Advances in Enzymology

Edited by

ALTON MEISTER

ADVANCES IN ENZYMOLOGY

AND RELATED AREAS OF MOLECULAR BIOLOGY

Founded by F. F. NORD

Edited by ALTON MEISTER

CORNELL UNIVERSITY MEDICAL COLLEGE,
NEW YORK, NEW YORK

VOLUME 55

AN INTERSCIENCE® PUBLICATI

JOHN WILEY & SONS
New York · Chichester · Brisbane · Toronto · Singapore

Library of Congress Catalog Card Number: 41-9213
ISBN 0-471-86866-4

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

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Volume 55

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PHYTOALEXINS: ENZYMOLOGY AND MOLECULAR BIOLOGY

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CONTENTS

I. Introduction

Phytoalexins are host-synthesized, low molecular weight, broad-spectrum antimicrobial compounds whose synthesis from distant precursors is induced in plants in response to microbial infection or treatment of plant tissues with a wide range of naturally occurring or synthetic, artificial compounds (biotic or abiotic elicitors). Phytoalexins were first implicated in the protection of susceptible potato cultivars against infection with a virulent race of *Phytophthora infestans* (potato late blight) as a result of pre-inoculation of tuber tissue with a race of the fungus to which the potato cultivar was resistant (1,2). It was a further 20 years before the first reported phytoalexin, pisatin, was isolated from fungally infected pea pods and its pterocarpanoid structure determined (3,4). During the two subsequent decades, many new phytoalexins have been isolated and identified from other plant species, these compounds belonging to such structurally diverse chemical classes as isoflavonoid, terpenoid, stilbene, polyacetylene, and dihydrophenanthrene. The reader is referred to several excellent reviews dealing wholly, or in part, with the structure, occurrence, and biological activity of phytoalexins (5-14).

Two important factors are implied in the definition of a phytoalexin. First, phytoalexins are believed to be crucial components of the overall expression of disease resistance in the plant cells in which they accumulate; although the evidence supporting this view is strong, it is nevertheless indirect (see Section V), and the study of phytoalexin induction is still, therefore, of much interest to plant pathologists. Second, phytoalexins are not found in healthy, uninfected tissues; as their induced synthesis has conclusively been demonstrated by radioisotopic labeling experiments, phytoalexin induction has recently become of great interest to the biochemist as a suitable experimental system for investigating the regulation of enzyme levels, and possibly *de novo* gene expression, in plant cells. A knowledge of the key enzymes of phytoalexin metabolism is therefore an important starting point for the study of the expression of disease resistance in plants at the molecular level.

Accumulation of phytoalexins is not the only induced biochemical defense mechanism conferring disease resistance to plants; the involvement of induced, physical, cell surface barriers (15-17), agglutinins (18), and protease inhibitors (18,19) has also been implicated in certain plant-pathogen interactions. It is only through detailed investigation of these and other possible resistance phenomena that we will eventually arrive at an understanding of the exact nature of the resistance genes whose manipulation by classical methods still plays an important role in plant breeding programs. With the advent of recombinant DNA technology, the opportunity now exists for significant advances to be made in the field of plant disease resistance in the coming years (20). In addition to its main theme of discussing the enzymological aspects of phytoalexin metabolism and its control, it is hoped that the present review will focus attention on areas where the tools of modern molecular biology can be most fruitfully applied.

There are many aspects of the phytoalexin induction process which appear outside the scope of this review, although they are so much a part of an integrated, temporal sequence of events that they cannot be wholly ignored in the discussions that follow. These areas include (*a*) initial recognition between host and pathogen, (*b*) the biochemical determination of race specificity, (*c*) the structure and mode of action of fungal elicitors, (*d*) the nature of the putative receptors for fungal elicitors and the sites of action of abiotic elicitors, and (*e*) possible inter- and intracellular transmission of elicitation. Detailed information on these and other aspects of the biochemistry of plant disease resistance is available in a number of recent review articles (10, 18, 21-26). In the present review, sufficient background information will be given to enable the reader who is approaching this field for the first time to place the discussions of biosynthesis, enzymology, and regulation in a wider plant pathological context.

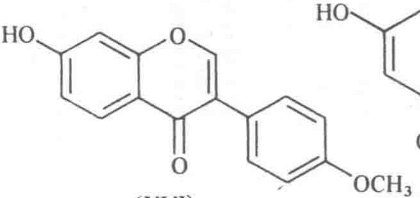
As attention has only recently been turned to biosynthetic aspects of phytoalexin accumulation, it is unavoidable that in some cases the enzymic reactions to be discussed have only been investigated in plant species other than those producing a particular class of phytoalexin; alternatively, in fewer cases, the enzymes may only have been well characterized in animal systems. It is hoped that the inclusion of information obtained from other organisms will be of value in directing attention to possible avenues for future research in the context of the phytoalexin pathways.

