Prostanoic Acid Chemistry

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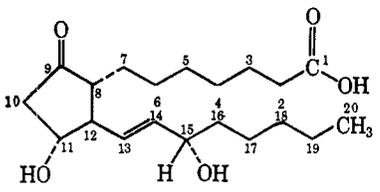
General chemical transformations of the prostaglandins are described on a preparative scale, and details are given for the chromatography and isolation of various derivatives of the prostanoic acids. Reductions of 9-oxo- and 15-oxoprostaglandins are described leading to both *R* and *S* isomers of the corresponding hydroxyls. Dehydration of the 11β-hydroxy-9-ketoprostanoic acids lead either with acid to the Δ^{10}-(PGA) unsaturated ketones or with base to $\Delta^{8(12)}$ (PGB) derivatives. 8-Isoprostaglandin E_1 obtained as a by-product from preparative biosynthesis can be isomerized under mild basic conditions in high yield to PGE_1; sodium borohydride reduction of 8-iso-PGE_1 leads to the corresponding two hydroxyl epimers at C-9. Selective protection of both the 9-oxo function and the carboxylic acid is described which allows regeneration of the functional groups under conditions which do not affect the other groups in the molecule.

The prostaglandins are a recently characterized group of naturally occurring oxygenated lipid acids with a remarkable range of biological properties and indications of a physiological role in several areas.[1] Typical members of the class are prostaglandin E_1 [15-(*S*)-11β,15-dihydroxy-9-oxoprost-13-*trans*-enoic acid or PGE_1] (**1**), prostaglandin $F_{1\alpha}$ (**2**), and prostaglandin E_2 (**4**). Several total synthetic approaches to these natural products have been recorded.[2,3] However, most of the elegant original work in Sweden by Bergstrom, Samuelsson, and coworkers on the structure elucidation was done on a microscale, especially employing gas chromatography and mass spectrometry, and little information is available on the general chemical transformations of these compounds on a preparative scale and the methods used in their handling and characterization. Now that preparative biosynthesis has made available larger quantities of the natural products,[4] a more extensive study of both the general chemistry and the physicoanalytical properties of the prostanoic acids is possible. A review which lists the spectral and related analytical properties of these compounds is available[5] and the present paper covers some aspects of their basic chemistry.



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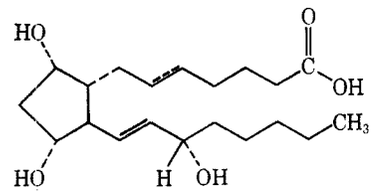
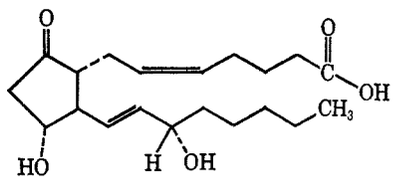


2, no 5,6 double bond
3, 5,6-*cis* bond present



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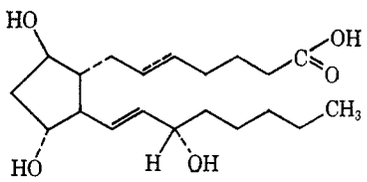


5, no 5,6 double bond
6, 5,6-*cis* double bond present

Reduction of PGE_1 and PGE_2 with sodium borohydride gives a mixture in both cases of the corresponding 9α- and 9β-hydroxy compounds, $PGF_{1\alpha}$ (**2**) and $PGF_{1\beta}$ (**5**) from PGE_1, and $PGF_{2\alpha}$ (**3**) and $PGF_{2\beta}$ (**6**) from PGE_2.[5] Experimental details are recorded here for the reductions on a preparative scale and the separation of the C-9 epimers. Noteworthy is the use of the more conventional acid-washed silica for the chromatographic separation in place of the reversed phase partition methods used in the earlier work.[6] It has been our experience that, although the reversed phase methods are excellent for small-scale separations, the use of silica gel or Amberlyst-15 (Ag^+ form)[4] is an improvement for larger amounts. In the case of both PGE_1 and PGE_2 the yields of the 9β-hydroxy isomers were somewhat greater than those of the natural 9α epimers.

Dehydration of both PGE_1 and PGE_2 with 90% acetic acid–water at 60° leads to the corresponding Δ^{10}-unsaturated ketones,[7] PGA_1 (**7**) and PGA_2 (**8**). These unsaturated ketones (earlier designated PGE_1-

(1) (a) S. Bergstrom, *Science*, **157**, 382 (1967); (b) "Nobel Symposium 2: Prostaglandins," S. Bergstrom and B. Samuelsson, Ed., Almqvist and Wiksell, Stockholm, and Interscience Publishers, Inc., New York, N. Y., 1967; (c) S. Bergstrom, L. A. Carlson, and J. R. Weeks, *Pharmacol. Rev.*, **20**, 1 (1968); (d) V. R. Pickles, *Biol. Rev.*, **42**, 614 (1967); (e) U. S. von Euler and R. Eliasson, "Prostaglandins. Medicinal Chemistry Monographs," Vol. 8, Academic Press, New York and London, 1967; (f) U. S. von Euler, *Clin. Pharmac. Ther.*, **9**, 228 (1968); (g) J. W. Hinman, *Bioscience*, **17**, 779 (1967).

(2) For a recent review, see U. F. Axen, "Annual Reports in Medicinal Chemistry," Vol. 3, 1968.

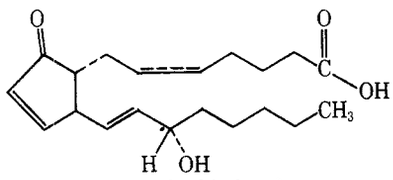
(3) E. J. Corey, N. H. Andersen, R. M. Carlson, J. Paust, E. Vedejs, I. Vlattas, and R. E. K. Winter, *J. Amer. Chem. Soc.*, **90**, 3245 (1968); E. J. Corey, I. Vlattas, N. H. Andersen, and K. Harding, *ibid.*, **90**, 3247 (1968).

(4) J. E. Pike, F. P. Kupiecki, and J. R. Weeks, ref 1b, p 161; E. G. Daniels and J. E. Pike, Proceedings of the Worcester Foundation Symposium on Prostaglandins, in press.

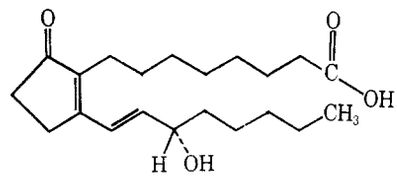
(5) P. W. Ramwell, J. E. Shaw, G. B. Clarke, M. F. Grostic, D. G. Kaiser, and J. E. Pike, *Progr. Chem. Fats Lipids*, **IX**, part 2, Chapter 7 (1968). For an earlier review of the chemistry, see B. Samuelsson, *Angew Chem. Intern. Ed. Engl.*, **4**, 410 (1965).

