concentrated, and distilled to give 28.6 g. (68%) of an oil, b.p. 87–89° (12 mm.); ν_{max} 3400, 965 cm.$^{-1}$.

Anal. Calcd. for $C_9H_{18}O$: C, 76.0; H, 12.8. Found: C, 76.4; H, 12.6.

The acetate of this alcohol, prepared using acetic anhydride in pyridine, had b.p. 92–94° (13 mm.); ν_{max} 1740, 1675, 1240, 965 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{20}O_2$: C, 71.7; H, 10.9. Found: C, 71.9; H, 11.1.

Acknowledgments.—We are grateful to Professor L. Crombie for helpful discussions, to Mr. I. H. M. Main for determination of biological activities and to Cynthia E. Coleman for technical assistance.

Hypocholesterolemic Agents. V.[1a] Isomeric Azacholesterols[1b]

R. E. COUNSELL,[1c] P. D. KLIMSTRA, L. N. NYSTED, AND R. E. RANNEY

Divisions of Chemical and Biological Research, G. D. Searle and Company, Chicago, Illinois 60680

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The potent hypocholesterolemic activity of certain diaza analogs of cholesterol prompted the synthesis of a series of cholesterol isosteres having only one nitrogen atom in the side chain. The hypocholesterolemic activity of these isomers was examined and certain rationalizations regarding structure and activity were presented.

One approach to the development of hypocholesterolemic agents has been the synthesis of compounds which will inhibit the endogenous synthesis of cholesterol. In this connection, investigations by several groups[2] have demonstrated that feeding cholesterol to laboratory animals promptly suppresses hepatic cholesterol synthesis. More recently, this negative feedback control of cholesterogenesis was found to be operative in man as well.[3]

Previous publications[4,5] from these laboratories described various diaza analogs of cholesterol which were synthesized as part of a program aimed at finding substances which would simulate cholesterol in the feedback mechanism. In these studies 20,25-diazacholesterol (X) was noted to be an extremely potent inhibitor of cholesterol synthesis in laboratory animals.[5] Subsequent clinical studies with this agent confirmed the high order of hypocholesterolemic activity in humans.[6]

Additional structure–activity relationship studies with the diazacholesterols tended to support the contention that these compounds were suppressing cholesterol synthesis in a "cholesteromimetic" fashion.[4,5] For example, replacing the isosteric dimethylamino end group with bulkier substituted amines markedly reduced the hypocholesterolemic activity. Lengthening the side chain by inserting one methylene group between the nitrogen atoms produced a similar effect. Shortening the side chain by one methylene group, however, produced little change in activity. These results implied that a receptor site with dimensions specific for cholesterol was involved and one must have an accurate fit of the substrate in order to get maximum activity. Accordingly, any structural change which tended to impede adsorption of the substrate molecule at the receptor site, produced a corresponding decrease in the hypocholesterolemic activity.

In an effort to obtain further insight as to the mode of action of these compounds, a series of cholesterol isosteres having only one nitrogen atom in the side chain was synthesized and biologically evaluated. Because of the disposition of the nitrogen atoms in the diazacholesterols described above, it was possible that these substances were exerting their hypocholesterolemic action *via* an intramolecular metal-chelating process which would tie up certain trace metals essential for cholesterol biosynthesis. Such a mechanism was proposed by Curran[7] to explain the inhibitory action of 8-quinolinol. The azacholesterols, on the other hand, would be incapable of acting in this manner. In addition, it was hoped that the study of the isomeric azacholesterols would provide further information regarding the electrical and topographical features of the receptor site.

20-Azacholesterol (IIIb, N-isohexyl-N-methyl-17β-aminoandrost-5-en-3β-ol) was readily obtained in several steps from 3β-acetoxyandrost-5-en-17-one (I). Condensation of I with isohexylamine in the presence of a catalytic amount of *p*-toluenesulfonic acid gave the expected 17-imine (II) as an oil. Reduction of II with lithium aluminum hydride afforded the corresponding amine (IIIa) which was methylated under Eschwiler–Clarke conditions[8] to give IIIb. Infrared and n.m.r.[9] analysis of the product clearly showed the characteristic absorption band for the N-methyl group at 3.6 μ[10] and 128 c.p.s.,[11] respectively.

