Syntheses and Potato Tuber-inducing Activities of Unnatural Long-chain OPC-9:0 and OPC-10:0

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The unnatural long-chain OPCs, OPC-9:0 and OPC-10:0, were respectively synthesized from the ethyl esters of OPC-7:0 and OPC-8:0. C2-carbon elongation was achieved via alkylation of the enolate of tert-butyl acetate. The potato tuber-inducing activity of OPC-10:0, as well as OPC-8:0, -6:0 and -4:0, was similar to that of jasmonic acid. OPC-9:0 also exhibited weak tuber-inducing activity.

Key words: OPC; 12-oxo-PDA; jasmonic acid; tuber-inducing activity

We have recently reported syntheses of the homologous OPC series, OPC-1:0, -3:0, -4:0, -5:0, -6:0, -7:0 and -8:0, as a racemic and trans-rich (ca. 95%) mixture, via 1,4-conjugate addition of zinc-copper reagents for 2-[(Z)-2-pentenyl]-2-cyclopenten-1-one. The esters of the homologous OPC series have been converted into 3-oxa-OPCs and 2-fluoro-OPCs. These results show that OPC derivatives are promising intermediates for other OPC analogues and homologues possessing various chain lengths that are required for studies on the biological activity of endogenous compounds related to the octadecanoid signalling pathway in higher plants. 12-Oxo-phytodiocenoic acid (12-oxo-PDA, 1), dihydro-12-oxo-PDA (OPC-8:0, 2), OPCs, and the oxa-analogues of OPCs have recently been shown to exhibit biological activities in some typical bioassay systems with 7-epi-jasmonic acid (7-epi-JA, 5) and jasmonic acid (JA, 6). These results suggest that not only 5 and 6 but also their biosynthetic intermediates (1, 2, 3 and 4) are biologically active without needing to be converted into 5 or 6 via enzymatic reduction and β-oxidation, and that there are some independent receptors corresponding to the carbon-chain length of the signalling molecules (Fig. 1). We describe here the syntheses and potato tuber-inducing activities of the unnatural long-chain OPCs, OPC-9:0 and OPC-10:0.

Two possible routes were planned for the syntheses of OPC-9:0 and OPC-10:0: (1) via 1,4-conjugate addition of the C2 and C10-zinc-copper reagents for 2-[(Z)-2-pentenyl]-2-cyclopenten-1-one, and (2) via C2-elongation as the enolate of tert-butyl acetate for the derivatives of OPC-7:0 and OPC-8:0. The former route was abandoned because the corresponding iodo-esters, which are not commercially available, must be prepared from appropriate compounds via several steps. In practice, ethyl 9-iodononanoate and ethyl 10-iododecanoate have been prepared from 9-decen-1-ol; however, generation of the corresponding zinc-copper reagents was more difficult than that from short-chain iodo-esters because purification by vacuum distillation at relatively high temperature would promote the elimination of hydrogen iodide. So the latter route was followed. Ethyl ester (7a), as a mixture of cis- and trans-isomers (cis, ca. 25%), has been previously converted into acetal 8a as a mixture of cis- and trans-isomers (cis, ca. 10%) with 2-ethyl-2-methyl-1,3-dioxorane and p-toluenesulfonic acid in a 94% yield. The ester of 8a was reduced with lithium aluminum hydride in tetrahydrofuran (THF) to give alcohol 9a in a 92% yield. Treatment of 9a with iodine, triphenylphosphine, and imidazole in benzene gave

Fig. 1. Structures of Jasmonic acid and its Biosynthetic Intermediates.

Abbreviations: 1A, jasmonic acid; PDA, phytodiocenoic acid; OPC-8:0, 8-[(3-oxo-2-cis-[(Z)-2-pentenyl]cyclopentyl)octanoic acid; the other OPC abbreviations follow the former and their numbers indicate corresponding carboxylic acids.
iodide 10a as an alkylation agent in a 69% yield. Alkylation of the enolate of tert-butyl acetate with 10a was next examined. When 3 eq. of the lithium enolate of tert-butyl acetate, which had been generated by treating with lithium diisopropylamide (LDA), in THF was used, alkylation proceeded slightly to give desired C2-elongated ester 11a in a 9% yield with the recovery of most of 10a. When 10 eq. of the lithium enolate was used in THF and hexamethylphosphoramide (HMPA), 11a was obtained in a 39% yield with a slight amount of 10a. Although the yield was significantly increased, even the use of excess lithium enolate did not result in a higher yield. The tert-butyl ester and acetal of 11a were finally hydrolyzed with 50% aq. trifluoroacetic acid at the same time to give OPC-9:0 (12a)9 as the predominantly trans-isomer (ca. 95%, based on the 13C-NMR spectrum)1,2 in an 87% yield. By following the same route, ethyl ester 7b was converted into OPC-10:0 (12b) as the predominantly trans-isomer (ca. 95%).1,2

