 210° (decomp.), which crystallised from ethyl acetate-light petroleum in needles and from methanol in plates, m.p. 230°,  $[\alpha]_{\text{D}}^{20} -80^\circ$  (*c* 0.1), of dehydrocurvularin (III) (lit.,<sup>6,7</sup> m.p. 230–232°,  $[\alpha]_{\text{D}}^{18} -83^\circ$ ) [Found: C, 66.2; H, 6.2%; *M* (mass spectrum), 290.1153. Calc. for  $C_{16}H_{18}O_5$ : C, 66.2; H, 6.25%; *M*, 290.1154 ( $C \equiv 12.0000$ ),  $\lambda_{\text{max}}$  225, 302, and 335 nm. ( $\epsilon$  14500, 5500, and 4700),  $\nu_{\text{max}}$  3420, 3310, 1727, 1635, 1600, and 845  $\text{cm}^{-1}$ ,  $\nu_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ ) 3660, 3540, 1730, 1620, and 1600  $\text{cm}^{-1}$ ,  $\tau$  ( $[\text{H}_6]\text{Me}_2\text{CO}$ ) 3.4 (ABX<sub>2</sub>, *J* 16 and 6 Hz, 2'- and 3'-H), 3.7 (s, 3- and 5-H), 5.3 (m, H-7'), 6.3 (AB, *J* 18 Hz, 1''-H<sub>2</sub>), 7.2 (2 × OH), 7.65 (m, 4'-H<sub>2</sub>), 8.0–8.5 (5'- and 6'-H<sub>2</sub>), 8.85 (d, *J* 6.5 Hz, Me). It gave an intense dark brownish-green colour with iron(III) chloride.

Fraction (iii) was extracted with boiling light petroleum.

<sup>10</sup> M. L. Wolfrom and J. M. Bobbitt, *J. Amer. Chem. Soc.*, 1956, **78**, 2489.

Concentration of the extract and recrystallisation of the solid product from light petroleum afforded prisms (45 mg.), m.p. 99°,  $[\alpha]_D^{22} > \pm 2^\circ$  ( $c$  0.5), of 2,3-dihydro-2-hydroxy-2,4-dimethyl-5-trans-propenylfuran-3-one (VI; R = H) [Found: C, 63.9; H, 7.3%;  $M$  (mass spectrum), 168.0788.  $C_9H_{12}O_3$  requires C, 64.3; H, 7.2%;  $M$ , 168.0786 ( $C = 12.0000$ )],  $\lambda_{max}$ . 215, 240, and 312 nm. ( $\epsilon$  5850, 9150, and 15,000),  $\nu_{max}$ . 3340, 1680, 1640, and 1575  $cm^{-1}$ ,  $\nu_{max}$  ( $CCl_4$ ; 1 mg./ml.) 3580, 3340, 1714, 1690, 1650, and 1582,  $\nu_{max}$  ( $CCl_4$ ; 0.25 mg./ml.) 3580, 1714, *ca.* 1690, 1650, and 1582  $cm^{-1}$ ,  $\tau$  3.15 (dq,  $J$  15.5 and 6.5 Hz, H-2'), 3.70 (dd,  $J$  15.5 and 1 Hz, H-1'), 5.15br (s, OH), 8.05 (dd,  $J$  6.5 and 1 Hz, Me), 8.32 (3H, s), 8.50 (3H, s). It was neutral (potentiometric titration) and gave no colour with iron(III) chloride or with concentrated sulphuric acid. It sublimed readily at 80°/10<sup>-1</sup> mm. It formed an intractable orange-red precipitate with 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid and slowly reduced ammoniacal silver nitrate at room temperature. The acetate (VI; R = Ac), prepared in pyridine with acetic anhydride at room temperature during 3 days, was purified by distillation at 100° (bath temp.)/10<sup>-1</sup> mm. giving an oil [Found: C, 63.2; H, 7.1%;  $M$  (mass spectrum), 210.  $C_{11}H_{14}O_4$  requires C, 62.8; H, 6.7%;  $M$ , 210],  $\nu_{max}$  (film) (OH absent) 1765, 1718, 1648, and 1600  $cm^{-1}$ ,  $\lambda_{max}$ . 212, 243, and 308 nm. ( $\epsilon$  7000, 7000, and 16,200).

Both the furanone (VI; R = H) and its acetyl derivative (VI; R = Ac) were unstable in air at room temperature and decomposed within 7 days with a hypsochromic shift of the chromophoric system and the formation of resinous polymeric products. The furanone (VI; R = H) could be stored in a stoppered tube at 0° in the dark for some months without deterioration.

