Preparative thin-layer and column chromatography of prostaglandins

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ABSTRACT Analytical and preparative chromatographic methods for monounsaturated prostaglandins are described. The systems were developed specifically for separation of the various hydroxy epimers of prostaglandin E_1 and F_1 but also offer superior separations for some of the known natural prostaglandins.

SUPPLEMENTARY KEY WORDS visualization methods · oxygenated fatty acids · stereochemical designations · diastereomers

Continuing studies on the synthesis of diastereomeric prostaglandins (1, 2) have forced us to reexamine the TLC systems suggested[1] in the literature. Many of these systems do not differentiate between PGE, PGF, and PGA compounds differing only in configuration at C-11 and (or) C-15. Thus the PGE-like compounds, with relative R_f 1.15 and 1.32 (PGE_1, relative R_f = 1.00) in the A-system of Nugteren, Vonkeman, and van Dorp (4), that can be isolated from the nonenzymic cyclization of eicosatrienoic acid are probably not, as Nugteren et al. suggested, hydroxyl diastereomers of the naturally-occurring PGE_1.

The recent reexamination (5) of the only synthesis of $PGF_1\alpha$ (and traces of PGE_1 methyl ester) published prior to ours (1, 2) again shows the need for better TLC systems. The materials isolated by the method of Just and Simonovitch (6) appear to be epimeric with the natural hormones.

Several TLC systems with high resolving power are described here. Knowledge of the chromatographic behavior of prostaglandins in these systems may facilitate identification of synthetic prostaglandins prepared by other workers, and the methods devised should prove useful to biochemists working with the natural prostaglandins as well. Some representative formulas are shown opposite.[2]

EXPERIMENTAL PROCEDURES AND RESULTS

Thin-Layer Chromatography

Standard and unknown samples [dissolved, about 1 $\mu g/\mu l$, in acetone, dioxane, or tetrahydrofuran (THF)] were applied as spots 1–2 mm in diameter on 5 × 10 or 5 × 15 cm plates with a fluorescent indicator. The plates were developed in glass tanks, 18 cm high, lined with filter paper. The development distance was 7–8 cm for neutral silica and alumina plates and 10 cm for acidic silica plates. Further development was prevented by scraping away a 1 mm strip of the adsorbent across the width of the plate.

The plates were viewed under UV radiation (PGB and, with heavy loading, PGA were visible as dark spots on the fluorescent background) and then made permanently visible by one of the following methods: (*a*) heating to 120°C on an electric hot plate and immediate dipping into a 1% solution of $SbCl_5$ in CCl_4–CH_2Cl_2 5:1 and then further heating, (*b*) vanillin–H_3PO_4–ethanol spray, (*c*) acidic ceric sulfate spray, (*d*)phosphomolybdic

The abbreviated nomenclature used for the prostaglandins in this article is explained in the Appendix. Abbreviations: TLC, thin-layer chromatography; PGA, prostaglandin A; PGB, prostaglandin B, etc; THF, tetrahydrofuran.

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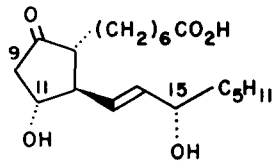
[1] No attempt at a complete literature survey is implied. The excellent review of the prostaglandins compiled by the Worcester and Upjohn groups (3) was available to us prior to publication and serves as an excellent starting point. In addition, several suggestions of W. P. Schneider of the Upjohn Co. were extremely helpful. Some of the TLC systems which we developed are closely allied or identical to those used by other workers.

