Synthesis of 6-(Methoxycarbonyl)prednisolone and Its Derivatives as New Antiinflammatory Steroidal Antedrugs

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Abstract □ The synthesis and pharmacological evaluation of 6-(methoxycarbonyl)prednisolone (**11**) (a 3:1 mixture of 6α-isomer **11a** and 6β-isomer **11b**), its 21-ol acetates **13a** (6α-isomer) and **13b** (6β-isomer), and 17,21-diol acetonide **14** (a 6:1 mixture of 6α-isomer **14a** and 6β-isomer **14b**) as local antiinflammatory steroidal antedrugs are described. The lead compound **11** was prepared via 12 steps from hydrocortisone (**1**). In the croton oil-induced ear edema assay, the topical antiinflammatory activity of **13a** was higher than that of its epimer **13b**. Except for **13a**, the compounds (**11**, **13b**, and **14**) showed less activity than prednisolone. The systemic activities were assessed after 5 days of consecutive administration of these compounds at equiactive doses. Neither **11** nor **14** depressed plasma corticosteroid levels or significantly altered adrenal weights. Thymic involution was absent for **14**, 15% for **11**, and 47% for prednisolone at the equiactive doses. Both **13a** and **13b** showed significant reduction of adverse systemic effects assessed as the increase of body weight and the decreases of adrenal and thymus weights. The putative metabolite, carboxylic acid **12**, showed 26 times less topical antiinflammatory activity than prednisolone. These results suggest that introduction of a labile methoxycarbonyl group at the C-6 position of prednisolone results in retention of antiinflammatory activity while reducing systemic effects noted following topical application of the parent compound prednisolone.

Introduction

The use of corticosteroids in the treatment of chronic inflammatory disorders has been limited due to their systemic adverse effects.[1] To overcome this limitation, efforts toward discovery of safer antiinflammatory steroids separating local antiinflammatory activity from potentially harmful side effects have been made in our laboratory. The prototypes steroidal 21-carboxylic ester [11β,17α-dihydroxy)-21-(methoxycarbonyl)-pregna-1,4-dien-3,20-dione] and epimeric 20-hydroxy 21-carboxylic esters [(20*R*)- and (20*S*)-11β,17α,20-trihydroxy-21-(methoxycarbonyl)pregna-1,4-dien-3-one] retained the significant local antiinflammatory activity of prednisolone, but were devoid of prednisolone-like systemic side effects, such as pituitary adrenal suppression and thymus involution.[5-7] A new generation of steroid acid esters possess a metabolically labile methoxycarbonyl moiety at the 16-position. 11α,17α,21-trihydroxy-16α-(methoxycarbonyl)pregna-1,4-diene-3,20-dione (P16CM) exhibited 14 times more topical activity than prednisolone in the rat croton oil-induced ear edema assay and showed significantly reduced systemic side effects.[2-4] The absence of systemic activities of those steroidal acid esters was ascribed to the biologically labile carboxy ester moiety.[2-8] The term antedrug was introduced by Lee and Soliman to define an active synthetic derivative which exerts its action locally at the target, but is rapidly metabolized to an inactive metabolite upon entry into the systemic circulation.[5] Thus a true antedrug acts only locally. The excellent separation of the systemic activity from topical activity was possible for 17α-(alkoxycarbonyl)prednisolone.[8] As an extension of the study, we introduced an alkoxycarbonyl group at the C-6 position of prednisolone. It is well-documented that glucocorticoids with a 6α-methyl or 6α-fluoro substituent usually exhibit modest increases of relative receptor affinity and antiinflammatory activity, and the substitution at the C-6 position of glucocorticoids also helps to increase their half-life by inhibiting C-6 hydroxylation, which is a primary metabolic pathway of glucocorticoids.[9] The current investigation is a part of our ongoing efforts to elucidate structure–activity relationships of steroidal carboxy esters designed on the basis of the "antedrug concept."

