CHAPTER II

INFORMATION

Pharmacology

Methyltestosterone does not occur naturally. It may be synthesized from cholesterol. Methyltestosterone is 17-α-methyl derivative of testosterone. Methyltestosterone has actions and uses similar to those of testosterone except that the former is active when administered orally or sublingually.

Methyltestosterone is an androgenic hormone but it is considered to be much less effective than long-acting esters of testosterone or testosterone pellets as replacement therapy in androgenic-deficient males. Although it is ineffective in producing full sexual maturation in patients with prepuberal testicular failure, but it may be effective when hypogonadism develops in adult life or after development of secondary sexual characteristics during parenteral therapy. As androgenic therapy depresses spermatogenesis, it should not be employed if sperm persist and particularly if preservation of fertility is desired. In the female, it is used for prevention of postpartum breast pain and engorgement in the nonnursing mother, and in the palliation of androgen-responsive; inoperable breast cancer in women who are more than 1 year, but less than 5 years, postmenopausal or who

have been proved to have a hormone-dependent tumor, as shown by previous beneficial response to castration.

Methyltestosterone also can be used for anabolic effects, increases protein synthesis in the body in both normal and pathological conditions. It causes retention of nitrogen in the body and a gain in weight. This action occurs even in the adrenalectomized animal; however it also inhibits formation of the catabolic 11-oxysteroids by the adrenal gland. Weight gain is associated with retention of nitrogen, phosphorus, sulfate, potassium, chloride and sodium. The retention of potassium is characteristic of the action. Methyltestosterone is useful to promote healing of fractures in old men and in combination with an estrogen, is prescribed in the treatment of postmenopausal osteoporasis. The combination exerts the anabolic action required to stimulate the formation of bone matrix, which is required for reminalization, and the smaller effective doses of each in the combination minimize the undesirable estrogenic and androgenic side effect(1).

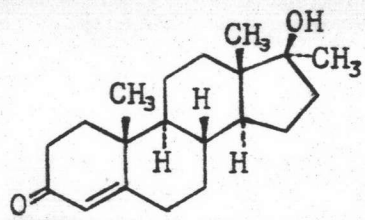
Untoward effects Jaundice of intrahepatic obstructive type, with little or no hepatocellular damage or fatty infiltration but with inspissated bile plugs in the canaliculi, may develop during therapy with methyltestosterone; patients recover on discontinuing therapy. Hepatic dysfunction develops in most persons recieving methyltestosterone in significant amounts for sufficient periods of time. Prolonged administration or excessive dosage may cause inhibition of testicular

function in the male, resulting in oligospermia and decrease in ejaculatory volume. It can produce virilization in females special attention should be given to any evidence of hoarseness or deepening of the voice, acne, hirsutism, enlarged clitoris, stimulation of libido, and menstrual irregularities. It should be used with caution in young boys to avoid possible premature epiphyseal closure or precocious sexual development. It may cautious use in patients with cardiac or renal disease, because of the retention of sodium and water, occasionally resulting in edema. It is contra-indicated in patients with liver disturbances(1,13).

The usual adult dose for androgen deficiency given orally in tablet form, is 10 mg three times daily, with a usual range of daily dose of 10 to 50 mg. For postpartum breast pain and engorgement the usual daily dose, given in divided portions, is 80 mg (for 3 to 5 days). For metastatic breast carcinoma in women the daily dose, given in divided portions, is 50 to 200 mg. For growth stimulation in boys, or anabolic effect, the daily dosage is 10 to 20 mg. Dosage by buccal or sublingual route is one-half the oral dosage(1).

Chemistry

1. Chemical name The chemical name of methyltestosterone is 17 β-hydroxy-17-methylandrost-4-en-3-one.



2. Preparation

Methyltestosterone was prepared by various methods:

2.1 17-Methyl-5,6-androstene-3,17-diol was oxidized with aluminum tert-butylate in acetone as H acceptor, methyltestosterone was obtained(15,16).

