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BIOSYNTHESIS OF THE SESQUITERPENOID PHYTOALEXIN RISHITIN FROM ACETATE VIA OXYLUBIMIN IN POTATO

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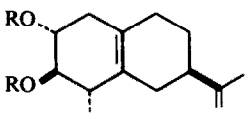
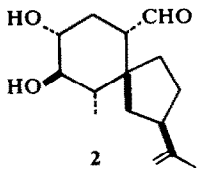
Key Word Index—*Solanum tuberosum* × *S. demissum*; Solanaceae; potato; sesquiterpenoid phytoatexins; biosynthesis; rishitin; oxylubimin.

INTRODUCTION

Since the isolation of rishitin (**1**) [1–3], many sesquiterpenoid phytoalexins and related compounds have been isolated from infected potatoes [3, 4]. Recently, attempts have been made to relate these compounds biogenetically. Stoessl *et al.* [4] proposed a pathway in which rishitin (**1**) and oxylubimin (**2**) [5, 6] were synthesized from farnesyl pyrophosphate via *trans*- and *cis*-eudesmanes, respectively. On the other hand, Kalan and Osman [7] proposed that **1** is synthesized through solavetivone, isolubimin, lubimin and **2**. However, there is no experimental evidence so far, which supports these hypothetical pathways. We report herein experimental evidence, indicating that oxylubimin (**2**) is converted to rishitin (**1**) and that the transformation from the spirovetivanes is a major pathway in the biosynthesis of the noreudesmane (**1**).



1 R = H
1a R = $(NO_2)_2C_6H_4CO$



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RESULTS AND DISCUSSION

The specific radioactivity of crystalline rishitin *bis* (3,5-dinitrobenzoate) (**1a**) is shown in Table 1. The radioactive **1** was obtained by addition of authentic cold **1** to the rishitin fraction extracted from tuber disks, which were infected by an incompatible race of *Phytophthora infestans* and treated with ^{14}C-labeled **2**. The specific activity of **1a** remained unchanged on repeated recrystallization. This result demonstrated that radioactivity of **2** is incorporated into **1**, indicating that **2** is a precursor of **1** in the infected potato tuber tissue.

Table 1. Effect of recrystallization on the specific radioactivity of *bis*(3,5-dinitrobenzoate) of rishitin-^{14}C extracted from the potato tissue treated with oxylubimin-^{14}C

Recrystallization	Radioactivity (10^{-3} dpm)	Wt of *bis*(3,5-dinitrobenzoate) (mg)	Specific activity (10^{-3} dpm/mg)
1st	420	17.6	23.8
2nd	299	12.7	23.5
3rd	197	8.4	23.5

If **2** is an intermediate on a main biosynthetic pathway to **1**, addition of cold **2** along with acetate-2-^{14}C should result in a significant decrease in incorporation of radioactivity into **1**. In both treatments 1 and 2 of experiment 2 (Table 2), acetate-2-^{14}C was first added to the infected potato disks and then, in treatment 2, unlabeled **2** was added, while in treatment 1, none of **2** was added. Preliminary experiments showed that addition of unlabeled **2** did not reduce the rate of uptake of labeled acetate by the tuber disks, and also that addition of 0.25, 0.5 and 1 mg of labeled **2** to the acetate (15 μg) reduced incor-

Table 2. Incorporation of radioactivity of ^{14}C labeled precursors into rishitin in potato tuber tissue

Treatment	Precursors: Acetate	Precursors: Oxylubimin (2)	Rishitin: Yield (μg)	Rishitin: Radioactivity (10^{-3} dpm)	Rishitin: Specific activity (10^{-3} dpm/μg)
1	22×10^6 dpm (15 μg)	—	59*	1322 (6%)†	22.4
2	22×10^6 dpm (15 μg)	Cold oxylubimin (1 mg)	189*	177 (0.8%)†	0.94
3	Cold acetate (15 μg)	113×10^4 dpm (1 mg)	189*	138 (12.2%)†	0.73 [1.55]‡

