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Enzymatic studies with 3-oxa n-3 DPA

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Dedicated to the living memory of Professor Maurizio Botta.

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ABSTRACT

Cyclooxygenase-2 and several lipoxygenases convert polyunsaturated fatty acids into a large variety of products. During inflammatory processes, these enzymes form several distinct families of specialized pro-resolving lipid mediators possessing potent anti-inflammatory and pro-resolving effects. These mediators have attracted a great interest as leads in drug discovery and have recently been the subject of biosynthetic pathway studies using docosahexaenoic and n-3 docosapentaenoic acid as substrates. Herein we present enzymatic studies with cyclooxygenase-2 and 5-, 12- and 15-lipoxygenase enzymes using 3-oxa n-3 DPA as a synthetic mimic of n-3 docosapentaenoic acid. Structural elucidation based on data from RP-HPLC UV and LC/MS-MS experiments enabled the identification of novel enzymatically formed products. These findings constitute the basis for further biosynthetic studies towards understanding the mechanisms regulating substrate utilization in the biosynthesis of specialized pro-resolving lipid mediators.

1. Introduction

Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA, **1**), eicosapentaenoic acid (EPA, **2**), docosahexaenoic acid (DHA, **3**) and n-3 docosapentaenoic acid (n-3 DPA, **4**), all play a major role in the physiology of living organisms [1]. All four PUFAs **1–4** are substrates for the stereoselective biosynthesis of several oxygenated endogenous [2] as well as marine products [3]. Biosynthetic pathway studies have revealed that individual lipoxygenases (LOXs) and cyclooxygenase-2 (COX-2) convert **1–4** into distinct families of the lipoxins, resolvins, protectins and maresins, collectively named specialized pro-resolving mediators (SPMs), see Fig. 1 [4].

SPMs are biosynthesized during the resolution phase of the acute-inflammatory response [5]. The biochemical pathways for some SPMs have been established [2c,2d,6] opening up new research areas related to the many disease states associated with inflammation and drug discovery efforts [7]. The biosynthesis [8,9] of protectin D1 has been established and is presented in Scheme 1.

The biosynthetic formation of and metabolism of $PD1_{n\text{-}3\ DPA}$, a congener of protectin D1 formed from n-3 DPA (**4**), was recently presented [10]. Of interest, an increased number of n-3 DPA derived SPMs have been reported [11,12] and submitted to biological testing [13] over the last five years. These and other SPMs have attracted attention in drug discovery towards developing small molecular resolution agents and immunoresolvents [7,14]. Moreover, the increased understanding that SPMs are actively biosynthesized products acting as resolution agonists is considered a biomedical paradigm shift [15]. However, the secondary metabolism of these potent pro-resolution agents has been less studied [2c,4a,16]. Hence, new knowledge on such metabolic enzymatic processes is central for the development of SPM based pro-resolution agonists as remedies in inflammation driven human diseases. As of today, it has been reported that protectin D1 undergoes enzymatic oxidation at C-22 to produce 22-OH-PD1 that carries potent anti-inflammatory and pro-resolving bioactions [17]. The eicosaoxido reductase C-10 keto product of PD1 has also been reported [2c,4a]. Moreover, SPMs, as all PUFAs, undergo β-oxidation metabolism [4a,18]. The double bond geometry of the specific classes of the SPMs reflects their biochemical origin, but also their stereospecific bioactions as agonists towards different G protein-coupled receptors (GPCRs) [19]. Hence, access to novel SPMs mimetic possesing pro-resolution and anti-inflammatory bioactions resistant to enzymatic oxidative metabolism or catabolic degradation is of great interest to the biomedical research

