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PROCESS FOR PREPARING HIGHER FATTY ACID SALTS OF NEOMYCIN

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This invention relates to compositions of matter known in the art of chemistry as organic salts of neomycin and more particularly to processes for making such compositions.

The invention here is described as residing in the concept of dissolving approximately equivalent quantities of neomycin base with the appropriate higher fatty acid in a lower alkanol solvent and at a final pH between 6.5–7.5 to form a chemical compound in which neomycin is associated with a higher fatty acid in the form of a true salt.

As used herein the term "higher fatty acid" means an aliphatic monocarboxylic acid containing 8 to 18 carbon atoms. The aliphatic portion of the acid may be straight chain or branched and saturated or unsaturated. The better known higher fatty acids are straight chain, saturated compounds containing 8 to 18 carbon atoms; the acids particularly useful in the practice of my invention can also be recognized as those whose alkali metal salts are generally known as soaps. My invention, however, is not so limited and contemplates all aliphatic fatty acids having 8 to 18 carbon atoms and which produce water-insoluble salts of neomycin. And as used herein the term "lower alkanol" means saturated alcohols having less than 3 carbon atoms, i.e., methyl and ethyl alcohols.

Neomycin, also referred to as neomycin base, is a naturally occurring antibiotic elaborated by a soil organism of the Streptomyces genus when cultured on the appropriate nutrient media. The neomycin thus produced is a basic, water-soluble compound active against many gram positive and gram negative micro-organisms. The antibiotic is often isolated and incorporated into pharmaceutical preparations in the form of its mineral acid salts, most commonly neomycin sulfate and chloride. Because of the very high water solubility of neomycin mineral acid salts (one gram of neomycin sulfate dissolves in one milliliter of water), these salts are readily leached from preparations containing them. Hence, the beneficial effects of neomycin are not fully realized in such preparations when topically applied.

The higher fatty acid salts of neomycin, unlike neomycin base or neomycin mineral acid salts, are wax-like and practically insoluble in water and thus may be more effectively utilized in ointments, lotions, creams, soap bases and other preparations designed for topical use. Replacement of the mineral acid anion by a higher fatty acid anion does not materially detract from the germicidal properties of the neomycin moiety. When neomycin sulfate and neomycin palmitate were compared in their anti-fungal and anti-bacterial properties against 34 representative micro-organisms including *C. diphtheria, S. typhosa, St. faecolis, Candida albicans, Trichophytin tonsurans* and *Rizopus nigricans,* the fatty acid salt was at least as effective as the sulfate; and in the case of 23 of the organisms tested, even smaller concentrations of the palmitate were sufficient to produce equivalent inhibition in the growth of the test bacteria and fungi.

Neomycin fatty acid salts have the added advantage of lessened toxicity per unit weight. Pharmacological studies of the acute intraperitoneal toxicity of neomycin palmitate showed that the LD_{50} at 48 hours after administration was 789 mg. kg. This is more than twice the LD_{50} for neomycin sulfate under the same conditions. In addition, the fatty acid salts are non-irritating. Animal mucous membrane irritation measured on rabbit's eyes and primary skin irritation measured on a modified Draize test on abraded and unabraded rabbit skin showed that neomycin palmitate passed the eye test and gave no apparent skin irritation. Thus the higher fatty acid salts of neomycin can be safely and effectively utilized in topical preparations for the treatment of dermatological conditions caused by neomycin susceptible organisms. These salts can also be incorporated into compositions such as hand creams which are used to obtain substantially antiseptic conditions. In many cases, the salts have the added advantage of producing their own emolient or demulcent effect.

