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BOTRYDIENAL, A NEW PHYTOTOXIN, AND ITS RELATED METABOLITES, DEHYDROBOTRYDIENAL AND DEACETYLDIHYDROBOTRYDIAL PRODUCED BY BOTRYOTINIA SQUAMOSA

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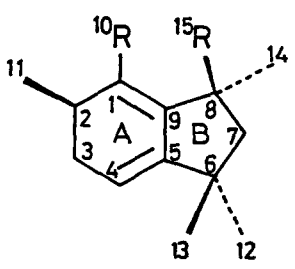
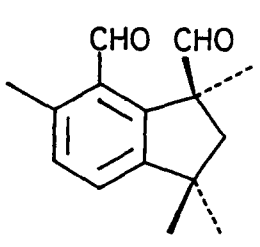
Summary: A new phytotoxin, botrydienal, and its two related metabolites, dehydrobotrydienal and deacetyldihydrobotrydial, were isolated from Botryotinia squamosa, and their structures were elucidated as 1, 2 and 3, respectively, on the basis of spectral analysis.

Recently, we reported that Botrytis cinerea, a phytopathogenic fungus, produces abscisic acid (ABA), a plant hormone, and that the ABA production by B. cinerea is enhanced by irradiating blue light on the culturing mycelium.[1] These findings prompted us to survey the possible production of ABA by other related fungi belonging to the genera Botrytis and Botryotinia, when cultured under blue light. Although ABA is not produced by these fungi, we found that Botryotinia squamosa, a pathogenic fungus, produces a phytotoxic substance. We here report the isolation and structure elucidation of the phytotoxin named botrydienal (1) and its two related metabolites, dehydrobotrydienal (2) and deacetyldihydrobotrydial (3).

B. squamosa IFO 9432 was cultured on a potato dextrose agar medium at 25°C for 7 days. The cultured mycelia and agar medium were macerated with acetone, and the acetone extract evaporated to an aqueous residue, which then was extracted with ethyl acetate at pH 9. Monitored by the bioassay on turnip seedlings, the phytotoxin was purified by column chromatography on silica gel followed by preparative TLC (silica gel) and HPLC (Develosil C-8), afford-



1 R=CHO
5 R=CH_2OH



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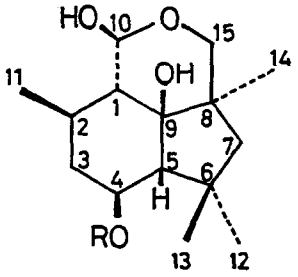


3 R=H
4 R=Ac

ing botrydienal (1) as the active principle (yield 10 mg from 6 liters of the cultured material). Two related metabolites, dehydrobotrydienal (2)[2] (5.8 mg) and deacetyldihydrobotrydial (3)[3] (25 mg) also were isolated from the same cultured material of B. squamosa.

The physicochemical properties of botrydienal (1) are colorless needles; mp 69-70°C; $C_{15}H_{20}O_2$ (M^+ obsd. m/z 232.1479, calcd. 232.1469); $[\alpha]_D^{30}$ +190° (c=0.18, n-hexane); UV λ_{max}^{EtOH} nm (ε) 245 (sh, 2670) and 324 (6940); IR ν_{max}^{KBr} cm^{-1} 1720, 1660 and 1635; ^{1}H-NMR[4] (100 MHz, $CDCl_3$) δ: 0.92 (d, $J_{2,11}$=7.1 Hz, 11-CH_3), 1.06, 1.25, 1.54 (s, 12-, 13- and 14-CH_3), 1.63, 2.24 (d, J_{gem}=13.9 Hz, 7-CH_2), 2.17, 2.48 (ddd, $J_{2,3\alpha}$=8.1, $J_{3\alpha,4}$=3.2, $J_{2,3\beta}$=2.0, $J_{3\beta,4}$=6.1 and J_{gem}= 18 Hz, 3α-, 3β-H), 2.98 (m, 2-H), 5.87 (ddd, $J_{2,4}$=0.5, $J_{3\alpha,4}$=3.2 and $J_{3\beta,4}$=6.1 Hz, 4-H), 9.67, 9.74(s, 15- and 10-CHO); ^{13}C-NMR (25 MHz, $CDCl_3$) δ: 18.3, 25.3, 28.7, 30.5 (q, CH_3 x 4), 30.5, 50.4 (t, CH_2 x 2), 24.2 (d, CH), 40.4, 56.3 (s, -Ċ- x 2), 123.5 (d, -ĊH=), 135.7, 149.2, 154.9 (s, -Ċ= x 3), 189.5, 199.5 (s, CHO x 2).

