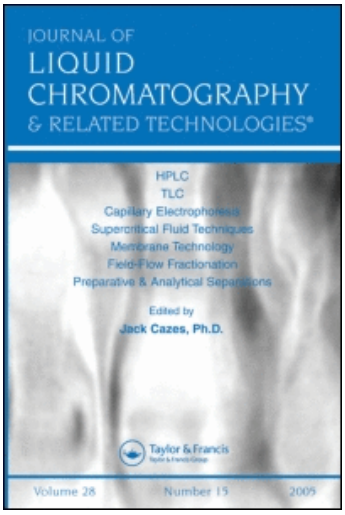

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Normal Phase High Performance Liquid Chromatography of Some Prostaglandin B_1 Derivatives

William F. Feely[ab]; Herman W. Shmukler[a]; Robert V. Dorman[cd]
[a] Naval Air Development Center, Warminster, Pennsylvania [b] Merck, Sharp and Dohme Research Labs, Three Bridges, N.J. [c] Department of Biological Sciences, Drexel University, Philadelphia, Pennsylvania [d] Department of Biolgical Sciences, Kent State University, Kent, Ohio

To cite this Article Feely, William F. , Shmukler, Herman W. and Dorman, Robert V.(1989) 'Normal Phase High Performance Liquid Chromatography of Some Prostaglandin B_1 Derivatives', Journal of Liquid Chromatography & Related Technologies, 12: 4, 515 — 527
To link to this Article: DOI: 10.1080/01483918908051754
URL: http://dx.doi.org/10.1080/01483918908051754

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(4), 515-527 (1989)

NORMAL PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF SOME PROSTAGLANDIN B_1 DERIVATIVES

WILLIAM F. FEELY[1], ROBERT V. DORMAN[2]
AND HERMAN W. SHMUKLER[1]
[1]*Naval Air Development Center*
Warminster, Pennsylvania 18974
[2]*Department of Biological Sciences*
Drexel University
Philadelphia, Pennsylvania 19104

ABSTRACT

15-Keto-13,14-trans-prostaglandin B_1 methyl ester, 13,14-trans-prostaglandin B_1 methyl ester, 13,14-cis-prostaglandin B_1 methyl ester, 13,14-dihydro-prostaglandin B_1 methyl ester and 13,14-dehydro-prostaglandin B_1 are organic intermediates used in the synthesis of prostaglandin B_x, a polymeric derivative of 15-keto-prostaglandin B_1 methyl ester. PGB_x has been shown to protect laboratory animals against cardiogenic shock, cerebral ischemia and hypoxia. A normal phase, high performance liquid chromatographic analysis is presented which permits the identification and quantitation of these PGB_1 intermediates.

INTRODUCTION

The prostaglandins (PGs) form a class of products with potent and diverse biological activities. The major PGs are derivatives of the twenty carbon parent

compound arachidonic acid. In mammals PGs affect the cardiovascular and pulmonary vascular systems. They are involved in shock, trauma, inflammation, neonatology, respiration and renal pathology. PGs also affect the nervous, endocrine, reproductive and gastrointestinal systems (1,2). The work described in this report involves the isolation, purification and identification of compounds similar to PGB_1 which is a 13,14 trans fatty acid. (Figure 1).

These PGB_1 intermediates are important in the synthesis of PGB_x, which has been shown to preserve oxidative phosphorylation in rat liver mitochondria (3). PGB_x treated monkeys survive cardiogenic shock (4), while treated rabbits survive cerebral ischemia (5) and PGB_x treated dogs survive "fatal" hypoxia (6). The total syntheses and structure determinations of PGB_1 related materials have been reported (7). The last few steps in the synthesis of PGB_1 intermediates used in this study are shown in Figure 2.

