the C-2 hydroxyl oxygen (O(3)) of topa in complex **1** is slightly longer than the above value, but Cu(II) can promote the oxidation of benzylamine. Although these preliminary experiments are not sufficiently extensive to be able to define the mechanism, the finding that Cu(II) is particularly essential for the acceleration of the oxidation of benzylamine is of interest in connection with the mechanism of amine oxidases. Complex **1** might provide significant information on the catalytic roles of the topa residue and copper in copper-containing amine oxidases. Further studies on the spectral and redox properties and the detailed catalytic reaction of complex **1** are in progress.

Acknowledgment. We are very grateful to Prof. Y. Kushi (this Institute), Prof. S. Kaizaki (Faculty of Science, Osaka University), and Prof. O. Yamauchi, Dr. A. Odani, and Dr. H. Masuda (Faculty of Science, Nagoya University) for helpful discussions. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, and Culture, Japan, for Research in Priority Areas (bioinorganic chemistry) (Grant 03241101).

Supplementary Material Available: Figure showing the stereoscopic view of the molecular packing for the complex **1** and tables of positional parameters and isotropic temperature factors for non-hydrogen atoms, interatomic distances and angles, positional parameters and isotropic temperature factors for hydrogen atoms, and anisotropic thermal parameters for non-hydrogen atoms (6 pages); listings of observed and calculated structure factors (9 pages). Ordering information is given on any current masthead page.

(16) McGuirl, M. A.; Brown, D. E.; McCahon, C. D.; Turowski, P. N.; Dooley, D. M. *J. Inorg. Biochem.* **1991**, *43*, 186.

Novel Tight-Binding Inhibitors of Leukotriene A_4 Hydrolase

Wei Yuan and Chi-Huey Wong*

Department of Chemistry
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, California 92037

Jesper Z. Haeggström, Anders Wetterholm, and Bengt Samuelsson*

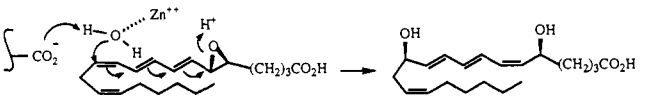
Department of Physiological Chemistry
Karolinska Institute, Box 60 400
S-104 01, Stockholm, Sweden

Received April 8, 1992

Leukotriene (LT) A_4 hydrolase (EC 3.3.2.6)[1] is a zinc-containing[2] monomeric enzyme (MW $\approx$ 70 kDa) which catalyzes the formation of LTB_4 (5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid) from its natural substrate LTA_4 ((5*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid),[3,4] one of the physiologically important processes in the arachidonic acid biosynthetic pathway.[1] It also catalyzes the hydrolysis of some L-amino acid amides.[3,4] The mechanisms of both enzymatic reactions, though not being elucidated, are believed to involve a general base (from a carboxylate) and a Lewis acid (from Zn^{2+}) (Schemes I and II). The zinc ion may coordinate to the nucleophilic water molecule to facilitate the general base catalysis. The peptidase and epoxide hydrolase activities, though they occur in the same active site,[3] may use a different general base (a carboxylate residue) as indicated in recent site-directed mutagenesis studies.[5]

Since LTB_4 is a proinflammatory mediator which stimulates adhesion of circulating neutrophils to vascular endothelium and directs their migration toward sites of inflammation, it is of interest to develop inhibitors of LTA_4 hydrolase as potential antiinflam-

Scheme I. Proposed Mechanism for the Hydrolysis of LTA_4 Catalyzed by LTA_4 Hydrolase



Leukotriene A_4 Leukotriene B_4

Scheme II. Proposed Mechanism for the Aminopeptidase Activity of LTA_4 Hydrolase

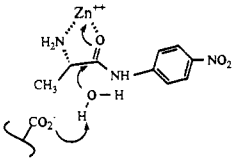
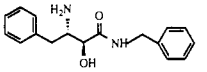
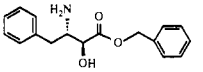
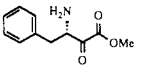
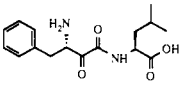
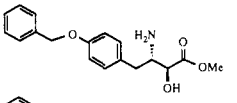
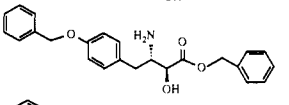
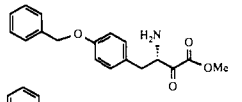
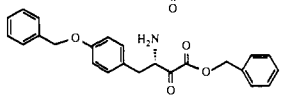


Table I. Inhibitors of LTA_4 Hydrolase

Entry	Compound	IC_{50} (μM)[a]
1		>100[b]
2		20
3		80
4		0.5
5		20
6		0.8
7		0.6
8		IC_{50} = 0.2 μM[c] K_i = 0.1 μM[c]

[a] All assays were performed in Tris-HCl buffer (0.05 M, pH 8.0) with L-alanyl *p*-nitroanilide (1.5 mM) as substrate unless otherwise indicated. LTA_4 hydrolase (1.4 μg) purified from human leukocytes was added for each assay (final volume = 1.0 mL). *p*-Nitroaniline formation was monitored spectrophotometrically at 405 nm. Dixon plot was used to determine the K_i value. [b] Less than 50% inhibition was observed at that concentration. [c] Slow binding behavior was observed. The inhibitor–enzyme mixture was incubated for 30 min prior to addition of the substrate.

