PROCESS FOR PRODUCING DIHYDRO-DESOXYSTREPTOMYCINS

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This invention relates to the production of dihydrodesoxystreptomycins which are very potent agents for the chemotherapy of tuberculosis having no side effects. More particularly, it relates to the process for producing dihydrodesoxystreptomycins which comprises charging a container, having anode and cathode compartments separated by a diaphragm, with an aqueous solution containing salt of streptomycins as the catholyte and an aqueous solution of acid or salt as the anolyte, dipping a proper electrode in each of the said electrolytes, maintaining the pH of the catholyte at 2.0–2.5 and also keeping the potential of the cathode approximately equal to the deposition potential of hydrogen while passing an electric current between the anode and the cathode until the salt of streptomycins in the catholyte is reduced, and recovering the corresponding dihydrodesoxystreptomycin of high quality.

The term "streptomycins" employed herein is intended to include "streptomycin," "hydroxystreptomycin" and "mannosidostreptomycin." The following are chemical structural formulae of these streptomycins:

Structural formula of streptomycin:

\[
\begin{align*}
R_1 & - O - CH_2 - \text{H} - OH \\
H & \text{CH} & \text{OH}
\end{align*}
\]

Structural formula of hydroxystreptomycin:

\[
\begin{align*}
R_1 & - O - CH_2 - \text{H} - OH \\
H & \text{CH} & \text{OH}
\end{align*}
\]

Structural formula of mannosidostreptomycin:

\[
\begin{align*}
R_1 & - O - CH_2 - \text{H} - OH \\
H & \text{CH} & \text{OH}
\end{align*}
\]

\(R_1\) denotes streptidine residue, i.e.,

\[
\begin{align*}
\text{NH} & \text{H} - \text{NH} - \text{C} - \text{NH} \\
\text{H} & \text{H} & \text{H} & \text{H}
\end{align*}
\]

\(R_2\) denotes N-methyl-L-glucosamine residue, i.e.,

\[
\begin{align*}
\text{CH} & \text{HOCH} & \text{CH} & \text{HOCH} & \text{CH} & \text{HOCH}
\end{align*}
\]

\(R_3\) denotes D-mannose residue, i.e.,

In accordance with the present invention, it is possible to obtain dihydrodesoxystreptomycin (IV) from streptomycin (I), dihydrodesoxyhydroxystreptomycin (V) from hydroxystreptomycin (II) and dihydrodesoxymannosidostreptomycin (VI) from mannosidostreptomycin (III). The chemical structural formulae of these reduction products are given below:

Structural formula of dihydrodesoxystreptomycin:

\[
\begin{align*}
R_1 & - O - CH_2 - \text{H} \\
H & \text{CH}_3
\end{align*}
\]

Structural formula of dihydrodesoxyhydroxystreptomycin:

\[
\begin{align*}
R_1 & - O - CH_2 - \text{H} \\
H & \text{CH}_3
\end{align*}
\]

Structural formula of dihydrodesoxymannosidostreptomycin:

\[
\begin{align*}
R_1 & - O - CH_2 - \text{H} \\
H & \text{CH}_3
\end{align*}
\]

It is an object of our invention to provide a new process for producing dihydrodesoxystreptomycins by means of electrolysis.

It is another object of our invention to provide a process for producing dihydrodesoxystreptomycins of high quality with high yield.

The above-mentioned dihydrodesoxystreptomycins have been discovered by the present inventors who have denominated its chemical structural formulae, and that invention was disclosed in the "Proceedings of the Japan Academy," vol. 32, 1956, pp. 48 and 53.

These dihydrodesoxystreptomycins were hereofore manufactured by treating an aqueous solution of streptomycin salt with amalgamated aluminum maintaining the
The two electrodes are connected to the direct current source and an electric current is passed between them to reduce streptomycin in the catholyte into dihydrodesoxy-streptomycin. To find out the most suitable condition for this purpose, the following experiments were made:

(a) During the reductive reaction, dilute sulfuric acid was added dropwise to the cathode compartment in order to maintain the pH within the following four ranges:

- (1) 2.0–2.5;
- (2) 2.5–4.0;
- (3) 4.0–5.0;
- (4) 5.0–7.0.

