Synthesis and Anabolic Activity of 3-Methylene- and 3-Methylsteroids of the Androstane and 19-Norandrostane Series

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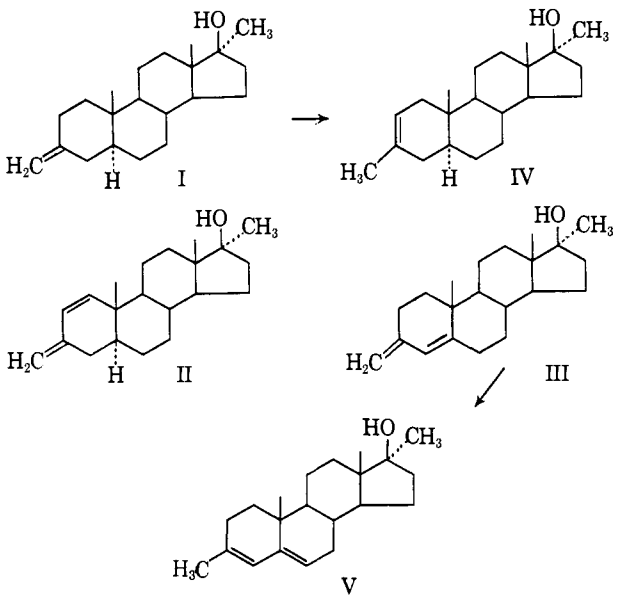
The preparation of 3-methylene-17α-methylandrostan-17β-ol, its Δ^1- and Δ^4-derivatives, as well as the isomeric 3-methyl compounds, is described. Syntheses of several other 3-methyleneandrost-4-enes and 3-methylene-19-norandrost-4-enes are also reported. 3-Methylene-17α-methylandrost-1-en-17β-ol displays a high anabolic activity with a favorable anabolic–androgenic ratio.

Recently de Winter, *et al.*,[1,2] found the surprising fact that the removal of the keto group of Δ^4-3-ketosteroids results in compounds of anabolic or progestational activity. Bowers and co-workers[3] prepared numerous androstane derivatives bearing on C-3 oxygen. They evaluated the influence of the position of the double bonds and of the substitution at C-2 on the biological activity. Segaloff and Gabbart[4] investigated saturated 3-deoxoandrostane derivatives of the 5α and 5β series.

Up to now it was unknown whether steroids having a C-3 carbon substituent instead of the oxygen function are biologically active. We studied the 3-methylenesteroids of the androstane and 19-norandrostane series and their 3-methyl isomers with endocyclic double bonds. Surprisingly, some of them showed considerable anabolic activity.



In search of the relation between constitution and anabolic activity we prepared the 17α-methylandrostane compounds I–V.

17α-Methyltestosterone and its 4,5α-dihydro derivative were transformed smoothly into the 3-methylene derivatives III and I by a Wittig reaction,[5] formerly applied by Sondheimer[6] in the preparation of 3-methylene derivatives of testosterone, 4,5α-dihydrotestosterone, and androst-4-en-3,17-dione. Similarly, 17α-methyl-5α-androst-1-en-17β-ol-3-one[7] yielded compound II. It was also prepared *via* a Wittig reaction of 5α-androst-1-en-17β-ol-3-one, subsequent oxidation in the 17 position with chromic acid–pyridine complex, and reaction with methyllithium.

Sondheimer[6] has shown that 3-methyleneandrost-4-enes can be isomerized to the corresponding 3-methylandrosta-3,5-dienes. Application of this reaction to III required milder conditions because of the easy elimination of the tertiary 17-hydroxyl group. Isomerization of III overnight at room temperature afforded V in good yield.

According to the results of Cookson, *et al.*,[8] in the cholestane series the isomerization of I was expected to yield the 3-methyl-5α-androst-2-ene derivative IV. The isomerization of the nonconjugated double bond required somewhat stronger conditions than that of diene III. Nevertheless, heating for a short period of time did not result in the elimination of the 17-hydroxyl group. It was interesting to note that neither II nor the corresponding more stable 17-ketone isomerized to the endocyclic 1,3-diene. This shows the increased thermodynamic stability of the diene with the exocyclic double bond in contrast to its homocyclic isomer.

