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SYNTHESIS AND POTATO TUBER-INDUCING ACTIVITY OF METHYL 5′,5′,5′-TRIFLUOROJASMONATE

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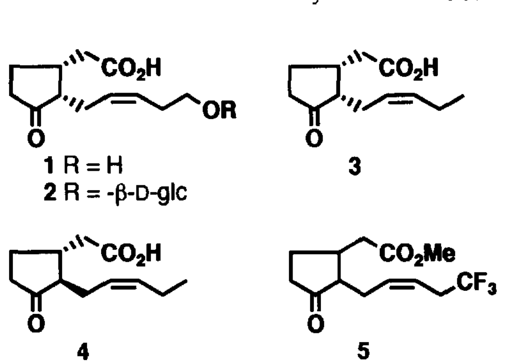
Key Word Index—*Solanum tuberosum*; Solanaceae; potato; tuber-inducing activity; radish germination; lettuce germination; rice seedling growth; methyl 5′,5′,5′-trifluorojasmonate; methyl jasmonate; tuberonic acid; epijasmonic acid.

Abstract—Methyl 5′,5′,5′-trifluorojasmonate was synthesized as an antimetabolic analogue of methyl jasmonate. It induced potato tuber formation more effectively than methyl jasmonate and inhibited the growth of rice seedlings and the germination of lettuce and radish seeds. These results suggest that epijasmonic acid itself has potato tuber-inducing activity and that the hydroxyl group of tuberonic acid is not necessary for this activity.

INTRODUCTION

Tuberonic acid (TA, **1**) and its glucoside (**2**) were isolated as potent potato tuber-inducing substances from potato leaves (*Solanum tuberosum* L.) [1, 2]. Recently, epijasmnic acid (EpiJA, **3**) was shown to be biosynthetic precursor for TA [3]. In potato leaves, the methyl group at the end of the pentenyl side chain of EpiJA is hydroxylated and glucosylated to give TA glucoside (**2**), then **2** is transmitted to the stolon. EpiJA, jasmonic acid (JA, **4**) and their methyl esters have various plant hormone-like activities and also show potato tuber-inducing activity [4]. However, it is unclear whether EpiJA itself has tuber-inducing activity or not, because this activity is thought to be exhibited after EpiJA is transformed into TA. Therefore, we designed a fluorinated analogue of EpiJA as an antimetabolite. The covalent bond length with carbon and the van der Waals' radius of fluorine are very similar to those of hydrogen, but the bond energy of C–F is larger than that of C–H. So a wide variety of fluorinated analogues of biologically active compounds have been used as antimetabolites.

Our synthetic target was a diastereomeric mixture of methyl 5′,5′,5′-trifluorojasmonate (**5**) for the following reasons. The unnatural stereoisomers of TA are inactive [5], and the unnatural stereoisomers of EpiJA are partially active [6]. The biological activities of the methyl esters of JA and TA are the same as those of derived acids. TA shows no plant growth inhibitory activities [5]. To ascertain that the analogue (**5**) did not function as a TA mimic, but an EpiJA mimic, the inhibitory effects of **5** on the growth of rice seedlings and the germination of lettuce and radish seeds were also investigated.



Scheme 1. Jasmonic acid and related compounds.

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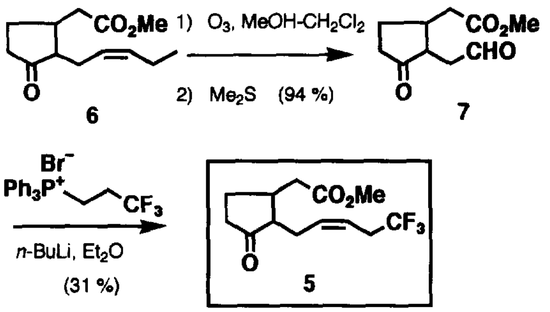
RESULTS AND DISCUSSION

Synthesis

The synthesis of **5** is shown in Scheme 2. Ozonolysis of methyl (±)-jasmonate (**6**, Zeppin®; containing 22% methyl (±)-epijasmonate) was followed by a reductive work-up with dimethyl sulphide to give the aldehyde **7** in 94% yield. In this step the *cis*/*trans* ratio of the two side chains with respect to the plane of the cyclopentanone ring was not changed. Treatment of the aldehyde **7** with the Wittig reagent prepared from 3,3,3,-trifluoropropyltriphenylphosphonium bromide [7] with *n*-butyllithium in diethyl ether furnished **5** in 31% yield. The *Z*/*E* ratio of the newly formed double bond was 25:1. The *cis*/*trans* ratio of the two side chains was determined by GC analysis: *cis*-**5**/*trans*-**5** = 7:43.



