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Qualitative and Quantitative Analysis of Endogenous Jasmonoids in Potato Plant (*Solanum tuberosum* L.)

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Qualitative and quantitative analyses of endogenous jasmonoids were done by liquid chromatography/selected ion monitoring (LC-SIM) using deuterium-labeled compounds as internal standards. To prove the practicality of this way of analyzing the contents of endogenous jasmonoids in plants, the method was used for estimating jasmonoids in potato plants.

Key words: *Solanum tuberosum* L.; potato microtuber inducing stimuli; jasmonoids; liquid chromatography/selected ion monitoring (LC-SIM)

Tuber development of potato plants (*Solanum tuberosum* L.) is controlled by environmental factors, mainly photoperiods. Short days stimulate this process, but long days have the opposite effect.[1-3] This phenomena mean that potato plants distinguish the day length. With grafting experiments, Gregory and Chapman demonstrated the existence of tuber-inducing stimuli which are synthesized in the leaflets and transported to underground parts to induce tuberization.[3,4] With these findings as triggers, a lot of researchers had made great efforts to find out potato tuber-inducing stimuli.

In 1988, Koda developed a method for detecting tuber-inducing stimuli *in vitro*,[5] and we reported potato tuber-inducing stimuli from the leaflets of potato (*Solanum tuberosum* L.)[6,7] and the leaves of Jerusalem artichoke (*Helianthus tuberosus*).[8] The structures of these active compounds were identified as 5′-hydroxyjasmonic acid glucoside (**1**, tuberonic acid β-D-glucoside: TAG), 5′-hydroxyjasmonic acid (**2**, tuberonic acid: TA), and methyl 5′-hydroxyjasmonate β-D-glucoside (MeTAG) as in Fig. 1, and it was also reported that jasmonic acid (**3**, JA) and JA′s biosynthetic intermediates, (1*S*, 2*S*) 3-oxo-2-[(*Z*)-2′-pentenyl]cyclopentanebutanoic acid (**4**, OPC 4:0), (1*S*, 2*S*) 3-oxo-2-[(*Z*)-2′-pentenyl]cyclopentanehexanoic acid (**5**, OPC 6:0), and (1*S*, 2*S*) 3-oxo-2-[(*Z*)-2′-pentenyl]cyclopentaneoctanoic acid (**6**, OPC 8:0), have potato tuber inducing activities.[9,10] Furthermore, we demonstrated that the ^{14}C-labeled jasmonic acid was metabolized to more polar compounds when applied to the leaflets of potato and transported to the other parts.[11]

Since there are no qualitative or quantitative analytical methods for TAG, TA, and other jasmonoids, the relationships between the day length and these active compounds are still unknown. In this paper, we report the synthese of deuterium-labeled (±)-*epi*-TAG, (±)-*epi*-TA, (±)-*epi*-OPC 4:0, (±)-*epi*-OPC 6:0, and (±)-*epi*-OPC 8:0. We also mention a qualitative and quantitative analytical method for endogenous TAG, TA, *epi*-JA, OPC 4:0, and OPC 6:0 by liquid chromatography/selected ion monitoring (LC-SIM) using deuterium-labeled compounds as internal standards.

Materials and Methods

General. Spectra were obtained with the following instruments: IR, Hitachi 285 spectrometer; NMR, Jeol JNM-EX 270 FT-NMR system; FD- and EI-MS, Jeol JMS-O1SG-2 and JMS-DX-300 mass spectrometers, respectively. LC-SIM was done using a M-1200AP LC-MS system (Hitachi, Japan) using a Wakosil 5C8 column (4 mm × 250 mm; Wako Pure Chemical Industries, Ltd.).

Chemicals. Methyl (±)-jasmonate was kindly given to us by Nippon Zeon Co., Ltd. (Tokyo, Japan). [1-^{2}H$_2$, 2-^{2}H$_2$, 3-^{2}H$_3$] Bromopropane and [1-^{2}H$_2$, 2-^{2}H$_2$, 3-^{2}H$_2$] 1,3-propanediol were purchased from MSD Isotopes (Canada). Bond Elut C_{18} and DEA are purchased from Varian (Harbor City,

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Canada), and β-D-glucosidase was purchased from Sigma (St. Louis, USA).

Plant material. Potato plants (*Solanum tuberosum* L. cv. Irish Cobbler) were grown in the experimental field of Hokkaido University. The mother tubers were planted on the first of May, and plants were harvested within June, July, and August 1997.

Preparation of **11**. A stirred mixture of [1-$^2H_2$, 2-$^2H_2$, 3-$^2H_3$] bromopropane (5 g, 44.6 mmol) and triphenyl phosphine (23.4 g, 89.3 mmol) in toluene (186 ml) was refluxed for 72 h. Filtration of the mixture gave deuterium-labeled triphenylphosphonium bromide as white crystals (**10**, 11.8g, 31.5 mmol, 70%), which was converted to [1-$^2H_2$, 2-$^2H_2$, 3-$^2H_3$] propylidenetriphenylphosphorane (**11**) by *n*-butyllithium (*n*-BuLi) in tetrahydrofuran (THF).

