on distillation gave a main fraction (9.2 g., 63.5%), b.p. 112–115° (2 mm.), $n^{26}D$ 1.4726. The bisphenylurethane melted at 160–161°. Lit.[10] b.p. for *trans*-3-hexene-1,6-diol, 88–90° (0.3 mm.), $n^{18}D$ 1.4747 (prepared by a different route). Lit. m.p. for the bisphenylurethane, 161–162°.

***trans*-3-Hexene-1,6-bistrimethylammonium Dibromide (I).**—A solution of 5.8 g. (0.05 mole) of *trans*-3-hexene-1,6-diol in 2.6 g. (0.063 mole) of dry pyridine was cooled to −30°, and 9 g. (0.033 mole) of phosphorus tribromide was added dropwise with stirring at −20 to −10°. After another hour at −10° the mixture was distilled and 7.0 g. (58%) of *trans*-1,6-dibromo-3-hexene, b.p. 84–85° (5 mm.); $n^{28}D$ 1.5228, was collected.[18] This oil was dissolved in 25 ml. of ether, cooled to −40°, 25 ml. of a 25% methanolic solution of trimethylamine was added, and the solution was allowed to stand in a pressure bottle at 25° for 5 days. The precipitated colorless solid was filtered off and recrystallized from ethanol. The colorless needles, m.p. 279–281° dec., represented *trans*-3-hexene-1,6-bis-trimethylammonium dibromide sesquihydrate (6.7 g., 71%).

Anal. Calcd. for $C_{12}H_{28}Br_2N_2 \cdot 1.5\ H_2O$: C, 37.23; H, 8.07. Found: C, 37.34, 37.07; H, 8.19, 8.14.

The **dipicrate** was prepared in aqueous solution and recrystallized from acetone; yellow needles, m.p. 263–264°.

Anal. Calcd. for $C_{24}H_{32}N_8O_{14}$: C, 43.90; H, 4.91. Found: C, 43.49; H, 5.11.

The dibromide (0.35 g.) was saturated by hydrogenation in methanolic solution over a palladium oxide catalyst at room temperature and pressure. One mole of hydrogen was absorbed, and the product was purified by crystallization from ethanol. Colorless needles (0.32 g., 91%), m.p. 272–273° dec. Hexamethonium dibromide melts at 272° dec.[2b]

***trans*-1,2-Cyclopropanedimethyl Ditosylate (VI).**—To a solution of 60 g. of *p*-toluenesulfonyl chloride in 120 ml. of dry pyridine at −5° was added a solution of 14 g. of *trans*-1,2-di-(hydroxymethyl)cyclopropane in 28 ml. of dry pyridine over a period of 30 min. After standing for another hour at −10 to −5°, the mixture was poured into ice water, most of the pyridine was neutralized with 25% sulfuric acid, and the precipitated white solid was filtered and recrystallized from methanol.[19]

***trans*-1,2-Cyclopropanediacetonitrile (VII).**—A solution of VI (40 g.) and 50 g. of sodium iodide in 280 ml. of acetone was stirred and refluxed for 1 hr., sodium *p*-toluenesulfonate was filtered off, the filtrate concentrated, and the residue dissolved in water and extracted with ether. The ether extracts were washed with sodium thiosulfate solution, dried, and evaporated. The residual oily *trans*-1,2-di(iodomethyl)cyclopropane (28 g.) was dissolved in 140 ml. of ethanol and treated, with stirring, with a solution of 22 g. of potassium cyanide in 35 ml. of water. The mixture was refluxed for 4 hr., concentrated *in vacuo* to 50 ml., and extracted with ten 50-ml. portions of ether.

***trans*-1,2-Di-(β-aminoethyl)cyclopropane (IX).**—(a) A saturated ammoniacal methanolic solution (350 ml.) of VII (4 g.) was hydrogenated with Raney nickel at 3 kg./cm.² for 12 hr.; the catalyst was filtered and the oily residue distilled. (b) To a solution of 1.2 g. of VII in absolute ethanol (100 ml.) was added sodium (2.5 g.) in small pieces, a steady evolution of hydrogen being maintained. Then concentrated hydrochloric acid was added to pH 1; the mixture was concentrated *in vacuo* and ether extracted. The ether solution was concentrated, neutralized with ethereal *p*-aminobenzoic acid, and the precipitated oil was triturated with ethanol. It crystallized after 1 week and was recrystallized from ethanol. A mixture melting point with a sample prepared by method (a) was undepressed, and the infrared spectra of the two salts were identical.

