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Research Article

Syntheses of deuterated jasmonates for mass spectrometry and metabolism studies

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Summary
Jasmonic acid and its metabolites play an essential role in the regulation of plant development and systemic defense responses. Isotopically labeled standards are required to quantify plant hormones for metabolism studies using mass spectrometry. A convenient method for the preparation of deuterated analogs of jasmonates is demonstrated. Modification of commercially available methyl jasmonate by base-catalyzed proton/deuterium exchange or Wittig reaction introduces either two or three heavy atoms into a molecule. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: jasmonates; 12-OH-jasmonates; ^{2}H-labeled standards; α-proton exchange; Wittig reaction

Introduction
(−)−Methyl jasmonate [(−)−MJA] **1** and (−)−jasmonic acid [(−)−JA] **2** are phytohormones widespread in the plant kingdom. Biosynthesis of jasmonates has been well studied.[1] The biosynthetic pathway starts from α-linolenic acid and yields (+)−7-iso-JA (**3**) as the final product. Because of greater steric hindrance of the *cis*-isomer, (+)−7-iso-JA epimerizes to a more stable *trans* configuration via the keto-enol tautomerization (Figure 1) during the isolation process or even within the plant. Consequently, JA is analyzed as the more stable epimer.

These natural compounds are known to be involved in many physiological and biochemical processes in plants, in particular in the response to biotic stress, promotion of senescence, inhibition of growth and chemical defense.[2–4] The volatility of MJA led to the suggestion that it is also

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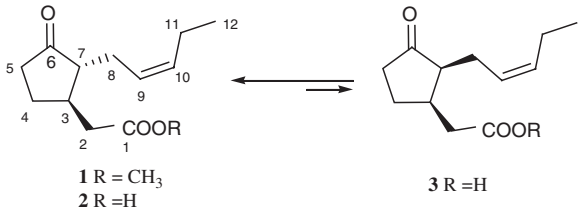


1 R = CH_3
2 R =H

3 R =H

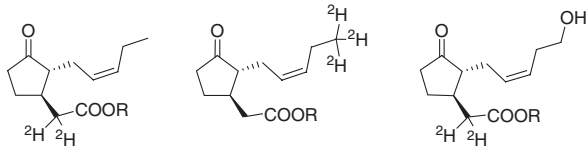
Figure 1. JA tautomerization

involved in plant to plant communication.[5] Whereas jasmonate biosynthetic pathway has been extensively studied, metabolism downstream of JA is less understood. A number of JA metabolites have been isolated such as cyclopentenone derivatives and amino acid conjugates.[4] Recent reports show that 11-hydroxy-JA and 12-hydroxy-JA are the predominant hydroxylated compounds observed in studies in which JA was fed to plant cell culture.[6]

Quantification of jasmonates in plant materials is an essential step for elucidation of their transport, distribution and metabolism. Several methods have been used to determine jasmonate levels in plant extracts. Earlier, bioassays[7] based on JA's biological properties and radioimmunoassays[8] were used. An HPLC method was developed based on the derivatization of JA with fluorescent hydrazide giving a stable fluorescent product.[9] The application of these methods, however, is limited because they lack specificity, sensitivity and ability to estimate recovery. More recent improved methods for jasmonates quantification involve the use of stable, isotope-labeled analogs as internal standards and analysis by GC-MS[10,11] or HPLC-MS.[12]

For jasmonates several methods of labeling have been developed so far.[13–18] For example Seto *et al.* incorporated deuterium atoms by catalytic semi-deuteriogenation of a 9,10-acetylene analog.[14] Ozonolysis of jasmonate and reconstruction of the pentenyl side chain by a Wittig reaction with [$^2H_5$]propylidenetriphenylphosphorane has been used to prepare [10-^{2}H, 11-$^2H_2$, 12-$^2H_3$]jasmonate.[13] Others[15–17] have prepared ^{13}C- and ^{14}C-labeled analogs.

In order to expand the plant hormone profiling method established earlier in our laboratory[19] to JA and its metabolites, we needed to develop simple and versatile synthesis of jasmonates, to be employed as internal and external standards. In this paper we present the synthesis of new deuterated racemic compounds (Figure 2) labeled at the C-2 and C-12 positions: MJA (**4**, **6**), JA (**5**, **7**) and 12-OH-JA derivatives (**8**, **9**) designed as internal standards for GC-MS and HPLC-coupled to electrospray ionization tandem mass spectrometry (ESI-MS/MS).



4 R = CH_3
5 R = H
6 R = CH_3
7 R = H
8 R = CH_3
9 R = H

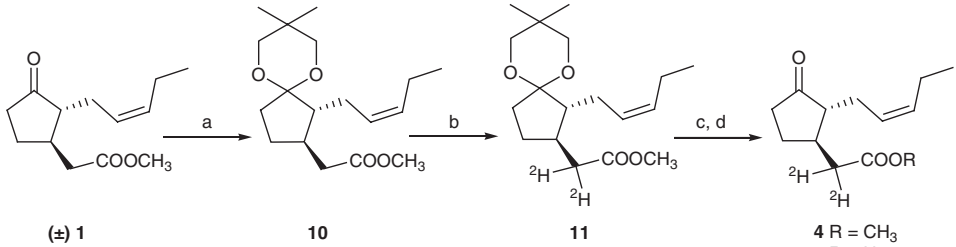
Figure 2. Deuterated jusmonates

Results and discussion

Two different approaches were applied to synthesize deuterium-labeled jasmonates with high isotopic incorporation: (a) base-catalyzed hydrogen/deuterium exchange in position α to the ester and (b) jasmonate ozonolysis and reconstruction by a Wittig reaction. Commercially available racemic MJA was used as a convenient starting material.

