

PATENT SPECIFICATION

(11) 1244 019

NO DRAWINGS

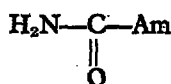
- (21) Application No. 57629/68 (22) Filed 4 Dec. 1968
 (31) Convention Application Nos. 687 498 and 687 499
 (32) Filed 4 Dec. 1967
 (31) Convention Application No. 744 627 (32) Filed 15 July 1968 in
 (33) United States of America (US)
 (45) Complete Specification published 25 Aug. 1971
 (51) International Classification C 07 d 21/00; C 07 g 11/00; A 61 k 21/00
 (52) Index at acceptance C2A 1C1B 1C1C 1C1D2 1C2C 2A3
 (72) Inventors EDDIE HERMAN MASSEY, ARTHUR PHILLIP RAUN and RICHARD MARTIN KLINE



(54) UREA-ANTIBIOTIC ADDUCTS AND PROCESS FOR PREPARING THE SAME

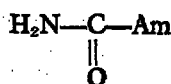
(71) We, ELI LILLY AND COMPANY, a Corporation organised and existing under the Laws of the State of Indiana, United States of America, of 740 South Alabama Street, City of Indianapolis, State of Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention provides an adduct of an aldehyde-containing macrolide antibiotic and urea, said adduct containing 2 moles of a urea per mole of antibiotic wherein said 2 moles of urea react with the aldehyde group of said antibiotic, said urea being represented by the formula:



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or R' N< wherein R' and R'' are independently hydrogen, C₁—C₄ alkyl or C₂—C₄ alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4.

The invention further provides a process for preparing an adduct of an aldehyde-containing macrolide antibiotic and a urea which comprises mixing the urea and the antibiotic in an aqueous or alcoholic solvent in amounts to provide at least two mols of the urea per mol of the antibiotic, said urea being represented by the formula



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or R' N< wherein R' and R'' are independently

R'' hydrogen, C₁—C₄ alkyl or C₂—C₄ alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4.

The antibiotic-urea adduct is suitable for use as the active ingredient of a veterinary composition for an animal feed composition comprising an animal feed and one or more of the adducts.

The invention contemplates the novel compounds dihydro(ketone - reduced)tylosin and dihydro(ketone - reduced)desmycosin which are prepared by acid hydrolysis of a urea adduct of dihydro(ketone - reduced)tylosin or dihydro(ketone - reduced)desmycosin. Dihydro(ketone - reduced)tylosin and dihydro(ketone - reduced)desmycosin are antibiotically active and are suitable for use in pharmaceutical and/or disinfectant compositions.

Several macrolide antibiotics contain an aldehyde group. These include tylosin (U.S. Patent 3,178,341, issued May 13, 1965), members of the leucomycin series (*Tetrahedron Letters*, 1967, page 609), spiramycins A, B, C, and D (U.S. Patents 3,000,785, issued September 19, 1961 and 3,105,794, issued October 1, 1963), carbomycin (U.S. Patent 2,796,379, issued June 18, 1957) and carbomycin B (U.S. Patent 2,785,104, issued March 12, 1957). These antibiotics also contain other reactive groups; for example, tylosin contains, in addition, a ketone group, a lactone group, amine groups, ether groups, and hydroxyl groups, as well as glycosidic linkages and ethylenic unsaturations. Because of the presence of such numerous reactive functions in each of the macrolide antibiotics, stability problems have arisen which have limited their therapeutic use. In particular, these macrolide

antibiotics undergo a series of acidic hydrolytic reactions and are also subject to oxidation degradation. Protection of the sites subject to chemical change in the various macrolides has been the subject of considerable research in recent years.

In addition, it has been found that the treatment of ruminants with antibiotics by the oral route is affected by the fact that the rumen flora can change the nature of the antibiotic molecule. For example, it has been found that ruminant organisms have the capability of reducing the aldehyde group present in many of these macrolide antibiotics to a primary alcohol group. With the antibiotic tylosin, this transformation product is known as tylosin D (also AM—684, disclosed in U.S. Patent 3,321,368, issued May 23, 1967). Tylosin D is much less active antibiologically than is tylosin and has a somewhat different antibiotic spectrum. Protection of the aldehyde group from transformations produced by rumen organisms during the therapeutic treatment of ruminants with macrolide antibiotics such as tylosin, would greatly enhance the therapeutic efficacy of these antibiotics.

