duction product gave a single spot on paper chromatography, positive to NIN and periodate–permanganate, negative to AHP, R_f 0.157 (BAW 2:2:1), while a simultaneous papergram of the starting diaminohexose showed one spot, positive to both NIN and AHP, R_f 0.201 (BAW 2:2:1).

The crude reduction product was converted to its dinitrophenyl derivative by the procedure described above for the preparation of N,N'-bis-(2,4-dinitrophenyl)-neosamine B. The deep yellow **N,N'-bis-(2,4-dinitrophenyl)-neosaminol B** isolated weighed 0.350 g. (30% from neosamine B dihydrochloride), had m.p. 118–122°, $[\alpha]^{26}D$ +30.5° (*c* 0.39, methanol), R_f (BEW 4:1:5) 0.912.

Anal. Calcd. for $C_{18}H_{20}N_6O_{12}$: C, 42.22; H, 3.95; N, 16.42. Found: C, 41.89; H, 3.86; N, 15.85.

Degradation Products of Framycetin.—Papergrams of framycetin sulfate[41] $[\alpha]^{26}D$ +47.4° (*c* 0.4, water), showed a single NIN-positive spot, R_f (BAW 2:2:1) 0.000, (PAW 10:1:9) 0.184; a simultaneous papergram of neomycin B sulfate had R_f (BAW 2:2:1) 0.000, (PAW 10:1:9) 0.184.

I. **Methyl neobiosaminide B** was obtained by methanolysis of 0.744 g. of framycetin under conditions identical to those employed above for methanolysis of neomycin B. Neamine hydrochloride obtained weighed 0.322 g. and had R_f (BAW 2:2:1) 0.131, (PAW 10:1:9) 0.485; on the same papergram a sample from methanolysis of neomycin B had R_f (BAW 2:2:1) 0.130, (PAW 10:1:9) 0.487. Methyl neobiosaminide B hydrochloride obtained weighed 0.404 g. and had $[\alpha]^{25}D$ +30° (*c* 1.3, water), R_f (BAW 2:2:1) 0.801, (PAW 10:1:9) 0.698. On the same papergram an authentic sample of methyl neobiosaminide B dihydrochloride from methanolysis of neomycin B had R_f (BAW 2:2:1) 0.804, (PAW 10:1:9) 0.699.

A sample of methyl neobiosaminide B hydrochloride from the framycetin methanolysis was purified by preparative descending paper chromatography, employing as solvent system BAW 2:2:1. The purified hydrochloride, $[\alpha]D$ +19.4° (*c* 0.35, 1 *N* aqueous hydrochloric acid), (21.7 mg., corresponding to 17.8 mg. of free base) was dissolved in 5 ml. of 1 *N* aqueous hydrochloric acid and heated in sealed tubes for varying lengths of time at 89°. Optical rotation data are summarized in the following table (*cf.* also Table V) and in Fig. 4. A paper chromatogram of the 360-minute hydrolysate showed one spot, positive to NIN and AHP, R_f (BAW 2:2:1) 0.187, (PAW 10:1:9) 0.541; on the same papergram neobiosamine B dihydrochloride, from neomycin B, had R_f (BAW 2:2:1) 0.190, (PAW 10:1:9) 0.540.

Time, min.	0	120	240	360
$[\alpha]^{25}D$	+19.4°	25	33.3	36

II. **D-Ribose.**—A solution of 50 mg. of methyl neobiosaminide B hydrochloride, from framycetin methanolysis, was deaminated and the deamination product hydrolyzed, precisely as described above for the identification of ribose from neomycin B. The pale yellow hygroscopic solid isolated was negative to NIN, strongly positive to AHP, R_f (BAW 4:5:1) 0.289, (BAW 2:2:1) 0.509, (PAW 10:1:9) 0.642. On the same papergrams the deamination–hydrolysis product from authentic methyl neobiosaminide B (neomycin B starting material) had R_f (BAW 4:1:5) 0.287, (BAW 2:2:1) 0.508, (PAW 10:1:9) 0.640, while authentic ribose had R_f (BAW 4:1:5) 0.290, (BAW 2:2:1) 0.510, (PAW 10:1:9) 0.644.

III. **Neosamine B** was prepared by hydrolysis of 105 mg. of methyl neobiosaminide B hydrochloride (from framycetin), previously purified by preparative descending paper chromatography (BAW 2:2:1). The hydrolysis and isolation procedures were precisely those employed to obtain neosamine B from methyl neobiosaminide B dihydrochloride (neomycin starting material; *cf.* above). The yield of neosamine B hydrochloride, $[\alpha]^{25}D$ +15.6° (*c* 1.07, water), was 46 mg. (70%). A papergram of the sample gave R_f (BAW 2:2:1) 0.250, (PAW 10:1:9) 0.604; on the same papergram an authentic sample of neosamine B dihydrochloride obtained from neomycin B gave R_f (BAW 2:2:1) 0.252, (PAW 10:1:9) 0.610. Both samples gave strongly positive tests with NIN and AHP. The infrared spectra of purified neosamine B (from neomycin B or framycetin) do not contain carbonyl absorption.

