Prostaglandin Prodrugs I: Stabilization of Dinoprostone (Prostaglandin E_2) in Solid State through Formation of Crystalline C_1-Phenyl Esters

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Abstract □ Dinoprostone *para*-substituted phenyl esters were synthesized in attempt to improve the solid-state stability of the parent prostaglandin. A phenol series covering a wide melting-point range was employed, and a linear relationship was observed between the phenol melting points and the resulting prostaglandin C_1-ester melting points. The crystalline esters showed improved solid-state stability over the parent compound, and many esters were biologically active.

Keyphrases □ Prostaglandins—dinoprostone, prodrugs, stabilization in solid state of crystalline C_1-phenyl esters, biological activity □ Prodrugs—dinoprostone, stabilization in solid state of crystalline C_1-phenyl esters, biological activity

Prostaglandins are naturally occurring long chain substituted carboxylic acids with various biological effects *in vivo* (1–3). These compounds are undergoing extensive molecular modification in attempts to increase potency and specificity and to improve pharmaceutical and biological properties. Chemical programs aimed at increasing potency or specificity involve the creation of analogs through substituent synthesis or skeletal modification, whereas biopharmaceutical properties are modified *via* prodrugs.

BACKGROUND

Following the identification of several highly active prostaglandin analogs (4–7), attention has been directed toward improving pharmaceutical or biological properties through the *in vivo* reversible modification known as the prodrug (8). Many highly active prostaglandin analogs do not have optimal pharmaceutical properties such as solubility, stability, and crystallinity nor optimal biological properties such as absorption and duration of action (9). These problems may be solved through prodrug synthesis.

Two pressing problems with prostaglandins are E-series prostaglandin instability and difficulty in handling many prostaglandins that occur as liquids (9). This report focuses on the utility of crystalline C_1-esters in improving the solid-state stability of dinoprostone (I).

Dinoprostone (I) instability is due to the 9,11β-ketol system in which the activated C_{11}-hydroxyl undergoes facile elimination to give prostaglandin A_2 (10). Attempts to stabilize this prostaglandin have included solvent stabilization (11), complex formation (12, 13), and bisulfite formation at C_9 (14).

Crystalline high-melting C_1-esters were synthesized to improve the solid-state stability of I based on the following reasoning. Compound I is a crystalline solid (mp 63°) stable at room temperature for short periods but liquefies and decomposes rapidly after a few months. High melting I C_1-esters might maintain crystal integrity and improve the solid-state stability. Prediction of the ester melting points was not possible *a priori* from structural formula inspection, but, intuitively, phenols capable of strong intermolecular interaction were expected to provide high melting esters. The melting point of the parent phenol used in ester formation reflects the intermolecular interaction strength; therefore, higher melting phenols would be expected to give higher melting esters.

