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5-Oxo-15-HETE: Total synthesis and bioactivity

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ABSTRACT

The first total synthesis of 6(*E*),8(*Z*),11(*Z*),13(*E*) 5-oxo-15-HETE **4** was accomplished. The synthetic material was evaluated with calcium mobilization assay and compared with 5-oxo-ETE the natural ligand for the OXE receptor.

Arachidonic acid **1** (AA) is converted to a large number of biologically active products (eicosanoids) that are important in a variety of pathological conditions including inflammatory and allergic diseases. Because of the complex array of products with different chiral centers and double bond configurations, chemical synthesis of eicosanoids has been critical for their structural identification and determination of their physiological and pharmacological properties.

Lipoxygenase (LO) enzymes catalyze the stereospecific oxidation of AA **1** to form hydroperoxyeicosatetraenoic acids (HPETEs); for example, 5(*S*)-HPETE is the major initial product formed by 5-LO, 12(*S*)-HPETE from 12-LO, and 15(*S*)-HPETE from 15-LO. However, in the last instance the use of soybean lipoxygenase, which is widely used as a source of 15-LO, generates a substantial amount (~15%) of the *R*-enantiomer.[1] 5-HPETE is also converted by peroxidase to 5-HETE which is further transformed to 5-oxo-ETE **2** by the $NADP^+$-dependent enzyme 5-hydroxy eicosanoid dehydrogenase (5-HEDH, Scheme 2). This enzyme is highly specific for eicosanoids containing a 5(*S*)-hydroxyl group followed by a 6,7-*trans* double bond. For example, neither 5(*R*)-HETE[2] nor LTB_4 which has a 6,7-*cis* double bond, is a substrate for this enzyme.[2] 5-HEDH is present in neutrophils,[3] monocytes,[4] lymphocytes,[4] eosinophils,[5] platelets,[6] and endothelial and epithelial cells, and keratinocytes.[7]

5-Oxo-ETE is the most potent eosinophil chemoattractant amongst lipid mediators and has similar effects on neutrophils[3] and monocytes. Its action is mediated by the highly selective OXE receptor.[8–10] 5-Oxo-ETE is hydroxylated by both lipoxygenase and cytochrome P_{450} pathways, being converted to 5-oxo-12-HETE and 5-oxo-15-HETE **4** by 12-LO[11] and 15-LO[12], respectively, and to 5-oxo-20-HETE **5**[13] and 5-oxo-19-HETE[14] by cytochrome P_{450} enzymes.

5-Oxo-15-HETE has an OH at C-15 with the *S*-stereochemistry and two sets of conjugated dienes (Scheme 1). The diene at C-6 is conjugated with carbonyl compound and show the appropriate UV at λ_{max} 281, and the diene at C-11 has a λ_{max} 229. Until now, 5-oxo-15-HETE has only been prepared for pharmacological testing from its biological precursors. For example, oxidation of AA with soybean lipoxygenase gave rise to 5-oxo-15-HETE as one of the minor products.[15] This enzyme also converts 5-oxo-ETE directly to 5-oxo-15-HETE[12] (Scheme 4). We have previously synthesized the latter product for biological testing by incubating 5,15-diHETE with neutrophil microsomes, which contain high levels of 5-HEDH, in the presence of $NADP^+$.[8] In addition, 5-oxo-15-HETE has also be synthesized by oxidation of 5,15-diHETE with 2,3-dichloro-5,6-dicyanobenzoquinone and separation from the other dehydrogenase products by HPLC.[16]

Although 5-oxo-15-HETE appears to activate the OXE receptor, there are some discrepancies in the literature on its potency compared to 5-oxo-ETE. Norgauer's group reported that these two eicosanoids are equipotent and have equivalent efficacies,[17,18] whereas we[5,8] and others[16] found it to be somewhat less potent and/or efficacious. It has been suggested that these differences could have been due to isomerization of 5-oxo-15-HETE to its less potent 8-*trans* form.[12]

We have invested substantial efforts in studying structure–activity relationships for the 5-oxo-ETE receptor.[19] To clarify the

