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995.032

Date of Application and filing Complete Specification: May 23, 1961.

No. 18544/61.

Application made in United States of America (No. 31236) on May 23, 1960.

Application made in United States of America (No. 106,146) on May 5, 1961.

Complete Specification Published: June 10, 1965.

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995.032

Index at acceptance:—C2 A (1C2A, 1C2C, 2A4)

Int. Cl.:—C 07 c

COMPLETE SPECIFICATION

Tetracycline Derivatives

We, CHAS. PFIZER & CO. INC., a corporation organized and existing under the laws of the State of Delaware, United States of America, located at 11 Bartlett Street, Brooklyn 6, State of New York, United States of America, 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:—

The present invention relates to new and useful tetracyclines and the process for producing the same. More particularly, the present invention relates to 6-epi-6-deoxytetracyclines, D-ring substituted analogs thereof, 11a-substituted analogs thereof, and D-ring-11a-substituted analogs thereof, as well as the method of producing these compounds.

In accordance with the present invention, it has been found that hydrogenation of 6-deoxy - 6 - demethyl - 6 - methylenetetracyclines, including various D-ring substituted and 11a-substituted analogs thereof, is an exceedingly valuable method for producing known 6-deoxytetracyclines and in addition, also produces a class of new and useful derivatives having chemical, physical and biological properties distinct from these known 6-deoxytetracyclines by virtue of an altered stereochemical configuration. These new tetracyclines possess a definite microbiological activity against a variety of Gram-positive and Gram-negative microorganisms and are appropriately designated as 6-epi-6-deoxytetracyclines since the steric configuration of the 6-methyl group is opposite to that of the known 6-deoxytetracyclines. Thus, the nomenclature "6-epi" as employed herein, is completely analogous to the accepted nomenclature for naming the known 4-epitetracyclines.

In accordance with the process of the present invention, an appropriate tetracycline compound as defined hereinafter, is dissolved

or suspended in the form of the free base, or as an acid addition salt or polyvalent metal salt complex thereof in a reaction inert solvent medium in the presence of a noble metal catalyst and contacted with hydrogen at an appropriate temperature and pressure until reduction of the 6-methylene group occurs. Thereafter, a 6-epi-6-deoxytetracycline and 6-deoxytetracycline mixture may be recovered by conventional procedure involving catalyst removal and recovery from the solvent medium. This mixture then may be in turn subjected to chromatographic procedure or other procedures to separate such mixture into its components.

As used herein "reaction inert solvent medium" refers to any media which is a solvent or suitable suspending agent for the antibiotic reactant, is stable under the hydrogenation conditions and does not interfere with the effectiveness of the catalyst or interact with the antibiotic reactants or products. Polar organic solvents are generally suitable and include the lower as hereinafter defined alkanols such as methanol, ethanol, and butanol, cyclic and straight chain water soluble ethers such as dioxane, tetrahydrofuran, diethylene glycol monomethylether, 2-ethoxyethanol, the lower as hereinafter defined alkanolic acids such as acetic acid, propionic acid, aqueous media including the foregoing solvents, and dilute aqueous hydrochloric acid. As will be appreciated these solvents and others are conventional in known hydrogenation techniques applicable to the tetracycline antibiotics and hence are not critical. Preferred results are obtained, however, if acidic media, as opposed to basic media, are employed since the latter may tend to promote decomposition and hence reduce the yield of the desired product. Preferred solvent usage is illustrated in the working examples appearing hereinafter.

The word "lower" whenever used in this specification referring to an alkanol means

having 5 or less carbon atoms, and when used referring to alkonic acid means having 6 or less carbon atoms.

The temperature is no more critical in the present process than it is in other known hydrogenations of the tetracycline antibiotics. Thus, the preferred temperature range is from 0°C. to 60°C., the preferred temperature within this range being from 10–50°C. and preferably room temperature. At temperatures below 0°C. the reaction is inordinately slow whereas at temperatures above about 60°C., decomposition of the starting material may occur. As is to be expected, the higher the temperature, the faster the reaction rate.

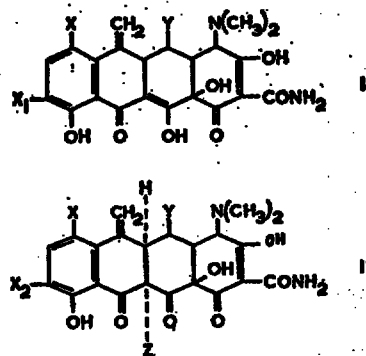
The noble metal catalysts as employed in the present invention include platinum, palladium, rhenium, rhodium and ruthenium, either of the supported or non-supported type, as well as the known catalytic compounds thereof such as the oxides, or chlorides. Examples of suitable catalyst supports include carbon, silica and barium sulfate. The catalysts may be preformed or formed *in situ* by pre-reduction of an appropriate salt of the catalytic compound. Examples of preferred catalysts are 5% palladium-on-carbon, 5% platinum-on-carbon, 5% rhodium-on-carbon, platinum chloride, palladium chloride, platinum oxide and ruthenium oxide. Materials such as the latter, where the metal is in a combined, non-elemental form, generally require pre-reduction before the hydrogenation can take place. This is accomplished simply by suspending the catalyst precursor in the hydrogenation solvent medium, hydrogenating it, adding the starting materials and continuing the hydrogenation. Alternatively, all of the components can be incorporated at once and hydrogenation commenced. The former procedure has the advantage of permitting the operator to separately determine the quantity of hydrogen absorbed during the catalyst pre-reduction and hydrogenation phase. The extent of hydrogenation of the antibiotic can then be more readily controlled. The maximum amount of hydrogen required in the process of the invention is 6 moles, for the reduction of a 7-11a-dihalo-9-nitro-6-methylenetetracycline starting compound.

Rhodium is the preferred catalyst for the process of the present invention since it produces the highest overall yield of 6-epi-6-deoxy- and 6-deoxytetracyclines. Additionally, use of rhodium permits retention of a 7-halo substituent where the starting compound is for example, 7-halo-6-deoxy-6-demethyl-6-methylene-5-oxytetracycline. Where retention of such 7-halo substituent is not desired, palladium is found to be a preferred catalyst. However, the other noble metal catalysts are entirely operative to obtain both 6-epi-6-deoxy- and 6-deoxytetracyclines.

The pressure employed during hydrogenation is not critical and is primarily depen-

dent upon apparatus availability. In general, pressures of from atmospheric to 2,000 p.s.i. are preferred. As is known, hydrogenation at atmospheric pressure is generally carried out in equipment wherein a measured volume of hydrogen contained in a reservoir is attached to a manometer in order to measure the volume of hydrogen consumed. Alternatively, a citrate of magnesia bottle and mechanical shaker with a calibrated pressure gauge, or a high pressure autoclave of the stirred or shaken variety may be employed.

For the purpose of the process of the present invention, suitable starting compounds are those represented by the following formulae:



wherein Y may be hydrogen or hydroxyl, X may be hydrogen, chloro, iodo or bromo, X₁ may be hydrogen, amino, and lower alkanoyl-amino, X₂ may be hydrogen or nitro and Z is chloro or fluoro. Additionally, the acid addition salts and the polyvalent metal salt complexes of these compounds may be employed. It will be noted that the steric configuration at the 5a and 11a positions is indicated in the second formula above.

Since the above described starting compounds for the process of the present invention are relatively new but known tetracycline compounds, Examples illustrating preparation thereof including necessary starting materials appear hereinafter as Examples III–XIX and XXII–XXXI inclusive. In general, the method of preparation involves treatment of an 11a-halo 6,12-hemiketal of an appropriate tetracycline compound with a strong acid of the dehydrating type such as sulfuric, trifluoroacetic, polyphosphoric, or perchloric, or hydrogen fluoride. Of these the preferred is hydrogen fluoride. Optimum conditions are readily determined by routine experimentation. Generally, the selected 11a-halo-6,12-hemiketal is merely added to the selected acid and allowed to react, most appropriately at a temperature within the range of from 0 to 50°C. and for a time of up to several hours. After reaction is complete the product is recovered in an appropriate manner, e.g. in the case of volatile acids by evapor-

ation of the same to obtain the residual product, and in other cases by standard procedure such as stirring with a non-solvent (e.g. diethyl ether) to precipitate the product.

5 These 6-methylene compounds may be converted to salts such as the hydrochloride by standard procedure prior to hydrogenation.

Substituent variation in the starting materials for the process of the present invention is also well illustrated in the referred to working examples. In brief, 7-halogenation of the starting material may be obtained by direct halogenation of an 11a-halo-6-deoxy-6-demethyl-6-methylene-5-oxytetracycline employing such well known reagents as N-chloro-, N-bromo-, and N-iodosuccinimide. For this purpose, liquid hydrogen fluoride has been found to be a preferred reaction medium and temperatures ranging from as low as -40°C. up to 60°C. are entirely suitable. The resulting 7,11a-dihalo compound may be used as such or may be converted to the corresponding 11a-deshalo compound by the procedures such as illustrated in Example VI hereof.

Direct nitration of the selected 6-deoxy-6-demethyl-6-methylenetetracycline serves as a convenient method for producing the 9-nitro starting materials of the present invention. In this method, the starting compound is reacted with nitric acid *per se* or formed *in situ*, e.g., potassium nitrate and sulfuric acid. For best results, nitration is carried out in a solvent such as liquid hydrogen fluoride, or a lower alkanolic acid. When hydrogen fluoride is used as solvent, it also acts as proton donor to form nitric acid *in situ* with nitrate salts. The use of high temperatures in excess of 50°C. should be avoided, preferred results being obtained at room temperature and even lower when liquid hydrogen fluoride is employed as solvent. Reaction times may vary as much as from 15 minutes to 12 hours.

When the desired starting compound is an 11a-deshalo-6-deoxy-6-demethyl-6-methylenetetracycline, 11a-dehalogenation may be accomplished by either chemical or catalytic reduction using the procedure well known to those in the art. Example XXXVII appearing hereinafter illustrates hydrogenation reduction of an 11a-chloro-6-deoxy-6-demethyl-6-methylenetetracycline hydrochloride to obtain the corresponding 11a-deschloro compound and also shows that the 11a-fluoro group is not susceptible to reduction by this method. Instead, 11a-fluoro groups are eliminated in the desired starting materials by reduction procedures such as illustrated in Example IV, method D, wherein chemical reduction employing zinc dust and dilute hydrochloric acid is employed.

The preparation of the 9-amino starting materials for the process of the present invention may be accomplished by reduction of the corresponding 9-nitro compound using

the methods generally employed for converting nitro compounds to amino compounds. Such procedures are well illustrated by Examples XIII, XIV and XXX. The acylated 9-amino starting compounds of the present invention are also prepared by the usual methods recognized by those in the art. The preferred method involves acylation of the 9-amino-6-deoxy-6-demethyl-6-methylenetetracycline with the desired acid anhydride or other acylating equivalent thereof such as the acid chloride in the presence of a base, e.g., a tertiary amine such as pyridine, and a reaction inert solvent. Examples XVIII and XIX illustrate such acylation techniques.

As indicated above, the reduction of the 6-methylene group of a selected starting compound in accordance with the present invention can result in the removal of a 7-halo substituent thereof and does result in the removal of an 11a-chloro substituent. Should such substituent be desired in the final mixed product or either of its components, namely the 6-epi-6-deoxytetracycline or 6-deoxytetracycline, they may be reintroduced by the chlorination technique described above and illustrated by Examples XLI-XLV. 11a-chloro and 11a-fluoro atoms may be introduced into 6-epi-6-deoxy-5-oxytetracyclines by the processes disclosed in Serial No. 995,051, application No. 16158/61, for introduction of such atoms into tetracyclines. Furthermore, and assuming the use of an 11a-fluoro substituted starting compound during reduction of the 6-methylene group, such group may be subsequently removed by the procedure described in Example XXXVIII.

