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SYNTHESIS OF TRITIUM LABELED ARBAPROSTIL AND RELATED 15-METHYL PROSTAGLANDINS

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SUMMARY

This report describes the synthesis of arbaprostil, a cytoprotective antiulcer agent, and a group of related 15-methylprostaglandins, labeled with tritium at the C-11 position, from 15(R)- and 15(S)-15-methylprostaglandin D_2 methyl esters. Radiolytic decomposition of the labeled product and some intermediates, and purification by preparative high performance liquid chromatography, and storage of [^{3}H]-arbaprostil are also discussed.

Keywords: Arbaprostil, tritium, synthesis, stability, purification, preparative HPLC

INTRODUCTION

Arbaprostil, 15(R)-15-methylprostaglandin E_2, [15(R)-15-methyl-PGE_2, **1**] is a gastric cytoprotection agent which also inhibits gastric secretion. The compound is under development for treating upper gastro-intestinal hemorrhage. Its utility for healing duodenal ulcer and other gastric lesions in humans has been reported and reviewed (1-4). As part of the program to investigate the biotransformation and disposition of arbaprostil in test animals and in man, we synthesized tritium labeled arbaprostil **2**. Because arbaprostil is believed to act as a prodrug which undergoes gastric acid catalyzed epimerization at C-15 to the 15(S)-isomer **3**, which is the biologically active entity (5-7), we also prepared tritium labeled 15(S)-15-methyl-PGE_2 (**4**) in order to study its metabolic transformations along with those of **2**. In addition, interest in the tris-(hydroxymethyl) aminomethane (THAM) salt **5** of 15-(S)-15-methyl-$PGF_{2\alpha}$ as a potential

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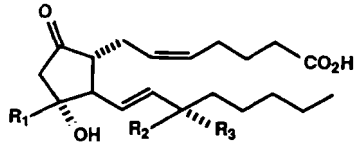
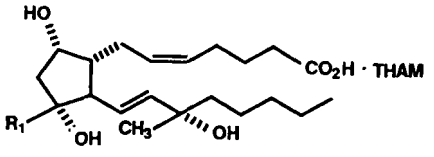
antifertility agent prompted us to obtain tritium labeled **6** as well, since its precursor, 15(S)-15-methyl-[11β-^{3}H]PGF$_{2\alpha}$ methyl ester, was also an intermediate for **4**.





