View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by DepositOnce

Biol. Chem., Vol. 381, pp. 741–748, August 2000 · Copyright © by Walter de Gruyter · Berlin · New York

Structure-Activity Relationships of Synthetic Analogs of Jasmonic Acid and Coronatine on Induction of Benzo[*c*]phenanthridine Alkaloid Accumulation in *Eschscholzia californica* Cell Cultures

Georg Haider[1,a], Thomas von Schrader[2], Martin Füßlein[2], Siegfried Blechert[2] and Toni M. Kutchan[1,3,*]

[1] Laboratorium für Molekulare Biologie, Universität München, Karlstr. 29, D-80333 München, Germany
[2] Institut für Organische Chemie, Technische Universität Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany
[3] Leibniz-Institut für Pflanzenbiochemie, Weinberg 3, D-06120 Halle, Germany

* Corresponding author

A facile test system based on the accumulation of benzo[*c*]phenanthridine alkaloids in *Eschscholzia californica* cell suspension culture (an indicator of defense gene activation) has been used to analyze a series of synthetic compounds for elicitor-like activity. Of the 200 jasmonic acid and coronatine analogs tested with this system, representative results obtained with 49 of them are presented here. The following can be summarized concerning structure-activity relationships: there is a large degree of plasticity allowed at the C-3 of jasmonic acid in the activation of defense genes. The carbonyl moiety is not strictly required, but exocyclic double bond character appears necessary. The pentenyl side chain at C-2 cannot tolerate bulky groups at the terminal carbon and still be biologically active. Substitutions to the C-1′ position are tolerated if they can potentially undergo β-oxidation. Either an alkanoic acid or methyl ester is required at C-1, or a side chain that can be shortened by β-oxidation or by peptidase hydrolysis. Coronatine and various derivatives thereof are not as effective as jasmonic acid, and derivatives in inducing benzo[*c*]phenanthridine alkaloid accumulation. Jasmonic acid rather than the octadecanoic precursors is therefore considered to be a likely signal transducer of defense gene activation *in planta*.

Key words: Alkaloid induction / Benzo[*c*]phenanthridine alkaloids / Coronatine / *Eschscholzia californica* / Methyl jasmonate / Octadecanoid analogs.

[a] Present address: Süd-Pharma GmbH, Research and Development, Osterseenstr. 2, D-82402 Seeshaupt, Germany.

Introduction

Jasmonic acid and its derivatives have been intensely investigated in recent years due to the multiple physiological responses in plants that can be effected by this molecule (Sembdner and Parthier, 1993; Weiler, 1997; Creelman and Mullet, 1997). The role of jasmonates in the induction of protease inhibitor proteins was demonstrated with tomato plants in the early 1990s (Farmer and Ryan, 1990, 1992). The influence of methyl jasmonate on the accumulation of secondary metabolites in plant cell suspension culture was first reported in 1992 (Gundlach *et al.*, 1992). Since then approximately 150 plant species have been demonstrated to respond to the addition of methyl jasmonate to the culture medium by accumulating secondary metabolites (M.H. Zenk, personal communication).

Induction of benzo[*c*]phenanthridine alkaloid biosynthesis in cell suspension cultures of the California poppy *Eschscholzia californica* provides a facile and sensitive assay for testing a large number of derivatives of jasmonic acid in order to establish structure-activity relationships for elicitation of secondary metabolites. This system has been used initially to demonstrate the biological activity of methyl jasmonate with respect to inducing alkaloid biosynthesis (Gundlach *et al.*, 1992). The jasmonate biosynthetic precursor 12-oxo-phytodienoic acid induced biosynthesis of benzo[*c*]phenanthridine alkaloids in a manner similar to methyl jasmonate (Kutchan, 1993). The *E. californica* test system was further exploited to show that β-oxidation of 10,11-dihydro-12-oxo-phytodienoic acid is not necessary for biological activity, implying that an octadecanoid-jasmonic acid biosynthetic precursor may be the *in planta* signal molecule (Blechert *et al.*, 1995). A wide range of derivatives, based on the structure of jasmonic acid, were tested for biological activity in various bioassays such as *E. californica* elicitation, *Bryonia dioica* tendril coiling and barley senescence. The results of this study showed that the jasmonate derivatives displayed in each of the assays an activity profile that was distinctly characteristic for the assays (Blechert *et al.*, 1997). These results implied that there are different structural requirements for each of the various physiological responses (such as induction of defense compound biosynthesis, mechanotransduction and cell senescence) that is regulated by jasmonates.

The *E. californica* alkaloid elicitation test system has also been used to demonstrate that the *Pseudomonas*

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

syringae phytotoxin coronatine is able to induce the biosynthesis of benzo[*c*]phenanthridine alkaloids without eliciting the accumulation of endogenous jasmonic acid (Weiler *et al.*, 1994). The results of Gundlach *et al.* (1992) clearly indicated that addition of a yeast cell wall elicitor preparation to *E. californica* cell suspension cultures led to an increase in endogenous jasmonic acid levels. Taken together, these results suggest that the phytotoxin coronatine mimics the octadecanoid signalling molecules of higher plants.

