cooled, as flocs of fine needles, in 70% yield. It was recrystallized from 100 parts of boiling water, from which it separated as fine silky needles which melted at 214–215° and rotated +80.3° in pyridine (*c*, 0.84). It is nearly insoluble in alcohol, chloroform, acetone, ether and cold water and moderately soluble in pyridine and hot water. It resembles D-*arabo*-hexose phenylosotriazole in ease of crystallization and low solubility in water, but the two substances can be distinguished readily by their melting points and rotations and of course their analyses are different.

Anal. Calcd. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80. Found: C, 53.02; H, 5.83.

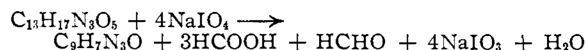
D-*gala*-**Heptose Phenylosotriazole Pentaacetate.**—The pentaacetate of D-*gala*-heptose phenylosotriazole was obtained in quantitative yield by warming 1 part of the phenylosotriazole with 0.25 parts of fused sodium acetate and 8 parts of acetic anhydride on the steam-bath for two hours and pouring the reaction mixture upon crushed ice. It crystallized from its solution in 5 parts of warm methyl alcohol as clusters of needles which melted at 134–135° and rotated +53.1° in chloroform solution (*c*, 0.92). It is soluble in warm methyl and ethyl alcohols, chloroform, acetone and ether and nearly insoluble in water and petroleum ether.

Anal. Calcd. for $C_{23}H_{27}N_3O_{10}$: C, 54.65; H, 5.38; CH_3CO, 42.6. Found: C, 54.78; H, 5.39; CH_3CO, 42.8.

D-*gala*-**Heptose Phenylosotriazole Pentabenzoate.**—The benzoylation of D-*gala*-heptose phenylosotriazole in pyridine solution with benzoyl chloride produced the pentabenzoate in quantitative yield. It was purified by crystallization from 20 parts of absolute alcohol and formed elongated prisms which melted at 134–135° and rotated +28.9° in chloroform solution (*c*, 0.81). It is soluble in ether, acetone, chloroform and pyridine and sparingly soluble in cold alcohol, water and hexane.

Anal. Calcd. for $C_{48}H_{37}N_3O_{10}$: C, 70.66; H, 4.57; C_6H_5CO, 64.4. Found: C, 70.85; H, 4.80; C_6H_5CO, 64.3.

Sodium Metaperiodate Oxidation of the Heptose Phenylosotriazoles.—The phenylosotriazoles from sedoheptulose, D-mannoheptulose, D-gluco-D-*gulo*-heptose and D-gala-L-*gluco*-heptose were oxidized by suspending a 0.4000 g. sample of each in 15 ml. of water and adding 4.5 molecular equivalents of 0.534 *M* sodium metaperiodate solution; the mixtures were shaken for three hours at 20°, cooled to 5° for two hours and the crystalline 2-phenyl-4-formyl-2,1,3-triazole recovered by filtration and washed with ice water; the yields in all cases were 92–96% of material melting at 68–69° and showing no depression of this value when admixed with an authentic sample of 2-phenyl-4-formyl-2,1,3-triazole. The aqueous solution and washings from the oxidation mixtures were diluted to 50 ml. and analyzed for formic acid, formaldehyde and consumed periodate; the results showed that the reaction for each heptose phenylosotriazole had followed the equation



$$C_{13}H_{17}N_3O_5 + 4NaIO_4 \longrightarrow C_9H_7N_3O + 3HCOOH + HCHO + 4NaIO_3 + H_2O$$

Accordingly, the structures of the four new phenylosotriazoles are those shown earlier in this article.

Summary

The D-*altro*-, D-*manno*-, D-*gluco*- and D-*gala*-heptose phenylosotriazoles and several of their acetyl and benzoyl derivatives are described. Their structures have been established through periodate oxidations.