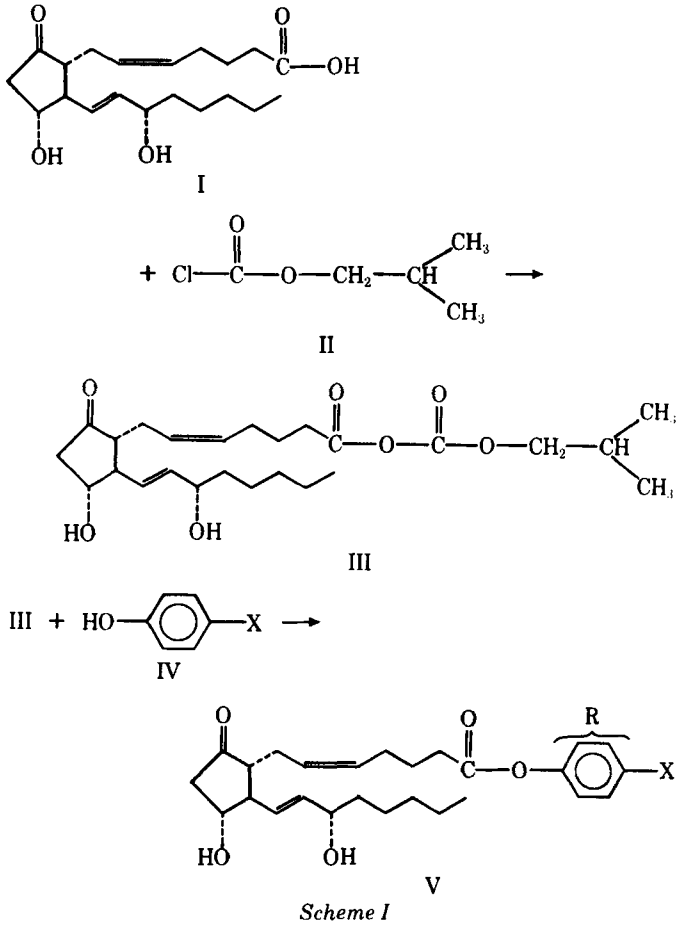
This work describes the synthesis of various I C_1-esters using a phenol series covering a wide melting-point range in an attempt to explore the: (*a*) relationship between the phenol and corresponding prostaglandin ester melting points, (*b*) utility of crystalline dinoprostone esters in improving the parent prostaglandin solid-state stability, and (*c*) influence of esterification on bioactivity.

RESULTS AND DISCUSSION

Synthesis—Prostaglandin C_1-esters have been synthesized by numerous routes including the *N,N*-dicyclohexylcarbodiimide procedure (15), carboxyl activation with pivaloyl chloride (16), and carboxyl activation with sulfonyl halides (17). In this work the isobutyl chlorocarbonate carboxyl activation route widely used in peptide synthesis (18) was chosen.

Reaction of I with isobutyl chlorocarbonate (II) gave the acylisobutylcarbonate mixed anhydride (III); upon reaction with 1.5–4 mole equivalents of the appropriate phenol in pyridine at room temperature, III gave the desired C_1-esters (V) (Scheme I). The use of a small excess (0.1–0.5 mole equivalents) of II generally gave quantitative I esterification in less than 15 min; for convenience, the reactions were allowed to stand at room temperature for up to 3 hr before workup.

The esters were purified by silica gel column chromatography and, upon recrystallization, they were shown to be pure by silica gel TLC. The structures were verified by combustion analyses (Table I) and, in some cases, by mass spectral analyses.



Scheme I

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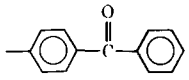
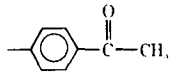
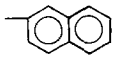
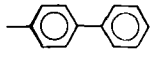
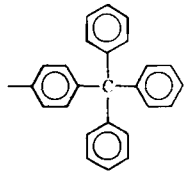
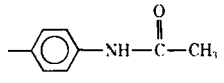
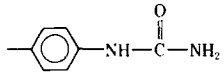
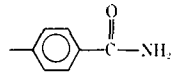
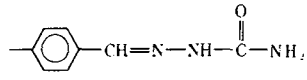
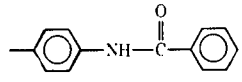
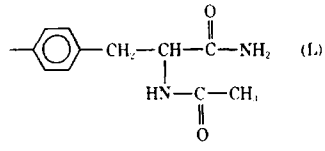
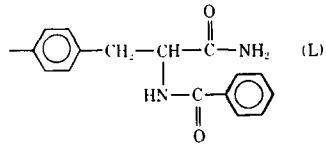
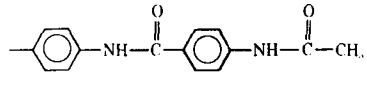
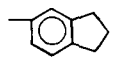
Table I—Physical Constants of Dinoprostone C_1-Esters