Several derivatives of III were synthesized in order to show the influence of further structural alterations on the anabolic activity. The investigations were extended to cover corresponding 17α-ethyl-, 19-nor-, and 9α-fluoro-11β-hydroxy compounds (VI–IX).

By the Wittig reaction with 17α-ethyltestosterone, 9α-fluoro-11β-hydroxy-17α-methyltestosterone, and 17α-ethyl-19-nortestosterone the 3-methylene derivatives VI, VII, and IX were obtained. Our synthesis of VIII started from 19-nortestosterone which was transformed into 3-methylene-19-norandrost-4-en-17β-ol *via* the Wittig reaction, then oxidized with chromic acid–pyri-

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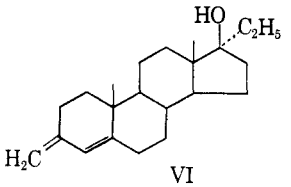
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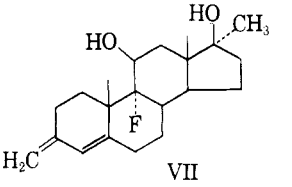
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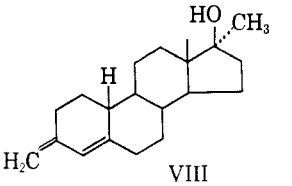
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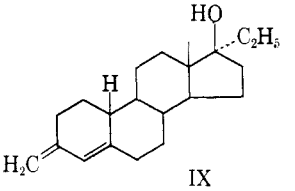
IX

dine complex and finally made to react with methyllithium. Isomerization to the $\Delta^{3,5}$-diene was avoided by exclusion of acids.

Biological Activity.—The compounds were assayed in immature castrated male rats (40–50 g.) by the method of Hershberger, *et al.*[9] The substances were administered daily by oral route over a period of 7 days and the animals sacrificed 24 hr. after the last administration. The anabolic (myotrophic) and androgenic activity was tested on the basis of the weights of the levator ani muscles and the seminal vesicles. From the results the relations of activity were calculated statistically according to Bliss.[10] Our preliminary findings are given in Tables I and II. They are merely approximate results.

TABLE I

BIOLOGICAL PROPERTIES OF 3-METHYLENE- AND 3-METHYLANDROSTANE DERIVATIVES

Compound	Myotrophic effect	Androgenic effect	Therapeutic ratio
17α-Methyltestosterone	1	1	1
I	0.59	0.11	5.36
II	2.66	0.12	22.10
III	0.53	0.28	1.89
IV	0.08	0.053	1.51
V	0.25	0.31	0.81

It can be seen that the conversion of 17α-methyltestosterone into I and III slightly reduces the myotrophic, but causes a considerable reduction of the androgenic, activity. This results in a good anabolic–androgenic ratio of I. A high myotrophic effect, however, and a decrease of the androgenic activity which result in an excellent anabolic–androgenic ratio can be observed with II. The conversion of I and III into their 3-methyl analogs (IV, V) led to a further decrease of activity.

The modifications of III show that the 17-ethyl (VI), the 19-nor derivative (VIII), and the 17-ethyl-19-nor compound (IX) have a decreased myotrophic activity compared to that of III. This applies especially to the compounds having a 17-ethyl side chain (VI, resp., IX). Even the introduction of the 9,11-fluorohydrin grouping (VII) does not enhance the myotrophic and the androgenic effects.

(9) L. G. Hershberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953).

(10) C. I. Bliss, "Vitamin Methods," Vol. II, P. György, Ed., 1951, p. 445 ff.

TABLE II

BIOLOGICAL PROPERTIES OF 3-METHYLENEANDROST-4-ENE AND -19-NORANDROSTENE DERIVATIVES

Compound	Myotrophic effect	Androgenic effect	Therapeutic ratio
17α-Methyltestosterone	1	1	1
III	0.53	0.28	1.89
VI	0.04	0.008	5.00
VII	0.30	0.32	0.94
VIII	0.42	0.35	1.20
IX	0.05	0.04	1.25

Experimental[11]

