STUDIES IN STEROID METABOLISM*

XIV. THE ISOLATION FROM HUMAN URINE OF Δ⁹-17-KETOSTEROIDS

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The isolation from human urine of Δ⁹-etiocholenolone (III) (Fig. 1) and Δ⁹-androstenolone (IV), both derived by dehydration of the adrenocortical metabolites 11-hydroxyetiocholanolone (I) and 11-hydroxyandrosterone (II), is described in this report. Since the presence of 11-hydroxyetiocholanolone has been established in a very high proportion of patients with neoplastic disease and is very rarely found in normal subjects (5-8), this investigation has a very significant bearing on cancer. The presence of this unusual steroid could easily have been overlooked, were it not for the systematic application of the powerful tool afforded by infra-red spectrometry. The investigation, therefore, is reported as an application of a methodical procedure to the elucidation of the chemical structure of the compounds. The more purely clinical aspects of the problem have been published elsewhere (5-8).

Infra-red spectrometry permits the positive identification of a compound by comparison of the spectrum with that of a known, pure substance (9, 10). For this purpose, it is not necessary that the substance be obtained in crystalline form, since, indeed, non-crystalline eluates from a chromatogram often show spectra identical with those obtained from authentic samples. Prior to the use of infra-red spectrometry, it was necessary to depend upon the more usual criteria of identity, such as the constancy of properties after repeated chromatography, melting point of a pure sample and of mixtures, rotation, and the like, together with the preparation of suitable derivatives. By these means we, as well as others, have isolated and characterized a number of urinary steroids, including androsterone and etiocholanolone, two of the more abundant ketosteroids.

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These substances follow immediately upon one another when chromato-graphed on alumina. When, therefore, slight but significant changes were observed in the infra-red spectra of successive eluates, which upon isolation seemed to be either androsterone or etiocholanolone by the usual criteria, it became highly probable that a new compound was present together with the known steroid.

A comparison of the spectrum of pure etiocholanolone (A) with that of an eluate (B), which, after crystallization, was identified as essentially pure etiocholanolone (m.p. 150–155°, \([\alpha]_d = 130° \pm 2°\), no depression in mixed melting point), is shown in Fig. 2. These alterations in the spectrum were interpreted as indicative of a mixture of etiocholanolone with a new component rather than a single new compound, since then a greater change in the spectrum would have been expected. When the new substance was isolated and identified as \(\Delta^2\)-etiocholenolone (III), the spectrum (Fig. 2, C) proved to be quite different from that of etiocholanolone.

Similar study of the "androsterone" fractions led to the conclusion that the "androsterone" was a mixture of that substance with \(\Delta^4\)-androstenolone (IV, Fig. 1 and Fig. 3). The unsaturated analogue of androsterone was found in the urine of all subjects examined, whether normal or diseased, whereas the \(\Delta^8\)-etiocholenolone was found when neoplastic disease had been diagnosed. Therefore, our initial chemical efforts were directed to the purification of "etiocholanolone," and the solution of this problem in turn afforded the essential information for the structural elucidation of the new component of the "androsterone" fraction.

1 It is likely, therefore, that most of the investigators who have attempted to measure the excretion of androsterone by isolation procedures have obtained mixtures of androsterone and \(\Delta^2\)-androstenolone.
FIG. 2. Infrared absorption spectra of (A) etiocholanol-3α-one-17; (B) eluate from urinary extract containing A and C; (C) Δ^9-etiocholenol-3α-one-17. The presence of the components A and C in the mixture is indicated by the arrows at the absorption bands specific for each compound.

FIG. 3. Comparison of the infra-red spectra of androsterone and Δ^9-androstenol-3α-one-17 with a urinary eluate containing both of these substances.
Repeated chromatography of the "etiocholanolone" fractions resulted in only a slight enrichment of the new component as judged by the infrared spectra. The various fractions had melting points slightly higher than authentic samples of etiocholanolone and showed a slightly higher dextro-rotation. An attempt was then made at Dr. L. C. Craig's suggestion to effect a separation by counter-current distribution (11, 12). Since no suitable system was found for the liquid-liquid technique, a liquid-solid system (13) consisting of ether and alumina was employed. Exhaustive partition between the two phases afforded enrichment but not a complete separation of the constituents. In view of the marked similarity in behavior, the conclusion was drawn that the unknown substance must be closely related to etiocholanolone. Since compounds that differ by only one double bond are eluted simultaneously and are most difficult to separate chromatographically (14, 15), the observations that the material gave a positive test with tetranitromethane indicated that the contaminant was probably an unsaturated analogue of etiocholanolone.

