purified by distillation or crystallization. In some cases, the crude products were used.

1,1-Diaryl-2-propynyl N,N-Disubstituted Carbamates. The following specific examples represent the three methods used for making the carbamates listed in Table I. They are designated as methods A, B, and C.

Method A. 1-(4-Bromophenyl)-1-phenyl-2-propynyl Carbamate. A solution of 57.4 g (0.2 mole) of 1-(4-bromophenyl)-1-phenyl-2-propyn-1-ol and 40 ml of pyridine in 200 ml of CH_2Cl_2 was cooled to 0° and 31.3 g (0.2 mole) of phenyl chloroformate was added dropwise with stirring over 30 min. Stirring and cooling were continued for 4 hr. Ice water (200 ml) and 500 ml of ether were added, and the organic layer was separated and washed with excess 5% HCl, saturated $NaHCO_3$, and water. After drying ($MgSO_4$), the solution was added dropwise to 400 ml of liquid NH_3 over a 15-min period. The resulting mixture was stirred for 16 hr and washed with 5% NaOH solution. After drying over $MgSO_4$, the solvent was removed and the residue was crystallized from benzene–petroleum ether (bp 35–60°). A white, crystalline solid was obtained: yield 6.5 g (25%) (see Table I, **45**).

Method B. 1-(4-Bromophenyl)-1-phenyl-2-propynyl 1-Pyrrolidinecarboxylate. The same procedure was used as above to make the phenyl carbonate intermediate on a 0.1 mole scale. The ether CH_2Cl_2 solution of the intermediate was added to 50 ml of pyrrolidine in an equal volume of ether, and the mixture was stirred for 16 hr at room temperature and washed with excess 5% HCl then 5% NaOH solution. After drying, the solvent was removed at reduced pressure. The residue was crystallized from benzene–petroleum ether in a yield of 20% (Table I, **49**).

Method C. 1,1-Diphenyl-2-propynyl N-Methylcarbamate. A solution of 0.1 mole of 1,1-diphenyl-2-propyn-1-ol, 0.25 mole of methyl isocyanate, and 1 g of triethylenediamine in 200 ml of $CHCl_3$ was left for 4 days at room temperature. All of the low-boiling materials were removed at reduced pressure and the residue was recrystallized twice from benzene–petroleum ether. The product was obtained in a 30% yield (Table I, **5**).

1,1-Diphenylallyl N,N-dimethylcarbamate. A solution of 27.9 g of 1,1-diphenyl-2-propynyl N,N-dimethylcarbamate in 200 ml of 4:1 benzene–petroleum ether (bp 85–100°) was hydrogenated at 40 psi using 0.5 g of 5% Pd–$BaSO_4$ and 0.5 g of KOH until 1 equiv of hydrogen was taken up. The catalyst was filtered and the solvent was removed at reduced pressure. The residue was crystallized twice from petroleum ether; yield 27% (Table I, **82**).

In a similar manner, 1-(4-chlorophenyl)-1-phenyl-2-propynyl carbamate was hydrogenated to **1-(4-chlorophenyl)-1-phenylallyl carbamate** (Table I, **83**).

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The 6-Deoxytetracyclines. VII. Alkylated Aminotetracyclines Possessing Unique Antibacterial Activity

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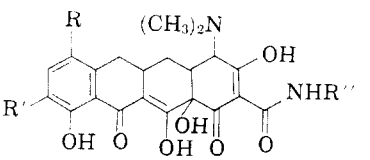
The reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline gives 7-dimethylamino-6-demethyl-6-deoxytetracycline (VI). Similarly, reductive alkylation of other nitro- or aminotetracyclines forms related derivatives. An *in vitro* spectrum of VI is presented and its unique activity against tetracycline-resistant staphylococci is discussed.

Nitration of 6-demethyl-6-deoxytetracycline (I) in strong acid results in electrophilic substitution at the 7 and 9 positions.[1] Subsequent reactions of these substances to form amino, diazonium, and further transformation products have been the subject of previous papers from these and other laboratories.[2]

In the course of new investigations into the chemistry of these modified antibiotics in the hope of further enhancing the pronounced antibacterial activity possessed by several members of this series, or to find new types of antibacterial activity (*i.e.*, broadened spectrum of activity) we had occasion to examine the reductive alkylation of these substances.

