Anal. Calcd. for $C_7H_9NO_3 \cdot HCl \cdot 0.5\ H_2O$: C, 42.1; H,5.55; N, 7.00; Cl, 17.7. Found: C, 42.0; H, 5.32; N, 6.86; Cl, 17.9.

3-Amino-4,5-bis(hydroxymethyl)pyridine Hydrochloride (III).—A suspension of 2.0 g. (0.011 mole) of 5-aminocinchomeronic acid in 30 ml. of methanol was treated with a solution of diazomethane in ether made from 5.6 g. (0.063 mole) of N-nitrosomethylurea. The reaction mixture was swirled occasionally and allowed to stand until no more nitrogen was evolved. The suspended solid was filtered off and found to be 1.0 g. of starting material. The ether filtrate was evaporated to a brown oil from which no solid product could be obtained. This 1.0 g. of crude ester in 50 ml. of anhydrous tetrahydrofuran was added slowly to a solution of 0.38 g. (0.010 mole) of lithium aluminum hydride in 100 ml. of tetrahydrofuran. The resulting orange mixture was refluxed for 8 hr. and allowed to stand overnight at room temperature. The excess hydride was destroyed with 2 ml. of water and the insoluble salts were filtered off. The solid was washed three times with 30 ml. of boiling methanol and the combined filtrates were evaporated *in vacuo* to a tarry residue. Recrystallization of this from ethanol–ethyl acetate (decolorizing charcoal) gave a tan solid; yield 0.25 g. (34% on the basis of reacted acid); m.p. 135–140° (dec.). As purification of the base was difficult the hydrochloride was prepared. Recrystallization of a 100 mg. sample of the hydrochloride from ethanol and alcoholic hydrogen chloride yielded 40 mg. of a light tan solid; m.p. 164–165° (dec.); λ_{max} 252.5, 322.5, log ϵ 3.988, 3.941.

Anal. Calcd. for $C_7H_{10}N_2O_2 \cdot HCl$: C, 43.8; H, 5.78; N, 14.6; Cl, 18.5. Found: C, 44.1; H, 6.12; N, 14.3; Cl, 18.7.

Acknowledgments.—We are indebted to Mr. L. Brancone and his staff for the microanalytical results, to Dr. H. Arlt and his associates for certain large scale preparations and to Mr. A. S. Piatt for the assistance in the care of the animals.

6-Deoxytetracyclines. II. Nitrations and Subsequent Reactions[1]

J. PETISI, J. L. SPENCER, J. J. HLAVKA, AND J. H. BOOTHE[1a]

Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

Received October 23, 1961

The nitration of 6-deoxytetracyclines and the proof of structure of the resulting nitro compounds are described. The nitro groups were reduced to form amino derivatives, and these in some cases were acylated. The *in vitro* antibacterial potencies of the new compounds are compared to tetracycline.

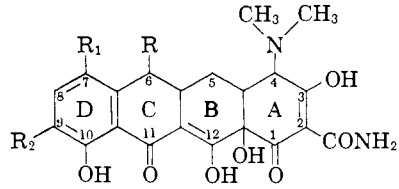
The tetracycline antibiotics are a family of potent, broad spectrum antibacterial substances elaborated by several members of the genus

(1) Paper I: J. H. Boothe, J. J. Hlavka, J. P. Petisi, and J. L. Spencer, *J. Am. Chem. Soc.*, **82**, 1253 (1960); *cf.* J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *ibid.*, **82**, 1003 (1960).

(1a) Inquiries should be addressed to this author.

Streptomyces. The importance of these antibiotics in the treatment of human infectious diseases has stimulated many attempts to modify them chemically in order to understand more clearly the relation between chemical structure and biological activity. However, the inherent chemical instability of the tetracyclines has often seriously limited the type of reactions that may be carried out at one position in the molecule without affecting some other functional group. One of the major contributors to this instability is the 6-hydroxyl group which eliminates with the 5a hydrogen in acidic media to form anhydrotetracyclines and participates with the 11 keto group in alkaline media to form isotetracyclines.[2,3] Of especial significance, then, was the discovery that the 6-hydroxyl group could be removed by catalytic hydrogenolysis, and, more important, that the resulting 6-deoxytetracyclines retained the characteristic antibacterial properties of the parent compounds.[4,5]

Actually the 6-deoxytetracyclines are remarkably stable to strong mineral acids, and therefore, lend themselves well to electrophilic substitution reactions. After the initial observation that these compounds were stable in concentrated sulfuric acid, the first electrophilic substitution reaction to be studied was nitration. This report deals with the nitration products of 6-demethyl-6-deoxytetracycline and 6-deoxytetracycline, their structure proofs, antibacterial activities, and subsequent reaction products.



