# 6-Substituted Indanoyl Isoleucine Conjugates Mimic the Biological Activity of Coronatine[‡]

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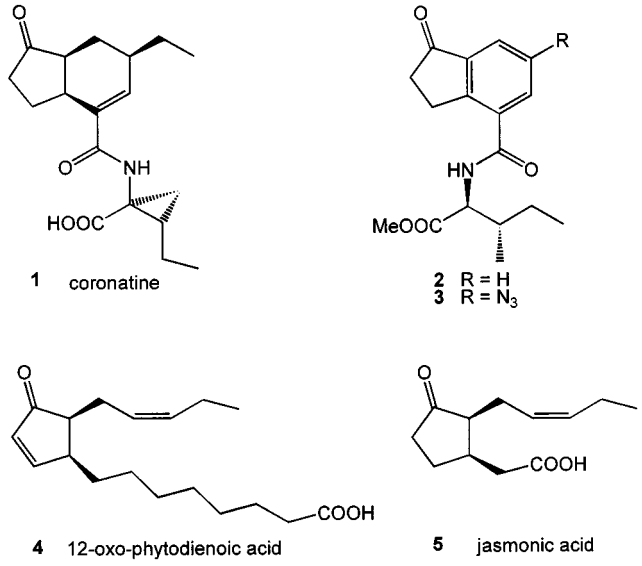
The 6-substituted indanoyl isoleucine conjugates of type **12** are potent elicitors of plant secondary metabolism and tendril coiling. The 6-substituted indanoyl carboxylic acid is available in four steps from 1,2,3,4-tetrahydronaphthalene (**6**). Key steps of the synthesis involve double acylation of **6** followed by oxidative cleavage and intramolecular Friedel–Crafts acylation of the resulting dicarboxylic acid **10**. The conjugate with isoleucine (**12**) triggers volatile biosynthesis in the Lima bean at 10 μm and coiling of the touch-sensitive tendrils of *Bryonia dioica* at 20 μm.

## Introduction

In recent years the phytotoxin coronatine (**1**; Scheme 1) has attracted considerable interest since it mimics many of the biological activities generally associated with jasmonic acid (**5**), one of the most powerful low molecular weight signalling molecules involved in plant stress responses.[4] Coronatine (**1**) is a conjugate of the polyketide coronafacic acid with the rare cyclopropyl amino acid coronamic acid. The phytotoxin is produced by several pathovars of *Pseudomonas syringae* (e.g. *tomato*, *glycinea*, *atropurpurea*) and was first isolated by Ichihara et al. in 1978 from a fermentation broth of *P. syringae* var. *atropurpurea*.[5]

In addition to coronatine, several other conjugates of coronafacic acid with, for example, norcoronamic acid, L-isoleucine, and L-valine have been isolated and found to be biologically active.[6] The application of **1** to higher plants elicited a spectrum of responses, especially diffuse chlorosis,[4] tendril coiling in *Bryonia dioica*[7] and emission of ethylene,[8] as well as the biosynthesis of terpenoids and other volatiles[9] that are often involved in the complex network of signalling among plants, herbivores, and their parasites.[10] Although distinct differences between the effects of coronatine (**1**), 12-oxophytodienoic acid (**4**), and jasmonic acid (**5**) (Scheme 1) were reported for some plants,[11] **1** generally appears to mimic the effect of bioactive compounds of the octadecanoid signalling pathway.[7]

Interestingly, and important for practical applications, in most assays **1** proved to be much more active than the genuine lipid-derived signals. For example, **1** stimulates the production of the antitumor agent paclitaxel in cell cultures of *Taxus media* more efficiently than jasmonic acid (**5**) or methyl jasmonate (JAMe).[12] Other inducible products are certain phytoalexins from rice.[13] Due to this scientific and economically interesting profile of biological activities, there is a need for efficient and high-yielding approaches to **1** and related structures. As yet, approximately 15 syntheses of **1** have been reported generally following one of three different protocols.[4] The coronafacic acid moiety has been assembled by inter- and intramolecular Diels–Alder reactions,[14–17] intramolecular ring closure of carbonyl compounds,[15,18,19] or by cleavage of tricylic precursors.[20,21] However, none of the published protocols is able to generate large quantities of coronafacic acid and related bioactive compounds in a few steps and in high overall yield.




