United States Patent [19]

Gaeumann, deceased et al.

[11] **3,852,424**

[45] **Dec. 3, 1974**

[54] **PURIFIED FERRIMYCIN AND PROCESS FOR OBTAINING SAME**

[75] Inventors: **Ernst Gaeumann, deceased,** late of Zurich, Switzerland; **Tino Gaeuman, legal representaitve,** Mont Sur Lausanne; **Vladimir Prelog,** Zurich; **Ernst Vischer,** Basel; **Hans Bickel,** Binningen, all of Switzerland

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[22] Filed: **Oct. 19, 1966**

[21] Appl. No.: **626,650**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 245,349, Dec. 11, 1962, abandoned, which is a continuation-in-part of Ser. No. 32,294, May 27, 1960, abandoned, which is a continuation-in-part of Ser. No. 749,616, July 21, 1958, abandoned.

[30] **Foreign Application Priority Data**

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July 8, 1958	Switzerland	61491/58
May 29, 1959	Switzerland	73755/59
Mar. 18, 1960	Switzerland	3062/60

[52] **U.S. Cl.** **424/118,** 195/80

[51] **Int. Cl.** **A61k 21/00**

[58] **Field of Search** 424/118; 195/80

[56] **References Cited**

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[57] **ABSTRACT**

The antibiotic ferrimycin A, its components ferrimycin A_1 and ferrimycin A_2 and the corresponding iron-free compounds desferrimycin A, desferrimycin A_1 and desferrimycin A_2, and also pharmaceutical preparations which contain these products, and a process for the manufacture of these substances and mixtures containing them. The antibiotic ferrimycin belongs to the sideramycins, a class of iron-containing or iron-binding antibiotics, to which also the antibiotics grisein, albomycin and A 1787 belong. The sideramycins are characterized by their antibiotic effect being antagonized by the ferrioxamines; see Bickel et al., Experientia 16 (1960) 129.

4 Claims, 8 Drawing Figures

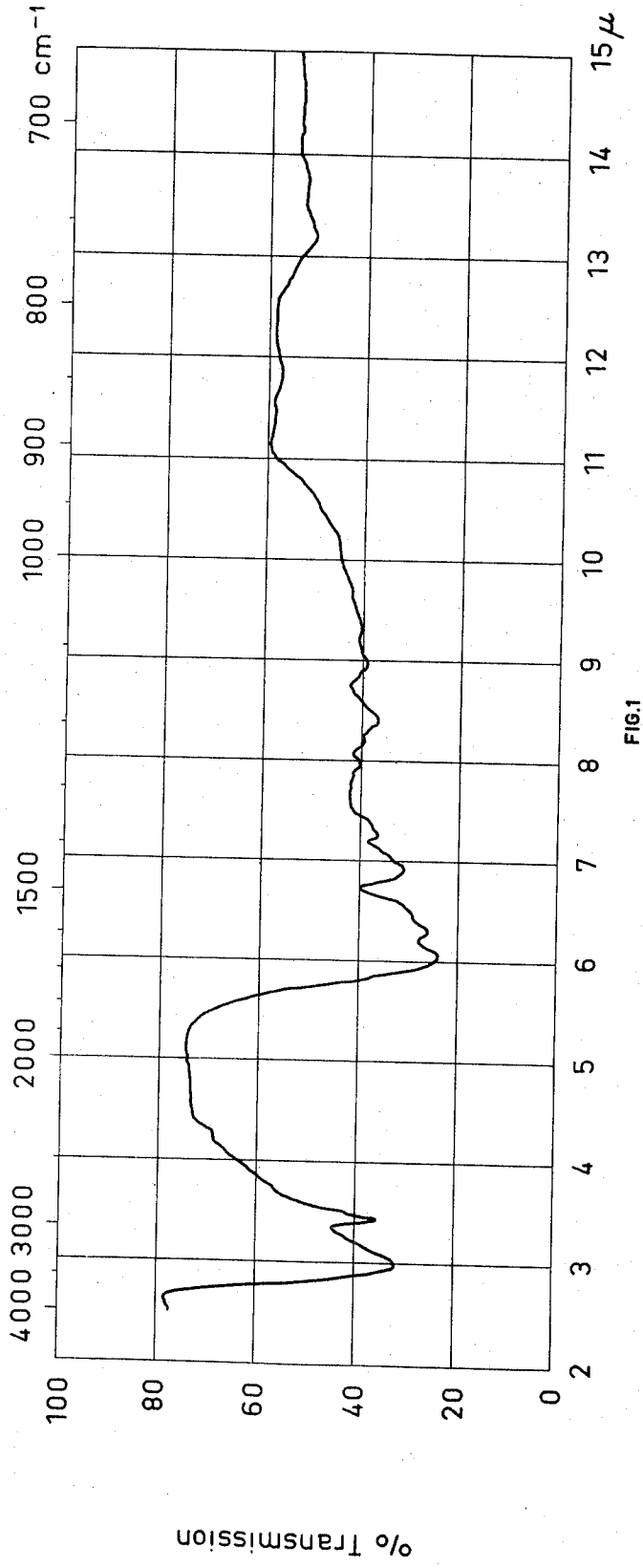
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FIG.1

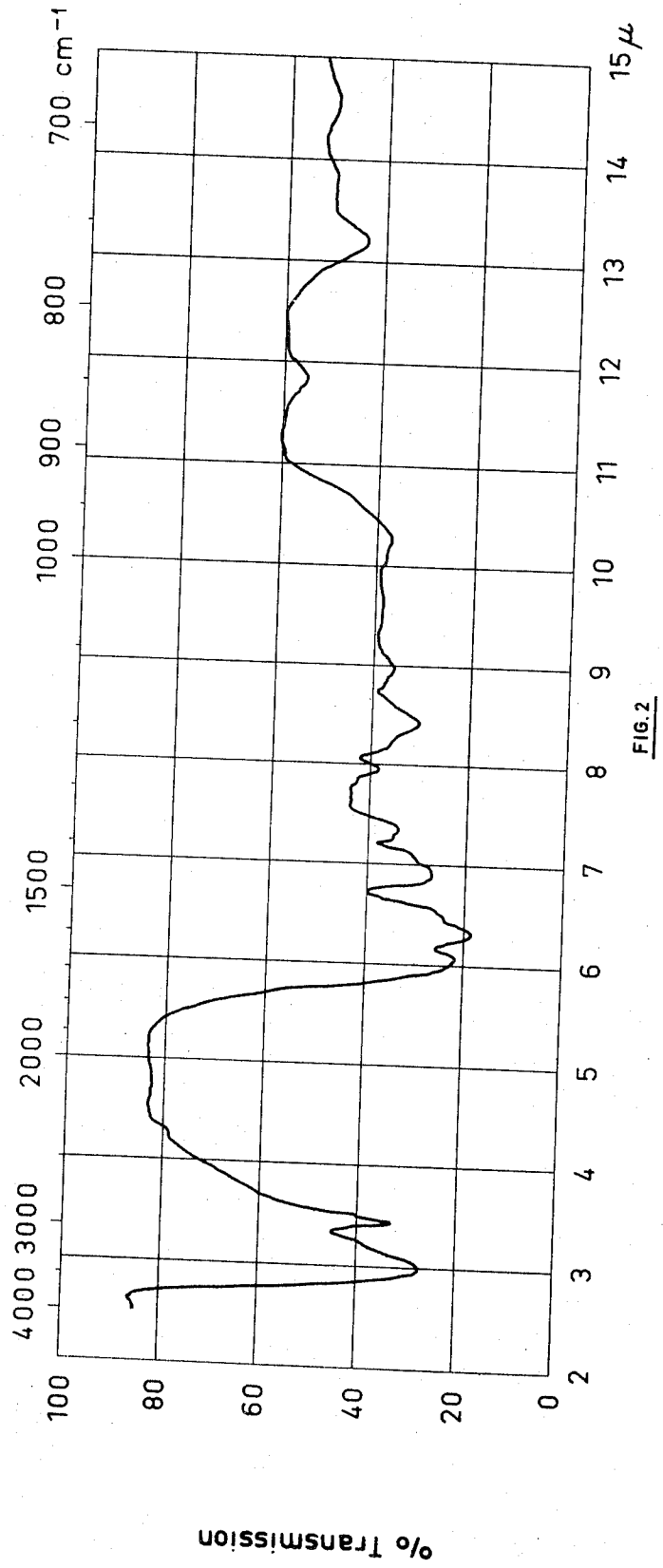
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FIG. 2

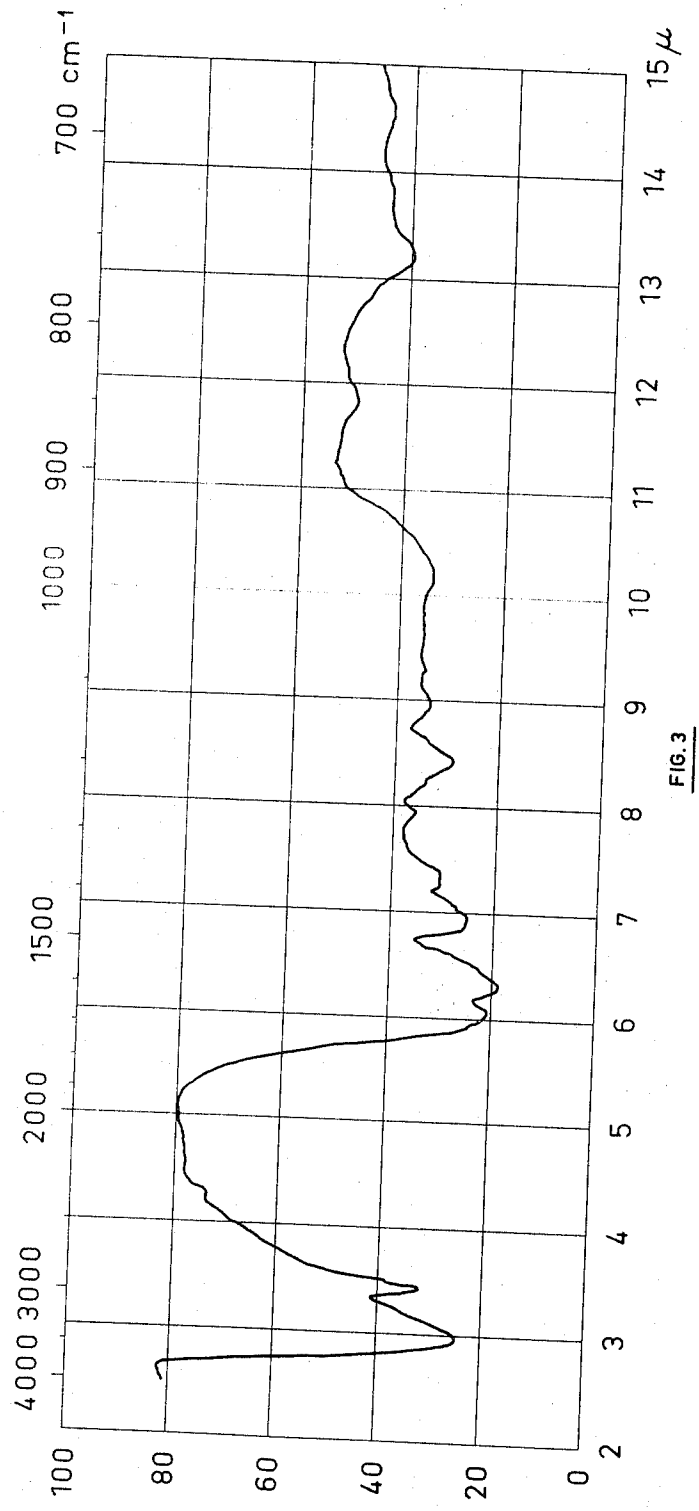
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FIG. 3

