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

Labilization of Ester Bonds in Aminocyclitol Derivatives. I. Derivatives of *myo*- and *scyllo*-Inositols and of Streptamine[1]

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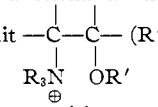
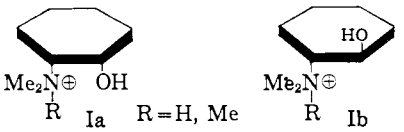
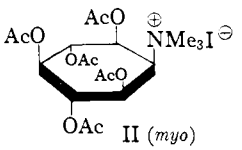
The neutral hydrolysis of acetate esters in the *scyllo* and *myo* series of tertiary and quaternary deoxyaminoinositol derivatives has been briefly studied as a function of over-all molecular conformation. A definite increment in ester labilization is imparted to one or more acetate functions by their incorporation into the *scyllo* conformation, as compared with *myo* structures. This additional lability extends to diamino cyclitols in the *scyllo* or streptamine series, in which all six substituents are also in the all-*trans* configuration. A possible explanation for increased ease of acetate hydrolysis as indicated by structure in the *scyllo* derivatives is thought to be centered about increased steric accessibility and/or internal assistance to hydrolysis by oriented ring intermediates. The *myo* structure II has been shown to be susceptible to acetylcholinesterase (AChE) catalyzed increments of hydrolysis rate, with an activity *vs.* [substrate] profile quite similar to those previously observed for the simple 1,2-difunctional aminoacetates in the cyclohexane and cyclopentane series. Interestingly, a multiplicity of the bifunctional unit  (R′ = Ac, H) does not lead to increased activity as substrate or inhibitor of AChE, or to augmented blocking power with respect to the propagated impulse in bullfrog sciatic nerve.

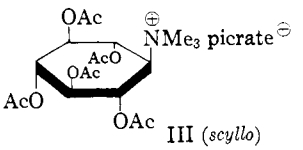
As a consequence of observations[2a,b] on the acetylcholinesterase (AChE) inhibitory properties displayed by cyclic amino alcohols of the general structure I, in which the stereochemical orientation of the two substituents markedly influences the enzymatic and pharmacological[2c] activities of these substances, it became a matter of considerable interest to extend these studies to *poly*functional aminocyclitol systems in which the *over-all* conformations of the molecules might furnish the structural parameter controlling activity. Indeed, preliminary studies[3] in the aminocyclohexitol series have shown that the molecular conformation of aminopolyacetates exercises striking control over the rate and extent of non-enzymatically catalyzed hydrolysis of the ester functions. It is the purpose of this paper to present these results and certain associated enzymatic phenomena in some detail. Also, since evidence for a limited ability to block impulse conduction in frog sciatic nerve and to produce neuromuscular blockade[2b] has been gathered for the simple structure Ia (R = H), it was of further interest to assay these aminopolyacetates for nerve-inhibitory properties as a general function of structure.



Results

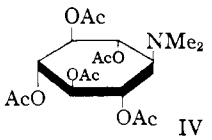
Non-enzymatic Hydrolysis at Neutral *p*H.—Initial studies of hydrolytic activity in purely aqueous medium were hampered by the low solubilities of certain of the cyclitol derivatives used. Consequently, many of these experiments were carried out at 25° in a supporting medium containing 0.088 *M* NaCl as electrolyte and 12.5% (by volume) in methanol to facilitate complete solution. Kinetic experiments began with a comparison of initial velocities of acetate hydrolysis for quaternary ammonium derivatives with *myo* and *scyllo* configurations, *i.e.*, II, trimethyl-(penta-O-acetyl-2-deoxy-2-*myo*-inosityl)-ammonium iodide, and III, trimethyl-(penta-O-acetyldeoxy*scyllityl*)-ammonium picrate,

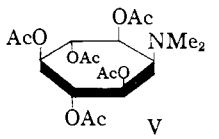




and were necessarily limited in scope by the milligram quantities of these materials available for study. Rates were followed by titration of liberated acetic acid with base to maintain constant *p*H. For comparative purposes the first-order rate constant derived from the initial slope of each hydrolysis curve is tabulated as an index of hydrolytic activity. For the *myo* and *scyllo* configurations I and II at 25° these rate constants, as summarized in Table I, show a minimum[4] factor of about 5 in favor of the *scyllo*.

