above stabilizing effect at any concentration. In this sense vitamin A,[17] phenothiazine tranquilizers,[9,18a] local anesthetics,[18a] alcohols, and steroids[18b] are nonspecific hemolysins. In particular Seeman[18b] considered testosterone to be a nonspecific hemolysin. Polyene antibiotics and saponins, on the other hand, are examples of specific hemolysins.

This is in agreement with the present data, which show the influence of the lipophilic character on the hemolytic activity of nonspecific hemolysins such as testosterone esters. The lipophilic character should exert its effect also in the case of specific hemolysins. In fact it should influence the penetration to their site of action.

Finally, Milborrow and Williams[19] recently reexamined Collander's contribution on the penetration of *Nitella* cells by nonelectrolytes.[13] Collander[13] had suggested a linear relationship between penetration and partition coefficient. Actually Milborrow and Williams[19] showed the existence of a parabolic relationship between penetration and partition coefficient. This provides further support for our findings of a quadratic relationship between hemolytic activity and R_m values.

Acknowledgment.—We are grateful to Dr. Hansch for his helpful suggestions.

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Steroidal Heterocycles. XIII.[1a] 4α,5-Epoxy-5α-androst-2-eno[2,3-*d*]isoxazoles and Related Compounds

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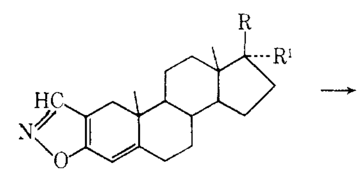
The preparation of a number of 4,5-epoxysteroido[2,3-*d*]isoxazoles from the corresponding Δ^4 compounds and their conversion into the corresponding 2-cyano derivatives and to 4,5-dihydroxy-, 5-hydroxy-4-oxo-, and 4-oxo derivatives are described. Several of these compounds were found to have unexpected adrenal cortical inhibitory activity.

Several compounds described in this paper, which were prepared as part of a continuing program in these laboratories involving the preparation and reactions of steroidal heterocycles,[1a] were found to inhibit adrenal cortical function. We have used them in attempts at molecular modification which has played such an important role in the development of better and safer drugs.[2]

Androsta-2,6-dieno[2,3-*d*]isoxazol-17β-ol (**1a**),[3] on treatment with either peracetic or perphthalic acid in C_6H_6, consistently yielded a mixture of 4α,5-epoxy-5α-androst-2-eno[2,3-*d*]isoxazol-17β-ol (**2a**) with starting material in a ratio of approximately 1:2. However, when a solution of isoxazole **1a** in $CHCl_3$ or CH_2Cl_2 was treated with either permaleic acid[4] or *m*-chloroperbenzoic acid[5] in the presence of a small amount of pyridine, **2a** was obtained in 80–90% yield.

Treatment of 4α,5-epoxy-5α-androst-2-eno[2,3-*d*]isoxazol-17β-ol acetate (**2b**) with aq Me_2CO–H_2SO_4 yielded 5α-androst-2-eno[2,3-*d*]isoxazole-4β,5,17β-triol 17-acetate (**5a**) in 76% yield which was converted in Ac_2O–pyridine into the corresponding 4β-acetoxy compound (**5b**), also obtained directly on treatment of **2b** with aq AcOH–H_2SO_4. When a solution of **2b** in



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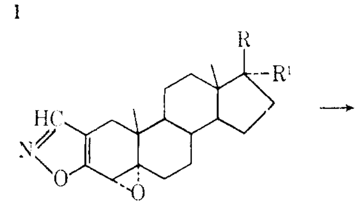

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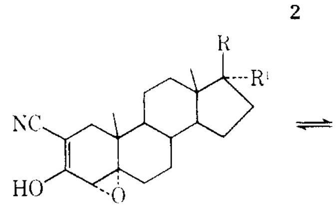

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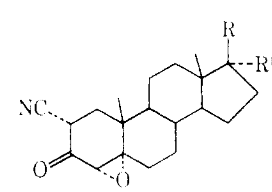

