

PATENT SPECIFICATION

NO DRAWINGS

995,031



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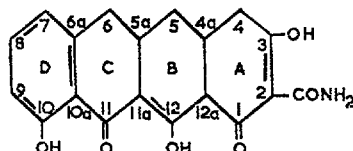
COMPLETE SPECIFICATION

6-Methylene-Tetracyclines and method of preparing same

We, PFIZER CORPORATION, a corporation of the Republic of Panama, of Calle, 15½ Avenidor Santa Isabel, Colon, Panama, 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:—

This invention relates to new 6 - methylene tetracycline compounds and processes for the preparation thereof. These and other objects of this invention are obvious from the following disclosure.

The tetracycline antibiotics comprise a group of biologically active perhydronaphthacene derivatives having the following essential structural features. The numbering system indicated is that employed by "Chemical Abstracts".



Among the biologically active members of this group are those containing the following substituent groups:

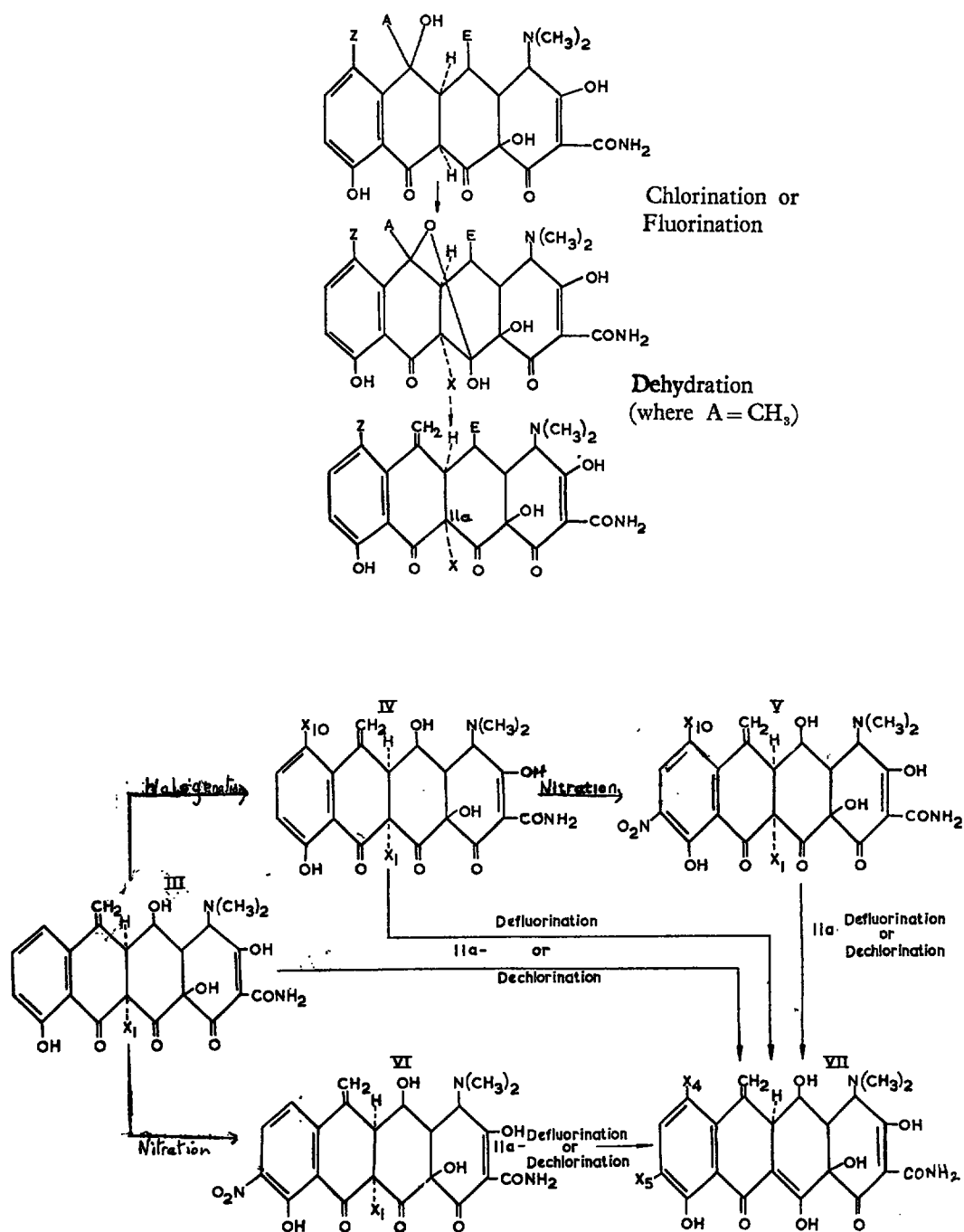
Substituents	Common Name
4 - N(CH ₃) ₂ , 6 - OH, 6 - CH ₃ , 12a - OH	tetracycline
4 - N(CH ₃) ₂ , 5 - OH, 6 - OH, 6 - CH ₃ , 12a - OH	5 - oxytetracycline
4 - N(CH ₃) ₂ , 6 - OH, 6 - CH ₃ , 7 - Cl, 12a - OH	7 - chlorotetracycline
4 - N(CH ₃) ₂ , 5 - OH, 6 - CH ₃ , 12a - OH	6 - deoxy - 5 - oxytetracycline
4 - N(CH ₃) ₂ , 6 - CH ₃ , 12a - OH	6 - deoxytetracycline
4 - N(CH ₃) ₂ , 12a - OH	6 - deoxy - 6 - demethyltetracycline
4 - N(CH ₃) ₂ , 6 - OH, 6 - CH ₃ , 7 - Br, 12a - OH	7 - bromotetracycline
4 - N(CH ₃) ₂ , 6 - OH, 7 - Cl, 12a - OH	6 - demethyl - 7 - chlorotetracycline
4 - N(CH ₃) ₂ , 6 - OH, 12a - OH	6 - demethyltetracycline

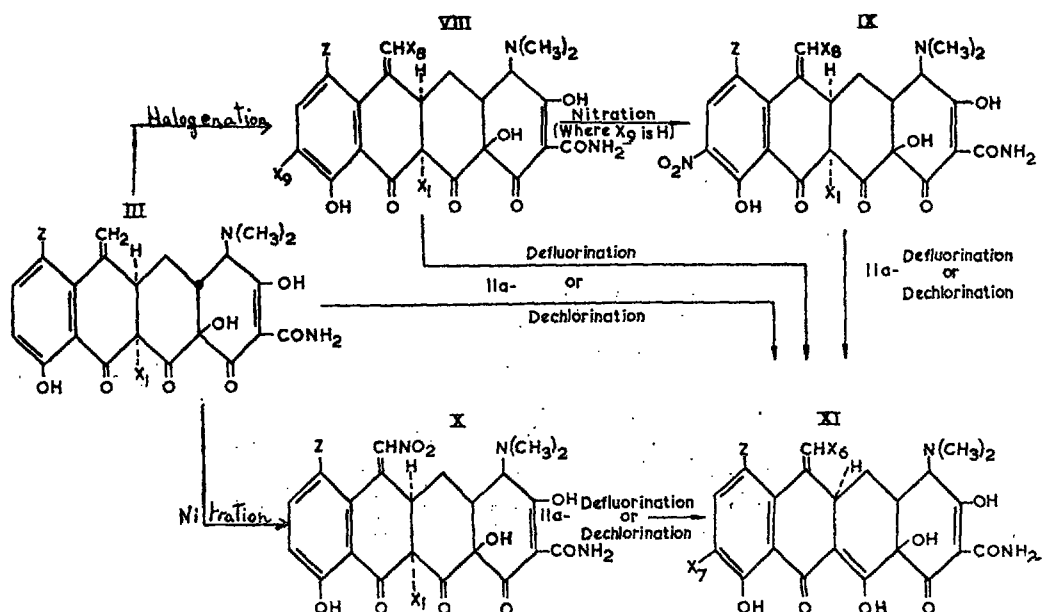
The new tetracycline compounds of this invention and processes for their preparation are illustrated by the following sequence of reactions and structural formulae, wherein: X is Cl or F

A is CH₃ or H;

Z is Cl, Br or H but when X is F and A is CH₃, Z is H;

E is H or OH, provided that when E is OH, A is CH₃ and Z is H.





Wherein:

Z and X₉ are as previously defined

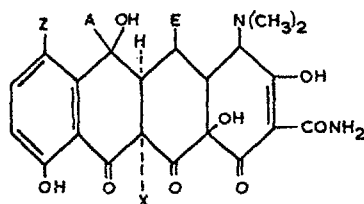
X₈ = Cl, Br, I

X₉ = Cl, Br, I, H

X₆ and X₇ = H, Cl, Br, I or amino provided that only one of X₆ or X₇ is amino, and when X₆ is H, X₇ must be H. When X₆ is amino X₇ must be H.