Results and Discussion

Chemistry—The side chain of hydrocortisone **1** was protected by treatment with formaldehyde and concentrated HCl in chloroform to afford cortisol bis(methylenedioxy) ether **2**.[10] The α,β-unsaturated enone of **2** was converted to the β,γ-unsaturated cyclic ketal to give compound **3**,[11] which was oxidized with *m*-chloroperbenzoic acid to give epoxide **4** as a 1:1 mixture of α- and β-epoxide (**4a** and **4b**).[12] The nucleophilic ring opening of the α-epoxide **4a** with vinylmagnesium bromide provided the corresponding alcohol **5**. The oxidative cleavage of the terminal alkene of **5** with $RuCl_3$ and $NaIO_4$ in a 2:1 mixture of acetone and water led to the carboxylic acid **6a** with a concomitant oxidation of 11-alcohol to 11-ketone.[14] The oxidation of **5** with $RuCl_3$ and $NaIO^4$ in a 2:2:3 mixture of CH_3CN–CCl_4–H_2O led to the corresponding aldehyde, and no further oxidation to the carboxylic acid occurred.[13] The crude acid was esterified with diazomethane in methanol to 6β-methyl ester **6b** in 80% yield. Deprotection of the cyclic ketal of **6b** with 2 N sulfuric acid in acetone gave the corresponding 3-ketone **7** in 87% yield.[15] Dehydration of the 5α-hydroxy ketone 7 in methanolic KOH followed by reesterification of the hydrolyzed 6-carboxylic acid gave a 2:1 epimeric mixture of γ-(methoxycarbonyl)-α,β-unsaturated ketones **8a** and **8b**.[16] The structures of the compounds **8a** and **8b** were assigned on the basis of the chemical shifts of vinyl hydrogen H_4. The signals of vinyl hydrogen H_4 for 6α- and 6β-(carboxymethyl)testosterone appear at δ 5.73 and 5.88, respectively.[14] Analogously, the upfield vinyl H_4 (δ 5.52) was assigned to compound **8a** and the downfield vinyl H_4 (δ 5.90) to compound **8b**. Treatment of the mixture of **8a** and **8b** with selenium dioxide and pyridine in *t*-BuOH afforded dienone **9** (2:1 mixture of **9a** and **9b**).[17] Reduction of the 11-ketone of the 2:1 mixture of **9a** and **9b** with $NaBH_4$ gave the 11β-hydroxy derivative **10** (2:1 mixture of **10a** and **10b**).[18] Deprotection of the side chain with aqueous formic acid gave a 3:1 mixture of **11a** and **11b** (P6CM **11**), which was inseparable (Scheme 1).[19]

The carboxylic acid **12**, a putative metabolite of **11**, was obtained by hydrolysis of **11**. The acylation of the 21-OH of **11** with pyridine and acetic anhydride gave a 3:1 mixture of 21-ol acetates **13a** and **13b** (P6CM-21Ac **13**).[20] The mixture was separated by flash column chromatography on silica gel (2:3 hexane–ethyl acetate). A treatment of P6CM **11** with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid in DMF gave a 6:1 mixture of the corresponding acetonides

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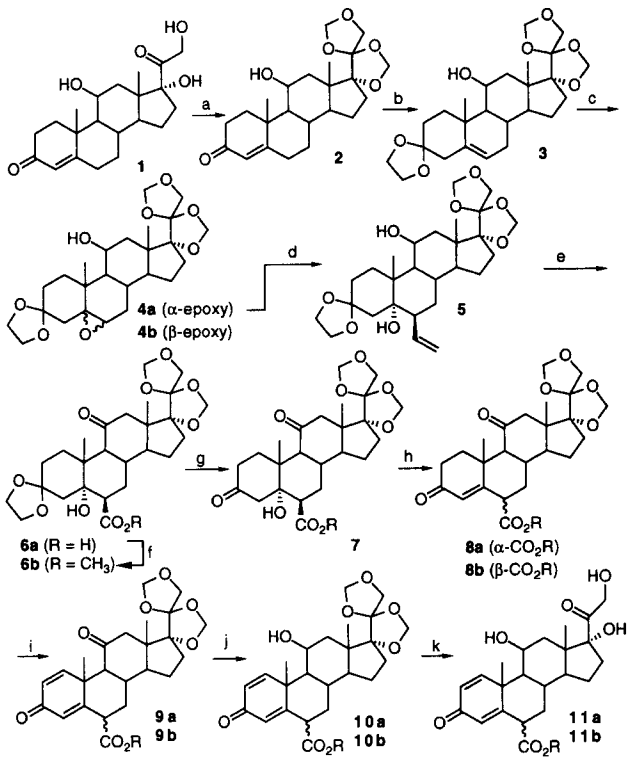
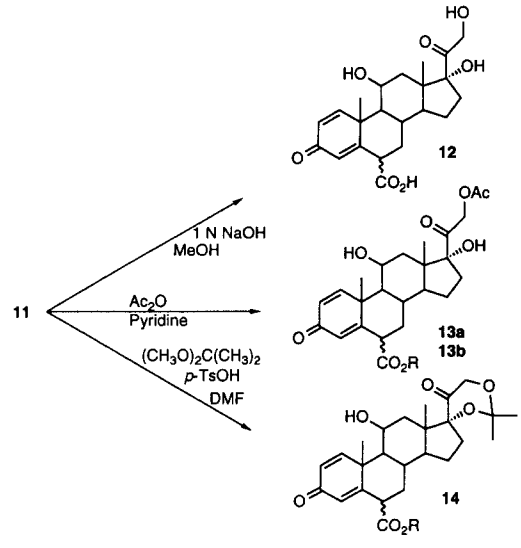