BETHESDA, MARYLAND RECEIVED DECEMBER 4, 1946

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Degradative Studies on Streptomycin. I.[1]

BY I. R. HOOPER,[2] L. H. KLEMM,[2] W. J. POLGLASE[2] AND M. L. WOLFROM

Folkers and co-workers[3] have advanced the formula $(C_{21}H_{37-39}O_{12}N_7 \cdot 3HCl)_2 \cdot CaCl_2$ for the crystalline calcium chloride compound of streptomycin trihydrochloride. Later work[4] on mercaptolysis products favored the possibility with the higher hydrogen content. Our analytical data on purified samples of the calcium chloride compound of streptomycin trihydrochloride and on didesoxydihydrostreptobiosamine tetraäcetate likewise substantiate the formula $C_{21}H_{39}O_{12}N_7$ for streptomycin. In addition we have established analytically the presence of one methyl group linked to carbon which must necessarily be a part of the portion $C_6H_8O_5<$ remaining after subtraction of the streptidine[5,6,7] and N-methyl-L-glucosamine[8] entities.

Hydrogenation of the calcium chloride compound of streptomycin trihydrochloride with Raney nickel catalyst at 150°, followed by methanolysis and acetylation yielded crystalline methyl dihydrostreptobiosaminide pentaacetate (m. p. 194–195° (cor.), $[\alpha]^{28}D$ − 123° in chloroform). Since methanolysis and acetylation of streptomycin[5] is known to yield a tetraacetate containing three methoxyl groups, it is evident that in the above compound a carbonyl group of streptomycin has been reduced to an alcohol group and this carbonyl group is the one that forms a dimethyl

(1) A preliminary notice of the work herein reported appeared in THIS JOURNAL, **68**, 2120 (1946).

(2) Bristol Laboratories Research Associate and Research Fellow (W. J. P.) of The Ohio State University Research Foundation (Project 224).

(3) R. L. Peck, N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, A. Walti and K. Folkers, THIS JOURNAL, **67**, 1866 (1945).

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

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

(6) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *ibid.*, **103**, 540 (1946).

(7) R. L. Peck, C. E. Hoffhine, Jr., Elizabeth W. Peel, R. P. Graber, F. W. Holly, R. Mozingo and K. Folkers, THIS JOURNAL, **68**, 776 (1946).

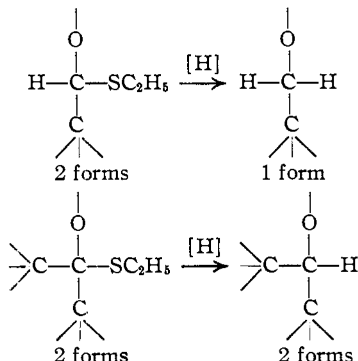
(8) F. A. Kuehl, Jr., E. H. Flynn, F. W. Holly, R. Mozingo and K. Folkers, *ibid.*, **68**, 536 (1946).

acetal on methanolysis. Methyl dihydrostreptobiosaminide pentaacetate has also been reported by Fried and Wintersteiner[9] and by Bartz and co-workers.[10] Peck, Hoffhine and Folkers[11] reported a crystalline helianthate of dihydrostreptomycin.

Mercaptolysis of the calcium chloride compound of streptomycin trihydrochloride with subsequent acetylation, yielded, after chromatographic purification, two anomeric forms of ethyl tetraacetylthiostreptobiosaminide diethyl thioacetal (A, m. p. 80.5–81° (cor.), $[\alpha]^{27}D$ −192° in chloroform; B, m. p. 111–111.5° (cor.), $[\alpha]^{28}D$ −29° in chloroform). Folkers and co-workers,[4] employing the general mercaptalation procedure of Brigl and Schinle[12] have reported a rather high yield of form A for which they record a melting point of 81–82° and a considerably different rotation ($[\alpha]^{25}D$ −178° in chloroform) than found by us. The formation of these two isomers indicates that the streptidine is attached to a glycosidic hydroxyl (cyclic hemiacetal) capable of existing in anomeric forms. The production of a thioglycoside from this cyclic hemiacetal rather than a thioacetal shows that the ring structure present has considerable stability.

Treatment of methyl tetraacetylstreptobiosaminide dimethyl acetal[5] with ethanethiol and hydrogen chloride led under our conditions to acetal exchange and partial deacetylation with the production of ethyl N-acetylthiostreptobiosaminide diethyl thioacetal, previously recorded by Folkers and co-workers.[4]

Hydrogenolysis of either thioacetal A or B above with Raney nickel catalyst[13] followed by reacetylation produced didesoxydihydrostreptobiosamine tetraacetate (m. p. 159–160° (cor.), $[\alpha]^{30}D$ −86° in chloroform), likewise reported by Folkers and co-workers.[4] The formation of a good yield of the same product from either A or B augurs for the identification as a potential aldehyde of the cyclic hemiacetal present in the $C_6H_8O_5$< portion of streptomycin. If this potential carbonyl were a ketone, two reduction products would be expected, as can be noted from the formulas.

