and Pharmacognosy, Purdue University. Thioethanol, cysteine, and glutathione were purchased from Sigma Chemical Co.

Equilibria Measurements. The stability constants were measured at 20 °C in 10-mm cells on a Perkin-Elmer 552 spectrophotometer, and the pH values were measured on a Radiometer PHM 64. The aqueous solutions contained 4×10^{-6} M alkaloids, 4×10^{-6} to 1.6×10^{-2} M thiols, 4×10^{-3} M hexadecyltrimethylammonium bromide, and phosphate buffers of ionic strength of 0.05.

The stability constant (K) of adduct formation of the thiol (S) with the alkaloid (A) (eq 1) was calculated by logarithmic transformation (eq 2), where ϵ_A and ϵ are the molar absorption

$$K = [AS]/[A][S] \quad (1)$$

$$\log \frac{\epsilon_A c_A - A}{A - \epsilon c_A} = \log K + \log \left(c_S - \frac{\epsilon_A c_A - A}{\epsilon_A - \epsilon} \right) \quad (2)$$

coefficients of the alkaloid and of its adduct with the thiol, c_A is the total concentration of the alkaloid (eq 3) and c_S that of the thiol (eq 4), and A is the absorbance of the solution in a 10-mm cell (eq 5). The measurements were carried out for wavelengths

$$c_A = [AS] + [A] \quad (3)$$

$$c_S = [AS] + [S] \quad (4)$$

$$A = \epsilon_A[A] + \epsilon[AS] \quad (5)$$

where the molar absorption coefficient of the alkaloid differed from that of its adduct with the thiol [sanguinarine (1): 275, 328, and 470 nm; chelerythrine (2): 268, 315, and 400 nm]. A medium of 4×10^{-3} M hexadecyltrimethylammonium bromide was used for protection of the adduct in the solution. At the selected pH, dissociation of the SH groups of thiols in the solution did not occur.[21]

Assay of Aminotransferase Activity. The effect of the alkaloids on L-alanine:2-oxoglutarate aminotransferase and L-aspartate:2-oxoglutarate aminotransferase activities was studied by using the postmitochondrial supernatant of rat liver. For all experiments, male Wistar rats, weighing ca. 200 g, were used. The rats were decapitated, and the liver was removed and homogenized after the addition of 0.1 M, pH 7.4 phosphate buffer (5 mL/g of liver) at 0 °C. The homogenate was centrifuged (15 min at 10000g) at 0 °C. For enzyme assays, the supernatant was diluted 2000× with a 0.1 M, pH 7.4 phosphate buffer. The enzyme assays were performed by the method of Reitman and Frankel.[22] Partially purified L-alanine:2-oxoglutarate aminotransferase was isolated from the liver of corticoid-treated rats.[23] To increase the high levels of the enzyme, prednison in doses of 10 mg/kg (suspended in a solution of tylose, 50 g/L) was orally administered to each rat for 5 days before decapitation. The purification procedure was that as described in ref 18. The purification was terminated after ammonium sulfate fractionation by precipitation. For storage, the enzyme solution was lyophilized. Prior to use, the enzyme was freshly diluted with 0.1 M, pH 7.4 potassium phosphate buffer. For the measurements of the partially purified alanine aminotransferase, the method[22] was modified, i.e., the substrate solution (8.33×10^{-2} M L-alanine; 1.67×10^{-3} M 2-oxoglutarate; 0.1 M, pH 7.4 phosphate buffer) (0.3 mL) was started with 10 μL of an enzyme solution or an enzyme–alkaloid mixture after a 5-min preincubation. All the measurements were carried out at 30 °C, the incubation period was varied to retain optimal levels of absorbancy changes, and then 2,4-dinitrophenylhydrazine reagent (0.25 mL) was added, left standing for 20 min, and made alkaline with 0.4 M NaOH (2.5 mL). After 10 min, the absorbancy at 505 nm was measured. For analysis of the effect of the thiols on enzyme inhibition, substrate solutions were used containing 10^{-2} M of the corresponding thiol.

