Food Chemistry **41** (1991) 195–217

Terpenoid Phytoalexins in Potatoes: A Review

S. J. Jadhav

Department of Sugar Chemistry and By-Products, Vasantdada Sugar Institute, Manjari (BK), Pune, Maharashtra, India 412307

G. Mazza*

Food Research Laboratory, Agriculture Canada, Research Station, Morden, Manitoba, Canada R0G 1J0

&

D. K. Salunkhe

Department of Nutrition and Food Sciences, Utah State University, Logan, Utah, USA 84322

(Received 16 May 1990; revised version received and accepted 25 July 1990)

ABSTRACT

Terpenoid phytoalexins are low molecular-weight, antimicrobial compounds that are synthesized by, and accumulate in, plants after exposure to microorganisms. The literature on the chemistry, including extraction, separation and assay, biosynthesis and metabolism of phytoalexins in potatoes is reviewed and summarized. The molecular structures and the physicochemical characteristics of the major and minor terpenoid phytoalexins of potatoes are presented and discussed with reference to their characterization and function.

INTRODUCTION

The concept of the phytoalexin theory of induced resistance in plants emerged from the early observations by Müller & Börger in 1940 with

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/91/$03·50

potato (Müller & Börger, 1940). According to this concept, a specific metabolic interaction between a plant host and microorganisms results in accumulation of chemical substances which interrupt infection. The concept of phytoalexins is summarized in several reviews (Uritani, 1967; Ingham, 1972; Kuć, 1976; Brishammar, 1987). The currently accepted definition of phytoalexins is the following: 'Phytoalexins are low-molecular weight antimicrobial compounds that are synthesized by, and accumulate in, plants after exposure to microorganisms' (Paxton, 1981). Formation of a particular group of these compounds seems to be associated with a taxonomic element. In general, the Leguminosae produce isoflavonoids, Solanaceae produce terpenoids, and the Compositae produce polyacetylenes. Furthermore, a plant tissue may provide more than one type of phytoalexin under certain conditions. In potato tubers, phenolics, glycoalkaloids and terpenoids have been implicated as phytoalexins. Earlier, we have published reviews on the glycoalkaloids (Jadhav & Salunkhe, 1975; Salunkhe & Wu, 1979; Jadhav *et al.*, 1981). This article deals with the chemical and biochemical aspects of the terpenoid phytoalexins in potatoes.

STRUCTURES AND CHEMISTRY

Terpenoid compounds that accumulate in potatoes as a result of stress can be conveniently classified into three groups namely (a) rishitin type, (b) vetispirane derivatives, and (c) phytuberin type. Rishitin (1) was originally isolated from the Rishira variety of potatoes by Tomiyama *et al.* (1968) and was assigned the structure of a bicyclic norsesquiterpene alcohol (Katsui *et al.*, 1968). The structure and configuration of the molecule were elucidated by spectral and chemical data and confirmed by synthesis (Katsui *et al.*, 1972). Molecular structures of major and minor terpenoid phytoalexins of potato are presented in Fig. 1.

Rishitin (1) and oxyglutinosone (2) have similar structural skeletons. The distinguishing feature of the latter is shown by α,β-unsaturation at C-2 and an hydroxyl group at C-5. Rishitinone (3) lacks a hydroxyl at C-3 while C-9 is carbonyl in nature. However, the angular methyl group is retained in it. Rishitinol (4a) is clearly a hydroxyoccidol which upon dehydration and acetylation can give rise to acetyldehydrorishitinol (5). The 8-*O*-acetyl derivative of rishitinol (4a) is known to occur in potatoes.

Lubimin (6) by virtue of change in configuration at C-2 and/or C-10, can exist in four isomeric forms. Thus, lubimin (6) and 10-epilubimin (6a) are epimeric aldehydes while 2-epilubimin (6b) and 2,10-epilubimin (6c) are epimeric too. Lubimin (6) and oxylubimin (hydroxylubimin) (7) differ from one another in an hydroxyl group which is present only in the latter at

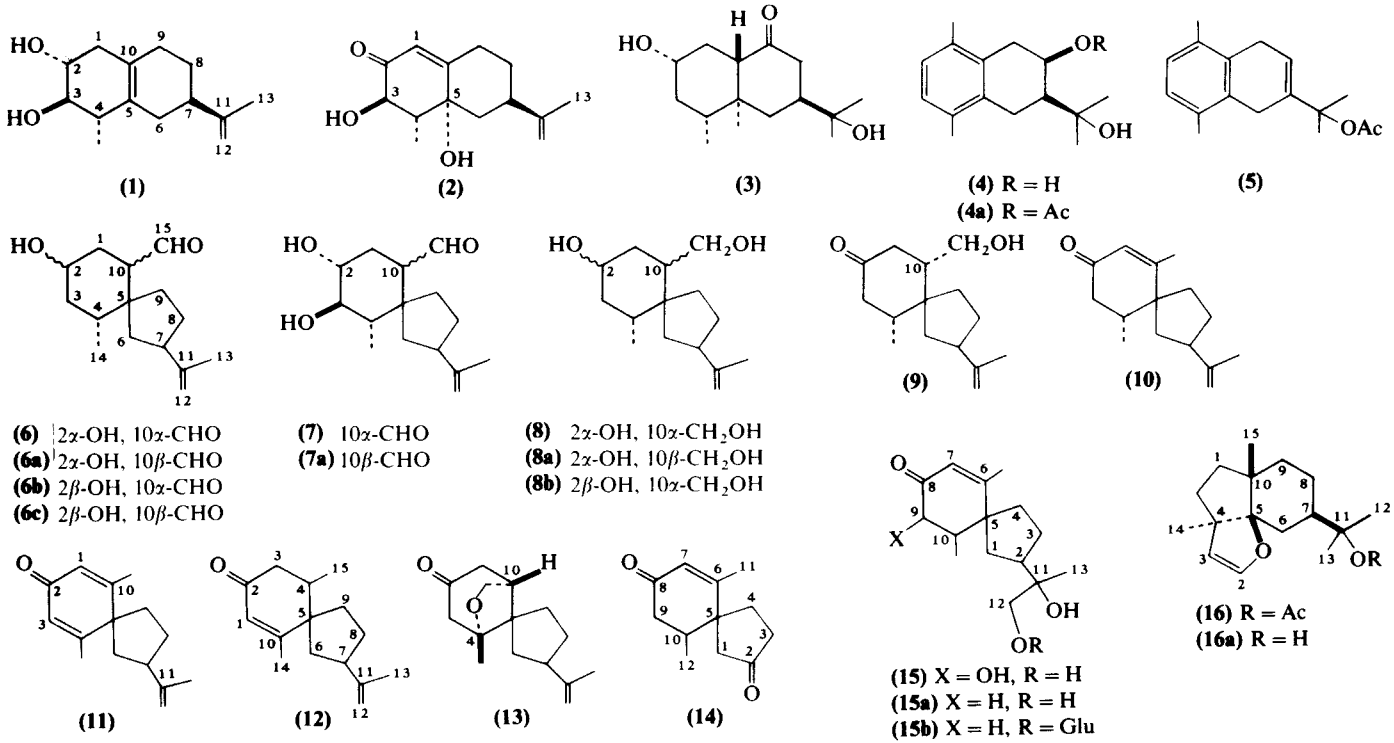

Fig. 1. Molecular structures of terpenoid stress metabolites of potato tuber. 1, Rishitin; 2, oxyglutinosone; 3, rishitinone; 4, rishitinol; 4a, 8-*O*-acetyl rishitinol; 5, acetyldehydrorishitinol; 6, lubimin; 6a, 10-epilubimin; 6b, 2-epilubimin; 6c, 2, 10-epilubimin; 7, oxylubimin (hydroxylubimin); 7a, epioxylubimin (epihydroxylubimin); 8, 15-dihydrolubimin; 8a, 15-dihydro-10-epilubimin; 8b, 15-dihydro-2-epilubimin; 9, isolubimin; 10, solavetivone (katahdinone); 11, anhydro-β-rotunol [spirovetiva-1(10), 3, 11-trien-2-one]; 12, spirovetiva-1(10), 11-dien-2-one; 13, cyclodehydroisolubimin; 14, 6, 10-dimethylspiro [4,5]dec-6-en-2,8-dione; 15,2-(11, 12-dihydroxy-11-methylethyl)-6, 10-dimethyl-9-hydroxyspiro [4,5] dec-6-en-8-one; 15a, 2-(11,12-dihydroxy-11-methylethyl-6,10-dimethylspiro[4,5]dec-6-en-8-one; 15b, 12-*O*-β-D-glucopyranoside of 15a; 16, phytuberin; 16a, phytuberol (desacetylphytuberin).

