THE ISOLATION OF 17-HYDROXYPROGESTERONE FROM THE ADRENAL GLAND

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During the past 6 years twenty-three steroids have been isolated from extracts of the adrenal glands of cattle. Among them is a series of four compounds which are allopregnane derivatives having 3 atoms of oxygen in the molecule, the so called C_{21}O_{3} series of adrenal steroids. Reichstein and his coworkers showed these four compounds to be allopregnane-3β,17β-diol-20-one, the two stereoisomeric allopregnane-3β,17β,20-triols, and Δ^{4}-pregnene-21-ol-3,20-dione (1). We have isolated a fifth member of this series from adrenal extracts. On examination it was found to be the hitherto unknown steroid, Δ^{4}-pregnene-17-ol-3,20-dione or 17-hydroxyprogesterone (I), a position isomer of desoxycorticosterone.

The compound was obtained in small amount from a ketonic fraction after the more reactive alcohols were separated with succinic anhydride. It crystallized from a concentrated acetone solution and was readily secured in pure form by repeated crystallization from acetone and ethanol. It melted at 212–215°. The elementary analyses and molecular weight data agreed with the formula C_{21}H_{30}O_{3}. Its specific rotation in chloroform was [α]_{D}^{27} = +102° ± 3°. A strong selective absorption in the ultraviolet region at 242 μ (ε_{max} = 18,600) indicated the presence of an α,β-unsaturated ketone group of the cholestenone type (Fig. 1). The compound did not react with acetic anhydride in pyridine at room temperature. Two carbonyl groups were shown to be present by the preparation of a disemicarbazone and a dioxime, both of which on analysis appeared to be the respective derivatives of a compound having the molecular composition C_{21}H_{30}O_{3}. Since

1 The observations on the ultraviolet absorption were made by Dr. D. T. Ewing, Michigan State College, East Lansing.
the 3rd oxygen atom was relatively inert, it appeared by analogy with the known adrenal steroids that the new compound might be either 11-hydroxy-, 11-keto-, or 17-hydroxyprogesterone. Oxidation with chromic acid in glacial acetic acid at room temperature yielded a crystalline neutral oxidation product which melted at 168-169° and which was found to be identical with Δ^4-androstenedione-3,17 (II) by analysis and mixture melting point. The structure of the new compound was thus demonstrated to be 17-hydroxyprogesterone. No direct evidence is available on the steric configuration around carbon atom 17. By analogy with the known adrenal steroids which have been shown by Reichstein to have the β configuration (2-4) the compound is most likely

![Graph showing ultraviolet absorption of 17-hydroxyprogesterone in ethanol.](http://www.jbc.org/)

**Fig. 1.** Ultraviolet absorption of 17-hydroxyprogesterone in ethanol.
17-α-hydroxyprogesterone. Neither of the 17-hydroxyprogester-
one has been prepared as yet by synthetic means. The com-
pound to which Ruzicka and Meldahl (5) ascribed the structure
of 17-α-hydroxyprogesterone was later recognized as a product of
rearrangement which no longer possessed the pregnane carbon
skeleton (6, 7).

Our sample of 17-hydroxyprogesterone failed to elicit any
progestational reaction in a series of three rabbits which received
intramuscularly 1.9, 2.5, and 5.0 mg. doses, respectively. A
slightly modified Clauberg technique was employed. Although
it cannot be concluded from these tests, which are necessarily

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of rats</th>
<th>Body weight (gm.)</th>
<th>Total dose (mg.)</th>
<th>Weight of seminal vesicles (mg.)</th>
<th>Weight of prostate (mg.)</th>
</tr>
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<tbody>
<tr>
<td>17-Hydroxyprogesterone</td>
<td>6</td>
<td>43</td>
<td>1.0</td>
<td>10</td>
<td>23</td>
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<tr>
<td>Adrenosterone</td>
<td>5</td>
<td>51</td>
<td>0.75</td>
<td>8.5</td>
<td>19</td>
</tr>
<tr>
<td>Androsterone</td>
<td>5</td>
<td>51</td>
<td>0.75</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>None</td>
<td>50</td>
<td>50</td>
<td>6.5</td>
<td>9</td>
<td>9</td>
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</table>

