# SYNTHESIS OF TRITIUM-LABELLED NATURAL PROSTAGLANDINS OF SERIES 1,2,3.

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## SUMMARY

The complete chemical synthesis of unsaturated precursors of eicosanoids of series 1,2,3 is described. Selective hydrogenation by gaseous tritium of eicosapolyenoic acid acetylenic analogues was used to introduce the label into dihomo-$\gamma$-linolenic, arachidonic and timnodonic acids, from which [$^3$H]PGE, [$^3$H]PGD and [$^3$H]$TXB_2$ were obtained by biosynthesis. From [$^3$H]PGE multiply labelled PGA, PGB, $PGF_{\alpha}$, $PGI_2ME$, 6-keto-$PGF_{1\alpha}ME$ were synthesized by chemical methods.

## INTRODUCTION

In order to obtain high-molar-radioactivity tritium-labelled prostaglandins one needs precursors that allow the maximum amount of tritium atoms to be introduced. Acetylenic analogues of polyenoic acids are well-suited to this purpose.

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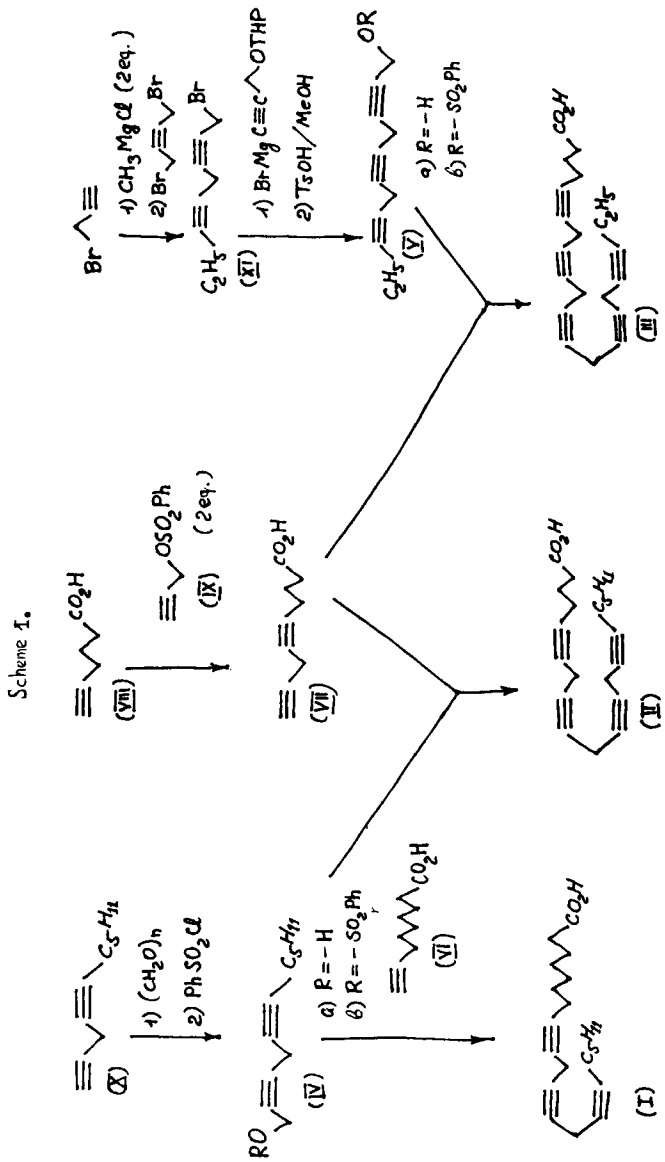
Therefore our first task was to carry out the complete chemical synthesis of the acetylenic analogues of dihomo-γ-linolenic, arachidonic and timnodonic acids: 8,11,14-eicosatriynoic (I), 5,8,11,14-eicosatetraynoic (II) and 5,8,11,14,17-eicosapentaynoic (III) acids.

