mg, 13%). Compound 3 was identified by comparison of its ^1H NMR with that of authentic 3. The ^1H NMR spectrum of an authentic sample of 7 was used to confirm its presence in the crude meDAST reaction mixture.

Biological Methods. [*methyl*-^3H]-*S*-Adenosylmethionine was purchased from Amersham Corp. (Arlington Heights, IL) and was converted to [*methyl*-^3H]MTA by the method of Schlenk et al.[19] Radiolabeled MTA was purified by TLC using acetone/ethyl acetate/H_2O (6:3:1) as solvent. 5'-Deoxy-5'-iodoadenosine (5'-IAdo) was purchased from Sigma Chemical Co. (St. Louis, MO).

MTA Phosphorylase. MTA phosphorylase was purified from mouse liver cytosol by acid precipitation to pH 5.0 with 1 M acetic acid. After removal of the precipitate by centrifugation, the supernatant was brought to pH 7.0 by titration with 0.5 M bicine, pH 9.2. The neutralized supernatant was heated to 65 °C and kept at this temperature for 2 min. After chilling, denatured protein was removed by centrifugation. The enzyme was then loaded on DEAE-Sephacel equilibrated with 10 mM HEPES, pH 7.4, and 1 mM dithiothreitol. The column was developed with a gradient of KCl in the above buffer and MTA phosphorylase eluted at ca 0.08 M KCl. Active fractions were pooled, concentrated with poly(ethylene glycol), and dialyzed against 10 mM HEPES, pH 7.4, and 1 mM dithiothreitol. This material, purified 43.5-fold with an 86% yield, was used as the source of enzyme.

Assay for MTA Phosphorylase Activity. Enzyme activity was determined by measuring the conversion of [*methyl*-^3H]MTA to [*methyl*-^3H]MTRP. The standard reaction mixture contained 4 μmol of potassium phosphate, pH 7.4, 0.1 μmol of dithiothreitol, 20 μg of bovine serum albumin, 50 nmol of MTA, and approximately 2×10^5 cpm [*methyl*-^3H]MTA in a final volume of 0.1 mL. After incubation at 37 °C for 10 min, the reaction was stopped by the addition of 0.1 mL of charcoal solution (1 g of norite, 0.5 g of dextran, 20 mL of 10% trichloroacetic acid). After 5 min on ice, the suspension was centrifuged and an aliquot of the clear supernatant was counted in a liquid scintillation spectrometer.

The ability of MFMTA and 5'-FMTA to act as substrates for MTA phosphorylase was tested by the spectrophotometric assay of Savarese et al.[20]

Cell Growth. Murine lymphocytic leukemia L1210 and L5178Y cell lines were grown in RPMI 1640 medium supplemented with 10% Nu Serum IV (Collaborative Research, Inc., Lexington, MA) and HEPES/MOPS buffer as previously described.[16] Cell cultures (0.3×10^5 cells/mL) were treated at 0 h with 5'-IAdo (Aldrich Chemical Co.), MFMTA, or MTA at concentrations up to 1000 μM and 5'-FMTA up to 100 μM. All compounds were dissolved in dimethyl sulfoxide and diluted in serum-free medium. After 48 h, cells were removed and cell number was determined by electronic particle counting (Cell-Dyne 300, Sequoia-Turner Corp., Mountain View, CA). The results were expressed as percent of cell growth in the absence of added inhibitors. The concentration that resulted in 50% growth inhibition (IC_{50} at 48 h) was determined.

Polyamine Pools. After cells were treated for 48 h with MFMTA or MTA at their approximate IC_{50} concentrations, a sample of cells was taken for polyamine determinations after extraction with 0.6 M perchloric acid (10^7 cells/0.5 mL of acid). The extract (50 μL) was analyzed by HPLC by using a system based on cation exchange and postcolumn derivatization with o-phthalaldehyde as described by Porter et al.[21]

Acknowledgment. This work was supported by Grants CA37606 and CA24538 from the National Cancer Institute.

Registry No. 3, 2457-80-9; 4, 3387-65-3; 5, 119771-16-3; 6, 119771-18-5; 7, 119771-17-4; (5*R*)-8, 119771-19-6; (5*S*)-8, 119771-20-9; 9, 118560-47-7; (5*R*)-10, 119771-21-0; (5*S*)-10, 119771-22-1; MTA phosphorylase, 61970-06-7.

(19) Schlenk, F.; Zydek-Cwick, C. R.; Hutson, N. K. *Arch. Biochem. Biophys.* **1971**, *142*, 144.

(20) Savarese, T. M.; Crabtree, G. W.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1981**, *30*, 189.

(21) Porter, C. W.; Cavanaugh, P. F., Jr.; Stolowich, N.; Ganis, B.; Kelly, E.; Bergeron, R. J. *Cancer Res.* **1985**, *45*, 2050.

18-Cycloalkyl Analogues of Enisoprost[1]

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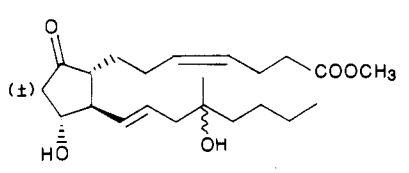
By use of standard cuprate methodology, a series of 18-cycloalkyl analogues of enisoprost was prepared in an effort to impede ω chain metabolism and prolong duration of gastric antisecretory activity. An initial product of ω chain oxidation, the C-20 hydroxy analogue, was also synthesized for pharmacological comparison. The cyclopropyl, cyclobutyl, and cyclopentyl analogues were approximately one-fourth as potent as enisoprost in inhibiting gastric acid secretion, while the cyclohexyl and cycloheptyl analogues showed very weak activity, and the 20-hydroxy compound was inactive at a dose 100 times the ED_{50} of enisoprost. The cyclobutyl compound had a longer duration of antisecretory action than enisoprost and the other cycloalkyl analogues. The cycloalkyl analogues unexpectedly possessed low diarrheogenic activity in rats.

