There is as yet no complete confirmation that the bleached compound is an oxidation product of chlorophyll. An alternative explanation is that complex formation with Fe^{+++} occurs.[4] The interpretation of the bleaching as formation of an intermediate oxidation product is supported by a similar transitory bleaching by bromine and iodine and the production of allomerized chlorophyll on allowing the ferric salt to react without reversion. The effect of non-oxidizing salts[4] is probably similar to the acceleration of allomerization by lanthanum chloride[3]; dissolved oxygen was necessary and again an initial partial bleaching was observed.

This investigation was sponsored by the O.N.R. (Contract NR-ori-212 for the year 1948) at the University of Minnesota.

(4) M. S. Ashkinazi, T. S. Glikman and B. J. Dain, *Compt. rend. Acad. Sci., U. R. S. S.*, **73**, 743 (1950).

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The Origin of 3,5-Cycloandrostan-6β-ol-17-one (i-Androsten-6β-ol-17-one) in Urinary Extracts

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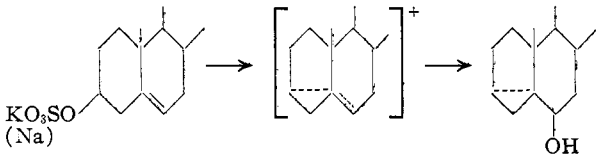
In 1948 Dingemanse, Huis in't Veld and Hartogh-Katz[1] reported the isolation from human urine of a new 17-ketosteroid, $C_{19}H_{28}O_2$, m.p. 140.5–141°, which shortly thereafter was identified[2–4] as 3,5-cycloandrostan-6β-ol-17-one (III).[5] Because III was readily converted even at room temperature by aqueous hydrochloric acid into 3-chloro-Δ^5-androsten-17-one (IV) and dehydroisoandrosterone (V), it was concluded[2] that the i-steroid was in fact the precursor of these familiar urinary products. III was isolated from urine only when neutral urine heated on a steam-bath was extracted continuously with benzene, a procedure which was postulated[6]



I II III

(1) E. Dingemanse, L. G. Huis in't Veld and S. Hartogh-Katz, *Nature*, **161**, 848 (1948).

(2) E. Dingemanse, L. G. Huis in't Veld and S. Hartogh-Katz, *ibid.*, **162**, 492 (1948).

(3) D. H. R. Barton and W. Klyne, *ibid.*, **162**, 494 (1948).

(4) E. Dingemanse and L. G. Huis in't Veld, *J. Biol. Chem.*, **195**, 827 (1952).

(5) It has been postulated on theoretical and molecular rotation considerations that the i-steroids formed by rearrangement of the 3-tosylates have a 6β-configuration; ((a) R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948); C. W. Shoppee, *Bull. soc. chim.*, **18**, 120 (1951)). In a recent comprehensive review of the stereochemistry and mechanism of this rearrangement convincing chemical evidence (b) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 3361 (1952)) has been presented which further substantiates this assignment. Based on reactions whose stereochemical course has not been clearly elucidated, Wallis and co-workers ((c) A. F. Wagner, N. E. Wolff and E. S. Wallis, *J. Org. Chem.*, **17**, 529 (1952)) have assigned the opposite configuration to the i-steroid.

(6) E. Dingemanse, L. G. Huis in't Veld and S. Hartogh-Katz, *J. Clin. Endocrin. and Metab.*, **12**, 66 (1952).

to cause the hydrolysis of a "certain conjugate" of III without effecting its subsequent conversion to IV or V. The failure of previous workers to isolate any i-steroid was ascribed to the use of methods which involved acid hydrolysis either at room or elevated temperatures.[2]

This paper reports the conversion of sodium and potassium dehydroisoandrosterone sulfate (I) to 3,5-cycloandrostan-6β-ol-17-one (III) using the conditions employed by Dingemanse, *et al.*,[1,6] for the isolation of III from urine, *i.e.*, heating a neutral aqueous solution covered with a layer of benzene and also by refluxing solutions buffered at various *p*H values under toluene. Since I has been isolated from urine[7] and is the most important known urinary conjugate of dehydroisoandrosterone, its conversion to III can satisfactorily account for all the observed results without postulating that the i-steroid is a naturally occurring urinary metabolite which serves as a precursor of dehydroisoandrosterone. The equivocal manner by which III was isolated from urine leaves the existence of the i-steroid as a genuine steroid metabolite in doubt.[8]