Compound **10**: EIMS *m/z* (rel. int.): 277.9 (6), 262.7 (100), 183 (61), 152 (9), 108 (24), 77 (5).

Preparation of deuterium-labeled (±)-epi-OPC 4:0, (±)-epi-OPC 6:0, and (±)-epi-OPC 8:0. To a stirred mixture of methyl (±)-jasmonate (39 g, 0.17 mol) in MeOH (500 ml) at −78°C was added excess O_3. After the color of the reaction mixture turned pale blue, dimethyl sulfide (Me_2S, 25 ml) was added to the reaction mixture. The reaction mixture was further stirred at room temperature for 24 h. The usual work-up followed by a purification using silica gel (Wakosil 200 g, $CHCl_3$) afforded an aldehyde (31 g, 156 mmol, 91%). Protections of the ketone and aldehyde moieties of the resulting compound (21 g, 106 mmol) were done by the usual method using a solution of ethylene glycol (58.6 ml, 1.06 mol) and pyridinium *p*-toluenesulfonate (PPTS, 80 mg) in toluene (300 ml), and the resulting product was purified by silica gel (Wakosil 250 g, MeOH–$CHCl_3$, 2:98, v/v) to afford a protected ester (26.7 g, 93.3 mmol, 92%). The ester was reduced by diisobutylaluminium hydride (DIBAL-H) in CH_2Cl_2 to give an aldehyde (70%), which was coupled with $(EtO)_2POCH_2CO_2Me$ and hydrogenated using Pd–C/H_2 to give **7** (69%, 2 steps). Compound **8** was obtained from **7** using the same manner (DIBAL-H, Horner Wadsworth Emmons reaction, hydrogenation, 66%, 3 steps), and compound **9** was obtained similarly from **8** (42%, 3 steps). After the 1,3-dioxolane moieties were removed by means of a solution of acetone-0.4 M HCl–H_2O (5:2:1, v/v/v) at 70–80°C , the deprotected derivatives of **7**–**9** were coupled with **11** to give methyl esters, which were hydrolyzed and purified by HPLC (GL Science Inc., Inertsil ODS 10 μm, 20 × 250 mm, A_{254nm}, 3 ml/min, MeOH–H_2O–AcOH, 90:10:0.1 for OPC 4:0 and OPC 8:0, 85:15:0.1 for OPC 6:0, v/v/v) to afford deuterium labeled (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 4:0 (6.3%, 3 steps from **7**), (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 6:0 (26%, 3 steps from **8**), and (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 8:0 (9.8%, 3 steps from **9**), respectively.

(±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 4:0. EIMS *m/z* (rel. int.): 244 (34), 226 (29), 171 (12), 157 (100), 130 (27), 96 (53), 84 (83), 83 (58); HREIMS *m/z*: $[M]^+$ 244.1956 (calcd. for $C_{14}{}^1H_{16}{}^2H_6O_3$: 244.1946); IR ν_{max} (film) cm^{-1}: 3700–2500, 2933, 1737, 1407, 1167; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.10 (1H, t, *J*= 7.6 Hz), 2.25 (5H, m), 2.00 (2H, m), 1.87–1.44 (5H, m), 1.27 (2H, m).

(±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 6:0. EIMS *m/z* (rel. int.): 272 (13), 254 (20), 199 (24), 157 (78), 130 (67), 129 (24), 96 (67), 84 (100), 83 (87); HREIMS *m/z*: 272.2252 $[M]^+$ (calcd. for $C_{16}{}^1H_{20}{}^2H_6O_3$: 272.2259); IR *ν* max (film) cm^{-1}: 3430, 2930, 1732, 1485, 1164; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.33 (1H, t, *J*=7.6 Hz), 2.45 (5H, m), 2.19 (2H, m), 1.90 (2H, m), 1.75 (3H, m), 1.45 (6H, m).

(±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 8:0. EIMS *m/z* (rel. int.): 300 (13), 282 (27), 227 (30), 157 (100), 130 (93), 129 (29), 96 (76), 84 (87), 83 (93); HREIMS *m/z*: 300.2588 $[M]^+$ (calcd. for $C_{18}{}^1H_{24}{}^2H_6O_3$: 300.2572); IR ν_{max} (film) cm^{-1}: 3437, 2856, 1739, 1710, 1408, 1163; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.33 (1H, t, *J*=7.6 Hz), 2.45 (5H, m), 2.19 (3H, m), 1.90 (2H, m), 1.73 (3H, m), 1.42 (10H, m).

