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ACTINOMYCIN-C AQUEOUS SOLUTIONS

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The present invention relates to the solubilization of insoluble or practically insoluble organic compounds and to the aqueous solutions thereby produced through the use of certain salts, particularly the hereinafter specified salts of cyclohexyl-salicylic acid, hippuric acid and cyclohexyl-salicyluric acid, and mixtures thereof, and their alkyl and cycloalkyl substitution products wherein the alkyl group contains 1 to 6 carbon atoms, i.e., is a lower alkyl group, especially those in which the cyclohexyl radical is in the 5-position.

Because of their insolubility in water, many organic compounds are prevented from finding their full range of uses and/or are utilizable only with partial effectiveness. In numerous instances it has been impossible to prepare true aqueous solutions of such compounds although such are greatly to be desired.

Typical water-insoluble organic compounds to which this invention is applicable are cytostatics such as actinomycin C or actinomycin F_1, antibiotics such as nystatin and the antibiotic from *Streptomyces resistomycificus* (Chemische Berichte 87 (1954) 1460–1469), sparingly soluble strpetomycin salts, sulfonamides such as 2-sulfanilamido-4,6-dimethylpyrimidine, diuretics such as diphenylmethane-4,4′-disulfonamide and 1,3-dimethyl-2,6-dihydroxypurine (theophylline), vitamins such as lactoflavin and vitamin A acetate, plant protectives such as phyllomycin, natural and synthetic dyestuffs such as alizarin, alkannin and p-dimethylaminoazobenzene, Sudan blue, Sudan red, cosmetics, pest control agents and disinfectants.

According to the invention there are used as solubilizers not only salts of cyclohexyl-salicylic acid, hippuric acid and cyclohexyl-salicyluric acid, but also alkyl or cycloalkyl substitution products of these compounds with alkyl or cycloalkyl radicals containing 1–6 carbon atoms, for example salts of cyclohexyl-o-cresotic acid. It is also possible to use mixtures of the said compounds, for example aqueous solutions containing, in addition to 20% of cyclohexyl-salicylic acid sodium salt, 5%, 10%, 15% or 20% of hippuric acid sodium salt, or, in addition to 20% of hippuric acid sodium salt, 5%, 10%, 15% of cyclohexylic acid sodium salt, or solutions containing sparingly water-soluble salts, for example cyclohexyl-salicylic acid magnesium salt, and readily water-soluble salts, for example hippuric acid sodium salt.

In order to produce aqueous solutions of the alkali metal (sodium and potassium), calcium, magnesium or lithium salts of cyclohexyl-salicylic acid, hippuric acid or cyclohexyl-salicyluric acid, the acids are dissolved in an equivalent amount of alkali or the corresponding bicarbonate with slight heating, and the solutions, after cooling, are adjusted to pH 7.0 ± 0.2 and filtered.

For producing true aqueous solutions of water-insoluble organic compounds, these compounds are stirred or shaken with an aqueous solution of the solubilizer for a prolonged time, if desired with slight heating; or the water-insoluble organic compounds are previously dissolved in a water-miscible organic solvent, e.g., ethanol, acetone, dioxane or tetrahydrofuran, and this solution is added to an aqueous solution of the solubilizer. For previously dissolving the water-insoluble organic compounds, organic solvents or solvent mixtures should preferably be

used in which the compounds are freely soluble so that after the addition of the pre-dissolved compounds to the aqueous solution of the solubilizer, the proportion by volume of the organic solvent or solvent mixture used for previously dissolving remains small.

The true aqueous solutions of water-insoluble organic compounds prepared with the use of alkali metal, calcium, magnesium or lithium salts of cyclohexyl-salicylic acid, hippuric acid or cyclohexyl-salicyluric acid are used directly or after removal of the solvent or solvents. The removal of the solvent or solvents is carried out by vacuum distillation, freeze-drying, spray drying or in a thin layer evaporator.

The following non-limitative examples illustrate the invention:

Example 1

5 g. of actinomycin C are dissolved in 30 ml. of tetrahydrofuran and to this solution are added 110 ml. of a 60% aqueous solution of the sodium salt of hippuric acid and the solution is freeze-dried. The residue after freeze-drying dissolves clearly in 125 ml. of water. The solution contains 40 mg. of actinomycin C per ml. In water, actinomycin C is soluble only to the extent of 0.5 mg. per ml.

Example 2

10 g. of actinomycin C are dissolved in 50 ml. of acetone. The acetone solution is added to 450 ml. of a 2% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid. The solution contains 20 mg. of actinomycin C per ml.

