

Research Article

Syntheses of deuterated jasmonates for mass spectrometry and metabolism studies

Patrycja W. Galka, Stephen J. Ambrose, Andrew R. S. Ross and Suzanne R. Abrams*

Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, Sask., Canada S7N 0W9

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; ²H-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

*Correspondence to: Suzanne R. Abrams, Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, Sask., Canada S7N 0W9. E-mail: sue.abrams@nrc.ca

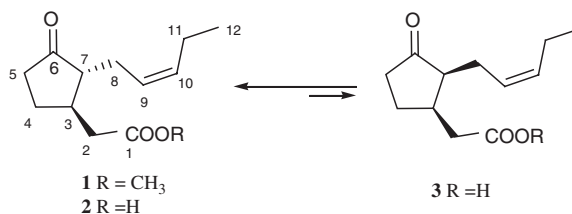


Figure 1. JA tautomerization

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, bioassays⁷ based on JA's biological properties and radioimmunoassays⁸ 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 [²H₅]propylidene triphenylphosphorane has been used to prepare [10-²H, 11-²H₂, 12-²H₃]jasmonate.¹³ Others^{15–17} have prepared ¹³C- and ¹⁴C-labeled analogs.

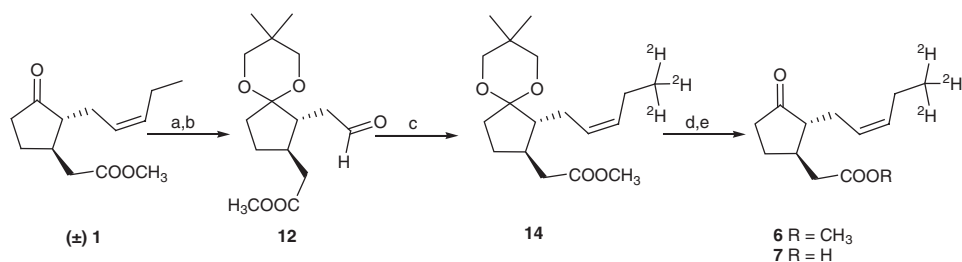
In order to expand the plant hormone profiling method established earlier in our laboratory¹⁹ to JA and its metabolites, we needed to develop simple and versatile synthesis of jasmonates, to be employed as internal and external standards. In this paper we present the synthesis of new deuterated racemic 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).

$^2\text{H}_2$ 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- $^2\text{H}_2$]-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- $^2\text{H}_3$]-MJA and [12,12,12- $^2\text{H}_3$]-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- $^2\text{H}_3$]-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- $^2\text{H}_3$]-MJA saponification with 1 M KOH gave [12,12,12- $^2\text{H}_3$]-JA (**7**). Both compounds **6** and **7** were examined for $^2\text{H}_3$ -incorporation by LC-MS and contained, respectively, 98% of 12,12,12- $^2\text{H}_3$ -MJA and 97% of [12,12,12- $^2\text{H}_3$]-JA. Positive ion ESI-MS of **6** generated a strong molecular ion $[\text{M} + \text{H}]^+$ ($m/z = 228$). 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°C ; (c) $\text{Br}^- + \text{Ph}_3\text{PCH}_2\text{CH}_2\text{C}^2\text{H}_3$ (**13**), $\text{KN}[\text{Si}(\text{CH}_3)_3]_2$, THF ; (d) 10% aq. HCl , acetone; (e) 1 M aq. KOH , CH_3OH , 45°C

analysis of these compounds by tandem mass spectrometry provided the information about their fragmentation patterns. A molecular ion of 12-OH-[2,2-²H₂]-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-[2,2-²H₂]-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-SealTM 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 ¹³C and H₃PO₄ for ³¹P. *J* values are given in Hertz (Hz). Merck silica gel 60 F₂₅₄ 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 UniplateTM Silica Gel (1000 μ m) 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 C₁₈ column (2.1 \times 100 mm, 4 μ m; 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-[(2*Z*)-pent-2-en-1-yl]-6,10-dioxo-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 MgSO₄ 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 , CDCl₃): 5.43 (1H, m, CH), 5.37 (1H, m, CH), 3.64 (3H, s, OCH₃), 3.52 (2H, m, OCH₂), 3.39 (2H, m, OCH₂), 2.61 (1H, dd $J_1 = 4.0$, $J_2 = 14.8$ Hz, CH₂), 2.36 (1H, m, CH₂), 2.18–1.84 (8H, m), 1.61 (1H, m, CH), 1.29 (1H, m, CH₂), 1.14 (3H, s, CH₃), 0.96 (3H, t $J = 7.5$ Hz, CH₃), 0.76 (3H, s, CH₃); ¹³C NMR (δ , CDCl₃): 172.5 (C=O), 132.0 (CH), 128.3 (CH), 108.2 (C), 72.2, (OCH₂), 71.1 (OCH₂), 53.1 (CH), 51.3 (OCH₃), 38.8 (CH₂), 38.7 (CH), 34.5 (C), 29.2 (CH₂), 28.0 (CH₂), 25.4 (CH₂), 22.8 (CH₃), 22.2 (CH₃), 20.6 (CH₂), 14.2 (CH₃); 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-[(2*Z*)-pent-2-en-1-yl]-6,10-dioxo-8,8-dimethylspiro[4.5]dec-2-yl} acetic acid-²H₂ (**11**)