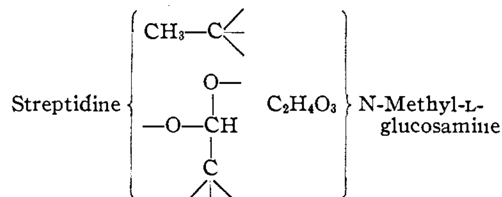


Since Prelog and co-workers[14] have demonstrated that olefinic linkages are not reduced under the hydrogenolysis conditions herein employed and since didesoxydihydrostreptobiosamine tetraacetate was recovered unchanged on attempted hydrogenation with Raney nickel catalyst at 150° and 100 atmospheres of hydrogen, it follows that the $C_6H_8O_5$< moiety contains no olefinic linkage.

N-Methyl-α-L-glucosamine pentaacetate was obtained on acetolysis of didesoxydihydrostreptobiosamine tetraacetate, a result likewise obtained by Folkers and co-workers[4] by acid hydrolysis followed by reacetylation. This finding establishes that the glycosidic hydroxyl of N-methyl-L-glucosamine is glycosidically joined to the central $C_6H_8O_5$< portion, which in turn is linked glycosidically to streptidine, since otherwise a reduced form of N-methyl-L-glucosamine would have resulted from the acetolysis.

Our analytical data established acetylated methyl dihydrostreptobiosaminide as a pentaacetate. This substance could not be further acetylated. The acetylated form of didesoxydihydrostreptobiosamine and the actylated anomers of ethyl thiostreptobiosaminide diethyl thioacetal are tetraacetates. Since four acetyl groups are required for the N-methyl-L-glucosamine entity, no chemical evidence is thus available for the existence of a hydroxyl group in the $C_6H_8O_5$< unit. A tertiary hydroxyl group in a sugar derivative has been acetylated under the conditions herein employed (sodium acetate and hot acetic anhydride).[15]

The formula of streptomycin may then be developed to the following partial form.



Experimental

Preparation and Properties of Streptomycin Trihydrochloride Calcium Chloride Compound, $(C_{21}H_{39}O_{12}N_7·3HCl)_2·CaCl_2$.—Amorphous streptomycin phosphate[16] of 400–600 units/mg. activity[17,18] and $[\alpha]^{25}D$ −50 to −65° (*c* 1, water) was converted to the crystalline helianthate by a modification of the procedure of Folkers and co-workers.[19,20] The streptomycin phosphate (5.00 g.)

(9) J. Fried and O. Wintersteiner, THIS JOURNAL, **69**, 79 (1947).

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

(11) R. L. Peck, C. E. Hoffhine, Jr., and K. Folkers, *ibid.*, **68**, 1390 (1946).

(12) P. Brigl and R. Schinle, *Ber.*, **65B**, 1890 (1932).

(13) M. L. Wolfrom and J. V. Karabinos, THIS JOURNAL, **66**, 909 (1944).

(14) V. Prelog, J. Norymberski and O. Jeger, *Helv. Chim. Acta*, **29**, 360 (1946).

(15) R. U. Lemieux and M. L. Wolfrom, forthcoming publication.

(16) Prepared by Bristol Laboratories, Syracuse, New York.

(17) Y. H. Loo, P. S. Skell, H. H. Thornberry, J. Ehrlich, J. M. McGuire, G. M. Savage and J. C. Sylvester, *J. Bact.*, **50**, 701 (1945).

(18) Assays performed by Bristol Laboratories, Syracuse, N. Y.

(19) F. A. Kuehl, Jr., R. L. Peck, A. Walti and K. Folkers, *Science*, **102**, 34 (1945).