Synthetic analogs of jasmonic acid have been synthesized in past years and monitored for biological activity in a number of bioassays (Yamane *et al.*, 1980; Weiler *et al.*, 1993; Ishikawa *et al.*, 1994; Taapken *et al.*, 1994; Krumm *et al.*, 1995; Kiyota *et al.*, 1996; Krumm and Boland, 1996; Holbrook *et al.*, 1997; Ward *et al.*, 1997; Zhang *et al.*, 1997; Blechert *et al.*, 1999; Miersch *et al.*, 1999). The general collective conclusion from a comparison of results obtained in all of these studies is that there are strict stereo- and regiochemical requirements for the biological activity of jasmonate analogs that vary with the bioassay used. This supports the conclusion of the study of Blechert *et al.* (1997) that the various physiological responses induced by jasmonate can be separated by structural modification of the inducer molecule.

In this work, we analyzed more precisely the structural requirements of octadecanoid signalling molecules necessary to effect benzo[*c*]phenanthridine alkaloid formation in cell suspension cultures of *E. californica*. The culture has been stable over a period of 20 years and is very well characterized with respect to the biosynthesis of the benzo[*c*]phenanthridine alkaloid macarpine (reviewed in Kutchan, 1998). The benzo[*c*]phenanthridine alkaloids have an antimicrobial activity and are thought to serve as part of the chemical defense system of this plant, as well as in other members of the Papaveracea (Dzink and Socransky, 1985; Cline and Coscia, 1988). Due to these advantages, *E. californica* serves as our model system for investigation of the regulation of defense-related alkaloid biosynthesis in plants. Representative results obtained for 49 of the more than 200 compounds tested are presented here.

Results

Jasmonic acid is a diastereomeric mixture of *cis* and *trans* isomers where the *trans* form is predominant at equilibrium (95:5 *trans:cis*) (Quinkert *et al.*, 1982). The 1*R*-isomers demonstrate more biological activity than the 1*S*-isomers (Koda *et al.*, 1992; Holbrook *et al.*, 1997). The *cis* diastereomer 1*R*,2*S* is more biologically active than the *trans* diastereomer 1*R*,2*R* (Koda *et al.*, 1992). The compounds that were used in this study were equilibrium mixtures, with the structures in the tables drawn as the predominant *trans* form.

The *E. californica* bioassay was performed hundreds of times during the course of this study. The initial evaluation can be made with the naked eye due to the intense red color of the benzo[*c*]phenanthridine alkaloids that are formed in response to elicitation. A typical response obtained for a comparison between methyl jasmonate **1** and coronatine **39** is given in Figure 1. Quantification is achieved by ethanol extraction of the cells followed by spectrophotometric determination of the total benzo[*c*]phenanthridine alkaloid content. A dose dependency curve for each test substance was determined at least three times in duplicate. To facilitate interpretation and to reduce data presentation, the results were scored relative to solvent controls as follows: ++++, 5 – 10 fold increase in activity as compared to solvent controls; +++, 3 – 5 fold increase in activity as compared to solvent controls; ++, 2 – 3 fold increase in ac-

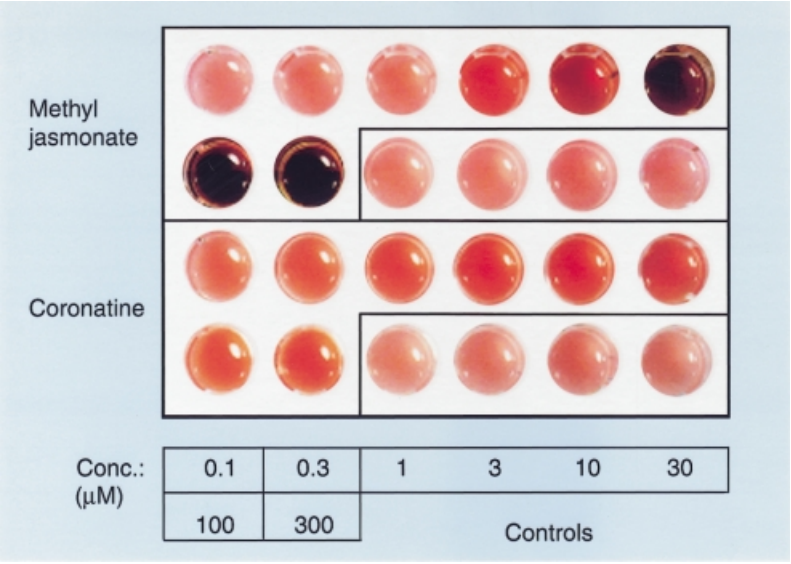


Fig. 1 Dose-Response of *E. californica* Cell Culture to Addition of Methyl Jasmonate or Coronatine in the Multiwell Assay. The pigments that are formed in the cells in response to addition of an elicitor substance are fluorescent benzo[*c*]phenanthridine alkaloids.

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

tivity as compared to solvent controls; +, 1.5 – 2 fold increase in activity as compared to solvent controls; –, no elicitation of alkaloid accumulation could be detected. All results in the tables are presented according to this scoring scheme.