II. Isoflavonoid Phytoalexins

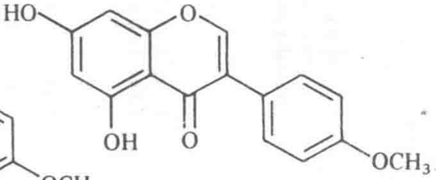
For historical and scientific reasons, the isoflavonoids are the most studied class of phytoalexins with respect to their occurrence and metabolism. Although potato, a producer of terpenoid phytoalexins, was the subject of the investigations that led to the formulation of the phytoalexin hypothesis (1,2), the first fully characterized phytoalexins were isolated from members of the Leguminosae; the garden pea (*Pisum sativum*), dwarf French bean (*Phaseolus vulgaris*), and soybean (*Glycine max*), therefore, soon became favored experimental material. Of equal importance is the fact that the biosynthesis of the isoflavonoid phytoalexins was known to share a common series of reactions with the general phenylpropanoid pathway leading to lignin formation, and much of the enzymology of this pathway had already been elucidated by the time chemists and biochemists turned their attention to phytoalexin biosynthesis. The following sections do not attempt to deal with all known isoflavonoid phytoalexins. The section on structure and occurrence presents only a general cross section of the different types of isoflavonoid compound encountered, whereas the discussion of enzymology and regulation is limited to those few systems where sufficient detailed work has been done to enable a coherent overall picture to be presented. As many reactions are common to the synthesis of different isoflavonoid phytoalexins, many general details of enzymology will be relevant to plants whose phytoalexin metabolism has not yet been investigated.

A. STRUCTURE AND OCCURRENCE

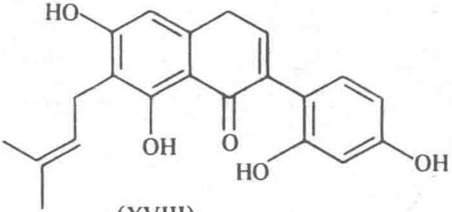
Most phytoalexins are chemically and biogenetically related to known classes of secondary products which may accumulate constitutively in the same, or taxonomically related, plant species. Table I summarizes the structures and sources of the most important postinfectionally formed isoflavonoid derivatives of the Leguminosae. Other strongly or weakly antifungal isoflavonoids may occur as preformed metabolites, for example, the isoflavone formononetin (XVI) in *Medicago sativa*, formononetin and biochanin A (XVII) in *Cicer arietinum* and *Trifolium pratense*, and luteone (XVIII) in the leaves of *Lupinus albus* (27). Most of the compounds in



(XVI)



(XVII)



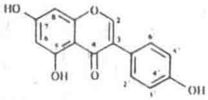
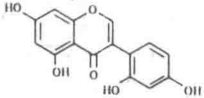
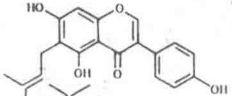
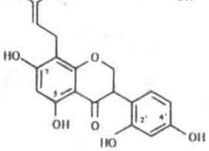
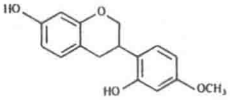
(XVIII)

Table I conform to the definition of phytoalexins as given earlier. Exceptions include genistein and 2′-hydroxygenistein which, although accumulating with phaseollin and kievitone in infected (28) and wounded (53) tissues of *Phaseolus vulgaris*, are only weakly antimicrobial. These two isoflavones also serve as precursors for the true phytoalexin kievitone (see Section II.B.4). Coumestrol is sometimes classed as a phytoalexin, although its biological activity is antibacterial rather than antifungal (51); it accumulates, along with related coumestans and true isoflavonoid phytoalexins, in several legumes including *Phaseolus vulgaris* (51, 52) and *Medicago sativa* (50). Maackiain occurs as a phytoalexin in *Pisum sativum* (43) and *Trifolium pratense* (41). It is, however, also present as the preformed glucoside trifolirhizin in heartwood, callus, or root tissue of several species including *Trifolium* and *Sophora* (7).