(b) The passing of an electric current was made in two different ways as follows:

- (1) The electric current was controlled in such a way as to keep the potential of the cathode in the neighborhood of the deposition potential of hydrogen.
- (2) The electric current was adjusted in such a way that hydrogen may deposit fairly actively from the surface of the cathode. The density of the electric current at that time was 0.03 ampere per 1 square centimeter of the mercury cathode, and the potential of the cathode was more negative than in (1).

Applying Ikeda's method (see "Journal of Scientific Research Institute," vol. 51, p. 234, 1957) and examining the percentage of dihydrostreptomycin that exists as by-product in the reduction product obtained from the catholyte during the experiments executed under the conditions mentioned above, it has been discovered that in the reduction product dihydrodesoxystreptomycin and dihydrostreptomycin co-exist in various proportions depending on the above-mentioned conditions. The variation is shown in the following Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Condition for passing an electric current</th>
<th>The current is passed so that hydrogen deposits actively from the surface of the cathode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrodesoxystreptomycin, percent</td>
<td>Dihydrostreptomycin, percent</td>
</tr>
<tr>
<td>pH of catolyte:</td>
<td></td>
</tr>
<tr>
<td>2.0–2.5</td>
<td>3–4</td>
</tr>
<tr>
<td>2.5–4.0</td>
<td>95–97</td>
</tr>
<tr>
<td>4.0–5.0</td>
<td>89–99</td>
</tr>
<tr>
<td>5.0–7.0</td>
<td>67–78</td>
</tr>
<tr>
<td></td>
<td>95–99</td>
</tr>
</tbody>
</table>

As is clearly shown by the above-mentioned experiment it has been found that, when streptomycin is reduced by electrolysis, dihydrostreptomycin and dihydrodesoxystreptomycin will come to coexist in the reduction product in various proportions depending on the pH of the catholyte and the way of passing electric current. Further, by making experiments with regard to streptomycins other than streptomycin under similar conditions, we have obtained reduction products in which dihydrostreptomycins and dihydrodesoxystreptomycins coexist in similar percentage as is shown in Table 1. We have also found that, in order to obtain dihydrodesoxystreptomycin of high purity (95% or more) as is shown in Table 1, it is absolutely necessary to maintain the pH of the catholyte at 2.0–2.5 and to pass a current to make the potential of the cathode approximately equal to the deposition potential of hydrogen.

Prior to the present invention, there were patent publications and papers regarding the method of producing dihydrostreptomycin by electrolytic reduction of streptomycin. However, the result of the investigations ex-
excuted by the present inventors has made it clear that these methods are quite different from the method of the present invention. Below, we shall explain about the grounds for this assertion.

First, we will give an explanatory criticism on the method of the U.S. Patent No. 2,717,236 (September 6, 1955) (Japanese Patent publication No. 1,034 (1952)). In the claim of the patent, it is stated that "the process essentially comprises charging an electrolytic cell, having anode and cathode compartments separated by a semi-permeable diaphragm, with a non-alkaline, electric-current-conducting aqueous solution of the corresponding streptomycin as the catholyte and an aqueous solution of a strong inorganic acid as the anolyte, passing an electric current between the anode and the cathode in the respective compartments until the streptomycin is substantially completely reduced, and recovering the dihydrostreptomycin from the catholyte." Also, in the lines 44-48 of the 2nd column, an explanation of the above-mentioned "non-alkaline" is given as follows: "Inasmuch as the catholyte tends to become alkaline during the electrolysis, dilute acid is added during electrolysis to maintain the catholyte non-alkaline, preferably, at a pH of about 4 to about 7." Furthermore, in all the examples, the catholyte is maintained at pH 7. Besides, no restrictions are put on the electric current to be passed between the anode and the cathode, and on the potential of the cathode.

