distilled, the reaction mixture was cooled, filtered and the solid washed with a 150-ml. portion each of ligroin and ether. After two recrystallizations of the crude IV from ethyl alcohol, a yield of 122 g. (55%) of light tan-colored crystals was obtained, m. p. 252–254°. The melting recorded by Conrad and Limpach[6] was 254°.

2-Phenyl-4-bromoquinoline (V).—This substance was prepared from 10 g. of IV according to the method used for the preparation of III; recrystallized from 70% ethyl alcohol, the yield was 10.8 g. (83.5%), m. p. 90–90.5°. The melting point reported by John[7] was 91°.

Using phosphorus tribromide, the yield of V was only 39%. When phosphorus pentabromide was used, the yield of V was 65% but a 7% yield of a by-product (VI) was obtained the analysis of which indicated it was a tribromophenylquinoline, m. p. 149.5–150°.

Anal. Calcd. for $C_{15}H_8Br_3N$: Br, 54.25. Found: Br, 54.32.

2-Phenyl-3-bromo-4-hydroxyquinoline (VIII).—This substance was prepared according to a method reported by Riegel.[8] A solution of 53 g. (0.25 mole) of IV in 300 ml. of hot glacial acetic acid, contained in a 500-ml. three-necked flask fitted with a stirrer, thermometer and a separatory funnel, was treated at 70° over a period of forty-five minutes with 46 g. (0.28 mole) of bromine. After the reaction mixture cooled, a dilute aqueous solution containing 40 g. of sodium hydroxide was added, the crude VII removed by filtration, washed thoroughly with water and dried in an air-bath. The substance was recrystallized three times from methyl alcohol, yielding 68 g. (94%) of fine white needles, m. p. 263–264°.

Anal. Calcd. for $C_{15}H_{10}BrNO$: Br, 26.62. Found: Br, 26.49.

2-Phenyl-6-bromo-4-hydroxyquinoline (VIII).—This substance was obtained from 24 g. of *p*-bromoaniline and 24 g. of ethyl benzoylacetate according to the method for the preparation of IV. After recrystallization of the crude VIII from butyl cellosolve, it was obtained in a 58% yield (24.5 g.) as a fine, white crystalline product, m. p. 331–333°.

Anal. Calcd. for $C_{15}H_{10}BrON$: Br, 26.62. Found: Br, 26.54.

2-Phenyl-4,6-dibromoquinoline (IX).—This substance was prepared from VIII by the action of phosphoryl tribromide, as described for 2-phenyl-4-bromoquinoline. After two recrystallizations from ligroin, the substance was obtained as silky white crystals in a 70% yield, m. p. 121.5–122°.

Anal. Calcd. for $C_{15}H_9Br_2N$: Br, 44.02. Found: Br, 44.10.

2-Phenyl-6-bromo-4-chloroquinoline.—This substance was obtained in 84% yield from 5 g. of VIII by the action of 20 g. of hot phosphoryl trichloride and recrystallization of the crude substance from ethyl alcohol; m. p. 113.5–114°.

Anal. Calcd. for $C_{15}H_9BrClN$: BrCl, 36.21. Found: BrCl, 36.13.

Action with Phosphorus Pentabromide.—Five grams (0.017 mole) of VIII was heated at 105–110° with 15 g. (0.037 mole) of phosphorus pentabromide for three hours, then the reaction mixture was cooled, warmed with water and finally made alkaline with sodium hydroxide. After drying the solid, it was extracted with ligroin (b. p. 60–90°), the solvent concentrated to a small volume and the solid recrystallized three times from ligroin. The yield of the fine white solid was 4.2 g., m. p. 178–179°.

Anal. Calcd. for $C_{15}H_7Br_4N$: Br, 61.37. Found: Br, 61.23.

When 2.5 g. of VII was treated with 5 g. of phosphorus pentabromide as in the case of VIII, there was obtained after extraction with ligroin and recrystallization from methanol, 2.2 g. of a white solid which melted at 166–167°.

Anal. Calcd. for $C_{15}H_7Br_4N$: Br, 61.37. Found: Br, 61.08.

Summary

Several new bromo-2-phenylquinolines have been reported; these are, namely, 2-(4′-bromophenyl)-4-hydroxyquinoline, 2-(4′-bromophenyl)-4-bromoquinoline, 2-phenyl-3-bromo-4-hydroxyquinoline, 2-phenyl-6-bromo-4-hydroxyquinoline, 2-phenyl-4,6-dibromoquinoline and 2-phenyl-6-bromo-4-chloroquinoline.

It has been shown that polybromination may occur when phosphorus pentabromide is used to convert the 2-phenyl-4-hydroxyquinolines to 2-phenyl-4-bromoquinolines.

BLOOMINGTON, INDIANA RECEIVED OCTOBER 13, 1949

(6) Conrad and Limpach, *Ber.*, **21**, 521 (1888).

(7) John, *J. prakt. Chem.*, **126**, 220 (1930).