Earlier we had developed preparative procedures for the synthesis of natural polyenoic acids based on intermediate polyacetylenic compounds [1-6]. In this paper we describe improved methods for obtaining acids (I)-(III) (SCHEME 1), with propargylic benzenesulphonates, 1-benzenesulphonyloxy-2,5-undecadiyne (IVb), 1-benzenesulphonyloxy-2,5,8-undecatriyne (Vb) and terminal acetylene acids, 8-nonynoic acid (VI)[1] and 5,8-nonadiynoic acid (VII) used as initial syntons. The polyacetylenic chain of compounds (I)-(III) and (VII) was constructed through condensation of acetylide-anions prepared from terminal acetylene acids (VI), (VII) and 5-hexynoic acid (VIII)[5] with benzenesulphonates (IVb), (Vb) and (1-benzenesulphonyloxy)-2-propyne (IV). The use of benzenesulphonates (IVb),(Vb) and (IX) instead of the corresponding bromides[1-6] enabled the reaction to proceed under milder conditions (20° C) with a 10-15% increase in yield. Diynic alcohol (IVa) was synthesized by condensing 1,4-decadiyne (X)[7] with paraformaldehide, and bromide (XI) by successively treating propargyl bromide with 2 equiv of $CH_3MgCl$ and 1,4-dibromo-2-butyne. A reaction with a bromomagnesium derivative of 1-tetrahydropyranyloxy-2-propyne followed by subsequent hydrolysis with TsOH catalysis was used to transform bromide into triynol (Va). Phenylsulphonylation of alcohols (IVa) and (Va) (acetone-water/KOH/$K_2CO_3$)produced benzenesulphonates (IVb), (Vb). Condensation of bis(bromomagnesium) derivatives of terminal acetylenic acids(VI)-(VIII) with 2 equiv. of propar-

Scheme 1.


(X)
1) (CH2O)n
2) PhSO2Cl
(VIII)
(IX) (2eq.)
(XI)
1) CH3MgCl (2eq.)
2) Br
Br
1) Br-Mg
2) TsOH/MeOH
OTHP
OR
a) R = H
b) R = -SO2Ph
(IV)
a) R = -H
b) R = -SO2Ph
(VI)
(VII)
(V)
(I)
(II)
(III)

gylic benzenesulphonates (VIb),(Vb) and (IX), followed by hydrolysis of intermediate propargyl esters for the target compounds(I)-(III) and (VII) with 2N sulphuric acid, produced polyacetylene acids (I)-(III) and (VII).

The tritium label was introduced into polyenoic fatty acids without the use of deactivating amines, which may catalyze the destruction of the original compound. When gaseous tritium was used with the Lindlar catalyst, after 25-30 min the appropriate acetylene precursors gave, according to GLC data, [$^3$H]dihomo-γ-linolenoic, [$^3$H]arachidonic and [$^3$H]timnodonic acids with 60-65% yields. The molar radioactivities of the resultant substances are listed in Table 1.

[$^3$H]PGE, [$^3$H]PGD and [$^3$H]TXB were obtained from multiply labelled polyenoic acids by enzymatic synthesis. Partially purified preparations of PGH synthetase and PGH-PGE isomerase were isolated from the microsomes of male sheep vesicular glands, and PGH-PGD isomerase was isolated from rat brain. The use of these purified enzymes increased the yield of labelled prostaglandins through reduction of nonspecific processes occurring during the synthesis, viz. the sorption of substrate and reaction products on inactive protein and nonspecific catalytic conversion of substrates into by-products. The yields of [$^3$H]PGE reached 80% with labelled dihomo-γ-linolenic and arachidonic acids as substrates, and 50% with timnodonic acid (Table 1).

The maximum yield of [$^3$H]PGD was attained when we used TLC-purified PGH treated with the cytosol fraction from rat brain (Table 2).

Labelled arachidonic acid was converted into [$^3$H]$TXB_2$ by means of two-step synthesis without isolating [$^3$H]$PGH_2$.

