[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA]

INVESTIGATIONS ON STEROIDS. XX. 6β- AND 6α-ACETOXY- AND HYDROXY-DERIVATIVES OF PROGESTERONE AND ANDROSTENEDIONE[1]*

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In a recent publication (1) the dehydration of 6β,21-diacetoxyallopregnane-5-ol-3,20-dione with dry hydrogen chloride was discussed. When carried out in carbon tetrachloride or in chloroform completely freed of alcohol, the reaction proceeded with retention of configuration at carbon atom 6. Thus 6β,21-diacetoxy-Δ^4-pregnene-3,20-dione was obtained and was smoothly deacetylated to 6β-hydroxy-11-desoxycorticosterone. In ordinary chloroform, containing about 0.7% of alcohol, inversion simultaneously took place at carbon atom 6 leading to the thermodynamically more stable 6α,21-diacetoxy- Δ^4-pregnene-3,20-dione which was deacetylated to 6α-hydroxy-11-desoxycorticosterone. It was further demonstrated that the 6β-acetoxy-α,β-unsaturated ketone could be readily epimerized to the 6α-acetoxy compound by treatment with dry hydrogen chloride in chloroform containing a small amount of alcohol. To explain this epimerization, the formation of an intermediary 6-acetoxy- $\Delta^{3,5}$-diene-3-ol was postulated.

The mentioned publication (1) not only presented interesting findings concerning the epimerization at carbon atom 6, but by chance established a link with biochemical experiments conducted in other laboratories. It was found (2, 3) that hog as well as beef adrenal brei contains an enzyme system capable of introducing a hydroxyl group not only in the β-position at carbon atom 11, but to some degree also in the β-position at carbon atom 6. Thus 11-desoxycorticosterone was biochemically oxidized mainly to corticosterone and, to a small extent, also to 6β-hydroxy-11-desoxycorticosterone. The identity of the latter was established (1, 2) by comparison of the infrared spectrum of its diacetate with that of an authentic sample of synthetic origin. It is considered likely that the bio-oxidation of other 3-keto- Δ^4-unsaturated steroids (*i.e.* structures of hormone type), by the action of the enzyme systems of adrenal glands and

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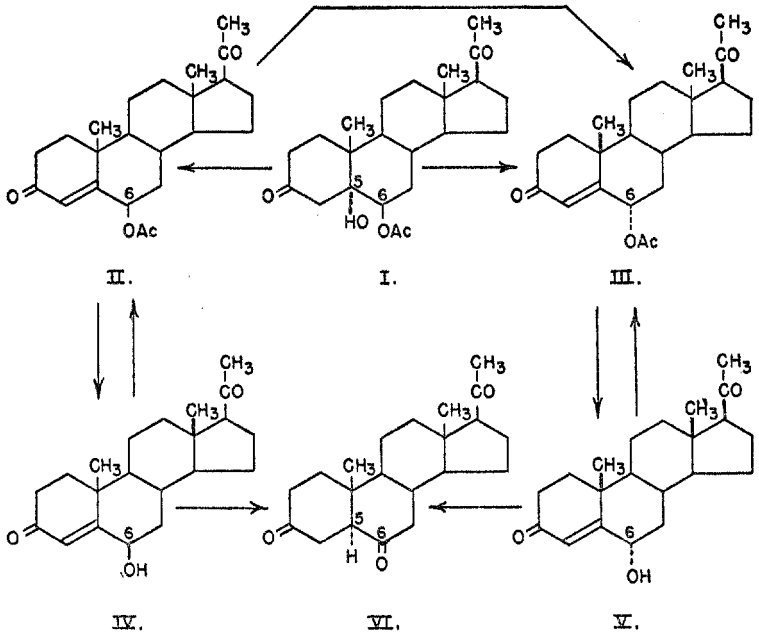
* The findings of this paper were presented on July 22, 1952 at the 2nd International Congress of Biochemistry in Paris (*cf.* M. R. Ehrenstein, "6-Hydroxy Derivatives of Steroid Hormones. Their Stereochemistry and Possible Biochemical Significance", *Symposium sur la Biochimie des Stéroides*, p. 13. *II^e Congrès International de Biochemie*. Société d'Edition d'Enseignement Supérieur, Paris-5^e, 1952).

[2] Predoctoral Fellow (1949–1951) of the American Cancer Society, Inc., upon recommendation of the Committee on Growth of the National Research Council. This paper is based on a section of a thesis submitted by Charles P. Balant to the Graduate School of Arts and Sciences of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiological Chemistry (June 1952).

possibly also of micro-organisms (*cf.* 4, 5), will lead in part to compounds hydroxylated in the 6-position. In order to facilitate the identification of such bio-oxidation products, the preparation of 6-hydroxy derivatives of various types of steroid hormones by way of the respective 6-acetoxy compounds was indicated. This appeared desirable also for theoretical reasons dealing with the general applicability of the epimerization reaction.

