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The Identification of Prostaglandins E_2, $F_{2\alpha}$ and A_2 from Rabbit Kidney Medulla

By J. B. LEE, K. CROWSHAW, B. H. TAKMAN* and KATHERINE A. ATTREP
Division of Endocrinology and Metabolic Research, St Vincent Hospital, Worcester, Mass. 01610, *U.S.A.*

and J. Z. GOUGOUTAS
Department of Chemistry, Harvard University, Cambridge, Mass. 02138, *U.S.A.*

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Rabbit kidney medulla (10kg.) was homogenized in 5mM-disodium hydrogen phosphate and deproteinized with ethanol, and the concentrated supernatant solution was extracted at pH 8 with light petroleum and at pH 2 with chloroform. The acidic lipids present in the chloroform phase were separated on silicic acid columns into three biologically active fractions. The first fraction contained only vasodepressor activity; the second fraction contained both vasodepressor and non-vascular-smooth-muscle-stimulating activity; the third fraction contained both vasopressor and non-vascular-smooth-muscle-stimulating activity. Purification of each fraction by reversed-phase partition and thick-layer chromatography yielded three pure acids. Thin-layer chromatographic, spectroscopic and mass-spectral analysis of the acids and their methyl esters established their structures as prostaglandins E_2, $F_{2\alpha}$ and A_2. Evidence is presented demonstrating that part or all of the prostaglandin A_2 is formed during the isolation procedures from endogenous prostaglandin E_2.

Previous investigations have shown that the vasodepressor and non-vascular-smooth-muscle-stimulating activity of homogenates of rabbit renal medulla (Lee, Hickler, Saravis & Thorn, 1962, 1963) were attributable to three acidic lipids: medullin, compound 1 and compound 2 (Lee, Covino, Takman & Smith, 1965). These substances exhibited pharmacological and chromatographic properties similar to those of the prostaglandins (Bergström & Samuelsson, 1965).

In the present paper we report the separation, purification and identification of PGE_2† and $PGF_{2\alpha}$ as major prostaglandins in rabbit renal medulla. In addition, evidence is presented indicating that medullin, the compound of principal interest in the earlier work and now identified as PGA_2, is formed at least in part during the isolation procedures.

A preliminary report of part of this work has been given (Lee, 1966; Lee *et al* 1966).

* Present address: Astra Pharmaceutical Products Inc., Worcester, Mass. 01610, U.S.A.

† Trivial names and abbreviations are based on the systematic nomenclature proposed by Bergström & Samuelsson (1967) [see Nugteren, van Dorp, Bergström, Hamberg, & Samuelsson (1966) for certain revisions]: PGE_1, prostaglandin E_1 (11,15-dihydroxy-9-oxoprost-13-enoic acid); PGE_2, prostaglandin E_2 (11,15-dihydroxy-9-oxoprosta-5,13-dienoic acid); PGE_3, prostaglandin E_3 (11,15-dihydroxy-9-oxoprosta-5,13,17-trienoic acid); $PGF_{1\alpha}$, 9,11,15-trihydroxyprost-13-enoic acid; $PGF_{2\alpha}$, 9,11,15-trihydroxyprosta-5,13-dienoic acid; PGA_1 (formerly PGE_1-217), 15-hydroxy-9-oxoprosta-10,13-dienoic acid; PGA_2 (formerly PGE_2-217), 15-hydroxy-9-oxoprosta-5,10,13-trienoic acid; PGB_1 (formerly PGE_1-278), 15-hydroxy-9-oxoprosta-8(12),13-dienoic acid; PGB_2 (formerly PGE_2-278), 15-hydroxy-9-oxoprosta-5,8(12),13-trienoic acid.

MATERIALS AND METHODS

Animals. For assays of vasodepressor activity, female albino Charles River C.D. strain rats were used (Charles River Laboratories, North Wilmington, Mass., U.S.A.). Fresh rabbit jejunal strips were obtained from rabbits of the New Zealand Flemish strain. Fresh rabbit kidney medullas were separated from cortex by scissor dissection as described by Lee *et al.* (1965). For large quantities (10kg.) this procedure was performed by Pel-Freeze Biologicals Inc., Rogers, Ark., U.S.A.; the fresh medullas were immediately frozen in solid CO_2 for shipment and stored at $-20°$ before use.

Chemicals. With the exception of 2-ethylhexan-1-ol (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.), all solvents were Spectro grade and redistilled before use (E. J. Curtis Co., North Wilmington, Mass., U.S.A.). Silicic acid (Bio Sil HA, −325 mesh) was obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A., and Johns-Manville Celite analytical filter aid from Fisher Scientific Co., Medford, Mass., U.S.A. Thin-layer chromatography was done on silica gel G or GF (E. Merck A.-G., Darmstadt,

West Germany). Dimethyldichlorosilane was obtained from Chemicals Procurement Laboratories Inc., College Point, N.Y., U.S.A.

Authentic PGE_1 and PGE_2 were given by Professor S. Bergström, Karolinska Institutet, Stockholm, Sweden, and $PGF_{1\alpha}$ and $PGF_{2\alpha}$ by Dr D. A. van Dorp, Unilever Laboratories, Vlaardingen, The Netherlands. PGA_1 and PGA_2 formed by acetic acid dehydration of PGE_1 and PGE_2 respectively (Daniels *et al.* 1965) were obtained from the Upjohn Co., Kalamazoo, Mich., U.S.A. Methyl esters of these authentic standards and of the renomedullary compounds were prepared with ethereal diazomethane.