The patent further states that the streptomycin is completely reduced and the dihydrostreptomycin is only recovered under the conditions mentioned above. However, as is clear from the result secured by the present inventors and mentioned in Table 1, under the pH of the catholyte maintained within the range of 4-7, a product will be obtained in which an indefinite proportion of 21-45% of dihydrostreptomycin and 55-79% of dihydrodesoxystreptomycin will co-exist when the electrolysis is carried out by passing an electric current keeping the cathode potential approximately equal to the deposition potential of hydrogen, and also a product will be obtained in which an indefinite proportion of 50-57% of dihydrostreptomycin and 43-50% of dihydrodesoxystreptomycin will co-exist when the electrolysis is carried out by passing an electric current in such a way that hydrogen may deposit actively from the surface of the cathode. In either case, it is absolutely impossible to obtain only dihydrodesoxystreptomycin of high purity (95% or more).

In this report, the equipment used for reduction by the electrolysis is not much different from that which is mentioned in the above-mentioned (1), or that employed in the method of the present invention. The reporters, Ueda et al., have investigated into the method adopted in the above-mentioned (1), especially into the conditions of having better current efficiency, and proposed the following as the best condition of recovering dihydrostreptomycin from streptomycin by electrolysis.

According to this proposal, an amalgamated lead is used as the most suitable cathode and sodium sulfate is added to the catholyte. Further, by maintaining the catholyte at pH 6 or more with the occasional addition of acid, the electrolytic reduction is executed. However, no restrictions are placed on the electric current to be passed or on the potential of the cathode. Under such a condition where the catholyte is maintained at pH 6 or more and the electric current is restricted, dihydrodesoxystreptomycin of high purity can never be obtained irrespective of whether or not sodium sulfate is employed, as is clear from the result secured by the present inventors, and the product obtained will be only a mixture of dihydrostreptomycin and dihydrodesoxystreptomycin of which the mixture percentage is not very much different from that of the method adopted in the aforementioned U.S. Patent 2,717,236.

(3) We will now give an explanatory criticism on the method of the Japanese Patent No. 3,978 (1952).

In the claim of the said patent is described as follows: "It essentially comprises preparing an aqueous solution of streptomycin salts as the catholyte, having either a plate covered beforehand with platinum black or palladium black by electric plating or cadmium as the cathode, suspending a very small quantity of platinum or palladium catalyser with or without carrier in the catholyte being kept neutral, and recovering the dihydrostreptomycin salts from the catholyte after conducting electrolytic reduction." This method is particularly and only different from the above-mentioned two methods in that the former has a catalyser on the surface of the cathode or in the catholyte. The principle of this is that, besides effecting the reduction on the surface of the cathode as is done in the general electrolytic reduction, the molecular hydrogen depositing from the cathode in this method is not allowed to disperse, but is added to the streptomycin by the action of catalyser. For the purpose of tracing this method, the present inventors maintained the catholyte at pH 6 and suspended platinum oxide in the catholyte, effecting reduction, and obtained a reduction product as a mixture which consisted of 70% of dihydrostreptomycin and 30% of dihydrodesoxystreptomycin.

It is a clear fact, as it is stated in the known reports and patents, that, when streptomycin is cathodically reduced by molecular hydrogen and catalyser, only dihydrostreptomycin will be obtained. When the electrolytic reduction is effected with the addition of catalyser as in the method in question, the percentage of the dihydrostreptomycin contained in the reduction product will be only a little larger than when catalyser is not added, as is clearly seen from the aforesaid result obtained by the present inventors. Thus, it will be absolutely impossible to obtain by this method salts of dihydrodesoxystreptomycin of high purity (95% or more).

(4) Here, we will explain and criticize the report on the electrolytic reduction of salts of streptomycin described in "Reports of the Scientific Research Institute," vol. 28 (1952), pp. 103, 118, 199 and 316.

