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Soft drugs 4. 3-Spirothiazolidines of hydrocortisone and its derivatives

Nicholas Bodor *, Kenneth B. Sloan *,**, Roy J. Little *,**, Sally H. Selk ** and Larry Caldwell **

* *Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, Box J-4, University of Florida, Gainesville, FL 32610 and ** INTER$_x$ Research Corporation, Lawrence, KS 66044 (U.S.A.)*

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Summary

The previously unknown thiazolidines of some α,β-unsaturated 3-ketone steroids were prepared and characterized as 4,5-double-bond isomers. The thiazolidines readily reverted to their parent steroidal ketones, thus meeting the requirements for a prodrug. Most of the thiazolidines were more lipophilic than their parent steroids thereby imparting the desired change in the physical properties to the derivatives of the steroids. The thiazolidines of hydrocortisone 21-acetate (I and II) were more active than hydrocortisone or hydrocortisone 21-acetate, but the thiazolidines of the 17-esters were not. Thiazolidines I and II were less toxic systemically and this appears, at least in the case of I, to be due to the fact that less hydrocortisone was delivered through the skin from the application of thiazolidine I as from hydrocortisone 21-acetate. On the other hand, there was no appreciable disassociation of the increased activity of I and II from their ability to cause local atrophy.

Introduction

In one of our earlier works (Bodor et al., 1980a), the concept of 'soft drugs' was introduced as biologically active, useful compounds which are characterized by a

* To whom correspondence should be addressed at the University of Florida.

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predictable in vivo destruction (metabolism) to non-toxic moieties, after they achieve their therapeutic role. It was mentioned that the soft drugs can be classified into 5 basic types, among which the soft analogues (soft antimicrobials (Bodor et al., 1980a), soft anticholinergics (Bodor et al., 1980b), and activated soft compounds (soft alkylating agents (Bodor et al., 1980c) and soft N-haloamines (Kaminski et al., 1976a; Kaminski et al., 1976b; Kaminski et al., 1976c; Kosugi et al., 1976)) were already exemplified. The present paper describes an example of a different class of soft drugs: endogenous substances as drugs. A number of endogenous substances, such as steroid hormones (hydrocortisone, progesterone, testosterone, estradiol) can be considered *natural soft drugs*, since the body has developed efficient, metabolic ways for their disposition. Thus, it is expected that endogenous substances used at *concentrations close to their natural levels* will not cause any unexpected toxicity. The metabolism of these compounds is, although predictable, not controllable, and often is so fast and efficient that they cannot directly be used as drugs. The solution in these cases is the use of specific chemical protecting techniques for their sustained release, such as a *prodrug–soft drug combination*. A clear cut explanation of the differences between a soft drug and a prodrug is in order at this stage. Formally, prodrugs and soft drugs are just opposite to each other: *a prodrug is inactive and it is activated by an enzymatic process* to the active drug in a predictable and controllable manner. On the other hand, *a soft drug is active as such and it is enzymatically deactivated* in a predictable and controllable way. The prodrug approach is, however, ideal to be used to deliver the natural soft drugs, i.e. the various useful endogenous compounds such as hydrocortisone.

Although hydrocortisone is an endogenous glucocorticoid, topical applications of hydrocortisone that result in higher than normal in vivo levels of the hormone can cause side-effects such as dermal atrophy, thymus involution and suppression of adrenal, hypothalamus and pituitary functions (Kligman and Kaidbey, 1978). Formulations of hydrocortisone or derivatives of hydrocortisone that merely increase the efficiency of the delivery of the steroid cannot realistically be expected to alleviate these side-effects. On the other hand, separation of activity from side effects should result from a derivative of hydrocortisone that is: (1) not active itself, i.e. a prodrug, but (2) accumulates in the skin and, (3) hydrolyzes slowly to give the parent drug at such a rate that the rate of systemic metabolism of the released parent drug to inactive, easily excreted substances more closely matches the rate of release of the parent drug from the skin.

Since the α,β-unsaturated 3-ketone group is essential for the activity of glucocorticoids, a bioreversible tetrahedral derivative of the 3-ketone appeared to be the ideal prodrug candidate. Although thiazolidine derivatives of α,β-unsaturated steroidal ketones were not known, and in fact were suggested to be unobtainable with known methods of synthesis (Lieberman et al., 1948), the properties of thiazolidines (Schubert, 1935) appeared to offer some unique advantages as prodrugs. Thiazolidines are known to undergo hydrolysis by a spontaneous S_N1 cleavage of the carbon–sulfur bond (Schubert and Motoyama, 1965) to give imines (e.g. XVII). Thus, since imine formation is biologically reversible (possibly by a hydration followed by a disassociation mechanism (Williams, 1959; Testa and

thiazolidine formation is also biologically reversible.

