Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Occurrence of 11-Hydroxyjasmonic Acid Glucoside in Leaflets of Potato Plants (Solanum tuberosum L.)

Hideyuki MATSUURA[a], Yasuhiro OHKUBO[a] & Teruhiko YOSHIHARA[a]
[a] Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University
Published online: 22 May 2014.

Biosci. Biotechnol. Biochem., **65** (2), 378–382, 2001

Occurrence of 11-Hydroxyjasmonic Acid Glucoside in Leaflets of Potato Plants (*Solanum tuberosum* L.)

Hideyuki MATSUURA, Yasuhiro OHKUBO, and Teruhiko YOSHIHARA†

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Received August 15, 2000; Accepted October 16, 2000

In order to examine the occurrence of 11-hydroxyjasmonic acid glucoside in potato plants, a synthesis of 11-hydroxyjasmonic acid was accomplished, and the synthetic compound was employed as a standard for an LC-SIM analysis. The existence of 11-hydroxyjasmonic acid glucoside was proved by the LC-SIM analysis.

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

In our previous paper,[1] we demonstrated qualitative and quantitative analyses of endogenous jasmonoids in *Solanum tuberosum* L. by means of liquid chromatography-selected ion monitoring (LC-SIM), using deuterium-labeled compounds as internal standards. However, there was an unknown ion peak which showed the ion of *m/z* 225 and had a retention time close to that of tuberonic acid (**1**, TA, 12-hydroxyjasmonic acid) in the LC-SIM profile for the analysis of tuberonic acid glucoside (**2**, TAG, 12-β-D-glucopyranosyloxyjasmonic acid). (Fig. 1) Since there are reports about the existence of 11-glucopyranosyloxyjasmonic acid (**3**, Fig. 1) in *Eschscholtzia*[2] and of 11-hydroxyjasmonic acid in *Solanum demissum*,[3] we assumed that the unknown peak was derived from 11-hydroxyjasmonic acid glucoside (**3**). In order to prove this hypothesis, 11-hydroxyjasmonic acid was synthesized, and the occurrence of 11-hydroxyjasmonic acid glucoside (**3**) in the potato plant (*Solanum tuberosum* L.) was proved. The ability for inducing potato tubers[4] was compared between jasmonic acid and 11-hydroxyjasmonic acid.

Materials and Methods

General. Spectral data 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 carried out by an M-1200AP LC-MS system (Hitachi, Japan) with a Wakosil 5C8 column (4 mm × 250 mm; Wako Pure Chemical Industries).

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

Preparation of (−)-jasmonic acid (4). Compound **4** was prepared from methyl (±)-jasmonate according to the method developed by Nohira and co-workers,[5] using (+)-1-(*p*-tolyl)ethylamine. Obtained **4** showed $[\alpha]_D^{23}$ − 55.4° (c 0.95, MeOH).

Preparation of 2-formylmethyl-1-O-tetrahydropyranyl-3-(2-tetrahydropyranyloxyethyl)-cyclopentanol (5). To a stirred mixture of compound **4**, which had been prepared from 1.18 g of the (+)-1-(*p*-tolyl)ethylamine salt conjugated with (−)-jasmonic acid, in MeOH was added excess CH_2N_2. The usual work-up followed by a rough purification procedure gave crude methyl (−)-jasmonate. This crude material was reduced by LAH in the usual manner, and the resulting two hydroxyl groups were protected by THP groups, using DHP and PPTS in CH_2Cl_2. To a stirred mixture of the protecting compound in MeOH (80 ml) at −78°C was added excess O_3. After the color of the reaction mixture had turned pale blue, dimethyl sulfide (6 ml, 82 mmol) was added, and the mixture was further stirred at room temperature for 1 h. The usual work-up followed by a purification procedure employing silica gel (Wakosil 100 g, *n*-hexane-EtOAc, 7:3, v/v) afforded an aldehyde (**5**, 470 mg, 1.38 mmol, 40% from the ethylamine salt); EIMS *m/z* (rel. int.): 277 (31), 257 (43), 256 (55), 239

