Pergamon

PII: S0031-9422(96)00530-1

Phytochemistry, Vol. 43, No. 6, pp. 1219–1225, 1996

0031-9422/96 $15.00 + 0.00

SYNTHESIS OF SYRINGOLIDE 1 KETALS AND ESTERS FOR RECEPTOR STUDIES

TETSU TSURUSHIMA,* SHARON L. MIDLAND, CHUN-MIN ZENG, CHENG JI, JAMES J. SIMS and NOEL T. KEEN†

Department of Plant Pathology, University of California, Riverside, CA 92521, U.S.A.

(*Received* 10 *June* 1996)

Key Word Index—*Pseudomonas syringae* pv. *tomato*; synthesis; syringolide derivative; elicitor activity; radioiodination; *avr*D; hypersensitive response; soybean cotyledon.

Abstract—The syringolides, specified by avirulence gene D from *Pseudomonas syringae*, elicit a disease defense reaction only in soybean cultivars carrying the *Rpg4* disease resistance gene. In order to identify structural features important for elicitor activity and construct highly labelled elicitor-active probes, several syringolide esters and ketals were synthesized. Alteration of the C-3 hemiketal group of syringolide 1 abolished elicitor activity, but ester derivatives based on the C-4′ hydroxy group all retained activity. [^{125}I]4′-(2-iodo-3,4,5-trimethoxyphenylacetyl) syringolide 1 was prepared for receptor studies. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Genetic studies in plant–pathogen interactions have established a gene-for-gene relationsip in which disease resistance is often governed by a single dominant gene for disease resistance in the host plant and a single dominant gene for avirulence in the pathogen [1, 2]. The elicitor-receptor hypothesis proposes that pathogen biotypes carrying an avirulence gene produce a specific elicitor that is, in turn, only recognized by plants carrying the matching resistance gene [3–5]. Although there have been several specific elicitors described from plant pathogens, nothing is known about the putative plant receptors.

Avirulence gene D (*avrD*) cloned from *Pseudomonas syringae* pv. *tomato* [6, 7] causes Gram-negative bacteria expressing the gene to elicit the hypersensitive defense response (HR) on soybean plants carrying the disease resistance gene, *Rpg4*. Bacteria expressing *avrD* produce syringolides, extracellularly secreted specific elicitors which initiate the HR only in soybean plants harbouring *Rpg4* [8]. It has therefore been speculated that syringolide receptors exist in soybean plants harbouring *Rpg4*.

Since receptors generally are present in low abundance in cells, receptor studies require that the ligand be suitably labelled [9]. For example, receptors for insulin and steroid hormones were first demonstrated using radioiodinated ligands in animal cells [10, 11]. Agonists and antagonists of altered biological activity are also useful to demonstrate the specificity of ligand binding [9, 11].

Considerable progress has been made in the detection of plant binding sites for pathogen produced elicitors. For example, Renelt *et al.* [12] showed ligand specific binding of a glycoprotein elicitor to parsley protoplasts and microsomal membranes and Cheong and Hahn [13] identified plasma membrane binding sites in soybean cells for a hepta-β-glucoside elicitor. Frey *et al.* [14] obtained data indicating that a 70 kDa membrane protein is involved in binding of the hepta-β-glucoside elicitor.

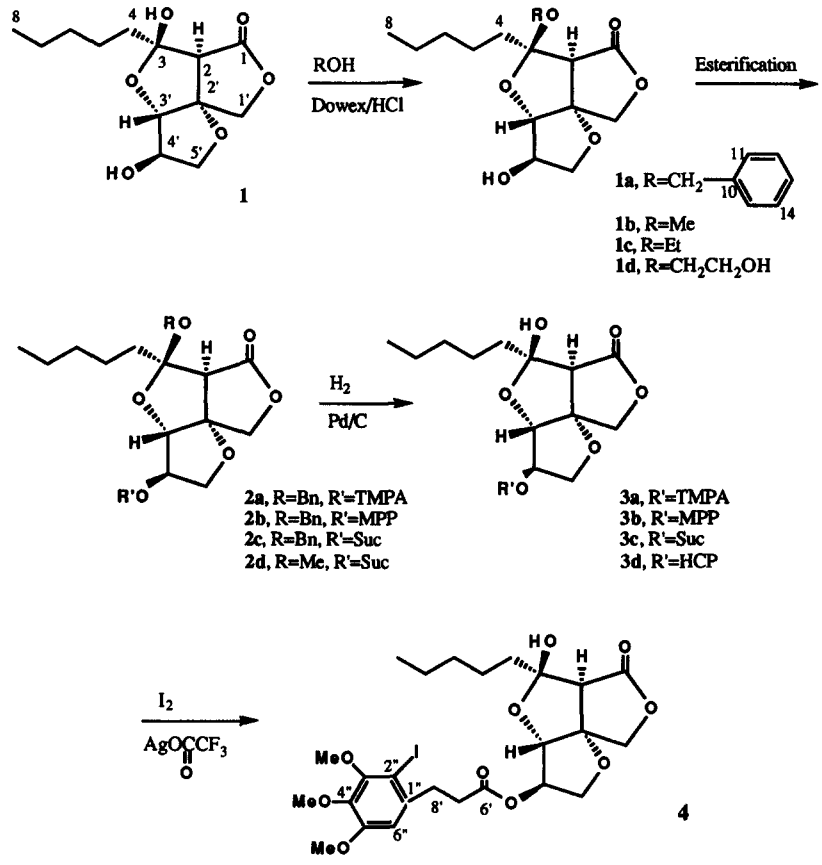
Unfortunately, little progress has occurred in assessing plant binding sites for specific elicitors that are active only in plants carrying a complementary disease resistance gene. As a prerequisite to these studies with the syringolide elicitors, we have synthesized several derivatives of syringolide 1 (**1**, Scheme 1) and determined their elicitor activities. We also report the preparation of a mono-iodinated syringolide derivative (**4**) which retains high elicitor activity and should accordingly be useful for the preparation of radioactive adducts for binding studies with soybean cell fractions.