Initial extraction and purification. The initial extraction of the active material was carried out as described by Lee *et al.* (1965). In the present studies, 10kg. of frozen rabbit kidney medullas was partially thawed and homogenized in a Waring Blendor for 3min. at room temperatures with 5mM-Na_2HPO_4 (1·5l./kg. of tissue). The homogenate was centrifuged at 10000*g* for 30min., the supernatant was decanted and combined with ethanol (final ethanol concn. 80%, v/v), and the solution was kept overnight at 5°. The large amounts of precipitate that separated were removed by filtration and the filtrate was concentrated to approx. 6l. by vacuum distillation at 35°.

The concentrate, essentially free of ethanol, was adjusted to pH8 with 5N-NaOH and partitioned three times against equal volumes of light petroleum (b.p. 35–60°). The light petroleum was discarded and the aqueous phase further concentrated to approx. 2l. by vacuum distillation. The concentrate was adjusted pH3 with 6N-HCl, extracted with three 1l. lots of chloroform and the aqueous phase discarded. The combined chloroform extracts were washed several times with distilled water to remove traces of acid, evaporated to dryness *in vacuo* and stored dry.

Silicic acid chromatography. Silicic acid chromatography was performed by the method of Hirsch & Ahrens (1958) with the same solvent systems as those devised by Samuelsson (1963) for the purification of prostaglandins in human seminal plasma. In the initial large-scale chromatography, silicic acid (500g.) was slurried in 1500ml. of benzene–ethyl acetate (9:1, v/v), poured into a 95cm. × 5cm. water-jacketed constant-temperature (20°) glass column and allowed to settle to a height of 45cm. Crude renomedullary extract (5g.) was dissolved in 30ml. of benzene–ethyl acetate (9:1, v/v) and introduced on the column. Fractions were collected by stepwise elution with various concentrations of ethyl acetate in benzene at a rate of approx. 5ml./min. (Table 1). In the large-scale chromatography, the entire elution volume at each solvent composition was pooled and distilled to dryness *in vacuo*. The residue from each pool was dissolved in 10ml. of chloroform–methanol (4:1, v/v); 0·1ml. samples were removed, dried under a stream of nitrogen and dissolved in 0·5ml. of 0·2M-sodium phosphate buffer, pH8, for biological assays.

The active fractions were combined and further purified by small-scale chromatography on silicic acid by the following procedure: silicic acid (20g.) was slurried in 65ml. of benzene–ethyl acetate (9:1, v/v) and poured into a 25 cm. × 1·6cm. water-jacketed constant-temperature (20°) glass column. Fractions (100ml.) were collected by stepwise elution with increasing concentrations of ethyl acetate in benzene (Table 1) at an elution rate of approx. 1ml./min. Each fraction was assayed for vasodepressor and non-vascular-smooth-muscle-stimulating activity. The active

Table 1. *Elution procedure utilized in silicic acid chromatography*

Elution sequence	Solvent composition	Elution volume (l.) Large scale	Small scale
1	Benzene–ethyl acetate (9:1, v/v)	2	0·4
2	Benzene–ethyl acetate (4:1, v/v)	2	0·8
3	Benzene–ethyl acetate (7:3, v/v)	—	0·9
4	Benzene–ethyl acetate (2:3, v/v)	—	1·1
5	Benzene–ethyl acetate (1:4, v/v)	20	1·8
6	Methanol	1	0·6

fractions were pooled and further purified by reversed-phase partition and thin-layer chromatography. To characterize the eluates further, samples (0·2ml.) of each fraction were treated with N-NaOH (0·2ml.) at 35° for 2hr. and assayed for the characteristic ultraviolet absorption at 278mμ that develops after alkali treatment of PGE-type but not PGF-type prostaglandins (Bergström, Ryhage, Samuelsson & Sjövall, 1963).

Reversed-phase partition chromatography. Reversed-phase partition chromatography was performed by the procedure of Howard & Martin (1960). Celite was washed with 0·01 N-HCl followed by distilled water, dried at 110° and kept overnight in a desiccator containing dimethyldichlorosilane vapour. After repeated washings with methanol [until a dilution of the methanol wash with distilled water (1:10) was neutral], the Celite was redried at 110° for 3hr. and stored *in vacuo*.

Solvent system C-45 of Samuelsson (1963) was used for the reversed-phase chromatography of all active fractions. Methanol–water–chloroform–2-ethylhexan-1-ol (9:11:1:1, by vol.) was kept for 24hr. at room temperature, resulting in the separation of an upper (mobile) phase and a lower (stationary) phase. Celite (4·5g.) was mixed with the stationary phase (4ml.), mobile phase (50ml.) was added and the slurry was agitated with a magnetic stirrer for 30min. The slurry was poured into a 20cm. × 0·9cm. column and allowed to settle under nitrogen pressure. The resultant column height was 12·5cm. The active material (10–15mg.) from the final silicic acid column was dissolved in 1ml. of mobile phase and applied on the column. About 190 fractions (4ml. each) were collected and samples of fractions were assayed for biological activity and for absorption at 278mμ as described above. When larger amounts of active material were processed, 9g. of support was slurried with 75ml. of mobile phase and poured into a 50cm. × 1·6cm. column to a height of 15cm.