† To whom correspondence should be addressed. Fax: 81-11-706-2505; E-mail: yosihara@chem.agr.hokudai.ac.jp

Abbreviations: TA, 12-hydroxyjasmonic acid; TAG, 12-β-D-glucopyranosyloxyjasmonic acid; LC-SIM, liquid chromatography/selected ion monitoring

(12), 155 (20), 137 (18), 111 (13), 85 (100), 67 (18), 41 (14); IR ν_{max} (film) cm^{-1}: 3414, 2941, 1724, 1455, 1441, 1352, 1323, 1261, 1120, 1077, 1033; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.83 (1H, t, J=3.0 Hz), 4.64 (1H, t, J=3.6 Hz), 4.55 (1H, t, J=3.3 Hz), 3.91–3.67 (3H, m), 3.53–3.38 (4H, m), 2.54–2.02 (3H, m), 1.96–1.52 (19H, m).

Preparation of 2-(3,3-dibromo-2-propenyl)-1-O-tetrahydropyranyl-3-(2-tetrahydropyranyloxyethyl)-cyclopentanol (6). To a stirred mixture of carbon tetrabromide (1.9 g, 5.9 mmol), triethylamine (1.6 ml, 11.8 mmol) and PPh_3 (3.1 g, 11.8 mmol) in CH_2Cl_2 (30 ml) at 0°C was added a mixture of **5** in CH_2Cl_2 (6 ml). The reaction mixture was further stirred at 0°C for 20 min and then poured into H_2O (100 ml). The organic layer was successively washed with sat. aq. $NaHCO_3$ (100 ml × 2) and sat. aq. NH_4Cl (100 ml × 2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel (Wakosil 80 g, *n*-hexane-EtOAc, 9:1, v/v) to give **6** (391 mg, 0.79 mmol, 57%); EIMS *m/z* (rel. int.): 394 (0.7), 310 (1), 308 (0.7), 199 (1.4), 197 (0.7), 131 (1), 101 (2), 85 (100), 67 (5), 41 (5); IR ν_{max} (film) cm^{-1}: 2940, 2361, 1508, 1439, 1323, 1260, 1034; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.40 (1H, t, J=7.26 Hz), 4.50 (2H, m), 3.80 (2H, m), 3.67 (2H, m), 3.42–3.20 (3H, m), 2.21–1.98 (3H, m), 1.85–1.32 (19H, m).

Preparation of 2-(4-acetoxy-2-pentynyl)-1-O-tetrahydropyranyl-3-(2-tetrahydropyranyloxyethyl)-cyclopentanol (7). To a stirred mixture of **6** (391 mg, 0.79 mmol) in THF (20 ml) at −78°C was added a mixture of *n*-BuLi in *n*-hexane (0.8 ml, 2 mmol). The reaction mixture was stirred at −78°C for 1 h. The mixture was allowed to warm to room temperature and stirred for a further 1 h, before acetaldehyde (2 ml, 36 mmol) was added. The reaction mixture was further stirred at room temperature for 1 h and poured into a solution of sat. aq. NH_4Cl (50 ml) and CH_2Cl_2 (50 ml), before the H_2O layer was extracted with CH_2Cl_2 (50 ml × 3). The combined organic layers were successively washed with $NaHCO_3$ (100 ml × 2) and sat. aq. NaCl (100 ml × 2), dried over Na_2SO_4, and concentrated under reduced pressure. To a stirred mixture of the residue in pyridine (3 ml) was added acetic anhydride (1 ml), and the reaction mixture was stirred for 24 h. The usual work-up was applied before purification procedure with silica gel (Wakosil 40 g, *n*-hexane-EtOAc, 4:1, v/v) to give **7** (300 mg, 0.71 mmol, 89%); EIMS *m/z* (rel. int.): 422 (0.5), 362 (5), 337 (6), 335 (4), 278 (2), 255 (4), 237 (5), 205 (4), 177 (11), 159 (4), 131 (3), 85 (100), 67 (7), 43 (9); HREIMS *m/z*: $[M-THP]^+$ 337.2009 (calcd. for $C_{19}H_{29}O_5$: 337.2015); IR ν_{max} (film) cm^{-1}: 3307, 2940, 1743, 1442, 1372, 1337, 1034; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.50 (1H, q, J=6.60 Hz), 4.72 (1H, m), 4.64 (1H, m), 3.96–3.70 (4H, m), 3.60–3.40 (3H, m), 2.54–2.25 (2H, m), 2.12 (3H, s), 2.20–1.52 (23H, m).

