Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 11

TRITIUM-LABELLING OF NATURAL AND MODIFIED PROSTAGLANDINS

V.P.Shevchenko, V.V.Bezuglov, I.Yu.Nagayev, N.F.Myasoyedov
Institute of Molecular Genetics, USSR Academy of Sciences,
Kurchatov Sq., Moscow 123182

Summary

The paper describes the synthesis of [5,6-$^3H_2$]PGE_1, 15-fluoro-15-deoxy-[5,6-$^3H_2$]PGE_1 and 11-deoxy-[5,6,10,11-$^3H_4$]PGE_1 through selective hydrogenation in the presence of heterogeneous and homogeneous catalysts. With heterogeneous catalysts, the yield of labelled compounds was 35%. If bulk substituents were introduced and $(Ph_3P)_3RhCl$ was used as the catalyst, the yield of labelled prostaglandins was increased to 70%.

KEY WORDS: Selective hydrogenation, tritium-labelling of prostaglandins and their drivatives.

INTRODUCTION

Introducing the tritium label directly into natural and modified prostaglandins is an effective way to obtain radioactively labelled derivatives of biologically active compounds. While natural prostaglandins with a high molar radioactivity can be synthesized by enzymic methods from tritium-labelled polyenic fatty acids, modified prostaglandins can be obtained either by isotopic exchange or by selective hydrogenation or by selective dehalogenation or by synthesis from labelled synthons.

0362–4803/89/111243–13$06.50

Received January 18, 1989
Revised June 2, 1989

The interest in labelling not only natural but also modified prostaglandins - fluoro-deoxy-prostaglandins (FPG), deoxy--prostaglandins, etc., is associated with their specific biological and pharmacological properties[1].

Procedures have been described for obtaining such compounds through isotopic exchange reactions. By this means tritium was introduced into methyl esters of 15-fluoro-15-deoxy-prostaglandins A_2, E_2 and $F_{2\alpha}$[2], into PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$[2-4], into methyl esters of $PGF_{2\alpha}$[2]. The yields were in the range 25-75%, and the molar radioactivity of the labelled compounds reached 0.1 TBq/mmol.

For modified prostaglandins with a halogenated phenyl core one can obtain labelled compounds with a high molar radioactivity through selective dehalogenation[5]. Upon the dehalogenation of bromophenacyl esters of 15-fluoro-15-deoxy $PGF_{2\alpha}$, 11,15-dideoxy -15-fluoro-PGE_2 labelled prostaglandins were synthesized with a yield of 15-25% and a molar activity of 0.22-0.26 TBq/mmol.

Another option for the synthesis of labelled prostaglandins modified at the carboxyl group is the condensation of the original compounds with labelled reagents. Thus, [^{3}H]valine with a molar radioactivity of 2 TBq/mmol was used to obtain $PGF_{2\alpha}$ valine amide by the mixed anhydride method with a 90% yield[6].

The most simple and widely used way of obtaining multiply labelled prostaglandins of the first series is selective hydrogenation of the C_5=C_6 bond by tritium[7,8]. Using 5% Pd/C as the catalyst, [5,6-$^3H_2$]PGE_1 was synthesized with a molar activity of 1.8 TBq/mmol, but the yield of the desired product did not exceed 6%. Hence the search for new, improved methods is necessary. This paper deals with the processes that occur when tritium is introduced into prostaglandins in the presence of heterogeneous catalysts.

