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Novel fluoro- and hydroxyl-containing jasmonate derivatives as highly efficient elicitors in suspension cultures of *Taxus chinensis*

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Abstract—To develop more effective abiotic elicitors for cell suspension cultures of *T. chinensis* to meet the needs for paclitaxel as anti-tumor drug, some fluoro- or hydroxyl-containing groups are introduced to the ester moiety of jasmonic acid by the esterification or acylation with bis(trichloromethyl) carbonate and corresponding alcohol. Some of them are found to be novel and effective elicitors, which can enhance the production of taxuyunnanine C (Tc) up to 60% more than that by methyl jasmonate (MJA) in *T. chinensis* cell cultures.

In *Taxus* plant cell culture, methyl jasmonate (MJA) is recognized as the most effective abiotic elicitor to obtain the valuable anti-tumor drug paclitaxel (commercial trade name: taxol) and a novel physiologically active substance taxuyunnanine C (Tc).[1–5] Tc was reported to have a neuron growth factor (NGF)-like activity and facilitate the treatment of Alzheimer's disease.[6]

Structure–bioactivity relationship of MJA suggests that structural moieties for high bioactivity are mainly acetyl side chain at C-1, pentenyl chain at C-2 and keto at C-3. Kiyota et al. synthesized two compounds 1,2- and 4,5-didehydrojasmonates and found methyl 1,2-didehydrojasmonate inhibited the germination of lettuce seed more strongly than that by MJA, and methyl 4,5-didehydrojasmonate was comparable to that by MJA in rice seedlings assays.[7] Another jasmonate derivative was methyl 5′,5′,5′-trifluorojasmonate synthesized also by Kiyota et al., which induced sessile microtubers 20% more effectively than that by MJA.[8] Blechert et al. synthesized a series of MJA derivatives with modification of chains on C-1 and C-2, some of them had stronger bioactivities than MJA.[9] For example, a compound with octanoic acid on C-1 rather than acetic acid of MJA mimicked hydrogenated 12-oxophytodienoic acid (OPDA), a lipid-derived signal molecule in higher plants.[10]

However, most of modifications on MJA are not successful and impractical for application to biological systems. In comparison with MJA, Miersch et al. investigated jasmonic acid (JA) and 66 structurally related compounds in gene expression of barley leaves, and found that all structural modifications on C-1, C-2, and C-3 moiety would decrease their bioactivities.[11] Koda et al. found that the introduction of locking methyl group at C-2 position considerably lowered tuber-inducing activities compared with MJA.[12]

In cell suspension cultures of *T. media*, Yukimune et al. investigated two elicitors derived from MJA, that is, methyl cucurbate, which was obtained by reducing the keto into hydroxyl at C-3 position of MJA, and *cis*-jasmone, which does not have the carboxyl group at C-1 position, and both of them had a weak elicitation activity or almost no activity.[1] Dong et al. tried HMJA whose 2-pentenyl was substituted by 2-pentyl and found that dihydro-methyl jasmonate (HMJA) had less inducing effect on the taxane accumulation than that of

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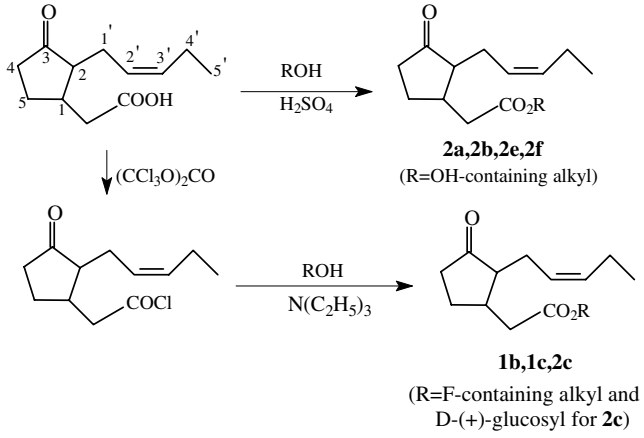
MJA.[13] In addition, stereo-configuration of MJA also affected production of paclitaxel and cell growth. (1*R*,2*S*)-MJA showed the strongest cell growth inhibition, while (1*R*,2*R*)-MJA had the highest activity in promoting paclitaxel biosynthesis.[14] This means that the configuration of C-1 and C-2 is important for its bioactivity.[7,12] Commercially available MJA is a mixture of about 5% each of (1*R*,2*S*)- and (1*S*,2*R*)-*cis* isomers, and 45% each of (1*R*,2*R*)- and (1*S*,2*S*)-*trans* isomers. MJA in our experiments was bought from Tokyo Kasei Kogyo Co., and it was also a mixture with its ratio of *cis*:*trans* at 6:44 as determined by GC analysis.

