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Detection of Prostaglandin $F_{2\alpha}$ as Pentafluorobenzyl Ester by Electron-Capture GLC

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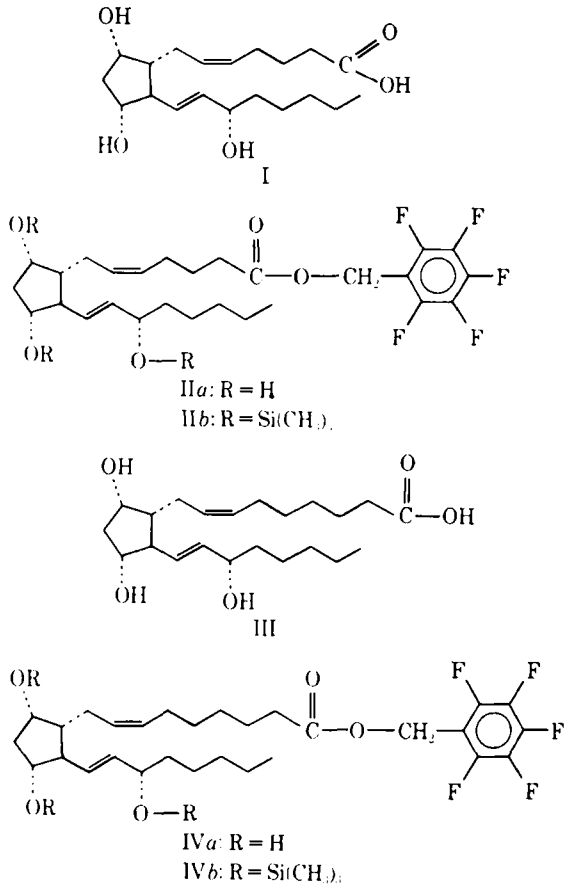
Abstract □ The pentafluorobenzyl ester of prostaglandin $F_{2\alpha}$ was synthesized on a preparative scale and was gas chromatographed as the tris(trimethylsilyl) ether. The latter was found to be stable during GLC and highly sensitive to electron-capture detection. The lower limit of detection was 12.5 pg. of the ester, injected on-column as the silylated product. The nanogram scale conversion of prostaglandin $F_{2\alpha}$ to the ester, under conditions amenable to electron-capture GLC detection, was developed. The electron-capture GLC response was linear over the 0.03–0.84-ng. range of the ester, injected as the tris(trimethylsilyl) ether.

Keyphrases □ Prostaglandin $F_{2\alpha}$—electron-capture GLC analysis as pentafluorobenzyl ester □ GLC, electron-capture detection—analysis, prostaglandin $F_{2\alpha}$ as pentafluorobenzyl ester

Prostaglandins are biologically important compounds that are active at very low concentrations, usually in the nanogram range. To study the physiological role and the mechanisms of action, as well as the absorption, metabolism, and excretion of these prostaglandins, simplified analytical methodology of high sensitivity and specificity is required. Among the many methods considered for the quantitation of prostaglandins are those based on biological responses (1, 2), enzymatic assay (3), fluorescence and UV spectroscopy (4–6), radioimmunoassay (7–11), and chromatography. The last includes: (*a*) high-pressure liquid chromatography for the detection of low levels of prostaglandins after conversion to the C-1-*p*-nitrobenzyl esters (12), and (*b*) GLC utilizing flame-ionization detection (13–15), mass spectrometric detection (16–22), or electron-capture detection (23–26). GLC methods utilizing flame ionization usually lack adequate sensitivity for the detection of low levels of prostaglandins incident in biological fluids. The GLC–mass spectrometric methods and the electron-capture GLC methods, which afford high sensitivity, also offer a high degree of specificity when combined with a preliminary TLC separation. In terms of general utility, the widely used and elegant GLC–mass spectrometric method has the disadvantage of requiring expensive and usually inaccessible instrumentation, as well as the synthesis of the isotopically labeled (deuterated) prostaglandins for internal standards and carriers.