Among other possibilities, Δ⁹-etiocholenolone (III) was given the most serious consideration because 11/β-hydroxyandrosterone (II) had already been isolated (16-20) from human urine and shown to be dehydrated to Δ⁹-androstenolone by heating with mineral acid. At that time, vigorous acid hydrolysis had been our usual first step in the isolation of urinary steroids and the suspicion that dehydration of an 11/β-hydroxyl group had led to an unsaturated analogue of etiocholanolone was strong in our minds. We, therefore, urged Dr. L. H. Sarett to prepare the then unknown Δ⁹-etiocholenolone for comparison with the urinary steroid. In the most generous manner Dr. Sarett made (21) the desired compound and furnished us with a sample for infra-red spectrometry. The spectrum was compared with that of the most highly purified product from urine as well as with that of pure etiocholanolone (Fig. 2). The spectrum of Δ⁹-etiocholenolone is markedly different from that of etiocholanolone, but it resembles the spectrum of the urinary material in the shape and positions of certain bands marked by arrows in Fig. 2. This was taken as evidence that the urinary material was a mixture of Δ⁹-etiocholenolone and etiocholanolone.

The preparation of a derivative of the unsaturated component was therefore undertaken, and the 9,11-epoxide seemed to offer good opportunity for separation and identification. The urinary material was oxidized with perbenzoic acid, and the epoxide was readily separated from etiocholanolone by chromatography. The epoxide was characterized and was identical in all respects, including the infra-red spectrum, with the same derivative prepared from Dr. Sarett's sample of Δ⁹-etiocholenol-3α-one-17.
Subsequently, from the urine of a patient with cancer of the breast, we obtained fractions whose infra-red spectra indicated the presence of essentially pure Δ9-etocholepensolone. It was possible to crystallize 2 mg. of pure Δ9-etocholepensolone from these fractions and thus confirm by direct isolation the identity of the compound.

The androsterone fractions from urinary extracts were found to give a yellow color with tetranitromethane. Repeated chromatography did not effect separation of the unsaturated component. The mixture was, therefore, oxidized with perbenzoic acid, and it was possible to achieve a ready separation of androsterone from an epoxide which was isolated and characterized as 3α-hydroxy-9α,11α-epoxyandrostan-17-one (V) (Fig. 4). The epoxide from the urinary material was identical in all respects, including the infra-red spectrum, with the epoxide V prepared from authentic Δ9-androstenolone, kindly furnished to us by Dr. H. L. Mason of the Mayo Foundation. A further proof of the structure of the epoxide V was obtained by oxidation to the epoxydiketone VI, which was identical with the compound prepared by Reich and Lardon (22), from allopregnane-3α,11β,17α,20,21-pentol, VII (Reichstein's Substance A). These authors had graciously prepared VI, in order to afford independent proof for the identity of the urinary steroid and kindly made their results and the product available to us for comparison. They oxidized Substance A (VII) with periodic acid and obtained the 17-ketone VIII which on dehydration gave the androstenolone IX. Epoxylation (IX, X) followed by chromic acid oxidation led to the same diketoepoxide VI obtained from urinary sources. The double bond in IX was definitely located at C9-C11 because oxidation of IX with chromic acid yielded the α,β-unsaturated 12-ketone, Δ9-androstene-3α-ol-12,17-dione (XI).

Wolfe, Fieser, and Friedgood (23) and we, at a later time (20), reported the isolation of another androstenolone from human urine.
originally suggested that the compound was the Δ^{11}-androsten-3α-ol-17-one. Although the infra-red spectra of this substance and its acetate are very similar to those of Δ^{a}-androsten-3α-ol-17-one and its acetate, the spectra of the epoxides and benzoates of both compounds show marked differences. Shoppee (24) converted the androstenolone isolated by Wolfe et al. (23) to an unsaturated diketo derivative which was not identical with Δ^{a}-androsten-3,17-dione. Fieser and Fieser (25) have recently reviewed the evidence bearing upon the structure of this androstenolone and have cast considerable doubt on the presence of an 11,12 double bond. It is apparent, therefore, that no certain conclusion about the structure of the compound in question can be drawn at this time and further investigation of the product will have to be undertaken.

