[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

Streptomycin. VI. Some Derivatives and Reactions of Dihydrostreptobiosamine

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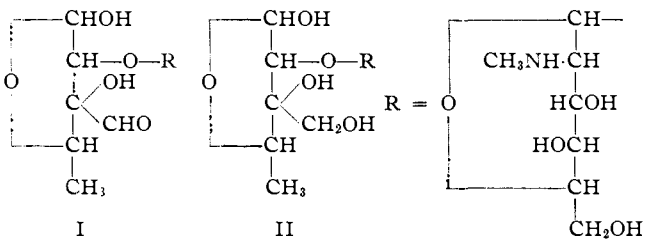
Dihydrostreptomycin[1,2,3,4] is cleaved by methanolic hydrogen chloride into streptidine dihydrochloride and methyl dihydrostreptobiosaminide hydrochloride. The latter compound is an amorphous product which yields on acetylation two crystalline pentaacetates differing by anomerism at the hemiacetalic carbon atom 1 of the streptose moiety (α-form,[2,3,4,5] β-form[5]). Another glycosidic derivative of dihydrostreptobiosamine is the amorphous ethyl thiodihydrostreptobiosaminide hydrochloride,[6] which has been characterized by the preparation of crystalline N-acetyl[6] and pentaacetyl[7] derivatives. The structure of dihydrostreptobiosamine (II) is implied in that of streptobiosamine (I) recently established by Kuehl, Flynn, Brink and Folkers.[8]



That the primary alcoholic group in II is derived from the free aldehyde group in I follows from the conversion of α-methyl streptobiosaminide dimethyl acetal hydrochloride to methyl pentaacetyldihydrostreptobiosaminide by selective hydrolysis of the dimethyl acetal group, catalytic reduction of the free aldehyde group thus liberated, and acetylation.[2,3]

In the present communication we describe a few additional derivatives of dihydrostreptobiosamine which were obtained in the course of early structure studies on this entity.

Acetylation of dihydrostreptobiosamine hydrochloride with acetic anhydride and pyridine yielded as the main product a hexaacetate m. p. 144°, $[\alpha]_D$ −108°, designated α, and smaller amounts of two other hexaacetates designated β (m. p. 107°, $[\alpha]_D$ −36.2°) and γ (m. p. 136°, $[\alpha]_D$ −175°). From the optical data it would appear that the α- and β-hexaacetates are anomers differing from each other by stereoisomerism at C_1 of the dihydrostreptose moiety, and that they correspond sterically to the α- and β-forms of methyl pentaacetyldihydrostreptobiosaminide,[5] the specific rotations of which are −117° and −34°, respectively. Since streptose has been shown to be an L-sugar,[9] this assignment of prefixes is in accord with the established conventions for designating anomers on the basis of their specific rotations.

The nature of the highly levorotatory γ-hexaacetate remains to be elucidated. There can be little doubt that it represents a definite, homogeneous entity, as the properties, especially the rotation values, of preparations from different runs showed excellent agreement, and remained unchanged on chromatographing. The analytical data for nitrogen, total and O-acetyl indicate that it is a hexaacetate derived from dihydrostreptobiosamine, but the carbon values were consistently 0.5–0.8% too high for a compound isomeric with the α- and β-hexaacetates. Furthermore, unlike the latter, it gives evidence of being unsaturated (tetranitromethane, permanganate) and decomposes with pigment formation on short heating at 100° in the dry state or in aqueous solution. This instability is not due to the presence of a free aldehyde group, since the Schiff test, as well as the more reliable spectrophotometric test with thiosemicarbazide,[10] by which the preformed aldehyde group in streptomycin and streptobiosamine can be readily demonstrated, was completely negative. The compound is being further investigated.

By hydrolysis with boiling water the α- and β-isomers of hexaacetyldihydrostreptobiosamine are both transformed, by loss of the acetyl group at carbon atom 1 of the dihydrostreptose portion, into an amorphous product which appears to be essentially an equilibrium mixture of anomeric pentacetates. Nevertheless the proportion of anomers in the crystalline products secured from these mixtures varied in dependence on the isomer serving as the starting product. The experiment starting from the α-hexaacetate yielded a pentaacetate m. p. 140–142° which, to judge from its rotation (−98°) and the fact that it could be reacetylated in good yield to the α-hexaacetate, is probably substantially pure α-pentaacetyldihydrostreptobiosamine. On the other hand, the crystalline pentaacetate fractions derived from the β-hexaacetate were anomeric mixtures having spe-

(1) R. L. Peck, C. E. Hoffhine and K. Folkers, THIS JOURNAL, **68**, 1390 (1946).

(2) Q. R. Bartz, J. Controulis, H. M. Crooks, Jr.; and M. C. Rebstock, *ibid.*, **68**, 2163 (1946).

(3) J. Fried and O. Wintersteiner, *ibid.*, **69**, 79 (1947).

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

(5) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn and K. Folkers, *ibid.*, **68**, 2557 (1946).

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

(7) R. U. Lemieux, W. J. Polglase, C. W. De Wall and M. L. Wolfrom, *ibid.*, **68**, 2747 (1946).

