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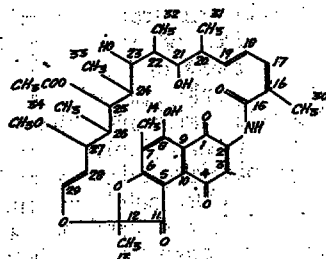
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COMPLETE SPECIFICATION

Antibiotically Active Derivatives of Rifamycin S and Rifamycin SV and Process for their Manufacture

5 We, CIBA LIMITED, a body corporate organised according to the laws of Switzerland, of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

10 The present invention provides anti-biotically active compounds which are 3-substituted derivatives of (a) rifamycin S of the following formula



15 or (b) rifamycin SV (the hydroquinone corresponding to the quinone of the above formula)

20 or (c) a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being a free or aliphatically substituted amino group, that is to say, an amino group substituted by one or two monovalent hydrocarbon radicals of aliphatic character, which may be the same or different, or by a divalent hydrocarbon radical of aliphatic character, which hydrocarbon radicals of aliphatic character may be interrupted in the carbon chain by one or more hetero atoms and may be unsubstituted or substituted. The expression "hydrocarbon radical of aliphatic character" is used in this

Specification to mean a hydrocarbon radical in which any carbon atom which is directly bound to the nitrogen atom of an amino group or an amine is not a member of an aromatic system.

The aliphatically substituted amino group in position 3 may be a secondary amino group, but it is preferably a tertiary amino group.

40 The said hydrocarbon radicals of aliphatic character may be interrupted in the carbon chain by one or more hetero atoms such as, for example, oxygen, nitrogen or sulphur atoms, and/or may be substituted by one, two or more identical or different functional groups, such as, for example, hydroxyl groups, halogen atoms, free or esterified carboxyl or sulphonic acid groups, aldehyde groups or nitrile groups or amino groups.

50 Advantageously, the hydrocarbon radicals of aliphatic character contain not more than 12 carbon atoms, and preferably contain from 1 to 8 carbon atoms.

In the following description the term "lower", whenever used in this Specification to describe an aliphatic radical such as, for example, an alkyl, alkenyl, or alkoxy radical, is intended to define such a radical as having up to 8 carbon atoms.

60 Particularly suitable hydrocarbon radicals of aliphatic character are saturated or mono-unsaturated radicals, for example lower alkyl, alkenyl, alkylene, cycloalkyl, cyclo-alkenyl, cycloalkyl-alkyl or cycloalkenyl-alkyl radicals, or phenylalkyl radicals such as, for example, phenyl - lower alkyl radicals in which the phenyl residue may be unsubstituted or monosubstituted or substituted by two or more identical or different substituents, such as lower alkyl, lower alkoxy groups, halogen atoms or carboxyl groups. As examples of hydrocarbon radicals of aliphatic character there may be mentioned

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methyl, ethyl, β - hydroxy - ethyl, allyl, methallyl, propyl, isopropyl groups; linear or branched butyl, pentyl, hexyl or heptyl groups which may be bound to the nitrogen atom of the main group through any one of their constituent carbon atoms; ethylene - (1,2), butylene - (1,4), pentylene - (1,5), hexylene - (1,6), hexylene - (1,5), hexylene - (2,5), heptylene - (1,7), heptylene - (2,7), heptylene - (2,6), cyclopentyl, cyclohexyl, cyclopentenyl, cyclohexenyl, cyclopentyl, cyclohexyl-, cyclopentenyl-, cyclohexenyl - methyl or -ethyl residues, benzyl, para-chlorobenzyl, para-methoxybenzyl, 1-phenylethyl and 2-phenylethyl groups. Hydrocarbon radicals of aliphatic character which are interrupted by hetero atoms are, for example, oxaalkyl and oxaalkylene residues, for example 3-oxabutyl, 3-oxapentyl, 3-oxaheptyl, 3 - oxapentylene - (1,5) and 3 - oxahexylene - (1,6).

Of special value are those compounds of the invention in which the aliphatic hydrocarbon radical or radicals of the amino groups in position 3 are interrupted by nitrogen, and more especially compounds in which two substituents of the main nitrogen in position 3 form together with it a diaza-cycloalkyl group comprising from 6 to 9 ring members, in which the nitrogen atoms are separated by at least 2 carbon atoms.

The diazacycloalkyl group may be substituted on the carbon atoms by one or more identical or different lower alkyl groups, preferably by methyl or ethyl, and/or on the N' - nitrogen atom by, for example, a lower alkyl or hydroxy-alkyl radical, such as, for example, a methyl, ethyl, propyl or isopropyl radical; or a linear or branched butyl, pentyl or hexyl residue which may be bound to the N' - nitrogen atom through any one of their constituent carbon atoms; or by a β - hydroxyethyl or β,γ - dihydroxypropyl group, by a lower alkoxyalkyl group such as, for example, a methoxy- or ethoxy-ethyl or -propyl group, by a carbalkoxy group or by a phenyl or phenyl-lower alkyl group such as, for example, a benzyl- or (1)- or (2)-phenylethyl residue which may be substituted in the aromatic rings by one or more identical or different substituents, for example alkyl or alkoxy groups and/or halogens. Advantageously, the diazacycloalkyl group is a piperazino group which may be substituted as indicated above, being more especially a methyl-, ethyl- or β - hydroxyethyl - piperazino group.