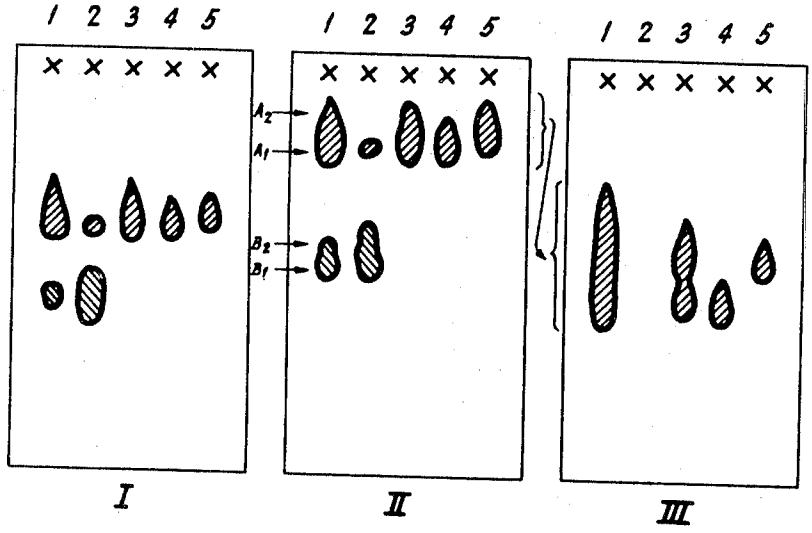


Fig. 4

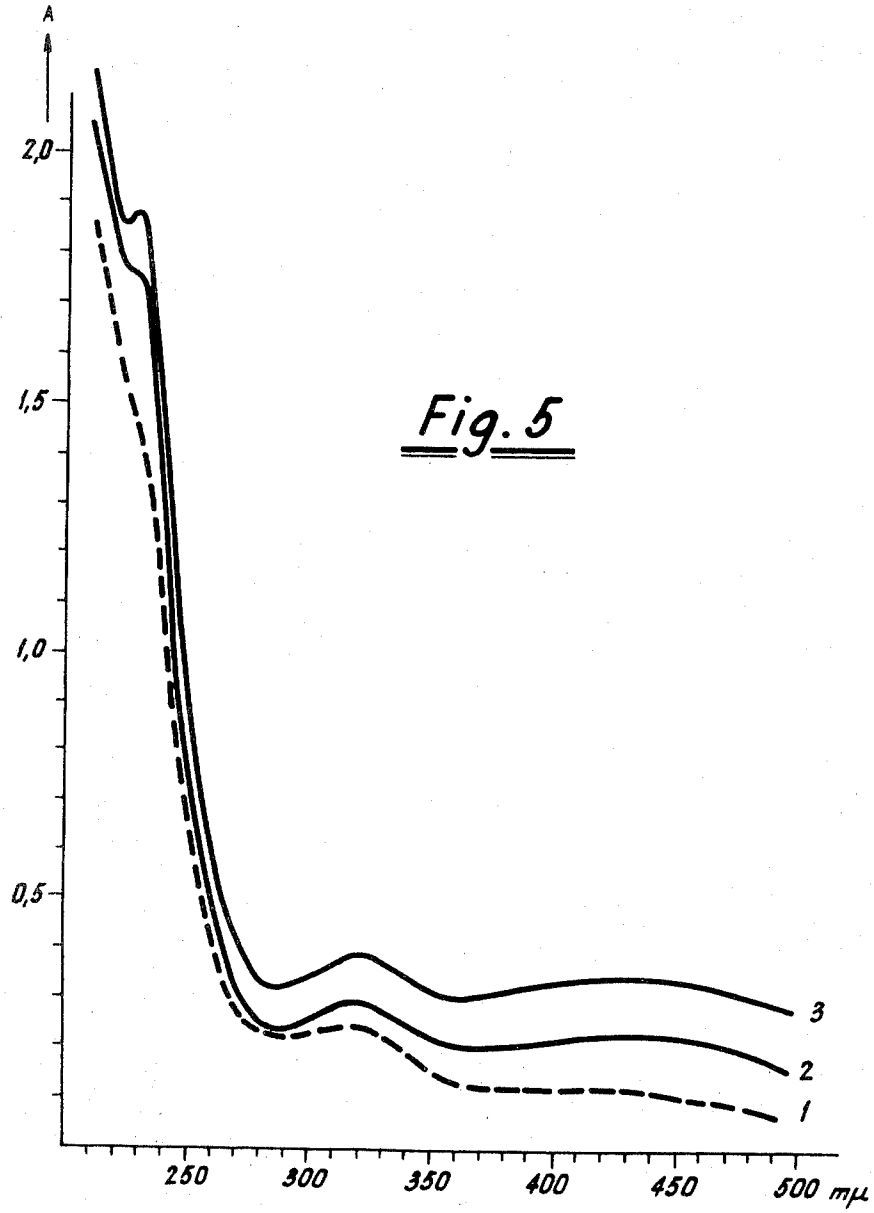
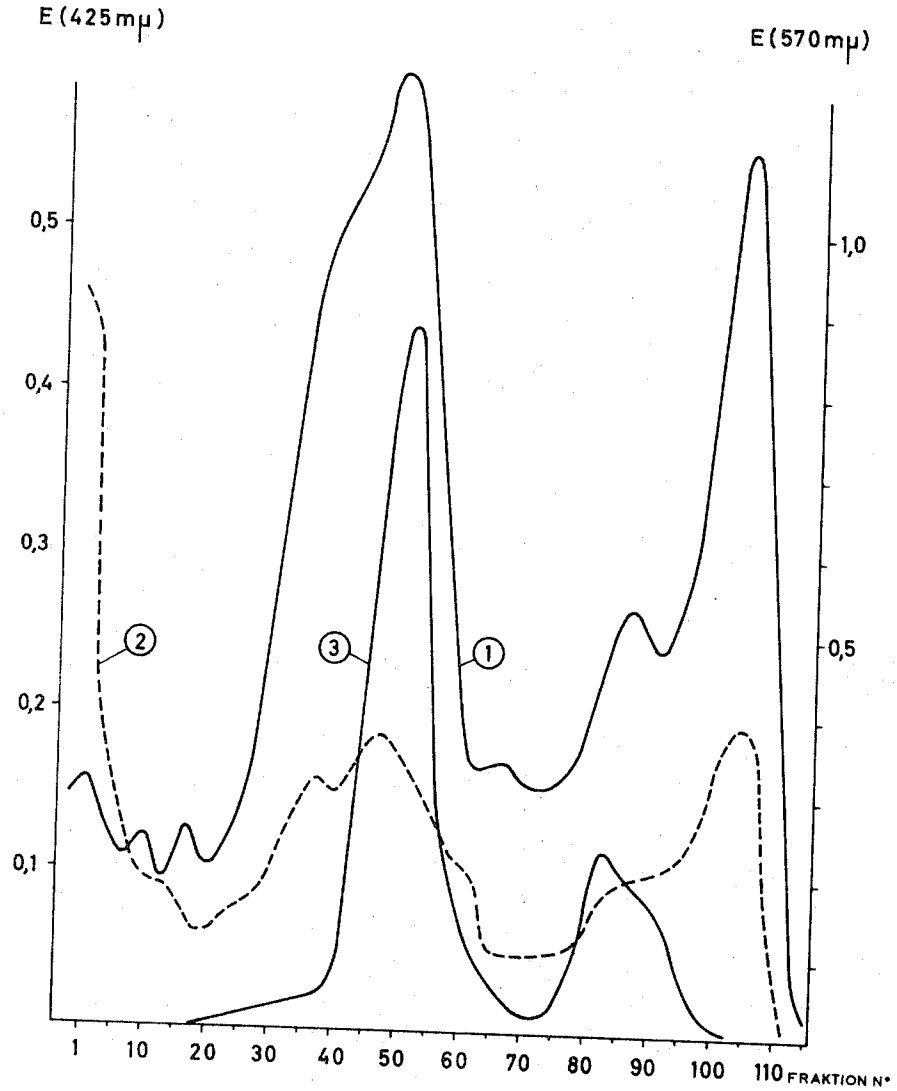


Fig. 5


E (425mμ)
E (570mμ)
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FRAKTION N°

FIG. 6

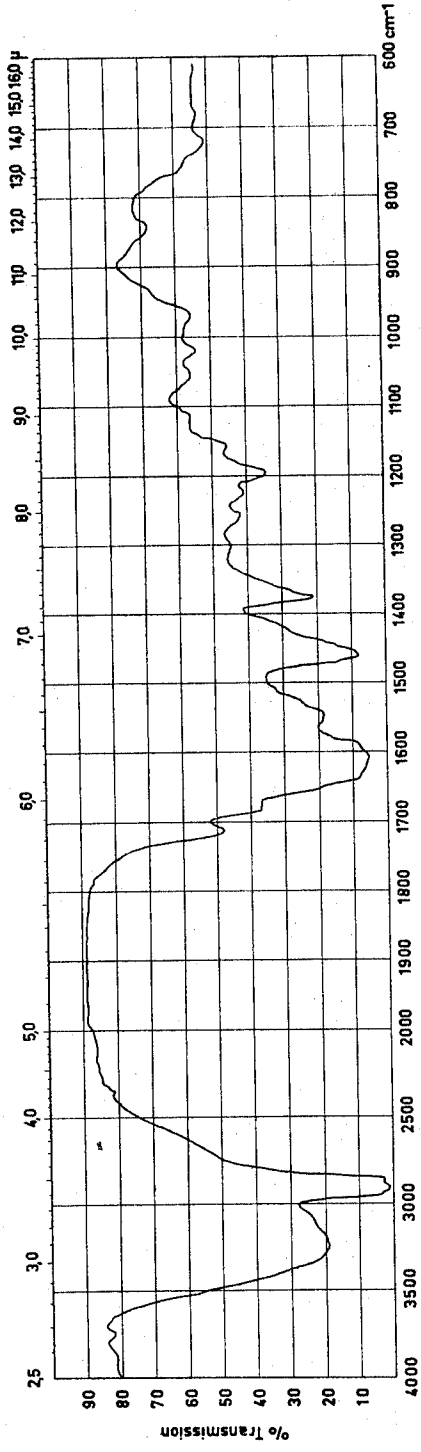

FIG. 7

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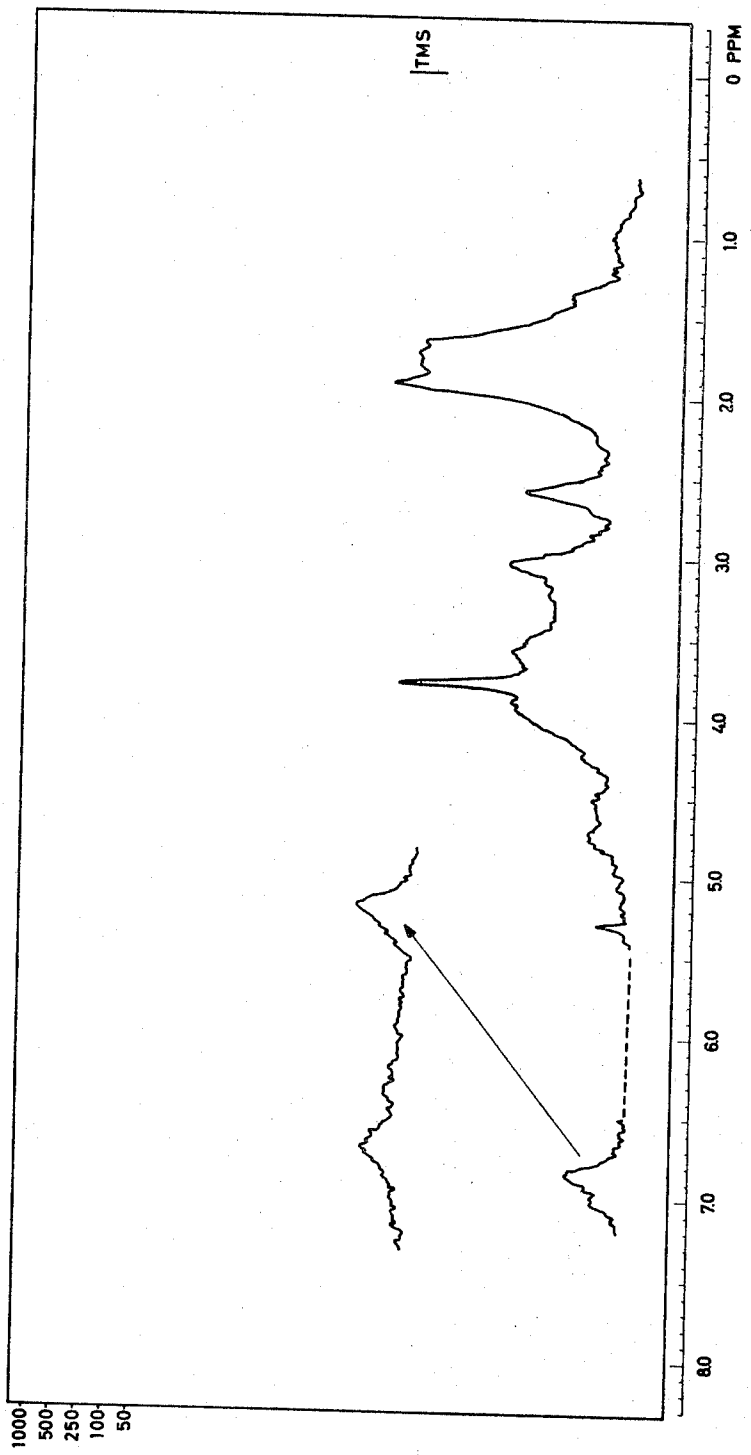

FIG. 8

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PURIFIED FERRIMYCIN AND PROCESS FOR OBTAINING SAME

This is a continuation-in-part of our application Ser. No. 245,349, filed Dec. 11, 1962 (now abandoned), which is itself a continuation-in-part of our application Ser. No. 32,294, filed May 27, 1960 (now abandoned), which is itself a continuation-in-part of application Ser. No. 749,616, filed July 21, 1958 (now abandoned), by Vladimir Prelog et al.

The invention relates to a new watersoluble antibiotic which we formerly designated as A 9578 or as pilosomycin and is now called ferrimycin A, its components ferrimycin A_1 and ferrimycin A_2 and the corresponding iron-free compounds desferrimycin A, desferrimycin A_1 and desferrimycin A_2, and also pharmaceutical preparations which contain these products, and a process for the manufacture of these substances and mixtures containing them.

The antibiotic ferrimycin belongs to the sideramycins, a class of iron-binding antibiotics, to which also the antibiotics grisein, albomycin and A 1787 belong. The sideramycins are characterized by their antiobiotic effect being antagonized by the ferrioxamines; see Bickel et al., Experientia 16 (1960) 129.

