2 hr., then concentrated under reduced pressure. The residual white solid was mixed with 200 ml. of ether and the slurry was shaken with 200 ml. of 30% potassium hydroxide solution. The ether layer was separated and the aqueous layer was extracted with three 200 ml. portions of ether. After drying over anhydrous magnesium sulfate, the combined ether solutions were distilled to give 4.3 g. (76%) of product which boiled at 91–92° at 14 mm.; $n^{25}D$ 1.4516. Exposure to CO_2 gas of a 3.2 g. (0.022 mole) sample of the base gave 3.8 g. (99%) of the carbonate which decomposed at 66–69°. Recrystallization from ethanol–ether (−70°) did not change the melting point.

Acknowledgments.—We wish to acknowledge the able technical assistance of D. B. Hooker in the synthesis of many of the described compounds. Analytical and spectral data were supplied by W. A. Struck, J. L. Johnson and staff of our Physical and Analytical Chemistry Department. The LD_{50} values in Table II were kindly supplied by William Veldkamp. Assistance in running the GABA-KGA transaminase evaluations both *in vitro* and *in vivo* was provided by Norman J. Crittenden and W. C. Bell, and is gratefully acknowledged.

Anabolic Agents: Derivatives of 2-Halo-5α-androst-1-ene

R. E. COUNSELL AND P. D. KLIMSTRA

Division of Chemical Research of G. D. Searle & Co., Chicago 80, Illinois

Received November 18, 1961

Various derivatives of 2-halo-5α-androst-1-ene were synthesized in the hope of obtaining compounds with high anabolic and minimal androgenic activity. Anabolic and androgenic activities are reported for a number of these compounds as well as for some of the synthetic intermediates.

In a previous publication,[1] the high anabolic activity exhibited by derivatives of 5α-androst-1-ene[2] was described. Since substitution of halogen for hydrogen at C-4 of testosterone produced a favorable effect on the anabolic/androgenic ratio,[3,4] the preparation of the 2-halo analogs of 17β-hydroxy-5α-androst-1-en-3-one (Ia) and its derivatives seemed inviting.

The preparation of these compounds involved the same sequence of reactions employed by other investigators to arrive at 4-halo-Δ^4-

(1) R. E. Counsell, P. D. Klimstra, and F. B. Colton, *J. Org. Chem.*, **27**, 248 (1962).

(2) The 1957 IUPAC rules on steroid nomenclature as set forth in *J. Am. Chem. Soc.*, **82**, 5577 (1960), have been followed.

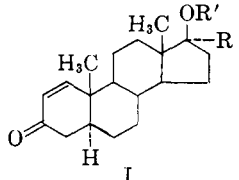
(3) B. Camerino, B. Patelli, and A. Vercellone, *ibid.*, **78**, 3540 (1956).

(4) G. Sala and G. Baldratti, *Proc. Soc. Exptl. Biol. Med.*, **95**, 22 (1957).

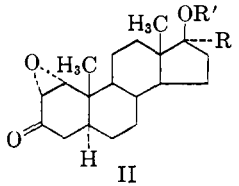
3-ketosteroids.[3,5] Thus, oxidation of the Δ^1-3-ketosteroids with alkaline hydrogen peroxide according to the method of Hoehn[6] afforded the intermediate 1α-2α-epoxy-3-ketosteroids (II) in excellent yield. The epoxides without further purification were converted to the desired 2-chloro or 2-bromo derivatives (III) by treatment with either hydrochloric acid or hydrobromic acid in acetone. The resulting products exhibited ultraviolet absorption maxima at 247.5 and 255 mμ which are characteristic for 2-chloro- and 2-bromo-Δ^1-3-ketosteroids, respectively.[7] Reduction of these products with lithium-tri-*t*-butoxyaluminohydride gave the corresponding 3β-hydroxy compounds (IV). The assignment of the 3β configuration was based on the known stereospecific course for hydride reduction of Δ^1-3-ketosteroids.[8]

