[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY, AND THE MEDICAL CLINIC OF THE PETER BENT BRIGHAM HOSPITAL]

Nature of the Androgens in Female Adrenal Tumor Urine[1]

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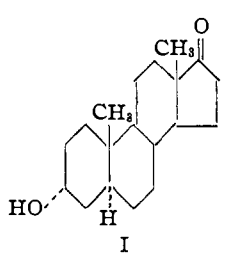
One of the most striking observations concerning the hormone content of different urines is that females suffering from adrenal virilism excrete excessive amounts of male hormones and that an increased output of oestrogenic hormones is associated with adrenal feminism in males.[2] The very high levels of hormone excretion occurring in cases of adrenal cortical tumor are not paralleled in other pathological conditions, and the recognition of a highly excessive hormone output is thus of definite diagnostic value and constitutes one of the most prominent of present clinical applications of hormone assay.[3] The problem of determining urinary steroids and interpreting the results is complicated by the appearance in the urine of several steroids associated with each of the two sex groups and by the fact that these substances are not themselves true hormones possessing characteristic physiological functions but are excretory transformation products of glandular hormones. A knowledge of the output of steroids in the urine nevertheless can be of distinct value when it provides an accurate if indirect index of the activity of the glands producing the true hormones. Since both the gonads and the adrenals appear to be involved in the production of hormones which are excreted in altered form in the urine, data concerning the output of excretory products can be translated in terms of the activity of a given gland only if determination is made of individual urinary steroids whose point of origin in the body is known or can be safely inferred.

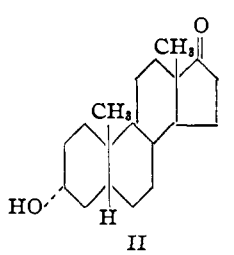
Certain of the urinary steroids retain the biological properties of glandular hormones, although in diminished potency, while others are wholly devoid of biological activity. Since the activity or lack of activity is purely incidental and has no physiological significance, bio-assays for androgenic and oestrogenic activity can provide only a very incomplete and unsatisfactory indication of glandular functioning. Methods of determination based upon specific chemical or physical properties are not subject to any such inevitable limitation and offer promise of being capable of elaboration to a point where all substances excreted in significant amounts can be characterized individually. For the improvement and extension of existing methods, particularly as applied to pathological urines, further information is required on the composition of the steroid mixtures and on the efficacy and validity of procedures for the hydrolysis and extraction of urine and for the fractionation of the extracted material. The present investigation of the nature of the androgens of adrenal tumor urine was undertaken partly with this objective in view and partly in the hope of gaining a further insight into intermediary steroid metabolism.

The neutral ketonic fraction obtained by removal of the phenolic oestrogens with alkali and separation of the ketones from the non-ketonic material with Girard's reagent may be designated the "neutral 17-ketosteroid fraction" or, since it includes all of the urinary steroids which possess androgenic activity, the "androgen fraction." The androgen fraction from the urine of normal males has been shown to contain androsterone[4,5] (I, rather strongly androgenic), 3α-hydroxyaetiocholanone-17[6] (II, inactive), and dehydroisoan-



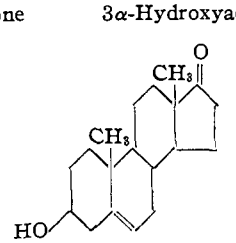
I

Androsterone



II

3α-Hydroxyaetiocholanone-17



III

Dehydroisoandrosterone

(1) This work was supported by grants from the Milton Fund of Harvard University and the National Advisory Cancer Council.

(2) Burrows, Cook, Roe and Warren, *Biochem. J.*, **31**, 950 (1937).

(3) R. K. Callow, *Proc. Roy. Soc. Med.*, **31**, 841 (1938).

(4) Butenandt and Tscherning, *Z. angew. Chem.*, **44**, 905 (1931).

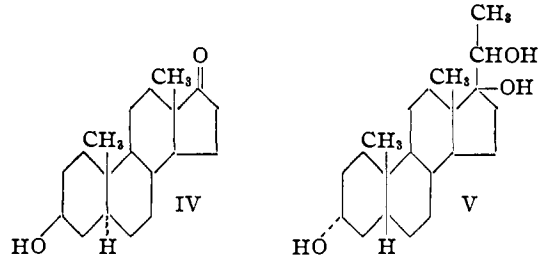
(5) Butenandt, Dannenbaum, Hanisch and Kudszus, *Z. physiol. Chem.*, **237**, 57 (1935).

(6) N. H. Callow, *Biochem. J.*, **33**, 559 (1939).

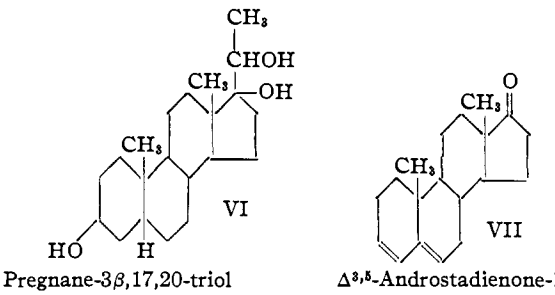
drosterone[7,8] (III, moderately androgenic). The amounts of these steroids which have been isolated from normal male urine in an identifiable form are in the order of 0.5–1.5 mg. per liter of urine[6,9]; still other substances may be present in small amounts but have not been identified. The same three steroids have been isolated from the urine of normal females[10] in amounts comparable with the yields from normal male urine.[11] That the substances can be produced elsewhere than in the genital organs has been established by the isolation of androsterone, 3α-hydroxyaetiocholanone-17, and dehydroisoandrosterone from the urine of eunuchs[9] and from the urine of ovariectomized women.[12] There is little doubt that the adrenal gland is responsible for the production of these steroids in castrates and, since in the case of ovariectomized women the output is only slightly below normal, it may be inferred that in the female organism the adrenal gland probably is the sole source of the components of the androgen fraction. Since castrated males excrete somewhat less androsterone and 3α-hydroxyaetiocholanone-17 than do normals, a part of the production of these two substances in males may be attributed to the testes. The evident precursor in the genital gland is testosterone, from which the two C_5-epimers are derivable by a process of partial reduction and disproportionation. Indeed administration of testosterone to males results in an increased output of androsterone and 3α-hydroxyaetiocholanone-17.[6,13] By analogy it would appear that the androsterone and 3α-hydroxyaetiocholanone-17 of adrenal origin are end-products of the metabolism of α,β-unsaturated ketones of the cortical steroid group.

The dehydroisoandrosterone found in the urine of males and females alike may all come from the adrenals,[14] but the nature of the precursor is still obscure. The suggestion that the substance is a reduction product of a Δ^4-3-ketosteroid[15] lacks any foundation of analogy[6] and seems to us unlikely; the process would require the migration of the double bond at 4,5, presumably after reduction of the carbonyl group, away from its position of conjugation with the oxygen atom.

The adrenal origin of dehydroisoandrosterone is further indicated by Callow's[16] isolation of an abnormally large amount of this steroid from the urine of a young girl having an adrenal tumor and showing symptoms of virilism. Butler and Marrian investigated the neutral steroids from the unhydrolyzed urine of women suffering from adrenal tumors[17] and from adrenal hyperplasia[18,19] and encountered no dehydroisoandrosterone but isolated four other crystalline products. One of these proved to be 3α-hydroxyaetiocholanone-17 (II), a steroid of normal female urine, and the others were characterized as isoandrosterone (IV), pregnane-3α,17,20-triol (V), and an isomer which was assigned tentatively the probable structure of pregnane-3β,17,20-triol (VI).



