rapid communication

On the structure and synthesis of neuroprotectin D1, a novel anti-inflammatory compound of the docosahexaenoic acid family

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Abstract Potato tuber lipoxygenase was shown to convert 17(*S*)-hydro(pero)xydocasahexaenoic acid in 10,17(*S*)-dihydro(pero)xydocosahexa-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-enoic acid [10,17(*S*)-diHDHA] which was formed apparently through a double lipoxygenation mechanism. No traces of 10,17(*S*)-dihydro(pero)xydocosahexa-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-enoic acid were found among the reaction products. It is very likely that a described earlier "neuroprotectin D1" [or "10,17(*S*)docosatriene"], a novel and potent anti-inflammatory compound derived from docosahexaenoic acid, was, in fact, 10,17(*S*)-dihydroxydocosahexa-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-enoic acid formed through a double lipoxygenation mechanism instead of a previously thought epoxidation/isomerization mechanism.—Butovich, I. A. **On the structure and synthesis of neuroprotectin D1, a novel anti-inflammatory compound of the docosahexaenoic acid family. *J. Lipid Res.* 2005.** 46: **2311–2314.**

Supplementary key words 10,17(*S*)-docosatriene • 10,17-dihydroxydocosahexaenoic acid • lipoxygenase • double lipoxygenation • nuclear magnetic resonance • *cis,trans*-geometry

Recently, several papers were published that described a novel class of potent anti-inflammatory lipid compounds formed from docosahexaenoic acid (ω-3 C22:6; DHA) by various cells and isolated enzymes (1–6). One of the derivatives, 10,17(*S*)-dihydroxydocosahexaenoic acid (10, 17-diHDHA), was postulated to be formed by lipoxygenase (LOX) with unidentified specificity (2–4) and/or in a LOX-like reaction through an epoxidation/isomerization of 17(*S*)-hydroperoxydocosahexaenoic acid (5, 6). The compound was termed 10,17(*S*)-docosatriene (and neuroprotectin D1) and was proposed to be 10,17(*S*)-dihydroxydocosahexa-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-enoic acid. Along with the various compounds formed in vivo, the authors of those papers mentioned that the same derivatives of DHA were produced in a "one-pot" reaction catalyzed by soybean and potato tuber LOXs (sLOX and ptLOX, respectively) (5, 7).

Considering that this potent bioregulatory compound is formed in vivo in extremely low quantities (1–7) and that it is not available commercially, it was important to develop and describe an effective method of 10,17(*S*)-diHDHA synthesis.

Recently, my colleagues and I published the results of our study of ptLOX-catalyzed transformations of DHA, 17(*S*)-hydroperoxydocosahexaenoic acid [17(*S*)-HPDHA], and 17(*S*)-hydroxydocosahexaenoic acid [17(*S*)-HDHA] (8). The ptLOX-catalyzed oxidation of DHA unexpectedly led to two novel oxylipins, 10(*S*)-HDHA and 10(*S*), 20-diHDHA, that were not described before, whereas in the reactions with 17(*S*)-HPDHA or 17(*S*)-HDHA, another pair of products, 7,17(*S*)-diH(P)DHA and 10,17(*S*)-diH(P)DHA, were formed, apparently through a double lipoxygenation mechanism. The epoxidation/isomerization mechanism proposed in the earlier papers (1–7) was ruled out, as ptLOX did not need the hydroperoxy group at C17 of the substrate to form 10,17(*S*)-diH(P)DHA, which would have been a prerequisite for epoxidation to occur. Instead, the enzyme swiftly transformed 17(*S*)-HDHA to 10,17(*S*)-diH(P)DHA. In keeping with the double lipoxygenation mechanism, the expected geometry of the conjugated triene fragment in 10,17(*S*)-diH (P)DHA was proposed to be 11*E*,13*Z*,15*E* (8), which obviously differed from the one postulated by Serhan and colleagues (1–5, 7) and Bazan and colleagues (6). At that time, we had no direct information on the geometrical features of 10,17(*S*)-diH(P)DHA formed by ptLOX. No new information on the structure of neuroprotectin D1 or 10,17(*S*)-docosatriene has been published since then, either.

Abbreviations: COSY, correlation spectroscopy; DHA, docosahexaenoic acid (ω-3 C22:6); LOX, lipoxygenase; ptLOX, potato tuber lipoxygenase; sLOX, soybean lipoxygenase; 10,17(*S*)-diHDHA, 10,17(*S*)-dihydroxydocosahexaenoic acid; 17(*S*)-HDHA, 17(*S*)-hydroxydocosahexaenoic acid; 17(*S*)-HPDHA, 17(*S*)-hydroperoxydocosahexaenoic acid.

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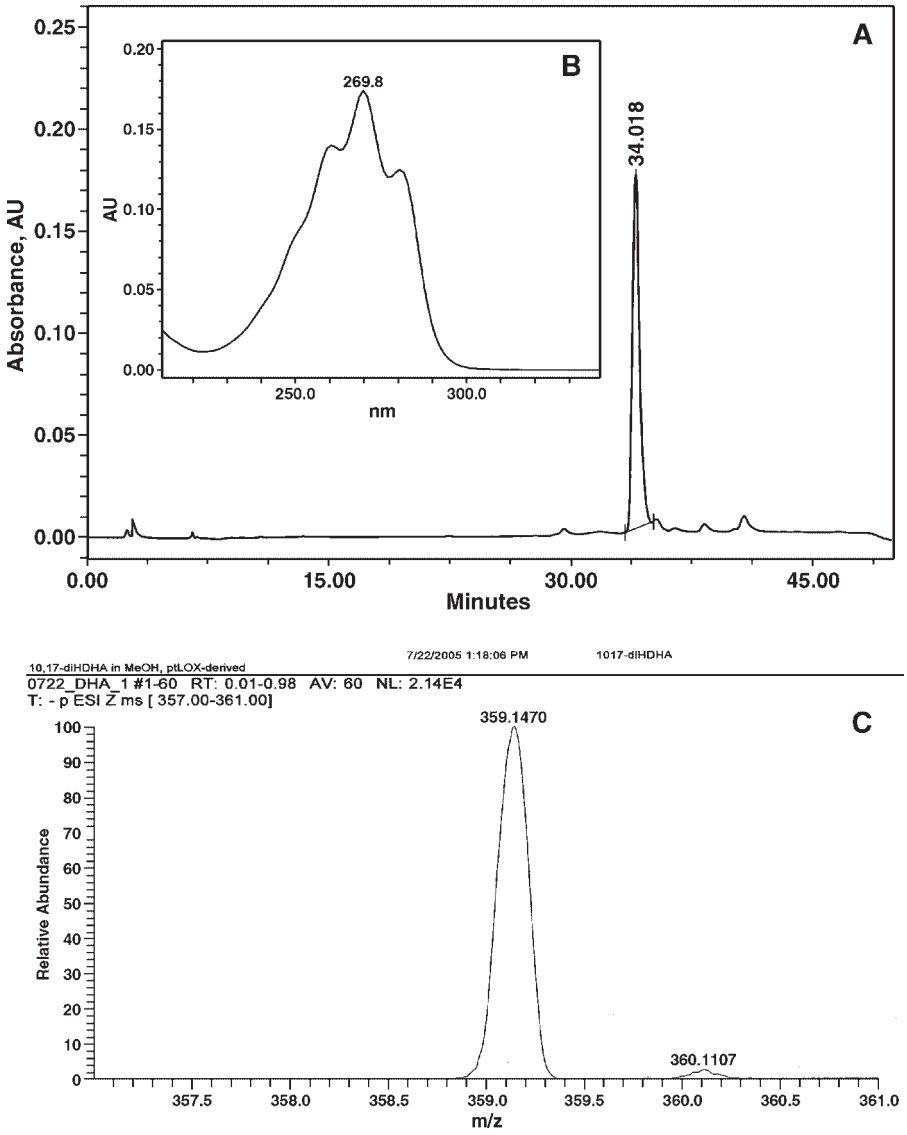


Fig. 1. Normal-phase HPLC purification (A), ultraviolet spectrum (B), and negative ion mode electrospray ionization mass spectrometry analysis (C) of 10,17(*S*)-dihydroxydocosahexa-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-enoic acid [10,17(*S*)-diHDHA]. AU, absorbance units.