Ferrimycin and its components are red-brown, basic substances which are readily soluble in acids and strongly polar solvents, such as water, methanol, dimethylformamide, glycol, methyl cellolsolve. They also dissolve in benzyl alcohol, in phenols or in mixtures of phenols and lipoid solvents. They consist of hydrocarbon, hydrogen, oxygen and nitrogen, and also contain iron or are capable of binding iron. Ferrimycin is a mixture of closely related compounds which consist of two main components, ferrimycin A and ferrimycin B. Ferrimycin A can be separated into two components, ferrimycin A 1 and ferrimycin A 2. The properties of these various components are described below.

It has not hitherto been possible to obtain ferrimycin or the above-mentioned components in a pure form. The special difficulty in purifying the ferrimycins arises principally from the fact that they are present in the culture filtrate in very small proportion in addition to large amounts of inactive substances from the nutrient solution and fermentation products having very similar physical-chemical properties. The culture filtrate contains on an average for every one part of ferrimycin 10,000–15,000 parts by weight of inactive substances which are hydrophilic, like the ferrimycins. The difficulty of enriching and purifying the ferrimycins also arises from their instability in a wide range of pH values. The separation of the antibiotic into its individual components is also rendered difficult owing to the fact that these components differ from one another only slightly in their R_f-values even in chromatographically favorable solvent systems.

Antibiotic ferrimycin is obtained by the culture of a new strain of actinomycetes, of the species Streptomyces griseflavus, which has been isolated from a sample of soil collected in Boston, Mass., and which is kept in our laboratories, and also in the Eidg. Technische Hochschule, Institut fur spezielle Botanik under the designation A 9578 and in the U.S. Department of Agriculture, Agricultural Research Service, Northern Utilization Research and Development Division, Peoria, Ill., under the designation NRRL 2717.

Streptomyces griseoflavus NRRL 2717 belongs to the species *Streptomyces griseoflavus* (Krainsky) Waksman and Henrici, and differs from other members of this species in that it forms a new water-soluble antibiotic. Hitherto only one strain of *Streptomyces griseoflavus* is known which produces an antibiotic. This is the so-called griseoflavine, which differs from the new antibiotic ferrimycin in its solubility in organic solvents. The air mycelium of *Streptomyces griseoflavus* NRRL 2717 is ash grey. The spore carriers are branched and form copious spirals generally having 2–5 turns. The spores themselves have a size of 1.0 to 1.3μ × 0.7 to 0.9μ, and have at their surface spines about 0.2 γ in length which are pointed and only slightly widened at their base. Their growth is relatively little dependent on temperature, so that the mould develops well at 18°C and also at 40°C, although the optimum temperature is between 25°C and 32°C.

For the purpose of identification there is described below the growth of *Streptomyces griseoflavus* NRRL 2717 on various nutrient media. The nutrient media numbers 1–7 and also 10 are prepared as described by W. Lindenbein, Arch. Mikrobiol. 17, page 361 (1952).

1) Synthetic agar:	Growth cloud-like colorless, no air mycellium.
2) Synthetic solution:	Sediment, reddish flocks, Well developed pellicle having cloud-like white-grey air mycellium. Substratum brown-yellow to deep yellow.
3) Glucose-agar:	Growth initially point-like, wrinkled after 12 days and pale yellow.
4) Glucose-asparagine-agar:	Growth initially point-like, later cloudy and yellowish. Air mycellium velvety, initially brownish grey, later ash grey. Substratum yellow.
5) Calcium malate-agar:	Growth cloud-like, white-yellow, later deep yellow, Air mycellium scanty, covered with flour-like dust, floury white. Substratum pale brown.
6) Gelatine stab culture (18°C):	Growth very scanty, liquefaction very slow (0.5 cm after 30 days).
7) Starch plate:	Growth cloud-like, milk-white, no air mycellium. Hydrolysis 1 cm after 5 days.
8) Potatoes:	Growth initially point-like, pale yellow, later pimply. Air mycellium velvety, white yellow to ashgrey. Substratum colored brownish grey.
9) Carrots:	Growth very scanty. Air mycellium scanty. Substratum not colored.
10) Litmus milk:	Ring growth and surface skin colorless. Air mycellium white-grey, hydrolysis slow without co-agulation, litmus blue.
Tyrosinase reaction:	Negative.

When tested by the technique of T.G. Pridham and D. Gottlieb, J.Bacteriology, Vol 56, page 107 (1948), *Streptomyces griseoflavus* NRRL 2717 grows well with the following sources of carbon: L-Xylose, L-arabinose, L-rahmnose, saccharose, raffinose, inulin, D-mannitol, D-sorbitol, mesoinositol, salicin, and D-fructose.

The process for producing antibiotic ferrimycin A in accordance with the present invention is not limited to

the use of Streptomyces griseoflavus NRRL 2717 or other strains corresponding to the description thereof, but also includes the use of variants such, for example, as are obtained by selection or mutation, especially under the action of ultra-violet rays or X-rays or nitrogen mustard oils.

In order to produce antibiotic ferrimycin A or its components a strain of Streptomycetes having the properties of Streptomyces griseoflavus NRRL 2717 is incubated aerobically, for example, in an aqueous nutrient solution containing a source of carbon and of nitrogen and inorganic salts until the solution exhibits a substantial antibacterial action, and the antibiotic ferrimycin is then isolated.

As source of carbon there may be used, for example, glucose, saccharose, lactose, mannitol, starches or glycerine. As nitrogenous nutrient substances and, if desired, growth promoting substances there may be mentioned amino-acids, peptides and proteins, and also their degradation products such as peptone or tryptone, and also meat extracts, water-soluble constituents of cereal grains, such as maize or wheat, or of the distillation residues from the manufacture of alcohol, or yeast, or beans, especially soya bean plants, or seeds, for example, those of cotton plants etc., and also ammonium salts and nitrates. Among other inorganic salts the nutrient solution may contain chlorides, carbonates, sulfates of alkali metals, alkaline earth metals, magnesium, iron, zinc and manganese.

The incubation is carried out aerobically, for example, in a quiescent surface culture or advantageously submerged while agitating or stirring with air or oxygen in agitated flasks or known fermenters. The temperature may be within the range of 18°C to 40°C. The nutrient solution generally exhibits a substantial antibacterial action after 1 ½ to 5 days.

In order to isolate antibiotic ferrimycin the following methods may be used: The mycelium is separated from the culture filtrate, and the greater part of the antibiotic is found in the culture filtrate. However, considerable quantities of the antibiotic are adsorbed on the mycelium. It is therefore of advantage to wash to mycelium well. For this purpose water or an aqueous organic solvent may be used, such as an alcohol, for example, aqueous methanol. In order to recover the antibiotic from the culture filtrate and to purify it, various methods may be used which can be employed singly or in combination with one another. It is of advantage during these operations to maintain the culture solution at pH value within the range of 3–5.

1. For isolating the crude ferrimycin from the culture filtrate, and adsorption medium may be used, for example, an active carbon such as "Norit," activated earths such as Fuller's earth or "Floridine"(aluminum magnesium silicates) or a resin adsorbant such as "Asmit" (a meta-phenylenediamine-formaldehyde decolorizing resin). The adsorbate is advantageously eluted with a mixture of water and an organic solvent miscible with water or an aqueous acid, for example, a mixture of water and methanol, water and pyridine, dilute acetic acid and methanol, or water, methanol, glacial acetic acid and butanol. Especially advantageous for the elution of a Norit-adsorbate is a mixture of 2 parts by volume of water, 1 part by volume of methanol, 1 part by volume of glacial acetic acid, and 2 parts by volume of butanol.

2. A second method for separating the antibiotic from the culture filtrate is to adsorb the antibiotic on a cation-exchanger, for which purpose resins containing acid groups, such as "Amberlite" IRC–50 (a carboxylic acid type ion exchanger) are especially suitable. The latter can be used either in the acid form or in the sodium form, although a mixture of both forms in the volumetric ratio 1:2 is particularly advantageous. Elution is advantageously carried out with a dilute acid, e.g. methanolic hydrochloric acid.

3. The basic antibiotic can also be precipitated directly from the culture filtrate, for example by reaction with an organic acid of the type of picric acid. By treating a precipitate so obtained with a salt of an organic base, for example, with triethyl-ammonium sulfate, or with a dilute acid, the antibiotic is obtained in the form of the corresponding salt. These operations may be carried out either in an aqueous medium or in a solvent miscible with water, such as methanol or acetone. The conversion of the sparingly soluble salts into the readily soluble salts of the antibiotic is carried out either by means of mineral acids or by treatment with ion exchanging resins, e.g. Amberlite IRA–400 (a polystyrene resin containing quaternary ammonium groups).

4. The antibiotic can be concentrated by adding to an aqueous or alcoholic-aqueous solution of the salt of the antibiotic an excess of an organic solvent miscible with water, such as acetone, dioxane etc., whereby the salt is precipitated in solid form.

5. Another method of enriching the antibiotic consists in extracting an aqueous solution thereof with a solution of phenol in chloroform in which operation both the pH of the aqueous solution and the phenol content of the chloroformic solution can be varied. As an example, in a distribution between a solution containing in 100 cc of chloroform 100 grams of phenol and an aqueous phase having a pH of 1 to 6, nearly all of the antibiotic is in the organic phase, whereas, when a solution is used which contains only 33 grams of phenol in 100 cc of chloroform, it can be extracted nearly completely from the aqueous phase only at a pH between 4 and 6. When the distribution coefficient of the antibiotic means the ratio of concentration in the organic phase to the concentration in the aqueous phase, the foregoing shows that the distribution coefficient increases as the phenol content of the organic phase increases, and decreases as the pH of the aqueous phase falls. It thus being possible to chose any desired distribution coefficient of the antibiotic in this system, a large proportion of inactive impurities can be eliminated by combining a small number of distributing operations.

6. There is still another method of enriching the antibiotic, namely, chromatography, such as adsorption chromatography on various materials, e.g. Norit (activated carbon), alumina, magnesium silicates, silica gel, calcium sulfate, or distribution chromatography with cellulose, starch, silica gel, "Celite" (an infusorial earth) or the like as carrier substances, or chromatography on ion exchangers, e.g., "Dowex"50 (a sulfonated polystyrene), Amberlite IRC–50 (a carboxylic acid type cation exchanger) or the like. Good results have been obtained for example with distribution chromatography on cellulose using the solvent system of 4 parts by volume of butanol, 1 part by volume of glacial acetic acid, and 5 parts by volume of water.

7. The antibiotic can also be enriched by the countercurrent distribution according to Craig between two immiscible solvent phases. The following solvent systems have proved particularly successful:

a. secondary butanol 1/10 N-ammonium acetate buffer having a pH of 4.68.

b. 1/10 N-ammonium acetate buffer having a pH of 4.6–10% solution of phenol in chloroform.

The distribution coefficient of the antibiotic in the system (b), and thus the location of the activity maximum in the distribution can be changed as desired on the one hand by changing the pH of the buffer solution and on the other hand by changing the phenol content of the organic phase.

The following combination of the enriching methods described above gives preparations of considerable purity. From the culture filtrate, the antibiotic is adsorbed on the buffered ion exchanger Amberlite IRC–50 and eluted by means of methanolic hydrochloric acid. At a pH of 5, the eluates are concentrated under reduced pressure, an aqueous concentrate of the antibiotic being obtained in an amount of about 1/100 of the volume of the nutrient solution. By method (5), above, the concentrate is distributed several times between phenol-chloroform mixtures and aqueous solutions of varying pH. Oh freeze-drying the resulting active solution there is obtained a preparation the specific activity of which is 500–1000 times higher than that of the lyophilized culture filtrate. A specific embodiment of the present invention consists in further purifying the crude ferrimycin as obtained according to the above mentioned methods by subjecting it to electrophoresis and/or countercurrent distribution with the use of benzyl alcohol and, if desired, chromatography.

The electrophoresis is carried out in the form of a high voltage electrophoresis at 500 to 4,000 volts, in the form of zonal electrophoresis, and especially countercurrent electrophoresis.

In the case of zonal electrophoresis in dilute acetic acid the activity of ferrimycin A is increased to 7,000 to 8,000 times the activity of the lyophilized culture filtrate.

An even higher increase in activity (up to 10,000 times) is obtained in the counter-current electrophoresis of ferrimycin A. In this method of separation the antibiotic is present as a locally fixed cation, in that the tendency for movement by an electric field is exactly counteracted by means of an oppositely directed stream of the electrolyte. Substances that undergo electrical movement in a different manner leave the apparatus at either one of the two ends, where the electrodes are fixed.

For chromatography there are used as adsorbents preferably strong acid ion-exchange resins, such as Dowex 50–WX_2 (a sulfonated polystyrene containing 2 percent of divinylbenzene). As eluting agents there are advantageously used basic buffer solutions of increasing concentrations, such as ammonium acetate buffers of pH value 4.6 in a molecular concentration of 0.2 to 2.0.