Scheme 1

To overcome these difficulties, we designed and developed the structurally simpler and readily available indanoyl isoleucine conjugates such as **2** as functional analogs

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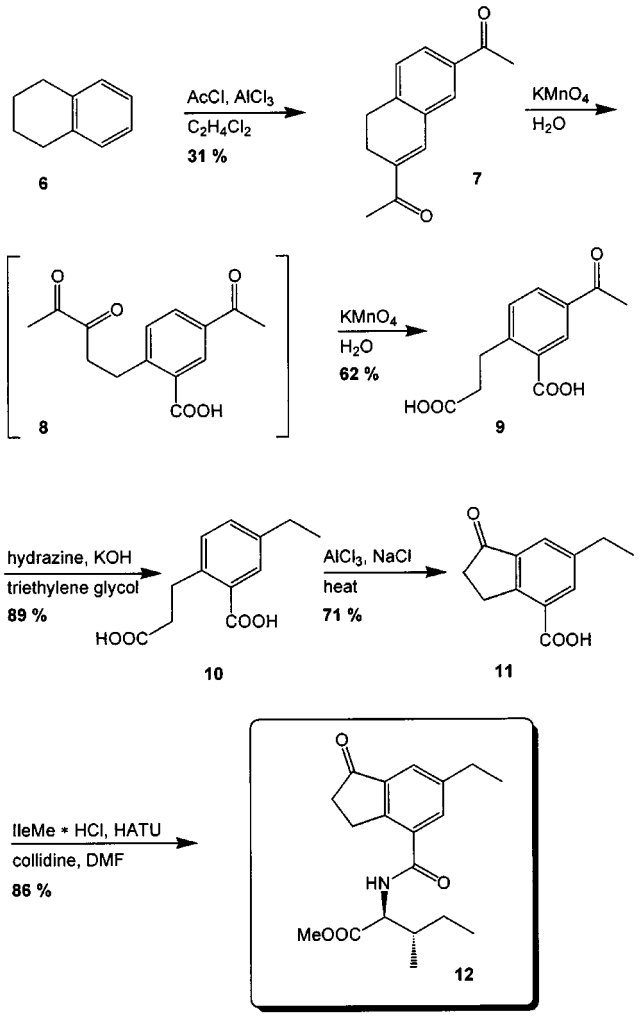
of **1**. Like **1**, the aromatic analogue **2** is an efficient elicitor of plant secondary metabolism.[1] The spectrum of the biological effects of **2** studied so far was, however, clearly more jasmonate-related than that of **1**, which stimulates a broader range of biological responses.[1] Recently such conjugates were successfully applied to analyse the signalling pathway of touch-sensitive tendrils of *Bryonia dioica*,[22] as well as to stimulate the production of benzo[*c*]phenanthridine alkaloids in cell cultures of *Eschscholzia californica*.[23]

Owing to its high biological activity and selectivity, the core structure of **2** was used to develop a photoreactive probe for tagging binding proteins and/or receptors.[24] Interestingly, the introduction of the photolabile azido group at C-6 of the indanoyl moiety not only strongly enhanced the biological activity of the compound but at the same time modified the biological activity of **3** (volatile induction) more in the direction of **1**.[24] This observation prompted us to synthesise 6-alkyl indanoyl isoleucine conjugates as potential elicitors of plant secondary metabolism adequate to the phytotoxin **1**. Here we report a novel and general approach to 6-substituted indanoyl amino acid conjugates which results in a rapid and high-yield synthesis of bioactive compounds that stimulate the secondary metabolism of plants.

## Results and Discussion

1-Oxoindan-4-carboxylic acid, the original mimic of coronafacic acid, is readily available by intramolecular Friedel−Crafts acylation of 2-(2-carboxyethyl)benzoic acid.[25] Attempts to functionalise the aromatic nucleus of **2** at C-6 by bromination, nitration, acylation etc. failed owing to the strong deactivation by the two adjacent carbonyl groups. An efficient alternative approach directly providing the required substitution pattern of the aromatic nucleus was found in a previous report on acylation of 1,2,3,4-tetrahydronaphthalene (**6**).[26] In the presence of $AlCl_3$ and acetyl chloride, the aromatic hydrocarbon suffers a sequence of reactions that leads to the diketone **7**. Subsequent oxidative cleavage of **7** with aqueous $KMnO_4$ proceeds smoothly with exclusive cleavage of the nonaromatic double bond to yield the triketone intermediate **8**, which is rapidly further oxidised to give the dicarboxylic acid **9** (Scheme 2).[26]

