# 17,18-Tritium Labeled Prostaglandins

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## SUMMARY

The triple bond in 3α,5α-dihydroxy-2β-(3α-hydroxy-*trans*-1-octen-5-ynyl)-1α-cyclopentylacetic acid γ-lactone *bis*-2-tetrahydropyranyl ether (I) was reduced with tritium gas in the presence of *tris*-(triphenylphosphine)-rhodium chloride to give 3α,5α-dihydroxy-2β-(3α-hydroxy-5,5,6,6-tetratritio-*trans*-1-octenyl)-1α-cyclopentylacetic acid γ-lactone (II). The diol-lactone II was converted in five steps *via* modified Corey synthesis to prostaglandin $E_2$-17,18-$^3H_4$ (VI). Reduction of VI gave prostaglandin $E_1$-17,18-$^3H_4$ (VII) and its 13,14-dihydro (VIII) and 13,14-dihydro-15-dehydro (IX) derivatives. The prostaglandin E's were dehydrated to afford the corresponding tritium labeled prostaglandins $A_2$ (X) and $A_1$ (XI).

Key Words: Prostaglandins; 17,18-tritiated; synthesis

## INTRODUCTION

The identification of a number of urinary metabolites following administration of prostaglandin $E_2$ ($PGE_2$) to animals and man has been reported by Samuelson and other investigators (1-7). Little information on urinary excretion rate and no information on fecal excretion are available. In order to quantify excretion rates and routes, radioactive compounds with metabolically stable labels are

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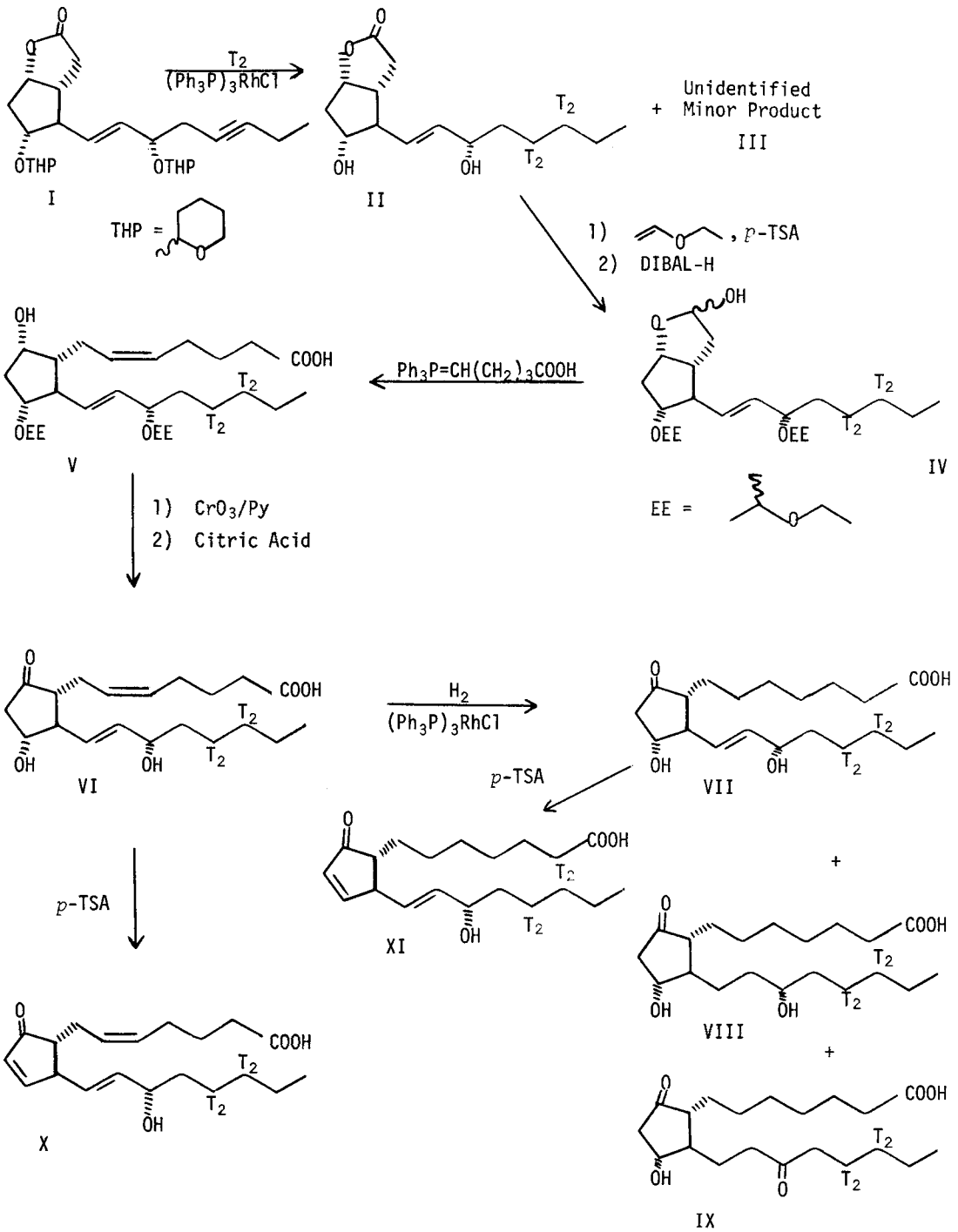
needed. It has been found that following the administration of $PGE_2$-5,6,8,11,12 14,15-$^3H_7$ to rats, approximately 30% of radioactivity is excreted as tritiated water (8), which makes quantification of the complex metabolism of $PGE_2$ difficult. $PGF_{2\alpha}$ is known to undergo metabolic degradation at the $\omega$-end of the molecule (9, 10) to give $\omega$-dinor and $\omega$-tetranor products. Whether these transformations are paralleled in the PGE and PGA series is not known. This communication describes the synthesis of prostaglandins labeled with tritium in the 17,18-positions to provide hopefully more suitable labeled materials for conducting metabolism studies.

