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METHOD OF TREATMENT USING  
17 $\beta$ -CYCLOPROPYLAMINOANDROSTENE DERIVATIVES

BACKGROUND OF THE INVENTION

5 A. Chemistry

European Patent Application 0 288 053, published October 26, 1988, describes the preparation of 17 $\beta$ -(cyclopropylamino)androst-5-en-3 $\beta$ -ol and related compounds and indicates that those compounds are useful in the treatment of androgen-dependent disorders. British Patent 1,027,746 describes a variety of steroids having a variety of 17-amine substituents. The most relevant substituent of this type would appear to be (mononuclear cycloalkyl)amine wherein the cycloalkyl group is defined as containing 3 to 8 carbon atoms.

10 However, the only compounds of this type specifically described in the patent contain a cyclohexyl group. No other cycloalkyl group is even named in the patent. In addition, the compounds in the patent are described as possessing central stimulant activity but there is no elaboration on that activity and there is no suggestion of the inhibition of aldosterone biosynthesis.

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B. Utility

Aldosterone is a steroidal hormone which is synthesized in the zona glomerulosa cells of the adrenal glands. The primary biological function of this compound is the regulation of salt retention and, in particular, aldosterone plays a major role in controlling the reabsorption of sodium ions

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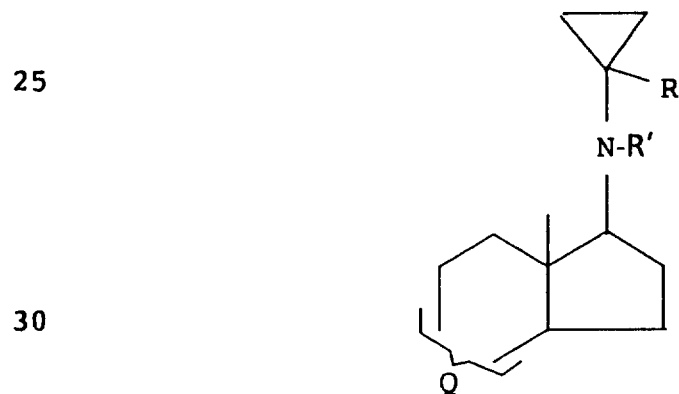
from the urine by the kidney. Thus, a deficiency of the enzyme responsible for the synthesis of aldosterone is a characteristic of patients with a salt-losing syndrome, while primary hyperaldosteronism can result from hyperbio-  
5 synthesis of aldosterone as caused by an adrenocortical tumor or the administration of certain drugs. The hyperaldosteronism may involve hypertension, hypokalemia, alkalosis, muscular weakness, polyuria and polydipsia. Thus, treatment of hyperaldosteronism and the conditions  
10 associated with it would be possible by blockage of the enzymatic synthesis of aldosterone.

#### SUMMARY OF THE INVENTION

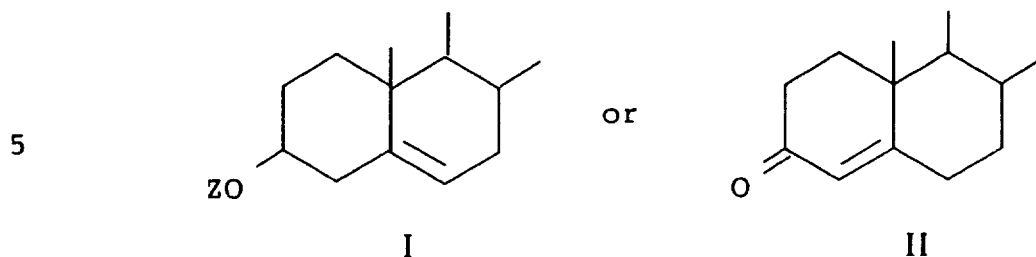
The present invention relates to the use of certain 17-  
15 cyclopropylamino derivatives of androstene as aldosterone inhibitors.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for treating  
20 hyperaldosteronism which comprises administering to a patient having said condition a therapeutically effective amount of a 17-cyclopropylaminoandrostene of the formula



wherein R is hydrogen or methyl; R' is hydrogen, C<sub>1</sub>-C<sub>4</sub>  
35 alkyl or cyclopropyl; and Q is



