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Synthesis of hydroperoxide and perketal derivatives of polyunsaturated fatty acids as potential antimalarial agents

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Abstract

Hydroperoxide derivatives of β-oxa-substituted polyunsaturated fatty acids were prepared by 15-lipoxygenase catalysed oxidation and perketal derivatives of fatty acid hydroperoxides were synthesized. The perketals are more stable than their parent fatty acid hydroperoxides, but less active as antimalarial agents in the *in vitro* growth inhibition of *Plasmodium falciparum*. © 1998 Elsevier Science Ltd. All rights reserved.

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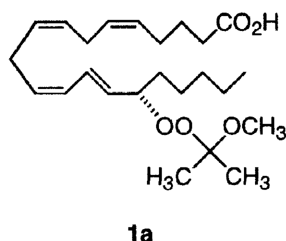
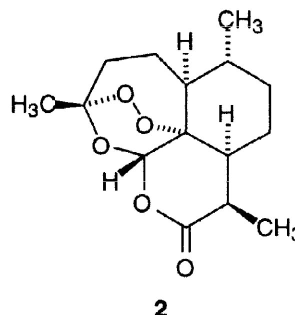
Polyunsaturated fatty acids (PUFAs) display antimalarial activity which has been attributed to their oxidized forms such as hydroperoxides [1,2]. The inherent instability of PUFA hydroperoxides lends complication to their potential use as antimalarial agents, and we therefore sought to prepare perketals, such as **1a**, of PUFA hydroperoxides, anticipating that these stable derivatives might serve as masked hydroperoxides *in vitro* and *in vivo*. In addition, perketal derivatives of PUFA hydroperoxides are of interest as they bear structural resemblance to the active 1,2,4-trioxane functionality of the potent antimalarial, Artemisinin (**2**) [3–6], and as such might be expected to possess antimalarial activity in their own right.



1a



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To provide a range of discrete PUFA hydroperoxides for the synthesis of perketal derivatives, chemoenzymatic oxidation of a range of PUFAs, including the novel non-β-oxidisible β-oxa-substituted PUFAs **3**, **6** and **9b** [7], was investigated. 15-Lipoxygenase catalysed oxidation [8,9] of arachidonic acid (**9a**), in oxygen-saturated pH 9.0 phosphate buffer for 1 h at 0°C regioselectively affords the 15-(*S*)-hydroperoxyeicosatetraenoic acid **10a** (15-HPETE) in a high degree of purity (*Scheme 1*). When arachidonyloxyacetic acid (**9b**) was similarly treated with 15-lipoxygenase a single oxidation product was obtained, namely the 15'-hydroperoxide derivative **10b** (*Scheme 1*). The structure of the 15'-hydroperoxyeicosatetraenyloxyacetic acid **10b**, thus obtained, was determined by *in situ* reduction with sodium borohydride [9,10] to the corresponding alcohol **11b**, which was stable for characterization[2]. The position of the hydroperoxide substituent on the carbon chain of **10b** was determined by negative ion tandem electrospray mass spectrometry of the alcohol **11b**, which displayed a molecular anion at *m/z* 363, and a daughter ion at *m/z* 263 corresponding to loss of a $C_6H_{13}O$ fragment, resulting from C14'-C15' bond cleavage. This characteristic fragmentation of the carbon chain at the bond adjacent to the hydroxyl group of **11b** then indicates C15' as the position of the hydroperoxy substituent of the original hydroperoxide **10b**.

15-Lipoxygense catalysed oxidation of the isomeric β-oxa fatty acids, γ-linolenyloxyacetic acid (**3**) and linolenyloxyacetic acid (**6**), gave the corresponding 13'-hydroperoxide derivatives **4** and **7**, respectively, resulting from regioselective oxidation at the ω-6 carbon in each case (*Scheme 1*). The structure of the hydroperoxides **4** and **7**, thus obtained, was confirmed as above, by reduction to the corresponding alcohols **5** and **8**, which upon mass spectrometric analysis, each yielded a molecular anion at *m/z* 337. In the case of the alcohol **5**, a daughter ion at *m/z* 237, corresponding to loss of a $C_6H_{13}O$ fragment, established C13' as the position of the hydroperoxy substituent in the hydroperoxide **4**, and in the case of the alcohol **8**, a daughter ion at *m/z* 239 corresponding to loss of a $C_6H_{11}O$ fragment similarly established C13' as the position of the hydroperoxy substituent in the hydroperoxide **7**.

Dussault and co-workers [11–14] have previously reported perketals as protecting groups for PUFA hydroperoxides, which are stable to normal or reverse-phase chromatography, and which may be removed under mildly acidic conditions. Accordingly, the perketals **1a** and **1b** were conveniently synthesized *via* derivatization of the respective fatty acid hydroperoxides **10a** and **10b** (*Scheme 1*). Each of the crude hydroperoxides **10a** and **10b** was esterified by treatment with diazomethane (CAUTION: explosive!) in diethyl ether at 0°C, and each of the resultant crude methyl esters **12a** and **12b** was then treated with approximately 2 equivalents of 2-methoxypropene and pyridinium *para*-toluenesulfonate (PPTS) catalyst in dichloromethane, to give the stable perketal derivatives **13a** and **13b**. After purification by flash column chromatography on silica, the perketal methyl esters **13a** and **13b** were obtained in yields of 35 and 33% from the fatty acids **9a** and **9b**, respectively. Saponification with lithium hydroxide of **13a** and **13b** at 0°C then afforded the desired perketals **1a** and **1b**, in 75 and 74% yield, respectively, after chromatography on silica. The perketals **1a** and **1b** were stable for extended periods upon storage under nitrogen at –20°C, and were fully characterized[3].