Selection of a 9-nitro-6-deoxy-6-demethyl-6-methylenetetracycline as a starting material for the reduction process of the present invention will result in reduction of the 9-nitro group to a 9-amino group. Accordingly, and if the desired product is a 9-nitro-6-deoxytetracycline of either normal or epimeric configuration, such 9-nitro group should be introduced into the product of the reduction process of the present invention by the same nitration means hereinbefore described and illustrated in Examples L, LIV, LV, LVI and LVIII.

In view of the acid stability of the 6-deoxy products of the process of the present invention, the 9-amino substituted derivatives thereof such as 6-epi-6-deoxy-9-amino-5-oxytetracycline may be converted to the corresponding 9-chloro compound. This procedure involves diazotization followed by conventional Sandmeyer reaction and is illustrated in Example LIII hereof. It will further be appreciated that this Sandmeyer reaction can be applied to the preparation of other halides, or nitriles.

Direct nitration of the final products or the starting materials of the process of the

present invention favors substitution at the 9-position, trace amounts of the 7-nitro derivatives being occasionally detected. For the purpose of preparing 7-amino starting materials or 7-amino final products of the present invention, the preferred method involves preparation of a corresponding 7-aromatic azo compound followed by reduction to produce the desired 7-amino compound, catalytic hydrogenation in the presence of a noble metal catalyst being preferred for such reduction. Generally speaking, any diazotizable aromatic primary amine may be employed for producing the 7-azo compounds, it being essential that an 11a-halogen blocking group or other appropriate blocking group be present in order to protect the 11,12-enolic position. During the coupling reaction, it is preferred to employ an excess of the diazonium salt and to utilize reaction temperatures of 0–10°C. An aqueous solvent system such as a 50% aqueous solution of a water soluble lower alkanol or ketone is suitable. Examples XLVI–XLVII herein illustrate diazonium coupling followed by reduction to obtain a 7-amino compound.

The above description of the process of the present invention indicates that D-ring substitution of the final products may be obtained through appropriate selection of a D-ring substituted starting material or by appropriate D-ring substitution of the selected 6 - epi - 6 - deoxytetracycline or 6-deoxytetracycline. In general, the preferred procedure involves D-ring substitution of the product of the hydrogenation process claimed herein since the resulting yields are normally higher.

In summary of the process of the present invention, it will be appreciated that it not only provides a convenient means for producing new and useful 6 - epi - 6 - deoxytetracyclines but in addition, also produces known 6-deoxytetracyclines. Whereas the latter compounds may be produced by hydrogenation of a parent tetracycline antibiotic, i.e., one containing both a 6-methyl and a 6-hydroxy substituent, the procedure of the present invention is preferred since the yields thereof are substantially higher than those obtained by the known procedure.

Hydrogenation of the 6 - deoxy - 6 - de-

methyl - 6 - methylenetetracycline starting materials of the present invention, when carried out under the preferred acidic conditions sometimes produces a minor proportion of 4-epi products as well. If desired, such 4-epi compounds could be separated from the compounds having a normal configuration at the 4-position by conventional paper chromatography. Additionally, the 4-desdimethylamino analogs of the previously defined starting compounds of the present invention, could be employed to produce 4-desdimethylamino analogs of the products thereof.

The products of the present invention include the 6 - epi - 6 - deoxy-, 6 - epi - 6 - deoxy - 7 - substituted-, 6 - epi - 6 - deoxy - 9 - substituted-, 6 - epi - 6 - deoxy-11a - chloro-, 6 - epi - 6 - deoxy - 11a-fluoro-, 6 - epi - 6 - deoxy - 7,11a - dichloro, 6 - epi - 6 - deoxy - 7 - substituted 9 - substituted-, and 11a - fluoro- and chloro-analogs of the latter compound and 6 - epi - 6 - deoxy - 7 - chloro - 11a - fluoro-tetracyclines, both with and without the 5-hydroxyl substituent, as well as acid addition salts thereof, pharmaceutically acceptable metal salts thereof, and polyvalent metal salt complexes thereof, the substituents of the 7- and 9-positions being selected from the group consisting of chloro, bromo, iodo, nitro, amino and lower alkanoyl amino. Of these, a preferred group of compounds are those including a 5-hydroxyl substituent, since they are found to have particularly good *in vivo* effectiveness.

The novel compounds described immediately above with the exception of the 9-nitro compounds, are useful by virtue of their high order of activity against a variety of microorganisms, both *in vivo* and *in vitro*. 9-Nitro compounds generally are of a lower order of activity. These nitro compounds are useful for the preparation of the corresponding 9-amino compounds as described herein.

The following table summarizes the *in vitro* activity of 6 - epi - 6 - deoxy - 5 - oxytetracycline (1) as compared with 6 - deoxy - 5 - oxytetracycline hydrochloride (2) against a variety of disease-causing microorganisms. The minimum inhibitory concentration (MIC) is determined by the well known serial dilution technique.

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Organism	MIC (mcg./ml)	
	(1)	(2)
<i>Micrococcus pyogenes</i> var. <i>aureus</i>	0.78	0.78
<i>Streptococcus pyogenes</i>	0.09	0.09
<i>Streptococcus fascialis</i>	0.39	0.78
<i>Diplococcus pneumoniae</i>	0.19 (p.i.)	0.39
<i>Erysipelothrix rhusiopathiae</i>	0.39	0.39
<i>Corynebacterium diphtheriae</i>	0.19	1.56
<i>Listeria monocytogenes</i>	0.19	1.56
<i>Bacillus subtilis</i>	0.19	0.19
<i>Bacterium ammoniagenes</i>	—	0.78
<i>Aerobacter aerogenes</i>	6.3	12.5
<i>Escherichia coli</i>	3.12	12.5
<i>Proteus vulgaris</i>	100	>200
<i>Pseudomonas aeruginosa</i>	12.5	>200
<i>Salmonella typhosa</i>	6.3	6.3
<i>Salmonella gallinarum</i>	3.12	12.5
<i>Klebsiella pneumoniae</i>	3.16	6.3
<i>Neisseria gonorrhoeae</i>	0.09	6.3
<i>Hemophilus influenzae</i>	0.09	0.39
<i>Shigella sonnei</i>	3.12	6.3
<i>Brucella bronchiseptica</i>	0.78	3.12
<i>Malleomyces mallei</i>	0.78	25
<i>Vibrio comma</i>	0.39	6.3
<i>Pasteurella multocida</i>	0.19	—
<i>Streptococcus agalactiae</i>	0.19	—

Antibiotic resistant strains of *Micrococcus pyogenes* var. *aureus*:

376	6.3 (p.i.)	>200
400	6.3 (p.i.)	200

(p.i.) = partial inhibition

The following tabulated data compares the properties of 6 - epi - 6 - deoxy - 5 - oxytetracycline (1) and 6 - oxy - 5 - oxy - tetracycline (2):

Properties of 6-Deoxy-5-Oxytetracyclines

Bioactivity vs. <i>K. pneumoniae</i> (oxytetracycline standard)	(1)	(2)
	Ca. 1500 u/mg.	Ca. 500 u/mg.
10 λ max HCl - methanol	267 ($\epsilon=17,300$)	266 ($\epsilon=18,100$)
	351 ($\epsilon=13,200$)	343 ($\epsilon=13,200$)
Rf *	0.5	0.3

- 15 The present new 6 - epi - 6 - deoxytetra-
cycline compounds may be formulated into
various compositions analogous to the parent
tetracycline from which they are derived.
They are useful therapeutically in feeds or
20 as growth stimulants, in veterinary practice
and in agriculture.

For human therapy, the usual oral dosage
of the present new compounds is from 0.1

* Chromatography on paper saturated with
pH 3.5 aqueous Mac Ilvaine buffer, developed
1 hour with 20:10:3 by volume nitromethane
: chloroform: pyridine.

to 2 g. per day for the average adult. The product is formulated into capsules or tablets containing from 25 to 250 mg. of antibiotic on an activity basis. Suspensions or solutions in various vehicles are prepared using concentrations ranging from 5 to 125 mg/ml. For parenteral administration intramuscularly or intravenously, the daily dose is reduced to .05 to 1.0 g. Intramuscular formulations comprise solutions of the antibiotic at concentrations ranging from 50 to 100 mg/ml. Intravenous administration is by means of isotonic solutions having antibiotic concentration of about 10 mg/ml. Both types of parenteral products are conveniently distributed as solid compositions for reconstitution. These products may also be used for topical applications in the usual extending media. In all instances, of course, the attending physician will indicate the dosage to fit the needs of a particular patient. For children, smaller doses are generally used.

The present invention embraces all salts, including acid-addition and metal salts, of the newly recognized amphoteric antibiotics. The well known procedures for preparing salts of tetracycline compounds are applicable here and are illustrated by examples appearing hereinafter. Such salts may be formed with both pharmaceutically acceptable and pharmaceutically unacceptable acids and metals. By "pharmaceutically acceptable" is meant those salt-forming acids and metals which do not substantially increase the toxicity of the amphoteric antimicrobial agent. The preferred salts are the acid addition salts and pharmaceutically acceptable metal salts.

The pharmaceutically acceptable acid addition salts are of particular value in therapy. These include salts of mineral acids such as hydrochloric, hydriodic, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, as well as salts of organic acids such as tartaric, acetic, citric, malic, benzoic, glycollic, gluconic, gulonic, succinic, and arylsulphonic, e.g., p-toluenesulfonic acids. The pharmaceutically unacceptable acid addition salts, while not useful for therapy, are valuable for isolation and purification of the newly recognized antibiotic. Further, they are useful for the preparation of pharmaceutically acceptable salts. Of this group, the more common salts include those formed with hydrofluoric and perchloric acids. Hydrofluoride salts are particularly useful for the preparation of the pharmaceutically accept-

able salts, e.g. the hydrochloride, by solution in hydrochloric acid and crystallization of the hydrochloride salt formed. The perchloric acid salts are useful for purification and crystallization of the new antibiotic.

Whereas all metal salts may be prepared and are useful for various purposes, the pharmaceutically acceptable metal salts are particularly valuable because of their utility in therapy. The pharmaceutically acceptable metals include more commonly sodium, potassium and alkaline earth metals of atomic number up to and including 20 i.e. magnesium and calcium, and additionally, aluminum, zinc, iron and manganese. Of course, the metal salts include complex salts, i.e. metal chelates, which are well recognized in the tetracycline art. The pharmaceutically unacceptable metal salts embrace most commonly salts of lithium and of alkaline earth metals of atomic number greater than 20; i.e. barium and strontium, which are useful for isolation and purifying the antibiotic. Since the new antibiotic is amphoteric, it also forms salts with amines of sufficient basicity.

It will be obvious that, in addition to their value in therapy, the pharmaceutically acceptable acid and metal salts are also useful in isolation and purification.

The following examples are given by way of illustration only.

EXAMPLE I

11a - Fluoro - 5 - oxytetracycline - 6,12-hemiketal

To a mixture of 6.9 g. of anhydrous oxytetracycline base dissolved in 285 ml. of methanol cooled in an ice bath is added 1 equivalent of 1N sodium methoxide-methanol solution. The yellow sodium salt precipitates. Perchloryl fluoride is bubbled in and the sodium salt redissolves. As the mixture nears neutrality a heavy precipitate starts to form. The excess perchloryl fluoride is swept out with a stream of nitrogen, the product filtered off, washed with cold methanol and dried under vacuum at room temperature to obtain 5.1 g. of pale yellow crystals. Infrared absorption shows no carbonyl absorption below 6 microns. Bioassay against *K. pneumoniae* shows an activity of 4 mcg./mg. on the tetracycline scale. Ultraviolet absorption shows maxima at 265 and 335 microns. Elemental analysis gives the following results after recrystallization of the product from water.