Preparation of **12**. Sodium hydride (2.66 g of a dispersion in oil, 66.4 mmol) was placed in a three-necked, round-bottomed flask with a stirring bar, septum cap, and N_2 balloon, and cooled to 0°C. The sodium hydride was then suspended in anhydrous THF (500 ml), and the mixture was allowed to warm to room temperature over 30 min. [1-$^2H_2$, 2-$^2H_2$, 3-$^2H_2$]-1,3-Propanediol (5 g, 60.4 mmol) was added dropwise to the stirred suspension over 10 min, and the reaction mixture was further stirred at room temperature for 40 min. The stirred reaction mixture was cooled with an ice bath, and *t*-butyldiphenylsilyl chloride (TBDPS-Cl, 17 g, 62 mmol) was added dropwise to the reaction mixture at 0°C. The reaction mixture was further stirred at room temperature for 24 h, and the usual work-up followed by a purification using silica gel (Wakosil 250 g, EtOAc-*n*-hexane, 25:75, v/v) afforded [1-$^2H_2$, 2-$^2H_2$, 3-$^2H_2$]-3-(*t*-butyldiphenylsilyloxy)propanol (18.2 g, 57 mmol, 95%). To a stirred mixture of the propanol derivative (16 g, 50 mmol) at 0°C was added at once a mixture of Ph_3P (15.7 g, 60 mmol) and carbon tetrabromide (CBr_4, 24.8 g, 75 mmol), and the reaction mixture was stirred for 5 min at 0°C, and then, sat. aq. $NaHCO_3$ (300 ml) was added to the reaction mixture. The mixture was washed with sat. aq. $NaHCO_3$ (300 ml × 2) and sat. aq. NaCl (300 ml × 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel (Wakosil 340 g, $CHCl_3$-*n*-hexane, 1:1, v/v) to give

[1-$^2H_2$, 2-$^2H_2$, 3-$^2H_2$]-1-bromo-3-(*t*-butyldiphenylsilyloxy)propane (**12**, 12.8 g, 67%): EIMS *m/z* (rel. int.): 327 (67), 325 (65), 295 (41), 293 (40), 263 (100), 261 (100), 213 (41), 181 (32), 93 (33), 77 (8).

Preparation of **13**. A mixture of **12** (11 g, 28 mmol) and Ph_3P in toluene (180 ml) was stirred at reflux for 24 h. After being collected with filter paper, the resulting white powders were dissolved with a mixture of *p*-toluenesulfonic acid (*p*-TsOH, 700 mg) in MeOH (150 ml), and the mixture was stirred over 48 h. The volatile components of the mixture were removed under reduced pressure to give a colorless oil, which was purified with silica gel (Wakosil 150 g, MeOH–$CHCl_3$, 5:95, v/v) to give a deprotected phosphonium bromide (10.5 g, 2 steps 92%). A mixture of the phosphonium salt (12 g, 29.6 mmol), 3,4-dihydropyran (DHP, 5.3 ml), and pyridinium *p*-toluenesulfonate (PPTS, 40 mg) in CH_2Cl_2 (300 ml) was stirred at reflux for 3 h. After additional DHP (10 ml) being added, the reaction mixture was further stirred for 10 h. The volatile components of the mixture were removed under reduced pressure to give a colorless oil, which was purified with silica gel (Wakosil 150 g, MeOH–$CHCl_3$, 5:95, v/v) to afford **13** (13.1 g, 26.7 mmol, 90%): EIMS *m/z* (rel. int.): 291 (44), 262 (100), 247 (36), 201 (29), 183 (56), 108 (25); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.88–7.68 (15H, m), 4.54 (1H, m), 3.79 (1H, m), 3.48 (1H, m), 1.75 (2H, m), 1.51 (4H, m).

Preparation of deuterium-labeled (±)-epi-TA. To a stirred suspention of **13** (4.6 g, 9.4 mmol) in THF (100 ml) at room temperature was added n-BuLi (11.3 mmol) dropwise, and the reaction mixture was stirred for 1 h. After the solution was cooled to −45°C, a solution of the aldehyde (2.3 g, 11.5 mmol), which was derived from methyl (±)-jasmonate as shown in scheme 3, was added to this solution, and the reaction mixture was stirred for 3 h at −45°C. The reaction mixture was poured into EtOAc (300 ml) and washed with 1 N HCl (100 ml), sat. $NaHCO_3$ (100 ml × 2), and sat. NaCl (200 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was purified using silica gel (100 g, EtOAc-hexane, 4:6, v/v) to afford methyl [3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-5′-*O*-tetrahydropyranyltuberonate (**14**, 760 mg, 24%). Deprotection of the THP group and purification of the resulting material using silica gel (Wakosil 80 g, EtOAc-hexane, 1:1, v/v) afforded methyl (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-tuberonate (**15**, 470 mg, 1.9 mmol, 82%), which was then hydrolyzed by 0.5 % NaOH/EtOH. The resulting product was purified by HPLC (GL Science Inc., Inertsil ODS 10 um, 20 × 250 mm, A_{254nm}, 3 ml/min, MeOH–H_2O–AcOH, 65:35:1, v/v/v) to afford (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TA (about 5% from **13**): EIMS *m/z* (rel. int.): 231 (3), 213 (3), 142 (71), 83 (100); HREIMS *m/z*: 231.1515 $[M]^+$ (calcd. for $C_{12}{}^1H_{13}{}^2H_5O_4$: 231.1519); IR ν_{max} (film) cm^{-1}: 3500–2500, 3431, 2961, 1732, 1407, 1272, 1170, 970, 626; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.45 (1H, br. t), 2.66 (1H, br. dd), 2.5–2.0 (7H, complex), 1.94 (1H, br. quintet), 1.53 (1H, br. quintet).

