[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, AND THE NAVAL MEDICAL RESEARCH INSTITUTE, BETHESDA 14, MD.]

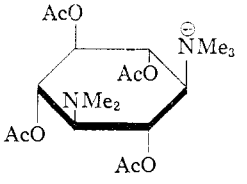
Labilization of Ester Bonds in Aminocyclitol Derivatives. II. Polyacetates of Deoxystreptamine[1]

BY J. DALY, R. C. DURANT, S. L. FRIESS, G. F. HOLLAND, H. KNY AND B. WITKOP

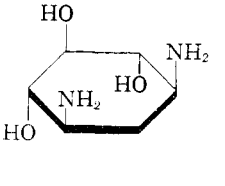
RECEIVED MAY 13, 1960

2-Deoxystreptamine has been converted *via* the N,N′-tetramethyl derivative to the ditertiary, mono- and diquaternary tri- and diacetates (Table I). The spontaneous hydrolysis of these esters in neutral buffer solutions surpasses that of *p*-nitrophenylacetate ($t_{1/2}$ = 3000 min.) by a factor of more than 100. The first step in the hydrolysis of the ditertiary triacetate IV ($t_{1/2}$ = 21 min.) approached the maximal hydrolytic activity of monoquaternary N,N′-tetramethyl-O-tetraacetylstreptamine (I, $t_{1/2}$ = 19 min.), the most active compound in the series. Mono- and diquaternization of N,N-tetramethyldeoxystreptamine O-acetates led to a decrease in the rates of spontaneous hydrolysis. Kinetic and chromatographic analysis was used to investigate the rate and sequences of the various stages in the stepwise controlled hydrolysis of the tri- and diacetates. These results are discussed in terms of over-all conformational effects peculiar to per- or polysubstituted cyclohexane derivatives.

Whereas acetates of tertiary and quaternary *cis*- and *trans*-2-aminocyclohexanols show spontaneous hydrolysis in aqueous buffer solutions only to the negligible extent of 1% of that of acetylcholine,[2] remarkable hydrolytic activities have been observed for polyacetates derived from tertiary and quaternary aminocyclitols. The fastest initial rate for spontaneous hydrolysis, namely, that of a monoquaternary N,N′-tetramethyltetra-O-acetylstreptamine (I), $k_1 = 36.3 \times 10^{-3}$ min.$^{-1}$ (t = 25° in phosphate buffer *p*H 6.89),[3] was more than hundredfold that of *p*-nitrophenyl acetate, $k_1 = 0.23 \times 10^{-3}$ min.$^{-1}$ (t = 25° in phosphate buffer *p*H 7.4),[4] the prototype of labile esters frequently used in model studies. The persubstitution of the cyclohexane in these aminocyclitols was thought to cause buttressing and compression of charges which resulted in an over-all conformational effect unknown in bifunctional cyclohexanes. In order to assess the magnitude and limitations of this new over-all conformational effect a pentasubstituted diaminocyclitol, namely, deoxystreptamine, generally formulated as 1,2,3-trideoxy-1,3-bisaminoscyllitol (II)[5-7] was used as the starting material for the preparation of the tri- and two position-isomeric diacetates derived from N,N′-tetramethyldeoxystreptamine (III) and its mono- and diquaternary ammonium salts.



I



II

Materials

Table I summarizes briefly the compounds prepared and their mode of synthesis. Infrared spectra of the acetyl derivatives show a strong absorption maximum at 5.68–5.72 μ, characteristic of normal ester carbonyls and excluding enol esters. The triacetyl derivative has an n.m.r. spectrum (Table I) in which the peaks for the N-methyl protons are not split, while the peaks for the methyl protons of the non-equivalent acetyl groups are split. The asymmetrical structure VI is tentatively assigned to the diacetate obtained by partial acetylation on the basis of the clear non-equivalence of the methyl protons (140 c.p.s., Table I) of the N-methyl groups. N,N′-Tetramethyl-2-deoxystreptamine and its various O-acetates were easily converted in acetonitrile with methyl iodide to diquaternary bases, while in the streptamine series only monoquaternary derivatives form under these conditions.

Chromatographic investigation of the hydrolysis at *p*H 7.4 of the triacetyl derivative (Table I) gave evidence for the formation of both symmetric and ⅓ asymmetric diacetates during hydrolysis. The symmetric diacetate was also, as stated above, isolated from the triacetate derivative after hydrolysis with alkali under controlled conditions. Total hydrolysis of the triacetate apparently led to material indistinguishable from authentic 2-deoxy-N,N′-tetramethylstreptamine to judge from comparison of cleavage rate with periodate and melting point determination.

Experimental[8]

2-Deoxystreptamine Dipicrate.—Neamine hydrochloride (neomycin A hydrochloride)[9] was hydrolyzed with boiling 48% hydrobromic acid by the procedure of Leach and Teeters.[10] The crude hydrolysis product, a viscous oil, was

(1) The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department.

(2) H. D. Baldridge, Jr., W. J. McCarville and S. L. Friess, THIS JOURNAL, **77**, 739 (1955).

(3) G. F. Holland, R. C. Durant, S. L. Friess and B. Witkop, *ibid.*, **80**, 6031 (1958).

(4) G. L. Schmir, Thesis, Yale University, 1958, p. 114; *cf.* T. C. Bruice and G. L. Schmir, THIS JOURNAL, **79**, 1663 (1957).

(5) *Cf.* F. A. Kuehl, M. N. Bishop and K. Folkers, *ibid.*, **73**, 881, (1951).

(6) *Cf.* J. R. Dyer, "The Chemisty of Neamine," Thesis, Univ. of Illinois, 1954, p. 127.

(7) The *scyllo* configuration of deoxystreptamine rests on the following four observations: (i) lack of optical activity; (ii) *cis*-position of the 1,3-amino groups (ref. 5); (iii) no hydroxyl *cis* to either amino group as evidenced by acyl migration studies (ref. 6); (iv) the rate of periodate cleavage of N,N,-dibenzoyldeoxystreptamine is even slower (ref. 6) than that of the comparable derivative of streptamine whose configuration has been proved by synthesis. Point IV is the weakest link in the chain of evidence. In the present studies the question of the *meso* structure with three *cis*-hydroxyls *trans* to the two amino groups is raised by the occurrence of three diacetates (Table I).