(8) Riegel, Lappin, Albisetti, Adelson, Dodson, Ginger and Baker, THIS JOURNAL, **68**, 1229 (1946).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

A Synthesis of Streptidine[1]

BY M. L. WOLFROM, S. M. OLIN[2] AND W. J. POLGLASE[2]

That component of streptomycin designated streptidine[3] was degradatively established[4] as 1,3-diguanidino-2,4,5,6-tetrahydroxycyclohexane by Carter,[5,6] Folkers,[3,7–9] Wintersteiner,[10,11] and

(1) The synthesis of streptamine from D-glucosamine was reported by M. L. Wolfrom and S. M. Olin before the Division of Sugar Chemistry and Technology and recorded in Abstracts of Papers, 113th Meeting, American Chemical Society, Chicago, Illinois, April, 1948, p. 5Q. A preliminary report by M. L. Wolfrom and W. J. Polglase, of the synthesis of streptidine from streptamine appeared in THIS JOURNAL, **70**, 1672 (1948).

(2) Bristol Laboratories Research Fellow of The Ohio State University Research Foundation (Project 224).

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

(4) R. U. Lemieux and M. L. Wolfrom, *Advances in Carbohydrate Chem.*, **3**, 337 (1948), review paper.

(5) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *J. Biol. Chem.*, **160**, 337 (1945); *Science*, **103**, 540 (1946).

(6) 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, *ibid.*, **103**, 53 (1946).

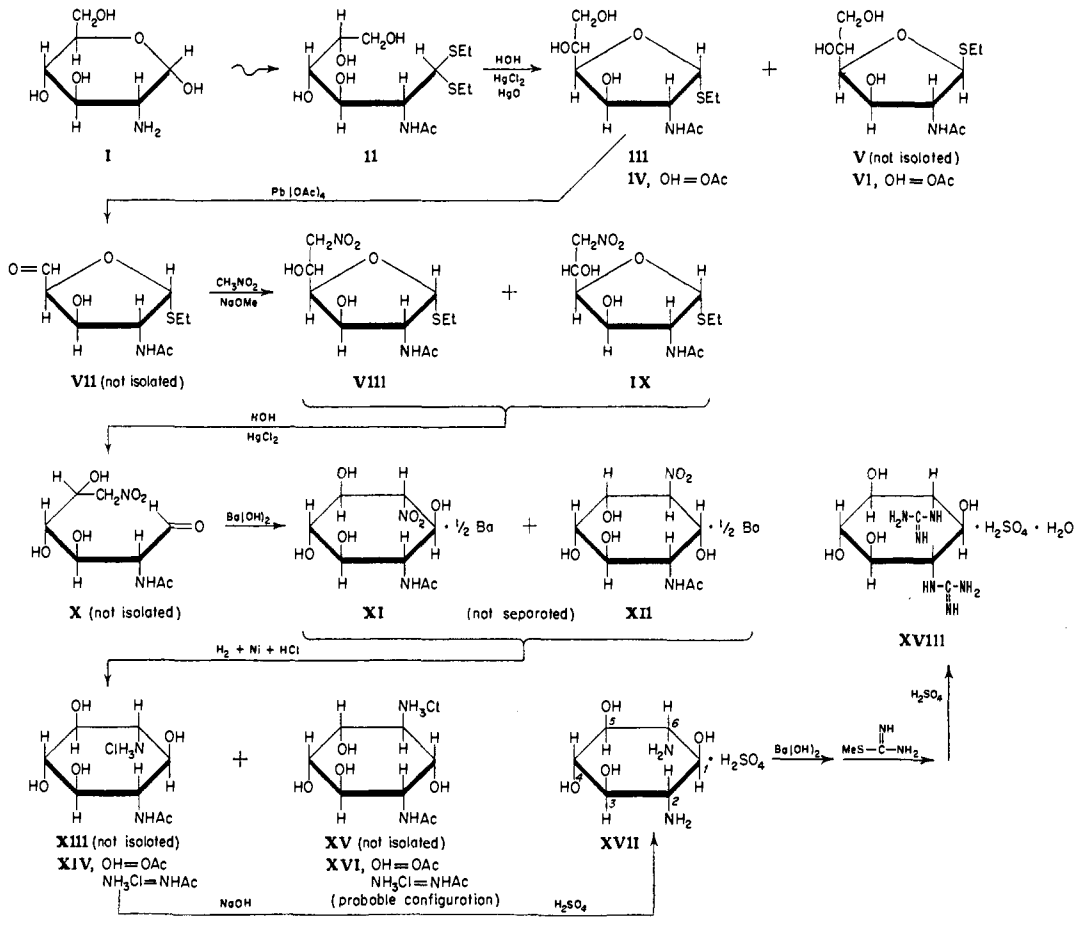
(7) R. L. Peck, R. P. Graber, A. Walti, Elizabeth W. Peel, C. E. Hoffhine, Jr., and K. Folkers, THIS JOURNAL, **68**, 29 (1946).

