THE PREPARATION, PROOF OF STRUCTURE, ANTI-ESTROGENIC AND ANTI-ANDROGENIC ACTIVITY OF 17,17-DIMETHYL-$\Delta^{4,13}$-GONADIEN-3-ONE *

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Received January 25, 1963

Abstract

The acid-catalyzed dehydration and rearrangement of three 17β-hydroxy-17α-methyl steroids to 17,17-dimethyl-Δ^{13}-steroids is described. One of the rearranged compounds, 17,17-dimethyl-$\Delta^{4,13}$-gonadien-3-one (V), which had been previously unreported was examined in detail for hormonal and anti-hormonal activity. It proved to be less than 1/2000th as active as estrone by subcutaneous injection in the immature female mouse and showed significant anti-estrogenic activity when administered either by injection or gavage. It was also an effective anti-androgen in the castrated mouse on the basis of its ability to inhibit the testosterone stimulation of the seminal vesicle. The ring-A phenolic analog (VII) of V which had been previously reported[10] in the literature as a Δ^{16}-compound has been shown to be 17,17-dimethyl-1,3,5(10),13-gonatetraen-3-ol (VII), and its 3-methoxy derivative which had previously been prepared[7] only in an impure state has been obtained homogenous and characterized by several physical constants.

Introduction

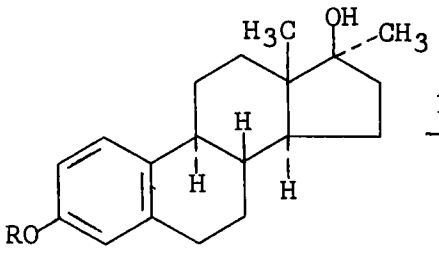
As part of an earlier study[1] of the isomerization of β,γ-unsaturated to α,β-unsaturated steroidal ketones, we had occasion to prepare 17α-methyl-19-nortestosterone (IV) and observed[1] that its formation[2] by acid-catalysis from 3-methoxy-17α-methyl-$\Delta^{2,5(10)}$-estradien-17β-ol (III) was accompanied by a previously

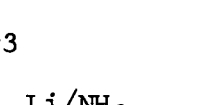
unrecognized byproduct which was chromatographically less polar than IV. This behavior suggested that elimination of the 17β-hydroxyl group had occurred.

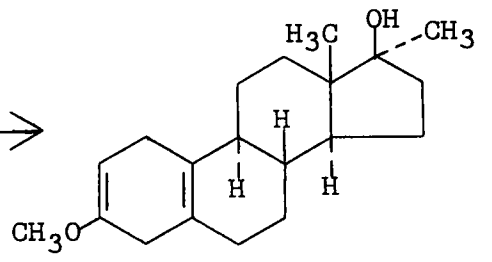
A consideration of the known[3] relationship of structure to biological activity of steroids reveals that hormonal behavior of the estrogenic and androgenic type is generally associated with an oxygen function at C-17, and removal of this group is very deleterious; thus, in the conversion of estrone to 17-desoxy estrone, estrogenic activity is reduced[4] to about one-third that of estriol. The possibility that our byproduct (V) was the desoxy derivative of (IV), which is a potent androgen[2] in the chick comb test and weakly androgenic in rats, prompted us to study its (V) preparation and structure as a prelude to determination of whether removal of the oxygenation at C-17 would reduce or abolish androgenicity. Furthermore, the concept of a close relative of a biologically active substance acting as a competitive inhibitor[5], or, in the lock-and-key analogy[5], of its acting as an "imperfect key" suggested to us that the byproduct might antagonize the effects of testosterone and/or perhaps of other hormonally active steroids.

Preparation and Structure

The enol ether (3-methoxy-17α-methyl-$\Delta^{2,5(10)}$-estradien-17β-ol, III) was prepared essentially as previously described[2] by Birch reduction of the methyl ether (I) of 17α-methylestradiol. When it (III) was submitted to acid (HCl) catalysis in refluxing methanol, the ratio of byproduct (V) to 17α-methyl-19-nortestosterone (IV) was time-dependent. After 15 min. of reaction, the