If a monovalent aliphatic hydrocarbon radical which substitutes the amino group in position 3 carries an amino group, especially a tertiary amino group, the amino group is advantageously a di-lower alkylamino-, pyrrolidino-, piperidino- or morpholino-group, or a piperazino-lower alkyl, e.g. an -ethyl-, -propyl or -butyl group (which may

be N-substituted as indicated above for the N'-atom of the diazacycloalkyl group). Therefore, the substituent in position 3 may be, for example, a dimethylamino - ethylamino, diethylamino - ethylamino, diisopropylamino-ethylamino, dipropylamino - ethylamino, dimethylamino - propylamino, diethylamino - propylamino, pyrrolidino - ethylamino, morpholino - propylamino or N - methyl - piperazino - ethylamino group.

The compounds may also be present in the form of their tautomers.

Most of the 3 - aminoquinones of the invention are crystalline compounds of violet red colour; they are insoluble in water, but readily soluble in most organic solvents, such as, for example, alcohols, halogenated hydrocarbons, esters, and dioxane. They can be reduced by reducing agents such as, for example, hydrogen sulphite, dithionite or, preferably, by ascorbic acid to form yellow-coloured hydroquinones which, generally, are crystalline. The hydroquinones can form metal salts, especially alkali metal salts whose aqueous solutions are of approximately neutral reaction. In an alkaline solution the hydroquinones are very readily transformed into the quinones.

The quinones may likewise be present in the form of their alkali metal salts, provided they carry acidic groups on the 3 - substituent. Quinones and hydroquinones that carry a basic group on the 3 - substituent form acid addition salts and quaternary ammonium salts on treatment with acids reactive derivatives of acids, especially on treatment with esters of lower alkanols with hydrohalic acids, sulphuric acid or sulphonic acids. Preferred addition salts are the physiologically tolerable salts. Examples of acids giving physiologically tolerable salts are hydrohalic acids, sulphuric and phosphoric acids, nitric and perchloric acid; aliphatic, alicyclic, aromatic or heterocyclic carboxylic or sulphonic acids such as, for example formic, acetic, propionic, succinic, glycolic, lactic, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic or pyruvic acid; phenylacetic, benzoic, para-amino-benzoic, anthranilic, para-hydroxy-benzoic, salicylic or para - aminosalicylic acid, embonic, methanesulphonic, ethanesulphonic, hydroxy-ethanesulphonic, ethylenesulphonic acid; halobenzenesulphonic, toluenesulphonic, naphthalenesulphonic acids or sulphanilic acid; methionine, tryptophan, lysine or arginine.

The afore-mentioned or other salts of the compounds of the invention, for example their picrates, may also be used for purifying the resulting bases, by converting the bases by converting the bases into salts, isolating the salts and liberating the bases again from them. In view of the close relationship between the bases in the free form and in the form of their salts what has been

said above and hereinafter with reference to the free bases concerns also the corresponding salts wherever this is possible and useful.

The compounds of the invention are distinguished by a high antibiotic activity. Thus, they are very potent antibacterial agents, for example, against gram positive bacteria, such as the staphylococci, as has been shown for example, in animal tests in mice. Furthermore, they display in animal tests, for example in mice, an antitubercular activity. The compounds of the invention are therefore useful for treating bacterial infections, especially tuberculosis. The compounds are also valuable intermediates for the manufacture of other useful substances, especially of pharmacologically active compounds.

Special mention should be made of 3 - (N' - lower alkyl or N' - hydroxyalkyl) - piperazino - rifamycin S and SV, especially 3 - (N' - methylpiperazino) - rifamycin S; furthermore 3 - cyclohexyl - amino - rifamycin SV, and 3 - cyclopropylamino - rifamycin S and SV, 3 - morpholino - rifamycin SV and 3 - lower alkyl - amino - rifamycin S and SV, especially 3 - methylamino-, 3 - ethyl - amino- and 3 - isopropylamino - rifamycin SV. The latter compound produces, for example, on oral administration to mice in doses of from 10 to 50 mg per kg bodyweight, a distinct tuberculostatic effect; the same result is also achieved with 3 - morpholino - rifamycin SV.

The compounds of the invention may also be used as additives to animal fodders and for preserving victuals.

The invention also provides a process for the manufacture of compounds which are 3 - substituted derivatives of

- a) rifamycin S, or
- b) rifamycin SV, or
- c) a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV,

the substituent in position 3 being a free or aliphatically substituted amino group (as hereinbefore defined), wherein rifamycin S, or a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative thereof, is reacted with ammonia or with a primary or secondary aliphatically substituted amine (as hereinafter defined) and the 3 - substituted derivative of rifamycin S and/or the 3 - substituted derivative of rifamycin SV so obtained (which 3 - substituted derivative will, if the starting material is a hydrogenated derivative, be the corresponding 3 - substituted hydrogenated derivative) is isolated, a hydroquinone obtained before or after the isolation being oxidised, if desired, to form a quinone and/or a quinone obtained before or after the isolation being reduced, if desired, to form a hydroquinone, and/or, if desired, a resulting compound is converted into a salt. As an "aliphatically substituted"

amine there is to be understood one whose substituents are hydrocarbon radicals of aliphatic character as hereinbefore defined.

