Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 2, pp. 105-108, 1997
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0960-894X/97 $17.00 + 0.00

PII: S0960-894X(96)00598-7

SYNTHESIS AND BIOLOGICAL EVALUATION OF 14,15-DEHYDRO-LTA$_4$ ANALOG.

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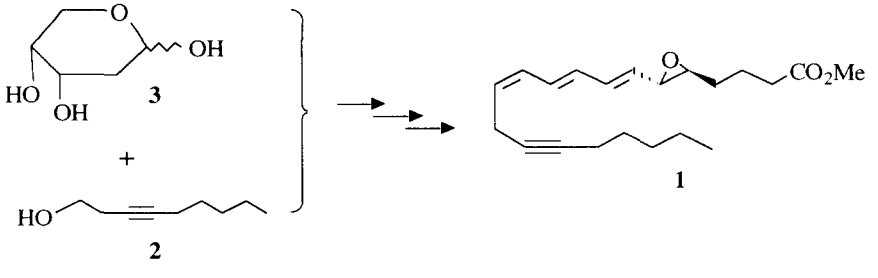
Abstract. New stable leukotriene A$_4$ analog with acetylenic function in positions 14,15 has been synthesized. Conversion of this analog to the corresponding LTC$_4$ derivative and the structural and biological characterization are presented. © 1997, Elsevier Science Ltd. All rights reserved.

Since the discovery of the leukotrienes[1] (LTs) there has been intensive effort to synthesize these products and analogs[2,3]. The cysteinyl leukotrienes C$_4$, D$_4$ and E$_4$ are sequential metabolites of leukotriene A$_4$ (LTA$_4$), itself produced by action of 5-lipoxygenase (5-LO) on arachidonic acid[4]. If a more selective LT intervention than 5-LO inhibition appears warranted in the future, there are several potential downstream targets. Inhibition of the enzyme LTA$_4$ hydrolase blocks the formation of LTB$_4$. Several inhibitors have been reported, but have not yet progressed to clinical evaluation. Significant research has been advanced in characterizing, cloning and expressing LTC$_4$ synthase[5], an enzyme unlike other glutathione transferases. Inhibition of LTC$_4$ synthase provides a selective blockade of the cysteinyl LT metabolites derived from LTA$_4$[5].

Our laboratories are interested by 14,15-dehydro-LTA$_4$ methyl ester **1** which is obtained according to the strategy described in Scheme 1. Conversion of this analog to the corresponding LTC$_4$ derivative as well as its structural and biological characterization are presented.



Scheme 1

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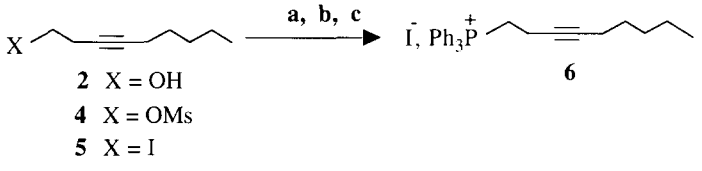
Chemistry

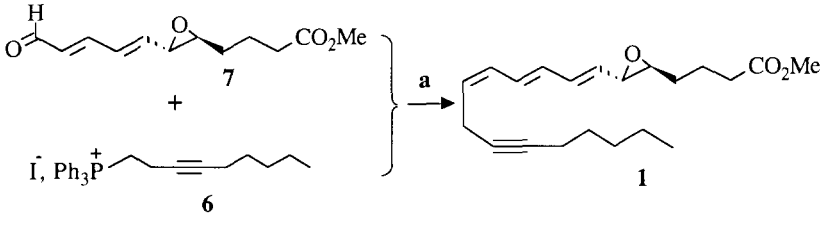
The phosphonium salt **6** was prepared from commercial non-3-yn-1-ol **2** in three steps (Scheme 2). The synthesis of epoxydienal **7** was carried out as described by Rokach[6] and Ernest[7].



Reagents: a: $MsCl/Et_3N/CH_2Cl_2$/-45°C 30 min 100%, **b**: NaI/acetone/reflux 2h 87%,
c: Ph_3P/CH_3CN 90°C 24h 92%.

Scheme 2

The 14,15-dehydro-LTA_4 methyl ester **1**[9] was obtained by coupling of epoxydienal **7** (1 eq) with non-3-ynyl-triphenyl phosphonium iodide **6** (3 eq.) in presence of *n*-butyllithium (3 eq) in a mixture THF/HMPT (5:1) at -78°C for 15 min in 80% yield after purification by flash chromatography (Scheme 3).



