

1

26,253

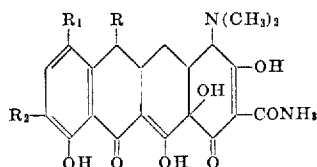
7- AND 9-ALKYLAMINO-6-DEOXYTETRACYCLINE
Joseph Petisi, Suffern, N.Y., and James Howard Boothe,
Montvale, N.J., assignors to American Cyanamid Com-
pany, Stamford, Conn., a corporation of Maine
No Drawing. Original No. 3,226,436, dated Dec. 28, 1965,
Ser. No. 281,349, May 17, 1963, which is a continua-
tion of Ser. No. 147,137, Oct. 24, 1961, and Ser. No.
161,412, Dec. 22, 1961, now Patent No. 3,148,212,
dated Sept. 8, 1964. Application for reissue Dec. 20,
1966, Ser. No. 606,500

3 Claims. (Cl. 260—559)

Matter enclosed in heavy brackets [] appears in the
original patent but forms no part of this reissue speci-
fication; matter printed in italics indicates the additions
made by reissue.

This application is a continuation of our copending
applications Serial No. 147,137, filed October 24, 1961,
now abandoned, and Serial No. 161,412 filed December
22, 1961, now U.S. Patent No. 3,148,212.

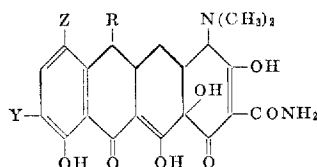
This invention relates to new compounds of the tetra-
cycline family and, more particularly, is concerned with
novel substituted 7-[and/or 9-]amino tetracyclines which
may be represented by the following general formula:



wherein R [is hydrogen or methyl and R₁] and R₂ are
hydrogen, [mono(lower alkyl)amino or] and R₁ is
di(lower alkyl)amino [with the proviso that R₁ and R₂
cannot both be hydrogen]. Typical compounds repre-
sented by the above general formula are, for example,

[7-methylamino-6-deoxy-6-demethyltetracycline,]
[7-ethylamino-6-deoxy-6-demethyltetracycline,]
[7-iso-propylamino-6-deoxy-6-demethyltetracycline,]
[9-methylamino-6-deoxy-6-demethyltetracycline,]
[9-ethylamino-6-deoxy-6-demethyltetracycline,]
[9-iso-propylamino-6-deoxy-6-demethyltetracycline,]
[7,9-di(ethylamino)-6-deoxy-6-demethyltetracycline,]
7-dimethylamino-6-deoxy-6-demethyltetracycline,
7-diethylamino-6-deoxy-6-demethyltetracycline,
[9-dimethylamino-6-deoxy-6-demethyltetracycline,]
[7-methylamino-6-deoxytetracycline,]
[9-ethylamino-6-deoxytetracycline,]
[7,9-di(methylamino)-6-deoxytetracycline,]
[7-diethylamino-6-deoxytetracycline,]
[9-diethylamino-6-deoxytetracycline,]
[7,9-di(methylethylamino)-6-deoxytetracycline,]
[7-methylamino-9-ethylamino-6-deoxytetracycline, and
9-methylamino-5-hydroxy-6-deoxytetracycline.]

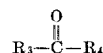
These new tetracycline derivatives may be prepared
by a novel reductive alkylation process comprising inter-
acting a compound of the following general formula:



wherein R [is hydrogen or methyl] and Y [and Z] are
hydrogen, and Z is amino, a substituent reducible to
amino, mono(lower alkyl)amino or a substituent reducible
to mono(lower alkyl)amino [with the proviso that

2

Y and Z cannot both be hydrogen], with [a carbonyl
compound of the general formula:



5 wherein R₃ is hydrogen or lower alkyl and R₄ is hydrogen
or lower alkyl,] formaldehyde or acetaldehyde, in the
presence of a reducing agent.

It is to be understood that when the term "lower alkyl"
is used throughout this specification, it is meant to include
[all lower alkyl groups having up to about 6 carbon
atoms. Accordingly, aldehydes and ketones useful in
carrying out this reductive alkylation include, for ex-
ample, formaldehyde, acetaldehyde, propionaldehyde, n-
butyraldehyde, iso-butyraldehyde, acetone, methyl ethyl
ketone, diethyl ketone, etc.] methyl and ethyl.