C-3 Analogs of Methyl Jasmonate

Comparison of the series of compounds **1**–**6** (Table 1) suggests that the carbonyl function at C-3 is not critically important for induction of benzo[*c*]phenanthridine alkaloid biosynthesis in *E. californica* cell suspension culture. Increasing the bulkiness of the C-3 substituent led to only a small decrease in biological activity. Only when the carbonyl oxygen is replaced by $=CBr_2$ (compound **4**) does the capacity to induce alkaloid accumulation become reduced by a factor of 3–5. The full activity obtained with **6** may be attributed to enol-keto tautomerization.

C-2 Analogs of Methyl Jasmonate

Variation of the substitution at C-2 also indicated that this position has relatively lax requirements for biological activity (Table 2). The results with **10** clearly indicate that a 3-carbon side chain is too short. The halogen substitutions present in **7**–**9** suggest that bulky groups are tolerated at this position, as for C-3. Interestingly, the nitrile present in compound **12** led to an abolishment of the biological activity, whereas ethylene **11** and isopropyl **13** moieties had no effect. Linearization of the side chain through introduction of a triple bond **14** reduced activity 3–5 fold. The cyclopropyl derivative **15** showed full activity as expected due to the double bond character of the cyclopropyl group. Addition of oxygen as heteroatom at the end of the C-2 side chain reduced the activity either marginally, as for **17**, or 3–5 fold, as for **16**. Nitrogen as heteroatom (**18**) again completely abolished the activity as was observed for **12**. Compound **19** is a methyl jasmonate dimer mimic. It was found to be inactive in this test system. The pentene side chain can accommodate a large amount of structural variation and still maintain the ability to induce alkaloid formation.

Table 1 Induction Activity of C-3 Analogs of Jasmonic Acid Methyl Ester and Related Compounds.

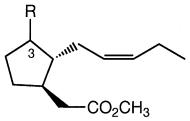
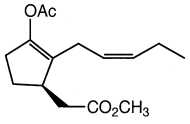
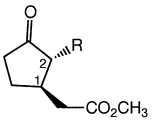
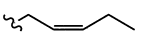
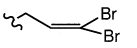
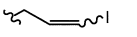
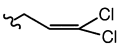
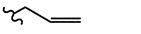
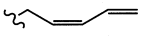
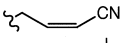
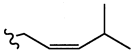
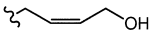
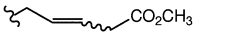
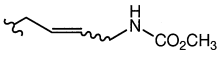
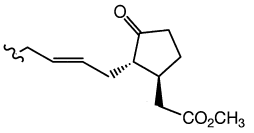
Structure	R	Compound number	Activity score
	=O	1	++++
	$=CF_2$	2	+++
	$=CH_2$	3	+++
	$=CBr_2$	4	++
		5	+++
		6	++++

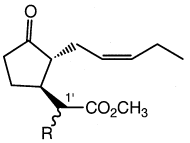
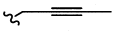
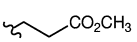
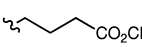
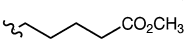
Table 2 Induction Activity of C-2 Analogs of Jasmonic Acid Methyl Ester.

Structure	R	Compound number	Activity score
		1	++++
		7	++++
		8	++++
		9	+++
		10	—
		11	++++
		12	—
		13	++++
		14	++
		15	++++
		16	++
		17	+++
		18	—
		19	—

C-1(1′) Analogs of Methyl Jasmonate

The C-1(1′) position was also found to be relatively flexible with respect to the ability to induce alkaloid formation in *E. californica* cell cultures (Table 3). The only change that

Table 3 Induction Activity of 1(1′)-Substituted Jasmonic Acid Methyl Ester.

Structure	R	Compound number	Activity score
		20	—
		21	+++
		22	++
		23	++++
		24	++++
		25	++++
		26	+++

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

was not tolerated was addition of an allylic moiety to C1′ (compound **20**). Introduction of a triple bond (**21**, **22**) reduced the activity not more than 3–5 fold. The addition of a carboxy methyl group such as methyl carbonate, proprionate, butyrate or pentanoate (compounds **23–26**) effected the biological activity only marginally, if at all. The ester moiety is presumably hydrolyzed *in planta* and the resultant fatty acid side chain could then be shortened through consecutive rounds of β-oxidation. This may explain the apparent tolerance of these modifications.

C-1 Analogs of Methyl Jasmonate

Variation of the length of the acyl side chain as C-1 did not markedly effect the biological activity of the methyl jasmonate analogs (Table 4). This is consistent with earlier reports on the ability of the jasmonic acid biosynthesis precursor 12-oxo-phytodienoic acid to induce alkaloid biosynthesis in *E. californica* (Kutchan, 1993). The kinetics, but not the magnitude of response, are similar between 12-oxo-phytodienoic acid and methyl jasmonate. In jasmonic acid biosynthesis, the octanoic acid side chain of 3-oxo-2(2′(Z)-pentenyl)-cyclopentane-1-octanoic acid is shortened by three rounds of β-oxidation to an acetic acid side chain. As indicated by the results obtained with compounds **27–32**, induction of alkaloid biosynthesis does not appear to be sensitive to even or odd numbered chain-length residues at C-1. Replacing the carboxyl function with a ketone does, however, strongly effect biological activity. The methyl butyryl ketone **33** is only marginally or completely (**34**) active. The presence of an aliphatic C_{10} aldehyde at C-1 (**35**) also abolished the activity. This suggests that either some degree of chain shortening of the C-1 side chain, or that a terminal carboxyl moiety in the C-1 side chain, is essential for the biological activity.