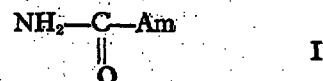
It has also been found that oral administration of antibiotics, including the macrolides and the tetracyclines, to ruminant animals either for therapeutic purposes or to increase feed efficiency or weight gain is usually accompanied by undesirable side effects. These antibiotics, when administered at rates at which they will be useful for such purposes, deleteriously affect the rumen flora. As a consequence, the digestive mechanism of the ruminant is upset and the animals customarily go off feed, with or without attacks of diarrhea, etc. For example, Bell et al. *J. Anim. Sci.* 9, 647 (1950) and *Proc. Soc. Exptl. Biol. and Med.* 76, 284 (1951) have found that administration of chlortetracycline depresses both crude fiber and dry matter digestibility in cows. The importance of the rumen in the digestion of fiber (consisting almost entirely of dietary cellulose) can hardly be over-emphasized and the depression of the cellulose digestion process slows the animals' growth, milk production, etc. Similar results were obtained by Luther et al. ("European Symposium on Antibiotics and New Growth Factors in Animal Nutrition", Rome, Italy, May 10-12, 1955) for the antibiotics polymyxin B, chloramphenicol, bacitracin, dihydrostreptomycin, oxytetracycline, penicillin G and carbomycin. Finally, in certain *in vitro* tests, Hungate et al., *J. Anim. Sci.* 14, 997 (1955) found that the presence of high levels of chlortetracycline reduced the total fermentation products with a typical rumen flora.

In addition to the above studies, it is well recognized that there is frequently a period of anorexia, with or without diarrhea, in the initial stages of the oral antibiotic treatment of mature ruminants. These undesirable side

effects, have also been found when the macrolide antibiotics, including tylosin, are administered to ruminants.

It is apparent from the above considerations that an improved method of administering antibiotics to ruminants via the oral route would be most desirable.

The antibiotic-urea adducts described herein contain two molecules of a urea for each molecule of aldehyde-containing macrolide antibiotic. Although referred to herein as adducts, it should be understood that these adducts are definite compounds and that a reaction takes place between the aldehyde group of the antibiotic and two molecules of a urea as evidenced by the disappearance of the characteristic aldehyde signal in the nuclear magnetic resonance spectrum of the adduct. Ureas useful in preparing our novel adducts in which two moles of the urea combine with each mole of macrolide antibiotic containing an aldehyde group, are represented by the following formula:



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or

$\text{N} \begin{smallmatrix} \text{R}' \\ \text{R}'' \end{smallmatrix}$ wherein R' and R'' are independently

hydrogen, C₁—C₄ alkyl or C₂—C₄ alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4. Groups which R can represent include *o*-tolyl, *m*-tolyl, *p*-tolyl, *o*-chlorophenyl, *m*-bromophenyl, or *p*-fluorophenyl. Groups which R' and R'' can represent include methyl, ethyl, allyl, *n*-propyl, iso-propyl, or *n*-butyl. Ureas useful in preparing the adducts of this invention include N-methylurea, N,N-diethylurea, N-*n*-butylurea, N-allylurea, N-crotylurea, N-methyl-N-allylurea, N-phenylurea, N-(*p*-chlorophenyl)urea, N-(*o*-tolyl)urea, and N-(*p*-trifluoromethylphenyl)urea.

Among the macrolide antibiotics containing an aldehyde group which react with two moles of a urea according to Formula I above are included tylosin, macrocin, desmycosin, lactenocin, carbomycin, carbomycin B, middamycin (desacetyl carbomycin B), spiramycins A, B, C and D, and members of the leucomycin series, leucomycin A₁—A₄.

The bis-urea adducts of this invention are prepared by dissolving the macrolide antibiotic in a saturated aqueous or alcoholic solution of urea. A thick syrup results which is allowed to remain at ambient temperature until analysis, preferably by thin layer chromatography, indicates that substantially all of the antibiotic product has been transformed into a bis-urea adduct. When the analytical procedure indicates that the formation of the

adduct is substantially complete, the product is isolated by diluting the reaction mixture with 1—2 volumes of water, adjusting the pH of the aqueous mixture to about 9.0, and then saturating the mixture with sodium chloride. The resulting precipitate is separated by filtration or by other conventional means and is purified by dissolution in an organic solvent, usually chloroform, washing the organic layer with water, separating the organic layer and removing the solvents therefrom at reduced pressure. The resulting solid amorphous residue can then be crystallized from an anhydrous organic solvent to yield the desired adduct as a white crystalline solid.

The following compositions are also provided by the present invention:—

(a) a veterinary composition which comprises a carrier and as active ingredient an adduct of an aldehyde-containing macrolide antibiotic and a urea of the present invention;

(b) an animal feed composition which comprises an animal feed and an adduct of an aldehyde-containing macrolide and a urea of the present invention;

(c) a pharmaceutical composition which comprises a carrier and as active ingredient dihydro (ketone-reduced) tylosin and/or dihydro (ketone-reduced) desmycin;

(d) a disinfectant composition which comprises a carrier and an active ingredient dihydro (ketone-reduced) tylosin and/or dihydro (ketone-reduced) desmycin.

The antibiotic-urea adducts described herein provide a method for administering an aldehyde-containing macrolide antibiotic to ruminants by the oral route in which the antibiotic is administered in the form of a bis-urea adduct which is substantially stable to chemical degradation in the rumen and also antibiotically inactive, but which, upon passing into the abomasum, is converted into an antibiotically-active substance, whereby said antibiotic is able to achieve its full therapeutic or growth-promoting effect without disturbing the microflora of the rumen.