Scheme 1[a]

[a] (a) aqueous HCl, aqueous CH_2O, $CHCl_3$; (b) pyridine hydrochloride, ethylene glycol, benzene; (c) *m*-CPBA, methylene chloride, (d) vinylmagnesium bromide, THF; (e) $RuCl_3$, $NaIO_4$, acetone–water; (f) CH_2N_2, methanol; (g) sulfuric acid, acetone; (h) KOH, MeOH; CH_2N_2, MeOH; (i) SeO_2, pyridine, *t*-BuOH; (j) $NaBH_4$, DMF-MeOH; (k) HCO_2H, H_2O.



Scheme 2

14a and 14b (Scheme 2), which was used without separation for pharmacological tests.[21] The structures of compounds **9a,b**–**14a,b** were assigned in a manner similar to those of compounds **8a** and **8b**.

Antiinflammatory Activity—Dose–response curves of the inhibitory effect of 11 and its 21-ol acetates (**13a** and **13b**) and 17,21-diol acetonide 14, in the rat croton oil-induced ear edema bioassay, are compared with that of prednisolone (**P**) in Figure 1. Following a single topical application, treatment with all compounds resulted in dose-dependent inhibition of edema.

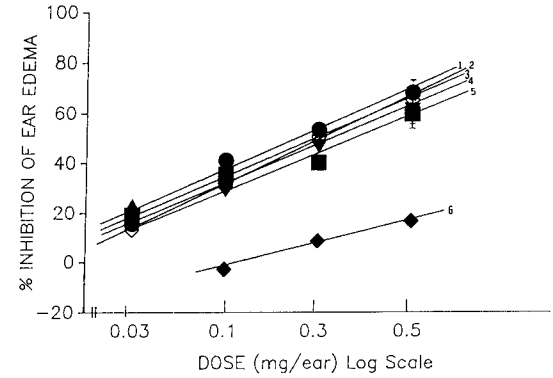


Figure 1—Inhibition of croton oil-induced ear edema in rats after a single topical application of **13b** (1, ▲), **14** (2, ◇), prednisolone (3, ●), **11** (4, ▼), **13a** (5, ■), and **12** (6, ◆). Each point represents the mean ± SEM of five animals.

From the dose–response profiles, the following ID_{50} values (nmol resulting in a 50% reduction of edema) were calculated: 540, 770, 720, 490, and 550 nmol/ear for **P**, **11**, **13a**, **13b**, and **14**, respectively. The topical antiinflammatory activity of **11**, **13a**, and **14** was slightly less than that of prednisolone. But the topical antiinflammatory activity of **13b** was higher than that of prednisolone. Compound **13b**, which has a 6β-methoxycarbonyl group, showed higher topical antiinflammatory activity than its C6-epimer **13a**. The carboxylic acid **12**, a putative metabolite of **11**, showed very weak antiinflammatory activity at 0.5 mg/ear. These results indicate that incorporation of the 6-methoxycarbonyl moiety into prednisolone neither increases the antiinflammatory potency nor significantly decreases the potency compared to the parent compound prednisolone.

Parameters of undesirable systemic effects such as a decrease in body weight gain, decreases in thymus and adrenal weights, and suppression of plasma corticosterone levels were assessed following topical application for 5 consecutive days at equiactive doses of prednisolone and the C-6 ester derivatives in the rat croton oil-induced ear edema assay model as shown in Table 1.

Prednisolone caused significant reduction in plasma corticosterone and adrenal and thymus weights and normal body weight increase. Alteration in body weight gain was only seen with **14** (14% vs 31% for prednisolone), and a reduction in thymic weight was noted only following treatment with **11** (15% vs 47% for prednisolone). Plasma corticosterone levels were decreased for **13a** and **13b** (50% and 60%, respectively, vs 87% for prednisolone). Taken together, these results indicate that **11**, **13a**, **13b**, and **14** show dramatic reduction of systemic side effects such as pituitary–adrenal axis suppression and thymus atrophogenic effects following multiple topical applications.

We have previously synthesized 16-(methoxycarbonyl)prednisolone (P16CM), which retained local antiinflammatory activity in various animal models but were devoid of systemic side effects.[2-4] Absence of side effects has been attributed to their rapid hydrolysis to steroid acids which are inactive and readily excreted in accord with our recently developed antedrug concept. The acid **12**, a proposed metabolite of **11**, was screened for topical antiinflammatory activity and found by extrapolation to be roughly 26 times less potent (topically) than the parent drug **11**. The metabolism of **11** is currently under investigation.