The potassium dehydroisoandrosterone sulfate employed in our experiments was prepared[9] by the the action of pyridine–sulfur trioxide[10] on dehydroisoandrosterone. To ensure that the product of this reaction was indeed the 3-sulfate of the normal steroid and not the 6-sulfate of the i-compound resort has been taken to the Method of Molecular Rotation Differences.[11] Earlier data indicate that a sulfate group makes only a small negative contribution to the M_D value. Thus, the $\Delta M_D(\text{M–O–SO}_2\text{O–})$ calculated from sodium cholestan-3β-ol sulfate (M_D +81)[9] and cholestan-3β-ol (M_D +113) is −32, and that calculated from sodium cholestan-3α-ol sulfate (M_D +74)[9] and cholestan-3α-ol (M_D +132) is −58. It seems certain, therefore, that the sulfate group in I is at C_3 since the $\Delta M_D(\text{M–O–SO}_2\text{O–})$ calculated from potassium dehydroisoandrosterone sulfate (M_D +28) and dehydroisoandrosterone (M_D +29) is −1. On the other hand, the M_D of the 6-sulfate of the i-steroid (III) would be expected to be a large positive value, only slightly less positive than that of the M_D of the corresponding alcohol (+351).

The hydrolysis of steroid sulfates has been studied under a variety of conditions and the results can be summarized as follows:

At *room temperature* the sulfates can be cleaved under acidic conditions and this has been shown[9] to involve the splitting of the S–O bond of the sulfate, affording the alcohol with retention of configuration.

When *elevated temperatures* are employed, the C–O bond of the sulfate ester linkage may dissociate to give a resonating hybrid (II), such as described by

(7) P. Munson, T. F. Gallagher and F. C. Koch, *J. Biol. Chem.*, **152**, 67 (1944).

(8) H. L. Mason and W. W. Engstrom (*Physiol. Rev.*, **30**, 321 (1950)) have already called attention to this and have suggested that the procedure used for extraction might be responsible for the conversion of I to III.

(9) S. Lieberman, L. B. Hariton and D. K. Fukushima, THIS JOURNAL, **70**, 1427 (1948).

(10) A. E. Sobel and P. E. Spoerri, *ibid.*, **63**, 1259 (1941).

(11) D. H. R. Barton and W. Klyne, *Chemistry and Industry*, 755 (1948).

Shoppee[12] and Winstein and Adams.[13] The driving force[14] for such a dissociation is provided by the participation of the 5,6-double bond as a neighboring group, and results in centers of low electron density at C_3 and C_6. When heat is applied, *under neutral conditions*, and continuous extraction with benzene, the thermodynamically less stable but more rapidly formed i-androstenolone results from the attack of the solvent, water, on II at C_6. The continuous removal of the i-steroid into the organic phase makes possible its isolation and may increase the extent to which it is formed.

When *elevated temperatures and hydrochloric acid* are employed for the cleavage of the sulfates, two other types of products can be isolated in addition to dehydroisoandrosterone itself; the 3-chloro compound[15] which would result from an attack of chloride ion on II and the diene[16] which would result from the loss of a proton from II, an elimination reaction which is known to be favored by elevated temperatures.[17] Whether the dehydroisoandrosterone is formed from the sulfate by cleavage of the S–O bond, by rearrangement of an intermediate i-derivative or by attack of the solvent, water, on II at C_3 cannot now be decided.

When a solution of sodium dehydroisoandrosterone sulfate[18] was heated to boiling under toluene at *p*H 1.2, and at *p*H 3.2 (0.1 *M* phthalate buffer) and the reaction product subjected to countercurrent distribution, dehydroisoandrosterone was the only product recognized. However, at *p*H 4.7 (0.1 *M* acetate buffer) equal amounts of dehydroisoandrosterone and 3,5-cycloandrostanolone were realized. These latter conditions were recommended by Bitman and Cohen[19] as suitable for the hydrolysis of Δ^5-3β-stenol sulfates without concomitant production of transformation products of dehydration and halide formation. The present data indicate that another type of artifact may be introduced under these conditions. At *p*H 6.8 (0.1 *M* phosphate buffer) the yield of the i-steroid was more than twice that of dehydroisoandrosterone (Table I).

