Tetrahedron, 1958, Vol. 3, pp. 43–48. Pergamon Press Ltd., London

ESTRANE DERIVATIVES—I*

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(*Received* 4 *October* 1957)

Abstract—Three of the four possible ring A/B isomeric estrane-17-ol-3-ones and their corresponding acetates, benzoates and diones were prepared for the purpose of biochemical studies. The positions of the hydrogens on C5 and C10 of these compounds have been assigned.

One of the estrane-3,17-diones prepared was, according to the double melting points, identical with one of the diones described by Marker and co-workers, obtained from non-pregnant urine source and and synthetic means.

SCHOELLER and associates[1] first accomplished the catalytic hydrogenation of estrone to a mixture of "octahydrofollicular hormones". They found this mixture of ring "A" saturated compounds, the estranes, to be active as androgens at 8–10 mg per capon unit. Such "A" ring hydrogenations were performed later by other investigators.[2–5]

Marker *et al.*[6] isolated two isomeric estranediols from human female non-pregnancy urine. One of the two isomers was identical with a compound prepared by hydrogenation of estrone in alcoholic solution over platinum.[5] On carrying out the hydrogenation in an acidic medium, Dirscherl obtained a third isomer.[4]

It seemed desirable to pursue the study of the estranediols and their derivatives further in regard to the influence of the angular methyl group C19 on their physiological properties, both in androgenic and in anabolic activities. The anabolic or myotrophic effects are of particular importance in view of recent biochemical developments.[7–9] From analogy with the metabolism of adrenal cortical hormones, androgenic hormones and progestrone, there are good reasons to believe that some metabolic products of 19-nortestosterone may be estranediols and derivatives. In the studies of the metabolism of estradiol-16-C^{14} in human female subjects, Beer and Gallagher[10,11] obtained up to 6 per cent "neutral fraction" from the total radioactivity of the urinary extract. It is possible that the "neutral fraction" contains ring "A" saturated compounds. Such a view would serve to explain the origin of the two estranediols isolated by Marker *et al.*[6]

Recently, it has been established that testosterone and cholesterol may be converted

* A part of the work described in this and subsequent papers was performed in 1940–1944 in Berlin, Germany, under the direction of Professor Walter Schoeller and in Basle, Switzerland, under the direction of Professor T. Reichstein.

The present work is in part supported by a grant-in-aid from the Atomic Energy Commission.

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biologically to estrogens.[12–14] No evidence is, however, available that such conversion is obligatory. Indeed, it may play only a relatively minor role in the biosynthesis of estrogens. It is then possible that estranes or their unsaturated counterparts are the precursors of estrogens.

In this paper three of the possible four isomeric series of estrane compounds that differ in the spatial arrangement at C5 and at C10 or rings A/B are presented.

Hydrogenation of estradiol with platinum catalyst was sluggish and caused extensive hydrogenolysis on one or both hydroxy groups. Raney nickel catalyst was therefore selected. Rapid hydrogenation of estradiol-17-acetate (I) in *cyclo*hexane solution took place at about 120°. The hydrogenation products were immediately oxidized and chromatographically separated through alumina. Two estrane-17-ol-3-one acetates (II and III) in about equal amounts were obtained. When the hydrogenation was performed in methanol solution, transesterification sometimes took place as evidenced by the isolation of two diones (VI and VII) corresponding to the two acetates.

It was thought, because of the probable thermodynamic stability of the 10 β-compounds, both of the estraneolone acetates II and III would be 10 β in orientation, but the huge difference in optical rotation of the two compounds indicated otherwise.

Hydrogenation of nortestosterone, which has the 10 β-configuration[15] or its esters (X), with palladium catalyst produced two estrane-17-ol-3-ones or their esters (III, XI, IX, and XVI). The acetate of the predominant isomer acetate was identical with III obtainable from the hydrogenation of estradiol-17-acetate. Compound III was assigned the 5 α-, 10 β-isomeric form as in the case of the catalytic hydrogenation of testosterone[16] and by contrast with the products of the catalytic hydrogenation of cholestenone.[17] The minor hydrogenation product (XI) was not identical with II and was designated to be the 5 β-, 10 β-isomer. The assigned steric configuration was supported by the slightly positive optical rotatory power of XI as compared to III, and by the thermodynamic consideration that the *trans–anti–trans* (5 α, 10 β) isomer has the lowest energy and should be favored in its formation.[18,19] It was thus established that II was a 10 α-isomer. The position of the hydrogen on C5 of II is tentatively assigned the α-position, again on the basis of thermodynamic.