(6) S. Bergstrom, L. Krabisch, B. Samuelsson, and J. Sjovall, *Acta Chem. Scand.*, **16**, 969 (1962).

(7) E. G. Daniels, J. W. Hinman, B. A. Johnson, F. P. Kupiecki, J. W. Nelson, and J. E. Pike, *Biochem. Biophys. Res. Commun.*, **21**, 413 (1965).



7, no 5,6 double bond
8, 5,6-*cis* double bond present



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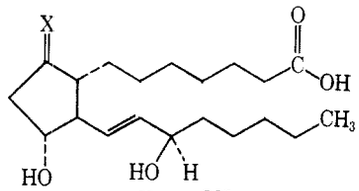
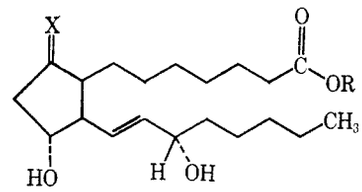
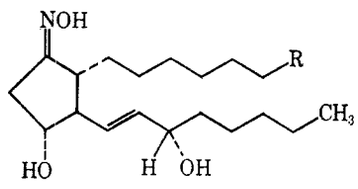


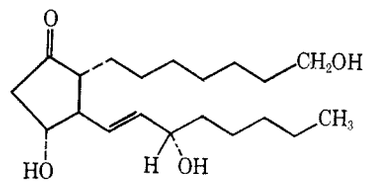
10, X = α-OH
11, X = ketone



12, X = ketone; R = H or CH_3
13, X = α-OH
14, X = α-OH



15, R = CO_2H
16, R = CH_2OH



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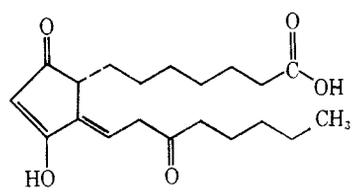

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217 and PGE_2-217 based on their uv absorption) are of special interest as they have been identified as natural products in human seminal plasma where it is thought they serve as substrates for a 19-hydroxylation enzyme.[8] Treatment of PGE_1 with sodium hydroxide gives the conjugated unsaturated ketone PGB_1[9] (**9**).

Oxidation of $PGF_{1\alpha}$ or PGE_1 with manganese dioxide gives the corresponding 15-ketones, selective reaction occurring with the allylic hydroxyl.[10] Reduction of 9α,11α-dihydroxy-15-oxoprost-13-*trans*-enoic acid (15-keto-$PGF_{1\alpha}$) with sodium borohydride gave two compounds the more polar of which (by tlc) was identical with $PGF_{1\alpha}$ [15-(*S*) configuration] and the less polar is assigned the 15-(*R*) structure (**10**) (or 15-epi-$PGF_{1\alpha}$). The 15-(*R*) compound was also obtained as a minor product (30% yield) when $PGF_{1\alpha}$ was treated with formic acid–sodium formate at room temperature followed by base treatment, isomerization presumably occurring through the intermediate allyl cation. The mass spectrum of the 15-(*R*)-$PGF_{1\alpha}$ was identical with that of natural $PGF_{1\alpha}$ but some minor differences were found in the nmr spectra. A similar treatment of PGE_1 with formic acid–sodium formate at 25° for 2 hr gave 15-(*R*)-PGE_1 (**11**) in 17% yield together with 52% of recovered PGE_1; again formate esters were hydrolyzed by treatment of the total product with sodium bicarbonate. When the experiment was run and the mild base treatment omitted a reasonable yield (*ca.* 40%) of the 15-formate of PGE_1 was obtained indicating some selectivity in the acylation of the allylic hydroxyl. Treatment of the 15-(*R*)-PGE_1 with 1 *N* sodium hydroxide at 30–40° for 1 hr gave 15-(*R*)-PGB_1, which had an optical rotatory dispersion curve which was the mirror image of that of the epimeric 15-(*S*)-PGB_1.

Chromatography of the mother liquors from the crystallization of PGE_1 obtained by preparative biosynthesis[4] gave a new crystalline prostaglandin, mp 87–88° with analytical properties consistent with the structure of 8-iso-PGE_1[11] (**12**, R = H). In particular the optical rotatory dispersion curve of **12** (R = H) was nearly the mirror image of that of PGE_1. The nmr spectrum showed differences in the absorptions associated with the 13,14-vinyl protons and the 8 and 12 protons which were consistent with the *cis* structure of the two side chains in **12**. Treatment of 8-iso-PGE_1 with sodium acetate in ethanol at room temperature for 5 days gave after recrystallization a 70% yield of PGE_1. The ratio of PGE_1 and 8-iso-PGE_1 present under equilibrating conditions has been determined as about 85:15, and the isolation of the *cis* isomer (**12**, R = H) reflects this relative stability rather than direct enzy-

(8) M. Hamberg and B. Samuelsson, ref 1b.
(9) S. Bergstrom, R. Ryhage, B. Samuelsson, and J. Sjovall, *J. Biol. Chem.*, **238**, 3555 (1963).
(10) E. Anggard and B. Samuelsson, *J. Biol. Chem.*, **239**, 4097 (1964).
(11) E. G. Daniels, W. E. Krueger, F. P. Kupiecki, J. E. Pike, and W. P. Schneider, *J. Amer. Chem. Soc.*, **90**, 5894 (1968).

matic formation of 8-iso-PGE_1. Reduction of **12** (R = CH_3) with sodium borohydride gave the two alcohols, **13** (R = CH_3) and **14** (R = CH_3), epimeric at C-9.

Conversion of PGE_1 to the 9-oxime offered a suitable method for protection of the ketonic function since regeneration of PGE_1 was possible by treatment of the oxime with nitrous acid, prepared *in situ* from sodium nitrite and acetic acid. An instance of the use of this protecting group was the preparation of the 1-alcohol (**17**) ("PGE_1-alcohol"). Reduction of the oxime (**15**) with lithium aluminum hydride gave the corresponding oxime alcohol (**16**) which could be converted into **17**, mp 106–108°, by treatment with nitrous acid. The preparation of 1-alcohols in the F series has been described earlier.[4,12]

A protective group for the carboxylic acid was needed in connection with studies on the total synthesis.[13] Preparation of the trichlorethyl ester[14] of PGE_1 proved suitable and this ester could be reconverted to the parent acid using zinc dust in acetic acid at room temperature. A comparable conversion of 8-iso-PGE_1 to the corresponding trichloroethyl ester and hydrolysis with zinc dust–acetic acid to 8-iso-PGE_1 was also effected.