For dialysis, the enzyme (0.25 mg) was dissolved in a pH 7.4 phosphate buffer (5 mL), containing 0.1 M L-alanine, for prolongation of enzyme stability, and the studied alkaloid was added (3.1×10^{-4} M). After standing for 30 min at room temperature, the solution was dialyzed against the same buffer for a period of 24 h at 4 °C. The enzyme activity and the protein contents were assayed in aliquot parts of sample at the beginning and at the end of the dialysis. For control, the dialysis of only the enzyme was carried out under the same conditions. The proteins were determined by the procedure of Lowry.[24]

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A Structure–Activity Relationship Study of Spirolactones. Contribution of the Cyclopropane Ring to Antimineralocorticoid Activity

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A series of spirolactones containing a cyclopropane ring in the molecule was examined for its effects on the mineralocorticoid receptor. The results were compared with those of a similar series of spirolactones in which the cyclopropane ring was replaced by a double bond. Insertion of a double bond or an α-cyclopropane ring into the 1,2 or the 6,7 position leads to a reduction in the binding affinity. The π-bonding system of the β-cyclopropane ring at C-6 and C-7 does not promote binding to the receptor. The presence of the 6β,7β-cyclopropane ring may deter metabolic inactivation to account for the enhanced in vivo activity.

Aldosterone plays an important role in maintaining the electrolyte balance in the body. Since it promotes the retention of sodium and the excretion of potassium, inhibition of its effects on gene expression would be an appropriate adjunct to the treatment of pathologic conditions which are sodium dependent or which are characterized by edema formation.

In the mid-1950's, Kagawa, Cella, and Van Arman discovered a series of spirolactones that inhibited the effects of aldosterone.[1] One of them, spironolactone (1a), was ultimately marketed. Since the discovery of Kagawa et

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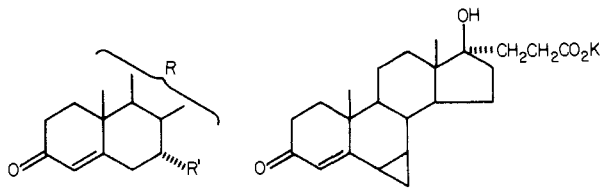
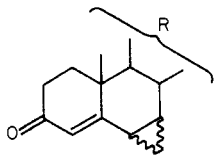
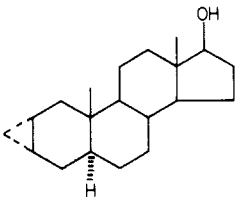


1a, R' = $CH_3C(=O)S$
b, R' = H

2



3a, 6β,7β-CH_2
b, 6α,7α-CH_2



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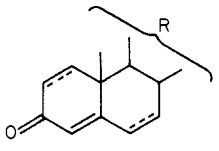
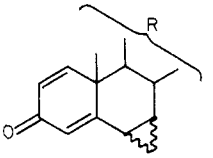
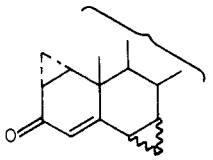


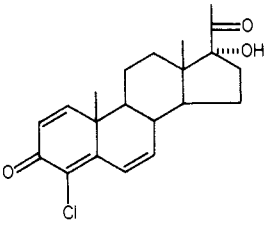
5a, Δ^6
b, Δ^1
c, $\Delta^{1,6}$



6a, 6β,7β-CH_2
b, 6α,7α-CH_2



7a, 6β,7β-CH_2
b, 6α,7α-CH_2



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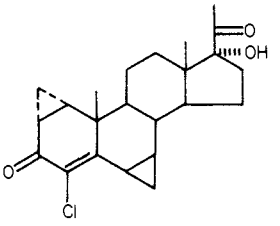

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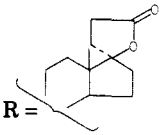

al., numerous attempts have been made to increase the potency of spironolactone.[2] Although these efforts have not achieved the remarkable success that attended the discovery of the therapeutic effects of cortisone and the subsequent enhancement of its potency through systematic structural modifications, a number of compounds have been observed to be more potent than spironolactone (**1a**) in various assays. One aldosterone antagonist found to be more potent than spironolactone in the several species in which it was tested was prorenoate potassium (**2**). When administered orally, it is approximately three and five times as potent as spironolactone in blocking the mineralocorticoid effects of aldosterone in the dog and the rat, respectively.[3] In man it is about three times as potent as **1a** in inhibiting the effects of the synthetic mineralocorticoid fludrocortisone.[4]