Synthesis of [2,2-$^2H_2$]-MJA and [2,2-$^2H_2$]-JA

When a NaOMe-CH_3O^2H solution of ketal **10** was refluxed for 5 days the α-hydrogen atoms were replaced by deuterium. Higher isotopic purity was obtained simply by repeating the reaction with a fresh portion of CH_3O^2H. Subsequent ketal deprotection led to [2,2-$^2H_2$]-MJA (**4**). Deuterium incorporation was examined by LC-MS analysis using positive ion ESI, which showed no response for the protonated molecular ion $[M+H]^+$ of unlabeled MJA ($m/z = 225$). However, the intensity of the corresponding ion for MJA $^2H_1$ ($m/z = 226$) was found to be 4% of that for the $^2H_2$-analog ($m/z = 227$). Thus the calculated value for $^2H_2$-incorporation in **4** was found to be 96%. The ^1H NMR spectrum of **4** was identical to that of unlabeled compound **1** except for the absence of the signal at 2.67 ppm, which was assigned to the C-2 proton. In order to obtain [2,2-$^2H_2$]-JA (**5**), the methyl ester (**4**) was hydrolyzed using porcine liver esterase[20] under slightly basic conditions (Scheme 1). The level of



(±) **1** **10** **11** **4** R = CH_3, **5** R = H

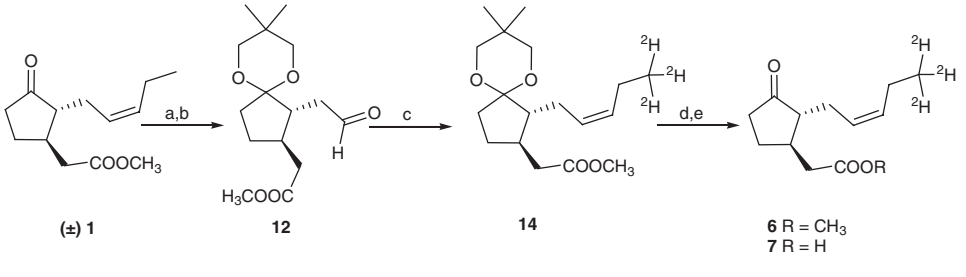
Scheme 1. Reagents and conditions: (a) 2,2-dimethyl-1,3-propanediol, pyridinium *p*-tosylate, C_6H_6; (b) $NaOCH_3$, CH_3O^2H; (c) 10% aq. HCl; (d) porcine liver esterase, phosphate buffer, pH 7.5, THF

$^2H_2$ incorporation at C-2 was determined to be 94%. Isotopically labeled and non-labeled JA analogs were examined for the corresponding fragmentation pathways using negative ion ESI-MS/MS. In multiple reaction monitoring (MRM) mode, each ionized compound gives a distinct precursor-to-product ion transition that is diagnostic for the presence of that particular compound. For [2,2-$^2H_2$]-JA (**5**) the most intense transition observed is from $m/z = 211$ to 61 ($211 > 61$), whereas for JA (**2**) it is $209 > 59$, due to the loss of a 2-pentenylcyclopentenone fragment through a McLafferty rearrangement. This shows that monitoring for specific precursor-to-product ion transitions, utilizing isotopically labeled internal standards, can provide selective quantification of jasmonates. This is especially advantageous when quantifying low levels of target compounds in the presence of a high level of background from sample matrix or co-extractives.

Synthesis of [12,12,12-$^2H_3$]-MJA and [12,12,12-$^2H_3$]-JA

The tri-deuterated jasmonate analogs (**6**, **7**) were prepared by a method similar to the procedure of Miersh (Scheme 2).[13]

Ozonolysis of a protected MJA **10** gave a convenient precursor **13** for the Wittig reaction. The Wittig reagent (ylide) required for the reaction was prepared by deprotonation of the triphenyl-[1,1,1-$^2H_3$]-propyl phosphonium bromide generated by the quaternization of the phosphine with organic halide. Thus, *Z*-selective Wittig reaction of **12** followed by the deprotection step led to MJA in its isotopically labeled form (**6**). GC-MS analysis of **6** indicated the presence of 7% of the *E*-isomer. [12,12,12-$^2H_3$]-MJA saponification with 1 M KOH gave [12,12,12-$^2H_3$]-JA (**7**). Both compounds **6** and **7** were examined for $^2H_3$-incorporation by LC-MS and contained, respectively, 98% of 12,12,12-$^2H_3$-MJA and 97% of [12,12,12-$^2H_3$]-JA. Positive ion ESI-MS of **6** generated a strong molecular ion $[M+H]^+$ ($m/z = 228$). Collision-induced



