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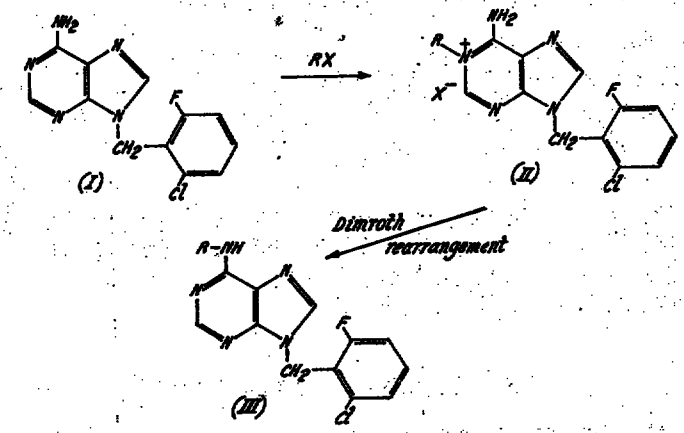
(71) We, MERCK & CO. INC., a corporation duly organized and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

Coccidiosis is a widespread poultry disease which is produced by infections of protozoa of the genus Eimeria which causes severe pathology in the intestines and ceca of poultry. Some of the most significant of these species are *E. tenella, E. acervulina, E. necatrix, E. brunetti* and *E. maxima.* This disease is generally spread by the birds picking up the infectious organism in droppings on contaminated litter or ground, or by way of food or drinking water. The disease is manifested by hemorrhage, accumulation of blood in the ceca, passage of blood in the droppings, weakness and digestive disturbances. The disease often terminates in the death of the animal, but the fowl which survive severe infections have had their market value substantially reduced as a result of the infection. Coccidiosis is, therefore, a disease of great economic importance and extensive work has been done to find new and improved methods for controlling and treating coccidial infections in poultry.

This invention is based on the discovery of certain novel 6-(N-alkylamino)-9-(2-chloro-6-fluorobenzyl)purines. The novel compounds of the present invention have the formula III below. Compounds of this formula have been found to possess high degree of activity against coccidiosis of poultry. Administering a small amount of the active compounds, preferably in combination with poultry feed, is effective in preventing or greatly reducing the incidence of coccidiosis. The compounds are effective against both the cecal form (caused by *E. tenella*) and the intestinal forms (principally caused by *E. acervulina, E. brunetti, E. maxima* and *E. necatrix*). The coccidiostats of this invention are particularly effective against the species that cause cecal damage. In addition to preventing the pathology caused by coccidia, these compounds also exert an inhibitory effect on the oocysts by greatly reducing the number and/or the sporulation of those produced.

The novel purine derivatives of this invention are prepared in two steps from the starting material, 6-amino-9-(2-chloro-6-fluorobenzyl)purine (I). This starting material is disclosed and claimed in U.K. Patent No. 1,338,520. The first step comprises the formation of the N^1-loweralkyl iodide derivative of 6-amino-9-(2-chloro-6-fluorobenzyl)purine (II). The resulting iodide, (II), is subjected to the Dimroth rearrangement to give the desired 6-(N-loweralkylamino)-9-(2-chloro-6-fluorobenzyl)purine product (III). The process for preparing the novel compounds of this invention is set forth in Table I below.

TABLE I



wherein R is C_{1-6} alkyl and X is a halogen such as chlorine, bromine or iodine, preferably iodine.

Also in accordance with this invention, coccidiosis in poultry is controlled or suppressed by administering to the poultry a non-toxic, anti-coccidially effective quantity of a compound of the structural formula III above, e.g. 6-(N-methylamino)-9-(2-chloro-6-fluorobenzyl)purine.

In a practical method of preparing the novel coccidiostats of this invention, in the first step the starting material 6-amino-9-(2-chloro-6-fluorobenzyl)purine, (I), is dissolved in a dialkylalkanoamide solvent, such as dimethylformamide or dimethylacetamide. To this solution is added a C_{1-6} alkyl halide, in which the halogen may be chlorine, bromine or iodine, preferably iodine.