RESULTS AND DISCUSSION

Syringolide derivatives based on the C-3 hemiketal and the C-4′ alcohol were examined. Ketals **1a** (benzyl), **1b** (methyl), **1c** (ethyl), and **1d** (2-hydroxyethyl) were readily prepared by treatment of **1** with the corresponding alcohols in the presence of dry Dowex/HCl. All ketal derivatives thus constructed by reaction at the C-3 position of **1** were devoid of detectable elicitor activity in soybean leaves (Table 1). It was

*Present address: Faculty of Business, Hannan University, Matsubara City, Osaka 580, Japan.

†Author to whom correspondence should be addressed.



Scheme 1. Synthesis of syringolide 1 derivatives. Bn = benzyl; TMPA = 3,4,5-trimethoxyphenyl acetyl; MPP = 3-(4-methoxyphenyl) propionyl; Suc = succinyl; HCP = 3-(4-hydroxycyclohexyl) propionyl.

particularly interesting that **1d**, in which the end of the (C-3)-O chain was hydroxyl, also had no elicitor activity. The results indicate that the C-3 hemiketal group is essential for syringolide elicitor activity and lead to the prediction that ketal derivatives should not function as competitors of syringolide binding to a putative receptor in soybean cells.

Syringolide 1 required protection of its hemiketal as

Table 1. Elicitor activity as measured by hypersensitive responses in leaves of various soybean cultivars infiltrated with syringolide 1 or its derivatives

Cpd	R at 3-*O*	R at 4'-*O*	Acme (*rpg4*)	Flambeau (*Rpg4*)	Merit (*rpg4*)	Harosoy (*Rpg4*)
1	H	H	−	+	−	+
1a	Bn	H	−	−	−	−
1b	Me	H	−	−	−	−
1c	Et	H	−	−	−	−
1d	$HO(CH_2)_2$	H	−	−	−	−
2a	Bn	TMPA	−	−	−	−
2b	Bn	MPP	−	−	−	−
2c	Bn	Succinyl	−	−	−	−
2d	Me	Succinyl	−	−	−	−
3a	H	TMPA	−	+	−	+
3b	H	MPP	−	+	−	+
3c	H	Succinyl	−	+	−	+
3d	H	HCP	−	+	−	+
4	H	Iodo-TMPA	−	+	−	+

Bn = benzyl; TMPA = 3,4,5-trimethoxyphenyl acetyl; MPP = 3-(4-methoxyphenyl) propionyl; HCP = 3-(4-hydroxycyclohexyl) propionyl. Water solutions of compounds (3.68×10^{-5} M) were infiltrated into primary soybean leaves. Reactions were observed after 24 hr: +, positive reaction; −, no reaction.

a ketal before esterification (Scheme 1). Direct esterification of C-4′ alcohol was not possible since common esterification methods using *N,N′*-dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI), 4-(dimethylamino)pyridine (DMAP) or pyridine destroyed the syringolide ring system. It was also destroyed by esterification attempts using acids (HCl, H_2SO_4) or cobalt(II) chloride. However, syringolide ketals were stable to esterification using base catalysis. Thus syringolide 1 ketal esters **2a**, **2b**, **2c** and **2d** were synthesized, in which the C-4′ alcohol of **1a** or **1b** was esterified with 3,4,5-trimethoxyphenylacetic acid, (3-(4-methoxyphenyl) propionic acid, or succinic anhydride (Scheme 1). The (C-3)-*O*-methyl ketal of **2d** was very resistant to deprotection by acid hydrolysis. However, the (C-3)-*O*-benzyl protecting groups in **2a**, **2b** and **2c** could be easily removed by hydrogenation to produce the desired syringolide hemiketal ester analogues **3a**, **3b**, and **3c**. Hemiketal esters of syringolide 1 elicited the HR only in soybean cultivars harbouring *Rpg4*, whereas the ketal esters were inactive (Table 1). Hemiketal esters **3a**, **3b** and **3c** were also active elicitors of glyceollin in soybean cotyledons, while ketals **1a** and **1b** were not active (Table 2). We conclude that the (C-4′)-hydroxyl group is not necessary for elicitor activity.

The hemiketal ester derivatives, **3a** and **3b**, were elicitor active and contained highly activated aromatic rings which could be iodinated using iodine and silver trifluoroacetate [15]. Compound **3a** was converted in 95% yield to a single product, **4**, which retained elicitor activity (Table 1). The DEI mass spectrum of **4** showed $[M]^+$ at *m/z* 606 and the ^1H NMR spectrum showed a single aromatic proton at δ 6.69, confirming monoiodination. Thus **4** was identified as 4′-(2-iodo-3,4,5-trimethoxyphenylacetyl) syringolide 1. The iodination reaction of **3b** was not useful since multiple iodinated compounds were produced in poor yield.

The high yield of the iodinated product **4** indicates that these iodination reagents are suitable for the production of [^{125}I]**4**. Radioiodination was accordingly carried out using the method of Fowler *et al.* [16] to generate [^{125}I]I_2. The availability of highly radioactive syringolide derivatives will facilitate the search for syringolide receptors in soybean leaves. Furthermore, syringolide succinate **3c** should be particularly useful in the preparation of affinity columns. The inactive C-3 and certain of the C-4′ derivatives of reduced activity will also be extremely valuable as competitors with radiolabelled syringolide 1 in ongoing binding studies.

Table 2. Soybean cotyledon assay of elicitor activity of syringolide 1 and its derivatives

Cpd	R at 3-*O*	R at 4′-*O*	Elicitor activity (EC_{50}, μM)
1	H	H	27
1a	Bn	H	>400
1b	Me	H	>400
3a	H	TMPA	94
3b	H	MPP	89
3c	H	Suc	31
4	H	Iodo-TMPA	89

EC_{50} is half maximum induction of phytoalexin accumulation in cultivar Harosoy cotyledons.

