C_{14} oxylipin glucosides isolated from *Lemna paucicostata*

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Abstract

Oxylipin glucosides (**2**−**4**) were isolated from *Lemna paucicostata*, with their structures and absolute configurations elucidated by spectroscopic and chemical methods. Compounds **2**−**4** were glucosides of C_{14}-oxylipin which were synthesized from α-linolenic acid via the 9-lipoxygenase pathway.

Keywords: *Lemna paucicostata*, Lemnaceae, oxylipin, oxylipin glucoside, 9-lipoxygenase pathway

1. Introduction

Plant cells develop oxidative processes including peroxidation of membrane-associated polyunsaturated fatty acids in response to stress. Both reactive oxygen species (ROS) and enzymes such as 9/13-lipoxygenase (LOX) and α-dioxygenase are involved in lipid oxidation processes, leading to production of bioactive molecules termed oxylipins (Feussner and Wasternack, 2002; Blee, 2002). During the past decade, interest in this field has focused on understanding the biosynthesis and physiological functions of jasmonic acid (JA) and its related compounds that were synthesized via 13-LOX pathway. These research efforts clearly indicated that JAs played an important role in development and defense of plants (Wasternack, 2007). Like other plant hormones, ubiquitin-regulated protein degradation by the 26S proteasome seemed to be central to JA responses (Zheng *et al.*, 2002; Sullivan *et al.*, 2003). By contrast, in most cases, knowledge on diversity and function of oxylipins other than JAs is rather scarce, and no general concept of their biological role has been established.

Previously, we found that *Lemna paucicostata*, a free-floating aquatic plant, secretes an α-ketol, (12*Z*,15*Z*)-9-hydroxy-10-oxooctadeca-12,15-dienoic acid (**1**) (Fig. 1) which is synthesized by 9-LOX and allene oxide synthase (AOS) into the surrounding culture in response to drought, heat, and osmotic stresses (Yokoyama *et al*., 2000). This suggested that the production of 9-LOX oxylipins would be an adaptation of *L. paucicostata* to the environment. α-Ketol **1** is also of interest since it induces flowering of *L. paucicostata* after reacting with norepinephrine (Yokoyama *et al*., 2000; Yamaguchi *et al*., 2001). These detailed studies established that all fatty acids containing a β,γ-unsaturated carbonyl moiety have the potential to form cycloadducts

with catecholamines and then induce flowering (Kai *et al*., 2008a,b). Although to this date we have not yet detected such adducts in *L. paucicostata*, the possibility remains that other oxylipins are involved in flowering. In both cases, it is of utmost importance to clarify the composition of various oxylipins in the plant. Therefore, we analyzed the extract of *L. paucicostata* using LC/MS/MS, and found three novel oxylipins apparently different from already known ones. Here we report the isolation and structural elucidation of three new oxylipins based on their spectroscopic and chemical characteristics.

2. Results and discussion

Whole plants of *L. paucicostata* were homogenized in liquid N_2 and extracted with MeOH. The extract was concentrated and successively subjected to solid-phase extraction after adjusting the pH to approximately 3.0. The eluate thus collected with 80% aq MeCN was analyzed by LC/MS/MS, which showed the presence of three new compounds **2**−**4** together with the known oxylipin **1**. Compounds **2**, **3**, and **4** were detected as $[M+NH_4]^+$ ion at 7.09, 7.58, and 6.90 min, respectively, in the selected-ion monitoring (SIM) chromatograms, and their fragmentations were different from those of previously known oxylipins of *L. paucicostata* (Fig. 2A, C, and E) (Kai *et al*., 2007). Endogenous levels of **2**−**4** were expected to be very low (<1% of **1**) from their peak intensities. The specific incorporation of [U-^{13}C]-**1** into **2**−**4** was confirmed by the substrate administration experiments followed by LC/MS/MS analysis, in which the *m/z* of their precursor and several fragment ions increased by 14 was observed (Fig. 2B, D, and F). This indicated that **2**−**4** should be metabolites of **1** and derived from two repetitive β-oxidations. In order to elucidate their structures, compound **1** was

administered to the plants and then the plants extracts were subjected to chromatography using an ODS column and then preparative HPLC to afford **2**−**4**.