From these considerations it is obvious that a change in any of the reaction conditions, such as temperature, acidity, nature of the anions present, and time can alter the character and the ratio of the products isolated and any conclusions concerning urinary steroid metabolites must take into account the nature of the hydrolytic procedure.

TABLE I

THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE HYDROLYSIS OF DEHYDROISOANDROSTERONE SULFATE

		Reflux	Dehydroisoandrosterone			3,5-Cycloandrostanolone		
Buffer	*p*H	time, min.	Mg.	Yield, %	K^a	Mg.	Yield, %	K^a
HCl	1.2	15	3.7	42	0.62[b]			
Phthalate	3.2	50	5.3	60	.67			
Acetate	4.7	240	3.0	34	.50	3.1	35	1.22[c]
Phosphate	6.8	360	2.5	28	.62	6.3	71	1.64

[a] Partition coefficient in cyclohexane/50% MeOH–50% H_2O. [b] Average K = 0.60. The previously determined value for the pure compound was 0.62. [c] The previously determined value for the pure compound was 1.7.

(12) C. W. Shoppee, *J. Chem. Soc.*, 1147 (1946). For further elaboration of the intimate details of the mechanism see ref. 5b.

(13) S. Winstein and R. Adams, THIS JOURNAL, **70**, 838 (1948).

(14) S. Winstein and E. Grunwald, *ibid.*, **70**, 828 (1948).

(15) A. Butenandt and H. Dannenbaum, *Z. physiol. Chem.*, **229**, 192 (1934).

(16) H. Burrows, J. W. Cook, E. M. F. Roe and F. L. Warren, *Biochem. J.*, **31**, 950 (1937).

(17) E. D. Hughes and C. K. Ingold, *Trans. Faraday Soc.*, **37**, 657 (1941).

(18) Prepared by the method of Levi, Holden and Bromley, THIS JOURNAL, **71**, 3844 (1949), and generously supplied by Ciba Pharmaceutical Products, Inc.

(19) J. Bitman and S. L. Cohen, *J. Biol. Chem.*, **191**, 35 (1951).

Experimental

The Conversion of Potassium Dehydroisoandrosterone Sulfate (I) to 3,5-Cycloandrostan-6β-ol-17-one (III).—One hundred milligrams of potassium dehydroisoandrosterone sulfate (m.p. 218–220°, $[\alpha]^{19}D$ +6.9 ± 2° (10.25 mg. in 2 cc. of a 1:3 propylene glycol–ethanol mixture); *Anal.* Calcd. for $C_{19}H_{27}O_5SK$: S, 7.88. Found: S, 8.13) was suspended in 90 cc. of water. The solution was brought to neutrality (glass electrode) with a few drops of 5% sodium carbonate solution and then covered with 30 cc. of benzene. The mixture was heated under reflux on a steam-bath with stirring for 5 hours. The benzene layer was separated, dried over sodium sulfate and evaporated to dryness leaving a crystalline product weighing 35 mg. (theoretical, 71 mg.). A second 5-hour heating with an additional 30 cc. of benzene resulted in a colorless crystalline product weighing 28 mg. These fractions were recrystallized once from ligroin (b.p. 90°)–ether and melted at 135–139°, $[\alpha]^{25}D$ +111 ± 2° (9.21 mg. in 2 cc. of chloroform), reported[3] m.p. of 3,5-cycloandrostan-6β-ol-17-one 139–140°, $[\alpha]D$ +122° (ethanol). On admixture with dehydroisoandrosterone (m.p. 145°, $[\alpha]D$ +11°), it melted at 96–104°.

Ten milligrams of the crystalline material was acetylated at room temperature with 0.1 cc. of acetic anhydride and 0.1 cc. of pyridine. The acetylated product, obtained by evaporation of the acetic anhydride and pyridine was purified by chromatography on silica gel. The acetate, eluted with ether–ligroin (1:3), was recrystallized once from acetone–ligroin (b.p. 30°) and melted at 110–113°. When mixed with an authentic sample of 3,5-cycloandrostan-6β-ol-17-one acetate (m.p. 115–116°), generously made available to us by Dr. W. Klyne, the sample melted at 113–116°. The infrared spectra[20] of the two samples were indistinguishable.