It will be seen from Table I that different plant species produce different groups of closely related phytoalexins; the main exception within the Leguminosae is the production, along with medicarpin, of the acetylenic

TABLE I

Structures and Sources of Postinfectional Isoflavonoid Compounds of the Leguminosae

Chemical class	Trivial name	Compound number	Structure	Species	References
Isoflavone	Genistein	(I)		*Phaseolus vulgaris*	(28)
Isoflavone	2'-Hydroxygenistein	(II)		*Phaseolus vulgaris* *Cajanus cajan*	(28) (29)
Isoflavone	Wighteone	(III)		*Glycine wightii*	(30)
Isoflavanone	Kievitone	(IV)		*Phaseolus vulgaris* *Vigna unguiculata* *Vigna sinensis*	(31) (32) (33)
Isoflavan	Vestitol	(V)		*Lotus corniculatus*	(34)

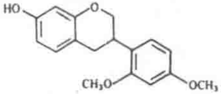
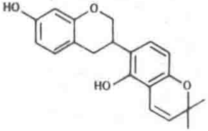
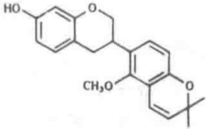
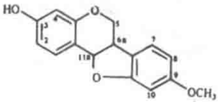
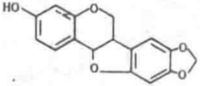
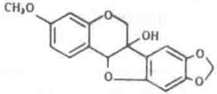
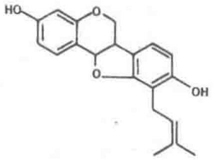
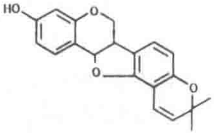
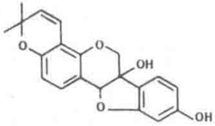
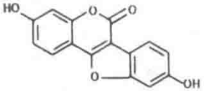
Isoflavan	Sativan	(VI)		*Lotus corniculatus* *Medicago sativa*	(34) (34)
Isoflavan	Phaseollinisoflavan	(VII)		*Phaseolus vulgaris*	(35)
Isoflavan	2′-Methoxyphaseollin-isoflavan	(VIII)		*Phaseolus vulgaris*	(36)
Pterocarpan	Medicarpin	(IX)		*Vicia faba* *Vigna unguiculata* *Canavalia ensiformis* *Medicago sativa* *Trifolium pratense* *Cicer arietinum*	(37) (38) (39) (40) (41) (42)
Pterocarpan	Maackiain	(X)		*Pisum sativum* *Trifolium pratense* *Cicer arietinum*	(43) (41) (42)
Pterocarpan	Pisatin	(XI)		*Pisum sativum*	(3,4)

TABLE I (CONTINUED)

Chemical class	Trivial name	Compound number	Structure	Species	References
Pterocarpan	Phaseollidin	(XII)		*Phaseolus vulgaris* *Vigna sinensis* *Psophocarpus tetragonolobus*	(44) (45) (46)
Pterocarpan	Phaseollin	(XIII)		*Phaseolus vulgaris* *Vigna sinensis*	(47) (45)
Pterocarpan	Glyceollin (Isomer I)	(XIV)		*Glycine max*	(48,49)
Coumestan	Coumestrol	(XV)		*Medicago sativa* *Phaseolus vulgaris*	(50) (51,52)

phytoalexin wyerone (see Section IV.A) by *Vicia faba* (54, 55). Also, the same phytoalexin may be found in a variety of different, but closely related species, this being particularly the case with medicarpin. The relative proportions of the different phytoalexins accumulating in a given plant species may depend upon the nature of the invading pathogen (56,57).

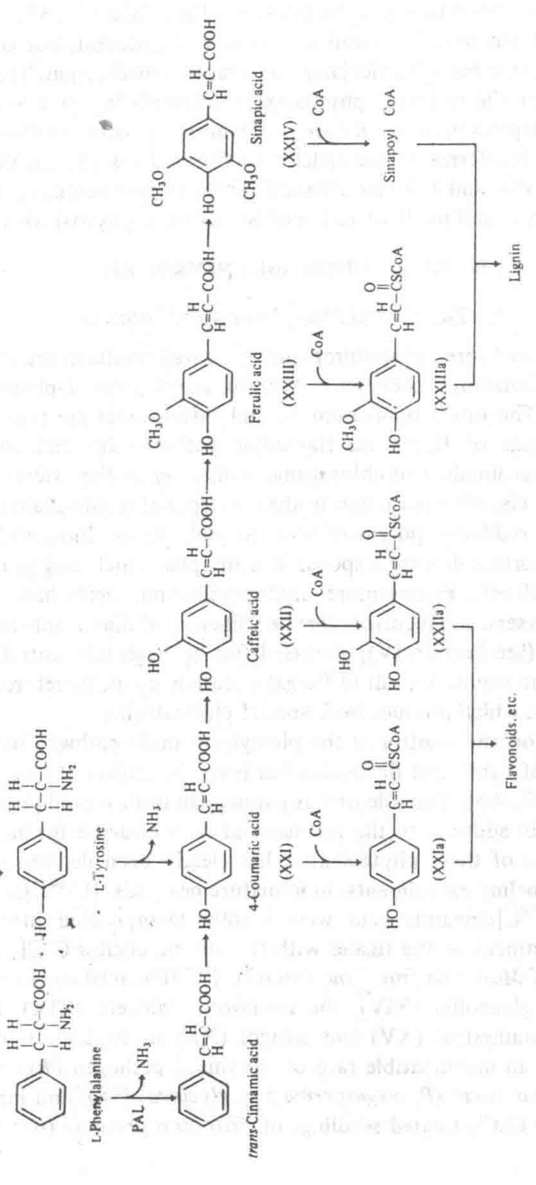
The reader is referred to the articles by Cruickshank (5,12), Van Etten and Peuppke (9), and Rizk and Wood (11) for further details of the structure, occurrence, and mode of action of isoflavonoid phytoalexins.