10 wherein Z is hydrogen, alkanoyl of 1-10 carbon atoms, cyclopentane-alkanoyl or benzene-alkanoyl wherein the alkanoyl portion of the cyclopentane-alkanoyl or benzene-alkanoyl contains up to 4 carbon atoms. Examples of alkanoyl groups are acetyl, propionyl, butanoyl, and decanoyl; examples of the cyclopentane- and benzene-alkanoyl groups are cyclopentanepropionyl and benzenepropionyl. Preferred compounds are those in which Q is structure II.

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Acid addition salts of the aforesaid compounds with pharmaceutically acceptable acids are equivalent to the above amines for the purposes of this invention. Illustrative of such salts are the salts with inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric and like acids; with organic carboxylic acids such as, for examples, acetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic and dihydroxymaleic, benzoic, phenylacetic, 4-aminobenzoic, 4-hydroxybenzoic, anthranilic, cinnamic, salicylic, 4-aminosalicylic, 2-phenoxybenzoic, 2-acetoxybenzoic, mandelic and like acids; and with organic sulfonic acids such as methanesulfonic acid and p-toluenesulfonic acids.

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A preferred embodiment of the present invention relates to a method for treating hyperaldosteronism which comprises administering to a patient having said condition 17 $\beta$ -(cyclopropylamino)androst-4-en-3-one or 17 $\beta$ -[N-methyl(cyclopropylamino)]androst-4-en-3-one.

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More specifically, the present invention relates to the use of the indicated compounds to inhibit the synthesis of aldosterone and thus for use in a method for the treatment of conditions in which such inhibition would be desired.

5 Thus, the indicated compounds are useful in a method for the treatment of hyperaldosteronism and various conditions wherein a reduction of the excessive amount of aldosterone responsible for the condition would be beneficial. That is, they are useful in a method for the general treatment of  
10 hyperaldosteronism and any associated hypertension, edema and/or sodium retention whether this is the result of some bodily disorder or whether it results from the administration of some agent. As a result of their effect on the factors responsible for edema and/or sodium retention, the  
15 indicated compounds would be useful in a method for treatment as diuretic agents.

The activity of the indicated compounds as aldosterone inhibitors and, thus, their utility in a method for treating  
20 hyperaldosteronism can be demonstrated by the following procedure which measures the inhibition of enzymes in the synthesis of aldosterone.

Young male Sprague-Dawley rats were maintained on a  
25 sodium-deficient diet for about two weeks prior to use. From these animals, adrenal capsule/glomerulosa homogenates were prepared (6 mg/ml) in pH 7.4 assay buffer [MgCl<sub>2</sub> 8.5 mM, CaCl<sub>2</sub> 2.7 mM, KCl 3.13 mM, NaCl 7.591 mM, TRIS 50 mM and 0.1% triethylamine] and centrifuged 500xg for 10 minutes.

30 Assays were conducted in 35 ml glass tubes maintained at 25°C in a Dubnoff shaker with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The tubes contained the following material: 100 µl of an NADPH<sup>+</sup> generating system, 300 µl of adrenal capsular/glomerulosa cytosol, and 50 µl of test compound or buffer (control). After  
35 initial preincubation intervals of 20 minutes, the 10-minute assay was started by the addition of 50 µl of tritium-labelled substrate, i.e., 1 µM [<sup>3</sup>H]-DOC. Reactions were

quenched by the addition of 5 ml of ethyl acetate and non-radiolabelled steroids were also added. The samples were extracted twice with 5 ml of ethyl acetate and the solvent evaporated under nitrogen at 30-40°C.

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Residues were redissolved in methanol:water (40:60) with 0.1% triethylamine and high performance liquid chromatography was used to separate products on a C18 reverse phase (5  $\mu$  ODS-Hypersil) column (4.6 x 250 mm, Shannon) with a 1 ml/min flow rate using an MeOH:H<sub>2</sub>O gradient (solvent A 10/90:solvent B 90/10).

