Androgenic Activity of Highly Purified 5α-Androstane and 5α-Androstan-17β-ol

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Highly purified 5α-androstan-17β-ol having steroid impurity of only 0.0005% and 5α-androstane showing steroid impurity level of less than 0.00002% were 128% (range 107–158% at $P = 0.95$) and 1.94% (range 1.49–2.50% at $P = 0.95$) as androgenic as testosterone in a chick's comb injection assay.

During the course of other studies, Jones and Leman had occasion to prepare 5α-androstan-17β-ol, and the sensitive methods of analysis now available, *e.g.*, gas–liquid partition chromatography (glpc), detected the presence of several impurities which were extremely difficult to remove by the normal chromatographic procedures. This observation prompted us to prepare a highly purified sample of the 17β-alcohol in order to ascertain whether or not the reports of previous assays[2-4] had been influenced by the possible presence of these impurities. In view of the very low level of activity reported for the parent hydrocarbon,[5,6] it also was decided to prepare highly purified 5α-androstane, since from the magnitude of the effect reported the possibility existed that the observed androgenicity might have also resulted from the presence of an active contaminant. From the experimental data given,[6] it was apparent that the sample of 5α-androstane evaluated previously had been carefully purified. However, the analytical methods available at that time were not able to detect levels of impurities below 0.1% and active contaminants at or below this concentration would only have required a potency equal to that of testosterone to have caused the observed androgenicity.

Experimental Section

Chemical.—An F and M Model 400 Biomedical unit fitted with 3.8% SE-30 on silanized Diatoport S and 1% QF-1 on silanized Chromosorb G columns was used for the glpc analyses. Alumina refers to BDH chromatographic alumina deactivated with 5% of its weight of 10% aqueous acetic acid. Thin layer chromatography (tlc) was carried out on silica gel G and the spots were visualized by spraying with 10% aqueous toluene-*p*-sulfonic acid followed by heating at 100°. Preparative layer chromatography was also effected on silica gel G and the steroids were detected by spraying with water, as fluorescent phosphor methods were found unsatisfactory. All solvents used were reagent grade and were redistilled before use. Melting points were determined on a Fisher-Johns block and are corrected. All the steroids described were purified until analysis by tlc and glpc was satisfactory.

5α-Androstan-17β-ol.—5α-Androstan-17β-ol was obtained in 72% yield by lithium in liquid ammonia reduction of 17β-

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TABLE I

ANDROGENIC ACTIVITY OF 5α-ANDROSTANE AND 5α-ANDROSTAN-17β-OL IN A CHICK COMB TEST BY INJECTION IN ABSOLUTE ETHANOL

Expt no.	Steroid	Total dose, μg	No. of chicks	Mean body wt, g	Mean comb ratio ± SE
I	0	0	10	78	0.78 ± 0.06
	Testosterone	7	10	85	1.20 ± 0.11
		70	10	88	2.62 ± 0.24
	5α-Androstane	7000	10	88	2.01 ± 0.13
	5α-Androstan-17β-ol	7000	10	90	2.82 ± 0.28
II	0	0	12	77	0.87 ± 0.06
	Testosterone	2	12	78	0.96 ± 0.06
		6	12	79	1.38 ± 0.09
		18	12	80	1.75 ± 0.16
		54	13	81	2.24 ± 0.19
	5α-Androstane	500	12	78	2.03 ± 0.21
		1500	11	76	2.16 ± 0.17
		4500	12	77	2.24 ± 0.11
	5α-Androstan-17β-ol	250	12	79	3.95 ± 0.29
		500	12	75	4.64 ± 0.35
		1000	12	76	4.54 ± 0.35
III	0	0	10	75	0.62 ± 0.06
	Testosterone	2	10	83	0.83 ± 0.06
		6	10	83	1.08 ± 0.06
		18	10	83	1.79 ± 0.14
		54	10	84	2.36 ± 0.15
	5α-Androstane	10	10	73	0.64 ± 0.04
		30	90	74	0.71 ± 0.03
		90	10	74	0.93 ± 0.06
		270	10	75	0.99 ± 0.05
	5α-Androstan-17β-ol	10	9	79	1.45 ± 0.11
		30	10	76	1.93 ± 0.13
		90	10	74	3.01 ± 0.20
		270	10	76	4.34 ± 0.36
IV	0	0	11	73	0.37 ± 0.02
	Testosterone	2	10	74	0.52 ± 0.04
		8	10	73	0.94 ± 0.09
		32	10	72	1.48 ± 0.13
		125	10	69	1.88 ± 0.20
		500	10	75	2.57 ± 0.20
	5α-Androstane	100	10	72	0.66 ± 0.04
		300	10	75	0.92 ± 0.05
		900	10	71	1.03 ± 0.08
	5α-Androstan-17β-ol	2	10	78	0.78 ± 0.06
		8	10	79	0.87 ± 0.06
		32	10	80	1.24 ± 0.07
		125	9	73	2.12 ± 0.18
		500	10	71	2.19 ± 0.15

hydroxyandrost-4-en-3-one followed by Wolff–Kishner reduction, as described by Brutcher and Bauer.[7] Attempts to effect complete purification *via* the 17β-acetate, mp 70° (lit.[8] mp 70–72°), and the 17β-toluene-*p*-sulfonate, mp 138–140° (lit.[9] mp 139–140°) were only partly successful. Treatment of the 17β-tosylate with $LiAlH_4$ in refluxing ether or dioxane for 12 hr regenerated the alcohol in 60% yield only.