Thin-layer chromatography. Thin-layer chromatography was done by the methods described in detail by Lee *et al.* (1965). In certain instances, $AgNO_3$ was incorporated into the adsorbent ($AgNO_3$/adsorbent ratio 1:30). Flaking of the $AgNO_3$-impregnated plates was minimized by reheating at 110° for 10min. and cooling immediately before use. The solvent systems utilized were: A-I, benzene–1,4-dioxan–acetic acid (20:20:1, by vol.); A-II, ethyl acetate–acetic acid – methanol – 2,2,4 - trimethylpentane – water (22:6:7:2:20, by vol.); III, chloroform–methanol–acetic acid (18:1:1, by vol.); IV, chloroform–methanol–acetic

acid (18:2:1, by vol.); V, 2,2,4-trimethylpentane–propan-2-ol–acetic acid (120:40:1, by vol.); M-I, benzene–1,4-dioxan (5:4, v/v); M-II, ethyl acetate–methanol–water (8:2:5, by vol.). Systems A-I, A-II, M-I and M-II were adopted from Gréen & Samuelsson (1964), system III from Wallach (1965) and system V from Eneroth (1963). Systems A-II and M-II were allowed to equilibrate for 1 hr. and the organic upper phase was used.

Spectroscopy. Ultraviolet spectra were recorded on a Zeiss PMQ–II spectrophotometer with micro quartz cells with a capacity of 0·3 ml. and a light-path of 1 cm. Infrared spectra were recorded on a Perkin–Elmer model 337 grating infrared spectrophotometer. Mass spectra were recorded on an AEI MS–9 double-focusing spectrometer.

Biological assays. Assays of vasodepressor activity were obtained by the methods described by Lee *et al.* (1963). The test material was dissolved in phosphate buffer, pH 8, and injected into the jugular vein of pentobarbital-anaesthetized vagotomized pentolinium-treated rats. Mean carotid blood pressure was recorded kymographically.

Studies on non-vascular smooth muscle were performed as described by Lee *et al.* (1965) with isolated segments of rabbit jejunum. The tissues were suspended in 10 ml. of oxygenated Tyrode solution at either 30° or 37°. All tissues were allowed to equilibrate with the media for 1 hr. before use; isotonic contractions were recorded at 1 g. resting tension and eightfold magnification.

RESULTS

Preliminary purification. The bulk of the biologically inactive lipids present in the crude lipid extract from 10 kg. of kidney medullas was extracted by the light petroleum, the biologically active material remaining in the pH 8 aqueous phase. Subsequent acidification and extraction with chloroform yielded a crude renomedullary acidic lipid extract (25 g.) that contained most of the original blood-pressure-lowering and smooth-muscle-stimulating activity.

Separation of prostaglandins by silicic acid chromatography. Earlier work on the isolation of the active lipids from rabbit kidney medulla by using DEAE-cellulose chromatography had indicated the presence of prostaglandins of the PGE, PGF (Lee *et al.* 1965) and PGA types (Lee *et al.* 1966). Samuelsson (1963) had previously reported that prostaglandins are also readily separated on silicic

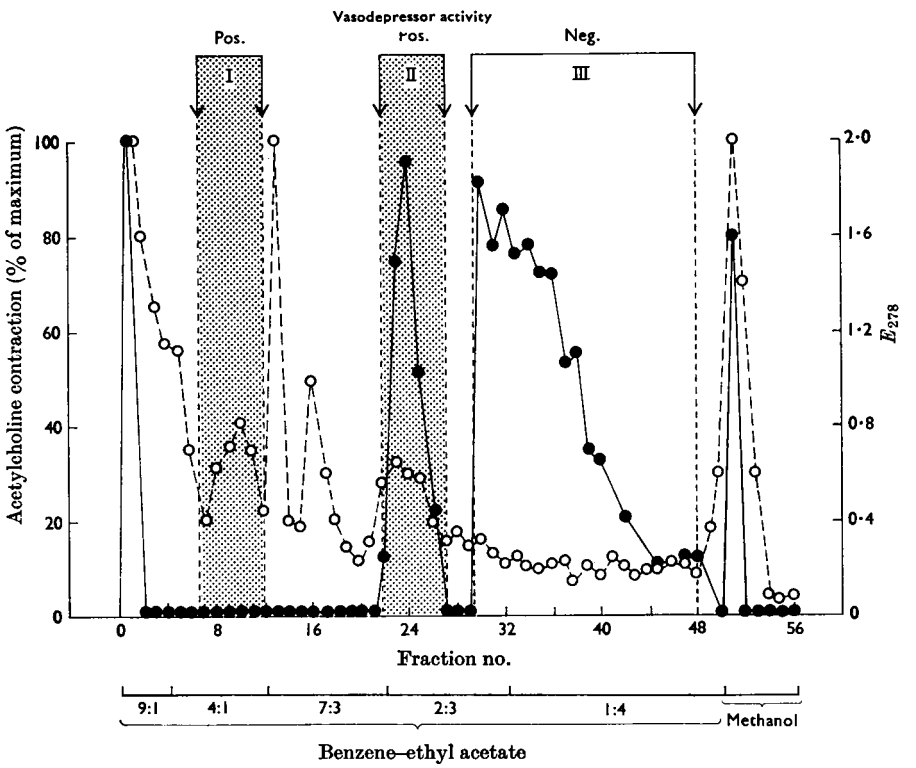


Fig. 1. Silicic acid chromatography of a lipid extract of rabbit renal medulla. The following conditions were used: column, 20 g.; extract, 250 mg.; temperature, 20°; fractions, 100 ml. ●, Acetylcholine contraction (% of maximum); ○, E_{278} measured after alkali treatment (see the Materials and Methods section).