Oxidation of PGE_1 with chromium trioxide (Jones reagent) gave the triketo acid (**18**). The structure of **18** was established mainly by the uv spectrum and nmr spectrum.

Experimental Section

Ir spectra were recorded with a Perkin-Elmer Model 221 IR spectrometer on Nujol mulls or on methylene chloride solutions.

The nmr spectra were run on a Varian A-60 spectrophotometer operating at 60 Mc, and employing tetramethylsilane as an internal standard.

Mass spectra were recorded on an Atlas CH-4 instrument equipped with a TO-4 source (ionization voltage 70 eV).

Uv spectra were taken on 95% ethanol solutions using a Cary Model 14 spectrophotometer.

We are grateful to Dr. W. A. Struck and associates for the analytical data, to Dr. M. F. Grostic for the mass spectra.

Reduction of PGE_1. Preparation of $PGF_{1\alpha}$ (2) and $PGF_{1\beta}$ (5).—An ice-cold solution of 300 mg of PGE_1 in 30 ml of methanol was treated portionwise during 2–3 min with a partial solution of 900 mg of sodium borohydride in 105 ml of cold methanol. After 20 min in the ice bath and 1 hr at room temperature the solution was concentrated, diluted with water, acidified, and extracted with ether. Evaporation of the washed and dried extract afforded 0.26 g of a partially crystalline mixture of $PGF_{1\alpha}$ and $PGF_{1\beta}$, which were separated first by reverse phase chromatography over siliconized Celite (Gas-Chrom CLZ 100–120 mesh with mobile and stationary phase composed of the upper and lower phase respectively derived from 516 ml of methanol, 684 ml of water, 60 ml of isooctanol, and 60 ml of chloroform), and then the still mixed fractions were separated by standard chromatography over acid-washed silica gel using ethyl acetate and ethyl acetate containing 2% methanol and 1% acetic acid elution. $PGF_{1\beta}$ is the least polar on the reverse phase column while $PGF_{1\alpha}$ is eluted first from the standard column. The β epimer predominated slightly. After two crystallizations from ethyl acetate–Skellysolve B the $PGF_{1\alpha}$ had mp 101–103° and $[\alpha]^{25}D$ (EtOH) +30°; the β epimer had mp 127–130° and $[\alpha]^{25}D$ (EtOH) −20°; for ir and nmr, see ref 5.

$PGF_{1\alpha}$—*Anal.* Calcd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.30; H, 9.92.

$PGF_{1\beta}$—*Anal.* Calcd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.04; H, 10.39.

Preparation of Triiodobenzoates.—A mixture of 50 mg of $PGF_{1\alpha}$ methyl ester, 3 ml of pyridine and 170 mg (5 equiv) of *p*-iodobenzoyl chloride was heated under reflux in a nitrogen atmosphere for 3 hr. Most of the excess pyridine was evaporated under a stream of nitrogen, the residue slurried with methylene chloride and filtered. The filtrate was concentrated somewhat and then poured directly over a column of 15 g of Florisil. The column was eluted with 5% acetone in Skellysolve B to give in the early fractions 53 mg of oily product. Crystallization (twice) from methanol gave powdery white crystals: mp 81–83°; ir (CH_2Cl_2) no OH, C=O (1730), C=C (1595); tlc R_f 0.58 on silica gel plate, development with 20% ethyl acetate in cyclohexane.

Anal. Calcd for $C_{42}H_{47}O_8I_3$: C, 47.56; H, 4.47; I, 35.90. Found: C, 47.93; H, 4.80; I, 34.68.

A mixture of 50 mg of $PGF_{1\beta}$ methyl ester, 3 ml of pyridine and 170 mg (5 equiv) of *p*-iodobenzoyl chloride was treated exactly as for the $PGF_{1\alpha}$ epimer. Chromatography gave 87 mg of product which was crystallized from methanol to afford silky needles: mp 82–83°; ir (CH_2Cl_2) no OH, C=O (1735), C=C (1600); tlc R_f 0.71 on silica gel plate, 20% ethyl acetate in cyclohexane development.

Anal. Calcd for $C_{42}H_{47}O_8I_3$: C, 47.56; H, 4.47; I, 35.90. Found: C, 47.67; H, 4.82; I, 36.20.

Preparation of Methyl Esters.—A mixture of 60 mg of $PGF_{1\beta}$, 5 ml of methylene chloride, 0.5 ml of methanol and an excess of ethereal diazomethane was allowed to stand 15 min, then evaporated. The crystalline residue was recrystallized from ether to yield leaflets, mp 107–108°.

Anal. Calcd for $C_{21}H_{38}O_5$: C, 68.07; H, 10.34. Found: C, 67.86; H, 10.31.

In the same manner $PGF_{1\alpha}$ methyl ester was obtained as silky needles, mp 49–50°.

Reduction of PGE_2. Preparation of $PGF_{2\alpha}$ (3) and $PGF_{2\beta}$ (6).—The reduction of 4.00 g of PGE_2 was carried out as described above for PGE_1 to afford a partially crystalline mixture of $PGF_{2\alpha}$ and $PGF_{2\beta}$. Recrystallization from ethyl acetate gave 2.8 g of a β rich fraction (crystals) and 1.2 g of an α rich fraction (filtrate). Each fraction was chromatographed separately over acid-washed silica gel. $PGF_{2\alpha}$ was eluted with ethyl acetate and ethyl acetate containing 2% methanol and 1% acetic acid. $PGF_{2\beta}$ was eluted with ethyl acetate containing 4% methanol and 2% acetic acid. In this manner 1.77 g of $PGF_{2\alpha}$, 0.12 g of mixture and 2.10 g of $PGF_{2\beta}$ was obtained. The materials were seen as separate distinct spots on silica gel tlc using system AIX[15] (2X) for development. The α epimer moved slightly faster. $PGF_{2\alpha}$ was a viscous colorless oil which could be made to crystallize with difficulty from ether, mp 30–35°. Analytical and spectral data were obtained on the oil: ir (neat) 3320, 2640, 1710, 1295, 1260, 1245, 1120, 1080, 1055, 1025 and 975 cm^{-1}; nmr (d_6 acetone) 4 H multiplet δ 5.48 (olefinic protons), 4 H singlet 5.12 (hydroxyl and carboxyl protons), 3 H multiplet 4.05 (carbinolic protons), 3 H distorted triplet 0.9 (terminal CH_3); mass spectrum 336 (M − 18), 318 (M − 36), 264 (M − 18 − 72).

