from Mössbauer studies[2] but differs from that determined from X-ray photoelectron experiments,[6] which suggest that the two iron atoms are low-spin Fe(III).

The X-band EPR spectra of $(TPPFe)_2N$ recorded at 50 K in a CS_2 glass is shown in Figure 2. The spectrum recorded at 10 K (not shown) showed slightly broader bands, but the salient features remained the same. The spectrum exhibits a signal at $g = 2.15$ which is split into a triplet with a spacing of 22 G. This fine structure is assigned as ^{14}N ($I = 1$) nuclear hyperfine splitting which arises from interaction of the unpaired electron with the nucleus of the bridging nitrogen. The EPR signal observed at $g = 2.01$ is split into a doublet with a separation of 44 G. This splitting could arise from a number of different sources, including nuclear hyperfine interaction or additional magnetic species. The g values which we observe for the EPR signals of $(TPPFe)_2N$ are identical with those reported by Summerville and Cohen.[2] However, in the previous EPR study, *no* nuclear hyperfine splittings were resolved. The origin of the difference between the EPR spectra reported here and those reported previously is not clear. However, a likely possibility is that in the earilier study, which was conducted on a solid sample, the nitrogen hyperfine interactions were rendered unobservable by broadening due to the increased dipolar interactions between the molecules in the solid. In any case, the observation of the large ^{14}N hyperfine interaction clearly indicates that the unpaired electron in $(TPPFe)_2N$ is localized in an orbital which has substantial nitrido character.

The resonance Raman and EPR results for $(TPPFe)_2N$ reported here should aid in better characterizing the electronic structure of the iron atoms in the complex. Additional resonance Raman studies at different excitation wavelengths will further elucidate the properties of the metal centers in the complex. It is also apparent that high-field EPR studies will be useful in order to better determine the origin of the splittings of the EPR signals observed near $g = 2.01$.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Cottrell Research Grants Program of the Research Corp. for support of this research. We thank Mr. R. H. Morse for aid in recording the EPR spectra and Professor S. I. Chan for the use of the EPR facility. We also acknowledge Professor M. F. Rettig for helpful discussions.

G. Alan Schick, David F. Bocian*
Department of Chemistry, University of California
Riverside, California 92521
Received August 11, 1980

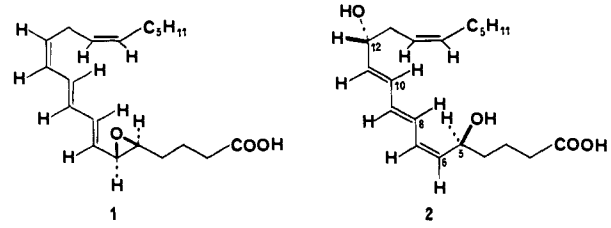
Leukotriene B. Total Synthesis and Assignment of Stereochemistry

Sir:

Arachidonic acid is transformed by incubation with polymorphonuclear leukocytes in low yield into a 5-(*S*),12-(*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid of undetermined stereochemistry with regard to the Δ^6, Δ^8, and Δ^{10} double bonds.[1] This substance was devoid of smooth muscle stimulating activity in the conventional guinea pig ileum assay and its biological role was initially unclear. More recently this compound, now termed leukotriene B (or LTB), has been shown to arise enzymically from the same progenitors, 5-(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HPETE) and 5-(*S*)-*trans*-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (leukotriene A),[2,3] which give rise to the slow reacting substances (leukotrienes C, D, and E).[4] Furthermore leukotriene B has been shown to be chemotactic for macrophages and neutrophils at concentrations of ~ 1 ng/mL[5] and as a result powerfully inflammatory. The detection of LTB in the synovia of patients with rheumatoid arthritis[5b] coupled with its chemotactic potency (greater than any other known lipid chemotactic factor) implies a major role for this substance in the underlying mechanisms of inflammatory and allergic states.[6]

The rarity of native LTB (available in only microgram amounts hitherto), the lack of a detailed structural assignment, and the obvious need for analytical methodology and radiolabeled LTB have prompted the synthetic studies described in this and the following communication. These studies have now led to the complete elucidation of the structure and the capability to prepare readily gram amounts of LTB by a stereoselective and effective process.