Calcd. for: $C_{22}H_{23}O_5N_2F_2H_2O$:

C, 51.4; H, 5.25, N, 5.5
C, 51.2; H, 5.3, N, 5.7.

EXAMPLE II

11a - Chloro - 5 - oxytetracycline - 6,12-hemiketal

Twenty-three grams of anhydrous oxytetracycline is dissolved in 250 ml. of 1,2 - di-

methoxyethane and 8 g. of N-chlorosuccinimide is next added. The mixture is stirred for two minutes and then poured into 1 liter of stirred water. The product which separates is collected by filtration water-washed and

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dried. Infrared analysis of the product (KBr at 1% concentration) shows no carbonyl absorption in the 5—6 micron region but shows the following principal peaks: 6.12, 6.35, 6.66, 6.85, 7.22, 7.55, 7.75, 7.92, 8.14, 8.36, 8.78, 9.18, 9.43 microns. Bioassay of the product shows a tetracycline activity of 4 mcg./mg.

EXAMPLE III

10 11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

Five grams of 11a - chloro - 5 - oxytetra-
cyclyne - 6,12 - hemiketal is added to 15 ml.
of dry, liquid hydrogen fluoride, and the mix-
15 ture is stirred for 3.5 hours at ice-bath tem-
perature. The hydrogen fluoride is evaporat-
ed off by warming under a flow of nitrogen
gas to obtain the product as the hydrofluoride
salt.

Calcd. for: $C_{22}H_{21}N_2O_5Cl$ HI: C, 43.7; H, 3.7; N, 4.6; Cl, 5.8
Found: C, 44.0; H, 4.0; N, 4.2; Cl, 5.5.

40 Ultraviolet analysis shows the following
maxima: 222, 270 and 372 m μ .

Infrared analysis shows principal peaks at
3.05, 3.2, 5.7, 6.02, 6.03, 6.22, 6.4, 6.88,
7.4, 7.8, 8.1, 8.9 and 9.1 microns. The
45 perchlorate salt on ultraviolet analysis shows
maxima at 237, 270 and 372 m μ .

11a - Fluoro - 6 - deoxy - 6 - demethyl-
6 - methylene - 5 - oxytetracycline is pre-
pared in the same manner using 11a - fluoro-
5 - oxytetracycline - 6,12 - hemiketal as the
starting compound.

EXAMPLE IV

6 - Deoxy - 6 - demethyl - 6 - methylene-
5 - oxytetracycline

55 Method A

To a solution of 5 g. of the Example III
product (as the hydriodide) in 125 ml. of
dilute hydrochloric acid (1 part conc. HCl in
55 parts of water) at 20°C. is added 2 g.
of zinc dust. After stirring for 10 minutes,
the zinc is filtered off, the filtrate adjusted
to pH 0.8 and extracted with butanol. The
butanol extract is concentrated under reduced
pressure to a residue which is triturated with
65 ether. The ether-insoluble residue is crystal-
lized from methanol-acetone-conc. HCl-ether

The crude hydrofluoride product is dis-
solved in water and conc. HCl or perchloric
acid (70%) is added dropwise to precipitate
the hydrochloride or perchlorate salts respec-
tively. The hydriodide salt is precipitated
from acetone solution of the crude hydro-
fluoride salt by addition of 47% hydriodic
acid.

Alternatively, the original reaction mixture
is diluted with 6—7 volumes of water and
ice and perchloric acid or naphthalene sul-
fonic acid (concentrated acid) is added to
precipitate the respective salt. Dilution with
acetone of the original reaction mixture
followed by addition of HI, precipitates the
hydriodide salt.

The hydriodide salt, on elemental analysis,
shows the following values:

to obtain the product as the hydrochloride-
monomethanolate (2.5 g.) melting at 205°C.
with decomposition. Ultraviolet analysis in
0.01 N HCl in methanol shows λ_{max} 252 m μ ,

E 1% 1cm, 450 and λ_{max} 345 m μ , E 1% 1cm, 302;

in 0.01N NaOH in methanol, λ_{max} 235 m μ ,
E 1% 1cm, 442; λ_{max} 254 m μ , E 1% 1cm, 408;

λ_{max} 385 m μ , E 1% 1cm, 329; λ_{inf} 280 m μ ,
E 1% 1cm, 329; in 0.01N MgCl₂ in methanol,

λ_{max} 240 m μ , E 1% 1cm, 461 λ_{max} 277 m μ ,
E 1% 1cm, 326 λ_{max} 351 m μ , E 1% 1cm, 282. In-

frared analysis shows principal peaks at 6.03,
6.2, 6.37 and 6.87 microns. Bioassay shows
a value of 2000 to 2400 mcg./mg. (K. pneumo-
niae turbimetric assay with oxytetracycline
as standard). Elemental analysis of the pro-
duct gives the following values: C, 55.0;
H, 5.2; N, 5.5; Cl, 7.0; OCH₃, 3.4. The
product shows Rf values of 0 and 0.35 re-
spectively in the following systems:

Mobile Phase

(1) 20:3 toluene-pyridine
saturated with pH 4.2 buffer

90 (2) 20:10:3 nitromethane,
chloroform pyridine saturated
with pH 3.5 buffer

Immobile Phase

pH 4.2 buffer (aqueous)

pH 3.5 buffer (aqueous)



Method B

A mixture of 1 g. of the Example III 11a-chloro product in 10 ml. of methanol containing 200 mg. of 5% rhodium on carbon is hydrogenated at room temperature and 1 atmosphere of hydrogen gas until an equimolar amount of hydrogen is taken up (2 hours). The catalyst is filtered, the filtrate evaporated to dryness and the residue crystallized as in Method A.

Method C

A mixture of 1 g. of the Example III 11a-chloro product in 70 ml. of water containing 1 g. of sodium hydrosulfite is stirred for 0.5 hours at room temperature. The mixture is then extracted with butanol and the butanol extract evaporated to dryness. The product is crystallized from the residue as in Method A.

Method D

Using the procedure of Method A, 11a-fluoro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline perchlorate salt is reduced to 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline.

The crystalline hydrochloride-methanolate product of this example may be recrystallized from isopropanol as 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline hydrochloride. The recrystallized material shows the following peaks on infrared analysis: 3.1, 3.75, 6.02, 6.23, 6.36, 6.55, 6.9, 7.35, 7.6, 7.8, 8.15, 8.26, 8.5, 9.27, 9.95, 10.55, 10.8, 11.53, 11.93 and 12.15 microns.

EXAMPLE V

7,11a - Dichloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

Method A

To 5 g. of 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline hydriodide in 15 ml. of liquid hydrogen fluoride cooled at ice bath temperature, is added 1.5 g. of N - chloro - succinimide. The solution is stirred at ice bath temperature for 1.5 hours. The crude product is precipitated by the addition of 500 ml. of ether and recovered by filtration.

The crude product is taken up in methanol at room temperature, the insoluble material is filtered, the filtrate treated with activated carbon, filtered and concentrated under reduced pressure. The remaining residue is taken up in dil. hydrochloric acid from which the product, as the hydrochloride, crystallizes. Ultraviolet analysis in 0.01N HCl in methanol shows $\lambda_{\max}$ 239 $m\mu$ $E_{1\text{cm}}^{1\%}$, 352 $\lambda_{\max}$ 378 $m\mu$, $E_{1\text{cm}}^{1\%}$, 60; $\lambda_{\text{inflection}}$ 258 $m\mu$, $E_{1\text{cm}}^{1\%}$ 324.

Infrared analysis shows principal bands at 5.7, 6.0 and 6.9 microns.

Method B

Five grams of 11a - chloro - 5 - oxytetracycline - 6,12 - hemiketal is added to 15 ml. of liquid hydrogen fluoride at ice-bath temperature. After stirring for 3.5 hours at ice bath temperature, the procedure in Method A is then followed after addition of the same weight of N-chlorosuccinimide to obtain the product.

An alternative and somewhat more convenient method of work up is as follows. After the removal of most of the liquid hydrogen fluoride by evaporation, 100 ml. of water is added and then 5 g. of β -naphthalene-sulfonic acid is added. The product precipitates as the β -naphthalenesulfonic acid salt and is collected by filtration.

A further method of work-up involves dilution of the original reaction mixture with 6-7 volumes of water and is followed by dropwise addition of concentrated acid to precipitate the perchlorate and β -naphthalene sulfonate salts as described in Example III. The crude perchlorate salt so obtained is crystallized as long needles from isopropanol, which on ultraviolet analysis shows maxima at 260 and 377 $m\mu$. Infrared analysis shows peaks at 5.7, 6.0, 6.26, 6.55, 6.88, 7.2, 7.25 and 8.35 microns.

EXAMPLE VI

7 - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

To a solution of 0.5 g. of the 7,11a-dichloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline perchlorate in 7.5 ml. of water is added 0.45 g. of sodium hydrosulfite and the resulting mixture is stirred for 12 minutes. The product separates and is collected by filtration. Bioassay of the product gives a value of 3400 mcg/mg. (K. pneumoniae turbidimetric assay with oxytetracycline (1000 mcg/mg.) as the standard.

Method B

Twenty grams of the β -naphthalene sulfonate salt of the previous example is suspended in 500 ml. of methanol containing 5 g. of 5% rhodium on carbon and the mixture hydrogenated at room temperature and 1 atmosphere of hydrogen gas. After the uptake of 700 ml. of hydrogen, the reaction is filtered and the filtrate evaporated to dryness to obtain 15.4 g. of residue.

A methanolic solution of 11 g. of the residue is then adjusted to pH 6.5 with triethylamine and passed onto an 8 x 100 cm. column containing 2 kg. of cellulose powder using water as stationary phase. The column is eluted with ethyl acetate saturated with water and 45 ml. fractions are collected. The elution pattern is followed by paper



60
65
70
75
80
85
90
95
100
105
110
115
120

and an inflection at 260 $m\mu$

METHOD A

chromatography and fractions 132 to 260 are pooled, evaporated to dryness, slurried in ether and filtered to give 2.74 g. of pure amorphous amphoteric product.

The product is crystallized by dissolving 1.6 g. in 40 ml. of warm methanol and scratching. Filtration gives 890 mg. of the product as the amphoteric base. Infrared analysis shows the following peaks: 2.96, 3.29, 3.42, 6.06, 6.18, 6.30, 6.58, 6.88, 7.19, 7.48, 7.70, 8.23, 9.06, 9.88, 10.63, 10.92, 11.55 and 11.76 microns. Ultraviolet analysis

shows the following: in 0.01N HCl in methanol, maxima at 247 m μ (log ϵ 4.28) and 346 m μ (log ϵ 4.02) and an inflection at 370 m μ (log ϵ 3.98); in 0.01N NaOH in methanol, maxima at 234 m μ (log ϵ 4.24), 253 m μ (log ϵ 4.22) and 389 m μ (log ϵ 4.12) and an inflection at 284 m μ (log ϵ 4.07); in 0.01M MgCl₂ in methanol, maxima at 241 m μ (log ϵ 4.32); 349 m μ (log ϵ 4.04; and 372 m μ (shoulder) (log ϵ 4.02).

