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Preparation of Prostaglandin F from Prostaglandin E

Prostaglandins and Related Factors 6 *

SUNE BERGSTRÖM, L. KRABISCH, B. SAMUELSSON and J. SJÖVALL

Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

Reduction of prostaglandin E (PGE) ($C_{20}H_{34}O_5$) with sodium borohydride yielded two isomeric reduction products PGF_1 and PGF_2, ($C_{20}H_{36}O_5$) of which PGF_1 was identical with a compound earlier isolated from sheep prostate glands and designated PGF. The data presented indicate that PGE is an unsaturated dihydroxyketocarboxylic acid and that PGF_1 and PGF_2 are stereoisomeric, unsaturated trihydroxycarboxylic acids.

Recently we reported the isolation of two crystalline biologically active compounds from sheep prostate glands, prostaglandin E ($C_{20}H_{34}O_5$) and prostaglandin F ($C_{20}H_{36}O_5$) [1–4]. It has now been found that reduction of PGE with sodium borohydride yielded two compounds one of which proved identical with PGF while the other had different physical and biological properties but the same elementary composition. The former compound is now designated PGF_1 and the new compound PGF_2.

EXPERIMENTAL

Reduction of PGE with sodium borohydride. PGE (100 mg, m.p. 115°) was dissolved in 10 ml of methanol and cooled in an ice bath. A solution of sodium borohydride (300 mg) in 35 ml of methanol was chilled and added. After 20 min at 0° the mixture was left at room temperature for one hour. Water was added and most of the methanol was taken off *in vacuo*. After acidification with hydrochloric acid the aqueous phase was extracted three times with ether, the combined ether extracts were washed with water and brought to dryness at room temperature.

Paper chromatography showed that two new compounds were present whereas the original spot due to PGE had disappeared.

Taking the relative mobility of PGE as 1.00 the values for the two new spots were 0.72 and 0.55, respectively, in a phase system consisting of equal parts of ethylene chloride and heptane as moving phase and 70 % acetic acid as stationary phase [5]. The relative mobility of PGF isolated from sheep prostate glands is 0.72 in the same phase system.

* For earlier papers in this series see Refs.[1-4,7].

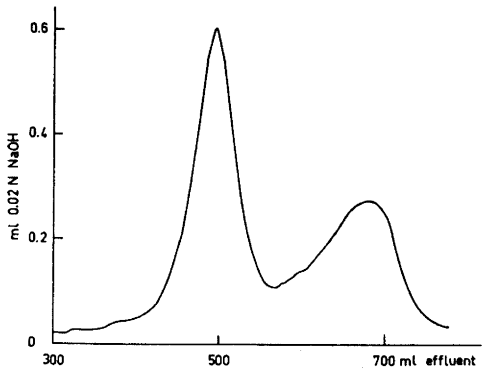

Fig. 1. Reversed phase partition chromatography of reaction product formed by reduction of prostaglandin with sodium borohydride (see text).

Table 1. Interplanar spacings in Ångström units as determined from Guinier powder photographs taken with Cu$K\alpha$ radiation.

P G F		*P G F*$_1$		*P G F*$_2$	
d Å	*I*	*d* Å	*I*	*d* Å	*I*
16.44	s	16.43	s	10.79	vw
9.46	w	9.49	vw	9.15	vw
8.16	w	8.22	vw	8.41	w
6.19	m	6.19	w	7.22	m
5.46	m	5.45	w	5.64	m
5.05	m	5.06	w(d)	4.78	vw
4.71	vw	4.73	w	4.66	vw
4.63	vs	4.62	s(d)	4.53	s
4.54	m	4.53	w	4.37	s
4.46	m	4.45	m(vd)	4.27	w
4.08	m	4.09	m(d)	4.17	m
4.02	s	4.02	m(d)	3.89	m
3.74	w	3.75	w	3.77	m
3.56	w	3.56	vw	3.64	vw
3.45	vw	3.45	vw(d)	3.58	vw
3.07	vw	3.07	w(vd)	3.51	vw
2.93	w	2.95	w(vd)	3.42	vw
2.80	w	2.67	vw(vd)	3.38	vw
2.67	vw	2.58	w(vd)	3.34	vw
2.56	vw	2.49	w(d)	3.12	vw(vd)
2.48	vw	2.39	vw(vd)	3.03	vw
2.39	vw	2.35	vw(vd)	2.96	vw
2.34	vw	2.25	vw(vd)	2.92	vw
2.02	w	2.17	vw(d)	2.83	vw
1.48	w	2.02	vw(vd)	2.70	vw(d)
		1.89	vw(vd)	2.60	w(d)
		1.70	vw(vd)	2.46	vw(d)
				2.40	vw
				2.35	vw
				2.32	vw
				2.25	m
				2.08	vw
				2.00	vw(vd)
				1.69	vw(vd)