Preparation of deuterium-labeled (±)-epi-TAG. To a stirred mixture of Drierite (3 g), HgO (1.08 g, 5 mmol), and $HgBr_2$ (1.08 g, 3 mmol) in $CHCl_3$ (60 ml) at 35–40°C was added the solution of **15** (470 mg, 1.9 mmol) in $CHCl_3$ (10 ml), and the mixture was stirred for 30 min. To the stirred mixture was added 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide (3.3 g, 8 mmol), and the mixture was stirred at 35–40°C for 24 h. The usual work-up followed by a rough purification using silica gel (Wakosil 50 g, MeOH-$CHCl_3$, 3:97, v/v) gave a crude material containing methyl (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyltuberonate. The crude material was hydrolyzed using 0.5% NaOH/EtOH, and the resulting product was purified by HPLC (GL Science Inc., Inertsil ODS 10 um, 20 × 250 mm, A_{254nm}, 3 ml/min, MeOH–H_2O–AcOH, 60:40:1, v/v/v) to afford (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TAG (192 mg, 0.49 mmol, 5% from 13): FDMS *m/z* (rel. int.): 416 $[M+Na]^+$ (100), 394 $[M+H]^+$ (42), 30 $[M]^+$ (100),; HRFDMS *m/z*: 394.2120 $[M+H]^+$ (calcd. for $C_{18}{}^1H_{24}{}^2H_5O_9$: 394.2125); ^{1}H-NMR (270 MHz, CD_3OD): δ 5.54 (1H, br. t), 4.40 (1H, br. d, J=7.9 Hz), 4.0 (1H, br. d, J=11.5 Hz), 3.80 (1H, br. dd, J=12.5, 4.3 Hz), 3.30 (1H, br. t, J=8.2 Hz), 2.79 (1H, m), 2.6–2.2 (7H, complex), 2.13 (1H, m), 1.66 (1H, m).

Extraction and purification of TA, epi-JA, OPC 4:0, OPC 6:0, and OPC 8:0. The plant material was frozen at −25°C, crushed, and soaked in 80% aq. MeOH (1:10, w/v) for 48 h. The mixture was filtered to give a crude extract. A portion (50 g fr wt equivalent) was used for the analyses of endogenous TA, *epi*-JA, OPC 4:0, OPC 6:0, and OPC 8:0. Before purification, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TA, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-JA, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-OPC 4:0, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-OPC 6:0, and (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-OPC 8:0 were added to the extracts to the concentration of 0.2 μg (g fr wt)$^{-1}$ as each internal standard. The volatile components of the extract were removed *in vacuo*. After being dissolved in H_2O (100 ml), the extract was adjusted to pH 2–3 with 4 N HCl and extracted with EtOAc (70 ml × 3). The combined organic layers were washed with sat. $NaHCO_3$ (70 ml × 3). The sat. $NaHCO_3$ layers were adjusted to pH 2–3 with 4 N HCl and extracted with EtOAc (70 ml × 3). The combined organic layers were concentrated, and the residue was dissolved in 1 ml of H_2O and put

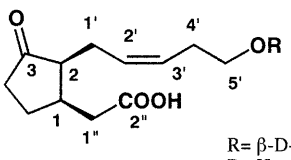
onto the cartridge column of Bond Elut C_{18}. The column was successively washed with H_2O (1 ml × 2) and MeOH–H_2O (4:1, v/v, 1 ml × 4). The volatile components of the MeOH–H_2O (4:1) eluents were removed *in vacuo*, and the residue was dissolved with MeOH (1 ml) and put onto the cartridge column of Bond Elut DEA. The column was successively washed with MeOH (1 ml × 2) and 1 N AcOH–MeOH (1 ml × 4). The volatile components of the AcOH–MeOH eluents were removed, and the residue was dissolved in MeOH–H_2O (4:1, 100 μl) and put onto a column of Wakosil-II 5C18 (10 mm × 300 mm; Wako Pure Chemical Industries, Ltd.). The column was eluted with a mixed solvent of MeOH (solvent A) and 0.2% AcOH–H_2O (solvent B) using a liner gradient mode with the flow rate of 3 ml min^{-1}. From 0 sec to 30 sec, the column was eluted with the mixed solvent of A:B = 70:30, and then from 30 sec to 25 min, the combination of A and B was linearly converted from 70:30 to 100:0. Finally the column was eluted with A:B (100:0) from 25 min to 30 min. To measure the relative retention time of each jasmonoid, indole-3-butyric acid was injected before injection of samples. The typical retention time for indole-3-butyric acid was 12 min, and the fractions that were eluted during 7–9 min, 12–15 min, 15–19 min, 20–24 min, and 25–29 min were due to TA, *epi*-JA, OPC 4:0, OPC 6:0, and OPC 8:0, respectively. Each fraction was concentrated *in vacuo*, dissolved with MeOH–H_2O (4:1, v/v, 20 μl), and analyzed by LC-SIM.