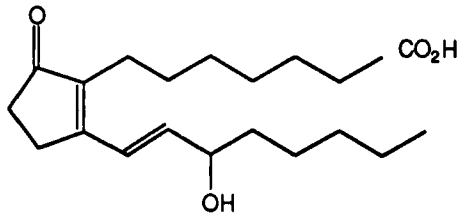

FIGURE 1. Structure of PGB_1

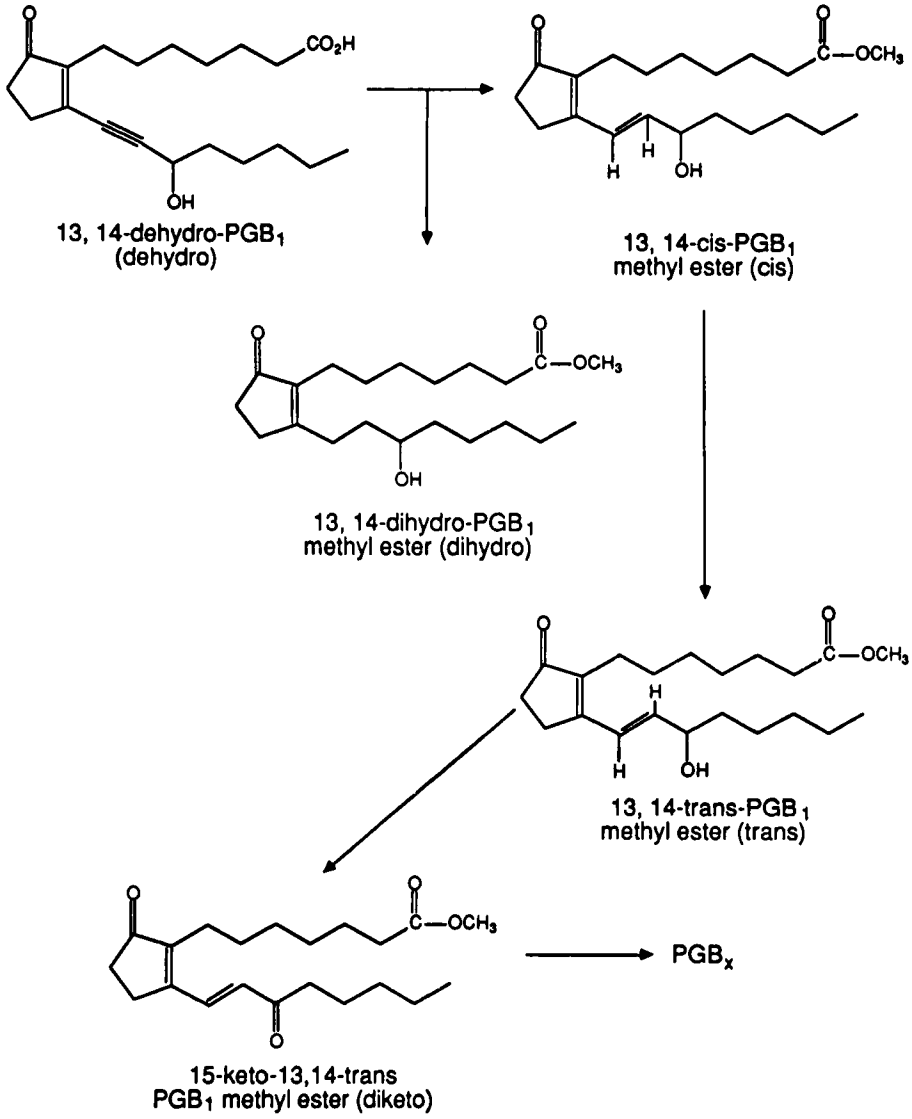

FIGURE 2. Final steps in synthesis of PGB_1 intermediates

Dehydro PGB_1 (dehydro) which has a triple bond at C-13,14 is first converted to its methyl ester ($-OCH_3$) with diazomethane. This derivative is reduced to cis-PGB_1-OCH_3 (cis) with palladium on barium sulfate, quinoline and methanol. Cis is isomerized to trans-PGB_1-OCH_3 (trans) by reaction with iodine in cyclohexane/chloroform. Trans is oxidized to 15-keto-PGB_1-OCH_3 (diketo) by addition of Jones reagent (8). Reduction of dehydro-PGB_1-OCH_3 also gives some dihydro-PGB_1-OCH_3 (dihydro) as a byproduct. The dihydro compound has a single bond at C-13,14.

