TREATMENT OF HYPERLIPIDAEMIA

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References Cited
UNITED STATES PATENTS
3,492,349 1/1970 Doyle et al....................... 424/324

FOREIGN PATENTS OR APPLICATIONS
1,208,710 10/1970 United Kingdom

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ABSTRACT
3-Tert.-butyl-4-hydroxyacetanilides are administered to mammal, especially human, subjects to reduce serum lipid, especially cholesterol, levels. 3,5-Di-tert.-butyl-4-hydroxyacetanilide is preferred and the invention has particular application to the treatment of hypercholesterolaemia in humans.

8 Claims, No Drawings
TREATMENT OF HYPERLIPIDAEMIA

The present invention relates to the reduction of serum lipid levels in animals and in particular provides a treatment for hyperlipidaemia, especially hypercholesterolaemia, i.e., excess of cholesterol in the blood.

U.K. Pat. Specification No. 1208710 (Aspro-Nicholas Limited), published on Oct. 14, 1970, discloses that certain 3,5-di-tet-butyl-4-hydroxyacetanilides, especially the 3,5-di-tet-butyl compound, possess a higher level of analgesic activity than paracetamol (i.e., 4-hydroxyacetanilide) and/or more slowly excreted than paracetamol. It has now been found that 3,5-di-tet-butyl-4-hydroxyacetanilide and other 3-tet-butyl-4-hydroxyacetanilides optionally substituted in the aromatic nucleus by C1 to C6 alkyl groups have anti-hyperlipidaemic activity as determined in a screening test hereinafter described.

According to the present invention therefore, there is provided a method of reducing serum lipid levels in a mammal, especially those having hyperlipidaemia and more especially hypercholesterolaemia, which comprises administering to the animal subject a lipid-reducing amount of a 3-tet-butyl-4-hydroxyacetanilide of the formula 1:

wherein R represents a hydrogen atom or an alkyl group containing one to four carbon atoms.

The alkyl group represented by R may be any straight or branched chain alkyl group containing from one to four carbon atoms and may be located in any one of the 2, 5 and 6 positions of the aromatic nucleus, preferably the 5 or 6 position. Alkyl groups having from one to four carbon atoms are methyl, ethyl, n- and iso-propyl, and n-, sec.- and tert.-butyl.

The presently preferred compound of formula 1 is 3,5-di-tet-butyl-4-hydroxyacetanilide. Other compounds of formula 1 include 3-tet-butyl-6-methyl-4-hydroxyacetanilide 3-tet-butyl-4-hydroxyacetanilide 3-tet-butyl-5-sec.-propyl-4-hydroxyacetanilide 3-tet-butyl-6-ethyl-4-hydroxyacetanilide 3-tet-butyl-2-methyl-4-hydroxyacetanilide 3,6-di-tet-butyl-4-hydroxyacetanilide

The alkyl group R and the aromatic nucleus of the compounds of formula 1 may be substituted with one or more substituents which are "therapeutically compatible" with the molecule being substituted.

The term "therapeutically compatible" as used in this specification in relation to a substituent means that the presence of that substituent neither destroys the pharmacological activity of the molecule nor so decreases said activity and/or increases the toxicity of the molecule that the therapeutic ratio is reduced to five or below. The therapeutic compatibility of a particular substituent may depend upon the intended site of substitution in the molecule and/or the presence in the molecule of other substituents. Hence a given substituent may be therapeutically compatible in respect of one molecule into which it is to be introduced but incompatible, i.e., inactivating, in respect of another molecule. The compatibility of any substituent in respect of any molecule having the basic formula 1 can be readily assessed by subjecting the relevant compound to standard screening tests such as that referred to hereinafter. It is well within the ability of the averagely skilled man concerned with the development of new drugs to ascertain which substituents may be present and at what positions in pharmacologically active compounds of formula 1.

The 3-tet-butyl-4-hydroxyacetanilides of formula 1 can be prepared by the methods described in U.K. Pat. Specification Nos. 1198059 (Aspro-Nicholas Limited) and 1208710 (Aspro-Nicholas Limited) from the corresponding 4-hydroxyanilines.

The effect of the 3-tet-butyl-4-hydroxyacetanilides of formula 1 (hereinafter referred to as "active compounds of the invention") on serum lipid levels and in particular their anti-hypercholesterolaemic activity (hereinafter referred to as "AHCA") has been assessed on the basis of antagonism of Triton WR 1339-induced hypercholesterolaemia in the rat.