To answer the question of whether the geometry of 10,17(*S*)-diHDHA was of 11*E*,13*Z*,15*E*-type or 11*E*,13*E*,15*Z*-type, the product was synthesized exactly as described previously (8). Briefly, an sLOX-generated 17(*S*)-HDHA was treated with ptLOX, and the resulting product was reduced with sodium borohydride to form 10,17(*S*)-diHDHA. The compound was purified by normal-phase HPLC on a 4.6 × 300 mm Waters μBondapak silica gel column in a hexane-i-propanol-acetic acid mobile phase (95:5:0.1, v/v/v; 1 ml/min flow rate) at 30°C on a Waters Alliance 2695 HPLC system with a Waters 2996 diode-array detector operating in scan mode (200–350 nm) (**Fig. 1A**). The purity of the compound was rechecked by analytical normal-phase HPLC on a silica gel column under the same conditions. The product showed an ultraviolet spectrum of a conjugated triene, with characteristic maxima at 260.5, 269.8, and 280.5 nm (Fig. 1B). The molecular weight of the product was determined by electrospray ionization mass spectrometry on an LCQ Deca XP Max MS^n ion trap mass spectrometer (Thermo Electron Corp.). The product was dissolved in methanol and directly infused with a syringe pump at a flow rate of 2 μl/min. A prominent parent peak with *m/z* ratio of 359.15 ($M-H^+$, consistent with the molecular formula $C_{22}H_{32}O_4$; Fig. 1C) and a chloride adduct (*m/z* 395.5, $M+Cl^-$) were observed in the negative ion zoom scan mode, whereas in the positive ion mode, the most prominent peak had an *m/z* value of 383.56 ($M+Na^+$). Mass spectrometric analysis of the parent ion with *m/z* 359.15 produced a daughter ion with *m/z* 341 ($M-H^+-H_2O$), whereas fragmentation of the latter gave an ion with *m/z* 323 ($M-H^+-2H_2O$).

A 400 MHz one-dimensional ^{1}H-NMR spectrum of the compound was taken in CD_3OD at room temperature (**Fig. 2A**). The spectrum was simulated with MestReC software version 4.5.1, which allowed us to determine the corresponding coupling constants (**Table 1**). The simulated spectrum showed a remarkable similarity to the experimental one (Fig. 2B, C). Importantly, the value of $J_{13,14}$ was found to be ~10, which is indicative of *cis*-geometry of the vinyl group, whereas $J_{11,12}$ and $J_{15,16}$ were ~15, clear evidence of the *trans*-nature of the C11-C12 and C15-C16 bonds (**Scheme 1**).

A 400 MHz ^{1}H,^{1}H-COSY experiment was conducted with ptLOX-formed 10,17(*S*)-diHDHA dissolved in CD_3OD

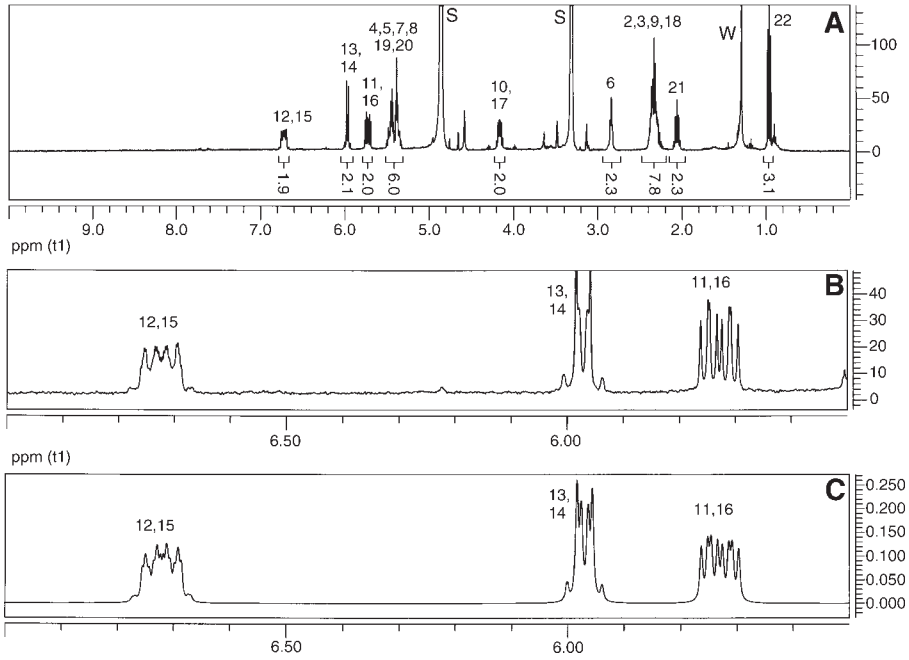


Fig. 2. One-dimensional ^{1}H-NMR spectrum of 10,17(*S*)-diHDHA. A: The full spectrum of the compound taken in CD_3OD (see Table 1 for more information on δ values and coupling constants). Bracketed are integrals of the proton resonances. Hydrogens at C10 and C17 were used as internal standards for integration. S, solvent peak; unlabeled peaks are impurities. B: Expansion of the vinyl region of the full spectrum. C: Partial spectrum of 10,17(*S*)-diHDHA simulated in the MestReC program using the parameters from Table 1.