Natural prostaglandins are susceptible to three major modes of metabolic inactivation: side chain hydroxyl oxidation, β-oxidation of the acid chain, and oxidation of the ω chain terminus. The oxidative events occur rapidly in animals and humans and have been a critical drawback for the potential therapeutic application of E-type prostaglandins to peptic ulcer disease.[2] Over the years the primary focus of our synthetic prostaglandin program has been the design of structural modifications that would prevent or slow these metabolic processes and thus provide analogues with an improved activity/duration profile compared to natural prostaglandins.

Enisoprost, 1, is a 16-hydroxy prostaglandin analogue currently under clinical study for the treatment of peptic ulcer disease and related conditions.[3] In addition to a



1

methyl group at C-16 to block the metabolic oxidation of the hydroxy moiety, enisoprost also contains a cis double bond at C-4,5 that reduces susceptibility to β-oxidation.[4]

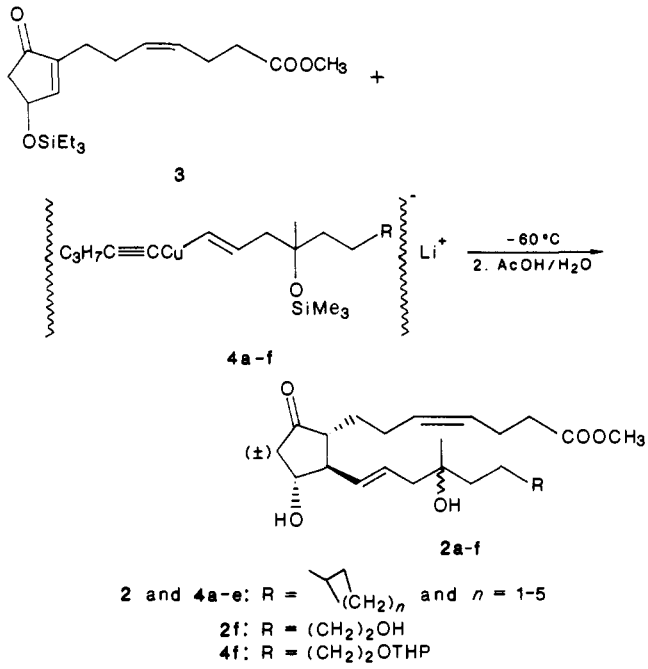
(1) This work was presented at the 192nd National Meeting of the American Chemical Society, September 1986, Anaheim, CA.

(2) Collins, P. W. *J. Med. Chem.* **1986**, *29*, 437.

(3) Howden, C. W.; Burget, D. W.; Hunt, R. H. *Clin. Sci.* **1986**, *71*(S15), 75.

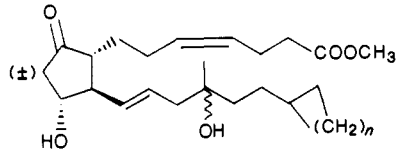
0022-2623/89/1832-1001$01.50/0

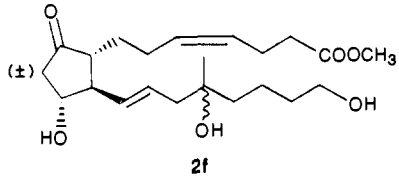
Scheme I



The improved gastric antisecretory activity and duration of action of enisoprost, relative to the saturated parent misoprostol,[5] is presumably due to its increased metabolic resistance.

We have also investigated strategies for blocking or impeding the third metabolic attack point, oxidation of the ω chain terminus (C-19,20). This report details one of our efforts toward this end. On the basis that substitution of a cycloalkyl moiety at the ω terminus of 1 might sterically impede enzymatic attack or that elimination of the primary terminal carbon would remove a requirement for the oxidative process, a series of 18-cycloalkyl analogues of enisoprost (**2a–e**) was prepared. In addition, to determine the effect of ω oxidation on gastric antisecretory activity, the C-20 hydroxyl analogue **2f**,[6] an initial product of the ω chain oxidative process, was also synthesized.

