versed-phase LC procedure recently published by the United States Food and Drug Administration (11, 12). In addition to being twice as fast, the selectivity of the adsorption LC system is such that I and II elute prior to aspirin, permitting a low limit of detection for I. The normal phase LC method avoids the use of hydroxylic solvents, which lead to degradation of aspirin and III preventing accurate determination of III by reversed-phase LC. This method uses an inexpensive fixed wavelength (254 nm) UV detector and has a lower limit of detection than any previously published normal phase LC procedure.

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Soft Drugs V: Thiazolidine-Type Derivatives of Progesterone and Testosterone

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Received April 8, 1981, from the **Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610* and the ‡*Inter$_x$ Research Corporation, Lawrence, KS 66044.* Accepted for publication August 5, 1981.

Abstract □ Progesterone and testosterone are natural soft drugs, but to be used as drugs, their fast and facile metabolism must be prevented and their delivery controlled. A prodrug–soft drug combination can serve this purpose. Thiazolidines of testosterone, testosterone 17-propionate and progesterone were synthesized from the reaction of cysteine alkyl esters, *N*-methylaminoethanethiol, and mercaptamine and their hydrochlorides with the appropriate steroids. The thiazolidines function as bioreversible derivatives of the parent steroids.

Keyphrases □ Soft drugs—thiazolidine-type derivatives of progesterone and testosterone □ Prodrugs—thiazolidine-type derivatives of progesterone and testosterone □ Progesterone—thiazolidine-type derivatives, prodrugs □ Testosterone—thiazolidine-type derivatives, prodrugs

Oral contraception is accomplished currently by products containing synthetic hormones, mostly as fixed combinations of synthetic estrogens and progestins (1). The contraceptive action of these products is mediated primarily by inhibition of ovulation through specific macromolecular receptors for each hormone. It is clear that if the natural hormones, *e.g.*, progesterone and estradiol, were delivered to the receptors, they would elicit the same contraceptive effect as the synthetic analogs. The advantage of this method is that the natural hormone might decrease or eliminate the side effects accompanying synthetic contraceptive agents. Some of these side effects are the result of oxidative metabolism (2), such as the one involving the 17α-ethinyl group in norethindrone (17β-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one) or norgestrel (13-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one), which leads to destruction of cytochrome P-450 (3).

Natural hormones such as progesterone, estradiol, and testosterone are natural soft drugs (4, 5); that is, due to their efficient and nontoxic metabolic disposition, they will not cause unexpected toxicity at concentrations close to their natural levels. On the other hand, natural hormones administered as drugs suffer from low physiological availability because, as natural substances, the body has developed efficient mechanisms for their metabolism and excretion. For example, the α,β-unsaturated ketone and the 20-ketone in progesterone are reduced in the liver to give pregnane-3α,20-diol, which can then be conjugated and excreted (1). In addition, the natural hormones are virtually insoluble in water which precludes their efficient dissolution and absorption from most formulations. Thus, to utilize progesterone as a contraceptive drug component, solutions to the problems of slow dissolution (water solubility) and rapid metabolism must be found.

In the case of hydrocortisone (4), the preferred approach to solving the described problems is through chemical modification, *i.e.*, a prodrug. However, progesterone is a difficult candidate for such an approach because the only functional groups available for reversible modification are the 3- and 20-ketones. Therefore, an extension of the spirothiazolidine approach (4) seemed the most attractive, as thiazolidines are unique examples of a bioreversible steroidal ketone derivative. The spontaneous S_N1 cleavage of the thiazolidine (6) to a β-thioethylene imine is followed by hydrolysis of the imine [possibly through a hydration–disassociation mechanism (7)] to regenerate the parent carbonyl compound (4). In addition to their potential bioreversibility, thiazolidines were attractive as prodrugs because they could be prepared from cysteine. Consequently, the hydrolysis products (steroid and cysteine) of the prodrugs would not present any unusual metabolic burden to the body. The carboxylic group of cysteine could also be easily esterified, thus providing a convenient method for changing the lipophilicity/hydrophilicity of the derivatives. In addition to progesterone, testosterone thiazolidines were also investigated[1].

[1] Part 4 of this series (N. Bodor, K. B. Sloan, R. J. Little, S. H. Selk, and L. Caldwell, *Int. J. Pharm;* in press).

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EXPERIMENTAL[2]

Biological—*In vivo* animal tests used standard procedures for the Clauberg and seminal vesicle and ventral prostate weight tests.

Plasma Hydrolysis Studies of Thiazolidines—Whole blood was drawn from female beagles immediately before the hydrolysis study. The whole blood was treated with edetate disodium as an anticoagulant. The blood was centrifuged at 2000 rpm for 10 min. The plasma was removed from the red blood cells and filtered through a 0.2-μm methylcellulose membrane filter. Concentrated spiking solutions of the thiazolidines (Table I) were prepared in dimethylformamide. Small volumes of these solutions (20 μl/ml of plasma) were added to the plasma to give the appropriate concentrations. Methyl testosterone (0.020 mg/ml) was added as an internal standard for chromatography. The samples were mixed thoroughly and placed in a water bath at 37°, equipped with a shaking mechanism. Samples (5 μl) were withdrawn at ~12-hr intervals for injection onto the high-pressure liquid chromatograph. The mobile phase was tetrahydrofuran–methanol–water (1:1:2) at a 2.0-ml/min flow rate. The eluate was monitored at 254 nm for progesterone. The progesterone concentration was determined by comparison of the peak areas of the sample and standard solutions, and corrected using the internal standard. Hydrolysis rates were determined by the rate of appearance of progesterone. Straight lines were determined using a least-squares fit.

Pharmacokinetics of XI in Dogs—Female beagle dogs were used for the study which included a crossover experiment. The dogs were placed in separate stainless steel cages 24 hr prior to administration of the drug. Water was available at all times and normal feeding schedules were maintained. The dogs were dosed with 2.5 μCi of drug by intravenous injection in the front leg over a 30 sec period. The ^{14}C-labeled progesterone was 1 μCi/mg. The ^{14}C-labeled XI was 0.446 μCi/mg. All drugs were dissolved in 100 μl of benzyl alcohol. Blood samples (~4 ml) were drawn from the jugular vein of the dog using tubes containing edetate disodium as an anticoagulant preservative. Blood samples were taken at 5, 10, 15, 30, 45, 60, and 90 min, 2, 4, 6, 8, 12, 24, 32, and 48 hr, and at other appropriate times after injection. The blood (3.5 ml) was transferred to a tared 25-ml heavy duty centrifuge tube with a polytef-lined screw cap. The samples were weighed accurately before extraction. Extractions were performed by shaking for 10 min with 10 ml of 10% ethylene dichloride in ether (v/v) on a wrist-shaker, centrifuging for 15 min at 2000 rpm, and transferring the organic phase to new liquid scintillation vials. Samples were extracted twice and the organic phases from each extraction were combined. The organic solvent was evaporated to dryness in a 50° water bath under dry nitrogen stream. The extracts were dissolved in 15 ml of scintillation cocktail and mixed thoroughly. The samples were stored overnight at 4° prior to counting to reduce chemiluminescence.

