Biochem. J. (1974) **139**, 381–389
Printed in Great Britain

Inhibitors of Rabbit Plasma Prostaglandin A Isomerase

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(*Received* 3 *October* 1973)

1. The potent inhibitory activities of three groups of prostaglandin analogues on the prostaglandin A isomerase of rabbit plasma were demonstrated. 2. Six of the compounds were prepared by $NaBH_4$ reduction of the C-9 oxo groups of prostaglandin A_2 and prostaglandin C_2 and their C-15 epimers. The remaining four were racemates and were synthesized in another laboratory. Unknown configurations at C-9 and at C-15 were assigned. 3. All the compounds were found to be competitive inhibitors of the isomerase *in vitro*. K_m/K_i ratios were determined and it was found that both the 15(*S*) and 15(*R*) epimers have potent inhibitory activity. 4. One of the inhibitors was used to study the reversibility of the isomerase. 5. It is suggested that these compounds may be useful for determining the biological significance of prostaglandin A isomerase. In view of its weak biological activity and possibly extended half-life *in vivo*, the reduction product of 15-epi-prostaglandin C_2 may be the most suitable agent for this purpose.

The purification and properties of an isomerase enzyme which catalyses the conversion of prostaglandins A into the corresponding prostaglandins C have been described (Jones, 1972*a*; Jones *et al.*, 1972; Jones & Cammock, 1973). The chemical change involved in the isomerization of PGA_2* to prostaglandin C_2 is shown in Fig. 1. The enzyme, which is found in the plasmas of cat, dog, rabbit, pig and rat, but not of man and guinea pig, is highly stable and requires no cofactors. Prostaglandins of the C series are particularly noteworthy in view of their high biological activity, especially on the cardiovascular system (Jones, 1972*b*; Jones & Cammock, 1973). They readily isomerize under mild alkaline conditions to prostaglandins B, which, in general, have feeble biological activity.

The physiological function of the plasma isomerase is unknown and to investigate this it was considered that a readily available, potent isomerase inhibitor would be a useful tool. The present paper describes the preparation and testing of a group of chemically stable compounds closely related to PGC_2 which show potent inhibitory activity of a competitive nature against rabbit plasma PGA isomerase *in vitro*.

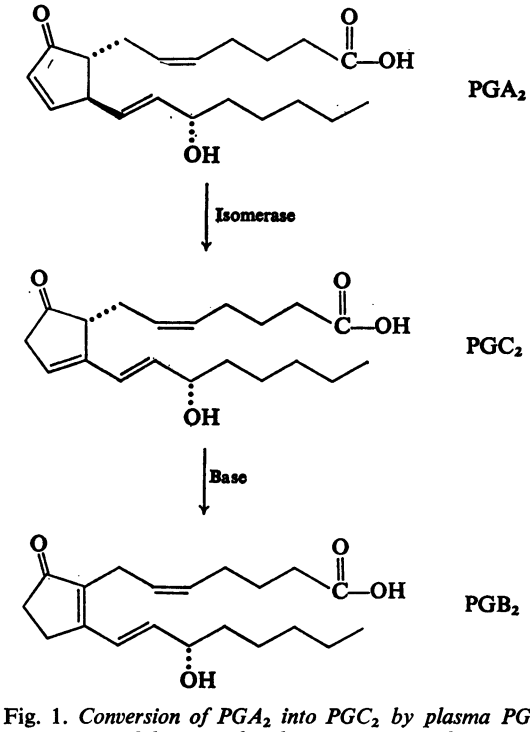

Fig. 1. *Conversion of PGA_2 into PGC_2 by plasma PGA isomerase and base-catalysed isomerization of PGC_2 to PGB_2*

* Abbreviations: PGA_1, prostaglandin A_1 [(−)-15(*S*)-hydroxy-9-oxoprosta-10,13(*trans*)-dienoic acid]; PGA_2, prostaglandin A_2 [(−)-15(*S*)-hydroxy-9-oxoprosta-5(*cis*), 10,13(*trans*)-trienoic acid]; PGB_2, prostaglandin B_2 [(−)-15(*S*)-hydroxy-9-oxoprosta-5(*cis*),8(12),13(*trans*)-trienoic acid]; PGC_2, prostaglandin C_2 [(−)-15(*S*)-hydroxy-9-oxoprosta-5(*cis*),11,13(*trans*)-trienoic acid]; PGE_2, prostaglandin E_2 [(−)-11α,15(*S*)-dihydroxy-9-oxoprosta-5(*cis*), 13(*trans*)-dienoic acid]; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$ [(−)-9α, 11α, 15(*S*)-trihydroxyprosta-5(*cis*), 13(*trans*)-dienoic acid]; $PGF_{2\beta}$, prostaglandin $F_{2\beta}$ [(−)-9β,11α,15(*S*)-trihydroxyprosta-5(*cis*),13(*trans*)-dienoic acid]. The term *PG* refers to the 15-hydroxyprosta-5(*cis*),13(*trans*)-dienoic acid moiety.

