Robert L. Ory

ANTINUTRIENTS AND NATURAL TOXICANTS IN FOODS

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Edited by

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PREFACE

At a recent seminar in Chicago, Dr. Philip L. White, Director, Department of Foods and Nutrition, American Medical Association, said, "Nothing has happened in the last 1, 5, 10, or 50 years that has suddenly made our food supply hazardous. If anything, it is much less hazardous than it was 75 years ago. Our food supply has never been better; never been safer." [Food & Nutrition News (1979) *50*(2):4]. Despite such statements, however, today's consumers are confused by contradictory reports on the wholesomeness and safety of our foods. During the past decade, changes in diets and food habits have helped make consumers more nutrition conscious. Food scientists and nutritionists have provided much data on wholesomeness and safety of our foods, but consumers want more information on both the nutrients and antinutrients that can affect their health.

New sources of edible protein will likely cause further changes in consumption patterns. Higher plants (legumes, oilseeds, cereals) are primary crops that will provide these new proteins. The quantities that can be eaten are limited, however, unless the foods are processed to remove or detoxify the antinutrients (toxicants). Various antinutrients are present in plants: hemagglutinins (lectins), protease inhibitors, cyanogens, adventitious pigments, factors that can bind essential minerals, allergens, flatus oligosaccharides, or vitamin antagonists. In addition to naturally occurring toxins, some toxicants are introduced indirectly during storage, processing, or cooking by microbial activity, principally fungi, but bacteria can also produce food toxins.

This book contains the papers presented in a Symposium on Antinutrients and Natural Toxicants in Foods, sponsored by the A.C.S. Division of Agricultural and Food Chemistry, held at the American Chemical Society/Chemical Society of Japan International Congress in Honolulu, Hawaii, April 1—6, 1979. The Symposium included the above-mentioned subjects. Although aflatoxins are probably the most widely published mycotoxins in oilseeds and cereals, they were not included because of adequate coverage in numerous other symposia and books. Instead, we included current research on toxins from sweet potatoes and spices, staphylococcal and tremorgenic toxins. Some authors were not able to present their research in the symposium but were invited to include it in this book, to provide better balance in covering this important subject.

One book cannot possibly cover all of the antinutrient/toxicant problems encountered in food chemistry. The purpose of the book, however, is not to call attention to the presence of these materials in our foods, but to emphasize the broad areas of research underway here and in Japan to understand the nature and actions of these agents, and to illustrate acceptable methods for removing or inactivating them. As Dr. White stated, "Our food supply has never been safer." This book will, I hope, support his statement. In sponsoring the Symposium and the book, the Division of Agricultural and Food Chemistry has not censored the views or data interpretations of the contributors. Views and conclusions expressed are those of the authors. I sincerely thank all of them for contributing their time and energy to present their research in the symposium and for preparing it for publication in the book.

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CONTENTS

CHAPTER

BIOCHEMISTRY OF FURANO-TERPENES PRODUCED IN MOLD-DAMAGED SWEET POTATOES

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INTRODUCTION

When tuberous roots of sweet potato, *Ipomoea batatas*, are damaged by many kinds of necrosis-inducing pathogenic fungi, such as *Ceratocystis fimbriata* (Hiura 1943) and *Fusarium solani* (Wilson 1973), and by some harmful weevil larvae, such as *Cylas formicarius* and *Euscepes postfasciatus* (Uritani *et al.* 1975), various kinds of furano-terpenes are accumulated 1 to 5% in the injured necrotic region adjacent to the non-injured tissue of sweet potato (Uritani 1976). Such an event is also induced in sweet potato root tissue by toxic chemicals, such as mercuric chloride (Uritani *et al.* 1960). These furano-terpenes are produced in leaves and stems in response to such injurious agents, but not in large amounts compared with the case of tuborous roots.

It was noticed that mold-damaged sweet potato roots were bitter, and many workers tried to isolate and characterize the causative entity. Finally Hiura (1943) isolated a main component of the entity, named ipomeamarone, and determined that it was a sesquiterpene ($C_{15}H_{22}O_3$). Kubota and Matsuura (1953) determined the chemical structure as a furano-terpene (Fig. 1.3).