	R	R_1	R_2		R	R_1	R_2
II	H	H	H	IX	CH_3	H	H
III	H	NO_2	H	X	CH_3	NO_2	H
IV	H	H	NO_2	XI	CH_3	H	NO_2
V	H	NH_2	H	XII	CH_3	H	NH_2
VI	H	H	NH_2	XIII	CH_3	H	$NHCOCH_3$
VII	H	NHCHO	H				
VIII	H	H	NHCHO				

(2) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, **76**, 3568 (1954).

(3) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard, and J. H. Williams, *ibid.*, **74**, 4981 (1952).

(4) C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover, and K. J. Brunings, *ibid.*, **80**, 5324 (1958).

(5) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *ibid.*, **82**, 3381 (1960).

Nitration of 6-demethyl-6-deoxytetracycline (II) in concentrated sulfuric acid with one equivalent of nitrate leads to a net over-all increase in antibacterial potency. This increase in antibiotic activity was found to be attributable to one very potent antibacterial substance formed in the nitration but which was accompanied by an equal amount of a second compound having little or no antibacterial properties. Separation and characterization of these two products showed that they were isomeric mononitro derivatives whose stability and spectral properties ruled out the possibility of aliphatic nitro compounds or nitrate esters. This left only the three positions on the aromatic D ring as possible sites of attachment, and since the orientation rules would predict electrophilic attack at the 7 and 9 position, the nitro groups were assigned to these positions.

The tentative decision as to which was the 7-isomer and which the 9-isomer was based on the absorption spectra. In 0.1 *N* sodium hydroxide, the spectrum of the biologically inactive nitro isomer showed a maximum at 430 mμ, much further into the visible region than was normal for the tetracyclines. This profound change in absorption characteristics was interpreted to reflect the influence of a nitro group adjacent to the phenolic hydroxyl causing a change in hydrogen bonding and thereby a change in enolization of the B, C, D chromophore. Thus the compound which lacked the typical antibacterial properties of the tetracyclines was tentatively assigned the structure 6-demethyl-6-deoxy-9-nitrotetracycline (IV) while the isomer possessing a high antibiotic potency and a normal absorption spectrum was designated as 6-demethyl-6-deoxy-7-nitrotetracycline (III).

Fortunately, the means were available to check the positional assignment of the nitro group in one of the isomers by an unequivocal method. 7-Chloro-6-demethyltetracycline is a naturally occurring antibiotic whose structure, including the position of the chlorine, has been well documented.[6-8] Replacement of the chlorine in this compound by tritium[9] was readily accomplished by means of a palladium catalyst, and further hydrogenation replaced the 6-hydroxyl group to provide 6-demethyl-6-deoxytetracycline-7-^{3}H. Nitration of this intermediate, tagged now in the 7-position, provided the two mono-

(6) J. R. D. McCormick, N. O. Sjolander, Ursula Hirsch, E. R. Jensen, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 4561 (1957).

(7) J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein, and C. F. Wolf, *ibid.*, **79**, 4565 (1957).

(8) J. H. Boothe, A. Green, J. P. Petisi, R. G. Wilkinson, and C. W. Waller, *ibid.*, **79**, 4564 (1957).

