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The Metabolism of 4-Chloro-2-Methylphenoxycetic Acid in Plants

By DAVID J. COLLINS and J. K. GAUNT. (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

The results described below were obtained from an investigation into the metabolism of the selective herbicide MCPA* in rape (*Brassica napus* var. *arvensis* var. Early Giant), a susceptible species, and in red campion (*Melandrium rubrum*) and peas (*Pisum sativum* var. Progress no. 9), two species that possess some resistance to the herbicide.

The presence of two ether-soluble metabolites, rigorously identified in peas as 4-chloro-2-hydroxymethylphenoxycetic acid (hydroxy-MCPA) (Collins & Gaunt, 1970) and *N*-(4-chloro-2-methylphenoxycetyl)-L-aspartic acid (MCPA-aspartic acid), was confirmed in rape and red campion by using radioactive-isotope-dilution analysis. A further ether-soluble metabolite, which was detected in peas, has not been identified.

A β -glycoside of hydroxy-MCPA was detected in all species and tentatively identified as 4-chloro-2-(β -D-glucopyranosidomethyl)phenoxycetic acid. A β -linked sugar ester of MCPA was detected in rape and tentatively identified as 4-chloro-2-methylphenoxycetyl- β -D-glucose. A further minor unidentified β -glycoside was detected in peas.

MCPA-aspartic acid, hydroxy-MCPA and the β -glucoside of the latter were synthesized and examined for auxin activity by using the *Avena* first-internode and pea third-internode bioassays. MCPA-aspartic acid showed growth-promoting effects similar to those of MCPA. Hydroxy-MCPA possessed some auxin activity at higher concentrations (10 mg/l), but its β -glucoside was virtually inactive. After foliar applications to intact plants of each species hydroxy-MCPA was completely inactive. MCPA-aspartic acid, however, produced phytotoxic effects comparable with those produced by MCPA itself.

Foliar applications of ^{36}Cl - or carboxy- ^{14}C -labelled MCPA were made to 21-day-old plants of each species and the distribution of MCPA and its metabolites was followed during the next 14 days. In rape about 50% of the label that entered the treated leaves was rapidly translocated to the rest

of the plant. In peas and red campion translocation was markedly less extensive.

Differences were also evident in the patterns of metabolism in the three species. Rape showed conversion of MCPA, with MCPA-aspartic acid as the major product. In peas and red campion hydroxy-MCPA and the β -glycosides were predominant.

It seems probable that the differences in phytotoxicity of MCPA with these plants can be largely attributed to differential translocation and to a smaller extent to differential metabolism.

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The Subcellular Distribution of Tocopherols in the Green Leaves of *Pisum sativum*

By CHRISTINE T. HUGHES, J. K. GAUNT and D. L. LAIDMAN. (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

Previous studies on the subcellular distribution of tocopherols in plant tissues (Bucke, 1969; Dilley & Crane, 1963; Lichtenthaler, 1966; Peake, 1970) have been restricted by the limited ability of differential centrifugation to resolve cellular organelles and by the low capacity of density-gradient centrifugation in conventional rotors. Also, the recoveries of tocopherols after homogenization and centrifugation have often been very low. This could be attributable to the destructive action of tocopherol oxidase, an enzyme found in many plant tissues (Barlow & Gaunt, 1968).

In the present work the zonal rotor has been used to achieve large-capacity density gradients. Effective inhibitors of tocopherol oxidase have been incorporated in homogenization and centrifugation media. Green-leaf homogenates were fractionated into three fractions in the zonal rotor: a nuclear fraction, chloroplast fraction and a mixture of mitochondrial, microsomal and supernatant fractions. The last was then subjected to differential centrifugation at 20000g and 100000g to separate the three parts. Each fraction was analysed for tocopherols and related isoprenoid lipids.

The recoveries of tocopherols from the homogenization and centrifugation procedure were as follows: α -tocopherol, 65%; γ -tocopherol, 82%; δ -tocopherol, 87%; α -tocopherolquinone, 66%.

The purity of each subcellular fraction was assessed by analysis of marker compounds and by

* Abbreviation: MCPA, 4-chloro-2-methylphenoxycetic acid.