It has been often reported in Japan since the beginning of the 20th century, that a number of cattle died when they were fed with *C. fimbriata*-damaged sweet potato roots. Hiura (1943) reported that the cause of the death was principally due to the toxicity of ipomeamarone. After World War II, in both Japan and USA, there occurred many accidental deaths

of cattle when fed with mold-damaged sweet potato roots. The events led several workers to elucidate the toxicological effect of the furano-terpenes (Kondo 1971; Wilson 1973).

On the other hand, the furano-terpenes were found to be toxic to *C. fimbriata* and other microorganisms (Hiura 1943; Uritani *et al.* 1947), and regarded as phytoalexins in sweet potato, whose name was defined as antimicrobial toxins produced by hosts in response to pathogenic infection by Muller and Borger (1940), through experimental data on *Phytophthora infestanse*-infected white potato.

This chapter deals with the biosynthesis and biological activities of the furano-terpenes and describes ideas to solve some problems which could be caused by the biosynthesis of the furano-terpenes.

BIOSYNTHESIS OF FURANO-TERPENES

Time-course of Biosynthesis

When sweet potato root tissue was inoculated on the cut surfaces with the endoconidial suspension of *C. fimbriata* and subjected to mycelial penetration, the tissue produced various kinds of furano-terpenes and accumulated them in the infected region adjacent to the non-infected tissue, after a lag of about 20 h. The amounts of the compounds produced reached a plateau after 2 to 3 days, then decreased slowly, as shown in Fig. 1.1 (Uritani 1978). TLC patterns show that those furano-terpenes were produced almost at the same time after a lag (Fig. 1.2) (Oguni and Uritani 1974).

Chemical Structures

Ipomeamarone, $C_{15}H_{22}O_3$ (in Fig. 1.3) was isolated from *C. fimbriata*-infected sweet potato roots by Hiura (1943), and its chemical structure was determined by Kubota and Matsuura (1953). This was the first example of the isolation and chemical characterization of phytoalexins.

Since then, ipomeamaronol, $C_{15}H_{22}O_4$ (Kato *et al.* 1971; Yang *et al.* 1971), dehydroipomeamarone, $C_{15}H_{20}O_3$ (Oguni and Uritani 1973), 4-hydroxymyoporone, $C_{15}H_{22}O_4$ (Burka *et al.* 1974), 7-hydroxymyoporone, $C_{15}H_{22}O_4$ (Burka *et al.* 1974), 4-hydroxydehydromyoporone, $C_{15}H_{20}O_4$ (Inoue *et al.* 1977), 1-(3'-furyl)-6,7-dihydroxy-4,8-dimethylnonan-1-one, $C_{15}H_{24}O_4$ (Burka 1978), component A_1, $C_{15}H_{18}O_3$ (Ito, I. *et al.* 1980; Bohlmann and Rao 1972) and component A_2, $C_{15}H_{20}O_2$ (Ito, I. *et al.* 1980; Bohlmann and Rao 1972) have been isolated from mold-damaged sweet potato roots, and their chemical structures determined (Fig. 1.3).

It should be emphasized that all of these stress compounds belong to furano-sesquiterpenes.