The second, rapid cleavage reaction of the vicinal diketone moiety **8** is of high synthetic value since it removes the complete carbon skeleton of the second acylation at the saturated ring moiety, resulting only in the C-5-acylated aromatic nucleus with the correct substitution pattern required for the envisaged intramolecular Friedel−Crafts cyclisation. Moreover, the sequence **6** → **9** is generally applicable, since acylation of **6** with other acyl halides proceeds by analogy. For example, acylation of **6** with butanoyl chloride and subsequent oxidative cleavage with $KMnO_4$ provided a dicarboxylic acid of type **9** in high yield bearing a 1-oxobutyl group at C-5. Attempts to achieve an intramolecular Friedel−Crafts acylation with **9** failed owing to the




Scheme 2

deactivating effect of the keto group. However, after reduction of the carbonyl group with hydrazine according to the procedure of Huang-Minlon in boiling triethylene glycol,[27] the resulting 5-ethyl dicarboxylic acid (**10**) could be smoothly cyclized. Heating with $AlCl_3$/NaCl furnished the 6-ethyl-1-oxoindan-4-carboxylic acid (**11**) in 71% yield. The protocol of Scheme 2 offers several advantages. The aromatic analogue of coronafacic acid is available in only four simple operations and has a good overall yield (34% from **7**). All transformations can be easily performed on a large scale (ca. 10 g) and do not require dedicated reaction conditions or reagents.

Conjugation of the indanoyl carboxylic acid **11** with the hydrochloride of the methyl ester of isoleucine was best achieved in DMF with HATU [*O*-(7-aza-1-benzotriazolyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate] for activation of the amino acid and the indanoyl moiety.[28] Yield (89%), workup, and purification of the product were superior to our previous protocol with 1-hydroxybenzotriazole (HOBT), ethylmorpholine, and dicylohexylcarbodiimide for condensation.[29] The amino acid conjugate **12** crystallised as monoclinic colourless prisms.

Two pairs of symmetry-independent molecules of the indanoyl conjugate **12** are jointly packed into a single unit

cell. The two aromatic systems are fixed in a sandwich-like fashion and kept in an *anti*-orientation by two hydrogen bonds between the weakly acidic amide N−H and the lone pairs of the oxygen atoms of the keto groups (Figure 1).

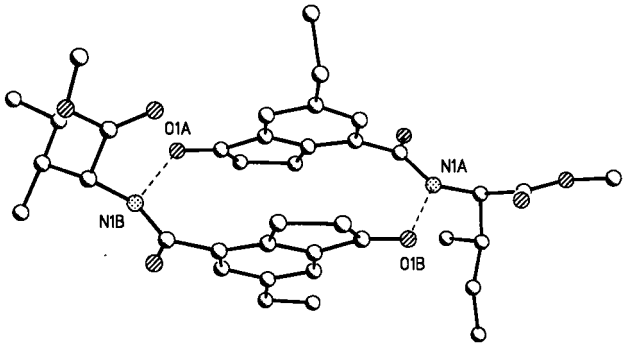



Figure 1. Crystal structure of the conjugate **12**; two pairs of two sandwich-packed molecules are present in a single unit cell; for clarity only a single pair is shown

### Induction of Volatile Biosynthesis and Tendril Coiling

Several microbial- or insect-derived high- and/or low-molecular-weight metabolites have been shown to induce the biosynthesis of volatiles in plants. Their elicitor activity is often based on up-regulation of the octadecanoid pathway.[2] Coronatine (**1**) apparently bypasses the activation of the lipid-based signalling pathway by interacting directly with the receptors or binding proteins of the genuine signals such as 12-oxo-phytodienoic acid (**4**) and/or jasmonic acid (**5**).[7,22] To assess the profile of activities of an elicitor, the analysis of a blend of induced volatiles is of particular value since the spectrum of emitted compounds comprises many metabolites from very different pathways (see Figure 2). Since a complex network of signals individually regulates the different pathways, differences in the elicitor activity of test compounds may be reflected in qualitative and/or quantitative composition of the volatile blends. This is clearly the case for the novel 6-ethyl indanoyl isoleucine **12** and the unsubstituted parent compound **2**. In contrast to **2**, the novel elicitor **12** proved to be ca. 30−50-fold more active with a threshold concentration of less than 10 μM. Significant qualitative differences of the volatiles elicited by the two compounds become apparent from Figure 2.