The organic syntheses used to make the five intermediates discussed above are complex and by-products are formed during these reactions. Obtaining pure samples and separating by-products is essential for high percentage yields in subsequent synthetic steps. Therefore, a quick reproducible means of analyzing these compounds is required. A high performance liquid chromatographic technique, utilizing UV detection and a silica gel column eluted with isopropanol/hexane, was developed which allows rapid quantitation of the PGB_1 analogues.

MATERIALS AND METHODS

Standard Preparation

PGB_1 intermediates were prepared by Dr. George Nelson (7) at St. Joseph's University (Philadelphia,

Pa.). Purification and identification of the compounds were performed at the Naval Air Development Center.

Preparative silica gel HPLC was used to obtain standards. Chromatographic conditions were as follows: u Porasil column (Waters Associates; 30 cm x 7.8 mm ID); Constametric II pump (Laboratory Data Control); solvent composition, 2-5% isopropanol/hexane; flow rate, 2 ml/min.; amount of sample injected, 20 mg; UV detector, Altex Model 153 with prep cell, eluate monitored at 254 nm. Like samples (as determined below) were combined and flash evaporated after peak shaving.

Previously, the purity of PGB_1 intermediates was determined by UV spectroscopy and thin layer chromatography according to Polis et al.(7). Therefore, these two methods were used to screen standards for purity prior to quantitative HPLC analysis.

Thin layer chromatography was performed on precoated 20 cm x 20 cm silica gel 60 fluorescent - 254 plates (E.M. Laboratories). Two ul of PGB_1 intermediates in ethanol were manually spotted at a concentration of .01 mg/ul 1.7 cm from the bottom of the plate. The plate was developed in a sealed glass tank (21 cm x 6cm x 21 cm) in 45% ethyl acetate/55% hexane (Fischer Scientific) until the solvent front moved 10 cm from the origin (approximately 33 minutes). The plate was removed, dried, and visualized in a

Chromato - Vue (Ultraviolet Products) broad wavelength UV viewer.

UV spectra and calculated molar extinction coefficients were obtained using a Cary 14 (Applied Physics) variable wavelength, double beam, scanning spectrophotometer. Quartz flow cells (Arthur H. Thomas) with dimensions 4.5 cm x 0.5 cm x 1 cm were used. Reference and sample cells were filled with 1.0 ml ethanol. One ul of PGB_1 intermediate was added to the sample cell to a concentration of 10 mg/ml.

Quantitative HPLC

Quantitative HPLC was performed on a Hewlett-Packard 1084A liquid chromatograph equipped with semi-automatic injection. HPLC grade isopropanol (Waters Associates) and hexane (Fischer Scientific) were premixed to 2% isopropanol/98% hexane (solvent B) and 50% isopropanol/50% hexane (solvent A). A Hewlett-Packard 5 um Si - 100 column (250 mm x 4.6 mm ID) was used for the separation. A Vari-chrom (Varian) variable wavelength detector was used for PG identification.

Samples of each PGB_1 intermediate were made at concentrations of 0.05, 0.1 and 0.2 mg/ml. Injected volume for each run was 10 ul. Other parameters were programmed as follows: flow, 3.0 ml/min.; maximum pressure, 400 bar; minimum pressure, 0 bar; solvent temperature A, 40° C; solvent temperature B, 40°

C; oven temperature, 40° C; chart speed, 0.30 cm/min.; chart zero, 10.0 cm; slope sensitivity, 0.2; area rejection, 1000; change to 100% A (50% isopropanol/50% hexane) between 29.9 and 30.0 minutes; integration setting 9 (a bunching factor which causes broad, spread out peaks to be read by the instrument as narrow ones) 33.5 minutes; and stop run, 40.0 minutes.

Diketo, cis, trans and dehydro PGB_1 intermediates were injected separately and detected at 270 nm. Dihydro was detected at 237 nm.