MATERIALS AND METHODS

Catalysts: I - 5%Pd/$BaSO_4$, 10% Pd/C, 5% Rh/C, 5% Pt/C, II - Lindlar catalyst (FLUKA,Switzerland), III - 5% Ni/$CaCO_3$, 5% Cu/$CaCO_3$, $LaNi_4Cu$; Solvents (reagend grade) and prostaglandins were commercial preparations. Butyl dimethyl silyl ethers of PGE_2 15-fluoro-15-deoxy-PGE_2 (2xBDMS 15FPGE$_2$) and PGA_2, were synthesized by V.V.Bezuglov at the Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences. The 5% Pd/$BaSO_4$ catalysts (Produced USSR (a), FRG (b), Switzerland (c)) were deactivated by lead diacetate according to the method of Lindlar[9], and solvents were purified by standard procedures. Mass spectra were obtained on the CH-5 mass spectrometer (Varian, USA) with the sample directly introduced into the ion source at the ionization chamber temperature of 250°C, ionizing voltage 70 eV, evaporation temperature 70-100°C. NMR spectra were registered on the WM-500 BRUKER spectrometer in deuterochloroform. Reaction mixture were analysed with a MILICHROM chromatograph (USSR) equipped with a 2x60mm column, stationary phase: NUCLEOSIL 5 C_{18}, eluent: acetonitrile-water (55:45); capacity factor for bromophenacyl esters of PGE_2, PGE_1 and PGE_0 was 7.0; 8.4; 9.6 respectively; detection at 260 nm. The preparative separation of the reaction mixture was performed on a GILSON liquid chromatograph (Gilson, France) equipped with a 4.6x250 mm column, stationary phase - Servachrom Octadecyl Si-100, 10μm; eluent: acetonitrile-water-acetic acid (33:67:0.1), 1 ml/min, detection at 210 nm. Capacity factors: 6.26, 7.13, 8.33 for PGE_2, PGE_1 and PGE_0 free acids, respectively. Methyl esters of PGE_1, 5,6-trans PGE_2 and 13,14-dihydro-PGE_2 were separated in acetonitrile-water (40:60), capacity factors of 7.55 , 6.49 and 7.05 respectively. Preparative thin-layer chromatography (TLC) was performed on plates with a layer of silica gel (Kieselgel-G) with 10% gypsum

(MERCK, FRG) impregnated with a 12% solution of silver nitrate. Analytical TLC was performed on Silufol plates (Kavalier, CSSR) impregnated immediately before use by a single passage of 12% silver nitrate solution followed by warm-air drying for 5 min. The reaction with diluted tritium (hydrogen-tritium mixture, 1000:1), hydrogen and deuterium was carried out by the method described elsewhere[10].

Synthesis of Bromophenacyl Esters of Prostaglandins (BPEPG).

To 5-50 μg of tested mixture evaporated to dryness was added a fourfold excess of p-bromo phenacyl bromide (0.5% solution in acetone) and a twofold molar excess of triethylamine (0.5% solution in acetone). The reaction mixture was kept for 2-3 h at room temperature. The yield of p-bromophenacyl esters was 85-90%.

Synthesis of $[5,6\text{-}^3H_2]PGE_1$ from PGE_2.

5 mg of PGE_2 15 mg of Lindlar catalyst and 1 ml of ethyl acetate were placed in a reaction ampoule. The mixture was frozen by liquid nitrogen, evacuated to a residual pressure of $1x10^{-3}$Torr and filled with gaseous tritium to 400 hPa. The contents of ampoule were thawed out, and the reaction mixture was stirred for 40 min at room temperature.

Then the ampoule was again frozen by liquid nitrogen, and excessive tritium was removed. Labile tritium was removed by triple evaporation (at lowered pressure) of the reaction solution with 5 ml of methanol. After purification by HPLC and TLC - $AgNO_3$ (chloroform-methanol-acetic acid-water, 95:7.5:1.0:0.6), $[5,6\text{-}^3H_2]PGE_1$ was obtained with a yield of 32%, a molar radioactivity of 1.8 TBq/mmol and a radiochemical purity of 95-97%.

Synthesis of $[5,6\text{-}^3H_2]PGE_1$ from 3xBDMS PGE_2.

5 mg of 3xBDMS PGE_2 and a solution of 5 mg of $(Ph_3P)_3RhCl$ in 0.3 ml of dioxane were placed in a reaction ampoule, frozen,

evacuated and filled with tritium up to a pressure of 333 hPa. The contents of ampoule were thawed out and the reaction mixture was stirred for 6 h at room temperature. Excess and labile tritium was then removed as described above. The labelled compound was purified by TLC (hexane-ether, 3:1 Rf 3xBDMS PGE_1 0.58). The butyl dimethyl silyl protecting group was removed through treatment of 4 mg of the substance dissolved in 1.5 ml of tetrahydrofuran with 0.5 ml of 50% $(_{aq})$ HF, stirring for 2 h at room temperature. Then the solution was diluted with 10 ml of chloroform and washed with saturated aqueous $NaHCO_3$ (15 ml x 2), water (5 ml x 3), dried by Na_2SO_4 and evaporated.