Example 3

10 g. of actinomycin C are dissolved in 50 ml. of acetone, the acetone solution is added to 100 ml. of a 10% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid and the solution freeze-dried. The residue after freeze-drying dissolves clearly in 100 ml. of water. The solution contains 100 mg. of actinomycin C per ml.

Example 4

10 g. of actinomycin C are dissolved in 50 ml. of tetrahydrofuran. The solution is added to 1380 ml. of a 4% aqueous solution of the sodium salt of 5-cyclohexyl-o-cresotic acid. The solution contains 7 mg. of actinomycin C per ml.

Example 5

6 g. of actinomycin C are dissolved in 50 ml. of tetrahydrofuran. The solution is added to 950 ml. of a 13% aqueous solution of the magnesium salt of hippuric acid. The solution contains 6 mg. of actinomycin C per ml.

Example 6

8.5 g. of actinomycin C are dissolved in 30 ml. of tetrahydrofuran and added to a 10% aqueous solution of the lithium salt of hippuric acid. The solution contains 17 mg. of actinomycin C per ml.

Example 7

5 g. of actinomycin C are dissolved in 25 ml. of tetrahydrofuran, the solution is added to 100 ml. of a 7% aqueous solution of the lithium salt of 5-cyclohexyl-salicylic acid and freeze-dried. The residue after freeze-drying dissolves clearly in 100 ml. of water. The solution contains 50 mg. of actinomycin C per ml.

Example 8

12 g. of actinomycin C are dissolved in 50 ml. of tetrahydrofuran and the solution is added to 950 ml. of an aqueous solution containing 130 g. of hippuric acid magnesium salt and 150 g. of hippuric acid sodium salt. The final solution contains 12 mg. of actinomycin C per ml.

Example 9

25 g. of actinomycin C are dissolved in 75 ml. of tetrahydrofuran and the solution is added to 925 ml. of an aqueous solution containing 25 g. of 5-cyclohexyl-salicylic acid magnesium salt and 150 g. of hippuric acid sodium salt. The solution contains 25 mg. of actinomycin C per ml.

Example 10

3.5 g. of actinomycin C are shaken with 500 ml. of a solution containing 20 g. of 5-cyclohexyl-salicyluric acid sodium salt (sodium salt of 2-hydroxy-5-cyclohexylbenzoyl-glycine) and 20 g. of 5-cyclohexyl-salicylic acid sodium salt in water until the actinomycin dissolves. The solution contains 70 mg. of actinomycin C per ml.

Example 11

2 g. of actinomycin F_1 are previously dissolved in 30 ml. of tetrahydrofuran and to this solution are added 170 ml. of a 4% aqueous solution of the sodium salt of 5-cyclohexyl-salicyluric acid (sodium salt of 2-hydroxy-5-cyclohexylbenzoyl-glycine). The solution contains 10 mg. of actinomycin F_1 per ml. The normal solubility of actinomycin F_1 in water is the same as actinomycin C.

Example 12

33.7 g. of crystalline nystatin (125×10^6 units of nystatin) are stirred with 30 ml. of dimethyl-sulphoxide and added to 970 ml. of a 10.4% aqueous solution of the sodium salt of 5-cyclohexylsalicylic acid. The solution contains 33.7 mg. of nystatin per ml. The normal solubility in water of nystatin is only 0.23 mg. per ml.

Example 13

260 mg. of the antibiotic from *Streptomyces resistomycificus* are dissolved in 5 ml. of tetrahydrofuran and 995 ml. of a 12.5% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid are added thereto. The solution contains 260γ of the antibiotic of *Streptomyces resistomycificus* per ml. The normal solubility in water is only 2γ per ml.

Example 14

30 g. of the streptomycin salt of 5-cyclohexyl-o-cresotic acid (1 mol of streptomycin per 3 mols of 5-cyclohexyl-o-cresotic acid) are stirred with 100 ml. of a 40% aqueous solution of the sodium salt of hippuric acid until the streptomycin salt dissolves. The solution contains 300 mg. of streptomycin salt per ml. The normal solubility in water is 0.8 mg. per ml.

Example 15

16 g. of the streptomycin salt of 5-cyclohexyl-o-cresotic acid (1 mol of streptomycin per 3 mols of 5-cyclohexyl-o-cresotic acid) are stirred with 100 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid until the streptomycin salt dissolves. The solution contains 160 mg. of streptomycin salt per ml. The normal solubility in water is 0.8 mg. per ml.