3-Methylene-17α-methyl-5α-androstan-17β-ol (I).—A stirred mixture of 88 g. of methyltriphenylphosphonium bromide and 1.23 l. of dry ether was heated with 200 ml. of 1.115 *N* ethereal *n*-butyllithium solution in a nitrogen atmosphere. After stirring for 2 hr. at room temperature a solution of 15 g. of 17α-methyl-5α-androstan-17β-ol-3-one in 1.2 l. of dry tetrahydrofuran was added during 15 min. The reaction mixture was stirred for 4 hr. more and then kept standing at room temperature overnight. While removing the ether by distillation an equal amount of tetrahydrofuran was added. After being refluxed for 6 hr. the mixture was poured into 7.5 l. of water and extracted three times with 2.6-l. portions of ether. The combined extracts were washed with three portions of 1.5 l. of water, dried over sodium sulfate, and evaporated. The residue was dissolved in 200 ml. of ether. After addition of 200 ml. of petroleum ether the mixture was filtered. The solution was concentrated and chromatographed on 450 g. of alumina (active, basic, heated at 110° (1 mm.) and cooled in a nitrogen atmosphere). The eluted portions were monitored by thin layer chromatography (t.l.c.) and the pure fractions combined. The solvent was removed and the remainder crystallized from methanol. There was obtained 9.98 g. of I, m.p. 180–182°. Additional amounts of 2.06 g., m.p. 176–178°, and 0.53 g., m.p. 167–172°, were isolated from the mother liquor. Further recrystallization of the main product yielded 7.7 g. of I, m.p. 182–184°; $[\alpha]^{24}D$ −3°.

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.4; H, 11.3. Found: C, 83.4; H, 11.4.

3-Methylene-17α-methyl-5α-androst-1-en-17β-ol (II).—A solution of methylenetriphenylphosphorane was prepared from 175 g. of methyltriphenylphosphonium bromide and 360 ml. of 1.2 *N* *n*-butyllithium in a total of 2.86 l. of ether as described in the previous experiment, and allowed to react with 29.66 g. of 17α-methyl-5α-androst-1-en-17β-ol-3-one, worked up, and chromatographed in the same manner. II (17.97 g.) was obtained after crystallization from acetone, λ_{max} 235 mμ (ε 21,100) which yielded 14.4 g. of II, m.p. 109–110°; $[\alpha]^{23}D$ +10°; λ_{max} 234.5 mμ (ε 21,800), shoulders at 228 and 244 mμ, after an additional chromatography and recrystallization from acetone.

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.9; H, 10.7. Found: C, 84.1; H, 10.7.

3-Methylene-5α-androst-1-en-17β-ol.—A solution of 15.9 g. of 5α-androst-1-en-17β-ol-3-one in 1.4 l. of tetrahydrofuran was added to a solution of methylenetriphenylphosphorane prepared from 97 g. of methyltriphenylphosphonium bromide and 232 ml. of 1.1 *N* *n*-butyllithium in 1.8 l. of ether, made to react, and worked up as usual. Chromatography on 450 g. of basic nitrogen-treated alumina yielded 14.4 g. of product, m.p. 101–103° (petroleum ether); $[\alpha]^{24}D$ +31°; λ_{max} 234.5 mμ (ε 20,900), shoulders at 228 and 243 mμ.

17-Acetate: from petroleum ether, m.p. 85–86°; $[\alpha]^{24}D$ +34°; λ_{max} 234.5 mμ (ε 23,500), shoulders at 228 and 244 mμ.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.4; H, 9.8. Found: C, 80.1; H, 9.9.

3-Methylene-5α-androst-1-en-17-one.—Chromium trioxide (34.9 g.) was added to 356 ml. of dry pyridine portionwise at 10° under nitrogen. After addition of a solution of 15.8 g. of 3-methylene-5α-androst-1-en-17β-ol in 85 ml. of pyridine at 8° the reaction mixture was stirred for 3 hr. with cooling and then

(11) Melting points are corrected. Ultraviolet spectra were taken in 96% ethanol, and rotations are for chloroform solutions, except where stated otherwise. Analyses were carried out by Dr. M. Hochenegger, Analytical Laboratory of the E. Merck AG, Darmstadt.

left overnight at room temperature. The work-up was carried out with petroleum ether and water. The product melted at 100–103° (methanol); $[\alpha]^{25}D$ +130°; λ_{max} 234.5 (ϵ 22,900), shoulders at 228 and 244 mμ.