Compound	C_1-Ester (R in V)	Recrystallization Solvent	Melting Point: C_1-Ester	Melting Point: Parent Phenol	Empirical Formula	Combustion Analyses, %: Calc.	Combustion Analyses, %: Found
Va		—	Liquid	43°	$C_{26}H_{36}O_5$	—[a]	—
Vb		—	Liquid	130–133°	$C_{33}H_{40}O_6$	—[b]	—
Vc		Ethyl acetate diluted with hexane	76.8–77.8°	108–110°	$C_{28}H_{38}O_6$	C 71.46 H 8.14	71.46 8.13
Vd		Ethyl acetate diluted with hexane	79.3–80.3°	122–124°	$C_{30}H_{38}O_5$	C 75.38 H 8.00	75.44 7.73
Ve		Ethyl acetate diluted with hexane	91.8–92.8°	165°	$C_{32}H_{40}O_5$	C 76.16 H 7.99	76.26 7.86
Vf		Ethyl acetate diluted with hexane	96.3–97.8°	283°	$C_{45}H_{50}O_5$	C 80.56 H 7.51	79.52 7.81
Vg		Ethyl acetate diluted with hexane	102.3–103.3°	167–169°	$C_{28}H_{29}NO_6$	C 69.25 H 8.09 N 2.88	69.36 8.27 2.87
Vh		Ethyl acetate diluted with hexane	105.3–108.3°	170–172°	$C_{27}H_{38}N_2O_6$	C 66.64 H 7.87 N 5.76	66.73 8.13 5.83
Vi		Acetonitrile diluted with water	106.3–108.3°	196–197°	$C_{27}H_{37}NO_6$	C 68.76 H 7.91 N 2.97	68.36 7.71 2.95
Vj		Hot acetonitrile	125.3–126.5°	224°	$C_{28}H_{39}N_3O_6$	C 65.48 H 7.65 N 8.18	65.62 7.76 8.27
Vk		Tetrahydrofuran diluted with hexane	132.8–135.0°	218°	$C_{33}H_{41}NO_6$	C 72.37 H 7.55 N 2.56	71.95 7.34 2.55
Vl	 (L)	Warm acetone diluted with hexane	137.3–140.8°	223–225°	$C_{31}H_{44}N_2O_7$	C 66.88 H 7.97 N 5.03	66.46 8.03 5.27
Vm	 (L)	Hot acetone	160.8–164.8°	205–208°	$C_{36}H_{46}N_2O_7$	C 69.88 H 7.49 N 4.53	69.80 7.80 4.89
Vn		Hot acetone	173.2–176.2°	275–277°	$C_{35}H_{44}N_2O_7$	C 69.51 H 7.33 N 4.63	69.30 7.25 4.69
Vo		—	44.5–48.0°[c]	51–53°	$C_{29}H_{39}O_5$	—	—

[a] Characterized by mass spectral analysis of the bis(trimethylsilyl) derivative showing a molecular ion at *m/e* 572 and a fragmentation pattern consistent with the structure. [b] Characterized by mass spectral analysis of the bis(trimethylsilyl) derivative. A partial list of fragment ions includes *m/e* 605 ($M^+ - 71$), 586 ($M^+ - 90$), 571 ($M^+ - 90 - 15$), and 515 ($M^+ - 90 - 71$). [c] Reference 19.

Table II—Biological Activity of Dinoprostone C_1-Esters

Compound	Hamster Antifertility Assay: Percent Nonpregnant	Hamster Antifertility Assay: Dose, μg/animal	Gerbil Colon[a]	Rat Blood Pressure (Depressor)[a]
I[b]	100	200[c]	2 (1.4–2.6)[d]	0.6 (0.4–1.1)[d]
V*a*	50	234[e]	1–3	0.1–0.3
V*b*	17	302[e]	1–3	0.1–0.3
V*c*	100	267	1–3	0.1–0.3
	17	134		
V*d*	0	272[e]	0.3–1	0.1–0.3
V*e*	17	286[e]	0.1–0.3	0.01–0.03
V*f*	17	381[e]	0.01–0.03	Inactive
	100	1000		
V*g*	0	100	0.01–0.03	0.1–0.3
V*h*	50	276[e]	1–3	0.1–0.3
V*i*	33	268[e]	0.3–1	0.1–0.3
V*j*	17	291[e]	0.3–1	0.1–0.3
V*k*	33	1000	0.1–0.3	0.1–0.3
V*l*	17	316[e]	1–3	0.3–1
V*m*	0	351[e]	0.3–1	0.1–0.3
V*n*	0	100	0.1–0.3	0.1–0.3