The product shows the following Rf values in the indicated solvent systems:

Rf Value	Mobile Phase	Solvent System
		Immobile Phase
0.35	ethyl acetate saturated with water	Aqueous phosphate buffer (pH 3)
0.33	ethyl acetate saturated with water	McIlvaine's buffer, pH 4.2

Bioassay (K. pneumoniae—oxytetracycline standard) gives a value of 600 mcg/mg.

The crystalline perchlorate salt of the product of the preceding example is hydrogenated to give this product which is crystallized from methanol and 70% perchloric acid. The perchlorate salt of the product shows identical ultra-violet spectra to that of the amphoteric base.

Method C

This product is also obtained by reduction of the starting compound of Method A with zinc and acid according to the procedure of Example IV.

EXAMPLE VII

7 - Bromo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

This product is prepared according to the procedures of Method A and B of Example V using an equivalent amount of N-bromosuccinimide in place of N-chlorosuccinimide.

EXAMPLE VIII

7 - Bromo - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

This product is obtained from Example VII product by the sodium hydrosulfite treatment described in Example VI.

The following compounds are prepared from the corresponding 11a-halo compounds by the procedure of example V:

7 - chloro - 11a - fluoro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

7 - iodo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

7 - bromo - 11a - fluoro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

These compounds are converted to the corresponding 11a-des halo compounds by the procedure of Example VI.

EXAMPLE IX

7 - Chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline

To a solution of 1.0 g. of 7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline base in 8 ml. of anhydrous liquid hydrogen fluoride (HF) at 0°C. is added 220 mg. of KNO₃. The mixture is stirred for 30 minutes at 0°C. and the HF evaporated off under nitrogen. The residue is slurried in dry ether, filtered and dried. The product shows the following Rf values in the solvent systems indicated:

Rf	Mobile Phase	Solvent System
		Immobile Phase
0.2	ethylacetate saturated with water	aqueous phosphate buffer (pH=3)
0.55	nitromethane, toluene, pyridine 20:10:3)	McIlvaine's buffer (ph=3.5)



4.32



65 demethyl

70

75

80

85

Ultraviolet analysis in 0.01M NaOH—MeOH shows maxima at 248, 341 and 447 m μ and a shoulder at 275 m μ . Bioassay shows a value of less than 100 mcg./mg. (*K. pneumoniae* turbimetric assay with oxytetracycline standard).

EXAMPLE X

9 - Nitro - 7,11a - dichloro - 6 - deoxy - 6-demethyl - 6 - methylene - 5 - oxytetracycline
 10 To a solution of 600 mg. of 7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6-methylene - 5 - oxytetracycline perchlorate in 1.5 ml. of anhydrous liquid hydrogen fluoride is added 100 mg. of KNO₃. The mixture is
 15 stirred at 5°C. for 15 minutes, and the product is then precipitated with ether and collected by filtration, with ether wash.

EXAMPLE XI

9 - Nitro - 6 - deoxy - 6 - demethyl - 6-methylene - 5 - oxtetracycline
 20 To a mixture of 500 mg. of 6 - deoxy - 6-demethyl - 6 - methylene - 5 - oxytetracycline in 1.5 ml. of anhydrous liquid hydrogen fluoride is added 100 mg. of KNO₃. The mixture is stirred at 15°C. for 30 minutes and
 25 then evaporated to dryness. The residue is slurried in ether and filtered to obtain the crude product which is slurried in 5% aqueous HCl and extracted with butanol. The
 30 butanol extract is concentrated to obtain the product.

EXAMPLE XII

9 - Nitro - 11a - chloro - 6 - deoxy - 6-demethyl - 6 - methylene - 5 - oxytetracycline

A solution of 5 g. of 11a - chloro - 5-oxytetracycline - 6,12 - hemiketal in 10 ml. of anhydrous hydrogen fluoride is allowed to stand for 3½ hours after which 1.2 g. of KNO₃ is added with stirring. Stirring is continued for one hour at ice bath temperature and the mixture is then added to 200 ml. of dry ether, filtered and washed with ether to obtain crude product.

EXAMPLE XIII

7 - Chloro - 9 - amino - 6 - deoxy - 6-demethyl - 6 - methylene - 5 - oxytetracycline

To a solution of 1.42 g. of 7 - chloro-9 - nitro - 6 - deoxy - 6 - demethyl - 6-methylene - 5 - oxytetracycline dissolved in 50 ml. of water and 2 ml. of conc. HCl is added 1.3 g. of zinc dust with stirring at 20°C. After 15 minutes, the mixture is filtered, the pH adjusted to 2.5 and the mixture is extracted with butanol (5 × 30 ml.). The butanol extract on concentration gives 760 mg. of the product (as dihydrochloride). Bioassay (*K. pneumoniae*) gives a value of 1680 mcg/mg. Ultraviolet analysis in 0.01N MeOH—HCl shows maxima at 262 and 348 m μ ; in 0.01N MeOH—NaOH, 264 and 380 m μ .

The product shows R_f values as follows:

	Solvent System		
	R _f	Mobile Phase	Immobile Phase
65	0.2	ethyl acetate saturated with water	Aqueous buffer (ph=3)
	0.35	nitromethane, chloroform, pyridine (20:10:3)	McIlvaine's pH 3.5 buffer

This product is also obtained by reduction (with aqueous sodium hydrosulfite) of 9-nitro - 7,11a - dichloro - 6 - deoxy - 6-demethyl - 5 - oxytetracycline.

EXAMPLE XIV

9 - Amino - 6 - deoxy - 6 - demethyl - 6-methylene - 5 - oxytetracycline

This product is obtained by reduction of the corresponding 9-nitro compound with sodium hydrosulfite in water or with SnCl₄ in aqueous hydrochloric acid.

EXAMPLE XV

9 - Nitro - 11a - chloro - 6 - deoxy - 6-demethyl - 6 - methylene - 5 - oxytetra-cycline

To 1 g. of 11a - chloro - 6 - deoxy - 6-demethyl - 6 - methylene - 5 - oxytetra-

cycline in 20 ml. of acetic acid is added 1 ml. of conc. HNO₃. The mixture is allowed to stand for 2 hours, then evaporated to ¼ of the original volume and 200 ml. of ether is then added. The product separates as the nitrate salt and is collected by filtration.

Similarly, the corresponding 11a-fluoro compound is prepared.

EXAMPLE XVI

9 - Amino - 6 - deoxy - 6 - demethyl - 6-methylene - 5 - oxtetracycline

The product of the previous example is reduced according to the procedures of Example IV to obtain this compound.

This product is also obtained by chemical or catalytic reduction of 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5-oxtetracycline.

EXAMPLE XVII

7,11a - Dichloro - 9 - nitro - 6 - deoxy-
6 - demethyl - 6 - methylene - 5 - oxytetra-
cycline

- 5 One gram of 7,11a - dichloro - 6 - deoxy-
6 - demethyl - 6 - methylene - 5 - oxytetra-
cycline hydrochloride is dissolved in 20 ml.
of acetic acid and 1 ml. of conc. HNO₃ is
added. The mixture is allowed to stand for
10 12 hours and then concentrated to $\frac{1}{4}$ of the
original volume. It is added dropwise to 200
ml. of ether with stirring at ice-bath tem-
perature. Stirring is continued for 3 hours
and the solid is then recovered by filtration.
15 The solid is slurried several times in ether
and then dried to obtain the product as the
hydrochloride salt.

- 20 Using the same procedure, the following
products are obtained from corresponding
starting compounds:

- 7 - chloro - 11a - fluoro - 9 - nitro - 6 -
deoxy - 6 - demethyl - 6 - methylene -
5 - oxytetracycline
7 - bromo - 11a - chloro - 9 - nitro - 6 -
deoxy - 6 - demethyl - 6 - methylene -
25 5 - oxytetracycline
7 - iodo - 11a - fluoro - 9 - nitro - 6 -
deoxy - 6 - demethyl - 6 - methylene -
5 - oxytetracycline

- 30 These products are converted to 7-bromo-,
7-iodo- and 7 - chloro - 9 - amino - 6 -
deoxy - 6 - demethyl - 6 - methylene - 5 -
oxytetracycline by the reduction procedures of
Examples IV and VI.

EXAMPLE XVIII

- 35 9 - Formylamino - 6 - deoxy - 6 - demethyl-
6 - methylene - 5 - oxytetracycline

- To a solution of 4.5 g. of 9-amino-6-de-
oxy - demethyl - 6 - methylene - 5 - oxytetra-
cycline base in 40 ml. of dry pyridine is
40 added 20 ml. of aceto-formic acid at ice bath
temperature. The mixture is stirred for 15
minutes at ice bath temperature and added
dropwise to one liter of dry ether. The prod-
uct is filtered, reslurried in ether and filtered.
45 The crude product is suspended in 100 ml.
of water providing a solution of Ph=4.2 and
stirred for ten minutes to hydrolyze O-formyl
groups. The pH of the mixture is then
50 adjusted to pH 5.5 and the solution freeze-
dried to get crude product which is chroma-
tographed on a cellulose column to obtain
purified product as the main fraction.

EXAMPLE XIX

- 55 9 - Acetyl amino - 6 - deoxy - 6 - demethyl-
6 - methylene - 5 - oxytetracycline

- To a solution of 4.2 g. of the starting
compound of Example XVIII in 200 ml. of
dry tetrahydrofuran and 200 ml. of dry
60 methanol is added 20 ml. of acetic anhydride

and the mixture is stirred for 45 minutes at
room temperature. The mixture is then
stripped of most of the solvent and dropped
into dry ether, filtered and the solid cake re-
slurried in ether, filtered and dried to obtain
crude product.

The crude product is chromatographed as
in Example XVIII to obtain purified product
as the main fraction.

Employing the procedures of Examples
XVIII and XIX, the following products are
prepared:

- 9 - formylamino - 7 - chloro - 6 - deoxy-
6 - demethyl - 6 - methylene - 5 - oxy-
tetracycline
9 - acetyl amino - 7 - chloro - 6 - deoxy - 6 -
demethyl - 6 - methylene - 5 - oxytetra-
cycline
9 - butyl amino - 7 - chloro - 6 - deoxy - 6 -
demethyl - 6 - methylene - 5 - oxytetra-
cycline
9 - propionyl amino - 6 - deoxy - 6 - de-
methyl - 6 - methylene - 5 - oxytetra-
cycline
9 - valeryl amino - 6 - deoxy - 6 - demethyl-
6 - methylene - 5 - oxytetracycline

EXAMPLE XX

11 - Chlorotetracycline - 6,12 - hemiketal

This compound is prepared in accordance
with the method of Example II by substi-
tuting an equivalent amount of tetracycline
for the oxytetracycline of Example II.

EXAMPLE XXI

7,11a - Dichlorotetracycline - 6,12 - hemiketal

This compound is prepared in accordance
with the method of Example XX by employ-
ing a molecular equivalent amount of chloro-
tetracycline, substituting ether or ether-hexane
instead of water to precipitate the product.

EXAMPLE XXII

11a - Chloro - 6 - deoxy - 6 - demethyl - 6 -
methylenetetracycline

11a - Chlorotetracycline - 6,12 - hemi-
ketal is dissolved in liquid hydrogen fluoride
(in a ratio of 2 g./15 ml.) at 0°C. The
mixture is maintained at this temperature for
10-15 minutes, after which the hydrogen
fluoride is evaporated off. The residue is tri-
tured in ether to obtain the solid product,
11a - chloro - 6 - deoxy - 6 - demethyl - 6 -
methylenetetracycline, as the hydrofluoride
salt which is recrystallized from methanol.