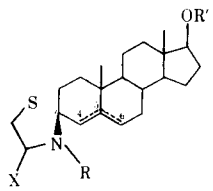
Urine samples from each dog were collected at 24 and 48 hr after dosing. Both samples were frozen separately until all the studies were completed and all samples were prepared at once. The urine was thawed and the total volumes measured. A small amount of well mixed urine from each sample was centrifuged to remove particulate matter. One milliliter of each sample was quantitatively transferred to a liquid scintillation vial containing 0.5 ml of 0.5 *N* hydrochloric acid and mixed. Fifteen milliliters of scintillation cocktail was added to each vial and mixed. Samples were then counted.

Recovery experiments were conducted using radioactive labeled drugs at three blood levels for each of the three compounds in whole dog blood. Extraction efficiencies for each compound at each blood level were determined using 10% ethylene dichloride in ether as a solvent. For each compound, studies were conducted at blood levels of 0.001, 0.0001, and 0.00005 μCi/3 ml of blood. For each blood level, 18 ml of blood was transferred to a tared, heavy-duty centrifuge tube and weighed accurately. Each pool of blood was spiked at the appropriate concentration using either 30 or 60 μl of a spiking solution (methanol). The spiked blood was shaken for 30 min to reach equilibrium. Five replicate 3-ml samples were drawn from each pool and transferred to tared 25-ml heavy-duty centrifuge tubes equipped with polytef-lined screw caps and weighed accurately.

Each sample was extracted twice by shaking for 15 min on a wrist-shaker with 10 ml of 10% ethylene dichloride in ether, centrifuging for 10 min at 2200 rpm, and transferring the supernate to a liquid scintillation vial. Combined extracts were evaporated to dryness under a dry nitrogen stream at 55°. The residues were dissolved in 15 ml of scintillation cocktail. Samples were stored overnight at 4° to reduce chemiluminescence and then counted.

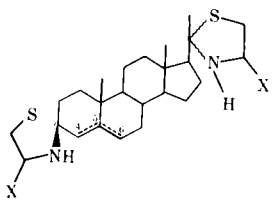
Distribution Studies in Rats—Approximately 1 μCi/mmole of progesterone, XI, and VIII*a* in 5 ml of benzyl alcohol were injected intravenously in the tail veins of six female rats (90–120 g). They were then placed in a clean metabolism cage and their urine and feces were collected.

Table I—Thiazolidines of Testosterone and Testosterone 17β-Propionate



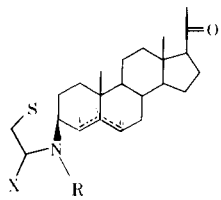
Compound	X	R	Δ	R′
I*a*	$CO_2C_2H_5$	H	5,6	COC_2H_5
I*b*	$CO_2C_2H_5$	H	4,5	COC_2H_5
II*a*	$CO_2C_2H_5$	H	5,6	H
II*b*	$CO_2C_2H_5$	H	4,5	H
III	$CO_2CH_2CH_2OH$	H	5,6	COC_2H_5
IV	$CO_2CH_2CH_2OH$	H	5,6	H
V	H	H	5,6	COC_2H_5
VI	H	H	5,6	H
VII	H	CH_3	5,6	H

Thiazolidines of Progesterone



Compound	X	Δ
VIII*a*	$CO_2C_2H_5$	5,6
VIII*b*	$CO_2C_2H_5$	4,5
X	$CO_2C_4H_9$	4,5
XI	$CO_2C_6H_{13}$	4,5:5,6(1:1)
XII	$CO_2C_{10}H_{21}$	4,5

Thiazolidines of Progesterone



Compound	X	R	Δ
IX*a*	$CO_2C_2H_5$	H	5,6
IX*b*	$CO_2C_2H_5$	H	4,5
XIII	H	H	5,6
XIV	H	COH	5,6
XV	H	$COCH_3$	5,6
XVI	H	$COCH_2NHCO_2$-$CH_2C_6H_5$	5,6
XVII	H	CH_3	5,6

[2] Radiolabeled counting was performed on a Beckman LS-100C Scintillation Counter. TLC were run on Brinkman Polygram Sil G/UV 254. Melting points (uncorrected) were taken with a Thomas–Hoover capillary apparatus. NMR spectra were recorded on a Varian T-60 (^1H spectra) or on a Bruker WP-80 (^{13}C spectra) spectrophotometer. Infrared spectra were obtained on a Beckman Acculab 4 infrared spectrophotometer, while UV spectra were determined on a Carey Model 14 spectrophotometer. Optical rotations were obtained using a Perkin Elmer 141 polarimeter. HPLC analyses were run on a Waters 6000A solvent delivery system U6K universal injector and a 440 Dual Channel UV detector. Microanalyses were performed by Midwest Microlab, Ltd, Indianapolis, Ind. Progesterone, testosterone, testosterone propionate, cysteine methyl, and ethyl ester hydrochlorides were obtained from Sigma. 2-Aminoethanethiol hydrochloride was obtained from Aldrich, the scintillation cocktails were Aquasol or Biofluor from New England Nuclear, the protein solubilizer was Protosol from New England Nuclear, while all other reagents were obtained from Mallinckrodt unless otherwise specified. The cysteine glycerol, butyl, hexyl, and decyl esters were prepared according to Voullie (8) *et al.*, and the *N*-acyl-thiazolidines were prepared according to Sloan *et al.*[3] The hairless mice (SKH-hr-1) were obtained from the Temple University Skin and Cancer Hospital. *In vivo* animal tests were conducted at the National Institutes of Health.

[3] K. B. Sloan, N. Bodor, and J. Zupan, *Tetrahedron*, in press.

Sixty minutes after injection each rat was anesthetized with ether, and its blood was collected in a preweighed test tube containing 4 drops of 15% edetate disodium. Each rat was then sacrificed with more ether and dissected. The fat deposits, spleen, kidneys, ovaries, liver, lungs, heart, brain, intestinal walls, GI contents, and the tail were all removed and each one placed in a preweighed test tube.

After each organ (except the tail) was weighed, it was homogenized with a known amount of water in a glass tissue grinder. The tail was wrapped in aluminum foil, frozen in liquid nitrogen, shattered with a hammer, and mixed thoroughly with a known volume of water. The feces were diluted with water and mixed thoroughly. Samples (0.5 ml) of the homogenates or suspensions and 1.0 ml of a protein solubilizer–ethanol mixture were placed in liquid scintillation counting vials. The vials containing the samples were heated at 55° for 60 min, and if necessary, the samples were decolorized by heating with 0.1–0.5 ml of 30% hydrogen peroxide. After the samples were cooled, 15.0 ml of scintillation cocktail and 0.5 ml of 0.5 *N* HCl were added to the samples, and they were counted. A quenching curve was obtained using tissue samples, protein solubilizer, hydrogen peroxide, and scintillation cocktail. The urine from each rat was collected directly in a scintillation vial, and counted in 15.0 ml of scintillation cocktail.

Percutaneous Distribution in Mice—For the percutaneous delivery study, the thiazolidine VIII*a* and progesterone were dissolved in ethanol–isopropyl myristate (90:10) and applied to the backs of six hairless mice using a bandage to form an occlusive application. The mice were then placed in metabolism cages for 24 hr and then sacrificed. The bandages were then removed and the surface of the skins were washed three times with 5 ml of ethanol. The washes were combined with the patches and counted. An epidermal plug, 2 cm in diameter, was taken from each carcass from directly beneath where the patches (3 cm^2) had been with a leather punch. The epidermal plugs, the remainder of the carcasses, the urine, and feces were processed as described for the rat distribution study.

