Synthesis, structural characterization and biological activity of two diastereomeric JA-Ile macrolactones
Synthesis, structural characterization and biological activity of two diastereomeric JA-Ile macrolactones†


Jasmonates (JAs) are a large family of lipid-derived plant metabolites that mediate responses to stress and regulate development. These compounds owe their name to the metabolites that mediate responses to stress and regulate metabolism, senescence, and defense. Jasmonoyl-L-isoleucine (JA-Ile), an amino acid conjugate of jasmonic acid (JA, 1), has been identified as a bioactive endogenous jasmonate. However, JA-Ile (2) analogues trigger different responses in the plant. ω-Hydroxylation of the pentenyl side chain leads to the inactive 12-OH-JA-Ile (3) acting as a "stop" signal. On the other hand, a lactone derivative of 12-OH-JA (5) (jasmine ketolactone, JKL) occurs in nature, although with no known biological function. Inspired by the chemical structure of JKL (6) and in order to further explore the potential biological activities of 12-modified JA-Ile derivatives, we synthesized two macrolactones (JA-Ile-lactones (4a) and (4b)) derived from 12-OH-JA-Ile (3). The biological activity of (4a) and (4b) was tested for their ability to elicit nicotine production, a well-known jasmonate dependent secondary metabolite. Both macrolactones showed strong biological activity, inducing nicotine accumulation to a similar extent as methyl jasmonate does in Nicotiana attenuata leaves. Surprisingly, the highest nicotine contents were found in plants treated with the JA-Ile-lactone (4b), which has (3S,7S) configuration at the cyclopentanone not known from natural jasmonates. Macrolactone (4a) is a valuable standard to explore for its occurrence in nature.

Introduction

Jasmonates (JAs) are a large family of lipid-derived plant metabolites that mediate responses to stress and regulate development. These compounds owe their name to the initial isolation and characterization of methyl jasmonate (MeJa, 7) from jasmine oil Jasminum grandiflorum in the early 1960s. Since then, many JAs have been detected and isolated from different plant species. JAs were first studied because of their properties as odorants, greatly appreciated in perfumery, and later – and more important – because of their role as phytohormones. JAs occur throughout the plant kingdom (algae, mosses, gymnosperms and angiosperms) and also in fungi. The capacity to produce or transform JAs is extraordinarily high in fungi. JA (1) is one of the key players of the JA family. It is biosynthesized by consecutive enzymatic reactions starting from linolenic acid (Fig. 1). JA (1) is produced as the cis isomer (with respect to the cyclopentanone ring) (3R,7S)-JA, but it can readily epimerize at C7 to the more stable trans form (3R,7R)-JA. Both isomers exist in equilibrium in the cell and have different biological activities; generally, the cis isomer is more active although it is often found in lower amounts. Several enzymes can act on JA (1) and transform it into numerous derivatives (Fig. 1). One of these metabolites is 12-OH-JA (5) which has been described as a potent tuber-inducing agent. The hydroxy acid (5) is believed to be the natural precursor of JKL (6), a naturally occurring 10-membered ring macrolactone. JKL (6) was the first jasmonate to be reported in the literature, interestingly only in its trans form and with no biological activity known to date. JA-Ile (2), an amino acid conjugate of JA (1), is a bioactive endogenous jasmonate. This was postulated in 1995 by Krumm et al. and later confirmed by the discovery that, in A. thaliana, JAR1 activates JA by conjugation with L-isoleucine. Accumulation of JA-Ile (2) is observed in different plant tissues in response to environmental stresses, but...
when the activating signal is no longer needed, JA-Ile (2) is converted to 12-OH-JA-Ile (3) by hydroxylation at C12.\(^2\) The structure of (3) possesses a free carboxylic acid group and a hydroxyl group in analogy to the molecule of 12-OH-JA (5). Since JKL (6) exists in nature, a macrolactone like JA-Ile-lactone (4), derived from 12-OH-JA-Ile (3), may also exist (Fig. 1). Moreover, JA-Ile (2) analogues have shown different biological activities. For instance, the phytotoxin coronatine (Fig. S1, ESI†), a structural mimic of JA-Ile (2), is considerably more active than (2) in promoting the interaction of the COI1 (coronatine-insensitive 1) receptor with the JAZ (jasmonate ZIM domain) repressors \(^{29}\). The stronger activity of coronatine compared with JA-Ile (2) can be explained by two reasons: (i) the larger surface area provided by the cyclohexene ring compared with the corresponding area of the pentenyl side chain of JA-Ile (2) to interact with the COI1 receptor,\(^{30}\) and (ii) the high stability of the cis-hydrindanone moiety of coronatine.\(^{31}\) The methyl oxime derivatives of JA-Ile (2) and coronatine (Fig. S1, ESI†) act as JA-perception antagonists by binding to the COI1 receptor and hindering the interaction with the JAZ repressors due to the oxime group.\(^{32}\) As the idea of tailoring jasmonate analogues for specific applications has been discussed,\(^{33}\) these findings suggest a means of manipulating the JA-signaling pathway by chemically modifying the ligand (JA-Ile, 2).