A third period of heating with 30 cc. of benzene gave 10 mg. of a brownish semi-crystalline residue. This material as well as the mother liquor from the recrystallization of the first two fractions was examined for absorption in the ultraviolet between 235 and 250 mμ. No absorption characteristic of $\Delta^{3,5}$-androstadien-17-one (maximum at 240 mμ) was observed.

An effort was made to determine whether small amounts of dehydroisoandrosterone were also formed by this procedure. The unacetylated fractions above were combined and carefully chromatographed on silica gel. The infrared spectrum of each eluate was determined. Only i-androstenolone could be detected; the characteristic spectrum of dehydroisoandrosterone was not observed.

Hydrolysis of Sodium Dehydroisoandrosterone Sulfate in Buffer Solutions.—Sodium dehydroisoandrosterone sulfate (12 mg.) was dissolved in 100-ml. portions of 0.1 *M* buffer solution of the required *p*H and refluxed under a layer of 25 ml. of toluene. After cooling, the toluene was removed and the aqueous solution was washed with four 20-ml. portions of ether which was added to the toluene solution. After washing with sodium bicarbonate solution and distilled water the organic phase was taken to dryness under reduced pressure. The residue was dissolved in 50% aqueous methanol (preequilibrated with cyclohexane) and transferred to a stainless steel 25-tube Craig counter-current distribution apparatus.[21] After 24 transfers had been accomplished, the contents of the tubes were removed and the solvents removed by evaporation from the frozen state. The residues were then analyzed for ketosteroids by the Zimmermann reaction[22,23] and the theoretical distribution curves calculated by the graphical method of Slaunwhite.[24] The contents of selected

(20) A Perkin–Elmer model 12C infrared spectrometer was used.

(21) L. C. Craig and H. O. Post, *Anal. Chem.*, **21**, 500 (1949).

(22) I. T. Nathanson and H. Wilson, *Endocrinology*, **33**, 189 (1943).

(23) H. Wilson and I. T. Nathanson, *ibid.*, **37**, 208 (1945).

(24) W. R. Slaunwhite, Jr., *Anal. Chem.*, **23**, 687 (1951).

tubes were also examined by infrared spectroscopy to confirm the identities of the materials present.[25]

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(25) The Perkin–Elmer Model 21 double beam infrared spectrophotometer was used.

(26) This work undertaken independently and simultaneously in the Columbia and Harvard laboratories is published here jointly.

(27) This work was supported in part by gifts from the Squibb Institute for Medical Research and the Damon Runyon Memorial Fund.

(28) This is publication 782 of the Cancer Commission of Harvard University. This work was supported in part by an Institutional Grant from the American Cancer Society, Inc., to the Massachusetts General Hospital and in part by a grant from the National Cancer Institute (U. S. Public Health Service).

The Preparation of β-Ketosulfonyl Chlorides[1]

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The conversion of a β-ketosulfonic acid or its salt to the corresponding sulfonyl chloride does not appear to have been reported previously. The action of chlorosulfonic acid on acetophenone produced the 2,ω-disulfonyl chloride, while acetophenone-4-sulfonic acid with chlorosulfonic acid probably formed the 4,ω-disulfonyl chloride.[3] Treatment of sodium ω-acetophenonesulfonate with the same reagent resulted in the 2,ω-disulfonyl chloride.[3] The preparation of γ- and δ-ketosulfonyl chlorides has been accomplished by conventional methods.[4,5]

When sodium ω-acetophenonesulfonate[6] was treated with phosphorus pentachloride or phosphorus oxychloride, charring occurred. The free sulfonic acid was then prepared by a modification of the procedure of Truce and Alfieri.[6] Separate portions were immediately treated with phosphorus pentachloride, thionyl chloride, or phosphorus oxychloride, but charring occurred in all cases. However, treatment of the acid with phosphorus trichloride,[7] resulted in the formation of ω-acetophenonesulfonyl chloride. The sulfonyl chloride was found to be too unstable to be submitted for analysis; it was necessary to prepare the sulfonamide for that purpose. The instability of the free acid and the sulfonyl chloride is probably due in part to the activity of the methylene group.[8] The activity of the methylene group is further demonstrated by the fact that the ester, ethyl ω-acetophenonesulfonate, can be dissolved in dilute aqueous sodium hydroxide and can be recovered by the addition of acid.