## DISCUSSION

The alkynyl lactone I is an intermediate in the total synthesis of $PGE_3$ (11). Reduction of I with hydrogen gas in the presence of *tris*-(triphenylphosphine) rhodium chloride Wilkinson catalyst (12) occurred exclusively at the triple bond to give the *bis*-2-tetrahydropyranyl ether of the non-radioactive form of II. This specificity as well as the presence in I of all the desired stereochemistry makes it an ideal starting material for preparing 17,18-tritiated prostaglandins. The analogous reduction of I with tritium gas (see Experimental section), however, inexplicably resulted in the total loss of the tetrahydropyranyl groups and yielded a 2:1 mixture of the diol lactone II and an unidentified radioactive product III. Purification of II was carried out by chromatography on Amberlyst A15 resin. Compound II proved to be highly unstable radiochemically and must be purified immediately prior to its use. The conversions leading from II to $PGE_2$-17,18-$^3H_4$, shown in Scheme 1, are modifications of the Corey prostaglandin synthesis (13,14).

The loss of tetrahydropyranyl groups during the reduction of I necessitated introduction of protecting groups for the hydroxyl functions in II. The ethoxyethyl protecting group (15) was used because of the ease of its introduction and eventual removal as a volatile by-product. Treatment of II with ethyl vinyl ether

Scheme 1. Synthesis of 17,18-Tritiated Prostaglandins



gave the *bis*-(1-ethoxy)-ethyl ether of II, which was reduced with diisobutyl aluminum hydride (DIBAL-H) to 3α,5α-dihydroxy-2β-(3α-hydroxy-5,5,6,6-tetratritio-*trans*-1-octenyl)-1α-cyclopentylacetaldehyde γ-lactol *bis*-(1-ethoxy)ethyl ether (IV). The lactol IV was treated with triphenylphosphonium 4-carboxybutyl ylide to afford the 17,18-tritiated 11,15-*bis*-(1-ethoxy)ethyl ether of $PGF_{2\alpha}$ (V). The 9α-hydroxy function of V was oxidized with chromic acid and the protecting ethoxyethyl groups were removed with mild acid treatment to produce $PGE_2$-17,18-$^3H_4$ (VI). Controlled hydrogenation of VI in the presence of *tris*-(triphenylphosphine)rhodium chloride according to known procedure (16) gave a readily separable mixture of $PGE_1$-17,18-$^3H_4$ (VII), 13,14-dihydro-$PGE_1$-17,18-$^3H_4$ (VIII), and 13,14-dihydro-15-dehydro-$PGE_1$-17,18-$^3H_4$ (IX). Acid-catalyzed dehydration of $PGE_2$-17,18-$^3H_4$ and $PGE_1$-17,18-$^3H_4$ produced $PGA_2$-17,18-$^3H_4$ (X) and $PGA_1$-17,18-$^3H_4$ (XI).