Compound **2** possessed the molecular formula $C_{20}H_{32}O_9$ as established by HRESI-MS (*m/z* 439.1944 $[M+Na]^+$), whereas the ^1H and ^{13}C NMR spectra of **2** were indicative of a glycoside of unsaturated C_{14}-fatty acid (Table 1). The ^1H–^1H COSY connectivities of C-1 to C-5 and C-7 to C-14, and HMBC correlations of H-5 (δ_H 4.07) and H-7 (δ_H 3.44, 3.56) to C-6 (δ_C 212.4) indicated that the fatty acid part is 5-hydroxy-6-oxotetradeca-8,11-dienoic acid (**6**). The sugar was deduced to be β-glucopyranose based on the ^1H–^1H coupling constants in the sugar moiety of **2** and its tetraacetate (**5**). The HMBC correlation of H-1′ (δ_H 4.33) to C-5 (δ_C 85.8) indicated that the glucose was connected to C-5 of the fatty acid. The geometries of the olefinic bonds at C-8 and C-11 were determined as *Z*/*Z* on the basis of the ^1H coupling constants between the olefinic protons, H-8 and H-9 (10.5 Hz), and H-11 and H-12 (10.6 Hz), respectively. Therefore, compound **2** was assigned to be (8*Z*,11*Z*)-5-β-glucopyranosyloxy-6-oxotetradeca-8,11-dienoic acid (Fig. 1). In order to determine the stereochemistry at C-5, we carried out enzymatic hydrolysis of **2** with β-glucosidase to afford the aglycone **6**. Chiral HPLC analysis of **6** gave a peak with an identical retention time to synthetic 5*R*-**6** (Kai *et al*., 2008). Therefore, the absolute configuration at C-5 of **2** was assigned as *R*.

HRESI-MS analysis established that the molecular formula of **3** was $C_{23}H_{34}O_{12}$ (*m/z* 525.1950 $[M+Na]^+$), indicating that **3** could be a malonate of **2**. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **2** except for the presence of the two carbonyl carbons (δ_C 170.2, 172.4) and the methylene (δ_H 3.40, δ_C 43.4) (Table 1). In addition, the HMBC correlations from singlet H-2″ to C-1″ and C-3″ indicated that a malonyl group was present in **3**. This malonyl moiety was found to be connected to C-6′ by the

HMBC correlation of H-6′ (δ_H 4.25, 4.42) to C-1″ (δ_C 170.2). The other signals in ^{1}H and ^{13}C NMR could be assigned as those of **2** as listed in Table 1. The geometric configurations around the two double bonds were determined to be *Z*/*Z* by the coupling constants of H-8 (10.6 Hz) and H-10 (10.6 Hz). To define the stereochemistry of **3**, we applied enzymatic hydrolysis of glucoside bond with β-glucosidase. As a result, **3** also afforded aglycone **6**, whose absolute configuration was shown to be 5*R*-**6** using chiral HPLC analysis. Therefore, **3** was assigned to be the 6′-*O*-malonate of **2** (Fig. 1).

Compound **4** exhibited an $[M+Na]^+$ at *m/z* 441.2096 corresponding to the molecular formula of $C_{20}H_{34}O_9$, suggesting that **4** was a reduced form of **2**. In the ^{1}H and ^{13}C NMR spectra of **4**, one carbonyl group observed in **2** was lacking, and additional signals assignable to an oxymethine moiety were newly observed. However, the completely overlapping signals of two oxymethine protons (δ_H 3.62−3.65) at C-5 and C-6 in the ^{1}H NMR spectrum prevented us from determining the position of glucosylation. Accordingly, **4** was methyl esterified with diazomethane and subsequently acetylated with acetic anhydride to afford the pentaacetylated derivative of **4** (**7**). The ^{1}H NMR spectrum of **7** in $CDCl_3$ gave the well-separated and sharp signals for these oxymethine protons making it possible to identify the HMQC and HMBC correlations from the respective proton signals. The anomeric proton at C-1′ (δ_H 4.60) in **7** showed an HMBC correlation to C-5 (δ_C 81.2), indicating that the position of glucosylation in **4** was identical to that of **2**. The geometric configurations around the two double bonds were determined to be *Z*/*Z* by the coupling constants of H-9 (10.6 Hz) and H-11 (10.4 Hz). Consequently, the structure of compound **4** was determined to be the keto-reduced analog of **2** (Fig. 1). The stereochemistry at C-5 was deduced to be *R* since the administration experiments with *R*-**1** afforded a product whose retention time on HPLC was identical to **4**. In this study, we did not determine the stereochemistry at C-6 of **4**

due to the difficulty in preparing its Mosher ester.

