DETECTION OF FUROCOUMARINS IN PLANTS AND PLANT PRODUCTS WITH AN ULTRASENSITIVE BIOLOGICAL PHOTOASSAY EMPLOYING A DNA-REPAIR-DEFICIENT BACTERIUM

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Abstract—The application of an ultrasensitive photobiological assay which detects photosensitizing furocoumarins with sensitivities as high as $1 \times 10^{-11}$ g is discussed in relation to these molecules as phytoalexins. Examples of the utilization of this technique, verified by both HPLC and TLC, are the analyses of healthy and diseased celery and carrots, dry seeds, plant extracts and oils, and whole plants and leaves. The usefulness of this method in following the metabolic detoxification of furocoumarins is also illustrated. The extreme sensitivity of the test has permitted the detection, for the first time, of both 5-methoxypsoralen and 8-methoxypsoralen in fresh carrot roots.

Key Words—Furocoumarins, psoralen, 5-methoxypsoralen, 8-methoxypsoralen, phototoxicity, photobiological assay, celery, carrot, analysis, umbelliferous seeds.

INTRODUCTION

The relationship between photosensitizing furocoumarins in a number of plant families and their properties as phytoalexins has been of considerable interest and comment (Scheel et al., 1963; Hahlbrock et al., 1981; Beier et al., 1983, Beier and Oertli, 1983). It is among members of the Rutaceae and Umbelliferae that the greatest variety and highest concentrations of furocoumarins have been reported, although these chemicals do occur in other families.

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The occurrence and distribution of furocoumarins have been used in the chemotaxonomy of plants (Crowden et al., 1969; Heywood, 1971), although often only the presence or absence of these compounds has been used for classification. The absence, for example, of furocoumarins in carrot roots, although present in leaf wax (Stadler and Buser, 1984) has always seemed surprising. However, using new ultrasensitive biological photoassay, backed by HPLC analysis, we have since confirmed the presence of furocoumarins in carrots (Ceska, 1986). Furocoumarins increase in plants when they are subjected to stress. These stresses can take many forms, including exposure to UV radiation, changes in temperature, increases in metal ion content, and infection with bacteria or fungi (Beier et al., 1983). In parsley tissue culture cells the responses to stress are very fast, and the available evidence suggests that gene activation, followed by the synthesis of specific mRNA, precedes increases in enzymes associated with the synthesis of psoralen from umbelliferone (Hahlbrock et al., 1981). Psoralen, which can also be considered the representative furocoumarin molecule, is the precursor of the linear furocoumarins, 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP). The structural formulas of key furocoumarins are illustrated in Figure 1.

The linear furocoumarins can form, in the presence of near UV (320–380 nm), both monoadducts with DNA and DNA interstrand cross-links; the angular furocoumarins such as angelicin can form only DNA monoadducts under normal circumstances (Ashwood-Smith and Grant, 1976). The consequences of these photoadditions to DNA are cell death and, in surviving cells, mutation, chromosome aberrations, and carcinogenicity in both animals and man (Ashwood-Smith et al., 1982; IARC, 1982). Berenbaum and Feeny (1981) have discussed the relative actions of angelicin and xanthotoxin in terms of toxicity to insects which feed on furocoumarin-containing plants. Ivie et al. (1983) and Ashwood-Smith et al. (1984) have both described insect larvae, the resistance of which to furocoumarins appears to be based on enzymatic detoxification.

In this paper we shall demonstrate how the application of an ultrasensitive and rapid photobiological assay for photosensitizing compounds can resolve problems of the occurrence, distribution, and metabolism of furocoumarins.

METHODS AND MATERIALS

The furocoumarins used in this study were carefully checked for purity by TLC, HPLC, and photobiological assay and were recrystallized from 95% ethanol before use. Stock solutions in methanol or ethanol were kept at 4°C in the dark. The sources for the furocoumarin standards have been described previously (Chaudhary et al., 1985). Conditions for both normal and reverse-phase HPLC are indicated in the figure legends together with details of tissue separation and preparation. TLC analysis was carried out on Merck K 60 silica