Pergamon

Tetrahedron Letters, Vol. 35, No. 24, pp. 4051-4054, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)E0762-M

Synthesis of 12-KETE and Its 8,9-*Trans*-Isomer

Steven S. Wang and Joshua Rokach*

Claude Pepper Institute and Department of Chemistry, Florida Institute of Technology, 150 W. University Blvd., Melbourne, FL 32901, USA

William S. Powell

Meakins-Christie Laboratories, 3626 St-Urbain St., Montreal, Que. H2X 2P2, Canada

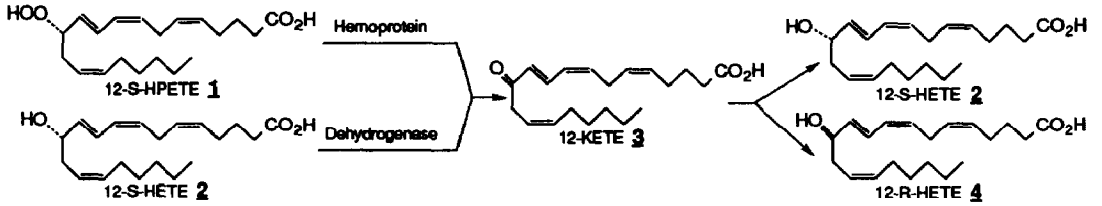
Catherine Dekle and Steven J. Feinmark

Department of Pharmacology, Columbia University, 630 W. 168th St., New York, NY 10032, USA

Abstract: The first total synthesis of the highly unstable biological mediator 12-ketoeicosatetraenoic acid (12-KETE) **3** and its 8,9-*trans* -isomer **20** is presented. The strategy focuses on the stable precursor dithiane **13** and its conversion to **3** and **20**. Biochemical experiments show that the two isomers are not interconverted *in vivo*, raising the possibility that the *trans*-isomer **20** may be formed by a primary biochemical mechanism.

12-R-Hydroxyeicosatetraenoic acid (12-R-HETE) **4** has been isolated from psoriatic skin lesions[1] and rabbit eye.[2] The properties described so far for this compound, e.g. chemotactic for PMNs, indicate a role in skin inflammation such as psoriasis. In addition, this compound is an inhibitor of Na^+/K^+ ATPase and causes a drop in intraoccular pressure in animal models.[2] These properties are quite distinct from 12-S-HETE **2** which is the enzymatic product of the 12-lipoxygenase enzyme. No lipoxygenase enzyme is known for the formation of 12-R-HETE. It would appear that in the eye it is a product of a cytochrome P-450 monooxygenase. Because of the important biological activity of this compound, we were intrigued by the biosynthetic mechanism of its formation.

We have proposed[3] that 12-R-HETE could be formed from the enzymatic reduction of 12-KETE, which itself could have been formed from 12-S-hydroperoxyeicosatetraenoic acid (12-S-HPETE) **1** or 12-S-HETE **2** by a dehydration or the action of a dehydrogenase (Scheme 1).



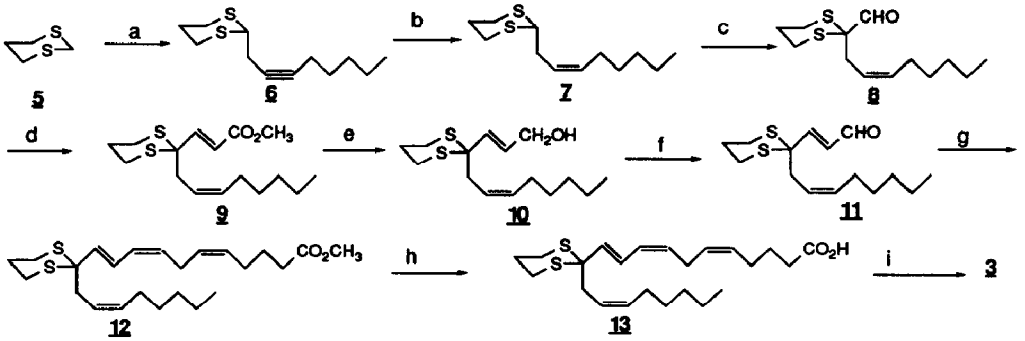
Scheme 1

12-KETE was then an unknown compound, and in order for us to test this hypothesis we decided to prepare it by the most expeditious way via Jones oxidation of 12-HETE, and we have shown that incubation of freshly prepared 12-KETE with liver microsomes led to the formation of 12-R-HETE and 12-S-HETE.[3] The ratio of 12-R to 12-S was very conveniently analyzed by HPLC on a chiral column. In the meantime, 12-KETE has been isolated from human platelets[4] and from porcine neutrophils.[5] It has also been isolated from *Aplysia* nervous tissue and reported to be a histamine-like neurotransmitter.[6] The 8,9-*trans*-isomer has also been isolated in these experiments. 12-KETE has also been implicated in the formation of 10,11-dihydro-12-S-