(8) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, *ibid.*, **68**, 2679 (1946).

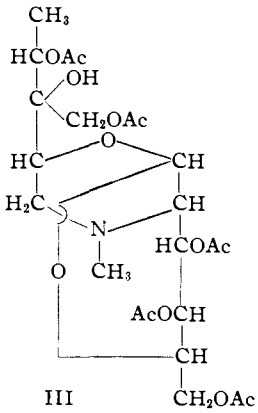
(9) J. Fried, D. E. Walz and O. Wintersteiner, *ibid.*, **68**, 2746 (1946).

(10) R. Donovick, G. Rake and J. Fried, *J. Biol. Chem.*, **164** 173 (1946).

cific rotations close to that attained at equilibrium in boiling water (about $-80°$). A product with similar properties was also obtained by hydrolytic removal, with mercuric chloride, of the ethylthio group of ethyl thiodihydrostreptobiosaminide pentaacetate.[7] The latter reaction demonstrates that the acetyl group eliminated from the anomeric hexaacetates by hydrolysis with water is indeed the one situated at carbon atom 1 of the dihydrostreptose moiety.

The ethyl thiodihydrostreptobiosaminide pentaacetate used in the above reaction was prepared by treating dihydrostreptobiosamine hydrochloride with concentrated hydrochloric acid and ethyl mercaptan and acetylating the resulting crude product, whereas Lemieux, *et al.*,[7] obtained it by acetylation of the amorphous but purified ethyl thiodihydrostreptobiosaminide hydrochloride[6] resulting from the mercaptolysis of dihydrostreptomycin with ethyl mercaptan and anhydrous hydrogen chloride. While in the latter case the formation of an ethyl thioglycoside might be expected, it is somewhat surprising that the same product was obtained from free dihydrostreptobiosamine, because aldo-pentoses and -hexoses generally react with ethyl mercaptan in the presence of aqueous hydrochloric acid to yield the diethyl thioacetals derived from their *aldehydo* forms. Evidently the furanose ring in the streptose moiety is more stable under these conditions than is the case in other furanoses, for instance in 1,2-isopropylidene-5-desoxy-L-arabinose,[11] which readily forms the diethyl thioacetal.

While pentaacetyldihydrostreptobiosamine in aqueous solution fails to take up hydrogen in the presence of platinum oxide, dihydrostreptobiosamine hydrochloride is reduced under these conditions with the consumption of one mole of hydrogen. Acetylation of the reduction product afforded a crystalline compound m. p. 154–155° which, however, was not the expected heptaacetyltetrahydrostreptobiosamine, but a base hydrochloride of the composition $C_{23}H_{20}O_8N(CH_3CO)_5 \cdot HCl \cdot C_2H_5OH$. The mole of ethanol, which cannot be removed by drying at 80°, is derived from the solvent used in the crystallization and is probably not an integral part of the molecule. Otherwise the change produced by the reduction corresponds to the addition of 2 hydrogen atoms and the loss of one molecule of water. Accordingly the term *tetrahydroanhydrostreptobiosamine* is proposed for the constituting free base. The unacetylated product, in contradistinction to dihydrostreptobiosamine, does not reduce Fehling solution. This fact, together with the retention of basic properties and the lack of an N-acetyl group after acetylation, suggests that the hydrogenation effects a reductive condensation of the aldehyde group of the dihydrostreptose moiety with the methylimino group, so that pentaacetyltetrahydroanhydrostreptobiosamine would be represented by the morpholine structure III.



III

Potentiometric titration of the pentaacetyl derivative revealed that the acid-binding group has a much lower basicity (pKa' 4.6) than would be expected from the tertiary amino group in III. However, the influence of environmental factors, that is of steric hindrance or of inductive effects exerted by the neighboring acetoxy groups, cannot be predicted, so that this fact *per se* does not necessarily argue against the above structure. More surprising was the observation that drastic acid hydrolysis, under conditions which are known to cleave the glycosidic linkage between the two moieties of dihydrostreptobiosamine, failed to liberate the reducing group of the N-methyl-L-glucosamine portion. That no extensive hydrolysis of any kind had occurred was evidenced by the recovery of some of the pentaacetyl derivative on acetylation of the acid-treated product. It would then have to be assumed that the acetalic linkages incorporated in the morpholine and pyranose rings of III are unusually resistant to acid. However, this is not without precedent in anhydro structures of this type. A similar stability toward acid hydrolysis is exhibited by difructopyranose anhydride[12] and difructofuranose anhydride[13] in which the reducing groups of the two fructose residues are parts of a dioxane ring as well as of the pyranose or furanose rings.

The hydrochloride, m. p. 155°, can be obtained in good yield from streptobiosamine hydrochloride directly in the same manner as from dihydrostreptobiosamine. The requisite two moles of hydrogen are consumed without a discernible break in the hydrogenation curve after the absorption of the first mole. This result tends to confirm the supposition that the aldehydic group of the dihydrostreptose moiety is involved in the addition of the second mole, because when this group is blocked by glycoside formation, as in methyl streptobiosaminide or streptomycin, the reduction stops at the stage of the dihydro derivatives. A

(11) P. A. Levene and J. Compton, *J. Biol. Chem.*, **116**, 189 (1936).