1 $R_1 = H, R_2 = OH, R_3 = CH_3$

2 $R_1 = {}^3H, R_2 = OH, R_3 = CH_3$

3 $R_1 = H, R_2 = CH_3, R_3 = OH$

4 $R_1 = {}^3H, R_2 = CH_3, R_3 = OH$

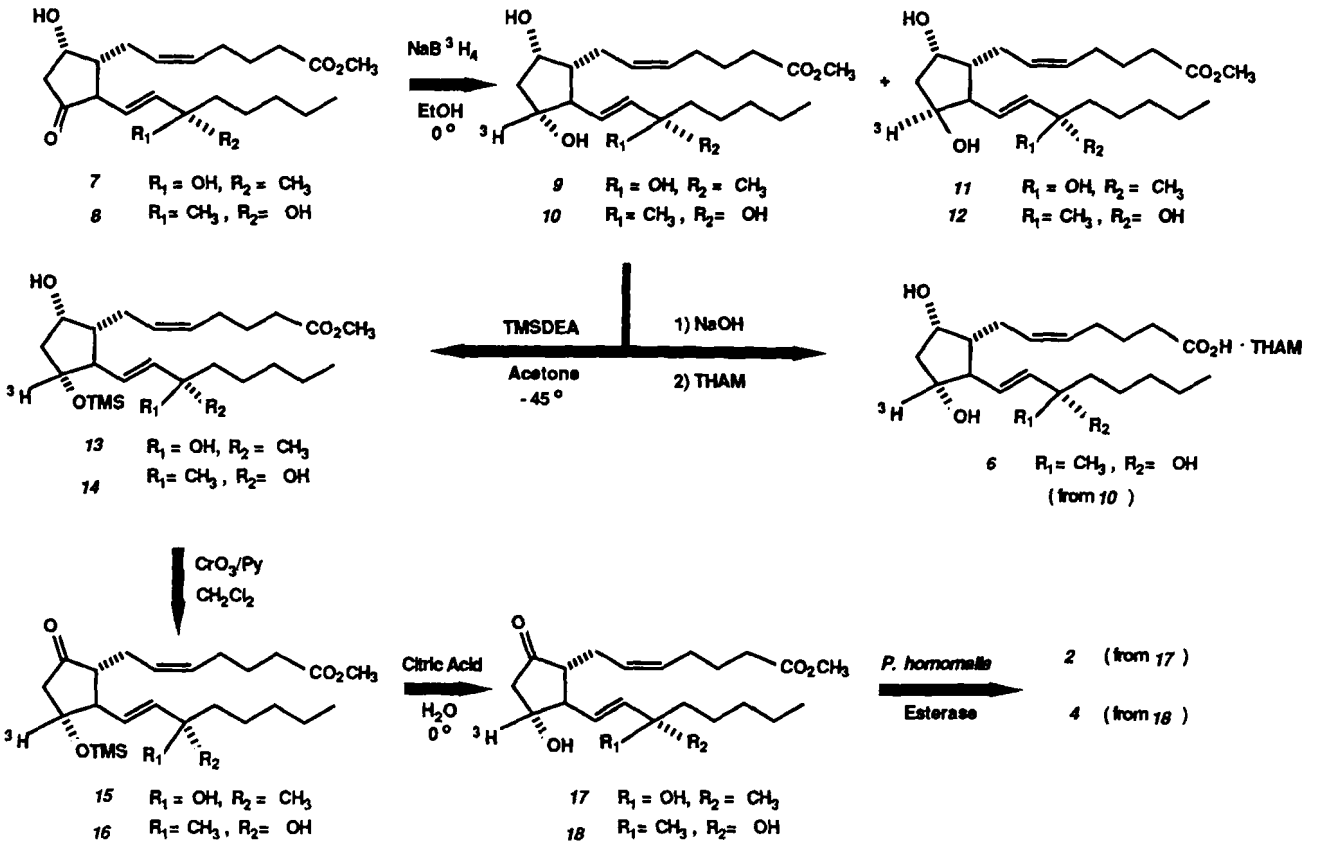
5 $R_1 = H$

6 $R_1 = {}^3H$

DISCUSSION AND RESULTS

[11β-^{3}H]Prostaglandin E$_2$ was synthesized by reduction of 9-acetyl-15-tetrahydropyranyl-PGD$_2$ with sodium borotritide followed by appropriate manipulations of functional groups at C-9 and C-15 (8). The C-15 epimers of 15-methyl-PGD$_2$ appeared to be convenient starting materials for 11-tritiated 15-methyl-prostaglandins F and E, since they would be obtainable from the corresponding C-15 epimers of 15-methyl-PGF$_{2\alpha}$, the synthesis of which had already been reported (9), as was the selective oxidation of 15(S)-15-methyl-PGF$_{2\alpha}$ to 15(S)-15-methyl-PGE$_2$ (10). Thus 15(S)-15-methyl-PGF$_{2\alpha}$ methyl ester was oxidized with Jones reagent to **8**, and similarly 15(R)-15-methyl-PGF$_{2\alpha}$ was converted to **7**. The sodium borotritide reduction of 9-acetyl-15-tetrahydropyranyl-PGD$_2$ was reported to occur with complete stereospecificity to afford the 11α-hydroxy product (8). In the reduction of PGD$_1$ (11) and PGD$_2$ (12) with sodium borohydride, again total stereospecificity at C-11 was claimed, although the PGD$_1$ reduction was also reported to afford a 9:1 epimeric mixture of 11α:11β hydroxy products (13). The borotritide reduction of PGD$_2$ methyl ester gave a ~4:1 mixture of 11α:11β in 72% total radiochemical yield (14). However,