The two substances reported here (Δ^{a}-androstenolone and Δ^{a}-etiocholenolone) are transformation products formed from the corresponding 11β-hydroxy compounds by dehydration during the acid hydrolysis of the urine. The isolation of 11-hydroxyetiocholanolone from human urine has been reported by us (15) and by Dingemanse and Huis in't Veld (26), and of 11-hydroxyandrosterone by us (20), by Mason and Kepler (17), and by Miller et al. (18). Both compounds are metabolites of adrenocortical hormones with 11-oxygen functions. This has been experimentally proved by their isolation after stimulation of the adrenals with adrenocorticotropic hormone and after administration of cortisone acetate (26–30). The occurrence of 11β-hydroxy derivatives of androsterone and etiocholanolone in urine thus affords, in part, a measure of the glandular production of adrenocortical hormones, and the abnormality of one of these excretory products in neoplasia clearly implies a key rôle for the adrenal gland in this syndrome of disease.

EXPERIMENTAL

The methods used to isolate the α-ketonic steroids from acid-hydrolyzed urine and to separate the individual components by fractional chromatographic analysis and the application of infra-red analysis have been described (10, 14).

Isolation and Identification of Δ^{a}-Etiocholenol-3α-one-17' (III)

Chromatographic Separation

Certain crystalline eluates obtained by chromatographic separation of urinary steroids were considered to be etiocholanolone by virtue of their

2 The microanalyses reported herein were performed by the courtesy of Dr. A. Elek, The Rockefeller Institute for Medical Research, New York. The melting points were taken in a Hershberg melting point apparatus and are correct to about ±1°.
position in the order of elution, their melting points, and mixed melting points. However, the infra-red spectra of these fractions were not identical with those given by pure etiocholanolone. Further purification a sample melting at 148-154° was recrystallized three times from acetone. The melting point of the product was 152-156°, with preliminary softening at 150°. This melting point was somewhat higher than the highest melting point of authentic etiocholanolone, 152-153°. The mixture of this material with authentic etiocholanolone did not show any depression in melting point. Analysis was in agreement with etiocholanolone.

C_{13}H_{18}O_2. Calculated, C 78.57, H 10.40; found, C 78.46, H 10.48

The infra-red absorption spectrum was different from that of pure etiocholanolone. The fractions containing the higher melting material gave a positive test with tetranitromethane, indicating the presence of an unsaturated material. The specific rotation of +132° ± 2° (10.23 mg. in 2.00 ml. of ethanol) was significantly higher than that of pure etiocholanolone, [α]_p = +111° ± 3°.

Further purification by preparation of derivatives failed to produce a uniform material. 10 mg. of the material were acetylated, and the acetate after several recrystallizations from ligroin (b.p. 30°) melted at 86-88°. It did not depress the melting point of an authentic sample of etiocholanolone acetate, m.p. 88-89°. The oxime also was prepared and after two recrystallizations from ethyl acetate^3 melted at 214-217°. Although the oxime melted at a lower temperature than an authentic sample of etiocholanolone oxime, m.p. 219-225°, there was no depression in melting point when the two were mixed.

**Counter-Current Distribution**

An attempt was made to employ the counter-current method of Craig et al. (11-13). Since it was difficult to find two liquid phases which would be suitable for steroids of closely related structure, we used, at Dr. Craig's suggestion, a solid-liquid pair, alumina and ether. In order to employ this system properly, it was first necessary to determine the ratio of alumina to ether which would distribute pure etiocholanolone equally between the two phases (K = (amount in ether supernatant)/(amount adsorbed on alumina) = 1). With 500 mg. of alumina and 50 ml. of anhydrous ether, it was found that this condition was satisfied and K was relatively constant when the amount of etiocholanolone varied from 5 to 50 mg. The distribution studies were carried out in 50 ml. glass-stoppered graduated

^3 The recrystallizations necessary to purify these derivatives undoubtedly effected a slight separation and resulted in the purification of the derivative of one component, i.e. etiocholanolone.
cylinders and equilibrium was established in about 1 minute by vigorous shaking. 44.4 mg. of the urinary "etiocholanolone" (m.p. 154-155.5°; \([\alpha]_D = +130° \pm 2°\)) were distributed between 500 mg. of alumina and 50 ml. of anhydrous ether. The ether solution was then transferred to Tube 2, which contained 500 mg. of alumina. 50 ml. of ether were then put into Tube 1 and both Tubes 1 and 2 were equilibrated. The process was repeated until thirteen distributions had been made. 0.5 ml. of methanol was added to the ether in each tube, and the material adsorbed on the alumina was eluted. The ether supernatant was filtered through a plug of cotton and evaporated to dryness and the residue weighed to the nearest 0.1 mg.