(20) F. A. Kuehl, Jr., R. L. Peck, C. E. Hoffhine, Jr., R. P. Graber and K. Folkers, THIS JOURNAL, **68**, 1460 (1946).

was dissolved in 100 ml. of water and added to a hot solution of 6.67 g. of methyl orange in 667 ml. of water. The solution was cooled and the precipitated streptomycin helianthate was removed by filtration and recrystallized from 1100 ml. of 50% methanol; yield 7.3 g. (2 crops). This crystalline helianthate was converted directly in good yield to the crystalline calcium chloride compound according to the procedure of Folkers and co-workers[3] with some modification. After removal of the calcium helianthate and concentration under reduced pressure to a volume of 40–50 ml., additional calcium helianthate was removed by filtration. The filtrate was made just acid to congo red with concentrated hydrochloric acid and free helianthine removed by filtration. The helianthine remaining in the filtrate was removed by three successive treatments with 250-mg. portions of decolorizing charcoal (Darco G-60). The colorless solution was concentrated under reduced pressure to 5–7 ml. volume and crystallized by allowing to stand (with occasional stirring) at icebox temperature for one to two days. The crystalline material was removed by filtration and washed with a minimum amount of cold methanol; yield 1–1.5 g., $[\alpha]^{25}D$ −70 to −76° (*c* 1.5, water). A further crop was obtainable on concentration of the mother liquor; yield 0.3 g., $[\alpha]^{25}D$ −65 to −75° (*c*, 1.5 water). Pure material was obtained on recrystallization from methanol; $[\alpha]^{25}D$ −81° (*c* 2, water).

Anal. Calcd. for $(C_{21}H_{39}O_{12}N_7 \cdot 3HCl)_2 \cdot CaCl_2$: C, 33.79; H, 5.67; N, 13.14; Cl, 19.00; Ca, 2.68; CH_3—C (one group, calcd. as % CH_3), 2.0. Found: C, 33.82; H, 5.69; N, 12.94; Cl, 18.64; Ca, 2.65; CH_3—C (as CH_3), 1.9.

Crystalline streptomycin trihydrochloride calcium chloride compound showed a biological potency of 750 units/mg.[18] which represents an activity of 960 units/mg. for the streptomycin molecule. We find that there is an approximately linear relation between the optical rotation and the bioassay in streptomycin preparations of 500 units/mg. or higher activity.

Methyl Dihydrosptreptobiosaminide Pentaacetate.—Crystalline streptomycin trihydrochloride calcium chloride compound (3.3 g.) was dissolved in 10 ml. of water and reduced with hydrogen and Raney nickel catalyst by heating at 150° for one hour at 2000 p. s. i. The residue obtained after catalyst and solvent removal was dissolved in 150 ml. of anhydrous methanol containing dry hydrogen chloride in 1 molar concentration and kept at room temperature for two days. The streptidine dihydrochloride was precipitated in amorphous form with 550 ml. of anhydrous ether (completeness of precipitation was verified by observing a negative Sakaguchi test[21] in the supernatant liquid). The mixture was filtered into a suction flask containing anhydrous calcium sulfate and the precipitate was washed four times with 60-ml. portions of methanol–ether (1:4). The amorphous streptidine dihydrochloride was converted to its crystalline sulfate monohydrate.[21a] The latter was characterized by its X-ray powder diagram (Table I). It was optically inactive in aqueous solution throughout the visible spectrum. Octaacetylstreptidine[22] was likewise optically inactive in chloroform solution throughout the visible spectrum.

The filtrate from the streptidine dihydrochloride separation was treated with an excess of silver carbonate and again filtered. The residue obtained on solvent removal was acetylated by standing for one day with pyridine (15 ml.) and acetic anhydride (30 ml.). The solution was then poured into a well-stirred mixture of ice, water and excess potassium bicarbonate. Crystalline material was obtained on solvent removal of a water-washed chloroform extract of the above mixture; yield 0.60 g., m. p. 193–195° (cor.). Pure material was obtained on further crystallization from ethyl acetate; m. p. 194–195° (cor.), $[\alpha]^{28}D$ −123° (*c* 3.2, chloroform). The substance was readily soluble in methanol, acetone, chloroform and hot ethyl acetate; somewhat soluble in water and ethyl acetate; and was insoluble in petroleum ether and ethanethiol.

Anal. Calcd. for $C_{13}H_{19}O_4(NCOCH_3)(OCOCH_3)_4$-$OCH_3$: C, 51.15; H, 6.61; N, 2.49; OCH_3, 5.51; Ac (total), 8.88 ml. of 0.1 *N* NaOH per 100 mg.; O-Ac, 7.10 ml. Found: C, 51.04; H, 6.93; N, 2.50; OCH_3, 5.86; Ac (total), 8.88 ml.; O-Ac,[23] 7.12 ml.