HETE, a potent proinflammatory agent.[7] As it turned out, 12-KETE was very unstable, its preparation and purification quite tedious, and storage was a chancy proposition. More importantly the synthesis of 12-HETE, which we and others have described earlier,[8,9] is itself not trivial and is hardly a convenient starting material for 12-KETE. We decided to undertake a proper synthesis for 12-KETE which would guarantee an uninterrupted supply for the studies of its biological properties and the evaluation of its pivotal role in the formation of 12-S-, 12-R-HETE and 10,11-dihydro-12-S-HETE. We report here on the first total synthesis of this biologically important mediator. We were also able to prepare the 8,9-*trans*-isomer **20**. This is of some importance since it is not known if the *trans* compound is formed by a primary mechanism or by an *in vivo* isomerization of 12-KETE or by an artifact of the isolation procedure. The availability of synthetic **20** will allow us to perform these experiments.

What we needed was 1) a stable precursor of 12-KETE so as to allow the accumulation of gram quantities, and 2) a one-step mild procedure to transform this precursor to 12-KETE. Scheme 2 describes the strategy we used to prepare the stable precursor **13** and its transformation to 12-KETE **3**. As can be seen, we selected the dithioacetal over other protecting groups because of an abundant choice of deprotection methods and conditions. We also avoided protective groups requiring basic conditions to regenerate the carbonyl function.

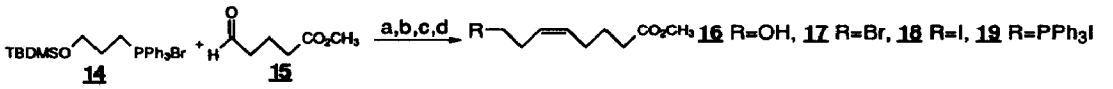
1,3-Dithiane **5** was reacted with 2-octyn-1-yl bromide to give **6** in 91% yield. Hydrogenation of **6** using nickel boride as catalyst afforded **7** with a yield of 94%. Formylation of **7** with DMF gave **8** in 77% yield. This was followed by a Wittig reaction with trimethyl phosphonoacetate to produce **9** in 91% yield. An attempted reduction of **9** to aldehyde **11** with DIBAL-H yielded a mixture of the aldehyde **11** and alcohol **10**. We decided instead to perform the two-step procedure, as shown in the scheme. Dithiane ester **9** was treated with DIBAL-H to give **10** in 96% yield. This was followed by an oxidation with PCC-SiO_2 to **11** in 76% yield. A second Wittig reaction of aldehyde **11** with synthon **19** gave the protected 12-KETE methyl ester **12** in 62% yield. Saponification of **12** afforded **13** in 92% yield. Removal of the dithiane protection group produced the final product, 12-KETE **3**[10] in 75-80% yield (15-20% isolated yield, *vide infra.*).



a) 1.1 eq n-BuLi/THF, -78°C to -20°C, 2hrs, 2-octyn-1-yl bromide, -78°C to room temperature. b) $Ni(AcO)_2{\cdot}4H_2O$, $NaBH_4$, 95%EtOH, H_2. c) 1.1 eq n-BuLi/THF -30°C, 2hrs, DMF, -78°C to room temperature. d) 1.1 eq LDA, 1.2 eq trimethyl phosphonoacetate, THF, -78°C to room temperature. e) 2.1 eq DIBAL-H, CH_2Cl_2, -78°C. f) 2.0 eq PCC-SiO_2, CH_2Cl_2. g) 1.2 eq synthon **19**, 1.1 eq $LiN(SiMe_3)_2$, THF/HMPA, -78°C to room temperature. h) 15 eq LiOH, THF/H_2O 3:1. i) NCS $AgClO_4$, 97% acetone/H_2O.

