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Prostaglandin Prodrugs III: Synthesis and Biological Properties of C_9- and C_{15}-Monoesters of Dinoprost (Prostaglandin $F_{2\alpha}$)

W. MOROZOWICH ˣ, T. O. OESTERLING, W. L. MILLER, C. F. LAWSON, J. C. CORNETTE, J. R. WEEKS, and S. L. DOUGLAS

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Abstract □ Methods are described for the synthesis of dinoprost C_9- and C_{15}-monoesters using protective groups. Esters at C_9 were synthesized by acylation of dinoprost 11,15-bis(tetrahydropyran-2-yl)ether followed by acid-catalyzed protective group removal. Esters at C_{15} were synthesized by initial formation of the protected intermediate, dinoprost 9,11-*n*-butylboronate, followed by acylation and hydrolytic protective group removal. Many esters were active *in vivo* in the hamster antifertility screen. Plasma hydrolysis studies showed that the C_{15}-esters were more readily cleaved than the C_9-esters. *In vivo* studies in the rat showed that both the C_9- and C_{15}-esters resulted in urinary excretion of 5α,7α-dihydroxy-11-ketotetranorprosta-1,16-dioic acid in amounts comparable to those obtained after dosing with dinoprost, indicating that ester hydrolysis occurred *in vivo*.

Keyphrases □ Dinoprost—prodrugs, C_9- and C_{15}-monoesters, synthesis, bioactivity □ Prostaglandins—dinoprost, prodrugs, C_9- and C_{15}-monoesters, synthesis, bioactivity □ Prodrugs—dinoprost, C_9- and C_{15}-monoesters, synthesis, bioactivity

Previously, methods were described for the synthesis of aromatic and aliphatic prostaglandins C_1-esters, and the utility of these prodrugs in improving solid-state stability and oral absorption was reported (1, 2). This report describes synthetic routes for dinoprost C_9- and C_{15}-monoesters and an evaluation of ester bioactivity.

EXPERIMENTAL

Materials and Methods—Dinoprost and dinoprost 11,15-bis(tetrahydropyran-2-yl)ether (I)[1] (3) were pure by silica gel TLC. The acid chlorides were obtained commercially[2]. Pyridine (analytical reagent) was dried over molecular sieves[3] for 1 week prior to use. All other solvents were glass-distilled quality[4].

Column chromatography was conducted on 0.063–0.2-mm silica gel[5]. Silica gel TLC was conducted on 250-μm layer plates[6], and visualization was by spraying the developed plates with aqueous 15% ammonium sulfate followed by charring on a hot plate. Prostaglandin ester mass spectra were obtained[7] for (*a*) the trimethylsilyl derivative, (*b*) the C_1-methyl ester of the trimethylsilyl derivative, and (*c*) the nonderivatized compound. The methyl esters were obtained using diazomethane, and the trimethylsilyl derivatives were obtained with a mixture of hexamethyldisilazane – trimethylchlorosilane – bis(trimethylsilyl)acetamide–pyridine (2:2:1:5).

Synthesis—Representative ester syntheses are described. The other esters were synthesized similarly.

Dinoprost 9-n-Butyrate (*III*b)—A solution of 100 mg of I (3) in a mixture of 0.5 ml of butyric anhydride and 1 ml of pyridine was allowed to stand at room temperature for 24 hr. Upon solvent removal at 45° under vacuum, the residue was dissolved in 50 ml of acetone–acetic acid–water (1:1:1). After 24 hr at 40°, the solvent was removed and the residue was subjected to column chromatography on 75 g of silica gel. Elution was achieved with ethyl acetate–acetic acid (97:3), and the product fractions were identified by silica gel TLC with the same solvent system. Evaporation of the pooled product fractions gave 51 mg of a colorless oil, which was pure by TLC.

Dinoprost 15-Acetate (*VIII*a)—A solution of 48.4 mg (0.137 mmole) of IV and 24 mg (0.236 mmole) of *n*-butylboronic acid in 2 ml of dry pyridine was allowed to stand at room temperature for 1 hr. Acetic anhydride, 2 ml, was added; after 24 hr at room temperature, 3 ml of water was added and the solvent was removed at 45° under vacuum. The residual oil was dissolved in 50 ml of ethyl acetate and extracted with 50 ml of 0.2 *M* citrate buffer, pH 3.0.