RESULTS AND DISCUSSION

The preparative HPLC samples were analyzed by thin layer chromatography and UV spectroscopy for sample purity. The results are summarized in Tables 1 and 2.

The cis-trans isomers have essentially the same R_f value in this TLC system (Table 1) and possess the same

TABLE 1

TLC R_f Values of the Five PGB_1 Intermediates

Compound	Rf
15-keto-13,14-trans PGB_1-OCH_3 (diketo)	0.61
13,14-trans-PGB_1-OCH_3 (trans)	0.39
13,14-cis-PGB_1-OCH_3 (cis)	0.38
13,14-dihydro-PGB_1-OCH_3 (dihydro)	0.31
13,14-dehydro-PGB_1 (dehydro)	0.09

TABLE 2

Calculated Molar Extinction Coefficients of the Five PGB_1 Intermediates at Various Wavelengths

Compound	at maximum absorbance	at 270 nm	at 237 nm
diketo	28,000 (296 nm)	11,800	2,100
trans	31,800 (278 nm)	28,300	1,400
cis	22,700 (278 nm)	21,300	2,100
dihydro	14,300 (237 nm)	350	14,300
dehydro	18,700 (270 nm)	18,700	2,000

UV absorbance maximum (Table 2). Previous to the method described here, these two compounds could be unambiguously identified only by ^{13}C-NMR (9). In addition, the cis compound isomerizes into the trans compound when it is not refrigerated. The diketo, dihydro and dehydro compounds can be identified by UV and TLC; time of sample preparation, however, is a disadvantage. Long analysis times are required when NMR is used and in complex samples the NMR scan is difficult and sometimes impossible to interpret.

The quantitative HPLC analysis is fast, requires little sample preparation and can be used to simultaneously qualitate and quantitate the five PGs. Quantitative evaluation of this method was made by injecting each pure sample in isopropanol at three concentrations covering a range from 500 to 2000 ng. Linear regression

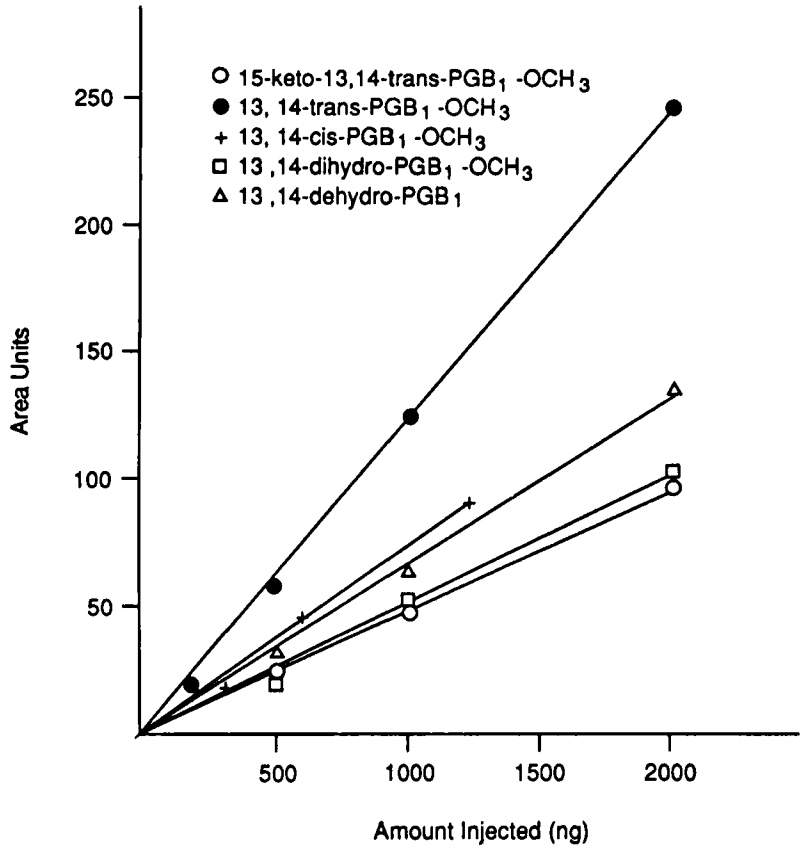

FIGURE 3. Standard curves for each PGB_1 intermediate (peak area vs. weight)