The report as a whole, which contains the method mentioned above (in 3), is based on the experiments where the catholyte is maintained at pH 6, and according, the product obtainable by this method is nothing but a mixture of dihydrostreptomycin and dihydrodesoxystreptomycin as shown in Table 1, and dihydrodesoxystreptomycin of high purity (95% or more) can not be obtained by this method.

As we have explained and criticized in detail in the foregoing passages, production of dihydrodesoxystreptomycins of high purity (95% or more) is absolutely impossible under the conditions stated in the patents and papers concerning the preparation of dihydrostreptomycin by the electrolytic reduction of streptomycin. The object of obtaining dihydrodesoxystreptomycin of high purity by the electrolytic reduction of streptomycin can be attained for the first time through the method with its necessary conditions furnished by the present inventors.

The method of the present invention being generally carried out may be described in the following way by way of example. An electrolytic cell is divided into anode and cathode compartments by means of an unglazed pottery plate. Streptomycin salt is put into the cathode compartment and an aqueous solution of strong inorganic acid is put into the anode compartment. As the cathode, any one of the materials which are generally employed as the cathode in the electrolytic reduction, such as mercury, lead, amalgamated lead, zinc or amalgamated zinc may be used. As the anode, any one of carbon plate,
platinum plate or lead plate coated with lead dioxide may be used. By maintaining the catholyte always at pH of 2.0–2.5, and keeping the potential of cathode approximately equal to 1% of the deposition potential of hydrogen in the same condition, and passing an electric current between the cathode and anode, streptomycin in the catholyte is reduced by electrolysis. During the process of reduction, a portion of catholyte is taken out, and if the quantity of streptomycin remaining in the catholyte becomes less than 1% of the beginning quantity, it shows that the reduction is substantially completed. Then the catholyte is taken out, and, by freeze drying or by other means, dihydrododesoxystreptomycin salt can be obtained with high yield (generally 95–98%).

During the execution of the process of the present invention, the pH of the catholyte which is an aqueous solution containing streptomycin salt inevitably rises up as the electrolysis proceeds. To prevent this rise, and to maintain the catholyte always at pH 2.0–2.5, there are several means about which we shall explain below in detail:

(1) Addition of acids during the whole period of reaction to prevent the rise of pH.

The most suitable acids to be employed for this purpose are inorganic strong acids such as sulfuric, hydrochloric or phosphoric acid.

(2) Addition of pH buffer at the beginning of the reaction.

With regard to (1) mentioned above, it is required to follow a troublesome procedure of properly adding acid with incessant supervision over the pH during the whole period of reaction. On the other hand, if the pH is maintained at about 2.0 by the addition of a predetermined quantity of buffer from the beginning of the reaction, the variation in the value of pH may occur only within a limited range, conveniently dispensing with the above-mentioned procedure. The desirable buffer is such one as to have the greatest buffer capacity

$$
\beta = \frac{\Delta B}{\Delta \text{pH}}
$$

within the range of pH 2.0–2.5 or in the neighborhood at which the pH is to be maintained. For instance, mono-basic phosphate ion buffer (addition of 49 g. of phosphoric acid and 10.4 g. of caustic soda per 1 l. of catholyte will make a buffer solution of pH 2.0, 0.5 mol/l., regarding phosphoric acid) is a suitable one. Besides this, any weak acid or weak base which is stable during the electrolysis and proper in dissociation constant may be used as a buffer in combination with these salts. As an example, there is a combination of tartaric acid or citric acid and their sodium salts. However, in each case, the resultant reduction products must be separated from these buffers and refined after completion of reduction.

(3) Addition of a predetermined quantity of aluminum sulfate or aluminum chloride at the beginning of the reaction.

In this method, aluminum sulfate or aluminum chloride is employed instead of a buffer mentioned in (2). By nature, these aluminum salts show an acid character because of their hydrolysis in water, and so it will be easy to maintain the pH at 2.0–2.5 by properly fixing the concentration. In addition, they have a certain extent of buffer capacity and are stable in the catholyte. Therefore, they can be effectively used as a substitute for buffer.