11 - a - Halogenation

The 11a - halo tetracyclines of structure (II) which form the subject matter of divisional application No. 31490/63 (Serial No. 952,656) are prepared by reaction of a suitable tetracycline (I) with a chlorinating or a fluorinating agent in a reaction — inert solvent. As is obvious from the structural formula, the chlorine or fluorine atom (X) at the 11a - position of the present compounds exists in both the *cis* and *trans* relationship to the hydrogen at the 5a - position of the tetracycline nucleus. Those compounds in which the halogen is *cis* are believed to exist predominantly in the structure designated, i.e. the structure containing an oxygen bridge between the 6 and 12 positions of the tetracycline nucleus, resulting from the formation of a hemi - ketal between the 12 - position carbonyl function and the hydroxy group at the 6 - position. In the crystalline state, these compounds appear to be predominantly in the form of hemi - ketal structure as is evidenced by the lack of carbonyl absorption on infrared analysis (no bands in the 5—6 micron region). Of course, such structures exist in equilibrium with the non - ketalized form:



particularly in solution and when reference is made herein to the hemi - ketal compounds, it is fully intended that this designation embraces *cis* 11a - halo non - ketalized compounds as well as the hemi - ketal. The present epoxy compounds are 11a - halo-1,4,4a,5,5a,6,11,11a,12,12a - decahydro - 3,10,12,12a - tetrahydroxy - 1,11 - dioxo - 2 - carboxamidonaphthacene - 6,12 - epoxides. For the sake of simplicity, the present halo compounds hereinafter will be referred to as tetracycline - 6,12 - hemi - ketals.

As stated chlorination or fluorination is accomplished by reaction of a suitable tetracycline (I) with a chlorinating or a fluorinating agent in a reaction — inert solvent. Suitable tetracycline starting compounds include tetracycline, oxytetracycline, chlortetracycline, 6 - demethyltetracycline and 6 - demethylchlortetracycline. The



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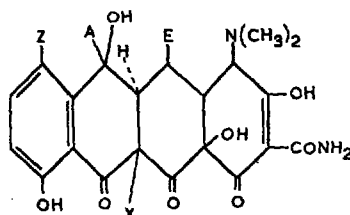
tetracycline starting compound, of course, should have a 6 - hydroxy substituent in order to form the hemi - ketal structure. For the production of 11a - chloro - tetracyclines in which the halogen is Cl, a variety of chlorinating reagents may be employed in a reaction — inert solvent. Suitable chlorinating agents include; chlorine, iodochloride; N - chloro, lower alkanolic acid amide, the term "lower" referring to a carbon content of 1 to 5 atoms, e.g. N - chloroacetamide; N-chlorinated hydrocarbon dicarboxylic acid imides, e.g. N - chlorosuccinimide, or phthalimide, and N-chlorinated lower - alkanoyl anilides e.g. N - chloroacetanilide, or N - chloropropionanilide; 3-chloro-, and 3,5 - dichloro 5,5 - dimethyl - hydantoin; and perchloride hydrohalides, e.g. pyridinium perchloride hydrohalide; and lower alkyl hypochlorites, e.g. tertiary butylhypochlorite. The term "lower" used in this specification has the meaning given above. It is obvious that, in general, any chlorinating commonly employed in the art is operable, but the above are preferred. In general, it is preferred to use from 1 to 1.2 mols of halogenating agent per mol of tetracycline starting compound.

By "reaction - inert solvent" as employed herein is meant a solvent which, under the conditions of the reaction, does not react in an undesired manner with either starting compounds or final products. A minimum of laboratory experimentation will permit the selection of suitable solvents for the present process. Exemplary of such solvents are dioxane, tetrahydrofuran, methyl ether of diethylene glycol (diglyme), and the methyl ether of ethylene glycol (monoglyme). Temperature does not appear to be critical in this process, temperatures of from 0° to 50°C. being found suitable. Temperatures above 50°C. should preferably be avoided due to the possible formation of 5a,6 - anhydro compounds which reduces the efficiency of the process. The selection of the best reaction conditions, e.g. temperature, solvent, or chlorinating agent, is a matter of routine experimentation. It is preferable to choose a solvent system from which the 11a - chloro product crystallizes on formation. It is usually preferable to prepare the chloro hemi - ketal compounds in a water - miscible solvent, such as tetrahydrofuran, dioxane, acetone, di - lower alkyl ethers of ethyleneglycol, or propyleneglycol. The product is obtained by mere dilution of the reaction mixture with water, which precipitates the 11a - chlorotetracycline - 6, 12 - hemi - ketal.

The 11a - chloro compounds do not appear to be as highly reactive as the corresponding 11a - bromo compound thus reducing the necessity of special handling in preparation. They are in fact quite stable in aqueous solutions, even at low pH values. For example, 11a - chlorotetracycline - 6,12 - hemi - ketal hydrochloride on standing in water, at pH of about 1 for 18 hours at room temperature shows no detectable decomposition.

The preparation of 11a - fluorotetracyclines is accomplished by contacting the starting tetracycline compound with perchloryl fluoride in the presence of a strong base, preferably an alkali metal hydroxide or alkoxide. The reaction is usually carried out by dissolving the starting compound in the selected solvent, preferably an alkanol containing up to 5 carbon atoms, containing at least a molar equivalent of the base and adding perchloryl fluoride, a gas at room temperature. As the reaction proceeds, the pH of the solution drops from alkaline to near neutral values, the product usually commencing to separate at a pH of approximately 8. The crystalline product is collected in the usual fashion and dried.

In addition to the 11a - halotetracycline - 6, 12 - hemi - ketals, the present process also yields 11a - halotetracyclines, i.e., those in which hemi - ketal formation has not occurred, in which it is presumed that the 11a - halo atom is on the reverse side of the 11a - carbon atom from the halogen of the hemi - ketals. It is thought that the stereochemical relationship of the former prevents formation of the hemi - ketal structure. These compounds are generally obtained by evaporation of the reaction mixture after precipitation of the 6,12-hemi-ketal compounds. The trans 11a-halo compounds are represented by the following formula, wherein Z, A, E and X are as previously defined:



The 11a - chloro and fluoro compounds of structure II are useful for the preparation of corresponding D-ring substituted tetracyclines by aromatic substitution reac-

tions to give compounds substituted in the 7 and/or 9 - positions. Exemplary of such aromatic substitution reactions are halogenation and nitration.

This halogenation is brought about by contacting the starting 11a - halo compound with a halogenating (chlorinating, brominating or iodinating) agent in a reaction inert solvent as previously described with respect to synthesis of the 11a - halo compounds.

The halogenated product obtained by this method is determined by the molar ratio of halogenating agent employed. With a substantially equimolar ratio of halogenating agent per mol of 11a - halo compound, monohalogenation occurs. With 2 or more molar equivalents of halogenating agent, dihalogenation occurs.

After the halogenation reaction is completed, the products are obtained by the usual methods. Generally, the method employed involves the addition of a non - solvent to the reaction mixture with subsequent collection of the precipitated product.

Nitration is accomplished by any of the standard methods used for this purpose. For example, the starting compound is reacted with nitric acid *per se* or formed *in situ*, e.g. potassium nitrate and sulfuric acid. For best results the nitration reaction is carried out in a solvent, such as a lower alkanolic acid, e.g. acetic acid, although a variety of other solvents may also be used. The preferred solvent is liquid hydrogen fluoride. A minimum of laboratory experimentation will permit the selection of other suitable solvents. While reaction conditions may vary appreciably, the use of high temperatures should be avoided. Satisfactory results are obtained over the range of from -25° to 50°C. with room temperature preferred as most convenient. Reaction time is not particularly critical, particularly in view of the stability of the starting compounds to the acid reaction conditions. To insure best yields it is preferred to use reaction periods of from as little as 15 minutes to about 12 hours.

Further, the present 11a - halotetracyclines are also useful as starting compounds in coupling reactions with diazonium salts. The coupling reaction with aryldiazonium salts is accomplished according to known methods. For example, the 11a - halo - tetracycline is dissolved in water or in a water soluble alcohol or ketone such as acetone to which is added an aqueous solution containing one to three molar equivalents of the selected aryldiazonium salt. The temperature of the mixture is maintained at 0—10°C. The coupling product usually commences to precipitate from the reaction mixture after about 15 minutes, but reaction times of the order of two hours are ordinarily employed to ensure complete reaction. The product usually separates as an amorphous material which is collected, washed and dried. If precipitation does not occur, the product may be obtained by evaporation of the solvent or by extraction of the reaction mixture with such solvents as n-butanol, or methyl isobutyl ketone. The azo products as obtained are generally suited for further chemical transformation or may be further purified by reprecipitation or crystallization from solvents, chromatography, or solvent extraction techniques.

The aryl diazonium salts which provide coupling products of the present type are myriad. The products obtained are 7- and/or 9 - arylazo - 11a - halotetracycline-6,12 - hemi - ketals. When Z in the starting compound is chloro or bromo, the product is a 9 - arylazo compound; when Z is hydrogen, the product is a mixture of the 7 and 9-isomers.