Scheme 2

The synthon **19** was prepared by a more convenient method, shown in Scheme 3, than the one we described earlier.[11] Phosphonium salt **14**, prepared in two steps from 3-bromopropanol in quantitative yield, was reacted with aldehyde **15**, followed by removal of the silyl group with Bu_4NF to give **16** in 83% yield. Bromination gave **17** in quantitative yield. The bromoester was converted to iodide **18** in 89% yield. The phosphonium salt **19** was formed in 92% yield by reacting **18** with triphenylphosphine in acetonitrile in 92% yield.

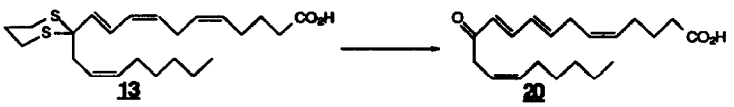


14 **15**

a) 1.2 eq **14**, 1.1 eq $LiN(SiMe_3)_2$, THF/HMPA, -78°C, 40min., 1.0 eq **15**, -78°C to room temperature, crude product reacted with 1.2 eq Bu_4NF, THF, room temperature. b) 1.0 eq DIPHOS, 2.0 eq CBr_4, CH_2Cl_2, 0°-5°C. c) 3.0 eq NaI, acetone, reflux. d) 1.5 eq PPh_3, acetonitrile, reflux.

Scheme 3

We found that ceric ammonium nitrate acetonitrile/H_2O, 80:20, R.T. was the most convenient reagent for the conversion of **13** to **20** as the 8,9-*trans*-KETE[12] was the sole product isolated, making the purification a much easier process.



13 **20**

Scheme 4

The dithiane deprotection step of **13** to yield the 12-KETE **3** deserves some comment. As anticipated based on our previous experience, the 12-KETE is very unstable. It is the most unstable molecule we have had to work with in the lipoxygenase field. Exposure to basic conditions results in complete decomposition. Mild acid catalysis is also detrimental to the molecule, leading to destruction of the molecule, albeit at a slower rate. By way of comparison, the peptido leukotrienes, which were considered very delicate molecules, are relatively stable in aqueous basic solution and in mild acidic medium.

We used a variety of conditions and reaction times in order to optimize conversion of **13** to 12-KETE. Short reaction times of 15-30 seconds were the best. Since most reagents were acidic, we found that acetone was a better solvent than acetonitrile; it probably protects the 12-KETE-carbonyl from protonation and subsequent isomerization and/or damage. Two products were formed under the optimized conditions: 12-KETE (85%) and 8,9-*trans*-12-KETE (15%). The reaction at this point was very clean, and the yield of the two isomers as analyzed by UV was practically quantitative. Work-up had to be rapid and consisted of a quick filtration over celite and silica followed by HPLC.[13] The pure 12-KETE eluate was passed through a short carbonate column[14] to remove the acetic acid, the solvents evaporated and the residue stored in ethanol. The isolated yield of the rigorously pure 12-KETE was 15-20%. We prepared a 5-mg batch by this procedure reproducibly. The pure 12-KETE was stored in ethanol at -20°C and is stable for prolonged periods of time.

Synthetic 12-KETE cochromatographed with biological 12-KETE from porcine leukocytes, prepared as previously described[5] by incubation of 12-S-HETE with neutrophil microsomes in the presence of NAD^+.

The two synthetic 12-KETE isomers were used to verify the earlier identification of these metabolites as products of *Aplysia* nervous tissue.[6] Furthermore, biochemical experiments in which each isomer was incubated with *Aplysia* neural homogenates clearly demonstrate that this preparation does not catalyze the isomerization of either 12-KETE. Thus, the initial identification of *trans*-12-KETE was not an artifact of the sample preparation as had been previously assumed. This finding raises two important questions for further research. First, what is the mechanism of formation for the *trans*-isomer *in vivo* and what is its immediate precursor. Second, does this isomer have any biological activity in tissues previously shown to respond to 12-KETE.[6]

Preliminary experiments show that 12-KETE stimulated cytosolic calcium levels in human neutrophils and the results will be published elsewhere in detail.

ACKNOWLEDGMENTS

We wish to thank the National Institutes of Health for support under NIDDK Grant No. 1 R01 DK44730-01A1, NINDS Grant No. NS-29832, the National Science Foundation for an AMX-360 NMR instrument (Grant CHE-9013145), and the Medical Research Council of Canada.