Epoxidation of Δ^1-3-ketosteroids gives predominantly the 1α,2α-epoxide.[6] The presence of any 1β,2β-epoxide in the crude product, however, did not necessitate further purification, since treatment of pure 1β,2β-epoxy-3-ketosteroids with hydrochloric acid was found to afford the same product (III). 1β,2β-Epoxy-5α-androstan-17β-ol-3-one acetate (VII) was prepared by utilizing the known directive influence of the hydroxyl group in peracid oxidation of allylic alcohols.[9] Treatment of 5α-androst-1-ene-3β,17β-diol 17-monoacetate (V) with perbenzoic acid in benzene gave the desired 1β,2β-epoxide (VI) which was transformed to VII by chromic acid oxidation in pyridine. Reaction of VII with hydrochloric acid in acetone resulted in opening of the epoxide ring and concomitant hydrolysis of the ester group to give 2-chloro-17β-hydroxy-5α-androst-1-en-3-one (IIIa). The ester group was retained when the reaction was performed in acetic acid. The ease with which both the α- and β-epoxides (IIb and VII) yielded the 2-halo-Δ^1-3-ketosteroids differs from the results obtained with the α- and β-epoxides of testosterone.[3,5] In the latter instance, treatment of the β-epoxide with hydrochloric acid in acetic acid gave 4-chlorotestosterone directly whereas similar treatment of the α-epoxide gave 4-chloroandrostan-5α,17β-diol-3-one which was converted to 4-chlorotestosterone only after prolonged heating.[3] It was later reported, however, that both epoxides furnished 4-chlorotestosterone when chloroform was used as the solvent.[10]

(5) H. G. Ringold, E. Batres, O. Mancera, and G. Rosenkranz, *J. Org. Chem.*, **21**, 1432 (1956).
(6) W. M. Hoehn, *ibid.*, **23**, 929 (1958).
(7) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corporation, New York, N. Y., 1959, p. 287.
(8) W. Bergmann, M. Kita, and D. J. Giancala, *J. Am. Chem. Soc.*, **76**, 4974 (1954).
(9) H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, 1958 (1957).
(10) B. Camerino, U. S. Patent 2,953,582 (1960).

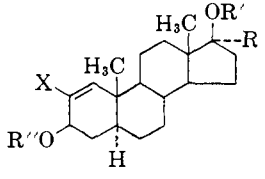


I



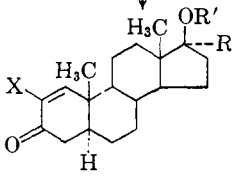
II

a, R = R′ = H
b, R = H, R′ = Ac
c, R = CH_3, R′ = H
d, R = C_2H_5, R′ = H



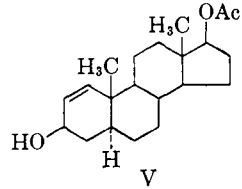
IV

a, R = R′ = R″ = H, X = Cl
b, R = H, R′ = R″ = Ac, X = Cl
c, R = R′ = R″ = H, X = Br
d, R = H, R′ = R″ = Ac, X = Br
e, R = CH_3, R′ = R″ = H, X = Cl
f, R = CH_3, R′ = H, R″ = Ac, X = Cl
g, R = CH_3, R′ = R″ = H, X = Br
h, R = C_2H_5, R′ = R″ = H, X = Cl
i, R = C_2H_5, R′ = H, R″ = Ac, X = Cl
j, R = C_2H_5, R′ = R″ = H, X = Br

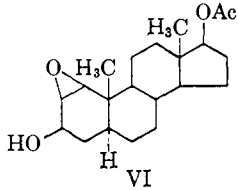


III

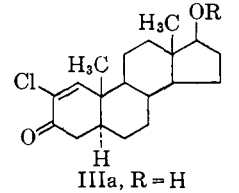
a, R = R′ = H, X = Cl
b, R = H, R′ = Ac, X = Cl
c, R = R′ = H, X = Br
d, R = H, R′ = Ac, X = Br
e, R = CH_3, R′ = H, X = Cl
f, R = CH_3, R′ = H, X = Br
g, R = C_2H_5, R′ = H, X = Cl
h, R = C_2H_5, R′ = H, X = Br



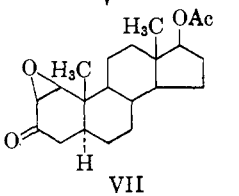
V



VI



IIIa, R = H
b, R = Ac



VII

Biological Activity.[11]—Table I shows the estimates of androgenic

(11) We are grateful to Dr. Francis J. Saunders and Mr. E. F. Nutting of our Endocrinology Division for furnishing us with this information.