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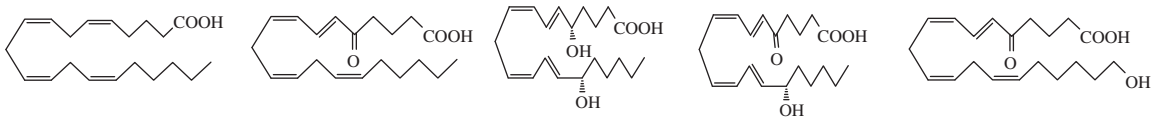
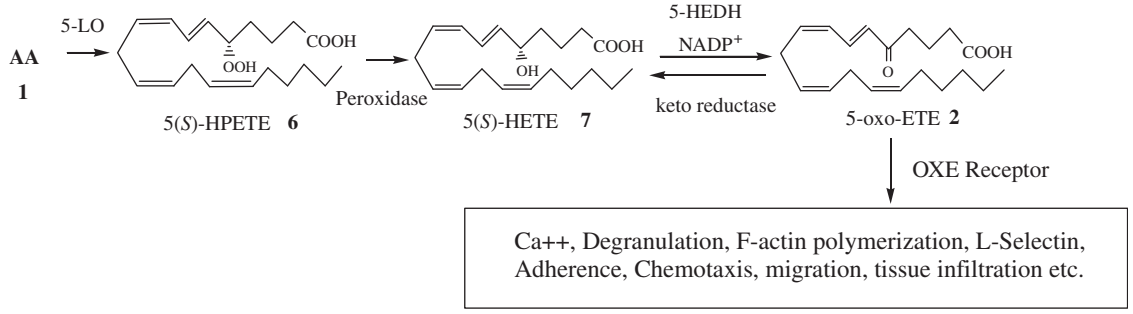


Scheme 1. Eicosanoids.



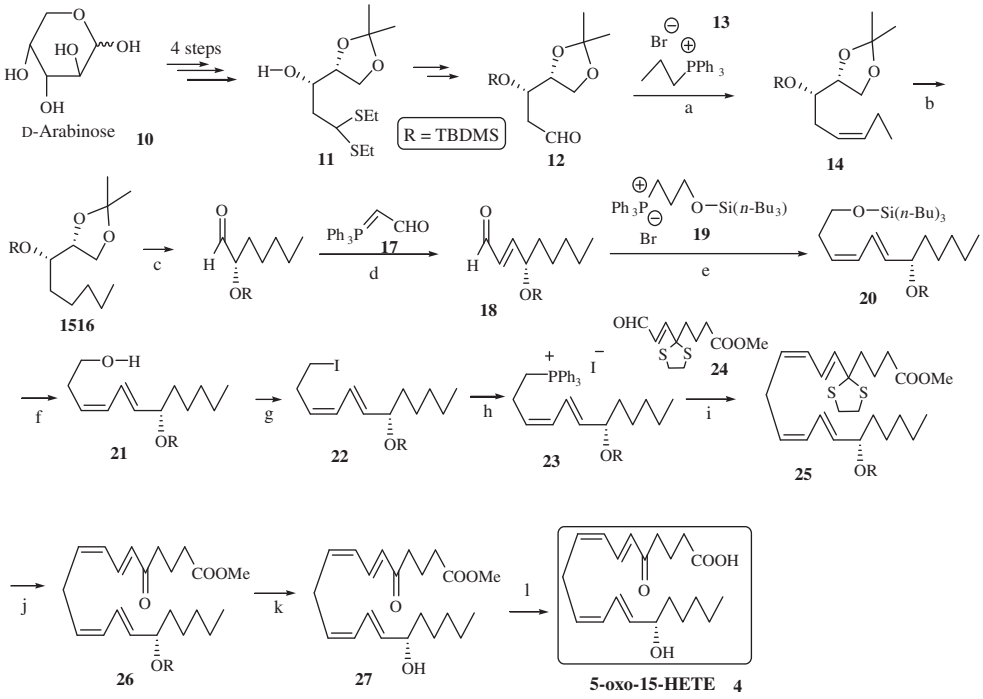
Scheme 2. Biosynthesis of 5-oxo-ETE.

degree of biological activity of 5-oxo-15-HETE and to avoid any ambiguity about its structure, we elected to prepare this mediator by total synthesis.

