Colletruncoic Acid Methyl Ester, a Unique Meroterpenoid from *Colletotrichum truncatum**

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Fungal Metabolites, Butane-2,3-diols, 2-Hydroxymethylhexa-2,4-dienol, Soybean Pathogen, NMR Studies

Metabolites isolated from the soybean pathogen *Colletotrichum truncatum* were identified as *meso*- and D-(−)-butane-2,3-diol and the previously unreported compound, colletruncoic acid methyl ester, to which structure **1** is assigned mainly on ^{1}H- and ^{13}CMR evidence. The compound has a skeleton unprecedented for a fungal polyketide. The previously unknown 2-hydroxymethylhexa-2,4-dienol was also isolated.

Introduction

Colletotrichum spp. have furnished a number of biologically active and structurally unusual metabolites, such as the colletotrichins [1], colletodiol [2] and gloeosporone [3]. As part of a program on mycotoxin production by fungi, we undertook a study of metabolites produced by *C. truncatum,* a species from which no metabolites appear to be known but which is of agricultural importance as a pathogen of soybean [4]. Chromatography of a culture filtrate obtained from the fungus yielded substantial amounts of partly resolved mixtures of *meso*- and D-(−)-butane-2,3-diol, and numerous fractions consisting of obviously closely related substances (^{1}HMR spectra) that were active in the brine shrimp bioassay [5]. These fractions, ranging in amount from 0.2 to at most a few mg, tended to decompose or rearrange during their separation and only one compound could be isolated in an essentially homogeneous but oily form. This also decomposed during the course of our studies; however, sufficient data were accumulated to define its structure as **1**. The compound is highly unusual, as a fungal metabolite, both in the absence of oxygenation of its benzenoid ring and as a contradiction of Turner's "rule" that "the uncyclized residues from the methyl ends of polyketide chains are never shorter than residues from the carboxyl end of the chain" [6].

Materials and Methods

Fungal culture

Colletotrichum truncatum (Schweinitz) Andrus *et* Moore, was obtained from the American Type Culture Collection, Rockville, Md., USA, as ATCC 18013 and maintained on potato-dextrose agar. Small portions of mycelium were used to inoculate potato-dextrose broth (cubed potato, 200 g fresh weight; dextrose, 20 g, boiled in 1 l H_2O for 1 h; strained and volume readjusted to 1 l) contained in 60 Roux bottles (100 ml each) which was then incubated at 27 °C in the dark in still culture for 6 weeks.

Isolation of metabolites

Cultures were harvested by filtration and the filtrate extracted with ethyl acetate (3 × 2 l). The material (700 mg) obtained on evaporation of the washed extract was chromatographed over a column of silica gel (SiO_2) (300 g; Camag DFO) with methanol–chloroform (MeOH–$CHCl_3$) (1:4 v/v) as irrigant, collecting 25 ml fractions. Fractions 19–21 (54 mg) were rechromatographed on the same adsorbent (50 g) with MeOH–$CHCl_3$ 5:95 v/v in 6 ml fractions. The material (17 mg) eluted in fractions 25–31 was further purified by preparative thin layer chromatography (TLC) on SiO_2 (Baker 7001-4; 8 plates, 20 × 20 cm) in MeOH–ethyl ether (5:95 v/v). The most strongly UV quenching band was scraped off and eluted with MeOH–ethyl acetate (1:4 v/v) to give colletruncoic acid methyl ester (7 mg) as a colourless, viscous syrup homogeneous by TLC, and

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used as obtained for ^{1}HMR spectroscopy. Decomposition occurred, fortunately only after extensive decoupling experiments had been performed, and the material was repurified (^{1}HMR spectrum unchanged) by preparative TLC as above, for obtention of the CMR spectrum. It again decomposed (during an attempted 2D NMR experiment) and could not be repurified.

Fractions 22–26 (125 mg) from the main separation consisted very largely, and fractions 27–36 entirely, of mixtures of the *meso*- and D-(−)-butane-2,3-diols in the approximate ratios 1:2 and 3:1 respectively. Rechromatography of fractions 22–26 (MeOH–$CHCl_3$, 5:95 and 1:9 v/v; SiO_2, Camag DFO, 55 g) achieved substantial separation of the isomers from each other.