The dose was evenly divided in six injections administered subcutane-
ously in 0.25 cc. of peanut oil. The rats were castrated at 25 days of age
and injected daily for the following 6 days. The animals were killed on the
7th day after castration. The seminal vesicles and the ventral lobe of the
prostate were dissected out and weighed.

limited by the quantities of the compound available, that 17-
hydroxyprogesterone has no progestational activity, it is clearly
less active than 21-hydroxyprogesterone. The latter compound
at a dose level of 5 mg. was strongly active. Progesterone itself
gave a comparable reaction at a dose level of 0.5 mg. As might be
expected, 17-hydroxyprogesterone was found inactive when tested
for cortical hormone activity. It was administered subcutane-
ously in a daily dose of 0.25 mg. to a series of six 30 day-old rats,
following adrenalectomy. They survived an average of 6 days,
just as did untreated, adrenalectomized, control animals. Cor-

2 We are indebted to Dr. D. A. McGinty of this laboratory for the assay
of progestational and androgenic activities.
Isolation of 17-Hydroxyprogesterone

testosterone was active at a dose level of 0.1 mg. per rat per day. The compound was also entirely inactive in the acute muscle work test of Ingle (8) in a series of four animals in doses of 1.0, 2.0, 2.0, and 3.0 mg., respectively. When examined for androgenic activity in the castrate rat, 17-hydroxyprogesterone exhibited an activity comparable to androsterone and adrenosterone, as is evident from the data summarized in Table I. No androgenic activity was observed in the capon when tested at a level of 200 γ per day. The standard daily dose of androsterone in the capon assay is 100 γ (9). Limited supplies of the compound prevented further tests at higher levels. The results on the castrate rats, although admittedly having limited quantitative significance, would indicate that 17-hydroxyprogesterone may play a rôle along with adrenosterone (1) in the adrenal-gonad relationship.

EXPERIMENTAL

The adrenal extract was prepared by the methods of Swingle and Pfiffner (10) and further fractionated into the so called first and second ether concentrates (11). The first ether concentrate was separated into ketonic and non-ketonic fractions by means of Girard's Reagent T (betaine hydrazide hydrochloride). The ketonic complex was fractionally hydrolyzed, a fractionation procedure first employed by Reichstein (12). The ketonic fraction liberated between pH 6 and pH 4 weighed 9.9 gm. from approximately 3 tons of beef adrenal glands. The highly pigmented sirup was dissolved in 320 cc. of methyl alcohol and 80 cc. of water were added, containing 8 gm. of KHCO₃. The mixture was refluxed for 1 hour, the methyl alcohol distilled off under reduced pressure, and the saponification mixture extracted six times with 500 cc. portions of ether. The ether-soluble fraction was a pale yellow sirup weighing 4.3 gm. It was dissolved in 30 cc. of py-

We wish to thank Dr. D. J. Ingle for his kindness in testing this compound.

The capon assay was made at the University of Chicago through the courtesy of Professor F. C. Koch.

All melting points were determined in a Berl block and are uncorrected. The microanalyses were made by Mr. Clark Chamberlain of this laboratory. For physiological assays the compounds were dissolved in peanut oil except in the case of the muscle work test by Dr. D. J. Ingle, when sesame oil was employed.
ridine; 5.5 gm. of succinic anhydride were added and the mixture warmed gently at 50° until the anhydride had dissolved. The mixture was allowed to stand overnight, the bulk of the pyridine removed by distillation under reduced pressure, the residue taken up in ether, and the remaining pyridine removed by washing with small quantities of dilute hydrochloric acid. The half esters were separated from the neutral fraction in the usual manner with half saturated sodium carbonate. The ether solution of the neutral fraction was dried with anhydrous sodium sulfate and the ether removed. The pale yellow residue weighed 1.4 gm. It was dissolved in 2.5 cc. of acetone. A crystalline deposit started to form promptly. After the solution had stood several days in the refrigerator, the crystalline fraction was filtered off and washed with cold acetone. It was dried and weighed 264 mg. The crude crystals melted at 190–195°, with softening from about 185°. Three recrystallizations from acetone and two from ethanol yielded 60 mg. of thin platelets melting at 212–215°. The melting point was not changed on further recrystallization. It is somewhat dependent on the rate of heating. Another 100 mg. of the same compound, m.p. 210–212°, was obtained when the mother liquors were worked up. The compound is readily soluble in chloroform but insoluble in ether. It crystallizes from ethyl acetate in platelets. It does not precipitate with digitonin either in 80 per cent ethyl alcohol or 50 per cent methyl alcohol. The specific rotation is \([\alpha]_D^{27} = +102° \pm 3° (c = 1.56 \text{ in chloroform})\). The compound was dried for analysis in vacuo at 110° for 3 hours.

