United States Patent

[11] 3,616,244

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[54] **PRODUCTION OF LINCOMYCIN SULFOXIDE**
6 Claims, No Drawings

[52] **U.S. Cl.** ..... **195/80,** 195/29, 195/81, 260/210 R
[51] **Int. Cl.** **C12d 9/00**
[50] **Field of Search** 195/80, 81, 29; 260/210

[56] **References Cited**

UNITED STATES PATENTS

2,602,769	7/1952	Murray et al.	195/51
2,735,800	2/1956	Murray et al.	195/51
3,086,912	4/1963	Bergy et al.	195/80 X
3,380,992	4/1968	Argoudelis et al.	195/80 X
3,382,230	5/1968	Magerlein	260/210

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ABSTRACT: A microbiological process for the oxygenation of lincomycins to obtain the corresponding lincomycin sulfoxides which are known active antibacterial agents.

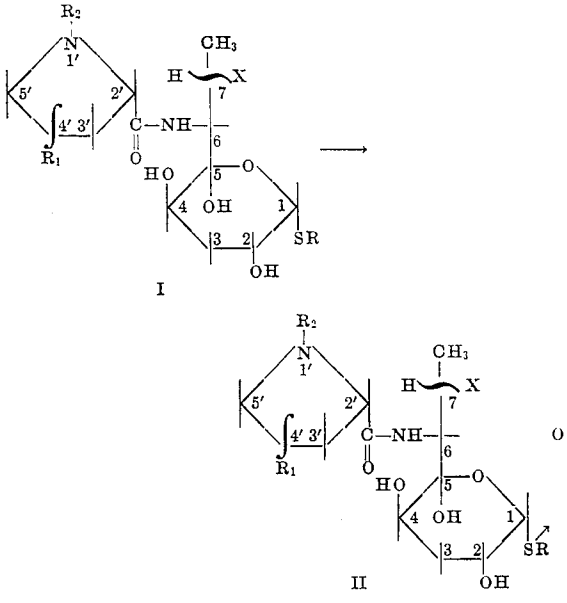
PRODUCTION OF LINCOMYCIN SULFOXIDE

SUMMARY OF THE INVENTION

This invention relates to a novel process for the oxygenation of lincomycins to obtain the corresponding lincomycin sulfoxides. More particularly, this invention comprises a process whereby lincomycins, e.g., lincomycin, 7-epilincomycin (the epimer of lincomycin wherein the 7-hydroxy substituent is in the 7(S) position rather than the 7(R) position as in lincomycin itself), 7(S)-chloro-7-deoxylincomycin and 7(R)-chloro-7-deoxylincomycin analogs thereof, and the corresponding acid addition salts thereof, are subjected to the oxygenating activity of certain micro-organisms to obtain the corresponding lincomycin sulfoxides, such as lincomycin sulfoxide, 7-epilincomycin sulfoxide, the respective 7(S)-chloro-7-deoxylincomycin sulfoxide, 7(R)- chloro-7-deoxylincomycin sulfoxide, analogs thereof, and the corresponding acid addition salts thereof.

DETAILED DESCRIPTION OF THE INVENTION

The microbiological process of this invention is represented by the following reaction scheme:



wherein R is methyl or ethyl, R_1 is alkyl of from two to eight carbon atoms, inclusive, R_2 is hydrogen or alkyl of from one to eight carbon atoms, inclusive, and X is hydroxy, chlorine or bromine.

Examples of alkyl of from one to eight carbon atoms, inclusive, are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, and octyl and isomeric forms thereof.

In this application, the wavy line ($\int$) appearing at the 4'-position of the pyrrolidine ring is used to indicate that the group R_1 can be position cis (below the plane of the ring) of trans (above the plane of the ring), with respect to the carbonyl group the wavy line appearing at the 7-position is used to indicate that both epimers are to be included, i.e., the (S) configuration wherein the hydroxy, chloro or bromo group is located to the right of the vertical line as drawn or the (R) configuration wherein the hydroxy, chloro or bromo group is located to the left. The wavy line to the oxygen atom is used to indicate that the oxygen can be up or down.