(9) We are indebted to Dr. E. F. Ullman for suggesting this unique method of determining the position of entering groups.

nitro isomers already described but in this case one of the isomers was radioactive due to the retained tritium while in the other the tritium had been displaced. Application of this method showed without question that the isomer with the high biological activity lost the tritium and thus was 6-demethyl-6-deoxy-7-nitrotetracycline (III). The isomeric derivative, on the other hand, retained the tritium and was assumed to be 6-demethyl-6-deoxy-9-nitrotetracycline (IV).[10]

The nitration of 6-deoxytetracycline (IX) was carried out in an analogous manner and similarly the reaction yielded 2 isomeric mononitro compounds. The properties of these two isomers, both chemical and biological, paralleled quite closely those of the 6-demethyl derivatives just described, and again the tritium labeling technique unequivocally pointed out the proper positional assignments. Moreover, the nitration of 6-deoxytetracycline produced about four times as much 9-nitro as 7-nitro isomer while the two isomers were formed in about equal amounts during the nitration of 6-demethyl-6-deoxytetracycline. This production of a significantly smaller amount of the 7-isomer was attributed to the steric interference of the 6-methyl group, thus making the 9-position relatively more favored for electrophilic attack.

6-Deoxy-9-nitrotetracycline (XI) proved especially easy to separate from its isomer since it was observed that if an excess of nitrate was added to the reaction, the 7-nitro isomer was converted to a product which was eliminated by the normal isolation procedure. Thus, if two equivalents of nitrate were added during the nitration, the normal isolation procedure yielded pure 6-deoxy-9-nitrotetracycline (XI). The 6-deoxy-7-nitrotetracycline (X), on the other hand, was isolated only with some difficulty by the use of pressurized paper column chromatography techniques.

Reduction of the various nitro compounds to the corresponding amines proceeded normally. It is noteworthy that reduction of the 9-nitro derivatives to the corresponding amino compounds was accompanied by a loss of the long wave length absorption maximum as well as by a concomitant reappearance of the antibacterial activity. This absorption spectral shift lends additional support to the assignment of the nitro group to the position *ortho* to the phenolic hydroxyl group, since reduction of the group eliminates hydrogen bonding

(10) The 6-demethyl-6-deoxy-9-nitrotetracycline-7-H^3 contained only about 80% as much radioactivity as did the unnitrated parent, and this loss was attributed to tritium–hydrogen exchange under the influence of sulfuric acid. This theory was confirmed readily by treating 6-demethyl-6-deoxytetracycline-7-H^3 with cold sulfuric acid but no nitrate, and observing a similar loss of radioactivity.

which was responsible for lengthening of the B, C, D ring chromophore. Certain of the amino compounds were acylated to provide some examples of acylamino substitution for microbiological comparisons.

The *in vitro* antibacterial comparisons of the new substituted tetracyclines with tetracycline itself are shown in Table I.

TABLE I

in Vitro ANTIBACTERIAL POTENCY[a] COMPARED TO TETRACYCLINE

No.	Name	Biological activity
I	Tetracycline	100
II	6-Demethyl-6-deoxytetracycline	160
III	6-Demethyl-6-deoxy-7-nitrotetracycline	640
IV	6-Demethyl-6-deoxy-9-nitrotetracycline	12
V	7-Amino-6-demethyl-6-deoxytetracycline	40
VI	9-Amino-6-demethyl-6-deoxytetracycline	160
VII	6-Demethyl-6-deoxy-7-formamidotetracycline	35
VIII	6-Demethyl-6-deoxy-9-formamidotetracycline	225
IX	6-Deoxytetracycline	72
X	6-Deoxy-7-nitrotetracycline	240[b]
XI	6-Deoxy-9-nitrotetracycline	1
XII	9-Amino-6-deoxytetracycline	60
XIII	9-Acetamido-6-deoxytetracycline	24

[a] Antibacterial activities were measured by the turbidimetric assay of E. Pelcak and A. C. Dornbush, *Ann. N. Y. Acad. Sci.*, **51**, 218 (1948), using *Staphylococcus aureus* as the test organism. [b] The conditions under which this compound was isolated caused epimeric equilibrium to occur at the 4-position (see Experimental). It is assumed that the pure natural epimer would have approximately double the activity indicated here.

