PERGAMON

Phytochemistry 50 (1999) 53–56

PHYTOCHEMISTRY

Elicitation of tropane alkaloid biosynthesis in transformed root cultures of *Datura stramonium*

I. Zabetakis [a], R. Edwards [b], D. O'Hagan [a,*]

[a] *Department of Chemistry, University of Durham, South Road, Durham DH1 3LE, UK*
[b] *Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK*

Revised 16 June 1998

Abstract

Hairy root cultures of *Datura stramonium* were treated with methyl jasmonate (MeJa), a cell wall preparation from baker's yeast and oligogalacturonides, respectively, and analysed for the accumulation of the tropane alkaloids, littorine, hyoscyamine and scopolamine, and their precursors, phenyllactate and tropine. The treatments increased alkaloid accumulation in the order MeJa > fungal elicitor > oligogalacturonide and, in all cases, this was associated with an increase in tropine but a decline in phenyllactate concentrations. Time–course studies following MeJa treatments confirmed that increased tropane alkaloid synthesis was due to the differential enhancement of tropine biosynthesis. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Datura stramonium*; Solanaceae; Hyoscyamine; Root cultures; Elicitation; Methyl jasmonate

1. Introduction

In plant cell cultures, the accumulation of antimicrobial terpenoid and phenolic phytoalexins is a characteristic response to treatment with elicitor preparations from the cell walls of fungi or plants (Whitehead & Threlfall, 1992). More recently, it has become apparent that the synthesis of alkaloids can be elicited similarly, with jasmonic acid and its esters playing a key role in regulating the response (Hashimoto & Yamada, 1994). It has also been reported that fungal cell-wall elicitors and methyl jasmonate (MeJa) can activate inducible secondary metabolism in soy bean cell cultures by different mechanisms (Enyedi, Yalpani, Silverman, & Raskin, 1992). Jasmonates, are known to have several biological activities, including promoting stomatal closure (Curtis, 1984) and accelerating leaf senescence in oats and barley (Weidhase et al., 1987), apparently mediating their effect by controlling gene expression (Blechert et al., 1997). Intracellular jasmonates have been shown to transiently accumulate in cell suspension cultures treated with a yeast elicitor preparation implicating a complex physiological role for jasmonates, possibly in the signal transduction system of the defence response (Kutchan, 1993). In cell cultures of *Rauvolfia canescens* and *Eschscholtzia californica*, elicitation of benzophenanthridine alkaloids was associated with the biosynthesis of MeJa (Gundlach, Muller, Kutchan, & Zenk, 1993). It has also been established that treatments with exogenous MeJa can elicit the accumulation of several classes of alkaloids in a range of plant species, including the benzophenanthridines (Gundlach et al., 1993), the *Vinca* alkaloids (Aerts, Schafer, Hesse, Baumann, & Slusarenko, 1996) and the tropane alkaloids (Saenz-Carbonell & Loyola-Vargas, 1996). In the latter case, concentrations of the tropane alkaloid, hyoscyamine **2**, increased by 100% in *Datura stramonium* root cultures after treatment with MeJa at 0.1 μM (Saenz-Carbonell & Loyola-Vargas, 1996). MeJa can also be highly selective in its elicitation of alkaloid synthesis, causing, for example, the enhancement of monomeric rather than dimeric alkaloids in *Catharanthus roseus* (Aerts et al., 1996).

Intrigued by the potential for the selective regulation of alkaloid biosynthesis by MeJa and fungal cell-wall elicitors, we have exposed root cultures of *Datura stramonium,* which were generated by treatment with *Agrobacterium rhizogenes* (Robins, Parr, Bent, &

* Corresponding author. Tel.: +44 191-374-3119; Fax: +44 191-384-4737; E-mail: david.o'hagan@durham.ac.uk.