No other homogeneous fractions were obtained by rechromatography of main fractions 19–22 (above) and 16–18 (36 mg). The mixtures obtained contained numerous UV quenching substances (R_f values 0.04–0.76 on TLC on Macherey-Nagel Polygram Sil G/UV in MeOH– $CHCl_3$, 3:97 v/v). In another, preliminary run, several such fractions were found to give rise to prominent ^{1}HMR resonances characteristic of structural features of compound **1**.

In a subsequent run, the filtrate from 120 Roux bottle cultures was chromatographed over SiO_2 (300 g) in MeOH–CH_2Cl_2 (5:95). Fractions (20 ml each) were monitored by TLC and those with R_f values near that of **1** were scanned by ^{1}HMR. No evidence for the presence of **1** was found while the butanediols were detected in only greatly reduced amounts. Fractions 116–129 (5.4 mg) yielded **5**, homogeneous by TLC, ^{1}H and ^{13}CMR, as an oil after rechromatography (SiO_2, BDH 60–120 mesh). The 0,0-diacetate was prepared by treatment with acetic anhydride (30 µl) in pyridine (50 µl) at room temperature overnight and isolated by evaporation in vacuo; m/e 212 (M^+), 152 ($M-CH_3CO_2H$), 137 ($M-CH_3CO_2H-CH_3$), 110 ($M-CH_3CO_2H-CH_2CO$), 97, 95, 93, 92 ($M-2CH_3CO_2H$), 81, 79, 77 ($M-2CH_3CO_2H-CH_3$), 67, 65, 43 (100%, CH_3CO). Exact mass calculated for $C_{11}H_{16}O_4$: 212.1048; found 212.1044.

Spectroscopy

Infrared spectra (IR) were determined on a Pye Unicam PU 9516 spectrometer and ultraviolet spectra (UV) with a Shimadzu UV-240 instrument. The mass spectra were obtained with a Varian MAT 311A system at 70 eV and an inlet temperature of 18 °C. A Varian XL-200 system was used for measuring ^{1}HMR spectra and ^{1}H-^{1}H decoupling experiments, and a Varian XL-300 system for ^{13}CMR spectra, with $CDCl_3$ as solvent and tetramethylsilane as internal reference.

Results and Discussion

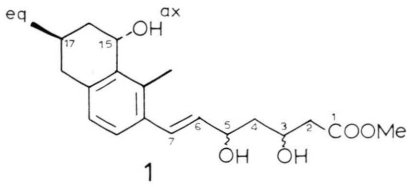
Butane-2,3-diols

The identity of the components of fractions 22–26 and 27–36 as the *meso*- and D-(−)-isomers of butane-2,3-diol followed without ambiguity from the IR spectrum (liq. film: strong broad OH absorption 3370 cm^{-1}, no carbonyl) and the NMR spectra which were in excellent agreement with literature data (^{1}HMR [7, 8], ^{13}CMR [8, 9]). It may be noted that the ^{1}HMR spectrum published in ref. [7]. is clearly that of the *meso* compound, containing a small amount of the other isomer. ^{1}HMR parameters for the D-(−)-isomer do not appear to have been reported previously; the sample obtained by rechromatography of fractions 22–26 was 90% pure (10% *meso*-isomer by ^{1}HMR) and had δ 3.52 (m, 2H) and 1.17 (d, J = 5.5 Hz), $[\alpha]_D$ − 6°.

The butane-2,3-diols are well known as bacterial metabolites [10] but a search of the literature revealed only one report of a previous isolation from a fungus [8].