Materials and Methods

Prostaglandins obtained from outside sources

PGA_2 was provided by Dr. J. E. Pike of Upjohn Co., Kalamazoo, Mich., U.S.A. For the kinetic studies of the isomerase, 20mg of this material was purified by LH-20 reversed-phase gel partition chromatography. Samples of (±)-15(*S*)-9α,10α-dihydroxy-11,12-dehydro-*PG* ($\lambda_{max.} = 238$nm, $\varepsilon_{max.} = 17670$) and the 15(*R*) epimer ($\lambda_{max.} = 238$nm, $\varepsilon_{max.} = 17100$) were obtained from Dr. P. Crabbé of Syntex S.A., Mexico (Crabbé, 1972; Crabbé *et al.*, 1972). The C-15 epimers of (±)-9α-hydroxy-11,12-dehydro-*PG*, separated by preparative t.l.c., were also supplied by Dr. Crabbé. The four racemates were subjected to LH-20 reversed-phase gel partition chromatography before use as test substrates for the isomerase.

Enzymic preparation of prostaglandins C

15-epiPGA_2 was isolated from a crude extract of the coral, *Plexaura homomalla*, as described by Jones *et al.* (1972). PGC_2 and 15-epiPGC_2 were prepared from the corresponding prostaglandins A by using rabbit plasma PGA isomerase bonded to a solid support. The isomerase was purified to a specific activity of 7.5munits/mg of protein by acetone precipitation and DEAE A-50 ion-exchange chromatography as described by Jones & Cammock (1973). A unit of isomerase activity is the amount of enzyme that will catalyse the formation of 1μmol of PGB_1/min under the following conditions: substrate, 0.2μmol of PGA_1; buffer, 0.1M-Tris–HCl, pH8.5; temperature, 25°C; end volume, 3.0ml. Protein (100mg), dry CNBr-activated Sepharose 4B (10g) (Pharmacia Fine Chemicals, Uppsala, Sweden) and 50ml of 0.1M-Na_2CO_3–$NaHCO_3$ buffer, pH8.5, containing 0.5M-NaCl were placed in a 100ml stoppered glass tube. The vessel was rotated end-over-end and the coupling reaction was allowed to proceed at room temperature for 2h. The suspension was filtered on a Büchner funnel and unchanged groups were de-activated with 1M-ethanolamine hydrochloride for 1h. Non-covalently bound enzyme was removed by alternate washing with 0.1M-sodium acetate buffer, pH4.0, and 0.1M-Tris–HCl buffer, pH8.0, both containing 1M-NaCl. The gel product was stored at 4°C in 0.1M-Tris–HCl buffer, pH6.5, containing 0.5% *n*-butanol as preservative.

A solution (50μM) of the PGA in 0.1M-Tris–HCl buffer, pH6.5, was pumped through a column (50mm×16mm) of the gel product at 1ml/min. The temperature was maintained between 22° and 24°C. The PGC content of the effluent was continuously monitored by recording its extinction at 242nm with a Cecil 212 single-beam u.v. spectrophotometer and a 10mm-path-length flow cell.

The effluent from the reactor was acidified to pH5.5 with HCl and the prostaglandins were extracted with diethyl ether. The ether phase was washed with water, dried over Na_2SO_4, filtered and evaporated to dryness *in vacuo*. The residue was purified by LH-20 reversed-phase gel partition chromatography.

Sodium borohydride reduction

The prostaglandin A or C was dissolved in methanol (1–5mg/ml) and cooled to −20°C. Solid $NaBH_4$ was added and the progress of the reduction was monitored by measuring the concentration of unchanged prostaglandin. This was performed by removing 20μl of the reaction mixture into a cuvette containing 2ml of 0.2M-KOH in methanol and recording the final extinction at 278nm caused by the production of the corresponding prostaglandin B. On completion of the reduction, the reaction mixture was left at room temperature for 1h and then diluted with 20vol. of water, acidified to pH4 with HCl and extracted with diethyl ether. The combined ether phases were washed with water, dried over Na_2SO_4, filtered and evaporated to dryness *in vacuo*. The residue was purified either by LH-20 reversed-phase gel partition chromatography or by preparative t.l.c.

Catalytic hydrogenation

Prostaglandin methyl esters (0.05–4mg) were dissolved in ethanol (0.7ml) in a 10ml pear-shaped flask. The solution was purged with H_2 gas for 10min and then 0.5mg of 10% Pd on charcoal in 0.1ml of ethanol was added. Bubbling with H_2 was continued for 60min, after which the catalyst was filtered off by using a Whatman no. 50 filter circle. Samples of the filtrate were subjected to both t.l.c. in the MI system and combined g.l.c.–mass spectrometry after trimethylsilyl ether formation (see below).

Gel partition chromatography

Reversed-phase gel partition chromatography was performed as described by Nyström & Sjövall (1973). The gel consisted of Sephadex LH-20 (Pharmacia Fine Chemicals) which had been size-graded to 40±5μm by continuous-flow differential sedimentation (Hamilton, 1958). The gel bed (12.5mm×350mm) was contained in a silicone-treated glass column, the lower end of which was attached by Teflon tubing to the flow cell (2mm path-length) of a Cecil 212 u.v. spectrophotometer. The moving phase consisted of water–methanol–*n*-butanol–chloroform (10:10:1:1, by vol.) with 0.1% acetic acid added. Prostaglandins were applied to the column in 0.5–2.5ml of moving phase containing 0.5% acetic acid. Elution was

performed at 8ml/h and 20min fractions were collected.