Table 1. Specific radioactivities of eicosanoids

| Compounds | Specific radioactivity, PBq/mol |
|---|---|
| [8,9,11,12,14,15(n)-$^3$H]dihomo-$\gamma$-linolenic acid | 5,55 |
| [8,11,12,14,15(n)-$^3$H]$PGE_1$ | 4,65 |
| [8,11,12,14,15(n)-$^3$H]$PGA_1$ | 4,06 |
| [11,14,15(n)-$^3$H]$PGB_1$ | 2,90 |
| [8,11,12,14,15(n)-$^3$H]$PGF_{1\alpha}$ | 4,58 |
| [5,6,8,9,11,12,14,15(n)-$^3$H]arachidonic acid | 7,03 |
| [5,6,8,11,12,14,15(n)-$^3$H]$PGE_2$ | 5,90 |
| [5,6,8,9,12,14,15(n)-$^3$H]$PGD_2$ | 5,98 |
| [5,6,8,9,11,12,14,15(n)-$^3$H]$TXB_2$ | 5,27 |
| [5,6,8,11,12,14,15(n)-$^3$H]$PGA_2$ | 5,15 |
| [5,6,11,14,15(n)-$^3$H]$PGB_2$ | 3,68 |
| [5,6,8,11,12,14,15(n)-$^3$H]$PGF_{2\alpha}$ | 5,80 |
| [5,8,11,12,14,15(n)-$^3$H]$PGI_2$ME | 5,02 |
| [5,8,11,12,14,15(n)-$^3$H]6-keto-$PGF_{1\alpha}$ME | 4,91 |
| [5,6,8,9,11,12,14,15,17,18(n)-$^3$H]timnodonic acid | 9,25 |
| [5,6,8,11,12,14,15,17,18(n)-$^3$H]$PGE_3$ | 7,35 |
| [5,6,8,11,12,14,15,17,18(n)-$^3$H]$PGA_3$ | 7,32 |
| [5,6,11,14,15,17,18(n)-$^3$H]$PGB_3$ | 5,69 |
| [5,6,8,11,12,14,15,17,18(n)-$^3$H]$PGF_{3\alpha}$ | 7,30 |

Table 2. The use of PGH-PGD isomerase in the synthesis of multiply labelled prostaglandins

| Reaction mixture | Prostaglandin content in % of total radioactivity | | |
|---|---|---|---|
| | $[^3H]PGF_{2\alpha}$ | $[^3H]PGE_2$ | $[^3H]PGD_2$ |
| 1. $[^3H]PGH_2^a$ + buffer | 28.0 | 16.5 | 7.0 |
| 2. $[^3H]PGH_2^a$ + PGH-PGD - isomerase | 18.5 | 7.0 | 26.0 |
| 3. $[^3H]PGH_2^b$ + PGH-PGD - isomerase | 27.0 | 10.0 | 14.5 |

a - purified TCX $[^3H]PGH_2$

b - non-purified extract of PGH-synthetase reaction products

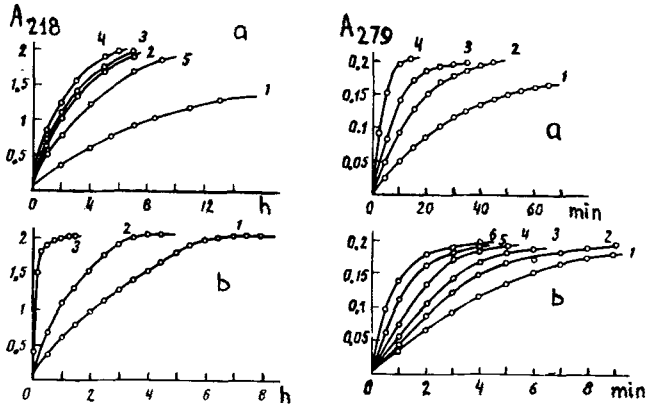



FIGURE 1. Kinetics of $PGE_2 \rightarrow PGA_2$ conversion depending on the amount of acid added (a) and on temperature (b). a-t20°C; 1N HCl content: 5 (1), 20 ( 2), 50 (3), 100 (4), 200μl(5); b - 20μl 1N HCl, temperature 20 (1), 30(2), 50°C (3).

FIGURE 2. Kinetics of $PGE_2 \rightarrow PGB_2$ conversion depending on the amount of alkali added (a) and on temperature (b). a-t30°C, 8N KOH Content: 5(1), 10(2), 15(3), 20μl(4); b-20μl 8N KOH, temperature 25(1), 30(2), 35(3), 40(4), 45(5), 50°C(6).