The reaction is advantageously carried out in an inert solvent that is free from hydroxyl groups, for example in chloroform, methyl cellosolve ("Cellosolve" is a Trade Mark), tetrahydrofuran or, advantageously, in a non-polar solvent, for example in an aromatic hydrocarbon such as benzene, or preferably in dioxane. When dioxane is used, the reaction is usually complete within about 5 to 10 minutes. It has been observed that the reaction speed also depends on the structure of the particular amine used. It is advantageous to use a large excess (5 to 10 mols) of amine. The reaction is advantageously performed at temperatures ranging from 20°C to 100°C, preferably at room temperature. When it progresses slowly more elevated temperatures may be chosen. The progress of the reaction can be checked by thin layer chromatography. In general, the reaction solution contains the reaction product partially in the form of the quinone and partially in the form of the hydroquinone. It is advantageous to oxidize the product in the reaction solution completely to the quinone and to isolate the latter. The oxidation is advantageously carried out with an inorganic oxidant, such as, for example, hydrogen peroxide or ammonium persulphate, or, preferably, with potassium ferricyanide. The quinone can be extracted with organic solvents.

The aliphatically substituted amines used as starting materials have the composition corresponding to the amino group defined above. Preferred use is made of amines that yield the above-mentioned, particularly potent final products.

The compounds of the invention may be used, for example, in the form of pharmaceutical preparations comprising the compounds in conjunction or admixture with an organic or inorganic, solid or liquid pharmaceutical carrier suitable for enteral, local or parenteral administration. Suitable carriers are substances that do not react with the compounds of the invention, such as, for example, water, gelatin, lactose, starches, stearylalcohol, magnesium stearate, talcum, vegetable oils, benzyl alcohols, gums, propylene glycol, polyalkyleneglycols, white petroleum jelly, cholesterol or other known medicinal carriers. The pharmaceutical preparations may be, for example, tablets, dragees, ointments, creams or capsules, or in liquid form solutions, suspensions or emulsions, e.g. oily solutions for injections in ampoules. They may be sterilized and/or contain assistants such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure or buffers. They may also contain other therapeutically valuable substances.

The preparations are formulated by the usual methods.

The compounds of the invention may also be used in veterinary medicine, for example in one of the forms mentioned above.

The following Examples illustrate the invention.

The hydroquinones of Examples 2—14 and 18—19 are prepared by reduction of the quinones as performed in Example 1.

The thin-layer chromatograms were carried out on silica gel in the following systems A, B and C:

System A: Benzene+acetone (4:1), plate impregnated with citric acid (52ml of 5% citric acid solution per 25g of silica gel)

System B: Chloroform+Methanol (9:1), plate not impregnated

System C: Benzene+acetone (2:1), plate impregnated with citric acid (52 ml of 5% citric acid solution per 25g of silica gel).

The R_f values are referred to the R_f value of rifamycin S=1 and are designated in the Examples as R_s(A), R_s(B) and R_s(C).

EXAMPLE 1

A solution of 50g of rifamycin S in 200 ml of dioxane is mixed with 12g of methylamine and the batch is kept for 10 minutes. Then chloroform, water and an aqueous solution of 50g of potassium ferricyanide are added and the whole is vigorously agitated for some time, then neutralized, and the residue is chromatographed on silica gel, the elution being performed with chloroform with a gradient of methanol. The resulting 3 - methylamino - rifamycin S, which is quickly eluted in a reddish brown solution, displays an R_s(A) value of 0.48; it is evaporated, crystallized from ether and then from methanol or aqueous alcohol; it has a melting point of 243 to 244°C (from aqueous methanol; dark red prisms).

The nuclear magnetic resonance spectrum of the compound does not contain the signal appearing in the case of rifamycin S for hydrogen on C₈. The mass spectrum of 16, 17, 18, 19, 28, 29 - hexahydro - 3 - methylamino - rifamycin S (obtained by catalytic hydrogenation, for example on 10% palladium charcoal in ethanol under atmospheric pressure at room temperature) corresponds to this formula.

Reduction with ascorbic acid furnishes yellow 3 - methylamino - rifamycin SV melting at 185°C.

5 Grams of 3 - methylamino - rifamycin S are dissolved in as little methanol as necessary, excess ascorbic acid is added and a sufficiency of water just to leave the solution still clear. The initially dark red solution gradually turns yellow. When the solution has become pure yellow, much water is added and

the batch is extracted with chloroform until the aqueous phase remains colourless. The combined chloroform extracts are washed with sodium chloride solution, dried and evaporated. The evaporation residue crystallizes from 90% aqueous methanol or methanol in yellow prisms. R_s(C)=0.62.

EXAMPLE 2

When in the reaction described in Example 1 methylamine is replaced by 16g of ethylamine, 3 - ethylamino - rifamycin S melting at 258 to 260°C is obtained (from methanol). R_s(A)=0.64. The corresponding hydroquinone has an R_s(C) value of 0.70.

EXAMPLE 3

When 50g of rifamycin S is reacted with 22g of ethanolamine according to the method described in Example 1, 3 - (2' - hydroxy - ethylamino) - rifamycin S melting above 340°C is obtained (from methanol+ether). R_s(A)=0.42. The corresponding hydroquinone decomposes above 185°C. R_s(C)=0.46.

EXAMPLE 4

A solution of 30g of rifamycin S in 200ml of dioxane is heated on a boiling waterbath, 12.5g of isopropylamine are added and heating is continued until the initially deep violet solution has turned yellowish brown. It is then rapidly cooled, then chloroform, water and an aqueous solution of 30g of potassium ferricyanide are added and the whole is vigorously agitated for some time. The batch is then neutralized, and the chloroform phase is isolated, dried and evaporated. The residue is chromatographed on silica gel, the elution being performed with chloroform with a gradient of methanol. The 3 - isopropylamino - rifamycin S, which displays in the thin-layer chromatogram an R_s(A) value of 0.72, is contained in the brownish red, rapidly travelling portions of the eluate. The product is crystallized from aqueous methanol, to yield 5.5g of dark red crystals melting at 168 to 170°C.