Reported electron-capture GLC methods based on the inherent electron-capturing properties of prostaglandin B (PGB) compounds (23, 24) have drawn considerable attention. This approach, however, is limited to the PGB compounds or to prostaglandins that can be readily and quantitatively converted to the PGB's. The most common derivatives of the prostaglandins examined for electron-capture detection involve derivatization of the hydroxy groups as, for example, conversion to a heptafluorobutyrate ester (25, 26) or bromomethyl–dimethylsilyl ether (24). The thermal instability of the heptafluorobutyrate derivative, resulting in the formation of multiple GLC peaks, renders it unsuitable for electron-capture GLC analysis (26).

Since all naturally occurring prostaglandins contain a C-1 carboxyl group that has been shown to undergo facile reaction with benzylic halides, the C_1-penta-



fluorobenzyl ester of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was prepared to examine its electron-capture detection properties.

EXPERIMENTAL

Preparative Scale Synthesis of $PGF_{2\alpha}$ Pentafluorobenzyl Ester (IIa)—A solution of $PGF_{2\alpha}$ (I) (40 mg.) in acetonitrile (2 ml.) was treated with pentafluorobenzyl bromide (0.2 ml.) and diisopropylethylamine (0.2 ml.). TLC examination [on silica gel in ethyl acetate–acetic acid (97:3)] indicated quantitative formation of the derivative (II*a*) (R_f 0.4) in 1 hr. with little evidence of side-products. The reaction mixture was diluted with 0.1 *M* citric acid (50 ml.) and extracted with ethyl acetate (50 ml.). The organic phase was washed with water (50 ml.) and dried with anhydrous sodium sulfate. The crude product was chromatographed[1], and the ester was eluted with ethyl acetate-acetone (6:4). Removal of the solvent gave a colorless oil (42 mg.) which showed a single spot with TLC and a single peak with GLC (flame-ionization detection) as the trimethylsilyl derivative (II*b*).

Submicrogram Scale Conversion of $PGF_{2\alpha}$ to IIa—$PGF_{2\alpha}$ (I) was treated with a solution of pentafluorobenzyl bromide in acetonitrile [10 μl. containing 5.8 mg. (0.022 mmole) of pentafluorobenzyl bromide] and a solution of diisopropylethylamine in acetonitrile [10 μl. containing 0.886 mg. (0.007 mmole) of diisopropylethylamine]. The reaction mixture was heated at 40° for 5 min. and then evaporated to dryness under nitrogen. The recovered sample was treated with the reagents again in a manner identical to that indicated previously and was evaporated to dryness under nitrogen.

Tris(trimethylsilyl) $PGF_{2\alpha}$ Pentafluorobenzyl Ester (IIb)—Electron-capture GLC analysis was carried out on the tris(trimethylsilyl) ether of the $PGF_{2\alpha}$ pentafluorobenzyl ester (II*a*).

On a preparative scale, II*a* (10 mg.) was dissolved in *N,O*-bis-(trimethylsilyl)acetamide–pyridine (1:1) (1 ml.), and the solution was heated at 60° for 15 min. Excess reagent was removed with a stream of nitrogen, and the residue was dissolved in tetrahydrofuran for GLC analysis. Appropriate dilutions of this stock solution were made with the same solvent. The gas chromatograms did not show any additional peaks or significant decrease in concentration on standing for 24 hr. at room temperature.

In a submicrogram scale analysis, II*a* residues from the esterification were treated with *N,O*-bis-(trimethylsilyl)acetamide (10 μl.) at room temperature (~23°) for 5 min. Aliquots (1 μl.) of the solution were directly analyzed by electron-capture GLC.