Some thiazolidines, such as cysteine ester derivatives, were more attractive than others as prodrugs because of the release of cysteine as a by-product.

Materials and methods

Melting points (uncorrected) were taken with a Thomas-Hoover capillary apparatus. NMR spectra were recorded on a Varian T-60 (^{1}H-spectra) or on a Bruker WP-80 (^{13}C-spectra) spectrophotometer. Infrared spectra were obtained on Beckman AccuLab 4 infrared spectrophotometer. UV spectra were determined on a Cary Model 14 spectrophotometer. Optical rotations were obtained using a Perkin Elmer 141 polarimeter. HPLC analyses were run on a Waters 6000 A solvent delivery system, U6K universal injector and a 440 Dual Channel UV detector. Microanalyses were performed by Midwest Microlab Indianapolis, IN. The hydrocortisone, hydrocortisone 21-acetate, cysteine ethyl and methyl esters and penicillamine were obtained from Sigma. 2-Aminoethanethiol hydrochloride was obtained from Aldrich. All other reagents unless otherwise specified were obtained from Mallinckrodt. Penicillamine ethyl ester hydrochloride and L-cysteine esters were prepared according to the method of Voullie et al. (Voullie et al., 1960).

Preparation of 4-pregnene-11β,17α-diol-21-acetyloxy-20-one-3-spiro-2'-(4'-carboethoxyl-1',3'-thiazolidine) (I)

Hydrocortisone 21-acetate (16.16 g, 0.04 mol) was mixed with 15 g (0.08 mol) of cysteine ethyl ester hydrochloride in 110 ml of pyridine and allowed to stir at room temperature for 16 h under a nitrogen atmosphere. The solution was then concentrated to dryness and the residue that resulted was partitioned between CH_2Cl_2 (300 ml) and water (200 ml). The CH_2Cl_2 layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from CH_2Cl_2–ethanol to give 15.4 g (m.p. 172–177°C, 72% yield) of I: IR (KBr) 1735 and 1715 cm^{-1} (s) (C=O); ^{1}H NMR ($CDCl_3$) δ 5.23 (broad s, 1, C*H*=C), 4.95 (ABq, $J_{AB} = 17$ Hz, $\Delta_{\nu AB} = 12$ Hz, 2, OC*H*$_2$C=O), 4.43 (m, 1, C*H*–OH), 4.27 (q, J = 7 Hz, 2, CH_3–C*H*$_2$O), 4.3–3.85 (m, 1, O_2CC*H*N), 3.55–2.8 (m, 2, C*H*$_2$S), 2.17 (s, 3, C*H*$_3$CO$_2$), 1.3 (s, 3, C*H*$_3$–C), 0.93 (s, 3, C*H*$_3$–C), 2.8–0.7 (m, 23, C*H*$_3$, C*H*$_2$ and C*H*); ^{13}C NMR ($CDCl_3$) δ 205.2 (C_{20} = 0), 172.0 and 171.0 (CO_2), 150.0 (C_5) and 121.4 (C_4).

Preparation of 4-pregnene-11β,17α-diol-21-acetyloxy-20-one-3-spiro-2'-(1',3'-thiazolidine)

To 4.0 g (0.01 mol) of hydrocortisone 21-acetate and 6.8 g (0.06 mol) of 2-aminoethanethiol hydrochloride was added 20 ml of pyridine. The suspension was stirred at room temperature under a nitrogen atmosphere overnight. The reaction mixture was then concentrated in vacuo and partitioned between CH_2Cl_2 and H_2O (100 ml: 100 ml). The CH_2Cl_2 layer was separated, dried over Na_2SO_4 and concentrated to give a foam. The foam was redissolved in 20 ml of CH_2Cl_2 and quickly diluted to 250 ml with heptane. The suspension that resulted was stirred at room

temperature for 6 h, then filtered and dried to give 2.60 g (mp 150–156°C, 56% yield) of the desired thiazolidine: IR (KBr) 1750 and 1730 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 5.27 (s, 1, C*H*=C), 4.77 (ABq, 2, $J_{AB} = 18$ Hz, $\Delta_{\nu AB} = 16$ Hz, O=C-C*H*$_2$O), 4.6–4.3 (m, 1, C*H*-OH), 2.14 (s, 3, C*H*$_3$CO$_2$), 1.3 (s, 3, C*H*$_3$-C), 0.9 (s, 3, C*H*$_3$-C), 3.6–2.8 (m, 4, N-C*H*$_2$C*H*$_2$S), 2.8–0.8 (m, 26, C*H*$_2$, C*H*, O*H* and N*H*); ^{13}C NMR ($CDCl_3$) δ 146.4 (C_5) and 122.4 (C_4).