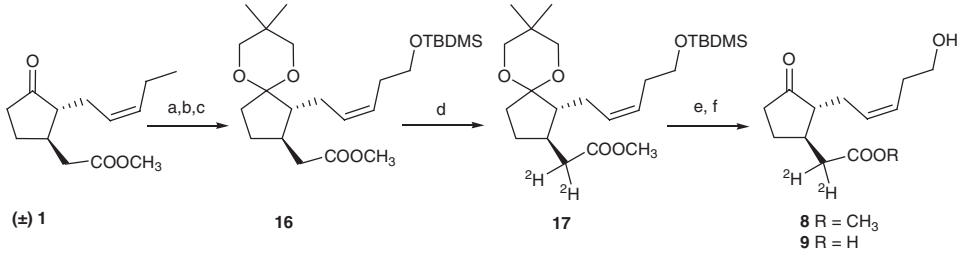
Scheme 2. Reagents and conditions: (a) 2,2-dimethyl-1,3-propanediol, pyridinium *p*-tosylate, C_6H_6; (b) O_3, CH_3OH, $-78^\circ C$; (c) $Br^- {}^+Ph_3PCH_2CH_2C^2H_3$ (13), $KN[Si(CH_3)_3]_2$, THF; (d) 10% aq. HCl, acetone; (e) 1 M aq. KOH, CH_3OH, $45^\circ C$

dissociation (CID) of this ion generated a fragment of $m/z = 154$ due to the loss of methyl acetate, whereas 225 > 151 was the predominant transition for the unlabeled MJA. These transitions could be used for MRM analysis. Similarly, MS analysis of [12,12,12-$^2H_3$]-JA and JA in the positive ion mode showed major transitions for each compound (214 > 154 and 211 > 151, respectively).

Synthesis of 12-OH-[2,2-$^2H_2$]-MJA and 12-OH-[2,2-$^2H_2$]-JA

Following our protocols, and applying a combination of both synthetic pathways, deuterated hydroxy-metabolites of JA **8**, **9** were synthesized for the first time (Scheme 3). As expected, compound **12** readily underwent Wittig reaction with the (3-*tert*-butyldimethylsilyloxy-propyl)(triphenyl)phosphonium bromide in the presence of a base, led to the pentenyl side chain with a TBDMS (*tert*-butyldimethylsilyl) protected 12-OH-group. Subsequent base-catalyzed α-proton exchange introduced two deuterium atoms into the molecule.

The attempts to obtain detailed analytical data for intermediate **17** failed because this compound is not stable in solution. Under these conditions, the ketone group is partially deprotected. Nevertheless, it can be stored neat at –20°C without decomposition. Mild acid hydrolysis of **17** readily removed ketone- and hydroxy-protecting groups, giving the desired 12-OH-[2,2-$^2H_2$]-MJA (**8**). GC-MS analysis of **8** showed that the ions at $m/z = 260$, 243 and 225 contained two deuterium atoms, which were, respectively, assigned to $[M + NH_4]^+$, $[M + H]^+$, and $[M - H_2O]^+$. These results were consistent with the ^1H NMR data. Hydrolysis of hydroxylated MJA derivative **8** with porcine liver esterase at pH 7.5 produced 12-OH-[2,2-$^2H_2$]-JA (**9**). The isotopic purity was established by LC-MS. The incorporation of $^2H_2$ was found to be 95% for 12-OH-[2,2-$^2H_2$]-MJA (**8**) and 96% for 12-OH-[2,2-$^2H_2$]-JA (**9**). Further



Scheme 3. Reagents and conditions: (a) 2,2-dimethyl-1,3-propanediol, pyridinium *p*-tosylate, C_6H_6; (b) O_3, CH_3OH, −78°C; (c) $Br^-{}^+Ph_3PCH_2CH_2CH_2OTBDMS$ (15), $KN[Si(CH_3)_3]_2$, THF; (d) $NaOCH_3$, CH_3O^2H; (e) 10% aq. HCl; (f) porcine liver esterase, phosphate buffer, pH 7.5, THF

analysis of these compounds by tandem mass spectrometry provided the information about their fragmentation patterns. A molecular ion of 12-OH-[2,2-$^2H_2$]-MJA (**8**) ($m/z = 243$) was examined using positive ion electrospray ionization, and peaks at $m/z = 225$, 193 and 165 were assigned to the fragments generated after loss of water, methanol and carbon monoxide. A similar pattern was observed for 12-OH-MJA ($m/z = 223$, 191, 163). Thus the transitions $243 > 225$, $243 > 193$ or $243 > 165$ could be used for MRM analysis of **8**. Negative ion ESI-MS of 12-OH-[2,2-$^2H_2$]-JA showed a strong molecular ion at $m/z = 227$ for which minimal fragmentation was observed under the experimental conditions. The product ion MS/MS spectrum showed fragments at $m/z = 147$ and 61. The fragment ion at $m/z = 61$ generated by CID of **9** corresponds to loss of a pentenylcyclopentenone fragment from the deprotonated molecular ion ($m/z = 227$). Because the deuterium labeling is retained in the observed fragment, this transition could also be used for MRM studies.

