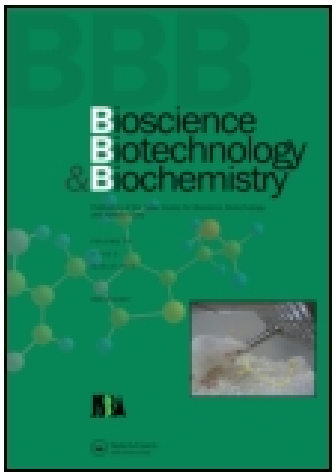

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Preparation of (±)-2-(2,3-$^2H_2$)Jasmonic Acid and Its Methyl Ester, Methyl (±)-2-(2,3-$^2H_2$)Jasmonate

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Note

Preparation of (±)-2-(2,3-$^2H_2$)Jasmonic Acid and Its Methyl Ester, Methyl (±)-2-(2,3-$^2H_2$)Jasmonate

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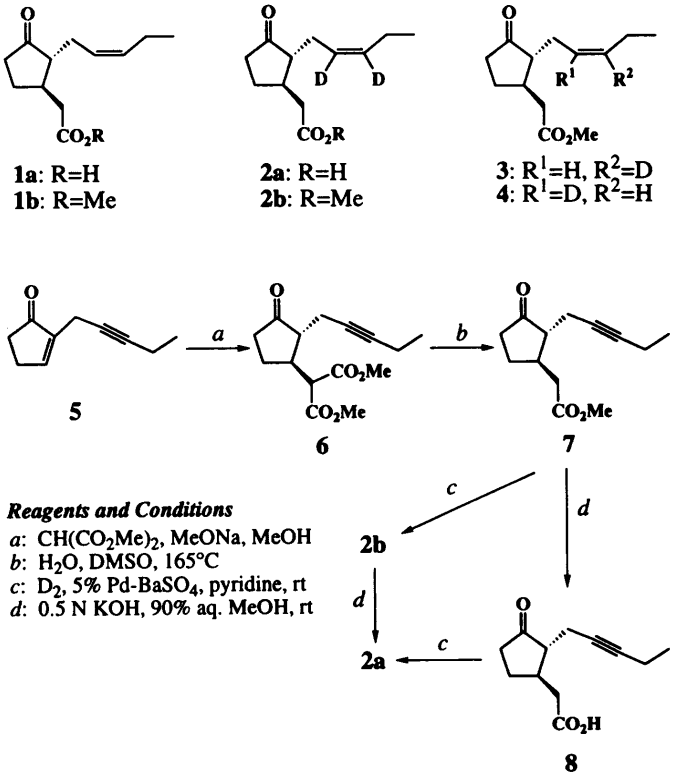
For use as the internal standards in a quantitative analysis of natural jasmonic acid (JA) and methyl jasmonate (JAMe) by gas chromatography-mass spectrometry-selected ion monitoring, (±)-2-(2,3-$^2H_2$)JA and its methyl ester, (±)-2-(2,3-$^2H_2$)JAMe, were efficiently prepared from 2-(2-pentyl)-2-cyclopentenone through catalytic semi-deuteriogenation of acetylenic intermediates with deuterium gas in pyridine.

Key words: jasmonic acid; methyl jasmonate; (±)-2-(2,3-$^2H_2$)jasmonic acid; methyl (±)-2-(2,3-$^2H_2$)jasmonate

We have previously established a method for the quantitative analysis of endogenous jasmonic acid (JA) (−)-**1a** in plant materials by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM), using (±)-2-(2,3-$^2H_2$)JA **2a** as an internal standard, in connection with an investigation to clarify the relationship between endogenous jasmonic acids and the development of onion bulbs.[1] We have subsequently been receiving a considerable number of requests for **2a** and its methyl ester, (±)-2-(2,3-$^2H_2$)JAMe **2b**, from plant physiologists, showing the usefulness of these deuterated compounds for quantifying the endogenous levels of JA and JAMe. These are currently important in plant physiology due to their hormonal properties[2] and involvement in the plant defense signaling pathway.[2,3] Since, to date, there has only been one labeled JA reported as an internal standard for GC-MS-SIM analysis, *i.e.*, (±)-1-(1-^{13}C)JA,[4] we describe here detailed procedures for the preparation of **2a** and **2b** from 2-(2-pentynyl)-2-cyclopentenone **5**, together with their isotopic purity.