A further demonstration of the greater hydrolytic lability resulting from the *scyllo* as compared with *myo* configuration is seen in the relative rates of neutral hydrolysis of the *tertiary* amino derivatives I (*scyllo*) and V (*myo*). Here, a hydrolysis rate ratio of 2.2 (see Table I) is displayed by the *scyllo* isomer over the *myo*. This comparison of initial rates appears to rest on the hydrolysis of a maximum of one OAc function, in the *myo* isomer V at least, since a long term hydrolysis run under kinetic





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

(2) (a) H. D. Baldridge, W. J. McCarville and S. L. Friess, THIS JOURNAL, **77**, 739 (1955); (b) S. L. Friess, *ibid.*, **79**, 3269 (1957); (c) S. L. Friess, F. G. Standaert and L. J. Reber, *Proc. Soc. Exptl. Biol. Med.*, 1958, in press.

(3) G. F. Holland, B. Witkop and S. L. Friess, *Experientia*, **14**, 129 (1958).

(4) That this factor would be even larger in experiments with II at *p*H levels higher than 5.4 is inferred from general observations in the series (Table I) that hydrolytic rates rise with increasing *p*H in the *p*H 6–7 range.

TABLE I

HYDROLYSIS RATES OF CYCLITOL DERIVATIVES

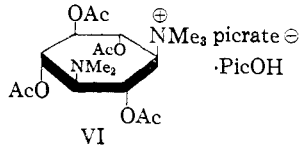
25°, 12.5% (vol.) methanol, [NaCl] = 0.088 *M*

Compound	*p*H	Initial rate k_1, min.$^{-1}$ × 10^3	Moles of base consumed[b] per mole of compound
II (*myo*)	7.33	1.17	
III (*scyllo*)	5.40	~6[a]	
IV (*scyllo*)	7.65	1.32	1.57
V (*myo*)	7.71	0.60	0.44
VI (strep.)	7.70	13.1	1.58
	7.08		1.38
VII (strep.)	6.22	7.45	
	6.33	14.5	
	6.72	29.1	
	6.89	36.3	
VIII (strep.)	7.40	2.05	0.77
	7.65	2.76	1.04
	7.80	3.62	0.87

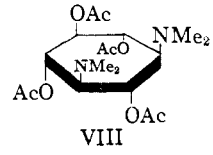
[a] A lower limit because of incomplete solubility in the supporting medium. [b] Measured when further spontaneous hydrolysis is negligible.

test conditions to the point of virtually zero residual rate resulted in a total release of HOAc amounting to 0.44 mole per mole of starting compound. In contrast, the *scyllo* derivative IV, after its initial rapid phase consuming nearly a mole of titrant base, proceeds at a slower pace ($k_1 = 9 \times 10^{-4}$ min.$^{-1}$) until coming to a virtual halt at a total base consumption for liberated acetic acid of 1.57 moles per mole of IV.

This relatively high lability of one or more acetate functions in molecules possessing the *scyllo* configuration is repeated in the streptamine series for derivatives in which the C-3 oxygen function of III and IV is replaced by a second $-NMe_2$ or $-\overset{+}{N}Me_3$ group. For example, hydrolysis at neutral *p*H of the monoquaternary streptammonium dipicrate VI proceeds at a very rapid rate in solutions near satu-



VI

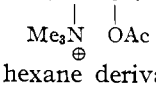


VIII

ration ($k_1 \sim 13 \times 10^{-3}$ min.$^{-1}$), while stoichiometric runs (permitted to proceed until diminution of rates to vanishing values occurred) gave ratios of base consumed/mole of VI in the numerical range 1.38–1.58. Essentially the same high rates were obtained with an incompletely acylated sample of the chloride–hydrochloride VII corresponding to the dipicrate VI. For this streptamine derivative VII, studied over the *p*H range 6.2–6.9 (see Table I) as a representative compound in the *scyllo* series, first-order rate constants increasing from 7 to 36 × 10^{-3} min.$^{-1}$ were observed.