The tuber-inducing activities of synthetic unnatural long-chain OPC-9:0 (12a) and OPC-10:0 (12b) were examined by monitoring the rate of tuberization.10 Both 12a and 12b exhibited activity in the range from a 10^{-6} to 10^{-4} M concentration. While the activity of 12b was similar to that of the JA standard [a mixture of minor (±)-5 (ca. 7%) and major (±)-6 that was used as a positive control],10 12a exhibited rather weak activity. The rate of tuberization of 12a was 46% at the relatively high concentration of 10^{-4} M. In previous bioassays on tuber-inducing activity, the following results have been obtained:1,2 (1) Even-numbered OPC-8:0, OPC-6:0, and OPC-4:0 exhibited activity at the same concentration (> 10^{-6} M) as that of the JA standard. (2) Unnatural odd-numbered OPC-7:0 exhibited slightly weaker activity than that of the JA standard. (3) Unnatural odd-numbered OPC-5:0 and 3-oxa-OPCs (8:0, 6:0, and 4:0) exhibited weak activities. Their rate of tuberization was 36–46% at a 10^{-4} M concentration when that of the JA standard was 100%. (4) Unnatural odd-numbered OPC-3:0 and OPC-1:0 are considered to have exhibited almost no activity, because their rate of tuberization was below 17% at a 10^{-4} M concentration.

fore, the order of the tuber-inducing activities of OPCs, including the unnatural odd-numbered and long-chain homologues was OPC-10:0, -8:0, -6:0, and -4:0 = JA standard > OPC-7:0 > OPC-9:0 ≈ OPC-5:0 > OPC-3:0 = OPC-1:0. It is not always considered that the activity of all even-numbered OPCs would only arise from 5 or 6 produced endogenously by β-oxidation in plant tissue because 3-oxa-OPCs exhibit activity as analogues of the corresponding OPCs. Furthermore, unnatural odd-numbered OPC-9:0, -7:0 and -5:0, which would undergo β-oxidation into OPC-3:0 and -1:0 in plant tissue, also exhibit activity. Considering the potent 5/6-like activity and the molecular size of the phytotoxin, coronatine,10 OPC-9:0 (12a, C_{19}) and -7:0 (C_{17}) might be recognized as mimics of OPC-8:0 (2, C_{18}) or its precursor, 12-oxo-PDA (1, C_{18}). Enzymatic reduction of 1 to 2 and β-oxidation of 2 to 5 might be not required to exhibit the various 5/6-like biological activities. It is necessary to consider the roles of 1, 2, 3 and 4 as biologically active endogenous compounds. Other structurally simple analogues of 1 and 2, which do not undergo β-oxidation but exhibit potent biological activities, are required in studies of the octadecanoid signalling pathway.

In conclusion, long-chain OPCs 12a and 12b were synthesized in five steps from the ethyl esters of OPC-7:0 and OPC-8:0, respectively. It was proved that the esters of the homologous OPC series are useful intermediates for other homologues. Both unnatural long-chain OPCs exhibited potato tuber-inducing activity. The order of activity of the present OPCs has been presented. Plant physiological studies related to the octadecanoid signalling pathway will be assisted by OPC homologues and analogues that provide significant information.

