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COMPLETE SPECIFICATION

New Derivatives of Tetracycline Antibiotics and their production

We, CARLO ERBA S.P.A., an Italian Body Corporate of Via Imbonati 24, Milan, Italy, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to new derivatives of tetracycline antibiotics and to their production. More particularly it relates to the preparation of new derivatives of tetracycline antibiotics which are easily soluble in water, with a pH value near to neutrality, and which are effective when administered orally, intravenously or intramuscularly.

The term "tetracycline antibiotics" whenever used in this specification is intended to mean chlortetracycline, oxytetracycline, tetracycline, 6-desmethyl-tetracycline and 6-desmethyl-chlortetracycline.

It is known that derivatives of tetracycline which are easily soluble in water are obtainable by reacting a tetracycline with formaldehyde and certain amino compounds such as a dialkylamine, piperidine, morpholine and piperazine. The aminomethyl group which is thereby introduced into the molecule of the tetracycline may be bound to a carbon atom of the tetracycline ring system (Specification No. 872,991) or to the nitrogen atom of the carbonamide group of the tetracycline molecule (British Patent Specification No. 809,585). The aminomethyl derivatives thus obtained are as effective as tetracycline in antibacterial activity when administered intravenously; however they are considerably inferior when administered orally (E. N. Ewald and G. S. Redin, Antibiotics and Chemotherapy, 1960, pages 419—421).

It has been found that many amino acids are not capable of reacting with tetracycline antibiotics and formaldehyde with the formation of water-soluble products which possess a sufficient stability to hydrolysis and which can be used for parenteral purposes. The product obtainable for instance by reacting 1 mol of tetracycline, 1 mol of formaldehyde and 1 mol of glycine is so unstable in aqueous solution that the difficultly soluble tetracycline precipitates after a short time. When using ornithine or arginine instead of glycine, the products obtained also possess poor stability in water.

It is an object of the present invention to provide new derivatives of tetracycline antibiotics (as hereinbefore defined) which possess good stability in water, and according to a first feature of the present invention there are provided compounds of the general structure A—CH_2—B where A represents the radical of a tetracycline antibiotic (as hereinbefore defined) and B represents the lysine radical, the methylene group being connected on the one hand to the nitrogen atom of the amide group in the 2-position of the tetracycline molecule and on the other hand to the ε-amino group of the lysine molecule, and the acid addition salts thereof.

According to a further feature of the invention new derivatives of tetracycline antibiotics (as hereinbefore defined) are prepared by reacting 1 mol of a said tetracycline antibiotic with 1 to 2 mols of formaldehyde and approximately 1 mol of lysine or an acid addition salt thereof. The products of the said structure, obtainable by the said process have good water-stability and are effective by parenteral and oral application.

The reaction between the three components mentioned above may be carried out in a one-stage process by reacting all three components simultaneously with each other. Alternatively formaldehyde may first be reacted with one of the other two components and the remaining component then reacted with the intermediate product thus obtained.

Of the tetracycline antibiotics it is preferred to use tetracycline in the form of the free base. The formaldehyde is preferably

[*Price 4s. 6d.*]

used in the form of its aqueous or alcoholic solution. Instead of formaldehyde there may be used such compounds as are capable of forming formaldehyde under the conditions of the reaction.

The reaction is preferably carried out in the presence of solvents or diluents. It is preferred to use organic solvents such as methanol or mixtures of water and organic solvents. Water alone cannot successfully be used as the solvent.

The one-stage reaction is preferably carried out at a slightly elevated temperature, for instance 30—40° C., using methanol or mixtures of methanol and up to 20% by weight of water as solvent.

When working in two stages, lysine or an acid addition salt thereof may be reacted with 1 mol of formaldehyde in aqueous or alcoholic solution or with 2 mols of formaldehyde in aqueous solution. The intermediate product thus formed may be isolated from the solution, dissolved in a mixture of methanol and water (80—95% methanol), and may then be added to a solution of a tetracycline antibiotic in methanol at a temperature of 30—40° C.

