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

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Received 17 October 1995; revised version received 16 November 1995

Abstract **One of the most intriguing plant defense reactions against herbivores is the emission of volatiles as potentially attractive signals for the natural enemies of the attacking species. Like many other low and high molecular weight chemical defenses, volatile production is under the control of the octadecanoid signalling pathway leading to jasmonic acid (2) (threshold concentration of jasmonic acid giving rise to volatile induction in *Phaseolus lunatus*: ≈100 nmol·ml^{-1}). A significantly more active compound is the phytotoxin coronatine (3) (threshold concentration: ≥1 nmol·ml^{-1}). Methyl esters of 1-oxo-indanoyl-isoleucine (4) or 1-oxo-indanoyl-leucine (5), designed as readily available analogues of coronatin (3), have also been shown to be active (threshold concentration: ≥20 nmol·ml^{-1}). Crucially, their component parts, i.e. 1-oxo-indan-carboxylic acid and the amino acids are completely inactive. The pattern of emitted volatiles, produced by plants treated with these analogues, is largely identical to that released from coronatine- or jasmonic acid-treated plants. While the reduction of the carbonyl group of jasmonic acid (2) results in an inactive molecule, namely cucurbic acid, the methyl ester of the 1-hydroxy-indanoyl-isoleucine conjugate (8) is at least as effective as the corresponding oxo-derivatives (4) and (5) (threshold concentration: ≥20 nmol·ml^{-1}). The results support the concept that *epi*-jasmonic acid (1) may be converted into a leucine or isoleucine conjugate at an early stage in the natural signal transduction pathway. Their later interaction with a macromolecular receptor apparently requires enolization of the carbonyl group in the jasmonate moiety, yielding a planar segment which is essential for successful binding with the macromolecule. The resulting hydroxy group is implicated in the formation of a hydrogen bond in the ensuing ligand/receptor complex.**

Key words: Induction of volatiles; Jasmonic acid; Coronatin; 1-oxo-indanoyl-(iso)leucine; 1-hydroxy-indanoyl-(iso)leucine; *Phaseolus lunatus*

1. Introduction

Plants have developed highly effective and complex chemical systems to defend themselves against herbivores and microbial pathogens [1]. Recent studies have established that plants, under attack from a herbivore, may release volatiles that attract the natural enemies of the herbivore. For example, Dicke et al. have shown that Lima beans (*Phaseolus lunatus*) infested by spider mites emit volatiles that attract predatory mites. Undamaged, or mechanically damaged plants do not release such chemicals [2,3]. Another tritrophic interaction was described by Turlings et al. who studied volatile production by corn seedlings and cotton plants after caterpillar damage [4,5]. Both plants became attractive to predatory wasps several hours after herbivore damage. Often, but not necessarily, the response is systemic, with undamaged leaves of the injured plants also emitting volatiles [3,6].

Recently we have shown that the biosynthesis and emission of the volatiles is, like many other chemical defenses, under the control of the octadecanoid signalling pathway, and is effectively stimulated by free jasmonic acid [7,8]. For example, treatment of corn plants with jasmonic acid (**2**) results in emission of the same volatiles produced after damage by the lepidopteran caterpillar *Spodoptora exigua* [4,5]. Other, well established, defenses which are under the control of the jasmonate family lead to altered gene expression programs as that of high molecular weight proteinase inhibitors [9], the biosynthesis of proteinaceous antifungals (e.g. the pathotoxic thionines [10] and some hydroxyproline-rich proteins for cell wall strengthening [11]), the induction of the key enzyme of the phenylpropanoid pathway, phenylalanine ammonia lyase (PAL) [12], as well as stimulation of the biosynthesis of low-molecular phytoalexins like certain alkaloids [13], terpenoids and, in addition, the emission of ethylene [14]. Even the mechanoreceptors of *Bryonia dioica* rely on jasmonic acid for signal transduction [15] (for reviews see [16–21]).

Interestingly, many of these responses, like for example ethylene emission [14], alkaloid biosynthesis [13], tendril coiling [15], and volatile production [7] can be also provoked by application of coronatin (**3**) to plants or plant cell cultures [8]. Coronatin (**3**) is a phytotoxin which can be isolated from the fermentation broth of certain strains of *Pseudomonas syringae* [22] and *Xanthomonas campestris* [23]. In most assay systems coronatin (**3**) proved to be significantly more active (ca. 100 fold) than jasmonic acid (**2**).

To account for the high activity of coronatin (**3**) it was originally claimed that (**3**) may mimic 12-oxo-phytodienoic acid (12-oxo-PDA, cf. Fig. 1), a biologically active, early biosynthetic precursor of jasmonic acid (**2**) [24]. However, an even more important structural feature is the incorporation of the labile *cis*-disubstitution pattern of the biologically more active *epi*-jasmonic acid (**1**) [25] into the rigid bicyclic system of (**3**), which cannot be significantly altered by acid/base catalysis [26]

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This is part three of a series; for part two refer to ref. [8].

SSDI 0014-5793(95)01398-9

or isomerisation by an enzyme. Such a process is assumed to inactivate (**1**) by isomerisation to jasmonic acid (**2**) [27].