Substrate remaining and products formed were monitored by UV absorbance at 246 nm and the amount of steroid compound present was quantified by [<sup>3</sup>H] radioactivity. The concentration of compound to produce half-maximal inhibition (IC<sub>50</sub>) of aldosterone formation from the 40 minute preincubation data was graphically estimated from a linear-log plot of percent inhibition vs. log of the compound concentration. The K<sub>i</sub> for time-dependent inhibition and the t<sub>1/2</sub> for enzyme inactivation at infinite inhibitor concentration (t<sub>50</sub>) was determined by the linear regression analyses of t<sub>1/2</sub> vs. 1/[I] data of Kitz-Wilson plots. Using this procedure, the following results were observed:

| <u>Test Compound</u>                                                | <u>IC<sub>50</sub> (<math>\mu</math>M)</u> | <u>K<sub>i</sub> (<math>\mu</math>M)</u> | <u>t<sub>50</sub> (min)</u> |
|---------------------------------------------------------------------|--------------------------------------------|------------------------------------------|-----------------------------|
| 17 $\beta$ -(Cyclopropyl-amino)androst-4-en-3-one                   | 0.18                                       | --                                       | --                          |
| 17 $\beta$ -(Cyclopropyl-amino)androst-5-en-3 $\beta$ -ol           | 1.10                                       | 4.85                                     | 9.7                         |
| 17 $\beta$ -[N-Methyl(cyclopropylamino)]androst-4-en-3-one          | 0.13                                       | 0.14                                     | 15.4                        |
| 17 $\beta$ -[N-Methyl(cyclopropylamino)]-androst-5-en-3 $\beta$ -ol | 0.35                                       | 0.35                                     | 9.0                         |

The above results demonstrate the effectiveness of 17 $\beta$ -cyclopropylaminoandrostenes as inhibitors of aldosterone

biosynthesis according to the method of the present invention. The 17 $\beta$ -[N-methyl(cyclopropylamino)]androst-4-en-3-one was obtained from the corresponding 5-en-3 $\beta$ -ol by the same procedure described in EP 0 288 053 for the preparation  
5 of 17 $\beta$ -(cyclopropylamino)androst-4-en-3-one.

To achieve a particular desired effect, such as a diuretic effect, in the method of the present invention, the compounds as described above can be administered orally or  
10 parenterally, for example, intramuscularly and subcutaneously, to a patient in need of treatment. The term patient is taken to mean a warm-blooded mammal/such as rats, mice, dogs, cats, horses, pigs, cows, sheep and humans. The compounds of the invention can be administered alone or suitably admixed in the form of a pharmaceutical preparation to  
15 the patient being treated. The amount of compound administered will vary with the severity of the condition and repetitive treatment may be desired. For oral and parenteral administration, the amount of compound administered, that  
20 is, the diuretic effective amount, is from 0.1 to 150 mg/kg of body weight per day and preferably from 1 to 50 mg/kg of body weight per day. Unit dosages for oral or parenteral administration may contain, for example, from 5 to 200 mg of the active ingredient. The compounds can be administered  
25 alone or in combination with one another, or in combination with other diuretics.

For oral administration, the compounds can be formulated into solid or liquid preparations, such as capsules, pills,  
30 tablets, troches, powders, solutions, suspensions or emulsions. The solid unit dosage forms can be a capsule which can be of the ordinary gelatin type containing the active compound and a carrier, for example, lubricants and inert filler such as lactose, sucrose and corn starch. In another  
35 embodiment, an active compound of the invention can be tableted with conventional tablet bases such as lactose, sucrose and corn starch in combination with binders such as acacia, corn starch or gelatin, disintegrating agents such

as potato starch or alginic acids and a lubricant such as stearic acid or magnesium stearate.

For parenteral administration, the compounds may be administered as injectable dosages of a solution or suspension of the compound in a physiologically acceptable diluent with a pharmaceutical carrier which can be a sterile liquid such as water-in-oil with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. Illustrative of oils which can be employed in these preparations are those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil and mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions, ethanol and glycols, such as, propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

The compounds can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants may employ inert materials such as biodegradable polymers and synthetic silicones, for example, Silastic, silicone rubber manufactured by the Dow-Corning Corporation. Sustained release can also be achieved by use of an appropriately formulated transdermal patch.