The organic phase was dried over sodium sulfate; upon solvent removal, the residue was subjected to column chromatography on 50 g of silica gel. Elution was achieved with ethyl acetate–acetic acid (97:3). The product fractions were identified by TLC; upon solvent removal, 25.3 mg of a colorless oil was isolated.

Biological Activity—The ester activity was studied in the gerbil colon assay, the rat pressor blood pressure assay, and the hamster antifertility assay (subcutaneous administration) as described previously (1, 4).

Plasma Hydrolysis—Standard aqueous solutions (0.1 ml) of dinoprost, the 9-acetate (IIIa), the 15-acetate (VIIIa), the 9-*n*-butyrate (IIIb), and the 15-*n*-butyrate (VIIIc) containing quantities in terms of IV ranging from 5 to 20×10^3 ng/ml were incubated separately with 0.05 ml of each of the following: (*a*) saline solution, (*b*) heparinized rat plasma, (*c*) heparinized monkey plasma, and (*d*) heparinized human plasma. After 2 hr at 37°, the affinity of each incubated sample for anti-dinoprost serum was determined using a double antibody radioimmunoassay (5). Relative cross-reactivity was calculated by comparing the amount of the compound required to displace 50% of the label with the amount of IV required. Increased antibody affinity occurred as ester hydrolysis proceeded to yield IV.

***In Vivo* Metabolism**—Mature Sprague–Dawley female rats were injected subcutaneously with one of the following: I, IIIa, VIIIa, IIIb, VIIIc, and the vehicle as the control. The compounds were administered in amounts equivalent to 1 mg of IV dissolved in 0.5 ml of vehicle consisting of polyethoxylated vegetable oil[8]–ethanol–5% aqueous dextrose (1:1:8).

The animals were kept in metabolism cages, and total urine volumes

[1] Supplied by the Research Division, The Upjohn Co.
[2] Aldrich Chemical Co., Milwaukee, Wis., and Eastman Kodak Co., Rochester, N.Y.
[3] Beads, 10–16 mesh, 4 Å, Davison Chemical Co., Baltimore, Md.
[4] Burdick & Jackson Co., Muskegon, Mich.
[5] Silica gel 60, EM Laboratories, Elmsford, N.Y.
[6] Uniplate, Analtech Inc., Newark, Del.
[7] LKB 9000 mass spectrometer.
[8] Emulphor EL-620, GAF Corp., New York, N.Y.

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Table I—Mass Spectral Data of Dinoprost Esters [a]

Compound	Formula	Derivative Employed for Mass Spectrometry[b]	Key Mass Spectral Ions (*m/e*)
IIIa	$C_{22}H_{36}O_6$	A	597 ($M^+ - CH_3$), 552 ($M^+ - CH_3COOH$), 537 ($M^+ - CH_3 - CH_3COOH$), 462 [$M^+ - CH_3COOH - (CH_3)_3SiOH$]
IIIb	$C_{24}H_{40}O_6$	A	625 ($M^+ - CH_3$), 552 ($M^+ - C_3H_7COOH$), 537 ($M^+ - CH_3 - C_3H_7COOH$), 462 [$M^+ - C_3H_7COOH - (CH_3)_3SiOH$]
IIIc	$C_{28}H_{48}O_6$	B	336 ($M^+ - C_7H_{15}COOH$), 318 ($M^+ - C_7H_{15}COOH - H_2O$)
IIId	$C_{32}H_{56}O_6$	A	752 (M^+), 737 ($M^+ - CH_3$), 681 ($M^+ - C_5H_{11}$), 662 [$M^+ - (CH_3)_3SiOH$]
VIIIa	$C_{22}H_{36}O_6$	A	612 (M^+), 552 ($M^+ - CH_3COOH$), 537 ($M^+ - CH_3 - CH_3COOH$), 462 [$M^+ - CH_3COOH - (CH_3)_3SiOH$]
VIIIb	$C_{23}H_{38}O_6$	B	336 ($M^+ - C_2H_5COOH$), 318 ($M^+ - C_2H_5COOH - H_2O$)
VIIIc	$C_{24}H_{40}O_6$	A	640 (M^+), 552 ($M^+ - C_3H_7COOH$), 537 ($M^+ - CH_3 - C_3H_7COOH$), 481 ($-C_3H_7COOH - C_5H_{11}$)
VIIId	$C_{25}H_{42}O_6$	B	336 ($M^+ - C_4H_9COOH$), 318 ($M^+ - C_4H_9COOH - H_2O$)
VIIIe	$C_{26}H_{44}O_6$	C	610 (M^+), 595 ($M^+ - CH_3$), 579 ($M^+ - OCH_3$), 520 [$M^+ - (CH_3)_3SiOH$], 494 ($M^+ - C_5H_{11}COOH$)
VIIIf	$C_{30}H_{52}O_6$	B	336 ($M^+ - C_9H_{19}COOH$), 318 ($M^+ - C_9H_{19}COOH - H_2O$), 300 ($M^+ - C_9H_{19}COOH - 2H_2O$)
VIIIg	$C_{36}H_{64}O_6$	B	318 ($M^+ - C_{15}H_{31}COOH - H_2O$), 300 ($M^+ - C_{15}H_{31}COOH - 2H_2O$)

