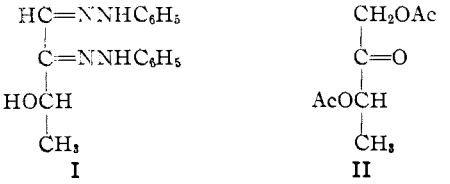
COMMUNICATIONS TO THE EDITOR

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 $C_{13}H_{25}O_7N\cdot HCl$: 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 — J. FRIED, DORIS E. WALZ
NEW BRUNSWICK, NEW JERSEY — 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).