The corresponding hydroquinone melts at 165 to 170°C (from aqueous methanol). R_s(C)=0.77.

EXAMPLE 5

The reaction of 50g of rifamycin S with 36g of n - hexylamine according to the method described in Example 1 gives rise to 3 - hexylamino - rifamycin S as an amorphous substance. R_s(A)=1.00. The corresponding hydroquinone displays an R_s(C) value of 0.86.

EXAMPLE 6

The reaction of 50g of rifamycin S with 36g of cyclohexylamine as described in Example 4 gives rise to 3 - cyclohexylamino -

rifamycin S as an amorphous substance.

$R_{s(A)}=1.00$.

The corresponding hydroquinone crystallizes from ether. It melts at 161 to 162°C.

$R_{s(O)}=0.85$.

EXAMPLE 7

50 Grams of rifamycin S are dissolved in 200ml of dioxane, 42g of N,N - diethyl - ethylenediamine are added and the whole is left to itself until the initially deep violet solution has turned yellowish brown. Then chloroform, water and an aqueous solution of 50g of potassium ferricyanide are added, and the whole is vigorously agitated for some time, then neutralized, and the chloroform phase is separated. The resulting 3 - (2' - diethylamino - ethylamino) - rifamycin S is repeatedly extracted from the chloroform solution by means of citric acid solution of 1% strength. The citric acid solution is neutralized, agitated with chloroform, and the chloroform extract is dried and evaporated under vacuum. Chromatography of the evaporation residue on silica gel with chloroform and a gradient of methanol yields at first red and then violet-brown eluates that contain the desired product in pure form. The batch is evaporated and crystallized from alcohol or aqueous methanol. It forms blackish brown crystals that do not melt up to 300°C. $R_{s(B)}=0.80$.

The corresponding hydroquinone has the following Rf values: $R_{s(O)}=0.03$, $R_{s(B)}=0.44$.

EXAMPLE 8

A solution of 50g of rifamycin S in 200ml of dioxane is mixed with 50g of amino - acetaldehyde diethylacetal, and the mixture is left to itself until the initially deep violet solution has turned yellowish brown. Then chloroform, water and an aqueous solution of 50g of potassium ferricyanide are added, and the batch is vigorously agitated for some time, neutralized, and the chloroform phase is separated, dried and evaporated. The evaporation residue is dissolved in a mixture of equal parts of dioxane and 2N - hydrochloric acid and left to itself for 15 minutes, then diluted with sodium chloride solution, extracted by agitation with ether, and the ether phase is dried and evaporated. For purification the evaporation residue is repeatedly chromatographed on silica gel, using for the elution ether or chloroform with a methanol gradient. The violet-brown fractions contain 3 - formylmethyl - amino - rifamycin S. $R_{s(A)}=0.80$.

$R_{s(O)}$ of the corresponding hydroquinone: 0.91.

EXAMPLE 9

30 Grams of rifamycin S are dissolved in 300ml of dioxane, 18g of piperidine are

added, and the mixture is heated on a water bath until all rifamycin S has reacted (checked by thin-layer chromatography). Then chloroform, water and an aqueous solution of 30g of potassium ferricyanide are added, and the whole is vigorously agitated for some time, then neutralized, and the chloroform phase is separated, dried and evaporated. The residue is purified by chromatography on silica gel with chloroform and a methanol gradient. The fractions of violet brown colour, which contain 3 - piperidino - rifamycin S of $R_{s(A)}=1.10$, are collected. On crystallization from ether the substance is obtained in coarse, black crystals melting at 200°C.

The corresponding hydroquinone, obtained by crystallization from methanol, melts at 190—191°C. $R_{s(O)}=1.11$.

EXAMPLE 10

100 Grams of rifamycin S are dissolved in 800ml of dioxane, 70g of N - methylpiperazine are added, and the mixture is heated on a water bath until all rifamycin S has reacted (checked by thin-layer chromatography). Then chloroform, water and an aqueous solution of 100g of potassium ferricyanide are added, and the whole is vigorously shaken for some time, neutralized, and the chloroform phase is separated. The desired reaction product is extracted from the chloroform solution by repeated agitation with citric acid solution of 5% strength. The citric acid solution is neutralized, extracted with chloroform, and the extract is dried and evaporated under vacuum. The residue is filtered through 500g of silica gel, using as solvent chloroform containing 5 to 10% of methanol. The filtrate is evaporated and the residue crystallized from ether, to yield 3 - (N' - methyl - piperazino) - rifamycin S in the form of red prisms melting at 169°C. $R_{s(B)}=0.66$. When the compound is suspended in water and citric acid is added until all has dissolved, the citrate, melting at 197 to 200°C, is obtained, which crystallizes from water or aqueous methanol.

When 3 - (N' - methylpiperazino) - rifamycin S is dissolved in methyl iodide, it gives rise to crystalline, dark reddish brown methiodide which decomposes above 200°C. When an aqueous solution of the methiodide is poured over a column of Amberlite IRA-400 (in the chloride form), the methochloride is obtained.

3 - (N' - methylpiperazino) - rifamycin SV crystallizes from aqueous methanol and melts at 208 to 209°C. $R_{s(O)}=0.00$, $R_{s(B)}=0.28$.

EXAMPLE 11

50 Grams of rifamycin S are reacted with 35g of morpholine according to the method described in Example 9. The resulting 3 -

morpholine - rifamycin S melts at 213°C (from methanol). $R_{s(A)}=0.80$.

The corresponding hydroquinone crystallizes from aqueous methanol and melts at 243 to 244°C. $R_{s(O)}=1.11$.