and myotrophic potencies obtained in a modification[12] of the levator ani assay.[13] As reported previously,[1] the Δ^1 isomer of testosterone (Ia) was found to be much more active than testosterone both androgenically and myotrophically. In contrast with the reported ability of 4-halo substitution to increase the anabolic/androgenic ratio of testosterone, this effect was not obtained by 2-halo substitution of the potent 5α-androst-1-ene derivatives. 2-Halo substitution in this series had a marked diminishing effect on both the myotrophic and androgenic response. Reduction of the C-3 carbonyl tended to produce a further decrease in activity. The intermediate epoxides (IIc and IId) were also found to possess less than 5% the myotrophic and androgenic potency of testosterone propionate. Some of the compounds, however, did exhibit antiestrogenic activity. For instance, 2-chloro-17α-methyl-5α-androst-1-en-3-one (IIIe) was found to be as active as progesterone as an estrogen antagonist.

TABLE I

ANABOLIC-ANDROGENIC ACTIVITIES OF 2-HALO-5α-ANDROST-1-ENE DERIVATIVES

	Myotrophic[a] potency	Androgenic[a] potency
Testosterone propionate	100	100
Testosterone	26	35
Methyltestosterone	26	24
Ia	100	50
IIIb	20	10
IIIc	10–20	10
IIIe	20	10
IIIf[b]	20–40	50
IVe	4–10	10
IVf	4	5

[a] Potencies are given in terms of per cent. of the activity of testosterone propionate and were determined from the minimal levels at which significant increases in seminal vesicle or levator ani muscle weights were obtained. The compounds were injected intramuscularly into castrated male rats. [b] Compounds IIIg, h, IVa, c, h, i, and j were essentially inactive in this assay.

Experimental[14]

1α,2α-Epoxy-17α-methyl-5α-androstan-17β-ol-3-one (IIc).[15]—To a cooled,

(12) F. J. Saunders and V. A. Drill, *Proc. Soc. Exptl. Biol. Med.*, **94**, 646 (1957).

(13) E. Eisenberg and G. S. Gordan, *J. Pharmacal. Exptl. Therap.*, **99**, 38 (1950).

(14) Optical rotations, spectra, and analytical data were furnished by our Analytical Department under the supervision of Drs. R. T. Dillon and H. W. Sause. Optical rotations were obtained in chloroform and ultraviolet spectra in methanol.

(15) The synthesis of 1α,2α-epoxy-5α-androstan-17β-ol-3-one (IIa) and its acetate (IIb) have been described previously.[6]

stirred solution of 17α-methyl-17β-hydroxy-5α-androst-1-en-3-one[1] (3.5 g.) in methanol (60 ml.) was added dropwise and simultaneously 30% hydrogen peroxide solution (4 ml.) and 10% sodium hydroxide in methanol (1 ml.). The solution was stirred for an additional 10 min. and water added dropwise. The resulting precipitate was removed by filtration and washed with water. After drying, the product weighed 3.45 g. and was suitable for subsequent reactions. Recrystallization from ethanol–water afforded an analytical sample, m.p. 129–130°/152–152.5°, $[\alpha]^{26}D$ +98°.

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.40; H, 9.55.

1α,2α-Epoxy-17α-ethyl-5α-androstan-17β-ol-3-one (IId).—Treatment of 17α-ethyl-17β-hydroxy-5α-androst-1-en-3-one[1] (3.0 g.) as described above gave the 1α,2α-epoxide (2.9 g.). Recrystallization from ethanol-water furnished an analytical sample, m.p. 188–189.5°, $[\alpha]^{24}D$ +85°.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.76; H, 9.34.

2-Halo-17β-hydroxy-5α-androst-1-en-3-ones (III)—General Method.—To a solution of the 1α,2α-epoxy-3-ketosteroids (II, 1 g) in acetone (20 ml.) was added either concd. hydrochloric acid (2 ml.) or 48% hydrobromic acid (2 ml.). The solution was allowed to stand at room temperature for 1 hr. and poured slowly into ice water. The resulting precipitate was collected by filtration, washed with water, and recrystallized from a suitable solvent (see Table II).