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[CONTRIBUTION FROM THE ORGANIC CHEMISTRY RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

Chemistry of the Tetracycline Antibiotics. II. Bromination of Dedimethylaminotetracyclines

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The reaction products obtained by brominating several different dedimethylaminotetracyclines are described as well as some further reactions of these bromo derivatives. The conversion of 12a-deoxydedimethylaminotetracycline to a known degradation product of oxytetracycline, dedimethylaminoterrarubein, is described.

In the course of chemical studies dealing with the tetracycline antibiotics, bromination reactions were carried out on three key tetracycline derivatives lacking the dimethylamino substituent. The compounds investigated were dedimethylaminotetracycline,[1] dedimethylamino-7-chlorotetracycline[1,2] and 12a-deoxydedimethylaminotetracycline.

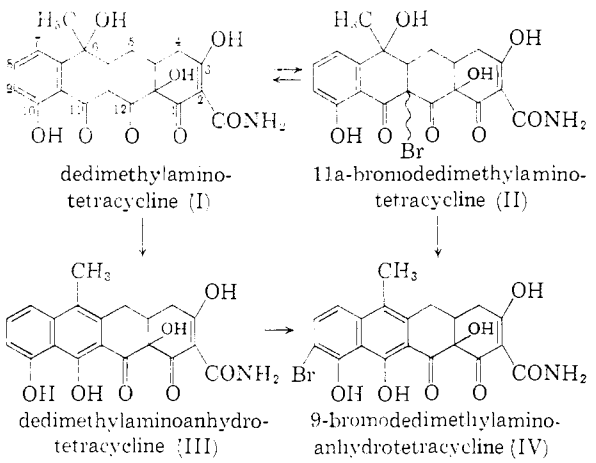
Bromination of a chloroform suspension of dedimethylaminotetracycline at room temperature with one equivalent of N-bromosuccinimide (NBS) led to a crystalline monobromosubstitution product. The same substance was also isolated in poorer yield from reaction of dedimethylaminotetracycline with bromine in acetic acid containing sodium acetate. Introduction of the bromine was observed to result in a 15 mμ hypsochromic shift in the ultraviolet maximum in 0.1 *N* hydrochloric acid to 350 mμ (log ε 3.494) and in the ap-

(1) J. H. Boothe, G. E. Bouvicino, C. W. Waller, J. P. Petisi, R. G. Wilkinson and R. B. Broschard, THIS JOURNAL, **80**, 1654 (1958).

(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, *ibid.*, **76**, 3568 (1954).

pearance of a new sharp maximum at 5.75 in the infrared. Catalytic hydrogenation of the product regenerated the starting dedimethylaminotetracycline, indicating that no cleavage or rearrangement of the molecule had taken place. On the basis of these data the bromination product is assigned structure II, wherein the shortened B–C–D chromophore and the isolated C-12 carbonyl group are in accord with the observed changes in the ultraviolet and infrared spectra, respectively.[3]

When dedimethylaminotetracycline was treated with bromine in acetic acid in the absence of sodium acetate the halogenation was accompanied by dehydration and the product is a bromoanhydrodedimethylaminotetracycline IV. The same compound was formed by dehydration of the previously described 11a-bromodedimethylaminotetracycline with hydrogen bromide in acetic acid or by the action of NBS on anhydrodedimethylaminotetracycline. Although the position of the bromine in this anhydro derivative is not established, its stability toward alkaline substances suggests that the halogen is bound to aromatic carbon. The chlorination of anhydrodedimethylaminotetracycline with N-chlorosuccinimide results in a monochlorosubstitution product which is not identical with anhydrodedimethylamino-7-chlorotetracycline, made by acid dehydration of the known dedimethylamino-7-chlorotetracycline.[2] If the chlorination product also possesses aromatic halogen, then by analogy the bromine atom in IV probably does not occupy position 7; we regard C-9 as the next most probable point of attachment.



Reaction of dedimethylamino-7-chlorotetracycline with NBS in chloroform took an unexpected course and gave as the only recognizable product a dibrominated dedimethylamino-iso-7-chlorotetracycline. With pyridinium bromide perbromide as the halogenating agent a monobromo variant of the dedimethylamino-iso-7-chlorotetracycline was isolated. Bromine in acetic acid afforded a bromoanhydrodedimethylamino-7-chlorotetracycline.