Colletruncoic acid methyl ester

The IR spectrum of this compound contained hydroxyl absorption at 3610 (s) and 3510 (br) cm^{-1} and a strong band at 1740 cm^{-1} indicative of a saturated ester or lactone. The ^{13}CMR spectrum established the presence of 20 nonequivalent carbons with signals at 20.7, 22.0, 51.9 (CH_3), 23.2, 38.5, 40.2, 41.5, 42.6 (CH_2), 65.2, 68.0, 72.7 (−CHOR−), 127.3, 128.0, 129.9, 137.6 (sp^2–CH), 133.7, 134.8, 135.2, 137.2 (quat sp^2–C) and 173.0 (−COOR). The lowest field signal is readily assigned to the carbonyl carbon of an ester and the methoxyl signal at δ 51.9 suggested a $-COOCH_3$ function. The ^{1}HMR spectrum revealed the presence of an aromatic nucleus bearing two *ortho* coupled protons (δ 6.91 and 7.04, J = 7.8 Hz), an aromatic methyl group (δ 2.25), a nonaromatic methoxyl (δ 3.70) and a *trans*-disubstituted olefinic bond. Alternative assignments for these groups are excluded by the presence of eight



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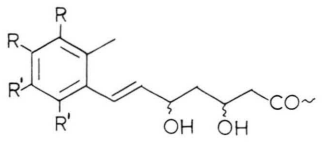
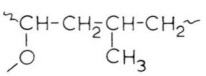


2 a R = C; R' = H
b R = H; R' = C



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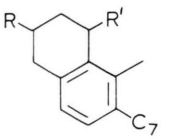

4 a R = Me; R' = OH
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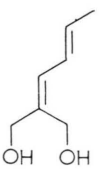

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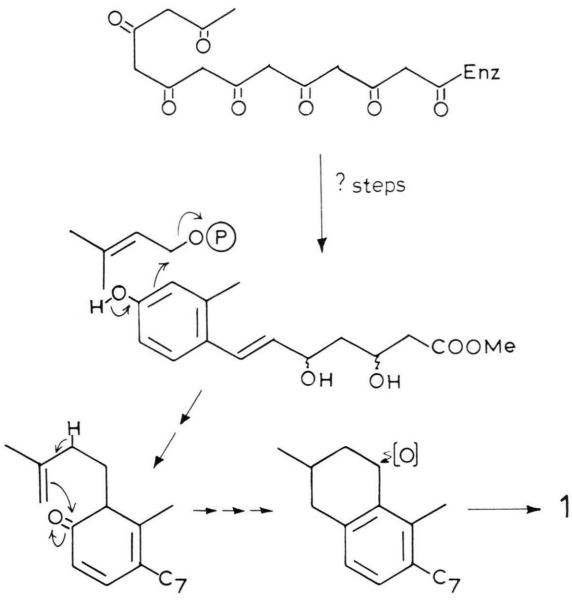
non-oxygenated sp^2-carbon signals in the range 127–137 ppm in the ^{13}C spectrum with no absorption for a methyl ketone. The olefinic protons gave rise to the AB part of an ABX pattern (δ_A 6.75, d, J = 16.3 Hz, H-7 and δ_B 5.79, dd, J = 6.5, 16.3 Hz, H-6) which was clearly indicative of a double bond adjacent to an aromatic ring (*cf.* styrene H_a δ 6.64 [11]). Irradiation of the H-6 pattern reduced the δ 6.75 (H-7) doublet to a singlet and a methine multiplet at δ 4.55 became a doublet of doublets (J = 4 and 6 Hz), thus identifying H-5 which must be bonded to carbon bearing oxygen. Irradiation of the H-5 pattern, collapsed the H-6 absorption to a clean doublet, J = 16.3 Hz and each of two partially overlapping multiplets at δ 1.70 and 1.78 were transformed to doublets of doublets with J = 3.5, 14 Hz and J = 8, 14 Hz respectively, thereby revealing the 4-methylene grouping. Analogous decoupling experiments showed that the latter methylene protons are adjacent to another methine proton at δ 4.32 which, in turn, is flanked by a second methylene group near δ 2.6 which appears as the AB part of an ABX pattern typical of a freely rotating methylene group adjacent to an asymmetric centre. The lack of further splitting and the chemical shift of these methylene protons require the second substituent to be deshielding and to lack α-protons. The only available grouping which fits these criteria is the oxycarbonyl function. Hence, at this juncture, the sum of these data led to part structure **2a** or **b** with the aryl methyl ortho to the C_7 side-chain because this fragment almost undoubtedly must be polyketide derived. Irradiation of the methyl signal at δ 2.25 slightly sharpened the aryl proton doublet at δ 7.04 suggesting that this methyl group is para to the lower field aryl proton as in **2a**, although the alternative formulation **2b** cannot be entirely dismissed.