The mere mixing of higher fatty acids with neomycin base in aqueous solution has not proven to be a satisfactory method for the preparation of fatty acid salts of neomycin. The reaction is hampered by the poor solubility of both the reactant fatty acid and the resultant fatty acid salt. Particles of the poorly soluble acid became coated with the even more insoluble salt giving a system from which it is extremely difficult to isolate the low yields of product obtained. Such a process is grossly unsuited for large scale production. If it be desired to operate in an aqueous system, one must utilize the reaction of a mineral acid salt of neomycin with an alkali metal salt of the reactant fatty acid. This approach is not completely satisfactory because of the fact that one of the reactants is a soap and the product is soap-like and, hence difficult to handle, isolate cleanly or purify. Drying may also be a problem. Inorganic salts such as sodium sulfate, potassium chloride and potassium sulfate are obtained as by-products and may contaminate the product.

I have discovered that neomycin reacts in alcoholic solution and under carefully controlled conditions with the appropriate higher fatty acid to give excellent yields of the desired neomycin fatty acid salt. As will be discussed below, the pH and the ratio of reactants used must be carefully controlled in order to obtain good yields of high grade product. Deviation from the optimum conditions will still give the fatty acid salt of neomycin but in a lessened degree of purity.

It is therefore, an object of the present invention to provide a convenient method for the preparation of higher fatty acid salts of neomycin free of inorganic and other contaminants.

The reaction of neomycin base with higher fatty acids is effected, according to my process, in a lower alkanol, preferably methanol or ethanol. Mixtures of those alcohols are also satisfactory. It is not necessary that the alcohol used be anhydrous; the usually available commercial grades of methyl or ethyl alcohol are most often employed. The order of mixing the reactants is not critical and all methods of mixing the reactants with each other and with the solvent are equivalent. Usually a solution of the acid is added to a solution of the neo-

mycin base but the reverse order may be employed. The fatty acid can be added directly to the alcoholic solution of the neomycin base. Temperature control is not required and the process is carried out at ambient temperatures. A certain amount of warming results when the reactants are mixed raising the temperature of the reaction mixture from room temperature to about 40–45 degree centigrade. Slight warming may be utilized to further solubility; this is especially the case when it is desired to clarify the solution with charcoal before recovery of the product.

In order to achieve consistently high yields of superior product, it is critical that the final pH of the reaction mixture and the ratio of reactants fall within the limits stated below. The final pH of the reaction mixture must be about 6.5 to 7.5. If the final pH is allowed to vary from the suggested range, either the fatty acid will precipitate from solution or certain other salts of neomycin will be found in conjunction with the desired neomycin fatty acid salt. While it is not an object of the present invention to prepare such compositions, they can be made by varying the general procedure of my process. However, the best way to obtain the proper final pH and pure product is to titrate the neomycin base in alcohol solution with the fatty acid dissolved in the same solvent or to add the calculated approximate stoichiometric amount of acid (directly or in solution) to a solution of the base.

A slight stoichiometric excess of acid is not harmful and in some cases may even be helpful; but the yield and quality of the product, as measured by its biological potency, fall off sharply at even slightly alkaline pH's in excess of 7.5. If necessary, the pH of the reaction mixture may also be adjusted by the addition of small amounts of mineral acid or inorganic base. Other variations will be obvious to one skilled in the art.

The higher fatty acid salts of neomycin have a desirable waxy consistency. The palmitate and higher salts are sufficiently insoluble in alcoholic solution so as to be capable of isolation directly by filtration, centrifugation, etc. Chilling, although not essential, may be utilized to diminish the solubility of the product. The presence of small amounts of water in the alcoholic solvent will accomplish the same result and may even be added for that purpose. The lower salts are too soluble to be isolated directly and are recovered by evaporation of the solvent, preferably by spray drying. This technique can be used successfully because of the absence of contaminating by-products; the presence of small amounts of water will not interfere. In certain situations, the presence of up to about 20 percent of water in the alcoholic solvent used will not be detrimental and can be tolerated.

The novel neomycin higher fatty acid salts of my invention have been shown by X-ray analysis to be true salts and not mere mixtures of neomycin with the reactant fatty acid.