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

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

(10) J. Fried, G. A. Boyack and O. Wintersteiner, *J. Biol. Chem.*, **162**, 391 (1946).

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



their co-workers. Streptidine and its salts have no definite melting points, are optically inactive, and are best characterized by X-ray diffraction pattern.[12] Two tasks remain in the establishment of the formula of streptidine: (1) the verification of its gross structure by synthesis and (2) the elucidation of its configuration.

In the work herein reported, N-acetyl-D-glucosamine diethyl thioacetal (synonym 2-acetamido-2-desoxy-D-glucose diethyl thioacetal, II)[13] was converted to the N-acetyl-α-D-thiofuranoside III, designated ethyl 2-acetamido-2-desoxy-α-D-glucothiofuranoside, by hydrolysis in the presence of mercuric chloride and mercuric oxide in a manner analogous to that described by Pacsu and Wilson[14] for the conversion of D-glucose diethyl thioacetal to ethyl α-D-glucothiofuranoside.[15] The new thiofuranoside was further characterized by its tetraacetyl derivative IV and the anomeric form (VI) of IV was obtained from the acetylated and chromatographed mother liquor material. In the anomeric pair IV and VI, the higher dextrorotation of IV establishes it and its parent body III as α-D forms. The furanoside structure of III was demonstrated by the fact that periodate assay showed the consumption of one mole (per mole of III) of oxidant with the formation of a like amount of formaldehyde.

Ethyl 2-acetamido-2-desoxy-α-D-glucothiofuranoside (III) was then subjected to glycol scission between C_5 and C_6 through treatment with lead tetraacetate in chloroform solution. The resultant dialdehyde derivative VII was not isolated but was immediately condensed with alkaline nitromethane, following the general procedures adapted to sugar derivatives by Fischer and co-workers,[16–18] and the two predictable isomeric products were isolated by chromatographic technics. These substances, designated ethyl 2-acetamido-6-nitro-2,6-didesoxy-α-D-glucothiofuranoside (VIII) and ethyl 2-acetamido-6-nitro-2,6-didesoxy-β-L-idothiofuranoside (IX), differ in the configuration of C_5. No data were obtained to establish which of the two crystalline isomers was

(12) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, THIS JOURNAL, **68**, 2120 (1946); **69**, 1052 (1947).

(13) M. L. Wolfrom, R. U. Lemieux and S. M. Olin, *ibid.*, **71**, 2870 (1949).

(14) E. Pacsu and E. J. Wilson, Jr., *ibid.*, **61**, 1450 (1939).

(15) M. L. Wolfrom, S. W. Waisbrot, D. I. Weisblat and A. Thompson, *ibid.*, **66**, 2063 (1944).

(16) J. C. Sowden and H. O. L. Fischer, *ibid.*, **66**, 1312 (1944).

(17) H. O. L. Fischer, *Harvey Lectures*, Ser. **40**, 156 (1945).

(18) J. M. Grosheintz and H. O. L. Fischer, *Abstracts Papers Am. Chem. Soc.*, **108**, 9R (1944); THIS JOURNAL, **70**, 1476, 1479 (1948).

TABLE I

POWDER X-RAY[a] DIFFRACTION PATTERNS OF NATURAL AND SYNTHETIC HEXAACETYLSTYREPTAMINE[b]

Hexaacetylstreptamine, from streptomycin		Hexaacetylstreptamine, synthetic	
Interplanar spacing, Å.	Relative intensity[c]	Interplanar spacing, Å.	Relative intensity[c]
7.09	0.7	7.05	0.6
4.55	1.0	4.54	1.0
3.94	0.7	3.94	0.8
3.54	.4	3.54	.5
3.28	.5	3.28	.5
3.09	.2	3.09	.2
2.87	.1	2.87	.1
2.57	.2	2.57	.2
2.38	.2	2.38	.1
1.88	.2	1.88	.2
1.77	.05	1.77	.05

[a] Copper K_α radiation. [b] Professor P. M. Harris and Mr. D. Tuomi of this Laboratory kindly assisted in obtaining these data. [c] Estimated visually.

TABLE II

POWDER X-RAY[a] DIFFRACTION PATTERN OF FRACTION B AND STREPTAMINE SULFATE[b]

Fraction B		Streptamine sulfate	
Interplanar spacing, Å.	Relative intensity[c]	Interplanar spacing, Å.	Relative intensity[c]
5.37	1.0	4.87	0.5
4.81	0.1	4.04	1.0
4.28	.9	3.03	0.5
2.87	.5	2.81	.4
2.69	.5	2.63	.2
2.43	.9	2.22	.3
2.19	.4	1.88	.4
2.09	.3		
1.71	.5		

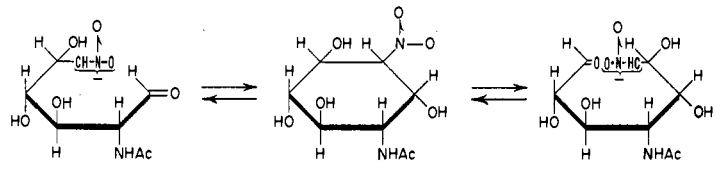
[a] Copper K_α radiation. [b] See footnote *b*, Table I. [c] Estimated visually.