Acid-catalyzed hydrolysis of leukotriene A (LTA) (**1**), studied



1 **2**

in these laboratories with pure synthetic LTA,[3] leads to two diastereomeric 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acids (rapidly cleaved by aqueous periodate) and two diastereomeric 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acids (unreactive to aqueous periodate, UV_{max} 268 nm), *neither of which* corresponds by reversed phase high performance liquid chromatography (RP-HPLC) to LTB; this is in accord with previous findings using uncharacterized in situ LTA generated from polymorphonuclear leukocytes.[7-9]

The diastereomeric 5,12-diols from LTA are clearly 6,8,10-*trans*-trienes on the basis of their mode of formation (carbonium ion formation and neutralization to give the kinetically more favored all-trans triene system), UV absorption,[1,7a] and comparison with totally synthetic reference compounds (vide infra). In LTB the most probable 6,8,10-triene geometry involves two trans and one cis double bond as previously pointed out.[1,7a] Our analysis of the transition states for cation formation leading to the three isomers of this type suggested that the most favored energetically should be that affording the 6-*cis*,8-*trans*,10-*trans*-triene (**2**) and the least favored should be that which leads to the 6-trans,8-trans,10-cis isomer (**3**) with the 6-trans,8-cis,10-trans geometry (**4**) intermediate. (Formation of **3** involves severe repulsion between HC(9) and $H_2C(13)$ groups; formation of **4** involves less steric repulsion (principally between HC(7) and HC(10) groups); formation of **2** entails relatively little steric repulsion.) On this basis we favored structure **2** for LTB, a surmise shown to be correct by its total synthesis (and that of the isomers **3** and **4** as well[10]).

(1) Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 2643.
(2) Rådmark, O.; Malmsten, C.; Samuelsson, B.; Clark, D. A.; Goto, G.; Marfat, A.; Corey, E. J. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 954.
(3) Corey, E. J.; Clark, D. A.; Goto, G.; Marfat, A.; Mioskowski, C.; Samuelsson, B.; Hammarström, S. *J. Am. Chem. Soc.* **1980**, *102*, 1436, 3663.
(4) For nomenclature of the leukotrienes see: Samuelsson, B.; Hammarström, S. *Prostaglandins* **1980**, *19*, 645.
(5) (a) Ford Hutchinson, A. W.; Bray, M. A.; Smith, M. J. H. "Inflammation: Mechanisms and Treatment. Proceedings of Future Trends in Inflammation IV"; MTP Press: Lancaster, England, 1980. (b) Klickstein, L. B.; Shapleigh, T.; Goetzl, E. J. *J. Clin. Invest.*, in press. (c) Palmer, R. M. J.; Stepney, R. J.; Higgs, G. A.; Eakins, K. E. *Prostaglandins* **1980**, *20*, 411.
(6) See Goetzel, E. J. *New Engl. J. Med.* **1980**, *303*, 822.
(7) (a) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.*, **1979**, *76*, 3213. (b) Studies in this laboratory were performed by Dr. D. A. Clark.
(8) Chromatographic comparison was performed by using very high selectivity columns prepared in these laboratories by bonding of dimethyloctadecylsilyl to μ-Porasil (Waters Associates) with 3:1 methanol/water containing 0.01% acetic acid as the mobile phase. Native LTB and all but one of the diastereomers prepared in this research could be distinguished in this system.
(9) Small amounts of other products arise from acid-catalyzed hydrolysis of LTA, but no LTB could be detected by RP-HPLC analysis.