The remaining thiazolidines were prepared in a similar manner except for III, which was soluble in the water layer during the partitioning between CH_2Cl–water. III was liberated from the water layer by adjusting the pH to 7.0 with bicarbonate then extracting with CH_2Cl_2.

Croton oil test

The ability of the steroids to reduce the irritation caused by croton oil was assessed in mice using a modification of the method of Tonelli et al. (1965). The right ears of male mice (20–25 g, n = 10) were treated with 50 µl of an acetone–2% croton oil solution of the steroidal thiazolidine; the left ear was not treated. Three hours later, the mice were sacrificed, both ears were removed, circular sections were taken using a leather punch and the sections were weighed. The results are shown in Table III. The percent reduction in edema was calculated at 4 doses using the left ear as an internal control to determine the weight increase in the right ear caused by the irritation and using a separate group of mice whose right ear was treated only with acetone–2% croton oil as the external control from the following formula:

% inhibition of edema =

$$100 \times \frac{\text{weight increase external control ear} - \text{weight increase right ear}}{\text{weight increase external control ear}}$$

Then, the ED_{50} was calculated from linear regression plots of the percent inhibition of the edema against the four doses.

Systemic toxicity

The systemic toxicity was determined using a modification of the method of Tonelli et al. (1965). Both sides of both ears of female weanling rats (n = 10) were treated with 50 µl of 0.03 M acetone–isopropyl myristate (90:10) solution of the steroids being tested. After 48 h the rats were sacrificed, weighed and their thymi were removed and weighed. The weights of thymi were expressed as mg/100 g of rat. The significance of the differences among the derivatives was determined using the Student's *t*-test.

Local toxicity

A modification of the method of Kirby and Munroe (Kirby and Munroe, 1976) was used to determine the local toxicity. The results of those experiments are presented in Table IV and Fig. 2. Mature female white Swiss mice (n = 10) were treated for 4 days on the left ear with 10 µl of an acetone–isopropyl myristate

(90:10) solution of the steroid to be tested; then the thickness of the left ear and the right ear were measured. An Ames, number 3, tabletop, lever-controlled micrometer was used to measure the thickness of the ears. A one pound weight was applied to the top of the micrometer during the measurements. The ears were held in the micrometer for 10 s each time before a measurement was taken. The mean of the first day pretreatment measurements of the left ear for each group of mice was used as a control value to calculate the percent thinning induced by the treatment. The right ear was used as an internal control to ensure that there was no significant systemic effect. The percent reduction in ear thickness was calculated from the following formula:

% reduction in thickness =

$$100 \times \frac{\text{thickness of left ear (day 1)} - \text{thickness of left ear (day 5)}}{\text{thickness of left ear (day 1)}}$$

Blanching studies in human volunteers

A modification of the vasoconstrictor test developed by McKenzie and Stoughton (1962) was used to establish the activity of the thiazolidine derivatives in man. The thiazolidines were dissolved in acetone–isopropyl myristate (90:10) and 100 μl of the solution were applied to bandaid-type strip tapes. The tapes were applied to the backs of volunteers who had been previously shown to give a good blanching response. Five compounds were tested, each at 4 different positions in an asymmetric array across and up and down the back in each study. Two compounds of known, but substantially different, blanching capacity were included in each screen to ensure that a spectrum of activity could be seen in each screen. The compounds were occluded for 4 h, then the tapes were removed and blanching scores were determined, using the scoring criteria of Barry and Woodford (Barry and Woodford, 1974) for 4–8 h afterwards. The blanching data presented in Fig. 1 are the average for the blanching scores for the 4 positions for only 4 subjects (average S.D. $\pm$ 0.25).