EXPERIMENTAL

NMR spectra were recorded at 300 MHz ^1H and 75 MHz ^{13}C in $CDCl_3$ and referenced to TMS = 0. TLC was carried out on silica gel F using phosphomolybdic acid visualization at 110°. HPLC samples were prepurified using silica Sep-Paks or vacuum liquid chromatography (VLC). Small samples were purified by HPLC on a Maxsil 5 (4.6 mm × 250 mm) and larger samples on a Dynamax (25.4 mm × 250 mm) silica column using refractive index detection. All solvents and benzyl alcohol were distilled before use. CH_2Cl_2 was dried by refluxing over CaH_2 before distillation. Anhydrous Dowex/HCl was prepared by washing HCl-treated Dowex with dry MeOH followed by evacuation to 1 mm Hg for 16 hr. Positive ion mass spectra were observed by desorption chemical ionization (DCI) using NH_3 or CH_4 or by desorption electron ionization (DEI) or fast atom bombardment (FAB) using *m*-nitrobenzyl alcohol (NBA). Polyethylene glycol (PEG) or perfluorokerosene (PFK) served as reference for high resolution measurements.

Isolation of syringolide 1 (**1**). Synthetic liquid M9 glucose (0.2%) medium [17] supplemented with thiamine (4 μg/ml), ampicillin (75 μg/ml) and 0.4 mM isopropyl-thiogalactoside was adjusted to pH 5.0 with conc HCl. One litre of medium in a 2 l Erlenmeyer was seeded with *E. coli* DH5α cells carrying expression plasmid pPADRI with the class II *avrD* allele from *Pseudomonas syringae* pv. *phaseolicola* G-50 [18]. The cultures were shaken at 28° for approximately 21 hr. The cell-free culture fluids were extracted three times with EtOAc (*ca* 1/4 volume). The combined organic phases were dried with $MgSO_4$ then filtered and taken to dryness at 40°. The crude extracts, further purified by VLC and HPLC on silica gel [8], afforded **1** in yields of about 7.5 mg per litre of culture. It was stored as a waxy solid.

Synthesis of syringolide 1 *ketals* **1a**, **1b**, **1c**, *and* **1d**. Syringolide 1 was stirred overnight with vacuum-dried Dowex 50W-X8/HCl and the appropriate alcohol in about 3-fold excess [8]. Dry CH_2Cl_2 was added as a solvent for **1a** and **1d**. The reaction mixtures were filtered, concentrated *in vacuo* and purified by HPLC.

3-O-*Benzyl syringolide* 1 (**1a**). Crude **1a** was purified on a Dynamax silica gel column (27 min) using *iso*-PrOH–EtOAc–hexane (1.5:43.5:55) at 6 ml/min. The yield was 80%. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, *J* = 8 Hz, H-8), 1.3 (4H, *bm*, H-6 and H-7), 1.45 (1H, *m*, H-5a). 1.63 (1H, *m*, H-5b), 1.9 (1H, *m*, H-4a), 2.05 (1H, *m*, H-4b), 3.15 (1H, *s*, H-2), 3.83 (1H, *dd*, *J* = 10.4, 2.7, H-5′a), 4.04 (1H, *dd*, *J* = 10.4, 0.8, *H*-5′b), 4.22 (1H, *s*, H-3′), 4.30 (1H, *d*, *J* = 2.7, H-4′),

4.40 (1H, *d*, $J = 10.4$, H-1′a), 4.55 (1H, *d*, $J = 10.4$, H-1′b), 4.58 (2H, *s*, H-9), 7.3 (5H, *m*, H-11, H-12, H-13, H-14, and H-15); ^{13}C NMR: δ 14.0 (C-8), 22.4 (C-7), 23.4 (C-5), 31.8 (C-6), 33.4 (C-4), 59.7 (C-2), 62.7 (C-9), 74.2 (C-5′), 74.7 (C-4′), 74.9 (C-1′), 91.3 (C-3′), 98.1 (C-2′), 111.5 (C-3), 126.7 (C-11 and C-15), 127.4 (C-13), 128.5 (C-12 and C-14), 137.8 (C-10), 171.8 (C-1); DCIMS (NH_3), *m/z*: 380 $[M + NH_4]^+$ (4), 272 (14), 256 (16), 255 $[M - C_6H_5CH_2O]^+$, (100), 211 (3), 151 (14), 108 (7), 99 (4), 91 (57); HRDCIMS (NH_3), 380.2075 ($C_{20}H_{30}NO_6 = 380.2073$, $\Delta = -0.5$ ppm).

3-O-*Methyl syringolide* 1 (**1b**). Using syringolide 1 (15 mg) in 3 ml of dry MeOH [8], **1b** was prepared in nearly quantitative yield. It had a retention time of 27.9 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.88 (3H, *t*, $J = 8.0$ Hz, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.45 (1H, *m*, H-5a), 1.60 (1H, *m*, H-5b), 1.78 (1H, *m*, H-4a), 1.92 (1H, *m*, H-4b), 3.10 (1H, *s*, H-2), 3.24 (3H, *s*, H-9), 3.82 (1H, *dd*, $J = 10.0$, 2.7, H-5′a), 4.03 (1H, *d*, $J = 10.0$, H-5′b), 4.30 (1H, *d*, $J = 2.7$, H-4′), 4.31 (1H, *s*, H-3′), 4.42 (1H, *d*, $J = 10.4$, H-1′a), 4.65 (1H, *d*, $J = 10.4$, H-1′b); ^{13}C NMR: δ 14.0 (C-8), 22.4 (C-7), 23.5 (C-5), 31.8 (C-6), 32.4 (C-4), 48.5 (C-9), 59.5 (C-2), 74.2 (C-5′), 74.7 (C-4′), 74.8 (C-1′), 91.1 (C-3′), 98.2 (C-2′), 111.3 (C-3), 171.8 (C-1); DCIMS (CH_4), *m/z*: 287 $[M + H]^+$ (4), 255 $[M - CH_3O]^+$ (100), 215 (52), 211 (15), 155 (11), 151 (16), 55 (10); HRDCIMS (CH_4), 287.1483 ($C_{14}H_{23}O_6 = 287.1495$, $\Delta = 4.1$ p.p.m.).