Anal. Calcd for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67. Found: C, 67.48; H, 9.76.

$PGF_{2\beta}$ formed colorless prisms from ethyl acetate: mp 96.5–97°; ir (Nujol) 3440, 3260, 3220, 2720, 2660, 2600, 1697, 1275, 1250, 1200, 1040, 977, and 968 cm^{-1}; nmr (d_6 acetone) 4 H multiplet δ 5.5 (olefinic protons), 4 H singlet 5.25 (hydroxyl and carboxyl protons), 3 H multiplet 4.0 (carbinolic protons), 3 H distorted triplet 0.9 (terminal CH_3); mass spectrum 336 (M − 18), 318 (M − 36), 300 (M − 54), 264 (M − 18 − 72); $[\alpha]^{25}D$ −4° (95% ethanol).

Anal. Calcd for $C_{20}H_{34}O_5$: C, 67.76; H, 9.67. Found: C, 67.63; H, 9.49.

Synthesis of 15-(*S*)-15-Hydroxy-9-oxoprosta-10,13-*trans*-dienoic Acid (PGA_1) (7).—Prostaglandin E_1 (3.0 g) was dissolved in 90 ml of glacial acetic acid, and then 10 ml of water was added to this solution. The mixture was then heated at 60° under nitrogen for 18 hr. At the end of this time, tlc on silica gel (AIX system)[15] indicated that no PGE_1 remained and that conversion to PGA_1 was essentially complete. The solvent was then removed *in vacuo* at <60° and the residue was dissolved in 20% ethyl acetate–cyclohexane together with methylene chloride and applied to a column of 300 g of acid-washed silica gel made up in

(12) H. J. J. Pabon, L. VanderWolf, and D. A. vanDorp, *Rec. Trav. Chim. Pays-Bas*, **85**, 1251 (1966).

(13) W. P. Schneider, U. F. Axen, F. H. Lincoln, J. E. Pike, and J. L. Thompson, *J. Amer. Chem. Soc.*, **91**, 5372 (1969).

(14) R. B. Woodward, *et al.*, *ibid.*, **88**, 852 (1966).

(15) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **241**, 257 (1965).

20% ethyl acetate–cyclohexane. Elution was effected with increasing percentages of ethyl acetate in cyclohexane, and finally with ethyl acetate. The following 75-ml fractions were obtained: fractions 1–74, 0.174 g; 75–115, 0.59 g. This product was assigned the structure PGA_1 15-acetate. The mass spectrum of the methyl ester was identical with that of an authentic sample prepared from PGA_1. The nmr spectrum showed two protons, each doublets of doublets, at δ 7.5 and 6.2 (10 and 11 vinylic protons); two protons, multiplet at 5.6 (13 and 14 vinylic protons); one-proton multiplet at 5.25 (15 proton showing downfield shift due to acetylation at 15); and strong absorption singlet at 2.1 (acetate methyl group).

Fractions 116–124 weighed 0.110 g, a mixture of PGA_1 and the 15-acetate. Fractions 125–150 weighed 1.68 g, PGA_1. Crystallization of a portion of this twice from ethyl acetate–pentane gave material: mp 42–44°; uv: λ_{max}^{EtOH} 217 mμ (ϵ 11,650); ν_{max}^{Nujol} 3420, 2740, 2700, 2660, 2600, 1715, 1700, 1585, 1275, 1200, 1180, 1020, 720 cm^{-1}. The nmr spectrum in $CDCl_3$ showed absorption peaks at δ 6.17 and 7.52 (10,11 vinyl H's, both doublets of doublets), at 5.6 (13,14 vinyl H's), at 4.1 (15 H) and 3.25 (12 H). The mass spectrum of the acid supported the assigned structure (7) [*m/e* 336 (M^+), 318 (M − 18), 300 (M − 36), 265 (M − 71, C_5H_{11}), 247, 219, 190].

Anal. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.11; H, 9.64.

In a later, similar conversion the total PGA_1 fraction (1.66 g) was crystallized from ethyl acetate–pentane to give 530 mg of crystalline PGA_1, mp 42–44°.

15-(*S*)-Hydroxy-9-oxoprosta-5-*cis*-10,13-*trans*-trienoic Acid (PGA_2) (8).—Prostaglandin E_2 (0.158 g) was dissolved in 9 ml of acetic acid and 1 ml of water added. The reaction mixture was heated at 65° under nitrogen for 18 hr. After cooling the solvent was removed *in vacuo* at <60° and the residue was partitioned between ether and water. The ether layer was dried sodium sulfate) and evaporated to give 135 mg of crude PGA_2. This material (130 mg) was applied to a 20-g column of acid-washed silica gel made up in 20% ethyl acetate–cyclohexane. Elution with increasing percentages of ethyl acetate in cyclohexane gave 60 mg of material which was essentially one spot by tlc on silica gel and on $AgNO_3$–silica gel GF_{254} (E. Merck Darmstadt incorporating a phosphor) (AIX system).[15] Final purification of this material was accomplished first, by applying 15 mg to each of four $AgNO_3$–silica gel plates (8 in. × 8 in.) and using the AIX system for elution (ascending). The PGA_2 was localized both by uv and spraying (vanillin–phosphoric acid)[6] a standard of PGA_1 and PGA_2 on the two edges of the plate. The silica with the correct R_f was collected, extracted with methanol and the insoluble material removed by filtration. The residue was then partitioned between ether and water, the aqueous layer was acidified to pH 2, and after separation of the organic layer and drying ($MgSO_4$) the solvent was removed to give 42 mg. A final purification of 20 mg of this by a similar preparative tlc gave 12 mg of PGA_2.

In a later run the total product (0.164 g) was purified directly by preparative tlc on $AgNO_3$–silica gel, omitting the column chromatography, to give 78 mg. Finally 40 mg of this was purified as before by tlc on ordinary silica gel (AIX) to give 20 mg of PGA_2.

The uv spectrum revealed λ_{max}^{EtOH} 217 mμ (ϵ 9900). The nmr spectrum in $CDCl_3$ confirmed the structure; principal absorption peaks were seen at δ 6.17 and 7.52 (10,11 vinyl H's), 5.2–5.7 (5,6 and 13,14 vinyl H's), 4.1 (15 H), and 3.26 (12 H). The R_f on silica gel thin layer chromatography of both PGA_1 and PGA_2 using the AIX system was 0.61; on $AgNO_3$–silica gel tlc the R_f's of PGA_1 and PGA_2 were, respectively, 0.40 and 0.29.

