Note

1. The term *image processing* was introduced in the electronic literature by L. S. G. Kovasznay and H. M. Joseph (*Proc. I.R.E.* 43, No. 5, 1955) to describe electronic techniques for sharpening photographic images. We have borrowed and expanded the term to describe the larger field defined in this communication.

10 October 1955

Scopoletin in Differentiating and Nondifferentiating Cultured Tobacco Tissue

Two morphologically distinct strains of cultured tobacco tissue, when extracted with ether, yield markedly different amounts of a fluorescent material. These two strains have been cultured for 5 years and are of a similar (but not identical) origin, being derived from root tumors on two separate seedlings of *Nicotiana affinis* (*1*). Both strains first grew as undifferentiated callus for 3 years. Strain 20-B then started to produce buds and has continued to do so for 2 years, while strain 3-S, which was cultured on the same medium and under the same conditions, has continued to grow as undifferentiated callus with occasional wound tracheids (Fig. 1). More recently, a substrain of 20-B, called 20-B-O, which has reverted to the original nondifferentiating condition, has been isolated (*2*). The tissues are all maintained in diffuse light at room temperature on a modified White's medium containing 3 g/lit of yeast extract.

The tissues were extracted with 2.5 ml of freshly distilled ethyl ether per gram of fresh weight for 16 hours in the cold room. Dry weights were determined on samples of tissue similar to those extracted. The materials present in the ether extracts were chromatographed on Whatman No. 1 paper with water as the ascending solvent. The fluorescent material, visible under ultraviolet light, moved as a distinct band and was eluted with ethyl alcohol. Optical densities of this eluted material were measured at 345 mμ in a Beckman spectrophotometer. For reasons given in a subsequent paragraph, the substance was presumed to be scopoletin, and concentrations were calculated using a molar extinction coefficient of 14,000 determined by Goodwin (*3*) for solutions of this compound.

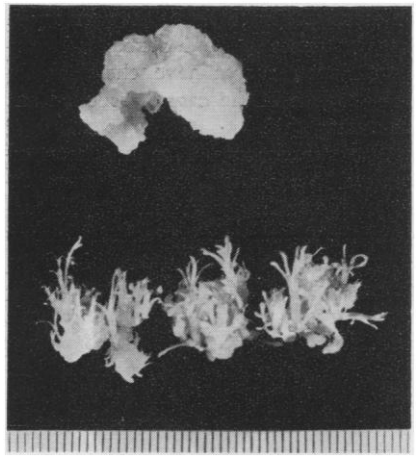

Fig. 1. Two strains of cultured tobacco tissue. (Top) Undifferentiated callus of strain 3-S; (bottom) differentiated shoots on strain 20-B. Scale in millimeters.

The results of the analyses are shown in Fig. 2. The figures are undoubtedly low estimates of the amounts of the compound present in the tissues, since the substance is light-labile, and other losses occur in the elution process. Each bar represents a mean of 12 extractions, with the standard deviation indicated. By the methods used, approximately 18 times as much of the fluorescent compound is extracted from tissue that produces organized structures (strain 20-B) as from tissue that has never so differentiated (strain 3-S). About 6 times as much is extracted from the differentiating strain as from its nondifferentiating substrain (20-B-O). Hot and cold acidic and basic aqueous extractions of macerated and unmacerated tissue yield similar relative differences.

On filter-paper chromatograms, the extracted fluorescent material moves with spots of known scopoletin (6-methoxy-7-hydroxy coumarin) giving R_f values of approximately 0.9 in butanol-acetic acid-water, and approximately 0.34 in butanol-ammonia-water (*4*). The fluorescent material, when eluted from the chromatograms with 95-percent ethanol, has an absorption spectrum that coincides with that reported by Goodwin (*3*) for scopoletin, and with the spectrum of known scopoletin determined simultaneously in alcoholic solution. Identical spectra were obtained for five separate preparations at various concentrations. Figure 3 shows one of these. The same absorption spectrum is observed in material prepared according to Best (*5*), followed by column chromatography on alumina (*3*). Four characteristic maxima occur at 230, 254, 300 and 345 mμ. Both known scopoletin and the extracted material give a green-fluorescent spot on paper chromatograms in basic solvents in daylight, but no daylight-visible spot in acidic solvents. These properties of the fluorescent material are accepted as good indirect evidence that it is indeed scopoletin.

Thus it appears that in these tissues, under the stated conditions of culture, scopoletin is associated with the presence of structures in which much differentiation exists. Similarly, Goodwin and Pollock (*6*) have observed that scopoletin occurs in *Avena* roots in relative abundance only in older parts where tissue differentiation is occurring. Whether the presence of scopoletin is a prerequisite for the formation of the organized structure or the organized structure produces the scopoletin is a question under investigation.

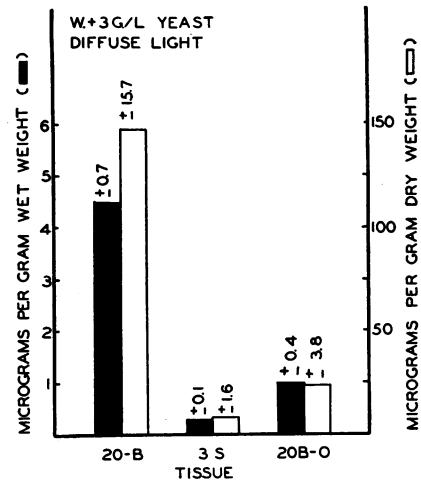

Fig. 2. Amounts of "scopoletin" extracted from three strains of tissue. The figures at the ends of the bars are standard deviations.

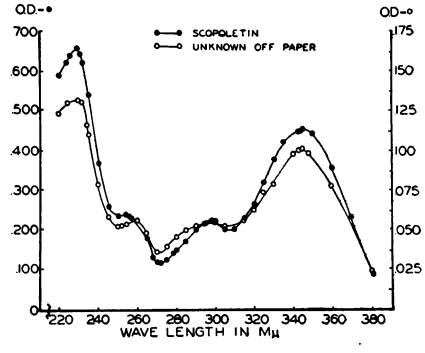

Fig. 3. Absorption spectra of known scopoletin (black dots, left-hand scale) and the fluorescent compound obtained from tobacco tissue (white dots, right-hand scale).

KATHARINE TRYON
Department of Botany,
University of Wisconsin, Madison

References and Notes

1. K. Tryon, *Am. J. Botany* 42, 604 (1955).
2. Work conducted as a fellow of the American Cancer Society, recommended by the Committee on Growth of the National Research Council. I am indebted to F. Skoog, in whose laboratory the work was done.
3. R. H. Goodwin and F. Kavanagh, *Bull. Torrey Botan. Club* 76, 255 (1949).
4. Samples of scopoletin were kindly supplied by R. H. Goodwin, Connecticut College, and by W. A. Andreae, Science Service Laboratory, London, Ontario.
5. R. J. Best, *Australian J. Exptl. Biol. Med. Sci.* 22, 251 (1944).
6. R. H. Goodwin and B. M. Pollock, *Am. J. Botany* 41, 516 (1954).

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