EXAMPLE 12

The reaction of 30g of rifamycin S with 31g of N - carbethoxy - piperazine according to the method described in Example 9 yields 3 - (N' - carbethoxy - piperazino) - rifamycin S which crystallizes from ether and does not melt at temperatures up to 340°C. $R_{s(A)}=0.70$. $R_{s(O)}$ value of the corresponding hydroquinone: 1.11.

EXAMPLE 13

The reaction of 30g of rifamycin S with 16g of pyrrolidine according to the method described in Example 9 gives rise to 3 - pyrrolidino - rifamycin S melting at 189°C (from ether). $R_{s(A)}=0.72$.

The corresponding hydroquinone has an $R_{s(O)}$ value of 0.91.

EXAMPLE 14

30g of rifamycin S are dissolved in just the required quantity of dioxan, and the solution treated at room temperature, while stirring vigorously, with 30g of N - (2 - aminoethyl) - morpholine. After 6 minutes, the reaction is interrupted by the addition of chloroform and excess citric acid (cooling, stirring). The batch is diluted with water and extracted with chloroform. The chloroformic extract is agitated for quite some time with excess aqueous potassium ferricyanide solution which is maintained alkaline with sodium bicarbonate. The chloroformic solution is then dried and evaporated. For purification, the residue is chromatographed on 1.5 kg of silica gel using chloroform as eluent. Rifamycin S is eluted first which is followed by the desired reaction product viz 3 - (2' - morpholino - ethyl) - Rifamycin S, in a dark wine-red solution. It is crystallized from ether, and re-crystallized twice from aqueous methanol to obtain 4.8g of coarse, dark red crystals which do not melt up to 350°C. $R_{s(B)}=0.90$.

The corresponding hydroquinone has an $R_{s(B)}$ value of 0.36.

EXAMPLE 15

A solution of 30g of rifamycin S in 30 ml of diethylamine is stirred for 30 minutes at room temperature. The reaction mixture is then evaporated, the residue dissolved in chloroform, and the chloroformic solution agitated for quite some time with an excess of aqueous potassium ferricyanide solution. After this, the chloroformic phase which meanwhile has assumed a brown-red colour is separated, dried with sodium sulphate, and evaporated. The residue is re-crystallized

once from ether and twice from methanol. There are obtained 5g of red crystals of 3 - diethylamino - rifamycin S of melting point 190—191°C. $R_{s(A)}=0.90$.

When a suspension of the red crystals of melting point 190—191°C in aqueous methanol is treated with excess Na - ascorbate and stirred for some time, a clear yellow solution is obtained from which on acidification with aqueous citric acid solution the hydroquinone of the above substance, the 3 - diethylamino - rifamycin SV crystallizes out. The crystals are filtered and washed with aqueous methanol containing some ascorbic acid. Yellow crystals of melting point 154°C. $R_{s(A)}=0.82$.

EXAMPLE 16

20 ml of cyclopropylamine are added at room temperature to a solution of 20g of rifamycin S in 20 ml of dioxan, and the mixture stirred and allowed to react for 3 minutes. The batch is then evaporated as rapidly as possible at 25°C, the residue dissolved in chloroform, and the solution reacted for a rather long time with an excess of an aqueous solution of potassium ferricyanide. The chloroform phase is then separated, washed with 3% citric acid solution, dried with Na_2SO_4 , and evaporated. The residue is chromatographed on the 30- to 50-fold quantity of silica gel that has been washed with acid. The initially yellow-coloured eluate contains unchanged rifamycin S. The succeeding red portions contain the desired reaction product. The latter fractions are evaporated and the residue recrystallized from aqueous methanol to obtain red crystals melting at 162—170°C constituting the 3 - (cyclopropylamino) - rifamycin S. Yield, 3g; $R_{s(A)}=0.58$.

For reduction of the quinone of melting point 162—170°C, the crystals are suspended in aqueous methanol and the suspension stirred while being treated with excess Na - ascorbate. After a short while a clear, yellow solution is obtained from which, after addition of citric acid and sodium chloride solution the desired hydroquinone can be extracted with chloroform. The hydroquinone 3 - (cyclopropylamino) - rifamycin SV obtained on crystallization from aqueous methanol melts at 193—195°C (decomposition); $R_{s(A)}=1.0$.

EXAMPLE 17

30g of rifamycin S are dissolved in as little dioxan as possible, and the solution treated at room temperature with 30 ml of benzylamine while stirring well. When but a slight quantity of rifamycin S can be detected by thin layer chromatography, the reaction is interrupted by the addition of ice, excess citric acid solution and chloroform. The chloroformic solution is vigorously stirred

for some time with excess aqueous potassium ferricyanide solution, then isolated, dried and evaporated. The residue is chromatographed on 1.5 kg of silica gel using a 9:1 mixture of benzene and acetone as eluant. Rifamycin S is eluted first, and then the desired reaction product, 3 - (benzylamino) - rifamycin S. For final purification, the latter is converted into the hydroquinone in the manner described in Example 16, and the hydroquinone re-crystallized several times from ether. In this manner 5g of 3 - (benzylamino) - rifamycin SV of melting point 151—152°C are obtained.

EXAMPLE 18

In a manner analogous to that of Example 9, 20g of rifamycin S in 10 ml of dioxan are reacted with 20 ml of homopiperidine. The resulting 3 - homopiperidino - rifamycin S, when re-crystallized from ether, forms wine-red crystals melting at 218—220°C (decomposition). The corresponding hydroquinone 3 - (benzylamino) - rifamycin SV crystallizes from methanol in the form of yellow prisms of melting point 190—191°C. $R_s(A) = 1.30$.

