Soft Drugs Based on Hydrocortisone: The Inactive Metabolite Approach and Its Application to Steroidal Antiinflammatory Agents

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Purpose. The soft drug approach was applied to the design of analogs of highly potent synthetic steroids but with a metabolically labile ester group which at the same time served as an activating group.
Methods. Several structural modifications of soft antiinflammatory steroids were synthesized and tested in several assays of biological activity. The hydrolytic stability of the compounds was also determined.
Results. One of the compounds synthesized was determined to be a very potent steroid and had a highly significant separation of topical from systemic activity. However, the compound demonstrated greater than expected stability in the hydrolysis studies.
Conclusions. The goal of the soft drug approach has been achieved with the development of a highly potent drug which displays little or no systemic activity as measured in the tests presented here. The anticipated hydrolytic instability of the compounds was not corroborated; however, in view of other results, the interpretation is allowed that the rapid hydrolysis of the unbound fraction of the drug is an important factor in its lack of systemic effects.

KEY WORDS: drug design; soft drugs; antiinflammatory steroids; structure-activity relations.

INTRODUCTION

The many goals of drug design can perhaps best be subsumed under the heading of the improvement of the therapeutic index, which is defined as the ratio of the toxic to the therapeutic dose. This issue is specifically addressed by the soft drug approach (1,2), in which the predicted metabolic fate of the potential drug is incorporated into the design stage of its development. The modern antiinflammatory steroids are based upon the activity of the natural hormone hydrocortisone. However, their design was based largely on the protection of the target compound from metabolic inactivation, resulting in what have been termed "hard" drugs. The design of new compounds using the soft drug approach is philosophically in direct contrast with this more traditional approach.

The existence of both glucocorticoid and mineralocorticoid receptors made possible the improvement of the therapeutic index in this series of compounds by increasing the relative receptor selectivity (3). However, since the glucocorticoid receptor is the effector of both topical antiinflammatory and systemic glucocorticoid activity (4), improvement of the therapeutic index in this series must be approached through the modulation of pharmacokinetic rather than pharmacodynamic parameters (5). In topical administration there exists a natural barrier (the skin) to the systemic effects of the steroids, but even this therapy is accompanied by systemic side effects (6).

Approximately 10–25% of the metabolism of hydrocortisone is through oxidation of the 17β-side chain to give carboxylic acid metabolites (7), which are inactive, water soluble, and rapidly eliminated. In designing soft drugs based on these metabolites, we chose to activate them through the formation of 17β-carboxylic esters which we anticipated would be sufficiently similar to the natural dihydroxyacetone group to impart activity as well as to hydrolyze rapidly upon reaching the systemic circulation. In consonance with the soft drug approach, we were careful to modify the rest of the hydrocortisone structure as little as possible, to avoid toxicity due to forcing the derivative to be metabolized through unpredictable routes. The biolabile groups which were added were the methylthiomethyl (8), the carboalkoxymethyl (9), and the chloromethyl (10). Compounds of this type of have been prepared before, for example, in the synthesis of the n-butyl ester of the 21-acid metabolite of Fluocortolone (11,12), the syntheses of haloalkyl esters of 17α-acyl-17β-carboxylic acids (13), as well as thiol esters (14). We were also interested in studying the analogous acidic derivatives of some other steroids, namely Prednisolone (Δ^1-unsaturation), Fluocinolone Acetonide (Δ^1-unsaturation, 6α,9-difluoro, 16α,17α-acetonide), and Hydrocortisone-17α-valerate (17α-ester).

The compounds were studied in the topical blanching assay and in the local granuloma pellet assay, to assess the local activity, as well as in a test of systemic activity following intraperitoneal injection. To obtain evidence that the soft drugs indeed owe their lack of systemic activity (toxicity) to their facile degradation to inactive metabolites, *in vitro* studies of hydrolytic decomposition in various media were performed.