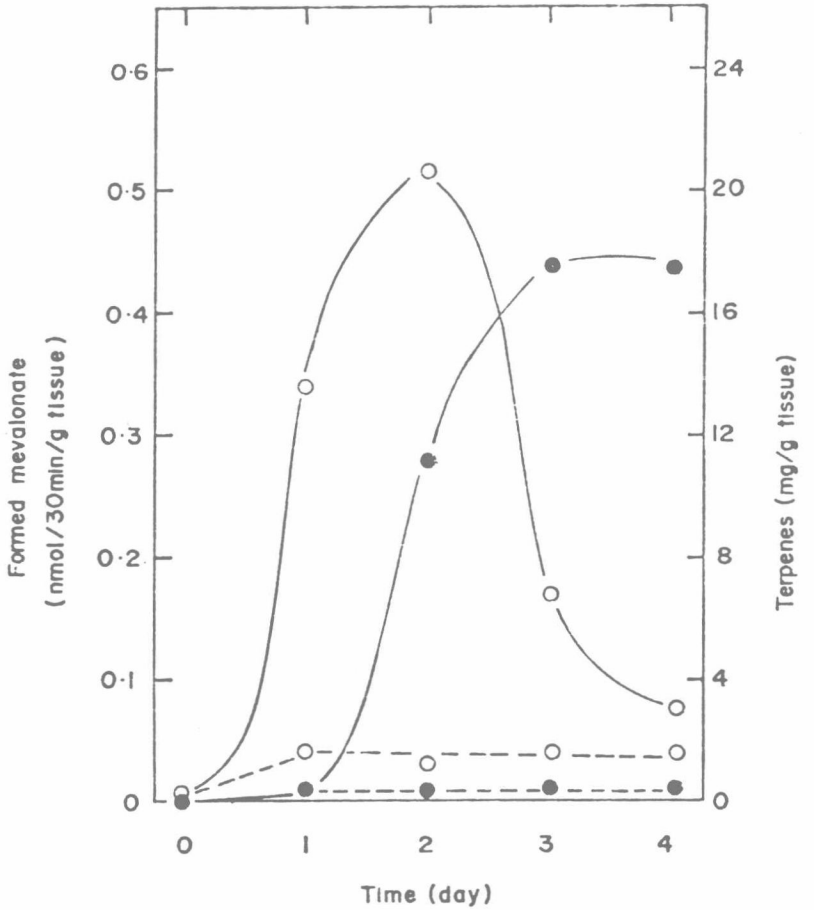


FIG. 1.1. CHANGES IN FURANO-TERPENE CONTENTS AND HMG-CoA REDUCTASE ACTIVITY AFTER *C. FIMBRIATA* INFECTION OR CUT INJURY

The infected region (about 0.5 to 1.5 mm thick) and tissue sliced (3 mm thick) from uninoculated tissue were used for determination of furano-terpenes in diseased and cut tissues, respectively. Particulate fractions from non-infected parts of diseased tissue and cut tissue incubated for various periods were used for the assay of HMG-CoA reductase. At zero time, fresh tissue was used. (●): Contents of furano-terpenes (mg/g tissue); (○): Activity of HHG-CoA reductase (nmole of formed mevalonate for 30 min/g tissue). (———), (— — —): Diseased and cut tissues, respectively (Suzuki *et al.* 1975).

Biosynthetic and Convertible Pathways

We have been investigating the biosynthetic and convertible pathways for the furano-terpenes in sweet potato root tissue, dividing