However, in this case too, for the purpose of refining reduction products, aluminum salt must be removed from the catholyte by adding barium hydroxide in case aluminum sulfate is used, or silver oxide in case aluminum chloride is used. These procedures are shown in the examples.

In carrying out the process of the present invention, it is necessary to keep the potential of cathode approximately equal to the deposition potential of hydrogen in the same condition. As the potential differs according to the kind and shape of the cathode employed and the composition of the catholyte, it is determined beforehand by the result of experiments. We shall explain below citing concrete examples. The cathode potential is determined against a saturated calomel electrode, its tip being placed close to the surface of the cathode. When 100 ml. of the catholyte of pH 2.0 containing 10 g. of streptomycin sulfate, 0.05 mole of phosphoric acid and 0.026 mole of caustic soda is to be electrolyzed under stirring with mercury cathode, it is advisable to maintain the cathode potential at 1.40 v. against the saturated calomel electrode. Again, if an aqueous solution of 10% streptomycin sulfate maintained at a pH 2.0–2.5 with the dropping addition of sulfuric acid is to be electrolyzed under stirring with the mercury cathode, the cathode potential is to be maintained at —1.47 v. This potential may be adjusted either by placing a resistance in the electric circuit or by changing bath voltage with other means, or by changing the surface area of electrode, or by employing an equipment for automatic adjustment, for instance, such as a controlled potential electrolyser.

As a diagram to be used in the execution of the process of the present invention, any material will be effective that prevents streptomycins in the catholyte from diffusing too much in the anolyte, and enables hydrogen ion and other ions to pass through it. In this respect, an unglazed porcelain plate, a bladder membrane or a sintered glass disk may represent an apt example.

As an electrode to be used in executing the process of the present invention, any electrode that is applicable in an ordinary electrolysis will be all right. However, proper materials for a cathode are mercury with a high hydrogen overvoltage, lead, zinc, amalgamated lead or amalgamated zinc, etc.; and the best materials for an anode are stable platinum, carbon, lead coated with lead dioxide etc.

For the process of the present invention, impure streptomycins with 50–60% of purity as well as those of high purity serve the purpose very well. In the actual industrial production, the fact that the material of impure quality can be used does offer a great advantage that the management of the factory can be facilitated and the cost of production can be decreased.

However, it should be considered that, although the use of streptomycins of high purity enables use to obtain dihydrododesoxystreptomycins in the catholyte by electrolysis over the reaction is over, the use of impure streptomycins as the starting material will necessitate the process of refinement of the crystals of free bases or basic salts of dihydrododesoxystreptomycins in the way.

When the concentration of pH of the catholyte is effected with the addition of sulfuric acid, the electrolysis shall be allowed to be continued after the completion of the reaction to let the union in the catholyte move into the anode compartment and, at the same time, raise the pH of the catholyte by consuming the hydrogen ion in the catholyte until pH 10 is attained. Then, by concentrating it, or adding acetone thereto, crystals of basic salts of dihydrododesoxystreptomycins can be obtained. Or, if the pH is raised up to pH 13 by electrolysis, either it may be concentrated or acetone may be added thereto and crystals of the free bases of dihydrododesoxystreptomycins will be obtained.

As described above, according to the process of the present invention, dihydrododesoxystreptomycins of high purity, potent agents for the chemotherapy of tuberculosis, can easily be obtained with high yield. Below, we shall illustrate the present invention by way of example in detail about the execution of examples. These examples show the various embodiments of the present invention, but it is to be understood that these examples are given by way of illustration and not of limitation.