Preparations of PGH synthetase and TX synthetase (from human blood platelets) were added in succession to the reaction mixture with an interval required for the maximum concentration of [$^3$H]$PGH_2$ to form. The yield of [$^3$H]$TXB_2$ was 10%. The multiply labelled eicosanoids produced by chemical and enzymatic synthesis were purified and analysed by TLC, GLC and HPLC[8].

There are well-known procedures for obtaining prostaglandins A,B,F,I and 6-keto-$F_{1\alpha}$[1,9-12]. However, to reduce the radiation dose in the case of multiply labelled compounds these procedures had to be modified so as to achieve the shortest possible duration of the reactions with the lowest concentration of the labelled component.

To determine the optimum conditions for the synthesis of multiply labelled PGA, a number of experiments were performed at different temperatures and different concentrations of hydrochloric acid (Figure 1).

As seen in Figure 1a, the rate of PGA formation from PGE grew with the amount of in HCl increasing from 5 to 100 μl. The distribution of radioactivity along analytical thin-layer plates showed the radiochemical purity of PGA to reach a maximum (85-90%) at 20 μl of 1N HCl. Therefore, to analyse the temperature dependence of the PGA formation rate, we added 20 μl of 1N HCl to the reaction mixture (Figure 1b). The degree of PGE - PGA conversion was monitored by absorption at 218 nm.

As seen in Figure 1b, when the reaction proceeded beyond 6 h, 4 h and 1 h at 20°C, 30°C and 50°C respectively, no more PGA was formed. When preparative synthesis was performed at 50°C (for 1 h) [$^3$H]PGE turned into [$^3$H]PGA with a yield of 73-75% (as determined after chromatographic purification).

As to the dependence of the PGE - PGB isomerization rate upon alkali concentration and temperature (Figure 2), the reaction rate was shown to increase with growing alkali concentration and temperature. Already at 30°C, with 10 μl or more of 8N KOH, the yield of PGB came to 85-95%. When the reaction was held at 35°C for 10 min in the presence of 20μl of 8N KOH, the yield of multiply labelled PGB after chromatographic purification came to 75-80%. The degree of PGE - PGB conversion was assessed by absorption at 279 nm.

The reduction of [$^3$H]PGE by L-selectride ($Li^+[HB(CH(CH_3)-C_2H_5)_3]^-$) for 40-50 min in tetrahydrofuran (THF) at -78°C produced [$^3$H]$PGF_\alpha$. When handling microgram quantities of tritium-labelled PGE (~0.5GBq) (to prevent appreciable radiolysis), we had to strongly dilute the reaction mixture with THF and to perform the reduction with a large excess of L-selectride, so as to accelerate the process. Not only PGE, but their methyl esters could be used as initial compounds (60-65% yield of [$^3$H]$PGF_\alpha$ and [$^3$H]$PGF_\alpha$ME). The direct conversion of [$^3$H]$PGE_2$ME into [$^3$H]$PGF_{2\alpha}$ME allowed it to be used, without any further purification, for the synthesis of [$^3$H]$PGI_2$ME and [$^3$H]6-keto-$PGF_{1\alpha}$ME (Table 1).

## MATERIALS AND METHODS

PGE, PGD, PGA, PGB, $PGF_\alpha$, $PGI_2$ME, 6-keto-$PGF_{1\alpha}$ME, $TXB_2$ were commercially supplied by SERVA (FRG) and the Tallin Experimental Plant (USSR). Eicosapolyenoic acids were obtained from the Institute of Marine Biology, USSR Academy of Sciences (Far-Eastern Branch) at Vladivostok and from FLUKA (Switzerland). Tween-20 by MERCK (FRG), Lubrol PX, hemin ($Fe^{3+}$protoporphyrin) by SIGMA (USA), DEAE cellulose by Whatman (UK), Blue Toyopearl 650M-I by TOYO SODA (Japan), L-adrenaline by SERVA (FRG), silica gel plates for TLC by FLUKA (Switzerland),

