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Identification of reaction products of 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid (KODA) and norepinephrine that strongly induce flowering in *Lemna*

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Abstract The reaction of 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid (KODA) and catecholamine in water (pH 8.0) in vitro generates many products, and we have shown that the major adduct FN1 among the reactants of KODA and norepinephrine is a potent inducer of floral differentiation in *Lemna* (*L.*) *paucicostat*a 151 [Yokoyama et al. (2000) Plant Cell Physiol 41: 110–113, Yamaguchi et al. (2001) ibid 42: 1201–1209]. Since such reactants may be involved in flower induction in vivo, it is of interest to elucidate the structure-activity relationships among the reactants to throw light on the nature of the putative active site. Here, we report two other adducts, designated as FN7 and FN10. They are structurally similar to FN1, having a tricyclic structure with 12-*cis*-olefin conjugated with noradrenochrome. FN10 was a more potent inducer than FN1. These results are consistent with the hypothesis that tricyclic KODA/norepinephrine adducts play an important role in flower-induction in *L. paucicostata*, strain 151.

Key words: Catecholamine, flower induction, FN, KODA, *Lemna paucicostata*.

Oxylipins are acyclic or cyclic oxidation products that are formed during the catabolism of fatty acids. Among them, jasmonic acid (JA) has been reported to be involved in plant flowering. It has been shown that mRNA of allene oxide cyclase, which catalyses the conversion of allene oxide to cis(+)-12-oxophytodienoic acid (OPDA), a crucial step in the biosynthesis of JA, accumulates in flower buds of *Licopersicon esculentum* (tomato) (Hause et al. 2000). Constitutive expression of allene oxide cyclase in tomato caused a dramatic elevation of the levels of JA and related oxylipins in flower organs, but not in leaves (Miersch et al. 2004). JA enhanced flower induction in *Lemna minor* under long-day conditions, but prevented flower induction at higher concentration under the same conditions (Krajncic et al. 2006).

Ives and Posner (1982) showed that in ammonium-free medium, propranolol, a β-adrenergic blocking agent, inhibited flowering of *Lemna paucicostata* 6746, and this inhibition was completely overcome by epinephrine, l-epinephrine, l-norepinephrine, and l-isoproterenol, which substantially promoted flowering under short-day conditions (Khurana et al. 1987).

The aqueous homogenate of *L. paucicostata*, strain 441, induces flowering of *L. paucicostata*, strain 151 (P151) (Takimoto et al. 1989; Takimoto and Kaihara. 1990). Two factors are involved in this flower-inducing activity, one being (−)-norepinephrine (NE) (detected in the supernatant after centrifugation of the plant homogenate), and the other being the α-ketol type oxylipin, 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid (KODA), which is induced by stresses, such as drought, heat and osmotic stress (Takimoto et al, 1994; Yokoyama et al, 2000). Neither alone was active, but their mixture was active after incubation (Yokoyama et al. 2000). We previously isolated two major reaction products of KODA and NE, and determined their absolute chemical structures as (9*R*)-11-{(2′*R*,8′*R*,10′*S*,11′*S*)-2′,8′-dihydroxy-7′-oxo-11′-[(*Z*)-2-pentenyl]-9′-oxa-4′-azatricyclo[6.3.1.0^{1,5}]dodec-5′-en-10′-yl}-9-hydroxy-10-oxoundecanoic acid (FN1) and its C-9 epimer (FN2). FN1 showed a strong flower-inducing activity per se

Abbreviations: JA, jasmonic acid; KODA, 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid; NE, (−)-norepinephrine

This article can be found at http://www.jspcmb.jp/

(Yamaguchi et al. 2001). However, other active components were also generated in smaller amounts in the incubation mixture of KODA and NE (Yamaguchi et al. 2001). Here we report the structures of two new components of the mixture.