Taking into account that peptide bonds containing α-branched or conformationally strained, cyclic amino acids are generally (more) resistant towards hydrolysis [28], one could also look upon (**3**) as a structural analogue of an amino acid conjugate of *epi*-jasmonic acid (**1**). From the various, well known, amino acid conjugates of jasmonic acid (**2**) [29,30], the one containing isoleucine appeared to be particularly interesting, since this structural element is easily derived from the coronamic acid building block of (**3**) by formal opening the C(2)–C(3) bond of the cyclopropyl amino acid (cf. Fig. 1). We have postulated that modification of the coronafacic portion of the molecule to a 1-oxo-indan-4-carboxylic acid moiety would give a readily available analogue of coronatin (**3**) (see Fig. 1).

The current work provides evidence that synthetic analogues of (**3**) like, for example, 1-oxo-indanoyl-isoleucin (**4**) do, indeed, function as powerful signals for the induction of volatile biosynthesis in leaves of *P. lunatus*. Functional group manipulations of these readily available compounds allows further insight into details of the interaction of *epi*-jasmonic acid or its corresponding amino acid conjugates with a macromolecular receptor.

2. Materials and methods

2.1. Chemicals

1-Oxo-indan-4-carboxylic acid was synthesised from commercial 2-carboxycinnamic acid (Aldrich) according to the protocol of [31] (Fig. 2). The double bond was hydrogenated by stirring a 0.25 M solution of the cinnamic acid in 0.6 N aqueous NaOH under a hydrogen atmosphere in the presence of a palladium catalyst (Pd/C, 10%). The resulting saturated diacid was then cyclised by simple heating of the crude diacid together with $AlCl_3$/NaCl to 150°C. Pure 1-oxo-indan-4-carboxylic acid is obtained by crystallisation from acetone. Yield: 90%, overall.

Amino acid conjugates of 1-oxo-indan-4-carboxylic acid (1-oxo-ICA): general procedure. A well stirred solution of 1-oxo-ICA (1.0 mmol), the amino acid ester (1.0 mmol; methyl or allylester) and 1-hydroxybenzotriazole (HOBT) (ca. 1.5 mmol) in THF (10 ml) was cooled (0°C) and dicyclohexylcarbodiimide (DCC, 1.1 mmol) was added [32]. The reaction was allowed to stir for 12 h at rt. The amino acid conjugates were purified by chromatography on silica gel using either ether/hexane (3:1, v/v) or ethyl acetate/hexane (1:1, v/v) for elution.

2.2. Removal of the ester moiety

Free acids were obtained in high yield simply by stirring a solution of the corresponding allylester in ethanol/water (9:1, v:v) in the presence of a catalytic amount of $[(C_6H_5)_3P]_3RhCl$ [33]. Recrystallisation from methanol/ether afforded pure products. Full experimental data will be published elsewhere.

1-Oxo-indanoyl-L-isoleucyl methylester (**4**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.88–0.95 (m, 6 H), 1.14–1.27 (m, 1 H), 1.40–1.52 (m, 1 H), 1.92–2.03 (m, 1 H), 2.64 (t, 2 H), 3.27–3.42 (m, 2 H), 3.72 (s, 3 H), 4.76 (dd, 1 H), 6.57 (d, 1 H), 7.39 (t, 1 H), 7.80 (d, 2 H); MS (EI, 70 eV): 303 (M^+, 2), 247 (4), 244 (13), 176 (15), 159 (100), 131 (23), 103 (32), 77 (15).

1-Oxo-indanoyl-D-isoleucyl methylester (**4a**): ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 0.90–1.01 (m, 6 H), 1.16–1.36 (m, 1 H), 1.42–1.60 (m, 1 H), 1.92–2.12 (m, 1 H), 2.64–2.72 (m, 2 H), 3.28–3.49 (m, 2 H), 3.78 (s, 3 H), 4.76–4.34 (dd, 1 H), 6.66 (d, 1 H), 7.42 (t, 1 H), 7.82 (d, 1 H), 7.83 (d, 1 H); MS (EI, 70 eV): 303 (M^+, 2), 247 (5), 244 (16), 176 (15), 159 (100), 131 (21), 103 (26), 77 (11).

1-Oxo-indanoyl-L-alloisoleucyl methylester (**4b**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.88 (d, 3 H), 0.92 (t, 3 H), 1.11–1.26 (m, 1 H), 1.39–1.52 (m, 1 H), 1.94–2.06 (m, 1 H), 2.60 (t, 2 H), 3.22–3.38 (m, 2 H), 3.71 (s, 3 H), 4.85 (dd, 1 H), 6.64 (d, 1 H), 7.35 (t, 1 H), 7.74 (d, 1 H), 7.78 (d, 1 H); MS (EI, 70 eV): 303 (M^+, 2), 247 (12), 244 (17), 176 (10), 159 (100), 131 (20), 103 (24), 77 (14).