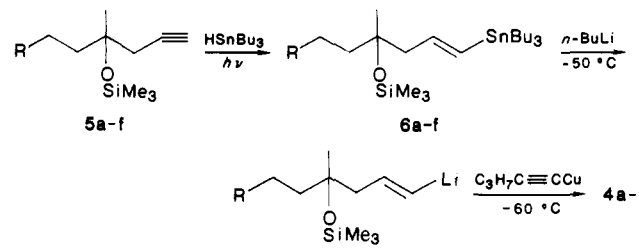




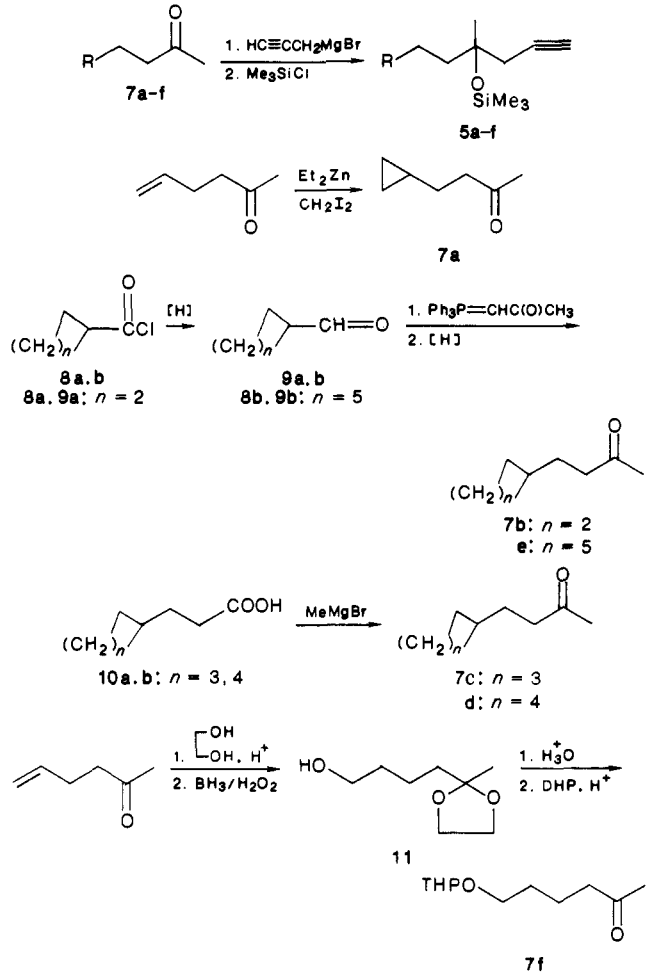
Chemistry

The key step in the synthesis of these compounds was the well-documented[2] stereospecific conjugate addition of the respective cuprate reagents (**4a–f**) to the cyclopentenone **3**[5] followed by acetic acid hydrolysis of protecting groups and chromatographic purification to give **2a–f**[6] (Scheme I). The cuprate reagents (**4a–f**) were prepared by previously described methodology.[5] Thus irradiation with a sunlamp of a mixture of the individual acetylenes **5a–f** (Scheme II) and tri-*n*-butyltin hydride produced the analogous (*E*)-vinylstannanes, **6a–f**. Treatment of the stannanes with *n*-butyllithium at −50 °C followed by addition of an ethereal solution of copper 1-pentyne solubilized with hexamethylphosphorous triamide gave the cuprate reagents **4a–f**. The acetylenic side chains (**5a–f**) were prepared as shown in Scheme III. Reaction of the respective methyl ketones **7a–f** with the Grignard reagent derived from propargyl bromide followed by treatment with trimethylchlorosilane and imidazole in DMF provided the protected acetylenic side chains **5a–f**. The necessary methyl ketones were obtained by several different pathways (Scheme III). The cyclopropyl compound **7a** was prepared by treatment of 5-hexen-2-one with diethylzinc and methylene iodide in benzene solution at

Scheme II



Scheme III



(4) Green, K.; Samuelson, B.; Magerlein, B. J. *Eur. J. Biochem.* **1976**, *62*, 527.
(5) Collins, P. W.; Dajani, E. Z.; Pappo, R.; Gasiecki, A. F.; Bianchi, R. G.; Woods, E. M. *J. Med. Chem.* **1983**, *26*, 786.
(6) All compounds described (1 and **2a–f**) are mixtures of two racemates.

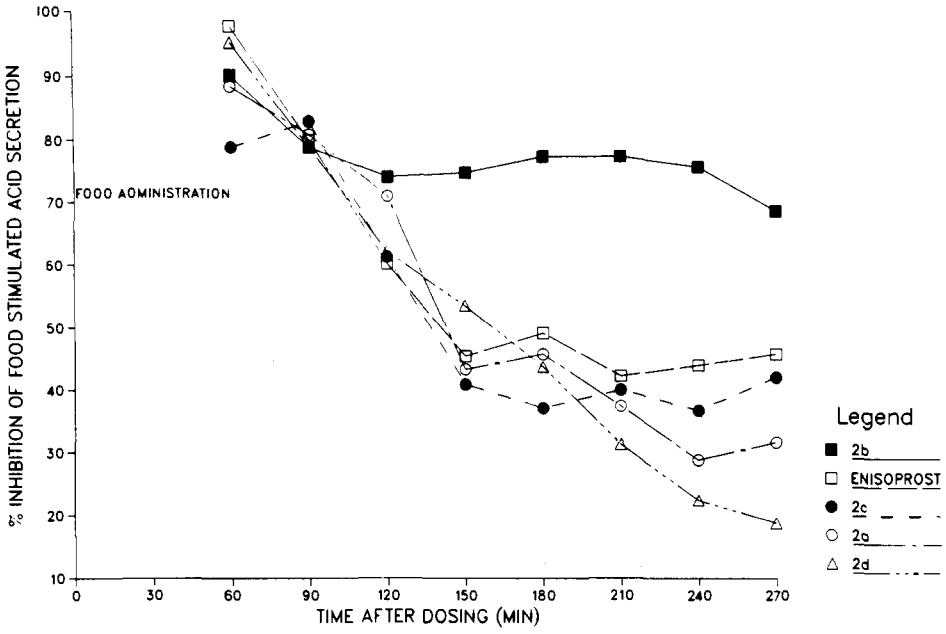


Figure 1. Time profile of the gastric antisecretory effects of approximately equipotent doses of compounds **2b** (0.2 μg/kg), enisoprost (0.03 μg/kg), **2c** (0.3 μg/kg), **2a** (0.3 μg/kg), and **2d** (10 μg/kg). Food stimulus for secretion given 30 min after compound and inhibition of acid secretion determined from 30 to 270 min after dosing. Compounds not underscored below with the same line exhibited significantly ($p < 0.05$) different antisecretory activity at 270 min:

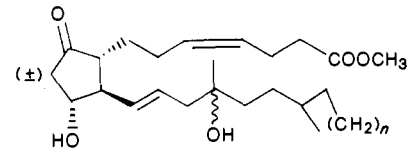
2b enisoprost 2c 2a 2d

room temperature. The cyclobutyl and cycloheptyl analogues **7b** and **7e** were obtained by Rosenmund reduction of the corresponding acid chlorides to give the aldehydes **9a,b**, condensation with 1-(triphenylphosphoranylidene)-2-propanone to provide the unsaturated methyl ketones and catalytic hydrogenation to produce the saturated methyl ketones **7b** and **7e**. The cyclopentyl and cyclohexyl analogues **7c** and **7d** were obtained in moderate yield (40%) by low-temperature (−15 °C) addition of 2 equiv of methylmagnesium bromide to the corresponding commercially available acids **10a,b**. The hydroxy analogue **7f** was obtained by hydroboration/oxidation of the ethylene ketal of 5-hexen-2-one to give the hydroxy ketal **11**. Acidic deketalization followed by acid-catalyzed protection of the alcohol with dihydropyran provided **7f**.

