Biosynthesis from Solavetivone of the Phytoalexin Rishitin in Potato. Implicit Role of Solavetivone as an Activator

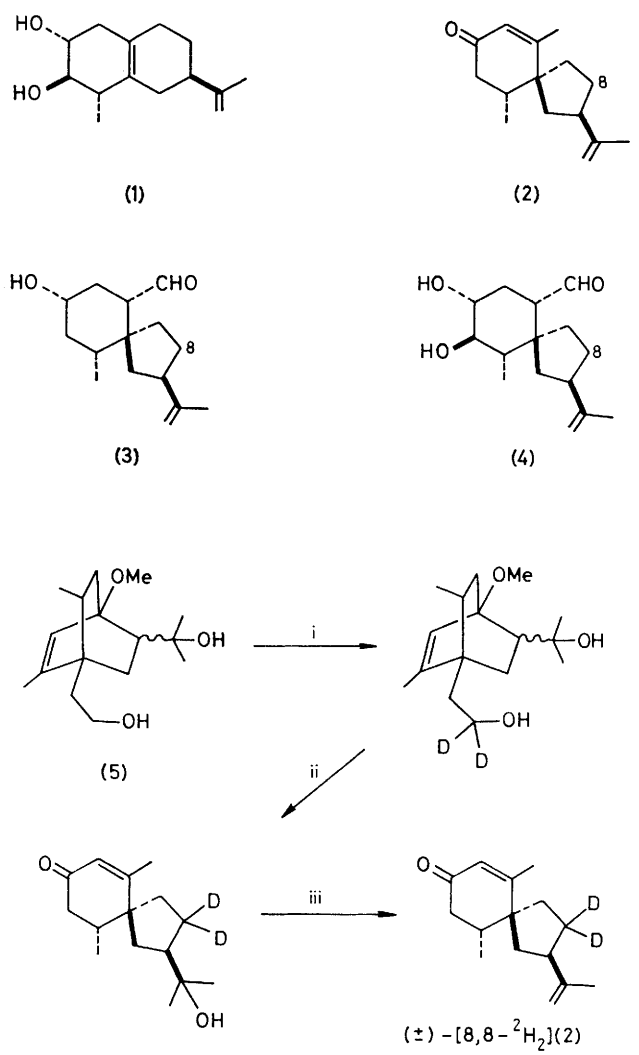
Akio Murai, Shingo Sato, Atsushi Osada, Nobukatsu Katsui, and Tadashi Masamune*

Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan

The biosynthetic production of rishitin from solavetivone *via* lubimin and oxylubimin has been established on the basis of experiments with (±)-[8,8-$^2H_2$]solavetivone.

Since the isolation of rishitin (**1**),[1] many sesquiterpenoid phytoalexins (stress compounds) have been isolated from infected potatoes, and attempts to elucidate the biosynthetic pathways leading to (**1**) have been made by several groups.[2–4] Stoessl *et al.*[3b] proposed that (**1**) and three major spirovetivane phytoalexins, solavetivone[5] (**2**), lubimin[6] (**3**), and oxylubimin[6] (**4**), would be separately biosynthesized from farnesyl pyrophosphate. Kalan *et al.*[4b] and we[2] reported almost the same biogenetic pathway, in which (**2**) would be transformed into (**1**) *via* (**3**) and (**4**) successively *in vivo*. While this hypothesis is based on our experiments[7] with ^{14}C-labelled (**4**), indicating that (**4**) is an intermediate on the main pathway to (**1**), the requisite studies with labelled (**2**) have not been performed yet. In this communication we present evidence confirming our proposed biogenesis on the basis of the experiments with (±)-[8,8-$^2H_2$]solavetivone.