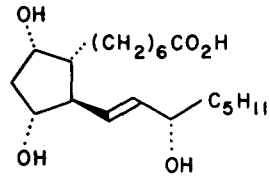
[2] The bracketed notations for these prostaglandins are explained in the Appendix.

or molybdic acid–ethanol spray, (*e*) exposure to iodine vapor followed by charring at 200–250°C, or (*f*) 3% cupric acetate in 15% aqueous phosphoric acid spray followed by heating at 120°C. The characteristic colors obtained with the vanillin spray (*b*) have been reported (3). The $SbCl_5$ reagent (*a*) also gives different colors with various prostaglandins: PGF, initial reddish cast becomes dark gray-brown; PGE_1, brown with slight reddish cast; PGA, brown with initial green cast; and PGB, lemon yellow. The acidic cupric acetate spray (*f*) also gives characteristic colors: green for PGA and PGE, yellow for PGB, and violet for PGF. Methods *a*, *b*, *d*, and *f* are the most sensitive, giving excellent results with 2–5 μg of prostaglandins and usable results with less. Methods *a* and *f* are the best, but the spots obtained by method *a* fade noticeably with time.

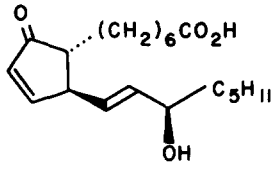
Adsorbents. Commercial adsorbent-coated glass plates were used throughout. The plates coated with neutral silica and alumina (Merck adsorbents) were supplied by Brinkmann Instruments Inc., Westbury, N. Y. The acidic silica plates were Mallinckrodt ChromAR 4GF plates. The plates with 2-mm layers of silica were also supplied by Brinkmann. Acidic silica for column chromatography was 100–200 mesh Mallinckrodt SilicAR CC-4.



PGE_1 [= PG(E$\alpha\alpha$)$_1$]



$PGF_{1\alpha}$ [= PG($\alpha\alpha\alpha$)$_1$]



15-epi-PGA_1 [= PG(A$\Delta\beta$)$_1$]

Solvent Systems. *P*-11 is the superficially dried[3] organic layer from ethyl acetate–hexane–water–methanol–acetic acid 4:2:2:1:1. *C*-I is chloroform–THF–acetic acid 10:2:1. *N*-I is hexane–THF–methylene dichloride 1:1:1. The *H*-series consists of hexane–methylene dichloride–THF–acetic acid in ratios as follows: *H*-I, 6:2:2:1; *H*-II, 30:10:3:3; and *H*-IV, 10:10:10:1. The *D*-series consists of benzene–dioxane–acetic acid in ratios as follows: *D*-I, 3:2:0; *D*-II, 40:10:1; *D*-III, 20:10:1; and *D*-IV, 20:20:1. *D*-IV is the same as Gréen and Samuelsson's *A*-I system (7). The *F*-series is based on ethyl acetate and more polar additives as indicated: *F*-I, ethyl acetate–formic acid 100:1; *F*-IV, ethyl acetate–formic acid 400:5; *F*-V, ethyl acetate–ethanol–acetic acid 100:1:1; *F*-VI, ethyl acetate–acetone–acetic acid 90:10:1; and *F*-VII, cyclohexane–ethyl acetate–acetic acid 60:40:2.

Apparent R_f Values. These are shown in Table 1 as $R_f \times 100$ values. Most of the difficult separations were accomplished by multiple development, and since R_f values are not reproducible after three or more developments, the $R_f \times 100$ values given should be used only as an indication of relative mobilities.

Preparative TLC. The first consideration here is to have nondestructive visualization. Iodine vapor and water spraying have been recommended (3, 7), but neither was satisfactory, particularly on the commercial plates carrying 2-mm layers of silica. PGB and PGA (at sufficiently high loading) offer no difficulties, since they quench fluorescence. PGF and PGE diastereomers can be located easily by exposing the layer to a glowing-hot

[3] Dried by brief shaking with crystalline sodium chloride.