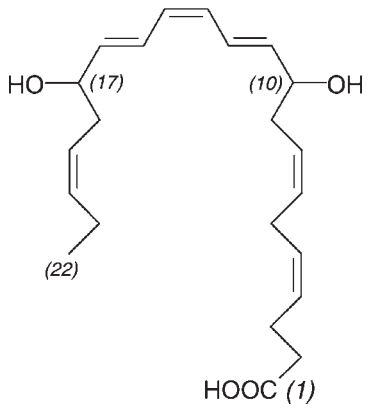
on a Varian 400 MHz spectrometer at room temperature. The resulting complete two-dimensional NMR spectrum and corresponding assignments are presented in **Fig. 3**. In agreement with our one-dimensional ^{1}H-NMR observations, it was apparent that 10,17(*S*)-diHDHA did not have a conjugated *E,E*-diene fragment in it, as it would have produced a resonance with a chemical shift δ of 6.15–6.24 ppm that is seen, for example, in *all-trans* isomers of 9(*S*)- and 13(*S*)-hydroxylinoleyl alcohols (9), 9(*S*)- and 13(*S*)-hydroxymonolinoleoyl glycerols (10), 5,6-dihydroxyeicosapenta-7*E*,9*E*,11*Z*,14*Z*,17*Z*-enoic acid (11), 5-oxo-eicosatetra-7*E*,9*E*,11*Z*,14*Z*-enoic acid (12), 14,15-dihydroxyeicosatetra-5*Z*,8*Z*,10*E*,12*E*-enoic acid (13), and 5,12(*S*)-dihydroxyeicosatetra-6*E*,8*E*,10*E*-enoic acid (14). Generally, a proton system similar to the 11*E*,13*E*,15*Z* conjugated triene would have produced at least six distinctive groups of signals of olefinic protons (13), whereas ptLOX-synthesized 10,17(*S*)-diHDHA gave only four (Figs. 2, 3), which is consistent with the highly symmetrical structure of 10, 17(*S*)-dihydroxydocosahexa-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-enoic acid (Scheme 1).

TABLE 1. One-dimensional ^{1}H-NMR analysis of 10,17(*S*)-dihydroxydocosahexa-4*Z*,7*Z*,11*E*,13*Z*,15*E*,19*Z*-enoic acid

Carbon	δ	Coupling Constants
	ppm	*Hz*
1	NA	
2, 3	2.3–2.4 (m)[a]	
4, 5	5.4 (m)[a]	
6	2.84 (t, 2H)	$J = 5.4$
7, 8	5.4 (m)[a]	
9	2.3–2.4 (m)[a]	$J_{9,10} = 15.5$
10	4.165 (1H)	$J_{10,11} = 7, J_{10,12} = 0.9$
10-OH		$J_{10\text{-OH},10} = 5, J_{10\text{-OH},11} = 4.5, J_{10\text{-OH},12} = 1$
11	5.73 (1H)	$J_{11,12} = 15, J_{11,13} = 1$
12	6.72 (1H)	$J_{12,13} = 11$
13	5.97 (1H)	$J_{13,14} = 10$
14	5.97 (1H)	$J_{14,15} = 11, J_{14,16} = 1$
15	6.72 (1H)	$J_{15,16} = 15$
16	5.73(1H)	$J_{16,17} = 7$
17	4.165 (1H)	$J_{17,18} = 15.5, J_{17,15} = 0.9$
17-OH		$J_{17\text{-OH},17} = 5, J_{17\text{-OH},16} = 4.5, J_{17\text{-OH},15} = 1$
18	2.3–2.4 (m)[a]	
19, 20	5.4, 5.45 (m)[a]	
21	2.06 (p, 2H)	$J = 7.2$
22	0.961 (t, 3H)	$J_{21,22} = 7.5$

Experimental conditions were as follows: frequency, 399.783 MHz; acquisition time, 3.744 s; spectral width, 15.01 ppm; temperature, 29°C; number of scans, 128. NA, not applicable.