This substance was recovered unchanged after treatment with acetic anhydride and sodium acetate at a temperature just short of reflux.

TABLE I

X-RAY POWDER DIFFRACTION PATTERN OF STREPTIDINE SULFATE MONOHYDRATE[a]

Interplanar spacing, Å.	Relative intensity[b]
5.71	1.00
4.48	1.00
4.00	1.00
3.56	0.35
3.00	.40
2.64	.30
2.20	.30

[a] Measurements made by Mr. R. K. Ness of this Laboratory under the supervision of Professor P. M. Harris. Copper $K\alpha$ radiation was employed. [b] Estimated visually.

Anomeric Forms A and B of Ethyl Tetraacetylthiostreptobiosaminide Diethyl Thioacetal.—Streptomycin trihydrochloride calcium chloride compound (2.50 g.), dissolved in 10 ml. of concentrated hydrochloric acid (sp. gr. 1.19), was stirred mechanically at 0° for four hours with 8 ml. of ethanethiol. At the end of this period the mixture was neutralized rapidly (under cooling) with concentrated ammonium hydroxide (sp. gr. 0.90) to *p*H 8 ± 0.5. The neutralized solution was extracted several times with chloroform (Fraction I); the extract was washed once with water and the washing combined with the original solution (Fraction II) which was then concentrated rapidly under good vacuum (oil pump) at 35°. The dry residue was acetylated at room temperature for two to three days with acetic anhydride (20 ml.) and pyridine (10 ml.). The acetylation mixture was poured into ice and water, extracted with chloroform and the extract washed with aqueous sodium bicarbonate and water. The residue (800 mg.) obtained on solvent removal was dissolved in 50 ml. of benzene and added to the top of a column of Magnesol–Celite[24] (5:1) 20 cm. in height and 4.2 cm. dia. The chromatogram was developed with 1000 ml. of benzene–alcohol (100:1) which moved a bright yellow band to the bottom of the column. The column was extruded and streaked with the alkaline permanganate indicator.[24] The lowest permanganate-reducing zone (10–12 cm. from the top) was cut out and eluted with acetone. The semi-crystalline residue obtained on acetone removal was recrystallized from ethanol–water; yield 200 mg., m. p. 104–106°. Pure material (designated form B) was obtained on further crystallization from ethanol–water; m. p. 111–111.5° (cor.), $[\alpha]^{28}D$ −29° (*c* 2.8, chloroform).

The substance crystallized in slender prisms that were soluble in ethanol, ether, benzene, ethyl acetate and chloroform; insoluble in water and petroleum ether. It did not reduce Fehling solution and liberated ethanethiol on warming with dilute hydrochloric acid.

(21) S. Sakaguchi, *J. Biochem. Japan*, **5**, 25 (1925).

(21a) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, J. S. Meek, P. S. Skell, W. A. Strong, J. T. Alberi, Q. R. Bartz, S. B. Binkley, H. M. Crooks, Jr., I. R. Hooper and Mildred C. Rebstock, *Science*, **103**, 53 (1946).

(22) R. L. Peck, R. P. Graber, A. Walti, Elizabeth W. Peel, C. E. Hoffhine, Jr., and K. Folkers, THIS JOURNAL, **68**, 29 (1946).

(23) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, THIS JOURNAL, **58**, 490 (1936).

(24) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67** 527 (1945).

Fraction I above was dried and the residue obtained on solvent removal was acetylated and chromatographed as described above. The sufficiency of column development could again be followed by moving a leading bright yellow band to the bottom of the column. A small amount of the B-form could be obtained from the lower reducing zone (10–12 cm. from the top). In a diffuse and poorly characterized zone above this there was obtained on acetone elution a yellow oil that was crystallized from ether–petroleum ether; yield 200 mg.; m. p. 75–79°. Pure material (designated form A) was obtained on further crystallization from benzene–petroleum ether; m. p. 80.5–81° (cor.), $[\alpha]^{27}D$ $-192°$ (c 2.3, chloroform). The substance crystallized in large, white needles.