VIII or IX. The thioethoxy group was hydrolyzed from the higher melting isomer (VIII or IX) in the presence of mercuric chloride[19] and the nitroaldehyde (X) formed was not isolated but was immediately subjected to alkaline condensation, following the general technic of Grosheintz and Fischer[18] for analogous ring closures to hexahydroxycyclohexane (inositol) derivatives. The mixture of nitro compounds so obtained was isolated as an amorphous mixture of barium salts (XI and XII) and these were hydrogenated in acid solution. The resultant diamino derivatives (XIII and XV) were transformed to their readily separable hexaacetyl derivatives (XIV and XVI). One of these (XIV) was shown to be identical with hexaacetylstreptamine[8,12] obtained from streptomycin, by its behavior on heating and by its powder X-ray diffraction diagram (Table I). The other product (XVI) was obtained in amounts insufficient for analytical characterization but its behavior on heating and its powder X-ray diffraction diagram (Table II, Fraction B) are recorded. It is presumably a stereoisomer of hexaacetylstreptamine (XIV) of the probable configuration shown in XVI.

Hexaacetylstreptamine (XIV) was then deacetylated and the resultant streptamine, isolated as the sulfate (XVII) and herein characterized by its powder X-ray diffraction pattern (Table II), was converted to the diguanidino derivative XVIII by reaction with methylisothiourea, following the general procedure of Rathke[20] for the synthesis of substituted guanidines. The product was isolated as the crystalline sulfate monohydrate (XVIII) and shown to be identical by its powder X-ray diffraction pattern,[12] with streptidine sulfate monohydrate obtained from the hydrolysis of streptomycin. Folkers and co-workers[21] have published an alternative route for the conversion of streptamine to streptidine.

A synthesis of streptidine sulfate monohydrate from D-glucosamine has thereby been effected. The nature of the consecutive reactions concerned in the sequence I → XVII is such as to confirm by synthesis the structure of streptamine degradatively established as 1,3-diamino-2,4,5,6-tetrahydroxycyclohexane.

The structure of D-glucosamine (chitosamine) has been definitely established as 2-amino-2-desoxy-D-glucose (I) by the work of Haworth, Lake and Peat.[22] Consider formula XVII for streptamine sulfate. It is known[17,18,23] that the alkaline condensation of nitro compounds with the carbonyl group is reversible and that therefore a mixture of XI and XII should be obtainable from either VIII or IX.



Therefore the configurations of C_5, C_6 and C_1 (following the original numbering of the D-glucosamine carbons) in formula XVII are not defined from the known configuration of D-glucosamine but those of C_2, C_3 and C_4 are and these three are configurationally all-*trans* to each other. There are eight theoretically possible isomers obtainable from the alkaline condensation of X, with subsequent transformation to the hexaacetyl derivative of the 1,3-diaminotetrahydroxycyclohexane. These eight isomers will exist as six optically active and two *meso* forms. The synthesis of inactive streptamine from active D-glucosamine without operating on the three surviving asym-

(19) E. Fischer, *Ber.*, **27**, 673 (1894).

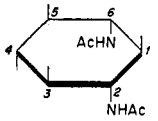
(20) B. Rathke, *ibid.*, **14**, 1774 (1881); *cf.* R. Phillips and H. T. Clarke, THIS JOURNAL, **45**, 1755 (1923).

(21) F. W. Holly, R. Mozingo and K. Folkers, *ibid.*, **70**, 3944 (1948).

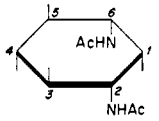
(22) W. N. Haworth, W. H. G. Lake and S. Peat, *J. Chem. Soc.*, 271 (1939).

(23) A. Lambert and A. Lowe, *ibid.*, 1517 (1947); N. Levy and J. D. Rose, *Quart. Revs. (London)*, **1**, 374 (1947).

metric atoms proves that the product must be a *meso* form. Furthermore, the occurrence in streptomycin of a racemic form of streptidine would be impossible as the remainder of the streptomycin molecule contains optically active centers which would lead to the resolution of a D,L-streptidine component. There remain then for consideration only the *meso* structures XIV and XIX, which differ in the configuration of C_1.



XIV

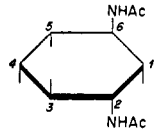


XIX

The configurations of five (2–6, inclusive) of the six asymmetric centers of hexaacetylstreptamine and of the related streptidine, are therefore rigorously established as all-*trans*. This deduction is confirmed by the work of Wintersteiner and Klingsberg,[24] who have shown that carbons 4,5 and 6 of streptidine, and thus of hexaacetylstreptamine (XIV or XIX) bear an all-*trans* configurational relationship to each other.

