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.

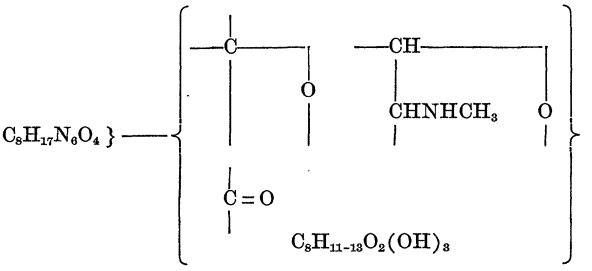
When streptomycin was treated with a variety of carbonyl group reagents, complete inactivation was observed under pH conditions which, in the absence of the reagents, caused only 50 per cent. or less inactivation. These experiments suggested that streptomycin possessed at least one carbonyl group. Streptomycin 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:



Acknowledgment. The authors wish to thank Dr. N. R. Trenner and Mrs. R. C. Anderson for the infrared and ultraviolet absorption measurements, Dr. J. B. Conn for the molecular weight determination, Mr. Richard N. Boos and his associates for microanalyses, and Mr. David Hendlin for assay data.

NORMAN G. BRINK
FREDERICK A. KUEHL, JR.
KARL FOLKERS

RESEARCH LABORATORIES,
MERCK & CO., INC.,
RAHWAY, N. J.

[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-

methane dye) has been related in a foregoing note.[1] The lability of these derivatives, however, makes it difficult to draw definitive conclusions concerning the relation between chemical structure and bacteriostatic activity.

We have now repeated these experiments with the diphenylmethane homologs of the above-mentioned derivatives. Although tetramethyl-diamino-diphenyl-methane dyes have a much weaker bacteriostatic activity than their triphenyl-methane homologs,[2] they offer, nevertheless, the advantage of forming more and stabler leucoderivatives, thus enabling the realization of more complete and more reliable comparative assays.

We have now found that the quinoid dye salts of tetramethyl-diamino-diphenylmethane (*i.e.,* dye salt of Michler's hydrol) and of tetramethyl-diamino-diphenyl (amino) methane (*i.e.,* auramine dye) are bacteriostatically active against *Staphylococcus aureus*, both of them practically at the same concentration (1:40,000).

The leucobases of both compounds, without quinoid structure, were inactive (to 1:5,000).

The methane-sulphonic derivative of Michler's hydrol, also a substance without quinoid structure, was equally inactive. This fact contrasts with our earlier observation according to which the triphenyl-methane homolog of the mentioned substance, *i.e.,* the bisulphite derivative of malachite green, showed a strong bacteriostatic activity in spite of its non-quinoid structure.[1] This bisulphite derivative is, however, an unstable substance ("vat dye"[3]), which transforms easily into the quinoid-structured dye salt, whereas its inactive diphenylmethane homolog has a stable non-quinoid character.[4]

Michler's ketone or tetramethyl-diamino-benzophenone, a non-quinoid substance, was inactive, and so was a series of other leucoderivatives of auramine and of Michler's hydrol (aminoethane nitril, aminoethanoilamide, hydroxyethanoilamide, aminoethanoic acid and hydroxyethanoic acid.[5]

Summary: Among various derivatives of tetramethyl-diamino-diphenylmethane only the quinoid-structured dye salts had bacteriostatic activity in our experiments, while the leucoderivatives were inactive.

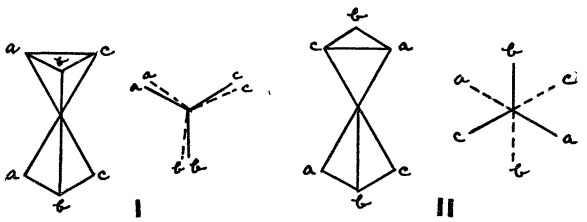
E. FISCHER
C. GARCÉS
A. LÓPEZ

INSTITUTO BACTERIOLÓGICO DE CHILE
AND S. A. ORGANA SANTILAGO

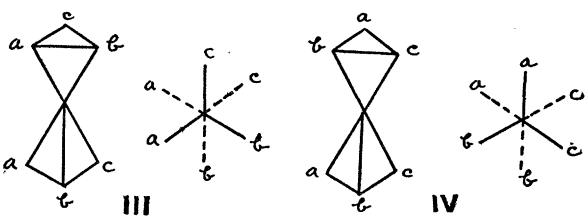
[1] E. Fischer, O. Hoffmann and E. Prado, SCIENCE, 100: 576, 1944.
[2] I. J. Kligler, *Jour. Exp. Med.*, 21: 463, 1918.
[3] H. Wieland, *Ber. deutsch. chem. Ges.*, 52: 880, 1919.
[4] H. Weil, *Ber. deutsch. chem. Ges.*, 27: 1403, 1894.
[5] K. Albrecht, *Ber. deutsch. chem. Ges.*, 27: 3294, 1894.

THE LACK OF MEANING OF THE PHRASE "INACTIVE BY INTERNAL COMPENSATION" AS APPLIED TO MESO COMPOUNDS

THE usual explanation of the inactivity of meso compounds given in both elementary and advanced texts is that the molecule consists of two asymmetric halves which are mirror images and hence rotate the plane of polarization equal amounts in opposite directions. The resulting compound is said to be "optically inactive by internal compensation." It frequently is recognized that the individual molecules would be inactive only when the groups occupy certain specified positions. For example, in the simplest case, the molecule Cabc Cabc is inactive only when the groups are in the positions corresponding to Figs. I and II and the molecules have, respectively, a



plane and a center of symmetry. In each case the mirror images are superimposable. In all other positions, however, for example, those illustrated by Figs. III and IV, there is no plane or center of sym-



metry, and the mirror image of the molecule is not superimposable. The explanation given for the non-existence of forms corresponding to III and IV is the same as that given for the non-existence of isomers of ethane and of 1,2-dichloroethane, namely, the assumption of "free rotation" about the single carbon-carbon bond. It is known, however, that in compounds such as 1,2-dichloroethane[1] and 1,2-dibromoethane,[2] rotation is not free, and that the mean

[1] Debye, *Physik. Z.*, 31: 142, 1930; Beach and Palmer, *Jour. Chem. Phys.*, 6: 639, 1938.
[2] Smyth and Kamerling, *Jour. Am. Chem. Soc.*, 53: 2988, 1931; Beach and Turkevitch, *ibid.*, 61: 303, 1939.