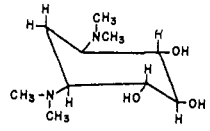
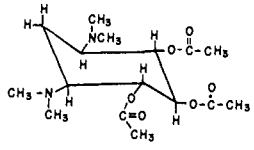
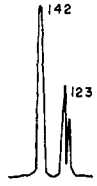
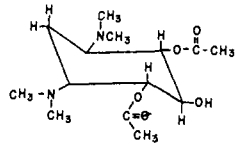
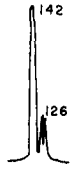
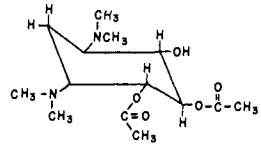
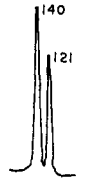
(8) All melting points are corrected, all boiling points are uncorrected. Analyses were carried out in the Institutes' Analytical Services Unit under the direction of Dr. W. C. Alford.

(9) We are greatly indebted to Dr. W. G. Jackson, The Upjohn Co., Kalamazoo, Mich., for his courtesy and coöperation in providing us with the neamine. The data for the neamine supplied to us were: Neamine (3961-DeV-26), m.p. 215–250°, $[\alpha]^{25}D$ +117° (water; reported +123°; *cf.* B. E. Leach and C. M. Teeters, THIS JOURNAL, **73**, 2794 (1951)), neut. equiv. 86 (calcd. 81).

(10) B. E. Leach and C. M. Teeters, THIS JOURNAL, **74**, 3187 (1952).

TABLE I

N,N'-TETRAMETHYL-2-DEOXYSTREPTAMINE AND ACETYL DERIVATIVES

COMPOUND	PRESUMABLE STRUCTURE	NUCLEAR MAGNETIC RESONANCE PEAKS[a]	M.P. R_F	PREPARATION	QUATERNARY SALTS
N,N'-TETRAMETHYL-2-DEOXY-STREPTAMINE (III)			158-159° R_F .20	FROM NEAMINE HYDROCHLORIDE	BISMETHIODIDE M.P. 270-275°
4,5,6-TRI-O-ACETYL-N,N'-TETRAMETHYL-2-DEOXYSTREPTAMINE (IV)			155° R_F .93	ACETYLATION WITH EXCESS OF Ac_2O AND NaOAc	BISMETHIODIDE M.P. 265° MONOMETHIODIDE M.P. 244-260°
SYMMETRIC (?) DI-O-ACETYL-N,N'-TETRAMETHYL-2-DEOXYSTREPTAMINE (V)			125-130° R_F .80	CONTROLLED HYDROLYSIS OF TRIACETATE	—
ASYMMETRIC (?) DI-O-ACETYL-N,N'-TETRAMETHYL-2-DEOXYSTREPTAMINE (VI)			137-140° R_F .60	ACETYLATION WITH 2 OR LESS EQUIVALENTS OF ACETYL CHLORIDE OR Ac_2O IN PYRIDINE	BISMETHIODIDE M.P. 223°
SYMMETRIC (?) DIACETATE OF UNKNOWN STRUCTURE			150° R_F .95	ACETYLATION WITH EXCESS Ac_2O AND NaOAc OF AN INHOMOGENEOUS III (CF. EXPERIMENTAL)	BISMETHIODIDE M.P. 215-225°

[a] NMR Spectra were measured on a 4300-C Varian high resolution spectrometer at 60 mc. Compounds were studied in concentrations of 0.3–0.5 molar in carbon tetrachloride. Shift values of principal peaks were determined by the audio-frequency side-band technique in cycles per second and were reproducible to approximately 1 c.p.s. Chemical shifts are expressed with reference to internal tetramethylsilane in c.p.s.

converted to the readily crystallizing picrate in aqueous solution. After recrystallization from aqueous methanol, the sample for analysis, m.p. 260° dec., was dried for 3 hours at 100° and then to constant weight at 20° *in vacuo*. Attempted recrystallization from non-aqueous solvents yielded only oily fractions.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2C_6H_3N_3O_7$: C, 34.85; H, 3.25; N, 18.06. Found: C, 34.79; H, 3.39; N, 17.67.

The free base was obtained by pouring a solution of the dihydrobromide through a carbonate-free column of Dowex I ion exchange resin in the OH^- form and elution with water. After evaporation of the eluates the colorless crystalline residue was recrystallized from ethanol. The analytical sample was dried at 65° for 16 hr. at 0.01 mm., m.p. 225–228°.

Anal. Calcd. for $C_6H_{14}N_2O_3$: C, 44.43; H, 8.70; N, 17.27. Found: C, 44.20; H, 8.63; N, 17.54.

N,N'-Tetramethyl-2-deoxystreptamine (III).—The free base of 2-deoxystreptamine (13.8 g., 0.085 mole), obtained as described above from the dihydrobromide by the use of ion exchange resin, was dissolved in 330 ml. of formic acid (98%) and 190 ml. of aqueous formaldehyde (37%) and refluxed for 20 hours. The reaction mixture was treated with concentrated hydrochloric acid and evaporated to dryness under reduced pressure. The crystalline, yellowish residue, which contains much polymeric formaldehyde, was extracted three times with 50-ml. portions of water. The combined extracts were brought to a volume of 250 ml. and passed through a column (36 × 200 mm.) of Dowex 50 ion exchange resin (H^+ form). The column was thoroughly washed with water. The amine was then obtained from the column by gradient elution with hydrochloric acid of increasing strength. The dihydrochloride of the ditertiary base appeared in the fractions resulting from elution with 2.0 *N* hydrochloric acid. After evaporation to dryness the crystalline residue (24.35 g.) showed m.p. 274–276°. The dihydrochloride was easily soluble in warm methanol and slowly crystallized from such a solution after the addition of four volumes of absolute ethanol. After two recrystallizations in this manner the colorless rosette-like crystals showed m.p. 277°. The dihydrochloride so obtained loses 10.7% of its weight on drying; calcd. for a loss of two molecules of water, 11.0%.

Anal. Calcd. for $C_{10}H_{22}N_2O_3 \cdot 2HCl$: C, 41.24; H, 8.31; N, 9.62; Cl, 24.38. Found: C, 41.06; H, 8.22; N, 9.76; Cl, 24.2.