The reaction time and temperature conditions are not unduly critical. The time of the reaction, however, decreases as the reaction temperature increases. The reaction is most conveniently conducted between the temperature in the range 0°C to 100°C. However, the preferred temperature is room temperature.

The relative proportions of the components of the reaction mixture of the first step may vary over a relatively wide range. The reactants may be used in stoichiometric amounts, i.e. equimolar amounts of the two reactants may be used, or an excess of the alkyl halide may be used. The preferred relative molar proportions of the alkyl halide to the purine compound is 3:1. The amount of the solvent used may also vary over a wide range. The solvent is used in a quantity sufficient to enable the reaction to proceed at a reasonable rate and facilitate isolation of the reaction product.

At the conclusion of the reaction the compound (II) is obtained by adding an excess of an organic solvent in which the product is not soluble and filtering out the precipitated compound (II). The preferred solvent is diethyl ether.

The second step in the process for making the novel compounds in the present invention is generally known in the art as the Dimroth rearrangement. This reaction is carried out by dissolving the alkyl halide purine derivative, II, in an aqueous solution containing an equal molar amount of an alkali metal hydroxide such as sodium hydroxide, potassium hydroxide or lithium hydroxide. The resulting solution is preferably heated under reflux in an inert atmosphere with stirring. The reaction time is not unduly critical and in most instances the reaction when carried out under reflux, is complete in one hour.

At the completion of the reaction, the reaction solution is cooled and the product, (III), crystallizes out. The product is then isolated by filtration, and if desired purified by conventional methods such as washing with water and recrystallizing from a suitable solvent such as ethanol, acetic acid, aqueous acetic acid, dimethylformamide or dimethylsulfoxide.

The novel compounds of this invention are orally administered to poultry for the control and prevention of coccidiosis. Any number of conventional methods are suitable for administering the coccidiostats of this invention to poultry, as for example, they may be given in the poultry feed or included in drinking water. The actual quantity of the coccidiostats administered to the poultry in accordance with this invention will vary over a wide range and be adjusted to individual needs, depending upon species of the coccidia involved and severity of the infection. The limiting criteria are that the minimum amount is sufficient to control coccidiosis and the maximum amount is such that the coccidiostat does not result in any undesirable effects.

A feed will typically contain from 0.0005, to 0.05%, preferably from 0.0025 to 0.01%, by weight of one of the coccidiostats of this invention. The optimum levels will naturally vary with the specific compound utilized and species of *Eimeria* involved, and can be readily determined by one skilled in the art. Levels of 6-(N-methylamino)-9-(2-chloro-6-fluorobenzyl)purine, which is the preferred coccidiostat of this invention, in poultry feed of from 0.004% to 0.008% by weight of the diet are especially useful.

Depending on the compound, levels of 0.005% to 0.05% possess the effects of reducing the number of oocysts passed in the droppings of infected chickens and/or inhibiting the subsequent division and maturation to infectivity, scientifically designated as the process of sporulation. Thus, the combination of prevention of pathology, coupled with the inhibiting effects on the reproductive product of these organisms, the oocysts, present a unique two-fold method for the control of coccidiosis in poultry.

The quantity or concentration of a novel coccidiostat of this invention in any admixture in which it is administered to the poultry will, of course, vary in accordance with the type of admixture utilized.

Of the various methods of administering the coccidiostats of this invention to poultry, they are most conveniently administered as a component of a feed composition. The novel coccidiostats may be readily dispersed by mechanical mixing in finely ground form with the poultry feedstuff, or with an intermediate formulation (premix) that is subsequently blended with other components to prepare the final poultry feedstuff that is fed to the poultry. Typical components of poultry feedstuffs include molasses, fermentation residues, corn meal, ground and rolled oats, wheat shorts and middlings, alfalfa, clover and meat scraps, together with mineral supplements such as bone meal and calcium carbonate and vitamins.