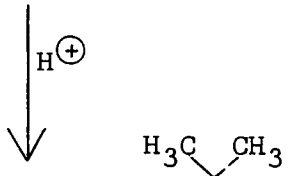


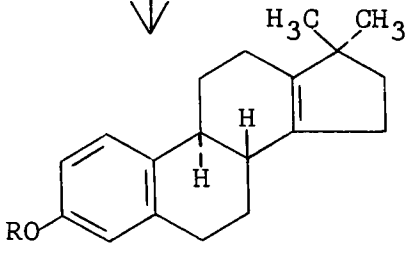




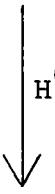
I, R = CH_3
II, R = H

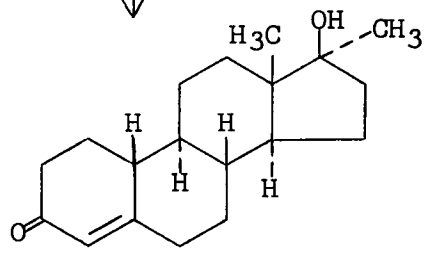
III



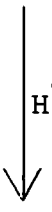


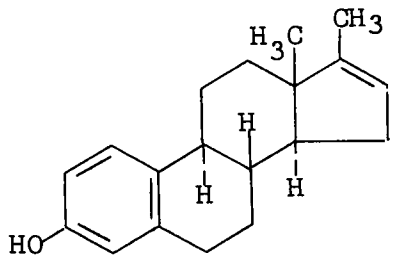
VI, R = CH_3
VII, R = H



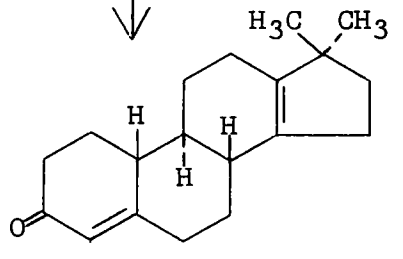


IV





VIII, R = CH_3
IX, R = H



V

ratio was 0.25, and after 90 min. it was 1.1. When the time was extended to 5.5 hrs., the amount of IV was insignificant, and the total weight of material in the chromatographic band corresponding to V was 92% of theory. Rechromatography and crystallization yielded pure V in 57% yield. Since IV was the major product after 15 min. of reaction and V the major product after 5.5 hrs., we believe the sequence of events must be the conversion of III to IV to V, or, in other words, dehydration and methyl migration must be slower than hydrolysis of the methoxyl group, ketonization, and migration of the $\Delta^{5(10)}$-bond into conjugation with the carbonyl group.

The structure of V follows from its physical properties and those of its analogs (VI and VII) bearing an aromatic ring-A which were prepared by direct acid-catalyzed rearrangement of I and II without interposition of reduction. The presence in the spectra of V of strong absorption maxima at 238 mμ and 5.95 μ proved the presence of a Δ^{4}-3-keto moiety. Absence of an sp^2-methyl band in the 8 tau region of the nuclear magnetic resonance spectra of all three rearranged compounds (V, VI, and VII) proved that simple dehydration had not occurred to give a Δ^{16}-compound, e.g., VIII or IX; in the case of V a positive test by the bromination procedure of Axelrod and Pulliam[6] which is diagnostic for ditertiary double bonds contraindicated the alternative $\Delta^{17(20)}$-structure and was in agreement with the presence of the fully substituted Δ^{13}-bond. The gemdimethyl grouping was apparent in a strong singlet at 9.0 tau in the nuclear magnetic spectrum of all three rearranged compounds, and the methoxyl group of VI consti-

tuted an internal reference for quantitation. The singlet (9.0 tau) for the gemdimethyl group with six hydrogen atoms should have possessed twice the integrated intensity of the singlet (6.23 tau) which arose from the methoxyl group with three hydrogen atoms. Experimentally the ratio was 2.03. Since the band at 9.0 tau was not a multiplet, both methyl groups must have been on carbon atoms which lacked a hydrogen atom, and this is not only consistent with a gemdimethyl group, but it is also inconsistent with an analog of VIII in which the Δ^{16}-bond has migrated leaving a hydrogen atom at C-17. These facts are interpretable only on the basis of structures in which C-18 has migrated to C-17.

There remains, however, one slight uncertainty, and this has to do with the position of the double bond resulting from dehydration. The most stable position should be one which is fully substituted, i.e., either the $\Delta^{8(14)}$- or Δ^{13}-arrangement, in the absence of resonance stabilization by conjugation. Of the two fully substituted positions, the $\Delta^{8(14)}$-arrangement would have to arise from the Δ^{13}-arrangement, but in the case of VI and VII a still more stable position should be the $\Delta^{8(9)}$-arrangement which allows conjugation with the aromatic nucleus. Now, if isomerization had occurred, we feel that for VI and VII the double bond would have proceeded all the way to the conjugated position. This did not happen, because the ultraviolet spectra of VI and VII showed only the benzenoid and not a styrene-type of absorption. Consequently, we assign the Δ^{13}-arrangement to VI and VII and by analogy to V.