Although the majority of the compounds are induced by **2** and **12** in a qualitatively and quantitatively comparable fashion (e.g. ocimene, linalool, and the two unknown monoterpenoids $C_{10}H_{14}$ and $C_{10}H_{16}O$), phenylacetonitrile, methyl salicylate and the $C_{16}$ terpenoid TMTT (see Figure 1) were induced only by treatment with **12** and coronatine (**1**). The $C_{11}$ terpenoid hydrocarbon 4,8-dimethylnona-1,3,7-triene (DMNT) is present in both volatile blends, but the up-regulation of its biosynthesis is more pronounced after treatment with **12**. In summary, the volatile blend resulting from elicitation with **12** is closer to the effect of coronatine than to that of **2** or jasmonic acid (**5**). Unlike coronatine (**1**), both indanoyl conjugates **2** and **12** can be applied as esters (reactive methyl- and allyl esters preferred)

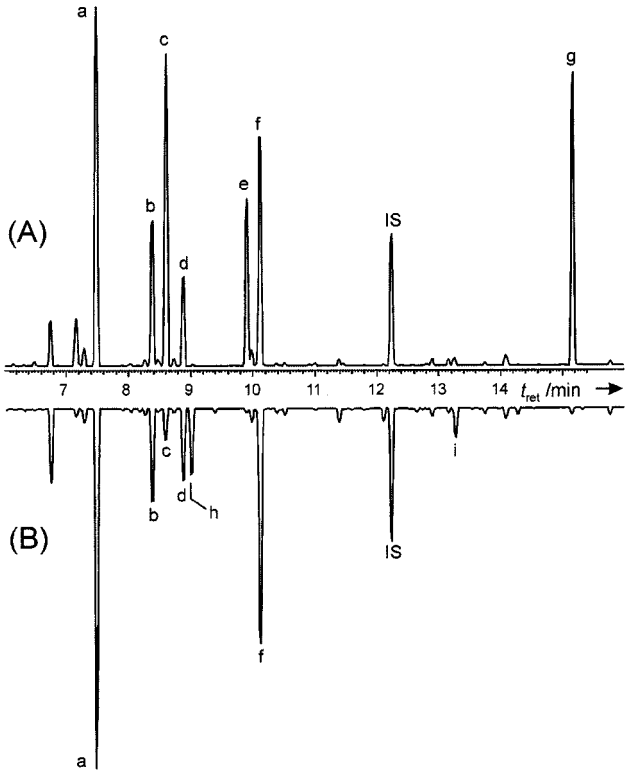



Figure 2. Volatile blends emitted from leaves of the Lima bean *P. lunatus* after treatment with the 6-ethyl conjugate **12** (Figure 2A) and the unsubstituted parent compound **2** (Figure 2B); identification of compounds: (a) β-ocimene, (b) linalool, (c) 4,8-dimethylnona-1,3,7-triene (DMNT), (d) $C_{10}H_{14}$, (e) methyl salicylate, (f) $C_{10}H_{16}O$, IS: internal standard (1-bromodecane), (g) 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), (h) phenyl acetonitrile, (i) caryophyllene; volatiles were collected by absorption onto carbon traps;[36] Separation and identification of the compounds were achieved by GLC-MS

since phytogenic esterases are apparently able to generate the free acids required for elicitation.

The emission of significant amounts of methyl salicylate after stimulation with **12** suggests that the novel elicitor may be also able to enhance the plant's resistance, which is generally mediated by salicylate. Indeed, analysis of the internal level of salicylic acid in plants pre-treated with **12** confirmed a strongly enhanced level of salicylic acid (eightfold increase of salicylate).

Like coronatine **12** is also a potent elicitor of the coiling reaction of touch-sensitive tendrils of *Bryonia dioica.* The coiling reaction was most efficiently induced by coronatine (**1**), with a threshold concentration at ca. 2 μM.[7] A 10-fold higher concentration of **12** was required to induce a comparable coiling reaction (for details, see Experimental Section). In contrast to **12**, the conjugate **2** induced a coiling response only at rather high concentrations in the range of ca. 1 mM.

In addition to the induction of volatile biosynthesis in the lima bean and tendril coiling in *Bryonia dioica*, **12** triggers a number of additional responses in other plants. Details of the broad spectrum of bioactivities of the compound and its application to enhance the production of valuable products in plant cell cultures will be reported in due course.

