Studies on the Degradation of α -Tocopherol by Plant Extracts

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Many plants contain a heat-labile system that will degrade α -tocopherol (Booth, 1962; Gaunt & Stowe, 1967). The presence of such a system can lead to inaccurate tocopherol analyses and invalidate studies on the intracellular localization of tocopherols in these plants. This investigation was directed towards establishing the nature of the system.

Etiolated pea shoots (*Pisum sativum* L. var. Progress No. 9), 8–12 days old, were homogenized in buffer. The supernatant after centrifugation at 1000g for 10 min. was used as the cell-free extract (C.F.E.). α -Tocopherol in ethanol was added to this giving a final ethanol concentration of 20% (v/v). Samples were removed from the incubation mixture and transferred to propan-2-ol to stop the reaction. Tocopherol was extracted with light petroleum (b.p. 40–60°) and determined as described by Gaunt & Stowe (1967). Breakdown rates of up to 37 μ g. of α -tocopherol/min./g. fresh wt. were observed. Degradation required oxygen and had a pH optimum of 5.5. The system was completely inhibited by boiling, cyanide and ascorbic acid, and partially inhibited by EDTA. Boiling ethanol did not stop the reaction instantly, although propan-2-ol, at a final concentration of 80% (v/v) and at room temperature, was an immediate and complete inhibitor. Exposure of the system to 20% (v/v) ethanol for 20 min. before the addition of α -tocopherol resulted in complete loss of activity.

Examination of the activity of the various subcellular fractions demonstrated that no individual fraction was active. However, combination of any particulate fraction with the 100000g supernatant restored full activity of the C.F.E. The heat- and ethanol-labile factor was found in the supernatant. The active factor in the particulate fraction proved to be phospholipid after extraction and purification by silica-gel column chromatography.

α -Tocopherol breakdown is known to be caused by lipid hydroperoxides (O'Brien & Titmus, 1967). However, pea lipoxidase is completely inhibited by 20% (v/v) ethanol, and the phospholipid, when added to the system, contained very low concentrations of hydroperoxides. Further, if the hydroperoxide concentration was increased by lipoxidase action before the phospholipid was added to the system, no increase in the rate of tocopherol breakdown was observed. The pro-

ducts of the system were examined by chromatography and found to differ clearly from those produced by the action of linoleate hydroperoxides on α -tocopherol.

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Cuticular Lipids in the Gramineae

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The cuticular lipids of higher plants are a complex mixture of many different classes of lipid. Such mixtures can be studied qualitatively and quantitatively by chromatography to assay the variation both between and within species. This variation may be used to interpret plant phylogeny, and also to assess plasticity with regard to environment and ontogeny. In the present study, this comparative biochemical technique has been applied to the grasses (Gramineae), by examining the composition patterns of a wide variety of genera, and the differences that occur in any one plant or population. All material was grown from seed or tillers under controlled conditions. Cuticular waxes were extracted by a chloroform spray whose duration was chosen to avoid contamination by intracellular components. Each extract was expressed as a percentage of fresh weight, and then treated by thin-layer chromatography to determine the pattern of lipid composition, followed by gas-liquid chromatography to analyse homologous series in selected classes (usually *n*-alkanes and *n*-alkanols). Infrared spectrophotometry and qualitative tests were used as an aid in the determination of classes. The classes of compounds present include alkanes, primary alkanols, aliphatic aldehydes and their polymers, fatty acids, aliphatic esters, flavonols and aliphatic ketones including β -diketones.

Initial studies centred on the effects of environment and ontogeny on cuticular wax composition, with seedlings of oat, *Avena sativa* L., and barley, *Hordeum vulgare* L. Results showed that although the specific pattern of lipid constituents remained constant under different environmental conditions, the total amount of cuticle was proportional to light intensity and inversely proportional to relative humidity. Age and part of the plant slightly affected the proportions of compounds present.

Consequently, for all comparative work waxes were extracted from leaves of equivalent age. The significance of the results from a study of about thirty widespread grass species will be discussed. Although the results indicate a broad agreement with the present classification of the Gramineae, some controversial relationships are also suggested, for example in the case of *Cortaderia*. The analysis of variation between adjacent populations of *Agrostis stolonifera* L. has revealed possible adaptive evolution related to environment. It also provides a demonstration in chemical terms of the differentiation that can occur over small areas as a response to natural selection.

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Induced Triglyceride Metabolism in Germinating Wheat Grains

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The ungerminated wheat grain contains large reserves of triglycerides in all of its tissues. During germination these reserves are progressively broken down. This communication describes investigations to determine the precise breakdown patterns in the various tissues and to elucidate some of the controlling biochemical mechanisms.

Wheat grains, var. Capelle, were germinated for up to 8 days in the dark at 24°, and dissected into embryo axis, scutellum, starchy endosperm and aleurone layer + pericarp (bran). Duplicate samples of each tissue were analysed respectively for triglyceride content and lipase activity. Although triglyceride breakdown in the embryo axis, endosperm and bran had commenced within 24 hr. of imbibition, that in the scutellum did not start until 48 hr. after imbibition. During germination, the total triglyceride content of the grain decreased from 59 mg./90 grains at day 0 to 18 mg./90 seedlings at day 8. Scutellar lipase activity remained constant at 5.3 units/90 grains for 2 days, and then increased to 60.5 units/90 grains after 6 days of germination, thereby correlating with the pattern of triglyceride breakdown in that tissue. The appearance of lipase activity in the embryo axis and endosperm also coincided with the initiation of triglyceride breakdown in those tissues. In the bran, however, lipase activity did not appear until after 24 hr. of germination, although triglyceride breakdown had started well before this time.

When endosperm halves were incubated alone,

the triglycerides in the bran decreased by 20% during the first 24 hr. and thereafter remained constant. The contents in the endosperm did not change. Lipase activity could not be detected in either tissue. Incubation of the endosperm halves in the presence of physiological concentrations of gibberellic acid (GA₃), kinetin, indolylacetic acid (IAA), or any pair of these hormones did not induce lipase activity in the bran or endosperm.

When bran tissue was incubated alone, its triglyceride content remained constant. Incubation in the presence of an aqueous extract of the endosperm or 0.1 μ M-kinetin, however, induced a 20% decrease exactly like that in the bran of incubated endosperm halves. IAA induced a decrease of 10%, but GA₃, kinetin + GA₃ and kinetin + IAA were without effect on triglyceride contents.

These results suggest that the induction processes involved in the breakdown of reserve materials in germinating cereals are more complex than has hitherto been anticipated and that hormones other than gibberellic acid are involved.

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Mineral Translocation in Germinating Wheat Grain

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The macro-nutrients, potassium, phosphorus, magnesium and calcium, present as phytin in the endosperm and aleurone tissues of ungerminated grain (Plimmer, 1913) are progressively translocated to the developing seedlings. During the first 5 days of germination the phytase activity of the whole grain increases some sevenfold (Peers, 1953). This communication describes experiments to elucidate biochemical mechanisms controlling these processes.

Estimation of phytase activities in individual tissues of germinating grain revealed that, in the bran, phytase activity rose from 26 units/25 grains at 0 days to 127 units/25 grains after 4 days, and decreased slowly thereafter. In the embryo axis, activity remained constant at 0 units/25 grains for 4 days, and increased rapidly to 26 units/25 grains by 7 days, but activities in the endosperm and scutellum remained at a low value for the period under study.

The bran phytase activity of endosperm halves incubated alone remained constant for up to 4 days. Incubation in the presence of physiological concentrations of gibberellic acid (GA₃) or indolylacetic acid (IAA) was without effect, but 1 μ M-kinetin