SILUFOL (Czechoslovakia), L-selectride by ALDRICH (USA). All syntheses were carried out in dry argon. IR spectra were recorded by a SHIMADZU IR-435 spectrometer (Japan) on film, NMR spectra by BRUKER WM250 (FRG) in $CDCl_3$ at 250 mHz, UV spectra by SPECORD M40 (GDR), the distribution of radioactivity over plates was determined by a BERTHOLD 2027 scanner (FRG), radioactivity was measured by a scintillation counter with 30% registration efficiency in dioxane scintillator. Apart from TLC, tritium-labelled eicosanoids and their acetylene precursors were analysed and purified by gas-liquid chromatography on a CHROM 5 chromatograph (Czechoslovakia) with a 3x1200mm column, 10% Silar, 10 CP on Chromosorb W-AW, 100-120 mesh, injector temperature 260°C, detector temperature 220°C, carrier gas: nitrogen, v=20 ml/min, flame-ionization detector, high-pressure liquid chromatography on a MILICHROM chromatograph (USSR) with a 2x60mm column, Nucleosil 5 C18 or Silasorb 7.5 C18, or on a GILSON chromatograph (France) with a 4.6x250mm column, Servachrom Octadecyl Si 100 Polyol or with a 3.3x150mm column. Separon 5 C18 equipped with radioactivity monitor with a cuvette of scintillation quartz glass. Labelled compounds were analysed both in the form of free acids and in the form of methyl and p-bromophenacyl esters; preparative separation was performed in the form of free acids as described in [8].

## THE ENZYME PREPARATIONS

PGH synthetase and PGH-PGE isomerase were obtained as described in [13,14] (all enzyme preparations were isolated and purified at 0-4°C). A microsomal fraction was obtained by centrifugation (60 min, 105000xg) from sheep vesicular gland homogenate, then treated with the Tween-20 detergent (1%, w/v) for the solubilization of membrane proteins. PGH synthetase and PGH-PGE isomerase were separated and purified

on a column with DEAE cellulose. The activity of the resultant PGH synthetase amounted to 5.0-7.5μmol of arachidonic acid (AA) in 1 min per 1 mg of protein ($[AA]_0$ - 0.2 mM, pH 8, 25°C), and that of PGH-PGE isomerase to 0.17μmol of $PGE_2$ in 30 min per 1 mg of protein ($[AA]_0$ - 0.1 mM, pH7.4, 32°C).

PHG-PGD isomerase was isolated from the brain of mongrel rat as described in[15]. The cytosol fraction obtained by homogenate centrifugation (90 min, 100000xg) was concentrated 10-fold by ultrafiltration and dialyzed. The activity of the resultant preparation of PGH-PGD isomerase was 4.2 nmol of $PGH_2$ in 1 min per 1 mg of protein ($[PGH]_0$ - 5μM, pH 9.5, 25°C).

To isolate thromboxane synthetase we used human platelets precipitated in the course of blood fractionation. The enzyme was purified by the procedure[16], which involved obtaining the microsomal fraction, solubilization of membrane proteins by the non-ionic detergent Lubrol PX (1%, w/v), chromatography on DEAE cellulose and chromatography on a column with Blue Toyopearl 650M-I. The thromboxane synthetase activity of the purified preparation came to 120 nmol of $PGH_2$ in 1 min per 1 mg of protein ( $[PGH]_0$ - 5μM, pH 7.4, 25°C). All enzyme preparations were kept at -40°C.

## KINETIC STUDIES

To obtain the kinetic curves of the PGE - PGA conversion we used a solution of 230μg of PGE in 3 ml of dioxane and allowed the reaction to proceed at different temperatures and HCl concentrations (Figure 1).

To obtain the kinetic curves of the PGE - PGB isomerization we used a solution of 8μg of PGE in 0.3 ml of ethanol and allowed the reaction to proceed at different temperatures and KOH concentrations (Figure 2).

