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

INVESTIGATIONS ON STEROIDS. XXV. 19-OXOPROGESTERONE AND 19-OXO-11-DESOXYCORTICOSTERONE*,[1]

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The synthesis, starting from strophanthidin, of 19-hydroxyprogesterone (III) and 19-hydroxy-11-desoxycorticosterone (VII) has been described (1, 2) and the spatial similarity of VII to corticosterone has been pointed out (2). The present paper is concerned with the extension of that synthesis to the preparation of 19-oxoprogesterone (IV) and 19-oxo-11-desoxycorticosterone (VIII).

In a brief investigation of methods for the oxidation of the 19-hydroxyl group, ethyl 19-hydroxy-3-oxo-Δ^4-etienate (I) (3, 4) served as the model. Ethyl 3,19-dioxo-Δ^4-etienate (II) was prepared successfully by oxidation of I with sodium dichromate dihydrate in glacial acetic acid as well as with chromic anhydride in 95% acetic acid or in glacial acetic acid. The best yield of II was obtained with the last combination. Surprisingly, compound I was not oxidized by one equivalent of chromic anhydride in either pyridine[2] or tertiary butanol.

Proceeding from this study, 19-hydroxyprogesterone (III) and 19-hydroxy-11-desoxycorticosterone 21-monoacetate (V) were oxidized with one equivalent of chromic anhydride in glacial acetic acid, yielding, respectively, 19-oxoprogesterone (IV) and 19-oxo-11-desoxycorticosterone acetate (VI). Hydrolysis with potassium bicarbonate of VI gave the free 19-oxo-11-desoxycorticosterone (VIII), the structure of which was confirmed by reacetylation to give VI.

The infrared studies pertaining to this paper were carried out on a Perkin-Elmer Model 21 double beam spectrometer in the Division of Steroid Metabolism of the Sloan-Kettering Institute for Cancer Research through the courtesy of Dr. Thomas F. Gallagher. The interpretation was done by Friederike Herling. The correlations are based upon those summarized in the publication of Jones and Herling (6). Only those bands are mentioned which appear to have a direct bearing upon the structure of the particular compound. Details of other correlations between spectrum and structure will be summarized at a later time by the group at the Sloan-Kettering Institute.

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[1] The findings of this paper were incorporated in reports given on July 22, 1955, at the XIVth International Congress of Pure and Applied Chemistry Covering Organic Chemistry in Zürich (*cf.* Maximilian R. Ehrenstein, "Analogs of Steroid Hormones Oxygenated in the 19-Position", Congress Handbook, p. 173 [No. 264]) and on August 5, 1955, at the 3rd International Congress of Biochemistry in Brussels (*cf.* Maximilian R. Ehrenstein, G. Winston Barber, and Max Dünnenberger, "Analogs of Steroid Hormones Oxygenated in the 19-Position and Their Biological Significance" Résumés des Communications, p. 4 [No. 1–26]).

[2] *Cf.* the apparently contrary finding in the case of 19-hydroxy-Δ^4-androstene-3,17-dione (5).

The infrared spectrum of 19-oxoprogesterone (IV)[3] was determined in chloroform and in carbon tetrachloride solution. There is no hydroxyl absorption. Carbonyl absorption at 1717 cm^{-1} ($CHCl_3$) suggests the presence of the 19-oxo group as influenced by the α,β-unsaturated system, absorption at 1703 cm^{-1} is due to the 20-ketone group, and bands at 1673 and 1618 cm^{-1} ($CHCl_3$) are due to the Δ^4-3-ketone system. The band at 1673 cm^{-1} is higher than the expected value for a Δ^4-3-ketone (1665 cm^{-1} in $CHCl_3$ solution). This shift seems to be due to the influence of the 19-oxo group. Absorption at 1421 cm^{-1} (CCl_4) is due to C—H scissoring vibration of the unsubstituted methylene group at C_2 next to the 3-ketone. There are C—H bending bands at 1387 cm^{-1} due to the angular C_{18}-methyl group, and at 1356 cm^{-1} (CCl_4) due to the C_{21}-methyl group (in 20-keto steroids).

The spectrum of 19-oxo-11-desoxycorticosterone (VIII)[3] was determined in chloroform solution. There are O—H stretching bands at 3490 cm^{-1}, which are due to an associated hydroxyl group. The carbonyl region shows a broad band at 1717–1708 cm^{-1}, which is due to the presence of the 19-oxo group and the 20-ketone group, and bands at 1673 and 1619 cm^{-1} are due to the Δ^4-3-ketone system.