The advantages of this invention are exemplified by the use of tylosin-bis-urea adduct, in treating ruminants for infections caused by gram positive organisms, or CRD-related diseases, or for increasing weight gains or feed efficiency or both. The inventive bis-urea adducts of the macrolide antibiotics are virtually inactive antibiotically and do not affect the growth or metabolism of important rumen microflora. In addition, they are chemically stable, being resistant to hydrolysis at the substantially neutral pH of the rumen. However, when these bis-urea adducts pass into the acidic abomasum, they are chemically unstable at pH's encountered there and are rapidly hydrolyzed to yield the original antibiotic, or in some cases, a mixture of the original antibiotic plus an antibiotic-

ally-active, acid degradation product of the original antibiotic. In other words, our invention makes the oral administration of macrolide antibiotics to ruminants similar to the oral administration of antibiotics to non-ruminant animals, in which latter case, the active antibiotic immediately passes into the stomach which has an acidic pH and from which it can be absorbed, either as such or as an antibiotic acid degradation product.

Thus, a macrolide antibiotic can be administered to a ruminant by the oral route without disturbing the microflora of the rumen and consequently without causing intestinal upset, anorexia or diarrhea, since it is probably the destruction of the microfloral balance in the rumen which leads to these various undesirable side effects accompanying antibiotic administration to ruminants, since the particular microflora which are disturbed invariably include the cellulose digesting organisms. By our invention, the full therapeutic and/or growth-promoting or feed efficiency effects of the macrolide antibiotic are achieved without any of the commonly occurring undesirable side effects.

Specifically, when the bis-urea adduct of tylosin is administered to cows at the rate of 250 mg./cow/day, there is little, if any, initial feed intake depression, such as occurs when tylosin alone is administered, nor is there any appreciable effect upon cellulose digestion nor upon non-protein nitrogen utilization. Furthermore, there is neither diarrhea nor loss of appetite.

In carrying out the process of this invention, using tylosin as an example of the antibiotic, tylosin-bis-urea adduct is fed to cows, sheep or goats at the rate of from 1 to 1000 mg./animal/day depending on whether a rapid therapeutic effect is desired or whether the antibiotic is simply being fed to the animal either to increase weight gains and general health or to increase feed efficiency or both as a part of an over-all feeding program. The ruminant animals to whom these levels of tylosin-bis-urea adduct are fed exhibit all the favorable results to be expected from administration of the antibiotic but without any of the side effects ordinarily associated with the administration of tylosin by itself.

In administering tylosin-bis-urea adduct or other macrolide bis-urea adducts to ruminant animals, the compounds are mixed with the feed as such. They can also be coated with gelatin or other similar material and then mixed with the feed. Finally, they can be administered in bolus form as is customary with administration of therapeutic doses of antibiotic to beef animals.

The following table indicates the lack of effect of tylosin-bis-urea adduct, an antibiotic urea adduct of the present invention upon the cellulose digestive organisms in the rumen. In this demonstration, a rumen inoculum was pre-

(12)

- 5 pared from rumen liquor collected from a fistulated steer maintained on a high roughage ration. In preparing this inoculum, the rumen contents were strained through 4 layers of cheesecloth; the residue remaining in the cheesecloth was re-suspended in an equal volume of phosphate-carbonate buffer at 37°C., and was again strained through the cheesecloth. The resulting rumen liquor was
- 10 which time the protozoa settled to the bottom while small amounts of particulate matter rose to the surface. The protozoa and liquid phase were separated and used as the rumen inoculum. Prior to use, the inoculum was diluted with an equal volume of a phosphate-bicarbonate buffer previously saturated with carbon dioxide having the following ingredients:
- 15

Composition of Nutrient Buffer Mixture

Ingredient	Gm./Liter
NaH ₂ PO ₄	.316
KH ₂ PO ₄	.152
NaHCO ₃	2.260
KCl	.375
NaCl	.375
MgSO ₄	.112
CuCl ₂	.038
FeSO ₄	.008
MnSO ₄	.004
ZnSO ₄	.004
CuSO ₄	.002
CuCl ₂	.001