5α-Androstan-17-one.—Jones' oxidation of 5α-androstan-17β-ol with chromic acid[7] afforded 5α-androstan-17-one in 95% yield. Rigorous purification by column chromatography on alumina and recrystallization from methanol gave the pure ketone as feathery plates, mp 124.8–125° (lit.[10] mp 119–121°), $\nu_{max}^{CHCl_3}$ 1738 cm.$^{-1}$

Purified 5α-Androstan-17β-ol.—A solution of pure 5α-androstan-17-one in methanol was reduced with $NaBH_4$ to give the 17β-alcohol (82% yield). Repeated layer chromatographic purification with benzene–diethyl ether (5 : 1) as the developing solvent yielded pure 5α-androstan-17β-ol, mp 166.8–168.0° (lit.[11] mp 165.5–166.5°). Glpc on SE-30 and QF-1 columns showed the steroid impurity level to be 0.0005%.

Purified 5α-Androstane.—Reduction of pure 5α-androstan-17-one with 85% hydrazine hydrate and KOH in ethylene glycol[6] gave a 95% yield of 5α-androstane. Column chromatography on alumina followed by several recrystallizations from methanol afforded the pure hydrocarbon as wafer thin plates, mp 48.2–48.9° (lit.[6] mp 50–51°). Glpc on SE-30 and QF-1 columns showed the steroid impurity level to be less than 0.00002%.

Biological.—The highly purified samples of 5α-androstan-17β-ol and 5α-androstane were assayed by inunction to the 1-day-old White Leghorn chick's comb. The details of the method have been published.[12] Briefly, the method consists in the application of the standard, testosterone, and the test compounds in 0.05 ml of absolute alcohol once daily for 7 days. Twenty-four hours after the last application the chicks were sacrificed, and comb weights and body weights were determined. The results are expressed as a comb ratio defined as the milligrams of comb per gram of body weight.

Table I lists the results of three experiments indicating the chick comb response to 5α-androstane, 5α-androstan-17β-ol, and testosterone applied directly to the comb. Statistically significant increases in comb ratio were found for total doses of 90, 270, 500, 1500, 4500, and 7000 μg of 5α-androstane. The two lowest doses of 10 and 30 μg were inactive. All total doses of 5α-androstan-17β-ol from 10 to 7000 μg were highly effective in increasing the comb ratios.

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Discussion and Conclusions

5α-Androstan-17β-ol was prepared by reduction of testosterone with lithium in liquid ammonia followed by Wolff–Kishner reduction.[7] The material obtained contained several impurities detectable by glpc. The 17β-alcohol is notoriously difficult to recrystallize and the removal of contaminants by column and layer chromatography proved extremely tedious. Attempted purification *via* the 17β-acetate and the 17β-toluene-*p*-sulfonate was also unsatisfactory. It is of interest to note that lithium aluminum hydride reduction of the tosylate in refluxing ether or dioxane regenerated the required 17β-alcohol only with difficulty.

Material of the required quality was eventually obtained by chromic acid oxidation of the above 5α-androstan-17β-ol to give the 17-ketone, which was rigorously purified by chromatography and by repeated recrystallization. Sodium borohydride reduction afforded the 17β-alcohol and preparative layer chromatography was carried out repeatedly until the steroidal impurity level, shown by glpc on SE-30 and QF-1 columns, was 0.0005%. This compound was a highly active androgen when applied directly to the comb. The calculated relative potency (testosterone = 100) was 128 (range 107–158 at $P = 0.95$).

A highly purified sample of 5α-androstane was obtained by Wolff–Kishner reduction of pure 5α-androstan-17-one[6] followed by column chromatography and repeated recrystallization. Glpc analysis on two columns showed steroid impurities in the hydrocarbon to be less than 0.00002%. The relative potency of this sample of 5α-androstane was 1.94 (range 1.49–2.50 at $P = 0.95$).

These observations confirm the previous findings of significant androgenic activity of the hydrocarbon 5α-androstane and demonstrate further that the relatively high androgenic activity of 5α-androstan-17β-ol and the androgenicity of 5α-androstane are due to the compounds *per se* and not to associated impurities.

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