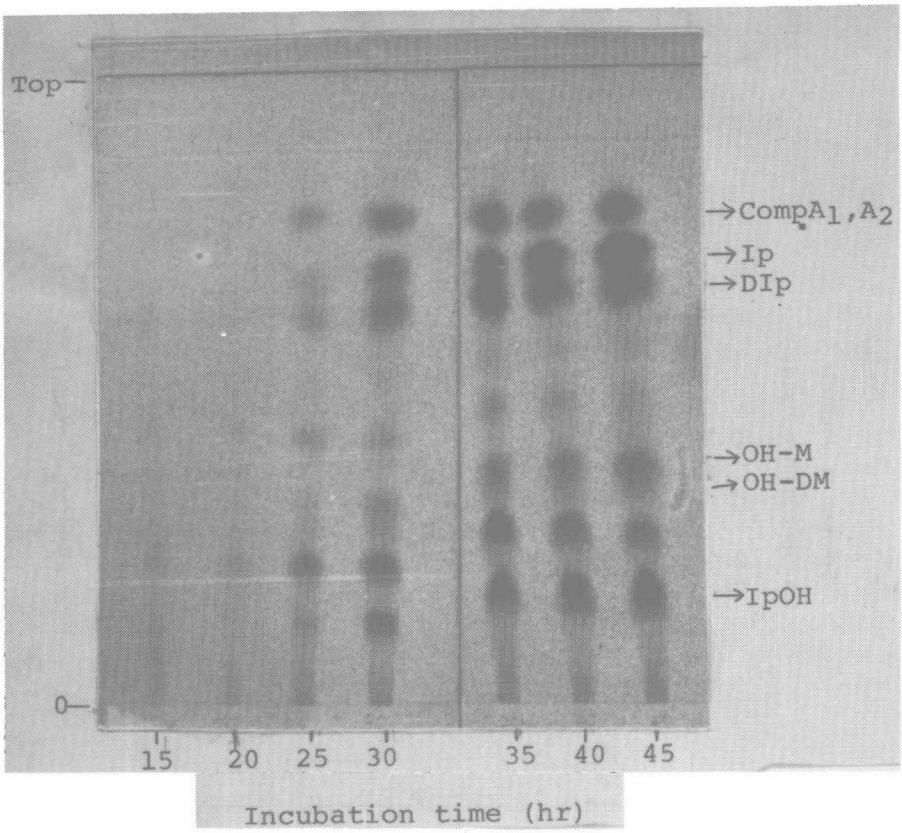


FIG. 1.2. TIME COURSE ANALYSIS OF FURANO-TERPENE PRODUCTION IN SWEET POTATO ROOT TISSUE INFECTED WITH *C. FIMBRIATA*

Crude oily substance was extracted with chloroform-methanol (1:1, v/v) from 8 discs prepared from tissue inoculated with *C. fimbriata* for each period. A portion of each crude oily substance was subjected to TLC using benzene-ethylacetate (8:2, v/v) as developing solvent. After developing, the thin layer plate was sprayed with Ehrlich's reagent. Comp. A_1, Comp. A_2,, Ip, DIp, OH-M, OH-DM and IpOH are component A_1, component A_2, ipomeamarone, dehydroipomeamarone, 4-hydroxymyoporone, 4-hydroxydehydromyoporone and ipomeamaronol, respectively.

the pathways into the first half and latter half steps. These are the step from pyruvate to farnesol (or farnesyl pyrophosphate), and that from farnesol to many furano-terpenes, respectively.

To elucidate the pathways, we have used both *in vivo* and *in vitro* systems. In the case of the *in vivo* system, ^{14}C-labeled compounds that might be regarded as intermediates were applied to thin discs (about 1 to 3 mm thick) with the *C. fimbriata*-infected or $HgCl_2$-injured region (about

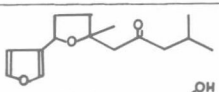
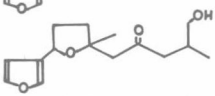
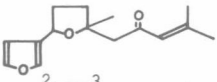
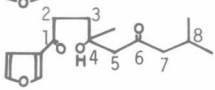
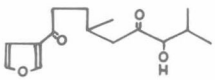
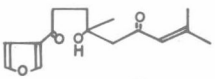
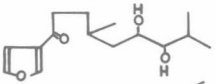
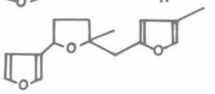
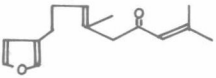
Name	M.W.	Structure	Year published
ipomeamarone	$C_{15}H_{22}O_3$		1943,1952
ipomeamaronol	$C_{15}H_{22}O_4$		1971
dehydroipomeamarone	$C_{15}H_{20}O_3$		1973
4-hydroxymyoporone	$C_{15}H_{22}O_4$		1974
7-hydroxymyoporone	$C_{15}H_{22}O_4$		1974
4-hydroxydehydro-myoporone	$C_{15}H_{20}O_4$		1977
1-(3'-furyl)-6,7-dihydroxy4,8-dimethyl-nonan-1-one	$C_{15}H_{24}O_4$		1978
component A_1	$C_{15}H_{18}O_3$		1979
component A_2	$C_{15}H_{20}O_2$		1979

FIG. 1.3. CHEMICAL STRUCTURES OF FURANO-TERPENES FROM MOLD-DAMAGED SWEET POTATO ROOTS

0.5 to 1.0 mm thick) where furano-terpenes were actively accumulated. The discs were incubated at 7 to 30 °C for definite periods and subjected to extraction of the furano-terpene fraction, which was divided into the individual components by TLC, GC and LC. Then the radioactivity of the components was measured (Oguni and Uritani 1974; Inoue and Uritani 1979; Ito and Uritani 1980).