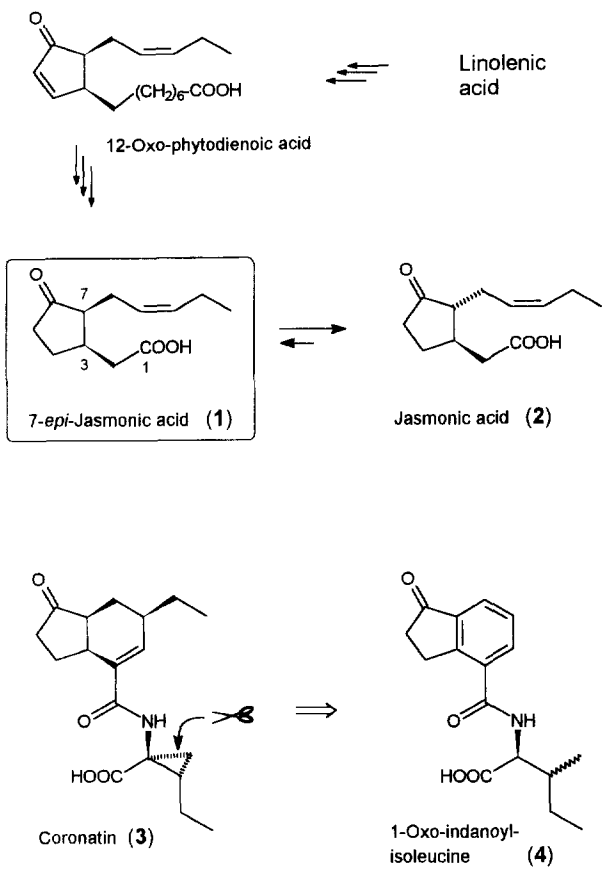

Fig. 1. Biosynthetic pathway from linolenic acid via 12-oxo-PDA and 7-epi-jasmonic acid (**1**) to jasmonate (**2**). The *cis*-disubstitution pattern of (**1**) is fixed in coronatin (**3**). Derivatives of 1-oxo-indanoyl(iso)leucine (**4**) are formally obtained from (**3**) by ring opening as indicated in the figure. The substituted cyclohexene moiety of (**3**) is simplified to an aromatic ring.

1-Oxo-indanoyl-L-leucyl methylester (**5**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.92 (d, 3 H), 0.94 (d, 3 H), 1.57–1.75 (m, 3 H), 2.62 (t, 2 H), 3.25–3.40 (m, 2 H), 3.71 (s, 3 H), 4.75–4.82 (m, 1 H), 6.53 (d, 1 H), 7.36 (t, 1 H), 7.78 (d, 2 H); MS (EI, 70 eV): 303 (M^+, 1), 247 (12), 244 (8), 159 (100), 144 (4), 131 (20), 103 (22), 77 (11).

1-Oxo-indanoyl-D-leucyl methylester (**5a**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.87 (d, 3 H), 0.90 (d, 3 H), 1.51–1.75 (m, 3 H), 2.59 (t, 2 H), 3.21–3.37 (m, 2 H), 3.68 (s, 3 H), 4.71–4.79 (m, 1 H), 6.43 (d, 1 H), 7.33 (t, 1 H), 7.74 (d, 2 H); MS (EI, 70 eV): 303 (M^+, 1), 247 (13), 244 (8), 159 (100), 144 (4), 131 (20), 103 (25), 77 (10).

1-Oxo-indanoyl-L-valyl methylester (**6**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.93 (d, 3 H), 0.97 (d, 3 H), 2.18–2.30 (m, 1 H), 2.62–2.66 (m, 2 H), 3.27–3.41 (m, 2 H), 3.73 (s, 3 H), 4.72 (dd, 1 H), 6.56 (d, 1 H), 7.39 (t, 1 H), 7.79 (d, 1 H), 7.81 (d, 1 H); MS (EI, 70eV): 289 (M^+, 4), 230 (14), 176 (15), 159 (100), 131 (21), 103 (26), 77 (13).

1-Oxoindanoyl-L-phenylalanyl methylester (**7**): ^{1}H-NMR (400 MHz, $CDCl_3$): 2.59 (t, 2 H), 3.11–3.31 (m, 4 H), 3.73 (s, 3 H), 4.99–5.05 (m, 1 H), 6.49 (d, 1 H), 7.05–7.10 (m, 2 H), 7.16–7.26 (m, 3 H), 7.34 (t, 1 H), 7.67 (d, 1 H), 7.77 (d, 1 H); MS (EI, 70 eV): 162 (87), 159 (100), 131 (42), 103 (33), 91 (24), 77 (14).

1-Hydroxy-indanoyl-L-isoleucyl methylester (**8**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.87–0.93 (m, 6 H), 1.10–1.30 (m, 1 H), 1.37–1.50 (m, 1 H), 1.80–2.00 (m, 2 H), 2.02–2.30 (s, br., 1 H), 2.38–2.48 (m, 1 H), 2.91–3.04 (m, 1 H), 3.16–3.31 (m, 1 H), 3.72 (s, 3 H), 4.74 (dd, 1 H), 5.16 (t, 1 H), 6.44 (d, 1 H), 7.24 (t, 1 H), 7.46 (d, 1 H), 7.51 (d, 1 H); MS (EI, 70 eV): 305 (M^+, 1), 287 (8), 161 (46), 143 (100), 133 (8), 115 (65), 89 (7), 77 (5).

