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.

REFERENCES

(1) T. O. Oesterling, W. Morozowich, and T. J. Roseman, *J. Pharm. Sci.*, **61**, 1861 (1972).

(2) J. R. Weeks, *Annu. Rev. Pharmacol.*, **12**, 317 (1972).

(3) J. W. Hinman, *Annu. Rev. Biochem.*, **41**, 161 (1972).

(4) W. P. Schneider, in "The Prostaglandins, Progress in Research," S. M. M. Karim, Ed., Medical and Technical Publishing, Oxford, England, 1972, p. 293.

(5) U. F. Axen, J. E. Pike, and A. W. Schneider, in "Progress in the Total Synthesis of Natural Products," vol. I, J. ApSimon, Ed., Wiley, New York, N.Y., 1973, p. 81.

(6) G. L. Bundy, *Annu. Rep. Med. Chem.*, **7**, 157 (1972).

(7) M. P. L. Caton, in "The Prostaglandins, Pharmacological and Therapeutic Advances," M. F. Cuthbert, Ed., William Heineman Medical Books, London, England, 1973, p. 1.

(8) A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).

(9) W. Morozowich, "Abstracts, 121st Annual Meeting of the American Pharmaceutical Association," 1974, p. 45.

(10) D. C. Monkhouse, L. V. VanCampen, and A. J. Aguiar, *J. Pharm. Sci.*, **62**, 576 (1973).

(11) R. G. Stehle and T. O. Oesterling (to The Upjohn Co.), U.S. pat. 3,749,800 (1974).

(12) Ono Pharmaceutical Co., South African pat. 3398 (1971).

(13) A. C. O'Rouke and J. S. Kent (to Syntex Inc.), U.S. pat. 3,826,823 (1974).

(14) D. C. Monkhouse (to Pfizer Inc.), U.S. pat. 3,851,052 (1974).

(15) J. E. Pike, F. H. Lincoln, and W. P. Schneider, *J. Org. Chem.*, **34**, 3552 (1969).

(16) Ono Pharmaceutical Co., Belgian pat. 775,106 (1972).

(17) Ono Pharmaceutical Co., Belgian pat. 776,294 (1972).

(18) A. Kapoor, *J. Pharm. Sci.*, **59**, 1 (1970).

(19) Pfizer Inc., German pat. 2,365,205 (1974).

(20) T. Gramstad and W. I. Fluglevik, *Acta Chem. Scand.*, **16**, 1369 (1962).

(21) J. R. Weeks, D. W. DuCharme, W. E. Magee, and W. L. Miller, *J. Pharmacol. Exp. Ther.*, **186**, 67 (1973).

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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Table I—Physical Constants and Biological Activity of Prostaglandin C_1-Esters