For countercurrent distribution there are used, for example, the following systems:

Benzyl alcohol (60 parts by weight) — methylisobutylketone (44 parts by weight) — aqueous sodium chloride solution of 15% strength (50 parts by weight) — 0.01N-hydrochloric acid (50 parts by weight); Benzyl alcohol (66 parts by weight) — methylisobutylketone (33 parts by weight) — aqueous sodium chloride solution of 5% strength (50 parts by weight) — 0.01N-hydrochloric acid (50 parts by weight). Also suitable is the system benzyl alcohol (200 parts by volume)-n-butanol (100 parts by volume) — water (300 parts by volume) — saturated, aqueous sodium chloride solution (60 parts by volume) — N-hydrochloric acid (6 parts by volume). By the use of of these systems the active substance is divided into the two components ferrimycin A and ferrimycin B.

The two components A and B ferrimycin are defined by paper-chromatography by a direct comparison of their Rf values with the Rf values of a series of known antibiotics (2–11) in the systems A – G. In the case of system H the figures represent in cm the distance travelled by the antibiotics after 16 hours. The antibiotics are detected autobiographically with *Staphylococcus aureus* or *Bacillus subtilis*.

System	1a	1b	2	3	4	5	6	7	8	9	10	11
A	0	0	0	0	0	0	0	0	0	0.92	0.07	0
B	0.49	0.63	0.05	0.05	0.17	0.02	0.02	0.66	0.55	0.92	0.32	0.22
C	0.34	0.58	0.22	0	0.02	0.22	0.03	0.72	0.62	0.93	0.39	0.11
D	0.05	0.15	0	0	0	0	0	X	X	0.92		0
E	0.32	0.32	0	0.10	0.22	0	0	X	X	0.86		0.12
F	0.47	0.47	0.22	0.14	0.12	0.04	0.05	0.49	0.42	0.91	0.43	0.36
G	0.74	0.74	0.07			0.02		X	X	0.94	0.61	0.69
H	2.7	7.6	0	0	0	0	0	(14.5)	(8.8)	27		1

X antibiotic distribution over the whole course
( ) position unsharp
A water-saturated butanol
B butanol-glacial acetic acid-water (4:1:5) (upper phase)
C water-saturated butanol + 2% para-toluene-sulfo acid
D water-saturated butanol + 2% piperidine
E butanol-pyridine-water (6:4:3)
F 80% ethanol + 1.5% NaCl. Whatman No. 4 impregnated with 0.95 molar Na_2So_4 + 0.05 molar $NaHSO_4$
G butanol-ethanol-water (1:1:2)
H butanol-butyl acetate-glacial acetic acid-water (10:3:1.3:14.3) (upper phase)
1a ferrimycin base A
1b ferrimycin base B
2 Streptomycin
3 Ristocetin A
4 Ristocetin B
5 Neomycin B
6 Viomycin
7 Chlorotetracyclin
8 Oxytetracyclin
9 Actinomycin I
10 Cycloserin
11 Grisein

During paper electrophoresis in an 0.1-molar acetate buffer having a pH value of 4.6 antibiotic ferrimycin migrates towards the cathode. The speed of migration is about half as great as that of Streptomycin.

For separation into the components A1 and A2 distribution chromatography on cellulose in the system n-butanol-0.5N-acetic acid (1:1) is especially suitable.

The resulting preparations have 10 to 20,000 times the acitivity of the lyophilized culture filtrate, whereas the products used as starting material have only 100 to 1,000 times the activity of the lyophilized culture filtrate. A ferrimycin A preparation purified in the described manner, a strongly acidic ion exchanger being used in the last purification step, shows, in the form of the dihydrochloride, the following chemical and physical properties:

Microanalysis:
C:48.65%, H:7.09%,
N:12.95%, Fe 4.56%,
Cl: 6.10%, (C)CH_3: 1.99% (Roth-Kuhn), Amino-N: 2.33% (Van Slyke).

Titration:
pK^*_{MCS} (Helv. 37,1872 (1954)):
4.18; 7.88
Equivalent weight: 1106.

Absorption spectrum:
λ max: 228 mμ, $E_{1cm}^{1\%}$ 282 max: 319 mμ, $E_{1cm}^{1\%}$ 28.2 max: 425 mμ, $E_{1cm}^{1\%}$ 22.6

Reduction value according to C. S. Hanes, Biochem. J.23, 99 (1929); 1.7 ml 1/100 N-sodium thiosulfate.

Bound hydroxylamine according to T. Emery and J. B. Neilands, Nature 184, 1632 (1959): 0.83 mol NH_2OH per atom Fe.

Ferrimycin A is an orange-yellow powder which dissolves very readily in water, methanol and mixtures of phenol and chloroform, dissolves in dimethylformamide, methyl cellosolve, benzyl alcohol and glacial acetic acid, dissolves sparingly in ethanol and is practically insoluble in pyrridine, propanol, butanol and the usual organic solvents, especially lipoid solvents. It can be precipitated from an aqueous solution by means of picric acid, picrolonic acid and ammonium reineckate.

The orange-red aqueous solutions of the antibiotic change color reversibly through claret red to pale yellow on the addition of mineral acid. Decoloration is also brought about with sodium hydroxide solution. Neutral solutions do not react with potassium ferrocyanide, and acidified solutions yield colorations or precipitates of Prussian blue. The trivalent iron bound in complex union is liberated when the pH value is decreased.

Ferric chloride causes a claret red coloration and ferric chloride plus potassium ferricyanide give a blue coloration. The following tests are negative: Molish, Anthron, Folin-Ciocalteu and Sakaguchi. Its ninhydrin reaction in a mixture of butanol and pyridine is only slightly positive after heating for a long time. Hydrolysis with 6N-hydrochloric acid yields a mixture of about 15 substances detectable by paper chromatography. Among these compounds the following can be identified: succinic acid, 1-amino-5-hydroxyamino-pentane, δ-aminovaleric acid, cadaverine, ammonia, proline, a crystalline substance ($C_7H_8O_3N.HCl$) with λmax 227 and 323 mμ and ferric chloride.

The purified base A can be split up by chromatography into components A_1 and A_2. As an adsorbent it is of advantage to use cellulose, and as the flow agent tertiary butanol/0.001 N-hydrochloric acid/saturated aqueous NaCl solution 2:1:1.

The components A_1 and A_2 are characterized as follows:

A_1-dihydrochloride: Elementary analysis (after drying for 50 hours at 25°C/10^{-3}mm over P_2O_5/KOH; average from three determinations) yields: C = 46.71%; H = 6.80%; N = 12.70%; Cl = 6.76%; Fe (color.) = 5.33%; 0 (calc. = 21.70%). Titration in 80% methyl-cellosolve; equivalent weight: 1078; pK = 4.11; 7.92; 11.4. These values suggest the empirical formula $C_{41}H_{65}O_{14}N_{10}Fe$, HCl, and the molecular weight 1051. Its ultraviolet spectrum shows maxima at 229 mμ ($E_{1cm}^{1\%}$ = 336), 319 mμ ($E_{1cm}^{1\%}$ = 37) and 425 mμ ($E_{1cm}^{1\%}$ = 27.6). Its infrared spectrum (in potassium bromide) is given in FIG. 2. It shows bands at 3.00; 3.44; 6.08; 6.31; 6.90; 7.35; 7.45; 7.95; 8.41; 9.00; 10.25; 11.85; 13.20μ.

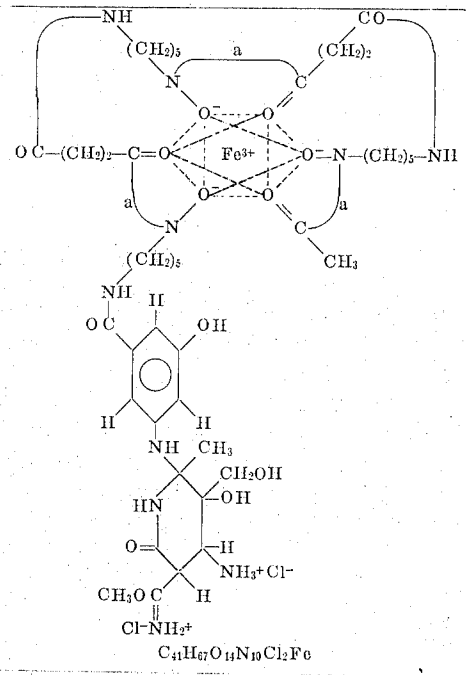
A_2-dihydrochloride: Elementary analysis (after drying) as above) gives: C =45.78%; H = 6.77%; N = 12.75%; Cl = 6.23%; Fe (color.) = 5.29%; titration in 80% methyl-cellosolve; molecular weight: 1086; pK = 4.04; 7.91; 11.05. These values suggest the empirical formula $C_{41}H_{68}O_{15}N_{10}Fe$, Hcl. Its ultraviolet spectrum shows maxima at 227 mμ ($E_{1cm}^{1\%}$ = 332), 319 mμ ($E_{1cm}^{1\%}$ = 37) and 425 mμ ($E^{1\%}_{1cm}$ 32 25). Its infra-red spectrum (in potassium bromide) is shown in FIG. 3. It shows bands at the same positions as the spectrum of component A_1.

In FIG. 5 are shown the ultraviolet spectra (in water: $c = 5 \times 10^{-3}$) of purified ferrimycin A (Curve 1, Extinction E = A), of the component A_2 (Curve 2, E = A − 0.1) and of the component A_1 (Curve 3, E = A −0.2).

The ferrimycins can be definitely distinguished by paper chromatography from Grisein (F. A. Kuehl, M. N. Bishop, L. Chaiet and K. Folkers, Am. Soc. 73, 1770 (1951)), Albomycin [G. F. Gause, Brit. Med. J. **1955**, 1177] and A 1787 [H. Thrum, Naturwiss. **44**, 561 (1957)]. In the system n-butanol-glacial acetic acid-water 4:1:5 (see FIG. 4) these antibiotics are still almost at the starting point after a running time of 10 hours. The test is carried out bio-autographically with Staphylococus aureus.

The purified ferrimycin A and its components A_1 and A_2 respectively react with 8-oxyquinoline in solution in methanol with the separation of a black colored crystalline precipitate of iron oxyquinoline. The iron-free ferrimycin A can be isolated from the solution as a yellowish powder. It is also obtained when ferrimycin A is treated with alkali or with strong mineralic acids. Desferrimycin A possesses one half of the activity in vitro of the starting material, and exhibits, apart from the absence of the iron band at 425 mμ, the same absorption spectrum as ferrimycin A. By the addition of ferric chloride its specific activity in vitro is doubled and the red color and iron absorption at 425 mμ return. Treatment of ferrimycin A with 2-m sodium acetate at room temperature for 24 hours leads to complete loss of the antibiotic activity. Acid hydrolysis of iron-free ferrimycin A_1 (desferrimycin A_1) yields, among other compounds, three moles of 1-amino-5-hydroxy-amino pentane, two moles of succinic acid and 1 mole of acetic acid. This means that the same products of hydrolysis found in desferrioxamine B occur also in desferrimycin A_1. Additionally desferrimycin A_1 contains a phenolic constituent. Upon hydrolysis of desferrimycin A_1 with 6-n. hydrochloric acid at room temperature there is obtained this phenolic compound bound to one 1 -amino-5-hydroxylamino-pentane residue, besides two moles of N-(5-hydroxylaminopentyl)-succinimide hydrochloride and one mol of acetic acid. The formula of

the phenolic hydrolysis product is $C_{21}H_{34}O_7N_6$, 3 HCl. It yields, on hydrolysis with 1-n. hydrochloric acid at 110°C, 1-amino-5-hydroxylamino-pentane-dihydrochloride, ammonium chloride and a phenolic aminocarboxylic acid hydrochloride of the formula $C_{16}H_{21}O_8N_3$, HCl. The latter yields, on hydrolysis with 6-n. hydrochloric acid at 110°C, the crystalline hydrochloride of 3-amino-5-hydroxybenzoic acid, $C_7H_7O_3N$, HCl. From the different hydrolysis products and their spectra the following formula can be deduced for ferrimycin A_1-dihydrochloride:



$C_{41}H_{67}O_{14}N_{10}Cl_2Fe$

Desferrimycin A_1-dihydrochloride gives the following values in elementary analysis:

C = 49.48%; H = 7.33%; N = 13.80%; CH_3CO = 7.33%; OCH_3 = 3.03%;

pK_{MCS} = 4.06 and 7.79; equivalent weight 1060 (potentiometric microtitration in methylcellusolve-water). $[\alpha]^{20}_D = -25°$ (in ethanol). On paperchromatography in the solvent system tertiary butanol-ethanol-0.01-N-hydrochloric acid (4:1:1) the Rf-value is 0.4.

These values, together with those for ferrimycin A_1, suggest the formula $C_{41}H_{68}O_{14}N_{10}$, 2HCl and the molecular weight 998 for the dihydrochloride of desferrimycin A_1. Desferrimycin A_1 shows in the UV-spectrum in ethanol maxima at $\lambda = 210$ (log $\epsilon = 4.57$); 233 (log $\epsilon = 4.38$) and 322 (log $\epsilon = 3.22$) mμ. In 0.01 N alcoholic sodium hydroxide there are maxima at 230 mμ (log $\epsilon^{1\%}_{1cm} = 2.61$) and 335 mμ (log $\epsilon^{1\%}_{1cm} = 1.54$) and a weak shoulder at 245 mμ (log $\epsilon^{1\%}_{1cm} = 2.48$). The IR-spectrum in liquid petrolatum shows bands at 845; 930 (shoulder); 975; 1002; 1022; 1057; 1125; 1161; 1195; 1225; 1259; 1305 (shoulder); 1378; 1466; 1550; 1612; 1677 (shoulder); 1715 cm^{-1} (cf. FIG. 7).