Straight-phase gel partition chromatography was performed by using Lipidex 5000 gel (Becker-Delft N.V., Groningen, Holland). This gel is equivalent to a 53.4% (w/w) substitution of the hydroxyl groups in Sephadex LH-20 with 1,2-epoxyheptadecane (see Nyström & Sjövall, 1973). The moving phase was *n*-heptane–chloroform (9:1, v/v) and a flow rate of 12ml/h was maintained.

All the above operations were performed in a room maintained between 22° and 24°C. For those prostaglandins exhibiting a strong chromophore between 220 and 250nm the column effluent was monitored for light-absorption at 240nm. The methyl esters of the borohydride-reduced prostaglandins A were detected and determined by g.l.c. $PGF_{2\beta}$ methyl ester (2.5μg) was added to a sample of each fraction and the trimethylsilyl ether derivatives were prepared. The ratio of the peak areas of the compound of interest and the $PGF_{2\beta}$ internal standard was calculated in each case. The elution volumes of the individual compounds are expressed as percentages of the total bed volume.

Thin-layer chromatography

Preparative t.l.c. was performed by using 200mm × 200mm grooved glass plates (May and Baker Ltd., Dagenham, Essex, U.K.) coated with a 0.75mm layer of 4GF Acidic Silica (Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.). The plates were activated at 110°C for 45min before use. The solvent system was ethyl acetate–acetone–acetic acid (95:5:1, by vol.), a modification of the F VI system of Andersen (1969). The prostaglandins were eluted from the silica with methanol.

For comparisons of the chromatographic mobilities of the different prostaglandins, 0.25mm-thick silica-gel plates (Merck, Darmstadt, W. Germany) were used. The solvent systems adopted were the F VI, ethyl acetate–acetone–acetic acid (90:10:1, by vol.), and the MI, benzene–dioxan (1:1, v/v), as described by Andersen (1969) and Gréen & Samuelsson (1964) respectively. The prostaglandins were detected by spraying with 10% (w/v) phosphomolybdic acid in ethanol followed by heating to 110°C for 15min.

Combined gas–liquid chromatography–mass spectrometry

This was performed on an LKB 9000 mass spectrometer. Methyl esters of the prostaglandins were prepared by treatment with diazomethane in methanol–diethyl ether (1:9, v/v) at room temperature for 15min. Trimethylsilyl ether derivatives were prepared by treatment with *NN*-bis(trimethylsilyl)-trifluoroacetamide [Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.] at room temperature for 3h or at 60°C for 15min. The g.l.c. column (1.5mm × 1.5m) was packed with 3% OV1 on 100–120 mesh Supelcoport (Supelco Inc., Bellefonte, Pa., U.S.A.) and operated at 200–205°C. Carbon values were computed by reference to the retention times of the methyl esters of the C_{18}–C_{24} straight-chain fatty acids.

Infrared spectroscopy

I.r. spectra were recorded with a Perkin–Elmer 237 i.r. spectrometer. The prostaglandin was dissolved in freshly redistilled chloroform and contained in a 0.1mm-path-length cell with NaCl windows.

Determinations of inhibitory activity

PGA isomerase was purified from rabbit plasma as described by Jones & Cammock (1973). The specific activity was 7.5munits/mg of protein. The assay system consisted of 0.4munit of isomerase, 300μmol of Tris–HCl buffer, pH7.0, and 9–75nmol of PGA_2 in a final volume of 3.0ml. The cuvettes were enclosed in the constant-temperature housing (25°C) of either an SP.800 or SP.8000 Pye Unicam u.v. spectrophotometer. The extinction reading was expanded to give a full-scale deflexion of 0.10 extinction unit on an auxiliary recorder. After addition of the substrate the extinction change at 242nm was recorded for 5–10min. Each record was analysed by extracting extinction values at either 20 or 40s intervals and determining the best-fitting quadratic regression line by the method of least squares by using a PDP8 digital computer. From this equation the reaction velocity at zero time was calculated.

Results

Preparation of inhibitors

The structural formulae of the three groups of inhibitors under study are shown in Fig. 2. All the compounds possess a *cis* double bond in the 5,6-position and a *trans* double bond in the 13,14-position and thus belong to the 2-series of prostaglandins. The orientations of the side chains attached to the cyclopentane ring are those found in the naturally occurring prostaglandins. The naming of these prostaglandin analogues poses certain problems since the full chemical names (see the footnote) are cumbersome both in speech and in print and the modification of existing trivial names is often not possible. The names of these compounds have therefore been based on the parent structure shown in Fig. 2, the proper name of which is 15-hydroxyprosta-5(*cis*),13(*trans*)-dienoic acid. This has been abbreviated to *PG*.