Compounds **2**−**4** are new type of oxylipins, in which a glucose moiety is conjugated to the hydroxy group in α-ketol or vicinal diol of C_{14}-fatty acid. These fatty acids, **6** and **8**, were previously identified as metabolites of **1** in *Pharbitis nil* (Kai *et al*., 2007) and were also detected in an extract of *L. paucicostata* by LC/MS. Therefore, we proposed the biogenetic pathway of these oxylipins as shown in Scheme 1. α-Ketol **1** produced from linolenic acid by the action of 9-LOX and AOS undergoes two repetitive cycles of β-oxidation. The reaction of **1** with the *Pseudomonas fragi* enzyme via β-oxidation exclusively leads to **6**, implying that **6** is no longer β-oxidized (unpublished data). The next step is glucosylation to yield 5-*O*-glucoside of **6** (**2**). The glucoside can be then converted by either malonylation or keto-reduction to give **3** and **4**. There is of course the possibility that carbonyl carbon at C-6 in **6** is reduced to form diol **8** before forming glucoside bond. If so, diol formed can be glucosylated at the 5-hydroxy group to yield **4**.

As α-ketol **1** showed flowering induction activity against *L. paucicostata* after reacting with norepinephrine, the effect of α-ketol glucoside **2** was also examined. The reaction product of **2** with norepinephrine induced 20% flowering at 10 μM, which was about 10^4 times weaker than that of **1**. Therefore, if α-ketol **1** or the derivatives play a role in the flowering process of *L. paucicostata*, glucoside **2** might be an inactive form of bioactive molecule. The relationship between oxylipin glucosides (**2**−**4**) and stress responses is being further investigated.

3. Concluding remarks

In plants, oxygenation of C_{18}-fatty acids via 9/13-LOX is a crucial point from where a

cascade of oxylipin pathway being (Scheme 1). In a previous study, we isolated 9-LOX oxylipin **1** as a stress-induced flowering factor from *L. paucicostata* (Yokoyama *et al*., 2000). It was also suggested that **1** could be related to the flowering induction in *Pharbitis nil* (Suzuki *et al*., 2003). In this process, we newly identified six oxylipins as metabolites of **1** (Kai *et al*., 2007). The present work provides the first example of the presence of glucosides of 9-LOX-derived oxylipins in plants. Glycosylation of bioactive molecules is generally thought to be due to storage and/or inactive forms (Gachon *et al*., 2005). To our knowledge, previously-known oxylipin glycosides were restricted to derivatives of JAs. Production of these diverse 9-LOX oxylipins might actually be the rule rather than the exception in *L. paucicostata* and *P. nil*. The study presented here strongly suggests that plants have developed more complicated oxylipin pathways than previously predicted.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a JNM λ500A spectrometer (JEOL, Tokyo, Japan). High-resolution mass spectra were obtained with a JMS-T100LC AccuTOF mass spectrometer (JEOL) using PPG as internal standard. LC/MS analysis was conducted with an LC-10VP system equipped with an LCMS 2010A mass spectrometer (Shimadzu, Kyoto, Japan). LC/MS/MS analysis was carried out with a NANOSPACE SI-2 system (Shiseido, Tokyo, Japan) equipped with a TSQ Quantum Discovery MAX (Thermo Fisher Scientific, Massachusetts, USA). HPLC separation was performed with a JASCO (Tokyo, Japan) LC system. Solvents for HPLC were purchased from Kanto

Chemical (Tokyo, Japan).

4.2. Plant material

Plants of *Lemna paucicostata* 151 were obtained from Prof. O. Tanaka (Konan University), and were planted on 1/2 Hutner's media and grown at 25ºC under continuous light conditions (Kaihara *et al.*, 1981).