The compounds of Formulas **1** and II exist either in the pronotated or nonprotonated forms. The free bases can be converted to stable acid-addition salts in accordance with methods known in the art, for example, by neutralizing the free base with the appropriate acid to below about pH 7.0, and advantageously to about pH 2 to pH 6. Suitable acids for this purpose include hydrochloric, sulfuric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, pamoic, chlolic, palmitic, mucic, camphoric, glutaric, glycolic, phthalic, tartaric,

lauric, stearic, salicyclic, 3-phenylsalicyclic, 5-phenylsalicyclic, 3-methylglutaric, orthosulfobenzoic, cyclopentanepropionic, 1, 2-cyclohexane-dicarboxylic, 4-cyclohexanecarboxylic, octadecenylsuccinic, octenylsuccinic, methanesulfonic, benzenesulfonic, helianthic, Reinecke's, dimethyldithiocarbamic, cyclohexylsulfamic, hexadecylsulfamic, octadecylsulfamic, sorbic, monochloroacetic, undecylenic, 4'-hydroxyazobenzene-4-sulfonic, octyldecylsulfuric, picric, benzoic, cinnamic, and like acids.

The lincomycin sulfoxides produced by the process of the present invention, represented by Formula **11**, above, and the acid addition salts thereof are known active antibacterial agents. For example see U.S. Pat. No. 3,382,230. They have in vitro and in vivo antibacterial activity and can be administered orally or parenterally to animals, including mammals, for the alleviation of bacterial infections especially those caused by Gram positive bacteria such as *Staphylococcus aureus* and *Streptococcus hemolyticus*. For example, 7(S)-chloro-7-deoxylincomycin sulfoxide has in vivo antibacterial activity comparable to that of lincomycin and can be used in vivo for the same purposes and in the same manner as lincomycin. The compounds of the invention are particularly useful because of the relative low toxicity and their effectiveness in rapidly establishing high blood levels.

The starting compounds of Formula **1**, above, and the acid addition salts thereof, are known in the art, see for example, U.S. Pat. No. 3,380,992, Belgium Pat. Nos. 676,202 and 705,345 issued Aug. 8, 1966 and Apr. 19, 1968 , respectively; French Pat. No. 1,474,237, issued Feb. 13, 1967; South African Pat. Nos. 66/0319 and 66/0320, issued Jan. 25, 1967 and Magerlein et al., "Chemical Modifications of Lincomycin", Antibiotics and Chemotherapy (1966), page 727 ff.

The microbiological process of this invention comprises subjecting a lincomycin of Formula **1** to the oxygenating activity of one of the following micro-organisms:

Streptomyces armentosus, NRRL 3176;
Streptomyces spectabilis, NRRL 2494;
Streptomyces spectabilis, NRRL 2792;
Streptomyces lincolnensis, NRRL 2936;
Anixiopsis multispora, CBS;
Aspergillus citricus, IMI 15954;
Aspergillus niger, ATCC 8740; and
Aspergillus niger, ATCC 9029;

which are available from known public sources such as the Northern Utilization Research and Development Branch, U.S. Department of Agriculture, Peoria, Ill. (NRRL); the American Type Culture Collection (ATCC), Washington, D.C.; Centraalbureau voor Schimmelcultures (CBS), Baarn, Holland; and The Commonwealth Mycological Institute (IMI), Kew, Surrey, England, as indicated above. In the practice of this invention the species *Streptomyces armentosus*, NRRL 3176 is especially advantageous.

The operational conditions and reaction procedures for the bioconversion process of this invention are advantageously those known in the art of bioconversion as illustrated in Murray et al., U.S. Pat. Nos. 2,602,769 and 2,735,800.

In the practice of this invention, the bioconversion can be effected by a growing or resting culture of the micro-organism or by spores, washed cells or enzymes of the micro-organism.

Culture of the micro-organism for the purpose and practice of this invention is in or on a medium favorable to its development. Sources of nitrogen and carbon should be present in the culture medium and an adequate sterile air supply should be maintained during the conversion, for example, by the conventional techniques of exposing a large surface of the medium to the air or by passing air through a submerged culture.