Reagents: a: BuLi/THF/HMPT, -78°C, 15 min, 80%

Scheme 3

Biological Results and Discussion

Washed human platelets were prepared according to Patscheke[10] as previously described[11]. LTA_4 and 14-15-dehydro-LTA_4 (dehydro-LTA_4) free acids were obtained through base hydrolysis of the corresponding methyl esthers (MEs) as previously described[12]. Purity was checked by RP-HPLC coupled to diode-array UV detection according to Fitzpatrick[13]. Free acids were added to human platelets (10^8 cells) at the final concentration of 1 µM.

LTA_4-derived metabolites were analysed by RP-HPLC coupled to diode-array UV detection[13], revealing similar chromatographic profiles for LTA_4 and dehydro-LTA_4, with all the chromatographic peaks observed moving to shorter retention times in the case of dehydro-LTA_4 (Fig. 1). The peak corresponding to putative dehydro-LTC_4 showed a UV spectra in agreement with the presence of a sulphur atom conjugated to a triene, with a λ_{max} of 279 nm and shoulders at 269 and 290 nm (Fig. 1, inset).

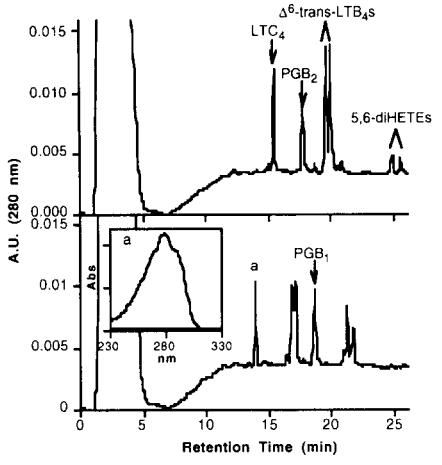


Figure 1. UV absorbance profile at 280 nm from the RP-HPLC of human platelets incubated with LTA$_4$ (Panel A) and 14,15-dehydro-LTA$_4$ (Panel B). Panel B inset: UV absorbance spectrum of peak a.

The HPLC fraction corresponding to the retention time of the postulated dehydro-LTC$_4$ was taken to dryness, reconstituted in PBS and incubated for 60 min at 37°C in presence of 0.5 U.I. of γ-glutamyl transpeptidase (Sigma). RP-HPLC analysis showed an increased retention time of the resulting compound, consistent with the removal of the γ-glutamyc residue from a glutathione conjugate (Fig. 2).

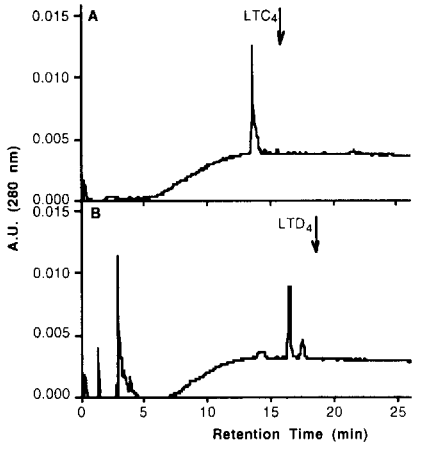


Figure 2. UV absorbance profile at 280 nm from the RP-HPLC of putative 14,15-dehydro-LTC$_4$ analog before (Panel A) and after (Panel B) treatment with γ-glutamyl transpeptidase. Arrows indicate the retention time of authentic LTC4 and its γ-glutamyl transpeptidase metabolite LTD$_4$.

Mass spectrometric analysis of putative dehydro-LTC$_4$ (5-10 μL) was performed by negative ion electrospray ionization using a Sciex API III$^+$ triple quadrupole (PE-Sciex, Thornhill, Ontario, Canada) with spectra acquired from 50-700 atomic mass units at 1 scan/5 sec, resulting in an observed molecular ion at m/z 622, two mass units lower than authentic LTC$_4$. Collision-induced dissociation (CID) and tandem mass spectrometric analyses, with a collision offset energy (E_{lab}) of 20 eV of the molecular ion resulted in a product

ion spectrum identical to that obtained using fast atom bombardment mass spectrometry (FAB-MS)[14], with major ions at m/z 604, 493, 306, 288, 272 and m/z 254 in agreement with the proposed structure.
Concentration of the postulated dehydro-LTC_4 was calculated using an extinction coefficient of 40,000 at 280 nm, and biological activity was assessed using the guinea-pig ileum assay, as previously described[15].
Dehydro-LTC_4 was assessed for concentration that effected contractions equal in amplitude to that of 3 nM LTC_4 and resulted in a contractile potency not different from that shown by the parent compound (relative potency 0.9 ±0.2).