In the second general formula set forth above, the
substituent[s] [Y and] Z [are] is defined as [hydro-
gen,] amino a substituent reducible to amino, mono
(lower alkyl)amino or a substituent reducible to mono
(lower alkyl)amino [with the proviso that Y and Z can-
not both be hydrogen]. Suitable substituents reducible
to amino may be, for example, nitro, nitroso, diazonium
halide, beneneazo, substituted beneneazo, etc. [Where]

25 R₁ [and/or R₂] in the first general formula set forth
above [are] being a disubstituted amino group[s], such
products may be prepared in either of two ways. In the
first situation, [Y and/or] Z may be amino or a sub-
stituent reducible to amino and reductive dialkylation on
30 an unsubstituted amino group occurs, whereby a disub-
stituted amino group is obtained. In the second situation,
[Y and/or] Z may be mono(lower alkyl)amino or a
substituent reducible to mono(lower alkyl)amino and
reductive monoalkylation on a monoalkyl-substituted
35 amino group occurs, whereby a disubstituted amino group
is obtained. Suitable mono(lower alkyl)amino groups
[may be, for example,] are methylamino[, and ethyl-
amino[, n-propylamino, iso-propylamino, n-butylamino,
iso-butylamino, t-butylamino, etc]. Suitable substituents
40 reducible to mono(lower alkyl)amino may be, for ex-
ample, formylamino, acetylamino, N-(lower alkyl)hy-
droxylamino, and the like. Specific starting materials oper-
able in this process include

7-nitro-6-deoxy-6-demethyltetracycline,
45 7-amino-6-deoxy-6-demethyltetracycline,
[9-nitro-6-deoxy-6-demethyltetracycline,]
[9-amino-6-deoxy-6-demethyltetracycline,]
[7,9-dinitro-6-deoxy-6-demethyltetracycline,]
[7,9-diamino-6-deoxy-6-demethyltetracycline,]
50 [7-nitro-6-deoxytetracycline,]
[7-amino-6-deoxytetracycline,]
[9-nitro-6-deoxytetracycline,]
[9-amino-6-deoxytetracycline,]
[7,9-diamino-6-deoxytetracycline,]
55 [9-amino-7-nitro-6-deoxy-6-demethyltetracycline,]
[9-amino-7-nitro-6-deoxytetracycline,]
7-formylamino-6-deoxy-6-demethyltetracycline,
[9-formylamino-6-deoxy-6-demethyltetracycline,]
[7-acetylamin-6-deoxytetracycline,]
60 [9-acetylamin-6-deoxytetracycline,]
[7,9-diacetylamin-6-deoxytetracycline,]
[7-nitro-9-acetylamin-6-deoxytetracycline,]
6-deoxy-6-demethyltetracycline-7-diazonium chloride,
[6-deoxy-6-demethyltetracycline-9-diazonium chloride,]
65 [6-deoxytetracycline-9-diazonium chloride,]
[9-nitro-5-hydroxy-6-deoxytetracycline,]
[9-amino-5-hydroxy-6-deoxytetracycline,]
and the like. The tetracycline starting materials for this

70 process may be prepared by following the procedures set
forth in Austrian Patent No. 212,308 to American Cyan-
amid Company, in the articles by Beereboom et al.

J.A.C.S. 82, 1003 (1960), and by Boothe et al., J.A.C.S. 82, 1253 (1960), and in the copending application of Petisi and Boothe, Serial No. 65,584, filed October 28, 1960. The tetracycline starting materials may be employed either in the form of their free bases or in the form of their salts with various organic and inorganic acids depending upon whether solubility in polar or non-polar solvent systems is desired.

The reductive alkylation process may be accomplished by either chemical or catalytic reduction using procedures well-known to those in the art. Catalytic reduction, which is especially suited for the reductive alkylation of the tetracycline starting compounds set forth above, may be accomplished in a solvent for the tetracycline starting compound in the presence of [a carbonyl compound] formaldehyde or acetaldehyde and a metal catalyst and hydrogen gas at pressures from atmospheric to super-atmospheric. Ordinarily, the reductive alkylation is conveniently carried out at hydrogen pressures of from about one to about four atmospheres. Temperature does not appear to be critical in the catalytic hydrogenation. Temperatures of from 0° C. to 50° C., and usually room temperature, are preferred since they generally give best results. The metal catalyst may be of the base metal type, such as nickel or copper chromite, or it may be of the noble metal type, such as finely divided platinum, palladium or rhodium. The noble metal catalysts are advantageously employed on a carrier such as finely divided alumina, activated charcoal, diatomaceous earth, etc., in which form they are commonly available. The hydrogenation is carried out until the desired amount of hydrogen gas is absorbed at which point the hydrogenation is stopped. The solvents selected for the catalytic reduction should be reaction-inert, that is, they should not be capable of reacting with the starting materials, product, or hydrogen under the conditions of the reaction. A variety of solvents may be used for this purpose and minimum laboratory experimentation will permit the selection of a suitable solvent for any specific tetracycline starting compound. Generally, the catalytic reductive alkylation may be carried out in solvents such as water, lower alkanols, e.g. methanol, ethanol; lower alkoxy lower alkanols, e.g. 2-methoxyethanol, 2-ethoxyethanol; tetrahydrofuran, dioxane, dimethylformamide, etc.