SCHEME 1. SYNTHESIS OF [^{3}H]ARBAPROSTIL AND ITS C-15 EPIMER



when analyzed with thin-layer chromatography (TLC), the isomers were distinguishable from one another only by using boric acid impregnated silica gel. In the present work, reduction of 15(S)-15-methyl-PGD_2 methyl ester (**8**) with sodium borotritide followed by sodium borohydride (to complete the reaction) led to a 5:1 mixture of 11α:11β epimers in 68% overall radiochemical yield. The epimers were again separated by chromatography on silica gel impregnated with boric acid. Purified **10** was obtained with a specific activity of 11 mCi/mg, which was subsequently isotopically diluted to 6 mCi/mg. In an earlier run we first encountered the radiolytic decomposition of **10**. The radiochemical purity of a crystalline sample of **10** at 1mCi/mg declined ~ 0.4% per day when stored at -20°C. Furthermore, we found subsequently that the radiolytic decomposition problem was even more serious with the 15(R)-epimer **9**. At 5.1 mCi/mg, it deteriorated at a rate of ~14% per day at -20°C. Although a 1 mCi/ml solution of **10** in methanol was found to be quite stable at -20°C, these conditions (high dilution and protic solvent) were incompatible with the reactions shown in Scheme 1 for transforming it into 15(S)-15-methyl-[11β-^{3}H]PGE_2 (**4**). It was therefore decided to carry out the purification of **10** and all subsequent transformations as rapidly as possible, i.e., non-stop over a 40 hour period, and immediately following analysis of the product, to store it in solution in 95% ethanol at -20°C. In a similar manner, **7** was reduced with sodium borotritide to give a 4:1 mixture of **9**:**11**. The epimer **9** was then transformed non-stop into [^{3}H]arbaprostil (**2**) through **13**, **15**, and **17**.

The reactions used to convert the C-15 epimers of 15-methyl-$PGF_{2\alpha}$ methyl ester to the C-15 epimers of 15-methyl-PGE_2 methyl ester were analogous to those reported earlier (8). Selective monosilylation at the more accessible hydroxyl group at C-11 of **9** and **10** followed by oxidation of the C-9 hydroxyl of 11-silylated $PGF_{2\alpha}$ esters**13** and **14** with Collins reagent gave the silylated PGE_2 esters **15** and **16**. Mild desilylation with citric acid afforded the PGE_2 esters **17** and **18**. To accomplish the hydrolysis of the PGE_2 esters without causing dehydration of the acid- and base-sensitive β-hydroxyketones, the esterase derived from the coral *P. homomalla* was used. 15(S)-15-methyl-$PGF_{2\alpha}$

methyl ester (**10**) was also hydrolyzed with sodium hydroxide and the resulting acid was converted to the THAM salt **6**.

All the tritium labeled intermediates and products described above have been stored in solutions in ethyl acetate, methanol, or 95% ethanol in concentrations ranging from 0.3 to 5 mCi/ml at -20°C and more recently at -70°C. Under these conditions, all of them have exhibited good radiochemical stability. Not surprisingly, greater physical dilution results in less radiolytic decomposition. Thus [^{3}H]arbaprostil (**2**) of 5.3 mCi/mg decomposed at a rate of 2.3% per month in a 25 mCi/ml ethanol solution at -20°C, while a 0.3 mCi/ml solution at the same temperature decomposed at only 0.18% per month. Table 1 shows some -20°C stability data on [^{3}H]arbaprostil which have been stored at various concentrations in 95% ethanol. After extended storage, [^{3}H]arbaprostil can be readily purified by preparation HPLC as described in the experimental section.

Table 1. Radiochemical Stability of [^{3}H]Arbaprostil

Concentration mCi/ml	Decomposition %/month*
25	2.31
10	1.48
5	1.07
2	0.80
1	0.71
0.3	0.18

*Data obtained after eight months of storage at -20°C as solutions in 95% ethanol at the indicated concentrations. Samples analyzed by thin-layer chromatography.