Alternatively, the crude hydrofluoride pro-
duct (10 g.) is dissolved in 350 ml. of H₂O
by warming and stirring. An equal volume of
conc. HCl is added to the clear solution and
the product crystallizes as the hydrochloride
salt. Elemental analysis of the hydrochloride
salt thus obtained gives the following results:

Calcd. for: $C_{22}H_{22}O_7N_2Cl_2$; C, 53.11; H, 4.56; chloride 7.13; N, 5.63
 Found: C, 52.62; H, 4.63; chloride 6.84; N, 5.54

Infrared analysis of the product as the hydrochloride salt in a KBr pellet at 1% concentration shows carbonyl absorption at 5.70 μ as well as the following significant peaks: 6.1, 6.23, 6.36, 6.45 (shoulder), 6.91, 7.85, 8.14, 8.55, 10.22, 10.55 and 10.89. Bioassay of

the product (*K. pneumoniae*) shows an oxytetracycline activity of 50–100 mcg./mg. Ultraviolet analysis of the sample in 0.01 N methanol-HCl shows maxima at 376, 278 and 242 m μ . The product shows an R_f value of from 0.2 to 0.3 in the following system:

15

Mobile Phase

20:3 toluene-pyridine
 saturated with pH 4.2 buffer

Immobile Phase

pH 4.2 buffer (aqueous)

On analysis of the papergram with ultraviolet lights, the product spot does not fluoresce strongly. However, on spraying, with aqueous sodium hydrosulfite, it shows strong fluorescence.

The C.4 epimer of the product, present as a minor impurity, shows R_f 0.1 in this system.

EXAMPLE XXIII

11a - Fluoro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

11a - Fluorotetracycline - 6,12 - hemiketal, 250 mg. is stirred in 2 ml. of 63% aqueous perchloric acid. The solid dissolved on warming to 60–65°C. for 15 minutes after which the mixture is cooled and water is added to obtain 11a - fluoro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline as the chlorate salt. The product shows similar absorption on ultraviolet analysis as that of Example XXII.

EXAMPLE XXIV

The following compounds are prepared from corresponding 11a - halotetracycline-6,12 - hemiketals, the 11a - fluoro compound by the procedure of Example XXIII and the remaining 11a-halo compounds by the procedure of Example XXII:

7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

7 - chloro - 11a - fluoro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline
 7 - bromo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline
 7 - iodo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

EXAMPLE XXV

6 - Deoxy - 6 - demethyl - 6 - methylenetetracycline

Method A

The product of Example XXII (5 mg.) is dissolved in 3 ml. of methanol and a freshly prepared solution of sodium hydrosulfite (20 mg. in 2 ml. of water) is added. The mixture is allowed to stand for 15 minutes at room temperature, after which it is stripped of methanol and extracted with butanol. The butanol extract is concentrated to obtain the product, 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline. The product is crystallized from water as the p-toluene-sulfonate salt by addition of p-toluenesulfonic acid. Alternatively, it is crystallized as the hydrochloride salt from water by addition of concentrated HCl.

On testing against *K. pneumoniae*, the product has an oxytetracycline activity of at least 1100 mcg./mg. The product shows an R_f value of 0.6 in the same system as described in Example XXII, and shows R_f 0.9 in the following system:

80

Mobile Phase

20:10:3 nitromethane:chloroform;
 pyridine

Immobile Phase

pH 3.5 buffer
 (aqueous)

The C.4 epimer of this substance show R_f's of approximately 0.4 and approximately 0.6 in the two systems.

Method B

A solution of 50 g. of 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline hydrochloride in 500 ml. of monomethyl ether of ethylene glycol is cooled to 4°C. in an ice bath and the rapidly stirred

solution is treated with 50 g. of zinc metal dust at a gradual rate over a period of about 10 minutes. The temperature rises to 12°C. during addition. After addition is complete, the temperature begins to fall. After a total reaction time of 15 minutes the zinc is removed by rapid filtration and washed with solvent. One liter of water is added gradually to the filtrate over a period of about 10 minutes. A yellow slurry of the

zinc complex of the product is formed. The pH of the solution is then adjusted to 6.8 with 10% aqueous sodium hydroxide.

The resulting slurry is digested for about 1.5 hours in an ice bath and filtered. The wet cake is then slurried in 750 ml. of water and conc. HCl is added dropwise until a clear solution is obtained. A slight excess of conc. HCl causes rapid crystallization of 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline hydrochloride as glistening needles. After digestion for one hour, the product is filtered and dried. The yield of product is 37.8 g. The product melts at 213.8—214.2°C. with decomposition.

EXAMPLE XXVI

The procedure of Example XXV is repeated to dechlorinate the corresponding 7-halo - 11a - chloro - 6 - methylenetetracyclines ~~of the~~ to provide the following products:

~~eyclines to provide the following products:~~

7 - bromo - 6 - deoxy - 6 - demethyl - 6 - methylefetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

C.4 epimers occur as impurities.

EXAMPLE XXVII

11a - Chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

A mixture of 1 g. of the Example XXII product in 20 ml. of acetic acid containing 1 ml. of conc. HNO₃ is allowed to stand for 12 hours after which the addition of 20 ml. of H₂O crystallizes the product as the nitrate salt. The product is collected by filtration water-washed and dried.

EXAMPLE XXVIII

9 - Nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

This product is obtained from the corresponding 11a-chloro compound (Example XXVII) by treating one gram in 70 ml. water with a molecular equivalent of sodium hydrosulfite. The reaction mixture is stirred at room temperature for a half hour, and the desired product recovered by butanol extraction and evaporation of the extract to dryness under reduced pressure.

EXAMPLE XXIX

7 - Chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

To a solution of 1.0 g. of 7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline in 8 ml. of anhydrous liquid hydrogen fluoride (HF) at 19°C. is added 220 mg. of KNO₃. The mixture is stirred for 30 minutes at 0°C. and the HF evaporated off

under nitrogen. The residue is slurried in dry ether, filtered and dried.

EXAMPLE XXX

7 - Chloro - 9 - amino - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

To a solution of 1.42 g. of 7 - chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline dissolved in 50 ml. water and 2 ml. of concentrated HCl is added 1.3 g. of zinc dust with stirring at 20°C. After 15 minutes, the mixture is filtered, the pH adjusted at 2.5 and the mixture is extracted with butanol 5 times employing 30 ml. per extraction. The butanol extract on concentration gives desired product as the dihydrochloride.

EXAMPLE XXXI

7,11a - Dichloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

One g. of 7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline hydrochloride is dissolved in 20 ml. of acetic acid and 1 ml. of concentrated nitric acid is added. The mixture is allowed to stand for 12 hours, then concentrated to $\frac{1}{4}$ of the original volume. It is added dropwise to 200 ml. of ether with stirring at ice-bath temperature. Stirring is continued for 3 hours and the solid is then recovered by filtration. The solid is slurried several times in ether and then dried to obtain the product as the hydrochloride salt.

Using the same procedure, the following products are obtained from corresponding starting compounds:

7 - chloro - 11a - fluoro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene-tetracycline

7 - bromo - 11a - chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene-tetracycline

7 - iodo - 11a - fluoro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

These products are converted to 7 - bromo-, 7-iodo- and 7 - chloro - 9 - amino - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline by the reduction procedures of Examples IV and VI.

EXAMPLE XXXII

6-Deoxy-6-demethyl-6-methylene - 5 - oxy-tetracycline (40 g.) was introduced into a conventional hydrogenation apparatus together with 1.5 l. of 0.01 N HCl and 40 g. of 5% rhodium on carbon. Hydrogen was then introduced into the apparatus and maintained at a pressure of 1800 p.s.i. at a temperature of 30°C. for 20 hours. Thereafter the catalyst was filtered off and the filtrate was freeze-dried to obtain 23.9 g. solids, a mixture of

6 - deoxy - 5 - oxytetracycline and 6 - epi - 6 - deoxy - 5 - oxytetracycline. This step yield of about 60% could be improved by employing 5% rhodium on barium sulfate carrier to thereby reduce absorption losses on the carbon catalyst carrier.

A mixture of methanol and water (25 ml. of each) was employed to dissolve 20.8 of the crude solids of the previous step. To this was added a solution of 20 g. sulfosalicylic acid in a mixture of methanol and water (50 ml. of each). The 6 - deoxy - 5 - oxytetracyclines crystallized readily in the form of the sulfosalicylate salts. The slurry was then filtered and the cake washed with 50% aqueous methanol and dried to obtain 24.2 g. of salt (a 75% step yield).

Countercurrent distribution was employed to separate the 6 - epi - 6 - deoxy - 5 - oxytetracycline from 6 - deoxy - 5 - oxytetracycline. For this purpose, 500 mg. of the salt of the previous step was dissolved in 30 ml. of ethyl acetate, plus 10 ml. 2M sodium acid phosphate. The resulting two phases were stirred and the pH adjusted to 6.8 by the addition of further Na_2HPO_4 . The aqueous phase was then separated in the funnel and equilibrated with 30 ml. fresh ethyl acetate in the second funnel. This operation was repeated two more times with fresh portions of ethyl acetate and the aqueous phase was then set aside. Aqueous



buffer at pH 6.8 (10 ml.) was equilibrated with each of the ethyl acetate layers in the four funnels and then set aside. This operation was repeated two more times with fresh pH 6.8 buffer. The four ethyl acetate layers were then combined and evaporated to dryness yielding 172 mg. of substantially pure 6 - epi - 6 - deoxy - 5 - oxytetracycline (about a 50% step yield). The remaining product in the aqueous buffer layers was concentrated to yield 6 - deoxy - 5 - oxytetracycline (about a 50% step yield).

The 6-epi-6-deoxy-5-oxytetracycline product of the preceding step was finally purified by dissolving in 1 ml. methanol and adding 3 drops concentrated HCl. It crystallized as a pure hydrochloride analyzing as follows:

Found: C, 53.69; H, 5.81
Calculated: C, 53.86; H, 5.70

This material when assayed against oxytetracycline in the *K. pneumoniae* assay showed 1400 units per milligram.

EXAMPLE XXXIII

Hydrogen was introduced into a standard hydrogenation vessel containing 10 g. 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline hydrochloride, 150 ml.

methanol and 5 g. 5% rhodium on carbon. The pressure was maintained at 50 p.s.i. while agitating at room temperature for 24 hours. The catalyst was then filtered off, the cake washed with methanol and the combined filtrates were evaporated to dryness. The dry solids were slurried in ether, filtered and the cake dried. The resulting solids exhibited a bioactivity of 1345 units per mg. v. *K. pneumoniae*.

Water (35 ml.) was employed to dissolve 8.5 g. of the above product and the pH was adjusted to 6.0 with triethylamine, sufficient dimethyl formamide being added to maintain the solids in solution. Cellulose powder (2 kg.) was slurried in water-saturated ethyl acetate and packed into a tower of about 3½ inches diameter, to a height of 3 ft. The product solution was then chromatographed over this column, developing with about 12 liters water-saturated ethyl acetate. The first product fraction to come from the tower yielded 1.85 g. 6 - epi - 6 - deoxy - 5 - oxytetracycline. The next fraction contained 2.0 g. of 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline. The third fraction yielded 0.8 g. 6 - deoxy - 5 - oxytetracycline.

EXAMPLE XXXIV

To a solution of 500 mg. of 7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline hydrochloride in 25 ml. of methanol is added 500 mg. of 5% palladium on carbon. The suspension is hydrogenated at atmospheric pressure until no further uptake of hydrogen occurs. The catalyst is removed by filtration and the filtrate evaporated to dryness *in vacuo*. Paper chromatography of the residue showed high concentrations of 6-epi-6-deoxy-5-oxytetracycline, and 6 - deoxy - 5 - oxytetracycline with traces of starting material.