Experimental

General Procedure for Nitration.—To 1 mmole of a 6-deoxytetracycline hydrochloride in 25 ml. of concentrated sulfuric acid at 0° was added 1 mmole of potassium nitrate with stirring. The reaction solution was stirred for 15 min. and then poured into 100 g. of chopped ice. The aqueous solution was extracted 5 times with 20 ml. of butanol each time. The butanol extracts were washed three times with 10 ml. of water each time, and concentrated *in vacuo* to a volume of 25 ml. The light yellow crystalline solid which precipitated was filtered, washed with 2 ml. of butanol and dried *in vacuo* at 60° for 2 hr. This solid was a mixture of the two mononitro isomers.

6-Demethyl-6-deoxy-9-nitrotetracycline (IV).—To 980 mg. of the nitration product from 6-demethyl-6-deoxytetracycline (a mixture of the 2 isomers) in 25 ml. of methanol was added enough triethylamine to dissolve the solid. The filtered solution (pH 9.0) was adjusted to pH 5.2 with concd. sulfuric acid. A crystalline yellow solid (236 mg.) was obtained (29% yield). The material at this point was quite pure and contained only small amounts of the 7-isomer. Final purification was accomplished by liquid partition chromatography using a diatomaceous

earth packed column and the solvent system: chloroform: butanol: 0.5 *M* phosphate buffer (pH 2) (16:1:10); $\lambda_{max.}^{0.1\ N\ HCl}$ 263 mμ (log ε 4.42), 360 mμ (log ε 4.42); $[\alpha]^{25}D$ −131°.

Anal. Calcd. for $C_{21}H_{21}N_3O_9$: C, 55.0; H, 4.62; N, 9.15. Found: C, 55.1; H, 5.2; N, 9.0.

6-Demethyl-6-deoxy-7-nitrotetracycline (III).—The methanol filtrate from the above solid was immediately adjusted to pH 1.0 with concd. sulfuric acid. The light yellow crystalline solid, which was obtained as the sulfate salt, weighed 243 mg. (25% yield). A purified free base was obtained by adjusting an aqueous solution of the sulfate salt (25 mg./ml.) to pH 5.2 with 2 *N* sodium carbonate. This material was recrystallized from water; $\lambda_{max}^{0.1\ N\ HCl}$ 262 mμ (log ε 4.35), 350 mμ (log ε 4.27); $[\alpha]^{25}D$ −442°.

Anal. Calcd. for $C_{21}H_{21}N_3O_9 \cdot 2H_2O$: C, 51.0; H, 5.1; N, 8.47. Found: C, 51.3; H, 5.8; N, 8.17.

Separation of 7- and 9-Nitro-6-deoxytetracycline.—The starting material, 6-deoxytetracycline, was nitrated by the general procedure given above. Paper strip chromatography showed the nitration product was composed of two components which were separated using a paper column chromatogram[11] (Chromax #3504) and the system butanol/phosphate buffer (pH 2.0). During the course of the separation, which required at least 24 hr., the 7-nitro derivative reached 4-epimeric equilibrium as evidenced by no further loss of biological activity on continued standing in pH 2 buffer.

6-Deoxy-7-nitrotetracycline and its 4-Epimer (X).—The 6-deoxy-7-nitrotetracycline sulfate obtained from several column runs was combined, dissolved in 0.1 *N* sulfuric acid, and extracted with butanol. The butanol extract was concentrated to a small volume and on standing a solid crystallized; $\lambda_{max}^{0.1\ N\ HCl}$ 260 mμ (log ε 4.81), 350 mμ (log ε 4.41); R_f, 0.80 (butanol-pH 2 phosphate buffer).

Anal. Calcd. for $C_{22}H_{23}N_3O_9 \cdot C_4H_9OH \cdot H_2SO_4$: C, 48.4; H, 5.5; N, 6.5; S, 5.0. Found: C, 48.3; H, 5.4; N, 6.7; S, 4.8.

6-Deoxy-9-nitrotetracycline (XI).—The 9-nitro-6-deoxytetracycline could also be separated from its isomer by pressurized paper column chromatography, but it was more conveniently prepared by the use of excess nitrate (2 moles) in the nitration reaction. Using the general nitration procedure outlined above but substituting two equivalents of nitrate, the isolated product was found to be pure 9-nitro-6-deoxytetracycline free of its 7-nitro isomer; $\lambda_{max}^{0.1\ N\ HCl}$ 260 mμ (log ε 4.43), 365 mμ (log ε 4.23); $[\alpha]^{25}D$ − 268°.