Reductive methylation of 7- or 9-nitro- (II or III) or -amino-6-demethyl-6-deoxytetracycline (IV or V) in methoxyethanol under restricted pH conditions using 10% palladium-on-charcoal catalyst at atmospheric pressure, gave 7- or 9-dimethylamino-6-demethyl-6-deoxytetracycline (VI or VII) and their 4-epimers. These compounds could be purified using liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth. The reaction was



I, R = R′ = R″ = H
II, R = NO_2; R′ = R″ = H
III, R = R″ = H; R′ = NO_2
IV, R = NH_2; R′ = R″ = H
V, R = R″ = H; R′ = NH_2
VI, R = $(CH_3)_2N$; R′ = R″ = H
VII, R = R″ = H; R′ = $(CH_3)_2N$
VIII, R = $(CH_3)_2N$; R′ = H, R″ = CH_2N
IX, R = $(C_2H_5)_2N$; R′ = R″ = H

(1) J. Petisi, J. L. Spencer, J. J. Hlavka, and J. H. Boothe, *J. Med. Pharm. Chem.*, **5**, 538 (1962); J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *J. Am. Chem. Soc.*, **82**, 1003 (1960).

(2) J. J. Hlavka, A. Sehneller, H. Krazinski, and J. H. Boothe, *ibid.*, **84**, 1426 (1962); J. J. Hlavka, H. Krazinski, and J. H. Boothe, *J. Org. Chem.*, **27**, 3674 (1962); J. L. Spencer, J. J. Hlavka, J. Petisi, H. M. Krazinski, and J. H. Boothe, *J. Med. Chem.*, **6**, 405 (1963); J. J. Hlavka and H. M. Krazinski, *J. Org. Chem.*, **28**, 1422 (1963); C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, K. Murai, R. K. Blackwood, and M. Schach, von Wittenau, *J. Am. Chem. Soc.*, **85**, 2643 (1963).

remarkably sensitive to the amount of acid used. Less than 2 equiv of acid resulted in up to 30% epimer formation.

Assignment of the structures was established primarily by the use of high-resolution mass spectrometry, radiolabeling experiments, and X-ray crystallographic structural analysis. Other attempts such as nmr to define the structure gave ambiguous results.

Nmr spectra of IV in $CDCl_3$ revealed a sharp singlet at τ 7.58 corresponding to the dimethylamino group. However VI, in the N-methyl region, showed a doublet. One peak at τ 7.42 was approximately twice the peak height of the other peak which was at 7.54 (return to base line was not achieved obviating precise integration). Similar results were obtained with VII as solution in hexadeuteriodimethyl sulfoxide.

High-resolution mass spectrometry provided a more definitive tool for assignment of the structures resulting from the reductive alkylation. A value of m/e 457.1818 was found for VI (as the hydrochloride) as opposed to a calculated value of 457.1849 for which only a molecular formula of $C_{23}H_{27}N_3O_7$ was consistent. A similar result was obtained for the 9-dimethylamino compound VII.

Additional support for the structure of VI was furnished through radiolabeling experiments. If the reductive alkylation were carried out on II using 2 equiv of ^{14}C-formaldehyde, the resultant product had a molar specific activity of twice that of the starting formaldehyde, demonstrating the fact that two methyl groups were incorporated into the molecule. As a final confirmation of structural assignment, a monohydrobromide of VI was prepared and a crystal was subjected to X-ray crystallographic analysis by Dr. J. Van den Hende of these laboratories. The results show the structure of VI to be as pictured and details of this study will be published elsewhere.