(1) For a review, see: Samuelsson, B.; Funk, C. D. *J. Biol. Chem.* **1989**, *264*, 19469.

(2) Haeggström, J. Z.; Wetterholm, A.; Shapiro, R.; Vallee, B. L.; Samuelsson, B. *Biochem. Biophys. Res. Commun.* **1990**, *172*, 965.

(3) Haeggström, J. Z.; Wetterholm, A.; Vallee, B. L.; Samuelsson, B. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 431. Minami, M.; Ohishi, N.; Mutoh, H.; Izumi, T.; Bito, H.; Wada, H.; Seyama, Y.; Toh, H.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 620.

(4) Yuan, W.; Zhong, Z.; Wong, C.-H.; Haeggström, J. Z.; Wetterholm, A.; Samuelsson, B. *Biomed. Chem. Lett.* **1991**, *1*, 551. Several L-amino acid amides such as L-Ala *p*-nitroanilide (K_m = 0.5 mM, V_{max} = 530 nmol/min/mg enzyme) and L-Pro *p*-nitroanilide (K_m = 0.1 mM, V_{max} = 130 nmol/min/mg enzyme) are good substrates. The D enantiomers are not substrates. Values of K_m and V_{max} in the ranges 7–30 μM and 1.7–3.0 μmol/mg/min, respectively, have been reported using LTA_4 as substrate: Radmark, O.; Haeggstrom, J. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1990**, *20*, 35–45. The complementarity components were designed on the basis of computer modeling using MACROMODEL.

(5) Haeggström, J. Z.; Samuelsson, B. Unpublished results.

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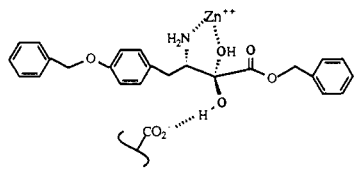
Scheme III. Proposed Inhibitor-Enzyme Complex



matory agents. LTA_4 and its analogs (e.g., LTA_3 and LTA_5)[6] are irreversible inhibitors, and some inhibitors of other Zn^{2+}-containing amino peptidases and angiotensin-converting enzymes are also reversible inhibitors of LTA_4 hydrolase,[7] supporting the proposed mechanism.

Our previous studies on the inhibition of this enzyme with more than 10 peptide-based, synthetic transition-state analog inhibitors (including α-hydroxy β-amino acids and their peptide derivatives, fluoro ketone and phosphoramidate derivatives)[4] have led us to develop another class of compounds (see Table I, **6-8**) which have proven to be better inhibitors than those simply based on the amidase activity. These inhibitors contain a transition-state mimic of the enzyme-catalyzed amide cleavage as a "core" and additional complementarity components (the aromatic moieties) which resemble the hydrophobic nature of the conjugated polyene system of the natural substrate LTA_4, which binds to the enzyme more tightly than the amide substrates.[4] We chose α-keto esters instead of α-keto amides[8] for further development because the ester derivative **2** binds to the enzyme more tightly than the amide **1**. The α-keto amide with a free carboxyl group (**4**) is, however, a better inhibitor (IC_{50} = 0.5 μM) than the corresponding α-(*S*)-OH derivative (IC_{50} = 20 μM; the α-(*R*)-OH derivative is not an inhibitor).[4]

Further adjustment of the inhibitor structure at the P1′ and P2-P3 sites led to the development of an α-keto β-amino ester (**8**) with $K_i = 0.1$ μM. The NMR spectra indicate that both α-keto β-amino amide and α-keto β-amino esters are completely hydrated in water and 60% hydrated in DMSO containing 5% H_2O. We therefore propose that the inhibitor exists as a *gem*-diol bound in the enzyme active site;[9] the free amino group and one of the hydroxyl groups may coordinate to the Zn^{2+} (as *N*-Boc and *N*-Cbz derivatives are not inhibitors) and the other hydroxyl group interacts with the general base (CO_2^-) via H-bonding (Scheme III).

In summary, the α-keto β-amino esters developed in this study are a new class of selective[10] inhibitors of LTA_4 hydrolase. The bound inhibitor seems to resemble the transition-state structure of the enzymatic amide cleavage and LTA_4 binding.[12] Work is in progress to determine the structure of the inhibitor-enzyme complex for mechanistic investigation and to develop better inhibitors.

Acknowledgment. The work at Scripps was supported by the NSF and NIH and that at Karolinska was supported by Swedish Medical Research Council, Magnus Bergvalls, and the O.E. & Edla Johanssons Foundations.

Supplementary Material Available: Listings of experimental procedures and physical data (mass and NMR data) for all of the inhibitors (5 pages). Ordering information is given on any current masthead page.