In summary, introduction of a methoxycarbonyl group at the C-6 position of prednisolone as in **11** affords retention of antiinflammatory activity concomitant with a desirable reduction of systemic side effects following topical applications.

Experimental Section

Melting points were determined on a Thomas capillary melting point apparatus and were uncorrected. The ^1H NMR spectra weer obtained

Table 1—Effects of Steroids in the Rat Croton Oil-Induced Ear Edema Assay following Multiple Topical Applications[d]

Drug	Dose (nmol/ear)	Body Weight (% of control)	Relative Weight (% of control)		Plasma Corticosterone (% of control)
			Thymus	Adrenal	
P[a]	540	68.6[c]	53.2[c]	82.6[c]	12.8[c]
11[a]	720	85.7	84.5[c]	91.3	92.6
13a[a]	770	97.1	95.5	100.0	50.0[c]
13b[a]	490	120.0	95.7	91.3	40.5[c]
14[b]	550	85.6[c]	93.0	116.3	90.8

[a] Control values: body weight gain, 35 ± 3 g; thymus weight, 601 ± 28 mg; adrenal weight, 23 ± 2 mg; and plasma corticosterone, 399 ± 50.4 ng/mL. [b] Control values: body weight gain, 38.8 ± 2.7 g; thymus weight, 663 ± 24 mg; adrenal weight, 22.7 ± 1.7 mg; and plasma corticosterone, 332.1 ± 83.2 mg/mL. [c] Significant difference at $P < 0.05$ (Student's t test). [d] Values indicate the average percent of control with six animals per group.

with a Bruker HX-270 spectrometer, and the chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnigan 4510 GCMS, using positive chemical ionization. Silica gel (Merck, 70–230 mesh) was used for flash column chromatography. The homogeneity of intermediates and products was determined by TLC on Merck 60F-254 plates with visualization under UV light. Elemental analysis were performed for the stated elements by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of calculated values for the stated empirical formulas unless otherwise indicated.

Syntheses. 11β-Hydroxy-17α,20:20,21-bis(methylenedioxy)pregn-4-en-3-one (2)[10]—To a solution of 20.0 g (55.2 mmol) of hydrocortisone (1) in 800 mL of chloroform was added 200 mL of formalin and 200 mL of concentrated HCl. The resulting solution was stirred at room temperature for 48 h. The $CHCl_3$ layer was separated, washed with 5% $NaHCO_3$ (3 × 200 mL) and with H_2O (3 × 200 mL), dried (Na_2SO_4), and concentrated *in vacuo* to gie a yellowish solid. Recrystallization with 100 mL of acetone gave 8.70 g (39%) of **2**. Mother liquor was evaporated and the resulting yellowish solid was recrystallized from acetone to give another 3.30 g (14.8%) of **2**: mp 217–222 °C; ^{1}H NMR δ ($CDCl_3$) 1.15 (s, 3H, 13-CH_3), 1.45 (s, 3H, 10-CH_3), 4.0 (m, 2H, 21-CH_2), 4.45 (m, 1H, 11-CH), 5.0, 5.22 (s, 4H, 2 CH_2 from bis(methylenedioxy)), 5.7 (s, 1H, 4-H).

3,3-(Ethylenedioxy)-17α,20:20,21-bis(methylenedioxy)pregna-5-en-11β-ol (3)—Ethylene glycol (25 mL) and pyridine hydrochloride (100 mg) were added to a solution of **2** (5 g, 12.4 mmol) in 200 mL of benzene and the mixture was heated at reflux with continuous removal of H_2O for 24 h. The reaction mixture was diluted with ethyl acetate, washed with 5% $NaHCO_3$ (3 × 150 mL) and H_2O (3 × 150 mL), and dried (Na_2SO_4). After evaporation of the solvent, the product was submitted to column chromatography on silica gel (2:1 hexane–ethyl acetate) to give 4.5 g (81%) of **3**, which was purified by recrystallization from MeOH: mp 167–169 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 3H, 13-CH_3), 1.30 (s, 3H, 10-CH_3), 4.0 (m, 6H, ethylenedioxy and 21-CH_2), 4.40 (m, 1H, 11-CH), 4.96, 5.20 (m, 5H, vinyl H and 2 CH_2 from bis(methylenedioxy)).