The three estrane-17-ol-3-one acetates II, III and XI were hydrolyzed to their parent compounds, estraneolones (IV, V and XII); they were in turn benzoylated and oxidized to their benzoates (VIII, IX and XIV) and estranediones (VI, VII and XIII).

It may be noted that of the three estranedions prepared only one, VII, could be identified with one of the two diketones described by Marker and associates[5,6]

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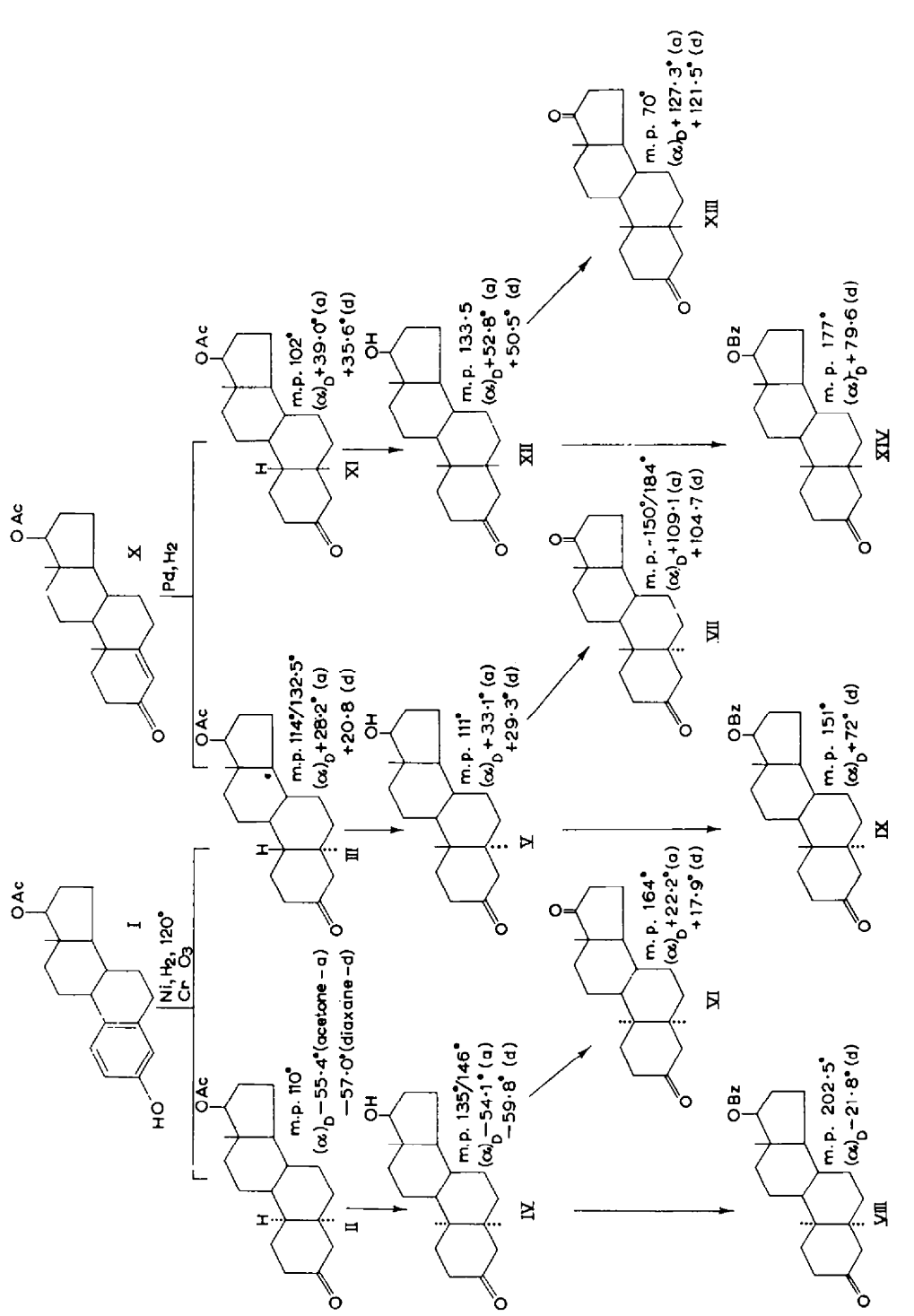
* A communication to the Editor has appeared since the preparation of this manuscript. A compound m.p. 130–132° (uncorrected), $[\alpha]_D$ + 60°, to which was assigned the structure V (5 α) and is apparently identical with our compound XII (5 β), m.p. 133·5–4°, $[\alpha]_D^{23.5}$ + 52·8° (acetone), $[\alpha]_D^{23.5}$ — 50·5° (dioxane).