Experimental section

All chemicals and reagents were used as received from the suppliers unless otherwise noted. Tetrahydrofuran was distilled from sodium metal/benzophenone. All other solvents were purchased from Aldrich in Sure-Seal™ containers. Melting points (m.p.) are uncorrected and were recorded on Electrothermal Digital Melting Point Apparatus IA 9000 Series. IR spectra were recorded using KBr pellets on Perkin-Elmer Paragon 1000. NMR spectra were recorded on a Bruker AMX 500 MHz spectrometer. Chemical shifts δ are given in ppm relative to the solvent peak (chloroform) of 7.24 for ^1H and 77.0 for ^{13}C and H_3PO_4 for ^{31}P. *J* values are given in Hertz (Hz). Merck silica gel 60 F_{254} plates (0.2 mm) with aluminum sheet backing were used in analytical TLC. The spots were visualized by dipping in a solution of phosphomolybdic acid and heating on a hot plate. Preparative TLC was performed on Uniplate™ Silica Gel (1000 μm) with UV254 indicator. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh). Low-resolution GC-MS spectra were obtained using a DB-5 MS column (30 m) and a 6890N Network GC system (Agilent, Palo Alto, CA) equipped with a 5973N mass selective detector, using either electron impact (EI) or chemical ionization (CI) mode. High-resolution mass spectra were acquired on a QSTAR hybrid quadrupole-time of flight mass spectrometer (ABI/Sciex, Concord, Ont.). GC was performed on a HP 6890 Series system with FID detector. LC-MS was performed on an Alliance 2695 HPLC (Waters, Milford, MA) equipped with a Genesis C_{18} column (2.1×100 mm, 4 μm; Jones Chromatography, Hengoed, UK) at various isocratic compositions with a flow rate of 0.2 ml/min, coupled to a Quattro Ultima quadrupole tandem mass spectrometer (Micromass, Manchester, UK) fitted with a Z-spray ESI source (Micromass).

*Methyl {1-[(2Z)-pent-2-en-1-yl]-6,10-dioxa-8,8-dimethylspiro[4.5]dec-2-yl} acetate (**10**)*

A round-bottomed flask, equipped with a Dean-Stark trap was charged under argon with MJA **1** (10.00 g, 44.6 mmol), neopentyl glycol (9.29 g, 89.0 mmol, 2 eq.), pyridinium *p*-tosylate (0.17 g, 0.7 mmol, 1.5% mol) and 300 ml of dry benzene. The reaction was completed after 18 h of reflux. After the solution was cooled to room temperature the organic layer was washed with 100 ml of water, dried over anhydrous $MgSO_4$ and filtered. The evaporation of the solvent under reduced pressure gave the crude product which was further purified by flash column chromatography (20% EtOAc in hexanes). Yield 13.26 g (96%) of colorless oil. IR (KBr) ν_{max} 2955, 2905, 2851, 1740, 1450, 1435, 1394, 1135, 1016, 908, 871 cm^{-1}; ^1H NMR (500 MHz, δ, $CDCl_3$): 5.43 (1H, m, CH), 5.37 (1H, m, CH), 3.64 (3H, s, OCH_3), 3.52 (2H, m, OCH_2), 3.39 (2H, m, OCH_2), 2.61 (1H, dd $J_1 = 4.0$, $J_2 = 14.8$ Hz, CH_2), 2.36 (1H, m, CH_2), 2.18–1.84 (8H, m), 1.61 (1H, m, CH), 1.29 (1H, m, CH_2), 1.14 (3H, s, CH_3), 0.96 (3H, t $J = 7.5$ Hz, CH_3), 0.76 (3H, s, CH_3); ^{13}C NMR (δ, $CDCl_3$): 172.5 (C=O), 132.0 (CH), 128.3 (CH), 108.2 (C), 72.2, (OCH_2), 71.1 (OCH_2), 53.1 (CH), 51.3 (OCH_3), 38.8 (CH_2), 38.7 (CH), 34.5 (C), 29.2 (CH_2), 28.0 (CH_2), 25.4 (CH_2), 22.8 (CH_3), 22.2 (CH_3), 20.6 (CH_2), 14.2 (CH_3); GCMS (EI) *m/z* (relative intensity) 310 ($[M]^+$, 5), 237 (10), 195 (26), 141 (100), 128 (31), 109 (15), 69 (47), 55 (34), 41 (32).

*Methyl {1-[(2Z)-pent-2-en-1-yl]-6,10-dioxa-8,8-dimethylspiro[4.5]dec-2-yl} acetic acid-$^2H_2$ (**11**)*

A solution of **10** (1.00 g, 3.2 mmol) in CH_3O^2H (10 ml) was added dropwise to a solution of CH_3ONa (0.43 g, 8.0 mmol) in 2 ml of CH_3O^2H. The mixture was refluxed for 5 days at 65°C. NH_4Cl was added, the pH was adjusted to 7 with 10% aqueous HCl and the organic solvent was evaporated. The residue was taken up in water (5 ml) and after the product was extracted with Et_2O the organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* after filtration. The product was purified by flash column chromatography (15% EtOAc in hexanes). Yield 0.82 g (82%). IR (KBr) ν_{max} 2940, 2870, 1736, 1462, 1434, 1257, 1136, 1064, 1002, 908; ^1H NMR (δ, $CDCl_3$): 5.43 (1H, m, CH), 5.37 (1H, m, CH), 3.64 (3H, s, OCH_3), 3.54 (2H, m, OCH_2), 3.39 (2H, m, OCH_2), 2.37 (1H, m, CH_2), 2.16–1.84 (7H, m), 1.61 (1H, m, CH_2), 1.30 (1H, m, CH_2), 1.14 (3H, s, CH_3), 0.95 (3H, t $J = 7.5$ Hz, CH_3), 0.76 (3H, s, CH_3); ^{13}C NMR (δ, $CDCl_3$): 173.4 (C=O), 132.1 (CH), 128.3 (CH), 108.2 (C), 72.3 (OCH_2), 71.1 (OCH_2), 53.1 (CH), 51.4 (OCH_3), 38.6 (CH), 30.1 (C), 29.3 (CH_2), 28.0 (CH_2), 25.8 (CH_2), 22.8 (CH_3), 20.8 (CH_3), 20.6 (CH_2), 14.1 (CH_3); GCMS (EI) *m/z* (relative intensity) 312 ($[M]^+$, 21), 283 (10), 237 (36), 195 (66), 151 (20), 141 (100), 128 (36), 109 (17), 69 (53), 55 (25).

