hepatotoxic, 2–3 times more potent than ipomeamarone. A detailed report of the toxicity of **2** is forthcoming.

EXPERIMENTAL

NMR spectra were obtained at 100 MHz in $CDCl_3$. MS were run via direct probe inlet at 70 eV. IR spectra were obtained as neat samples. HPLC was carried on a Waters Associates instrument using two 30 cm C-18 μbondapak columns. Column chromatography was carried out using the so-called short column technique of Hunt and Ribgy [12].

Sweet potato roots, cut into 0.5 cm slices, were dipped into a 1% soln of $HgCl_2$ and placed in covered pans for 1 week. At this time the slices were homogenized with MeOH and $CHCl_3$. The $CHCl_3$ extract was dried ($MgSO_4$) and concd *in vacuo*. The residue was chromatographed on a 5 × 7 cm column of Merck Sigel G(Type 60) for TLC. For the isolation of myoporone, 1:5 EtOAc–hexane was used as the eluent: for isolation of **2** and **3**, 1:1 EtOAc–hexane was used.

Myporone was isolated using the C-18 column with 2:1 MeOH–H_2O at a flow rate of 1.7 ml/min as the eluent. Compounds **2** and **3** were isolated by HPLC using the C-18 column with 3:2 MeOH–H_2O at 1.7 ml/min as the eluent. MS of **2**: *m/e* (rel. int.) 252 [M^+] (3), 195 [cleavage at C-6, C-7] (4), 177 [195 $-H_2O$] (9), 166 [cleavage at C-5, C-6] (11), 149 [cleavage at C-4, C-5] (11), 123 [cleavage at 3-C, C-4] (20), 110 [McLafferty at C-2, C-3] (64) and 95 [cleavage at C-1, C-2] (100). MS of **3**: *m/e* (rel. int.) 252 [M^+] (91), 234 [$M^+ - H_2O$] (8), 195 [cleavage at C-6, C-7] (11), 177 [195 $-H_2O$] (8), 127 [cleavage at G3, C-4] (100), 100 (McLafferty at C-4, C-5] (32) and 97 [cleavage at C-1, C-2] (83).

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ISOLATION AND IDENTIFICATION OF PHYTUBERIN FROM *NICOTIANA TABACUM* PREVIOUSLY INFILTRATED WITH AN INCOMPATIBLE BACTERIUM*

RAYMOND HAMMERSCHMIDT and JOSEPH KUĆ
Department of Plant Pathology, University of Kentucky, Lexington, KY 40506, U.S.A.

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Key Word Index—*Nicotiana tabacum*; Solanaceae; incompatible bacteria; sesquiterpene; phytuberin; stress metabolite.

Twenty structurally-related sequiterpenes have been isolated and characterized from Solanaceous species [1, 2]. Three of these compounds, rishitin, lubimin and capsidiol, have been found in more than one species after stress. To date, only capsidiol has been shown to be a stress metabolite in *Nicotiana tabacum* L. [3, 4]. In this paper, we report the isolation of phytuberin, a sesquiterpene previously only reported in potato [5, 6], from tobacco leaf tissue infiltrated with the bacterium *Pseudomonas lachrymans*, a nonpathogen of tobacco.

The presence of phytuberin in extracts from infected tobacco tissue was first suggested by TLC analysis which revealed a compound that had the same R_f and produced the same characteristic color as authentic phytuberin with the vanillin–H_2SO_4 reagent [5]. The unknown was determined to be phytuberin by direct comparison of the unknown with an authentic sample [5], by TLC, GLC, chemical ionization MS and IR. Phytuberin was not detected in healthy leaf tissue.

EXPERIMENTAL

Leaves of 6- to 10-week-old *Nicotiana tabacum* L., cv KY 16, were infiltrated with H_2O or a suspension of the incompatible bacterium *Pseudomonas lachrymans* (10^8 cells/ml) prepared from 24-hour-old cultures. The leaves which were infiltrated

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with the bacterium exhibited a necrotic hypersensitive response within 96 hr of infiltration, whereas the control plants remained symptomless. The leaf tissues were harvested and extracted by a modification of Keen's facilitated diffusion technique [7]. Leaf tissue was submerged in 40% EtOH (*ca* 15 ml/g fr. wt) in 500 ml flasks and then vacuum infiltrated. The flasks were shaken for 5 hr at which time the solvent was replaced with fresh 40% EtOH for an additional 5 hr. After the extraction period, the extracts were combined and concd to one half the original vol. *in vacuo* at 40°. The resulting aq. fraction was partitioned 3× (v/v) against $CHCl_3$. The $CHCl_3$ extracts were combined, concd to dryness, and redissolved in MeOH (10 g fr. wt/ml).

Chromatographic techniques. TLC (Si gel) of the crude extract revealed a compound that reacted to chromogenic spray reagents and had R_f values identical to authentic phytuberin. Both compounds produced a heliotrope color with the vanillin–H_2SO_4 reagent [5] and an orange color with conc H_2SO_4. The orange substance fluoresced yellow-green under 366 nm UV light. Phytuberin and the unknown had R_f 0.70 (cyclohexane–EtOAc, 1:1), 0.34 (cyclohexane–EtOAc, 4:1), 0.59 (hexane–dioxane, 9:1), 0.69 ($CHCl_3$ MeOH, 95:5) and 0.12 C_6H_6–$CHCl_3$, 9:1). The unknown was purified by PLC (500 μm layers of Si gel) using the first and third solvents listed above.

The identity of the unknown was further checked by GLC on a 200 cm × 2 mm Pyrex glass column packed with 3% OV-225 at 180° with N_2 as the carrier gas at 40 ml/min. Phytuberin and the unknown were both found to have a retention time of 4.9 min. Addition of authentic phytuberin to the unknown did not change the elution pattern of the unknown although the area under the peak was greatly enlarged.

Spectral analyses. IR spectra of the unknown and authentic phytuberin were run as thin liquid films between AgCl plates. Major bands found for both: ν_{max} cm^{-1}: 2960, 2880, 1742, 1632, 1475, 1380, 1260, 1160 and 1045. Chemical ionization MS (CH_4 as reagent gas) major peaks found for both: *m/e* (rel. int.) 295 (M^+ + H, 6.6), 235 (M^+ − acetate, 100), 234 (M^+ − 60, 4.8), 233 (M^+ − 61, 7.4), 205 (M^+ − 89, 6.2), 193 (M^+ − 101, 12.0), 191 (M^+ − 103, 8.3) and 149 (M^+ − 144, 13.0).

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