TABLE 1
Physical Constants of Sesquiterpenoid Stress Metabolites (SSM) in Potato

Sr. no.	*Compound*	*Formula*	*MP* (°C)	$[\alpha]_D$	*Spectroscopic and chromatographic data*	*Reference*
1.	Rishitin (1)	$C_{14}H_{22}O_2$	65–7	−29 (EtOH)	IR, UV, NMR, MS	Tomiyama *et al.* (1968); Katsui *et al.* (1968)
			65–7	−35·1	IR, UV, NMR	Masamune *et al.* (1977)
			64–6	−30·1 (c 2·5)	IR, NMR, TLC, GLC	Coxon *et al.* (1977)
2.	Oxyglutinosone (2)	$C_{14}H_{20}O_3$	—	+150	IR, NMR, MS	Katsui *et al.* (1978)
3.	Rishitinone (3)	$C_{15}H_{24}O_3$	72–5	+10·1	IR, NMR, MS	Katsui *et al.* (1982)
4.	Rishitinol (4)	$C_{15}H_{22}O_2$	127–9	+47 ($CHCl_3$)	IR, UV, NMR, MS	Katsui *et al.* (1971)
			128–9	+47 ($CHCl_3$)	IR, UV, NMR, MS, TLC	Katsui *et al.* (1972)
5.	8-*O*-Acetyl rishitinol (4a)	$C_{17}H_{24}O_3$			IR, UV, NMR	Katsui *et al.* (1981)
6.	Acetyldehydrorishitinol (5)	$C_{17}H_{22}O_2$			IR, UV, NMR, GC–MS	Alves *et al.* (1984)
7.	Lubimin (6)	$C_{15}H_{24}O_2$	Oil	+36	IR, NMR, MS	Katsui *et al.* (1974)
					MS	Stoessl *et al.* (1978)
					ORD, NMR, MS	Stoessl *et al.* (1975)
			Oil	+36	IR, NMR, MS, GLC, TLC	Katsui *et al.* (1977)
				+39 (EtOH, c 1%)	NMR, MS	Stoessl *et al.* (1974)
			Oil		IR, GC–MS	Uegaki *et al.* (1981)
8.	10-Epilubimin (6a)	$C_{15}H_{24}O_2$			IR, UV, MS	Stoessl *et al.* (1978)
9.	2-Epilubimin (6b)	$C_{15}H_{24}O_2$	Oil		IR, NMR, MS	Stoessl & Stothers (1980)
10.	2, 10-Epilubimin (6c)	$C_{15}H_{24}O_2$			IR, UV, NMR	Katsui *et al.* (1981)
11.	Oxylubimin (hydroxylubimin) (7)	$C_{15}H_{24}O_3$	85–6	+27 ($CHCl_3$)	IR, NMR, MS	Katsui *et al.* (1974)
			85–6	+27	IR, NMR, MS, TLC	Katsui *et al.* (1977)
			96–8	+55 (EtOH)	IR, UV	Stoessl *et al.* (1975)
			96·5–9·6		NMR	Birnbaum *et al.* (1976)
12.	Epioxylubimin (epihydroxylubimin) (7a)	$C_{15}H_{24}O_3$	123–4	−12·1	IR, NMR, MS	Katsui *et al.* (1978)
13.	15-Dihydrolubimin (8)	$C_{15}H_{26}O_2$			NMR, MS, TLC	Stoessl *et al.* (1978)
14.	15-Dihydro-10-epilubimin (8a)	$C_{15}H_{26}O_2$	138–40		MS, TLC	Stoessl *et al.* (1978)

15.	15-Dihydro-2-epilubimin (8b)	$C_{15}H_{26}O_2$	Viscous oil		NMR, MS, TLC	Stoessl & Stothers (1980)
16.	Isolubimin (9)	$C_{15}H_{24}O_2$	Syrup		IR, NMR, MS	Stoessl *et al.* (1978)
			Oil	+34·4	IR, NMR	Katsui *et al.* (1978)
17.	Solavetivone (Katahdinone) (10)	$C_{15}H_{22}O$	Oil		IR, GC–MS	Uegaki *et al.* (1981)
				−96 (EtOH, c 0·5)	IR, UV, MS, TLC	Stoessl *et al.* (1978)
18.	Anhydro-β-rotunol (spirovetiva-1 (10), 3, 11-trien-2-one) (11)	$C_{15}H_{20}O$	44–4·5	+57 (EtOH)	IR, UV, NMR, MS, GC, TLC	Coxon *et al.* (1974)
19.	Spirovetiva-1 (10), 11-dien-2-one (12)	$C_{15}H_{22}O_2$	Oil	−119	IR, UV, NMR, MS, GC, TLC	Coxon *et al.* (1974)
20.	Cyclodehydroisolubimin (13)	$C_{15}H_{22}O_2$			IR, NMR, MS	Coxon *et al.* (1979)
21.	6, 10-Dimethylspiro [4, 5] dec-6-en-2, 8-dione (14)	$C_{12}H_{16}O_2$	Amorphous	−11 (MeOH) (c 0·14)	IR, UV, NMR, MS	Malmberg (1982)
22.	2-(11, 12-Dihydroxy-11-methylethyl)-6, 10-dimethyl-9-hydroxyspiro [4,5] dec-6-en-8-one (15)	$C_{15}H_{24}O_4$	Amorphous	+88·7 (MeOH, c 0·15)	UV, NMR, MS	Malmberg (1982)
23.	2-(11, 12 Dihydroxy-11-methylethyl)-6, 10-dimethyl-spiro [4,5] dec-6-en-8-one (15a)	$C_{15}H_{24}O_2$	Amorphous	−55 (EtOH, c 0·1)	IR, UV, NMR, MS	Malmberg & Theander (1980)
24.	12-*O*-β-D-Glucopyranoside of compound 15a (15b)	$C_{21}H_{34}O_8$	Amorphous	−75·7 (EtOH, c 0·1)	IR, UV, NMR, MS	Malmberg & Theander (1980)
25.	Phytuberin (16)	$C_{17}H_{26}O_4$	Oil		IR, NMR, MS	Coxon *et al.* (1974)
					IR, GC, MS	Uegaki *et al.* (1981)
					IR, MS, GC, TLC	Hammerschmidt & Kuć (1979)
					IR, NMR, MS, TLC	Stoessl *et al.* (1978)
			Oil	−35·9 (c 1·34)	IR, NMR, X-ray, MS, GC, TLC	Coxon *et al.* (1977)
					X-ray	Hughes & Coxon (1974)
26.	Phytuberol (desacetylphytuberin) (16a)	$C_{15}H_{24}O_2$	Oil		IR, GC, MS	Uegaki *et al.* (1981)
					IR, NMR, MS	Takagi *et al.* (1979)

the C-3 position. The configuration of the remaining functional groups is identical in these two compounds. Oxylubimin (7) and epioxylubimin (epihydroxylubimin) (7a) are another pair of epimeric aldehydes. Reduction of the aldehyde group in lubimin (6) to a hydroxymethyl group can afford 15-dihydrolubimin (8) which is epimeric to 15-dihydro-10-epilubimin (8a) with respect to the —CH_2OH group. The change in configuration of the C-2-hydroxyl group of 15-dihydrolubimin (8) would convert it into 15-dihydro-2-epilubimin (8b). Oxidation of the secondary hydroxyl of 15-dihydrolubimin (8) could provide isolubimin (9) from which solavetivone (katahdinone) (10) could arise by dehydration and double bond isomerization to the conjugated position. Introduction of another double bond into solavetivone (10) at the C-3–C-4 position resembles the structure of anhydro-β-rotunol (11). Out of these two double bonds, one double bond is absent is spirovetiva-1(10),11-dien-2-one (12) as shown in Fig. 1. In cyclodehydroisolubimin (13), the third ring is derived by a linkage between C-4 and C-15 through an oxygen atom. The spiro compounds which are either without the isopropenyl side chain or in the hydroxylated side chain form are represented in potatoes by 6,10-dimethylspiro [4,5] dec-6-en-2,8-dione (14) or 2-(11,12-dihydroxy-11-methylethyl)-6,10-dimethyl-9-hydroxyspiro [4,5] dec-6-en-8-one (15) and its 9-deoxy compound (15a), respectively. The primary hydroxyl group in the side chain seems to be the site for glycosylation (15b) (Malmberg & Theander, 1980).

Phytuberin (16) and its deacetylated form, phytuberol (16a) are tricyclic compounds containing two furan rings. These structures are unusually different from those belonging to rishitin (1) or spiro type compounds.

Structural elucidation of the above stress metabolites has been achieved by IR, UV, NMR, and mass spectroscopy, X-ray diffraction, ORD and by other physical constants (Table 1) in conjunction with different chemical reactions.