The viscous residue dissolved in chloroform was placed on a column filled with 2.5 g of silica gel L (LaChema, CSSR) and the column was eluted successively with chloroform (40 ml) and a chloroform-methanol mixture, 95:5 (20 ml), 90:10 (40 ml), taking 2 ml fractions. The fractions containing $[5,6\text{-}^3H_2]PGE_1$ (TLC-$AgNO_3$ test, see above) were combined, evaporated and dissolved in 70% aqueous methanol. The labelled prostanoid was obtained with a yield of 60-70%, molar radioactivity 1.8-2.0 TBq/mmol and radiochemical purity 95-97%.

Synthesis of 15-fluoro-15-deoxy-$[5,6\text{-}^3H_2]PGE_1$.

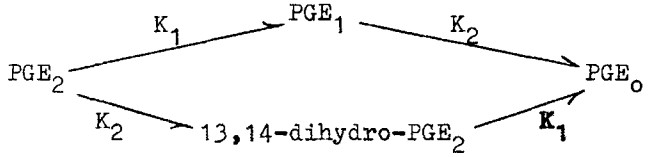
The labelled compound was synthesized from 2xBDMS 15FPGE_2 by the procedure described above with a yield of 50-70%, molar radioactivity 0.9-1.1TBq/mmol and radiochemical purity 95-97% (for details of analysis and purification see reference[11]).

Synthesis of11-deoxy$[5,6,10,11,\text{-}^3H_4]PGE_1$.

The labelled compound was synthesized from 2xBDMS PGA_2 by the procedure described above with a yield of 40-45%, molar radioactivity 2.6-3.0 TBq/mmol and radiochemical purity 95-97% (for details of analysis and purification see reference[12]).

RESULTS AND DISCUSSION

The possible reactions involved in the hydrogenation of PGE_2 (with no allowance for cis-trans isomerization or double--bond migration) are illustrated in the following diagram:



The effect of the choice of catalyst upon the selectivity of reduction of the 5,6-double bond in PGE_2 was examined using active hydrogenation catalysts (I), Lindlar catalysts A-D (II), and copper-and nickel-based catalysts (III). The reaction was carried out for 3-180 min in dioxane. The catalysts of group I were found to cause a faster hydrogenation of PGE_2: already after 3 min there was found 12% PGE_2 in the reaction mixture in the presence of 10% Pd/C or 5% Pt/C, and only 4% PGE_2 in the presence of 5% Rh/C. The hydrogenation was somewhat slower in the presence of 5% $Pd/BaSO_4$ (Figure 1), but in this case the reduction of PGE_2 to PGE_1 was not selective.

In the presence of the catalysts of group III no hydrogenation of PGE_2 was observed. The most interesting results were obtained for Lindlar catalysts (Table 1). In this experiment a mixture of PGE_2 and $[5,6,8,11,14,15\text{-}^3H_7]PGE_2$ (37MBq per 10 mg of prostaglandin) was used as a model compound, and hydrogenation was performed under hydrogen.

The largest yield of PGE_1 (Tables 1,2) was obtained with catalyst D and with ethyl acetate used as solvent. The use of other solvents (acetone, benzene, dioxane, chloroform, methanol) led to a reduced yield of PGE_1(Table 2).