Anal. Calcd. for $C_{13}H_{18}O_4(C_2H_5S)_3(NCOCH_3)(OCOCH_3)_3$: C, 49.44; H, 6.92; N, 2.14; S, 14.66; CH_3CO (total), 6.09 ml. of 0.1 *N* NaOH per 100 mg.; O-Ac, 4.57 ml.; mol. wt., 655.9. Found for A: C, 49.63; H, 6.89; N, 2.21; S, 14.82; A-Oc,[23] 4.44 ml.; mol. wt. (Rast), 620. Found for B: C, 49.54; H, 6.71; N, 2.15; S, 14.75; CH_3CO (total), 6.09 ml.; O-Ac,[23] 4.27 ml.; mol. wt. (Rast), 651.

Ethyl N-Acetylthiostreptobiosaminide Diethyl Thioacetal from Methyl Tetraacetylstreptobiosaminide Dimethyl Acetal.—Methyl tetraacetylstreptobiosaminide dimethyl acetal[5] (498 mg., m. p. 122° (cor.), $[\alpha]^{23}D$ $-127°$ (c 2.6) in chloroform) was treated with ethanethiol and hydrogen chloride as described by Brigl and Schinle.[12] The reaction mixture was protected from moisture and allowed to stand at room temperature for several days. The separated crystals (293 mg.) were recrystallized from hot ethyl aectate; m. p. 171.5–172.5° (cor.), $[\alpha]^{27}D$ $-248°$ (c 2.0, chloroform), $[\alpha]^{22}D$ $-248°$ (c 0.4, methanol). Folkers and co-workers[4] record for ethyl N-acetylthiostreptobiosaminide diethyl thioacetal: m. p. 173–175°, $[\alpha]^{25}D$ $-250°$ (c 1.5, methanol). The formula for this substance reported by Folkers and co-workers is incorrect in respect to its oxygen content but their calculated values of percentage composition are correct.

Anal. Calcd. for $C_{19}H_{36}O_7S_3(NCOCH_3)$: N, 2.64; CH_3CO, 1.89 ml. of 0.1 *N* NaOH per 100 mg. Found: N, 2.64; CH_3CO, 1.93 ml.

Didesoxydihydrostreptobiosamine Tetraacetate.—Ethyl tetraacetylthiostreptobiosaminide diethyl thioacetal (Form B, 737 mg.) was reduced with Raney nickel in 70% aqueous ethanol according to the general procedure described by Wolfrom and Karabinos.[13] The resultant partially crystalline product was reacetylated with acetic anhydride and pyridine by the procedure described above in the synthesis of the starting material. The last traces of pyridine were removed by washing the chloroform solution of the product in a separatory funnel with an excess of a saturated aqueous solution of cadmium chloride,[25] removing the separated cadmium chloride–pyridine compound, and washing the chloroform solution with water. The oil obtained on solvent removal from the dried chloroform extract was crystallized from methyl ethyl ketone by the addition of petroleum ether; yield 414 mg. (78%), m. p. 148–153°. Pure material was obtained on further crystallization effected in the same manner or from benzene–petroleum ether; m. p. 159–160° (cor.), $[\alpha]^{30}D$ $-86°$ (c 1.9, chloroform).

The substance crystallized as fine, colorless needles that were soluble in the common solvents except water and petroleum ether. It did not reduce Fehling solution.

Anal. Calcd. for $C_{13}H_{21}O_4(NCOCH_3)(OCOCH_3)_3$: C, 53.04; H, 7.00; N, 2.94; O-Ac, 6.32 ml. of 0.1 *N* NaOH per 100 mg. Found: C, 53.06; H, 7.02; N, 3.04; S, absent; O-Ac,[23] 6.00 ml.

The same product was obtained in 70% yield from the hydrogenolysis of Form A of ethyl tetraacetylthiostreptobiosaminide diethyl thioacetal effected in the same manner; m. p. 158.5–159° unchanged on admixture with the hydrogenolysis product of form B, $[\alpha]^{31}D$ $-86°$ (c 3, chloroform).

This substance was recovered unchanged on treatment with Raney nickel and hydrogen at 150° and 2000 p. s. i. as described above in the synthesis of methyl dihydrostreptobiosaminide pentaacetate.

