

PATENT SPECIFICATION

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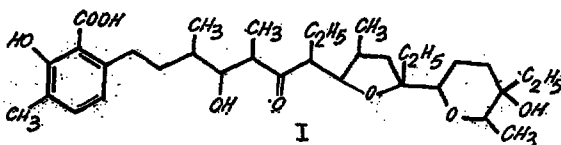
(54) NOVEL ANTIBIOTICS AND A PROCESS FOR THE MANUFACTURE THEREOF

(71) We, F. HOFFMANN-LA ROCHE & CO., AKTIENGESellschaft, a Swiss Company of 124—184 Grenzacherstrasse, Basle, Switzerland, 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:—

This invention relates to novel antibiotics and to a process for the manufacture thereof. More particularly, the invention relates to derivatives of antibiotic X-537A prepared by substitution on the phenyl ring of the antibiotic or substitution at the ketone position of the antibiotic molecule. These novel derivatives exhibit activity as coccidiostatic agents and antibacterial agents.

Antibiotic X-537A is the designation given to a crystalline antibiotic produced by a Streptomyces organism isolated from a sample of soil collected at Hyde Park, Massachusetts. Lyophilized tubes of the culture bearing the laboratory designation X-537 were deposited with the United States Department of Agriculture, Agricultural Research Service, Northern Utilization Research and Development Division, Peoria, Illinois. The culture, given identification number NRRL 3382 by the Agricultural Research Service, has been made available to the public through NRRL. The culture is also available to the public from the International Center of Information in collaboration with W.H.O. in Belgium.

The antibiotic material, heretofore identified as antibiotic X-537A, upon laboratory analysis has been found to be 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5-dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6-methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl] - salicylic acid, i.e., a compound of the formula



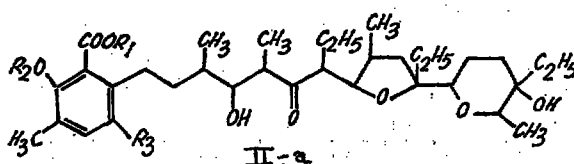
Antibiotic X-537A is prepared by growing the Streptomyces organism in an aerated submerged culture, with the pH of the broth adjusted to about neutral, i.e., 6.5 to 7.5. The medium utilized contains a nitrogen source such as yeast, a yeast derived product, corn meal, bean meal and the like, with soybean meal being the most preferred, and a carbohydrate source such as sugar, molasses and the like, with brown sugar being the most preferred. The fermentation is carried out at slightly elevated temperatures, i.e., between about 25°C and 35°C, with the preferred incubation temperature being about 28°C. After an incubation of about 4 to 6 days, the fermentation broth is filtered and the antibiotic recovered by extraction.

As indicated earlier, the present invention relates to novel derivatives of antibiotic X-537A and to a process for the manufacture thereof. The novel derivatives to which the invention relates are compounds of the formula

Examples of compounds of formula II and III and their salts are:

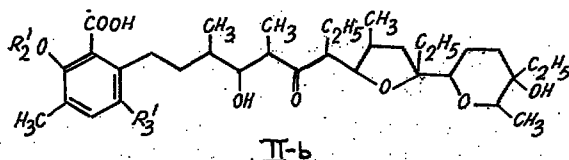
- 3-Methyl-6-[4,6-dihydroxy-3,5-dimethyl-7-ethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]-salicylic acid; 5
- (-)-3-Methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt; 5
- (-)-3-Methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid hydroxyl-ammonium salt; 10
- (+)-3-Methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt; 10
- 3-Methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid methyl ester (isomer A); 15
- 3-Methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid methyl ester (isomer B); 15
- 3-Methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]-acetyl salicylic acid methyl ester; 20
- 2-Methoxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid methyl ester; 20
- Ammonium 3-methyl-2-(2-methyl-propionyloxy)-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoate; 25
- Ammonium 3-methyl-2-nicotinoyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoate; 30
- 5-Nitro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt; 30
- 5-Amino-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid; 35
- 5-Acetamido-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid; 40
- 5-Acetamido-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid; 40
- 5-Diazo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid; 45
- 5-Phenylazo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid; 45
- 2-Benzoyloxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid.

A preferred group of compounds falling within formula II are those of the formula



wherein R₁, R₂ and R₃ have the significance given earlier, and pharmaceutically acceptable salts of those compounds which are acids.

A most preferred group of compounds falling within formula II are those of the formula



wherein R_2' represents hydrogen or lower alkanoyl, R_3' represents hydrogen or halogen and at least one of R_2' or R_3' represents other than hydrogen, and pharmaceutically acceptable salts thereof.

Examples of compounds of formula II-b and their salts are:

3-Methyl-6-[7-ethyl-4-hydroxy-3,5e-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]-acetyl salicylic acid sodium salt;

3-Methyl-2-propionyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt;

2-Butyryloxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt;

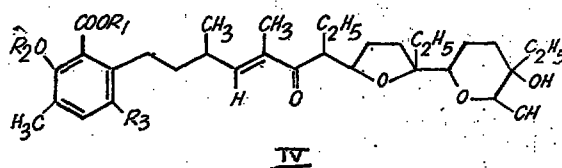
5-Bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt;

5-Iodo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt;

5-Chloro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid;

5-Bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid sodium salt.

As mentioned earlier, the invention is also concerned with dehydration derivatives of antibiotic X-537A. These dehydration derivatives are compounds of the formula



wherein R_1 , R_2 and R_3 have the significance given earlier.

Further, the pharmaceutically acceptable salts of those compounds of formula IV which are acids also form part of this invention.

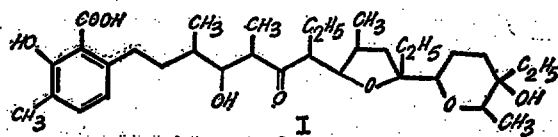
Examples of compounds of formula IV and their salts are:

3-Methyl-6-[7-ethyl-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]-4-heptenyl]-salicylic acid sodium salt (Isomer A);

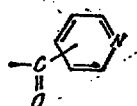
3-Methyl-6-[7-ethyl-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]-4-heptenyl]-salicylic acid sodium salt (Isomer B);

5-Bromo-3-methyl-6-[7-ethyl-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]-4-heptenyl]salicylic acid sodium salt.