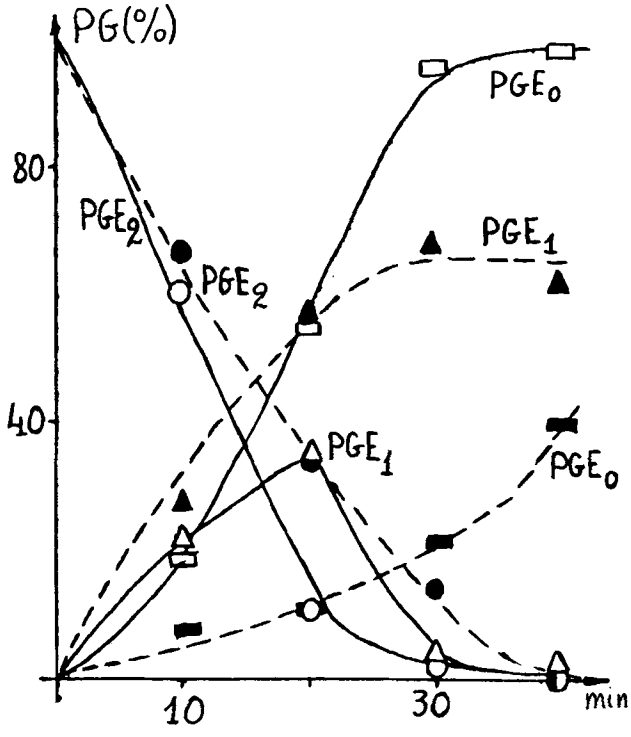


FIGURE 1. Products of PGE_2 reduction: - catalyst 5% $Pd/BaSO_4$, solvent dioxane; --- cat.D, solv. ethyl acetate; o and ● - PGE_2; Δ and ▲ - PGE_1; ▭ and ▬ - PGE_0

TABLE 1. Proportion (%) of reaction products upon selective hydrogenation of PGE_2 (solvent: ethyl acetate, hydrogen pressure 400 hPa, reaction time 30 min, analysis by TLC-$AgNO_3$) using different Lindlar catalysts.

Compound	Catalyst			
	A	B	C	D*
PGE_2	50	62	92	22
PGE_1	39	33	5	67
PGE_0	11	5	3	11

*D - Lindlar catalyst (FLUKA)

TABLE 2. Proportion of reaction products upon selective hydrogenation of PGE_2 in different solvents (catalyst D, hydrogen pressure 400 hPa, reaction time 30 min, analysis by TLC-$AgNO_3$).

Compound	Solvent					
	Ethyl acetate	Acetone	Benzene	Dioxane	Chloro form	Methanol
PGE_2	28	43	45	74	67	19
PGE_1	52	48	43	24	23	17
PGE_0	20	9	8	2	10	64

Kinetic studies were performed in ethyl acetate in the presence of catalyst D and in dioxane in the presence of 5% $Pd/BaSO_4$ (Figure 1). The highest yield of products carrying one reduced double bond was observed after 30-40 min with catalyst D, and after 20 min with 5% $Pd/BaSO_4$. A study of the correlation between the prostaglandin/catalyst ratio and the PGE_1 yield showed the best results were achieved with a ratio of 1:3.

Reaction mixtures were separated by HPLC (Figure 2a). For a more detailed study of fraction composition, the prostaglandins were methylated by diazomethane and re-analysed by HPLC (Figure 2b).

The products reduced by deuterium under similar conditions were analysed by mass spectrometry[13] and NMR[14]. The resulting compounds had characteristic peaks in their mass spectra due to the separation of H_2O, C_5H_{11}, OCH_3, CH_3OH fragments in a varying sequence. From the NMR spectra was drawn conclusions about the presence of double bonds in the prostaglandin molecules and their configuration ($-C_{13}H=C_{14}H-$, $-C_5H=C_6H-$).

As the study demonstrated the reduction of PGE_2 produced a mixture of products (fractions I-VI, Figure 2a). The first

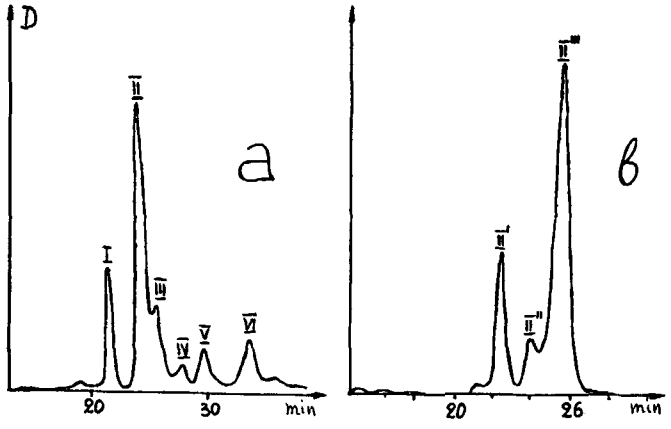


FIGURE 2. PGE_2 reduction products (ethyl acetate, Lindlar catalyst, catalyst/substance ratio 3:1): a - analysed in the form of free acids (GILSON Chromatograph), b - analysed in the form of methyl esters, fraction II (MILICHROM Chromatograph).