The NMR-spectrum in trifluoracetic acid is given in FIG. 8.

The antibiotic ferrimycin A and its components ferrimycin A_1 and ferrimycin A_2 and the corresponding iron-free compounds can easily be obtained in the form of the free base from a salt thereof, for example, from the sulfate, for example, by reaction in an aqueous medium with barium hydroxide, neutralization of the excess of baryta with carbon dioxide, separation of the precipitate of barium carbonate and barium sulfate, and isolation of the free base by freeze drying. A simple method of producing the base from its salts is to use a strongly basic anion-exchanger, for example, the OH-form of the product known in commerce as "Dowex"-2 (a polystyrene resin containing dimethylethanolamine).

One of the most remarkable properties of ferrimycin A and components is its pronounced stability minimum between pH values of 7 and 11. Solutions of the antibiotic at 20°C and at a pH value of 8–10 lose their activity in the course of 48 hours, whereas at the same temperature and at pH values of 1–5 they remain fully and at pH values 6–7 or greater than 11 partially active.

Salts of antibiotic ferrimycin A and its components and the corresponding iron-free compounds can be obtained from the known inorganic and organic acids, for example, hydrochloric acid, sulfuric acids and phosphoric acids, acetic acid, propionic acid, valeric acid, palmitic acid, or oleic acid, succinic acid, citric acid, mandelic acid, pantothenic acid, glutamic acid or other amino acids. They are neutral or acid salts. They can be prepared by the action of the corresponding acids on the free base or by the double decomposition of salts, for example, of ferrimycin-sulfate with calcium pantothenate.

Antibiotic ferrimycin A, ferrimycin A_1 and ferrimycin A_2 and the corresponding iron-free compounds have a very high antibiotic activity against various test organisms. In the so-called agar cross streak test they are active against the following test organisms: *Micrococcus pyogenes* var. aureus, *Streptococcus pyogenes*, *Streptococcus viridans*, *Streptococcus faecalis*, *Corynebacterium diphteriae*, *Escherichia coli*, *Bacillus megatherium* and *Bacillus subtilis*.

The bacteriostatic activity of the ferrimycins, for instance against Staphylococcus aureus, can be used as a measure for the activity of preparations of different degrees of purity. One unit is defined as the activity which in 20 ml of meat extract agar (contained in a Petri-plate of 9 cm diameter) inoculated with about 200 germs of Staph. aureus ATCC 6538 and incubated for 24 hours at 37°C suppresses 50% of the growth. The culture filtrate has an activity of 2,000–5,000 units/ml. The activity of the purest preparations of ferrimycin A was found to be 900,000 units/mg; that of ferrimycin A_1 1,000,000 ± 100,000 units/mg and that of ferrimycin A_2 1,100,000 ± 100,000 units/mg.

In the following Table are given the activities in vitro of purified ferrimycin products. There are given in millimeters the diameters of the zones of inhibition which are obtained in the agar plate test with paper discs of 6 millimeters diameter when impregnated with solutions of 0.1 percent strength:

Strain	Ferrimycin A	Ferrimycin A_1	Ferrimycin A_2
Staph. aur.	26	27	26.5
Strepto. faec.	8	9	9.5
Esch. coli	10.5	10.5	11
Shigella sonnei	13.5	14	14
Klebs.typ.A	23*	22.5*	22.5*
Past.pestis	12	12	12
Bac.megatherium	20	20	20
Ustilago sphaerogena	28	—	—
Ustilago scabiosae	24	—	—

*Cloudy zones of inhibition

Table I

	Infection with Staph. aureus s.c.	Staph. aureus p.o.	Strept. haemol. s.c.	Strept. haemol. p.o.	Pneumococc. III s.c.	Pneumococc. III p.o.
Ferrimycin A, A_1, A_2 *	0.1	5	0.1	3.3	0.33	3.3
Penicillin-G	1	3.3	5	3.3		
Erythromycin	5	100		10	50	

* or corresponding iron-free compound

Table 1: Doses (in mg/kg) of different antibiotics which cure 75–100% of the infected animals with five subcutaneous or oral doses within 30 hours (observation period at least 10 days).

In the paper disc test the diameters of the zones of inhibition of highly purified products were linearly dependent on the logarithm of the concentration of the antibiotic over a wide range of dilutions.

Desferrimycin A, desferrimycin A_1 and desferrimycin A_2 exhibit the same activities as the corresponding ferrimycins in a culture medium that contains small amounts of iron (III) salt whereby the iron-free compounds are converted to the ferriferous compounds.

Ferrimycin A, ferrimycin A_1 and ferrimycin A_2 and the corresponding iron-free compounds are also active in vivo. Mice were infected with virulent strains of *Staphylococcus aureus*, *Streptococcus haemolyticus* and *Diplococcus pneumoniae* Type III which is chemotherapeutically difficult to attack. As infection dose approximately 100 lethal doses were administered intraperitoneally in each case; in the untreated control animals this led in 100% to sepsis followed by death within 24–48 hours. Treatment was carried out

a. five times within 30 hours (first administration ½ hour before infection, then 3, 5, 21 and 30 hours after infection),
b. with single doses which were given immediately after infection.

In both series of experiments the preparation was administered both subcutaneously and per os. In general, at least 10 animals were used in each group. The result of the treatment was checked on the tenth day after infection and expressed as a percentage per group of the surviving animals. The results are put together in Tables 1 and 2.

In experiments on at least ten animals per group the difference between a treatment effect of 75–100% survival and a control group mortality of 100% (no survivors) fulfils the requirements of the 2×2 test for a significance of $P \leq 0{,}01$ (Mainland & Murray, 1952, Science 116:591–594). Thus, in both series of the above experiments, statistically significant data were obtained at the 1% level ($P \leq 0.01$).

From the above Table it is clear that of the three antibiotics investigated the ferrimycins or desferrimycins respectively are the most effective against *Staphylococcus aureus* and *Streptococcus haemolyticus* when administered subcutaneously, being quantitatively 10 to 50 times superior to penicillin and 50 to 100 times superior to erythromycin. When administered orally, the new antibiotics have about the same effect as penicillin, but are definitely superior to erythromycin.

Even when administered only once, the ferrimycins or desferrimycins respectively show a superior effect.

Table 2

	Staph. aureus s.c.	Staph. aureus p.o.	Strept. haemol s.c.	Strept. haemol p.o.	Pneumococc. III s.c.	Pneumococc. III p.o.
Ferrimycin A, A_1, A_2 *	0.33	10	10	10	10	33
Penicillin	10	33	25	50		
Erythromycin	100	100	25	100		

* or corresponding iron-free compound

Table 2: Doses (in mg/kg) of different antibiotics which effect a 75–100% cure when administered once.

The toxicity of the ferrimycins and desferrimycins is low. Thus, for example, mice tolerated the subcutaneous administration of 1000 mg or the oral administration of 3,000 mg per kilogram of body weight without suffering harm. Higher doses have not been tested.

Because of their good antibacterial activity and their low toxicity ferrimycin A, ferrimycin $A_1$and ferrimycin A_2 can be used as medicaments against infections caused by the above-mentioned microorganisms, especially infections caused by Staphylococci, Streptococci or Pneumococci. The compounds can be applied orally or parenterally, for instance subcutaneously or intramuscularly. Antibiotic ferrimycin A, its components ferrimycin A_1 and and ferrimycin A_2, its salts and derivatives are useful as medicaments, for example, in the form of pharmaceutical preparations. They can also be used as additives for animal feedstuffs or as disinfectants or preserving agents, The pharmaceutical preparations contain the active compounds in admixture with a pharmaceutical organic or inorganic carrier suitable for enteral, parenteral or local administration. For making the carrier there are used substances which do not react with the new compounds, for example, gelatine, lactose, starches, magnesium stearate, talc, vegetable oils, benzyl alcohols, gums, polyalkylene glycols, white petroleum jelly, cholesterol or other known carrier for medicaments. The pharmaceutical preparations

may be, for example, in the form of tablets, dragees, powders, salves, creams, suppositories or in liquid form as solutions, suspensions or emulsions. If desired, they may be sterilized and/or may contain auxiliary substances, such as preserving, stabilizing, wetting or emulsifying agents. They may also contain other therapeutically valuable substances.

FIG. 1 shows the IR-spectrum in potassium bromide of crude ferrimycin A - monohydrochloride;

FIG. 2 shows the IR-spectrum in potassium bromide of ferrimycin A_1 - dihydrochloride;

FIG. 3 shows the IR-spectrum in potassium bromide of ferrimycin A_2 - dihydrochloride;

FIG. 4 shows paper chromatography of various enriched ferrimycin products (1 = product of Ex. 7; 2 = product of Example 10; 3 = product of Example 13; 4 = ferrimycin A_1; 5 = ferrimycin A_2) in the systems

I butanol-glacial acetic acid-water 4:1:5; 10 hours

II butanol-butyl acetate-glacial acetic acid-water 100:13:143; 24 hours

III butanol-butyl acetate-glacial acetic acid-water 100:30:13:143; 60 hours;

FIG. 5 shows the UV-spectra (in water, $c = 5.10^{-3}$) of

ferrimycin A (Curve 1, Extinction E = A)

ferrimycin A_2 (Curve 2, Extinction E = A0.1)and

ferrimycin A_1 (Curve 3, Extinction E = A-0.3);

FIG. 6 shows the countercurrent distribution of crude ferrimycin (containing ferrimycin A and ferrimycin B) over 115 stages according to Craig. Extinctions at 425 mμ (iron color. curve 1) and at 570 mμ (ninhydrin color, curve 2) and in vitro activity (against Staph. aureus, curve 3) are indicated;

FIG. 7 shows the IR-spectrum in liquid petrolatum of desferrimycin A_1;

FIG. 8 shows the NMR-spectrum in trifluoracetic acid of desferrimycin A_1,

The following examples illustrate the invention:

EXAMPLE 1

Streptomyces griseoflavus NRRL 2717 is incubated by the submersion method. There is used a nutrient solution containing, per liter of tap water, 20 grams of soya bean meal and 20 grams of mannitol. The nutrient solution is sterilized in the inoculation flasks or fermenters for 20–30 minutes under 1 atmosphere pressure. The sterilized nutrient solution has a pH value of 7.5–8.0. The nutrient solution is inoculated with up to 10 percent of a partially sporulating vegetative culture of the organism. Incubation is carried out while stirring or shaking well at 27°C, the cultures in the fermenters being aerated with about 2 parts by volume of sterile air per volume of solution per minute. After incubating for 48–120 hours the culture solution has a high inhibiting value against test organisms (B. subtilis, B.megatherium, micrococcus pyogenes, var.aureus). The culture is interrupted and the pH value is adjusted to 4.5 by the addition of dilute sulfuric acid, and the mycelium and any other solid material is separated from the main body of the solution containing the antibiotic by filtration or centrifuging, 1% of a filtration assistant, for example, "Hyflo Supercel," being added if desired to the culture solution before filtration. The filter residue is washed with water and with aqueous methanol, and the washings are united with the culture filtrate.

By using instead of the above nutrient solution, solutions which contain, per liter of tap water, the following nutrient substances culture filtrates of similarly high antibiotic activity are obtained by incubation and working up in an analogous manner.

a)	Glucose	10 grams
	Soya bean meal	10 grams
	Sodium chloride	5 grams
	Sodium nitrate	1 gram
b)	Glycerine	20 grams
	Soya bean meal	10 grams
	Sodium chloride	5 grams
	Sodium nitrate	1 gram
	Calcium carbonate	10 grams
c)	Glucose	10 grams
	Soya bean meal	10 grams
	Corn steep liquor	20 grams
	Sodium chloride	5 grams
	Sodium nitrate	1 gram
	Calcium carbonate	10 grams
d)	Lactose	20 grams
	Distillers solubles	20 grams
	Sodium chloride	5 grams
	Sodium nitrate	1 gram

EXAMPLE 2

3 Liters of a culture filtrate obtained as described in Example 1, are adjusted to a pH value of 7.5 by the addition of a dilute solution of caustic soda, and 50 grams of active carbon (Norit A) are added. The whole is mechanically stirred for one hour, during which the whole of the antibiotically active substance is adsorbed by the carbon. The carbon is separated from the completely inactive solution by filtration, advantageously with a small amount of a filtration assistant, for example "Hyflo Supercel" (an infusorial earth). The carbon is then introduced into 500cc of a mixture of 4 parts by volume of water and 1 part by volume of pyridine, the mixture is mechanically stirred for ½ hour, and then filtered, the carbon residue being extracted once more in the same manner. The eluate contains the whole antibiotic activity.