Triton WR 1339 is an oxyethylated tertiary octylphenol polymethylene polymer supplied by Ruger Chemical Co. Inc. It has been found that if rats which have been fasted overnight (about 16 hours) are injected intravenously with Triton WR 1339 in an amount of 200 mg/kg body weight, the serum cholesterol levels after 6 hours are some 2 to 3 times those of untreated control rats. Certain drugs, with the notable exception of Atromid-S (i.e., clofibrate), which are known to reduce serum cholesterol levels in humans antagonise said Triton-induced hypercholesterolaemia.

In the screening test, used to assess AHCA the test compound was suspended in 0.5% w/w aqueous gum tragacanth and then administered orally in an amount of 1 ml suspension per 100 g body weight to a first group of six male albino rats which had been fasted overnight. One hour later each of the rats was injected via its tail vein with 0.2 ml per 100 g body weight of a 10% w/v solution of Triton WR 1339; the rats having first been warmed to about 30°C for 15 minutes. After the lapse of 6 hours from injection, the rats were bled by direct heart puncture under either anaesthetic and serum cholesterol levels measured using the method of Zlatkis et al (Journal of Laboratory and Clinical Medicine, 41, 1953, 486). Each of a second group of six male albino rats was injected with Triton WR 1339 as above but without the prior oral administration of the test compound.

A third group of six male albino rats was used as a control.

In each group, the rats were of the Carworth Europe CFHB strain and of a weight in excess of 300 g.

The mean value of serum cholesterol level in each group was determined from a standard curve constructed from the individual results of the group and the percentage inhibition was calculated according to the formula.

% Inhibition = 100 \( \left[ 1 - \frac{C_0 - C_d}{C_0 + C_d} \right] \)
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where

$C_1$ represents the mean value for the first group;

$C_2$ represents the mean value for the second group; and

$C_3$ represents the mean value for the third group.

When a typical active compound of formula 1, viz. 3,5-di-tert.-butyl-4-methyl-4-hydroxyacetanilide, was submitted to the aforementioned screening test, the percentage inhibition at a dose level of 100 mg/kg was calculated to be 19.3. On repeated submission of the preferred compound, viz. 3,5-di-tert.-butyl-4-hydroxyacetanilide to said screening test, the percentage inhibition at a dose level of 100 mg/kg was found to be in the range 25.8 to 33.4. These results compare with a percentage inhibition at the same dose level of 34 calculated from a comparative test with 3-methylsalicylic acid. The latter compound was known to have a high AHCA from the work of Hyams and Howard (Modern Geriatrics, July 1971, 336/342).

Another comparative test was carried out using 100 mg/kg of 3,5-di-iso-propyl-4-hydroxyacetanilide as the test compound. This compound was selected because of its close structural resemblance to the active compound of the present invention and, as far as we are aware, its AHCA had not previously been assessed. In this test, no antagonism of the Triton-induced anti-hypercholesterolaemia occurred.

The 3,5-di-tert.-butyl-4-hydroxyacetanilides of formula 1 are usually insoluble in most pharmaceutically acceptable solvents and hence usually will be administered rectally or, more usually, orally. Generally they will be administered in association with a pharmaceutically acceptable carrier. For example, they can be mixed with a carrier, suspended in a carrier or enclosed or encapsulated by a carrier in the form of a capsule, sachet, cachet, paper or other container. The carrier may be a solid, semi-solid or liquid material which serves as a vehicle, excipient or diluent for the active material. Some examples of the carriers which may be employed are lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, liquid paraffin, cocoa butter, oil of theobroma, alginites, gum tragacanth, gelatin, Syrup B.P., methyl cellulose, polyethylene sorbitan monolaurate, and methyl and propyl hydroxybenzoates. In particular the formulations containing the active compounds of the present invention may be in the form of tablets, capsules and suspensions.

The dosage range within which a therapeutically useful reduction in serum lipid levels is produced by administration of the 3,5-di-tert.-butyl-4-hydroxyacetanilides of formula 1 will vary according to inter alia the species and weight of the animal to be treated and the route of administration as is well known in the art. Such a range will usually fall within the limits of 0.01 to 250 mg/kg. A preferred range for rats is 100 to 200 mg/kg. In the case of humans it is expected that said dosage for adult humans will be within the range of 1 to 4 g per day.