In the case of 12a-deoxydedimethylaminotetracycline both positions C-11a and C-12a are potentially reactive toward electrophilic halogenation. Treatment of 12a-deoxydedimethylaminotetracycline with one equivalent of NBS in chloroform resulted in the selective introduction of bromine at the 12a-position to give crystalline 12a-bromo-12a-deoxydedimethylaminotetracycline (VII). The position of the substituent was indicated by the fact that the ultraviolet spectrum of the product was very similar to that of dedimethylaminotetracycline and distinctly different from that of 12a-deoxydedimethylaminotetracycline[4] or of the previously described 11a-bromo compound. Reaction of 12a-deoxydedimethylaminotetracycline with two equivalents of NBS resulted in 11a,12a-dibromo-12a-deoxydedimethylaminotetracycline (VIII). As expected, this dibromo compound exhibited the infrared peak at 5.75 μ and ultraviolet absorption maximum at 350 mμ previously observed for 11a-bromodedimethylaminotetracycline. Upon treatment of the 11a,12a-dibromo compound with hydrogen bromide the molecule was dehydrated and rearranged to give a crystalline anhydro derivative assumed to have the structure 9,12a-dibromodedimethylaminoanhydrotetracycline (X).

The bromine atom in position 12a of VII readily participates in E2 elimination in the presence of weak bases. Thus, brief treatment of 12a-bromo-12a-deoxydedimethylaminotetracycline with warm triethylamine or pyridine resulted in loss of hydrogen bromide to give 4a,12a-anhydrodedimethylaminotetracycline (XI) which was also encountered in the course of an unrelated investigation described in an accompanying publication.[5]

This intermediate, in which the A and D rings of the molecule are aromatic, underwent the usual acid-catalyzed dehydration to give the bright red dedimethylaminoterrarubein (XII). This fully aromatic compound was proven to be identical with a sample obtained by the method of Hochstein, *et al.*,[6] from degradation of 5-hydroxytetracycline. Thus, through a series of transformations, tetracycline, 7-chlorotetracycline and 5-hydroxytetracycline have been modified and related through a common tetracyclic degradation product.

Experimental[7]

11a-Bromodedimethylaminotetracycline (II).—(1) To a suspension of 25.25 g. (0.0313 *M*) of dedimethylaminotetracycline (I) in 1750 cc. of reagent chloroform, was added 10.10 g. (0.0313 *M*) of N-bromosuccinimide dissolved in 100 cc. of the same solvent. After 10 minutes the slightly turbid solution was clarified, and the reddish-amber filtrate

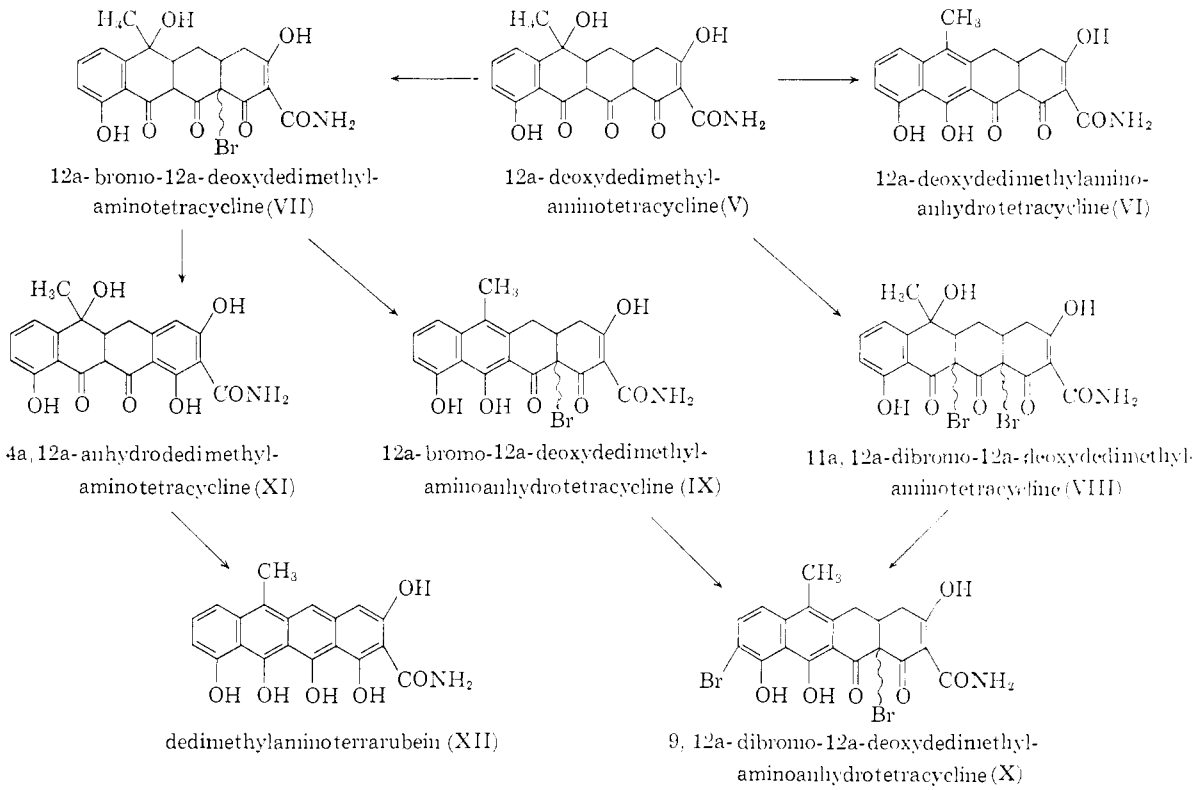
(3) The blocking at position 11a of the potentially enolic 11,12β-dicarbonyl system rationalizes the observation that the bromination product undergoes an irreversible transformation in sodium borate solution (*p*H 9) accompanied by generation of ultraviolet maxima characteristic of a substituted 1,8-naphthalene-diol. Such a moiety could arise by cleavage of the bond between C-11a and C-12 and subsequent displacement and prototropy leading to the elimination of the oxygen at C-6.