fraction contained the original PGE_2, the second one contained the trans isomers PGE_2 (II'), 13,14-dihydro-PGE_2 (II") and PGE_1 (II"'), the fourth-PGE_0, the sixth-13,14-dihydro-15-keto--PGE_2 and 15-keto-PGE_0, while fractions 3 and 5 contained non-prostaglandin low-molecular compounds.

The kinetic data were mathematically processed according to the above diagram, using first-order equations to describe each step. The rate constants of the 5,6-double bond hydrogenation were assumed to equal k_1 for both PGE_2 and 13,14--dihydro-PGE_2. Likewise, for both PGE_2 and PGE_1 the rate constants of 13,14-double bond hydrogenation were assumed to equal k_2. Obviously, the yield of naturally structured PGE_1 depends on the k_1/k_2 ration.

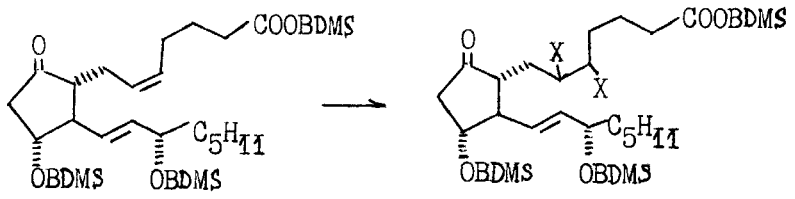
The use of active catalysts of group I did not appreciably change the rate constants k_1 and k_2, resulting in low yields of the desired PGE_1. For instance, with 5% Pd/$BaSO_4$ (Figure 1)

we obtained $k_1 = 0.14 \pm 0.02$ min^{-1} and $k_2 = 0.15 \pm 0.03$ min^{-1}. In this case the maximum yield of the desired product (PGE_1) was only 18-19%, almost equal to that of isomeric by-product, 13,14-dihydro-PGE_2.

The reduction of the 5,6-double bond in PGE_2 was more selective on Lindlar catalysts. Under optimum conditions (Figure 1) the values of k_1 and k_2 were 0.06 ± 0.01 min^{-1} and 0.03 ± 0.006 min^{-1} respectively. The yield of PGE_1 at the kinetic curve maximum was 35% in comparison with 14% for 13,14 dihydro-PGE_2, in good agreement with the theoretically expected results (38% and 15% respectively). It is interesting that the hydrogenation rate decreased with the use of different hydrogen isotopes in the protium-deuterium-tritium series (the ratio of PGE_2 to reduction products changed from 0.04 to 0.25 to 0.89 respectively), while the selectivity of the process remained almost unchanged (the yield of PGE_1 was 32%, 34% and 30% respectively).

Thus, as a result of present study, we succeeded in synthesizing $[5,6\text{-}^3H_2]PGE_1$ with a molar radioactivity of 1.7-1.8TBq /mmol and a yield of 30-35% by reducing PGE_2 with 80% tritium on a heterogeneous catalyst. To obtain a labelled preparation with a radiochemical purity of 95-97%, $[5,6\text{-}^3H_2]PGE_1$ was purified from the isomeric products (fraction II) by chromatography on argentized silica gel[3]. The PGE_2 reduction products were analysed by HPLC of their p-bromophenacyl esters.

To raise selectivity and to simplify the separation of the desired product we also used PGE_2 protected at the hydroxyl and carboxyl groups. Large substituents at positions 11,15 were used to reduce the likelihood of the 13,14-double bond of PGE_2 being adsorbed. 1,11,15-tris-t-Butyldimethyl silyl-PGE_2 (3xBDMS PGE_2) served as the starting compound:



X = ^2H or ^3H

It is very convenient to analyse such derivatives by mass spectrometry because the degradation under the electron impact chiefly involves the abstraction of the tertiary butyl radical C_4H_9 and the formation of the intense $(M - 57)^{+\cdot}$ ion. By analysing the relative intensities of the ion peaks in the $(M - 57)^{+\cdot}$ region it was possible to evaluate the proportion of the initial compound and products with varying deuterium content[15].