As was reported from this laboratory earlier (6), dehydration of 6β-acetoxyallopregnane-5-ol-3,20-dione (I) with dry hydrogen chloride in "alcohol-free chloroform" led to a crystalline product interpreted[3] as 6β-acetoxyprogesterone (II) (7). On repeating the experiment in "redistilled dried chloroform," an amorphous α,β-unsaturated ketone of the same composition was obtained which resisted all attempts at crystallization. It was considered an "amorphous



modification" of the same compound (8). That the slight difference in the solvent would account for this discrepancy, was not evident at that time. In the light of the present knowledge (1), it became necessary to reinvestigate this matter and, at the same time, to supplement the experimental data.

When I was treated with dry hydrogen chloride in dry ethanol-free chloroform, a crystalline α,β-unsaturated ketone resulted, which was assigned the structure of 6β-acetoxyprogesterone (II). The physical constants of this compound are in good agreement with those of the crystalline product described earlier (6). The same crystalline compound (II) was also obtained when a suspension of I in carbon tetrachloride was treated with dry hydrogen chloride.[4]

[3] Originally (6) this compound was considered to represent the 6 α-epimer.

[4] On refluxing I with glacial acetic acid for ¾ hour, a 50% yield of II and 30% of unchanged I were obtained after chromatography (Experiment by Mr. Klaus G. Florey).

Saponification of II with potassium hydroxide (1.1 equiv.) in absolute ethanol at room temperature gave the crystalline 6β-hydroxyprogesterone (IV) which by acetylation was reconverted into II. When the dehydration of I was performed in a solution of chloroform containing 0.7% of ethanol, an amorphous α,β-unsaturated ketone resulted which resisted all attempts at crystallization even after chromatography. It was recognized as 6α-acetoxyprogesterone (III). The amorphous product also resulted when crystalline II was treated with dry hydrogen chloride in a solution of chloroform containing 0.7% of ethanol. Saponification of the amorphous III with potassium hydroxide in methanol gave the crystalline 6α-hydroxyprogesterone (V) which by acetylation could be reconverted into the amorphous III. It follows from this investigation that the previously described crystalline (6) and amorphous (8) 6-acetoxyprogesterone are not two modifications of the same compound, but represent the 6β- and the 6α-epimers respectively.

In accordance with expectations[5] heating II in methanol-water (1:2) with 2.2 equivalents of potassium hydroxide for one hour gave no IV, but exclusively allopregnane-3,6,20-trione (VI) (6, 9). The latter compound (VI) was obtained also by rearrangement of V with glacial acetic acid in the presence of a small amount of sulfuric acid.[6]

COMPOUND	M.P., °C.	$[\alpha]_D$	M_D	$M_D^\beta - M_D^\alpha$	λ_{max}^{alc}	ϵ_{max}
6β-Acetoxyprogesterone (II)	147–148	+100.9°	+376	−141	235	13,970
6α-Acetoxyprogesterone (III)	amorphous	+138.8°	+517		235.5	14,910
6β-Hydroxyprogesterone (IV)	178–179	+106.8°	+353	−142	235.5	12,390
6α-Hydroxyprogesterone (V)	192–193	+149.9°	+495		240	15,570

As was stated in an earlier publication (6, p. 323), the compound now recognized as 6β-acetoxyprogesterone (II) is approximately one-third as active as progesterone (Corner-Allen test). On the other hand, the compound now assigned the structure of 6α-acetoxyprogesterone (III) possesses one-tenth or less of the activity of progesterone.[7,7a]

In 1941 there was described (10) the dehydration of 6β-acetoxyandrostane-5-ol-3,17-dione (VII) with dry hydrogen chloride in a solution of supposedly "alcohol-free redistilled chloroform." The resulting product, melting at 174–176°

[5] *Cf.* the analogous case (1, pp. 1053, 1059).