Isoandrosterone — Pregnane-3α,17,20-triol



Pregnane-3β,17,20-triol — $\Delta^{3,5}$-Androstadienone-17

Burrows, Cook, Roe and Warren[2] processed the acid-hydrolyzed urine of a male patient with a malignant tumor of the adrenal gland and

(7) Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 197 (1934).

(8) Nomenclature: Fieser, "The Chemistry of Natural Products Related to Phenanthrene," 2nd edition, p. 233, Reinhold Publishing Corp., New York, N. Y., 1937. The substance is also referred to in the literature as dehydroandrosterone (Butenandt) and *trans*dehydroandrosterone (Ruzicka). The former name seems incorrect because the compound is the dehydro derivative not of androsterone but of its isomer (C_3-OHβ). The latter name to be strictly descriptive should be changed to dehydro*trans*androsterone.

(9) N. H. Callow and R. K. Callow, *Biochem. J.*, **34**, 276 (1940).

(10) (a) N. H. Callow and R. K. Callow, *ibid.*, **32**, 1759 (1938); (b) **33**, 931 (1939).

(11) According to a preliminary report by Pearlman, *J. Biol. Chem.*, **136**, 807 (1940), normal female urine also contains small amounts of isoandrosterone.

(12) Hirschmann, (a) *ibid.*, **130**, 421 (1939); (b) **136**, 483 (1940).

(13) Dorfman, Cook and Hamilton, *ibid.*, **130**, 285 (1939).

(14) N. H. Callow, R. K. Callow, Emmens and Stroud, *J. Endocrinology*, **1**, 76 (1939).

(15) Marker, THIS JOURNAL, **60**, 1725 (1938).

(16) R. K. Callow, *J. Soc. Chem. Ind.*, **55**, 1030 (1936); Crooke and R. K. Callow, *Quart. J. Med.*, **8**, 233 (1939).

(17) Butler and Marrian, *J. Biol. Chem.*, **119**, 565 (1937).

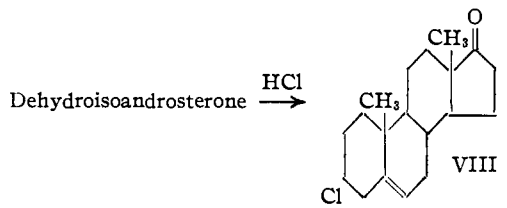
(18) Butler and Marrian, *ibid.*, **124**, 237 (1938).

(19) Marrian and Butler, *Nature*, **142**, 400 (1938).

isolated $\Delta^{3,5}$-androstadienone-17 (VII) and two other substances which were not fully characterized.

The urine investigated in the present work was that of a girl suffering from a recurrent corticoadrenal tumor and exhibiting marked signs of virilism.[20] Colorimetric determinations of the neutral 17-ketosteroid content were made by a modification of the Zimmermann method[21] and the results calculated as androsterone equivalents. Just prior to excision of the tumor the average output was 83 mg. per twenty-four hour urine. The excretion of androgens dropped sharply after operation, and within two weeks was equivalent to about 1 mg. of androsterone per day. An increased excretion was evident five months after operation and before recurrence of the tumor growth had been observed. The urine investigated was collected in the period twenty-two to twenty-seven months after operation, at which time the patient was in her twelfth year; death occurred five months later. During the period of collection the average daily excretion as determined by Zimmermann assay was 285 mg. equivalents. Polarographic determinations[22] were made only on samples collected just prior to the patient's death. We are indebted to Dr. Hugh H. Darby for carrying out bio-assays of twenty-four hour urine samples collected at the same time as the material used for the isolation work. His results indicated a daily excretion of 350 capon units (I. U.) of male hormone and 31 rat units of oestrogenic hormone. It is of interest that the oestrogen level is in the normal range even though the androgen output is extremely high.

The urine was submitted to hydrolysis with hydrochloric acid at the boiling point and the steroids extracted with benzene[23] or, in the case of the last batches, with carbon tetrachloride.[24] After removal of the phenolic oestrogens by extraction from ether with 2 *N* alkali, considerable colored matter was eliminated by washing with alkaline hydrosulfite solution. The residual neutral steroid mixture was processed twice with Girard's reagent (T) and the non-ketonic fraction was set aside and not investigated. The neutral ketonic or androgen fraction was processed differently in two separate experiments, as indicated in the flow sheet. In Experiment A the material (7.4 g.) from 28.5 l. of urine was treated with succinic anhydride in pyridine to separate the alcoholic and non-alcoholic steroids. The non-carbinol fraction was passed through an alumina adsorption tower in carbon tetrachloride solution to remove highly colored gums and converted to a mixture of semicarbazones. One crystalline product proved to be very sparingly soluble in alcohol and could be separated easily from the mixture, and this on hydrolysis gave a substance which was identified by analysis and properties as 3-chloro-Δ^5-androstenone-17 (VIII). This chloro



3-Chloro-Δ^5-androstenone-17

ketone was isolated by Butenandt and Dannenbaum[25] from an acid-hydrolyzed urinary extract and recognized as arising from the action of hydrochloric acid on dehydroisoandrosterone during the hydrolysis step.[26] The amount of material isolated is thus translated in terms of an equivalent amount of hydroxy compound in the original urine sample. The material in the semicarbazone mother liquor did not crystallize satisfactorily and gave only an oily product on hydrolysis; this may have consisted of a mixture of the chloroketone and the ketone isolated in Experiment B.

The material which had undergone succinoylation was separated from the non-alcoholic fraction by extraction with soda solution. A part of the product crystallized nicely and was identified as the half-succinate of dehydroisoandrosterone by saponification and identification of the steroid, by analysis of the methyl ester, and by direct comparisons with samples of the acid and methyl ester prepared from dehydroisoandrosterone. The remaining half-succinate fraction was saponified and the keto alcohol mixture was treated with digitonin in order to separate the α and β 3-hy-

(20) For a description of the case, see Friedgood and Gargill, *J. Clin. Investigation*, **17**, 504 (1938).

(21) Friedgood and Whidden, *Endocrinology*, **25**, 919 (1939); Friedgood and Berman, publication in press.