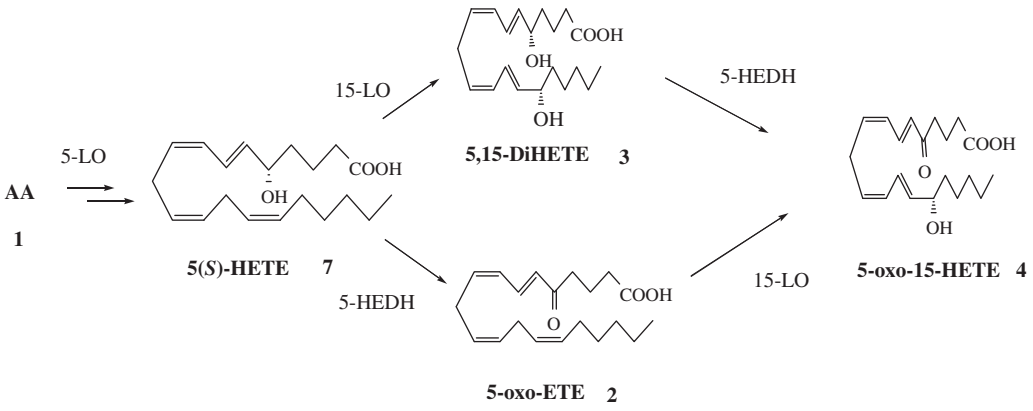
We approached the synthesis of 5-oxo-15-HETE **4** as described in detail in Scheme 3. We opted for a convergent synthesis in which the 5-oxo component and the 15-hydroxy synthon were prepared and then connected. Synthon **12** was previously synthesized by us using a known procedure.[20] We used a protected 5-oxo group **24** in order to avoid complications later in the synthesis and was prepared in three very efficient steps, as described previously.[21] The synthon **20,** which contained the 15-hydroxy component, was prepared from D-arabinose as shown in Scheme 3.

The synthetic 6(*E*),8(*Z*),11(*Z*),13(*E*) 5-oxo-15-HETE shows a UV absorption at λ_{max} 281 for the dienone at C-6 and λ_{max} 229 for the diene at C-11. The NMR shows the coupling constant for the proton at C-6 (*J* = 15.5 Hz, 1H), C-7 (*J* = 15.1, 11.1 Hz, 1H), C-9 (*J* = 10.5, 7.8 Hz, 1H), C-11 (*J* = 10.4, 7.4 Hz, 1H), and C-13 (*J* = 15.1, 11.1 Hz, 1H).

Human neutrophils were purified from whole blood from healthy individuals as described previously using dextran 500 to



Scheme 3. Total synthesis of 5-oxo-15-HETE. Reagents and conditions: (a) LiHMDS, **13,** THF/HMPA, rt to −78 °C to rt, 12 h, 97%; (b) Pd/C, H_2, ethanol, rt, 5 h, 100%; (c) H_5IO_6, THF/Ether, rt, over night, 94%; (d) **17**, Benzene, 65 °C, 12 h, 85%; (e) **19**, LiHMDS, THF/HMPA, −78 °C to rt, 2 h, 93%; (f) AcOH/THF/H_2O (3/2/1), rt, 8 h, 82%; (g) TPP, Im, I_2, CH_2Cl_2, 0 °C to rt, 3 h, 90%; (h) TPP, CH_3CN, 60 °C, 3 days, 100%; (i) **23**, *n*- BuLi, THF/HMPA, 0 °C to −78 °C to rt, 2 h, 85%; (j) $PhI(OCOCF_3)_2$, CH_3OH/H_2O, 0 °C, 2 min, 46%; (k) PPTS, THF/H_2O, rt, 2 days, 78%; (l) LiOH, THF/H_2O, rt, 1 h, 72%.



Scheme 4. Biosynthesis of 5-oxo-15-HETE.

remove red blood cells, centrifugation over Ficoll–Paque to remove mononuclear cells and hypotonic lysis to remove any remaining red blood cells.[2] The neutrophils were suspended in phosphate-buffered saline (PBS) and loaded with indo-1 acetoxymethyl ester (Invitrogen, as previously described).[13] Five minutes prior to data acquisition, $CaCl_2$ and $MgCl_2$ were added to give final concentrations of 1.8 and 1 mM, respectively. Fluorescence was measured using a spectrofluorometer with a temperature-controlled cuvette holder equipped with a magnetic stirrer. After stabilization of the baseline, various concentrations of 5-oxo-ETE or 5-oxo-15-HETE were added, followed 1.5 min later by 5-oxo-ETE (10 nM) to evaluate receptor desensitization by the initial agonist. After another 0.5 min, digitonin (0.1% final concentration) was added to permit measurement of the maximal fluorescence.

The response of neutrophils to 10 nM 5-oxo-ETE was unaffected by the addition of vehicle 90 s earlier (Fig. 1A, top panel). In contrast, prior addition of 100 nM 5-oxo-15-HETE, which itself strongly stimulated Ca^{2+} mobilization, completely blocked the response to 5-oxo-ETE (Fig. 1A, lower panel). The ability of 5-oxo-15-HETE to desensitize neutrophils to 5-oxo-ETE is consistent with its activity being mediated by the 5-oxo-ETE receptor.