(12) H. H. Schlubach and C. Behre, *Ann.*, **508**, 16 (1934).

(13) W. N. Haworth and H. R. L. Streight, *Helv. Chim. Acta*, **15**, 693 (1932).

further requirement for the occurrence of the second reduction step is the presence of the imino hydrogen atom, as is evident from the unreactivity of pentaacetyldihydrostreptobiosamine, in which the nitrogen atom instead of the aldehydic group is blocked.

In experiments designed to convert α-methyl pentaacetyldihydrostreptobiosaminide to α-hexacetyldihydrostreptobiosamine by the acetolysis procedure of Hann and Hudson,[14] the methyl glycoside was treated with a mixture of acetic anhydride and acetic acid containing catalytic amounts of sulfuric acid. The ensuing reaction was accompanied by a fall of the levorotation to low levels. The mixture was worked up in the usual manner (bicarbonate–chloroform). The crystalline material extractable with chloroform consisted for the most part of N-methyl-L-glucosamine α-pentaacetate. From the amount of material recovered in this fraction it appeared that the disaccharide had been nearly quantitatively cleaved by acetolysis into the component sugars. Since the dihydrostreptose portion apparently had remained in the aqueous phase, the procedure of working up the reaction mixture was modified in various ways so as to render feasible the isolation of this moiety. These attempts were unsuccessful, largely on account of the difficulty encountered in separating the water-soluble products derived from the starting product from the sulfoacetic acid, SO_3HCH_2COOH, which is formed almost quantitatively from the sulfuric acid present in the acetolysis mixture by reaction with acetic anhydride,[15] and which inconveniently forms a water-soluble barium salt. When acid conditions prevailed during the decomposition of the acetic anhydride in the acetolysis mixture, the yield of chloroform-extractable material was generally much lower. Reacetylation of the water-soluble products in two such experiments yielded additional chloroform-soluble material from which, by chromatographing, the hitherto undescribed N-methyl-L-glucosamine β-pentaacetate was isolated. It appears that the acids present in the aqueous phase effected the hydrolytic removal of at least the 1-acetyl group in this moiety as well as the subsequent inversion to the β-form.

It should be mentioned that the β-pentaacetate is also formed, besides the preponderant α-isomer,[16] in the acetylation of the crude N-methyl-L-glucosamine resulting from the reduction of synthetic N-methyl-L-glucosaminic acid lactone.[16,16a]

Finally we describe in the experimental part a crystalline, high-melting product of unknown structure which was obtained in early attempts to prepare an acetone derivative of methyl dihydrostreptobiosaminide. The method used was that of Dangschat and Fischer[17] by which these authors succeeded in preparing monoisopropylidene-meso-inositol. The oily product obtained by this procedure from methyl dihydrostreptobiosaminide hydrochloride was acetylated and chromatographed. The isolated crystalline substance (m. p. 289° dec.) lacks the methoxyl group of the starting product, but is undoubtedly a derivative of dihydrostreptobiosamine and not a fragment. The analytical data are not quite conclusive, but fit best for a dimer $C_{42}H_{64}O_{21}N_2$ containing two isopropylidene groups and five acetyl groups, one of which is bound to nitrogen.

Experimental

Acetylation of Dihydrostreptobiosamine Hydrochloride.—The disaccharide was prepared by hydrolysis of dihydrostreptomycin trihydrochloride with 1 *N* sulfuric acid at 45° as previously described,[3] except that in later runs the hydrolysis period was shortened to fifteen hours.

Dihydrostreptobiosamine hydrochloride (4 g.) was shaken with acetic anhydride (8 cc.) and pyridine (2 cc.) until it was dissolved (three hours) and the solution was then kept at 45° overnight. The solvents were removed *in vacuo*, and the residue was extracted three times with benzene. The benzene solution was concentrated to about 20 cc. and passed through a column of aluminum oxide 1 × 20 cm. The column was washed with benzene and then with benzene–ether 7:3. On trituration of the residues with ether crystallization occurred in the later benzene and most of the benzene–ether eluates. The crystalline material was purified by treating it in acetone solution with norit, and then by recrystallization from acetone–ether or ethyl acetate–hexane. The **α-hexaacetate** thus obtained forms rosets of prisms m. p. 143–144°,[18] $[\alpha]^{23}D$ −108° (*c*, 1.0 in chloroform). Yield was 0.8–1.0 g.

Anal. Calcd. for $C_{13}H_{19}O_9N(CH_3CO)_6$: C, 50.76; H, 6.30; N, 2.37; $6CH_3CO$, 43.7; O-Acetyl, 36.4. Found: C, 50.80; H, 6.58; N, 2.44; CH_3CO, 42.30; O-Acetyl, 36.0.

The β- and γ-hexaacetates were obtained only after milder conditions for the acetylation had been adopted. Though a part of the acetylated material then crystallized directly, these crystals proved to be a mixture from which the preponderant α-isomer could not be obtained in pure form by fractional crystallization. Chromatographing can therefore not be dispensed with for securing the pure isomers.