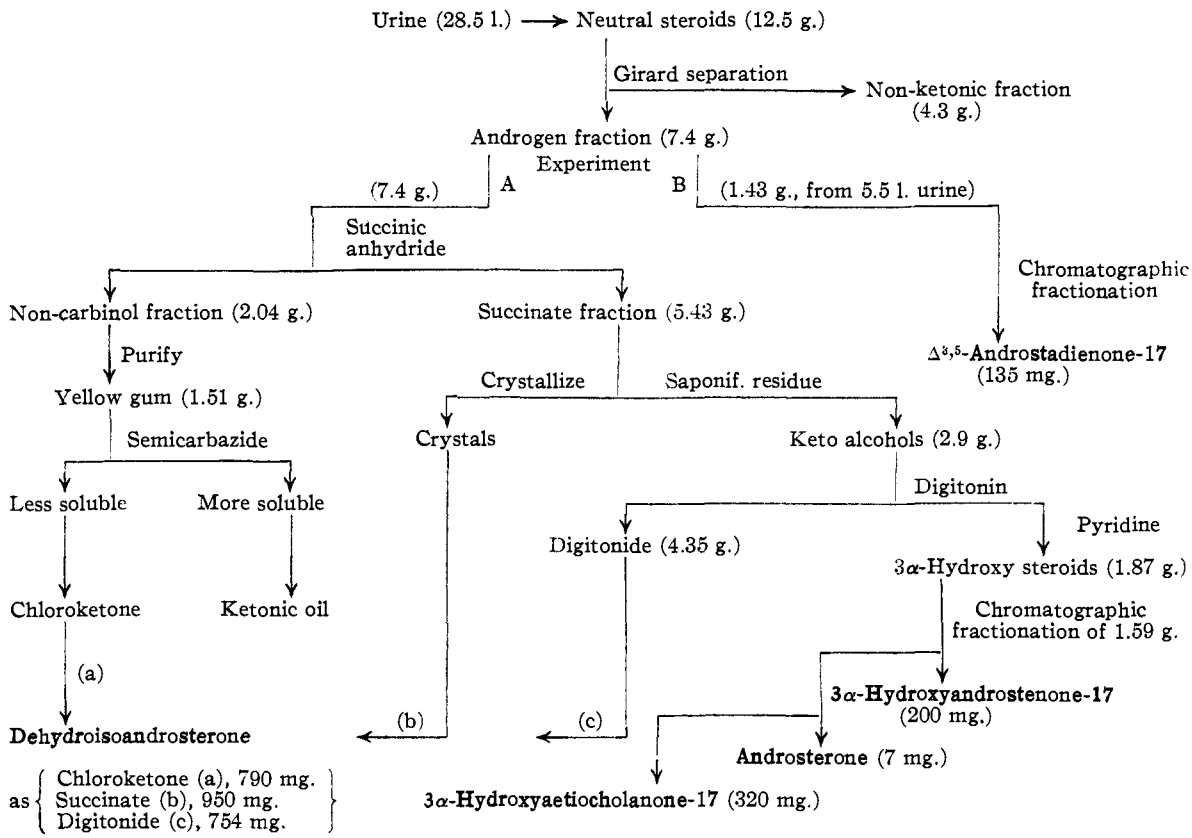
(22) Wolfe, Hershberg and Fieser, *J. Biol. Chem.*, **136**, 653 (1940).

(23) G. V. S. Smith and O. W. Smith, *Am. J. Physiol.*, **112**, 340 (1935).

(24) Hershberg and Wolfe, *J. Biol. Chem.*, **133**, 667 (1940).

(25) Butenandt and Dannenbaum, *Z. physiol. Chem.*, **229**, 192 (1934).

(26) Butenandt and Grosse, *Ber.*, **69**, 2776 (1936).



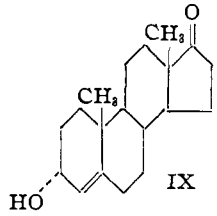
droxysteroids. This proceeded efficiently, giving a digitonide precipitate which on cleavage with pyridine according to Bergmann[27] afforded in 91% yield a steroid identified as dehydroisoandrosterone by conversion to the benzoate. Direct comparisons with authentic samples were made with the benzoate, the acetate, the free hydroxy compound and its oxime.

The 3α-hydroxy steroid fraction recovered from the mother liquor remaining after the precipitation of the digitonide was a gum, but a separation into at least some of the components was accomplished rather easily by application of the efficient method of chromatographic fractionation described by Callow and Callow.[6,10b] The material was adsorbed from carbon tetrachloride solution onto alumina and then eluted in fourteen fractions, using carbon tetrachloride at the start and then carbon tetrachloride containing increasing, small amounts (0.1–0.3%) of ethanol. As is shown in the chart, three crystalline products were isolated, two of which are known compounds. The small amount of androsterone was identified, after recrystallization, by mixed melting point determination. 3α-Hydroxyaetiocholanone-17 was identified by analysis and by the preparation of the acetate and benzoate; the melting points of these derivatives and of the substance itself corresponded with the values given in the literature.

The substance obtained from the first eluate appears to be a new compound. The steroid melts at 181–183°, corr., is strongly dextrorotatory, $[\alpha]^{26.5}_{D}$ +122° in alcohol, and analyses indicate the formula $C_{19}H_{28}O_2$. It may be noted that Hirschmann isolated from the same fraction of an extract of the urine of ovariectomized women an unidentified compound melting at 184°. Our substance forms an acetate, a benzoate and a semicarbazone, and therefore is a keto alcohol. The hydrogen content and the production of a faint yellow color on treatment of the substance with tetranitromethane suggested that it is unsaturated and isomeric with dehydroisoandrosterone. Indeed it was found that the substance can be hydrogenated readily as the acetate in the presence of Adams catalyst. After acetylation of the product there was isolated a substance identified by melting point and mixed melting

(27) Bergmann, *J. Biol. Chem.*, **132**, 471 (1940).

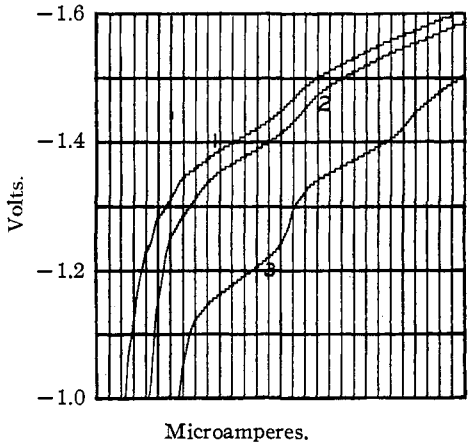
point determinations as androstanediol diacetate. This establishes the nature of the ring system and the positions of the oxygen atoms. Since the compound is not precipitated by digitonin, a hydroxyl group if present at C_3 must have the same configuration (α) as that of dehydroandrosterone,[28] and the lack of correspondence with this substance indicates that the double bond probably is not located at the 5,6-position. The Δ^4-structure IX was considered at least a possibility, but such a formulation is rendered very unlikely by the observation that the substance can be refluxed with 1 *N* alcoholic hydro-



IX

(Hypothetical)

chloric acid without undergoing alteration. Sterols[29] and hormone derivatives[30] having an allylic alcohol grouping as in IX are extremely sensitive to acids and undergo dehydration under even milder conditions.

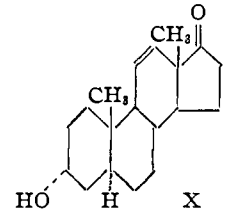


Microamperes.

Fig. 1.—Steroids (1 mg.) submitted to Oppenauer oxidation and the products (0.1 mg.) polarographed as the Girard derivatives: Curve 1, 3α-hydroxyandrostenone-17; Curve 2, androsterone; Curve 3, dehydroisoandrosterone.

It has been shown that the method of polarographing a ketosteroid in the form of the Girard derivative[22] distingushes between C_3 and C_{17} saturated ketones and between these substances and α,β-unsaturated ketones. When submitted to the standard procedure[22] and polarographed, the new androstenolone gave a wave of potential and span characteristic of the 17-ketosteroids. The substance is therefore a 3-hydroxyandrostenone-17. Further information was obtained by oxidizing a sample by the Oppenauer procedure,[31,32] processing the resulting androstenedione (not isolated) with Girard's reagent, and running the polarographic analysis. Samples of androsterone and dehydroisoandrosterone were similarly oxidized and polarographed for comparison, and the results for the three compounds are recorded in Fig. 1. The waves for the dione from the new substance (Curve 1) and for androstanedione (Curve 2) are similar and occur at a potential indicative of the C_{17}-carbonyl group; the discharge associated with this group is not influenced by the presence of the C_3-carbonyl group or by non-conjugated double bonds. Curve 3 for Δ^4-androstenedione shows a wave at a potential characteristic of the α,β-unsaturated ketones and is clearly distinguishable from the other two. The double bond of the new 3-hydroxyandrostenone-17 therefore cannot be located at the 1,2-, 4,5-, or 15,16-position, for conjugation would then result. Furthermore, since it is known that in the Oppenauer oxidation a double bond will migrate to a position which is conjugated with a carbonyl group,[31] positions 5,6 and 14,15 can likewise be ruled out.