## SYNTHESIS OF EICOSAPOLYYNOIC ACIDS

2,5-Undecadiynol-1 (IVa). To the reagent prepared from 2.4 g (0.1 mol) of magnesium, 10.9 g (0.1 mol) of ethyl bromide and 13.4 (0.1 mol) of 1,4-decadiene in 70 ml of tetrahydrofuran 9.0 g (0.3 mol) of paraformaldehyde were added. The reaction mass was kept for 40 min at 60°C, then cooled down to 20°C, acidified with 2N $H_2SO_4$, extracted with ether (3x20ml), dried with $Na_2SO_4$, whereupon the solvent was evaporated and the residue distilled. The yield came to 12.1 g (74%). B.P.98-101°C/0.1 Torr; $n_D^{20}$ 1,4857; IR spectrum ($\nu$,$cm^{-1}$): 3300-3400(OH), 2282(C≡C), 1307($CH_2$-C≡C); NMR spectrum ($\delta$,ppm): 0.98(t,3H,$CH_3$), 1,38(m,6H,$CH_2$),2,14(t,2H,$CH_2C{\equiv}C$), 3.11 (m,2H, $C{\equiv}CCH_2{\equiv}C$), 4.37(t,2H, $CH_2O$), 4.61 (S,H,OH).

1-Bromo-2,5-octadiyne (XI). To the Grignard reagent obtained from 4.8 g (0.2 mol) of magnesium and an excess of methyl chloride in 100 ml of tetrahydrofuran 0.1g of copper chloride (I) and 11.9 g (0.1 mol) of propargyl bromide in 20 ml of tetrahydrofuran were added during 1h at 5-10°C. The mixture was kept for 1.5 h at 50°C, cooled down to 5°C, then 0.1 g of copper chloride (1) and 25.4 g (0.12 mol) of 1,4-dibromobut-2-yne were added. The reaction mass was kept for 6 h at 60°C, acidified with 2N $H_2SO_4$, extracted with ether, dried with $CaCl_2$, then the solvent was evaporated and the residue distilled. The yield came to 9.4g (51%). B.P.68-70°C /0.42Torr; $n_D^{20}$ 1.5294; IR spectrum ($\nu$,$cm^{-1}$):2223(C≡C), 1319 ($C{\equiv}CCH_2$), 620(CBr); NMR spectrum ($\delta$,ppm):1.10(t,3H,$CH_3$), 2.14(m,2H,$CH_2C{\equiv}C$), 3.14(m,2H,$C{\equiv}CCH_2C{\equiv}C$), 4.15(t,2H,$C{\equiv}CCH_2Br$).

2,5,8-Undecatriynol-1 (Va). To the reagent prepared from 0.96 g (0.040 mol) of magnesium, 4.36 g (0.040 mol) of ethyl bromide and 5.6 g (0.040 mol) of tetrahydropyranyloxypropargyl alcohol in 100 ml of tetrahydrofuran was added 0.1 g

of copper chloride (I) and 3.7 g (0.020 mol) of 1-bromo-2,5-octadiyne. The reaction mass was kept for 1.5 h at 20°C, acidified with 2N $H_2SO_4$, extracted with ether, evaporated and the residue dissolved in 40 ml of methanol. After adding 1.0 g of p-toluenesulphonic acid, the mixture was stirred for 1h at 20°C, dissolved with water (100 ml) and extracted with ether. The extract was dried with $Na_2SO_4$, evaporated and the residue was chromatographed on a column packed with 100/160 μm silica gel elution with ether:hexane (1.0:1.5). The yield was 2.1 g (65%). M.P. 27-28°C; IR spectrum ($\nu$,$cm^{-1}$):3400(OH), 2400(C≡C),1320($CH_2C{\equiv}C$);NMR spectrum ($\delta$,ppm): 1.06(t,3H,$CH_3$), 2.10(m,2H,$CH_2C{\equiv}C$), 3.04(m,2H,$C{\equiv}CCH_2C{\equiv}C$),3.12(m,2H,$C{\equiv}CCH_2C{\equiv}CCO$), 4.10(t,2H,$CH_2O$).

Propargylic benzenesulphonates. To a mixture of 0.01 mol of propargyl alcohol and 0.015 mol of benzenesulphonyl chloride in 10 ml of acetone, stirred and cooled down to 5°C, was added 0.015 mol of KOH and 0.005 mol of $K_2CO_3$ in 5 ml of water. The reaction mass was kept for 1.5 h at 20°C, diluted with 50 ml of water, extracted with ether, dried with $Na_2SO_4$, evaporated and the residue was chromatographed on a column packed with 40/100 μm silica gel elution with ether:hexane (1:2). 1-Benzenesulphonyloxy-2,5-undecadiyne (IVb). The yield was 88%. IR spectrum ($\nu$, $cm^{-1}$):2301(C≡C), 1320($CH_2C{\equiv}C$), 1365, 1198, 1094 ($OSO_2$), 1584, 687($C_6H_5$). 1-Benzenesulphonyloxy-2,5,8-undecatriyne (Vb). The yield was 79%. IR spectrum ($\nu$,$cm^{-1}$): 2340(C≡C),1325($CH_2C{\equiv}C$), 1370, 1200, 1096($OSO_2$) 1588, 690($C_6H_5$).

