Presence of 6-Methoxybenzoxazolinone in Uninjured Corn Tissue

Sir:

Wahlroos and Virtanen (1) have reported the isolation of a glucoside from rye seedlings which, after enzymatic transformation to the aglucone, is capable of being converted to benzoxazolinone. Virtanen finds that aglucone formation occurs when the rye seedlings are crushed. He stated that benzoxazolinone and 6-methoxybenzoxazolinone are not originally present in the rye, wheat, or maize plants, but arise only after the plant tissue is injured (2). In a subsequent paper Wahlroos and Virtanen (3), who used maize seedlings, isolated the glucosidic precursor to 6-methoxybenzoxazolinone by placing the intact seedling in boiling water in order to destroy the glycosidase.

On the basis of the above reports, it appeared that 6-methoxybenzoxazolinone (resistance factor A, RFA) which had been isolated in these laboratories (4) would only arise when corn tissue was injured. The following experiments were conducted in order to prove or disprove the presence of 6-methoxybenzoxazolinone in uninjured corn tissue.

Six young, high-resistance corn plants, 12 inches high, were cut and immediately immersed in liquid air. The frozen plants were powdered readily on placing them between two boards and applying pressure. The powdered material was immediately added to 2,500 ml. of 95% ethanol. Six more plants were cut at the same time and were allowed to stand at room temperature for 8 hours after being cut into small pieces.

Virtanen and co-workers found it necessary to hydrolyze the glucoside precursor to 6-methoxybenzoxazolinone by boiling the tissue for 2 hours in 2 *N* hydrochloric acid to effect *in vitro* cleavage to the aglucone, since the glucoside does not hydrolyze in boiling water alone. Plant material from the liquid air treatment and that obtained by our usual procedure were dried separately at 40–45° and extracted with ether in Soxhlet extractors for 48 hours. The ether solutions were concentrated, and an amount equivalent to 1.5 Gm. of fresh plant material was chromatographed (5).

The absorption maxima (228 and 285 mμ) and the extinction coefficients of the material eluted from the columns with 80:20 ether-ethanol were superimposable. The maxima were identical with those of authentic 6-methoxybenzoxazolinone.

Since no enzymatic or *in vitro* hydrolysis of the glucoside could take place in the tissue treated with liquid air and ethanol, it must be concluded, contrary to Virtanen's reports, that 6-methoxybenzoxazolinone does exist in uninjured corn tissue.

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