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One-pot synthesis of bioactive cyclopentenones from α-linolenic acid and docosahexaenoic acid

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ABSTRACT

Oxidation products of the poly-unsaturated fatty acids (PUFAs) arachidonic acid, α-linolenic acid and docosahexaenoic acid are bioactive in plants and animals as shown for the cyclopentenones prostaglandin 15d-PGJ_2 and PGA_2, *cis*-(+)-12-oxophytodienoic acid (12-OPDA), and 14-A-4 neuroprostane. In this study an inexpensive and simple enzymatic multi-step one-pot synthesis is presented for 12-OPDA, which is derived from α-linolenic acid, and the analogous docosahexaenoic acid (DHA)-derived cyclopentenone [(4*Z*,7*Z*,10*Z*)-12-[[-(1*S*,5*S*)-4-oxo-5-(2*Z*)-pent-2-en-1yl]-cyclopent-2-en-1yl] dodeca-4,7,10-trienoic acid, OCPD]. The three enzymes utilized in this multi-step cascade were crude soybean lipoxygenase or a recombinant lipoxygenase, allene oxide synthase and allene oxide cyclase from *Arabidopsis thaliana*. The DHA-derived 12-OPDA analog OCPD is predicted to have medicinal potential and signaling properties *in planta*. With OCPD in hand, it is shown that this compound interacts with chloroplast cyclophilin 20-3 and can be metabolized by 12-oxophytodienoic acid reductase (OPR3) which is an enzyme relevant for substrate bioactivity modulation *in planta*.

1. Introduction

2-Cyclopentenones are potent biologically active compounds as shown for α-linolenic acid (LeA; Fig. 1; **1**)-derived 12-OPDA (Fig. 1; **4**), docosahexaenoic acid (DHA; **5**)-derived neuroprostane A4-14 (Fig. 2; **10**) and arachidonic acid (ARA)-derived prostaglandin 15d-PGJ_2 (Fig. 2; **12**).[1–3] The plant signaling molecule 12-OPDA (Fig. 1; **4**) is synthesized in the plant plastid. Following lipase-mediated release of LeA from membrane-bound phospholipids, three consecutive enzymatic steps catalyzed by 13-lipoxygenase (13-LOX), allene oxide synthase (AOC) and allene oxide cyclase (AOS) convert LeA (**1**) to 12-OPDA (Fig. 1; **4**).

12-OPDA is involved in redox regulation and signaling and stress acclimation,[4] e.g. during heat shock, high light and pathogen response.[5] *In planta* further processing of 12-OPDA occurs outside the plastid. The old yellow enzyme homolog 12-oxo-phytodienoic acid reductase isoenzyme 3 (OPR3) reduces the double bond in the cyclopentenone ring in the peroxisome.[6,7] Subsequent shortening of the carbon skeleton by six carbon atoms in three cycles of β-oxidation generates the plant hormone jasmonic acid leading to the enormous spectrum of LeA-derived oxylipins.[8]

For long, 12-OPDA was exclusively considered to act as precursor for jasmonic acid synthesis. However the oxylipin 12-OPDA displays signaling functions in plant defense on its own.[1] Recently, 12-OPDA was established as effector of plant defense. Following its wound- or light-induced synthesis from LeA in the plastid 12-OPDA binds to cyclophilin 20-3 and this complex activates cysteine synthase complex consisting of serine acetyl transferase and O-acetylserine thiol lyase.[4] This causes up-regulation of cysteine and glutathione synthesis which is absent in plants lacking cyclophilin 20-3.[4]

Pharmacological application of 12-OPDA to mammalian cell systems suppresses growth of breast cancer cells,[9] inhibits LPS-