The aromatic azo coupling products as well as corresponding nitro compounds produced in accordance with the foregoing description are particularly useful for the production of corresponding aminotetracyclines. The azo compounds are reduced by electrolytic or chemical methods, but preferably by catalytic hydrogenolysis.

If desired, the 11a - halogen atom of the ring D - substituted - 11a - halotetracyclines obtained from Structure II compounds may be selectively removed by reduction, preferably using chemical means. The 11a - chloro atoms may be readily removed by treatment with sodium hydro - sulfite in aqueous media. This latter reduction, is a particularly facile process and is especially preferred with 11a-chloro compounds containing other reducible groups. Accordingly, 7,11a - dichlorotetracycline - 6,12 - hemi - ketal is reduced to 7 - chlorotetracycline by treatment with sodium hydrosulfite in aqueous dimethylformamide. The 11 - a fluoro compounds appear to be more difficult to reduce selectively since they require more vigorous reaction conditions. For example, the present 11a - fluorotetracyclines are converted to corresponding 11a-desfluoro compounds by reaction with zinc in mineral acid according to standard practice. The 11a-fluorine may also be removed by hydrogenolysis over noble metal catalysts, preferably at superatmospheric pressures of hydrogen gas, e.g. up to about 1000 atmospheres. The use of temperatures higher than room temperature (20—25°C.) may also be required in some cases to permit substantial yield of the desired product. Under such conditions of hydrogenolysis, other reduc-

ible groups, e.g. the 6 - hydroxy group and 7 or 9 - halogen or 7 or 9 nitro groups, will also be susceptible to reduction. For example, 7 - nitro - 11a - fluorotetracycline - 6, 12 - hemi - ketal on treatment with hydrogen gas over palladium on carbon at a pressure of 1500 psi yields *inter alia*, 7 - amino - 6 - deoxytetracycline as a product.

5 The present 11a - halotetracyclines are also useful as intermediates for the synthesis of 6 - deoxytetracyclines. On treatment with hydrogen gas in the presence of a mineral acid over a noble metal catalyst, both the 11a - halogen and the 6 - hydroxy group are hydrogenolyzed. For this purpose, pressures of hydrogen gas from atmospheric to superatmospheric pressures may be used, although it is usually preferred to employ high pressures, particularly with 11a - fluoro - compounds which are found to be less susceptible to hydrogenolysis and are preferably hydrogenolyzed at elevated temperatures, e.g. up to 100°C. Except in the case of 11a - fluoro compounds, temperatures does not appear to be critical in this reaction, the use of high temperatures (up to 50°C.) mainly serving to shorten reaction time. The 11a - halotetracyclines appear to be of only slight biological activity when tested *in vitro* against test organisms such as *Klebsiella pneumoniae*.

Employing the procedures hereinbefore described, the above 11a-halo compounds are converted to the following products:

7 - bromo - 11a - chlorotetracycline - 6,12 - hemi - ketal
 7 and 9 - nitro - 11a - chlorotetracycline - 6,12 - hemi - ketal
 7 - iodo - 11a - chlorotetracycline - 6,12 - hemi - ketal
 7 - chloro - 6 - demethyltetracycline
 7,11a - dichlorotetracycline - 6,12 - hemi - ketal
 7, - fluoro - 6 - demethyltetracycline

25 and corresponding non - ketalized compounds where applicable. The 11a - halogen atoms may be removed by the methods described herein.

DEHYDRATION

Of particular value are the 11a - halo hemi - ketals of Structure II in which substituent A is a methyl group and X is chlorine or fluorine. These compounds are converted to substances of a surprisingly high *in vivo* biological activity against disease causing microorganisms which makes them valuable for use in human therapy. These compounds on *in vitro* testing show only moderate albeit significant activity. The conversion is accomplished by treating the starting compound (II) with a strong acid of the dehydrating type. Although a number of such acids may be used for this purpose, it is preferred to use mineral acids, such as concentrated sulfuric acid, phosphoric acid, polyphosphoric acid, perchloric acid, as well as other dehydrating acids, such as glacial acetic acid (containing boron trifluoride), hydrogen fluoride (preferably in liquid form), and trihalo acetic acids, e.g. trifluoroacetic acid. It is also preferred to employ the mineral acids in concentrated form, e.g. at least 60% aqueous acids. When the mineral acids are employed, particularly advantageous results are obtained with acids having the following concentrations:

sulfuric acid 90 to 95%
 phosphoric acid 80 to 85%
 perchloric acid 60 to 70%

45 Reaction time and temperature do not appear to be critical. In carrying out this process, the starting compound is added to the selected acid and allowed to stand for relatively short periods of time. For example, the starting compound is added to hydrogen fluoride, preferably liquid hydrogen fluoride, at 0° to 50°C. and allowed to stand for 5 to 7 minutes, and even longer, e.g. up to several hours, after which the hydrogen fluoride is allowed to evaporate. The residue is then treated by standard procedures to obtain a crystalline product as the hydrofluoride salt, e.g. stirred in a non - solvent and recrystallized from solvents such as lower alkanols. When sulfuric acid is used it is preferred to employ temperatures below 20°C. When perchloric acid is employed, the reaction is preferably carried out at temperatures of from about 50°C. and higher preferably from 60°C. to 70°C. More highly concentrated perchloric acid may be used in this process but its use is not preferred because of the explosion hazard with which those skilled in the art are familiar. The reaction time does not appear to be critical since the reaction appears to be almost instantaneous. For example, the reaction time of from 5 to 15 minutes is usually found to give excellent results when the reaction temperature is in the range of 60 to 70°C. Heating for longer periods of time, while not harmful, provides no appreciable advantage and for this reason is not used. At lower temperatures, slightly longer reaction periods

discuss



may be required to obtain substantial yield of the product. When trifluoro acetic acid or acetic acid are used, the reaction is preferably carried out at room temperature for a period of about 24 hours. After the reaction is complete, the product is obtained by standard procedures. For example, it is most convenient to merely dilute the reaction mixture with non-solvent, e.g. water which results in precipitation of the product as the resulting acid addition salt. The product of the acid dehydration may be converted from the resulting acid addition salt to the free base or any desired salt in the usual manner. When the starting compound for this process is an 11a-fluoro compound, best results are obtained using perchloric acid.

The products of the dehydration process are 11a-halotetracyclines of Structure III which are named 6-deoxy-6-demethyl-6-methylene-11a-halotetracyclines. The compound in which Z is H, as the mineral acid salt on infrared analysis (KBr pellet) shows carbonyl absorption at or near 5.70 microns. This absorption as previously mentioned is lacking in the 11a-halo hemi-ketal starting compound. Further, there is a dramatic shift in ultraviolet absorption maxima (measured in 0.01N methanol HCL) from the vicinity of 345 m μ (which is characteristic of the hemi-ketal starting compounds) to the vicinity of 375 m μ (which is characteristic of the dehydrated products). The dehydrated products also show two additional ultraviolet absorption maxima which are respectively about 4.2 and 5 times more intense than that at 375 m μ . The two additional peaks are found at or about 240 and 277 m μ respectively. The previously mentioned characteristic peak at about 375 m μ in the case of compounds in which Z is H occurs at about 380-390 m μ in the case of compounds in which Z is chloro or bromo.

Additional 11a-halo-6-methylenetetracyclines are prepared from D-ring substituted 11a-halotetracycline-6, 12 hemi-ketals in the same manner. The D-ring substituted hemi-ketals are prepared by methods hereinbefore described. Thus are prepared 7 and/or 9-substituted-6-methylene-11a-halotetracyclines in which the substituent is halogen, nitro or amino.

The 11a-halo-6-demethyltetracyclines on reaction with hydrogen fluoride, perchloric acid or other strong mineral acid (hydrochloric or sulfuric) are converted to 11a-halo-5a,6-anhydro-6-demethyltetracyclines which may also be prepared by direct halogenation of 5a,6-anhydro-6-demethyltetracyclines by the halogenation methods previously described. The 11a-halo-6-demethyl-5a,6-anhydrotetracyclines are particularly useful intermediates which may be reduced to 11a-halo-6-deoxy-6-demethyltetracyclines, for example, by controlled reduction with hydrogen over platinum catalysts such as platinum oxide in a lower alkanolic acid solvent e.g. acetic acid. The 11a-chloro and fluoro products are then subject to reduction, chemical or catalytic, as herein described to obtain 6-deoxy-6-demethyltetracyclines. 11a-Fluoro-6-demethyltetracyclines are preferred for this reaction sequence. The 11a-halo-6-methylene tetracyclines of structure III are useful for further substitution reactions, e.g. D-ring substitution reactions such as those previously described for the 11a-halo hemi-ketals of structure II, i.e. nitration and halogenation. Further, compounds of structure III, in which E is hydrogen, also undergo substitution reactions in the 6-methylene group. In all of such substitution reactions where mixtures of compounds are produced, the mixtures may be separated into their components and the desired products recovered by conventional procedures, e.g. countercurrent distribution, column chromatography or combinations thereof.