**Experimental**

**General methods.** 1H- and 13C-NMR spectra were recorded with a JEOL JNM-EX-270 spectrometer (1H at 270 MHz; 13C at 67.5 MHz). In the 1H-NMR spectra, chemical shifts are reported as δ (ppm) values relative to the residual proton (δ 7.26 ppm) of CDCl3. In the 13C-NMR spectra, chemical shifts are reported as δ (ppm) values relative to the carbon signal (δ 77.0 ppm) of
CDCl₃. IR spectra were measured with a Perkin Elmer System 2000 FT-IR spectrometer, and mass spectra were recorded with a JEOL JMS-AX500 or JEOL JMS-SX102A spectrometer. Column chromatography was carried out with silica gel 60 (spherical, 70-140 mesh ASTM; Kanto Chemical Co., Japan).

**Ethyl 7-[(Z)-2-penteny]l-1,4-dioxaspiro[4.4]non-7-yl] heptanoate (8a).** The synthesis and spectral data of 8a have been described. Since there are some errors in the assignment of the 1H-NMR spectrum, the corrected data are described here. 1H-NMR (CDCl₃) δ: 0.96 (3H, t, J = 7.6 Hz, CH₂Me), 1.25 (3H, t, J = 7.2 Hz, CO₂CH₂Me), 1.23-1.38 (6H, m), 1.52-1.80 (10H, m), 2.00-2.06 (4H, m, allyl-CH₂×2), 2.28 (2H, t, J = 7.3 Hz, CH₂CO₂Et), 3.84-3.89 (4H, m, OCH₂CH₂O), 4.12 (2H, q, J = 7.2 Hz, CO₂CH₂Me), 5.37 (2H, m, vinyl-H×2).

**Ethyl 8-[(Z)-2-penteny]l-1,4-dioxaspiro[4.4]non-7-yl] octanoate (8b).** According to the same synthetic method as that for 8a and the corresponding short-chain esters, 7b (1.05 g, 3.30 mmol) was converted into 8b (705 mg, 73%, based on consumed 7b) as a colorless oil. IR νmax (film) cm⁻¹: 2932, 2874, 1738, 1653, 1464, 1373, 1250, 1163, 1117, 1040; 1H-NMR (CDCl₃) δ: 0.96 (3H, t, J = 7.6 Hz, CH₂Me), 1.23 (3H, t, J = 7.2 Hz, CO₂CH₂Me), 1.23-1.39 (8H, m), 1.52-1.79 (10H, m), 2.03-2.09 (4H, m, allyl-CH₂×2), 2.28 (2H, t, J = 7.3 Hz, CH₂CO₂Et), 3.87-3.90 (4H, m, OCH₂CH₂O), 4.12 (2H, q, J = 7.2 Hz, CO₂CH₂Me), 5.37 (2H, m, vinyl-H×2); EI-MS m/z: 366 (23, M⁺), 321 (17 M-C₂H₅O₂), 195 (26), 99 (100). HR-MS m/z (M⁺): calcd. for C₂₈H₄₅O₄: 436.2770; found, 436.2753.

7-[(Z)-2-penteny]l-1,4-dioxaspiro[4.4]non-7-yl] heptanol (9a). According to the same synthetic method as that for the corresponding short-chain alcohols, 2a (865 mg, 2.70 mmol) was converted into 9a (770 mg, 92%) as a colorless oil. IR νmax (film) cm⁻¹: 3356, 2929, 2856, 1652, 1462, 1324, 1153, 1043; 1H-NMR (CDCl₃) δ: 0.96 (3H, t, J = 7.6 Hz, CH₂Me), 1.19-1.40 (8H, m), 1.52-1.80 (10H, m), 2.01-2.06 (5H, m, allyl-CH₂×2 and OH), 3.63 (2H, m, CH₂OH), 3.84-3.89 (4H, m, OCH₂CH₂O), 5.36 (2H, m, vinyl-H×2); EI-MS m/z: 310 (11, M⁺), 195 (18), 99 (100). HR-MS m/z (M⁺): calcd. for C₆₇H₇₃O₃: 1336.5127; found, 1334.5142.