Octadecanoid Analogs and Coronatine

Induction of alkaloid biosynthesis was stringently sensitive to the presence of bulky, presumably non-hydrolyzable, modifications at the C-1 side chain. A series of phenolate esters was tested (Table 5) and each the *ortho*- and *meta*-hydroxybenzoates (**36**, **37**, respectively) were completely inactive in the *E. californica* test system. Analogous to this series was a jasmonic acid analog **38** that contains an *ortho*-hydroxyaniline amide functionality. This compound was fully active. It has been previously shown that coronatine **39** induces alkaloid biosynthesis in *E. californica* cell cultures, but slightly less efficiently than methyl jasmonate (Weiler *et al.*, 1994). The results obtained here with coronatine **39** (Table 6) are indeed consistent with those original observations. It becomes of interest whether **38** and **39** can be hydrolyzed *in planta* by action of a peptidase, which would result in a free acid side chain at C-1. Biological activity afforded by compounds **40**, **41** and **42** all correspond to that of coronatine (**39**). Again, peptidase action on these molecules may be a likely cause for release of a biologically active molecule. Aromatization of the six-membered ring of the annulated C_5-C_6 ring system of coronatine **39** forms a more planar molecule (compounds **43**, **44**) that is rendered biologically inactive. This is in all cases true regardless of the position of the amide side chain on the benzene ring system (compounds **45–49**).

Table 4 Induction Activity of 3-Oxo-2(2′(Z)-Pentenyl)-Cyclopentane-1-Alkanoic Acid Methyl Ester.

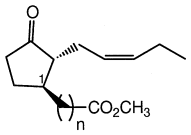
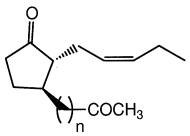
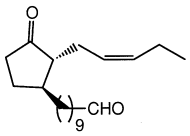
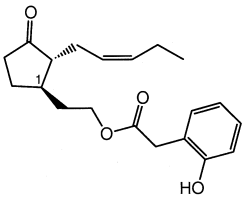
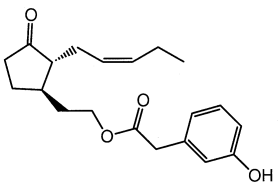
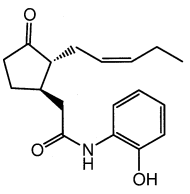
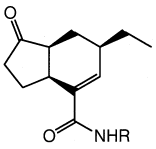
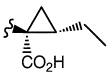
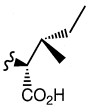
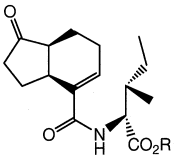
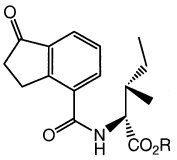
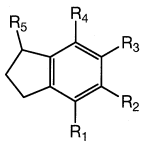
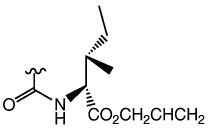
Structure	n	Compound number	Activity score
	1	**1**	++++
	2	**27**	+++
	3	**28**	++++
	4	**29**	++++
	5	**30**	++++
	6	**31**	++++
	7	**32**	+++
	3	**33**	+
	4	**34**	—
		**35**	—

Table 5 Induction Activity of Analogs of 3-Oxo-2(2′(Z)-Pentenyl)-Cyclopentane-1-Octanoic Acid Methyl Ester.

Structure	Compound number	Activity score
	**36**	—
	**37**	—
	**38**	++++

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

Table 6 Induction Activity of Analogs of Coronatine.

Structure	R	Compound number	Activity score
		39	+++
		40	+++
		41	++
	H	42	+++
	CH_3	43	—
	H	44	—
	R_1 R_2 R_3 R_4 R_5		
	X H H H =O	45	—
	H X H H =O	46	—
	H H X H =O	47	—
	H H H X =O	48	—
	X H H H H	49	—
X = 			

Discussion

The biosynthesis of antimicrobial benzo[*c*]phenanthridine alkaloids in plants is induced by a series of biotic and abiotic elicitor substances. Central to the elicitation process in California poppy cell suspension culture is likely transcriptional activation of several biosynthetic genes. The biosynthesis of the benzo[*c*]phenanthridine alkaloid macarpine (Figure 2) in *E. californica* is largely understood at the enzyme level (reviewed in Kutchan, 1998). The biosynthetic pathway begins with two molecules of L-tyrosine and involves two broad categories of enzymes – those that are elicitor-responsive and those that are constitutively expressed. The enzyme activities of seven cytochromes P-450 as well as the vesicular berberine bridge enzyme increase after addition of an elicitor substance to the culture medium. cDNAs encoding the berberine bridge enzyme (Dittrich and Kutchan, 1991), the cytochrome P-450 monooxygenase (*S*)-*N*-methylcoclaurine 3′-hydroxylase (Pauli and Kutchan, 1998) and a cytochrome P-450 reductase (Rosco *et al.*, 1997) have been isolated. RNA blot analyse using these three cDNAs as hybridization probes indicate that gene transcript accumulates after elicitor addition, suggesting that transcriptional activation underlies benzo[*c*]phenanthridine alkaloid accumulation (Pauli and Kutchan, 1998).

