sodium methoxide, m.p. 84–86°, yield 25%. The infrared spectrum of VIIIc was essentially similar to those of VIIIa and VIIIb.

Base-catalyzed Reaction of Propargyl Alcohol with Phenyl Isothiocyanate.—During a period of 1.3 hr., a solution of 13.5 g. (0.1 mole) of phenyl isothiocyanate in 15 ml. of ether was added to a solution of 5.6 g. (0.1 mole) of propargyl alcohol and 0.1 g. of sodium methoxide in 50 ml. of anhydrous ether. Periodic cooling was needed to maintain the reaction temperature at 25–30° during addition. The mixture was stirred at room temperature for 2 days and then was added to 200 g. of water. The organic layer was dried over anhydrous sodium sulfate. An oil (15.5 g.) remained when the ether was removed. The products IX and X were separated by fractional crystallization from an ether–petroleum ether (30–60°) mixture. First there was obtained 3.0 g.(16%) of X which was purified by recrystallization from diethyl ether, m.p. 109–110°.

Anal. Calcd. for $C_{10}H_9OSN$: C, 62.9; H, 4.71; N, 7.34; S, 16.75; mol. wt. 191. Found: C, 62.8; H, 4.76; N, 7.47; S, 16.66; mol. wt. (ebullioscopic in acetone), 195.

Infrared absorptions: 1660 (>C=CH_2), 1600 and 1500 (aromatic C=C), 1455 (CH_2), 1400, 1340, 1165, 840 (>C=CH_2) 692 cm.$^{-1}$ (monosubstituted benzene).

IX was then isolated and purified by recrystallization from an ether–petroleum ether (30–60°) mixture, m.p. 45–47°.

Anal. Calcd. for $C_{10}H_9OSN$: C, 62.9; H, 4.71; N, 7.34; S, 16.75; mol. wt., 191. Found: C, 62.4; H, 4.89; N, 74.7; S, 16.41; mol. wt. (ebullioscopic in acetone), 200.

Infrared absorptions: 1675 (C=N), 1630 (>C=CH_2) 1600, and 1500 (aromatic C=C), 1455 (CH_2), 1400, 1340, 1115, 1040, 875 (>C=CH_2), 695 cm.$^{-1}$ (monosubstituted benzene).

A 0.20-g. sample of IX and X was titrated potentiometrically (crystal violet indicator also used) with 0.1 *N* perchloric acid in glacial acetic acid.[14] Compound IX required 8.1 ml. (Found, 4.1 meq./g.; Calcd., 5.2 meg./g.); compound X took less than 0.5 ml.

Acknowledgment.—The authors wish to thank Mr. Sandy H. Venable for supplying the infrared spectra; Mr. Clyde W. Nash, under whose direction the elemental analyses were performed; Dr. Keith S. McCallum and Mr. Francis L. Jackson, for producing the n.m.r. spectra and helping with their interpretation; and Mr. Harry Mason for the molecular weight determinations. We are particularly grateful to Dr. Newman M. Bortnick for many helpful suggestions during the course of this work.

(14) S. Siggia and H. J. Stolten, "An Introduction to Modern Organic Analysis," Interscience Publishers, Inc., New York, N. Y., 1956, p. 37.

Nystatin. III. Mycosamine: Preparation and Determination of Structure

JAMES D. DUTCHER, DAVID R. WALTERS, AND OSKAR WINTERSTEINER

The Squibb Institute for Medical Research, New Brunswick, New Jersey

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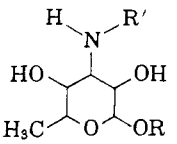
The preparation of mycosamine, the amino sugar component of nystatin and several other polyenic antifungal antibiotics, and of various derivatives is described. Periodate degradation has shown that mycosamine is a 3,6-dideoxy-3-amino-hexose belonging to the D-series.

In a preliminary communication[1] we have briefly presented the evidence which has let us assign structure I to mycosamine, the amino sugar obtained by hydrolysis of the antifungal antibiotics, nystatin and amphotericin B.[2] The present paper describes in detail the



I. R = R′ = H
II. R = H, R′ = $COCH_3$
III. R = H, R′ = $COOCH_2C_6H_5$
IV. R = CH_3, R′ = $COCH_3$
VII. R = CH_3, R′ = H + HCl
VIII. R = CH_3, R′ = C_2H_5

procedures used for the hydrolysis of nystatin, the isolation of the amino sugar, the preparation of its derivatives, and the degradation reactions by which its structure was established. The elucidation of its stereochemistry, as that of 3,6-dideoxy-3-amino-D-mannopyranose, by degradative means and its synthesis have been reported separately.[3,4]

Hydrolytic cleavage of amino sugar glycosides usually requires more vigorous conditions than that of ordinary glycosides because of the presence in the former of the positively charged amino group which shields the glycosidic linkage from the approach of the proton.[5] Conversion of the amino group to the neutral amido group by acylation restores normal susceptibility to hydrolysis to the glycosidic linkage.[6] Thus the cleavage of nystatin into mycosamine and aglycone was initially achieved by acetolysis (acetic acid, acetic anhydride, sulfuric acid), and the amino sugar moiety was isolated as the polyacetate. Subsequently, however, it was found that mycosamine could be cleaved from the nystatin molecule by vigorous aqueous acid hydrolysis or by methanolysis. It was possible in this manner to obtain mycosamine in the form of its hydrochloride or as the α-methyl glycoside hydrochloride. All these measures resulted in extensive degradation of the aglycone portion.