Preparation of 2-(4-acetoxy-cis-2-pentenyl)-3-(2-hydroxyethyl)-cyclopentanol (8). To a stirred mixture of **7** (300 mg, 0.71 mmol) in MeOH (20 ml) was added an excess of the Lindlar reagent, and the suspension was stirred under an H_2 atmosphere for 12 h. The Lindlar reagent was filtered off by using Celite, and the filtered mixture was concentrated under reduced pressure. To a mixture of the residue in MeOH (4 ml) was added TsOH (10 mg), and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure to give a residue which was purified with silica gel (Wakosil 60 g, MeOH–$CHCl_3$, 7:93, v/v) to afford **8** (135 mg, 0.53 mmol, 75%); EIMS *m/z* (rel. int.): 178 (11), 151 (12), 145 (13), 134 (61), 119 (23), 111 (19), 105 (28), 93 (51), 83 (100), 68 (50), 67 (51), 43 (55); IR ν_{max} (film) cm^{-1}: 3335, 2930, 1715, 1434, 1368, 1156, 1056, 924, 752; ^{1}H-NMR (270 MHz, CD_3OD) δ: 5.40 (2H, m), 4.55 (1H, m), 3.76 (1H, m), 3.51 (2H, m), 2.4–1.2 (10H, m), 1.32 (3H, s), 1.14 (3H, d, J=6.4 Hz).

Preparation of 11-hydroxyjasmonic acid (9). To a stirred suspension of pyridinium dichromate (300 mg) and 3A molecular sieves (400 mg) in CH_2Cl_2 (2 ml) was added a mixture of **8** (77.6 mg, 0.3 mmol) in CH_2Cl_2 (0.2 ml), and the mixture was stirred for 20 min at room temperature. The reaction mixture was filtered off by using Celite and then purified by silica gel (Wakosil 50 g, *n*-hexane-EtOAc, 3:2, v/v) to afford oxidized compound **8**. To a mixture of this oxidized compound (42 mg, 0.16 mmol) in CH_3CN (1 ml) was added 8% aq. NaH_2PO_4 (0.3 ml) at 5°C. To the stirred mixture were added H_2O_2 (0.1 ml) and $NaClO_2$ (116 mg), and the reaction mixture was stirred for 1 h at 5°C. The usual work-up and subsequent removal of the acetyl moiety with a solution of 1 M KOH in EtOH afforded 11-hydroxyjasmonic acid (**9**, 30 mg, 0.13 mmol, 36%) as a colorless oil; FDMS *m/z* (rel. int.): 227 $[M+H]^+$ (55), 209 (100); EIMS *m/z* (rel. int.): 208 (33), 179 (18), 149 (20), 142 (25), 123 (11), 105 (16), 91 (19), 83 (100), 79 (22), 69 (25), 55 (17), 43 (40); HREIMS *m/z*: $[M-H_2O]^+$ 208.1099 (calcd. for $C_{12}H_{16}O_3$: 208.1100); IR ν_{max} (film) cm^{-1}: 3419, 2967, 2930, 1732, 1408, 1272, 1059, 762; ^{1}H-NMR (270 MHz, CD_3OD) δ: 5.35 (2H, m), 4.54 (1H, m), 2.60 (1H, dd, J=10.9, 3.6 Hz), 2.4–1.8 (7H, m), 1.50 (1H, m), 1.25 (1H, m), 1.13 (3H, d, J=6.3 Hz).