EXTRACTION, SEPARATION AND ASSAY

Extraction

Aqueous alcohol is the most widely used solvent for extraction of sesquiterpenoids from fresh or freeze-dried potato tissue (Kasui *et al.*, 1972; Masamune *et al.*, 1977). However, diffusates from a large quantity of potatoes can be conveniently extracted with a suitable solvent such as ether (Stoessl & Stothers, 1980). The concentrated aqueous alcohol extract can be partitioned against chloroform. The extract is treated with acetone and then hexane to remove the respective solvent-insoluble materials. The resulting

hexane soluble fraction is evaporated and the residue in ether is made free from acidic and basic components by washing. Removal of the solvent gives a crude extract which may be oily or syrupy.

The method of extraction and nature of solvent are likely to influence the type and recovery of the sesquiterpenes. The water-soluble sesquiterpene glycosides (Malmberg & Theander, 1980) might escape detection if only hydrophobic organic solvents are preferred and no systematic approach for investigation of such compounds is undertaken. Hence the possibility of revealing more types of glycosides and similar other compounds would also be diminished. It may also be difficult to select a sample of uniform quality that contains healthy and dead cells as well as cells undergoing degradation (Brishammar, 1987). In spite of very thin slices from cut (infected) tubers, the samples are composed of a variety of tissues whose biosynthetic activities are out of phase with each other. The isolation of infected tissues of interest located at the centre of the tuber pulp becomes critical when the concentration of rishitin (1) for instance, has to be determined as a function of time. During extraction, any loss and induced degradation or other molecular change may lead to decreased yields. Also, production of artifacts (Kuć, 1983) during extraction may render the compound biologically ineffective although the native compound exhibits activity *in situ.*

Separation

It is customary to separate the components of the crude extract by conventional column chromatography using silica gel and a certain amount of celite while eluting with solvent by gradual increase in its polarity. The different fractions so collected contain terpenoid stress compounds which can be separated and characterized by TLC. A number of workers have published articles relating to use of TLC for separation of potato sesquiterpenoids (Table 2). These compounds can be located on TLC plates after exposure of plates to spray reagents which show characteristic colours (Table 3). Solavetivone (10) produced a purple-brown spot, but the other compounds gave no colour change reactions when the plates were sprayed with vanillin–sulfuric acid reagent (Price *et al.*, 1976). In spite of very similar R_f values, phytuberin (16) and solavetivone (10) could be distinguished because of their distinct colours. In the case of epimeric compounds, TLC impregnated with silver nitrate may become useful in clear separation. With the advent of HPLC, separation of many natural products occurring in small amounts has been greatly facilitated and its applications to stress compounds may prove to be fruitful (Heisler *et al.*, 1981). Separation of some stress compounds has also been achieved by GLC on SE-30 (Stoessl *et al.*, 1976) and capillary (PEG-20M Ultra Bond) columns (Uegaki *et al.*, 1981). A

TABLE 2
Qualitative TLC of Sesquiterpenoid Stress Metabolites

Sr. no.	*Compound*	*Adsorbent*	*Developer*	*$R_f \times 100$*	*Visualization*	*Reference*
1.	(a) Rishitin (1)	x	$CHCl_3$: ether, 1:1	(a) 63	—	Katsui *et al.* (1972)
2.	(a) Lubimin (6)	Silica gel (camag (DFS)), 300 μ, activated	x	x	—	Stroessl *et al.* (1975)
3.	(a) Rishitinol (4) (b) Lubimin (6) (c) Rishitin (1) (d) Oxylubimin (7)	Silica gel	Ether	(a) 53 (b) 39 (c) 29 (d) 14	Ceric sulfate and Ehrlich reagent	Masamune *et al.* (1977)
4.	(a) Phytuberin (16)	Merck Kieselgel G	Cyclohexane–EtOAC, 1:1	(a) 65, (b) 25	I_2 vapor, vanillin/H_2SO_4	Coxon *et al.* (1977)
	(b) Rishitin (1)		$CHCl_3$–AcOH–MeOH, 85:2:13	(a) 80, (b) 60		
5.	(a) Lubimin (6) (b) Oxylubimin (7)	Silica gel (Merck) GF-254 or Wakogel (B-5)	Benzene–Ether, 1:2 Ether	x (b) 50	$SbCl_3/CHCl_3$ satd Ceric sulfate, vanillin/H_2SO_4 or Ehrlich reagent	Katsui *et al.* (1977)
6.	(a) 2-Epilubimin (6b) (b) 15-Dihydro-2-epilubimin (8b)	Silicagel (0·2 mm; Macherey–Nagel Polygram sil N-HR/UV)	i-PrOH–EtOAC, 5:95 MeOH–$CHCl_3$, 5:95	(a) x, (b) 26 (a) x, (b) 17	Vanillin/H_3PO_4 and heating at 110°C	Stoessl & Stothers (1980)
7.	(a) Phytuberin (16)	Silica gel	Cyclohexane–EtOAc, 1:1 Cyclohexane–EtOAc, 4:1 Hexane–Dioxane, 9:1 $CHCl_3$–MeOH, 95:5 Benzene–$CHCl_3$, 9:1	(a) 70 (a) 34 (a) 59 (a) 69 (a) 12	Vanillin/H_2SO_4, conc. H_2SO_4	Hammerschmidt & Kuć (1979)

8.	(a) Rishitin (1) (b) Lubimin (6) (c) Phytuberin (16) (d) Oxylubimin (7) (e) Anhydro-β-rotunol (11) (f) Solavetivone (10)	Silica gel (Camag DF5), 0·3 mm	MeOH–$CHCl_3$, 1:19 MeOH–Ether, 1:19 Ether	(a) 22, (b) 41, (c) 69, (d) 18, (e) 62, (f) 68 (a) 46, (b) 53, (c) 71, (d) 30, (e) 55, (f) 68 (a) 24, (b) 33, (c) 62, (d) 13, (e) 40, (f) 60	Phosphomolybdic acid, H_3PO_4	Stoessl *et al.* (1976)
9.	(a) Rishitin (1) (b) Phytuberin (16) (c) Lubimin (6) (tentatively)	Silica gel 300 u	Cyclohexane–EtOAc, 1:1	(a) 22 (b) 72 (c) 34	Conc. H_2SO_4, Vanillin/H_2SO_4	Corsini & Pavek (1980)
10.	(a) Rishitin (1) (b) Phytuberin (16)	Silica gel pre-coated plate (Analtech Inc.)	Cyclohexane–EtOAC, 1:1	(a) 21 (b) 70	Carr–Price reagent	Shih *et al.* (1973)
11.	(a) Rishitin (1) (b) Lubimin (6) (c) Desacetyl phytuberin (16b) (d) Anhydro-β rotunol (11) (e) Solavetivone (10) (f) Phytuberin (16)	Silica gel 60 Pre-coated plate (Merck)	Cyclohexane–EtOAC, 1:1	(a) 23 (b) 27 (c) 36 (d) 42 (e) 60 (f) 63	Vanillin/H_2SO_4	Price *et al.* (1976)
12.	(a) Rishitin (1) (b) Lubimin (6) (c) Oxylubimin (7) (d) Solavetivone (10) (e) Phytuberin (16)	Silica gel G	Cyclohexane–EtOAC, 1:1	(a) 22 (b) 30 (c) 10 (d) 44 (e) 58		Brindle *et al.* (1988)

x = Not reported.

TABLE 3
Colour Reactions of Some Sesquiterpenoid Stress Metabolites on TLC Plates

	Compounds	*Color*	*Reagent*	*Reference*
1.	Rishitin (1)	Dark blue	Exposed to I_2 and vanillin/H_2SO_4 spray (120°C, 10 min)	Price *et al.* (1976)
	Lubimin (6)	Dark blue		
	Phytuberol (16a)	Purple		
	Anhydro-β-rotunol (11)	Blue		
	Solavetivone (10)	Turquoise		
	Phytuberin (16)	Purple		
2.	Rishitin (1)	Gray	Ehrlich reagent	Masamune *et al.* (1977)
	Lubimin (6)	Pink		
	Oxylubimin (7)	Magenta		
	Rishitinol (4)	Pink		
3.	Phytuberin (16)	Heliotrope	Vanillin/H_2SO_4	Hammerschmidt & Kuć (1979)
		Orange	Conc. H_2SO_4	
4.	15-Dihydro-2-epilubimin (8b)	Red	Vanillin/H_2SO_4 (110°C)	Stoessl & Stothers (1980)
5.	Rishitin (1)	Blue	Vanillin/H_2SO_4	Brindle *et al.* (1988)
	Lubimin (6)	Turquoise		
	Oxylubimin (7)	Purplish blue		
	Solavetivone (10)	Buff		
	Phytuberin (16)	Reddish pink		

typical scheme (Malmberg & Theander, 1980) for separation of both non-polar and polar terpenoids is shown in Fig. 2.