The improved metabolic stability of the tritium labels in 17,18-tritiated prostaglandins has been demonstrated by excretion and metabolism studies carried out in test animals dosed with $PGA_1$-17,18-$^3H_4$ and $PGE_2$-17,18-$^3H_4$. Only trace amounts of tritiated water were found in the rat excreta after intravenous administration of $PGA_1$-17,18-$^3H_4$ (17). In contrast, urinary excretion of tritiated water accounted for 10-15% of the radioactivity administered as $PGA_1$-5,6-$^3H_4$ (18). Following oral dosing of rats with $PGE_2$-17,18-$^3H_4$ (19) 4% of the radioactivity was excreted in the urine as tritiated water, in comparison to 30% after dosing with $PGE_2$-5,6,8,11,12,14,15-$^3H_7$.

## EXPERIMENTAL

Radioactivity determinations were carried out with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer, using Diotol and the external standard method. Thin layer chromatography (tlc) plates were analyzed with a Vanguard Model 880 Autoscanner equipped with Model 885 Glass Plate Scanner. Ultraviolet spectrum was obtained with a Cary Model 15 spectrometer. The tlc plates used were glass plates with a 250 μm thick coat of silica gel GF (Analtech) which was plain or impregnated with $AgNO_3$ or $FeCl_3$. The $AgNO_3$ impregnated plates

were prepared by dipping the plates in 10% $AgNO_3$ in MeOH and air drying. Visualization of these and also plain silica gel GF plates was accomplished by spraying with 1% vanillin in 1:1 v/v phosphoric acid in EtOH followed by charring on a hot plate. The $FeCl_3$ impregnated plates (20) were prepared by dipping the plates in a solution of 100 g of $FeCl_3 \cdot 6H_2$ in 1 liter of $Me_2CO$ with air drying for 15 min., followed by 20 min. of drying at 100°C. The dark brown plates were stored over $CaSO_4$ (Drierite). Visualization was by spraying with 10% phosphomolybdic acid in EtOH followed by charring.

Reduction of I to II

A solution of the alkyne I (2.46 g, 5.7 mmoles) in 37 ml of $Me_2CO$ and 25 ml of PhH was stirred with a mixture of hydrogen gas and 100 Ci of tritium gas* in the presence of 0.23 g of *tris*-(triphenylphosphrine)rhodium chloride at room temperature and atmospheric pressure. The crude reduction product was subjected to cursory purification by column chromatography on buffered silica gel** eluted with 1:1 v/v EtOAc-cyclohexane to give 972 mg (33 Ci) of crude product. This material was shown by tlc (silica gel-$AgNO_3$, 4:1 v/v EtOAc in cyclohexane, developed twice) to consist mainly of II ($R_f$ 0.26) and III ($R_f$ 0.37) in the ratio of approximately 2:1. Separation of III from II was carried out using a 1.9 x 104 cm column of Amberlyst A15 resin (Ag+ cycle) eluted with 1.75 liters of 95% EtOH***, which was collected in 10 ml fractions at 15 min per fraction, after a forerun of 70 ml. The pooled residues of fractions 29-71, containing primarily II and free of III, was further chromatographed on 2.8 x 30 cm columns of 90 g of silica gel (Brinkman, 70-230 mesh) eluted either with 3:1 v/v EtOAc in

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* The reduction with tritium gas was carried out by the Radiochemical Centre, Amersham, England according to procedure developed by Dr. R. C. Kelly of The Upjohn Company.

** Buffered silica gel was prepared by treating silica gel with a phosphate buffered solution, pH 4.5, filtering, washing with distilled water and drying.

*** This separation procedure was developed by Dr. E. G. Daniels of The Upjohn Company.

cyclohexane followed by EtOAc, or with 2:3 v/v $Me_2CO$ in cyclohexane, collected in 10 ml fractions at 2.5 min. per fraction. The diol lactone II proved to be radiochemically unstable either as an oil or in solution in EtOAc, THF, MeOH, EtOH, or $Me_2CO$, and the material must be freshly purified immediately prior to being used.