*Methyl {3-oxo-2-[(2Z)-pent-2-en-1-yl]cyclopentyl}acetate-$^2H_2$ (**4**)*

The crude compound **11** (0.40 g, 1.3 mmol) was dissolved in acetone followed by the addition of aqueous 10% HCl (1 ml). The reaction mixture was stirred at room temperature for 0.5 h and then neutralized with 10% aqueous NaOH. The organic product was extracted with Et_2O and the etheral layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (20% Et_2O in hexanes). Yield 0.19 g (65%). IR (KBr) ν_{max} 3024, 2937, 2913, 1735, 1450, 1259, 1156, 1067, 991, 820; ^{1}H NMR (δ, $CDCl_3$): 5.45 (1H, m, CH), 5.25 (1H, m, CH), 3.69 (3H, s, OCH_3), 2.39–2.02 (8H, m), 1.89 (1H, m, CH_2), 1.50 (1H, m, CH_2), 0.96 (3H, t $J = 7.5$ Hz, CH_3); ^{13}C NMR (δ, $CDCl_3$): 219.0 (C=O), 172.9 (C=O), 134.1 (CH), 124.9 (CH), 53.9 (CH), 51.6 (OCH_3), 37.9 (CH), 37.7 (CH_2), 27.1 (CH_2), 25.5 (CH_2), 20.6 (CH_2), 14.1 (CH_3); GCMS (EI) *m/z* (relative intensity) 226 ($[M]^+$, 77), 195 (29), 151 (81), 133 (33), 95 (47), 83 (100), 67 (24), 55 (25).

*{3-Oxo-2-[(2Z)-pent-2-en-1-yl]cyclopentyl}acetic acid-$^2H_2$ (**5**)*

[2,2-$^2H_2$]-MJA **4** (90 mg, 0.39 mmol) was dissolved in THF (1 ml) and KH_2PO_4/K_2HPO_4 buffer (pH 7.5) was added (1 ml) followed by the addition of a solution of porcine liver esterase in 3 ml of the phosphate buffer. The reaction mixture was stirred for 2 h at room temperature, acidified to pH 5 with 10% aqueous HCl solution and extracted with EtOAc. After the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated the product was purified by preparative TLC plate (30% EtOAc in hexanes). Yield 67 mg (80%) of colorless oil. IR (KBr) ν_{max} 3250 (br), 3028, 2908, 1734, 1541, 1406, 1295, 1156, 1070, 1028, 827; ^{1}H NMR (δ, $CDCl_3$): 5.46 (1H, m, CH), 5.25 (1H, m, CH), 2.45–2.25 (5H, m), 2.16–2.03 (3H, m), 1.92 (1H, m, CH_2), 1.53 (1H, m, CH_2), 0.96 (3H, t $J = 7.5$ Hz, CH_3); ^{13}C NMR (δ, $CDCl_3$): 218.8 (C=O), 177.7 (C=O), 134.2 (CH), 124.8 (CH), 53.8 (CH), 37.7 (CH), 37.6 (CH_2), 27.1 (CH_2), 25.5 (CH_2), 20.6 (CH_2), 14.1 (CH_3); GCMS (EI) *m/z* (relative intensity) 212 ($[M]^+$, 51), 151 (56), 144 (44), 133 (20), 109 (31), 95 (37), 83 (100), 67 (19), 55 (21).

*Methyl [1-(2-oxoethyl)-6,10-dioxa-8,8-dimethylspiro[4.5]dec-2-yl]acetate (**12**)*

A solution of **10** (7.00 g, 22.5 mmol) in methanol (250 ml) was cooled to –78°C. Ozonized oxygen was passed through for 2 h. Dimethyl sulfide (2.25 ml, 30.6 mmol, 1.36 eq.) was then added dropwise to quench the resulting ozonide. The reaction mixture was brought to room temperature and stirred for another 1.5 h. After the solvent was removed the product was purified by flash column chromatography (20% EtOAc in hexanes). Yield 4.51 g (71%) of colorless oil that solidifies when stored in the fridge. IR (KBr) ν_{max} 2938, 2847,

2723, 2019, 1734, 1436, 1137, 1018 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 9.81 (1H, s, CHO), 3.65 (3H, s, OCH_3), 3.52 (2H, m, OCH_2), 3.37 (2H, m, OCH_2), 2.71 (1H, m, CH_2), 2.51 (1H, m, CH_2), 2.37 (1H, m, CH_2), 2.26–1.92 (6H, m), 1.39 (1H, CH_2), 1.11 (3H, s, CH_3), 0.72 (3H, s, CH_3); ^{13}C NMR (δ, $CDCl_3$): 218.9 (C=O), 172.8 (C=O), 109.2 (C), 72.4 (OCH_2), 70.8 (OCH_2), 51.6 (CH), 50.0 (OCH_3), 38.7 (CH), 38.4 (CH_2), 37.3 (CH_2), 34.2 (C), 33.1 (CH_2), 27.4 (CH_2), 23.1 (CH_3), 21.9 (CH_3); GCMS (EI) *m/z* (relative intensity) 284 ($[M]^+$, 4), 283 (20), 211 (28), 181 (30), 167 (54), 129 (98), 128 (100), 115 (98), 97 (39), 83 (30), 69 (99), 55 (47).

