6-Substituted Indanoyl Isoleucine Conjugate Induces Tobacco Plant Responses in Secondary Metabolites

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To characterize the role of the phytotoxin mimic 6-substituted indanoyl isoleucine conjugate **1** in plant secondary metabolism, tobacco (*Nicotiana tabacum* L. K326) was treated with compound **1**. The volatile compounds of tobacco leaves were analyzed by GC-MS. In contrast to the control, three compounds, farnesene (**2**), santalol (**3**) and tetradecanal (**4**), were induced by treatment with 1 mM of compound **1**. Concurrently other volatile compounds were also regulated.

Key words: 6-Substituted Indanoyl Isoleucine Conjugate, Coronatine, Secondary Metabolism

Introduction

The phytotoxin coronatine (**5**) is produced by several pathogenic strains of *Pseudomonas syringae* and was first isolated from a fermentation broth of *P. syringae* var. *atropurpurea* (Ichihara *et al.*, 1977). This compound and its analogs act as strong inducers of defense responses in many plants (tomato, corn, potato), and have attracted considerable interest. It mimics many biological activities associated with jasmonic acid, a well-known signaling molecule (Ichihara and Toshima, 1999). Compound **5** was applied to higher plants to elicit a wide spectrum of responses, especially diffuse chlorosis (Ichihara *et al.*, 1999), tendril coiling in *Bryonia dioica* (Weiler *et al.*, 1994), emission of ethylene (Greulich *et al.*, 1995), and the biosynthesis of terpenoids and other volatiles (Boland *et al.*, 1995). Recently the structurally simpler 6-substituted indanoyl isoleucine conjugate **1** was synthesized in high yield by a rapid procedure (Schueler *et al.*, 2001). The conjugate with isoleucine triggers volatile biosynthesis in the Lima bean and coiling of the touchsensitive tendrils of *Bryonia dioica.*

Treating freshly harvested leaves of tobacco (*Nicotiana tabacum* L. K326) with compound **1** three volatile compounds in contrast to the control were induced. These were identified as farnesene (**2**), santalol (**3**) and tetradecanal (**4**) by GC-MS analysis. At the same time some other volatile compounds were also up- or down-regulated.

Results and Discussion

Several microbial- or insect-derived high- and/or low-molecular-weight metabolites have been shown to induce the biosynthesis of volatiles in plants. Their elicitor activity is often based on up-regulation of the octadecanoid pathway (Piel *et al.*, 1997). Coronatine (**5**) apparently makes a detour to avoid the activation of the lipid-based signaling pathway by interacting directly with the receptors or binding proteins of the genuine signals such as 12-oxo-phytodienoic acid and/or jasmonic acid (Weiler *et al.*, 1994; Blechert *et al.*, 1999). To evaluate the activities of an elicitor, the analysis of a mixture of induced volatiles is of particular interest since the spectrum of the produced compounds comprises many metabolites from very different pathways. Since a complex network of signals individually regulates the different pathways, differences in the elicitor activity of test compounds will show up in the qualitative and/or quantitative composition of the volatile compounds. Previously it was reported that compound **1** triggered the volatile biosynthesis in the Lima bean (Schueler *et al.*, 2001), and this is confirmed in the present work. In contrast to the control, compound **1** induces farnesene (**2**), santalol (**3**) and tetradecanal (**4**) at significant levels (Fig. 1; Table I).

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Table I. Comparison of aroma of *Nicotiana tabacum*.

No.	Component	Sample 1 (Treated)	Sample 2 (Control)
1	Toluene	0.069	0.070
2	Hexanal	0.062	0.072
3	2-Methyl-tetrahydrofuran-3-one	0.026	0.036
4	4-Methyl-3-valerenal	0.065	0.056
5	2-Furfural	0.487	0.713
6	2-Furanmethanol	0.172	0.308
7	4-Cyclopenten-1,3-dione	0.028	0.129
8	2-Acetyl-furan	0.044	0.032
9	γ-Butyrolactone	0.037	0.064
10	6-Methyl-2-heptanone	0.281	0.241
11	5-Methyl-furfural + benzaldehyde	0.093	0.125
12	Maltol hydrate	0.031	0.009
13	6-Methyl-5-hepten-2-one	0.129	0.091
14	Benzyl alcohol	1.381	0.693
15	Phenylacetaldehyde	0.980	3.299
16	2-Acetyl-pyrrol	0.492	0.669
17	2-Methyl-1,4-benzenediol	0.196	0.327
18	Guaiacol	0.006	0.006
19	Linalool	0.203	0.150
20	Nonanoical	0.229	0.202
21	Phenylethyl alcohol	0.320	0.300
22	Iso-phorone	0.031	0.023
23	Iso-phorone oxide	0.038	0.069
24	2,6-Nonadienal	0.705	0.168
25	2-Nonene-al	0.257	0.146
26	2,6,6-Trimethyl-1,4-cyclohexanedione	0.032	0.055
27	α-Terpilenol	0.041	0.034
28	Safranal	0.081	0.084
29	β-Cyclocitral	0.332	0.200
30	Ethyl-citronellol	0.057	0.047
31	Indole	0.179	0.110
32	4-Ethenyl-2-methoxyphenol	1.510	2.215
33	Solanone	8.242	7.289
34	β-Damascenone	3.179	3.134
35	Caryophyllene oxide	1.192	1.452
36	β-Damascone	0.467	0.416
37	Geranyl acetone	0.668	0.713
38	Norsolanadione + nicotyrine	6.422	5.116
39	β-Ionone	0.381	0.345
40	1,3,7,7-Tetramethyl-2-oxabicyclo[4.4.0]deca-5-en-9-one	0.076	0.075
41	5,6-Expo-β-ionone	0.167	0.179
42	2,6-Ditertiarybutyl-4-methyl-phenol	0.198	0.165
43	Farnesene	2.607	– –
44	2,3-Dihydro-7-hydroxy-3-methyl-1*H*-inden-1-one	1.607	2.064
45	Dihydroactinidiolide	0.546	0.364
46	Megastigmatrienone	2.819	3.336
47	Pseudoionone	0.116	0.159
48	3-Hydroxy-β-damascone	0.314	0.394
49	4-Hydroxy-β-damascone	0.361	0.366
50	Tetradecanal	0.752	– –
51	Santalol	2.550	– –
52	4-Oxo-α-ionol	3.549	4.198
53	Malto-oxazine	4.010	3.984
54	Nookatone	1.070	0.102
55	Pentadecanal	14.625	2.233
56	Anthracene	0.707	0.503
57	Vetivone	1.047	0.617
58	Neophytadiene	390.155	570.913