The ditertiary streptamine derivative VIII also displays enhanced hydrolytic activity as compared with the *myo* tertiary analog (*cf.* a rate ratio of 6.0 for VIII compared with V near *p*H 7.8), but this activity apparently extends only to the lability of a maximum of one OAc function, since values for moles of base consumed/mole of compound range upward to about 1.0.

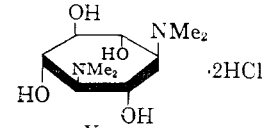
Enzymatic (AChE) Results.—The availability of adequate amounts of the quaternary salt II in the *myo* series, with the added feature of good solubility in aqueous buffer systems, made it possible to study this compound as an AChE substrate. In a sense, this salt is a twofold analog (*i.e.*, possesses a twofold *cis* >C—C< unit) of the *cis* difunctional cyclo-



hexane derivative IX, which has previously been shown[2a] to surpass the natural substrate acetylcholine chloride (AC) in certain aspects of AChE catalyzed hydrolysis, and it was of some significance to assess the potency of II for comparison with IX. Results at *p*H 7.32 and 25.14° are summarized in Fig. 1. It is seen that the activity *vs.* [substrate] profile is essentially of the same character as that observed for the bifunctional cyclohexane[1a] and cyclopentane[5] derivatives, with a nearly horizontal section of the curve at low substrate levels and an inflection point before proceeding to a substrate optimum, followed by marked inhibition of activity in the excess substrate region. Several quantitative features of the curve include: (1) the maximum in activity which occurs at 4.20×10^{-3} *M* (as compared with 3.35×10^{-3} *M* for the acetylcholine optimum under present conditions); (2) a steady-state rate constant $k_1 = 3.64 \times 10^{-3}$ min.$^{-1}$ at the substrate optimum; and (3) the observation that at the two optima for II and acetylcholine, respectively, under these conditions, the measured rates of splitting of the esters, are virtually identical. Result (2) above indicates that the relative rate produced by AChE operating on II (at optimum substrate level) as compared with the non-enzymatic hydrolysis rate at *p*H 7.3 is of the order of 3.1. However, II is inferior to both IX and the acetylcholine ion as an AChE substrate, in the sense that it requires a higher initial concentration to reach its optimum activity than does either IX or AC, and at its optimum it does not exceed the activities observed for IX or AC at their respective optima.

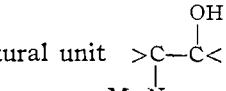
A second set of enzymatic experiments was carried out with the ditertiary streptamine derivative X, in runs designed to test the nature and strength

IX



X

of its inhibitory power *vs.* the AChE–AC system at 25.14°. Selection of X was based on the anticipation that a twofold *trans* structural unit



would lead to inhibition *via* bifunctional interaction[6] with the enzymatic catalytic site of the same order of strength as that unexpectedly observed[2b] for Ia (R = H). The results of these inhibition tests at two substrate levels are summarized in Table II. It is seen that, within the limits of reliability im-

(5) S. L. Friess and H. D. Baldridge, THIS JOURNAL, **78**, 2482 (1956).

(6) D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).

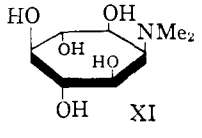
TABLE II

INHIBITION OF THE AChE-AC SYSTEM BY STREPTAMINE DERIVATIVE X

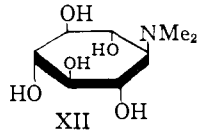
$[AC]_0$, $M \times 10^3$	*p*H	Competitive $K_1 \times 10^5$
1.69	6.5–7.0	7.3 ± 0.5
3.35	6.2–6.7	8.7 ± 0.6

posed by imperfect *p*H control as the concentrations of the dihydrochloride X were varied in a weakly poised buffer, the inhibition is of the competitive type with an enzyme–inhibitor dissociation constant K_1 of $(8.0 \pm 0.7) \times 10^{-5}$.