A solution of **10** (1.00 g, 3.2 mmol) in CH₃O²H (10 ml) was added dropwise to a solution of CH₃ONa (0.43 g, 8.0 mmol) in 2 ml of CH₃O²H. The mixture was refluxed for 5 days at 65°C. NH₄Cl 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 Et₂O the organic layer was dried over anhydrous MgSO₄ 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 , CDCl₃): 5.43 (1H, m, CH), 5.37 (1H, m, CH), 3.64 (3H, s, OCH₃), 3.54 (2H, m, OCH₂), 3.39 (2H, m, OCH₂), 2.37 (1H, m, CH₂), 2.16–1.84 (7H, m), 1.61 (1H, m, CH₂), 1.30 (1H, m, CH₂), 1.14 (3H, s, CH₃), 0.95 (3H, t $J = 7.5$ Hz, CH₃), 0.76 (3H, s, CH₃); ¹³C NMR (δ , CDCl₃): 173.4 (C=O), 132.1 (CH), 128.3 (CH), 108.2 (C), 72.3 (OCH₂), 71.1 (OCH₂), 53.1 (CH), 51.4 (OCH₃), 38.6 (CH), 30.1 (C), 29.3 (CH₂), 28.0 (CH₂), 25.8 (CH₂), 22.8 (CH₃), 20.8 (CH₃), 20.6 (CH₂), 14.1 (CH₃); 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-²H₂ (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 Et₂O and the ethereal layer was dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (20% Et₂O in hexanes). Yield 0.19 g (65%). IR (KBr) ν_{\max} 3024, 2937, 2913, 1735, 1450, 1259, 1156, 1067, 991, 820; ¹H NMR (δ , CDCl₃): 5.45 (1H, m, CH), 5.25 (1H, m, CH), 3.69 (3H, s, OCH₃), 2.39–2.02 (8H, m), 1.89 (1H, m, CH₂), 1.50 (1H, m, CH₂), 0.96 (3H, t $J = 7.5$ Hz, CH₃); ¹³C NMR (δ , CDCl₃): 219.0 (C=O), 172.9 (C=O), 134.1 (CH), 124.9 (CH), 53.9 (CH), 51.6 (OCH₃), 37.9 (CH), 37.7 (CH₂), 27.1 (CH₂), 25.5 (CH₂), 20.6 (CH₂), 14.1 (CH₃); 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-²H₂ (5)

[2,2-²H₂]-MJA **4** (90 mg, 0.39 mmol) was dissolved in THF (1 ml) and KH₂PO₄/K₂HPO₄ 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 MgSO₄, 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; ¹H NMR (δ , CDCl₃): 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, CH₂), 1.53 (1H, m, CH₂), 0.96 (3H, t $J = 7.5$ Hz, CH₃); ¹³C NMR (δ , CDCl₃): 218.8 (C=O), 177.7 (C=O), 134.2 (CH), 124.8 (CH), 53.8 (CH), 37.7 (CH), 37.6 (CH₂), 27.1 (CH₂), 25.5 (CH₂), 20.6 (CH₂), 14.1 (CH₃); 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-dioxo-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 ($[\text{M}]^+$, 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- $^2\text{H}_3$ -propyl)phosphonium bromide (13)

A pressure tube with threaded Teflon cap (Ace Glass) was charged with [3,3,3- $^2\text{H}_3$]-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); $^{31}\text{P}\{^1\text{H}\}$ NMR (δ , CDCl_3): 24.6 (s); HRMS calculated for $\text{C}_{21}\text{H}_{19}^2\text{H}_3\text{P} [\text{M}]^+$ m/z 308.1647 found 308.1647; m.p. 238.5–238.8°C.

Methyl {1-[(2Z)-pent-2-en-5,5,5- $^2\text{H}_3$ -1-yl]-6,10-dioxo-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, CH₂), 2.61 (1H, m, CH₂), 2.37 (1H, m, CH₂), 2.18–1.84 (8H, m), 1.61 (1H, m, CH₂), 1.29 (1H, m, CH₂), 1.14 (3H, s, CH₃), 0.76 (3H, s, CH₃).