Extraction and purification of TAG. The plant material was frozen at −25°C, crushed, and soaked with 80% aq. MeOH (1:10, w/v) for 48 h. The mixture was filtered to give a crude extract. A portion (10 g fr wt equivalent) was used for an analysis. Before purification, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TAG were added to the extract to the concentration of 5 μg (g fr wt)$^{-1}$ for an internal standard. The volatile components of the extract were removed *in vacuo*, and the residue was dissolved in H_2O (100 ml). The mixture was washed with $CHCl_3$ (100 ml), and the H_2O phase was concentrated *in vacuo*. The residue was dissolved in 1 ml of H_2O and put onto the cartridge column of Bond Elut C_{18}. The column was washed with H_2O (2 ml × 2) and then with MeOH–H_2O (1:1, v/v, 1 ml × 4). The volatile components of the MeOH–H_2O (1:1) eluents were removed *in vacuo*, and the residue was dissolved in MeOH (1 ml) and put onto the Bond Elut DEA. The column was washed with MeOH (1 ml × 2) and then with 1 N AcOH MeOH (1 ml × 4). The volatile components of the AcOH MeOH eluents were removed, and the residue was dissolved in 3 ml of 0.1 N $AcONH_4$-AcOH buffer (pH 5.6). To the mixture was added β-glucosidase (5 mg), and the mixture was shaken (100 rpm) at 37°C for 24 h. The mixture was put directly onto the cartridge column of Bond Elut C_{18}. The column was washed with H_2O until the pH of the eluents reached pH 6–7 and then with 80% aq. MeOH (2 ml × 4). The volatile components of the 80% aq. MeOH eluents were removed, and the residue was purified with a column by Wakosil-II 5C18, 10 mm × 300 mm; Wako Pure Chemical Industries, Ltd. by the same procedure used for the purification of TA, because enodogenous and deuterium-labeled TAGs were converted to their aglycone forms.

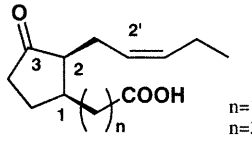
LC-SIM analysis. LC-SIM was done using M-1200AP LC-MS system (Hitachi, Japan). The analytical conditions for HPLC were as follows: column, Wakosil 5C8 (4 mm × 250 mm; Wako Pure Chemical Industries, Ltd.); solvent system, MeOH: 0.2% aq. AcOH, 25:75 for TA, 50:50 for *epi*-JA, 60:40 for OPC 4:0, 70:30 for OPC 6:0 and OPC 8:0, v/v; flow rate, 0.5 ml min^{-1}. The analytical conditions for MS were as follows: Nebulizer, 170°C; Desolvator, 400°C; Aperature 1 Htr, 120°C; Aperature 2 Htr; heater ON; Needle-2700 V; Polarity, negative; Multiplier, 2300 V; Drift, −40 V; Needle Voltage, 2700 V; Focus, −120 V. Because the system was run under the condition of negative mode, each jasmonoid was detected by the m/z of $[M-H]^-$. The amounts of endogenous jasmonoid were found from the ratios of the peak areas of the ions that corresponded to the endogenous and deuterium labeled jasmonoids. All experiments were run in duplicate, and the presented results were evaluated from the data of the plants which were independently harvested within June, July, and August in 1997, Hokkaido.

Result and Discussion

The synthesis of (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-JA was reported by Miersch,[12] and based on his report, the synthese of deuterium-labeled (±)-*epi*-TAG, (±)-*epi*-TA, (±)-*epi*-OPC 4:0, (±)-*epi*-OPC 6:0, and (±)-*epi*-OPC 8:0 were accomplished (Fig. 1, Schemes 1–3). The deuterium-labeled positions of (±)-*epi*-OPC 4:0, (±)-*epi*-OPC 6:0, and (±)-