[a] Prostaglandin E = 1. [b] Reference compound. [c] Minimal effective dose (MED_{100}) for 100% inhibition of pregnancy. [d] Relative potency and 95% confidence interval in parenthesis. [e] Amount equivalent to 200 μg of I.

Melting Points—Plotting the I C_1-ester melting points *versus* the starting phenol[1] melting points (Fig. 1) showed that higher melting phenols generally gave higher melting esters. The lowest melting ester on the plot was that of 5-indanol (19).

The least-squares line is shown without the most deviant point (V*f*, the *p*-tritylphenyl ester). The correlation coefficient was 0.937 (slope, 0.57; intercept, 10.1°). Inclusion of V*f* decreased the correlation coefficient to 0.780 (slope, 0.43; intercept, 31.1°). The reason for the large deviation of V*f* is not clear but could be the isolation of an unusually low melting polymorph or a different crystal packing arrangement caused by exceptional ester bulkiness.

Structurally, the amido-substituted phenyl esters provided higher melting points than the acyl- or aromatic-substituted phenyl esters, presumably due to strong amido moiety hydrogen bonding (20). The melting points increased progressively from the *p*-acetamidophenyl ester (V*g*) to the *p,p'*-acetamidobenzamidophenyl ester (V*n*). The latter ester is remarkably effective in raising the melting point of I and is the highest melting ester (173.2–176.2°) recorded to date.

Stability—Storage of crystalline esters V*c*–V*e*, V*g*–V*l*, and V*n* at room temperature for 23–30 months resulted in no detectable degradation as shown by silica gel TLC; the esters remained as white solids. The free acid I, on the other hand, underwent 44–59% degradation in 12 months at room temperature with partial liquefaction. Since the liquid esters V*a* and V*b* underwent decomposition as well as discoloration upon storage at room temperature, the improved solid-state stability of the crystalline esters apparently is due to the stabilization by the crystal lattice.

Biological Activity—The ester biological activities in three test screening systems are summarized in Table II. Significant activity was observed in the antifertility assay for all compounds except V*d*, V*m*, and V*n*. Full activity was attained with V*c*. Some esters were nearly as active as I in the gerbil colon assay, but all compounds were less active than I in the rat blood pressure assay.

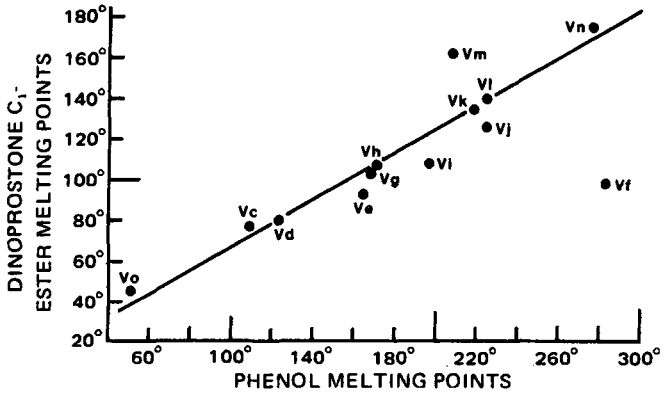


Figure 1—*Plot of the melting points of starting phenols* versus *the melting points of resulting dinoprostone C_1-esters. The least-squares line is shown omitting ester V*f.

[1] The melting-point range midpoint was used.

EXPERIMENTAL

Materials and Methods—Compound I[2] (mp 63°) purity was verified by silica gel TLC using ethyl acetate–acetic acid (97:3). The phenols, except those synthesized below, were obtained commercially[3]. Pyridine (analytical reagent) was dried over molecular sieves[4] for 1 week before use. All other solvents were glass-distilled quality[5]. Acetone and tetrahydrofuran were dried by stirring 100 ml of the solvent with 50 g of alumina[6] for 15 min followed by filtration under nitrogen.