The process for the manufacture of the novel derivatives provided by the invention comprises subjecting a compound of the formula



or a pharmaceutically acceptable salt thereof, in any desired sequence, to one or more of the steps: (1) treatment with an esterifying agent to yield an ester group R_1 ; (2) treatment with a lower alkylating agent to form a compound of formula V in which R_1 and/or R_2 represents lower alkyl; (3) treatment with an agent yielding lower alkanoyl,



benzoyl, halo-benzoyl, nitro-benzoyl or (lower alkyl)-benzoyl group to form a compound of formula V in which R_2 has the corresponding meaning; (4) treatment with a halogenating agent to form a compound of formula V in which R_3 represents halogen; (5) treatment with a nitrating agent to form a compound of formula V in which R_3 represents nitro; (6) treatment with a diazonium salt of aniline or an aniline substituted by halogen, nitro, lower alkyl or lower alkoxy to form a compound of formula V in which R_3 represents phenylazo or phenylazo substituted by halogen, nitro, lower alkyl or lower alkoxy; (7) treatment with an acid addition salt of hydroxylamine to form a compound of formula V in which R_4 represents hydroxyimino or reduction to form a compound of formula V in which R_4 represents hydroxy or treatment with a dehydrating agent to form a corresponding dehydration product; whereupon, if desired, a nitro group present is reduced to an amino group or, in the presence of a lower alkyl aldehyde, to a lower alkylamino group; if desired, the so-formed amino group is lower alkylated, lower alkanoylated or converted into a diazo group by treatment with nitrous acid or a lower alkyl nitrite; and, if desired, an acid of formula V obtained is converted into a pharmaceutically acceptable salt thereof.

The derivatives of antibiotic X-537A form a variety of pharmaceutically acceptable salts. These salts are prepared from the free acid form of the derivatives of the antibiotic by methods well known in the art; for example, by washing the free acid in solution with a suitable base or salt. Examples of such pharmaceutically acceptable basic substances capable of forming salts for the purpose of the present invention include alkali metal bases such as sodium hydroxide, potassium hydroxide, lithium hydroxide and the like, alkaline earth metal bases such as calcium hydroxide, barium hydroxide and the like and ammonium hydroxide. Alkali metal or alkaline earth metal salts suitable for forming pharmaceutically acceptable salts include anions such as carbonates, bicarbonates and sulfates.

The compounds of formula V, that is to say, the compounds of formulae II, III and IV, may be manufactured following a variety of procedures. The choice of procedure will depend upon the site or sites upon the antibiotic molecule where substitution is to take place.

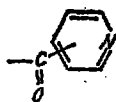
For example, the compound of formula I or compounds of formulae II and IV in which R_1 represents hydrogen can be converted into the corresponding compounds of formulae II or IV in which R_1 represents lower alkyl, phenyl or substituted phenyl (as hereinbefore defined) by conventional esterifying techniques. Examples of agents which can be used to effect the esterification are lower alkanols such as ethanol, methanol, propanol, and the like and aromatic alcohols such as phenol, p-halo-phenols, p-nitrophenol, o-nitrophenol, trimethoxyphenol and the like.

The compound of formula I or compounds of formulae II and IV in which R_1 and/or R_2 represents hydrogen can be converted into the corresponding compounds in which R_1 and/or R_2 represents lower alkyl by treatment with an alkylating agent. Preferably, the alkylation is accomplished by dissolving a compound of formula I or a compound of formula II or IV in which R_1 and/or R_2 represents hydrogen in an

inert organic solvent such as N,N-dimethylformamide. To the resulting solution there is added an alkylating agent such as a lower alkyl halide (e.g. methyl iodide, ethyl iodide, propyl iodide and the like) or a di(lower alkyl) sulfate (e.g. dimethyl sulfate, diethyl sulfate and the like). The mixture is allowed to stand at a temperature of from about 20°C to about 50°C, most preferably at room temperature. When advantageous, the alkylation is effected in the presence of a catalyst, which may be a noble metal oxide such as silver oxide and the like.

The compound of formula I or a compound of formula II or IV in which R₂ represents hydrogen can be lower alkanoylated with any conventional and conveniently available lower alkanoyl group providing agents. Examples of such lower alkanoyl group providing agents are acid anhydrides such as acetic anhydride, propionic anhydride and the like, and acid chlorides such as acetyl chloride, propionyl chloride, butyryl chloride and the like.

Benzoyl groups R₂ (which may be substituted by halogen, nitro and lower alkyl) and



groups R₂ can be introduced in an analogous manner.

Compounds of formulae II or IV in which R₃ represents halogen can be manufactured from the compound of formula I or a compound of formula II or IV in which R₃ represents hydrogen by any conveniently available halogenating technique. Among the many procedures suitable for this purpose there may be included bromination utilizing bromine, chlorination utilizing chlorine or iodination utilizing iodine monochloride and the like.

Compounds of formula II or IV in which R₃ represents nitro can be manufactured by treating the compound of formula I or a compound of formula II or IV in which R₃ represents hydrogen with a nitrating agent such as nitric acid in the presence of glacial acetic acid. The resulting nitro compound can be hydrogenated in the presence of any suitable reducing system such as one which includes hydrogen and Raney nickel to yield the corresponding compound in which R₃ represents amino. It will, of course, be understood that when a compound of formula IV is involved, careful selection of the reducing system should be made so as to reduce the nitro group R₃ while leaving the double bond present in the heptenyl radical unaffected.

If desired, the compounds of formula II in which R₃ represents amino, manufactured as described in the preceding paragraph, may be converted to the corresponding diazonium salt of formula III by treatment with nitrous acid or lower alkyl nitrites (e.g. amyl nitrite), if desired, followed by a cuprous halide (e.g. cuprous chloride). The formation of the diazonium salt can be effected by first preparing a solution of a compound of formula II in which R₃ represents amino in a dilute mineral acid such as aqueous sulfuric acid, aqueous hydrochloric acid and the like. This solution is then treated with nitrous acid. Preferably, the nitrous acid is provided by adding to said solution an aqueous solution of an alkali metal nitrile, preferably sodium nitrite. The nitrous acid treatment is preferably carried out at or below room temperature so that the reaction does not proceed too energetically. Thus, temperatures between -5°C and 25°C are preferred.

Compounds of formula II or IV in which R₃ represents amino can be converted to the corresponding compounds in which R₃ represents lower alkylamino by conventional alkylation procedures. For example, the sodium salt of a compound of formula II or IV in which R₃ represents amino can be reacted with a molar equivalent of a lower alkyl halide to obtain a compound of formula II or IV in which R₃ represents lower alkylamino. For the purposes of this embodiment of the process, lower alkyl halides such as methyl iodide or ethyl bromide, or sulfates such as dimethyl sulfate, diethyl sulfate and the like may be used.

According to an alternate embodiment, compounds of formula II and IV in which R₃ represents lower alkylamino can be manufactured by reducing the nitro group of compounds of formula II and IV in which R₃ represents nitro in the presence of a lower alkyl aldehyde and preferably in the presence of hydrogen and a hydrogenation catalyst such as Raney Nickel.

Compounds of formulae II and IV in which R₃ represents amino can also be

converted into the corresponding compounds in which R_3 represents lower alkanoyl-amino by treatment with a lower alkanoyl group providing agent. Examples of lower alkanoyl group providing agents are acid anhydrides such as acetic anhydride, propionic anhydride, butyric anhydride and the like and acid chlorides such as acetyl chloride, propionyl chloride, butyryl chloride and the like. Conveniently, this treatment is effected in the presence of an inert organic solvent such as pyridine and the like.