Alternatively, when the tetracycline antibiotic and formaldehyde are reacted in the first stage, the reaction is preferably carried out by suspending the tetracycline antibiotic in an organic solvent or diluent such as butanol, adding the formaldehyde at normal temperature and heating the mixture thus obtained to boiling under reflux for about 30 minutes. The reaction product is precipitated by cooling and is isolated. The isolated product and the equivalent quantity of lysine or an acid addition salt thereof are dissolved in an organic solvent such as methanol and the solution is heated to a slightly elevated temperature as mentioned above.

Analysis indicates that the reaction products obtained are tetracycline/methylene/lysine compounds of the general formula

$$A—CH_2—B$$

as indicated above. The optical rotation of the reaction products is different from the values obtained for tetracycline and for lysine. The reaction product is comparatively stable in water, i.e., aqueous solutions of the reaction products remain clear at room temperature for about 24 hours. This renders it possible to isolate the reaction products by lyophilising their aqueous solutions.

The therapeutic properties of the tetracycline derivatives of the present invention are illustrated in tabular form in the accompanying drawings.

Patients who were submitted to treatment some with tetracycline-methylene-L.-lysine (a product according to the present invention), some with pyrrolidine-methyl-tetracycline and some with tetracycline-hydrochloride exhibited blood levels with respect to the three compounds as shown in Figure 1. The products in question were administered intramuscularly. From Figure 1 it can be seen that the product of the invention affords higher blood levels than the two other products. Each patient received 100 mg. (calculated on tetracycline base). This dose was given only once in each case.

From Figure 2 it can be seen that a dosage level of 250 mg. (calculated on tetracycline base) tetracycline-methylene-L.-lysine affords higher blood levels than pyrrolidine-methyl-tetracycline. In this case the products in question were administered intramuscularly and each patient received only one dose.

Figure 3 shows the blood levels which were obtained from tetracycline - methylene - L.-lysine and tetracycline-hydrochloride after 6, 12, 30 and 54 hours. The antibiotics were administered *per os*. Each patient received 150 mg. (calculated on tetracycline base) every 6 hours. The blood levels which were obtained with the product according to the invention are higher than the blood levels obtained with tetracycline-hydrochloride.

The following table shows the toxicities on mice of tetracycline - hydrochloride, pyrrolidine - methyl - tetracycline and tetracycline-methylene - L. - lysine. The symbol DL_{50} defines the acute toxicity in mg./kg., i.e., the dose which kills half the mice treated, determined statistically. The products were administered intravenously. The volume of each dose amounted to 0.4—0.6 ml/20 g. The doses were administered at a rate of 1 ml/min. It can be seen from the Table that the toxicity of the product according to the invention is considerably smaller than the toxicities of the two other products.

TABLE

	Tetracycline-hydrochloride	Pyrrolidine-methyl-tetracycline	Tetracycline-methylene-L.-lysine
DL_{50} of the product	220	125	320
DL_{50} of the corresponding hydrochloride	220	115	253

The curves shown in Figures 1, 2 and 3 demonstrate that a smaller dose of tetracycline-methylene-L.-lysine is necessary in order to obtain the same blood levels and the same therapeutic effect as that given by pyrrolidine - methyl - tetracycline of tetracycline - hydrochloride.

The following Examples will serve to illustrate the production of tetracycline derivatives according to the present invention:—

EXAMPLE I

5 g. of tetracycline base are dissolved in 100 ml. of methanol. To this solution are added 2.25 g. of L.-lysine hydrochloride in 7 ml. of water and 1 ml. of 38% formaldehyde.

The solution is allowed to stand at room temperature for three hours and is concentrated in vacuo at a low temperature to almost dry state. The residue is dissolved in 25 ml. of distilled water and lyophilised. It is finally dried in vacuo over P_2O_5.

The light yellow substance thus obtained melts at 190—195° C.

Anal. Calc'd for $C_{29}H_{38}N_4O_{10}.HCl$: C, 54.49; H, 5.99; N,8.76.

Found: C,55.18; H, 6.71; N, 8.58.

Moisture 7.78 (determined by the Karl Fischer method).

EXAMPLE II

1 ml. of 38% aqueous formaldehyde is dissolved in a solution of 5 g. tetracycline base in 160 ml. of methanol; while stirring at 30° C. 1.75 g. of DL-lysine in 3 ml. of water and 10 ml. of methanol are added. Precipitation begins after a few minutes. Stirring is continued for about two hours. The precipitate is filtered off and washed with a small amount of methanol and diethyl ether. After drying in vacuo at 60° C. a yellow powder, highly soluble in water, is obtained; it melts above 200° C. with a slow decomposition.

Anal. Calc'd for $C_{29}H_{38}N_4O_{10}$: C, 57.79; H, 6.35; N, 9.25.

Found: C, 57.59; H, 6.82; N, 8.52.

The product may be converted into an acid addition salt (e.g., an inorganic salt such as the hydrochloride, or sulphate, or an organic salt such as the formate, acetate, oxalate, citrate or ascorbate) by dissolution in water followed by the addition of a stoichiometric equivalent of the acid of which the salt is desired.

EXAMPLE III

2.4 ml. of 38% aqueous formaldehyde are added to a solution of 6 g. of tetracycline in 200 ml. methanol at a temperature of 30° C. 2 g. of L.-lysine dissolved in a mixture of 3.4 ml. of water and 12 ml. of methanol are added.

A similar procedure to that described in Example II is then applied. The product is a yellow powder, soluble in water and beginning to decompose slowly above 190° C. $[\alpha]_D^{25} \sim -195°$ (0.5% water).

EXAMPLE IV

To a solution of 5 g. of tetracycline base in 200 ml. of methanol are added slowly, while stirring at room temperature, 1.78 g. of L - "methylene"- lysine dissolved in 1.5 ml. of water and 2 ml. of methanol. After 90 minutes, the precipitated product is filtered off, washed with diethyl ether and dried in vacuo at 50° C.

The product thus obtained is a yellow powder which decomposes slowly above 200° C.

Anal. Calc'd for $C_{29}H_{38}N_4O_{10}$: C, 57.79; H, 6.35; N, 9.25.

Found: C, 57.12; H, 6.33 N, 9.44.

Moisture 6.7.

The L-"methylene"-lysine can be obtained as follows:

1 mol of L-lysine is dissolved in a small quantity of aqueous methanol. To the solution thus obtained a solution of 1 mol of formaldehyde in methanol is added. The reaction product is precipitated, filtered off and washed with a small quantity of methanol.

EXAMPLE V

1 ml. of 38% aqueous formaldehyde is added to a solution of 5 g. tetracycline in 160 ml. of methanol, 1.75 g. of L.-lysine base in 3 ml. of water and 10 ml. of methanol are added while stirring at 30° C. After a few minutes, precipitation sets in. The stirring is continued for about two hours. The precipitate is filtered off and washed with a small amount of methanol and diethyl ether. After drying in vacuo at 60° C. a yellow powder, highly soluble in water, is obtained; it melts above 200° C. with a slow decomposition.

The yield amounts to 82%; $[\alpha]_D^{25} \sim -200°$ (0.5% water).

Anal. Calc'd for $C_{29}H_{38}N_4O_{10}$: C, 57.79; H, 6.35; N, 9.25.

Found: C, 57.30; H, 5.95; N, 9.02.

Moisture 10.49.

EXAMPLE VI

1.1 ml. of 35% alcoholic formaldehyde are added to a solution of 5 g. of tetracycline in 200 ml. of methanol. While stirring at 35° C., 1.65 g. of L.-lysine dissolved in a mixture of 5 ml. of water and 7 ml. of methanol are added to the solution. The suspension is stirred for 90 minutes and then filtered. The precipitate is washed with a small amount of diethyl ether. The yield amounts to 5.2 g. of a yellow powder which begins to decompose above 200° C.