Structures Leading to Blockade of the Nerve Impulse.—Brief studies[7] of the effects of 10 m*M* solutions of V, X and XI, and saturated solutions of VIII and XII (all at *p*H 8.1) on the propagated impulse in desheathed bullfrog sciatic nerve were



XI



XII

prompted by the observation that Ia (R = H) is demonstrably active in attenuating the spike potential under these same conditions. Solutions (10 m*M*) of Ia at *p*H 8.1 produce 13 ± 1% attenuation of the spike height in an action reversible on washing of the nerve; lowering of the *p*H of the solution to 6.7 resulted in complete loss of activity. In comparison with this behavior, negligible attenuation of the spike was produced at *p*H 8.1 by the compounds V, X, VIII and XII. However, compound XI displayed virtually the same power in attenuation as that shown by Ia, producing 13 ± 3% decrease in height of the spike potential in a reversible type of action, with essentially no effect on the velocity of impulse propagation. Accordingly, a twofold *cis* >C(OH)—C(NMe$_2$)< unit (in XI) results in no measurable increase in attenuating power over that produced by the single bifunctional unit (in Ia).

Discussion

A major point of curiosity arising from the neutral hydrolysis of acetate esters in the *myo* and *scyllo* series resides in the nature of the constraints imposed by molecular architecture that lead to relative lability in the *scyllo* (and streptamine) derivatives. Gross examination of molecular models of the cations II and III offers some preliminary enlightenment. It is difficult to accommodate six bulky substituents on the ring, but Briegleb models do permit the construction of both cations in the chair form. In the all-*trans* and all-equatorial *scyllo* derivative III the acetate functions adjacent to the quaternary nitrogen atom appear less crowded and present greater solid angle of exposure of the carbonyl carbon atoms to the attack of a potential nucleophilic agent, *e.g.*, a solvent molecule or an intramolecular anionoid center, than in the highly compact *myo* structure II.

(7) Measurements made by Mr. E. Whitcomb, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Md., according to techniques described previously: J. W. Moore, S. L. Friess and E. Whitcomb, *J. Cell. Comp. Physiol.*, 1958, in press.

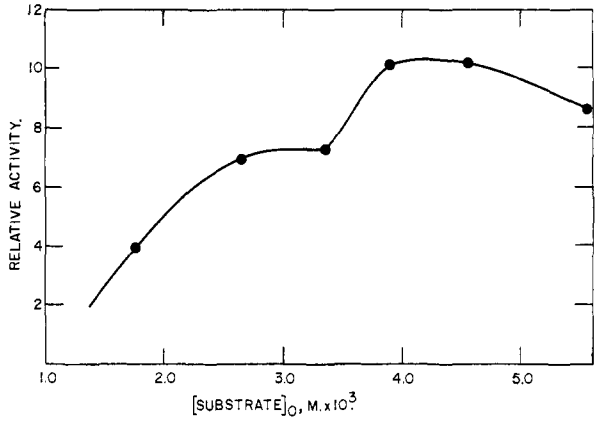
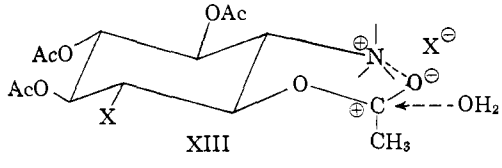


Fig. 1.—Enzymatic hydrolysis of the trimethyl-(penta-O-acetyl-2-*myo*inosityl)-ammonium iodide (II) at *p*H 7.32 and 25.14 ± 0.03°.

If such a difference in steric accessibility produced by molecular conformation can indeed be taken as a prime factor in accounting for acetate group lability in these aminodeoxyinositol derivatives, it is of some concurrent interest to consider the possibility of neighboring group interactions that might augment lability, similar to those postulated by Garrett[8a] in the hydrolysis of scopolamine methyl bromide, to the analogies of Henbest and Lovell,[8b] and in part formally similar to the proposals on hydrolysis of salicylic phosphate esters made by Sobotka, Chandley and co-workers.[9] Such a representative labilization of a quaternary derivative in the *scyllo* (or streptamine) series *via* some quasi cyclic state, which could perhaps achieve greater planarity and stability in the less sterically crowded *scyllo* conformation as compared with *myo*, is pictured in structure XIII. Here, H_2O entry would be facilitated by orientation in the cycle, whose stability in turn depends upon the



XIII

ability to achieve ionic interaction (as dictated by over-all conformation) between the charged N atom and the negative end of the carbonyl dipole.