(4) The preferential enolization of the 12a-deoxydedimethylamino compounds toward C-12a rather than C-11a, which is borne out by the hypsochromic shift to *ca.* 330 mμ relative to 365 mμ for the 12a-hydroxylated compounds, may be a major factor in the observed selectivity of the halogenation.

(5) A. Green and J. H. Boothe, THIS JOURNAL, **82**, 3950 (1960).

(6) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **75**, 5455 (1953).

(7) We are indebted to Mr. L. Brancone and staff for analytical data and to Mr. W. Fulmor and staff for spectral data.



was left to stand at room temperature for 3 to 4 hours during which time a crystalline product was deposited. After cooling overnight the product was filtered, washed with chloroform, ether and dried in vacuum for 4 hours; wt. 21.40 g., yield 70.3%.

(2) One gram (0.0025 *M*) of dedimethylaminotetracycline (II) was dissolved in 20 cc. of glacial acetic acid by heating to 75°, and after cooling 0.205 g. of sodium acetate (0.0025 *M*) was dissolved in it. At 10–12°, 0.128 cc. of bromine (0.4 g., 0.0023 *M*) dissolved in 5 cc. of glacial acetic acid was added to the above solution over a 30-minute period. After stirring for 2 hours, the solution was added to 100 cc. of water and an amorphous precipitate was filtered off and dried; wt. 0.72 g. This solid was washed three times by slurrying in 10 cc. of ethyl acetate which removed most of the color, leaving a light gray solid; wt. 0.35 g.

Purification was accomplished by crystallizing 500 mg. from 25 cc. of hot acetic acid and then drying at 60° *in vacuo;* wt. 280 mg., m.p. 250° dec.

Anal. Calcd. for $C_{20}H_{18}NO_8Br$: C, 50.0; H, 3.75; N, 2.92; Br, 16.7. Found: C, 50.2; H, 4.17; N, 2.81; Br, 16.47.

The ultraviolet absorption spectra show $\lambda_{max}^{0.1N\ HCl}$ 265 mμ (log ε 4.260), 350 mμ (log ε 3.494). $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 238 mμ (log ε 4.685), 315 mμ (log ε 3.872), 328 mμ (log ε 3.885), 342 mμ (log ε 3.899).

Purification was also accomplished by crystallizing 1 g. from 20 cc. of 2-methoxyethanol by adding 80 cc. of 0.1 *N* hydrochloric acid. Material of analytical purity was obtained after two such purifications.

Dedimethylaminotetracycline by Hydrogenation of II.—A solution of 480 mg. of 11a-bromodedimethylaminotetracycline in 10 cc. of 2-methoxyethanol was hydrogenated in the presence of 100 mg. of 10% palladium-on-charcoal for 15 minutes. During this time 1 g. of triethylamine in 10 cc. of 2-methoxyethanol was dripped into the reaction flask, and 1 mol. of hydrogen was absorbed. After filtering off the catalyst, the filtrate and wash were diluted with 10 volumes of water and clarified by filtration. The filtrate was extracted with ethyl acetate (4 × 20 cc.) and the extracts were dried and concentrated to dryness. The residue was boiled with ether and then filtered off and dried; wt. 140 mg., yield 35%. This material was identical by all criteria with dedimethylaminotetracycline.[7]

Anhydrodedimethylaminotetracycline (III).—A solution of 4.01 g. (0.01 *M*) of dedimethylaminotetracycline in 75 cc. of hot acetic acid was treated with 5 cc. of 31% hydrobromic acid in acetic acid. After heating on the steam-bath, the anhydro compound crystallized as flat needles, and was filtered, washed twice with acetic acid and ether, and finally dried in vacuum at 60° for 2 hours; wt. 2.96 g., yield 77.3%. The anhydrodedimethylaminotetracycline was dissolved in 20 cc. of dimethylformamide which was diluted with 100 cc. of methanol. The bright yellow compound which crystallized after cooling was filtered, washed twice with methanol, ether and dried in vacuum; wt. 2.65 g. The ultraviolet absorption spectra show $\lambda_{max}^{0.1N\ HCl}$ 272 mμ (log ε 4.731), 425 mμ (log ε 3.922); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 268 mμ (log ε 4.658), 420 mμ (log ε 3.994).