The mass spectrum of PGA_2 showed peaks at *m/e* 334 (M^+); *m/e* 316 (M − 18); and *m/e* 190.

The ir spectrum showed λ_{max}^{Nujol} 3400, 1705, 1580, 1255, 1115, 1070, 1015 cm^{-1}.

9α,11α-Dihydroxy-15-oxoprost-13-*trans*-enoic Acid.—A solution of 500 mg of $PGF_{1\alpha}$ in chloroform (400 ml) was stirred for 3 days at room temperature with 5.0 g of activated manganese dioxide. At the end of this period the insoluble material was removed by filtration through Celite, and the collected solid washed thoroughly with hot methanol–ethyl acetate. The solvent was removed *in vacuo* and the residue (0.421 g) chromatographed on 50 g of acid-washed silica made up in ethyl acetate. Elution with increasing percentages of methanol in ethyl acetate, and analysis of the fractions by tlc gave 0.18 g of the desired 15-ketone and fractions containing recovered $F_{1\alpha}$ mixed with the 15-ketone. The 15-ketone was less polar than $F_{1\alpha}$ by tlc: ir ν_{max}^{Nujol} 3380, 1690, 1665, 1615 cm^{-1}; uv λ_{max}^{EtOH} 233 mμ (ϵ 10,950).

Anal. Mass calcd for $C_{20}H_{34}O_5$: 368.2562. Found: 368.2573.

A sample of 15-(*R*)-$PGF_{1\alpha}$ methyl ester (32 mg) was shaken in 3 ml of ethyl acetate with 600 mg of activated manganese dioxide overnight at 25°. The sample was filtered, washed with not ethyl acetate, and evaporated. Chromatography on 5 g silica gel and elution with 50% and 100% ethyl acetate–cyclohexane gave a major fraction, 10 mg, λ_{max} 235 mμ.

15-(*R*)-$PGF_{1\alpha}$ or 15-(*R*)-9α,11α,15-Trihydroxyprost-13-*trans*-enoic Acid (10).—A solution was prepared of 32 mg of 15-keto-$PGF_{1\alpha}$ in 10 ml methanol. After cooling in an ice bath a solution of 90 mg of sodium borohydride in 10.5 ml of cold methanol was added with stirring in portions. After 45 min at 0° another 90 mg of sodium borohydride in 10.5 ml methanol was added to the solution and the reaction was allowed to proceed in the cold for 10 min and then at room temperature for 30 min. The mixture was then concentrated to about two-thirds volume and then additional water was added and the concentration continued until all the methanol had been removed. After acidification with cold dilute hydrochloric acid, the mixture was extracted three times with ether, and the organic extracts were washed with water until neutral. The extracts were dried ($MgSO_4$) and evaporated; a tlc of the total product showed two spots both more polar than the starting 15-ketone, one of which had the same R_f as $PGF_{1\alpha}$. A combined crude sample of the total product from two similar runs (30 mg and 32 mg of starting material) was chromatographed on 15 g of acid-washed silica gel made up in ethyl acetate. Elution with increasing percentages of methanol in ethyl acetate gave two main peaks from the chromatogram. The first (19 mg) which was less polar is assigned the 15-(*R*) $PGF_{1\alpha}$ structure. The second (22 mg) was more polar and has the same R_f as $PGF_{1\alpha}$. The 15-(*R*)-$PGF_{1\alpha}$ was crystallized from ether, mp 67.5–69°.

Anal. Mass Calcd for $C_{20}H_{32}O_3$ ($M^+ - 2H_2O$): 320.2351. Found: 320.2332.

These two isomers were further purified by preparative tlc on silica gel. A portion of the less polar or 15-(*R*)-$F_{1\alpha}$ (8 mg) was converted to the methyl ester with diazomethane and applied to a 4 in. × 8 in. silica plate. The plate was developed with ethyl acetate with $F_{1\alpha}$ methyl ester as a standard on the edge of the plate. Visualization of the plate on the edges was accomplished by spraying with vanillin–phosphoric acid. The 15-(*R*)-$F_{1\alpha}$ methyl ester was eluted from the silica with methanol, and the insoluble material was removed by filtration. After removal of the methanol *in vacuo* the residue was taken up in chloroform. Evaporation gave a pure sample of 15-(*R*)-$PGF_{1\alpha}$ methyl ester. The mass spectrum of this ester was the same as that of $PGF_{1\alpha}$ methyl ester: *m/e* 370 (M^+), *m/e* 352 (M − 18), *m/e* 334 (M − 2 × 18), *m/e* 280 (M − 18 − 72, C_5H_{12}).

Preparation of 15-(*R*)-Prostaglandin $F_{1\alpha}$ (10) from $PGF_{1\alpha}$.—To 200 mg of prostaglandin $F_{1\alpha}$ was added a solution of 120 mg sodium carbonate in 20 ml ice-cold formic acid (100%). After stirring for about 15 min at 0°, the mixture was warmed to room temperature and stirred for 2 hr. The formic acid was removed *in vacuo;* the residue was extracted with ethyl acetate, which was washed with water, saturated salt, dried with sodium sulfate, and evaporated. To this residue was added 30 ml methanol and 30 ml of 1 *N* sodium hydroxide (aqueous). This was stirred 2 hr at room temperature to hydrolyze formate esters. It was diluted with water, acidified to pH 2 with hydrochloric acid, and concentrated *in vacuo* to remove methanol. The products were extracted with ethyl acetate, which was washed with saturated salt, dried with sodium sulfate, and evaporated. The residue was chromatographed on 40 g of acid-washed silica gel and eluted with 300 ml each of 50% ethyl acetate–cyclohexane, ethyl acetate, 2% methanol–ethyl acetate, and 2% methanol with 2% acetic acid in ethyl acetate. Fifty-milliliter fractions were collected.

Fractions 11–13 contained 18 mg of oily material, λ_{max} 235 mμ, which was not further investigated. Fractions 15 and 16 contained 60 mg of crystalline material, identical in thin layer behavior with the 15-(*R*)-$PGF_{1\alpha}$ from the preceeding experiment, mp about 63°. This was recrystallized from ether to give material, mp 67.5–69°.

Anal. Calcd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 66.99; H, 10.57.

The nmr spectrum in d_6 acetone showed protons at δ 5.6, multiplet, two protons at C-13, C-14; 5.1, multiplet, four

hydroxylic protons; 4.2, multiplet, three carbinol protons at C-9, C-11, and C-15.

The ir spectrum indicated hydroxylic and carboxylic absorptions, and a band at 970 cm^{-1} indicative of a *trans* double bond.