Extraction and purification of TAG and 11-hydroxyjasmonic acid glucoside. The plant material was frozen at −25°C, crushed, and then soaked in 80% aq. MeOH (1:10, w/v) for 48 h. The mixture was filtered to give a crude extract. A portion (10 g fr

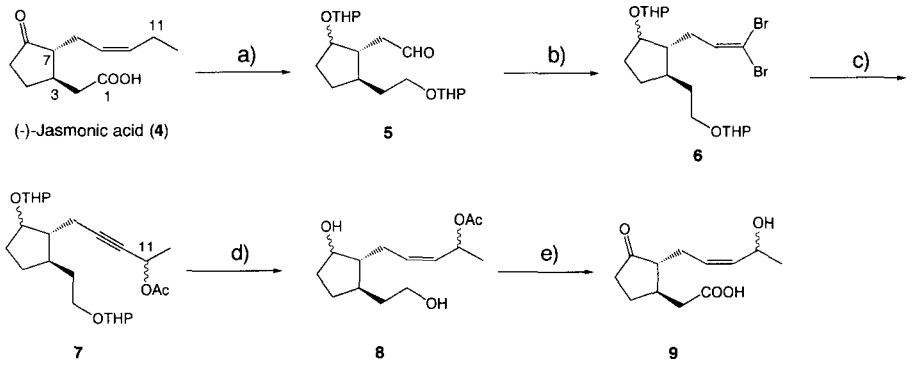
wt equivalent) was used for an analysis. Before purification, (±)-[3′-$^2H_1$, 4′-$^2H_2$, 5′-$^2H_2$]-*epi*-TAG were added to the extract to a concentration of 5 μg (g fr wt)$^{-1}$ as an internal standard. The volatile components of the extract were removed *in vacuo*, and the residue was dissolved 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 loaded into a 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) eluate were removed *in vacuo*, and the residue was dissolved in MeOH (1 ml) and then loaded into a Bond Elut DEA column. The column was washed with MeOH (1 ml × 2) and then with 1 N AcOH in MeOH (1 ml × 4). The volatile components of the AcOH–MeOH eluate were removed, and the residue was dissolved in 3 ml of a 0.1 N $AcONH_4$–AcOH buffer (pH 5.6). To the mixture was added β-glucosidase (5 mg), and the resulting mixture was shaken (100 rpm) at 37°C for 24 h. The mixture was directly loaded into the Bond Elut C_{18} cartridge column. The column was washed with H_2O until the pH value of the eluate reached 6–7 and then with 80% aq. MeOH (2 ml × 4). The volatile components of the 80% aq. MeOH eluate were removed, and the residue was dissolved in MeOH–H_2O (4:1, 100 μl), before being loaded into a column of Wakosil-II 5C18 (10 mm × 300 mm; Wako Pure Chemical Industries). The column was eluted with a mixed solvent of MeOH (solvent A) and 0.2% AcOH–H_2O (solvent B), using a linear gradient 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. The column was finally eluted with A:B = 100:0 from 25 min to 30 min. In order to determine the relative retention times, indole-3-butyric acid was injected before injecting the sample. The typical retention time for indole-3-butyric acid was 12 min, the fraction which was eluted during 7–9 min being due to the eluate which contained TA and 11-hydroxyjasmonic acid. This fraction was concentrated *in vacuo*, dissolved in MeOH–H_2O (4:1, v/v, 20 μl), and subjected to an LC-SIM analysis.

LC-SIM analysis. LC-SIM was carried out with an 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); flow rate, 0.5 ml min^{-1}. The solvent systems were MeOH:0.2% aq. AcOH = 30:70 (v/v) for the co-injection experiments and 25:75 (v/v) for estimating the amounts of the compounds. The analytical conditions for MS were as follows: nebulizer, 170°C; desolvator, 400°C; aperture 1 heater, 120°C; aperture 2 heater, ON; needle, −2700 V; polarity, negative; multiplier, 2300 V; drift, −40 V; needle voltage, 2700 V; focus, −120 V. Since the system was run in the negative mode, TA and 11-hydroxyjasmonic acid were detected by the *m/z* value of $[M-H]^-$. The amounts of endogenous TAG and 11-glucopyranosyloxyjasmonic acid were determined from the ratios of the peak areas of the ions that corresponded to the compounds and deuterium labeled TAG. All experiments were run in duplicate, and the presented results were evaluated from the data for plants which had been independently harvested within June, July, and August 1997 in Hokkaido.