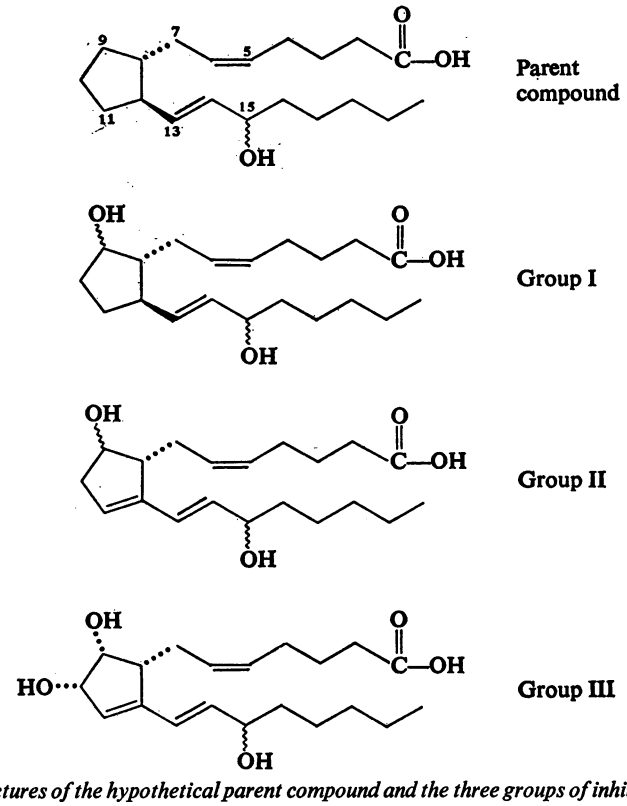

Fig. 2. *Structures of the hypothetical parent compound and the three groups of inhibitors studied*

The orientations of the hydroxyl groups at C-9 and C-15 have been left unspecified where there are two compounds epimeric at one of these positions or where the orientation was unknown at the start of the study.

Table 1. *T.l.c. and g.l.c. analyses of the isomerase inhibitors*

For experimental details see the text.

Group	Origin and t.l.c. polarity of compound where applicable			Original free acid forms: F VI t.l.c.	Original free acid forms: Methyl ester–trimethylsilyl ether derivative on g.l.c.	Pd/H_2 reduction of methyl esters: M I t.l.c.	Pd/H_2 reduction of methyl esters: Trimethylsilyl ether derivative on g.l.c.	Configurations at C-15 and C-9
I	$NaBH_4$ reduction products of:	PGA_2	Less	0.480	22.70	0.615	23.55	*S** α
			More	0.445	22.65	0.585	23.40	*S** β
		15-epiPGA_2	Less	0.515	22.70	0.590	23.55	*R** α
			More	0.455	22.65	0.575	23.40	*R** β
II	$NaBH_4$ reduction products of:	PGC_2		0.490	23.00	0.610	23.55	*S** α
		15-epiPGC_2		0.455	22.80	0.590	23.60	*R** α
	Syntex racemates		Less	0.490	23.05	0.615	23.55	*S* α*
			More	0.455	22.75	0.590	23.55	*R* α*
III	Syntex racemates		Less	0.400	24.30	—	—	*S** α*
			More	0.360	24.10	—	—	*R** α*
Other reference compounds	$PGF_{2\alpha}$			0.140	24.25	—	—	*S** α*
	$PGF_{2\beta}$			0.100	23.80	—	—	*S** β*

* Configurations that were known at the outset of the study.