Results and Discussion

The compounds were evaluated for gastric antisecretory activity in Pavlov pouch dogs by intrapouch administration and for diarrheogenic side effects in rats by intragastric administration. The results and comparison with the parent compound enisoprost (1) are presented in Table I. Not listed in Table I is the 20-hydroxy analogue **2f**, which was prepared to determine the effect of ω oxidation on gastric antisecretory activity. As anticipated, **2f** was significantly less active than enisoprost. In fact, **2f** showed no inhibition of acid secretion at 3.0 μg/kg,[7] a dose 100 times the ED_{50} value for enisoprost. Thus metabolic oxidation of the ω chain of enisoprost and related 16-hydroxy prostaglandins to the corresponding 20-hydroxy derivatives virtually eliminates gastric antisecretory activity, and blockage of this process is clearly an important strategy for improving activity and duration of action in this series of compounds. The cyclopropyl (**2a**), cyclobutyl (**2b**), and cyclopentyl (**2c**) compounds were each approximately one-fourth as potent as enisoprost in inhibiting gastric acid secretion (Table I). In contrast, the larger cycloalkyl analogues, **2d** and **2e**, were very weakly active as gastric antisecretory agents. Compounds **2a–d** were also examined for duration of gastric antisecretory effects relative to enisoprost. Each compound was given at a dose that caused approximately 80% inhibition of acid secretion for the second collection period (60–90 min after dosing, the time of peak acid secretion in control experiments) and were followed for a 4.5-h period after dosing (Figure 1). Only the cyclobutyl analogue **2b** exhibited significantly ($p < 0.05$) greater antisecretory activity than enisoprost 4.5 h after administration. In contrast, the cyclopentyl compound **2c** showed antisecretory activity similar to that of enisoprost while **2d** was significantly less active for the last 60 min of the study. The activity of the cyclopropyl analogue **2a** was intermediate between **2c** and **2d** and not

Table I. Comparative Oral Gastric Antisecretory and Diarrheal Effects of Enisoprost Analogues



2a-e: $n = 1$-5

compound	n	ED_{50}, μg/kg, and 95% confidence limits: gastric antisecretory activity in dogs[a]	diarrheal efects in rats[b]
enisoprost (1)		0.023 (0.017–0.032)[13]	49 (37–77)
2a	1	0.09 (0.083–0.094)	273 (106–698)
2b	2	0.09 (0.069–0.096)	316 (141–1134)
2c	3	0.09 (0.06–0.12)	1310 (947–1848)
2d	4	70 (63.2–76.9)	>3160
2e	5	≈95[c]	>3160

[a] Determined in food-stimulated Pavlov dogs by intrapouch administration. ED_{50} values for new compounds were generated with 2–12 dogs/dose and four to six doses/compound. [b] Determined in adult male rats by intragastric administration. [c] An exact ED_{50} and 95% confidence limit were not determined because of insufficient compound supply.

(7) Compound **2f** was not tested at higher doses than 3.0 μg/kg so an ED_{50} value could not be determined.

statistically different from either compound. Thus, on the basis of the existing data, the order of duration of action in this series is cyclobutyl > enisoprost ≥ cyclopentyl ≥ cyclopropyl ≥ cyclohexyl.[8] Since the only structural differences between these compounds are in the ω chain, the variations in activity profiles suggest that ω oxidation does occur in this series of compounds and that it may be the process governing their duration of action. However, the reasons for metabolic rate differences between the compounds are unclear and the precise metabolic events are not known. An alternative explanation for the variations in duration of action is that the cycloalkyl groups differentially influence other metabolic pathways, for example β-oxidation of the α chain. Detailed metabolic studies are obviously needed to clarify the metabolic role of the cycloalkyl groups.

The distinguishing feature of these compounds, however, was their unexpectedly low diarrheogenic activity relative to that of enisoprost. As seen in Table I, all of the cycloalkyl analogues have higher ED_{50} values for diarrheogenic activity in rats than enisoprost, and the values increase with the size of the cycloalkyl ring. Since diarrhea is a significant clinical side effect with antiulcer prostaglandins,[2,9] this is an important finding and the first indication that ω chain modification can differentiate receptor affinities of 16-hydroxy prostaglandins. Although the ω chain terminus probably does not play a key role in receptor binding affinity, the increased ω chain bulk in the series must be interfering with interaction of the molecules at receptors involved in the diarrheogenic response. In contrast, the parietal cell antisecretory receptor must be more accommodative of larger ω chains. We are currently exploring this lead with the goal of discovering a nondiarrheogenic prostaglandin analogue and also of extending the activity separation to other undesirable biological effects of prostaglandins.

Experimental Section

The NMR spectra were obtained on either a Varian FT-80A, a Varian XL-200, or a GE-QE-300 spectrometer in $CDCl_3$ with Me_4Si as internal standard. The ^{13}C NMR spectrum was determined on a Varian XL-200 spectrometer at 75 MHz with the APT pulse technique. Infrared spectra were recorded on a Perkin-Elmer 685 spectrometer in $CHCl_3$. Elemental analyses were within ±0.4% of the theoretical values. Solvents were removed under reduced pressure on a rotary evaporator. TL chromatograms were run on Polygram Sil G/UV plastic sheets (Macherey-Nagel Co.) with PMA as visualization agent.

4-Cyclopropyl-2-butanone (7a). To a solution of 4.9 g (50 mmol) of 5-hexen-2-one in 275 mL of benzene under argon was added 75 mL of diethylzinc (25% solution in toluene; 150 mmol) over 15 min at room temperature. The reaction mixture was stirred for 5 min and then was treated over a 15-min period with a solution of 40 g (150 mmol) of methylene diiodide in 50 mL of benzene.[10] The reaction mixture was allowed to stand at room temperature for 36 h and then was poured into a mixture of hexane and 1 N HCl and shaken well. The layers were separated, and the aqueous layer was extracted with hexane twice, and the combined organics were washed with water three times and once with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The residue was distilled at water aspirator pressure (15 mmHg) to afford 3.5 g (62.5%) of a clear liquid: bp 57–60 °C; ^1H NMR δ 0.1–0.6 (complex band, 4 H's, cyclopropyl protons), 0.81 (m, C-5H), 1.48 (q, J = 7 Hz, C-4H), 2.13 (s, C-1H), 2.63 (t, J = 7.5 Hz, C-3H); IR 1710 cm^{-1}.