4

a. R = OH; R¹ = H
b. R = OCOCH₃; R¹ = H
c. R = OH; R¹ = CH₃
d. R = OH; R¹ = C≡CH
e. R = OCOCH₂CH₂COOH; R¹ = H
f. R = OCOCH₃; R¹ = C≡CH

(1) (a) Paper XII: T. C. Miller, *J. Heterocycl. Chem.*, **3**, 338 (1966); (b) Albany Medical Center Hospital, Albany, N. Y.
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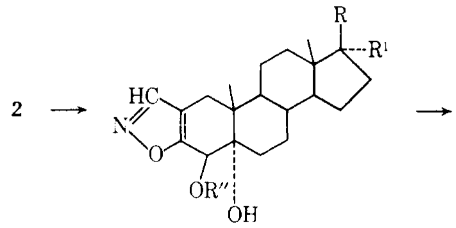
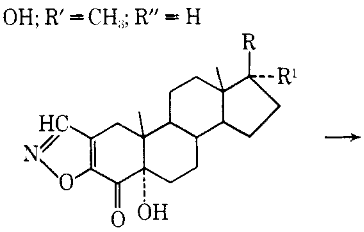


5

a, R = $OCOCH_3$; R' = H; R'' = H

b, R = $OCOCH_3$; R' = H; R'' = $OCOCH_3$

c, R = OH; R' = CH_3; R'' = H



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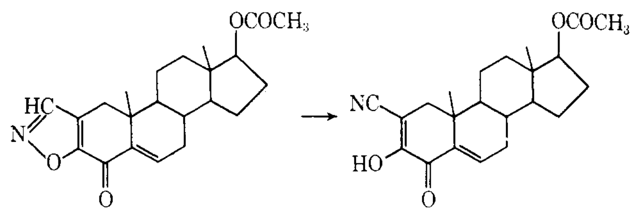

7 8

C_6H_6–Et_2O was subjected to treatment with either BF_3–Et_2O or HF,[6] unchanged starting material was recovered.

Treatment of **5a** with CrO_3 yielded 5,17β-dihydroxy-5α-androst-2-eno[2,3-*d*]isoxazol-4-one 17-acetate (**6a**) which on treatment with pyridine–$SOCl_2$[7] yielded 85% of 17β-hydroxyandrosta-2,5-dieno[2,3-*d*]isoxazol-4-one 17-acetate (**7**).

Ring opening of the isoxazoles **2a** and **2b** in the presence of NaOMe yielded 4α,5-epoxy-3,17β-dihydroxy-5α-androst-2-ene-2-carbonitrile (**3a**) and its 17-acetate **3b**, respectively.

The nmr spectrum of **3b** in $CDCl_3$ exhibited 2 peaks of about equal intensity at 102 and 104 Hz, attributable to the C-19 Me, indicating the presence of approximately a 1:1 mixture of the keto–enol tautomers **4b** and **3b**. In deuteropyridine, the nmr spectrum indicated the presence of only the enol **3b**.

In view of the interesting biological activity of **3a**, a number of structurally related compounds, varying only at C-17, were prepared using procedures similar to those described above. These compounds and their derivatives are listed in Tables I, II, and III below. In the case of the hemisuccinate isoxazole **2e**, treatment with NaOMe in dry THF led to the precipitation of its Na salt before ring opening to the cyano ester derivative **3e** was affected. Upon addition of H_2O to the reaction mixture there was obtained a mixture of unreacted starting compound **2e** and cyano alcohol **3a**. However, when KO-*t*-Bu in dry THF was used, a 40% yield of the cyano ester **3e** was obtained along with starting compound **2e** and cyano alcohol **3a**.

Hydroxylic solvents (alcohols) had to be avoided for the recrystallization of 2-cyano-3-keto steroids in view of poor yields presumably resulting from decomposition (an HCN-type odor was usually noted). The nature of the decomposition products has not been investigated, but the uv spectrum of a solution of **3a** in EtOH showed a decrease in the absorption maximum (ϵ) from 8100 to 7000 on standing at 25° for 3 days. During the same period, a study of this solution by tlc showed almost complete disappearance of the original 2-cyano-3-ketone and appearance of several new spots.