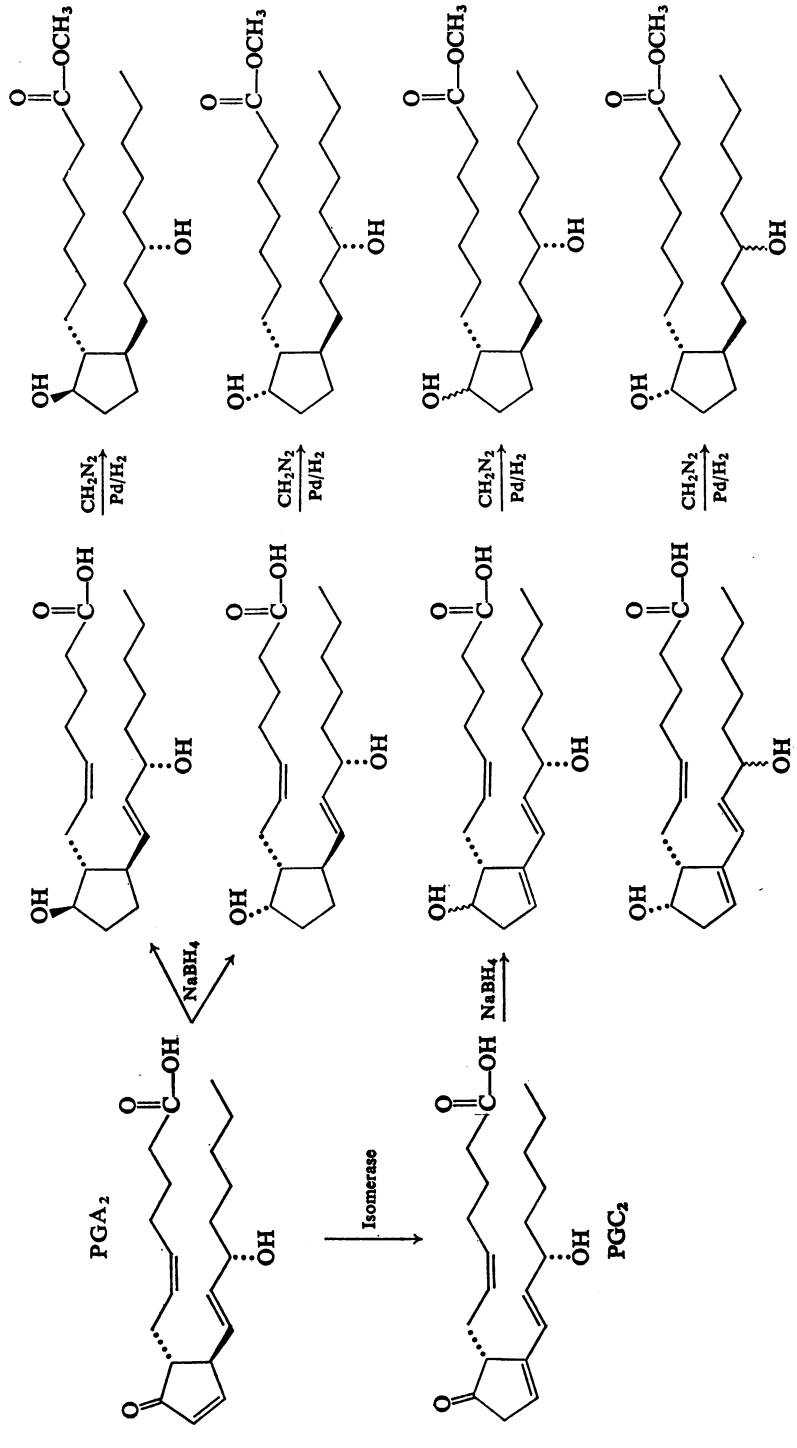

Fig. 3. *Transformations involved in the preparation and structural elucidation of the inhibitors in Groups I and II*

The whole sequence from PGA_2 was repeated with the starting material having the 15(*R*) configuration (15-epiPGA_2).

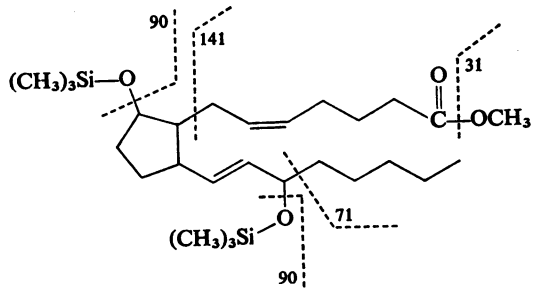

Fig. 4. *Major fragmentations of the methyl ester–trimethylsilyl ether derivatives of the PGA_2 and 15-epiPGA_2 borohydride-reduction products*

Fragment ions were present at *m/e* values of 496 [*M*], 425 [*M*−71], 406 [*M*−90], 375 [*M*−(90+31)], 335 [*M*−(90+71)], 316 [*M*−(90+90)] and 265 [*M*−(141+90)].

Thus one of the Group II inhibitors studied will be referred to as 15(*S*)-9α-hydroxy-11,12-dehydro-*PG*, emphasizing three factors, namely the configuration at the C-15 asymmetric centre, the orientation(s) of the ring hydroxyl group(s) and the unsaturation within the cyclopentane ring.

The steps involved in the preparation of compounds in Groups I and II are shown in Fig. 3. The Group I compounds were derived from the $NaBH_4$ reduction of PGA_2 and 15-epiPGA_2. Each prostaglandin yielded two products (9α and 9β epimers) as revealed by t.l.c. in the F VI solvent system (Table 1). The PGA_2 reduction products were not separated by LH-20 reversed-phase partition chromatography and so preparative t.l.c. was attempted. By using acidic silica and a modified F VI solvent system both the PGA_2 and 15-epiPGA_2 mixtures were resolved.

The four diastereoisomers were analysed by combined g.l.c.–mass spectrometry after the preparation of methyl ester–trimethylsilyl ether derivatives. Each compound produced a single peak on g.l.c., the C values for the less and more polar isomers (as judged by t.l.c.) being 22.70 and 22.65 respectively. A mass spectrum taken during each peak showed a molecular ion at *m/e* 496, suggesting that four H atoms had been incorporated into the molecule during the $NaBH_4$ reduction. Prominent peaks were also present at *m/e* 265. This fragment ion is due to the loss of a trimethylsilyl ether grouping and the complete α side chain [*M*−(90+141)] as shown in Fig. 4. If the 5,6 double bond had been reduced then the side chain would have been lost as a fragment of mass 143. An i.r. spectrum of the methyl ester of the more polar PGA_2 reduction product showed a strong extinction at 970 cm^{-1}. This feature establishes that the 13,14-*trans* double bond has remained intact during the metal-hydride reduction: 13,14-*cis*-PGB_1 shows no absorption in the 950–980 cm^{-1} region whereas PGB_1 does (Klok *et al.*, 1968).

Thus it is evident that the reduction of the C-9 oxo function has been accompanied by saturation of the conjugated 10,11 double bond.