***trans*-1,2-Di-(β-dimethylaminoethyl)cyclopropane (X).**—To 10.5 ml. of 95% formic acid was added 2.6 g. of the diamine IX, then 10 ml. of formaldehyde solution, and the mixture was heated on a steam bath for 9 hr. After cooling and addition of 20 ml. of 4 *N* hydrochloric acid, it was concentrated to about 12 ml. under reduced pressure, made basic with 20% sodium hydroxide solution, ether extracted, and worked up.

***cis*-1-(β-Bromoethyl)-2-(β-trimethylammoniumethyl)cyclopropane Bromide.**—*cis*-1,2-Di-(hydroxyethyl)cyclopropane[11] (3.2 g.) was treated with phosphorus tribromide as described for *trans*-1,6-dibromo-3-hexene above. The resulting *cis*-1,2-di-(bromoethyl) cyclopropane (2.1 g., 33%) had b.p. 102–104° (4 mm.), $n^{28}D$ 1.5245. A solution of 2 g. of this dibromo compound in 10 ml. of ether was added to 15 ml. of a 25% ethanolic solution of trimethylamine at −50° and allowed to stand in a pressure bottle for 14 days. Evaporation furnished an oil which crystallized to a colorless solid on treatment with ethyl acetate and acetone. Recrystallization from ethanol gave 1.8 g. (73%) of needles, m.p. 287–289° dec.

Anal. Calcd. for $C_{10}H_{21}Br_2N$: C, 38.12; H, 6.72. Found: C, 38.51; H, 7.02.

When this salt (0.5 g.) was heated with 30 ml. of a 12.5% anhydrous methanolic solution of trimethylamine in a sealed tube at 100–110° for 7 days and the reaction mixture was evaporated, a waxy residue was obtained from which 0.1 g. (20%) of tetramethylammonium bromide was elaborated by trituration with 30 ml. of dry acetone. It sublimed above 200°.

Anal. Calcd. for $C_4H_{12}BrN$: C, 31.18; H, 7.85. Found: C, 31.05; H, 7.46.

The residual oil was converted to a **dipicrate** which crystallized from ethanol, m.p. 175.5–177°.

Anal. Calcd. for $C_{23}H_{30}N_8O_{14}$: C, 43.00; H, 4.71; N, 17.44. Found: C, 42.90; H, 4.61; N, 18.06.

(18) This dibromo compound has been mentioned without any description by LeR. W. Clemence and M. T. Leffler, U. S. Patent 2,545,876 (March 20, 1951).

(19) A. T. Bloomquist and D. T. Longone, *J. Am. Chem. Soc.*, **81**, 2012 (1959).

6-Deoxytetracyclines. V.[1a] 7,9-Disubstituted Products

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The preparation and antibacterial activity of a number of 7 and 9 disubstituted 6-deoxytetracyclines are described.

The successful introduction of various functional groups into the 7 or 9 positions of the aromatic ring of the 6-deoxytetracyclines[2] and the effects of these substituents on *in vitro* antibacterial activity have been previously reported.[3,4] The retention of broad spectrum antibiotic properties by many of these new derivatives as well as a marked increase in antibacterial potency in some cases was noted. In order to under-

(1) (a) For the previous paper in this series see J. Hlavka, H. Krazinski, and J. Boothe, *J. Org. Chem.*, **27**, 3674 (1962); a preliminary report of this material has been published in *J. Am. Chem. Soc.*, **82**, 1253 (1960). (b) Please address reprint requests to J. H. Boothe.

(2) Recently it has been shown that the stereochemistry of the 6 position in the 6-deoxytetracyclines is the unnatural or *epi* configuration (β), M. Schach von Wittenau, J. Beereboom, R. Blackwood, and C. Stephens, *J. Am. Chem. Soc.*, **84**, 2645 (1962).

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

(4) J. J. Hlavka, A. Schneller, H. Krazinski, and J. H. Boothe, *ibid.*, **84**, 1426 (1962).