Of the remaining possibilities, the bridgehead positions 8,9 and 8,14 are unlikely because of the ease with which the substance undergoes hydrogenation. Thus the evidence indicates that the new steroid is a 3α-hydroxyandrostenone-17 with the double bond at the 6,7-, 7,8-, 9,11- or 11,12-position. The $\Delta^{11,12}$-structure X represents one of these four possibilities, and this allocation of the



X

nuclear double bond was postulated by Shoppee[33] for a 3β-hydroxyandrostenone-17 resulting from

(28) Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 1407 (1936).
(29) Schoenheimer and Evans, *J. Biol. Chem.*, **114**, 567 (1936).
(30) Butenandt and Heusner, *Ber.*, **71**, 198 (1938).
(31) Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).
(32) Analytical method: Hershberg, Wolfe and Fieser, THIS JOURNAL, **62**, 3516 (1940).
(33) Shoppee, *Helv. Chim. Acta*, **23**, 740 (1940).

the dehydration of a substance derived from one of the cortical steroids having a hydroxyl group at the 11-position. This investigator found the C_{11}-hydroxyl group to be eliminated very easily under the influence of mineral acids and, since in the present work the acid hydrolysis of the urine was sufficiently drastic to convert much of the dehydroisoandrosterone into the chloride, the 3α-hydroxyandrostenone-17 isolated may well be the dehydration product of an androstadiolone precursor. Our compound resembles Shoppee's dehydration product in the ease with which the double bond can be saturated with hydrogen, and it may, indeed, be the C_3-epimer of the Shoppee compound.

A fifth steroid was isolated in the separate Experiment B starting with the androgen fraction from 5.5 l. of urine; the yield of crude neutral 17-ketosteroids was exactly the same as in Experiment A. When the entire fraction was adsorbed from carbon tetrachloride solution onto alumina it was observed that elution with a small volume of the same solvent removed a portion of the total material very easily, while the chief constituents were very strongly adsorbed and could be left completely on the alumina. The sharp separation became understandable when it was found that the substance so easily eluted is nonhydroxylic and contains only one oxygen atom, present as a carbonyl group as shown by the formation of an oxime. The compound melts at 87–88.5°, has the formula $C_{19}H_{26}O$, and Dr. R. N. Jones found that it shows an intense ultraviolet absorption at about 235 mμ. The substance corresponds in all of these properties, and in the melting point of the oxime, with $\Delta^{3,5}$-androstadienone-17 (VII), as described by Burrows, *et al.*,[2] and direct comparison with material prepared from dehydroisoandrosterone by the procedure of these investigators established the identity.

Table I lists the amounts of the steroids isolated in this investigation in a substantially pure form either as such or in the form of derivatives. Although the total weight of the five crystalline products isolated represents only slightly more than half the weight of the crude androgen fraction, the losses attending the separations seemed to be rather evenly distributed and no particular concentration of unidentified material was noted. It is now evident, however, that considerable improvement can be made in the

TABLE I

NEUTRAL STEROID CONTENT OF THE ADRENAL TUMOR URINE

	Mg. per liter	
Non-ketonic fraction (not investigated)	151	
Total crude 17-ketosteroid fraction	260	
Androsterone (I)	0.3	134 mg.
3α-Hydroxyaetiocholanone-17 (II)	13	
Dehydroisoandrosterone (III)	88	
$\Delta^{3,5}$-Androstadienone-17 (VII)	25	
3α-Hydroxyandrostenone-17 (X?)	8	

method of processing the urine. The fact that about one-third of the total dehydroisoandrosterone initially present was converted into the corresponding chloroketone shows that the conditions of acid hydrolysis were too drastic. Quite possibly the 3α-hydroxyandrostenone-17 and the $\Delta^{3,5}$-androstadienone were formed by the dehydration of hydroxylated precursors in the course of the acid hydrolysis (see below), and there is no assurance that such reactions do not proceed only partially, as in the case of the formation of the chloroketone, and give a mixture far more complex than that present in the urine. Since the method of hydrolysis is that currently used in most assay laboratories, it is evident that the assay results are subject to complicating uncertainties on the score of a probable alteration of some of the original steroids. Fortunately a method of hydrolysis is now available which avoids at least some of the difficulties. Talbot, *et al.*,[34] have shown that when hydrolysis and extraction are conducted simultaneously in the rapid extractor developed in this Laboratory,[24] dehydroisoandrosterone added to the urine can be recovered quantitatively in unaltered form. Whether or not this method will be found to provide adequate protection to other sensitive steroids, it represents a distinct advance over older procedures.

Burrows, *et al.*,[2] expressed the view that the $\Delta^{3,5}$-androstadienone-17 (VII) which they encountered probably was not present as such in the original urine. They offered no suggestion as to the nature of the precursor other than to point out that this is unlikely to be dehydroisoandrosterone or its epimer dehydroandrosterone, for neither substance suffers dehydration under conditions comparable with those of the acid hydrolysis to which the urine was subjected. We may comment further that when the acid strength

(34) Talbot, Butler, MacLachlan and R. N. Jones, *J. Biol. Chem.*, **136**, 365 (1940).

is increased to a point where alteration does occur, dehydroisoandrosterone does not suffer dehydration but is converted into the chloroketone. We agree with Burrows, *et al.*, that the $\Delta^{3,5}$-androstadienone-17 may well be a transformation product and suggest that the most likely precursor is a keto alcohol of the allylic type, namely, Δ^4-dehydroisoandrosterone[35] or the epimer Δ^4-dehydroandrosterone (IX). Either substance should be highly sensitive to mineral acids and could yield the dienone VII by the 1,4-elimination of the elements of water.

Our results confirm and extend those of others who have studied adrenal tumor urines. The doubly unsaturated ketone VII which Burrows, *et al.*,[2] isolated from the acid-hydrolyzed urine of a male patient has been obtained in abundant quantity from the urine of a female. Like Callow,[16] we find urine of the latter type to contain huge amounts of dehydroisoandrosterone. Our experiments are not comparable with those of Butler and Marrian,[17,18] for they worked only with the neutral, ether-soluble fraction obtained from unhydrolyzed urine. The two non-ketonic triols V and VI which they isolated, in the first case in yields as high as 40 mg. per liter, would not have been encountered in the present work because the non-ketonic fraction was separated (151 mg. per liter) but not investigated. While Butler and Marrian isolated two saturated 3-hydroxy-17-keto steroids (II and IV), we obtained from hydrolyzed urine only one of these substances (3α-hydroxyaetiocholanone-17, II). In view of the very large amounts of dehydroisoandrosterone which we found present after hydrolysis, the fact that Butler and Marrian[18] encountered no trace of this substance in the digitonin precipitated 3β-hydroxyketone fraction from unhydrolyzed urine strongly suggests that the entire amount of dehydroisoandrosterone is excreted in a conjugated, ether-insoluble form. If certain steroids are present entirely in a conjugated form while others are wholly in the free state, it would seem of distinct advantage to investigate separately the material extractable before and after hydrolysis.