Scheme 2. Synthesis of methyl 5′,5′,5′-trifluorojasmonate.

Bioassays

The biological activities of **5** were assessed in the following bioassays: tuber formation of potato, growth of rice seedlings and germination of radish and lettuce seeds. The potato tuber-inducing test was carried out according to the previously reported procedure [8]. As shown in Table 1, **5** induced sessile microtubers more effectively than **6** (at a concentration of 10^{-7} M, the tuberization rate of **6** was below 20%) [4]. In the rice seedling growth test, **5** inhibited the growth of the second leaf sheath five times more effectively than methyl jasmonate (containing 14% **6**) (Fig. 1). In the root elongation and the seed germination tests for radish, the analogue **5** showed growth inhibitory activity comparable to that of **6** (Figs 2 and 3). In the case of the lettuce seed germination assay, the analogue **5** was less active than **6** (Fig. 4). These results show that the plant growth inhibitory activities of **5** are comparable to those of **6**. Since TA shows no plant growth inhibitory activities [5], the analogue **5** did not function as a TA mimic but a JA mimic. Consequently, EpiJA itself has potato tuber-inducing activity.

EXPERIMENTAL

General. IR: film; ^{1}H and ^{13}C NMR: 270 and 67.5 MHz, respectively, using TMS as int. standard.

Methyl 2-formyl-3-oxo-1-cyclopentaneacetate (**7**). Ozone was passed through a cooled soln of **6** (1.00 g, 4.46 mmol) in CH_2Cl_2–MeOH (1:3, 20 ml) at −80°. The mixt. was stirred for 90 min at −80°, before O_2 was bubbled into the mixt. to remove the excess O_3. To this was added Me_2S (1.5 ml, 1.3 g, 20 mmol) and the mixt. was stirred for *ca* 12 hr, during which time it was allowed to warm to room temp. The reaction mixt. was neutralized with $NaHCO_3$ and concd *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane–EtOAc (10:1–2:1) gave **7** (0.830 g, 4.19 mmol, 93.9%) as an oil. This aldehyde was

Table 1. The effect of methyl trifluorojasmonate (**5**) and methyl jasmonate (**6**) on the ratio of potato tuberization

	Ratio		
Compound	0 M	10^{-5} M	10^{-7} M
5	0	100	100 (%)
6	0	100	20*
		Strong induction (sessile tubers)	Weak induction (tuberized at the end of elongated lateral shoots)

*See ref. [4].

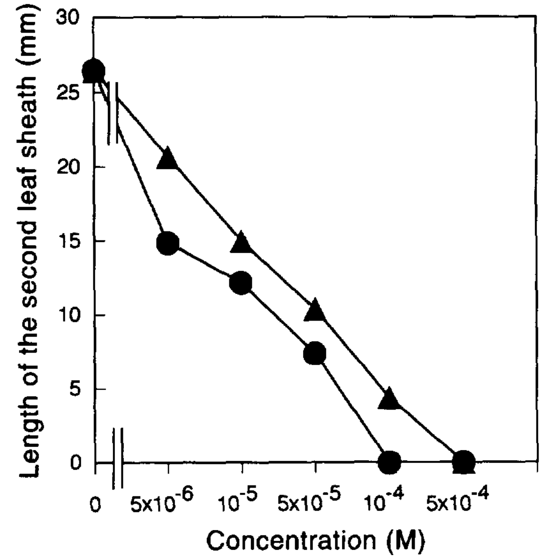


Fig. 1. The growth inhibitory activities of methyl trifluorojasmonate (**5**) (●) and methyl jasmonate (**6**) (▲) on rice seedlings (*Oryza sativa* cv. Satohonami).

