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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF METHYL 3,7- AND 4,5-DIDEHYDROJASMONATES

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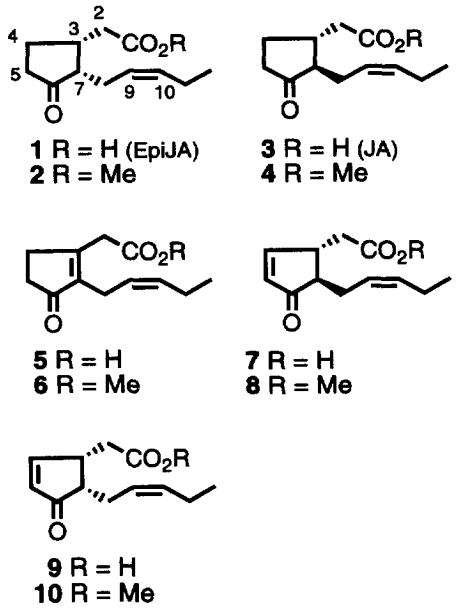
Key Word Index—*Oryza sativa*; *Lactuca sativa*; *Raphanus sativus*; radish germination; lettuce germination; rice seedling growth; methyl 3,7-didehydrojasmonate; methyl 4,5-didehydrojasmonate; methyl jasmonate; epijasmonic acid.

Abstract—Methyl 3,7-didehydrojasmonate was synthesized as an analogue of methyl epijasmonate. This analogue inhibited the germination of lettuce seed more strongly than methyl jasmonate. Methyl 4,5-didehydrojasmonate was also synthesized and its biological activities were comparable to those of methyl jasmonate.

INTRODUCTION

Recently compounds related to jasmonic acid have been found to play important roles in plant growth regulation [1–3]. Epijasmonic acid [EpiJA, **1** or its methyl ester (Me-EpiJA, **2**)], which is thought to be a natural product [4], shows strong plant growth inhibitory activities. However, this compound easily isomerizes to its thermodynamically stable *trans*-isomer jasmonic acid [JA, **3** or its methyl ester (Me-JA, **4**)], the activities of which are weaker than those of Me-EpiJA [5]. Some attempts have been made to fix the *cis*-configuration of the two side chains of Me-EpiJA by introducing methyl [6–8] or fluoro [8] substituents at the 7-position. However, introduction of the substituents diminished the activities. In relation to these attempts, we thought that the introduction of a double bond would fix the side chains in the same plane as found in Me-EpiJA without epimerization. Thus, methyl 3,7-didehydrojasmonate (**6**) may function as a non-epimerizing analogue of Me-EpiJA and we intended to synthesize **6** to compare some plant growth inhibitory activities with Me-JA. Miersch *et al.* [9] reported the isolation of 3,7-didehydrojasmonic acid (**5**) from an immature fruit *Vicia fava*. During the process of the synthesis of Me-EpiJA analogue **6**, we also prepared methyl 4,5-didehydrojasmonate (**8**). Compound (**8**) and methyl 4,5-didehydroepijasmonate (**10**) were isolated from jasmine absolute oil (*Jasminum grandiflorum* L.) [10]. The corresponding acids **7** and **9** were also isolated from the fungus *Botryodiplodia theobromae* Pat. [11] and from fertile fronds of *Equisetum sylvaticum* [12], respectively. The synthesis of the compound **8** was reported by Torii *et al.* [13] and Hatanaka *et al.* [14]. The synthesis of **6** was only reported by Ducos and Rouessac [15] as an unseparated mixture with **8**. In this paper, synthesis and some plant growth inhibitory activities of **6** and **8** are described.



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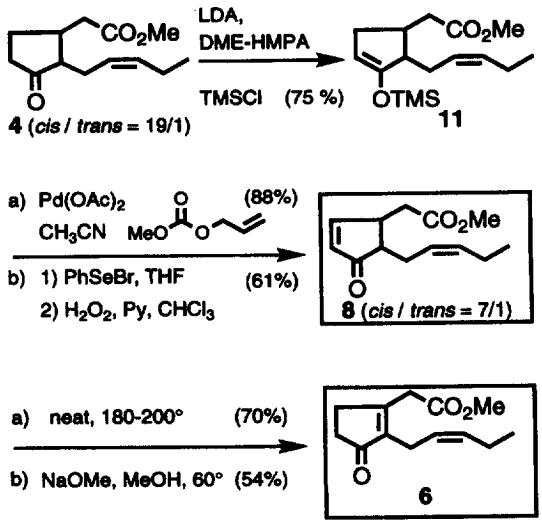

Scheme 1. Synthesis of methyl 4,5- and 3,7-didehydrojasmonates.

