Synthesis and Biological Activity of D-Bishomo Steroids†

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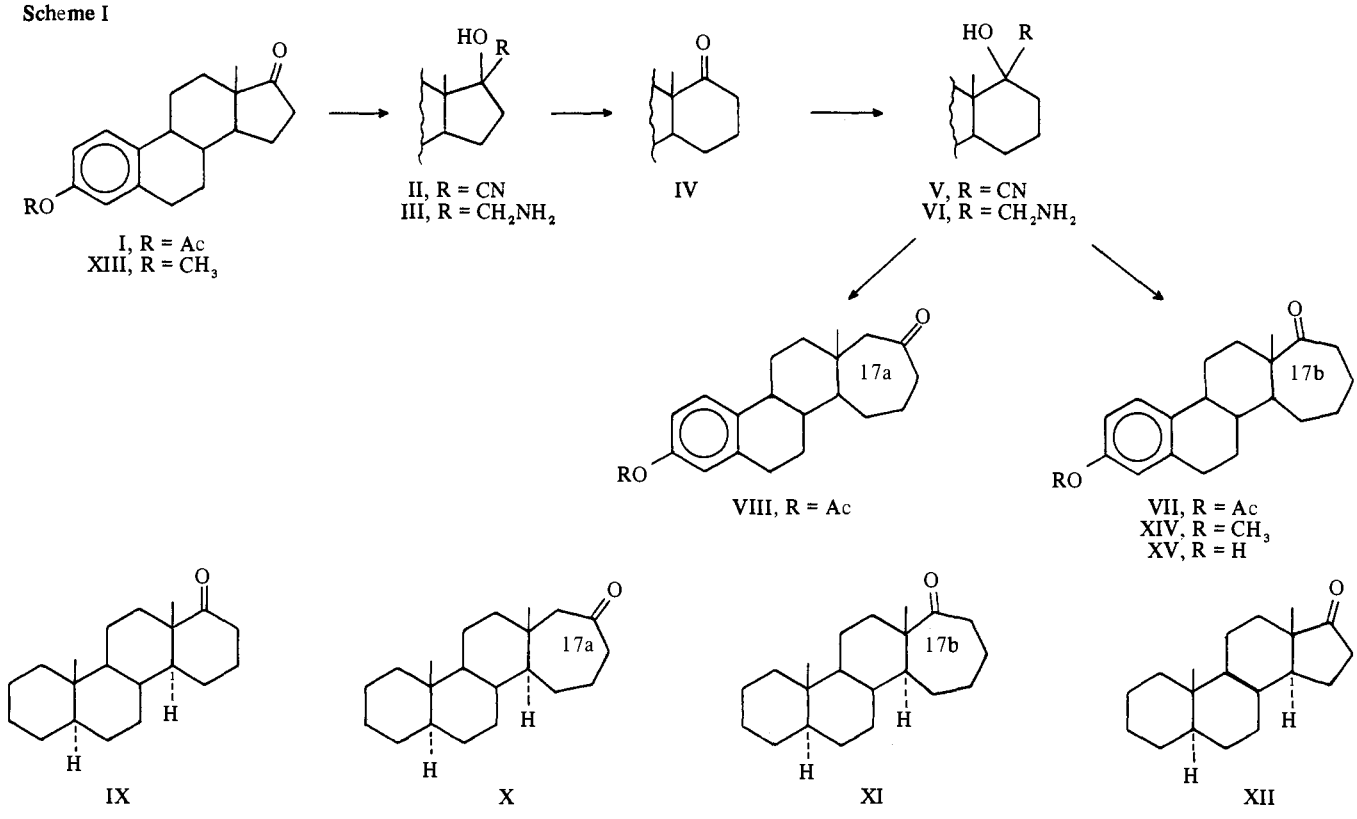
The $AlCl_3$-catalyzed homologation of 17-keto steroids with CH_2N_2 offers a one-step route to the corresponding D-bishomo-17b-keto steroids. Earlier multistep syntheses of D-bishomo-17b-keto steroids apparently produced instead the corresponding 17a-keto steroids. By this procedure analogs of testosterone, estrone, and estradiol were prepared in which ring D is 7 membered. This molecular change resulted in greatly diminished biological activity.