Column chromatography was conducted on 0.063–0.2-mm silica gel[7]. In most cases, the support was deactivated by stirring 1 kg of silica gel with 4 liters of ethyl acetate containing 30 ml of water. After the slurry settled for about 2 min, the supernate was decanted and the remaining thick slurry was poured into glass columns. Silica gel TLC was conducted on 250-μm layer plates[8]. The compounds were visualized by spraying the developed plates with aqueous 15% ammonium sulfate followed by charring on a hot plate.

Synthesis—p-*Benzamidophenol*—A solution of 20 g of *p*-aminophenol in 200 ml of dry pyridine was treated with 20 g of benzoic anhydride. After 4 hr at room temperature, the solvent was removed under vacuum at 45°, and the residue was dissolved in 200 ml of hot methanol. Dilution with 300 ml of water gave 22 g of tan crystals. An 11.2-g portion of the product was recrystallized from 350 ml of hot acetonitrile, affording 8.5 g of white crystals, mp 218.0–218.5°. The product was soluble in alkali, indicative of a phenol, and the mass spectrum revealed a molecular ion at *m/e* 213.

Anal.—Calc. for $C_{13}H_{11}NO_2$: C, 73.22; H, 5.20; N, 6.56. Found: C, 73.49; H, 5.30; N, 6.47.

p,p'-*Acetamidobenzamidophenol*—A solution of 12.5 g of *p*-acetamidobenzoic acid in 250 ml of dry tetrahydrofuran was treated with 11.1 ml of triethylamine, and partial crystallization of the triethylammonium salt occurred. Isobutyl chlorocarbonate (10.4 ml) was added to the suspension with stirring, and a voluminous precipitate occurred. After 5 min of stirring at room temperature, a solution of 13.3 g of *p*-aminophenol in 80 ml of dry pyridine was added. The suspension was stirred for 40 min and then diluted with 2 liters of water, affording 14.3 g of a brown solid. Then the product was dissolved in 500 ml of hot methanol. After partial decolorization with 1 g of charcoal, the solution was diluted with 300 ml of water, giving 5.9 g of white crystals, mp 275.0–277.0°.

Anal.—Calc. for $C_{15}H_{14}N_2O_3$: C, 66.69; H, 5.18; N, 10.36. Found (corrected for 3.87% H_2O): C, 66.08; H, 5.36; N, 10.33.

The compound was rendered anhydrous by drying under vacuum at 110° overnight.

[2] Supplied by the Research Division, The Upjohn Co.
[3] Aldrich Chemical Co., Milwaukee, Wis., and Eastman Kodak Co., Rochester, N.Y.
[4] Beads, 10–16 mesh, 4 Å, Davison Chemical Co., Baltimore, Md.
[5] Burdick & Jackson, Muskegon, Mich.
[6] Woelm alumina, ICN Pharmaceuticals, Cleveland, Ohio.
[7] Silica gel 60, EM Laboratories, Elmsford, N.Y.
[8] Uniplate, Analtech Inc., Newark, Del.

p-*Benzamidophenyl Ester of Dinoprostone* (*Vk*)—A solution of 260 mg (0.74 mmole) of I in 20 ml of dry acetone was treated with 0.206 ml (1.48 mmoles) of triethylamine. While under a positive nitrogen atmosphere, the solution was cooled to −5°, and 0.194 ml (1.47 mmoles) of isobutyl chlorocarbonate was added. Rapid crystallization of triethylamine hydrochloride occurred. After 3 min at −5°, a solution of 0.8 g (3.76 mmoles) of *p*-benzamidophenol in 8 ml of dry pyridine was added.