Surprisingly, several attempts to carry out a Leuckart reductive amination of I with isohexylamine were unsuccessful. This was in marked contrast with the ease

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(2) R. G. Gould and C. B. Taylor, *Federation Proc.*, **9**, 179 (1950); G. M. Tomkins, H. Sheppard, and I. L. Chaikoff, *J. Biol. Chem.*, **201**, 137 (1953); I. D. Frantz, Jr., H. S. Schneider, and B. T. Hinkelman, *ibid.*, **206**, 465 (1954).

(3) E. P. Madhava Bhattathiry and M. D. Siperstein, *J. Clin. Invest.*, **42**, 1613 (1963).

(4) R. E. Counsell, P. D. Klimstra, R. E. Ranney, and D. L. Cook, *J. Med. Pharm. Chem.*, **5**, 720 (1962).

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(6) J. M. Martt and C. R. Talbert, *Circulation*, **28**, 763 (1963).

(7) G. L. Curran, *Proc. Soc. Exptl. Biol. Med.*, **88**, 101 (1955).

(8) M. L. Moore, *Org. Reactions*, **5**, 301 (1949).

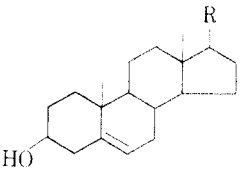
(9) The n.m.r. spectrum was obtained in deuteriochloroform with a Varian A-60 spectrometer and is reported in c.p.s. downfield from tetramethylsilane which was used as the internal standard.

(10) K. Nakaniski, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p. 40.

(11) L. M. Jackman, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 56.

TABLE I

ISOMERIC AZACHOLESTEROLS



Compd.	R	M.p., °C.	$[\alpha]^{29}D$, deg.	% found[a] C	H	N	MED
III	CH_3>N—$CH_2CH_2CH_2$—$CH(CH_3)_2$	121–125	−48.5	80.54	11.52	3.93	Inactive
VI	CH_3>CHNH$CH_2CH_2CH(CH_3)_2$	120–121	−33.5	80.79	11.78	4.04	Inactive
IXa	CH_3>$CHCH_2NHCH_2CH(CH_3)_2$	129–131	−39	80.41	11.54		0.60
IXb	CH_3>$CHCH_2CH_2NHCH(CH_3)_2$	164–166	−38	80.38	11.66	3.68	0.15
IXc	CH_3>$CHCH_2CH_2CH_2N(CH_3)_2$	147.5–149.5[c]	−41	80.60	11.79	3.73	0.03
X	CH_3>$NCH_2CH_2CH_2N(CH_3)_2$						0.30

[a] *Anal.* Calcd. for $C_{26}H_{45}NO$: C, 80.56; H, 11.70; N, 3.61. [b] MED = minimal effective dose (mg./kg.) as defined in text. All compounds were evaluated in the form of their hydrochloride salts. [c] Lit.[13] m.p. 153.5–154°.

by which dimethylaminopropylamine condensed with I under these conditions.[5]

3β-Acetoxy-20α-aminopregn-5-ene (IV), previously described by Julian and co-workers,[12] was utilized for the synthesis of 22-azacholesterol (VI, N-isoamyl-20α-aminopregn-5-en-3β-ol). Acylation of IV with isovaleryl chloride gave the amide V which readily underwent metal hydride reduction to VI.

The appropriate steroidal carboxylic acids (VIIa–c) served as starting materials for the synthesis of 23-, 24-, and 25-azacholesterol (IXa–c). The standard method of converting the acids to the amides (VIIIa–c) *via* the acid chlorides and subsequent metal hydride reduction furnished the desired amines in good over-all yield. The preparation of IXc by this method was previously described by Louw, Strating, and Backer.[13]

Preliminary Biological Results.[14]—The oral hypocholesterolemic activities of the azacholesterol analogs were assayed in male rats made hypercholesterolemic with 6-propylthiouracil according to the procedure described previously.[15] The minimal effective dose (MED) was the criterion of activity, and was estimated from dose–response curves as that dose which would induce a 10% reduction in serum cholesterol level after a 10-day treatment period.

Although the substitution of nitrogen for carbon at positions 20 and 22 (III and VI, Table I) resulted in compounds that were inactive at the standard dose (10 mg./kg.), successive substitution at positions 23, 24, and 25 produced azasterols with progressively increasing hypocholesterolemic activity. 25-Azacholesterol (IXc), the most potent member of the series, showed an activity of about 10 times that of 20,25-diazacholesterol (X). This is good evidence that these substances as well as the diazacholesterols are not exerting their hypocholesterolemic action by some metal-chelating process.

