Synthesis and Gastrointestinal Pharmacology of the 4-Fluoro Analogue of Enisoprost[1]

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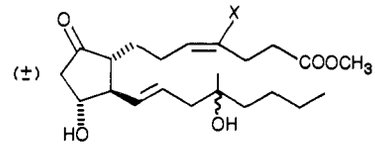
A 4-fluoro analogue of enisoprost was prepared and evaluated for gastric antisecretory and mucosal protective activity in animals. The synthesis centered upon cuprate chemistry but also involved a Wittig reaction to produce a cis fluoro olefinic moiety, a furan rearrangement/isomerization reaction to provide the necessary hydroxycyclopentenone, and a two-carbon-homologation procedure. The fluoro analogue was much less potent as a gastric antisecretory and mucosal protective agent than enisoprost.

Enisoprost,[2] **1a**, is a synthetic 16-hydroxy prostaglandin currently under clinical study for the treatment of peptic ulcer disease.[3] In comparison to the saturated parent compound, misoprostol,[2] enisoprost has increased gastric antisecretory activity and a longer duration of action in laboratory animals. The enhanced activity and duration

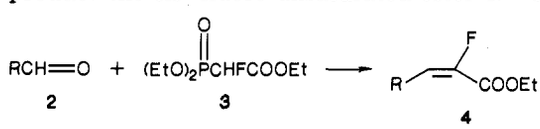


1a, X = H
b, X = F

is presumably due to the presence of the cis double bond at C-4,5, a modification that reduces susceptibility to β-oxidation.[4] On the basis of the theory[5] that Δ^4 unsaturated fatty acids must be enzymatically reduced to the saturated acids before β-oxidation can occur, we reasoned that replacement of one of the hydrogen atoms of the Δ^4 double bond of **1a** with a fluorine atom might interfere with the enzymatic reduction and thus further improve the activity/duration profile of enisoprost. For synthetic reasons the 4-fluoro analogue **1b**[6] was selected as the initial target.

Chemistry

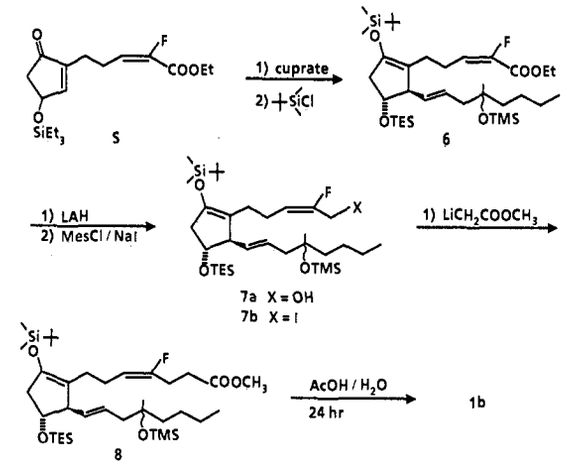
A primary consideration in the synthesis of **1b** was the stereoselective preparation of the cis fluoro olefinic functionality. A feasible method for formation of this moiety is an ylide reaction of the phosphonate derivative **3**[7] of ethyl bromofluoroacetate[8] with an appropriate aldehyde **2** to produce the cis[9] fluoro unsaturated ester **4**. Utili-