We hypothesized that a lactone such as the JA-Ile-lactone (4) analogous to JKL (6) may exist in nature. Furthermore, such a lactone retains the important moieties required for jasmonate perception and therefore may be biologically active.\(^{16}\) Herein, we present a brief and efficient synthesis to JA-Ile-lactone (4). The biological activity of this new synthetic jasmonate was evaluated together with its diastereoisomer (4b). Since JKL (6) has been only described in the trans form, we prepared the (3R,7R)-isomer of the lactone (4a).

Results and discussion

Synthesis of the JA-Ile-lactone (4)

Our synthetic approach is based on two previously reported studies of jasmonates.\(^{14,35}\) This route allows not only the synthesis of JA-Ile-lactone (4), but also the preparation of other 12-modified jasmonates which are of great biological interest.
(e.g., compounds (5), (5a), (5b) and (3)). Furthermore, this synthetic route provides the opportunity to prepare enantiomerically pure amino acid conjugates of JA (1) starting from a racemic mixture of commercially available MeJA (7). Although most of the bioassays were carried out with commercially available or synthetic JAs consisting of mixtures of isomers, inhibition by the non-natural isomers has not been reported to date.

The synthesis of JA-Ile-lactone (4) starts from MeJA (7) as depicted in the retrosynthetic analysis (Fig. 2). Key steps are the Wittig reaction to generate the cis olefin (9) (Fig. 3, step b) and the final macro lactonization to JA-Ile-lactone (4). Ozonolysis of (7) as previously reported (CH2Cl2, 1 : 1) produced a mixture of the aldehyde (8) and the dimethyl acetal. To avoid acetal formation, the reaction was conducted in CH2Cl2, and the aldehyde (8) was obtained in 94% yield after flash chromatography. A regioselective Wittig reaction of the aldehyde (8) with the Wittig reagent (15) directly afforded the keto ester (9). The conditions employed in the reaction produced the cis isomer of (9) with high selectivity (>95%, estimated by 1H and 13C NMR, ESI†) and yield (70%). This is a reliable procedure to obtain 12-modified jasmonates enriched with the Z-isomer. Long synthetic routes to these important metabolites are no longer needed. Saponification of (9) afforded the free acid (10) quantitatively, which was employed in the next step without purification. Conjugation of L-Ile to (10) was carried out according to the procedure described for the linolenic acid/L-Ile conjugate. This procedure was more straightforward than the procedure reported by Kramell et al. The amino acid conjugate (11) (86% crude yield) was directly deprotected with p-TsOH in EtOH to afford the seco-acid 12-OH-JA-Ile (3) in 90% yield.