4.3. Feeding experiments

Three-week-old plants (1 g fresh wt) were transferred to 1/2 Huntner's media containing 80 μM [U-^{13}C]-**1** and incubated for 48 h under the continuous light at 25ºC. The plants were washed with H_2O, homogenized in liquid N_2, and extracted two times with MeOH (5 mL). The aqueous concentrate was subjected to the solid-phase extraction cartridge (Sep-Pak Plus C18, Waters, Massachusetts, USA) and the eluate with CH_3CN-H_2O (8:2, v/v) was analyzed by LC/MS/MS. HPLC conditions: column, HyPURITY C18 2.1×150 mm (Thermo Fisher Scientific): flow rate, 200 μL/min: solvent A, 50 μM ammonium acetate: solvent B, CH_3CN: gradient profile, 25−70 % B/(A+B) within 10 min.

4.4. Extraction and isolation

Three-week-old plants (80 g fresh wt) were transferred to 1/2 Hutner's media containing 80 μM **1**. After 48 h under the continuous light at 25ºC, the plants were homogenized using liquid N_2 and subsequently extracted with MeOH (40 mL) for 2 h.

After the extraction procedure had been repeated, the combined extract was concentrated under reduced pressure. The aqueous concentrate was subjected to an ODS column (Cosmosil 140C_{18}-OPN, Nacalai Tesque, Kyoto, Japan) after adjusting pH to 3.0. The column was washed with distilled H_2O and then eluted with an increasing concentration of CH_3CN in H_2O (20% increments from 20 to 100% CH_3CN). The fractions collected were checked by LC/MS. The fraction containing target compounds was further purified by HPLC [CAPCELL PAK C_{18} UG120 10×250 mm (Shiseido), CH_3CN−0.05% aq TFA (3:7), 4 mL/min] to afford **2**−**4**.

4.5. (5*R*,8*Z*,11*Z*)-5-β-Glucopyranosyloxy-6-oxotetradeca-8,11-dienoic acid (**2**).

Colorless oil (14 mg). HRMS (ESI^+) *m/z* 439.1944 $[M+Na]^+$ (calcd for $C_{20}H_{32}NaO_9$, 439.1944). For ^1H and ^{13}C NMR data see Table 1.

4.6. (5*R*,8*Z*,11*Z*)-5-(6′-*O*-Malonyl-β-glucopyranosyloxy)-6-oxotetradeca-8,11-dienoic acid (**3**).

Colorless oil (1 mg). HRMS (ESI^+) *m/z* 525.1950 $[M+Na]^+$ (calcd for $C_{23}H_{34}NaO_{12}$, 525.1948). For ^1H and ^{13}C NMR data, see Table 1.

4.7. (5*R*,8*Z*,11*Z*)-5-β-Glucopyranosyloxy-6-hydroxytetradeca-8,11-dienoic acid (**4**).

Colorless oil (1 mg). HRMS (ESI^+) *m/z* 441.2096 $[M+Na]^+$ (calcd for $C_{20}H_{34}NaO_9$, 441.2101). For ^1H and ^{13}C NMR data, see Table 1.

4.8. Tetraacetate of **2** (**5**)

A solution of **2** (3 mg) in Ac_2O−pyridine (3:2, 1 mL) was stirred for 4 h at room temperature. After removing solvent, the residue was subjected to preparative HPLC [CAPCELL PAK C_{18} UG120 10×250 mm, MeCN−0.05% aq TFA (1:1), 4 mL/min] to yield **5** (3 mg). HRMS (ESI^+) *m/z* 607.2363 $[M+Na]^+$ (calcd for $C_{28}H_{40}NaO_{13}$, 607.2367). ^1H NMR (500 MHz, $CDCl_3$) δ 0.99 (3H, t, J = 7.6 Hz, H-14), 1.68 (2H, m, H-3), 1.72 (2H, m, H-4), 2.01 (3H, s, Ac), 2.02 (3H, s, Ac), 2.06 (3H, s, Ac), 2.07 (3H, s, Ac), 2.08 (2H, m, H-13), 2.36 (2H, t, J = 6.9 Hz, H-2), 2.76 (2H, m, H-10), 3.34 (1H, dd, J = 5.9, 18.4 Hz, H-7), 3.50 (1H, dd, J = 5.5, 18.4 Hz, H-7), 3.67 (1H, ddd, J = 2.1, 5.5, 9.6 Hz, H-5′) 3.83 (1H, m, H-5), 4.02 (1H, dd, J = 2.1, 12.3 Hz, H-6′), 4.19 (1H, dd, J = 5.5, 12.3 Hz, H-6′), 4.54 (1H, d, J = 7.9 Hz, H-1′), 5.05 (1H, t, J = 9.6 Hz, H-4′), 5.10 (1H, dd, J = 7.9, 9.6 Hz, H-2′), 5.21 (1H, t, J = 9.6 Hz, H-3′), 5.28 (1H, ddt, J = 1.5, 7.0, 10.6 Hz, H-11), 5.41 (1H, ddt, J = 1.5, 7.0, 10.6 Hz, H-12), 5.52 (1H, dt, J = 6.2, 10.5 Hz, H-8), 5.54 (1H, dt, J = 6.2, 10.5 Hz, H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2 (C-14), 20.4 (C-3), 20.5 (Ac×4), 20.7 (C-13), 25.8 (C-10), 31.3 (C-4), 31.3 (C-4), 33.8 (C-2), 35.9 (C-7), 61.8 (C-6′), 68.4 (C-4′), 71.3 (C-2′), 72.0 (C-5′), 72.7 (C-3′), 86.5 (C-5), 101.6 (C-1′), 120.9 (C-8), 126.4 (C-11), 131.7 (C-9), 132.5 (C-12), 169.4−170.6 (Ac×4), 209.3 (C-6). The ^{13}C signal at C-1 could not be observed.