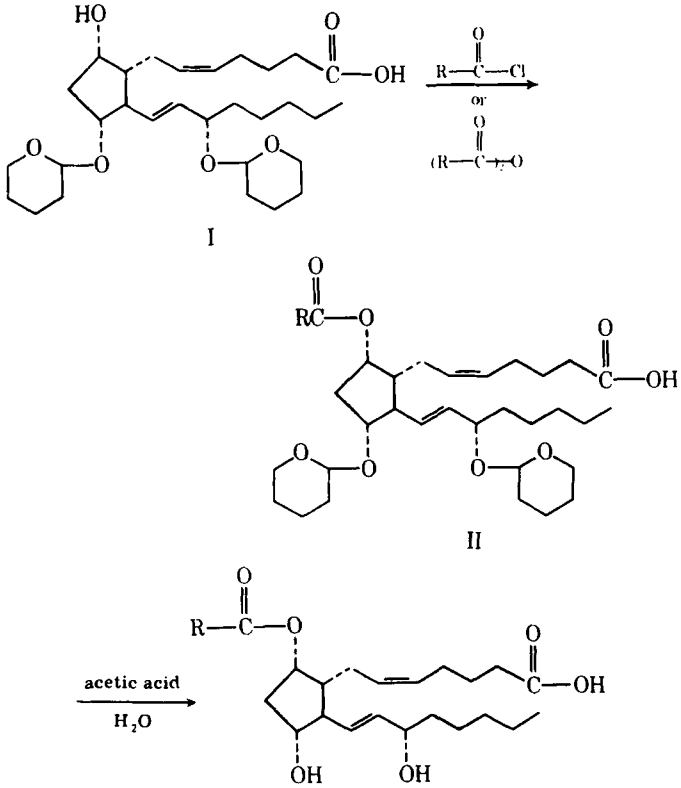
[a] The compounds were liquids, except IIIc (mp 55.9–60.5°). [b] A = trimethylsilyl derivative, B = no derivatization, and C = C_1-methyl ester trimethylsilyl derivative.

were collected over 2 days. Each 24-hr urine sample was assayed for 5α,7α-dihydroxy-11-ketotetranorprosta-1,16-dioic acid (IX), an ultimate major urinary IV metabolite (6), using the radioimmunoassay and the antibody specific for this compound (7).