EXAMPLE 19

In a manner analogous to that of Example 9, 30g of rifamycin S in 20 ml of dioxan are reacted with 20 ml of N - methyl - tetrahydrofurfurylamine. Crystallization from ether yields more than 20g of 3 - (N - methyltetrahydrofurfurylamino) - rifamycin S in the form of black-violet crystals which melt at 211—212°C. The corresponding hydroquinone has an $R_s(A)$ value of 1.10.

EXAMPLE 20

A pharmaceutical antibiotic preparation for parenteral use containing 3 - morpholino - rifamycin SV as active ingredient is prepared as follows: 50 mg of 3 - morpholino - rifamycin SV-sodium salt are dissolved in 1 ml of distilled water. The solution is lyophilized at -30°C. The dry compound thus obtained is diluted with e.g. 50 ml of distilled water or 100 ml of physiological sodium chloride solution. These solutions are used for the parenteral administration of the antibiotic.

EXAMPLE 21

A pharmaceutical preparation containing 3 - morpholino - rifamycin in the form of hard-gelatin capsules is prepared as follows:

Composition	
3-morpholino rifamycin SV	150 mg
ethyl cellulose	3 mg
stearic acid	3 mg
	156 mg

Manufacture

- 1) The ethyl cellulose and the steric acid are dissolved in 20 times their weight of methylene chloride.
- 2) 3 - Morpholino - rifamycin SV is mixed with the solution resulting from 1) to form a homogeneous mass and this is passed through a sieve of 3—5 mm mesh at a temperature not exceeding 40°C.
- 3) The granulate resulting from 2) is passed through a sieve of 0.5 mm mesh and poured into hard gelatine capsules of size 1 (=0.5 cm³).

In the same manner other similar pharmaceutical preparations can be obtained with any of the 3 - amino rifamycin S or SV derivatives of the present invention, if desired, varying the doses in a suitable manner.

WHAT WE CLAIM IS:—

1. A process for the manufacture of compounds which are 3 - substituted derivatives of

- a) rifamycin S, or
- b) rifamycin SV, or
- c) a 16, 17, 18, 19-tetrahydro or 16, 17, 18, 19, 28, 29-hexahydro derivative of rifamycin S or of rifamycin SV,

the substituent in position 3 being a free or aliphatically substituted amino group (as hereinbefore defined), wherein rifamycin S, or a 16, 17, 18, 19-tetrahydro or 16, 17, 18, 19, 28, 29-hexahydro derivative thereof, is reacted with ammonia or with a primary or secondary aliphatically substituted amine (as hereinbefore defined) and the 3 - substituted derivative of rifamycin S and/or the 3 - substituted derivative of rifamycin SV so obtained (which 3 - substituted derivative will, if the starting material is a hydrogenated derivative, be the corresponding 3 - substituted hydrogenated derivative) is isolated, a hydroquinone obtained before or after the isolation being oxidised, if desired, to form a quinone and/or a quinone obtained before or after the isolation being reduced, if desired, to form a hydroquinone, and/or, if desired, a resulting compound is converted into a salt.

2. A process as claimed in claim 1, wherein the reaction is performed in an inert solvent free from hydroxyl groups.

3. A process as claimed in claim 1 or claim 2, wherein the reaction is performed in dioxan.

4. A process as claimed in any one of claims 1 to 3, wherein the reaction is performed at a temperature in the range of from 20 to 100°C.

5. A process as claimed in any one of claims 1 to 4, wherein a hydroquinone is oxidized by means of hydrogen peroxide, ammonium persulphate or potassium ferricyanide to form a quinone.

6. A process as claimed in any one of claims 1 to 4, wherein a quinone is reduced

for some time with excess aqueous potassium ferricyanide solution, then isolated, dried and evaporated. The residue is chromatographed on 1.5 kg of silica gel using a 9:1 mixture of benzene and acetone as eluant. Rifamycin S is eluted first, and then the desired reaction product, 3 - (benzylamino) - rifamycin S. For final purification, the latter is converted into the hydroquinone in the manner described in Example 16, and the hydroquinone re-crystallized several times from ether. In this manner 5g of 3 - (benzylamino) - rifamycin SV of melting point 151-152°C are obtained.

EXAMPLE 18

In a manner analogous to that of Example 9, 20g of rifamycin S in 40 ml of dioxan are reacted with 20 ml of homopiperidine. The resulting 3 - homopiperidino - rifamycin S, when re-crystallized from ether, forms wine-red crystals melting at 218-220°C (decomposition). The corresponding hydroquinone 3 - (benzylamino) - rifamycin SV crystallizes from methanol in the form of yellow prisms of melting point 190-191°C. $R_s(A) = 1.30$.

EXAMPLE 19

In a manner analogous to that of Example 9, 30g of rifamycin S in 20 ml of dioxan are reacted with 20 ml of N - methyl - tetra - hydrofurfurylamine. Crystallization from ether yields more than 20g of 3 - (N - methyltetrahydrofurfurylamino) - rifamycin S in the form of black-violet crystals which melt at 211-212°C. The corresponding hydroquinone has an $R_s(A)$ value of 1.10.

EXAMPLE 20

A pharmaceutical antibiotic preparation for parenteral use containing 3 - morpholino - rifamycin SV as active ingredient is prepared as follows: 50 mg of 3 - morpholino - rifamycin SV-sodium salt are dissolved in 1 ml of distilled water. The solution is lyophilized at -30°C. The dry compound thus obtained is diluted with e.g. 50 ml of distilled water or 100 ml of physiological sodium chloride solution. These solutions are used for the parenteral administration of the antibiotic.