After 3 hr at room temperature, the solvent was removed under vacuum at 45°, and the resulting oily residue was purified by column chromatography on 80 g of silica gel. Elution was achieved with 150 ml of ethyl acetate followed by acetonitrile. TLC showed the product was in tubes 25–30 (15 ml each); upon solvent removal under vacuum at 45°, a white solid was obtained. The product was dissolved in 20 ml of tetrahydrofuran and diluted with 20 ml of hexane, affording 269 mg of white crystals, mp 132.8–135.0°, after drying under vacuum at 50°. The product was pure by silica gel TLC with ethyl acetate as the developing solvent.

Anal.—Calc. for $C_{33}H_{41}NO_6$: C, 72.37; H, 7.55; N, 2.56. Found: C, 71.95; H, 7.34; N, 2.55.

Mass spectral analysis showed a peak at *m/e* 529 corresponding to $M^+ - H_2O$, and the fragmentation pattern supported the structure.

The other I C_1-esters were synthesized by similar methods.

Biological Assays—*Hamster Antifertility*—The esters were evaluated for their ability to inhibit pregnancy in adult female hamsters, as reported previously (21). The compounds were administered subcutaneously in 0.5 ml of a vehicle containing 30% ethanol and 70% physiological saline. (In some cases, the ethanol concentration was increased slightly to achieve dissolution.) The solutions were stored at −30° until used. The ester doses were equivalent to 200 μg of I/animal unless otherwise indicated. The minimum I dose giving 100% pregnancy inhibition was 200 μg. The percentage inhibition of pregnancy was determined from the number of animals pregnant in a six to 12 animal group.

Isolated Gerbil Colon—The male gerbil ascending colon was used to evaluate smooth muscle stimulating activity, as previously described (21). Generally, two colons were used for each test. The response to 3.2 and 10 ng/ml of prostaglandin E_1 was obtained, and increasing ester concentrations were tested until a response was obtained between the two standard concentrations. The standard concentration to test concentration ratio was calculated, and the activity was expressed as the range of these two ratios.

Rat Blood Pressure—Direct blood pressure measurement was made from a common carotid artery of anesthetized, mature female rats as described previously (21). Generally, only one rat was used for each test. The depressor response of 1 and 3.2 μg/kg of prostaglandin E_1 was determined following intravenous administration, and increasing test compound doses were administered intravenously until a response was obtained between the two standard doses. Activity was expressed as indicated for the gerbil colon.

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Prostaglandin Prodrugs II: New Method for Synthesizing Prostaglandin C_1-Aliphatic Esters

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Abstract □ A new method for synthesizing C_1-aliphatic esters of dinoprost and dinoprostone without using hydroxyl protective groups is described. Reaction of the prostaglandin with an alkyl halide in the presence of the sterically hindered amine *N,N*-diisopropylethylamine proceeds smoothly to give C_1-esters in various solvents at ambient or slightly elevated temperatures. Polar solvents were strongly catalytic, and even the hindered *tert*-butyl esters were synthesized by employing solvents such as dimethylformamide or dimethyl sulfoxide. Biological evaluation in the hamster antifertility assay showed that some esters maintained high bioactivity.

Keyphrases □ Prostaglandins—prodrugs, dinoprost, dinoprostone, C_1-aliphatic esters, synthesis, biological activity □ Prodrugs–dinoprost and dinoprostone, C_1-aliphatic esters, synthesis, biological activity □ Dinoprost—prodrugs, C_1-aliphatic esters, synthesis □ Dinoprostone—prodrugs, C_1-aliphatic esters, synthesis

The conversion of prostaglandins to C_1-methyl esters has resulted in increased or more rapid intestinal absorption (1, 2), as well as in greater potency following intravenous administration (3). Since few prostaglandin C_1-aliphatic esters have been reported, various aliphatic esters of dinoprost (I) and dinoprostone (II) were synthesized for evaluation of their utility as prodrugs in continuation of our effort on prostaglandin prodrugs (4).

The S_N2 reaction of inorganic metal salts of carboxylic acids and alkyl halides produces C_1-alkyl esters (5), but the

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