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SYNTHESIS OF 12-HYDROPEROXYEICOSATETRAENOIC ACID (12-HPETE). ON THE STEREOCHEMISTRY IN THE CONVERSION OF 12(S)-HETE TO 12-HPETE

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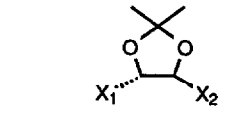
Summary: 12(S)-hydroxyeicosatetraenoic acid methyl ester 1 was synthesized. 1 was converted to phosphite 17 which upon treatment with hydrogen peroxide afforded the corresponding hydroperoxide 18 (12-HPETE methyl ester) with partially retained configuration.

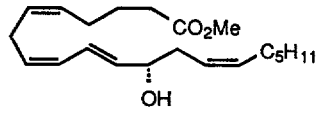
Although 12(S)-hydroperoxyeicosatetraenoic acid (12(S)-HPETE) and the corresponding alcohol 12(S)-HETE have been found in several mammalian sources as metabolites of arachidonic acid,[1] these physiological roles are still unclear. The recent finding that a metabolite of 12-HPETE is a second messenger in *Aplysia* neurons,[2] however, indicates a general importance of 12-HPETE in a variety of biological systems. In order to obtain information about the metabolites of 12-HPETE in various biological sources, we required sufficient amounts of 12-HPETE. We describe here a new synthesis of 12(S)-HETE methyl ester **1**[3] based on Wittig olefination strategy and the direct transformation of **1** into 12-HPETE methyl ester in which an unusual nucleophilic displacement of phosphite group by hydrogen peroxide with a partially retained configuration was observed.

(+)-2,3-O-isopropylidene-L-threitol **2**[4] was converted to monoprotected alcohol **3**[5] in 87% yield by reaction with KH (1 equiv) followed by TBDMSCl (1 equiv) in THF at 0 °C. Swern oxidation of **3** (DMSO-oxalyl chloride, -78 °C, then excess triethylamine) produced crude aldehyde **4** which was directly treated with the anion from phosphine oxide **6**[6] (1.5 equiv) and *n*-BuLi (1.2 equiv) at 0 °C in THF in the presence of HMPA followed by silica gel column chromatography (3 : 1 to 1 : 1 hexane-ethyl acetate) to afford aldehyde **5** in 83% overall yield from alcohol **3** together with adduct **7** (14%). Aldehyde **5** was also generated from adduct **7** in 80% yield by treatment with KH in THF[6] at room temperature for 1.5 h and then silica gel column chromatography. *Cis*-olefination of **5** to **9** was achieved in 81% yield by treatment with the ylid from phosphonium bromide **8** (1.5 equiv) and potassium hexamethyldisilazide (1.2 equiv) in toluene at -78 °C for 10 min and then -78 to 0 °C over 30 min. After desilylation with tetra-*n*-butylammonium fluoride in THF at 0 °C (98%), the resulting alcohol **10** was again converted to aldehyde **12** (70%) along with adduct **13**[7] (17%) via **11** in a similar manner. Direct conversion of **12** to enal **14**[8] was effected by exposure to activated alumina (200 mg of **12**/3 g) in acetonitrile at room temperature for 30 min in 63% yield based on 71% substrate consumption.[9] Finally, Wittig reagent from phosphonium iodide **15**[10] (3 equiv) and potassium hexamethyldisilazide (2.5 equiv) at -20 °C for 20 min in THF was allowed to react with the enal **14** in THF in the presence of HMPA at -78 °C to room temperature over 2 h to afford 12(S)-HETE methyl ester **1**[11] in 57% yield. In order to determine

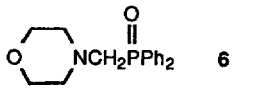
the optical purity at C-12, **1** was converted to menthoxycarbonate derivative of dimethyl malate **16** by the following sequence:[12] 1) conversion of **1** to its menthoxycarbonate derivative with (-)-menthylchloroformate and pyridine in toluene; 2) ozonolysis at -78 °C for 10 min in dichloromethane followed by treatment with 90% hydrogen peroxide in acetic acid at 60 °C for 16 h; and 3) esterification with excess diazomethane. The optical purity of the resulting dimethyl malate derivative **16** was established to be 95% as determined by capillary GC analysis (ULBON HR-1701, 25 m, 190 °C, S isomer: 24.0 min, R isomer: 24.6 min).