Didesoxystreptobiosamine tetraacetate (207 mg.) was dissolved in 10 ml. of a solution prepared by adding at 0°, 0.5 ml. of concentrated sulfuric acid (sp. gr. 1.83) to a mixture of 17.5 ml. of acetic anhydride and 7.5 ml. of glacial acetic acid. The resultant solution was kept at room temperature for one day, whereupon it was poured upon cracked ice, partially neutralized with sodium bicarbonate and extracted with chloroform. The residue obtained on chloroform removal was dissolved in a small amount of benzene and chromatographed[24] on a column (14 cm. long, 3.5 cm. dia.) of Magnesol-Celite (5:1), previously wet with benzene. Development was effected with 220 ml. of benzene–ethanol (100:1). Extrusion of the column and streaking with the alkaline permanganate indicator[24] located a zone about 35–50 mm. from the top of the column that on acetone elution yielded crystalline material (58 mg. crude yield) that was further crystallized from benzene–petroleum ether; m. p. 154–156° (cor.); mixed melting point with a synthetic specimen[26] of N-methyl-α-L-glucosamine pentaacetate (m. p. 155–157°), 154–156°; mixed melting point with didesoxydihydrostreptobiosamine tetraacetate (m. p. 157–158°), 130–146°; $[\alpha]^{29}D$ $-99 \pm 2°$ (c 0.8, chloroform).

Acknowledgment.—Mrs. Mary H. Klotz purified most of the streptomycin used in this work. Acknowledgment is also made to the assistance of Dr. Velma E. Nichols, Dr. S. M. Moffett and Mr. C. W. DeWalt.

Summary

1. Analytical data on streptomycin trihydrochloride calcium chloride compound and on didesoxydihydrostreptobiosamine tetraacetate support the formula $C_{21}H_{39}O_{12}N_7$ for streptomycin.

2. Streptomycin contains one CH_3-C group.

3. Hydrogenation of the calcium chloride compound of streptomycin trihydrochloride with Raney nickel catalyst followed by methanolysis and acetylation yields crystalline methyl dihydrostreptobiosaminide pentaacetate.

4. Mercaptolysis of the calcium chloride compound of streptomycin trihydrochloride with subsequent acetylation yields, after chromatographic purification, two forms of ethyl tetraacetylthiostreptobiosaminide diethyl thioacetal, both of which produce good yields of didesoxydihydrostreptobiosamine tetraacetate on hydrogenolysis with Raney nickel catalyst.

5. Didesoxydihydrostreptobiosamine tetraacetate is not further hydrogenated at 150° with Raney nickel catalyst and on acetolysis yields N-methyl-α-L-glucosamine pentaacetate.

6. The above data indicate that the moiety $C_6H_8O_5<$ of streptomycin contains one CH_3C-group but no olefinic linkage and is attached to N-methyl-L-glucosamine through the glycosidic group of the latter. It is likewise in glycosidic union with streptidine (herein characterized by the X-ray diagram of its sulfate monohydrate), the glycosidic hydroxyl being very probably aldehydic

(25) A procedure devised by Mr. D. O. Hoffman of this Laboratory.

(26) Prepared by Dr. A. Thompson of this Laboratory.

in nature and in the form of a cyclic hemiacetal that is resistant to acid hydrolysis but is capable of existing in anomeric forms. In dihydrostreptobiosamine, a carbonyl group has been reduced to an alcohol group.

COLUMBUS, OHIO RECEIVED DECEMBER 12, 1946

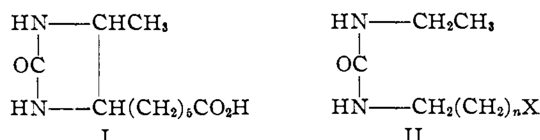
[FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, MEDICAL RESEARCH DIVISION, SHARP AND DOHME, INC.]

The Reaction of Aminoesters with Ethyl Isocyanate: Open Chain Models of Desthiobiotin

BY EVERETT M. SCHULTZ

It has been proposed that in order for a compound to resemble biotin in avidin combinability, two structural features are essential: an imidazolidone nucleus and an ω-alkylcarboxylic acid side-chain.[1] It has been demonstrated also that for certain organisms pimelic acid acts as a precursor[2] in the biosynthesis of biotin. More recently, Tatum has shown that for some organisms pimelic acid may act as the precursor for desthiobiotin.[3]

The work described in this paper is the preparation of some 1,3-disubstituted ureas (II) that may be considered as open chain analogs of desthiobiotin (I). When $n = 4$ (IIA) the urea has the carboxyl side chain that occurs in desthiobiotin. When $n = 5$ (IIB), the urea becomes a true acyclic model of desthiobiotin. In view of the



IIA ($n = 4$, X = CO_2H)
IIB ($n = 5$, X = CO_2H)
IIC ($n = 3$, X = H)
IID ($n = 4$, X = $CO_2C_2H_5$)
IIE ($n = 4$, X = $CONH_2$)
IIF ($n = 5$, X = $CO_2C_2H_5$)

biosynthesis of biotin and desthiobiotin from pimelic acid, it was thought possible that the same organisms that can bring about such transformations might also utilize such a compound as IIB as precursor. It also seemed of interest to test such open chain desthiobiotin analogs for avidin combinability.