EXPERIMENTAL

Radioactivity determinations were carried out in Diotol with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer by means of the external standard method. TLC analyses were carried out on 1" x 4" glass plates coated with a 250 μm thick layer of silica gel GF (Analtech). Developed plates were scanned with a Vanguard Model 880 Autoscanner equipped with a Model 885 Glass Plate Scanner. Developed zones were visualized by spraying with vanillin-H_3PO_4 and charring. HPLC analyses and preparative separations were carried out with a Spectra Physics Model 8700 solvent delivery system with a Supelcosil LC-18 5 μm column (4.6 mm ID x 25 cm).

15(R)-15-Methyl-[11β-^{3}H]PGF$_{2\alpha}$ Methyl Ester (9)

To a chilled (0°) solution of 1.00 g of 15(R)-15-methyl-PGD_2 methyl ester (2.63 mmol) in 10 ml of 95% EtOH was added 18 mg of sodium borotritide* (0.475 mmol). The mixture was stirred at 0° for 1.25 hr, and 89 mg of sodium borohydride (2.35 mmol) was added to complete the reduction. After 2 hours TLC (95:4:1 v/v $CHCl_3$:MeOH:AcOH), analysis of the reaction mixture on H_3BO_3 impregnated silica gel plates** showed the reduction was complete. The mixture was transferred to a 25 ml 2-neck round bottom flask fitted with silicon rubber septums through which were inserted two needles for gas inlet and outlet. To the stirred mixture at 0° was added, dropwise at first, 1 ml of a 1:1 mixture of HOAc and H_2O, while the flask was flushed with a gentle stream of N_2. After the evolution of gases had subsided and the flask had been thoroughly flushed with N_2 (~5 min), the mixture was partitioned with 50 ml of Et_2O and 25 ml of saturated $NaHCO_3$. The aqueous phase was extracted with 2 x 40 ml of Et_2O. The combined Et_2O

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* Supplied by DuPont/NEN Products, nominally 5 Ci.

** The boric acid impregnated silica gel plates were prepared by dipping neutral silica gel plates in a mixture of 50 ml of saturated solution of H_3BO_3 in MeOH, 495 ml of MeOH, and 660 ml of $CHCl_3$, and allowing the plates to air-dry at RT.

extracts were washed with 50 ml of brine and dried over Na_2SO_4 at room temperature for 1 hr. The solution was filtered, concentrated at room temperature and 30 torr, the residue dissolved in 5 ml of absolute EtOH and 15 ml of CH_2Cl_2, and the solution again concentrated at 30° and 30 torr. The residual oil in 4 ml of CH_2Cl_2 was chromatographed on a 100 g column of H_3BO_3 impregnated Silic AR CC-4* (Mallinckrodt Chemical Works, 60-100 mesh) eluted at 8 ml/min with 95:5 v/v $CHCl_3$:MeOH saturated with H_3BO_3.**

The eluate was collected for 85 fractions of 10 ml each. Fractions were pooled as follows: 17-29 (**9** and by-products), 30-38 (**9**, **11**, and by products), and 39-70 (**11** and by-products). The residue from fractions 17-29 was dissolved in 100 ml of Et_2O and extracted with 50 ml + 25 ml of saturated $NaHCO_3$. The combined aqueous extracts were back washed with 50 ml of Et_2O. The combined Et_2O solutions were washed with 50 ml of brine and dried over Na_2SO_4 overnight. Removal of Et_2O gave crude **9**, 3.06 Ci. The residue from fractions 39-70 was similarly treated to remove H_3BO_3 eluted from the column to give 0.662 Ci of crude **11**. The residue from fractions 30-38 (0.29 Ci) consisted of approximately equal amounts of **9** and **11**. Therefore the ratio of **9** to **11** in the reduction mixture was estimated to be 4:1.

The crude **9** was chromatographed on a 75 g column of Silic AR CC-4 packed in, and eluted with, 49:1 v/v CH_2Cl_2:MeOH. The eluate was collected for 70 fractions at 10 ml/fraction/2 min. The fractions were pooled as follows: 34-37, 0.529 Ci, **9** and by-products; 38-70, 1.96 Ci, pure **9**. The residue from fractions 34-37 was rechromatographed on a 50 g column of Silic AR CC-4 to give in fractions 26-36 0.240 Ci of pure **9**. The total radiochemical yield of **9** from 5 Ci of NaB^3H_4 was 44%. The

* This packing material was prepared by slurrying 250 g of CC-4 Silic AR with a mixture of 50 ml of a saturated solution of H_3BO_3 in MeOH, 495 of MeOH and 660 ml of $CHCl_3$. Excess solvent was filtered and solids were dried *in vacuo* at 50-60° for 18 hr.