0031-9422/98/$ - see front matter
PII: S0031-9422(98)00490-7

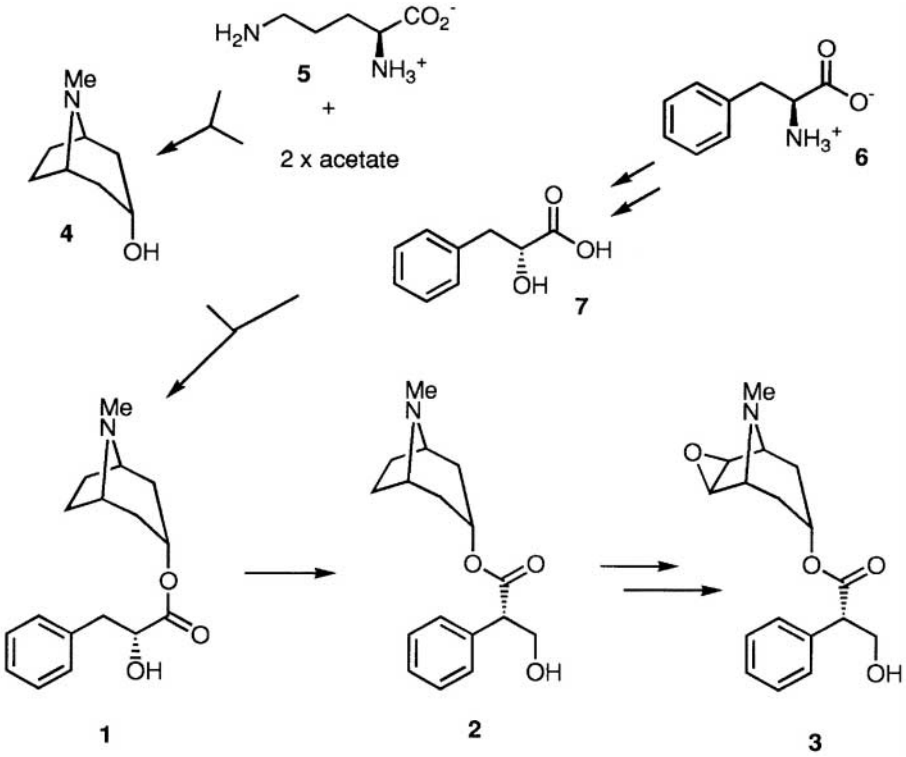

Fig. 1. Biosynthetic pathways for tropane alkaloids.

Rhodes, 1991), to MeJa and a range of biotic elicitors. These roots accumulate the tropane alkaloids, littorine **1**, hyoscyamine **2** and scopolamine **3**, alkaloids which are derived from the combination of two biosynthetic pathways (Robins et al., 1991). Tropine **4** is derived from ornithine **5** and the tropate ester moiety is derived from L-phenylalanine **6** via D-phenyllactate **7** (O'Hagan & Robins, 1998; Wong, Hamilton, O'Hagan, & Robins, 1998) (Fig. 1). In view of the divergent nature of these biosynthetic precursors, a key focus of the investigation was to assess the effect of elicitors on tropine and phenyllactate acid concentrations in order to determine if there is differential elicitation of the pathways providing these precursors.

2. Results and discussion

Datura stramonium root cultures were incubated for 4 days with the various elicitors and then analysed for changes in fr. wt and alkaloid content. The cell wall elicitor preparations used were derived from baker's yeast and citrus pectin, respectively, both preparations being known to elicit phytoalexin synthesis in other plant cell cultures (Hahn et al., 1992). Between days 7 and 11, the fr. wt. of the untreated cultures increased from 0.9 ± 0.05 to 1.9 ± 0.09 g. None of the elicitation treatments significantly inhibited root growth, suggesting that any associated changes in alkaloid content was not due to non-specific phytoxicity. Although glutathione has been shown to be an elicitor of the phytoalexin response in other plant root cultures (Aerts, Gisi, De Carolis, De Luca, & Baumann, 1994), it failed to perturb alkaloid synthesis in *D. stramonium*, relative to the controls (Table 1). However, all of the other treatments tested stimulated alkaloid production and, at the concentrations tested, were active in the order MeJa > fungal elicitor > oligogalacturonide. With the yeast and oligogalacturonide elicitors, the concentrations of all of the tropane alkaloids increased relative to the controls. Of the precursors, the amounts of tropine **4** increased significantly, whereas those of phenyllactate **7** remained unaltered.