The following Examples illustrate typical formulations in which the active compound of the present invention can be administered to humans. All parts are by weight unless otherwise stated.

**EXAMPLE 1**

Tablets containing the following components are made by the following procedure:

The 3,5-di-tert.-butyl-4-hydroxyacetanilide is mixed with 45 parts of the starch and compressed into slugs. These slugs are reduced to granules by passage through a B.S. No. 40 sieve, the granules mixed with the remainder of the starch, and the talc and magnesium stearate added. The resultant mixture is compressed into tablets weighing (a) 110 mg, (b) 275 mg, and (c) 550 mg, to provide tablets containing respectively 100, 250 and 500 mg of 3,5-di-tert.-butyl-4-hydroxyacetanilide.

Two of the 500 mg tablets administered three times daily will reduce the cholesterol level in patients suffering from hypercholesterolaemia. Other dosage regimes of between 1 to 4 g daily may readily be maintained using the 100, 250 and 500 mg tablets separately or in combination. Control of blood lipid levels is usually necessary over a prolonged if not indefinite period and hence an appropriate dosage regime may be established for the individual patient.

**EXAMPLE 2**

Capsules each containing the following components are made:

| 3,5-di-tert.-butyl-4-hydroxyacetanilide | 100 parts |
| Calcium phosphate | 20 parts |

The powders are thoroughly mixed together and filled into hard gelatin capsules so that each capsule contains 250 mg of 3,5-di-tert.-butyl-4-hydroxyacetanilide.

**EXAMPLE 3**

Suppositories each having the following composition are made up as follows:

| 2,5-di-tert.-4-hydroxyacetanilide | 10 parts |
| Oil of Theobroma | 30 parts |

The finely powdered 3,5-di-tert.-butyl-4-hydroxyacetanilide is triturated with the molten oil of theobroma and the mixture so produced poured into suppository moulds of a nominal capacity of 1 g or 2 g as desired to produce suppositories each containing respectively 250 mg or 500 mg of 3,5-di-tert.-butyl-4-hydroxyacetanilide.

The capsules and suppositories of Examples 2 and 3 may be used instead of the tablets of Example 1 to maintain the dosage regimes referred to in that Example. Having regard to the long term treatment required for most patients, the oral forms of Examples 1 and 2 are preferred to the rectal form of Example 3.

Having regard to the foregoing disclosure, the following is claimed as the inventive and patentable embodiments thereof:

1. A method of reducing serum cholesterol in a mammal which comprises administering to the mammal in need thereof a cholesterol-reducing effective amount of a 3,5-di-tert.-butyl-4-hydroxyacetanilide of the formula:
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3. The method according to claim 1 wherein the 3-tert-butyl-4-hydroxyacetanilide is 3-tert-butyl-6-methyl-4-hydroxyacetanilide.
4. The method according to claim 1 wherein the 3-tert-butyl-4-hydroxyacetanilide is administered orally.
5. The method according to claim 1 wherein the 3-tert-butyl-4-hydroxyacetanilide is administered rectally.
6. The method according to claim 1 wherein the mammal is human having hypercholesterolaemia.
7. The method according to claim 6 wherein the 3-tert-butyl-4-hydroxy acetanilide is administered in a daily amount of from 1 to 4 g via doses containing from 100 to 500 mg of said compound.
8. The method according to claim 6 wherein the 3-tert-butyl-4-hydroxyacetanilide is 3,5-di-tert-butyl-4-hydroxyacetanilide.

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CERTIFICATE OF CORRECTION

Patent No. 3,899,590 Dated August 12, 1975

Inventor(s) Seymour Jeffrey Corne

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, line 13: after "and/or" please insert --are--

Column 2, line 68:

\[ \% \text{ Inhibition} = 100 \frac{1 - \left( \frac{C_1 - C_2}{C_1 - C_3} \right)}{1 - \left( \frac{C_1 - C_2}{C_2 - C_3} \right)} \]

should be

\[ \% \text{ Inhibition} = 100 \left( \frac{C_1 - C_2}{C_2 - C_3} \right) \]

Signed and Sealed this twentieth Day of April 1976

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks