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SUBSTITUTED 7- AND/OR 9-AMINO-6-DEMETHYL-6-DEOXYTETRACYCLINES

Joseph J. Hlavka, Tuxedo, Panayoto Bitha, New York, and Adma Schneller Ross and Michael Joseph Martell, Suffern, N.Y., assignors to American Cyanamid Company, Stamford, Conn.

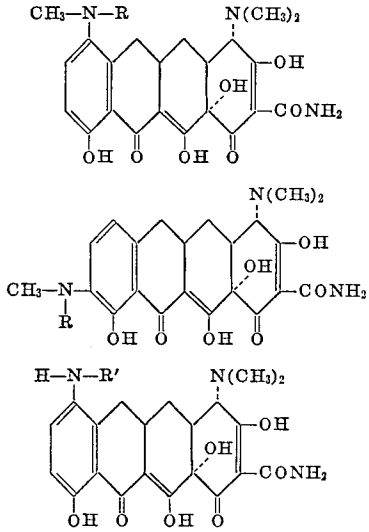
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ABSTRACT OF THE DISCLOSURE

This disclosure describes compounds of the class of 7- or 9-di(lower alkyl)amino-6-demethyl-6 - deoxytetracycylines and 7-monoalkylamino - 6 - demethyl-6-deoxytetracyclines useful as antibacterial agents.

BRIEF SUMMARY OF THE INVENTION

This invention relates to new organic compounds and, more particularly, is concerned with novel 7- or 9-di (lower alkyl)amino-6-demethyl-6-deoxytetracyclines and 7-monoalkyl - amino-6 - demethyl-6 - deoxytetracyclines which may be represented by the following general formulae:



wherein R is ethyl or isopropyl and R' is cyclohexyl, 1,3-dimethylbutyl or lauryl.

DETAILED DESCRIPTION OF THE INVENTION

The novel compounds of the present invention are typical tetracyclines, being yellow crystalline materials having characteristic melting points and absorption spectra. The free bases are appreciably soluble in many organic solvents such as lower alkanols, ethyl acetate, and the like, but are relatively insoluble in water.

The novel compounds of the present invention are amphoteric compounds and hence form both anionic and cationic salts with a variety of organic and inorganic salt-forming reagents. Thus, acid-addition salts, formed by admixture of the organic free base with a non-toxic pharmaceutically acceptable acid, suitably in a neutral solvent, are formed with such acids as sulfuric, phosphoric, hydrochloric, hydrobromic, sulfamic, citric, lactic, malic, succinic, tartaric, acetic, benzoic, gluconic, ascorbic, and the like. Base-derived salts, formed by admixture of the organic free base with base, suitably in a neutral solvent, are formed with such bases as sodium hydroxide, calcium hydroxide, magnesium hydroxide, tetraethylammonium hydroxide, etc. For purposes of this invention, the organic free bases are equivalent to their anionic and cationic salts. These new compounds of the present invention also form typical Mannich derivatives with organic aldehydic compounds and nitrogen bases.

The novel compounds of the present invention are biologically active and have been found to possess antibacterial activity. The antibacterial spectrum of the compounds of this invention, representing the amount required to inhibit the growth of various typical bacteria, was determined by the agar dilution streak technique using a special nutrient agar at pH 6.0 (Difco No. 634) supplemented with 0.5% of neopeptone. The initial solution of each compound was made in 0.1 N hydrochloric acid. Serial two-fold dilutions were then made using distilled water and one milliliter portions of the initial soluton and each dilution was mixed with measured volumes of warm fluid nutrient agar. After the agar had hardened overnight, broth cultures of the various test organisms were applied to the surface and the plates were incubated. End-points of activity were read after 24 hours. In a representative operation, and merely by way of illustration, the minimal inhibitory concentrations, expressed in micrograms per milliliter, of 7-methylethylamino-6-demethyl-6-deoxytetracycline (1) and 7-methylisopropylamino - 6 - demethyl - 6 - deoxytetracycline (2) against various test organisms are set forth in Table I below as compared with tetracycline hydrochloride (3) and 7-dimethylamino-6-demethyl-6-deoxytetracycline hydrochloride (4) as reference standards.

TABLE I

Organism	Minimal inhibitory conc. (mcg./ml.) (1)	(2)	(3)	(4)
Staphylococcus aureus, 4050B122-3	3.1	1.6	100	1.6
Staphylococcus aureus, 4050B122-9	3.1	1.6	100	3.1
Staphylococcus aureus, 4050B122-11	3.1	1.6	100	3.1
Staphylococcus aureus, 4050B122-14	3.1	1.6	50	3.1
Staphylococcus aureus, 4050B122-20	3.1	1.6	50	1.6
Staphylococcus aureus, Rose, ATCC 14154	3.1	1.6	50	1.6
Staphylococcus aureus, Smith, ATCC 13709	0.8	0.8	0.8	0.4
Staphylococcus aureus, Smith (TC-R)	0.8	0.8	100	0.8
Staphylococcus aureus, Smith (M-R)	6.2	6.2	25	6.2
Streptococcus pyogenes, C203	0.4	0.2	0.4	0.2
Entereococcus aerogenes, 75	12.5	12.5	6.2	6.2
Escherichia coli, 311	6.2	6.2	3.1	3.1
Escherichia coli, 311 (TC-R)	12.5	6.2	25	6.2
Escherichia coli, 311 (RB)	25	12.5	100	25
Escherichia coli, 311 (DA)	25	12.5	100	25
Escherichia coli, 311 (DY)	25	12.5	100	25
Klebsiella pneumoniae, AD	3.1	3.1	1.6	0.8
Proteus morganii, ATCC 8019	12.5	50	1.6	3.1
Proteus vulgaris, ATCC 6380	12.5	12.5	6.3	3.1
Proteus vulgaris, ATCC 9484	3.1	6.2	3.1	0.8
Pseudomonas aeruginosa, ATCC 10145	25	12.5	25	6.2
Salmonella cholerasuis, ATCC 10708	6.2	6.2	3.1	6.2
Salmonella typhosa, ATCC 6539	6.2	6.2	1.6	6.2
Shigella flexneri, I	3.1	3.1	1.6	1.6

The high in vitro antibacterial activity of the novel compounds of the present invention makes them useful as additives to materials which are subject to microbial deterioration such as cutting oils and fuel oils. They are also useful in soaps, shampoos, and topical compositions for the treatment of wounds and burns.

The novel compounds of the present invention have also been found to be highly active in vivo against *Stapylococcus aureus*, strain Smith, ATCC 13709.

Staphylococcus aureus, strain Smith, ATCC 13709 has been studied at the Rockefeller Institute and has been described by J. M. Smith and R. J. Dubos in the Journal of Experimental Medicine 108, 87 (1956). This organism 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 nontypable.

Staphylococcus aureus, strain Rose, ATCC 14154 was isolated clinically from an abscess of a patient who did

not respond to treatment with the tetracyclines and has been described in G. S. Redin and M. E. McCoy in Antibiotics Annual 1959–1960, pp. 213–219. This organism has been found to be resistant to the clinically used tetracyclines in vitro and in vivo. *Staphylococcus aureus,* strain, Rose ATCC 14154 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, ATCC 14154 has been phagetyped with the following results: Phage pattern 80/81.

The in vivo activity was demonstrated by experiments carried out as follows:

Unit test groups consist of 5 or 10 Carworth Farms CF1 female mice approximately 6 weeks old and averaging 18 to 21 grams of body weight per mouse. Infections are produced by intraperitoneal injections of 0.5 milliliter volumes of a 10^{-2} trypticase soy broth (TSP) dilution of a 5 hour blood broth culture of *Staphylococcus aureus,* strain Smith, ATCC 13709 or *Staphylococcus aureus,* strain Rose, ATCC 14154, containing

$$15 \pm 10 \times 10^6$$

viable units as determine by plate counts. The test compound mixed in 0.2% aqueous agar is administered by a single oral tubing dose in 0.5 ml. volumes to each test mouse one-half hour after infection. Control mice received 0.5 ml. volumes of 0.2% aqueous agar without test compound. By way of illustration, the results obtained when 7 - methylethylamino - 6 - demethyl-6-deoxytetracycline (1) and 7-methylisopropylamino-6-demethyl-6-deoxytetracycline (2) were so tested are set forth in Table II and Table III below:

TABLE II

Staphylococcus aureus, strain Smith ATCC 13709 infections in mice

Dose, mg./kg. of body weight	Mice alive/mice tested 5 days after infection [1]	
	(1)	(2)
32	10/10	5/5
16		5/5
8	9/10	15/15
4		5/10
2	7/10	4/15
1		1/10
0.5	0/10	0/15

[1] 167/170 untreated infected control mice died within one day.

TABLE III

Staphylococcus aureus, strain Rose, ATTC 14154 infections in mice

Dose, mg./kg. of body weight	Mice alive/mice tested 5 days after infection [1]	
	(1)	(2)
32	10/10	5/5
16		5/5
8	6/10	13/15
4		1/10
2	3/10	5/15
1		0/10
0.5	0/10	

[1] 203/210 untreated infected control mice died within one day.

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

EXAMPLE 1

Preparation of 7-monomethylamino-6-demethyl-6-deoxytetracycline

The 7-amino-6-demethyl-6-deoxytetracycline free base (400 mg.; 0.93 mmole; previously chromatographed) was dissolved in ethylene glycol monomethyl ether (28 ml.) containing 2 N sulfuric acid (2 ml.) and 37% formaldehyde solution (1.5 ml.). The palladium-on-carbon (10%) catalyst (100 mg.) was added and the mixture was reduced for about 20 minutes until 26 ml. of hydrogen was taken up (1 equiv. +36 ml. for the catalyst). The catalyst was then filtered, the filtrate poured into 600 ml. of anhydrous diethyl ether and the precipitate isolated by filtration. The crude product was purified by partition column chromatography after it was neutralized in methanol to pH 6.0 with the basic resin Amberlite IR–45®. The R_f value in a methyl ethyl ketone-water system (pH 7.7) was 0.65 (strong blue fluorescence after developing the dried sheet by dipping it in an acidic methanolic solution of magnesium acetate).

EXAMPLE 2

Preparation of 9-monomethylamino-6-demethyl-6-deoxytetracycline

To a solution of 1.28 g. (2.7 mmoles) 9-amino-6-demethyl-6-deoxytetracycline hydrochloride in 75 ml. of methyl Cellosolve plus 3.0 ml. of 4 N sulfuric acid was added 0.21 ml. (2.7 mmoles) 37% formaldehyde and 300 mg. 10% palladium-on-carbon. The solution was hydrogenated at room temperature and atmospheric pressure; uptake was 73 ml. in 40 minutes. The catalyst was filtered off through a Celite® pad and the filtrate was poured slowly into 750 ml. diethyl ether. The yellow precipitate was filtered off, washed with diethyl ether, dried under vacuum, and dissolved in 500 ml. methanol. Methanol washed Amberlite IR–45® was added to pH 6.3, the resin was filtered off, and the filtrate was evaporated to dryness, yield 0.9 g. Six hundred milligrams of this crude material was purified by partition column chromatography on neutral (acid washed) Celite® using a solvent system heptane:ethyl acetate:methyl Cellosolve:water

(60:40:15:4)

9-monomethylamino-6-demethyl-6-deoxytetracycline was obtained in hold back volumes 3.0–4.0, 180 mg.