The methyl ester of the product (prepared with ethereal diazomethane) moved faster on thin layer chromatograms than $PGF_{1\alpha}$ methyl ester.

Fractions 18–23 of the above chromatogram contained 95 mg of crystalline $PGF_{1\alpha}$ (47% recovery), identified by thin layer chromatography and ir spectrum.

15-(*R*)-Prostaglandin E_1 or (15*R*)-11α,15-Dihydroxy-9-oxo-prost-*trans*-13-enoic Acid (11).—To 500 mg prostaglandin E_1 in a flask under nitrogen was added a nitrogen-purged solution of 300 mg sodium bicarbonate in 15 ml formic acid (mp 7°). The solution was stirred at 25° 2 hr, and then evaporated with a vacuum pump. Benzene was added and removed *in vacuo* to complete removal of formic acid. To the residue was added 25 ml methanol and 5 ml saturated aqueous sodium bicarbonate. This was stirred at 25° 1 hr under nitrogen, then stored in the refrigerator overnight. It was concentrated *in vacuo*, water was added, and the pH adjusted to 2–3. The product was extracted with ethyl acetate, which was washed, dried, and evaporated. The residue showed thin layer spots (AIX system)[15] corresponding to E_1, 15-(*R*)-E_1, PGA_1 and a spot slightly less polar than this, probably 15-(*R*)-PGA_1. It was chromatographed on 100 g of acid-washed silica gel and eluted with 3 l. of 25–100% ethyl acetate–Skellysolve B.

The first peak eluted, 129 mg total, consisted of the mixture of 15-(*R*)- and 15-(*S*)-PGA_1, which were not well separated. A small amount (*ca.* 5%) of PGB_1 was also evident in the uv spectrum.

The second peak eluted, 84 mg, consisted of 15-(*R*)-PGE_1.

Anal. Calcd for $C_{20}H_{34}O_4$ ($M^+ - 18$): mass, 336.2300. Found: mass, 336.2263.

The third peak eluted, 259 mg, was crystalline PGE_1.

This experiment yielded 25.8% mixed PGA_1 and 15-(*R*)-PGA_1, 16.8% 15-(*R*) PGE_1, 6.8% mixed PGE_1 and 15-(*R*)-PGE_1, and 51.8% PGE_1.

15-(*R*)-Prostaglandin B_1.—A mixture of 11 mg of 15-(*R*)-PGE_1 in 4 ml 95% ethanol and 4 ml 1 *N* sodium hydroxide was stirred under nitrogen at 30–40° for 1 hr. It was concentrated *in vacuo* to remove ethanol, acidified with 5 ml 1 *N* hydrochloric acid and extracted with ethyl acetate. The extracts were washed, dried, evaporated, and chromatographed on 3 g acid-washed silica gel. Elution was with 20, 30, 40, 50, and 100% ethyl acetate–cyclohexane. Fractions 4 and 5 contained 6 mg of noncrystalline material showing a uv peak at 278 mμ, and moving on thin layer plates like PGB_1. The optical rotary dispersion curve showed a negative Cotton-effect curve which was the mirror image of that of PGB_1. The sample was then converted to its methyl ester with diazomethane. The mass spectrum of the methyl ester was consistent with the PGB_1 structure, showing a molecular ion at 350, and also 332 ($M - 18$); 301 ($M - 31 + 18$); 25 ($M - 99$); 247 ($M - 103$); 220 ($251 - 31$); 219 ($251 - 32$), etc.

Prostaglandin E_1 15-Formate and 15-(*R*)-Prostaglandin E_1 15-Formate.—To 250 mg of prostaglandin E_1 was added a solution of 50 mg sodium carbonate in 7.5 ml of dry formic acid. This was stirred under nitrogen at 25° for 2 hr, and the formic acid was then removed with a vacuum pump. Benzene was added and removed *in vacuo* to complete removal of formic acid. The residue was chromatographed on 50 g of acid-washed silica gel, eluting with a gradient of 2.5 l. of 25 to 75% ethyl acetate–Skelly B, collecting 100-ml fractions. Fractions 3 and 4, (56 mg) contained a noncrystalline 15-formate of PGA_1, showing nmr peaks at δ 8.2 (one proton, formate ester), doublet of doublets at 7.55 and 6.2 (two conjugated olefinic protons at C 10,11), multiplet at 5.7 (two 13,14 vinyl protons), multiplet at 5.4 (15-carbinol proton of formate ester), and 3.25 (the diallylic 12 proton ?). This is probably a mixture of 15 epimers. Fractions 7 and 8 contained 68 mg of a formate ester which moved slightly faster on tlc plates than the 15-formate of PGE_1 described below, and consisted of the 15-formate of 15-(*R*)-PGE_1.

Fractions 9–11 contained 99 mg of the 15-formate of PGE_1. The nmr spectrum had peaks at δ 8.15 (one formate proton), 6.1 (the carboxyl and hydroxyl protons), 5.75 (two vinyl protons), 5.45 (15-carbinol proton of the formate ester), and 4.15 (11-carbinol proton). When a small sample of this material was dissolved in methanol and a very small crystal of *p*-toluenesulfonic acid was added, hydrolysis of the formate ester occurred, giving a large spot on tlc corresponding to PGE_1 in mobility and color with vanillin–phosphoric acid spray.[5]

Hydrolysis of Prostaglandin E_1 15-Formates.—A solution of 100 mg of a mixture of 15-(*R*)- and 15-(*S*)-prostaglandin E_1 15-formates (prepared as above but incompletely separated by chromatography) was stirred 2.5 hr under nitrogen in 10 ml of methanol with 2.5 ml of saturated aqueous sodium bicarbonate. Then 5 ml of water and 2 ml of 1 *N* hydrochloric acid was added, and the methanol was removed *in vacuo*. The aqueous residue was adjusted to pH 2–3 and extracted with ethyl acetate. The extracts were washed, dried, evaporated, and the residue chromatographed on 20 g of acid-washed silica gel, eluting with 60, 80, and 100% ethyl acetate–cyclohexane, and 5% methanol–ethyl acetate. Three peaks were eluted. Fractions 5 and 6 (6 mg) had a uv peak at 218 mμ, and evidently consist of PGA-like materials.

Fractions 13–17, (13 mg), noncrystalline, consisted of 15-(*R*)-PGE_1. The nmr spectrum contained peaks at δ 5.7 (two olefinic protons), 5.35 (three hydroxylic and carboxylic protons), 4.15 multiplet (two carbinol protons), quite similar to that of PGE_1.