Acetylenic compound **7**, a common intermediate in the earlier syntheses of **1a** and/or methyl jasmonate (JAMe) **1b** reported by other workers,[5,6] should be considered to be the most suitable synthetic precursor of **2a** and **2b**. Thus, **7** was prepared from cyclopentenone **5**, which we had previously employed in the synthesis of **1b** from adipic acid.[7] When **5** was reacted with the sodium enolate of dimethyl malonate, generated by treatment of dimethyl malonate with sodium methoxide, in methanol at temperatures ranging from the initial 40°C to the final −10°C, Michael adduct **6** was obtained in 89% yield. Ending the reaction at −10°C was critical to attain a high yield of **6** because of the reversible nature of the Michael addition reaction: at higher temperatures, the yield was lower and a considerable amount of starting material **5** was recovered, *e.g.*, at 0°C the yield of **6** was reduced to 75%. Diester **6** was then subjected to decarbomethoxylation with H_2O in dimethyl sulfoxide[8] at 165°C to give monoester **7** in 71% yield.

Catalytic semi-deuteriogenation of methyl (±)-dehydroJAMe **7** to **2b** was carried out by following the procedure for the semi-hydrogenation of **7** to **1b** reported by Johnson *et al.*[6] When **7** was deuteriogenated over 5% Pd–$BaSO_4$ catalyst, using deuterium gas (≥99.5 D-mol%) at room temperature (rt) and at atmospheric pressure in pyridine, (±)-2-(2,3-$^2H_2$)JAMe **2b** was obtained in 86% yield, after purification by column chromatography and subsequent HPLC.



The ^{1}H-NMR spectrum of **2b** was identical to that of unlabeled compound **1b**, except for the olefinic region. The tiny signals at δ 5.26 and 5.45, with each integral value corresponding to *ca.* 0.05 protons, were assigned to the olefinic protons of monodeuterated compounds (±)-2-(2-^{2}H)JAMe **3** and (±)-2-(3-^{2}H)JAMe **4**, respectively, because none of them possess the vicinal coupling characteristic of *cis*-olefinic protons, which was observed in the signals at δ 5.26 and 5.46 of **1b** with J_{vic} 10.8 Hz. This indicated that the ratio of **3** to **4** was 1 : 1, and that the total amount of contaminants **3** and **4** in **2b** was *ca.* 10%; the olefinic signals of **1b** may have overlapped those of **3** and **4**.

The MS of obtained **2b** showed that the ions at *m/z* 226, 195, and 153 contained two deuterium atoms, which were respectively assigned to M^+, M^+-OMe, and $M^+-CH_2CO_2Me$, by comparison with the spectrum of the unlabeled compound **1b** possess-

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ing these ions at *m/z* 224, 193, and 151. In addition, the relative intensity of the ions at 226, 225, and 224 showed that sample **2b** contained 1.4% of **1b** and 9.2% of monodeuterated compounds **3** and **4**; thus the total amount of contaminants estimated by MS was well consistent with that estimated by ^{1}H-NMR spectrum (*vide supra*).

Methyl ester **2b** was then hydrolyzed with 0.5 N KOH in 90% aqueous MeOH at rt to give (±)-2-(2,3-$^2H_2$)JA **2a** in 91% yield. Acid **2a** was also prepared from **7** *via* (±)-dehydroJA **8** by alkali hydrolysis and subsequent catalytic semi-deuteriogenation in 57% overall yield. HPLC analysis of **2b**, derived from **2a** by treatment with ethereal diazomethane, showed that **2a** contained 5.1% of the epimer at the 2-position, which would have resulted from epimerization under alkali hydrolysis conditions.