The enzymatic observations also present several points of intrinsic interest. First, the fact that AChE-catalyzed hydrolysis of a *myo*-acetate (II) at the optimum of its activity–*p*S curve only increases cleavage rates by a factor of 2 to 3, and thereby approaches the more elevated velocities found in the *scyllo* series, points to the relative potencies of molecular conformation *vs.* enzymatic catalysis in mediating ester lability in these cyclic compounds. The enzymatic hydrolysis of II is also particularly instructive in the sense that the shape of the activity profile and the maximum activity evoked are essentially those of the simple *cis* bi-

(8) (a) E. R. Garrett, THIS JOURNAL, 79, 1071 (1957); (b) H. B. Henbest and B. J. Lovell, *J. Chem. Soc.*, 1965 (1957).

(9) J. D. Chanley, E. M. Gindler and H. Sobotka, THIS JOURNAL, 74, 4347 (1952); J. D. Chanley and E. Feageson, *ibid.*, 77, 4002 (1955).

functional molecule IX. This behavior is certainly consonant with the assumption[1a] that AChE operates on these compounds by two-point interaction with only two polar functional groups; any multiplicity in occurrence of these two bifunctional units, as in IX, appears incapable of evoking greater response from the enzyme by even a statistical factor of two through an increased probability of two-pointed, oriented interaction of enzyme with substrate.

A comparison in much the same vein emerges from the observations on X as an inhibitor of the AChE–AC system. As contrasted with its *cis* analog Ia (R = H) with a single bifunctional unit, which has previously been shown to be non-competitive in action as an AChE inhibitor, the double barreled *trans* derivative X is essentially competitive in its inhibitory behavior with respect to the AChE–AC system, but displays a decrease in strength by about one order of magnitude from that shown by Ia. The implication here is that *trans* configuration in a given bifunctional unit leads to lower activity than the *cis* (*cf.* the *cis vs. trans* activities in bifunctional quaternary compounds of the cyclohexane[2a] and cyclopentane[5] series), and that a twofold multiplicity of the unit in a given molecule leads to no dramatic increase in inhibitory power.

Finally, and quite in accord with the same notion of the ineffectiveness of multiplicity of a given molecular unit in enhancing biological activities in this series, it is of passing interest that the twofold *cis*

$$\begin{array}{c} >C-C< \\ \quad | \quad | \\ Me_2N \quad OH \end{array}$$

unit in XI, and its unitary analog in the ring structure Ia (R = H), display essentially the same activity with respect to blockade of the propagated impulse in bullfrog sciatic nerve.

Experimental[10]

2-Keto-*myo*-inositol[11] was prepared by the oxidation of *myo*-inositol with *Acetobacter suboxydans*.[12,13] 2-Amino-2-deoxy-*myo*-inositol (inosamine SA) and aminodeoxyscyllitol (inosamine SB) were prepared by the Raney nickel reduction of the phenylhydrazone obtained from 2-keto-*myo*-inositol.[14]

Penta-O-acetyl-2-deoxy-2-dimethylamino-*myo*-inositol (V).—A mixture of 1.6 g. (6.6 mmoles) of 2-deoxy-2-dimethylamino-*myo*-inositol hydrochloride (XI, m.p. 217°)[15], 45 ml. of acetic anhydride and 0.45 g. of sodium acetate was heated under reflux for 90 minutes. After this time the solution was concentrated under reduced pressure and the residue dried *in vacuo*. The residue was dissolved in a mixture of chloroform–water and the chloroform layer separated and dried over sodium sulfate. The product obtained after removal of the solvent was recrystallized from methanol as colorless prisms (1.2 g., 44%), m.p. 130°. An analytical sample was prepared by two more recrystallizations from methanol; m.p. 132–133°.

Anal. Calcd. for $C_{18}H_{27}NO_{10}$: C, 51.79; H, 6.52; N, 3.36. Found: C, 51.50; H, 5.30; N, 3.34.

The picrate was prepared from a very dilute acidic solution; it separated as fine needles and was recrystallized from methanol; m.p. 252°.