MATERIALS AND METHODS

Chemistry

Initial efforts in developing the synthetic strategy used in the present work were reported previously (15). Melting points were determined using a Fisher-Johns Hot Stage and are uncorrected. Elemental analyses were performed by Atlantic Microlab of Atlanta, Georgia. TLC analyses were done using prepared plates, EM brand, SiO_2, thickness 200 μ, with UV_{254} indicator. Spots were visualized both by UV quenching and H_2SO_4/methanol charring. NMR spectra were obtained either on a Varian A-60 or EM-390 spectrometer. Infrared spectra were obtained on a Beckman Acculab 4 spectrometer. Hydrocortisone was purchased from Sigma Chemical Co., St. Louis, Missouri. With the exception of trimethyl orthovalerate and chloroiodomethane, which were prepared according to the literature procedures (16,17), all other chemical precursors and general laboratory reagents were purchased from either Sigma, Aldrich Chemical Co., Milwaukee, Wisconsin or from Fisher Scientific of Ocala, Florida.

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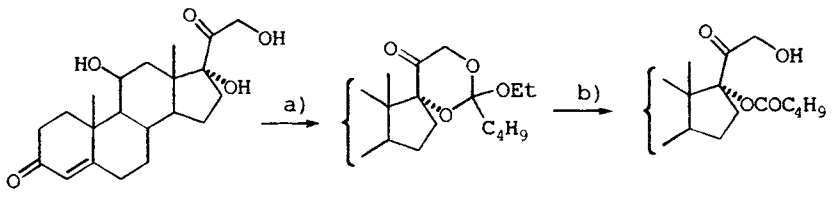


Hydrocortisone

Reagents: a) *n*-BuC(OEt)$_3$, *p*-TsOH, Φ-H; b) 0.1N NaOAc/HOAc, pH 3.0

Fig. 1. Synthesis of Compound 1, hydrocortisone-17α-valerate.

11β, 21-Dihydroxy-17α-pentanoyloxy-pregn-4-ene-3,20-dione, 1

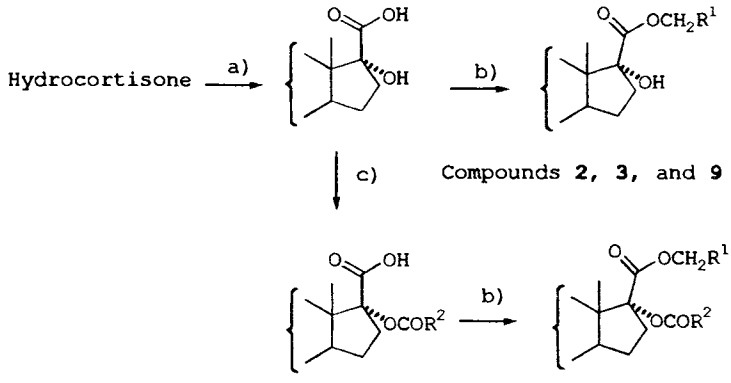
This compound was prepared according to the literature procedure (18). The intermediate crystalline orthoester weighed 6.62 g, 87% theor. The orthoester was then selectivley hydrolyzed in 0.1*N* sodium acetate buffer, pH 3.0, for 18 h. The reaction was concentrated *in vacuo* to give a residue which was partitioned between CH_2Cl_2 and water. The organic layer was separated and dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness *in vacuo* to give a crystalline residue which was recrystallized from a solvent mixture consisting of CH_2Cl_2, diethyl ether, and hexane. Yield was 4.72 g (74% theor., mp 152–155° C[lit(19) 159–161° C]). ^{1}H-NMR (DMSO-d_6) δ5.50(s,1,C = C*H*), 1.35(s,3,19-C*H*$_3$), 0.81(s,3,18-C*H*$_3$).

11β-17α-Dihydroxy-3-oxo-androst-4-ene-17β- carboxylic acid (Cortienic acid)

Hydrocortisone was oxidized using sodium *meta*-periodate ($NaIO_4$) in a homogenous solution consisting of H_2O, tetrahydrofuran, and methanol at room temperature for 30 min. The organic solvent was removed by evaporation which caused the crude acid to precipitate. This acid was purifed by extraction with aqueous bicarbonate and CH_2Cl_2. The aqueous layer was separated and acidified with 1*N* HCl. The precipated acid was collected by filtration and washed with water, then dried *in vacuo* over P_2O_5. The yield was 20.2 g (85% theor., mp 229–231°C [lit(20)235–245° C(d)]). ^{1}H-NMR (DMSO-d_6) δ5.51 (s,1,C = C*H*), 1.41(s,3,19-C*H*$_3$), 0.92(s,3,18-C*H*$_3$). Anal. Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found C, 69.02, 69.00; H, 8.13, 8.11. IR (KBr) 1720, 1620 cm^{-1}.