The starting material, (±)-[8,8-$^2H_2$](**2**), was prepared from bicyclo-octenediol[8] (**5**) as shown in Scheme 1 and showed spectra which differed markedly from those of natural (**2**) only in the following: *m/e* 220 (M^+, 57%) and 218 (*ca.* 0%) ([8,8-$^2H_2$]-content, *ca.* 100%); ν_{max} 2200 and 2100 cm^{-1}. Thin slices of aged potato (Rishiri, *Solanum tuberosum* × *S. demissum*) were incubated with (±)-[8,8-$^2H_2$](**2**) and related compounds, and extracted with methanol–chloroform (1 : 1). The chloroform extracts were fractionated sequentially by column and thin-layer chromatography, giving the deuteriated products shown in Table 1. These stress compounds were not detected on a thin-layer chromatogram of the corresponding chloroform extracts of the aged potato slices treated only with water–methanol (1 : 1) in the same manner. In view of the fact that substitution of the hydrogen atoms at C-8 by deuterium atoms affects the optical rotations of the respective compounds to some extent, the results summarized in Table 1 indicate that (i), (−)-[8,8-$^2H_2$](**2**) and (+)-[8,8-$^2H_2$]-(**3**) are incorporated efficiently into potato slices, (ii), natural (−)-[8,8-$^2H_2$](**2**) is metabolized into (+)-[8,8-$^2H_2$](**4**) *via* (+)-[8,8-$^2H_2$](**3**), while synthetic (+)-[8,8-$^2H_2$](**2**) does not undergo such enzymatic reactions, (iii) the [8,8-$^2H_2$]-content of the stress metabolites decreases slightly (but definitely) (2—7%) on incubation of (±)-[8,8-$^2H_2$](**2**) (run 1), but remains unchanged on that of (+)-[8,8-$^2H_2$](**3**) (run 3). In summary, the present result, coupled with our previous one,[7] establishes the main biosynthetic pathway leading to (**1**), the sequence (−)-(**2**) → (+)-(**3**) → (+)-(**4**) → (−)-(**1**), and also suggests that natural (−)-(**2**) might be an activator of the



Scheme 1. i, Jones oxidation, then CH_2N_2, then $LiAlD_4$, 99% yield, ii, $MeSO_2Cl$, —78 °C, then $(CO_2H)_2$, 33% aq. acetone, 85 °C, 4 h, 84% yield, iii, py-Al_2O_3, 220 °C, 8 min, 64% yield.

Table 1. Transformation of (±)-[8,8-$^2H_2$](**2**) and related compounds in potato tubers.[a]

Run	Precursors	[8,8-$^2H_2$]-Products[b]	Yield[c]	$[\alpha]_D$[d]	[$^2H_2$]-content[e]
1	(±)-[8,8-$^2H_2$](**2**)	(+)-(**2**)	74%[f]	+82 (−119)	*ca.* 100%
	$[\alpha]_D$ ± 0,	(+)-(**3**)	29%[h]	+31 (+36)	97·8%
	(*ca.* 100%)[g]	(+)-(**4**)[i]	7%[h]	+17 (+22)	97·7%
		(−)-(**1**)	10%[h]	−20 (−35)	93·5%
2	(+)-[8,8-$^2H_2$](**2**)	(+)-(**2**)	71%	+71 (+119)	99·0%
	$[\alpha]_D$ + 82,				
	(*ca.* 100%)[g]				
3	(+)-[8,8-$^2H_2$](**3**)	(+)-(**3**)	9%	+42 (+36)	97·7%
	$[\alpha]_D$ + 31,	(+)-(**4**)[i]	11%	+12 (+22)	97·8%
	(97·8%)[g]	(−)-(**1**)	10%	−30 (−35)	97·2%

[a] All reactions were carried out by incubation of the precursors in water–methanol (1 : 1) to thin slices of aged potato tubers at 23—24 °C for 6 h. [b] The known phytoalexins other than the products described in Table 1 could not be detected on a thin-layer chromatogram. [c] Isolated yields. [d] Measured in ethanol. The figures in parentheses denote $[\alpha]_D$ of the corresponding unlabelled samples. [e] Determined by comparison of intensities of peaks corresponding to (M^+) and ($M^+ - 2$) in the respective mass spectrum. [f] Based on the (+)-enantiomer. [g] [8,8-$^2H_2$]-content. [h] Based on the (−)-enantiomer. [i] Isolated as its diacetate.

enzyme system for formation of these stress metabolites from acetic acid.

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