Anal. Calcd. for $C_{18}H_{27}NO_{10}\cdot C_6HN_3O_7$: C, 44.59; H, 4.69; N, 8.66. Found: C, 44.57; H, 4.59; N, 8.52.

Trimethyl-(penta-O-acetyl-2-deoxy-2-*myo*-inosityl)-ammonium Iodide (II).—To a solution of 0.05 g. (1.2 mmoles) of penta-O-acetyl-2-deoxy-2-dimethylamino-*myo*-inositol (V) in 3 ml. of ethyl acetate was added 0.15 ml. of methyl iodide. After heating under reflux for 20 minutes, an additional 0.15 ml. of methyl iodide was added and again refluxed for 20 minutes. The fine colorless needles which formed on storage in a refrigerator were centrifuged and washed three times with ethyl acetate. There was obtained 0.035 g. (52%), m.p. 255–257°. An analytical sample of the quaternary iodide was prepared by recrystallization from methanol; m.p. 256–257°.

Anal. Calcd. for $C_{19}H_{30}NO_{10}I$: C, 40.79; H, 5.37; N, 2.51; I, 22.71. Found: C, 40.64; H, 5.29; N, 2.61; I, 22.76.

Smaller yields were obtained when the conditions for quaternization were varied. For example in refluxing methyl iodide 45%, and in a mixture of ethyl acetate and methyl iodide at 25°, 40% of the iodide were formed.

Dimethylaminodeoxyscyllitol (XII) Hydrochloride. Aminodeoxyscyllitol hydrochloride (0.75 g., 3.5 mmoles) was treated with aqueous alcoholic ammonia to liberate the free base (0.6 g., 94%). The latter was dried in *vacuo* and then heated on a steam-bath for 2 hours in a mixture of 0.6 ml. of 37% formaldehyde and 0.8 ml. of 98% formic acid. On cooling it was acidified with concentrated hydrochloric acid and methanol and ether were added to the cloud point. After considerable scratching and two days in a refrigerator 0.7 g. (88%) of crystalline material, m.p. 221–223°, was collected. The sample for analysis was obtained by two recrystallizations from methanol; m.p. 222–223°

Anal. Calcd. for $C_8H_{17}NO_5\cdot HCl$: C, 39.44; H, 7.39; N, 5.75. Found: C, 39.40; H, 7.09; N, 5.87.

In subsequent preparations, due to the difficulty in obtaining this hydrochloride crystalline, the product after methylation with formaldehyde and formic acid was not acidified but instead concentrated to an oil, dried *in vacuo* and then acetylated.

Penta-O-acetyldimethylaminodeoxyscyllitol (IV).—Dimethylaminodeoxyscyllitol hydrochloride (0.2 g., 0.82 mmole) was refluxed for one hour in a mixture of 5 ml. of acetic anhydride and 0.1 g. of sodium acetate. After the excess anhydride was removed and the residue dried *in vacuo*, water–chloroform was added and the chloroform layer separated and dried. The powder remaining after evaporation of the solvent was recrystallized from methanol and yielded 0.163 g. (48%) of colorless crystals, m.p. 210–213°. An analytical sample, m.p. 213–214°, was prepared by two recrystallizations from methanol.

Anal. Calcd. for $C_{18}H_{27}NO_{10}$: C, 51.79; H, 6.52; N, 3.36. Found: C, 51.91; H, 6.45; N, 3.32.

Trimethyl-(penta-O-acetyldeoxyscyllityl)-ammonium picrate (III).—To a solution of 0.2 g. (0.48 mmole) of penta-O-acetyldimethylaminodeoxyscyllitol (IV) in 5 ml. of freshly distilled acetonitrile was added 2.5 ml. of methyl sulfate. After 24 hours at room temperature, the solvent was evaporated under reduced pressure and the residue treated with chloroform and water. The aqueous extract was concentrated to a small volume. Aqueous picric acid was added. The yellow needles of the picrate, after storage in a refrigerator overnight, were collected and dried *in vacuo*. After recrystallization from ethanol fine needles, m.p. 193–194°, were obtained.