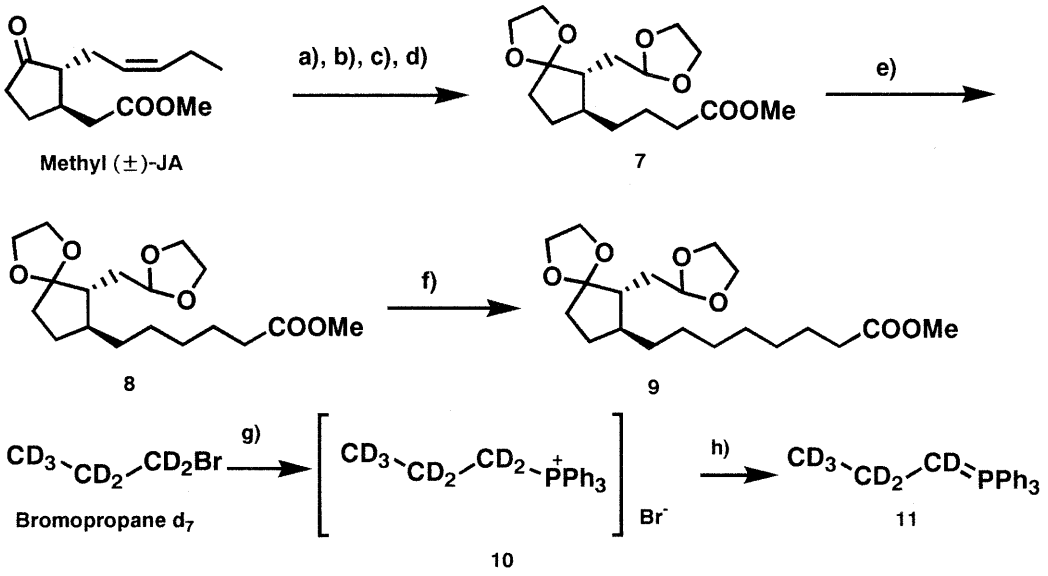


R= β-D-glc: tuberonic acid glucoside (TAG, **1**)
R= H: tuberonic acid (TA, **2**)

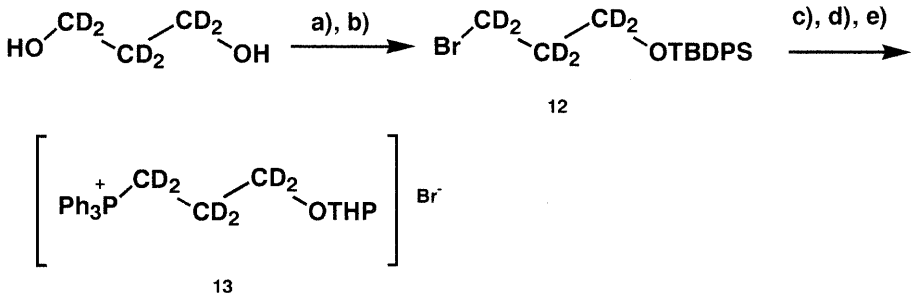


n=1: *epi*-jasmonic acid (JA, **3**)
n=3: OPC 4:0 (**4**)
n=5: OPC 6:0 (**5**)
n=7: OPC 8:0 (**6**)

Fig. 1. Potato Tuber-inducing Compounds.



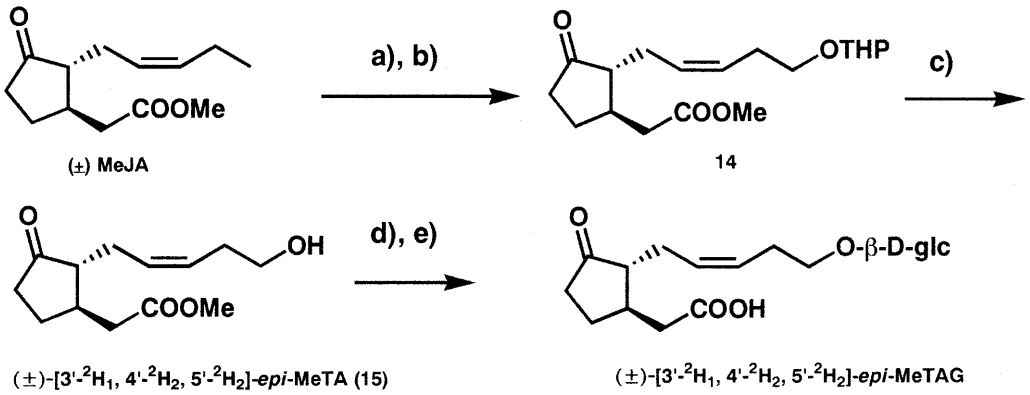
Scheme 1



Scheme 2

epi-OPC 8:0 were originated from [1-$^2H_2$, 2-$^2H_2$, 3-$^2H_3$] bromopropane (Scheme 1) as in the case of deuterium-labeled (±)-JA. The deprotected intermediates of **7**, **8**, and **9**, which were prepared from methyl (±)-jasmonate, were coupled with **11** to give methyl esters, which were hydolyzed and purified by HPLC to afford deuterium-labeled (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 4:0, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 6:0, and (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_3$]-*epi*-OPC 8:0, respectively. The syntheses of deuterium-labeled (±)-*epi*-TAG and (±)-*epi*-TA were done according to Schemes 2 and 3. The phosphorane derived from **13** (Scheme 2) were coupled with the aldehyde prepared from methyl (±)-jasmonate to give methyl (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-5′-*O*-tetrahydropyranyltuberonate (**14**). The protective group for the hydroxyl moiety of **12** was changed from TBDPS to THP because the phosphorane protected by a silyl ether group did not give the desired product. Acidic hydrolysis of **14** afforded methyl (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-tuberonate (**15**), which were saponified and purified by HPLC to give (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TA. The glucosylation of **15** gave methyl (±)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-tuberonate, which was hydolyzed and purified by HPLC to give (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TAG (Scheme 3).