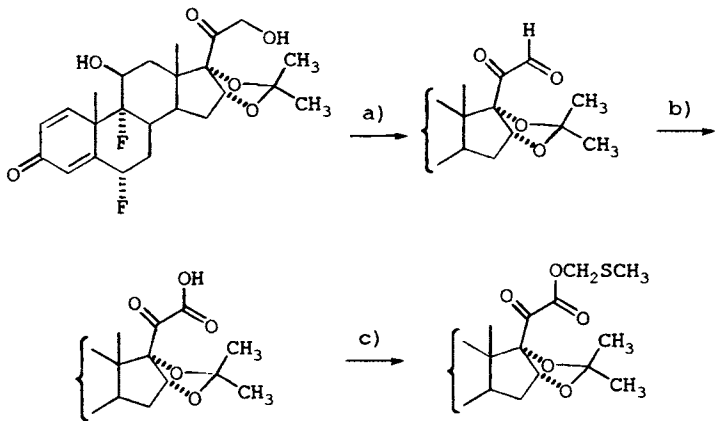
Chloromethyl 11β-17α-dihydroxy-3-oxo-androst-4-ene-17β-carboxylate, 2

A solution of the dry sodium salt of cortienic acid (prepared by the reaction between cortienic acid and $NaHCO_3$ in water and evaporating and drying over P_2O_5, 4.69 g, 12.7 mmol) in 25 ml of hexamethylphosphoric triamide was treated with chloroiodomethane 8.92 g, 50.6 mmol). The reaction was stirred at room temperature for 18 h and was then diluted with 150 ml of ethyl acetate. The resulting mixture was extracted successively with 5% $Na_2S_2O_3$, 3% $NaHCO_3$, and H_2O. The organic layer was separated and dried over $MgSO_4$, and then filtered and concentrated *in vacuo* to a crystalline solid weighing 3.1 g, (62% theor.). The product had mp 182–184° C[lit(21) 190–



Reagents: a) Aqueous $NaIO_4$; b) XCH_2R^1, aprotic solvent; c) R_2COCl, NEt_3, CH_2Cl_2

Fig. 2. Syntheses of cortienic acid mono-and diesters.



Reagents: a) $Cu(OAc)_2$, O_2; b) *m*-ClPBA, CH_2Cl_2; c) $ClCH_2SCH_3$, NEt_3, CH_3CN

Fig. 3. Synthesis of methylthiomethyl fluocinolone-21-oate.

191°C] and ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ5.81 (s,1,C=C*H*), 5.56(s,2,-OCH_2Cl), 1.41(s,3,19-CH_3), 0.89(s,3,18-CH_3).

Methylthiomethyl 11β, 17α-dihydroxy-3-oxo-androst-4-ene-17β-carboxylate, 3

Cortienic acid (0.61 g, 1.76 mmol), triethylamine (0.18 g, 1.76 mmol), and chloromethyl methyl sulfide (0.17 g, 1.76 mmol) were dissolved in acetonitrile and heated to reflux for 2h. The reaction was concentrated *in vacuo* to a residue which was triturated with a small amount of tetrahydrofuran and filtered to remove NEt_3•HCl. The filtrate was concentrated *in vacuo* and the crude product (0.6 g, 83% theor.) was purified by column chromatography. The purified product had mp 152–155°C; IR(KBr) 1721, 1620, 1200 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ5.66(s,1,C = C*H*), 5.21(s,2,-OCH_2S-), 2.28 (s,3,-SCH_3), 1.44(s,3,19-CH_3), 1.06(s,3,18-CH_3). Anal. Calcd for $C_{22}H_{32}SO_5$: C,64.68;H,7.89. Found: C,64.30;H,7.70.