Compound	C_1-Ester	Halide Used	Conditions	Formula	Melting Point	Characteristic Mass Spectra Ions (m/e)[a]					Hamster Antifertility Assay[b]	
						M^+	$M^+ - 15$	$M^+ - 31$	$M^+ - 71$	$M^+ - 90$	Percent Non-pregnant	Dose, μg/animal, sc
IIIa	$-CH_3$	Iodide	Acetonitrile, room temperature, 1 hr	$C_{21}H_{36}O_5$	Liquid	—[c]	—	—	—	—	100	100
IIIb	$-C_2H_5$	Iodide	Dimethyl sulfoxide, room temperature, overnight	$C_{22}H_{38}O_5$	Liquid	598	583	—	508	493	100	50
IIIc	$-n-C_3H_7$	Iodide	Acetonitrile, room temperature, overnight	$C_{23}H_{40}O_5$	Liquid	612	597	—	541	522	83	50
IIId	$-iso-C_3H_7$	Bromide	Dimethyl sulfoxide, 50°, 4 hr	$C_{23}H_{40}O_5$	Liquid	612	597	—	541	522	83	50
IIIe	$-n-C_4H_9$	Iodide	Dimethyl sulfoxide, room temperature, overnight	$C_{24}H_{42}O_5$	Liquid	626	611	—	555	536	100	1000
IIIf	$-iso-C_4H_9$	Iodide	Dimethyl sulfoxide, room temperature, overnight	$C_{24}H_{42}O_5$	Liquid	626	611	—	555	536	40	100
IIIg	$-sec-C_4H_9$	Iodide	Dimethyl sulfoxide, room temperature, overnight	$C_{24}H_{42}O_5$	Liquid	626	611	—	555	536	67	100
IIIh	$-CH(C_2H_5)_2$	Iodide	Dimethyl sulfoxide, room temperature, overnight	$C_{25}H_{44}O_5$	Liquid	640	—	—	569	550	33	100
IIIi	$-n-C_6H_{13}$	Bromide	Dimethyl sulfoxide, room temperature, overnight	$C_{26}H_{46}O_5$	Liquid	654	639	—	583	564	33	100
IIIj	$-n-C_{10}H_{21}$	Iodide	Acetonitrile, room temperature, 32 hr	$C_{30}H_{54}O_5$	43.5–44.0°[d]	—[e]	—	—	—	—	83	200
IIIk	$-CH_2-C_6H_5$	Bromide	Acetonitrile, room temperature, overnight	$C_{27}H_{40}O_5$	Liquid	660	545	—	589	570	100	1000
IIIl	$-CH_2-C_6H_4-p-NO_2$	Bromide	Acetonitrile–dimethyl sulfoxide, room temperature, 3 hr	$C_{27}H_{39}NO_7$	Liquid	705	690	—	634	615	—	—
IVa	$-C_2H_5$[f]	Iodide	Dimethyl sulfoxide, room temperature, 3 hr	$C_{22}H_{36}O_5$	Liquid	553	538	522	482	463	83	1000
IVb	$-n-C_3H_7$	Iodide	Acetonitrile, room temperature, 24 hr	$C_{23}H_{38}O_5$	Liquid	567	552	536	496	477	66	1000
IVc	$-iso-C_3H_7$	Bromide	Dimethyl sulfoxide, room temperature, 24 hr	$C_{23}H_{38}O_5$	Liquid	567	552	536	496	477	100	200
IVd	$-n-C_4H_9$[f]	Iodide	Acetonitrile, room temperature, 24 hr	$C_{24}H_{40}O_5$	Liquid	581	566	550	510	491	100	1000
IVe	$-sec-C_4H_9$	Iodide	Acetonitrile–dimethyl sulfoxide (1:1), room temperature, 24 hr	$C_{24}H_{40}O_5$	Liquid	581	566	550	510	491	100	1000
IVf	$-tert-C_4H_9$	Iodide	Dimethyl sulfoxide, 40°, 3 hr	$C_{24}H_{40}O_5$	Liquid	581	566	550	510	491	17	100
IVg	$-n-C_{10}H_{21}$[f]	Iodide	Acetonitrile, room temperature, 24 hr	$C_{30}H_{52}O_5$	44.9–47.3°[d]	636	621	—	565	546	17	200[g]
IVh	$-CH_2-C_6F_5$	Bromide	Acetonitrile–dimethyl sulfoxide (1:1), room temperature, 1 hr	$C_{27}H_{33}F_5O_5$	50.8–51.8°[d]	676	661	—	605	586	17	100
IVi	$-CH_2-C_6H_4-p-NO_2$	Bromide	Acetonitrile–dimethyl sulfoxide (2:1), room temperature, 3 hr	$C_{27}H_{37}NO_7$	40.0–45.0°[d]	660	645	629	589	—	20	100

[a] Conducted on the trimethylsilyl derivatives of the II esters and on the trimethylsilyl–methoxime derivatives of the IV esters unless otherwise indicated. [b] The minimum effective doses for 100% pregnancy inhibition are 100 μg of I/animal and 200 μg of II/animal. The dose is stated as the equivalent amount of the parent prostaglandin. [c] Characterized by silica gel TLC and GLC as identical to authentic I methyl ester prepared by diazomethane (13). [d] Compound crystallized upon storage in a freezer. [e] Mass spectral analysis was conducted on nonderivatized ester. Key fragmentation pattern: m/e 476 ($M^+ - 18$), 458 ($M^+ - 36$), and 405 ($M^+ - 18 - 71$). [f] Prepared previously by the diazoalkane route (8). [g] Administered in 95% ethanol. Compound was inactive in the standard vehicle.

reaction generally is slow due to the use of a heterogeneous medium. A recent communication reported that prostaglandins could be converted rapidly to C_1-*p*-nitrophenacyl esters by reaction of the prostaglandin with *p*-nitrophenacyl bromide in the presence of a small excess of the sterically hindered amine *N,N*-diisopropylethylamine (6). The competing quaternary ammonium side reaction between this sterically hindered amine and the phenacyl halide was slower than the esterification reaction, thereby allowing successful phenacyl ester formation.