Internal Standard for GLC—The tris(trimethylsilyl) pentafluorobenzyl ester of 9-{3α,5α-dihydroxy-2β-[(3*S*)-3-hydroxy-*trans*-1-octenyl]-1α-cyclopentyl}-*cis*-7-nonenoic acid or 2*a*,2*b*-dihomo-$PGF_{2\alpha}$ (III) was used as an internal standard for GLC.

*Pentafluorobenzyl Ester of 9-{3α,5α-dihydroxy-2β-[(3S)-3-hydroxy-*trans*-1-octenyl]-1α-cyclopentyl}-cis-7-nonenoic Acid (III)*—A solution of III (31.91 mg.) in acetonitrile (2 ml.) was treated with pentafluorobenzyl bromide (0.2 ml.) and diisopropylethylamine (0.2 ml.) at room temperature. After stirring for 1 hr., the reaction mixture was concentrated (to about 0.5 ml.) by evaporation under nitrogen, diluted with 0.1 *M* citric acid (10 ml.), and extracted with ethyl acetate (2 × 10 ml.). The combined organic phase was washed with water (10 ml.), dried with anhydrous sodium sulfate, filtered, and evaporated. The crude product was purified by sequential preparative TLC on silica gel in solvent systems of: (*a*) ethyl acetate–acetic acid (97:3), and (*b*) ethyl acetate–acetic acid (95:5). The pentafluorobenzyl ester (IV*a*) zone was located by exposure of the reference spots on discrete lanes to iodine vapor in the manner indicated previously. Then IV*a* was extracted from the silica gel using ether (2 × 5 ml.). The purified ester (IV*a*) (9.48 mg.) was observed to be homogeneous by TLC on silica gel in ethyl acetate–acetic acid (95:5).

Tris(trimethylsilyl) Ether of Pentafluorobenzyl Ester (IVa)—An aliquot of IV*a* (2.04 mcg.) was converted to the tris(trimethylsilyl) derivative (IV*b*) by treatment with *N,O*-bis-(trimethylsilyl)acetamide (1 ml.) and examined by electron-capture GLC. A GLC peak corresponding to a minor impurity (retention time ~12 min.) was observed in addition to the peak due to the major product tris-(trimethylsilyl) pentafluorobenzyl ester (IV*b*). The identity of the latter was confirmed by GLC–mass spectrometry.

A solution of IV*b* in the silylating agent (65.174 mcg./ml.) was employed as the internal standard for GLC analysis. Aliquots (10 μl.) of this solution were added to submicrogram quantities of II*a* and mixed well[2]. Samples (1 μl.) of this solution were utilized for electron-capture GLC analysis.

Electron-Capture GLC—*Method A*—A chromatograph[3], equipped with a ^{63}Ni electron-capture detector, was operated with a constant (d.c.) polarizing voltage. Spiral glass columns [0.61 m. (2 ft.) × 0.28 cm. (0.125 in.) i.d.] packed with 3% OV-1 on 80–100-mesh Gas Chrom Q were utilized. High purity nitrogen was used as the carrier gas at a flow rate of 40 ml./min. The column oven was operated isothermally at 255°, the flash heater at 275°, and the detector at 290°. Samples of 1 μl. were chromatographed. Under these conditions, II*b* and the internal standard (IV*b*) had retention times of 8.9 and 16 min., respectively.

Method B—A second chromatograph[4] was equipped with a ^{63}Ni electron-capture detector (pulse interval of 5 μsec.). A glass column [0.77 m. (2.5 ft.) × 0.28 cm. (0.125 in.) i.d.] packed with 1% OV-1 on 100–120-mesh Gas Chrom Q was arranged for on-column injection. Argon (95%)–methane (5%) was used as the carrier gas at a flow rate of 60 ml./min. The column oven was operated isothermally at 250°, the flash heater at 250°, and the detector at 280°. Samples of 1 μl. were chromatographed. Under these conditions, the tris(trimethylsilyl) $PGF_{2\alpha}$ pentafluorobenzyl ester (II*b*) had a retention time of 4 min.