The analyses of several crystalline derivatives of mycosamine established the composition of the base as $C_6H_{13}NO_4$. No methoxy or N-methyl groups were present. One C-methyl group was demonstrable by the Kuhn–Roth determination, as well as by a positive iodoform reaction. Since in nystatin, as well as in mycosamine itself, the nitrogen atom is basic and reacts with ninhydrin, it must be present as a primary amino group. The observation that mycosamine is weakly but definitely reducing towards Tollen's and Fehling's reagents and gives a positive Morgan–Elson reaction indicated that it probably was a 6-deoxyaminoaldohexose. The absence of any absorption bands in the carbonyl region of the infrared spectrum suggested that a pyranose or furanose ring was present.

(1) D. R. Walters, J. D. Dutcher, and O. Wintersteiner, *J. Am. Chem. Soc.*, **79**, 5076 (1957).

(2) J. D. Dutcher, M. B. Young, J. H. Sherman, W. E. Hibbits, and D. R. Walters, "Antibiotics Annual, 1956–1957," Medical Encyclopedia, Inc., New York, N. Y., 1957, p. 866.

(3) M. H. von Saltza, J. Reid, J. D. Dutcher, and O. Wintersteiner, *J. Am. Chem. Soc.*, **83**, 2785 (1961).

(4) M. H. von Saltza, J. D. Dutcher, J. Reid, and O. Wintersteiner, *J. Org. Chem.*, **28**, 999 (1963).

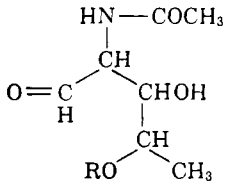
(5) P. W. Kent and M. W. Whitehouse, "Biochemistry of the Amino Sugars," Academic Press, Inc., New York, N. Y., 1955, p. 235.

(6) K. L. Rinehart, Jr., P. W. K. Woo, A. D. Argoudelis, and A. M. Giesbrecht, *J. Am. Chem. Soc.*, **79**, 4567 (1957); H. E. Carter, J. R. Dyer, P. D. Shaw, K. L. Rinehart, Jr., and M. Hichens, *ibid.*, **83**, 3723 (1956); and A. B. Foster, J. Lehmann, and M. Stacey, *J. Chem. Soc.*, 1398 (1962).

From the acetolysis reaction there was obtained, by chloroform extraction and chromatography over alumina, crystalline tetraacetylmycosamine. Mild hydrolysis of this compound yielded the crystalline triacetyl derivative. Hydrolysis of either the tri- or tetracetate with barium or sodium methoxide afforded crystalline N-acetylmycosamine (II). To secure free mycosamine, nystatin was hydrolyzed with aqueous methanolic sulfuric acid and the reaction mixture freed from tarry material and pigmented decomposition products of the aglycone by extraction with 1-butanol. Since direct recovery of the crystalline amino sugar from the aqueous phase proved difficult, the mycosamine was converted to the N-carbobenzyloxy derivative (III) which readily crystallized. Hydrogenolytic removal of the carbobenzyloxy group was smoothly accomplished with palladium black in the presence of hydrochloric acid and crystalline mycosamine hydrochloride thus obtained.

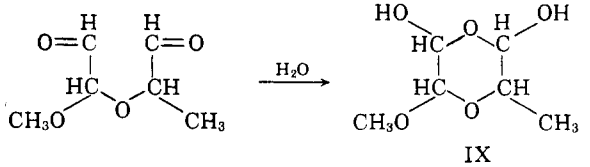
The crystalline α-methyl glycosides of mycosamine, N-acetyl- and N-carbobenzyloxymycosamine could be prepared by treatment with anhydrous methanolic hydrogen chloride. When the latter reagent was used on nystatin directly, crystalline α-methylmycosaminide hydrochloride (VII) could be isolated after removal of the pigmented decomposition products.

The periodate oxidation studies by which the position of the amino group was determined were carried out on N-acetylmycosamine (II) and its methyl glycoside (IV). IV consumed no periodate. II consumed one mole of periodate and yielded an amorphous product (V) which gave a positive Morgan–Elson test (for 2-acetamidoaldoses), reduced Fehling's solution and consumed one mole of base to yield formic acid and a crystalline product, $C_7H_{13}NO_4$, which was obviously a 2-acetamido-2,5-dideoxypentose (VI). Only structure I



V. R = HC=O
VI. R = H

would account for this result. Confirmatory evidence for I was adduced by the periodate oxidation (uptake 2 moles) of methyl N-ethylmycosaminide (VIII) (prepared by lithium aluminum hydride reduction of IV) and of α-methylmycosaminide (VII) to yield the known D′-methoxy-D-methyldiglycolic aldehyde (IX).[7]



IX

These results permitted the unequivocal assignment of structure I to mycosamine and, in addition, proved that C-5 had the D configuration. Subsequent studies[3,4] have shown that mycosamine is 3,6-dideoxy-3-amino-D-mannose.