PGE_1 Trichloroethyl Ester.—To a stirred mixture of 5 ml methylene chloride, 1 ml trichloroethanol, and 0.5 ml pyridine was added 100 mg of PGE_1 and 100 mg dicyclohexylcarbodiimide, while stirring at room temperature. After 1 hr most of the PGE_1 had disappeared (as judged by tlc) and was replaced by a less polar material. After 2 hr, the whole reaction mixture was poured onto a column of 100 g silica gel and eluted with 1400 ml of 20–50% ethyl acetate–Skellysolve B.

Fractions 32 and 33, eluted with 100% ethyl acetate, 138 mg, had ester absorption (1760 cm^{-1}) as well as OH (3350 cm^{-1}) and two broad peaks at 805 and 725 cm^{-1} indicative of the trichloroethyl group in the ir. The nmr spectrum showed two olefinic protons at 335 cps, two protons of the trichloroethyl group at 284, two protons on carbon bearing OH at 242, two hydroxyl protons (removed by D_2O at 219) and about six protons either allylic or α to a carbonyl group.

Regeneration of PGE_1 from Its Trichloroethyl Ester.—PGE_1 trichloroethyl ester (fractions 32 and 33) from the above experiment, 130 mg, in 5 ml 90% aqueous acetic acid was cooled in an ice bath and 750 mg of zinc dust was added. The mixture was stirred magnetically in the cold room (5°) for 18 hr (work-up after 2.3 and 5 hr had shown that much starting trichloroethyl ester was remaining). The mixture was filtered and washed well with ethyl acetate, and the filtrate was washed well with dilute hydrochloric acid, water, and saturated salt, dried, and evaporated. Thin layer chromatography showed no ester remaining, and the residue crystallized from ethyl acetate–Skellysolve B to obtain 67 mg of colorless prisms, mp 108–112° (Kofler), whose ir spectrum (Nujol mull) was identical with that of an authentic sample of PGE_1.

8-Iso-PGE_1-trichloroethyl Ester.—To 20 mg of 8-iso-PGE_1 in 2 ml of methylene chloride was added 0.33 ml of trichloroethanol, 0.18 ml of pyridine, and 50 mg of dicyclohexylcarbodiimide. After stirring under nitrogen at 25° for 2 hr, the reaction mixture was poured onto a dry column of 10 g of silica gel. Elution with 50 ml each of 25, 40, 55, 75, and 100% ethyl acetate–Skelly B mixtures and collection of 10-ml fractions gave in 18–26, 26 mg of noncrystalline material. The nmr spectrum of this was consistent with the formulation as the trichloroethyl ester of 8-iso-PGE_1. The 13 and 14 olefinic protons showed the characteristic overlapping quartets of 8-iso-PGE_1 centered at δ 5.6 and 5.4, respectively, a two-proton singlet at 4.75 for the protons of the trichloroethyl group, and two protons as multiplets between 4.0 and 4.5, representing the C-11 and C-15 protons.

Conversion of 8-Iso-PGE_1-trichloroethyl Ester into 8-Iso-PGE_1.—The above 26 mg of 8-iso-PGE_1-trichloroethyl ester was dissolved in 1 ml of 90% acetic acid and stirred at 25° with about 50 mg of zinc dust for 2 hr. Ethyl acetate was added, and the solution decanted into a separatory funnel. It was washed several times with water containing a little 1 *N* hydrochloric acid, then with saturated salt, dried with sodium sulfate, and evaporated to leave a residue, 15 mg. This, by tlc (AIX system), consisted largely of 8-iso-PGE_1, with a faint spot corresponding in mobility to PGE_1. Recrystallization from ethyl acetate–Skelly B gave 8-iso-PGE_1 partially melting at 65–70°, partially resolidifying, and remelting at 87–88°, mixture melting point undepressed by authentic 8-iso-PGE_1. The nmr spectrum was identical with that of the natural material. None of the crystals was observed on the Kofler hot stage to melt around 110°, as would be characteristic of PGE_1.

Prostaglandin E_1 Oxime (15).—A solution of hydroxylamine hydrochloride (0.2 g) and sodium acetate (0.25 g) in 4 ml of aqueous methanol (1:1) was added to a solution of 0.2 g of prostaglandin E_1 in 2 ml of methanol and the reaction allowed to proceed at room temperature for 18 hr. At the end of this time the methanol was removed at room temperature in a nitrogen stream. Further water was added and the crystalline solid, which formed, was collected by filtration, washed with water, and dried *in vacuo* to give **4**, 0.19 g. Crystallization from aqueous methanol gave 0.15 g, mp 122–124° (sintered at 113°). A mixture melting point with PGE_1 was depressed to 91–92° (PGE_1 mp 115°). On tlc (AIX) system the oxime was less polar than PGE_1.

Anal. Calcd for $C_{20}H_{35}O_5N$: C, 65.01; H, 9.51; N, 3.79. Found: C, 64.87; H, 9.68; N, 3.74.

The uv showed only end absorption. The ir showed ν_{max}^{Nujol} 3420, 3340, 3080, 3030, 2750, 2680, 2560, 1715, 1665, 1255, 1240, 1230, 1065, 945 cm^{-1}.

15-(*S*)-1,11α,15-Trihydroxy-9-oxo-13-*trans*-prostene Oxime (16).—A solution of PGE_1 oxime (250 mg) in dry tetrahydrofuran (17 ml) was added to a stirred suspension of lithium aluminum hydride (1.2 g) in ether (119 ml) under nitrogen. After 2 hr at room temperature the excess $LiAlH_4$ was decomposed by the successive addition of 50 ml ethyl acetate and water. After filtration the organic layer was washed with water and dried (Na_2SO_4) and the solvent removed *in vacuo*. A crystalline residue was obtained (248 mg) which was triturated with ether and crystallized from ethyl acetate–Skellysolve to give the alcohol, 70 mg, mp 118–124°.

Anal. Calcd for $C_{20}H_{37}O_4N$: C, 67.57; H, 10.49; N, 3.94. Found: C, 66.83; H, 10.44; N, 3.40.

"Prostaglandin E_1 Alcohol," or 15-(*S*)-1,11α,15-Trihydroxy-9-oxo-13-*trans*-prostene (17).—A solution of the oxime (**16**, 146 mg) in 10 ml of 90% acetic acid was cooled to 10° and treated with 5 ml of a 10% aqueous solution of sodium nitrite. After 1 hr at 10° the reaction mixture was allowed to warm to room temperature and then treated with an additional 5 ml of 10% sodium nitrite for 30 min. Excess water was then added and the organic material was extracted with ethyl acetate. The organic extracts were washed with sodium bicarbonate solution, water, and dried (Na_2SO_4). Removal of the solvent gave an oil (163 mg) which was dissolved in 1:1 ethyl acetate–cyclohexane and applied to a 20-g column of acid-washed silica gel made up in 50% ethyl acetate–cyclohexane. Elution successively with 50% ethyl acetate–cyclohexane, ethyl acetate, 2% methanol–ethyl acetate, and 5% ethanol–ethyl acetate gave crystalline fractions (67 mg) which were combined. Crystallization from ethyl acetate gave "PGE_1-alcohol" (**17**), mp 106–108°.

Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.54; H, 10.66. Found: C, 70.35; H, 11.08.

The mass spectrum was consistent with structure (*m/e* 340, 322, 304, 269, 208).

The ir spectrum showed ν_{max}^{Nujol} 3470, 3360, 1725, 1160, 1125, 1080, 1055, 1020, 990, 975, 970 cm^{-1}.

Conversion of 8-Iso-PGE_1 (12) into PGE_1.—A solution of 16 mg of 8-iso-PGE_1 (**12**) in 8.8 ml of 3A ethanol was stirred at room temperature with 352 mg of anhydrous potassium acetate for 110 hr. At the end of this time a trace of 8-iso-PGE_1 remained and in addition to PGE_1 a less polar material was formed. The ethanol was removed in a nitrogen stream and the residue partitioned between ethyl acetate and water. After adjustment to pH 3 the ethyl acetate extracts were separated, washed with water, and dried ($MgSO_4$). Removal of the solvent *in vacuo* and trituration with ether followed by crystallization of the residue from ethyl acetate gave material, mp 108–112°. A mixture melting point with PGE_1 was 108–112°. The nmr spectrum of this material was identical with that of authentic PGE_1 (100 Mc; CAT).

A solution of 100 mg of 8-iso-PGE_1 in 50 ml of 95% ethanol containing 2.2 g of potassium acetate stood under nitrogen for 92 hr. Work-up as above gave a crude residue which was partially crystalline. Recrystallization from ethyl acetate–Skellysolve B gave 57 mg of PGE_1, mp 112–114°. The mother liquors still contained some 8-iso-PGE_1 by tlc and was retreated as above with one-half the amounts of ethanol and potassium acetate. Similar work-up and crystallization gave a further 12 mg of crystalline PGE_1, mp 112–115°, making the total yield 69%.

Reduction of 8-Isoprostaglandin E_1 Methyl Ester.—A solution of 100 mg of 8-isoprostaglandin E_1 methyl ester (**12**, R = CH_3) in 5 ml of isopropyl alcohol was treated with cooling with a solution of 50 mg of sodium borohydride in 1 ml of water. The mixture was stirred in the melting ice bath for 2.5 hr after which 1 ml of acetone was added, the mixture then neutralized with dilute acetic acid and concentrated *in vacuo* and the product extracted with ethyl acetate. The gummy residue obtained after evaporation was chromatographed over 15 g of silica gel to afford 15 mg of a less polar gummy product (ethyl acetate elution) and 61 mg of a more polar partially crystalline product (ethyl acetate containing 5% methanol elution). Intervening fractions (13 mg) consisted mainly of the latter. On an Analtech 2 × 8 in. silica gel plate developed three times with ethyl acetate the less polar product exhibited as R_f of 0.36, the more polar 0.27. On the same plate $PGF_{1\alpha}$ methyl ester had an R_f of 0.36; $PGF_{1\beta}$ methyl ester 0.20. Ir and nmr spectrums were barely distinguishable, if at all, from these afforded by $PGF_{1\alpha}$ or $PGF_{1\beta}$ methyl esters. The mass spectrums were likewise similar. The more polar epimer crystallized from ether–Skellysolve B to give waxy crystals, mp 60–61°.

Anal. Calcd for $C_{21}H_{38}O_5$: C, 68.07; H, 10.34. Found: C, 67.63; H, 10.44.

On the basis of chromatographic mobility the less polar epimer is assigned the 9β-hydroxy structure (**14**, R = CH_3) and the more polar epimer the 9α-hydroxy structure.

9,15-Diketo-11-hydroxy-10,12-prostadienoic Acid (18) and Methyl 9,15-Diketo-11-methoxy-10,12-prostadienate.—A solution of 150 mg PGE_1 in 30 ml acetone was cooled to 0° and treated with stirring with 0.5 ml Jones reagent for 20 min. Then 1.5 ml methanol was added, followed by 15 ml water, and the solution was concentrated *in vacuo*. The aqueous mixture was extracted with ethyl acetate, washed with water and saturated salt, dried, and evaporated. The residue was chromatographed on 25 g of acid-washed silica gel and eluted with 150 ml each of 1:2, 1:1, 2:1, and 100% ethyl acetate–Skellysolve B, collecting 25-ml fractions. Fractions 18–21, 50 mg, had λ_{max}^{EtOH} at 277 mμ, and showed carboxylic and enolic absorptions at 2500–3500 cm^{-1}, also carbonyl absorptions occurred at 1750, 1700 (shoulder) cm^{-1} and a very strong enolic double bond at 1580 cm^{-1}.

The crude product from a duplicate run was treated with excess ethereal diazomethane for 15 min. After evaporation, the residue was chromatographed on 20 g of silica gel, eluting with 100 ml each of 5, 10, 15, 20, 40, 50, 75, and 100% ethyl acetate–Skellysolve B, collecting 25-ml fractions. The main peak (45 mg) had carbonyl absorptions at 1750, 1720, 1680 cm^{-1} and strong enolic double bond at 1595 cm^{-1}; λ_{max}^{EtOH} 272 (17800) and 207 mμ (8450). The nmr absorptions showed terminal methyl at 53 cps, the C-14 hydrogens, doublet, at 198, OCH_3 at 219 and 233, the C-10 hydrogen at 325, and the C-13 hydrogen, triplet, at 377.

Registry No.—**2**, 21562-44-7; **2** methyl ester, 21562-45-8; **2** methyl ester (tri-*p*-iodobenzoate), 21562-46-9; **3**, 551-11-1; **5**, 21562-48-1; **5** methyl ester, 21562-49-2; **5** methyl ester (tri-*p*-iodobenzoate), 20986-28-1; **6**, 4510-16-1; **7**, 14152-28-4; **8**, 13345-50-1; **10**, 21562-54-9; **13**, R = CH_3, 21562-59-4; **15**, 21562-55-0; **16**, 21562-56-1; **17**, 21562-57-2; 9α,11α-dihydroxy-15-oxoprost-13-*trans*-enoic acid, 21562-58-3.