*bis*-(1-Ethoxy)ethyl Ether of II

Non-radioactive diol lactone II was prepared by treating a solution of 3α, 5α-dihydroxy-2β-(3α-hydroxy-*trans*-1-octenyl)-1α-cyclopentylacetic acid (666 mg, 2.33 mmoles) in 25 ml of dry tetrahydrofuran (THF) with 0.35 ml of 1% solution of *p*-toluenesulfonic acid (*p*-TSA) in THF (1g/100 ml). The mixture was stirred under nitrogen at 60°C for 1 hr and 3 μl of $Et_3N$ was added. The solvent was removed at 30°C and 50 mm Hg pressure to give the diol lactone as an oil. This oil was combined with 2.25 Ci of II (45 mg, 0.17 mmole) and thoroughly dried by azeotropic distillation* (bath temperature 45-55°C) with $CH_2Cl_2$, until 100 ml of distillate was collected. The $CH_2Cl_2$ solution, 50 ml, was cooled to -10°C under dry $N_2$ and 2.5 ml of ethyl vinyl ether was added, followed by 0.35 ml of *p*-TSA in THF (1g/100 ml). The mixture was stirred under $N_2$ at -10°C to -4°C for 2.5 hrs, at which time tlc (silica gel, 3:2 v/v EtOAc in cyclohexane) showed that reaction was complete ($R_f$ 0.09 for II, 0.59 for the *bis*-ether).

Lactol IV

The above mixture was cooled to -70°C and 4.5 ml of 10% DIBAL-H in toluene was added with stirring under $N_2$ in 3 min from a syringe. After 20 min, the cooling bath was removed and 4.8 ml of 2:1 v/v THF in $H_2O$ was added in 3 min. The mixture was stirred at room temperature for 1 hr, filtered to remove solids which were washed with $CH_2Cl_2$. The combined filtrate and washings were dried

* The molar equivalent of $H_2O$, generated during the preparation of non-radioactive diol lactone II, was still in the mixture at this point, and must be removed to ensure success of addition of ethyl vinyl ether to the hydroxyl groups in II.

over $Na_2SO_4$ and concentrated at 25°C and 50 mm Hg pressure to give IV as an oil; tlc (silica gel, 3:2 v/v EtOAc in cyclohexane) showed the material ($R_f$ 0.49) was >90% pure radiochemically.

$PGF_2\alpha$-17,18-$^3H_4$ 11,15-*bis*-(1-Ethoxy)ethyl Ether (V)

To 17 ml of warm (65°C) dimethylsulfoxide (DMSO) was added 360 mg (8.5 mmoles) of NaH (57% mineral oil dispersion). Mixture was heated to 78°C over 15 min with evolution of gases. The resulting clear brown solution was stirred under $N_2$ at room temperature for 15 min and 1.88 g (4.24 mmoles) of 4-carboxybutyltriphenylphosphonium bromide was added in 3 min. The wine red solution was stirred at room temperature under $N_2$ for 40 min and compound IV from above in a mixture of 1.8 ml of DMSO and 1 ml of toluene was added in 3 min. The mixture was stirred for 3.25 hr and poured with stirring into a cold (0°C) mixture of 10 ml of EtOAc, 5 ml of benzene and 10 ml of $H_2O$. The pH of the mixture was adjusted to 2.95 with the addition of 7.5 ml of 1*N* $KHSO_4$. The phases were separated and the aqueous layer containing solids was extracted with 20 ml + 10 ml of EtOAc. The extracts were combined with the organic layer and washed with 3 x 5 ml of 2.5% $NH_4HCO_3$. The combined washings were extracted with 2 x 20 ml of $CH_2Cl_2$. The extracts were combined with the organic layer, washed with 2 x 25 ml of brine, and dried over $Na_2SO_4$. Removal of solvents at 30°C and 50 mm Hg pressure gave V as an oil; ∿90% radiochemically pure by tlc (silica gel, EtOAc containing 0.15% v/v of HOAc, $R_f$ 0.44).