The compounds in Group II derive from two sources. First, there are the $NaBH_4$-reduction products of PGC_2 and 15-epiPGC_2. The latter two compounds were prepared from the corresponding prostaglandins A by using rabbit plasma PGA isomerase bonded to a solid support. The prostaglandins C were separated from the A and B prostaglandins by LH-20 reversed-phase gel partition chromatography. The elution volumes for the A, B and C isomers were 555, 610 and 680% of the total column volume respectively and for the 15(*R*) epimers 500, 520 and 580% respectively. Reduction of both prostaglandins C resulted in a shift of the wavelength maximum in methanol from 234 to 239 nm and t.l.c. of each reaction product in the F VI solvent system revealed a single spot. Thus it seemed likely that the reduction of prostaglandin C yields a single product. This was examined further by preparing the methyl ester of the PGC_2 reduction product and subjecting it to Lipidex 5000 straight-phase gel partition chromatography. This system efficiently separates prostaglandin methyl esters which differ in the orientation of the C-9 hydroxyl group. Thus the methyl esters of 15(*S*)-9 α-hydroxy-*PG* and 15(*S*)-9β-hydroxy-*PG* (PGA_2 borohydride-reduction products) have elution volumes of 460 and 600% respectively. By using a more polar moving phase with the same gel a complete separation between $PGF_{2\alpha}$ and $PGF_{2\beta}$ methyl esters can also be obtained (A. R. Brash & R. L. Jones, unpublished work). A single peak only (660%) was detected with the PGC_2 borohydride-reduction product.

The other two compounds in Group II were racemates, prepared by total synthesis in the Syntex S. A. research laboratories. The C-9 hydroxyl group has the α orientation and the two compounds are 15(*S*) ($\lambda_{max.} = 239$ nm, $\varepsilon_{max.} = 15500$) and 15(*R*) ($\lambda_{max.} = 239$ nm, $\varepsilon_{max.} = 17300$) epimers. The u.v. spectra of these two compounds and the PGC_2 and 15-epiPGC_2 reduction products were identical.

Establishment of configuration at C-9 and C-15

The configuration at C-15 was known in the compounds prepared by borohydride reduction of PGA_2, 15-epiPGA_2, PGC_2 and 15-epiPGC_2. In all these compounds the configuration of the newly formed C-9 hydroxyl group was unknown. In the Group II racemates, the configuration of the C-9 hydroxyl group was known to be α, whereas the configurations at C-15 had not been assigned (see Fig. 3).

A comparison of the behaviour of the PGA borohydride-reduction products with that of $PGF_{2\alpha}$ and $PGF_{2\beta}$ on F VI t.l.c. (Table 1) suggested that the less polar isomers possessed a 9α-hydroxyl group and the more polar isomers a 9β-hydroxyl group. In the

Group II prostaglandins, the PGC_2 borohydride-reduction product [15(*S*) known] had identical chromatographic indices to the less polar Syntex racemate (9α known) and hence both could have the 15(*S*)-9α-hydroxy configuration. Similarly, the 15-epi-PGC_2 borohydride-reduction product [15(*R*) known] was indistinguishable from the more polar Syntex racemate (9α known) and hence both could have the 15(*R*)-9α-hydroxy configuration.

The validity of the above assumptions was tested by reducing the methyl ester derivative of each of the eight compounds in Groups I and II with Pd and H_2 to form the corresponding methyl 9,15-dihydroxy-prostanoates (Fig. 3) and comparing the behaviour of these products on both t.l.c. and g.l.c. (Table 1). The less polar borohydride-reduction product of PGA_2 [15(*S*) known], the PGC_2 borohydride-reduction product [15(*S*)] and the less polar Syntex racemate (9α) showed identical R_F values in the MI solvent system and identical C values on g.l.c. Thus one may assign the 15(*S*)-9α-hydroxy configuration to these three compounds. Also the less polar borohydride-reduction product of 15-epiPGA_2 [15(*R*)], the 15-epiPGC_2 borohydride-reduction product [15(*R*)] and the more polar Syntex racemate (9α) showed identical chromatographic properties; these may therefore be designated 15(*R*)-9α-hydroxy. The remaining two compounds, namely the more polar isomers of borohydride reduced PGA_2 [15(*S*)] and 15-epiPGA_2 [15(*R*)], exhibited different chromatographic properties from the other three 15(*S*) and 15(*R*) compounds and must therefore have the β-hydroxy configuration at C-9.

Supporting evidence for these assignations comes from the mass spectra of the trimethylsilyl ether derivatives of the methyl 9,15-dihydroxyprostanoates. The same major fragment ions were present in all eight compounds and there was very little difference between the percentage abundances of these ions in the three compounds designated 15(*S*)-9α-hydroxy and the three designated 15(*R*)-9α-hydroxy. The mass spectra of the two compounds in which the C-9 hydroxyl group is postulated to be β orientated were almost identical, but showed considerable differences from the mass spectra of the other six compounds.

On completion of this part of the study the preparation of the PGA_2 borohydride-reduction products [15(*S*)] was independently reported (Lincoln *et al.*, 1973). On the basis of nuclear-magnetic-resonance studies, these workers have assigned the 9α-hydroxy and 9β-hydroxy structures respectively to the less and more polar compounds as judged by silica-gel column chromatography and t.l.c.

