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Synthesis of 16-Oxygenated steroids

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Thesis

Synthesis of 16-Oxygenated Steroids

by

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To Mother and Dad

I wish to express my sincere appreciation to Dr. Herbert H. Wotiz for his aid and suggestions regarding my thesis, and for the interest he has shown in my medical career. I am indebted to Dr. Peter Ofner for many tangible and intangible qualities learned while first starting my graduate studies. My thanks go to Mrs. Erika Smakula for the determination and interpretation of infrared data. Also, Dr. Herbert Mescon has been most helpful in providing laboratory space and financial assistance during my studies.

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INTRODUCTION

This thesis deals with methods for the preparation of steroids substituted at carbon 16 of the steroid nucleus. These compounds have been prepared with the possibility in mind that certain *in vitro* metabolic products of testosterone may prove to be oxygenated at position 16. Compounds have been prepared in both the 5α and 5β-androstane series of steroids, by following the method of Leeds, Fukushima and Gallagher (1) for the synthesis of the 16-oxygenated compounds. In addition, a method is presented for the chromatographic separation of 5β-androstane-3,17-dione from its 5α stereoisomer, as well as the separation of 3α-hydroxy-5β-androstan-17-one from its 3β epimer.

HISTORICAL SURVEY

Samuels, in 1947, developed a method for studying the tissue catabolism of 17β-hydroxy-androst-4-en-3-one (testosterone) and found various animal livers capable of transforming this steroid (2, 3). Diphosphopyridine nucleotide, DPN, was shown to be an essential co-factor in this reaction (4, 5). The importance of the role of DPN in the destruction of testosterone was also shown by the fact that the addition of nicotinamide, a known inhibitor of nucleotidase, to the incubation medium considerably increased the transformation of testosterone by normal rat-liver tissue (4). Thus, the requirement for DPN, a known co-enzyme, indicates the enzymatic nature of the reaction and supports the hypothesis of the stepwise breakdown involving the intermediate formation of 17-ketosteroids (4). Citrate also speeds up the destruction of testosterone, however, without the production of 17-ketosteroids (4).

Further work by Sweat et al. (5) demonstrated that DPN, in minces and crude extracts, increased the destruction of the α,β-unsaturated ketone group and enhanced the formation of 17-ketosteroids, while the more purified extracts affected only the C-17 group. A possible explanation of this finding could be that an enzyme acting on the conjugated system in ring A works more effectively on a compound having a ketone at C-17, such as androst-4-ene-3,17-dione (androstenedione) than on a 17-hydroxy compound. It therefore seemed likely that the favored route of breakdown is through androst-4-ene-3,17-dione formed by the DPN-activated enzyme. Enzyme systems involved in the catabolism of testosterone were demonstrated in kidney tissue from dog, rabbit, and guinea pig. The principal action of the kidney enzymes in all these species, was to oxidize the hydroxyl group at C-17 to a ketone, using DPN as a cofactor, in both the dog and guinea pig, but not in the rabbit (6). Liver tissue of various species destroyed the conjugated system in ring A of both testosterone and 17α-methyltestosterone (7). With the addition of DPN to the incubation mixtures, the formation of 17-ketosteroids from testosterone and the destruction of the Δ^4-3-ketone moiety were both increased in all species except the rabbit. The nucleotide had no effect on methyltestosterone, indicating that the methyl group on carbon-17 prevents the action of an additional enzyme acting on ring A. When citrate was added as cofactor, the destruction of the conjugated double bond system in ring A was increased similarly in both steroids, with no elevation of 17-ketosteroid levels.

Schneider and Mason (8) reported that rat liver slices were able to effect both 16α-hydroxylation and reduction of 17-ketone to a β-oriented hydroxyl, as evidenced by the transformation of 3β-hydroxy-androst-5-en-17-one to androst-5-ene-3β,16α,17β-triol, which had previously been isolated from normal urine (9) and from urine of patients with adrenal tumors (10, 11). Testosterone was shown to be transformed by rabbit-liver slices to androstenedione, 17α-hydroxy-androst-4-en-3-one and to small amounts of other partially identified steroids, including a $C_{19}H_{28}O_3$ compound (12).

When 5β-androstane-3α,16α,17β-triol and 5α-androstane-3α,16α,17β-triol were isolated from human urine (13), Lieberman stated that "the hydroxylation of C-16 appears to be a general pattern in the metabolism of steroids and that the introduction of a 16-hydroxy group into a C_{19}-steroid is not dependent upon the presence of a 17-oxygen function, since several 16-hydroxylated C_{21} compounds have been isolated."

Wotiz et al. (14) demonstrated that benign hypertrophic human prostate metabolized testosterone and identified androstenedione as one of the transformation products. Lemon, Wotiz and Robitscher (15) further showed a rapid enzymatic interaction in vitro between benign or malignant prostatic tumors and testosterone. Carcinomatous human prostate tends to metabolize testosterone more quickly than does benign hypertrophic prostate. It was also shown by these authors that normal skin and malignant breast tissue metabolized far greater amounts of testosterone than did any of the other tissues. Prostatic and testicular tissues were able to reduce androstenedione to testosterone (14). On paper-chromatographic separation of the transformation products it was seen that all tissues incubated with testosterone formed androstenedione with the exception of the testis (14). In some cases additional Zimmermann-positive material of a mobility different from testosterone and androstenedione was detected. It is significant to note that these investigators found substances more polar than testosterone in all cases.

In a preliminary investigation (16), Ofner presented further evidence that compounds more polar than testosterone are major transformation products in rat liver. Hydroxylation reactions were postulated to account for the formation of the polar 17-ketosteroids. As was mentioned previously Lieberman isolated 16-hydroxy steroids from human urine. Marrian et al. (17) isolated $3\beta,16\alpha$-dihydroxy-androst-5-en-17-one from the urine of normal men and concluded that it probably was a metabolic product of 3β-hydroxy-androst-5-en-17-one. He felt that the 16α-hydroxy compound may have the same metabolic relationship to 3β-hydroxy-androst-5-en-17-one and androst-5-ene-3β, $16\alpha,17\beta$-triol as $3,16\alpha$-dihydroxy-estra-1,3,5(10)-trien-17-one may have to 3-hydroxy-estra-1,3,5(10)-trien-17-one (estrone) and estra-1,3, 5(10)-triene-$3,16\alpha,17\beta$-triol (estriol).

Ofner (16) speculated that 16-hydroxy-17-ketosteroids may have been formed under the conditions described in his experiment. If this is the case, he stated, rupture of the steroid D-ring may give rise to further compounds.

This same author (18) also found that 17β-hydroxy-androst-1-en-3-one was a product of the incubation of testosterone-4-C^{14} with human benign hypertrophic prostate. Of twenty-five steroid metabolites obtained in a dog liver perfusion study, six products were identified, one of which was $16\alpha,17\beta$-hydroxy-androst-4-en-3-one (19). Axelrod feels that two of the transformation products isolated are especially significant when considered in connection with the suggestion that the principle pathway of testosterone metabolism in dog liver is rupture of the ring system. These two compounds are $16\alpha,17\beta$-dihydroxy-androst-4-en-one and $2\beta,17\beta$-dihydroxy-androstan-3-one (19).

From the neutral fraction of equine pregnancy urine, Heard and McKay (20) had isolated an hydroxy ketone. The parent hydrocarbon was obtained by Clemmensen reduction of the diketone and identified as androstene. Huffman and Lott (21) later showed that Heard's ketone was 3β-hydroxy-androstan-16-one. Marrian et al. (22) have isolated 16α-hydroxyestrone from the urine of pregnant women. The group suggests the possibility that 16α-hydroxyestrone may be the metabolic intermediate between estrone and estriol. 3,17β-diol-estra-1,3,5(10)-trien-16-one (16-ketoestradiol-17β) was shown to be a metabolite of estradiol-17β (23). In recent publications Layne and Brown have reported the isolation of 3,16β-dihydroxy-estra-1,3,5(10)-trien-17-one (16β-hydroxyestrone) from the urine of pregnant women (24, 25).

In 1939, Butenandt et al. (26) were able to synthesize 16β-17β-dihydroxy-androst-4-en-3-one (16β-hydroxytestosterone) in poor yield. Stodola and coworkers (27) found that the androst-5-3,16,17-triol required in the synthesis of 16β-hydroxytestosterone by the Butenandt method could easily be prepared by the reduction of the isonitroso derivative of 3β-hydroxy-androst-5-en-17-one with zinc dust and acetic acid. In this reduction, an α ketol was formed instead of the expected α-amino ketone. Thus a very satisfactory method became available for the introduction of an hydroxyl group adjacent to a keto group in the steroid nucleus.

Huffman (28) converted estrone to the 16-isonitroso derivative (III), and of Stodola reduction obtained a mixture of α-ketols. From this mixture he isolated 16β-hydroxyestrone (Fig. 1). In 1944, estra-1,3,5(10)-triene-3,16α,17α-triol was synthesized (29). This compound was epimeric with naturally occurring estriol. Huffman (28) and Huffman and Lott (30) accomplished the partial synthesis of estriol, 3,17β-dihydroxy-estra-1,3,5(10)-trien-16-one and 3-hydroxy-estra-1,3,5(10)-triene-16,17-dione. The 3,17β-dihydroxy-estra-1,3,5(10)-

trien-16-one resulted from the zinc-acetic acid reduction of either 16-oximinoestrone or 16-ketoestrone. Further work by these authors concerning the steric structure of steroidal 16,17-ketols and 16,17-glycols (31) showed that the 16,17-ketols are actually 16-keto, 17β-hydroxysteroids, and that no rearrangement occurs upon acetylation following the Stodola reduction. By the Clemmensen reduction of $3\beta,17\beta$-dihydroxy-5α-androstan-16-one prepared by the sequence of nitrosation and Stodola reduction of 3β-hydroxy-5α-androsta-17-one, 3β-hydroxy-5α-androstan-16-one was prepared. The compound proved to be identical with the hydroxy-ketone isolated by Heard and McKay in 1939 (20). An interesting point to note is that transference of the carbonyl from position C_{17} to C_{16} on the 3-hydroxy-estratriene nucleus followed by Clemmensen reduction of $3,17\beta$-dihydroxy-estra-1,3,5(10)-trien-16-one resulted in a change of molecular rotation of 689° in a negative direction. The molecular rotation of Heard's ketone was 700° more negative than that of 3β-hydroxy-5α-androstan-17-one.

In 1955 Huffman and Lott described a new synthetic approach to 16-ketosteroids (32) (Fig. 2) more elegant than their original method which had always yielded a mixture of 16- and 17-ketosteroids. These mixtures were often inseparable. Furthermore, the Clemmensen reduction is too drastic for some unsaturated compounds in the steroid series. The new method takes advantage of the fact that 16-hydroxy-17β-toluene sulfonoxy steroids readily lose toluene sulfonic acid from the molecule upon mild saponification. Using this method, the authors have prepared compounds in the estrogen series as well as 3β-hydroxy-androst-5-en-16-one.