The concentration–response relationships for 5-oxo-ETE and 5-oxo-15-HETE are shown in Figure 1B. 5-Oxo-15-HETE (EC_{50}, of 56 ± 10 nM) was about one-eighth as potent as 5-oxo-ETE (EC_{50}, 7 ± 4 nM) in inducing Ca^{2+} mobilization and also displayed lower efficacy, as it induced a maximal response 34 ± 7% lower than that for 5-oxo-ETE. These results compare very favorably with our previous finding that biologically derived 5-oxo-15-HETE was about one-sixth as potent as 5-oxo-ETE and about 75% as efficacious in inducing calcium mobilization in neutrophils.[8] This suggests that the lower potency we observed for 5-oxo-15-HETE in inducing this response was not due to its isomerization to its less potent 8-*trans* isomer. Moreover, we found that 5-oxo-15-HETE could be stored at −80 °C for up to three years without any significant degradation, as evaluated by reversed-phase-HPLC.

Despite its lower potency in stimulating Ca^{2+} mobilization in neutrophils, we previously found that 5-oxo-15-HETE is approximately equipotent with 5-oxo-ETE in stimulating both neutrophil[8] and eosinophil[5] chemotaxis, but is only about 40–60% as efficacious, consistent with its being a partial agonist. It is possible that the increased relative potency of 5-oxo-15-HETE in the longer 2 h chemotaxis assay could be due to resistance to metabolism (compared to 5-oxo-ETE) by cytochrome P_{450} and 5-HEDH, which reduces 5-oxoeicosanoids to 5(*S*)-hydroxy products.[2] Both pathways result in dramatic losses in biological activity.[22] Thus both 5-oxo-ETE and 5-oxo-15-HETE could play roles as inflammatory mediators in allergic diseases such as asthma, which are characterized by tissue infiltration of eosinophils.

The biosynthesis of 5-oxo-15-HETE is more complicated than that of 5-oxo-ETE, as it requires an additional step to introduce the 15-hydroxyl group. It could arise in vivo either by the 15-LO-catalyzed oxidation of 5-oxo-ETE or by the 5-HEDH-catalyzed oxidation of 5,15-diHETE, a product of the combined actions of 5-LO and 15-LO (Scheme 4). Eosinophils contain all of these

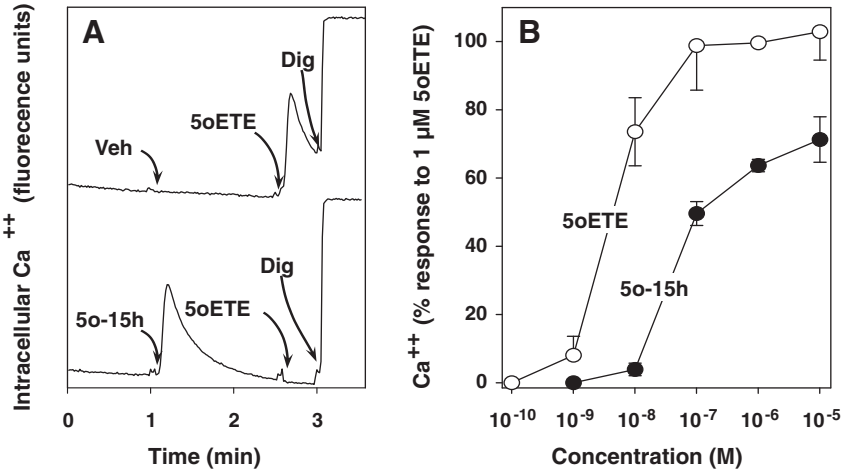


Figure 1. Effects of 5-oxo-15-HETE and 5-oxo-ETE on Ca^{2+} mobilization in human neutrophils. (A) Either vehicle (top panel) or 5-oxo-15-HETE (5o–15h; 100 nM; bottom panel) were added to a suspension of indo-1-loaded neutrophils whil measuring fluorescence as described above. After 1.5 min, 5-oxo-ETE (5oETE; 10 nM) was added, followed by digitonin (dig, 0.1%). (B) Concentration–response curves for 5-oxo-ETE (o) and 5-oxo-15-HETE (•) The results are expressed as percentages of the response to 1 μM 5-oxo-ETE; $n = 4$).

enzymes and produce small amount of both 5-oxo-ETE and 5-oxo-15-HETE when incubated with calcium ionophore and AA.[5] However, cysteinyl leukotrienes are the major 5-LO products formed by these cells. 5-Oxo-15-HETE could also be synthesized by transcellular biosynthesis, with 5-HETE or 5-oxo-ETE being provided by inflammatory cells and then further metabolized by 15-LO in other cells such as epithelial cells, which contain both 15-LO[23] and 5-HEDH,[24] but little 5-LO activity.