19-Oxo-11-desoxycorticosterone acetate (VI) was measured in both chloroform and carbon tetrachloride solution. Weak O—H stretching bands indicate the presence of a trace of impurity, possibly VIII. The spectrum shows carbonyl absorption at 1748 and 1724 cm^{-1} ($CHCl_3$) which is evidence for a 21-acetoxy-20-ketone group. The intensity of the band at 1724 cm^{-1} is increased with reference to the 1748 cm^{-1} band. This can be interpreted as indicative of the presence of the 19-oxo group. Bands at 1673 and 1619 cm^{-1} ($CHCl_3$) are due to the Δ^4-3-ketone system. There are C—H scissoring bands at 1421 and 1408 cm^{-1} (CCl_4) which are probably due to the unsubstituted methylene groups at C_2 adjoining the 3-ketone group, and at C_{21} in 21-acetoxy-20-ketones. C—H bending vibration at 1390 cm^{-1} is due to the C_{18}-methyl group and at 1373 cm^{-1} (CCl_4) is due to the acetate methyl group.

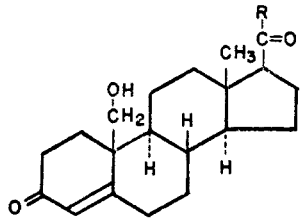
The previous paper (2) reports molecular rotation data in support of the then unverified assignment of normal configurations at positions 14 and 17 in III and VII. Subsequent to that report, the synthetic 19-hydroxy-11-desoxycorticosterone (VII) was found to be identical with a compound obtained by the perfusion of progesterone through bovine adrenal glands (7), as well as with a substance resulting from incubating 11-desoxycorticosterone with a twice washed homogenate residue of beef adrenals (8).[3a, b] Furthermore, the identity of synthetic VII with a substance isolated from beef adrenal extract has been established (9). These findings tend to support the assignment of configurations in III and VII. Further confirmation has been obtained in this laboratory by

[3] The fingerprint region of the spectrum of this compound is different from that of any steroid in the collection of the Sloan-Kettering Institute.

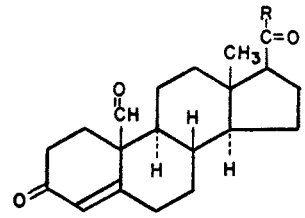
[3a] Addition, June 6, 1955. The C-19 hydroxylation of 11-desoxycorticosterone by incubation of this steroid with beef adrenal breis has just been described by Zaffaroni, *et al.* (22). The yield in the conversion to 19-hydroxy-11-desoxycorticosterone ranged from 3.5 to 5%. The view is expressed that this may be of considerable significance in connection with the production of estrogens by the adrenal gland, the introduction of the 19-hydroxyl group being the first step in the oxidative removal of the C-19 methyl group prerequisite to aromatization of ring A. Similar interpretations have recently been advanced by Wettstein and co-workers (23, p. 1242).—Addition, Sept. 10, 1955. The conversion of 19-hydroxy-Δ^4-androstene-3,17-dione to estrone by endocrine tissue has just been demonstrated (24).

[3b] Addition, June 6, 1955. Dr. A. Wettstein (Basel) informed us that his group obtained appreciable quantities of 19-hydroxy-11-desoxycorticosterone by aerobic incubation of 11-desoxycorticosterone with adrenal homogenates (adrenal breis or supernatant solution of centrifugates) (23).

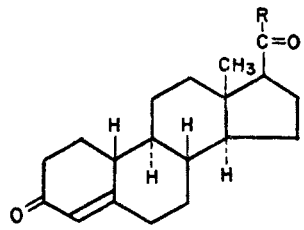
conversion of III to 19-norprogesterone (IX) with normal configurations at all centers of asymmetry.



I. R = OC_2H_5
III. R = CH_3
V. R = CH_2OAc
VII. R = CH_2OH



II. R = OC_2H_5
IV. R = CH_3
VI. R = CH_2OAc
VIII. R = CH_2OH



IX. R = CH_3
X. R = CH_2OH

Since 19-hydroxyprogesterone (III) is a vinyl analog of a primary β-ketol which cannot dehydrate easily, treatment of III with base might be expected to cause elimination of formaldehyde, yielding IX.[4] When III was allowed to stand with aqueous alcoholic potassium hydroxide, there was obtained, in addition to unchanged III, a very small amount of IX. The infrared spectrum of IX (CS_2) showed carbonyl absorption at 1706 cm^{-1} (20-ketone group) and 1676 cm^{-1} (carbonyl group of the Δ^4-3-ketone system). The fingerprint region was identical with the spectrum of authentic 19-norprogesterone (10). This identity establishes the normal (α) configuration at position 14 in all compounds of this series. The normal (β) configuration at position 17 follows from the previously (2) cited molecular rotation data.