4.9. Pentaacetate of **4** (**7**).

To a solution of **4** (1 mg) in MeOH (1 mL), a solution of (trimethylsilyl)diazomethane (2 M in hexane, 100 μL) was added dropwise and stirred for 5 min at room temperature. After removing the solvent and reagent under vacuum,

the resulting oil was dissolved in acetic Ac_2O−pyridine (3:2, 1 mL) and stirred at room temperature for 4 h. The reaction was quenched with H_2O with the mixture extracted three times with EtOAc (5 mL). After removing the solvent, the residue was purified by preparative HPLC [CAPCELL PAK C_{18} UG120 10×250 mm, CH_3CN−0.05% aq TFA (1:1), 4 mL/min] to yield **7** (1 mg). HRMS (ESI^+) *m/z* 665.2779 $[M+Na]^+$ (calcd for $C_{31}H_{46}NaO_{14}$, 655.2785). ^1H NMR (500 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.6 Hz, H-14), 1.50−1.64 (4H, H-3,4), 1.99−2.08 (15H, Ac×5), 2.05 (2H, m, H-13), 2.30 (2H, t, J = 7.0 Hz, H-2), 2.32 (2H, m, H-7), 2.77 (2H, m, H-10), 3.66 (1H, m, H-5), 3.67 (3H, s, OMe), 3.74 (1H, ddd, J = 2.4, 5.4, 9.6 Hz, H-5′) 4.14 (1H, dd, J = 2.4, 12.2 Hz, H-6′), 4.19 (1H, dd, J = 5.4, 12.2 Hz, H-6′), 4.60 (1H, d, J = 8.0 Hz, H-1′), 4.99 (1H, dd, J = 8.0, 9.6 Hz, H-2′), 5.04 (1H, t, J = 9.6 Hz, H-4′), 5.12 (1H, dt, J = 4.0, 9.8 Hz, H-6), 5.19 (1H, t, J = 9.6 Hz, H-3′), 5.28 (1H, m, H-11), 5.39 (1H, m, H-12), 5.42 (1H, m, H-9), 5.48 (1H, m, H-8). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2 (C-14), 20.6 (Ac×5), 21.0 (C-3), 21.1 (C-13), 25.6 (C-10), 26.2 (C-7), 28.7 (C-4), 33.8 (C-2), 51.6 (OMe), 62.2 (C-6′), 68.4 (C-4′), 71.7 (C-2′), 71.9 (C-5′), 73.0 (C-3′), 73.9 (C-6), 81.2 (C-5), 101.6 (C-1′), 124.9 (C-8), 126.9 (C-11), 130.9 (C-9), 132.1 (C-12), 169.3−170.7 (Ac×5), 173.4 (C-1).

4.10. Chiral HPLC analysis

A solution of oxylipin glucosides and β-glucosidase (from Almond, Wako Pure Chemical) in 0.1 M citrate buffer (pH 5.0) was stirred for 2 h at 30ºC. The mixture was diluted with H_2O and extracted with EtOAc. After removing solvent, the residue was dissolved in MeOH and analyzed on a CHIRALCEL OD-RH (Daicel Chemical, Tokyo, Japan) eluting with CH_3CN−0.05% aq HCOOH (35:75).