17α,20:20,21-Bis(methylenedioxy)-5α,6α-epoxy-3,3-(ethylenedioxy)-11β-hydroxypregnane (4)—To a solution of **3** (5.0 g, 11.2 mmol) in 100 mL of CH_2Cl_2 at 5 °C was added a solution of *m*-chloroperbenzoic acid (4.21 g, 13.44 mmol) in 30 mL of CH_2Cl_2. When the addition was complete, the reaction mixture was allowed to stand at 5 °C for 16 h, warmed to room temperature and diluted with ethyl acetate. The ethyl acetate solution was washed with 5% $NaHSO_3$ (3 × 100 mL), and with water (3 × 150 mL) and dried (Na_2SO_4). After evaporation of the solvent, the crude product obtained was purified by flash column chromatography on silica gel (2:1 hexane–ethyl acetate). The laser polar product was 5,6β-epoxy-3,3-(ethylenedioxy)-11β-hydroxy-17α,20:20,21-bis(methylenedioxy)pregnane (**4b**) (2.4 g, 41.6%): ^{1}H NMR ($CDCl_3$) δ 1.08 (s, 3H, 13-CH_3), 1.30 (s, 3H, 10-CH_3), 3.08 (m, 1H, 6α-CH), 3.90 (m, 6H, 2 CH_2 from ethylenedioxy, 21-CH_2), 4.25 (m, 1H, 11-CH), 4.98–5.25 (m, 4H, 2 CH_2 from bis(methylenedioxy)). The more polar product was 5,6α-epoxy-3,3-(ethylenedioxy)-11β-hydroxy-17α,20:20,21-bis(methylenedioxy)pregnane (**4a**) (2.4 g, 41.6%): mp 196–198 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 3H, 13-CH_3), 1.35 (s, 3H, 10-CH_3), 2.78 (m, 1H, 6β-CH), 3.95 (m, 6H, 2 CH_2 from ethylenedioxy, 21-CH_2), 4.30 (bs, 1H, 11-CH), 5.0–5.25 (m, 4H, 2 CH_2 from bis(methylenedioxy)).

17α,20:20,21-Bis(methylenedioxy)-5α,11β-dihydroxy-3,3-(ethylenedioxy)-6β-vinylpregnane (5)—To a solution of **4a** (5.0 g, 10.7 mmol) in 80 mL of anhydrous THF was added dropwise a solution of vinylmagnesium bromide in THF (1.0 M, 40 mL, 40 mmol). When the addition was complete, the mixture was heated at reflux overnight. To the reaction mixture was added 20 mL of cold saturated NH_4Cl solution. The resulting solution was extracted with ethyl acetate (3 × 150 mL). The combined organic layer was washed with 5% $NaHCO_3$ solution (3 × 100 mL) and with water (3 × 100 mL) and dried (Na_2SO_4). After evaporation of the solvent, the crude product was purified by flash column chromatography on silica gel (2:1 hexane–EtOAc) to give crystaline solid which was recrystallized to give pure **5** (4.0 g, 75%): mp 177–183 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (s, 3H, 13-CH_3), 1.18 (s, 3H, 10-CH_3), 3.95 (m, 6H, 2 CH_2 from ethylenedioxy, 21-CH_2), 4.25 (m, 1H, 11-CH), 4.30 (s, 1H, 5-OH), 4.90–4.96 (m, 2H, CH=CH_2), 5.00–5.25 (m, 4H, 2 CH_2 from bis(methylenedioxy)), 6.00 (m, 1H, CH=CH_2); MS, *m/e* (relative intensity) 475.4 (36.94, $M^+ - H_2O$), 456.9 (100, $M^+ - 2H_2O$).

17α,20:20,21-Bis(methylenedioxy)-3,3-(ethylenedioxy)-5α-hydroxy-6β-(methoxycarbonyl)pregnan-11-one (6)—To a solution of **5** (5.0 g, 10.2 mmol) in 60 mL of acetone was added a yellow solution of RuO_4–$NaIO_4$ (prepared from 150 mg of $RuCl_3$, 6.0 g of $NaIO_4$, and 30 mL of water). After 1 h, a second portion of the catalyst solution was added. After an additional 1 h, 5 mL of isopropyl alcohol was added. The reaction mixture was stirred for 15 min and then filtered and the solvent was evaporated below 40 °C *in vacuo*. The residue was diluted with water and extracted with ethyl acetate (3 × 150 mL). The combined ethyl acetate solution was washed with water (3 × 100 mL) and dried (Na_2SO_4). The solvent was removed under vacuum to provide a yellow oil which was purified by flash column chromatography on silica gel (2:1 hexane–ethyl acetate) to give a white solid 4.0 g (77%) of **6a**. The solid was dissolved in 100 mL of methanol and esterified with diazomethane in ether (20 mL) at 0 °C. After stirring for 30 min, the solvent was removed under vacuum to produce a yellowish solid. The solid was purified by flash column chromatography on silica gel (2:1 hexanee–ethyl acetate) to give 2.5 g (61%) of **6b** as a white amorphous solid: mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 0.8 (s, 3H, 13-CH_3), 1.05 (s, 3H, 10-CH_3), 3.65 (s, 3H, $COOCH_3$), 3.97 (m, 6H, 2 CH_2 from ethylenedioxy, 21-CH_2), 4.65 (s, 1H, 5-OH), 5.00–5.25 (m, 4H, 2 CH_2 from bis(methylenedioxy)); MS, *m/e* (relative intensity) 505 (100, $M^+ - H_2O$).