The rearrangement of 17α-methylestradiol methyl ether (I) was

first achieved and, on the basis of dehydrogenation experiments of the analog in the equilenin series, interpreted in terms of methyl migration by Cohen, Cook and Hewett[7]. These investigators carried out the reaction at 160 - 165° in the presence of potassium hydrogen sulfate, and the product (VI) was obtained by distillation (0.2 mm. pressure) at an oil bath temperature of 190 - 200°. While their final sample was probably not homogeneous as indicated by an indefinite melting point ranging from 58° to 80°, our material (m.p. 73 - 75°) and theirs are presumably the same except for purity.

Subsequently by the use of formic acid at 100° Miescher and Kagi[8] achieved methyl migration of secondary 17-alcohols, e.g., of androstan-3β,17α-diol to the 17-methyl-$\Delta^{13(17)}$-derivatives, and various investigators, most recently Johns[9] whose paper also serves as a key to the literature, have exploited this rearrangement.

In 1958 Nicholas[10] introduced the technique of refluxing a tertiary 17-alcohol with ethanolic hydrogen chloride, but on the assumption that these conditions were too mild to induce methyl migration he assumed dehydration alone occurred. Thus, from 17α-methylestradiol (II) he obtained a compound to which he assigned the structure of a Δ^{16}-compound (IX). While he eliminated a $\Delta^{17(20)}$-structure on the basis of infrared data, no further information was presented in support of the Δ^{16}-assignment. We have precisely repeated the conditions of Nicholas[10] and have found that the product, as previously discussed, possesses a nuclear magnetic resonance spectrum in accord with the presence of a gemdimethyl group. We believe, therefore, that it is correct to reassign

structure VII to his compound. The products of hydrogenation[10] must then also be 17,17-dimethyl compounds rather than the 17α-methyl and 17β-methyl epimers which were suggested[10] on the basis of structure IX.

In agreement with our bioassay of V which showed that the gemdimethyl group at C-17 was inconsistent with estrogenic or androgenic behavior, Nicholas[10] found that VII possessed little or no estrogenic activity.

Experimental

All melting points were taken on a Kofler heating block. Ultraviolet spectra were determined on a Cary Model 14 recording spectrophotometer and infrared spectra on a Perkin-Elmer infracord. Rotations were measured at room temperature (ca. 21°) in 1% solutions on a Rudolph High Precision polarimeter equipped with a photoelectric cell. Nuclear magnetic resonance spectra were determined on a Varian Associates instrument, Model V4302, by Mr. T. Wittstruck working under the direction of Dr. N. McNiven. Combustion analyses were performed by Schwarzkopf Microanalytical Laboratories.

17,17-Dimethyl-4,13-Gonadien-3-one (V). - A solution of 2.8 g. of 3-methoxy-17α-methyl-2,5(10)-estradien-17β-ol (III), which was prepared from 17α-methylestradiol methyl ether (I) as previously described,[2] in 138 ml. of methanol and 83 ml. of 3 N hydrochloric acid was refluxed for 5.5 hrs. After being cooled, the solution was concentrated under reduced pressure and then diluted with water. The product was extracted into ether and the ether solution was washed (aq. $NaHCO_3$, H_2O), dried (anhydrous Na_2SO_4), and evaporated to dryness. The residue was chromatographed on 70 g. of silica gel. Crude 17,17-dimethyl-4,13-gonadien-3-one (V) which was

eluted with benzene containing 3% of ether weighed 2.3 g. (92%). While material obtained at this stage in other runs could be crystallized, it was found greater purity was obtained by an additional chromatography. Consequently, the 2.3 g. was rechromatographed on silica gel and yielded 2.0 g. of a cream colored solid which after crystallization from aq. ethanol yielded 1.5 g. of colorless elongated crystals; m.p. 91 - 93°; λ_{max}^{EtOH} 238 mμ (ε 16,000); $\lambda_{max}^{CS_2}$ 5.95 μ; $[\alpha]_D$ -12°; n.m.r. maxima in tau values at 4.133 and 9.042; calcd. for $C_{19}H_{26}O$: C, 84.44, H, 9.67; found: C, 84.73, H, 9.95.

When the reaction was carried out with 800 mg. of I for 15 min. and the products were chromatographed, 133 mg. of anhydro derivative (V) was eluted with 5% ether in benzene and 527 mg. of 17α-methyl-19-nortestosterone (IV) (after crystallization, m.p. 155 - 156°, lit.[2] 156 - 158°) was eluted with 2% methanol in benzene. A similar experiment with 90 min. of reaction yielded 329 mg. of V and 312 mg. of IV.