EXAMPLE 21

A pharmaceutical preparation containing 3 - morpholino - rifamycin in the form of hard-gelatin capsules is prepared as follows:

Composition	
3-morpholino rifamycin SV	150 mg
ethyl cellulose	3 mg
stearic acid	3 mg
	156 mg

Manufacture

- 1) The ethyl cellulose and the steric acid are dissolved in 20 times their weight of methylene chloride.
- 2) 3 - Morpholino - rifamycin SV is mixed with the solution resulting from 1) to form a homogeneous mass and this is passed through a sieve of 3-5 mm mesh at a temperature not exceeding 40°C.
- 3) The granulate resulting from 2) is passed through a sieve of 0.5 mm mesh and poured into hard gelatine capsules of size 1 (=0.5 cm³).

In the same manner other similar pharmaceutical preparations can be obtained with any of the 3 - amino rifamycin S or SV derivatives of the present invention, if desired, varying the doses in a suitable manner.

WHAT WE CLAIM IS:—

1. A process for the manufacture of compounds which are 3 - substituted derivatives of
 - a) rifamycin S, or
 - b) rifamycin SV, or
 - c) a 16, 17, 18, 19-tetrahydro or 16, 17, 18, 19, 28, 29-hexahydro derivative of rifamycin S or of rifamycin SV,
 the substituent in position 3 being a free or aliphatically substituted amino group (as hereinbefore defined), wherein rifamycin S, or a 16, 17, 18, 19-tetrahydro or 16, 17, 18, 19, 28, 29-hexahydro derivative thereof, is reacted with ammonia or with a primary or secondary aliphatically substituted amine (as hereinbefore defined) and the 3 - substituted derivative of rifamycin S and/or the 3 - substituted derivative of rifamycin SV so obtained (which 3 - substituted derivative will, if the starting material is a hydrogenated derivative, be the corresponding 3 - substituted hydrogenated derivative) is isolated, a hydroquinone obtained before or after the isolation being oxidised, if desired, to form a quinone and/or a quinone obtained before or after the isolation being reduced, if desired, to form a hydroquinone, and/or, if desired, a resulting compound is converted into a salt.
2. A process as claimed in claim 1, wherein the reaction is performed in an inert solvent free from hydroxyl groups.
3. A process as claimed in claim 1 or claim 2, wherein the reaction is performed in dioxan.
4. A process as claimed in any one of claims 1 to 3, wherein the reaction is performed at a temperature in the range of from 20 to 100°C.
5. A process as claimed in any one of claims 1 to 4, wherein a hydroquinone is oxidized by means of hydrogen peroxide, ammonium persulphate or potassium ferricyanide to form a quinone.
6. A process as claimed in any one of claims 1 to 4, wherein a quinone is reduced





- to a hydroquinone by means of ascorbic acid.
7. A process as claimed in any one of claims 1 to 6, wherein the reaction is performed with a primary or secondary amine whose amino group is substituted by one or two hydrocarbon radicals of aliphatic character each containing not more than 12 carbon atoms.
8. A process as claimed in claim 7, wherein the hydrocarbon radicals are interrupted in the carbon chain by one or more hetero atoms and/or are substituted by one or more identical or different functional groups, selected from the group consisting of hydroxyl groups, halogen atoms, free or esterified carboxyl or sulphonic acid groups, aldehyde groups, nitrile groups and amino groups.
9. A process as claimed in any one of claims 1 to 7, wherein the reaction is performed with an alkylamine or cycloalkylamine having not more than 8 carbon atoms.
10. A process as claimed in any one of claims 1 to 7, wherein the reaction is performed with an alkyleneamine having from 5 to 7 ring carbon atoms.
11. A process as claimed in any one of claims 1 to 7, and 9, wherein the reaction is performed with cyclopropylamine.
12. A process as claimed in claim 9, wherein the reaction is performed with methylamine, ethylamine, isopropylamine or cyclohexylamine.
13. A process as claimed in any one of claims 1 to 8, wherein the reaction is performed with an optionally substituted diazacycloalkane which has 6-9 ring members and in which the nitrogen atoms are separated by at least 2 carbon atoms.
14. A process according to claim 13, wherein the reaction is performed with a piperazine which may be substituted on the N' atom by alkyl or hydroxyalkyl groups containing not more than 8 carbon atoms.
15. A process as claimed in claim 14, wherein the reaction is performed with N'-methylpiperazine.
16. A process as claimed in any one of claims 1 to 9, wherein the reaction is performed with morpholine.
17. A process as claimed in any one of claims 1 to 7, wherein the reaction is performed with N,N - diethyl - ethylene - diamine.
18. A process as claimed in any one of claims 1 to 9, wherein the reaction is performed with N - carbethoxy - piperazine.
19. A process as claimed in claim 10, wherein the reaction is performed with pyrrolidine.
20. A process as claimed in any one of claims 1 to 7, wherein the reaction is performed with diethylamine.
21. A process as claimed in any one of claims 1 to 8, wherein the reaction is performed with N - (2 - aminoethyl -) - morpholine.
22. A process as claimed in any one of claims 1 to 7, wherein the reaction is performed with benzylamine.
23. A process as claimed in any one of claims 1 to 8, wherein the reaction is performed with homopiperidine.
24. A process as claimed in any one of claims 1 to 8, wherein the reaction is performed with piperidine.
25. A process as claimed in any one of claims 1 to 7, wherein the reaction is performed with N - methyl - tetrahydrofurfuryl - amine.
26. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being a free or aliphatically substituted amino group as hereinbefore defined, a salt thereof, or a quaternary ammonium compound thereof.
27. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being a secondary or tertiary amino group the nitrogen atom of which is substituted by hydrocarbon radicals of aliphatic character having at most 12 carbon atoms, or a salt thereof, or a quaternary ammonium compound thereof.
28. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being an amino group whose nitrogen atom is substituted by one or two hydrocarbon radicals of aliphatic character as hereinbefore defined, which may be interrupted in the carbon chain by one or more hetero atoms and/or may be substituted by one or more identical or different functional groups selected from the group consisting of hydroxyl groups, halogen atoms, free or esterified carboxyl or sulphonic acid groups, aldehyde groups, nitrile groups and amino groups, a salt thereof, or a quaternary ammonium compound thereof.
29. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being an alkylamino group of cycloalkyl - amino group with at most 8 carbon atoms, a salt thereof, or a quaternary ammonium compound thereof.
30. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 -