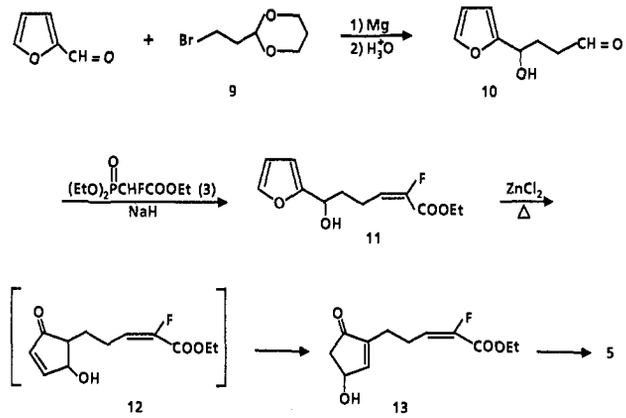
2 **3** **4**

zation of this reaction requires a two-carbon-chain homologation to place the olefin at the proper location in the α chain of **1b**. Therefore our strategy centered upon synthesis of the cyclopentenone **5** (Scheme I) and its conversion to **6** via a selective cuprate addition of the ω side chain to the enone portion of **5** and in situ enolate capture with *tert*-butyldimethylchlorosilane.[10] We further reasoned that intermediate **6** would possess sufficient stability to permit execution of the chain-extension sequence outlined in Scheme I.

Scheme I



Scheme II



The requisite cyclopentenone **5** was prepared via furan rearrangement chemistry[11-13] (Scheme II). Condensation of 2-furaldehyde with the Grignard reagent derived from 2-(bromoethyl)-1,3-dioxane[14] (**9**) followed by acidic hydrolysis of the acetal produced the hydroxy aldehyde **10**. Although **10** existed primarily as an internal hemiacetal,

(1) This work was presented at the 20th National Medicinal Chemistry Symposium, June 3–6, 1986, Chapel Hill, NC.
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(3) Howden, C. W.; Burget, D. W.; Hunt, R. H. *Clin. Sci.* **1986**, *71*(S15), 75.
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(5) Kunau, W-H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 61.
(6) Both **1a** and **1b** are mixtures of two racemates.
(7) Machleidt, H.; Wessendorf, R. *Liebigs Ann. Chem.* **1963**, *674*, 1.
(8) Ethyl bromofluoroacetate was purchased from SCM Speciality Chemicals, Gainesville, FL.
(9) Under the priority rules of *E*/*Z* nomenclature, the cis fluoro ester is the *E*. isomer.
(10) Collins, P. W.; Gasiecki, A. F.; Jones, P. H.; Bauer, R. F.; Gullikson, G. W.; Woods, E. M.; Bianchi, R. G. *J. Med. Chem.* **1986**, *29*, 1195.
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(13) Collins, P. W.; Kramer, S. W.; Gasiecki, A. F.; Weier, R. M.; Jones, P. H.; Gullikson, G. W.; Bianchi, R. G.; Bauer, R. F. *J. Med. Chem.* **1987**, *30*, 193.
(14) Purchased from Fluka Chemical Corp.

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Table I. Comparative Oral Gastric Antisecretory and Mucosal Protective Effects of Enisoprost and Its 4-Fluoro Analogue

compound	gastric antisecretory effects in dogs:[a] ED_{50}, μg/kg, ig (95% confidence limits)	mucosal protective activity in rats: MED,[b] μg/kg, ig
enisoprost (1a)	0.023 (0.017–0.032)	1
1b	9.7 (6.2–15.3)	50

[a] Determined in food-stimulated Pavlov pouch dogs by intrapouch administration. [b] Minimal effective dose.