Anal. Calcd. for $C_{20}H_{17}NO_7$: C, 62.7; H, 4.43; N, 3.65. Found: C, 62.93; H, 4.44; N, 3.56.

9-Bromoanhydrodedimethylaminotetracycline (IV).—(a) A solution of 280 mg. of 11a-bromodedimethylaminotetracycline (II) in 22 cc. of hot acetic acid was treated with 0.3 cc. of 31% hydrobromic acid in acetic acid. After heating a short time on the steam-bath, the product crystallized and was filtered off and washed with acetic acid and then ether. The product after drying at 60° weighed 140 mg., yield 53%. This material was quite pure but was recrystallized from 3 cc. of dimethylformamide by adding 6 cc. of methanol; m.p. 240° dec. The ultraviolet absorption spectra show $\lambda_{max}^{0.1N\ HCl}$ 228 mμ (log ε 4.628), 274 mμ (log ε 4.716), 430 mμ (log ε 3.908); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 232 mμ (log ε 3.664), 435 mμ (log ε 4.062).

Anal. Calcd. for $C_{20}H_{16}O_7NBr$: C, 52.0; H, 3.47; N, 3.04; Br, 17.3. Found: C, 52.69; H, 3.48; N, 3.20; Br, 17.62.

(b) A suspension of 477 mg. (0.00125 *M*) of anhydrodedimethylaminotetracycline (III) and 202 mg. of N-bromosuccinimide (0.00125 *M*) in 50 cc. of chloroform was shaken for 10–15 minutes, then filtered. Some undissolved starting material was filtered off in clarifying the solution. The amber yellow filtrate was left to stand at room temperature and after 45 minutes a crystalline precipitate had formed, which was filtered, washed with chloroform, ether and dried *in vacuo* at 60° for 1 hour; wt. 140 mg., yield 24.5%. The compound was twice recrystallized from dimethylform-

amide and methanol, and dried at 60° in vacuum; wt. 60 mg. The product was identical to that described under (a).

Anal. Found: Br, 17.17.

(c) A solution of 1.0 g. of dedimethylaminotetracycline in 20 cc. of hot acetic acid was cooled and stirred just above its freezing point. A solution of 0.4 g. of bromine in 1 cc. of acetic acid was dripped slowly in which caused a precipitate to form. About 2 hours after all the bromine was added, the product was filtered off, washed with ether and dried; wt. 0.8 g. After recrystallization from dimethylformamide and methanol the compound was identical to that described under a and b.

9-Chloroanhydrodedimethylaminotetracycline.—A suspension of 373 mg. of anhydrodedimethylaminotetracycline (III) and 238 mg. of N-chlorosuccinimide in 50 cc. of chloroform was shaken for 1 hour at room temperature. An additional 50 cc. of chloroform was added and the mixture was refluxed 1 hour and filtered hot. The filtrate was kept at room temperature for 8 days after which a dark red precipitate was removed by filtration. The mother liquor on further standing yielded a crystalline product which was isolated, washed with chloroform, ether and dried *in vacuo* at 60°; wt. 30 mg. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 240 mμ (log ε 4.404), 275 mμ (log ε 4.471), 335 mμ (shoulder) (log ε 3.716), 455 mμ (log ε 3.920); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 272 mμ (log ε 4.508), 345 mμ (log ε 3.766), 465 mμ (log ε 4.074). A comparison of the spectral data of this product and 7-chloroanhydrodedimethylaminotetracycline showed that they were not identical.

Anal. Calcd. for $C_{20}H_{16}NO_7Cl$: C, 57.5; H, 3.84; N, 3.36; Cl, 8.52. Found: C, 57.22; H, 4.16; N, 3.05; Cl, 8.66.

12a-Deoxydedimethylaminotetracycline (V).—A solution of 5 g. of tetracycline base in 70 cc. of acetic acid and 30 cc. of water was reduced by stirring with 10 g. of zinc dust over a 72-hour period. The apparatus was kept filled with nitrogen and the zinc dust was added in 3 equal portions during the 72 hours. The reaction was kept at 20–25° (especially important in larger scale preparations) during this time by immersion in a water-bath. The reaction mixture was filtered and the filtrate was poured into 350 cc. of cold water containing 5 cc. of concentrated hydrochloric acid. After cooling a short time the light yellow precipitate was filtered off, washed well with water, and dried; wt. 2.82 g., yield 65%.

This product was partially purified by dissolving 2.0 g. in 75 cc. of chloroform, filtering off some insoluble material (5–10%) and concentrating the filtrate to dryness. This material was sufficiently pure for further reactions, but to obtain an analytical sample the residue was dissolved in 50 cc. of boiling ethanol which on cooling in ice 2 hours deposited a yellow material which was filtered off and discarded. The filtrate was reheated, 50 cc. of water was added and after cooling, 2 cc. of concentrated hydrochloric acid was added. After cooling well in ice, the yellow amorphous product was filtered off and dried at 60° *in vacuo*. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 262 mμ (log ε 4.324), 327 mμ (log ε 4.128); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ (after 30 min.) 270 mμ (log ε 4.140), 375–400 mμ (shoulder) (log ε 3.946), 465 mμ (log ε 4.340).

