Scytalidin: a New Fungitoxic Metabolite produced by a *Scytalidium* Species

By **G. M. Strunz,* M. Kakushima,** and **M. A. Stillwell,** Canadian Forestry Service, P.O. Box 4000, Fredericton, New Brunswick, Canada

A new fungitoxic metabolite, designated scytalidin, was isolated from culture medium which had supported growth of *Scytalidium* sp., an imperfect fungus. The structure (3) of scytalidin (10-butyl-5,9,10,11-tetrahydro-10-hydroxy-4-pentyl-4*H*-cyclonona[1,2-*c*:5,6-*c*']difuran-1,3,6,8-tetraone) was established on the basis of spectrometric and chemical investigations. The metabolite is related structurally and biosynthetically to the nonadrides.

A SPECIES of *Scytalidium*, FY strain, was observed by Ricard and his collaborators [1,2] to be antagonistic to *Poria carbonica*, a wood-rotting organism responsible, *inter alia*, for heartwood decay in Douglas-fir utility poles in western North America. Metabolites produced by *Scytalidium* sp. were isolated, and included yellow and red pigments, as well as a colourless compound, *m/e* 348·42809. Antifungal activity was found to be associated with the crystalline red and colourless substances.[1,2]

Scytalidium sp., FY strain, has been further investigated in our laboratory. When grown in a synthetic medium in liquid-shake culture, it produced at least two major metabolites which displayed antifungal properties.[3] Chemical investigation of one of these, a new compound for which the name scytalidin is proposed, forms the subject of this report.

The molecular formula of scytalidin was established as $C_{22}H_{28}O_7$ by elemental analysis and high resolution mass spectrometry.[4] I.r. absorption ($CHCl_3$ solution) at 3575 cm^{-1} was attributed to alcohol functionality, further characterized as a single tertiary hydroxy-group on the basis of the following observations. Scytalidin was recovered unchanged after treatment with Jones reagent.[5] The metabolite was resistant to acetylation with acetic anhydride–pyridine; it was, however, readily converted into a monoacetate by the action of acetic anhydride in the presence of zinc chloride.[6] Comparison of the n.m.r. spectra of scytalidin and its acetate did not reveal any differences which could be attributed to a proton geminal to the hydroxy-group.[7] The n.m.r. spectrum of scytalidin in [$^2H_6$]dimethyl sulphoxide showed a downfield shift of the OH signal from δ 2·14 to 4·58 p.p.m., and, in accord with the tertiary nature of the hydroxy-function, the signal retained its singlet character.[8,9]

Scytalidin displayed i.r. bands (KBr) at 1850, 1827, and 1773 cm^{-1}, and u.v. absorption [$\lambda_{max.}$ (acetonitrile)] at 250 nm (ε 9700), the combined data demonstrating the presence of dialkylmaleic anhydride functionality.[10,11] Comparison of the u.v. characteristics with those of models [cyclohex-1-ene-1,2-dicarboxylic anhydride,[12] $\lambda_{max.}$ (acetonitrile) 250 nm (ε 4280), and 2,3-dimethylmaleic anhydride, $\lambda_{max.}$ (acetonitrile) 250 nm (ε 4900)] indicated that two dialkylmaleic anhydride groups are present. The spectrum indicated furthermore that the anhydride functions are not mutually conjugated. Additional evidence that the metabolite is a bis-anhydride was obtained by acid-catalysed reaction with methanol, which afforded a product, $C_{26}H_{40}O_9$, whose analytical and spectral properties were appropriate for a tetramethyl ester.

No vinyl protons were evident in the n.m.r. spectrum. Two maleic anhydride functions account for eight of the nine sites of unsaturation indicated by the molecular formula: subsequent observations require, in addition, the presence of a carbocycle. The presence of two alkyl chains was manifested in the 220 MHz n.m.r. spectrum as two three-proton triplets at δ 0·88 and 0·97 p.p.m. (*J* 6 and 7 Hz, respectively) for the terminal methyl groups.

The base peak in the mass spectrum, *m/e* 85 (see Experimental section) corresponds to the ion $CH_3{\cdot}[CH_2]_3{\cdot}CO^+$, and it is apparent that it arises from fragmentations in the vicinity of the carbon atom bearing the tertiary hydroxy-group. This indicates an n-butyl chain attached to the latter carbon atom, an assignment substantiated by the presence of a significant ion at *m/e* 347 $(C_{18}H_{19}O_7)^+$, which results from loss of C_4H_9 from the molecular ion.

