

Biotransformation of potato stress metabolites rishitin, lubimin, and 15-dihydro lubimin by potato and soybean cell cultures

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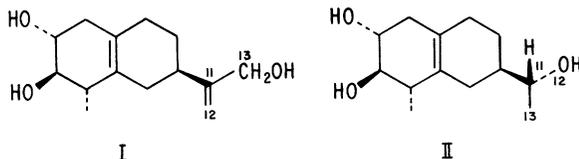
ABSTRACT

Potato callus and cell suspensions of potato and soybean were exogenously supplied with potato phytoalexin rishitin, much of which was converted by both species to an unknown tentatively identified as glutinosone. Exogenous lubimin was unaffected by the potato cell culture, but was transformed to 15-dihydro lubimin by the soybean cell suspensions. The stability of the exogenous lubimin may be ascribed to a second block in the rishitin pathway of the potato cell culture.

ABBREVIATIONS: Murashige and Skoog (1962), MS; 1-naphthaleneacetic acid, NAA; 2,4-dichlorophenoxyacetic acid, 2,4-D; Tobacco Mosaic Virus, TMV; thin layer chromatography, TLC; gas chromatography, GC; gas chromatography/mass spectrometry, GC/MS; nuclear magnetic resonance, NMR.

INTRODUCTION

Rishitin and lubimin in addition to other stress metabolites are produced by potato tuber tissue on interaction with incompatible races of *Phytophthora infestans* or cell-free mycelial wall homogenates of the fungus (fungal elicitor). Horikawa et al. (1976) gave evidence that healthy potato tissue transformed rishitin to other compounds, which might explain its virtual absence therein and the accumulation of rishitin in necrotic tissue. On the other hand, Ishiguri et al. (1978) concluded that intact potato tissue had little activity in metabolizing rishitin, but this activity was induced by wounding. Two metabolites identified by Murai et al. (1977) from the interaction of potato slices and rishitin are 13-hydroxyrishitin (I) and 12-hydroxyrishitin (II). I, an oxidative product of rishitin, was also identified by Ward et al. (1977) following incubation of rishitin with potato cell suspensions. II was shown to be a derivative of rishitin hydrated in the isopropenyl group (II) (Murai et al. 1977).



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Although Ward et al. (1977) observed the decline of lubimin during incubation with potato cell cultures, no detectable amount of a metabolite derived from ^{14}C -labeled lubimin was evident.

Failure to induce sesquiterpenoids on a regular and predictable basis in potato cell cultures with biotic stress and failure to observe solavetivone in these cultures (Zacharius and Kalan 1984), regarded as a key precursor in the phytoalexin pathway (Kalan and Osman 1976; Masamune et al. 1978), led us to examine the response of potato suspension cells to exogenous solavetivone (Zacharius and Kalan 1984). In the present report, rishitin, lubimin, and 15-dihydro lubimin were incubated with the aforementioned potato cell suspension lines to examine their ability to metabolize these compounds. In view of our findings with non-solanaceous plant cell cultures acting on solavetivone (Zacharius and Kalan 1984), the activity of soybean cultures on the above three potato metabolites also was studied.

MATERIALS AND METHODS

Plant Cell Cultures

Calluses of *Solanum tuberosum* cv. Kennebec and cv. Merrimack were initiated from tuber tissue and maintained on solid MS media containing 6 mg/l NAA, 2 mg/l 2,4-D and 0.21 mg/l kinetin. Cell suspensions were derived from the calluses and maintained on LB5C media as described by Zacharius and Kalan (1984). Callus and suspension cell cultures of *Glycine max* (L) Merr. cv. Mandarin (culture line Sb-2) were initiated and maintained on LB5 media (Gamborg 1975) as reported earlier (Fett and Zacharius 1982); an Sb-1 cell line originating at the Prairie Regional Laboratory, Saskatoon, Sask., was maintained similarly.

Interaction, Extraction, and Analysis

Rishitin and lubimin were isolated and purified on silica gel columns and by silica gel TLC as described for solavetivone (Zacharius and Kalan 1984) after extraction from potato tubers infected with *P. infestans*. Purity was determined by TLC and GC. 15-Dihydro lubimin was isolated and characterized from an earlier incubation of tuber slices with isolubimin and also by chemical reduction (NaBH_4) of isolated lubimin (Kalan et al. 1976). Rishitin was injected at several points into Kennebec callus (0.53 mg/10 g) with a sterilized Hamilton syringe and incubated at

27°C up to 92 h. Rishitin and lubimin were supplied separately to the suspension cell cultures of potato and all three compounds separately to the soybean cultures in less than 1 ml of methanol, by dispersing in the cell media with a sonifier before cell addition at a level of 0.53 mg rishitin, 0.50 mg lubimin, and 0.05 mg dihydro lubimin per 10 ml of cell culture. Soy cells were incubated in air at 27°C with constant shaking at 150 oscillations/min. The potato cell culture was maintained in atmospheric air and in one containing 12 ppm ethylene in air (Alves et al. 1979; Zacharius and Kalan 1984). Following a zero time sample, 10 ml aliquots of cells and media were withdrawn at 24 h intervals, extracted, concentrated, and components of the extract separated by TLC on Silica gel G plates with cyclohexane:ethyl acetate (1:1) solvent. Quantitation of sesquiterpenoids was performed by the GC method of Heisler et al. (1978).

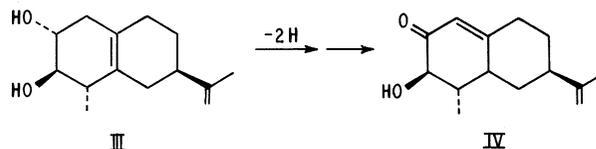
Quantities sufficient for identification were isolated by preparative TLC and purified by GC. Identification was made through comparison with authentic compounds of spectral data obtained on a Hewlett Packard GC/MS Model #5992b and a Jeol NMR Model FX60Q².