HALOGENATION OF STRUCTURE III COMPOUNDS

For example, halogenation of structure III compounds, by methods previously described under "11a-Halogenation" of compounds of structure I, produces compounds of structures IV and VIII. Starting with compounds of structure III, in which E is OH, halogenation occurs principally at the 7-position, with some substitution also occurring at the 9-position, and to a lesser extent at both the 7 and 9 positions. Starting with compounds of structure III in which E is hydrogen, halogenation occurs principally in the 6-methylene group, and to a lesser extent at the 9-position and/or both positions.

NITRATION OF STRUCTURE III COMPOUNDS

Nitration of structure III compounds produces compounds of structures VI and X. The nitration is accomplished using the same procedures previously described for nitration of structure II compounds. Nitration of structure III compounds in which E is OH produces principally 9-nitro compounds, and to a lesser extent 7-nitro and 6-nitromethylene compounds. In nitration of structure III compounds in which

E is hydrogen, nitration occurs principally on the 6 - methylene group, as well as at the 9 - position, and to a lesser extent at the 7 - position.

NITRATION OF STRUCTURE IV AND VIII COMPOUNDS

Nitration of structure IV and VIII compounds produces compounds of structures V and IX, respectively. The nitration is accomplished using the same procedures hereinbefore described.

11a - DEHALOGENATION

The compounds of structures III, IV, V, VI, VIII, IX and X, which form the subject matter of divisional application No. 36489/63 (Serial No. 995,033) and are hereinafter referred to as 6 - methylene - 11a - chloro and fluoro tetracyclines are intermediates for the production of corresponding 11a - deschloro and fluoro, i.e. 6 - methylenetetracyclines (compounds of structures VII and XI), the compounds of this invention.

Although such structures of some of these compounds appear to be isomeric with known 5a,6 - anhydrotetracyclines, they appreciably differ, particularly in their broader spectrum of activity against varieties of microorganisms, higher activity against individual organisms and particularly against antibiotic resistant organisms. Further, the present compounds provide animal protection against disease - causing organisms which property is not shared by the known 5a,6 - anhydro compounds as is well known.

The chlorination or defluorination reaction may be accomplished by standard methods used for the removal of chlorine or fluorine including both chemical and catalytic reduction methods. One such catalytic reduction is accomplished by treatment with hydrogen gas over a noble metal catalyst, e.g. palladium or rhodium catalysts at pressures ranging from atmospheric to 1000 atmospheres and temperature ranges from 0 to 100°C. A variety of chemical methods may be used. One such method involves reduction with alkali metal hydrosulfites. The starting compound is added to an aqueous solution of the selected hydrosulfite and allowed to stand at room temperature which is found most convenient for this reaction. Additional methods for removal of 11a - chlorine and fluorine suggest themselves to those skilled in the art, including:

- (a) treatment with dilute aqueous hydriodic acid,
- (b) treatment with zinc metal in the presence of a proton donor, such as aqueous acetic acid or hydrochloric acid.

It has been surprisingly found that 11a - chloro and fluorotetracyclines may be readily dehalogenated by simultaneous treatment of the 11a - chloro and fluoro compound with zinc and sodium iodide in a halogen - acceptor solvent (preferably a lower alkanone, e.g. acetone optionally in the presence of zinc at room to reflux temperature, using from equivalent amount up to excesses of 1000 mole - percent and higher of the reagents. At reflux temperature, reaction is complete in as little as 30 minutes. After reaction is completed, the product is obtained by filtration and concentration of the reaction mixture. Under the conditions of the reductive removal of 11a-chlorine or fluorine as above described, nitro groups are reduced to amino groups to produce the corresponding amino derivatives defined in structures VII and XI.

In the conversion of the 11a - halotetracycline - 6,12 - hemi - ketals to the 11a - halo - 6 - methylenetetracyclines and thence to 6 - methylenetetracyclines the 11a - chlorotetracycline hemi - ketals are preferred, since the 11a - chloro - 6 - methylene tetracycline compounds generally appear to be quite stable and readily lend themselves to dehalogenation. The 11a - fluoro compounds are less readily dehalogenated.

The 11a - deshalo compounds, i.e. 6 - methylenetetracyclines are of a surprisingly high order of activity when tested *in vitro* against a wide variety of disease causing organisms and are found to be particularly effective against antibiotic resistant strains of microorganisms.

The following table summarizes the activity of 6 - methylenetetracycline against a variety of disease - causing microorganisms including antibiotic - resistant strains. The minimum inhibitory concentration (MIC) is determined by the well known serial-dilution technique. Included in the table are the MIC'S of 11a - chloro - 6 - methyl - enetetracycline and 6 - deoxytetracycline. It is noted that the MIC values for 6-methylenetetracycline are generally lower than those of 6 - deoxytetracycline indicating greater activity, especially against *Micrococcus pyogenes* var. *aureus* 400, a tetra - cycline - resistant organism.



Amounts

Chlorotetracycline
tetracyclines



TABLE I

1. 6-methylenetetraacycline
2. 6-deoxytetraacycline
3. 11a-chloro-6-methylenetetraacycline

Organism	MIC (mcg./ml.)		
	1.	2.	3.
<i>Micrococcus pyogenes</i> var. <i>aureus</i>	<0.19	0.78	50 (25 p.)
<i>Streptococcus pyogenes</i>	<0.19	0.39	12.5
<i>Streptococcus faecalis</i>	<0.19	0.78	25
<i>Diplococcus pneumoniae</i>	<0.19	3.12	25
<i>Erysipelothrix rhusiopathiae</i>	<0.19	0.39	—
<i>Corynebacterium diphtheriae</i>	0.78	3.12	>100
<i>Listeria monocytogenes</i>	0.19	6.25	25
<i>Bacillus subtilis</i>	<0.19	0.01	3.12
<i>Lactobacillus casei</i>	0.78	25	100 (50 p.)
<i>Bacterium ammoniagenes</i>	<0.19	0.78	12.5
<i>Aerobacter aerogenes</i>	3.12	6.3	>100
<i>Escherichia coli</i>	1.56	6.3	>100
<i>Proteus vulgaris</i>	12.5	100	>100
<i>Pseudomonas aeruginosa</i>	25.0	100	>100
<i>Salmonella gallinarum</i>	3.12	12.5	>100
<i>Salmonella pullorum</i>	0.78	3.12	100 (p)
<i>Klebsiella pneumoniae</i>	1.56	6.3	>100
<i>Neisseria gonorrhoeae</i>	<0.19	0.78	12.5
<i>Hemophilus influenzae</i>	<0.19	0.09	6.25
<i>Shigella sonnei</i>	3.12	3.12	—
<i>Brucella bronchiseptica</i>	<0.19	0.19	6.25
<i>Malleomyces mallei</i>	0.39	3.12	25
<i>Vibrio comma</i>	<0.19	0.19	6.25
<i>Pasteurella multocida</i>	<0.19	0.39	25
<i>Streptococcus adalactiae</i>	<0.19	—	12.5
<i>Mycobacterium 607</i>	<0.19	0.19	0.78
<i>Mycobacterium berolinense</i>	<0.19	100	0.39
<i>Candida albicans</i>	50	100	>100
<i>Sarcina lutea</i>	<0.19	—	100
	1.	2.	3.

Antibiotic resistance strains of *Micrococcus pyogenes* var. *aureus*

376 *	6.25	6.3	>100
400 **	0.78	3.12	50

* Resistant to tetracycline at a concentration below 100 mcg./ml.

** Resistant to tetracycline at a concentration below 50 mcg./ml.

The following table summarizes the *in vitro* activity of further representative compounds of the present invention.

TABLE II

1. 6-Deoxy-6-demethyl-6-methylene-5-oxytetracycline
2. 7-Chloro-6-deoxy-6-demethyl-6-methylene-5-oxytetracycline

Organism	MIC (mcg/ml)	
	1	2
<i>Micrococcus pyogenes</i> var. <i>aureus</i> 5	0.78	0.25
<i>Streptococcus pyogenes</i>	0.78	0.03
<i>Streptococcus faecalis</i>	0.39	0.6
<i>Diplococcus pneumoniae</i>	0.39	0.06
<i>Erysipelothrix rhusiopathiae</i>	0.39	0.125
<i>Corynebacterium diphtheriae</i>	3.12	1.0
<i>Listeria monocytogenes</i>	0.19	0.19
<i>Bacillus subtilis</i>	0.09	0.01
<i>Lactobacillus casei</i>	0.78	—
<i>Bacterium Ammoniagenes</i>	0.39	0.19
<i>Streptococcus pyogenes</i> 98	0.09	0.01
<i>Micrococcus pyogenes</i> var. <i>aureus</i> 209p	0.25	0.25
<i>Aerobacter aerogenes</i>	6.3	1.56
<i>Escherichia coli</i>	3.12	0.78
<i>Pseudomonas aeruginosa</i>	100	3.12
<i>Salmonella gallinarum</i>	12.5	1.56
<i>Salmonella pullorum</i>	3.15 (p)	0.78
<i>Klebsiella pneumoniae</i>	3.15 (p)	0.78
<i>Neisseri gonorrhoeae</i>	0.19	0.06
<i>Hemophilus influenzae</i>	0.19	0.03
<i>Shigella sonnei</i>	3.12	0.39
<i>Brucella bronchiseptica</i>	0.39	0.25
<i>Malleomyces mallei</i>	1.56	0.25
<i>Vibrio comma</i>	0.78	3.12
<i>Pastuerella multocida</i>	0.19	0.5
<i>Sarcina lutea</i>	0.78	—
<i>Streptococcus agalactiae</i>	0.39	—
Antibiotic resistant strains of <i>Micrococcus pyogenes</i> var. <i>aureus</i> :		
376	100	100
400	50	100
(p) = partial inhibition		

