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SESQUITERPENOID STRESS COMPOUNDS FROM *NICOTIANA* SPECIES

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Key Word Index—*Nicotiana*; Solanaceae; stress compounds; solavetivone; 3-hydroxysolavetivone; solanascone; phytuberin; phytuberol; glutinosone; tobacco mosaic virus; tobacco rattle virus.

Abstract—Solavetivone, 3-hydroxysolavetivone, solanascone, phytuberin and phytuberol were identified as stress compounds in leaves of *Nicotiana tabacum* cv Samsun NN. *N. sylvestris*, which is the maternal progenitor of *N. tabacum*, produced all the above compounds except 3-hydroxysolavetivone. In the F_1 hybrid of *N. tabacum* and *N. glutinosa*, all the stress compounds produced by *N. tabacum* and *N. glutinosa*, respectively, were accumulated.

INTRODUCTION

The Solanaceae has been known to produce many structurally-related sesquiterpenoid stress compounds [1, 2]. To date, five sesquiterpenoid stress compounds, capsidiol [3], solavetivone [4, 5], phytuberin [6, 7], phytuberol [7, 8] and glutinosone [9], have been isolated from plants of the genus *Nicotiana*. In response to virus, only solavetivone accumulates in leaves of *Nicotiana tabacum* [4, 5], glutinosone in *N. glutinosa* [9] and capsidiol in *N. clevelandii* and *N. tabacum* [3].

In this paper, we report the occurrence of stress compounds in leaves of *N. tabacum* cv Samsun NN inoculated with tobacco mosaic virus (TMV), *N. sylvestris* inoculated with TMV and tobacco rattle virus (TRV) and the F_1 hybrid of *N. tabacum* and *N. glutinosa* inoculated with TMV.

RESULTS AND DISCUSSION

N. tabacum *cv Samsun NN inoculated with TMV*

The steam-distillate of the CH_2Cl_2 extract from TMV-infected leaves contained solavetivone (**1**), 3-hydroxysolavetivone (3-hydroxyspirovetiva-1(10),11-dien-2-one) (**2**), solanascone (**3**), phytuberin (**4**) and phytuberol (**5**), which was revealed by capillary GC/MS analysis. These compounds were isolated as oils by preparative GLC. The mass, ^1H NMR and IR spectra of 3-hydroxysolavetivone (**2**) and solanascone (**3**) were in accord with those of the reported data [10, 11]. The mass and IR spectra and retention times of the remaining compounds agreed with those of authentic samples. TMV stimulated maximum accumulations of 1.11 mg of solavetivone (**1**) and 0.68 mg 3-hydroxysolavetivone (**2**)/g dry wt of inoculated leaves during 7 days after inoculation. Concentrations of other compounds were as follows: solanascone (**3**) 0.06 mg, phytuberin (**4**) 0.14 mg and phytuberol (**5**) 0.21 mg/g dry wt of inoculated leaves.

N. sylvestris *inoculated with TMV and TRV*

Both steam distillates of the CH_2Cl_2 extracts from leaves inoculated with TMV and TRV contained solavetivone (**1**), solanascone (**3**), phytuberin (**4**) and phytuberol (**5**). The presence of these compounds was suggested by capillary GC/MS analysis and these compounds were identified by the mass spectra and retention times. **1** was present as the major metabolite in these inoculated leaves. Up to 1.52 (1.86*) mg **1**/g dry wt of TMV inoculated leaves and 1.02 (1.25*) mg/g dry wt of TRV inoculated leaves were found. The accumulations of **3**–**5** were very low compared with that of **1**. Thus, *N. sylvestris*, which is the maternal progenitor of *N. tabacum*, produced all the stress compounds of *N. tabacum* except **2**.

N. tabacum × N. glutinosa *inoculated with TMV*

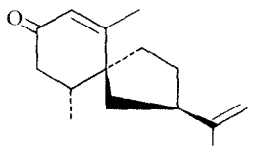
The presence of **1**–**6** in the steam distillate of the CH_2Cl_2 extract from TMV-infected leaves were recognized by capillary GC/MS analysis. **1**, **2** and **6** were the major stress compounds in inoculated leaves. Up to 0.13 mg **1**, 0.05 mg **2** and 0.58 mg **6**/g dry wt of inoculated leaves were found. The amounts of **3**–**5** were very low compared with the above three compounds. **6** in leaves of *N. glutinosa* inoculated with TMV was found at the level of 0.47 mg/g dry wt of inoculated leaves. Thus, the F_1 hybrid of *N. tabacum* and *N. glutinosa* appeared to accumulate all the stress compounds produced by *N. tabacum* and *N. glutinosa* [9], respectively.

Uninoculated plants

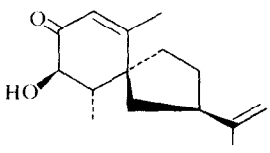
Sesquiterpenoidal stress compounds were not detected by capillary GC/MS analysis in healthy leaves of *N. tabacum* cv Samsun NN, *N. sylvestris* and the F_1 hybrid of *N. tabacum* and *N. glutinosa*.