Corresponding products were prepared using oxytetracycline, 6 - desmethylchlortetracycline or 6 - desmethyltetracycline in place of tetracycline in Example 6.

EXAMPLE VII

10.5 g. of tetracycline base is suspended in 160 ml. of tertiary butanol; to the mixture thus obtained, 4.1 ml. of 38% aqueous formaldehyde and a few crystals of sodium carbonate are added while stirring.

The mixture is stirred for 15 minutes at room temperature, then heated to boiling and refluxed for 30 minutes. The solution is filtered while hot to remove traces of insoluble material. The filtrate is cooled down to room temperature and the precipitate thus obtained is filtered off and dried in vacuo at 50° C.

The product is a light yellow powder, highly soluble in water, and begins to decompose above 190° C.

To 2 g. of this substance dissolved in 60 ml. of methanol is added a solution of 0.618 g. L.-lysine in 1 ml. of water and 4 ml. of methanol.

Precipitation rapidly sets in and the stirring is continued for 1 hour. The precipitate is filtered and dried in vacuo at 50° C. The product is a yellow powder, highly soluble in water, which exhibits the same characteristics as that described in Example V. $[\alpha]_D^{25} \sim -200°$ (0.5% water).

Anal. Calc'd for $C_{29}H_{38}N_4O_{10}$: C, 57.79; H, 6.35; N, 9.25.

Found: C, 57.42; H, 6.67; N, 9.47.

Moisture 5.47.

EXAMPLE VIII

To a solution of 3 g. of chlortetracycline in 150 ml. of methanol are added, at room temperature, 0.55 ml. of 38% aqueous formaldehyde and a solution of 1.15 g. of L-lysine hydrochloride in 3 ml. of water. The mixture is stirred for 4 hours at room temperature. The precipitate is filtered off and washed with methanol and diethyl ether.

The product thus obtained, after drying in vacuo at 60° C., is a yellow powder which begins to decompose above 190° C.

Anal. Calc'd for $C_{29}H_{37}ClN_4O_{10}.HCl$: C, 51.70; H, 5.68; N, 8.31.

Found: C, 52.04; H, 6.07; N, 7.58.

Moisture 0.99.

WHAT WE CLAIM IS:—

1. Compounds of the general structure A—CH_2—B where A represents the radical of a tetracycline antibiotic as hereinbefore defined and B represents the lysine radical, the methylene group being connected on the one hand to the nitrogen atom of the amide group in the 2-position of the tetracycline molecule and on the other hand to the ε-amino group of the lysine molecule, and the acid addition salts thereof.

2. A compound according to claim 1 wherein A represents a tetracycline radical.

3. A compound according to claim 1 or 2 wherein B represents the L.-lysine radical.

4. A process for the production of derivatives of tetracycline antibiotics as defined in claim 1 which comprises reacting 1 mol of a tetracycline antibiotic as hereinbefore defined with 1 to 2 mols of formaldehyde and approximately 1 mol of lysine or an acid addition salt thereof.

5. A process according to claim 4 wherein the reaction is effected in the presence of methanol or a mixture of methanol with up to 20% by weight of water at a temperature of 30—40° C.

6. A process according to claim 4 or 5 wherein the tetracycline antibiotic as hereinbefore defined is reacted with the formaldehyde, the product is isolated and this is reacted with the lysine.

7. A process according to claim 4 or 5 wherein the lysine is reacted with the formaldehyde, the product is isolated and this is reacted with the tetracycline antibiotic (as hereinbefore defined).

8. A process according to any of claims 4—7 wherein the tetracycline antibiotic is tetracycline.

9. A process according to any of claims 4—8 wherein the lysine used is L-lysine or an acid addition salt thereof.

10. A process according to claim 4 substantially as set forth in any one of the foregoing specific Examples I—VIII.

11. Derivatives of tetracycline antibiotics (as hereinbefore defined) whenever prepared or produced by any of the processes hereinbefore described and claimed.

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