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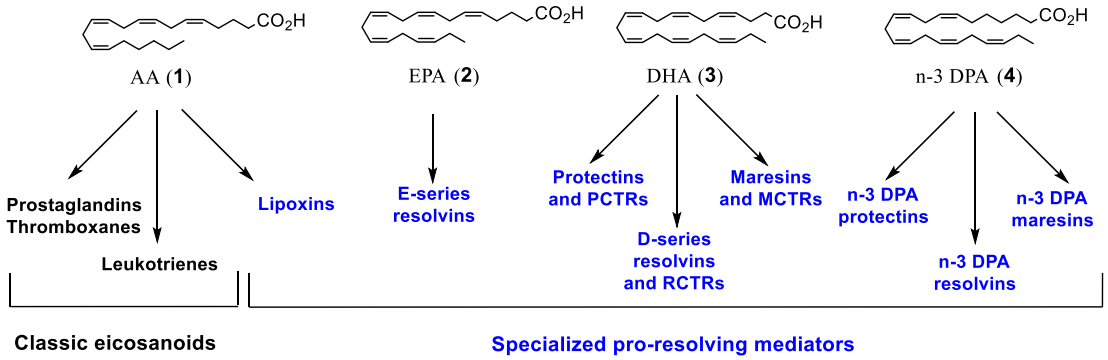
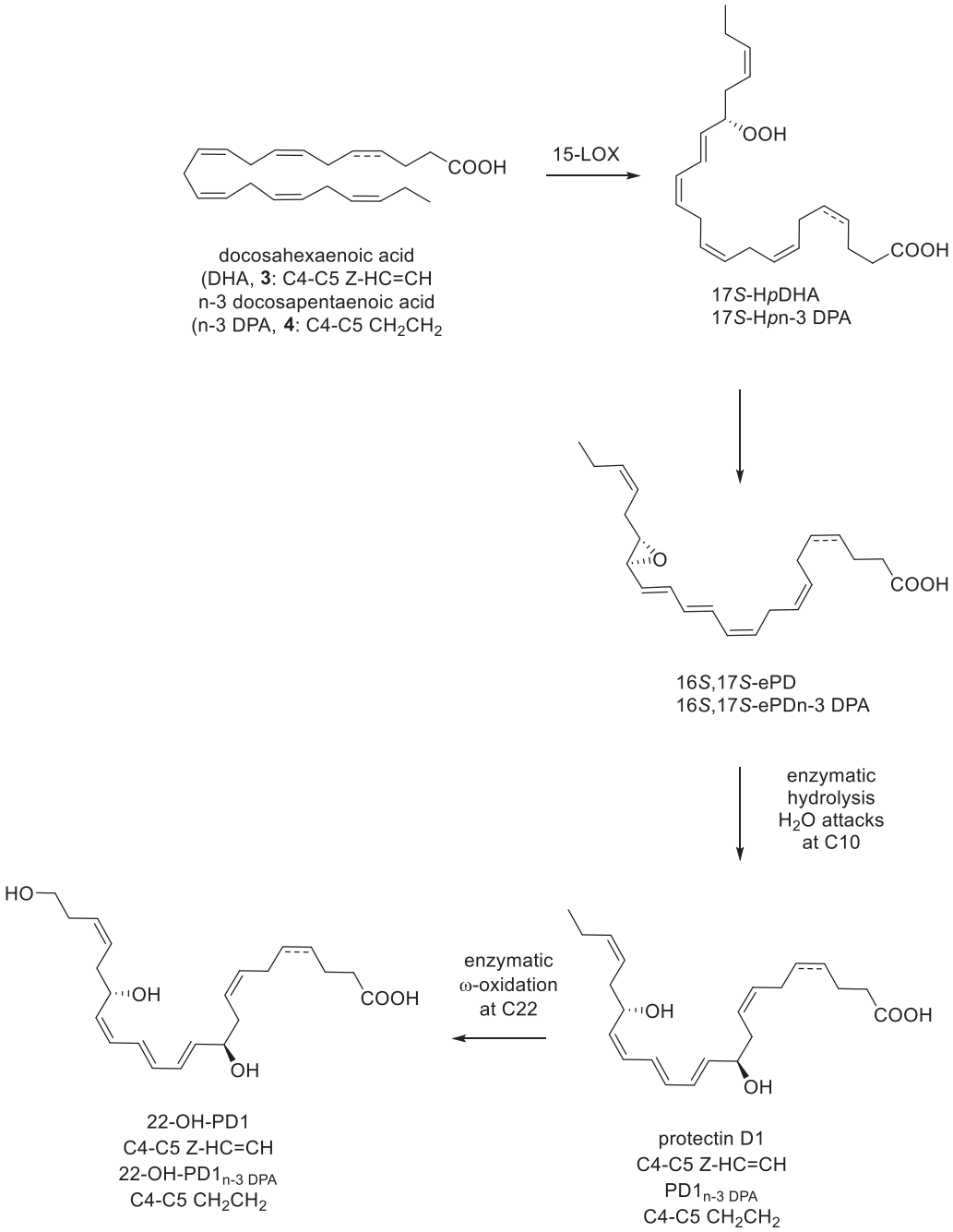


Fig. 1. Chemical structures of PUFAs, outline of families of SPMs and classical eicosanoids.



Scheme 1. Outline of established biosynthetic pathways of protectin D1 and $PD1_{n\text{-}3\ DPA}$.

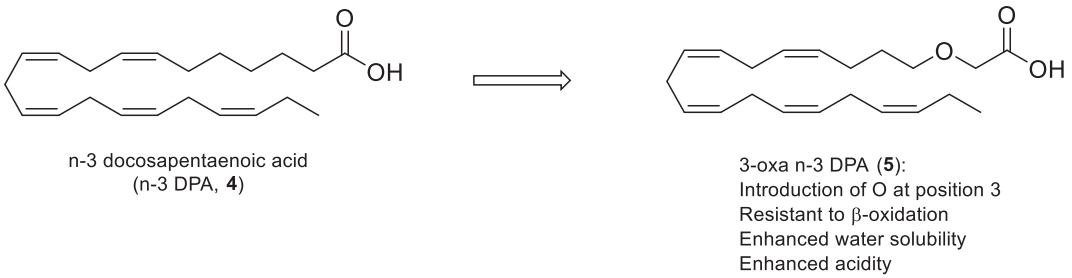


Fig. 2. Structure and design of the n-3 DPA (**4**) mimetic 3-oxa n-3 DPA (**5**).

community [13,14]. Towards such aims we prepared the n-3 DPA (**4**) mimetic **5**, named 3-oxa n-3 DPA, see Fig. 2. Earlier Hamberg and co-workers have shown that 3-oxalinolenic acid is efficiently oxygenated by soybean lipoxygenase-1 into 3-oxa-oxylipins [20]. These studies provided inspiration for the design as well as the synthetic and enzymatic results presented herein.

2. Results and discussion

2.1. Chemistry

2.1.1. Stereoselective synthesis of 3-oxa n-3 DPA

Based on our experience in using commercially available EPA and DHA in stereoselective synthesis [21,22], we prepared 3-oxa n-3 DPA (**5**) (Scheme 2).

Starting from commercially available ethyl ester of DHA, the C18 aldehyde **6** was made as previously reported [21]. Aldehyde **6** was then reduced to the corresponding alcohol **7** ($NaBH_4$, MeOH) and then converted to the C19 nitrile **8** over two steps (MsCl, THF, Et_3N, then NaCN, DMSO). The nitrile functionality in **8** was reduced to the aldehyde **9** (DIBAL-H, THF, −78 °C) that was converted to the C19 alcohol **10** ($NaBH_4$, MeOH). Reacting **10** with *tert*-butyl bromoacetate under phase-transfer conditions afforded ester **11** that was saponified with LiOH in aqueous THF. This gave 3-oxa n-3 DPA (**5**) in 15% yield from **6**. UV and ^{13}C NMR spectral data analyses confirmed that the all-*Z* double bond configuration was present in **5** [23].

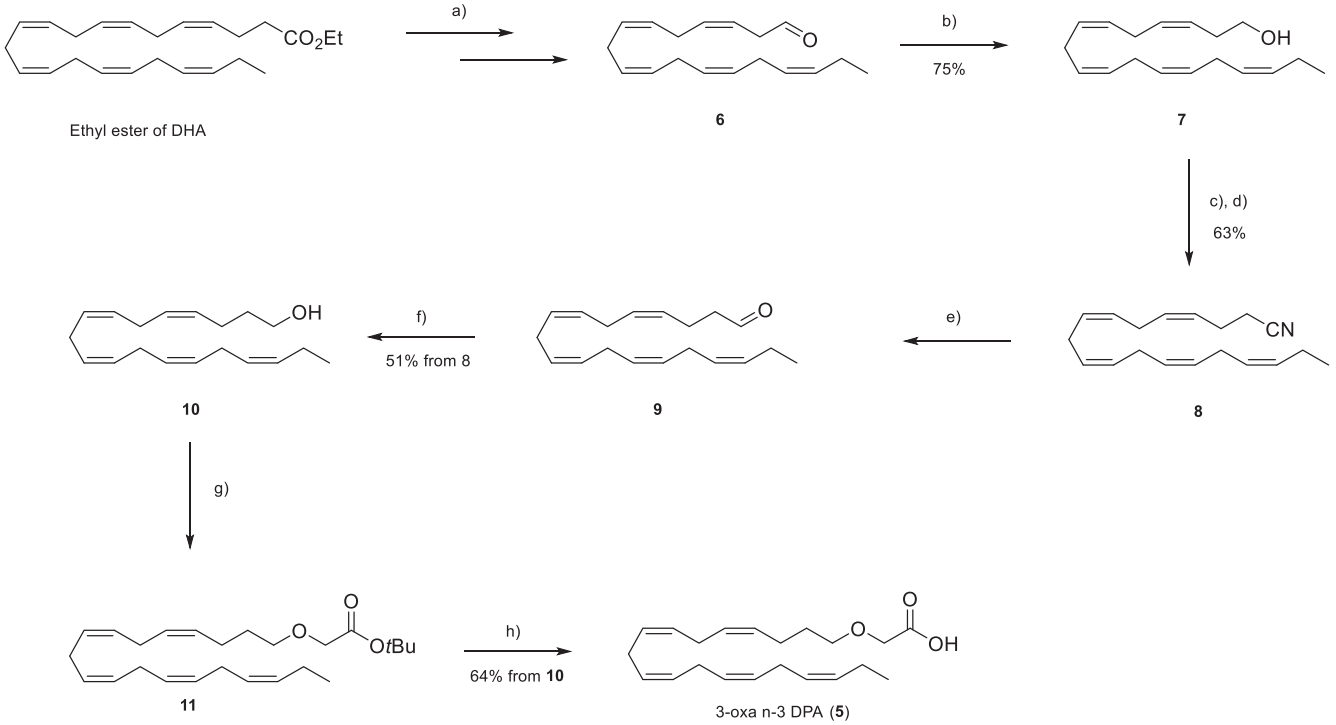
2.2. Biological evaluations

2.2.1. Enzymatic studies and LC/MS-MS identification of products

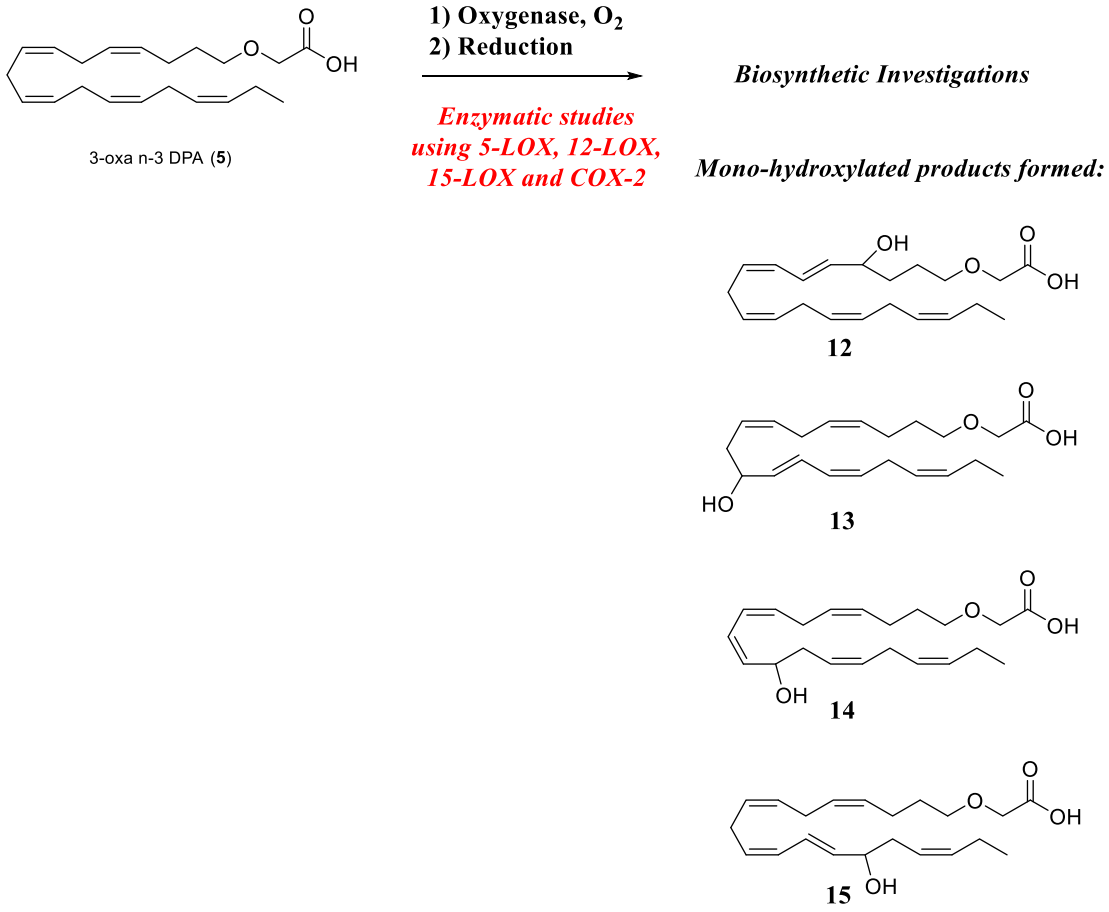
The enzymatic studies with different oxygenase enzymes were then investigated, Scheme 3.