0002-7863/80/1502-7984$01.00/0

The C(1)–C(6) segment for the construction of **2** was synthesized as follows: Reaction of 2-deoxyribose with 1.3 equiv of 2-methoxypropene and 0.02 equiv of pyridinium tosylate in ethyl acetate at 23 °C for 3 h followed by stirring with pH 5.5 phosphate buffer at 23 °C for 10 min furnished after isolation of 60% of the deoxyribopyranose 3,4-acetonide (**5**), $[\alpha]^{25}_D$ −18.5° (*c* 4.2, $CHCl_3$).[11] Treatment of **5** with 1.1 equiv of [(methoxycarbonyl)methylene]triphenylphosphorane in dimethoxyethane (DME) containing a trace of benzoic acid[3] at reflux under argon for 6 h effected Wittig coupling to an α,β-unsaturated ester which upon hydrogenation (1 atm) in ethyl acetate at 23 °C for 4 h over 10% palladium-on-charcoal catalyst afforded the hydroxy ester **6** [95% from **5**; $[\alpha]^{23}_D$ +21.8° (*c* 2.7, $CHCl_3$)]. Reaction of **6** with 1.2 equiv of tosyl chloride in pyridine at 23 °C for 12 h gave the corresponding monotosylate ketal, mp 78 °C, $[\alpha]^{25}_D$ +23.7° (*c* 3.7, $CHCl_3$), which was deketalized (2% dry HCl in methanol and 3.5 mL/g of ketal at 23 °C for 7 h) to a glycol which upon exposure to 2 equiv of potassium carbonate in dry methanol at 23 °C for 30 min provided the epoxy ester **7**, $[\alpha]^{23}_D$ +18.9° (*c* 2.6, $CHCl_3$) (90% from **6**). Benzoylation of the hydroxy group in **7** (1.2 equiv of benzoyl chloride in pyridine and 10 mL/g of **7** at 23 °C for 1 h) and subsequent oxirane → 1,2-glycol cleavage (1:0.3:0.03 dimethyl carbonate/water/70% perchloric acid, 13 mL/g of substrate, at 23 °C for 4 h) proceeded smoothly to form methyl 5-benzoyloxy-6,7-dihydroxyheptanoate which by treatment with 1.05 equiv of lead tetraacetate in methylene chloride containing 2 equiv of powdered sodium carbonate at −40 °C for 10 min led to the aldehyde ester **8**, $[\alpha]^{25}_D$ −33.3° (*c* 2.5, $CHCl_3$) (74% overall from **7**).

The C(7)–C(20) segment for the synthesis was prepared in a stereochemically unambiguous manner starting with the cyclic hemiacetal **9** which is easily available in 57% yield from inexpensive D-(+)-mannose as previously described.[12] Wittig reaction of **9** with 2 equiv of *n*-hexylidenetriphenylphosphorane in 9:1 tetrahydrofuran (THF)/hexamethylphosphoric triamide (HMPA) at −78 °C for 0.5 h, from −78 to −20 °C for 3 h, and −20 °C for 72 h under argon occurred stereospecifically to give the *cis*-olefin **10**, R = H, $[\alpha]^{25}_D$ +1.35° (*c* 1.7, cyclohexane) (70%), further converted by exposure to tosyl chloride (2 equiv)–pyridine at 23 °C for 3–4 days to **10**, R = Ts, $[\alpha]^{25}_D$ +13.4° (*c* 1.7, cyclohexane) (97%). Deprotection of **10**, R = Ts (10% dry HCl in methanol at 23 °C for 8 h), and subjection of the resulting tosylate triol to the sequence (1) reaction with 1.1 equiv of phenyl chloroformate and 2 equiv of pyridine in methylene chloride at 0 °C for 1 h and (2) reaction with 5 equiv of diazabicyclo[4.3.0]nonene in THF at 75 °C for 9 h provided the *cis*-epoxide **11** (66% overall from **10**, R = Ts, $[\alpha]^{25}_D$ −5.6° (*c* 1.75, $CHCl_3$). Hydrolysis of the carbonate **11** by using 5 equiv of lithium hydroxide in 5:1 DME/H_2O at 23 °C for 2 h gave 95% of the corresponding 1,2-diol[11] which upon glycol cleavage (1.1 equiv of lead tetraacetate in methylene chloride at −45 °C for 30 min) afforded an aldehyde (95%) which was transformed by reaction with 2 equiv of allylidenetriphenylphosphorane in THF (from −40 to −10 °C for over 30 min under argon) into the epoxy triene **12** (70%), $[\alpha]^{25}_D$ +40.37° (*c* 3.5, $CHCl_3$). Treatment of **12** with 1.0 equiv of dry hydrogen bromide in methylene chloride at 23 °C for 30 min effected stereospecific conversion to the bromo alcohol **13**, X = Br, which upon reaction with 3 equiv of triphenylphosphine in the same solvent at 23 °C for 18 h gave the crystalline phosphonium bromide **13**, X = $Ph_3P^+Br^-$, in 70% yield from **12**.

