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ISOLATION OF THE TOXIN, ALTENUISOL, FROM THE FUNGUS, ALTERNARIA TENUIS AUCT.

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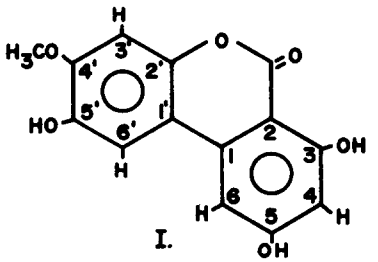
Our study of the toxigenic Alternaria spp. has led to the discovery of a new metabolite, 3,5,5'-trihydroxy-4'-methoxy-dibenzo-α-pyrone (I), trivially called altenuisol. Several other dibenzo-α-pyrone metabolites of the Alternaria have been structurally elucidated[1,2] and a number of others have been isolated but only partially characterized[3]. Altenuisol resembles one of these partially characterized structures, altertenuol[4], both in melting point and empirical formula. However, lack of published spectral data and an authentic sample of altertenuol has limited our progress in solving this apparent anomaly[5].



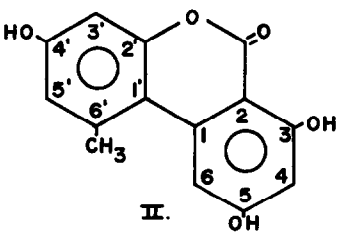
I.

The biological activity of the dibenzo-α-pyrones produced by the Alternaria seem to be quite diverse. Alternariol ($C_{14}H_{10}O_5$), alternariol monomethyl ether (AME, $C_{15}H_{12}O_5$) and altenuene ($C_{15}H_{16}O_6$) have been shown to have general cytotoxicity[1,2,6]. The antibiotic effects of alternariol can be synergized by AME[7], and AME can cause chlorosis in tobacco leaves[8]. Alternariol and altenuene also show some acute and chronic toxicity in mice (Unpublished data, authors). The toxicity of altenuisol has been evaluated against human tissue culture and bacterial cells by previously reported methods[9,10]. The ID_{50} dose with Hela cells was 8 μg/ml and zones of inhibition with Bacillus mycoides were noted from

200-5 μg/assay disc. This bioactivity and the near ubiquitous presence of Alternaria in agricultural commodities have indicated the potential importance of these compounds in contaminating our food supply.

Altenuisol was isolated from the 70% acetone extract of 28-day old cultures of A. tenuis grown on rice[2]. Column chromatography using silica gel G and an increasing tetrahydrofuran in benzene elution series afforded separation of altenuisol as a blue fluorescent band (Ca. 1 mg/gm crude extract). This semi-pure preparation was further purified by rechromatography on silica gel G using ethyl acetate as the eluant.

Altenuisol crystallized from hot acetic acid as slender needles, m.p. 277-282°. It was soluble in ethanol, acetone, and ethyl acetate, but only slightly soluble in chloroform or diethyl ether, and was insoluble in benzene and hexane. The empirical formula, $C_{14}H_{10}O_6$, was determined by high resolution mass spectroscopy (Obs.: 274.0495, Calc.: 274.0476). The uv absorption maxima in ethanol were 216, 256, 278 (shoulder) nm (ε: 9,000, 11,000, 3,500) and with an added drop of 20% NaOH the λ_{max} were 208 and 264 nm (ε: 27,400, 9,400). The major ir bands (KBr) were at 3380, 3280, 1653, 1620, 1600, 1560, 1530, 1510, 1440, 1370, 1275, 1200, 1180, 1170, 1160, 1095, 1035, 985, 915, 857, 830, 815 and 780 cm^{-1}. These spectral data indicate the presence of hydroxy groups (3380, 3280 cm^{-1}), the presence of a hydrogen-bonded lactone (1653 cm^{-1} and uv shifts in base) and a substituted aromatic ring system (aromatic C=C 1620-1440 cm^{-1}, out of plane C-H 857-780 cm^{-1}). A hydroxylated dibenzo-α-pyrone moiety was also supported by comparison of the ir pattern for alternariol[3] (II) (3440, 3180, 1657, 1623, 1608, 1573, 1510, 1457, 1418 cm^{-1}).



II.

The nmr spectrum in tetrahydrofuran-d_8 indicated the presence of four aromatic protons (singlet 7.38 δ, doublet 6.92 δ, singlet 6.70 δ, doublet 6.47 δ) and three methoxyl protons at 3.89 δ. A peak at 11.62 δ suggested a proton from a hydroxyl group hydrogen bonded to a

carbonyl. The aromatic doublet protons were assigned to the unprimed ring as meta-coupled, and the singlets were located on the primed ring as uncoupled para-protons. The methoxyl group was placed at C-4' by comparison of altenuisol and its triacetate derivative with scopoletin and its acetate (Fig. 1). Since the greatest downfield shift is observed in the proton at C-6', then acetylation must have taken place at C-5'. The ortho-nature of the oxygen function of the primed ring was verified by the fact that altenuisol reacted with ammonium heptamolybdate, which forms color complexes with ortho-phenols, only after hydrolysis in 57% hydriodic acid.

Altenuisol has an R_f value of 0.33 (alternariol = 0.54, alternariol methyl ether = 0.72, altenuene = 0.11) in 20% tetrahydrofuran in benzene on 250μ silica gel G thin layer plates. It appears as a streaking blue fluorescent spot under both long and short wave ultraviolet light. The fluorescence fades very rapidly (within 10 minutes on dried plates) and is somewhat characteristic of this particular compound.

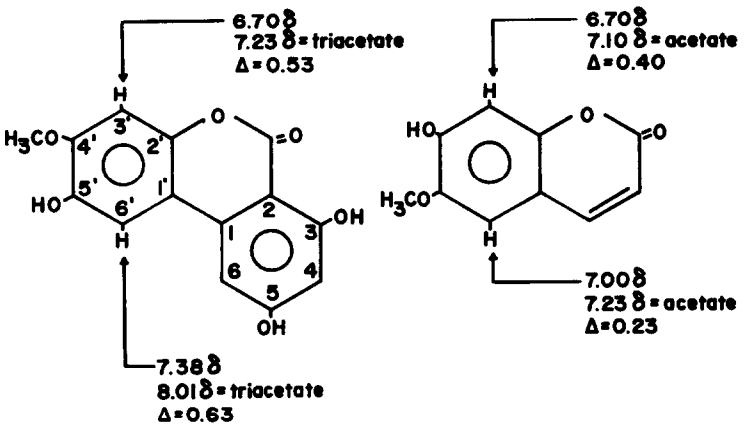


Fig. 1

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