We have described the synthesis of 14,15-dehydro-leukotriene A_4 methyl ester **1** and found that the free acid of this compound is metabolized by human platelet to the corresponding 14,15-dehydro-LTC_4 as efficiently as authentic LTA_4. This compound possesses biological activities undistinguishable from authentic LTC_4. These data provide the information that the double bond at carbon 14,15 does not represent an important structural requirement neither for the recognition of the substrate by the specific human LTC_4-synthase nor for the myotropic activity on the guinea pig ileum, according to previous data on biological activity of a 14,15-dihydro analog of LTC_4[15-16]. Further investigation are under course to establish the suitablity of 14,15-dehydro-leukotriene A_4 as a substrate for the LTA_4-hydrolase.

Acknowledgment. We wish to thank the "Ministere de l'Enseignement et de la Recherche" (MESR) for financial support of (M.G.).

References and Notes

1. Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 2643.
2. Delorme, D.; Guindon, Y.; Lau, C.K.; Zamboni, R. *J. Org. Chem.* **1988**, *53*, 267. Tolstikov, G.A.; Miftakhov, M.S.; Tolstikov, A.G. *Tetrahedron Lett.* **1985**,*26*, 3867. Rokach, J.; Guindon, Y.; Young, R.N.; Adams, J.; Atkinson, J.G. in *Synthesis of Natural Products;* Apsimon, J.; Ed.; Wiley-Interscience: New York, 1988, vol. 7, pp 141-273 .
3. This compound has been prepared in racemic form according to Bestmann, H.J.; Röder, T. *Eicosanoids* **1992**, Suppl., S1.
4. Samuelsson, B. *Adv. prostaglandin, Thromboxane, and leukotriene Res.* **1983**, *11*, 1-13.
5. Lam, B.K.; Penrose, J.F.; Freeman, G.J.; Austen. K.F. *Proc. Natl. Acad. Sci.* U.S.A. **1994**, *91*, 7663. Welsch, D.J.; Creely, D.P.; Hauser, S.D.; Mathis, K.J.; Krivi, G.G.; Isackson, P.C. *Proc. Natl. Acad. Sci.* U.S.A. **1994**, *91*, 9745. Austen, K.F. *Int. Arch.Allergy Immunol.* **1995**,*107*,19. Lam, B.K.; Penrose, J.F.; Xu, K.; Austen, K.F. *J. Lip. Med. Cell. Signalling* **1995**, *12*, 333 . Brooks, C.D.; Summers, J.B. *J. Med. Chem.* **1996**, *39*, 2629 and references herein.
6. Rokach, J.; Zamboni, R.; Lau, C.K.; Guindon, Y. *Tetrahedron Lett.* **1981**,*22*, 2759.
7. Ernest, J.; Main, A.J.; Menasse, R. *Tetrahedron Lett.* **1982**,*23*, 167-170.
8. The structure assigned is consistent with spectroscopic data (360 MHz, ^{31}P, ^{1}H and ^{13}C NMR).
9. The structure assigned is consistent with spectroscopic data (UV, elemental analysis, 360 MHz, ^{1}H and ^{13}C NMR)
10. Patscheke H. *Haemostasis* **1980***10*: 14-22.
11. Sala, A.; Folco, G.; Henson, P.; Murphy, R.C. *Biochem Pharmacol*, **1996**, *in press.*
12. Sala, A.; Bolla, M.; Zarini, S.; Müller-Peddinghaus, R.; Folco, G. *J Biol Chem.* **1996**, *271*, 17945-17948.
13. Fitzpatrick, F.A.; Morton, D.R.; Wynalda, M.A. *J. Biol. Chem.* **1982**,*257*, 4680-4683.
14. Raftery, M.J.; Thorne, G.C.; Orkiszewski, R.S.; Gaskell, S.J. *J. Biomed. Environ. Mass. Spectrom.* **1990**, *19*, 465-474.
15. Sala, A., Civelli, M., Oliva, D., Spur, B., Crea, A.E.G., Folco, G.C., Nicosia, S. *Eicosanoids* **1990**, *3*, 105-110.
16. Drazen, J.M., Lewis, R.A.; Austen, K.F.; Toda, M.; Brion, F.; Marfat, A.; Corey, E.J. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3195-3198.

(*Received in Belgium* 18 *September* 1996; *accepted* 28 *November* 1996)