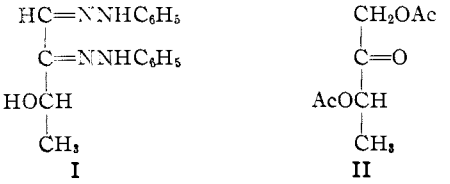
COMMUNICATIONS TO THE EDITOR

STREPTOMYCIN. III. 4-DESOXY-L-ERYTHROSE (THREOSE) PHENYLOSAZONE FROM STREPTOBIOSAMINE

Sir:

We have found that the action of phenylhydrazine base on streptobiosamine results in the formation of a crystalline phenylosazone which has been identified by synthesis as 4-desoxy-L-erythrose (threose) phenylosazone, I.



I II

Streptobiosamine hydrochloride[1] (0.5 g.) was dissolved in water (16 ml.) and phenylhydrazine base (0.5 ml.) was added. The solution was allowed to stand at 24° under nitrogen for seventy-two hours. The resulting crystalline precipitate was collected and adsorbed on alumina from benzene solution. Continued washing with benzene eluted amorphous material followed by a crystalline fraction, which was recrystallized from benzene–hexane to a constant melting point of 145–146° (cor., no dec.); fine yellow prisms $[\alpha]^{25}D$ +113° (*c*, 0.81, pyridine); +52° after twenty-two hours. *Anal.* Calcd. for $C_{16}H_{18}ON_4$: C, 68.07; H, 6.43; N, 19.8. Found: C, 68.11; H, 6.37; N, 19.6. The ultraviolet absorption spectrum (maxima at 255 mμ, ε 18,000; 310 mμ, ε 10,200; 390 mμ, ε 19,700; in ethanol) was identical with that of glucose phenylosazone.

1,3-Diacetyl-4-desoxy-L-erythrulose, II, was synthesized from acetyl-*l*-lactyl chloride via 3-acetyl-1,4-bisdesoxy-1-diazo-L-erythrulose. The diacetyl derivative II was hydrolyzed with dilute ammonia and the hydrolysate was treated with acetic acid and phenylhydrazine. The resulting oil after chromatographic purification yielded the desired phenylosazone I, shown to be identical with the compound from streptobiosamine by melting point (144–145°, mixed m.p. no depression), rotation ($[\alpha]^{25}D$ +116°; +50° after twenty-two hours), absorption spectrum, and analysis (C, 68.26; H, 6.52; N, 19.9).

Since a C-methyl group has been demonstrated in streptomycin[2] and methyl dihydrostreptobiosaminide,[3] it appears probable that the isolated phenylosazone is derived from a fragment of streptonose[3] representing carbon atoms 3 to 6 of this dicarbonyl sugar. On this premise, the asymmetric carbon atom in the phenylosazone is identical with carbon atom 5 of streptonose. Since this carbon atom has now been shown to have the *l*-configuration, streptonose by convention must be designated an *l*-sugar.

The preparation of the phenylosazone from streptobiosamine is well reproducible, with yields of 25–30% of the theoretical in terms of chromatographed material. From this and other considerations it appears likely that the C_4-fragment was formed from streptobiosamine under the influence of phenylhydrazine base rather than as a by-product in the preparation of the disaccharide. The unusual lability of the bond between C_2 and C_3 of streptonose is undoubtedly connected with its dicarbonyl nature, since dihydrostreptobiosamine,[3] in which the aldehyde group of streptonose is reduced, failed to yield the osazone under similar conditions.

(1) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, THIS JOURNAL, **68**, 2096 (1946). The streptobiosamine hydrochloride used in our work was prepared directly by hydrolysis of streptomycin trihydrochloride with 1 *N* H_2SO_4 at 45° for fifteen hours. It was obtained as an amorphous reddish powder containing about 5% of streptidine; $[\alpha]^{25}D$ −96° in water. *Anal.* Calcd. for $C_{13}H_{25}O_9N \cdot HCl$: C, 41.8; H, 6.49; N, 3.76; Cl, 9.50. Found: C, 41.1; H, 6.88; N, 5.15; Cl, 8.84.

(2) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, THIS JOURNAL, **68**, 2120 (1946).

(3) J. Fried and O. Wintersteiner, THIS JOURNAL, in press.

DIVISION OF ORGANIC CHEMISTRY
SQUIBB INSTITUTE FOR MEDICAL RESEARCH
NEW BRUNSWICK, NEW JERSEY
J. FRIED
DORIS E. WALZ
O. WINTERSTEINER

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ELECTROPHORETIC INHOMOGENEITY OF CRYSTALLINE BETA-LACTOGLOBULIN

Sir:

The fact that different values for the molecular weight[1,2] and in its content of certain amino acids[3,4] have been reported for crystalline β-lactoglobulin suggests that the crystalline protein may not be a single substance. Our electrophoretic studies with this protein indicate that this is indeed the case.

Pedersen[5] employed the light absorption technique to observe the moving boundary of a 0.2% crystalline β-lactoglobulin solution in electrophoresis and believed that he had shown that the protein is essentially homogeneous. It is now generally agreed that the protein concentration in a solution should be at least 1% for electrophoretic homogeneity studies and that the Schlieren methods are more sensitive than the light absorption procedure. We employed a 1.5% solution of crystalline β-lactoglobulin in the

(1) Pedersen, *Biochem. J.*, **30**, 948 (1936).

(2) Bull, THIS JOURNAL, **68**, 742 (1946).

(3) Brand, Saidel, Goldwater, Kassell and Ryan, *ibid.*, **67**, 1524 (1945).

(4) Chibnall, *J. I. S. L. T. C.*, **30**, 1 (1946).