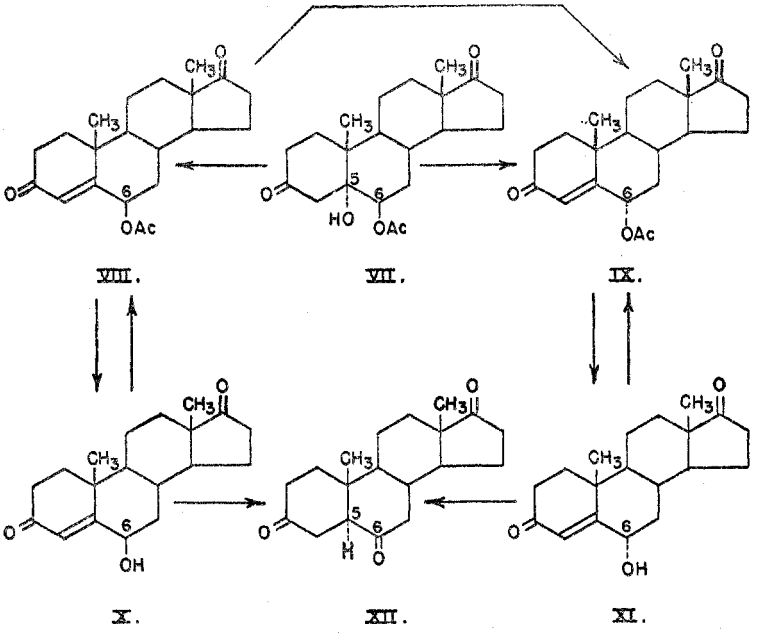
[6] *Cf.* the analogous case (1, pp. 1054, 1060).

[7] According to findings of Drs. George W. Corner and S. Reynolds in 1942 which had remained unpublished.

[7a] Addition to proof, Dec. 2, 1952. Recently II and III were simultaneously reassayed (Corner-Allen test) by Drs. Willard M. Allen and Arthur Haskins of the Department of Obstetrics and Gynecology, Washington University School of Medicine, Saint Louis, Missouri. The tests were done on sexually mature castrated rabbits. On a dosage level of 7.0 mg. both compounds failed to produce proliferation. (1 mg. of progesterone gives a positive response.) Thus II appeared less active than in the bioassays conducted in 1940 (6). Tests on higher dosage levels are under way.

was originally (10) assigned the structure of 6α-acetoxy- Δ⁴-androstene-3,17-dione (IX), but was later (7) interpreted to be 6β-acetoxy- Δ⁴-androstene-3,17-dione (VIII). Depending on the character of the solvent, one would expect the formation of two epimers. Therefore a reinvestigation of this reaction was undertaken.

On dehydrating VII with dry hydrogen chloride in alcohol-free chloroform or in carbon tetrachloride, an α,β-unsaturated ketone with a melting point of 201–201.5° was obtained. In analogy with the cases discussed above, it was assigned the structure VIII. Treatment of this compound with potassium hydroxide in absolute ethanol yielded 6β-hydroxy- Δ⁴-androstene-3,17-dione (X) which in turn could be reacetylated to VIII. When VII was dehydrated



with dry hydrogen chloride in chloroform containing a small amount of alcohol, a product melting at 176.5–177.5° resulted which was identical with the compound obtained previously. In spite of the statement to the contrary, the chloroform in the previous experiment (10, p. 641) must have contained some alcohol. Therefore, the resulting product was IX. The same compound was also obtained by treatment of VIII with dry hydrogen chloride in chloroform containing 0.7% of ethanol. Deacetylation of IX with methanolic potassium hydroxide gave 6α-hydroxy- Δ⁴-androstene-3,17-dione (XI) which could be reacetylated to IX.

In analogy with the experiments in the pregnane series (*vide supra*), heating of VIII with 2.2 equivalents of potassium hydroxide in methanol-water (3:25) yielded androstane-3,6,17-trione (XII) (11, 12). The same compound (XII)

was obtained also by rearrangement of XI with glacial acetic acid in the presence of small amounts of sulfuric acid and of water.

COMPOUND	M.P., °C.	$[\alpha]_D$	M_D	$M_D^\beta - M_D^\alpha$	λ_{max}^{alc}	ϵ_{max}
6β-Acetoxy-Δ⁴-androstene-3,17-dione (VIII)	201–201.5	+110.7°	+381	−185	235	12,480
6α-Acetoxy-Δ⁴-androstene-3,17-dione (IX)	176.5–177.5	+164.2°	+566		235	15,520
6β-Hydroxy-Δ⁴-androstene-3,17-dione (X)	193.5–194.5	+109.2°	+330	−220	235.5	13,550
6α-Hydroxy-Δ⁴-androstene-3,17-dione (XI)	229–230	+181.9°	+550		239.5	16,170

The epimeric 6-acetoxy compounds (VIII and IX) as well as the free 6-hydroxy compounds (X and XI) have been assayed for androgenic activity in the laboratory of Ralph I. Dorfman of the Worcester Foundation for Experimental Biology. Male white chicks (11 animals in each series), 2 to 3 days old, were injected with 0.05 cc. of oil solution daily for 7 days. The compounds did not produce any androgenic effect $\left(\text{comb ratio} = \frac{\text{comb wt.}}{\text{body wt.}}\right)$ at total dosage levels of 150 μg. (VIII and IX) and 300 μg. (X and XI) respectively.

It is noteworthy that previous tests in capons both by injection adn inunction showed that the compound now known to be IX exhibited one-fifth the activity of androsterone (10, p. 630).