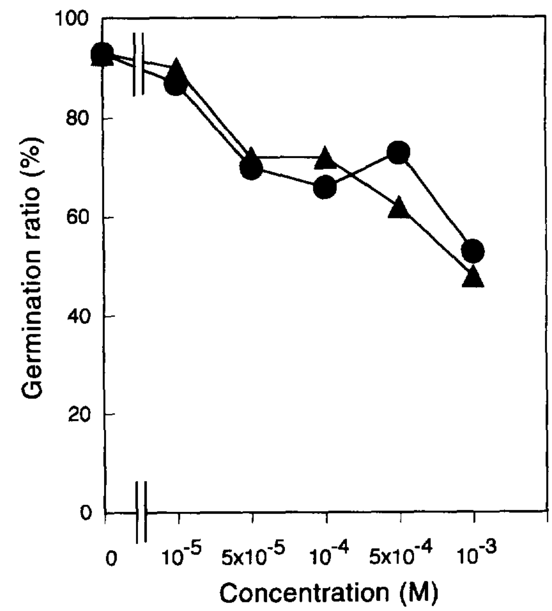


Fig. 2. The inhibitory activities of methyl trifluorojasmonate (**5**) (●) and methyl jasmonate (**6**) (▲) on the germination of radish seeds (*Raphanus sativus* cv. Radicula DC).

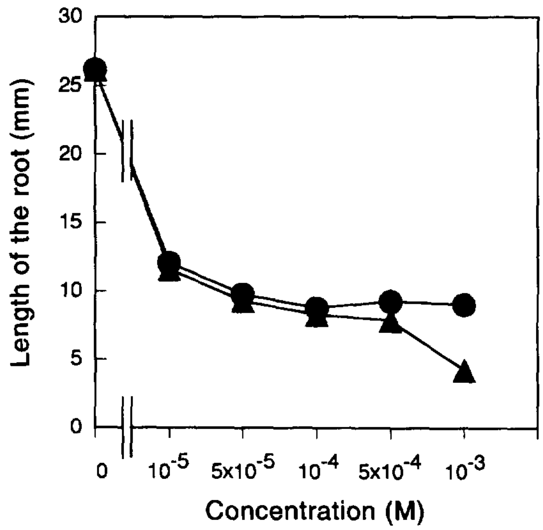


Fig. 3. The growth inhibitory activities of methyl trifluorojasmonate (**5**) (●) and methyl jasmonate (**6**) (▲) on the root elongation of germinating radish seeds (*Raphanus sativus* cv. Radicula DC).

unstable and was used in the next step without further purification. IR ν_{max}^{film} cm^{-1}: 2950 (*s*), 2845 (*w*, H–CO), 2730 (*m*, H–CO), 1735 (*s*, O=COMe), 1720 (*s*, O=CH, O=CCH), 1440 (*m*), 1340 (*m*), 1200 (*s*), 1170 (*s*), 890 (*m*); ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.5–1.7 (1H, *m*), 2.2–2.5 (6H, *m*), 2.5–2.65 (1H, *m*), 2.72 (1H, *dd*, J = 19.0, 5.0 Hz), 2.91 (1H, *dd*, J = 19.0, 4.0 Hz), 3.68 (3H, *s*, Me), 9.74 (0.8H, *s*, CHO), 9.82 (0.2H, *s*, CHO).

Methyl (2′Z)-3-oxo-2-(5′,5′,5′-trifluoro-2′-pentenyl)-1-cyclopentaneacetate (**5**). *n*-BuLi in hexane (1.66 M, 1.37 ml, 2.27 mmol) was added to a cooled suspension of 3,3,3-trifluoropropyltriphenylphosphonium bromide (1.00 g, 2.28 mmol) in Et_2O (10 ml) at −40° under N_2. After generation of the reddish-yellow solid, **7** (0.410 g, 2.07 mmol) in Et_2O (5 ml) was added dropwise to the suspension at −40°, and the resulting mixt. was stirred for 3 hr at this temp. The reaction was then quenched with H_2O. The organic layer was sepd, and the H_2O layer was extracted with Et_2O. The combined organic layers were washed with brine, dried ($MgSO_4$) and concd *in vacuo*. The residue was chromatographed (×2) on silica gel. Elution with hexane–EtOAc (10:1) and hexane–CH_2Cl_2–Et_2O (20:6:1) gave **5** (0.180 g, 0.647 mmol, 31.3%) as an oil; $n_D^{23.5}$ = 1.4412. IR ν_{max}^{film} cm^{-1}: 3030 (*w*), 2950 (*s*), 1735 (*s*, C=O), 1435 (*s*), 1345 (*s*), 1255 (*s*), 1135 (*m*), 1060 (*m*), 910 (*m*), 840 (*m*); ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.45–1.6 (1H, *m*), 1.9–2.0 (1H, *m*), 2.0–2.5 (7H, *m*), 2.6–2.7 (1H, *m*, 2-H), 2.8–3.0 (2H, *m*, 4′-H), 3.70 (3H, *s*, Me), 5.49 (1H, *dtt*, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 7.0, J_{HF} = 1.5 Hz, 3′-H), 5.70 (1H, *pseudo dt*, $J_{2',3'}$ = 10.5, $J_{1',2'}$ = 7.5 Hz, 2′-H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 126.04 (*g*, $^1J_{CF}$ = 276 Hz, C-5′), 119.29 (*g*, $^3J_{CF}$ = 3.5 Hz, C-3′), 32.15 (*g*, $^2J_{CF}$ = 29.7 Hz, C-4′), etc. Found: C, 56.01; H, 6.13; calc. for $C_{13}H_{17}O_3F_3$: C, 56.11; H, 6.16%.