EXAMPLE 3

3 Liters of a culture filtrate obtained as described in Example 1 are adjusted to a pH value of 7.5 by the addition of a dilute solution of caustic soda, and then the solution is immediately supplied at the rate of 0.5 liter per hour to a column of "Amberlite" IRC–50 (H-form) having a length of 30 centimeters and a diameter of 5 centimeters. The antibiotic is completely adsorbed by the column. The column is washed with 3 liters of water. For the purpose of elution there is used one liter of 0.4N-hydrochloric acid, the first 500 cc of the eluate are antibiotically inactive, whereas the second 500 cc contain the whole activity. In order to remove the excess of hydrochloric acid this active eluate is percolated through a column of Amberlite IR–4B (a weakly basic polystyrene resin containing polyamine exchange groups). By freeze drying the percolate the enriched antibiotic ferrimycin is obtained in the form of a highly active amorphous powder.

EXAMPLE 4

6 Liters of a culture filtrate obtained as described in Example 1 are adjusted to a pH of 7.5 by the addition of dilute caustic soda solution, and then immediately

percolated through a column of "Äsmit" 173, 14 cm long and having a diameter of 4.5 centimeters. The antibiotic is completely adsorbed. The adsorbant resin is then washed with 3 liters of water and the antibiotic is eluted, with 1 liter of a mixture of methanol and 1N-acetic acid (1:1). The eluate, which contains the whole of the active substance, is concentrated in vacuo at 35°C to 15 cc. 75 cc of a 0.25N-solution of hydrogen chloride in methanol is then added to the solution, and the mixture is poured into 10 liters of acetone, whereby the hydrochloride of the antibiotic is precipitated. The antibiotic is filtered off and washed with acetone. For the purpose of further purification it is dissolved in 150 cc of methanol, and the somewhat turbid solution is filtered with the addition of "Celite" (an infusorial earth). By evaporating the filtrate in vacuo at 25°C, the hydrochloride of antibiotic ferrimycin is obtained in the form of an amorphous powder.

EXAMPLE 5

30 Liters of a culture filtrate obtained as described in Example 1 are adjusted to a pH value of 4.5, and then concentrated to 2 liters in a thin-layer evaporator. The concentrate is adjusted to a pH value of 8 by the addition of dilute caustic soda solution, and the mixture is then filtered with the addition of Celite. The clear filtrate is adjusted to a pH value of 5, and 1.5 liters of a hot aqueous solution of picric acid of 5 percent strength are added while stirring. The precipitate so formed, after being allowed to stand for several hours is filtered off at 0°C with the addition of 50 grams of Celite. The filtrate has only a very slight antibiotic activity. The filter residue is then stirred three times with 800 cc of cold acetone each time and filtered. The filtrate is concentrated in vacuo to 80 cc, whereupon the picrate of the antibiotic and excess of picric acid precipitate out. After separation there are obtained 8.5 grams of dry substance.

In order to isolate the antibiotic in the form of its hydrochloride 2.5 grams of the aforesaid dry substance are dissolved in 30 cc of methanol. There are then added first a mixture of 1 cc of concentrated hydrochloric acid and 10 cc of acetone, and subsequently 500 cc of ether, whereupon the hydrochloride precipitates out quantitatively. By repeated dissolution of the precipitate in methanol acidified with hydrochloric acid and precipitation with ether the last residues of picric acid are removed. Finally, the hydrochloride is dissolved in as small a quantity as possible of methanol, and the solution is filtered and evaporated in vacuo. There are obtained 714 mg of the hydrochloride of antibiotic ferrimycin.

EXAMPLE 6

600 liters of a culture filtrate obtained as described in Example 1 are stirred with 5.5 kilograms of "Hyflo Supercel," adjusted to a pH value of 4 with 2.5 liters of 2N-HCl and then filtered. The filter residue is washed with 100 liters of water. The clear filtrate is stirred with 7 kilograms of pretreated Norit for 45 minutes. The pretreatment of the Norit is carried out by stirring several times with 1N-HCl and then washing neutral with water. The Norit loaded with antibiotic is filtered. The filtrate contains no antibiotic activity. The Norit adsorbate is twice washed with 200 liters of water by stirring and filtered each time. The washings contain no activity. Elution is carried out by stirring the Norit adsorbate twice for one hour with a mixture of n-butanol-methanol-glacial acetic acid and water (2:1:1:2), the active charcoal being separated by filtration. The combined eluates (140 liters + 46 liters) are mixed well with 96 liters of butyl acetate. The aqueous phase is separated and the organic phase is washed with 1.2 liters of water. The combined aqueous phase (65.5 liters) are washed in succession by stirring with 72 liters of a mixture of n-butanol-n-butyl-acetate (1:2), 48 liters of ethyl acetate and finally with 24 liters of ether. The organic phases are discarded. The remaining aqueous phase (44 liters) which contains the whole antibiotic activity is concentrated to a volume of 5.45 liters at a temperature of 30°C at the most. From this highly active, black-brown colored concentrate the antibiotic is obtained in the form of 509 grams of a brown powder by freeze drying. This materialhas a 30–50 times more specific antibiotic activity when compared with the culture filtrate (activity per weight unit of dry substance).

EXAMPLE 7

For the purpose of isolating antibiotic ferrimycin from 300 liters of a culture filtrate obtained as described in Example 1, 1 part by volume of Amberlite IRC 50 in the H-form is mixed mechanically with 2 parts by volume of Amberlite IRC 50 in the Na-form. 6.3 liters of this mixture are poured into a glass column. The ratio of the height to diameter of the resin filing is 8:1. The culture filtrate is adjusted to pH 4 with 2N HCl and percolated through the resin at a rate of flow of 0.2 liter per minute per liter of resin, an orange-brown zone being formed in the upper two-thirds of the column. The resin is then washed with 30 liters of water and with 60 liters of methanol of 80% strength. The runnings and the washings contain only little antibiotic activity. Elution is carried out in two portions with a total amount of 37 liters of a mixture of 8 parts by volume of methanol and 2 parts by volume of 1N HCl. Both portions are adjusted to pH 5 with 5N NaOH, combined and then concentrated to 2 liters at a temperature of at the most 30°C in a circulation evaporator. The aqueous concentrate is adjusted to pH 5.6 and filtered through Hyflo Supercel for the purpose of removing any insoluble material. The filtrate (2.3 liters) contains approximately the whole antibiotic activity of the culture solution. It is extracted in 4 portions with a total amount of 500 ml of a phenol-chloroform mixture containing 100 grams of phenol in 100 ml of chloroform. The aqueous phase is discarded. The phenol-chloroform extract (500 ml) is diluted with 1 liter of chloroform and extracted three times with 500 ml of 1/10 N-ammonium acetate buffer of pH 4.60 each time, colored antibiotically inactive impurities thus being removed from the organic phase. The chloroform phase is then extracted first with 300 ml and then twice with 100 ml of 1/10N HCl each time. The deep red colored acid extract containing the antibiotic is adjusted to pH 3.5 with potassium bicarbonate and re-extracted with four 50 ml portions of a phenol-chloroform mixture (100 grams : 100 ml). The phenol-chloroform extract is filtered through Celite. To the clear, red-colored filtrate (200 ml) 50 ml of water, 500 ml of ether and 300 ml of petroleum ether are added with vigorous agitation. After separating the aqueous phase, the organic phase is washed twice with 50 ml of water each time. The combined aqueous extracts are ex-

tracted twice with 500 ml of ether each time and once with 500 ml of benzene and then lyophilized. There are obtained 2.60 grams of an antibiotically highly active orange-brown colored powder. This material shows 500–1,000 times more specific antibiotic activity compared with the starting material (activity per weight unit of dry substance). Paper-chromatography of this material on Whatman No. 1 paper in n-butanol: n-butyl acetate: glacial acetic acid:water (10:3:1. 3:14.3) system reveals two spots after autobiographic development with Staphylococcus aureus. The slowly travelling antibiotic substance is designated Base A, the substance travelling 2.5 times more quickly Base B. Base A gives a blue color reaction on paper on being sprayed with ferric chloride and potassium ferricyanide.

EXAMPLE 8

338 Grams of an antibiotic preparation obtained as described in Example 6 are dissolved in 1.5 liters of water. 150 Grams of crystalline ammonium sulfate are added and the solution is extracted first with 1 liter and then twice with 500 ml each time of a phenol-chloroform mixture containing 100 grams of phenol in 100 ml of chloroform. The combined phenol chloroform extracts are extracted with 750 ml of 1n hydrochloric acid and then filtered through a layer of Celite. To the clear red-brown filtrate there are added 600 ml of water, 4 liters of ether and finally 4 liters of petroleum ether with stirring. The aqueous phase is separated and the organic phase extracted with 200 ml of water twice. The combined aqueous phases (1 liter) are washed twice with 1 liter of ether and then lyophilized, 136 grams of a brown powder are obtained which has a specific antibiotic activity twice as high as the starting material.

EXAMPLE 9

550 mg of highly active antibiotic preparation (Base A) obtained as described in Example 11 are dissolved in 55 ml of a 1/10N-ammonium acetate buffer having a PH value of 4.6 and extracted 4 times with 20 ml of a phenol-chloroform mixture containing 100 grams of phenol in 400 ml of chloroform. The organic extracts are re-washed twice with 15 ml buffer solution. The organic extract (80 ml) containing the antibiotic is diluted with 40 ml of chloroform, washed once again with 60 ml of buffer solution and then filtered through a double pleated filter. The deep red-colored filtrate is extracted successively with 30, 20, and 10 ml of 0.2N-hydrochloric acid. The acid solution containing the antibiotic is diluted with 50 ml of water and exhaustively extracted twice with 20 and then with 10 ml of a mixture of phenol and chloroform containing 100 grams of phenol in 100 ml of chloroform. The combined phenol-chloroform extracts are filtered through a double pleated filter and agitated with 20 ml of water, 200 ml of ether and 100 ml of petroleum ether. After separating the aqueous phase, the organic phase is reextracted twice with 15 ml of water. The combined aqueous extracts are washed twice with 50 ml of ether and once with 50 ml of benzene and then lyophilized. There are obtained 117 mg of a brown-red powder which has approximately five times the amount of antibiotic activity compared with the starting material.

EXAMPLE 10

700 mg of the antibiotic preparation obtained as described in Example 7 are chromatographed over 127 grams of Whatman No. 1 cellulose powder. For the purpose of elution there is used the upper phase of a mixture of 4 parts of butanol, 1 part of glacial acetic acid and 5 parts of water, to which are added 10% by volume of butanol. The substance is triturated with ten times the quantity of cellulose powder and put on the column as powder. The rate of running through is 15–20 ml per hour. Fractions of 40 ml are collected. The separate fractions are agitated with 50 ml of petroleum ether. The separated aqueous phase is washed with benzene and lyophilized. The major portion of the antibiotic activity is in the fractions 7–8 (121 mg) and 10–13 (142 mg). On being examined by paper-chromatography it is found that the fractions 7–8 contain chiefly Base B and fractions 10–13 primarily Base A.

EXAMPLE 11

3 Grams of an antibiotic preparation obtained as described in Example 8 are distributed over a hundred stages in a Craig's distributing apparatus in the secondary butanol —0.1N-ammonium acetate buffer system having a pH value of 4.68, each unit contains 100 ml upper phase and 100 ml lower phase and the substance being charged into unit No. 3. After distribution the content of each unit is worked up by adding double the volume of petroleum ether to the mixture of the two phases and freeze-drying the aqueous phase. The dark-colored fractions 3–11 show only little antibiotic activity. The orange-yellow colored fractions 12–20 (631 mg) contain the major portion of the antibiotic activity (chiefly Base A). The yellow-colored fractions 21–40 are less active and contain a mixture of Base A and Base B in which the latter predominates.

EXAMPLE 12

220 mg of an antibiotic preparation (Base A) obtained as described in Example 10 are distributed over 29 stages in a Craig's distributing apparatus in the 1/10N-ammonium acetate buffer (pH value 4.58) — 10% phenol in chloroform. Each stage contains 10 ml each of upper and lower phase. The major portion of the antibiotic activity is in fractions 6 – 15. The latter are combined (about 200 ml), 400 ml of ether and 300 ml of petroleum ether are added and the whole agitated. The separated, orange-red colored aqueous phase is washed with chloroform and extracted successively with 20 and three times with 10 ml of a mixture of 100 grams of phenol in 100 ml of chloroform; 20 ml of water, 300 ml of ether and 200 ml of petroleum ether are added to the phenol-chloroform extracts with agitation. The red-colored aqueous phase is separated, washed with much ether and benzene and lyophilized. There are obtained 80.4 mg of Base A in the form of a yellow water-soluble powder. Color reactions: $FeCl_3$: brown-red; $FeCl_3 + K_3Fe(CN)_6$: blue;

Ninhydrin: weakly positive.

Negative: Sakaguchi, Maltol, Elson-Morgan.