3-O-*Ethyl syringolide* 1 (**1c**). The ethyl ketal of syringolide 1 was obtained in nearly quantitative yield. It had a retention time of 20.5 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, $J = 8.0$ Hz, H-8), 1.17 (3H, *t*, $J = 7.7$, H-10), 1.30 (4H, *bm*, H-6 and H-7), 1.45 (1H, *m*, H-5a), 1.60 (1H, *m*, H-5b), 1.78 (1H, *m*, H-4a), 1.92 (1H, *m*, H-4b), 3.08 (1H, *s*, H-2), 3.47 (1H, *dq*, $J = 16.6$, 7.7, H-9a), 3.58 (1H, *dq*, $J = 16.6$, 7.7, H-9b), 3.84 (1H, *dd*, $J = 10.4$, 2.7, H-5′a), 4.04 (1H, *dd*, $J = 10.4$, 1.0, H-5′b), 4.30 (2H, *m*, H-3′ and H-4′), 4.45 (1H, *d*, $J = 10.3$, H-1′a), 4.65 (1H, *d*, $J = 10.3$, H-1′b); ^{13}C NMR: δ 14.0 (C-8), 15.3 (C-10), 22.4 (C-7), 23.4 (C-5), 31.8 (C-6), 33.2 (C4), 56.4 (C-9), 59.7 (C-2), 74.1 (C-5′), 74.5 (C-4′), 75.0 (C-1′), 91.1 (C-3′), 98.3 (C-2′), 111.1 (C-3), 171.8 (C-1), CIMS (NH_3), *m/z*: 318 $[M + NH_4]^+$ (5), 272 (35), 256 (15), 255 $[M - CH_3CH_2O]^+$ (100), 229 (17), 151 (6), 136 (5), 55 (7); HRCIMS (NH_3), 318.1929 ($C_{15}H_{28}NO_6 = 318.1917$, $\Delta = 4.1$ p.p.m.).

3-O-(2-*Hydroxyethyl*) *syringolide* 1 (**1d**). By reaction of ethylene glycol with **1** (30 mg) a nearly quantitative yield of **1d** was obtained. Its retention time was 29 min on the Dynamax silica column (iso-PrOH–EtOAc–hexane, 1.5:43.5:55) at 6 ml/min. ^1H NMR ($CDCl_3$, 300 MHz) δ 0.90 (3H, *t*, $J = 8.0$, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.45 (1H, *m*, H-5a), 1.60 (1H, *m*, H-5b), 1.78 (1H, *m*, H-4a), 1.92 (1H, *m*, H-4b), 3.13 (1H, *s*, H-2), 3.57 (1H, *m*, H-9a), 3.63 (1H, *m*, H-9b), 3.73 (2H, *m*, H-10), 3.85 (1H, *dd*, $J = 10.5$, 2.5, H-5′a), 4.05 (1H, *d*, $J = 10.5$, *H*-5′b), 4.32 (1H, *d*, $J = 2.5$, H-4′), 4.43 (1H, *s*, H-3′), 4.45 (1H, *d*, $J = 10.4$, H-1′a), 4.68 (1H, *d*, $J = 10.4$, H-1′b); C^{13} NMR: δ 14.0 (C-8), 22.4 (C-7), 23.3 (C-5), 31.8 (C-6), 33.1 (C-4), 59.1 (C-10), 59.3 (C-2), 63.1 (C-9), 74.2 (C-5′), 74.7 (C-4′), 74.9 (C-1′), 91.1 (C-3′), 98.2 (C-2′), 112.0 (C-3), 171.5 (C-1); CIMS (NH_3), *m/z*: 334 $[M + NH_4]^+$ (3), 272 (17), 256 (20), 255 $[M - OCH_2CH_2OH]^+$ (100), 151 (12), 87 (69); HRCIMS (NH_3), 334.1879 ($C_{15}H_{28}NO_7 = 334.1866$, $\Delta = -3.9$ p.p.m.).

Synthesis of 3-O-*benzyl*-4′-(3,4,5-*trimethoxyphenylacetyl*) *syringolide* 1 (**2a**). To a solution of **1a** (14 mg, 0.04 mmol) in 500 μl dry CH_2Cl_2 were added 3,4,5-trimethoxyphenylacetic acid (9 mg, 0.04 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI, 15 mg) and 4-dimethylaminopyridine (DMAP, 1.8 mg) and stirred at room temperature. Reaction progress was monitored by TLC ($CHCl_3$/MeOH 19:1). After 16 hr the solution was diluted with 5 ml of $CHCl_3$ and washed with 1% HCl. The $CHCl_3$ phase was collected and dried with $MgSO_4$. The crude ketal ester, **2a**, was prepurified on a silica Sep-Pak by elution with 10 ml of 20% EtOAc–hexane; the yield was 87%. It had a retention time of 12.5 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, $J = 8$, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.45 (1H, *m*, H-5a), 1.63 (1H, *m*, H-5b), 1.90 (1H, *m*, H-4a), 2.06 (1H, *m*, H-4b), 3.18 (1H, *s*, H-2), 3.56 (2H, *d*, $J = 0.8$, H-7′), 3.83 (6H, *s*, C-3″-OMe and C-5″-OMe), 3.86 (3H, *s*, C-4″-OMe), 3.91 (1H, *dd*, $J = 11.1$, 2.8, H-5′a), 4.11 (1H, *dd*, $J = 11.1$, 0.8, H-5′b), 4.24 (1H, *d*, $J = 0.8$, H-3′), 4.32 (1H, *d*, $J = 10.4$, H-1′a), 4.41 (1H, *d*, $J = 10.4$, H-1′b), 4.58 (2H, *s*, H-9), 5.18 (1H, *d*, $J = 2.8$, H-4′), 6.46 (2H, *s*, H-2″), 7.30 (5H, *m*, H-11, H-12, H-13, H-14 and H-15); ^{13}C NMR: δ 14.0 (C-8), 22.4 (C-7), 23.4 (C-5), 31.8 (C-6), 33.3 (C-4), 41.2 (C-7′), 56.1 (C-3″-OCH_3 and C-5″-OCH_3), 59.6 (C-2), 60.9 (C-4″-OMe), 62.8 (C-9), 71.4 (C-5′), 74.4 (C-1′), 77.2 (C-4′), 89.0 (C-3′), 98.4 (C-2′), 106.2 (C-2″ and C-6″), 111.8 (C-3), 126.6 (C-11 and C-15), 127.5 (C-13), 128.5 (C-12 and C-14), 128.7 (C-1″), 137.6 (C-4″), 137.6 (C-10), 153.3 (C-3″ and C-5″), 170.4 (C-1), 171.3 (C-6′); DEIMS, *m/z*: 570 $[M]^+$ (38), 463 $[M - C_6H_5CH_2O]^+$ (12), 226 (27), 181 (51), 165 (28), 107 (15), 91 (100), 55 (22); HRDEIMS, 570.2470 ($C_{31}H_{38}O_{10} = 570.2465$, $\Delta = -0.9$ p.p.m.).