Methyl {3-oxo-2-[(2Z)-pent-2-en-5,5,5-²H₃-1-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 Et₂O and the organic layer was dried over anhydrous MgSO₄, 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 , CDCl₃): 5.44 (1H, m, CH), 5.25 (1H, m, CH), 3.69 (3H, s, OCH₃), 2.70 (1H, m, CH₂), 2.44–1.83 (10H, m), 1.48 (1H, m, CH₂); ¹³C NMR (δ , CDCl₃): 219.0 (C=O), 172.5 (C=O), 134.1 (CH), 125.0 (CH), 54.0 (CH), 51.6 (OCH₃), 38.8 (CH₂), 38.0 (CH), 37.7 (CH₂), 27.2 (CH₂), 25.4 (CH₂), 20.33 (CH₂); 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-5,5,5-²H₃-1-yl]cyclopentyl}acetic acid (7)

[12,12,12-²H₃]-MJA (**6**) (40 mg, 0.18 mmol) was dissolved in CH₃OH (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 MgSO₄, filtered and concentrated under vacuum. [12,12,12-²H₃]-JA was purified by preparative TLC plate (AcOH:Et₂O: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 , CDCl₃): 5.46 (1H, m, CH), 5.25 (1H, m, CH), 2.77 (1H, m, CH₂), 2.43–1.87 (10H, m), 1.52 (1H, m, CH₂); ¹³C NMR (δ , CDCl₃): 218.8 (C=O), 177.2 (C=O), 134.2 (CH), 124.8 (CH), 53.8 (CH), 38.5 (CH₂), 37.7 (CH), 37.3 (CH₂), 27.2 (CH₂), 25.4 (CH₂), 20.3 (CH₂); GCMS (EI) m/z (relative intensity) 213 ([M]⁺, 15), 154 (30), 142 (28), 95 (27), 83 (100), 67 (14), 55 (17).

[(3-Tert-butyl)dimethyl)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 Et₂O. The combined organic extracts were dried over anhydrous MgSO₄, filtered and concentrated *in vacuo*. Yield 3.60 g (99%). Without further purification the product was used in the next step. ¹H NMR (δ , CDCl₃): 3.71 (2H, t J = 5.7 Hz, CH₂), 3.49 (2H,

$t J = 6.5$ Hz, CH₂), 2.01 (2H, m, CH₂), 0.87 (9H, s, CH₃), 0.04 (6H, s, CH₂). 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 (CH₂Cl₂/Et₂O). 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; ¹H NMR (δ , CDCl₃): 7.73 (9H, m, CH), 7.63 (6H, m, CH), 3.76 (2H, m, CH₂), 3.69 (2H, m, CH₂), 1.82 (2H, m, CH₂), 0.77 (9H, s, CH₃), -0.05 (6H, s, CH₃); ¹³C NMR (δ , CDCl₃): 134.9 (C), 133.4 (CH), 130.3 (CH), 118.0 (d, CH₂), 61.45 (OCH₂), 25.71 (CH₃), 18.8 (CH₂), 17.9 (C), -5.5 (SiCH₃); ³¹P{¹H} NMR (δ , CDCl₃): 25.0 (s); HRMS calculated for C₂₇H₃₆OPSi [M]⁺ m/z 435.2273 found 435.2267; m.p. 127.3–128.2°C.

Methyl {2-[(2*Z*)-5-(*tert*-butyldimethyl)silyloxy-pent-2-en-1-yl]-6,10-dioxo-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 Et₂O. Combined organic extracts were washed with brine, dried over anhydrous MgSO₄ 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; ¹H NMR (δ , CDCl₃): 5.56 (1H, m, CH), 5.38 (1H, m, CH), 3.63 (3H, s, OCH₃), 3.60 (2H, t $J = 7.1$ Hz, OCH₂), 3.52 (2H, m, OCH₂), 3.38 (2H, m, OCH₂), 2.59 (1H, m, CH₂), 2.38 (1H, m, CH₂), 2.30 (2H, m, CH₂), 2.17–1.83 (6H, m), 1.61 (1H, m, CH₂), 1.29 (1H, m, CH₂), 1.13 (3H, s, CH₃), 0.88 (9H, s, CH₃), 0.75 (3H, s, CH₃), 0.04 (6H, s, CH₃); ¹³C NMR (δ , CDCl₃): 173.3 (C=O), 130.9 (CH), 126.1 (CH), 108.1 (C), 72.3 (OCH₂), 71.1 (OCH₂), 62.9 (OCH₂), 53.1 (CH), 51.3 (OCH₃), 39.9 (CH₂), 38.7 (CH), 31.3 (CH₂), 30.1 (C), 29.2 (CH₂), 28.1 (CH₂), 26.0 (CH₂), 25.9 (CH₃), 22.8 (CH₃), 22.2 (CH₃), 18.3 (C), -5.30 (SiCH₃); 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-²H₂ (8)