First, synthetic 3-oxa n-3 DPA (**5**) was incubated with human 5-LOX as this oxygenase is central for the biosynthesis of several resolvins and other oxygenated PUFA products [4]. The product profile was analyzed using LC/MS-MS metabololipidomics as earlier reported [24] that allowed the identification of the novel enzymatic product 2-(((5*E*,7*Z*,10*Z*,13*Z*,16*Z*)-4-hydroxynonadeca-5,7,10,13,16-pentaen-1-yl) oxy)acetic acid, named 7-OH-3-oxa n-3 DPA (**12**), see Fig. 3A. The MS-MS spectra for biosynthesized **12** gave the following fragments: *m/z* 347 = M-H, *m/z* 329 = M-H-H_2O, *m/z* 303 = M-H-CO_2 as well as both *m/z* = 201 and *m/z* = 145; the latter at the secondary hydroxyl group at position 7.

These experiments were repeated using human recombinant COX-2. This enzyme is the initiating enzyme in the biosynthesis of the novel 13-series resolvins [6c,12], the 17*R*-protectins [25] and 17*R*-resolvins [26]. Again, a novel monohydroxylated product was isolated and structurally elucidated, this time as 2-(((4*Z*,7*Z*,11*E*,13*Z*,16*Z*)-10-



Scheme 2. Stereoselective synthesis of 3-oxa n-3 DPA (**5**). (a) See reference 21a; (b) $NaBH_4$, MeOH, 0 °C; (c) MsCl, Et_3N, rt; (d) KCN, DMSO, 70 °C; (e) DIBAL-H, hexane, −78 °C; to rt; (f) $NaBH_4$, MeOH, 0 °C; (g) n-Bu_4NHSO_4, *tert*-butyl bromoacetate, NaOH, toluene, 0 °C; (h) THF, MeOH, H_2O, LiOH, 0 °C.



Scheme 3. Enzymatic investigations using 3-oxa n-3 DPA (**5**) as substrate.

hydroxynonadeca-4,7,11,13,16-pentaen-1-yl)oxy)acetic acid, 13-OH-3-oxa n-3 DPA (**13**), Fig. 3B. The M-H *m/z* value of 347 was detected and the same *m/z* fragmentation pattern was observed for **13** as for **12**, i.e. *m/z* = 329, 303, 225, 207 and 197.