Reaction of Testosterone 17β-propionate with Cysteine Ethyl Ester Hydrochloride—To a mixture of testosterone 17β-propionate (1.0 g, 0.0029 mole) and cysteine ethyl ester hydrochloride (3.70 g, 0.02 mole) was added 10 ml of pyridine. The solution was allowed to sit at room temperature overnight under a nitrogen atmosphere in a tightly closed flask. The solution was then partitioned using methylene chloride–water (100:100) and the methylene chloride layer was separated, dried over sodium sulfate, and concentrated to give a solid residue. The residue was crystallized from 10 ml of hot ethanol to give 0.35 g (mp 144–147°, 26% yield) of 5-androstene-17β-propionyloxy-3-spiro-2′-(4′-ethoxycarbonyl-1′,3′-thiazolidine), I*a*: TLC (silica gel, ether) R_f 0.45; IR (KBr): 1735 cm^{-1} (s, C=O); ^{1}H-NMR ($CDCl_3$): δ 5.53–5.2 (m, 1, C*H*=C), 4.6 (t, *J* = 8 Hz, 1, C*H*O_2C), 4.21 (q, *J* = 7 Hz, 2, CH_3CH_2O), 4.2–3.8 (m, 1, O_2CC*H*N), 3.4–2.7 (m, 2, CH_2S), 1.29 (t, *J* = 7 Hz, 3, CH_3CH_2O), 1.01 (s, 3, CH_3—C), 0.8 (s, 3, CH_3—C), and 2.7–1.0 (m, 24, N*H*, CH_2 and C*H*); ^{13}C-NMR ($CDCl_3$): δ 174.7, 171.9 (CO_2), 140.7 (C-5), and 122.4 (C-6); $[\alpha]_D^{25}$ −55° (*C* = 0.55, ethanol).

Anal.—Calc. for $C_{27}H_{41}NO_4S$: C, 68.17; H, 8.69; N, 2.95. Found: C, 68.46; H, 8.78; N, 2.70.

The mother liquor was concentrated to 5 ml and allowed to crystallize further. This gave 0.51 g (mp 100–104°, 37% yield) of a mixture containing primarily 4-androstene-17β-propionyloxy-3-spiro-2′-(4′-ethoxycarbonyl-1′,3′-thiazolidine), I*b*, but with some I*a* (ratio ~9:1, I*b*:I*a*). TLC and IR were the same as for I*a*. The ^{1}H-NMR spectrum was the same except that the major (90%) C*H*=C absorption was a singlet as δ 5.3; ^{13}C-NMR ($CDCl_3$): δ 174.7, 171.9 (CO_2), 148.7 (C-5), and 123.0 (C-4); $[\alpha]_D^{25}$ = +10 (*C* = 0.49, ethanol).

Anal.—Calc. for $C_{27}H_{41}NO_4S$: C, 68.17; H, 8.69; N, 2.95. Found: C, 68.28; H, 8.79; N, 2.75.

Compounds II*a*, II*b*, III, and IV were prepared in a similar manner and had NMR and IR spectra consistent with the assigned structures.

5- and 4-Androstene-17β-ol-3-spiro-2′-(4′-ethoxycarboxyl-1′,3′-thiazolidine)—These are II*a* and II*b*, respectively.

II*a*: mp 136–140° from ethanol, 32% yield; $[\alpha]_D^{25}$ = −60° (*C* = 0.47, $CHCl_3$).

Anal.—Calc. for $C_{24}H_{37}NO_3S$: C, 68.69; H, 8.89; N, 3.34. Found: C, 68.58; H, 8.79; N, 3.18.

II*b*: An amorphous powder from water; $[\alpha]_D^{25}$ = +2° (*C* = 0.45, $CHCl_3$).

Anal.—Calc. for $C_{24}H_{37}NO_3S$: C, 68.69; H, 8.89; N, 3.34. Found: C, 67.87; H, 8.79; N, 3.54.

The 4-androstene-3-thiazolidine could be crystallized from 1 ml of ethanol to give a light yellow fibrous solid (0.28 g, mp 67–75°), which was identical in all respects with the crude product and gave a low carbon analysis also.

5-Androstene-17β-propionyloxy-3-spiro-2′-(4′-β-hydroxyethoxycarbonyl-1′,3′-thiazolidine)—III: mp 127–130° from ether; $[\alpha]_D^{25}$ = −49° (*C* = 0.5, $CHCl_3$).

Anal.—Calc. for $C_{27}H_{41}NO_5S$: C, 66.00; H, 8.34, N, 2.84; S, 6.52. Found: C, 65.90; H, 8.55; N, 2.68; S, 6.69.

5-Androstene-17β-ol-3-spiro-2′-(4′-β-hydroxyethoxycarbonyl-1′,3′-thiazolidine)—IV: mp 135–140° from ether.

Anal.—Calc. for $C_{24}H_{37}NO_4S$: C, 66.22; H, 8.50, N, 3.21; S, 7.36. Found: C, 66.16; H, 8.77; N, 3.39; S, 7.48.

Reaction of Progesterone with Cysteine Ethyl Ester Hydrochloride—Progesterone (1.0 g, 0.0032 mole) was mixed with increasing amounts of cysteine ethyl ester hydrochloride (0.65 g, 0.0035 mole, one equivalent; 1.30 g, 0.0070 mole, two equivalents; 2.60 g, 0.014 mole, four equivalents; 3.90 g, 0.021 mole, six equivalents) and dissolved in pyridine (10 ml) in four separate reactions. The reactants were kept at room temperature overnight under nitrogen in a tightly closed flask. Each reaction was then concentrated *in vacuo* (0.1 mm, 1 hr, 45°) to give a gummy solid which was suspended in 50 ml of ethylene dichloride. The ethylene dichloride suspensions were extracted with 10 ml of water, dried over sodium sulfate and concentrated *in vacuo* to give yellow solids which were analyzed by TLC and NMR spectroscopy. Only the reaction with six equivalents of cysteine ethyl ester hydrochloride appeared to give a homogeneous product. All of the reaction products were suspended in 25 ml of boiling ethanol and filtered. In the case of the reactions with four and six equivalents, there was a considerable amount of the ethanol insoluble material. The ethanol insoluble fraction from the reaction with four equivalents of cysteine ethyl ester hydrochloride was a mixture, but that from six equivalents was homogeneous and it was identified as 17β-(4″-ethoxycarbonyl-2″-methyl-1″,3″-thiazolidine-2″-yl)-5-androstene-3-spiro-2′-(4′-ethoxycarbonyl-1′,3′-thiazolidine)VIII*a* (1.2 g, mp 167–170°, 67% yield); IR (KBr): 1735 cm^{-1} (s, C=O); ^{1}H-NMR ($CDCl_3$): δ 5.6–5.2 (m, 1, C*H*=C), 4.20 (q, *J* = 7 Hz, 4, OCH_2CH_3), 4.1–3.65 (m, 2, O_2CC*H*—N), 3.5–2.6 (m, 4, CH_2—S), 1.57 (s, 3, CH_3—C(N)—S), 1.03 (s, 3, CH_3—C), 0.85 (s, 3, CH_3—C), 1.3 (t, *J* = 7 Hz, 6, CH_3CH_2O), 2.6–0.6 (m, 19, CH_2 and C*H*); ^{13}C-NMR ($CDCl_3$): δ 171.9 (CO_2), 140.6 (C-5) and 122.6 (C-4); $[\alpha]_D^{25}$ = −92° (*C* = 0.56, $CHCl_3$); TLC (silica gel, ether) R_f 0.42.

