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ISOLATION OF PHYTOTOXIC SUBSTANCES PRODUCED BY *CEPHALOSPORIUM GREGATUM* ALLINGTON & CHAMBERLAIN

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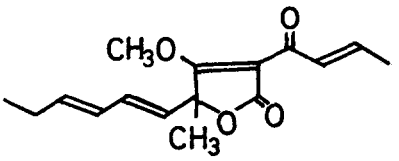
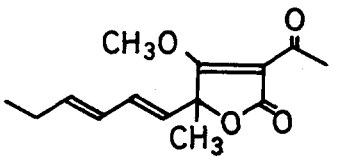
Cephalosporium gregatum is a root infecting fungus which causes brown stem rot of azuki beans (*Phaseolus radiatus* L., *var. aurea*, Prain).

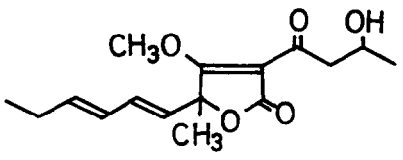
In the course of our researches on the metabolites produced by genus *Cephalosporium*, we isolated five new phytotoxic compounds closely related to Aspertetronin A and B, which have been obtained as the metabolites of *Aspergillus rugulosus* by Ballantine et al.[1]



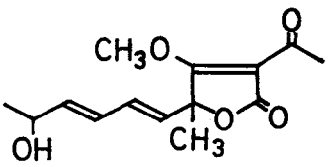
I



II



III



IV

They gave a characteristic lilac color on the thin layer chromatograms sprayed with diazotized o-dianisidine followed by ammonia. The present paper describes the isolation and structural elucidation of these compounds.

Cephalosporium gregatum was grown in azuki bean stem medium containing 5 % dextrose for 30 days at 25°. New phytotoxic compounds, (A, B, C, D, E), were isolated by repeated silica-gel column chromatography and preparative TLC of the ethyl acetate extract from the culture filtrate.

The compound A, $C_{16}H_{20}O_4$; m/e 276 (M^+), 245 (M^+-OCH_3), was recrystallized from petr-ether as colorless needles, mp 72°, $[\alpha]_D$ -140° (C=0.94 in $CHCl_3$) and was determined to be an optical antipode of Aspertetronin A[2] by comparisons of the spectral data with those in literature[1] (Formula 1).

The compound B, $C_{14}H_{18}O_4$; m/e 250 (M^+), 235 (M^+-15), 218 (M^+-MeOH), 207 (M^+-COCH_3), 176 ($M^+-C_3H_6O_2$), mp 80 – 81°, $[\alpha]_D$ +207° (C=0.84 in $CHCl_3$), was obtained as a minor product.

The IR [ν_{max}^{KBr} 1740sh, 1705] , UV [λ_{max}^{EtOH} 235 (33000), 265 (11000)] and NMR spectra (90 MHz, τ, CCl_4) [3.6 – 4.7 (4H, m, olefinic H), 6.26 (3H, s, $OC\underline{H}_3$), 7.4 (3H, s, $COC\underline{H}_3$), 7.8 – 8.0 (2H, quintet, $=CHC\underline{H}_2CH_3$), 8.52 (3H, s, $-\overset{|}{C}-C\underline{H}_3$), 9.0 (3H, t, J=7, $CH_2C\underline{H}_3$)] of this compound were similar to those of A.

From these spectral data it is obvious that this compound have an acetyl group instead of a crotony group in A.

Therefore the compound B was represented as formula 11.