In the present study, the effect of MeJa was dose-dependent and maximal at 0.1 μM, as previously reported in root cultures of *D. stramonium* (Saenz-Carbonell & Loyola-Vargas, 1996). At this concentration, MeJa had a greater effect than the fungal elicitor in enhancing the concentrations of littorine **1**, hyoscyamine **2** and tropine **4**, but gave a similar enhancement of **3**. Notably, unlike the yeast elicitor, MeJa treatment resulted in significant reduction in amounts of phenyllactate **7**, suggesting that the biosynthesis of tropine **4** was selectively stimulated, subsequently influencing the amounts of the tropane alkaloids **1** and **2**. To explore this further, 7-day-old cultures of *D. stramonium* were supplemented with 0.1 μM MeJa and the concentrations of the alkaloids and their precursors monitored at various intervals over an 8 day period (Figs. 2–4).

Table 1
Amounts (μmol g^{-1} dry wt) of compounds **1**–**4** and **7** on day 11 in *D. stramonium* roots after elicitor treatments on day 7

Treatment	Fr. wt (g)	Littorine **1**	Hyoscyamine **2**	Scopolamine **3**	Tropine **4**	Phenyllactate **7**
Control	1.86	1.19 ± 0.15	12.16 ± 1.10	2.45 ± 0.18	0.65 ± 0.18	2.10 ± 0.08
Yeast extract	1.60	2.71 ± 0.09	17.34 ± 1.06	6.13 ± 0.47	1.52 ± 0.12	2.00 ± 0.07
GSH	1.70	1.32 ± 0.10	11.11 ± 0.72	2.52 ± 0.19	0.95 ± 0.11	2.01 ± 0.19
MeJa (0.1 μM)	1.65	3.70 ± 0.09	22.14 ± 0.81	6.14 ± 0.21	2.53 ± 0.21	1.21 ± 0.12
Oligogalacturonides	1.80	1.81 ± 0.05	14.07 ± 0.21	2.71 ± 0.06	1.08 ± 0.09	1.84 ± 0.15

All values represent the mean of duplicate independent experiments ± the variation in the replicates from the mean.

Relative to the controls, the concentrations of the tropane alkaloids **1**, **2** and **3** and tropine **4** increased steadily during the first 4 days following MeJa treatment. The amounts of these compounds then reduced gradually to the amounts in controls during the next 4 days. In contrast, the concentrations of phenyllactate **7** fell over the first three days of MeJa treatment and remained depressed for the remainder of the incubation period. These results reinforce the data in Table 1 and suggest that the accumulation of the alkaloids **1**, **2** and **3** following MeJa treatment, results from increased contents of tropine **4**, rather than phenyllactate **7**. The decline in the amount of **7** over the period is presumably due to the fact that the demand for **4** is exceeding its rate of synthesis.

It is noteworthy that MeJa stimulates selectively the biosynthesis of **4** over **7**. There is evidence that MeJa exerts its effect on alkaloid accumulation through both an increase of the alkaloid precursor pool and an enhancement of several enzymes involved in alkaloid biosynthesis (Aerts et al., 1994). In particular, enzymes linking primary with secondary metabolism can be upregulated by MeJa (Aerts et al., 1994). Arginine decarboxylase and putrescine methyltransferase have been shown to be important regulatory enzymes in tropane alkaloid biosynthesis (Robins et al., 1991); the