Nitrogen in assimilable form can be provided by sources normally employed in such processes, such as corn steep liquor, cotton seed meal, soybean meal, yeast extracts, peptone, soluble or insoluble vegetable or animal protein, lactalbumin, casein, whey, distillers solubles, amino acids, nitrates and ammonium compounds, such as ammonium tartrate, nitrate, sulfate and the like.

Available carbon can also be provided by sources normally used in bioconversions such as carbohydrates, e.g., glucose,

fructose, sucrose, lactose, maltose, dextrines, starches; meat extracts, peptones, amino acids, proteins, fatty acids, glycerol, whey and the like. These materials may be used either in a purified state of as concentrates such as whey concentrate, corn steep liquor, grain mashes, cotton seed meal, and the like, or as mixtures of the above. Many of the above sources of carbon can also serve as a source of nitrogen.

The medium can desirably have a pH before inoculation of between about pH 4 to about 8 , though a higher or lower pH can be used. A temperature between about 25° to 32° C. is preferred for growth of the micro-organism, but higher or lower temperatures within a relatively wide range are suitable.

The substrate compound (1) can be added to the culture during the growth period of the micro-organism as a single feed or by intermittent addition during the conversion period, or it can be added to the medium before or after sterilization or inoculation making appropriate adjustments for effects of pH and/or temperature upon the stability of the substrate used. The preferred, but not limiting, range of concentration of the substrate in the culture medium is about 50 –500 mg. per liter. In the practice of this invention, it is convenient to add the substrate to the medium in the form of a water-soluble acid addition salt.

The temperature during the fermentation can be the same as that found suitable for growth of the micro-organism. It need be maintained only within such range as supports life, active growth or the enzyme activity of the micro-organism. A range of 20° to 35° C. is preferred. A pH of about 6 to 8 is generally preferred for growth of the micro-organism during the bioconversion. Aeration can be effected by surface culture or preferably by use of submerged fermentation conditions with air sparging, in accordance with methods well-known in the art. The time required for oxygenation by the enzymatic system of the micro-organism employed can vary considerably. The range of about 2 to 120 hours is practical but not limiting; 24 –72 hours is generally satisfactory. The progress of the bioconversion and its completion are conveniently determined by paper-strip chromatography, or thin-film chromatography [Heftman, Chromatography (1961) Reinhold Publishing Co., New York, N.Y.].

Alternatively, oxygenation of the selected substrate can be effected under aerobic conditions by subjecting it to the activity of enzymes prepared from the micro-organism, to the action of spores of the micro-organism. Isolated enzyme preparations can be prepared in accordance with the general procedures disclosed by Zuidweg et al., Biochim, Biophy. Acta, 58, 131–133 (1962). The bioconversion can be effected with spores in accordance with the general process disclosed in U.S. Pat. Nos. 3,031,379 and 3,031,382. The separation of washed cells from the fermentation medium is well-known in the art, see for example, U.S. Pat. No. 2,831,789.

In the case of the bioconversion of lincomycin itself to lincomycin sulfoxide by use of the oxygenating activity of *Streptomyces lincolnensis*, the bioconversion may be conducted according to the principles discussed above, with addition of preformed lincomycin or an acid addition salt thereof to the oxygenating milieu, as borne out by oxygenations in which added radioactive lincomycin is found to be bioconverted to radioactive lincomycin sulfoxide.

Lincomycin in produced *S. lincolnensis* as described in U.S. Pat. No. 3,086,912. It has been found in the present invention that lincomycin thus accumulated from the lincomycin-biosynthesis activities of *S. lincolnensis* can be bioconverted to lincomycin sulfoxide by the simple agency of extending the time of the lincomycin fermentation beyond the time necessary for maximal lincomycin accumulation, for example, by use of a total fermentation time of about 12 days as compared to a time of about 6 days for maximal lincomycin production.

The term "oxygenating activity" as used throughout this specification means the enzymatic action of a growing or resting culture of the micro-organism which effects introduction of oxygen into the molecule of the substrate under fermentation conditions.