(7) W. D. Maclay, R. M. Mann, and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1660 (1939).

Experimental[8]

Acetolysis of Nystatin. 1,2,4,N-Tetraacetylmycosamine and 2,-4,N-triaceTylmycosamine.—To a mixture of 70 ml. of acetic anhydride, 30 ml. of glacial acetic acid, and 2 ml. of concd. sulfuric acid, chilled in an ice bath, there was slowly added with stirring 5.0 g. of nystatin. As the temperature rose to about 20°, the solution became very dark brown and after a time dark tarry material separated. The reaction mixture was allowed to stand at room temperature for about 70 hr. and was then worked up by pouring it on cracked ice, neutralizing with sodium carbonate to pH 5.0, filtering and extracting the filtrate with several portions of chloroform. The combined chloroform extracts were washed with water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The dark yellow sirup (1.6 g.) was dissolved in benzene and chromatographed over a column containing acid-washed alumina (15 g., Merck). Some sirupy, pigmented material (0.35 g.) ran through the column with the benzene and the subsequent benzene washings. The column was then washed with benzene containing increasing amounts of chloroform. The material eluted chiefly by benzene-chloroform (1:1) crystallized as long needles when the dry residue was moistened with benzene (0.5 g.). It was recrystallized from warm benzene and dried *in vacuo* at 78°; yield, 0.5 g., m.p. 159–161°; $[\alpha]^{23}D$ +39.3° (*c*, 1.0, ethanol). The analyses and the absence of a hydroxyl band in the infrared absorption spectrum showed that this product was 1,2,4,-N-tetraacetylmycosamine.

Anal. Calcd. for $C_6H_9NO_4\cdot(CH_3CO)_4$: C, 50.75; H, 6.39; N, 4.23; CH_3CO, 52.0. Found: C, 50.40; H, 6.44; N, 4.26; CH_3CO, 50.9.

The eluting solvent was next changed to chloroform and then to chloroform containing increasing amounts of acetone. The fractions eluted with chloroform or chloroform containing the first increments of acetone were solids, and when moistened with ethanol readily crystallized (0.7 g.). Recrystallization of this material from warm ethanol or acetone yielded large, colorless prisms which were freed from solvent *in vacuo* at room temperature, yield 0.63 g.; m.p. 185–187° after softening at 180°, $[\alpha]^{22}D$ +85° (*c*, 1.0, ethanol). Although this product gave only extremely faint Fehling's and Tollen's reaction, the analytical data showed that it was 2,4,N-triacetylmycosamine.

Anol. Calcd. for $C_6H_{10}NO_4\cdot(CH_3CO)_3$: C, 49.8; H, 6.62; N, 4.81; CH_3CO, 44.7. Found: C, 50.1; H, 6.56; N, 4.80; CH_3CO, 42.8.

The yield of the tri- and tetraacetates taken together was approximately 60%. Rechromatography of pure tetraacetate over alumina always showed a definite amount of hydrolysis to the triacetate and warming a solution of the tetraacetate in 0.1 *N* aqueous hydrochloric acid to 50° for 30 min. also hydrolyzed it to the triacetate. Treatment of the triacetate with pyridine–acetic anhydride or with the acetolysis solvent mixture of acetic acid, acetic anhydride, sulfuric acid gave an amorphous tetraacetyl product which could not be crystallized probably because of its being an anomeric mixture.

N-Acetylmycosamine (II).—Either tetraacetyl- or triacetylmycosamine could be saponified with barium methylate in methanol solution to yield crystalline N-acetylmycosamine. To a solution of 0.5 g. of triacetylmycosamine (1.73 mmoles) in 25 ml. of absolute methanol there was added 0.25 ml. of a 1.5 *N* barium methylate solution in methanol. The mixture was allowed to stand at 4° for 18 hr. After addition of a slight excess of sulfuric acid and removal of the barium sulfate by centrifugation the solution was slightly alkalinized with aqueous ammonia and evaporated *in vacuo* to constant weight. The partially crystalline residue, recrystallized from warm dioxane–methanol, afforded fine needles (342 mg., 96%), m.p. 190–192°. Recrystallization from methanol–acetone raised the m.p. to 195–196°, $[\alpha]^{22}D$ −46° (*c*, 1.0, ethanol).

Anal. Calcd. for $C_6H_{12}NO_4\cdot CH_3CO$: C, 46.82; H, 7.37; N, 6.83; CH_3CO, 21.0. Found: C, 46.58; H, 7.22; N, 7.07; CH_3CO, 21.2.

Methyl N-Acetylmycosaminide (IV).—The methyl glycoside of N-acetylmycosamine was prepared either from the tetraacetylmycosamine by the method of Moggridge and Neuberger[9] (refluxing 6 hr. in 2.7% methanolic hydrogen chloride) or from N-

(8) Melting points were determined in a capillary and are uncorrected. The microanalyses were performed by Mr. J. F. Alicino, Analytical Chemistry Division, The Squibb Institute for Medical Research, New Brunswick, N. J.