*Triphenyl(3,3,3-$^2H_3$-propyl)phosphonium bromide (***13***)*

A pressure tube with threaded Teflon cap (Ace Glass) was charged with [3,3,3-$^2H_3$]-bromopropane (1.00 g, 7.9 mmol) and triphenylphosphine (1.04 g, 4.0 mmol).

The threaded cap on a pressure tube was finger tight and the tube was placed in an oil bath (110°C). After the reaction mixture was stirred for 48 h the white precipitate appeared. The tube was cooled to ambient temperature and the phosphonium salt was washed with Et_2O and dried under high vacuum. Yield 1.32 g (85%) of white solid. IR (KBr) ν_{max} 3043, 2850, 2670, 2467, 2212, 1914, 1829, 1586, 1490, 1439, 1343, 1112, 1022, 943, 822; ^1H NMR (δ, $CDCl_3$): 7.72 (9H, m, CH), 7.63 (6H, m, CH), 3.61 (2H, m, CH_2), 1.61 (2H, m, CH_2); ^{13}C NMR (δ, $CDCl_3$): 134.9 (C), 133.41 (CH), 130.7 (CH), 117.9 (d, CH_2), 24.2 (d, CH_2), 16.0 (C^2H_3); $^{31}P\{^1H\}$ NMR (δ, $CDCl_3$): 24.6 (s); HRMS calculated for $C_{21}H^2_{19}H_3P$ $[M]^+$ *m/z* 308.1647 found 308.1647; m.p. 238.5–238.8°C.

*Methyl {1-[(2Z)-pent-2-en-5,5,5-$^2H_3$-1-yl]-6,10-dioxa-8,8-dimethylspiro[4.5]-dec-2-yl}acetate (***14***)*

A phosphonium salt **13** (0.90 g, 2.3 mmol) was suspended in freshly distilled THF (150 ml). 0.5 M solution of potassium *bis*(trimethylsilyl)-amide in toluene (4.6 ml, 2.3 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 1 h and then was cooled to –78°C. After a solution of aldehyde **12** (0.44 g, 1.5 mmol) in THF was added, the mixture was warmed up to –40°C and the stirring was continued for another 1.5 h. Next the reaction was brought to room temperature, water was added and the organic material was extracted with Et_2O. Combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* after filtration. Purification of the crude material by flash column chromatography (10% EtOAc in hexanes) gave 0.28 g (61%) of colorless oil. ^1H NMR (δ, $CDCl_3$): 5.43 (1H, m, CH), 5.36 (1H, m, CH), 3.64 (3H, s, OCH_3), 3.53 (2H, m, OCH_2),

3.39 (2H, m, CH_2), 2.61 (1H, m, CH_2), 2.37 (1H, m, CH_2), 2.18–1.84 (8H, m), 1.61 (1H, m, CH_2), 1.29 (1H, m, CH_2), 1.14 (3H, s, CH_3), 0.76 (3H, s, CH_3).

*Methyl {3-oxo-2-[(2Z)-pent-2-en-5,5,5-$^2H_3$-1-yl]cyclopentyl}acetate (**6**)*

To a solution of **14** (0.28 g, 0.9 mmol) in acetone (25 ml), aqueous 10% HCl (1 ml) was added at room temperature. After the reaction mixture was stirred for 0.5 h the solution was neutralized with aqueous 10% NaOH. The product was extracted with Et_2O and the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Yield 120 mg (60%). IR (KBr) ν_{max} 3028, 2936, 2909, 2217, 2076, 1738, 1437, 1335, 1163, 986 cm^{-1}; ^1H NMR (δ, $CDCl_3$): 5.44 (1H, m, CH), 5.25 (1H, m, CH), 3.69 (3H, s, OCH_3), 2.70 (1H, m, CH_2), 2.44–1.83 (10H, m), 1.48 (1H, m, CH_2); ^{13}C NMR (δ, $CDCl_3$): 219.0 (C=O), 172.5 (C=O), 134.1 (CH), 125.0 (CH), 54.0 (CH), 51.6 (OCH_3), 38.8 (CH_2), 38.0 (CH), 37.7 (CH_2), 27.2 (CH_2), 25.4 (CH_2), 20.33 (CH_2); GCMS (EI) *m/z* (relative intensity) 227 ($[M]^+$, 74), 196 (27), 154 (81), 135 (39), 109 (25), 95 (44), 83 (100), 67 (22), 55 (19).