The investigations on compounds of the described type will be continued.[8]

EXPERIMENTAL

The melting points were determined with the Fisher-Johns melting point apparatus. The readings are sufficiently near the true melting points so that no corrections have been made. Unless stated otherwise, the microanalyses were carried out by Dr. E. W. D. Huffman, Denver 2, Colorado, on samples which were dried *in vacuo* over phosphorus pentoxide at 80–90°.

SECTION I

6β-Acetoxyprogesterone [6β-acetoxy-Δ⁴-pregnene-3,20-dione] (II). *A. From 6β-acetoxyallopregnane-5-ol-3,20-dione* (I)[9] *by the action of dry hydrogen chloride in ethanol-free chloroform.* Through a solution of 100 mg. of I in 25 cc. of ethanol-free, dry chloroform[10] was passed a moderate stream of dry hydrogen chloride for a period of two hours. The temperature was kept between −2 to +2°, except during the last 20 minutes when the reaction mixture was

[8] The preparation of 6β,17α-dihydroxy-11-desoxycorticosterone 6,21-diacetate (m.p. 191–192°. $[\alpha]_D^{20}$ + 62.1° in chloroform; $\lambda_{max}^{alc.}$ 235 mμ; ε 13,300) has been completed and will be published separately (with Klaus G. Florey). Confirmatory observations have also been made in the cholestenone series (Louis F. Fieser, private communication).

[9] Prepared from pregnenolone as described previously (6, 8). This material was kindly provided by Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J. and by Dr. C. R. Scholz of Ciba Pharmaceutical Products, Summit, N. J.

[10] Chloroform Baker Reagent, shaken four times with conc'd sulfuric acid, washed to neutrality with water, dried over calcium chloride, and distilled.

allowed to assume room temperature. After pouring into 30 cc. of ice-cold water, the colorless chloroform layer was washed with cold *N* sodium bicarbonate and with water. After drying over sodium sulfate and evaporating the solvent, a resin resulted which crystallized from ether-petroleum ether; yield: 67.3 mg. of colorless plates, m.p. 140–141°. Recrystallization from ether gave 49.7 mg. of m.p. 147–148°. $[\alpha]_D^{32}$ +100.9° (20.0 mg. in 2 cc. of chloroform; l, 2 dm.; α +2.02°). λ_{max}^{alc} 235mμ; ϵ 13,970. [Lit. (6): m.p. 145–146°. $[\alpha]_D^{17.5}$ +89.7° (ethanol). λ_{max}^{alc} 232 mμ; ϵ 14,000].

B. From 6β-acetoxyallopregnane-5-ol-3,20-dione (I)[9] *by the action of dry hydrogen chloride in carbon tetrachloride.* Through a suspension of 200 mg. of I in 70 cc. of purified carbon tetrachloride (13, p. 365) was passed (temp. between −5 and +5°) a moderate current of dry hydrogen chloride for a period of 1½ hours. The material dissolved quickly during the course of the reaction. The subsequent treatment was the same as under *A*. From the crude reaction product there resulted by crystallization from ether-petroleum ether 20.7 mg. of m.p. 140–141°; identical with II, as obtained under *A* (mixture m.p.). The mother liquor yielded additional II after chromatography on aluminum oxide [activity III–IV (14)] and elution by petroleum ether-benzene 1:9 and by benzene alone.

C. By acetylation of 6β-hydroxyprogesterone (IV). To 15 mg. of IV (*vide infra*) in 0.3 cc. of dry pyridine was added 0.15 cc. of acetic anhydride. After keeping the mixture at room temperature for 17 hours and subsequently pouring it into 10% sulfuric acid, the resulting white crystalline precipitate was recrystallized from acetone-petroleum ether, yielding 9.2 mg. of crystals, m.p. 141–142°; no depression of m.p. when mixed with a sample of II as obtained under *A*.

The crystals of 6β-acetoxyprogesterone, in contrast with those of 6β-hydroxy-11-desoxycorticosterone 6,21-diacetate (1, p. 1057) and of 6β-acetoxy-Δ⁴-androstene-3,17-dione (VIII) (*vide infra*), do not turn yellow upon exposure to light. On adding conc'd sulfuric acid, a green fluorescence develops slowly in the yellow solution.

6β-Hydroxyprogesterone [*Δ⁴-pregnene-6β-ol-3,20-dione*] (IV) *from 6β-acetoxyprogesterone* (II). To 80 mg. of II in 3.0 cc. of absolute ethanol was added in the cold 12.0 mg. of potassium hydroxide in 1.2 cc. of absolute ethanol (approx. 1.1 equiv.). The mixture was kept at room temperature for 20 minutes and was then made neutral to litmus by the addition of 10% sulfuric acid in the cold. After evaporating to dryness *in vacuo* at room temperature, the crystalline residue was dissolved in ethyl acetate and a little water. After washing with water and drying over sodium sulfate, the organic phase yielded a crystalline residue. Recrystallization from acetone-petroleum ether gave 47.2 mg. of clusters of thin colorless needles; m.p. 178–179°; no change of m.p. after another recrystallization. $[\alpha]_D^{28}$ +106.8° (19.2 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +2.05°). λ_{max}^{alc} 235.5 mμ; ϵ 12,390.