EXAMPLE 13

8 grams portions of an enriched ferrimycin product (main component A: activity in relation to the lyophilized culture filtrate = 2,000 to 3,000) such as is obtained for example, according to Example 10 were subjected in a vertical glass column having a length of 1 meter and a diamter of 6 centimeters which was filled

with cellulose powder and provided with a cooling jacket, to zonal electrophoresis by the method of J. Porath [Biochimica et Biophysica Acta, Vol. 22 page 151 (1956)]. As an electrolyte solution there was used ⅓ N-acetic acid. The product was dissolved in 160 ml of water, and the brown-red solution was poured on to the upper anode end of the column. At a voltage of 1,000 and a current of 100 milliamperes the orange antibiotic zone about 20 cm wide migrated towards the cathode at a speed of 3.3 cm per hour. In order to increase the separating action of the column this electric movement was exactly compensated by a stream of the electrolyte moving the opposite direction at the rate of 81 ml per hour, and in this way the antibiotic was held stationary in the same place in the column. Brownish colored inactive accompanying substances having higher or lower electrical migration speeds than that of the antibiotic were moved to the cathode zone or anode zone, and wahsed away from the zone either continuously or periodically. After a period of electrophoresis lasting 5–6 days the antibiotic had moved through a liquid column 4–5 meters long. The substance on the column was then eluted with electrolyte solution, and the eluate fractions collected in an automatic fraction receiver were tested biologically. The deep red colored biologically active fractions were combined (1.3 liters). From the acetic acid solution the antibiotic could be extracted after the addition of 75 ml of a saturated aqueous solution of sodium chloride, with 250 ml of a mixture of phenol and chloroform (1g:1ml). After filtering the extract through Celite, the antiobiotic could be precipitated as an orange colored precipitate on 16 grams of Hyflo Supercel by the addition of 1 liter of ether and 500 ml of petroleum ether. The mixture of the precipitate and filtration assistant was washed well with acetone and finally extracted with a small amount of cold methanol. From the methanol extract the crude ferrimycin A monohydrochloride was precipitated with acetone in the form of an orange-colored powder. After being dried at room temperature under 0.001 mm pressure for two days the product contained approximately 100% of the original activity in a form enriched four to six times. Analysis: C = 50.68%; H = 6.99%; N = 13.45%; Fe (gravimetric) = 3.55%; Fe (colorimetric) = 3.66%; Cl = 2.75%; 2.46% drying loss at 120°C under 0.01 mm pressure.

Infra-red spectrum in potassium bromide; see FIG. 1. It shows bands at 2.97; 3,43; 6.05; 6.30; 6.92; 7.25; 7.98; 8.25; 8.40; 8.95; 13.20 μ.

Ultra-violet-spectrum in water; maximum at 318 mμ ($E^{1\%}_{1cm} = 47.2$); inflexions at 229 mμ and at 400 mμ. Solubility: dissolves very well in water, methanol and mixtures of phenol and chloroform, soluble in dimethylformamide, methyl-cellosolve, benzyl alcohol and glacial acetic acid, sparingly soluble in ethanol and practically insoluble in pyridine, propanol, butanol and the usual organic solvents, especially lipoid solvents.

Precipitation reactions: precipitatable from aqueous solution by means of picric acid, picrolonic acid, and ammonium reineckate.

Color reactions: orange-red aqueous solutions change color upon the addition of mineral acid reversibly through claret red to pale yellow. Decoloration is likewise caused by caustic soda solution. Neutral solutions do not react with potassium ferrocyanide. Acidified solutions give colorations or precipitations of Prussian blue. Trivalent iron bound in complex union is liberated as the pH-value decreases.

Ferric chloride produces a claret red coloration and a mixture of iron chloride and potassium ferricyanide gives a blue coloration. The following tests are negative: Molish, Anthron, Folin Ciocalteu, Sakaguchi. The ninhydrin reaction in butanol-pyridine is weakly positive after heating for a long time. Hydrolysis with 6N-hydrochloric acid yields a mixture of about 15 substances detectable by paper chromatography.

Iron-free ferrimycin A:

100 mg of the purified ferrimycin A so obtained were dissolved in 1 ml of methanol. 334 mg of o-oxyquinoline in 2ml of methanol were added and the whole was allowed to stand at room temperature for 8 hours. After allowing the whole to stand for a further 15 hours at 0°C the precipitated black-green crystals of iron 8-oxyquinoline were separated. The solution was diluted with a small amount of water and extracted by thorough agitation with chloroform and benzene to remove the excess of oxyquinoline. The remaining pale yellow aqueous phase, which contains the iron-free antibiotic was lyophilised: 90 mg of a beige powder. The iron-free antibiotic exhibited the same absorption spectrum in the ultraviolet region as the starting material, but in the visible region it lacked the flat band at 400–430 mμ. It had only ⅓ of the activity in vitro of the starting material. A colorless solution of the iron-free antibiotic is instantaneously colored deep red on the addition of ferric chloride, whereupon the iron absorption at 400–430 mμ in the spectrum returns and the specific antibiotic activity is increased.

EXAMPLE 14

800 mg of a preparation of ferrimycin A obtained as described in Example 13 were chromatographed over a cellulose column measuring 3 × 65 cm (198 grams) at 12°C. As flowing agent there was used the system tertiary butanol/0.001N hydrochloric acid/saturated aqueous NaCl-solution (2:1:1). The fractions collected in an automatic fraction receiver amounted to 30–40 ml and were examined biologically, spectroscopically and by paper chromatography. The fractions 60–104 contained 191 mg of ferrimycin A_1, fractions 105–200 contained 92 mg of ferrimycin A_2. The correspondingly unified fractions were agitated with an equal volume of petroleum ether and with about 10% by volume of water, whereby the antibiotic substance was driven into the aqueous phase. From the latter it was worked up by the method described in Example 13 with a mixture of phenol and chloroform and was obtained in the form of an orange powder. After being dried at 25°C under 0.001 mm pressure for 50 hours over P_2O_5/KOH the product had the following properties: Ferrimycin A_1 dihydrochloride: Analysis (average from three determinations): C = 46.71%; H = 6.80%; Fe = 5.33%; N = 12.70%; Cl = 6.76%; O (calc.) = 21.70%. Titration in 80% methylcellosolve; equivalent weight: 1078; pK. = 4.11; 7.92 and 11.4. Ultra-violet spectrum:λmax 229 mμ ($E^{1\%}_{1cm} = 336$), 319 mμ ($E^{1\%}_{1cm} = 37$) and 425 mμ ($E^{1\%}_{1cm} = 27.6$). Infra-red spectrum potassium bromide: see FIG. 2. It shows bands at 3.00; 3.44; 3.51; 6.08; 6.31; 6.90; 7.10; 7.35; 7.45; 7.95; 8.41; 9.00; 10.25; 11.85; 13.20 μ.

Ferrimycin A_2-dihydrochloride: Analysis: C = 45.78%; H = 6.77%; N = 12.75%; Cl = 6.23%; Fe = 5.29%; titration in 80% methyl-cellosolve; molecular weight: 1086; pK: 4.04, 7.91 and 11.05. Ultra-violet spectrum:λ max. 227 mμ ($E^{1\%}_{1cm} = 332$), 319 mμ ($E^{1\%}_{1cm} = 37$) and 425 mμ ($E^{1\%}_{1cm} = 25$). Infra-red

spectrum (in potassium bromide): see FIG. 3. The paper chromatography of various enriched and purified ferrimycin products on Whatman No. 1-paper is shown in FIG. 4. The test was made bio-autographically with Staphylococcus aureus.

In FIG. 4 the symbols have the meanings:

I: System butanol glacial acetic acid-water 4:1:5; 10 hours

II: System butanol-butyl acetate-glacial acetic acid-water 100:30:13:143; 24 hours

III: System butanol-butyl acetate-glacial acetic acid-water 100:30:13:143; 60 hours.

1: Antibiotic ferrimycin, base A+B, according to Example 7 (1 μg)

2: Antibiotic ferrimycin B, according to Example 10 (5 μg)

3: Antibiotic ferrimycin A according to Example 13 (0.1 μg)

4: Antibiotic ferrimycin Al, according to this Example (0.05 μg)

5: Antibiotic ferrimycin A2, according to this Example (0.05 μg).

EXAMPLE 15

6 grams of ferrimycin product having about 1,000 times the antibiotic activity of the lyophilized culture filtrate and containing ferrimycin A as well as ferrimycin B, are distributed by countercurrent over 115 stages according to Craig. The apparatus consists of 120 units. It is filled per unit with 100 cc of upper phase and 100 cc of lower phase of a mixture, equilibrium at 19°C, of benzyl alcohol (200 parts by volume), n-butanol (100 parts by volume), N-hydrochloric acid (6 parts by volume), water (300 parts by volume) and aqueous sodium chloride solution (60 parts by volume) saturated at 19°C. The first two units merely contain solvent. In each of the following three units 2 grams of substance are introduced and the whole distributed 115 times at 19°C. The number of shakes per distribution is 30, the duration of intervals 15 minutes.

When the distribution is complete, the resulting 118 fractions are kept at −10°C. From every third fraction there are taken for test purposes 10 cc of the upper and 10 cc of the lower phase which are agitated with 50 cc of petroleum ether. The separated aqueous phase (10 cc) which now contains all the hydrophilic material is freed from any adhering organic solvent by brief evacuation. The resulting test solutions are used on the one hand for colorimetric evaluation (extinction at 425 mμ in 1 cc cuvette. Compare FIG. 6, curve 1) and for the ninhydrin color reaction. To carry out the latter 0.5 cc of test solution and 0.5 cc of ninhydrin reagent, prepared is described by S. Moore and W. H. Stein, J. Biol. Chem. 211, 907 (1954), are mixed, heated at 100° C for 15 minutes, diluted with 5 cc of a mixture of alcohol and water (1:1) and then measured in a spectrophotometer at 570 mμ (curve 2). For biological tests the test solutions are diluted 1:50. In curve 3 there are shwon the in vitro activities against *Staphylococcus aureus* in the plate diffusion test in relation to an arbitrary standard. The active fractions 40–70 contain, as can be shown by paper chromatography, ferrimycin A, whilst ferrimycin B is in fractions 75–100. A considerable enrichment can be achieved. The antibiotically active fractions 25–39, 40–48, 49–55, 56–70 and 71–95 are combined and agitated with the same volume of petroleum ether. The red colored substances are driven into the aqueous phases. From the latter they are isolated with phenol-chloroform in the manner already described. The quantities obtained and the enrichment achieved in relation to the starting material and yields of antibiotic activity are put together in the following table:

Fraction	Quantity in mg	Enrichment factor	Yield of activity	Ferrimycin
25–39	492	0.55	4.6 %	
40–48	254	4.77	20.4 %	
49–55	211	9.22	32.8 %	A
56–70	257	3.49	15.1 %	
71–95	938	1.72	27.2 %	B

The starting material is obtained as follows:

30 liters of an aqueous eluate concentrate obtained as described in Example 7 by elution of ferrimycin from Amberlite IRC 50 and subsequent removal of the methanol in vacuo, are mixed with 1 kg of Hyflo with stirring and then in the course of 2 hours with 1.8 liters of a mixture of 11 parts of phenol and 1 part of water. Stirring is then continued for half an hour, and the mixture is then suction-filtered. The filtrate contains about 2–5 percent of the activity and is discarded. The well squeezed filter cake is stirred twice with 4 liters of ether each time and once with a mixture of 8 liters of acetone and 2 liters of ether and suction-filtered on each occasion, whereupon the residue is washed with succession on the filter with 5 liters of acetone and 4 liters of chloroform. All the washings are practically inactive. The chloroform-moist filter-cake is then introduced into 3 liters of a mixture of phenol and chloroform 1:1 (weight/volume) and stirred for 1 hour. In the course of 1 hour 15 liters of chloroform are added in a uniform current. The Hyflo is suction-filtered and washed twice with 2 liters of phenol-chloroform 1:11 (weight-/volume) and once with 2 liters of chloroform. The residue contains 5–10% of the activity.

The combined active filtrate (22 liters) are concentrated to 4 liters at 25°C and the concentrate added dropwise in the course of 30 minutes to a mixture of 4 liters of ether, 8 liters of petroleum ether and 400 grams of Hyflo. After another 30 minutes the mixture is suction-filtered. The filter-cake is washed with 2 liters of ether and twice with 1 liter of acetone. All the filtrates contain only traces of activity.

The filter-cake is stirred three times for 10 minutes with 1.5 liters of methanol each time, suction-filtered and finally washed with 0.5 liter of methanol on the filter. The washed Hyflo does not contain any activity.

The dark brown filtrates (4 – 4.5 liters) are evaporated to dryness cautiously at 20°–30°C in a water-jet vacuum. The still sticky residue is dried in a high vacuum for 20 hours. 75–88 grams of a red-brown strongly active ferrimycin product are obtained. The yield of activity calculated on the starting material is 90%.

EXAMPLE 16

2 grams of a ferrimycin A product (fractions 49–55) obtained as described in Example 15 which has about 9,000 times the antibiotic activity of the lyophilized culture filtrate, are chromatographed on a 70 cm × 7.14 cm^2 column of a strongly acid ion exchanger Dowex 50 WX_2 (100/200 mesh). The ion exchanger is first purified according to Hirs et al., J.Biol.Chem 219,

(1956), 623 and after conversion into the ammonium form equilibrated for 3 days with 0.2 molar ammonium acetate buffer having a pH value of 4.50 at 17°C at a flow speed of 100 cc/h. The substance is then dissolved in 20 cc of a buffer solution, cautiously poured on to the column and then eluted with the same buffer solution and at the same speed. After an elution time of 20 hours, gradient elution is performed. The 0.2 molar ammonium acetate buffer entering the column is continuously concentrated in a 1 liter mixing chamber by an equal addition of 2-molar ammonium acetate buffer of pH 4.50 and thus continuously strengthened in its eluting effect. The eluate is collected in fractions of 40 cc in an automatic fraction collector. The evaluation of the chromatogram is carried out by measuring the extinction at 425 mμ, by paper chromatography and biological tests. The fractions combined after this evaluation are worked up with phenol-chloroform as described in Example 13, with the variation that the phenol-chloroform extracts were washed with 0.01 N-hydrochloric acid. This resulted in the formation of dihydrochloride instead of monohydrochloride: From fractions 195–216, 440 mg of ferrimycin A in the form of a brown-red powder are obtained which contains about 16,000 times the antibiotic activity of the lyophilized culture filtrate.

