ican Medical Association invites applications for grants of money to aid in research in problems bearing more or less directly on clinical medicine. Preference is given to requests for moderate amounts to meet specific needs. As a rule grants are not made for the purchase of equipment or apparatus of a permanent nature. For application forms and further information, the committee should be addressed at 535 North Dearborn St., Chicago 10, Ill.

THE Psycho-Acoustic Laboratory, initiated at Harvard University in 1940 as a war research unit, will continue its activities directly under the Faculty of Arts and Sciences. Funds available under a contract with the U. S. Navy, Office of Research and Inventions, will provide for basic research in experimental psychology, with special emphasis on problems of communication (speech, hearing and electronics). There will be a research and teaching staff of about twelve members, including S. S. Stevens, director, and E. B. Newman, associate director.

THE twentieth Exposition of Chemical Industries will be held from February 25 to March 2 in Grand Central Palace, New York. The exposition, on a reduced scale due to war conditions, was last convened in 1943.

THE Long Island College of Medicine, Brooklyn, N. Y., will give the fourth postgraduate course in industrial medicine from January 14 to February 1.

SPECIAL ARTICLES

STREPTOMYCES ANTIBIOTICS. III. DEGRADATION OF STREPTOMYCIN TO STREPTOBIOSAMINE DERIVATIVES

EXPERIMENTAL results indicate that streptomycin has the general constitution of a hydroxylated base (streptidine)[1] attached through a glycosidic linkage to a nitrogen-containing disaccharide-like molecule. The latter moiety of the streptomycin molecule contains a free or potential carbonyl group and a methylamino group.

The hydrolytic cleavage of streptomycin in acid solution and the isolation and characterization of the basic fragment streptidine are described in a forthcoming publication.[1] When the present formula of streptomycin, $C_{21}H_{37-39}N_7O_{12}$,[2] and the formula of streptidine, $C_8H_{18}N_6O_4$,[1] are used in an equation (I)

I. $C_{21}H_{37-39}N_7O_{12} + H_2O \longrightarrow C_8H_{18}N_6O_4 + C_{13}H_{21-23}NO_9$

involving the reaction with one molecule of water, it is seen that a product might be formed which is rich in oxygen and contains a nitrogen atom. The formula, $C_{13}H_{21-23}NO_9$, of such a product, the insolubility of streptomycin in water-immiscible organic solvents, the formation of a streptomycin trihydrochloride-calcium chloride double salt,[2] and the cleavage of streptomycin in acid solution at 25°,[1] are suggestive of the general constitution of a hydroxylated base linked glycosidically to a disaccharide-like molecule. The following experiments yielded further evidence in support of this formulation.

When streptomycin hydrochloride was treated with methanol containing hydrogen chloride, the biological activity decreased markedly. The mixture of products was separated chromatographically into streptidine hydrochloride[1] and the amorphous hydrochloride of a base, methyl streptobiosaminide dimethyl acetal hydrochloride[3]; $(\alpha)\frac{25}{D} - 143°$ (methanol).

Anal. Calcd. for $C_{13}H_{20}NO_7(OCH_3)_3 \cdot HCl$: C, 44.49; H, 7.00; N, 3.24; OCH_3, 21.6. Calcd. for $C_{13}H_{22}NO_7(OCH_3)_3 \cdot HCl$: C, 44.29; H, 7.57; N, 3.19; OCH_3, 21.5. Found: C, 44.35; H, 7.13; N, 4.00; OCH_3, 19.1; amino-nitrogen (van Slyke), none.

The cleavage of streptomycin to give a product of the formula $C_{13}H_{20-22}NO_7(OCH_3)_3 \cdot HCl$, is shown in equation II.

II. $C_{21}H_{37-39}N_7O_{12} \cdot 3\,HCl + 3\,CH_3OH \rightarrow$
$C_8H_{18}N_6O_4 \cdot 2\,HCl + C_{13}H_{20-22}NO_7(OCH_3)_3 \cdot HCl + H_2O$

In the infrared, methyl streptobiosaminide dimethyl acetal hydrochloride in tetrachloroethane solution absorbed in the 3 μ (–OH, > NH) region; no carbonyl absorption could be detected. Since, as will be discussed below, the disaccharide-like portion of the molecule in streptomycin contains a free or potential carbonyl group, it seems likely that in the derivative of streptobiosamine described here, the original carbonyl group has been converted to a dimethyl acetal. The third methoxyl group is presumably that of a methyl glycoside.

Acetylation of methyl streptobiosaminide dimethyl acetal hydrochloride gave a crystalline acetyl derivative, m.p. 124.5–126°, $(\alpha)\frac{25}{D} - 124°$ (chloroform). Analytical and molecular weight data on material recrystallized to constant properties were in agreement with a composition $C_{13}H_{16-18}NO_7(CH_3CO)_4(OCH_3)_3$, or methyl tetra-acetylstreptobiosaminide dimethyl acetal.

[1] Peck, Graber, Walti, Peel, Hoffhine and Folkers, *Jour. Am. Chem. Soc.* (In press.)

[2] Peck, Brink, Kuehl, Flynn, Walti and Folkers, *Jour. Am. Chem. Soc.*, 67: 1866, 1945.