The nature of the second alkyl chain was clarified by subjecting scytalidin to a modified Kuhn–Roth oxidation.[13] The detection of n-hexanoic acid as the highest homologue in a series of volatile n-alkanoic acids indicated the presence of an n-pentyl chain.

The fourteen methylene protons of the two side-chains

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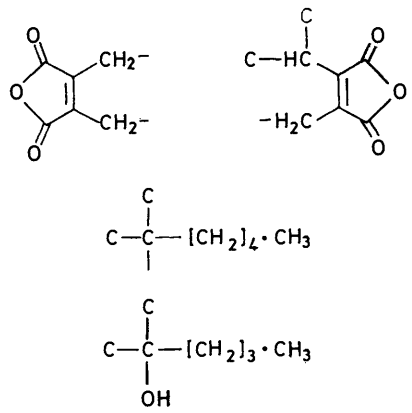
account for all signals constituting a multiplet at δ 1·1—1·9 p.p.m. in the n.m.r. spectrum. Signals corresponding to seven protons absorbing downfield from δ 2·5 remain to be interpreted and, in view of the functionality established for the metabolite, they can most readily be assigned to hydrogens allylic with respect to the maleic anhydride functions. Thus, a multiplet, δ 2·5—3·2 p.p.m., accounts for six allylic methylene protons, and a multiplet (comprising at least six lines) at δ 3·31—3·53 p.p.m. represents a single allylic methine proton.[12]

The presence of the structural elements depicted as (1) is inferred from the data presented.

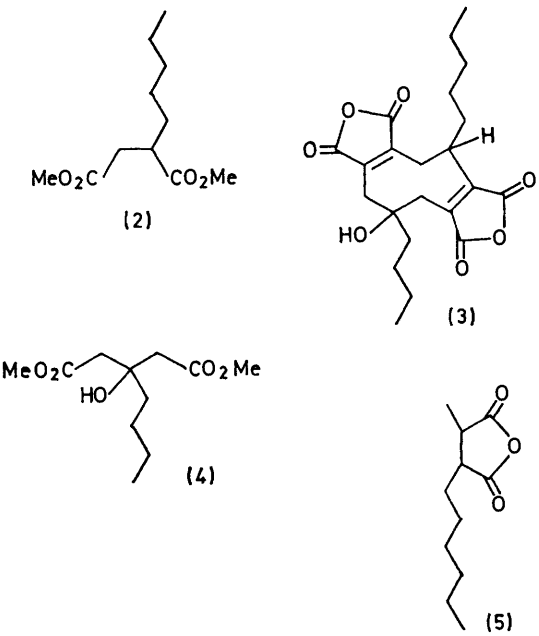


(1)

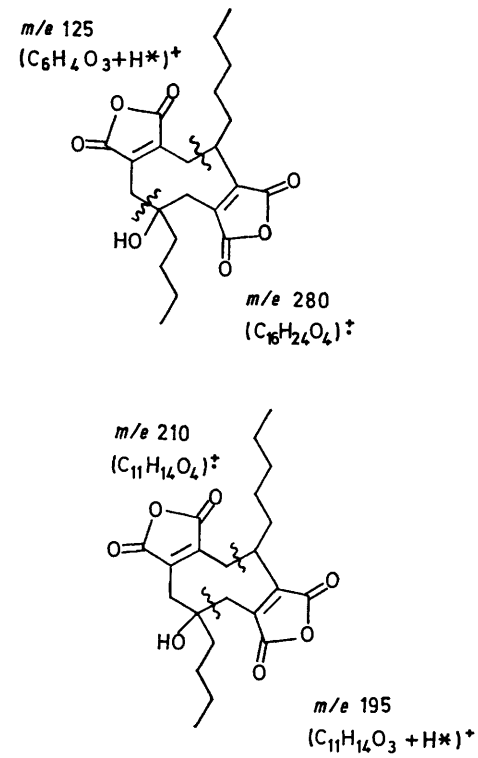
A study of the products of oxidative cleavage of a scytalidin derivative allowed the assembly of these elements, establishing a complete and unique plane structure for the metabolite. Thus, ozonolysis of the pentaol derived from scytalidin by reduction of the anhydride groups with sodium bis-(2-methoxyethoxy)-aluminium hydride, afforded, after appropriate work-up, a mixture of acids, which was treated with diazomethane. Among the products, dimethyl n-pentylsuccinate (2) was isolated and identified by comparison with an authentic sample. Of the possible structures for scytalidin which accommodate the features represented in (1), the formulation (3) alone accounts for the isolation of n-pentylsuccinic acid as an ozonolysis product.