We have used here a facile test system based on the accumulation of benzo[*c*]phenanthridine alkaloids in *E. californica* cell suspension culture as an indicator of defense gene activation. Of the 200 jasmonic acid and coronatine analogs tested as part of this study, the representative results obtained with 49 were presented. The following can be summarized concerning structure-activity relationships.

There is a large degree of plasticity allowed at position C-3 of jasmonic acid in the activation of defense genes. The carbonyl moiety is not strictly required, but exocyclic double bond character appears necessary. The pentenyl side chain at C-2 cannot tolerate bulky groups at the terminal carbon and still be biologically active. Substitutions to the C-1′ position are tolerated if they can potentially undergo β-oxidation. Either an alkanoic acid or methyl ester is required at C-1, or a side chain that can be shortened by β-oxidation or by peptidase hydrolysis. Coronatine and various derivatives thereof are not as effective as jasmonic acid and derivatives in inducing benzo[*c*]phenanthridine alkaloid accumulation.

When compared to the results obtained by Blechert *et al.* (1999) with similar and identical test compounds, a striking observation is that activation of defense genes and activation of genes for mechanotransduction appear to be regulated by different compounds that lie along the jasmonic acid biosynthetic pathway. Coronatine, the close structural analog of 12-oxo-phytodienoic acid, and the octadecanoids are strong inducers of tendril coiling. On the other hand, methyl jasmonate is much more effective in activating benzo[*c*]phenanthridine alkaloid accumulation than are the octadecanoids or coronatine. These results add support to the hypothesis that the various physiological responses, such as to herbivory or pathogens, and mechanotransduction among others, that are

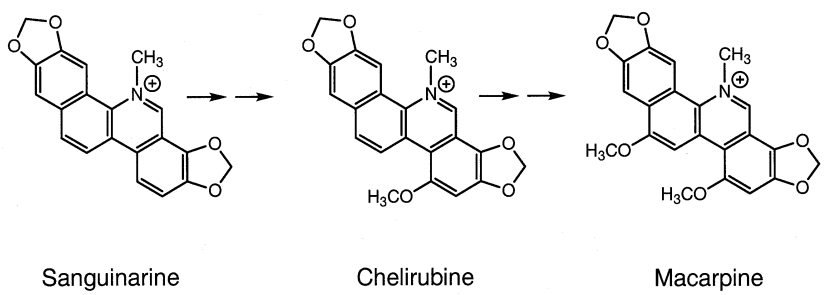


Fig. 2 Structures of Benzo[*c*]phenanthridine Alkaloids Produced by *E. californica* Cell Cultures in Response to Elicitor Addition.

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

activated by the octadecanoid pathway can be separated by chemical derivatization of the inducer molecule (Blechert *et al.*, 1997). This is strongly demonstrated by the different results obtained for defense gene activation herein with, for example, compounds **6, 7** or **29** (all strong inducers of benzo[*c*]phenanthridine alkaloid accumulation in *E. californica*) and the failure of those same compounds to induce mechanotransduction in *Bryonia* by Blechert *et al.* (1999). In order to facilitate the comparison of these two studies, the compound numbers have been retained as closely as possible in the present report.

These differential induction effects suggest the existence of branched signal transduction pathways in plants. A plant may also not respond to just one signal compound, but to a mixture of closely related structures. This is consistent with the idea of signal signatures proposed by Weber *et al.* (1997) and supported by Blechert *et al.* (1999) that would control the very complex processes initiated in plants upon elicitation. Selected compounds from this study may eventually serve as biochemical tools for the dissection of this complex signaling system in plants.

Materials and Methods

Plant Cell Cultures

Plant cell suspension cultures of *E. californica* were provided by the cell culture laboratory of the Lehrstuhl für Pharmazeutische Biologie, Universität München, Germany. Cultures were routinely grown in 1 liter conical flasks containing 400 ml of Linsmaier-Skoog medium (Linsmaier and Skoog, 1965) over 7 days at 23 °C on a gyratory shaker (100 rev/min) in diffuse light (750 lux).

Elicitation of *E. californica* Cell Culture

Each well of a 24-well plate was inoculated under sterile conditions with 1 ml of a 3–4 day old cell suspension culture grown under the conditions described above. Each well was then treated with either 1 or 3 μl of elicitor solution (stock solution, 100 mM, from which three additional dilutions, 10^{-1}, 10^{-2}, 10^{-3}, were prepared). In this manner, end concentrations of elicitors ranging from 0.1–300 μM were achieved. Each dose-dependency experiment contained negative controls that comprised cells treated only with the appropriate amount of solvent as used to dissolve the test substance. Each multiwell plate also contained methyl jasmonate treated cells as positive control. The multiwell plates were then sealed with parafilm and incubated 4 days at 23 °C on a gyratory shaker (200 rev/min) in diffuse light (750 lux). Each determination was performed at least three times in duplicate.