Anal. Calc'd for $C_{21}H_{30}O_3$ (330.45); C, 76.32; H, 9.15.

Found:[11] C, 76.57; H, 9.17.

On adding conc'd sulfuric acid to this substance, a green fluorescence develops slowly in the yellow solution.

6α-Acetoxyprogesterone [*6α-acetoxy-Δ⁴-pregnene-3,20-dione*] (III). *A. From 6β-acetoxyallopregnane-5-ol-3,20-dione* (I)[9] *by the action of dry hydrogen chloride in chloroform containing 0.7% of ethanol.* Through a solution of 150 mg. of I in 40 cc. of dry chloroform[10] to which 0.25 cc. of ethanol had been added was passed a moderate stream of dry hydrogen chloride at a temperature of approx. −5° for a period of 1½ hours. After pouring the reaction mixture into cold water, the solution was washed with ice-cold *N* sodium bicarbonate and with water. After drying over sodium sulfate and removal of the solvent *in vacuo*, 140.5 mg. of resin was obtained. A second experiment was performed with 150 mg. of I under similar conditions (temp. during the reaction approx. +5°). The final material (136.5 mg.) was likewise resinous. The product resulting from both experiments (*i.e.* from 300 mg. of I) was chromatographed over 12 g. of aluminum oxide [activity III (14); length of column: 11 cm.]. This yielded two major fractions: (a) 146.0 mg. of resin, eluted with

[11] As a precautionary measure special drying (15) was performed though it proved unnecessary.

petroleum ether-benzene 1:4. This material resisted all attempts at crystallization. It represented III, as was proven by its deacetylation to the crystalline 6α-hydroxyprogesterone (V) (*vide infra*). (b) 41.7 mg. of resin, eluted with benzene-ether 9:1. This could be crystallized and was identified as II.

B. By rearrangement of 6β-acetoxyprogesterone (II) *by the action of dry hydrogen chloride in chloroform containing 0.7% of ethanol.* Through a solution of 20 mg. of II in 25 cc. of chloroform[10] to which 0.2 cc. of ethanol had been added was passed a moderate stream of dry hydrogen chloride for one hour (temperature below −5°). After processing the reaction mixture as under *A*, 22.8 mg. of resin was obtained which could not be crystallized, representing crude III. Saponification with methanolic potassium hydroxide gave crystalline 6α-hydroxyprogesterone (V) (*vide infra*), identified by mixture m.p. determination.

C. By acetylation of 6α-hydroxyprogesterone (V). To 16.3 mg. of V (*vide infra*) in 0.3 cc. of dry pyridine was added 0.15 cc. of acetic anhydride and the mixture was kept at room temperature for 19 hours. After bringing it to dryness *in vacuo* at room temperature, the residue was dissolved in ethyl acetate and the solution was washed to neutrality with *N* hydrochloric acid, *N* sodium bicarbonate, and water. After drying over sodium sulfate and evaporating to dryness, 21.8 mg. of resin resulted which was chromatographed over 3 g. of aluminum oxide [activity II–III (14); length of column: 11 cm.]. Several consecutive resinous fractions, amounting to 9.5 mg., were eluted with a total of 700 cc. of petroleum ether-benzene 4:6. They resisted all attempts at crystallization and were finally pooled to be used for the determination of the optical constants. $[\alpha]_D^{28.5}$ +138.8° (6.7 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +0.93°). λ_{max}^{alc} 235.5 mμ; ε 14,910. [Lit. (8): $[\alpha]_D^{22.5}$ +106.7° (ethanol). λ_{max}^{alc} 233 mμ; ε 16,000].

6α-Hydroxyprogesterone [*Δ⁴-pregnene-6α-ol-3,20-dione*] (V) *from 6α-acetoxyprogesterone* (III). To 115.9 mg. of the amorphous acetate III [preced. expt. *A*, chromatographic fraction (a)] in 2 cc. of methanol was added 1.9 cc. of a 1% solution of potassium hydroxide in methanol. The deep yellow solution was kept at room temperature for 2¾ hours and, after the addition of 30 cc. of saturated sodium chloride solution, was extracted repeatedly with ethyl acetate. After washing the extract with water, drying over sodium sulfate, and evaporating to dryness, 89.5 mg. of resin was obtained. Crystallization was achieved by dissolving the residue in a small volume of acetone and slowly adding petroleum ether. A total of 70.1 mg. of colorless needles resulted in three successive crops; melting points between 184 and 188.5°. Recrystallization from aqueous methanol and again from acetone-petroleum ether raised the melting point to 192–193°. $[\alpha]_D^{27}$ +149.9° (15.0 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +2.25°). λ_{max}^{alc} 240 mμ; ε 15,570.