Although D-homo steroids have been the object of considerable interest, hormone analogs of practical medicinal use have not yet been uncovered.[1] On the other hand, the physiological properties of D-bishomo steroids remain largely unexplored, presumably because they are not so easily accessible synthetically. Previous syntheses have involved the homologation of the corresponding D-homo steroid, itself prepared from the normal ring D steroid (Scheme I).[2,3] Although it was suggested that the ultimate product of the multistep synthesis was the 17b-ketone (*e.g.*, VII), no evidence was presented to exclude the alternative structure, the 17a-ketone (VIII).[2,3]

Scheme I



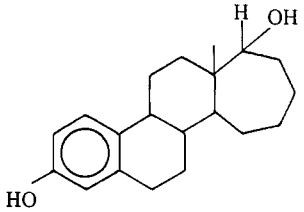
This ambiguity has been resolved by work performed in these laboratories in connection with another investigation.[4,5] Homologation of the model compound D-homo-5α-androstan-17a-one (IX) *via* the Tiffeneau rearrangement gives D-bishomoandrostan-17a-one (X) and not the expected D-bishomoandrostan-17b-one (XI). The structural assignment is based on nmr evidence [angular CH_3 at 0.75 and 0.80 ppm, a pair of doublets centered at 2.75 and 2.71 ppm (1 proton each), and a multiplet at 2.73 ppm (2 protons)], and on the observation that exhaustive base-catalyzed exchange resulted in the incorporation of 4 D atoms. This observation proves that the earlier syntheses[2,3] had, in fact, produced D-bishomo-17a-ones instead. Thus, it appeared desirable to prepare a few authentic D-bishomo-17b-keto hormonal analogs in order to investigate their utility as potential medicinal agents.

Model studies were initiated on 5α-androstan-17-one (XII). $AlCl_3$-catalyzed homologation of androstan-17-one with CH_2N_2 produced D-bishomoandrostan-17b-one (XI) *in a single step* and in fair yield (25–35%). The structural assignment is based on nmr evidence (angular CH_3 at 0.77 and 1.06 ppm) and on the observation that exhaustive base-catalyzed deuteration resulted in the incorporation of only 2 D atoms.

Analogous reaction of the Me ether of estrone (XIII) gave D-bishomo-3-methoxyestra-1,3,5(10)-trien-17b-one (XIV); cleavage of the Me ether yielded D-bishomo-3-hydroxyestra-1,3,5(10)-trien-17b-one (XV) whose melting point and rotation differed from those reported earlier for this compound.[3] Thus, the conclusion must be drawn that homologation of D-homo-3-acetoxyestra-1,3,5(10)-trien-17a-one

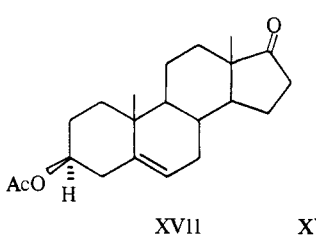
†Financial support by the National Institutes of Health (AM 12758) is gratefully acknowledged.
‡National Institutes of Health Predoctoral Fellow, 1968–1971.

(IV) *via* the Tiffeneau rearrangement gives the *D*-bishomo-17a-one (VIII), while CH_2N_2 homologation of estrone methyl ether (XIII) yields the corresponding *D*-bishomo-17b-one (XIV). Estrogen activity was assayed according to the procedure of Dorfman, *et al.*[6] *D*-Bishomoestra-1,3,5(10)-triene-3,17b-dione (XV) in sesame oil (0.1 ml) was injected once daily for 3 consecutive days into 10- to 21-day-old female mice (total dose 1 and 10 μg); autopsy on the fourth day indicated that the ketone possessed about 0.04 times the potency of estrone itself. The corresponding diol, *D*-bishomoestra-1,3,5(10)-triene-3,17b-diol (XVI) exhibited 0.01 times the potency of estrone when assayed in a similar manner.§