s = strong
m = medium
w = weak
vw = very weak

d = diffuse
vd = very diffuse

The residue (100 mg) was then subjected to reversed phase partition chromatography on 18 g of hydrophobic kieselguhr. The solvent system was prepared through mixing of methanol (516 ml), water (684 ml), isooctanol (60 ml) and chloroform (60 ml). 16 ml of the chloroform phase was supported on the kieselguhr. The titration values of the fractions are plotted in Fig. 1.

PGF_1. Fractions 95—133 (600—750 ml effluent) were combined and taken to dryness and crystallized from ethyl acetate-pentane. Yield 37 mg, m.p. 101° not depressed by admixture of PGF isolated from sheep prostate glands. X-Ray diffraction powder pattern, mass spectra and IR-spectra were also identical (see Table 1) with the data published earlier [1].

PGF_2. Fractions 60—85 (440—540 ml effluent) were also combined and yielded 47 mg, m.p. 128°, when crystallized from ethyl acetate-pentane. (Found: C 67.2; H 10.2. Calc. for $C_{20}H_{36}O_5$: C 67.4; H 10.2).

The results of the X-ray diffractions of earlier isolated PGF and PGF_1 and PGF_2 prepared now are listed in Table 1. The mass spectrum of the methyl ester of PGF_2 was practically identical with that of the methyl ester of PGF_1.

Microdetermination of the ratio between the acylable groups and the carboxyl group of PGE, PGF_1 and PGF_2. The method is based on the preparation of the methyl ester with ^{14}C-labeled diazomethane followed by acylation with *p*-nitrobenzoylchloride in pyridine at room temperature. The ratio between the light absorption of the nitrobenzoylgroup at 257 mμ and the radioactivity is then determined.

About one mg of the substance was treated with an excess of diazomethane-^{14}C prepared from nitrosomethyl-^{14}C-urea and then taken to dryness *in vacuo* and dried in a desiccator. The ester was then dissolved in 0.3 ml of dry pyridine and 0.25 ml of a solution of *p*-nitrobenzoylchloride in pyridine (60 mg per ml) was added. After standing overnight at room temperature the solution was cooled in ice and one drop of 50 % aqueous pyridine was added. After 15 min more water was added and the mixture extracted with ether. The combined ether extracts were carefully washed three times with dilute hydrochloric acid followed by 0.2 M sodium carbonate and water. The residue was dissolved in 10 ml of absolute ethanol. The light absorption at 257 mμ was determined after dilution (1:5) and the ^{14}C-content was determined by counting aliquots, plated on aluminium planchets, in a gas flow counter (Frieseke-Hoepfner FH 51) (Table 2).

Table 2.

	(1) c.p.m.	(2) E_{257}	(2)/(1) × 0.475 × 10^3
2-Hydroxystearic acid	3 141	0.69	1.05
9,10-Dihydroxystearic acid	3 293	1.35	1.96
PGE	3 020	0.99	1.53
PGF_1	2 822	1.68	2.8
PGF_2	2 834	1.75	2.95

The data on the mono- and dihydroxystearic acids are reasonably in agreement with one respectively two hydroxyls per methyl ester group. Using these as standards PGF_1 and PGF_2 have approximately three hydroxyl groups in agreement with their preparation from PGE through reduction of a carbonyl group with sodium borohydride. The data for PGE varied in different runs (1.4—1.8) and the reason is apparently that a partial dehydration takes place. The structural implications of these findings will be discussed in a future publication.

Preparation of the methyl ethers of methyl esters of PGE, PGF_1 and PGF_2. For the mass spectrometric analysis the methylation of the methyl esters was carried out essentially as described recently by Müller and Rundel [6].