**Example 1**

An electric cell is divided into anode and cathode compartments by means of an unglazed pottery diaphragm.
The cathode compartment is charged with the catholyte comprising 100 ml. of water in which 10.0 g. of streptomycin sulfate (750 u./mg.) is dissolved. At the bottom of the catholyte is placed mercury as the cathode which is connected with the cathode by a platinum wire enclosed in a glass tube. At the point a few millimeters apart from the surface of the cathode, an opening of the saturated calomel electrode is placed as a standard for regulating the cathode potential. The surface of mercury is mechanically stirred with a glass stirrer. The anode compartment is charged with 100 ml. of 2% sulfuric acid into which a carbon plate is inserted as the anode. Both the anode and cathode electrodes are connected to the electric source of the controlled potential electrolyzer. The cathode potential is controlled at —1.45 v. against the above-mentioned calomel electrode, and an electric current is passed. During the electrolysis, the pH of the catholyte is maintained at pH 2.0–2.5 with the dropwise addition of 20% sulfuric acid. The amperage of the current, which is of several hundred milliamperes at the beginning, is gradually diminished and in 3 hours reaches a very low fixed value. After continuing electrolysis for another hour, 1 ml. of the catholyte is taken out and the residual unconverted streptomycin is determined by the malot assay to be less than 1% of the starting quantity. Then the dropwise addition of sulfuric acid is ceased and by making the cathode potential more negative, an electric current is continued to be passed for some time so that the pH of the catholyte rises gradually. When the pH of 6 is attained, the current is cut off, and the catholyte is taken out and concentrated to 40 ml. under reduced pressure at below 40° C. The solution is then added dropwise to five volumes of methanol under stirring, and dihydrodesoxystreptomyein of high purity precipitates in white amorphous form. The yield is 8.7 g. The dihydrodesoxystreptomycin content determined by Ikeda method after drying the product is 3.9% of the whole, and the unit of the biological assay is 830 u./mg.

**EXAMPLE 2**

As the catholyte aqueous solution of 10.0 g. of streptomycin hydrochloride-calcium chloride complex salt is employed. Instead of sulfuric acid added for maintaining pH at 2.0–2.5 in Example 1, 5% hydrochloric acid is employed. In other points, the same procedure as described in Example 1 is pursued. The reduction being completed, the pH of the catholyte is raised to pH 6 by passing an electric current, and, with the addition of a proper quantity of silver carbonate the solution is filtered. Then the filtrate is freeze-dried, and 8.2 g. of dihydrodesoxystreptomyein hydrochloride of high purity is obtained. By applying the analytical method as described in Example 1, the content of dihydrodesoxystreptomyein is found to be 3.8%. 

**EXAMPLE 3**

In this example, instead of adding dropwise dilute sulfuric acid to the catholyte to maintain its pH 2.0–2.5 as in Example 1, 1.96 g. of phosphoric acid and 0.416 g. of caustic soda are added to the catholyte prior to the electrolysis. In other points, the same procedure as pursued as in Example 1 except that the cathode potential is controlled at —1.4 v. against the saturated calomel electrode. When streptomycin is certified by malot reaction to have become about 1% of its starting quantity, the catholyte is taken out. The pH of the catholyte upon completion of the electrolysis is 2.4. Aqueous solution of caustic soda is added to the catholyte to make it a pH 8.5, and by adding an excess amount of sodium pentachlorophenate, dihydrodesoxystreptomyein salt of high purity which is reduction product precipitates as its pentachlorophenate. The precipitate is filtered off, washed with water and dissolved in aqueous n-butanol. To this solution, 10% sulfuric acid is added under stirring, and dihydrodesoxystreptomyein of high purity moves to a separating water layer. Sulfuric acid is added dropwise to the water layer until its pH becomes 5.0. The water layer is separated and added dropwise to five volumes of methanol, and dihydrodesoxystreptomyein sulfate of high purity precipitates. The yield after drying the product is 8.2 g. By applying the analytical method as described in Example 1, the content of dihydrostreptomycin is found to be 3.2%, and the result of biological assay is 840 u./mg.

