Isolation of Urinary Metabolites of 17α-Methyltestosterone*

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Methyltestosterone (17α-methyl 17β-hydroxyandrost-4-ene-3-one) is an androgen which is orally effective and has had wide clinical use. We have treated patients with methyltestosterone for their advanced breast cancer (1). The compound led to a substantial decrease in urinary gonad-stimulating hormone similar to that seen after the parenteral administration of testosterone propionate. However, unlike testosterone propionate, methyltestosterone did not increase the urinary 17-ketosteroid excretion. Instead, it produced an increase in pregnanediol chromogen that was proportionate to the amount of pionate, methyltestosterone administered.

Previous work by Levedahl and Samuels (2) indicated that 17α-methyltestosterone was not converted in vitro to 17-ketosteroids by dog, rat, rabbit, or human livers, whereas ring A was metabolized. Van Stewart and Samuels (3) demonstrated that CF-17α-methyltestosterone was metabolized in vitro to 17α-methyltestosterone by man.

This lack of knowledge concerning the fate of methyltestosterone stimulated our interest in the metabolism in vivo of methyltestosterone by man.

EXPERIMENTAL PROCEDURE

A patient with a nonfunctional papillary adenocarcinoma of the ovary was given methyltestosterone orally, 1 g daily for 4 days. All of the urine passed during these 4 days was refrigerated immediately after voiding and was subsequently pooled. No preservative was added.

β-Glucuronidase Hydrolysis—Aliquots of 850 ml of urine (total volume, 3500 ml) were adjusted to pH 7.0. To each aliquot 170 ml of phosphate buffer, 0.3 M, pH 7.0, containing 17,000 units of bacterial β-glucuronidase and 170 ml of chloroform were added. After the mixture was incubated for 24 hours at 37°C, 8,500 additional units of bacterial β-glucuronidase were added in 85 ml of buffer, and the mixture was incubated for an additional 48 hours.

Extraction Procedure—The urine was separated from the chloroform, readjusted to pH 7.0 if necessary, and extracted four successive times with 2 volumes of ethyl acetate. The residual urine was saved. The ethyl acetate was concentrated in a vacuum to 250 ml. This concentrate was then extracted three times with 0.1 volume of distilled water and dried with anhydrous Na2SO4. The Na2SO4 was then washed with 25 ml of ethyl acetate, and this washing was added to the main body of ethyl acetate. The ethyl acetate was then distilled under reduced pressure.

RESULTS

Purification of Ethyl Acetate Residue—The ethyl acetate residue from the β-glucuronidase hydrolysate was dissolved in ethyl acetate and chromatographed on 35 g of silica gel prepared in ethyl acetate. After 600 ml of ethyl acetate had passed through the column, the remaining material was eluted with methanol. These two fractions were dried under reduced pressure. The ethyl acetate residue was then rechromatographed on 35 g of silica gel prepared in ethyl acetate. After 600 ml of ethyl acetate had passed through the column, the remaining material was eluted with methanol. These two fractions were dried under reduced pressure. The ethyl acetate residue was then rechromatographed on 35 g of silica gel prepared in ethyl acetate. After 600 ml of ethyl acetate had passed through the column, the remaining material was eluted with methanol.

Semicrystalline materials eluted in the petroleum ether-chloroform mixture (1:3) were combined and rechromatographed on 3 g of silica gel prepared in petroleum ether-chloroform (1:3) eluates. The petroleum ether-chloroform (1:3) fractions were combined and rechromatographed on 3 g of silica gel prepared in petroleum ether-chloroform (1:3) mixture. The column was developed as described above. Semicrystalline materials eluted in the petroleum ether-chloroform mixture (1:3) were combined and rechromatographed on 1 g of silica gel prepared in petroleum ether-chloroform (1:1) eluates. The petroleum ether-chloroform (1:1) fractions were combined and rechromatographed on 1 g of silica gel prepared in petroleum ether-chloroform (1:1) eluates. The petroleum ether-chloroform (1:1) eluates were combined and rechromatographed on 1 g of silica gel prepared in petroleum ether-chloroform (1:1) eluates. Crystalline material was obtained in the petroleum ether-chloroform (3:1) eluates. This recrystallized from aqueous ethanol and yielded 10 mg of crystalline compound which melted at 189-192°C. The compound was identified as 17α-methyl-5α-androstan-3α,17β-diol by its melting point and infrared spectra. 17α-Methyl-5α-androstan-3α,17β-diol, melting point 189-190°C, was obtained from the Grignard reaction of androstan-4-one acetate and methyl-magnesium iodide (5), and the melting point of a mixture of this compound with the substance above was not depressed.