Materials and methods

Culture of Lemna paucicostata

Flower-inducing activity was measured as described previously (Takimoto et al. 1994), using *Lemna paucicostata* (P151). The plants were subcultured aseptically on half-strength Hutner's medium (Hutner 1953) containing 1% sucrose, under continuous light provided by cool-white fluorescent tubes (20 W m^{-2}) at 25°C. For the assay, the plants were cultured initially in the assay medium (one-tenth strength E medium containing 1 μM benzyladenine, but no sucrose), and then in the same medium, but in the presence of a reactant of KODA and NE, or purified FN7 or FN10.

Analyses of FN7 and FN10

KODA was prepared using lipoxygenase from rice germ and allene oxide synthase from flax seed (Yokoyama et al. 2000). In order to obtain the reactants, KODA (6.45 mmol) in distilled water (3.3 L) was added to NE (6.45 mmol in 645 mL of water) in 1 M Tris-HCl buffer (pH 8.0, 320 mL) and the solution was incubated for 160 hr at 25°C. The reaction mixture was analyzed by means of HPLC with a UV (210 nm) detector and a CAPCELLPAK C_{18} column (250 mm×4.6 mm I.D., Shiseido, Tokyo, Japan). Elution was done with 50% acetonitrile [0.1% trifluoroacetic acid (TFA)] at 1 ml/min. The following instruments were used to obtain physical data: specific rotations, SEPA-300 digital polarimeter (L=5 cm, Horiba, Kyoto, Japan); UV spectra, UV-2200 spectrometer (Shimadzu, Kyoto, Japan); IR spectra, FT/IR-5300 spectrometer (Jasco, Tokyo, Japan); fast atom bombardment mass spectrometry (FAB-MS) and high-resolution FAB-MS, MAT95Q mass spectrometer (Finnigan MAT, MA, U.S.A.); ^{1}H-nuclear magnetic resonance (NMR) spectra, ECP-400 (400 MHz) spectrometer (JEOL, Tokyo, Japan); ^{13}C-NMR spectra, ECP-400 (100 MHz) spectrometer (JEOL); HPLC, LC100 system (Yokogawa, Tokyo, Japan) or NANOSPACE SI-1 chromatograph (Shiseido).

Physico-chemical properties

FN7-1:

FAB-MS: *m/z* 494 $[M+H]^+$ (positive), *m/z* 492 $[M-H]^-$ (negative), HR-FAB-MS *m/z* 494.2751 (found), (−0.3 mmu) 494.2754 (calc. for $C_{26}H_{40}O_8N$) $[M+H]^+$; (positive). UV λmax (MeOH, nm) 295 nm.

^{1}H-NMR (400 MHz, DMSO-*d*6): δ 0.99 (3H, t, *J*=7.3, 5″-H_3), 1.25–1.46 (8H, 4,5,6,7-H_2), 1.25, 1.46 (both 1H, m, 3-H_2),1.40, 1.89 (both 1H, m, 8-H_2), 1.50 (1H, m, 11′-H), 1.94, 1.98 (both 1H, m, 12′-H_2), 2.09 (2H, m, 4″-H_2), 2.12 (2H, t-like, *J*=7.3, 2-H_2), 2.13, 2.50 (both 1H, m, 1″-H_2), 3.00 (1H, m, 9′-H), 3.18 (1H, m), 3.63 (1H, dd, *J*=3.4, 12.0, 3′-H_2), 4.08 (1H, dd, *J*=4.3, 7.8, 9-H), 4.12 (1H, d, *J*=3.4, 2′-H), 4.23 (1H, m, 10′-H), 4.98 (1H, s, 6′-H), 5.27 (1H, m, 2″-H), 5.30 (1H, m, 3″-H), 8.11 (1H, s, NH).

^{13}C-NMR (100 MHz, DMSO-*d*6) δc: 13.9 (C-5″), 20.2 (C-4″), 22.6 (C-1″), 24.5 (C-3), 25.0 (C-7), 28.5, 28.7, 28.8 (C-4,5,6), 31.7 (C-8), 33.7 (C-2), 35.5 (C-12′), 42.0 (C-11′), 53.6 (C-3′), 55.3 (C-9′), 55.4 (C-1′), 67.3 (C-10′), 71.0 (C-2′), 74.4 (C-8′), 77.9 (C-9), 91.9 (C-6′), 130.2 (C-3″), 131.0 (C-2″), 173.1 (C-5′), 174.5 (C-1), 192.0 (C-7′), 211.4 (C-10).