To dihydrostreptobiosamine hydrochloride (4.8 g.) pyridine (38 cc.) and acetic anhydride (19 cc.) were added. The mixture was placed into the refrigerator and shaken occasionally. After about one hour all the material except a small amount of a dark brown sediment was dissolved. After forty-six hours of standing at 4° the solvents were removed *in vacuo*. The residue was taken up in chloroform, and the filtered solution after chilling was washed successively with ice-cold water, dilute hydrochloric acid, sodium bicarbonate solution and water. The residue obtained on evaporation of the dried chloroform solution (7.4 g.) was dissolved in ethyl acetate (3.5 cc.). The crystalline product formed on addition of ether (50 cc.) and seeding with the α-hexaacetate was collected and washed with ether (2.17 g., m. p. 135–140°). After several recrystallizations from ethyl acetate–ether the product, to judge from appearance and physical properties (m. p. 140–143°, $[\alpha]^{23}D$ −97.5°), was still inhomogeneous.

The mother liquor material from the original crystals

(14) R. M. Hann and C. S. Hudson, THIS JOURNAL, **56**, 2465 (1934).

(15) Franchimont, *Compt. rend.*, **22**, 1054 (1881); *Rec. trav. chim.*, **7**, 25 (1888); T. F. Murray and W. O. Kenyon, THIS JOURNAL, **62**, 1230 (1940).

(16) F. A. Kuehl, Jr., K. H. Flynn, F. W. Holly, R. Mozingo and K. Folkers, *ibid.*, **68**, 536 (1946).

(16a) Addendum to proof (November 1, 1947).—The preparation of the β-pentaacetate by this procedure has meanwhile been reported by Wolfrom and Thompson, *ibid.*, **69**, 1817 (1947).

(17) G. Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **30**, 116 (1942).

(18) All melting points reported are corrected (capillary).

was dissolved in benzene and adsorbed on a column (25 × 200 mm.) of alumina. The column was washed successively with benzene (1.5 liters), benzene–ether 4:1 (1.5 liters), benzene–ether 1:1 and ether (1.2 liters), and the effluent collected in 150-cc. portions. All the residues inclusive of the first benzene–ether 1:1 eluate crystallized on trituration with dry ether. The first three benzene eluates yielded only a small amount of streptidine octaacetate. The following benzene fractions and the first two benzene–ether 4:1 fractions contained the **γ-hexaacetate.** The crystalline material in these fractions was freed from oil by washing with ether, combined and recrystallized from acetone–ether (344 mg., m. p. 126–132°), from which the crude compound forms large, block-shaped crystals selectively adsorbing a brown pigment present. The pigment was removed by treatment with norit in ethyl acetate solution. After repeated recrystallization from ethyl acetate–ether the product melted at 135–136°, $[\alpha]^{23}D$ −175° (*c* 1.05 in chloroform). In contradistinction to the α- and β-hexaacetates, the substance is fairly readily soluble in cold water. It gives a pale yellow color with tetranitromethane in chloroform solution. It reduces aqueous permanganate on short standing, and Fehling solution on warming. The analytical sample was dried *in vacuo* at 56°, since at 100° some decomposition took place.

Anal. Calcd. for $C_{13}H_{19}O_9N(CH_3CO)_6$: C, 50.76; H, 6.31; N, 2.37; $6CH_3CO$, 43.7; O-acetyl, 36.4. Found: C, 51.25; H, 6.56; N, 2.70; CH_3CO, 43.6; O-Acetyl, 35.9.

Two other, separately prepared specimens ($[\alpha]^{24}D$ −175 and −174°, respectively) gave the following figures: (1) C, 51.57; H, 6.45; (2) C, 51.58; H, 6.62; N, 2.84; CH_3CO, 43.2; O-Acetyl, 35.7.

The third benzene–ether 4:1 eluate (680 mg.) after crystallization from acetone–ether consisted mainly of small needles interspersed with some larger crystals of the α-hexaacetate. The latter were removed mechanically, and the remainder (210 mg., m. p. 100–104°) was recrystallized several times from acetone–ether. The **β-hexaacetate** thus obtained melted at 105–107°, $[\alpha]^{23}D$ −36.2° (*c*, 1.10 in chloroform). It reacted readily with hot Fehling reagent.

Anal. Calcd. for $C_{13}H_{19}O_9N(CH_3CO)_6$: C, 50.76; H, 6.31; N, 2.37; $6CH_3CO$, 43.7; O-Acetyl, 36.4. Found C, 50.72; H, 6.42; N, 2.34; CH_3CO, 43.3; O-Acetyl, 36.5.

The remainder of the benzene–ether 4:1 eluates and the crystalline benzene–ether 1:1 eluates were combined and recrystallized from ethyl acetate–ether (378 mg., m. p. 139–141.5°). After two more recrystallizations the melting point remained constant at 142–143° and was not depressed by admixture of the α-hexaacetate, $[\alpha]^{23}D$ −107° (*c*, 1.07 in chloroform).