TABLE 1 APPARENT R_f X 100 FOR SOME PROSTAGLANDINS

	Neutral Silica														Acidic Silica						Al_2O_3	
	P-II (1x)	*C*-I (2x)	*H*-I (2x)	*H*-I (4x)	*H*-II (5x)	*D*-I (1x)	*D*-I (2x)	*D*-II (3x)	*D*-III (2x)	*D*-IV (1x)	*F*-I (2x)	*F*-I (4x)	*N*-I (4x)	*F*-IV (2x)	*F*-IV (1x)	*F*-IV (1x)	*F*-V (1x)	*F*-VI (1x)	*F*-VII (2x)	*D*-III (1x)	*H*-IV (1x)	*H*-II (2x)	New Designation
$PGF_{1\beta}$	18	5							17	23				15		12	10	14	3	15	8		PG($\beta\alpha\alpha$)$_1$
$PGF_{1\alpha}$	22	12							26	30				25		23	18	24	7	25	17		PG($\alpha\alpha\alpha$)$_1$
PGE_1	34	25	24	38	15			15	42	37	32	57		37	24	37	32	39	10	38	27	39	PG(E$\alpha\alpha$)$_1$
11-epi-PGE_1	35	29	31	48	17			21				66		47	29	49	45	54	11	52	39		PG(E$\beta\alpha$)$_1$
15-epi-PGE_1	40	37	36	56	21			28	56	46	48	76		57	36	58	54	62	15	58	48	42	PG(E$\alpha\beta$)$_1$
11,15-epi-PGE_1	42	40	41	59	22			29				76		56	34	59	55	64	19	60	50		PG(E$\beta\beta$)$_1$
PGA_1	57	78	69	82	50			69	78	57	62			76	50	79	77	79	62	75	81	62	PG(A$\Delta\alpha$)$_1$
15-epi-PGA_1	59	80	72	84	57			73	80	58				77	51	83	81	86	68	81	87		PG(A$\Delta\beta$)$_1$
PGB_1	56	79	67	81	50				77					74		79	76	78	60	76	85		PG(B–α)$_1$
CH_3-PGE_1	47	38	34	49	20	27	42			45			38		30								CH_3-PG(E$\alpha\alpha$)$_1$
CH_3-11-epi-PGE_1				57			51						51										CH_3-PG(E$\beta\alpha$)$_1$
CH_3-15-epi-PGE_1				63			58						64										CH_3-PG(E$\alpha\beta$)$_1$
CH_3-11,15-epi-PGE_1				65			57						63										CH_3-PG(E$\beta\beta$)$_1$
CH_3-PGA_1	64	84	79		69	58				65	76				67							82	CH_3-PG(A$\Delta\alpha$)$_1$
CH_3-PGB_1	63	85	79			58																	CH_3-PG(B–α)$_1$

wire for 1–2 sec. The hot wire is touched to the plate in a series of lines perpendicular to the solvent front. After this treatment the PGF and PGE zones quench fluorescence. Further exposure leads to charring but is definitely not necessary. This treatment is essentially nondestructive since only the very top layer in the narrow exposed zones is partially converted to the material containing the chromophore responsible for the observed quenching. The exposed areas of the zones are eluted with the entire zone and in no case have artifacts been detectable by TLC of the eluted materials. 15–150 mg of prostaglandins can be separated on one layer that is 100 mm square and 2 mm thick; the hot wire visualization works well in this entire range.

The free acids are eluted from the powdered zones with methanol. The residue from the methanol solution is partitioned between water and ether (containing added ethyl acetate or methylene chloride) after the addition of several drops of acetic acid. The organic phase is washed with several portions of water and then with saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered, and concentrated on a rotary evaporator.

PGE isomers purified in this way are invariably contaminated with traces of PGA resulting from dehydration during chromatography and reisolation. The degree of contamination is reduced significantly when acetic acid is used in the extraction instead of the mineral acids usually employed. Oxalic acid can also be used. PGE diastereomers from preparative TLC are best purified on small columns of acidic silica (see below).

