tion times for **1** and **2** were 61.6 and 63.2 min on an OV-101 glass capillary column (50 m × 0.27 mm i.d.; 100–240°, 2°/min; carrier gas: He, 0.5 ml/min) and 56.8 and 58.8 min on a PEG-20M fused Si capillary column (50 m × 0.20 mm i.d.; 100–210°, 2°/min; carrier gas: He, 0.5 ml/min), respectively.

(−)-1,2-*Dehydro-α-cyperone* (**1**). Colourless oil; GC/MS 70 eV, *m/z* (rel. int.): 216.1532[M]$^+$ ($C_{15}H_{20}O$, 4%), 201(24), 188(7), 173(34), 159(28), 145(40), 105(80), 91(100), 79(50), 77(33), 67(51), 65(45) and 41(59); IR ν_{max}^{film} cm^{-1}: 1658, 1629, 1603, 886, 829; ^{1}H NMR(100 MHz, $CDCl_3$): δ 6.76(1H, *d*, *J* = 10 Hz, H-2), 6.24(1H, *d*, *J* = 10 Hz, H-1), 4.82(2H, *m*, $W_{1/2}$ = 3 Hz, H-12), 1.92(3H, *s*, H-14), 1.80(3H, *s*, H-13), 1.25(3H, *s*, H-15); UV λ_{max}^{MeOH} nm (log ε): 238(3.66), 264(sh, 3.53), 312(1.72); $[\alpha]_D$ − 172.4°(MeOH; *c* 2.9×10^{-3}); ORD: $[\phi]_{234}$ + 5268, $[\phi]_{265}$ 0, $[\phi]_{281}$ − 1580.

Preparation of (−)-1,2-*dehydro-α-cyperone*. According to the method described [1], (+)-*α*-cyperone (**3**) was reacted with DDQ to give (−)-1,2-dehydro-α-cyperone(**1a**) as colourless needles after purification by prep. GC. The IR, NMR, UV and MS data and the GC retention time were identical with those of natural **1**. $[\alpha]_D$ − 144° (EtOH; *c* 3.40×10^{-2}); ORD: $[\phi]_{233}$ + 14 294, $[\phi]_{264}$ 0, $[\phi]_{280}$ − 9212.

Acknowledgements—We thank Dr. A. Murai, Hokkaido University, Sapporo, for generously supplying (+)-α-cyperone and Dr. Y. Harigaya, Kitazato University, Tokyo, for ORD determination.

REFERENCES

1. Piers, E. and Cheng, K. F. (1967) *Can. J. Chem.* **45**, 1591.
2. Coxon, D. T., Price, K. R., Howard, B., Osman, S. F., Kalan, E. B. and Zacharius, R. M. (1974) *Tetrahedron Letters* **34**, 2921.
3. Fujimori, T., Kasuga, R., Kaneko, H. and Noguchi, M. (1977) *Phytochemistry* **16**, 394.

Phytochemistry, Vol. 21, No. 12, pp. 2987–2988, 1982.
Printed in Great Britain.

0031-9422/82/122987-02$03.00/0

ACCUMULATION OF SIX SESQUITERPENOID PHYTOALEXINS IN TOBACCO LEAVES INFILTRATED WITH *PSEUDOMONAS LACHRYMANS**

M. E. M. GUEDES, J. KUĆ, R. HAMMERSCHMIDT and R. BOSTOCK

Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, U.S.A.

(*Received* 12 *March* 1982)

Key Word Index—*Nicotiana tabacum*; Solanaceae; sesquiterpene stress metabolites; phytoalexins.

Abstract—Tobacco leaves inoculated with *Pseudomonas lachrymans* accumulated capsidiol, rishitin, lubimin, solavetivone, phytuberin and phytuberol.