The procedure of the previous paragraph is repeated employing as starting materials, the following as the hydrochlorides:

7 - bromo - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline
7 - iodo - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline
7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline

to obtain separable mixtures of the corresponding 7 - deshalo - 6 - epi - 6 - deoxy - tetracyclines and 7 - deshalo - 6 - deoxy - tetracyclines.

In a similar fashion, the hydrochlorides of 7 - chloro - 9 - amino - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline, 7 - chloro - 9 - acetylamino - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline and 7 - chloro - 9 - amino -

6 - deoxy - 6 - demethyl - 6 - methylene-tetracycline are reduced to obtain separable mixtures of the corresponding 7 - deshalo-9 - substituted - 6 - epi - 6 - deoxytetracyclines and 7 - deshalo - 9 - substituted-6 - deoxytetracyclines.

In a similar fashion, the hydrochlorides of 7,11a - dichloro - 9 - nitro - 6 - deoxy-6 - demethyl - 6 - methylenetetracycline and 7 - bromo - 11a - chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline are reduced to separable mixtures of 9 - amino - 6 - epi - 6 - deoxytetracycline and 9 - amino - 6 - deoxytetracycline.

EXAMPLE XXXV

To a solution of 500 mg. of 7 - chloro-6 - deoxy - 6 - demethyl - 6 - methylene-5 - oxytetracycline hydrochloride and 25 ml. of methanol is added 500 mg. of 5% rhodium on carbon. The suspension is hydrogenated at a pressure of 2,000 p.s.i. until 1 mol of hydrogen has been consumed. The catalyst is removed by filtration and the filtrate evaporated to dryness *in vacuo*. Preparative paper chromatography using an ethyl acetate-water system is employed to isolate 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline.

EXAMPLE XXXVI

A suspension of 1 g. of platinum dioxide in 50 ml. of methanol is saturated with hydrogen. To it is added a solution of 15 g. of 9 - nitro - 6 - methylene - 5 - oxytetracycline in 200 ml. of methanol and 1 ml. of concentrated hydrochloric acid. The suspension is hydrogenated under atmospheric conditions until 3 mols of hydrogen have been consumed. Removal of the catalyst by filtration and evaporation of the filtrate yields a residue of 9 - amino - 6 - methylene - 5 - oxytetracycline.

EXAMPLE XXXVII

To separate solutions of 500 mg. 11a-chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline hydrochloride and the corresponding 11a-fluoro compound in 25 ml. of methanol, is added 500 mg. of 5% palladium on carbon. Each suspension is then hydrogenated at atmospheric pressure until one mol of hydrogen has been consumed. The catalyst is removed by filtration and the filtrate evaporated to dryness *in vacuo*. The compounds separated are respectively 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline and a mixture of 11a - fluoro - 6 - deoxytetracycline and 11a - fluoro - 6 - epi - 6 - deoxytetracycline.

EXAMPLE XXXVIII

To 50 mg. of the mixed 11a - fluoro - 6 - deoxytetracycline and 11a - fluoro - 6 - epi - 6 - deoxytetracycline product of Example XXXVII dissolved in 1.25 ml. of 1% aque-

ous HCl, is added 30 mg. zinc dust and the mixture is stirred 15 minutes at 20°C. The zinc is removed by filtration and the product extracted with butanol from the mother liquor. On evaporation to dryness, a mixture of 6 - deoxytetracycline and 6 - epi - 6 - deoxytetracycline is obtained.

EXAMPLE XXXIX

Acid Addition Salts of 6 - epi - 6 - deoxy-5 - oxytetracycline

6 - Epi - 6 - deoxy - 5 - oxytetracycline is dissolved in methanol containing an equimolar amount of hydrogen chloride. The hydrochloride salt is then precipitated by the addition of ether, and is collected by filtration and dried. Butanol is employed to recrystallize the hydrochloride.

In a similar manner, acid addition salts of 6 - epi - 6 - deoxy - 5 - oxytetracycline are prepared using, in lieu of hydrochloric acid, sulfuric, nitric, perchloric, hydrobromic, phosphoric, hydrofluoric, p-toluenesulfonic, hydriodic, tartaric, acetic, citric, malic, benzoic, glycollic, gluconic, gulonic, succinic, and sulfosalicylic acids.

In like manner, the acid addition salts of the products of the examples appearing hereinafter are prepared. Those acid addition salts which are pharmaceutically unacceptable may be employed in conventional fashion for purification purposes to produce either the amphoteric compounds or pharmaceutically acceptable acid addition salts thereof. Additionally, the pharmaceutically unacceptable acid addition salts are useful as intermediates for producing other products of the present invention as will be apparent on considering the examples presented hereinafter.

EXAMPLE XL

Metal Salts of 6 - epi - 6 - deoxy - 5 - oxytetracycline

The sodium salt of 6 - epi - 6 - deoxy-5 - oxytetracycline is prepared by dissolving the amphoteric compound in methanol containing an equimolar amount of sodium hydroxide and evaporating the resulting mixture *in vacuo*. In this fashion, other metal salts are prepared including potassium, calcium, barium, lithium and strontium salts.

Metal salt complexes of 6 - epi - 6 - deoxy-5 - oxytetracycline are prepared by dissolving it in a lower aliphatic alcohol, preferably methanol, and treating with an equimolar amount of the selected metal salt, preferably dissolved in the selected alcohol. The insoluble metal salt complexes may be isolated by simple filtration. Generally, however, most of them are alcohol soluble and recovery is effected by evaporation of the solvent or addition of a non-solvent such as diethyl ether.

In this fashion, metal salt complexes of 6 - epi - 6 - deoxy - 5 - oxytetracycline con-

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sisting primarily of compounds containing 1:1 ratio of metal to 6 - epi - 6 - deoxy - 5 - oxytetracycline are prepared employing the following metal salts: calcium chloride, cobalt chloride, magnesium sulfate, magnesium chloride, stannous chloride, zinc chloride, cadmium chloride, barium chloride, silver nitrate, stannous nitrate, strontium nitrate, magnesium acetate, palladium chloride, manganous chloride, cerium chloride, titanium chloride, platinum chloride, vanadium chloride, plumbous acetate, stannous bromide, zinc sulfate, chromous chloride and nickelous chloride.

In the same fashion, the same metal salt complexes of the compounds of the examples appearing hereinafter are prepared.

EXAMPLE XLI

6 - Epi - 6 - deoxy - 11a - chloro - oxytetracycline

To a cold, stirred, solution of 1 g. 6 - epi - 6 - deoxy - 5 - oxytetracycline in 15 ml. of dimethoxyethane there is added 0.35 g. of N-chlorosuccinimide. After 20 minutes at 0—5°C. the product is precipitated with ether and collected by filtration with ether wash.

EXAMPLE XLII

6 - Epi - 6 - deoxy - 11a - fluoro - 5 - oxytetracycline

A suspension of 4.8 g. of 6 - epi - 6 - deoxy - 5 - oxytetracycline hydrochloride in 100 ml. of methanol is cooled at 0°C. and nitrogen gas is bubbled through. A solution of 20 ml. of a normal solution of sodium methoxide in methanol is added and the mixture is saturated with perchloryl fluoride. The mixture is then maintained in a refrigerator for 24 hours during which time crystals of the chlorate salt of the desired product form. The filtrate yields a second crop of crystals of less pure product.

EXAMPLE XLIII

6 - Epi - 6 - deoxy - 7,11a - dichloro - 5 - oxytetracycline

To 5 g. of 6 - epi - 6 - deoxy - 11a - chloro - 5 - oxytetracycline hydrochloride in 15 ml. of liquid hydrogen fluoride cooled at ice bath temperature, is added 1.5 g. of N-chlorosuccinimide. The solution is stirred at ice bath temperature for 1.5 hours. The crude product is precipitated by the addition of 500 ml. of ether and recovered by filtration.

EXAMPLE XLIV

6 - Epi - 6 - deoxy - 7 - chloro - 11a - fluoro - 5 - oxytetracycline

To 4.6 g. of 6 - epi - 6 - deoxy - 11a - fluoro - 5 - oxytetracycline in 20 ml. of trifluoroacetic acid, there is added 1.4 g. of N-chlorosuccinamide and the mixture is allowed to stand at room temperature for 4 hours.

After cooling, the mixture is added dropwise to stirred ether at 0°C., the product separates and is filtered and slurried several times with ether.

EXAMPLE XLV

6 - Epi - 6 - deoxy - 7 - chloro - 5 - oxytetracycline

(1) The product of Example XLIII (1 g.) is added to 50 ml. of methanol containing 100 mg. of 5% rhodium on carbon. The mixture is treated at a pressure of 3 atmospheres hydrogen gas at a temperature of 32°C. Hydrogenation is stopped after the uptake of hydrogen ceases, the mixture is filtered and the resulting solution is evaporated to dryness at reduced pressure. The residue is slurried in 25 ml. of ether, filtered and dried to obtain the desired product.

(2) Molecular equivalent amounts of 6 - epi - 6 - deoxy - 7,11a - dichloro - 5 - oxytetracycline, sodium iodide and powdered zinc metal are refluxed in acetone for 30 minutes. The reaction mixture is cooled, filtered and concentrated under reduced pressure to obtain the desired product.

(3) To a solution of 0.45 g. of 6 - epi - 6 - deoxy - 7,11a - dichloro - 5 - oxytetracycline perchlorate in 7.5 ml. of water is added 0.45 g. of sodium hydrosulfite and the resulting mixture is stirred for 12 minutes. The product separates and is collected by filtration.

EXAMPLE XLVI

6 - Epi - 6 - deoxy - 7 - phenylazo - 11a - chloro - 5 - oxytetracycline

One molecular proportion of aniline is dissolved in 2N HCl, employing about 20 ml. thereof per gram of aniline, and the solution treated with 1 molecular proportion of sodium nitrite at a temperature of 0°C. The resulting benzene diazonium chloride solution is mixed at 0 to 20°C. with an aqueous solution of 1 molecular proportion of 6 - epi - 6 - deoxy - 11a - chloro - 5 - oxytetracycline and a molecular equivalent quantity of sodium hydroxide, and containing sufficient sodium carbonate to neutralize the excess HCl in the diazotized aniline solution. The pH of the resulting solution is in the range 8—10. Stirring is continued at 0°C. for approximately 2 hours during which time crude 6 - epi - 6 - deoxy - 7 - phenylazo - 11a - chloro - 5 - oxytetracycline separates. This product is collected on a filter, washed and dried.

EXAMPLE XLVII

6 - Epi - 6 - deoxy - 7 - amino - 5 - oxytetracycline

One part by weight of 6 - epi - 6 - deoxy - 7 - phenylazo - 11a - chloro - 5 - oxytetracycline is mixed with 20 parts by weight of methanol and 1/5 part by weight of 5% palladium on carbon hydrogenation catalyst

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is added to the mixture. The mixture is then hydrogenated at 30—45 p.s.i. in a conventional apparatus at 30°C. until consumption of hydrogen ceases. The catalyst is then filtered and the filtrate evaporated to dryness. The residue comprises a mixture of aniline and 6 - epi - 6 - deoxy - 7 - amino - 5 - oxytetracycline. The aniline is removed from the residue by washing with ether.

EXAMPLE XLVIII

6 - Epi - 6 - deoxy - 7 - nitro - 5 - oxytetracycline

6 - Epi - 6 - deoxy - 7 - amino - 5 - oxytetracycline, 4.58 g., is dissolved in 20 ml. of 6N nitric acid at 0°C. treated with 0.7 g. of sodium nitrite. The diazonium nitrate solution is then mixed with a suspension of cuprous oxide in water, which is prepared by reducing an aqueous solution of copper sulfate (approximately 5 g. in 50 ml. of water) with an alkaline glucose solution which is subsequently neutralized with acetic acid. A reaction ensues with the evolution of nitrogen. The mixture is strongly acidified to decompose the copper complexes and the 6 - epi - 6 - deoxy - 7 - nitro - 5 - oxytetracycline is extracted from the solution with methyl isobutylketone.