TABLE I

In Vitro INHIBITORY POTENCIES AGAINST *Staphylococcus aureus*,[a] R_f VALUES,[b] AND ULTRAVIOLET SPECTRA

Derivative of 6-deoxytetracycline	Biological activity	R_f values	0.1 *N* HCl λ_{max}	log ϵ
III. 6-Demethyl-7,9-dinitro	60	0.40	255	4.50
			365	4.61
IV. 7-Chloro-6-demethyl-9-nitro	21	.68	263	4.47
			380	4.16
V. 7-Bromo-6-demethyl-9-nitro	15	.63	370	4.09
			450	3.70
VI. 9-Amino-7-chloro-6-demethyl	525	.20	260	4.25
			348	4.11
VII. 9-Amino-7-bromo-6-demethyl	320	.20	275	3.13
			380	3.03
VIII. 9-Amino-6-demethyl-7-nitro	275	.25	261	4.37
			348	4.16
IX. 9-Amino-7-nitro	160	.48	261	4.32
			348	4.14
X. 9-Acetamido-7-nitro	15	.60	252	4.41
			297	4.23
XI. 9-Amino-7-bromo	140	.80	265	4.31
			348	4.07
XII. 9-Acetamido-7-bromo	75	.75	250	4.36
			345	4.16

[a] Antibacterial activities measured by the turbidimetric assay of E. Pelcak and A. C. Dornbush, *Ann. N. Y. Acad. Sci.*, **51**, 218 (1948), using *Staph. aureus* as the test organism. Tetracycline, having a biological activity of 100, is used as standard. [b] R_f values determined on Whatman No. 1 paper buffered at pH 2.0 using the system butanol:0.2 *M* phosphate buffer (pH 2.0).

of 6-deoxytetracycline (II) with one equivalent of nitrate yielded a mixture of 7 and 9 nitro products. This was in contrast to the nitration procedure using two equivalents of nitrate, in which case only the 9-nitro isomer was isolated. This result was interpreted to mean that the 7-nitro derivative was quickly nitrated to a dinitro compound which was then lost during isolation while the 9-nitro isomer was not nitrated under the reaction conditions and was readily isolable. Although this reaction was not pursued any further in the 6-deoxytetracycline series, it was subsequently shown that 6-demethyl-6-deoxy-7-nitrotetracycline[3] could be easily nitrated to give the 7,9-dinitro derivative (III) while under identical nitrating conditions the 6-demethyl-6-deoxy-9-nitrotetracycline[3] was recovered unchanged.

Similar nitrations of 7-chloro[5]- and 7-bromo[4]-6-demethyl-6-deoxytetracyclines yielded the corresponding 7-halo-9-nitro derivatives (IV and V) which have the characteristic absorption spectra of the 9-nitro compounds.[3] Careful reduction of these products yielded 9-amino-7-chloro-6-demethyl-6-deoxytetracycline (VI) and 9-amino-7-bromo-6-demethyl-6-deoxytetracycline (VII), respectively.