Free **N,N'-tetramethyl-2-deoxystreptamine** was prepared by passing a solution of 1.0 g. of the dihydrochloride in 15 ml. of water through a column of Dowex-1 ion exchange resin (OH^- form) and by subsequent elution with 50 ml. of

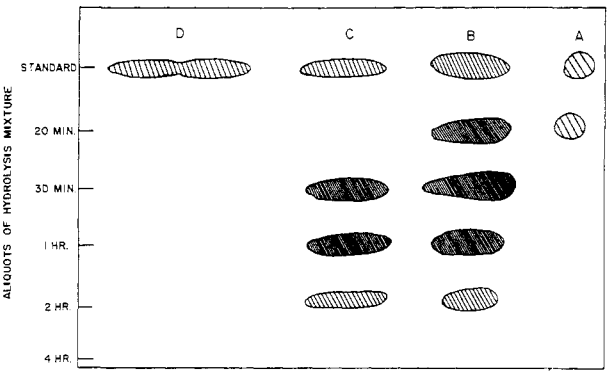


Fig. 1.—Chromatographic investigation of the hydrolysis of 4,5,6-tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine: standard: (A) 4,5,6-tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine IV, (B) symmetric (?) di-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine VI, (C) asymmetric (?) di-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine V, (D) symmetric and asymmetric monoacetates. Descending chromatography on partially acetylated paper (Schleicher and Schuell No. 2495) using chloroform–benzene as stationary and benzene as mobile phase. Spots were made visible with iodine vapor. Shading suggests relative amounts of hydrolysis products.

water. The eluate, which was free of halogen, was evaporated to dryness, and the crystalline residue was recrystallized from ethanol. The analytical sample, m.p. 158–159°, was dried at 65° and 0.01 mm. for 6 hours.

Anal. Calcd. for $C_{10}H_{22}N_2O_3$: C, 55.02; H, 10.16; N, 12.83. Found: C, 54.98; H, 10.15; N, 12.60.

N,N′-Tetramethyl-2-deoxystreptamine dipicrate, prepared in aqeous solution, had m.p. 257–258° after recrystallization from water.

Anal. Calcd. for $C_{10}H_{22}N_2O_3 \cdot 2C_6H_3O_7N_3$: C, 39.06; H, 4.17; N, 16.57. Found: C, 39.16; H, 4.00; N, 17.67.

N,N′-Tetramethyl-2-deoxystreptamine Bismethiodide.—To 50 mg. of N,N′-tetramethyl-2-deoxystreptamine in 20 ml. of acetonitrile was added 500 mg. of methyl iodide. After 3 days the rosette-shaped crystals were filtered, washed with methylene chloride and dried *in vacuo* at 100°. The yield was 89 mg., m.p. 270–237° dec.

Anal. Calcd. for $C_{10}H_{22}N_2O_3 \cdot 2CH_3I$: C, 28.70; H, 5.62; N, 5.58; I, 50.54. Found: C, 28.07; H, 5.72; N, 6.18; I, 50.32.

The dipicrate prepared in aqueous solution had m.p. 295–297° dec.

Anal. Calcd. for $C_{12}H_{28}N_2O_3 \cdot 2C_6H_2N_3O_7$: C, 40.91; H, 4.58; N, 15.90. Found: C, 40.95; H, 4.42; N, 15.60.

Isomers of N,N′-Tetramethyl-2-deoxystreptamine Dihydrochloride, $C_{10}H_{22}N_2O_3 \cdot 2HCl \cdot H_2O$, M.p. 205°, of Unknown Structure.—When both in the preparation of free deoxystreptamine from the salt (dihydrobromide or dipicrate) and in the work-up of the reaction mixture after treatment with formic acid and formaldehyde chemical methods as described below were used for purification, a much lower melting dihydrochloride was obtained which was not homogeneous. In the event, 8.8 g. (0.014 mole) of 2-deoxystreptamine dipicrate was dissolved in acetone and treated with a slight excess of sulfuric acid. The oily precipitate that formed was washed well with acetone and then dissolved in water. The aqueous solution was extracted 4 times with ether and then neutralized dropwise with a hot concentrated solution of barium hydroxide until the *p*H of the solution was close to 8. The barium sulfate was removed by filtration and the clear filtrate was treated with a dilute solution of barium hydroxide until no more precipitation occurred. Again the barium sulfate was removed and the clear filtrate was titrated with 1 *N* sulfuric acid and dilute barium hydroxide until the solution was free of inorganic ions. After concentration of the clear filtrate the free diamine was dissolved in 20 ml. of 37% formaldehyde and 45 ml. of 98% formic acid and refluxed for 20 hours. The reaction mixture was acidified with concentrated hydrochloric acid and concentrated under reduced pressure. The residue was washed with water, filtered and the filtrate evaporated *in vacuo* to an oil. After standing overnight at 25° in methanol the concentrated solution deposited fine colorless needles of the dihydrochloride (2.8 g.), m.p. 205–215°. This material is not homogeneous and gives several fractions on acetylation.

Anal. Calcd. for $C_{10}H_{22}N_2O_3 \cdot 2HCl \cdot H_2O$: C, 38.85; H, 8.43; N, 9.07; Cl, 22.95. Found: C, 39.17; H, 8.29; N, 8.83; Cl, 22.74.

Via the Dihydrobromide.—The crude dihydrobromide (20 g., 0.062 mole) from the hydrolysis of neamine was dissolved in a minimum amount of concentrated ammonium hydroxide and enough water added to obtain complete solution. A light yellow oil formed on addition of absolute ethanol; more absolute ethanol was added until no further material separated. The mother liquor was decanted and the residue washed with absolute ethanol. The free diamine without further purification was dissolved in a mixture of 140 ml. of 37% formaldehyde and 240 ml. of 98% formic acid and refluxed as above. Following the procedure outlined above there was obtained 9 g. (47%) of the dihydrochloride (m.p. 205–215°) of the ditertiary base.

O-Diacetyl-N,N′tetramethyl-2-deoxystreptamine, M.p. 150°, of Unknown Structure.—Two grams (0.007 mole) of the dihydrochloride of m.p. 205°, 2.0 g. of sodium acetate and 200 ml. of acetic anhydride was heated on the steam-bath for 1 hour. The solution was filtered, concentrated under vacuum to an oil and dried. Ether was added and the residue removed by centrifugation. The ether was allowed to evaporate leaving a light brown powder. A second filtration and evaporation again using ether gave material of m.p. 148–150°.

The infrared spectrum in carbon disulfide had a tiny peak at 2.89 and a *single ester carbonyl at 5.71 μ*. Active hydrogen determination by the Zerewitinoff method slowly liberated 1.1 moles of methane per molecular weight.