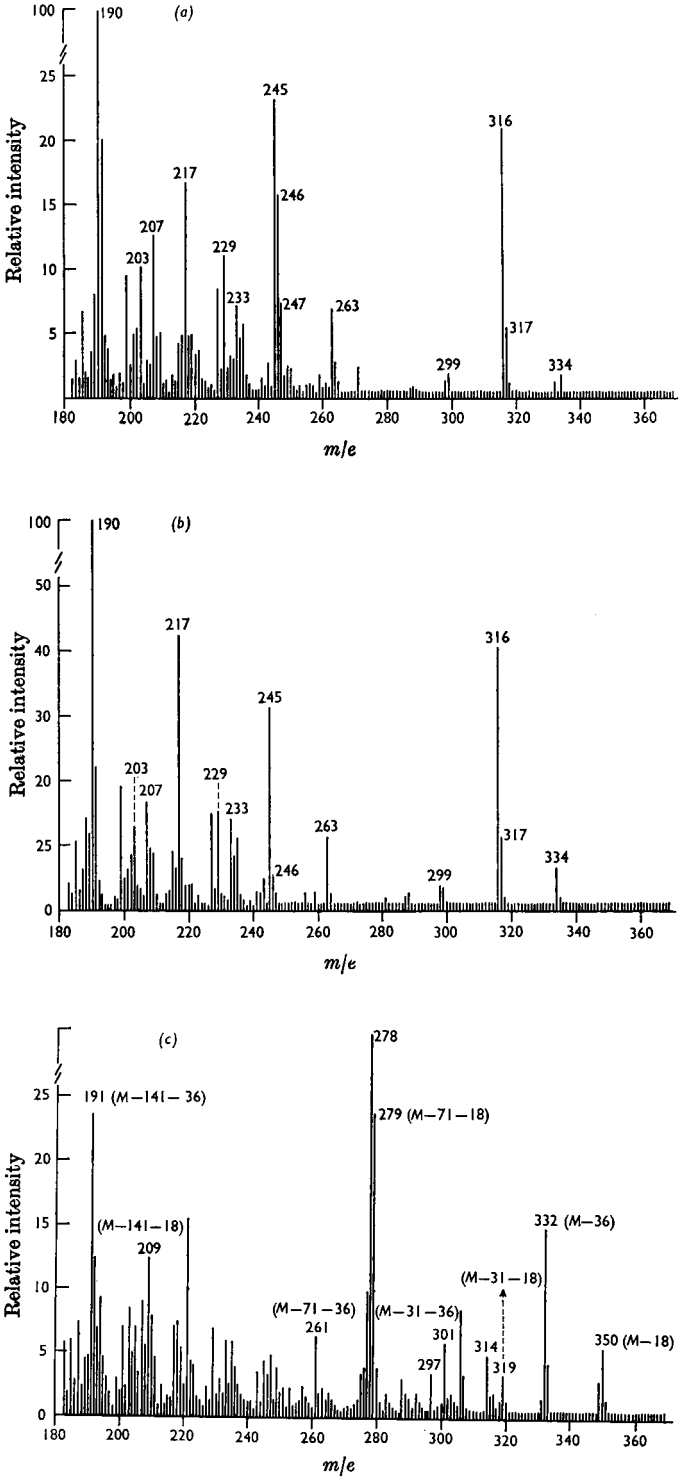


Fig. 2. Mass spectra of the isolated prostaglandins: (*a*) medullin (PGA_2); (*b*) compound 2 (PGE_2); (*c*) methyl ester of compound 1 (methyl $PGF_{2\alpha}$).

Table 2. *Major peaks in the mass spectra of medullin and compound 2*

For comparison, authentic samples of PGA_1, PGA_2 and PGE_2 were also submitted to mass-spectral analyses. Fragments arise by breakdown of the molecule as shown in Fig. 5.

Fragment	Medullin	PGA_2	PGA_1	Fragment	Compound 2	PGE_2
				M	–	–
M	334	334	336	$M-18$	334	334
$M-17$	317	317	319	$M-(18+17)$	317	317
$M-18$	316	316	318	$M-(2\times18)$	316	316
$M-(18+17)$	299	299	301	$M-(2\times18+17)$	299	299
				$M-71$	281	281
$M-71$	263	263	265	$M-(71+18)$	263	263
$M-(71+17)$	246, 247*	247*	248, 249*	$M-(71+18+17)$	246	247*
$M-(71+18)$	245	245	247	$M-(71+2\times18)$	245	245
				$M-(87+18)$	—	247
$M-(87+18)$	229	229	—	$M-(87+2\times18)$	229	229
$M-101$	233	233	235	$M-(101+18)$	233	233
$M-(101+17)$	217*	217*	219*	$M-(101+18+17)$	217*	217*
$M-(113+18)$	203	203	—	$M-(113+2\times18)$	203	—
$M-127$	207	207	—	$M-(127+18)$	207	207
$M-(127+18)$	190*	190*	—	$M-(127+2\times18)$	190*	190*
$M-129$	—	—	207			
$M-(129+18)$	—	—	190*			