On treatment with acid, prorenoate potassium is converted into the spirolactone prorenone (**3a**), which is its major metabolite.[5] The presence of the cyclopropane ring at the 6β,7β position enhances the antimineralocorticoid activity. Earlier, we suggested that the unusual electronic characteristics of the cyclopropane ring[7] may promote binding to the aldosterone receptor in the manner postulated by Wolff et al.,[8] to account for the high degree of androgenicity of the 2α,3α-cyclopropano steroid **4**.

Method. In seeking to establish whether π bonding was involved, we determined the binding affinities of a series of spirolactones containing a cyclopropane ring in the molecule and compared these values to the binding affinities of the corresponding spirolactones in which the cyclopropane ring was replaced by a double bond. The mineralocorticoid receptor utilized was obtained from the adrenalectomized rat kidney cytosol.[9] Competitive studies conducted with this receptor preparation revealed that the linear portions of the log dose–response curves of the spirolactones and that of aldosterone are more or less parallel.[9] In our study, 2×10^{-9} M [1,2-$^3H_2$]aldosterone was allowed to be bound to the receptor in the presence of dexamethasone. The competitiveness of each of the spirolactones to displace the radioligand was determined at a concentration of 8×10^{-8} M. At this concentration, unlabeled aldosterone generally displaces 92 ± 1% of the tritiated aldosterone from the steroid–receptor complex. The amount of labeled aldosterone that remained bound to the receptor after incubation at 25 °C for 40 min in the presence of the competitor was used to calculate the binding affinity of a particular spirolactone, relative to aldosterone (Table I).

Results

The affinity of spironolactone (**1a**) for the mineralocorticoid receptor was found to be 0.24 that of aldosterone, and that of dihydrocanrenone (**1b**), the parent spirolactone, was 0.22. When a double bond was introduced into the 6,7 position to furnish canrenone (**5a**), binding was reduced to 0.13. This result is in accord with the conclusion of Funder et al.[9a] that unsaturation at the 6,7 position produces a decrease in the affinity for the receptor. Their conclusion was based on a three-point analysis, while a single-point analysis was used in our study. If the double bond is inserted instead into the 1,2 position to give **5b**, a similar decrease in binding affinity can be observed. Insertion of double bonds into both the 1,2 and the 6,7 positions results in a further decrease in binding. The

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Table I. Binding to the Mineralocorticoid Receptor

compound	% binding of [^{3}H]aldosterone[c] (n = 2)	rel binding affinity[a,c]
aldosterone	7.8 ± 1.0	1.00
3-(17β-hydroxy-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (1b)	36.0 ± 5.0	0.22 ± 0.06
3-(17β-hydroxy-3-oxo-4,6-androstadien-17α-yl)propionic acid γ-lactone (5a)	60.0 ± 0.5	0.13 ± 0.02
3-(17β-hydroxy-3-oxo-1,4-androstadien-17α-yl)propionic acid γ-lactone (5b)	53.5 ± 4.6	0.15 ± 0.03
3-(17β-hydroxy-3-oxo-1,4,6-androstatrien-17α-yl)propionic acid γ-lactone (5c)	76.6 ± 1.2	0.10 ± 0.02
3-[7α-(acetylthio)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (1a; spironolactone)	32.2 ± 2.0	0.24 ± 0.05
3-(17β-hydroxy-6α,7α-methylene-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (3b)	66.0 ± 2.6	0.12 ± 0.02
3-(17β-hydroxy-6β,7β-methylene-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (3a)	31.7 ± 0.7	0.25 ± 0.03
3-(17β-hydroxy-6β,7β-methylene-3-oxo-1,4-androstadien-17α-yl)propionic acid γ-lactone (6a)	53.0 ± 5.5	0.15 ± 0.04
3-[1α,2α,6β,7β-bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (7a)	55.0 ± 4.7	0.14 ± 0.03
3-[1α,2α,6α,7α-bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (7b)	77.6 ± 1.9	0.10 ± 0.02
3-(17β-hydroxy-6α,7α-methylene-3-oxo-1,4-androstadien-17α-yl)propionic acid γ-lactone (6b)	(77.0 ± 4.0[b])	(0.10 ± 0.02[b])