17α,20:20,21-Bis(methylenedioxy)-5α-hydroxy-6β-(methoxycabonyl)pregnane-3,11-dione (7)—To a solution of **6b** (1.0 g, 1.91 mmol) in 30 mL of acetone was added to 1 mL of 2 N H_2SO_4. After stirring for 1 h at room temperature, the reaction mixture was diluted with distilled water (300 mL), neutralized with 5% $NaHCO_3$ solution, and then extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with water (3 × 100 mL) and dried (Na_2SO_4). Evaporation of the solvent gave 0.79 g (87%) of **7** as a white solid: mp 239–241 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3H, 13-CH_3), 1.26 (s, 3H, 10-CH_3), 3.72 (s, 3H, $COOCH_3$), 3.97 (m, 2H, 21-CH_2), 5.00–5.25 (m, 4H, 2CH_2 from bis(methylenedioxy)); MS, *m/e* (relative intensity) 479.4 (17.85, $M^+ + 1$), 460.9 (100, $M^+ - H_2O$).

17α,20:20,21-Bis(methylenedioxy)-6-(methoxycarbonyl)pregn-4-ene-3,11-dione (8)—To a suspension of **7** (300 mg, 0.63 mmol) in 10 mL of methanol was added 5 mL of 0.5% KOH solution in methanol. The reaction mixture was heated at reflux for 1 h. The solution was diluted with water (90 mL), acidified with 1 N HCl, and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with water and dried (Na_2SO_4). The solvent was evaporated *in vacuo* to give a white solid. The solid was dissolved in 10 mL of methanol and reesterified with diazomethane in ether (5 mL) at 0 °C for 30 min. The solution was evaporated *in vacuo* and the residue was purified by flash column chromatography on a silica gel column (2:1 hexane–ethyl acetate) to

give 27.4 mg (85%) of 8 (a 2:1 mixture of **8a** and **8b**): mp 128–131 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3H, 13-CH_3), 1.23 (s, 3H, 10-CH_3), 3.67 (s, 3H, $COOCH_3$ of **8a**), 3.74 (s, 3H, $COOCH_3$ of **8b**), 3.97 (m, 2H, 21-CH_2), 4.95–5.15 (m, 4H, 2 CH_2 from bis(methylenedioxy)) 5.52 (s, 1H, 4- CH of **8a**), 5.90 (s, 1H, 4-CH of **8b**); MS, *m/e* (relative intensity) 460.9 (100, $M^+ + 1$).

17α,20:20,21-Bis(methylenedioxy)-6-(methoxycarbonyl)pregna-1,4-diene-3,11-dione (9)—To a solution of 8 (300 mg, 0.65 mmol) in 20 mL of anhydrous *t*-BuOH were added selenium dioxide (180 mg, 1.62 mmol) and pyridine (0.06 mL, 0.74 mmol). The solution was heated at reflux for 30 h under nitrogen, cooled, and filtered through Celite and the residue was washed with hot ethyl acetate. The combined filtrate was evaporated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with water. The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give a sticky solid, which was purified by flash column chromatography on silica gel (2:1 hexane–ethyl acetate) to give 180 mg (60.3%) of **9** (a 2:1 mixture of **9a** and **9b**) as a white amorphous solid: mp 240–241 °C dec; ^{1}H NMR ($CDCl_3$) 0.87 (s, 3H, 13-CH_3), 1.30 (s, 3H, 10-CH_3), 3.70 (s, 3H, $COOCH_3$ of **9a**), 3.79 (s, 3H, $COOCH_3$ of **9b**), 3.97 (m, 2H, 21-CH_2), 5.00–5.20 (m, 4H, 2 CH_2 from bis(methylenedioxy)), 5.9 (s, 1H, 4-CH of **9a**), 6.22 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH), 6.25 (s, 1H, 4-CH of **9b**), 7.85 (d, J = 10.0 Hz, 1H, 1-CH); MS, *m/e* (relative intensity) 459.0 (100, $M^+ + 1$).