- 20 Cellulose at the rate of 5 g./liter and urea at the rate of 1 g./liter were added to the diluted inoculum. The mixture was incubated under a carbon dioxide atmosphere for 30 minutes. Twenty-five milliliter aliquots were
- 25 then removed and placed in small incubation flasks equipped with one-way gas valves to allow for excess gas escape and for maintenance of anaerobic conditions. Various antibiotic materials at various levels were added to the incubation flask, and the incubation
- 30 carried out for 24 hours. At the end of the incubation, the pH was recorded. Five milliliters of 20 percent metaphosphoric acid were then added to each flask. The contents of the flasks were frozen, then thawed and centrifuged. A sample of the supernatant was recovered to determine short chain volatile fatty acids by gas chromatography. The
- 40 remaining contents of the flask were mixed with 20 ml. of 80 percent acetic acid and 2 ml. of nitric acid, and the mixture heated at 130°C. for 30 minutes in order to digest all material except cellulose. After the heating period, 20 ml. of ethyl alcohol were added to the contents of each flask and the insoluble material, predominantly cellulose, centrifuged.
- 45 Tylosin, chlortetracycline and oxytetracycline were added to the flasks at a concentration of 1 mg./ml Tylosin-bis-urea adduct was added to the flask at a level which would provide 1 microgram of tylosin/ml. after hydrolysis of the adduct. One flask was kept as a control. Table 1 below gives the results of this experiment. In the table, column 1 gives the treatment and column 2 the average percent cellulose digested (3 incubations).
- 50
- 55

TABLE 1

Treatment	Average Percent Cellulose Digested
Tylosin-bis-urea adduct	69.6
Tylosin	30.3
Chlortetracycline	12.9
Oxytetracycline	16.8
Control	76.3

Table 2 gives the effect of the same antibiotics upon volatile fatty acid production. In Table 2, column 1 gives the treatment and

column 2 the average number of micro-moles/ml. of volatile fatty acids. 5

TABLE 2

Treatment	Average Volatile Fatty Acids Produced, μ M/ml.
Tylosin-bis-urea adduct	27.0
Tylosin	8.6
Chlortetracycline	3.4
Oxytetracycline	2.0
Control	29.2

10 It can be seen from the results of the above experiments that tylosin-bis-urea adduct has little or no effect upon the percent cellulose digested or upon the volatile fatty acid production by rumen flora, whereas the other antibiotics, including tylosin alone, have a deleterious effect upon both of these rumen activities. 15

Tylosin-bis-urea adduct has also proved to be very effective in actual use in the field. The

results listed in Table 3 were obtained in a 28-day feeding experiment with yearling steers on a high roughage ration. In the table, column 1 gives the number of steers on each feeding regimen, column 2 daily antibiotic level, if any, per steer, column 3 pounds per steer per day, column 4 pounds of feed per steer per day and column 5 pounds of feed per pound of gain. 20 25

TABLE 3

No. Steers in Each Group	Daily Antibiotic Level in Feed	Pounds Gained Per Steer Per Day	Pounds of Feed per Steer Per Day	Pounds of Feed Pounds of Gain
11	None	1.33	20.7	15.5
11	Tylosin, 250 mg.	1.38	21.1	15.3
11	Tylosin-bis-urea adduct containing 250 mg. tylosin activity	1.50	21.2	14.1

As can be seen from the data in the above table, steers receiving tylosin-bis-urea adduct gained more weight per day than either steers receiving an equivalent amount of tylosin or a control group and that the feed efficiency (feed per pounds gained) was also increased. Furthermore, the steers receiving tylosin-bis-urea adduct did not exhibit any of the undesirable side effects discussed above.

Tylosin-bis-urea adduct can be used to treat ruminant animals suffering from various common pathogenic conditions including the following:

abscess of the liver, caused by *Sphaerophorus necrophorus*;

blackleg, caused by *Clostridium chauvoei*;

enterotoxemia, caused by *Clostridium perfringens*;

malignant edema, caused by *Clostridium septicum*;

tetanus, caused by *Clostridium tetani*; and

pneumonia, probably caused by a complex of virus, mycoplasma and bacteria.

Dosages of tylosin-bis-urea adduct up to 250 mg. per head per day of prophylactic as to all of the above organisms, and in addition will prevent pneumonia. Higher dosages, up to 2000 mg. per animal per day, may be required to treat pathological disease.

In addition, tylosin-bis-urea adduct can be used to eliminate coccidia from cattle and sheep. These coccidia are parasites which occupy epithelial cells of the intestinal tract in which they first multiply asexually and then undergo sexual reproduction within the host cells. Again, dosages up to 2000 mg. per animal per day may be required to eliminate all signs of the organism and its debilitating effect upon the animal. The most pathogenic coccidia for cattle are *Eimeria bovis* and *E. zurnii*, although *E. cylindrica*, *E. ellipsoidalis*, *E. bukidonensis*, *E. auburnensis* and *E. albaniensis* may also be pathogenic but to a lesser degree. In sheep the most pathogenic organisms are *Eimeria ninakohlyakimovae*, *E. arloingi* and *E. ahsata*, although *E. faurei*, *E. intricata*, and *E. parva* may also be pathogenic but to a lesser degree.

Bis-urea adduct of other macrolide antibiotic containing an aldehyde group including spiramycin A, macrocin, desmycosin, lactenocin, carbomycin, carmycin B, niddamycin, other members of the spiramycin series and

members of the leucomycin series, can be prepared in analogous fashion. These adducts can be administered orally to ruminants for therapeutic purposes or to increase weight gain and/or feed efficiency in a standard ruminant fattening program in the same way that tylosin-bis-urea adduct is. These other bis-urea adducts are also substantially stable at the rumen pH and antibiotically inactive, but on passing into the abomasum, they are hydrolyzed to the original antibiotic which then can act as such. As with tylosin-bis-urea adduct, there are minimal side-effects accompanying their administration. The dosage range is the same as that for tylosin-bis-urea adducts.