Indanoyl-L-isoleucyl methylester (**9**): ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 0.91–1.01 (m, 6 H), 1.14–1.36 (m, 1 H), 1.40–1.59 (m, 1 H), 1.93–

2.16 (m, 3 H), 2.92 (t, 2 H), 3.16 (t, 2 H), 3.76 (s, 3 H), 4.82 (dd, 1 H), 6.45 (d, 1 H), 7.19 (t, 1 H), 7.33 (d, 1 H), 7.46 (d, 1 H); MS (EI, 70 eV): 289 (M^+, 10), 230 (5), 172 (6), 161 (10), 145 (100), 115 (37), 91 (9).

3-Acetyl-benzoyl-L-isoleucyl methylester (**10**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.83–0.91 (m, 6 H), 1.13–1.29 (m, 1 H), 1.39–1.51 (m, 1 H), 1.88–1.99 (m, 1 H), 2.52 (s, 3 H), 3.68 (s, 3 H), 4.71 (dd, 1 H), 7.10 (d, 1 H), 7.41 (t, 1 H), 7.90 (dt, 1 H), 7.96 (dt, 1 H), 8.27 (t, 1 H); MS (EI, 70 eV): 232 (27), 203 (13), 164 (21), 147 (100), 119 (9), 91 (20), 76 (12).

7-Methyl-jasmonoyl-L-isoleucyl methylester (**11**): ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 0.88–1.02 (m, 12 H), 1.06–1.22 (m, 1 H), 1.31–1.72 (m, 2 H), 1.81–2.52 (m, 11 H), 3.73 (s, 3 H), 4.56–4.67 (m, 1 H), 5.17–5.29 (m, 1 H), 5.40–5.51 (m, 1 H), 5.91–6.02 (m, 1 H); MS (EI, 70 eV): 222 (6), 187 (13), 164 (26), 146 (42), 128 (40), 109 (23), 86 (100), 69 (27), 55 (35).

2.3. Rearing of plant material

Lima beans (*Phaseolus lunatus*, 'Ferry Morse' var. *Jackson Wonder Bush*, kindly provided by the BASF AG, Ludwigshafen) were grown from seeds in unsterilized garden soil. Individual plants were grown in a pot (H=5.5 cm) at 25–27°C using daylight fluorescent tubes at ca. 270 $\mu E \cdot m^2 \cdot s^{-1}$ and a photophase of 12 h. Experiments were conducted with 10- to 15-day-old seedlings showing two fully developed leaves.

2.4. Induction experiments

Freshly cut shoots of *P. lunatus* were immediately placed into aqueous solutions of the test substance (1 $\mu mol \cdot ml^{-1}$). The shoots were preincubated at 25–27°C for 12 h and then transferred into a closed system (small desiccator, 750 ml) for volatile collection (24 h) [34] (see below). Illumination was maintained over the whole period of the experiment. Ca. 1–2 ml of the aqueous solution were consumed per shoot corresponding to a total uptake of 1–2 μmol of the test substance under standard conditions. Control experiments were made by placing freshly cut shoots into pure water, followed by volatile collection (see below). For each test compound the induction experiments and volatile collection was repeated at least six times.

2.5. Collection and analysis of headspace volatiles

The pre-treated plants were placed into a closed system (small desiccator, ca. 750 ml; illumination continued), and the emitted volatiles were continuously collected over a period of 24 h on small charcoal traps (1 mg charcoal, CLSA-Filter, Winterthur, Switzerland) using air circulation as described [34]. After desorption of the volatiles from the charcoal trap with dichloromethane (2 × 15 μl), addition of an internal standard (1-bromo-decane, 5 μl of a 7.2 mmolar solution in CH_2Cl_2) and adjustment to a total volume of 40 μl, the compounds were directly analysed by GC/MS. GC-conditions: DB 1 fused-silica capillary column (15 m × 0.25 mm) under programmed conditions (50°C for 2 min, then at 10°/min to 200°). MS: Fisons MD 800; GC-interface at 260°C, scan range 35–300 Da. Individual compounds were quantified by comparison of their peak areas to that of the internal standard.

3. Results

3.1. Induction of volatiles with 1-oxo-indanoyl-amino acid conjugates

The treatment of Lima bean shoots with jasmonic acid (**1**) (at 1 $\mu mol \cdot ml^{-1}$) results after 24 h in a massive emission of volatiles from the leaves [7,8]. The phytotoxin coronatin (**3**) is even more active (threshold $\geq$1 $nmol \cdot ml^{-1}$), but the pattern of the induced volatiles is largely identical. As shown in Fig. 3A the major compounds are fatty acid derived fragments (LOX-route; (3*Z*)-hex-3-enol acetate and (2*E*)-hex-2-enol acetate), and terpenoids (e.g. ocimene, linalool and 4,8-dimethylnona-1,3,7-triene; cf. Fig. 3A). Fig. 3B demonstrates, that the emission of most of the above volatiles can be, in fact, also triggered by application of the methyl ester of 1-oxo-indanoyl-L-isoleucin (**4**) to the plant (standard assay at 1 $\mu mol \cdot ml^{-1}$). However, (**4**) does not induce the biosynthesis of the C_{16} homoterpene (4,8,12-trimethyltrideca-1,3,7,11-tetraene). Moreover, instead