The following are illustrative pharmaceutical formulations suitable for oral or parenteral administration which may be employed in practicing the present invention:

TABLET

|    |                                                                 |          |
|----|-----------------------------------------------------------------|----------|
| 35 | (a) 17 $\beta$ -[N-Methyl(cyclopropylamino)]-androst-4-en-3-one | 75.0 g   |
|    | (b) Lactose                                                     | 1.216 kg |
|    | (c) Corn starch                                                 | 0.3 kg   |

Mix the active ingredient, the lactose, and the corn starch uniformly. Granulate with 10% starch paste. Dry to a moisture content of about 2.5%. Screen through a No. 12 mesh screen. Add and mix the following:

- 5 (a) Magnesium stearate 0.015 kg  
(b) Corn starch qs ad 1.725 kg

Compress on a suitable tablet machine to a weight of 0.115 g/tablet.

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IM INJECTIONS (Oil Type)

- (a)  $17\beta$ -[N-Methyl(cyclopropylamino)]-androst-4-en-3-one 25.0 mg  
(b) BHA, BHT aa 0.01 % w/v  
15 (c) Peanut oil or sesame oil qs 1.0 ml

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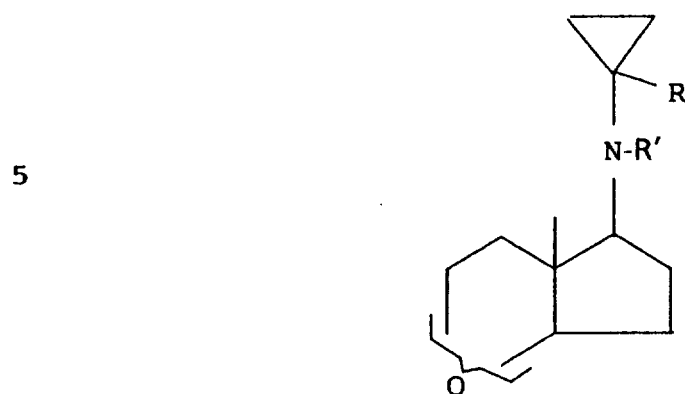
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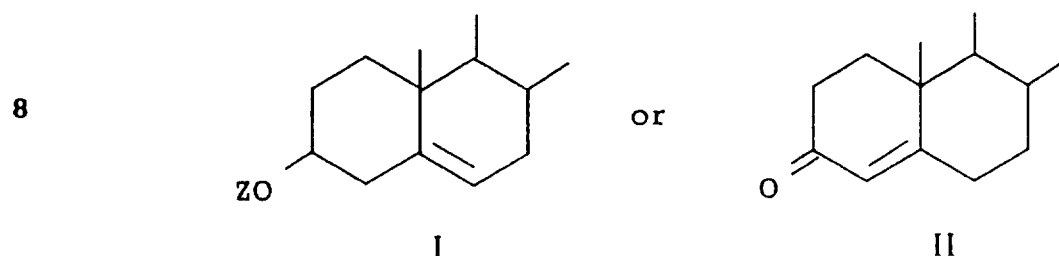
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WHAT IS CLAIMED IS:

1        1. A method for treating hyperaldosteronism which  
2        comprises administering to a patient having said condition a  
3        therapeutically effective amount of a compound of the  
4        formula

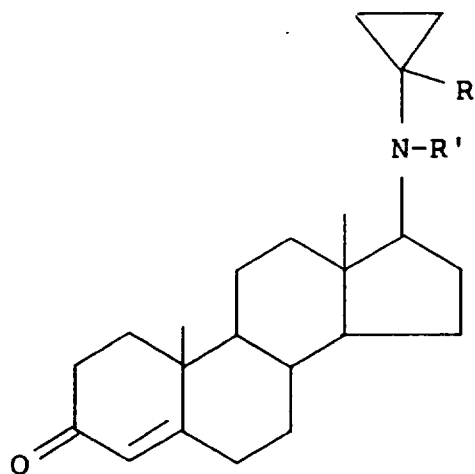


6 wherein R is hydrogen or methyl; R' is hydrogen, C<sub>1</sub>-C<sub>4</sub>  
7 alkyl or cyclopropyl; and Q is



9 wherein Z is hydrogen, alkanoyl of 1-10 carbon atoms,  
10 cyclopentane-alkanoyl or benzene-alkanoyl wherein the  
11 alkanoyl portion of the cyclopentane-alkanoyl or benzene-  
12 alkanoyl contains up to 4 carbon atoms.