** Boric acid-saturated 95:5 v/v $CHCl_3$-MeOH was prepared by mixing 950 ml of $CHCl_3$ with 50 ml of a saturated solution of H_3BO_3 in MeOH. Precipitates were removed by filtration through a medium frit sintered glass disc Buchner funnel to give ~ 1 l of solvent mixture.

calculated sp. act. of **9** was 5.10 mCi/mg or 1.95 Ci/mmol, i.e., the same molar sp. act. as **2** (see below), since no isotopic dilution was carried out in the conversion of **9** to **2**. TLC analysis of **9** showed a single component which co-chromatographed with an authentic sample of 15(R)-15-methyl-$PGF_{2\alpha}$ methyl ester.

15(R)-15-Methyl-[11β-^{3}H] $PGF_{2\alpha}$ Methyl Ester 11-Trimethylsilyl Ether (**13**)

A solution of freshly prepared **9** (1.35 Ci, nominally 265 mg, 0.696 mmol) in 10 ml of Me_2CO was cooled to -45° in a dry ice/CH_3CN bath. To the stirred cold solution (-43° to -48°) was added 2 ml of N-trimethylsilyldiethylamine (TMSDEA, Pierce Chemical Co.). The reaction mixture was analyzed by TLC (1:1 and 3:1 v/v EtOAc-Hexane) at 60, 120, and 150 min, and fresh TMSDEA was added to the stirred mixture, 1 ml at 70 min and 0.5 ml at 130 min. At 150 min the reaction was essentially complete and none of the disilylation product was observed. At 160 min., 40 ml of precooled (-78°) Et_2O was added and the mixture was partitioned with 45 ml of half-saturated $NaHCO_3$. The aqueous layer was extracted with 2 x 40 ml of Et_2O. The Et_2O solutions were combined and washed with 50 ml of brine, and dried over Na_2SO_4 for 30 min. The solution was filtered, concentrated at 30 torr, and dried at room temperature at 0.02 torr for 30 min to give **13** as a light straw colored oil, which was used without further purification in the next step.

15(R)-15-Methyl-[11β-^{3}H]PGE_2 Methyl Ester 11-Trimethylsilyl Ether (**15**)

Collins reagent was prepared while **13** was being worked up and dried. A mixture of 0.8 ml of dry pyridine and 25 ml of CH_2Cl_2 was cooled to 0° and 1 g of Celite 545 (Johns-Manville Corp.) was added with stirring, followed by 0.5 g of dry CrO_3. The reddish suspension was stirred at 0° for 5 min and at room temperature for 1.75 hr, and again cooled to 0°. To this suspension was added a solution of **13** from above in 10 ml of CH_2Cl_2. TLC (1:1 and 3:1 v/v EtOAc:Hexane) analysis of the mixture after 45 min of stirring at 0° showed the oxidation was complete. The mixture was filtered through a

40 g column of neutral silica gel, packed in EtOAc, under a slight vacuum. The column was thoroughly washed with 400 ml of EtOAc in portions. The combined eluate was evaporated at 30° and 30 torr and the residue (**15**) was dissolved in 16 ml of MeOH, filtered, and used without further purification in the next step.