Biological Evaluation.—Certain members of this series of compounds blocked the corticotrophin- (ACTH) induced catabolic and thymolytic responses of castrated male rats. It is well established that treatment with ACTH results in an increased excretion of N in the urine and a reduction in the weight of the thymus and that these effects are brought about as a result of an increased output of corticoids by the adrenal cortex. The presumptive evidence that the epoxy steroids were exerting their effect at the level of the adrenal cortex was based substantially on their inability to block the catabolic and thymolytic effects produced by cortisone acetate in adrenalectomized-castrated male rats. Adrenal blocking activity was determined using a modification of the method of Beyler, *et al.*[8] Male rats of the Sprague–Dawley strain weighing 90–100 g were castrated. One week postoperatively the rats were given daily weighed portions (15 g) of coarsely ground laboratory chow. Tap water was provided *ad libitum*. After a 4-week equilibration period the rats were placed on an overnight fast, then transferred to individual metabolism cages for a 24-hr experimental period during which time free access to H_2O but no feed was permitted. ACTH (45 I.U./rat) was administered as 3 subcutaneous injections evenly spaced over the first 8 hr of the 24-hr experimental period. The steroids were administered sc at either 30 or 60 mg/kg concurrently with the ACTH. Urine collections (24 hr) were analyzed for N content by a micro-Kjeldahl procedure. At the end of the 24-hr period the rats were returned to their individual cages, placed on a daily ration of 15 g (as above) and held for autopsy 4 days later. At autopsy, the thymus was recovered and weighed on a microtorsion balance. The percentage inhibition of the ACTH-induced N excretion in the urine and thymolysis were used to assess adrenal blocking activity.

It can be seen from Table IV that sc administration of 4α,5-epoxy-17β-hydroxy-3-oxo-5α-androstane-2α-carbonitrile (**4a**) at 30 mg/kg completely inhibited the ACTH-induced urinary N loss and thymolysis. Derivatives of **4a** with changes at position 17, such as the acetate **4b**, 17α-methyl (**4c**), 17α-ethinyl (**4d**), hemisuccinate (**4e**), vary in degree of lipid and water solubility, but possess marked adrenal blocking activity.

Experimental Section

All melting points are corrected and were taken in a Hershberg-type apparatus. Except as noted, optical rotations were determined in $CHCl_3$ at 25°, C~1. Uv were determined in 95% EtOH (Cary 15) and ir in KBr disks (Perkin-Elmer 21). Nmr spectra were measured with Me_4Si as internal standard (Varian A60) in $CDCl_3$ unless otherwise stated. Where analyses are indicated only by symbols of the elements or functions, analytical

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Table I

Steroidal 4α,5-Epoxy[2,3-*d*]isoxazoles

No.	4α,5-Epoxy[2,3-*d*]isoxazole	Mp, °C	[α]D in $CHCl_3$	λ_{max}^{EtOH}, mμ (ε)	λ_{max}^{KBr}, μ	Formula
2a	17β-Hydroxyandrost-2-eno-	205–207	107.8	237 (6460)	3.00, 6.13, 6.75	$C_{20}H_{27}NO_3$
2b	17β-Acetoxyandrost-2-eno-	229–230	76.5	237 (6350)	3.28, 5.78, 6.12, 7.95	$C_{22}H_{29}NO_4$
2c	17β-Hydroxy-17-methylandrost-2-eno-	214–218	80.7	237 (6260)	2.95, 6.13, 6.20, 6.74	$C_{21}H_{29}NO_3$
2d	17-Hydroxy-17α-pregn-2-en-20-yno-	237–243	32.0	238 (6500)	2.99, 3.05, 6.11, 6.71[a]	$C_{22}H_{27}NO_3$
2f	17-Acetoxy-17α-pregn-2-en-20-yno-	226–228	23.9	238 (6900)	3.06, 3.25, 4.24, 5.76, 6.11, 6.74, 7.95, 8.10	$C_{24}H_{29}NO_4$
2e	17β-Hemisuccinoxyandrost-2-eno-	210–212	71.9	237 (6950)	3.15–4.00,[b] 5.80, 6.04, 6.72, 8.15	$C_{24}H_{31}NO_6$