In conclusion, (±)-2-(2,3-$^2H_2$)JA **2a** and (±)-2-(2,3-$^2H_2$)JAMe **2b** were efficiently prepared from acetylenic compound **5** *via* semi-deuteriogenation, both of which contained the corresponding nondeuterated compound (1.4% calculated by an analysis of the MS of **2b**) and monodeuterated compounds (9.2% total). Dideuterated compounds **2a** and **2b** with high isotopic purity should be more suitable than the known (±)-1-(1-^{13}C)JA[4] for quantifying the endogenous levels of (−)-**1a** and (−)-**2b**, because the standard peak of **2b** used for GC-MS-SIM, *m/z* 226, corresponds to $M^+ + 2$ of (−)-**1b**, while that of the methyl ester of (±)-1-(1-^{13}C)JA, *m/z* 225, corresponds to $M^+ + 1$ of (−)-**1b**. It is noteworthy that tritium-labeled JA and JAMe could be prepared from acetylenic compounds **7** and **8**, respectively, by using tritium gas, which are also useful for physiological and metabolic studies on JA and JAMe.

Experimental

Melting point (mp) and boiling point (bp) data are uncorrected. NMR spectra were measured for $CDCl_3$ solutions with a JEOL JNM-EX90 spectrometer operated at 89.5 MHz for ^{1}H and at 22.5 MHz for ^{13}C, and with a Bruker AC-300 Plus instrument at 300 MHz for ^{1}H. Chemical shifts were recorded as δ values in parts per million (ppm) and were referenced to TMS as an internal standard for ^{1}H and the solvent signal of 77.0 ppm for ^{13}C. A Hitachi M-80 mass spectrometer was used to obtain MS spectra, and column chromatography was performed with Wakogel C-300 (Wako Pure Chemical Industries).

(2R,3S*)-3-Bis(methoxycarbonyl)methyl-2-(2-pentynyl)cyclopentanone* **6**. Dimethyl malonate (37 ml, 324 mmol) was added slowly to a stirred solution of MeONa in MeOH, prepared from sodium metal (750 mg, 32.6 mg-atom) and dry MeOH (15 ml), at rt under nitrogen atmosphere. The reaction temperature was allowed to warm to 40°C, and then 2-(2-pentynyl)cyclopent-2-enone **5**[7] (30 g, 160 mmol, 79% purity) was slowly added. After stirring for 1.5 h, the reaction mixture was gradually cooled to −10°C at the rate of *ca.* 25°C/h, and stirring was continued for 1 h. The solution was neutralized with 1 N HCl and extracted with Et_2O. The organic layer was successively washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated. The residual oil was subjected to fractional distillation to afford Michael adduct **6** (40 g, 89%) as a pale yellow oil. bp 137–145°C/0.2 mmHg; R_f value of 0.47 on TLC (hexane–AcOEt, 2:1); ^{1}H-NMR (89.5 MHz): δ 1.08 (3H, t, J=7.4 Hz, ω-CH_3), 1.57–2.58 (10H), 2.85 (1H, m), 3.77 (1H, d, J=5.9 Hz, C<u>H</u>$(CO_2CH_3)_2$), 3.77, and 3.78 (2×3H, each s, 2×CO_2CH_3); MS *m/z*: 280 (M^+, 0.51%), 251 (39), 189 (11), 149 (30), 148 (100), 133 (38), 122 (29), 108 (17), 106 (12), 92 (12).