α-Pentaacetyldihydrostreptobiosamine.—Samples of α-hexaacetyldihydrostreptobiosamine (about 100 mg.) were dissolved in boiling water to give 1% solutions. The solutions were refluxed for varying intervals of time, cooled, freeze-dried and analyzed for total acetyl. The values found were 40.9, 39.9 and 39.4 after 30, 70 and 120 minutes, respectively. $C_{13}H_{20}O_9N(CH_3CO)_5$ requires 39.2%.

In a preparative experiment a solution of 409 mg. of the α-hexaacetate in 10 cc. of water was refluxed for two hours and then lyophilized. The white amorphous residue weighed 376 mg. and had $[\alpha]^{24}D$ −84° in water. A portion of this material was reacetylated, but the resulting crystalline product was a mixture from which no pure α-hexaacetate could be obtained. The remainder of the pentaacetate mixture (310 mg.) was dissolved in ethyl acetate (2 cc.). On addition of dry ether (4 cc.) and scratching a crystalline product slowly deposited (199 mg., m. p. 137–139.5°), which after several recrystallizations from the same solvents melted at 140–142°. The melting point of a mixture with the starting material was strongly depressed (125–137°), $[\alpha]^{24}D$ −98° (*c*, 0.90 in chloroform).

Anal. Calcd. for $C_{13}H_{20}O_9N(CH_3CO)_5$: C, 50.27; H, 6.42; N, 2.55; $5CH_3CO$, 39.2; O-Acetyl, 31.3. Found: C, 49.93; H, 6.37; N, 2.62; CH_3CO, 39.2; O-Acetyl, 31.8.

Reacetylation of 33 mg. of the compound with acetic anhydride pyridine yielded 25 mg. of crystals which after one recrystallization melted at 143–144° and did not depress the melting point of the α-hexaacetate, $[\alpha]^{23}D$ −109° (*c*, 0.87 in chloroform). Additional amounts of the latter isomer (18 mg., m. p. 141–143°, $[\alpha]D$ −107°) could be obtained by reacetylation of the mother liquor material (50 mg.) from the crude crystalline pentaacetate.

When the α-pentaacetate is dissolved in water, the equilibrium value of rotation is established almost immediately. The observed values for $[\alpha]^{26}D$ in water of a preparation showing an initial value of −94° (in chloroform) were: five minutes, −84.7°; one hour, −84.1° (constant for twenty-four hours). The addition of a catalytic amount of ammonia at this point caused a slow rise to −94° in the following twenty-four hours. In dry chloroform free of ethanol and hydrochloric acid $[\alpha]D$ remained at the initial level for three hours, and then slowly fell to −82° within twenty-four hours.

Hydrolysis of β-Hexaacetyldihydrostreptobiosamine with Water.—The β-hexaacetate (69 mg.) was hydrolyzed with boiling water (4 cc.) for two hours. The lyophilized material ($[\alpha]^{24}D$ −79°, in chloroform) on treatment with ethyl acetate–ether yielded large rosets of prisms (15 mg., m. p. 136–138.5°) and, on concentration of the mother liquor, microscopic needles (22 mg., m. p. 135–137°). The melting point of neither fraction was materially improved by two recrystallizations. The specific rotations $[\alpha]^{23}D$ in chloroform were −83 and −76°, respectively. The O-acetyl determination showed the presence in both fractions of four such groups, as required for pentaacetyldihydrostreptobiosamine (calcd. 31.3. Found: 31.8, 31.2).

Ethyl Thiopentaacetyldihydrostreptobiosaminide from Dihydrostreptobiosamine Hydrochloride.—A solution of dihydrostreptobiosamine hydrochloride (1.42 g.) in ice-cold concentrated hydrochloric acid (1.5 cc.) was shaken with ethyl mercaptan (1.5 cc.) in an ice-bath for one hour, and then at room temperature for two-and-a-half hours. The solvents were removed *in vacuo*, and the oily red residue was acetylated with pyridine (10 cc.) and acetic anhydride (5 cc.) at 5° overnight. The mixture was worked up, and the acetylated material (1.9 g.) chromatographed, as described above for the acetylation products from dihydrostreptobiosamine. (Direct crystallization yields a product which is difficult to purify.) The combined crystalline fractions yielded practically pure material on one recrystallization from ethyl acetate–ether (950 mg., m. p. 114–116°). The analytical sample melted at 115–116°, $[\alpha]D$ −165° (*c*, 0.87 in chloroform).

Anal. Calcd. for $C_{15}H_{24}O_8NS(CH_3CO)_5$: N, 2.36; S, 5.40; O-Acetyl, 29.0. Found: N, 2.44; S, 5.40; O-Acetyl, 30.7.