4.11. Flower induction assay

The flower induction assay was performed according to the previous study (Kai *et al*., 2008a,b). A three-frond colony of *L. paucicostata* was placed on M medium containing test sample and 6-benzylaminopurine, and incubated on for 10 days at 25ºC under continuous light. The percentage of fronds with flowers was determined.

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Figure and scheme legends

Fig. 1. Structures of oxylipin glucosides (**1**−**4**).

Fig. 2. LC/MS/MS analysis of compounds **2**−**4** in extracts of *L. paucicostata* from control (A, C, and E) and substrate administration (B, D, and F) experiments. Metabolites were detected as $[M+NH_4]^+$ in the SIM mode (left). Subsequently, their MS/MS spectra were measured (right).

Scheme 1. 9-LOX oxylipins in *L. paucicostata*.

Figure(s)

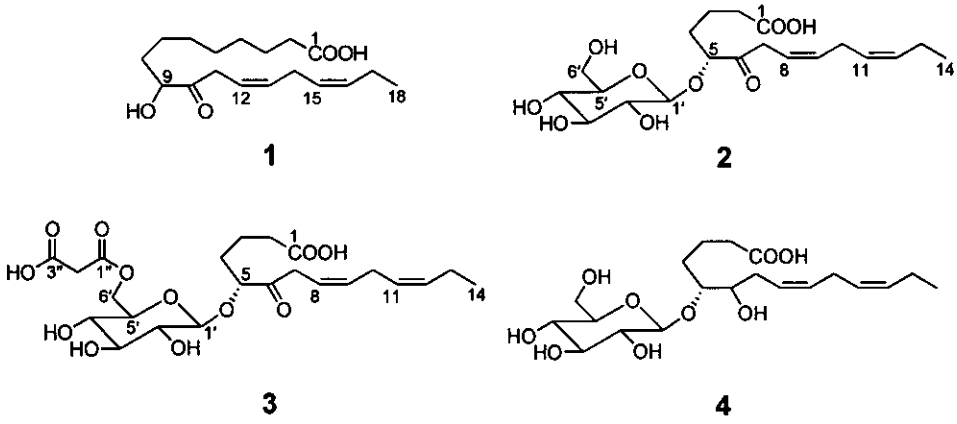


Fig. 1. Kai et al.