Of these six compounds, 3-hydroxysolavetivone (**2**) and solanascone (**3**) have not been reported before as stress compounds in tobacco plants. 3-Hydroxysolavetivone (**2**) has been isolated as the aglycone of glucoside from flue-cured Virginia tobacco [10] and solanascone (**3**) has been obtained from air-cured Burley tobacco [11] and *N. sylvestris* [12] in very small amount.

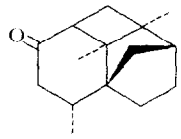
* 1/g dry wt of leaves excluding the midrib.



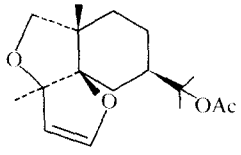
1 Solavetivone



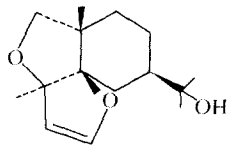
2 3-Hydroxysolavetivone



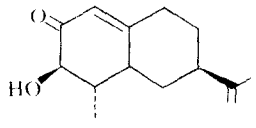
3 Solanascone



4 Phytuberin



5 Phytuberol



6 Glutinosone

EXPERIMENTAL

N. tabacum cv Samsun NN was grown in a greenhouse at 24°. Fully expanded leaves of 2-month-old tobacco were inoculated with TMV-OM (0.5 μg/ml in 0.1 M phosphate buffer, pH 7.0). Carborundum was used as an abrasive. When brown lesions had been produced (7 days), the leaves were harvested. The harvested leaves (320 g fr. wt) which had been inoculated with TMV were freeze-dried. The dried materials (37 g) were extracted with CH_2Cl_2 (2 l. × 3) and evapd to dryness. The crude extract (2.20 g) was steam-distilled and extracted with Et_2O by simultaneous distillation and extraction [13]. The ether extract was evapd to dryness to give 218 mg of the volatile.

Similarly, the leaves of *N. sylvestris* (2-month-old), inoculated with a tomato strain of TMV (10 μg/ml in 0.1 M phosphate buffer, pH 7.0) or with the buffer extract (1:20) of TRV-infected tobacco leaves, were harvested. The harvested leaves were 550 g (TMV-infected) and 160 g (TRV-infected). The dried materials (TMV-infected 50 g and TRV-infected 27 g), crude extract (3.65 and 1.58 g) and volatile (158 and 89 mg) were obtained.

Tobacco leaves of *N. tabacum* × *N. glutinosa* (2-month-old) were inoculated with TMV (0.3 μg/ml in 0.1 M phosphate buffer, pH 7.0). Six days later, the leaves were harvested. The harvested leaves (210 g fr. wt) were treated in the same manner described above. The dried materials (26 g), crude extract (1.60 g) and volatile (91 mg) were obtained.

The leaves of 2-month-old *N. glutinosa* were inoculated with TMV (0.5 μg/ml in 0.5 M phosphate buffer). The fresh leaves (600 g), dried materials (70.5 g), CH_2Cl_2 extract (4.4 g) and steam distillate (151 mg) were obtained.

1–**6** were isolated as an oil from the volatile of virus-inoculated tobacco leaves by prep. GLC (5% FFAP 3 mm × 1 m, 100–240°, 5°/min). The following spectral data of **2** and **3** were obtained. 3-Hydroxysolavetivone (**2**): MS *m/z* (rel. int.): 234 (M^+, 5), 205 (9), 176 (100), 161 (32), 133 (49), 109 (70), 108 (70), 91 (53), 68 (88); ^1H NMR (100 MHz, $CDCl_3$): δ 1.21 (3 H, *d*, *J* = 7 Hz), 1.75 (3 H, *s*), 2.02 (3 H, *s*), 3.80 (1 H, *d*, *J* = 12.5 Hz), 4.75 (2 H, *s*), 5.81 (1 H, *m*); IR ν_{max}^{film} cm^{-1}: 3480, 3080, 1680, 1645, 890. Solanascone (**3**): MS *m/z* (rel. int.): 218 (M^+, 28), 203 (10), 190 (62), 121 (49), 120 (100), 105 (37), 91 (31), 41 (45); ^1H NMR (100 MHz, $CDCl_3$): δ 1.03 (3 H, *s*), 1.11 (3 H, *d*, *J* = 7 Hz), 1.31 (3 H, *s*), 2.43 (2 H, *m*); IR ν_{max}^{film} cm^{-1}: 1710, 1460, 1426, 1382, 1300.

Characteristic retention times on capillary GLC (PEG-20M Ultra Bond 0.27 mm × 60 m, 100–215°, 2°/min) were **1** 43.6 min, **2** 51.6 min, **3** 33.2 min, **4** 40.0 min, **5** 43.1 min and **6** 47.7 min. The amounts of **1** and **6** were estimated by the mass fragmentography procedure and those of the other compounds were evaluated by integration of the peaks in the capillary gas chromatograms.

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