it reacted smoothly with the phosphonate carbanion of 3 to provide the cis fluoro ester 11[9] in good yield. Only a trace of the undesired trans isomer was detected by NMR analysis. The cis stereochemistry of 11 was assigned on the basis of a J_{HF} value of 22 Hz and comparison with model compounds.[15] In this reaction, it was necessary to add a solution of the phosphonate carbanion dropwise to 10 because large amounts of the dehydration product of 11 were obtained when the addition process was reversed. Rearrangement–isomerization of 11 was accomplished in a one-pot procedure using $ZnCl_2$ as the sole reagent.[12] Thus a solution of 11 in a dioxane–water mixture was refluxed for 32 h in the presence of 10 equiv of $ZnCl_2$ to give 13 directly. A small amount of the intermediate enone 12 remained, but was easily separated from 13 by chromatography. Treatment of 13 with triethylchlorosilane and imidazole in DMF produced the protected enone 5. The key intermediate 6 (Scheme I) was formed by treating 5 with the cuprate reagent derived from (*E*)-(tri-*n*-butylstannyl)-4-methyl-4-[(trimethylsilyl)oxy]-1-octene[2] followed by enolate capture with *tert*-butyldimethylchlorosilane.[10] There was no evidence of 1,4 cuprate addition to the unsaturated fluoro ester of 5. LAH reduction of 6 proceeded smoothly without conjugate reduction to give the alcohol 7a. Treatment of 7a with mesyl chloride in pyridine at 0 °C for 30 min[16] followed by NaI in acetone yielded the allylic iodide 7b. Reaction of 7b with the lithium isopropylcyclohexylamide (LICA) generated lithium salt of methyl acetate at −60 °C provided the desired homologated intermediate 8 in modest yield (47%). The major side product was the corresponding β-keto ester formed by addition of a second acetate residue to 8. No attempts were made to optimize the yield of 8 in this reaction. Overnight exposure of 8 to a 3:1:1 mixture of acetic acid, water, and THF at room temperature provided the desired compound 1b in good yield (70%). A small amount (5–10%) of the corresponding 8-epimer was formed by hydrolysis of the silyl enol ether but was easily separated from 1b by chromatography.

Results and Discussion

The 4-fluoro analogue 1b was evaluated for gastric antisecretory activity in Pavlov pouch dogs by intrapouch administration and for mucosal protective activity in the ethanol rat model.[17] The results of the assays are presented in Table I and compared with the activities of the parent compound, enisoprost (1a). Both enisoprost and 1b produced nearly complete (88%) inhibition of total acid output at doses of 0.3 and 100 μg/kg, respectively, but 1b was approximately 400 times less potent than enisoprost on the basis of ED_{50} values. In a similar manner, both compounds provided almost complete protection for the rat gastric mucosa against ethanol-induced damage at high doses (256–500 μg/kg). Enisoprost, however, still demonstrated significant protection at 1.0 μg/kg while the minimally effective dose for 1b was 50 μg/kg. Thus 1b was much less potent as an antisecretory and mucosal protective agent than enisoprost.

These findings are surprising and difficult to explain. The lesser potency of 1b cannot reasonably be attributed to an inability to meet receptor spacial requirements because of the small size difference between hydrogen and fluorine atoms. One possible explanation is that hydrophobic bonding of the α chain of prostaglandins is an important component of their overall receptor binding affinity, and the presence of polarized centers in the α chain, such as the fluoro-olefin moiety of 1b, interferes with the hydrophobic bonding. The fact that substitution of other electronegative atoms in the α chain of prostaglandins also reduces biological activity[18,19] provides some support for this hypothesis. Alternatively, the lower potency of 1b might be due to hydrogen bonding between the fluorine atom and the 16-hydroxy group. Inspection of molecular models of 1b indicates that the two centers are sufficiently close to one another to permit this type of interaction. By involving the 16-hydroxy group in an intramolecular interaction, the fluorine atom may be preventing effective interplay of this pivotal hydroxy group with its receptor binding site.

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 50 MHz using the APT pulse technique. Except where indicated, elemental analyses were within ±0.4% of the theoretical values. Solvents were removed under reduced pressure on a rotary evaporator.