The preparations of bis-urea adducts of specific macrolide antibiotics are illustrated below.

EXAMPLE 1.

Tylosin-Bis-urea Adduct.

One hundred grams of tylosin tartrate are dissolved in a solution containing 100 g. of urea in 100 ml. of water. The resulting mixture is allowed to stand until thin layer chromatography indicates that the formation of the adduct is substantially complete. In carrying out the thin layer chromatography, silica gel is used as the substrate with a 1:9 diethylamine-ethyl acetate solvent system. In this system, the tylosin-bis-urea adduct remains at the origin while tylosin moves closely behind the solvent front.

The tylosin-bis-urea adduct is isolated from the reaction mixture by adding two volumes of water thereto and then adjusting the pH of the solution to about pH=9 with 10 percent aqueous sodium hydroxide. Solid sodium chloride is next added until the crude tylosin-bis-urea adduct precipitates as a yellowish, gummy solid, which solid is separated by filtration. The filter cake is dissolved in chloroform and the chloroform solution washed with water and dried. The chloroform is removed therefrom at reduced pressure to yield tylosin-bis-urea adduct as a pale yellow amorphous solid which is thoroughly dried at reduced pressure to remove as much residual chloroform as possible. The dried solid is then dissolved in dry acetone at the rate of 5 ml. per gram of solid and the resulting solution is cooled. Tylosin-bis-urea adduct crystallizes as white needles which are separated by filtration. MP=220—222°C.

Analysis, Calc. for $C_{47}H_{88}N_5O_{18}$: C, 56.22; H, 8.39; N, 6.96
Found: C, 56.05; H, 8.32; N, 6.72

A nuclear magnetic resonance spectrum of the adduct shows no proton attributable to the aldehyde group. The adduct is microbiologically inactive against organisms which are routinely used to assay tylosin.

Tylosin is chemically regenerated from its bis-urea adduct by dissolving the adduct in

aqueous solution maintained at a pH in the range 4—7 by means of standard buffers. Some desmycosin, an acidic hydrolysis product of tylosin, is also obtained.

Tylosin - bis - (N - methylurea) adduct is prepared by substituting N-methylurea for urea in the above procedure.

Other ureas represented by Formula I above can be used in place of urea or N-methylurea in the above example to form bis-urea adducts with macrolide antibiotics. Among such ureas which form a bis-urea adduct are allylurea, N,N-diethylurea, ethylurea, and N-methyl-N-n-propylurea. These ureas are all soluble in water and the above procedure can be used to prepare the bis-urea adduct without modification. With other ureas, such as N-(*m*-tolyl)urea, N-phenylurea, N-(*t*-butyl)urea, and N-(*p*-trifluoromethylphenyl)urea, alcoholic solutions of the urea and of the tylosin salt are used instead of aqueous solutions.

EXAMPLE 2.

Carbomycin-Bis-urea Adduct.

Two grams of carbomycin are dissolved in 5.0 ml. of saturated aqueous urea solution maintained at a pH in the range 5.0–5.5 by the addition of 10 percent aqueous hydrochloric acid when needed. After standing at room temperature for 12 days, the solution is diluted with 4 volumes of water. The aqueous solution is extracted with 40 ml. portions of chloroform at the following pH's: 5.0, 5.5, 6.0, 6.5 and 7.0. The pH of the solution is adjusted as desired prior to each extraction by the addition of 10 percent aqueous sodium hydroxide. The urea adduct is present in each of these extracts, but only the extracts taken at pH=6.5 and 7.0 are substantially free from carbomycin. The extracts at these higher pH's are combined and carbomycin-bis-urea adduct isolated and purified by the method employed for tylosin-bis-urea adduct in Example 1. 0.5 G. of a white solid is obtained having a typical carbomycin spectrum in the ultraviolet ($\epsilon_{240m\mu}^{max} = 13,000$). The NMR spectrum of the adduct lacks the aldehyde proton signal. Bioassay of the bis-urea adduct shows it to be inactive against organisms employed in a standard carbomycin assay procedure.

Carbomycin is recovered from carbomycin-bis-urea adduct by autoclaving the adduct at 120°C. for 20 minutes at pH=5.

EXAMPLE 3.

Spiramycin-Bis-urea Adduct.

Two grams of spiramycin A are dissolved in 50 ml. of saturated aqueous urea solution which is maintained in the range pH 4.5–5.0 by addition of 10 percent aqueous hydrochloric acid as needed. After standing at room temperature for 12 days, the solution is diluted with 4 volumes of water and subjected to a gradient pH extraction procedure as in Example 2, except that the extracts are obtained at pH=6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Forty milliliters of chloroform are employed for each extraction. The extracts at pH=8.0, 8.5 and 9.0 are combined to give 1.0 gram of spiramycin-bis-urea adduct as a

white solid. The ultraviolet spectrum of the adduct is substantially the same as that of unreacted spiramycin A ($\epsilon_{230m\mu}^{max} = 23,000$).