Eicosapolyynoic acids. To the reagent prepared from 0.01 mol of magnesium, 0.01 mol of ethyl bromide and 0.005 mol of $\omega$-acetylenic acid in 10 ml of tetrahydrofuran were added 0.02 g of copper cyanide (1) and 0.02 mol of propargylic benzenesulphonate in 5 ml of tetrahydrofuran. The reaction mass was stirred for 1.5 h at 20°C, acidified with 2N $H_2SO_4$, stirred

for 15 min at 20°C, extracted with ether, then the extract was dried with $Na_2SO_4$, evaporated and the residue was purified on the silica gel column, elution with ether:hexane (1:1). 8,11,14-Eicosatriynoic acid(I): The yield was 78%. M.P. 51-52°C; NMR spectrum ($\delta$,ppm):1,05(t,3H,$CH_3$),1.41(m,12H,$CH_2$), 1.79(m,2H,$CH_2CCOO$), 2.13(m,4H,$CH_2C{\equiv}C$), 2.23(t,2H,$CH_2COO$), 3.15(m,4H,$C{\equiv}CCH_2C{\equiv}C$). 5,8,11,14-Eicosatetraynoic acid (II): The yield was 74%. M.P. 80-81°C; NMR spectrum ($\delta$,ppm): 0.90(t,3H,$CH_3$), 1.40(m.6H,$CH_2$),1.75(m,2H,$CH_2CCOO$),2.03(m,2H, $CH_2C{\equiv}C$), 2.27(m,2H,$CH_2CCCOO$),2.40(t,2H,$CH_2COOH$),3.15(m,6H, $C{\equiv}CCH_2C{\equiv}C$). 5,8,11,14,17-Eicosapentaynoic acid (III): The yield was 71%. M.P.114-116°C; NMR spectrum ($\delta$ppm): 1,12(t,3H,$CH_3$), 1.82(m,2H,$CH_2CCOO$), 2.18 (m,2H,$CH_2C{\equiv}C$), 2.26(m,2H,$C{\equiv}CCH_2CCCOO$),2.49(t,2H,$CH_2COO$),3.14 (m,8H, $C{\equiv}CCH_2C{\equiv}C$).

SYNTHESIS OF MULTIPLY LABELLED POLYENOIC ACIDS

Selective hydrogenation by gaseous tritium was performed in the following manner. A dioxane solution of the appropriate polyynoic acid was placed in a reaction ampoule with a 2:1 (mg/mg) ratio of Lindlar catalyst (FLUKA) to the substance, the ampoule was frozen with liquid nitrogen, evacuated to a pressure of $1x10^{-3}$ hPa and filled with tritium to 400 hPa, thawed out and stirred while the reaction proceeded for 25-30 min. Then the ampoule was frozen again and excess tritium was removed through evacuation. The catalyst was filtered off, washed with methanol (3mlx3), the filtrate was thrice evaporated with methanol to remove labile tritium. The desired compound was purified by chromatographic procedure[8]. The yield of [$^3H$]polyenoic acids came to 60-65%. The molar radioactivities of these and other labelled eicosanoids obtained by chemical synthesis are listed in Table 1.

ENZYMATIC SYNTHESIS OF [$^3$H]$PGD_2$

1.85 GBq of [$^3$H]AA was converted into [$^3$H]$PGH_2$ using PGH synthetase as described in[17]. [$^3$H]$PGH_2$ was purified by preparative TLC at -20°C in ethyl acetate-hexane-iso-propanol--acetic acid (15:9:1:0.05, v/v/v/v). The yield of [$^3$H]$PGH_2$ in terms of radioactivity came to 25-30%. Then to 450 MBq of [$^3$H]$PGH_2$ was added 20 ml of PGH-PGD isomerase solution (2.5 g/ml) in 0.1 M tris-HCl (pH 9.5) pre-incubated at 25°C. After 20 min's incubation at 25°C 0.8 ml of 2M citric acid solution was added, and the reaction products were extracted with ethyl acetate (4x100ml). The yield of [$^3$H]$PGD_2$, after chromatographic purification came to 20-25%.