Compounds of formula II or IV in which R_3 represents a phenylazo group or a substituted phenylazo group (as hereinbefore defined) can be manufactured from the compound of formula I or a compound of formula II or IV in which R_3 represents hydrogen by treatment with a diazonium salt obtained from an acid addition salt of aniline or an appropriately substituted derivative thereof such as *p*-chloraniline, *p*-nitroaniline, a *p*-lower alkylaniline, a *p*-lower alkoxyaniline and the like. Preferably, this treatment is effected by adding an acidic solution of the aniline or the substituted derivative thereof to an aqueous solution of sodium nitrite whereby to form the diazonium salt and then adding the resulting solution containing the diazonium salt to an alcoholic solution of the compound of formula I or a compound of formula II or IV in which R_3 represents hydrogen. Conveniently, the treatment is effected in the cold, for example, at temperatures of from -10°C to $+15^\circ\text{C}$, most preferably from -5°C to $+5^\circ\text{C}$.

Compounds of formula II in which R_4 represents a $=\text{N}-\text{OH}$ group can be manufactured from the compound of formula I or a corresponding compound of

formula II in which R_4 represents a O group by treatment with an acid addition salt of hydroxylamine in a molar excess. Preferably the hydrochloride salt is used. The procedure is preferably conducted in the presence of pyridine.

The keto group present in the compound of formula I or a compound of formula II in which R_4 represents $=\text{O}$ may be reduced to the corresponding compound of formula II in which R_4 represents $-\text{OH}$. The keto compound can, for example, be treated with an alkali metal borohydride such as sodium borohydride, potassium borohydride and the like. Preferably, the reduction is effected in the presence of an inert organic solvent. Among the inert organic solvents suitable for this purpose there may be mentioned lower alkanols such as methanol, ethanol, propanol, butanol and the like, ethers such as diethyl ether, methyl ethyl ether, dioxane and the like and organic solvents of a similar type.

Compounds of formula IV can be manufactured from the compound of formula I or a compound of formula II in which R_4 represents $=\text{O}$ by treatment with a dehydrating agent. Dehydrating agents suitable for this purpose include acidic dehydrating agents; for example, mineral acids such as hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid. In addition, inorganic bases such as alkali metal hydroxides (for example sodium hydroxide, potassium hydroxide or lithium hydroxide) and alkali earth metal hydroxides (e.g. calcium hydroxide, barium hydroxide and the like) may be used as dehydrating agents. An inorganic alkali metal base such as sodium hydroxide is preferred.

According to one embodiment of the dehydration, the compound of formula I or a compound of formula II in which R_4 represents $=\text{O}$ is added to an inert organic solvent such as *p*-dioxane. To the resulting mixture there is added an aqueous solution of the dehydrating agent, preferably aqueous sodium hydroxide and the mixture is permitted to stand. Although temperature is not a critical aspect of this embodiment, it is preferable to carry out the dehydration at a temperature of from 20°C to 50°C , most preferably at room temperature. The mixture can be maintained under such conditions for a period of one to several hours, with a duration of about seven hours being preferred. Thereafter, the desired product can be isolated and purified from the resulting mixture, which contains both isomers, employing conventional techniques such as recrystallization or chromatography.

The novel derivatives provided by the invention (e.g. in crystalline form) are useful both for the prophylaxis and therapy of coccidiosis in poultry. Coccidiosis is a disease caused by a microscopic protozoal parasite belonging to the genus *Eimeria*. The compounds of the present invention are especially useful because of their high activity against single and multiple infections, that is to say, infections caused by a single species or by a combination of species. Following standard procedures for ascertaining coccidiostatic activity, the compounds of formulae II, III and IV were administered to chickens at varying levels in their feed, with the result that these compounds were shown to be active coccidiostats. For example,

- 3-Methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]-acetyl salicylic acid sodium salt;
- 5 3-Methyl-2-propionyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt; 5
- 2-Butyryloxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt; and
- 10 5-Bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt 10

show coccidiostatic activity in chickens at a feed level of 0.03 per cent.

- Further, the novel derivatives provided by the invention (e.g. in crystalline form) are useful as antibacterial agents. Following standard procedures for determining antibacterial activity,
- 15 15

- (-)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid hydroxylammonium salt;
- 20 5-bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt; 20
- 5-nitro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt; and 25
- 5-iodo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-2-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt 25

show *in vitro* activity against *E. Bacillus*.

- The invention is further illustrated by the following Examples. All temperatures given are in degrees Centigrade.
- 30 30

Example 1

- Preparation of 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid (crystalline antibiotic X-537A)
- 35 35

- The *Streptomyces* organism was grown in aerated submerged culture in shaken flasks. The pH of the broth was adjusted by the addition of KOH solution to 6.5—7.5 and then the broth was sterilized. A tank fermentation was used wherein a 5—10% inoculum consisting of 3 day old submerged growth from aerated bottles was used in the tank. The medium contained 2% soybean flour, 2% brown sugar, 0.5% K_2HPO_4 . The fermentation was carried out at 28° under positive air pressure with air-flows of 5—10 cubic feet of air per minute per 40 to 80 gallon liquid charge. The broth was harvested after 4 to 6 days fermentation, filtered and the antibiotic was recovered by extraction. The extraction was carried out as follows:
- 40 40

- 204 liters of broth were filtered and the wet filter cake was suspended in 100 liters of butyl acetate and the mixture was stirred overnight at room temperature. The mixture was then filtered and the water layer was separated and discarded. The butyl acetate solution, assaying 30 million *Bacillus E* units, was concentrated *in vacuo* to 3 liters, washed with 10% sodium carbonate solution and dried with anhydrous sodium sulfate.
- 45 45

- On further concentration to 300 ml and dilution with 350 ml of petroleum ether (boiling point 50—60°), 41 g of solid material assaying 25 million *Bacillus E* units separated. This solid material was then extracted in a Soxhlet apparatus with 4 liters of petroleum ether (boiling point 50—60°) for 40 hours. The extract was taken to dryness *in vacuo*, the crystalline residue suspended in petroleum ether and filtered, yielding 24.49 g of a mixture of the salt and free acid form of 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid. The mother liquor of the solid yielded an additional 5.73 grams of the antibiotic.
- 50 50

- After recrystallization from ether/petroleum ether, this material, which con-
- 55 60

tained sodium, was dissolved in ether and washed with dilute sulfuric acid to convert it to the free acid. Removal of the ether left an oily residue which crystallized from ethanol to yield pure 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid. Several recrystallizations from ethanol did not change the melting point which remained unsharp at 100—109°.