A solution of 5—10 mg of the methyl ester of PGE, PGF_1 and PGF_2 in 10 ml of dry ether was cooled to —40°. Freshly prepared diazomethane was

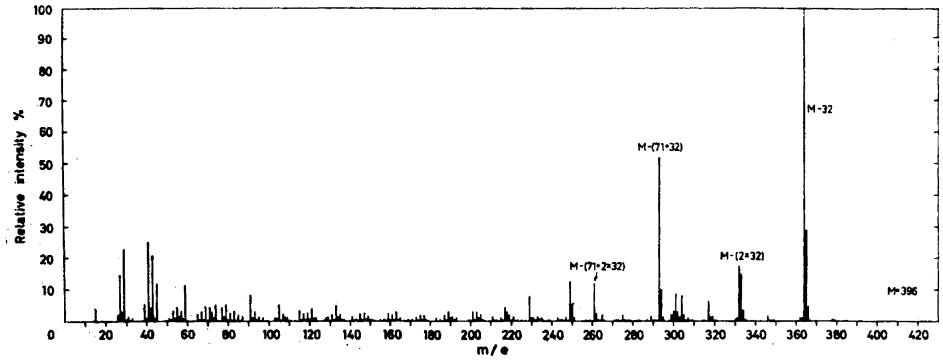

Fig. 2. Masspectrum of the dimethylether methyl ester of prostaglandin E. (PGE).

dried over potassium hydroxide for 2 h, cooled to −40° and added to the solution of the methyl ester immediately after addition of 0.05 ml of boron trifluoride methyl etherate. Diazomethane was added until the yellow colour persisted. After 15 min at −40° and 1 1/2 h at room temperature the solution was filtered from polymethylenes, washed with sodium bicarbonate and water, dried over sodium sulfate and evaporated under reduced pressure.

Gas-liquid chromatography and mass spectrometric analysis of methyl ethers of PGE, PGF_1 and PGF_2 methyl esters. A Pye chromatograph with an argon ionization detector was used. The stationary phase (Apiezon L, Shell Chemicals Ltd.) was supported on 100—120 mesh Celite in the ratio stationary phase: Celite 1:6.4. The retention times for these derivatives of PGE, PGF_1 and PGE_2 at 245° with an argon pressure of 1 kg/cm² are given below.

	Retention time in min.
PGE	48.3
PGF_1	40.5
PGF_2	35.0

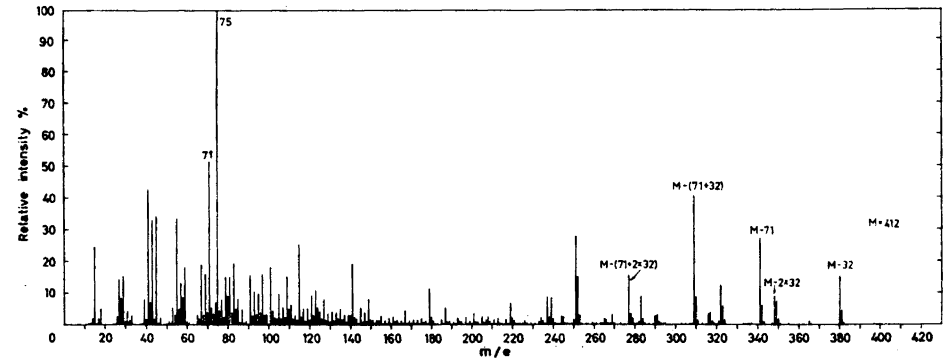

Fig. 3. Masspectrum of the trimethylether methyl ester of prostaglandin F_1 (PGF_1).

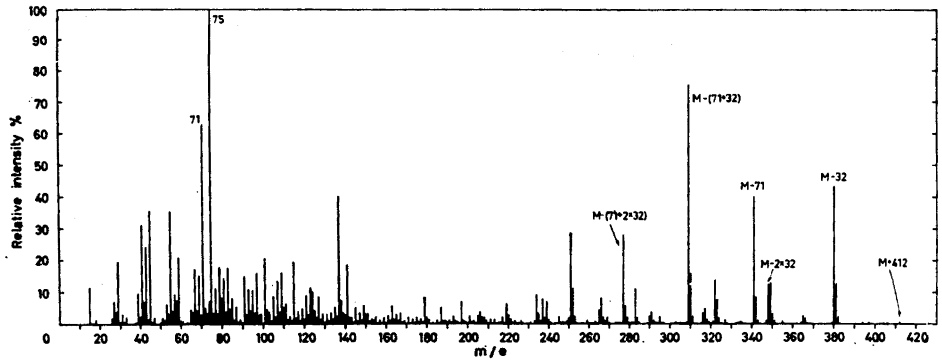

Fig. 4. Masspectrum of the trimethylether methyl ester of prostaglandin F_2 (PGF_2).