## Conclusion

The structurally simple and easily accessible conjugates of 1-oxoindan-4-carboxylic acid with isoleucine as the amino-acid building block are efficient mimics of the phytotoxin coronatine (**1**). The introduction of an additional substituent at C-6 of the indanoyl moiety further enhances the activity of the compounds (ca. 30−50-fold with respect to **2**) and at the same time tunes the activity profile of the compounds more to that of coronatine (**1**). The novel approach provides a rapid and convenient access to large quantities of the highly active compounds. The protocol is flexible and allows the introduction of different substituents at C-6 of the indanoyl moiety, eventually leading to elicitors with different activity profiles.

## Experimental Section

**General:** Reactions were performed under argon. Solvents were dried according to standard methods. Melting points were determined with Büchi B-540 apparatus. IR: Bruker Equinox 55 FTIR Spectrophotometer. $^1$H and $^{13}$C NMR: Avance DRX 500 spectrometer; $CDCl_3$ or [D$_6$]DMSO as solvent. Chemical shifts of $^1$H and $^{13}$C NMR are given in ppm (δ) downfield relative to TMS as internal standard. GLC-MS (70 eV): Finnigan GCQ, equipped with a fused silica capillary, coated with DB5 (30m × 0.25 mm); helium served as carrier gas. HR-MS: Micromass MasSpec 2. Silica gel: Si 60 (0.200−0.063 mm, E. Merck, Darmstadt, Germany) was used for chromatography. Thin layer chromatography was performed with silica gel plates from Merck (60 $F_{256}$).

**1-(7-Acetyl-5,6-dihydronaphthalen-2-yl)ethanone (7):** Acetyl chloride (19.0 g, 240 mmol) was added slowly at room temp. to a well-stirred solution of $AlCl_3$ (43.0 g, 320 mmol) in ethylene dichloride (20.0 mL) followed by slow addition of a solution of 1,2,3,4-tetrahydronaphthalene (10.0 g, 75.6 mmol) in $C_2H_4Cl_2$ (8.0 mL). When the vigorous reaction had ceased, the solvent was removed under reduced pressure, and the residue was heated to 100° C for 2 h. Pure product was obtained by distillation at reduced pressure. B.p.: 190−200° C /8 × $10^{-3}$ bar. Yield: 5.1 g, 23.8 mmol (31%). The highly viscous oil solidified while standing to give a colourless crystalline solid. M.p.: 76 °C. $^1$H NMR ($CDCl_3$, 500 MHz): δ = 2.36 (s, 3 H), 2.50−2.55 (m, 5 H), 2.80 (t, 2 H, *J* = 8.2 Hz), 7.20 (d, *J* = 7.9 Hz, 1 H), 7.35 (s, 1 H), 7.74 (s, 1 H), 7.76 (d, *J* = 7.9 Hz, 1 H). − $^{13}$C NMR ($CDCl_3$, 125 MHz): δ = 20.5 ($CH_3$), 25.2 ($CH_3$), 26.4 ($CH_2$), 27.5 ($CH_2$), 127.9 ($CH_{arom}$), 128.1 ($CH_{arom}$), 129.6 ($CH_{arom}$), 132.8 (C=C), 135.9 ($C_{arom}$), 136.0 ($C_{arom}$), 138.8 (C=C), 142.8 ($C_{arom}$), 197.1 (C=O), 198.1 (C=O). − IR (KBr): ṽ = 3055, 3004, 2954, 2894, 2839, 1682, 1657, 1622, 1592, 1411, 1386, 1350, 1285, 1205, 833 cm$^{-1}$. − MS (70 eV): *m*/*z* (%) = 214 (100) [M$^+$], 199 (80), 171 (82), 156(11), 141 (7), 128 (50). − HR-MS [M$^+$]: calcd. 214.0994; found 214.0990

