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PHYTUBERIN AND PHYTUBEROL, SESQUITERPENES FROM *NICOTIANA TABACUM* TREATED WITH ETHREL

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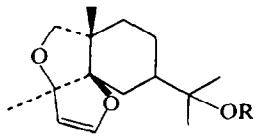
INTRODUCTION

The terpenoid stress compounds capsidiol [1], glutinosone [2], solavetivone [3, 4] and phytuberin [5] have previously been found in leaves of *Nicotiana* species. These compounds have been produced in response to tobacco mosaic virus (TMV), tobacco necrosis virus (TNV) or the bacterium *Pseudomonas lachrymans*. In the case of potato, which belongs to the same family (Solanaceae), the terpenoid stress compounds, such as rishitin, lubimin, solavetivone, phytuberin and phytuberol, have been found in the tubers inoculated with micro-organisms [6].

The alteration of plant metabolism by the ethylene-releasing compound ethrel, 2-chloroethylphosphonic acid, has been extensively studied. Potato slices treated with ethrel and subsequently inoculated with *Helminthosporium victoriae*, *H. carbonum* or an incompatible race of *Phytophthora infestans* accumulated considerably more phytuberin and phytuberol than did slices treated with water followed by inoculation. But ethrel alone did not elicit the accumulation of the terpenoids [7].

It has been reported that treatment of tobacco leaves with ethrel induced the formation of necrotic spots [8], changes in protein constitution and peroxidase isoenzyme patterns [8], and the accumulation of scopoletin [9], but the accumulation of terpenoid stress compounds has not yet been studied.

In this paper, we report the accumulation of terpenoid stress compounds phytuberin (**1**) and phytuberol (**2**) in tobacco leaves treated with ethrel. This is the first report of phytuberol (**2**) occurring in tobacco as a stress compound.



1 R = Ac
2 R = H

RESULTS AND DISCUSSION

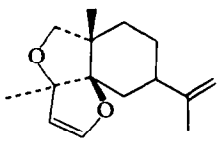
On leaves pricked with needles moistened with 0.05 M ethrel, small light brown spots developed around the punctures within 1 hour. During the following days, these spots slightly enlarged and turned dark brown, whereas the centres became whitish. These spots resembled the virus-induced local lesions.

The CH_2Cl_2 extract from the treated leaves contained phytuberin (**1**) and phytuberol (**2**) which were isolated by preparative GLC. From a fraction eluted with hexane–Et_2O (4:1) on a silicic acid column, phytuberin (**1**) was isolated as an oil (2.0 mg) and identified by comparison with an authentic sample (GLC, MS, IR, ^{1}H NMR and CD spectra). Phytuberol (**2**) was isolated as an oil (1.0 mg) from a fraction eluted with Et_2O. The GLC retention time and spectral data were in accord with those of an authentic sample.

In control leaves, which were pricked with water instead of ethrel, **1** and **2** were not detected even by the very sensitive analytical method of mass fragmentography.

The occurrence of **1** and **2** is the first evidence for terpenoid stress compound accumulation in response to ethrel treatment of tobacco leaves. On the other hand, in the case of potato tubers, ethrel did not increase the accumulation of these compounds in non-inoculated tubers [7].

Up to 0.18 mg phytuberin (**1**) and 0.04 mg phytuberol (**2**)/g dry wt of treated leaves were found in an analysis by mass fragmentography. Phytuberin (**1**) was unstable to heat. During the isolation by preparative GLC, **1** was mostly converted to dehydroxyphytuberol (**3**). It is considered that elimination of



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acetic acid was caused by the high temperature at the TCD (300°). In fact, **1** did not undergo such thermal degradation when the temperature of the TCD was lowered to 270°. Dehydroxyphytuberol (**3**) was identified by the measurement of MS, IR and ^{1}H NMR spectra. The molecular ion at *m/e* 234 indicated the loss of acetic acid from **1** or water from **2** to give an isopropenyl group. The ^{1}H NMR methyl signal at δ1.74 and the methylene signal at 4.73, and the IR absorption at 893 cm^{-1} supported this conclusion. The other ^{1}H NMR signals and IR absorptions were very

similar to the corresponding spectral data of phytuberin (**1**) and phytuberol (**2**). These facts suggested dehydroxyphytuberol had structure **3**.

EXPERIMENTAL

GC-MS was on a 3 mm × 1 m column of 5% OV 101 on Chromosorb W(AW-DMCS), IR spectra were measured as a film, ^{1}H NMR spectra were measured at 100 MHz in $CDCl_3$ and mass fragmentograms were determined using a 3 mm × 1 m column of 2% OV-1 on Chromosorb W(AW-DMCS) at 165°.