OAc
HO
I
Ni, H2, 120°
Cr O3
m.p. 110°
(α)D −55·4° (acetone – a)
−57·0° (dioxane – d)
II
OAc
H
O
m.p. 135°/146°
(α)D −54·1° (a)
−59·8° (d)
IV
OH
X
Pd, H2
m.p. 114°/132·5°
(α)D +28·2° (a)
+20·8 (d)
III
m.p. 111°
(α)D +33·1° (a)
+29·3° (d)
V
m.p. 102°
(α)D +39·0° (a)
+35·6° (d)
XI
m.p. 133·5
(α)D +52·8° (a)
+50·5° (d)
XII
m.p. 164°
(α)D +22·2° (a)
+17·9° (d)
VI
m.p. ~150°/184°
(α)D +109·1 (a)
+104·7 (d)
VII
m.p. 70°
(α)D +127·3° (a)
+121·5° (d)
XIII
OBz
m.p. 151°
(α)D +72° (d)
IX
m.p. 177°
(α)D +79·6 (d)
XIV
m.p. 202·5°
(α)D −21·8° (d)
VIII

from non-pregnant urine source, as judged by its double melting points (lit. 144–6°/179–80°; found: ~150°/184–5°).

It should be emphasized here that our knowledge concerning the hormonal activities of steriods is limited to the 10 β-compounds. The synthesis of a 10 α-steroid may offer an opportunity for the investigation of spatial configuration and biological properties of this family of compounds.

EXPERIMENTAL*

Hydrogenation of estradiol-17-acetate (I).† Estradiol-17-acetate (1·62 g) in 50 ml *cyclo*hexane was hydrogenated for 4½ hr under 120 atmospheric pressure at 120° with 1 g of freshly prepared Raney nickel that had been washed carefully with methanol and *cyclo*hexane. After the solvent was removed, the glassy residue was oxidized with 250 mg chromic oxide in 37 ml glacial acetic acid pre-treated with chromic oxide. After standing at room temperature for 3 hr, all of the chromic acid was consumed. More chromic acid solution was added until there was an excess of chromic acid after 5 hr standing at room temperature. In all a total of 562 mg of chromic oxide was used. Three milliliters of methanol was added and the solution concentrated to dryness in vacuum at 30°. The residue was taken up in ether and washed with dilute sulfuric acid, sodium carbonate and water until neutral. After the ether solution was dried over anhydrous sodium sulfate and concentrated to dryness, 1·62 g glassy materal remained in the flask.

From an ether and pentane mixture, some crystals, m.p. 108–12°, could be obtained. However, the yield of the pure crystals was too small to be practical. The mixture was next subjected to chromatographic separation through 50 g aluminum oxide and developed with 150 ml portions of solvent or solvent mixture. From pentane–benzene (9 : 1) eluates, 508 mg of the first compound, estrane-17-ol-3-one acetate (II) was obtained. Rectangular or hexagonal crystals were obtained from ether and pentane mixture, m.p. 110–11°.

$[\alpha]_D^{23}$ −55·4°, M_D −176·3° (acetone); $[\alpha]_D^{23}$ −57·0°, M_D −181·5° (dioxane).

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75·43; H, 9·50. Found: C, 75·58; H, 9·66.

From the benzene eluate a second estrane-17-ol-3-one-acetate (III) appeared. After two recrystallizations from ether pentane mixture, 137 mg of fine needles were obtained, m.p. 132–4°. Occasionally, it gave a double melting point of 114–16°/132·5–134·5°.

$[\alpha]_D^{16}$ +28·2°, M_D +89·8° (acetone); $[\alpha]_D^{28·5}$ +20·8°, M_D +65·1° (dioxane).

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75·43; H, 9·50. Found: C, 75·05; H, 9·35.

Similar runs using methanol instead of *cyclo*hexane gave occasionally two diketones: m.p. 164° (VI) which appeared after II in the chromatogram and m.p. 184° (VII) which appeared after III in the chromatogram. Both compounds will be described later.