GLC–Mass Spectrometry—GLC–mass spectra were recorded on a gas chromatograph–mass spectrometer[5], utilizing a column [0.77 m. (2 ft.)] packed with 3% OV-1 on Diatoport S (60–80 mesh). The column was maintained at a temperature of 225° and the flash heater at 270°.

Tris(trimethylsilyl) $PGF_{2\alpha}$ Pentafluorobenzyl Ester (IIb)—A sample of $PGF_{2\alpha}$ (I) was converted to the pentafluorobenzyl ester (II*a*) on a microscale. The product was treated with *N,O*-bis-(trimethylsilyl)-acetamide, and an aliquot was analyzed.

[1] On Silicar CC-4, 50 g., prepacked in ethyl acetate.

[2] Using a Vortex mixer.
[3] Varian Aerograph model 1740.
[4] Hewlett-Packard model 5750.
[5] LKB-9000.

A GLC peak with a retention time of ~2.2 min. was observed. A mass spectrum of this material showed an M^+ ion at *m/e* 750 and prominent peaks at: 735 ($M^+ - 15$) due to loss of CH_3, 679 ($M^+ - 71$) due to loss of C_5H_{11}, 660 ($M^+ - 90$) due to loss of $(CH_3)_3$—Si—OH, 645 ($M^+ - 105$) due to loss of $(CH_3)_3$—Si—OH + CH_3, 589 ($M^+ - 161$) due to loss of C_5H_{11} + $(CH_3)_3$—Si—OH, 570 ($M^+ - 180$) due to loss of 2× [$(CH_3)_3$—Si—OH], 237 ($M^+ - 513$), and 181 ($M^+ - 569$) due to CH_3—C_6F_5.

Tris(trimethylsilyl) Pentafluorobenzyl Ester (IVb)—A sample of the pentafluorobenzyl ester (IV*a*) prepared was treated with *N,O*-bis-(trimethylsilyl)acetamide, and an aliquot was analyzed.

A GLC peak with a retention time of ~16.5 min. was observed. A mass spectrum of the material showed an M^+ ion at *m/e* 778 and prominent peaks at: 763 ($M^+ - 15$) due to loss of CH_3, 707 ($M^+ - 71$) due to loss of C_5H_{11}, 688 ($M^+ - 90$) due to loss of $(CH_3)_3$—Si—OH, 673 ($M^+ - 105$) due to loss of $(CH_3)_3$—Si—OH + CH_3, 617 ($M^+ - 161$) due to loss of C_5H_{11} + $(CH_3)_3$—Si—OH, 598 ($M^+ - 180$) due to loss of 2× [$(CH_3)_3$—Si—OH], 237 ($M^+ - 541$), and 181 ($M^+ - 597$) due to CH_3—C_6F_5.

RESULTS AND DISCUSSION

The preparative scale synthesis of $PGF_{2\alpha}$ C_1-pentafluorobenzyl ester (II*a*) was conducted in acetonitrile using excess pentafluorobenzyl bromide (0.2 ml.), diisopropylethylamine (0.2 ml.), and $PGF_{2\alpha}$ (40 mg.). Under these conditions, the reaction was virtually quantitative in less than 1 hr. at room temperature on the basis of TLC examination. The ester was purified by silica gel column chromatography[6], and GLC-mass spectroscopy of the tris(trimethylsilyl) derivative confirmed the structure.

Only two reports appear in the literature for the preparation of pentafluorobenzyl esters of carboxylic acids, and the reported methods are slower than the method employed herein. Kawahara (27) refluxed carboxylic acids in acetone with potassium carbonate and excess pentafluorobenzyl bromide for 3 hr. to effect ester formation. Ehrsson (28) developed an ion-pair extraction process employing methylene chloride and an aqueous alkaline solution of tetrabutylammonium sulfate. However, only long-chain esters gave quantitative reaction in less than 20 min. Formation of the *n*-butyrate and *n*-valerate esters was incomplete after 2 hr. The use of diisopropylethylamine in the present study allowed homogeneous reaction conditions, and the high steric requirement of the amine served to minimize quaternary ammonium salt formation with pentafluorobenzyl bromide.

