The Biosynthesis of Prostaglandin E_1 Studied with Specifically ^3H-Labelled 8,11,14-Eicosa-trienoic Acids

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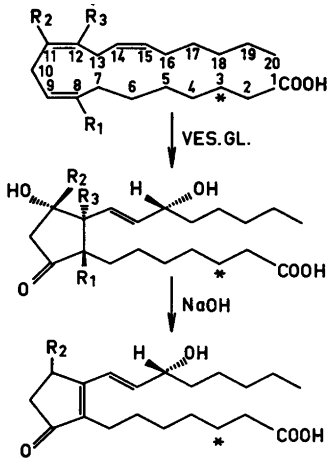
It was recently shown that 5,8,11,14-eicosa-tetraenoic acid is a precursor of prostaglandin E_2 (PGE_2) in homogenates of the vesicular gland of sheep.[1,2] The conversion of 8,11,14-eicosa-trienoic acid [3,4] and 5,8,11,14,17-eicosapentaenoic acid [3] into PGE_1 and PGE_3, respectively, was also demonstrated later using the same system. In homogenates of guinea pig lung, however, 5,8,11,14-eicosa-tetraenoic acid was transformed into both PGE_2 and $PGF_{2\alpha}$ and in addition into metabolites of these two compounds.[5]

The transformation of 8,11,14-eicosa-trienoic acid into PGE_1 (Fig. 1) can be summarized to involve: a) introduction of hydroxyl groups at C-11 and C-15 and of a keto group at C-9, b) isomerization of the Δ^{14}-double bond to the Δ^{13}-position and c) formation of a new bond between C-8 and C-12.

As a basis for further studies of the enzymatic mechanisms of these transformations, we have now followed the fate of the hydrogens at C-8, C-12, and C-11 of 8,11,14-eicosa-trienoic acid during the conversion to PGE_1. These positions are of specific interest since the ring closure as shown in Fig. 1 should occur between C-8 and C-12 and since one of the hydroxyl groups should be introduced at C-11.

The preparation of the specifically tritium labelled 8,11,14-eicosa-trienoic acids was based on the following procedure. The keto stearic acid (6-, 9-, or 10-keto stearic acid) was reduced with tritium labelled sodium borohydride and the labelled hydroxy-stearic acid was converted into the tosylate with *p*-toluene sulfonylchloride in pyridine. The tosylate was subjected to hydrogenolysis with $LiAlH_4$ and the resulting octadecanol was oxidized with chromic acid to yield stearic acid (specific activity 13.4 μC/mg) containing the tritium label at C-6, C-9, or C-10.

The specifically labelled stearic acids were converted into 6,9,12-octadeca-trienoic acids utilizing *Tetrahymena pyriformis*.[6] (We are indebted to Dr. J. Law for generous help with these experiments). The tritium labelled acids were mixed with 1-^{14}C-6,9,12-octadeca-trienoic acid, converted into the methyl esters, and elongated by two carbon atoms using a malonic ester synthesis.[7] In this way, 8,11,14-eicosatrienoic acids containing ^{14}C at C-3 and tritium at C-8, C-11, or C-12 (Ia-c, Fig. 1) were obtained.



8,11,14-EICOSA-TRIENOIC ACID

Ia: $R_1 = {}^3H$, $R_2 = H$, $R_3 = H$
Ib: $R_1 = H$, $R_2 = {}^3H$, $R_3 = H$
Ic: $R_1 = H$, $R_2 = H$, $R_3 = {}^3H$

PGE_1

IIa: $R_1 = {}^3H$, $R_2 = H$, $R_3 = H$
IIb: $R_1 = H$, $R_2 = {}^3H$, $R_3 = H$
IIc: $R_1 = H$, $R_2 = H$, $R_3 = {}^3H$

15-HYDROXY-9-KETO-PROSTA-8(12),13-DIENOIC ACID

IIIa: $R_2 = H$
IIIb: $R_2 = {}^3H$
IIIc: $R_2 = H$

* = ^{14}C

Fig. 1. Transformation of specifically tritium labelled 8,11,14-eicosa-trienoic acids into PGE_1.

Table 1.

Compound	Incubation No.	^3H:^{14}C	^3H Retained %
8,11,14-Eicosa-trienoic acid-3-^{14}C-8-^3H (Ia)	1.2	4.2	100
PGE_1 (IIa)	1	3.9	93
PGE_1 (IIa)	2	4.0	95
15-Hydroxy-9-ketoprosta-8(12),13-dienoic acid (IIIa)	1	0.08	2
8,11,14-Eicosa-trienoic acid-3-^{14}C-11-^3H (Ib)	3.4	3.3	100
PGE_1 (IIb)	3	3.3	100
PGE_1 (IIb)	4	3.2	97
15-Hydroxy-9-ketoprosta-8(12),13-dienoic acid (IIIb)	3	3.2	97
8,11,14-Eicosa-trienoic acid-3-^{14}C-12-^3H (Ic)	5.6	3.2	100
PGE_1 (IIc)	5	3.2	100
PGE_1 (IIc)	6	3.1	97
15-Hydroxy-9-ketoprosta-8(12),13-dienoic acid (IIIc)	5	0.23	7

The doubly labelled trienoic acids were transformed into PGE_1 (IIa-c, Fig. 1) using homogenates of the vesicular gland of sheep.[3] Part of the isolated PGE_1 was also converted into 15-hydroxy-9-keto-prosta-8(12),13-dienoic acid (IIIa-c, Fig. 1) by treatment with sodium hydroxide.[8]

The ^3H : ^{14}C ratios of the precursors and products are given in Table 1. These data show that there is practically complete retention of tritium in the formation of PGE_1 from 8,11,14-eicosa-trienoic acid labelled with tritium at C-8, C-11, or C-12. Furthermore, only 2 or 7 % of the tritium label was retained in the prostadienoic acid derived from the C_{20}-precursors having tritium at C-8 or C-12, whereas 97 % was retained with tritium at C-11 in the precursors.

These results demonstrate that the hydrogens at C-8 and C-12 remain in their original positions during the formation of the new bond between these carbon atoms. Furthermore, the hydroxyl group at C-11 is introduced with retention of the hydrogen at this carbon atom.

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