Estrane-17-ol-3-one (IV). Estrane-17-ol-3-one acetate (40 mg) (II, m.p. 110–11°) was hydrolyzed by refluxing for 45 min in a solution of 35 mg potassium hydroxide in 1 ml methanol. After the addition of 100 mg potassium bicarbonate in 2 ml water,

* The melting points were taken on a Kofler block and were corrected to ± 1·5;. The author is indebted to Dr. E. Thommen, Basle, Switzerland, for the microanalysis, as well as to Mr. Terry Yamauchi and Mrs. Bojana Kabalin for their technical assistance.

† The author wishes to express his appreciation for the assistance rendered by Professor Herman Pines of this University in the high pressure hydrogenation of one batch of the material in his laboratory.

the methanol present was removed in vacuum. The residue was extracted with ether. The ether extract was washed to neutral with water, dried over anhydrous sodium sulfate and concentrated to dryness, yielding 36 mg crystalline mass. Recrystallization from ether–pentane mixture gave 29 mg granular crystals, m.p. 135–36°/146–7·5°. $[\alpha]_D^{23}$ −54·1°, M_D −150·0° (acetone); $[\alpha]_D^{23\cdot5}$ −59·8°, M_D 165·3° −(dioxane).

Anal. Calcd. for $C_{18}H_{28}O_2$: C, 78·20; H, 10·20. Found: C, 78·22; H, 10·05.

Estranedione (VI). Estrane-17-ol-3-one (19 mg) (IV m.p. 133–34°) was dissolved in 0·35 ml purified glacial acetic acid and treated with 7 mg chromic oxide in 0·35 ml acetic acid for 14 hr at room temperature. After the reaction mixture was concentrated to dryness in vacuum at 30°, the residue was taken up in ether and washed with sulfuric acid, sodium carbonate and water until neutral, dried over anhydrous sodium sulfate and concentrated to dryness. Residue of 16 mg was obtained which crystallized from ether : pentane mixture as leaflets, m.p. 162–64°. The admixture of this product and the compound previously isolated from the mixture of oxidized hydrogenation products gave no depression. Upon further recrystallization, the m.p. rose to 164–66°. $[\alpha]_D^{13}$ +22·2°, M_D +60·9° (acetone); $[\alpha]_D^{23}$ +17·9°, M_D +49·1° (dioxane).

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 78·79; H, 9·55. Found: C, 78·70; H, 9·62.

Estrane-17-ol-3-one (V). Compound III (480 mg) (m.p. 129°) was hydrolyzed in similar manner as in VI, yielded 465 mg material, which upon two recrystallizations from acetone–hexane gave 420 mg silky crystals that melted at 111–12°. $[\alpha]_D^{23}$ + 33·1° M_D +91·4°; $[\alpha]_D^{23\cdot5}$ +29·3°, M_D +81·0°.

Anal. Calcd. for $C_{18}H_{28}O_2$: C, 78·20; H, 10·20. Found: C, 78·10; H, 10·21.

Estrane-3, 17-dione (VII). Estrane-17-ol-3-one (60 mg) (V, m.p. 111°) was oxidized as in VI and yielded, after recrystallizations from *n*-hexane, 40 mg rod-shaped crystals, m.p. 184–85·5° which underwent, first a change of crystal form and sometimes actually melted at about 150°. $[\alpha]_D^{17}$ +109·1°, M_D +299·36° (acetone); $[\alpha]_D^{23}$ +104·7° M_D +287·2° (dioxane).

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 78·79; H, 9·55. Found: C, 78·79; H, 9·70.

Estrane-17-ol-3-one benzoate (VIII). Estrane-17-ol-3-one (55 mg) (IV, m.p. 133°) was reacted with 0·1 ml benzoyl chloride and 0·5 ml pyridine at room temperature for 16 hr. One milliliter of water was added and the solution kept at room temperature for ½ hr. It was then taken up in ether and washed with 2 N hydrochloric acid, 2 N sodium carbonate and water until neutral. The semi-oily material was chromatographed on 1·5 g alumina. All the *n*-hexane eluates were combined and recrystallized from acetone. Oblong or rectangular crystals thus obtained (37 mg) melted at 201–3°. For analysis the crystals were repeatedly recrystallized from methylene chloride–methanol and from acetone–hexane, irregular prisms, m.p. 204·5–6°, sweating began at 201°. $[\alpha]_D^{25}$ −21·8°, M_D −82·95° (dioxane).