[a] The ir spectrum showed weak bands at 5.75, 5.87 μ, indicative of a trace amount of oxo compound (probably 17-oxo). [b] 17β-Hydroxy impurity.

Table II

Miscellaneous Steroidal [2,3-*d*] Isoxazoles

No.	[2,3-*d*]Isoxazole	Mp, °C	[α]D in $CHCl_3$	λ_{max}^{EtOH}, mμ (ε)	λ_{max}^{KBr}, μ	Formula
5a	4β,5α-Dihydroxy-17β-acetoxy-androst-2-eno-	283–284 dec	107.9[a]	227 (5000)	2.89, 3.01, 5.84, 6.10, 7.93	$C_{22}H_{31}NO_5$
5c	4β,5α,17β-Trihydroxy-17-methyl-androst-2-eno-	257–259 dec	103.0[a]	229 (4500) 270 (100)[b]	2.95, 3.25, 6.08, 6.75	$C_{21}H_{31}NO_4$
5b	5α-Hydroxy-4β,17β-diacetoxyandrost-2-eno-	309–312 dec	98.0	227 (4600) 257, 264 (310)[c]	2.88, 5.76, 5.80, 6.05, 6.80, 7.94, 8.17	$C_{24}H_{33}NO_6$
6b	5α-Hydroxy-17β-acetoxy-4-oxo-androst-2-eno-	241–246	−26.0	233 (3800) 259 (6800) 340 (80)	2.93, 3.24, 5.85, 7.85	$C_{22}H_{29}NO_5$
6c	5α,17β-Dihydroxy-17-methyl-4-oxo-androst-2-eno-	254–255 dec	−21.2	230 (3900) 260 (5500)	2.79, 2.98, 5.85 6.17	$C_{21}H_{29}NO_4$
7	17β-Acetoxy-4-oxoandrosta-2,5-dieno-	244–245	−29.0	273 (8100) 290 (9100)	3.23, 5.78, 5.91 6.16, 6.22, 7.99	$C_{22}H_{27}NO_4$

[a] In pyridine. [b] Impurity. [c] Low intensity peaks probably due to the presence of some 5-dehydration product.

Table III

Steroidal 4α,5-Epoxy-3-oxo-2α-carbonitriles

No.	4α,5-Epoxy-3-oxo-2α-carbonitrile	Mp, °C	[α]D	λ_{max}^{EtOH}, mμ (ε)	λ_{max}^{KBr}, μ	Formula
4a	17β-Hydroxyandrostane-	258–270 dec	137.4[b]	252 (8300)	2.93, 3.70, 4.54, 5.78, 6.00	$C_{20}H_{27}NO_3$
4b	17β-Acetoxyandrostane-	195–198	116.2[b] −21.2[a]	253 (9300)	3.24, 4.56, 5.77, 5.88, 6.11, 7.80	$C_{22}H_{29}NO_4$
4c	17β-Hydroxy-17-methylandrostane-	246–247 dec	122.9[b]	254 (9300)	2.98, 3.79, 4.48, 4.57, 5.84, 6.06	$C_{21}H_{29}NO_3$
4d	17-Hydroxy-17α-pregn-20-yne-	238–240 dec	59.0[b]	253 (8900)	2.95, 3.06, 3.74, 4.54, 4.75, 5.80, 6.00	$C_{22}H_{27}NO_3$
4e	17β-Hemisuccinoxyandrostane-	145–150	−14.3[a]	252 (8700)	2.90–3.20, 3.75, 4.30, 4.56, 5.80 6.01, 6.13, 6.43 7.28, 8.05, 8.28	$C_{24}H_{31}NO_6$
8	17β-Acetoxy-4-oxoandrost-5-ene-	216–220	4.5[a]	280 (6900) 318 (8200)	3.03, 4.53, 5.78, 6.01, 6.19, 8.02	$C_{22}H_{27}NO_4$

[a] $CHCl_3$. [b] Pyridine.

results obtained for those elements or functions were within ±0.4% of the theoretical values.