Example 2

Preparation of 3-methyl-6-[7-ethyl-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]-4-heptenyl]-salicylic acid sodium salt (Isomer A)

To a solution of 10 g of crystalline antibiotic X-537A in 100 ml of p-dioxane were added 200 ml of 10% aqueous sodium hydroxide and the mixture was stirred at room temperature. After 7 hours, the mixture, which had separated into two phases, was extracted twice with an equal volume of ether. The ether extracts were dried (Na_2SO_4) and concentrated to a viscous oil which partially crystallized on standing. The crystals were removed by the addition of ether and filtration. Recrystallization of a sample from ether gave colourless needles of 3 - methyl - 6 - [7 - ethyl - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - 4 - heptenyl]salicylic acid sodium salt (isomer A) of melting point 220°; $[\alpha]_D^{25} - 19.96^\circ$ (1%, CH_3OH).

Example 3

Preparation of 3-methyl-6-[7-ethyl-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]-4-heptenyl]salicylic acid sodium salt (isomer B).

The ether filtrate obtained in Example 2 was evaporated to dryness, dissolved in hexane and chromatographed on a Florisil (Registered Trade Mark) column (200 g) using gradient elution from 2 liters hexane/ether (1:1) to 2 liters ether/acetone (1:1). The first fraction eluted was discarded. The second fraction was a mixture which, on concentration and treatment with ethyl acetate, gave an additional yield of 3 - methyl - 6 - [7 - ethyl - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - 4 - heptenyl]salicylic acid sodium salt (isomer A). Chromatography of the filtrate on silica gel using gradient elution from methylene chloride to acetone gave the second component of the mixture as an oil which crystallized on standing to yield 3 - methyl - 6 - [7 - ethyl - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - 4 - heptenyl]salicylic acid sodium salt (isomer B) of melting point 125°; $[\alpha]_D^{25} + 27.64^\circ$ (1%, CH_3OH).

Example 4

Preparation of 5-bromo-3-methyl-6-[7-ethyl-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]-4-heptenyl]salicylic acid sodium salt

To 1.34 g of crystalline antibiotic X-537A in carbon disulfide (25 ml.) was added dropwise a solution of bromine (0.12 ml) in carbon disulfide (10 ml). The mixture was left overnight under a stream of nitrogen to remove the CS_2 . The residue which contained the free acid form of the desired end product was dissolved in ethyl acetate. After washing successively with aqueous sodium bisulfite and saturated sodium carbonate, the ethyl acetate solution was dried (Na_2SO_4) and evaporated under reduced pressure. Crystallization from methylene chloride/hexane gave colourless needles of 5 - bromo - 3 - methyl - 6 - [7 - ethyl - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - 4 - heptenyl]salicylic acid sodium salt of melting point 235°; $[\alpha]_D^{25} + 9.63^\circ$ (1%, DMSO).

Example 5

Preparation of 5-bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt

To a solution of 1.53 g of the sodium salt of antibiotic X-537A in 30 ml carbon disulfide was added dropwise a solution of 0.14 ml bromine in 10 ml carbon disulfide at -5° over 10 minutes. The solvent was removed under a stream of nitrogen at room temperature (approximately 2 hours). The residue was purified by dissolving it

in ethyl acetate and the solution washed successively with aqueous sodium bisulfite and saturated sodium carbonate. After drying (Na_2SO_4), the solution was evaporated under reduced pressure and the residue crystallized from ethyl acetate to give 5 - bromo-3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid sodium salt as colourless cubes of melting point 185° ; $[\alpha]_D^{25} - 7.36^\circ$ (1% in methanol).

Example 6

Preparation of 3-methyl-6-[4,6-dihydroxy-3,5-dimethyl-7-ethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid
To a solution of 10 g of crystalline antibiotic X-537A in 500 ml of absolute ethanol were added 1.36 g of NaBH_4 . The reaction was followed by assaying with thin layer chromatography (silica gel — CHCl_3 /acetone 8:2) and the reaction was continued overnight after adding an additional 1.0 g of NaBH_4 . The following morning the solvent was removed under reduced pressure and the heavy oil diluted with methylene chloride, washed with dilute HCl , dried (Na_2SO_4) and concentrated to a solid foam. The slightly pink solid was chromatographed on a 50 g silica gel column eluting with 2 liters of hexane/methylene chloride (1+1), 2 liters of methylene chloride and 2 liters of methylene chloride/ether (1+1). The 250 ml fractions were assayed by TLC and fractions 13—17 pooled, concentrated to a yellow solid and rechromatographed on a 150 g Florisil column eluting with a gradient from hexane to acetone. The fractions containing 3 - methyl - 6 - [4,6 - dihydroxy - 3,5 - dimethyl - 7 - ethyl - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid were pooled and concentrated to a small volume from which pure 3 - methyl - 6 - [4,6 - dihydroxy - 3,5 - dimethyl - 7 - ethyl - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid crystals were recovered; melting point $151-153^\circ$. From the mother liquor, additional material was crystallized; $[\alpha]_D^{25} + 0.83$ (0.60%, CH_3OH).

Example 7

Preparation of 2-methoxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid methyl ester
A solution of 5 g of crystalline antibiotic X-537A in 50 ml of N,N-dimethylformamide was stirred overnight with 6 g of silver oxide and 50 ml of methyl iodide. After assay (TLC silica gel — benzene/acetone, 9:1) an additional 5 g of silver oxide and 10 ml of methyl iodide were added. After 48 hours the solids were removed by filtration and the filtrate diluted with water and methylene chloride. The solvent was separated, removed under reduced pressure and the remaining N,N-dimethylformamide removed by water washing of an ether solution. The ether layer was chromatographed on a 140 g Florisil column eluting with a gradient between hexane (1 liter) to acetone (1 liter). The fractions containing the product were pooled and concentrated to a clear oil which could be distilled at 165° and 0.05 mm yielding pure 2 - methoxy - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]benzoic acid methyl ester; $[\alpha]_D^{25} - 10.79$ (1% CH_3OH).

Example 8

Preparation of 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid sodium salt
To a solution of 6.12 g of crystalline antibiotic X-537A in 10 ml dry pyridine were added 2 ml of acetic anhydride. After 2 hours, 10 g of ice were added to the solution and the resulting mixture was washed into a separating funnel with ethyl acetate and 1N HCl . The ethyl acetate solution was washed with 1N HCl until all the pyridine had been removed. The ethyl acetate solution was then washed with saturated sodium carbonate solution, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was dissolved in methylene chloride and the solution evaporated on a steam bath with additions of hexane until crystallization started. Cool-

ing to 0° gave 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydrofuryl)]heptyl]acetyl salicylic acid sodium salt as white needles of melting point 186—187°; $[\alpha]_D^{25} -18.75^\circ$ (1%, CH₃OH).

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Example 9

Preparation of (—)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid, hydroxylammonium salt

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A mixture of 10 g of crystalline antibiotic X-537A and 5 g of hydroxylamine hydrochloride in 50 ml ethanol containing 5 ml pyridine was heated under reflux for 60 hours. The solution was evaporated to dryness and 5 ml of water were added. The solid product was triturated and filtered and then recrystallized from aqueous ethanol to give (—)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid hydroxylammonium salt of melting point 168—170°; $[\alpha]_D^{25} -12.34^\circ$ (1%, DMSO).