Six bicyclic sesquiterpenes are established as stress metabolites of *Nicotiana* spp.: Capsidiol in *N. tabacum* and *N. clevelandii* foliage infected with TNV [1] or *P. tabacina* [2] or callus tissue infected by *Phytophthora parasitica* var. *nicotianae* [3]; glutinosone in *N. glutinosa* foliage infected with TMV [4]; rishitin in *N. tabacum* callus tissue infected with *P. parasitica* var. *nicotianae* [5]; phytuberin and phytuberol in *N. tabacum* foliage infected with *Pseudomonas lachrymans* [6] or treated with Ethrel [7]; and solavetivone in *N. tabacum* foliage infected by TNV [8]. In this paper we report the accumulation of the sesquiterpene stress metabolites (SSM) capsidiol, rishitin, lubimin, solavetivone, phytuberin and phytuberol (identified by TLC (Table 1), GC and GC/MS) as a function of time after inoculation of tobacco leaves with *Pseudomonas lachrymans*. Unlike studies with pepper fruit [9] and potato tuber [10] in which maximum accumulation of SSM was reported 48–96 hr after infection, maximum accumulation in response to infection with *P. lachrymans* in tobacco occurred 12–24 hr after infection (Fig. 1). Although a 19 hr lag was observed for rishitin accumulation in potato tuber [11], the data in this paper and in ref. [9] indicate that SSM accumulation can be detected as early as 6 hr after infection. The rapid maximum in SSM accumulation in tobacco foliage may explain the inability to detect appreciable quantities of SSM in foliage of potato and tomato 24 hr or longer after infection with incompatible races of *Phytophthora infestans* or *Cladosporium fulvum*, respectively.

*Journal paper 82-10-21 of the Kentucky Agricultural Experiment Station, Lexington, KY 40546, U.S.A.

Table 1. R_f values of sesquiterpenoid stress metabolites in different solvent systems after TLC*

Compound‡	R_f values†				
	A	B	C	D	E
Capsidiol	0.12 G§	0.18	0.11	0.00	0.68
Rishitin	0.22 DB	0.26	0.20	0.00	0.73
Lubimin	0.28 B	0.48	0.23	0.00	0.80
Phytuberol	0.34 H	0.48	0.30	0.03	0.80
Phytuberin	0.68 H	0.80	0.58	0.31	0.93

*Compounds were separated on glass plates precoated with Si gel G (250 μm) in tanks which were equilibrated with solvent. Origin to front *ca* 15 cm.

†A, Cyclohexane–ethyl acetate (1:1); B, chloroform–methanol (19:1); C, *n*-hexane–acetone (3:1); D, *n*-hexane–dioxane (9:1); E, methanol–diethyl ether (1:19).

‡Standards and isolated compounds.

§G, Gray; DB, dark blue; B, blue; H, heliotrope after spraying with vanillin–sulfuric acid reagent and heating for 2–3 min.

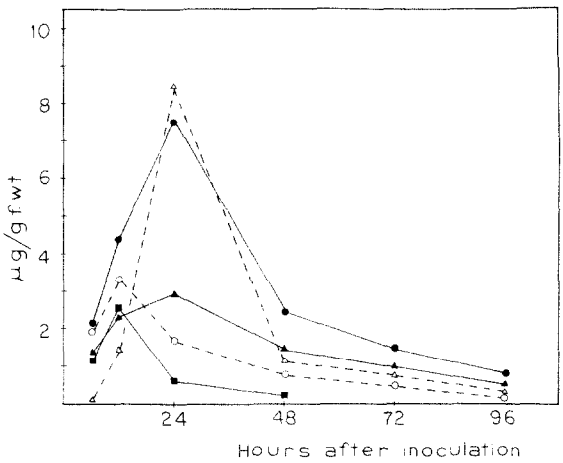

Fig. 1. The accumulation of sesquiterpene stress metabolites in tobacco foliage (cv K16) at varying time intervals after inoculation with *Pseudomonas lachrymans*. (●) Capsidiol, (▲) rishitin, (■) lubimin, (△) phytuberin, (○) phytuberol.