In the case of the *in vitro* system, the enzyme extracts were prepared from non-injured tissue closely adjacent to the injured region where furano-terpenes were actively accumulated, and assayed for activities of the enzymes pertaining to furano-terpene biosynthesis. Time-course changes in activities of the respective enzymes and their purification and characterization were performed in connection with the biosynthesis of

furano-terpenes (Oba *et al.* 1976; Suzuki *et al.* 1975).

The First Half Step. In response to the infection, carbohydrates such as starch and sucrose are vigorously consumed with a lag of several hours, accompanied with the increase in respiration and production of secondary metabolites, such as phenylpropanoids and furano-terpenes (Kato and Uritani 1976).

Pyruvate thus formed is converted to acetyl-CoA, perhaps in two ways (Takeuchi *et al.* 1977). As one way, pyruvate is oxidized to acetyl CoA by pyruvate dehydrogenase complex in mitochondria, then acetyl CoA should be synthesized to citrate in mitochondria, which might be transported to cytosol and converted to acetyl-CoA by ATP citratelyase in the cytosol. In fact, the activity is revealed in response to the infection. In this case, a large part of acetyl CoA should be completely oxidized through the TCA cycle in mitochondria, generating ATP from ADP and monophosphate.

As the alternative way, pyruvate is converted to acetaldehyde by pyruvate decarboxylase in cytosol (Oba and Uritani 1975). Acetaldehyde is then converted to acetate by NAD-dependent acetaldehyde dehydrogenase (Takeuchi *et al.* 1980). It should be emphasized that activities of the above two enzymes were activated by the stress of the infection. Acetate thus formed should be converted into acetyl CoA by acetyl CoA synthetase (Takeuchi *et al.* 1977). These three enzymes were localized in cytosol.

In response to the infection, the enzyme system from acetyl CoA to 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) and HMG-CoA reductase were formed, possibly cytosol and microsomes, respectively, prior to the production of furano-terpenes (Fig. 1.1) (Suzuki and Uritani 1975).

Acetate-2-^{14}C was converted to geraniol and farnesol, as well as to furano-terpenes, when it was applied to discs with the injured region. Furthermore, the enzyme system from mevalonate (MVA) to isopentenyl pyrophosphate (IPP) was formed in response to the infection. The precise investigations elucidated the formation of MVA-kinase, phospho-MVA-kinase and pyrophospho-MVA decarboxylase preceding the production of furano-terpenes (Oba *et al.* 1976).

All of those data indicate that, in response to the infection or any continuous injury such as $HgCl_2$-treatment, some part of pyruvate yielded from carbohydrates is converted to acetyl CoA in cytosol, then acetyl CoA is utilized to form farnesol under the same pathway as that of the first half step of cholesterol biosynthesis (Richards and Hendrickson 1964), as shown in Fig. 1.4.

The Latter Half Step. Farnesol-2-^{14}C was easily converted to many kinds of furano-terpenes, such as ipomeamarone, when it was applied to the discs with the injured region. ^{14}C-labeled dehydroipomeamarone was