8-[(Z)-2-penteny]l-1,4-dioxaspiro[4.4]non-7-yl] octanol (9b). According to the same synthetic method as that for the corresponding short-chain alcohols, 8b (492 mg, 1.34 mmol) was converted into 9b (419 mg, 96%) as a colorless oil. IR νmax (film) cm⁻¹: 3357, 2928, 2856, 1653, 1458, 1325, 1152, 1044; 1H-NMR (CDCl₃) δ: 0.96 (3H, t, J = 7.6 Hz, CH₂Me), 1.19-1.39 (10H, m), 1.52-1.80 (10H, m), 2.01-2.06 (5H, m, allyl-CH₂×2 and OH), 3.63 (2H, m, CH₂OH), 3.84-3.89 (4H, m, OCH₂CH₂O), 5.36 (2H, m, vinyl-H×2); EI-MS m/z: 324 (11, M⁺), 195 (18), 99 (100). HR-MS m/z (M⁺): calcd. for C₅₉H₆₅O₃: 1324.2665; found, 1324.2663.

1-Iodo-7-[(Z)-2-penteny]l-1,4-dioxaspiro[4.4]non-7-yl] heptane (10a). To a stirred solution of 9a (760 mg, 2.45 mmol) in benzene (10 ml) were successively added imidazole (417 mg, 6.13 mmol), triphenylphosphine (1.60 g, 6.13 mmol), and iodine (622 mg, 4.90 mmol) at room temperature. The mixture was stirred for 19 h, and the reaction was quenched by adding sat. aq. Na₂SO₄ (10 ml). After diluting with EtOAc (10 ml), the reaction mixture was successively washed with a 1:1 mixture of sat. aq. Na₂SO₄ and sat. aq. NaHCO₃ (20 ml), sat. aq. NaHCO₃ (20 ml), and brine (20 ml). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The resulting insoluble materials in hexane were filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (hexane:Et₂O = 10:1) to give 10a (706 mg, 69%) as a colorless oil. IR νmax (film) cm⁻¹: 2959, 2927, 2854, 1741, 1461, 1307, 1158, 1044; 1H-NMR (CDCl₃) δ: 0.96 (3H, t, J = 7.3 Hz, CH₂Me), 1.17-1.44 (8H, m), 1.52-1.84 (10H, m), 2.01-2.06 (4H, m, allyl-CH₂×2), 3.19 (2H, t, J = 6.9 Hz, CH₂I), 3.84-3.89 (4H, m, OCH₂CH₂O), 5.36 (2H, m, vinyl-H×2); EI-MS m/z: 420 (20, M⁺), 195 (28), 99 (100). HR-MS m/z (M⁺): calcd. for C₅₀H₆₃O₃I: 420.1525; found, 420.1548.

tert-Butyl 9-[(Z)-2-penteny]l-1,4-dioxaspiro[4.4]non-7-yl] nonanoate (11a). To a stirred solution of diisopropylamine (2.20 ml, 15.7 mmol) in dry THF (15.7 ml) and dry HMPA (1.48 ml, 8.51 mmol) was added dropwise n-BuLi (a 1.60 M solution in hexane, 9.81 ml, 15.7 mmol) at −10°C under an argon atmosphere, and the mixture was stirred for 30 min at 4°C. To the resulting LDA solution was added dropwise tert-butyl acetate (2.12 ml, 15.7 mmol) at −78°C. The mixture was stirred for 40 min at the same temperature. To the resulting enolate solution was added dropwise 10a (656 mg, 1.57 mmol) in dry THF (1.0 ml) at −78°C. The temperature was gradually allowed to warm to room temperature over 60 min. After adding sat. aq. NH₄Cl (30 ml), the reaction mixture was extracted with Et₂O (30 ml × 3). The combined organic layers were successively washed with water (30 ml) and brine (30 ml), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane:Et₂O = 2:1) to give 11a (250 mg, 39%) as a colorless oil. IR νmax (film) cm⁻¹: 2928, 2856, 1732, 1457, 1367, 1154, 1044; 1H-NMR (CDCl₃) δ: 0.96
tert-Butyl 10-[(Z)-2-pentenyl]-1,4-dioxaspiro[4.4]non-7-yl]decenoate (11b). According to the same synthetic method as that for 11a, 10b (404 mg, 0.93 mmol) was converted into 11b (172 mg, 44%) as a colorless oil. IR $\nu_{\text{max}}$ (film) cm$^{-1}$: 2929, 2856, 1732, 1458, 1367, 1154, 1044; $^1$H-NMR (CDCl$_3$) $\delta$: 0.96 (3H, t, $J=7.6$ Hz, CH$_2$Me), 1.23–1.34 (12H, m), 1.44 (9H, s, CMe$_3$), 1.51–1.83 (10H, m), 2.01–2.08 (4H, m, allyl-CH$_2$), 2.80–2.83 (5H, m), 5.27 (1H, m, vinyl-H). HR-MS m/z (M$^+$): calcd. for C$_{26}$H$_{40}$O$_4$, 422.3247; found, 422.3235.