15(R)-15-Methyl-[11β-^{3}H]PGE_2 Methyl Ester (**17**)

To the MeOH solution of **15** at 0° was added 4 ml of 2.5% solution of citric acid in H_2O. The slightly yellow mixture was stirred at 0°and TLC (1:1 and 3:1 v/v EtOAc:Hexane) analysis of the mixture at 30 min showed complete desilylation. At 45 min the mixture was partitioned with 30 ml of cold saturated $NaHCO_3$ and 40 ml of EtOAc. The aqueous layer was extracted with 40 ml + 25 ml of EtOAc. The combined EtOAc solutions were washed with 50 ml of brine and dried over Na_2SO_4 at room temperature for 30 min. The solution was filtered and concentrated at 30° and 28 torr, and the residue in 2 ml of EtOAc was chromatographed on a 50 g column of neutral silca gel packed in 1:3 v/v EtOAc:Hexane. The column was eluted with 3:1 v/v EtOAc:Hexane at 10 ml/fraction/2 min for 75 fractions. Fractions 29-60 were combined and concentrated at 30° and 28 torr to give 508 mCi of **17** as an oil, radiochromatographically pure by TLC (3:1v/v EtOAc:Hexane), used immediately in the next step.

15(R)-15-Methyl-[11β-^{3}H]PGE_2 ([11-^{3}H]Arbaprostil, **2**)

A suspension of 16 g of pulverized coral *P. homomalla* in 100 ml of H_2O was stirred at room temperature for 24 hr. The slurry was poured into 800 ml of Me_2CO with vigorous stirring. The mixture was stirred at room temperature for 30 min and filtered. The solids were thoroughly washed with portions of Me_2CO and air-dried. The dry solids were sieved through 3 inch #20 and #40 sieves. The material collected on the #40 sieve, 10 g, 20-40 mesh, particle size 0.42-0.84 mm, was used to hydrolyze the methyl ester **15**.

A solution of **15** in 0.25 ml of MeOH was added dropwise to a vigorously stirred suspension of 1 g of 20-40 mesh ground coral *P. homomalla* in 10 ml of H_2O. The suspension had been adjusted to pH 6.3-6.5 by the addition of 5 drops of diluted H_3PO_4 (5 ml of 85% H_3PO_4 in 95 ml of H_2O). Transfer of the MeOH solution of **15** was completed with the aid of another 0.25 ml of MeOH followed by 5 ml of H_2O The mixture was stirred at room temperature for 12 hr whereupon TLC analysis (EtOAc, AIX*) showed complete hydrolysis. The slurry was filtered through a cake of Celite 545 and the solids were washed with 120 ml of Me_2CO. The combined filtrates were concentrated at 30° and 28 torr and the resulting milky mixture was partitioned with 40 ml of EtOAc and 10 ml of 0.2 M $NaHSO_4$. The aqueous phase was extracted with 2 x 40 ml of EtOAc. The combined EtOAc solutions were washed with 50 ml of brine and dried over Na_2SO_4. The solution was filtered and concentrated at 30° and 28 torr and the residue in 2 ml of EtOAc was chromatographed on a 20 g column of Silic AR CC-4 packed in 1:3 v/v EtOAc:Hexane. The column was eluted with 3:1 v/v EtOAc:Hexane at 5 ml/fraction/1.3 min for 45 fractions. Fractions 16-28 were combined and concentrated to give 373 mCi of oily **2**. This crude material was dissolved in 0.6 ml of Et_2O, the solution cooled to 0°, and 0.2 ml of hexane was added dropwise with stirring and cooling. The slightly cloudy mixture was seeded with arbaprostil. When crystallization occurred within minutes, another 0.3 ml of hexane was added dropwise to the stirred cold mixture. After 30 min, the crystals were filtered, washed with cold 1:1 v/v Et_2O:hexane, followed by hexane, and dried at 0.02 torr to give 51 mg of crystalline **2**, sp. act. 5.32 mCi/mg or 1.95 Ci/mmol, λmax in EtOH 278 nm (ε 25,800), radiochemically pure by TLC (AIX, 95:4:1 v/v $CHCl_3$:MeOH:HOAc).

11-Epi-15(R)-15-methyl-[11α-^{3}H]$PGF_{2\alpha}$ Methyl Ester (**11**)

*AIX TLC solvent mixture is the upper phase of a mixture of 90:20:50:100 v/v EtOAc:HOAc:isooctane:H_2O.