γ-Hydroxy-2-furanbutanal (10). To a suspension of Mg turnings (9.73 g, 0.40 mol) under argon in dry THF were added a few I_2 crystals and about 200 mg of $HgCl_2$. After the purple color had disappeared, a solution of 2-(bromoethyl)-1,3-dioxane[14] (68.3 g, 0.35 mol) in 350 mL of THF was added over 75 min to the reaction flask. After the solution was stirred for 1 h at room temperature, a solution of freshly distilled 2-furaldehyde (30.8 g, 0.32 mol) in 50 mL of THF was added to the solution with rapid stirring, using a cold-water bath to maintain the reaction temperature between 15 and 25 °C during addition. After being stirred at room temperature for 30 min, the reaction mixture was quenched with saturated NH_4Cl solution and extracted three times with EtOAc. The extracts were combined, washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated. After chromatographic purification of the residue, 60.7 g of pure product (97%) was obtained as a pale yellow liquid. ^1H NMR: δ 4.71 (t, CHO_2), 4.60 (t, CHOH), 6.28, 7.37 (furan). Fifteen grams of the product above was mixed with 150 mL of acetone, 50 mL of water, and 25 mL of 2 N HCl and the resultant mixture stirred at room temperature for 28 h. The solution was neutralized with solid K_2CO_3 and evaporated. The residue was partitioned between EtOAc and saturated NaCl solution. The aqueous layer was reextracted with EtOAc, and the combined extracts were washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The remaining dark oil was purified by chromatography (silica gel, 25% EtOAc, 75% hexane) to give 4.5 g (30%) of 10 as a golden

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oil. Compound **10** exists predominately as an internal hemiacetal. ^{1}H NMR: δ 1.8–2.5 (m, CH_2CH_2), 5.00 (dd, J = 8, 7 Hz, CHOH), 5.22 (t, J = 7 Hz, CHOH), 5.58 (dd, J = 8, 4 Hz; dd, J = 12, 4 Hz, CHOR), 6.25, 7.35 (3 H, furan). Anal. ($C_8H_{10}O_3$) C, H.

Ethyl 2-Fluoro-6-(2-furanyl)-6-hydroxy-2(*E*)-hexenoate (11).[9] To a suspension of 0.38 g of sodium hydride (60% in mineral oil prewashed with hexane under argon) in dry THF under argon was added portionwise 2.27 g (9.6 mmol) of ethyl [(diethoxyphosphinyl)oxy]fluoroacetate (**3**).[7] After being stirred at room temperature for 1 h, the solution was added dropwise to a THF solution of the aldehyde **10** (1.10 g, 8 mmol) at −40 °C. After being stirred for 30 min at −40 °C, the mixture was poured into water and extracted twice with EtOAc. The combined extracts were washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The brown oil was chromatographed (silica gel, 15% EtOAc, 85% hexane) to give 1.26 g of pure **11** (84%) as a light yellow oil. ^{1}H NMR: δ 4.69 (t, J = 7 Hz, CHOH), 5.92 (dt, J_{HF} = 21 Hz, J_{HH} = 8 Hz, CH=CF), 6.23, 6.32, 7.35 (3 H, furan). Anal. ($C_{12}H_{15}O_4F$) C, H, F.

Ethyl 2-Fluoro-5-(hydroxy-5-oxo-1-cyclopenten-1-yl)-2(*E*)-pentenoate (13). A mixture of **11** (9.0 g, 37 mmol), $ZnCl_2$ (50.7 g, 370 mmol), 90 mL of H_2O, and 180 mL of dioxane was heated to reflux under nitrogen for 32 h. The dark brown mixture was cooled to room temperature, poured into a dilute solution of NaCl, and extracted three times with EtOAc. The combined extracts were washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The remaining brown oil was chromatographed (silica gel, 40% EtOAc, 60% hexane) to give 2.4 g (27%) of pure **13** as an oil. ^{1}H NMR (assignments based on prostaglandin numbering system): δ 2.35 (br t, J = 8 Hz, 2 H, C-8 H), 2.32 (dd, J = 18, 2 Hz, C-10 H), 2.82 (dd, J = 18, 6 Hz, C-10 H), 2.74 (qd, J_{HH} = 8 Hz, J_{HF} = 2 Hz, 2 H, C-6 H's), 4.95 (br s, C-11 H, before D_2OX), 4.95 (dt, J = 6, 2 Hz, C-11 H), 5.89 (dt, J_{HF} = 21 Hz, J_{HH} = 8 Hz, CH=CF), 7.22 (dt, J = 2, 1 Hz, C-12). Anal. ($C_{12}H_{15}O_4F$) C, H, F.