	X_1	X_2
2:	$-CH_2OH$	$-CH_2OH$
3:	↓	$-CH_2OTBDMS$
4:	$-CHO$	↓
5:	$-CH_2CHO$	↓
7:	**A**	↓
9:	**B**	↓
10:	↓	$-CH_2OH$
11:	↓	$-CHO$
12:	↓	$-CH_2CHO$
13:	↓	**A**



1 12(S)-HETE methyl ester



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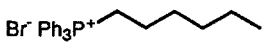

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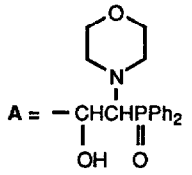
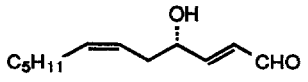
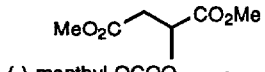




14

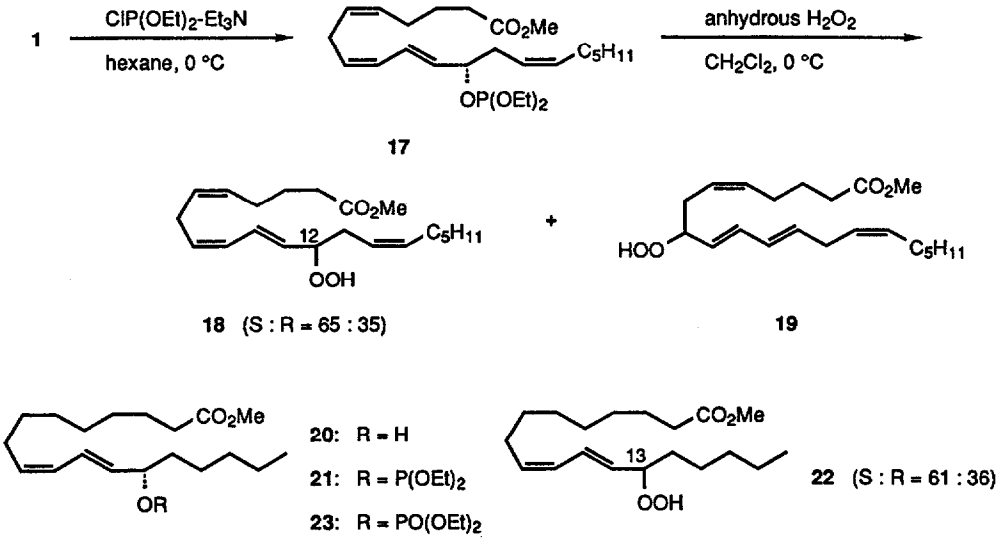
15



16

The reported procedure for the conversion of HETE to HPETE (MesCl-triethylamine in dichloromethane at -65 °C followed by treatment with hydrogen peroxide at -100 °C) is known to result in a complete racemization.[13] Therefore, we explored a new procedure for the transformation of HETE methyl ester into the corresponding hydroperoxide. 12(S)-HETE methyl ester **1** was treated with chlorodiethylphosphite (2 equiv) and triethylamine (3 equiv) in hexane at 0 °C for 30 min. The precipitate formed was filtered off and the filtrate was evaporated under a nitrogen stream to afford phosphite **17** (quantitative) which was allowed to react with anhydrous hydrogen peroxide (30 equiv) in dichloromethane at 0 °C for 1.5 h. Silica gel column chromatography of the reaction mixture (5 : 1 hexane-ethyl acetate) provided a mixture of desired 12-HPETE methyl ester **18**[14] and isomeric hydroperoxide **19**[14] in a 2 : 1 ratio in 74% overall yield from **1**. Pure **18** could be obtained by further purification using straight phase

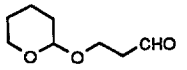
HPLC (COSMO SIL 4.6 mm x 25 cm, 2 mL/min, 100 : 0.3 hexane-isopropanol, **18**: 12.9 min and **19**: 20.9 min).[15] **18** was reduced with trimethylphosphite and the resulting alcohol was derivatized to menthoxycarbonate **16** as mentioned above. Capillary GC analysis showed that the ratio of S malate to R malate is 65 : 35, indicating that *the transformation of 17 into 18 proceeds with a partially retained configuration.*[16] A similar result was also obtained in the conversion of 13(S)-hydroxyoctadecadienoic acid (13(S)-HOD) methyl ester **20** to the corresponding hydroperoxide via phosphite **21** in which **22**[17] was produced in 54% yield with a S/R ratio of 61 : 39 at C-13.[18] In order to learn more about this reaction, the phosphite **21** was oxidized to the phosphate **23** with bis(trimethylsilyl) peroxide.[19] Treatment of **23** with excess anhydrous hydrogen peroxide in dichloromethane at 0 °C also afforded **22** (44%). However, the S/R ratio (56 : 44) was somewhat smaller than that obtained by the original procedure. S-enriched 12-HPETE methyl ester **18** thus obtained could be hydrolyzed to the corresponding free acid with 0.1 N aqueous lithium hydroxide-dimethoxyethane.