From the results now available it is evident that women with adrenal tumors excrete certain normal neutral steroids in abnormal amounts, and that they excrete several additional substances of this group. The androsterone excretion is at about the normal level (Table I), the amount of 3α-hydroxyaetiocholanone-17 excreted is considerably above normal (about 10-fold), and the output of dehydroisoandrosterone represents an increase above normal in the order of 100-fold. Of the ketonic steroids found in pathological but not in normal female urine, namely, IV, VII and X, two are unsaturated substances (VII and X) which may come from hydroxylated precursors. Whether or not an unsaturated steroid is derived from such a precursor, it is properly considered as being in a higher state of oxidation than a saturated substance. $\Delta^{3,5}$-Androstadienone-17 (VII) and 3α-hydroxyandrostenone-17 (X), which may be described as very abundant, and abundant, components of the mixture, respectively, are equivalent in the state of oxidation and stand above dehydroisoandrosterone. The latter substance, which is excreted in such excessive amounts, is in a more highly oxidized condition than the other two components of the normal androgen fraction. Thus excessive activity of the adrenal gland appears to result in the pouring into the urine of large amounts of steroids in an abnormally highly oxidized or dehydrogenated condition. In those cases investigated (cattle) the steroids which are produced normally in the adrenal gland but not excreted are, on the average, in a perhaps even more highly oxidized condition, for the nucleus is often unsaturated at C_{4-5} and hydroxylated at C_{11} and C_{17}, while in many of the compounds isolated from the complex mixture the oxygen at C_3 is present in the oxidized ketonic form. The general picture of biogenesis which this suggests is that the cortical steroids arise in a synthetic process involving highly hydroxylated or otherwise oxidized intermediates, as in the interesting scheme postulated by Reichstein,[36] and are normally excreted in a reduced form. A tumor-invaded adrenal gland, however, produces such a flood of oxidized products that the mechanism for their reduction is overtaxed, and partially processed materials are dumped into the urine.

The as yet wholly hypothetical but at least conceivable conversion of a steroid into a carcinogen of the cholanthrene type by a process of abnormal metabolism[37] would require an extensive dehydrogenation of the hydroaromatic rings. The closest

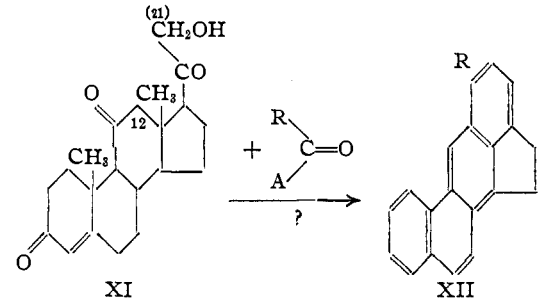
(35) Ruzicka, Fischer and Meyer, *Helv. Chim. Acta*, **18**, 1483 (1936).

(36) Reichstein, *Helv. Chim. Acta*, **20**, 978 (1937).

(37) Cook and Haslewood, *J. Chem. Soc.*, 428 (1933).

analogy is in the appearance in the urine of pregnant mares of the oestrogen equilenin, which probably arises from a steroid precursor and which contains two aromatic rings. This suggests that an abnormal process leading to the formation of a carcinogen may be only slightly differentiated from normal sex hormone metabolism. Suspicion as to the point of origin of a "degenerated biocatalyst"[38] centers around the adrenal cortex partly because steroids of the adrenal cortical type appear to be the likely precursors of all the sex hormones[35] and partly because this gland, particularly when hyperactive, appears to maintain conditions favorable for dehydrogenative processes. A second essential step in the formation of a carcinogen is the removal of the steroid oxygen atom at the 3-position, for if this were to remain in the form of a phenolic hydroxyl group the aromatized product very probably would be non-carcinogenic.[39] Once the end ring A of the molecule has been aromatized, as in the case of all of the oestrogenic hormones, there is little likelihood that the deactivating phenolic hydroxyl group can be eliminated, and this may be advanced as an argument for discounting the possibility that any of the known oestrogens can undergo biological transformation into a carcinogenic hydrocarbon. An indication that the oxygen atom can be eliminated in the course of steroid metabolism while the terminal ring is still in the hydroaromatic condition is furnished by the isolation from male and female adrenal tumor urines of $\Delta^{3,5}$-androstadienone-17 (VII), in which the C_3-oxygen is missing, and the argument will hold good whether this is formed in the body or arises from a very easily dehydrated precursor such as IX.

The adrenal gland is again indicated as a likely point of origin of a possible carcinogen. If one of the potent carcinogens of the 1,2-benzanthracene series were to be formed from a known cortical steroid as precursor, an additional six-membered ring would have to be built onto the molecule. It may be noted that with utilization of the two-carbon side chain, only one more carbon atom would be required, and that a cortical steroid with carbonyl groups at the 11 and 20 positions would offer points of fixation of the carbon residue. Thus dehydrocorticosterone (XI), a substance isolated from the adrenal cortex[40] and known to possess cortin activity,[41] might condense with a carbonyl compound (RCOA) at the two activated positions 12 and 21 to give a six-ring. The carbonyl component might be a



XI XII

simple aldehyde, an α-keto acid, or an easily degraded derivative of one of the ketols of the adrenal cortex. By elimination of oxygen and dehydrogenation, the intermediate might afford cholanthrene (XII, R=H) or a 23-alkyl cholanthrene.

Experimental Part[42]

Hydrolysis and Extraction. The urine was collected, treated with toluene (5 cc. per liter) as a preservative, and processed in 1-l. portions, usually within one or two days after collection. A 1-l. portion was acidified with 10 cc. of concentrated hydrochloric acid and the mixture was boiled for ten minutes, cooled, and extracted with benzene in a Smith and Smith continuous extractor[24] for twenty-two to twenty-four hours. The last few extractions were made, after similar hydrolysis, with carbon tetrachloride in the Hershberg–Wolfe apparatus[25]; a one-hour extraction period in this case was adequate. The solvent was then removed under reduced pressure and the resulting gum taken up in ether and washed several times with saturated sodium bicarbonate solution, the washings being discarded. Oestrogens were next removed by several washings with 2 *N* sodium hydroxide. Considerable color was eliminated at this point by washing the ethereal solution twice with portions of a solution of 10 g. of sodium hydrosulfite in 1 l. of 2 *N* sodium hydroxide, the mixture being shaken each time for about five minutes. After repeated washing with water, the ethereal solution was evaporated to dryness. For purposes of colorimetric assay by the Zimmermann reaction,[22] the residue was dissolved in 95% alcohol and an aliquot portion removed for analysis. For the chemical investigation the alcoholic solutions of the extracts from a total of 28.5 l. of urine were combined and evaporated under reduced pressure, giving a dark gum weighing 12.5 g.

Separation of the Ketonic Fraction.—The entire gum (12.5 g.) was dissolved in 60 cc. of 95% alcohol, 55 cc. of glacial acetic acid and 12 g. of Girard's reagent T were

(38) Butenandt, *Angew. Chem.*, **51**, 621 (1938).

(39) Compare the inactive 3-hydroxy-20-methylcholanthrene, synthesized by Cook and de Worms, *J. Chem. Soc.*, 1825 (1937); Fieser and Riegel, THIS JOURNAL, **59**, 2561 (1937).

(40) Mason, Myers and Kendall, *J. Biol. Chem.*, **114**, 613 (1936).

(41) Reichstein, *Helv. Chim. Acta*, **20**, 953 (1937).