*{3-Oxo-2-[(2Z)-pent-2-en-5,5,5-$^2H_3$-1-yl]cyclopentyl}acetic acid (**7**)*

[12,12,12-$^2H_3$]-MJA (**6**) (40 mg, 0.18 mmol) was dissolved in CH_3OH (5 ml) and the solution was adjusted to pH 9 with aqueous 1 M KOH. The reaction mixture was warmed up to 45°C and was stirred for 2 h. Then it was acidified with 10% aqueous HCl to pH 3 at 0°C and the organic product was extracted with EtOAc. Combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. [12,12,12-$^2H_3$]-JA was purified by preparative TLC plate (AcOH:Et_2O:hexanes 1:39:60). Yield 31 mg (84%). IR (KBr) ν_{max} 3250 (br), 3028, 2935, 2218, 2077, 1735, 1406, 1166, 1057; ^1H NMR (δ, $CDCl_3$): 5.46 (1H, m, CH), 5.25 (1H, m, CH), 2.77 (1H, m, CH_2), 2.43–1.87 (10H, m), 1.52 (1H, m, CH_2); ^{13}C NMR (δ, $CDCl_3$): 218.8 (C=O), 177.2 (C=O), 134.2 (CH), 124.8 (CH), 53.8 (CH), 38.5 (CH_2), 37.7 (CH), 37.3 (CH_2), 27.2 (CH_2), 25.4 (CH_2), 20.3 (CH_2); GCMS (EI) *m/z* (relative intensity) 213 ($[M]^+$, 15), 154 (30), 142 (28), 95 (27), 83 (100), 67 (14), 55 (17).

*[(3-Tert-butyldimethyl)silyloxy-propyl](triphenyl)phosphonium bromide (**15**)*

To an ice-water bath cooled solution of 3-bromopropanol (2.00 g, 14.4 mmol), *tert*-butyl-dimethylsilylchloride (2.38 g, 15.8 mmol) and imidazole (1.18 g, 17.2 mmol) were added. After the ice bath was removed, the mixture was stirred at room temperature overnight. Next water was added and the product was extracted with three portions of Et_2O. The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Yield 3.60 g (99%). Without further purification the product was used in the next step. ^1H NMR (δ, $CDCl_3$): 3.71 (2H, t $J = 5.7$ Hz, CH_2), 3.49 (2H,

t $J = 6.5$ Hz, CH_2), 2.01 (2H, m, CH_2), 0.87 (9H, s, CH_3), 0.04 (6H, s, CH_2). A pressure tube with threaded Teflon cap (Ace Glass) was charged with 1-bromo-3-*tert*-butyl-dimethylsilyloxypropane (3.60 g, 14.2 mmol) and triphenylphosphine (3.73 g, 14.2 mmol). The threaded cap on a pressure tube was finger tight and the tube was placed in an oil bath (120°C). After the reaction mixture was stirred for 48 h a white solid appeared. The mixture was then cooled to ambient temperature and a glass-like solid was formed. The product was purified by crystallization (CH_2Cl_2/Et_2O). Yield 4.24 g (58%) of a white solid. IR (KBr) ν_{max} 3318, 2940, 2867, 2428, 2218, 1992, 1918, 1838, 1618, 1586, 1485, 1253, 1116, 940, 836; ^{1}H NMR (δ, $CDCl_3$): 7.73 (9H, m, CH), 7.63 (6H, m, CH), 3.76 (2H, m, CH_2), 3.69 (2H, m, CH_2), 1.82 (2H, m, CH_2), 0.77 (9H, s, CH_3), −0.05 (6H, s, CH_3); ^{13}C NMR (δ, $CDCl_3$): 134.9 (C), 133.4 (CH), 130.3 (CH), 118.0 (d, CH_2), 61.45 (OCH_2), 25.71 (CH_3), 18.8 (CH_2), 17.9 (C), −5.5 ($SiCH_3$); ^{31}P{^{1}H} NMR (δ, $CDCl_3$): 25.0 (s); HRMS calculated for $C_{27}H_{36}OPSi$ $[M]^+$ m/z 435.2273 found 435.2267; m.p. 127.3–128.2°C.

*Methyl {2-[(2Z)-5-(tert-butyldimethyl)silyloxy-pent-2-en-1-yl]-6,10-dioxa-8,8-dimethylspiro[4.5]dec-2-yl}acetate (***16***)*

A phosphonium salt **15** (1.54 g, 3.0 mmol) was suspended in freshly distilled THF (90 ml). 0.5 M solution of potassium *bis*(trimethylsilyl)-amide in toluene (6.0 ml, 3.0 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 1 h and then was cooled to –78°C. After a solution of aldehyde **13** (0.56 g, 2.0 mmol) in THF was added, the mixture was warmed up to –40°C and the stirring was continued for another 1.5 h. Next the reaction was brought to room temperature, water was added and the organic material was extracted with Et_2O. Combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* after filtration. Purification of the crude material by flash column chromatography (10% EtOAc in hexanes) gave 0.60 g (68%) of colorless oil. IR (KBr) ν_{max} 3021, 2938, 2853, 1740, 1451, 1361, 1252, 1094, 1006, 927, 835; ^{1}H NMR (δ, $CDCl_3$): 5.56 (1H, m, CH), 5.38 (1H, m, CH), 3.63 (3H, s, OCH_3), 3.60 (2H, t $J = 7.1$ Hz, OCH_2), 3.52 (2H, m, OCH_2), 3.38 (2H, m, OCH_2), 2.59 (1H, m, CH_2), 2.38 (1H, m, CH_2), 2.30 (2H, m, CH_2), 2.17–1.83 (6H, m), 1.61 (1H, m, CH_2), 1.29 (1H, m, CH_2), 1.13 (3H, s, CH_3), 0.88 (9H, s, CH_3), 0.75 (3H, s, CH_3), 0.04 (6H, s, CH_3); ^{13}C NMR (δ, $CDCl_3$): 173.3 (C=O), 130.9 (CH), 126.1 (CH), 108.1 (C), 72.3 (OCH_2), 71.1 (OCH_2), 62.9 (OCH_2), 53.1 (CH), 51.3 (OCH_3), 39.9 (CH_2), 38.7 (CH), 31.3 (CH_2), 30.1 (C), 29.2 (CH_2), 28.1 (CH_2), 26.0 (CH_2), 25.9 (CH_3), 22.8 (CH_3), 22.2 (CH_3), 18.3 (C), −5.30 ($SiCH_3$); GCMS (EI) m/z (relative intensity) 440 ($[M]^+$, 21), 383 (97), 367 (30), 325 (59), 297 (63), 265 (25), 223 (86), 149 (30), 141 (100), 128 (44), 119 (22), 105 (20), 89 (59), 73 (66), 55 (24).