Diffusion cell experiments

In vitro percutaneous absorption was measured at 32°C using freshly excised hairless mouse skin (12–14-week-old SKH-hr-1 female mice from the Animal Services Division, Skin and Cancer Hospital, Temple University, 3222 Broad Street, Philadelphia, PA) stretched over the lower opening of an 8.0 cm^2 diffusion cell equipped with teflon lids (obtained from Kersco Engineering Consultants, 3248 Kipling Street, Palo Alto, CA) and a receptor compartment volume of 45 cm^3. The receptor phase was maintained at pH 7.0 with a phosphate buffer containing thimerosal (0.01%) or gentamycin sulfate (200 ppm) and was stirred magnetically. Samples (1.0 ml) of the receptor fluid were removed and replaced with fresh buffer each sampling time. The samples were analyzed using HPLC on a Brownlee RP-8 column with a 40% tetrahydrofuran/water mobile phase flowing at 2.0 ml/min. The eluate was monitored at 254 nm and recorded on a strip chart.

Results and discussion

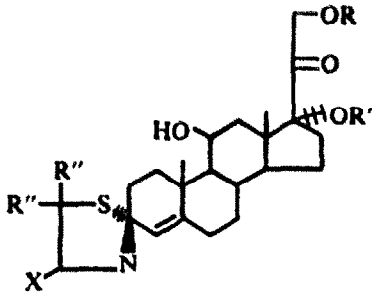
In spite of the fact that the literature reported that thiazolidines of α,β-unsaturated ketones could not be prepared by a simple base-catalyzed condensation reaction (Lieberman et al., 1948), we found that the thiazolidine I was formed in high yield in the presence of pyridine and an excess of cysteine ethyl ester hydrochloride (2 eq.) from hydrocortisone 21-acetate. Other amines such as triethylamine (either as a solvent or in benzene), or collidine, or inorganic bases such as potassium carbonate, were not as effective as pyridine, although some I was formed each time based on analyses of the position and intensity of the steroidal C*H*=C absorption in the NMR spectra of crude mixtures. In each case, only one isomer was observed. Since the thiazolidines were observed to decompose on standing on TLC plates and during column chromatography, analyses of the ^{1}H-NMR spectra of the isomers was selected for following the extent of the reactions. Under similar conditions, II was prepared from hydrocortisone 21-acetate and aminoethanethiol hydrochloride.

The structures of the thiazolidines I and II were established based on their optical rotations and from their ^{1}H- and ^{13}C-NMR spectra by comparison of their spectra with those of similar spectra for the progesterone and testosterone thiazolidines where two series of isomers could be isolated (Sloan et al., 1981). These two series of isomers were determined to be double-bond positional isomers and I was found to belong to the 4,5-double-bond isomer series. A complete description of the details of the determination of the structures of I, II and the progesterone and testosterone thiazolidines has been presented elsewhere (Sloan et al., 1981). The position of the double-bond has biological as well as chemical significance. If the double-bond is in the 4,5-position, the hydrolysis of the thiazolidine immediately regenerates the parent steroid. On the other hand, if the double-bond is in the 5,6-position, then an intermediate isomerization step by Δ^5-3-ketosteroid isomerase (Talalay, 1963) may be necessary to regenerate the parent steroid.

Tables I and II list the 3-spiro-thiazolidines that have been synthesized from cysteine esters, penicillamine ethyl ester and aminoethanethiol and hydrocortisone and its 21- and 17α-esters. Notable by their absence are any free cysteine thiazolidines, since the reaction product could not be separated from the excess cysteine. Since the thiazolidines were all prepared in a similar manner, and the shape and position of their steroidal C*H*=C NMR absorptions were identical with I and II, the remaining thiazolidines were assigned the same structure as I and II. All of the thiazolidines except III and XI were much more lipophilic than hydrocortisone or hydrocortisone 21-acetate as they were all soluble to the extent of >50 mg/ml in $CHCl_3$.