Ethoxycarboxymethyl 11β,17α-dihydroxy-3-oxo-androst-4-ene-17β-carboxylate, 4

This compound was prepared using a procedure similar to that used in the synthesis of **2**, using the sodium salt of cortienic acid (1.0 g, 2.7 mmol) and ethyl bromoacetate (1.8 g, 10.8 mmol). The product was isolated as crystals from diethyl ether. The product weighed 650 mg (mp 147–149°C; 56% theor.) and had ^{1}H-NMR ($CDCl_3$) δ5.61(s,1,C = C*H*), 4.64(s,2,-$CO_2CH_2CO_2$-), 4.19(q,J = 7Hz,2,-OC$H_2$$CH_3$), 1.44(s,3,19-C$H_3$), 1.27(t,J = 7Hz,3,-OCH$_2CH_3$), 1.07(s,3,18-C$H_3$). Anal. Calcd for $C_{24}H_{34}O_7$: C,66.34;H,7.89. Found: C,66.58;H,7.80.

Methylthiomethyl 11β-Hydroxy-17α-pentanoyloxy-3-oxo-androst-4-ene-17β-carboxylate, 5

A suspension of cortienic acid (3.0 g, 8.61 mmol), in CH_2Cl_2 at 0°C was treated with triethylamine (1.74 g, 17.2 mmol) and valeryl chloride (2.08 g, 17.2 mmol). The reaction was allowed to warm to room temperature and was washed with H_2O, 3% $NaHCO_3$, 1*N* HCl, and H_2O again. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo* to give 2.99 g (67% theor. yield) of the intermediate mixed anhydride, which was solvolyzed in 50% aqueous pyridine at room temperature for 0.5 h. The free acid was isolated from the aqueous solution by acidification with 6*N* HCl. The product was dried *in vacuo* over P_2O_5 and weighed 1.81 g (49% theor., mp 130–135°C, d.). A mixture consisting of cortienic acid 17α-valerate (1.0 g, 2.35 mmol), triethylamine (0.24 g, 2.35 mmol) and chloromethyl methyl sulfide (0.23 g, 2.35 mmol) was heated, at 75°C, in acetonitrile for 18 h. The product was isolated as in the synthesis of **3**. The crude product weighed 1.13 g (mp 126–128°C; 98% theor.) and had ^{1}H-NMR ($CDCl_3$) δ5.62(s,1,C = C*H*), 5.18(s,2,-CO_2CH_2S–), 2.24(s,3,-SCH_3), 1.43(s,3,19-CH_3), 1.01(s, 3,18-CH_3). Anal. Calcd for $C_{27}H_{39}O_6$: C,65.96;H,8.00. Found: C,65.78;H,7.88.

Ethoxycarboxymethyl 11β-Hydroxy-17α-hexanoyloxy-3-oxo-androst-4-ene-17β-carboxylate, 6

Cortienic acid 17α-hexanoate was prepared in a fashion similar to that used in the preparation of the valerate, using cortienic acid (3.35 g, 9.61 mmol), hexanoyl chloride (2.59 g, 19.2 mmol), and triethylamine (1.98 g, 19.6 mmol). The yield of the intermediate mixed anhydride was 5.05 g (96% theor.). The anhydride was solvolyzed with diethylamine/acetone. After aqueous workup, the pure isolated acid weighed 2.28 g (mp 165–167°C; 56% theor.). The sodium salt of cortienic acid 17α-hexanoate (1.03 g, 2.30 mmol) was treated with ethyl bromoacetate (1.53 g, 9.17 mmol), neat. The ester was crystallized from diethyl ether. The product weighed 0.33 g(33% theor.) and had mp 115–117°C. ^{1}H-NMR ($CDCl_3$) δ5.65(s,1,C = C*H*), 4.65(s,2,-$CO_2CH_2CO_2$-), 4.19(q,J = 7Hz,2,-OC$H_2$$CH_3$), 1.44(s,3,19-C$H_3$), 1.27(t,J = 7Hz,3,-OCH$_2CH_3$), 1.01(s,3,18-C$H_3$). Anal. Calcd for $C_{30}H_{44}O_8$: C, 67.64; H, 8.33. Found: C,67.78; H,8.11.

