

NOTE

CYCLONERODIOL, A
SESQUITERPENE METABOLITE
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Although *Gibberella fujikuroi* (Saw.) Wr. is noted for the variety of gibberellins and related diterpenes it produces¹, to our knowledge no other isoprenoid metabolites have been isolated from this fungus. We have examined the neutral fraction extracted from cultures of *G. fujikuroi* strain ACC 917 grown on a corn steep-glucose-ammonium succinate-salts medium (medium A) as described previously². Potassium DL-mevalonate (2.4 mmoles/liter) and calcium carbonate (12 g/liter) were added after 128 hours incubation and the cultures harvested after a further 160 hours.

The culture filtrate (7.9 liters) was treated with activated charcoal (79 g). The charcoal cake, air dried to approximately 40 % moisture, was extracted with acetone and the product partitioned between ethyl acetate and 0.5 M aqueous sodium carbonate. The non-acidic fraction (2.88 g) was chromatographed on a column (4.4 × 37 cm) of silicic acid developed with a 0~100 % gradient of ethyl acetate in petroleum ether (b.p. 30~60°C). The 45 fractions (200 ml each) collected were examined by thin-layer chromatography on silica gel G, developed with diisopropyl ether - acetic acid (95 : 5), sprayed with sulfuric acid, and heated at 110°C. An unidentified metabolite was detected in fractions 25~27 (30~35 % ethyl acetate) as an orange-red spot at Rf 0.65. The oil (302

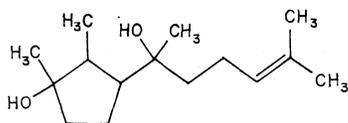
mg) obtained from these fractions contained traces of minor components at Rf 0.45 and 0.88. 7-Hydroxykaurenolide (66 mg) and 7, 18-dihydroxykaurenolide (235 mg) were isolated from fractions 20 (23 % ethyl acetate) and 33 (70 % ethyl acetate) respectively.

The crude product was rechromatographed on a column of neutral alumina (activity grade II) developed with an ethyl acetate-petroleum ether (b.p. 30~60°C) gradient, from which it was obtained as a colorless neutral oil, n_D^{25} 1.493, $[\alpha]_D^{25}$ -19.5° (c 2.7, chloroform). Its mass spectrum showed peaks up to m/e 225. Because of the relative intensities of peaks at m/e 225 and 222 (2 % and 12 %, respectively, of the most intense peak at m/e 109) they were suspected to be fragment ions from a compound of molecular weight 240. Loss of a methyl radical from the undetected molecular ion would give rise to m/e 225, and loss of a water molecule to m/e 222. The presence of a second oxygen function was indicated by the peak at m/e 204 (4 % of m/e 109) presumably arising from the loss of the elements of water from m/e 222. These conclusions from low resolution mass spectrometry were reinforced by high resolution data. The peak at m/e 222 was found to have an accurate mass of 222.1982 indicating an elemental composition of C₁₅H₂₆O (Calculated : 222.1984). Thus the parent compound should be C₁₅H₂₈O₂ and must have two double bonds or rings since a fully saturated alicyclic molecule would have four additional hydrogen atoms. The presence of at least one hydroxyl group was indicated from the infrared absorption spectrum which showed O-H and strong C-H, but no C=O stretching vibrations. The proton magnetic resonance spectrum contained only a broad triplet at τ 4.93, J=7 Hz, attributed to the olefinic proton in a grouping (CH₂-CH=C), and a multiplicity of signals above τ 7.8, of which those at τ 8.98 (doublet, J=6 Hz; CH₃-C-H), 8.87 and 8.78 (singlets; (CH₃)₂C=), and 8.36

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Fig. 1. Cyclonerodiol.



and 8.33 (singlets; $\text{CH}_3\text{-C-O}$) could be assigned to methyl groups.

While this work was in progress Nozoe *et al.*⁹⁾ reported the isolation from a species of *Trichothesium* of a new sesquiterpene, cyclonerodiol (Fig. 1), with properties closely resembling those of the *Gibberella* metabolite. Direct comparison of samples (infrared, proton magnetic resonance and mass spectra) established that the two substances were identical. Cyclonerodiol was labeled with approximately the same efficiency as the kaurenolides when cultures were supplemented with potassium ($2\text{-}^{14}\text{C}$) DL-mevalonate. A scan of radioactivity after thin-layer chromatography of the neutral extract from such cultures showed one-fifth of the total radioactivity to be associated with the sesquiterpene.

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