Next we used mouse recombinant platelet 12-LOX since it is pivotal for the enzymatic formation of the maresins [6]. LC/MS-MS metabololipidomics experiments provided evidence for the formation of 2-(((4*Z*,7*Z*,9*Z*,13*Z*,16*Z*)-11-hydroxynonadeca-4,7,9,13,16-pentaen-1-yl) oxy)acetic acid, 14-OH-3-oxa n-3 DPA (**14**), Fig. 3C. As for the novel mono-hydroxylated PUFAs **12** and **13**, the *m/z*-fragmentation pattern was in accord with the assigned structure with *m/z* = 347 (M-H), 329 (M-H-H_2O) and 303 (M-H-CO_2). Of note, *m/z* = 209 was also detected.

The 15-LOX enzyme is involved in the biosynthesis of the protectins [9,10] and some resolvins [2c,2d,4a]. Hence, we investigated the formation of mono-hydroxylated products in the presence of soybean 15-LOX. LC/MS-MS metabololipidomics paved the way for the identification of a mono-hydroxylated PUFA product, based on the *m/z* fragmentation pattern. In the MS/MS-spectrum fragments associated with *m/z* = 347 (M-H), 329 (M-H-H_2O) and 303 (M-H-CO_2) were observed, along with peaks for *m/z* = 277 and 249 (Fig. 3D). The UV chromophores (λ_{max} = 237 nm) observed for **12–15** were all characteristic for a *E*,Z-diene conjugated double bond system, see right panels in Fig. 3.

Human lipoxygenases catalyze the incorporation of molecular oxygen at a 1(*Z*),4(*Z*)-pentadienyl moiety in PUFAs resulting in the formation of an *S*-configured hydroperoxy *E*,*Z*-conjugated fatty acid that is reduced to the corresponding alcohol [26]. Based on this, the configuration of the secondary alcohols in **12**, **14** and **15** should all be *S*. On the other hand, COX-2 enables oxygenation of DHA and n-3 docosapentaenoic to produce *R*-configured PUFA-alcohols [4a,12]. Future studies will establish the absolute configuration of the four novel PUFA-products presented herein.

Recently it was reported that protectin D1 undergoes rapid β-oxidation metabolism *in vitro* by human hepatoma cells [27]. Sala and coworkers identified two products formed 30 min after incubation of protectin D1, as the result of one cycle or two cycles of β-oxidation metabolism, respectively. These authors confirmed the presence of minor amounts of the known 22-OH-PD1 metabolite [9], but no further ω-metabolism of 22-OH-PD1 was observed [27]. Interestingly, the tetranor C18 metabolite of protectin D1 was the only metabolite that maintained the bioactivity of the protectin D1 [4], inhibiting neutrophil chemotaxis *in vitro* and neutrophil LTB_4-induced chemotaxis *in vivo*. Overall, these studies support the design, synthesis and enzymatic studies on 3-oxa n-3 DPA (**5**) reported herein, but also provide a rational for protectin D1 analogs resistant to β-oxidation metabolism. Towards such aims, the biosynthesis of 17-OH-3-oxa n-3 DPA (**15**) presented herein, is of particular interest.

3. Conclusions

To summarize, 3-oxa n-3 DPA (**5**) was stereoselectively synthesized in multi-milligram amounts and in chemical purity of > 97%. We have shown that 3-oxa n-3 DPA (**5**) is a substrate for 5-LOX, 12-LOX, 15-LOX and COX-2, yielding mono-hydroxylated products as identified using LC/MS-MS analysis. These enzymes are essential in the biosynthesis of all SPMs reported to date. SPMs [7,13], as well as other oxygenated PUFA-products [28], exhibit potent and interesting pharmacological actions. These lipids are of interest as lead compounds in resolution pharmacology and drug development.