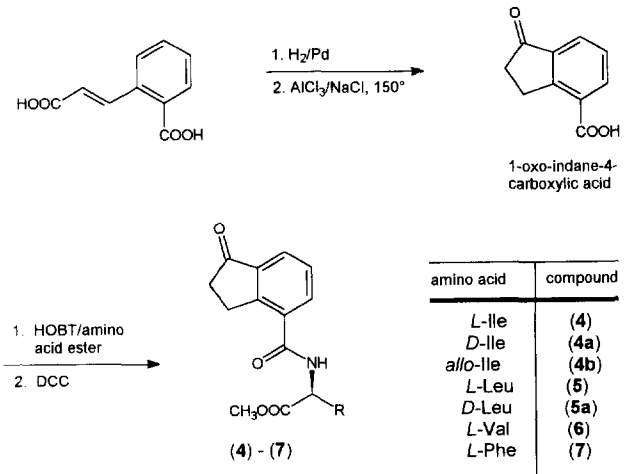

Fig. 2. Synthesis of amino acid conjugates of 1-oxo-indan-carboxylic acid [31,32].

of methyl salicylate (**g**) its nitrogen analogue methyl anthranilate (**k**) is scented; sometimes in quite high amounts (up to ≈5% of the total volatiles). As previously shown for jasmonic acid (**2**) (threshold concentration for volatile induction in *P. lunatus* ca. 100 $nmol \cdot ml^{-1}$ [7]), the extent of volatile production after treatment with the amino acid conjugate (**4**) is dose-dependent. Solutions with ca. ≈20 $nmol \cdot ml^{-1}$ are still effective in triggering the biosynthesis of the monoterpenoid $C_{10}H_{16}$ (**f**) and the corresponding alcohol $C_{10}H_{16}O$ ((**h**), Fig. 3A/B). Unlike coronatin (**3**), which is only active as the free acid [24], the 1-oxo-indanoyl-(iso)leucine conjugates (**4**) and (**5**) proved to be active as their methyl-, ethyl- and allyl esters or as free acids, indicating that substantial ester hydrolysis may take place under physiological conditions.

In contrast to the amino acid conjugates, their building blocks, namely free 1-oxo-indane-4-carboxylic acid and (iso)leucine, are completely inactive and, hence, prove that the intact conjugates are the only biologically active species.

The very high biological activity of this class of compounds prompted us to investigate next whether or not there is a specific interaction between a macromolecular receptor and the low-molecular weight amino acid conjugates of the 1-oxo-indan type. As to be taken from Fig. 4, there is, indeed, a high structure-activity dependence on the amino acid component of the molecule. Only conjugates containing leucine (**5**) or isoleucine (**4**), with a preference for isoleucine, proved to be biologically active. Conjugates with phenylalanine or even valine, which has only one CH_2 group less than leucine, turned out to be inactive. The same observation is made, if L-(iso)leucine is replaced by D-(iso)leucine. None of the conjugates (**4a**) and (**5a**) containing the unnatural D-amino acids were able to induce the production of volatiles. Hence, it follows that the leucine- or isoleucine conjugates of the 1-oxo-indane-carboxylic acid somehow resemble an endogenous, most likely jasmonate derived, signal which acts in the volatile-based plant defense against herbivores.

3.2. Modification of the indanoyl moiety

In order to investigate the interaction between the macromolecular receptor and the indanoyl moiety of the conjugate, several isoleucine conjugates, with modifications in the 1-oxo-