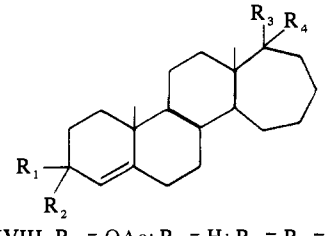


XVI

$AlCl_3$-catalyzed homologation of 3β-acetoxyandrost-5-en-17-one (XVII) with CH_2N_2 gave *D*-bishomo-3β-acetoxyandrost-5-en-17b-one (XVIII). Hydrolysis of the acetate group and Jones oxidation yielded *D*-bishomoandrost-4-ene-3,17b-dione (XIX). Protection of the 3-keto group as the pyrollidine enamine,[7] reduction of the 17b-keto group with LAH, and hydrolysis of the protecting group[7] gave the testosterone homolog 17b-hydroxy-*D*-bishomoandrost-4-en-3-one (XX).§ The androgenic and anabolic activity of XX was assayed according to the procedure of Dorfman and Dorfman.[8] Daily injection of the testosterone homolog into castrated 21- to 28-day-old rats with autopsy on the eighth day (total dose 1 mg) indicated that XX was inactive in rats. Androgen assay in white Leghorn chicks according to the procedure of Dorfman and Dorfman[9] (total dose 10 and 100 μg) indicated that XX possessed slight activity; however, its potency was less than 0.03 times that of testosterone itself.



XVII



XVIII, R_1 = OAc; R_2 = H; R_3 = R_4 = O
XIX, R_1 = R_2 = R_3 = R_4 = O
XX, R_1 = R_2 = O; R_3 = OH; R_4 = H

§ The stereochemistry of the OH group at C-17b in *D*-bishomotestosterone and *D*-bishomoestradiol remains undefined. In the 5-membered ring D series, both estrone and estradiol exhibit pronounced estrogenic activity; since *D*-bishomoestrone is of lesser potency, it is justified to state that bishomologation leads to a decrease in estrogenic activity in these compds. The lessened anabolic and androgenic activity of *D*-bishomotestosterone could be attributed either to bishomologation or to the configuration of the OH group at C-17b. The LAH reduction of a C-17 carbonyl group in the 5-membered ring D series produces predominantly the 17β-alcohol. Consideration of molecular models suggests a similar stereochemistry for reductions in the bishomo-17b-one series. It therefore appears likely that the decreased potency of *D*-bishomotestosterone and *D*-bishomoestradiol is due to *D*-bishomologation, and not the stereochemistry of the C-17b OH.

Experimental Section#

D-**Bishomo-3β-acetoxyandrost-5-en-17b-one (XVIII).** A soln of 2.0 g of 3β-acetoxyandrost-5-en-17-one (dehydroisoandrosterone acetate, XVII) in 50 ml of anhyd Et_2O was cooled to 0° in an ice bath. Addn of 2 ml of an ethereal soln of CH_2N_2[10] produced a yellow soln; addn of a catalytic amt of anhyd $AlCl_3$ discharged the color and caused the vigorous evolution of N_2. The dropwise addn of CH_2N_2 was contd until the yellow color persisted for longer than 1 min; addl anhyd $AlCl_3$ was then added. The process was repeated until analysis by vpc (0V-25 column operated at 280°) indicated the consumption of 80% of the starting material (20 min). The Et_2O soln was then washed with 10% HCl, H_2O, 10% aq KOH, and H_2O and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a pale yellow oil (2.1 g). Column chromatog, and recrystn of the ketonic fractions from acetone gave 608 mg of XVIII: mp 153–154°; [α]D–27° ($CHCl_3$); ir, ν_{max} 1750,1700 cm^{-1}; whose mass spectrum exhibits a base peak at *m/e* 298 (M^+–HOAc); the nmr spectrum exhibits angular Me signals at 1.08 and 0.89 ppm. *Anal.* ($C_{23}H_{34}O_3$) C, H.

D-**Bishomo-3β-hydroxyandrost-5-en-17b-one.** The 3β-acetate (XVIII) was dissolved in 50 ml of MeOH contg 5 ml of satd aq KOH. The soln was stirred at room temp under N_2 for 30 min and then worked up in the usual manner. Recrystn (Me_2CO) gave *D*-bishomo-3β-hydroxyandrost-5-en-17b-one: mp 167–168.5°; [α]D–21° ($CHCl_3$); ir ν_{max} 1700 cm^{-1}; M^+ at *m/e* 316.