Potato single node segment assay. The potato micro-tuber forming activity was examined by using cultures of single-node segments of potato stem *in vitro* as previously described.[4] Briefly, single-node segments of the stem, which had been prepared from etiolated potato shoots (*Solanum tuberosum* L. cv Irish Cobbler), were sterilized with 1% NaClO for 1 h. Three segments were planted horizontally in a 100-ml flask that contained 10 ml of a basal medium (usually White's medium) supplemented with the compound to be tested. Five replicates were prepared. The concentration of sucrose in the medium was 2% by wt, the medium being adjusted to pH 5.6 and solidified with 0.6% Bacto agar before being autoclaved. The culture was maintained at 25°C in the dark for 3 weeks, before the rate of tuberization was calculated as the number of tuberized laterals divided by the total number of laterals that had emerged.

Results and Discussion

(−)-Jasmonic acid (**4**) was prepared according to the method developed by Nohira and co-workers,[5] and the synthesis of 11-hydroxyjasmonic acid was accomplished according to Scheme 1 by using the obtained (−)-jasmonic acid (**4**, $[\alpha]_D^{23} - 55.4°$) as the starting material. In Scheme 1, compound **4** was reduced with LAH, and the resulting hydroxyl groups were protected by THP groups with DHP and PPTS in CH_2Cl_2. The protected compound was treated with excess O_3 and then by dimethyl sulfide to give compound **5**. Aldehyde **5** was converted to dibromo-olefin **6**, because the desired phosphonium salt for the Wittig reaction could not be prepared from 2-hydroxypropanebromide. Dibromo-olefin **6** was coupled with acetaldehyde and treated with pyridine and acetic anhydride to give compound **7**. Deprotection of the THP groups and subsequent oxidation of the resulting hydroxyl groups afforded 11-acetyloxyjasmonic acid. Compound **9** was obtained according to the manner in Scheme 1 for deprotection of the hydroxyl group. Since compound **7** had been synthesized by using acetaldehyde, compound **9** was a mixture of (11*R*)- and (11*S*)-diastereoisomers. The

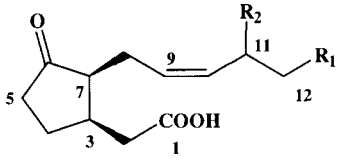


Reagents and conditions: a) (i) excess CH_2N_2, MeOH; (ii) LAH, Et_2O, 0°C; (iii) DHP, PPTS, CH_2Cl_2; (iii) O_3, MeOH, -78°C, and Me_2S (40%, 4 steps); b) CBr_4, PPh_3, Et_3N, CH_2Cl_2 (57 %); c) (i) n-BuLi, THF, -78 °C and acetaldehyde; (ii) Ac_2O, pyridine (89%, 2 steps); d) (i) Lindlar, H_2, MeOH; (ii) TsOH, MeOH (75%, 2 steps); e) (i) PDC, 3A mol. sieves, CH_2Cl_2; (ii) NaH_2PO_4, H_2O_2, $NaClO_2$, 5°C, CH_3CN; (iii) 1M KOH/EtOH (36%, 3 steps).

Scheme 1

mixture could not be separated by the LC/SIM conditions described in the experimental section.