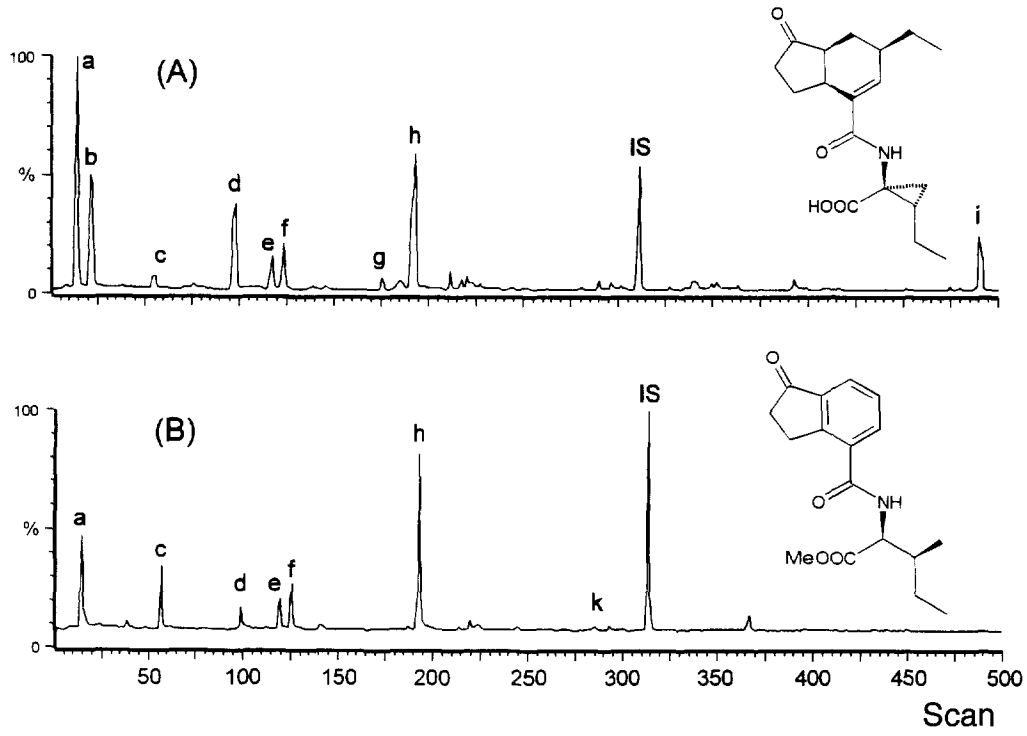

Fig. 3. Gas chromatographic profiles of the induced volatiles from Lima beans (*Phaseolus lunatus*) after pre-treatment of the plantlets for 12 h with coronatin at 1.0 μmol·ml^{-1} (trace (**A**)) or the methylester of 1-oxo-indanoyl-isoleucine at 1.0 μmol·ml^{-1} (trace (**B**)). After collection of the volatiles compounds were separated and identified by combined gas chromatography and mass spectrometry using a fused-silica column DB1 (15 m × 0.25 mm) under programmed conditions from 50°C (2 min) at 10°C·min^{-1} to 200°C, then at 30°C min^{-1} to 280°C. Identical numbers in the two volatile profiles denominate identical compounds. Identification of compounds: (**a**) (3*Z*)-hexenol acetate, (**b**) (2*E*)-hexenyl acetate, (**c**) β-ocimene, (**d**) linalool, (**e**) 4,8-dimethylnona-1,3,7-triene, (**f**) $C_{10}H_{16}$, (**g**) methyl salicylate, (**h**) $C_{10}H_{16}O$, (**i**) 4,8,12-trimethyltrideca-1,3,7,11-tetraene, (**k**) methyl anthranilate, (**IS**) internal standard: 1-bromodecane.

indan segment were studied (Fig. 4). If the cyclopentanone ring system is removed and replaced by a simple acetyl group, allowing rotation of the substituent around the single bond next to the ring, the biological activity is lost. Thus, planarity, or at least rigidly fixing the carbonyl group into a distinct orientation is essential for the ligand-receptor interaction. Unexpectedly, and in sharp contrast to the inactive cucurbic acid [35], reduction of the carbonyl group of (**4**) yielding racemic 1-hydroxy-indanoyl-isoleucin (**8**) does not result in an inactive molecule. As a matter of fact, the alcohol (**8**) appears to be at least as effective as (**4**). Activity is clearly bound to the hydroxy group, since complete removal of the oxygen functionality results in the very weakly active indanoyl-isoleucine (**9**). Another interesting, but completely inactive compound is the conjugate of racemic 7-methyl-7-*epi*-jasmonate with L-isoleucine (**11**). Similar results were previously obtained with the free 7-methyl-7-*epi*-jasmonic acid [36] which failed to induce the biosynthesis of volatiles in Lima beans or willow (*Salix alba*) as well (T. Koch and W.B., unpublished).

4. Discussion

Although the role of jasmonic acid and that of some early biosynthetic precursors has been established as an important control element for secondary pathways, until now, virtually nothing was known about the molecular events after the initial release of free jasmonic acid, following plant damage. For example, the action of *S. littoralis* caterpillars feeding on leaves of *Vicia faba* leads to a transiently enhanced level of free jasmonic acid in the plant, reaching a maximum after 45 min and subsequently returning to background levels within about 2 hours [35]. Early transient levels of jasmonic acid, in response to damage, have been also observed in wounded soybean (*Glycine max*) [37], in cell cultures of the grass *Agrostis tenuis* after treatment with an elicitor from the yeast *Phytophthora megasperma* [35] and in *Nicotiana tabacum* shoots [38]. Initial, exploratory investigations have revealed some of the structural elements of jasmonic acid which are required for its function as a signal molecule. The disubstituted cyclopentanone and the double bond of the C_5-side chain are essential for biological activity [35]. Furthermore, the higher substituted 7-methyl-7-*epi*-jasmonate turned out to be inactive in all previous bioassays [36].