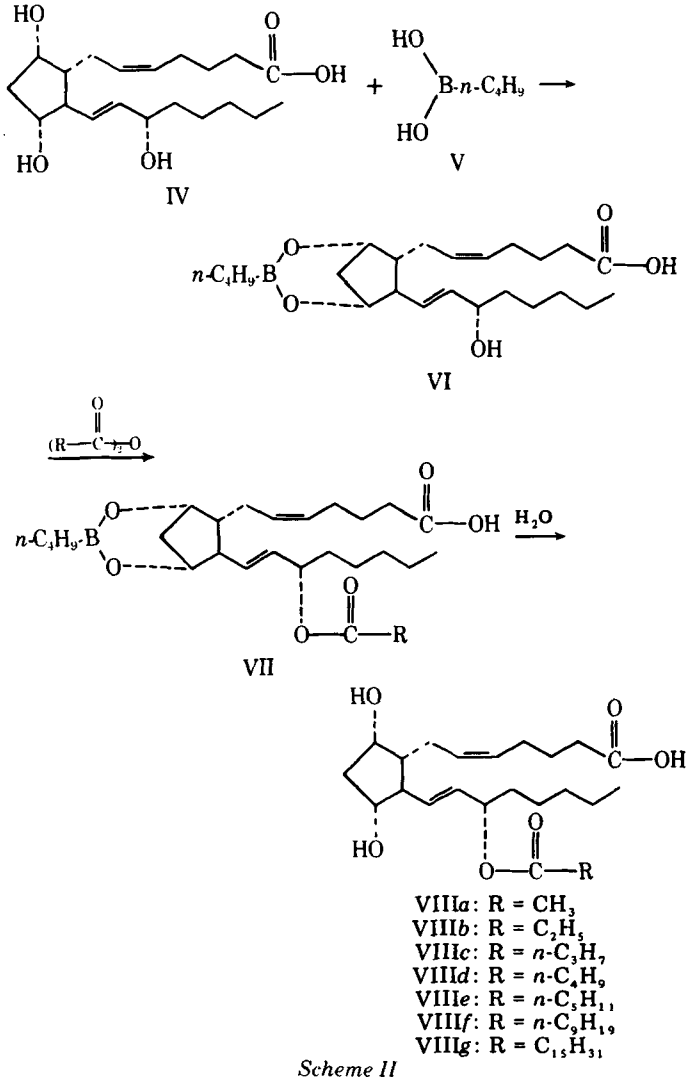
RESULTS AND DISCUSSION

Synthesis—Since selective monoesterification of dinoprost (IV) with acid anhydrides or acid chlorides in pyridine was not possible, protective groups were necessary (8).

The C_9-esters (III) were synthesized by reacting the 11,15-bis(tetrahydropyran-2-yl)ether (I) (3) with an acid anhydride or an acid chloride in pyridine at room temperature (Scheme I). Only the acetate ester could be synthesized *via* the acid anhydride in high yields (>90%) at room temperature in 24 hr, and acid chlorides were required for the synthesis of the higher esters at room temperature.



Scheme I



Scheme II

Tetrahydropyranyl protective group removal from ester II was achieved with aqueous acetic acid, and the resulting C_9-esters (III) were purified by column chromatography.

The C_{15}-esters (VIII) were synthesized by initial protection of the C_9- and C_{15}-hydroxyl groups with *n*-butylboronic acid (V) (9) (Scheme II). The reaction proceeded rapidly at room temperature (15–60 min) in the presence of a 1–2-mole excess of *n*-butylboronic acid to give the cyclic 9,11-boronate ester (VI).

Compound VI formation generally was quantitative, as evidenced by GLC upon trimethylsilylation of a reaction mixture aliquot. Occasionally,

Table II—Biological Activity of Dinoprost Esters

Compound	Hamster Antifertility[a] Percent Non-pregnant	Dose, μg/animal	Gerbil Colon[b]	Rat Blood Pressure (Depressor)[c]
IIIa	50	125	0.03–0.1	0.01–0.03
IIIb	75	125	0.03–0.1	0.01–0.03
IIIc	100	200	<0.001	0.003–0.01
IIId	17	100	0.01–0.03	0.03–0.1
VIIIa	75	125	0.1–0.3	0.3–1
VIIIb	0	100	0.01–0.3	0.3–1
VIIIc	83	125	0.03–0.1	0.3–1
VIIId	17	100	0.1–0.3	0.3–1
VIIIe	33	100	0.01–0.03	0.3–1
VIIIf	0	100	0.03–0.1	0.3–1
VIIIg	0	100	0.001–0.003	0.3–1

[a] Administered subcutaneously. The minimum effective dose for 100% inhibition of pregnancy was 100 μg of IV/animal. [b] The dinoprost (IV) activity score in this assay was 2. Prostaglandin E_1 = 1. [c] Dinoprost = 1.

a larger (3–5 mole) excess of *n*-butylboronic acid had to be employed to provide a quantitative reaction, presumably due to the inhibition by trace moisture contamination. Silica gel TLC was ineffective in monitoring the reaction since severe streaking occurred due to apparent VI hydrolysis during chromatography.

Compound VI esterification with an acid anhydride proceeded smoothly to give the protected C_{15}-esters (VII). After hydrolysis with water, the C_{15}-esters (VIII) were isolated by column chromatography.