EXAMPLE 3

Preparation of 7-monoisopropylamino-6-demethyl-6-deoxytetracycline

The 7-nitro-6-demethyl-6-deoxytetracycline sulfate salt (400 mg.) was dissolved in a mixture of ethanol (17 ml.) and water (16 ml.) containing 2 N H_2SO_4 (0.72 ml.) acetone (0.5 ml.). The catalyst PtO_2 (50 mg.) was added and the mixture was reduced under atmospheric pressure and room temperature for 1 hour and 45 minutes. Hydrogen taken up—79 ml. (theory: $0.72 \times 4 \times 24 = 69$ ml.+5 ml. for catalyst=74 ml.). The catalyst was filtered through a Celite® precoated funnel under nitrogen and filtrate evaporated to dryness. It was purified by partition chromatography after neutralized (pH 6.3) in methanol with Amberlite IR–45®.

EXAMPLE 4

Preparation of 9-monoisopropylamino-6-demethyl-6-deoxytetracycline

A mixture of 400 mg. of 9-amino-6-demethyl-6-deoxytetracycline hydrochloride, 0.5 ml. of acetone, and 0.43 ml. of 2 N sulfuric acid in 36 ml. of a 50% ethanol-water mixture was reduced at S.T.P. with 75 mg. of PtO_2 and 35 ml. of hydrogen for 1½ hours. The mixture was filtered and the filtrate evaporated to dryness, yield 280 mg. R_f=0.9.

EXAMPLE 5

Preparation of 7-methylethylamino-6-demethyl-6-deoxytetracycline

The chromatographed 7-monomethylamino-6-demethyl-6-deoxytetracycline free base (100 mg.) was dissolved in ethylene glycol monomethyl ether (6.3 ml.) containing 2 N H_2SO_4 (0.48 ml.) and acetaldehyde (0.225 ml.). The palladium - on - carbon (10%) catalyst (22.5 mg.) was added and the mixture reduced under atmospheric pressure and room temperature for two hours. Catalyst was then filtered, filtrate poured into 100 ml. of diethyl ether and precipitated solid filtered and dried. Yield—124 mg.

This material was neutralized in methanol with Amberlite IR–45® (to pH 6.1) and chromatographed. The R_f value in the methyl ethyl ketone-water pH 7.7 system was 0.87 while starting material has an R_f 0.65 in the same system.

EXAMPLE 6

Preparation of 9-methylethylamino-6-demethyl-6-deoxytetracycline

To a solution of 111 mg. (0.25 mmole) 9-monomethylamino-6-demethyl-6-deoxytetracycline in 7 ml. of methyl Cellosolve plus 0.26 of 4 N sulfuric acid was added 0.25 ml. of acetaldehyde and 25 mg. of 10% palladium-on-carbon. The solution was hydrogenated at room temperature and atmospheric pressure. The uptake (5.3 ml.) ceased after 30 minutes, but the hydrogenation was continued for and additional 90 minutes. The catalyst was filtered through a Celite® pad and the filtrate was poured slowly into 200 ml. of rapidly stirred diethyl ether. The precipitate was filtered off, washed with diethyl ether, dried under vacuum, and dissolved in 60 ml. methanol. Amberlite IR–45® was added and the solution was stirred until the pH was 6.3. The resin was filtered off and the filtrate was evaporated to dryness. Yield 72 mg. The above crude material (55 mg.) was purified by partition column chromatography on neutral (acid washed) Celite® using a solvent system heptane:ethyl:acetate:methyl Cellosolve water (55:45:15:4). The product was eluted in hold back volumes 1.9–2.3, 23 mg.

EXAMPLE 7

Preparation of 7-methylisopropylamino-6-demethyl-6-deoxytetracycline

The purified 7-monoisopropylamino-6-demethyl-6-deoxytetracycline free base (100 mg.) was dissolved in ethylene glycol monomethyl ether (7 ml.) containing 2 N H_2SO_4 (0.5 ml.) and 37% solution of formaldehyde (0.75 ml.). The palladium-on-carbon (10%) catalyst (25 mg.) was added and the mixture reduced under atmospheric pressure and room temperature for one hour. Catalyst was then filtered, filtrate poured into diethyl ether and precipitated solid was isolated by filtration. It was neutralized (pH 6.4) in methanol with Amberlite IR–45® and chromatographed.