Ethyl 2-Fluoro-5-[5-oxo-3-[(triethylsilyl)oxy]-1-cyclopenten-1-yl]-2(*E*)-pentenoate (5). A solution of **13** (3.7 g, 15.3 mmol) in 30 mL of DMF was treated with imidazole (1.7 g, 26 mmol) and triethylchlorosilane (2.88 g, 19 mmol) and stirred at room temperature for 90 min. The mixture was poured into 250 mL of ice water and extracted twice with ether. The ether extracts were combined, washed twice with dilute NaCl solution, dried (Na_2SO_4), and evaporated. The residue was chromatographed (silica gel, 10% EtOAc, 90% hexane) to give 5.4 g (99%) of **5** as a colorless oil. ^{1}H NMR: δ 0.65 (q, J = 6 hz, CH_2Si), 0.99 (t, J = 6 Hz, CH_3CH_2Si). Anal. ($C_{18}H_{29}O_4SiF$) C (calcd, 60.65; found, 60.02), H, F.

(±)-Ethyl 5-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-5β-[4-methyl-4-[(trimethylsilyl)oxy]-1(*E*)-octenyl]-4α-[(triethylsilyl)oxy]-1-cyclopenten-1-yl]-2-fluoro-2(*E*)-pentenoate (6). A solution of (*E*)-(tri-*n*-butylstannyl)-4-methyl-4α-[(trimethylsilyl)oxy]-1-octene[2] (0.88 g, 1.75 mmol) in 4 mL of dry THF was treated under argon with 1.1 mL of a 1.6 M solution of *n*-butyllithium (1.76 mmol) in hexane at −60 °C. After being stirred for 90 min at −60 °C, a solution of pentynylcopper (0.23 g, 1.75 mmol) dissolved in 8 mL of dry ether containing hexamethylphosphorous triamide (0.56 g, 3.5 mmol) was added, and the reaction mixture was stirred for 15 min at −60 °C. A solution of **5** (356 mg, 1 mmol) in 2 mL of dry ether was added dropwise to the mixture at −60 °C and stirred for 30 min. A solution of *tert*-butyldimethylchlorosilane (0.32 g, 2.1 mmol) in 2 mL of ether and 3 mL of hexamethylphosphoric triamide were added, and the reaction mixture was stirred at −15 °C for 45 min. The mixture was poured into saturated NH_4Cl solution and stirred vigorously for 30 min and extracted twice with ether. The ether extracts were combined and shaken successively with cold 0.5 N HCl, dilute $NaHCO_3$ solution, and saturated NaCl solution, dried (Na_2SO_4), and evaporated. The residue was chromatographed (silica gel, 2% EtOAc, 98% hexane) to give 0.47 g (69%) of **6** as a colorless oil. ^{1}H NMR (assignments based on prostaglandin numbering system): δ 0.11 (s, Me_3Si), 0.15 (s, Me_2-*t*-BuSi), 0.95 (s, Me_2-*t*-*Bu*Si), 1.16 (s, C-16 Me), 1.34 (t, OCH_2CH_3), 2.16 (d, J = 7 Hz, C-15 H's), 2.94 (dt, J = 8, 3 Hz, C-12 H), 3.97 (br dt, J = 7, 3 Hz, C-11 H), 4.28 (q, OCH_2CH_3), 5.12 (dd, J = 16, 8 Hz, C-13 H), 5.50 (dt, J = 16, 7 Hz, C-14 H), 5.87 (dd, J_{HF} = 22 Hz, J_{HH} = 8 Hz, CH=CF). Anal. ($C_{36}H_{69}O_5Si_3F$) C (calcd, 63.11; found, 62.51), H, F.