* Data from the experiment 3, the other data from experiment 2.
† % incorporation into rishitin.
‡ Dilution value.

poration of the radioactivity from the acetate into **1** by 20, 67 and 87%, respectively. Addition of unlabeled **2** (1 mg) resulted in a 7-fold decrease in the relevant incorporation of radioactivity of acetate-2-^{14}C into **1**. In treatment 3 of experiment 2 (Table 2), ^{14}C-labeled **2** was administered along with cold acetate to give 189 μg of **1** (measured by GLC analysis). Calculation from the radioactivity of produced **1** revealed that 122 μg of accumulated **1** resulted from 2 applied. These results established that oxylubimin (**2**) with a spirovetivane skeleton is an intermediate on the main biosynthetic pathway to rishitin (**1**) with a noreudesmane skeleton. In view of the currently accepted pathway in which spirovetivanes are produced from eudesmanes [8], the aforementioned result is noteworthy. The efficient incorporation and low dilution value (1.55) also exclude incorporation resulting from fragmentation of **2** followed by synthesis of **1** from some of the fragments.

EXPERIMENTAL

Oxylubimine-^{14}C (2.8×10^6 dpm/mg) was prepared by treating potato tuber tissue with acetate-2-^{14}C according to the method of ref. [9] for prepn of rishitin-^{14}C. Oxylubimin-^{14}C was extracted with EtOAc from soln in the holes made in the tuber tissue of potato cv Rishiri (*Solanum tuberosum* × *S. demissum*). The holes have previously been inoculated with zoospore suspension and then filled with acetate-2-^{14}C. The extracts were separated by TLC over Si gel (Merck Si gel G plate, 0.5 mm thick) with C_6H_6–EtOAc (2:3), and then purified by recrystallization from isopropyl ether. After sterilizing the surface with 0.1% NaClO, tubers of Rishiri were cut into disks, 1 mm thick, 16 mm dia, washed with streaming H_2O and then incubated at 18° for 20 hr. Zoospore suspension of *P. infestans*, race 0 containing antibiotic ceporan (4 mg/ml) was inoculated onto both surfaces of the disks. Inoculated disks were incubated at 18°.

Experiment 1. 20 hr after inoculation, 76 disks were placed in 4 ml of sterile H_2O containing 1 mg oxylubimin-^{14}C, ceporan (4 mg/ml) and 10% aq. Me_2CO, and incubated at 18° for 24 hr. Rishtin was isolated from the incubated sample with MeOH followed by separation by TLC and was crystallized as the *bis*(3,5-dinitrobenzoate) according to the method of ref. [10]. After recrystallization, the radioactivity was determined in the usual way. The recrystallization and radioactivity counting were repeated.

Experiment 2. 25 hr after inoculation, 11 disks (*ca* 2.2 g) were placed in 1 ml of sterile H_2O containing, in treatment 1, acetate-2-^{14}C (10 μCi/15 μg), in treatment 3, 1 mg oxylubimin (1.13×10^6 dpm/mg) and cold NaOAc (15 μg), and then incubated at 18° for 12 hr. Each soln for the treatment contained 4 mg ceporan and 10% aq. OAc. Rishitin-^{14}C was extracted and crystallized as described above.

Experiment 3. The coordinated experiment with experiment 2 was carried out. The disks were treated with unlabeled OAc or unlabeled oxylubimin as in experiment 2, and the content of rishitin was determined by GLC using a modified method of ref. [11], after separating the rishitin fraction by the method described above. Gaschromatograph (JEOL 1100, Nihondenshi Co.) fitted with a 300 × 0.03 cm i-d., pyrex glass column packed with 3% silicone OV-225 on chromosorb W (80–100 mesh) was used. Operating conditions were: temp., column 190°, injector 220°, detector 215°: carrier gas N_2 45 ml/min: sample size 1 μl.

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