Anal. Calcd. for $C_{20}H_{19}O_7N$: C, 62.4; H, 5.0; N, 3.63. Found: C, 62.52; H, 5.27; N, 3.38.

Anhydro-12a-deoxydedimethylaminotetracycline (VI).—A solution of 500 mg. of 12a-deoxydedimethylaminotetracycline (V) in 10 cc. of acetic acid at 70° was treated with 1 cc. of 31% hydrobromic acid in acetic acid. A crystalline product formed which was filtered off after one hour at room temperature and washed with acetic acid and ether and then dried at 60° *in vacuo;* wt. 400 mg. The compound was recrystallized from dimethylformamide and methanol; m.p. 228–231° with dec. The ultraviolet absorption spectra show $\lambda_{max}^{0.1N\ HCl}$ 221 mμ (log ε 4.478), 271 mμ (log ε 4.478), 435 mμ (log ε 4.189); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 225 mμ (log ε 4.358), 268 mμ (log ε 4.465), 425 mμ (log ε 4.138).

Anal. Calcd. for $C_{20}H_{17}NO_6$: C, 65.5; H, 4.7; N, 3.8. Found: C, 65.36; H, 4.69; N, 3.71.

12a-Bromo-12a-deoxydedimethylaminotetracycline (VII).—A solution of 385 mg. (0.001 mole) of 12a-deoxydedimethylaminotetracycline (V) and 177 mg. (0.001 mole) of N-bromosuccinimide in 25 cc. of reagent chloroform was stoppered and kept at room temperature for 3 days. The yellow crystalline precipitate which formed was filtered, washed with chloroform and ether, then dried at 60° *in vacuo* several hours; wt. 360 mg, yield 77.5%. The product was combined with other samples of similar runs to give a total of 1.18 g., which was recrystallized twice by dissolving in hot 2-methoxyethanol, treating with charcoal, filtering and diluting the filtrate with slightly more than an equal volume of water. There was still complete solution and three drops of 6 *N* hydrochloric acid was added. The solution was cooled in ice whereupon the product crystallized and the weight after isolation and drying at 60° in vacuum was 760 mg. A further recrystallization was accomplished by suspending 760 mg. previously obtained in 120 cc. of ethanol and heating the mixture to reflux. 2-Methoxyethanol was added slowly, keeping the alcohol at constant boiling until there was complete solution. The hot solution was treated with charcoal and then 75 cc. of water was added along with 2 cc. of 6 *N* hydrochloric acid. When the light yellow solution was cooled the product crystallized, and was filtered, washed with water and dried in vacuum for 2 hours without heat, then for 2 hours at 60°; wt. 660 mg. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 254 mμ (log ε 4.178), 365 mμ (log ε 4.185); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 250 mμ (log ε 4.129), 270 mμ (log ε 4.090), 385 mμ (log ε 4.279).

Anal. Calcd. for $C_{20}H_{18}NO_7Br$: C, 51.7; H, 3.88; N, 3.02; Br, 17.25. Found: C, 52.09; H, 4.27; N, 2.86; Br, 17.34.

12a-Bromo-12a-deoxydedimethylaminoanhydrotetracycline (IX).—To a solution of 130 mg. of the 12a-bromo-12a-deoxydedimethylaminotetracycline (VII) in 15 cc. of acetic acid at 95° was added 1 cc. of 31% hydrobromic acid in acetic acid. The color of the solution changed immediately to an orange-red, and after heating on the steam-bath for several minutes the anhydro (IX) compound crystallized out. The product was filtered, washed with acetic acid and ether, then dried at 60° *in vacuo;* wt. 110 mg., yield 88%. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 227 mμ (log ε 4.402), 270 mμ (log ε 4.352), 440 mμ (log ε 4.085); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 232 mμ (log ε 4.338), 270 mμ (log ε 4.352), 337 mμ (log ε 3.757), 430 mμ (log ε 4.085).

Anal. Calcd. for $C_{20}H_{16}NO_6Br$: C, 53.8; H, 3.6; N, 3.14; Br, 17.9. Found: C, 53.82; H, 3.73; N, 3.42; Br, 17.69.

4a,12a-Anhydrodedimethylaminotetracycline (XI).—A solution of 5.0 g. of 12a-bromo-12a-deoxydedimethylaminotetracycline in 40 cc. of pyridine was heated on a steam-bath for 15 minutes. A crystalline precipitate formed which was filtered off after cooling the suspension in ice. The solid then was washed successively with pyridine, water, dilute hydrochloric acid, and again with water, after which it was dried to constant weight *in vacuo;* wt. 2.7 g. The product was recrystallized by dissolving 300 mg. in 25 cc. of warm dimethylformamide, treating with charcoal, and diluting the solution with 25 cc. of methanol. After cooling, the product was filtered, washed with methanol and dried at 60° *in vacuo;* m.p. above 250°, dec. slowly. The ultraviolet absorption spectra show $\lambda_{max}^{0.1N\ HCl}$ 247 mμ (log ε 4.396), 408 mμ (log ε 4.361), 425 mμ (log ε 4.342); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 248 mμ (log ε 4.236), 430 mμ (log ε 4.458).