Conversion of Ethyl Thiodihydrostreptobiosamine Pentaacetate to Pentaacetyldihydrostreptobiosamine.—To a solution of ethyl thiodihydrostreptobiosamine pentaacetate (95 mg.) in 50% acetone (2 cc.) cadmium carbonate (205 mg.) and a solution of mercuric chloride (44 mg.) in a few drops of acetone were added. The mixture was boiled under reflux with stirring for forty minutes and then cooled and filtered. The filtrate was freed from acetone *in vacuo* and extracted with chloroform. The residue from the dried chloroform solution (89 mg.) could be crystallized only with difficulty. The crystals eventually obtained from ethyl acetate ether (23 mg., m. p. 133–136°) after two recrystallizations melted at 141–143° and did not depress the melting point of α-pentaacetyldihydrostreptobiosamine (m. p. 140–142°), but showed a considerably lower rotation value ($[\alpha]^{24}D$ −83°, *c*, 0.65 in chloroform); O-Acetyl, calcd.: 31.3. Found: 31.7.

In an identical experiment in which 425 mg. of the starting product was used, the total chloroform-soluble material (394 mg.) was chromatographed from benzene

solution on alumina. Benzene–ether 3:1 eluted some unchanged starting material. The following fractions, eluted with benzene–ether 1:1, yielded 165 mg. of partly crystalline material which after purification melted at 135–137° and showed $[\alpha]^{25}D$ −79° in chloroform. The melting point was not depressed by admixture of similar material obtained by water hydrolysis of β-hexaacetyldihydrostreptobiosamine. The chromatographic method used does not appear to be suitable for the separation of the anomeric pentaacetates. Moreover, in this as in other similar chromatograms carried out on pentaacetate mixtures recoveries were poor, as a considerable portion of the material resisted elution even by solvents such as acetone and methanol.

Pentaacetyltetrahydroanhydrostreptobiosamine Hydrochloride.—Dihydrostreptobiosamine hydrochloride (2.0 g.) was dissolved in water (40 cc.) and hydrogenated in the presence of platinum oxide (150 mg.) for eighteen hours. The hydrogen uptake was 1.03 moles per mole of substance. The filtered solution yielded on lyophilizing a white amorphous product which did not reduce hot Fehling solution. A portion (750 mg.) was acetylated with acetic anhydride–pyridine (1:1, 20 cc.) for twenty-four hours at room temperature. The solvents were removed *in vacuo* and the chloroform solution of the residue washed with dilute acid, bicarbonate solution and water. Since previous experience had shown that the resulting free base could not be satisfactorily crystallized, the hydrochloride was prepared in the following way: The acetylated product (1.0 g.) was dissolved in absolute ethanol (3 cc.) and a solution of ethanolic hydrogen chloride was added to acidic reaction (congo). A crystalline product deposited which was collected after several hours of standing at 4° (450 mg.). Recrystallization from absolute ethanol afforded stout prisms which melted with decomposition at 154–155° after sintering at 130°; $[\alpha]^{25}D$ +6.1° (*c*, 1.48 in water).

Anal. Calcd. for $C_{13}H_{20}O_8N(CH_3CO)_5 \cdot HCl \cdot C_2H_5OH$: C, 48.74; H, 6.81; N, 2.27; $5CH_3CO$, 34.9; Cl, 5.76; OC_2H_5, 7.31. Found: C, 48.71; H, 6.77; N, 2.19; CH_3CO, 35.3; Cl, 5.66; OC_2H_5, 7.92; O-Acetyl determination according to Kunz and Hudson, 58.7 mg. consumed 5.63 cc. of 0.1 *N* sodium hydroxide; calcd. for 5 acetyl groups and 1 HCl, 5.71 cc.

The titration curve showed a single span with a midpoint at *p*H 4.6.

A product of identical properties was obtained in about the same yield when the above procedure was applied to streptobiosamine hydrochloride (2.0 g. in 40 cc. of water, 125 mg. platinum oxide, 1.84 molar equivalents of hydrogen consumed in fifteen hours).

A solution of the unacetylated reduction product from dihydrostreptobiosamine hydrochloride (1.0 g.) in 5.5 *N* hydrochloric acid (10 cc.) was boiled under reflux for sixteen hours. The dark solution was decolorized with norit and after filtering evaporated to dryness *in vacuo*. The residue, which showed no reducing power toward Fehling solution or Tollens reagent, on reacetylation and treatment with ethanolic hydrogen chloride yielded the crystalline pentaacetyl base hydrochloride described above (280 mg.).

Acetolysis of α-Methyl Pentaacetyldihydrostreptobiosaminide.—The starting material (213 mg.) was dissolved in 21 cc. of a mixture consisting of 70 cc. of acetic anhydride, 30 cc. of acetic acid and 1.0 cc. of concentrated sulfuric acid. $[\alpha]^{21}D$ remained constant at the starting level (−100°) for forty-five minutes, whereupon it began to fall fairly rapidly (−65° at five hours). After twenty-two hours the value was −15.8° and decreased only very slightly within the following three hours. The solution was poured onto crushed ice, and sodium bicarbonate was added until the reaction was alkaline. Extraction with chloroform yielded substantially pure N-methyl-L-glucosamine α-pentaacetate (148 mg.) which after recrystallization from acetone–ether melted at 155–157° and gave no melting point depression with a reference sample, $[\alpha]^{25}D$ −100° (*c*, 0.80 in chloroform).