Compound VII could be crystallized easily from methanol but VI could be obtained only as a bright yellow-orange solvated amorphous solid although a crystalline monohydrochloride dihydrate could be made. A crystalline "Mannich" derivative of VI was prepared. This pyrrolidinomethyl derivative VIII which analyzed well for the assigned structure was prepared from VI and molar quantities of formaldehyde and pyrrolidine in methoxyethanol. Curiously VIII was quite water insoluble and resistant to acid hydrolysis which is contrary to the behavior of related substances of this type, which find dosage advantage in their extreme water solubility. This derivative was also very insoluble in almost all organic solvents as well.

Reductive alkylation with acetaldehyde instead of formaldehyde gave 7-diethylamino-6-demethyl-6-deoxytetracycline (IX) which was also purified by liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth.

The biological activity of VI, VIII, and IX is outstanding and unique among the known tetracyclines. Table I illustrates the *in vitro* spectrum of VI as compared to tetracycline. It should be noted that against tetracycline-sensitive organisms, 7-dimethylamino-6-demethyl-6-deoxytetracycline (VI) shows a somewhat greater activity than tetracycline. However, the unique difference is illustrated by the infinitely greater activity against the tetracycline-resistant staphylococci.

TABLE I

ACTIVITY OF TETRACYCLINE AND DERIVATIVES

Organism	Min inhib concn, μg/ml: Tetracycline·HCl	VI
Tetracycline sensitive		
Bacillus cereus ATCC 10702	0.5	0.25
Bacillus subtilis ATCC 6633	1.0	0.25
Escherichia coli ATCC 9637	15.0	8.0
Mycobacterium ranae	2.0	0.5
Mycobacterium smegmatis ATCC 607	2.0	0.5
Proteus vulgaris ATCC 9484	15.0	2.0
Pseudomonas aeruginosa ATCC 10145	31.0	31.0
Salmonella galinarium Lederle 604	15.0	15.0
Streptococcus faecalis ATCC 8043	2.0	1.0
Streptococcus pyogenes C203	0.5	0.5
Staphylococcus aureus ATCC 6538P	2.0	1.0
Staphylococcus aureus strain Smith	1.0	0.5
Tetracycline resistant[a]		
Staphylococcus aureus		
strain Rose ATCC 14154	250	4
strain No. 3	250	8
5	250	4
9	>250	4
15	>250	8
20	>250	4
23	>250	8

[a] These strains of *S. aureus* are pathogenic clinical isolates resistant to tetracycline and several other antibiotics. All of these resistant strains are virulent for mice.

In addition, the development of resistance by repeated growth cycles in the presence of sublethal amounts of VI of different strains of staphylococci was markedly slower and to much less a degree than a number of antibiotics including tetracycline. For example, against *S. aureus* strain Smith after seven serial transfers, the minimum inhibitory concentration of VI had increased by 64-fold (from 0.03 to 2.0 μg/ml), while the corresponding concentrations of tetracycline and novobiocin had increased by 1024- and 512-fold, respectively. This low level of resistance to VI remained stable for at least 25 transfers.

The *in vivo* activity of VI closely parallels that of the *in vitro* results, especially against the resistant staphylococci and the mycobacteria. In addition, oral dosage in animals produces higher blood levels than does tetracycline.[3]

The *in vitro* and *in vivo* activity of VIII and IX is similar to but less than VI while VII is devoid of unique antibacterial activity.

Experimental Section

Descending paper chromatography was determined on 16-hr preequilibrated Whatman No. 1 paper buffered with phosphate–citrate buffer pH 3.4 and run at 0° for about 3 hr in a system of nitromethane–benzene–pyridine–pH 3.4 buffer (40:20:6:6). The spots were developed by dipping the dried sheet in an acidic methanolic solution of magnesium acetate. Liquid–liquid partition chromatography was carried out on neutral (acid-washed) diatomaceous earth (Celite). Nmr spectra were determined on a Varian Associates HR-60 or A-60 instrument using tetramethylsilane as the internal standard. Analyses were performed by Mr. L. Brancone and staff and by Schwarzkopf Microanalytical Laboratory of Woodside, N. Y. Rotations were determined in 0.1 *N* HCl. Mass spectra were determined on an M.S.-9 or a CEC-21-110 instrument.

(3) G. S. Redin, 6th Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct 26–28, 1966, Philadelphia, Pa., paper 152.