Anal. Calcd. for $C_{14}H_{26}N_2O_5$: C, 55.61; H, 8.61; N, 9.27; acetyl, 28.5. Found: C, 55.73; H, 8.57; N, 8.96; acetyl, 28.4.

On further reaction of this diacetate with refluxing acetic anhydride in the presence of sodium acetate the unreactive hydroxyl was acetylated only partially. The fractionation of the reaction mixture on silica gel gave a variety of fractions whose kinetic analysis showed them to be mixtures as given in Table II.

TABLE II

FRACTIONS OBTAINED ON FURTHER ACETIC ANHYDRIDE TREATMENT OF DI-O-ACETYL-N,N′-TETRAMETHYL-2-DEOXYSTREPTAMINE OF UNKNOWN STRUCTURE

M.p., °C.	*p*H of buffer system	Init. rates k_1 of spontaneous hydrol., min.$^{-1}$ × 10^4	Characterization according to kinetic data and titration values
150–151	7.7	0.31	Inert diacetate
148–150	7.7	4.79	Mixts. of inert and act. diacetates obtained on fractn. on silica gel
157–158	7.7	7 23	
143–144	7.6	18.8	
161–162	7.75	11.3	Mixt. of very act. triacetate and inact. diacetate

Dimethiodide.—By quaternization of the diacetate of m.p. 150° with methyl iodide in acetonitrile (see above) there was obtained after dilution with methyl iodide 70 mg. of colorless crystals, m.p. 215–225° (with evolution of gas).

Anal. Calcd. for $C_{14}H_{26}N_2O_5 \cdot 2CH_3I$: C, 32.78; H, 5.50; I, 43.30. Found: C, 34.43; H, 5.51; I, 37.83.

4,5,6-Tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine: A. Unstable Form (M.p. 98–100°).—A solution of 2.9 g. (10 mmoles) of N,N′-tetramethyl-2-deoxystreptamine dihydrochloride (m.p. 277°) in 80 ml. of acetic anhydride was heated with 3.4 g. of freshly fused potassium acetate for 16 hours at 100–110°. The mixture was then refluxed for

2 hours, filtered and evaporated to dryness under reduced pressure. The crystalline residue was extracted with benzene in a Soxhlet apparatus. The benzene extract was evaporated to dryness and the brownish, crysta line residue again extracted with hexane in a Soxhlet apparatus. After 6 hours, 2.20 g. of a slightly off-white crystalline material was collected, washed with hexane, and recrystallized from dibutyl ether, m.p. 98–100°.

Anal. Calcd. for $C_{16}H_{28}N_2O_6$: C, 55.80; H, 8.20; N, 8.13; acetyl, 37.48. Found: C, 54.32; H, 8.24; N, 7.54; acetyl, 36.8.

The analytical sample, m.p. 98–100°, after drying for 64 hours at 20° and 0.02 mm. over P_2O_5 melted at 148–244°. In order to explore the nature of this process 116.2 mg. of freshly recrystallized triacetate (m.p. 98–100°) was filled into one leg of a U-shaped tube which was then evacuated (0.001 mm.) and sealed. While the leg with the sample remained at room temperature, the empty leg was cooled in Dry Ice–acetone for 10 days. A crystalline material, m.p. 10°, had formed in the cold part by that time. The tube was then opened, 3 ml. of a 0.1 *N* solution of *m*-nitroaniline in chloroform was added to the distillate. After 17 hours at 60° the reaction mixture was chromatographed on paper treated with formamide, using benzene as mobile phase. By the use of reference compounds (*m*-nitroaniline, R_f 0.6) the presence of acet-*m*-nitroanilide (R_f 0.18) was easily spotted under ultraviolet light.[11] The residual crystals in the other leg of the U-tube weighed 88.9 mg., corresponding to the weight loss of 23.5%, and showed a melting range of 149–240°. The acetyl content of this material surprisingly was still as high as 35.8% (3 acetyls require 37.48%).

4,5,6,-Tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine (IV): B. Relatively Stable Form (m.p. 150°).—After an additional 20 hours of extraction 1.3 g. of crystalline material was obtained which by fractional recrystallization from dibutyl ether was separated into 0.4 g. of a fraction, m.p. 100°, and 0.8 g., m.p. 125–130°. On drying *in vacuo* at room temperature for several days the melting points of both fractions rose to, and broadened their range from, 148–244°. During the drying process acetic acid as well as acetic anhydride were released into the gas phase (see above). Further purification was achieved by chromatography on a column made up of a 50-fold amount of silica gel in the following way: 0.57 g. of the acetylation mixture containing the fractions m.p. 100° and 125–130° was dissolved in 4 ml. of benzene; about 20 mg. of an insoluble colorless crystalline powder remained, which after recrystallization from ethanol had m.p. 265–268°. The analysis of this halogen-free material was unexpectedly low in carbon (C, 45.00; H, 7.91; N, 6.82; acetyl, 29.0). After chromatography on 15 g. of silica gel in hexane–benzene (8:2) the pure triacetate (330 mg.) was found in fractions 2–6 (each 20 ml.). After recrystallization from benzene–hexane colorless needles were obtained, m.p. 149–150°. The infrared spectrum was taken in carbon disulfide. There was only one sharp peak in the ester region at 5.68 mμ in the infrared spectrum, and no peak in the NH,OH region. After storage in a desiccator over P_2O_5 for 2.5 months one third of the most labile acetate (half-life time 17 min.) had been lost according to kinetic analysis.

Anal. Calcd. for $C_{16}H_{28}N_2O_6$: C, 55.80; H, 8.20; N, 8.13; acetyl, 37.48. Found: C, 55.89; H, 8.18; N, 7.87; acetyl, 37.0.

Purification of Triacetate on Alumina.—A sample of 0.1 g. of the acetylation mixture, containing the fractions m.p. 100° and 125–130°, was dissolved in 5 ml. of benzene, filtered from undissolved material, and passed over a column containing 3 g. of *neutral* aluminum oxide (Woelm, activity 1) prepared from a slurry of alumina in hexane. Portions of 5 ml. of benzene were used for elution. The first fraction (19 mg.) was not crystalline. Fractions 2–4 contained 26 mg. of crystals, m.p. 153°, after recrystallization from chloroform–hexane, m.p. 155°.