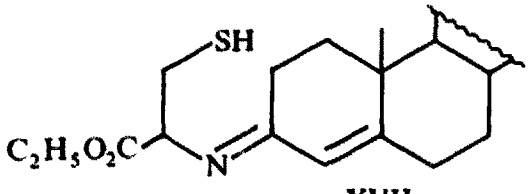
Although the hydrolysis of some simple thiazolidines to their parent carbonyl compounds has already been amply documented (Pesek and Frost, 1975; Luhowy and Meneghini, 1979), the rates of hydrolyses of selected thiazolidines were determined by UV (Table III) at pH 4.2, since the pH of the skin is also mildly acidic. Each determination was characterized by the immediate formation of a chromophore at 280 nm which decayed in pseudo-first-order fashion to give the chromophore of the parent ketone at 245 nm. The initial chromophore was attributed (by

TABLE I



	X	R	R'	R''
I	$CO_2C_2H_5$	$COCH_3$	H	H
II	H	$COCH_3$	H	H
III	H	H	H	H
IV	$CO_2C_2H_5$	H	H	H
V	$CO_2C_2H_5$	H	COC_4H_9	H
VI	$CO_2C_2H_5$	H	COC_2H_5	H
VII	H	H	COC_3H_7	H
VIII	H	H	COC_2H_5	H
IX	H	H	$COCH_3$	H
X	$CO_2C_2H_5$	H	$COCH_3$	H
XI	$CO_2CH_2CH_2OH$	$COCH_3$	H	H
XII	$CO_2C_2H_5$	$COCH_3$	H	CH_3
XIII	$CO_2C_{10}H_{21}$	$COCH_3$	H	H
XIV	$CO_2C_6H_{13}$	$COCH_3$	H	H
XV	$CO_2C_4H_9$	$COCH_3$	H	H
XVI	CO_2CH_3	$COCH_3$	H	H

analogy (Johnson et al., 1956)) to the imine intermediate XVII. Hydrocortisone 21-acetate was recovered quantitatively from the hydrolyses of the thiazolidines thus establishing that they had readily reverted to their parent steroid.



XVII

The hydrolysis data show that at pH 4.2 the spiro-thiazolidines of the cysteine ethyl and butyl esters are more quickly hydrolyzed than those of the hexyl and decyl esters, while the substitution of the 5'-hydrogens with methyls in the penicillamine derivative XII resulted in a slower release of hydrocortisone 21-acetate from XII than from I. It appears that pK_a of the thiazolidines is less than that of pyridine, since the free base of the thiazolidine was obtained from cysteine ethyl ester or aminoethanethiol hydrochlorides.

TABLE II

PHYSICAL AND SPECTROSCOPIC PROPERTIES OF SPIROTHIAZOLIDINES

Compound	Yield (%)	O.R. (25°C)	NMR ($CDCl_3$)		
			CH_3(s)	CH_3(s)	CH=C (Broad s)
I	72	+85°C (C=0.5, $CHCl_3$)			
		+103°C (C=0.6, ethanol)	1.3	0.93	5.23
II	56	+154°C (C=0.58, $CHCl_3$)	1.3	0.90	5.27
III	58	+151°C (C=0.5, ethanol)	1.2	0.73	5.2 [a]
IV	50	+46°C (C=0.5, $CHCl_3$)	1.3	0.90	5.21
V	58	0°C (C=0.54, $CHCl_3$)	1.33	0.95	5.23
VI	58	0°C (C=0.5, $CHCl_3$)	1.30	0.90	5.2
VII	61	+83°C (C=0.58, $CHCl_3$)	1.3	0.93	5.27
VIII	50	+72°C (C=0.52, $CHCl_3$)	1.3	0.91	5.23
IX	32	+73°C (C=0.56, $CHCl_3$)	1.3	0.90	5.27
X	75	+44°C (C=0.5, ethanol)			
		−6°C (C=0.63, $CHCl_3$)	1.31	0.93	5.23
XI	10	+86°C (C=0.56, ethanol)	1.07	0.77	5.33 [a]
XII	50	+125°C (C=0.55, ethanol)		0.89	
XIII	26	+73°C (C=0.5, ethanol)	1.30	0.93	5.23
XIV	42	+78°C (C=0.5, ethanol)	1.33	0.92	5.23
XV	53	+76°C (C=0.5, ethanol)	1.29	0.92	5.21
XVI	42	+102°C (C=0.5, ethanol)	1.30	0.93	5.21
HC-acetate			1.43	0.98	5.70
HC			1.53	0.75	5.55 [a]

[a] Run in DMSO-d_6.