Cyclobutanecarboxaldehyde (9a). Ten grams (84.3 mmol) of cyclobutylcarbonyl chloride (**8a**) in 120 mL of THF was hydrogenated at 5 psi for 3.5 h at room temperature with 1.5 g of a 10% Pd/C as catalyst. The reaction mixture was filtered and used directly in the next step due to the volatile nature of the product.

4-Cyclobutyl-2-butanone (7b). The hydrogenation solution from the previous experiment was mixed with 2 volumes of benzene and 26.8 g (84.3 mmol) of 1-(triphenylphosphoranylidene)-2-propanone. The mixture was mechanically stirred and refluxed under N_2 for 8 h. The reaction mixture was allowed to stand at room temperature overnight and filtered, and the filtrate was carefully concentrated on a rotary evaporator at 5 °C to a small volume. The residue was treated with 300 mL of hexane and chilled in an ice bath to precipitate triphenylphosphine oxide. The solid was removed by filtration and the filtrate evaporated. The crude product was chromatographed on silica gel (10% EtOAc, 90% hexane) to yield 7.6 g (73%) of light yellow liquid: ^1H NMR δ 1.70–2.40 (complex band, 6 H, cyclobutane protons), 2.23 (s, C-1H), 3.07 (br m, 1 H, cyclobutane methine), 5.94 (dd, J = 1, 16 Hz, 1 H, C-3H), 6.86 (dd, J = 16, 7 Hz, 1 H, C-4H). This material in 75 mL of absolute EtOH was hydrogenated at 5 psi for 5 h at room temperature with 1.0 g of 5% Pd/C as catalyst. The reaction mixture was filtered and the filtrate carefully evaporated at 5 °C to yield 7.4 g (98%) of a light yellow liquid: ^1H NMR δ 2.12 (s, C-1H's); IR 1710 cm^{-1}.

4-Cyclopentyl-2-butanone (7c). A solution of 10.0 g (70 mmol) of 3-cyclopentylpropionic acid (**10a**) in 150 mL of anhydrous ether was chilled to −20 °C with mechanical stirring under N_2 and treated dropwise with 50 mL (140 mmol) of a 2.8 M solution of methylmgnesium bromide in ether over a 30-min period while the temperature was maintained below −15 °C. After the addition was completed, the reaction mixture was allowed to warm to room temperature and then poured into a mixture of ether and 1 N HCl and shaken well. The layers were separated, and the aqueous portion was extracted successively with ether and EtOAc. The organic extracts were combined and washed with water twice and saturated NaCl solution once, dried (Na_2SO_4), and evaporated. The crude product was chromatographed on silica gel (10% EtOAc, 90% hexane) to give 4.2 g (42%) of a clear liquid: ^1H NMR δ 0.8–2.0 (complex band, 11 H's), 2.13 (s, C-1H's), 2.43 (t, J = 7 Hz, C-3H). Anal. ($C_9H_{16}O$) C, H.

In a similar manner compound **7d** was prepared from 3-cyclohexylpropionic acid in 39% yield. Anal. ($C_{10}H_{18}O$) C, H.

Cycloheptanecarbonyl Chloride (9a). To a solution of 10.0 g (70 mmol) of cycloheptanecarboxylic acid in 125 mL of benzene was added 17.8 g (150 mmol) of thionyl chloride in one portion at room temperature. The reaction mixture was refluxed for 4 h and then allowed to stand at room temperature overnight. The reaction mixture was evaporated and the residue was vacuum distilled to yield 7.0 g (62%) of a clear liquid: bp 44–45 °C (1.0 mm).

4-Cycloheptyl-2-butanone[11] (7e). In a manner similar to the preparation of **7b** from **8a**, the title compound was obtained from **9a**. Anal. ($C_{11}H_{20}O$) C, H.

2-Methyl-1,3-dioxolane-2-butanol (11). A solution of 25 g (255 mmol) of 5-hexen-2-one and 20 g (322 mmol) of ethylene glycol in 250 mL of toluene and a catalytic amount of *p*-toluenesulfonic acid was refluxed until no more water was collected in a Dean-Stark trap. The reaction mixture was cooled and poured into a mixture of EtOAc and 5% K_2CO_3 solution and shaken well. The layers were separated, and the organic portion was washed with water three times and saturated NaCl solution once, dried (Na_2SO_4), and evaporated. The residue was vacuum distilled to give 33.2 g (92%) of a clear liquid, bp 30-31 °C (1.0 mm).

A borane solution (1 M, 120 mL, 120 mmol) in THF was chilled in an ice bath under argon and a solution of 32 g (234 mmol) of the above ketal in 50 mL of THF was added dropwise with stirring over 1 h. The reaction mixture was allowed to warm to room temperature over 2 h and then 50 mL of 3 N NaOH solution was added dropwise. After the addition of the hydroxide was completed, 40 mL of 30% H_2O_2 was added portionwise at room

(8) The relative durations of action are tentatively assigned because antisecretory activity was not followed to cessation.
(9) Bauer, R. F.; Collins, P. W.; Jones, P. H. *Ann. Rep. Med. Chem.* **1987**, *22*, 191.
(10) Furukawa, J.; Kuwabata, N.; Nishimura, J. *Tetrahedron* **1968**, *24*, 53.
(11) Garnick, R. L.; LeQuesne, P. W. *J. Am. Chem. Soc.* **1978**, *100*, 4213.

temperature. The reaction mixture was stirred for 1 h and then poured into a mixture of EtOAc and saturated NaCl solution and shaken well. The layers were separated, and the aqueous portion was extracted with EtOAc three times. The organic extracts were combined and washed twice with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The residue was vacuum distilled to yield 27 g (72%) of a clear liquid: bp 98–100 °C (0.2 mm); ^{1}H NMR δ 1.29 (s, C-1H's), 3.62 (q, J = 6 Hz, CH_2OH), 3.90 (s, 4 H's, ethylene ketal). Anal. ($C_8H_{16}O_3$) C, H.