Anal. Calcd. for $C_{16}H_{28}N_2O_6$: C, 55.80; H, 8.20; N, 8.13; acetyl, 37.48. Found: C, 56.05; H, 8.32; N, 7.88; acetyl, 36.0.

Total Hydrolysis of the Triacetate: A. Acid Hydrolysis.—A solution of 0.17 g. of triacetyl ester (m.p. 148–150°) in 10 ml. of 1.0 *N* hydrochloric acid was refluxed for 2 hours, evaporated to dryness and the residue dissolved in 10 ml. of water. This solution was passed through a column containing 5 g. of Dowex 2 ion exchange resin (OH^- form). The eluate was evaporated to dryness and the residue recrystallized from aqueous ethanol yielding colorless crystals, m.p. 156–157°. The picrate of this base had m.p. 257–258° dec., undepressed on admixture with authentic N,N′-tetramethyl-2-deoxystreptamine dipicrate.

B. Hydrolysis with Barium Hydroxide.—A solution of 35 mg. of the triacetate in 10 ml. of aqueous 0.05 *N* baryta was heated for 2 hours on a steam-bath. To the hot mixture was added 5.0 ml. of 0.1 *N* sulfuric acid and the barium sulfate was removed by filtration. After evaporation of the filtrate the residue, a colorless glass, was dissolved in aqueous ethanol, and a concentrated solution of 50 mg. of picric acid in methanol added. There was isolated 61 mg. of picrate, m.p. after recrystallization from 90% ethanol, 255–256°. A solution of this picrate in 10 ml. of 50% ethanol was poured through a column containing 3 g. of Dowex 1 ion exchange resin (OH^- form). The eluates on evaporation to dryness left 10.3 mg. of colorless crystals. This residue was dissolved in 0.95 ml. of 0.1 *N* sulfuric acid and brought to a volume of 10.0 ml. (0.0047 molar) by addition of water.

Comparison of the rates of periodate oxidation of tetramethyldeoxystreptamine and the hydrolysis product from the triacetyl ester: A 0.001 *M* solution of sodium iodate and a 0.046 *M* stock solution of sodium metaperiodate were prepared and standardized with sodium thiosulfate. The consumption of periodate was followed by the decrease in absorbancy[12] at 223 mμ using a Cary model 14 recording spectrophotometer. Measurements were taken consecutively in a set of three cells containing the control mixture, the standard for reference and the hydrolysis product, as follows: A, Control: The mixture of 0.25 ml. of 0.1 *N* acetate buffer (*p*H 4.7), 1.23 ml. of $NaIO_4$ 4.06×10^{-2} *M* and 0.50 ml. of $NaIO_3$ 1.0×10^{-3} *M* was brought to a total volume of 25.0 ml. B, Reference sample: The above mixture contained in addition 1.25 ml. of a 0.1 *M* solution of N,N′-tetramethyl-2-deoxystreptamine sulfate in a total volume of 25.0 ml. C, Test sample: Same solution as in A containing in addition 2.66 ml. of the 0.0047 molar hydrolysate solution. The three solution were kept at 20° in the dark; 1.0-ml. aliquots were taken and diluted to 10.0 ml. and the periodate absorbance measured at 223 mμ. The absorbancy of the control blank A was constant over 71 hours within the experimental error. Samples B and C showed the same rates of periodate consumption, *i.e.*, 0.5 mole after 5.5 hours, 1.0 mole after 30 hours, and 1.4 moles after 71 hours.

4,5,6-Tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine Monomethiodide.—To 69 mg. (0.2 mmole) of triacetyl-N,N′-tetramethyl-2-deoxystreptamine in 10 ml. of dry methylene chloride was added 310 mg. (2 mmoles) of methyl iodide in 5 ml. of methylene chloride. The mixture was kept in the dark at room temperature. A voluminous mush of fine needles appeared in the course of 20 hours and was collected after 3 days. After washing several times with methylene chloride the hygroscopic residue was dried at 65° and 0.01 mm. for 20 hours. The methiodide melted over a range of 244–260° without decomposition.

Anal. Calcd. for $C_{16}H_{28}N_2O_6 \cdot CH_3I$: C, 41.98; H, 6.43; N, 5.76; I, 26.10; acetyl, 26.55. Found: C, 41.88; H, 6.46; N, 5.55; I, 25.74; acetyl, 24.7.

4,5,6-Tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine Bismethiodide.—When the same proportions of triacetate and methyl iodide were allowed to react in acetonitrile as solvent, the product cyrstallized more slowly and came out in transparent cubes, which were collected after 3 days, washed with methylene chloride and dried as before; m.p. 265° dec.

Anal. Calcd. for $C_{16}H_{28}N_2O_6 \cdot 2CH_3I$: C, 34.41; H, 5.45; N, 4.46; I, 40.40; acetyl, 20.55. Found: C, 33.85; H, 5.35; N, 4.56; I, 39.93; acetyl, 19.36.

Symmetric (?) O-Diacetyl-N,N′-tetramethyl-2-deoxystreptamine (VI). A. By Partial Hydrolysis of the Triacetate.—To 100 mg. of tri-O-acetyl-2-deoxy-N,N′-tetramethylstreptamine in 3 ml. of dioxane was added 1 equiva-

(11) Only acetic anhydride, but not glacial acetic acid, will form acet-*m*-nitroanilide, in contrast to aniline which forms acetanilide with both reagents.

(12) J. D. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954); G. O. Aspinall and R. S. Ferrier, *Chem. & Ind.*, 1216 (1957); *cf.* G. V. Marinetti and G. Rouser, THIS JOURNAL, **77**, 5345 (1955).

lent of sodium hydroxide (11 mg.) in 10 ml. of water. After standing for 20 minutes at room temperature, the solution was concentrated almost to dryness *in vacuo* and then extracted with hot chloroform. The chloroform was washed with water, dried, and evaporated to dryness *in vacuo*. The residue was extracted with boiling *n*-hexane which yielded on concentration 20 mg. of diacetyl compound m.p. 125–130°.

Anal. Calcd. for $C_{14}H_{26}N_2O_6$: C, 55.61; H, 8.67; N, 9.27; acetyl, 28.48. Found: C, 55.36; H, 8.22; N, 9.10; acetyl, 30.46.