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Example 10

Preparation of (+)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid sodium salt and (—)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid sodium salt

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A mixture of 10 g of crystalline antibiotic X-537A and 5 g of hydroxylamine hydrochloride in 50 ml ethanol containing 5 ml pyridine was heated under reflux for 60 hours. The solution was evaporated to dryness and treated with a mixture of 1N HCl and ethyl acetate. The ethyl acetate layer was washed with 1N HCl until all the pyridine and hydroxylamine had been removed. It was then treated with a saturated aqueous solution of sodium carbonate, separated and dried (Na₂SO₄). After evaporation to dryness, the residue was fractionally crystallized from ethanol. The first fraction was shown by TLC on silica gel using benzene/methanol (9:1) as solvent to be the sodium salt of the oxime previously isolated in Example 9 as its hydroxylammonium salt. Recrystallization from CH₂Cl₂/hexane gave white prisms of (—)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid sodium salt of melting point 173—174°; $[\alpha]_D^{25} -11.99^\circ$ (1%, methanol). The second crop isolated by fractional crystallization of the crude sodium salt of the oxime was found to be an isomer of (—)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid sodium salt. Recrystallization from CH₂Cl₂/hexane gave white needles of (+)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid sodium salt of melting point 220°; $[\alpha]_D^{25} +27.16^\circ$ (1%, methanol).

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Example 11

Preparation of 3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid methyl ester (Isomer A).

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A solution of 3.55 g of (—)-3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid sodium salt in 100 ml methylene chloride was treated with 1N HCl. The solvent layer was separated, washed with water and concentrated under reduced pressure to a solid. A portion (1.3 g) of the solid, equal to 2.15 millimoles, was dissolved in ether and treated with 4.25 millimoles of an ethereal solution of diazomethane. At the end of one hour the solvent was removed at reduced pressure leaving an oil which upon standing under a high vacuum solidified to yield 3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydrofuryl)]heptyl]salicylic acid methyl ester (isomer A); $[\alpha]_D^{25} +5.77$ (1.03%, CH₃OH).

Example 12

Preparation of 3-methyl-6-[7-ethyl-4-hydroxy-6-hydroxyimino-3,5-dimethyl-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid methyl ester (Isomer B).

5 A solution of 1.77 g of (+) - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 6 - hydroxyimino - 3,5 - dimethyl - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid sodium salt in 100 ml of methylene chloride was washed with 1N HCl. The solvent layer was separated, washed with water and concentrated at reduced pressure to a solid. A portion (1.48 g) of the solid, equal to 2.45 millimoles, was dissolved in ether and treated with 5 millimoles of a solution of diazomethane in ether. After one hour the solvent was removed under reduced pressure leaving an oil which upon standing under strongly reduced pressure solidified to yield 3 - methyl - 6 - [7-ethyl - 4 - hydroxy - 6 - hydroxyimino - 3,5 - dimethyl - 7 - [5 - ethyl - 3 - methyl-10 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid methyl ester (Isomer B); $[\alpha]_D^{25} +32.63^\circ$ (1.01%, CH₃OH). 15

Example 13

Preparation of 5-iodo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-2-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt

20 A solution of 1.8 g of crystalline antibiotic X-537A in 50 ml of glacial acetic acid was treated with 980 mg of iodine monochloride (freshly distilled 97—99° fraction). The ICI was mixed with 5 ml of acetic acid and added to the reaction mixture slowly over a period of 15 minutes. After an additional 15 minutes, water (100 ml) was slowly added and the mixture extracted with ether. The solvent layer was separated and washed successively with aqueous solutions of sodium bisulfite, sodium bicarbonate and sodium carbonate. The solvent was dried (Na₂SO₄). After concentration and crystallization, the product, 5 - iodo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 2 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid sodium salt was recovered by filtration. Recrystallization from ethyl acetate gave 5 - iodo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 2 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid sodium salt of melting point 222.5—223°; $[\alpha]_D^{25}$ -48.7° (1%, CHCl₃). 25 30 35

Alternatively, 5 - iodo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl-40 6 - oxo - 7 - [5 - ethyl - 2 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid sodium salt can be prepared by treatment of crystalline antibiotic X-537A (1 millimole) with iodine (1 millimole) and morpholine (3 millimoles). The reaction is usually complete in 5 days and the product, purified by chromatography, is identical to that described in the preceding paragraph. 45

Example 14

Preparation of 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid methyl ester

1.344 g (2 mmole) of 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl-45 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]acetyl salicylic acid sodium salt were treated with ether (30 ml) and 1N HCl (30 ml). The mixture was shaken in a separating funnel until all the starting material had dissolved. The ether layer was then washed twice with water and treated with 20 ml of a solution of ethereal diazomethane (5 mmole). After drying (Na₂SO₄), the solution was evaporated to give 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]acetyl salicylic acid methyl ester as an oil; $[\alpha]_D^{25} +0.99^\circ$ (1% in methanol). 50 55

Example 15

Preparation of 5-bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid sodium salt

60 To a solution of 2.11 g of the sodium salt of 5 - bromo - 3 - methyl - 6 - [7-ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5-ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]- 65

salicylic acid in 20 ml of dry pyridine was added 0.66 ml of acetic anhydride. After 4 hours at room temperature, the mixture was diluted with ice-water containing 40 ml of HCl and ethyl acetate. The ethyl acetate was separated, washed with 1N HCl until the pyridine was removed and then washed with a saturated aqueous solution of sodium carbonate and dried over Na_2SO_4 . The ethyl acetate was evaporated to 25 ml from which 5-bromo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid sodium salt was recovered by filtration. Melting point 213—215°; $[\alpha]_D^{25} -3.5^\circ$ (1% methanol). An additional yield of the desired product was recovered from the mother liquor after crystallization from methylene chloride/hexane.

Example 16

Preparation of 5-nitro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt

To a solution of 2 g of crystalline antibiotic X-537A in 75 ml of glacial acetic acid was added 1 ml of concentrated nitric acid and after stirring for 0.5 hour, an equal volume (75 ml) of water was added. The resulting yellow amorphous precipitate was extracted with ether. The ether layer was washed with sodium carbonate solution until all the acetic acid had been removed. After drying (Na_2SO_4), the ether was removed under reduced pressure to give 2.4 g of a yellow solid. Treatment with methylene chloride followed by filtration gave a crystalline product which was discarded. The methylene chloride filtrate was concentrated under reduced pressure and chromatographed on a silica (20 g) column using methylene chloride containing 1% methanol as eluant. The first fraction eluted gave 5-nitro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid sodium salt which, on recrystallization from methylene chloride/hexane, gave pale yellow plates of melting point 214—215°; $[\alpha]_D^{25} 98.05^\circ$ (1%, DMSO).