Anal.—Calc. for $C_{31}H_{48}N_2O_4S_2$: C, 64.54; H, 8.39; N, 4.86. Found: C, 64.61; H, 8.44; N, 4.63.

The crystals obtained from the 25-ml ethanol solutions contained mixtures except for the product from the reaction of progesterone with one equivalent of cysteine ethyl ester hydrochloride. Those crystals were identified as 5-pregnene-20-one-3-spiro-2′-(4′-ethoxycarbonyl-1′,3′-thiazolidine) (IX*a*) with about 10% of the 4-pregnene IX*b* as a contaminant (0.45 g, mp 127–134°, 33% yield); IR (KBr): 1735 and 1700 cm^{-1} (s) (C=O); ^{1}H-NMR ($CDCl_3$): δ 5.6–5.2 (m, 1, C*H*=C), 4.26 (q, *J* = 7 Hz, 2, CH_3CH_2O), 4.2–3.8 (m, 1, O_2CC*H*N), 3.6–2.8 (m, 2, CH_2S), 2.11 (s, 3, CH_3—C=O), 1.02 (s, 3, CH_3—C), 0.63 (s, 3, CH_3—C), 1.3 (t, *J* = 7 Hz, 3, CH_3CH_2O), 2.8–0.6 (m, 19, CH_2 and C*H*); ^{13}C-NMR ($CDCl_3$): δ 209.7 (C-20 = O), 171.9 (CO_2), 140.6 (C-5) and 122.5 (C-6); $[\alpha]_D^{25}$ = +11° (*C* = 0.58, $CHCl_3$); TLC (silica gel, ether) R_f 0.34.

Anal.—Calc. for $C_{26}H_{39}NO_3S$: C, 70.07; H, 8.82; N, 3.14. Found: C, 69.84; H, 8.80; N, 3.05.

In the case where six equivalents of cysteine ethyl ester hydrochloride was used, a precipitate formed after the reaction was allowed to run overnight. In a separate reaction, that precipitate was filtered to give VIII*a* (mp 164–170, 61% yield).

The following were prepared in a similar manner. Their NMR and IR spectra were consistent with the assigned structures.

17β-(4″-Butoxycarbonyl-2″-methyl-1″,3″-thiazolidine-2″-yl)-4-androstene-3-spiro-2′-(4′-butoxycarbonyl-1′,3′-thiazolidine)— mp 149–150°, from ethanol; $[\alpha]_D^{25}$ = −38° (*C* = 0.58, $CHCl_3$).

Anal.—Calc. for $C_{35}H_{50}N_2O_4S_2$: C, 66.42; H, 8.92; N, 4.43. Found: C, 66.90; H, 9.20; N, 4.30.

17β-(4″-Hexoxycarbonyl-2″-methyl - 1″,3″ - thiazolidine-2″-yl)-4- and 5-androstene-3-spiro-2′-(4′-hexoxycarbonyl-1′,3′-thiazolidine) (*XI*)—mp 113–115°, from ethanol; $[\alpha]_D^{25}$ = −25° (*C* = 0.51, $CHCl_3$).

Anal.—Calc. for $C_{39}H_{64}N_2O_4S_2$: C, 67.98; H, 9.36; N, 4.07. Found: C, 68.40; H, 9.48; N, 3.90.

17β-(4″-Decoxycarbonyl-2″-methyl-1″,3″-thiazolidine-2″-yl)-4-androstene-3-spiro-2′-(4′-decoxycarbonyl-1′,3′-thiazolidine) (*XII*) —mp 100–102°, from ethanol.

Anal.—Calc. for $C_{47}H_{80}N_2O_4S_2$: C, 70.45; H, 10.66; N, 3.50. Found: C, 70.62; H, 10.20; N, 3.40.

Preparation of 17β-(4″-Ethoxycarbonyl-2″-methyl-1″,3″-thiazolidine-2″-yl)-4-androstene-3-spiro-2′-(4′ - ethoxycarbonyl - 1′,3′-

Table II—Hydrolyses of Thiazolidines [a]

	$t_{1/2}$ [b], hr
Mono-thiazolidines	
IX*b*	22 h
IX*a*	77 h
XIII	16 h
Bis-thiazolidines	
VIII*b*	41 h
VIII*a*	106 h
XI	164 h

[a] Time for one-half the theoretical amount of progesterone to form. [b] Concentration of thiazolidines in plasma was 0.040 mg/ml.

thiazolidine) (VIII*b*)—Cysteine ethyl ester hydrochloride (11.2 g, 0.06 mole) was suspended in 40 ml of triethylamine with vigorous stirring for 1 hr at room temperature. The triethylamine hydrochloride was filtered and the filtrate was concentrated to give cysteine ethyl ester as an oil. The oil was dissolved in 30 ml of pyridine and allowed to react with 3.2 g (0.01 mole) of progesterone at room temperature for 3.5 days. The suspension that resulted was filtered and crystallized from ethyl acetate to give 2.7 g (mp 162–165°, 47% yield) of the desired product: IR (KBr): 1740 cm^{-1} (s, C=O); ^{1}H-NMR ($CDCl_3$): δ 5.23 (s, 1, C*H*=C), 4.2 (q, *J* = 7 Hz, 4, CH_3CH_2O), 4.2–3.6 (m, 2, O_2CCHN), 3.50–2.65 (m, 4, CH_2S), 1.55 (s, 3, CH_3—C(N)S), 1.3 (t, *J* = 7 Hz, 6, CH_3CH_2O), 1.03 (s, 3, CH_3—C), 0.85 (s, 3, CH_3—C), 2.6–0.6 (m, 19, CH_2 and C*H*); ^{13}C-NMR ($CDCl_3$): δ 171.9 and 172.0 (CO_2), 148.9 (C-5) and 122.9 (C-4); $[\alpha]_D^{25} = -24°$ (*C* = 0.63, $CHCl_3$).

Anal.—Calc. for $C_{31}H_{48}N_2O_4S_2$: C, 64.54; H, 8.39; N, 4.86. Found: C, 64.59; H, 8.46; N, 4.88.

Preparation of 4-Pregnene-20-one-3-spiro-2′-(4′-ethoxycarbonyl-1′,3′-thiazolidine) (IX*b*)—Compound VIII*a* (1.0 g) was heated in 25 ml of 80% acetic acid at 65° for 3.5 min then quickly neutralized with 400 ml of ice cold water containing 25 g of sodium bicarbonate. The suspension that resulted was filtered and dried to give 0.41 g of a white solid which was about 65% pure. The solid was crystallized from ethanol (7 ml) to give 0.22 g (mp 131–135°, 28% yield) of the desired product; IR (KBr): 1735 and 1700 cm^{-1} (s, C=O); ^{1}H-NMR ($CDCl_3$): δ 5.30 (s, 1, C*H*=C), 4.23 (q, *J* = 7 Hz, 2, CH_3CH_2O), 4.4–3.9 (m, 1, O_2CCHN), 3.6–2.8 (m, 2, CH_2S), 2.15 (s, 3, CH_3—C=O), 1.3 (t, 3, *J* = 7 Hz, CH_3CH_2O), 1.03 (s, 3, CH_3—C), 0.63 (s, 3, CH_3—C), 2.8–0.6 (m, 19, CH_2 and C*H*); ^{13}C-NMR ($CDCl_3$): δ 209.7 (C-20), 171.9 (CO_2), 148.7 (C-5), and 123.1 (C-6); $[\alpha]_D^{25}$ = +98° (*C* = 0.47, $CHCl_3$).