The remaining ^1H absorptions are uniquely accommodated by part structure **3** with methine patterns at δ ~ 2.1 and 4.92, attributable to H-17 and -15, respectively. The former is responsible for the doublet splitting of the methyl signal at δ 1.04 and is also coupled to one pair of methylene protons at δ 2.79 (dd, J = 3.5, 16.0 Hz) and 2.29 (dd, J = 11.5, 16.0 Hz) and a second pair at δ 1.96 and 1.40, each of which appear as doublets of doublets (J = 2.5, 14.0 and J = 12.5, 14.0 Hz, respectively) upon irradiation of the δ 4.92 (dd, J = 2.5, 3.5 Hz) methine pattern.

The aryl ring must be substituted with the terminal carbons of the five-carbon fragment as in **4a** or **4b**, with which the chemical shifts of the benzyl methine and methylene protons are in excellent accord. It follows that the methoxyl group is bonded to the carbonyl group and the oxygen functions are hydroxyl groups, as inferred from the methine proton and carbon chemical shifts, in agreement with the IR absorption. For biosynthetic reasons, structure **4a** is clearly preferred (Scheme 1) since it conforms to the expected mode of addition of the dimethylallyl pyrophosphate. The relative stereochemistry of the 17-methyl and 15-hydroxyl groups follows from the coupling constants of the corresponding methine protons.

2-Hydroxymethylhexa-2,4-dienol

An attempt to obtain a further supply of compound **1** from a fresh batch of cultures was unsuccessful but, instead, furnished a small amount of material which was identified as 2-hydroxymethylhexa-2,4-dienol on the following evidence. The ^1HMR spectrum revealed the absorption for a methyl group (δ 1.78, dd, 3H) vicinally coupled (J = 6.8 Hz) to an adjacent olefinic proton (δ 5.80, dq, 1H, H-5) and long-range coupled (J = 1.5 Hz) to another olefinic



Scheme 1. Proposed biosynthetic route to methyl colletruncoate.

proton (δ 6.31, ddq, 1H, H-4). These olefinic protons, H-4 and H-5, exhibited a common coupling of 14.6 Hz and, therefore, could be assigned to a *trans* double bond. The remaining coupling of H-4, J = 10.8 Hz, showed its vicinal relationship to a third olefinic proton (δ 6.07, broadened d, 1H, H-3). The spectrum also contained two singlets (δ 4.24, 2H; δ 4.39, 2H) which could be readily assigned to two oxymethylene groups. The singlet at δ 4.24 was distinctly broad ($\nu_{1/2}$ ~ 2 Hz) indicating long-range coupling to H-3. The ^{13}C spectrum consisted of seven signals: δ_c 135.6 (quat sp^2 C), 126.1, 129.6, 132.8 (sp^2CH), 60.2, 67.5 ($-CH_2O-$) and 18.5 (CH_3) as established by the DEPT sequence [12]. These data led directly to the formulation of the compound as **5**, in excellent accord with the UV spectrum, λ_{max} 232 nm (ε 19,500) which is that of a trialkyl-substituted *trans*-1,3-butadiene [13]. The presence of hydroxyl groups was indicated by the IR spectrum, ν_{max} 3600 (s) and 3450 (br) cm^{-1}, and was confirmed by acetylation to the 0,0-diacetate; δ_H 1.80 (dd, J = 6.75, 1.5 Hz, 3H, CH_3), 2.05 (s, 6H, $2CH_3CO$), 4.59 (s, 2H, $-CH_2O-$), 4.72 (s, 2H, $-CH_2O-$), 5.87 (dq, J = 13.5, 6.75 Hz, 1H, H-5), 6.24 (J = 11.0 Hz, 1H, H-3), 6.35 (ddq, J = 13.5, 11.0, 1.5 Hz, 1H, H-4) and the requisite molecular constitution $C_{11}H_{16}O_4$ as shown by high resolution mass spectrometry.

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