Attempts to reduce the sulfonyl chloride to ω-acetophenonesulfinic acid by conventional methods failed. Friedel–Crafts reactions with the sulfonyl chloride were unsuccessful due to the decomposition of that substance with the evolution of sulfur dioxide.

In an attempt to obtain a more stable β-ketosulfonyl chloride, isobutyrophenone-α-sulfonyl chloride was synthesized. Isobutyrophenone-α-sulfonic acid was prepared by the dioxane-sulfotrioxide method, and the sulfonyl chloride was prepared by means of phosphorus trichloride. These two substances were found to be much more stable than the analogous derivatives of acetophenone, *e.g.*, isobutyrophenone-α-sulfonic acid has been stored in a paraffin sealed bottle for a year with little apparent change. Despite its greater stability, attempts to reduce the sulfonyl chloride to the sulfinic acid and to carry out Friedel–Crafts reactions with it met with failure.

Experimental[9]

ω-Acetophenonesulfonyl Chloride.—Acetophenone (159 g., 1.34 moles) was added to 1.34 moles of the dioxane sulfotrioxide reagent[6] in 400 ml. of ethylene chloride. The reaction mixture was cooled with an ice-bath, whereupon the sulfonic acid separated out. The product was removed by filtration and placed in a vacuum desiccator for three hours. A portion of the acid was recrystallized twice from ethyl acetate; m.p. 75–78°, lit.[8,10] 73–75°, 77–78°. The crude acid and 361 g. (2.62 moles) of phosphorus trichloride were heated on the steam table under gentle reflux for 14 hours. The excess phosphorus trichloride was removed at a water aspirator and the product was recrystallized from chloroform; conversion 122 g. (42% of theory), m.p. 87.5–88.2°.

Dry ammonia was passed into an ether solution of ω-acetophenonesulfonyl chloride until the ammonia odor persisted. The resulting product was recrystallized from ethanol; m.p. 158–159°.

Anal. Calcd. for $C_8H_9O_3NS$: N, 7.04. Found: N, 7.02.

Thirteen grams (0.060 mole) of ω-acetophenonesulfonyl chloride was dissolved in 46 g. (1.0 mole) of absolute ethanol. Heat was liberated and after an hour the solution was cooled with an ice-bath, whereupon crystals of the product separated out; conversion 9.0 g. (64% of theory), m.p. 44.5–45.5° after recrystallization from petroleum ether. The neutral equivalent was obtained by titrating an alcoholic solution of the ester with standard aqueous sodium hydroxide solution to a phenolphthalein end-point; calcd. 228; found, 229 and 227.

Anal. Calcd. for $C_{10}H_{12}O_4S$: C, 52.62; H, 5.29. Found: C, 52.88; H, 5.29.

Isobutyrophenone-α-sulfonyl Chloride.—Using the same procedure as previously described, 84 g. (0.57 mole) of isobutyrophenone (b.p. 217–220° (746 mm.)) was converted to 81 g. (63% of theory) of isobutyrophenone-α-sulfonic acid. A portion of the acid was recrystallized from ethyl acetate and melted at 69.5–72.0°.

Isobutyrophenone-α-sulfonic acid (51 g., 0.22 mole) and 21 g. (0.15 mole) of phosphorus trichloride were heated on a steam-bath overnight. The reaction mixture was decomposed with ice water and extracted with chloroform. After drying over sodium sulfate, chloroform was removed by distillation and the resultant yellow oil was taken up in boiling petroleum ether. White needles separated out; weight 25 g. (44% of theory), m.p. 40.0–41.5°.

Anal. Calcd. for $C_{10}H_{11}ClO_3S$: C, 48.64; H, 4.49. Found: C, 48.75; H, 4.42.

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(1) An abstract of a portion of a thesis submitted by Calvin W. Vriesen to the Faculty of Purdue University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, August, 1952.

(2) Purdue Research Foundation Fellow.

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(6) W. E. Truce and C. C. Alfieri, THIS JOURNAL, **72**, 2740 (1950).

(7) W. A. Proell, C. F. Adams and B. H. Shoemaker, *Ind. Eng. Chem.*, **40**, 1129 (1938).

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