The $PGF_{2\alpha}$ C_1-pentafluorobenzyl ester (II*a*) was converted to the tris(trimethylsilyl) derivative (II*b*) prior to GLC.

Electron-capture GLC was conducted on one of two chromatographs[3,4]. Under the GLC conditions employed (see *Experimental* section), the lower limit of detection was 12.5 pg. of II*a* injected on-column in one instrument[3]. The lower limit of detection on the second instrument[4] was not determined. Typical chromatograms at the 1- and 10-ng. levels of II*a* are shown in Fig. 1. The GLC peak height response due to II*b* was linear for the 0.5–10-ng. range of II*a* injected on-column on the first instrument[3] (Fig. 2) and for the 0.03–0.84-ng. range of II*a* on the second[4].

The reaction conditions for the submicrogram scale conversion of $PGF_{2\alpha}$ (I) to the pentafluorobenzyl ester (II*a*) were explored. Since poor yields of the pentafluorobenzyl ester were obtained on attempting to derivatize nanogram quantities of $PGF_{2\alpha}$ adopting the conditions described earlier, the influence of reagent concentrations, temperature, and reaction time on the submicrogram pentafluorobenzyl esterification of $PGF_{2\alpha}$ was studied. The formation of the $PGF_{2\alpha}$ pentafluorobenzyl ester (II*a*) was monitored by electron-capture GLC analysis of the trimethylsilyl ether of the isolated product. Maximum conversion with minimum background interference was achieved when a picogram or nanogram quantity of $PGF_{2\alpha}$ (or the tromethamine salt of $PGF_{2\alpha}$) was treated with pentafluorobenzyl bromide (0.022 mmole) in acetonitrile (10 μl.) and diisopropylethylamine (0.007 mmole) in acetonitrile (10 μl.) and heated at 40° for 5 min. Maximum consistency of reaction was realized when the product was treated with the reagents again under identical conditions.

Initial attempts to isolate the pentafluorobenzyl ester product from the acidified reaction mixture by solvent extraction presented difficulties. Traces of citric acid used for the acidification caused high backgrounds on electron-capture analysis, probably as a result of pentafluorobenzyl esterification. Furthermore, under these extraction conditions, recoveries of the submicrogram quantities of the ester II*a* were erratic. Isolation of the product by direct evaporation of the solvent and excess reagents under nitrogen at room temperature was found to be ideal. Electron-capture detection background interference was minimal, and no sample loss was involved. Avoiding a conventional "workup" has the obvious advantages of saving time and effort. The $PGF_{2\alpha}$ pentafluorobenzyl ester product (II*a*) was converted to the tris(trimethylsilyl) ether (II*b*) by treatment with *N,O*-bis-(trimethylsilyl)acetamide. Hydrolysis of the trimethylsilyl groups of the submicrogram quantity of the derivative by traces of moisture was prevented, and maximum consistency of sample response was achieved by injection of the samples directly in the silylating agent. Typical gas chromatograms of the reagent blank and of II*b* formed by the reaction of a nanogram quantity of $PGF_{2\alpha}$ (I) are illustrated in Fig. 3. Although conversion of the $PGF_{2\alpha}$ ester (II*a*) to a bromomethyldimethylsilyl ether of enhanced electron-capture response is feasible, the excess halogen-containing silylating reagent would have to be completely eliminated, since even traces may cause "swamping" of the electron-capture detector (or high backgrounds). Attempts to remove rigorously all traces of the excess halogen-containing reagent prior to dissolving in a dry solvent (*e.g.*, ether) invariably result in partial hydrolysis of the timethylsilyl groups of the submicrogram sample and a consequent loss in sample response. To select a suitable "internal standard" for GLC, several prostaglandin analogs were converted to their pentafluorobenzyl esters on a microscale, and the GLC properties of the tris(trimethylsilyl) ethers of these esters were examined. Among those investigated, the tris(trimethylsilyl) pentafluorobenzyl ester of 9-{3α,5α-dihydroxy-2β-[(3*S*)-3-hydroxy-*trans*-1-octenyl]-1α-cyclopentyl}-*cis*-7-nonenoic