Vick and Zimmerman established the biosynthetic pathway of jasmonic acid, which gave (1*R*, 2*S*) jasmonic acid,[13] and the existence of allene oxide cyclase, which was the key enzyme for this biosynthesis, was demonstrated by Hamberg.[14] But, in the usual experimental cases, jasmonic acid tends to epimerize at the C-2 position during the isolation procedure to give an equilibrated mixture of (1*R*, 2*R*)-*trans* and (1*R*, 2*S*)-*cis* isomers. The major stereoisomer is (1*R*, 2*R*)-*epi*-form, because this isomer is energetically more stable.[15,16] Based on this fact, we decided to use the synthetic jasmonoides, which have *trans*-relative configuration dominantly, as internal standards for analyses of endogenous jasmonoids. The leaflets of potato (*Solanum tuberosum* L.), which were harvested in July 2, 7, and 10, 1997, were purified by a modification of the method for the qualitative and quantitative analysis of gibberellins.[17] All experiments were run in duplicate, and before purification, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-



Scheme 3

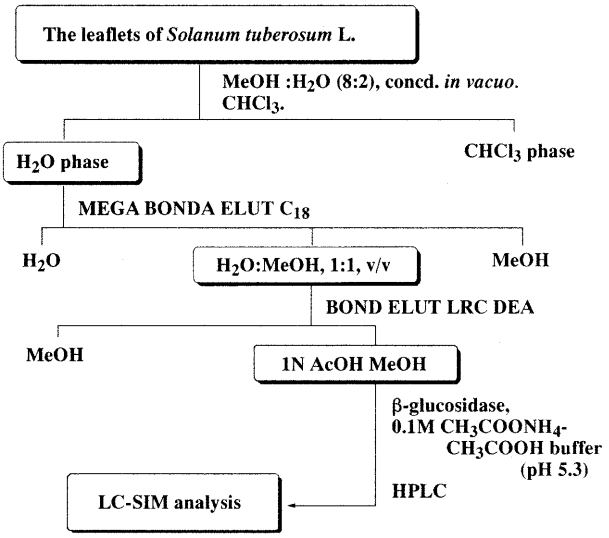


Fig. 2. Purification Procedure for TAG.

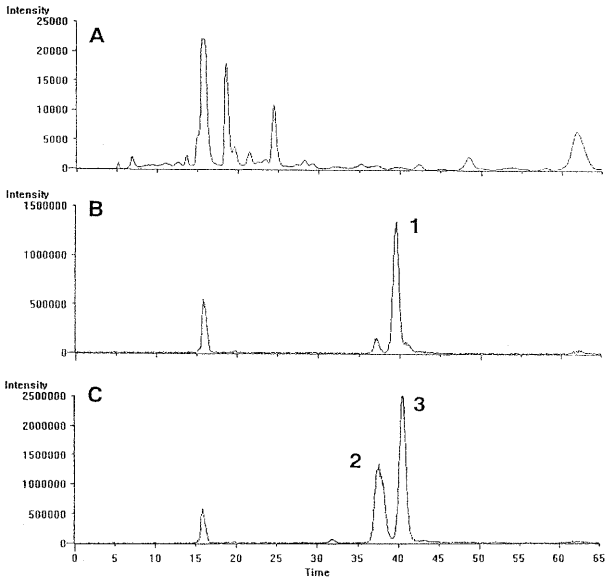


Fig. 3. LC-SIM Profile for the Analysis of TAG.
(A) Peaks were monitored by the UV absorbance at 210 nm. (B) Ion peaks were monitored at m/z 230. (C) Ion peaks were monitored at m/z 225. The peaks were numbered 1-3 for the jasmonoids; from 1 to 3, the peaks contained deuterium-labeled TA (Rt. 39.68 min), unknown compound (Rt. 37.67 min), and *epi*-TA (Rt. 40.59 min), respectively.

TAG were added to the extract to the concentration of 5 μg (g fr wt)-1 for an internal standard, and (±)-[3'-$^2H_1$, 4'-$^2H_2$, 5'-$^2H_2$]-*epi*-TA, (±)-[3'-$^2H_1$, 4'-$^2H_2$, 5'-$^2H_3$]-JA, (±)-[3'-$^2H_1$, 4'-$^2H_2$, 5'-$^2H_3$]-*epi*-OPC 4:0, (±)-[3'-$^2H_1$, 4'-$^2H_2$, 5'-$^2H_3$]-*epi*-OPC 6:0, and (±)-[3'-$^2H_1$, 4'-$^2H_2$, 5'-$^2H_3$]-*epi*-OPC 8:0 were added 0.2 μg (g fr wt)$^{-1}$ as each internal standard. The amounts of endogenous jasmonoid were found from the ratios of the peak areas of the ions that corresponded to the endogenous and deuterium labeled jasmonoids. The recovery rates were represented using the ratios of the peak areas of the ions due to the deuterium-labeled compound added before the purification and injected independently into the LC-SIM system.