17α,20:20,21-Bis(methylenedioxy)-11α-hydroxy-6-(methoxycarbonyl)pregna-1,4-dien-3-one (10)—To a solution of **9** (968 mg, 2.11 mmol) in a mixture of DMF (26 mL), methanol (30 mL) and water (3 mL) under nitrogen was added $NaBH_4$ (0.212 g, 5.6 mmol) at 0–2 °C. After 20 min, 1 N HCl (6 mL) was added and the reaction mixture was added to the saturated NaCl solution. The reaction mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash column chromatograph on silica gel (2:1 hexane–ethyl acetate) to give 729 mg (75%) of **10** (a 2:1 mixture of **10a** and **10b**) as a white amorphous solid: mp 123–125 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H, 13-CH_3), 1.24 (s, 3H, 10-CH_3), 3.67 (s, 3H, $COOCH_3$ of **10a**), 3.75 (s, 3H, $COOCH_3$ of **10b**), 3.97 (m, 2H, 21-CH_2), 4.45 (bs, 1H, 11-CH), 5.00–5.20 (m, 4H, 2 CH_2 from bis(methylenedioxy)), 5.82 (s, 1H, 4-CH of **10a**), 6.20 (s, 1H, 4-CH of **10b**), 6.30 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH) and 7.24 (d, J = 10.0 Hz, 1H, 1-CH); MS, *m/e* (relative intensity) 460.9 (100, $M^+ + 1$).

11β,17α,21-Trihydroxy-6-(methoxycarbonyl)pregna-1,4-diene-3,20-dione (11, P6CM)—A suspension of **10** (99.6 mg, 0.216 mmol) in 9 mL of 40% formic acid was heated at 70 °C under nitrogen for 2.5 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated *in vacuo*. The residue was diluted with 100 mL of distilled water and the aqueous solution was extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid which was purified by flash column chromatography (2:1 hexane–ethyl acetate) to give 81.8 mg (90%) of **11** (a 3:1 mixture of **11a** and **11b**): mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3H, 13-CH_3), 1.50 (s, 3H, 10-CH_3), 3.65 (s, 3H, $COOCH_3$ of **11a**), 3.76 (s, 3H, $COOCH_3$ of **11b**), 4.25 (d, J = 18.8 Hz, 1H, 21-CH_2), 4.70 (d, J = 18.8 Hz, 1 H, 21-CH_2), 4.45 (bs, 1H, 11-CH), 5.82 (s, 1H, 4-CH of **11a**), 6.20 (s, 1H, 4-CH of **11b**), 6.25 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH), 7.24 (d, J = 10.0 Hz, 1H, 1-CH); MS, *m/e* (relative intensity) 419.1 (71.15, $M^+ + 1$), 359.0 (100, $M^+ - C_2H_3O_2$).

6-Carboxy-11β,7α,21-trihydroxypregna-1,4-diene-3,20-dione (12)—To a solution of 11 (30 mg, 0.07 mmol) in 5 mL of methanol was added 0.1 mL of 1 N NaOH under nitrogen. The mixture was stirred for 1 h at room temperature, acidified with 0.15 mL of 1 N HCl, and poured into 100 mL of distilled water. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with distilled water (2 × 50 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give a yellowish solid which was purified by flash column chromatography on silica gel (3:5 benzene–ethyl acetate) to give 25 mg (74%) of **12** (a 3:1 mixture of **12a** and **12b**): mp 197–200 °C; ^{1}H NMR (CD_3OD) δ 1.03 (s, 3H, 13-CH_3), 1.52 (s, 3H, 10-CH_3), 4.35 (bs, 1H, 11-CH), 4.7–4.95 (2 d, J = 18.8 Hz, 2H, 21-CH_2), 5.75 (s, 1H, 4-CH of **12a**), 6.15 (s, 1H, 4-CH of **12b**), 6.25 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH), 7.45 (d, J = 10.0 Hz, 1H, 1-CH), 7.90 (s, 1H, COO*H*).

21-Acetoxy-11β,17α-dihydroxy-6-(methoxycarbonyl)pregna-1,4-diene-3,20-dione (13)—To a mixture of pyridine (1.0 mL, dried over 4A molecular sieve) and acetic anhydride (0.1 mL) was added 100 mg (0.24 mmol) of **11**. The reaction mixture was stirred for 2 h at room temperature, poured into 30 mL of 0.1 M HCl, and extracted with ethyl acetate. The organic phase was washed with 0.1 M HCl (20 mL) and with distilled water (2 × 20 mL), dried (Na_2SO_4), and evaporated *in vacuo* to give a white solid which was purified by flash column chromatography on silica gel (2:3 hexane–ethyl acetate) to give 76 mg (69%) of **13a** (less polar) and 25.3 mg (23%) of **13b** (more polar).