Asymmetric (?) O-Diacetyl-N,N′-tetramethyl-2-deoxystreptamine (V). A. By Acetylation with Acetic Anhydride in Pyridine.—To a solution of 240 mg. of N,N′-tetramethyl-2-deoxystreptamine and 90 mg. of pyridine in 125 ml. of chloroform was added 130 mg. of acetic anhydride in 75 ml. of chloroform. After 18 hours at room temperature, the solution was concentrated to dryness *in vacuo* and then freed of volatile compounds in a vacuum of 0.001 mm. for 24 hours. The product was recrystallized from cyclohexane or *n*-hexane to afford 70 mg. of a diacetate of m.p. 129–131°. The infrared spectrum in chloroform solution shows a small peak at 2.73 and a broad band at 2.92 μ with a shoulder at 2.80. There is a single ester carbonyl at 5.72 μ.

Anal. Calcd. for $C_{14}H_{26}N_2O_6$: N, 9.27; acetyl, 28.48. Found: N, 8.94; acetyl, 25.73.

B. By Acetylation with Acetyl Chloride in Benzene.—To a solution of 0.985 g. (4.5 mmoles) of N,N′-tetramethyl-2-deoxystreptamine in 200 ml. of dry benzene at 70° was added dropwise a solution of 0.354 g. (4.5 mmoles) of acetyl chloride in 50 ml. of benzene under stirring in the course of 45 min. The reaction mixture became turbid immediately and a fine precipitate was formed. After an additional 2 hours the mixture was filtered under exclusion of moisture. The residue melted above 265° under slight decomposition. The filtrate was evaporated and left 0.63 g. of a crystalline, halogen-free residue which melted at 129–131°. After two recrystallizations from benzene–pentane the m.p. rose to 137–140°.

Anal. Calcd. for $C_{14}H_{26}N_2O_6$: N, 9.27; acetyl, 28.48. Found: N, 9.37; acetyl, 27.2.

Bismethiodide.—To 52 mg. of (asymmetric) di-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine of m.p. 129–131° in 10 ml. of acetonitrile was added 280 mg. of methyl iodide in 5 ml. of acetonitrile. After standing for 3 days the crystals were filtered off and washed with methylene chloride. There was obtained 65 mg. of stout colorless needles, m.p. 223°, with evolution of gas.

Anal. Calcd. for $C_{14}H_{26}N_2O_6 \cdot 2CH_3I$: C, 32.78; H, 5.50; I, 43.30. Found: C, 32.72; H, 5.46; I, 40.69.

Characterization of N,N′-Tetramethyl-2-deoxystreptamine and of its O-Acetates by Paper Chromatography.—Descending chromatography of N,N′-tetramethyl-2-deoxystreptamine and the O-acetyl derivatives was carried out with Schleicher and Schuell No. 2495 paper (a partially acetylated paper), using chloroform–benzene as the stationary, and benzene as the mobile phase. The chromatograms were allowed to equilibrate with benzene and chloroform vapors for at least 6 hours before developing. The detection of the streptamine derivatives was accomplished with iodine vapor. The N,N′-tetramethyl-2-deoxystreptamine showed considerable trailing in this system, its approximate R_f being 0.2, while the asymmetric diacetate V had a R_f of 0.60 (trailing), and the symmetric diacetate VI obtained by partial hydrolysis had an R_f of 0.80 (trailing). The triacetate of N,N′-tetramethyl-2-deoxystreptamine had an R_f in this system of 0.93 (no trailing). The diacetate of unknown structure obtained from non-homogeneous N,N′-tetramethyl-2-deoxystreptamine as explained above had an R_f in this system of 0.95 (no trailing).

Investigation of the Hydrolysis of 4,5,6-Tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine by Paper Chromatography.—The hydrolysis of 10 mg. of 3,4,5-tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine was carried out at *p*H 7.40 at room temperature in 12 ml. of 0.1 *M* NaCl containing 1.5 ml. of dioxane to ensure solubility. Aliquots were taken at intervals, extracted with chloroform, the chloroform extracts dried with sodium sulfate and evaporated to dryness under nitrogen. The residue was taken up in benzene and chromatographed as above. The spot (R_f 0.91) corresponding to the triacetyl compound rapidly diminished in the course of the hydrolysis being replaced by two spots (R_f 0.80 and 0.59) corresponding, respectively, in R_f to the symmetric and asymmetric diacetates. The symmetric diacetate appeared to be formed to a greater extent. After 4 hours the amounts of diacetates were also greatly diminished. Apparently under the conditions of extraction monoacetates were not extracted into the chloroform phase since no additional compounds were detected by paper chromatography. The chromatograms are reproduced in Table I.

Kinetic Studies.—The experimental techniques and apparatus for subjecting the small amounts of materials available to hydrolysis at constant *p*H have been described in preceding communications.[3,13] The hydrolyses were carried out in a 40-ml. reaction volume, at *p*H 7.40 and 25.14 ± 0.03°, under a nitrogen atmosphere. The medium contained a fixed proportion (12.5% by volume) of purified dioxane, to ensure complete solubility in certain instances. In the only instrumental innovation as compared with the previous work, a *p*H stat (International Instrument Co., Canyon, Calif.) was used to monitor and record *p*H continuously, with a buzzer system to initiate the manual addition of micro-sized increments of titrant base or acid as the reactions progressed.

Results and Discussion

A summary of comparative kinetic data for spontaneous hydrolysis in the series is presented in Table III. As in the previous work[3] involving a comparison of kinetic results from multi-stage hydrolytic reactions in polyfunctional aminocyclitol esters, the results have been expressed as first-order rate constants, even though sufficient material was not available to establish the molecularity of each step by variation of concentration parameters. In practice, a given kinetic determination was carried out to the point of negligible further reaction, and the total rate plot arbitrarily split into segments corresponding to stoichiometric equivalents of acid produced per mole of initial compound. The first rate constant was then evaluated from the initial 50% of its reaction segment, and the subsequent k_1 values calculated from the centers of their respective segments on the assumption that contributions to the rates in these intervals from previous and subsequent hydrolysis stages are negligible.

The assumption of discrete hydrolysis stages in multistep reactions is in general not justified, unless the values for successive rate constants differ significantly from those predicted from probability considerations.[13a] This limitation must be borne in mind in a proper evaluation of the data presented in Table III.