The NMR spectrum is comparable to that of spiramycin A, except for the disappearance of the aldehyde proton signal.

Spiramycin-bis-urea adduct is converted back to spiramycin by dissolving 20 mg. of the adduct in 1 ml. of ethanol, adding 4 ml. of pH=5.5 buffer, and autoclaving the mixture for 20 minutes at 120°C.

Bis-urea adducts of other macrolide antibiotics containing an aldehyde group including macrocin, desmycosin, lactenocin, carbomycin, carbomycin B, niddamycin, other members of the spiramycin series and members of the leucomycin series, can be prepared in analogous fashion.

The compounds of this invention are useful as intermediates in the preparation of derivatives of the various macrolide antibiotics listed above. For example, reaction of the tylosin aldehyde group with urea to form a bis-urea adduct which is stable to alkali makes possible the reduction of the tylosin ketone group with sodium borohydride to produce, after acid hydrolysis of the two urea molecules, a novel dihydrodesmycosin or novel dihydrotylosin, depending upon the pH and time of the acid hydrolysis step. The above reduction cannot, of course, be carried out on tylosin or desmycosin since the aldehyde group present in these two antibiotics will reduce preferentially over the ketone group with sodium borohydride. Dihydro(ketone-reduced)desmycosin thus prepared have antibiotic spectra which are comparable to those of tylosin and desmycosin. These new antibiotic substances are used in the same way as tylosin and desmycosin in controlling growth of microorganisms such as *S. aureus*, *Sarcina lutea* and *B. subtilis*. Thus, for example, solutions of dihydro(ketone-reduced)-tylosin and dihydro(ketone-reduced)desmycosin can be used to disinfect hospital areas, laboratory glassware, etc.

EXAMPLE 4.

Bis-urea Adduct of Dihydro(ketone-reduced)Tylosin.

Seventeen grams of tylosin-bis-urea adduct and 650 mg. of sodium borohydride are dissolved in 450 ml. of 95 percent ethanol. The reaction mixture is allowed to stand overnight and is then diluted with 400 ml. of water. The ethanol is removed by evaporation *in vacuo* and the resulting aqueous solution is extracted with 100 ml. of chloroform. The chloroform layer is separated and dried, and the chloroform evaporated therefrom *in vacuo* to yield an amorphous, white solid as a residue. This white solid gives a single spot on thin layer chromatography. Recrystallization of the white solid from acetone yields crystalline dihydro(ketone-reduced)tylosin-bis-urea adduct.

EXAMPLE 5.

Dihydro(ketone-reduced)Desmycosin.

- 9.1 Grams of dihydro(ketone-reduced) tylosin-bis-urea adduct are dissolved in 300 ml. of water. The pH of the solution is adjusted to about 2.8 by the addition of 10 percent aqueous hydrochloric acid. The hydrolysis mixture is allowed to stand for 4 days at room temperature. Extraction of the aqueous reaction mixture with methylene dichloride is then carried out at the following pH's: 6.0, 6.5 7.0, 8.0 and 9.0. The pH of the solution is adjusted upward for each ex-

traction by the addition of 10 percent aqueous sodium hydroxide. Extracts at pH=7.0 and pH=8.0 are combined and show only a single spot on thin layer chromatography. Evaporation of the combined extracts yields 5.2 g. of dihydro(ketone-reduced)desmycosin as an amorphous white solid. Dissolution of the solid in the minimal amount of hot chloroform followed by slow cooling of the solution yields 2.2 g. of crystalline dihydro(ketone-reduced)desmycosin, decomposing in the range 70—80°C.

Analysis, Calc. for $C_{88}H_{56}NO_{14}$: C, 60.05; H, 8.62; N, 1.84
Found: C, 59.76; H, 8.48; N, 1.86

- Dihydro(ketone-reduced)desmycosin can also be prepared from dihydro(ketone-reduced) tylosin-bis-urea adduct by hydrolysis at pH=3.6 at 100°C. for 1.5 hours. Extraction of the hydrolysis mixture with methylene dichloride yields the desired compound directly.

- Dihydro(ketone-reduced)desmycosin inhibits the growth of *S. aureus*, *Sarcina lutea* and *B. subtilis* when present in the culture medium at a concentration of 10 micrograms per milliliter.

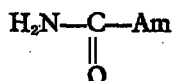
EXAMPLE 6.

Dihydro(ketone-reduced)Tylosin.