It should be kept in mind that the release of jasmonic acid is, so far as examined in cell cultures or attacked plants, an early and transient event, while the synthesis of low molecular weight defensive compounds like, for example, alkaloids and volatiles is a relatively long term reaction, which usually occurs about 10–24 h after jasmonate treatment or herbivore damage [7,13,24,35]. The data, presented here, for odour induction which represents only one of the many jasmonate mediated defense strategies, are consistent with a functional model which assumes that the transiently released jasmonic acid is rapidly metabolised, e.g. for inactivation of the signal, and that at least a certain fraction of the acid is converted into amino acid conjugates with leucine and isoleucine. The conjugates or even

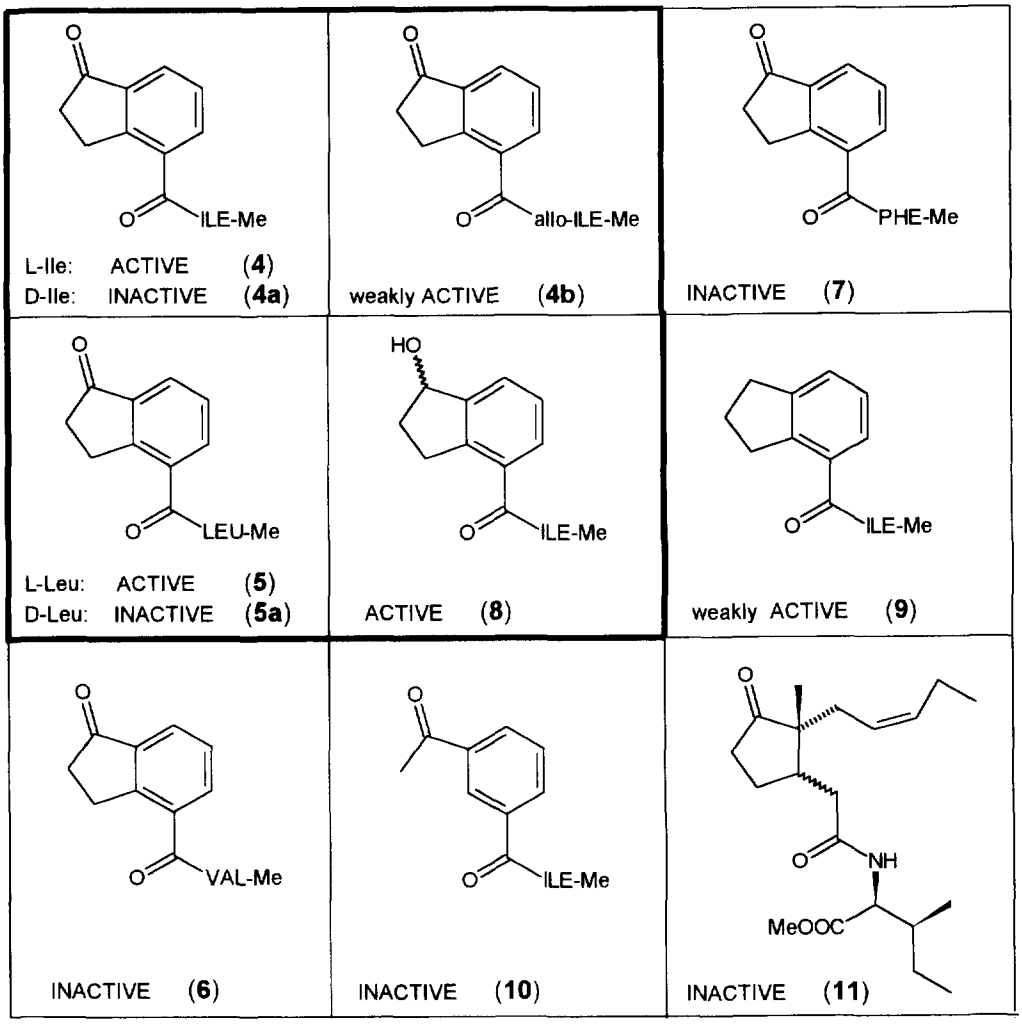

Fig. 4. Biological activities of the various amino acid conjugates of 1-oxo-indan-carboxylic acid. All active compounds are derived from L-(iso)leucine; compounds framed by the bold lines. Derivatives from D-amino acids are generally inactive.

further processed derivatives thereof are assumed to be, besides or together with jasmonic acid, the next elements in the signal transduction chain. The evidence is as follows: (i) neither 1-oxo-indan-carboxylic acid nor leucine or isoleucine are able to trigger the biosynthesis of volatiles; (ii) only the intact amino acid conjugates (**4**) and (**5**) are biologically active, showing a threshold level (≥ 20 nmol·ml^{-1}) which is lower than that reported for jasmonic acid (ca. 100 nmol·ml^{-1}) [7]; (iii) the patterns of induced volatiles after treatment with free jasmonic acid (**2**), coronatin (**3**) or the (iso)leucine conjugates are largely identical (cf. also ref. [7]); (iv) the interaction of the receptor with the amino acid part of conjugate is highly specific, discriminating between D- and L-(iso)leucine, and is apparently optimised for the side chain of (iso)leucine, since the conjugate with the closely related amino acid valine is inactive; (v) the indanoyl conjugates are structurally related to coronatine (**3**), a known mimic of jasmonate action [24,39].