[a] Relative binding affinity = mean of percent binding of [^{3}H]aldosterone in the presence of 8×10^{-8} M aldosterone/ mean of percent binding of [^{3}H]aldosterone in the presence of 8×10^{-8} M competitor. [b] Tested at a 2×10^{-7} M concentration as the etherate ($C_{23}H_{28}O_3 \cdot 0.25C_4H_{10}O$). [c] ±SD.

Table II. Chemical Shifts of the C-4 and C-19 Protons

compound	τ C-4	τ C-19	Δ C-4[a]	Δ C-19[b]
3-(17β-hydroxy-6β,7β-methylene-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (3a)	3.98	8.89		
3-[1α,2α,6β,7β-bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (7a)	4.15	8.87	0.17	−0.02
3-(17β-hydroxy-6α,7α-methylene-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (3b)	4.03	8.83		
3-[1α,2α,6β,7β-bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (7b)	4.27	8.75	0.24	−0.08

[a] Change in the chemical shift of the C-4 proton as a result of the added substituent at C-1 and C-2. [b] Change in the chemical shift of the C-19 protons as a result of the added substituent at C-1 and C-2.

Table III. Molecular Rotation Differences

compound	$[\alpha]_D$ ($CHCl_3$), deg	M_D, deg	Δ M_D, deg
3-(17β-hydroxy-6β,7β-methylene-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (3a)	−191.7	−679.5	
3-[1α,2α,6β,7β-bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (7a)	−10.7	−39.1	+640.4
3-(17β-hydroxy-6α,7α-methylene-3-oxo-4-androsten-17α-yl)propionic acid γ-lactone (3b)	+117.1	+415.1	
3-[1α,2α,6α,7α-bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic acid γ-lactone (7b)	+235.6	+863.4	+448.3

product **5c** had a value of 0.10.

The effect which the cyclopropane ring exerts on binding to the receptor depends on the orientation of the methylene group. If this group is oriented α, as in **3b**, the binding affinity of the parent spirolactone **1b** is reduced to the same extent as when a double bond is inserted into this position (**5a**). In contrast, if the methylene group is oriented β, as in prorenone (**3a**), there is no effect on the binding affinity. Thus, the binding affinity of **3a** is comparable to that observed for **1b**, as well as for spironolactone (**1a**). Similarily, **6a** (the Δ^1 analogue of **3a**) and **5b** (the $\Delta^{1,4}$ dienone) have indistinguishable binding affinities.

Further evidence that the cyclopropane ring directed below the plane of the steroid nucleus produces a reduction in binding, as with a double bond, is found in comparing the binding affinities of **6b** and **7b** with that of **5c** and the affinity of **7a** with that of **6a**. At a concentration of 8×10^{-8} M, **6b** showed no indication of displacing [^{3}H]aldosterone from its receptor. However, when the concentration was increased to 2×10^{-7} M, marginal binding of **6b** to the receptor was observed. From these results we conclude that if in a spirolactone the π-bond system of either the double bond or the cyclopropane ring interacts with the mineralocorticoid receptor, it does so at the α side of the steroid nucleus. Significantly, this interaction does not enhance binding; instead, it results in a decrease in binding.

Synthesis and Assignment of Configuration. Compounds **7a** and **7b** were prepared by methyleneation of the Δ^1-steroids **6a** and **6b**, respectively, with dimethyloxosulfonium methylide.[10] The configurations at C-1 and C-2

Table IV. Effects Produced by a 1α,2α-Methylene Group on the Chemical Shift and Molecular Rotation[a]

compound	C-4 H (τ)	Δ C-4[b]	C-19 H (τ)	Δ C-19[c]	M_D ($CHCl_3$), deg	Δ M_D, deg
16α,17α-dihydroxyprogesterone acetonide	4.27		8.80		+518	
16α,17α-dihydroxy-1α,2α-methyleneprogesterone acetonide	4.46	+0.19	8.71	−0.09	+1060	+542