After completion of the fermentation, the resulting oxygenated products **11** are recovered from the fermentation beer by conventional methods. For example, the whole beer can be extracted with a water-immiscible organic solvent such as methylene chloride, chloroform, carbon tetrachloride, ethylene chloride, trichlorethylene, ether, amyl acetate, benzene, and the like, or the beer and mycelium can be separated by conventional methods such as centrifugation or filtration, and then separately extracted with suitable solvents. The mycelium can be extracted with either water-miscible or water-immiscible solvents or in cases where little or no product is contained in the mycelium, it can be merely washed with water and the wash water added to the beer filtrate. The beer, free of mycelium, can then be extracted with water-immiscible solvents such as those listed above. The extracts are combined, dried over a drying agent such as anhydrous sodium sulfate, and the solvent removed by conventional methods such as evaporation or distillation at atmospheric or reduced pressure.

Alternatively, the products can be adsorbed from the beer on an adsorbent resin or on carbon and the products eluted with a polar organic solvent such as methanol, ethanol, acetone, ethyl acetate, methyl ethyl ketone, aqueous mixtures thereof, and the like.

The products **11** obtained by either the extraction or elution procedures can be isolated and further purified by conventional methods, e.g., chromatography and/or crystallization, and the like. The products **11** can be obtained as free base or as acid addition salts in accordance with procedures hereintofore disclosed.

The following examples are intended to illustrate the process of this invention. The examples are for the purpose of illustrating the best mode contemplated of carrying out the invention and to supplement the foregoing disclosure with additional descriptions of the manner and process of carrying out the invention so as to further enable workers skilled in the art to do so, but they are not to be construed as limiting.

Example 1 7(S)-Chloro-7-deoxylincomycin sulfoxide

A vegetative seed from a culture of *Streptomyces armentosus*, NRRL 3176, is prepared by growing the culture for 3 days at about 28° C. in reciprocating shake flasks (250 r.p.m., stroke 2.5 cm.) on a medium consisting of 25 g. per liter of Cerelose (glucosemonohydrate) and 25 g. per liter of Pharmamedia (Cotton Seed Flour, Traders Milling Co., P. O. Box 1837, Fort Worth, Tex. 76100) in tap water. The medium is sterilized prior to inoculation. The pH prior to sterilization is 7.2.

The resulting seed is used to inoculate a medium consisting of 20 g. per liter of black strap molasses, 30 g. per liter of starch, 15 g. per liter of fish meal and 15 g. per liter of Pharamamedia. Lard oil (5 ml. per liter) is added to prevent foaming. The medium is adjusted to pH 7.2 and 40 shake flasks (500 ml.) are prepared each containing 100 ml. of the medium. The shake flasks are sterilized (pH after sterilization is 6.2) and are inoculated with 5 ml. of the vegetative seed (prepared above) per 100 ml. of medium.

The culture is then allowed to grow for 48 hours on a rotary shaker (250 r.p.m., stroke 2.5 cm.) at about 28° C. At the end of the 48 hour growth period, 50 mg. of 7(S)-chloro-7-deoxylincomycin hydrochloride is added to each shake flask and the fermentation is continued for an additional period of about 72 hours. The contents of the flasks are then pooled (about 4 liters), diatomaceous earth is added and the beer and mycelium are separated by filtration. The filter cake is washed with about 1 l. of water and the wash water is added to the clear beer.

The combined clear beer and wash thus obtained (4.8 L.) is adjusted to pH 10.0 with aqueous sodium hydroxide and extracted three times with 1,600 ml. portions of methylene chloride. The combined extracts are evaporated to dryness to give 1.0 g. of crude 7(S)-chloro-7-deoxylincomycin sulfoxide, which is purified by conventional methods such as chromatography.

An aliquot (200 mg.) of the dried residue thus obtained is dissolved in 5 ml. of 1 N methanolic hydrogen chloride and the solution thus obtained is mixed with ether to give a precipitate which is removed by filtration, washed with ether

and dried. The dried product thus obtained is assayed by thin layer chromatography [silica gel G., methyl ethyl ketone-acetone water (140:40:22 Vol./Vol.)] and shows the presence of 7(S)-chloro-7-deoxylincomycin hydrochloride and 7(S)-chloro-7-deoxylincomycin sulfoxide hydrochloride as the main components.