FID-GC. CBP1 (silicone bonded, 25 m × 0.25 mm), programmed from 150° to 200° at 4° min^{-1}, temp.: 200°, injector temp.: 200°: t_R = 8.4 min [4.4%, (2′*E*)-**4**], 8.9 min (82.4%, **4**), 9.3 min (13.2%, epi-**4**).

Potato tuber-inducing assay. The bioassay for tuber-inducing activity was carried out using cultures of single-node segments of potato stem (*S. tuberosum* L. cv. Irish Cobbler) *in vitro*, as reported previously [8].

Lettuce germination assay. The inhibitory effect on the germination of lettuce seeds was tested in the same manner as described in ref. [9]. A group of 20 lettuce seeds (*Lactuca sativa* cv. Green Lake 2B61) was placed in a 4 cm Petri dish with 4 ml of a test soln of 0.7% agar medium. After incubation at 27° under fluorescent light (2000 lux) for 3 days, the root lengths of seedlings were measured on two replicates.

Radish germination assay. The inhibitory effect on germinating radish (*Raphanus sativus* cv. Radicula DC) seeds was tested according to the above mentioned lettuce assay. A group of 15 radish seeds was placed in a 4 cm Petri dish with 0.7% agar medium and incubated for 3 days at 27° under fluorescent light (2000 lux). The lengths of the roots and the germination ratios were then measured on two replicates.

Rice seedling assay. The inhibitory effect on rice seedling growth was carried out in the same manner as described previously [10] under non-sterile conditions. A group of 10 rice seedlings (*Oryza sativa* cv. Satohonami), germinated in H_2O for 3 days at 30°, was transplanted to a test tube (36ϕ × 100 mm) with 10 ml 0.7% agar medium and incubated for 5 days at 25° under fluorescent light (2000 lux). The lengths of the second leaf sheaths were then measured on two replicates.

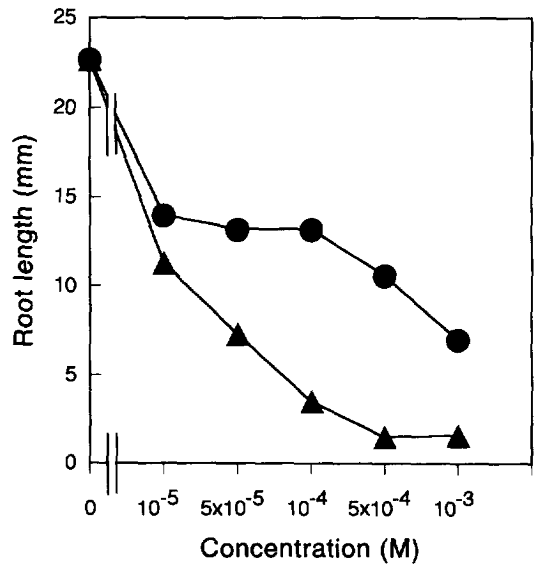


Fig. 4. The growth inhibitory activities of methyl trifluorojasmonate (**5**) (●) and methyl jasmonate (**6**) (▲) on the germination of lettuce seeds (*Lactuca sativa* var. Green Lake 2B61).

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