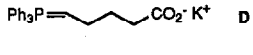
References and Notes

1. a) M. Hamberg and B. Samuelsson, *Proc. Natl. Accad. Sci. U. S. A.*, **71**, 3400 (1974); b) D. H. Nugteren, *Biochim. Biophys. Acta*, **380**, 299 (1975); For 12(R)-HETE: c) P. M. Woollard, *Biochem. Biophys. Res. Commun.*, **136**, 169 (1986); d) D. J. Hawkins and A. R. Brash, *J. Biol. Chem.*, **262**, 7629 (1987).
2. a) D. Piomelli, A. Volterra, N. Dale, S. A. Siegelbaum, E. R. Kandel, J. H. Schwartz, and F. Belardetti, *Nature*, **328**, 38 (1987); b) D. Piomelli, S. J. Feinmark, E. Shapiro, and J. H. Schwartz, *J. Biol. Chem.*, **263**, 16591 (1988).
3. Other syntheses of 12-HETE: a) E. J. Corey, K. Kyler, and N. Raju, *Tetrahedron Lett.*, **25**, 5115 (1984); b) Y. Leblanc, B. J. Fitzsimmons, J. Adams, F. Perez, and J. Rokach, *J. Org. Chem.*, **51**, 789 (1986); c) P. Yadagiri, S. Lumin, P. Mosset, J. Capdevila, and J. R. Falck, *Tetrahedron Lett.*, **27**, 6039 (1986); d) T. Shimazaki, Y. Kobayashi, and F. Sato, *Chemistry Lett.*, **1988**, 1785.

4. **2** was prepared from diethyl (+)-tartrate by protection with 2,2'-dimethoxypropane in the presence of pyridinium *p*-toluenesulfonate (96%) followed by reduction with sodium borohydride (65%).
5. Satisfactory ^{1}Hnmr, ir, and mass spectral data were obtained for all new compounds.
6. N. L. J. M. Broekhof, F. L. Jonkers, and A. van der Gen, *Tetrahedron Lett.*, **26**, 2433 (1979).
7. **13** could be converted to aldehyde **12** by a similar procedure to that described for **7**.
8. ^{1}Hnmr (200 MHz, chloroform-*d*) δ 0.87 (t, 3 H, J = 6.6 Hz), 1.20 - 1.42 (m, 6 H), 2.04 (q, 2 H, J = 6.7 Hz), 2.19 (bs, 1 H), 2.41 (t, 2 H, J = 7.1 Hz), 4.42 - 4.51 (m, 1 H), 5.37 (dt, 1 H, J = 12.0, 7.3 Hz), 5.63 (dt, 1 H, J = 12.0, 7.2 Hz), 6.31 (ddd, 1 H, J = 15.6, 7.8, 1.6 Hz), 6.84 (dd, 1 H, J = 15.7, 4.4 Hz), 9.59 (d, 1 H, J = 7.8 Hz). See ref. 3c.
9. Since **14** was slowly decomposed under these conditions, the reaction should be stopped before 100% substrate consumption.
10. **15** was prepared by the following sequence: 1) coupling of THP ether of 3-hydroxy-1-propanal (**C**) with Wittig reagent **D**; 2) esterification with diazomethane followed by deprotection of THP group to free alcohol with Amberlite® IR-120 (sulfonic acid form); and 3) iodination with iodine-imidazole-triphenylphosphine followed by condensation with triphenylphosphine in acetonitrile.