This compound can also be prepared together with 6 - epi - 6 - deoxy - 9 - nitro - 5 - oxytetracycline by direct nitration of 6 - epi - 6 - deoxy - 5 - oxytetracycline. Thus, 0.96 g. of 6 - epi - 6 - deoxy - 5 - oxytetracycline hydrochloride is dissolved in approximately 10 ml. of concentrated sulfuric acid at 0—5°C. and treated with 1.4 ml. of a 10:1 concentrated sulfuric acid - 70% nitric acid solution in dropwise fashion with stirring at the same temperature during a 15 minute period. The solution is then poured into 400 ml. of anhydrous ether and the nitro - 6 - epi - 6 - deoxy - 5 - oxytetracyclines precipitate as the acid sulfate salts. The precipitate is cooled, washed and dried. This crude nitration product is crystallized from about 5 ml. of methanol to yield a mixture of 7-nitro- and 9 - nitro - 6 - epi - 6 - deoxy - 5 - oxytetracyclines. The mixture is then separated by column partition chromatography using the toluene-pyridine-water system. Since the 7-nitro product is the less polar component, it is the first to be eluted.

The diazonium replacement method has the advantage of producing the 7-nitro isomer and thus obviates the described separation process.

EXAMPLE XLIX

6 - Epi - 6 - deoxy - 7 - acetyl amino - 5 - oxytetracycline

To a solution of 4.58 g. of 6 - epi - 6 - deoxy - 7 - amino - 5 - oxytetracycline in 200 ml. of tetrahydrofuran and 200 ml. of dry methanol is added 20 ml. of acetic

anhydride and the mixture is stirred for 45 minutes at room temperature. The mixture is then stripped of most of the solvent and dropped into dry ether, filtered and the solid cake reslurried with ether, filtered and dried to obtain crude product. The crude product is chromatographed on a cellulose column to obtain purified product as the main fraction.

Employing the procedure of this example, the following products are prepared:

6 - epi - 6 - deoxy - 7 - formyl amino - 5 - oxytetracycline 75

6 - epi - 6 - deoxy - 7 - butyl amino - 5 - oxytetracycline 70

6 - epi - 6 - deoxy - 7 - propionyl amino - 5 - oxytetracycline 80

6 - epi - 6 - deoxy - 7 - valeryl amino - 5 - oxytetracycline 85

EXAMPLE L

6 - Epi - 6 - deoxy - 9 - nitro - 5 - oxytetracycline 85

To a mixture of 450 mg. of 6 - epi - 6 - deoxy - 5 - oxytetracycline in 5.0 ml. of anhydrous liquid hydrogen fluoride is added 100 mg. of potassium nitrate. The mixture is stirred at 15°C. for 30 minutes and then evaporated to dryness. The residue is slurried in ether and filtered to obtain the crude product which is slurried in 5% aqueous HCl and extracted with butanol. The butanol extract is concentrated to obtain the product which is recrystallized from water with hydrochloric acid. 90

EXAMPLE LI

6 - Epi - 6 - deoxy - 9 - amino - 5 - oxytetracycline 100

To a solution of 2.5 g. of 6 - epi - 6 - deoxy - 9 - nitro - 5 - oxytetracycline dissolved in 50 ml. of water and 2 ml. of concentrated HCl is added 1.3 g. of zinc dust with stirring at 20°C. After 15 minutes the mixture is filtered, the pH is adjusted to 2.5 and the mixture is extracted with butanol. The butanol extract on concentration gives the desired product as the dihydrochloride. 105

EXAMPLE LII

6 - Epi - 6 - deoxy - 9 - formyl amino - 5 - oxytetracycline 110

To a solution of 7.0 g. of 6 - epi - 6 - deoxy - 9 - amino - 5 - oxytetracycline in 40 ml. of dry pyridine is added 20 ml. acetoformic acid at ice bath temperature. The mixture is stirred for 15 minutes at this temperature and added dropwise to 1 liter of dry ether. The product is filtered, reslurried in ether and filtered again. The crude product is suspended in 100 ml. of water providing a solution of pH of 4.2 and stirred for 10 minutes to hydrolyze O-formyl groups. The pH mixture is then adjusted to pH 5.5 and 120

the solution is freeze-dried to obtain crude product which is chromatographed on a cellulose column to obtain purified product as the main fraction.

- 5 Employing the procedure of this example, the following products are prepared:

6 - epi - 6 - deoxy - 9 - acetylamino - 5 - oxytetracycline

6 - epi - 6 - deoxy - 9 - butylamino - 5 - oxytetracycline

- 10 6 - epi - 6 - deoxy - 9 - propionylamino - 5 - oxytetracycline

6 - epi - 6 - deoxy - 9 - valerylamino - 5 - oxytetracycline

- 15 **EXAMPLE LVII**

6 - Epi - 6 - deoxy - 9 - chloro - 5 - oxytetracycline

- 20 To 1.04 gram of 6 - epi - 6 - deoxy - 9 - amino - 5 - oxytetracycline hydrochloride dissolved in 5 ml. water and 2 ml. concentrated hydrochloric acid cooled in an ice bath is added below the surface of the liquid 150 mg. sodium nitrite in 2 ml. water. After stirring 20 minutes, 50 mg. urea is added to destroy the excess nitrous acid. Cuprous chloride/hydrogen chloride is added and the mixture stirred at room temperature for five minutes. It is then immersed in a 50°C. water bath for 20 minutes, then cooled and extracted with n-butanol. The extract is dried with sodium sulfate and evaporated to dryness.

- 25 The cuprous chloride is prepared by adding a hot solution of 600 mg. copper sulfate pentahydrate, and 160 mg. sodium chloride in 2 ml. water to 160 mg. sodium bisulfite in 1.7 ml. of 1.1 N. sodium hydroxide. The mixture is cooled, centrifuged and the solid washed with 1 ml. water. It is then dissolved in 1 ml. concentrated HCl and used above.

The crystalline p-toluenesulfonate is prepared by standard procedure.

EXAMPLE LVIII

- 45 7 - Chloro - 9 - nitro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

- 50 To a solution of 1.0 g. of 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline base in 8 ml. of anhydrous liquid hydrogen fluoride at 0°C. is added 220 mg. of KNO₃. The mixture is stirred for 30 minutes at 0°C. and the hydrogen fluoride is evaporated off under nitrogen. The residue is slurried in dry ether, filtered and dried to obtain the desired product.

EXAMPLE LV

9 - Nitro - 7,11a - dichloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

- 60 To a solution of 600 mg. 7,11a - dichloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline perchlorate in 1.5 ml. of anhydrous liquid

hydrogen fluoride is added 100 mg. KNO₃. The mixture is stirred at 15°C. for 30 minutes and then evaporated to dryness. The residue is slurried in ether and filtered to obtain the crude product which is slurried in 5% aqueous HCl and extracted with butanol. The butanol extract is concentrated to obtain the product which is recrystallized from water with hydrochloric acid.

EXAMPLE LVI

9 - Nitro - 11a - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

A solution of 5 g. of 11a - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline in 10 ml. of anhydrous hydrogen fluoride is allowed to stand for 3½ hours after which 1.2 g. of KNO₃ is added with stirring. Stirring is continued for 1 hour at ice bath temperature and the mixture is then added to 200 ml. of dry ether, filtered and washed with ether to obtain crude product.

EXAMPLE LVII

7 - Chloro - 9 - amino - 6 - epi - 6 - deoxy - 5 - oxytetracycline

To a solution of 1.42 g. of 7 - chloro - 9 - nitro - 6 - epi - 6 - deoxy - 5 - oxytetracycline is dissolved in 50 ml. of water and 2 ml. of concentrated HCl is added 1.3 g. of zinc dust with stirring at 20°C. After 15 minutes, the mixture is filtered, the pH adjusted to 2.5 and the mixture is extracted 5 times with 30 milliliters per extraction of butanol. The butanol extract on concentration gives the desired product as the dihydrochloride.

This same product is also obtained by reduction with aqueous sodium hydrosulfite of 9 - nitro - 7,11a - dichloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline.

EXAMPLE LVIII

7,11a - Dichloro - 9 - nitro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

One gram of 7,11a - dichloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline hydrochloride is dissolved in 20 ml. of acetic acid and 1 ml. of concentrated HNO₃ is added. The mixture is allowed to stand for 12 hours and then concentrated to ¼ of its original volume. It is added dropwise to 200 ml. of ether with stirring at ice bath temperature. Stirring is continued for 3 hours and the solid is then recovered by filtration. The solid is slurried several times in ether and then dried to obtain the product as the hydrochloride salt.

Using the same procedure, the following products are obtained from corresponding starting compounds:

7 - chloro - 11a - fluoro - 9 - nitro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

- 7 - bromo - 11a - chloro - 9 nitro - 6 - epi - 6 - deoxy - 5 - oxytetracycline
 7 - iodo - 11a - fluoro - 9 - nitro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

5 These products are converted respectively to the 7-chloro, 7-bromo-, and 7 - iodo - 9-amino - 6 - epi - 6 - deoxy - 5 - oxytetracyclines by the reduction procedures of Examples IV and VI.

EXAMPLE LIX

- 10 9 - Formylamino - 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

75 To a solution of 4.5 g. of 9 - amino - 7-chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline base in 40 ml. of dry pyridine is added 20 ml. of acetoformic acid at ice bath temperature. The mixture is stirred for 15 minutes at that temperature and added dropwise to 1 liter of dry ether. The product is filtered, reslurried in ether and slurried again. The crude product is then suspended in 100 ml. of water providing a solution of pH 4.2 and stirred for 10 minutes to hydrolyze O-formyl groups. The pH of the mixture is then adjusted to pH 5.5 and the solution freeze-dried to get crude product which is chromatographed on a cellulose column to obtain purified product as the main fraction.

EXAMPLE LX

- 30 9 - Acetylamino - 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

35 To a solution of 4.2 g. of 7 - chloro - 9-amino - 6 - epi - 6 - deoxy - 5 - oxytetracycline base in 200 ml. of dry tetrahydrofuran and 200 ml. of dry methanol is added 20 ml. of acetic anhydride and the mixture is stirred for 45 minutes at room temperature. The mixture is then stripped of most of the solvent and introduced into dry ether, filtered and the solid cake reslurried in ether, filtered and dried to obtain crude product. The crude product is chromatographed as in Example LIX to obtain purified product as the main fraction.

45 Employing the procedures of Example LIX and the present example, the following products are prepared:

- 50 9 - butyrylamino - 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

- 9 - propionylamino - 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

- 9 - valerylamino - 7 - chloro - 6 - epi - 6 - deoxy - 5 - oxytetracycline

EXAMPLE LXI

55 A suspension of 6 - epi - 6 - deoxy - 5 - oxytetracycline is prepared with the following compositions:

Antibiotic	31.42	g.
70% aqueous sorbitol	714.29	g.
Glycerine U.S.P.	185.35	g.
Gum acacia (10% solution)	100	ml.
Polyvinyl pyrrolidone	0.5	g.
Butyl parahydroxybenzoate (preservative)	0.172	g.
Propyl parahydroxybenzoate (preservative)	0.094	g.
Water, distilled to make	1.	liter

60 To this suspension, various sweetening and flavoring agents, as well as acceptable coloring agents, may be added to choice. The suspension contains approximately 25 mg. of antibiotic activity per milliliter.