The methanol residue from the preliminary ethyl acetate column was dissolved in chloroform and chromatographed on 35 g of silica gel prepared in chloroform. The residue was eluted from the column by using chloroform and graded chloroform-ethanol eluates. All of the chloroform eluates were combined

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and dried. This residue was rechromatographed on 3 g of silica gel prepared in a petroleum ether-chloroform mixture (3:1). The column was developed in the normal manner. Crystalline material (100 mg) was obtained in the petroleum ether-chloroform (3:1) fractions which melted at 165-168°. The compound was identified as 17α-methyl-5β-androstane-3α, 17β-diol by its melting point, by the fact that the melting point of a mixture with authentic 17α-methyl-5β-androstane-3α, 17β-diol was not depressed, and by its infrared spectra.

17α-Methyl-5β-androstane-3α, 17β-diol, melting point 164-166°, was obtained by the reduction of 17β-hydroxy-17α-methyl-5β-androstan-3-one (6) in methanol by aqueous solutions of sodium borohydride, a stereoselective reagent which reduces 3-ketones of 5β-steroids to alcohols of 3α-configuration (7).

Dehydration and Rearrangement of 17α-Methyl-5β-androstane-3α, 17β-diol—17α-Methyl-5β-androstane-3α, 17β-diol (20 mg) was dissolved in 20 ml of ethanol and diluted to 50 ml with distilled water. Then 0.1 volume of concentrated hydrochloric acid was added, and the mixture was refluxed for 15 minutes, cooled to room temperature, and extracted three times with 2 volumes of ethyl acetate. The ethyl acetate was dried over anhydrous sodium sulfate and distilled under reduced pressure. The dry residue was crystallized from aqueous methanol, and fine needles were obtained which melted at 114–116°. The compound was identified as 18-nor-17-dimethyl-5β-androstane-3α, 17β-diol by its infrared spectra, and the melting point of a mixture of this compound with authentic 18-nor-17-dimethyl-5β-androstane-3α, 17β-diol was not depressed, and by its infrared spectra.

The metabolism in vivo of 17α-methyltestosterone has been partially elucidated. The substantial increase in urinary pregnanediol-like chromogens after methyltestosterone administration (1) suggests that it may affect the metabolism of pregnanediol in some manner, or that some metabolite of 17α-methyltestosterone may give a color similar in intensity to that of pregnanediol.

We have isolated two metabolites, 17α-methyl-5β-androstane-3α, 17β-diol and 17α-methyl-5α-androstane-3α, 17β-diol, from the urine of a patient given 17α-methyltestosterone. It is of interest that the 5β isomer was isolated in greater amount.

The small amounts of metabolites isolated may be explained by excretion in the stool instead of the urine, or by failure to hydrolyze the urinary conjugates completely. Loss during chromatography is unlikely, since recoveries of known compounds by this method range from 90 to 95%.

The increase in pregnanediol-like chromogens can hardly be explained by 17α-methyltestosterone, since it produces a color approximately one-twentieth (1) of that produced by an equivalent weight of pregnanediol.

Since the previous urinary pregnanediol studies (1) were carried out on urine that was treated with hydrochloric acid, it was considered that rearrangement of the metabolites may be the cause of the high pregnanediol values. When 17α-methyltestosterone is refluxed in a mixture of hydrochloric acid and acetic acid (1:1), a rearrangement occurs in which 18-nor-17-dimethylandrost-4,13-diene-3-one is formed. With this information in mind, 17α-methyl-5β-androstane-3α, 17β-diol was refluxed with acid, and 18-nor-17-dimethyl-5β-androst-13-ene-3α-ol was isolated.

The color produced by sulfuric acid on 18-nor-17-dimethyl-5β-androst-13-ene-3α-ol is approximately one-third of that produced by an equivalent amount of pregnanediol. This artifact, due to acid hydrolysis, appears to be a source of the increased pregnanediol-like chromogens in the previous studies. Isolation of the artifact from urine after acid hydrolysis is being attempted at the present time.

SUMMARY

17α-Methyl-5β-androstane-3α, 17β-diol and 17α-methyl-5α-androstane-3α, 17β-diol in a ratio of 10:1 were isolated from the urine of a patient after the administration of 17α-methyltestosterone. Refluxing 17α-methyl-5β-androstane-3α, 17β-diol with 10% hydrochloric acid produced 18-nor-17-dimethyl-5β-androst-13-ene-3α-ol which gives a color with sulfuric acid that is approximately one-third as intense as an equivalent amount (weight) of pregnanediol. The acid hydrolysis artifacts are thought to be the cause of the increase of pregnanediol-like chromogens found after administration of 17α-methyltestosterone.

REFERENCES


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