Extraction of Alkaloids from *E. californica* Cell Culture

The cell suspension culture contained in each well of the multiwell plate was transferred to a 1.5 ml conical plastic tube. The cells were collected by centrifugation for 10 min at room temperature and 14 000 *g*. The supernatant was removed and to the pellet was added 1 ml of an 80% ethanol solution containing 0.1% HCl. The suspension was incubated for 2 h at 60 °C. Insolubles were removed by centrifugation for 10 min at room temperature and 14 000 *g*. The clear supernatant was used for spectrophotometric quantitation of benzo[*c*]phenanthridine alkaloids at 490 nm on a Microplate Reader (MR600, Dynatech). To facilitate interpretation and to reduce data presentation, the results were scored relative to solvent controls as follows: ++++, 5–10 fold increase in activity as compared to solvent controls; +++, 3–5 fold increase in activity as compared to solvent controls; ++, 2–3 fold increase in activity as compared to solvent controls; +, 1.5–2 fold increase in activity as compared to solvent controls; -, no elicitation of alkaloid accumulation could be detected.

Origin of Test Substances

The various jasmonic acid and coronatine analogs used in this study were synthesized as part of a larger collaborative project designed to elucidate the structural requirements necessary for defense gene activation in plants as compared to other physiological responses that are effected by octadecanoids.

The synthesis of compounds **2**, **3**, **5–11**, **13**, **14**, **16–20**, **23–26**, **39–49** has already been described by Blechert *et al.* (1999). Compound **4** was synthesized from methyl jasmonate (**1**) by the Corey-Fuchs reaction. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and mass spectrometry (MS)-chemical ionization (CI) spectral data for **4**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 5.54-5.21 (m, 2H), 3.68 (s, 3H), 2.75-1.22 (m, 12H), 0.97 (t, J = 7.5 Hz, 3H); MS (CI): 381 (M^++H, 1), 289 (10), 287 (10), 245 (10), 243 (30), 207 (100).

Compound **12** was synthesized according to Brümmer *et al.* (1997); the ketal was removed according to the procedure described by Blechert *et al.* (1997). The synthesis of compound **15** starts from ketalized methyl jasmonate using the Simmons-Smith reaction followed by cleavage of the ketal as described. Spectral data for **15**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 3.7 (s, 3H), 2.76 (td, J = 15/4 Hz, 1H), 2.32-2.1 (m, 5H), 1.92-1.7 (m, 2H), 1.36-1.12 (m, 4H), 0.98 (t, 8 Hz, 3H), 0.9-0.54 (m, 3H), -0.24 (m, 1H); MS-electron impact (EI): 238 (M^+, 5), 207 (4), 165 (75), 123 (15), 109 (15), 83 (100); exact mass calculated for $C_{14}H_{22}O_3$ = 238.1568, mass found 238.1566.

The preparation of compounds **21** and **22** starts from the lithium enolate of ketalized methyl jasmonate, generated by treatment with lithium diisopropylamide as described by Blechert *et al.* (1997). The enolate was directly alkylated with propargylic halides and the ketal was removed as described. Exemplary spectral data are given for **21**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.45 (dtt, 11/7/1.5 Hz, 1H), 5.21 (dtt, 11/7/1.5 Hz, 1H), 3.73 (s, 3H), 2.77 (ddd, 9/6/6 Hz, 1H), 2.61 (ddd, 16/9/3 Hz, 1H), 2.50-2.02 (m, 10H), 2.01 (t, 3 Hz, 1H), 1.63 (m, 1H), 0.96 (t, 7.5 Hz, 3H); MS (EI): 262 (M^+, 1), 233 (10), 205 (15), 151 (100), 134 (20), 109 (25), 91 (27), 83 (41); exact mass calculated for $C_{16}H_{22}O_3$ = 262.1568, mass found = 262.1567.

Compounds **33** and **34** were prepared using a Kolbe reaction. The electrolysis was performed with jasmonic acid and commercially available keto acids as described by Blechert *et al.* (1997). Spectral data are given for **33**: ^{1}H-NMR (270 MHz, $CDCl_3$): δ = 5.4 (dtt, J = 11/7/1.5 Hz, 1H), 5.24 (dtt, J = 11/7/1.5 Hz, 1H), 2.44 (m, 2H), 2.3 (m, 3H), 2.12 (s, 3H), 2.0 (m, 3H), 1.88-1.46 (m, 6H), 1.36 (m, 1H), 1.22 (m, 1H), 0.92 (t, J = 8 Hz, 3H); MS (EI): 236 (M^+, 30), 218 (10), 168 (35), 151 (80), 83 (85), 43 (100); exact mass calculated for $C_{15}H_{24}O_2$ = 236.1776, mass found = 236.1776.

