REFERENCES

1. Sehgal, C. K., Taneja, S. C., Dhar, K. L. and Atal, C. K. (1982) *Phytochemistry* **21**, 363.
2. Takeda, Y., Nishimura, H. and Inouye, H. (1977) *Phytochemistry* **16**, 1401.
3. Choudhary, R. K., Affi-yazak, F. U. and Sticher, O. (1980) *Tetrahedron* **30**, 2317.
4. Bojthe, K., Horvath, A., Kocsis, Parkanyi, L. and Simon, K. (1982) *Tetrahedron Letters* **23**, 965.

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STRESS COMPOUNDS IN TOBACCO CALLUS INFILTRATED BY *PSEUDOMONAS SOLANACEARUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; callus; stress compounds; phytuberin; phytuberol; *Pseudomonas solanacearum*.

Abstract—Two sesquiterpenoids, phytuberin and phytuberol, have been identified in tobacco callus infiltrated by *Pseudomonas solanacearum*.

Seven sesquiterpenoidal stress compounds, solavetivone, 3-hydroxysolavetivone, solanascone, phytuberin, phytuberol, glutinosone and capsidiol, have been isolated from *Nicotiana* species [1]. Of these seven, phytuberin and phytuberol have been obtained in tobacco leaves treated with ethrel [2]. Phytuberin has also been isolated from tobacco leaves infiltrated with the bacterium *Pseudomonas lachrymans*, a nonpathogen of tobacco [3].

In this paper, we report the occurrence of two of these compounds in the tobacco callus challenged by *Pseudomonas solanacearum* U-7. Strain U-7 was highly pathogenic to tobacco plants. The methylene chloride extract from the callus contained phytuberin (**1**) and phytuberol (**2**). Both **1** and **2** were absent from healthy callus tissues.

EXPERIMENTAL

Callus tissues were induced from the pith of tobacco plants (*Nicotiana tabacum* cv Burley 21) by standard procedures on Linsmaier–Skoog agar medium [4], containing 3 mg indoleacetic acid, 3 mg naphthaleneacetic acid and 0.1 mg kinetin per l. Callus tissues were subcultured every 4 weeks on 20 ml medium for five or six times before use.

Pseudomonas solanacearum U-7 was grown on Kelman's TZC medium [5] for 2 days at 30°. Inoculums were prepared by suspending the bacteria in H_2O (10^7 cells/ml).

Well-grown callus tissues (7 g fr. wt/flask) were infiltrated with 1 ml/flask of the bacteria suspension. The infected callus exhibited a necrotic response within 2 days of infiltration. The callus tissues, incubated for 2 days after infiltration of *P. solanacearum*, were harvested and freeze-dried. The dried material (3.90 g dry wt) was extracted with CH_2Cl_2. The extract was evaporated to dryness to give 57.2 mg of yellow oil. The oil was analysed with capillary GC (OV-101, 0.2 mm × 50 m, 100–230°, 2°/min and PEG 20 M 0.2 mm × 25 m, 100–210°, 2°/min) and capillary GC/MS (OV-101 0.27 mm × 50 m, 100–230°, 2°/min). The presence of **1** and **2** was suggested by GC/MS and these compounds were identified with authentic samples by the MS and retention times (**1**: OV-101 45.0 min and PEG 20 M 39.0 min; **2**: OV-101 38.9 min and PEG 20 M 41.6 min). MS of **1**: *m/z* (rel. int.) 294 $[M]^+$ (8), 249 (10), 234 (14), 205 (100), 189 (61), 149 (41), 107 (46), 95 (38), 93 (39) 91 (37) and 67 (29). MS of **2**: *m/z* (rel. int.) 252 $[M]^+$ (12), 237 (6), 234 (3), 205 (41), 149 (36), 107 (37), 95 (36), 77 (35), 59 (51), 55 (37), 43 (100) and 41 (75). Up to 38.3 μg **1** and 3.83 μg **2** per g dry wt of callus tissues was recognized by capillary GC analysis.

REFERENCES

1. Uegaki, R., Fujimori, T., Kubo, S. and Katō, K. (1981) *Phytochemistry* **20**, 1567.
2. Uegaki, R., Fujimori, T., Kaneko, H., Kubo, S. and Katō, K. (1980) *Phytochemistry* **19**, 1543.
3. Hammerschmidt, R. and Kuć, J. (1979) *Phytochemistry* **18**, 874.
4. Linsmaier, E. M. and Skoog, F. (1965) *Physiol. Plant.* **18**, 100.
5. Kelman, A. (1954) *Phytopathology* **44**, 693.