Nicotiana tobacum cv Samsun NN was grown in a greenhouse at 24°. Tobacco plants (2 months old) with 6 to 8 fully expanded leaves were treated with 0.05 M ethrel. The leaves were pricked by stainless steel needles moistened with the treatment soln into, and occasionally through, the leaf. The leaves pricked with H_2O served as control. Six days later, the leaves were harvested. The harvested leaves (434 g fr. wt) were frozen at −20° for 2 days and then freeze-dried. The dried materials (48 g) were extracted with CH_2Cl_2 (2 l. × 3). The solvent was removed from the crude extract which was then introduced onto a column of Si gel (80 g) and eluted with hexane–Et_2O.

Phytuberin (**1**) was contained in a hexane–Et_2O 4:1 fraction and isolated by prep. GLC as an oil (2.0 mg). The retention time on GLC was 13.0 min (5% OV-101 on Chromosorb W(AW), 3 mm × 1 m, 100–240°, 5°/min, 60 ml He/min). MS *m/e* (rel. int.): 294 (M^+, 8), 249 (10), 234 (14), 205 (100), 189 (61), 149 (41), 107 (46), 95 (38), 93 (39), 91 (37), 67 (29); IR ν_{max}^{Film} cm^{-1}: 1735, 1620, 1375, 1260, 1155, 1088, 1040, 737; ^{1}H NMR: δ1.02 (3H, *s*), 1.42 (3H, *s*), 1.45 (3H, *s*), 1.57 (3H, *s*), 1.97 (3H, *s*), 3.32 (1H, *d*, *J* = 9 Hz), 3.47 (1H, *d*, *J* = 9 Hz), 4.65 (1H, *d*, *J* = 3 Hz), 6.42 (1H, *d*, *J* = 3 Hz); CD: $[\theta]_{206}$ −67 700 (EtOH, *c* 0.03).

Phytuberol (**2**) was contained in an Et_2O fraction and isolated by prep. GLC as an oil (1.0 mg). The retention time on GLC was 10.3 min (5% OV-101 on Chromosorb W(AW), 3 mm × 1 m, 100–240°, 5°/min, 60 ml He/min). MS *m/e* (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), 41 (75); IR ν_{max}^{Film} cm^{-1}: 3455, 1623, 1470, 1379, 1152, 1085, 1038, 915, 859, 810, 798, 731; ^{1}H NMR: δ1.00 (3H, *s*), 1.21 (6H, *s*), 1.55 (3H, *s*), 3.26 (1H, *d*, *J* = 9 Hz), 3.40 (1H, *d*, *J* = 9 Hz), 4.65 (1H, *d*, *J* = 3 Hz), 6.42 (1H, *d*, *J* = 3 Hz).

Dehydroxyphytuberol (**3**) was obtained as an oil (0.5 mg) by preparative GLC of the fraction containing phytuberin (**1**) at high TCD temp. (300°). MS *m/e* (rel. int.): 234(M^+, 15), 219 (5), 205 (51), 189 (79), 107 (74), 93 (83), 91 (70), 81 (91), 79 (74), 67 (74), 55 (100), 53 (84); IR ν_{max}^{Film} cm^{-1}: 1620, 1469, 1440, 1370, 1155, 1087, 1037, 893, 730; ^{1}H NMR: δ1.03 (3H, *s*), 1.58 (3H, *s*), 1.74 (3H, *s*), 3.33 (1H, *d*, *J* = 9 Hz), 3.49 (1H, *d*, *J* = 9 Hz), 4.64 (1H, *d*, *J* = 3 Hz), 4.73 (2H, *s*), 6.42 (1H, *d*, *J* = 3 Hz).

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REFERENCES

1. Bailey, J. A., Burden, R. S. and Vincent, G. G. (1975) *Phytochemistry* **14,** 597.
2. Burden, R. S., Bailey, J. A. and Vincent, G. G. (1975) *Phytochemistry* **14,** 221.
3. Ito, T., Takahashi, T., Oshima, Y., Takusari, H. and Odagiri, S. (1979) *Agric. Biol. Chem.* **43,** 413.
4. Fujimori, T., Uegaki, R., Takagi, Y., Kubo, S. and Kato, K. (1979) *Phytochemistry* **18,** 2032.
5. Hammerschmidt, R. and Kuć, J. (1979) *Phytochemistry* **18,** 874.
6. Stoessl, A., Stothers, J. B. and Ward, E. W. B. (1976) *Phytochemistry* **15,** 855.
7. Henfling, J. W. D. M., Lisker, N. and Kuć, J. (1978) *Physiol. Biochem.* **68,** 857.
8. Loon, L. C. van (1977) *Virology* **80,** 417.
9. Reuveni, M. and Cohen, Y. (1978) *Physiol. Plant Pathol.* **12,** 179.