A solution of **17** (0.45 g, 1.0 mmol) in CH₃O²H (5 ml) was added dropwise to a solution of CH₃ONa (0.13 g, 2.5 mmol) in 2 ml of CH₃O²H. The mixture was refluxed for 5 days at 65°C. NH₄Cl 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 Et₂O the organic layer was dried over anhydrous MgSO₄ 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 Et₂O then the organic layer was dried over anhydrous MgSO₄, 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; ¹H NMR (δ , CDCl₃): 5.47 (2H, m, CH), 3.70 (3H, s, OCH₃), 3.67 (2H, t J = 6.2 Hz, OCH₂), 2.46–2.07 (8H, m), 1.92 (1H, m, CH₂), 1.50 (1H, m, CH₂); ¹³C NMR (δ , CDCl₃): 219.1 (C=O), 174.5 (C=O), 128.6 (CH), 128.3 (CH), 62.1 (OCH₂), 54.0 (CH), 51.7 (OCH₃), 37.8 (CH₂), 37.7 (CH), 30.9 (CH₂), 27.2 (CH₂), 25.4 (CH₂); GCMS (CI) m/z (relative intensity) 260 ([M+NH₄]⁺, 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-²H₂ (9)

12-OH-2,2-²H₂-MJA **8** (12 mg, 0.39 mmol) was dissolved in THF (0.2 ml) and KH₂PO₄/K₂HPO₄ 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 MgSO₄, 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; ¹H NMR (δ , CDCl₃): 5.76 (1H, brs, OH), 5.44 (2H, m, CH), 3.66 (2H, m, OCH₂), 2.55–2.24 (7H, m), 2.12 (1H, m, CH₂), 1.94 (1H, m, CH₂), 1.52 (1H, m, CH₂); ¹³C NMR (δ , CDCl₃): 219.1 (C=O), 176.6 (C=O), 128.7 (CH), 128.3 (CH), 62.1 (OCH₂), 54.1 (CH), 37.8 (CH₂), 37.6 (CH), 30.7 (CH₂), 27.3 (CH), 25.4 (CH₂); 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 (\pm)-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.

Acknowledgements

We would like to thank Brock Chatson for his contributions to the spectroscopy.

References

1. Mueller MJ. *Physiol Plant* 1997; **100**: 653–663.
2. Beale MH, Ward JL. *Nat Prod Rep* 1998; **15**: 533–548.
3. Creelman RA, Mullet JE. *Annu Rev Plant Physiol Plant Mol Biol* 1997; **48**: 355–381.
4. Sembdner G, Parthier B. *Annu Rev Plant Physiol Plant Mol Biol* 1993; **44**: 569–589.
5. Farmer EE, Ryan CA. *Proc Natl Acad Sci USA* 1990; **87**: 7713–7716.
6. Swiatek A, Van Dongen W, Esmans EL, Van Onckelen H. *Plant Physiol* 2004; **135**: 161–172.
7. Yamane H, Takagi H, Hisato A, Yokota T, Takahashi N. *Plant Cell Physiol* 1981; **22**: 689–697.
8. Knofel HD, Bruckner C, Miersch O, Sembdner G. *Biochem Physiol Pflanz* 1990; **186**: 387–394.
9. Anderson JM. *J Chromatogr* 1983; **330**: 347–355.
10. Creelman RA, Tierney ML, Mullet JE. *Proc Natl Acad Sci USA* 1992; **89**: 4938–4941.
11. Mueller MJ, Brodschelm W. *Anal Biochem* 1994; **218**: 425–435.
12. Wilbert SM, Ericsson LH, Gordon MP. *Anal Biochem* 1998; **257**: 186–194.
13. Miersch O. *Z Naturforsch, Teil B* 1991; **46**: 1727–1729.
14. Seto H, Fujioka S, Fujisawa H, Goto K, Nojiri H, Yamane H, Yoshida S. *Biosci Biotech Biochem* 1996; **60**: 1709–1711.
15. Creelman RA, Mullet JE. *Proc Natl Acad Sci USA* 1995; **92**: 4114–4119.
16. Baldwin IT, Zhang Z-P, Diab N, Ohnmeiss TE, McCloud ES, Lynds GY, Schmetz EA. *Planta* 1997; **201**: 397–404.
17. Knofel HD, Gross D. *Z Naturforsch, Teil C* 1988; **43**: 29–31.
18. Loreau O, Boullais C, Noel J-P, Mioskowski C. *J Label Compd Radiopharm* 1997; **XLI**: 159–170.
19. Chiwocha SDS, Abrams SR, Ambrose SJ, Cutler AJ, Loewen M, Ross ARS, Kermod AR. *Plant J* 2003; **35**: 405–417.
20. Nelson LAK, Shaw AC, Abrams SR. *Tetrahedron* 1999; **47**: 3259–3270.