Mass spectrometric analysis of the methyl ethers was done after purification with gas chromatography. The analysis was done by Dr. R. Ryhage of this department with the technic described earlier [8]. The mass spectrogram of PGE methyl ester was consistent with the presence of a dimethyl ether with a molecular weight of 396 (= M) with large peaks at M-32, M-2 × 32, M-(32 + 71) and M-(2 × 32 + 71). The mass spectrograms for the two PGF compounds were practically identical, *i.e.* corresponded to trimethyl ethers and showed a molecular weight of 412 and had the same series of marked peaks at M-32, M-2 × 32, M-3 × 32, M-71 + 32 etc., *cf.* Figs. 2, 3 and 4.

The data are in agreement with the successive loss of one, two respectively three molecules of methanol (mol.wt. 32) from the dimethylether of PGE-methyl ester and the trimethylethers of the PGF-methyl esters. All three compounds apparently contain a relatively easily detached grouping with a molecular weight of 71 ($C_5H_{11}^+$?). Further data of the mass spectra will be discussed at a later time.

Biological activity. PGF_2 was found to have 10—20 % of the smooth muscle stimulating activity shown by PGF_1 when tested on the duodenum of the rabbit. The physiological data will be presented in greater detail in a later publication.

DISCUSSION

The formation of the originally isolated prostaglandin F_1 ($C_{20}H_{36}O_5$) when prostaglandin E ($C_{20}H_{34}O_5$) was reduced with sodium borohydride indicates that the two compounds are identical except for the presence of a carbonyl grouping in PGE that can be reduced to the corresponding alcohol in PGF_1. At the same time the other stereoisomeric alcohol PGF_2 is formed.

In this connection it might be mentioned that PGE gave a positive spot test on paper with nitrophenylhydrazine for the presence of a carbonyl whereas the two PGF's gave negative tests.

From the IR-spectra of PGE [2] earlier published it is apparent that only one strong band is present in the carbonyl region at 5.77 μ (1 733 cm^{-1}) indicating that the carbonyl group absorbed at the same wave length as the ester

carbonyl. As aldehydes and alifatic ketones as well as cyclohexanones absorb at longer wave lengths these data indicate that a cyclopentanone might be present that is known to absorb in this region.

As mentioned in earlier publications both PGE and PGF_1 absorbed one mole of hydrogen when reduced with hydrogen and platinum in neutral alcohol. The same was found to take place on reduction of PGF_2. All three compounds showed a band at 10.30 μ (971 cm^{-1}) that disappeared after reduction in a neutral medium indicating that the double bond had the *trans* configuration.

PGE did not show any absorption in the region 210—250 mμ indicating that the original double bond was not in conjugation with the carbonyl or carboxyl group.

From the quantitative acylation experiments it appears that PGF_1 and PGF_2 both contain three hydroxyls. The data for PGE varied somewhat between one and two and apparently some dehydration did occur.

The conclusion that one ring is present is based on the fact that all three compounds contain two hydrogens less than alifatic compounds with these substituents would require. The IR data indicating the presence of a cyclopentanone in PGE supports this conclusion.

The data reported so far thus indicate that the prostaglandins contain the following groups:

PGE	PGF_1 and PGF_2
($C_{20}H_{34}O_5$)	($C_{20}H_{36}O_5$)
1 carboxyl	1 carboxyl
1 carbonyl (cyclopentanone)	
2 hydroxyls	3 hydroxyls
1 double bond (*trans*)	1 double bond (*trans*)
1 ring	1 ring

The work is being continued.

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REFERENCES

1. Bergström, S. and Sjövall, J. *Acta Chem. Scand.* **14** (1960) 1693.
2. Bergström, S. and Sjövall, J. *Acta Chem. Scand.* **14** (1960) 1701.
3. Bergström, S., Eliasson, R., v. Euler, U. S. and Sjövall, J. *Acta Physiol. Scand.* **45** (1959) 133.
4. Bergström, S., Dunér, H., v. Euler, U. S., Pernow, B. and Sjövall, J. *Acta Physiol. Scand.* **45** (1959) 145.
5. Sjövall, J. *Clin. Chim. Acta* **4** (1959) 652.
6. Müller, E. and Rundel, W. *Angew. Chem.* **70** (1958) 105.
7. Bergström, S., Krabisch, L. and Sjövall, J *Acta Chem. Scand.* **14** (1960) 1706.
8. Ryhage, R. *Arkiv Kemi* **16** (1960) 19.

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