$PGE_2$-17,18-$^3H_4$ 11,15-*bis*-(1-Ethoxy)ethyl Ether

To a stirred suspension of 40 ml of $CH_2Cl_2$, 2.7 ml of pyridine and 2.5 g of Celite was added 1.65 g of $CrO_3$ in portions in 3 min. The mixture was stirred at room temperature for 30 min under $N_2$ and the above oil V in 10 ml of $CH_2Cl_2$ was added in 5 min. The mixture was stirred at 30°C under $N_2$ for 4 hr and filtered through a pad of Celite to remove solids which were washed with 50 ml of $CH_2Cl_2$ in several portions. The combined filtrate and washings were shaken with 50 ml of 5:4:6 v/v brine-MeOH-$H_2O$. The aqueous layer was extracted with 2 x 25 ml of

$CH_2Cl_2$. The combined organic layers were extracted with a mixture of 50 ml of 0.5 N HCl, 8 ml of brine and 18 ml of MeOH. The aqueous acid layer was extracted with 25 ml of $CH_2Cl_2$. All organic layers were combined and washed with 50 ml of 5:4:6 v/v brine-MeOH-$H_2O$. The aqueous layer was again back-extracted with 25 ml of $CH_2Cl_2$ and the extracts combined with the organic layer, and filtered through a pad of $Na_2SO_4$. The combined filtrate and washings were concentrated at 30°C and 50 mm Hg pressure to give a brown syrup, which was shown by tlc (silica gel, upper phase of 9:2:5:10 v/v EtOAc-HOAc-isooctane-$H_2O$, hereafter referred to as solvent system "A-IX") to consist of four major radioactive components, of which the desired product ($R_f$ 0.60) accounted for ~50% of the total radioactivity.

PGE$_2$-17,18-$^3H_4$ (VI)

The above brown syrup was dissolved in a mixture of 35 ml of THF and 5 ml of $H_2O$, and 12.5 ml of 2.4 M citric acid was added. The brown solution was stirred at room temperature under $N_2$ for 16 hr and 39°C for 4 hr. The resulting purplish solution was cooled to 0°C, the pH was adjusted to 3.85 with 2 ml of 1 N $NaHCO_3$, and the mixture was concentrated at 30°C and 50 mm Hg pressure. The aqueous residue was extracted with 180 ml of EtOAc in 3 portions. The extracts were washed with 2 x 50 ml of brine, dried briefly over $Na_2SO_4$ and concentrated at 30°C and 50 mm Hg pressure to give a dark brownish purple syrup. The crude was chromatographed on a 2.2 x 45 cm column of 70 g of Silic AR CC-4 (Mallinckrodt, 100-200 mesh) eluted with 1:4 v/v $Me_2CO$ in cyclohexane. After a forerun of 50 ml, the eluate was collected in 10.7 ml fractions at 2.75 min/fraction. The fractions were pooled as follows (groups, fractions, mCi): A, 6-15, 88; B, 17-30, 155; C, 94-135, 647; D, 136-160, 67. Tlc (silica gel, A-IX) of the pooled materials showed that groups A and B were mixtures of the starting material and a $PGA_2$ like material in terms of tlc motility, possibly a mono-(1-ethoxy)ethyl ether of $PGE_2$. Groups C and D were $PGE_2$ of >95% and >90% radiochemical purities, respectively, total radiochemical yield 31.7% based on II used. From group C, the following

crops of crystalline $PGE_2$-17,18-$^3H_4$ were obtained from $Et_2O$-hexane mixtures: 183 mg, sp. act. 1.58 mCi/mg; 45 mg, 1.55 mCi/mg (these two crops were undiluted); 103 mg, 0.420 mCi/mg; 118 mg, 0.206 mCi/mg; from group D: 122 mg, 0.198 mCi/mg, mp 65-67.5°C (uncorrected), anal: C, H, ± <0.4% of theory; 73 mg 0.207 mCi/mg. All above samples were >98% radiochemically pure by tlc (silica gel, A-IX, $R_f$ 0.26).

Hydrogenation of $PGE_2$-17,18-$^3H_4$

A solution of 286 mg (0.81 mmole, 72 mCi) of $PGE_2$-17,18-$^3H_4$ and 27 mg of *tris*-(triphenylphosphine)rhodium chloride in 6 ml of 3:2 v/v $Me_2CO$ in benzene was stirred under $H_2$ at room temperature and atmospheric pressure. Samples of the mixture were withdrawn at 0, 2, 4, and 6 hrs for radiochromatographic (tlc, silica gel-$AgNO_3$, A-IX twice) analyses to observe the disappearance of VI and formation of VII, VII, and IX. The reaction was terminated at 7.5 hrs after an uptake of 17.8 ml. The mixture was concentrated at 30°C and 50 mm HG pressure to give a brown syrup which was crystallized from $CH_2Cl_2$ to give 163 mg (56.8% yield) of $PGE_1$-17,18-$^3H_4$ (VII), sp. act. 254 μCi/mg, radiochemically pure and identical to an authentic sample of $PGE_1$ by tlc (silica gel-$AgNO_3$, A-IX twice, $R_f$ 0.38). This material was readily recrystallized from EtOAc-hexane.