(5) Pedersen, *Biochem. J.*, **30**, 961 (1936).

Tiselius electrophoretic apparatus with the Longsworth scanning method. The protein was crystallized three times as prepared by the method of Palmer.[6] Results showed that the material behaved as a single component in acetate buffer of ionic strength 0.10 at *p*H 5.3 and 5.6 with mobilities of -1.4×10^{-5} and -2.5×10^{-5} sq. cm. per sec. per volt at 1.5°, respectively. But, when the same preparation was electrolyzed at *p*H 4.8 and 6.5, it appeared to consist of three components with the following mobilities and relative concentrations:

*p*H	Mobility (10^5), sq. cm./sec./volt	Relative conen., %
4.8	+2.3	68
	+1.9	22
	+1.2	10
6.5	−5.6	48
	−5.2	25
	−4.5	27

Further crystallizations of the protein did not alter this electrophoretic behavior. It may be noted that the relative concentration of each component at *p*H 4.8 is not the same as that for the same components at *p*H 6.5; this disagreement may be caused by certain interactions between the components occurring in the mixture. However, it is clear that the fastest boundary is formed by the protein which is in the highest concentration. From the plot of values of mobility against *p*H's, the isoelectric point of the main component can be shown to be at *p*H 5.1. This value does not differ greatly from that obtained by Pedersen[5] as the isoelectric point of β-lactoglobulin.

Whether or not the demonstrated electrophoretic inhomogeneity of the crystalline β-lactoglobulin depends upon the method of preparation is now being investigated.

(6) Palmer, *J. Biol. Chem.*, **104**, 359 (1934); the author is greatly indebted to Dr. E. F. Jansen who kindly prepared the crystalline protein for these experiments.

INSTITUTE OF EXPERIMENTAL BIOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA CHOH HAO LI

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DEGRADATIVE STUDIES ON STREPTOMYCIN

Sir:

Acetylation of ethyl dihydrothiostreptobiosaminide hydrochloride[1] yields a pentaacetate (I): m. p. 116–116.5°, $[\alpha]^{23}D$ −172° (*c*, 1, chloroform).

Anal. Calcd. for $C_{13}H_{19}O_4(C_2H_5S)(NCOCH_3)(OCOCH_3)_4$: C, 50.58; H, 6.62; N, 2.36; S, 5.39; CH_3CO (O-acetyl), 6.74 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 50.69; H, 6.43; N, 2.30; S, 5.42; CH_3CO (O-acetyl[2]), 6.41 cc.

(1) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, THIS JOURNAL, **68**, 2096 (1946).

(2) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *ibid.*, **58**, 490 (1936).

Hydrogenolysis[3] of I followed by reacetylation yields desoxydihydrostreptobiosamine pentaacetate (II); m. p. 131°, $[\alpha]^{23}D$ −87° (*c*, 1, chloroform).

Anal. Calcd. for $C_{13}H_{20}O_4(NCOCH_3)(OCOCH_3)_4$: C, 51.77; H. 6.61; N, 2.63; CH_3CO (O-acetyl), 7.49 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 51.75; H, 6.56; N, 2.73; CH_3CO (O-acetyl[2]), 7.58 cc.

A refined assay for terminal methyl groups[4] in several derivatives of streptomycin yields (moles terminal methyl per mole): methyl streptobiosaminide dimethyl acetal tetraacetate[5] 5.0; methyl dihydrostreptobiosaminide pentaacetate (III,[3,6,7] 6.0; II, 6.0; didesoxydihydrostreptobiosamine tetraacetate (IV),[3,1] 5.9. The fact that the total number of CH_3-C groups present in the first three is one greater than the known number of acetyl groups, confirms the presence of a CH_3-C group in streptomycin.[3] III is therefore thus established as a pentaacetate. IV shows a preponderance of two CH_3-C groups over those required by its known acetyl content, thus establishing the presence of an aldehyde group in the central moiety of the original streptomycin molecule (C-CHO → C-CH$(SC_2H_5)_2$ → C-CH_3), a finding confirmatory of the work of Fried and Wintersteiner[6] based upon the isolation of an amorphous bromine oxidation product of streptomycin. This aldehyde group must be the one which undergoes thioacetal formation since the mercaptolysis product (I) of dihydrostreptomycin contains only one thioethoxyl group. It must be more than one carbon atom away from the terminal methyl group originally present since otherwise no enhancement of the CH_3-C assay would result by its reduction to the hydrocarbon stage. Moreover, the reducing group liberated on hydrolysis of the streptidine portion is likewise doubtless aldehydic (cyclic hemiacetal) in nature since the two anomeric forms[3] of ethyl thiostreptobiosaminide diethyl thioacetal tetraacetate produce on hydrogenolysis good yields of the same reduction product, R—CH_2—O.

Further proof for the presence of two aldehyde groups other than that of the hexosamine portion is provided by quantitative measurements of hypoiodite oxidation.[8] Although this reagent caused some general oxidation, time curves showed definite breaks at the following consumptions expressed in atoms of oxygen: streptomycin, 1; streptomycin hydrolyzate, 2; dihydrostrepto-

(3) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, *ibid.*, **68**, 2120 (1946).

(4) R. U. Lemieux and C. B. Purves, *Can. J. Research*, in press.

(5) N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **102**, 506 (1945).

(6) J. Fried and O. Wintersteiner, Abstracts of Papers 110th Meeting, Am. Chem. Soc., Chicago, September 9–13, p. 15B (1946).

(7) Q. R. Bartz, J. Controulis, H. M. Crooks, Jr., and Mildred C. Rebstock, THIS JOURNAL, **68**, 2163 (1946).

(8) H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 131 (1942).