Anal. Calcd. for $C_{20}H_{17}NO_7$: C, 62.7; H, 4.5; N, 3.66. Found: C, 62.57; H, 4.63; N, 3.50.

Dedimethylaminoterrarubein (XII).—A solution of 500 mg. of 4a,12a-anhydrodedimethylaminotetracycline (XI) in 70 cc. of hot dimethylformamide and 10 cc. of glacial acetic acid was treated with 4 cc. of 31% hydrobromic acid in glacial acetic acid and the reaction was heated on the steam-bath for several minutes before the product crystallized. After heating 10 minutes the mixture was cooled in ice, filtered, and the orange-red crystals were washed with ether and dried *in vacuo* at 60°; wt. 350 mg., yield 73.5%. One hundred mg. of the above crude was recrystallized from 22 cc. of dimethylformamide; wt. 70 mg. By comparison of spectra, this compound was shown to be identical with that described by Hochstein, *et al.*[6]

Anal. Calcd. for $C_{20}H_{15}NO_6$: C, 65.8; H, 4.15; N, 3.83. Found: C, 65.88; H, 4.37; N, 3.90.

11a,12a-Dibromo-12a-deoxydedimethylaminotetracycline (VIII).—After shaking at room temperature for 15 minutes, 2.31 g. of 12a-deoxydedimethylaminotetracycline (V) was

almost completely dissolved in 75 cc. of reagent chloroform, and 2.124 g. of N-bromosuccinimide (0.012 mole) was added. Within a few minutes a heavy but flocculent precipitate formed which, with continuous stirring, returned almost completely into solution. After clarification the reaction filtrate yielded the dibromo compound after standing at room temperature overnight. The almost white crystalline product was filtered, washed with ether and dried at 60° *in vacuo;* wt. 1.39 g. The filtrate yielded a second crop after further standing which was isolated in the same manner; wt. 153 mg., total yield 53.3%. This compound crystallizes either from 2-methoxyethanol and 0.1 *N* hydrochloric acid or from glacial acetic acid in the same way as did compound II; m p. 250° with dec. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 255–275 mμ (flat max.) (log ε 4.174), 350 mμ (log ε 3.579); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 240 mμ (log ε 4.598); 285 mμ (log ε 4.132); 315, 330, 345 mμ (log ε 3.978).

Anal. Calcd. for $C_{20}H_{17}NO_7Br_2$: C, 44.2; H, 3.13; N, 2.57; Br, 29.4. Found: C, 44.45; H, 3.46; N, 2.58; Br, 28.51.

Dibromo-12a-deoxydedimethylaminoanhydrotetracycline (X).—(a) A solution of 1.468 g. (0.004 *M*) of anhydro-12a-deoxydedimethylaminotetracycline (VI) and 1.416 g. (0.008 *M*) of N-bromosuccinimide at room temperature deposited crystalline precipitate within 1.5 hours. The compound was isolated and dried for 3 days without heat in vacuum; wt. 1.2 g., yield 46.5%. This orange-red compound contains one mole of chloroform of crystallization which is lost upon recrystallization from methanol. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 228 mμ (log ε 4.544), 277 mμ (log ε 4.615), 440 mμ (log ε 3.903); $\lambda_{max}^{0.1\ M\ Na_2B_4O_7}$ 232 mμ (log ε 4.458), 275 mμ (log ε 4.538), 440 mμ (log ε 4.021).

Anal. Calcd. for $C_{20}H_{17}NO_6Br_2$: C, 45.6; H, 2.86; N, 2.69; Br, 30.5. Found: C, 45.55; H, 3.02; N, 2.49; Br, 29.67.

(b) A solution of 250 mg. of 11a,12a-dibromo-12a-deoxydedimethylaminotetracycline (VIII) in 15 cc. of hot acetic acid was cooled and 30 mg. of the starting material crystallized and was filtered off. To the filtrate was added 0.5 cc. of 31% hydrobromic acid in acetic acid, and after heating on the steam-bath for some time a small amount of solid precipitate formed. After filtering off the solid (30 mg.), the filtrate was diluted with 35 cc. of water. The orange precipitate was filtered off and dried; wt. 70 mg. This product was shown by spectral evidence to be identical with that described under (a).