The scope and utility of my invention is further illustrated by the following examples:

Example I

This example illustrates the preparation of neomycin palmitate from neomycin base and palmitic acid in alcohol solution. In a smaller scale run, 80 grams of palmitic acid was added to methanolic solution containing 20 grams of neomycin base. Sufficient methanol was added to produce a clear solution at 50–55 degrees centigrade. The final solution contained about 500 milliliters of methanol and had a pH of 6.8. After chilling to ice temperature, 95 grams of neomycin palmitate having a potency of 205 mcg./mg. was obtained on filtration.

In a larger scale run, a solution of 7–8 percent of neomycin base in 22–24 gallons of methyl alcohol was used as the starting material. This solution was obtained by the methanol extraction of crude neomycin. Palmitic acid, amounting to about three times the weight of neomycin base solids present, was added to the neomycin extract, and the temperature raised to effect complete solution (about 45–50 degrees centrigrade). The clear solution (pH of about 7) was cooled to ice temperature and the resultant solid collected by filtration. There was obtained about 20 kilograms of neomycin palmitate having a potency of about 200 mcg./mg. The product was an off-white powder soluble in methanol, sparingly soluble in ethanol, slightly soluble in propylene glycol and insoluble in glycerin or water.

Example II

This example illustrates the preparation of neomycin pelargonate from neomycin base and pelargonic acid. Using the general procedure of the first example, the weight ratio of reactants was about 2.1 parts of acid to 1 part of neomycin base. The final solution had a pH of about 7 also but the product was too soluble to recover by filtration. A quantitative yield of neomycin pelargonate was obtained by spray drying the reaction mixture. The product was an off-white powder soluble in methyl alcohol but insoluble in water.

Example III

This example illustrates the preparation of neomycin undecylenate from neomycin base and undecylenic acid. A solution was prepared containing 277 grams of neomycin base in 7740 milliliters of methanol. After the addition of about 550 grams of undecylenic acid, the solution had a final pH of 7.1. Spray drying gave 640 grams of neomycin undecylenate having a potency of 250 mcg./mg. The product was an off-white powder soluble in methyl alcohol, ethyl alcohol or propylene glycol and practically insoluble in glycerin or water.

Example IV

This example illustrates the preparation of neomycin caprylate from neomycin base and caprylic acid. Using the general procedure of Example III, 446 grams of caprylic acid was added to a solution of 323 grams of neomycin base in 9020 milliliters of methanol. The final reaction mixture (pH about 7.1) was spray dried to yield 610 grams of neomycin caprylate having a potency of 405 mcg./kg. The product was an off-white powder soluble in methyl alcohol and insoluble in water.

The germicidal properties of my novel neomycin fatty acid salts were illustrated by the comparison of a typical compound, neomycin palmitate, with neomycin sulfate. The general procedure is described as follows: Neomycin sulfate was dissolved in pH 8.0 buffer to a concentration of 1000 mcg./mg. and neomycin palmitate was dissolved in absolute ethanol to the same activity. Each solution was sterilized by filtration through sterile glass filters. The anti-bacterial sensitivity tests were performed using a two-fold serial dilution tube technique for all cultures except *Hemophilus influenza* and *pertussis* which were done by the plate dilution method. The final concentration of the antibiotics were adjusted so that the first tube in the dilution series contained 100 mcg./ml. and the first agar plate 50 mcg./ml. For the bacterial tube sensitivities, trypticase soy broth was used for *Strep. viridans* and *Cl. perfrigens* where thioglycollate broth was employed. The antifungal activity of the drugs were determined using Sabouraud's broth. Bordet-Gengou agar plus 15 percent rabbit blood was used for the plate sensitivities. All cultures were obtained from the American Type Culture Collection. The 24 hour broth cultures were diluted 1:1000 in broth prior to their addition to the serially diluted drugs but those cultures which grew profusely were diluted 1:10,000. All bacterial tube and plate sensitivities were incubated at 37 degrees centigrade for 18 hours while the antifungal sensitivities were incubated at 25 degrees centrigrade for 72 hours. The minimal inhibitory concentration of the test compounds were recorded as the lowest concentration in mcg./ml. necessary to inhibit growth.