(±)-5-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-5β-[4-methyl-4-[(trimethylsilyl)oxy]-1(*E*)-octenyl]-4α-[(triethylsilyl)oxy]-1-cyclopenten-1-yl]-2-fluoro-2(*E*)-pentenol (7a). A dry THF solution (40 mL) of **6** (0.90 g, 1.3 mmol) was cooled to 0 °C under argon and treated with 0.13 g of LAH. After 30 min at 0 °C, the mixture was poured into saturated NH_4Cl solution and extracted twice with ether. The ether extracts were combined and washed successively with H_2O and saturated NaCl solution, dried (Na_2SO_4), and evaporated. Chromatographic purification (silica gel, 7% EtOAc, 93% hexane) provided 0.63 g (76%) of **7a** as a colorless oil. ^{1}H NMR: δ 4.12 (dd, J_{HF} = 21 Hz, $J_{H,OH}$ = 6 Hz, CH_2OH), 5.08 (J_{HF} = 20 Hz, CH=CF). Anal. ($C_{34}H_{67}O_4Si_3F$) C, H, F.

(±)-1-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-2-(4-fluoro-5-iodo-3-pentenyl)-3α-[4-methyl-4-[(trimethylsilyl)oxy]-1(*E*)-octenyl]-4β-[(triethylsilyl)oxy]cyclopentene (7b). To a solution of **7a** (640 mg, 1 mmol) in 7 mL of pyridine at 0 °C was added dropwise (230 mg, 2 mmol) of methanesulfonyl chloride. The solution was stirred at 0 °C for 30 min and then was poured into a 1:1 mixture of ether–hexane, washed twice with H_2O and once with saturated NaCl solution, and dried (Na_2SO_4). The solvent was evaporated to give 700 mg of crude product, which was immediately added to a solution of NaI (400 mg) in 25 mL of acetone under argon. The mixture was stirred at room temperature for 75 min and poured into hexane. The solution was washed successively with dilute sodium sulfite solution and saturated NaCl solution, dried (Na_2SO_4), and evaporated. Chromatographic purification (silica gel, 2% EtOAc, 98% hexane) gave 560 mg (75%) of **7b** as a light yellow oil. ^{1}H NMR: δ 4.11 (d, J_{HF} = 21 Hz, CH_2I), 5.25 (J_{HF} = 19 Hz, CH=CF). Anal. ($C_{34}H_{66}O_3SiIF$) C (calcd, 54.23; found, 55.01), H, F.

(±)-Methyl 9-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4-fluoro-16-methyl-11α-[(trimethylsilyl)oxy]-16-[(trimethylsilyl)oxy]prosta-4(*E*),8,13(*E*)-trien-1-oate (8). To a THF solution (15 mL) of *n*-isopropylcyclohexylamine (700 mg, 5.3 mmol) under argon at 0 °C was added 3.12 mL of a 1.6 M solution of *n*-butyllithium in hexane (5 mmol). After being stirred for 30 min, the solution was cooled to −60 °C and treated dropwise with a solution of methyl acetate (450 mg, 6 mmol) in 10 mL of THF. The reaction mixture was stirred for 30 min and then a solution of **7b** (480 mg, 0.64 mmol) in 10 mL of THF was added slowly. After being stirred at −60 °C for 1 h, the reaction mixture was poured into a mixture of ether–hexane (1:1) and 0.5 N HCl. The organic layer was washed twice with H_2O, dried (Na_2SO_4), and evaporated. Chromatographic purification (silica gel, 5% EtOAc, 95% hexane) afforded 221 mg (47%) of pure **8** as a colorless oil. ^{1}H NMR: δ 1.15 (s, C-16 Me), 2.15 (d, J = 7 Hz, C-15 H's), 2.91 (br d, J = 8 Hz, C-12 H), 3.65 (s, OCH_3), 3.95 (m, C-11 H), 4.14 (dd, J = 15, 8 Hz, C-13 H), 5.00 (dt, J_{HF} = 22 Hz, J_{HH} = 8 Hz, CH=CF), 5.49 (dt, J = 15, 7 Hz, C-14 H). Anal. ($C_{37}H_{71}O_5Si_3F$) C, H, F.