Table I. (cont.)

No.	Component	Sample 1 (Treated)	Sample 2 (Control)
59	Hexahydro-farnesyl acetone	1.933	1.943
60	Dihydro-farnesol	1.022	0.923
61	3-Hydroxy-vetivone	4.849	4.392
62	Methyl linolenate	3.839	0.751
63	Farnesyl acetone 1	3.814	3.900
64	Methyl palmitate	0.566	0.505
65	Palmitic acid	28.871	15.765
66	Ethyl palmitic acid	0.289	0.453
67	Farnesyl acetone 2	0.237	0.236

Mean values with the same letter in a row are not significantly different ($P < 0.05$). Results are mean from two separate trials.

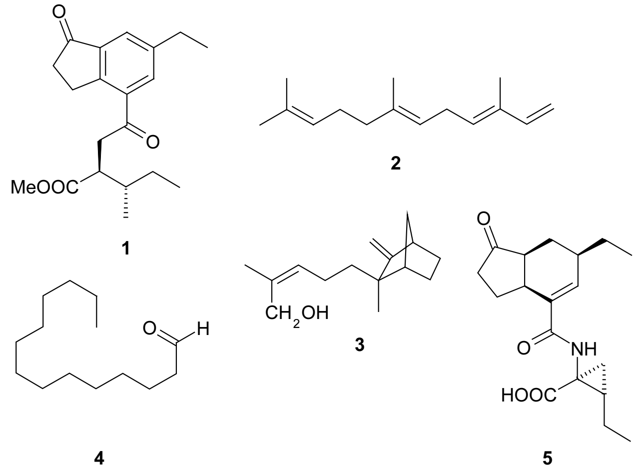

Fig. 1. Structures of the 6-substituted indanoyl isoleucine conjugate **1**, farnesene (**2**), santalol (**3**), tetradecanal (**4**), and coronatine (**5**).

The investigation of the volatile patterns induced in tobacco (*Nicotiana tabacum* L. K326) by the coronatine analog presented in this work illustrates the effectiveness to control metabolic activities in some plants, and also helps to evaluate the extent of selective manipulations of plant defense responses. It is also important that the responses could be used as a process to enhance the aroma of tobacco and improve the quality of tobacco. Furthermore, it could even be used for the processing of other commercially available products such as black tea.

Experimental

Induction experiments

Freshly harvested leaves of tobacco *Nicotiana tabacum* L. K326 (3000 g) were randomly divided into two groups (each 1500 g). Leaves of the two groups were sprayed with 300 ml of compound **1** prepared by dissolving 100 mg compound **1** in 100 ml ethyl alcohol and the volume made up to 300 ml with water (final concentration: 1 mM, sample 1) and same amount of solvent (sample 2), respectively. Leaves with and without treatment with compound **1** were kept at room temperature for 18 h. Leaves without compound **1** treatment were used as control. After 18 h the samples were heated as usual.

Analysis of volatile components of tobacco leaves

Leaves obtained from both treated and control (samples 1 and 2) were analyzed for the aroma components by GC-MS. Tobacco aroma concentrates were prepared by extractive distillation of volatiles, using the Likens-Nickerson method (Nickerson and Likens, 1966). The aroma volatiles from tobacco leaves were extracted using a micro Likens- Nickerson unit. The unit consists of a reflux and an extraction unit. 20 g of samples were placed in the round bottom flask of the reflux unit and 500 ml of distilled water was added. In the extraction unit, 20 ml of dichloromethane was used to trap the volatiles. Both flask contents were boiled after they were allowed to reflux for another 60 min. Then, the samples were allowed to cool, the organic layer was separated, dried over sodium sulphate and concentrated by nitrogen sparging. These concentrated samples were directly analyzed by GC (HP6890)-MS (HP5972). GC-conditions: Fused-silica capillary (50 m × 0.25 mm) coated with DB 5 (0.25 μm); helium served as carrier gas; separation of the compounds was under programmed conditions (50 °C for 2 min, then at 3 °C min^{-1} to 230 °C, finally at 12 °C min^{-1} to 250 °C and held for 5 min). Individual compounds were identified by comparison with standards of a mass spectrum database (Wiley and NIST). Peaks were quantified according to the peak area of the internal standard.

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