Anal. Calcd. for $C_{22}H_{23}N_3O_9 \cdot C_4H_9OH \cdot H_2SO_4$: C, 48.37; H, 5.46; N, 6.51; S, 4.96. Found: C, 48.2. H, 5.2; N, 6.7; S, 4.9.

9-Amino-6-demethyl-6-deoxytetracycline (VI).—A mixture of 2.3 g. (5 mmoles) of 9-nitro-6-demethyl-6-deoxytetracycline, 5.2 ml. of concd. hydrochloric acid, and 230 mg. of platinum oxide catalyst in 200 ml. of methanol was shaken with hydrogen. The theoretical amount of hydrogen was absorbed in less than 1 hr. After the catalyst was removed by filtration, a yellow solid was obtained on evaporation of the solvents. The product, which was obtained as a dihydrochloride, weighed 2.52 g. and was converted to the free base by adjusting an aqueous solution (75 mg./ml.) to pH 6.2 by the addition of a saturated solution of sodium sul-

(10a) Optical rotations were determined at a concentration of 0.1% to 0.5% in 0.1 *N* sulfuric acid.

(11) The equipment for this type of chromatography was obtained from Ivan Sorvall, Inc., Norwich, Connecticut.

fite. A light yellow crystalline solid was obtained; $\lambda_{max}^{0.1\ N\ HCl}$ 265 mμ (log ε 4.33), 350 mμ (log ε 4.25); $[\alpha]^{25}D$ − 212°.

Anal. Calcd. for $C_{21}H_{23}N_3O_7 \cdot 1.5H_2O$: C, 55.2, H, 5.74; N, 9.19. Found: C, 54.8; H, 5.83; N, 8.92.

7-Amino-6-demethyl-6-deoxytetracycline Dihydrochloride (V).—A catalytic reduction of 6-demethyl-6-deoxy-7-nitrotetracycline was accomplished as described above for the 9-nitro derivative. The yield of product, as a dihydrochloride, was quantitative. The product was purified by dissolving it in ethanol with addition of the least amount of concentrated hydrochloric acid and then pouring the solution into an excess of ether. A light tan solid was obtained; $\lambda_{max}^{0.1\ N\ HCl}$ 265 mμ (log ε 4.34), 350 mμ (log ε 4.21); $[\alpha]^{25}D$ −191°.

Anal. Calcd. for $C_{21}H_{23}N_3O_7 \cdot 2HCl \cdot 3H_2O$: C, 45.34, H, 5.62, N, 7.55. Found: C, 45.38; H, 6.01; N, 7.31.

6-Demethyl-6-deoxy-7-formamidotetracycline (VII).—A solution of 4.5 g. (9 mmoles) of 7-amino-6-demethyl-6-deoxytetracycline dihydrochloride in 20 ml. of 98% formic acid was refluxed for 1.5 hr. A yellow solid which was obtained by pouring the reaction mixture into an excess of ether weighed 4.52 g. This crude product was purified by adjusting a methanolic solution (100 mg./ml.) to pH 6.0 with triethylamine; $\lambda_{max}^{0.1\ N\ HCl}$ 250 mμ (log ε 4.29), 338 mμ (log ε 4.16); $[\alpha]^{25}D$ − 151°.

Anal. Calcd. for $C_{22}H_{23}N_3O_8 \cdot 2H_2O$: C, 53.53; H, 5.52; N, 8.52. Found: C, 53.74; H, 6.35; N, 8.52.

6-Demethyl-6-deoxy-9-formamidotetracycline (VIII).—The above reaction was repeated on a similar quantity of 9-amino-6-demethyl-6-deoxytetracycline hydrochloride. A light yellow solid which was obtained in theoretical yield was purified in the manner described above; $\lambda_{max}^{0.1\ N\ HCl}$ 250 mμ (log ε 4.36), 347 mμ (log ε 4.19); $[\alpha]^{25}D$ − 69°.

Anal. Calcd. for $C_{22}H_{23}N_3O_8 \cdot H_2O$: C, 55.57; H, 5.31; N, 8.84. Found: C, 55.08; H, 5.55; N, 9.03.