Macrolactonization to JA-Ile-lactone (4) was the most challenging step of the synthesis. Classical Yamaguchi–Yonemitsu conditions (Et3N, Cl3C6H2COCl, PhMe or PhH, DMAP) did not work at all, but we obtained excellent yields of the macro lactone (4) employing the two-step sequential reaction described by Ohba et al. Using the ethoxyvinyl-ester (EVE) method to activate the acid group of (3), the macro lactone (4) was obtained (64% total yield) after flash chromatography. The JA-Ile-lactone (4) (mixture of isomers) was chromatographed on silica gel with AcOEt–n-hexane (7 : 3) and afforded two major products, JA-Ile-lactone (4a) (11.6 mg, 46%, TLC Rf = 0.26) and JA-Ile-lactone (4b) (13.7 mg, 54%, TLC Rf = 0.19). Finally, recrystallization from AcOEt–n-hexane and from EtOH–acetone afforded diastereomERICALLY pure JA-Ile-lactones (4a) and (4b) respectively, as determined by NMR (ESI†).

**Crystallography and structural characterization**

Lactones (4a) and (4b) crystallize as orthorhombic colorless prisms. Both are packed in chain-like structures with the proton of the N–H group hydrogen bonding with the ketoamide group of the adjacent molecule (Fig. S2, ESI†). This orientation differs from the packing observed for indanoyl-isoleucine derivatives, which crystallize as dimers (sandwich-like) with two hydrogen bonds involving the keto group of the cyclopentanone ring and the N–H protons. The absolute configuration of JA-Ile-lactone (4a) was assigned by reference to a chiral center of the L-Ile moiety (C14, Fig. 4). The absolute configuration of JA-Ile-lactone (4b) was additionally confirmed by anomalous-dispersion effects in diffraction measurements on the crystal using the intensity quotients method.

**JA-Ile-lactones (4a) and (4b) induce nicotine biosynthesis**

Naturally occurring and syntheticJA analogs may have diverse biological backgrounds and activities. These facts have made JAs the target of several synthetic studies that examined the relationship between the molecular structure and their activity. Inspired by the structure of JKL (6) and other JA-Ile analogues, like coronatine, we designed and synthesized JA-Ile-lactones (4a) and (4b). The structures of these lactones possess all moieties known to be necessary for the bioactivity of JAs (e.g., pentenyl side chain on C7, cyclopentanone ring, L-Ile moiety). In addition, the macrocycle may confer a certain rigidity to the structure, in analogy to the cyclohexene ring to the molecule of coronatine or the aromatic ring in coronalon (Fig. S1, ESI†).

Nicotine is a typical direct defense stimulated by JAs in tobacco plants. When MeJA (7) is applied to the leaves it is rapidly converted into bioactive JAs that induce the accumulation of nicotine in *N. attenuata* plants and served as a positive control in our experiments. To test the potential
biological activity of the JA-Ile-lactones (4a) and (4b), we determined their ability to induce nicotine production in N. attenuata plants.

Both lactones induced nicotine accumulation in N. attenuata leaves similarly to MeJA (7) (Fig. 5). Strikingly, the JA-Ile-lactone (4b) induced the highest nicotine content although this molecule has a non-natural configuration at C3. To our knowledge, this is the first time that a jasmonate having the (3S,7S) configuration (not present in planta) is reported to strongly induce a secondary metabolite.
The precursor of the JA-Ile-lactones, the hydroxy acid 12-OH-JA-Ile (3), is a well-known jasmonate that acts as a stop signal in JA-signaling. However, the lactones (4a) and (4b) derived from (3) are strongly active at inducing nicotine production. These results suggest that, similarly to JA-Ile (2), the JA-Ile-lactones may activate nicotine production via a COI1-dependent mechanism. Further studies are needed to test this hypothesis.