3-Methoxy-17,17-Dimethyl-1,3,5(10),13-Gonatetraene (VI). - A solution of 500 mg. of the 3-methyl ether (I) of 17α-methylestradiol in 30 ml. of methanol and 15 ml. of 3 N hydrochloric acid was refluxed for 15 min. After extraction and chromatography as described above, 101 mg. of crystalline VI was obtained by elution with benzene. Recrystallization twice from aqueous acetone gave a sample melting at 73 - 75°; λ_{max}^{EtOH} 278 and 287 mμ (ε 2,400 and 2,100); $[\alpha]_D$ -32° ($CHCl_3$); n.m.r. maxima in tau values at 2.833, 2.883, 3.259, 3.317, 3.423, 6.295, and 9.000; calcd. for $C_{20}H_{26}O$:

C, 85.05, H, 9.28; found: C, 85.39, H, 9.34.

When 2.0 g. of I was refluxed for 24 hrs. in 115 ml. of ethanol containing sufficient hydrochloric acid to bring the concentration to 1 N and the product was crystallized (without chromatography) once from acetone at -70° and three times from acetone-hexane, 560 mg. of VI, m.p. 73 - 75°, was obtained. The infrared spectrum was the same as that of the sample of VI described above.

17,17-Dimethyl-1,3,5(10),13-Gonatetraen-3-ol (VII). - A solution of 1.8 g. of 17α-methylestradiol (II) (m.p. 194.5 - 195.5°, lit.[11] 195 - 196°, prepared from estrone acetate by the Grignard reaction, hydrolysis and chromatography) in 100 ml. of ethanol containing sufficient (8.3 ml.) concentrated hydrochloric acid to make a 1 N solution was refluxed as described by Nicholas[10] for 24 hrs. After dilution of the reaction mixture with water, the product was extracted and chromatographed on silica gel (75 g.). Elution with 5% ether in benzene yielded 1.6 g. of material which was crystallized once from methanol-water and then once from acetone-water. The resulting crystals, after being dried in a vacuum, melted at 166.0 - 166.5°; lit.[10] 162 - 162.5°; λ_{max}^{EtOH} 279 mμ (ε 2,900); lit.[10] λ_{max} 280 mμ ($E_{1\ cm.}^{1\%}$ 80); $[\alpha]_D$ -37° ($CHCl_3$); lit.[10] $[\alpha]_D$ +36.6° ($CHCl_3$); n.m.r. maxima in tau values at 2.750, 2.880, 3.442, 3.480, 3.550, 3.590, 5.417 and 9.000. Our physical constants agree reasonably well with those of Nicholas[10] except for the sign of the specific rotation. However, our rotation is of the same sign as the corresponding methyl ether (VI, $[\alpha]_D$ -32°)

which would be expected from, for instance, the data[12] for estrone ($[\alpha]_D$ +165°) and its methyl ether ($[\alpha]_D$ +171°). In all probability the positive rotation[10] is a typographical error, because Nicholas[10] found that both the acetate and benzoate ($[\alpha]_D$ -66° and -74°) had markedly negative rotations. Since VII and estrone have the same molecular weight within 1%, the increment (+37°) in specific rotation for deacetylation of estrone (estrone acetate, $[\alpha]_D$ +128°)[12] can be applied to the acetate of VII. This yields a rotation of -29° for VII. We observed -37° which is considerably closer to the calculated value than is the reported[10] +36.6°.

Biological Activity

Methods:

Estrogenic activity of 17,17-dimethyl-4,13-gonadien-3-one (V) was studied by subcutaneous injection into the immature Swiss albino mouse using uterine growth as the end-point.[13] Anti-estrogen activity was assessed by the methods described by Dorfman et al.[14,15] The anti-estrogenic activity was evaluated by the ability of a compound to inhibit the uterotrophic activity of the estrone. Anti-androgenic activity was determined in the testosterone stimulated castrated mouse.[16] The end-points were the weights of the seminal vesicles and prostate.