Chloromethyl 11β-Hydroxy-17α-propanoyloxy-3-oxo-androst-4-ene-17β-carboxylate, 7

Cortienic acid 17α-propionate was synthesized using a procedure analogous to those used in the syntheses of the valerate and hexanoate, using cortienic acid (3.98 g, 11.4 mmol) propionyl chloride (4.23 g, 46.0 mmol), and triethylamine (4.72 g, 47.0 mmol). The isolated crystalline acid weighed 5.84 g (100% theor., mp 215–218°C). The potassium salt of cortienic acid 17α-propionate (1.09 g, 2.51 mmol) was treated with chloroiodomethane (1.66 g, 9.38 mmol) in hexamethylphosphoric triamide. The product was isolated from diethyl ether and hexane and weighed 0.45 g (40% theor.) and had mp 215–217°C; ^{1}H-NMR ($CDCl_3$) δ5.67(AB$_q$,J = 5.5Hz, Δν = 13.4Hz,2,-OC*H*$_2$Cl), 5.63(s,1,C = C*H*), 1.43(s,3,19-C*H*$_3$), 1.12(t,3,J = 6.5Hz,-COCH$_2$C*H*$_3$), 1.03(s,3,18-C*H*$_3$). IR (KBr) 1767, 1728 cm^{-1}. Anal. Calcd for $C_{24}H_{33}ClO_6$: C,63.64; H,7.34; Cl,7.83. Found: C,63.76; H,7.35; Cl,7.89.

Chloromethyl 11β-hydroxy-17α-methoxycarbonyloxy-3-oxo-androst-4-ene-17β-carboxylate, 8

Cortienic acid 17α-methoxycarboxylate was prepared using a procedure analogous to that used in the synthesis of the propionate, using cortienic acid (3.0 g, 8.61 mmol), methyl chloroformate (1.63 g, 17.2 mmol), and triethylamine (1.74 g, 17.2 mmol). The 17α-ester was isolated according to the procedure used in the synthesis of compound **5**. The product was dried *in vacuo* over P_2O_5 and weighed 0.89 g (mp 194–196°C (d), 25% theor.). The chloromethyl ester was prepared according to the procedure used in the preparation of compound **7**, using cortienic acid 17α-methoxycarboxylate (0.26 g, 0.64 mmol), KOH in ethanol (0.32 ml of 2.0 M), and chloroiodomethane (0.45 g, 2.5 mmol). The product was purified by column chromatography using a solvent mixture consisting of ethyl acetate, CH_2Cl_2, and hexane as eluent. The product had mp 183–184°C; ^{1}H-NMR ($CDCl_3$) δ5.72(ABq, J = 6Hz, Δν = 11.5Hz, 2, -OCH2Cl), 5.67(s,1,C = CH), 3.75(s,3,-OCH$_3$), 1.44(s,3,19-CH$_3$), 1.07(s,3,18-CH$_3$). IR (KBr) 1765,1749 cm^{-1}. Anal. Calcd for $C_{23}H_{31}ClO_7$: C,60.72; H,6.87; Cl,7.79. Found: C,60.50; H,6.91; Cl,7.83.

Methylthiomethyl 11β,17α-dihydroxy-3-oxo-androst-1,4-diene-17β-carboxylate, 9

To a solution of 11β,17α,21-trihydroxy-3,20-dioxo-pregn-1,4-diene (1.0 g, 2.79 mmol) in 40 ml of a solvent mixture consisting of equal volumes of methanol and tetrahydrofuran was added a solution of H_5IO_6 (1.84 g, 8.07 mmol) in 3 ml of H_2O. The reaction was allowed to proceed at room temperature for 2 h and was then concentrated *in vacuo*. The residue was triturated with 100 ml of H_2O and filtered to give a residue which was dried *in vacuo* over P_2O_5 to give 1.01 g (100% theor.) of a white powder. The methylthiomethyl ester was prepared according to the procedure used in the preparation of compounds **3** and **5**, using 11β,17α-dihydroxy-3-oxo-androst-1,4-diene-17β-carboxylic acid (0.49 g, 1.41 mmol), chloromethyl methyl sulfide (0.14 g, 1.41 mmol), and triethylamine (0.14 g, 1.41 mmol). The crude product weighed 0.55 g (98% theor.). The chromatographically purified product had mp 171–174°C; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ7.29 (d,J = 9.5 Hz, 1, 1-CH), 6.16 (q, J_{12} = 9.5 Hz, J_{24} = 2 Hz, 1, 2-CH), 5.93 (m, 1, 4-CH), 5.18 (s, 2,-OCH$_2$S-), 2.26 (s, 3, -SCH$_3$), 1.46 (s, 3, 19-CH$_3$), 1.05 (s, 3, 18-CH$_3$). Anal. Calcd for $C_{22}H_{30}SO_5$ C, 65.00; H, 7.44; S, 7.89. Found: C, 64.76;H,7.50; S,7.71.