Although the amino groups might be expected to

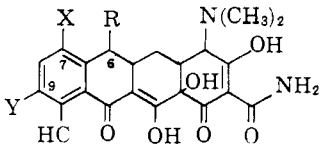
TABLE II

PREPARATIVE AND ANALYTICAL DATA OF DISUBSTITUTED TETRACYCLINES

Product	Starting compound 6-deoxytetracycline	Method	Yield, %	Product formula	Elemental analyses, % — Calculated — C	H	N	X	S	Found — C	H	N	X	S
III	6-DM[a]-7-NO_2·H_2SO_4[b]	B,45 min.	15	$C_{21}H_{20}N_4H_{11}\cdot H_2O$	48.3	4.2	10.7			48.4	4.3	10.8		
IV	7-Cl-6-DM·H_2SO_4	B,15 min.	60	$C_{21}H_{20}ClN_3O_9\cdot 1.5H_2O$	48.4	4.5	8.1	6.8		48.4	4.6	8.2	6.9	
V	7-Br-6-DM·H_2SO_4	B,10 min.	60	$C_{21}H_{20}BrN_3O_9$			7.8	15.1				7.5	15.4	
VI	7-Cl-6-DM-9-NO_2	D	85	$C_{21}H_{22}ClN_3O_7\cdot 2H_2SO_4$	38.2	4.0	6.4	5.4	9.7	39.0	4.5	6.0	5.0	9.3
VII	7-Br-6-DM-9-NO_2·H_2SO_4	D[c]	7.5	$C_{21}H_{22}BrN_3O_7$	49.6	4.4	8.3			49.1	4.6	8.2		
VIII	9-NH_2-6-DM·HCl	C	85	$C_{21}H_{22}N_4O_9\cdot 1.75H_2SO_4$	39.1	4.6	8.7		8.7	39.0	4.8	8.3		8.7
VIII	7,9-diNO_2·H_2SO_4	D[d]	10											
IX	9-NH_2·H_2SO_4	B,2 min.	60	$C_{22}H_{24}N_4O_9\cdot 2H_2SO_4$	38.6	4.1	8.2		9.4	38.8	4.7	8.0		9.1
X	9-CH_3CONH·H_2SO_4	C	75	$C_{24}H_{26}N_4O_{10}\cdot 0.5H_2O\cdot H_2SO_4$	45.2	4.6	8.8		5.0	45.1	5.2	8.4		5.2
XI	9-NH_2·$2H_2SO_4$	A,30 min.	90	$C_{22}H_{24}BrN_3O_7\cdot H_2O\cdot 2H_2SO_4$	35.9	4.1	5.7	10.9	8.7	36.0	4.5	5.8	10.9	8.6
XII	9-CH_3CONH·H_2SO_4[e]	A,15 min.	12	$C_{24}H_{28}BrN_3O_8\cdot H_2O\cdot H_2SO_4$	42.4	4.4	6.2	11.7	4.7	42.4	4.6	6.3	12.2	5.2

[a] DM = Demethyl. [b] Under identical reaction conditions, 6-DM-9-NO_2·H_2SO_4 was recovered unchanged. [c] No sulfuric acid was added in this case. [d] The selective catalytic reduction of the 9-NO_2 group was accomplished by using 20% of 1 equiv. of concd. sulfuric acid. The identity was determined by ultraviolet spectra and paper chromatography. [e] This product was obtained also by acetylation of the 7-Br-9-NH derivative.

stand more clearly the structure–activity relationships in this series, a number of derivatives substituted in both the 7 and 9 positions were prepared and tested for antibacterial activity.



I, R = X = Y = H
II, R = CH_3, X = Y = H
III, R = H, X = Y = NO_2
IV, R = H, X = Cl, Y = NO_2
V, R = H, X = Br, Y = NO_2
VI, R = H, X = Cl, Y = NH_2
VII, R = H, X = Br, Y = NH_2
VIII, R = H, X = NO_2, Y = NH_2
IX, R = CH_3, X = NO_2, Y = NH_2
X, R = CH_3, X = NO_2, Y = CH_3CONH—
XI, R = CH_3, X = Br, Y = NH_2
XII, R = CH_3, X = Br, Y = CH_3CONH—

The first indication that both the 7 and 9 positions could be readily substituted came during the early nitration studies[3a] with the observation that nitration

deactivate the ring under strongly acidic conditions, the nitration of both 9-amino-6-demethyl-6-deoxytetracycline[3a] and 9-amino-6-deoxytetracycline[3a] yielded 9-amino-6-demethyl-6-deoxy-7-nitrotetracycline (VIII) and 9-amino-6-deoxy-7-nitrotetracycline (IX), respectively. In VIII the proof of the position of the entering nitro group was shown by the displacement of tritium from the 7 position.[3a,4] Compound VIII was also prepared by selective catalytic reduction of the 9-nitro grouping in 6-demethyl-6-deoxy-7,9-dinitrotetracycline (III). The N-acetyl derivative of 9-amino-6-deoxytetracycline[3] was similarly nitrated to yield 9-acetamido-6-deoxy-7-nitrotetracycline (X).

Bromination of either 9-amino-6-deoxytetracycline[3] or 9-acetamido-6-deoxytetracycline[3] under the previously described conditions[4] resulted in the formation of the corresponding 7-bromo derivatives XI or XII.

The *in vitro* antibacterial comparisons of these new disubstituted tetracyclines are shown in Table I.

(5) J. R. D. McCormick and E. R. Jensen, German Patent, 1,082,905 (June 9, 1960); *cf.* T. L. Fields, A. S. Kende, and J. H. Boothe, *J. Am. Chem. Soc.*, **83**, 4617 (1961).

Experimental

A. Bromination.—A 15% molar excess of N-bromosuccinimide was added to a cold concd. sulfuric acid solution of the tetracycline, stirred in an ice bath, and the reaction solution was then poured into cold anhydrous ethyl ether. The resulting precipitate was purified by dissolving in alcohol and precipitating with ether.