Traditionally, the modifications on the ester group of MJA do not affect bioactivity too much, and the replacement of methyl by other groups usually lowered their bioactivities.[11] Until now, little work on the ester modification has been reported. Our previous work indicated that MJA could strongly stimulate taxanes biosynthesis by enhancing activities of key enzymes such as taxadiene synthase.[13] Besides the special structure and stereo configuration of MJA, the introduction of H-bond on it should affect its interaction with these catalyzing enzymes. We suppose that H-bond acceptor fluorine, H-bond donor and acceptor OH may change the above interaction and the lipophilicity/hydrophilicity for permeation of plant cell membranes, which might be beneficial to their bioactivity. Therefore, a series of jasmonate derivatives with fluoro- or hydroxyl-containing groups were synthesized in this paper, and their eliciting activities on cell suspension cultures of *T. chinensis* were studied.

Fluoro-containing compounds **1b**, **1c**, and **2c** (Scheme 1) were prepared through the esterification of jasmonate chloride, which resulted from JA and bis-(trichloromethyl) carbonate,[15] with fluoro-containing alcohol or D-(+)-glucose in the presence of triethylamine. JA was obtained from alkaline hydrolysis of MJA. The compound **2a** and the hydroxyl-containing compounds **2b**, **2e**, and **2f** were prepared through the esterification of JA and corresponding alcohol. All compounds were separated and purified by gel silica chromatography. The structures of compounds were identified by ^{1}H NMR and HR-MS.[19]



Scheme 1. Preparation of MJA derivatives. The details of R groups are shown in Table 2.

Suspension cultures of *T. chinensis* cells were performed in a 250-mL Erlenmeyer flask containing 50-mL medium as described elsewhere.[13,16,17] The elicitation activity of MJA and synthesized elicitors on Tc was dose dependent, and each elicitor at 100 μM was found to be optimal concentration for Tc production as a typical example shown in Table 1. An elicitor was added to the cell cultures in 1 μL of ethanol per 1 mL of culture medium on day 7 after inoculation, and an equal volume of ethanol was also added to control cultures. All experiments were performed in triplicate, and the data are expressed as the mean of three independent samples with standard deviations (i.e., mean ± S.D.). Tc content was measured by a reverse phase HPLC (Hewlett–Packard series 1100 HPLC system, Agilent Palo Alto, CA). The maximum Tc content and production obtained in 21 days are summarized in Table 2.

MJA has four stereoisomers and the isomeric ratios of novel jasmonate derivatives prepared in this paper were the same as those of MJA, which was determined by GC. Therefore, it is reasonable to compare the eliciting activity of new jasmonate derivatives with that of MJA.[11,14]

In cell culture experiments, novel jasmonate derivatives all displayed a certain degree (around 5–10%) of cell growth inhibition at their optimal concentration of 100 μM for Tc biosynthesis as a typical example shown in Table 1. As the dynamic profiles shown in Figure 1,

Table 1. Effect of elicitor concentrations on the maximum cell concentration (12 days), Tc content and Tc production (21 days) in cell cultures of *T. chinensis* treated by newly synthesized MJA derivatives on day 7 by taking the compound **2b** as a typical example

Concentration of **2b** (μM)	Cell concentration (g DW/L)	Tc content (mg/g DW)[a]	Tc production (mg/L)
0	18.3 ± 0.2[b]	9.4 ± 0.7	128 ± 5
1	18.5 ± 0.3	11.6 ± 1.3	160 ± 8
10	17.3 ± 0.2	27.0 ± 1.8	358 ± 19
100	16.6 ± 0.2	43.2 ± 2.1	544 ± 12
500	14.9 ± 0.6	49.8 ± 0.8	522 ± 7

[a] mg/g DW: Tc amount (mg) per gram dry cells.
[b] All data are expressed as an average ± S.D.