Figure 1

Huffman (28)

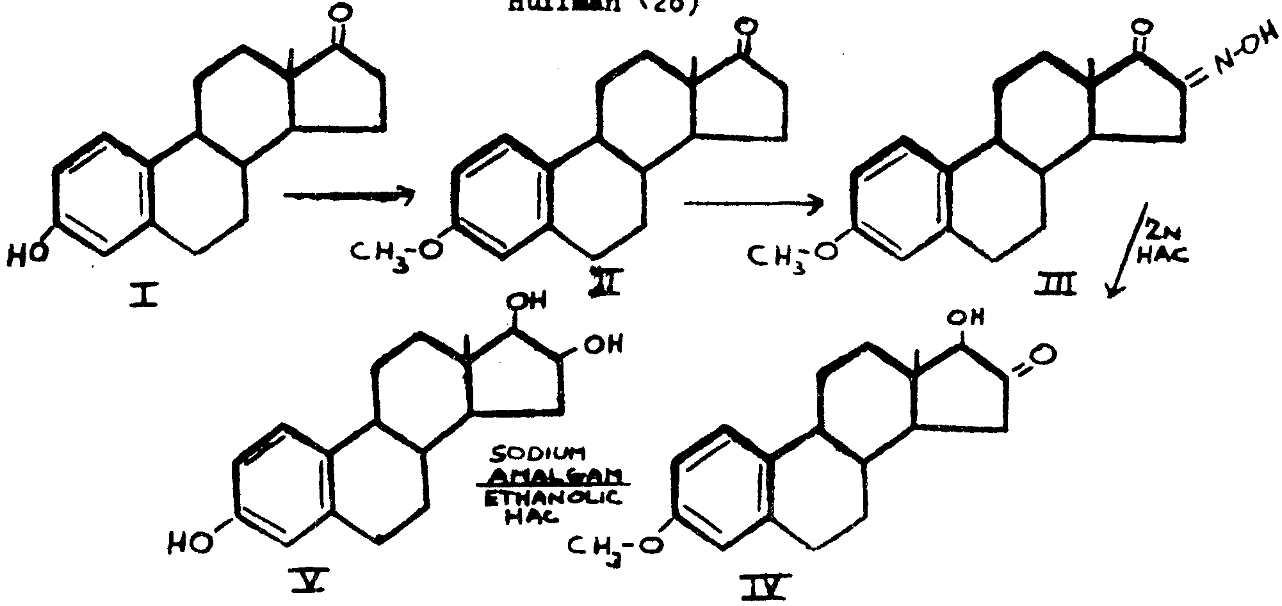
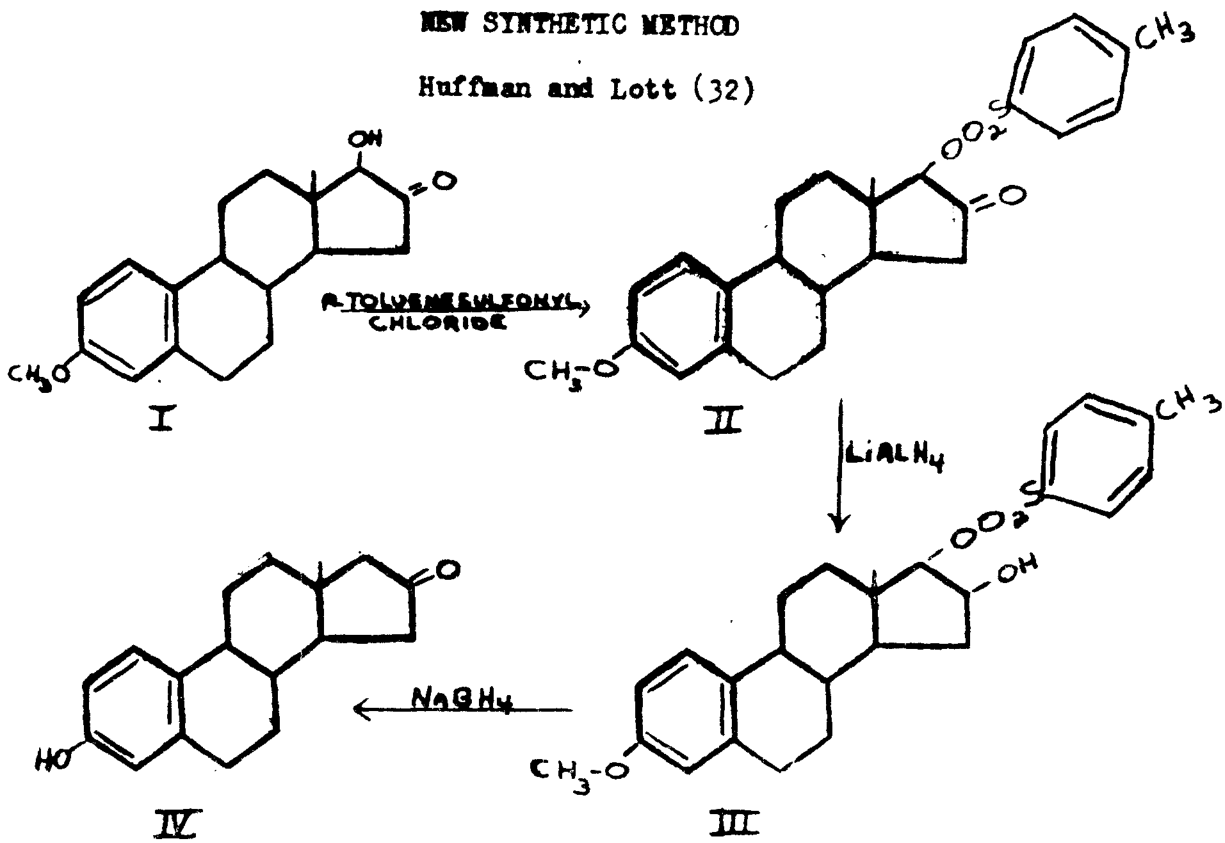


Figure 2

NEW SYNTHETIC METHOD

Huffman and Lott (32)



THEORETICAL ASPECTS OF THE PROBLEM

Since compounds of the 5β -androstane series were needed, it was necessary first to obtain 5β -androstane-3,17-dione as a starting material. This compound was prepared by the catalytic reduction of androst-4-ene-3,17-dione. Djerassi employed 0.6 g. of potassium hydroxide in 10 ml. of ethanol, and 0.15 g. of palladium on charcoal catalyst at room temperature and atmospheric pressure when he reduced 2 g. of a steroidal sapogenin to its 5β derivative in 50% yield (33). At other times ethyl acetate was employed with 10% lead and barium sulfate at room temperature to produce a 5β isomer in 70% yield from cortisone (34). Marker (35) found that a Δ^4 sterol could be reduced to its 5β derivative in 60% yield when a ratio of 2 g. of lead and barium sulfate to 200 mg. of sterol, at room temperature and 10 pounds of pressure for 10 hours in an ether solution were allowed to react. Originally potassium hydroxide was used to provide the alkaline medium. However, because of the difficulties in obtaining anhydrous potassium, potassium metal dissolved in absolute ethanol was used. The exact percentage of alkali necessary for the reduction was not obtainable from the literature, so that conditions varied for the several hydrogenations.

In each hydrogenation, formation of 5β -androstane-3,17-dione, as well as 5α -androstane-3,17-dione occurred. Separation of these stereoisomers was effected by alumina absorption chromatography and elution with various solvent mixtures. The 5α compound usually was eluted between 8:2 and 7:3 n-hexane:benzene, while its 5β analog was eluted between 6:4 n-hexane:benzene and 8.5:1.5 benzene:ether.

After chromatography, various fractions were subjected to infra-red and ultraviolet absorption spectroscopy in order to determine their purity. As a further means of detecting the presence or absence of α, β unsaturation, the thiosemicarbazones of several chromatographic fractions were prepared (16). With α, β unsaturation in the presence of a 17-ketone, absorption maxima at 300 and 270 mμ are expected. With the reduction of α, β unsaturation, only the maximum at 270 mμ appears.

The yields of the 5β isomer were low, even under the most favorable reaction conditions. Yields of 7-22% of 5β-androstandione and 2-5% of 5α-androstanedione were obtained.

With the last series of reductions, consisting of four lots of three grams each, an overall yield of 11.7% was obtained. A possible explanation for the decreased yield may stem from an incorrect adjustment of the hydrogenation apparatus, with the result that insufficient hydrogen was available for complete reduction of the androst-4-ene-3,17-dione.

The following step required the differential reduction of the 3-ketone by sodium borohydride (36). This reduction provided a mixture of 3α and 3β-hydroxy compounds. Separation was effected on alumina, with the 3β-hydroxy compound being eluted before the 3α-hydroxy isomer.

From this point on, preparation of compounds in both the 5α and 5β series followed the method of Leeds et al. (1). It was not necessary to prepare 3α-hydroxy-5α-androstan-17-one (androsterone) as this was obtained commercially. The Leed's procedure is initiated by the preparation of an enol acetate to form a Δ^{16} compound. The next step requires the formation of a $16\alpha,17\alpha$ epoxide, which is carried out with perbenzoic acid. Acid hydrolysis then produces a 16α-hydroxy, 17-oxo compound (Fig. 3). The 5α-androstane series was carried through this step. Difficulty was encountered with the preparation of the enol acetate of 3α-hydroxy-5β-androstan-17-one and enough material was obtained to produce its epoxide only.

The rearrangement of various 16, 17-epoxides has been the subject of several papers. These rearrangements have been effected by chromatography on silica gel, acid hydrolysis, or by heat alone (1). One of two mechanisms proposed by Leeds involved the postulation of the 16β-acetoxy ketone as an intermediary which was assumed to be unstable, readily undergoing epimerization to the 16α-isomer (Fig. 4, A). Further studies by Johnson (37) showed that this mechanism was not correct and that the alternative mechanism for the acetoxyl mechanism without inversion provides a reasonable pathway for rearrangement of the acetoxy group with the retention of configuration at C-16 (Fig. 4, B).

Figure 5 represents a mechanism for the enolization of the steroid at C-16,17. Isopropenyl acetate, in the presence of an acid catalyst becomes an enolate ion which undergoes cleavage into an acylium ion and acetone. The acylium ion can then react with the enol form of the 17-ketone giving an enol acetate, after the loss of a proton.

Figure 3

ANDROSTANE SERIES

Leeds (1)