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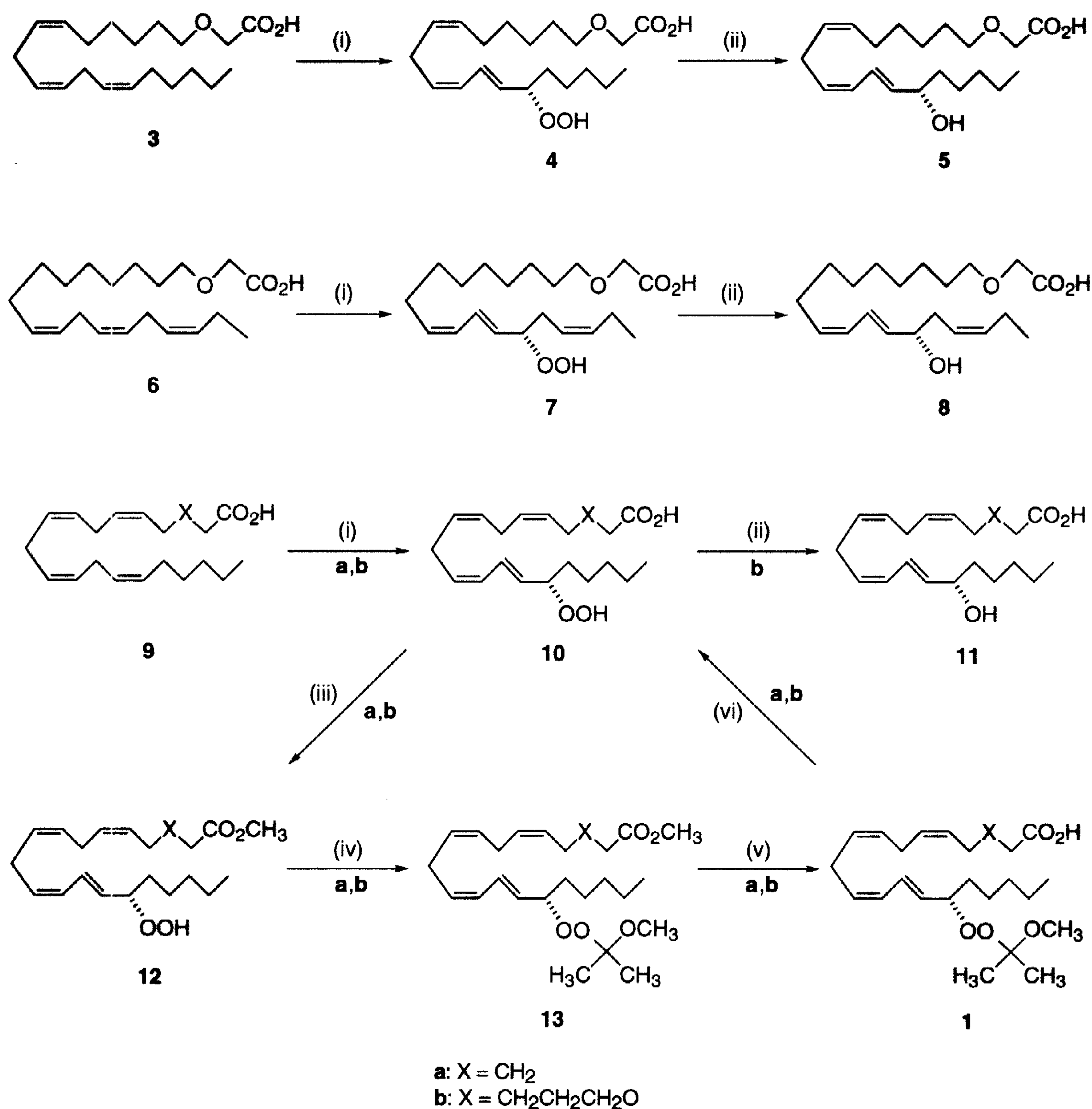
[2] *15'-(S)-Hydroxy-(Z,Z,Z,E)-eicosa-5',8',11',13'-tetraenyloxyacetic acid (**11b**):* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (3H, t, *J* = 6.8 Hz), 1.39 (9H, m), 1.64 (3H, m), 2.10 (2H, m), 2.82 (2H, m), 2.97 (2H, m), 3.55 (2H, t, *J* = 6.6 Hz), 4.10 (2H, s), 4.20 (1H, dt, *J* = 6.4, 6.2 Hz), 5.39 (5H, m), 5.70 (1H, dd, *J* = 15.0, 6.4 Hz), 6.00 (1H, dd, *J* = 11.0, 10.9 Hz), 6.53 (1H, dd, *J* = 15.0, 11.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.75, 136.17, 130.31, 129.79, 128.72, 128.06, 127,43, 125.46, 71.88, 67.68, 37.21, 31.73, 28.90, 26.78, 26.09, 25.82, 25.68, 25.05, 22.56, 14.02; ESI MS/MS *m/z* (%): 363 (100, $[M - H]^-$), 345 (11, $[M - H - H_2O]^-$), 263 (5, $[M - C_6H_{13}O]^-$), 75 (13, $[OCH_2CO_2H]^-$).