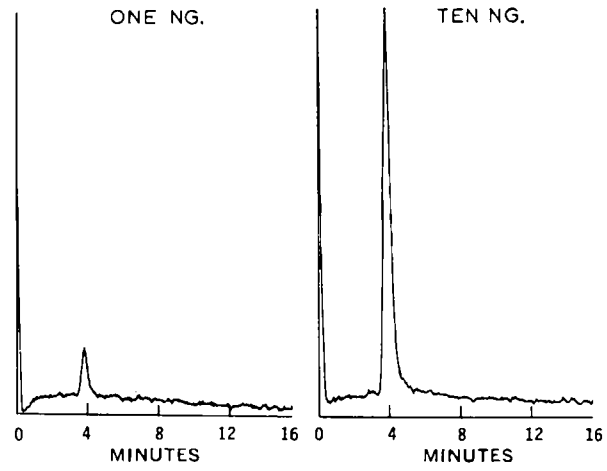


Figure 1—*Typical gas chromatograms of tris(trimethylsilyl) $PGF_{2\alpha}$ pentafluorobenzyl ester using electron-capture detection.*

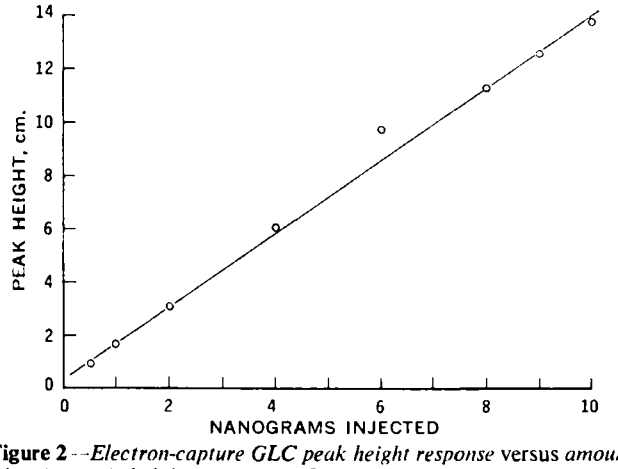


Figure 2—*Electron-capture GLC peak height response* versus *amount of tris(trimethylsilyl) $PGF_{2\alpha}$ pentafluorobenzyl ester injected.*

[6] Silicar CC-4, ethyl acetate–acetone (1:1).