Further studies with IXc showed that desmosterol accumulated in serum and liver of treated rats and that the amount of this sterol appearing in the tissue was directly related to the intensity of treatment. A similar effect has been previously reported for 20,25-diazacholesterol.[16] Thus, like the 20,25-[16,17] and 22,25-diaza[15,18] analogs, IXc appears to have an inhibitory action on desmosterol reductase. The fact that administration of these substances causes an over-all reduction in total sterols, however, indicates that a site of action prior to the cyclization of squalene is also involved. This latter site of action remains to be elucidated.

It is generally accepted that the mechanism of action of structurally specific drugs involves interaction of the drug with certain regions or receptor sites on cellular or enzyme surfaces.[19] Although it is hazardous to interpret *in vivo* data as indicating the mode of action of inhibitors at a molecular level, certain relationships between structure and activity of the isomeric azacholesterols are noteworthy. If these substances are presumed to have similar tissue distribution and membrane penetrability properties, then the degree with which they interact with the receptor site could account for the observed variations in potency. Accordingly, the potent hypocholesterolemic activity for IXc would tend to indicate the presence of an anionic site on the receptor surface complimentary to the C-25 position of cholesterol. This would account for the decrease in activity when the isosteric group is placed at lower numerical positions in the hydrocarbon side chain.

Moreover, the sudden loss in activity in going from 23- to 22-azacholesterol suggests that steric factors

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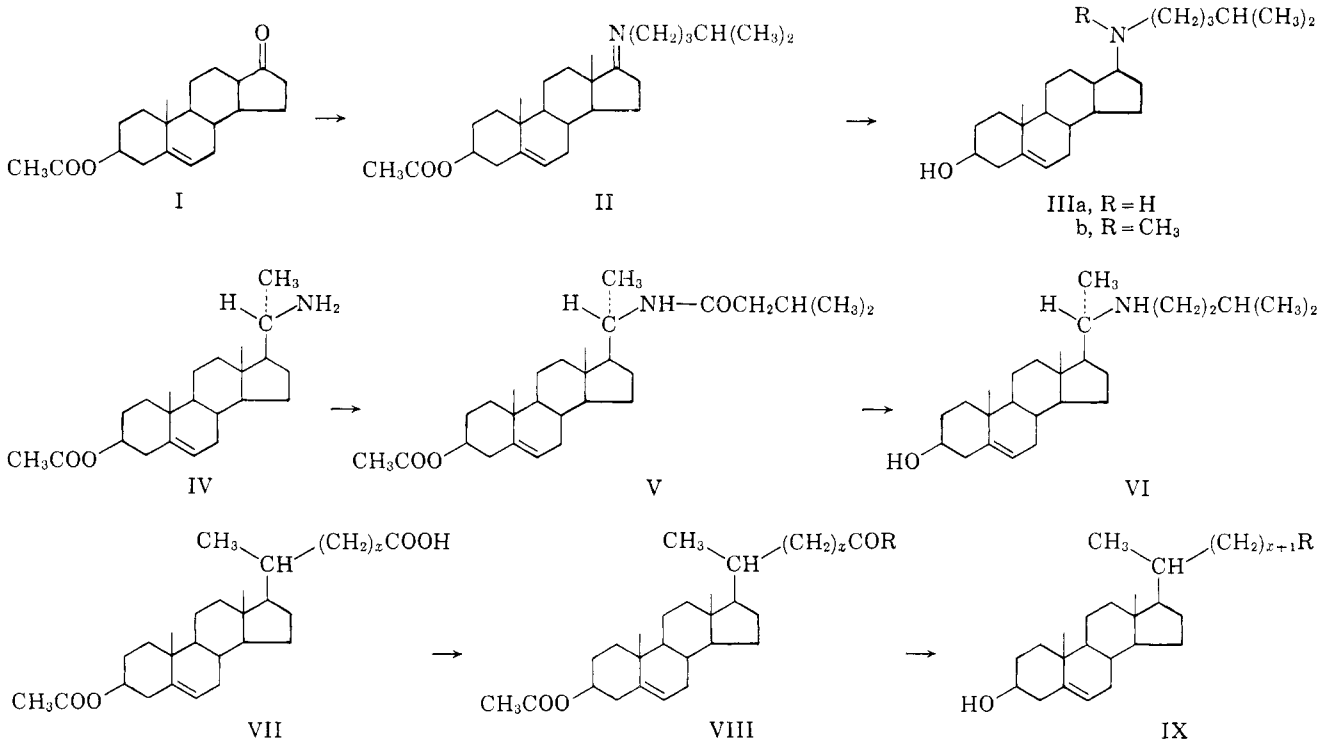

a, $x = 0$, R = $-NHCH_2CH(CH_3)_2$; b, $x = 1$, R = $-NHCH(CH_3)_2$; c, $x = 2$, R = $-N(CH_3)_2$