Conclusions

We have developed a short (7-step) and efficient synthesis (33% overall yield) of JA-Ile-lactones (4) from commercially available MeJA (7). A mixture of the synthesized JA-Ile-lactone (4) can be chromatographically resolved into the diastereomERICALLY pure lactones (4a) and (4b). Furthermore, enantio-merically enriched C12-modified JA and JA-Ile derivatives (e.g., 5a and 5b) can be prepared following our procedure. Both lactones are potent inducers of nicotine accumulation in the leaves of N. attenuata plants. The presence of such compounds in nature can now be explored with the synthetic JA-Ile-lactone (4a) as a reference. Furthermore, the rigid structure of these lactones makes them valuable molecules (templates) to study their interaction with the jasmonate receptor complex COI1/JAZ. Understanding the mechanism of action of these new synthetic jasmonates will shed light on the JA-signaling pathway and therefore on plant–insect herbivore interactions.
Concentrated under reduced pressure and chromatographed on silica gel (n-hexane–AcOEt, 9:1) to afford the protected alcohol (6.01 g, 75.0%, TLC Rf = 0.26) as a colorless oil. GC-MS (EI): m/z (%): 41(79), 56(40), 85(100), 120(15), 221(50), 223(53) [M+]. The alkyl bromide (3.04 g, 13.44 mmol) was dissolved in 15 mL of acetonitrile and triphenylphosphine (4.23 g, 16.13 mmol), and K2CO3 (1.01 g, 7.24 mmol) was added and the mixture was refluxed overnight (ca. 14 h). The precipitate was filtered off and the filtrate was poured into 150 mL of Et2O to obtain the solid Wittig salt. After filtration the solid was washed with 100 mL of fresh Et2O. The product (15) (5.81 g, 89%) was dried under vacuum and kept in a desiccator over CaCl2 until use. 1H NMR (DMSO-d6, 400 MHz): δ = 7.63–8.12 (m, 15H), 4.53 (br. s., 1H), 3.56–3.78 (m, 4H), 3.49 (dt, J = 9.9, 6.3 Hz, 1H), 3.43–3.80 (m, 6H), 1.65–1.87 (m, 3H), 1.54–1.64 (m, 1H), 1.34–1.52 ppm (m, 4H); 13C NMR (DMSO-d6, 100 MHz): δ = 135.9, 135.8, 134.2, 134.1, 130.9, 130.8, 119.0, 118.1, 100.5, 64.2, 30.9, 25.3, 20.0 ppm.

Synthesis of methyl (Z)-2-(3-oxo-2-(5-(tetrahydro-2H-pyran-2-yloxy)pent-2-en-1-yl)cyclopentyl)acetate (9). A 100 mL flask equipped with a rubber septum was charged with (15) (5.23 g, 10.78 mmol, 1.1 equiv.) and flushed with dry argon. Potassium bis(trimethylsilyl)amide (KHMDS, 17 mL, 0.7 M in toluene, 10.78 mmol, 1.1 equiv.) and flushed with dry argon. Potassium tert-butoxide (1.94 g, 16.13 mmol) was then added. The reaction was stirred at 78 °C for an additional hour. The mixture was then filtered through a short pad of silica and eluted with AcOEt. After evaporation of the solvent and flash chromatography (AcOEt–2-propanol–AcOH, 32:2:1), compound 3 (70 mg, 90%) was obtained as a thick pale yellow oil. HRMS (ESI-TOF): m/z = 338.1974 [M – H] (calc. for C18H27NNaO4, 338.1968); 1H NMR (CDCl3, 500 MHz): δ = 6.73 (d, J = 8.3 Hz, 1H), 6.49 (br. s., 1H), 5.44 (m, 2H), 4.57 (dd, J = 8.3, 5.0 Hz, 1H), 3.68 (m, 2H), 2.70 (m, 1H), 2.25–2.44 (m, 6H), 2.20 (m, 2H), 2.11 (m, 1H), 1.92 (m, 2H), 1.48 (m, 2H), 1.20 (m, 1H), 0.95 (d, 6 Hz, 3H), 0.92 ppm (t, 7.3 Hz, 3H); 13C NMR (CDCl3, 125 MHz): δ = 220.0, 174.9, 172.7, 128.9, 128.6, 62.1, 56.7, 54.2, 41.5, 38.9, 38.1, 37.6, 30.8, 27.4, 25.7, 25.3, 15.7, 11.7 ppm.