The remaining residue (800 mg.) is distributed in an all glass countercurrent distribution apparatus using a solvent system consisting of equal volumes of n-butanol and water. The pH of the lower phase of the first 20 tubes is adjusted to 4.0 using aqueous hydrochloric acid. After about 500 transfers, the distribution is analyzed by thin layer chromatography. Those fractions which show 7(S)-chloro-7-deoxylincomycin sulfoxide hydrochloride to be the major product are triturated with acetone to give crystalline 7(S)-chloro-7-deoxylincomycin sulfoxide hydrochloride (about 440 mg.). The product is further identified by comparison with an authentic sample of the same compound using NMR spectra, mass spectra and thin layer chromatography.

An analytical sample of the 7(S)-chloro-7-deoxylincomycin sulfoxide hydrochloride product from the above bioconversion gives the following analysis:

Anal. Calcd. for	$C_{18}H_{33}N_2O_6SCl\cdot H_2O\cdot HCl$	
	C, 43.63; H, 7.27; N, 5.66; Cl, 14.34	S, 6.46;
Found	C, 42.62; H, 7.38; N, 5.91; Cl, 14.25	S, 6.32;

Mol. Wt. calcd. for the free base: 440

Mol. Wt. found for the free base: 440 (by mass spectrometry)

Other acids such as those hereintofore listed can be used in place of hydrochloric acid to obtain the corresponding acid addition salt of 7(S)-chloro-7-deoxylincomycin sulfoxide.

In the same manner, the following micro-organisms:

Streptomyces spectabilis, NRRL 2494;
Streptomyces spectabilis, NRRL 2792;
Streptomyces lincolnesis, NRRL 2936;
Anixiopsis multispora, CBS;
Aspergillus citricus, IMI 15954;
Aspergillus niger, ATCC 8740; and
Aspergillus niger, ATCC 9029;

are used in place of *Streptomyces armentosus*, NRRL 3176 to obtain 7(S)-chloro-7-deoxylincomycin sulfoxide or one of its acid addition salts.

In the same manner following the procedure of example 1, other lincomycins of formula I can be substituted as the substrate in place of 7(S)-chloro-7-deoxylincomycin hydrochloride to obtain the corresponding lincomycin sulfoxide of formula II. The following conversions are representative of further substrate materials which can be used, either as free bases or acid addition salts thereof, with reference to formula I, in this invention to obtain the corresponding compounds of formula II as free bases or acid addition salts thereof.

R_1	7-X	R	R_2
trans-ethyl	(S)-Cl	Methyl	methyl
trans-ethyl	(S)-Cl	methyl	hydrogen
trans-ethyl	(R)-OH	methyl	methyl
trans-ethyl	(R)-Cl	methyl	methyl
cis-ethyl	(S)-Cl	methyl	methyl
cis-ethyl	(R)-Cl	methyl	methyl
trans-n-propyl	(S)-Cl	methyl	methyl
trans-n-propyl	(S)-Cl	methyl	ethyl
trans-n-propyl	(S)-Cl	methyl	hydrogen
trans-n-propyl	(S)-Br	methyl	methyl
trans-n-propyl	(R)-OH	methyl	methyl
trans-n-propyl	(R)-OH	methyl	ethyl
trans-n-propyl	(R)-OH	methyl	hydrogen
trans-n-propyl	(S)-Cl	ethyl	methyl
trans-n-propyl	(S)-Cl	ethyl	ethyl
trans-n-propyl	(S)-Cl	ethyl	hydrogen
trans-n-propyl	(R)-OH	ethyl	methyl
trans-n-propyl	(R)-Cl	methyl	methyl
cis-n-propyl	(S)-Cl	methyl	methyl
cis-n-propyl	(R)-OH	methyl	methyl
trans-i-propyl	(S)-Cl	methyl	methyl
trans-n-butyl	(S)-Cl	methyl	methyl
trans-n-butyl	(R)-OH	ethyl	methyl
trans-n-butyl	(R)-Cl	methyl	methyl
cis-n-butyl	(S)-Cl	methyl	methyl
trans-n-pentyl	(S)-Cl	methyl	methyl
trans-n-pentyl	(S)-Cl	methyl	ethyl
trans-n-pentyl	(S)-Cl	methyl	hydrogen
trans-n-pentyl	(S)-Br	methyl	methyl
trans-n-pentyl	(R)-OH	methyl	methyl
trans-n-pentyl	(R)-OH	methyl	ethyl
trans-n-pentyl	(R)-OH	methyl	hydrogen
trans-n-pentyl	(S)-Br	ethyl	methyl
trans-n-pentyl	(R)-Cl	methyl	methyl
trans-n-pentyl	(S)-OH	methyl	methyl
cis-n-pentyl	(S)-Cl	methyl	methyl
trans-n-hexyl	(S)-Cl	methyl	methyl
trans-n-hexyl	(S)-Cl	methyl	ethyl
trans-n-hexyl	(S)-Cl	methyl	hydrogen
trans-n-hexyl	(S)-Cl	ethyl	methyl
trans-n-hexyl	(R)-Cl	methyl	methyl
cis-n-hexyl	(S)-Cl	methyl	methyl
trans-i-heptyl	(R)-OH	methyl	methyl
trans-n-heptyl	(S)-Cl	methyl	methyl
trans-n-heptyl	(R)-Cl	methyl	methyl
cis-n-heptyl	(S)-Cl	methyl	methyl
cis-n-heptyl	(R)-Br	methyl	methyl
trans-n-octyl	(S)-Cl	methyl	methyl
trans-n-octyl	(S)-Cl	ethyl	methyl
trans-n-octyl	(R)-OH	ethyl	methyl
trans-n-octyl	(R)-Cl	methyl	methyl
trans-i-octyl	(S)-Cl	methyl	methyl
cis-n-octyl	(S)-Cl	methyl	methyl