Anal. Calcd. for $C_{20}H_{28}O$: C, 84.5; H, 9.9. Found: C, 84.5; H, 10.1.

3-Methylene-17α-methyl-5α-androst-1-en-17β-ol (II).—3-Methylene-5α-androst-1-en-17-one (6.3 g.) was dissolved in 380 ml. of sodium-dried ether and treated with a solution of 2.44 g. of methyllithium in 270 ml. of ether within 20 min., while being cooled with ice. After stirring for 4 hr. at room temperature the reaction mixture was poured into 1 l. of iced water and extracted 3 times with 500-ml. portions of ether. The combined extracts were washed with 3 portions of 300 ml. of water, dried over sodium sulfate, and evaporated. The crude product, dissolved in petroleum ether–ether (4:3), was chromatographed on 180 g. of basic nitrogen-treated alumina. The first eluates contained 1.53 g. of starting material, the following (eluted with petroleum ether–ether (1:1)) 4.18 g. of II, m.p. 109–111° (acetone); $[\alpha]^{23}D$ + 10°; λ_{max} 235 mμ (ϵ 21,800), shoulders at 228 and 245 mμ. Infrared spectra and t.l.c proved to be identical with those of II obtained by Wittig reaction.

3-Methylene-17α-methylandrost-4-en-17β-ol (III).—The reaction of 21 g. of methyltriphenylphosphonium bromide and 48.7 ml. of 1.3 *N* phenyllithium solution in 350 ml. of dry ether resulted in methylenetriphenylphosphorane. To this was added a solution of 4.45 g. of 17α-methyltestosterone in 400 ml. of absolute tetrahydrofuran which was allowed to react and worked up as described above. The benzene solution of the crude product was chromatographed on basic alumina. The first fractions yielded 2 g. of III after recrystallization from methanol, m.p. 134–135°; $[\alpha]^{20}D$ +135.5°; λ_{max} 237–239 mμ (ϵ 27,600).

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.9; H, 10.7. Found: C, 83.7; H, 10.9.

3,17α-Dimethyl-5α-androst-2-en-17β-ol (IV).—3-Methylene-17α-methyl-5α-androstan-17β-ol (4.48 g.) was refluxed with 240 ml. of ethanol and 7.5 ml. of concentrated hydrochloric acid for 30 min. The cooled mixture was poured into 2.4 l. of water and extracted with chloroform. The organic phase was washed with water, dried, and evaporated. The crude product was chromatographed on 120 g. of basic alumina. Elution with petroleum ether–ether (2:1) yielded 700 mg. of a nonpolar side product; further elution with petroleum ether–ether (1:1) resulted in 3.4 g. of IV. After preparative t.l.c. and recrystallization from acetone the melting point was 146–148°; $[\alpha]^{24}D$ +34°.

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.4; H, 11.3. Found: C, 83.0; H, 11.3.

3,17α-Dimethylandrosta-3,5-dien-17β-ol (V).—A solution of 1.8 g. of 3-methylene-17α-methylandrost-4-en-17β-ol in 96 ml. of ethanol was treated with 3 ml. of concentrated hydrochloric acid. The mixture remained overnight at room temperature. It was poured into 800 ml. of water, the precipitate filtered, and recrystallized from methanol; 1.2 g. of V resulted, m.p. 148–150°; $[\alpha]^{20}D$ −184° (dioxane); λ_{max} 232 mμ (ϵ 21,700); λ_{max} 238.5 mμ (ϵ 23,100); λ_{max} 247 mμ (ϵ 15,000).

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.9; H, 10.7; 5.3. Found: C, 83.9; H, 10.8; O, 5.4.

3-Methylene-17α-ethylandrost-4-en-17β-ol (VI).—Methyltriphenylphosphonium bromide (21.3 g.) was allowed to react with 49.5 ml. of 1.3 *N* phenyllithium solution as usual and the resulting methylenetriphenylphosphorane was treated with 5 g. of 17α-ethyltestosterone. The crude product was chromatographed on basic alumina and eluted with benzene–chloroform. The first fractions gave 3 g. of VI after recrystallization from methanol, m.p. 88–89°; $[\alpha]^{20}D$ +144°; λ_{max} 237–239 mμ (ϵ 26,200).