Abbreviations: ARA, arachidonic acid; AOC, allene oxide cyclase; AOS, allene oxide synthase; Cyp20-3, cyclophilin 20-3; DHA, docosahexaenoic acid; EDH, 16,17-epoxy-4,7,10,13,15,19-docosahexaenoic acid; EOT, 12,13-epoxy-9,11,15-octadecatrienoic acid; LeA, linolenic acid; HPDH, 17-hydroperoxy-4,7,13,15,19-docosahexaenoic acid; LOX, lipoxygenase; LOX6, LOX6 of *Arabidopsis thaliana*; OCPD, ((4*Z*,7*Z*,10*Z*)-12-[[-(1*S*,5*S*)-4-oxo-5-(2*Z*)-pent-2-en-1yl] cyclopent-2-en-1yl] dodeca-4,7,10-trienoic acid); 12-OPDA, *cis*-(+)-12-oxo-phytodienoic acid; PUFAs, poly unsaturated fatty acids; RP-HPLC, reverse phase high-performance liquid chromatography; SLOX, soybean lipoxygenase.

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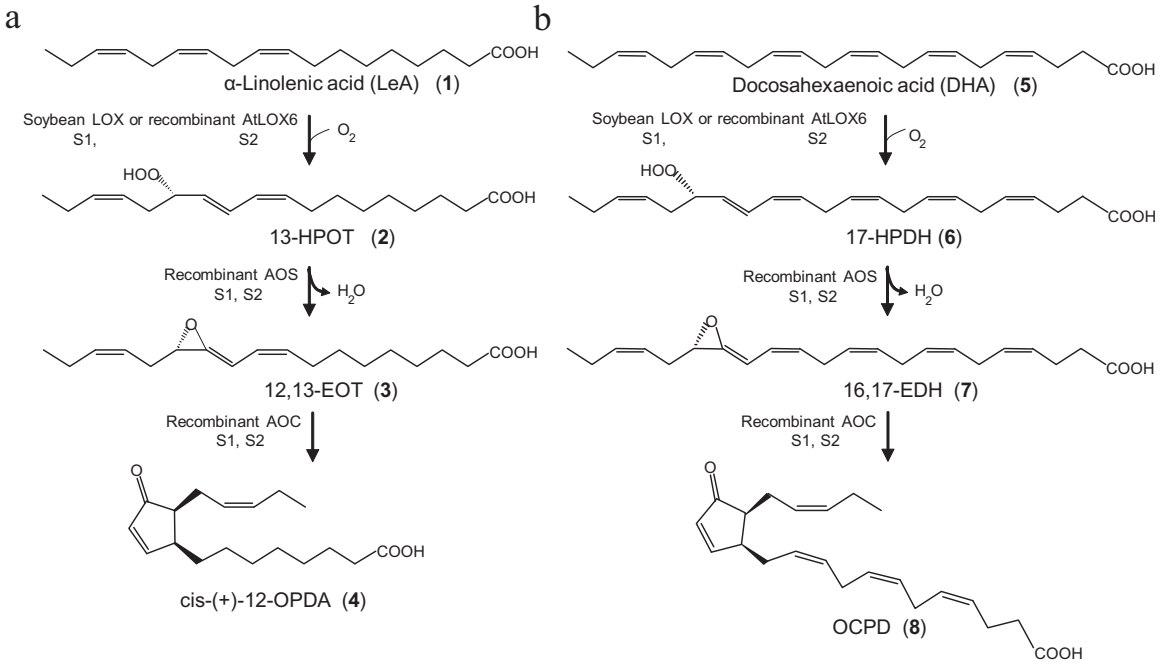