Assay

The method used by Shih *et al.* (1973) for quantitative determination of rishitin (1) was based on spectrophotometry of its chromogen. The procedure involved dissolution of freeze-dried tuber tissue in 1 ml of cyclohexane followed by addition of 2 ml concentrated sulfuric acid to the solution. The mixture was agitated and centrifuged at low speed for 2–3 min. The red color of the lower sulfuric acid layer was measured at 500 nm 10–20 min after the addition of sulfuric acid. Concentrated sulfuric acid served as blank. Since colored products are developed by a mixture of terpenoids in the tissue with the reagent the results would represent total terpenoid content when expressed against a suitable standard such as rishitin (1).

Henfling & Kuć (1979) developed a semi-micro GLC method for the quantitation of potato terpenes from samples as small as 0·1 g. The upper 2-mm portions of infected slices were extracted with methanol without

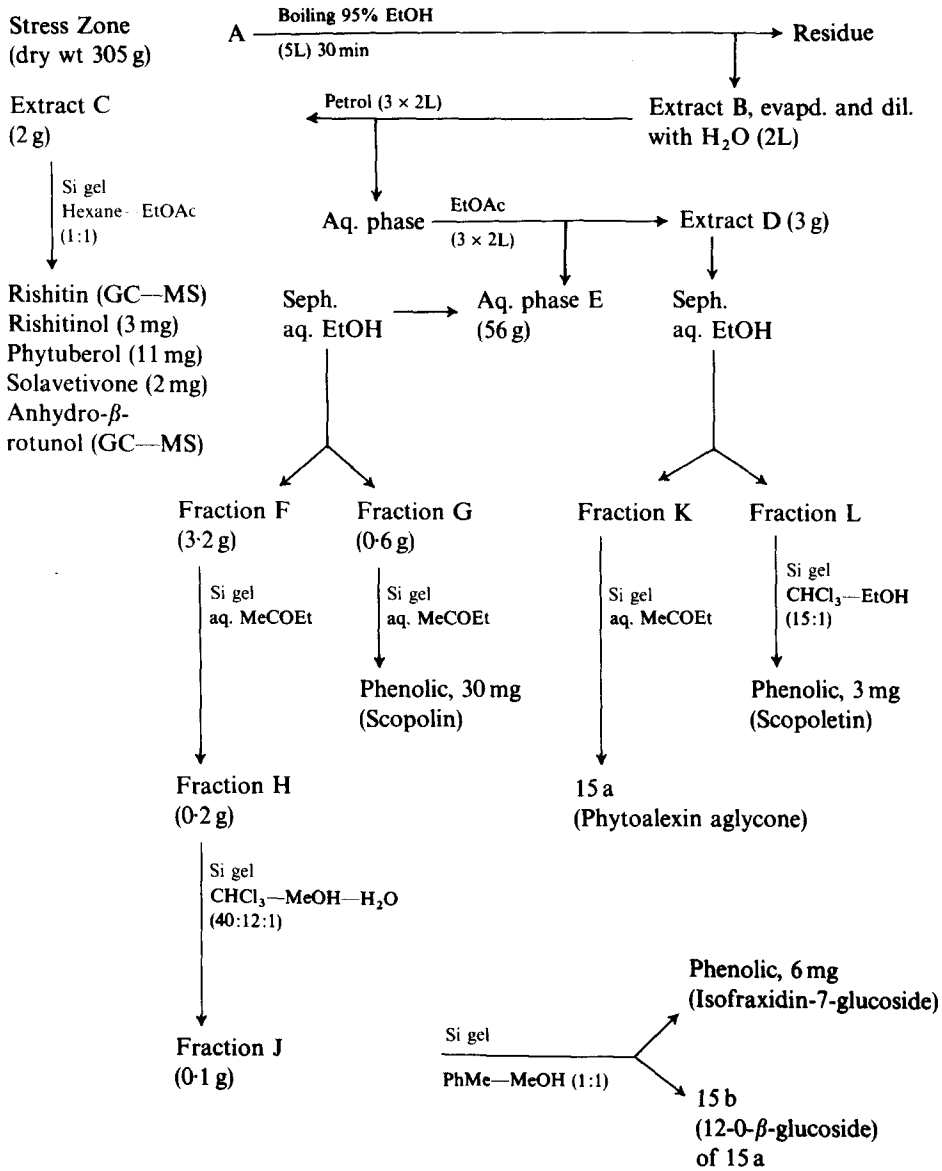

Fig. 2. Separation of sesquiterpene glycoside from *Phoma*-infected potato tuber. Seph. and Si gel = Column chromatograph on sephadex LH-20 and silica gel, respectively, aq. EtOH = elution with H_2O with increasing EtOH content (0–95%). aq. MeCoEt = 2-butanone satd with H_2O. From Malmberg, A. G. & Theander, O. (1980). *Phytochemistry*, **19**, 1739–42. With permission.

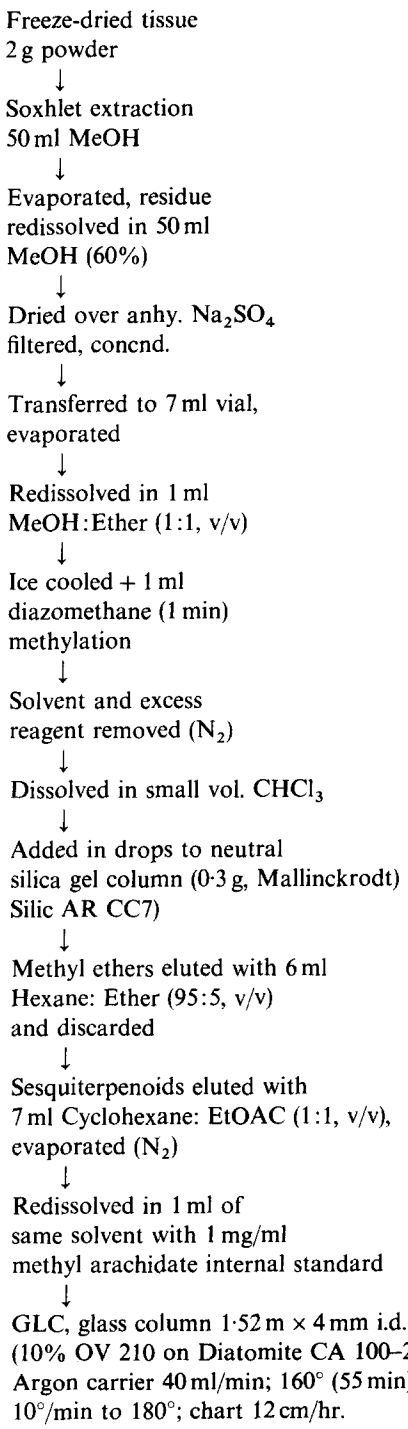

Fig. 3. Sample preparation and quantitative analysis of terpenoids by GLC. From Price, K. R., Howard, B. & Coxon, D. T. (1976). *Physiol. Plant Pathol.*, **9**, 189–97. With permission.

homogenization. The residue after evaporation of the solvent was partitioned in a test tube between water and ethyl acetate. The dry residue from the organic layer was dissolved in methanol and the terpenes were determined by GLC using a glass column packed with 3% OV-225 on supelcoport (FID, N_2 as carrier gas). The recoveries of added standards, such as phytuberol (16a), phytuberin (16), rishitin (1) and lubimin (6) were 85–95%. For quantitative analysis of six terpenoids, Price *et al.* (1976) followed a procedure as outlined in Fig. 3. Chloroform extract of 10 g fresh tissue obtained by the procedure described by Lyon (1972) was similarly treated in this experiment. Although qualitative and quantitative results can be achieved with the packed columns, capillary columns would be quite effective in rapid and reliable GC analysis. Reverse phase HPLC has been employed (Heisler *et al.*, 1981) for the analysis of four major sesquiterpenes, namely phytuberin (16), katahdinone (10), rishitin (1) and lubimin (6). The separation of these compounds on a Bondpack C_{18} column (30 cm × 3·9 mm) was achieved with a MeOH–H_2O (7:3) solvent. It is claimed that the method is suitable for routine analysis of these metabolities in infected potato tissue since it provides good precision in terms of standard deviation and recovery data.

BIOSYNTHESIS AND METABOLISM

Biosynthesis

Sesquiterpene stress metabolites of potatoes originate from the acetate–mevalonate pathway. However, the biosynthetic mechanisms associated with the formation of many such stress compounds are quite intriguing. The progress that has been made in this area on potato phytoalexins is significant (Stoessl *et al.*, 1976, 1977, 1978). It is expected, however, that new members of the terpenoid family will be found in potato tissue and the composition and structural features of these metabolites will be governed by the nature of the inducing agent.