FN7-2:

FAB-MS: *m/z* 494 $[M+H]^+$ (positive), *m/z* 492 $[M-H]^-$ (negative), HR-FAB-MS *m/z* 494.2746 (found), (−0.8 mmu) 494.2754 (calc. for $C_{26}H_{40}O_8N$) $[M+H]^+$; (positive). UV λmax (MeOH, nm) 295 nm.

^{1}H-NMR (400 MHz, DMSO-*d*6): δ 0.95 (3H, t, *J*=7.3, 5″-H_3), 1.22-1.48 (8H, 4,5,6,7-H_2), 1.30, 1.50 (both 1H, m, 3-H_2), 1.48, 1.88 (both 1H, m, 8-H_2), 1.60 (1H, m, 11′-H), 1.94, 1.99 (both 1H, m, 12′-H_2), 2.09 (2H, m, 4″-H_2), 2.18 (2H, t-like, *J*=7.3, 2-H_2), 2.10, 2.50 (both 1H, m, 1″-H_2), 3.27 (1H, m, 9′-H), 3.34 (1H, m), 3.62 (1H, dd, *J*=3.4, 12.0) (3′-H_2), 3.79(1H, dd, *J*=4.3, 7.8, 9-H), 4.13 (1H, d, *J*=3.4, 2′-H), 4.19 (1H, m, 10′-H), 4.99 (1H, s, 6′-H), 5.28 (1H, m, 2″-H), 5.30 (1H, m, 3″-H), 8.08 (1H, s, NH).

^{13}C-NMR (100 MHz, DMSO-*d*6) δc: 13.9 (C-5″), 20.2 (C-4″), 22.5 (C-1″), 24.5 (C-3), 24.8 (C-7), 28.5(C-5), 28.7 (C-6, 7), 31.7 (C-8), 33.6 (C-2), 34.6 (C-12′), 42.2 (C-11′), 53.4 (C-3′), 54.2 (C-9′), 55.1 (C-1′), 67.9 (C-10′), 71.0 (C-2′), 74.5 (C-8′), 77.4 (C-9), 91.5 (C-6′), 130.0 (C-3″), 131.0 (C-2″), 171.1 (C-5′), 174.4 (C-1), 191.7 (C-7′), 212.4 (C-10).

FN10-1:

FAB-MS: *m/z* 494 $[M+H]^+$ (positive), *m/z* 492 $[M-H]^-$ (negative), HR-FAB-MS *m/z* 494.2738 (found), (−1.6 mmu) 494.2754 (calc. for $C_{26}H_{40}O_8N$) $[M+H]^+$; (positive). (FN10-1, 2 mix). UV λmax (MeOH, nm) 293 nm.

^{1}H-NMR (400 MHz, DMSO-*d*6): δ 0.93 (3H, t, *J*=7.3, 5″-H3), 1.20, 1.45 (both 1H, m, 3-H_2), 1.29-1.40 (8H, 4,5,6,7-H_2), 1.35, 1.63 (both 1H, m, 8-H_2), 1.36 (1H, m, 11′-H), 1.79, 1.85 (both 1H, d, *J*=12, 12′-H_2), 2.02, 2.20 (both 1H, m, 1″-H_2), 2.10 (2H, m, 4″-H 2), 2.17 (2H, t-like, *J*=7.3, 2-H_2), 2.42 (2H, m, 11-H_2), 3.25 (1H, d, *J*=12.2), 3.72 (1H, dd, *J*=3.4, 12.2) (3′-H_2), 3.82 (1H, dd, *J*=4.3, 7.8, 9-H), 4.15 (1H, d, *J*=3.4, 2′-H), 4.17 (1H, m, 10′-H), 5.19 (1H, s, 6′-H), 5.20 (1H, m, 2″-H), 5.25 (1H, m, 3″-H), 8.58 (1H, s, NH).