Figure 1.

also may be involved. An examination of molecular models (Figure 1) reveals that the β-orientation of the C-17–C-20 bond would decrease the ability of the 20- and 22-positions to interact with a receptor surface if adsorption occurred by the less hindered α-face of the steroid molecule.[20] Positions 23, 24, and 25, on the other hand, can readily assume a conformation which will allow them to rest on such a surface when approach is from the α-face (see Figure 1). Studies with isolated enzyme systems are now in progress in an effort to obtain more information regarding the action of these substances at a molecular level.

Experimental[21]

N-Isohexyl-17β-aminoandrost-5-en-3β-ol (IIIa).—A solution of 3β-acetoxyandrost-5-en-17-one (I, 25 g.), isohexylamine (15.5 g.), and *p*-toluenesulfonic acid (2.8 g.) in benzene (400 ml.) was refluxed, and the water was removed with the aid of a Dean–Stark trap. When the reaction was complete, the reaction mixture was allowed to cool to room temperature. The solution was washed with three 100-ml. portions of water and dried over a mixture of Na_2SO_4 and Darco. Removal of the solvent *in vacuo* afforded 3β-acetoxy-17-(N-isohexyl)iminoandrost-5-ene (II, 28 g.) as an oil ($[\alpha]^{25}D$ −34°) which resisted crystallization from a variety of solvents. The infrared spectrum displayed the characteristic absorption band for 17-imino steroids at 5.94 μ.[5]

Anal. Calcd. for $C_{27}H_{45}NO_2$: C, 78.02; H, 10.91; N, 3.37. Found: C, 78.40; H, 10.48; N, 3.45.

The imine (23 g.) was dissolved in dioxane[22] (200 ml.) and added dropwise with stirring to a slurry of lithium aluminum hydride (10.3 g.) in dioxane (400 ml.). The addition required 30 min., whereupon the mixture was refluxed with stirring for 5 hr. The excess hydride was decomposed by the successive dropwise addition of water (10.3 ml.) in dioxane (25 ml.), 20% NaOH solution (8 ml.), and water (34 ml.). The inorganic salts were removed by filtration and washed with dioxane. Concentration of the filtrate *in vacuo* and crystallization of the residual oil from acetone–water gave crude II (15.5 g.). Recrystallization from the same solvent system gave pure material (10.5 g.), m.p. 89–91°, $[\alpha]^{26}D$ −50°.

Anal. Calcd. for $C_{25}H_{43}NO$: C, 80.37; H, 11.60; N, 3.75. Found: C, 80.37; H, 11.16; N, 3.69.

N-Isohexyl-N-methyl-17β-aminoandrost-5-en-3β-ol (IIIb).—A solution of IIIa (7.0 g.) in formic acid (4.0 ml.) and formalin (4.0 ml.) was refluxed for 7 hr. The mixture was poured into methanol (150 ml.) and 30% NaOH solution (10 ml.) was added slowly at the reflux temperature. The solution was allowed to cool and poured into ice–water (1 l.). The precipitate was collected by filtration, washed with water, and allowed to dry. Recrystallization of the crude product from acetone–water gave pure IIIb (5.3 g.), m.p. 121–125°, $[\alpha]^{26}D$ − 48.5°.

Anal. Calcd. for $C_{26}H_{45}NO$: C, 80.56; H, 11.70; N, 3.61. Found: C, 80.54; H, 11.52; N, 3.93.