Rishitin (1) was the first terpenoid whose biosynthesis was studied in potato tuber slices (Shih & Kuć, 1973; Shih *et al.*, 1973). The two groups of compounds, steroidal glycoalkaloids and terpenoids, which accumulate in cut or otherwise wounded potato tuber tissue are biosynthetically derived through the acetate–mevalonate route. When cut tissue is subjected to different stimuli, rishitin (1) is accumulated in large amounts (Shih *et al.*, 1973). The phenomenon is consistently associated with the suppression of alkaloid accumulation. When labelled acetate and mevalonate are fed to potato tuber slices inoculated with *Phyptophthora infestans*, a high degree of

incorporation of mevalonate takes place. The marked inhibition of alkaloid accumulation suggests that either a block in the acetate–mevalonate pathway and/or the synthesis or activation of key enzymes at a branch point in the pathway are necessary for accumulation of rishitin (1). The labelled studies have also indicated a diversion of the steroidal alkaloids to sesquiterpene biosynthesis with the branching point at some point after mevalonic acid (Shih & Kuć, 1973). It is generally believed that the glycoalkaloids accumulated over a much wider area of tissue than rishitin production, may be attributed to wound periderm formation. This implies that the biosynthesis of glycoalkaloids and rishitin (1) are regarded as under separate and independent control (Ishizaka & Tomiyama, 1972).

Numerous stress compounds can be shown to originate from cyclization of farnesyl pyrophosphate to germacrene (decalin) precursors which upon further cyclization afford eudesmane skeletons (Stoessl *et al.*, 1976, 1978). It was proposed (Stoessl *et al.*, 1976) that the bicyclic sesquiterpenoid stress compounds which are structurally related to one another can be derived from eudesmanes by plausible rearrangements and biotransformations. These rearrangements, except in rishitinol (4), take place by migration of an electron pair from C-9–C-10 to C-9–C-5. The majority of sesquiterpenoidal stress compounds in potato tissue belong to this group derived by rearrangements and are known as spirovetivanes. According to Stoessel *et al.* (1976), the above two generalizations can serve as guidelines in structure elucidations of newly isolated stress compounds. Nevertheless, the possibility of by-passing eudesmanes or rearranged eudesmane structures by new stress compounds cannot be ruled out. It is coincidental that the bicyclic compounds, except rishitinol (4), possess oxygen on one or more carbon atoms 1–4 but none on carbon atoms 6–9—with the exception of the two spiro compounds in Fig. 1.

As indicated in Figs 4 and 5, the decalin skeleton can adopt a variety of conformations which favorably explain the formation of certain sesquiterpenoidal stress metabolites. Stoessl *et al.* (1978) proposed these two schemes while studying the biosynthetic mode of formation of ten terpenoids produced by invasion of potato tubers fed with doubly labelled sodium acetate–$^{13}C_2$. Since the *de novo* synthesis of these metabolites was certain under these stress conditions, a high level of incorporation was noted. In the isoprenoidal pathway of terpenes, the incorporation of acetate–$^{13}C_2$ into mevalonate units yields mevalonate with two intact acetate units and one carbon (C_2) which has lost its original neighbor. In the event of sesquiterpenes formed without rearrangement or cleavage of the farnesyl chain, the ^{13}C NMR spectrum of the compound would reveal six pairs of signals having ^{13}C satellites for each of the six intact acetate units and three enhanced signals lacking prominent satellites which arise from C-2 of the

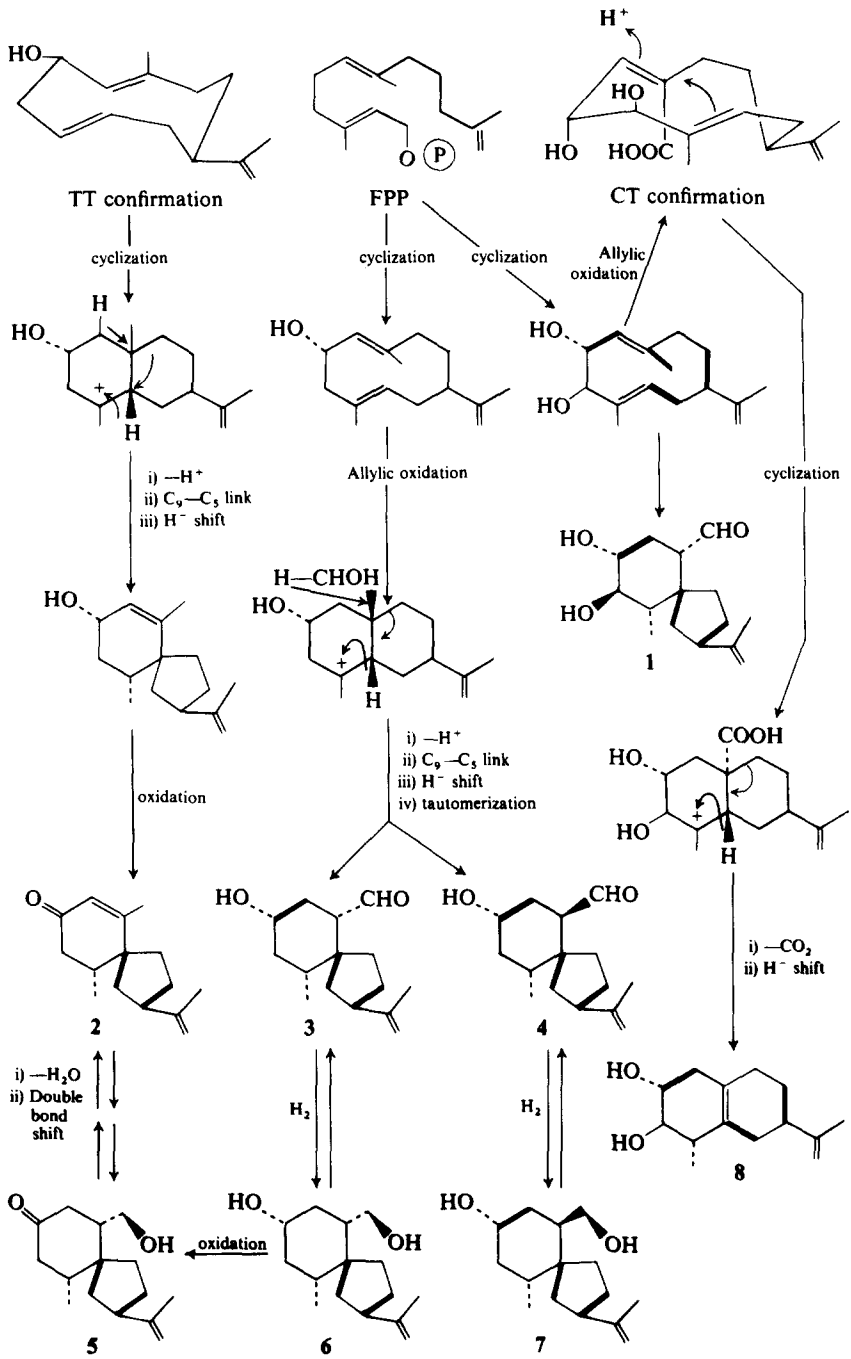

Fig. 4. A proposed scheme for biogenesis of some stress metabolites in potatoes. 1, Hydroxylubimin; 2, solavetivone; 3. lubimin; 4. 10-epilubimin; 5, isolubimin; 6, 15-dihydrolubimin; 7, 15-dihydro-10-epilubimin; 8. rishitin. From Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1978). *Can J. Chem.*, **56**, 645. With permission.