Anal.—Calc. for $C_{26}H_{39}NO_3S$: C, 70.07; H, 8.82; N, 3.14. Found: C, 70.00; H, 8.89; N, 2.55.

Preparation of 5-Andostrene-17β-ol-3-spiro-2′-(3′-methyl-1′,3′-thiazolidine) (VII)—*N*-Methylaminoethanethiol (1.3 g, 0.014 mole) was warmed to its melting point, 2.0 g (0.0069 mole) of testosterone was added, and the mixture was heated at ~130° for 40 min. The mixture was dissolved in methylene chloride (80 ml), and the solution was washed with water (40 ml), separated, dried over sodium sulfate, and concentrated to give an orange gum. The gum was extracted with 50 ml of refluxing cyclohexane. The cyclohexane was concentrated to 15 ml and cooled to give 0.67 g (mp 112–123°, 27% yield) of the desired thiazolidine: TLC (silica gel, ether) R_f 0.30; IR (KBr): 3400 cm^{-1} (m, OH); ^{1}H-NMR ($CDCl_3$): δ 5.5–5.3 (m, 1, C*H*=C), 3.67 (t, 1, *J* = 7 Hz, C*H*—OH), 3.5–3.17 (m, 2, CH_2—N), 3.17–3.77 (m, 2, CH_2—S), 2.37 (s, 3, CH_3—N), 1.05 (s, 3, CH_3—C), 0.78 (s, 3, CH_3—C), and 2.7–0.8 (m, 20, O*H*, CH_2— and C*H*); ^{13}C-NMR ($CDCl_3$): δ 140.6 (C-5) and 122.5 (C-6).

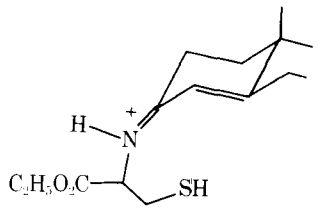
Anal.—Calc. for $C_{22}H_{35}NOS$: C, 73.07; H, 9.75; N, 3.87. Found: C, 73.19; H, 9.41; N, 3.88.

The filtrate was concentrated to 5 ml, the cyclohexane was decanted from some gum that precipitated, and the cyclohexane was concentrated to dryness to give an additional 0.80 g (32% yield) of the desired product as a foam which was identical with the crystalline material by TLC, NMR, and IR.

5-Pregnene-20-one-3-spiro-2′-(3′-methyl-1′,3′-thiazolidine) (XVII) was prepared in a similar manner (mp 149–151°, from hexane, 25% yield); IR (KBr): 1710 cm^{-1} (s, C=O); ^{1}H-NMR ($CDCl_3$): δ 5.5–5.2 (m, 1, C*H*=C), 2.8–3.5 (m, 4, CH_2—N), 2.33 (s, 3, CH_3—N), 2.13 (s, 3, CH_3CO), 1.03 (s, 3, CH_3—C), and 0.63 (s, 3, CH_3C).

Anal.—Calc. for $C_{24}H_{37}NOS$: C, 74.36; H, 9.62; N, 3.61. Found: C, 74.60; H, 9.80; H, 3.45.

Preparation of 5-Pregnene-20-one-3-spiro-2′-(1′,2′-thiazolidine) (XIII)—A mixture of 3.14 g (0.01 mole) of progesterone and 6.7 g (0.06 mole) of 2-aminoethanethiol hydrochloride was suspended in 20 ml of pyridine overnight at room temperature under a nitrogen atmosphere.



XVIII

The suspension was concentrated *in vacuo*. The residue that resulted was partitioned between methylene chloride–water (100:150 ml). The methylene chloride layer was dried over sodium sulfate, concentrated, and the residue triturated with 10 ml of warm methanol. Almost all of the residue went into solution. After the suspension had cooled, it was filtered to give 1.72 g (mp 127–136°, 46% yield) of the desired compound: TLC (silica gel, ether) R_f 0.20; IR (KBr): 3300 (w, N—H), and 1700 cm^{-1} (s, C=O); ^{1}H-NMR ($CDCl_3$): 5.5–5.2 (m, 1, C*H*=C), 3.6–2.9 (m, 4, SCH_2—CH_2—N), 2.11 (s, 3, CH_3C=O), 1.03 (s, 3, CH_3—C), 0.63 (s, 3, CH_3—C) and 2.9–0.6 (m, 21, CH_2, C*H* and N*H*); $[\alpha]_D^{25}$ = +104° (*C* = 0.51, $CHCl_3$).

Anal.—Calc. for $C_{23}H_{35}NOS$: C, 73.94; H, 9.44; N, 3.75. Found: C, 73.69; H, 9.80; N, 4.00.

Compounds V and VI were prepared in a similar manner. Their NMR and IR spectra were consistent with the assigned structures.

5-Androstene-17β-ol-3-spiro-2′-(1′,3′-thiazolidine) (VI)—TLC (silica gel, ether) R_f 0.24; $[\alpha]_D^{25}$ = +83° (*C* = 0.57, $CHCl_3$).

Anal.—Calc. for $C_{21}H_{33}NOS$: C, 72.57; H, 9.57; N, 4.03. Found: C, 72.56; H, 9.23; N, 4.03.

5-Androstene - 17β-propionyloxy-3-spiro-2′-(1′,3′-thiazolidine):V—mp 152–157° (from methanol, 71% yield); TLC (silica gel, ether) R_f 0.16; $[\alpha]_D^{25}$ = +69° (*C* = 0.52, $CHCl_3$).

Anal.—Calc. for $C_{24}H_{37}NO_2S$: C, 71.41; H, 9.24; N, 3.47. Found: C, 71.32; H, 9.38; N, 3.15.

Preparation of [4-^{14}C]-VIII*a* and XI—[4-^{14}C]Progesterone (100 mg, 100 μCi) was mixed with 1 g of cysteine ethyl or hexyl ester hydrochloride in 2 ml of pyridine. The systems were purged with nitrogen and then stirred overnight under nitrogen. The reaction mixtures were dissolved in 50 ml of ether and extracted with 25 ml of water. The water layers were extracted with an additional 50 ml of ether. The two ether layers were combined, dried over magnesium sulfate and evaporated. Methanol (10 ml) was added to each residue and after they were cooled the solutions gave 47 mg of VIII*a* from the reaction with the ethyl ester and 114 mg of XI from the reaction with hexyl ester. Both thiazolidines were analyzed by radiochromatograph and found to be homogeneous (VIII*a*, R_f = 0.8; XI, R_f = 0.7) using silica gel and ether.