Anal. Calc'd for $C_{21}H_{30}O_3$ (330.45): C, 76.32; H, 9.15.

Found:[12] C, 76.19; H, 9.14.

Upon adding conc'd sulfuric acid to this compound, a green fluorescence develops slowly in the yellow solution.

Allopregnane-3,6,20-trione (VI). *A. From 6β-acetoxyprogesterone* (II). To 160 mg. of II in 10 cc. of methanol was successively added 53 mg. of potassium hydroxide (approx. 2.2 equiv.) in 5 cc. of methanol and 30 cc. of water. After heating the yellow mixture on the water-bath for one hour and subsequently saturating it with sodium chloride, it was extracted with chloroform and the extract was washed to neutrality, dried over sodium sulfate, and evaporated to dryness. Crystallization of the residue from acetone-petroleum ether gave 86.6 mg. of thin, colorless needles, m.p. 226–227°. This material showed no absorption of ultraviolet light between 222 and 300 mμ. A second crop of 22.5 mg. of crystals with slightly lower m.p. was obtained from the mother liquor. Recrystallization of the first crop from acetone-petroleum ether raised the m.p. to 232.5–233°. $[\alpha]_D^{28}$ +61.0° (20.0 mg. in 2.0 cc. of chloform; l, 2 dm.; α +1.22°) [Lit. (9): m.p. 227–230.5°. $[\alpha]_D^{23}$ +52.9° (dioxane)].

B. From 6α-hydroxyprogesterone (V). To 22 mg. of V in 1.0 cc. of glacial acetic acid was added 0.2 cc. of 3% sulfuric acid. After keeping the mixture at room temperature for 44 hours, it was neutralized with *N* sodium carbonate. The resulting white precipitate was

[12] Special drying (15) was essential.

filtered, dried, and recrystallized from ether-petroleum ether. Yield: 13.8 mg. of small thin needles; m.p. 223–227.5°; no depression of m.p. when mixed with sample of V as obtained under *A*.

SECTION II

6β-Acetoxy-Δ⁴-androstene-3,17-dione (VIII). *A. From 6β-acetoxyandrostane-5-ol-3, 17-dione* (VII)[13] *by the action of dry hydrogen chloride in ethanol-free chloroform.* Through a solution of 748 mg. of VII in 85 cc. of ethanol-free chloroform[10] was passed at −10° a moderate stream of hydrogen chloride for 2¾ hours. After washing the solution to neutrality with cold *N* sodium bicarbonate and with water, it was dried over sodium sulfate and the solvent was removed *in vacuo*, leaving a white crystalline residue. This was recrystallized by dissolving it in a small amount of acetone and adding some ether and much petroleum ether. A first crop of 588.6 mg. of large colorless plates, melting between 177 and 193°, was obtained. Two subsequent recrystallizations from acetone-petroleum ether yielded 372.9 mg. of constant m.p. 201–201.5°. There was no depression of the m.p. upon admixture with a sample of VIII as obtained under *B* (*vide infra*).

B. From 6β-acetoxyandrostane-5-ol-3,17-dione (VII)[13] *by the action of dry hydrogen chloride in carbon tetrachloride.* Through a solution of 105 mg. of VII in 50 cc. of purified carbon tetrachloride (13, p. 365) was passed at approx. −15° a moderate stream of dry hydrogen chloride for two hours. By processing the reaction mixture as under *A*, a first crop of crystals (46.2 mg.) of m.p. 190–193° was obtained. Additional material with a slightly lower m.p. resulted from the mother liquor. After recrystallization of the first crop from acetone-petroleum ether, the m.p. was 199–120°. $[\alpha]_D^{27}$ +110.7° (20.0 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +2.21°). λ_{max}^{alc} 235 mμ; ε 12,480.

Anal. Calc'd for $C_{21}H_{28}O_4$ (344.43): C, 73.23; H, 8.19.

Found: C, 73.29; H, 8.31.

The crystals of VIII turn deep yellow upon exposure to direct sunlight. The color change is not reversible in the dark,[14] but recrystallization gives the colorless material back. On adding to VIII conc'd sulfuric acid, a green fluorescence develops slowly in the yellow solution.