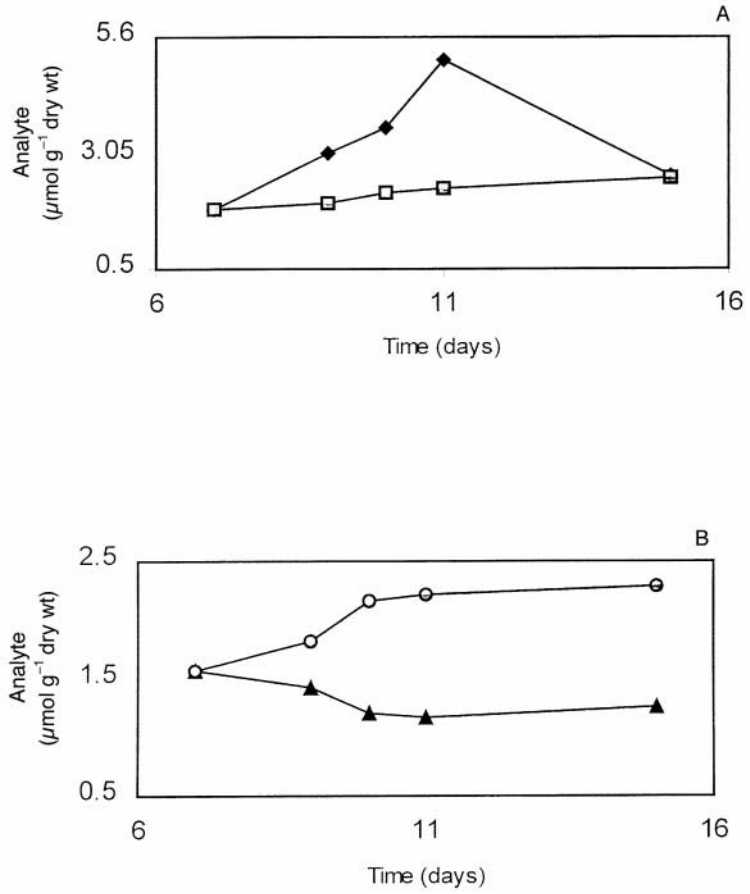

Fig. 2. Amounts of tropine **3** (A) and phenyl lactate **4** (B) during an 8 day incubation period; ◆ = tropine, ■ = tropine (control), ▲ = phenyl lactate and ● = phenyllactate (control).

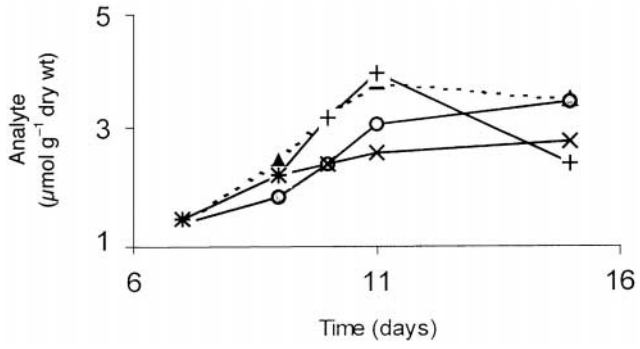

Fig. 3. Amounts of littorine **2** and scopolamine **7** during an 8 day incubation period. ▲ = littorine, ● = littorine (control), + = scopolamine and × = scopolamine (control).

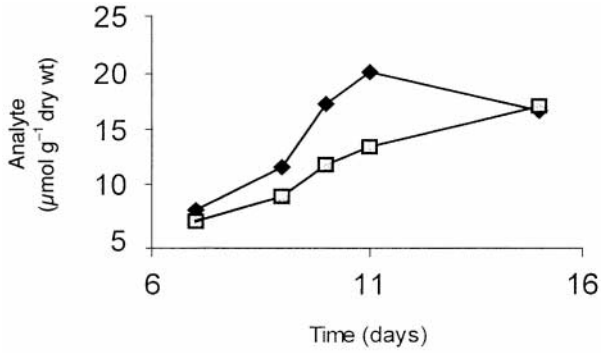

Fig. 4. Amounts of hyoscyamine **1** during an 8 day incubation period. ◆ = hyoscyamine and ■ = hyoscyamine (control).

expression of one of these may be regulated by MeJa in *D. stramonium*. In other species, MeJa stimulates phenylpropanoid biosynthesis following the induction of PAL (Gundlach et al., 1993). However, PAL is not involved in the biosynthesis of D-phenyllactate **7**. It will now be of interest to determine the effect of MeJa and other elicitors on the specific activity of the enzymes involved in the biosynthesis of **4** in *D. stramonium*.