20α-Isovaleramidopregn-5-en-3β-ol Acetate (V).—To a warm solution of 3β-acetoxy-20α-aminopregn-5-ene[12] (IV, 18 g.) in benzene (300 ml.) and triethylamine (15 ml.) was added dropwise with stirring a solution of isovaleryl chloride (10 ml.) in benzene (50 ml.). The addition funnel was rinsed with benzene (25 ml.) and the mixture refluxed for 30 min. The reaction mixture was allowed to cool, water (100 ml.) was added, and the organic phase was separated. The latter was washed successively with 2 *N* HCl (100 ml.), 5% Na_2CO_3 solution (two 100-ml. portions), and water (100 ml.). The benzene solution was dried over a mixture of anhydrous potassium carbonate and Darco, and the solvent was removed by distillation *in vacuo*. Crystallization of the residue from acetone afforded crude V (13.1 g.), m.p. 189–194°. Recrystallization from acetone–water gave needles, m.p. 191–194°, $[\alpha]^{26}D$ −17°.

Anal. Calcd. for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22; N, 3.16. Found: C, 75.60; H, 10.24; N, 3.39.

3β-Acetoxypregn-5-ene-20-carboxylic Acid Isobutylamide (VIIIa).—To a stirred solution of crude 3β-acetoxypregn-5-ene-20-carbonyl chloride[12] (10 g.) in anhydrous benzene (100 ml.) was added isobutylamine (25 ml.). The reaction mixture was heated to reflux, cooled slightly, stoppered, and placed in a steam

(20) Studies showing the preferential interaction of the α-face of steroids with protein structures have been reviewed by U. Westphal, "Mechanism of Action of Steroid Hormones," C. A. Villee and L. L. Engel, Ed., Pergamon Press, New York, N. Y., 1961, pp. 65–70.

(21) The elemental analyses, optical rotations, and infrared and n.m.r. spectra were furnished by Dr. R. T. Dillon, Mr. E. Zelinski, and Mr. J. Damascus of our analytical department. The optical rotations and infrared spectra were obtained in chloroform. The melting points were obtained on a Fisher-Johns apparatus and are corrected.

(22) Purified dioxane as obtained from Pierce Chemical Co. was used in these experiments.

oven at 65° for 1 hr. The solvent was removed *in vacuo*, and the residue was taken up in ether–ethyl acetate (1:1). This solution was washed successively with dilute HCl, water, and 5% potassium carbonate solution. The washed extract was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residual solid was recrystallized from acetone–hexane to give pure VIIIa (9.2 g.), m.p. 194–197°, $[\alpha]^{25}D$ −57°.

Anal. Calcd. for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22; N, 3.16. Found: C, 75.54; H, 10.21; N, 3.13.

3β-Acetoxy-23-nor-5-cholenic Acid Isopropylamide (VIIIb).—Thionyl chloride (1 ml.) was added dropwise with stirring to a solution of 3β-acetoxy-23-nor-5-cholenic acid[23] (2.0 g.) in anhydrous benzene (25 ml.). The solution was refluxed for 90 min., and the solvent was removed under reduced pressure. Traces of thionyl chloride were removed by the repeated addition and distillation of anhydrous benzene. The crude acid chloride was dissolved in anhydrous benzene (15 ml.) and added dropwise with stirring to a cooled solution of isopropylamine (2 ml.) in anhydrous benzene (20 ml.). The mixture was stirred at room temperature for 3 hr. and water (25 ml.) and ether were added (50 ml.). The organic layer was separated, washed with water, and dried over a mixture of Na_2SO_4 and Darco. Removal of the solvent *in vacuo* afforded a white crystalline solid (2.2 g.), m.p. 187–194°. Recrystallization from acetone–heptane gave pure VIIIb, m.p. 197–199°, $[\alpha]^{25}D$ −41°.

Anal. Calcd. for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22. Found: C, 75.92; H, 10.21.

3β-Acetoxy-5-cholenic Acid Dimethylamide (VIIIc).—The crude acid chloride of 3β-acetoxy-5-cholenic acid (15 g.) was prepared in a manner similar to that described above. This product was dissolved in anhydrous benzene (130 ml.) and a solution of dimethylamine in toluene (26.6% w./w., 30 ml.) was added with stirring and external cooling. Benzene (40 ml.) was used to rinse the addition funnel and this was added to the reaction mixture. The mixture was stirred at room temperature for 2 hr. and ether (200 ml.) was added. The solution was washed successively with water, 2 *N* HCl, and 5% $NaHCO_3$ solution and dried over a mixture of anhydrous potassium carbonate and Darco. Removal of the solvent under reduced pressure gave crude VIIIc (17.3 g.), m.p. 177–183°, which was satisfactory for subsequent use. A sample was purified by adsorption onto silica gel and elution with ethyl acetate–benzene (1:9). Alternate recrystallization from ethyl acetate–heptane and benzene–heptane gave a pure sample, m.p. 184–186.5°, $[\alpha]^{26}D$ −41° (lit.[13] m.p. 189.5–190°, $[\alpha]D$ −40.9°).