C. By acetylation of 6β-hydroxy-Δ⁴-androstene-3,17-dione [*Δ⁴-androstene-6β-ol-3,17-dione*] (X). To 15.5 mg. of X (*vide infra*) in 0.3 cc. of pyridine was added 0.15 cc. of acetic anhydride. The mixture was kept at room temperature for 17 hours and was then treated with four times its volume of 10% sulfuric acid. After standing for one-half hour in the refrigerator, the crystalline precipitate was filtered, washed, dried, and recrystallized from acetone-petroleum ether. Yield: 8.9 mg. of crystals, m.p. 194–195°; no depression of m.p. when mixed with sample of VIII as obtained under *B* (*vide supra*).

6β-Hydroxy-Δ⁴-androstene-3,17-dione [*Δ⁴-androstene-6β-ol-3,17-dione*) (X) *from 6β-acetoxy-Δ⁴-androstene-3,17-dione* (VIII). To 70 mg. of VIII in 10 cc. of absol. ethanol was added 12.5 mg. of potassium hydroxide (approx. 1.1 equiv.) in 6 cc. of absol. ethanol and the mixture was kept at room temperature for 35 minutes. After making it neutral to litmus by adding 10% sulfuric acid in the cold, the alcohol was removed *in vacuo* at room temperature. The residue, which crystallized upon the addition of a little water, was dissolved in ethyl acetate and the extract was washed with water and with a saturated sodium chloride solution. After drying over sodium sulfate and evaporating the solvent, the crystalline residue was recrystallized from acetone-petroleum ether. A first crop of 44.3 mg. of colorless plates was obtained, m.p. 190–191°. From the mother liquor there resulted additional material with a somewhat lower m.p. Renewed recrystallization of the first crop raised the m.p. to 193.5–194.5°. $[\alpha]_D^{28}$ +109.2 (19.4 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +2.12°). λ_{max}^{alc} 235.5 mμ; ε 13,550.

[13] Prepared from dehydroepiandrosterone as described previously (10). This material was kindly donated by Dr. C. R. Scholz of Ciba Pharmaceutical Products, Summit, N. J.

[14] The color change in the analogous case of 6β-hydroxy-11-desoxycorticosterone 6,21-diacetate (1, p. 1057) is reversible.

Anal. Calc'd for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67.
Found:[11] C, 75.47 H, 8.66.

Upon dissolving a sample of this substance in conc'd sulfuric acid, a green fluorescence develops slowly in the yellow solution.

6α-Acetoxy-Δ⁴-androstene-3,17-dione (IX). *A. From 6β-acetoxyandrostane-5-ol-3,17-dione* (VII)[18] *by the action of dry hydrogen chloride in chloroform containing ethanol.* Through a solution of 150 mg. of VII in 30 cc. of dry chloroform[10] to which 1 cc. of ethanol had been added was passed a moderate stream of dry hydrogen chloride at approx. 0° for a period of two hours. After the first ten minutes, a light greenish coloration was observed which persisted until the end. The color disappeared on pouring the reaction mixture into cold water. After washing the chloroform layer neutral with *N* sodium bicarbonate and drying over sodium sulfate, evaporation of the chloroform yielded 143.7 mg. of resin. Crystallization from very little acetone and much petroleum ether gave 76.6 mg. of clusters of thin, colorless needles; m.p. 172–173°. Additional crystalline material (11.3 mg.) with somewhat lower m.p. resulted from the mother liquor. Repeated recrystallizations from the same solvents and also from aqueous methanol raised the m.p. to 176.5–177.5°. $[\alpha]_D^{32}$ +164.2° (20.0 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +3.28°). λ_{max}^{alc} 235 mμ; ϵ 15,520. [Lit. (10): m.p. 174–176°. $[\alpha]_D^{26.7}$ +153.5° (acetone). λ_{max}^{alc} 235 mμ; ϵ 16,020].

B. By rearrangement of 6β-acetoxy-Δ⁴-androstene-3,17-dione (VIII) *by the action of dry hydrogen chloride in chloroform containing 0.7% of ethanol.* A sample of 46 mg. of VIII was dissolved in 50 cc. of chloroform[10] to which 0.35 cc. of ethanol had been added previously. The conditions of this experiment as well as the subsequent manipulations were otherwise the same as under *A*. The product of the reaction, when dissolved in a minute amount of acetone to which petroleum ether was subsequently added, crystallized upon seeding with a sample of IX (preced. expt.). A total of 31.0 mg. of crystalline material with melting points between 173 and 176° was obtained. The m.p. of the first crop (23.4 mg.) was 175.5–176° and was not depressed upon admixture with a sample of IX as obtained under *A*.

C. By acetylation of 6α-hydroxy-Δ⁴-androstene-3,17-dione [*Δ⁴-androstene-6α-ol-3,17-dione*] (XI). To 15 mg. of XI (*vide infra*) in 0.3 cc. of dry pyridine was added 0.15 cc. of acetic anhydride. After keeping the solution at room temperature for 18 hours and diluting it with three times its volume of cold 10% sulfuric acid, the resulting crystalline precipitate was recrystallized from acetone-petroleum ether. Yield: 9.4 mg. of crystals; m.p. 176–177°. There was no depression of the m.p. when mixed with a sample of IX (*cf.* expt. under *A*).