TABLE III

Compound	Croton oil irritation test ED_{50} (M) [a]	Hydrolyses rate constants of spirothiazolidines $k \times 10^3$ min^{-1}
I	0.0033	34.3 ± 1.46 [b]
XII	0.0055	9.8 ± 1.2
XIII	0.0036	1.8 ± 0.03
XIV	0.0039	5.3 ± 2.9
XV	0.0027	43.0 ± 4.4
XVI	0.0035	28.7 [b,c]
Hydrocortisone 17-butyrate	0.0011	
Hydrocortisone [d]	0.0084	
Hydrocortisone [d] 21-acetate	0.016	
Hydrocortisone [d] 17-butyrate	0.00086	

[a] Based on linear regression analyses of data obtained at 3×10^{-5}, 3×10^{-4}, 3×10^{-3} and 3×10^{-2} M. Data generated at the Laboratories of Kanebo Pharmaceuticals.

[b] pH 4.07, all the rest were run at pH 4.21.

[c] One run, all the rest were the average of 3 runs in 10% ethanol-phosphate buffer at 37°C, μ=0.272.

[d] Separate run.

m.p.	Formula	Anal.					
		Calcd.			Found		
		C	H	N	C	H	N
172–177°C	$C_{28}H_{40}NO_7S$	62.89	7.54	2.62	62.90	7.76	2.30
150–156°C	$C_{25}H_{37}NO_5S$	64.76	8.05	3.02	64.20	8.05	2.65
135–152°C	$C_{23}H_{35}NO_4S \cdot H_2O$	62.84	8.48	3.19	62.81	8.20	3.42
111–129°C	$C_{26}H_{39}NO_6S$	63.26	7.96	2.84	62.87	7.97	2.59
foam	$C_{31}H_{47}NO_7S$	64.44	8.20	2.42	63.33	8.38	2.20
109–113°C	$C_{29}H_{43}NO_7S$	63.36	7.88	2.55	63.26	7.86	2.48
foam	$C_{27}H_{41}NO_5S$	65.95	8.40	2.85	66.26	8.36	2.49
foam	$C_{26}H_{39}NO_5S$	65.37	8.23	2.93	65.18	8.00	2.81
foam	$C_{25}H_{37}NO_5S$	64.76	8.05	3.02	64.85	7.93	2.54
foam	$C_{28}H_{41}NO_7S$	62.78	7.72	2.23	63.00	7.58	2.53
174–177°C	$C_{28}H_{41}NO_8S$	60.95	7.49	2.54	60.97	7.33	2.35
105–120°C	$C_{30}H_{45}NO_7S$	63.91	8.05	2.48	63.81	8.10	2.50
142–154°C	$C_{36}H_{57}NO_7S$	66.73	8.87	2.16	66.95	9.25	1.95
149–151°C	$C_{32}H_{49}NO_7S$	64.94	8.35	2.37	65.10	8.50	2.15
165–168°C	$C_{30}H_{45}NO_7S$	63.91	8.05	2.48	63.86	8.13	2.25
161–164°C	$C_{27}H_{39}NO_7S$	62.16	7.54	2.68	62.15	7.63	2.41

The anti-inflammatory activities of the thiazolidines were determined in two tests. The first one was the well-known Tonelli mouse ear test (Tonelli et al., 1965) using croton oil as an irritant. Table III indicates that all the thiazolidines of hydrocortisone 21-acetate were somewhat more active than their parent compound, hydrocortisone 21-acetate, and that there is no clear correlation between activity and stability of the thiazolidine.

The general trend observed in the animal model was also found in the human blanching studies (McKenzie and Stoughton, 1962). However, none of the thiazolidines of the 17α-esters, either substituted or unsubstituted in the 4'-position, were as active as the parent compounds. Fig. 1 shows the results of a typical blanching study which exemplifies the kind of qualitative data that was obtained using this test. However, the results were very reproducible in terms of identifying the rank order of activity of series of compounds. Clearly, thiazolidine II is more active than either hydrocortisone or hydrocortisone 21-acetate in this test.

The activity of thiazolidines XII–XVI could not be differentiated from I. On the other hand, the more hydrophilic thiazolidines XI and III (based on solubility in $CHCl_3$) were much less active than hydrocortisone 21-acetone both in the blanching test and in the croton oil irritation test.