(2R,3R*)-3-Methoxycarbonylmethyl-2-(2-pentynyl)cyclopentanone* [(±)-*dehydroJAMe* **7**]. A mixture of diester **6** (30 g, 107 mmol), DMSO (90 ml) and water (3 ml) was heated at 165°C with stirring in a nitrogen gas stream for 2 days. After cooling, water was added, and the mixture was extracted with Et_2O. The ether solution was successively washed with water and brine, dried over $MgSO_4$, and concentrated. After fractional distillation, (±)-dehydroJAMe **7** (16.8 g, 71%) was obtained as a pale yellow oil. bp 115–120°C/0.4 mmHg; R_f value of 0.50 on TLC (hexane–AcOEt, 2:1); ^{1}H-NMR (300 MHz): δ 1.09 (t, J=7.5 Hz, ω-CH_3), 1.52 (1H, m), 1.94 (1H, m), 2.03–2.71 (9H), 2.85 (1H, dd, J=15.2 and 4.4 Hz), 3.72 (s, CO_2CH_3); ^{13}C-NMR (22.5 MHz): δ 13.7 (ω-<u>C</u>H_3), 11.9, 17.0, 26.7, 37.2, 38.1 (5×<u>C</u>H_2), 37.5, 52.4 (C-2 and -3), 51.1 (CO_2<u>C</u>H_3), 75.6, 83.0 (<u>C</u>≡<u>C</u>), 172.0 (<u>C</u>O_2CH_3), 216.6 (C-1); MS *m/z*: 222 (M^+, 1.6%), 193 (59), 149 (14), 147 (11), 134 (15), 123 (11), 122 (100), 108 (37), 92 (20), 79 (15).

(2R,3R*)-3-Methoxycarbonylmethyl-2-[(2,3-$^2H_2$)-2-pentenyl]cyclopentanone* [(±)-*2-(2,3-$^2H_2$)JAMe* **2b**]. A solution of **7** (200 mg) in pyridine (4 ml) was deuteriogenated at rt and atmospheric pressure over 5% Pd–$BaSO_4$ (10 mg) by deuterium gas (≥99.5 D-mol%, purchased from Showa-Denko Co.). The absorption of gas (*ca.* 22 ml, 22 ml theoretical) ceased completely after 20 min. After filtration to remove the catalyst, the filtrate was concentrated and subjected to column chromatography. Elution with hexane–AcOEt, 5:1, afforded an oil (198 mg), which was further purified by preparative HPLC (Senshu Pak Silica-5251-N, 20 mm i.d. × 25 cm). Elution with hexane–AcOEt, 10:1, at a flow rate of 8 ml/min gave (±)-2-(2,3-$^2H_2$)JAMe **2b** (176 mg, 86%) as a colorless oil. The R_f value of 0.56 on TLC (hexane–AcOEt, 2:1) and the t_R value of 48 min by HPLC were identical to those of authentic (±)-JAMe **1b**, and the ^{1}H-NMR spectrum was well consistent with that of **1b**, except for the olefinic region. ^{1}H-NMR (300 MHz): δ 0.96 (3H, t, J=7.5 Hz, ω-CH_3), 1.49 (1H, m), 1.89 (1H, m), 1.93–2.42 (9H), 2.71 (1H, dm, J=11.0 Hz), 3.70 (3H, s, CO_2CH_3), 5.26 (0.05H, tm, J=7.3 Hz, –CH=CD-Et of contaminant **3** overlapping with –C<u>H</u>=CH-Et of contaminant **1b**), 5.45 (0.05H, tm, J=7.1 Hz, –CD=CH-Et of contaminant **4** overlapping with –CH=C<u>H</u>-Et of contaminant **1b**); MS *m/z*: 226 (M^+, 87%), 225 ($M^+ - 1$, 9.2), 224 ($M^+ - 2$, 1.4), 195 (27), 156 (34), 153 (97), 152 (28), 110 (23), 98 (25), 85 (36), 84 (100), 83 (31). HRMS *m/z* (M^+): calcd. for $C_{13}H_{18}D_2O_3$, 226.1536; found, 226.1533. HRMS *m/z* ($M^+ - 1$): calcd. for $C_{13}H_{19}DO_3$, 225.1474; found, 225.1477. For reference, see the spectra for **1b**. ^{1}H-NMR (300 MHz): δ 5.26 (1H, dtm, J=10.8 and 7.5 Hz, –C<u>H</u>=CH-Et), 5.46 (1H, dtm, J=10.8 and 7.2 Hz, –CH=C<u>H</u>-Et); MS *m/z*: 224 (M^+, 100%), 223 (0.3), 193 (29), 156 (43), 151 (84), 135 (18), 110 (26), 96 (27), 94 (18), 84 (87), 83 (22).