A second product of the oxidative cleavage was isolated in low yield: on the basis of its i.r. and mass spectral characteristics (see Experimental section), it was identified tentatively as dimethyl 3-n-butyl-3-hydroxyglutarate (4), adding further support to the formulation (3) for its progenitor.

The results of mass spectral analysis of scytalidin are in excellent accord with the structure (3). In addition to the fragmentations already discussed, the principal ions in the higher mass region of the spectrum (see Experimental section) can result readily from cleavages indicated in the Scheme.



(2) (3) (4) (5)



SCHEME Deuterium labelling shows H* is derived from OH

Like rubratoxins A and B,[12,14,15] scytalidin possesses features which relate it structurally and biosynthetically to the nonadride metabolites[11,16] glauconic,[17-19]

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glaucanic,[18,19] and byssochlamic [20,21] acids. Its biosynthesis appears to be closely analogous to that proposed for byssochlamic acid [11,16,22] and accordingly can be considered to involve the coupling of two C_{11} units possessing identical carbon skeletons (5) at the appropriate oxidation level. The C_{11} precursor may be derived by decarboxylation–dehydration of the condensation product of an octanoic acid derivative with oxaloacetate.

EXPERIMENTAL

M.p.s were determined on a hot-stage apparatus. I.r. spectra were recorded on a Beckmann IR-10 spectrophotometer. U.v. spectra were obtained on either a Beckmann DK-2A or a Perkin-Elmer 402 spectrophotometer. The 220 MHz n.m.r. spectra were recorded for solutions in deuteriochloroform on a Varian HR-220 instrument at the Canadian 220 MHz N.M.R. Center, Ontario Research Foundation; 60 MHz spectra were measured on a Varian T-60 instrument. Accurate mass measurements were obtained with a CEC 21-110 B mass spectrometer; the other mass spectra were determined on a Hitachi–Perkin-Elmer RMU-6D spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan, U.S.A.

Scytalidin (10-*Butyl*-5,9,10,11-*tetrahydro*-10-*hydroxy*-4-*pentyl*-4H-*cyclonona*[1,2-c:5,6-c′]*difuran*-1,3,6,8-*tetraone*).—The production and isolation of scytalidin are described in detail elsewhere.[3] Material extracted with chloroform from filtered culture medium which had supported growth of *Scytalidium* sp., FY strain, was chromatographed on a column of silica gel (Grace Davidson, grade 923, 100—200 mesh; deactivated with 5% water). Scytalidin was eluted with methylene chloride and further purified by crystallization from ether–cyclohexane to give colourless lustrous *platelets*, m.p. 142—146°, $[\alpha]_D^{21}$ −66·6° (*c* 0·4745 in EtOAc). Spectral data are given in the Discussion section. Accurate mass measurements (*cf.* Scheme): *m/e* 404·1830 (*M*, $C_{22}H_{28}O_7$ requires 404·1835), 347·1135 ($C_{18}H_{19}O_7$ requires 347·1131), 280·1666 ($C_{16}H_{24}O_4$ requires 280·1675), 210·0887 ($C_{11}H_{14}O_4$ requires 210·0892), 195·1018 ($C_{11}H_{15}O_3$ requires 195·1021), 125·0242 ($C_6H_5O_3$ requires 125·0239), and 85·0655 (C_5H_9O requires 85·0653). Peaks at *m/e* 262 and 192 appear to correspond to the ions $(C_{16}H_{24}O_4 - H_2O)^{\cdot+}$ and $(C_{11}H_{14}O_4 - H_2O)^{\cdot+}$, respectively (Found: C, 65·95, 65·35; H, 7·0, 6·95; O, 26·85. $C_{22}H_{28}O_7$ requires C, 65·35; H, 7·0; O, 27·7%).