Example 2 7(S)-Chloro-7-deoxylincomycin sulfoxide

A medium is prepared consisting of 2 g. of malt extract, 0.1 g. of peptone and 2 g. of Cerelose diluted to 100 ml. Five 20-ml. portions of this medium in 125-ml. flasks are sterilized and each is inoculated with a 72 hour vegetative growth of one of the following micro-organisms:

Streptomyces spectabilis, NRRL 2494;
Streptomyces spectabilis, NRRL 2792;
Anixiopsis multispora, CBS;
A*Aspergillus citricus*, IMI 15954;
Aspergillus niger, ATCC 8740; and
Aspergillus niger, ATCC 9029.

After 2 to 5 days when a heavy growth of mycelium is apparent by visual observations 50 to 100 mg. of 7(S)-chloro-7-deoxylincomycin hydrochloride are added to each flask and the incubation of each flask is continued for an additional period of from 3 to 5 days.

The samples are then checked for bioconversion by paper chromatography. Aliquots of 2 to 5 μl of the filtered beer from each of the bioconversions are applied on filter paper (Whatman No. 3 HR, 18 × 13 mm.). The bioconversion products are separated from the starting material by ascending chromatography using a mixture of ethyl acetate; acetone; water (8:5:1 vol./vol.) as solvent system. Comparison of these papergrams with a papergram made in the same manner from an authentic mixture of 7(S)-chloro-7-deoxylincomycin and 7(S)-chloro-7-deoxylincomycin sulfoxide shows that each of the bioconversions produce 7(S)-chloro-7-deoxylincomycin sulfoxide as the bioconversion product.

Likewise, using the micro-organisms listed above, the other lincomycins of formula I, above, or acid addition salts thereof, for example, those compounds listed in example 1, above, can be converted to the corresponding sulfoxides of formula II or the acid addition salts thereof.

Example 3 Lincomycin sulfoxide

Sterile preseed medium consisting of 10 g. per liter Cerelose, 5 g. per liter Yeastolac (Partial autolysate of brewer's yeast cells, blended with milk solids, Vico Products Co., Chicago, Ill.) and 5 g. per liter N-Z Amine B (Casein hydrolysate, Sheffield Division of National Dairy Products, Norwich, N.Y.), adjusted to pH 7.2 with NaOH, is dispensed in 100ml. portions in 500-ml. widemouthed Earlenmeyer shake flasks and sterilized at 121° C. for 30 minutes. The cooled flasks are inoculated with *Streptomyces lincolnensis*, NRRL2936 and incubated on a reciprocating shaker at 28° C. for 2 days to yield vegetative preseed.