(42) Microanalyses by Lyon Southworth. The melting points are all corrected.

added, and the solution was refluxed for one hour, cooled and poured into 600 cc. of ice water containing 3 g. of sodium hydroxide. The mixture was cooled to 0–5° and extracted with six 100-cc. portions of ether. The ethereal extract was washed several times with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to a gum (a). The aqueous solution remaining after the ether extraction was acidified with a cold solution of 10 cc. of concentrated sulfuric acid in 50 cc. of ice water and then covered with a layer of ether and allowed to stand overnight at room temperature. The mixture was then extracted with six 150-cc. portions of ether and the ether extract was washed with water, dried and evaporated to a slightly yellow gum. The gum (a) which had not reacted with Girard's reagent was then treated afresh with 3 g. more of the reagent, and the entire process was repeated. A small amount of additional ketonic material was obtained and this with the first batch gave a total of 7.4 g. in the ketonic fraction. The non-ketonic fraction weighed 4.3 g.

Experiment A: Separation of the Alcoholic and Non-alcoholic Steroids.—The ketonic gum (7.4 g.) was treated with 10 g. of succinic anhydride and 40 cc. of dry pyridine and the mixture heated on the steam-bath for one hour. The resulting solution was then poured into 500 cc. of ice water and extracted with six 100-cc. portions of ether. The total extract was washed with six 50-cc. portions of 10% sulfuric acid and the carbinol fraction was then removed by extraction with six 50-cc. portions of 10% sodium carbonate solution. The carbonate solution was washed thrice with ether, acidified in the cold with concentrated hydrochloric acid, and extracted with four 100-cc. portions of ether. The ether solution was dried and evaporated under reduced pressure, giving 1.05 g. of a reddish gum.

The unreacted neutral residue was treated again with succinic anhydride in pyridine, and this time the solution was refluxed for three hours. On working up the mixture as before a batch of half-succinate was obtained which was lighter colored and partly solid (4.58 g.), and this was kept separate from the above gummy product. The non-carbinol fraction weighed 2.04 g.

Dehydroisoandrosterone Half-Succinate. (a) Isolation from the Mixture.—The second batch of acid ester (4.58 g.) was taken up in alcohol, and on standing the solution deposited slightly brown crystals, m. p. 250–255°. After several crystallizations from alcohol the substance (1.28 g.) was obtained in a pure condition in the form of colorless prisms, m. p. 257–259°.

Anal. Calcd. for $C_{23}H_{31}O_5$: C, 71.28; H, 8.06. Found: C, 71.20; H, 8.57.

The mother liquors and washings were combined and evaporated under reduced pressure to a gum which when added to the first batch made a total of 4.0 g. of crude carbinol half-succinate.

(b) The **methyl ester** was prepared by treating 50 mg. of the pure half-succinate with excess diazomethane in dry ether, evaporating the solvent and crystallizing the residue from dilute methanol; m. p. 154–156°. Two further crystallizations gave 40 mg. of colorless prisms, m. p. 155.5–156.5°.

Anal. Calcd. for $C_{24}H_{33}O_5$: C, 71.78; H, 8.28. Found: C, 71.77; H, 8.67.

(c) **Saponification.**[43]—A solution of 750 mg. of the half-succinate in 25 cc. of methanol was treated with a solution of 1 g. of potassium bicarbonate in 12 cc. of water and the solution was refluxed for one and one-half hours, diluted just short of turbidity, and cooled. The crystallizate which separated was recrystallized from aqueous methanol and gave 400 mg. of dehydroisoandrosterone. This melted at 138–139°, solidified, and remelted at 147–148°, and there was no depression on admixture with authentic material. The aqueous filtrate on acidification gave 150 mg. of unsaponified material, hence the reaction was only 80% complete under the conditions used. The yield of the free steroid is 90%, based upon ester consumed.

(d) **Preparation from Pure Dehydroisoandrosterone.**—A solution of 100 mg. of dehydroisoandrosterone and 500 mg. of succinic anhydride in 5 cc. of pyridine was refluxed for two hours, cooled and poured into water. The mixture was worked up as described above and the product after repeated crystallization from alcohol formed prisms melting constantly at 257–259° and showing no depression when mixed with the isolated sample (a). The methyl ester melted at 156–157°.

(e) **Saponification of Gummy Residue.**—A solution of this material (4.0 g.) in 50 cc. of *n*-propanol was refluxed with a solution of 15 g. of potassium hydroxide in 45 cc. of water for four hours. After being cooled and diluted with an equal volume of water, the solution was extracted with four 50-cc. portions of ether, and the ethereal extract was washed with 10% sodium hydroxide containing 10% of sodium hydrosulfite and then with water, dried, and evaporated at diminished pressure, giving 2.9 g. of residual gum of keto alcohol mixture.

Digitonin Separation.—The above gum (2.9 g.) was dissolved in 50 cc. of 50% ethanol, a hot solution of 12 g. of digitonin in 450 cc. of 50% ethanol was added and the mixture allowed to stand overnight. The precipitate was collected, washed twice with 50% ethanol and dried, giving 4.35 g. of digitonide.

Since the material in the filtrate and washings could not be extracted satisfactorily with organic solvents, the combined solutions were evaporated to dryness in a stream of warm air. Following Bergmann's procedure,[27] the residue was warmed in pyridine solution at 80–90° for one hour, the pyridine was evaporated under diminished pressure, and the residue was dried in a vacuum desiccator over sulfuric acid, pulverized, and extracted with ether in a Soxhlet apparatus. The dry powder was again treated with pyridine and the process repeated. Evaporation of the ether extracts gave 1.87 g. of crude α-hydroxy steroids in the form of a yellow gum.

Decomposition of the Digitonide.—In order to determine quantitatively the amount of dehydroisoandrosterone present, a 256-mg. portion of the dried precipitate was warmed with 3 cc. of pyridine for thirty minutes at 100° and the cooled mixture was treated with 25 cc. of dry ether and centrifuged. The residue was washed by centrifugation with two 25-cc. portions of dry ether and then subjected to a repetition of the above treatment with pyridine. The ethereal solutions were combined and washed with three 25-cc. portions of 15% sulfuric acid and two 25-cc.

(43) Method of Reichstein and Euw, *Helv. Chim. Acta*, **21**, 1184 (1938).

portions of water. After drying over anhydrous sodium sulfate the solution was evaporated to dryness. The crystalline residue melted at 132–135° but was not easily recrystallized without appreciable losses, and the material was therefore converted to the benzoate by treating it with 1 cc. of pyridine and 3 drops of benzoyl chloride. The mixture was warmed at 100° for five minutes, diluted with 2 cc. of water, and warmed again for ten minutes. After cooling to 0° the crystalline product was collected (m. p. 246–252°) and recrystallized several times from ethyl acetate. There was obtained 63.2 mg. of dehydroisoandrosterone benzoate in the form of needles, m. p. 251–253°; this showed no depression when mixed with an authentic sample. The amount of pure benzoate collected represents a 90.6% recovery from the digitonide and this figure has been used in calculating the yield of the steroid.

Identification of Dehydroisoandrosterone.—A sample of the benzoate (86.2 mg.) obtained from the digitonide was refluxed for one hour with 5 cc. of 1 *N* potassium hydroxide in methanol and the solution was diluted with water and cooled. The product (64.7 mg.) when crystallized several times from aqueous methanol gave needles melting at 148–149° (upper, more characteristic m. p.) and giving no depression when mixed with dehydroisoandrosterone. The acetate (needles, m. p. 168–169°) and the oxime (plates, m. p. 192–194°) likewise were found identical with authentic samples by mixed melting point determinations.