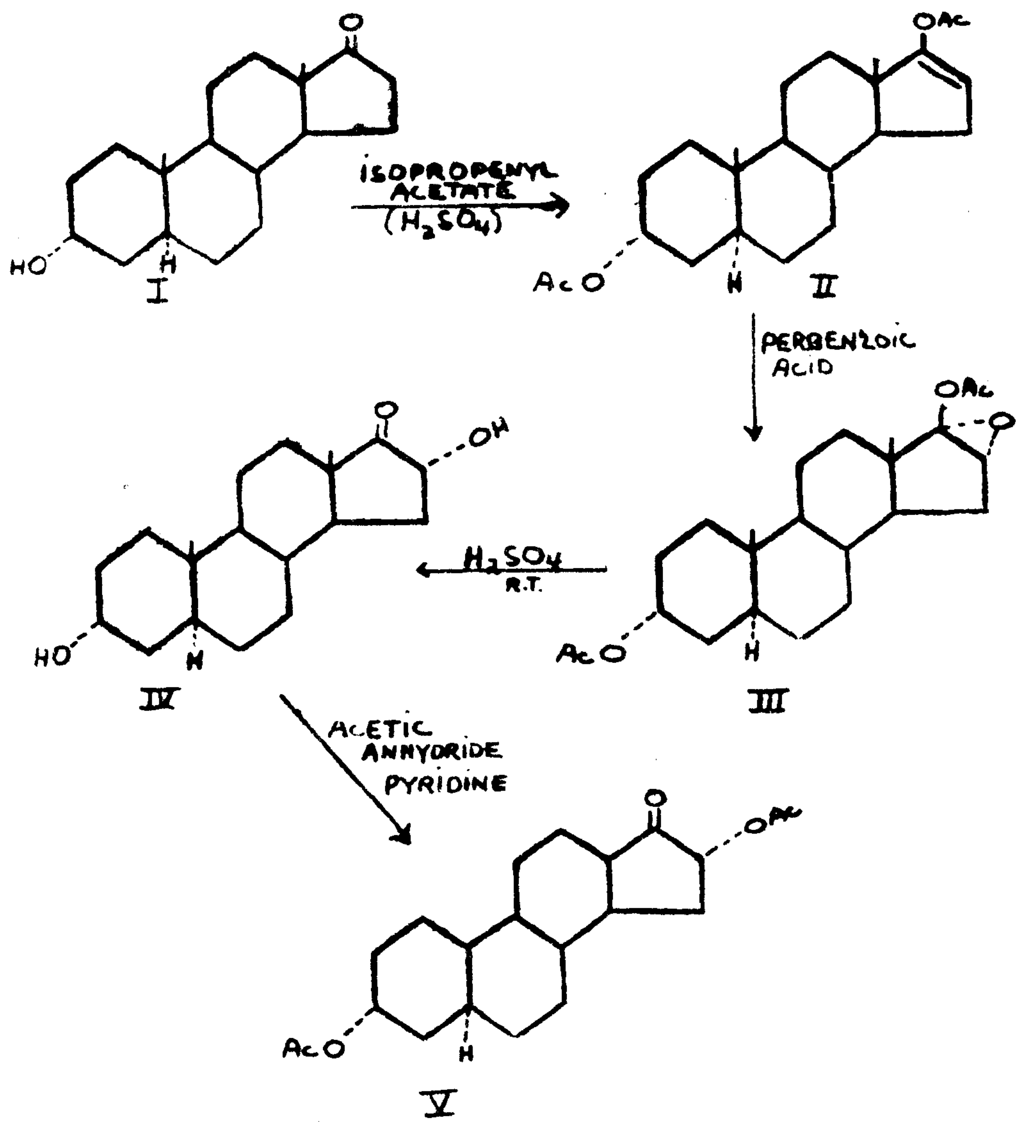
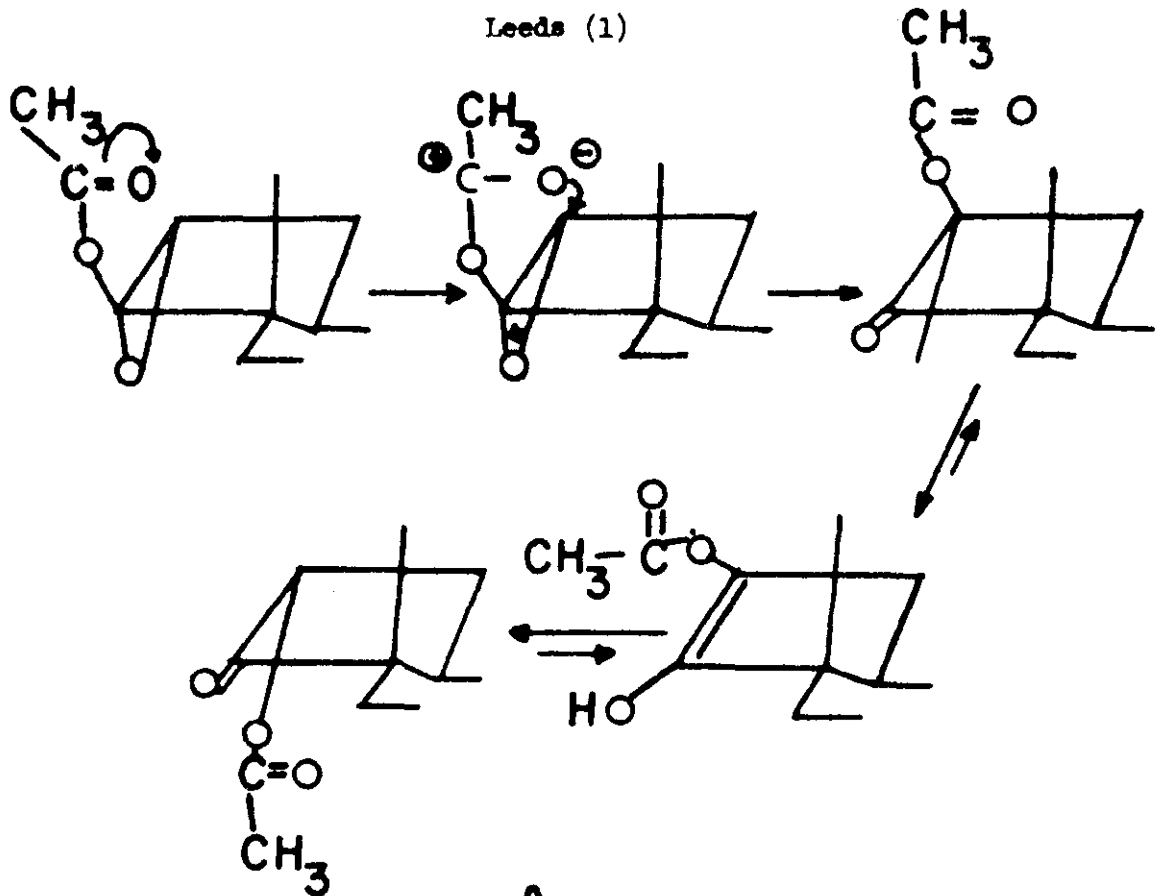


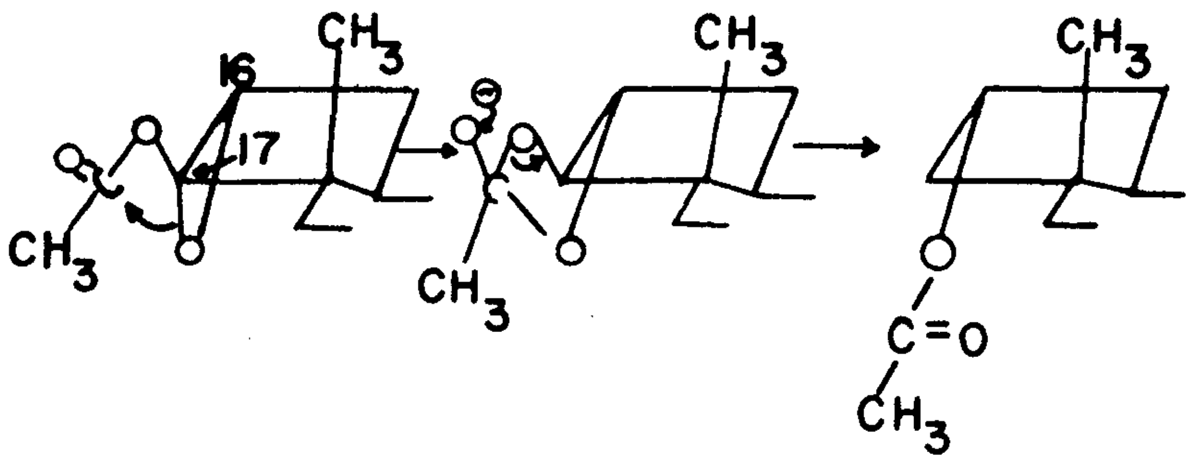
Figure 4

MECHANISMS FOR EPOXIDE REARRANGEMENTS

Leeds (1)



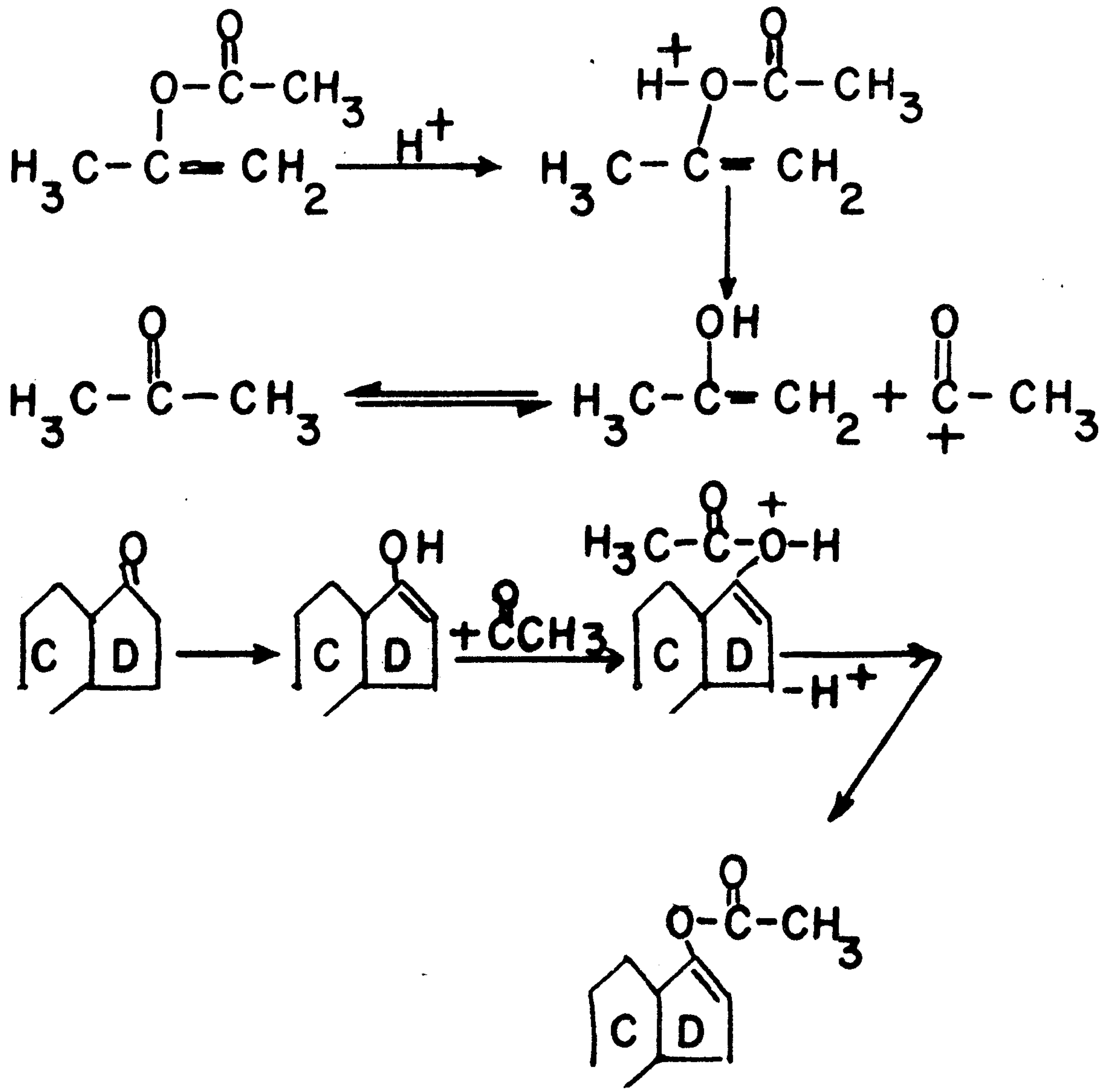
A



B

Figure 5

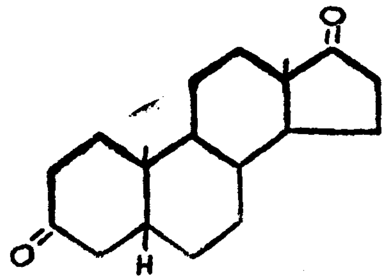
MECHANISM FOR ENOLIZATION AT C-16,17



EXPERIMENTAL

All alumina used for chromatography was Merck acid washed alumina. The solvents used in all cases were redistilled. Melting points given are uncorrected and were taken on a Fisher-Johns apparatus.