Methylthiomethyl 6α,9-difluoro-11β-hydroxy-3,20-dioxo-16α,17-isopropylidenedioxypregn-1,4-diene-21-carboxylate, 10

The free 21-oic acid of Fluocinolone Acetonide was prepared according to a modification of the literature procedure using Fluocinolone Acetonide (1.0 g, 2.21 mmol) and cupric acetate monohydrate (0.25 g, 1.25 mmol). The intermediate aldehyde weighed 0.85 g (1.9 mmol) and was treated with *m*-chloroperbenzoic acid (0.33 g, 1.9 mmol) for 18 h. The desired product precipitated from the reaction and was isolated by filtration and was washed with as small as practicable an amount of CH_2Cl_2. The product weighed 0.61 g (70% theor.) and had mp 292–294°C. The methylthiomethyl ester was prepared according to the procedure which was used in the preparation of compounds **3**, **7**, and **9**, using the 21-carboxylic acid (0.50 g, 1.07 mmol), chloromethyl methyl sulfide (0.10 g, 1.07 mmol), and triethylamine (0.11 g, 1.07 mmol). The chromatographically isolated product had mp 268–270°C; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ7.17 (d, J = 10 Hz, 1, 1-CH), 6.34 (s, 1,4-CH), 6.16 (d, J = 10 Hz, 1, 2-CH), 5.24 (s, 2, -OCH$_2$S-), 2.28 (s, 3, -SCH$_3$), 1.53 (s, 3, 19-CH$_3$), 1.40 (s, 3, -OC(CH$_3$)$_2$O-), 1.22 (s, 3, -OC(CH$_3$)$_2$O-), 1.05 (s, 3, 18-CH$_3$). Anal. Calcd for $C_{26}H_{32}F_2SO_7$ C,59.30; H,6.12; S,6.09. Found: C,59.39; H,6.31; S,6.27.

Relative Blanching Activities (22)

The standard used to evaluate the other compounds for the blanching activity was hydrocortisone-17α-valerate, compound **1**. An aliquot of a solution of the test compound (50 μl of a 0.05M solution in 10% isopropyl myristate/ethanol) was applied to a 1.5 cm diameter patch, backed with plastic. This patch was applied to the flexor surface of the forearm and left 6 h. The patches were removed, and, one hour later, under artificial (fluorescent) light, the spots were scored according to the pallor produced on a scale of 1 to 4 with respect to the standard, which was given a score of 3. At least four compounds were studied at a time, in duplicate.

Stabilities in Various Media

The rates of disappearance from various media were determined for compounds **2**, **3**, **7**, **8**, and **9** by an HPLC method in which aliquots of the reaction under study were removed at time intervals, quenched in methanol, and frozen for later analysis. These rates were determined in acidic (0.1 N HCl, pH 1.13 at 51°C), neutral (0.01 M Phosphate, pH 7.4 at 37°C), and basic (0.01 N NaOH, pH 12 at 23°C) aqueous media, as well as in human plasma (80%, with citrate anticoagulant) and rat liver homogenate (freshly prepared with 32 g of rat liver and 81 ml of 0.25% sucrose), both at 37°C. In all cases, only hydrolysis of the 17β-ester was observed.