Bromodedimethylaminoanhydro-7-chlorotetracycline.—To a solution of 9.44 g. of dedimethylamino-7-chlorotetracycline[2] in 50 ml. of dimethoxyethane was added 100 ml. of glacial acetic acid and 11.05 ml. of bromine at room temperature. Orange crystals rapidly deposited and after standing for 4 hours the mixture was heated on the steam-bath for 1 hour and cooled. The orange hexagonal plates were filtered and dried to yield 8.54 g., m.p. 250–260° dec. After one recrystallization from dimethylformamide and methanol the m.p. remained the same. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 232 mμ (log ε 4.448), 276 mμ (log ε 4.527), 440 mμ (log ε 4.527); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 237 mμ (log ε 4.545), 273 mμ (log ε 4.638), 350 mμ (log ε 3.829), 444 mμ (log ε 4.110).

Anal. Calcd. for $C_{20}H_{15}NO_7BrCl$: C, 48.3; H, 3.04; N, 2.82; Cl, 7.14. Found: C, 48.20; H, 3.34; N, 2.99; Cl, 7.59.

Bromodedimethylaminoiso-7-chlorotetracycline.—To a cooled solution of 0.94 g. (0.002 *M*) of dedimethylamino-7-chlorotetracycline dissolved in 10 ml. of dimethoxyethane and 10 ml. of acetic acid was added 0.69 g. (0.00216 *M*) of pyridinium bromide perbromide dissolved in 10 ml. of dimethoxyethane. The solution stood in an ice-bath 10 minutes when 0.95 ml. of 25% aqueous sodium acetate (0.0023 *M*) was added. This solution stood at room temperature for 40 minutes after which some of the solvent was removed under an air-jet. Upon adding 80 ml. of water a flocculent yellow precipitate was obtained. This was filtered, washed well with water and dried at 60° for 1 hour *in vacuo;* wt. 0.85 g. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 220 mμ (log ε 4.369), 247 mμ (log ε 4.210), 263 mμ (log ε 4.218), 312 mμ (log ε 3.573), 365 mμ (log ε 3.255); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 223 mμ (log ε 4.366), 256 mμ (log ε 4.151), 281 mμ (log ε 4.230), 344 mμ (log ε 3.754). The infrared spectrum showed the peak at 5.72 μ characteristic of the phthalide carbonyl of isotetracyclines.

Anal. Calcd. for $C_{20}H_{17}O_8NClBr$: N, 2.72; Cl, 6.89. Found: N, 2.75; Cl, 6.92.

Dibromodedimethylaminoisochlorotetracycline.—A suspension of 888 mg. (0.0025 *M*) of dedimethylamino-7-chlorotetracycline in 25 cc. of chloroform was treated with 404 mg. of N-bromosuccinimide (0.0025 *M*) in 5 cc. of chloroform. The complete solution which formed was permitted to stand at room temperature overnight during which time a crystalline product deposited. The reaction flask was cooled for 2 days and the almost white precipitate was filtered, washed with chloroform and ether, and then dried in vacuum at 60°; wt. 380 mg., yield 36%. The compound was recrystallized first from 15 cc. of acetic acid and 30 cc. of water, and then from 15 cc. of acetic acid and 80 cc. of water; wt. 180 mg. The compound may also be crystallized from 2-methoxyethanol and water; m.p. 205–210° dec. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 220 mμ (log ε 4.494), 241 mμ (log ε 4.285), 260–263 mμ (shoulder) (log ε 4.171), 315 mμ (log ε 3.774); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 228 mμ (log ε 4.345), 260 mμ (log ε 4.171), 284 mμ (log ε 4.228), 350 mμ (log ε 3.834).

Anal. Calcd. for $C_{20}H_{16}NO_8ClBr_2$: C, 40.4; H, 2.69; N, 2.36. Found: C, 40.32; H, 2.81; N, 2.26.

[CONTRIBUTION FROM THE ORGANIC CHEMISTRY RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

Chemistry of the Tetracycline Antibiotics. III. 12a-Deoxytetracycline

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The preparation of a 12a-deoxytetracycline by means of a reduction with zinc and ammonium hydroxide is described. The proof of structure by means of spectral data and chemical transformations is discussed. The conversion of 12a-deoxytetracycline to a known oxytetracycline[1] degradation product, dedimethylaminoterrarubein, is described.

Part of our work on the chemistry of the cycline antibiotics has been designed to attempt the selective removal of single functional groups or atoms without affecting the remainder of the molecule. The purpose of such work was to determine which of the various functional groups are necessary for the biological activity of these antibiotics. A number of examples of tetracyclines which differ from each other by only one group or atom are known, prepared either by means of chemical transformations or fermentations. In addition to the well known tetracycline, chlorotetracycline and oxytetracycline,[1] mutant strains of *Streptomyces aureo-*

(1) The trademark of American Cyanamid Co. for tetracycline is Achromycin and the trademark of Chas. Pfizer and Co., Inc., for this compound is Tetracyn. The trademark of American Cyanamid Co. for chlorotetracycline is Aureomycin. The trademark of Chas. Pfizer and Co., Inc., for oxytetracycline is Terramycin.