CHEMISTRY LETTERS, pp. 1207-1208, 1978. Published by the Chemical Society of Japan

ISOLATION OF RISHITIN-M-1 FROM DISEASED POTATO TUBER TISSUE SLICES[1)]

Tadashi MASAMUNE,* Akio MURAI,* Yu-koh TAKAHASHI,* Fujio YAGIHASHI,*
Nobukatsu KATSUI,* Noriyuki DOKE,** and Kohei TOMIYAMA**

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

** Plant Pathology Laboratory, Faculty of Agriculture, Nagoya University, Nagoya 464

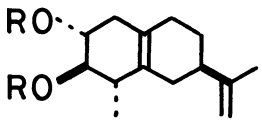
Isolation of rishitin-M-1, a metabolite of rishitin produced in healthy tissues of white potato, from diseased potato tuber slices and its convenient preparation from rishitin are described.

Rishitin[2)] (1), a representative phytoalexin produced in diseased potato tuber tissues, has recently been reported to be metabolized rapidly to rishitin-M-1[3)] (2), when it is incubated exogenously with healthy tissues of white potato (Solanum tuberosum × S. demissum). We report herein that the metabolite (2) was isolated from the tuber tissue slices infected with an incompatible race of Phytophthora infestans, though only in poor yield. In view of the currently accepted hypothesis for phytoalexin production that phytoalexins are biosynthesized in non-infected living cells surrounding hypersensitive areas, and exported into the infected tissues, consisting mainly of dead cells and intercellular spaces colonized by the pathogen, and hence accumulate there,[4)] the present result (isolation of 2 in low yield) suggests strongly that the rate of movement of rishitin-M-1 (2) from living cells into the adjacent infected areas is very slow as compared with those of rishitin (1), oxylubimin,[5)] and others, though the conditions and techniques for the isolation are not always appropriate. This presumption is also supported by the fact that the metabolite (2), when incubated with the healthy tissues, is not easily metabolized in the living cells.[6)]

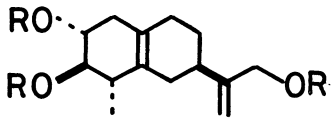
White potato tuber slices (338 kg, 1.5-2.0 mm in thickness), whose both sides were inoculated with the fungus, were extracted with methanol, and the methanol extracts were concentrated and again extracted with chloroform.[7)] A part (31.9 g) of the chloroform extracts (ca. 110 g) was treated with ethyl acetate (2.8 l) and water (10 × 0.9 l). The aqueous solution was concentrated to 1.25 l, saturated with sodium chloride, and again extracted with ethyl acetate (13 × 0.3 l). The acetate solution gave oily substance (0.74 g), which was separated by column chromatography (Merck, Kieselgel 60, 40 g) with ether, ether-methanol, and methanol to yield a rishitin-M-1-rich fraction (53 mg). The fraction was treated with acetic anhydride and pyridine and then purified by repeated chromatography over the silica gel (hexane-ethyl acetate) to give triacetate (9 mg), oil, $[\alpha]_D^{20}$ -16.4° (EtOH), in pure state: m/e 304 (M^+ - 60) and 244; $\nu_{max}^{CCl_4}$ 3100, 1753, 1653, 1245, 1228, 1063, 1033, 1028, and 910 cm^{-1}; δ ($CDCl_3$) 1.05 (3H, d, J = 6.5), 2.02, 2.06,

and 2.09 (each 3H, s), 4.58 (2H, s), 4.91 and 5.10 (each 1H, s), and 4.98 (2H, m). These properties were completely identical with those of an authentic sample of rishitin-M-1 triacetate[3] (2a).

In connection with this, we searched for a convenient preparative method of the compound (2) in order to study the metabolism of rishitin-related compounds. Rishitin diacetate[2] (1a) was oxidized by the van Tamelen procedure[8] to give a 11,12-epoxide mixture (3), oil, $[\alpha]_D^{23}$ +0.6° (EtOH); m/e 238 (M^+), 220, 202, and 144 (base); $\nu_{max}^{CHCl_3}$ 3400, 1237, 1070, 1050, 1016, 900, and 837 cm^{-1}; δ 1.13 (3H, d, J = 6), 1.27 and 1.29 (total 3H, each s, 13-H), 2.59 (2H, m, 12-H), 3.17 (1H, t, J = 9), and 3.36 (1H, br do d, J = 9 and 7). Treatment of the mixture (3) with lithium diethylamide (5 mol equiv) in a 17:1 mixture of ether and hexamethylphosphoramide (HMPA) (reflux, 48 h) afforded rishitin-M-1 (2), oil, $[\alpha]_D^{23}$ -31.8° (EtOH), in 35% yield from 1, which was identical with natural rishitin-M-1 in all respects.



1 R=H
1a R=Ac



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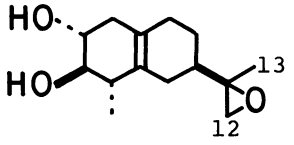
2a R=Ac



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(Received August 21, 1978)