^{1}H-NMR spin decoupling experiments revealed that 1 has a partial structure of $^{11}CH_3$-^{2}ĊH-$^3CH_2$-^{4}CH=^{5}Ċ- (the ring A). An α,β,γ,δ-unsaturated aldehyde group whose presence was indicated by UV (λ_{max} 324 nm) and IR (ν_{max} 1660 cm^{-1}) spectra, should be connected to this partial structure, because only one 4-vinyl proton present in the molecule was commonly contained in both moieties. The conjugated aldehydic carbon that appeared as a double-doublet (δ_C 189.5, J=172 and 3 Hz) in the gated decoupled ^{13}C-NMR spectrum indicated that one proton should be located three bonds away from the aldehydic carbon. Thus ring A was constructed as 1. Further structural information on 1 was obtained from dehydrobotrydienal (2).

Dehydrobotrydienal (2) has the following properties: colorless oil; $C_{15}H_{18}O_2$ (M^+ at m/z 230); $[\alpha]_D^{30}$ -22° (c=0.14, n-hexane); UV λ_{max}^{EtOH} nm (ε) 260 (6390) and 315 (2080); IR ν_{max}^{KBr} cm^{-1} 1725, 1685 and 1585; ^{1}H-NMR (200 MHz, $CDCl_3$) data shown in Fig. 2; ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 19.2, 22.1, 30.5, 31.4 (q, CH_3 x 4), 51.3 (t, CH_2), 42.4, 57.9 (s, -Ċ- x 2), 128.3, 132.2 (d, -ĊH= x 2), 129.7, 140.8, 141.2, 153.1 (s, -Ċ= x 4), 191.3, 200.5 (d, CHO x 2).

The molecular formula indicates 2 to be a dehydro-derivative of 1 and its UV spectrum suggests that an aromatic ring is contained in 2. Upon irradiation of aromatic 11-CH_3, large NOE enhancement was observed on 10-CHO and 3-H, and the 3-H was meta-coupled to 4-H, the ring A structure being constructed. The partial structure surrounding ring B was constructed similarly by measuring NOE enhancements (Fig. 2). The planar structure of 2 thus was clarified, and the planar structure of 1 deduced.

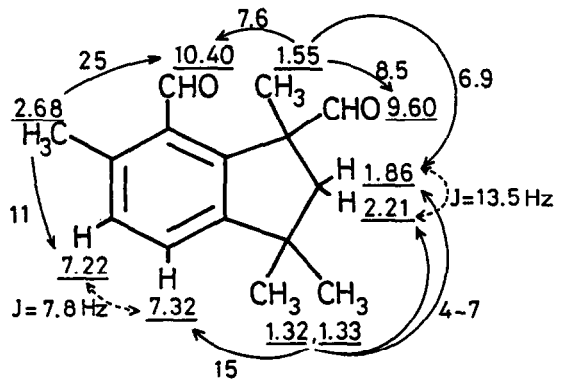


Fig. 2 ^{1}H-NMR data and NOE(→ , %) values of 2

The physicochemical properties of deacetyldihydrobotrydial (3) are colorless prisms; mp 163-165°C; $C_{15}H_{26}O_4$[5]; $[\alpha]_D^{28}$ +22.4° (c=1.2, MeOH); IR ν_{max}^{KBr} cm^{-1} 3460, 3370 and 1000. ^{1}H- (200 MHz, pyridine-d_5) and ^{13}C-NMR (50 MHz, CD_3OD) spectra were analyzed by proton spin decoupling experiments involving the difference spectral technique and ^{13}C-[^{1}H] selective decoupling experiments, clarifying that the molecule is constructed of the four partial structures shown