Anal. Calcd. for $C_{17}H_{25}O_{10}N$: N, 3.47; Found: N, 3.32.

In a similar experiment starting with 607 mg. the acetolysis mixture (60 cc.) was poured with stirring into 500 cc. of ice-water containing barium hydroxide equivalent to the sulfuric acid originally present. After decomposition of the acetic anhydride by continued stirring at ice temperature the mixture was extracted with chloroform. The dried and washed chloroform extract in this case yielded only 198 mg. of grossly impure N-methyl-L-glucosamine-α-pentaacetate. The residue obtained from the aqueous phase (1.87 g.), after removal of the barium sulfate (1.1 g.) consisted largely of the barium salt of sulfoacetic acid. It could be in part removed by digesting the residue with ice-cold water, in which it is sparingly soluble (a sample recrystallized from hot water was analyzed. Calcd. for $C_2H_2O_5SBa \cdot H_2O$: C, 8.18; H, 1.36; Ba, 45.8. Found: C, 8.40; H, 1.57; Ba, 46.3). The remainder of the water-soluble fraction, after some further manipulation which need not be described, was quantitatively freed from the barium still present, and the resulting strongly acidic product was acetylated in the usual manner. The chloroform-soluble material thus obtained (174 mg.) on chromatographing on alumina yielded in the first benzene eluates crude **N-methyl-L-glucosamine-β-pentaacetate** (100 mg.) which after repeated recrystallization from ethyl acetate–ether melted at 151–152°, $[\alpha]^{23}D$ −14.6° (*c*, 0.41 in chloroform).

Anal. Calcd. for $C_{17}H_{25}O_{10}N$: C, 50.62; H, 6.25; N, 3.47; $5CH_3CO$, 53.3. Found: C, 50.66; H, 6.25; N, 3.56; CH_3CO, 53.1.

The compound did not depress the melting point of a synthetic specimen which melted at 152–153° and had $[\alpha]^{23}D$ −11.6° (*c*, 0.71 in chloroform).[19] Another experiment, in which the acetic anhydride in the reaction mixture was converted to methyl acetate by reaction with methanol prior to the distribution between chloroform and aqueous barium hydroxide, yielded an even smaller proportion of chloroform-soluble material, presumably because the sugar components had been partly deacetylated by transesterification to methanol. Again the only product which could be isolated from the water-soluble fraction after reacetylation was N-methyl-L-glucosamine-β-pentaacetate.

Product from Reaction of Methyl Dihydrostreptobiosaminide Hydrochloride with Acetone–Acetic Acid–Zinc Chloride.—Methyl dihydrostreptobiosaminide hydrochloride (1.0 g.) was dissolved in a solution of zinc chloride (20 g.) in acetone (160 cc.) and acetic acid (20 cc.) which had been freed from precipitated zinc oxide by centrifuging. The solution was refluxed for five hours. After standing overnight dry pyridine (75 cc.) was added to the dark brown mixture. The resulting zinc chloride–pyridine complex was filtered off and the filtrate was concentrated *in vacuo* until all of the acetone was removed. The material was acetylated with acetic anhydride and pyridine (2:1) and worked up in the usual manner. The residue obtained by evaporation of the chloroform phase was chromatographed in benzene solution on a column of alumina. Washing with benzene was continued till the filtrate was nearly free of solids. On subsequent washing with benzene–ether a product was eluted which crystallized on rubbing with hexane. It was recrystallized three times from benzene–hexane (yield *ca.* 100 mg.). The substance melted at 289° after progressive darkening from 274° on, $[\alpha]^{25}D$ −191° (*c*, 1.5 in benzene). It is readily soluble in benzene and chloroform and moderately soluble in ether and ethanol. Its insolubility in dilute hydrochloric acid and the fact that it remained unchanged on treatment with ethanolic hydrogen chloride argue against the presence of a basic group.

Anal. Found: C, 54.16, 54.81; H, 6.92, 7.00; N, 2.83, 2.87; CH_3CO, 22.2, 23.2; O-Acetyl, 16.6, 17.7; isopropylidene, 10.78; OCH_3, 0; mol. wt. (Rast), 918.

(19) The synthetic β pentaacetate was prepared by Dr. A. E. O. Menzel by fractional crystallization of the crude product resulting from the reduction and subsequent acetylation of N-methyl-L-glucosaminic acid lactone.

The duplicate figures represent analyses of two independently prepared specimens.

The isopropylidene determination was carried out by the method of Kuhn and Roth.[20]

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Summary

The preparation and properties of three hexaacetates, and of a pentaacetate of dihydrostreptobiosamine are described.

Catalytic reduction of the hydrochlorides of either dihydrostreptobiosamine or streptobiosamine yields an anhydro base, tetrahydroanhydrostreptobiosamine hydrochloride, which on acetylation forms a crystalline O-pentaacetyl base hydrochloride. A structural formula is suggested for this compound.

N-Methyl-L-glucosamine-β-pentaacetate, isolated in the course of acetolysis studies on methyl pentaacetyldihydrostreptobiosaminide, and a dimeric product of unknown structure obtained by acetonation of methyl dihydrostreptobiosaminide and subsequent acetylation, are described.