Determination of isomerase inhibition

The kinetics of rabbit plasma PGA isomerase were investigated as described for the cat plasma isomerase (Jones *et al.*, 1972; Jones & Cammock, 1973). The method involves the measurement of PGC_2 formation by u.v. spectroscopy. The spectrophotometer is set to a fixed wavelength of 242nm and the initial rate of increase of extinction is measured. At the pH (7.0) of the assay system there is very little isomerization of PGC_2 to PGB_2 and hence the PGC_2 concentration is a valid measure of isomerase function.

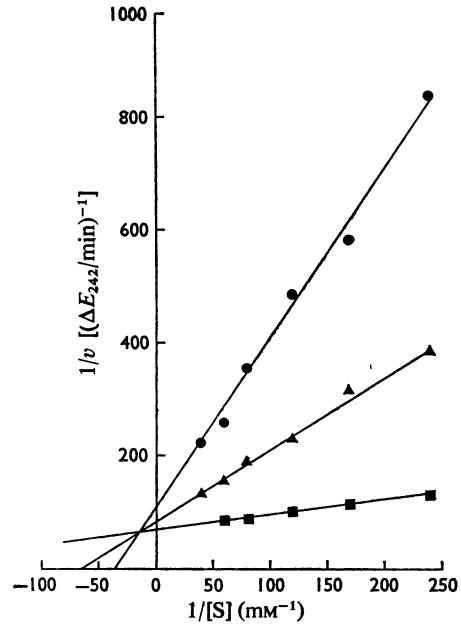

Fig. 5. *Double-reciprocal plot showing the inhibition of rabbit plasma PGA isomerase by* (±)-15(*S*)-9α,10α-*dihydroxy*-11,12-*dehydro-PG*

Prostaglandin A_2 was used as the substrate. The conditions of the assay system are described in the Materials and Methods section. Concentrations of inhibitor used were: ■, 0μM; ▲, 4μM; ●, 10μM.

The Groups II and III isomerase inhibitors exhibit strong extinctions at 242nm owing to the 11,13-diene grouping. This did not interfere with the PGC_2 measurement, since at the highest concentration of any inhibitor used (30μM) the extinction did not exceed 0.5. These compounds, unlike PGC_2, are stable in alkaline solution and therefore no problems with changes of extinction during the assay were experienced. Owing to the low extinction changes involved in the measurements, each determination at a particular substrate concentration and inhibitor concentration was carried out twice and the mean initial velocity calculated.

Plots of reciprocal velocity versus reciprocal substrate concentration were made. The K_m for PGA_2 was 4.0×10^{-6}M. A competitive type of inhibition

Table 2. *K_m/K_i values for inhibitors of rabbit plasma PGA isomerase*

For experimental details see the text.

Inhibitor group	Nature of cyclopentane ring	Configuration at C-15 (*S*)	Configuration at C-15 (*R*)
I	9α-Hydroxy	1.0	2.7
	9β-Hydroxy	0.59	1.9
II	9α-Hydroxy-11,12-dehydro	5.2	3.6
	(±)-9α-Hydroxy-11,12-dehydro	4.8	1.7
III	(±)-9α,10α-Dihydroxy-11,12-dehydro	4.2	1.3

was seen with all the inhibitors except for the (±)-15(*S*)-9α,10α-dihydroxy-11,12-dehydro-*PG*, where there was evidence of a mixed type of inhibition. However, on the double-reciprocal plot (Fig. 5) the point of intersection of the regression lines is near to the reciprocal-velocity axis and the inhibition can be considered to be predominantly competitive for this compound. The relative potencies of the different inhibitors were determined from plots of V_m/V_i versus inhibitor concentration, and from the slopes of the regression lines the K_m/K_i ratios were calculated (Thorn, 1953). These results are shown in Table 2.

Reversibility of PGA isomerase

When PGC_2 is added to rabbit plasma PGA isomerase at pH 7 there is a rapid initial decrease in extinction at 242 nm. After 10–15 min a much slower decrease of extinction at this wavelength occurs. The extinction at 283 nm caused by PGB_2 production increases at virtually the same rate from zero time. The initial rate of change at 242 nm was dependent on the concentration of isomerase. From these results it was postulated that the major part of the initial change in the 242 nm extinction is due to the conversion of PGC_2 into PGA_2, thus implying that the isomerase is reversible, and that this is superimposed on a much slower extinction change owing to the base-catalysed conversion of PGC_2 into PGB_2.

Additional evidence was obtained in experiments using the most potent of the inhibitors, the 15(*S*)-9α-hydroxy-11,12-dehydro-*PG*. Concentrations of 10 and 25 μM of this compound decreased the initial rate of decrease of extinction at 242 nm by 33 and 72% respectively when a PGC_2 concentration of 50 μM was used.

By making the isomerase react with mixtures of PGA_2 and PGC_2 and recording the initial extinction change at 242 nm an estimate of the equilibrium point of the enzyme system was obtained. Thus at a total prostaglandin concentration of 50 μM, no change at 242 nm occurred when the PGA_2/PGC_2 ratio was 1:5.

