

ISOLUBIMIN: A POSSIBLE PRECURSOR OF LUBIMIN IN INFECTED POTATO SLICES

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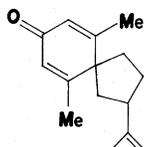
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Key Word Index—potato stress metabolites; biosynthesis; phytoalexins; isolubimin; lubimin; rishitin.

Abstract—Potato slices treated with spirovetiva-1(10),11-diene-2-one yield lubimin and rishitin within 24 hr. A vetispirane which has not been detected in fungally infected potatoes was also isolated. This compound, isolubimin, appears to be an intermediate in the conversion of the above spirovetivone to lubimin.

INTRODUCTION

Potato tuber slices, when infected with the fungus, *Phytophthora infestans*, produce a variety of sesquiterpenes which are either fungitoxic or fungistatic; these compounds may be considered to be phytoalexins[1]. Included in this class of compounds are the vetispirane sesquiterpenes lubimin (**1a**)[2], oxylubimin (**1b**) [2], spirovetiva-(10),11-diene-2-one (**2**), and spirovetiva 1(10)3,11-triene-2-one (**3**)[3].



(3)

We now wish to report the isolation of a new vetispirane sesquiterpene that appears to be an intermediate in the biosynthesis of lubimin (**1a**) and the norsesquiterpene, rishitin (**4**) which has been associated with the incompatible fungus-tuber interaction. This compound is produced in significant quantities in tuber slices when **2** is applied to slices which are then incubated.

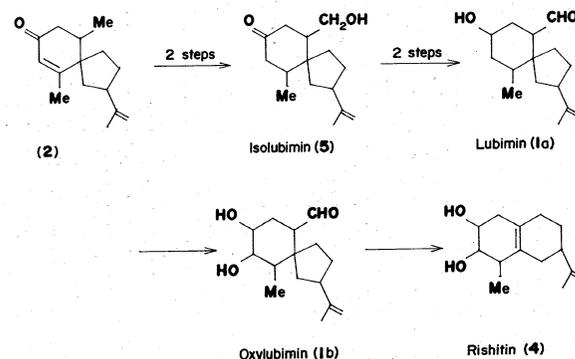
RESULTS AND DISCUSSION

The hitherto unknown compound resulting from the application of **2** to potato slices was characterized as follows. The compound (MW 236 by MS) had no significant UV absorption above 210 nm. The IR spectrum indicated the presence of carbonyl (unconj., 1710 cm^{-1}) and terminal unsaturation (885 cm^{-1} , 3080 cm^{-1}). The following structural features were evident from the NMR spectrum (90 MHz): an isopropenyl group (2H, 4.68 δ (s); 3H, 1.69 δ (broad s)); $\text{CH}_3\text{-C}$ (3H, 0.90 δ (d)); and $\text{CH}_2\text{-OH}$ (2H, 3.70 δ (m)). The mass spectrum was strongly suggestive of an alcohol (intense M-18) and otherwise contained fragment ions common to terpenes (M-43, the ion series 91, 93, 105, 107, 119, 121, 133, 135). In the light of this information and also the fact that

2 is an apparent precursor (see below), the unknown compound was tentatively assigned structure **5** (spirovetiva 14-hydroxy-11-ene-2-one) which we refer to as isolubimin.

The structure of this compound was confirmed by relating it to lubimin (**1a**) via NaBH_4 reduction of both **5** and **1a**. Reduction of **5** yielded two products, in a 7/3 ratio as determined by GLC, with identical mass spectra (MW 238; ions corresponding to diol: M-18, M-36); these must represent the diols isomeric about C-2. The mass spectrum of reduced lubimin was identical to reduced **5**. The Reduced lubimin co-chromatographed (GLC) with the minor product from the NaBH_4 reduction of **5**. One would expect lubimin to be isomerically pure with regard to the C-2 hydroxyl since it is of biological origin. The diacetates from the diols of **1a** and **5** were also identical by GLC and MS.

The presence of this compound as an intermediate in the conversion of **2** to lubimin and rishitin is based on the following observations) a) isolubimin is formed when **2** is added to the surface of potato slices which are then incubated at 20°; concomitant decrease in concentration of **2** is noted with increased concentration of isolubimin; (b) isolubimin is formed prior to lubimin and rishitin and decreases with increasing rishitin and lubimin concentration. The isolation of isolubimin and its possible role as an intermediate in lubimin and rishitin formation from **2** suggest the following pathway:



This pathway is tentative and further evidence is being sought regarding the various interconversions involved.

EXPERIMENTAL

Mass spectra were obtained on an LKB-9000 GC-MS. NMR spectra were obtained at 90 MHz. Analytical GLC was carried out on a 1.82 mm \times 3.2 mm glass column packed with 3% QF-1 on Gas Chrom Q.

For purposes of isolation, 0.3 mg **2** was applied to each of 400 potato slices (3–5 mm thick) from a 50% H₂O–MeOH solution. Control slices were treated with 50% H₂O–MeOH. The slices were incubated at 20° in petri dishes for 24 hr. The slices each weighing 15–20 g were then ground in a blender with CHCl₃–MeOH (1:1), 30 cm³ per slice, the solution filtered and the CHCl₃ layer removed and concentrated. The residue was taken up in MeOH and analyzed by TLC on

silica plates (Analtech, 250 μ m) using EtOAc–cyclohexane, 1:1. Compounds of interest were purified by using 5% increments of EtOAc in hexane followed by preparative TLC of the pertinent column fractions. Isolubimin and lubimin were reduced with NaBH₄ in EtOH at room temperature within 2 hr. The diacetates of the reduced products were prepared by the method of Schwartz[4].

REFERENCES

1. Kuc, J. (1972) *Microbial Toxins*, Vol. 8 (Kadis, S., ed.), pp. 211–247, Academic Press, New York.
2. Katsui, N., Matsunaga, A. and Masamune, T. (1974) *Tetrahedron Letters* 4483.
3. Coxon, David T., Price, Keith R., Howard, Barbara, Osman, S. F., Kalan, E. B. and Zacharius, R. M. (1974) *Tetrahedron Letters* 2921.
4. Schartz, D. P. (1976) *Anal. Biochem.* (in press).