Results and Conclusions:

Compound V was inactive in the estrogenic assay up to a total dose of 100 µg whereas 0.05 µg of estrone produced a significant increase in the uterine ratio. Thus, Compound V was less than 1/2000th as active as estrone, if active at all (Table 1). The compound, however, was anti-estrogenic when administered either by gavage or by injection. In the former test, as little as

TABLE 1

ESTROGENIC ACTIVITY OF COMPOUND V ADMINISTERED BY INJECTION

Exp.	Material Administered	Total Dose µg	No. of Mice	Mean Uterine Ratio[a] ± S.E.
A	0	0	15	0.76 ± 0.07
	Estrone	0.05	14	1.98 ± 0.11
		0.10	15	3.45 ± 0.16
		0.20	15	5.06 ± 0.22
	V	10	15	0.86 ± 0.04
		50	15	0.80 ± 0.04
		100	8	0.72 ± 0.05
B	0	0	10	1.50 ± 0.07
	Estrone	0.05	10	2.42 ± 0.24
		0.10	10	3.19 ± 0.29
		0.20	10	4.79 ± 0.34
	V	10	10	1.21 ± 0.08

a) Mg. of uterus per gram of body weight

TABLE 2

THE ANTI-ESTROGENIC ACTIVITY OF COMPOUND V

Route of Administration	No. of Mice	Dosage Range µg	Minimum Inhibitory Dose, µg	Maximum Inhibition %
Injection	125	5 - 1500	160	49
Gavage	93	25 - 2000	100	42

TABLE 3

THE ANTI-ANDROGENIC ACTIVITY OF COMPOUND V IN THE CASTRATED MOUSE

Exp. No.	TEST COMPOUND			No. of Mice	Mean Body Wt. g.	TISSUE RATIO[a] ± S. E.	
	Desig-nation	Total Dose mg.	Total Dose of Testos-terone mg.			Prostate	Seminal Vesicles
C	0	0	0	10	15	0.06 ± 0.008	0.22 ± 0.018
	0	0	0.8	10	16	0.19 ± 0.025	1.26 ± 0.091
	V	20	0.8	6	20	0.18 ± 0.013	0.73 ± 0.077
		40	0.8	7	17	0.17 ± 0.016	0.99 ± 0.069
D	0	0	0	11	17	0.10 ± 0.009	0.21 ± 0.031
	0	0	0.8	9	19	0.29 ± 0.022	1.01 ± 0.111
	V	20	0.8	9	18	0.33 ± 0.076	0.75 ± 0.085
		40	0.8	10	19	0.21 ± 0.036	0.62 ± 0.071
E	0	0	0	10	17	0.09 ± 0.008	0.17 ± 0.016
	0	0	0.8	8	19	0.15 ± 0.015	0.94 ± 0.134
	Proges-terone	40	0.8	9	18	0.20 ± 0.030	0.41 ± 0.049
	A-Nor-proges-terone	10	0.8	8	21	0.18 ± 0.015	0.94 ± 0.091
		20	0.8	9	17	0.15 ± 0.016	0.58 ± 0.034
		40	0.8	9	19	0.15 ± 0.009	0.67 ± 0.063
	I	40	0.8	9	18	0.19 ± 0.031	0.58 ± 0.030

a) Mg. of tissue per gram of body weight

100 μg of compound was effective, while by injection 160 μg was effective as an inhibitor of estrogenic action (Table 2).

Compound V in total doses of 20 and 40 mg. was anti-androgenic in three consecutive experiments. The results of Experiment C

clearly illustrate that significantly lower seminal vesicle ratios were found when Compound V at total doses of 20 to 40 mg. was administered together with 0.8 mg. of testosterone than when only the androgen was injected. The prostate ratio was not modified. The results of Experiments D and E confirmed those of Experiment C (Table 3). In Experiment D a dose of 20 mg. decreased the seminal vesicle ratio from 1.01 ± 0.111 to 0.75 ± 0.085 while the 40 mg. dose depressed the seminal vesicle ratio to 0.62 ± 0.071 which represented a depression in seminal vesicle inhibition of about 50%. A total dose of 40 mg. of compound in Experiment E produced a 47% inhibition. Progesterone at 40 mg. and A-norprogesterone were included for comparison in Experiment E. Significant inhibitions were found for the 40 mg. dose of the former compound and for the 20 and 40 mg. doses of the latter steroid.

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ACKNOWLEDGEMENTS

We should like to thank Dr. R. Jerussi for carrying out some large-scale preparations which allowed biological evaluation. The support of this investigation through research grants RG-6019 and CY-2193 of the National Institutes of Health is gratefully acknowledged.

FOOTNOTES

*The use of the symbol Δ in the paper is in keeping with the Tentative Recommendation of the meeting in Munich, 1959, of the Joint Subcommittee of the IUPAC Commission on Nomenclature of Organic Chemistry and of Biological Chemistry (see page 57 of Appendix B).