*Reduction of the Keto-lactone (m.p. 252°) with Sodium Borohydride.*—The keto-lactone (5 mg.) in methanol (2 ml.) at 0° was treated with sodium borohydride (5 mg.) and the solution was allowed to warm to room temperature during 30 min. After removal of most of the methanol *in vacuo*, the neutral solution obtained by adding water and acetic acid was extracted with ethyl acetate. Distillation of the product at 190° (bath temp.)/10<sup>-1</sup> mm. furnished the glassy tetrahydro-derivative (Found: C, 61.6; H, 7.1. Calc. for  $C_{16}H_{24}O_6$ : C, 61.5; H, 7.75%),  $\nu_{max}$  ( $CCl_4$ ) 3610 (OH) and 1750  $cm^{-1}$  (lactone C=O).

*Catalytic Reduction of the Furan-3-one (VI; R = H).*—The furan-3-one (12.3 mg.) in ethyl acetate (5 ml.) took up 1.03 mol. hydrogen in 4 min. at 20° in the presence of 5% palladium-charcoal (10 mg.). There was no further uptake in 15 min., and, after removal of the catalyst by filtration, recovery gave a yellow gum which was distilled at 100° (bath temp.)/10<sup>-1</sup> mm., giving the oily 2,3-dihydro-2-hydroxy-2,4-dimethyl-5-propylfuran-3-one (VII) (Found: C, 63.0; H, 8.3.  $C_9H_{14}O_3$  requires C, 63.5; H, 8.3%),  $\nu_{max}$  (film) 3350, 1690, and 1610  $cm^{-1}$ ,  $\lambda_{max}$ . 281 nm. ( $\log \epsilon$  4.1),

$\tau$  6.1br (s, OH), 7.5 (2H, t), 8.4 (2H, m), 8.4 (3H, s), and 9.05 (3H, t). The furanone (VII) was more stable than the furanone (VI; R = H) and was kept at room temperature for some days without deterioration.

*Oxidation of the Furan-3-one (VII) with Periodate.*—The furanone (17 mg.) in N-hydrochloric acid (5 ml.) containing potassium periodate (50 mg.) was heated at 100° with stirring under efficient reflux in a stream of nitrogen subsequently passed through traps containing 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid, and, finally, saturated barium hydroxide. Carbon dioxide (0.80 mol.), collected in the usual way as barium carbonate, and a volatile carbonyl compound which formed a dinitrophenylhydrazone, were evolved during 30 min., after which no further reaction took place. After 18 hr. at room temperature, the crude dinitrophenylhydrazone was collected and extracted with hot methanol. The insoluble portion crystallised from ethyl acetate in orange-red prisms (3 mg.), m.p. 245—246° (decomp.) (lit.,<sup>11</sup> 247—249°) of hexane-2,3-dione bisdinitrophenylhydrazone (Found: C, 45.4; H, 4.0; N, 23.5. Calc. for  $C_{18}H_{18}N_8O_8$ : C, 45.6; H, 3.8; N, 23.6%), identified by mixed m.p. and comparison of i.r. spectra with those of an authentic specimen.

Under these conditions hexan-3-one was not oxidised to the 2,3-dione.

When the oxidation of the furanone (VII) was carried out at 70°, no volatile products were detected after 10 min. When the furanone was heated at 100° in N-hydrochloric acid, no volatile products were observed and the furanone (VII) was recovered (by ether extraction).

*Oxidation of Ethyl  $\alpha$ -Ethylacetoacetate with Periodate.*—Ethyl  $\alpha$ -ethylacetoacetate (25 mg.) in N-hydrochloric acid (5 ml.) containing potassium periodate (50 mg.) was heated under reflux as just described. A volatile carbonyl compound and carbon dioxide were slowly evolved, and after 2 hr., when barium carbonate (0.35 mol.) had been collected, the reaction was stopped. The dinitrophenylhydrazone was collected and crystallised from ethyl acetate in orange-red prisms (3 mg.), m.p. 273—275° (decomp.), of pentane-2,3-dione bisdinitrophenylhydrazone, identified by mixed m.p. and comparison of i.r. spectra with those of an authentic specimen.<sup>2</sup>

When the potassium periodate was omitted, the hydrolysis took the same course giving, after 2 hr., barium carbonate (0.35 mol.), but the dinitrophenylhydrazone crystallised from ethanol in yellow needles (6 mg.), m.p. 142—143° of pentan-2-one dinitrophenylhydrazone (lit., m.p. 141°).

This work was supported by the Agricultural Research Council during the tenure of a Comyns Berkeley Bye-Fellowship from Gonville and Caius College. I thank Mr. B. Chrystell for the n.m.r. spectra, Miss T. Prior and Miss E. Gray for the mass spectra, and Miss M. M. Blight and Mrs. B. Brown for harvesting a fermentation carried out at the Chemical Laboratory, University of Sussex.

<sup>11</sup> M. Stoll, M. Winter, F. Gautschi, I. Flament, and B. Willhalm, *Helv. Chim. Acta*, 1967, **50**, 628.