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COMPLETE SPECIFICATION

Process for Producing Derivatives of Chloramphenicol and of the Pantothenates

I, JOÃO EMERICO VILAX, formerly known as IVAN VILLAX, now a Portuguese citizen, of 69, Rua Nova do Almada, Lisbon, Portugal, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to the preparation of complex compounds of chloramphenicol with a strongly reduced toxicity.

The detoxicating action of the derivatives of pantothenic acid is a known fact. A number of patents refer to the preparation of pantothenic salts of antibiotic compounds, having at least one amino group. But up to now there is no patent or description using these derivatives to reduce the toxicity of chloramphenicol which does not contain in its molecule an amino group able to form salts but an amido-group only.

Actually chloramphenicol is one of the most effective broad spectrum antibiotics, but its high toxicity, especially the chronic one, restricts its application to special cases.

My observations, which led me to conclude that pantothenic derivatives greatly reduce the toxic effects of chloramphenicol without interfering with its antibacterial action, are very important.

The processes related here concern the preparation of complex compounds of chloramphenicol, which possess not only a reduced toxicity but also other favourable pharmacological properties, such as higher and more prolonged blood levels and a practically complete absence of secondary reactions.

During the preparation of pantothenic esters of chloramphenicol, I observed the formation of intermediary reaction products and was able to isolate them. I consider them according to their properties as complex compounds. Thus dissolving chloram-

phenicol and an alkali or alkaline-earth metal salt of D-pantothenic acid in an inert solvent where one of the reagents is only slightly soluble but it dissolves upon the action of the other reagent, at 100°C in an adequate solvent and distilling over immediately in vacuo the solvent, one obtains the complex compounds of chloramphenicol and a salt of pantothenic acid, as an oily residue. The complex can be crystallised by stirring this reaction product in a mixture of a lower halogenated hydrocarbon such as 1, 2-dichlorethane, and petrol ether (boiling range 40-60°C) and letting it stand at a temperature below 0°C. I have isolated among others the complex compounds: one mole of sodium pantothenate and two moles of chloramphenicol; one mole of calcium pantothenate and two moles of chloramphenicol; and one mole of calcium pantothenate and four moles of chloramphenicol. This last one offers a particular interest, as it has very favourable pharmaceutical properties.

These complex compounds have, besides their different pharmaceutical properties, solubilities, crystal shape and infra-red spectra different from those of the simple mixture of the two components. Comparing the infra-red spectra of the complex compound of chloramphenicol and of calcium pantothenate in the proportion of 4 : 1 moles ("P") and of the mixture of one part of chloramphenicol and of 0.368 part of calcium pantothenate in mineral oil mull, one observes three essential differences:

(a) The mixture gives a shoulder at 5.95 μ and a strong maximum at 6.05 μ . "P" gives a strong maximum at 5.95 μ and only a shoulder at 6.05 μ .

(b) At 6.6 μ the mixture has no band, but both chloramphenicol and "P" have one.

(c) A large twinned band appears for the mixture from 9.6 μ to 9.8 μ and for "P"

9.4 μ to 9.7 μ .

As is already known, chloramphenicol has a relatively low acute-toxicity. The DL_{50} is about 245 mg/kg i. v.; at the same time, the DL_{50} of chlorotetracyclin is about 140-170 mg/kg. But after a prolonged administration, the toxic action of chloramphenicol appears in a cumulative manner. Therefore, these new compositions and derivatives were tested specially for their chronic toxicity.

A prolonged administration of chloramphenicol may provoke a blood dyscrasia, such as aplastic anaemia with a sometimes fatal result.

The chronic toxicity of chloramphenicol can manifest itself, apart from reactions, by the development of blood dyscrasia, such as aplastic anaemia, often fatal.

For chronic toxicity tests I used rabbits divided into groups of 4. The rabbits of one group were administered daily, except for Sundays, intramuscularly, with 200 mg/kg of chloramphenicol, and the rabbits of the other groups with the pantothenic ester of chloramphenicol and with the molecular complex of calcium pantothenate and chloramphenicol 1:4 moles in a quantity equivalent to 200 mg/kg of chloramphenicol - base, for 45 days. Weights and blood counts were controlled before and during the administration.