Table 2. Eliciting activities of novel MJA derivatives

No.	R (in Scheme 1)	Tc content (mg/g DW)	Tc production (mg/L)
MJA	CH_3	29.2 ± 0.6	386 ± 10
1b	CH_2CF_3	34.94 ± 0.1	440.3 ± 5.9
1c	$CH_2CF_2CF_3$	38.22 ± 0.9	480.5 ± 0.6
2a	$CH_2CH_2CH_3$	27.9 ± 1.6	357 ± 7
2b	$CH_2CHOHCH_2OH$	47.2 ± 0.5	550 ± 13
2c	$C_6H_{11}O_5$(2-)	40.5 ± 0.0	492 ± 6
2e	CH_2CH_2OH	38.1 ± 0.9	464 ± 20
2f	$CH_2CH_2OCH_2CH_2OH$	31.4 ± 1.5	417 ± 2

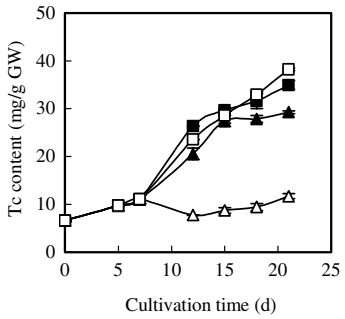


Figure 1. Time course of Tc content as elicited by fluorine-containing jasmonate derivatives. The bar in the figure shows standard deviations calculated from three independent samples. Symbols: ctrl-△; MJA-▲; **1b**-■; **1c**-□.

the Tc content induced on day 7 by the novel fluorine-containing compounds was higher than that by MJA, especially around the harvest time of cell cultivation (i.e., day 21). Also, all chemically synthesized hydroxyl-containing compounds had strong Tc stimulation activities than that by MJA (Fig. 2).

It was reported that a fluoro-containing compound methyl 5′,5′,5′-trifluorojasmonate at 10^{-7} M induced sessile microtubers tuberization rate 20% more effectively than that by MJA.[8] Compared with MJA, trifluoroethyl jasmonate **1b** increased Tc content and production by about 20% and 15% higher, and pentafluoropropyl jasmonate **1c** increased Tc content and production by about 30% and 25% higher, respectively. The effect of fluorine introduction could be observed by comparing **2a** and **1c**, and about 35% increase in Tc content and production was observed by introducing five fluorine atoms (Fig. 1 and Table 2). With the calculation of A log P_{98} by Cerious[2] 4.8 (Accelrys Inc., San Diego, CA, USA), fluorine introduction markedly increased lipophilicity as represented by $A\log P_{98}$[18] (the A log P_{98} values of MJA, **1b** and **1c** are estimated to be 2.22, 3.22, and 3.72, respectively). Interestingly, the bioactivities elicited by MJA, **1b** and **1c** seem to be quantitatively correlated with the lipophilicity properties of these compounds, because the bioactivity was enhanced (Fig. 1 and Table 2) with an increase of the fluoro-groups introduced into MJA (an increase in their lipophilicity).

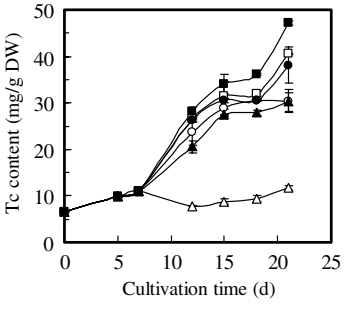


Figure 2. Time profiles of Tc content as elicited by hydroxyl-containing jasmonate derivatives. The bar in the figure shows standard deviations calculated from three independent samples. Symbols: ctrl-△; MJA-▲; **2b**-■; **2c**-□; **2e**-●; **2f**-○.