7-Dimethylamino-6-demethyl-6-deoxytetracycline.—A solution of 2.23 g of 7-nitro-6-deoxy-6-demethyltetracycline monosulfate, 6 ml of 40% aqueous formaldehyde, 110 ml of reagent methoxyethanol, 8.4 ml of 1 *N* H_2SO_4, and 0.4 g of 10% Pd–C catalyst was reduced at atmospheric pressure and room temperature. Uptake was complete (425 ml) in 45 min. The catalyst was removed by filtration on a pad of diatomaceous earth and the filtrate was poured slowly into 3 l. of dry ether. The precipitate was filtered off, washed well with dry ether, and dried in a vacuum desiccator; 2.41 g.

The material was converted to its neutral form by dissolving in absolute methanol, adjusting the pH to an apparent reading of 5.0 with a solution of sodium methoxide in absolute methanol, filtering off the Na_2SO_4, and evaporating to dryness.

Liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth using a system heptane–ethyl acetate–methanol–water (50:50:15:6) gave the product in the second hold-back volume as a yellow-orange amorphous solid: $[\alpha]^{25}D$ − 166° (*c* 0.524); $\lambda_{max}^{0.1\ N\ HCl}$ 352 mμ (log ε 4.16), 263 mμ (log ε 4.23); $\lambda_{max}^{0.1\ N\ NaOH}$ 380 mμ (log ε 4.30), 243 mμ (log ε 4.38). Paper chromatography showed a spot representing the product at R_f 0.65 with the 4-epimer having an R_f of 0.25. Fluorescent quantitative scans of the paper strips revealed less than 5% of epimer present.

Anal. Calcd for $C_{23}H_{27}N_3O_7$: C, 60.38; H, 5.95; N, 9.19; NCH_3(as CH_3), 13.15; mol wt, 457. Found: C, 60.55; H, 6.44; N, 9.45; NCH_3, 10.04, 10.33, 9.92; mol wt (thermistor), 487, 496.

The monohydrochloride dihydrate could be prepared in 90% yield by dissolving the neutral form in four times its weight of water and adjusting the pH to 3.8 with concentrated hydrochloric acid.

Anal. Calcd for $C_{23}H_{27}N_3O_7 \cdot HCl \cdot 2H_2O$: C, 52.12; H, 6.09; N, 7.93; Cl, 6.69; NCH_3, 11.34; H_2O, 6.85; mol wt (base), 457.1849. Found: C, 52.15; H, 6.19; N, 7.79; Cl, 6.72; NCH_3, 9.01; H_2O, 6.50 (vpc), 6.47 (l.o.d.); mol wt (mass spectroscopy), 457.1818.

7-^{14}C-Dimethylamino-6-demethyl-6-deoxytetracycline.—A mixture of 1.3925 g (2.5 mmoles) of 7-nitro-6-demethyl-6-deoxytetracycline monosulfate, 50 ml of methoxyethanol, 250 mg of 10% palladium on carbon, and 5 ml of 1.5 *N* aqueous sulfuric acid solution was reduced at atmospheric pressure and room temperature. Uptake was complete in 45 min (173 ml, theory 192 ml). At this point two ampoules containing 5.48 ml each of ^{14}C-formaldehyde solution [75 mg (2.5 mmoles) of formaldehyde in each vial containing 25 mcuries of activity for each] (New England Nuclear Corp. lot 212-209-5a, 5b) were added and washed in with 15 ml of methoxyethanol. Reduction was resumed; another 74 ml of hydrogen was consumed in the next 2 hr. The catalyst was filtered off on a pad of diatomaceous earth and the pad was washed with 4 ml of methoxyethanol. The filtrate was slowly added to a solution of 250 ml of ether and 750 ml of *t*-butyl alcohol. The resultant precipitate was filtered off, washed well with ether, and dried. The solid was dissolved in 75 ml of dry methanol and neutralized to pH 5.5 by the addition of *ca.* 4.5 ml of 10% sodium methoxide in methanol. After filtration, the solution was evaporated to dryness. The crude material was chromatographed on a 100-g column of diatomaceous earth using a system heptane–ethyl acetate–methanol–water (70:30:15:4) and the bright yellow band was collected: 323 mg; specific activity, 47 μcuries/mg (average of two); molar specific activity, 20.4 mcuries/mmole.