*Arising from fragment + 1.

acid by stepwise elution with benzene–ethyl acetate mixtures. In the present study, separation of the crude mixture obtained as described above was first carried out on large-scale silicic acid columns (500 g.). Most of the biologically inactive lipid was eluted with 4l. of benzene–ethyl acetate (9:1, v/v). Subsequent elution with 20l. of benzene–ethyl acetate (1:4, v/v) resulted in recovery of material with vasodepressor and smooth-muscle-stimulating activity. In some runs, further elution with ethyl acetate–methanol (1:1, v/v) yielded material with only smooth-muscle-stimulating activity, and this was combined with the benzene–ethyl acetate (1:4, v/v) fractions.

On rechromatography of the combined active fractions on small silicic acid columns, three different biologically active classes of compounds were obtained (Fig. 1). Elution with benzene–ethyl acetate (4:1, v/v) resulted in the recovery of material (peak I) with vasodepressor and only weak non-vascular-smooth-muscle-stimulating activity. In addition, this material exhibited ultraviolet absorption at 278mμ after alkali treatment. Elution with benzene–ethyl acetate (2:3, v/v) yielded material (peak II) exhibiting both vasodepressor and non-vascular-smooth-muscle-stimulating activity as well as 278mμ absorption after alkali treatment. After the appearance of peak II, a third biologically active material appeared in the benzene–ethyl acetate (2:3 and 1:4, v/v) fractions. This material (peak III) exhibited non-vascular-smooth-muscle-stimulating activity without vasodepressor activity or 278mμ absorption after alkali treatment. The two peaks of non-vascular-smooth-muscle-stimulating activity eluted with 90% benzene and methanol respectively were not further characterized.

Identification of material from peak I. The biologically active material from peak I (14mg.) was further purified by reversed-phase partition chromatography (4·5g. of hydrophobic Celite; temperature, 20°; fractions, 4ml.; solvent system, C-45). Fractions 60–100 exhibited vasodepressor activity with only weak non-vascular-smooth-muscle-stimulating activity similar to that shown by medullin, originally isolated from rabbit kidney medulla by DEAE-cellulose chromatography. Final purification of the combined fractions was effected by thick-layer chromatography on silver nitrate-impregnated silica gel (system IV). The principal band, which alone exhibited biological activity, yielded a colourless viscous oil that appeared to be homogeneous on thin-layer chromatography in a number of different solvent systems. Attempts to crystallize this material were unsuccessful. Comparison of the biological, spectral and chromatographic properties of this compound and medullin showed them to be identical. Since solutions of medullin (especially in chloroform) appeared to be unstable at room temperature, the material was stored either dry or as a methanolic solution under nitrogen at −20°.

An ethanolic solution of medullin obtained from peak I exhibited a principal ultraviolet absorption

maximum at 215mμ (ε 6200), which was suggestive of a cyclopentenone structure similar to that of PG-220 (Bergström *et al.* 1963) and of PGA_1 and PGA_2 (Daniels *et al.* 1965; Hamberg & Samuelsson, 1966). These latter compounds were reported to exhibit ultraviolet absorption at 217mμ (ε 11650) and their base-catalysed conversion into PGB_1 and PGB_2 respectively parallels the reactivity of medullin under the same alkaline conditions. Samples of medullin obtained after final purification exhibited a rather tenuous absorption at 275mμ suggesting the presence of trace quantities of PGB compounds.

The mass spectrum of medullin (Fig. 2*a*) gave an apparent molecular ion at *m/e* 334 and a fragmentation pattern consistent with that expected for PGA_2. Authentic samples of PGA_1 and PGA_2 were also subjected to mass spectroscopy. The mass spectrum of PGA_2 was essentially identical with that of medullin (Table 2), both giving significant peaks 2 mass units lower than those obtained with PGA_1. Additional confirmation was obtained by

Table 3. *Major peaks in the mass spectra of medullin methyl ester and compound 2 methyl ester*

Principal peaks obtained from authentic samples of methyl PGA_2 and methyl PGE_2 are also listed. The fragmentation of the esters is similar to that shown for the free acids (Fig. 5).

Fragment	Medullin (methyl ester)	Methyl PGA_2	Fragment	Compound 2 (methyl ester)	Methyl PGE_2
			M	336	—
M	349*	348	*M*−18	348	348
M−18	330	330	*M*−(2×18)	330	330
M−31	317	317	*M*−(31+18)	317	317
M−(31+18)	299	299	*M*−(31+2×18)	299	299
			M−71	295	295
M−71	277	277	*M*−(71+18)	277	277
			M−(71+32)†	263	—
M−(71+32)†	245	245	*M*−(71+32+18)†	—	—
M−101	—	247	*M*−(101+18)	—	247
M−(101+18)	229	229	*M*−(101+2×18)	—	—
M−(101+31)	217*	217*	*M*−(101+18+31)	217*	217*
M−(127+18)	203	203			
			M−(127+31)	208	208
M−(127+31)	190	190	*M*−(127+31+18)	190	190
			M−(141+18)	208*	208*
M−(141+18)	190*	190*	*M*−(141+36)	190*	190*

* Arising from fragment+1.