5β-ANDROSTANE-3,17-DIONE



Catalytic Reduction of Androst-4-ene-3,17-dione

To a solution of potassium hydroxide dissolved in absolute ethanol (170 mg. in 100 ml.) was added 1.00g. of androst-4-ene-3,17-dione and 10% palladium on charcoal catalyst. The weight of the catalyst was unrecorded, noting only that two full microspatulas of material were used. (Observed total absorption of hydrogen was 88 ml. Calculated: 85 ml.).

After completion of the reduction, the mixture was filtered with suction through Hyflo-Supercel to remove the catalyst. The yellow solution was acidified to pH 3.5, added to 500 ml. of redistilled ether and washed with 1% sodium bicarbonate solution. The ether layer was washed with water, and dried for 48 hours over anhydrous sodium sulfate. The ethereal solution was evaporated, leaving a crude amorphous product, brownish orange in color, weighing 1.19 g. The material was then chromatographed on alumina.

Several modifications were made in the process beginning with the fourth reduction. To obtain ethanol as anhydrous as possible, the alcohol was refluxed over lithium aluminum hydride for one half hour and distilled from it before the addition of alkali. Furthermore, potassium metal was now used in place of the potassium hydroxide. The potassium obtainable was not anhydrous enough for the reduction, making desirable the change to potassium metal. To reduce 2 g. of steroid, an 0.8% potassium ethoxide solution (100 ml.) was used. For the reduction of 5 g. of steroid, 100 ml. of a 1.6% solution was used. In addition, 0.08 g. of 10% palladium on charcoal catalyst were used for each gram of steroid. One further change to be noted was the evaporation of the reaction mixture to half its original volume before the material was extracted.

Column Chromatography of Reduction Mixture

All alumina columns were prepared as a slurry, and a ratio of 16 parts of alumina to 1 part of steroid was used. The diameter of the column for all chromatography of more than one gram of steroid was one inch. A one piece glass column, with a fitted sintered glass filter was used, and the flow rate varied from 50 ml. to 300 ml. per hour. The alumina column was slurried in the solvent mixture which was used to dissolve the steroid. These ratios ranged from 2:1 to 9:1 n-hexane:benzene.

The steroid mixtures were dissolved in the smallest amount of benzene, and then n-hexane was added until precipitation barely began, at which time a drop or two of benzene was added to bring the precipitate back into solution.

The steroid was applied to the column in the smallest possible amount of solvent, using a hand pipette to transfer the material from the flask on to the column. After allowing the material to settle into the column, the solvent chosen to develop the chromatogram was run through the column. A solvent was used until a minimum of 3-5% of the total material applied was eluted from the column. The next eluent was then applied. This process was repeated until approximately the weight of the starting material was recovered. Fractions of 25-50 ml. of material were collected, evaporated to dryness in vacuo and the material was transferred to tared vials. The solvents used were n-hexane, benzene, ethyl ether, and chloroform in the order of polarity. Mixtures usually employed were n-hexane:benzene 9:1, 8:2, 7:3, 6:4, and 1:1. 100% benzene was used next, then benzene:ether of various ratios followed by ether.

Evaluation of a Typical Chromatogram

Column Chromatography of Hydrogenation Run IV

Fourty grams of alumina were slurried in 6:4 n-hexane:benzene. The reaction mixture (2.0 g.) was dissolved in 5 ml. of 6:4 n-hexane: benzene and 50 ml. fractions of solvent were collected.

FRACTION	SOLVENT	ML. COLLECTED	WT. (mg.)	COMMENT
1	6:4 n-hexane: benzene	350	1200	Semi-crystalline material (Rechromatographed)
2	1:1 n-hexane: benzene	200	0	
3	Benzene	150	0	
4	8.5:1.5 benzene: ether	200	329	White crystalline material
5	8:2 benzene: ether	200		
6	Ether	120	x	Yellowish oil
7	Chloroform	100		

Rechromatography of Fraction I of Hydrogenation Run IV

The steroid mixture (1.2 g.) was dissolved in 20 ml. of 9:1 n-hexane:benzene, and applied to a 40 gram column of alumina (packed by slurry in 9:1 n-hexane:benzene). The resulting column height was three inches and the diameter one inch.

FRACTION	SOLVENT	Ml. COLLECTED	WEIGHT Mg.	COMMENT	
1	9:1 n-hexane:benzene	240	0		
2	8:2 n-hexane:benzene	250	0		
3	7:3 n-hexane:benzene	65	71.0	Yellow oil	Androstane-dione
4	7:3 n-hexane:benzene	75	47.0	Yellow oil	
5	7:3 n-hexane:benzene	90	45.0	Yellow oil	
6	7:3 n-hexane:benzene	90	43.0		
7	7:3 n-hexane:benzene	50	36.0		
8	7:3 n-hexane:benzene	50	20.0	Semi-crystalline	
9	7:3 n-hexane:benzene	60	24.0	Semi-crystalline	Combined and re-crystallized
10	7:3 n-hexane:benzene	110	29.0	Crystalline	
11	6:4 n-hexane:benzene	45	18.0	Crystalline	
12	6:4 n-hexane:benzene	105	53.0	Crystalline	
13	6:4 n-hexane:benzene	200	55.0		
14	6:4 n-hexane:benzene	150	28.0		
15	1:1 n-hexane:benzene	210	44.0		
16	Benzene	200	174.0	White, crystalline MP (126°) 132-134°	
17	8:2 benzene:ether	200	287.0	Yellow oil	
18	Ether	250	75.0		
	Total	2440 ml.	1059.0 mg.		

SUMMARY OF CHROMATOGRAMS I AND II OF HYDROGENATION IV

FRACTION	CHROMATOGRAPHY	SOLVENT	ML. COLLECTED	WT. (mg.)	M. P.		COMMENT
4	I	8.5:1.5 benzene: ether	200	329.0	(108°)	128-134°	Mostly 5α-androstane-3,17-dione by I. R.
3-7	II	7:3 n-hexane: benzene	850	242.0			Little 5α-androstane-3,17-dione
10	II	7:3 n-hexane: benzene	105	28.8			5β-androstane-3, 17-dione
15	II	1:1 n-hexane: benzene	120	44.0	(124°)	130-133°	5β-androstane-3, 17-dione by I. R.
16	II	Benzene	200	173.5	(126°)	132-134°	5β-androstane-3, 17-dione

Total 5β-androstane-3,17-dione isolated---- 444 mg.

SUMMARY OF CATALYTIC HYDROGENATIONS

Gm. reduced	1.00		1.00		2.00		5.00		5.00		1.00	12.00
Alumina (Gm.)	30		20		40		80		80		20	50
Solvent for slurry	4:1 n-hexane:benzene		9:1 n-hexane:benzene		6:4 n-hexane:benzene		6:4 n-hexane:benzene		2:1 n-hexane:benzene		9:1 n-hexane:benzene	
Solvent	Ml.	Wt. mg.	Ml.	Wt. mg.	Ml.	Wt. mg.	Ml.	Wt. mg.	Ml.	Wt. mg.	Ml.	Wt. mg.
9:1 n-hexane: benzene	300	138	300	2.4	-	-	-	-	-	-	420	230
8:2 n-hexane: benzene	200	157.3	190	5.0	-	-	-	-	-	-	100	30
7:3 n-hexane: benzene	250	145.7	215	50.7	-	-	-	-	355	2185	100	24.2
6:4 n-hexane: benzene	300	130	260	170	350	1200	664	3608	2340	1088	100	34.4
1:1 n-hexane: benzene	350	80.2	450	170.3	200	0	185	26.5	410	25	570	49.7
Benzene	300	151	200	113.3	150	0	250	71.2	100	1055	155	43.1
Benzene:15% ether	-	-	-	-	200	329	350	463	800	671		
Ether	100	245	140	367.1	320	-	435	960			200	437.4
$CHCl_3$ + MeOH	500	48.2	120	38.2	100	-	-	-	-	-	400	54
TOTALS	Ml.	Wt. (g.)	Ml.	Wt. (g.)	Ml.	Wt. (g.)	Ml.	Wt. (g.)	Ml.	Wt. (g.)	Ml.	Wt. (g.)
	2300	1.096	1875	0.917	1420	1.529	1885	5.129	4000	5.024	1.845	0.903

SUMMARY OF CATALYTIC HYDROGENATIONS (continued)

Wt. of 5 -androstanedione (mg.)	75.0	113.3	444	957	1102	163	1400
% Yield	7.5%	11.3%	22.2%	19.1%	22.4%	16.3%	11.7%

Sodium Borohydride Reduction of Etiocholanedione

Etiocholane-3,17-dione (229 mg.) was dissolved in methanol (15 ml.). To this solution was added sodium borohydride (10 mg.) dissolved in water (0.5 ml.) and methanol (0.5 ml.). After being allowed to react at room temperature for twenty-five minutes, distilled water (50 ml.) and ether (50 ml.) were added to the reaction mixture. The mixture was transferred to a separatory funnel, and extracted four more times with 50 ml. of ether. The ether was washed with a 2% sodium bicarbonate solution, followed by washing with water. The organic layer was dried over anhydrous sodium sulfate. After evaporation of the ether, the solid residue was chromatographed on alumina.

Chromatography of Reduction Mixture

Alumina (7 g.) was slurried in 3:2 n-hexane:benzene. (0.5 cm. diameter column; height of 11.5 cm.) Eluates were collected in 10 ml. fractions.

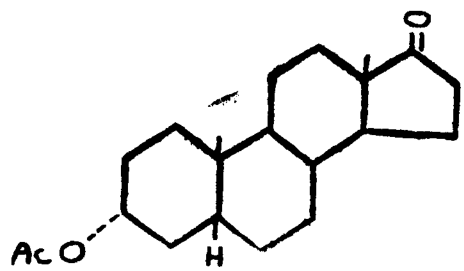
FRACTION	SOLVENT	ML.	WT. OF MATERIAL (mg.)	COMMENT
0	3:2 n-hexane:benzene	65	0	
1	1:1 n-hexane:benzene	20	6.5	
2	1:1 n-hexane:benzene	30	10.0	Starting material
3	1:1 n-hexane:benzene	50	18.0	3β compound
4	Benzene	20	16.3	3β plus 3α compound
5	Benzene	50	21.4	3β plus 3α compound
6	9.5:5 benzene:ether	20	10.7	
7	9.5:5 benzene:ether	25	15.3	3α-hydroxy-5β-androstan-16-one
8	8.5:1.5 benzene:ether	50	110.8	144.6 mg. M. P. (149°) 151-152°
9	8.5:1.5 benzene:ether	20	7.8	
Total		350	216.8	

Confirmation of the separation of the 3α and 3β hydroxy compounds was obtained by paper chromatography and infra-red spectroscopy.

Summary of Sodium Borohydride Reductions

Weight of 5β-androstane-3,17-dione	Weight of 3α-hydroxy-5β-androstan-17-one	Per cent Yield
295 mg.	74 mg.	25.0%
869 mg.	306 mg.	35.0%
290 mg.	144 mg.	50.0%
1147 mg.	636 mg.	54.0%
150 mg.	61 mg.	41.0%
228.5 mg.	144.6 mg.	64.0%
2.970 g.	1.366 g.	46.0% (weighted)

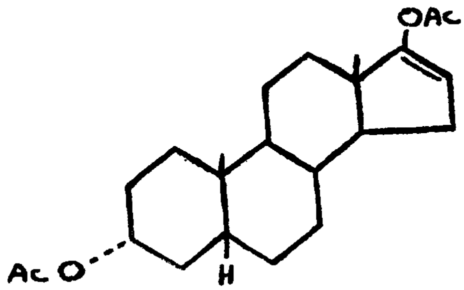
17-OXO-5β-ANDROSTAN-3α-YL-ACETATE



In order to have a reference compound with which to compare the 5β-androst-16-en-3α,17β-ylene acetate by infra-red spectroscopy, the 3α monoacetate was prepared.