Synthesis of (4S,2)-4-((S)-sec-buty1)-3,4,8,11,13,14,14a-octahydro-1H-cyclopentan-9-oxa-4azacyclotridecine-2,5,12-(7H)-trione, JA-Ile-lactone (4). Activation of the carboxyl group: ethoxycetyleth (48 µL, 0.718 g·mL–1 in hexanes, 0.49 mmol, 4 equiv.) and dichloro[p-cymene]ruthenium(ii) dimer (1 mg, 0.002 mmol) were dissolved in dry acetone (3 mL) under an atmosphere of argon at 0 °C. 12-OH-JA-Ile (3) (42 mg, 0.12 mmol) dissolved in acetone (3 mL) was added slowly and the mixture was stirred for 1 h at RT. The reaction was then filtered through a short pad of silica and eluted with AcOEt. Evaporation of the solvent afforded the desired EVE derivative. Macroactinization: p-TsOH (230 µL, 0.05 M in EtOH) was diluted in 1,2-dichloroethane (DCE, 20 mL), the solution was warmed to 50 °C and the previously obtained EVE (in 5 mL of DCE) was injected dropwise for 2 h. The mixture was stirred for another 6 h and worked up. Flash chromatography (AcOEt–n-hexane, 7:3) afforded JA-Ile-lactone (4a) (11.6 mg) and JA-Ile-lactone (4b) (13.7 mg). The total yield was 64% (25.3 mg). Recrystallization was carried out as described above in the results and discussion section.

JA-Ile-lactone (4a). Silica gel TLC Rf = 0.26; m.p. (from AcOEt–n-hexane, uncorrected) 188–189 °C; HRMS (ESI-TOF): m/z = 344.1846 [M + Na]+ (calc. for C18H27NNaO4, 344.1832); 1H NMR (CDCl3, 500 MHz): δ = 5.71 (d, J = 8.8 Hz, 1H), 5.43 (m, 1H), 5.18 (m, 1H), 4.57 (ddd, J = 10.8, 4.3, 3.4 Hz, 1H), 4.49 (dd, J = 8.9, 6.7 Hz, 1H), 3.90 (td, J = 11.0, 1.7 Hz, 1H), 2.63 (dd, J = 12.1, 2.2 Hz, 1H), 2.58–2.38 (m, 4H), 2.36–2.04 (m, 6H), 1.91 (m, 1H), 1.66 (m, 1H), 1.47 (ddg, J = 13.4, 7.5 kHz, 1H), 1.16 (m, 1H), 0.94 (dd, J = 7.0 Hz, 3H), 0.92 ppm (t, J = 7.3 Hz, 3H); 13C NMR (CDCl3, 125 MHz): δ = 220.0, 170.9, 170.6, 128.9,
Crystal structure determination

The intensity data for the compounds were collected on a Nonius KappaCCD diffractometer using graphite-monochromated Mo-Kα radiation. Data were corrected for Lorentz and polarization effects but not for absorption effects. The structures were solved by direct methods (SHELXS) and refined by full-matrix least-squares techniques against F² (SHELXL). All hydrogen atoms were refined anisotropically. Mercury 3.5.1 (Cambridge Crystallographic Data Centre, Build RC5) software was used for structure representations.