The data for **13a**: mp 113–115 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, 13-CH_3), 1.45 (s, 3H, 10-CH_3), 2.22 (s, 3H, 21-O*Ac*), 3.75 (s, 3H, $COOCH_3$), 4.45 (m, 1H, 11-CH), 4.85 (d, J = 18.8 Hz, 1H, 21-CH_2), 4.95 (d, J = 18.8 Hz, 1H, 21-CH_2), 5.82 (m, 1H, 11-CH), 6.22 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH), 7.24 (d, J = 10.0 Hz, 1H, 1-CH); MS, *m/e* (relative intensity), 461 (100, $M^+ + 1$).

The data for **13b**: mp 115–117 °C; ^{1}H NMR($CDCl_3$) 0.93 (s, 3H, 13-CH_3), 1.32 (s, 3H, 10-CH_3), 2.15 (s, 3H, 21-O*Ac*), 3.62 (s, 3H, $COOCH_3$), 4.45 (m, 1H, 11-CH), 4.85 (d, J = 18.8 Hz, 1H, 21-CH_2), 4.95 (d, J = 18.8 Hz, 1H, 21-CH_2), 6.15 (m, 1H, 4-CH), 6.25 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH) and 7.24 (d, J = 10.0 Hz, 1H, 1-CH); MS, *m/e* (relative intensity 460.9 (100, $M^+ + 1$).

11β-Hydroxy-6-(methoxycarbonyl)pregna-1,4-diene-3,20-dione 17α,21-Acetonide (14)—To a solution of 11 (50 mg, 0.12 mmol) in 5 mL of DMF was added 0.5 mL of 2,2-dimethyloxypropane and 2.5 mg of *p*-toluenesulfonic acid under nitrogen. The mixture was stirred at 80 °C for 2 h, concentrated *in vacuo*, and then poured into 100 mL of distilled water. The solution was extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with distilled water, dried (Na_2SO_4), and evaporated *in vacuo* to give crude product which was purified by flash column chromatography on silica gel (2:1 hexane–ethyl acetate) to give 44.9 mg (82%) of **14** (a 6:1 mixture of **14a** and **14b**) as a crystaline solid: mp 132–135 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3H, 13-CH_3), 1.47 (s, 3H, 13-CH_3), 1.35 (s, 3H, CH_3 from acetonide), 1.40 (s, 3H, CH_3 from aceton ide), 3.75 (s, 3H, $COOCH_3$), 4.03 (d, J = 18.8 Hz, 1H, 21-CH_2), 4.28 (d, J = 18.8 Hz, 1H, 21-CH_2) 4.50 (bs, 1H, 11-CH), 5.85 (s, 1H, 4-CH of **14a**), 6.20 (s, 1H, 4-CH of **14b**), 6.30 (dd, J = 10.0 Hz, 2.1 Hz, 1H, 2-CH), 7.24 (d, J = 10.0 Hz, 1H, 1-CH); MS, *m/e* (relative intensity) 459 (100, $M^+ + 1$).

Croton Oil-Induced Ear Edema Assay—Effects of topically applied steroids on edema formation were assessed, using a modified version of the croton oil-induced ear edema assay of Tonneli et al. as described by Heiman et al.[4] Briefly, initial ear thicknesses of 100-g Sprague–Dawley male rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were measured with a spring-loaded micrometer (Lux Scientific Instruments). Then, 25 μL of indicated amounts of steroids, dissolved in acetone, was applied to the right ears of rats and 25 μL of vehicle applied to the left ears. Thirty minutes later, a 5% solution of croton oil in acetone was applied in the same manner. Five hours later, at peak inflammation, ear thicknesses were remeasured. Percent inhibition of edema formation was determined by comparing the ear thickness of steroid-treated animals with that of control animals. The dose which inhibited edema formation by 50% (ID_{50}) was determined from the linear portion of log dose versus percent inhibition plots by best-fit regression analysis.

For multiple topical application studies, drugs were applied as above to the animals' right ears for 5 days. Five hours following the final treatment, right ears were measured. Blood samples were collected by cardiac puncture for plasma corticosterone measurements and the thymus glands were removed and weighed as a measure of thymolytic activity.

Statistical Analysis. All data are presented as mean values of five or six samples ± SEM. The ANOVA analysis, followed by least-squares differences between means subtest, was used to determine significant differences between groups at $p < 0.05$.

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