in Fig. 3. The presence of three OH groups was shown by disappearing signals (δ_H 5.60, 5.82 and 8.31) when D_2O was added.

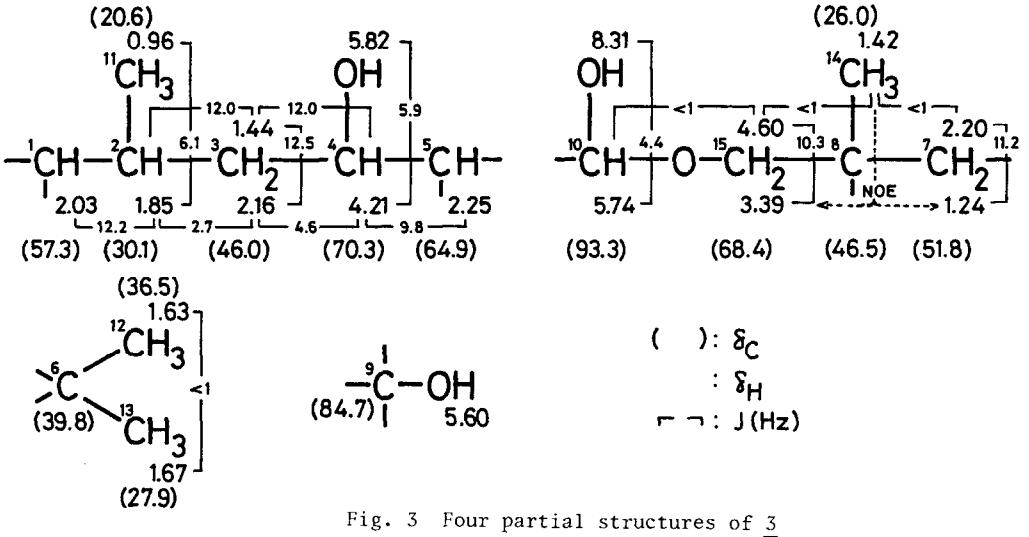
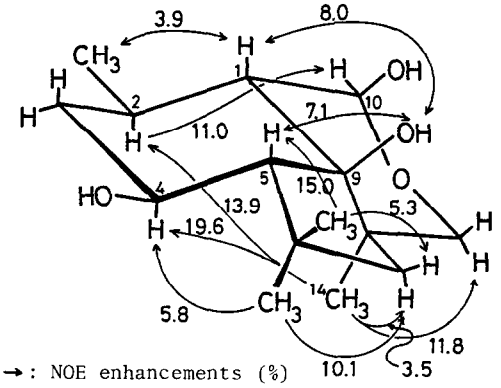


Fig. 3 Four partial structures of 3

In the ^{13}C-NMR spectrum measured with a coaxial dual tube of CD_3OD/CD_3OH,[6] the upfield shifted carbons at δ_C 70.3 ($\Delta\delta_C$ 0.131), 84.7 (0.136) and 93.3 (0.112) were assigned to the three respective OH-bearing carbons at 4-C, 9-C and 10-C. Interestingly, the two carbons vicinal to the OH-bearing carbons at 1-C and 5-C (δ_C 57.3 and 64.9 or vice versa) showed large upfield values ($\Delta\delta_C$ 0.136 and 0.141, respectively), clearly indicative that they are sandwiched between the two OH-bearing carbons.[7] Three of the four partial structures in Fig. 3 could be combined, and the carbon sequence of $^4C(OH)$-^5C-$^9C(OH)$-^1C-$^{10}C(OH)$ reasonably connected.