Discussion

The inhibitors investigated in this study are closely related to PGA_2 and PGC_2, the major transformation being the reduction of the ring ketone to a secondary alcohol group. Borohydride reduction has been used previously in the prostaglandin field, in particular to produce PGF_α and PGF_β isomers from prostaglandins E. The reduction of PGE_1 and PGE_2 has been reported to give a slight excess of the β isomers (Pike *et al.*, 1969). In my laboratory reduction of PGE_2 yielded $PGF_{2\alpha}$ and $PGF_{2\beta}$ in a 7:3 ratio. In the present study borohydride reduction of PGA_2 and 15-epiPGA_2 yielded an excess of the 9β-hydroxy isomers over the 9α-hydroxy, whereas reduction of PGC_2 and 15-epiPGC_2 produced solely the 9α-hydroxy isomers. These effects can be explained on the basis of steric-approach control (see House, 1965). Steric hindrance to the approach of the metal hydride from the α face of the ring is present in the PGC compounds owing to the α-orientation of the carboxylic acid side chain. Attack therefore occurs preferentially from the β-face and a 9α-hydroxylated product results. With PGA and PGE compounds there is hindrance to approach from both the α and β faces and hence a mixture of isomers is obtained.

All the compounds exhibited a competitive type of inhibition and several may be considered to be potent inhibitors, since their K_i values are considerably lower than the K_m value for PGA_2 (thus giving K_m/K_i ratios greater than unity). The racemic 15(*S*)- and 15(*R*)-9α-hydroxy-11,12-dehydro-*PG* analogues show somewhat different K_m/K_i ratios than the optically active forms. It is probable that with the 15(*S*) racemate the optical antipode contributes to the total inhibitory effect. The latter will have the 15(*R*)-9β-hydroxy-11,12-dehydro-8-iso*PG* structure. Inversion of configuration at the C-8 position is known to produce considerable changes in the biological activities of the prostaglandins. Thus 8-isoPGE_1, in which the side chains have a *cis* orientation to the cyclopentane ring (Daniels *et al.*, 1968), is less active than PGE_1 as a vasodilator in the dog and exhibits PGF_α-like properties on the pulmonary vasculature (Nakano & Kessinger, 1970). Thus it may be of interest to investigate certain optically active 8-isoprostaglandins for their inhibitory action on PGA isomerase.

One of the aims of the present study was to find a potent inhibitor of PGA isomerase and to use this compound as a tool to investigate the physiological function (if any) of this enzyme. In this context the configuration at the C-15 position is an important factor to be considered.

Prostaglandins possessing the 15(*R*) configuration can be isolated from the coral *Plexaura homomalla* (Weinheimer & Spraggins, 1969), but there have been no reports of these compounds being present in mammalian tissues. The stereochemistry at C-15 markedly affects the metabolism and biological activity of the prostaglandins in mammalian systems. For example, PGE_1 and a number of other 15(*S*) prostaglandins are good substrates for the 15-hydroxyprostaglandin dehydrogenase of pig lung, whereas 15-epiPGE_1 is not a substrate but a competitive inhibitor (Nakano *et al.*, 1969; Shio *et al.*, 1970). The prostaglandin dehydrogenase has a wide tissue distribution and plays an important part in the rapid inactivation of prostaglandins which occurs *in vivo*. On mammalian smooth muscle the 15(*S*) epimers have been found to be more active than the 15(*R*) forms. PGA_2, for instance, is 30 and more than 1000 times more potent than 15-epiPGA_2 in lowering the blood pressure of the cat (Jones *et al.*, 1973) and dog (Nakano, 1969) respectively. Also $PGF_{2\alpha}$ is 12 times more active than 15-epi$PGF_{2\alpha}$ on the gerbil colon (Bundy *et al.*, 1973) and finally the 15(*S*)-9α-hydroxy-11,12-dehydro-*PG* prepared in the present study was found to be 22 times more active than the 15(*R*) epimer on the isolated guinea-pig ileum (R. L. Jones, unpublished work).

In the light of the previous findings, the comparable inhibitory activities of the 15(*S*) and 15(*R*) analogues on PGA isomerase becomes of considerable importance. Thus one of the 15(*R*) epimers (e.g. 15(*R*)-9α-hydroxy-11,12-dehydro-*PG*) may be expected to inhibit PGA isomerase effectively *in vivo*, to exert little agonistic action on prostaglandin receptor sites and, since prostaglandin dehydrogenase will only metabolize 15(*S*) prostaglandins, to have a prolonged duration of action.

I thank Dr. J. E. Pike of Upjohn Co., Kalamazoo, Mich., U.S.A., and Dr. P. Crabbé of Syntex S.A., Mexico, for gifts of prostaglandins. The samples of Lipidex 5000 from Dr. V. Tarkkanen of Becker-Delft N.V., Groningen, Holland, and size-graded Sephadex LH-20 from Mr. A. R. Brash are gratefully acknowledged. Assistance with the g.l.c.–mass-spectrometric analyses was provided by Mr. C. G. Marr and Mr. C. N. Goode. This work was supported by grants to Professor E. W. Horton from the Medical Research Council.

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