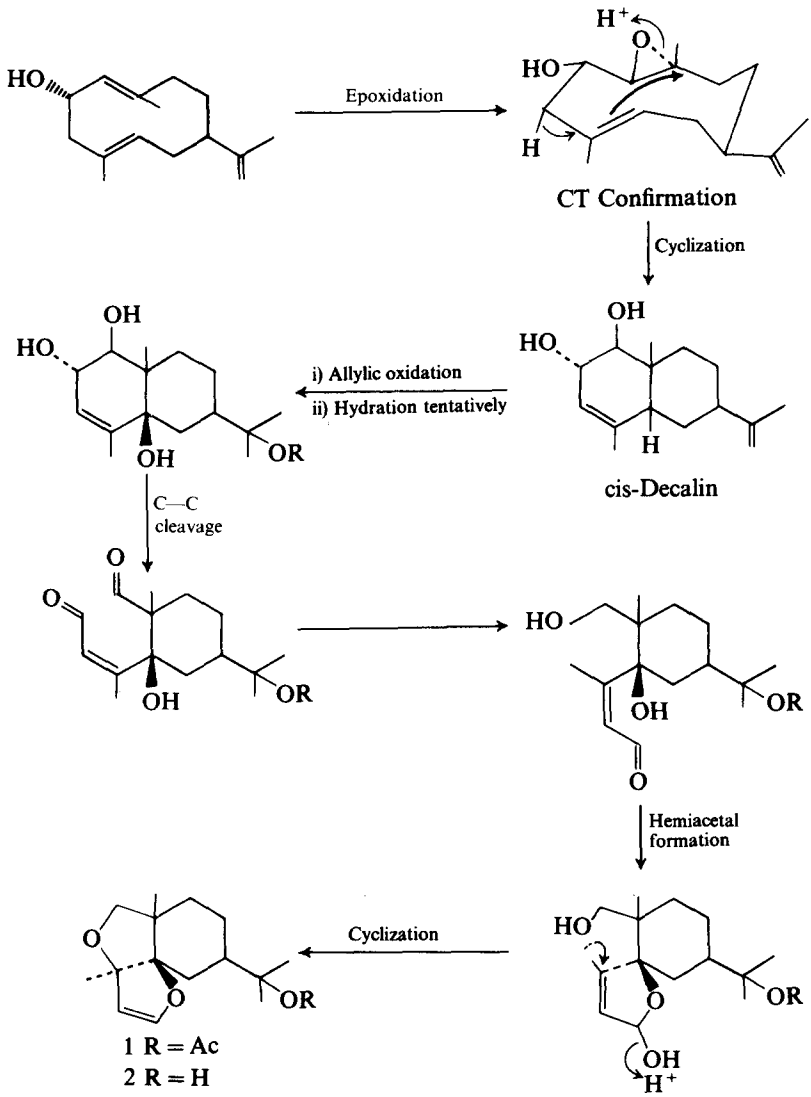

Fig. 5. A proposed scheme for biogenesis of 1, phytuberin and 2, phytuberol. From Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1978). *Can. J. Chem.*, **56**, 645–53. With permission.

mevalonate unit. Thus,with the exception of rishitin (1), phytuberin (16) and phytuberol (16a), the remaining metabolites contained six intact acetate units.

The formation of the spirovetivane skeleton from the farnesyl chain involves ring contraction by cleavage of the C-9–C-10 bond with formation of a C-9–C-5 linkage (Fig. 4). In this case, there is no rupture of the intact acetate unit as C-9 is derived from the C-2 of the mevalonate unit. The biogenesis of rishitin (1) involves allylic oxidation of cyclodecadiene to an

intermediate which upon cyclization yields the *trans*-decalin skeleton. Finally, decarboxylation and hydride shift result in the rishitin (1) formation. This loss of carbon dioxide responsible for cleavage of a C—C bond of an intact acetate unit reflects four signals in the ^{13}C NMR spectrum of rishitin (1) lacking prominent ^{13}C sattelites. The hybrid shift from C-5 to C-4 was shown by incorporation of $D_3{}^{13}CCOONa$ in tubers inoculated with *Monilinia fruticola* and by NMR studies (Stoessl & Stothers, 1982). The hydroxyl oxygen atom of lubimin (6), rishitin (1) and two metabolites of rishitin (1) originated from molecular oxygen (Brindle *et al.*, 1985).

The ^{13}C NMR spectra of the labelled samples of phytuberin (16) and phytuberol (16a) reported by Stoessl *et al.* (1978), clearly indicated only five intact acetate units. A logical sequence of biotransformations leading to the phytuberin skeleton is shown in Fig. 5.

It is interesting to note that the spiro compound oxylubimin (7) is shown to be a precursor of rishitin (1) while isolubimin (9) is a precursor of lubimin (6) and rishitin (1) (Kalan & Osman, 1976; Sato *et al.*, 1978). Also, the pattern of accumulation of phytoalexins in potato cell suspension cultures in the presence or absence of a saturating concentration for sterol synthesis of either [2-^{14}C] mevalonic acid (3·3 mM) or [2-^{14}C] acetate (1 mM) were in agreement with the partial biosynthetic sequence of lubimin (6), oxylubimin (7) and rishitin (1) (Brindle *et al.*, 1988). In a similar situation, the bioconversion of another spiro compound (±)-solavetivone-8-8-$^2H_2$ established that the main phytoalexin rishitin (1) was formed from (−)-solavetivone (10) via (+)-lubimin (6) and (+)-oxylubimin (7) in potato (Murai *et al.*, 1982). Also, it was suggested that (−)-solavetivone (10) might be an inducer of the enzyme system for the formation of these stress metabolites from acetic acid. Katsui *et al.* (1981) predicted that *in vivo* transformation of solavetivone (10) to rishitin (1) would be inhibited to some extent by *P. infestans* with the race 1 gene. Capsidiol, a stress compound of sweet-pepper which is similar to rishitin (1) in structural features, was proposed to be derived from a series of spiro-rearrangements (Fig. 6) as an alternative route to the hypothesis of the angular methyl group migration from C-10 from a precursor of the eudesmanoid skeleton type (Baker & Brooks, 1976). Murai *et al.* (1987) also extended the spiro compound theory to phytuberin (16) which is considered to be biosynthesized from solavetivone (10).

Metabolism

Rishitin (1) metabolizes to rishitin M-1 and rishitin M-2 in potato tuber by wounding (Ishiguri *et al.*, 1978). These metabolites possess a C-13—OH group which is absent in rishitin (1) (Murai *et al.*, 1977), while the rishitin (1)

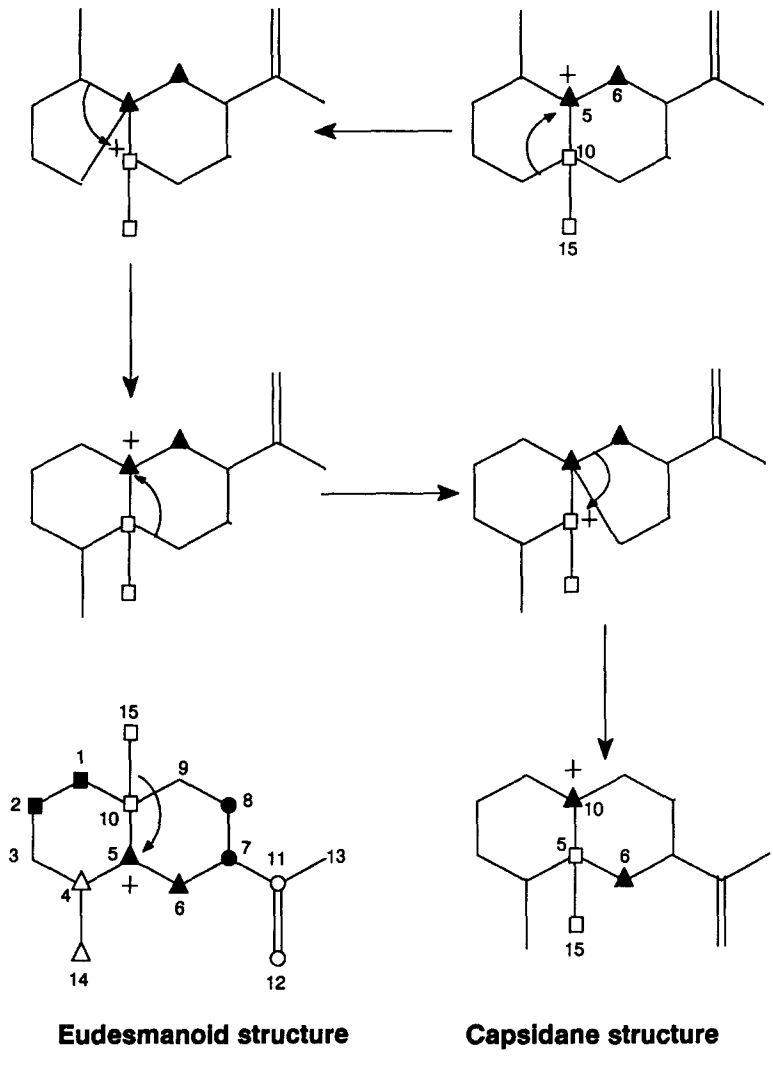

Fig. 6. Hypothetical formation of capsidiol skeleton from two alternate routes: (A) eudesmanoid structure favoring vicinal methyl shift and (B) series of spiro-rearrangements leading to capsidane structure. From Baker, F. C. & Brooks, C. J. W. (1976). *Phytochemistry*, **15**, 689–94. With permission.

has one double bond between C-11 and C-12. Ward *et al.* (1977) reported that this compound was metabolized to 13-hydroxy derivatives by potato tissue cultures. It appears that 13-hydroxylation may be a general metabolic process in Solanaceae. Further steps such as glucosylation may function as detoxification mechanisms. Also, they could increase the number of water-soluble phytoalexins, thus adding to the complexity of their isolation and

characterization. Potato cell culture was able to convert rishitin (1) to an unknown compound, tentatively characterized as glutinosone (Zaharius *et al.*, 1985). On the other hand, exogenous lubimin (6) was unaffected by the potato cell culture. The stability of the exogenous lubimin (6) may be ascribed to a second block in the rishitin pathway of the potato cell culture.