The hydrogenation of 3xBDMS PGE_2 in the presence of heterogeneous catalysts (5% $Pd/BaSO_4$, 5% PdO/Al_2O_3) proved ineffective, the yield of 3xBDMS PGE_1 did not exceed 10%. Meanwhile with $(Ph_3P)_3RhCl$ used as catalyst the yield of [5,6-$^3H_2$]PGE_1 reached 60-70%. In spite of the need to remove the protective groups, the procedure for separating the labelled products was simpler than in the previous case.

The proposed approach has the major advantage of permitting the tritium-labelling of prostaglandins with non-natural structures, which are not available by enzymic methods. Using the procedure elaborated for the synthesis of doubly labelled PGE_1, from 3xBDMS PGE_2, we obtained 15-fluoro-15-deoxy [5,6-$^3H_2$]PGE_1 with a yield of 50-70% and 11-deoxy[5,6,10,11-$^3H_4$]PGE_1 from 2xBDMS PGA_2 with a yield of 40-45%.

REFERENCES

1. Bezuglov V.V., Manevich E.M., Bergelson L.D., Ovchinnikov Yu.A. -Prostaglandins and Cardio-vascular Diseases, T.Ozawa, K.Yama-

da, S.Yamamoto. (eds.), Japan Sci. Soc. Press Press, 1985; p. 191-200.

2. Bezuglov V.V., Bergelson L.D., Shevchenko V.P., and Myasoedov N.F. - Bioorg. Khim. 7:448 (1981).
3. Shevchenko V.P., Myasoedov N.F., Bezuglov V.V., and Bergelson L.D. - Khim. Prir. Soedin. 2:148 (1980).
4. Shevchenko V.P., Bezuglov V.V. and Myasoedov N.F. - Radiokhimia 25:757 (1983).
5. Shevchenko V.P., Bezuglov V.V., Lazurkina T.Yu., Potapova A.V. and Myasoedov N.F. - Radiokhimia 28:376 (1986).
6. Kogteva G.S., Bezuglov V.V., Bergelson L.D., Shevchenko V.P. and Myasoedov N.F. - in "Biologically Active Compounds labelled with radioactive isotopes". Tezisy dokladov I Vsesoyuznogo Soveshania, Zvenigorod, 1985, p.13.
7. Samuelsson B. - J.Biol.Chem. 239:4091 (1964).
8. Koch G.K. and Dalenberg G.V. - J.Label.Compounds 6:395 (1970).
9. Lindlar H. - Helv.Chim. Acta 35:446 (1952).
10. Shevchenko V.P., Myasoedov N.F. and Bergelson L.D. - Bioorg. Khim. 5:730 (1979).
11. Shevchenko V.P., Lazurkina T.Yu., Myasoedov N.F., Bezuglov V.V. and Bergelson L.D. - in "Synthesis and Research of Prostaglandins". Tezisy dokladov I Vsesoyuznogo soveshania, Riga, 1982, p.59.
12. Shevchenko V.P., Myasoedov N.F., Freimanis Ya.F., Bezuglov V.V. and Bergelson L.D. - in "Synthesis and Research of Prostaglandins". Tezisy dokladov I Vsesoyuznogo soveshania, Riga, 1982, p.60.
13. Shevchenko V.P., Nagayev I.Yu., Vrzhesh P.V., Ershov D.E., Zaitzev S.V., Varfolomeev S.D. and Myasoedov N.F. - Bioorg. Khim. 14:1075 (1988).

14. Ahrem A.A., Koroleva E.V. - Izvestiya AN BSSR 6:103 (1978).
15. Shevchenko V.P., Myasoedov N.F., Kogtev L.S., Bezuglov V.V. and Bergelson L.D. - in "Synthesis and Research of Prostaglandins". Tezisy dokladov Vsesoyuznogo simpoziuma, Tallin, 1986, p.57.