19-Oxoprogesterone (IV) was examined for progestational activity by Dr. Roy Hertz of the National Cancer Institute. The Corner-Allen test was negative with 1.0 mg. of IV in two rabbits. In this test a maximal effect is obtained with 1.0 mg. of progesterone. On the other hand, in the Clauberg test 2.5 mg. of IV produced a plus two reaction in each of two rabbits. This degree of response could have been expected from as little as 250 micrograms of progesterone. Thus

[4] The same reaction was successfully carried out with 19-hydroxy-Δ^4-androstene-3,17-dione, yielding 19-nor-Δ^4-androstene-3,17-dione (5).

19-oxoprogesterone (IV) produces 10% of the progestational activity of progesterone.

In bioassays conducted by Drs. John A. Luetscher, Jr., and Robert H. Curtis, Stanford University School of Medicine (*cf.* 11, 12), the effects of 19-oxo-11-desoxycorticosterone (VIII) on the sodium excretion and on the potassium-sodium ratio were, respectively, 50% and 40% of those produced by DOCA. In preliminary liver glycogen deposition tests performed by Drs. R. O. Stafford and L. E. Barnes of the Research Division of the Upjohn Company (*cf.* 13), 19-oxo-11-desoxycorticosterone (VIII) was found to be practically inactive.[5]

It is of interest to compare the relative activities of this series of compounds. The 19-nor compounds have the highest activity and are followed in decreasing order by the parent hormones, the 19-oxo derivatives and finally the 19-hydroxy derivatives which show little or no activity.[6] Thus, using progesterone as standard, the progestational activity of 19-norprogesterone (IX) (10) is four to eight times greater (16); the activity of 19-oxoprogesterone (IV), as reported in this paper, is reduced to one-tenth, whereas 19-hydroxyprogesterone (III) (2) is practically inactive. Similarly, with 11-desoxycorticosterone acetate (DOCA) as standard,[7] the sodium-retaining activity of 19-nor-11-desoxycorticosterone (X) (18) is approximately twice as great (18, *cf.* also 19), the potency of 19-oxo-11-desoxycorticosterone (VIII), as reported in this paper, is one-half, while 19-hydroxy-11-desoxycorticosterone (VII) (2) is almost inactive (4%) (2, *cf.* also 19).

The synthesis of 19-hydroxy and 19-oxo analogs of other steroid hormones is in progress in this laboratory.

EXPERIMENTAL

Melting points were determined with the Fisher-Johns melting point apparatus and are uncorrected. Ultraviolet spectra were determined in 95% ethanol with a Beckman Model DU spectrophotometer. Microanalyses were performed by Dr. E. W. D. Huffman, Wheatridge, Colorado, on samples which were dried to constant weight *in vacuo* (P_2O_5; 80°) according to Milner and Sherman (20). The percentage loss of weight on drying is recorded. To determine the optical rotation, the sample was dissolved in chloroform to make 2 cc. of solution and the rotation was measured in a 2-dm. semi-micro tube. The alumina and silica gel used as adsorbents for chromatography have been described (2). The "Florisil" (60–100 mesh, Floridin Company, Warren, Pa.) was washed with methanol and water and dried at 180° for 48 hours.

Ethyl 3,19-dioxo-Δ^4-etienate (II). Five trial oxidations were carried out. In each experiment, 40 mg. of ethyl 19-hydroxy-3-oxo-Δ^4-etienate (I), m.p. 179–183°, was dissolved in 5 cc. of the indicated solvent and the calculated amount of a 1 mg. per cc. solution of the oxidant was added dropwise. After standing for the indicated time, the reaction mixture was evaporated to dryness. In those cases where acetic acid had not served as a solvent, the residue

[5] When administered at a dose of 4 mg. to two rats, there was no glycogen deposition at all. Since hydrocortisone gives a response at a dosage level of 0.2 mg., the glucocorticoid activity of VIII is certainly less than 5% of that produced by the former.