2-Carboxamido-N-pyrrolidinomethyl-7-dimethylamino-6-demethyl-6-deoxytetracycline.—To a solution of 640 mg of 7-dimethylamino-6-demethyl-6-deoxytetracycline disulfate in 6 ml of methoxyethanol was added 92 mg of 40% aqueous formaldehyde in 0.5 ml of methoxyethanol and 360 mg of redistilled pyrrolidine in 0.5 ml of methoxyethanol. The brown solution was allowed to stir at room temperature for 1 hr during which time yellow crystals appeared. The crystals were filtered off and washed with 2 ml of methoxyethanol and then with 50 ml of dry ether. The solid was dried in a vacuum desiccator; weight 300 mg. Paper chromatography showed a single spot at R_f 0.0. A sample was allowed to stand overnight in 0.1 *N* HCl and paper chromatography showed about 50% hydrolysis to starting material.

Anal. Calcd for $C_{28}H_{36}N_4O_7$: C, 62.20; H, 6.71; N, 10.36. Found: C, 62.36; H, 6.73; N, 10.18.

7-Diethylamino-6-demethyl-6-deoxytetracycline.—A solution of 278 mg (0.5 mmole) of 7-nitro-6-demethyl-6-deoxytetracycline sulfate, 14 ml of reagent methoxyethanol, 1.05 ml of 1 *N* H_2SO_4, and 0.5 ml of acetaldehyde was reduced with 50 mg of 10% Pd–C as described above. The disulfate salt which resulted was dried, 290 mg. The R_f of the material was at 0.72 and that of its 4-epimer was at 0.31.

Anal. Calcd for $C_{25}H_{31}N_3O_7 \cdot 2H_2SO_4$: N, 6.16; S, 9.41. Found: N, 5.98; S, 9.32.

The neutral form was prepared as described above and subjected to liquid–liquid partition chromatography on neutral (acid-washed) diatomaceous earth using a system heptane–ethyl acetate–methanol–water (70:30:15:6), the product appearing in the second hold-back volume; $[\alpha]^{25}D$ −182° (*c* 0.528); $\lambda_{max}^{0.1\ N\ HCl}$ 352 mμ (log ε 4.08), 263 mμ (log ε 4.20); $\lambda_{max}^{0.1\ N\ NaOH}$ 380 mμ (log ε 4.25), 242 mμ (log ε 4.33); molecular weight 485 (mass spectroscopy) (calcd 485).

9-Dimethylamino-6-demethyl-6-deoxytetracycline.—A solution of 233 mg (0.5 mmole) of 9-amino-6-demethyl-6-deoxytetracycline monohydrochloride in 14 ml of methoxyethanol, 0.75 ml of 40% aqueous formaldehyde, 0.5 ml of 2 *N* H_2SO_4, and 50 mg of 10% Pd–C was reduced as described above. The mixed salt so prepared (1 g) was dissolved in 75 ml of methanol and adjusted to an apparent pH of 6.0 with triethylamine. Crystals appeared, which after several minutes were collected by filtration, washed with 10 ml of methanol, and dried, 309 mg; R_f 0.45; $[\alpha]^{25}D$ −210° (*c* 0.533); $\lambda_{max}^{0.1\ N\ HCl}$ 352 mμ (log ε 4.16), 265 mμ (log ε 4.23); $\lambda_{max}^{0.1\ N\ NaOH}$ 377 mμ (log ε 4.30), 245 mμ (log ε 4.38).

Anal. Calcd for $C_{23}H_{27}N_3O_7 \cdot 0.5H_2O$: C, 59.22; H, 6.05; N, 9.01; NCH_3, 12.89. Found: C, 59.09; H, 6.17; N, 9.35; NCH_3, 10.03; mol wt (mass spectroscopy), 457.

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