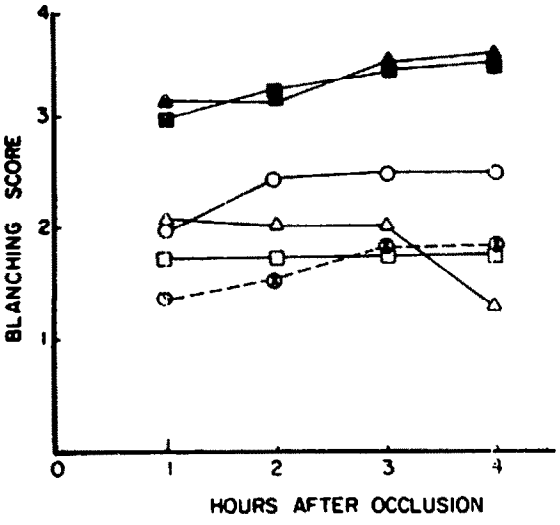


Fig. 1. Blanching in human volunteers after topical application of steroids. ▲, hydrocortisone 17-valerate; ■, Hydrocortisone 17-butyrate; ○, Thiazolidine II; △, Hydrocortisone 21-acetate; □, Hydrocortisone; ⊗, Thiazolidine I.

Hydrocortisone 17α-butyrate was used as a standard for activity in both activity test. In addition, hydrocortisone or hydrocortisone 21-acetate was used with hydrocortisone 17α-butyrate as standards in the blanching studies to ensure that the expected spectrum of activity under consideration could be observed in each study. The difference in activity between hydrocortisone 17α-butyrate and 21-acetate of about tenfold (Table III) that we observed in the mouse ear-croton oil model, was consistent with the results reported by others using similar models (Young et al., 1977).

In addition to the anti-inflammatory activity tests, two different types of toxicity measurements were assessed for the thiazolidines. The first test measured the effect of topically applied steroids on the involution of the thymus, i.e. a systemic effect, using weanling rats (45–50 g) (Table IV).

Only the spirothiazolidines I and II showed decreases in the side-effects observed. Table V shows that, although this effect is real at higher concentrations, there is little difference between thiazolidine I and its parent compound at lower doses; the dose–response curves are not parallel.

In the results for the ear thinning model for local toxicity (Kirby and Munroe, 1976), shown in Table VI, most of the steroids exhibited parallel dose–response curves except for hydrocortisone 21-acetate and the thiazolidines I and II. Again, the general trend seems to be that at higher doses thiazolidine I was less toxic than its parent compound, but that this advantage was clearly not maintained at all concentrations. Thiazolidine II, the more active thiazolidine in the blanching studies, exerted greater local toxicity than the less active thiazolidine I, hydrocortisone or hydrocortisone 21-acetate.

The reason for the decreased systemic toxicity of I and II appeared to be the

TABLE IV

THYMUS INVOLUTION [a] IN RATS AFTER TOPICAL APPLICATION OF STEROIDS

Compound	mg of thymus / 100 g of rat ± S.D.				% Reduction in Thymus [b]			
Blank [c]	268 ± 25	197 ± 42						
Vehicle	257 ± 59	210 ± 44	316 ± 58	271 ± 32				
I	204 ± 25 [d]	161 ± 31		196 ± 37	22	21		27
II	233 ± 40 [d]				11			
III			236 ± 28				25	
IV			192 ± 26				39	
VI		123 ± 27				39		
VII				163 ± 28				40
VIII			191 ± 18				40	
X			224 ± 39				29	
XI			225 ± 52				29	
Hydrocortisone	168 ± 30	140 ± 42	209 ± 29		36	33	34	
Hydrocortisone 21-acetate	167 ± 18			165 ± 42	36			39
Hydrocortisone 17-acetate		140 ± 25				31		
Hydrocortisone 17-propionate		120 ± 26				41		
Hydrocortisone 17-butyrate		120 ± 19	208 ± 37			41	34	
Hydrocortisone 17-valerate	206 ± 20 [d]	159 ± 29			21	22		

[a] All compounds were administered in a total of 50 µl as a 0.03 M acetone/IPM (90 : 10) solution, except where specified, to 10 rats each, 100 rats at a time.
[b] Compared to vehicle.
[c] Not treated or handled.
[d] $P < 0.001$ compared to hydrocortisone 21-acetate.
[e] THF/IPM, 90 : 10.

TABLE V

THYMUS INVOLUTION IN RATS AFTER TOPICAL APPLICATION OF STEROIDS

Compound	Concentration (M) [a]	mg of thymus / 100 g of rat ± S.D.	% Reduction in thymus [b]
Hydrocortisone 21-acetate	0.03	165 ± 42	39
	0.01	241 ± 30	11
	0.003	262 ± 28	3
I	0.03	196 ± 37	27
	0.01	238 ± 27	12
	0.003	265 ± 37	2
vehicle, THF/IPM, 90 : 10		271 ± 32	

[a] All compounds were delivered in a total of 50 µl to 10 rats each.
[b] Compared to vehicle.