Smaller quantities (1 μg–10 mg) are best separated on a ChromAR 4GF plate (0.25 mm layer). The free acids, eluted with acetone in nearly quantitative yield, do not contain dehydration products when nonacidic systems (generally cyclohexane, ethyl acetate–acetone) or weakly acidic systems (1–2% acetic acid) are used. The prostaglandin samples obtained in this way need not be subjected to reisolation from acidic aqueous solution. The only further purification needed (if any) is removal of fine particles by filtration or centrifugation after the sample has been dissolved in chloroform, ether, or ethyl acetate. As an example, the $PGF_1\alpha$ and $PGF_1\beta$ mixture obtained from 10.6 mg of PGE_1 by the method of Bergström, Krabisch, Samuelsson, and Sjövall (8) was separated on a 20 × 20 cm ChromAR 4GF plate. The plate was developed to 8 cm and then 16 cm with *F*-VI. $PGF_1\alpha$ (4.5 mg, mp 97–100°C) and $PGF_1\beta$ (6 mg, mp 127–128°C) were obtained from the two zones (R_f for $PGF_1\alpha$ 0.5–0.6 and for $PGF_1\beta$ 0.32–0.48) by elution with acetone.

Column Chromatography on Acidic Silica

PGE diastereomers contaminated with PGA were purified on 5 × 60 mm columns of acidic silica (packed in cyclohexane) by elution with ethyl acetate–cyclohexane 1:2 after prior elution with the same solvent in the proportions 3:2. Such columns can be used for as much as 20 mg of contaminated PGE obtained by preparative TLC.

The same method can be used directly for separation of the classes as well as separation of the various diastereomers. The column is packed with SilicAR CC-4 (100–200 mesh) as a cyclohexane slurry. The prostaglandin mixture is applied to the top in a small volume of ethyl acetate or cyclohexane–ethyl acetate and eluted with cyclohexane–ethyl acetate mixtures: PGA and PGB are eluted with a 2:1 mixture, PGE diastereomers with a 1:1 mixture, and PGF diastereomers with pure ethyl acetate (sometimes acetone was added). PGE_1, $PG(E\beta\alpha)_1$, and $PG(E\alpha\beta)_1$ can be separated from each other in this way. A 100 mg sample of PGE mixture can be separated on a 17 × 300 mm column with more than 80% of the material eluted in pure form. The mixed fractions can be rechromatographed to improve the yields of separated components. Recovery of PGE is greater than 97% as measured by UV absorption of PGB formed on base treatment, and conversion to PGA is insignificant (<3%).

DISCUSSION

These results indicate: (*a*) that 1 μg–1 g of prostaglandins can be purified by chromatographic methods with little or no losses due to irreversible adsorption or rearrangement if the proper precautions and solvent systems are employed; (*b*) that TLC serves as an excellent analytical method for distinguishing diastereomers of prostaglandins; and (*c*) that the relative mobilities of the various hydroxy diastereomers are not altered by the solvent systems or by esterification. These relative mobilities[4] are summarized below.

R_f values for PGF diastereomers:

$(\beta\alpha\alpha) < (\alpha\alpha\alpha) \sim (\alpha\beta\beta) \lesssim (\alpha\beta\alpha) \sim (\beta\beta\alpha) < (\beta\beta\beta) \sim (\beta\alpha\beta) < (\alpha\alpha\beta)$

R_f values for PGE diastereomers:

$(E\alpha\alpha) < (E\beta\alpha) < (E\alpha\beta) \lesssim (E\beta\beta)$

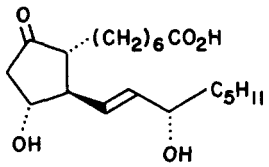
R_f values for PGA diastereomers:

$(A\Delta\alpha) < (A\Delta\beta)$

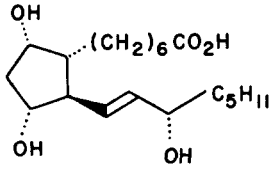
NOMENCLATURE APPENDIX

A new, convenient extension of the presently used abbreviations for the prostaglandins has been used in this article. In this extension, the configurations at C-9, C-11, and C-15 (the usual positions for oxygen functional groups) are indicated, in

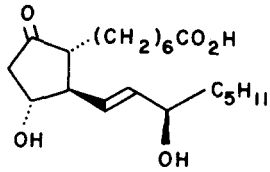
[4] N. H. Andersen, unpublished observations.