2.2 5-Chloroandrostane-17-one on treatment with methylmagnesiumiodide gave 5-chloro-17-methylandrostane-17-one which was reacted with potassium hydroxide to give Δ^4-17-methylandrosten-17-ol. Methyltestosterone was obtained from the reaction of Δ^4-17-methylandrosten-17-ol with chromic acid(17).

2.3 Androstenedione-3-enol ethylether was allowed to react with methylmagnesiumbromide, the 3-enol ethyl ether of 17-methyltestosterone was obtained, which could easily be hydrolyzed to methyltestosterone(18).

2.4 The 3-ethylene mercaptole of androstenediol was reacted with methylmagnesiumbromide in diethyl ether, and then treated with methylene and alkali metal in liquid ammonia. The product, 3-ethylene mercaptole of 17-methyltestosterone was hydrolyzed to methyltestosterone(19).

2.5 Methyltestosterone could be prepared from Δ^5-androsten-3β-ol-17-one (dehydroisoandrosterone) by treatment with methylmagnesiumiodide to give the 17-α-methyl compound which was oxidized to yield methyltestosterone by an Oppenauer oxidation(20,21).

3. Description

Methyltestosterone occurs as white or creamy-white crystal or as crystalline powder that is odorless and stable in air but is affected by light. Melts between 163° and 168°. It is sensitive to alkali(1,20,22).

4. Solubility

Methyltestosterone is practically insoluble in water; soluble 1 in 5 of alcohol, 1 in 10 of acetone, and 1 in 160 of arachis oil; freely soluble in chloroform and dioxane; soluble in methyl alcohol; sparingly soluble in fixed oils; slightly soluble in diethyl ether(13,22).

Method of analysis

1. Spectrophotometric method

1.1 Ultraviolet absorption method

Ultraviolet spectrophotometry is one of the most general quantitative analytical method of bulk steroid hormones and their dosage forms. Methyltestosterone can be determined, after dissolving in absolute ethanol directly(4) or after thin-layer separation(3), by UV-spectrophotometer. The solution is diluted to a concentration of about 10 mcg per ml, and the maximum absorption is determined at about 241 nm. For tablet, the finely powdered tablet is suspended in water and extracted with chloroform. After filtration through a plug of cotton-wool, the combined extracts are evaporated to dryness in a stream of air on a boiling water-bath. The residue is dissolved in ethanol and the absorbance

of the resulting solution is measured at the maximum at about 241 nm. A standard methyltestosterone solution in the same medium having a known concentration of about 10 mcg per ml is compared(3).

Very valuable data can be obtained by direct measurement in relatively inexpensive equipment and the extreme simplicity of this method are advantages, but its selectivity is rather poor. Therefore, this method cannot be used in the combined drugs of vitamin-hormone preparations.

1.2 Colorimetric method

The most widely used colorimetric method based on the reaction of methyltestosterone with 2,4-dinitrophenylhydrazine in acid medium forming 2,4-dinitrophenylhydrazone derivative. Methyltestosterone in aldehyde-free ethanol was mixed with a hot, freshly prepared and previously filtered of 0.25 per cent w/v solution of 2,4-dinitrophenylhydrazine in 2 M hydrochloric acid. The mixture was treated on a water-bath for 30 minutes, allowed to stand overnight and filtered. The precipitate was washed with hydrochloric acid solution and water, dried at 105° for 30 minutes and dissolved in chloroform. The maximum absorbance of the resulting solution was measured at 390 nm against blank prepared by the same procedure(4).

Hosangadi and Farias(5) also described two methods for the colorimetric determination of methyltestosterone tablets with 2,4-dinitrophenylhydrazine. In the first method, methyltestosterone solution in carbonyl-free methanol was

treated with 0.1 per cent solution of 2,4-dinitrophenylhydrazine in methanol in acid medium and heated under reflux for 30 minutes. The reaction mixture was transfered into the column packed with acidic cation exchange resin, Indion-225 resin. The solution was carried out with methanol and the resulting solution was measured at 360 nm against a reagent blank. In the second method, the reaction mixture was extracted with hexane. Then the hexane extract was measured at 360 nm.