**Analysis—C_{21}H_{26}O_{8}**. Calculated. C 76.3, H 9.2, mol. wt. 330

<table>
<thead>
<tr>
<th>Found</th>
<th>&quot; 76.0, &quot; 9.3, &quot; 363</th>
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<tr>
<td></td>
<td>76.0, &quot; 9.1</td>
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27 mg. of the compound were allowed to stand overnight in 1 cc. of acetic anhydride and 0.7 cc. of pyridine. The mixture was distilled to dryness and the residue recrystallized twice from ethanol. The product weighing 25 mg. melted at 211–213°, had the same crystal form as the starting material, and failed to depress the melting point of the original compound. It was dried for analysis in vacuo at 110° for 3 hours.

**Analysis—C_{21}H_{26}O_{8}**. Calculated. C 76.3, H 9.2

<table>
<thead>
<tr>
<th>Found</th>
<th>&quot; 76.0, &quot; 9.4</th>
</tr>
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<tr>
<td></td>
<td>75.8, &quot; 9.2</td>
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Disemicarbazone—15 mg. of the compound were refluxed for 30 minutes in 1 cc. of ethanol with 30 mg. of semicarbazide acetate. On dilution with water the product separated promptly in crystalline form. It was collected, washed thoroughly with water and alcohol, and dried. The product weighed 19.8 mg. It did not melt below 360°. It darkened at about 240° and sintered markedly at 280–290°. Because of its extreme insolubility in ethanol it was not recrystallized for analysis. The compound was dried for analysis at 110° in vacuo.

Analysis—C_{22}H_{30}O_{5}N_{6}. Calculated. C 62.1, H 8.2, N 18.9
“ 61.4, “ 8.1

Dioxime—14 mg. of the compound were refluxed for 4 hours in 3 cc. of 95 per cent alcohol with 100 mg. of hydroxylamine acetate. The mixture was boiled down to 1 cc. and 2 cc. of water were added. The product separated promptly. It was filtered off and recrystallized from 50 per cent ethanol. It separated in thin plates which melted with decomposition at 250–251°, with sintering from about 240°. The compound weighing 9 mg. was dried for analysis at 110° in vacuo for 2 hours.

Analysis—C_{21}H_{28}O_{5}N_{2}. Calculated, N 7.8; found, N 7.9

Oxidation with Chromic Acid—41 mg. of the compound were dissolved in 1 cc. of glacial acetic acid and 25 mg. of chromic acid in 0.5 cc. of 90 per cent acetic acid added. The mixture was allowed to stand overnight and the excess chromic acid was reduced with sodium sulfate. After the mixture was distilled to dryness under reduced pressure at 50°, the residue was taken up in water and ether and the ether-soluble fraction freed of acids with aqueous sodium carbonate. The dried neutral ether-soluble fraction weighed 32 mg. It was taken up in 2 cc. of benzene and filtered through 200 mg. of aluminum oxide. The filter was washed well with benzene, the filtrate evaporated to dryness, and the residue recrystallized twice from dilute ethanol, yielding 13 mg. of rosettes of microscopic needles, m.p. 168–169°. A mixture melting point with Δ^{4}-androstenedione-3, 17, m.p. 167–168°, showed no depression. The product was dried for analysis at 80° in vacuo for 4 hours.

Analysis—C_{19}H_{24}O_{5}. Calculated. C 79.7, H 9.2
SUMMARY

The isolation of a new steroid ketone from the adrenal glands of cattle is described. Its structure is shown to be that of a 17-hydroxyprogesterone.

BIBLIOGRAPHY

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