EXAMPLE 8

Preparation of 9-methylisopropylamino-6-demethyl-6-deoxytetracycline

By replacing the 7-monoisopropylamino-6-demethyl-6-deoxytetracycline employed in Example 7 with a like amount of 9-monoisopropylamino-6-demethyl - 6 - deoxytetracycline and following substantially the same procedure described in Example 7, there is obtained the 9-methylisopropylamino-6-demethyl-6-deoxytetracycline.

EXAMPLE 9

Preparation of 7-cyclohexylamino-6-demethyl-6-deoxytetracycline

A mixture of 200 mg. of 7-nitro-6-demethyl-6-deoxytetracycline sulfate, 0.36 ml. of 2 N sulfuric acid, 0.3 ml. of cyclohexanone and 60 mg. of PtO_2 in 7 ml. of ethanol and 4 ml. of water was reduced at S.T.P. until the system absorbed 45 ml. of hydrogen. The catalyst was filtered and the filtrate was evaporated to dryness, yield 225 mg. R_f in methyl ether ketone system buffered at 7.7=0.97.

EXAMPLE 10

Preparation of 7-(1,3-dimethylbutyl)amino-6-demethyl-6-deoxytetracycline

Five hundred milligrams (0.9 mmole) of 7-nitro-6-demethyl-6-deoxytetracyline sulfate was dissolved in 21 ml. ethanol and 20 ml. water, and 450 ml. of 4 N sulfuric acid, 1.125 ml. of methyl isobutyl ketone, and 70 mg. of platinum oxide were added. The solution was hydrogenated at room temperature and atmospheric pressure for 2½ hours, uptake 84 ml. The catalyst was filtered off through a Celite® pad and the filtrate evaporated to dryness. The product obtained from two such reactions was dissolved in 700 ml. methanol. Amberlite IR–45® was added with stirring until the pH was 6.3. The resin was filtered off and the filtrate was evaporated to dryness, yield 700 mg. The above crude material was purified by partition column chromatography on neutral (acid washed) Celite® using a solvent system heptane:ethyl acetate:methyl Cellosolve:water (70:30:15:4). The product was eluted in hold back volumes 1.9–2.4, yield 52 mg.

EXAMPLE 11

Preparation of 7-laurylamino-6-demethyl-6-deoxytetracycline

A suspension of 500 mg. of 7-nitro-6-demethyl-6-deoxytetracycline sulfate in 30 ml. of methyl Cellosolve with 2 ml. lauraldehyde, 450 ml. of 4 N sulfuric acid, and 70 mg. of platinum oxide was hydrogenated on a Parr shaker for 2½ hours. The initial pressure was 29 pounds and dropped to 18 pounds. The catalyst was filtered off and the filtrate was poured into 1200 ml. of diethyl ether. Since the precipitate was too fine to be filtered off with a medium porosity funnel, the precipitate was allowed to settle and almost all the ether was removed with a filter stick. The remaining slurry was evaporated to an oil which was dissolved in 600 ml. of methanol. The solution was adjusted to pH 6.3 with triethylamine and then evaporated to an oil. The oil was triturated with ethyl acetate and the ethyl acetate was evaporated to an oil. The above crude material was purified by partition column chromatography on neutral (acid washed) Celite® using a solvent system heptane:ethyl acetate:methyl Cellosolve:water (85:15:15:4). The product, an orange solid, was obtained in hold back volumes 1.1–1.7, yield 64 mg.

We claim:

1. A compound selected from the group consisting of 7-cyclohexylamino-6-deoxy - 6 - demethyltetracycline and the acid-addition, alkali metal and alkaline earth metal salts thereof.

2. A compound selected from the group consisting of 7-laurylamino-6-deoxy - 6 - demethyltetracycline and the acid-addition, alkali metal and alkaline earth metal salts thereof.

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ALEX MAZEL, Primary Examiner

A. M. T. TIGHE, Assistant Examiner

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