RESULTS AND DISCUSSION

We chose a commercially available methyl (±)-jasmonate [**4**, containing 5% methyl (±)-epijasmonate] as the starting material (Scheme 1). Methyl (±)-jasmonate (**4**) was converted to the corresponding kinetic silyl enol ether (**11**), which was oxidized with palladium catalyst to give the desired 4,5-didehydro product (**8**) in 88% yield [16]. The *cis*:*trans* ratio of the two side chains with respect to the plane of the cyclopentenone ring was determined to be 1:7 by HPLC analysis. We also attempted selenenylation and oxidative elimination protocols [17]. However, the yields were low. Finally, thermal isomerization of **8** at 180–200° afforded methyl 3,7-didehydrojasmonate (**6**) in 70% yield [18]. Overheating lead to *Z-E* isomerization of the 9,10-double bond. An alternative method, base-catalysed isomerization, gave a poor yield [19]. The ^{1}H NMR spectral data supported the structures of **6** and **8**.

The biological activities of **6** and **8** were assessed on the growth of rice seedlings and the germination of radish and lettuce seeds. Table 1 shows the IC_{50} values.

Table 1. The plant growth inhibitory activities of methyl jasmonate (**4**), methyl 3,7-didehydrojasmonate (**6**) and methyl 4,5-didehydrojasmonate (**8**)

Compound	IC_{50} in assay (μM) Rice*	Lettuce†	Radish‡
4	35	>2000	89
6	≈1000	46	≈1000
8	13	86	630

* Elongation of rice seedlings (*Oryza sativa* cv. Sasaminori). The second leaf sheaths were measured after 5 days.

† Germination of lettuce seeds (*Lactuca sativa* cv. Grate Lake). The germination rates were measured after 5 days.

‡ Germination of radish seeds (*Raphanus sativus* cv. Sakuranbo). The germination rates were measured after 4.5 days.

Methyl (±)-jasmonate [Me-JA, **4**, containing *ca* 5% methyl (±)-epijasmonate] was used as a reference. In the rice seedlings assay, the analogue **8** was rather more active than Me-JA. This result seems to reflect the *cis*-isomeric composition of each compound; **8** contained *ca* 13% of methyl (±)-4,5-didehydro-epijasmonate (**10**). The analogue **6** was less active in this assay. On the other hand, the unsaturated analogues (**8**, and especially **6**) inhibited lettuce germination more strongly than did Me-JA. In the case of radish seed germination, the analogues were less active than Me-JA. In the rice and radish assays, our results were in accord with Koda's conclusion [5]; though there may exist different receptors for each species, the natural isomer Me-EpiJA has the highest activities. We also found that introduction of the double bond at the 4,5-position does not lead to a considerable change of the conformation at the 3,7-position, hence the growth inhibitory activities on rice and radish are little affected. In addition to this, the low activity of **6** indicates that the dihedral angle between the cyclopentenone ring and the base of the side chains is important, or the π-electrons of the 3,7-double bond are inhibitory. For the lettuce germination, the coplanarity of the two side chains is more important than the dihedral angle with the cyclopentenone ring. Furthermore, it seems that the π-electrons of the 4,5-double bond are beneficial while those of the 3,7-double bond are not significant, for the recognition of the receptor of lettuce.

EXPERIMENTAL

General. IR spectra were recorded as a film on a Jasco IR-810 spectrometer. ^{1}H NMR spectra were recorded on a Jeol JNM GSX-400 spectrometer (400 MHz) and a Varian Gemini 2000 spectrometer (300 MHz) in $CDCl_3$, TMS as int. standard. ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 spectrometer (125 MHz) in $CDCl_3$, $CDCl_3$ as int. standard.