Suitable compositions also include feed premixes in which the active ingredient is present in relatively large amounts and which are suitable for addition into the feed either directly or after an intermediate dilution or blending step. Such compositions may also be added to the animals feed in the form of a top dressing. Typical carriers or diluents suitable for such compositions include for example, distillers dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corncob meal, edible bean mill feed, soyagrits and crushed limestone. The active compound is intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from 0.1 to 50% by weight, especially from 0.5 to 25% by weight of the compound are particularly suitable as feed premixes.

The following non-limiting example further illustrates the invention.

EXAMPLE

Step A

Preparation of 6-Amino-9-(2-chloro-6-fluorobenzyl)purine

The compound 6-amino-9-(2-chloro-6-fluorobenzyl)purine is prepared by slowly adding 67.5 grams (0.5 mole) of dry adenine to a solution of 57.0 grams (0.5 mole) potassium *t*-butoxide in 1 liter of absolute ethanol. This mixture is stirred for 2 hours, and then 89.5 grams (0.5 mole) of 2-chloro-6-fluorobenzyl chloride is added over a period of 4 hours. The resulting mixture is heated to reflux with stirring and allowed to react for about 42 hours, after which it is cooled to −10°C. The crude 6-amino-9-(2-chloro-6-fluorobenzyl)purine thus formed is isolated by filtration and then washed with ice-cold ethanol and ice-cold water to yield the crude product. After recrystallization from a mixture of acetic acid and water the m.p. is 245.5—246.5°C.

Step B

Preparation of 6-Amino-9-(2-chloro-6-fluorobenzyl)purine-N^1-methiodide

6-Amino-9-(2-chloro-6-fluorobenzyl)purine, 27.8 grams (0.1 mole), prepared by the process set forth in Step A, is suspended in 300 ml. dimethylacetamide. To this suspension is added 18.7 ml. (0.3 mole) methyl iodide and the solution is stirred at room temperature for 26 hours. The reaction solution is poured into 1 liter of ether with stirring. The product separates as a white solid. The product is collected by filtration, washed with ether and dried to give 42.6 g. of the named compound, m.p. 278—9°C. (dec.). NMR showed CH_3-N^+ at 3.8δ.

Calculated for $C_{13}H_{12}ClFIN_5$:	C, 37.21;	H, 2.88;	N, 16.69
Found:	C, 36.87;	H, 2.93;	N, 16.69

Step C

Preparation of 6-(N-Methylamino)-9-(2-chloro-6-fluorobenzyl)purine

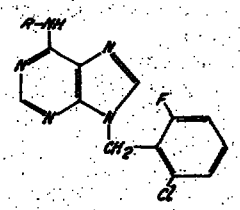
6-Amino-9-(2-chloro-6-fluorobenzyl)purine-N^1-methiodide, 21.0 g. (50 mmole) prepared by the process set forth in Step B, is dissolved in a solution of 250 ml. of water

containing 2.0 g. (50 mmole) NaOH. The solution is heated under reflux in a nitrogen atmosphere with magnetic stirring for 1 hour. The reaction solution is cooled and the product precipitates as white crystals. The product is collected by filtration, washed with water and sucked dry. The damp product is recrystallized from ethanol to give 9.79 g. of the named compound, m.p. 180—5°C. The product is purified by TLC on Silica-Gel using the solvent 10% methanol in chloroform.

Calculated for $C_{13}H_{11}ClFN_5$: C, 53.53; H, 3.80; N, 24.01
Found: C, 53.37; H, 4.04; N, 23.78

WHAT WE CLAIM IS:—

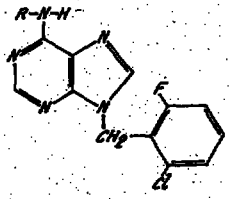
1. A compound of the structural formula:



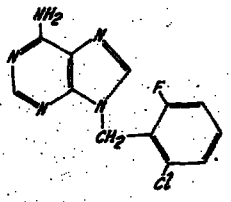
where R is alkyl containing 1 to 6 carbon atoms.