C



D

11. ^{1}Hnmr (400 MHz, chloroform-*d*) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.22 - 1.39 (m, 6 H), 1.70 (5et, 2 H, J = 7.4 Hz), 2.05 (q, 2 H, J = 7.3 Hz), 2.11 (q, 2 H, J = 7.3 Hz), 2.28 - 2.39 (m, 2 H), 2.33 (t, 2 H, J = 7.4 Hz), 2.93 (t, 2 H, J = 6.5 Hz), 3.67 (s, 3 H), 4.23 (q, 1 H, J = 6.3 Hz), 5.34 - 5.44 (m, 4 H), 5.57 (dtt, 1 H, J = 11.0, 7.2, 2.0 Hz), 5.73 (dd, 1 H, J = 15.0, 6.2 Hz), 5.99 (t, 1 H, J = 10.7 Hz), 6.56 (dd, 1 H, J = 15.2, 11.1 Hz).
12. M. Hamberg, *Analytical Biochemistry*, **43**, 515 (1971).
13. a) E. J. Corey, J. O. Albright, A. E. Barton, and S. Hashimoto, *J. Am. Chem. Soc.*, **102**, 1435 (1980); b) R. Zamboni and J. Rokach, *Tetrahedron Lett.*, **24**, 999 (1983).
14. ^{1}Hnmr (400 MHz, chloroform-*d*) for **18**: δ 0.88 (t, 3 H, J = 7.0 Hz), 1.24 - 1.38 (m, 6 H), 1.70 (5et, 2 H, J = 7.5 Hz), 2.03 (q, 2 H, J = 7.4 Hz), 2.12 (q, 2 H, J = 7.2 Hz), 2.31 (dt, 1 H, J = 14.4. 7.1 Hz), 2.33 (t, 2 H, J = 7.4 Hz), 2.45 (dt, 1 H, J = 14.3, 7.1 Hz), 2.87 -3.02 (m, 2 H), 3.67 (s, 3 H), 4.44 (q, 1 H, J = 7.1 Hz), 5.33 - 5.46 (m, 4 H), 5.49 (dtt, 1 H, J = 11.0, 7.4, 2.0 Hz), 5.62 (dd, 1 H, J = 15.3, 7.4 Hz), 6.01 (t, 1 H, J = 10.9 Hz), 6.62 (dd, 1 H, J = 15.3, 11.0 Hz), 8.13 (s, 1 H); for **19**: δ 0.88 (t, 3 H, J = 6.9 Hz), 1.24 - 1.40 (m, 6 H), 1.70 (5et, 2 H, J = 7.3 Hz), 2.03 (q, 2 H, J = 7.3 Hz), 2.08 (q, 2 H, J = 7.3 Hz), 2.30 (dt, 1 H, J = 13.5, 7.1 Hz), 2.32 (t, 2 H, J = 7.4 Hz), 2.42 (dt, 1 H, J = 13.5, 7.1 Hz), 2.84 (t, 2 H, J = 6.9 Hz), 3.67 (s, 3 H), 4.37 (q, 1 H, J = 7.0 Hz), 5.35 - 5.49 (m, 4 H), 5.52 (dd, 1 H, J = 15.3, 7.5 Hz), 5.74 (dt, 1 H, J = 15.4, 6.8 Hz), 6.07 (dd, 1 H, J = 15.3, 10.3 Hz), 6.29 (dd, 1 H, J = 15.4, 10.4 Hz), 7.87 (s, 1 H). The position attached to hydroperoxy group was determined by mass spectral analysis of methyl hydroxyeicosanoate after reduction and hydrogenation.
15. A small amount of 8-*trans*-12-HPETE methyl ester (ca. 2-3%) was also detected.
16. A similar nucleophilic substitution with a retained configuration has been previously reported. K. Okamoto, K. Takeuchi, and T. Inoue, *J. Chem. Soc. perkin II*, **1980**, 842.
17. Spectral data of **22** was identical with those of authentic 13(S)-HPOD methyl ester prepared from linoleic acid with Soybean lipoxygenase.
18. The product was converted to menthoxycarbonate derivative of 2-hydroxyheptanoic acid methyl ester in a similar manner to the case of **16** and analyzed by capillary GC (ULBON HR-1701, 25 m, 200 °C, S isomer: 12.9 min; R isomer: 13.5 min).
19. Y. Hayakawa, M. Uchiyama, and R. Noyori, *Tetrahedron Lett.*, **27**, 4191 (1986). Direct preparation of **23** from **20** with chlorodiethylphosphate and triethylamine was sluggish.

20. This work was supported by Grant-in-Aid from Ministry of Education and Asahi Glass Foundation. R. N. gratefully acknowledges Japan Society for the Promotion of Science for a financial support.

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