[a] Total numbers of Hs with δ 2.3–2.4 and 5.3–5.45 were 7.76 (n = 8) and 5.96 (n = 6), respectively. In total, 29 protons of 32 were quantified. Protons 10-OH, 17-OH, and –COOH could not be reliably quantified because of the H-D exchange with the solvent and peak broadening.



Scheme 1. Structure of 10,17-dihydroxydocosahexaenoic acid.

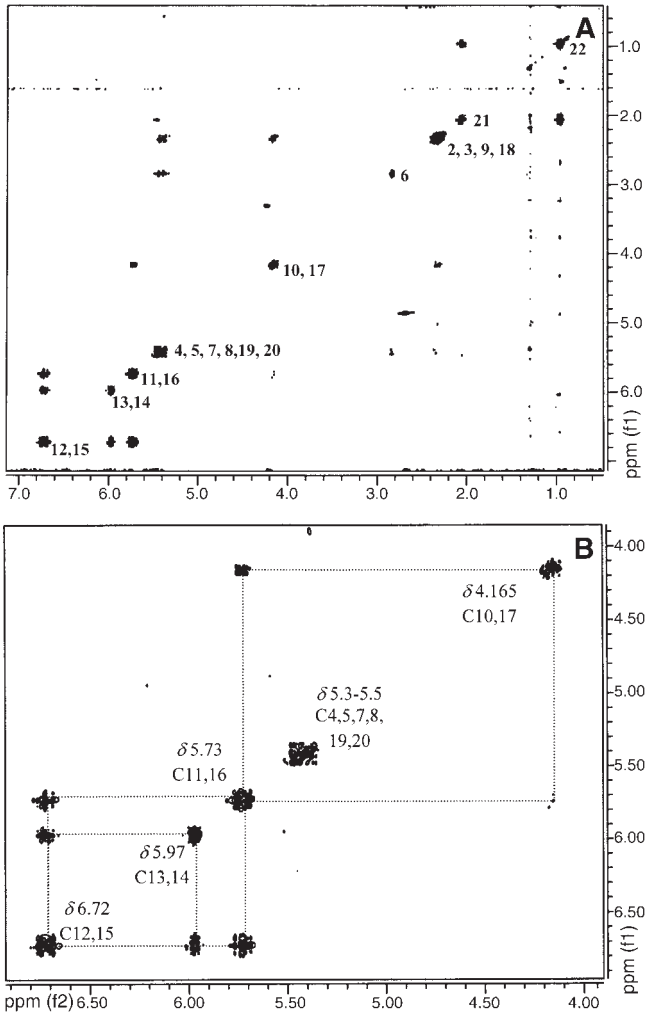


Fig. 3. Two-dimensional $^{1}H,^{1}H$-COSY analysis of 10,17(*S*)-diHDHA. A: Full proton two-dimensional spectrum of 10,17(*S*)-diHDHA in CD_3OD. Solvent peaks (d ~3.31 and 4.86) were suppressed with the help of the high-pass filter in the MestReC software. Numbers 2 through 22 indicate carbons C2 to C22 of 10,17(*S*)-diHDHA. B: An expanded portion of the vinyl region of the spectrum and connectivities of the protons at C10 through C17.

Additional evidence that confirms our hypothesis comes from the fact that C10 and C17 methine protons of the =C**H**—OH group (δ 4.165) of our compound produced cross peaks with *trans*-olefinic protons (δ 5.73, C11 and C16) but not with *cis*-olefinic protons (δ 5.97, C13 and C14). This observation suggests an 11*E*,13*Z*,15*E* arrangement of the product's triene fragment as well, as both the resonances would have been seen in its 11*E*,13*E*,15*Z* or 11*Z*, 13*E*,15*E* derivatives. The last observed resonance (δ 6.72) belongs to protons at C12 and C15.

Interestingly, the same compound was obtained through sLOX-only catalyzed oxidation of *1*) DHA, *2*) 17(*S*)-HPDHA, and *3*) 17(*S*)-HDHA (unpublished data). No traces of the 11*E*,13*E*,15*Z* isomer were found among the products of any of these reactions, either.

Therefore, under the tested conditions potato LOX produced only 11*E*, 13*Z*, 15*E* isomer of 10,17(*S*)-dihydroxydocosahexaenoic acid that was apparently formed through the double lipoxygenation mechanism. These findings need to be considered when making this and similar compounds involves plant LOXs (5, 7, 15) as the compounds made in vitro may differ from the compounds biosynthesized in living cells.

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