TOXICITY IMPLICATIONS

There is no evidence that the terpenoid phytoalexins can cause toxic reactions in humans or animals consuming potatoes as food or feed. As previously stated, phytoalexins are generally restricted to the diseased parts of tubers, which are usually small. Moreover, no harmful effects were noted when mice were exposed to phytuberin (16) (Renwick, 1972), and no embryotoxic or teratogenic effects appeared when pregnant mice were exposed to either rishitin (1) or phytuberin (16) (Neudecker & Schober, 1984).

Rishitin (1) and phytuberin (16) are phytotoxic, causing the death of plant protoplasts and isolated cells (Lyon & Mayo, 1978; Lyon, 1980), although there is no experimental evidence concerning the extent to which their phytotoxicity may contribute to plant cell death during the infection process. Lyon (1989) suggested that rishitin (1) affected membrane permeability by increasing fluidity and permitting an increased passage of low molecular-weight compounds through the membrane. Secondary effects on membrane-bound proteins could also occur when membrane integrity has been affected by compounds such as rishitin (Lyon & Mayo, 1978). Very high concentrations of phytoalexins are, however, required to cause such adverse reactions, and it is uncertain that they actually occur in nature.

FUTURE PERSPECTIVE

The induction of the terpenoid phytoalexins accumulation in response to infections is generally believed to be a part of the resistance mechanism against pathogens. Phytoalexin research has been centered around some major compounds such as rishitin (1), lubimin (6), solavetivone (10), phytuberin (16), and phytuberol (16a). However, the occurrence of a wide range of terpenoids in potatoes would require further characterization in order to provide a more complete assessment of the role of individual compounds in disease resistance. The emergence of several new compounds, particularly glycosidic terpenes, should stimulate increased interest in extending the chemical, biochemical and biological investigations on these compounds. Toxic effects of potato phytoalexins to humans and animals

also received very little attention. However, no harmful effects were noted when mice were exposed to phytuberin (16) and no embryotoxic or teratogenic effects appeared when pregnant mice were exposed to rishitin (1) or phytuberin (16).

The elucidation of existing relationships between three-dimensional chemical structure and biological activity may become useful in establishing definite trends in phytoalexin research. There is a general consensus to substantiate research findings involving selection of experimental plant material, biological interactions, biosynthetic schemes, mechanism of pathogen inhibition, toxicological effects and bioassays, purification and determination of phytoalexins.

REFERENCES

Alves, L. M., Kirchner, R. M., Lodato, D. T., Nee, P. B., Zappia, J. M., Chichester, M. L., Stuart, J. D., Kalan, E. B. & Kissinger, J. C. (1984). Acetyldehydrorishitinol, a rishitinol-related potato stress metabolite. *Phytochemistry*, **23**, 537–8.

Baker, F. C. & Brooks, C. J. W. (1976). Biosynthesis of the sesquiterpenoid capsidiol in sweet pepper fruits inoculated with fungal spores. *Phytochemistry*, **15**, 689–94.

Birnbaum, G. I., Huber, C. P., Post, M. L., Stothers, J. B., Robinson, J. R., Stoessl, A. & Ward, E. W. B. (1976). Sesquiterpenoid stress compounds of *Datura stramonium*: biosynthesis of the three major metabolites (1,2- ^{13}C) acetate and the X-ray structure of 3-hydroxylubimin. *J. Chem. Soc. Chem. Comm.*, **9**, 330–1.

Brindle, P. A., Coolbear, T., Kuhn, P. J. & Threlfall, D. R. (1985). A molecular oxygen-18 study of the biosynthesis and metabolism of rishitin. *Phytochemistry*, **24**, 1219–22.

Brindle, P. A., Kuhn, P. J. & Threlfall, D. R. (1988). Biosynthesis and metabolism of sesquiterpenoid phytoalexins triterpenoids in potato cell suspension cultures. *Phytochemistry*, **27**, 133–50.

Brishammar, S. (1987). Critical aspects of phytoalexins in potato. *J. Agric. Sci. Fin.*, **59**, 217–30.

Corsini, D. L. & Pavek, J. J. (1980). Phenylalanine ammonia lyase activity and fungitoxic metabolites produced by potato cultivars in response to *Fusarium* tuber rot. *Physiol. Plant Pathol.*, **16**, 63–72.

Coxon, D. T., Curtis, R. F., Price, K. R. & Howard, B. (1974). Phytuberin: a novel antifungal terpenoid from potato. *Tetrahedron Lett.* **27**, 2363–6.

Coxon, D. T., Price, K. R., Howard, B., Osman, S. F., Kalan, E. B. & Zacharius, R. M. (1974). Two new vetispirane derivatives: stress metabolites from potato (*Solanum tuberosum*) tubers. *Tetrahedron Lett.*, **34**, 2921–4.

Coxon, D. T., Price, K. R., Howard, B. & Curtis, R. F. (1977). Metabolism from microbially infected potato. Part 1. Structure of phytuberin. *J. Chem. Soc. Perkin*, **1**, 53–9.

Coxon, D. T., Price, K. R., Stothers, J. B. & Stoessl, A. (1979). Cyclodehydroisolubimin: a new tricyclic sesquiterpene from potato tubers inoculated with *Phytophthora infestans*. *J. Chem. Soc. Chem. Comm.*, **7**, 348–9.

Hammerschmidt, R. & Kuć, J. (1979). Isolation and identification of phytuberin from *Nicotiana tabacum* previously infiltrated with an incompatible bacterium. *Phytochemistry*, **18**, 874–5.

Heisler, E. G., Siciliano, J., Kalan, E. B. & Osman, S. F. (1981). High performance liquid chromatographic determination of stress-induced sesquiterpenes of the potato (*Solanum tuberosum*). *J. Chromatogr.*, **210**, 365–9.

Henfling, J. W. D. M. & Kuć, J. (1979). A Semimicro method for the determination of sesquiterpenoid stress metabolites in potato tuber tissue. *Phytopathology*, **69**, 609–12.

Hughes, D. L. & Coxon, D. T. (1974). Phytuberin, revised structure from the X-ray crystal analysis of dihydrophytuberin, *J. Chem. Soc. Chem. Comm.*, **20**, 822–3.

Ingham, J. L. (1972). Phytoalexins and other natural products as factors in plant disease resistance. *Bot. Rev.*, **38**, 343–424.

Ishiguri, Y., Tomiyama, K., Doke, N., Murai, A., Katsui, N., Yagihashi, F. & Masamune, T. (1978). Induction of rishitin-metabolizing activity in potato tuber disks by wounding and identification of rishitin metabolites. *Phytopathology*, **68**, 720–5.

Ishizaka, N. & Tomiyama, K. (1972). Effect of wounding or infection by *Phytophthora infestans* on the contents of terpenoids in potato tubers. *Plant and Cell Physiol.*, **13**, 1053–63.

Jadhav, S. J. & Salunkhe, D. K. (1975). Formation and control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum* L. and evaluation of glycoalkaloid toxicity. *Adv. Food Res.*, **21**, 307–54.

Jadhav, S. J., Sharma, R. P. & Salunkhe, D. K. (1981). Naturally occurring toxic alkaloids in foods. *CRC Crit. Rev. Toxicol.*, **9**, 21–104.

Kalan, E. B. & Osman, S. F. (1976). Isolubimin—a possible precursor of lubimin in infected potato slices. *Phytochemistry*, **15**, 775–6.

Katsui, N., Murai, A., Takasugi, M., Imaizumi, K. & Masamune, T. (1968). The structure of rishitin, a new antifungal compound from diseased potato tubers. *J. Chem. Soc. Chem. Comm.*, **1**, 43–4.

Katsui, N., Matsunaga, A., Imaizumi, K., Masamune, T. & Tomiyama, K. (1971). The structure and synthesis of rishitinol, a new sesquiterpene alcohol from disease potato tubers. *Tetrahedron Lett.*, **2**, 83–6.

Katsui, N., Matsunaga, A., Imaizumi, K., Masamune, T. & Tomiyama, K. (1972). Phytoalexins VII. Structure and synthesis of rishitinol sesquiterpene alcohol from diseased potato tubers. *Bull. Chem. Soc. Jap.*, **45**, 2871–7.

Katsui, N., Matsunaga, A. & Masamune, T. (1974). The structure of lubimin and oxylubimin, antifungal metabolites from diseased potato tubers, *Tetrahedron Lett.*, **51/52**, 4483–6.