[6] Meyer (5) refers to the relative ease of aromatizing 19-hydroxy steroids, demonstrated in this laboratory (*cf.* 14, 15), and speculates that they may be intermediates in the biological formation of compounds of estrogen type.

[7] DOCA rather than the slightly more active free 11-desoxycorticosterone is used as standard in the tests of Luetscher (11, 12) and of Simpson and Tait (17).

was dissolved in glacial acetic acid-methanol and the solution was again brought to dryness. The residue was taken up in ether and the solution was shaken with *N* sulfuric acid, *N* sodium carbonate, and water, and the ether was evaporated. The neutral residue so obtained was purified by direct crystallization or by chromatography over Florisil.

A. Reaction medium, 95% acetic acid; 7.40 mg. of chromic anhydride; 3 hours at room temperature; neutral fraction, 35.4 mg.; 4 g. of Florisil; chromatographic peak, 26.9 mg., eluted by chloroform-ether; crystalline II as rods from methanol-water, 17.3 mg.; m.p. 114–121°.

B. Reaction medium, glacial acetic acid; 11.0 mg. of sodium dichromate dihydrate; 18 hours at 5°; neutral fraction, 32.6 mg.; 2 g. of Florisil; chromatographic peak, 28.0 mg., eluted by chloroform-ether; crystalline II as prisms from ether-petroleum ether, 13.6 mg.; m.p. 120–121°.

C. Reaction medium, glacial acetic acid; 7.40 mg. of chromic anhydride; 2½ hours at room temperature; neutral fraction, 35.8 mg.; crystalline II as prisms from ether-petroleum ether, 23.3 mg.; m.p. 118–120°.

D. Reaction medium, pyridine; 7.40 mg. of chromic anhydride; 40 hours at room temperature; neutral fraction, 39.4 mg. of crystalline I, m.p. 180–183°.

E. Reaction medium, *tert*-butyl alcohol; 7.40 mg. of chromic anhydride; 40 hours at room temperature; neutral fraction, 39.4 mg. of crystalline I, m.p. 168–178°.

The yields of II from *A*, *B*, and *C* above were combined and recrystallized from ether-petroleum ether and methanol-water to give small prisms melting sharply at 121°. $[\alpha]_D^{22}$ +205° (10.91 mg., α +2.24° ±0.02°). λ_{max}^{alc} 241 mμ; ϵ 12,600.

Anal. Calc'd for $C_{22}H_{30}O_4$ (358.46): C, 73.71; H, 8.44.

Found: C, 73.45; H, 8.34; Residue, 0.11.

19-Oxoprogesterone (IV). To 61.0 mg. of 19-hydroxyprogesterone (III), m.p. 169–171°, in 5 cc. of glacial acetic acid there was added during one hour 12.31 cc. of a 1 mg. per cc. solution of chromic anhydride in glacial acetic acid. After standing for two additional hours at room temperature, the reaction mixture was evaporated to dryness *in vacuo*. The green residue was suspended in ether and extracted successively with *N* sulfuric acid, *N* sodium carbonate, and water. Drying and evaporation of the ether left 56.8 mg. of crystalline neutral residue which was chromatographed on 5 g. of alumina (activity I–II; 6 x 150 mm.). Pure benzene eluted 30.3 mg. of crystalline IV, and recrystallizatioa from methanol-water yielded 24.5 mg. of long, slender needles, m.p. 137–141°. The ether-methanol eluates gave 13.1 mg. of III, m.p. 164–169°.

When IV was recrystallized by dissolving in methanol and adding water very slowly, short, stout rods melting at 150–152° resulted, but when this material was dissolved in methanol and an excess of water was added quickly, long, slender needles were obtained once more, m.p. 144–145°. $[\alpha]_D^{23}$ +259° (11.65 mg., α +3.02° ±0.02°). λ_{max}^{alc} 241 mμ; ϵ 11,250.

Anal. Calc'd for $C_{21}H_{28}O_2$ (328.43): C, 76.79; H, 8.59.

Found: C, 76.84; H, 8.76.