Conversion of the hydroxy phosphonium salt **13**, X = $Ph_3P^+Br^-$, to the corresponding oxidophosphonium ylide was carried out using 2 equiv of *n*-butyllithium in THF at −40 °C for 30 min. After the solution was cooled to −78 °C, 15 equiv of HMPA and 1 equiv of the aldehyde ester **8** were added sequentially and the reaction was allowed to proceed at −78 °C (30 min), from −78 to −20 °C (30 min), and from −20 to 0 °C (30 min). Extractive isolation and thin-layer chromatography furnished ~32% of the 5-benzoyl derivative of **2** methyl ester, UV_{max} 260, 270, 281 nm, $[\alpha]^{25}_D$ +164.4° (*c* 1.4, $CHCl_3$). Hydrolysis of this product by using 10 equiv of potassium carbonate in methanol (25 °C, 6.5 h), followed directly by lithium hydroxide in methanol–water (2:1) at 23 °C for 1 h, acidification to pH 5–6 with acetic acid and purification by RP-HPLC[8] proceeded cleanly and afforded leukotriene B (**2**), UV_{max} (CH_3OH) 260, 270.5, 281 nm (ε 38 000, 50 000, 39 000) (identical with native LTB), along with ~15% of the 6-trans isomer of **2**,[13] UV_{max} (CH_3OH) 258, 268, 279.5 nm. RP-HPLC[8] retention volumes of native LTB and synthetic **2** were identical (5.0) and different from those for the 6-trans isomer of **2** (4.0) and the isomer **3** (4.4). Although RP-HPLC did not distinguish between **2** (or LTB) and the stereoisomer **4**, isomer **4** was clearly differentiated from **2** (or LTB) by bioassay[14] and by its UV spectrum [UV_{max} (CH_3OH) 258, 268, 278 nm].[10]

Synthetic **2** was indistinguishable from native LTB with regard to chemotactic activity toward neutrophils (measurements in two different laboratories[14a]) and effect on pulmonary tissue.[14b,15]

The identity of **2** with native LTB and the observed differences in properties of **3**, **4**, or the 6-trans isomer of **2**, in comparison with native LTB, allow the unambiguous assignment of structure **2** to LTB. This fact and the ready availability of synthetic LTB provide a basis for forthcoming biological and medical studies which are bound to be of major significance.[16]

Supplementary Material Available: Experimental details (21 pages). Ordering information is given on any current masthead page.

(10) See: Corey, E. J.; Hopkins, Paul B.; Munroe, John E.; Marfat, Anthony; Hashimoto, Shun-ichi *J. Am. Chem. Soc.* **1980**, *102*, following paper in this issue. Isomers **3** and **4** referred to in the text above are shown in the following communication as structures **2** and **3**.

(11) Satisfactory spectroscopic data (^1H NMR, IR, UV, and mass) were obtained on a chromatographically homogeneous sample of each stable intermediate.

(12) Corey, E. J.; Goto, G. *Tetrahedron Lett.*, **1980**, *21*, 3463.

(13) The 6-trans isomer of **2** prepared in this way was identical with one of the diastereomeric 5,12-diols (that which elutes earlier in RP-HPLC) produced by (nonenzymic) acid-catalyzed hydrolysis of LTA (**1**). UV measurements, made using a Perkin-Elmer 559-A spectrometer carefully calibrated using aqueous holmium trichloride or holmium oxide glass, are precise to at least 0.5 nm.

(14) (a) We are indebted to Professors E. J. Goetzl, K. F. Austen, and R. A. Lewis of the Harvard Medical School and Dr. Ivan Otterness of the Chas. Pfizer Co. for these bioassays. (b) Bioassays performed by Dr. Pierre Sirois, University of Sherbrooke. Detailed biological data on **2**, **3**, and **4** and native LTB (**2**) will be published separately.

(15) We are grateful to Drs. E. J. Goetzl, Pierre Borgeat, and Bengt Samuelsson for microgram amounts of native LTB.

(16) This research was assisted financially by the National Science Foundation and the National Institutes of Health.

E. J. Corey,* Anthony Marfat
Giichi Goto, Francis Brion
Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

Received November 3, 1980