[a] G. W. Krakower and H. A. Van Dine, *J. Org. Chem.*, 31, 3469 (1966). [b] Change in the chemical shift of the C-4 proton as a result of the added substituent at C-1 and C-2. [c] Change in the chemical shift of the C-19 protons as a result of the added substituent at C-1 and C-2.

were established by NMR spectroscopy (Table II) and molecular rotation differences (Table III). Krakower and Van Dine[11] had demonstrated that introduction of a 1α,2α-methylene group into 16α,17α-dihydroxyprogesterone acetonide [16α,17α-(dimethylmethylenedioxy)-4-pregene-3,20-dione] resulted in an upfield shift in the NMR position of the C-4 proton of 0.19 ppm, a downfield shift of the C-19 protons of 0.09 ppm, and an increase in the molecular rotation of +542 (Table IV). In agreement with their results, the NMR signals for the C-4 protons in **7a** and **7b** are shifted upfield by 0.17 and 0.24 ppm, respectively, when compared to the signals of the corresponding protons in **3a** and **3b**. In addition, the signals of the C-19 protons in **7a** and **7b** are shifted downfield by 0.02 and 0.08 ppm, respectively. As previously observed,[11] introduction of the 1α,2α-methylene group leads to a substantial dextrarotatory shift in the optical rotation. The difference in molecular rotation between **7a** and **3a** is +640 and that between **7b** and **3b** is +448. When the cross-conjugated trienone **5c** was treated with dimethyloxosulfonium methylide, bismethylenation occurred. The only crystalline product which could be readily isolated proved to be **7a**. Methylenation proceeded from the α side of the molecule at C-1 and C-2 but from the β-side at C-6 and C-7 to furnish **7a**. The stereoselectively observed is consistent with the finding of Wiechert et al.[12] that the 4-chlorotrienone 8 undergoes methylenation to give preferentially **9**, in which the methylene group at the 1,2 position is α oriented while that at the 6,7 position is β. The conversion of **5c** to **7a** demonstrates that the addition of methylene groups to both double bonds occurs even in the absence of a chloro group at C-4, contrary to previous experience.[12] Admittedly, a longer period of time is required for this reaction.

Conclusion

In vivo, prorenoate potassium (**2**) is converted mainly into prorenone (**3a**).[5] The binding affinity of **3a** is nearly the same as that of spironolactone (**1a**). The latter is metabolized to give principally canrenone (**5a**)[13] whose binding affinity is consideraly less than that of prorenone. Thus, relative to spironolactone, the greater activity of prorenoate potassium in altering sodium/potassium metabolism can be ascribed in part, if not entirely, to its being metabolized to a product that binds to the receptor with a greater affinity than does canrenone (**5a**). The cyclopropane ring per se does not promote binding to the receptor. It contributes to the activity of **2** possibly by inhibiting metabolic inactivation. Conceivably, inactivation could result from dehydrogenation or hydroxylation in the vicinity of the 6,7 position of the steroid nucleus in the absence of the 6β,7β-methylene group. In the presence of the 6β,7β-methylene group, insertion of a double bond would be impossible and introduction of a hydroxyl group would be with considerable difficulty. Alternatively, the presence of the β-cyclopropane ring at C-6 and C-7 may induce a subtle conformational change in prorenone, which could either render the latter less susceptible to inactivation or produce a favorable effect on the kinetics of interaction between the aldosterone antagonist and the mineralocorticoid receptor.[14]

Experimental Section

All melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. ^1H NMR spectra were determined in deuteriochloroform on a 60-MHz Varian A-60 spectrometer with tetramethylsilane employed as an internal standard.