(9) R. C. G. Moggridge and A. Neuberger, *J. Chem. Soc.*, 745 (1938).

acetylmycosamine by treatment with 0.2 *N* methanolic hydrogen chloride. In some cases, chromatography over alumina, which probably effected the separation of the anomers, was necessary in order to obtain a crystalline product. A solution of 1.3 g. of N-acetylmycosamine in 70 ml. of 0.2 *N* hydrogen chloride in absolute methanol was refluxed for 2 hr., at which time the optical rotation became constant. The solution was neutralized by passing it over a column of Amberlite MB-3,[10] and the combined eluate and washes were evaporated to dryness *in vacuo* (790 mg.). The pale yellow sirupy residue dissolved in a mixture of 2 ml. of acetone and 10 ml. of chloroform, was adsorbed on a column of acid washed alumina (20 g.), and then eluted with chloroform containing increasing amounts of acetone. The bulk of the material was eluted with the solvent mixture containing 17% acetone. The residue crystallized when moistened with a small volume of acetone (650 mg.). After recrystallization from acetone by the addition of chloroform, dense prisms, m.p. 168–170° after softening at 155°, $[\alpha]^{23}D$ +47° (*c*, 0.9, ethanol) were obtained; yield 617 mg., 44% of theory.

Anal. Calcd. for $C_9H_{17}NO_5$: C, 49.30; H, 7.82; N, 6.39; OCH_3, 14.31. Found: C, 49.00; H, 7.56; N, 6.13; OCH_3, 13.5.

That this product was the α-anomer was later confirmed by its unambiguous synthesis.[3,4]

Another crystalline product (needles) could on occasion be isolated from fractions of the chromatogram. This product, which is probably the β-anomer, was not characterized further.

α-Methyl 2,4,N-Triacetylmycosaminide.—A solution of 100 mg. of IV in 2.0 ml. of dry pyridine was treated with 0.5 ml. of acetic anhydride and allowed to stand at 24° for 48 hr. The solvents were removed *in vacuo* and the residue was recrystallized from acetone or a mixture of acetone and chloroform (1:1); yield 105 mg.; m.p. 140–141°; $[\alpha]^{23}D$ +33° (*c*, 1.0, ethanol).

Anal. Calcd. for $C_{13}H_{21}O_7N$: C, 51.48; H, 6.98; N, 4.62; OCH_3, 10.23. Found: C, 51.32; H, 6.86; N, 4.46; OCH_3, 10.44.

α-Methyl Mycosaminide Hydrochloride (VII).—Since attempts to secure free mycosamine or its hydrochloride in crystalline form by vigorous acid hydrolysis of the acetylated derivatives remained fruitless, the methanolysis of nystatin was investigated in the hope that means could be found for separating the small amount of methyl mycosaminide present from the bulk of complex degradation products of the aglycone moiety. In this effort the use of paper chromatography for the detection of the ninhydrin positive mycosamine moiety was most helpful. Ultimately, it was found that refluxing of a solution of nystatin in 3 *N* hydrogen chloride in absolute methanol for 2 hr., or allowing it to stand at 22° for 24 hr., cleaved the glycosidic linkage with the formation of methyl mycosaminide. After removal of the bulk of solvent by concentration *in vacuo*, cold water was added and the aqueous solution, after decanting from tarry solids, was extracted repeatedly with 1-butanol which removed most of the pigmented decomposition products. The aqueous phase was then neutralized to approximately pH 5.0 with Amberlite IRA 400 (OH) resin,[10] freed from butanol by concentration to a small volume *in vacuo*, and lyophilized. The pale yellow solid was then dissolved in a minimum volume of ethanol and induced to crystallize by the gradual addition of ether with chilling and scratching. Even after several recrystallizations, paper chromatography in the system 1-propanol–water (7:3) always showed the presence of a trace of the β-anomer. Once seed crystals of α-methyl mycosaminide hydrochloride were available it was possible to isolate this compound, by the above procedure, in amounts sufficient for characterization from as small an amount of nystatin as 100 mg. In a representative experiment in which 1.0 g. of nystatin was subjected to methanolysis the nystatin was suspended in 10 ml. of absolute methanol at room temperature, and 10 ml. of methanolic hydrogen chloride (6 *N*) at 4° was added. The nystatin readily dissolved, and the solution, which quickly darkened, was slowly brought to reflux temperature at which point some dark, tarry solids precipitated. After refluxing for 2 hr. the solution was allowed to stand at 24° for 18 more hours. The papergram showed the presence of the characteristic ninhydrin positive spot for methyl mycosaminide and a weak spot for free mycosamine. Removal of the methanol *in vacuo*, followed by the addition of 50 ml. of water, and repeated extraction with 1-butanol, yielded a pale yellow aqueous solution which was concentrated to dryness *in vacuo* at the lowest possible temperature. The sirupy residue when moistened with ethanol set into a mass of crystals. Recrystallization from ethanol by the gradual addition of ether yielded clusters of colorless needles, m.p. 188–190° dec., $[\alpha]^{23}D$ +54° (*c*, 0.5, methanol); yield, 190 mg., 80% of theory.