B. BIOSYNTHESIS AND ENZYMOLOGY

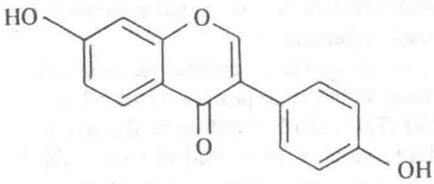
1. The Central Phenylpropanoid Pathway

The central or "core" phenylpropanoid pathway results in the formation of hydroxycinnamoyl coenzyme A thiol esters from L-phenylalanine (Scheme 1). The esters of 4-coumaric and caffeic acids are precursors in the biosynthesis of flavonoids (including anthocyanins and condensed tannins), isoflavonoids, and chlorogenic acid, whereas the esters of ferulic and sinapic acids, after reduction to the corresponding side-chain alcohols, may undergo oxidative polymerization to yield lignin. Induced lignification is an important defense response in many plants including potato (58) and wheat (59,60). Furthermore, hydroxycinnamic acids have recently been shown to serve as precursors for the stilbene and dihydrophenanthrene phytoalexins (see Section IV); increased flux through the central phenylpropanoid pathway as a result of fungal infection is not, therefore, limited to those plants which produce isoflavonoid phytoalexins.

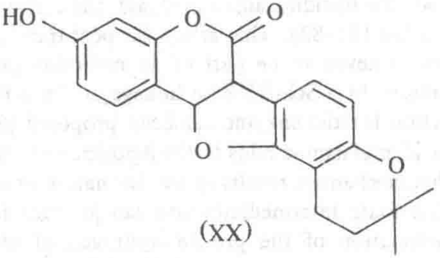
The operation and control of the phenylpropanoid pathway during the biosynthesis of lignin and flavonoids has been the subject of several excellent reviews (61–65). The role of this pathway in isoflavonoid phytoalexin biosynthesis, in addition to the provision of early evidence for induced *de novo* synthesis of these phytoalexins, has clearly been demonstrated by radioactive labeling experiments. In immature pea pods, [U-^{14}C]phenylalanine and [1-^{14}C]cinnamic acid were readily incorporated into pisatin following treatment of the tissues with the abiotic elicitor $CuCl_2$ or spore suspensions of *Monilinia fructicola* (66,67). [^{14}C]Phenylalanine was incorporated into glyceollin (XIV), the isoflavone daidzein (XIX), and the coumestans coumestrol (XV) and sojagol (XX) in soybean hypocotyls infected with an incompatible race of the fungal pathogen *Phytophthora megasperma* var *sojae* (*P. megasperma* f.sp.*glycinea*) (68), and into medicarpin (IX) in $CuCl_2$-treated seedlings of *Trifolium pratense* (69). Phenyl-



Scheme 1. The central phenylpropanoid pathway.



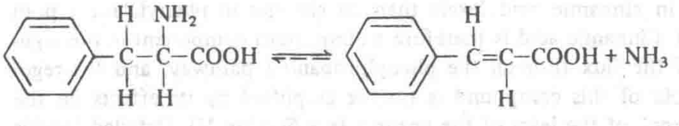
(XIX)



(XX)

alanine and cinnamic acid were similarly good precursors of phaseollin (XIII) in excised pods (70,71) and tissue cultures (72,73) of *Phaseolus vulgaris*.

L-Phenylalanine ammonia-lyase (PAL, E.C.4.3.1.5) catalyzes the first committed step in the biosynthesis of phenylpropanoid compounds: the elimination of ammonia from L-phenylalanine to yield *trans*-cinnamic acid (reaction 1).



$$C_6H_5-\overset{H}{\underset{H}{C}}-\overset{NH_2}{\underset{H}{C}}-COOH \rightleftharpoons C_6H_5-\overset{H}{C}=\underset{H}{C}-COOH + NH_3 \quad (1)$$

Since its discovery in 1961 by Koukol and Conn (74), a great deal of attention has been given to the regulation of the enzyme in response to light, plant growth substances, wounding, and fungal, bacterial, or viral infection. It has been the subject solely, or in part, of several useful reviews (61,75–77). In this section the general properties of the enzyme are summarized, and its role in the phytoalexin response is discussed. The