2-Halo-5α-androst-1-ene-3β,17β-diol Derivatives (IV)—General Method.—To a cooled solution of III (1.5 g.) in purified tetrahydrofuran (10 ml.) was added lithium tri-*t*-butoxyaluminohydride (3.0 g.) in purified tetrahydrofuran (30 ml.). The solution was stirred for 1–2 hr. and the excess reagent decomposed by the addition of 5% aqueous acetic acid (250 ml.). The mixture was extracted with ether (3 × 50 ml.) and the ether extract washed with 5% sodium bicarbonate solution (2 × 50 ml.) and water (50 ml.). After drying the ether extract over anhydrous potassium carbonate, the solvent was removed by distillation. The resulting residue was crystallized from a suitable solvent (see Table III). The acetates were prepared by treating the halohydrins with acetic anhydride in pyridine for 15–20 hr. at room temperature. The solution was poured into ice water and the resulting oil extracted with ether. The ether extract was washed successively with 2 *N* hydrochloric acid, 5% sodium bicarbonate solution, and water. After drying the ether solution over anhydrous sodium sulfate, the solvent was removed by distillation and the residue crystallized from a suitable solvent (see Table III).

1β,2β-Epoxy-5α-androstan-3β,17β-diol 17-Monoacetate (VI).—To a cooled solution of 5α-androst-1-ene-3β,17β-diol 17-monoacetate[1] (V, 1.0 g.) in anhydrous benzene (10 ml.) was added with stirring 0.32 *N* perbenzoic acid in benzene (30 ml.). The solution was allowed to stand overnight at room temperature and then washed well with 5% sodium carbonate solution and water. After drying the solution over anhydrous sodium sulfate, the solvent was removed *in vacuo*. Crystallization of the residue from acetone–heptane gave VI (0.5 g.), m.p. 185–190°. Recrystallization from ethanol–water afforded an analytical sample, m.p. 189–191.5°, $[\alpha]^{26}D$ +40°.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.26; H, 9.04.

1β,2β-Epoxy-5α-androstan-17β-ol-3-one Acetate (VII).—Chromium trioxide (0.5 g.) was added in portions to pyridine (5 ml.) with cooling and stirring. To this mixture was added dropwise with stirring a solution of VI (0.4 g.) in pyridine (7.5 ml.). The reaction mixture was allowed to stand overnight at room temperature. Methylene chloride (50 ml.) was added and the solution washed with 5%

TABLE II: 2-HALO-17β-HYDROXY-5α-ANDROST-1-EN-3-ONE DERIVATIVES

III	Recrystallized from	Yield, %	M.p., °C	$[\alpha]^{22}D$	Formula	Calcd. C	Calcd. H	Found C	Found H
a	EtOH	82.5	224–226	+37	$C_{19}H_{27}ClO_2$	70.68	8.43	70.49	8.55
b	Me_2CO-hexane	79.5[a]	155.5–156.5	+33	$C_{21}H_{29}ClO_3$	69.12	8.01	69.09	8.40
c	$CHCl_3$-EtOAc	99[b]	231–233.5	+32.5	$C_{19}H_{27}BrO_2$	62.13	7.41	62.26	7.70
d	MeOH	67[c]	179–180	+28	$C_{21}H_{29}BrO_3$	61.61	7.14	61.79	7.35
e	Me_2CO-heptane	79	173–176	+15	$C_{20}H_{29}ClO_2$	71.30	8.68	71.53	8.77
f	MeOH-H_2O	61.5	175–177	+10.5	$C_{20}H_{29}BrO_2$	62.99	7.69	63.14	7.68
g	EtOH-H_2O	81	164–166	+20	$C_{21}H_{31}ClO_2$	71.87	8.91	72.22	9.09
h	MeOH-H_2O	75.5[d]	162–164	+15	$C_{21}H_{31}BrO_2$	63.79	7.90	63.86	7.83

[a] Prepared by acylating IIIa with acetic anhydride in acetic acid containing a catalytic amount of *p*-toluenesulfonic acid. [b] Prepared by acid-catalyzed methanolysis of IIId. [c] Acetic acid was used in place of acetone as solvent. [d] This compound was previously isolated as a by-product in the bromination–dehydrobromination of 17α-ethyl-5α-androstan-17β-ol-3-one.[1]

