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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-EETE 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-EETE **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*)-HETE² nor LTB₄ 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-EETE is the most potent eosinophil chemoattractant amongst lipid mediators and has similar effects on neutrophils³ and monocytes. Its action are mediated by the highly selective OXE receptor.^{8–10} 5-oxo-EETE is hydroxylated by both lipoxygenase and cytochrome P₄₅₀ pathways, being converted to 5-oxo-12-HETE and 5-oxo-15-HETE **4** by 12-LO¹¹ and 15-LO¹², respectively, and to 5-oxo-20-HETE **5**¹³ and 5-oxo-19-HETE¹⁴ by cytochrome P₄₅₀ enzymes.

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5-oxo-15-HETE has an OH at C-15 with the *S*-stereochemistry and two sets of conjugated dienes. 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-EETE directly to 5-oxo-15-HETE¹² (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 been 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-EETE. Norgauer's group reported that these two eicosanoids are equipotent and have equivalent efficacies,^{17,18} whereas we^{5,8} and others¹⁶ 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-EETE receptor.¹⁹ To clarify the 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 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, CaCl₂ and MgCl₂ 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-EETE or 5-oxo-15-HETE were added, followed 1.5 min later by 5-oxo-EETE (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-EETE 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 Ca²⁺ 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 Fig. 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 $34 \pm 7\%$ 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 $-80^{\circ}C$ 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 neutrophil⁸ and eosinophil⁵ chemotaxis, but is only about 40 to 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-hour chemotaxis assay could be due to resistance to metabolism (compared to 5-oxo-ETE) by cytochrome P450 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 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-LO²³ 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Falck JR, Manna S, Jacobson HR, Estabrook RW, Chacos N, Capdevila J. J. Am. Chem. Soc 1984;106:3334.
2. Powell WS, Gravelle F, Gravel S. J. Biol. Chem 1992;267:19233. [PubMed: 1326548]
3. O'Flaherty JT, Cordes J, Redman J, Thomas MJ. Biochem. Biophys. Res. Commun 1993;192:129. [PubMed: 8386504]
4. Zhang Y, Styler A, Powell WS. J. Leukoc. Biol 1996;59:847. [PubMed: 8691070]
5. Powell WS, Chung D, Gravel S. J. Immunol 1995;154:4123. [PubMed: 7706749]
6. Praticò D, Ferro D, Iuliano L, Rokach J, Conti F, Valesini G, FitzGerald GA, Violi F. Blood 1999;93:3401. [PubMed: 10233892]
7. Cossette C, Patel P, Anumolu JR, Sivendran S, Lee GJ, Gravel S, Graham FD, Lesimple A, Mamer OA, Rokach J, Powell WS. J. Biol. Chem 2008;283:11234. [PubMed: 18287092]
8. Powell WS, Gravel S, MacLeod RJ, Mills E, Hashefi M. J. Biol. Chem 1993;268:9280. [PubMed: 8387490]
9. Powell WS, MacLeod R, Gravel S, Gravelle F, Bhakar A. J. Immunol 1996;156:336. [PubMed: 8598482]
10. O'Flaherty JT, Taylor JS, Thomas MJ. J. Biol. Chem 1998;273:32535. [PubMed: 9829988]
11. Powell WS, Gravel S, Khanapure SP, Rokach J. Blood 1999;93:1086. [PubMed: 9920859]
12. Schwenk U, Schroder JM. J. Biol. Chem 1995;270:15029. [PubMed: 7797484]
13. Powell WS, MacLeod RJ, Gravel S, Gravelle F, Bhakar A. J. Immunol 1996;156:336. [PubMed: 8598482]
14. Hevko JM, Bowers RC, Murphy RC. J. Pharmacol. Exp. Ther 2001;296:293. [PubMed: 11160610]
15. Schwenk U, Morita E, Engel R, Schroder JM. J. Biol. Chem 1992;267:12482. [PubMed: 1618754]
16. O'Flaherty JT, Cordes JF, Lee SL, Samuel M, Thomas MJ. Biochim. Biophys. Acta 1994;1201:505. [PubMed: 7803484]
17. Norgauer J, Barbisch M, Czech W, Pareigis J, Schwenk U, Schroder JM. Eur. J. Biochem 1996;236:1003. [PubMed: 8665888]
18. Czech W, Barbisch M, Tenscher K, Schopf E, Schroder JM, Norgauer J. J. Invest. Dermatol 1997;108:108. [PubMed: 8980298]
19. Patel P, Cossette C, Anumolu JR, Gravel S, Lesimple A, Mamer OA, Rokach J, Powell WS. J. Pharmacol. Exp. Ther 2008;325:698. [PubMed: 18292294]
20. Spur B, Crea A, Peters W, König W. Arch. Pharm 1985;318:225.
21. Khanapure SP, Shi XX, Powell WS, Rokach J. J. Org. Chem 1998;63:337.
22. Powell WS, Rokach J. Prog. Lipid Res 2005;44:154. [PubMed: 15893379]
23. Hunter JA, Finkbeiner WE, Nadel JA, Goetzel EJ, Holtzman MJ. Proc. Natl. Acad. Sci. USA 1985;82:4633. [PubMed: 3927287]
24. Erlemann KR, Cossette C, Gravel S, Lesimple A, Lee GJ, Saha G, Rokach J, Powell WS. Free Radic. Biol. Med 2007;42:654. [PubMed: 17291989]

Figure 1. Effects of 5-oxo-15-HETE and 5-oxo-EETE 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 while measuring fluorescence as described above. After 1.5 min, 5-oxo-EETE (5oETE; 10 nM) was added, followed by digitonin (dig, 0.1%). **B:** Concentration-response curves for 5-oxo-EETE ($\circ$) and 5-oxo-15-HETE ($\bullet$). The results are expressed as percentages of the response to 1 μ M 5-oxo-EETE; n = 4).

Scheme 1.
Eicosanoids

Scheme 2.
Biosynthesis of 5-oxo-ETE

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) $\text{PhI}(\text{OCOCF}_3)_2$, $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, 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