There remains for consideration the configuration of C_1 (XIV or XIX). Fischer and co-workers[17,18,25] have adduced considerable evidence that alkaline carbonyl condensations of the type herein employed, when effected with optically active compounds, lead to *trans* configurations only. This would eliminate XIX, since in this structure the configurations of C_6 and C_1 are *cis*. By this reasoning, hexaacetylstreptamine is XIV or the all-*trans* isomer. Since streptamine is derived from streptidine by procedures[8,10] which do not change the configurations of the asymmetric centers, then streptidine is likewise the all-*trans* isomer. It is of interest that an all-*trans* hexahydroxycyclohexane (inositol), designated scyllitol, occurs in nature.[26,27]

Formula XVI represents the only other possible substance derivable from D-glucosamine and possessing an all-*trans* configuration on the centers, C_5, C_6 and C_1, involved in the carbonyl condensation reaction. It is probable that this then represents the structure of the other isomer predictable from the reactions employed.



XVI

An incidental result to be noted is that the value of A_{SEt} (chloroform) = 35,500, obtainable from the molecular rotations of the anomeric forms (IV and VI) of ethyl 2-acetamido-2-desoxytriacetyl-D-glucothiofuranoside by solution[28] of equations (1) and (2) is in sensible agreement with that of

$$A_{SEt} + B = (391)(+140°) = 54{,}700 \quad (1)$$
$$-A_{SEt} + B = (391)(-42°) = -16{,}400 \quad (2)$$

34,500 obtainable from the rotations[14] of the analogous derivatives of D-glucose.

Experimental[29]

Ethyl 2-Acetamido-2-desoxy-α-D-glucothiofuranoside (III).—This substance was prepared essentially according to the procedure of Pacsu and Wilson[14] for preparing ethyl α-D-glucothiofuranoside from D-glucose diethyl thioacetal. The washed yellow mercuric oxide prepared from 5 g. of mercuric chloride and 2 g. of sodium according to Pacsu and Wilson[14] was added to a solution of 4.65 g. of N-acetyl-D-glucosamine diethyl thioacetal[13] in 60 ml. of water. To this suspension was added, dropwise and under vigorous mechanical stirring, over a period of twenty minutes, a solution of 2.01 g. (0.5 equiv.) of mercuric chloride in 150 ml. of water and stirring was maintained for fifteen minutes after the addition. Pyridine (3 ml.) was added and the solution was filtered through a precoat of Celite.[30] The filtrate was concentrated under reduced pressure and the resultant sirup was crystallized from ethanol–ether (one drop of pyridine added); yield 2.01 g., m. p. 116–118°, $[\alpha]^{23}D$ + 153° (*c* 3, water). Pure material was obtained on further crystallization effected in the same manner; m. p. 119–121°, $[\alpha]^{22}D$ + 170° (*c* 3, water).

Anal. Calcd. for $C_{10}H_{19}O_5NS$: C, 45.27; H, 7.22; N, 5.28; S, 12.07. Found: C, 45.44; H, 7.17; N, 5.11; S, 11.87. Periodate assay (moles per mole of substance): oxidant consumed, 1.0; formaldehyde, 0.8.

A product with the same constants was obtained by the partial deacetylation of ethyl 2-acetamido-2-desoxytriacetyl-α-D-glucothiofuranoside (see succeeding section) with methanolic ammonia according to the procedure employed for the preparation of N-acetyl-D-glucosamine diethyl thioacetal.[13]

Ethyl 2-Acetamido-2-desoxytriacetyl-α-D-glucothiofuranoside (IV).—N-Acetyl-D-glucosamine diethyl thioacetal (10.0 g.) was transformed to the thiofuranoside as described in the preceding section except that the resultant crude sirup was not crystallized from ethanol–ether-pyridine but was acetylated by dissolving in pyridine (60 ml.), adding acetic anhydride (60 ml.) and allowing the mixture to stand at room temperature for eighteen hours. The reaction mixture was then poured into an excess of ice and water and the product was isolated by chloroform extraction. A crystalline mixture was obtained on solvent removal from the washed (water and aqueous sodium bicarbonate) and dried extract; m. p. 80–90°. One recrystallization from ethanol–water produced a relatively pure substance; yield 2.51 g., m. p. 119–122°, $[\alpha]^{23}D$ + 123° (*c* 4, chloroform). Pure material was obtained on further recrystallization effected in the same manner; m. p. 124.5–125.5°, $[\alpha]^{23}D$ + 140° (*c* 4, chloroform).

Anal. Calcd. for $C_{16}H_{25}O_8NS$: C, 49.09; H, 6.44; N, 3.58; S, 8.19. Found: C, 49.22; H, 6.72; N, 3.77; S, 8.03.

Ethyl 2-Acetamido-2-desoxytriacetyl-β-D-glucothiofuranoside (VI).—The mother liquors from the above-described crystallization of ethyl 2-acetamido-2-desoxytriacetyl-α-D-glucothiofuranoside were concentrated to a sirup; yield 6 g. An amount of 4.0 g. of the sirup in 75 ml. of benzene was added at the top of a 200 × 55 mm.