The weight of substances in each of the thirteen tubes was as follows (the values in parentheses are the optical rotations): Tube 1, 2.1 mg. (+111°); Tube 2, 3.1 mg.; Tube 3, 5.0 mg. (117°); Tube 4, 5.8 mg. (121°); Tube 5, 6.3 mg. (129°); Tube 6, 6.8 mg. (124°); Tube 7, 5.9 mg. (131°); Tube 8, 4.2 mg. (142° ± 0.5°); Tube 9, 2.6 mg.; Tube 10, 1.1 mg.; Tube 11, 0.5 mg.; Tube 12, 0.6 mg.; and Tube 13, 1.1 mg.

These results indicated that the mixture had been resolved only slightly. The higher specific rotations of the material in Tubes 6 to 8 and the infrared spectrum indicated that the unsaturated component was faster moving. The material in Tubes 3 to 11 was combined and redistributed in a similar manner between ether and alumina. The results obtained revealed that further distribution by this technique could not, with the small amount of material on hand, succeed in complete separation because the partition coefficients of the two components were too nearly alike. Therefore, this approach was abandoned in favor of the method involving the preparation of the epoxide.

**Isolation As the Epoxide**

Fractions which gave positive tests with tetranitromethane were combined (157 mg.) and treated with 5 ml. of a chloroform solution containing 40 mg. of perbenzoic acid per ml. After 48 hours in the refrigerator, the solution was diluted with ethyl acetate and the extract washed with solutions of sodium bisulfite, sodium carbonate, and water. After drying over sodium sulfate and evaporation of the solvent *in vacuo*, 161 mg. of an oil remained and this was purified by chromatography on magnesium silicate-Celite. The column was developed with the following solvents: *Fraction I*, carbon tetrachloride (300 ml.), carbon tetrachloride + 10 per cent benzene (300 ml.), carbon tetrachloride + 20 per cent benzene (300 ml.), carbon tetrachloride + 30 per cent benzene (300 ml.), carbon tetrachloride + 40 per cent benzene (450 ml.), carbon tetrachloride + 50 per cent benzene (300 ml.), benzene (450 ml.), benzene + 3 per cent ether
(300 ml.); Fraction II, benzene + 5 per cent ether (300 ml.), benzene + 10 per cent ether (300 ml.), benzene + 20 per cent ether (150 ml.), benzene + 30 per cent ether (300 ml.); Fraction III, benzene + 40 per cent ether (300 ml.), benzene + 50 per cent ether (300 ml.), ether (300 ml.), ether + 5 per cent acetone (300 ml.); Fraction IV, ether + increasing amounts of acetone and finally acetone.

Very little material was eluted in Fraction I. The crystalline material found in the eluates of Fraction II was identified by infra-red spectroscopy as pure etiocholanolone. The crystalline eluate (39 mg.) from Fraction III contained the epoxide, characterized by its infra-red spectrum. The material eluted in Fraction IV (54 mg.) was rechromatographed on magnesium silicate-Celite and furnished only small additional amounts of the epoxide.

Fraction III (39 mg.) was purified further by rechromatographing on alumina. The crystalline epoxide was eluted with ether-benzene (1:1) and after two recrystallizations from ether-pentane melted at 182-184° (rods) (Kofler block); \([\alpha]_D^{19} = +116° \pm 5°\) (6.62 mg. in 1.01 ml. of ethanol).

Pure \(\Delta^2\)-etiocholenol-3α-one-17, from Sarett's partial synthesis, was treated with perbenzoic acid, and after three recrystallizations from acetone-ligroin (b.p. 30°) the epoxide melted at 175-177°; \([\alpha]_D^{23} = +123° \pm 7°\) (3.08 mg. in 2.00 ml. of ethanol). There was no depression of the melting point when the epoxide obtained from urinary material was admixed with this sample, and the infra-red spectra of both products were identical.

The acetate of 9,11-epoxyctiocholanolone was prepared from 23 mg. of the epoxide obtained from urine, and after purification by chromatography on alumina two recrystallizations from pentane yielded crystals melting at 114° (Kofler block); \([\alpha]_D^{18} = +128° \pm 2°\) (10.37 mg. in 1.01 ml. of ethanol).