13C-NMR (100 MHz, DMSO-*d*6) δc: 13.7 (C-5″), 20.3 (C-4″), 22.7 (C-1″), 23.5 (C-4), 24.4 (C-3), 28.4, 28.5, 28.6 (C-5,6,7), 31.9 (C-12′), 33.6 (C-8), 36.5 (C-2), 38.4 (C-11′), 40.1 (C-11), 53.7 (C-3′), 56.2 (C-1′), 69.0 (C-10′), 71.4 (C-2′), 73.1 (C-9), 92.3 (C-6′), 93.1 (C-8′), 129.8 (C-2″), 130.8 (C-3″), 174.4 (C-1), 174.4 (C-5′), 185.6 (C-7′), 212.9 (C-10).

FN10-2:

^{1}H-NMR (400 MHz, DMSO-*d*6): δ 0.91 (3H, t, *J*=7.3, 5″-H3), 1.20, 1.45 (both 1H, m, 3-H_2), 1.29-1.40 (8H, 4,5,6,7-H_2), 1.35, 1.63 (both 1H, m, 8-H_2), 1.36 (1H, m, 11′-H), 1.79, 1.85 (both 1H, d, *J*=12, 12′-H_2), 2.02, 2.20 (both 1H, m, 1″-H_2), 2.10 (2H, m, 4″-H 2), 2.18 (2H, t-like, *J*=7.3, 2-H_2), 2.42 (2H, m, 11-H_2), 3.25 (1H, d, *J*=12.2), 3.72 (1H, dd, *J*=3.4, 12.2) (3′-H_2), 3.82 (1H, dd, *J*=4.3, 7.8, 9-H), 4.15 (1H, d, *J*=3.4, 2′-H), 4.17 (1H, m, 10′-H), 5.19 (1H, s, 6′-H), 5.20 (1H, m, 2″-H), 5.25 (1H, m, 3″-H), 8.58 (1H, s, NH).

^{13}C-NMR (100 MHz, DMSO-*d*6) δc: 13.7 (C-5″), 20.4 (C-

4″), 22.7 (C-1″), 23.4 (C-4), 24.4 (C-3), 28.4, 28.5, 28.6 (C-5,6,7), 31.7 (C-12′), 33.6 (C-8), 36.0 (C-2), 38.4 (C-11′), 39.9 (C-11), 53.7 (C-3′), 56.2 (C-1′), 69.1 (C-10′), 71.4 (C-2′), 72.7 (C-9), 92.3 (C-6′), 92.9 (C-8′), 129.7 (C-2″), 131.1 (C-3″), 173.6 (C-1), 174.4 (C-5′), 185.5 (C-7′), 214.0 (C-10).