Leaflets of potato (*Solanum tuberosum* L. cv. Irish Cobbler) harvested within June, July, and August 1997 were extracted and analyzed according to the method described in the experimental section. Since 11-hydroxyjasmonic acid glucoside (**3**, Fig. 1) could not be synthesized, the extract containing the glucosides was treated with β-glucosidase. One of the LC-SIM profiles for the analysis of TAG and 11-hydroxyjasmonic acid glucoside is given in Fig. 2, and the other LC-SIM profiles showing very similar features. The ion peaks of m/z 225 represent the amount of endogenous TAG and 11-hydroxyjasmonic acid glucoside, because the extract which contained the glucosides had been treated with β-glucosidase and the ions were observed as $[M-H]^-$ by the LC/SIM system run under the condition described in the experimental section. In order to identify each peak (Fig. 2, profile C), co-injection experiments were carried out. When synthetic 11-hydroxyjasmonic acid and synthetic TA were co-injected with the extract, the ion peaks showing the retention time of 27.40 min (Fig. 2, LC-MS profile B) and 29.33 min (Fig. 2, LC-MS profile A) respectively increased. Since the occurrence of 11-hydroxyjasmonic acid glucoside was proved, the contents of the glucosides were estimated. In Fig. 3, the ion peak of m/z 230 (profile A) was due to deuterium-labeled TAG, and the ion peaks of m/z 225 (profile B) due to endogenous 11-hydroxyjasmonic acid glucoside and TAG. The amounts of endogenous 11-hydroxyjasmonic acid glucoside and TAG were determined from the ratios of the peak areas of the ions that corresponded to the endogenous compounds and deuterium-labeled TAG. The contents of 11-hydroxyjasmonic acid glucoside and TAG are given in Table 1.



R_1= OH, R_2= H: tuberonic acid (1, TA)
R_1= O-β-D-glc, R_2= H: O-β-D-glucopyranosyltuberonic acid(2, TAG)
R_1= H, R_2= O-glucoside: 11-hydroxyjasmonic acid glucoside (3)

Fig. 1. Structures of TA (12-Hydroxyjasmonic Acid), TAG (12-Glucopyranosyloxyjasmonic Acid), and 11-Hydroxyjasmonic Acid Glucoside.

The co-injection experiments proved the occurrence of 11-hydroxyjasmonic acid glucoside, and the amounts were estimated as in Table 1. This is the first report on the occurrence of 11-hydroxyjasmonic acid glucoside in potato plants (*Solanum tuberosum* L. cv. Irish Cobbler) and an estimate of the amount of the compound. The ability for inducing potato tubers was also examined by a potato single-node segment assay.[4] Jasmonic acid showed its activity at 10^{-6} M, and in case of 11-hydroxyjasmonic acid, a concentration of 10^{-5} M–10^{-4} M was needed to have the same activity as that of jasmonic acid.

Acknowledgments

The authors thank Mr. K. Watanabe and Dr. E. Fukusi for EIMS and HRMS measurements, and Nippon Zeon Co. for kindly providing us methyl (±)-jasmonate.

Table 1. Contents of TAG and 11-Hydroxyjasmonic Acid Glucoside in Potatoes Harvested within June, July, and August 1997

Harvesting data	TAG (μg/g)		11-hydroxyjasmonic acid glucoside (μg/g)	
	Exp. I (%[a])	Exp. II (%[a])	Exp. I	Exp. II
June 29	5.2(22)	4.6(30)	4.4	4.2
July 2	5.0(25)	4.9(47)	3.5	4.2
7	7.9(23)	3.5(14)	6.3	4.3
10	9.8(19)	8.9(25)	6.9	6.4
14	5.3(50)	5.3(60)	4.9	5.4
17	6.0(31)	5.6(32)	3.4	3.9
Aug. 5	10.9(20)	8.7(13)	2.6	5.0

The amounts of endogenous TAG and 11-hydroxyJA glucoside were determined from the ratios of the peak areas of the ions that corresponded to the endogenous and deuterium-labeled TAG. All experiments were run in duplicate (Exp. I and Exp. II), and the presented results were evaluated from plants which had been independently harvested within June, July, and August 1997 in Hokkaido.

[a] Recovery rate of the internal standard.