A solution of 3α-hydroxy-5β-androstan-17-one (95.5 mg.) in pyridine (5 ml. dried over potassium hydroxide) and acetic anhydride (2 ml.) was allowed to react at room temperature for 90 hours. The reaction was stopped by adding the mixture to ice and water (150 ml.). The ethereal layer was extracted five times with 50 ml. portions of 2% sodium bicarbonate and then washed to neutral with water. The organic layer was dried over sodium sulfate and the ethereal layer was evaporated to dryness. The mixture was chromatographed on 4 g. of alumina, slurried in 1:1 n-hexane:benzene, yielding 63 mg. (65% yield) of material melting at 94-96.5°C. This material was shown to be 17-oxo-5β-androstan-3α-yl-acetate by infrared spectroscopy.

5β-ANDROST-16-EN-3α,17β-YLENE ACETATE



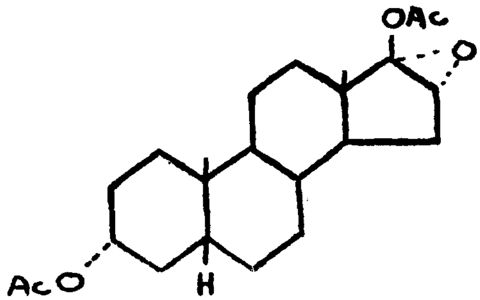
To a solution of 3α-hydroxy-5β-androstan-17-one (100 mg.) in redistilled isopropenyl acetate (10 ml.) was added 0.2 ml. of catalyst solution composed of 5 ml. of isopropenyl acetate and 0.1 ml. of concentrated sulfuric acid.

The mixture was refluxed for two hours under nitrogen. At the end of this time, 15 ml. more of isopropenyl acetate and 3 ml. of catalyst solution were added and refluxing was continued for another hour. Fifteen milliliters of solvent were then distilled off in a fifty minute period. After cooling, the mixture was added to ether (200ml.), and washed twice with 100 ml. of ice cold 2% sodium bicarbonate solution. After washing the ether layer with water until neutral, the organic layer was dried over sodium sulfate.

The reaction mixture was chromatographed on 3 g. of alumina, in a 1 cm. diameter column and eluted with n-hexane. Recrystallization from n-hexane yielded 43 mg. of material.

M. P. 90.5°-92° C.
Optical Rotation $[\alpha]_D^{22°} = 54.07$
Infrared Maxima at: 1240, 1205, 1090 cm.$^{-1}$

16α,17α-EPOXY-5β-ANDROSTAN-3α,17β-YLENE ACETATE



A solution of 5β-androst-16-en-3α,17β-ylene acetate (17 mg.), benzene (1 ml.) and perbenzoic acid (0.3 ml.) were allowed to react at room temperature for 23 hours. The material was extracted with ether, washed first with cold 1% sodium hydroxide, and then was washed to neutral with water. The material was dried for 72 hours over anhydrous sodium sulfate. After evaporating to dryness, the compound still retained the odor of perbenzoic acid. It was therefore rewashed with 2% sodium hydroxide. The compound obtained melted from 153-156° C. and infrared studies established the presence of an epoxide, by showing maxima at 1155, 1225, 980 and 110 $cm.^{-1}$

Preparation of Perbenzoic Acid (38, 39)

Recrystallization of 60 g. of benzoyl peroxide from 2000 ml. of chloroform and 400 ml. of absolute methanol yielded 30 grams of purified material. To a solution of 4 g. of metallic sodium dissolved in 80 ml. of absolute methanol at -25°C. (dry ice and acetone) were added slowly 15-20 ml. of benzoyl peroxide dissolved in benzene while keeping the reaction mixture at about 0°C. After the complete addition of the benzoyl peroxide, 150 ml. of water and ice were added, and the mixture was transferred to a separatory funnel. An additional 350 ml. of ice and water were added, and the benzene layer was drawn off. The water layer was extracted twice with 100 ml. of ice-cold benzene and then neutralized with 225 ml. of ice-cold 1N sulfuric acid. The liberated perbenzoic acid was removed by extraction with 300 ml. of ice-cold benzene. This fraction was dried over sodium sulfate (anhydrous) for 36 hours, filtered and stored in a dark bottle.

Quantitative determination of perbenzoic acid:

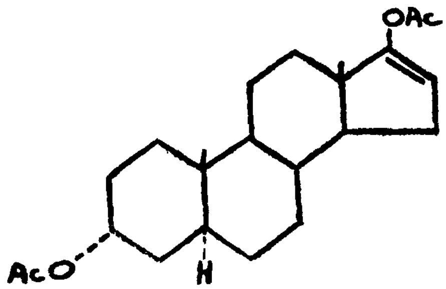
(1) 1.5 grams of sodium iodide were dissolved in 50 ml. of water and transferred to a 250 ml. flask.

(2) 5 ml. of glacial acetic acid were added.

(3) 3 ml. of perbenzoic acid solution were introduced.

(4) The liberated iodine was titrated with 0.1 N sodium thiosulfate. One milliliter of sodium thiosulfate (0.1 N)= 0.0069 g. of perbenzoic acid. Three titrations showed that each three milliliters of solution contained 0.1684 g. of perbenzoic acid.

5α-ANDROST-16-EN-3α,17β-YLENE ACETATE

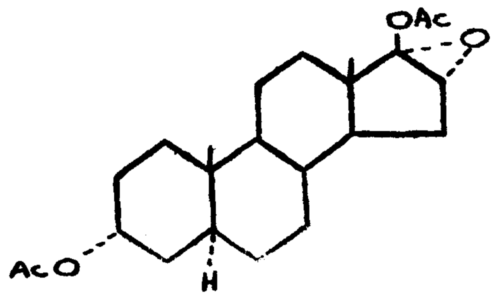


To a solution of 3α-hydroxy-androstan-17-one (5 g.) in isopropenyl acetate (25 ml.) was added 1 ml. of catalyst solution (see pages 25-27).

The mixture was refluxed for 2.5 hours, at the end of which time 20 ml. of isopropenyl acetate and 1.5 ml. of fresh catalyst solution were added. Refluxing was continued for another half hour. Twenty milliliters of solvent were distilled off over a two hour period, and the mixture was extracted as before. The mixture was chromatographed on 4 g. of alumina, using light petroleum ether as the eluent. Material obtained from the column weighed 0.767 g. (15.3% yield). Material recovered from the mother liquor yielded 0.314 g. (6.3%) of white crystals.

M. P. 170-172° C.
$[\alpha]_D^{23°} = 74.5°$
Analysis calculated for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15
Found: C, 73.40; H, 9.08
Infared Maxima: 1769, 1208, 1099 cm.$^{-1}$ (spectrum D).

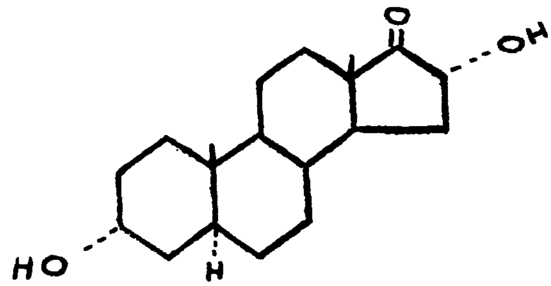
16α,17α-EPOXY-5α-ANDROSTAN-3α,17β-YLENE ACETATE



To a solution of perbenzoic acid (0.617 g.) in benzene (11 ml.) was added 500 mg. of 5β-androst-16-en-3α,17β-ylene acetate. The mixture was allowed to stand at room temperature for twenty hours. The solution was then transferred to a separatory funnel containing 300 ml. of ice-cold ether. This was washed with 200 ml. of ice-cold sodium hydroxide (0.1N). The ether layer was then washed twice with 100 ml. of distilled water. The organic phase was dried overnight over anhydrous sulfate. After the ether layer had been filtered through glass wool, it was evaporated <u>in vacuo</u>. The residue was recrystallized from acetone, yielding 228 mg. (45.3%).

M. P. 172-173°C.
Analysis: Calculated for $C_{23}H_{34}O_5$: C, 70.74; H, 8.77
Found : C, 70.9; H, 9.0
Infrared Maxima at: 1764, 1199, 1145, 1062 $cm.^{-1}$ (Spectrum E).

3α,16α-DIHYDROXY-5α-ANDROSTAN-17-ONE



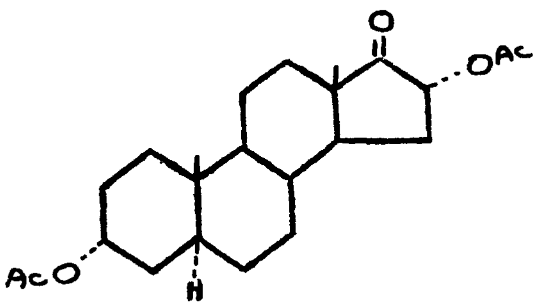
A mixture of 16α,17α-epoxy-5α-androstan-3α,17β-ylene acetate (100 mg.) dissolved in absolute methanol (14 ml.) and sulfuric acid (3.2 ml.) was allowed to react for four days at room temperature. The mixture was then partitioned between ethyl acetate (200 ml.) and ice-cold sodium hydroxide (300 ml., 2 g./500 ml.). The organic phase was dried over anhydrous sodium sulfate. After evaporation of the ethyl acetate, the material was recrystallized from acetone, yielding 42 mg. of white crystals.

A second run yielded a white crystalline material melting from 200-202°C., with infrared spectra similar to the first preparation. However, the C H determination was more accurate.

	Run 1	Run 2
	M. P. 191-193° C.	M. P. 200-202° C.
	$[\alpha]_D^{23°} = +124.5°$ (ethanol)	—
Analysis: Calc'd. for $C_{19}H_{30}O_3$:	C, 74.54; H, 9.80	C, 74.54; H, 9.80
Found:	C, 75.1; H, 10.4	C, 74.09; H, 9.57

Infrared Maxima at: 3530, 3375, 999 $cm.^{-1}$ (Spectrum F)

17-OXO-5α-ANDROSTAN-3α,16α-YLENE ACETATE



To a solution of absolute methanol (30 ml.) and 6 N sulfuric acid (7 ml.) was added 16α,17α-epoxy-5α-androstane-3α,17β-ylene acetate (220 mg., 0.001 M). After standing for four days at room temperature the material was extracted with ethyl acetate and worked up as described for the preceeding compound. The residue remaining, after evaporation of the ethyl acetate, was dissolved in 6 ml. of pyridine and 5 ml. of acetic anhydride and reacted for 72 hours. The material was then partitioned between ether and ice-cold water. After drying over anhydrous sodium sulfate and evaporating the solvent, the residue was dissolved in acetone. Following the addition of petroleum ether, a tan colored crystalline material was obtained. Infrared spectroscopy confirmed the structual assignment (spectrum F).