Fig. 1. Pathway for one-pot synthesis of 12-OPDA and OCPD with three enzymes. (a) Biosynthetic pathway of 12-OPDA (**4**) in the plastid starting with linolenic acid (LeA, **1**) which is converted to (13*S*,9*Z*,11*E*,15*Z*)-13-hydroperoxy-9,11,15-octadecatrienoic acid (13-HPOT, **2**) by 13-lipoxygenase (13-LOX) and further to 12,13*S*-epoxy-9*Z*,11,15*Z*-octadecatrienoic acid (12,13-EOT, **3**) by allene oxide synthase (AOS) and finally to OPDA by allene oxide cyclase (AOC). The strategy 1 (S1) for synthesis of **4** from **1** used commercial crude soybean LOX preparation and lysates of AOS- and AOC-expressing *E. coli*. Strategy 2 (S2): The novel strategy involved the cloned AtLOX6 of Arabidopsis realizing the complete synthesis with recombinant proteins. (b) Based on the structural similarity, docosahexaenoic acid (DHA, **5**) was introduced into the enzyme cascade with the expectation to produce (4*Z*,7*Z*,10*Z*)-12-[[-(1*S*,5*S*)-4-oxo-5-(2*Z*)-pent-2-en-1yl] cyclopent-2-en-1yl] dodeca 4, 7, 10-trienoic acid (OCPD, **8**) employing both strategies S1 and S2. Synthesis scheme is proposed from literature.[13,20] For successful OCPD formation the LOX-catalyzed hydroperoxidation at ω-6 of **5** leading to (17*S*,4*Z*,7*Z*,13*Z*,15*E*,19*Z*)-17-hydroperoxy-4, 7, 13, 15, 19 docosahexaenoic acid (**5**) is essential. **5** transforms into the epoxide, 16, 17 (S)-epoxy-4*Z*,7*Z*,10*Z*,13*Z*,15,19*Z* docosahexaenoic acid (**6**) through AOS and finally gets converted into (**8**) by AOC.

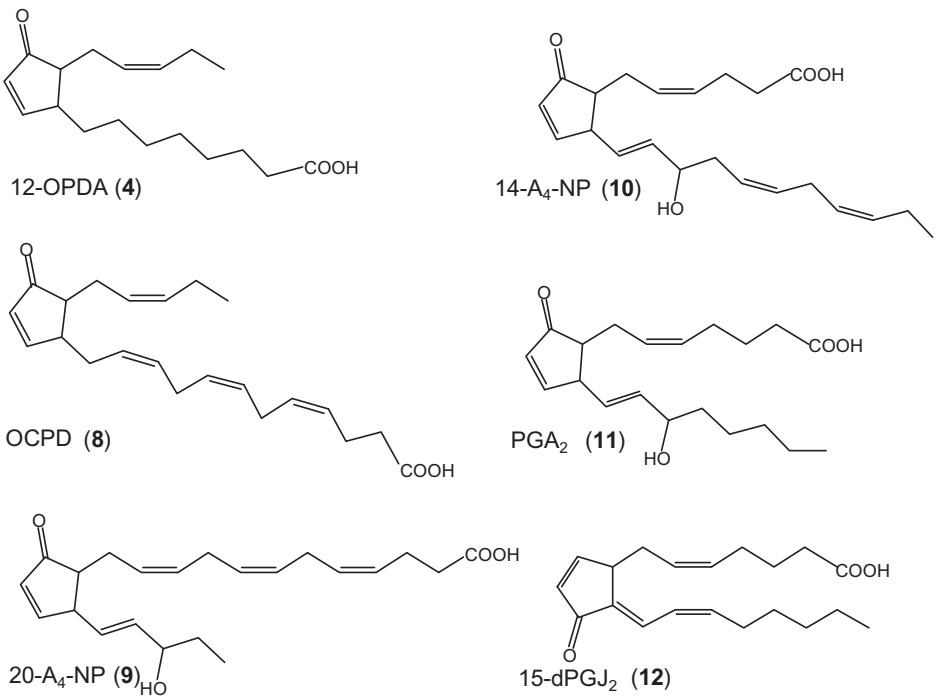

Fig. 2. 12-OPDA (4) and OCPD (8) analogs. For simplicity, stereochemistry is not indicated. 9: neuroprostane 20-A_4-NP; **10**: neuroprostane 14-A4; **11**: prostaglandin A2; **12**: ARA-derived prostaglandin 15d-PGJ_2.

induced cell inflammation in mice cells[10] and suppresses H_2O_2-induced cytotoxicity in human neuroblastoma cells.[11] It is suggested that the activity of the mentioned lipid derivatives is partly due to their α,β-unsaturated carbonyl moiety that reacts with thiol-containing proteins involved in defense mechanisms. As shown for the transcription factor nuclear factor κB (NF-κB), its proinflammatory action is suppressed through covalent adduct formation with 15d-PGJ_2 (**12**) or neuroprostane 14-A4 (14-A4-NP;