Twenty liters of sterile seed medium consisting of 10 g. per liter Cerelose, 10 g. per liter torula yeast (St. Regis Paper Co., Lake States Division, Rhinelander, Wis.), 5 g. per liter distillers solubles (National Distillers Products Co., New York, N.Y.) and 4 g. per liter of NaCl is made up in a 40-liter seed tank and adjusted to pH 7.2 with NaOH. One g. per liter of $CaCO_3$ and 2 ml. per liter of lard oil (antifoam) are added and the medium is sterilized at 121° C. for 30 minutes. Upon cooling by use of cold jacket water it is inoculated with 100 mls. of the above preseed and incubated for 2 days at 28° C. with stirring by flat-bladed impellor at 400 r.p.m. and sparging with sterile air at a rate of 10 standard liters per minute, to yield vegetative seed.

Two hundred fifty liters of the following sterile fermentation medium are prepared in a fermentor tank of about 380 liters total volume: Cerelose, 3.75 kg.; cornstarch, 10 kg.; Pharmamedia (an industrial grade of cottonseed flour produced by Traders Milling Co., Fort Worth, Tex.), 6.25 kg.; blackstrap molasses, 5.0 kg.; K_2SO_4 (industrial grade) 0.5 kg.; water to about 225 liters;

NaOH to pH 7.25; water to give 250 liters after sterilization. The medium is sterilized at 123° C. for 30 minutes and cooled by cold water jacket cooling. Poststerilization pH 7.30. This sterile fermentation medium is inoculated with 12.5 liters (5percent vol./vol.) of the above seed. The fermentation is run for 282 hours at 29° C. with agitation by flat-bladed impellor at 280 r.p.m. and sparging with sterile air at 250 liters per minute.

Whole fermented beer (186.5 kg.) obtained as above is suction-filtered with the use of 4percent (wt./vol.) Celatom FW-40 (Eagle Pitcher Industries, Cincinnati, Ohio) filter aid. The resulting clear beer is adjusted to pH 4 with concentrated H_2SO_4, let stand for nearly 2 hours and refiltered with suction upon addition of 1percent (wt./vol.) Celatom FW-40 , to give polished beer.

The polished beer (205 liters) is extracted three times with methylene chloride (about 100 liters each time) to yield 290 liters of pooled extract and 200 liters of half-spent beer. The half-spent beer is adjusted to pH 10 with NaOH and extracted three times with n-butanol (about 75 liters each time) to yield 210 liters of pooled butanol extract. The butanol is evaporated, the residue dissolved in water and concentrated to a dry solid I, 437.4 g. Paper chromatogram analysis indicates the presence of lincomycin sulfoxide lincomycin, and other impurities.

About 400 g. of dry solid I is triturated with 8 liters of acetone. Insoluble materials are filtered off. The filtrate is mixed with an equal volume of diethyl ether. A precipitate which appears is filtered off. The filtrate is concentrated to dryness to give solid II.

Further purification is achieved by counter-double-current distribution in the solvent system n-butanol:water 1:1 (vol./vol.). Typically, 20 g. of solid II is dissolved in 100 ml. of the lower phase of this solvent system. The pH is adjusted to 4.2 with HCl. Upper phase is added and the mixture is placed in four tubes of an all glass counter-double-current distribution apparatus, each tube having an operating liquid capacity of 25 mls. After 140 transfers, the lincomycin sulfoxide is found by papergrams to be contained primarily in tubes 20 to 40. These are pooled and evaporated, and the resulting solid is redistributed in a second counter-double-current distribution as above. Papergram analysis shows that lincomycin sulfoxide is segregated in tubes 20 to 37. These are pooled and evaporated to yield solid III. Solid III is now dissolved in a minimal volume of methanol and distributed in a countercurrent distribution apparatus (10 ml. per phase, solvent water:n-butanol 1:1 vol./vol.) for 1,000 transfers. Lincomycin sulfoxide is found to be segregated in tubes 70 to 99. These tubes are pooled and evaporated to dryness. The residue is dissolved and mixed with diethyl ether. A precipitate appears which is isolated by filtration and dried. This solid is dissolved in a minimal volume of methanol and redistributed in the countercurrent apparatus as above for 1,900 transfers. Tubes 85 to 100 are now found to contain only lincomycin sulfoxide by papergram examination. These tubes are pooled and the pools are evaporated to dryness to yield about 600 mg. of lincomycin sulfoxide.