These methods are time-consuming. The spectrophotometric determination cannot be carried out in the presence of excess 2,4-dinitrophenylhydrazine, because it exhibits appreciable absorbance at the maximum of its 2,4-dinitrophenylhydrazone derivative. These methods are specific for the reaction of carbonyl group, therefore it cannot be used to analyzed the drug in combined preparations.

1.3 Infrared spectrophotometric method

Carol(6) determined methyltestosterone in tablets using infrared spectrophotometer. Powdered tablet was extracted with diethyl ether. The combined extracts were washed with saturated sodium hydrogen carbonate solution and water, and evaporated to dryness. The residue was dissolved in 1 ml of carbon disulfide and the extinction of this solution was measured at 935 cm^{-1} against carbon disulfide using 1 mm cells.

Ito and Amakasu(7) developed the assay method in the similar manner, but used the more intensive $\nu C=O$ band at 1,678 cm^{-1} and the $\nu C-O$ band at 1,085 cm^{-1} for the analysis.

This method took time and needed a special technique. Methyltestosterone should be preliminary purified, but the purification of methyltestosterone in vitamin-hormone preparations is difficult.

2. Gas chromatographic method

Pawelczyk et al.(8) presented a gas chromatographic method for methyltestosterone preparations using a glass column 1.2 m long packed with 10 per cent SE-30 on chromosorb W(HMDS).

Bruschi(9) described gas chromatographic procedure for the assay of methyltestosterone in multivitamin preparations. A chloroform extract of the sample was injected into a 2-m long column packed with a support containing 1 per cent of silicone gum on silanised chromosorb W, at 250°. Tocopheryl acetate in the preparation was also analyzed with evaluable peak in the chromatogram.

For quantitative analysis of methyltestosterone using gas chromatography, the optimum experimental conditions must be strictly adhered to. In various vitamin-hormone formulations, the determinations of optimum conditions are rather difficult.

3. Densitometric method

Jarzebinski et al.(10) described the densitometric determination of methyltestosterone in pharmaceutical preparations. Methyltestosterone that combined with dieneestrol was dissolved in the mixture of chloroform and methanol (1:1).

Five microliters of the resulting solution, containing 2 mg per ml of methyltestosterone, was applied on thin-layer chromatographic plate. The plate was developed in suitable mobile phase and sprayed with the methanolic solution of isonicotinohydrazide. The colored spots were measured at 380 nm with a densitometer.

Ivanova and Sokolov(11) presented the chromatospectrophotometric method for the determination of methyltestosterone. Silufol UV-254 plates were used for TLC analysis in the developing solvent of chloroform-methanol-water 475:25:1. The absorbance was measured at 242 nm.

These methods were simple and rapid, but not sensitive, especially in the vitamin-hormone preparations which contain small amount of methyltestosterone.

The proposed method

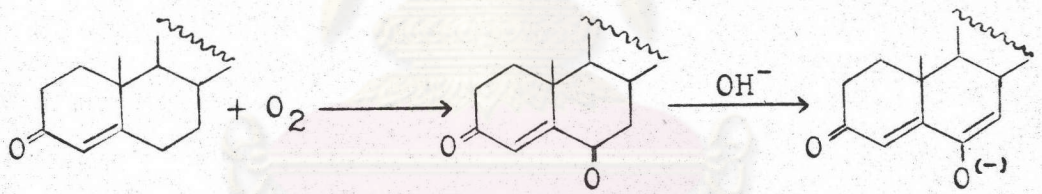
The purpose of this work is to develop a convenient, rapid and precise method which could be employed in quality control of methyltestosterone in vitamin-hormone preparations.

Many methods for the determination of methyltestosterone in pharmaceutical preparations have been described, including ultraviolet spectrophotometry, colorimetry, infrared spectrophotometry, gas chromatography and densitometry. These methods are lacking in either sensitivity or specificity. Because of small amount of methyltestosterone in vitamin-hormone preparations, fluorescence measurement combined with thin-layer chromatographic separation are now being used with advantage. Thin-layer chromatography gives a sig-

nificant increase in specificity and fluorescence measurement enhances sensitivity.