Anal. Calcd. for $C_7H_{15}NO_4 \cdot HCl$: C, 39.35; H, 7.55; N, 6.56; Cl, 16.60; OCH_3, 14.51. Found: C, 39.28; H, 7.49; N, 6.55; Cl, 16.50; OCH_3, 14.60.

α-Methyl mycosaminide hydrochloride with identical properties was obtained by treating mycosamine hydrochloride (see later) with 0.2 *N* methanolic hydrogen chloride at 24° for 18 hr.

Aqueous Hydrolysis of α-Methyl Mycosaminide.—The conditions required for the complete cleavage of the methyl glycoside by acid hydrolysis were established in small scale experiments in which the strength of the acid and the reflux time were varied and the composition of the resulting mixture was ascertained by paper chromatography in the system 1-butanol–ethanol–water–ammonium hydroxide (4:1:4.9:0.1). It was found that after 3-hr. boiling in 0.1 *N* hydrochloric acid practically all the methyl mycosaminide had been hydrolyzed to mycosamine.

N-Carbobenzyloxymycosamine (III).—When nystatin was hydrolyzed with aqueous acid and the reaction mixture worked up essentially as described for the methanolysis reaction, the residue from the aqueous phase could not be induced to crystallize although the papergram showed it to consist essentially of mycosamine hydrochloride. To secure the latter in pure form it was found necessary to convert the crude amino sugar to the N-carbobenzyloxy derivative and hydrogenolyze the latter in the presence of palladium catalyst as described in this and the following section.

For the aqueous hydrolysis of nystatin sulfuric acid was used since it gave less tarry and pigmented products from the aglycone portion than did hydrochloric acid. Because of the insolubility of nystatin in the aqueous acid a mixture of the latter with methanol was used. A suspension of 5.0 g. (5.38 mmoles) of nystatin in 35 ml. of methanol was treated with 50 ml. of 2.5 *N* aqueous sulfuric acid. The solid readily dissolved on mixing. The solution was placed into a round bottom flask fitted with a short air condenser which was connected in turn with a take-off condenser, so that the methanol could be slowly distilled while the reaction mixture was boiled under reflux. The solution quickly darkened, and as the methanol was slowly distilled some tarry solids separated. The rate of heating was such as to effect the removal of nearly all the methanol in 2 hr. The residual aqueous solution, after cooling, was decanted from the tarry solid and extracted five times with 20-ml. portions of wet 1-butanol. The aqueous phase was freed of butanol by concentration *in vacuo* while the volume was maintained at approximately 50 ml. by the addition of water, and then made alkaline with solid sodium carbonate. Carbobenzyloxy chloride (1.0 ml.) was added dropwise with stirring, together with additional increments of sodium carbonate over an hour, at which time the reaction mixture no longer gave a positive ninhydrin reaction. Stirring was continued at room temperature overnight, whereupon the mixture was neutralized and then extracted with ethyl acetate in a continuous liquid–liquid extraction for 8 hr. The ethyl acetate extract was washed with small portions of water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The nearly colorless residue was dissolved in the minimum amount of warm ethanol and chilled and scratched. Colorless needles in clusters soon deposited. Additional material was obtained by concentration of the mother liquor. Recrystallization could be best accomplished with glacial acetic acid; yield 1.2 g. (75%), m.p. 190–193°, $[\alpha]^{23}D$ −15.5° (*c*, 3.0, methanol).

Anal. Calcd. for $C_{14}H_{19}NO_6$: C, 56.56; H, 6.44; N, 4.71. Found: C, 56.68; H, 6.20; N, 4.70.

α-Methyl N-Carbobenzyloxymycosaminide.—Treatment of III (1.0 g.) with 100 ml. of 0.2 *N* hydrogen chloride in absolute methanol at 24° for 48 hr. yielded, after careful removal of the excess acid with Amberlite IRA-400 (OH^-)[10] and evaporation of the solvent, 0.927 g. of crystalline methyl N-carbobenzyloxymycosaminide. On recrystallization from aqueous ethanol it formed needles, m.p. 120–121°, $[\alpha]^{23}D$ +33.7° (*c*, 2.9, in methanol).

Anal. Calcd. for $C_{15}H_{21}NO_6$: C, 57.87; H, 6.80; N, 4.50; OCH_3, 9.99. Found: C, 58.06; H, 6.91; N, 4.40; OCH_3, 10.04.

Mycosamine Hydrochloride (I).—A suspension of 500 mg. of III in a mixture of 20 ml. of 0.1 *N* aqueous hydrochloric acid and 5 ml. of methanol was shaken with 50 mg. of palladium black in an atmosphere of hydrogen at 24° and atmospheric pressure.

(10) Amberlite is the trade name of the Rohm & Haas Co., Philadelphia, Pa., for ion-exchange resins.