Synthesis of compounds **36** and **37** started with a lithium aluminum hydride reduction of ketalized methyl jasmonate. The resulting alcohol was converted to the target compounds using a Mitsunobu esterification reaction with the corresponding phenols. Exemplary spectral data are given for **36**: ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 7.25–7.05 (m, 2H), 7.0-6.82 (m, 2H), 5.44 (dtt, 11/7/1.5 Hz, 1H), 5.22 (dtt, 11/7/1.5 Hz, 1H), 4.23 (dd, 7/6 Hz, 2H), 3.66 (s, 2H), 2.4-2.26 (m, 3H), 2.22-1.20 (m, 9H), 0.96 (t, 8 Hz, 3H); MS (EI): 330 (M^+, 18), 262 (7), 151 (89), 134 (91), 107 (44), 83 (100).

The amide **38** was synthesized starting from jasmonic acid by preparation of the mixed anhydride and coupling with 2-aminophenol. Spectral data for **38**: ^{1}H-NMR (400 MHz, $CDCl_3$):

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

δ = 9.0 (s, 1H), 8.6 (s, 1H), 7.34 (dd, J = 8/1 Hz), 7.08 (m, 1H), 6.96 (dd, J = 8/1 Hz), 6.86 (ddd, J = 8/7/1 Hz, 1H), 5.42 (dtt, 11/7/1.5 Hz, 1H), 5.22 (dtt, 11/7/1.5 Hz, 1H), 2.8 (m, 1H), 2.26-1.84 (m, 10H), 1.5 (m, 1H), 0.9 (t, 8 Hz, 3H); MS (EI): 301 (M^+, 8), 193 (4), 181 (10), 151 (12), 109 (100); exact mass calculated for $C_{18}H_{23}NO_3$ = 301.1677, mass found = 301.1669.

The purity of the synthetic compounds was rigorously checked by HPLC or GC-MS. The absence of jasmonic acid and methyl jasmonate was carefully verified, since the presence of even trace amounts of these compounds would give false positive results.

Acknowledgements

This work was supported by the Bundesminister für Bildung und Wissenschaft (BMBF), Bonn, and by Fonds der Chemischen Industrie, Frankfurt. Coronatine was the kind gift of Prof. E.W. Weiler, Bochum, and 12-oxo-phytodienoic acid was the kind gift of Prof. M.H. Zenk, Munich.

References

Blechert, S., Brodschelm, W., Hölder, S., Kammerer, L., Kutchan, T.M., Mueller, M. J., Xia, Z.-Q., and Zenk, M. H. (1995). The octadecanoic pathway – signal molecules for the regulation of secondary pathways. Proc. Natl. Acad. Sci. USA *92*, 4099–4105.

Blechert, S., Bockelmann, C., Brümmer, O., Füßlein, M., Gundlach, H., Haider, G., Hölder, S., Kutchan, T.M., Weiler, E.W., and Zenk, M.H. (1997). Structural separation of biological activities of jasmonates and related compounds. J. Chem. Soc. Perkin Trans. *I* 3549–3560.

Blechert, S., Bockelmann, C., Füßlein, M., v. Schrader, T., Stelmach, B., Niesel, U., and Weiler, E.W. (1999). Structure-activity analyses reveal the existence of two separate groups of active octadecanoids in elicitation of the tendril-coiling response of *Bryonia dioica* Jacq. Planta *207*, 470–479.

Brümmer, O., Rückert, A., and Blechert, S. (1997). Olefin cross-metathesis with monosubstituted olefins. Chem. Eur. J. *3*, 441–446.

Cline, S.D., and Coscia, C.J. (1988). Stimulation of sanguinarine production by combined fungal elicitation and hormonal deprivation in cell suspension cultures of *Papaver bracteatum*. Plant Physiol. *86*, 161–165.

Creelman, R.A., and Mullet, J.E. (1997). Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. *48*, 355–381.

Dittrich, H., and Kutchan, T.M. (1991). Molecular cloning, expression and induction of berberine bridge enzyme, an enzyme essential to the formation of benzophenanthridine alkaloids in the response of plants to pathogenic attack. Proc. Natl. Acad. Sci. USA *88*, 9969–9973.

Dzink, J.L., and Socransky, S.S. (1985). Comparative *in vitro* activity of sanguinarine against oral microbial isolates. Antimicrob. Agents Chemother. *27*, 663–665.

Farmer, E.E., and Ryan, C.A. (1990). Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc. Natl. Acad. Sci. USA *87*, 7713–7716.

Farmer, E.E., and Ryan, C.A. (1992). Octadecanoid precursors of methyl jasmonate activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell *4*, 129–134.

Gundlach, H., Müller, M.J., Kutchan, T.M., and Zenk, M.H. (1992). Jasmonic acid as a signal transducer in elicitor-induced plant cell cultures. Proc. Natl. Acad. Sci. USA *89*, 2389–2393.

Holbrook, L., Tung, P., Ward, K., Reid, D.M., Abrams, S., Lamb, N., Quail, J.W., and Moloney, M.M. (1997). Importance of the chiral centers of jasmonic acid in the response of plants – activities and antagonism between natural and synthetic analogs. Plant Physiol. *114*, 419–428.