† 32 is loss of $CH_3 \cdot OH$ (Nugteren & van Dorp, 1965).

Table 4. *R_F values of kidney prostaglandins after thin-layer chromatography*

Chromatography of each compound (4–10μg.) was done on a 0·25mm. layer of silica gel G. Plates were developed with solvent for 10cm. in well-equilibrated tanks. Spots were detected by iodine vapour or by spraying with 10% phosphomolybdic acid in ethanol. In certain systems (*) $AgNO_3$ was incorporated into the silica-gel layer ($AgNO_3$/silica gel ratio 1:30).

Compound	R_F values of free acids						R_F values of methyl esters	
	A-I	A-II*	III	III*	IV*	V	M-I	M-II*
Medullin	0·64	—	0·55	0·49	0·74	0·30	—	0·82
Compound 2 (fr. 50–70, Fig. 3)	0·38	0·68	0·17	0·11	0·36	0·20	0·29	0·56
Compound 1 (fr. 13–37, Fig. 4)	0·25	0·46	0·07	—	0·15	0·17	0·18	0·26
PGA_1	0·64	—	0·55	0·60	0·78	0·30	—	0·86
PGA_2	0·64	—	0·55	0·49	0·73	0·30	—	0·83
PGE_1	0·38	0·77	0·17	0·18	0·46	0·20	0·29	0·65
PGE_2	0·38	0·68	0·17	0·11	0·36	0·20	0·29	0·56
$PGF_{1\alpha}$	0·25	0·59	0·07	—	0·24	0·16	0·19	0·41
$PGF_{2\alpha}$	0·25	0·47	0·07	—	0·15	0·16	0·18	0·26

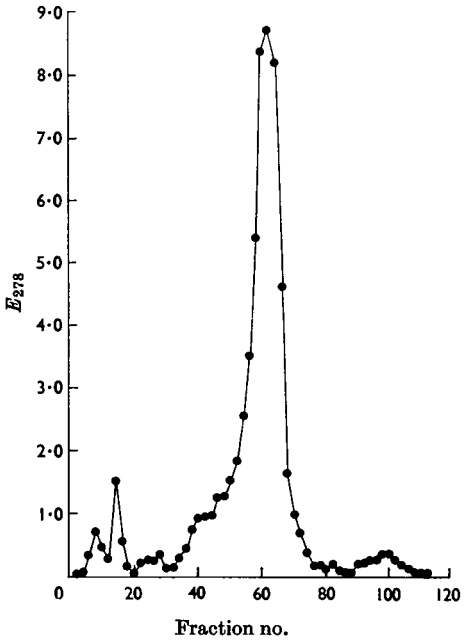


Fig. 3. Reversed-phase partition chromatography of the material from peak II after silicic acid chromatography (Fig. 1). The following conditions were used: column, 4·5g. of hydrophobic Celite; extract, 17mg.; temperature, 20°; fractions, 2ml.; solvent system, C-45 (see the Materials and Methods section). A sample (0·2ml.) of every other fraction was incubated with N-NaOH (0·2ml.) at 35° for 2hr. and the E_{278} value measured.

comparison of the mass spectra of the methyl esters of medullin and PGA_2, which again were indistinguishable (Table 3). Final evidence demonstrating that medullin is PGA_2 was obtained by direct comparison of their chromatographic behaviour on silver nitrate-impregnated silica-gel plates, conditions that separate derivatives of prostaglandins according to the degree of unsaturation. Both the free acids and both the methyl esters of these compounds exhibited identical mobilities in a number of solvent systems (Table 4).

Identification of material from peak II. The biologically active material from peak II was further purified by reversed-phase partition chromatography (Fig. 3). A major peak of material developing a chromophore at 278mμ with alkali was eluted in fractions 50–70. These fractions revealed both vasodepressor and non-vascular-smooth-muscle-stimulating activity. The active fractions were pooled and evaporated to dryness, and the residue was separated by preparative thick-layer chromatography (silver nitrate) in the A-II system. The major band, found in the zone corresponding to PGE_2, contained the original biological activity and was homogeneous on subsequent thin-layer chromatography (silver nitrate) in systems A-II and III. The biological activity, spectral characteristics and chromatographic behaviour of this material and compound 2 previously isolated from rabbit kidney (Lee *et al.* 1965) were directly compared and found to be identical.

An infrared spectrum of compound 2 (in chloroform solution) showed principal absorption peaks at 3350 (–OH), 1735 (C=O), 1700 (acid C=O) and 970cm.$^{-1}$ (*trans*-double bond). An ethanolic solution of compound 2 showed ultraviolet absorption ($\lambda_{max.}$ 278mμ) only after alkali treatment.

The mass spectrum of compound 2 (Fig. 2*b*) exhibited major peaks consistent with a structure of PGE_2. Further, the mass spectra of compound 2 and authentic PGE_2 (Table 2) as well as their methyl esters (Table 3) were practically identical. Additional confirmation for the presence of PGE_2 in renal medulla was provided by comparative thin-layer chromatography of compound 2 and authentic PGE_2. The free acids and methyl esters of these compounds, pairwise, gave identical R_F values in several solvent systems on silica-gel plates with and without silver nitrate (Table 4).

During the final purification of PGE_2 by thick-layer chromatography, a minor component with R_F value corresponding to PGE_1 was isolated. However, significant differences in R_F values in subsequent thin-layer-chromatographic studies with a variety of solvent systems indicated that this component was not PGE_1. The material was not further characterized.