3. Experimental

3.1. General

Compounds **2**, **3**, **4** and **7** were obtained from Aldrich. Racemic **1** was prepd as described previously (Zabetakis, Edwards, Hamilton, & O'Hagan, in press). MeJa was purchased from Sigma. Root cultures of *D. stramonium*, which were previously generated after treatment of roots with *A. rhizogenes* (Robins et al., 1991), were maintained in B50 medium as described previously (Robins et al., 1991) and sub-cultured every 21 days.

3.2. Feeding of elicitors

All treatments were added to cultures (50 ml) 7 days after subculturing and worked up on day 11. MeJa was added as a soln in MeOH (0.2 ml) to the flasks. A fungal cell wall prep., composed of both glucan and chitin elicitors, was prepared from baker's yeast and the sugar content determined using glucose as standard (Schumacher, Grundlach, Fiedler, & Zenk, 1987). The yeast elicitor was added to the flasks (0.5 ml) at a final concn of 100 mg of glucose equivalents l^{-1} of medium. An oligogalacturonide elicitor was prepd from citrus pectin (Hahn et al., 1992) and added to the culture medium at a final concn of 200 mg of glucose equivalents l^{-1} of medium. After adjusting to pH 7, a solution of glutathione in H_2O (2.5 ml) was added to the flasks to give a final concn of 5 mM. Corresponding controls were prepd in order to correct for the effect of the addition of carrier solvent. At harvest, roots were freeze-dried and analysed for tropane alkaloids by GC-FID as described previously (Zabetakis et al., in press).

Acknowledgements

We would like to thank the Leverhulme Trust for a grant in support of this work.

References

Aerts, R. J., Gisi, D., De Carolis, E., De Luca, V., & Baumann, T. W. (1994). *Plant J., 5,* 635.
Aerts, R. J., Schafer, A., Hesse, M., Baumann, T. W., & Slusarenko, A. (1996). *Phytochemistry, 42,* 417.
Blechert, S., Bockelmann, C., Brummer, O., Fublein, M., Gundlach, H., Haider, G., Holder, S., Kutchan, T. M., Weiler, E. W., & Zenk, M. H. (1997). *J. Chem. Soc. Perkin Trans. I,* 3549.
Curtis, R. W. (1984). *J. Plant Growth Regul., 3,* 157.
Enyedi, A. J., Yalpani, N., Silverman, P., & Raskin, I. (1992). *Cell, 70,* 879.
Gundlach, H., Muller, M. J., Kutchan, M. J., & Zenk, M. H. (1993). *Proc. Natl. Acad. Sci. USA, 89,* 2389.
Hahn, M. G., Darvill, A., Albersheim, P., Bergmann, C., Cheong, J. J., Koller, A. & Lo, V. M. (1992). In J. J. Gurr, M. J. McPherson and D. J. Bowles (Eds.), Molecular Plant Pathology: A Practical Approach (Vol 11, pp. 103–147). IRL Press, Oxford.
Hashimoto, T., & Yamada, Y. (1994). *Annu. Rev. Plant. Physiol. Plant Mol. Biol., 45,* 257.
Kutchan, T. M. (1993). *J. Plant Physiol., 142,* 502.
O'Hagan, D., & Robins, R. J. (1998). *Chem. Soc. Rev., 27,* 207.
Robins, R. J., Parr, A. J., Bent, E. G., & Rhodes, M. J. C. (1991). *Planta, 183,* 196.
Saenz-Carbonell, L., & Loyola-Vargas, V. M. (1987). *Appl. Biochem. Biotechnol., 61,* 321.
Schumacher, H. M., Grundlach, H., Fiedler, F., & Zenk, M. H. (1987). *Plant Cell Rep., 6,* 410.
Weidhase, R. A., Kramell, H. M., Lehmann, J., Liebisch, H.-W., Lerbs, W., & Parthier, B. (1987). *Plant Sci., 51,* 177.
Whitehead, I. M., & Threlfall, D. R. (1992). *J. Biotechnol., 26,* 63.
Wong, C. W., Hamilton, J. T. G., O'Hagan, D., & Robins, R. J. (1998). *Chem. Comm., 0,* 1045.
Zabetakis, I., Edwards, R., Hamilton, J. T. G. & O'Hagan, D. Plant Cell Rep. (in press).