- Ten grams of dihydro(ketone-reduced) tylosin-bis-urea adduct are dissolved in a mineral amount of warm ethanol, and the ethanol solution diluted with 450 ml. of a pH=4.8 phosphate-citrate buffer. The pH of the solution is adjusted to 5.0 by the addition of citric acid. The solution is heated to about 90°C. for 30 minutes. Extraction of the reaction mixture with methylene dichloride at a pH in the range 5.0—5.5 (the pH being adjusted as needed with dilute sodium hydroxide) yields 5.7 g. of dihydro(ketone-reduced)tylosin as a white amorphous solid. The solid gives one spot on thin layer chromatography, and the spot moves at a different rate than that of tylosin in the same system. Dihydro(ketone-reduced)tylosin inhibits the growth of *S. aureus*, *Sarcina lutea* and *B. subtilis* when present in the culture medium at a concentration of 10 micrograms per milliliter.

WHAT WE CLAIM IS:—

1. An adduct of an aldehyde-containing macrolide antibiotic and urea, said adduct containing 2 moles of a urea per mole of antibiotic wherein said 2 moles of urea react with the aldehyde group of said antibiotic, said urea being represented by the formula:



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or R'

N< where R' and R'' are independently R''

hydrogen, C_1-C_4 alkyl or C_3-C_4 alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4.

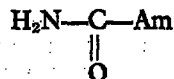
2. A bis-urea adduct of a macrolide antibiotic according to claim 1 wherein said macrolide antibiotic is tylosin, macrocin, desmycosin, lactenocin, carbomycin, carbomycin B, niddamycin, one of the spiramycine A, B, C, or D or leucomycin A₁₋₈.

3. The bis-urea of tylosin.

4. Dihydro (ketone-reduced) tylosin.

5. Dihydro (ketone-reduced) desmycosin.

6. A process for preparing an adduct of an aldehyde-containing macrolide antibiotic and a urea which comprises mixing the urea and the antibiotic in an aqueous or alcoholic solvent in amounts to provide at least two mols of the urea per mol of the antibiotic, said urea being represented by the formula



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or R'

N< where R' and R'' are independently R''

hydrogen, C_1-C_4 alkyl or C_3-C_4 alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4.

7. The process of claim 6 which comprises dissolving the macrolide antibiotic in a saturated aqueous or alcoholic solution of urea, holding the saturated solution at ambient temperature until reaction is complete, diluting the reaction mixture with water, adjusting the pH of the mixture to about 9, and precipitating, separating and purifying the product.

EXAMPLE 5.

Dihydro(ketone-reduced)Desmycosin.

- 9.1 Grams of dihydro(ketone-reduced) tylosin-bis-urea adduct are dissolved in 300 ml. of water. The pH of the solution is adjusted to about 2.8 by the addition of 10 percent aqueous hydrochloric acid. The hydrolysis mixture is allowed to stand for 4 days at room temperature. Extraction of the aqueous reaction mixture with methylene dichloride is then carried out at the following pH's: 6.0, 6.5 7.0, 8.0 and 9.0. The pH of the solution is adjusted upward for each ex-

traction by the addition of 10 percent aqueous sodium hydroxide. Extracts at pH=7.0 and pH=8.0 are combined and show only a single spot on thin layer chromatography. Evaporation of the combined extracts yields 5.2 g. of dihydro(ketone-reduced)desmycosin as an amorphous white solid. Dissolution of the solid in the minimal amount of hot chloroform followed by slow cooling of the solution yields 2.2 g. of crystalline dihydro(ketone-reduced)desmycosin, decomposing in the range 70—80°C.

Analysis, Calc. for $C_{33}H_{56}NO_{14}$: C, 60.05; H, 8.62; N, 1.84
Found: C, 59.76; H, 8.48; N, 1.86

- Dihydro(ketone-reduced)desmycosin can also be prepared from dihydro(ketone-reduced)-tylosin-bis-urea adduct by hydrolysis at pH=3.6 at 100°C. for 1.5 hours. Extraction of the hydrolysis mixture with methylene dichloride yields the desired compound directly.

- Dihydro(ketone-reduced)desmycosin inhibits the growth of *S. aureus*, *Sarcina lutea* and *B. subtilis* when present in the culture medium at a concentration of 10 micrograms per milliliter.

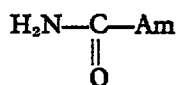
EXAMPLE 6.

- Dihydro(ketone-reduced)Tylosin.

- Ten grams of dihydro(ketone-reduced)-tylosin-bis-urea adduct are dissolved in a mineral amount of warm ethanol, and the ethanol solution diluted with 450 ml. of a pH=4.8 phosphate-citrate buffer. The pH of the solution is adjusted to 5.0 by the addition of citric acid. The solution is heated to about 90°C. for 30 minutes. Extraction of the reaction mixture with methylene dichloride at a pH in the range 5.0—5.5 (the pH being adjusted as needed with dilute sodium hydroxide) yields 5.7 g. of dihydro(ketone-reduced)tylosin as a white amorphous solid. The solid gives one spot on thin layer chromatography, and the spot moves at a different rate than that of tylosin in the same system. Dihydro(ketone-reduced)tylosin inhibits the growth of *S. aureus*, *Sarcina lutea* and *B. subtilis* when present in the culture medium at a concentration of 10 micrograms per milliliter.