6α-Hydroxy-Δ⁴-androstene-3,17-dione [*Δ⁴-androstene-6α-ol-3,17-dione*] (XI) *from 6α-acetoxy-Δ⁴-androstene-3,17-dione* (IX). To 100 mg. of IX in 2 cc. of methanol was added 1.8 cc. of 1% methanolic potassium hydroxide (approx. 1.1 equiv.). The mixture was kept at room temperature for 2¾ hours and, after the addition of a saturated sodium chloride solution, was extracted repeatedly with ethyl acetate. After washing the solvent with a saturated sodium chloride solution and drying over sodium sulfate, evaporation to dryness gave a white crystalline residue. This yielded from acetone-petroleum ether 68.7 mg. of colorless needles; m.p. 225–228°. Repeated recrystallization raised the m.p. to 229–230°. $[\alpha]_D^{27}$ +181.9° (21.1 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +3.84°). λ_{max}^{alc} 239.5 mμ; ϵ 16,170.

Anal. Calc'd for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67.
Found: C, 75.33; H, 8.73.

Androstane-3,6-17-trione (XII). *A. From 6β-acetoxy-Δ⁴-androstene-3,17-dione* (VIII). To 100 mg. of VIII in 3 cc. of methanol were successively added 36 mg. of potassium hydroxide (approx. 2.2 equiv.) in 3 cc. of methanol and 50 cc. of water. After heating the yellow mixture on the water-bath for one hour and then saturating it with sodium chloride, it was extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The white crystalline residue was recrystallized from acetone-petroleum ether, yielding 58.0 mg. of long thin colorless needles, m.p. 190–191°. After renewed crystallization from the same solvents the m.p. was 191.5–192.5°. This material did not absorb ultraviolet light between 222 and 300 mμ. The m.p. was depressed more than 40° when mixed with a sample of X. $[\alpha]_D^{25}$ +71.0° (20.0 mg. in 2.0 cc. of chloroform; l, 2 dm.; α +1.42°) [Lit. (12): m.p. 191–192°].

B. From 6α-hydroxy-Δ⁴-androstene-3,17-dione [*Δ⁴-androstene-6α-ol-3,17-dione*] (IX). To 40 mg. of IX in 4 cc. of glacial acetic acid was added 0.25 cc. of 10% sulfuric acid and 0.5 cc. of water. The mixture was kept at room temperature for 44 hours and was then made slightly alkaline by the addition of a solution of sodium carbonate. After extracting repeatedly with ethyl acetate, the extract was washed with water, dried over sodium sulfate, and freed from solvent. The residue was crystallized from acetone-petroleum ether, yielding 21.2 mg. of crystalline material; m.p. 171–175°. After two additional crystallizations from the same pair of solvents, 12.1 mg. of thin colorless needles was obtained. The m.p. was 188–189.5° and was not depressed upon admixture with a sample of XII (*cf.* preced. expt.).

SUMMARY

1. Dehydration of 6β-acetoxyallopregnane-5-ol-3,20-dione (I) furnished 6β-acetoxyprogesterone (II) (m.p. 147–148°) which was rearranged to the thermodynamically more stable 6α-acetoxyprogesterone (III) (amorphous). The previously described crystalline (6, 7) and amorphous (8, 7) 6-acetoxyprogesterone represent the 6β- and the 6α-epimers respectively. Saponification of II and III gave 6β-hydroxyprogesterone (IV) (m.p. 178–179°) and 6α-hydroxyprogesterone (V) (m.p. 192–193°) respectively. Both II and V were rearranged to allopregnane-3,6,20-trione (VI). The progestational activities of I and II have been discussed.

2. Dehydration of 6β-acetoxyandrostane-5-ol-3,17-dione (VII) gave 6β-acetoxy- Δ⁴-androstene-3,17-dione (VIII) (m.p. 201–201.5°) which was rearranged to the thermodynamically more stable 6α-acetoxy- Δ⁴-androstene-3,17-dione (IX) (m.p. 176.5–177.5°). The latter compound had been obtained previously (10, 7), though its true configuration at carbon atom 6 had not been recognized. Saponification of VIII and IX furnished 6β-hydroxy- Δ⁴-androstene-3,17-dione (X) (m.p. 193.5–194.5°) and 6α-hydroxy- Δ⁴-androstene-3,17-dione (XI) (m.p. 229–230°) respectively. Both VIII and XI were rearranged to androstane-3,6,17-trione (XII). The androgenic activities of VIII, IX, X, and XI have been discussed.

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