Katsui, N., Matsunaga, A., Kitahara, H., Yogihashi, F., Murai, A., Masamune, T. & Sato, N. (1977). Lubimin and oxylubimin. The structure elucidation. *Bull. Chem. Soc. Jap.*, **50**, 1217–25.

Katsui, N., Yagihashi, F., Murai, A. & Masamune, T. (1978). Studies on the phytoalexins XVIII. Structure of oxyglutinosone and epioxylubimin, stress metabolites from diseased potato tumbers. *Chem. Lett.*, **11**, 1205–6.

Katsui, N., Takahashi, Y., Sato, N., Murai, A. & Masamune, T. (1981). Studies of the phytoalexins. 26. Phytoalexins produced by potato variety Rishiri inoculated with a compatible race of *Phytophthora infestans*. *Nippon Kagaka Kaishi*, **5**, 659–64.

Katsui, N., Yagihashi, F., Murai, A. & Masamune, T. (1982). Studies on the phytoalexins. XXXV. Structure of rishitinone, a valencane stress metabolite in diseased potato. *Bull. Chem. Soc. Jap.*, **55**, 2428–33.

Kuć, J. (1976). Phytoalexins. In *Physiological Plant Pathology*, ed. R. Heitefuss & P. H. Williams. Springer Verlag, NY. pp. 632–52.

Kuć, J. (1983). Phytoalexins from the Solanaceae. In *Phytoalexins*, ed. J. A. Bailey & J. W. Mansfield. Blackie, Glasgow and London, p. 81–105.

Lyon, G. D. (1972). Occurrence of rishitin and phytuberin in potato tubers inoculated with *Erwinia caratovora* var. *astroseptica*. *Physiol. Plant Pathol.*, **2**, 411–6.

Lyon, G. D. (1980). Evidence that the toxic effect of rishitin may be due to membrane damage. *J. Experim. Botany*, **31**, 957–66.

Lyon, G. D. (1989). The biochemical basis of resistance of potatoes to soft rot, *Erwinia* spp.—a review. *Plant Pathology*, **38**, 313–39.

Lyon, G. D. & Mayo, M. A. (1978). The phytoalexin rishitin affects the viability of isolated protoplasts. *Phytopathologische Zeitschrift*, **92**, 298–304.

Malmberg, A. G. (1982). Two new isoprenoid spiro compounds from potato tubers infected with *Phoma exigua*. *Phytochemistry*, **21**, 1818–9.

Malmberg, A. G. & Theander, O. (1980). Two phytoalexin glycosides from potato tubers infected with *Phoma*. *Phytochemistry*, **19**, 1739–42.

Masamune, T., Murai, A., Takasugi, M., Matsunaga, A., Katsui, N., Sato, N. & Tomiyama, K. (1977). Studies on the phytoalexins. XIII. Rishitin I. The isolation and structure elucidation. *Bull. Chem. Soc. Jap.*, **50**, 1201–5.

Müller, K. & Börger, H. (1940). Experimentelle Untersuchungen die *Phytophthora-resistenz* der Kartoffel. *Arb. Biol. Reichsanst. Land-und Forstu* (Berlin), **23**, 189–231.

Murai, A., Katsui, N., Yagihashi, F., Masamune, T., Ishiguri, Y. & Tomiyama, K. (1977). Structure of rishitin M-1 and M-2, metabolites of rishitin in healthy potato tuber tissues. *J. Chem. Soc. Chem. Comm.*, **19**, 670–1.

Murai, A., Sato, S., Osada, A., Katsui, N. & Masamune, T. (1982). Biosynthesis from solavetivone of the phytoalexin rishitin in potato: implicit role of solavetivone as an activator. *J. Chem. Soc. Chem. Comm.*, **1**, 32–3.

Murai, A., Yoshizawa, Y., Miyazaki, H., Masamune, T. & Sato, N. (1987). Studies on the phytoalexins. Part XLIV. Biosynthesis of phytuberin. *Chem. Lett.*, **7**, 1377–8.

Neudecker, C. & Schober, B. (1984). Fütterungsversuche zum Teratogenen Potential Phytoalexinhaltiger Kartoffeln. *EAPR Abstr. Conf. Papers*, **372**.

Paxton, J. D. (1981). Phytoalexins—a working definition. *Phytopath. Z.*, **101**, 106–9.

Price, K. R., Howard, B. & Coxon, D. T. (1976). Stress metabolite production in potato tubers infected by *Phytophthora infestans*, *Fusarium avenaceum* and *Phoma exigua*. *Physiol. Plant Pathol.*, **9**, 189–97.

Renwick, J. H. (1972). Hypothesis: Anencephaly and bifida are usually preventable by avoidance of a specific but unidentified substance present in certain potato tubers. *Brit. J. Prev. Soc. Med.*, **26**, 67–88.

Salunkhe, D. K. & Wu, M. T. (1979) Control of postharvest glycoalkaloid-formation in potato tubers. *J. Food Prot.*, **42**, 519–25.

Sato, K., Ishiguri, Y., Ioke, N., Tomigama, K., Yagihashi, F., Murai, A., Katsui, N. & Masamune, T. (1978). Biosynthesis of the sesquiterpenoid phytoalexin rishitin from acetate via oxylubimin in potato. *Phytochemistry*, **17**, 1901–2.

Shih, M. & Kuć, J. (1973). Incorporation of ^{14}C from acetate and mevalonate into rishitin and steroid glycoalkaloids by potato slices inoculated with *Phytophthora infestans*. *Phytopathology*, **63**, 826–9.

Shih, M. J., Kuć, J. & Williams, E. B. (1973). Suppression of steroid glycoalkaloid accumulation as related to rishitin accumulation in potato tubers. *Phytopathology*, **63**, 821–6.

Stoessl, A. & Stothers, J. B. (1980). 2-Epi and 15 dihydro-2-epilubimin: new stress compounds from potato. *Can. J. Chem.*, **58**, 2069-72.

Stoessl, A. & Stothers, J. B. (1982). The betahop: (2-$^{2}H_3$, 2-$^{13}C_1$) as a probe for 1,2-hydride shifts in the biosynthesis of natural products. *J. Chem. Soc. Chem. Comm.*, **15**, 880–1.

Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1974). Lubimin: A phytoalexin of several Solanaceae. Structure, revision and biogenetic relationship. *J. Chem. Soc. Chem. Comm.*, **17**, 709–10.

Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1975). A 2,3-dihydroxygermacrene and other stress metabolites of *Datura stramonium*. *J. Chem. Soc. Chem. Comm.*, **11**, 431–2.

Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1975). The structure of some stress metabolites from *Solanum melongena*. *Can. J. Chem.*, **53**, 3351–8.

Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1976). Sesquiterpenoid stress compounds of the Solanaceae. *Phytochemistry*, **15**, 855–72.

Stoessl, A., Ward, E. W. B. & Stothers, J. B. (1977). Biosynthetic relationships of sesquiterpenoidal stress compounds from the solanaceae. In *Host Plant Resistance to Pests*, ed. P. A. Hedin. Amer. Chem. Soc., Washington, DC, p. 61–77.

Stoessl, A., Stothers, J. B. & Ward, E. W. B. (1978). Biosynthetic studies of stress metabolites from potato: incorporation of sodium acetate—$^{13}C_2$ into 10 sesquiterpenes. *Can. J. Chem.*, **56**, 645–53.

Takagi, Y., Fugimori, T., Kaneko, H. & Kato, K. (1979). Phytuberol from Japanese domestic tobacco, *Nicotiana tabacum* cv. Suifu. *Agric. Biol. Chem.*, **43**, 2395–6.

Tomiyama, K., Sakuma, T., Ishizaka, N., Sato, N., Takasugi, M. & Katsui, T. (1968). A new antifungal substance isolated from potato tuber tissue infected by pathogens. *Phytopathology*, **58**, 115–6.

Uegaki, R., Fujimoro, T., Kubo, S. & Kato, K. (1981). Sesquiterpenoid stress compounds from *Nicotiana* species. *Phytochemistry*, **20**, 1567–8.

Uritani, I. (1967). Abnormal substances produced in fungus contaminated foodstuffs. *J. Assoc. Off. Anal. Chem.*, **50**, 105–14.

Ward, E. W. B., Stoessl, A. & Stothers, J. B. (1977). Metabolism of sesquiterpenoid phytoalexins capsidiol and rishitin to their 13-hydroxy derivatives by plant cells. *Phytochemistry*, **16**, 2024–5.

Zacharius, R. M., Kalan, E. B. & Kimotoa, W. I. (1985). Biotransformation of potato stress metabolites, rishitin, lubimin, and 15-dihydrolubimin by potato and soyabean cell cultures. *Plant Cell Rep.*, **4**, 1–3.