6-[(Tetrahydro-2*H*-pyran-2-yl)oxy]-2-hexanone (7f). A solution of 27 g (169 mmol) of 11 in a mixture of 100 mL of THF and 30 mL of 1 N HCl was allowed to stand at room temperature overnight. The reaction mixture was evaporated to remove most of the THF and then poured into a mixture of EtOAc and water and shaken well. The layers were separated, and the aqueous portion was extracted with EtOAc three times. The organic extracts were combined and washed twice with saturated NaCl solution, dried (Na_2SO_4), and evaporated to give 17 g (87%) of 6-hydroxy-2-hexanone.[12]

A solution of 1.2 g (10.3 mmol) of 6-hydroxy-2-hexanone, 883 mg (10.5 mmol) of freshly distilled dihydropyran, a catalytic amount of *p*-toluenesulfonic acid, and 10 mL of ether was allowed to stand at room temperature for 4 h. The reaction mixture was poured into a mixture of ether and 5% K_2CO_3 solution and shaken well. The layers were separated, and the organic portion was washed with water three times and saturated NaCl solution once, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (30% EtOAc, 70% hexane) to afford 945 mg (46%) of a clear liquid: ^{1}H NMR δ 1.0–2.0 (complex band, 10 H's), 2.09 (s, C-1H's), 3.1–4.1 (complex band, 4 H's, $-CH_2O-$), 4.52 (br t, 1 H). Anal. ($C_{11}H_{20}O_3$) C, H.

[[1-(2-Cyclopropylethyl)-1-methyl-3-butynyl]oxy]trimethylsilane (5a). In a flame-dried apparatus under N_2 were suspended 850 mg (35.0 mmol) of magnesium turnings and a catalytic amount of $HgCl_2$ in 50 mL of dry THF. To this suspension was added 10 mL of a solution containing 4.2 g (35 mmol) of propargyl bromide and 3.5 g (31.3 mmol) of **7a** in 50 mL of dry THF. After reaction began, the remainder of the solution was added at a rate sufficient to maintain a gentle reflux. After the addition was completed, the reaction was stirred for 1 h and poured into a mixture of ether and 1 N HCl and shaken well. The layers were separated, the aqueous portion was extracted with ether twice, and the combined organics were washed with water three times and once with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The crude product was chromatographed on silica gel (15% EtOAc, 85% hexane) to give 2.44 g (51%) of a clear liquid: ^{1}H NMR δ 0.05–1.00 (complex band, 5 H's, cyclopropane protons), 1.31 (s, CH_3), 2.13 (t, J = 2.5 Hz, C-1H), 2.42 (d, J = 2.5 Hz, C-3H's).

To a solution of 2.40 g (15.8 mmol) of the above alcohol in 25 mL of DMF under N_2 at room temperature was added 2.0 g (29.4 mmol) of imidazole followed by 1.90 g (17.5 mmol) of trimethylchlorosilane. The reaction mixture was stirred for 30 min and then poured into a mixture of ether and water and shaken well. The layers were separated, and the aqueous portion was extracted three times with a 1:1 mixture of ether and hexane. The organic extracts were combined and washed with water three times and saturated NaCl solution once, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (2% EtOAc, 98% hexane) to give 3.2 g (90%) of a clear liquid: ^{1}H NMR δ 0.11 (s, 9 H's, Me_3Si); IR 2125, 3315 cm^{-1}. Anal. ($C_{13}H_{24}OSi$) C, H.

[[1-(2-Cyclobutylethyl)-1-methyl-3-butynyl]oxy]trimethylsilane (5b). The title compound was prepared from **7b** by the same procedure described for **5a**: ^{1}H NMR δ 0.11 (s, 9 H's, Me_3Si), 1.4–2.3 (complex band, 11 H's), 1.31 (s, CH_3), 1.97 (t, J = 3 Hz, C-1H), 2.34 (d, J = 3 Hz, C-3H's). Anal. ($C_{14}H_{26}OSi$) C, H.

Compounds **5c–e** were prepared in a similar manner. Anal. **5c** ($C_{15}H_{28}OSi$) C, H; **5d** ($C_{16}H_{30}OSi$) C, H; **5e** ($C_{17}H_{32}OSi$) C, H.

4-Methyl-4-[(trimethylsilyl)oxy]-8-[(tetrahydro-2*H*-pyran-2-yl)oxy]-1-octyne (5f). In a similar manner, the title compound was prepared from **7b** in 91% yield: ^{1}H NMR δ 0.10 (s, 9 H's, Me_3Si), 1.4–1.9 (complex band, 12 H's), 3.2–4.1 (complex band, 4 H's, $-CH_2O-$), 4.59 (m, 1 H, CHO_2). Anal. ($C_{15}H_{28}O_3Si$) C, H.

(*E*)-1-(Tri-*n*-butylstannyl)-4-methyl-4-[(trimethylsilyl)oxy]-6-cyclopropyl-1-hexane (6a). A mixture of 1.50 g (6.68 mmol) of **5a** and 1.95 g (6.68 mmol) of freshly distilled tri-*n*-butyltin hydride contained in a Pyrex flask was irradiated under argon with a GE sunlamp for 8 h at approximately 55–60 °C (heat generated by the lamp when placed at a distance of about 8 in. from the reaction vessel). The resulting product was used directly in the next step: ^{1}H NMR δ 0.02, 0.39, 0.61 (m's cyclopropyl protons), 1.16 (s, C-4 CH_3), 2.27 (d, J = 5.5 Hz, C-3H), 5.39 (d, J = 19 Hz, C-2H), 5.48 (dt, J = 19, 5.5 Hz, C-1H). Anal. ($C_{25}H_{52}OSiSn$) C, H.

Compounds **6b–f** were prepared in a similar manner. Anal. **6b** ($C_{26}H_{54}OSiSn$) C, H; **6c** ($C_{27}H_{56}OSiSn$) C, H **6d** ($C_{28}H_{58}OSiSn$) C, H; **6e** ($C_{29}H_{60}OSiSn$) C, H; **6f** ($C_{28}H_{58}O_3SiSn$) C, H.