WHAT WE CLAIM IS:—

1. An adduct of an aldehyde-containing macrolide antibiotic and urea, said adduct containing 2 moles of a urea per mole of antibiotic wherein said 2 moles of urea react with the aldehyde group of said antibiotic, said urea being represented by the formula:



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or R'

N<' wherein R' and R'' are independently R''

hydrogen, C_1 — C_4 alkyl or C_2 — C_4 alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4.

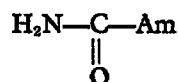
2. A bis-urea adduct of a macrolide antibiotic according to claim 1 wherein said macrolide antibiotic is tylosin, macrocin, desmycosin, lactenocin, carbomycin, carbomycin B, niddamycin, one of the spiramycine A, B, C, or D or leucomycin A₁₋₈.

3. The bis-urea of tylosin.

4. Dihydro (ketone-reduced) tylosin.

5. Dihydro (ketone-reduced) desmycosin.

6. A process for preparing an adduct of an aldehyde-containing macrolide antibiotic and a urea which comprises mixing the urea and the antibiotic in an aqueous or alcoholic solvent in amounts to provide at least two mols of the urea per mol of the antibiotic, said urea being represented by the formula



wherein Am is —NH—R wherein R is phenyl, tolyl, halophenyl or trifluoromethylphenyl; or R'

N<' wherein R' and R'' are independently R''

hydrogen, C_1 — C_4 alkyl or C_2 — C_4 alkenyl, the sum of the carbon atoms in R' and R'' not to exceed 4.

7. The process of claim 6 which comprises dissolving the macrolide antibiotic in a saturated aqueous or alcoholic solution of urea, holding the saturated solution at ambient temperature until reaction is complete, diluting the reaction mixture with water, adjusting the pH of the mixture to about 9, and precipitating, separating and purifying the product.



8. The process of claim 7 wherein the macrolide antibiotic is in the form of a salt.
9. The process of claims 6, 7 or 8 wherein the macrolide antibiotic is tylosin, macrocin, desmycosin, lactenocin, carbomycin, carbomycin B, niddamycin, one of the apiramycins, A, B, C or D, or leucomycin A₁₋₃.
10. The process of claim 9 wherein the macrolide antibiotic is tylosin.
11. The process of claim 9 wherein the macrolide antibiotic is desmycosin.
12. A process for preparing a urea adduct of dihydro (ketone-reduced) tylosin or dihydro (ketone-reduced) desmycosin which comprises reducing with sodium borohydride the ketone group of the antibiotic moiety of the urea adduct of tylosin or desmycosin produced by the process of claims 6, 7, or 8.
13. A process for preparing dihydro (ketone-reduced) tylosin or dihydro (ketone-reduced) desmycosin which comprises hydrolyzing in an acid medium a urea adduct of dihydro (ketone-reduced) tylosin or dihydro (ketone-reduced) desmycosin produced by the process of claim 12.
14. A veterinary composition which comprises a carrier and as active ingredient an adduct of an aldehyde-containing macrolide antibiotic and a urea as defined in claims 1, 2 or 3.
15. An animal feed composition which comprises an animal feed and an adduct of an aldehyde-containing macrolide antibiotic and a urea as defined in claims 1, 2 or 3.
16. A pharmaceutical composition which comprises a carrier and as active ingredient dihydro (ketone-reduced) tylosin and/or dihydro (ketone-reduced) desmycosin.
17. A disinfectant composition which comprises a carrier and as active ingredient dihydro (ketone-reduced) tylosin and/or dihydro (ketone-reduced) desmycosin.
18. A method of administering an aldehyde-containing macrolide antibiotic substance to ruminants disturbing the rumen microflora therein, while obtaining full antibiotic effects thereof, which comprises orally administering said antibiotic in the form of a bis-urea adduct defined in claims 1, 2 and 3 which is substantially chemically stable and antibiotically inactive in the rumen, but which adduct on passing from the rumen into the abomasum is reconverted to an antibiotically active substance, capable of exerting its full antibiotic effect.
19. The method of claim 18, wherein the rumen-stable, antibiotically-active substance is tylosin bis-urea adduct.
20. An adduct of an aldehyde-containing macrolide antibiotic and urea substantially as hereinbefore described with particular reference to any of Examples 1 to 4.
21. A process for preparing an adduct of of aldehyde-containing macrolide antibiotic and a urea substantially as hereinbefore described with particular reference to any of the Examples 1 to 4.
22. A process for preparing dihydro (ketone-reduced) tylosin or dihydro (ketone-reduced) desmycosin substantially as hereinbefore described with particular reference to Examples 5 or 6.

BOULT, WADE & TENNANT,
111 & 112, Hatton Garden, London, E.C.1,
Chartered Patent Agents,
Agents for the Applicants.