Results

Isolation of FN7 and FN10

The HPLC profile of the reaction products of KODA and NE is shown in Figure 1. Fractions were collected at 5-minute intervals and assayed for flower-inducing activity; every fraction showed distinct activity (Yamaguchi et al. 2001). We planned to isolate the components in peaks FN6, FN7, and FN10, in addition to FN1, because they seemed to show the highest activities. The whole reaction mixture was lyophilized and divided into 5 fractions by chromatography with on a reverse-phase silica gel column (Chromatorex DM1020T, Fuji Silysia Chemical, Aichi, Japan) (Fr. 1: 100% H_2O, Fr. 2: 20% MeOH, Fr. 3: 40% MeOH, Fr. 4: 60% MeOH, Fr. 5: 100 % MeOH). Fr. 3 (2 g) was further subjected to normal-phase chromatography (Silica gel 60, Kanto Chemical, Tokyo, Japan) [$CHCl_3$-methanol-H_2O (8:3:1 lower layer)] and then purified by HPLC on an ODS column [CAPCELLPAK C_{18}, 250 mm×10 mm I.D. with 25% CH_3CN (0.1% TFA), Shiseido, Tokyo, Japan] to give FN10 (8.1 mg). The fraction containing FN7 was further separated by HPLC on an ODS column [identical with the one described above, except for the use of 30% CH_3CN (0.1% TFA)], affording two epimers, FN7-1 (5.4 mg) and FN7-2 (1.6 mg). FN7-1 showed molecular-related ion peaks at *m/z* 506 $(M-H)^-$ and 508 $(M+H)^+$ in negative and positive FAB-MS, respectively, and its molecular formula was established as $C_{26}H_{37}O_9N$ through high-resolution MS analysis of $(M+H)^+$. The UV spectrum was similar to that of FN1, showing an absorption maximum at 295 nm. Comparison of the ^{1}H- and ^{13}C-NMR spectra of FN7-1 with those of FN1 revealed that the C-9′ carbon is that of a methine group (δ3.08, δc55.3) instead of methylene (C-11 of FN1). The correlation between C-8′ and C-9′ suggested that the pyran ring of FN1 has been replaced with a cyclohexane ring. The coupling constant between 9′-H and 10′-H was J=11.0 Hz, and the 10′-H signal was a double doublet. In addition, NOE was observed between 9′-H and 12′-H, 3′-H and 11′-H. Thus, the structure of FN7-1 was presumed to be as shown in Figure 2. In order to identify the epimers of FN7, 9*R*- and 9*S*-KODA, isolated on a Chiralcel OD-R column (Daicel Chemical Industries, Ltd, Tokyo) (Yamaguchi et al. 2001), were separately reacted with NE. Reverse-phase HPLC (30% CH_3CN, 0.1% TFA) comparison of the products with the epimers of FN7 indicated that FN7-1 is the 9R-epimer and FN7-2 is the 9S-epimer. FN10 showed the same molecular weight as FN1 (493), with molecular-related ion peaks at *m/z* 492 $(M-H)^-$ and 494 $(M+H)^+$. The molecular formula was $C_{26}H_{39}NO_8$, based on high-resolution MS analysis. The UV spectrum was similar to that of FN1, with an absorption maximum at 293 nm. The ^{1}H and ^{13}C-NMR spectra indicated that FN10 is a mixture of components with the same molecular formula in a ratio of about 1 : 1. The acetylation product of FN10 showed 2 peaks upon HPLC. These data suggested that FN10 is an enantiomeric mixture. The ^{1}H and ^{13}C-NMR spectra of FN10 were very similar to those of FN1 and FN2, except

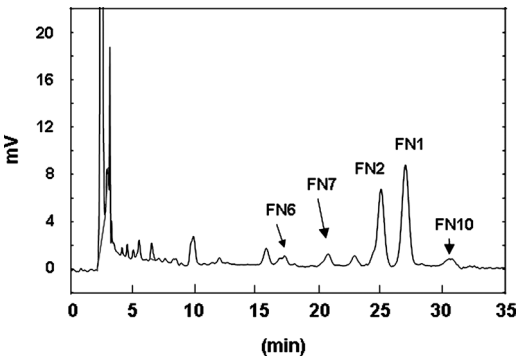

Figure 1. HPLC profile of the products of the reaction between KODA and NE. A mixture of KODA and NE was incubated at pH 8 for 160 h, extracted with chloroform and evaporated to dryness. Reversed-phase HPLC was performed with 25% acetonitrile (containing 0.1% TFA), monitored at 300 nm. Peaks of FN6, 7 and 10 showed high activities, as did that of FN1. The structures and properties of FN1 and 2 have already been reported (Yamaguchi et al. 2001).