When these *in vitro* tests were repeated in the presence of human serum, similar results were observed. The following table summarizes the activity of the aforementioned compounds of Table II when tested in 20% human serum:

	MIC (mcg/ml)	
	1	2
<i>Micrococcus pyogenes</i> var. <i>aureus</i> 5	0.78	0.39
<i>Streptococcus pyogenes</i>	0.78	0.09
<i>Streptococcus pyogenes</i> 98	—	0.19
<i>Micrococcus pyogenes</i> var. <i>aureus</i> 209p	—	0.39

It is noted that 7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline has greater *in vitro* activity against a large number of organisms than 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline.

When 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline was tested *in vivo* through both the oral and parenteral route, it showed activity greater than tetracycline or 5 - oxytetracycline against infection produced with tetracycline - sensitive microorganisms. The PD_{50} (PD = protective dose) for the present new compound against an infection produced with *Micrococcus pyogenes* var. *aureus* 5 is 3.2 mg/kg. (orally) and 0.34 mg/kg. (parenterally). The corresponding PD_{50} for tetracycline is 6.4 mg/kg. (orally) and 0.78 mg/kg. (parenterally).

The present new 6 - methylene compounds of structure VII and XI may be formulated into various therapeutic compositions each comprising the compound in a pharmaceutically acceptable carrier therefore. They are useful in human therapy, and they are useful therapeutically in feeds or as growths stimulants, in veterinary practice and in agriculture.

For human therapy, the usual oral dosage of the present 6 - methylene tetracyclines is from 0.1 to 2 g. per day for the average adult. The products are 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 having concentrations ranging from 5 to 125 mg./ml. For parenteral administration intramuscularly or intravenously, the daily dose is reduced to 0.1 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 product are conveniently distributed as solid compositions for reconstitution. 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.

It is specifically intended to include within the purview of this invention the acid and base salts of the present new amphoteric tetracyclines.

In the case of the therapeutically active tetracyclines, salts formed with pharmaceutically acceptable acids and bases are suitable for administration to a host in suitable dosage form. Those salts formed with pharmaceutically unacceptable acids and bases are of value in the isolation and purification of the present new tetracyclines and also in the preparation of pharmaceutically acceptable salts.

The following examples are given by way of illustration of the preparation of the starting materials and products of this invention.

EXAMPLE I

11a - Fluorotetracycline - 6,12 - hemi - ketal

To a suspension of 20 g. of tetracycline base in 800 ml. of water cooled to 0°C. is added 45 ml. (2 equivalents) of 2N Sodium hydroxide solution. The tetracycline dissolves to yield a solution, the pH being approximately 11. Perchloryl fluoride is then bubbled through the stirred solution (maintained under a nitrogen atmosphere) until the pH of the mixture approximates 7. A heavy precipitate starts to form between pH 8 and 8.5. The excess perchloryl fluoride is flushed out with a stream of nitrogen and the nearly white crystalline material is filtered off, washed with water and dried under vacuum at room temperature to obtain 7.9—8.5 g. of product. Ultraviolet absorption shows maxima at 267 and 340 m μ ; infrared shows no carbonyl absorption below 6 microns. Bioassay against *K. pneumoniae* shows an activity of 8 mcg./mg. on the tetracycline scale. Elemental analysis gives the following results:

Calcd. for: $C_{22}H_{23}N_2O_8F.H_2O$; C, 54.95, H; 5.20, N, 5.83
Found: C, 54.97, H; 5.19, N; 5.85.

EXAMPLE II

11a - Fluoro - 5 - hydroxytetracycline - 6,12 - hemi - ketal

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 336 m μ . Elemental analysis gives the following results after recrystallization of the product from water.

Calcd. for: C₂₂H₂₃O₉N₂F.2H₂O: C, 51.4; H, 5.25; N, 5.5

Found: C, 51.2; H, 5.3; N, 5.7.

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EXAMPLE III

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11a - Chlorotetracycline - 6,12 - hemi - ketal

To a solution of 2.2 g. of anhydrous tetracycline in 25 ml. of monoglyme (dimethyl ether of ethylene glycol) is added 800 mg. of N - chlorosuccinimide with stirring to dissolve the reagent. The mixture is allowed to stand for seven minutes and then diluted with water (25 ml.). The product, 873 mg., crystallizes as white needles. Bioassay of the product shows a tetracycline activity of about 4 mcg./mg. against *K. pneumoniae*. Infrared analysis shows no carbonyl bands between 5 and 6 microns.

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Ultraviolet absorption shows maxima at 267 and 340—342 m μ .

~~Ultraviolet absorption shows maxima at 267 and 340—342 m μ .~~

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On treatment of this product with sodium hydrosulfite in aqueous dimethylformamide at room temperature, tetracycline is regenerated. Bioassay of the reaction mixture shows a tetracycline activity of 520—665 mcg./mg. (*K. pneumoniae*).

The crystalline hydrochloride of this product is obtained by dissolving it in excess aqueous HCl (pH ca. 1) and freeze drying the mixture.

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EXAMPLE IV

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7,11a - Dichlorotetracycline - 6,12 - hemi - ketal

A mixture of 2.4 g. of anhydrous 7 - chlorotetracycline, 800 mg. of N - chlorosuccinimide, and 25 ml. of 1,2 - dimethoxyethane is stirred for 2 1/2 minutes, after which 100 ml. of ether is added, followed by 300 ml. of hexane. The precipitate thus formed is collected by filtration, washed with hexane and dried.

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In similar fashion, 7 - bromo - 11a - chlorotetracycline - 6,12 - hemi - ketal is prepared from 7 - bromotetracycline.

EXAMPLE V

11a - Chloro - 5 - hydroxytetracycline - 6,12 - hemi - ketal

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Twenty - three grams of anhydrous oxytetracycline is dissolved in 250 ml. of 1,2 - dimethoxyethane 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 dried. Infrared analysis of the product (KBr at 1% concentration) shows no carbonyl absorption in the 5—6 micro 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 and 9.43 microns. Bioassay of the product shows a tetracycline activity of 4 mcg./mg.

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EXAMPLE VI

Acid salts of 11a - halotetracycline - 6,12 - hemi - ketals

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The hydrochloride salts of the above - described 11a - halo compounds are prepared by dissolving the free base in water containing an equimolar amount of hydrogen chloride and freeze - drying the resulting solution.

In similar fashion, salts are formed with sulfuric, hydrobromic and phosphoric acids.

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EXAMPLE VII

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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 triturated in ether to obtain the solid product, 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline, as the hydrofluoride salt which is recrystallized from methanol.

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Alternatively, the crude hydrofluoride product (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:

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Calcd. for:

C₂₂H₂₂O₇N₂Cl₂; C, 53.11; H, 4.56; N, 5.63; chloride 7.13

Found: C, 52.62; H, 4.63; N, 5.54; chloride 6.84.

- Infrared analysis of the product as the hydrochloride salt in a KBr pellet at 1% concentration shows carbonyl absorption at $5.70\ \mu$ 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 $242\ m\mu$. The product shows an R_f value of from 0.2 to 0.3 in the following system:

	<i>Mobile Phase</i>	<i>Immobile Phase</i>	
10	20:3 toluene - pyridine saturated with pH 4.2 buffer	pH 4.2 buffer (aqueous)	10

- On analysis of the papergram with ultraviolet light, the product spot does not fluoresce strongly. However, on spraying with aqueous sodium hydrosulfite, it shows strong fluorescence.
- When the product is boiled in methanolic - conc. HCl, mixture the unchanged product is recovered. Under the same conditions, the starting compound is converted to a compound presumed to be 11a - chlorisotetracycline which on treatment with aqueous sodium hydrosulfite yields isotetracycline.

EXAMPLE VIII

- 11a - Fluoro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline
- 11a - Fluorotetracycline - 6,12 - hemi - ketal, 250 mg. is stirred in 2 ml. of 63% aqueous perchloric acid. The solid dissolved on warming to $60-65^\circ\text{C}$. 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 perchlorate salt. The product shows the same absorption on ultraviolet and infrared analysis as that of Example XI.

