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SHORT COMMUNICATION

THE BIOSYNTHESIS OF PISATIN*

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Abstract—The biosynthesis of pisatin, an antifungal compound produced in *Pisum sativum* L. tissue infected with various parasitic organisms or treated with chemicals, was investigated. L-phenylalanine-[U-^{14}C]methionine-methyl-^{14}C, cinnamic acid-COOH-^{14}C and acetate-1-^{14}C administered to excised pea pods were incorporated into pisatin. Evidence is presented that implicates acetate and cinnamic acid as precursors of pisatin.

INTRODUCTION

THE antifungal compound, pisatin (I), was isolated from peas (*Pisum sativum* L.) and characterized by Cruickshank and Perrin[1] (see Fig. 1). These authors suggested that this compound

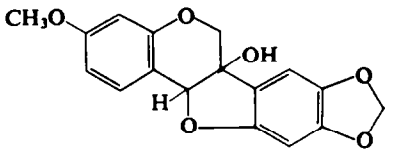

FIG. 1.

is of direct significance in the natural disease resistance of the pea. Pisatin is structurally related to various other antifungal compounds (phytoalexins)[2] such as orchinol,[3] phaseollin,[4] and trifolirhizin[5] which occur as abnormal metabolites in various plant genera after attack by certain parasitic organisms. This study was initiated to determine which metabolic pathway of the plant is altered in the production of these abnormal metabolites. The biosynthesis of pisatin was chosen for preliminary study since it is reasonably simple to isolate and determine quantitatively. Also, its production can be induced both chemically and biologically.

RESULTS AND DISCUSSION

Pisatin labelled with ^{14}C was isolated from excised split pea pods 48 hr after L-phenylalanine-[U-^{14}C], methionine-methyl-^{14}C, cinnamic acid-1-^{14}C, acetate-1-^{14}C, D-glucose-[U-^{14}C] and L-tyrosine-[U-^{14}C] were administered (Table 1). Production of pisatin was induced by addition of $3{\cdot}0 \times 10^{-3}$ M $CuCl_2$ to the pea pods. The relatively high incorporation

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[1] I. A. M. CRUICKSHANK and D. R. PERRIN, *Australian J. Biol. Sci.* **14**, 336 (1961).
[2] I. A. M. CRUICKSHANK, *Ann. Rev. Phytopathol.* **1**, 351 (1963).
[3] E. GAUMANN and H. KERN, *Phytopathol. Z.* **36**, 1 (1959).
[4] I. A. M. CRUICKSHANK and D. R. PERRIN, *Life Sci.* **9**, 680 (1963).
[5] J. B. BREDENBERG and P. K. HIETALA, *Acta Chem. Scand.* **15**, 696 (1961).

obtained for cinnamic acid and phenylalanine indicates the normal shikimic acid pathway is operative in forming part of the pisatin molecule. The greater percent incorporation of phenylalanine can be attributed to its higher specific activity, but since cinnamic acid shows a much lower isotope dilution than phenylalanine, the latter may be converted to pisatin by way of cinnamic acid. A large portion of the radioactivity in the labelled pisatin from methionine-methyl-^{14}C would presumably reside in the *O*-methyl carbon and the methylenedioxy carbon. Although no degradations were carried out, the labelling pattern would expectedly be similar to the well-documented findings of Watkin[6] *et al.* and other workers.[7, 8] That is, the *A* ring is derived from acetate units, with the carbon atoms of cinnamic acid forming the *B* ring and C-2, 3 and 4 of pisatin.

TABLE 1. COMPARISON OF CARBON-14-LABELLED PRECURSORS IN THE BIOSYNTHESIS OF PISATIN

Precursor			Pisatin extracted			
	Spec. act. mc/m-mole	Uptake dpm* × 10^7	dpm × 10^3	μmole	Incorp. (%)	Isotope dilution
L-Phenylalanine-[U-^{14}C]	375	1·01	135	0·551	1·34	3399
Methionine-methyl-^{14}C	11·07	0·90	117	0·404	1·30	85
Cinnamic acid-COOH-^{14}C	0·89	1·07	40·4	0·330	0·38	16
D-Glucose-U-^{14}C	3·49	1·02	8·75	0·441	0·086	391
Acetate-1-^{14}C	2·0	1·07	5·45	0·207	0·051	1694
L-Tyrosine-[U-^{14}C]	375	1·00	0·27	0·44	0·0027	1·25 × 10^{-6}

* Disintegration per min.

Pisatin has been reported to have extremely effective antifungal properties even at the relatively low levels occurring in pea tissues.[1] Being an abnormal metabolite, its biological or chemical induction apparently alters in some way the normal metabolism of phenolic compounds subsequent to the production of cinnamic acid. The point at which the metabolism of cinnamic acid differs from that of the healthy plant is presently under investigation.

EXPERIMENTAL

Materials

L-phenylalanine-[U-^{14}C], methionine-methyl-^{14}C, D-glucose-[U-^{14}C], acetate-1-^{14}C and L-tyrosine-[U-^{14}C] were obtained from the New England Nuclear Corporation, Boston, Massachusetts. Cinnamic acid-COOH-^{14}C was kindly supplied by Dr. H.-G. Floss of the Organic Chemistry Institute of the Technical University, Munich, Germany. The pisatin used for carrier was isolated and purified from pea pods and was compared with an authentic sample supplied by Dr. I. A. M. Cruickshank, Division of Plant Industry, Canberra, Australia.

Procedure

Alaska peas were grown in the field for large-scale pisatin extractions, and in the greenhouse for isotope incorporation experiments. Six immature pods (approximately 1 g) were

[6] J. E. WATKIN, E. W. UNDERHILL and A. C. NEISH, *Can. J. Biochem. Physiol.* **35**, 229 (1957).
[7] H. GRISEBACH and N. DOERR, *Naturwissenschaften* **17**, 514 (1959).
[8] H. GRISEBACH and W. D. OLLIS, *Experientia* **17**, 4 (1961).

excised, split and 2 μc of each isotope was administered to the exposed endocarps by a series of injections with a micro-syringe. After the isotopic solution appeared to have been taken into the pod, 1 ml of $3{\cdot}0 \times 10^{-3}$ M $CuCl_2$ was added for each pod. The pods were placed in the dark for 48 hr. The method for extracting pisatin was essentially that of Cruickshank and Perrin.[1] The $CuCl_2$ solution was drained off and combined with two subsequent water washes of the pods. This aqueous portion was extracted twice with two-fold volumes of light petrol (b.p. 30–60°). The petrol fraction was taken to dryness and the residual pisatin was dissolved in 0·4 ml of ethanol. Three 0·1 ml aliquots were removed. One aliquot was counted in a Packard "tri-carb" liquid scintillation spectrometer. Percent uptake was determined by subtracting the counts per minute recorded for water washes of the pods after the ether extraction from the total counts per minute administered. Carrier pisatin (15 mg) was added to the second aliquot and was re-crystallized to constant specific activity. The remaining aliquot was dissolved in 5 ml of ethanol and the pisatin was quantitatively determined by its absorbance at 309 mμ. Freshly harvested pods and pods incubated with sterile deionized water produced no detectable quantities of pisatin.

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