Synthesis of 3-O-*benzyl*-4′-3-(4-*methoxyphenylpropionyl*) *syringolide* 1 (**2b**). Ketal ester **2b** was synthesized from **1a** (18 mg) by the same method as **2a** except substituting 3-(4-methoxyphenyl) propionic acid for 3,4,5-trimethoxyphenylacetic acid. The yield was 78%. It had a retention time of 6 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, $J = 8.0$, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.45 (1H, *m*, H-5a), 1.63 (1H, *m*, H-5b), 1.90 (1H, *m*, H-4a), 2.06 (1H, *m*, H-4b), 2.60 (2H, *m*, H-7′), 2.86 (2H, *t*, $J = 7.5$, H-8′), 3.16 (1H, *s*, H-2), 3.70 (3H, *s*, C-4″-OMe), 3.88 (1H, *dd*, $J = 11.1$, 3.0, H-5′a), 4.04 (1H, *d*, $J = 11.1$, H-5′b), 4.17 (1H, *s*, H-3′), 4.29 (1H, *d*, $J = 10.3$, H-1′a), 4.35 (1H, *d*,

J = 10.3, H-1′b), 4.58 (2H, *s*, H-9), 5.13 (1H, *d*, J = 2.8, H-4′), 6.78 (2H, *d*, J = 8.6, H-3″ and H-5″), 7.08 (2H, *d*, J = 8.6, H-2″ and H-6″), 7.30 (5H, *m*, H-11, H-12, J-13, H-14 and H-15); ^{13}C NMR: δ 14.0 (C-8), 22.4 (C-7), 23.4 (C-5), 30.0 (C-8′), 31.8 (C-6), 33.3 (C-4), 35.9 (C-7′), 55.2 (C-4″-OMe), 59.6 (C-2), 62.9 (C-9), 71.8 (C-5′), 74.5 (C-1′), 77.2 (C-4′), 89.1 (C-3′), 98.4 (C-2′), 111.7 (C-3), 113.9 (C-3″ and C-5″), 126.8 (C-11 and C-15), 127.5 (C-13), 128.5 (C-12 and C-14), 129.2 (C-2″ and C-6″), 131.9 (C-1″), 137.7 (C-10), 158.3 (C-4″), 171.5 (C-1), 171.9 (C-6′); DCIMS (NH_3), *m/z*: 542 $[M + NH_4]^+$ (6), 417 $[M - C_6H_4CH_2]^+$ (68), 198 (17), 180 (30), 134 (13), 121 $[CH_3OC_6H_4CH_2]^+$ (100), 108 (15), 91 (62), 78 (16), 65 (10), HRDCIMS (NH_4), 542.2761 ($C_{30}H_{40}NO_8$ = 542.2754, Δ = −1.3 p.p.m.).

Synthesis of 3-O-*benzyl*-4′-*succinyl syringolide* 1 (**2c**). Succinic anhydride (5 mg) and DMAP (2 mg) were added to **1a** (10 mg) in 600 μl of $CDCl_3$. Followed by NMR, the esterification was complete after 72 hr. The crude mixture was diluted with 6 ml of $CHCl_3$ and washed with 6 ml of 2% HCl. The $CHCl_3$ phase was collected and dried with $MgSO_4$. After evaporation 12 mg (96%) of **2c** was obtained and purified by HPLC. It had a retention time of 13 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, J = 8 H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.40 (1H, *m*, H-5a), 1.60 (1H, *m*, H-5b), 1.90 (1H, *bm*, H-4a), 2.03 (1H, *m*, H-4b), 2.63 (4H, *m*, H-7′ and H-8′), 3.17 (1H, *s*, H-2), 3.90 (1H, *dd*, J = 11.1, 2.9, H-5′a), 4.11 (1H, *d*, J = 11.1, H-5′b), 4.27 (1H, *s*, H-3′), 4.37 (1H, *d*, J = 10.4, H-1′a), 4.51 (1H, *d*, J = 10.4, H-1′b), 4.58 (2H, *s*, H-9), 5.18 (1H, *d*, J = 2.8, H-4′), 7.3 (5H, *m*, H-11, H-12, H-13, H-14 and H-15); ^{13}C NMR: 14.0 (C-8), 22.4 (C-7), 23.4 (C-5), 28.4 (C-8′), 28.8 (C-7′), 31.8 (C-6), 33.3 (C-4), 59.6 (C-2), 62.9 (C-9), 71.7 (C-5′), 74.6 (C-1′), 77.6 (C-4′), 88.9 (C-3′), 98.5 (C-2′), 112.0 (C-3), 126.8 (C-11 and C-15), 127.4 (C-13), 128.5 (C-12 and C-14), 137.7 (C-10), 171.1 (C-1), 171.6 (C-6′), 177.3 (C-9′); FABMS (NBA/NaCl), *m/z*: 485 $[M + Na]^+$ (34), 445 (10), 413 (15), 355 $[M - C_6H_5CH_2O]^+$ (100), 255 (21), 219 (20), 193 (21), 123 (82); HRFABMS (NBA/NaCl/PEG) 485.1801 ($C_{24}H_{30}O_9Na$ = 485.1788, Δ = −2.8 p.p.m.).