(20) R. Kuhn and H. Roth, *Ber.*, **65**, 1285 (1932).

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Steric Inhibition of Resonance. The Reactivities of Some Halogenated Primary and Tertiary Aromatic Amines

BY REUBEN B. SANDIN AND JACK R. L. WILLIAMS[1]

During the past decade many important contributions have been made to the topic of steric inhibition of resonance and reaction rates.[2] Derivatives of aromatic tertiary amines have been examined extensively from this point of view. A careful study of hydrogen–deuterium exchange in aromatic amines has been carried out by Brown, Widiger and Letang.[3] Westheimer and Metcalf[4] have made an equally careful study of the rates of saponification of the esters of aminobenzoic acids. The authors of the present paper have examined in a qualitative way the behavior of *p*-iodoaniline, *p*-iododimethylaniline, 2,4,6-triiodoaniline and 2,4,6-triiododimethylaniline toward several electrophilic reagents (reactions with acid stannous chloride, bromine, and nitrous acid). The assumption was made that only in the case of 2,4,6-triiododimethylaniline should "damping" of resonance be marked and therefore should lead to a lowered reactivity of the halogen atoms, in the above reactions.

All the compounds needed for the investigation were known with the exception of 2,4,6-triiododimethylaniline. This substance was readily made from 2,4,6-triiodoaniline by the methylation procedure of Clarke, Gillespie and Weisshaus.[5] This process has also been used successfully by Emerson and co-workers.[6]

(1) Present address: Noyes Chemical Laboratory, University of Illinois, Urbana, Illinois.

(2) See the excellent discussions on this subject by Wheland in Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 272–279; and by Bartlett in Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, pp. 211–213; see also Birtles and Hampson, *J. Chem. Soc.*, 10 (1937); Ingham and Hampson, *ibid.*, 981 (1939).

(3) Brown, Widiger and Letang, THIS JOURNAL, **61**, 2597 (1939); see also Brown, Kharasch and Sprowls, *J. Org. Chem.*, **4**, 442 (1939).

(4) Westheimer and Metcalf, THIS JOURNAL, **63**, 1339 (1941).

(5) Clarke, Gillespie and Weisshaus, *ibid.*, **55**, 457 (1933).

(6) (a) Emerson, Neumann and Moundres, *ibid.*, **63**, 972 (1941); (b) Emerson, *ibid.*, **63**, 2023 (1941).

Experimental

Materials.—2,4,6-Triiodoaniline,[7] m. p. 185°, *p*-iodoaniline,[8] m. p. 62–63°, and *p*-iododimethylaniline,[9] m. p. 79–80°, were prepared without difficulty and in good yield.

2,4,6-Triiododimethylaniline.—Ten grams of 2,4,6-triiodoaniline, 300 cc. of 90% formic acid and 25 cc. of formalin was boiled under reflux for two hours. After the addition of 3–5 cc. of concentrated hydrochloric acid and removal of most of the formic acid under reduced pressure, the residue was made alkaline and distilled with steam. The methylated product passed over very slowly and was collected as a heavy oil. On long standing it solidified. It was found that much time could be saved by not doing a steam distillation. Instead, the reaction mixture after refluxing was diluted with 3 liters of water. It was then seeded with some of the solid compound and after standing for twelve hours, the material was collected. The yield of crude substance, m. p. 60–68°, was 9.2 g. After crystallization from acetic acid a 54% yield (6 g.) of pure compound was obtained as pale yellow needles, m. p. 69–70°.

Anal. Calcd. for $C_8H_8NI_3$: I, 76.32. Found: I, 76.14, 76.23.

Reaction with Hydrochloric Acid and Stannous Chloride.—The method employed was similar to the one used by Nicolet[10] and co-workers for the determination of so-called "positive" halogen. It was essential that the acid concentrations should be the same for all runs, since it is known that the rate of halogen removal is directly proportional to the acid concentration but is independent of the stannous chloride concentration.

In this work 0.001 mole of compound was dissolved in a boiling mixture of 50 cc. of glacial acetic acid and 10 cc. of concentrated hydrochloric acid. To this was added 0.025 mole of stannous chloride and the solution was refluxed for one hour. On boiling, some hydrogen chloride was lost. However, since all experiments were carried on as nearly as possible under the same conditions, no attempt was made to avoid this loss.

After refluxing, the reaction mixture was made alkaline, extracted with carbon tetrachloride and steam distilled.

(7) Jackson and Whitmore, *ibid.*, **37**, 1522 (1915).

(8) Brewster, "Organic Syntheses," Coll. Vol. II, 1943, p. 347.

(9) Reade and Sim, *J. Chem. Soc.*, 157 (1924).

(10) (a) Nicolet, THIS JOURNAL, **43**, 2081 (1921); (b) Nicolet and Sampey, *ibid.*, **49**, 1706 (1927); (c) Nicolet and Ray, *ibid.*, **49**, 1801 (1927); (d) Nicolet and Sandin, *ibid.*, **49**, 1806 (1927); (e) Nicolet, *ibid.*, **49**, 1810 (1927).