analysis of these data gave correlation coefficients of 1.00 (Figure 3). Analysis of the cis sample used to make the standard curve confirms the ^{13}C-NMR analysis that the cis isomerizes into the trans compound. This cis sample is actually 37% trans. Therefore, the amount of cis in the sample is calculated from the amount of

known trans injected, since trans PG is not isomerized. Accordingly, a fourth point (.02 mg/ml) has been added to the standard curve for the trans compound. Representative chromatograms are shown in Figure 4. PGs were subjected to dual wavelength analysis at 270 nm and 237 nm in order to optimize sensitivity, as shown in Table 2. All five PGs were separated using this method.

In order to test the method, a sample of unknown PGB_1 analogue composition was made by a colleague. The unknown was dried, weighed and brought to a concentration of 0.2 mg/ml. The sample (10 ul) was injected as described. Examination of the chromatograms and comparison to the standard concentration curves revealed that this sample contained 28% diketo, 24% trans, 25% dihydro and 23% of a non-standardized compound. ^{13}C-NMR identified the last compound as 13,14-dihydro-15-keto-PGB_1-OCH_3 (diketo dihydro), which is similar to the diketo compound except for a saturated C-13,14 bond.

The diketo dihydro had been used in the past for structural studies (10). It can be incorporated into the analysis since it eluted at a different retention time than the other compounds (7.65 min.). As a result, at least six PGB_1 intermediates can be examined by this method.

The main disadvantage of this system is the need for analysis at two UV wavelengths. This problem can be

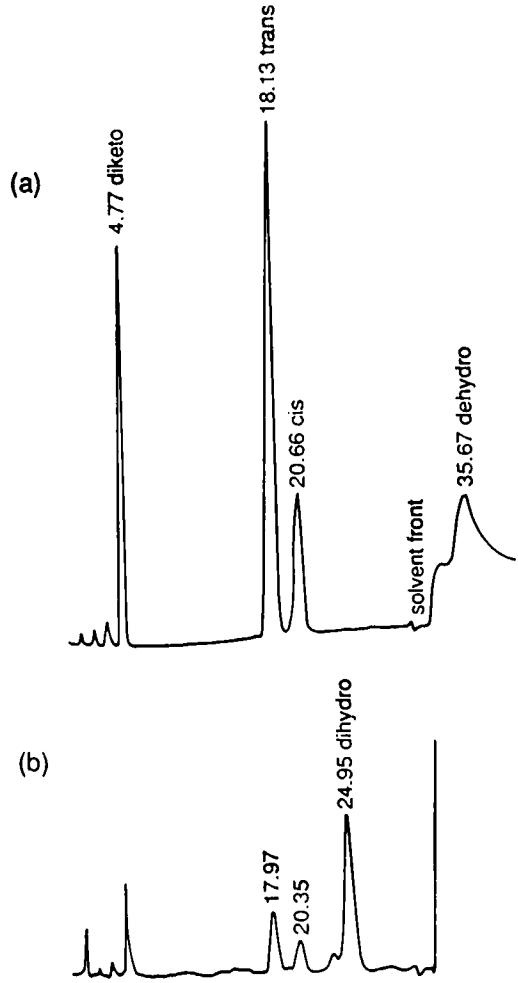

FIGURE 4. Normal phase HPLC of the five PGB_1 intermediates. A mixture of the compounds was applied to a Hewlett-Packard 5 um Si-100 column (250 mm x 4.6 mm) and eluted isocratically with premixed solvents consisting of 2% isopropanol/98% hexane; solvent change to 50% isopropanol/50% hexane for elution of last component. Flow rate: 3.0 ml/min. UV detection: 270 nm for chromatogram (a) (a blank chromatogram of solvent only indicated that after the appearance of the solvent front at approximately 31 min., a flat baseline was obtained and therefore the dehydro compound could be integrated); 237 nm for chromatogram (b) (dehydro not shown; has minimal absorbance at 237 nm; see Table 2). Amount injected: 1000 ng of each component in isopropanol.

overcome by using two detectors and two recorders in sequence or by using a photodiode array detector. Other investigators have quantitated PGs by using detectors which allow analysis at 193 nm where all PGs absorb (11,12).