Synthesis of 3-O-*methyl*-4′-*succinyl syringolide* 1 (**2d**). This synthesis was analogous to that of **2c** but started with **1b** instead of **1a**. The yield was 94%. It had a retention time of 8.5 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, J = 8, H-8), 1.30 (4H, *m*, H-6 and H-7), 1.40 (1H, *m*, H-5a), 1.60 (1H, *m*, H-5b), 1.80 (1H, *m*, H-4a), 1.93 (1H, *m*, H-4b), 2.68 (4H, *m*, H-7′ and H-8′), 3.12 (1H, *s*, H-2), 3.23 (3H, *s*, H-9), 3.90 (1H, *dd*, J = 11.1, 2.9, H-5′a), 4.11 (1H, *d*, J = 11.1, H-5′b), 4.37 (1H, *s*, H-3′), 4.40 (1H, *d*, J = 10.4, H-1′a), 4.60 (1H, *d*, J = 10.4, H-1′b), 5.18 (1H, *d*, J = 2.9, H-4′); ^{13}C NMR: δ 14.0 (C-8), 22.4 (C-7), 23.2 (C-5), 28.6 (C-8′), 28.8 (C-7′), 31.8 (C-6), 32.3 (C-4), 46.8 (C-9), 59.5 (C-2), 71.7 (C-5′), 74.5 (C-1′), 77.2 (C-4′), 88.7 (C-3′), 98.5 (C-2′), 111.6 (C-3), 171.1 (C-1), 171.6 (C-6′), 177.3 (C-9′); DEIMS, *m/z*: 386 $[M]^+$ (2), 355 $[M - MeO]^+$ (44), 315 (71), 257 (7), 165 (29), 155 (18), 139 (14), 125 (10), 109 (35), 101 $[COCH_2CH_2COOH]^+$ (100), 81 (13), 73 (27), 55 (53); HRDEIMS, 386.1567 ($C_{18}H_{26}O_9$ = 386.1577, Δ = 2.5 p.p.m.).

Synthesis of 4′-(3,4,5-*trimethoxyphenylacetyl*) *syringolide* 1 (**3a**). Benzyl ketal ester **2a** (6 mg in 10 ml of EtOAc) was hydrogenated by adding 20 mg of 10% Pd/C catalyst and stirring under H_2 atmosphere at room temp. for 3 hr. The resulting soln. of hemiketal ester **3a** was then filtered through Whatman No. 2 paper and solvents were removed using a rotavap. The reaction was quantitative. It had a retention time of 21 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, J = 8, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.50 (2H, *m*, H-5), 1.93 (2H, *t*, J = 7.4, H-4), 3.09 (1H, *s*, H-2), 3.58 (2H, *s*, H-7′), 3.84 (3H, *s*, C-4″-OMe), 3.86 (6H, *s*, C-3″-OMe and C-5″-OMe), 3.90 (1H, *dd*, J = 11.2, 2.8, H-5′a), 4.12 (1H, *d*, J = 11.2, H-5′b), 4.51 (1H, *s*, H-3′), 4.36 (1H, *d*, J = 10.4, H-1′a), 4.54 (1H, *d*, J = 10.4, H-1′b), 5.16 (1H, *d*, J = 2.8, H-4′), 6.49 (2H, *s*, H-2″ and H-6″); ^{13}C NMR: δ 14.0 (C-8), 22.5 (C-7), 23.1 (C-5), 31.5 (C-6), 38.7 (C-4), 41.4 (C-7′), 56.2 (C-3″-OMe and C-5″-OMe), 58.9 (C-2), 60.9 (C-4″-OMe), 71.8 (C-5′), 74.3 (C-1′), 77.2 (C-4′), 89.1 (C-3′), 98.0 (C-2′), 106.3 (C-2″ and C-6″), 108.5 (C-3), 128.7 (C-1″), 137.6 (C-4″), 153.4 (C-3″ and C-5″), 170.5 (C-6′), 171.8 (C-1); DEIMS, *m/z*: 480 $[M]^+$ (8), 226 (100), 211 (31), 181 (64), 167 (20), 137 (10), 121 (15), 99 (19), 82 (11), 71 (25), 55 (50); HRDEIMS, 480.1990 ($C_{24}H_{32}O_{10}$ = 480.1995, Δ = 1.1 p.p.m.).

Synthesis of 4′-[3-(4-*methoxyphenyl*) *propionyl syringolide*] 1 (**3b**). From hydrogenation of **2b** (12 mg) using 10% Pd/C catalyst (20 mg), **3b** was obtained by the procedure described for **3a**. The reaction, followed by silica TLC ($CHCl_3$–MeOH 19:1), was complete in 3 hr. and the yield of **3b** was quantitative. It had a retention time of 26 min. on Maxsil eluted with 25% EtOAc in hexane at 1.5 ml/min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.91 (3H, *t*, J = 8.3, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.50 (2H, *m*, H-5), 1.93 (2H, *t*, J = 7.6, H-4), 2.64 (2H, *m*, H-7′), 2.90 (2H, *t*, J = 7.2, H-8′), 3.08 (1H, *s*, H-2), 3.79 (3H, *s*, C-4″-OMe), 3.87 (1H, *dd*, J = 11.0, 2.9, H-5′a), 4.06 (1H, *d*, J = 11.0, H-5′b), 4.37 (1H, *d*, J = 10.4, H-1′a), 4.44 (1H, *s*, H-3′), 4.54 (1H, *d*, J = 10.4, H-1′b), 5.12 (1H, *d*, J = 2.9, H-4′), 6.85 (2H, *d*, J = 8.5, H-3″ and H-5″), 7.11 (2H, *d*, J = 8.5, H-2″ and H-6″); ^{13}C NMR: δ 14.0 (C-8), 22.5 (C-7), 23.1 (C-5), 30.1 (C-8′), 31.5 (C-6), 36.0 (C-7′), 38.7 (C-4), 55.3 (C-4″-OCH_3), 58.9 (C-2), 71.9 (C-5′), 74.4 (C-1′), 77.1 (C-4′), 89.2 (C-3′), 97.9 (C-2′), 108.4 (C-3), 114.0 (C-3″ and C-5″), 129.3 (C-2″ and C-6″), 131.9 (C-1″), 158.3 (C-4″), 171.8 (C-1), 172.0 (C-6′); DEIMS, *m/z*: 434 $[M]^+$ (1), 180 (21), 134 (14), 121 $[CH_3OC_6H_4CH_2]^+$ (100), 91 (26), 77 (17), 71 (11), 55 (15); HRDEIMS 434.1928 ($C_{23}H_{30}O_9$ = 434.1941, Δ = 2.9 p.p.m.).