19-Oxo-11-desoxycorticosterone acetate (VI). To a solution of 49.8 mg. of 19-hydroxy-11-desoxycorticosterone 21-monoacetate (V), m.p. 193–194°, in 5 cc. of glacial acetic acid, 8.55 cc. of a 1 mg. per cc. solution of chromic anhydride in glacial acetic acid was added dropwise during two hours. After standing for one hour more, the reaction mixture was frozen in Dry Ice and evaporated to dryness *in vacuo*. The residue was suspended in ether and shaken with *N* sulfuric acid, *N* sodium carbonate, and water. Evaporation of the ether left 40.0 mg. of crystalline neutral material. Recrystallization from ether-petroleum ether yielded 32.6 mg. of VI as colorless granular crystals, m.p. 119–120°. Two recrystallizations from methanol-water gave the analytical sample, needles, m.p. 122°. $[\alpha]_D^{22}$ +237° (8.68 mg., α +2.06° ±0.02°). λ_{max}^{alc} 243 mμ; ϵ 12,000.

Anal. Calc'd for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82.

Found: C, 71.25; H, 7.77.

19-Oxo-11-desoxycorticosterone (VIII). A mixture of 221.8 mg. of 19-oxo-11-desoxycorticosterone acetate (VI), m.p. 120–122°, in 10 cc. of methanol and 10 cc. of aqueous *N* potassium

bicarbonate was left under nitrogen for 18 hours. Removal of most of the methanol *in vacuo* below room temperature was followed by dilution with water to a volume of 50 cc. and extraction with chloroform. Drying and evaporation of the chloroform left 180.7 mg. of yellow foam. Repeated crystallization from acetone-petroleum ether and methanol-water yielded 74.3 mg. of VIII as colorless plates, m.p. 157–160°. One more recrystallization from methanol-water gave 54.7 mg. of VIII as minute needles, m.p. 158–160°. $[\alpha]_D^n$ +239° (8.04 mg., α +1.92° ±0.02°). λ_{max}^{alc} 245 mμ; ϵ 12,000.

Anal. Calc'd for $C_{21}H_{28}O_4$ (344.43): C, 73.22; H, 8.19.

Found: C, 73.23; H, 8.22; Residue, 0.1; Weight loss, 0.82.

The material remaining in the mother liquors was chromatographed on 20 g. of silica gel (16 x 140 mm.). Chloroform-ether mixtures eluted 52.9 mg. of crystalline material, followed by 28.0 mg. of colorless resin. Recrystallization of the former fraction from methanol-water gave 40.1 mg. of VIII, m.p. 140–150°. By recrystallization from acetone-hexane the m.p. was raised to 157–160°.

Acetylation of 10 mg. of VIII in pyridine-acetic anhydride and recrystallization of the product from methanol-water yielded 7.2 mg. of VI as needles, m.p. 119–120°, undepressed on admixture with authentic VI.

19-Norprogesterone (IX). To 30 mg. of 19-hydroxyprogesterone (III), m.p. 169–171°, in 20 cc. of methanol was added a solution of 1.3 g. of potassium hydroxide in 10 cc. of water (21). After standing for 18 hours at room temperature, the reaction mixture was concentrated *in vacuo* below 0° to a volume of approximately 10 cc. Dilution with 20 cc. of water and extraction with ether yielded 21 mg. of yellow resin which was chromatographed on 2 g. of alumina (activity I–II, 6 x 80 mm.). Pure benzene eluted traces of crystalline material which were combined and recrystallized from methanol-water to give 0.9 mg. of IX as slender needles, m.p. 130–134°, undepressed on admixture with an authentic specimen of 19-norprogesterone (10). For infrared spectrum *cf.* the introduction. Ether-methanol mixtures eluted 11.4 mg. of crystalline III, followed by 6.5 mg. of resinous material. Acidification of the alkaline phase followed by extraction with ether gave 10.2 mg. of a product which was not investigated.

SUMMARY

1. By oxidation with chromic acid, ethyl 19-hydroxy-3-oxo-Δ^4-etienate (I) was converted into ethyl 3,19-dioxo-Δ^4-etienate (II). In analogous fashion, 19-hydroxyprogesterone (III) was transformed into 19-oxoprogesterone (IV). In the same way, 19-hydroxy-11-desoxycorticosterone 21-monoacetate (V) was converted into 19-oxo-11-desoxycorticosterone acetate (VI) which by saponification yielded the free 19-oxo-11-desoxycorticosterone (VIII).

2. The conversion, though in small yield, of 19-hydroxyprogesterone (III) into "normal" 19-norprogesterone (IX) lends further support to the assignment of normal configurations to the compounds of this series.

3. The bioassays of 19-oxoprogesterone (IV) and of 19-oxo-11-desoxycorticosterone (VIII) are reported.

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