EXAMPLE IX

- 11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline
- Five grams of 11a - chloro - 5 - oxytetracycline - 6,12 - hemiketal is added to 15 ml. of dry, liquid hydrogen fluoride, and the mixture is stirred for 3.5 hours at ice-bath temperature. The hydrogen fluoride is evaporated off by warming under a flow of nitrogen gas to obtain the product as the hydrofluoride salt.
- The crude hydrofluoride product is dissolved in water and conc. HCl or perchloric acid (70%), is added dropwise to precipitate the hydrochloride or perchlorate salts respectively. The hydriodide salt is precipitated from acetone solution of the crude hydrofluoride 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 sulfonic acid (concentrated acid) is added to precipitate the respective salt. Dilution with acetone of the original reaction mixture followed by addition of HCl, precipitates the hydriodide salt.
- The hydriodide salt, on elemental analysis, shows the following values:
- Calcd. for: $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_8\text{Cl HI}$: 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.
- Ultraviolet analysis shows the following maxima: 222, 270 and $372\ m\mu$.
- 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 perchlorate salt on ultraviolet analysis shows maxima at 237, 270 and $372\ m\mu$.

EXAMPLE X

- 11a - Fluoro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline is prepared in the same manner as in Example IX using 11a - fluoro - 5 - oxytetra - cycline - 6,12 - hemiketal as the starting compound.

EXAMPLE XI

- 11a - Chloro - 6 - Methylenetetracycline
- The starting compound of Example VII is dissolved in 94% sulfuric acid (1 gram in 10 ml.) and the mixture allowed to stand for 2 hours at 10°C . The product is obtained as the sulfate salt by pouring the mixture into several volumes of diethyl ether and filtering.

EXAMPLE XII

11a - Chloro - 6 - Methylenetetraacycline

The product of Example XI is obtained as the phosphate salt by using 85% phosphoric acid in lieu of sulfuric acid under the same conditions as described in that example.

EXAMPLE XIII

Preparation of Mineral Acid Salts of

11a - halo - 6 - methylenetetraacyclines

11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline hydrofluoride is dissolved in water and the solution adjusted to pH 5. The resulting amphoteric compound which precipitates is filtered and dried. The amphoteric compound is dissolved in methanol containing a molar equivalent of hydrogen chloride and the hydrochloride salt is precipitated by the addition of ether. This hydrochloride salt is also prepared by the method of Example XI. Other mineral acids may be substituted for hydrochloric acid in these procedures to obtain the corresponding salt.

Using similar procedures, the hydrochloride, hydrobromide, sulfate, hydriodide, nitrate, and phosphate salts of the 11a - halo - 6 - methylene - tetraacyclines of the previous examples are prepared.

In like manner, other salts are formed using a variety of acids, for example, organic carboxylic acids such as tartaric, citric, malic, benzoic, glycolic, gluconic, gulonic, succinic, or acetic acid. The salts formed with pharmaceutically - acceptable acids are useful for therapy; those with pharmaceutically - unacceptable acids are useful in the purification of the products and in the preparation of pharmaceutically acceptable salts.

EXAMPLE XIV

Dehalogenation of Example XI product

The product of Example VII (5 mg.) is dissolved in 3ml. of methanol and a freshly prepared solution of sodium hydrosulfite (20 mg. in 20 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 - methylenetetraacycline. The product is crystallized from acetonitrile. 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 XI, and follows the solvent front in the following system:

Mobile Phase

20:10:3 nitromethane:chloroform:pyridine

Immobile Phase

pH 3.5 buffer (aqueous)

EXAMPLE XV

The procedure of Example XIV repeated to dechlorinate the corresponding 11a-chloro - 6 - methylenetetraacyclines to provide the following products which correspond to that of Example XIX on ultraviolet and infrared analysis (infrared analysis shows a lack of carbonyl absorption by virtue of no maximum at or about 5.7 microns):

7 - Bromo - 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline

7 - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline

5 - Hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline

EXAMPLE XVI

6 - Methylenetetraacycline

A mixture of 250 mg. of the Example VII product (11a - chloro - 6 - methylenetetraacycline - hydrochloride) and 100 mg. of zinc in 10 ml. of acetone is stirred for 15 minutes at room temperature, after which 5 ml. of 5% HCl is added. The mixture is filtered and stripped of acetone. 6 - methylenetetraacycline - hydrochloride separates from the concentrated reaction mixture and is collected by filtration.

This procedure was repeated using, as the solvent system, 5 ml. of HCl and 5 ml. of acetone, with comparable results.

EXAMPLE XVII

6 - Methylenetetraacycline

250 mg. of the Example VII product (11a - chloro - 6 - methylenetetraacycline - hydrochloride) is refluxed for 2 hours. The reaction mixture is cooled, filtered, and concentrated to obtain the product,



2



1 product of

of

1 and 250 mg of sodium iodide in 25ml

EXAMPLE XVIII

6 - Methylene-tetracycline

250 mg. of the Example VII product (11a - chloro - 6 - methylenetetracycline - hydrochloride) is refluxed in 50 ml. of acetone for 24 hours. The mixture is cooled, filtered clear, and concentrated under reduced pressure to obtain the product as the hydrochloride salt.

EXAMPLE XIX

The hydrochloride, hydrobromide, sulfate, hydriodide and phosphate salts of the 6 - methylenetetracyclines are prepared according to the methods described in Example XIII.

EXAMPLE XX

The sodium salt of 6 - methylenetetracycline is prepared by dissolving the tetracycline compound in water containing a molar equivalent amount of sodium hydroxide. The solution is freeze - dried to obtain the sodium salt.

Similarly, other alkali and alkaline earth metal salts of the present new 6 - methylene compounds are prepared; including the potassium, lithium, barium, calcium, strontium and magnesium salts.

In similar fashion, other salts are formed using a variety of acids and bases, for example, organic carboxylic acids such as tartaric, citric, malic, benzoic, glycollic, gluconic, gulonic, succinic, or acetic acid, as well as both organic (amines) and inorganic bases. The salts formed with pharmaceutically - acceptable acids or bases are useful for therapy; those with pharmaceutically unacceptable acids or bases are useful in the purification of the new products and in the preparation of pharmaceutically acceptable salts.

EXAMPLE XXI

A suspension of 6 - methylenetetracycline is prepared with the following composition:

antibiotic	31.42 g.
70% aqueous sorbitol	714.29 g.
Glycerine, USP	187.35 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

To this suspension, various sweetening and flavouring agents, as well as acceptable colors, may be added by choice. The suspension contains approximately 25 mg. of antibiotic activity per milliliter.

EXAMPLE XXII

A solution of 6 - methylenetetracycline is prepared with the following composition:

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

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

EXAMPLE XXIII

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

Into this base there is blended sufficient 6 - methylenetetracycline to provide tablets containing 25, 100 and 250 mg. of active ingredient.

EXAMPLE XXIV

A blend is prepared containing the following ingredients:

		Grams	
5	Calcium carbonate USP	17.6	
	Dicalcium phosphate	18.8	5
	Magnesium trisilicate USP	5.2	
	Lactose USP	5.2	
	Potato starch	5.2	
	Magnesium stearate A	0.8	
10	Magnesium stearate B	0.35	10

To this blend is added sufficient 6 - methylenetetraacycline to provide capsules containing 25, 100, and 250 mg. of active ingredient respectively.

EXAMPLE XXV

One thousand grams of 6 - methylenetetraacycline are intimately mixed and ground with 2500 grams of ascorbic acid. 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.

The procedures of Examples XXI to XXV are repeated with other 6 - methyl - enetetraacyclines described in the preceding examples to form corresponding dosage forms.

EXAMPLE XXVI

6 - Deoxy - 6 - demethyl - 6 - methylenetetraacycline

A solution of 50g. of 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - tetraacycline 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 50g. 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. 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 - methylenetetraacycline 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 XXVII

6 - Deoxy - 6 - demethyl - 6 - methylenetetraacycline

11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline is also effectively dechlorinated to produce 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline with the following respective reagents:

50	Aqueous phenylhydrazine acetate	
	Aqueous ferrous sulfate	
	Sodium formaldehyde sulfoxalate	
	Hypophosphorous acid and palladium black	
	Iron powder in dimethylformamide	50
	Raney nickel in 50% aqueous acetic acid.	

As would be apparent to those skilled in the art, the 6 - methylenetetraacyclines and the 11a - chloro and fluoro - 6 - methylenetetraacyclines may be partially converted to their C.4 epimers under many conditions, particularly at pH's between 2 and 6 and in such solvents as glacial acetic acid. In practice, 6 - methylenetetraacycline and 11a - halo - 6 - methylenetetraacycline as isolated by the procedures described herein may contain small amounts — i.e. less than 20% — of their C.4 epimers. 5 - Hydroxy - methylenetetraacyclines are more resistant to C.4 epimeriza-

tion. The C.4 epimers contained in the compounds of this invention may be isolated from mixtures using standard procedures such as by paper chromatography or by counter - current distribution techniques. The essentially pure C.4 epi compounds may be reconverted to the normal, more active form by procedures well known to the art, for example, by treatment with glacial acetic acid.