Anal. Calcd. for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22; N, 3.16. Found: C, 76.13; H, 9.90; N, 3.33.

Reduction of Amides. General Method.—A solution of V (4.4 g., 0.01 *M*) in dioxane (75 ml.) was added dropwise with stirring to a suspension of lithium aluminum hydride (3.8 g., 0.1 *M*) in dioxane (75 ml.) at the reflux temperature. The mixture was refluxed with stirring for 18 hr., whereupon the excess hydride was decomposed by the successive dropwise addition of water (4 ml.) in dioxane (50 ml.), 20% NaOH solution (3 ml.), and water (20 ml.). The inorganic salts were removed by filtration and washed with dioxane. The filtrate was concentrated to dryness *in vacuo* and the residue was crystallized from acetone. This gave pure N-isoamyl-20α-aminopregn-5-en-3β-ol (VI, 2.7 g.), m.p. 120–121°, $[\alpha]^{25}D$ −33.5°.

Anal. Calcd. for $C_{26}H_{45}NO$: C, 80.56; H, 11.70; N, 3.61. Found: C, 80.79; H, 11.78; N, 4.04.

Hydrochloride Salts.—The crystalline amines were dissolved in isopropyl alcohol and sufficient 7 *N* HCl in isopropyl alcohol was added dropwise with agitation. The mixture was allowed to stand at room temperature for a few minutes and the precipitate was collected by filtration. The salts were recrystallized from either aqueous isopropyl alcohol or a mixture of methanol and isopropyl alcohol and gave satisfactory elemental analyses.

(23) K. J. Sax and W. Bergmann, *J. Am. Chem. Soc.*, **77**, 1910 (1955).

Anabolic Agents. A-Ring Conjugated Enone Androstane Derivatives[1a]

P. D. KLIMSTRA AND R. E. COUNSELL

Division of Chemical Research, G. D. Searle and Company, Chicago, Illinois 60680

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Various A-ring modified derivatives of testosterone were prepared in the hope of obtaining compounds with high anabolic and minimal androgenic activity. A comparison of the biological activity of these A-ring isomers is discussed. The synthesis of some of these isomers is described in detail.

In a previous publication,[1b] it was noted that shifting the double bond of testosterone and 17α-methyltestosterone from the 4,5- to the 1,2-position enhanced parenteral anabolic and, to a lesser extent, androgenic activity. Moreover, a comparison of oral myotrophic activity of the 4,5- and 1,2-ene systems indicated that the latter had a more favorable anabolic–androgenic ratio. These oral activities (Table I) were much greater than would have been expected from an examination of the original biological parenteral data.[1b]

The potent activity found for the 1-dehydro isomers of testosterone (I) prompted interest in other A-ring isomers (Table II) in which the position of not only the double bond, but also the carbonyl group was altered. This paper will discuss the chemistry and biology of these modifications.

The observation by Wharton and Bohlen[2] that hydrazine caused rearrangement of steroidal α,β-epoxy ketones to allylic alcohols prompted investigation of this reaction when applied to the 1,2α-epoxy-3-keto-

TABLE I
ANABOLIC–ANDROGENIC ACTIVITIES[a]

	I.m.		Oral	
Compd.	Myotrophic	Androgenic	Myotrophic	Androgenic
Testosterone propionate	100	100		
Testosterone	26	35		
Ie	200	100		
Ia	400	100		
IVa	5	1		
VIIIa	4	1		
XIIa	200	25		
17α-Methyltestosterone	26	24	100	100
Ib	50	25	1600	100
IVb	20	10	150	20
VIIIb	≤5	<1	<25	≤20
XIIb	100	10–25	100	15

[a] Potencies are given in terms of per cent of the activity of testosterone propionate and 17α-methyltestosterone and were determined from the lowest levels at which significant increases in seminal vesicle or levator ani muscle weights were obtained.

(1a) Presented in part before the Division of Medicinal Chemistry at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964. (1b) R. E. Counsell, P. D. Klimstra, and F. B. Colton, *J. Org. Chem.*, **27**, 248 (1962).

(2) P. S. Wharton and D. H. Bohlen, *ibid.*, **26**, 3615 (1961).