**5-Acetyl-2-(2-carboxyethyl)benzoic acid (9):** The diketone **7** (4.5 g, 21 mmol) was dissolved in the minimum amount of 1,2-dichloroethane (ca. 5 mL) and slowly added while stirring to a chilled aqueous solution of $KMnO_4$ (9.0 g, 57 mmol in 225 mL water). Stirring was continued at 0 °C for 3 h. NaOH (4.0 g, 100 mmol) was then added and the solid $MnO_2$ was filtered off. The aqueous solution was acidified with 12 N HCl until pH 2 to precipitate the product **9**. The diacid was filtered off and washed with a small amount of ice-cold ethanol (2 mL). Crystallisation from water afforded the pure dicarboxylic acid **9** as a colourless solid. Yield: 3.1 g, (62%). M.p.: 76 °C. $^1$H NMR ([D$_6$]DMSO, 500 MHz): δ = 2.55 (t, *J* = 7.7 Hz, 2 H), 2.59 (s, 3 H), 3.21 (t, *J* = 7.7 Hz, 2 H), 7.50 (d, *J* = 8.2 Hz, 1 H), 8.03 (d, *J* = 8.2 Hz, 1 H), 8.33 (s, 1 H), 12.66 (s). − $^{13}$C NMR ([D$_6$]DMSO, 125 MHz): δ = 26.7 ($CH_2$), 29.1 ($CH_2$), 34.9 ($CH_3$), 129.9 ($CH_{arom}$), 130.9 ($C_{arom}$−COOH), 131.2 ($CH_{arom}$), 131.3 ($CH_{arom}$), 135.0 ($C_{arom}$−$COCH_3$), 147.1 ($C_{arom}$), 168.0 (*C*OOH), 173.5 (*C*OOH), 197.0 (C=O). − IR (KBr): ṽ = 3079, 3000, 2931, 1717, 1695, 1656, 1603, 1429, 1359, 1289, 1193, 1071, 835, 660 cm$^{-1}$. − MS (70 eV): *m*/*z* (%) = 236 (11) [M$^+$], 221 (56), 218 (82), 203 (21), 190 (97), 175 (100), 147 (21), 91 (34), 77 (33), 65 (17). − HR-MS [M$^+$]: calcd. 236.0685; found 236.0688.

**2-(2-Carboxyethyl)-5-ethylbenzoic acid (10):** A suspension of the dicarboxylic acid **9** (3.0 g, 12.7 mmol), hydrazine hydrate (98%, 2 mL, 42 mmol) and finely powdered KOH (3.0 g, 53 mmol) in triethylene glycol (75.0 mL) was refluxed for 2 h. Hydrazine and water were continuously removed by distillation until the temperature in the reaction flask remained constant at 195° C. After 4 h the suspension was allowed to cool to room temp. and the same volume of water was added. Acidification with conc. HCl precipitated the dicarboxylic acid **10**. The product was extracted with ether (3 × 5 mL). Recrystallisation from water afforded the dicarboxylic acid as a white solid. Yield: 2.5 g, 11.3 mmol (89%). M.p.: 174 °C. $^1$H NMR ([D$_6$]DMSO, 500 MHz): δ = 1.17 (t, *J* = 7.6 Hz, 3 H), 2.50 (t, *J* = 7.8 Hz, 2 H), 2.60 (q, *J* = 7.6 Hz, 2 H), 3.11 (t, *J* = 7.8 Hz, 2 H), 7.25 (d, *J* = 7.8 Hz, 1 H), 7.31 (d, *J* = 7.8 Hz, 1 H), 7.65 (s, 1 H), 12.45 (s). − $^{13}$C NMR ([D$_6$]DMSO, 125 MHz): δ = 15.4 ($CH_3$), 27.5 ($CH_2$), 28.8 ($CH_2$), 35.4 ($CH_2$−COOH), 129.5 ($CH_{arom}$), 130.3 ($C_{arom}$−COOH), 130.8 ($CH_{arom}$), 131.2 ($CH_{arom}$), 139.1 ($CH_{arom}$), 141.7 ($C_{arom}$), 168.8 (COOH), 173.8 (COOH). − IR (KBr): ṽ = 3031, 2967, 2933, 2638, 1688, 1403, 1305, 1275, 1211, 907, 828 cm$^{-1}$. − MS (70 eV): *m*/*z* (%) = 222 (3) [M$^+$], 204 (29), 176 (100), 159 (16), 148 (9). − HR-MS [M$^+$]: calcd. 222.0892; found 222.0885.