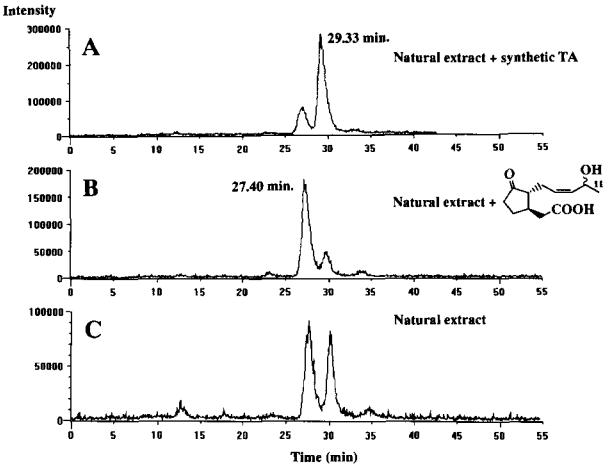


Fig. 2. LC-SIM Profile for the Analysis of TAG and 11-Hydroxyjasmonic Acid Glucoside.

(A) Ion peaks due to the natural extract with synthetic TA monitored at m/z 225. (B) Ion peaks due to the natural extract with 11-hydroxyjasmonic acid monitored at m/z 225. (C) Ion peaks due to the natural extract monitored at m/z 225. The analytical conditions for HPLC were as follows: column, Wakosil 5C8 (4 mm × 250 mm; Wako Pure Chemical Industries); solvent system, MeOH:0.2% aq. AcOH, 30:70 (v/v); flow rate, 0.5 ml min^{-1}.

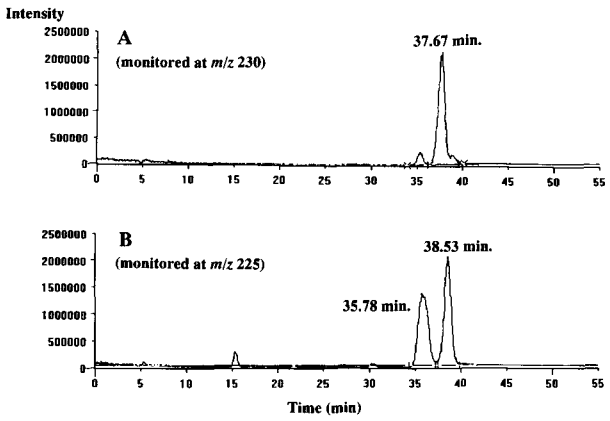


Fig. 3. LC-SIM Profile for the Analysis of the Leaflets of Potato Plants Harvested on June 29th 1997.

(A) Ion peak due to deuterium-labeled TA monitored at m/z 230. (B) Ion peaks due to the natural extract monitored at m/z 225. The analytical conditions for HPLC were as follows: column, Wakosil 5C8 (4 mm × 250 mm; Wako Pure Chemical Industries); solvent system, MeOH:0.2% aq. AcOH, 25:75 (v/v); flow rate, 0.5 ml min^{-1}.

References

1) Matsuura, H., Ohmori, F., Kobayashi, M., Sakurai, A., and Yoshihara, T., Qualitative and quantitative analysis of endogenous jasmonoids in potato plant (*Solanum tuberosum* L.), *Biosci. Biotechnol. Biochem.*, **64**, 2380–2387 (2000).

2) Xia, Z.-Q. and Zenk, M. H., Metabolism of jasmonic acid in suspension culture of *Eschscholtzia*. *Planta Medica*, **59**, 575 (1993).

3) Helder, H., Miersch, O., Vreugdenhil, D., and Sembdner, G., Occurrence of hydroxy jasmonic acids in leaflets of *Solanum demissum* plants grown under long- and short-day conditions. *Physiologia Plantarum*, **88**, 647–653 (1993).

4) Koda, Y., Omer, E. A., Yoshihara, T., Shibata, H., Sakamura, S., and Okazawa, Y., Isolation of a specific tuber-inducing substance from potato leaves. *Plant Cell Physiol.*, **29**, 1047–1051 (1988).

5) Nohira, H. and Sho, Y., Japan Kokai Tokkyo Koho, JP 03261743 (Dec. 21, 1991).