The introduction of hydroxyl groups gave us excellent results. Novel elicitors **2b**, **2c**, and **2e** had much improvement in the Tc accumulation. Compared with MJA, the compound **2b** could even increase Tc content and production up to 62% and 43% more, respectively. For **2a**, **2f**, **2e**, **2b**, and **2c**, their number of hydroxyl groups may contribute remarkably to their bioactivities. For example, the obvious difference in structure between **2a** and **2b** is that the latter has two hydroxyl groups, and the Tc eliciting activity for **2b** was about 1.7-fold that of **2a**. However, although **2c** has four hydroxyl groups, its eliciting activity was lower than that of **2b**. The reason might be that the lipophilicity of **2c** is too low for it to transport cell membrane effectively (A log P_{98} of **2b**, **2c**, **2e**, and **2f** is 1.17, 0.06, 1.68 and 1.55, respectively as calculated by Cerius[2]). Thus, the optimal lipophilicity and the number of hydroxyl groups on jasmonate derivatives are two key factors for high eliciting activity.

This work demonstrates that the introduction of fluoro- and hydroxyl-containing groups on jasmonate esters is a successful strategy to promote Tc production by *T. chinensis* cell cultures. Pentafluoropropyl jasmonate (**1c**) and 2,3-dihydroxypropyl jasmonate (**2b**) are the most effective jasmonate derivatives. It was found that the number of fluorine, hydroxyl and optimal lipophilicity are beneficial to their eliciting activities. The potential applications of these novel and effective elicitors in *Taxus* plant cell cultures may be helpful for obtaining a novel physiologically active substance Tc and the valuable anti-tumor drug paclitaxel.