The compounds, C and D, $C_{16}H_{22}O_5$, were isolated as unstable colorless viscous oils by repeated preparative TLC. The Mass [m/e 294 (M^+), 276 (M^+-H_2O), 263(M^+-OCH_3)], IR [ν_{max}^{film} 3450 (OH), 1740sh, 1705], UV [λ_{max}^{EtOH} 235 (26000), 265sh (10000)] and NMR spectra [3.5 – 4.7 (4H, m, olefinic H), 5.7 (1H, q, J=7, $-\underset{OH}{C\underline{H}}-CH_3$), 5.95 (1H, br, $O\underline{H}$, disappeared on addition of D_2O), 6.26 (3H, s, $OC\underline{H}_3$), 6.85 (2H, d, J=7, $-COC\underline{H}_2CH=$), 7.86 (2H, quintet, J=7, $-CH=C\underline{H}_2CH_3$), 8.50 (3H, s, $-\overset{|}{C}-C\underline{H}_3$), 8.70 (3H, d, J=7, $\underset{OH}{CH}-C\underline{H}_3$), 9.0 (3H, t, J=7, $CH_2-C\underline{H}_3$)] of these compounds were superimposable with those of Aspertetronin B (Formula 111). However, their optical rotations were different[3].

This indicates that the compounds, C and D, are stereo isomers of Aspertetronin B.

The compound E, $C_{14}H_{18}O_5$; m/e 266 (M^+), 251 (M^+-15), 235 (M^+-OCH_3), 22[illegible] (M^+-$COCH_3$), 217 (M^+-H_2O-OCH_3), 192 (M^+-$C_3H_6O_2$), $[\alpha]_D$ +144° (C=0.18 in $CHCl_3$) ; mp 89 – 91°, was obtained as colorless needles. The IR [ν_{max}^{KBr} 3300 (OH), 1740sh, 1705 cm^{-1}], UV [λ_{max}^{EtOH} 235 (25000), 265sh (11000)] and NMR spectra [3.5 – 4.5 (4H, m, olefinic H), 5.7 (1H, q, J=7, $\underset{OH}{CH}-CH_3$), 6.26 (3H, s, OCH_3), 7.40 (3H, s, $COCH_3$), 8.15 (1H, br, OH, disappeared on addition of D_2O), 8.50 (3H, s, $-\overset{|}{C}CH_3$), 8.91 (3H, d, J=7, $\underset{OH}{CH}CH_3$)] indicated that this compound differed from B in only one structural feature.

The NMR spectrum exhibited new signals due to a secondary methyl group and a hydroxyl group, while signals due to a ethyl group in B disappeared. It was also found that signals due to olefinic protons shifted to slightly lower field (0.1 ppm) than those of B, suggesting that E possesses a partial structure of $CH_3\underset{OH}{C}HCH=CHCH=CH-$ instead of $CH_3CH_2CH=CHCH=CH-$ in B.
Therefore the structure of the compound E can be assigned as formula IV.

This structure was further confirmed by spin decoupling experiments. Irradiation at τ4.49 caused the methyl doublet at τ8.91 to collapse to a singlet and irradiation at τ8.91 caused the quartet at τ5.70 to collapse to a doublet (J=6 Hz), indicating the presence of $CH_3\underset{OH}{C}HCH=$ group. The E, E geometry of the hexadiene portion in the compounds, A, B, C, D, and E, was also confirmed through comparisons of the NMR spectral data with those in Aspertetronin A and B.

The compounds, A, C, and D, induced the characteristic vascular browning against cutting of azuki bean seedlings and also caused brown necrotic spot on the leaves of azuki bean at a concentration of 50 ppm. They also inhibited at a low concentration (<25 ppm) the growth of many fungi and bacteria.

The compounds, B and E, showed no phytotoxic activity at a concentration of 400 ppm or more, but they showed a significant antimicrobial activity. Biological properties and details of this study will be presented elsewhere.

REFERENCES AND FOOTNOTES

1. J. A. Ballantine, V. Ferrito, C. H. Hassall and V. I. P. Jones, J. chem. soc., (C), 56 (1969)
2. Optical rotation of Aspertetronin A ; $[\alpha]_D$ +133° (C=0.3 in $CHCl_3$)
3. Optical rotations of compound C, D and Aspertetronin B ; $[\alpha]_D$ +152° (C=0.93 in $CHCl_3$), +76.9° (C=1.14 in $CHCl_3$), -70.6° (C=5.25 in $CHCl_3$)