[3] *15-(S)-[(1-Methoxy-1-methylethyl)dioxy]-(Z,Z,Z,E)-eicosa-5,8,11,13-tetraenoic acid (**1a**):* ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88



Reagents and conditions: (i) 15-lipoxygenase, O_2, $KHPO_4$, pH 9.0, 0°C, 1 h.; (ii) $NaBH_4$, 0°C, 1 h.; (iii) CH_2N_2, Et_2O, 0°C, 5 min.; (iv) 2-methoxypropene, PPTS, CH_2Cl_2, r.t., 5 min.; (v) LiOH, DME, H_2O, 0°C, 30 min.; (vi) AcOH/H_2O (95:5), 0°C, 2 h.

Scheme 1

(3H, t, J = 6.5 Hz), 1.38 (7H, m), 1.39 (6H, s), 1.71 (3H, m), 2.14 (2H, m), 2.37 (2H, t, J = 7.5 Hz), 2.82 and 2.97 (each 2H, m), 3.30 (3H, s), 4.42 (1H, dt, J = 7.2, 7.0 Hz), 5.39 (5H, m), 5.65 (1H, dd, J = 15.1, 7.2 Hz), 6.02 (1H, dd, J = 11.1, 10.8 Hz), 6.53 (1H, dd, J = 15.1, 11.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.93, 133.52, 130.16, 128.87, 128.51, 128.20, 127.62, 127.52, 104.64, 84.58, 49.26, 33.07, 31.77, 26.43, 26.10, 25.61, 25.07, 24.46, 22.99, 22.84, 22.50, 14.03; Anal. calcd for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. found: C, 70.22; H, 10.20. *15-(S)-[(1-Methoxy-1-methylethyl)dioxy]-(Z,Z,Z,E)-eicosa-5',8',11',13'-tetraenyloxyacetic acid (1b):* ^1H NMR (300 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.8 Hz), 1.38 (9H, m), 1.39 (6H, s), 1.66 (3H, m), 2.11 (2H, m), 2.82 and 2.97 (each 2H, m), 3.31 (3H, s), 3.57 (2H, t, J = 6.6 Hz), 4.10 (2H, s), 4.42 (1H, dt, J = 7.3, 7.0 Hz), 5.39 (5H, m), 5.65 (1H, dd, J = 15.3, 7.3 Hz), 6.02 (1H, dd, J = 11.0, 11.0 Hz), 6.52 (1H, dd, J = 15.3, 11.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.72, 133.48, 130.15, 129.79, 128.66, 128.13, 127.93, 127.45, 104.59, 84.58, 71.65, 67.65, 49.17, 32.98, 31.70, 28.95, 26.82, 26.05, 25.84, 25.57, 25.02, 22.93, 22.76, 22.45, 13.98; Anal. calcd for $C_{26}H_{44}O_6$: C, 68.99; H, 9.80. found: C, 68.77; H, 10.00.

The antimalarial activities of the hydroperoxy fatty acids **10a** and **10b** and their respective perketal derivatives **1a** and **1b** were investigated by determination of *in vitro* growth inhibition of *Plasmodium falciparum* infected red blood cells, using a radiometric assay, described previously [1,15,16]. At an assay concentration of 40 μM, 15-HPETE (**10a**) induced a 96% growth inhibition of *P. falciparum*, whereas the 15'-hydroperoxide **10b** was less active, inducing 46% growth inhibition. At 20 μM concentration, the hydroperoxides **10a** and **10b** induced 92 and 24% growth inhibition, respectively. The perketal derivatives **1a** and **1b** were less active than their corresponding hydroperoxides, inducing only 36 and 14% growth inhibition, respectively, at 40 μM concentration, and 20 and 10% growth inhibition, respectively, at 20 μM concentration.

The perketals **1a** and **1b** may be hydrolysed under acidic conditions to the corresponding hydroperoxides **10a** and **10b** in high yield, by treatment with 95:5 acetic acid/water, as reported previously [17] (*Scheme 1*). It is presumable that hydrolysis of the perketals **1a** and **1b** might occur during the assays described above, to release the hydroperoxides **10a** and **10b**, and it is then not clear whether the perketals **1a** and **1b** themselves possess antimalarial activity or whether their observed activity is due to the parent hydroperoxides **10a** and **10b**. Either way, further development of the perketal strategy outlined herein will provide convenient methodology for the storage and administration of a range of oxidised PUFA derivatives as potent antimalarial agents.

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