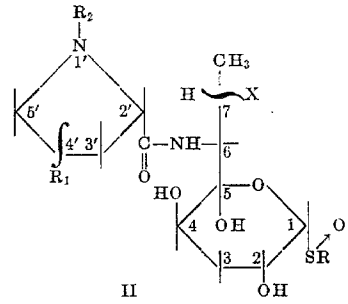
The lincomycin sulfoxide thus obtained is identical to an authentic sample on thin-layer chromatography [Silica gel G., (Merck Darmstadt), methyl ethyl ketone:acetone:water 140:40:22 by volume]; by infrared chromatography and by nuclear magnetic resonance. Elemental analysis (as hydrochloride acid addition salt) is:

Calculated: C, 45.33; H, 7.83; N, 5.87; S, 6.75; Cl, 7.44
Found: C, 45.22; H, 7.88; N, 6.28; S, 4.43; Cl, 6.07
(Calculated for $C_{18}H_{34}N_2O_7S \cdot HCl \cdot H_2O$)

Alternatively, the following sterile synthetic fermentation medium may be used: Per liter, glucose, 30 g.; sodium citrate, 3 g.; $ZnSO_4 \cdot 7H_2O$, 0.001 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; $MGGSO_4$, 1 g.; K_2HPO_4, 2.5 g.; NaCl, 0.5 g. and NH_4NO_3, 2 g. This medium, dispensed in 100-ml. portions in shake flasks, is advantageous for the making of small amounts of lincomycin sulfoxide. After inoculation such flasks can be shaken on a 250 r.p.m. rotary shaker, 2.5 inch stroke, at 28° C. for about 12 days.

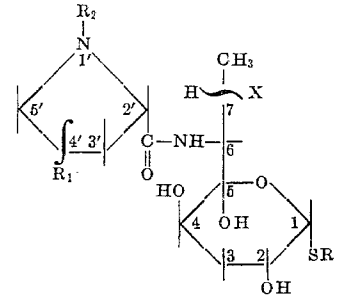
We claim:

1. The process for the production of a lincomycin sulfoxide of the formula:



II

wherein R is methyl or ethyl, R_1 is an alkyl of from two to eight carbon atoms, inclusive, R_2 is hydrogen or alkyl of from one to eight carbon atoms, inclusive, and X is hydroxyl, chlorine or bromine, or an acid addition salt thereof, which comprises subjecting a compound of the formula:



wherein R, R_1, R_2 and X have the meanings given above, or an acid addition salt thereof, to the oxygenating activity of:

Streptomyces armentosus, NRRL 3176,
Streptomyces spectabilis, NRRL 2494,
Streptomyces spectabilis, NRRL 2792,
Streptomyces lincolinesis, NRRL 2936,
Anixopsis multispora, CBS;
Aspergillus citricus, IMI 15954;
Aspergillus niger, ATCC 8740; or
Aspergillus niger, ATCC 9029 and isolating the oxygenated product.

2. The process of claim 1, wherein the oxygenation is carried out under submerged fermentation conditions and the bioconversion is continued until a substantial amount of oxygenated product is produced.

3. The process of claim 1, wherein the oxygenation is carried out in an aqueous nutrient medium under aerobic fermentation conditions.

4. The process of claim 1, wherein the oxygenation is carried out in an aqueous nutrient medium under submerged fermentation conditions with *Streptomyces armentosus*, NRRL 3176.

5. The process of claim 1, wherein 7(S)-chloro-7-deoxylincomycin hydrochloride is subjected to the oxygenating activity of *Streptomyces armentosus*, NRRL 3176, in an aqueous nutrient medium under submerged fermentation conditions to obtain 7(S)-chloro-7-deoxylincomycin sulfoxide.

6. The process of claim 1, wherein lincomycin hydrochloride is subjected to the oxygenating activity of *Streptomyces lincolnensis*, NRRL 2936, in an aqueous nutrient medium under submerged fermentation conditions to obtain lincomycin sulfoxide.

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