*Methyl {2-[(2Z)-5-hydroxypent-2-en-1-yl]-3-oxocyclopentyl}acetate-$^2H_2$ (**8**)*

A solution of **17** (0.45 g, 1.0 mmol) in CH_3O^2H (5 ml) was added dropwise to a solution of CH_3ONa (0.13 g, 2.5 mmol) in 2 ml of CH_3O^2H. The mixture was refluxed for 5 days at 65°C. NH_4Cl was added, the pH was adjusted to 7 with 1% aqueous HCl and the organic solvent was evaporated. The residue was taken up in water (5 ml) and after the product was extracted with Et_2O the organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* after filtration. Without delay, the crude product was dissolved in acetone (25 ml) and 1 ml of aqueous 10% HCl was added at room temperature. After the reaction mixture was stirred for 0.5 h the solution was brought to pH 5–6 with aqueous 10% NaOH. The products were extracted with Et_2O then the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification by preparative TLC plate (EtOAc:hexanes 1:1) gave pure **7**. Yield 10 mg. IR (KBr) ν_{max} 3458, 3025, 2930, 2883, 1734, 1435, 1261, 1048, 798; ^{1}H NMR (δ, $CDCl_3$): 5.47 (2H, m, CH), 3.70 (3H, s, OCH_3), 3.67 (2H, t $J = 6.2$ Hz, OCH_2), 2.46–2.07 (8H, m), 1.92 (1H, m, CH_2), 1.50 (1H, m, CH_2); ^{13}C NMR (δ, $CDCl_3$): 219.1 (C=O), 174.5 (C=O), 128.6 (CH), 128.3 (CH), 62.1 (OCH_2), 54.0 (CH), 51.7 (OCH_3), 37.8 (CH_2), 37.7 (CH), 30.9 (CH_2), 27.2 (CH_2), 25.4 (CH_2); GCMS (CI) *m/z* (relative intensity) 260 ($[M+NH_4]^+$, 100), 258 (21), 243 (88), 242 (31), 228 (24), 225 (68), 223 (24), 176 (47).

*{2-[(2Z)-5-hydroxypent-2-en-1-yl]-3-oxocyclopentyl}acetic acid-$^2H_2$ (**9**)*

12-OH-2,2-$^2H_2$-MJA **8** (12 mg, 0.39 mmol) was dissolved in THF (0.2 ml) and KH_2PO_4/K_2HPO_4 buffer (pH 7.5) was added (1.0 ml) followed by the addition of a solution of esterase in 3.0 ml of the phosphate buffer. The reaction mixture was stirred for 12 h at room temperature, acidified to pH 5 with 1% aqueous HCl solution and extracted with EtOAc. After the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated the product was purified by preparative TLC (EtOAc:hexanes 6: 4). Yield 4.7 mg (40%). IR (KBr) ν_{max} 3300 (br), 3021, 1730, 1543, 1403, 1260, 1049, 867; ^{1}H NMR (δ, $CDCl_3$): 5.76 (1H, brs, OH), 5.44 (2H, m, CH), 3.66 (2H, m, OCH_2), 2.55–2.24 (7H, m), 2.12 (1H, m, CH_2), 1.94 (1H, m, CH_2), 1.52 (1H, m, CH_2); ^{13}C NMR (δ, $CDCl_3$): 219.1 (C=O), 176.6 (C=O), 128.7 (CH), 128.3 (CH), 62.1 (OCH_2), 54.1 (CH), 37.8 (CH_2), 37.6 (CH), 30.7 (CH_2), 27.3 (CH), 25.4 (CH_2); LCMS (negative ion ESI) *m/z* 228 ($[M]^-$, 19), 227 ($[M–H]^-$, 100), 221 (48), 217 (12), 125 (19), 120 (16), 116 (11), 107 (10).

Conclusions

Deuterated jasmonates were efficiently prepared from commercially available (±)-MJA in high isotopic purity. The analogs are two and three mass units

higher than the naturally occurring compounds and are used as standards for mass spectrometry analysis. Volatility of the methyl ester analogs (**4**, **6**, **8**) makes them useful standards for GC-MS as well. Because the molecular weight of the deuterated derivatives are different from the natural compounds they can be clearly distinguished from the endogenous jasmonates by mass spectrometry and used for quantification of jasmonates in plant material.

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