Fractionation of the 3α-Hydroxy Steroids.—A 1.59-g. portion of the total yellow gum (1.87 g.) recovered from the mother liquors of the digitonin precipitation was dissolved in 25 cc. of carbon tetrachloride (dried over anhydrous sodium carbonate) and adsorbed on a 30 × 1.2 cm. chromatographic column of activated alumina prepared according to Brockmann. Fractional elution by the method of Callow and Callow[6,10b] was then carried out, using pure carbon tetrachloride at first and then mixtures of this solvent with small amounts of ethanol, as indicated in Table II. The solvent was run through under a slight head of mercury at a rate of about 10 cc. per minute. The fractions were evaporated to dryness at reduced pressure and each residue examined.

3α-Hydroxyandrostenone-17.—The first fraction, obtained by elution with a large volume of carbon tetrachloride, gave a residue (480 mg.) which partially crystallized on standing in contact with a small volume of methanol. When collected and washed, the crystalline product, m. p. 162–171°, amounted to 50 mg. Since the material retained some color it was sublimed at a pressure of 0.09 mm. and 115–120° (three hours) and the colorless sublimate was crystallized six times from aqueous methanol, when the substance formed prisms of the constant melting point 181–183° (depression with androsterone, m. p. 183–184°); $[\alpha]^{26.5}D$ +122° (±2°, *c* = 0.364 in 95% ethanol).

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.13; H, 9.79. Found: C, 79.15, 79.12; H, 10.07, 9.92.

On allowing the mother liquors to stand with seed at 0° for several weeks a second crop of material was obtained which, when recrystallized, afforded 60 mg. more of the same product. The mother liquor from the crystallization was evaporated and the residual gum (127 mg.) was dissolved in 200 cc. of petroleum ether (30–65°), adsorbed on alumina (Brockmann), and eluted with 100-cc. portions of benzene, dry ether, acetone, methanol, and 95% ethanol. The acetone eluate yielded 45 mg. of crystals which when recrystallized melted at 181–183°. The other fractions could not be induced to crystallize. The total amount estimated to be present in Fraction 1 is 200 mg.

Characterization of 3α-Hydroxyandrostenone-17. (a) **Action of Hydrochloric Acid.**—A solution of 8 mg. of the steroid in 3 cc. of 95% ethanol containing sufficient concentrated hydrochloric acid to give a *N*/30 solution was refluxed for two hours and diluted; the material which crystallized melted at 181–183° and gave no depression with the starting material. The experiment was repeated using a solution 1 *N* in hydrogen chloride with the same result.

(b) **Derivatives.**—For the preparation of the acetate, a solution of 10 mg. of 3α-hydroxyandrostenone-17 (m. p. 181–183°) in 0.5 cc. of acetic anhydride was treated with a few drops of pyridine and warmed at 100° for thirty minutes and diluted with water. The product which separated on cooling yielded on recrystallization from aqueous acetic acid 7 mg. of the acetate in the form of needles, m. p. 178–180°; $[\alpha]^{23.6}D$ +114° (±5°, *c* = 0.314 in 95% ethanol). The melting point was depressed markedly on mixture with the hydroxy compound.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.31; H, 9.15. Found: C, 75.91, 76.07; H, 8.97, 9.49.

TABLE II

CHROMATOGRAPHIC FRACTIONATION OF THE 3α-HYDROXY STEROIDS

Fraction	Solvent	Eluate, cc.	Substance obtained	
1	CCl_4	1000	3α-Hydroxyandrostenone-17 (200 mg.)	
2	CCl_4 + 0.1% C_2H_5OH	250	Gum	
3	CCl_4 + 0.1% C_2H_5OH	250	Androsterone (7 mg.)	
4	CCl_4 + 0.1% C_2H_5OH	250	Gum	
5	CCl_4 + 0.1% C_2H_5OH	250	3α-Hydroxyaetiocholanone-17	320 mg.
6	CCl_4 + 0.1% C_2H_5OH	250	3α-Hydroxyaetiocholanone-17	
7	CCl_4 + 0.1% C_2H_5OH	250	3α-Hydroxyaetiocholanone-17	
8	CCl_4 + 0.1% C_2H_5OH	300	3α-Hydroxyaetiocholanone-17	
9	CCl_4 + 0.1% C_2H_5OH	250	3α-Hydroxyaetiocholanone-17	
10	CCl_4 + 0.2% C_2H_5OH	200	3α-Hydroxyaetiocholanone-17	
11	CCl_4 + 0.2% C_2H_5OH	450	3α-Hydroxyaetiocholanone-17	
12	CCl_4 + 0.2% C_2H_5OH	200	3α-Hydroxyaetiocholanone-17	
13	CCl_4 + 0.3% C_2H_5OH	1000	Gum	
14	95% C_2H_5OH	200	Gum	

The **benzoate** was obtained from 8 mg. of the steroid in 0.5 cc. of pyridine and two drops of benzoyl chloride, heated at 100° for one-half hour and diluted with water. The substance crystallized from aqueous acetone in plates melting at 162–164°. The **semicarbazone** prepared from 10 mg. of the steroid in alcoholic solution formed small crystals from 95% ethanol and melted at 279–280°, decomp.

(c) **Hydrogenation of the Acetate.**—Five milligrams of 3α-hydroxyandrostenone-17 was placed in a 5-cc. hydrogenation flask together with 1 cc. of glacial acetic acid and 10 mg. of Adams catalyst. Hydrogenation was conducted at room temperature and pressure until the reaction had apparently stopped. The catalyst was removed by filtration and washed with acetic acid, the solvent was removed at reduced pressure and the residue dried overnight over sodium hydroxide in a vacuum desiccator. The product, which had crystallized on standing, was treated with 1 cc. of acetic anhydride and 3 drops of pyridine at 100° for three hours. When water was added slowly and the solution allowed to cool, the product crystallized. Recrystallization from aqueous acetone yielded 3 mg. of plates, m. p. 158–160°. The substance showed no depression in melting point when mixed with an authentic sample of androstanediol diacetate, m. p. 158–160°, prepared from androsterone acetate by the procedure of Butenandt.[44]

(d) **Polarographic Analysis.**—A polarogram of 3α-hydroxyandrostenone-17 in the form of the Girard derivative by the standard procedure[22] gave a curve of half-wave potential of about −1.45 v. and with a wave span within 2% of that found for an equivalent weight of dehydroisoandrosterone. The results charted in Fig. 1 were obtained as follows. One-milligram samples of 3α-hydroxyandrostenone-17, androsterone and dehydroisoandrosterone were each treated with 50 mg. of aluminum *t*-butoxide in 0.6 cc. of acetone and 1 cc. of benzene. After refluxing for fifteen hours, the mixtures were decomposed with dilute acid and extracted in the usual way. The solvent was removed and the samples were evacuated overnight in a desiccator in order to remove oderiferous condensation products of acetone. Each residue was then taken up in 1 cc. of isopropanol and a 0.1-cc. portion treated with Girard's reagent and polarographed by the standard procedure.

Isolation of Androsterone.—Fraction 3 (Table II) crystallized on long standing at 0°. After rubbing the material with a little aqueous methanol, 7 mg. of crystalline product was collected. Repeated recrystallization from dilute methanol gave 3 mg. of plates which melted at 182–183° and showed no depression when mixed with authentic androsterone.

Isolation of 3α-Hydroxyaetiocholanone-17.—Fractions 5 to 12 inclusive all gave crystallizates of the same appearance and showing a melting point in the range 125–137° and a second one at 133–142°. Mixed melting point determinations showed that they all consisted of the same compound and the fractions were therefore combined and crystallized from aqueous methanol, giving 149 mg. of material which melted at 138–139° and then at 147–148°. A further crop of 171 mg. was obtained from the filtrates and washings, bringing the total isolated to 320 mg.