(±)-Methyl 4-Fluoro-11α,16-dihydroxy-16-methyl-9-oxoprosta-4(*E*),13(*E*)-dien-1-oate[6,9] (1b). To 160 mg (0.23 mmol) of **8** was added 4 mL of a 3:1:1 mixture of AcOH, THF, and H_2O. The mixture was stirred until homogeneous and then allowed to stand at room temperature overnight. The solution was diluted with ether, washed with water three times, dried (Na_2SO_4), and evaporated. The residue was chromatographed (silica gel, 80% EtOAc, 20% hexane) to give 65 mg (71%) of pure **1b** as a viscous, colorless oil. ^{1}H NMR: δ 1.19 (s, C-16 CH_3), 2.36 (dt, J = 12, 9 Hz, C-12 H), 2.74 (dd, J = 19, 8 Hz, C-10β H), 3.70 (s, OCH_3), 4.07 (td, J = 9, 8 Hz, C-11 H), 4.99 (dt, J_{HF} = 22 Hz, J_{HH} = 8 Hz, CH=CF), 5.41 (dd, J = 15, 9 Hz, C-13 H), 5.65, 5.66 (dt, J = 15, 7 Hz, C-14 H, diastereomers). ^{13}C NMR: δ 51.8 (OCH_3), 172.9, C-1; 30.6, C-2; 23.4 (J = 28 Hz, C-3); 158.4 (J = 247 Hz, C-4); 106.1 (J = 21 Hz, C-5); 22.8 (J = 9 Hz, C-6 may be reversed with C-3 assignment); 28.1, C-7; 53.6, C-8; 214.8, C-9; 46.0, C-10; 71.8,C-11; 55.1, 55.2, C-12, diastereomers; 133.2, C-13; 130.0, 130.1, C-14; 44.8, C-15; 72.5, C-16; 26.2, 27.0, C-16 Me; 41.3, 42.4, C-17; 26.0, 26.2, C-18; 23.2, C-19; 14.1, C-20. Anal. ($C_{22}H_{35}O_5F$) C, H, F.

Gastric Antisecretory Studies. The prostaglandins were dissolved in absolute ethanol stock solution (1 mg/mL) and stored at −10 °C when not in use. Appropriate dilutions of the stock solution were carried out with an isosmotic phosphate buffer (pH 7.4) so that the final ethanol concentration did not exceed 20%.

Adult female beagles, 6–11 kg body weight, were surgically prepared with innervated (Pavlov) gastric pouches that were drained by Thomas-type gastric cannulae.[20] Following surgical recovery, each dog was trained to stand quietly in a dog restraining sling and was conscious for all studies. Experiments began at least 3 weeks after surgery, and no dog was used more than once per week.

Dogs were food-deprived with access to water for 24 h prior to experiments. Following a 30-min basal collection period, the prostaglandins or vehicle were administered into the gastric pouch in a 2–3-mL volume. Thirty minutes later the gastric pouch was emptied and gastric secretion was stimulated by feeding 10–12 oz of dog food (Fromm All Beef, Federal Foods Inc., Thiensville, WI). Gastric juice samples were collected from the pouch by gravity drainage over a 4-h period at 30-min intervals. The volume of secretion was measured (mL/30 min), and the acidity (mequiv/L) was determined by electrometric titration to pH 7.0 with 0.1 N NaOH. These two parameters were multiplied to obtain the total acid output (mequiv/30 min) for each collection period.