9-[(3-oxo-2-[(Z)-2-pentenyl]cyclopentyl]nonanonic acid (OPC-9:0, 12a). According to the same synthetic method as that for 3-oxa-OPCs, 11a (250 mg, 0.61 mmol) was hydrolyzed with 50%aq. TFA to give 12a (164 mg, 87%) as a colorless oil. IR $\nu_{\text{max}}$ (film) cm$^{-1}$: 3450–2670, 3010, 2923, 2856, 1733, 1715, 1652, 1456, 1162, 941; $^1$H-NMR (CDCl$_3$) $\delta$: 0.96 (3H, t, $J=7.6$ Hz, CH$_2$Me), 1.26–1.40 (12H, m), 1.62–1.82 (5H, m), 2.01–2.07 (4H, m, allyl-CH$_2$), 2.28–2.35 (5H, m), 5.57 (1H, m, vinyl-H), 5.40 (1H, m, vinyl-H); $^{13}$C-NMR (CDCl$_3$) $\delta$: 14.1, 20.5, 24.6, 25.3, 26.8, 26.9, 28.9, 29.1, 29.2, 29.6, 34.0, 34.6, 38.0, 40.1, 55.0, 125.3, 133.4, 179.9, 221.1; EI-MS m/z: 308 (10, M$^+$), 290 (29, M$^+-$H$_2$O) 151 (69), 83 (100). HR-MS m/z (M$^+$): calcd. for C$_{17}$H$_{32}$O$_3$, 308.2352; found, 308.2314.

10-[(3-oxo-2-[(Z)-2-pentenyl]cyclopentyl]decanoic acid (OPC-10:0, 12b). According to the same synthetic method as that for 3-oxa-OPCs, 11b (171 mg, 0.41 mmol) was hydrolyzed with 50%aq. TFA to give 12b (110 mg, 84%) as a colorless oil. IR $\nu_{\text{max}}$ (film) cm$^{-1}$: 3450–2660, 3009, 2927, 2855, 1740, 1710, 1652, 1463, 1163, 941; $^1$H-NMR (CDCl$_3$) $\delta$: 0.96 (3H, t, $J=7.6$ Hz, CH$_2$Me), 1.29–1.39 (14H, m), 2.01–2.07 (4H, m, allyl-CH$_2$), 2.28–2.38 (5H, m), 5.27 (1H, m, vinyl-H), 5.40 (1H, m, vinyl-H); $^{13}$C-NMR (CDCl$_3$) $\delta$: 14.1, 20.5, 24.6, 25.3, 26.9, 27.0, 29.0, 29.2, 29.3, 29.4, 29.7, 34.0, 34.6, 38.0, 40.1, 55.0, 125.4, 133.4, 179.9, 221.2; EI-MS m/z: 322 (10, M$^+$), 304 (29, M$^+-$H$_2$O) 151 (69), 83 (100). HR-MS m/z (M$^+$): calcd. for C$_{26}$H$_{40}$O$_3$, 322.2508; found, 322.2462.

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References and Notes

6) Ethyl 9-iodononanoate was prepared in 3 steps: (1) tosylolation, (2) oxidation with RuO$_4$ and NaO$_4$, and (3) ethyl esterification with Et$_2$O and K$_2$CO$_3$. Ethyl 10-iododecanoate was prepared in 4 steps: (1) tosylolation, (2) hydroboration with 9-borabicyclo[3.3.1]nonane (9-BBN) and then alkaline H$_2$O$_2$, (3) oxidation with RuO$_4$ and NaO$_4$, and (4) ethyl esterification with Et$_2$O and K$_2$CO$_3$.
8) OPC-9:0 has been synthesized via mixed Kolbe electrolysis in a low yield (see ref. 4c).
9) Racemic and trans-rich JA (JA standard) was prepared via alkaline hydrolysis of commercially available racemic methyl jasmonate.