Test Bacterial Organism		Minimal Inhibitory Concentration	
Species	Culture No.	Palmitate	Sulfate
A. aerogenes	100	1.56	3.12
C. diphtheriae	311	0.10	0.10
S. typhosa	401	3.12	12.5
E. coli	536	6.25	12.5
H. influenzae	554	25.0	6.25
H. pertussis	558	6.25	1.56
K. pneumoniae	602	1.56	3.12
Pr. vulgaris	720	25.0	6.25
Pr. aeruginosa	813	12.5	12.5
S. schott. (Para B)	910	6.25	25.0
Sh. paradysenteriae	959	25.0	12.5
M. pyogenes (albus)	1200	0.78	0.78
M. pyogenes (aureus)	1209	1.56	3.12
M. pyogenes (citreus)	1206	3.12	6.25
M. pyogenes (epidermidis)	1216	1.56	0.39
St. viridans	1300	3.12	100
St. hemolyticus	1307-3	0.78	6.25
St. Pyogenes	1307-4	0.20	1.56
St. faecalis	1341	50	100
D. pneumoniae	1900	1.56	12.5
Cl. perfringens	1054.3	3.12	250

Test Fungal Organism		Minimal Inhibitory Concentration	
Species	Culture No.	Palmitate	Sulfate
Candida albicans	M63	500	500
Cryptococcus neoformans	M260	500	500
Microsporum adouini	M80	15.6	125
Microsporum gypseum	M82	15.6	500
Sporotrichum schenckii	17161	62.5	500
Trichophyton mentagrophytes	17302	31.2	250
Trichophyton rubrum	17309	62.5	500
Trichophyton tonsurans	17304	500	500
Hormodendrum pedrosoi	17200	15.6	500
Allescheria boydii	M270	62.5	500
Microsporum canis	M83	31.2	500
Torulopsis utilis	M408	62.5	500
Rhizopus nigricans	M414	62.5	500

In summary, it can be said that the higher fatty acid salts of neomycin are white solids practically insoluble in water. They possess a waxy consistency and germicidal properties which render them useful in pharmaceutical compounding especially in preparations designed for topical application. These salts are conveniently prepared by the reaction of neomycin base and the appropriate fatty acid in lower alkanol solution. In addition to giving high yields of good quality product, the process has the added advantage of being able to utilize alcoholic extracts of neomycin base directly as obtained from Streptomyces fermentation beers.

Having described my invention, I claim:

1. The process for preparing higher fatty acid salts of neomycin which comprises: dissolving approximately stoichiometrically equivalent quantities of neomycin base and a higher fatty acid in a lower alkanol so that the final pH of the reaction mixture is between about 6.5 and about 7.5.

2. The process for preparing higher fatty acid salts of neomycin which comprises dissolving approximately stoichiometrically equivalent quatities of neomycin base and a higher fatty acid in a lower alkanol so that the final pH of the reaction mixture is between about 6.5 and about 7.5 and recovering the higher fatty acid salt of neomycin thus formed from the reaction mixture.

3. The process according to claim 2 wherein the fatty acid is undecylenic acid.

4. The process according to claim 2 wherein the fatty acid is pelargonic acid.

5. The process according to claim 2 wherein the fatty acid is caprylic acid.

6. The process according to claim 2 wherein the higher fatty acid is present in slight excess of the stoichiometric quantity.

7. The process according to claim 2 wherein the lower alcoholic solvent contains up to 20 percent of water.

8. The process for preparing neomycin palmitate which comprises dissolving approximately stoichiometrically equivalent quantities of neomycin base and palmitic acid in methyl alcohol at a final pH of about 7 and recovering the neomycin palmitate thus formed from the reaction mixture.

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