Example 17

Preparation of 3-methyl-2-propionyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt

To a solution of 3.0 g (49 mmol) of the crystalline antibiotic X-537A in 75 ml of pyridine, 0.7 ml (54 mmol) of propionic anhydride was added and the mixture kept at room temperature for 16 hours. Most of the solvent was then removed by concentration at reduced pressure below 35°. The residue was dissolved in methylene chloride, washed with dilute hydrochloric acid, dilute sodium carbonate solution and water and dried (Na_2SO_4). The solvent was then removed by distillation under reduced pressure. The residue crystallized from hexane to yield 3-methyl-2-propionyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt of melting point 193—195°. Recrystallization did not alter the melting point; $[\alpha]_D^{25} -20.3^\circ$ (1.3% MeOH).

Example 18

In a similar fashion as that disclosed in Example 17, the following compounds may be prepared from the indicated starting materials:

2-Butyryloxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt of melting point 191—193°; $[\alpha]_D^{25} -20.1^\circ$ (1%, MeOH); from 3.0 g of crystalline antibiotic X-537A and 0.88 ml of butyric anhydride.

2-Benzoyloxy-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt of melting point 177—180°; from crystalline antibiotic X-537A and benzoic anhydride.

Example 19

Preparation of the Ammonium Salt of Antibiotic X-537A

15.0 g of the sodium salt of antibiotic X-537A were dissolved in ethyl acetate (500 ml) and washed three times with water and three times with 200 ml portions of 2.8% ammonium hydroxide solution. Removal of solvent from the ethyl acetate phase

left a residue which was crystallized from methylene chloride/hexane to afford white crystals of the ammonium salt of antibiotic X-537A of melting point 195—196.5° (decomposition); $[\alpha]_D^{25} -50.84^\circ$ (1.34% in methanol). Concentration of the mother liquors afforded additional material of melting point 194—196°.

Example 20

Preparation of the Sodium Salt of Antibiotic X-537A

The sodium salt was prepared by shaking an ether solution of the free acid of antibiotic X-537A with aqueous sodium carbonate solution. The salt which remained in the ether was recrystallized twice from benzene/ligroin and dried. It had a melting point, taken in an open capillary tube, of 191—192° (decomposition).

Example 21

Preparation of the Lithium Salt of Antibiotic X-537A

5.0 g of the sodium salt of antibiotic X-537A were dissolved in ethyl acetate (200 ml). The solution was washed in turn with 100 ml portions of 1N hydrochloric acid (twice), water (twice), barium hydroxide solution (twice) and lithium sulfate solution (twice). A white solid was recovered by evaporation of the organic phase and, on recrystallization from methylene chloride/hexane, yielded the lithium salt as white crystals, of melting point 161—162° (decomposition); $[\alpha]_D^{25} -8.27^\circ$ (1.23% methanol).

Example 22

Preparation of 3-methyl-2-propionyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt

6.12 g of the sodium salt of antibiotic X-537A were dissolved in pyridine (10 ml) by warming slightly. The solution was cooled in an ice bath and treated with propionyl chloride, then allowed to stand at room temperature for 2 hours. The mixture was poured into water, extracted with ethyl acetate and the organic phase washed in succession with 1N hydrochloric acid (several times) and sodium carbonate solution (twice). Evaporation of the ethyl acetate solution left a residue which was crystallized from methylene chloride/hexane to afford 3-methyl-2-propionyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoic acid sodium salt of melting point 185—186.5°; $[\alpha]_D^{25} -20.00^\circ$ (1.00%, methanol).

Example 23

Preparation of ammonium 3-methyl-2-(2-methyl-propionyloxy)-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoate

A solution of the sodium salt of antibiotic X-537A (6.12 g) in pyridine (10 ml) was cooled in an ice bath and treated with isobutyric anhydride (1.74 g in 3 ml of pyridine). After stirring at room temperature for 2 days, the mixture was poured into water, extracted with ethyl acetate and the organic phase washed with 1N hydrochloric acid and 2.8% ammonium hydroxide solution. The residue obtained on evaporation of the ethyl acetate solution was ammonium 3-methyl-2-(2-methyl-propionyloxy)-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoate in solid form of melting point ca 98° (decomposition); $[\alpha]_D^{25} -23.33^\circ$ (1.25%, methanol).

Example 24

Preparation of ammonium 3-methyl-2-nicotinoyloxy-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]benzoate

A solution of the sodium salt of antibiotic X-537A (6.12 g) in pyridine (10 ml) was stirred at room temperature under nitrogen with 3 g of nicotinoyl chloride hydrochloride for 13 days. Extraction and purification as described in Example 22 gave a yellow residue which resisted crystallization. Attempted crystallization from hexane gave a gum which was dried on a clay plate to an amorphous solid of melting point 81—92° (decomposition); $[\alpha]_D^{25} -14.64^\circ$ (1.04%, methanol).

Example 25

Preparation of 5-phenylazo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid

To a solution of 0.18 ml of aniline in 8 ml of N HCl, cooled to 0—5° in an ice-bath there were slowly added 150 mg of NaNO₂ in water chilled to the same temperature. To the resulting solution there was added a cold methanolic solution of 550 mg of crystalline antibiotic X-537A. The mixture was kept at 3 to 5° for 15 minutes and then allowed to slowly reach room temperature. At the end of 2 hours, the mixture was diluted with water and extracted with ethyl acetate. The solvent extract was washed with a saturated solution of Na₂CO₃, dried over Na₂SO₄ and concentrated to a solid. The solid was chromatographed on a 30 g silica gel column eluting with a gradient between 300 ml methylene chloride and 500 ml ether. The product was eluted in the first peak and crystallized from methylene chloride/hexane; melting point 212—215°; $[\alpha]_D^{25}$ -27.30° (0.586%, CH₃OH).

Example 26

Preparation of 5-amino-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid

A solution of 5.0 g (7.6 millimole) of 5-nitro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid in ethanol (450 ml) was hydrogenated over 2 g of Raney-nickel with an uptake of 22.2 mm of hydrogen. The mixture was filtered (over N₂) and 17.2 ml of N HCl was added. The solvent was removed under reduced pressure and the residue was crystallized from aqueous acetone to give 5-amino-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid of melting point 223—225°; $[\alpha]_D^{25}$ +17.92° (1%, MeOH).

Example 27

Preparation of 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid methyl ester

To a solution of 6.2 g of crystalline antibiotic X-537A in 125 ml of methylene chloride there were added 4.6 g of silver oxide and 14 g of methyl iodide. After stirring for 18 hours, the mixture was filtered and the filtrate concentrated to 7.07 g of a light yellow oil which was decolorized with activated carbon giving 6.09 g of a colourless foam. A portion (4 g) of the foam was chromatographed on a Florisil column (150 g) eluting with a gradient between methylene chloride (1 liter) to ether (1 liter) and then ether (500 ml) to acetone (500 ml). Rechromatography of the slightly yellow oil recovered from the Florisil column on a 75 g silica gel column and eluting with a gradient between methylene chloride (1 liter) and 15% ether/methylene chloride gave 200 mg of a clear viscous oil; boiling point 170°/0.005 mm; $[\alpha]_D^{25}$ -7.19°, (1.1%, CH₃OH).