REFERENCES AND NOTES

1. Woollard, P.M. *Biochem. Biophys. Res. Commun.*, **1986**, *136*, 169-176.
2. Schwartzman, M.L.; Balazy, M.; Masferrer, J.; Abraham, N.G.; McGiff, J.C.; Murphy, R.C. *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 8125-8129.
3. Falgueyret, J.P.; Leblanc, Y.; Rokach, J.; Riendeau, D. *Biochem. Biophys. Res. Commun.*, **1988**, *156*, 1083-1089.
4. Fruteau de Laclos, B.; Maclouf, J.; Poubelle, P.; Borgeat, P. *Prostaglandins*, **1987**, *33*, 315-335.
5. Wainwright, S.L.; Powell, W.S. *J. Biol. Chem.*, **1991**, *266*, 20899-20906.
6. Piomelli, D.; Feinmark, S.; Shapiro, E.; Schwartz, J. *J. Biol. Chem.*, **1988**, *263*, 16591-16596.
7. Murphy, R.; Falck, J.; Lumin, S.; Yadagiri, P.; Zirrolli, J.; Balazy, M.; Masferrer, J.; Abraham, N.; Schwartzman, M. *J. Biol. Chem.*, **1988**, *263*, 17197-17202.
8. Leblanc, Y.; Fitzsimmons, B.J.; Adams, J.; Perez, F.; Rokach, J. *J. Org. Chem.*, **1986**, *51*, 789-793.
9. Fretland, D.J.; Djuric, S.W. *Prostaglandins*, **1989**, *38*, 215-228.
10. UV: λ_{max}:280 nm; **^{1}H-NMR** (360 MHz, $CDCl_3$) δ: 7.55 (dd, J=14.9, 12.0 Hz, 1H, C-10), 6.25 (d, J=15.2 Hz, 1H, C-11), 6.15 (t, J=11.0 Hz, 1H, C-9), 5.90 (dt, J=10.1, 8.0 Hz, 1H, C-8), 4.90-5.20 (m, 4H, C-5, C-6, C-14, C-15), 3.32 (d, J=6.3 Hz, 2H, C-13), 3.08 (t, J=6.5 Hz, 2H, C-7), 2.38 (t, J=7.1 Hz, 2H, C-2), 2.15 (dt, J=7.1, 6.9 Hz, 2H), 2.05 (dt, J=6.9, 6.8 Hz, 2H), 1.70 (m, 2H), 1.20-1.40 (m, 6H), 0.90 (t, J=6.5 Hz, 3H); **^{13}C-NMR** (360 MHz, $CDCl_3$) δ: 199.94, 177.23, 140.63, 137.58, 134.19, 130.41, 128.73, 127.14, 126.79, 120.93, 40.89, 33.13, 31.60, 29.13, 27.67, 26.88, 26.56, 24.63, 22.65, 14.16.
11. Zamboni, R.; Milette, S.; Rokach, J. *Tetrahedron Lett.*, **1983**, *24*, 4899-4902.
12. UV: λ_{max}:272 nm; **^{1}H-NMR** (360 MHz, C_6D_6) δ: 7.28 (dd, J=10.9, 15.4 Hz, 1H, C-10), 6.09 (d, J=15.4 Hz, 1H, C-11), 6.01 (dd, J=11.0, 15.2 Hz, 1H, C-9), 5.85 (m, 1H), 5.76 (dt, J=15.2, 6.4 Hz, 1H, C-8), 5.60 (m, 1H), 5.25 (m, 2H), 3.20 (d, J=6.5 Hz, 2H, C-13), 2.65 (t, J=6.2 Hz, 2H, C-7), 2.12 (t, J=7.3 Hz, 2H, C-2), 2.04 (dt, J=6.9, 6.8 Hz, 2H), 1.92 (dt, J=7.5, 6.9 Hz, 2H), 1.62 (m, 2H), 1.20-1.40 (m, 6H), 0.93 (t, J=6.8 Hz, 3H).
13. Waters μ-porasil 10 μm 3.9x300 mm column; solvent: hexane:*i*-propanol:acetic acid 99:1:0.05; retention time: 12-KETE, 15.74 min., *trans*-12-KETE, 23.37 min.
14. We found the sodium carbonate treatment quite convenient even at the expense of losing some 12-KETE in the process.

(*Received in USA* 17 *March* 1994; *accepted* 15 *April* 1994)