TLC analysis (95:4:1 v/v $CHCl_3$:MeOH:AcOH on neutral and H_3BO_3 impregnated silica gel) of the crude **11** obtained during the sodium borotritide reduction of **9** showed that the material had undergone extensive decomposition after storage in MeOH (~33 mCi/ml) at -20° for 10 days. Chromatography on a 75 g column of Silic AR CC-4 packed in 49:1 v/v $CHCl_3$:MeOH. The column was eluted with the same solvent mixture at 10 ml/fraction/2 min for 70 fractions. Fractions 47-53 were combined and concentrated to give 56.1 mCi of **16** as an oil. This material was found to contain 4.2% of **9** (TLC, H_3BO_3 impregnated silica gel, 95:4:1 v/v $CHCl_3$:MeOH:HOAc) but free of other impurities (TLC, neutral silica gel, 95:4:1 v/v $CHCl_3$:MeOH:HOAc). It has a calculated sp. act. of 5.10 mCi/mg, assuming a molar sp. act. of 1.95 Ci/mM, identical to that of **2** since no isotopic dilution was carried out during the transformations.

15(S)-15-Methyl-[11B-^{3}H]PGE_2 (**4**)

The synthesis of **4** from 15(S)-15-methyl-PGD_2 methyl ester (**8**) paralleled the preparation of [^{3}H]arbaprostil (**2**) from 15(R)-15-methyl-PGD_2 methyl ester (**7**). Reduction of **8** (579 mg, 1.52 mmol) in 7 ml of 95% EtOH at 0° with 5 Ci of sodium borotritide (9.1 mg, 0.24 mmol, 20.8 Ci/mmol) followed by 48.4 mg of sodium borohydride (1.28 mmol) afforded a 5:1 mixture with the 11α-hydroxy product predominating. Chromatographic separation on 60 g of H_3BO_3 impregnated Silic AR CC-4 (95:5 v/v $CHCl_3$: MeOH) followed by further purification on 75 g of plain Silic AR CC-4 (98:2 v/v CH_2Cl_2 : MeOH) gave pure **10** and **12** in 68% total radiochemical yield, sp. act. ~11 mCi/mg. Compound **10** was crystallized with isotopic dilution to 6 mCi/mg and immediately selectively silylated at the C-11 hydroxy group to give **14**, oxidized with Collins reagent to **16**, desilylated to afford the ester **18** which was hydrolyzed with pulverized coral *P. homomalla* to **4**, purified by column chromatography on silic gel with 3:1 v/v EtOAc:MeOH and on silic AR CC-4 with 96:4 v/v $CHCl_3$:MeOH, sp. act. 6.25 mCi/mg (calculated value based on sp. act. of **10**), 50% overall yield from **10**, stored at -20^0 in 95% EtOH at 0.5 mCi/ml.

15(S)-15-Methyl-[11β-^{3}H]$PGF_{2\alpha}$ THAM Salt (**6**)

To a solution of 174 mg of **10** (0.45 mmol, 180 mCi) in 3 ml of MeOH was added 1.5 ml of N KOH. The mixture was stirred at room temperature for 3.5 hr and partitioned with 12 ml of H_2O and 20 ml of Et_2O. The aqueous phase was extracted with 2 x 15 ml of Et_2O, covered with 20 ml of Et_2O, and 8.5 ml of 0.2 M $KHSO_4$ was added in portions with stirring at 7-9°, so that the final pH of the aqueous phase was ~3. The aqueous layer was extracted with 2 x 15 ml of Et_2O and the extracts combined with the Et_2O phase, washed with 20 ml each of H_2O and saturated NaCl solution, and dried over Na_2SO_4. Removal of Et_2O gave 145 mCi of oil which was dissolved in 1.5 ml of CH_3CN and treated with stirring with 36 mg (0.30 mmol) of THAM in 0.3 ml of dry DMSO. After being stirred for 20 min, the clear solution was diluted with 2.5 ml of CH_3CN, which gave gummy precipitates. Seeding, further stirring for 1.25 hr, and dilution with 15 ml of CH_3CN produced fine, hard crystalline **6**, 123 mg after drying *in vacuo*, 65.8% yield based on **10** used, sp. act. 0.802 mCi/mg, or 393 mCi/mmol, radiochemically homogeneous and identical to an authentic sample of **5** by TLC (95:4:1 v/v $CHCl_3$:MeOH:AcOH, AIX, 95:5 v/v EtOAc:AcOH, 8:5:1 v/v EtOAc:Me_2CO:H_2O).