ENZYMATIC SYNTHESIS OF [$^3$H]$TXB_2$

The initial reaction mixture (1.5 ml) containing a preparation of PGH synthetase (0.09 mg/ml), adrenalin (2 mM), hemin (2 mM) in 20 mM potassium-phosphate buffer (pH 7.9) with 0.1 Lubrol PX was pre-incubated for 5 min at 25°C. Then 0.84 GBq of [$^3$H]AA was added, the reaction mixture incubated for 1.5 min at 25°C and 6 ml of TX synthetase preparation was quickly added (to a final concentration of 0.025 mg/ml) in 20 mM potassium-phosphate buffer (pH 7.4). The mixture was incubated for 60 min at 25°C, and the [$^3$H]$TXB_2$ product was isolated by chromatographic procedure. The yield of [$^3$H]$TXB_2$ was 10%.

ENZYMATIC SYNTHESIS OF [$^3$H]PGE

The enzymic synthesis of [$^3$H]PGE was performed as described earlier[18,19] using partially purified preparations of PGH synthetase and PGH-PGE isomerase. The yield, after chromatographic purification, was 70-80% for [$^3$H]$PGE_1$ and [$^3$H]$PGE_2$, and 45-50% for [$^3$H]$PGE_3$.

MULTIPLY LABELLED PGA

23.0 μg of [$^3$H]PGE in 0.3 ml of dioxane was treated with

20 μl of 1 M HCl and kept at 50°C for 1 h. After this the reaction mixture was evaporated several times with methanol at room temperature until the HCl was removed, the dissolved in 0.1 ml of ethanol and purified by chromatography. The yield of [$^3$H]PGA came to 73-75%.

MULTIPLY LABELLED PGB

To 8 μg of [$^3$H]PGE in 0.3 ml of ethanol was added 20 μl of 8 M KOH and kept for 10 min at 35°C. Then the solution was acidified to pH 2 with 1 M acetic acid, evaporated several times with methanol and purified by chromatographic procedure. The yield of [$^3$H]PGB came to 75-80%.

MULTIPLY LABELLED PGF$_\alpha$

A solution of 36 μg of [$^3$H]PGE in 1 ml of tetrahydrofuran (THF) freshly distilled over sodium was stirred at -78°C while being treated with 0.3 ml of a $Li^+[HB(-CH(CH_3)-C_2H_5)_3]^-$ solution in THF (1 mmol/ml) for 40-50 min under argon. The reaction mixture was decomposed with water (0.3 ml) at -78°C, then heated to 20°C and purified as in [8]. The yield of [$^3$H]PGF$_\alpha$ came to 60-65%.

MULTIPLY LABELLED METHYL ESTERS OF PROSTACYCLIN AND 6-KETO-PGF$_{1\alpha}$

Highly labelled I-ether of PGI$_1$ME was obtained by treating 0.37 GBq of [$^3$H]PGF$_{2\alpha}$ME dissolved in 1 ml of ether at 0°C in the presence of 0.2 ml of saturated aqueous sodium bicarbonate with 0.1 ml of 2,5% iodine solution in ether. After the reaction mixture was stirred for 12 h at 0°C the excess iodine was removed with 5% aqueous solution of sodium thiosulphate. The yield of the labelled product after purification was 55-56%. To a solution of [$^3$H]I-ether of PGI$_1$ME (0.15 GBq) in 0.1 ml of absolute toluene was added 0.1 ml of DBU, and the mixture was kept for 3 h at 110°C. The yield of [$^3$H]PGI$_2$ME after puri-

fication was 40-50%. When 75 MBq of [$^3$H]$PGI_2ME$ was treated for 30 min with 1 M HCl (pH 3), an 80% yield of [$^3$H]6-keto-$PGF_{1\alpha}ME$ was obtained.

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