Crystal data for JA-Ile-lactone (4a). C₁₈H₂₇NO₄, M = 321.41 g mol⁻¹, colourless prism, size 0.075 × 0.054 × 0.048 mm³, orthorhombic, space group P2₁2₁2, a = 15.6931(3), b = 21.8725(4), c = 5.0383(1) Å, V = 1729.38(6) Å³, T = −140 °C, Z = 4, μ(Mo-Kα) = 0.86 cm⁻¹, F(000) = 696, 12,151 reflections in h(−20/20), k(−28/27), l(−6/6), measured in the range 2.27° ≤ Θ ≤ 27.48°, completeness Θ_max = 99.8%, 3985 independent reflections, R_int = 0.0599, 3378 reflections with Fo > 4σ(Fo), 316 parameters, 0 restraints, R² = 0.0466, wR² = 0.0946, GOF = 1.164, Flack parameter −1.4(8), largest difference peak and hole: 0.207/−0.192 e Å⁻³.

Crystal data for JA-Ile-lactone (4b). C₁₈H₂₇NO₄, M = 321.41 g mol⁻¹, colourless prism, size 0.06 × 0.06 × 0.04 mm³, orthorhombic, space group P2₁2₁2₁, a = 5.7917(3), b = 15.4852(7), c = 18.6976(9) Å, V = 1676.91(14) Å³, T = −140 °C, Z = 4, μ(Mo-Kα) = 1.237 g cm⁻³, μ(Mo-Kα) = 0.89 cm⁻¹, F(000) = 696, 27,196 reflections in h(−5/7), k(−18/19), l(−23/24), measured in the range 2.65° ≤ Θ ≤ 27.52°, completeness Θ_max = 99.7%, 3401 independent reflections, R_int = 0.0326, 3299 reflections with F_o > 4σ(F_o), 316 parameters, 0 restraints, R² = 0.0296, wR² = 0.0749, R¹_all = 0.0309, wR²_all = 0.0759, GOF = 1.031, Flack parameter −0.1(2), largest difference peak and hole: 0.219/−0.190 e Å⁻³.

Supporting information available. Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 1004515 for JA-Ile-lactone (4a) and CCDC 1004516 for JA-Ile-lactone (4b).

Plant material and planting conditions

In the present study, we used wild-type N. attenuata Torr. Ex. Watson plants of the 31st inbred generation derived from seeds collected at the Desert Inn Ranch in Utah, UT, USA in 1988. Before planting, the seeds were surface sterilized and germinated on Gamborg's B5 media as described by Krügel et al. Ten-day-old seedlings were transferred to Teku pots for another ten days (Pöppelmann GmbH & Co. KG, Lohne, Germany) before planting them into 1 L pots filled with washed sand. Twenty days later, 0.8 µmol of each compound (per plant) dissolved in lanolin paste was applied to the petals of rosette-stage plants. The treatments were repeated every other day for five days to obtain nine treated leaves in total. Lanolin-treated plants were used as a negative control (n = 6). The leaves were harvested 24 h after the last treatment, flash frozen in liquid nitrogen and stored at −80 °C until analyzed. Nicotine was quantified as previously described. Plants were grown at 45–55% relative humidity and 24–26 °C during days and 23–25 °C during nights under 16 h of light. Plants were watered twice every day by an automatic irrigation system.

Statistics

The statistical tests were carried out with Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA) using analysis of variance. Levene’s and Shapiro–Wilks’ tests were applied to determine error variance and normality. The Holm–Sidak post hoc test was used for multiple comparisons. To fulfill the assumptions for ANOVA, the data set was row-standard-transformed prior to analysis.

Acknowledgements

We thank Prof. L. Wessjohan and Prof. B. Westermann (Leibniz Institute of Plant Biochemistry, Halle (Saale)). Funding granted by the Max Planck Society, the Global Research Lab programme (2012055456) of the National Research Foundation of Korea and the Human Frontier Science Program (RGPP0002/2012) to ITB is gratefully acknowledged. We thank Kerstin Ploss for HRMS measurements and Emily Wheeler for editorial assistance.

References