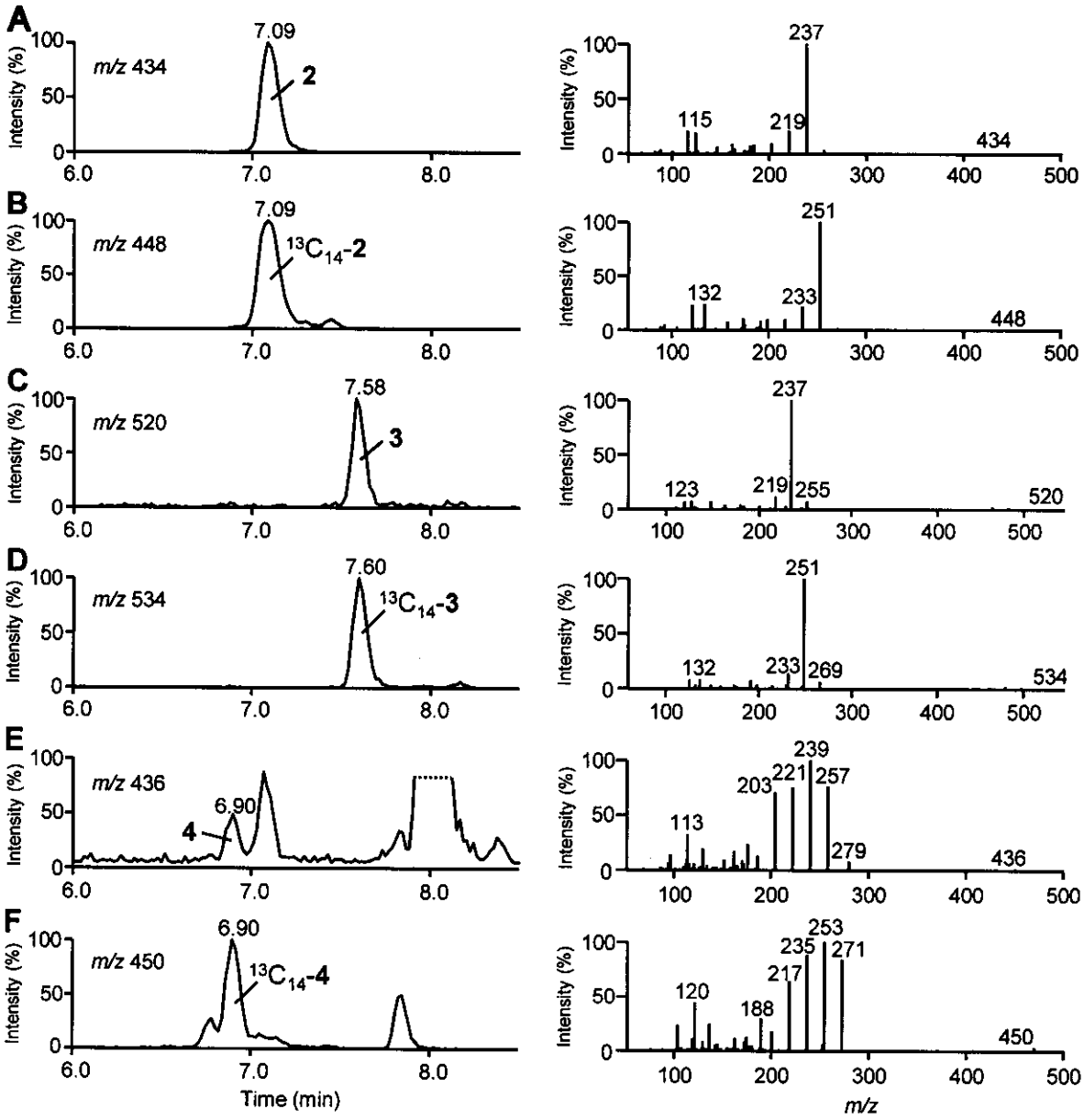
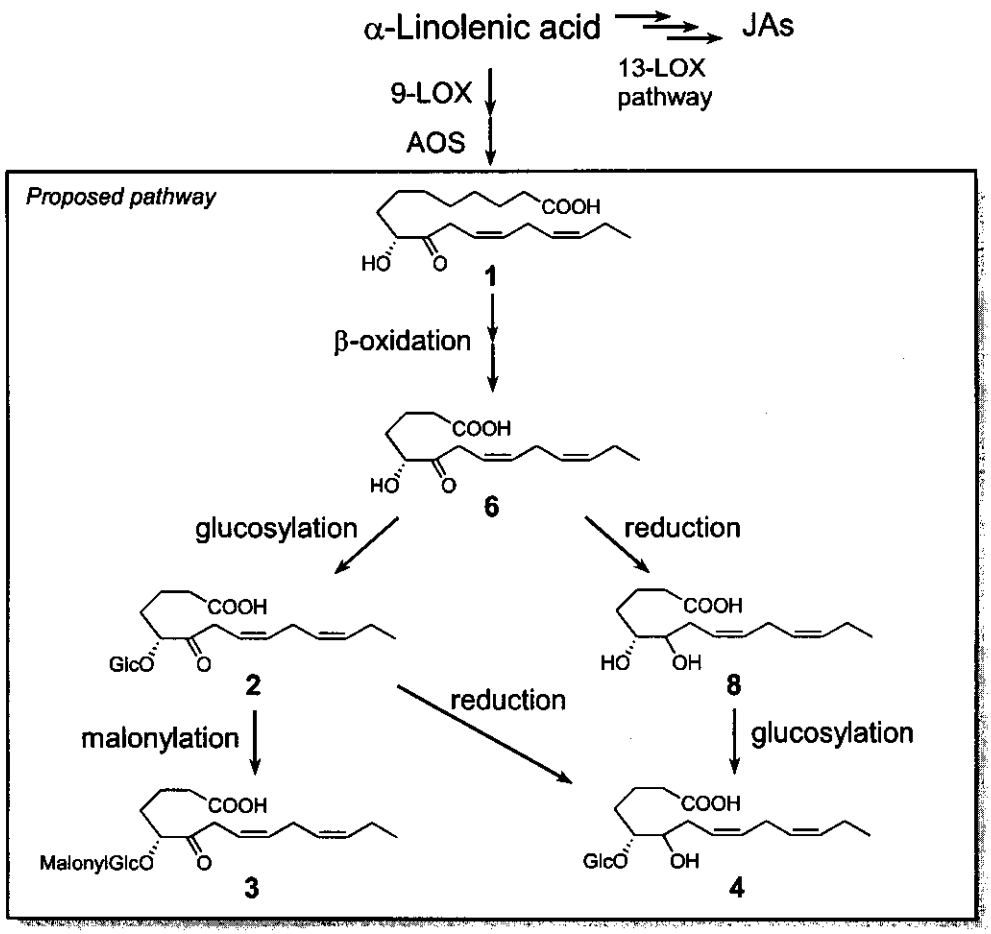