Ishikawa, A., Yoshihara, T., and Nakamura, K. (1994). Structure-activity relationships of jasmonates in the induction of expression of two proteinase inhibitor genes of potato. Biosci. Biotechnol. Biochem. *58*, 544–547.

Kiyota, H., Saitoh, M., Oritani, T., and Yoshihara, T. (1996). Synthesis and potato tuber-inducing activity of methyl 5′,5′,5′-trofluorojasmonate. Phytochemistry *42*, 1259–1262.

Koda, Y., Kikuta, Y., Kitahara, T., Nishi, T., and Mori, K. (1992). Comparisons of various biological activities of stereoisomers of methyl jasmonate. Phytochemistry *31*, 1111–1114.

Krumm, T., and Boland, W. (1996). Leucine and isoleucine conjugates of 1-oxo-2,3-dihydro-indene-4-carboxylic acid: mimics of jasmonate type signals and the phytotoxin coronatine. Molecules *1*, 23–26.

Krumm, T., Bandemer, K., and Boland, W. (1995). Induction of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*) by leucine- and isoleucine conjugates of 1-oxo- and 1-hydroxyindan-4-carboxylic acid: evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signalling pathway. FEBS Lett. *377*, 523–529.

Kutchan, T.M. (1993). 12-Oxo-phytodienoic acid induces accumulation of berberine bridge enzyme transcript in a manner analogous to methyl jasmonate. J. Plant Physiol. *142*, 502–505.

Kutchan, T.M. (1998). Molecular genetics of plant alkaloid biosynthesis. In: The Alkaloids Vol. 50, G. Cordell, ed. (San Diego, USA: Academic Press), pp. 257–316.

Linsmaier, E.M., and Skoog, F. (1965). Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. *18*, 100–127.

Miersch, O., Kramell, R., Pathier, B., and Wasternack, C. (1999). Structure-activity relations of substituted, deleted or stereospecifically altered jasmonic acid in gene expression of barley leaves. Phytochemistry *50*, 353–361.

Pauli, H.H., and Kutchan, T.M. (1998). Molecular cloning and functional heterologous expression of two alleles encoding (*S*)-*N*-methylcoclaurine 3′-hydroxylase (CYP80B1), a new methyl jasmonate-inducible cytochrome P-450 dependent monooxygenase of benzylisoquinoline alkaloid biosynthesis. Plant J. *13*, 793–801.

Quinkert, G., Adam, F., and Dürner, G. (1982). Asymmetrische Synthese von Methyljasmonat. Angew. Chemie *94*, 866–867.

Rosco, A., Pauli, H.H., Priesner, W., and Kutchan, T.M. (1997). Cloning and heterologous expression of cytochrome P450 reductases from the Papaveraceae. Arch. Biochem. Biophys. *348*, 369–377.

Sembdner, G., and Parthier, B. (1993). The biopchemistry and the physiology and molecular actions of jasmonates. Annu. Rev. Plant Physiol. Plant Mol. Biol. *44*, 569–589.

Taapken, T., Blechert, S., Weiler, E.W., and Zenk, M.H. (1994). Stereoselective synthesis of 7-substituted jasmonic acid derivatives and investigation of their biological activity. J. Chem. Soc. Perkin Trans. *I*, 1439–1440.

Ward, J.L., Gaskin, P., Beale, M.H., Sessions, R., Koda, Y., and Wasternack, C. (1997). Molecular modelling, synthesis and biological activity of methyl 3-methyl jasmonate and related derivatives. Tetrahedron *53*, 8181–8194.

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54

Weber, H., Vick, B.A., and Farmer, E.E. (1997). Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate family. Proc. Natl. Acad. Sci. USA *94*, 10473–10478.

Weiler, E.W., Albrecht, T., Groth, B., Xia, Z.-Q., Luxem, M., Liss, H., Andert, L., and Spengler, P. (1993). Evidence for the involvement of jasmonates and their octadecanoid precursors in the tendril coiling response of *Bryonia dioica*. Phytochemistry *32*, 591–600.

Weiler, E.W., Kutchan, T.M., Gorba, T., Brodschelm, W., Niesel, U., and Bublitz, F. (1994). The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. FEBS Lett. *345*, 9–13.

Weiler, E.W. (1997). Octadecanoid-mediated signal transduction in higher plants. Naturwissenschaften *84*, 340–349.

Yamane, H., Sugawara, J., Suzuki, Y., Shimamura, E., and Takahashi, N. (1980). Synthesis of jasmonic acid related compounds and their structure-activity relationships on the growth of rice seedlings. Agric. Biol. Chem. *44*, 2857–2864.

Zhang, Z.-P., Krumm, T., and Baldwin, I.T. (1997). Structural requirements of jasmonates and mimics for nicotine induction in Nicotiana sylvestris. J. Chem. Ecol. *23*, 2777–2789.

Received June 6, 2000; accepted July 7, 2000

Bereitgestellt von | Technische Universität Berlin
Angemeldet
Heruntergeladen am | 11.10.18 16:54