The purification procedure for endogenous TAG in potato is given in Fig. 2, for example, and the feature of LC-SIM analysis for endogenous TAG is given in Fig. 3. The molecular ions of jasmonoids were monitored as $[M-H]^-$ ions because of the negative mode of LC-SIM analyses. The ion peaks of m/z 225 (Rt. 40.59 min) and 230 (Rt. 39.68 min) represented those of endogenous and deuteium labeled TAG, respectively, because the extracts were treated with β-glucosidase during the purification procedure. We checked the ion peaks of m/z 225 by co-injection experiments using synthetic unlabeled *epi*-TA because there was a little difference in the retention times between the endogenous compound and the internal standard, which might be ascribable to isotope effects. When the authentic *epi*-TA was injected with the purified extract, the area of the peak which showed a retention time at 40.59 min was increased. The origin of the peak which showed a retention time at 37.67 min still remains to be solved. But it was likely that the origin was due to 4'-glucopyranosyloxyJA, because there are the reports

Table 1. The Contents of TAG, TA, and JA in Potatos Hervested within June, July, and August in 1997

Dates of collecting plants		TAG (μg/g)		TA (ng/g)		JA (ng/g)	
		Exp. I (%)[a]	Exp. II (%)[a]	Exp. I (%)[a]	Exp. II (%)[a]	Exp. I (%)[a]	Exp. II (%)[a]
June	29	5.2 (22)	4.6 (30)	78 (43)	88 (54)	101 (69)	123 (48)
July	2	5.0 (25)	4.9 (47)	58 (21)	62 (46)	67 (39)	66 (52)
	7	7.9 (23)	3.5 (14)	56 (49)	68 (56)	35 (46)	39 (37)
	10	9.8 (19)	8.9 (25)	36 (78)	45 (69)	66 (18)	75 (17)
	14	5.5 (50)	5.3 (60)	[b]	[b]	[b]	[b]
	17	6.0 (31)	5.6 (32)	101 (49)	111 (38)	22 (61)	22 (67)
Aug.	5	10.9 (20)	8.7 (13)	63 (49)	41 (42)	10 (60)	4 (78)

The amounts of endogenous jasmonoid were determined from the ratios of the peak areas of the ions that corresponded to the endogenous and deuterium labeled jasmonoids. All experiments were run in duplicate (Exp. I and Exp. II), and the presented results were evaluated from the plants which were independently harvested within June, July, and August in 1997, Hokkaido.

[a] recovery rates of internal standards.

[b] not determined.

about the existence of the 4′-glucopyranosyloxyJA in *Eschscholtzia* and 4′-hydroxyJA in *Solanum demissum*.[19] In the same manner, we checked the ion peaks for other jasmonoids, TA, JA, OPC 4:0, OPC 6:0, and OPC 8:0. The recovery rate of the internal standards of TAG, TA, JA, OPC 4:0, and OPC 6:0 were enough to estimate the contents, but that of OPC 8:0 was very row (less than 10%). Because sufficient recovery rates were obtained, we estimated that the contents of jasmonoids in the leaflets of potato (*Solanum tuberosum* L. cv. Irish Cobbler) which were harvested in July 2, 7, and 10, 1997, as in Table 1. The contents of OPC 4:0 and OPC 6:0 were below the level of marginal detection of this LC-SIM system. In order to get a sufficient peak area, more than 100 ng of jasmonoids has to be injected to LC system, since the precise peak area of the ions could not be obtained due to the noises of the base line. In case of the experiment extracting from 50 g of plant material, purifying, and analyzing according to the designed procedure, the contents of jasmonoides should be above 10 ng (g fr wt)$^{-1}$. The accurate analysis of OPC 8:0 could not be accomplished because of the low recovery rate of the internal standard. But we could roughly estimated that the content might be below the marginal detection-level, because the peaks of endogenous OPC 8:0 were not detected in the charts obtained.

In order to check the accuracy of the method, the contents of TAG, TA, and JA in the plants, which were harvested on other days, were also examined, and the result is given in Table 1. The analyses of the contents of OPC 4:0, OPC 6:0, and OPC 8:0 were not done because the contents were thought to be below the level of marginal detection of this LC-SIM system. Since the day length is thought to affect the contents of the jasmonoids, the amounts were not the same, but the recovery rates were sufficient to give enough area to calculate the amounts of endogenous jasmonoids, and the evaluated amounts between duplicates were close except for the datum for TAG of July 7th. Finally, we came to the conclusion that this protocol could be applied to qualitative and quantitative analyses for endogenous jasmonoids.

This is the first report that mentioned the qualitative and quantitative analyses of endogenous jasmonoids in plant by liquid chromatography/selected ion monitoring (LC-SIM) using deuterium-labeled compounds as internal standards, and our study provided basic information about the levels of endogenous jasmonoids in potato plants.

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