[3] Consideration of a convenient trivial name for this product led to the selection of streptobiosamine for the parent disaccharide-like compound. The name streptobiose would imply a neutral material rather than a base, and streptosamine would imply a nitrogen-containing hexose (similar to glucosamine).

Anal. Calcd. for $C_{13}H_{16}NO_7(CH_3CO)_4(OCH_3)_3$: C, 51.15; H, 6.62; N, 2.49; CH_3CO, 30.6; OCH_3, 16.5; mol. wt., 563. Calcd. for $C_{13}H_{18}NO_7(CH_3CO)_4(OCH_3)_3$: C, 50.97; H, 6.95; N, 2.48; CH_3CO, 30.5; OCH_3, 16.5; mol. wt., 565. Found: C, 50.88, 51.20; H, 7.09, 6.95; N. 2.55; CH_3CO, 29.7; OCH_3, 15.4; mol. wt., 530 (ebullioscopic in benzene).

A differential acetyl determination[4] showed that three of the acetyl groups were attached to oxygen and the fourth to nitrogen. The ultraviolet absorption spectrum of this compound in methanol solution showed only a low end absorption, with no maximum.

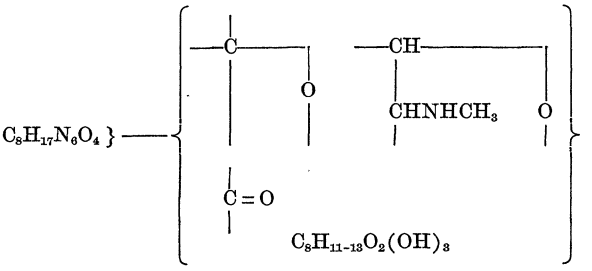
When streptomycin was treated with a variety of carbonyl group reagents, complete inactivation was observed under pH conditions which, in the absence of the reagents, caused only 50 per cent. or less inactivation. These experiments suggested that streptomycin possessed at least one carbonyl group. Streptomycin reacted with hydroxylamine to give an amorphous product having a composition in fair agreement with that of a streptomycin oxime hydrochloride. Similarly, the treatment of streptomycin with semicarbazide yielded an amorphous streptomycin semicarbazone hydrochloride. When streptomycin hydrochloride was treated with an excess of hydroxylamine hydrochloride in the presence of pyridine, an acidimetric determination of the pyridine hydrochloride formed[5] indicated that streptomycin contained a single carbonyl group. Since streptidine[1] is unreactive towards carbonyl reagents, it may be concluded that the free or potential carbonyl group of streptomycin resides in the disaccharide-like (streptobiosamine) moiety.

The failure of methyl streptobiosaminide dimethyl acetal hydrochloride to yield nitrogen in the van Slyke determination indicated that the basic nitrogen atom in streptobiosamine is not present as a primary amino group. Treatment of this compound with silver nitrite yielded an amorphous product with the properties and nitrogen content of an N-nitroso derivative. The presence of an N-acetyl group in methyl tetraacetylstreptobiosaminide dimethyl acetal afforded further evidence of the secondary character of the amino group. When methyl streptobiosaminide dimethyl acetal hydrochloride was subjected to drastic hydrolysis by alkali, methylamine was liberated. The methylamine was characterized by conversion to 2,4-dinitro-N-methylaniline. Since a methyl group might have migrated from oxygen to nitrogen under the influence of alkali,[6] it seemed advisable to carry out the alkaline hydrolysis after prior removal of the methoxyl groups from methyl streptobiosaminide dimethyl acetal hydrochloride by mild acid hydrolysis. This was done, and methylamine was again isolated and characterized. It may be concluded that the nitrogen atom in streptobiosamine is present as a methylamino group.

An examination of the reaction solutions of acid hydrolyses of streptomycin salts for the presence of low molecular weight cleavage products (*i.e.*, other than streptidine and streptobiosamine) did not yield positive results. A search for a volatile acid after alkaline hydrolysis revealed no acidic products other than those which could be accounted for by extensive decomposition of streptobiosamine.

The presence of a methyl group upon the nitrogen atom of streptobiosamine signifies a residual C_{12} structure, which is compatible with the disaccharide-like formulation. Since in all the known naturally occurring amino sugars the nitrogen atom is attached at position 2,[7] it seems likely that the methylamino group in streptobiosamine is at the 2-position of one hexose fragment.

These data and interpretations concerning the structure of streptomycin may be represented graphically as follows:



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[4] Kunz and Hudson, *Jour. Am. Chem. Soc.*, 48: 1982, 1926.

[5] Bryant and Smith, *Jour. Am. Chem. Soc.*, 57: 57, 1935.

[6] *Cf.* Irvine and Hynd, *Jour. Chem. Soc.*, 101: 1128, 1912.

[7] Gilman, "Organic Chemistry, An Advanced Treatise" (2nd Ed.), Vol. II, p. 1615. New York: John Wiley and Sons, Inc., 1943.

QUINOID STRUCTURE AND BACTERIOSTATIC ACTIVITY

BACTERIOSTATIC activity of certain leucoderivatives of malachite green (tetramethyl-diamino-triphenyl-