RESULTS AND DISCUSSION

Chemistry—The synthesis and biological activity of the thiazolidines of hydrocortisone and hydrocortisone derivatives have been described recently (4). Similar reaction conditions were used in this study except for the preparation of the *N*-methylthiazolidines and the 4,5-double bond isomers of the mono- and dithiazolidines of progesterone, where special reaction conditions were necessary. As in the hydrocortisone series, the product from the reaction of cysteine with the steroids could not be isolated. However, as opposed to the hydrocortisone series where only one thiazolidine isomer was obtained from the reaction with the cysteine esters or an aminoethanethiol, testosterone and progesterone gave two series of thiazolidine isomers. The simplest case was the testosterone series. The reaction between testosterone 17β-propionate and cysteine ethyl ester hydrochloride gave two isomers, I*a* and I*b* (Table I) which were separated by fractional crystalization from ethanol. The elucidation of the structures of these isomers was reported recently[4], and it was shown that the isomers are the 4,5- and 5,6-double bond isomers as indicated by their optical rotations and ^{1}H- and ^{13}C-NMR spectra when compared with known 4,5- and 5,6-double bond isomers of 3-ethylene ketals and with each other. Although testosterone also gave two double bond isomers from its reaction with cysteine ethyl ester hydrochloride, only the 5,6-double bond isomer II*a* could be isolated in pure form.

Similar reactions of progesterone could be directed to give either the 5,6-double bond mono-3-thiazolidine IX*a* or di-3,20-thiazolidine VIII*a* by using either one or six equivalents, respectively, of cysteine ester hy-

[4] K. B. Sloan, N. Bodor, and R. J. Little, *Tetrahedron*, in press.

Table III—Total Radioactivity (Disintegrations per Minute) in Blood [a] after Intravenous Administration [b] to Dogs of 2.5 μCi of Progesterone [c] and XI [d]

Dog	Drug	Time, hr												
		0.08	0.16	0.25	0.5	0.75	1.0	1.5	2	4	6	8	12	24
1	Progesterone	1084	564	564	394	384	264	172	144	80	27	—	34	10
1	XI	85	16	0	0	11	3	6	20	13	10	0	7	0
2	Progesterone	1177	917	666	509	387	365	278	273	80	105	78	62	33
2	XI	156	58	49	57	68	43	57	60	55	29	—	50	27

[a] 4-ml sample. [b] In 100 μl of benzyl alcohol. [c] 8×10^{-6} mole. [d] 8.14×10^{-6} mole.

drochloride. No significant amounts of the 4,5-double bond isomers VIII*b* and IX*b* were observed in these crude reaction mixtures based on an analysis of the shape and position of the C*H*=C absorption in the ^{1}H-NMR spectra. Since the thiazolidines decomposed on TLC plates and during column chromatography, analysis of the ^{1}H-NMR spectra of the isomers was the only reliable means of qualitatively determining the extent of the reactions and ratio of isomers. On the other hand, when butyl, hexyl, or decyl cysteine ester hydrochlorides were used instead of the ethyl ester, mixtures of the double bond isomers were isolated, or the 4,5-double bond isomer was the predominant isomer in the mixture (X, XI, and XII, Table I).

The preparation of the 4,5-double bond isomer VIII*b* from the reaction of cysteine ethyl ester with progesterone was accomplished without an acid catalyst necessary for the 4,5 → 5,6 migration by using the free base rather than the hydrochloride of cysteine ethyl ester.

In the second case (IX*b*), the successful synthesis was based on the observation of the iminium ion XVIII (9) as an intermediate in the UV monitored hydrolyses of the 4,5-double bond thiazolidines (4). This suggested that generation of the iminium ion followed by quenching of the reaction with base would result in recyclization of the imine to the thiazolidine, without concomitant reisomerization of the double bond to the 5,6-position. Indeed, when VIII*a* was treated with aqueous acetic acid at 65° for 3.5 min, then with cold aqueous bicarbonate, a mixture of progesterone and IX*b* was obtained from which IX*b* was easily isolated by crystallization from ethanol.

The success of the partial hydrolysis reaction to generate IX*b* was based in part on the observation that the 20-ketone thiazolidine hydrolyzed faster than the 3-ketone thiazolidine. This was not surprising since in addition to suggesting that the thiazolidine of α,β-unsaturated steroidal ketones could not be isolated, a previous report (10) suggested that thiazolidines of 17- and 20-steroidal ketones could not be obtained from the base catalyzed reaction of cysteine with the steroid. Thus, it was expected that both ketones would be difficult to derivatize for progesterone. In addition, the formation of the iminium ion XVIII from the 5,6-double bond isomer VIII*a* obviously could not be instantaneous because the double bond had to isomerize to the 4,5-position. Hence, the reaction time of 3.5 min was not only essential to ensure the complete conversion of VIII*a* to XVIII, but it also allowed more time for the complete hydrolysis of the 20-ketone thiazolidine to take place.

Since the 4′-ethoxycarboxyl group is electron withdrawing, the 4′ unsubstituted thiazolidines were prepared in an attempt to improve the hydrophilicity of the thiazolidines by increasing the pKa of the amine group, thus increasing the concentration of the conjugate acid of the thiazolidine at any pH. Since the rate of hydrolysis of thiazolidines is directly related to the pKa of the amine group (11), a 4′-unsubstituted thiazolidine (*e.g.*, XIII) was expected to be more easily hydrolyzed than the 4′-alkoxycarbonyl thiazolidines (*e.g.*, IX*a*). However, it was also expected that if the 4′-position was left unsubstituted, and an alkyl group was introduced into the 3′-position of the thiazolidine, it would be as soluble as the 4′-unsubstituted thiazolidine, and would also be more stable (11).

The 4′-unsubstituted thiazolidine XIII was prepared from the reaction of progesterone with six equivalents of aminoethanethiol hydrochloride in pyridine. Only the monothiazolidine was obtained even when 12 equivalents of the aminoethanethiol was used. Inspection of the positions of the 18-CH_3 and 19-CH_3 absorptions in the NMR spectra of the crude reaction mixtures showed that XIII was the only product present, even before work-up. The 18-CH_3 absorption shifted from 40 to 38 to 51 Hz in going from progesterone to the 3-mono IX*a* to the 3,20-dithiazolidine VIII*a* while the 19-CH_3 absorption shifted from 71 to 62 Hz in going from progesterone to the mono-3-IX*a* and the di-3,20-thiazolidine VIII*a*. Thus, the position of the 18- and 19-CH_3 absorptions at 38 and 62 Hz and the 20-CH_3 at 127 Hz in the NMR spectrum of XIII indicated only mono-3-thiazolidine formation.

Use of the aminoethanethiol rather than the hydrochloride in the reaction did not result in the preferential formation of the 4,5-double bond isomer. In fact, it was difficult to determine that XIII was the 5,6-double bond isomer. Because of the large number of scans necessary to obtain the ^{13}C-NMR spectra, deuterochloroform samples of XIII decomposed significantly before a satisfactory spectrum could be obtained. The structure of XIII was established from its ^{1}H-NMR spectrum, which showed the broad multiplet expected for 5,6-double bond isomers[4], and from the ^{13}C-NMR spectrum of its *N*-acyl derivative (XV: 141.0, C-5; 121.7, C-6)[3]. Although it is conceivable that XIII was actually the 4,5-double bond isomer which then underwent isomerization upon acylation, none of the 4′-substituted or unsubstituted 4,5-double bond thiazolidine isomers gave any *N*-acylthiazolidine (only hydrolysis products)[3] suggesting that XIII was not the 4,5-double bond isomer. It was also possible that XIII was a mixture of the two isomers and that the 4,5-double bond isomer hydrolyzed and the 5,6-double bond isomer was acylated. This would explain the rather low yields of the *N*-acylthiazolidine from these reactions. However, the ^{1}H-NMR spectrum of XIII is consistent with the presence of only the 5,6-double bond isomer.