D-**Bishomoandrost-4-ene-3,17b-dione (XIX).** Jones oxidation of the alcohol gave, after work-up in the normal manner,[11] and prep tlc [CH_2Cl_2–Et_2O eluent (4:1)] XIX as a clear oil: [α]D–3° ($CHCl_3$); ir ν_{max} 1680 cm^{-1}; M^+ at *m/e* 314. *Anal.* ($C_{21}H_{30}O_2$) C, H.

D-**Bishomo-17b-hydroxyandrost-4-en-3-one (XX).** The dione XIX (200 mg) was dissolved in the minimum vol of hot MeOH. The addn of 5 equiv of pyrollidine[7] was followed almost immediately by the formation of orange crystals. The crude *D*-bishomo-3-pyrollidinyl-3,5-androstadien-17b-one was collected by filtration, and after thorough drying under vacuum, was reduced in Et_2O to the corresponding alcohol with LAH. Hydrolysis of the enamine in refluxing 95% EtOH (40 min)[7] gave XX: mp 226–228° (acetone); [α]D+44° (MeOH); ir ν_{max} 1680 cm^{-1}.

D-**Bishomo-3-methoxyestra-1,3,5(10)-trien-17b-one (XIV).** Homologation of 1.0 g of 3-methoxyestra-1,3,5(10)-trien-17-one (estrone methyl ether, XIII) with CH_2N_2 according to the procedure described above gave XIV (320 mg): mp 130–131°; ir ν_{max} 1700 cm^{-1}; nmr angular Me at 1.10 ppm; M^+ at *m/e* 312. *Anal.* ($C_{21}H_{28}O_2$) C, H.

D-**Bishomo-3-hydroxyestra-1,3,5(10)-trien-17b-one (XV).** Cleavage of the Me ether XIV was accomplished according to the procedure of Huffman and Lott.[12] The ether (220 mg) was dissolved in 20 ml of glac AcOH, treated with 20 ml of 47% HI, and refluxed under N_2 for 7 min. The soln was cooled below 100°, and treated with satd $NaHSO_3$, chilled, and filtered. The solid was purified by prep tlc [CH_2Cl_2–Et_2O eluent (1:1)] to give, after recrystn from MeOH, XV (140 mg): mp 215–217°, [α]D+113° ($CHCl_3$); ir ν_{max} 1700 cm^{-1}; M^+ at *m/e* 298. *Anal.* ($C_{20}H_{26}O_2$) C, H.

D-**Bishomoestra-1,3,5(10)-triene-3,17b-diol (XVI).** To a 4-fold excess of LAH suspended in THF was added 100 mg of the ketone XV dissolved in THF. After 1 hr at reflux, the excess hydride was decompd by the addn of EtOAc, the soln was acidified and extd with $CHCl_3$. After washing the org layer with 10% HCl and H_2O, the soln was dried ($MgSO_4$) and concd. Prep tlc [C_5H_6–Et_2O eluent (1:1)] permitted the isolation of XVI: mp 230–232°; [α]D+45° (MeOH), which displayed no ir carbonyl absorption. *Anal.* ($C_{20}H_{28}O_2$) C, H.

#Melting points (uncor) were detd on a Thomas-Hoover melting point apparatus. The ir spectra were recorded in $CHCl_3$ soln with a Perkin-Elmer 137 Infrared Spectrophotometer, and nmr spectra in $CDCl_3$ soln (TMS) on a Varian HA-100 instrument. Mass spectra were detd on an Atlas CH-4 mass spectrometer, using the direct inlet procedure. Prep tlc was performed on silica gel HF_{254}. Where elemental analyses are indicated by the symbols of the elements, analytical results obtd for the elements are within ±0.3% of the calcd values. The authors are grateful to Syntex Research, Palo Alto, Calif., for supplying the steroid hormones utilized as starting materials in this study, and to Mr. W. R. Rooks of that organization for determining the biological activities discussed herein.