B. Nitration.—One equivalent of potassium nitrate was added with stirring to a cold concd. sulfuric acid solution of the tetracycline, and the solution was stirred for the required time. The product, isolated as the sulfate salt by pouring into cold anhydrous ether, was purified by dissolving it in water and precipitating the amphoteric form by adjusting the pH to about 4–5.

C. Nitration.—One equivalent of a 10% (by vol.) solution of concd. nitric acid in sulfuric acid was added to a cold concd. sulfuric acid solution of the tetracycline. This solution was stirred for 3 to 5 min., poured into cold anhydrous ethyl ether, and the precipitate collected. Purification was accomplished by dissolving in alcohol, filtering, and precipitating the product with ether.

D. Catalytic Reduction.—A solution of tetracycline in methanol (5 mg./ml.) containing a molar excess of concd. sulfuric acid and 10% by weight of platinum oxide was shaken in an atmosphere of hydrogen. When the theoretical amount of hydrogen necessary for reduction of the nitro group was absorbed the hydrogenation was stopped. The reduction solution was filtered from the catalyst and the filtrate was concentrated to about 20% of the original volume and added to cold anhydrous ethyl ether. In cases where the amphoteric form is reported, the sulfate was dissolved in water, the solution adjusted to pH 5–6, and the product collected.

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Analogs of the Aminonucleoside Derived from Puromycin. The Synthesis of 3′-Amino-3′-deoxyguanosine and 3′-Amino-3′-deoxycrotonoside

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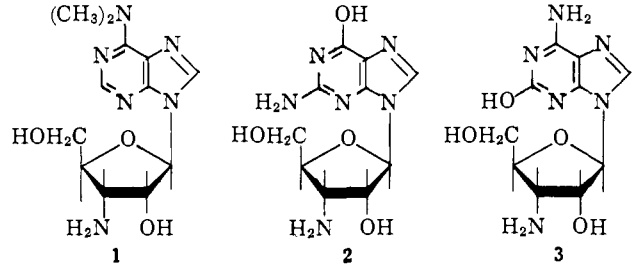
The title compounds were prepared by adaptations of known procedures. These nucleosides were inactive in several standard antitumor assays in mice.

Analogs of the aminonucleoside (**1**)[1] derived from puromycin are of interest because of the trypanocidal[2] and carcinostatic[3] activities exhibited by **1** in experimental animals. It has already been shown that replacement of the 6-dimethylaminopurine moiety of **1** by adenine[4] and hypoxanthine[5] as well as by various 6-alkylamino- and 6-dialkylaminopurines[6] gives nucleosides having considerable biological activity. On the other hand, replacement of the 3-aminoribofuranosyl moiety with the natural ribofuranosyl sugar results in a loss of biological effectiveness.[7] Therefore, we have been interested in the preparation of 3-aminoribofuranosyl derivatives of other naturally-occurring bases[8] and in this paper we wish to report the synthesis of 3′-amino-3′-deoxyguanosine (**2**) and 3′-amino-3′-deoxycrotonoside (**3**).

3′-Amino-3′-deoxyguanosine (**2**) was prepared by an adaptation of the procedure reported by Davoll and Lowy[9] for the synthesis of guanosine. Condensation



of chloromercuri-2,6-diacetamidopurine (**4**)[9,10] with the blocked 1-chloro-3-aminoribofuranose **5**[11] gave the blocked nucleoside **6** in 56% yield. Preferential cleavage of the 6-N-acetyl group (and of the sugar benzoates) was accomplished by heating with diisopropylamine[5,12] in refluxing methanol. The resulting **7** was obtained in 36% yield. Nitrous acid deamination of this partially blocked intermediate then gave the guanosine derivative **8** in 63% yield. In practice, however, it was found more convenient to isolate the deaminated product as the 2,5-di-*O*-acetate **9**. Finally, complete removal of the blocking groups by treatment of **9** with butylamine[6] in refluxing methanol furnished the desired 3′-amino-3′-deoxyguanosine (**2**) in 44% over-all yield from **7**.

For the synthesis of 3′-amino-3′-deoxycrotonoside (**3**),[13] the intermediate nucleoside **6** was treated with

(1) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **77**, 1 (1955).
(2) R. I. Hewitt, A. R. Gumble, W. S. Wallace, and J. H. Williams, *Antibiot. and Chemotherapy*, **4**, 1222 (1954).
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