The authors are indebted to Dr. Rudolph K. Kullnig and staff for spectral determinations and Mr. K. D. Fleischer and staff for analytical services.

The steroidal [2,3-*d*]isoxazoles used as starting material for the preparation of the compounds described in this paper were reported by Manson, *et al.*[3]

Androsta-2,4-dieno[2,3-*d*]isoxazol-17β-ol Hemisuccinate (1e).—A solution of androsta-2,4-dieno[2,3-*d*]isoxazol-17β-ol (**1a**) (44.9 g) and succinic anhydride (40 g) in pyridine (200 ml) was kept at room temperature for 2 days, heated in a steam bath for 1 hr, and quenched in ice containing concentrated HCl (275 ml). The resulting solid was collected, washed with H_2O, and slurried in CH_2Cl_2. There was obtained on filtration of the slurry 24.4 g of **1e** mp 200–210°. Another 17.5 g was recovered from the CH_2Cl_2 filtrate, mp 191–205°. A recrystallized sample had mp 207–210° (THF-*i*-PrOH); $[\alpha]_D$, +97.7°; λ_{max} 285 mμ (11,000). *Anal.* ($C_{24}H_{31}NO_5$) C, H, N.

The steroidal 4α,5-epoxy[2,3-*d*]isoxazoles (Table I) were prepared by a method similar to that described in the following example.

4α,5-Epoxy-5α-androst-2-eno[2,3-*d*]isoxazol-17β-ol (2a).—An ice-cooled solution of **1a** (20 g) in CH_2Cl_2 (200 ml) was added to

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TABLE IV
PERCENTAGE INHIBITION OF ACTH-INDUCED URINARY NITROGEN LOSS AND THYMOLYSIS

No.	Dose[a] mg/kg	% inhibition of ACTH-induced: Thymolysis	Urinary nitrogen loss
2a	30	0	0
2b	30	0	0
2c	30	0	0
5b			
4a	30	100	100
5a	30	0	0
6b	30	0	0
5c	30	0	0
7	30	0	0
2d	30	0	0
6c	30	0	0
2f	30	0	0
4b	30	84	100
4c	30	100	98
4d	30	100	61
8	30	61	100
1e	30	0	0
2e			
4e	60	57	100

[a] Seven rats per group.

a stirred, ice-cooled solution of permaleic acid prepared from maleic anhydride (10 g) and 88% H_2O_2[9] (2.5 ml) in CH_2Cl_2 (300 ml).[4] The clear, cold solution was treated with 10 drops of pyridine and allowed to stand at 5° for 16 hr. It became turbid as maleic acid settled out. The excess peracid was reduced with Na_2SO_3 solution and the mixture washed with $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated to a small volume. EtOAc was added and the solution again concentrated and cooled to give 18.3 g of **2a** mp 203–210°. A small sample was recrystallized (EtOAc) to mp 205–207°. *Anal.* ($C_{20}H_{27}NO_3$) C, H, N.

The following examples illustrate the procedures used for the preparation of the 4β,5-diols (Table II) and subsequent chemical changes carried out at those positions.

5α-Androst-2-eno[2,3-*d*]isoxazole-4β,5,17β-triol 17-Acetate (5a).—To a stirred slurry of **2b** (3.3 g) in Me_2CO (50 ml) was added 25% H_2SO_4 (5 ml) and stirring was continued for 2 hr during which time a clear solution formed followed by formation of a precipitate. The mixture was diluted with H_2O and filtered to give 2.5 g of crystals, mp 283–287° dec, contaminated by what probably was the Δ^4 compound (not isolated). Recrystallization yielded 1.18 g of **5a**, mp 283–284° dec (DMF–H_2O). *Anal.* ($C_{22}H_{31}NO_5$) C, H, N.