(6) Evans, J. F.; Nathaniel, D. J.; Zamboni, R. J.; Ford-Hutchinson, A. W. *J. Biol. Chem.* **1985**, *260*, 10966-10970. Nathaniel, D. J.; Evans, J. F.; Leblanc, Y.; Leveille, C.; Fitzsimmons, B. J.; Ford-Hutchinson, A. W. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 827-835. Ohoshi, N.; Izumi, T.; Minami, M.; Kitamura, S.; Seyama, Y.; Ohkawa, S.; Terao, S.; Yotsumoto, H.; Takaku, F.; Shimizu, T. *J. Biol. Chem.* **1987**, *262*, 10200-10205.

(7) Orning, L.; Krivi, G.; Fitzpatrick, F. A. *J. Biol. Chem.* **1991**, *266*, 1375.

(8) α-Keto amide inhibitors of aminopeptidase were reported: Ocain, T. D.; Rich, D. H. *J. Med. Chem.* **1992**, *35*, 451. For other types of keto ester inhibitors, see: Hori, H.; Yasutake, A.; Minematsu, Y.; Powers, J. C. In *Peptides: Structure and Function*, Proceedings of the 9th American Peptide Symposium; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 819. Angelastroc, M. R.; Mehdi, S.; Burkhart, J. P.; Peet, N. P.; Bey, P. *J. Med. Chem.* **1990**, *33*, 11. Hu, L. Y.; Abeles, R. H. *Arch. Biochem. Biophys.* **1990**, *281*, 271.

(9) All α-keto compounds were made as the HCl salt (**3**, **7**, and **8**) or trifluoroacetate salt (**4**). The chemical shifts of β-protons are ~3.8 ppm in D_2O for the hydrate forms and ~5.0 ppm in DMSO for the keto forms. The ^{13}C chemical shift for the α-carbonyl carbon of **8** is 188 ppm in DMSO and 92 ppm in D_2O. These active keto esters or keto amides are similar to fluoro ketones, which are easily hydrated in aqueous solution.[8]

(10) Compounds **6-8** are very weak inhibitors of aminopeptidases; the IC_{50} values are >100, 80, and >100 μM for aminopeptidase M, and 80, 50, and >100 μM for cytosolic leucine aminopeptidase, respectively.

(11) For transition-state structures of Zn^{2+} proteases, see: Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, *235*, 569. Tronrud, D. E.; Holden, H. M.; Matthews, B. W. *Science* **1987**, *235*, 571. Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62. Breslow, R.; Chin, J.; Hilvert, D.; Trainor, G. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4585. Izquierdo-Martin, M.; Stein, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 325. Burley, S. K.; David, P. R.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6917.

(12) The procedures for the synthesis of compounds in Table I are essentially the same as described previously.[4] The keto esters or keto amides were prepared from the corresponding OH compounds via Swern oxidation.

Substituent Effects on Diazomethanes and Diazirines by ab Initio Molecular Orbital Calculations

Michael A. McAllister and Thomas T. Tidwell*

Department of Chemistry, University of Toronto
Toronto, Ontario, Canada M5S 1A1

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Diazo compounds (**1**)[1] and the isomeric diazirines (**2**)[2] are important substrates in synthetic and mechanistic studies, particularly as precursors of carbenes,[3] and substituent effects on all these species is a topic of major current interest.[1-3] Although many substituted derivatives of these compounds have been prepared, there is considerable uncertainty[1,2] as to how substituents affect the ground-state stability of **1** and **2**.[4] The effect of substituents on the diazomethane/diazirine equilibrium[4b-e] and on reactions of these species such as carbene formation cannot be properly evaluated in the absence of an understanding of ground-state substituent effects on **1** and **2**.

We have recently reported ab initio molecular orbital studies on the effect of substituents on ketenes (**3**),[5a] which are isoelectronic to diazomethanes, and on diazocyclopolyenes such as diazocyclopentadiene (**4**),[5b] for which there is evidence for aromatic

(1) (a) Regitz, M.; Maas, G. *Diazo Compounds. Properties and Synthesis*; Academic Press: New York, 1986. (b) *The Chemistry of Diazonium and Diazo Groups*; Patai, S., Ed.; Wiley: New York, 1978.

(2) (a) *Chemistry of Diazirines*; Liu, M. T. H., Ed.; CRC Press, Inc.: Boca Raton, FL, 1987; Vols. I and II. (b) Creary, X. *Acc. Chem. Res.* **1992**, *25*, 31-38. (c) Moss, R. A. *Acc. Chem. Res.* **1989**, *22*, 15-12. (d) Liu, M. T. H. *Chem. Soc. Rev.* **1982**, *11*, 127-140. (e) Cameron, T. S.; Bakshi, P. K.; Borecka, B.; Liu, M. T. H. *J. Am. Chem. Soc.* **1992**, *114*, 1889-1890.

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(5) (a) Gong, L.; McAllister, M. A.; Tidwell, T. T. *J. Am. Chem. Soc.* **1991**, *113*, 6021-6028. (b) McAllister, M. A.; Tidwell, T. T. *J. Am. Chem. Soc.* **1992**, *114*, 5362-5368.

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