TABLE VI

EAR THINNING IN MICE AFTER TOPICAL APPLICATION OF STEROIDS

Compound	Concentration (M)	Ear thickness [a] ± S.D.						% Reduction in ear thickness
		First day		Third day		Fifth day		
		Left	Right	Left	Right	Left	Right	
Hydrocortisone	3×10^{-3}	86±5	86±5	72±4	88±9	70±4	89±4	18.6
	1×10^{-3}	86±5	86±5	78±4	85±4	76±4	88±3	11.6
	3×10^{-4}	88±6	88±7	82±5	89±7	83±6	91±3	5.7
Hydrocortisone 21-acetate	3×10^{-3}	90±7	87±5			81±6	86±5	10.0
	1×10^{-3}	87±4	84±3			79±5	83±3	9.2
	3×10^{-4}	88±3	87±4			80±5	83±6	9.1
Thiazolidine I	3×10^{-3}	88±6	90±6			82±4	87±6	6.8
	1×10^{-3}	87±4	86±5			78±3	88±7	10.3
	3×10^{-4}	89±7	90±7			84±5	88±6	5.6
Thiazolidine II	3×10^{-3}	87±6	86±6			71±5	87±7	18.4
	1×10^{-3}	88±6	88±7			74±3	88±5	15.9
	3×10^{-4}	88±9	89±6			77±5	84±5	12.5
Hydrocortisone 17-butyrate	1×10^{-3}	90±4	92±5	74±4	87±5	69±4	86±4	23.3
	3×10^{-4}	91±5	90±7			75±4	87±5	17.6
	1×10^{-4}	90±5	89±6			78±5	90±3	13.3
	3×10^{-5}	89±6	89±7			83±7	89±4	6.7
Hydrocortisone 17-valerate	3×10^{-3}	89±5	88±6			68±3	82±4	23.6
	1×10^{-3}	87±5	89±5			72±4	88±6	17.2
	3×10^{-4}	88±5	88±8			75±7	89±7	14.8
Triamcinoline acetonide	3×10^{-4}	87±4	89±6	70±4	82±2	66±3	86±5	24.1
	1×10^{-4}	84±4	88±6	73±5	85±6	70±5	85±5	16.7
Vehicle		90±7	92±5	89±6	89±3	91±4	88±4	

[a] 1×10^{4} inches.

TABLE VII

STEROID DIFFUSION THROUGH FRESH HAIRLESS MOUSE SKIN

Compound [c]	Mole % hydrocortisone [a] diffused ± S.D. [b] (h after application)				
Hydrocortisone		2.1 ± 0.8 (4)	4.1 ± 1.3 (9)	7.5 ± 1.8 (18)	8.4 ± 3.0 (24)
Hydrocortisone 21-acetate		1.7 ± 0.6 (4)	3.5 ± 1.3 (9)	6.2 ± 1.3 (18)	14.1 ± 5.9 (24)
Thiazolidine I	0.1 ± 0.05 (2)	0.6 ± 0.1 (4)	1.2 ± 0.2 (7.5)	1.8 ± 0.3 (12)	3.2 ± 0.7 (24)

[a] Only hydrocortisone was found on the receptor side of the membrane under HPLC conditions where hydrocortisone 21-acetate could have been detected. Samples for thiazolidine I were split and one-half was analyzed directly while the other half was treated with acid to hydrolyze any intact thiazolidine; there was no observable difference in the two results.

[b] n = 3.

[c] *50 μl of 0.03 M* acetone–IPM 90:10 was applied to the membranes.

lower levels of steroid being delivered to the systemic circulation. Thus, hydrocortisone, hydrocortisone 21-acetate and thiazolidine I were applied to acetone–isopropyl myristate (IPM) 90:10 solutions to the epidermal side of hairless mouse skin membranes and the rate of appearance of hydrocortisone on the reservoir side of the membrane was monitored by HPLC. The results given in Table VI show that about half as much hydrocortisone was delivered across hairless mouse skin from the thiazolidine I than from its parent compound.

The analogous radiolabeled cysteine ethyl ester thiazolidines of progesterone have been studied in vivo. The results are described elsewhere (Bodor and Sloan, 1980d), but briefly they showed that more than twice as much of the radiolabeled steroid remained in the skin after a topical application of the thiazolidine derivative than after a topical application of progesterone itself.

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