1 2. A method according to Claim 1 for treating hyperal-  
2 dosteronism which comprises administering to a patient  
3 having said condition a therapeutically effective amount of  
4 a compound of the formula

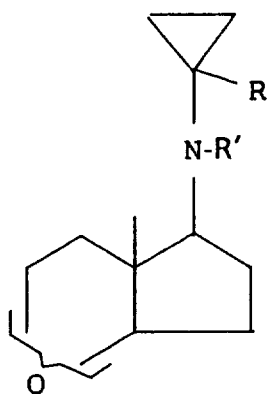


6 wherein R is hydrogen or methyl; and R' is hydrogen or C<sub>1</sub>-4  
7 alkyl.

1 3. A method according to Claim 1 for treating hyperal-  
2 dosteronism which comprises administering to a patient  
3 having said condition a therapeutically effective amount of  
4 17β-[N-methyl(cyclopropylamino)]androst-4-en-3-one.

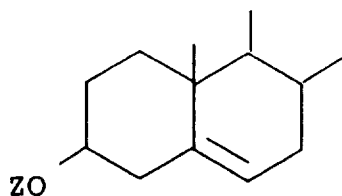
1 4. A method for producing a diuretic effect which com-  
2 prises administering to a patient in need of such treatment  
3 an effective amount of a compound of the formula

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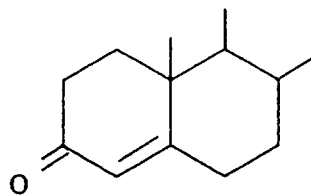
5 wherein R is hydrogen or methyl; R' is hydrogen, C<sub>1</sub>-C<sub>4</sub>  
6 alkyl or cyclopropyl; and Q is

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or

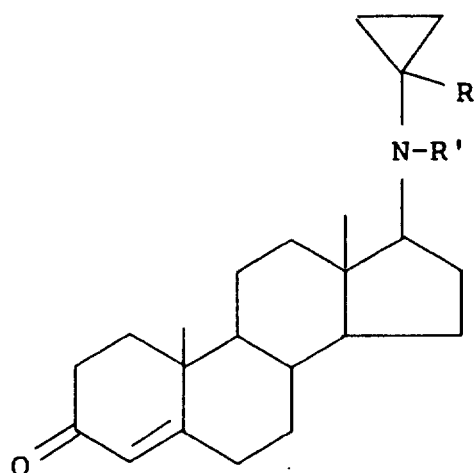


II

8 wherein Z is hydrogen, alkanoyl of 1-10 carbon atoms,  
9 cyclopentane-alkanoyl or benzene-alkanoyl wherein the  
10 alkanoyl portion of the cyclopentane-alkanoyl or benzene-  
11 alkanoyl contains up to 4 carbon atoms.

1 5. A method according to Claim 4 for producing a  
2 diuretic effect which comprises administering to a patient  
3 in need of such treatment a therapeutically effective amount  
4 of a compound of the formula

5



6 wherein R is hydrogen or methyl; and R' is hydrogen or C<sub>1-4</sub>  
7 alkyl.

1 6. A method according to Claim 4 for producing a  
2 diuretic effect which comprises administering to a patient  
3 in need of such treatment a therapeutically effective amount  
4 of 17 $\beta$ -[N-methyl(cyclopropylamino)]androst-4-en-3-one.

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ABSTRACT OF THE DISCLOSURE

The present invention is directed to a method for the treatment of hyperaldosteronism and related disorders which  
5 comprises administering to an appropriate patient an appropriate 17 $\beta$ -cyclopropylaminoandrostene.

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