It has been argued that the biological activity of coronatin (**3**) may be due to its similarity to 12-oxo-PDA [24], an early precursor of jasmonic acid (cf. Fig. 1), but this interpretation clearly not accounts for volatile induction, since 12-oxo-PDA induces a volatile pattern which is completely different from that obtained after treatment with jasmonic acid, coronatin (**3**) or the title compounds (J. Hopke and W.B., to be published). Hence, it follows that coronatin, as well as the 1-oxo-indanoyl-(iso)leucine conjugates (**4**) and (**5**) may represent analogues of a jasmonate derivative which occurs later in the plant's signal transduction chain. At this point it must be emphasised that the induction of volatiles can be also provoked by application of jasmonoyl-(iso)leucine to leaves of the Lima been (U. Ritgen and W.B., unpublished), but the results need to be independently verified, since an enzymatic hydrolysis of the amide bond yields jasmonic acid which may act in turn as a signal. Furthermore, derivatives of jasmonic acid which do not allow the formation of an amide bond are inactive (J. Hopke and W.B., unpublished).

Further insights into the interaction of the low-molecular weight ligand with a receptor stem from the functional group modifications of the indanoyl moiety. As mentioned, the 1-hydroxy-indanoyl-isoleucine conjugate (**8**) is at least as active than the 1-oxo-derivative (**4**). In the case of jasmonic acid this transformation leads to the completely inactive cucurbic acid

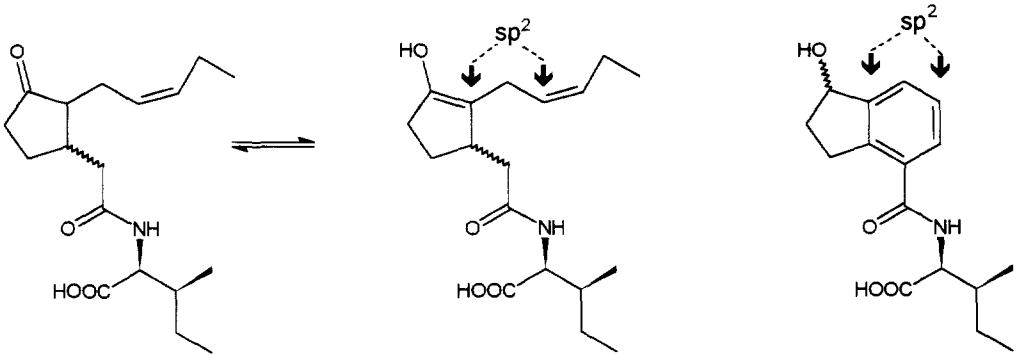

Fig. 5. Illustration of the enolisation process of a jasmonic acid conjugate with L-isoleucine during or prior to its interaction with the functional groups of the receptor. This reaction creates a new sp^2-centre and planarizes the 'upper half' of the molecule; the corresponding 1-hydroxy-indan (**8**) is intrinsically planar due to the presence of the aromatic ring.

[35]. We assume that the pronounced biological activity of 1-hydroxy-indanoyl-isoleucine (**8**) reflects a peculiar structural feature of the jasmonates during their interaction with the macromolecular host. The data support the idea that the jasmonate enolizes during or prior to its interaction with the functional groups of the receptor. This process creates a new sp^2-centre and planarizes the 'upper half' of the molecule (Fig. 5).

Such a structural modification is clearly not possible with 7-methyl-7-*epi*-jasmonic acid or cucurbic acid and, hence, these and related derivatives are inactive. In the case of 1-hydroxy-indanoyl-isoleucine (**8**) the planar element is intrinsically present and the molecule is able to interact with the macromolecular receptor. The role of the hydroxy group (or the enol from jasmonic acid) might be seen in the formation of a hydrogen bond with an appropriate functional group at the receptor site. The very low activity of the deoxygenated, but planar indanoyl-isoleucine (**9**) supports this view. Moreover, directionality of the mutualistic interactions and steric constraints, as exerted by the planar segment of the molecule are essential for a successful signal transduction. The monocyclic isoleucine conjugate of the 3-acetylbenzoic acid (**10**), having a side chain which is able to rotate, is inactive although all the required functional groups are present.

While the enolization and the formation of a hydrogen bond between the jasmonate moiety and macromolecular receptors may be a general phenomenon (7-methyl-7-*epi*-jasmonic acid appears to be generally inactive), the overall importance of amino acid conjugates in the octadecanoid signalling pathways of plants remains to be established. As a word of caution, even high concentrations of 1-oxo-indanoyl-isoleucine did not induce coiling in the touch sensitive tendrils of *B. dioica* (E. Weiler, personal commun.). Also, certain amino acid conjugates of jasmonic acid induce gene expression in barley, apparently without being hydrolysed, but the corresponding derivatives of the 1-oxoindan-4-carboxylic acid do not (K. Wasternack et al., unpublished).

For the future it remains to be established which, and to what extent, other high and/or low molecular weight chemical defenses are triggered by the above conjugates in the Lima bean and, most important, in other plants. Taking into account, however, that coronatine (**3**) induces many of the jasmonate based reactions, the phytotoxin and, now, the readily available 1-oxo- and 1-hydroxy-indanoyl conjugates (**4**) and (**8**) promise to become valuable tools for unravelling the functional significance of amino acid conjugates in plant signal transduction pathways.

Acknowledgements: Financial support by the Deutsche Forschungsgemeinschaft (Bonn) and the Fonds der Chemischen Industrie (Frankfurt am Main) is gratefully acknowledged. We also thank the BASF (Ludwigshafen) and the Bayer AG (Leverkusen) for generous supply with chemicals and solvents.

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