2. A compound in accordance with claim 1 where R is methyl.

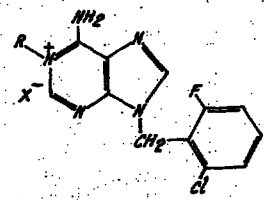
3. The process for preparing a compound of the structural formula:



where R is alkyl containing 1 to 6 carbon atoms which comprises reacting the compound having the structural formula:



with an alkyl halide, RX, where R is an alkyl group containing 1 to 6 carbon atoms and X is chlorine, bromine or iodine to obtain a compound of the structural formula:



and rearranging that compound in the presence of aqueous alkali metal hydroxide to obtain the desired product.

4. A process as claimed in claim 3, substantially as hereinbefore described in the Example.

5. A compound as claimed in claim 1 when prepared by a process as claimed in claim 3 or 4.

6. A composition for the treatment of coccidiosis comprising an inert carrier and the compound as claimed in claim 1, 2 or 5.

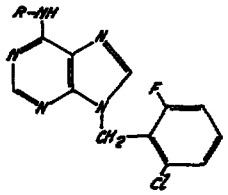
7. A composition according to claim 6 containing from 0.0005% to 0.05% by weight of the said compound.

containing 2.0 g. (50 mmole) NaOH. The solution is heated under reflux in a nitrogen atmosphere with magnetic stirring for 1 hour. The reaction solution is cooled and the product precipitates as white crystals. The product is collected by filtration, washed with water and sucked dry. The damp product is recrystallized from ethanol to give 9.79 g. of the named compound, m.p. 180—5°C. The product is purified by TLC on Silica-Gel using the solvent 10% methanol in chloroform.

Calculated for $C_{13}H_{11}ClFN_5$: C, 53.53; H, 3.80; N, 24.01
Found: C, 53.37; H, 4.04; N, 23.78

WHAT WE CLAIM IS:—

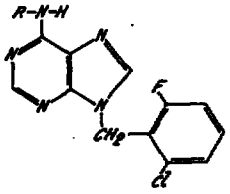
1. A compound of the structural formula:



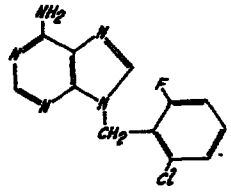
where R is alkyl containing 1 to 6 carbon atoms.

2. A compound in accordance with claim 1 where R is methyl.

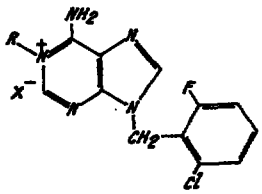
3. The process for preparing a compound of the structural formula:



where R is alkyl containing 1 to 6 carbon atoms which comprises reacting the compound having the structural formula:



with an alkyl halide, RX, where R is an alkyl group containing 1 to 6 carbon atoms and X is chlorine, bromine or iodine to obtain a compound of the structural formula:



and rearranging that compound in the presence of aqueous alkali metal hydroxide to obtain the desired product.

4. A process as claimed in claim 3, substantially as hereinbefore described in the Example.

5. A compound as claimed in claim 1 when prepared by a process as claimed in claim 3 or 4.

6. A composition for the treatment of coccidiosis comprising an inert carrier and the compound as claimed in claim 1, 2 or 5.

7. A composition according to claim 6 containing from 0.0005% to 0.05% by weight of the said compound.

8. A composition according to claim 7 containing from 0.0025% to 0.01% by weight of the said compound.

9. A composition according to claim 6 in which said composition is a feed premix containing from 0.1 to 50% by weight of the said compound.

10. A composition according to claim 9 containing from 0.5 to 25% by weight of the said compound.

11. A method for treating coccidiosis comprising administering to poultry a compound as claimed in claim 1, 2 or 5.

12. A method according to claim 11 in which the said compound is administered orally as a feed component making up from 0.0005% to 0.05% of the feed.

13. A method according to claim 12 in which the compound makes up from 0.0025% to 0.01% of the feed.

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