Scytalidin Acetate.—Zinc chloride (40 mg) was partially dissolved in acetic anhydride (3 ml). Scytalidin (100 mg, 0·247 mmol) was added and the mixture was stirred at 25° under anhydrous conditions for 1 h, after which it was poured into ice–water (*ca.* 100 ml). The resulting mixture was extracted with ether and the extracts were washed successively with saturated aqueous solutions of sodium hydrogen carbonate and sodium chloride. The organic phase, after drying ($MgSO_4$) and removal of solvent under reduced pressure afforded crude crystalline product (117 mg). Recrystallization from ether–cyclohexane yielded *crystals*, m.p. 88—92°, $\nu_{max.}$ ($CHCl_3$) 1850infl, 1835, 1775, and 1750 cm^{-1} (no OH), $\lambda_{max.}$ (MeCN) 248 nm (ε 10,000), δ ($CDCl_3$; 220 MHz) 0·88 and 0·95 (6H, overlapping triplets, *J* 6 and 7 Hz, respectively), 1·2—1·8 (14H, m), 1·96 (3H, s), and 2·1—3·5 (7H, m) (Found: C, 64·65; H, 6·75%; M^+, 446. $C_{24}H_{30}O_8$ requires C, 64·55; H, 6·75%; M^+, 446).

Methanolysis of Scytalidin.—A solution of scytalidin (200 mg, 0·494 mmol) in dry methanol (40 ml) containing concentrated sulphuric acid (0·1 ml) was refluxed under anhydrous conditions for 48 h. The volume was then reduced *in vacuo* to *ca.* 1 ml and ether was added. The ethereal solution was washed successively with saturated aqueous solutions of sodium hydrogen carbonate and sodium chloride, dried ($MgSO_4$), and evaporated under reduced pressure to afford crude yellow crystalline product (226 mg). Recrystallization from ether–cyclohexane yielded a tetramethyl ester as needles, m.p. 130·5—131°, $\nu_{max.}$ (CCl_4) 3440 and 1728 cm^{-1}, $\nu_{max.}$ (KBr) 1738infl, 1720, 1705, 1270, and 1147 cm^{-1}, $\lambda_{max.}$ (MeCN) 228 nm (ε 8300) (Found: C, 63·0; H, 8·1. Calc. for $C_{26}H_{40}O_9$: C, 62·9; H, 8·1%).

Kuhn–Roth Oxidation of Scytalidin.—To a solution of chromium trioxide (8·4 g) in water (50 ml), concentrated sulphuric acid (12·5 ml) was added cautiously. This reagent (31·0 ml) was added to scytalidin (300 mg, 0·742 mmol). The mixture was heated at 150—160° for 2 h while oxygen was passed in; as distillate was removed, the volume was maintained roughly constant by dropwise addition of water. The collected distillate was extracted continuously with ether; the extracts were dried ($MgSO_4$) and the solvent was carefully removed by distillation. The residue was esterified by treatment with a small excess of ethereal diazomethane, and the resulting mixture was analysed by g.l.c. Comparison of retention times and co-chromatography with authentic samples demonstrated the presence of the methyl esters of a homologous series of n-alkanoic acids, whose highest member was n-hexanoic acid. No material with retention time longer than that of the latter was observed.

Reduction of Anhydride Groups of Scytalidin.—To a solution of scytalidin (404 mg, 1 mmol) in dry ether (300 ml) was added sodium bis-(2-methoxyethoxy)aluminium hydride * (10 ml of 70% solution in benzene; 0·0715 g atom hydrogen). The mixture was refluxed under anhydrous conditions for 46 h. Excess of reagent was then decomposed by cautious addition of wet ether, after which the mixture was poured into water and extracted thoroughly with ether. The extracts were washed with 10% hydrochloric acid, dried ($MgSO_4$), and evaporated *in vacuo* to yield a gum (390 mg), $\nu_{max.}$ (CCl_4) 3300s cm^{-1} (negligible CO absorption).

Ozonolysis of Reduction Product.—The crude pentaol (390 mg, 1·01 mmol) obtained from reduction of scytalidin was dissolved in ethyl acetate (130 ml). An ozone–oxygen mixture was passed into the solution at 25° for 8 h (Welsbach model T-23 ozonator, operating at 90 V, 8 lb pressure, flow rate 0·02 ft^3 min^{-1}). The system was then flushed with nitrogen, and the solution was stirred under hydrogen at atmospheric pressure with a prehydrogenated 10% palladium–charcoal catalyst (250 mg) until absorption of hydrogen ceased. The oily product obtained after filtration and removal of solvent *in vacuo* was dissolved in methanol (*ca.* 2 ml) and a solution of sodium periodate (900 mg, 4·21 mmol) in water (10 ml) was added dropwise. The

* Vitride ™ Reducing Agent: Eastman Organic Chemicals, Rochester, New York.