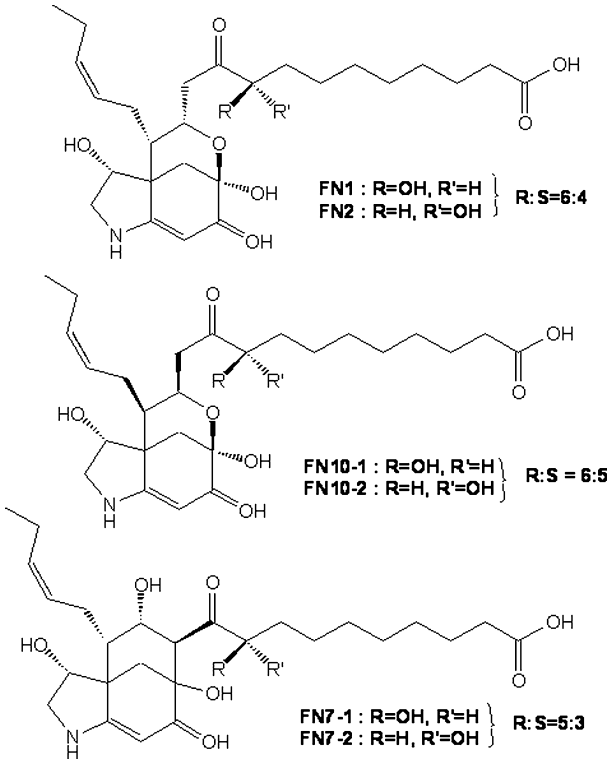

Figure 2. Structures of FN7-1 and 2, FN10-1 and 2, compared with those of FN1 and 2.

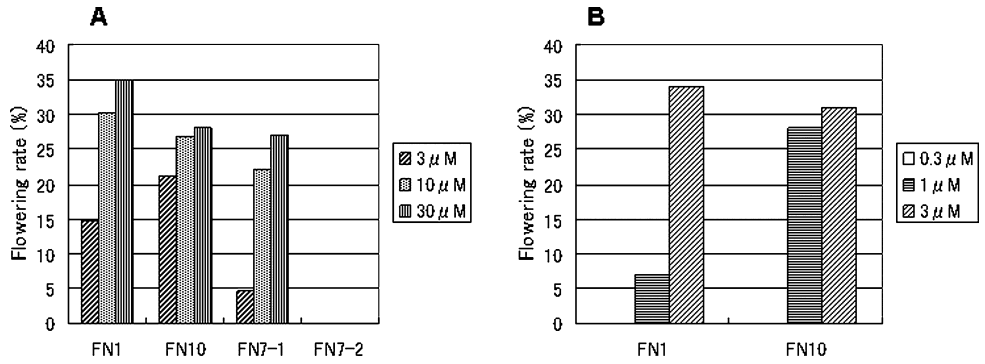

Figure 3. Flower-inducing activity of FN1, FN7-1 and 2, and FN10. FN1, FN7-1 and 2, and FN10 at 3, 10 or 30 μM were added to the assay medium at 1,000-fold dilution (3, 10 or 30 nM), and the flowering response of *Lemna* was examined (A). The flower-inducing activity of FN10 was compared with that of FN1 at 1 and 3 nM in another experiment (B). Data are percentage of flowering. The standard deviation was within 15% in all cases.

for the signals of the ring structure. The NOESY spectrum was also analyzed, and finally, FN10-1 and -2 were concluded to be isomers of FN1 and FN2, with the tricyclic part linked to the C_{18} chain at a different position (Figure 2). We could not isolate FN6 because of its instability: the HPLC profile of the peak changed readily during the purification process, although the FN6-containing fraction showed stronger activity than FN7 or FN10, and FN6 also seemed to be composed of stereoisomers, judging from the HPLC profile.

Biological activities of FN7 and FN10

The flower-inducing activities of FN7-1, FN7-2 and FN10 (as an epimeric mixture) were examined. FN7-1 and FN10 were active, while FN7-2 (9S-epimer) showed no activity, like FN2 (9S-epimer) (Figure 3). At lower concentration, FN10 had the strongest activity of them all (including FN1). The activities were in the order of FN10>FN1>FN7-1.