As to weight, at the start of the treatment, a slight increase was noticed, but this was short-lived, as, after twenty days of administration the rabbits had lost it. In the meantime, starting from the 20th day, the weight of the rabbits treated with chloramphenicol began to diminish slightly, and at the end of the 34th day this loss of weight attained an average of 10%. The rabbits treated with pantothenate began to gain weight after the 30th day, and at the end of 45 days, this gain was up to 13%.

The blood counts during the first 30 days, in both groups, suffered the following variations:

Group treated with chloramphenicol:

Haemoglobin: unchanged

Red corpuscles: a certain decrease (in one case 30% in proportion to the original count)

White corpuscles: slight increase

Platelets: normal

Group treated with pantothenate:

Haemoglobin: slight increase

Red corpuscles: slight decrease (between -18% and +6% in proportion to the initial count)

White corpuscles: no change

Platelets: normal

At the end of 34 days, the first rabbit treated with chloramphenicol died, with all the characteristics of anaemia (haemoglobin: 27%, red corpuscles: 1.900.000, white corpuscles: 6.050.000)

It must be noted that in the last 4 days, the

red corpuscles count lowered from 4.000.000 to 1.900.000.

At the end of 45 days, the second rabbit treated with chloramphenicol died, with a relatively normal number of red corpuscles (3,310.000), and 50% haemoglobin, but the number of platelets decreased in 8 days from 347.000 to 40.000.

In the groups of rabbits treated with chloramphenicol pantothenate complex, there were no deaths during the 45 days of observation, nor did the blood counts vary very much in proportion to the results obtained at the end of 30 days treatment.

Thus, the administration of 200 mg/kg of chloramphenicol per day caused the death of two rabbits out of four, in a period of 45 days, and in the two remaining, 10% loss of weight was verified.

The daily administration of chloramphenicol-pantothenate complexes at a quantity equivalent to 200 mg/kg of chloramphenicol-base caused no deaths, and the rabbits benefited by a weight increase of 13%.

The following examples are representative of the procedures embodied in the present invention but it is to be understood that these examples are given by way of illustration and not of limitation.

Example 1: One adds 71.1 grams of calcium pantothenate to a solution of 194 grams of chloramphenicol in 1,250 ml of dimethylformamide at 100° C. One stirs till the whole calcium pantothenate is dissolved. Then one cools it to 50° C and the solvent is distilled over in vacuo at, at least, 1mm. To the residual oil one adds 600 grams of 1,2-dichlorethane and stirs. The "pantophenicol" begins to crystallise. Afterwards one adds 950 ml of petrol ether (boiling range 58°-68° C) and allows it to stand overnight at a temperature of -3° C. The product is filtered in vacuo, washed with petrol ether and dried. The melting point is of 143°-148° C.

Example 2: One proceeds as in Ex. 1 but, after dissolving the whole calcium pantothenate, one maintains the reaction mixture at 100° C during 3 hours. The final product has a melting point of 158-163° C.

Example 3: One proceeds as in Ex. 1 but, instead of calcium pantothenate, one uses 144 grams of sodium pantothenate. The melting point of the final product is 142-148° C.

Example 4: One proceeds as in Ex. 3 but, instead of 144 grams, one uses 72 grams of sodium pantothenate (molar proportion 2:1).

WHAT I CLAIM IS:—

1. Process to produce new derivatives of chloramphenicol as complex compounds with reduced toxicity and with prolonged action, characterised by the fact that one reacts by heating for a few minutes an alkali metal or an alkaline-earth metal salt of D-pantothenic

9.4 μ to 9.7 μ .

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acid with chloramphenicol in an inert solvent where one of the reagents is rather insoluble but it dissolves upon the action of the other reagent.

- 5 2. Process for the crystallisation of the complex compounds obtained as in Claim 1. characterised by the fact that one stirs the amorphous complex compound with a mixture of organic solvents consisting of a lower
10 halogenated hydrocarbon and a light petrol ether and then lets it stay at a temperature

below 0° C.

3. Process as in Claims 1 and 2, characterised by the fact that one reacts 1 mole of D- calcium pantothenate with 4 15 moles of chloramphenicol.

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