The planar structure and the relative stereochemistry of 3 was established by NOE experiments (Fig. 4). NOE enhancements between 9-OH and 1-H or 5-H indicated that the ring juncture should be cis. Large NOE enhancements on 2-H and 4-H upon irradiation of 14-CH_3 revealed that they are axial on the same side. Evidence that 10-H is equatorial was obtained from the facts that the dihedral angle between 1-H and 10-H must be ca. 90° based on the zero value of $J_{1\text{-H},10\text{-H}}$ and that the 11% NOE enhancement on 10-H upon irradiation of 2-H. Thus the relative



→: NOE enhancements (%)

Fig. 4 Relative stereochemistry of 3

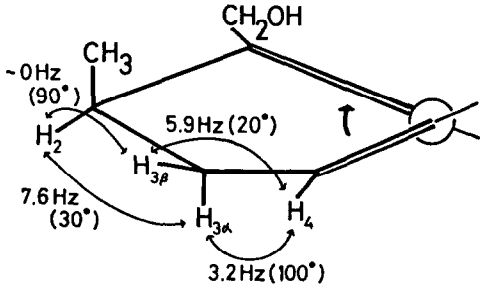


Fig. 5 Possible conformation estimated from the J values (in CD_3OD) of 5

stereochemistry of 3 was established. Because the three fungal metabolites, 1, 2 and 3, of B. squamosa should possess the same stereochemistry from the biosynthetic considerations, the relative stereochemistries of 1 and 2 were concluded to be the same as that of 3.

The absolute stereochemistry of botrydienal (1) was determined as follows: 1 was reduced with $LiAlH_4$ to the diol (5). Analysis of the dihedral angles between each two of 2-, 3α-, 3β-, and 4-H on the basis of the J values in ^{1}H-NMR spectrum indicated that ring A should have the conformation shown in Fig. 5.[8] The CD spectrum of 5 (MeOH) showed a positive Cotton effect at λ_{ext} 270 nm ($\Delta\varepsilon$ +3.0), requiring that the diene moiety in ring A has a right-handed helicity.[9] Thus, the absolute stereochemistry of 5, and therefore, the stereochemistries of 1, 2 and 3 have been determined as shown in Fig. 1.

Phytotoxic activity was bioassayed on turnip seedlings. The respective 50% inhibitory concentrations (IC_{50}) of 1, 2 and 3 were 10, 40 and 1000 μg/ml. 1 and 2 also showed antibacterial activity, the respective MICs against Escherichia coli B 110 being 10 and 20 μg/ml, and those against Staphyrococcus aureus IFO 12732 50 and 50 μg/ml.

GLC analysis revealed that botrydienal (1) was produced in a 60% higher yield under blue light (λ_{max} 460 nm, intensity 3000 erg sec^{-1} cm^{-2}) than in a dark culture.

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REFERENCES AND FOOTNOTES

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2) Botrydienal (1) was gradually converted into dehydrobotrydienal (2) in chloroform solution at room temperature.

3) The name of 3 was given because 3 was shown to be the 4-deacetyl derivative of dihydrobotrydial (4) that has been isolated from Botrytis cinerea. H.-W. Fehihaber, R. Geipel, H.-J. Mercker, R. Tschesche, K. Welmar and F. Schonbeck, Chem. Ber., 107, 1720 (1974).

4) Chemical shifts of all ^{1}H- and ^{13}C-NMR spectra are given in δ values using TMS as the internal standard.

5) The molecular formula of 3 was determined from the (M^+-H_2O) peak in the high resolution mass spectrum (obsd. m/z 252.1738, calcd. 252.1725) and ^{1}H- and ^{13}C-NMR spectral data.

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