From an analytical point of view, Δ^4-3-keto group is the most important functional group of steroid hormones, methyltestosterone. Spectrophotometric method used in the determination of methyltestosterone both directly measured (3,4) and previously been made derivatives(4,5), were based on this functional group.

Δ^4-3-keto group is rather sensitive, especially in alkaline media, towards oxidative effects. Its product is the Δ^4-3,6-dione, which exhibits fluorescence in alkaline media, the wavelength of maximum excitation is 385 nm and the wavelength of maximum emission is 580 nm.



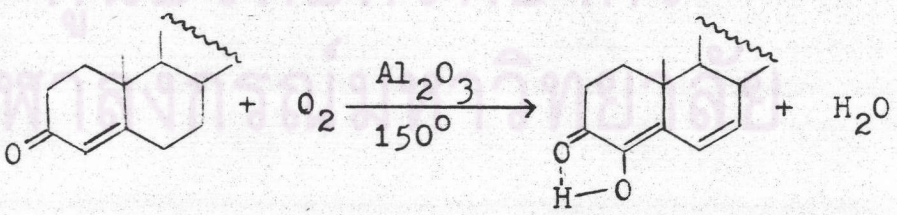
Abelson and Bondy(23,24) described the fluorimetric determination of Δ^4-3-ketosteroids in biological samples by carrying out the reaction in potassium tert-butoxide dissolved in tert-butyl alcohol. The method of Bush(25,26) for the detection of Δ^4-3-ketosteroids on paper-chromatograms with methanolic sodium hydroxide was based on the same reaction.

Egg and Huck(27) presented new fluorimetric determination of urinary testosterone by thin-layer densitometry. This method based on the specific reaction of Δ^4-3-keto

group of testosterone using TLC plate aluminum oxide F_{254} (type T) for separation. After heating the plate for 20 minutes at 180°, testosterone and other Δ^4-3-ketosteroids were characterized in long-wavelength ultraviolet light (366 nm) by a light blue fluorescence at maximum at about 440 nm. This new reaction was very sensitive and allowed the determination of small amounts of urinary testosterone by means of thin-layer densitometry without preliminary purification. The lowest amount of testosterone that could be detected was about 5 ng.

Egg(28) described the method for the determination of progesterone in plasma by thin-layer densitometry. The same specific reaction was carried out as described in the method for the determination of urinary testosterone(27).

This specific reaction called " aluminum oxide fluorescence " was taken place by heating of Δ^4-3-ketosteroids applied on alumina TLC plate in which aluminum oxide reacted as a catalyst. The blue fluorescent compound produced could be identified as 4-hydroxy-3-oxo-$\Delta^{4,6}$ steroids(12).



It was reported(12) that the sensitivity remained constant in the 150° to 180°, with a heating time of 20 minutes. However, the selectivity of the reaction increased with decreasing temperature, as the sensitivity for the

3-hydroxy-Δ^5-steroids decreased considerably at the same time. Therefore, the heating temperature at 150° for 20 minutes was used by Egg(28). The fluorescence intensity has been found remaining constant for a few days(28).

This method was modified in the determination of contraceptive hormones available in Thailand by Sittisomwong(29).

The outline of this research could be stated as follows:

1. The optimum mobile phase for the separation of methyltestosterone in vitamin-hormone preparations was selected by running thin-layer chromatography in different kinds and ratios of solvents. The optimum conditions such as a wavelength of excitation and a wavelength of emission; stability of fluorescence on time and sensitivity were studied. Interference of other substances such as hormone, vitamins, minerals, alkaloids and excipients were determined.

2. The accuracy and precision of the proposed method were determined.

3. The proposed method was compared with an official USP method, and applied to determine the contents of methyltestosterone in various commercial methyltestosterone preparations.

<u>The usefulness of the proposed method</u>

1. The new method is suitable for the assay of methyltestosterone in vitamin-hormone preparations. It is

the sensitive and less time-consuming method.

2. The new method is carried out by using a technique of thin-layer chromatography for the separation of very small quantities of methyltestosterone from mixtures.

3. The new method shows selectivity towards certain type of ketosteroids, which has advantage in quality control works of other Δ^4-3-ketosteroids.