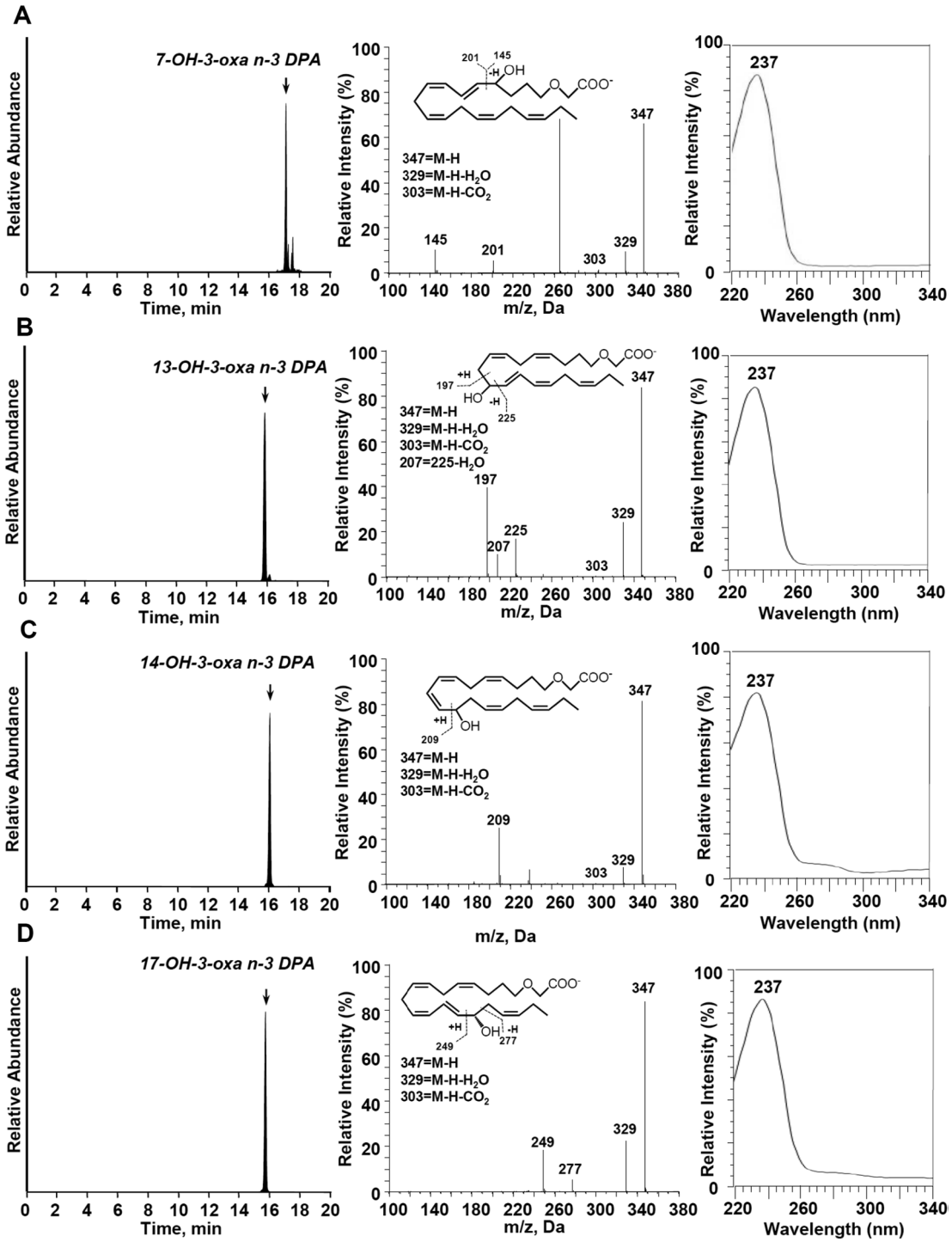


Fig. 3. 3-oxa n-3 DPA is substrate for lipoxygenases and cyclooxygenase-2. Illustrative chromatograms derived from experiments where 3-oxa n-3 DPA (10 μM) was incubated with (A) human 5-LOX; (B) human recombinant COX-2; (C) mouse recombinant platelet 12-LOX; (D) soybean 15-LOX for 15 min at 37 °C. Incubations were quenched with ice-cold methanol and products assessed using LC-MS/MS (for results depicted in left and central panels) or RP-UV-HPLC (for results depicted in right panels). Results are representative of n = 3 distinct incubations.

4. Experimental section

4.1. (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenal (6)

The aldehyde was prepared as earlier described with the spectral data in accord with those earlier reported [21]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.67 (t, J = 1.8 Hz, 1H), 5.68 (m, 1H), 5.60 (m, 1H), 5.45–5.27 (m, 8H), 3.22 (d, J = 7.2 Hz, 2H), 2.85–2.79 (m, 8H), 2.07 (quint, J = 7.4 Hz, 2H), 0.97 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.28 (CO), 133.13 (CH), 132.07 (CH), 128.86 (CH), 128.64 (CH), 128.45 (CH), 127.81 (CH), 127.78 (CH), 127.11 (CH), 126.97 (CH), 118.68 (CH), 42.50 (CH_2), 25.98 (CH_2), 25.65 (CH_2), 25.64 (CH_2), 25.55 (CH_2), 20.56 (CH_2), 14.26 (CH_3).

4.2. (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaen-1-ol (7)

The aldehyde **6** (3.39 g, 13.1 mmol) in MeOH (40 mL) was cooled to 0 °C and then $NaBH_4$ (1.29 g, 34.19 mmol) in MeOH (50 mL) was

portionwise added to the solution of **6**. After 30 min (TLC, hexane:EtOAc 80:20) showed complete conversion of **6**. Then HCl (aqueous, 1.4 M, 51 mL) was dropwise added and the product was extracted with hexane:Et_2O (2:1, 3 × 50 mL), the combined organic phases washed with brine (2 × 50 mL) and dried ($MgSO_4$). The product was purified by column chromatography (SiO_2, hexane:EtOAc 90:10). After removal of solvent the alcohol **7** was obtained as a colorless oil (2.56 g, 75%). The spectral data were in accord with those earlier reported [21]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.57 (m, 1H), 5.47–5.31 (m, 9H), 3.68 (t, J = 6.4 Hz, 2H), 2.90–2.83 (m, 8H), 2.39 (q, J = 6.8 Hz, 2H), 2.10 (quint, J = 7.2 Hz, 2H), 1.46 (s, 1H), 1.00 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.06 (CH), 131.14 (CH), 128.60 (CH), 128.34 (CH), 128.31 (CH), 128.04 (CH), 127.99 (CH), 127.87 (CH), 127.02 (CH), 125.66 (CH), 62.22 (CH_2), 30.83 (CH_2), 25.77 (CH_2), 25.66 (CH_2), 25.64 (CH_2), 25.55 (CH_2), 20.57 (CH_2), 14.28 (CH_3).