EXPERIMENTAL

Leaves on 6–10 week old *Nicotiana tabacum* L. cv Ky 16 plants grown in a greenhouse at 23–28° were infiltrated with H_2O or a suspension of *P. lachrymans* as previously described [6]. At varying intervals after infiltration, leaves were excised from the plants, and infiltrated areas, including a 3–5 mm margin of uninfiltrated tissue, were cut out and extracted as described previously [6]. The onset of necrosis was evident in leaves infiltrated with the bacterium 6–12 hr after infiltration and reached a maximum in 24 hr. The necrosis was largely confined to the infiltrated areas.

Chromatographic and spectral analyses. TLC of the crude MeOH extracts was performed on Analtech glass plates precoated with Si gel G (250 μm) using five solvent systems, and R_f values of SSM were compared to those of known standards (Table 1). Compounds were detected on thin-layer plates by spraying with vanillin–H_2SO_4 (2.8 g vanillin in 100 ml MeOH containing 0.5 ml conc H_2SO_4), $CHCl_3$ satd with $SbCl_3$ or conc H_2SO_4. Plates were observed before and after heating at 110° for 2–3 min. The identity of the SSM was further verified by GC as previously described using methyl arachidate as int. standard [12], and with the exception of solavetivone, by GC/MS using a Finnigan Automated GC/MS System, Model 3300-6100. The GC retention time for methyl arachidate was 14.7 min and the retention times relative to methyl arachidate for phytuberol, phytuberin, solavetivone, rishitin and lubimin were 0.32, 0.36, 0.58, 0.78 and 1.34, respectively. Quantitation of the terpenoids was by GC as previously described [12]. Capsidiol, rishitin and phytuberin reached a maximum of accumulation 24 hr after infiltration, whereas lubimin and phytuberol reached a maximum 12 hr after infiltration (Fig. 1). A trace of a compound believed to be solavetivone was detected by GC 12 and 24 hr after infiltration.

Infected tobacco foliage accumulated capsidiol, also reported in infected pepper fruit and foliage, as well as rishitin, lubimin, phytuberin, phytuberol and perhaps solavetivone, which were previously reported in infected potato tubers. The accumulation was associated with lesion development and it was not detected in plants infiltrated with H_2O. The SSM were not detected in apparently healthy tissue 5 mm or more from the edge of lesions. The rapid decrease in accumulation of SSM 24 hr after infection is evidence that SSM accumulation is determined by the rate of synthesis and degradation as is true for all SSM, isoflavonoid, furanoterpenoid and phenolic phytoalexins reported.

Acknowledgements—This work was supported in part by a grant from the Kentucky Tobacco and Health Research Institute and Grant 58-7B30-0-185 of the USDA/SEA.

REFERENCES

1. Bailey, J. A., Burden, R. S. and Vincent, G. G. (1975) *Phytochemistry* **14**, 597.
2. Cruickshank, I. A. M., Perrin, D. R. and Nadryk, M. (1976) *Annu. Rep. Plant Ind. CSIRO Aust.* 1975 65.
3. Helgeson, J. P., Budde, A. D. and Haberlach, G. T. (1978). *Plant Physiology* **61** (suppl.), 53.
4. Bauden, R. S., Bailey, J. A. and Vincent, G. G. (1975) *Phytochemistry* **14**, 221.
5. Budde, A. D. and Helgeson, J. P. (1980) *Phytopathology* **71**, 206.
6. Hammerschmidt, R. and Kuć, J. (1979) *Phytochemistry* **18**, 874.
7. Uegaki, R., Fujimori, T., Kaneko, H., Kubo, S. and Kato, K. (1980) *Phytochemistry* **19**, 1543.
8. Fujimori, T., Uegaki, R., Takogi, Y., Kubo, S. and Kato, K. (1979) *Phytochemistry* **18**, 2632.
9. Stoessl, A., Unwin, C. H. and Ward, E. B. (1973) *Phytopathology* **63**, 1225.
10. Kuć, J. (1975) *Recent Advances in Phytochemistry* **9**, 139.
11. Sato, N., Kitazawa, K. and Tomiyama, K. (1971) *Physiol. Plant Pathol.* **1**, 289.
12. Henfling, J. W. D. M. and Kuć, J. (1979) *Phytopathology* **69**, 609.