All C_9- and C_{15}-esters were isolated as oily liquids, except C_9-*n*-octanoate (IIIc), which was a solid, mp 55.9–60.5°. The ester structures were confirmed by mass spectral analysis of the trimethylsilyl derivatives, and the fragmentation patterns supported the structures (Table I).

Biological Activity—The biological activity of the esters in three test systems (1, 4) is summarized in Table II. In the hamster antifertility assay, the minimum effective dose for 100% pregnancy inhibition was 100 μg/animal for IV; the assay results indicate significant C_9-ester activity at the dosage level tested. In the C_{15}-ester series, VIIIa and VIIIc–VIIIe showed significant activity; VIIIb, VIIIf, and VIIIg were inactive.

In the gerbil colon assay, IV had an activity score of 2. The biological data show that the C_9- and C_{15}-esters displayed activities significantly less than the parent compound.

In the rat blood pressure assay, the pressor activity of VIIIa–VIIIg approached that of IV whereas IIIa–IIId were significantly less active.

***In Vitro* and *In Vivo* Hydrolysis**—The cleavage of the acetate and *n*-butyrate esters IIIa, IIIb, VIIIa, and VIIIc was studied both *in vitro* and *in vivo*. The *in vitro* studies were conducted by equilibrating the esters with rat, monkey, and human plasma for 2 hr at 37°. Hydrolysis was measured by the radioimmunoassay using the antibody specific for IV (5). The percent hydrolysis was determined by comparing the assay response of the esters to that of an equimolar amount of IV treated similarly. The results (Table III) showed that the 9-acetate (IIIa) and 9-butyrate (IIIb) esters were resistant to cleavage by the three plasma samples.

Table III—Plasma Hydrolysis (Percent in 2 hr at 37°) of Dinoprost Esters[a]

Compound	Saline Control	Rat Plamsa	Monkey Plasma	Human Plasma
IIIa	1.0	0.9	1.0	0.6
IIIb	1.3	1.5	0.7	0.5
VIIIa	22.0	50.8	28.6	9.1
VIIIc	5.0	40.0	6.7	2.6

[a] Solutions of the compounds in 0.1 ml of water diluted with 0.05 ml of plasma or saline control. The amount of IV liberated was determined by the radioimmunoassay.

Table IV—Total Daily Urinary Metabolite IX Excretion (Micrograms) in Rats Dosed Subcutaneously[a]

Compound	Day 1	Day 2
(Vehicle control)	(0.8)	(0.8)
IV	138	1.4
IIIa	116	2.5
IIIb	155	5.0
VIIIa	195	5.0
VIIIc	132	1.6

[a] Determined by the radioimmunoassay.

The 15-acetate (VIIIa) and 15-butyrate (VIIIc) esters were appreciably hydrolyzed by rat plasma but less efficiently by monkey plasma. Compound VIIIa hydrolysis by human plasma was unusual since partial hydrolysis occurred in the saline control, presumably due to the esterases present in the antiserum; C_{15}-ester hydrolysis did not occur in water at 37° for 2 hr. The decreased hydrolysis by human plasma may be due to an hydrolysis-inhibiting component present in human plasma or to extensive ester protein binding.

In vivo ester hydrolysis was studied in rats by monitoring the urinary excretion of the major urinary IV metabolite, 5α,7α-dihydroxy-11-ketotetranorprosta-1,16-dioic acid (IX) (6), using the radioimmunoassay (7). The esters were injected subcutaneously in rats, and the daily urinary IX excretion was determined using the antibody specific for this compound. Thus, if ester hydrolysis occurred *in vivo*, the resulting IV would be converted to IX.

Table IV shows that the acetate and butyrate esters at both the 9- and 15-positions yielded approximately the same amount of urinary metabolite IX as was found upon administration of IV, indicating complete ester hydrolysis *in vivo*. The data imply that all four esters should be as active as IV in the antifertility assay, but IIIa was somewhat less active. *In vivo* IIIa hydrolysis probably occurs competitively with metabolism at C_1 or C_{15}, and the low antifertility activity of this ester could be due to preferential metabolism to yield an inactive ester metabolite. Ultimate ester hydrolysis and metabolism to IX would account for the full bioavailability of the metabolite from IIIa.

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