(±)-Methyl 7-[2β-(6-Cyclopropyl-4-hydroxy-4-methyl-1-(*E*)-hexenyl)-3α-hydroxy-5-oxo-1α-cyclopentyl]-4(*Z*)-heptenoate (2a).[6] A solution of 500 mg (0.975 mmol) of **6a** in 3 mL of dry THF was cooled to −50 °C under argon and treated with 5.7 mL of a 1.7 M solution (0.975 mmol) of *n*-BuLi in hexane. The solution was stirred for 45 min at −50 °C, cooled to −60 °C, and treated with a solution of 127 mg (0.975 mmol) of copper 1-pentyne and 318 mg (1.95 mmol) of hexamethylphosphorous triamide in 3 mL of ether. The reaction mixture was stirred for 30 min at −60 °C and then a solution of 176 mg (0.50 mmol) of **3**[5] in 2 mL of ether was added in one portion. The solution was stirred for 30 min and then poured into a mixture of ether and 1 N HCl and shaken well. The layers were separated, and the aqueous portion was extracted with ether and then EtOAc. The organic extracts were combined and washed with water three times and saturated NaCl solution once, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (10% EtOAc, 90% hexane) to afford 175 mg of a viscous oil. The oil was dissolved with stirring in 8 mL of a 3:1:1 mixture of AcOH, THF, and water and allowed to stand at room temperature under N_2 for 1 h. The solution was diluted with ether, washed with water four times, dried (Na_2SO_4), and evaporated. The resulting oil was chromatographed on silica gel (80% EtOAc, 20% hexane) to give 117 mg (60%) of a colorless, viscous oil: ^{1}H NMR δ 0.02 (q, J = 5 Hz, 2 H, cyclopropane), 0.42 (m, 2 H, cyclopropane), 0.65 (m, J = 5 Hz, 1 H, cyclopropane), 1.16, 1.17 (s, C-16 CH_3, diastereomers[6]), 2.00 (dt, J = 12, 6 Hz, C-8H), 2.21 (d, J = 7 Hz, C-15 H's), 2.23 (dd, J = 18.5, 10 Hz, C-10αH), 2.34 (m, 4 H, C-2,3H's), 2.72 (dd, J = 18.5, 7.5 Hz, C-10βH), 3.67 (s, OCH_3), 4.05 (q, J = 8 Hz, C-11H), 5.34 (complex band, C-4,5H's), 5.43, 5.45 (dd, J = 15, 8 Hz, C-13H), 5.73, 5.74 (dt, J = 15, 7 Hz, C-14H); ^{13}C NMR δ 51.5 (OCH_3), 174 (C-1), 33.9 (C-2), 22.7 (C-3), 128.2, 130.4 (C-4,5), 24.4 (C-6), 27.5 (C-7), 53.8 (C-8), 215.1 (C-9), 46.1 (C-10), 71.7 (C-11), 54.8, 54.9 (C-12), 133.5 (C-13), 129.5, 129.6 (C-14), 44.9 (C-15), 72.2 (C-16), 26.0, 26.9 (C-16 CH_3, 2 peaks due to diastereomers[6]), 41.1, 42.3 (C-17), 28.9, 29.1 (C-18), 11.1 (cyclopropane methine), 4.5 (cyclopropane C's). Anal. ($C_{23}H_{36}O_5$) C, H.

(±)-Methyl 7-[2β-(6-Cyclobutyl-4-hydroxy-4-methyl-1-(*E*)-hexenyl)-3α-hydroxy-5-oxo-1α-cyclopentyl]-4(*Z*)-heptenoate (2b).[6] In a similar manner **2b** was prepared from **6b** in 62% yield from **3**: ^{1}H NMR δ 1.18 (s, C-16 CH_3), 2.73 (dd, J = 19, 8 Hz, C-10H), 3.66 (s, OCH_3), 4.04 (q, J = 8 Hz, C-11H), 5.32 (m, C-4,5H's), 5.37 (dd, J = 15, 8 Hz, C-13H), 5.74 (dt, J = 15, 7 Hz, C-14H). Anal. ($C_{24}H_{38}O_5$) C, H.

(±)-Methyl 7-[2β-(6-Cyclopentyl-4-hydroxy-4-methyl-1-(*E*)-hexenyl)-3α-hydroxy-5-oxo-1α-cyclopentyl]-4(*Z*)-heptenoate (2c).[6] In a similar manner, **2c** was prepared from **6c** in 66% yield from **3**. Anal. ($C_{25}H_{40}O_5$) C, H.

(±)-Methyl 7-[2β-(6-Cyclohexyl-4-hydroxy-4-methyl-1-(*E*)-hexenyl)-3α-hydroxy-5-oxo-1α-cyclopentyl]-4(*Z*)-heptenoate (2d).[6] In a similar manner, **2d** was prepared from **6d** in 70% yield from **3**. Anal. ($C_{26}H_{42}O_5$) C, H.

(±)-Methyl 7-[2β-(6-Cycloheptyl-4-hydroxy-4-methyl-1-(*E*)-hexenyl)-3α-hydroxy-5-oxo-1α-cyclopentyl]-4(*Z*)-heptenoate (2e).[6] In a similar manner, **2e** was prepared from **6e** in a 67% yield from **3**. Anal. ($C_{27}H_{44}O_5$) C, H.

(±)-Methyl 11α,16,20-Trihydroxy-16-methyl-9-oxoprosta-4(*Z*),13(*E*)-dienoate (2f).[6] In a similar manner, **2f** was prepared from **6f** after hydrolysis of the protecting groups with a mixture

(12) Heilbron, I.; Jones, E. R. H.; Toogood, B.; Weedon, B. C. L. *J. Chem. Soc.* **1949**, 1827.

of 3:1:1 HOAc/H_2O/THF at 45 °C for 3.5 h. The crude product was chromatographed on silica gel (2% MeOH, 98% EtOAc) to give **2f** in 50% yield from **3**: ^1H NMR δ 1.18 (s, C-16 CH_3), 2.71 (dd, J = 19, 8 Hz, C-10H), 3.60 (m, C-20H's), 3.67 (s, OCH_3), 4.01 (q, J = 8 Hz, C-11H), 5.31 (m, C-4,5H's), 5.35 (dd, J = 15, 8 Hz, C-13H), 5.72, 5.74 (2 dt, J = 15, 7 Hz, C-14H, diastereomers[6]). Anal. ($C_{22}H_{36}O_6$) C, H.