Microanalysis:

C: 48.65%, H: 7.09%, N: 12.95%,
Fe: 4.56%, Cl: 6.10%, (C)CH_3:1.99%
(Roth-Kuhn, Amino-N: 2.33% (Van Slyke).

Titration:

pK^*_{MCS} (Helv. 37. 1872 (1954): 4.18; 7.88
Equivalent weight: 1106

Absorption spectrum:

λ max 228 mμ, $E^{1\%}_{1cm}$ 282
max 319 mμ, $E^{1\%}_{1cm}$ 28.2
max 425 mμ, $E^{1\%}_{1cm}$ 22.6

Reduction value according to C.S. Hanes, Biochem. J.23, 99 (1929): 1.7 ml/1/100 N-sodium thiosulfate/mg
Bound hydroxylamine according to T. Emery and J. B. Neilands, Nature 184, 1632 (1959): 0.83 mol NH_2OH per atom Fe.

Hydrolysis: with 6N HCl 14 hours at 110°C; detected or isolated products: HN_3, cadaverin, 1-amino-5-hydroxylamino-pentane, 6-aminovaleric acid, proline, succinic acid, crystalline substance ($C_7H_8O_3N.HCl$) with λ max 227 and 323 mμ and traces of further ninhydrin-positive substances and $FeCl_3$.

From the fractions 115–145 of the chromatogram 135 mg of a mixture of violet-red colored substances having the activity of vitamin B_{12} can be isolated. The remainder of the starting material is still where it was originally placed in the form of brown colored bands and is not eluted under the conditions given.

EXAMPLE 17

1.142 g of ferrimycin A_1 are dissolved in 10 ml of water and the solution mixed with 5 ml of a saturated solution of 8-hydroxyquinoline in methanol. The mixture is stirred for 20 hours at room temperature, the black precipitate formed (iron oxinate) filtered off, and the yellowish filtrate evaporated under reduced pressure, 107 mg of the crude product is purified by chromatography over a column of 40 g of cellulose powder, a 4:1:1 mixture of n-butanol/glacial acetic acid/water being used as eluant. The main fraction moves as a blue, fluorescent band and can be separated from a small amount of impurities. It yields 0.046 g of a colorless amorphous powder. This compound, which according to paper chromatography is a unitary compound, is dissolved in N-hydrochloric acid and evaporated for conversion into the dihydrochloride, which is likewise amorphous.

Analysis of the latter reveals the following data: C 49.48%; H 7.33%; N 13.80%; OCH_3 3.03%; CH_3CO 7.33%. Paper chromatography with n-butanol/glacial acetic acid/water 4:1:1, coloration with Pauly reagent Rf 0.43. Potentionmetric microtitration in methylcellosolve+water: pK^*_{MCS} 4.06 and 7.79; equivalent weight: 1060; UV absorption spectrum in rectified alcohol:

210 mμ : high end absorption,	log ε = 4.57
233 mμ : pronounced shoulder	log ε = 4.38
322 mμ : maximum	log ε = 3.22

UV absorption spectrum in 0.01N-alcoholic NaOH:

230 mμ	: maximum	log	$E^{1\%}_{1cm}$	2.61
245 do.	: weak shoulder	do.	do.	2.48
335 do.	: maximum	do.	do.	1.54

IR spectrum in liquid petrolatum; bands at 845; 930 (shoulder); 975; 1002; 1022; 1057; 1125; 1161; 1195; 1225; 1259; 1305 (shoulder); 1378; 1466; 1550; 1612; 1677 (shoulder); 1715 cm^{-1}, see FIG. 7.

NMR spectrum in trifluoroacetic acid see FIG. 8.

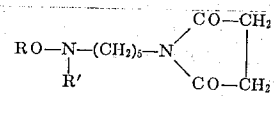
Weakly acid hydrolysis

2.4 g of desferri-ferimycin A_1 are heated to 95°C for 20 minutes with 50 ml of N-hydrochloric acid. After this time, the initially strong iron-chloride reaction of the solution (violet coloration) disappears completely. The hydrolysis mixture is evaporated to dryness under reduced pressure and the residue chromatographed over cellulose powder.

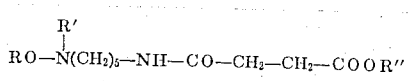
Column: 350 g of cellulose powder "Whatman Standard grade." Diameter of column 4 cm; height, 70 cm. Eluant: tertiary butanol/0.006N-hydrochloric acid/saturated aqueous NaCl solution 4:1:1.

The packed column is washed with the eluant 48 hours before use. The substance is then dissolved in about 20 ml of the eluant, poured over the column and eluated at the rate of about 15 ml/h. Fractions of about 8 ml are collected.

The first fractions yield about 100 mg of crystalline succinic acid. After that, 860 mg of a mixture is obtained which consists of a little N-(5-hydroxylaminopentyl)-succinimide (I) and a large amount of substance III.



I : R = R′ = H
II: R = R′ = CH_3CO

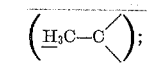


III: R = R′ = R″ = H
IV : R = R′ = CH_3CO; R″ = CH_3

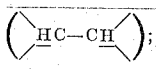
Stepwise hydrolysis of desferrimycin A_1

A solution of 1.75 grams of desferrimycin A_1-dihydrochloride in 100 ml of 6-n. hydrochloric acid is left at room temperature for 14 hours and then evapo-

rated at about 10 mm Hg. The residue is subjected to chromatography on cellulose powder with tert. butanol-ethanol-0.01-n. hydrochloric acid (4:1:1). 0.8 gram of N-(5-hydroxylaminopentyl)-succinimide hydrochloride, Rf = 0.6 (colored with triphenyltetrazolium chloride) are obtained. Further elution with the same solvent system yields 0.96 gram of the phenolic product of the formula $C_{21}H_{34}O_7N_6$, 3 HCl; Rf = 0.08 (coloration with Pauly reagent); $[\alpha]^{20}_D = -45°$ (in ethanol); pK in 80% methylcellosolve = 3.88; 5.57; 7.79; UV-spectrum in ethanol: λ_{max} = 216 (log ϵ = 4.44); 234 (log ϵ = 4.52); 324 (log ϵ = 3.41) mμ; IR-spectrum in potassium bromide: main bands at 3,420(vs); 2,940(vs); 1,715(vs); 1,635(sh); 1,595(vs) cm^{-1}; NMR-spectrum in D_2O: δ = 1.77

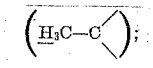


3.74 (H_3CO—); 3.82 (—H_2CO—); 1.0–2.3; 3.0–3.6; 3.75–4.22

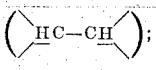


6.5–6.9 (3HC_{aryl}) ppm.

0.50 g of product $C_{21}H_{34}O_7N_6$,3 HCl is heated in a fused tube with 10 ml of 1-n. hydrochloric acid for 18 hours to 110°C. The residue is chromatographed on cellulose powder with the above-mentioned solvent system. There are eluted first 105 mg of 1-amino-5-hydroxylamino-pentane-dihydrochloride (Rf = 0.13, colored with ninhydrin), then 35 mg of ammonium chloride and finally 115 mg of a phenolic aminocarboxylic acid $C_{16}H_{21}O_8N_3$, HCl (Rf = 0.28, coloration with Pauly-reagent); $[\alpha]^{20}_D = -58°$ (in ethanol); elementary analysis: C = 46.22%; H = 5.78%; N = 9.84%; Cl = 8.02%; O (cal.) = 30.1%; UV-spectrum in ethanol: λ_{max} = 216 (log ϵ = 4.23); 236 (log ϵ = 4.26); 335 (log ϵ = 3.30) mμ; IR-spectrum in potassium bromide, main bands at 3,400(vs); 2,950(sh); 1,740–1,770(s); 1,680–1,710(m); 1,595–1,620(s) cm^{-1}; NMR-spectrum in D_2O: δ = 1.69

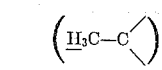


3.66 (H_3CO—); 3.78 (—H_2C-O—); 3.52–4.10

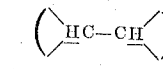


6.6–7.0 (3 HC_{aryl}) ppm.

100 mg of compound $C_{16}H_{21}O_8N_3$, HCl are esterified in methanolic solution with diazomethane, the solvent evaporated and the residue acetylated with acetic anhydride in pyridine. The crude product obtained on evaporation is dissolved in chloroform and the solution washed with sodium bicarbonate and with water. After evaporation of the solvent the residue is chromatographed on silica gel. The **0,0'**-diacetyl-N-acetyl-methyl ester of the formula $C_{23}H_{25}O_{11}N_3$ is eluted with 8% ethanol in methylene chloride. It crystallizes from methanol and ether. F. 249°–251°C. Elementary analysis: C = 53.07%; H = 5.85%; N = 8.04%; O (calc.) = 33.04%; UV-spectrum in ethanol: λ_{max} = 231 (log ϵ = 4.36); 256 (log ϵ = 3.98); 337 (log ϵ = 3.34). IR-spectrum in heavy petrolatum: main bands at 3,350(s); 3,260(s); 3,070(m); 1,770(vs); 1,705(vs); 1,655(s); 1,610(s); 1,590(m) cm^{-1}. NMR-spectrum (in trifluoracetic acid): δ = 1.75



2.09 (H_3CCON); 2.24 (H_3CCOOC_{aliph}); 2.51 (H_3CCOOC_{aryl}); 3.77 (H_3COOCC_{aliph}); 4.18 (H_3COOCC_{aryl}); 4.4 (—H_2CO); 4.4–5.4



7.5 – 7.9 (3 HC_{aryl}); 8.0 (—HN-CO—); 8.9 (HN-CO) ppm.

When compound $C_{16}H_{21}O_8N_3$, HCl is hydrolysed with 6-n. hydrochloric acid for 18 hours at 110°C and the crude hydrolysis product chromatographed on cellulose powder with tert. butanol-saturated sodium chloride solution-0.07-n. hydrochloric acid (4:2:1), there is obtained 3-amino-5-hydroxybenzoic acid in the form of needles (from ethanol and ethylacetate) which decompose at 200°–230°C.

EXAMPLE 18

For oral administration, capsules are prepared having the composition:

ferrimycin A_1-dihydrochloride	25 mg
stearic acid	7 mg
talc	10 mg
lactose ad	100 mg

EXAMPLE 19

An injection vial is prepared containing 50 mg of ferrimycin A_1-dihydrochloride.

Method:

The ferrimycin-dihydrochloride is dissolved in water, the solution filtered under sterile conditions, put into sterilized injection vials under aseptic conditions and lyophilized in the conventional manner. The vials are sealed under aseptic conditions.

What is claimed is:

1. Ferrimycin A_1 of the formula $C_{41}H_{65}O_{14}N_{10}Fe$ having pK_{MCS} values of 4.11, 7.87 and 11.4 and exhibiting the following maxima in the ultraviolet spectrum: λ_{max} 229 mμ($\epsilon_{1cm}^{1\%}$ = 336), 319 mμ($\epsilon_{1cm}^{1\%}$ = 37) and 425 mμ($\epsilon_{1cm}^{1\%}$ = 27.6) and the IR-spectrum in potassium bromide shown in FIG. 2.

2. Ferrimycin A_2 of the formula $C_{41}H_{68}O_{14}N_{10}Fe$ having pK_{MCS} values of 4.04, 7.91 and 11.05 and exhibiting in the ultraviolet spectrum the following maxima: λ_{max} 227 mμ($\alpha_{1cm}^{1\%}$ = 332), 319 mμ($\epsilon_{1cm}^{1\%}$ = 37) and 425 mμ($\epsilon_{1cm}^{1\%}$ = 25) and the IR-spectrum in potassium bromide in FIG. 3.

3. Desferrimycin A_1 having the empirical formula $C_{41}H_{68}O_{14}N_{10}$, having pK_{MCS} values of 4.06 and 7.79 and $[\alpha]_D^{20} = -25°$ (in ethanol) and exhibiting in the ultraviolet spectrum the folloiwng maxima: 210 mμ(log ϵ = 4.57), 233 mμ(log ϵ = 4.38) and 322 mμ(log ϵ = 3.22) and having the IR-spectrum in liquid petrolatum shown in FIG. 7 and the NMR-spectrum in trifluoracetic acid shown in FIG. 8.

4. A process for producing ferrimycin A, which comprises cultivating Streptomyces griseoflavus NRRL 2717 in an aqueous nutrient medium containing a source of carbon and nitrogen and inorganic salts under aerobic conditions at 18°–40°C until the nutrient medium exhibits a substantial antibacterial activity, and then isolating the ferrimycin A from the culture filtrate.

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