A variety of chemical reducing agents may be used in the reductive alkylation process. These include reduction with active metals in mineral acids, e.g. zinc, tin, or iron in hydrochloric acid; reduction with metal couples such as the copper-zinc couple, the tin-mercury couple, aluminum amalgam, or magnesium amalgam; and reduction with formic acid. Of these, reduction with zinc and hydrochloric acid and reduction with formic acid are preferred. When aqueous systems are used in the aforementioned chemical reductive alkylations, it is at times desirable to utilize a water-miscible organic solvent, particularly when the tetracycline starting compound is of limited solubility in the reaction mixture. The water-miscible solvent does not alter the course of the reduction but merely provides for more efficient reduction, e.g. a shorter reaction time by providing more intimate contact of the reagents. A large number of such solvents are available for this purpose and include, among others, dimethylformamide, dimethoxyethane, methanol, ethanol, dioxane, tetrahydrofuran, and the like.

The novel products of the present invention are obtained from the reductive alkylation reaction mixtures by standard procedures. For example, the products may be isolated from the catalytic hydrogenation reaction mixtures, after filtration of the catalyst, by precipitation with a solvent such as ether or hexane or by concentration, usually under reduced pressure, or by a combination of these. Work-up of the chemical reductive alkylation reaction mixtures to obtain the desired products may also be accomplished by known procedures such as precipitation, concentration, solvent extraction, or combinations of these procedures. After isolation, the products may

be purified by any of the generally known methods for purification of tetracycline compounds. These include recrystallization from various solvents and mixed solvent systems, chromatographic techniques, and counter current distribution, all of which are usually employed for this purpose.

The novel substituted 7-[and/or 9-]amino tetracyclines of the present invention are biologically active and possess the broad-spectrum antibacterial activity of the previously known tetracyclines. In particular, the [7-methylamino] 7-dimethylamino-6-deoxy-6-demethyltetracycline possesses extraordinary activity both orally and parenterally against *Staphylococcus aureus*, strain Smith, and *Staphylococcus aureus*, strain Rose, infections in mice.

Staphylococcus aureus, strain Smith, has been studied and described by J. M. Smith and R. J. Dubos in Journ. Expt. Med. 103, 87 (1956), at the Rockefeller Institute. *Staphylococcus aureus*, strain Smith, is coagulase positive, tellurite negative and is sensitive to tetracycline, penicillin, streptomycin, erythromycin, carbomycin, neomycin, chloramphenicol and novobiocin in vitro. Attempts have been made for phage typing of this strain, but it has been determined that it is non-typable.

Staphylococcus aureus, strain Rose (ATCC No. 14,154) was isolated clinically from an abscess of a patient who did not respond to treatment with the tetracyclines. This organism has been found to be resistant to the clinically used tetracyclines in vitro and in vivo. *Staphylococcus aureus*, strain Rose, is coagulase and tellurite positive and is resistant to tetracycline, penicillin, streptomycin, and erythromycin. It is sensitive to carbomycin, neomycin, chloramphenicol and novobiocin in vitro. *Staphylococcus aureus*, strain Rose, has been phage-typed with the following results:

Staphylococcus aureus, strain Rose—Phage pattern 80/81.

It has been determined that intravenously against *Staphylococcus aureus*, strain Smith, infections in mice, [7-methylamino]7 - dimethylamino - 6 - demethyl - 6-deoxytetracycline is about 4 times as potent as tetracycline. When administered in a single oral tubing dose, against *Staphylococcus aureus*, strain Smith, infection, [7-methylamino]7 - dimethylamino - 6 - demethyl - 6 - deoxytetracycline is 17 times more effective than tetracycline. Additionally, after single oral tubing doses in mice, plasma levels of [7-methylamino]7-dimethylamino-6-demethyl-6-deoxytetracycline are about 5 times higher than those obtained with demethylchlortetracycline.