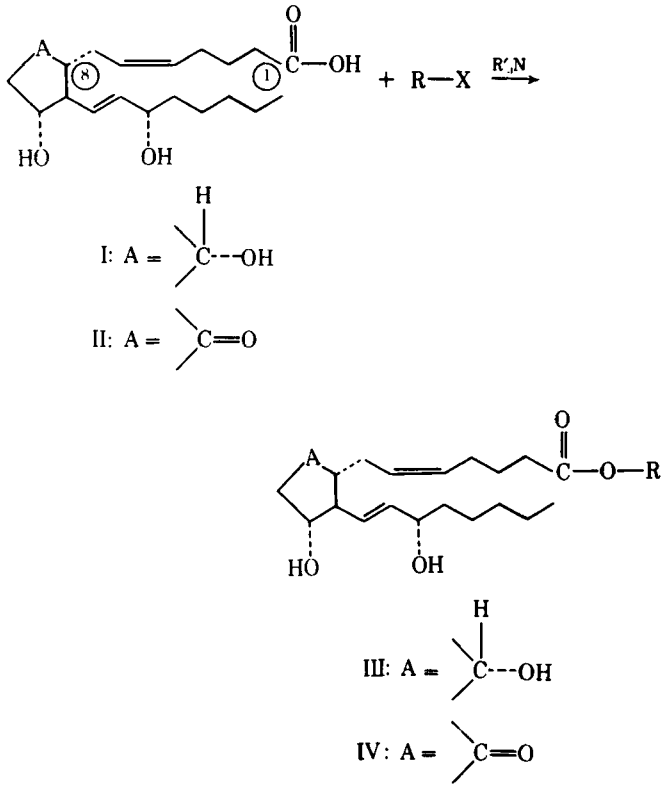
This report shows that prostaglandin C_1-aliphatic esters can be synthesized rapidly at ambient or slightly elevated temperatures using the prostaglandin, *N,N*-diisopropylethylamine, and an alkyl halide in a suitable solvent.

RESULTS AND DISCUSSION

Synthesis—The general method for synthesizing the aliphatic I and II C_1-esters involved reaction of the parent prostaglandin in a suitable solvent with a 3–10-mole alkyl halide excess in the presence of a 2–4-mole *N,N*-diisopropylethylamine excess (Scheme I).

The reaction was monitored by silica gel TLC, and greater than 90% esterification generally occurred with the linear alkyl halides in 1–24 hr at room temperature; in some cases, mildly elevated temperatures (40–50°) were employed. Polar solvents showed a remarkable catalytic effect as illustrated by the facile synthesis of the *tert*-butyl II ester. Reaction of II in dimethyl sulfoxide with *tert*-butyl iodide and *N,N*-diisopropylethylamine gave about 70% of the *tert*-butyl ester in 3 hr at 40°, as evidenced by silica gel TLC; in acetonitrile, about a 10% reaction occurred in the same time period.

Esterification of II in polar solvents generally gave a small amount (<10%) of a side product slightly less polar than the desired C_1-ester (IV). The by-product subsequently was removed by column chromatography and was identified tentatively as the II 8-iso C_1-ester since the II 8-isomer is slightly less polar than II by silica gel TLC. Previously, it was shown that prostaglandin E_1 undergoes isomerization at C_8 under mildly basic conditions (7). Presumably, the excess amine in the esterification medium was responsible for isomerization of either II or the resulting C_1-ester. Indeed, in the absence of an alkyl halide, treatment of II with a 3–20-mole excess of *N,N*-diisopropylethylamine in dimethyl sulfoxide or dimethylformamide gave about 5% of the II 8-isomer in 24 hr at room tempera-



Scheme I

ture. The II 8-isomer was identified by TLC in comparison with the authentic compound.

No II isomerization occurred in acetonitrile under the same conditions, in accord with similar observations reported previously (6).

Excepting the branched esters, the crude ester yield as shown by TLC generally was quantitative with I and about 90% with II due to formation of by-products. The esters were purified by conventional column chromatography using silica gel, and purity was verified by TLC. Table I lists the esters synthesized along with some of the mass spectral fragment ions observed. The only esters isolated as crystalline solids were the *n*-decyl esters (IV*g* and III*j*), the pentafluorobenzyl ester (IV*h*), and the *p*-nitrobenzyl ester (IV*i*).

The simplicity of the alkyl halide esterification method makes it an attractive alternative to previous methods that employed diazoalkanes (8), *N,N*-dicyclohexylcarbodiimide (9), dimethylsulfate (10), and acid chloride–carboxyl activation (11).

Biological—Preliminary prostaglandin C_1-ester screening in the subcutaneous hamster antifertility assay showed that all compounds were active in the range of 50–1000 μg (weight basis)/animal (Table I). Some of the esters, such as the isopropyl ester (III*d*), appeared to be more active than I, for which the minimal effective dose for 100% inhibition of pregnancy was 100 μg/animal.

Initial evaluation of orally administered ethyl ester (III*b*) and propyl ester (III*c*) in hamsters showed that both esters gave 33% inhibition of pregnancy at 500 μg/animal, but I was ineffective in inhibiting pregnancy at this dose. The results indicate improved oral absorption.