Anal. Calcd. for $C_{25}H_{32}O_3$: C, 78·91; H, 8·48. Found: C, 79·13; H, 8·67.

Estrane-17-ol-3-one benzoate (IX). Estrane-17-ol-3-one (65 mg) (V, m.p. 109–11°) was reacted with benzoyl chloride and pyridine in the same manner as in VIII. The gummy material obtained was subjected to chromatogram. Rod-shaped crystals obtained from an alumina column eluted with *n*-hexane–benzene (8 : 2) were combined

* The author wishes to thank Dr. John Babcock of the Upjohn Company for a generous gift of this material.

and recrystallized twice from acetone–*n*-hexane mixture; 50 mg, m.p. 151–53·5°, $[\alpha]_D^{26}$ +71·95°, M_D +273·77° (dioxane).

Anal. Calcd. for $C_{25}H_{32}O_3$: C, 78·91; H, 8·48. Found: C, 79·00; H, 8·71

Estrane-17-*ol*-3-*one acetates* (III *and* XI). A 1·0 g sample of nortestosterone* was hydrogenated in 10 ml ethanol with 50 mg of palladium catalyst on charcoal at room temperature and slightly above atmospheric pressure. The hydrogenation was completed in 1 hr. After the removal of the catalyst and solvent, the glassy material was acetylated with pyridine and acetic anhydride at room temperature for 18 hr. The resulting acetate mixture was worked up in the usual manner. The oily residue was allowed to crystallize from a *n*-hexane solution. Repeated recrystallization from the same solvent afforded 619 mg estraneolone acetate (III), m.p. 114–15°/ 132·5–4·5°. The admixture of this compound with III, previously described, gave no melting point depression.

The mother-liquor was chromatographed once through 15·0 g alumina. The benzene–*n*-hexane (1 : 9) eluate, weighing 210 mg, was rechromatographed through 7·0 g alumina. From the benzene–*n*-hexane (1 : 9) eluate, 58 mg oblong crystals of estraneolone acetate (XI) were obtained. It was recrystallized once from *n*-pentane, m.p. 102–3°. The oblong crystals changed to fine needles at about 90°. $[\alpha]_D^{23·5}$ + 39·0°, M_D +121·7° (acetone); $[\alpha]_D^{24}$ +35·6°, M_D +111·3° (dioxane).

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75·43; H, 9·50. Found: C, 75·15; H, 9·47.

Estrane-17-*ol*-3-*one benzoates* (IX *and* XIV). Hydrogenation of a 378 mg sample of nortestosterone benzoate, m.p. 178–9°, in glacial acetic acid with palladium catalyst, yielded 46 mg estraneolone benzoate (XIV). It crystallized in clusters of needles from acetone–*n*-hexane solution, m.p. 177–8·5°. $[\alpha]_D^{23·5}$ +79·6°, M_D +302·8° (dioxane).

Anal. Calcd. for $C_{25}H_{32}O_3$: C, 78·91; H, 8·48. Found: C, 78·77; H, 8·64.

From the same hydrogenation mixture, 194 mg estraneolone benzoate (IX) was obtained.

Similar hydrogenation of nortestosterone acetate (X) instead of nortestosterone benzoate gave approximately the same quantity of 5 α (III)- and 5 β (XI)-isomers.

Estrane-17-*ol*-3-*one* (XII). Both estraneolone acetate XI and estraneolone benzoate XIV gave, upon hydrolysis with sodium hydroxide in methanol, the free compound. Upon recrystallization from acetone–*n*-hexane mixture, hexagonal crystals were obtained, m.p. 133·5–4°. $[\alpha]_D^{23·5}$ +52·8°, M_D +146·0° (acetone); $[\alpha]_D^{23·5}$ +50·5°, M_D +139·5° (dioxane).

Anal. Calcd. for $C_{18}H_{28}O_2$: C, 78·20; H, 10·20. Found: C, 78·22; H, 10·05.

Estrane-3, 17-*dione* (XIII). Sample of estraneolone (XII) (48 mg) was oxidized with chromic acid in acetic acid as in VI. This gave an oily material, which upon chromatographic purification yielded clusters of needles. Two recrystallizations from *n*-pentane gave 22 mg pure crystals, m.p. 70–70·5°. $[\alpha]_D^{23·5}$ +127·3°, M_D +349·2° (acetone); $[\alpha]_D^{23·5}$ +121·5°, M_D +333·4° (dioxane).

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 78·79; H, 9·55. Found: C, 78·83; H, 9·49.