**6-Ethyl-1-oxoindan-4-carboxylic Acid (11):** The dicarboxylic acid **10** (1.0 g, 4.5 mmol) was thoroughly mixed with anhydrous $AlCl_3$ (4.2 g, 31.5 mmol) and sodium chloride (0.7 g, 12.1 mmol). The solid was heated with occasional stirring for 2 h to ca. 160 °C, resulting in a dark, viscous oil. After cooling, the complex was hydrolysed by stirring for 8 h with ice water (4 mL) and 6 N HCl (12 mL). The solid product was collected by filtration, washed thoroughly with water and dried. Yield: 0.66 g (71%). M.p.: 172 °C. $^1$H NMR ([D$_6$]DSMO, 500 MHz): δ = 1.22 (t, *J* = 7.5 Hz, 3 H), 2.63 (m, 2 H), 2.73 (q, *J* = 7.5 Hz, 2 H), 3.32 (m, 2 H), 7.65 (s, 1 H), 8.05 (s, 1 H), 13.03 (s). − $^{13}$C NMR ([D$_6$]DMSO, 125 MHz): δ = 15.3 ($CH_3$), 26.6 ($CH_2$), 27.4 ($CH_2$), 36.0 ($CH_2$−CO), 125.8 ($C_{arom}$−COOH), 128.8 ($CH_{arom}$), 135.8 ($CH_{arom}$), 138.2 ($CH_{arom}$−CO), 143.6 ($CH_{arom}$−$C_2H_5$), 153.9 ($C_{arom}$−$CH_2$), 166.9 (*C*OOH), 205.8 (*C*O). − IR (KBr): ṽ = 2966, 2931, 2871, 2579, 1715, 1675, 1581, 1432, 1303, 1234, 1124, 906, 827 cm$^{-1}$. − MS (70 eV): *m*/*z* (%) = 204 (100) [M$^+$], 189 (31), 186 (24), 176 (46), 161 (29), 143 (16), 133 (27), 115 (26), 91 (16), 77 (14). − HR-MS [M$^+$]: calcd. 204.0786; found 204.0787.

**2-[(6-Ethyl-1-oxoindane-4-carbonyl)amino]-3-methylpentanoic Acid Methyl Ester (12):** A chilled and well-stirred solution of 6-ethyl-1-oxoindan-4-carboxylic acid (**11**; 0.4 g, 1.96 mmol), the hydrochloride of L-isoleucine methyl ester (0.407 g, 2.24 mmol) and 2,4,6-collidine (0.63 mL, 4.8 mmol) in dry DMF (25.0 mL) was gradually treated with *O*-(7-aza-1-benzotriazolyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)[28] (0.81 g, 2.16 mmol). Stirring was continued for 2 h at 0 °C and for another 10 h at room temp. Then, a soln. of sat. aq. $NaHCO_3$ (10 mL) was

added, and, after 10 min., the mixture was extracted with diethyl ether (3 × 5 mL). The organic layer was washed with a sat. soln. of NaCl (5 mL) and dried ($MgSO_4$). After removal of the solvent in vacuo, the crude isoleucine conjugate was purified by chromatography on silica gel with ethyl acetate/hexane (2:1, v/v) for elution. Yield: 0.56 g, (86%). M.p.: 79 °C. $^1$H NMR ($C_6D_6$, 500 MHz): δ = 0.92 (t, $J$ = 7.4 Hz, 3 H), 0.94 (d, $J$ = 7.0 Hz, 3 H), 1.21 (t, $J$ = 7.65 Hz, 3 H), 1.47 (m), 1.61 (m), 1.98 (m), 2.69 (q, $J$ = 7.65 Hz, 2 H), 3.30 (m, 1 H), 3.73 (s, 3H), 4.78 (dd, $J$ = 8.1/4.6 Hz, 1H), 6.50 (d, $J$ = 8.1 Hz, NH), 7.20 (s), 7.65 (s). − $^{13}$C NMR ($CDCl_3$, 125 MHz): δ = 11.9 ($CH_3$), 15.6 ($CH_3$), 15.8 ($CH_3$), 25.7 ($CH_2$), 26.1 ($CH_2$), 28.6 ($CH_2$), 36.7 (CH), 38.5 ($CH_2$), 52.6 ($OCH_3$), 57.0 (CH), 125.6 ($CH_{arom}$), 133.1 ($CH_{arom}$), 133.3 ($C_{arom}$), 138.8 ($C_{arom}$), 144.6 ($C_{arom}$), 151.5 ($C_{arom}$−Et), 167.0 (CONH), 172.8 (*C*OOMe), 206.7 (CO). − IR (KBr): $\tilde{\nu}$ = 3335, 3312, 2965, 2923, 2872, 1756, 1738, 1701, 1659, 1585, 1525, 1298, 1196, 1150, 983, 831 cm$^{-1}$ − MS (70 eV): *m/z* (%) = 331 (21) [M$^+$], 299 (2), 272 (15), 243 (3), 203 (16), 187 (100), 186 (52), 159 (13), 131 (5), 115 (8), 91 (8), 71 (7), 57 (11). − HR-MS [M$^+$]: calcd. 331.1784; found 331.1785.