Discussion

KODA is an oxylipin that is common in green plants (Vick and Zimmerman. 1987). It is formed from linolenic acid by 9-specific lipoxygenase; this is different from the pathway to jasmonic acid, which involves a 13-specific lipoxygenase (Howe and Schilmiller. 2002). The total structure of KODA, including the α-ketol, carboxylic acid and two cis-olefin moieties, is strictly required for flower-inducing activity in *Lemna* (Yokoyama et al. 2000). In this report, we showed that two other reactants of KODA and norepinephrine, which also exhibit flower-inducing activity, retain the essential structure of KODA. The structures of FN7 and FN10 resemble that of FN1, but, in FN7, the oxide of NE, noradrenochrome, does not bond at the C12 double bond of KODA. As KODA is rather unstable, especially under alkaline conditions, our present findings are consistent with the idea that the KODA structure is required for flower-inducing activity and that its degradation results in loss of the activity.

Catecholamine structure is also important for flower-inducing activity in *Lemna* (Yamaguchi et al. 2001). Catecholamines are widespread in the plant kingdom, as well as in mammals (Smith 1977). Catecholamine was confirmed to be involved in flowering in *Lemna* in an experiment using a catecholamine pathway-blocking agent, propranolol (Khurana and Tamot 1987). Catecholamine also has other activities, such as ethylene production, nitrogen fixation, and defense against herbivores and injury (Dai et al. 1993; Kuklin and Conger 1995). Recently, it was established that catecholamine synthesis is activated in potato leaves under stress, through induction of gene expression of synthetic enzymes, such as tyrosine decarboxylase, when the plant is exposed to high salt, drought or UV irradiation (Swiedrych et al. 2004). Catecholamines are proposed to exert their activities through DoH-CB proteins, which retain the cytochrome b561 domain (CB) and the catecholamine-binding regulatory domain of dopamine-β-hydroxylase (DoH) (Verelst and Asard 2004). Cytochrome b561 catalyses ascorbate-mediated trans-membrane electron transport, and hence may be involved in ascorbate regeneration (Wakefield et al. 1986; Asard et al. 1998). These facts imply that catecholamines may be involved in ascorbate regeneration. We have observed that catecholamines, such as epinephrine and norepinephrine, and not only KODA, are released when *Lemna* is exposed to drought stress (unpublished data). Thus, both KODA and catecholamine appear to be stress-induced substances that react to generate compounds that directly induce flowering in *L. paucicostata*. We have tried to detect adducts such as FN1, FN7 and FN10 in *L. paucicostata* by means of LC-MS analysis, but so far without success. Based on the observed activity of the reactants of KODA and NE, the effective concentrations of these adducts in vivo could be at picomolar levels, and this may be the

reason why they were not detected. However, we can not rule out the possibility that compounds other than FN1, FN7 or FN10 are the active agents in *L. paucicostata* in vivo. Other active adducts may be generated by the reactions of other catecholamines, such as dopamine and epinephrine (Yamaguchi et al. 2001). Structure-activity analyses of many adducts reveal the essential structural requirements for flowering-inducing activity in *L. paucicostata* in nature, and thereby provide clues as to the mechanism involved and the development of a flower-regulating agent.

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References

Asard H, Horemans N, Preger V, Trost P (1998) Plasma membrane b-type cytochromes. In: Asard H, Berczi A, Caubergs RJ (eds) *Plasma Membrane Redox Systems and Their Role in Biological Stress and Disease*. Kluwer Academic Publishers, Dordrecht, pp 1–31

Dai YR, Michaels PJ, Flores HE (1993) Stimulation of ethylene production by catecholamines and phenylethylamine in potato cell-suspension cultures. *Plant Growth Regul* 12: 219–222

Hause B, Stenzel I, Miersch O, Maucher H, Kramell R, Ziegler J, Wasternack C (2000) Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. *Plant J* 24: 113–126

Howe GA, Schilmiller AL (2002) Oxylipin metabolism in response to stress. *Curr Opin Plant Biol* 5: 230–236

Hutner SH (1953) Comparative physiology of heterotropic growth in higher plants. In: Loomis WE (ed) *Growth and Differentiation in Plants.* Iowa State College Press, Ames, IA, pp 417–447

Ives JM, Posner HB (1982) Epinephrine, propranolol, and the sucrose-ammonium inhibition of flowering in *Lemna paucicostata* 6746. *Plant Physiol* 70: 311–312