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Cardiovascular Activity of Some Substituted 2-Aminobenzoquinolizines

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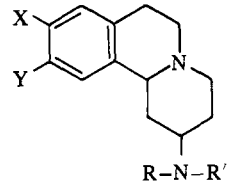
A series of substituted 2-aminobenzoquinolizines was synthesized and evaluated for antihypertensive and coronary dilator activity. Maximum antihypertensive activity was found in the trans isomer of *N*-phenyl-*N*-(1,3,4,6,7,11b-hexahydro-2*H*-benzo[*a*]quinolizin-2-yl)propionamide, which was selected for further evaluation.

In our search for novel antihypertensive compounds, we have synthesized a series of 2-aminobenzoquinolizines. The compounds chosen for study are illustrated by the general formula I where X and Y = H or methoxy; R = H, alkyl, aryl or aralkyl; R′ = H or acyl.



I

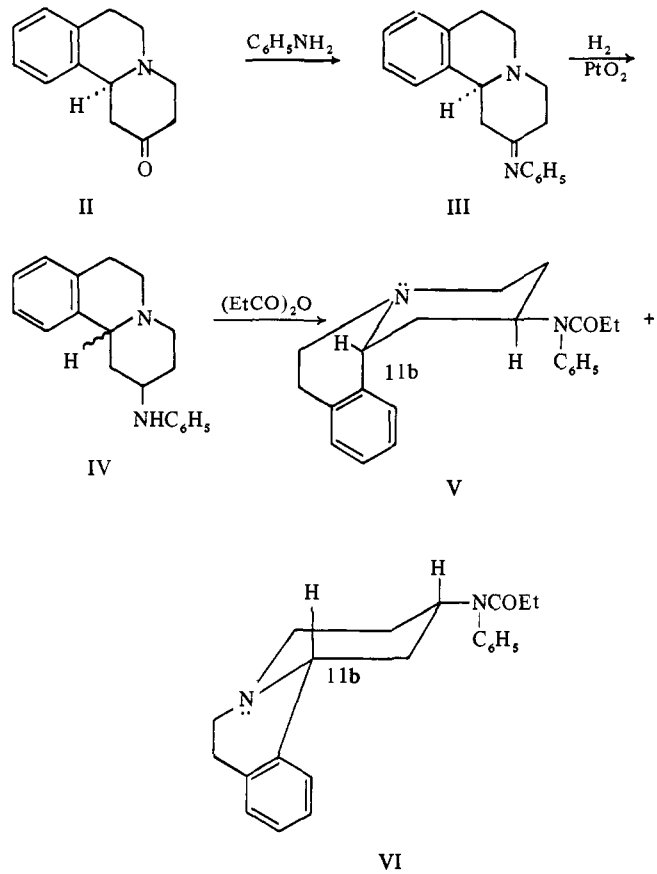
Compounds V (1, Table I) and VI (2, Table I) were originally prepared by treating 1,3,4,6,7,11b-hexahydro-2*H*-benzo[*a*]quinolizin-2-one (II)[1] with aniline to form the Schiff base III. The anil III was then hydrogenated with PtO_2 to give the amine IV which was propionylated to give the amides V (mp 157–158°) and VI (mp 126–127°)† in a ratio of approximately 1:2.

The proof that the amide V has the cis‡ configuration and the amide VI has the trans configuration can be obtained by examining the ir and nmr spectra. The ir spectrum of VI exhibits strong "Bohlmann bands"[2] at 2750 cm^{-1} and 2800 cm^{-1}. These bands are characteristic for the trans isomer and are not present in the ir spectrum of the amide V.

The nmr spectra of these isomers indicated that the conformations assigned to them on the basis of their ir spectra were correct. Uskokovic, *et al.*,[3] have shown that the angular proton in a cis fused configuration in benzo[*a*]quinolizines is shifted to lower field (below δ 3.8). Nmr also allows one to distinguish between the two alternative cis forms (V and VII) by the splitting pattern of the angular proton. Since our compound shows a 1:2:1 triplet at δ 4.10 (J = 4 cps) and not a 1:1:1:1 quartet, it was assigned conformation V. The nmr spectrum of VI showed no signal from δ 3.5 to 4.5.

Our findings are in complete agreement with those of Gootjes, *et al.*,[4] who have described in detail the stereochemistry of the benzoquinolizine system.

Scheme I



†A crystalline modification melting at 134–135° can also be obtained.

‡Cis and trans refer to the ring fusion.