Purification of [^{3}H]Arbaprostil (**2**) by Preparative HPLC

A partially decomposed sample of **2** (~35 mCi, ~12 mg) which had been stored as an ethanolic solution of 0.3 mCi/ml at -20°C was purified by preparative HPLC on a Supelcosil LC-18 (5μ) reverse phase column (4.6 mm I.D. x 25 cm) eluted with 40:60 v/v CH_3CN:H_2O pumped isocratically at 1.5 ml/min. The eluate was monitored with UV detector set at 190 nm and also with radioactivity flow detector. In a round bottom flask which had been rinsed with IN NH_4OH, H_2O, EtOH, and Me_2CO in that order and dried with N_2 purge, the ethanolic solution of **2** was mixed with 100 ml of H_2O and concentrated at 38° and 50 torr until the volume was ~ 125 ml. The remaining mixture was partitioned with 100 ml of water and 150 ml of ethyl acetate. The aqueous phase was extracted with 100 ml of ethyl acetate. The combined organic layers were washed

with 150 ml of brine and dried over anhydrous Na_2SO_4. The dried solution was filtered and concentrated. The residue was dissolved in 25 ml of CH_3CN, filtered to remove traces of insoluble material, and again concentrated. The residue was dissolved in 200 µl of CH_3CN, and 50 µl portions of the clear solution was chromatographed at 1.5 ml/min for 7.5 min, then at 3 ml/min for 18 min to rid the column of non-polar decomposition products prior to the subsequent run. The portion of eluate containing **2** from each run was collected. The pooled collections were concentrated at 38° and 50 torr, and the aqueous residue was extracted twice with 20 ml of EtOAc. The combined extracts were washed with brine and dried over Na_2SO_4. The solvent was removed and the residue was dissolved in 63 ml EtOAc. Analyses showed this solution of **2** had a radiochemical purity in excess of 99% by HPLC and stored at -70° a sp. act. of 0.50 mCi/ml; solution stored at -70°.

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REFERENCES

1. Fung W.P. and Karvin M.M. - Med. J. Aust. 2: 127 (1976).
2. Kollberg B., Nordemar R. and Johansson C. - Scand. J. Gastroenterol. 16: 1005 (1981).
3. Vantrappen G., Janssens J., Popiela T., Kulig J., Tytgat G.N.J., Huibregtse K., Lambert R., Pauchard J.P., and Robert A. - Gastroenterolgy 83: 357 (1982).
4. Gilbert D.A., Surawicz C.M., Silverstein F.E., Winberg C.R., Saunders D.R., Feld A.P., Sanford R.C., Bergman D. and Washington P. - Gastroenterology 86: 338 (1974).
5. Merritt M.V. and Bronson G.E. - J. Am. Chem. Soc. 100: 1891 (1978).
6. Robert A. and Yankee E.W. - Proc. Soc. Exp. Biol. Med. 148: 1155 (1975).
7. Reele S.B. - Dig. Dris. Sci. 30: 612 (1985).
8. Tanouchi T. and Hayashi M. - Prostaglandins 6: 381 (1974).
9. Yankee E.W., Axen U. and Bundy G.L. - J. Am. Chem. Soc. 96: 5865 (1974).

10. Bundy G. L., Morton D. R., Peterson D.C., Nishizaqa E.E. and Miller W.L. - J. Med. Chem. 26: 790 (1983).

11. Granström E., Lands W.E.M. and Samuelsson B. - J. Biol. Chem. 243: 4104 (1968).

12. Wlodawer P. and Samuelsson B. - *ibid.* 248: 5673 (1973).

13. Foss P. S., Sih C.J., Takeguchi C. and Schnoes, H. - Biochem. 11: 2271 (1972).

14. Hsi R.S.P., Stolle W.T., McGrath J.P. and Morton D.R. - J. Labelled Compds. and Radiopharm. 18: 1437 (1980).