Synthesis of 4′-*succinyl syringolide* 1 (**3c**). Hydrogenation of **2c** (10 mg), by the same method as **3a**,

gave **3c** in quantitative yield. It had a retention time of 43 min. on Maxsil with 25% EtOAc in hexane flowing at 1.5 ml/min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, $J = 8$, H-8), 1.30 (4H, *m*, H-6 and H-7), 1.50 (2H, *m*, H-5), 1.92 (2H, *t*, $J = 8.1$, H-4), 2.67 (4H, *m*, H-7′ and H-8′), 3.10 (1H, *s*, H-2), 3.89 (1H, *dd*, $J = 11.1$, 3.1, H-5′a), 4.14 (1H, *d*, $J = 11.1$, H-5′b), 4.44 (1H, *t*, $J = 10.5$, H-1′a), 4.59 (1H, *s*, H-3′), 4.64 (1H, *d*, $J = 10.5$, H-1′b), 5.18 (1H, *d*, $J = 3.1$, H-4′); ^{13}C NMR: δ 14.0 (C-8), 22.5 (C-7), 23.2 (C-5), 28.8 (C-7′), 28.9 (C-8′), 31.6 (C-6), 38.6 (C-4), 59.0 (C-2), 71.6 (C-5′), 74.6 (C-1′), 77.1 (C-4′), 89.0 (C-3′), 98.1 (C-2′), 108.5 (C-3), 171.3 (C-6′), 172.2 (C-1), 176.8 (C-9′); FABMS (NBA), *m/z*: 371 $[M-H]^+$ (62), 325 (51), 269 (51), 255 (100), 222 (49)' HRFABMS (NBA/PFK), 371.1344 ($C_{17}H_{23}O_9 = 371.1342$, $\Delta = -0.5$ p.p.m.).

Synthesis of 4′-[3-(4-*hydroxycyclohexyl*)*propionyl*] *syringolide* 1 (**3d**). When 50 mg of Pd/C was stirred under H_2 atmosphere with **2b** (10 mg, 0.019 mmol) in 10 ml of EtOAc overnight, **3d** was synthesized. After the soln was filled and evapd, **3d** was isolated by HPLC (13.4 min) using 40% EtOAc in hexane on a Maxsil silica column at 1.5 ml/min. The yield was 3 mg (37%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.91 (3H, *t*, $J = 8.4$, H-8), 1.30 (4H, *m*, H-6 and H-7), 1.40–1.80 (9H, *m*, H-1″, H-2″, H-3″, H-5″ and H-6″), 1.50 (2H, *m*, H-5), 1.60 (2H, *m*, H-8′), 1.93 (2H, *t*, $J = 7.6$, H-4), 2.38 (2H, *t*, $J = 7.2$, H-7′), 3.10 (1H, *s*, H-2), 3.90 (1H, *dd*, $J = 11.1$, 3.0, H-5′a), 4.00 (1H, *m*, H-4″), 4.10 (1H, *d*, $J = 11.1$, H-5′b), 4.45 (1H, *d*, $J = 10.4$, H-1′a), 4.60 (1H, *s*, H-3′), 4.69 (1H, *d*, $J = 10.4$, H-1′b), 5.14 (1H, *d*, $J = 3.0$, H-4′); ^{13}C NMR: δ 14.0 (C-8), 22.5 (C-7), 23.2 (C-5), 26.5 (C-2″ and C-6″), 31.1 (C-8′), 31.6 (C-6), 31.8 (C-1″), 32.2 (C-3″ and C-5″), 35.9 (C-7′), 38.7 (C-4), 58.9 (C-2), 66.7 (C-4″), 72.0 (C-5′), 74.5 (C-1′), 77.2 (C-4′), 89.3 (C-3′), 98.0 (C-2′), 109.5 (C-3), 171.5 (C-1), 171.8 (C-6′); DCIMS (NH_3), *m/z*: 444 $[M+NH_4]^+$ (4), 409 (19), 391 (10), 274 (14), 255 $[M-COCH_2CH_2C_6H_{10}OH]^+$ (95), 237 (63), 225 (20), 211 (44), 190 (75), 155 $[COCH_2CH_2C_6H_{10}OH]^+$ (77), 136 (61), 126 (16), 108 (25), 94 (100), 81 (52), 69 (22), 55 (75), HRDCIMS (NH_3), 444.2609 ($C_{22}H_{38}NO_8 = 444.2597$, $\Delta = -2.6$ p.p.m.).

Synthesis of 4′-(2-*iodo*-3,4,5-*trimethoxyphenylacetyl*) *syringolide* 1 (**4**). Iodine (3.1 mg) and $AgOCOCF_3$ (4.3 mg) were added to **3a** (6.9 mg) in 0.4 ml dry $CHCl_3$. They were stirred 10 min at room temp. then diluted with 8 ml EtOAc and washed successively with 8 ml of 5% HCl and satd NaCl. The organic layer was dried over $MgSO_4$, filtered and evapd *in vacuo* to afford **4** in 95% yield. The compound eluted from a Maxsil silica column in 14.5 min using eluant 25% EtOAc in hexane and flow rate 1.5 ml/min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (3H, *t*, $J = 8$, H-8), 1.30 (4H, *bm*, H-6 and H-7), 1.50 (2H, *m*, H-5), 1.93 (2H, *t*, $J = 7.0$, H-4), 3.09 (1H, *s*, H-2), 3.82 (2H, *s*, H-7′), 3.88 (6H, *s*, C-3″-OMe and C-5″-OMe), 3.84 (3H, *s*, C-4″-OMe), 3.90 (1H, *dd*, $J = 11.2$, 2.7, H-5′a), 4.18 (1H, *d*, $J = 11.2$, H-5′b), 4.62 (1H, *s*, H-3′), 4.35 (1H, *d*, $J = 10.4$, H-1′a), 4.58 (1H, *d*, $J = 10.4$, H-1′b), 5.20 (1H, *d*, $J = 2.7$, H-4′), 6.69 (1H, *s*, H-6″); ^{13}C NMR, δ 14.0 (C-8), 22.5 (C-7), 23.1 (C-5), 31.5 (C-6), 38.7 (C-4), 46.3 (C-7′), 56.2 (C-3″-OMe), 59.0 (C-2), 60.8, (C-4″-OMe), 61.0 (C-5″-OMe), 71.8 (C-5′), 74.3 (C-1′), 77.2 (C-4′), 89.0 (C-3′ and C-2″), 97.9 (C-2′), 108.5 (C-3), 110.1 (C-6″), 132.5 (C-1′), 141.8, (C-4″), 153.4 (C-5″), 153.7 (C-3″), 169.6 (C-6′), 171.8 (C-1); CIMS (NH_3), *m/z*: 606 $[M]^+$ (9), 353 (13), 352 (100), 334 (14), 307 (60), 226 (38), 211 (15), 181 (40), 167 (17), 165 (20), 149 (17), 121 (36), 99 (34), 55 (50); HRCIMS (NH_3), 606.0970 ($C_{24}H_{31}O_{10}I = 606.0962$, $\Delta = -1.3$ p.p.m.).