EXAMPLE LXII

65 A solution of 6 - epi - 6 - deoxy - 5 - oxytetracycline is prepared with the following compositions:

Antibiotic	30.22	g.
Magnesium chloride hexahydrate	12.36	g.
Monoethanolamine	8.85	ml.
Propylene glycol	376	g.
Water	94	ml.

EXAMPLE LXIII

70 The solution has a concentration of 50 mg./ml. and is suitable for parenteral and especially for intramuscular administration.

75 A tablet base is prepared by blending the following ingredients in the proportion by weight indicated:

Sucrose U.S.P.	80.3
Tapioca starch	13.2
Magnesium stearate	6.5

- 5 Into this base there is blended sufficient 6-epi - 6 - deoxy - 5 - oxytetracycline to provide tablets containing 25, 100 and 250 mg. of active ingredients.

EXAMPLE LXIV

- 10 A blend is prepared containing the following ingredients:

Calcium carbonate U.S.P.	17.6
Dicalcium phosphate	18.8
Magnesium trisilicate U.S.P.	5.2
Lactone U.S.P.	5.2
15 Potato starch	5.2
Magnesium stearate A	0.8
Magnesium stearate B	00.35

- 20 To this blend is added sufficient 6 - epi - 6 - deoxy - 5 - oxytetracycline to provide capsules containing 25, 100 and 250 mg. of active ingredients.

EXAMPLE LXV

- 25 One thousand grams of 6 - epi - 6 - deoxy - 5 - oxytetracycline are intimately mixed and ground with 2500 grams of sodium ascorbate. The ground, dry mixture is filled into vials, sterilized with ethylene oxide and the vials sterilely stoppered. For intravenous administration sufficient water is added to the vials to form a solution containing 10 mg. of active ingredient per milliliter.

WHAT WE CLAIM IS:—

- 35 1. A process for the preparation of a mixture of a 6-deoxytetracycline and a 6-epi-6-deoxytetracycline which includes the step of hydrogenating a compound of formula I or II as herein defined or acid addition salt or polyvalent metal complex salt thereof in the presence of a noble metal catalyst till reduction of the methylene group, when X₂ is on a nitro group it being reduced to an amino group and when Z is a chloro group it being replaced by hydrogen.

- 45 2. A process as claimed in claim 1 for producing a mixture of a 6 - deoxy - 5 - oxytetracycline and a 6 - epi - 6 - deoxy - 5 - oxytetracycline in which the substituent Y is OH.

- 50 3. A process according to claim 1 or claim 2 wherein the noble metal catalyst is rhodium, or palladium or platinum.

- 55 4. A process according to any of the preceding claims wherein the hydrogenation is carried out at a pressure of from one atmosphere to 2000 pounds per square inch.

5. A process according to any of the preceding claims wherein the temperature employed is from about 0°C. to 60°C.

- 60 6. A process according to any of the preceding claims wherein the resulting 6-deoxy-

and 6-epi-6-deoxytetracycline compounds are separately recovered.

7. A process according to any of the preceding claims wherein the hydrogenation is continued until reaction of from one to 6 moles of hydrogen per mole of starting compound.

8. A process in any of claims 1 to 7 where the starting compound is an 11a fluoro compound, the 11a fluoro atom is subsequently removed by reaction with a chemical reducing agent.

9. A process according to claim 8 in which the chemical reducing agent is zinc and aqueous hydrochloric acid or sodium hydro-sulphite.

10. A process for producing 11a-chloro or fluoro 6-epi-6-deoxy-5-oxytetracyclines which includes the step of contacting 6-epi-6-deoxy-5-oxytetracycline with a halogenating agent in a reaction-inert solvent.

11. A process according to claim 10, wherein the halogenating agent is chlorine; N-chloro-alkanoic acid amides containing 1—6 carbon atoms, -hydrocarbon dicarboxylic acid imides or -N-alkanoyl anilides containing 1—5 carbon atoms; 3-chloro- or 3,5-dichloro-5,5-di-methyl-hydantoin; pyridinium perchloride hydrohalides; lower alkyl hypochlorites containing 1—5 carbon atoms or perchloryl fluoride.

12. A process according to claim 10 or 11, wherein the halogenating agent is employed in an amount of from 1 to 1.2 mols per mol of the tetracycline compound.

13. A process according to any of claims 10 to 12, wherein the halogenation is carried out in the presence of a water-miscible solvent.

14. A process according to claim 13, wherein the halogenating agent is perchloryl fluoride and the solvent is a lower alkanol.

15. A process according to claim 14, wherein the fluorination is carried out in the presence of a base.

16. A process according to any of claims 10 to 15, wherein the halogenation is carried out at a temperature below 50°C.

17. A process according to claim 10 or claim 11, wherein the halogenating agent is employed in an amount of at least 2 mols per mol of the tetracycline compound.

18. A process according to any of the preceding claims which includes the further step of treating the product attained with a nitrating agent to obtain a nitro compound.

19. A 7 and/or 9 D-ring substituted 6-epi-6-deoxy-5-oxytetracycline in which the substituent is chloro, hydrogen, nitro, amino or lower alkanoylamino containing 1—5 carbon atoms.

20. An 11a-chloro or fluoro derivative of the compound of claim 18.

21. 6 - Epi - 6 - deoxy - 5 - oxytetra-cycline.



Sucrose U.S.P.	80.3
Tapioca starch	13.2
Magnesium stearate	6.5

5 Into this base there is blended sufficient 6-epi - 6 - deoxy - 5 - oxytetracycline to provide tablets containing 25, 100 and 250 mg. of active ingredients.

EXAMPLE LXIV

10 A blend is prepared containing the following ingredients:

Calcium carbonate U.S.P.	17.6
Dicalcium phosphate	18.8
Magnesium trisilicate U.S.P.	5.2
Lactone U.S.P.	5.2
15 Potato starch	5.2
Magnesium stearate A	0.8
Magnesium stearate B	00.35

20 To this blend is added sufficient 6 - epi - 6 - deoxy - 5 - oxytetracycline to provide capsules containing 25, 100 and 250 mg. of active ingredients.

EXAMPLE LXV

25 One thousand grams of 6 - epi - 6 - deoxy - 5 - oxytetracycline are intimately mixed and ground with 2500 grams of sodium ascorbate. The ground, dry mixture is filled into vials, sterilized with ethylene oxide and the vials sterilely stoppered. For intravenous administration sufficient water is added to the vials
30 to form a solution containing 10 mg. of active ingredient per milliliter.

WHAT WE CLAIM IS:—

35 1. A process for the preparation of a mixture of a 6-deoxytetracycline and a 6-epi-6-deoxytetracycline which includes the step of hydrogenating a compound of formula I or II as herein defined or acid addition salt or polyvalent metal complex salt thereof in the presence of a noble metal catalyst till
40 reduction of the methylene group, when X₂ is on a nitro group it being reduced to an amino group and when Z is a chloro group it being replaced by hydrogen.

45 2. A process as claimed in claim 1 for producing a mixture of a 6 - deoxy - 5 - oxytetracycline and a 6 - epi - 6 - deoxy - 5 - oxytetracycline in which the substituent Y is OH.

50 3. A process according to claim 1 or claim 2 wherein the noble metal catalyst is rhodium, or palladium or platinum.

55 4. A process according to any of the preceding claims wherein the hydrogenation is carried out at a pressure of from one atmosphere to 2000 pounds per square inch.

5. A process according to any of the preceding claims wherein the temperature employed is from about 0°C. to 60°C.

60 6. A process according to any of the preceding claims wherein the resulting 6-deoxy-

and 6-epi-6-deoxytetracycline compounds are separately recovered.

7. A process according to any of the preceding claims wherein the hydrogenation is continued until reaction of from one to 6 moles of hydrogen per mole of starting compound.

8. A process in any of claims 1 to 7 where the starting compound is an 11a fluoro compound, the 11a fluoro atom is subsequently removed by reaction with a chemical reducing agent.

9. A process according to claim 8 in which the chemical reducing agent is zinc and aqueous hydrochloric acid or sodium hydro-sulphite.

10. A process for producing 11a-chloro or fluoro 6-epi-6-deoxy-5-oxytetracyclines which includes the step of contacting 6-epi-6-deoxy-5-oxytetracycline with a halogenating agent in a reaction-inert solvent.

11. A process according to claim 10, wherein the halogenating agent is chlorine; N-chloro-alkanoic acid amides containing 1—6 carbon atoms, -hydrocarbon dicarboxylic acid imides or -N-alkanoyl anilides containing 1—5 carbon atoms; 3-chloro- or 3,5-dichloro-5,5-di-methyl-hydantoin; pyridinium perchloride hydrohalides; lower alkyl hypochlorites containing 1—5 carbon atoms or perchloryl fluoride.

12. A process according to claim 10 or 11, wherein the halogenating agent is employed in an amount of from 1 to 1.2 mols per mol of the tetracycline compound.

13. A process according to any of claims 10 to 12, wherein the halogenation is carried out in the presence of a water-miscible solvent.

14. A process according to claim 13, wherein the halogenating agent is perchloryl fluoride and the solvent is a lower alkanol.

15. A process according to claim 14, wherein the fluorination is carried out in the presence of a base.

16. A process according to any of claims 10 to 15, wherein the halogenation is carried out at a temperature below 50°C.

17. A process according to claim 10 or claim 11, wherein the halogenating agent is employed in an amount of at least 2 mols per mol of the tetracycline compound.

18. A process according to any of the preceding claims which includes the further step of treating the product attained with a nitrating agent to obtain a nitro compound.

19. A 7 and/or 9 D-ring substituted 6-epi-6-deoxy-5-oxytetracycline in which the substituent is chloro, hydrogen, nitro, amino or lower alkanoylamino containing 1—5 carbon atoms.

20. An 11a-chloro or fluoro derivative of the compound of claim 18.

21. 6 - Epi - 6 - deoxy - 5 - oxytetra-cycline.

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oxytetracycline.10 26. 6 - Epi - 6 - deoxy - 9 - acetyl amino-
5 - oxytetracycline.27. 6 - Epi - 6 - deoxy - 11a - fluoro - 5-
oxytetracycline.28. 6 - Epi - 6 - deoxy - 11a - chloro-
5 - oxytetracycline.29. Acid addition salts, metal salts, and
polyvalent metal salt complexes of compounds
of any of claims 19 to 26.30. A product according to claim 19 sub-
stantially as shown and described.31. A product according to claim 20 sub-
stantially as shown and described. 2032. A process according to claim 1 sub-
stantially as shown and described.33. A process according to claim 8 sub-
stantially as shown and described. 25MARKS & CLERK,
Chartered Patent Agents,
Agents for the Applicants. RgLeamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press
(Leamington) Ltd.—1965. Published by The Patent Office, 25 Southampton Buildings,
London, W.C.2, from which copies may be obtained.

ERRATA

SPECIFICATION No. 995,032
Amendment No. 1Page 4, line 14, for "producing" read "pro-
ducting"Page 5, line 4, for "-oxy-" (first occurrence)
read "-deoxy-"Page 8, line 87, after "377" insert "m μ and
an inflection at 260"

Page 8, line 89, for "7.25" read "7.85"

Page 8, line 93, after "line 92" insert "Method
A"

Page 9, line 21, for "432" read "4.32"

Page 9, line 32, for "600" read "6000"

Page 9, line 65, for "methyl" read "demethyl"

Page 11, line 88, for "11" read "11a"

Page 13, line 22, delete whole line

Page 15, line 103, for "prpeared" read "pre-
pared"

Page 18, line 69, for "ih" read "is"

Page 20, line 88, for "di-methyl" read
"dimethyl"THE PATENT OFFICE
20th July 1965