Anal. Calcd. for $C_{19}H_{30}NO_{10}\cdot C_6H_2N_3O_7$: C, 45.45; H, 4.89. Found: C, 45.21; H, 4.94.

No noticeable quaternization was observed with methyl iodide at room temperature, at 42°, in refluxing benzene, in refluxing chloroform or even in a sealed tube at 100°.

1,3-Dideoxy-1,3-bisdimethylaminoscyllitol (N,N′-Tetramethylstreptamine) Dihydrochloride (X).—1,3-Diamino-1,3-dideoxyscyllitol (streptamine) dihydrochloride[16] (0.6 g., 2.4 mmoles) was converted to the free base (0.37 g., 88°)

(10) All melting points are corrected. The microanalyses were performed by Dr. W. C. Alford and his associates of the Analytical Services Unit of this Laboratory.

(11) With regard to nomenclature *cf.* H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(12) H. E. Carter, *et al.*, *J. Biol. Chem.*, **174**, 415 (1948).

(13) We are indebted to Dr. Laura C. Stewart for generous assistance in this operation.

(14) H. E. Carter, R. K. Clark, B. Lytle and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1948).

(15) G. H. Latham, Jr., E. L. May and E. Mosettig, THIS JOURNAL, **74**, 2684 (1952).

(16) We are greatly indebted to Dr. O. Wintersteiner, The Squibb Institute for Medical Research, for a liberal amount of streptamine hydrochloride.

with aqueous alcoholic ammonia. The dry streptamine was dissolved in a mixture of 2.0 ml. of 37% formaldehyde and 4.0 ml. of 98% formic acid and heated on a steam-bath for 20 hours. The mixture was cooled, acidified with concentrated hydrochloric acid and evaporated under reduced pressure. After trituration of the residual oil with absolute alcohol complete crystallization occurred. One obtained 0.45 g. (70%), m.p. 272–275°. The sample for analysis, m.p. 275–276°, was prepared by two recrystallizations from methanol–ether.

Anal. Calcd. for $C_{10}H_{22}N_2O_4 \cdot 2HCl$: C, 39.09; H, 7.82; N, 9.12. Found: C, 39.02; H, 7.96; N, 9.11.

Tetra-O-acetyl-1,3-dideoxy-1,3-bisdimethylaminoscyllitol (N,N'-Tetramethyltetra-O-acetylstreptamine. VIII).—A solution of 2.5 g. of the ditertiary dihydrochloride X in a mixture of 125 ml. of acetic anhydride and 3.0 g. of sodium acetate was heated under reflux for 3 hours. The acetic anhydride was evaporated under reduced pressure and the residue dried *in vacuo*. The residue was taken up in chloroform–water. The chloroform extract was dried over sodium sulfate and taken to dryness. The crystalline residue was recrystallized from absolute alcohol yielding fine, colorless needles, 1.44 g. (30% from 1,3-diamino-1,3-dideoxyscyllitol dihydrochloride), m.p. 190–200° (subl.). An analytical sample was prepared by two recrystallizations from absolute ethanol.

Anal. Calcd. for $C_{18}H_{30}N_2O_8$: C, 53.72; H, 7.53; N, 6.96; CH_3CO, 42.6. Found: C, 53.71; H, 7.59; N, 6.91; CH_3CO, 42.5.

D,L-Tetra-O-acetyl-1,3-dideoxy-1-dimethylamino-3-trimethylammonium-scyllitol Dipicrate (VI).—To a solution of 1.04 g. (2.6 mmoles) of tetra-O-acetyl-1,3-bis-(dimethylamino)-scyllitol (VIII) in 40 ml. of distilled acetonitrile was added 25 ml. of dimethyl sulfate. After 24 hours at 25° the acetonitrile was removed *in vacuo* and the product taken up into chloroform–water. The aqueous layer was separated and concentrated to a small volume. On addition of aqueous picric acid clusters of yellow needles formed. The picrate, after storage in a refrigerator overnight, was collected and dried *in vacuo*. There was obtained 1.04 g. (46%). The analytical sample, prepared by recrystallization from methanol, had m.p. 212–214°.