Example 16

12 g. of diphenylmethane-4,4'-disulphonamide are dissolved in 30 ml. of acetone, and 970 ml. of a 20% aqueous solution of the sodium salt of hippuric acid are added. The solution contains 12 mg. of diphenylamide-4,4'-disulphonamide per ml. It is filtered under sterile conditions through a Seitz filter, filled under sterile conditions into 8.4 ml. phials and freeze-dried. The normal water-solubility of diphenylmethane-4,4'-disulphonamide is 0.2 mg. per ml.

Example 17

10 g. of diphenylmethane-4,4'-disulphonamide are dissolved in 30 ml. of acetone, and 470 ml. of a 12.5% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid are added. The solution contains 20 mg. of diphenylmethane-4,4'-disulphonamide per ml.

Example 18

30 g. of 2-sulphonamido-4,6-dimethylpyrimidine are shaken with 2000 ml. of a 40% aqueous solution of the sodium salt of hippuric acid at room temperature until the 2-sulphonamido - 4,6 - dimethylpyrimidine dissolves completely. The solution contains 15 mg. of 2-sulphonamido-4,6-dimethylpyrimidine per ml. The normal water-solubility of 2-sulphonamido-4,6-dimethylpyrimidine is 0.6 mg. per ml.

Example 19

50 g. of theophylline (1,3-dimethyl - 2,6 - dihydroxypurine) are shaken with 500 ml. of a 40% aqueous solution of the sodium salt of hippuric acid at room temperature until the theophylline dissolves completely. The solution contains 100 mg. of theophylline per ml. The solubility of theophylline in water is 4 mg. per ml.

Example 20

15 g. of lactoflavin (vitamin B_2) are kneaded with 1000 ml. of a 40% solution of the sodium salt of hippuric acid until the lactoflavin dissolves completely. The solution contains 15 mg. of lactoflavin per ml. The normal solubility in water is 0.2 mg. of lactoflavin per ml.

Example 21

17 g. of lactoflavin (vitamin B_2) are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid until the lactoflavin dissolves completely. The solution contains 17 mg. of lactoflavin per ml.

Example 22

1.1 g. of vitamin A acetate are dissolved in 10 ml. of acetone and slowly introduced into 190 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid. The solution contains 5.5 mg. of vitamin A acetate per ml., i.e., 16,000 immunization units of vitamin per ml. About 0.05 mg. of vitamin A acetate per ml. are normally soluble in water.

Example 23

1.2 g. of vitamin A acetate are dissolved in 10 ml. of acetone and the solution is added to 90 ml. of a 20% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid in which 13.5 g. of sodium hippurate are also dissolved. The solution contains 12 mg. of a vitamin A acetate per ml., i.e., 35,000 I.U. of vitamin A per ml.

Example 24

10.7 g. of phyllomycin are dissolved in 100 ml. of dioxane and diluted with 15 ml. of 1 N sodium hydroxide solution. This solution (93 mg. of phyllomycin per ml.) is poured into 2480 ml. of a 10% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid. The solution contains 4.12 mg. of phyllomycin per ml. Phyllomycin is practically water-insoluble.

Example 25

4.92 g. of phyllomycin are dissolved in 150 ml. of dioxane and diluted with 75 ml. of 0.1 N sodium hydroxide solution and 25 ml. of water. This solution (19.7 mg. of phyllomycin per ml.) is poured into 2250 ml. of a 2.2% aqueous solution of the sodium salt of 5-cyclohexyl-salicyluric acid (sodium salt of 2-hydroxy-5-cyclohexyl-benzoyl-glycine). The solution contains 1.97 mg. of phyllomycin per ml.

Example 26

10.2 g. of phyllomycin are dissolved in 300 ml. of dioxane and diluted with 150 ml. of 0.1 N sodium hydroxide solution and 50 ml. of water. This solution (20.4 mg. of phyllomycin per ml.) is poured into a second solution containing 350 g. of hippuric acid sodium salt and 150 g. of 5-cyclohexyl-salicylic acid sodium salt in 4500 ml. of water. The final solution contains 7% of hippuric

acid sodium salt, 3% of 5-cyclohexyl-salicylic acid sodium salt and 2.04 mg. of phyllomycin per ml.

Example 27

33 g. of alizarin (1,2-dihydroxyanthraquinone) are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of hippuric acid until the alizarin dissolves completely. The solution contains 33 mg. of alizarin per ml. The solubility of alizarin in distilled water is 0.08 mg. per ml.

Example 28

1.1 g. of alizarin (1,2-dihydroxyanthraquinone) are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid, pH 7.3±0.1, until the dyestuff dissolves completely. The solution contains 11 mg. of alizarin per ml.

Example 29

3.5 g. of alkannin [3-(2-methoxy-5-hydroxy-pentene-2-yl)-5,8,-dihydroxy-naphthoquinone-1,4] are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid until the dyestuff dissolves completely. The solution contains 3.5 mg. of alkannin per ml. Alkannin is insoluble in water.