Anal. Calcd. for $C_{22}H_{34}O$: C, 84.0; H, 10.9. Found: C, 83.7; H, 10.7.

9α-Fluoro-3-methylene-17α-methylandrost-4-en-11β,17β-diol (VII).—9α-Fluoro-17α-methylandrost-4-en-11β,17β-diol-3-one (5 g.) was treated with a solution of methylenetriphenylphosphorane prepared from 31.5 g. of methyltriphenylphosphonium bromide and 66 ml. of 1.0 *N* *n*-butyllithium in ether analogous to the preparation of I. The isolated crude material was chromatographed in benzene–chloroform on 150 g. of silica gel. First, a fluorine-free side-product (m.p. 127–132°) was eluted, then VII. The recrystallization of the latter product from aqueous ethanol yielded 1.3 g. of VII, m.p. 226–231°; $[\alpha]^{24}D$ +164°; λ_{max} 238 mμ (ϵ 23,600).

Anal. Calcd. for $C_{21}H_{31}FO_2$: C, 75.4; H, 9.3; F, 5.7. Found: C, 75.2; H, 9.5; F, 5.5.

3-Methylene-19-norandrost-4-en-17β-ol.—19-Nortestosterone (5 g.) was reacted with methylenetriphenylphosphorane in analogy to the preparation of I. The crude product was chromatographed in petroleum ether–ether (2:1) on 300 g. of alumina and recrystallized from methanol–water, which gave 4.2 g. of a material, m.p. 75–83°. Another 2 recrystallizations from methanol were followed by chromatography. Recrystallization of the product isolated from methanol under exclusion of oxygen yielded a pure sample, m.p. 103–105°; $[\alpha]^{21}D$ +147°; λ_{max} 237 mμ (ϵ 25,300).

Anal. Calcd. for $C_{19}H_{28}O$: C, 83.8; H, 10.4. Found: C, 83.5; H, 10.4.

3-Methylene-19-norandrost-4-en-17-one.—To a suspension of 47.1 g. of chromium trioxide in 480 ml. of dry pyridine a solution of chromatographed but noncrystalline 3-methylene-19-norandrost-4-en-17β-ol, prepared from 20 g. of 19-nortestosterone as described previously, was added dropwise at 10°. Stirring was continued for 3 hr. and the mixture left overnight at room temperature. Work-up was carried out with petroleum ether and water under nitrogen. After recrystallization from methanol 7.45 g. of product was obtained; m.p. 114–116°; $[\alpha]^{24}D$ +234°; λ_{max} 237–238 mμ (ϵ 25,200).

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.4; H, 9.7. Found: C, 83.9; H, 10.0.

3-Methylene-17α-methyl-19-norandrost-4-en-17β-ol (VIII).—3-Methylene-19-nor-androst-4-en-17-one (3.5 g.) was dissolved in 210 ml. of absolute ether and treated dropwise with a solution of 1.45 g. of methyllithium in 160 ml. of dry ether under nitrogen and external cooling with ice. After stirring for 3 hr. at room temperature the excess of methyllithium was decomposed by slow addition of 70 ml. of water. The crude product was chromatographed on 90 g. of neutral, nitrogen-treated alumina after the usual work-up with ether and water. Petroleum ether–ether (2:1) eluted 0.7 g. of starting material; ether eluted 2.63 g. of VIII, m.p. 103–105° after recrystallization from acetone. $[\alpha]^{24}D$ +110°; λ_{max} 237–238 mμ (ϵ 26,000).

Anal. Calcd. for $C_{20}H_{30}O$: C, 83.9; H, 10.6. Found: C, 84.1; H, 10.8.

3-Methylene-17α-ethyl-19-norandrost-4-en-17β-ol (IX).—The reaction of 5 g. of 17α-ethyl-19-nortestosterone with methylene triphenylphosphorane was effected as described under the preparation of I. The crude product was chromatographed on 150 g. of basic alumina and eluted with benzene. Recrystallization from ethanol–water gave 3.56 g. of IX, m.p. 123–125°; $[\alpha]^{24}D$ +109°; λ_{max} 238 mμ (ϵ 26,200).

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.9; H, 10.7. Found: C, 83.3; H, 10.8.