Gastric Antisecretory Studies. Prostaglandins were dissolved in absolute ethanol (1 mg/mL) and stored at -10 °C. Dilutions for administration were made in all studies with phosphate buffer (pH 7.4) so that the final ethanol concentration did not exceed 20%.

Antisecretory studies were done as previously described for enisoprost.[13] Briefly, adult female beagles, weighing 6-11 kg, with innervated (Pavlov) gastric pouches, were food deprived with access to water 24 h prior to experiments. Following a 30-min basal collection period, prostaglandin in isosmotic phosphate buffer (pH 7.4) or vehicle was administered into the pouch through a Thomas cannula. Thirty minutes later the gastric pouch was emptied and gastric secretion was stimulated by feeding 10-12 oz of canned dog food (Evanger's Dog and Cat Food Co. Inc., Wheeling, IL). Gastric juice samples were collected over a 4-h period at 30-min intervals. Total acid output (mequiv/30 min) was determined for each collection period by multiplying the volume of secretion (mL/30 min) and the acidity (mequiv/L). For new compounds, percent reduction of total acid output from control was calculated over each 4-h experiment for four to six doses and 2-12 dogs were used for each dose. ED_{50} values and 95% confidence limits were determined from inhibition of secretion curves.

Single doses of prostaglandins that caused approximately 80% inhibition of secretion for the second collection period (60-90 min after dosing, the time of peak acid secretion in control experiments) were used to determine duration of effect. Statistical differences among prostaglandins at each collection time were determined by ANOVA after transformation of percent inhibition values (PIV) by the function arcsine ($\sqrt{PIV}$).[14]

Diarrheal Studies. Adult Charles River male rats weighing 210-230 g were individually housed and fasted with water available ad libitum for 24 h prior to the test. The animals (N = 6-12) received logarithmically graded prostaglandin doses orally. Immediately after administration, the animals were returned to their cages, and diarrhea, if any, was assessed on an all or none basis for 8 h after drug treatment. The ED_{50} and relative potency values were calculated by the logistic method of Berkson.[15,16]

Acknowledgment. We thank C. Anglin and J. Casler for technical assistance in the antisecretory studies, R. Bianchi and C. Ponte for performing the diarrheal studies, the group of E. Hajdu for spectral data, L. Swenton for assistance in interpretation of spectral data, the group of E. Zielinski for microanalyses, L. Householder for artwork, and D. Weiman and J. Fraser for typing the manuscript.

(13) Collins, P. W.; Kramer, S. W.; Gullikson, G. W. *J. Med. Chem.* **1987**, *30*, 1952.

(14) Snedecor, G. W., Cochran, W. *Statistical Methods*, 7th ed.; Iowa State University Press: Ames, 1980; p 290.

(15) Finney, D. J. *Statistical Methods in Biological Assay*, 2nd ed.; Harper Publishing Co.: New York, 1964; Chapters 4-6.

(16) Berkson, J. *Am. Stat. Assoc. J.* **1953**, *48*, 565.

2,3-Dihydro-5-benzofuranols as Antioxidant-Based Inhibitors of Leukotriene Biosynthesis

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The enzymes that catalyze the oxidative metabolism of arachidonic acid have provided fertile ground for the development of useful therapeutic agents for nearly a quarter century. Inhibitors of the enzyme cyclooxygenase prevent the formation of the prostaglandins and thromboxanes and are clinically useful antiinflammatories and peripheral analgesics. More recently it has been discovered that the enzyme 5-lipoxygenase is the first step in the formation of a series of biologically important metabolites of arachidonic acid, the leukotrienes. Evidence suggests that an inhibitor of 5-lipoxygenase may be a useful therapeutic agent in the treatment of asthma, immediate hypersensitivity, and inflammation. Various antioxidants have been examined as inhibitors of 5-lipoxygenase in vitro. We were intrigued by recent reports that the 2,3-dihydro-5-benzofuranol ring system maximizes the stereoelectronic effects necessary for efficient hydrogen atom abstraction by peroxyl radicals. In this study we describe the synthesis of over 50 new 2,3-dihydro-5-benzofuranols and their biological evaluation as inhibitors of leukotriene biosynthesis in isolated human polymorphonuclear leukocytes. We show that the 2,3-dihydro-5-benzofuranol ring system, although not a potent inhibitor of leukotriene biosynthesis in itself, can provide a useful template for the design of antioxidant-based inhibitors of leukotriene biosynthesis. Furthermore, within a structural class the potency of a given analogue can be predicted on the basis of its overall calculated lipophilicity (log P). The data are interpreted in terms of a model in which the observed inhibition by this class of inhibitors is dependent on the intrinsic ability of the antioxidant to reduce the enzyme and on the fraction of the inhibitor that is partitioned into the membrane.

The enzymes that catalyze the oxidative metabolism of arachidonic acid have provided fertile ground for the development of useful therapeutic agents for nearly a quarter century. Inhibitors of the enzyme cyclooxygenase prevent the formation of the prostaglandins and thromboxanes and are clinically useful antiinflammatories and peripheral analgesics.[1] More recently it has been discovered that the enzyme 5-lipoxygenase is the first step in the formation of a series of biologically important metabolites of arachidonic acid, the leukotrienes[2] (Figure 1). In particular leukotriene C_4 (LTC_4) and LTD_4 have been identified as the slow reacting substance of anaphylaxis (SRSA) and cause a prolonged contraction of bronchial smooth muscle.[3]

(1) Shen, T. Y. In *Discoveries in Pharmacology, Volume 2: Haemodynamics, Hormones, and Inflammation*; Parnham, M. J., Bruinvels, J., Eds.; Elsevier: Amsterdam, 1984; pp 523-553.

(2) For a recent reviews on the leukotrienes, see: (a) Taylor, G. W.; Clarke, S. R. *Trends Pharmacol. Sci.* **1986**, *7*, 100. (b) Sirois, P. *Adv. Lipid Res.* **1985**, *21*, 79.

(3) Dahlen, S.; Hedqvist, P.; Hammarstrom, S.; Samuelsson, B. *Nature (London)* **1980**, *288*, 484.

0022-2623/89/1832-1006$01.50/0