3-(17β-Hydroxy-6β,7β-methylene-3-oxo-1,4-androstadien-17α-yl)propionic Acid γ-Lactone (6a). A solution of 10.0 g (28 mmol) of **3a** and 10.0 g (44 mmol) of dichlorodicyanobenzoquinone in 500 mL of dioxane was heated under reflux in an atmosphere of nitrogen for 24 h. The reaction mixture was cooled to room temperature, and the precipitate was removed by filtration and washed with dioxane. The combined filtrate and wash were diluted with EtOAc. The EtOAc solution was washed successively with 5% NaOH and H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residue was chromatographed on silica gel. Elution with 5% EtOAc–C_6H_6 gave a product which was crystallized from CH_2Cl_2–ether to yield 5.9 g (51%) of **6a**: mp 215–218 °C; UV λ_{max} (MeOH) 242–243 nm (ε 12 497), 283–285 (9974); λ_{min} 263–264 nm (6626); IR ($CHCl_3$) 1767, 1660, 1620, 1595 cm^{-1}. Anal. ($C_{23}H_{28}O_3$) C, H.

3-[1α,2α,6β,7β-Bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic Acid γ-Lactone (7a). Procedure A. To 200 mL of 0.47 N dimethyloxosulfonium methylide in Me_2SO, under a nitrogen atmosphere, was added 2.0 g (5.7 mmol) of **6a**. The reaction mixture was stirred at room temperature for 1 h. After 2 mL of acetone was added, stirring at room temperature was continued for an additional 0.5 h. The mixture was poured into ice–water. The resultant mixture was extracted with EtOAc. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residue was crystallized from CH_2Cl_2–ether–hexane to afford 1.22 g (59%) of **7a**: mp 204–208 °C; $[\alpha]^{25}_D$ −10.7° (*c* 0.93, $CHCl_3$); NMR τ 4.15 (s, C-4 H), 8.87 (s, C-19 H's), 9.02 (s, C-18 H's). The NMR spectrum was identical with that of **7a** prepared by precedure B (vide infra).

Procedure B. To a solution of dimethyloxosulfonium methylide prepared from 1.2 g of a 60% dispersion of NaH in mineral

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oil, 4.5 g (22 mmol) of trimethyloxosulfonium iodide, and 25 mL of Me_2SO was added a solution of 1.5 g (4.4 mmol) of the trienone **5c** in 40 mL of Me_2SO. The reaction mixture was stirred at room temperature in an atmosphere of nitrogen for 63 h. Then it was diluted with a large volume of water. The mixture was made slightly acidic with the addition of glacial HOAc. The solid was collected, washed with H_2O, and dried. It was chromatographed on silica gel. Elution with 20% EtOAc in C_6H_6 gave 0.62 g of a product, which was crystallized from EtOAc–hexane to furnish 0.15 (mp 208–211 °C), 0.27 (mp 204–211 °C), and 0.05 g of **7a** (mp 201–211 °C). The sample melting at 208–211 °C was submitted for analysis: UV λ_{max} (MeOH) 259–260 nm (ϵ 14 100); NMR τ 4.13 (s, C-4 H), 8.86 (s, C-19 H's), 9.01 (s, C-18 H's). Anal. ($C_{24}H_{30}O_3$) C, H.

3-(17β-Hydroxy-6α,7α-methylene-3-oxo-1,4-androstadien-17α-yl)propionic Acid γ-Lactone (6b). A 5.0 g (14.1 mmol) sample of **3b** was dehydrogenated with 5.0 g (22 mmol) of dichlorodicyanobenzoquinone in 250 mL of dioxane as described for the preparation of **6a**. The crude product was chromatographed on silica gel. Elution with 15% EtOAc–toluene gave fractions which were combined and crystallized from CH_2Cl_2–ether to afford 2.6 g (52%) of **6b**: mp 133–135 °C; UV λ_{max} (MeOH) 242–244 nm (ϵ 12 970); λ sh 267–270 nm (7401); IR ($CHCl_3$) 1770, 1660, 1618, 1600 cm^{-1}; NMR τ 3.07 (d, C-1 H, $J_{1,2}$ = 10 Hz), 3.75 [d (?), C-4 H, $J_{2,4}$ = 1.5 Hz (?)], 368 (dd, C-2 H, $J_{1,2}$ = 10 Hz, $J_{2,4}$ = 1.5 Hz), 6.53 (q, CH_3CH_2O, J = 7 Hz), 8.73 (s, C-19 H's), 8.80 [t (?), CH_3CH_2O, J = 7 Hz (?)], 8.95 (s, C-18 H's). Anal. ($C_{23}H_{28}O_3 \cdot 0.25C_4H_{10}O$) C, H.