Treatment of **5a** with Ac_2O–pyridine yielded the corresponding 4β-acetoxy compound **5b**, mp 309–312° dec (dioxane–$CHCl_3$). *Anal.* ($C_{24}H_{33}NO_6$) C, H, N.

5,17β-Dihydroxy-5α-androst-2-eno[2,3-*d*]isoxazol-4-one 17-Acetate (6a).—To a stirred, ice-cooled solution of **5a** (4.56 g) in glacial AcOH (100 ml) was added a solution of CrO_3 (1.25 g) in H_2O (5 ml). Cooling was discontinued, EtOH (2 ml) was added after 2 hr, and the solution was concentrated *in vacuo* to a small volume. On addition of H_2O, crystals precipitated which, on crystallization from EtOAc, yielded 1.3 g starting compound **5a**. The mother liquor was evaporated to dryness *in vacuo* and the residue chromatographed on SiO_2 (200 g) to yield on elution with 5% EtOAc in C_6H_6, 1.16 g of **6a**; mp 229–248°, recrystallized to mp 241–246° (MeOH). *Anal.* ($C_{22}H_{29}NO_5$) C, H, N.

17β-Hydroxyandrosta-2,5-dieno[2,3-*d*]isoxazol-4-one 17-Acetate (7).—A cold solution of **6a** (0.95 g) in pyridine (20 ml) was slowly added to a cold solution of $SOCl_2$ (1.0 ml) in pyridine (20 ml) with stirring. A H_2O and $NaHCO_3$ solution was added after 2 hr and the resulting precipitate was filtered and washed with H_2O to give 0.75 g of crystals. Recrysallization yielded 0.70 g of **7**, mp 244–245° (Me_2CO). *Anal.* ($C_{22}H_{27}NO_4$) C, H, N. The following two examples illustrate the procedures used for the preparation of the 3-hydroxy-Δ^2-2-carbonitriles.[3]

4α,5-Epoxy-3,17β-dihydroxy-5α-androst-2-ene-2-carbonitrile (3a).—To a stirred solution of **2a** (23.4 g) in dry tetramethylurea[10] (400 ml) under N_2 was added NaOMe (8 g). After 16 hr, Et_2O (500 ml) was added and the resulting Na salt was filtered and washed with Et_2O. The salt was dissolved in H_2O with slight warming and dilute HCl was added dropwise until precipitation ceased. The solid was collected and washed with H_2O to give 22.8 g of white powder. Recrystallization yielded 13.1 g of **3a**, mp 258–270° dec (DMF–H_2O). *Anal.* ($C_{20}H_{27}NO_3$) C, H, N.

4α,5-Epoxy-3,17β-dihydroxy-5α-androst-2-ene-2-carbonitrile 17-Hemisuccinate (3e).—To a stirred, cooled solution of **2e** (10 g) in THF (200 ml) under N_2 was added KO-*t*-Bu (5.6 g). The resulting slurry was stirred at room temperature for 16 hr, Et_2O was added, and the K salt was collected and washed with Et_2O and THF. A solution of the K salt in H_2O (300 ml) was treated successively with excess NaH_2PO_4 solution and dilute HCl (10 ml) to give, on filtration, 3.96 g of crystals, mp 140–162°. Exhaustive crystallization yielded 40 mg of **3e**, mp 1 0–157° (Me_2CO). *Anal.* ($C_{24}H_{31}NO_6$) C, H, N. The recrystallized material was contaminated by a trace amount of **3a**; the bulk of the material containing greater amounts of **3a** according to tlc.

(9) H_2O_2 (90%) of varying age was used, titrated before use. It was not used if assay had dropped below 75%.

(10) John Deere Chemical Co., Pryor, Okla. Dry THF was used in all of the other preparations.