Khurana JP, Tamot BK, Maheshwari N, Maheshwari SC (1987) Role of catecholamines in promotion of flowering in a short-day duckweed *Lemna* paucicstata 6746. *Plant Physiol* 85: 10–12

Krajncic B, Kristl J, Janzekovic I (2006) Possible role of jasmonic acid in the regulation of floral induction, evocation and floral differentiation in *Lemna minor* L. *Plant Physiol Biochem* 44: 752–758

Kuklin AI, Conger BV (1995) Catecholamines in plants. *Plant Growth Regul* 14: 91–97

Miersch O, Weicher H, Stenzel I, Hause B, Maucher H, Feussner I, Wasternack C (2004) Constitutive overexpression of allene oxide cyclase in tomato (*Lycopersicon esculentum* cv. Lukullus) elevates levels of some jasmonates and octadecanoids in flower organs but not in leaves. *Phytochem* 65: 847–856

Smith YA (1977) Phenethylamine and related compounds in plants. *Phytochem* 16: 9–18

Suzuki M, Yamaguchi S, Iida T, Hashimoto I, Teranishi H, Mizoguchi M, Yano F, Todoroki Y, Watanabe N, Yokoyama M (2003) Endogenous α-ketol linolenic acid levels in short day-induced cotyledons are closely related to flower induction in *Pharbitis nil*. *Plant Cell Physiol* 44: 35–43

Swiedrych A, Lorec-Kukula K, Skirycz A, Szopa J (2004) The catecholamine biosynthesis route in potato is affected by stress. *Plant Physiol Biochem* 42: 593–600

Takimoto A, Kaihara S, Hirai N, Koshimizu K, Hosoi Y, Oda Y, Sakakibara N, Nagakura A (1989) Flower-inducing activity of water extract of *Lemna*. *Plant Cell Physiol* 30: 1017–1021

Takimoto A, Kaihara S (1990) Production of the water-extractable flower-inducing substance(s) in *Lemna*. *Plant Cell Physiol* 31: 887–891

Takimoto A, Kaihara S, Yokoyama M (1994) Stress-induced factors involved in flower formation in *Lemna*. *Physiol Plant* 92: 624–628

Verelst W, Asard H (2004) Analysis of an Arabidopsis thaliana protein family, structurally related to cytochromes b561 and potentially involved in catecholamine biochemistry in plants. *J Plant Physiol* 161: 175–181

Vick BA, Zimmerman DC (1987) Oxidative systems for modification of fatty acids: The lipoxygenase pathway. In: Stumpf PK (ed) T*he Biochemistry of Plants, A Comprehensive Treatise*, *Vol. 9*. Academic Press, New York, pp 53–90

Wakefield LM, Cas AEG, Radda GK (1986) Functional coupling between enzymes of the chromatin granule membrane. *J Biol Chem* 261: 9739–9745

Yokoyama M (2005) Roles of 9,10-α-ketol linolenic acid (KODA), one of the oxylipins formed with 9-lipoxygenase in plant growth regulation. *Regul Plant Growth Dev* 40: 90–100

Yokoyama M, Yamaguchi S, Iida T, Suda A, Saeda T, Miwa T, Ujihara K, Nishio J (2005) Transient accumulation of α-ketol linolenic acid (KODA) in immature flower buds of some ornamental plants. *Plant Biotechnol* 22: 201–205

Yokoyama M, Yamaguchi S, Inomata S, Komatsu K, Yoshida S, Iida T, Yokokama Y, Yamaguchi M, Kaihara S, Takimoto A (2000) Stress-induced factor involved in flower formation of *Lemna* is an α-ketol derivative of linolenic acid. *Plant Cell Physiol* 41: 110–113

Yamaguchi S, Yokoyama M, Iida T, Okai M, Tanaka O, Takimoto A (2001) Identification of a component that induces flowering of *Lemna* among the reaction products of α-ketol linolenic acid (FIF) and norepinephrine. *Plant Cell Physiol* 42: 1201–1209