3-[1α,2α,6α,7α-Bis(methylene)-17β-hydroxy-3-oxo-4-androsten-17α-yl]propionic Acid γ-Lactone (7b). Methylenation of 1.0 g (2.8 mmol) of **6b** with 100 mL of 0.47 N dimethyloxosulfonium methylide in Me_2SO was carried out as described for the preparation of the 6β,7β isomer **7a** from **6a**. The product, which did not need to be chromatographed, was crystallized from CH_2Cl_2–hexane to afford 0.69 g (66%) of **7b**: mp 251–253 °C; $[\alpha]^{25}_D$ +235.6° (c, 1.01, $CHCl_3$); NMR τ 4.27 (m, C-4 H), 8.75 (s, C-19 H's), 8.97 (s, C-18 H's); UV λ_{max} (MeOH) 257–258 nm (ϵ 11 654); IR ($CHCl_3$) 1765, 1645, 1614 cm^{-1}. Anal. ($C_{24}H_{30}O_3$) C, H.

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Synthesis and Evaluation of 1,2,3,4-Tetrahydro[1]benzothieno[2,3-*h*]isoquinolines as Dopamine Antagonists

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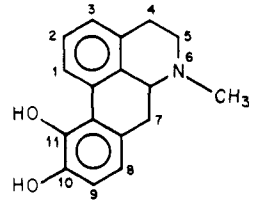
1,2,3,4-Tetrahydro[1]benzothieno[2,3-*h*]isoquinolines were prepared and evaluated as dopamine antagonists and for potential neuroleptic activity. These compounds, which are analogues of the dopamine agonist apomorphine (**2**) in which the C-7 methylene has been removed, were found to be dopamine antagonists by in vitro receptor binding studies. However, in vivo evaluation did not suggest potential antipsychotic activity.

In previous reports[1,2] we have described the synthesis and biological evaluation of some 8-aryl-1,2,3,4-tetrahydroisoquinolines, **2**, which are ring-opened analogues of



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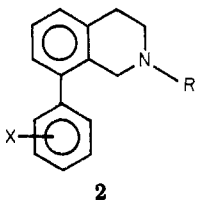

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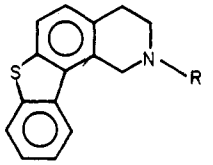

3

apomorphine (**1**). In contrast to **1**, the 8-aryl group of **2** is free to rotate out of general planarity with the rest of the molecule. Those compounds were found to be potent inhibitors of dopamine by in vitro dopamine receptor assays. In vivo evaluation of their biological effects did not indicate potential usefulness as antipsychotic agents, however.

This report describes the synthesis and evaluation of 1,2,3,4-tetrahydro[1]benzothieno[2,3-*h*]isoquinoline derivatives, **3**, which are also analogues of **1** in which the C-7 methylene group is absent. The two aromatic rings of **3** are held in a planar orientation by a sulfur bridge and, therefore, the overall geometry may more closely resemble **1**. Our objective was to determine if planar molecules such as **3** would have greater in vivo effects than the freely rotating compounds, **2**.

Chemistry. Synthesis of the title compounds is outlined in Scheme I. Dibenzothiophene-1-carboxylic acid (**4**) was prepared from 2-allylbenzo[*b*]thiophene and ethyl dichloroethoxyacetate[3] according to published methods.[4] It was converted to the acid chloride and reacted with 2-amino-2-methylpropanol, yielding an hydroxyamide. Cyclization with thionyl chloride produced the oxazoline **5**. Treatment of **5** with *n*-butyllithium, followed by ethylene oxide, produced **6**. The alcohol was converted to the lactone **7** by refluxing with 6 N hydrochloric acid. Heating **7** with benzylamine hydrobromide in benzylamine at 160 °C opened the lactone to the hydroxyamide **8**. The alcohol of **8** was converted to the mesylate, which on treatment with sodium hydride was cyclized to the dihydroisoquinolinone **9**. Lithium aluminum hydride reduction of **9** produced *N*-benzyl-1,2,3,4-tetrahydro[1]benzothieno-[2,3-*h*]isoquinoline **10**.

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