Some interesting features emerge from the comparative data of Table III. First, as a function of the total molecular conformation of the N,N′-tetramethyl-2-deoxystreptamine moiety, a rather high hydrolytic lability is imparted to ester linkages in this molecule. This lability approaches and in part equals that previously observed[3] for streptamine derivatives, which rather implies that the oxygen function in the 2-position of the streptamine nucleus is not a necessary factor for eduction of the facile hydrolysis phenomenon. Further, this labilization of ester groups is not restricted to a single function of a given molecule, since it is seen (*e.g.*, in the ditertiary triacetate IV) that suc-

(13) D. S. Masterson, S. L. Friess and B. Witkop, THIS JOURNAL, **80**, 5687 (1958).

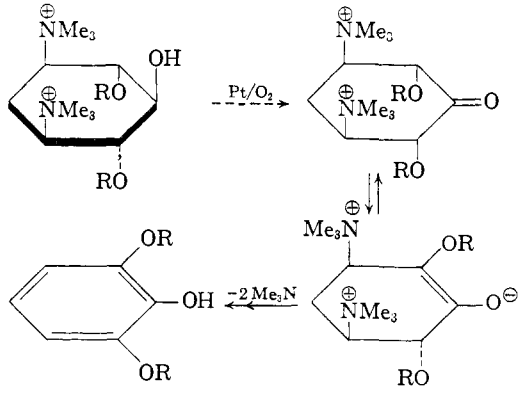
(13a) J. Greenspan, *Chem. Rev.*, **12**, 339 (1933).

TABLE III

HYDROLYSIS RATES FOR N,N'-TETRAMETHYL-2-DEOXYSTREPTAMINE O-ACETATES AT 25.14 ± 0.03°, *p*H 7.40 IN H_2O-DIOXANE (12.5% BY VOL.), PHOSPHATE BUFFER SOLUTION CONTAINING 0.083 *M* NaCl AND 0.013 *M* PHOSPHATE

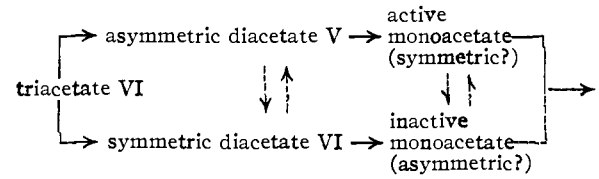
Compound	Moles of base consumed per mole of compd.[a]	k_1 values (min.$^{-1}$) for stoichiometric stages of hydrolysis: Triacetate → stage 1	Diacetate → stage 2	Monoacetate → stage 3
Tertiary bases				
Triacetate IV	2.5–2.9	$3.3 \pm 0.2 \times 10^{-2}$	$1.1 \pm 0.3 \pm 10^{-2}$	$3.0 \pm 0.7 \times 10^{-3}$
Asymmetric diacetate V	2.1		$2.2 \pm .2 \times 10^{-2}$	$3.2 \pm 0.3 \times 10^{-3}$
Symmetric diacetate VI	1.05		$1.0 \pm .1 \times 10^{-2}$	—0
Diacetate of unknown structure (R_f 0.95)	1.2		$1.4 \pm .2 \times 10^{-2}$	$1.6 \pm 0.2 \times 10^{-4}$
Quaternary bases				
Triacetate (IV) monomethiodide	2.4	$6.1 \pm 0.6 \times 10^{-3}$	$4.7 \pm 0.5 \times 10^{-3}$	$7.3 \times 0.7 \pm 10^{-4}$
Triacetate (IV) bismethiodide	[d]	Base production[b]	$6.4 \pm 1.2 \times 10^{-3}$; 8.30×10^{-4})[d]	
Asymmetric diacetate (V) bismethiodide	1.3		2.3×10^{-3} 7.1×10^{-4}	$2.2 \pm 0.2 \times 10^{-3}$
Diacetate of unknown structure (R_f 0.95) bismethiodide	0.14[c]		$4.8 \pm 0.5 \times 10^{-4}$	

[a] The total consumption was determined after the (asymptotic) termination of spontaneous hydrolysis. [b] The first stage of hydrolysis is characterized by a brief initial burst of acid production followed by a sustained and slower hydrolysis. The second stage showed an increased rate. [c] Only one stage of very slow hydrolysis was observed. [d] This bis-quaternary compound produced two moles of *base*, with the indicated first-order rate constants, before turning to a slow acid production with an apparent k_1 of $\sim 3.5 \times 10^{-3}$ min.$^{-1}$. In other experiments it was found necessary to add peroxide-containing dioxane in order to initiate base production. No volatile base such as trimethylamine which would result from a facile Hofmann elimination could be positively identified by gas as well as paper chromatography (*cf.* M. Steiner and E. Stein v. Kamienski, *Naturwiss.*, **40**, 483 (1953)). The stoichiometry of the base production was erratic varying from 0 to almost 2 moles of base per mole of bias-quaternary compound. In view of the catalytic effect of peroxide-containing dioxane on the generation of base from tri-O-acetyl-N,N'-tetramethyl-2-deoxystreptamine bismethiodide, the oxidation of compounds of this series was investigated. Catalytic oxidation with platinum and oxygen in aqueous acetone as used for the preparation of (*epi*)muscarone (*cf.* C. H. Eugster, F. Häfliger, R. Denss and E. Girod, *Helv. Chim. Acta*, **41**, 205 (1958)) in the case of N,N'-tetramethyl-2-deoxystreptamine bismethiodide and its symmetric O-diacetate led to uptake of oxygen, but the action of base on the reaction mixture failed to show the presence of a pyrogallol chromophore expected *via* the 5-ketone and subsequent epimerizations and double Hofmann elimination.



cessive hydrolytic stages, after one equivalent of reaction, are still characterized by sizable rate constants in the numerical range 10^{-2} to 10^{-3} min.$^{-1}$. Hence, the prime element for production of lability of all *three* ester linkages in this series may lie in the over-all conformation of a penta-substituted all-*trans* structure.

However, superimposed on this major element of conformation-dictated lability are some secondary and rather subtle elements of control over labilization. The first of these is seen on inspection of the rate data (Table III) for the two tertiary diacetates V and VI and comparison with the stage-by-stage rates of the tertiary triacetate IV. Stage 2 hydrolysis of both V and VI corresponds nicely to stage 2 hydrolysis of the triacetate IV, but with respect to stage 3 hydrolysis, compound V clearly follows the behavior of IV in the terminal hydrolysis step and contrasts strongly with the complete lack of stage 3 hydrolysis displayed by the symmetric diacetate VI. This observation raises the distinct probability that one of the two kinetically distinct monoacetates is labile, with a k_1 value $\sim 3 \times 10^{-3}$, and that the other is non-labile, with a net degradation scheme (starting with triacetate) of the form



N,N'-tetramethyl-2-deoxystreptamine

The chromatographic evidence for the formation of symmetric and asymmetric diacetates V and VI during the hydrolysis of triacetate and the isolation of N,N'-tetramethyl-2-deoxystreptamine after complete hydrolysis lend support to this scheme.

