Preparation of $(\pm)$-2-(2,3,2'-H$_2$)-Jasmonic Acid and Its Methyl Ester, Methyl $(\pm)$-2-(2,3,2'-H$_2$)-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. $(\pm)$-2-(2,3,2'-H$_2$)JA and its methyl ester. $(\pm)$-2-(2,3,2'-H$_2$)JAME, were efficiently prepared from 2-(2-pentyl)-2-cyclopentene through catalytic semi-deuteriation of acetylenic intermediates with deuterium gas in pyridine.

Key words: jasmonic acid; methyl jasmonate; $(\pm)$-2-(2,3,2'-H$_2$)jasmonic acid; methyl $(\pm)$-2-(2,3,2'-H$_2$)jasmonate

We have previously established a method for the quantitative analysis of endogenous jasmonic acid (JA) $(\pm)$-1a in plant materials by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM), using $(\pm)$-2-(2,3,2'-H$_2$)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. We have subsequently been receiving a considerable number of requests for 2a and its methyl ester, $(\pm)$-2-(2,3,2'-H$_2$)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 and involvement in the plant defense signaling pathway. Since, to date, there has only been one labeled JA reported as an internal standard for GC-MS-SIM analysis, i.e., $(\pm)$-1-(1-13C)JA, we describe here detailed procedures for the preparation of 2a and 2b from 2-(2-pentenyl)-2-cyclopentene 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, 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. 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 addition 6 was obtained in 89% yield. 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 decarboxymethylation with H$_2$O in dimethyl sulfoxide at 165°C to give monoester 7 in 71% yield.

Catalytic semi-deuteriation of methyl $(\pm)$-dehydroJAME 7 to 2b was carried out by following the procedure for the semi-deuteriation of 7 to 1b reported by Johnson et al. When 7 was deuteriated over 5% Pd-BaSO$_4$ catalyst, using deuterium gas (99.5 D%-mol%) at room temperature (rt) and at atmospheric pressure in pyridine, $(\pm)$-2-(2,3,2'-H$_2$)JAME 2b was obtained in 86% yield, after purification by column chromatography and subsequent HPLC.

Reagents and Conditions

- a: CH(CO$_2$Me)$_2$, MeONa, MeOH
- b: H$_2$O, DMSO, 165°C
- c: D$_2$, 5% Pd-BaSO$_4$, pyridine, rt
- d: 0.5 N KOH, 90% aq. MeOH, rt

The 1H-NMR spectrum of 2b was identical to that of unlabeled compound 1b, except for the olefinic region. The tiny signals at $\delta$ 5.26 and 5.45, with each integral value corresponding to ca. 0.05 protons, were assigned to the olefinic protons of monodeuterated compounds $(\pm)$-2-(2-2'-H$_2$)JAME 3 and $(\pm)$-2-(2-3'-H$_2$)JAME 4, respectively, because none of them possess the vicinal coupling characteristic of cis-olefinic protons, which was observed in the signals at $\delta$ 5.26 and 5.46 of 1b with $J_{\alpha\beta}$ 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 deuteron atoms, which were respectively assigned to M$^+$, M$^+$ - OMe, and M$^+$ - CH$_2$CO$_2$Me, 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 1H-NMR spectrum (vide supra).

Methyl ester 2b was then hydrolyzed with 0.5N KOH in 90% aqueous MeOH at rt to give (+)-(2-(2,3-H2)JJA 2a in 91% yield. Acid 2a was also prepared from 7 via (±)-dehydroJA B 8 by alkalai hydrolysis and subsequent catalytic semi-deuteriation 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 alkalai hydrolysis conditions.

In conclusion, (+)-(2-2,3-H2)JA 2a and (+)-(2-2,3-H2)JAMe 2b were efficiently prepared from acetylenic compound 5 via semi-deuteriation, 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-(1-13C)JA 8 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-(1-13C)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 CDC13 solutions with a JEOL JNM-E90X spectrometer operated at 89.5 MHz for 1H and at 22.5 MHz for 13C, and with a Bruker AC-300 Plus instrument at 500 MHz for 1H. Chemical shifts were recorded as δ values in parts per million (ppm) and were referenced to TMS as an internal standard for 1H and the solvent signal of 7.7 ppm for 13C. 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*,3R*)-3-Bis(3-methoxyacyl)methyl-2-(2-pentenyl)cyclopentanone [(±)-dehydroJA 4a]. Methyl ester 7 (50 mg) was dissolved in 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 1N HCl, and extracted with AcOEt. The extract was successively washed with water and brine, and then dried over Na2SO4. After removal of the solvent, the residue was subjected to column chromatography on silica gel (100-200 mesh). The elution with AcOEt gave (±)-dehydroJA 4a (38 mg, 82%) as a colorless oil. Rf value of 0.33 on TLC (AcOEt-H2O: 20:1, δ 0.91 (3H, t, J = 7.5 Hz, -OCH3), 1.55 (1H, m), 1.96 (1H, m), 2.07-2.63 (9H), 2.96 (1H, dd, J = 5.4 and 3.8 Hz), MS m/z: 208 (M+*, 0.9%), 179 (51), 149 (14), 123 (13), 122 (100), 108 (52), 106 (44), 92 (21), 79 (18).

(2R*,3R*)-3-Carbomethoxy-2-(2-pentenyl)cyclopentanone [(±)-dehydroJA 4a]. (a) From 2b. Essentially according to the same procedure as described above for the alkali hydrolysis of 7b. 2b (134 mg) afforded (±)-(2-2,3-H2)JJA 2a (114 mg, 91%) as a colorless oil. Rf value of 0.33 on TLC (AcOEt-H2O: 20:1, δ 0.91 (3H, t, J = 7.5 Hz, -OCH3), 1.52 (1H, m), 1.85-2.55 (10H), 2.78 (1H, dm, J = 11.7 Hz), 5.26 (0.03H, tt, J = 7.4 Hz, -CH=CH=Et of monodeuterated JA overlapping with -CH=CH=Et of 1a), 5.47 (0.05H, tm, J = 7.0 Hz, -CH=CH=Et of monodeuterated JA overlapping with -CH=CH=Et of 1a); MS m/z: 212 (M+*, 100%), 211 (M+−1, 10), 210 (M+−2, 2.2), 153 (88), 143 (24), 142 (41), 120 (115), 98 (21), 96 (15), 85 (30), 84 (10), 83 (25). For reference, see the MS spectrum of 1a: MS m/z: 210 (M+*, 74%), 151 (77), 142 (43), 121 (22), 96 (26), 94 (16), 84 (100), 83 (21).

(b) From 8. Acetylic compound 8 (10 mg) in pyridine (0.2 ml) was deuteriated at rt and at atmospheric pressure over 5% Pd-BaSO4 (1 mg) by deuteration 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 1N HCl, water and brine, and then dried over Na2SO4. After removal of the solvent, the residue was subjected to column chromatography. Elution with AcOEt gave (±)-(2-2,3-H2)JJA 2a (7 mg, 69%) as a colorless oil.

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