Pure PGE_2 isolated from rabbit renal medulla was obtained as a colourless oil, which was stored as a methanolic solution under nitrogen at −20°. Despite these precautions, material stored in this manner for any appreciable time gave rise to a second compound, which was indistinguishable from PGA_2 on subsequent thin-layer chromatography. This instability is consistent with the suggestion by D. A. van Dorp (personal communication) that medullin is an artifact arising from the dehydration of PGE_2 during our isolation procedure. To examine this possibility further, two fresh rabbit renal medullas were homogenized in 5mM-disodium hydrogen phosphate and the supernatants separated into two equal portions, *A* and *B*. PGE_2 (1·65mg.) in ethanol (2ml.) was added to portion *B*, both solutions were deproteinized with ethanol (final concn. 80%, v/v) and subjected to solvent extraction and small-scale silicic acid chromatography in the usual manner (see the Materials and Methods section). Both were analysed concurrently for prostaglandin content at the various stages of

isolation. Thin-layer chromatographic and biological analysis of the acid chloroform phase of both extracts *A* and *B* failed to reveal any PGA_2.

After silicic acid chromatography of extract *A*, no PGA_2 was detected in the benzene–ethyl acetate (7:3, v/v) fraction. However, material from the benzene–ethyl acetate (2:3, v/v) fraction was characterized as PGE_2 by both thin-layer chromatography and biological assay. From the intensity of the phosphomolybdic acid-reacting spot on thin-layer chromatography and the observed degree of vasodepression, the concentration of PGE_2 was estimated to be at least 15μg./g. wet wt. of renal medulla. After small-scale silicic acid chromatography of extract *B*, small amounts of PGA_2 (approx. 100–150μg.) were identified in the benzene–ethyl acetate (7:3, v/v) fraction. From the benzene–ethyl acetate (2:3, v/v) eluates, PGE_2 (0·9mg.) was also isolated by preparative thin-layer chromatography.

The above experiments indicate that the PGA_2 isolated from extract *B* must arise by dehydration of a portion of the added PGE_2, since no PGA_2 was detected in extract *A*. Thus, during the lengthy isolation procedures involved in the purification of the prostaglandins from 10kg. of renal medulla, it is likely that at least part of the PGA_2 isolated from this source is formed by dehydration of endogenous PGE_2. With a single medulla, however (extract *A*), the amounts of endogenous PGE_2 are too small to give rise to detectable quantities of PGA_2.

Identification of material from peak III. The pooled material from peak III was further purified by reversed-phase partition chromatography (Fig. 4) from which most of the biological activity was eluted in fractions 13–37. The combined fractions were evaporated and the residue was separated by preparative thin-layer chromatography (silver nitrate) in system A-II. Material with smooth-muscle-stimulating activity but without vasodepressor activity was recovered from a zone corresponding to $PGF_{2\alpha}$; no other prostaglandin-like material was recovered from the plate. The biological activity and chromatographic behaviour of this material and compound 1 (Lee *et al.* 1965) were identical. Interestingly, on every occasion that the material was tested for blood-pressure effects, a sustained pressor response was observed.

Pure compound 1 obtained as above did not absorb in the ultraviolet (200–300mμ) even after treatment with alkali. After compound 1 had been kept in concentrated sulphuric acid for several hours,

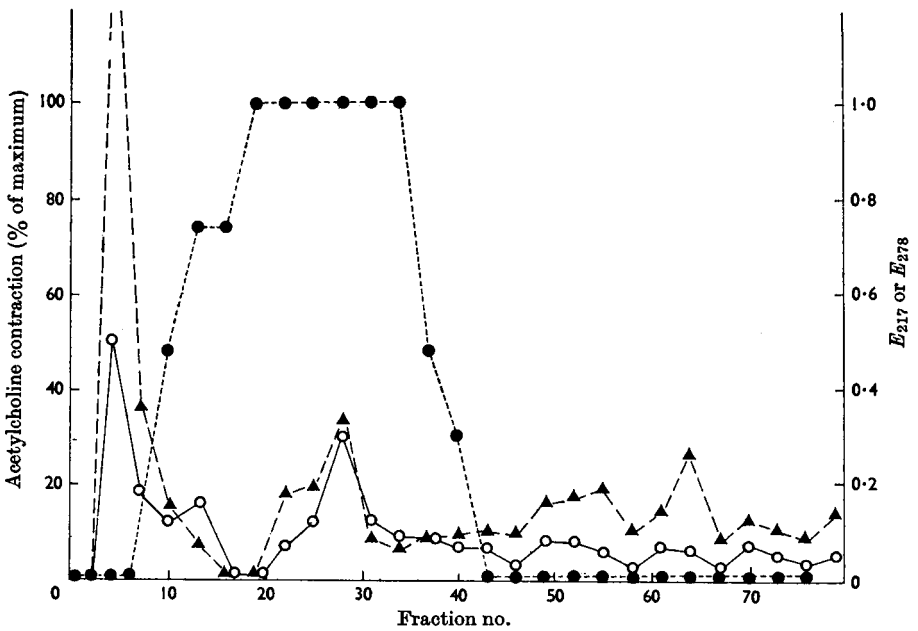
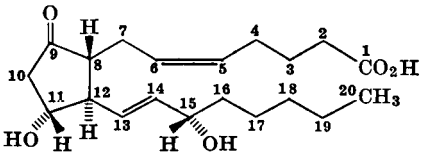
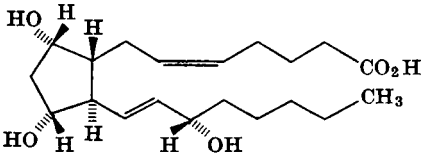