The availability of authentic 5-oxo-15-HETE with the correct stereochemistry and double bond configuration will be an important asset in determining the biological role of this proinflammatory mediator.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.032.

References and notes

1. Falck, J. R.; Manna, S.; Jacobson, H. R.; Estabrook, R. W.; Chacos, N.; Capdevila, J. *J. Am. Chem. Soc.* **1984**, *106*, 3334.
2. Powell, W. S.; Gravelle, F.; Gravel, S. *J. Biol. Chem.* **1992**, *267*, 19233.
3. O'Flaherty, J. T.; Cordes, J.; Redman, J.; Thomas, M. J. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 129.
4. Zhang, Y.; Styler, A.; Powell, W. S. *J. Leukoc. Biol.* **1996**, *59*, 847.
5. Powell, W. S.; Chung, D.; Gravel, S. *J. Immunol.* **1995**, *154*, 4123.
6. Praticò, D.; Ferro, D.; Iuliano, L.; Rokach, J.; Conti, F.; Valesini, G.; FitzGerald, G. A.; Violi, F. *Blood* **1999**, *93*, 3401.
7. Cossette, C.; Patel, P.; Anumolu, J. R.; Sivendran, S.; Lee, G. J.; Gravel, S.; Graham, F. D.; Lesimple, A.; Mamer, O. A.; Rokach, J.; Powell, W. S. *J. Biol. Chem.* **2008**, *283*, 11234.
8. Powell, W. S.; Gravel, S.; MacLeod, R. J.; Mills, E.; Hashefi, M. *J. Biol. Chem.* **1993**, *268*, 9280.
9. Powell, W. S.; MacLeod, R.; Gravel, S.; Gravelle, F.; Bhakar, A. *J. Immunol.* **1996**, *156*, 336.
10. O'Flaherty, J. T.; Taylor, J. S.; Thomas, M. J. *J. Biol. Chem.* **1998**, *273*, 32535.
11. Powell, W. S.; Gravel, S.; Khanapure, S. P.; Rokach, J. *Blood* **1999**, *93*, 1086.
12. Schwenk, U.; Schroder, J. M. *J. Biol. Chem.* **1995**, *270*, 15029.
13. Powell, W. S.; MacLeod, R. J.; Gravel, S.; Gravelle, F.; Bhakar, A. *J. Immunol.* **1996**, *156*, 336.
14. Hevko, J. M.; Bowers, R. C.; Murphy, R. C. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 293.
15. Schwenk, U.; Morita, E.; Engel, R.; Schroder, J. M. *J. Biol. Chem.* **1992**, *267*, 12482.
16. O'Flaherty, J. T.; Cordes, J. F.; Lee, S. L.; Samuel, M.; Thomas, M. J. *Biochim. Biophys. Acta* **1994**, *1201*, 505.
17. Norgauer, J.; Barbisch, M.; Czech, W.; Pareigis, J.; Schwenk, U.; Schroder, J. M. *Eur. J. Biochem.* **1996**, *236*, 1003.
18. Czech, W.; Barbisch, M.; Tenscher, K.; Schopf, E.; Schroder, J. M.; Norgauer, J. *J. Invest. Dermatol.* **1997**, *108*, 108.
19. Patel, P.; Cossette, C.; Anumolu, J. R.; Gravel, S.; Lesimple, A.; Mamer, O. A.; Rokach, J.; Powell, W. S. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 698.
20. Spur, B.; Crea, A.; Peters, W.; Konig, W. *Arch. Pharm.* **1985**, *318*, 225.
21. Khanapure, S. P.; Shi, X. X.; Powell, W. S.; Rokach, J. *J. Org. Chem.* **1998**, *63*, 337.
22. Powell, W. S.; Rokach, J. *Prog. Lipid Res.* **2005**, *44*, 154.
23. Hunter, J. A.; Finkbeiner, W. E.; Nadel, J. A.; Goetzl, E. J.; Holtzman, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4633.
24. Erlemann, K. R.; Cossette, C.; Gravel, S.; Lesimple, A.; Lee, G. J.; Saha, G.; Rokach, J.; Powell, W. S. *Free Radic. Biol. Med.* **2007**, *42*, 654.