In about 30 min. a slow uptake of hydrogen began and as the uptake continued the suspension went into solution. When all the solids had dissolved (*ca.* 8 hr.) and the uptake of hydrogen had ceased, the solution was filtered from the catalyst and evaporated to dryness *in vacuo*. The remaining hydrochloric acid was removed by repeated addition of water and evaporation. The colorless, sirupy residue slowly crystallized when moistened with a little ethanol and scratched. A first crop was obtained by suspending the crystals in a small volume of ethanol–ether (1:1) solvent and separating in the centrifuge; yield 285 mg., 85%. Additional small amounts were obtained by allowing the supernatant solvents to evaporate again and repeating the seeding and scratching. Recrystallization could be best accomplished by dissolving the crude product in the minimum volume of warm ethanol and adding ether gradually until the solution became slightly turbid, and after crystallization began, repeating the addition of ether from time to time. The thin, prismatic rods obtained in this manner melted at 162°; $[\alpha]^{24}D$ −11.5° (*c* 1.0, water).

Anal. Calcd. for $C_8H_{13}NO_4 \cdot HCl$: C, 36.10; H, 7.07; N, 7.02; Cl, 17.76. Found: C, 36.08; H, 7.10; N, 7.0; Cl, 17.60.

Hydrogenolysis of Methyl N-Carbobenzyloxymycosaminide.—To a solution of 100 mg. of methyl N-carbobenzyloxymycosaminide in 5.0 ml. of 80% methanol there was added 1 ml. of *N* aqueous hydrochloric acid and 40 mg. of palladium black catalyst. The mixture was stirred in an atmosphere of hydrogen until no further uptake of hydrogen occurred. After filtering off and washing the catalyst with aqueous methanol, the combined filtrates were evaporated to dryness *in vacuo*. The pale sirupy residue spontaneously crystallized. Moistening with a small volume of ethanol permitted the collection of a crop of colorless material. Recrystallization from ethanol or ethanol–ether yielded clusters of needles; yield 0.060 g., 87%; m.p. 189–190° with decomposition, $[\alpha]^{23}D$ +54° (*c*, 0.5, methanol), identical with α-methyl mycosaminide hydrochloride (VII) obtained by the methanolysis of nystatin.

Periodate Oxidation of N-Acetylmycosamine (II): 2,5-Dideoxy-2-acetamido-D-arabinose (VI).[3]—Quantitative measurement showed the rapid uptake of 1 mole of metaperiodate by N-acetylmycosamine (0.96 mole in 45 min.) and only slight further oxidation on longer standing. For the preparative experiment, 1.2 g. of N-acetylmycosamine (5.85 mmoles) was dissolved in 200 ml. of water and treated with 1.35 g. (5.87 mmoles) of potassium metaperiodate dissolved in 100 ml. of water. After standing at room temperature for 2 hr. no more periodate was present, and the solution was lyophilized. Extraction with several portions of dry methanol and evaporation of the latter yielded 1.212 g. of a pale yellow amorphous solid. This product readily reduced Fehling's solution and gave a positive Morgan–Elson test for 2-acetamido-aldoses. The analysis was in agreement with the composition $C_8H_{13}NO_5$.

Anal. Calcd. for C, 47.29; H, 6.45; N, 6.85. Found: C, 47.22; H, 6.84; N, 6.93.

The product was not acidic, but when a small portion was treated with excess alkali and the solution was back-titrated after some time, an amount of alkali equivalent to the mole weight 203, was consumed. The acidic product formed was identified as formic acid by steam distillation and titration of the distillate with bromine. The bulk of the oxidation product [2-acetamido-3-hydroxy-4-formoxypentanal (V)] was saponified with 0.1 *N* sodium hydroxide solution, the excess base neutralized with hydrochloric acid and the solution evaporated to dryness. Digestation of the solid residue with several portions of warm acetone and evaporation of the latter yielded a sirup which slowly crystallized. After two recrystallizations from warm acetone, 669 mg. of colorless needles of VI, m.p. 128–130°, were obtained (65% yield), $[\alpha]^{23}D$ −81° (*c*, 3.0, in ethanol).

Anal. Calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00. Found: C, 47.86; H, 7.41; N, 8.09.

Methyl N-Ethylmycosaminide (VIII).—To a boiling solution of 330 mg. of lithium aluminum hydride in 50 ml. of tetrahydrofuran there was slowly added a solution of 520 mg. of methyl N-acetylmycosaminide in 20 ml. of tetrahydrofuran. Another portion (80 mg.) of lithium aluminum hydride was added, and boiling was continued for a total of 22 hr. The excess of lithium aluminum hydride was decomposed by the addition of saturated sodium sulfate solution, and the inorganic salts were removed by centrifugation and washed with tetrahydrofuran. Evaporation of the solvent *in vacuo* left a colorless, viscous oil which was soluble in chloroform, benzene, acetone, ether or methanol, but insoluble in hexane. The moist sirup slowly crystallized and after recrystallization from chloroform–hexane, yielded 285 mg. (50% yield) of colorless needles, m.p. 90.5°, $[\alpha]^{23}D$ +25° (*c*, 3.0, in water). Some additional crystalline material could be obtained from the mother liquors. Titration with perchloric acid in glacial acetic acid solution showed that this product was a base with neutralization equivalent 211.