Anal. Calcd. for $C_{31}H_{38}N_8O_{22}$: C, 42.56; H, 4.39; N, 12.80; CH_3CO, 19.7. Found: C, 42.13; H, 4.19; N, 13.09; CH_3CO, 18.5, 19.5.

A sample of this material was titrated with sodium methoxide in absolute methanol and showed the presence of one acidic hydrogen. Titration with perchloric acid indicated the presence of two moles of picric acid.[17]

Similarly as with penta-O-acetyldimethylaminodeoxyscyllitol (IV) no noticeable quaternization occurred with methyl iodide under a variety of conditions.

Kinetics.—The non-enzymatic hydrolyses were carried out in a total volume of 40 ml., essentially as described previously.[18] Constant stirring was effected by a magnetic stirrer in the closed titration cell, with hundredth normal base being added from a microburet with tip immersed below the fluid level of the reaction mixture. Reactions were continuously monitored for *p*H change, with scheduling such that *p*H drops of 0.05 unit or less occasioned the addition of increments of base to readjust *p*H to the initial level. A typical plot of the initial rate in a neutral hydrolysis run at 25° for compound VIII at *p*H 7.40 is given in Fig. 2.

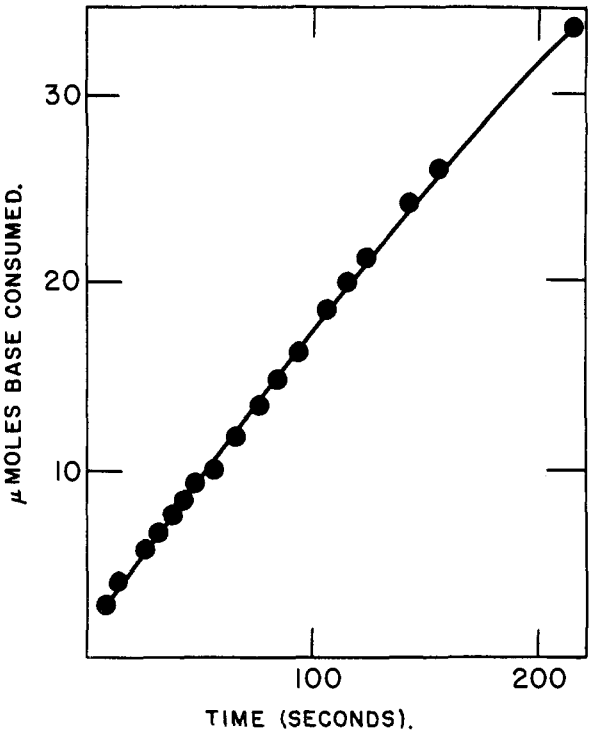


Fig. 2.—Initial rate of spontaneous non-enzymatic hydrolysis for tetra-O-acetyl-1,3-dideoxy-1,3-bisdimethylaminoscyllitol (VIII, 30.6 mg. of compound suspended in a 40 ml. reaction volume) at *p*H 7.40 and 25°.

The enzymatic hydrolysis rates were measured at 25.14° in the micro cell apparatus as previously described,[2,5] with a poorly-poised phosphate buffer medium used in a total reaction volume of 6.40 ml. Recrystallized acetylcholine chloride was employed at its substrate optimum of 3.35×10^{-3} *M* for comparison of rates with the aminopolyacetate hydrolyses, with an over-all reproducibility of ±5% in the enzymatic rates and better than ±10% in those polyester experiments in which sufficient amounts of compound permitted repeated runs. The enzyme employed was a purified preparation derived from electric eel tissue, with an activity (assayed at the substrate optimum) of 4.71×10^3 μmoles AC hydrolyzed/hr./mg. protein. Inhibition of the AChE–AC system by compound X was analyzed in terms of competitive and non-competitive K_I values according to the techniques of Wilson.[19]

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(17) These titrations were kindly performed by Dr. Abraham Patchornik in this Institute.

(18) S. L. Friess, A. A. Patchett and B. Witkop, THIS JOURNAL, **79**, 459 (1957).

(19) P. W. Wilson in "Respiratory Enzymes," H. A. Lardy, ed., Burgess Publishing Co., Minneapolis, Minn., 1949, p. 24.