(44) Butenandt and Tscherning, *Z. physiol. Chem.*, **234**, 224 (1935).

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.51; H, 10.40. Found: C, 78.38; H, 10.11. Callow[6] found 3α-hydroxyaetiocholanone-17 to melt at 140–141° and then at 151°.

The **acetate**, prepared from a 20-mg. sample with acetic anhydride and pyridine as above, was obtained by recrystallization from aqueous methanol in the form of needles, m. p. 94.5–96° (15 mg.). Butler and Marrian's[18] sample melted at 95–97°. The **benzoate** crystallized from aqueous methanol in the form of needles, m. p. 161–163° (Callow and Callow,[10b] 161.5–163.5°).

The Ketonic Non-Carbinol Fraction.—The residual ketonic material which had failed to react with succinic anhydride was a dark gum (2.04 g.). This was dissolved in 25 cc. of carbon tetrachloride and passed through a 15 × 135 mm. column of activated alumina (Brockmann). The column was eluted with 500 cc. of carbon tetrachloride in fractions, but since no separation was observed these were combined, giving 1.51 g. of light yellow gum. Elution with 95% alcohol then removed a highly colored gum from the tower, but no product was obtained from it. The purified gum (1.51 g.) was treated in 15 cc. of alcohol with 1.3 g. of semicarbazide hydrochloride in 5 cc. of water followed by 1.3 g. of anhydrous sodium acetate. The mixture was refluxed for four hours, during which time crystals appeared, and then cooled. There was obtained 1.34 g. of crystalline semicarbazone, m. p. 262.5–265°. This was digested with 25 cc. of alcohol at the boiling point and the mixture filtered while hot. The undissolved residue was digested again in the same way, when all of the color was removed and the **3-chloro-Δ⁵-androstenone-17 semicarbazone** was obtained as a white solid which melted with decomposition at 278.5–280.5° when heated at the rate of 5° per minute (271–273° when heated at the rate of 1°/minute). No pure product could be isolated from the mother liquor and the hydrolyzed material was an oil which failed to solidify.

3-Chloro-Δ⁵-androstenone-17.—The sparingly soluble semicarbazone (637 mg.) was boiled for twenty minutes with a mixture of 10 cc. of concentrated sulfuric acid, 15 cc. of water, and 75 cc. of alcohol. The acid was neutralized with sodium carbonate solution and the product was extracted with three 50-cc. portions of ether. After evaporation of the ether the product was taken up in methanol, from which there separated on cooling crystals contaminated with considerable yellow material. The substance was therefore sublimed at 0.001 mm. and 100–110° (one hour). The liquid sublimate solidified readily when rubbed with a rod moistened with methanol. After repeated crystallization from methanol the substance was obtained as colorless needles, m. p. 154.5–156° (Butenandt and Grosse,[26] 155–157°).

Anal. Calcd. for $C_{19}H_{27}OCl$: C, 74.36; H, 8.89. Found: C, 73.96; H, 9.10.

Experiment B: Isolation of Δ³,⁵-Androstadienone-17.—In this separate experiment 5.5 l. of urine from the same patient was hydrolyzed, extracted, and put through the Girard separation as before, and the androgen fraction (1.43 g.) was dissolved in 25 cc. of carbon tetrachloride and passed through a 15 × 20 cm. column of activated alumina (Brockmann). The column was then eluted with 200 cc. of carbon tetrachloride and the fraction evaporated under diminished pressure. On standing for several days

at 0° the oily residue partially crystallized. By rapid manipulation with methanol, the crystals could be washed free of oil on a filter, and in this way 135 mg. of crystalline product was obtained, m. p. 55–70°. The material was again leached with methanol and the colorless product was recrystallized from aqueous methanol, giving plates, m. p. 87–88.5°; $[\alpha]^{25.5}D$ −30.8° (±2°, c = 0.287 in 95% ethanol).

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 83.99, 84.55; H, 9.74, 9.56.

The substance showed no depression when mixed with a sample of $\Delta^{3,5}$-androstadienone-17 (m. p. 88–89°) prepared by the dehydration of dehydroisoandrosterone according to Burrows, *et al.*[2]

The oxime was prepared by warming 6.5 mg. of the ketone with 20 mg. of hydroxylamine hydrochloride and 20 mg. of anhydrous sodium acetate in 3 cc. of methanol at 60° for two hours and diluting with water. Recrystallized from aqueous methanol, the substance melted at 164–166°, which agrees with the value previously reported.[2]

Anal. Calcd. for $C_{19}H_{27}ON$: C, 79.80; H, 9.55. Found: C, 79.00; H, 9.56.

Summary

Five substances, and a transformation product of one of them, have been isolated from the neutral 17-ketosteroid fraction from the acid-hydrolyzed urine of a girl with a corticoadrenal tumor. Androsterone was found present in small amounts (0.3 mg./l.) corresponding to the level in normal female urine, while the quantity of 3α-hydroxyaetiocholanone-17 isolated (13 mg./l.) is about 10 times the normal amount, and the dehydroisoandrosterone encountered (88 mg./l.) represents approximately a 100-fold increase above normal. About one-third of the total dehydroisoandrosterone had been transformed into 3-chloro-Δ^5-androstenone-17 in the acid hydrolysis. The other two steroids isolated have not been encountered in the urine of normal males or females. One has been identified as $\Delta^{3,5}$-androstadienone-17 (25 mg./l.), which Burrows, Cook, Roe and Warren had isolated from the urine of a male patient having an adrenal tumor. It is suggested that the substance may have been produced in the acid hydrolysis from a precursor of the allylic alcohol type, namely, Δ^4-dehydroisoandrosterone or Δ^4-dehydroandrosterone. The other substance, found present to the extent of 8 mg./l., is a new steroid which has been characterized as a 3α-hydroxyandrostenone-17 with the double bond at either the 6,7-, 7,8-, 9,11-, or 11,12-position. It may arise from an easily dehydrated precursor, for example one with a hydroxyl group at C_{11}.

These and other observations regarding excretory steroids associated with pathological conditions suggest certain tentative inferences concerning steroid metabolism and the possible metabolic production of carcinogens in the body.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASSACHUSETTS

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Identification of Organic Compounds. IV. Trityl Ethers of Cellosolves, Carbitols and Related Glycols[1,2]

BY MARGARET K. SEIKEL[3] AND ERNEST H. HUNTRESS

The studies of this Laboratory on the systematic identification of organic compounds have now been extended to the preparation of trityl (*i. e.*, triphenylmethyl) ethers of certain important organic solvents of the classes commonly called "Cellosolves" and Carbitols" and also of related glycols. Previous work on the preparation of solid derivatives of these classes is well summarized and new instances reported in two papers[4] published while the present paper was being written.

The general method of tritylation has here been greatly simplified in comparison with that previously suggested for ethylene glycol,[5,6] methyl cellosolve[7] and cellosolve.[6] For example, ordinary rather than especially dehydrated reagents can be used, the reaction time is shortened to a few minutes, and one recrystallization of the initially crystallized products often suffices for purification.

Detailed methods of procedure are as follows.

(1) For Article II, see THIS JOURNAL, **62**, 750 (1940).

(2) Presented at the Detroit Meeting of the American Chemical Society, September, 1940.

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(4) Mason and Manning, THIS JOURNAL, **62**, 1635–1640, 3136–3169 (1940).

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