9-Amino-6-deoxytetracycline (XII).—A slurry of 2.00 g. of 6-deoxy-9-nitrotetracycline sulfate in 200 ml. of ethanol containing 0.4 ml. of sulfuric acid and 200 mg. of platinum oxide was hydrogenated at room temperature and atmospheric pressure. The theoretical hydrogen uptake was achieved in 15 min. The catalyst was removed by filtration and the reduction solution was evaporated to a small volume. The product which was precipitated by addition of excess ether was collected, washed with ether, and vacuum dried. Recrystallization from butanol yielded the disulfate butanolate, $\lambda_{max}^{0.1\ N\ HCl}$ 265 mμ (log ε 4.26), 350 mμ (log ε 4.17); $[\alpha]^{25}D$ − 211°.

Anal. Calcd. for $C_{22}H_{25}N_3O_7 \cdot C_4H_9OH \cdot 2H_2SO_4$: C, 43.76; H, 5.51; N, 5.89. Found: C, 43.7; H, 5.4; N, 6.2.

9-Acetamido-6-deoxytetracycline (XIII).—A solution of 5.0 g. of sodium acetate in 25 ml. of water was added to an aqueous solution of 6.4 g. of 9-amino-6-deoxytetracycline disulfate. The turbid solution was stirred while 2.0 ml. of acetic anhydride was added slowly. After 10 min., 1.0 ml. of concentrated ammonium hydroxide was added, the solution was stirred for 5 min., and extracted with butanol after 5.0 ml. of concentrated sulfuric acid had been added. The butanol extract was washed with water and concentrated under vacuum. The resulting solid was collected after overnight refrigeration, washed with butanol and chloroform, and vacuum dried; yield 2.9 g. An analytical sample was obtained as the

sulfate by recrystallization from butanol; $\lambda_{max}^{0.1\ N\ HCl}$ 245 mμ (log ε 4.33), 343 mμ (log ε 4.20); $[\alpha]^{25}D$ − 215°.

Anal. Calcd. for $C_{24}H_{27}N_3O_8 \cdot H_2SO_4$: C, 49.40; H, 5.01; N, 7.20; S, 5.40. Found: C, 49.5; H, 5.9; N, 7.2; S, 5.4.

Tritium Labeled Material. Tetracycline 7-^{3}H.[12]—A mixture of 1.0 g. of chlortetracycline and 100 mg. of 10% palladium-on-carbon in 4 ml. of dimethylformamide was shaken first with 10 curies of tritium and then the reduction was completed with hydrogen. After filtering off the catalyst, the reaction solution was diluted with 250 ml. of water and lyophilized. The material weighed about 850 mg. and contained radioactivity equivalent of 1–2 curies/g. A portion of this material (500 mg.) was diluted with 100 g. of tetracycline by crystallization of the two from a mixture of 400 ml. of butanol and 100 ml. of 2-methoxyethanol. Solution was effected by adding 22 ml. of triethylamine and after filtration the tetracycline was crystallized as the hydrochloride by the addition of 36 ml. of concd. hydrochloric acid. The radioactivity of this product, 90 g., was 2.5 curies/mole.[13]

6-Deoxytetracycline-7-^{3}H Hydrochloride.[14]—A solution of 5.1 g. of the above tetracycline-7-^{3}H in 120 ml. of dimethylformamide containing 0.5 ml. of concentrated sulfuric acid and 5.0 g. of 30% rhodium-on-carbon was hydrogenated at room temperature and 3.15 kg./cm.^{2} pressure for 3 hr. After filtration the reduction solution was poured into 3 l. of anhydrous ether. The resulting sticky solid was collected with the aid of diatomaceous earth filter aid. The product was extracted from the filter aid with 100 ml. of methanol which was washed with an additional 50 ml. of methanol. The combined methanol solutions were evaporated under vacuum to 10 ml. The crystallization which was initiated by scratching was completed by overnight refrigeration. After adding 10 ml. of butanol the product was collected; yield, 1.7 g. This product was crystallized twice from 17 ml. of butanol by adding triethylamine to cause solution and then acidifying with concentrated hydrochloric acid; yield 1.3 g; radioactivity 2.3 curies/mole.