The testosterone and testosterone 17β-propionate 4′-unsubstituted thiazolidines were prepared in the same manner as the progesterone analogs. The structures of the testosterone and testosterone propionate derivatives were determined from their ^{1}H-NMR spectra and the ^{13}C-NMR spectra of their *N*-acyl derivatives[3].

Attempts to prepare the *N*-methyl thiazolidines of progesterone and testosterone using the conditions previously used to prepare the other thiazolidines[1,4] were unsuccessful. In addition, although azeotropic distillation using *p*-toluenesulfonic acid in benzene as a catalyst was partially successful, an acid catalyst failed to drive the reaction between the steroid and *N*-methylaminoethanethiol (12) to completion. Furthermore, it was impossible to separate the unreacted starting material from the thiazolidine by crystallization or chromatography since excessive decomposition of the thiazolidine occurred in both cases. Finally, it was discovered that the reaction could be driven to completion by heating a mixture of the steroid with *N*-methylaminoethanethiol (two equivalents) at about 130° for 30 or 40 min. The products could then be conveniently isolated by crystallization. As in the other 4′-unsubstituted case, the only isomer obtained was the 5,6-double bond isomer VII, determined by comparison of its ^{13}C-NMR spectrum (carbon absorptions at δ 140.6 and 122.5) with that of other 5,6-double bond isomers[4]. The ^{1}H-NMR spectrum of the crude reaction product was identical to that of the pure product.

The preparation of the *N*-acyl thiazolidines XIV, XV and XVI *via* direct acylation has been described separately[3]. Thiazolidines III, IV, X, XI, and XII were prepared by substituting the appropriate cysteine ester for cysteine ethyl hydrochloride in the usual reaction conditions.

Although the hydrolysis of thiazolidines in buffers has been carefully studied already (11), their hydrolysis in plasma has not been reported. Table II shows the hydrolysis rates of some selected thiazolidines. The rates were obtained by following the appearance of progesterone so the values are qualitative, especially when considering the dithiazolidines VIII*a*, VIII*b*, and XI, where two separate rates corresponding to the hydrolyses of the 3- and 20- thiazolidines are involved. However, the data does show that the 4,5-double bond isomers release progesterone 2.5 to 3.5 times faster than the 5,6-double bond isomers and the 4′-unsubsti-

Table IV—Excretion of Progesterone and Its Metabolites in Urine after Intravenous Administration of [4-^{14}C]Progesterone [a] and [4-^{14}C]-XI [b] to Dogs

Dog	Drug	μCi			
		0–24 hr	24–48 hr	Total μCi	(% Dose)
1	Progesterone	0.71	0.13	0.84	(34%)
1	XI	0.027	0.036	0.063	(2.5%)
2	Progesterone	0.84	0.015	0.85	(34%)
2	XI	0.033	0.038	0.071	(2.8%)

[a] 2.5 mg of 1 μCi/mg progesterone (2.5 μCi) 100 μl benzyl alcohol; 8×10^{-2} *M* progesterone. [b] 5.6 mg of 0.446 μCi/mg XI (2.5 μCi) in 100 μl benzyl alcohol; 8.14×10^{-2} *M* XI.

Table V—Distribution of [4-^{14}C]Progesterone, XI, and VIII in Rats [a] 1 hr after Intravenous Tail Administration [b]

Compound	Dose, mg/kg	Dosage Equivalents of Progesterone, mg/kg	Organ	% Dose/Organ Mean ± *SE*
Progesterone	0.98	0.98[c]	Liver	16.25 ± 1.33
			Lungs	0.50 ± 0.07
			Blood	2.91 ± 0.39
			Feces	35.15 ± 2.97
XI	2.21	1.01[c]	Liver	14.22 ± 1.13
			Lungs	79.22 ± 4.14
			Blood	3.55 ± 0.51
			Feces	0.24 ± 0.06
VIII*a*	1.96	1.07[c]	Liver	14.39 ± 1.38
			Lungs	60.73 ± 1.12
			Blood	2.07 ± 0.49
			Feces	2.39 ± 0.27
VIII*a*	0.135	0.074[c]	Liver	40.57 ± 1.56
			Lungs	22.15 ± 1.62
			Blood	18.56 ± 2.68
			Feces	16.18 ± 4.5
VIII*a*	0.143	0.078[d]	Liver	34.41 ± 2.14
			Lungs	20.20 ± 1.76
			Blood	5.49 ± 0.56
			Feces	5.69 ± 0.94

[a] $n = 6$. [b] 5 μl benzyl alcohol vehicle. [c] Activity of progesterone 1 μCi/mg. [d] Activity of progesterone 9.47 μCi/mg, $n = 4$.

tuted thiazolidine about 5 times faster than the 4′-ethoxycarbonyl thiazolidine.

The low solubility of the thiazolidines in water was very difficult to determine accurately. An approximate value was obtained by dissolving the thiazolidines in acetonitrile and adding the acetonitrile solution to a pH 7.4 buffer solution until turbidity was produced. The suspension was then filtered through a millipore 0.2-μm filter and the filtrates were decomposed with acid and analyzed by UV for progesterone. No measurable water solubility was observed for the 3,20-dithiazolidines using this technique. The mono-3-thiazolidine XIII was about as soluble as progesterone (5 mg/liter) and the mono-3-thiazolidine IX*b* was about one-third as soluble.

Biology—Preliminary pharmacokinetic studies on the thiazolidines were concerned with determining their bioavailability and their distribution. 4-^{14}C-Labeled VIII*a* and XI were synthesized for this purpose from [4-^{14}C]progesterone. Initially, XI was given intravenously as a benzyl alcohol solution (0.1 ml) to two female beagle dogs in a crossover study. The results are shown in Tables III and IV. Table III shows that the level of [4-^{14}C]steroid found in the dog's blood after administration of progesterone itself was usually at least one order of magnitude higher than that after administration of [4-^{14}C]-XI in the first few minutes after injection. Samples that were taken over several days after injection showed little consistent variation in the level of radiolabeled steroid from either progesterone or XI. Moreover, Table IV shows that the higher blood levels of [4-^{14}C]steroid obtained from progesterone compared to XI resulted in higher levels (about one order of magnitude) of excreted [4-^{14}C]steroid.

Obviously, the pattern of distribution of XI and its parent compound were very much different. Consequently, whole animal distribution studies were run to determine where the labeled steroid was going. Table V shows the distribution results in rats ($n = 6$) from intravenous tail injections of labeled progesterone VIII*a* and XI as benzyl alcohol solutions; benzyl alcohol was used as a vehicle to be consistent with the dog study. Only the data for the four major distribution sites are given in Table V, but the total recovery of labeled material was 66% for progesterone, 75% for VIII*a*, and 100% for XI.