Of special interest is the fact that [7-methylamino]7-dimethylamino-6-demethyl-6-deoxytetracycline is uniquely active among the tetracyclines in possessing unexpected high oral activity against the tetracycline-resistant strain *Staphylococcus aureus*, strain Rose. This is the only tetracycline so far known having oral activity against this tetracycline-resistant infection in mice.

Thus with oral doses as low as 16 mg./kg., 16 out of 20 mice infected with *Staphylococcus aureus*, strain Rose, and treated with [7-methylamino]7-dimethylamino-6-demethyl-6-deoxytetracycline were alive 7 days after infection, whereas 19 out of 20 of the infected non-treated control mice died within 1 day. Under the same test conditions tetracycline is ineffective at the maximum tolerated dose of 2,048 mg./kg.

The new tetracyclines of this invention are amphoteric compounds and hence acid-addition salts, that is both mono- and di-salts, may be readily prepared. The preferred acids are the non-toxic pharmaceutically acceptable acids, e.g. the mineral acids such as hydrochloric, sulfuric, and the like although organic acids such as trichloroacetic may also be used. The acid-addition salts may be prepared by treating the new tetracyclines with approximately two equivalents or more of the chosen acid in a suitable solvent.

The invention will be described in greater detail in conjunction with the following specific examples.

5

EXAMPLE 1

Reductive methylation of 7-amino-6-deoxy-6-demethyl-tetracycline to yield [7-methylamino]7-dimethylamino-6-deoxy-6-demethyltetracycline

A solution of 792.9 mg. (1.5 millimoles) of 7-amino-6-deoxy-6-demethyltetracycline sulfate in 90 ml. of methyl Cellosolve, 1.5 ml. of 40% aqueous formaldehyde solution, and 300 mg. of 10% palladium-on-carbon catalyst was hydrogenated at room temperature and atmospheric pressure. Uptake of between one and one and one-half equivalents of hydrogen was complete in one to two hours. The catalyst was filtered off and the orange solution poured into 1.5 liters of dry ether. The precipitate was filtered off, washed well with dry ether and dried, weight 391 mg. The turbidimetric assay was 852 grammas per milliliter. Eighty-three milligrams of the material was chromatographed on neutral diatomaceous earth using a partition system of heptane, ethyl acetate, methanol, water in the ratio 45:55:15:6. The compound was run as a free base, pH of sample was 5.0. The product was eluted in the second hold back volume and was 15 mg. in weight.

The turbidimetric assay was 2190 gammas per milliliter. R_f 0.65 (nitromethane:benzene:pyridine:pH 3,4, buffer—20:10:3:3).

EXAMPLE 2

Reductive methylation of 7 - amino-6-deoxy-6-demethyl-tetracycline to yield [7-methylamino]7-dimethylamino-6-deoxy-6-demethyltetracycline

A solution of 527 mg. of 7-amino-6-deoxy-6-demethyl-tetracycline sulfate, 0.25 ml. of 97% formic acid, and 0.2 ml. of 37% formaldehyde solution was heated to reflux for 2 hours. The cooled mixture was taken up in 100 ml. of water. Paper chromatography revealed the presence of [7 - methylamino]7 - dimethylamino-6-deoxy-6-demethyltetracycline and its 4-epimer.

EXAMPLE 3

Reductive methylation of 7 - nitro-6-deoxy-6-demethyl-tetracycline to yield [7-methylamino]7-dimethylamino-6-deoxy-6-demethyltetracycline

A solution of 278.7 mg. of 7-nitro-6-deoxy-6-demethyl-tetracycline sulfate in 15 ml. of 93% methyl Cellosolve (7% water), 0.75 ml. of 40% aqueous formaldehyde solution and 50 mg. of 10% palladium-on-carbon catalyst was hydrogenated at room temperature and atmospheric pressure. Uptake was [complete (1) four equivalents(1)] in two hours. After removal of the catalyst by filtration, the solution was poured into 300 ml. of dry ether. The precipitate was collected by filtration, washed well with dry ether, and dried; weight, 200 mg.

EXAMPLE 4

[Reductive methylation of 9-amino-6-deoxy-6-demethyl-tetracycline to yield 9-methylamino-6-deoxy - 6 - demethyltetracycline]

[A solution of 233.5 mg. of 9-amino-6-deoxy-6-demethyltetracycline hydrochloride in 8 ml. of 0.1 N methanolic hydrochloric acid, 15 ml. of methyl Cellosolve, 0.5 ml. of 40% aqueous formaldehyde solution, and 100 mg. of 10% palladium-on-carbon catalyst was hydrogenated at room temperature and atmospheric pressure. Uptake of one to one and one-half equivalents of hydrogen was complete in less than one hour. The catalyst was filtered off, and the solution poured into 250 ml. of dry ether. The precipitate was washed well with dry ether and dried; weight, 171 mg.]