Percent reduction of total acid output from control was calculated over each 4-h experiment for doses of prostaglandin. ED_{50} values and 95% confidence limits were determined from inhibition of secretion curves.[21]

Ethanol-Induced Gastric Lesion Studies.[17] Male Charles River rats weighing 180–210 g were food-deprived for 24 h before the experiment but had access to drinking water. Doses of prostaglandin from 0.5 to 500 μg/kg were administered intragastrically to groups of six rats in a volume of 10 mL/kg. A separate control group of animals received only phosphate buffer vehicle. Thirty minutes following administration of the prostaglandins, each rat received an intragastric 1-mL dose of absolute ethanol. One hour after ethanol administration, the animals were sacrificed by CO_2 asphyxiation, and the stomachs were removed, opened, and gently rinsed with tap water. The gastric mucosa was visually inspected for lesions and scored on a severity scale of 1 to 7 with 7 indicating the complete absence of lesions. Statistically significant reduction in the formation of ethanol lesions compared to control animals was determined by a χ^2 test using the method of maximum likelihood.[22]

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Registry No. **1a**, 81026-63-3; **1b** (*R* isomer), 109976-44-5; **1b** (*isomer*), 109976-45-6; **3**, 2356-16-3; **5**, 109976-39-8; **6**, 109976-40-1; **7a**, 109976-41-2; **7a** (methylsulfonyl deriv.), 109996-15-8; **7b**, 109976-42-3; **8**, 109976-43-4; **9**, 33884-43-4; **10**, 109976-36-5; **10** (hemiacetal), 38299-92-2; **11**, 109976-37-6; **13**, 109976-38-7; **2**-furaldehyde, 98-01-1; **2**-[1-hydroxy-3-(1,3-dioxan-2-yl)propyl]furan, 109976-35-4; (*E*)-(tri-*n*-butylstannyl)-4-methyl-4α-[(trimethylsilyl)oxy]-1-octene, 69442-81-5; methyl acetate, 79-20-9.

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Cardiotonic Agents. 7. Inhibition of Separated Forms of Cyclic Nucleotide Phosphodiesterase from Guinea Pig Cardiac Muscle by 4,5-Dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones and Related Compounds. Structure–Activity Relationships and Correlation with in Vivo Positive Inotropic Activity

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The structure–activity relationships of a series of 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones and related compounds were investigated for the in vivo inhibition of different forms of cyclic nucleotide phosphodiesterase (PDE) isolated from guinea pig ventricular muscle. With few exceptions, these 4,5-dihydropyridazinones were potent inhibitors of cardiac type III phosphodiesterase, which is a low K_m, cyclic AMP specific form of the enzyme. The inhibitory effects on cardiac type I and type II phosphodiesterase, both of which hydrolyze cyclic AMP as well as cyclic GMP, were minimal. The most selective PDE III inhibitor was CI-930 (**10**), the 5-methyl analogue of imazodan (CI-914, **1**), with an IC_{50} of 0.6 μM. The most potent inhibitor of PDE III was the 4,5,6,7-tetrahydrobenzimidazole analogue of **10** (**31**), with an IC_{50} of 0.15 μM. This paper describes the structural features that impart both selectivity for inhibiting type III phosphodiesterase and potency of inhibition. In addition, correlations between in vitro PDE inhibitory potency, in vivo positive inotropic potency, and physicochemical properties are discussed.

Within the last decade a number of novel non-glycoside, non-catechol cardiotonic agents have been identified as potential replacements for digitalis in the treatment of congestive heart failure. These agents include amrinone, milrinone, carbazeran, sulmazole, enoximone, piroximone, imazodan (CI-914), and CI-930.[1] Until recently the mechanism responsible for the increase in cardiac contractility produced by these agents was not known. Early studies suggested that the inotropic response was unrelated to direct effects on cardiac β receptors, sarcoplasmic reticulum, or mitochondria or to the modulation of adenylate cyclase or Na^+,K^+-ATPase.[2,3]

Recently, however, it has been demonstrated that all of these novel cardiotonic agents exert an inhibitory effect on cardiac phosphodiesterase (PDE) activity.[2] Subsequent studies in which the inhibitor effects on the different molecular forms of phosphodiesterase present in cardiac

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