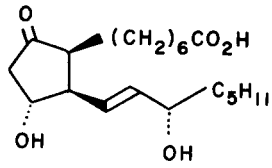
11α,15α-dihydroxy-9-oxo-13-*trans*-prostenoic acid
$PG(E\alpha\alpha)_1$



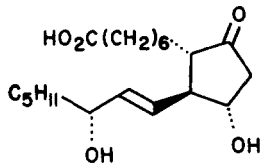
9α,11α,15α-trihydroxy-13-*trans*-prostenoic acid
$PG(\alpha\alpha\alpha)_1$



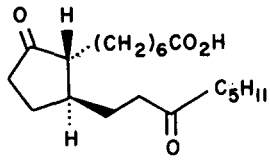
naturally derived 15-epi-PGE_1
= $PG(E\alpha\beta)_1$



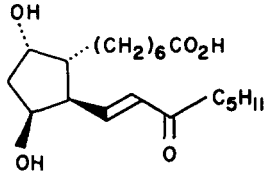
naturally derived 8-epi-PGE_1
= iso-$PG(E\alpha\alpha)_1$



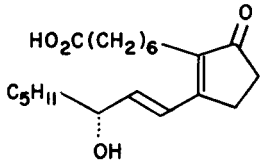
antipode of PGE_1
= ent-$PG(E\alpha\alpha)_1$



15-keto-dihydro-PGE_1
= $PG(E\alpha K)_0$



15-keto-11-epi-$PGF_1\alpha$
= $PG(\alpha\beta K)_1$



antipode of PGB_1
= ent-$PG(B\text{–}\alpha)_1$

that order, by α or β within parentheses. The hydroxyl groups in natural PGE_1 and $PGF_1\alpha$ are α-oriented in the projection most commonly used for the prostaglandins.

This system retains the use of subscripts to indicate the degree of unsaturation in the corresponding E-type prostaglandin. When no configuration assignment is required, the designations are: K = keto, Δ = unsaturation, and – (dash) for no substituent. In the case of 9-oxo compounds, these are further distinguished as E-, A-, or B-type by using these letters in place of K. Prostaglandins having *cis*-oriented side chains are designated by prefixing iso- to the abbreviation used for the C-8 epimer. Further, we designated the antipodes and racemates by the prefixes *ent-* (or *enantio*) and *rac-*, respectively.

We have found these configuration-indicating designations particularly useful in discussing the effects of stereochemistry on pharmacological activities, relative mobilities, and relative rates of biological degradation of these substances.

The examples below, and in the text and table above, illustrate the system.

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References

1. Corey, E. J., N. H. Andersen, R. M. Carlson, J. Paust, E. Vedejs, I. Vlattas, and R. E. K. Winter. 1968. *J. Amer. Chem. Soc.* **90:** 3245.
2. Corey, E. J., I. Vlattas, N. H. Andersen, and K. Harding. 1968. *J. Amer. Chem. Soc.* **90:** 3247.
3. Ramwell, P. W., J. E. Shaw, G. B. Clarke, M. F. Grostic, D. G. Kaiser, and J. E. Pike. 1968. *In* Progress in the Chemistry of Fats and Other Lipids. R. T. Holman, editor. Pergamon Press, New York. **9** (Pt. II): 231–273.
4. Nugteren, D. H., H. Vonkeman, and D. A. van Dorp. 1967. *Rec. Trav. Chim. Pays-Bas.* **86:** 1237.
5. Holden, K. G., B. Hwang, K. R. Williams, J. Weinstock, M. Harman, and J. A. Weisbach. 1968. *Tetrahedron Lett.* 1569.
6. Just, G., and C. Simonovitch. 1967. *Tetrahedron Lett.* 2093.
7. Gréen, K., and B. Samuelsson. 1964. *J. Lipid Res.* **5:** 117.
8. Bergström, S., L. Krabisch, B. Samuelsson, and J. Sjövall. 1962. *Acta Chem. Scand.* **16:** 969.