**Crystal Structure Determination:** The intensity data for the compound were collected on a Nonius KappaCCD diffractometer, using graphite-monochromated Mo-$K_\alpha$ radiation. Data were corrected for Lorentz and polarization effects, but not for absorption.[30,31] The structure was solved by direct methods (SHELXS[32]) and refined by full-matrix least-squares techniques against $F_o^2$ (SHELXL-97[33]). The hydrogen atoms of the structure were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms were refined anisotropically.[33] XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations.

**Crystal Data for Conjugate 12:**[34] $C_{19}H_{25}NO_4$, $M_r$ = 331.40 g·mol$^{-1}$, colourless prism, size 0.18 × 0.12 × 0.10 mm$^3$, monoclinic, space group $P2_1$, $a$ = 8.7528(4), $b$ = 22.2904(9), $c$ = 10.0250(3) Å, β = 115.620(2)°, $V$ = 1763.6(1) Å$^3$, $T$ = −90 °C, $Z$ = 4, $\rho_{calcd.}$ = 1.248 gcm$^{-3}$, μ(Mo-$K_\alpha$) = 0.87 cm$^{-1}$, $F$(000) = 712, 6577 reflections in $h$ (−11/11), $k$ (−26/28), $l$ (−11/11), measured in the range 3.77° ≤ Θ ≤ 27.50°, completeness $\Theta_{max}$ = 95.7%, 6577 independent reflections, 5495 reflections with $F_o > 4\sigma(F_o)$, 441 parameters, one restraint, $R1_{obs}$ = 0.057, $wR2_{obs}^2$ = 0.102, $R1_{all}$ = 0.075, $wR2_{all}^2$ = 0.108, GOOF = 1.049, Flack parameter 0.2(9), largest difference peak and hole: 0.171/−0.191 e·Å$^{-3}$.

**Plant Material:** Induction experiments were performed with plantlets of the Lima bean *Phaseolus lunatus* (Ferry Morse cv. *Jackson Wonder Bush*). Individual plants were grown from seed in a plastic pot (ϕ = 5.5 cm) at 23 °C and 80% humidity using daylight fluorescent tubes at ca. 270 μE m$^2$ s$^{-1}$ with a photophase of 14 h. Experiments were conducted with 12−16-day-old seedlings showing two fully developed leaves.[35]

**Induction Experiments:** Plantlets of *P. lunatus* with two fully developed primary leaves were cut with razor blades and immediately transferred into Eppendorf vials containing a solution of the test substance in tap water (100 μL). Solutions of indanoyl-L-isoleucine (**2**) and 6-ethyl indanoyl-L-isoleucine (**12**) were applied at 10, 100 and 1000 μM. Coronatine was used as a 100 μM aqueous solution. After uptake of the solution, the plantlets were placed into vials with tap water (5 mL) and were enclosed in small desiccators (750 mL) and maintained at 25 °C for 24 h. Control experiments were conducted under identical conditions by placing freshly cut plantlets into tap water. All experiments were carried out in triplicate.

**Collection and Analysis of Headspace Volatiles:** The volatiles emitted from the pre-treated plants were collected continuously on small charcoal traps (1 mg charcoal, CLSA-Filter, Le Ruisseau de Montbrun, 09350 Daumazan sur Arize, France) over a period of 24 h using air circulation as described previously.[36] After desorption of the volatiles from the carbon trap with 40 μL of a solution of 1-bromodecane (internal standard, 50 μM) in dichloromethane, the extracts were directly analysed by GC/MS. GC conditions: Fused-silica capillary (30 m × 0.25 mm) coated with DB-5 (0.25 μm). Helium at 40 cm min$^{-1}$ served as carrier gas. Separation of the compounds was achieved under programmed conditions (50 °C for 2 min., then at 10 °C min$^{-1}$ to 200 °C, finally at 35 °C min$^{-1}$ to 280 °C). MS: Finnigan GCQ; GC interface at 265 °C; scan range 35−450 Da. Individual compounds (peak area) were quantified with respect to the peak area of the internal standard.

**Tendril Coiling:** To test the ability of the conjugates to induce tendril coiling, shoots of *Bryonia dioica* with the youngest, most well-developed tendrils were cut and immediately placed into vials containing the test solution (2 mL) with the indanoyl conjugates (5−1000 μM) or coronatine (5−100 μM), and the extent of coiling was followed over a period of 20 h.[37]

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