Local Granuloma Assay

Cotton pellets, containing or not the test steroid, were implanted subcutaneously in juvenile male Sprague-Dawley

Table I. Substituent Groups of Cortienic Acid Derivatives[a]

Compound	R^1	R^2	Δ^1	X
2	$-Cl$	–	–	I
3	$-SCH_3$	–	–	Cl
4	$-CO_2C_2H_5$	–	–	Cl
5	$-SCH_3$	$-n-C_4H_9$	–	Cl
6	$-CO_2C_2H_5$	$-n-C_5H_{11}$	–	Cl
7	$-Cl$	$-C_2H_5$	–	I
8	$-Cl$	$-OCH_3$	–	I
9	$-SCH_3$	–	+	Cl

[a] Refer to Figure 2 for reagents and conditions.

Table III. Cotton-Pellet Granuloma Study

Compound	N[a]	Weight gain (gms.)	Granulation tissue (mg/100 g. body wgt.)	Thymus weight (mg/100 g. body wgt.)
Control	**10**	40.5 ± 0.8	43.7 ± 4.2	326 ± 22
1	**8**	33.4 ± 1.4	32.2 ± 5.0	73 ± 5
8	**7**	33.4 ± 1.3	24.6 ± 2.6	218 ± 15
Betamethasone 17α-valerate	8	16.6 ± 1.9	35.4 ± 7.3	47 ± 2

[a] N is number of subjects. Cotton pellets were impregnated with the test substance before implantation.

rats, weighing 90–110 g. After six days the pellets were excised, dried and weighed. The formation of the granuloma tissue was quantified by a comparison of the weight of the pellets before and after the implantation period. The assay measures the local antiinflammatory activity of the test compound. At the same time, the thymus weight was determined, as well as the difference between starting and final body weight, to obtain information about possible systemic effects.

Glycogen Deposition and Thymus Involution (23,24)

Male Sprague-Dawley rats, weighing 160–190 g, were aderenalectomized and maintained on salt water and laboratory chow. The test steroid was injected as a suspension in carboxymethylcellulose/water on the fourth and fifth days of the experiment. On the sixth day the animals were sacrificed and the livers removed. The glycogen content of the livers was determined by the anthrone method. The assay measures the systemic glucocorticoid activity of the test compound, both by its effect on liver glycogen and thymus involution.

RESULTS AND DISCUSSION

The synthetic strategy employed in the esterification of the normally inaccessible 17α-position took advantage of the 17β-mixed anhydride which transfers the acyl group through a five-membered cyclic intermediate to the α-position. This strategy is similar to that used in the esterification of the equivalent position in hydrocortisone (synthesis of compound **1**), but with greater flexibility in the type of acyl group which may be transferred.

In applying the soft drug approach we are mainly interested in the metabolic inactivation of the drug, but obviously, it must also possess the desired activity; a drug without activity is not a drug. Consideration of the results presented in Table II shows that two of the compounds tested, **2** and **3**, which possess only one activating group, namely the carboxyl ester at the 20-position, do not possess antiinflammatory activity. Therefore, at least two activating groups are necessary to produce a compound with activity, and these groups must be of a particular nature, since the combinations of either the methylthiomethyl or carboalkoxymethyl with a 17α-ester in compounds **5** and **6**, or the methylthiomethyl with Δ^1-unsaturation in compound **9**, respectively, did not produce active compounds. Thus, the combination of the 17β-chloromethyl ester with a 17α-ester was found to be the best combination. The only non-17β-chloromethyl ester derivative that shows activity is that based on fluocinolone acetonide, compound **10**, a structure with five other activating groups present.

The data presented in Table III are from the local cotton-pellet granuloma activity assay (25) and show that compound **8**, which was the most potent in the blanching assay, is also the most potent in inhibiting the formation of granulation tissue. There is here a rather striking contrast between this local activity (inhibition of granuloma formation) and systemic activity (thymus involution) when compounds **1** and Betamethasone ester are compared with compound **8**. An even more striking result is seen in the data of Table IV in which the compounds were administered systemically. There is still a notable difference between compounds **8** and **1** and Betamethasone 17α-Valerate with respect to the systemic activity.

Based only on the above evidence, one could conclude that the soft drug concept has been successfully applied in the present case: the preparation of a compound that has a high degree of *in vivo* potency coupled with a nearly complete lack

Table II. Biological Activity (Blanching[a])

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Activity	3	0	0	0	0	0	3	4	0	2

[a] Relative pallor was scored against the standard, compound **1**, which was given a score of 3.