In order to prepare the diketoepoxide from the small amount of material available, the acetoxy group was removed by mild saponification at room temperature with potassium carbonate in aqueous methanol solution. 14 mg. of the product (m.p. 182–184°) were dissolved in 0.25 ml. of glacial acetic acid and treated with 0.30 ml. of a 2 per cent solution of chromic acid in 90 per cent acetic acid (2 atoms of oxygen). After standing 2½ hours at room temperature, the acetic acid was removed under diminished pressure at 50°. The residue was taken up in water and ether, the ether extract was washed several times with 5 per cent sodium carbonate solution and with water and dried over sodium sulfate, and the solvent removed. The oily residue (18 mg.) was chromatographed on 700 mg. of alumina. Crystalline 9,11-epoxyctiocholanedione-3,17 was eluted with benzene and after several recrystallizations from ether-pentane yielded
rods, m.p. 155-157° (Kofler block); $[\alpha]_{D}^{15} = +85.2° \pm 6°$. For analysis, the sample (2.394 mg.) was dried at 100° at 0.01 mm. for 2 hours.

$C_{19}H_{24}O_{3}$. Calculated, C 75.46, H 8.67; found, C 76.04, H 9.05.

From the urine of a patient with cancer, fractions were subsequently obtained which, from the infra-red spectra, contained $\Delta^2$-etiocholenolone in nearly pure form. These fractions were combined (138 mg.) and were chromatographed on alumina, with benzene and increasing amounts of ether as the eluents. The benzene + 30 to 50 per cent ether fractions yielded about 20 mg. of crystalline material which, on recrystallization from acetone, gave 2 mg. of the $\Delta^2$-etiocholenolone, m.p. 167-169.5°, with preliminary softening at 162°. When admixed with Dr. Sarett’s sample of $\Delta^2$-etiocholenolone, melting at 168-170°, there was no depression in melting point. From the mother liquor, a second crop (14 mg.) melting at 159-166°, with softening at 152°, was obtained. Recrystallization of this fraction from acetone gave 5 mg. of $\Delta^2$-etiocholenolone, m.p. 166-169.5°; $[\alpha]_{D}^{25} = +151° \pm 5°$ (2.25 mg. in 2.00 ml. of acetone). Sarett (21) reported $[\alpha]_{D}^{25} = +155.5° \pm 2°$.

Isolation and Identification of $\Delta^2$-Androstenol-3α-one-17 (IV)

Detection and Estimation

Crystalline eluates were obtained which were considered to be androsterone by the ordinary criteria; i.e., one typical fraction melted at 177-179°, with a specific rotation of $[\alpha]_{D}^{23} = +107° \pm 3.5°$. A mixture with authentic androsterone (m.p. 184-185°; $[\alpha]_{D} = +97.7°$) melted at 180-183°. However, comparison of the infra-red spectrum with that of authentic androsterone (Fig. 3) clearly demonstrated that the crystalline material was not pure androsterone. Since these fractions also gave a positive test with tetranitromethane, the presence of $\Delta^2$-androstenolone was suspected.

Perbenzoic acid titrations were carried out on those fractions which gave strongly positive tetranitromethane tests in order to estimate quantitatively the amount of unsaturated compound present in the mixtures. Two 15 mg. samples of a crude androsterone mixture were treated with a chloroform solution of perbenzoic acid. After standing in the refrigerator for 48 hours, an acidified potassium iodide solution was added and the liberated iodine was titrated with 0.0536 N sodium thiosulfate. One sample required 3.30 ml. of thiosulfate and the other 3.35 ml., average 3.33; two blank solutions of perbenzoic acid under similar conditions required 3.87 ml. Consequently, 15 mg. of the urinary steroid consumed 2.0 mg. of perbenzoic acid (equivalent to 0.54 ml. of 0.0536 N thiosulfate).
Assuming an empirical formula of C$_{19}$H$_{28}$O$_3$ (mol. wt. 288) for the unsaturated compound, this component comprised 28 per cent of the mixture.

Isolation of the Epoxide (V)

A fraction (760 mg.), which by perbenzoic acid titration contained 60 per cent of the unsaturated compound, was treated with perbenzoic acid as in the preceding experiment. The oxidation product (826 mg.) was dissolved in 300 ml. of carbon tetrachloride and chromatographed on magnesium silicate-Celite (weight, 2:1). The column was developed with benzene-carbon tetrachloride, benzene, and benzene containing 3, 5, and 10 per cent of ether. Crystalline androsterone, the identity of which was established by infra-red analysis, was eluted with ether-benzene. In the fractions obtained with benzene containing 20, 30, 40, and 50 per cent of ether and with ether alone, a new crystalline substance was found whose infra-red spectrum was different from that of androsterone. The crystalline material (94 mg.) was rechromatographed on alumina. The compound (V) eluted with ether-benzene (1:3) was recrystallized several times from acetone-ether to a constant melting point of 196-198° (Kofler block); $[\alpha]_b^{19}$ = +90.4° ± 1.50° (14.4 mg. in 1.01 ml. of ethanol).