None of the ureas described in this paper showed either biotin or antibiotin activity when tested microbiologically against *Lactobacillus casei* or *Lactobacillus arabinosis*. None was avidin combinable nor did any one act as a precursor for the biosynthesis of desthiobiotin or biotin.[3,4]

Since *n*-butylamine combined readily with ethyl isocyanate to give 1-ethyl-3-butyl-urea (IIC), the chosen method of synthesis was the reaction of ω-amino acids with ethyl isocyanate. It was found that, while the free acids were inert to ethyl isocyanate, their ethyl esters reacted vigorously. The 1-ethyl-3-(ω-carbethoxyalkyl)-ureas were easily saponified by cold dilute sodium hydroxide solution to form the free acids and could be converted to amides by shaking with cold concentrated aqueous ammonia. The stability of the urea linkage is such that the compounds are completely unchanged by autoclaving at 120° for fifteen minutes in water, in which all are sufficiently soluble for microbiological assay.

For the synthesis of IIF, 7-aminoheptanoic acid was required. It was prepared by the reduction of 6-cyanocaproic acid (III), a compound that apparently has not been reported in the literature. The cyanoacid was prepared from cyclohexanone through ethyl 6-hydroxycaproate (V) and 6-bromocaproic acid (VI). The structure of III was demonstrated by hydrolysis to pimelic acid.

Experimental

6-Cyanocaproic Acid (III).—6-Bromocaproic acid,[5a,b,c] m. p. 32°,[6] (71.17 g., 0.37 mole) was suspended in water (125 cc.) and sodium carbonate (22.6 g., 0.185 mole) was added slowly with stirring. Then sodium cyanide (96%) (121 g., 0.409 mole) was added with shaking. The mixture was heated to 54° and the reaction flask was wrapped with a cloth. The internal temperature rose to 57° over a period of one-half hour. The mixture was then heated quickly to 100° and boiled for five minutes. The black opaque reaction mixture was cooled to 30° and acidified (hydrogen cyanide evolved) with concentrated hydrochloric acid. The free hydrogen cyanide was removed under reduced pressure at room temperature. After saturating the aqueous mixture with ammonium sulfate, the product was extracted with ether. The ether solution was filtered and dried over anhydrous sodium sulfate. Upon evaporation of the ether, there remained an oily residue that was distilled to give a liquid boiling at 158–160° (2.5–3 mm.) The yield was 56%.

One gram of the above nitrile was added to a solution of 13 g. of potassium hydroxide in 107 cc. of ethanol and the mixture was refluxed for twenty hours. Upon working up the reaction mixture there was obtained a good yield of pimelic acid, identified by melting point and mixed melting point.

7-Aminoheptanoic Acid (IV).—Dry potassium 6-cyanocaproate (17 g., 0.095 mole) (prepared by adding the calculated amount of 40% potassium hydroxide solution to an aqueous suspension of 6-cyanocaproic acid, and evaporating the water in a vacuum desiccator over solid potassium

(1) Dittmer and du Vigneaud, *Science*, **100**, 130 (1944).

(2) du Vigneaud, Dittmer, Hague and Long, *ibid.*, **96**, 186 (1942); Eakin and Eakin, *ibid.*, 187 (1942).

(3) Tatum, *J. Biol. Chem.*, **160**, 455 (1945).

(4) Tests were carried out by Dr. Lemuel D. Wright, of these laboratories.

(5) (a) Robinson and Smith, *J. Chem. Soc.*, 373 (1937); (b) Barger, Robinson and Smith, *ibid.*, 722 (1937); (c) Brown and Partridge, THIS JOURNAL, **66**, 839 (1944).

(6) Marvel, *et al.*, *ibid.*, **46**, 2838 (1934).