Methyl (2′Z)-2-(2′-pentenyl)-3-trimethylsilyloxy-3-cyclopentenylacetate (**11**). A soln of methyl jasmonate (1.00 g, 4.46 mmol) in dry 1,2-dimethoxyethane (DME, 10 ml) and dry hexamethylphosphoric triamide (HMPA, 1 ml) was added dropwise to a stirred soln of lithium diisopropylamide [prepd from diisopropylamine (0.75 ml, 0.58 g, 5.7 mmol) and *n*-butyllithium in hexane (1.6 M, 3.6 ml, 5.8 mmol)] in dry DME (10 ml) at −78° under nitrogen, stirring being continued for 2.5 hr. To this was added trimethylsilyl chloride (1.24 ml, 1.06 g, 9.8 mmol), and this was stirred for 2.5 hr at −78°. The reaction mixt. was diluted with Et_2O and filtered through a Celite pad. The filtrate was diluted with Et_2O, washed with H_2O, satd aq. NH_4Cl soln, satd aq. $NaHCO_3$ soln and brine, dried with $MgSO_4$ and concd *in vacuo* below 40°. The residue was chromatographed on silica gel (50 g, hexane–EtOAc = 10:1–5:1) to give **11** (9:1 mixt. of kinetic and thermodynamic enolate, 988 mg, 3.33

mmol, 74.8%, 83.0% taking account recovered methyl jasmonate) and methyl jasmonate (0.100 g, 0.446 mmol, 10.0%) as colourless oils.

Compound **11**. IR ν_{max}^{film} cm^{-1}: 3060 (w), 3005 (w), 1740 (s, C=O), 1645 (s, C=C), 1250 (s), 850 (s). ^1H NMR (300 MHz): δ 0.17 [0.9H, *s*, $(CH_3)_3Si$, thermodynamic enolate (te)], 0.20 [8.1H, *s*, $(CH_3)_3Si$, kinetic enolate (ke)], 0.959 (2.7H, *t*, J = 7.4 Hz, CH_3CH_2, ke), 0.964 (0.3H, *t*, J = 7.4 Hz, CH_3CH_2, te), 1.85 (0.9H, *m*, ke), 2.0–2.6 (10H, *m*), 2.75–2.95 (0.2H, *m*, te), 3.65 (3H, *s*, OMe), 4.51 (0.9H, *dd*, J = 2.2, 1.4 Hz, enol H, ke), 5.2–5.5 (2H, *m*, 2′ and 3′-H). This compound was used in the next step without further purification.

Methyl 4,5-*didehydrojasmonate* (**8**). A mixt. of **11** (8.00 g, 27.0 mmol), palladium(II) acetate (320 mg, 1.4 mmol) and allyl methyl carbonate (6.2 g, 54 mmol) in dry CH_3CN (100 ml) was stirred at 80° for 3 hr. The reaction mixt. was filtered through a Celite pad. The filtrate was diluted with hexane and concd *in vacuo*. The remaining CH_3CN was sepd as the hexane azeotrope. The residue was chromatographed on silica gel (hexane–EtOAc, 10:1) and distilled to give **8** (5.29 g, 23.8 mmol, 88.2%) as a pale yellow oil; bp 118–120° at 0.2 mmHg, n_D^{26} 1.4871. IR ν_{max}^{film} cm^{-1}: 3000 (w, C=C), 1735 (s, 1-C=O), 1705 (s, 6-C=O), 1585 (w, C=C), 1435 (m), 1160 (m), 1170 (m). ^1H NMR (400 MHz): δ 0.97 (3H, *t*, J = 7.5 Hz, CH_3CH_2), 2.02–2.12 (3H, *m*), 2.32 (1H, *m*), 2.47 (1H, *dd*, J = 15.8, 8.4 Hz, 2-H), 2.45–2.60 (1H, *m*), 2.59 (1H, *dd*, J = 15.8, 7.0 Hz, 2-H), 3.01 (1H, *ddddd*, J = 8.4, 7.0, 2.6, 2.5, 2.3 Hz, 3-H), 3.71 (3H, *s*, OCH_3), 5.25 (1H, *dtt*, J = 10.8, 7.4, 1.5 Hz, 9-H), 5.48 (1H, *dtt*, J = 10.8, 7.4, 1.5 Hz, 10-H), 6.19 (1H, *dd*, J = 5.7, 2.3 Hz, 5-H), 7.63 (1H, *dd*, J = 5.7, 2.6 Hz, 4-H). ^{13}C NMR (125 MHz): δ 14.0 (12-C), 20.4, 27.4, 37.9, 43.0, 50.7, 51.7, 124.2, 133.5, 134.3, 165.4 (4-C), 171.7 (1-C), 210.1 (6-C). Found: C, 70.12; H, 8.02; calcd for $C_{13}H_{18}O_3$: C, 70.25; H, 8.16%.