6-Demethyl-6-deoxytetracycline-7-^{3}H.—This material was prepared in the same manner as 6-deoxytetracycline-7-^{3}H starting with 7-chloro-6-demethyltetracycline. The material used for further reactions had a radioactivity of 2.73 curies/mole.

6-Demethyl-6-deoxy-9-nitrotetracycline-7-^{3}H.—The nitration of 6-demethyl-6-deoxytetracycline-7-^{3}H was carried out as described for the nontritiated material and the isolation and purification of the 9-nitro isomer was accomplished as previously described; radioactivity, 2.07 curies/mole.[10] The 7-nitro derivative was also isolated from this reaction mixture and was radioactive to the extent of only 0.11 curie/mole.

Nitration of 6-Deoxytetracycline-7-^{3}H.—The nitration and isolation of the product was carried out as described above for the non-tritiated material. The product, a mixture of the two isomers having a radioactivity of 2.3 curies/mole,

(12) A similar preparation was first described by T. André and S. Ullberg, *J. Am. Chem. Soc.*, **79**, 494 (1957).

(13) All of the tritium labeled compounds described herein were examined by paper chromatography coupled with scanning of the chromatograms for radioactive zones using sensitive Radiochromatogram Scanner designed by Dr. M. Bullock of these laboratories. This technique was of particular importance in the case of the nitro-6-deoxytetracyclines since each isomer was not isolated in a pure form.

(14) We are indebted to J. R. D. McCormick and E. R. Jensen for supplying the specific directions for preparing these 6-deoxytetracyclines (see German Patent 1,082,905, June 9, 1960).

was separated on a paper strip chromatogram using the system butanol:phosphate buffer (pH 2.0) into 6-deoxy-7-nitrotetracycline and 6-deoxy-9-nitrotetracycline-7-^{3}H. A radio-scan[13] of this chromatogram showed very little radioactivity for the 7-nitro and a great deal of radioactivity for the 9-nitro-7-^{3}H derivative.

Acknowledgments.—We wish to express our appreciation to Mr. A. C. Dornbush for his coöperation with microbiological assays and to Dr. D. A. Buyske for obtaining the radioactivity determinations. We are also indebted to Mr. L. Brancone for microanalyses and to Mr. W. Fulmor for spectroscopic results.

Synthetic Amebicides. VI. Benzo[b][1,8]phenanthrolines, Benzo[b][1,10]phenanthrolines, Dibenzo[b,h][1,6]naphthyridines, and Benzo[h]quino[4,3-b]quinolines[1]

EDWARD F. ELSLAGER AND FRANK H. TENDICK

Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan

Received November 17, 1961

Various 7-(mono- and dialkylaminoalkylamino)-benzo[b][1,8]phenanthrolines, -benzo[b][1,10]phenanthrolines and -dibenzo[b,h][1,6]naphthyridines have been prepared for antiamebic evaluation. These compounds were prepared by the condensation of the appropriate 7-chloroheterocyclic compound with a mono or dialkylaminoalkylamine in phenol. When tested against *Entamoeba histolytica in vitro* and against experimentally induced intestinal amebiasis in the rat, several of these compounds exhibited good antiamebic activity. Attempts to prepare 3-chloro-7-(3-diethylaminopropylamino)benzo[h]quino[4,3-b]quinoline were unsuccessful.

The preparation of various 7-(mono and dialkylaminoalkylamino)-benz[c]acridines as potential antiamebic agents has been reported previously.[2-4] Many of these compounds are more potent than 4-

(1) For previous paper in this series, see E. F. Elslager, and L. M. Werbel, *J. Org. Chem.*, **26**, 1337 (1961).

(2) E. F. Elslager, A. M. Moore, F. W. Short, M. J. Sullivan, and F. H. Tendick, *J. Am. Chem. Soc.*, **79**, 4699 (1957).

(3) F. W. Short, E. F. Elslager, A. M. Moore, M. J. Sullivan, and F. H. Tendick, *ibid.*, **80**, 223 (1958).

(4) E. F. Elslager, F. W. Short, M. J. Sullivan, and F. H. Tendick, *ibid.*, **80**, 451 (1958).