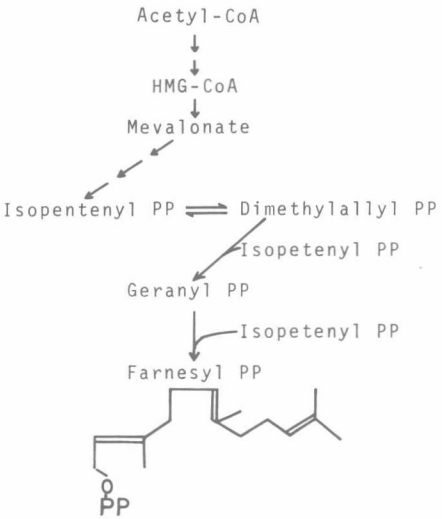


FIG. 1.4. THE FIRST HALF STEP OF THE PATHWAY OF FURANO-TERPENE BIOSYNTHESIS

very effectively incorporated into ipomeamarone as well as ipomeamaronol, and component A_1 (Oguni and Uritani 1974; Ito and Uritani 1980). Ipomeamarone was then converted into ipomeamaronol, but not component A_1. Finally, ipomeamaronol was decomposed to other substances that showed negative color-reaction to Ehrlich's reagent.

When ^{14}C-labeled component A_2 was applied to the discs, the label was rapidly incorporated into dehydroipomeamarone, ipomeamarone, ipomeamaronol and component A_1.

When ^{14}C-labeled 4-hydroxydehydromyoporone was given to the discs; the label was easily incorporated into 4-hydroxymyoporone, but not into either dehydroipomeamarone or ipomeamarone (Inoue and Uritani 1979). We also think that component A_2 is converted to 4-hydroxydehydromyoporone, then to 4-hydroxymyoporone.

When ^{14}C-labeled component A_1 was administrated to the discs, the label was incorporated into the two unknown components; the one seemed to be hydrophylic but the other hydrophobic, according to the moving behavior on TLC development (Ito and Uritani 1980).

Burka and Kuhnert (1977) proved that ipomeamarone was oxidized to 4-hydroxymyoporone by $HgCl_2$-treated sweet potato tissue, using ^{14}C-labeled ipomeamarone. The similar reaction might be involved in the oxidation of dehydroipomeamarone to 4-hydroxydehydromyoporone.

According to the above data, the latter half step of the biosynthetic and convertible pathways of furano-terpenes is possibly schematized as Fig. 1.5.

Ipomeamarone is not normally produced in cut tissue itself, but decomposed by cut tissue, and the decomposition is inhibited by fungal