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mixture was stirred at 25° for 21 h, after which it was diluted with water and extracted thoroughly with ether. The extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting orange oil was treated with excess of ethereal diazomethane, and the product was chromatographed on silica gel. Material (145 mg) eluted with benzene–ether (7 : 3) was subjected to preparative g.l.c. [Varian Aerograph model 90-P gas chromatograph, equipped with thermal conductivity detector, 10 ft × ¼ in (o.d.) aluminium column, packed with 30% SE 30 Chromosorb W, column temperature 212°, helium carrier gas at 100 ml min^{-1}]. Material from the two principal peaks was collected: 7 mg with retention time 14 min 46 s, and 3 mg at 20 min 35 s. These compounds were identified as the dimethyl esters (2) and (4) of n-pentylsuccinic acid and 3-n-butyl-3-hydroxyglutaric acid, respectively.

Dimethyl n-Pentylsuccinate (2).—This was an oil, $\nu_{max.}$ (CCl_4) 1740 cm^{-1}, M^+ (*m/e* 216) not detected, *m/e* 185 $(M - OCH_3)^+$ and 114 (base peak), and was identified by comparison (i.r. and mass spectra, g.l.c.) with an authentic sample synthesized as follows. To a solution of sodium ethoxide [from sodium (1·15 g, 0·050 g atom) in anhydrous ethanol (30 ml)] was added ethyl malonate (7·67 ml, 8·10 g, 0·051 mol). The solid sodium salt which separated was redissolved by addition of absolute ethanol (5 ml) and warming the mixture at 50° with swirling. To the resulting solution was added methyl 2-bromoheptanoate (11·16 g, 0·05 mol) [prepared (b.p. 101—103° at 15 mmHg) by acid-catalysed reaction of the corresponding acid with methanol]. The mixture was heated under reflux in a nitrogen atmosphere for 5 h. The precipitated sodium bromide was filtered off and the solvent was evaporated from the filtrate under reduced pressure.

A portion (4·268 g) of the crude oily triester thus obtained was dissolved in glacial acetic acid (25 ml). Water (25 ml) and 12N-hydrochloric acid (50 ml) were added and the mixture was heated under reflux for 72 h. It was then poured into ice–water and extracted thoroughly with ether. The extracts were washed with water and saturated sodium chloride solution, dried ($MgSO_4$), and evaporated *in vacuo* to afford an oil (2·72 g) which slowly crystallized. Recrystallization from hexane afforded n-pentylsuccinic acid, m.p. 77—81° (lit.,[23] 77°), $\nu_{max.}$ (CCl_4) 3400—2400 and 1717 cm^{-1} (Found: C, 57·45; H, 8·45. Calc. for $C_9H_{16}O_4$: C, 57·45; H, 8·55%).

Crystalline n-pentylsuccinic acid (684 mg, 3·63 mmol) was dissolved in anhydrous methanol (7 ml), and benzene (10 ml) and concentrated sulphuric acid (5 drops) were added. The mixture was refluxed under anhydrous conditions for 2·5 h, then poured into ice–water. The organic layer was separated, and the aqueous phase was extracted thoroughly with benzene. The combined organic solutions were washed with saturated aqueous solutions of sodium hydrogen carbonate and sodium chloride, dried ($MgSO_4$), and evaporated *in vacuo* to afford oily dimethyl n-pentylsuccinate (700 mg).

Dimethyl 3-n-butyl-3-hydroxyglutarate (4). This was an oil, $\nu_{max.}$ (CCl_4) 3510 and 1742 cm^{-1}, *m/e* (tentative assignments) [M^+ (*m/e* 232) not detected] 201 $(M - OCH_3)^+$, 183 $(M - OCH_3 - H_2O)^+$, 175 $(M - C_4H_9)^+$, 159 $(M - CH_2CO_2CH_3)^+$, 143 (base peak) $(M - OCH_3 - H - C_4H_9)^+$, 127 $(M - OCH_3 - H - CH_2CO_2CH_3)^+$, 101 $(M - C_4H_9 - H - CH_2CO_2CH_3)^+$, 85 $(CH_3{\cdot}[CH_2]_3{\cdot}CO)^+$, and 74 $(CH_2{\cdot}CO_2CH_3,H)^+$.

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