Table IV. Liver Glycogen Deposition and Thymus Involution (Parenteral Administration[a])

Compound	Dose (mg/Kg)	Thymus (mg)	Glycogen (mg/100g)
Control	—	674 ± 28	24.0 ± 2.6
8	0.1	787 ± 25	21.2 ± 2.1
"	1.0	654 ± 32	19.0 ± 0.9
"	10.0	631 ± 52	20.3 ± 1.6
1	1.0	495 ± 43	20.2 ± 2.5
"	10.0	108 ± 12	2853 ± 197
Betamethasone-17α-valerate	1.0	322 ± 31	23.0 ± 1.6
"	10.0	298 ± 20	118 ± 55

[a] The test compound was injected into animals on the 5th and 6th days after adrenalectomy.

Table V. Hydrolytic Stability[a] ($t_{1/2}$, h)

Compound	Aqueous media			Biological media	
	pH 1.13	pH 7.40	pH 12.0	Plasma	Liver
2	2.2 ± 0.07	7.1 ± 0.19	0.01	2.2 ± 0.07	0.12 ± 0.00
3	0.49 ± 0.01	14.6 ± 0.34	0.24 ± 0.00	38 ± 4.3	1.8 ± 0.38
7	5.8 ± 1.0	27.6 ± 0.9	1.1 ± 0.03	5.3 ± 0.41	4.6 ± 0.61
8	10 ± 0.3	50.1 ± 4.1	0.85 ± 0.01	16 ± 0.36	4.9 ± 0.11
9	0.47 ± 0.00	11.8 ± .2	0.19 ± 0.00	35 ± 1.7	—

[a] Rates are based on disappearance of ester. Only the 17β-esters were hydrolyzed; no 17α-OH acids were found.

of systemic activity, surprisingly even when the compound is administered systemically. However, consideration of the data in Table V would indicate that compound **8** may not be sufficiently labile to explain its lack of systemic activity on a facile hydrolysis to inactive metabolites *in vivo*. More recent studies on Loteprednol Etabonate (26), a compound closely related to **8**, having a Δ^1 double bond and an ethyl instead of methyl carbonate moiety at the 17α-position, have confirmed that the 17β-chloromethyl ester is indeed susceptible to rapid and complete hydrolysis in rat blood and plasma, although in later experiments to determine its pharmacokinetic profile, it was found that a large proportion of the dosage was recovered unmetabolized after intravenous infusion in dogs (27). In studies on Fluocortin Butyl Ester (12), Mützel also noted the surprising stability of the ester after intravenous injection in man, when it had been demonstrated to hydrolyze rapidly *in vitro* in plasma and buffer. Mützel's conclusion was that the *n*-butyl ester must be taken up rapidly by the tissues or be bound to plasma proteins with high avidity, thus avoiding hydrolysis, as well as systemic activity, *in vivo*, although the unbound fraction is indeed rapidly degraded, as we would also argue in our case.

A recent controversy (28,29) has arisen around the issue of the conceptual correctness of the present applicaction of the soft drug concept, which laid the foundation for the work on Loteprednol Etabonate. It is often the case in the field of drug design that the initial intellectual motivation, though eminently rational, does not fulfill its expectations precisely in the manner that it was initially formulated. The apparent stability of the soft drugs *in vitro* is far from being an invalidation of this application of a rational method of drug design in which the goal has been obviously achieved: a highly potent topical therapeutic agent lacking systemic side effects.

For a better understanding of the reasons for the successful application of the soft drug concept in this case we would have to go further in analyzing the factors responsible, which would be properly studied on the basis of structure-activity relationships, such as have been presented in part here. The biological activity we have reported is a multivariable observation, incorporating antiinflammatory activity as well as two measures of systemic toxicity, glycogen deposition and thymus involution. This multivariate activity then should be related to the properties of the molecules, of course including hydrolytic stability, as well as other tests, such as protein and tissue binding. The use of multivariate analysis of structure-activity relations is recently receiving a great deal of interest (30) in the field of drug design.

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