C$_{19}$H$_{28}$O$_3$. Calculated, C 74.96, H 9.27; found, C 74.10, H 9.07

14 mg. of V were treated with benzoyl chloride in pyridine solution at room temperature. The product was purified by chromatography on alumina, and after several recrystallizations from ether-ligroin (b.p. 30°) the monobenzoate melted at 186-188°; $[\alpha]_b^{19}$ = +85.4° ± 2° (5.42 mg. in 1.01 ml. of ethanol).

C$_{23}$H$_{32}$O$_4$. Calculated, C 76.44, H 7.89; found, C 76.62, H 7.91

The epoxy benzoate did not depress the melting point of androsterone benzoate, m.p. 178-180°; $[\alpha]_b$ = +3.4°.

The acetate of the epoxide was prepared with acetic anhydride in pyridine solution and melted at 160-162° (from ether-ligroin) (b.p. 30°); $[\alpha]_b^{17}$ = +82.7° ± 2° (7.17 mg. in 1.01 ml. of ethanol). It depressed the melting point of androsterone acetate, m.p. 164.5-166.5°.

C$_{23}$H$_{32}$O$_4$. Calculated, C 72.81, H 8.72; found, C 72.61, H 8.84

18 mg. of V were dissolved in 0.25 ml. of acetic acid and treated with 0.38 ml. of 2 per cent chromic acid solution in acetic acid (2 atoms of oxygen). After the mixture was kept for 1½ hours at room temperature, the acetic acid was removed under diminished pressure and the residue was extracted several times with ether. After washing the ether solution with sodium carbonate solution and water, the solvent was removed and the crystalline diketoepoxide VI was recrystallized several times from ace-
tone-ether, m.p. 213–215°; \([\alpha]^{14}_D = +116° \pm 3°\) (8.06 mg. in 1.01 ml. of chloroform).

C_{19}H_{26}O_3. Calculated, C 75.46, H 8.67; found, C 75.47, H 8.56

The mixed melting point of this substance with the diketoepoxide prepared by Reich and Lardon (22) from Reichstein's Substance A showed no depression.

We were subsequently able to detect by infra-red analysis and isolate a pure sample of \(\Delta^8\)-androstenolone (IV) from the urine of a woman with cancer of the breast. It crystallized from ligroin (b.p. 60°) as needles melting at 187–187.5°; \([\alpha]^{23}_D = +136° \pm 5°\) (4.11 mg. dissolved in 2.00 ml. of ethanol). When mixed with a pure sample of \(\Delta^8\)-androstenol-3\(\alpha\)-one-17 (m.p. 188–189°; \([\alpha]_D = +140° \pm 2°\), generously made available to us by Dr. H. L. Mason (16), there was no depression of the melting point; \(\Delta^8\)-androstenolone did not depress the melting point of androsterone (m.p. 184–185°) upon admixture. 8 mg. of the urinary \(\Delta^8\)-androstenol-3\(\alpha\)-one-17 (IV) were oxidized with perbenzoic acid in chloroform solution. The product was purified by chromatography on alumina and crystallized twice from acetone-ligroin. The epoxide melted at 193–195° (with softening at 191°) and did not depress the melting point of V. The infra-red spectra of the two samples were identical.

**SUMMARY**

Infra-red spectrometry disclosed the presence of components previously unrecognized in the chromatographic fractions, obtained from hydrolyzed human urine, that were characterized as "androsterone" and "etiocholanolone" by conventional chemical criteria. The compounds were shown to be the unsaturated analogues \(\Delta^9\)-androstenol-3\(\alpha\)-one-17 and \(\Delta^9\)-etiocholenol-3\(\alpha\)-one-17, produced by dehydration during acid hydrolysis. The urinary precursors are the corresponding 11\(\beta\)-hydroxy steroids. 11\(\beta\)-Hydroxyandrosterone is found in the urine of normal and diseased subjects, whereas its isomer, 11\(\beta\)-hydroxyetiocholanolone, is rarely found in the urine of normal people but is significantly associated with neoplastic disease.

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