TABLE III: 2-HALO-5α-ANDROST-1-ENE-3β,17β-DIOL DERIVATIVES

IV	Recrystallized from	Yield, %	M.p., °C.	$[\alpha]^{25}D$	Formula	Calcd. C	Calcd. H	Found C	Found H
a	Me_2CO-hexane	75[a]	165–167	+23	$C_{19}H_{29}ClO_2$	70.24	9.00	70.54	9.14
b	Me_2CO-hexane	66	191.5–193.5	+19	$C_{23}H_{33}ClO_4$	67.55	8.13	67.90	7.78
c	Me_2CO-heptane	93[b]	181–183	+17	$C_{19}H_{29}BrO_2$	61.78	7.91	61.74	7.92
d	EtOH-H_2O	53	179–182	+12	$C_{23}H_{33}BrO_4$	60.92	7.34	61.23	7.79
e	Me_2CO-heptane	76.5	162–164.5	0	$C_{20}H_{31}ClO_2$	70.87	9.22	71.19	9.33
f		80	oil[c]	−1	$C_{22}H_{33}ClO_3$	69.36	8.73	69.06	8.90
g	Me_2CO-heptane	67	186–188	−3	$C_{20}H_{31}BrO_2$	62.65	8.15	62.73	8.30
h	Me_2CO-heptane	92.5	183.5–184.5	+5	$C_{21}H_{33}ClO_2$	71.46	9.42	71.61	9.46
i	MeOH	70	78–80	+4	$C_{23}H_{35}ClO_3$	69.94	8.93	70.01	9.05
j	Me_2CO-heptane	80	155–157	−0.5	$C_{21}H_{33}BrO_2$	63.46	8.37	63.74	8.64

[a] Prepared from 2-chloro-5α-androst-1-ene-3,17-dione.[16] [b] Prepared from 2-bromo-5α-androst-1-ene-3,17-dione.[16] [c] Resisted crystallization from a variety of solvents.

sodium bicarbonate solution. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure. The resulting brown residue was taken up in ether containing a little acetone and the solution decolorized with Darco. Filtration and removal of the solvent yielded a crystalline residue. Recrystallization from heptane afforded VII (225 mg.) as needles, m.p. 175–178°. One additional recrystallization from heptane gave an analytical sample, m.p. 179–180°, $[\alpha]^{25}D$ +38.5°.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.73; H, 8.58.

Reaction of VII with Hydrochloric Acid.—To a solution of VII (0.47 g.) in acetone (10 ml.) was added concd. hydrochloric acid (1 ml.). The solution was allowed to stand overnight at room temperature and then poured slowly into a mixture of ice and water. The precipitate was collected by filtration and washed with water. Recrystallization of the product (0.42 g.) from acetone–heptane gave IIIa, m.p. 223–226° which was identical with the material obtained previously from IIa by treatment with hydrochloric acid. Substitution of acetic acid for acetone in this experiment yielded the corresponding acetate (IIIb).

(16) R. E. Counsell and P. D. Klimstra, U. S. Patent 2,980,710 (1961).

The Metabolites of Ergometrine and Lysergic Acid Diethylamide in Rat Bile

M. B. SLAYTOR AND S. E. WRIGHT

Pharmacy Department, University of Sydney, Sydney, Australia

Received August 21, 1961

Administration of ergometrine (1 mg./kg.) to rats results in the biliary excretion, within six hours, of two metabolites which are more polar than ergometrine. It is suggested that these metabolites are the β-glucuronides of 12-hydroxyergometrine and 12-hydroxyergometrinine. Under the same conditions and at the same dose level lysergic acid diethylamide (LSD) is converted to two metabolites, which are β-glucuronides of a hydroxy-LSD and a hydroxy-*iso*LSD, hydroxylation occurring in the benzene ring. At 45 mg./kg. ergometrine is metabolized to give a number of additional metabolites two of which are the glucuronide ethers of ergometrine and ergometrinine, conjugation apparently having occurred on the hydroxyl group of the aminopropanol side chain.

Although the metabolism of simpler substituted indoles such as tryptophan[1] and skatole[2] has been studied in detail few examples of the metabolism of more complex indoles, such as are found in alkaloids, have been recorded. Because of their clinical importance and

(1) I. M. Dibner, A. Feldstein, H. Freeman, and H. Hoagland, *Fed. Proc.*, **19**, 3 (1960).

(2) E. C. Horning, C. C. Sweeley, C. E. Dalgliesh, and W. Kelly, *Biochem. Biophys. Acta*, **32**, 566 (1959).