- 5 hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being a methylamino, ethylamino, isopropyl-amino or cyclohexylamino group, a salt thereof, or a quaternary ammonium compound thereof.
- 10 31. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being an optionally substituted N - diazacycloalkyl group having from 6 to 9 ring members and at least 2 carbon atoms between the nitrogen atoms, a salt thereof, or a quaternary ammonium compound thereof.
- 15 32. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being a piperazino group which may be substituted on the N' atom by an alkyl or hydroxylalkyl group having not more than 8 carbon atoms, a salt thereof, or a quaternary ammonium compound thereof.
- 20 33. A 3 - substituted derivative of rifamycin S or of rifamycin SV or of a 16, 17, 18, 19 - tetrahydro or 16, 17, 18, 19, 28, 29 - hexahydro derivative of rifamycin S or of rifamycin SV, the substituent in position 3 being an N' - methyl - piperazino group, a salt thereof, or a quaternary ammonium compound thereof.
- 25 34. 3 - Morpholino - rifamycin S.
- 30 35. 3 - Morpholino - rifamycin SV.
36. 3 - Pyrrolidino - rifamycin S and 3 - pyrrolidino - rifamycin SV.
- 40 37. 3 - diethylamino - rifamycin S and 3 - diethylamino - rifamycin SV.
38. 3 - (2' - morpholino - ethyl) - rifamycin S and 3 - (2' - morpholino - ethyl) - rifamycin SV.
- 45 39. 3 - Benzylamino - rifamycin S and 3 - benzylamino - rifamycin SV.
40. 3 - Piperidino - rifamycin S and 3 - piperidino - rifamycin SV.
- 50 41. 3 - Cyclopropylamino - rifamycin S and 3 - cyclopropyl - amino - rifamycin SV.
42. 3 - Methylamino - rifamycin S and 3 - methylamino - rifamycin SV.
43. 3 - Ethylamino - rifamycin S and 3 - ethylamino - rifamycin SV.
- 55 44. 3 - Isopropylamino - rifamycin S and 3 - isopropylamino - rifamycin SV.
45. 3 - Cyclohexylamino - rifamycin S and 3 - cyclohexylamino - rifamycin SV.
- 60 46. 3 - (N' - methyl - piperazino) - rifamycin S and 3 - (N' - methyl - piperazino) - rifamycin SV.
47. An acid addition salt of a compound as claimed in any one of claims 33 to 46.
48. A metal salt of a rifamycin SV- derivative as claimed in any one of claims 33 to 46.
- 65 49. A pharmaceutical preparation comprising a compound as claimed in claim 26, in admixture or conjunction with a pharmaceutically suitable carrier.
50. A pharmaceutical preparation comprising a compound as claimed in claim 27, in admixture or conjunction with a pharmaceutically suitable carrier.
- 70 51. A pharmaceutical preparation comprising a compound as claimed in claim 31, in admixture or conjunction with a pharmaceutically suitable carrier.
- 75 52. A pharmaceutical preparation comprising a compound as claimed in claim 32, in admixture or conjunction with a pharmaceutically suitable carrier.
- 80 53. A pharmaceutical preparation comprising a compound as claimed in any one of claims 36 to 47, in admixture or conjunction with a pharmaceutically suitable carrier.
- 85 54. A pharmaceutical preparation comprising 3 - morpholino - rifamycin S, or a salt thereof, in admixture or conjunction with a pharmaceutically suitable carrier.
55. A pharmaceutical preparation comprising 3 - morpholino - rifamycin SV, or a salt thereof, in admixture or conjunction with a pharmaceutically suitable carrier.
- 90 56. A pharmaceutical preparation as claimed in any one of claims 49 to 55 in the form of hard gelatine capsules.
- 95 57. A pharmaceutical preparation as claimed in any one of claims 49 to 55 in the form of oily injection solution in ampoules.
58. Animal fodder comprising a compound as claimed in any one of claims 26 to 47, in admixture or conjunction with a carrier suitable for use in animal fodder.
- 100 59. A compound as claimed in any one of claims 26 to 33 and described in any one of Examples 1 to 13 herein.
- 105 60. A compound as claimed in any one of claims 26 to 33 and described in any one of Examples 14 to 19 herein.
61. A process as claimed in claim 1 conducted substantially as described in any one of Examples 1 to 13 herein.
- 110 62. A process as claimed in claim 1 conducted substantially as described in any one of Examples 14 to 19 herein.
- 115 63. A pharmaceutical preparation as claimed in claim 49 and described in Example 20 or Example 21 herein.

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