Anal. Calcd. for $C_9H_{19}NO_4$ (205.25): C, 52.66; H, 9.33; N, 6.83. Found: C, 52.51; H, 9.19; N, 6.84.

Periodate Oxidation of Methyl N-Ethylmycosaminide.—A solution of 101.0 mg. of methyl N-ethylmycosaminide (0.493 mmole) and 350.1 mg. of sodium metaperiodate (1.64 mmoles) in total volume of 10 ml. of water at 24° was allowed to stand at room temperature. After 2.5 hr., $[\alpha]D$ had become constant, and an aliquot taken for titration with thiosulfate showed that 1.98 moles of periodate per mole of base had been consumed. The reaction mixture was stirred with Amberlite MB-3 resin[10] to remove inorganic ions, and the deionized solution was evaporated to dryness *in vacuo*. The colorless sirupy residue which spontaneously crystallized on moistening with ether, yielded after recrystallization from ether–hexane 56 mg. (82% yield) of D′-methoxy-D-methyldiglycolic aldehyde (IX), m.p. 99–102°, $[\alpha]^{23}D$ +131° (*c*, 0.5, in water).

Anal. Calcd. for $C_6H_{10}O_4 \cdot H_2O$: C, 43.88; H, 7.37; OCH_3, 18.9. Found: C, 44.40; H, 7.21; OCH_3, 18.3.

Direct comparison of this product with a sample of D′-methoxy-D-methyldiglycolic aldehyde (IX) prepared from α-methyl-6-deoxyglucopyranoside[7] confirmed its identity (m.p., $[\alpha]D$, and infrared spectrum).

Periodate Oxidation of α-Methyl Mycosaminide.—A solution of 500 mg. (2.34 mmoles) of α-methyl mycosaminide hydrochloride (VII) in 50 ml. of water was brought to pH 5.0 with 0.1 *N* aqueous sodium hydroxide solution and then treated with a solution of 2.0 g. (9.4 mmoles) of sodium metaperiodate in 10 ml. of water. The mixture was allowed to stand at 24° for 4 hr. At that time slightly more than 2 moles of periodate per mole of glycoside had been consumed. The solution was deionized with Amberlite MB-3 resin[10] and evaporated to dryness. After two recrystallizations of the residual solids from ether-hexane mixture there was obtained 310 mg. of D′-methoxy-D-methyldiglycolic aldehyde (IX) identical in all its properties with that previously obtained.

Methyl 2,4-Di-O-acetylmycosamine Hydrochloride.—This compound was prepared in connection with an abortive attempt to convert the amino group of mycosamine to hydroxyl by deamination with nitrous acid. To a solution of 1.0 g. of methyl N-carbobenzyloxymycosamine in 10 ml. of dry pyridine 5 ml. of acetic anhydride were added, and the mixture was allowed to stand at room temperature for 48 hr. The solvents were removed *in vacuo*, by repeated evaporation after the addition of water. The sirupy residue was dissolved in 15 ml. of 80% methanol, and after the addition of 3 ml. of 1 *N* hydrochloric acid, shaken with 200 mg. of palladium black catalyst in an atmosphere of hydrogen. When the uptake of hydrogen had ceased the catalyst was filtered off, the filtrate was neutralized to pH 5.0 with Amberlite IR-4B[10] resin and concentrated to dryness *in vacuo*. The residue (950 mg.) was moistened with ethanol, and the resulting crystalline mass was recrystallized from ethanol by the gradual addition of ether. There was obtained 448 mg. of methyl 2,4-di-O-acetylmycosamine hydrochloride, m.p. 185–186° with dec. after darkening and softening at 170°, $[\alpha]^{24}D$ +15.2° (*c*, 0.5, in methanol).

Anal. Calcd. for $C_{11}H_{19}O_6N \cdot HCl$ (297.743): C, 44.37; H, 6.77; N, 4.70; Cl, 11.91; OCH_3, 10.41; 2 CH_3CO, 28.91. Found: C, 44.28; H, 6.72; N, 4.6; Cl, 12.00; OCH_3, 10.14; CH_3CO, 28.76.

Deamination Studies.—Under a variety of conditions of solvent, concentration, and temperature an effort was made to deaminate this product with nitrous acid. In no case did the examination of the products by paper chromatographic procedures indicate that a nitrogen-free sugar moiety had been formed. This approach was abandoned as a method of deducing stereochemical information in the light of the many uncertainties reported as to the course of this reaction.[11,12]

Isonicotinylhydrazones.—Although no nicely crystalline phenylhydrazones of mycosamine derivatives could be prepared, the hydrazones obtained by reaction with isonicotinylhydrazide could

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be readily crystallized and characterized. These were best prepared by treating a solution of the sugar derivative in glacial acetic acid with slightly more than the calculated amount of isonicotinylhydrazide dissolved in a minimum amount of water, and warming the mixture in a water bath for 1 hr. The hydrazones of N-carbobenzyloxymycosamine and N-benzoylmycosamine[4] separated on cooling and could be recrystallized from warm methanol.