4.3. 4Z,7Z,10Z,13Z,16Z)-nonadeca-4,7,10,13,16-pentaenenitrile (8)

Alcohol **7** (2.56 g, 9.80 mmol) was dissolved in dry CH_2Cl_2 followed by the addition of Et_3N (2.75 mL, 19.7 mmol). The solution was cooled to 0 °C. Methanesulfonyl chloride (1.52 mL, 19.7 mmol) was added dropwise and the reaction mixture was left stirring for 10 min at this temperature. The solution was allowed to warm to room temperature and stirred at this temperature for three hours when brine (20 mL) was added dropwise. The resulting mixture was extracted with EtOAc (3 × 30 mL) and the combined organic extracts were washed with a saturated $NaHCO_3$-solution (2 × 20 mL) and brine (2 × 10 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give crude **8** (3.13 g) as a brown oil, which was used directly in the next reaction. The crude mesylate of **7** was dissolved in DMSO (25 mL) and KCN (0.90 g, 13.9 mmol) was added. The mixture was heated to 70 °C for two hours. Water (90 mL) was added and the mixture was extracted with EtOAc (3 × 50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting crude oil was purified by column chromatography (SiO_2; hexane:EtOAc 15:1) to afford nitrile **8** (1.7 g, 6.2 mmol; 63% over two steps) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.54 (m, 1H), 5.45–5.28 (m, 9H), 2.86–2.80 (m, 8H), 2.38 (m, 4H), 2.08 (quint, J = 7.3 Hz, 2H), 0.97 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.08 (CH), 131.48 (CH), 128.74 (CH), 128.64 (CH), 128.45 (CH), 127.85 (CH), 127.80 (CH), 127.42 (CH), 126.99 (CH), 125.51 (CH), 119.26 (C), 25.65 (3 × CH_2), 25.55 (CH_2), 23.30 (CH_2), 20.57 (CH_2), 17.51 (CH_2), 14.27 (CH_3); IR: 3013, 2963, 2932, 2246 cm^{-1}; HRMS: Exact mass calc for $C_{19}H_{27}N$ 269.2144, found: 269.2124.

4.4. (4Z,7Z,10Z,13Z,16Z)-nonadeca-4,7,10,13,16-pentaen-1-ol (10)

Nitrile **8** (0.28 g, 1.0 mmol) was dissolved in hexane (5 mL) and cooled to −78 °C. DIBAL-H (1 M in hexane, 1.4 mL, 1.4 mmol) was added dropwise. One hour later, more DIBAL-H (1 M in hexane, 0.7 mL, 0.7 mmol) was added. After a total reaction time of three hours, the reaction was quenched by addition of 1.4 M HCl to pH 2. The solution was left stirring for another 30 min at −78 °C, then allowed to warm to room temperature. Extraction with hexane (4 × 20 mL), drying (Na_2SO_4) and removal of solvents under reduced pressure, gave the corresponding aldehyde of **9** (0.24 g; 83%) as a colorless oil. The aldehyde proved to be labile and was thus dissolved in MeOH (2.6 mL) and cooled to 0 °C. Then $NaBH_4$ (0.085 g, 2.24 mmol) in MeOH (3.5 mL) was added. After 30 min, the reaction was quenched with HCl (1.4 M, 3.5 mL). The mixture was extracted with hexane: Et_2O (2:1) and the organic layer was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The crude oil was purified by column chromatography (silica gel; hexane:EtOAc 95:5) to afford alcohol **9** (0.14 g, 0.51 mmol; 51% over two steps) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45–5.28 (m, 10*H*), 3.65 (t, J = 6.48 Hz, 2H), 2.86–2.79 (m, 8H), 2.17 (q, J = 7.4 Hz, 2H), 2.07 (quint, J = 7.8 Hz, 2H), 1.64 (quint, J = 7.1 Hz, 2H), 1.35 (s, 1H), 0.97 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.33 (CH), 129.70 (CH), 128.84 (CH), 128.76 (CH), 128.55 (CH), 128.53 (CH), 128.40 (CH), 128.37 (CH), 128.15 (CH), 127.29 (CH), 62.78 (CH_2), 32.76 (CH_2), 25.91 (CH_2), 25.90 (CH_2), 25.87 (CH_2), 25.81 (CH_2), 23.85 (CH_2), 20.82 (CH_2), 14.53 (CH_3); IR: 3338 (broad), 3012, 2963, 2932 cm^{-1}; HRMS: Exact mass calc for $C_{19}H_{30}O$ 274.2297, found: 274.2295.