M. P. 175-176°C.
Infrared Maxima at: 1250, 1238, 1750 $cm.^{-1}$

New Compounds Prepared

	M. P.	Optical Rotation	C H Determination		I. R. Maxima
			Calc.	Found	
5 α-androst-16-en-3 α,17 β-ylene acetate	170-171.5°C.	$[\alpha]_D^{23°} = +74.5°$	C, 73.76 H, 9.15	C, 73.40 H, 9.08	1769, 1208, 1099 $cm.^{-1}$
5 β-androst-16-en-3 α,17 β-ylene acetate	90.5-92°C.	$[\alpha]_D^{22°} = +54.1°$	---	---	1240, 1205, 1090 $cm.^{-1}$
16 α,17 α-epoxy-5 α-androstan-3 α, 17 β-ylene acetate	172-173°C.	$[\alpha]_D^{23°} = +52.3°$	C, 70.74 H, 8.77	C, 70.9 H, 9.0	1764, 1199, 1145, 1062 $cm.^{-1}$
16 α,17 α-epoxy-5 β-androstan-3 α, 17 β-ylene acetate	153-156°C.	---	---	---	1225, 1155, 980, 710 $cm.^{-1}$
3 α,16 α-dihydroxy-5 α-androstan-17-one	191-193°C.	$[\alpha]_D^{23°} = +124.5°$	C, 74.54 H, 9.80	C, 75.1 H, 10.4	3530, 3375, 999 $cm.^{-1}$
3 α,16 α-dihydroxy-5 α-androstan-17-one	200-202°C.	---	C, 74.54 H, 9.80	C, 74.09 H, 9.57	3530, 3375, 999 $cm.^{-1}$
17-oxo-5 α-androstan-3 α,16 α-ylene acetate	175-176°C.	---	---	---	1250, 1238, 1750 $cm.^{-1}$

DISCUSSION

Two major problems involved in the preparation of 5β-androstane-3,17-dione were solved. Although yields were poor, conditions were derived through which 5β-androstane-3,17-dione could be prepared from androst-4-ene-3,17-dione by catalytic reduction. Associated with this reduction was the separation of the isomers, 5α-androstane-3,17-dione and 5β-androstane-3,17-dione. The separation was effected using an alumina adsorption column, from which 5α-androstane-3,17-dione was eluted first, with a higher percentage of n-hexane. The 5β-androstane-3,17-dione was eluted with a solvent composed of equal parts of n-hexane and benzene. Infrared absorption spectra proved to be the only feasible method of determining the purity of the separated fractions, since both stereoisomers have the same melting point and cannot be separated by paper chromatography.

The first reduction utilized potassium hydroxide in absolute ethanol. An attempt was made to produce an anhydrous form by heating it sufficiently previous to dissolving the hydroxide. However this procedure proved to be inadequate as the temperatures attained were not high enough, and the crucibles available were inefficient. The yield of 5β-androstane-3,17-dione from the first reduction was 7.5%. Additional information (46) obtained after the reduction stated that as little as 2% water present in the reaction mixture would greatly reduce the yields of 5β compound. In addition to the possibility of too much water, the question of the concentration of alkali necessary was raised. The second reaction was run under the same conditions, resulting in a yield of 11.3%.

With the third reduction, several new procedures were employed. The absolute ethanol was refluxed over lithium aluminum hydride for one half hour before being used in the reduction. In addition, metallic potassium was used in place of potassium hydroxide. These steps increased the yield to 16.3%. The next experiment employed 0.8 to 2.0 grams of potassium metal. With these procedures, the yields increased to 22.4%. These conditions produced the best yields when a ratio of 12 parts of steroid to one part of catalyst was used. However, the possibility that a greater proportion of catalyst should have been employed occurs.

Application of the principle of adsorption chromatography was the most feasible method for separating the 5α and 5β stereoisomers after catalytic reduction of androst-4-ene-3,17-dione. Initial separations usually did not produce pure substances. The major reason here was that the very polar component of the reduction would not allow a large enough proportion of n-hexane to be used when applying the material to the column. As a result, with many of the first chromatograms, the relatively high percentage of benzene caused 5α and 5β components to be developed at approximately the same rate. When the yellow polar component was eliminated, after the first chromatogram, the eluted material was redissolved in a greater percentage of n-hexane and a separation of 5α and 5β components was effected. The 5α component was eluted in a solvent range from 8:2 n-hexane:benzene to the beginning fractions of 6:4 n-hexane:benzene. The 5β compound was eluted from 6:4 n-hexane: benzene. This method of separation was very useful, as fractional crystallization was not feasible in separating these isomers. As was mentioned, the method for determining completeness of separation was a comparison of infrared spectra. The method of column adsorption chromatography has been shown to be feasible for macro-quantities of material. Its application with microgram quantities is undetermined. However, with the use of a fraction cutting apparatus, and collection of one to five milliliter volumes, separation of the 5 α and 5 β components may be possible with the same solvent system.

Preparation of 3 α-hydroxy-5 β-androstan-17-one was accomplished by a differential reduction of 5 β-androstane-3,17-dione with sodium borohydride as described by Elisberg et al. (36). Reported yields of 70% 3 α-hydroxy-5 β-androstan-17-one were higher than the overall 46% yields obtained in our experiments. This compound was identical in melting point with that reported in the literature. In addition, the travel rate on paper chromatography was the same as a standard sample of 3 α-hydroxy-5 β-androstan-17-one.

Difficulty was encountered with the preparation of 5 β-androst-16-ene-3 α,17 α-ylene acetate. Enolization at the 16, 17 position was attempted numerous times. The reaction was successful twice, but many repeated attempts to reproduce the results proved unsuccessful. One of the possible sources of difficulty arose in the distillation procedure associated with this step. This distillation was conducted under reduced pressure. After many attempts at enolization, the starting material was exhausted, and this particular step was subjected to further experimentation using estrone and androsterone. It was found that enolization would successfully occur at the 16,17 position if the distillation of solvent was not carried out under reduced pressure, but at a higher temperature and with a longer time of distillation. That enolization of the etiocholane series at the desired position might be successful utilizing the higher temperature of distillation now seems likely.

Preparation of 16 α,17 α -epoxy-5 β -androstane-3 α ,17 β -ylene acetate was accomplished without any difficulties. Infrared evidence for this compound occurs under infrared studies. Preparation of further compounds in this series was discontinued when the starting material was depleted.

Preparation of compounds in the 5 α-androstane series began with 3 α -hydroxy-5 α -androstan-17-one (androsterone) as starting material. The preparation of the enol diacetate was accomplished, using an increased temperature and a longer time of distillation. Discussion of the presence of the enol formation is included under infrared studies.

Preparation of 17-oxo-5 α -androstane-3 α ,16 α -diol was accomplished by the acid hydrolysis of 16α ,17α -epoxy-5 α -androstane-3 α ,17 β -ylene acetate. Many factors have been considered regarding the assigned structure. Formation of the 16 β-hydroxyl compound had to be considered because of the possible rearrangement occurring with the cold sodium hydroxide wash used. However, Leeds (1) previously had employed a sodium hydroxide wash after an epoxide rearrangement without noting transformation to the 16 β -configuration. Our compound was prepared again without using the alkaline wash. This compound had a melting range from 200-202°C., as compared with the first alkaline washed preparation of 191-193°C. A mixed melting point showed no appreciable depression, the mixture had an optical rotation of $[\alpha]_D^{23^{\circ}} = +124^{\circ}$. Various authors (1, 21, 28) have reported that the substitution of a steroid in the C-16 position produced a rotation in amore negative direction than the parent compound. This is not evident with the final compound prepared for this thesis. There is some descrepancy noted in the carbon-hydrogen determination. The calculated: C, 74.54; H, 9.80, as compared with that found: C, 75.1; H, 10.4. However, a carbon-hydrogen determination of the compound melting from 200-202°C. gave results: C, 74.09; H, 9.57. The possibility of an acetoxy group still being present was considered, but this seems unlikely from further calculations. Infrared data provides evidence for a monoketone, dihydroxy compound. The normal C-17 ketone transmission bands have been altered by the presence of an hydroxyl group, presumably at C-16. In addition, preparation of the diacetoxy derivative provides further infrared evidence for a 3 α,16 α-dihydroxy-17-keto compound. Thus, most of the evidence points to the assigned structure, but a definite statement of structural proof can not be made.

Analysis of Infrared Spectra

5β-Androstane Series:

The 5β-androstane-3,17-dione (see Spectrum A) prepared by the catalytic reduction of androst-4-ene-3,17-dione was identical with the spectrum of 5β-androstane-3,17-dione presented in Jones (40). Two bands, characteristic of the 3,17 diketone were noted at 1745-42 cm.$^{-1}$ and 1710 cm.$^{-1}$ respectively (40, 41). Important bands differentiating 5β-androstane-3,17-dione from its 5α isomer are the double bands noted at 1015 cm.$^{-1}$ and 995 cm.$^{-1}$ for the 5β compound, while the 5α compound has a single band at 1015 cm.$^{-1}$ (40, 41). In addition, characteristic bands for the 3-keto-5β compound occur at 1267-1264 cm.$^{-1}$ and 1250-1247 cm.$^{-1}$ (41, 44).

5β-androst-16-ene-3α,17β-ylene acetate (see Spectrum B) was characterized by the following vibrational bands. Two strong bands denoting a 3α-acetoxy-5β compound were noted at 1240 cm.$^{-1}$ and 1025 cm.$^{-1}$ (41, 42, 43). Characteristic for a Δ^{16}-17 acetoxy compound were the bands of fairly strong intensity found at 1205 cm.$^{-1}$ and 1090 cm.$^{-1}$ (41). In addition, with a Nujol mull preparation, a band of strong intensity was noted at 1614 cm.$^{-1}$, indicative of the existence of a double bond between carbon 16 and 17 of the D ring (41).

The 17-acetoxy group was characterized by the band occurring at 1742-37 cm.$^{-1}$ (40, 41). However, overlapping of the band for the 17-ketone may occur if the ketone is present. Other bands characteristic of the 3α-acetoxy-5β compound were seen at 1318 cm.$^{-1}$, 1170-1168 cm.$^{-1}$ and 890-885 cm.$^{-1}$ (41, 42, 43), while bands at 1155-1151 cm.$^{-1}$, 834-829 cm.$^{-1}$ and 813 cm.$^{-1}$ indicate a Δ^{16}-17-acetoxy compound (41).

Evidence for the structure of the 16α,17α-epoxy-5β-androstane-3α,17β-ylene acetate (see Spectrum C) resulted from the following spectral findings. Disappearance of the 1610 cm.$^{-1}$ band, characteristic of the 16,17 double bond was noted. The band at 1095 cm.$^{-1}$ was reduced in intensity. It appears that the epoxy group has the same influence upon the acetoxy group at C-17 as the enol form at C-16,17 has; that is an increased C=O frequency and a lowered C-O frequency. Bands at 834-829 cm.$^{-1}$ and 813 cm.$^{-1}$ have disappeared, while the strong band at 1155-1151 cm.$^{-1}$ has remained. A new band was seen at 1225-1221 cm.$^{-1}$ Bands pertaining to the 3α-acetoxy-5β structure were still present (41, 42), while a band at 1055 cm.$^{-1}$ of much greater intensity than seen with the enol compound was noted with the epoxide. Also a band of increased intensity in the spectrum of the epoxide compound occurred at 980 cm.$^{-1}$ and 1110 cm.$^{-1}$

Evidence for the synthesis of 5β-androst-16-ene-3α,17β-ylene acetate (see Spectrum D) was noted as follows. Bands occurring at 1769-1765 cm.$^{-1}$, 1208-1204 cm.$^{-1}$, and 1099-1096 cm.$^{-1}$ are characteristic of a Δ^{16},17β-acetoxy compound (41). Bands present at 1259-1255 cm.$^{-1}$, 1252-1244 cm.$^{-1}$, 1238-1236 cm.$^{-1}$, 1165-1160 cm.$^{-1}$, 1020-1016 cm.$^{-1}$, and 979-977 cm.$^{-1}$ indicate the 3α-acetoxy-5α compound (41, 42, 43). These results therefore verify the presence of both acetoxy groups. The characteristic band for the C-16, C-17 double bond is split into two bands, one occurring at 1624 cm.$^{-1}$ and the other at 1610 cm.$^{-1}$ (41). This raises the question of the possible shift of the acetoxy group from C-17 to C-16. Further evidence to indicate the enol formation is the disappearance of the 1408 cm.$^{-1}$ band, which when present is indicative of H_2C-16 (41). Findings relating to the formation of the 16α,17α-epoxy-5β-androstane-3α,17β-ylene acetate point to the verification of this compound (see Spectrum E). Notable are the disappearance of the C-17 enol acetate bands, and the appearance of new bands believed to be characteristic of the 16α,17α epoxy, 17β acetoxy groups. These new bands are found at 1764 cm.$^{-1}$ (low intensity), 1212 cm.$^{-1}$, 1199 cm.$^{-1}$, 1145 cm.$^{-1}$, 1062 cm.$^{-1}$ and 990 cm.$^{-1}$ A band near 1405 cm.$^{-1}$ may be characteristic of H_2C-15 adjacent to an epoxide group.