**EXAMPLE 4**

As the cathode, an amalgamated lead plate is employed, and, as the anode, a lead plate coated with lead dioxide is employed. In 10 ml. of the catholyte, 10.0 g. of streptomycin sulfate and 33 g. of aluminum sulfate $\text{Al}_2\left(\text{SO}_4\right)_3\cdot18\text{H}_2\text{O}$ are involved, and its pH is 2.2 at 30° C. In other points, the conditions are same as those of Example 1 and under which the electrolytic reduction is effected. During the process of reduction, when the pH of the catholyte has become 2.5, sulfuric acid or aluminum sulfate is added to the solution so that the pH of the catholyte may be maintained within the range of 2.0–2.5. Upon completion of the reduction, the catholyte is taken out and an aqueous solution of barium hydroxide is added thereto to raise the pH to 8. The precipitate is filtered off, the filtrate is concentrated at reduced pressure to 40 ml. and dihydrodesoxystreptomyein sulfate of high purity is precipitated as in Example 1. The yield is 8.2 g. The result of analysis shows that the dihydrostreptomycin sulfate involved occupies 3.4% of the total amount, and the result of biological assay is 830 u./mg.

**EXAMPLE 5**

Employing an aqueous solution containing 10.0 g. of streptomycin hydrochloride and 10.0 g. of aluminum chloride as the catholyte, and following the same procedure as in Example 1 in other points, the electrolytic reduction is executed. When the pH has become 2.5 during the process of reduction, an aqueous solution of aluminum chloride is further added. After the reduction is completed, excess silver oxide is added and stirred. The filtrate is concentrated under reduced pressure and freeze-dried, and dihydrodesoxystreptomyein hydrochloride of high purity is obtained. The yield is 8.1 g. The content of dihydrodesoxystreptomyein hydrochloride is 5.0%.

**EXAMPLE 6**

Employing 100 ml. of an aqueous solution containing 10.0 g. of mannolidsstreptomyein sulfate as the catholyte, and under quite the same conditions as in Example 1 in other points, the electrolytic reduction is executed. By pursuing the same method as in Example 1, 8.7 g. of dihydrodesoxymannolidsstreptomyein sulfate is obtained. The content of dihydromannolidsstreptomyein is 3.1%.

**EXAMPLE 7**

As the catholyte, an aqueous solution containing 10.0 g. of hydroxystreptomyein phosphate is employed, and the pH of the solution during the process of reduction is maintained at 2.0–2.5 with the addition of aqueous solution of 20% phosphoric acid. As the cathode, amalgamated zinc is used. Then, by pursuing the method under the same conditions as in Example 1 and regulating the cathode potential at —1.35 v., the electrolysis is executed, and 8.3 g. of dihydrodesoxyhydroxystreptomyein phosphate of high purity is obtained. The content of dihydrodesoxyhydroxystreptomyein phosphate is 3.7%.

**EXAMPLE 8**

(a) Employing an aqueous solution containing 15 g. of impure streptomycin sulfate (500 u./mg.) as the catholyte, and under the same conditions as in Example 1, the electrolytic reduction is executed. After the reduction is over, passing of electric current is continued at the more negative cathode potential, and the pH of the catholyte rises gradually. When the pH reaches about 10, the catholyte is taken out and concentrated quickly.
under reduced pressure at below 30° C. to 50 ml. After
the solution is cooled, acetone is added thereto, and left
to stand in an ice chamber. Then, basic sulfate of di-
hydrodesoxystreptomycin of high purity is crystallized
out. The yield is 7.5 g. The result of analysis shows
that the content of basic sulfate of dihydrostreptomycin
is 3.5%.

(a) The procedure for electrolytic reduction is fol-
lowed just as in Example 7. After the reduction is over,
the pH of the catholyte is raised to 13 by the passing
of an electric current, and the catholyte is concentrated
quickly to 30 ml. and left to stand in an ice chamber.
Then free base of dihydrodesoxystreptomycin is crystal-
lized out. The yield is 7.1 g. The result of analysis shows
that the content of free base of dihydrostrepto-
mycin is 2.7%

Modifications may be made in carrying out the pres-
ent invention without departing from the spirit and scope
thereof, and our invention is to be limited only by the
 appended claims.