Example 30

7.6 g. of alkannin [3-(2-methoxy-5-hydroxy-pentene-2-yl)-5,8-dihydroxynaphthoquinone-1,4] are shaken with 1000 ml. of an aqueous solution containing 200 g. of 5-cyclohexyl-salicylic acid sodium salt and 150 g. of hippuric acid sodium salt until the dyestuff dissolves completely. The final solution contains 20% of 5-cyclohexyl-salicylic acid sodium, 15% of hippuric acid sodium salt and 7.6 mg. of alkannin per ml.

Example 31

0.7 g. of p-dimethylaminoazobenzene are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid until the dyestuff dissolves completely. The solution contains 700 mg. of p-dimethylaminoazobenzene/litre. The normal solubility of p-dimethylaminoazobenzene in water is 1 mg./litre.

Example 32

125 mg. of Sudan blue are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid until the dyestuff dissolves completely. The solution contains 125 mg. of Sudan blue/litre. The normal solubility in water is 0.4 mg./litre.

Example 33

80 mg. of Sudan red B are shaken with 1000 ml. of a 15% aqueous solution of the sodium salt of 5-cyclohexyl-salicylic acid until the dyestuff dissolves completely. The solution contains 80 mg. of the Sudan red/litre. The normal solubility in water is 0.5 mg./litre.

Example 34

15 g. of 3-methoxy-4-N,N-diethyl-carbamidomethoxy-phenylacetic acid-n-propyl ester (B.Pt. 210–212° C./0.7 mm. Hg) are shaken with 100 ml. of an aqueous solution containing 20 g. of 5-cyclohexyl-salicylic acid sodium salt and 15 g. of hippuric acid sodium salt until the whole dissolves. The solution contains 150 mg. of 3-methoxy - 4 - N,N - diethyl-carbamidomethoxyphenyl-acetic acid-n-propyl ester per ml. The normal solubility of 3 - methoxy - 4 - N,N - diethyl - carbamidomethoxy-phenylacetic acid-n-propyl ester in water is 0.5 mg. per ml.

Example 35

5 g. of 3 - methoxy - 4 - N,N - diethyl - carbamido-methoxyphenylacetic acid-n-propyl ester are dissolved while shaking in 66.7 ml. of a 60% aqueous solution of the sodium salt of hippuric acid and the volume is made up with water to a total of 100 ml. The solution contains 50 mg. of 3-methoxy-4-N,N-diethyl-carbamidomethoxyphenylacetic acid-n-propyl ester per ml.

Example 36

5 g. of 3-methoxy-4-N,N-diethyl-carbamidomethoxy-phenylacetic acid-n-propyl ester are dissolved while shaking in 50 ml. of a 60% aqueous solution of the sodium salt of hippuric acid, 10 ml. of 1,2-propane-diol are added and the volume is made up with water to a total of 100 ml. The solution contains 50 mg. of 3-methoxy-4-N,N-diethyl - carbamidomethoxyphenylacetic acid - n - propyl ester per ml.

The invention thus makes it possible to prepare true, clear, aqueous solutions of many water-insoluble or sparingly soluble organic compounds in a comparatively simple but highly effective manner, thereby widening the range and efficacy of usefulness of such compounds, particularly since relatively concentrated solutions can be produced. True aqueous solutions of the kind provided by this invention have manifold advantages not heretofore available as will be understood. The solubilized compounds have enhanced or increased utility for the purposes for which said compounds are intended to be used. As mentioned above, the aqueous solutions of the invention may or may not contain an organic solvent in which the organic compound is readily soluble and which solvent is miscible with water. Where the organic solvent is not desired in the final solution, it is removed in any suitable or convenient manner and its removal does not throw the dissolved organic compound out of solution. The expression, true solution, as used herein means that the organic compound is actually dissolved as distinguished from a colloidal solution or a suspension.

What is claimed is:

1. An aqueous solution of actinomycin C containing at least about 12 times as much actinomycin C in solution as is normally soluble in the same amount of water, said solution comprising, in addition to water and actinomycin C, a solubilizing agent selected from the group consisting of the sodium, potassium, calcium, magnesium and lithium salts of cyclohexyl-salicylic acid, hippuric acid and cyclohexylsalicyluric acid and their alkyl and cycloalkyl substitution products wherein the alkyl group has 1 to 6 carbon atoms, and mixtures of said solubilizing agents.

2. An aqueous solution according to claim 1, wherein there is present an organic solvent in which the actinomycin C is readily soluble and which solvent is miscible with water.

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