Table VI—Androgenic Test [a]

Compound	Total Dose, mg	Seminal Vesicle Weight, mg	Ventral Prostate Weight, mg
Testosterone	16.0	14.0 ± 2.0	38.5 ± 2.5
	32.0	13.4 ± 1.2[b]	45.7 ± 2.0
	64.0	28.9 ± 3.2	67.1 ± 2.5
VII	4.0	8.2 ± 0.4	39.9 ± 1.9
	8.0	9.9 ± 0.6	45.7 ± 2.5
	16.0	15.8 ± 0.8	50.9 ± 3.4
Vehicle		6.4 ± 0.3	8.3 ± 0.5

[a] The compounds were given orally as a suspension in sesame oil daily for 10 days starting on day of castration using 10 rats/dose. [b] Unusually low value compared to data at same concentration in all other control experiments (17.0 ± 1.7 mg).

The finding of extremely high concentrations in the lung when the thiazolidines were injected is rather surprising. It did not seem to be an artifact and, if real, might have specific importance in site-specific drug delivery. The reason for the high concentration can be related to the fact that it was found that in a number of species (rabbit, rat, hamster); the mixed function oxygenase activity is at least one order of magnitude higher in the lung than in the liver (13). On the other hand, the importance of cysteine residue (14) in some slow reacting substance of anaphylaxis implies its more complex involvement in drug binding (through its —SH function) than originally believed.

None of the progesterone derivatives which were given orally or subcutaneously had even one-tenth the activity of a subcutaneous dose of progesterone in the Clauberg test. Although some of the testosterone thiazolidines had marginal activity in androgenic tests, their dose response curves were not parallel with those of the standard (*e.g.*, the parent steroid), so no estimation of their potency was possible. However, one derivative, VII, the *N*-methylthiazolidine of testosterone, was definitely more active than its parent steroid. The data for VII are presented in Table VI. It is representative of the data for the other thiazolidines in that it shows that the two dose response curves are not parallel.

Compound VIII*a* also was applied topically to hairless mice to see what effect the thiazolidine had on the residence time of the steroid in a biological membrane, as progesterone was found to show activity in controlling acne (15). Thus, a stronger binding pro-progesterone would have longer effect and reduced systemic activity. The results of the comparative percutaneous absorption and distribution of labeled steroid from doses of VIII*a* and progesterone are shown in Table VII. Those results show a remarkable tendency of the thiazolidine VIII*a* to increase the relative amount of labeled steroid in the skin. Whether this tendency is due to the greater lipophilicity of the thiazolidines or to the possible formation of disulfide bonds from the interaction of the ring-opened

Table VII—Dermal Delivery of Progesterone and VIII*a*

	Progesterone[a], % Dose Mean ± *SE*[c]	VIII*a*[b], % Dose Mean ± *SE*
Feces	16.65 ± 1.75	0.95 ± 0.13
Urine	7.00 ± 1.14	0.40 ± 0.09
Skin circle[d]	1.98 ± 0.10	4.52 ± 1.18
Intestine/fat	2.28 ± 0.47	0.09 ± 0.00
Liver	0.75 ± 0.06	0.08 ± 0.01
Blood	0.08 ± 0.02	0.28 ± 0.04
Kidney/spleen	0.06 ± 0.01	0.03 ± 0.01
Lung	0.04 ± 0.02	0.01 ± 0.00
Subtotal	28.74	6.36
Patch[e]	54.06 ± 1.6	83.46 ± 1.4
Total	82.80	89.82

[a] 75 μl of a solution of 0.9 mg in 675 μl in ethanol–isopropylmyristate (90:10). [b] 75 μl of a solution of 1.85 mg in 675 μl ethanol–isopropylmyristate (90:10). [c] $n = 6$. [d] 2-cm diameter plug of epidermis taken directly under the bandage patch (3 cm^2). [e] Includes the contents of an ethanolic wash of the skin circle after the bandage patch was removed.

thiazolidine with thiol groups in the skin cannot be determined at this time.

Thus, a series of thiazolidine derivatives of progesterone and testosterone were prepared and characterized. Except for VII the thiazolidines were found to be inactive or less active than their parent steroids in animal models. This lack of activity can be explained by the rapid distribution of the thiazolidines out of blood followed by a slow release of the parent, such that the biological concentration of steroid is always too low to be biologically effective.

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High-Performance Liquid Chromatographic Assay for Bumetanide in Plasma and Urine

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Abstract □ A new high-performance liquid chromatographic (HPLC) method was developed for the analysis of bumetanide in plasma and urine. A reversed-phase column was fitted to the instrument and fluorescent (excitation λ = 338 nm, emission λ = 433 nm) and UV (254 nm) detectors were utilized to monitor simultaneously bumetanide and the internal standard, acetophenone, respectively. The assay is rapid, sensitive, and specific. Plasma bumetanide concentrations can be detected as low as 5 ng/ml using a 0.20-ml sample. Time-consuming extraction and/or derivatization steps are not required. The only clean-up procedure involved is the precipitation of plasma proteins with acetonitrile.

Keyphrases □ Bumetanide—high-performance liquid chromatographic determination in plasma and urine □ High-performance liquid chromatography—determination of bumetanide in plasma and urine □ Diuretics—bumetanide, determination in plasma and urine by high-performance liquid chromatography

Bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamoylbenzoic acid) is a new, high-ceiling diuretic with a pharmacological action similar to furosemide (1–4). However, on a molecular weight basis, bumetanide is 40–60 times more potent (1–4). Studies describing the pharmacokinetics and disposition of bumetanide have been sparse due to the lack of a sensitive and specific assay method. Fluorimetric (1), GLC (5), radioactive (6, 7), radioimmune (8), and high-performance liquid chromatographic (HPLC) (9) assays currently are available for the determination of bumetanide in biological fluids. However, all of these methods have inherent disadvantages, including time-consuming extraction, derivatization, or incubation steps (1, 5–9), large sample requirements (1, 5–7, 9), poor sensitivity (1, 5), or lack of specificity (1, 8).

Therefore, a rapid, sensitive, and specific HPLC assay was developed, without requiring prior extraction and/or derivatization, for the measurement of bumetanide in plasma and urine. The analytical method is suitable for bioavailability and pharmacokinetic studies in dogs and human subjects.

EXPERIMENTAL

Chemicals—Bumetanide[1], acetophenone[2], acetic acid[3], and phosphoric acid[4] were used as received. The methanol[3], acetonitrile[5] (glass-distilled), and deionized water[6] were filtered and degassed prior to use.

Standard Solutions—Bumetanide (4.12 mg) was dissolved in 50% acetonitrile–distilled water to yield a stock solution of 41.2 μg/ml. This stock solution was diluted 20- (2.06 μg/ml) and 100-fold (0.412 μg/ml) to give the working standard solutions for urine and plasma, respectively. Acetophenone was diluted in 50% acetonitrile–distilled water to yield a 0.50 mg/ml stock solution for urine and a 0.25 mg/ml stock solution for plasma.

Instrumentation—Samples were analyzed using a high-performance liquid chromatograph[7] equipped with a U6-K universal injector[8], a flu-

[1] Hoffmann-La Roche, Nutley, N.J.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Baker Analyzed Reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Certified ACS, Fisher Scientific Co., Fair Lawn, N.J.
[5] MCB Manufacturing Chemists, Cincinnati, Ohio.
[6] Milli-Q Reagent-Grade Water System, Millipore Corp., Bedford, Mass.
[7] Model 6000A, Waters Associates, Milford, Mass.
[8] Model U6-K, Waters Associates, Milford, Mass.

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