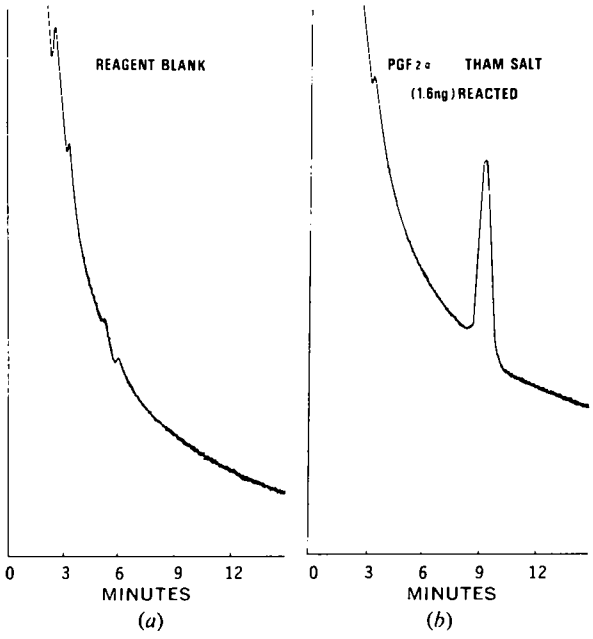


Figure 3—*Typical gas chromatograms of:* (a) *the reagent blank, and* (b) *$PGF_{2\alpha}$-tromethamine salt (1.6 ng.) reacted to the tris(trimethylsilyl) pentafluorobenzyl ester.*

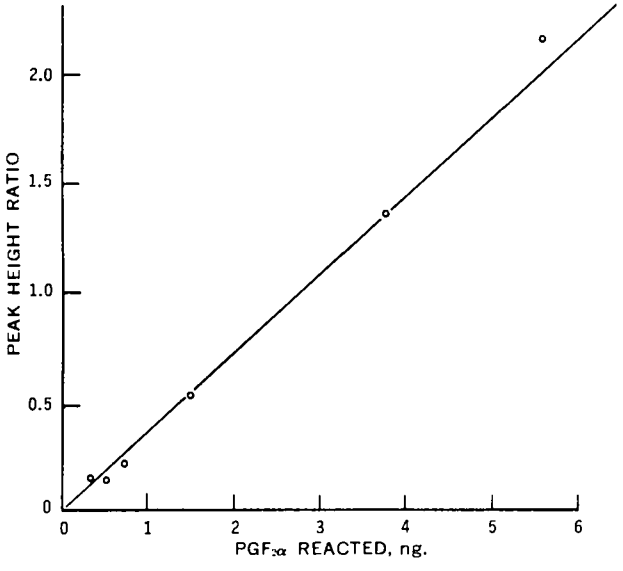


Figure 4—*Electron-capture GLC response of the tris(trimethylsilyl) $PGF_{2\alpha}$ pentafluorobenzyl ester* versus *quantity of $PGF_{2\alpha}$ (in nanograms) reacted (10% of each sample injected on-column).*

acid (III) proved to be the most suitable. This compound (IV*b*) had a retention time of 16.0 min. Compound II*b* had a retention time of 8.9 min. under the same GLC conditions on the first instrument[3]. The pentafluorobenzyl ester (IV*a*) was synthesized on a preparative scale by reaction of III (31.9 mg.) in acetonitrile with pentafluorobenzyl bromide and diisopropylethylamine (see *Experimental* section). The product was isolated from the acidified reaction mixture using ethyl acetate and was purified by sequential preparative GLC. Compound IV*b* was characterized by GLC–mass spectrometry (see *Experimental* section). Microgram and submicrogram scale syntheses of IV*a* were carried out by treatment with pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile in the manner described for the derivatization of $PGF_{2\alpha}$ (I). Excess reagents were removed by evaporation under nitrogen at room temperature. The pentafluorobenzyl ester product (IV*a*) was dissolved in the silylating agent *N,O*-bis-(trimethylsilyl)-acetamide and added to the $PGF_{2\alpha}$ pentafluorobenzyl ester (II*a*) prior to electron-capture GLC analysis. The electron-capture GLC response due to II*b* resulting from the submicrogram scale reaction of $PGF_{2\alpha}$ (I) was found to be linear over the concentration range of 0.2–5.6 ng. $PGF_{2\alpha}$ (Fig. 4) (determined on the first chromatograph[3]).

Thus, the pentafluorobenzyl ester of $PGF_{2\alpha}$ (II*a*) is easily formed, and the corresponding trimethylsilyl derivative (II*b*) allows quantitation of $PGF_{2\alpha}$ (I) in the picogram and nanogram range using electron-capture detection. The application of this methodology to the quantitation of $PGF_{2\alpha}$ in biological fluids is currently being developed.

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