Example 28

Preparation of 5-acetamido-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid

To a suspension of 1.21 g (2 mmole) of 5-amino-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid in 50 ml of glacial acetic acid there was added 1 ml of acetic anhydride (N 10 mmoles). On warming the mixture to 70° on a steam bath, the amine dissolved and after a further half an hour at room temperature, the solution was poured on to crushed ice. The mixture was carefully made alkaline with sodium carbonate solution and extracted twice with 400 ml ether. After drying (Na₂SO₄) the ethereal solution was evaporated to approximately 100 ml. The resulting microcrystalline product had a melting point of 189—190°; $[\alpha]_D^{25}$ -17.45° (1%, MeOH).

Example 29

Preparation of 5-diazo-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid

5 A solution of 5-amino-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid (1.28 g) in 10 ml of methanol and 2 ml of aqueous N HCl was cooled to 0° and a chilled solution of 150 mg of NaNO₂ in 1 ml water was slowly added. To the solution there were 10 added 320 mg of CuCl in 1 ml of concentrated HCl. The mixture was allowed to slowly warm up to room temperature and then diluted with water and extracted with ether. The ether extract was concentrated to a small volume and the crystallized product was collected by filtration; melting point 137—138°; [α]_D²⁵ -13.5° (0.7%, CH₃OH).

Example 30

Preparation of 5-acetamido-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid

20 To a solution of 5-amino-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid (1.21 g) in 20 ml of pyridine there were added 0.56 ml of acetic anhydride. At the end of 4 hours, the mixture was poured into 100 ml ice water containing 20 ml of concentrated HCl. The solution was extracted twice with ether and the solvent concentrated to dryness. The residue was dissolved in ethyl acetate from which a semicrystalline material 25 was recovered after addition of hexane. Recrystallization of this material from methylene chloride/ether gave the crystalline product of melting point 190—192°; [α]_D²⁵ -18.38° (1.1%, CH₃OH).

Example 31

30 Preparation of 5-chloro-3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]salicylic acid

35 To a cold solution (~3°) of 6.12 g of the sodium salt of antibiotic X-537A in 50 ml of chloroform/3 ml of carbon tetrachloride there were slowly added 34 ml of carbon tetrachloride saturated with 10 millimoles of chlorine gas. The reaction was continued for 0.5 hour after which the solution was filtered and the filtrate treated with a saturated aqueous solution of Na₂CO₃. The solvent layer was dried over Na₂SO₄ and concentrated to a small volume from which, after addition of hexane, the crystalline product was recovered. The crystalline material, which had a tendency 40 to incorporate the solvent of crystallization, was twice recrystallized from methylene chloride/ether and acetone/hexane; melting point 183—185°; [α]_D²⁵ -44.31° (0.99%, CHCl₃).

45 The following Example illustrates the utilization of a representative member of the derivatives of the present invention as a coccidiostatic agent in an animal feed. In a manner similar to that described hereinafter, the remaining derivatives provided by the present invention may also be incorporated as active coccidiostatic agents into animal feeds.

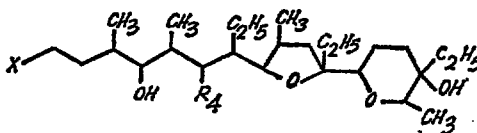
50 A medicated poultry feed intended as a starter feed for broiler is prepared by blending 0.005 per cent by weight of 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]heptyl]acetyl salicylic acid sodium salt in a basic poultry ration consisting of:

		Pounds per Ton	
55	Corn meal, No. 2 yellow, ground	1123	
	Stabilized grease or vegetable oil	60	55
	Soybean oil meal (low fiber content 50% protein)	480	
	Corn Gluten meal	50	
	Fish meal, antioxidant treated, 60% protein	30	
	Fish solubles, dried basis	10	
60	Meat and bone scraps (dehydrated), 50% protein	140	60
	Corn distillers dried soluble	50	
	Alfalfa meal, 17% protein 100,000 A/lb	30	

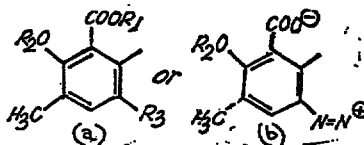
		Pounds per Ton	
	Salt, iodized	5	
	Manganese sulfate, feed grade	0.75	
	Zinc carbonate or oxide	0.25	
5	Riboflavin, Grams	3	5
	Vitamin B ₁₂ , mg	6	
	Calcium pantothenate, gms	5	
	Niacin, gm	30	
	Stabilized vitamin A USP units	6,000,000	
10	Vitamin D ₃ , IC units	650,000	10
	Vitamin E acetate, IU	5,000	
	Vitamin K, (menadione sodium bisulfite) gms	2	
	DL-methionine or hydroxy analog, pound	1	
	Antioxidant (ethoxyquin or butylated hydroxy toluene) lb	0.25	
15	Similar feeds can be prepared containing the active ingredient 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - heptyl]acetyl salicylic acid sodium salt at other concentrations; for example, containing from 0.001 per cent to 0.0125 per cent by weight of said salt.		
15			15

20 WHAT WE CLAIM IS:— 20

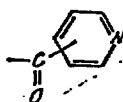
1. Compounds of the formula



wherein X represents a group of the formula

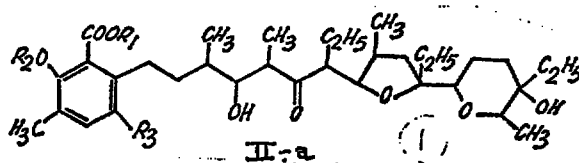


25 in which R₁ represents hydrogen, lower alkyl, phenyl or phenyl substituted by 25
halogen, nitro or lower alkyl, R₂ represents hydrogen, lower alkyl, lower alkanoyl,



30 benzoyl or benzoyl substituted by halogen, nitro or lower alkyl, R₃ represents 30
hydrogen, halogen, nitro, amino, lower alkanoylamino, lower alkylamino, phenyl-
azo or phenylazo substituted by halogen, nitro, lower alkyl or lower alkoxy, R₄
represents —OH, =O or =N—OH (provided that at least one of R₁, R₂ and
R₃ represents other than hydrogen or R₄ represents other than =O),
35 dehydration derivatives of those compounds of formula V in which X represents the 35
group (a) and R₄ represents =O and pharmaceutically acceptable salts of those com-
pounds and dehydration derivatives which are acids.

2. A compound of the formula



wherein R₁, R₂ and R₃ have the significance given in claim 1,
and pharmaceutically acceptable salts of those compounds which are acids.