(2R,3R*)-3-Carboxymethyl-2-(2-pentynyl)cyclopentanone* [(±)-*dehydroJA* **8**]. Methyl ester **7** (50 mg) was dissolved in 90% aqueous MeOH containing 0.5 N KOH (1 ml), and the solution was stirred at rt for 5 h. After removal of the solvent, the residue was dissolved in water, acidified with 1 N HCl, and extracted with AcOEt. The extract was successively washed with water and brine, and then dried over Na_2SO_4. After removal of the solvent, the residue was subjected to column chromatography. Elution with AcOEt gave (±)-dehydroJA **8** (38 mg, 82%) as a colorless oil. R_f value of 0.33 on TLC (AcOEt); ^{1}H-NMR (300 MHz): δ 1.09 (3H, t, J=7.5 Hz, ω-CH_3), 1.55 (1H, m), 1.96 (1H, m), 2.07–2.63 (9H), 2.96 (1H, dd, J=15.4 and 3.8 Hz); MS *m/z*: 208 (M^+, 0.9%), 179 (51), 149 (14), 133 (13), 123 (13), 122 (100), 108 (52), 106 (14), 92 (21), 79 (18).

(2R,3R*)-3-Carboxymethyl-2-[(2,3-$^2H_2$)-2-pentenyl]cyclopentanone* [(±)-*2-(2,3-$^2H_2$)JA* **2a**].

(a) *From* **2b**. Essentially according to the same procedure as described above for the alkali hydrolysis of **7**, **2b** (134 mg) afforded (±)-2-(2,3-$^2H_2$)JA **2a** (114 mg, 91%) as a colorless oil. R_f value of 0.33 on TLC (AcOEt); ^{1}H-NMR (300 MHz): δ 0.96 (3H, t, J=7.5 Hz, ω-CH_3), 1.52 (1H, m), 1.85–2.55 (10H), 2.78 (1H, dm, J=11.7 Hz), 5.26 (0.05H, tm, J=7.4 Hz, –CH=CD-Et of monodeuterated JA overlapping with –C<u>H</u>=CH-Et of **1a**), 5.47 (0.05H, tm, J=7.0 Hz, –CD=CH-Et of monodeuterated JA overlapping with –CH=C<u>H</u>-Et of **1a**); MS *m/z*: 212 (M^+, 100%), 211 ($M^+ - 1$, 10), 210 ($M^+ - 2$, 2.2), 153 (88), 143 (24), 142 (42), 110 (15), 98 (21), 96 (15), 85 (30), 84 (100), 83 (25). For reference, see the MS spectrum of **1a**: MS *m/z*: 210 (M^+, 74%), 151 (77), 142 (43), 110 (22), 96 (26), 94 (16), 84 (100), 83 (21).

(b) *From* **8**. Acetylenic compound **8** (10 mg) in pyridine (0.2 ml) was deuteriogenated at rt and at atmospheric pressure over 5% Pd–$BaSO_4$ (1 mg) by deuterium gas for 1 h. The catalyst was filtered off by silica gel on a glassfilter and washed with AcOEt. The filtrate was concentrated, and the residue was dissolved in AcOEt. The solution was successively washed with 1 N HCl, water and brine, and then dried over Na_2SO_4. After removal of the solvent, the residue was subjected to column chromatography. Elution with AcOEt gave (±)-2-(2,3-$^2H_2$)JA **2a** (7 mg, 69%) as a colorless oil.

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