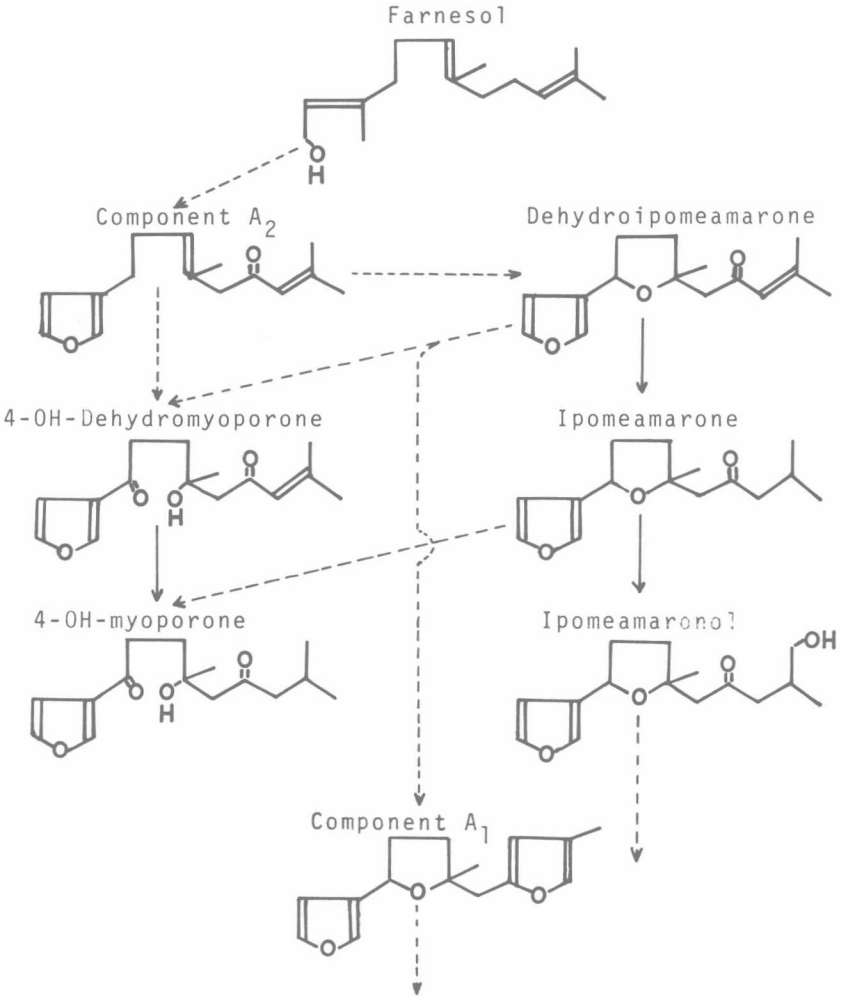


FIG. 1.5. THE LATTER HALF STEP OF THE POSSIBLE PATHWAY OF FURANO-TERPENE BIOSYNTHESIS

——→, – – →: The steps involving single enzymatic reaction and several enzyme reactions, respectively.

infection or $HgCl_2$-treatment (Oba *et al.* 1980), as shown in the decomposition of rishtin by cut white potato (Ishiguri *et al.* 1978) or of glyceollin by cut soybean hypocotyls (Yoshikawa *et al.* 1979).

Modification of 4-Hydroxymyoporone to C_9-Furano-terpenes by the Fungus

Boyd *et al.* (1973) found the production of four kinds of C_9-furano-terpenes in sweet potato roots following *F. solani* infection, in addition to C_{15}-furano-terpenes previously mentioned. They are 4-ipomeanol, 1-ipomenol, ipomeanine (Kubota and Ichikawa 1954) and 1,4-ipomeanol (Fig. 1.6). It was demonstrated that these compounds were produced from 4-hydroxymyoporone by *F. solani* (Burka *et al.* 1977), as shown in Fig. 1.6. Ipomeanine had been first isolated from *C. fimbriata*-infected sweet potato roots by Kubota and Ichikawa (1954), but Burka *et al.* (1977) failed to find the component from *C. fimbriata*-infected tissue and they thought that ipomeanine might have been produced non-enzymatically from 4-hydroxymyoporone during the distillation used to isolate ipomeanine (Kubota and Ichikawa 1954).

Mechanism of Furano-terpene Induction

The furano-sesquiterpenes are accumulated in the injured region of sweet potato root tissue in response to $HgCl_2$-treatment (Uritani *et al.* 1960), as well as in response to *C. fimbriata* infection. This indicates that the furano-sesquiterpenes shown in Fig. 1.3 are produced by sweet potato root tissue itself, but not by the fungus.

Elicitors. The furano-terpene inducing factors were extracted either from the mycelia or from endoconidia of *C. fimbriata* by 0.02 M KCl solution (Kim and Uritani 1974). They were heat stable, organic solvent-insoluble, dialyzable and non-ionic. Such factors are normally called phytoalexin elicitors (Keen 1976).

The furano-terpenes were also accumulated in sweet potato roots when infested by the larvae of sweet potato weevils, such as *C. formicarius* and *E. postfasciatus* (Uritani *et al.* 1975). The extracts from both larvae induced the furano-terpene production. The elicitor(s) of *C. formicarius* was further investigated. It was composed of a proteinacious moiety and a low-molecular weight cofactor (Sato *et al.* 1977). The former was heat-labile, inactivated by pronase, and adsorbed by concanavalin A Sepharone. The adsorbed fraction was eluted by methyl α-mannoside.

Enzyme Induction. As previously described, some of the enzymes involved in furano-terpene biosynthesis were formed *de novo*. For example, formation of HMG-CoA reductase in $HgCl_2$-treated tissue was inhibited by cycloheximide. However, when cycloheximide was administrated with