As seen from the n.m.r. spectra (Table I) the four N-methyl peaks of the triacetate IV as well as of one diacetate are coincident, whereas in the other diacetate they are markedly separated. Since this difference would appear to be due either to asymmetric shielding or hydrogen bonding, it is reasonable to assign the symmetric structure V to the diacetate of m.p. 125–130° and the asymmetric structure VI to the diacetate of m.p. 137–140°.

However, no final and binding assignments of structures to the intermediates and no exact correlation of rate of hydrolysis with particular O-acetyl groups are possible at this time, because acyl migrations prior to, or concomitant with, hydrolysis have not been excluded. In fact, even

TABLE IV

Compound	Color with $CuCl_2$[14] After 1 min.	Color with $CuCl_2$[14] After 12 hr.	Color with $CoCl_2$ After 1 min.
N,N′-Tetramethyl-2-deoxystreptamine (III)	Bright emerald green	Green ppt. formed	Deep blue violet
Tri-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine (IV)	Pale yellow green (negative)	No change	Pink (negative)
Symmetric di-O-acetyl-N,N′-tetramethyl-2-deoxytreptamine (V)	Pale green	Green, ppt. formed	Pale violet
Asymmetric di-O-acetyl-N,N′-tetramethyl-2-deoxystreptamine (VI)	Pale yellow green	Pale green, ppt. formed	Pink violet
Diacetate of unknown structure (R_f 0.95)	Pale yellow green (negative)	No change	Pink (negative)

epimerization, although thermodynamically not favored in a pentasubstituted all-*trans* and equatorial cyclohexane system, is a definite possibility, since a third diacetate of N,N′-tetramethylstreptamine of unknown configuration has been obtained and is described in the Experimental part.

Table IV describes the color phenomena observed in the complex formation of several deoxystreptamine derivatives with methanolic cupric chloride, a tool which has been used for configurational assignments of streoisomeric amino alcohols.[14]

The study of the lability of acetate esters derived from simpler model systems, such as 3α (and β)-N-dimethyl-1α,2β(and α)-cyclohexanediols[15] will be of interest.

Another element of relatively minor significance in the control of ester group lability in this series is seen in the comparison of the hydrolytic steps of tertiary esters with their corresponding mono- or diquaternary derivatives. Quaternization leads to a definite decrement in hydrolysis velocity at each stage of reaction. This result affords a curious contrast with the previously observed[3] behavior in the fully acetylated streptamine series; an acetoxy function in the 2-position causes the quaternary derivative I to surpass the tertiary parent compound in initial hydrolysis rate by a factor of about 5, at *p*H 7.7. Apparently two effects are operative: a *field effect* in the quaternary compounds accelerates nucleophilic attack without being able to transfer directly a proton to the ester site, whereas a *charge transfer effect* is operative in the protonated tertiary amines which are capable of transferring their charge to a neighboring ester[15a] aided by over-all conformational factors. In the monoquaternary streptamine derivative I both effects may be additive. If this picture is correct future studies might show that rates of hydrolysis in the diquaternary series are less dependent on *p*H than in the ditertiary series. That neighboring ammonium ions may have a protective influence on labile groups is known from 6-deoxy-6-aminomethyl-α-glucoside which is stable to acid hydrolysis.[16]

The new concept of labilization through over-all conformational effects should add to the interest in cyclic aminopolyols, their partial symmetric and asymmetric esters and acetals such as are present in kanamycin, a 4,6-disubstituted deoxystreptamine,[17] and paromomycin, a 5,6(4,5)-disubstituted deoxystreptamine.[18]

Acknowledgment.—We are indebted to Dr. Louis Cohen of this Institute for his assistance to us in the interpretation of n.m.r. spectra, to Mr. Robert Badley for the measurement of the n.m.r. spectra and to Dr. Edward Garrett for his valuable comments.

(14) *Cf.* G. Drefahl, *Ber.*, **93**, 509, 514 (1960).

(15) *Cf.* R. A. Barnard and L. R. Hawkins, *Can. J. Chem.*, **36**, 1241 (1958).

(15a) *Cf.* E. R. Garrett, THIS JOURNAL, **79**, 5206 (1957), **80**, 4049 (1958).

(16) F. Cramer, H. Otterbach and H. Springmann, *Ber.*, **92**, 384 (1959).

(17) M. J. Cron, D. L. Evans, F. M. Palermiti, D. F. Whitehead, I. R. Hooper, Paul Chu and R. U. Lemieux, THIS JOURNAL, **80**, 4741 (1958).

(18) Th. H. Haskell, J. C. French and Q. R. Bartz, *ibid.*, **81**, 3482 (1959).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC., GROTON, CONN.]

2-Acetyl-2-decarboxamidoöxytetracycline

BY F. A. HOCHSTEIN, M. SCHACH VON WITTENAU, FRED W. TANNER, JR., AND K. MURAI

RECEIVED MAY 14, 1960

A new antibiotic, $C_{23}H_{25}NO_9$, has been isolated from the fermentation beers of a strain of *Streptomyces rimosus*. Consideration of its physical properties, and acid hydrolysis to water, acetic acid, dimethylamine and decarboxamidoterrinolide (V) shows it to be 2-acetyl-2-decarboxamidoöxytetracycline (VIII). The new antibiotic resembles oxytetracycline in its antibacterial spectrum, though it is less active.

2-Acetyl-2-decarboxamidoöxytetracycline (ADOT)[1] was first detected as a minor compound in the broth of an oxytetracycline-producing strain of *Streptomyces rimosus*. Further mutation of this actinomycete yielded a strain which produces predominantly ADOT and only low levels of oxytetracycline. The new antibiotic was isolated as a yellow crystalline hydrochloride, m.p. 200–203° dec., with the composition $C_{23}H_{25}NO_9 \cdot HCl$; ADOT resem-

(1) We have chosen 2-acetyl-2-decarboxamidoöxytetracycline as a simple generic name for this substance. The abbreviated form, ADOT, is used henceforth in this article.