Isolation of Ergosterol from *Gibberella zea* Mycelium

Sir:

Gibberella zea Schwabe is the perfect stage of *Fusarium graminearum*, a plant pathogen causing stalk, ear, and root rot of corn and scab diseases on barley. This organism has been implicated in many reports of animal toxicities. Two different types of effects have been reported from studies of animals having ingested infested grains. Christensen *et al.* (1) and Stob *et al.* (2) have isolated a fungal product having uterotrophic activity. Curtin and Tuite (3) have reported on the emetic principle contained in *G. zea* infested corn. Christensen *et al.* (1) have also given evidence that ergosterol is produced by the fungus when cultured on cracked corn.

The authors wish to report the isolation of ergosterol as the major sterol product of *G. zea* when grown in an agitated, submerged, completely defined fermentation medium.

A growth medium composed of 3% glucose and 0.5% succinic acid plus salts [a modification of a synthetic medium described by Pacifici *et al.* (4) for the production of *Claviceps purpurea*] was inoculated with a liquid culture of *G. zea*, incubated at 26° for 5 days, and the mycelium removed by filtration. The mycelium was frozen immediately, freeze-dried, and powdered. This was extracted in a Soxhlet apparatus for 36 hr. with Skelly B.

The solvent was removed from the extract by evaporation *in vacuo*. The extract (0.69 Gm.), representing 34 Gm. freeze-dried mycelium, was extracted twice with 5 ml. of 90% ethanol each time. To the combined extracts was added 4 ml. of 1% digitonin solution. The resultant precipitated digitonide was recovered by filtration and dissolved in 6 ml. dimethylsulfoxide and treated according to the procedure of Issidorides *et al.* (5). The solution was heated for 15 min. on a steam bath, then cooled to room temperature and extracted once with 15 ml. and then 4 times with 6 ml. of *n*-hexane. The hexane solution was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The sterol isolated (0.11 Gm.) was recrystallized twice from ethanol.

This sterol was chromatographed on Silica Gel G (250 μ layer) buffered (pH 9.3) thin-layer plates using a solvent composed of methylene chloride–carbon tetrachloride–methanol (64:32:5). The hR_f values of the *G. zea* sterol and a recrystallized sample of authentic ergosterol were identical. The same results were obtained when both substances were chromatographed on Silica Gel G thin-layer nonbuffered plates with a solvent composed of cyclohexane–chloroform (1:1). The melting point was not depressed with mixtures of authentic ergosterol and the *G. zea* sterol. The ultraviolet spectrum of the *G. zea* sterol was identical in all respects to the spectrum of an authentic sample of ergosterol (maxima at 262, 271, 282, and 293.5 mμ). Infrared spectra of the *G. zea* sterol and ergosterol were also identical in every respect.

Although earlier reports of ergosterol as a product of *G. zea* were based upon extraction of not only the mycelium of this fungus but also the substrate upon which it was produced, the authors have conclusively proven that ergosterol is in fact the major sterol of *G. zea*, and that this organism can produce ergosterol when grown in a simple medium containing glucose and succinic acid as the sole source of carbon.

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