RESULTS AND DISCUSSION

No differences were observed in the response of potato cell suspensions incubated in air vs. those in 12 ppm ethylene in air. Rishitin, supplied exogenously to either potato callus or suspension cells of potato and soybean suspension cells, declined with the concomitant appearance of an unidentified "blue" spot on TLC (when reacted with SbCl₃) with an R_f value of 0.43, slightly greater than that of rishitin (R_f 0.39). This metabolite did not occur when potato slices were incubated with rishitin; moreover, rishitin was stable in cell-free culture media shaken over 72 h.

Metabolites I and II produced by healthy tuber slices from rishitin were not observed with our in vitro cells and calluses, whereas Ward et al. (1977) were able to oxidize exogenously supplied rishitin to I with potato cell suspensions. Brindle et al. (1983) were also unable to find compound I in their Kennebec cell suspensions which had been induced to produce rishitin, lubimin, and solavetivone. Conceivably, insufficient endogenous levels of rishitin are present on phytoalexin induction to activate cellular oxidation processes leading to compound I.

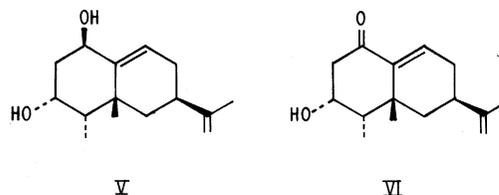
In this study, rishitin-injected Kennebec callus showed a 55% decline in rishitin after 20 h with the appearance of the "blue" compound; the latter persisted after 92 h. With Kennebec suspension cells, the "blue" unknown formed with a 25% decrease in rishitin concentration within 72 h. This metabolite presumably derived from rishitin has not been observed in potato slices undergoing fungal interaction with concurrent rishitin accumulation or in potato cell suspensions responding to fungal elicitor. When Sb-1 or Sb-2 soy cells were incubated with rishitin, there followed a decline in the latter and the occurrence of the "blue" compound within 48 h, persisting at 72 h. The "blue" compounds resulting from soybean and potato interactions with rishitin appear to be identical by TLC and GC (a peak with 15.5 min retention time vs. rishitin at 16 min). Although insufficient amounts of purified unknown could be obtained for a definitive characterization, its apparent molecular weight by mass spectrometer, its color reaction with SbCl₃ (blue), and



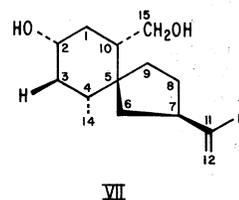
reaction with 2,4-dinitrophenyl hydrazine reagent (orange-red) suggests the compound to be glutinosone (IV) which could result from a loss of two hydrogens and a subsequent migration of the 5,10 ring double bond (III) into conjugation. This may be an alternative oxidation by the suspension culture to that in the isopropenyl group.

Burden et al. (1975) reported the induction of glutinosone in *Nicotiana glutinosa* following TMV infection. Murai et al. (1980) chemically transformed III into IV and have also isolated oxyglutinosone from late-blighted potatoes. An oxidation pathway common to soy, potato, and tobacco would be consistent with the non-specific behavior of a wide spectrum of cell species as earlier reported (Zacharius and Kalan 1984).

Capsidiol (V), a sesquiterpene structurally related to rishitin, is fungally induced by *Capsicum frutescens* L. and is oxidized to capsenone (VI) by four *Fusarium* species (Stoessel et al. 1973). On the other hand, pepper fruit and cell suspensions transformed capsidiol to its 13-hydroxylated derivative (Ward et al. 1977), as is rishitin by potato tuber tissue and by Ward's potato suspension cells.



Both soybean cell suspension lines produced 15-dihydro lubimin (V) from lubimin by a reduction of the aldehyde group. The diol was confirmed by TLC, GC, and mass spectral data in comparison with authentic VII. This biotransformation is the reverse of the reaction observed in potato tuber tissue where the diol is oxidized to lubimin and is formed by the reduction of isolubimin (Kalan et al. 1976). Diol VII is generally stable in soy cell suspensions, although during one incubation solavetivone was additionally observed at 21 h and also observed as a trace at 79 h accompanied by a lowered diol level. The solavetivone was isolated and its identity was confirmed by comparison of



its mass spectrum with that of authentic solavetivone. Most likely solavetivone did not arise by de novo synthesis from soy cells but rather through enzymatic conversion of the diol. Previously, Zacharius et al. (1981) had reported the production of glyceollin (soybean phytoalexin) on two occasions with the incubation of solavetivone (katahdinone) and

²Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

soy suspension cultures. In those cases the relatively high level of exogenous solavetivone may have acted as a stressing agent to induce glyceollin production.

Lubimin was not transformed on incubation with our potato suspension culture—most of the compound was recovered. This is in contrast to the finding (Ward et al. 1977) that lubimin was rapidly metabolized by potato cell suspensions although no metabolites were detected from the ¹⁴C-labeled lubimin in that study.

In this and our related report (1984), the findings that microorganism-free cell cultures are capable of modifying phytoalexin compounds provides further evidence that these modifications are not mediated by fungi or bacteria as once considered (Ward and Stoessl 1977). The evidence indicates that biochemical competence exhibited by cell cultures of the same species/cultivar may differ at times. Notably in this study, lubimin was converted to 15-dihydro lubimin by a non-solanaceous cell culture whereas it appeared to be unaffected by the potato cell culture. The latter indicates another potential block (Zacharius and Kalan 1984) in the proposed rishitin pathway (Kalan and Osman 1976; Murai et al. 1982) of this potato cell culture.

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