The spectrum of 3α,16α-dihydroxy-5α-androstane-17-one (see Spectrum F) yielded the following information. A crystalline melt showed two bands at approximately 3530 cm.$^{-1}$, and 3375 cm.$^{-1}$ These bands correspond to a broad hydroxyl association band at approximately 3440 cm.$^{-1}$, which was obtained from an amorphous melt. A ketone group is represented by the band at 1743 cm.$^{-1}$ Verification of the axial 3α hydroxyl 5α portion of the compound was obtained with an amorphous melt giving a band of strong intensity at 999 cm.$^{-1}$ Bands obtained at 910 cm.$^{-1}$, 1115 cm.$^{-1}$, 1167 cm.$^{-1}$, and 1206 cm.$^{-1}$ are also indicative of the 3α hydroxy 5α compound (41, 32, 43). The ring D ketone appears to be different from a simple ketone. The poor solubility and the two hydroxyl bands point to a second hydroxyl group in the molecule. Further evidence that this substance was the desired compound was furnished from the spectrum of its diacetate.

Following preparation of the derivative, characteristic epoxide bands of the starting compound were no longer present. Bands characteristic of the 3α-acetoxy 5α compound were present at 1238-1236 cm.$^{-1}$, 1165-1160 cm.$^{-1}$ and 979-977 cm.$^{-1}$ (41, 42, 43). Three bands instead of one appear near 1250 cm.$^{-1}$, which is characteristic of the 3α-acetoxy group. However, there appears to be overlapping by a single band of the C-16 acetoxy group. The band near 1015 cm.$^{-1}$ is also broadened by overlapping bands of both acetoxy groups. The high intensity band at 1760 cm.$^{-1}$ does not have the characteristic 1210 cm.$^{-1}$ low intensity band present with a 17-acetoxy group (41, 43). Therefore, the group at C-17 must be considered to be a 17-ketone, with its intensity raised by the presence of the C-16 acetoxyl group. A band characteristic of the 16α-acetoxy-17-ketone theoretically occurs at 1750-1748 cm.$^{-1}$, while two or three strong bands in the region from 1260-1220 cm.$^{-1}$ denote axial acetates of the 3α-acetoxy 5α variety (41).

LEGEND OF SPECTRA

CHART A

"b" is of lower concentration than "a"

CHART B

Heavy line = Nujol mull

Thin line = CS_2

"X" indicates 3α acetoxy, 5β component

● indicates Δ^{16}, 17β acetoxy component

■ indicates 16,17 double bond

CHART C

Heavy line = a melt

Thin line = CS_2

■ indicates new bands or disappearance of old bands

CHART D

■ indicates Δ^{16} double bond

● indicates Δ^{16},17β acetoxy

Heavy line = a melt

Thin line = CS_2

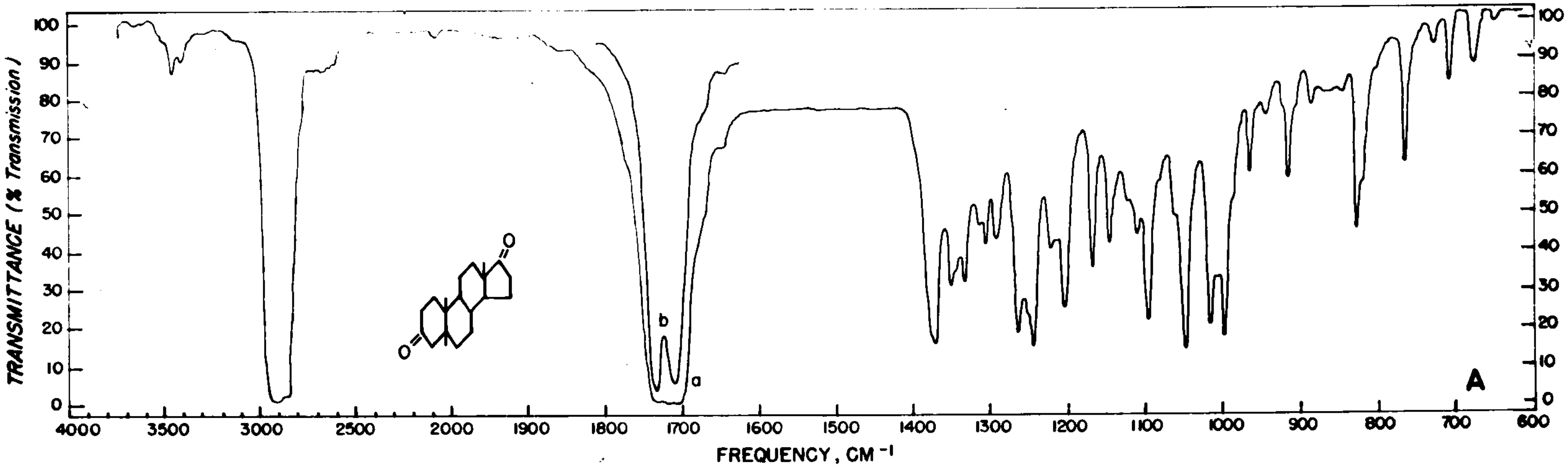
CHART E

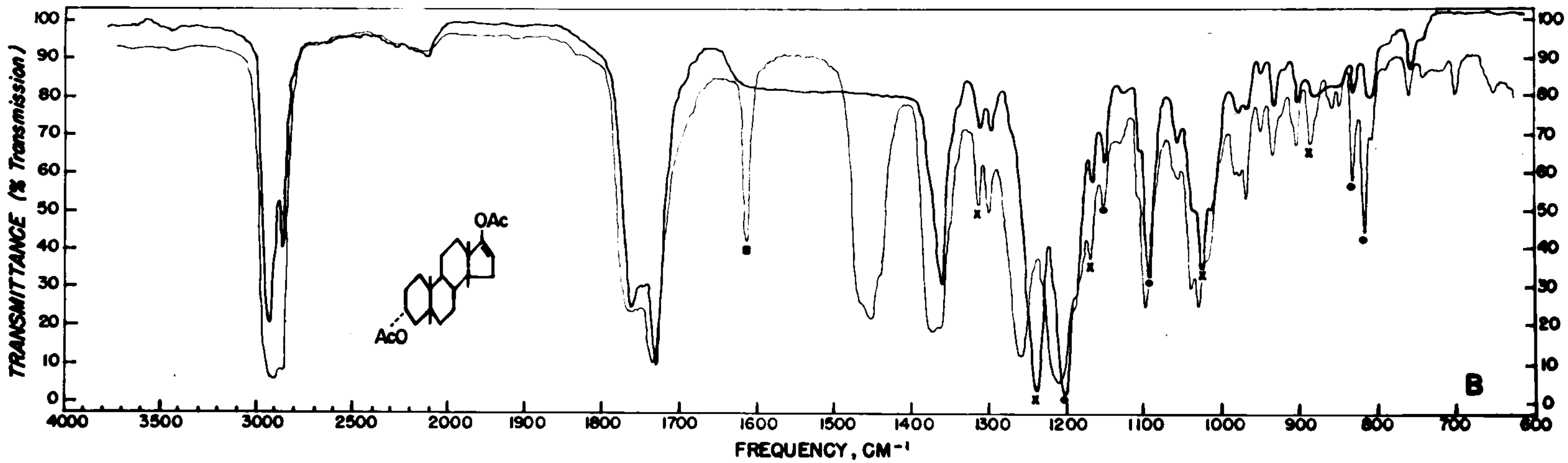
■ indicates disappearance of Δ^{16} double bond