EXAMPLE 5

[Reductive methylation of 9-nitro-6-deoxy-6-demethyl-tetracycline to yield 9 - methylamino-6-deoxy-6-demethyltetracycline]

6

[A solution of 114.8 mg. of 9 - nitro-6-deoxy-6-demethyltetracycline in 20 ml. of methanol containing 2.5 ml. of 0.1 N methanolic hydrochloric acid, 0.4 ml. of 40% aqueous formaldehyde solution, and 50 mg. of 10% palladium-on-carbon catalyst was hydrogenated at room temperature and atmospheric pressure. Uptake was complete (85% of theory) in one hour. After filtration of the catalyst, the solution was evaporated to dryness; weight, 115 mg.]

EXAMPLE 6

Reductive ethylation of 7-amino-6-deoxy-6-demethyltetracycline to yield [7-ethylamino]7-diethylamino - 6-deoxy-6-demethyltetracycline

A solution of 396 mg. of 7-amino-6-deoxy-6-demethyl-tetracycline sulfate in 50 ml. of methyl Cellosolve was hydrogenated at room temperature and atmospheric pressure using 2 ml. of acetaldehyde and 150 mg. of 10% palladium-on-carbon. Uptake stopped after 3 hours and the catalyst was removed by filtration and the solution poured into 750 ml. of dry ether. The precipitate was filtered off, washed well with dry ether and dried; weight, 64 mg.

EXAMPLE 7

Reductive ethylation of 7-nitro-6-deoxy-6-demethyltetracycline to yield [7-ethylamino]7-diethylamino-6-deoxy-6-demethyltetracycline

In 30 ml. of methyl Cellosolve was suspended 560 mg. of 7-nitro-6-deoxy-6-demethyltetracycline sulfate, 2.1 ml. of 1 N sulfuric acid, 1.0 ml. of acetaldehyde, and 100 mg. of 10% palladium-on-charcoal. The mixture was shaken with hydrogen for 1.5 hours and the catalyst was then removed by filtration. The filtrate was poured into about 400 ml. of ether which precipitated a light colored solid. The product after filtering and drying weighed 582 mg. and consisted mostly of [7-ethylamino]7-diethylamino-6-deoxy-6-demethyltetracycline.

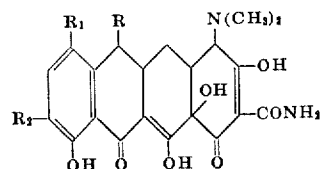
EXAMPLE 8

[Reductive iso-propylation of 7-nitro-6-deoxy-6-demethyl-tetracycline to yield 7 - iso-propylamino-6-deoxy-6-demethyltetracycline]

[A solution of 200 mg. of 7-nitro-6-deoxy-6-demethyl-tetracycline sulfate and 0.15 ml. of acetone in a mixture of 5.5 ml. of water and 5.5 ml. of ethanol was reduced in a Paar shaker with hydrogen and platinum oxide as a catalyst. The system absorbed 42 ml. of hydrogen during a two hour period. The catalyst was filtered and the filtrate evaporated to dryness in vacuo. The residue was dissolved in 3 ml. of MeOH and diluted with 150 ml. of ether. The solid product that separated weighed 155 mg.]

What is claimed is:

1. A compound of the group consisting of tetracyclines of the formula:



wherein R [is selected from the group consisting of hydrogen and methyl, and R₁] and R₂ are each [selected from the group consisting of] hydrogen, and [mono (lower alkyl)amino] R₁ is selected from the group consisting of dimethylamino and diethyl amino [with the proviso that R₁ and R₂ cannot both be hydrogen]; and the non-toxic acid-addition salts thereof.

2. [7 - methylamino]7 - dimethylamino-6-deoxy-6-demethyltetracycline.

3. 9-methylamino-6-deoxy-6-demethyltetracycline.]
4. [7-ethylamino]7 - diethylamino - 6 - deoxy-6-demethyltetracycline.
5. 7-isopropylamino - 6 - deoxy - 6 - demethyltetracycline.]

References Cited

The following references, cited by the Examiner, are of record in the patented file of this patent or the original patent.

UNITED STATES PATENTS

3,148,212 9/1964 Boothe et al. ----- 260—559

OTHER REFERENCES

- 5 Boothe et al.: Journal American Chemical Society, vol. 82, pages 1253-1255 (1960).

NICHOLAS S. RIZZO, *Primary Examiner*.