The *cis-trans* ratio of the two side chains with respect to the plane of the cyclopentenone ring was estimated to be about 1:7 by HPLC analysis [detected at 230 nm, column: Erma ERC Silica 1181, 6 × 230 mm; solvent, hexane–2-propanol (20:1); flow rate, 1.0 ml min^{-1}]: R_t = 9.5 min (12.7%) and 11.4 min (84.2%).

Methyl 3,7-*didehydrojasmonate* (**6**). The compound **8** (724 mg, 3.26 mmol) was heated at 180–200° for 2 hr as a neat liquid. The resulting mixt. was chromatographed on silica gel (100 g, hexane–EtOAc, 20:1–10:1) to give **6** (508 mg, 2.29 mmol, 70.2%) as a pale yellow oil. An analytical sample was distilled; bp 110–115° at 0.5 mmHg, n_D^{24} 1.4987. IR ν_{max}^{film} cm^{-1}: 3010 (w, C=C), 1740 (s, 1-C=O), 1700 (s, 6-C=O), 1645 (m, C=C), 1435 (m), 1350 (m), 1190 (m), 1170 (m), 1050 (w). ^1H NMR (400 MHz): δ 0.99 (3H, *t*, J = 7.5 Hz, CH_3CH_2), 2.15 (2H, pseudo *quint*, J = 7.5 Hz, CH_3CH_2), 2.40–2.45 (2H, *m*), 2.60–2.65 (2H, *m*), 2.97 (2H, *d*, J = 7.1 Hz, 8-H), 3.48 (2H, *s*, 2-H), 3.73 (3H, *s*, OCH_3), 5.20 (1H, *dtt*, J = 10.6, 7.1, 1.5 Hz, 9-H), 5.41 (1H, *dtt*, J = 10.6, 7.0, 1.5 Hz, 10-H). ^{13}C NMR (125 MHz): δ 13.9 (12-C), 21.0, 25.8, 29.7, 34.0, 36.3, 52.1 (OMe), 124.1, 132.7, 141.5 (7-C), 164.1 (3-C), 169.3 (1-C), 208.5 (6-C). Found: C, 70.03; H, 8.13; calc. for $C_{13}H_{18}O_3$: C, 70.25; H, 8.16%.

Lettuce germination assay. The inhibitory effect on the germination of lettuce seeds was tested in the same manner as described [20]. A group of 20 lettuce seeds (*Lactuca sativa* cv. Great Lake) was placed in a 4 cm Petri dish with filter paper moistened with 4 ml of a test soln of 0.7% agar medium. After incubation at 25° under fluorescent light (1700 cd sr m^{-2}) for 5 days, the germination rates were measured on two replicates.

Radish germination assay. The inhibitory effect on germination of radish (*Raphanus sativus* cv. Sakuranbo) 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 this was incubated for 4.5 days at 25° under fluorescent light (1700 cd sr m^{-2}). Then the germination rates were measured on two replicates.

Rice seedling assay. The inhibitory effect on rice seedling growth was carried out in the same manner as described previously [21] under non-sterile conditions. A group of 10 rice seedlings (*Oryza sativa* cv. Sasaminori) that had been germinated in water at 30° for 3 days was transplanted to a test tube (36 × 100 mm) with 7 ml of 0.7% agar medium and this was incubated for 5 days at 25° under fluorescent light (1700 cd sr m^{-2}). Then the lengths of the second leaf sheaths were measured on two replicates.

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