The $CH_2Cl_2$ mother liquor of VII was concentrated at 30°C and 50 mm Hg pressure. The residue, 31 mCi, was chromatographed on a 2.2 x 25 cm column of 40 g of Silic AR CC-4 eluted with 1 liter of 1:3 v/v $Me_2CO$ in cyclohexane. After a forerun of 30 ml, the eluate was collected in 11 ml fractions at 2.75 min/fraction. The fractions were pooled as follows (group, fractions, mCi): A, 15-20, 8.13; B, 25-31, 6.57; C, 36-55, 12.15. The residue from group A, ∿30 mg, was shown to be radiochemically pure and identical to an authentic sample of IX (16) by tlc (silica gel-$AgNO_3$, A-IX twice, $R_f$ 0.66). Group B residue, 26 mg, was shown by tlc to be radiochemically pure VIII (16), $R_f$ 0.53, and group C residue, 46 mg of $PGE_1$-17,18-$^3H_4$ (VII).

$PGA_2$-17,18-$^3H_4$ (X)

The mother liquors of the various crops of crystalline $PGE_2$-17,18-$^3H_4$ described above were combined and concentrated, and the residue was chromatographed on a 1.9 x 38 cm column of Silic AR CC-4 (40 g) eluted with 1:4 v/v $Me_2CO$ in cyclohexane to give 36.5 mCi, 150 mg, of $PGE_2$-17,18-$^3H_4$. The recovered material in 10 ml of THF was stirred with 5 ml of *p*-TSA in THF (1g/100 ml) under $N_2$ at 45°C for 3 hrs. After addition of 40 µl of $Et_3N$, the mixture was concentrated at 30°C and 50 mm Hg pressure and the residue was chromatographed on a 1.9 x 43 cm column of Silic AR CC-4 (45 g) eluted with 1:4 v/v $Me_2CO$ in cyclohexane. After a forerun of 30 ml, the eluate was collected in 5 ml fractions at 1.5 min/fraction. The pooled residue, 18.3 mCi, from fractions 35-60 was purified by preparative tlc (silica gel, 30:19:1 v/v EtOAc-hexane-HOAc) to give 42 mg, 10.5 mCi, of $PGA_2$-17,18-$^3H_4$ (X) as an oil, $\lambda_{max}^{EtOH}$ 217 nm (ε 9,900), radiochemically pure by tlc (silica gel-$FeCl_3$, 30:19:1 v/v EtOAC-hexane-HOAc, $R_f$ 0.24).

$PGA_1$-17,18-$^3H_4$ (XI)

A solution of 71 mg, 18.1 mCi, of $PGE_1$-17,18-$^3H_4$ (VII) and 25 mg of *p*-TSA in 7.5 ml of THF was stirred under $N_2$ at 50°C for 24 hrs. The solvent was removed at 25°C and 50 mm Hg pressure and the residue was chromatographed on a 2.2 x 25 cm column (40 g) of Silic AR CC-4 eluted with 15% v/v $Me_2CO$ in cyclohexane. After a forerun of 25 ml, the eluate was collected in 4.5 ml fractions at 1.5 min/fraction. The pooled residue from fractions 47-83, 13.9 mCi, was further purified by preparative tlc (silica gel, 30:19:1 v/v EtOAc-hexane-HOAC) to give 12.0 mCi, 38 mg, of product, which was crystallized along with 25 mg of non-radioactive $PGA_1$ from $Et_2O$-hexane to afford 44 mg of $PGA_1$-17,18-$^3H_4$ (XI), sp. act. 153 µCi/mg; $\lambda_{max}^{EtOH}$ 217 nm (ε 10,100); radiochemically pure by tlc (silica gel-$AgNO_3$, A-IX, $R_f$ 0.50; silica gel-$FeCl_3$, 30:19:1 v/v EtOAc-hexane-HOAc, $R_f$ 0.26); anal: C, H, ± <0.4% of theory.

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