Figure 2. Kai et al.



Scheme 1. Kai et al.

Table(s)

Table 1. NMR data of compounds **2**–**4** (500 MHz)[a]

No.	2		3		4	
	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz
1	177.3		178.7		181.3	
2	34.5	2.33, 2H, t, 7.0	33.9	2.42, 2H, t, 6.7	36.4	2.34, 2H, t, 6.7
3	21.5	1.67–1.78[b]	20.4	1.67–1.80[b]	22.7	1.50–1.64[b]
4	32.3	1.67–1.78[b]	30.9	1.67–1.80[b]	31.9	1.50–1.64[b]
5	85.8	4.07, 1H, m	84.8	4.18, 1H, m	84.8	3.62–3.65[b]
6	212.4		214.0		74.9	3.62–3.65[b]
7	37.8	3.44, 1H, dd, 6.4, 17.7 3.56, 1H, dd, 6.7, 17.7	37.1	3.40–3.52, 2H, m	32.7	2.19–2.26, 2H, m
8	122.5	5.54[b]	120.7	5.69, 1H, dt, 7.5,10.6	128.5	5.42, 1H, m
9	132.4	5.54[b]	133.0	5.46–5.52[b]	133.8	5.47, 1H, dt, 7.3, 10.6
10	26.8	2.80, 2H, m	25.7	2.78, 2H, t, 7.5	28.1	2.73, 2H, m
11	127.8	5.30, 1H, m	126.8	5.37, 1H, dt, 7.5, 10.6	130.2	5.30, 1H, ddt, 1.5, 7.2, 10.4
12	133.7	5.40, 1H, m	133.3	5.46–5.52[b]	135.7	5.37, 1H, ddt, 1.5, 7.2, 10.4
13	21.6	2.09, 2H, m	20.4	2.08, 2H, m	22.9	1.97, 2H, m
14	14.6	0.98, 3H, t, 7.6	13.9	0.96, 3H, t, 7.6	16.4	0.84, 3H, t, 7.5
1'	104.9	4.33, 1H, d, 7.9	103.2	4.50, 1H, d, 7.9	104.4	4.40, 1H, d, 7.9
2'	75.2	3.28, 1H, m	73.5	3.42, 1H, dd, 7.9, 9.0	76.1	3.19, 1H, dd, 7.9, 9.2
3'	77.9	3.36, 1H, t, 8.9	76.0	3.51, 1H, t, 9.0	78.6	3.38, 1H, t, 9.2
4'	71.7	3.31, 1H, m	70.0	3.46, 1H, t, 9.0	72.4	3.28, 1H, t, 9.2
5'	78.1	3.25, 1H, ddd, 2.4, 5.5, 9.2	73.7	3.62, 1H, ddd, 2.0, 5.8, 9.0	78.6	3.32, 1H, ddd, 2.0, 5.2, 9.2
6'	62.8	3.62, 1H, dd, 5.5, 11.7 3.79, 1H, dd, 2.4, 11.7	64.2	4.25, 1H, dd, 5.8, 12.1 4.42, 1H, dd, 2.0, 12.1	63.5	3.60, 1H, dd, 5.2, 12.2 3.78, 1H, dd, 2.0, 12.2
1"			170.2			
2"			43.4	3.40, 2H, s		
3"			172.4			

[a] CD_3OD (**2**) and D_2O (**3**, **4**).

[b] Overlapping signals.