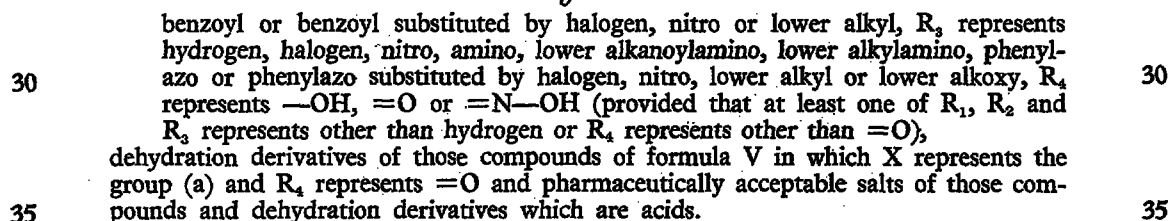


Similar feeds can be prepared containing the active ingredient 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - heptyl]acetyl salicylic acid sodium salt at other concentrations; for example, containing from 0.001 per cent to 0.0125 per cent by weight of said salt.

1. Compounds of the formula



wherein X represents a group of the formula



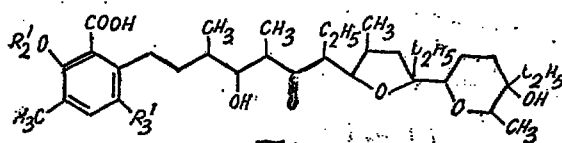
wherein R₁, R₂ and R₃ have the significance given in claim 1, and pharmaceutically acceptable salts of those compounds which are acids.



3, 5 - Nitro - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - heptyl]salicylic acid and pharmaceutically acceptable salts thereof.

5 4. A compound of the formula

5



II-b

wherein R_2' represents hydrogen or lower alkanoyl, R_3' represents hydrogen or halogen (at least one of R_2' or R_3' representing other than hydrogen), and pharmaceutically acceptable salts thereof.

10 5. A compound as claimed in claim 4, wherein R_2' represents lower alkanoyl and R_3' represents hydrogen.

10

15 6. 3 - Methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]acetyl salicylic acid and pharmaceutically acceptable salts thereof.

15

20 7. 3 - Methyl - 2 - propionyloxy - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]benzoic acid and pharmaceutically acceptable salts thereof.

20

25 8. 2 - Butyryloxy - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]benzoic acid and pharmaceutically acceptable salts thereof.

25

30 9. A compound as claimed in claim 4, wherein R_2' represents hydrogen and R_3' represents halogen.

35 10. 5 - Bromo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid and pharmaceutically acceptable salts thereof.

30

40 11. 5 - Chloro - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid and pharmaceutically acceptable salts thereof.

35

45 12. 5 - Iodo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid and pharmaceutically acceptable salts thereof.

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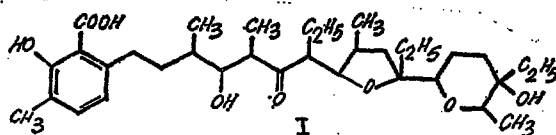
13. 5 - Diazo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl]heptyl]salicylic acid.

45

14. 5 - Bromo - 3 - methyl - 6 - [7 - ethyl - 4 - hydroxy - 3,5 - dimethyl - 6 - oxo - 7 - [5 - ethyl - 3 - methyl - 5 - (5 - ethyl - 5 - hydroxy - 6 - methyl - 2 - tetrahydropyranyl) - 2 - tetrahydrofuryl] - 4 - heptenyl]salicylic acid and pharmaceutically acceptable salts thereof.

15. A process for the manufacture of the derivatives claimed in claim 1, which process comprises subjecting a compound of the formula

45

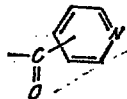


I

or a pharmaceutically acceptable salt thereof, in any desired sequence, to one or more of the steps: (1) treatment with an esterifying agent to yield an ester group R_1 ; (2)



treatment with a lower alkylating agent to form a compound of formula V in which R_1 and/or R_2 represents lower alkyl; (3) treatment with an agent yielding a lower alkanoyl,



- 5 benzoyl, halo-benzoyl, nitro-benzoyl or (lower alkyl)-benzoyl group to form a compound of formula V in which R_2 has the corresponding meaning; (4) treatment with a halogenating agent to form a compound of formula V in which R_3 represents halogen; (5) treatment with a nitrating agent to form a compound of formula V in which R_3 represents nitro; (6) treatment with a diazonium salt of aniline or an aniline substituted by halogen, nitro, lower alkyl or lower alkoxy to form a compound of formula V in which R_3 represents phenylazo or phenylazo substituted by halogen, nitro, lower alkyl or lower alkoxy; (7) treatment with an acid addition salt of hydroxylamine to form a compound of formula V in which R_4 represents hydroxyimino or reduction to form a compound of formula V in which R_4 represents hydroxy or treatment with a dehydrating agent to form a corresponding dehydration product; whereupon, if desired, a nitro group present is reduced to an amino group or, in the presence of a lower alkyl aldehyde, to a lower alkylamino group; if desired, the so-formed amino group is lower alkylated, lower alkanoylated or converted into a diazo group by treatment with nitrous acid or a lower alkyl nitrite; and, if desired, an acid of formula V obtained is converted into a pharmaceutically acceptable salt thereof.
- 10 16. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is subjected, in any desired sequence, to one or more of the steps (1), (2), (3), (4), (5) and (6), whereupon, if desired, a nitro group present is reduced to an amino group or, in the presence of a lower alkyl aldehyde, to a lower alkylamino group; if desired, the so-formed amino group is lower alkylated or lower alkanoylated; and if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 15 17. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a nitrating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 20 18. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a halogenating agent and/or lower alkanoylating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 25 19. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a lower alkanoylating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 30 20. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with an acetylating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 35 21. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a propionylating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 40 22. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a butyrylating agent, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 45 23. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a halogenating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 50 24. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a brominating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 55 25. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with a chlorinating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
- 60 26. A process according to claim 15, wherein the compound of formula I or a pharmaceutically acceptable salt thereof is treated with an iodinating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.
27. A process according to claim 17, wherein the nitro derivative obtained is reduced into the corresponding amino derivative which is converted into the corresponding diazo derivative by treatment with nitrous acid or a lower alkyl nitrite.

28. A process according to claim 24, wherein the resulting product is treated with a dehydrating agent and, if desired, a resulting acid is converted into a pharmaceutically acceptable salt.

5 29. A process for the manufacture of the derivatives claimed in claim 1, substantially as hereinbefore described with reference to the foregoing Examples.

30. Derivatives as claimed in claim 1, when manufactured by the process in any one of claims 15 to 29 inclusive.

10 31. A preparation having coccidiostatic or antibacterial activity comprising a derivative as claimed in any one of claims 1 to 14 inclusive in association with a compatible carrier material.

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