EXAMPLE XXVIII

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

Method A

To a solution of 5 g. of the Example IX 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 ether. The ether - insoluble residue is crystallized from methanol - acetone - conc.HCl - ether 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 $\lambda_{\max}$ 252 m μ , $E_{1\text{cm}}^{1\%}$ 450 and $\lambda_{\max}$ 345 m μ , $E_{1\text{cm}}^{1\%}$ 302; in 0.01N NaOH in methanol, $\lambda_{\max}$ 235 m μ , $E_{1\text{cm}}^{1\%}$ 442; $\lambda_{\max}$ 254 m μ , $E_{1\text{cm}}^{1\%}$ 408; $\lambda_{\max}$ 385 m μ , $E_{1\text{cm}}^{1\%}$ 329; and λ_{infl} 280 m μ , $E_{1\text{cm}}^{1\%}$ 329; in 0.01N MgCl₂ in methanol, $\lambda_{\max}$ 240 m μ , $E_{1\text{cm}}^{1\%}$ 461; $\lambda_{\max}$ 277 m μ , $E_{1\text{cm}}^{1\%}$ 326; and $\lambda_{\max}$ 351 m μ , $E_{1\text{cm}}^{1\%}$ 282.

Infrared 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. pneumoniae turbidimetric assay with oxytetracycline as standard). Elemental analysis of the product 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 respectively in the following systems:

Mobile Phase	Immobile Phase
(1) 20:3 toluene - pyridine saturated with pH 4.2 buffer	pH 4.2 buffer (aqueous)
(2) 20:10:3 nitromethane, chloroform pyridine saturated with Ph 3.5 buffer	pH 3.5 buffer (aqueous)

Method B

A mixture of 1 g. of the Example XIII 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 XIII 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 XXIX

7,11a - Dichloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - hydroxytetracycline
Method A

To 5 g. of 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxy - tetracycline hydriodide 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.

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 resulting 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

$\lambda_{\max} 239 \text{ m}\mu$, $E_{1\text{cm}}^{1\%}$, 352 $\lambda_{\max} 378 \text{ m}\mu$, $E_{1\text{cm}}^{1\%}$, and inflection $258 \text{ m}\mu$, $E_{1\text{cm}}^{1\%}$, 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 of 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, 100 ml. of water is added and then 5 g. of β - naphthalenesulfonic 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 hereinbefore. The crude perchlorate salt so obtained is crystallized as long needles from isopropanol, which on ultraviolet analysis shows maxima at 260 and 377 $\text{m}\mu$ and an inflection at 260 $\text{m}\mu$.

Infrared analysis shows peaks at 5.7, 6.0, 6.26, 6.55, 6.88, 7.2, 7.85 and 8.35 microns.

EXAMPLE XXX

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

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 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 mixture 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 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, 7.43, 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 $\text{m}\mu$ (log ϵ 4.28) and 346 $\text{m}\mu$ (log ϵ 4.02) and an inflection at 370 $\text{m}\mu$ (log ϵ 3.98); in 0.01N NaOH in methanol, maxima at 234 $\text{m}\mu$ (log ϵ 4.24), 253 $\text{m}\mu$ (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:

5	Rf Value	Solvent System		5
		Mobile Phase	Immobile Phase	
	0.35	ethyl acetate	aqueous phosphate	
	0.33	saturated with water	buffer (pH—3)	
		ethyl acetate	McIlvaines buffer,	
10		saturated with water	pH 4.2	10

Bioassay (K. pneumonia - oxytetracycline standard) gives a value of 6000 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 ultraviolet 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 XXVIII.

EXAMPLE XXXI

20 7 - Bromo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline 20

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

EXAMPLE XXXII

25 7 - Bromo - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline 25

This product is obtained from the Example XXXI product by the sodium hydro-sulfite treatment described in Example XXX.

30 The following compounds are prepared from the corresponding 11a-halo compounds by known procedures, such as methods A and B of Example XXIX 30

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 - oxytetra - cycline

35 These compounds are converted to the corresponding 11a-deshalo compounds by the aforementioned procedures. 35

EXAMPLE XXXIII

40 11a - Chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetra- cycline 40

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 12 hours, then evaporated to $\frac{1}{2}$ of the original volume and 200 ml. of ether is then added. The product separates as the nitrate salt and is collected by filtration.

45 Similarly, the corresponding 11a - fluoro compound is prepared. 45

EXAMPLE XXXIV

50 9 - Amino - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline 50

The product of the previous example is hydrogenated, according to the reduction procedures previously described, to obtain the 9 - amino - 6 - deoxy - 6 - demethyl- 6 - methylene - 5 - oxytetracycline.

EXAMPLE XXXV

55 7,11a - Dichloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 methylene - 5 - oxytetra - cycline 55

One gram of 7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline hydrochloride is dissolved in 20 ml. of acetic acid and 1 ml. of conc. HNO₃ is added. The mixture is allowed to stand for 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 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.

5 Using the same procedure, the following products are obtained from corresponding starting compounds: 5

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 - 5 - oxytetracycline
 10 7 - chloro - 11a - fluoro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline 10

The products are converted to 7 - bromo and 7 - chloro - 9 - amino - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline by the reduction procedures previously described. 15

The compounds are chemically (Zn in dil. HCl) or catalytically (Rhodium and hydrogen) reduced to 7 - chloro, and 7 - bromo - 9 - amino - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline. 15

EXAMPLE XXXVI

20 7 - Chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline 20
 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 R_f values in the solvent systems indicated: 25

R _f	Solvent System	
	Mobile Phase	Immobile Phase
0.2	ethylacetate saturated with water	aqueous phosphate buffer (pH=3)
0.55	nitromethane, toluene, pyridine (20:10:3)	McIlwain's buffer (pH=3.5)
30		30

Ultraviolet analysis in 0.01N NaOH — MeOH shows maxima at 248, 341 and 447 mμ and a shoulder at 275 mμ. Bioassay shows a value of less than 100 mcg/mg (K. pneumoniae turbidimetric assay with oxytetracycline standard). 35

EXAMPLE XXXVII

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

40 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 stirred at 5°C. for 15 minutes, then poured into 75 ml. of ice/water and finally filtered. The filtrate is evaporated to dryness to give the product. 40

EXAMPLE XXXVIII

45 9 - Nitro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline 45
 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 then evaporated to dryness. The residue is slurried in ether and filtered to obtain the crude product which is slurried in 5% aqueous HCl, filtered and extracted with butanol. The butanol extract is concentrated to obtain the product which is recrystallised from water with hydrochloric acid. 50

EXAMPLE XXXIX

55 Nitration of 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetra - cycline 55

A solution of 5g. of 11a - chlorotetracycline - 6,12 - hemiketal in 10 ml. of anhydrous hydrogen fluoride is allowed to stand for 15 minutes 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 6.1 g. of crude product.

Four grams of the crude product is slurried in 60 ml. of water and 4 ml. of conc. HCl is added with stirring. The crystalline precipitate is filtered, water - washed (part of precipitate dissolves). The water - insoluble product (1.9 g.) is 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - nitromethylene - tetracycline hydrochloride. The water washings are then concentrated to obtain 1.5 g. of a nitro compound which is isomeric with the aforementioned product.

EXAMPLE XL

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 (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 mμ; in 0.01N MeOH—NaOH, 264 and 380 mμ.

The product shows R_f values as follows:

Solvent System

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

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

EXAMPLE XLI

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 or SnCl₂ in hydrochloric acid.

EXAMPLE XLII

11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

To a mixture of 1.2 g. of the Example VII product in 15 ml. of trifluoroacetic acid is added 350 mg. of N - chlorosuccinimide and the mixture is heated at 60°C. After about 3 hours, the mixture gives a negative KI/starch test. The cooled mixture is added dropwise to 500 ml. of cold ether with stirring at ice - bath temperature. After stirring for 3 hours, the precipitated product is filtered, twice slurried in ether and dried to obtain 1 g. of product.

The product is dissolved in 300 ml. of hot methanol, filtered to remove a small amount of insoluble material and concentrated to a volume of 100 ml. Then 3 ml. of p - toluenesulfonic acid dissolved in methanol is added and the mixture permitted to stand at room temperature until crystallization is completed. The product is obtained as the p - toluene - sulfonate salt by filtering the precipitate, washing with methanol and dried.

Infrared analysis of the product shows a clear sharp curve with a band of 5.69μ.

Ultraviolet analysis in 0.01N methanolic HCl shows maxima 245 and 378 mμ.

Elemental analysis gives the following results:

Calcd. for:

C₂₉H₂₈O₁₀N₂Cl₂S (as the p - toluenesulfonate salt): C, 52.18; H, 4.2; N, 4.2; Cl, 10.6, S, 4.8

Found:

C, 51.8 ; H, 4.3; N, 4.3; Cl, 10.3; S, 4.8

EXAMPLE XLIII

11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetracycline

To a mixture of 4.8 g. of the Example VII product in 40 ml. of trifluoroacetic

acid is added a solution of 0.54 ml. of bromine in 10 ml. of acetic acid. The mixture is warmed at 40°—60°C. for one hour and then allowed to stand for 12 hours at room temperature. The crude product is obtained by the same workup as in the previous example. It is crystallized from methanol as the p - toluenesulfate salt (3.54 g.).