In summary, the quantitative HPLC method presented here has several advantages over UV, TLC and ^{13}C-NMR analysis of PG intermediates. The HPLC method completely resolves the five PGs used in the final synthesis steps of PGB_x and it can be used to identify other intermediates. The analysis is rapid (80 minutes total); improved hardware can reduce this time to 40 minutes. The method can quantitate nanogram quantities of complex mixtures of synthetic reaction products and requires little sample preparation.

ACKNOWLEDGMENTS

The authors wish to thank S. Kwong for performing the NMR experiments and interpreting the spectra.

CURRENT ADDRESSES

W.F.F.: Merck, Sharp and Dohme Research Labs, P.O. Box 450, Hillsborough Rd., Three Bridges, N.J. 08887.

R.V.D.: Department of Biological Sciences, Kent State University, Kent, Ohio 44242.

REFERENCES

(1) Samuelsson, B., Ramwell, P.W. and Paoletti, R., eds., Advances in Prostaglandin and Thromboxane Research, Raven Press, New York, Vol. 7., 1980.

(2) Samuelsson, B., Ramwell, P.W. and Paoletti, R., eds., Advances in Prostaglandin and Thromboxane Research, Raven Press, New York, Vol. 8., 1980.
(3) Polis, B.D., Polis E. and Kwong, S., Protection and reactivation of oxidative phosphorylation in mitochondria by a stable free-radical prostaglandin polymer (PGB_x), Proc. Natl. Acad. Sci. USA, 76, 1598, 1979.

(4) Angelakos, E.T., Riley, R.L. and Polis, B.D., Recovery of monkeys after myocardial infarction with ventricular fibrillation. Effects of PGB_x, Physiol. Chem. Phys., 12, 81, 1980.

(5) Kolata, R.J. and Polis, B.D., Facilitation of recovery from ischemic brain damage in rabbits by polymeric prostaglandin PGB_x, a mitochondrial protective agent, Physiol. Chem. Phys., 12, 545, 1980.

(6) Moss, G., Immediate restoration of CNS autonomic cardiopulmonary control. Survival of lethal cerebral hypoxia by treatment with PGB_x, Surg. Forum, 29, 535B, 1978.

(7) Polis, B.D., Kwong, S., Polis, E., Nelson, G. and Shmukler, H.W., Studies on PGB_x, a polymeric derivative of prostaglandin B_1: I Synthesis and purification of PGB_x, Physiol. Chem. Phys., 11, 109, 1979.

(8) Fieser, L.F. and Fieser, M., Reagents for Organic Synthesis, John Wiley and Sons, New York, 1967, p. 142.

(9) Kwong, S., Unpublished results.

(10) Polis, B.D., Polis, E. and Kwong, S.F., Carbon-13 Nuclear Magnetic Resonance Studies of Prostaglandin B Monomers as an Approach to the Elucidation of the Structure of PGB_x, a Polymer of Prostaglandin B, Physiol. Chem. Phys., 13, 111, 1981.

(11) Van Rollins, M., Ho, S.H.K., Greenwald, J.E., Alexander, M., Dorman, N.J., Wong, L.K. and Horrocks, L.A., Complete separation by high performance liquid chromatography of metabolites of arachidonic acid from incubation with human and rabbit platelets, Prostaglandins, 20, 571, 1980.

(12) Terragno, A., Rydzik, R., and Terragno, N.A., High performance liquid chromatography and ultra violet detection for the separation and quantitation of prostaglandins, Prostaglandins, 21, 101, 1981.