We claim:

1. A process for producing dihydrodesoxystreptomycins
which comprises charging a container, having anode and
cathode compartments separated by a diaphragm, with
an aqueous solution containing acid addition salt of
streptomycins as a catholyte and an aqueous solution of
strong inorganic salt as an electrolyte, positioning an elec-
trode in each of the electrolytes, characteristically main-
taining the pH of the catholyte at 2.0-2.5 and keeping
the cathode potential approximately equal to the deposit
ion potential of hydrogen while passing an electric cur-
rent between the anode and the cathode until the salt of
streptomycins in the catholyte is reduced, and recover-
ing the corresponding dihydrodesoxystreptomycin.

2. The process as defined in claim 1, wherein the
streptomycin employed is a substance selected from the
group consisting of streptomycin, hydrostreptomycin
and mannooxidestreptomycin.

3. The process as defined in claim 1, wherein the acid
addition salt of streptomycins employed is a substance
selected from the group consisting of sulfate, hydrochlor-
ide and hydrochloride-calcium chloride complex salt.

4. The process as defined in claim 1, wherein a strong
inorganic acid is added to the catholyte during the whole
period of reduction in order to maintain the pH of the
catholyte always at 2.0-2.5.

5. The process as defined in claim 1, wherein a pre-
determined quantity of monobasic phosphate ion buffer
is added to the catholyte at the beginning of the reduc-
tion in order to maintain the pH of the catholyte always
at 2.0-2.5.

6. The process as defined in claim 1, wherein a pre-
determined quantity of a substance selected from the
group consisting of aluminum sulfate and aluminum chlo-
ride is added to the catholyte at the beginning of the
reduction in order to maintain the pH of the catholyte
always at 2.0-2.5.

7. The process as defined in claim 1, wherein an
aqueous solution containing impure acid addition salt of
streptomycin is employed as the catholyte, the reduction
is carried out always maintaining the pH of the solution
at 2.0-2.5 with the addition of a strong inorganic acid
thereof, and, after the reduction is over, the pH of the
solution is made about 10 by continuously the passing of
gas electric current, the solution is then concentrated,
or acetone is added thereto, and crystals of basic salt of di-
hydrodesoxystreptomycins of pure quality are obtained.

8. The process as defined in claim 1, wherein an
aqueous solution containing impure acid addition salt of
streptomycin is employed as the catholyte, the reduction
is carried out maintaining the pH of the solution at
2.0-2.5 with the addition of a strong inorganic acid there-
to, and, after the reduction is over, the pH of the solu-
tion is made about 13 by continuing the passing of an
electric current, the solution is then concentrated, or
acetone is added thereto, and free base of dihydrodesoxy-
 streptomycins of pure quality is obtained.

9. The process as defined in claim 1 wherein said
diaphragm employed is made of a substance selected
from the group consisting of unglazed porcelain, bladder
membrane and sintered glass disk.

10. The process as defined in claim 1, wherein said
cathode employed is made of a substance selected from
the group consisting of mercury, lead, amalgamated lead,
zinc and amalgamated zinc.

11. The process as defined in claim 1, wherein said
anode employed is made of a substance selected from the
group consisting of carbon plate, platinum plate and
lead plate coated with zinc oxide.

12. A process for the production of dihydrodesoxy-
 streptomycin which comprises subjecting streptomycin
sulfate to electrolytic reduction in a diaphragm cell em-
ploying an aqueous solution of about 10% streptomycin
sulfate as catholyte and a dilute aqueous sulfuric acid
solution as anolyte, the electrolytic reduction being af-
fected at a pH maintained at about 2.0-2.5, at a cathode
potential of about 1.4 volts, and at room temperature.

13. A process for the production of dihydrodesoxy-
 streptomycin which comprises subjecting streptomycin
sulfate to electrolytic reduction in a diaphragm cell em-
ploying an aqueous solution of about 10% streptomycin
sulfate as catholyte and a dilute aqueous sulfuric acid
solution as anolyte, the electrolytic reduction being af-
fected at a pH maintained at about 2.0-2.5 and at a
cathode potential of about 1.4 volts.

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