Synthesis of [^{125}I]**4**. To 120 μl of a 5% solution of KIO_3, 100 μl of [^{125}I]NaI (1.1 mCi of radioactivity) and 500 μl of $CHCl_3$ was added 40 μl of 9N H_2SO_4. After 10 min the resulting $CHCl_3$ solution of [^{125}I]I_2 was dried by filtration through a 0.5 cm × 0.5 cm column of Sephadex G-25. To 2 μg of **3a** and 2 μg of $AgO(CO)CF_3$ (previously dissolved in 150 μl of ether and blown dry in the presence of **3a**) was added the dry $CHCl_3$ solution of [^{125}I]I_2. This was stirred at 40° for about 10 min. Free [^{125}I]I_2 was removed by washing with 100 μl of 5% $Na_2S_2O_3$. The [^{125}I]**4** was purified on a silica Sep-Pak using $CHCl_3$ as the eluting solvent, and after removal of $CHCl_3$ it was stored at −20° in a glass-lined lead container.

Determination of the soybean hypersensitive response. The elicitor activity of synthesized derivatives of syringolide 1 was qualitatively determined by the soybean leaf bioassay [17, 18]. The derivatives were dissolved at known concentrations in EtOH and diluted with water to concentration of 3.68×10^{-5} M for comparison of activity with syringolide 1. Water with the same conc of EtOH was used as a negative control. At 24 hr. after infiltration, the leaves were scored for the appearance of necrosis which occurred only in cultivars carrying the *Rpg4* disease resistance gene.

Soybean cotyledon bioassay. The soybean cotyledon bioassay was performed as previously described [19]. This assay monitors phytoalexin production as an indicator of the hypersensitive defence response and permits quantitative determination of elicitor activity. Assay solutions were applied to wounded cotyledons of cultivar Harosoy, and the amounts of phytoalexin glyceollin were measured after 24 hr. at room temperature. Water was used as a negative control in all cotyledon assays.

Acknowledgements—Mass spectra were determined by Ron New of the UCR ACIF. We thank the U.S. Department of Agriculture (grant 9501093) for financial support.

REFERENCES

1. Ellingboe, A. H., in *Active Defence Mechanisms in Plants*, ed. R. K. S. Wood. Plenum Press, London, 1982, p. 179.
2. Flor, H. H., *Phytopathology*, 1942, **32**, 653.

3. Dixon, R. A. and Lamb, C. J., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1990, **41**, 339.
4. Ebel, J. and Grisebach, H., *Trends in Biochemical Sciences*, 1988, **13**, 23.
5. Keen, N. T. and Dawson, W. O., in *Genes Involved in Plant Defence. Plant Gene Research*, Vol. 8, eds T. Boller and F. Meins. Springer-Verlag, New York, 1992, p. 76.
6. Kobayashi, D., Tamaki, S. and Keen, N. T., *Molecular Plant–Microbe Interactions*, 1990, **3**, 94.
7. Kobayashi, D. Y., Tamaki, S. J., Trollinger, D. J., Gold, S. and Keen, N. T., *Molecular Plant–Microbe Interactions*, 1990, **3**, 103.
8. Midland, S. L., Keen, N. T., Sims, J. J., Midland, M. M., Stayton, M. M., Burton, V., Smith, M. J., Mazzola, E. P., Graham, K. J. and Clardy, J., *Journal of Organic Chemistry*, 1993, **58**, 2940.
9. Hahn, M. G., in *Signal Molecules in Plants and Plant–Microbe Interactions*, ed. B. J. J. Lugtenberg. Springer-Verlag, Heidelberg, 1990, p. 1.
10. Cuatrecasas, P., *Proceedings of the National Academy of Science, U.S.A.*, 1971, **68**, 1264.
11. Sheridan, P. J., Blum, K. and Trachtenberg, M. C. (eds)., *Steroid Receptors and Disease: Cancer, Autoimmune, Bone, and Circulatory Disorders*. Marcel Dekker, Inc., New York, 1988.
12. Renelt, A., Colling, C., Hahlbrock, K., Nürnberger, T., Parker, J. E., Sacks, W. R. and Scheel, D., *Journal of Experimental Botany*, 1993, **44**, 257.
13. Cheong, J-J. and Hahn, M. G., *Plant Cell*, 1991, **3**, 137.
14. Frey, T., Cosio, E. G. and Ebel, J., *Phytochemistry*, 1993, **32**, 543.
15. Brown, E. and Robin, J.-P., *Tetrahedron Letters*, 1977, **23**, 2015.
16. Fowler, J. S., MacGregor, R. R., Wolf, A. P. Ansari, A. N. and Atkins, H. L. (1976) *Journal of Medicinal Chemistry*, 1976, **19**, 356.
17. Keen, N. T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D. and Staskawicz, B., *Molecular Plant–Microbe Interactions*, 1990, **3**, 112.
18. Yucel, I., Midland, S. L., Sims, J. J. and Keen, N. T., *Molecular Plant–Microbe Interactions*, 1994, **7**, 148.
19. Keen, N. T., Yoshikawa, M. and Wang, M. C., *Plant Physiology*, 1983, **71**, 466.