(24) O. Wintersteiner and Anna Klingsberg, THIS JOURNAL, **70**, 885 (1948).

(25) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **19**, 519 (1936).

(26) G. Staedeler and F. T. Frerichs, *J. prakt. Chem.*, [1] **73**, 48 (1858).

(27) T. Posternak, *Helv. Chim. Acta*, **25**, 746 (1942).

(28) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).

(29) Unless otherwise noted, all experimental work was performed by Mr. S. M. Olin.

(30) No. 535, a siliceous filter-aid manufactured by Johns-Manville Corp., New York, N. Y.

(diam.) column of a mixture (170 g.) of Magnesol-Celite[31] (5:1 by wt.). The chromatogram was developed with 2 liters of benzene–ethanol (100:1 by vol.). The column was extruded and a broad zone 28–60 mm. from the top was detected by the alkaline permanganate streak reagent.[31] The sectioned zone was eluted with acetone; yield 2.5 g., m. p. 160–170°. Pure material was obtained on recrystallization from ethanol-water; m. p. 179–180°, $[\alpha]^{22}D$ −42° (*c* 2, chloroform).

Anal. Calcd. for $C_{16}H_{25}O_8NS$: C, 49.09; H, 6.44; N, 3.58; S, 8.19. Found: C, 49.21; H, 6.59; N, 3.46; S, 8.10.

Ethyl 2-Acetamido-6-nitro-2,6-didesoxy-α-D-glucothiofuranoside and Ethyl 2-Acetamido-6-nitro-2,6-didesoxy-β-L-idothiofuranoside (VIII and IX).—Ethyl 2-acetamido-2-desoxytriacetyl-α-D-glucothiofuranoside (2.51 g.) was dissolved in absolute methanol (100 ml.) and cooled to −10°. Anhydrous ammonia was passed into the solution for thirty minutes at a rate which maintained the temperature between −5 and 0°. The deacetylation mixture was then allowed to stand at room temperature for ninety minutes. The solvent was removed under reduced pressure and resulting crude sirup was dissolved in methanol (3 ml.) and chloroform (ethanol-free, 50 ml.) and this solution was employed for the succeeding step. In other experiments the ethyl 2-acetamido-2-desoxy-D-glucothiofuranoside was obtained crystalline as described above. The substance crystallizes with such difficulty, however, that a better over-all yield is obtained if the crude product is used. Lead tetraacetate (4.43 g.) in chloroform (ethanol-free, 100 ml.) was added to the above solution and the reaction mixture was warmed to 50–55°. After fifteen minutes the slight remaining color was removed by the addition of ethylene glycol (one drop). The chloroform solution was cooled in an ice-bath and the lead diacetate was removed by filtration. The chloroform solution was extracted with six 2-ml. portions of cold water to remove lead diacetate and the combined water extracts were extracted with chloroform. The combined chloroform solutions were dried and concentrated under reduced pressure. The resultant sirup was dissolved in 20 ml. of 95% ethanol and 10 ml. of nitromethane was added. The cooled (0°) solution was made just basic to litmus by the dropwise addition of 2 *N* sodium methoxide in methanol (*ca.* 5 ml.) and the reaction mixture was maintained at ice-box temperature for eighteen hours. The solution was then concentrated under reduced pressure to a thin sirup and chloroform (150 ml.) was added. The solution was concentrated under reduced pressure to a volume of 100 ml. and extracted with cold water. A yellow crystalline residue (0.45 g.) was obtained on solvent removal from the dried chloroform solution. An amount of 100 mg. of the solid residue was dissolved in 5 ml. of warm chloroform (containing 2% ethanol) and added at the top of a 135 × 35 mm. (diam.) column of Magnesol-Celite[31] (5:1 by wt.).

The Magnesol had been previously washed with hydrochloric acid in the following manner. Magnesol (360 g.) was stirred with 1000 ml. of 12 *N* hydrochloric acid until the heat of reaction ceased (*ca.* ninety minutes). The suspension was washed with water in a large Büchner funnel until free of chloride, about 6 liters of water being required. The water in the solid was then displaced with acetone and the solid was dried for forty-eight hours at 110°. The dried product was passed through an 80-mesh (80 meshes per linear inch) screen.

The chromatogram was developed with 650 ml. of chloroform (containing 2% ethanol). Application of the alkaline permanganate streak indicator[31] to the extruded column located two zones at 35 and 50 mm. from the column top which on sectioning and elution with acetone yielded 36 mg. (16%, basis of ethyl 2-acetamido-2-desoxytriacetyl-α-D-glucothiofuranoside) and 42 mg. (18%), respectively.

The material from the upper zone crystallized from chloroform–benzene–*n*-butyl ether (2:1:2 by vol.); m. p. 114–115°, $[\alpha]^{18}D$ + 160° (*c* 2, methanol). It was soluble in cold methanol, ethanol and chloroform.