Ultraviolet analysis in 0.01N methanolic HCl shows maxima at 249 and 379 m μ .

EXAMPLE XLIV

11a - Chloro - 9 - bromo - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetraacycline
To a mixture of 280 mg. of the Example VII product in 5 ml. of trifluoroacetic acid is added 1.05 ml. of a solution of 0.53 ml. of bromine in 10 ml. of acetic acid. A heavy orange precipitate forms in the mixture, which is then stirred and warmed at 60°C. for 2 hours. The mixture is then allowed to stand for 48 hours and the product (287 mg.) then obtained by work - up with ether as previously described. The product is crystallized as the sulfate salt by dissolving in 5 ml. of methanol and adding 6 drops of conc. H₂SO₄.

Ultraviolet analysis of the product in 0.01N methanolic HCl shows maxima at 251 and 384 m μ .

EXAMPLE XLV

9,11a - Dichloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetraacycline

This product is prepared according to the procedure of Example XLII by using two molar equivalents of N - chlorosuccinimide.

EXAMPLE XLVI

Using the procedures of the previous examples, the following compounds are prepared from corresponding 11a - halo - 7 - chloro (or bromo) - 6 - deoxy - 6 - demethylenetetraacycline starting compounds:

7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetraacycline
7 - bromo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetraacycline
7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetraacycline
7 - chloro - 11a - fluoro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetraacycline
7 - bromo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - iodo methylenetetraacycline

EXAMPLE XLVII

11a - Chloro - 9 - ~~chloro~~ - 6 - deoxy - 6 - demethyl - 6 - methylenetetraacycline

A mixture of 1 g. of the Example VII 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 XLVIII

11a - Chloro - 6 - deoxy - 6 - demethyl - 6 - nitromethylenetetraacycline

One gram of the Example VII product is heated in slurry in 20 ml. of 5% aqueous nitric acid at 60°C. with stirring. The product crystallizes from the reaction solution as the nitrate salt and is collected by filtration followed by water - washing and drying.

EXAMPLE XLIX

9,11a - Dichloro - 6 - deoxy - 6 - demethyl - 6 - nitromethylenetetraacycline

This product is obtained from that of the previous example by chlorination using the procedure of Example XLII.

EXAMPLE L

11a - Chloro - 9 - nitro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetraacycline
This product is obtained from that of Example XLVII by the chlorination procedure of Example XLII.

EXAMPLE LI

Employing the procedures of Examples XLIX and L the following products are obtained from corresponding 11a-halo nitro compounds by the procedure of Example XLIX.

9 - bromo - 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - nitromethylenetetraacycline
9 - nitro - 11a - fluoro - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetraacycline
7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - nitromethylenetetraacycline





EXAMPLE LII

6 - Deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

To 1 mole of the Example XLII product in 25 ml of methanol is added 100 mg. of 5% rhodium on carbon. The mixture is hydrogenated with shaking at room temperature and one atmosphere of hydrogen gas until 1 mmole of hydrogen is taken up. After filtering off the catalyst, the solution is evaporated to dryness under reduced pressure. The residue is slurried in ether filtered and dried to obtain the product.

Using this procedure, the following compounds are prepared from the corresponding 11a - halo compounds described in previous examples. It is obvious that nitro compounds are reduced to amino compounds in this reaction, thus necessitating more than 1 mmole of hydrogen.

6 - deoxy - 6 - demethyl - 6 - bromomethylene tetracycline

9 - bromo - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetracycline

9 - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

7 - bromo - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

6 - deoxy - 6 - demethyl - 6 - aminomethylenetetracycline

9 - amino - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

9 - amino - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - aminomethylenetetracycline

WHAT WE CLAIM IS:—

1. A compound of structure VII or XI as set forth in the specification hereof.

2. 6 - Deoxy - 6 - demethyl - 6 - methylenetetracycline.

3. 7 - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

4. 7 - Chloro - 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

5. 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

6. 9 - amino - 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

7. 7 - Chloro - 9 - amino - 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

8. Salts of compounds of any of claims 1—7 with acids or bases.

9. A process for the preparation of a 6 - methylenetetracycline of structure VII or XI herein which includes the step of removing halogen from the 11a - position of an 11a - halo - 6 - methylenetetracycline, of structures III, IV, V, VI, VIII, IX or X herein.

10. A process according to claim 9 wherein the halogen is removed by treating the 11a - halo - 6 - methylenetetracycline starting compound with aqueous alkali metal hydrosulfite.

11. A process according to claim 9 wherein the halogen is removed by treatment with zinc in the presence of a proton donor.

12. A process according to claim 9 wherein the halogen is removed by catalytic hydrogenolysis.

13. A process according to claim 11, wherein the proton donor is an acid in aqueous solution.

14. A process according to claim 12 wherein halogen is removed by contacting the starting compound with hydrogen over a noble metal catalyst, preferably a rhodium or a palladium catalyst.

15. A process according to any one of claims 12, or 14, wherein hydrogenation is carried out at a temperature ranging from 0°C. to 100°C. and pressures ranging from atmospheric to superatmospheric pressures of hydrogen gas.

16. A process according to any one of claims 9—15 wherein the starting compound is 11a - fluoro or 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline and the product is 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline.

17. A process according to any one of claims 9—15 wherein the starting compound is 11a - fluoro or 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - tetracycline and the product is 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

18. A process according to any one of claims 9—15 wherein the starting compound is 7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline and the product is 7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline.



EXAMPLE LII

6 - Deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

To 1 mole of the Example XLII product in 25 ml of methanol is added 100 mg. of 5% rhodium on carbon. The mixture is hydrogenated with shaking at room temperature and one atmosphere of hydrogen gas until 1 mmole of hydrogen is taken up. After filtering off the catalyst, the solution is evaporated to dryness under reduced pressure. The residue is slurried in ether filtered and dried to obtain the product.

Using this procedure, the following compounds are prepared from the corresponding 11a - halo compounds described in previous examples. It is obvious that nitro compounds are reduced to amino compounds in this reaction, thus necessitating more than 1 mmole of hydrogen.

6 - deoxy - 6 - demethyl - 6 - bromomethylene tetracycline

9 - bromo - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetracycline

9 - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

7 - bromo - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

6 - deoxy - 6 - demethyl - 6 - aminomethylenetetracycline

9 - amino - 6 - deoxy - 6 - demethyl - 6 - chloromethylenetetracycline

9 - amino - 6 - deoxy - 6 - demethyl - 6 - bromomethylenetetracycline

7 - chloro - 6 - deoxy - 6 - demethyl - 6 - aminomethylenetetracycline

WHAT WE CLAIM IS:—

1. A compound of structure VII or XI as set forth in the specification hereof.

2. 6 - Deoxy - 6 - demethyl - 6 - methylenetetracycline.

3. 7 - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

4. 7 - Chloro - 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

5. 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

6. 9 - amino - 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

7. 7 - Chloro - 9 - amino - 5 - hydroxy - 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

8. Salts of compounds of any of claims 1—7 with acids or bases.

9. A process for the preparation of a 6 - methylenetetracycline of structure VII or XI herein which includes the step of removing halogen from the 11a - position of an 11a - halo - 6 - methylenetetracycline, of structures III, IV, V, VI, VIII, IX or X herein.

10. A process according to claim 9 wherein the halogen is removed by treating the 11a - halo - 6 - methylenetetracycline starting compound with aqueous alkali metal hydrosulfite.

11. A process according to claim 9 wherein the halogen is removed by treatment with zinc in the presence of a proton donor.

12. A process according to claim 9 wherein the halogen is removed by catalytic hydrogenolysis.

13. A process according to claim 11, wherein the proton donor is an acid in aqueous solution.

14. A process according to claim 12 wherein halogen is removed by contacting the starting compound with hydrogen over a noble metal catalyst, preferably a rhodium or a palladium catalyst.

15. A process according to any one of claims 12, or 14, wherein hydrogenation is carried out at a temperature ranging from 0°C. to 100°C. and pressures ranging from atmospheric to superatmospheric pressures of hydrogen gas.

16. A process according to any one of claims 9—15 wherein the starting compound is 11a - fluoro or 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline and the product is 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline.

17. A process according to any one of claims 9—15 wherein the starting compound is 11a - fluoro or 11a - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - tetracycline and the product is 6 - deoxy - 6 - demethyl - 6 - methylenetetracycline.

18. A process according to any one of claims 9—15 wherein the starting compound is 7,11a - dichloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetra-cycline and the product is 7 - chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 - oxytetracycline.



19. A therapeutic composition comprising a compound of any of claims 1—8 in a pharmaceutically acceptable carrier therefor.

20. A compound as claimed in claim 1, substantially as herein described.

5 21. A process according to claim 9 for the preparation of a compound of Structure VII or XI as set forth in the specification hereof, substantially as herein described. 5

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