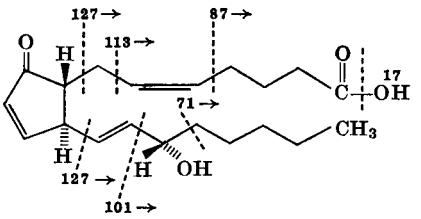
Fig. 4. Reversed-phase partition chromatography of the material from peak III after silicic acid chromatography (Fig. 1). The following conditions were used: column, 9·0g. of hydrophobic Celite; extract, 45mg.; temperature, 20°; fractions, 5ml.; solvent system, C-45 (see the Materials and Methods section). The E_{217} (▲) and E_{278} (○) values of a sample (0·3ml.) of every third fraction were measured. These samples were then evaporated and the activity of each of the residues was tested on isolated jejunal strips (●).



PGE_2 (compound 2)



$PGF_{2\alpha}$ (compound 1)



PGA_2 (medullin)

Fig. 5. Structures of the isolated prostaglandins. Suggested fragmentations for all three compounds are indicated in the structure of PGA_2.

three absorption maxima developed ($\lambda_{max.}$ 263, 306 and 394 mμ). This is similar to but not identical with the reported sulphuric acid spectrum of $PGF_{1\alpha}$ (Bergström & Sjövall, 1960). Direct comparison of the mobilities of compound 1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ and their methyl esters on thin-layer chromatography (Table 4) showed that compound 1 and $PGF_{2\alpha}$ were chromatographically indistinguishable. Final evidence for the identification of $PGF_{2\alpha}$ in rabbit kidney was obtained by comparison of the mass spectra of the methyl esters of compound 1 and of authentic $PGF_{2\alpha}$. The two spectra were practically identical. Although the parent molecular ion at 368 was absent, the peaks at *m/e* 350, 332 and 314 probably arise by loss of one, two and three molecules of water respectively from the parent ion. Other principal fragments are indicated in Fig. 2(*c*).

DISCUSSION

In the present study, three prostaglandins (Fig. 5) were isolated from frozen rabbit kidney medulla (10kg.) and identified as PGE_2 (10mg.), $PGF_{2\alpha}$ (3·2mg.) and PGA_2 (3·5mg.). Although an intensive effort was made to isolate and identify any additional prostanoic acids, no other known naturally occurring prostaglandins were detected. In particular, the absence of PGE_1 or PGE_3, which have been shown to be derived from homo-γ-linolenic acid and eicosapentaenoic acid respectively (van Dorp, Beerthuis, Nugteren & Vonkeman, 1964*a*; Bergström, Danielsson, Klenberg & Samuelsson, 1964), suggests some biochemical specificity with regard to the biosynthesis or distribution of renomedullary prostaglandins.

Since PGE_2 was shown to dehydrate readily during the extraction procedures, it was not possible to determine what proportion, if any, of the isolated PGA_2 is naturally occurring. The detection of only PGE_2 in homogenates prepared from a single fresh rabbit kidney medulla suggests that the concentration of any endogenous PGA_2 present in this tissue is relatively low. Strong, Boucher, Nowaczynski & Genest (1966) were also unable to detect any PGA-type of material in similar medulla extracts. However, they did isolate a vasodepressor lipid (VDL) that had biological and chromatographic properties similar to those reported for compound 2 by Lee *et al.* (1965). Both groups tentatively identified this lipid as PGE_1. In our present studies we have been unable to detect any PGE_1 and we therefore believe that the major PGE-type material in rabbit renal medulla is PGE_2. In addition, $PGF_{2\alpha}$ is also present in smaller concentrations. The isolation of a third prostaglandin, PGA_2 (medullin), is attributed at least in part to dehydration of the endogenous PGE_2 during the large-scale isolation and purification procedures.

It is noteworthy that these three prostaglandins, all derived from arachidonic acid (van Dorp, Beerthuis, Nugteren & Vonkeman, 1964*b*; Bergström, Danielsson & Samuelsson, 1964), have markedly different biological properties. These striking biological differences are the result of relatively simple structural modifications. As stated above, mildly acidic or alkaline conditions are sufficient to dehydrate PGE_2 to PGA_2, a

compound that exhibits only weak non-vascular-smooth-muscle-stimulating properties. Stereospecific reduction of the 9-oxo group of PGE_2 to yield $PGF_{2\alpha}$ produces a more polar compound without any vasodepressor activity. In fact, this latter compound was found to give a sustained pressor response in the pentolinium-treated rat, a result in agreement with observations by DuCharme & Weeks (1967).

Both PGE_2 and PGA_2 have pharmacological properties consistent with a possible antihypertensive (Muirhead, Jones & Stirman, 1960; Lee, 1967) and vasodepressor (Lee *et al.* 1962, 1963) renal endocrine function. However, the diverse biological activities of the three renomedullary prostaglandins suggest they may also participate in more complex intrarenal regulatory mechanisms.

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