Anal. Calcd. for $C_{10}H_{18}O_6N_2S$: C, 40.80; H, 6.16; N, 9.52. Found: C, 40.48; H, 6.26; N, 9.23.

The material from the lower zone crystallized from methanol–chloroform–*n*-butyl ether (1:4:5 by vol.) or absolute ethanol; m. p. 190–193° (dec.), $[\alpha]^{26}D$ + 171° (*c* 2, methanol). It was soluble in cold methanol and, on heating, in water, chloroform and ethanol.

Anal. Calcd. for $C_{10}H_{18}O_6N_2S$: C, 40.80; H, 6.16; N, 9.52. Found: C, 40.59; H, 5.99; N, 9.48.

Hexaacetylstreptamine (XIV) and an Apparently Isomeric Substance (XVI ?).—An amount of 165 mg. of the above-described high melting isomer (m. p. 190–193° dec.) of the ethyl 2-acetamido-6-nitro-2,6-didesoxyhexothiofuranoside was dissolved in 40 ml. of warm (50–60°) water and to the warm solution was added, in one portion, a solution of 150 mg. of mercuric chloride in 10 ml. of water. The reaction mixture was allowed to stand at room temperature for two hours whereupon the separated $Hg(SC_2H_5)Cl$ was removed by filtration. Silver acetate (200 mg.) was added to the filtrate and the reaction mixture was maintained at room temperature overnight. The silver chloride and excess silver acetate were then removed by filtration and hydrogen sulfide was passed into the filtrate. The filtered solution was concentrated at room temperature to a clear sirup.

To 80 mg. of the above sirup, dissolved in 1 ml. of water, was added 1.60 ml. of 0.194 *N* barium hydroxide and the solution was maintained at room temperature for twenty-four hours. Absolute ethanol (10 ml.) was then added to the straw-colored solution and a brown flocculent precipitate was removed by filtration. An amorphous barium salt was obtained on solvent removal at room temperature. An amount of 50 mg. of the barium salt was dissolved in 4 ml. of 0.1 *N* hydrochloric acid and hydrogenated for four hours at room temperature and at one atmosphere pressure, in the presence of Raney nickel (200 mg.). The catalyst was removed by centrifugation and washed with ethanol. The centrifugate and washings were concentrated to a sirup at room temperature and this was acetylated by heating under gentle reflux for one hour with 3 ml. of acetic anhydride–pyridine (2:1 by vol.). The reaction mixture was evaporated to dryness with a stream of dry air and the residue was extracted with warm chloroform. The chloroform-soluble material will be designated Fraction B and the chloroform-insoluble residue will be designated Fraction A.

Fraction A was treated with warm absolute ethanol, filtered and placed in the ice-box. Clusters of needles separated; yield 4 mg., transition point 245–250°, m. p. 345–348° (dec., sealed capillary). This behavior on heating was identical with that of an authentic sample of hexaacetylstreptamine. Within the error of measurement, the powder X-ray diffraction diagram (Table I) was identical with that of an authentic specimen of hexaacetylstreptamine. We did not obtain any chloroform-soluble form of hexaacetylstreptamine that was crystalline, such as was described by Folkers and co-workers.[8] Amorphous preparations were chloroform-soluble.

Fraction B was obtained in the form of elongated prisms on concentration of its chloroform solution with a stream of dry air; yield 5 mg., m. p. 350–355° (dec.). Table II shows the powder X-ray diffraction lines exhibited by this substance. It is different from hexaacetylstreptamine. Insufficient material was obtained for analytical characterization but the product is presumably a stereoisomer of hexaacetylstreptamine.

Streptamine Sulfate (XVII) from Hexaacetylstreptamine (XIV).[32]—Hexaacetylstreptamine (1.14 g.) was dissolved, with heating, in 20.0 ml. of *N* sodium hydroxide. The solution was refluxed for two hours, then cooled and acidified with *N* sulfuric acid. Streptamine sulfate separated during sixteen hours at 0 to 5°; yield 0.65 g. (88%).

(31) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(32) Experimental work by W. J. Polglase.

The X-ray diffraction pattern of this material (Table II) was identical with that of streptamine sulfate prepared by the alkaline degradation of streptidine according to the procedure of Folkers and co-workers.[8]