Acknowledgements

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19. Compound **1b**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.89 (3H, t, $J_{5',4'}$ = 7.50 Hz, 5′-H), 1.42–1.52 (1H, m), 1.97–2.10 (5H, m), 2.20–2.26 (4H, m), 2.43–2.46 (1H, m), 2.78–2.81 (1H, m, 2-H), 4.75 (2H, q, J = 9.11 Hz, OCH_2CF_3), 5.16 (1H, dt, $J_{3',4'}$ = 7.68 Hz, $J_{3',2'}$ = 10.56 Hz, 3′-H), 5.38 (1H, dt, $J_{2',1'}$ = 7.34 Hz, $J_{2',3'}$=10.55 Hz, 2′-H). HRMS (EI, *m/e*), Found M^+ = 292.1273; $C_{14}H_{19}O_3F_3$ requires 292.1286. **1c**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.89 (3H, t, $J_{5',4'}$ = 7.47 Hz, 5′-H), 1.42–1.49 (1H, m), 1.96–2.09 (5H, m), 2.17–2.27 (4H, m), 2.43–2.46 (1H, m), 2.78–2.82 (1H, m, 2-H), 4.83 (2H, t, J = 13.89 Hz, $OCH_2CF_2CF_3$), 5.20 (1H, dt, $J_{3',4'}$ = 7.56 Hz, $J_{3',2'}$ = 10.67 Hz, 3′-H), 5.38 (1H, dt, $J_{2',1'}$ = 7.31 Hz, $J_{2',3'}$ = 10.65 Hz, 2′-H). HRMS (EI, *m/e*), Found M^+ = 342.1218; $C_{15}H_{19}O_3F_3$ requires 342.1254. **2a**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.86–0.91 (6H, m, $2CH_3$), 1.41–1.48 (1H, m), 1.53–1.61 (2H, m), 1.91–2.08 (5H, m), 2.16–2.25 (4H, m), 2.29–2.34 (1H, m), 2.62–2.66 (1H, m, 2-H), 3.98 (2H, t, J = 6.58 Hz, OCH_2), 5.21 (1H, dt, $J_{3',4'}$ = 7.50 Hz, $J_{3',2'}$ = 10.80 Hz, 3′-H), 5.38 (1H, dt, $J_{2',1'}$ = 7.28 Hz, $J_{2',3'}$ = 10.81 Hz, 2′-H). **2b**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.90 (3H, t, $J_{5',4'}$ = 7.55 Hz, 5′-H), 1.39–1.49 (1H, m), 1.93–2.09 (5H, m), 2.16–2.25 (4H, m), 2.30–2.35 (1H, m), 2.63–2.67 (1H, m, 2-H), 3.30–3.38 (2H, m), 3.61–3.64 (1H, m), 3.90–3.94 (1H, m), 4.04–4.07 (1H, m), 4.63 (1H, s, OH), 4.86 (1H, s, OH), 5.22 (1H, dt, $J_{3',4'}$ = 7.56 Hz, $J_{3',2'}$ = 10.64 Hz, 3′-H), 5.38 (1H, dt, $J_{2',1'}$ = 7.28 Hz, $J_{2',3'}$ = 10.63 Hz, 2′-H). HR-MS (EI, *m/e*), Found M^+ = 284.1618; $C_{15}H_{24}O_5$ requires 284.1624. **2c**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.89 (3H, t, $J_{5',4'}$ = 7.50 Hz, 5′-H), 1.42–1.52 (1H, m), 1.97–2.10 (5H, m), 2.20–2.26 (4H, m), 2.43–2.46 (1H, m), 2.78–2.81 (1H, m, 2-H), 3.09–3.44 (4H, m), 3.57–3.76 (2H, m), 4.45–4.54 (2H, m), 4.80–4.88 (3H, m, OH), 5.16 (1H, dt, $J_{3',4'}$ = 7.68 Hz, $J_{3',2'}$ = 10.56 Hz, 3′-H), 5.38 (1H, dt, $J_{2',1'}$ = 7.34 Hz, $J_{2',3'}$ = 10.54 Hz, 2′-H). MS (EI, *m/e*), Found M^+ = 372; $C_{18}H_{28}O_8$ requires 372.42. **2e**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.89 (3H, t, $J_{5',4'}$ = 7.50 Hz, 5′-H), 1.39–1.48 (1H, m), 1.95–2.07 (5H, m), 2.20–2.25 (4H, m), 2.30–2.35 (1H, m), 2.63–2.67 (1H, m, 2-H), 3.55 (2H, t, J = 5.06 Hz, OCH_2), 4.03 (2H, t, J = 5.06 Hz, OCH_2), 5.22 (1H, dt, $J_{3',4'}$ = 7.60 Hz, $J_{3',2'}$ = 10.69 Hz, 3′-H), 5.38 (1H, dt, $J_{2',1'}$ = 7.30 Hz, $J_{2',3'}$ = 10.68 Hz, 2′-H). HR-MS (EI, *m/e*), Found M^+ = 254.1517; $C_{14}H_{22}O_4$ requires 254.1518. **2f**: colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.90 (3H, t, $J_{5',4'}$ = 7.53 Hz, 5′-H), 1.42–1.46 (1H, m), 1.92–2.08 (5H, m), 2.15–2.25 (4H, m), 2.31–2.36 (1H, m), 2.64–2.68 (1H, m, 2-H), 3.41 (2H, t, J = 5.00 Hz, OCH_2), 3.47 (2H, t, J = 4.69 Hz, OCH_2), 3.59 (2H, t, J = 4.99 Hz, OCH_2), 4.14 (2H, t, J = 4.70 Hz, OCH_2), 5.21 (1H, dt, $J_{3',4'}$ = 7.51 Hz, $J_{3',2'}$ = 10.77 Hz, 3′-H), 5.37 (1H, dt, $J_{2',1'}$ = 7.28 Hz, $J_{2',3'}$ = 10.78 Hz, 2′-H). HR-MS (EI, *m/e*), Found M^+ = 298.1794; $C_{16}H_{26}O_5$ requires 298.1790.