4.5. 2-(((4Z,7Z,10Z,13Z,16Z)-nonadeca-4,7,10,13,16-pentaen-1-yl)oxy) acetic acid (3-oxa n-3 DPA, 5)

The alcohol **9** (0.21 g, 0.77 mmol) in toluene (10 mL) was cooled to 0 °C. NaOH (50% aqueous solution, 10 mL) was added, followed by addition of n-Bu_4NHSO_4 (0.026 g, 0.077 mmol). The mixture was left stirring for one hour at 0 °C. *tert*-Butyl bromoacetate (0.17 mL, 1.2 mmol) was added dropwise and the reaction mixture was left stirring overnight at room temperature. Hexane:H_2O (1:1, 40 mL) was added and the phases were separated. The aqueous phase was extracted with Et_2O (4 × 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. This gave *tert*-butyl ester **11** (0.34 g, 0.87 mmol) that was used directly in the next reaction. The ester was dissolved in THF:MeOH:H_2O (2:2:1) (43 mL) and cooled to 0 °C. LiOH·H_2O (1.3 g, 30 mmol) was added. All the starting material was consumed after three hours, and the mixture was allowed to heat to room temperature. HCl (37%) was added until pH 2. The solution was diluted with CH_2Cl_2, and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting oil was purified by column chromatography (SiO_2, 100% CH_2Cl_2, followed by CH_2Cl_2:MeOH 97.5:2.5), giving the pure target molecule (0.16 g, 64% yield over two steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43–5-30 (m, 10*H*), 4.10 (s, 2H), 3.57 (t, J = 6.6 Hz, 2H), 2.88–2.78 (m, 8H), 2.15 (q, J = 6.4 Hz, 2H), 2.07 (quintet, J = 7.4 Hz, 2H), 1.71 (quintet, J = 7.2 Hz, 2H), 0.98 (t, J = 7.5 Hz, 3H); ^{13}C NMR (600 MHz, CD_3OD): δ 174.18 (CO), 132.78 (CH), 130.33 (CH), 129.48 (CH), 129.43 (CH), 129.32 (CH), 129.14 (CH), 129.13 (CH), 129.00 (CH), 128.92 (CH), 128.18 (CH), 71.97 (CH_2), 68.81 (CH_2), 30.52 (CH_2), 26.57 (CH_2), 26.55 (CH_2), 26.51 (CH_2), 26.44 (CH_2), 24.67 (CH_2), 21.50 (CH_2), 14.68 (CH_3); IR: 3500–2500 (broad), 3012, 2963, 1729 cm^{-1}; HRMS: Exact mass calc for $C_{21}H_{32}O_3$ 332.2351, found: 332.2369. The spectral data of **5** were in accord with those already published [29].

4.6. Biological evaluations

4.6.1. 3-oxa n-3 DPA conversion to monohydroxylated products

3-oxa n-3 DPA (**5**, 0.3, 1, 10, 30 or 100 μM) was incubated with soybean 15-LOX (Borate buffer, 4 °C, pH 9.2) for 17-OH-oxa n-3 DPA, mouse recombinant 12-LOX (Tris-HCl 0.1 M, pH8, EDTA 5 mM) for 14-OH-oxa n-3 DPA, human 5-LOX (Phosphate buffer 0.1 M, 0.08% Tween-20, pH6.3) for 7-OH-oxa n-3 DPA or human recombinant COX-2 (Tris-HCl 0.1 M, hematin porcine 20 μM, liquid phenol 0.67 mM, pH8) for 13-OH-oxa n-3 DPA. After 45 min the reaction was quenched using 2 volumes ice-cold methanol, products reduced using sodium borohydride, and extracted using C18 SPE [30]. Products were isolated using UV-RP-HPLC (Infinity 1260; Agilent Technologies). Online UV was obtained for each. Here, an Agilent Poroshell 120 EC-C18 column (100 mm × 4.6 mm × 2.7 μm) was kept at 50 °C and products isolated with a mobile phase consisting of methanol-water-acetic acid of 50:50:0.01 (vol/vol/vol) that was ramped to 80:20:0.01 (vol/vol/vol) from 2 min to 11 min, maintained till 14.5 min and then rapidly ramped to 98:2:0.01 (vol/vol/vol) for the next 0.1 min. This was subsequently maintained at 98:2:0.01 (vol/vol/vol) for 5.4 min, and the flow rate was maintained at 0.5 mL/min.

4.7. LC-MS/MS–based lipidomics

Collected fractions were analyzed using a Shimadzu LC-20AD HPLC and a Shimadzu SIL-20AC autoinjector, paired with a QTrap 5500 (ABSciex) using the same column and gradient as above. QTrap 5500 was operated in negative ionization mode using a multiple reaction monitoring (MRM) method coupled with information-dependent acquisition and enhanced product ion scan as previously described [30] using specific MRM transitions (7-OH-oxa n-3 DPA: 347 > 145, 13-OH-oxa n-3 DPA: 347 > 197, 14-OH-oxa n-3 DPA: 347 > 209 and 17-OH-oxa n-3 DPA: 347 > 249)

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103653.

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