EXPERIMENTAL

Materials and Methods—The purity of I[1] and II[1] was verified by silica gel TLC using ethyl acetate–acetic acid (97:3). The alkyl halides were obtained commercially[2]. All solvents were glass-distilled quality[3].

Column chromatography was conducted on 0.063–0.2-mm silica gel[4]. Silica gel TLC was conducted on 250-μm layer plates[5], and visualization was achieved by spraying the developed plates with aqueous 15% ammonium sulfate followed by charring on a hot plate. The mass spectra[6] of the I esters were obtained after conversion to the trimethylsilyl derivatives using a mixture of bis(trimethylsilyl)acetamide–trimethylchlorosilane–hexamethyldisilazane–pyridine (1:1:1:3). The II esters were converted initially to the C_9-methoxime derivatives by reaction of ~1 mg of the ester with 0.2 ml of a saturated solution of hydroxylamine hydrochloride in pyridine for 6 hr at 50°. The methoxination reaction mixture was treated with 0.5–1 ml of the silylating mixture followed by mass spectral analysis.

Ester Synthesis—Representative examples of the methods for synthesizing C_1-esters are given here. All other esters were synthesized by similar procedures.

Dinoprostone Ethyl Ester (IV*a*)—A solution of 181 mg of II in a mixture of 2 ml of dimethyl sulfoxide, 0.2 ml of *N,N*-diisopropylethylamine, and 1 ml of ethyl iodide was allowed to stand at room temperature for 3 hr. Silica gel TLC (ethyl acetate–acetic acid, 97:3) showed greater than 95% conversion of II to the less polar ester. The solution was diluted with 100 ml of ethyl acetate and extracted with 100 ml of aqueous 3% citric acid, and the organic phase was dried with sodium sulfate. After solvent removal at 40° under vacuum, the brown oily residue was chromatographed over 50 g of silica gel with ethyl acetate–methanol (99:1) as the eluant. The product fractions were identified by silica gel TLC. After solvent removal, 75 mg of a colorless oil was isolated.

Dinoprost Benzyl Ester (III*k*)—A solution of 116 mg of I in a mixture of 0.2 ml of *N,N*-diisopropylethylamine, 0.3 ml of benzyl bromide, and 3 ml of acetonitrile was stored at room temperature overnight. Silica gel TLC (ethyl acetate–acetic acid, 97:3) showed quantitative esterification. After extraction and column chromatography as for IV*a*, 85 mg of a colorless oil was isolated.

Biological: Hamster Antifertility Assay—The esters were evaluated for their ability to inhibit pregnancy following subcutaneous injection in adult female hamsters in estrus as reported previously (4, 12) using an ethanol–physiological saline (3:7) vehicle.

REFERENCES

(1) W. E. Magee, S. B. Armour, and O. V. Miller, *Biochim. Biophys. Acta*, **306**, 270 (1973).

(2) A. Robert, W. E. Magee, O. V. Miller, and J. E. Nezamis, *ibid.*, **348**, 269 (1974).

(3) N. Wiqvist, J. N. Martin, M. Bygdeman, and K. Green, *Prostaglandins*, **9**, 255 (1975).

(4) W. Morozowich, T. O. Oesterling, W. L. Miller, C. F. Lawson, J. R. Weeks, R. G. Stehle and S. L. Douglas, *J. Pharm. Sci.*, **68**, 833 (1979).

(5) H. Henecka, in "Methoden Der Organischen Chemie, Houben-Weyl," vol. 8, E. Müller, Ed., G. Thieme Verlag, Stuttgart, West Germany, 1952, p. 503.

(6) W. Morozowich and S. L. Douglas, *Prostaglandins*, **10**, 19 (1975).

(7) E. G. Daniels, W. C. Krueger, F. P. Kupiecki, J. E. Pike, and W. P. Schneider, *J. Am. Chem. Soc.*, **90**, 5894 (1968).

(8) Ono Pharmaceutical Co., Ltd., German pat. 2,118,242, 1973.

(9) J. E. Pike, F. H. Lincoln, and W. P. Schneider, *J. Org. Chem.*, **34**, 3552 (1969).

(10) M. F. Jones, *J. Pharm. Pharmacol.*, **25**, 900 (1973).

(11) M. Kurono, F. Lomoto, T. Chiba, and M. Hayashi (to Ono Pharmaceutical Co.), U.S. pat. 3,821,279 (1974).

(12) J. R. Weeks, D. W. DuCharme, W. E. Magee, and W. L. Miller, *J. Pharmacol. Exp. Ther.*, **186**, 67 (1973).

(13) E. J. Corey, N. M. Weinshenker, T. K. Schaaf, and W. Huber, *J. Am. Chem. Soc.*, **91**, 5675 (1969).

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[5] Uniplate, Analtech Inc., Newark, Del.

[6] LBK 9000 spectrometer.