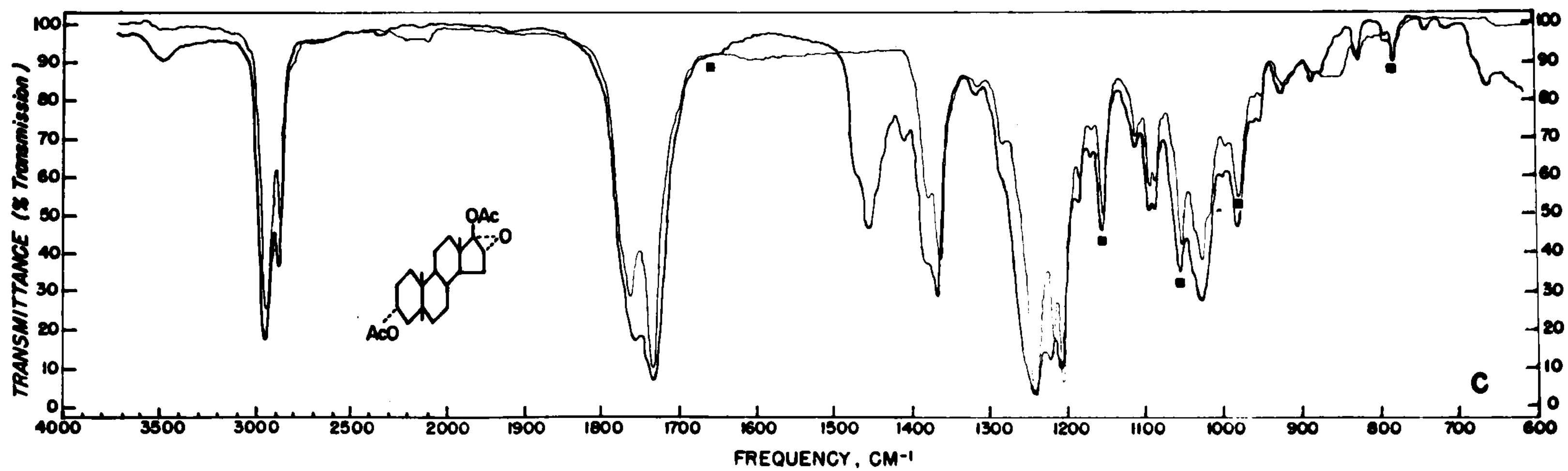
▲ indicates new bands

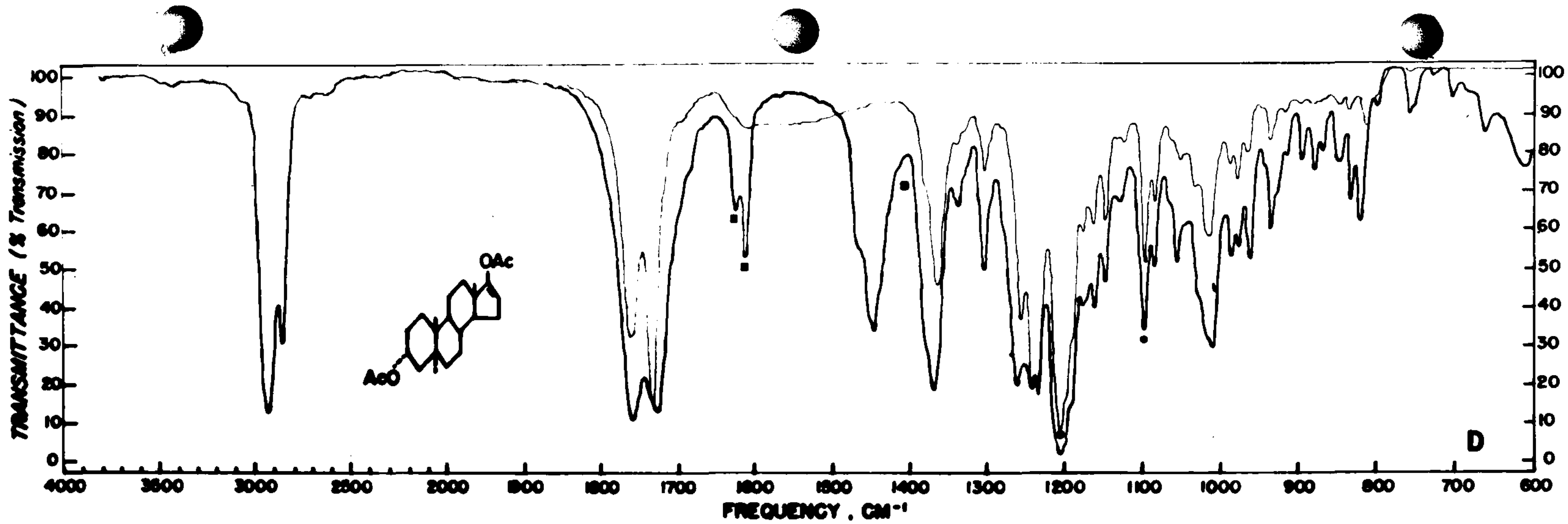
CHART F

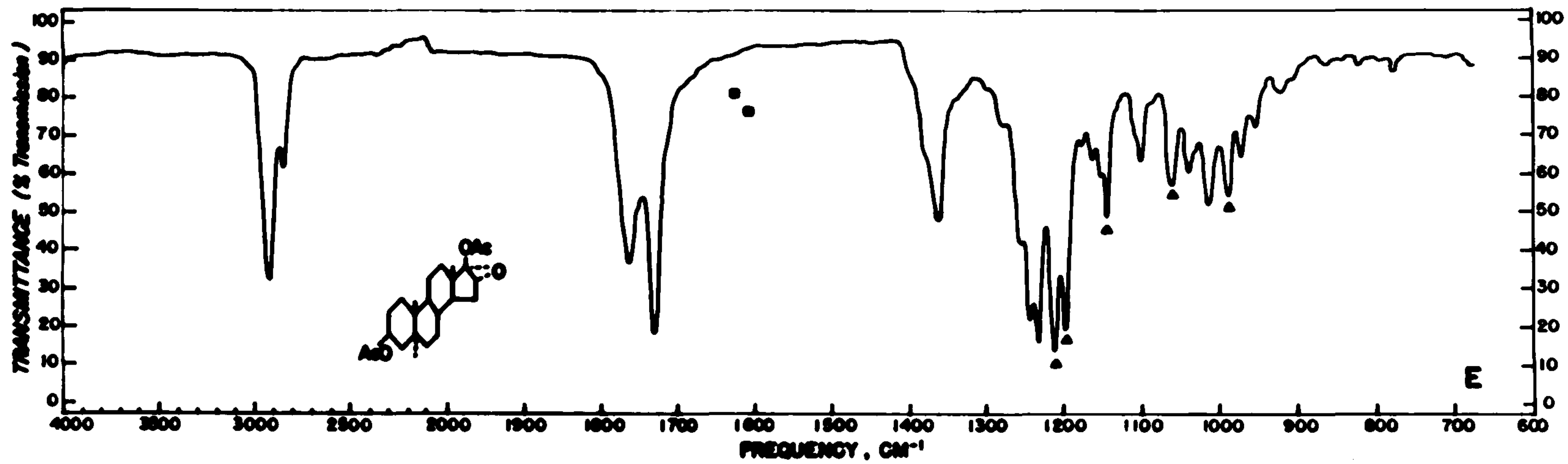
As indicated

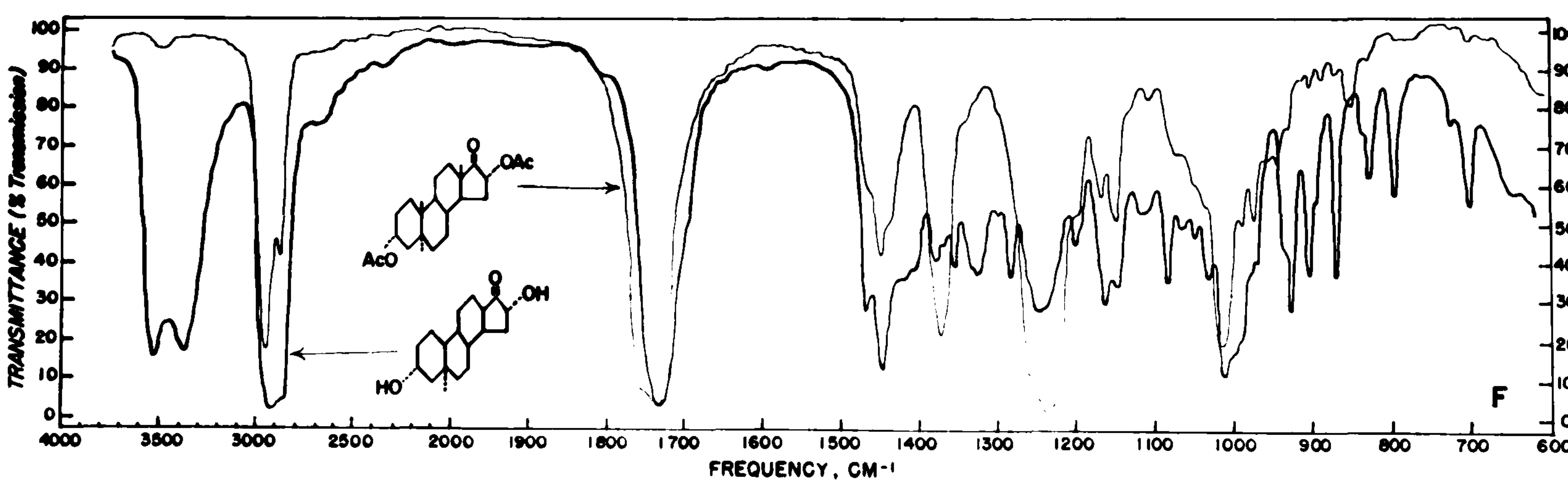

TRANSMITTANCE (% Transmission)
FREQUENCY, CM⁻¹
A
a
b
O
O


TRANSMITTANCE (% Transmission)
FREQUENCY, CM⁻¹
B
OAc
AcO


TRANSMITTANCE (% Transmission)
FREQUENCY, CM⁻¹
C
OAc
O
AcO


TRANSMITTANCE (% Transmission)
FREQUENCY, CM⁻¹
OAc
AcO
D


TRANSMITTANCE (% Transmission)
FREQUENCY, CM⁻¹
OAc
O
AcO
E


TRANSMITTANCE (% Transmission)
FREQUENCY, CM⁻¹
O
OAc
AcO
O
OH
HO
F

SUMMARY

In this thesis methods are presented for the introduction of a C-16 hydroxyl group into the 3 α-hydroxy-androstan-17-one molecule. Procedures are presented for the catalytic reduction of androst-4-ene-3,17-dione to 5 β-androstane-3,17-dione, for the preparation of 3α-hydroxy-5β-androstan-17-one by the differential reduction of 5β-androstane-3,17-dione with sodium borohydride, and for the preparation of 5 β-androst-16-ene-3 α,17β-ylene acetate and 16α,17α-epoxy-5 β-androstane-3 α,17β-ylene acetate and 16 α,17 α-epoxy-5 β-androstane-3α,17β-ylene acetate. Physical constants and infrared spectra, along with the analysis of the spectra are included.

A method has been presented for the chromatographic separation of 5 β-androstane-3,17-dione from its stereoisomer 5 α-androstane-3,17-dione. The system involves the use of acid-washed alumina, and a solvent system using various compositions of n-hexane and benzene. In addition, a chromatographic separation of 3 α-hydroxy-5 β-androstan-17-one from 3 β-hydroxy-5β-androstan-17-one has been offered.

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ABSTRACT

Since the development of methods for studying the tissue metabolism of testosterone (8), many investigators attempted to isolate and identify the catabolic products of testosterone degradation by various tissues. Reported isolation of a 16α-hydroxylated compound (14) opened a new pathway of investigation. Various investigators (5, 14, 17, 19, 22, 24) have since either reported the isolation of other C-16 oxygenated compounds, or have speculated their presence. In view of these observations, it was felt that an attempt should be made to prepare 16-oxygenated derivatives of 17-ketosteroids with the thought that some of the polar compounds isolated in the metabolic studies of Wotiz, Wotiz and Lemon, and Ofner might be steroids substituted at this position. Various syntheses for substitution at the C-16 position have been developed, particularly by Huffman and Lott (26, 33, 34, 35, 36, 37). Recently, Leeds et al. presented a method for the introduction of a C-16 ketone or hydroxyl group (1). This method was the one followed in this work.

Originally, attempts at substitution were carried out in the 5β-androstane series. The first step was the preparation of 5β-androstane-3,17-dione from androst-4-ene-3,17-dione to obtain starting material. Various conditions were investigated for the catalytic hydrogenation of androst-4-ene-dione. Variations in the concentrations of alkali and the amount of catalyst used were checked, since these facts were not found in any published reports.

The catalytic reduction of the androst-4-ene-3,17-dione produced a mixture of the stereoisomers 5β-androstane-3,17-dione

(20% yield) and 5α-androstane-3,17-dione (5% yield).

A method has been presented for their separation by adsorption chromatography on alumina using various mixtures of n-hexane and benzene. Proof of their separation was provided by infrared spectroscopy.

The reduction of the C-3 ketone of 5β-androstanedione was effected by sodium borohydride (6). Forty percent of the mixture was reduced to the 3α-hydroxy steroid, while a smaller percentage was reduced to the 3β-hydroxy compound. Separation was effected on alumina, and purity established by infrared studies and paper chromatography. The 3α-hydroxy-5β-androstan-17-one was then subjected to the Leeds procedure (1). This requires the formation of an enol acetate at the C-16,17 positions, followed by an epoxide formation at the C-16,17 positions on the nucleus. Formation of either the C-16α-hydroxy compound or the C-16 ketone is effected by acid hydrolysis to produce the former compound, or acid hydrolysis followed by alkaline hydrolysis to produce the latter. (See Scheme, page 7).

Difficulty was encountered with the enolization. Reactions were carried out until the starting material was depleted. The experiments were continued with 3α-hydroxy-5α-androstan-17-one (androsterone) which was obtained commercially. Synthesis was completed through the formation of 5β-androstane-17-oxo-3α,16α-diol. The thesis offers methods leading to its formation and physical data providing evidence for its assigned structure.