Streptidine Sulfate Monohydrate (XVIII) from Streptamine Sulfate (XVII).[32]—Streptamine sulfate (1.61 g., 5.8 millimoles) was suspended in 25.0 ml. of 0.46 *N* barium hydroxide (5.8 millimoles). The mixture was digested on the steam-bath for two minutes, filtered and washed with hot water. The filtrate and washings were concentrated under reduced pressure to about 5 ml. This solution was heated to 70–80° and maintained at this temperature during the addition of methylisothiourea sulfate. An amount of 1.02 g. of this reagent was added initially, 0.50 g. after twenty-four hours and 0.09 g. (total 1.61 g., 5.8 millimoles) after thirty-six hours. Heating was maintained for an additional twelve hours. The solution was then cooled to 0° and acetone added to incipient turbidity. The separated crystalline product was triturated with three 2-ml. portions of *N* ammonium hydroxide and the crystalline residue of streptidine sulfate monohydrate was washed with water and acetone and dried under reduced pressure (*ca.* 0.5 mm.) at 100°; yield 24 mg. The material was identified as streptidine sulfate monohydrate by its powder X-ray diffraction pattern (identical with the published[12] data), nitrogen analysis (calcd., 22.2; found, 22.1) and octaacetyl derivative. The latter was prepared from 5 mg. of the product by refluxing for one hour with sodium acetate (5 mg.) and acetic anhydride (1 ml.). The acetic anhydride was evaporated in a stream of dry air, the residue was triturated with water and the insoluble portion was crystallized from ethanol-water; m. p. 259–261°, unchanged on admixture with an authentic specimen of octaacetylstreptidine of like melting point. Folkers and co-workers[7] record the value 260–262° (micro-block) for this substance.

Summary

Reaction of N-acetyl-D-glucosamine diethyl thioacetal (II) with mercuric chloride in the presence of mercuric oxide gave ethyl 2-acetamido-2-desoxy-α-D-glucothiofuranoside (III). The furanoside structure of III was established by the fact that on periodate oxidation it yielded one mole (per mole of III) of formaldehyde with the consumption of one mole of oxidant. Oxidation of III with lead tetraacetate yielded a dialdehyde derivative (VII, not characterized) which on alkaline condensation with nitromethane gave ethyl 2-acetamido-6-nitro-2,6-didesoxy-α-D-glucothiofuranoside and ethyl 2-acetamido-6-nitro-2,6-didesoxy-β-L-idothiofuranoside (VIII and IX). The configuration of C_5 in each of the two isomers (m. p. 114–115° and 190–193° with dec.) was not determined. The thioethoxy group was hydrolyzed, in the presence of mercuric chloride, from the higher melting isomer and the resultant nitro sugar (X, not characterized) was cyclized by alkali and the product (XI and XII) was hydrogenated in acid solution with Raney nickel catalyst. The resultant mixture of diamino compounds (XIII and XV) was separated through the hexaacetyl derivatives. One of these (XIV) was identical with hexaacetylstreptamine from streptomycin.

Deacetylation of hexaacetylstreptamine (XIV) yielded streptamine, isolated as the sulfate (XVII), which produced streptidine, isolated as the sulfate monohydrate (XVIII), on reaction with methylisothiourea.

This synthesis of streptidine, a *meso* compound, from D-glucosamine rigorously establishes as all-*trans* the configuration of five of the asymmetric centers in streptidine and streptamine. From the known nature of the cyclization reactions, it is highly probable that the remaining center (C_1, XVII) is *trans* with respect to the adjacent nitrogen atoms. On this basis, streptidine and streptamine appear to possess the all-*trans* configurations.

It is probable that the other isolated product of the cyclization reaction has the structure and configuration shown in XVI.

The anomeric forms of ethyl 2-acetamido-2-desoxytriacetyl-D-glucothiofuranoside (IV and VI) are described.

COLUMBUS, OHIO RECEIVED JUNE 11, 1949

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 1313, PASADENA, CALIFORNIA]

A New Series of Substrates for the Evaluation of Chymotrypsin Activity[1]

BY B. M. ISELIN, H. T. HUANG, R. V. MACALLISTER AND CARL NIEMANN

Bergmann and Fruton[2] have recommended the use of glycyl-L-phenylalaninamide as a substrate for the estimation of chymotrypsin activity and more recently Kaufman, Neurath and Schwert[3–5] have suggested the use of benzoyl- and acetyl-L-tyrosinamide for the same purpose. While the above substrates may be satisfactory for some purposes all three possess characteristics which limit their general usefulness, *i. e.*, the first is relatively unstable,[6,7] the second is too insoluble in aqueous media[8] and the third, in common with acetyl-L-phenylalaninamide (*cf.* Table I), is hydrolyzed at too slow a rate. In contrast it has

(1) Supported in part by a grant from Eli Lilly and Co. Request for information relative to this article should be addressed to Dr. C. Niemann.

(2) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **145**, 253 (1942).

(3) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

(4) S. Kaufman and H. Neurath, *Arch. Biochem.*, **21**, 245 (1949).

(5) *Idem.*, *J. Biol. Chem.*, **180**, 181 (1949).

(6) H. T. Huang and Carl Niemann, THIS JOURNAL, in press.

(7) It is probable that the "spontaneous hydrolysis" of the analogous glycyl-L-tyrosinamide acetate noted by Kaufman, *et al.*,[3] is actually spontaneous conversion into the corresponding diketopiperazine.

(8) The practice of using aqueous methanol systems[3] does not appear to be as attractive as the use of water soluble substrates principally because of the introduction of an additional variable in the former case.