N-Carbobenzyloxymycosamine isonicotinylhydrazone, m.p. 199–203° dec.

Anal. Calcd. for $C_{20}H_{24}N_4O_6$ (416.42): C, 57.68; H, 5.81; N, 13.45. Found: C, 57.59; H, 5.79; N, 13.28.

N-Benzoylmycosamine isonicotinylhydrazone, m.p. 212–213° dec.

Anal. Calcd. for $C_{19}H_{22}N_4O_5$ (386.40): C, 59.06; H, 5.74; N, 14.50. Found: C, 59.09; H, 5.50; N, 14.45.

Nystatin. IV. The Stereochemistry of Mycosamine

MALCOLM VON SALTZA, JAMES D. DUTCHER, JOYCE REID, AND OSKAR WINTERSTEINER

The Squibb Institute for Medical Research, New Brunswick, New Jersey

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It has been shown that mycosamine, a 3-amino-3,6-dideoxy-D-aldohexose,[1] corresponds configurationally to D-mannose. Degradation of N-benzoyl-1-deoxymycosaminol (VI), obtained in three steps from mycosamine diethyl mercaptal (II), to N-benzoyl-D-allothreonine (VIIa), established the L-configuration for C-2 and C-3. The D-configuration of C-4 followed from the finding that the 1-phenylflavazole derivative of mycosamine (XIIIa) was the enantiomorph of that of L-rhamnose (XIIIb).

In preceding papers of this series[1,2] it has been shown that mycosamine, the amino sugar occurring in nystatin and several other polyenic antibiotics, is a 3-amino-3,6-dideoxy-D-aldohexose. In the present paper we give an account of the degradative reactions which proved that mycosamine corresponds configurationally to D-mannose, and thus furnished the basis for its recently reported synthesis from D-glucose.[3]

Our scheme for determining the configuration of C-2 and C-3 entailed conversion of carbon atom 1 to a methyl group, protection of the amino group by acylation followed by scission of the chain between C-4 and C-5 with periodate and oxidation of the resulting α-acylamino-β-hydroxy-*n*-butyraldehyde to the corresponding acid, which on hydrolysis was then expected to yield one of the stereoisomers of threonine or allothreonine. This sequence was originally carried out[4] with N-acetylmycosamine as the starting material, and a very small amount of crystalline amino acid, which appeared to be D-allothreonine, was obtained as the end product. In view of the minute yield, and also of the fact that intermediates were amorphous and of doubtful purity, it was decided to repeat this sequence in the N-benzoyl series. This change also promised to do away with the final hydrolysis step in the above scheme, as the optically active N-benzoylthreonines and N-benzoylallothreonines, in contrast to the N-acetyl derivatives, are crystalline, well characterized compounds.

The degradative scheme in its original form envisaged the preparation of a suitable mercaptal of N-benzoylmycosamine which was then to be desulfurized with Raney nickel to the N-benzoyl-1-deoxo compound. However, no pure mercaptals could be isolated from the mixtures resulting from the treatment of N-benzoylmycosamine with ethyl or isopropyl mercaptan, or ethylene dithiol, the reaction apparently leading preponderantly to β-thioglycosides (*cf.* Experimental). In contrast, the crystalline diethylmercaptal hydrochloride (II) of mycosamine itself could be readily obtained from the hydrochloride of the base (I) and separated from the accompanying, likewise crystalline, α- and β-thioglycosides (IIIa and b). The mercaptal was benzoylated with benzoyl chloride in pyridine and the resulting amorphous product directly subjected to Raney nickel desulfurization. There was obtained a crystalline product, m.p. 82–85°, showing approximately the composition of the desired 2,4,5,N-tetrabenzoyl-1-deoxymycosaminol (IV). However, further examination of this material showed that it was a mixture of true IV, m.p. 137–138°, and of a tribenzoate (V), m.p. 95–97°, which differed from IV, as will be shown below, by the lack of the 2-benzoyloxy group.

The tetrabenzoyl derivative IV could be O-debenzoylated with sodium methoxide in methanol to give in good yield N-benzoyl-1-deoxymycosaminol (VI), m.p. 120–121°, which consumed, as expected, one molar equivalent of periodate. In the preparative experiment the mixture from the oxidation was freed of acetaldehyde by sweeping with nitrogen, the aldehydic four-carbon fragment extracted with chloroform and the residue from the latter treated in carbonate–bicarbonate buffer with sodium hypoiodite, according to the procedure of Willstätter and Schudel,[5] until one molar equivalent of the oxidizing reagent was consumed. The crystalline acid, obtained in 45% over-all yield (from VI), was identified as N-benzoyl-D-allothreonine (VIIa) by comparison (m.p., $[\alpha]_D$ and infrared spectrum) with an authentic specimen. Carbon atoms 2 and 3 of mycosamine have therefore the L-configuration as in D-mannose and D-talose.

Unexpectedly, some nonacidic crystalline material, which was shown to be N-benzoyl-D-allothreonine methyl ester (VIIb), was also produced in the last oxidation step. The source of the O-methyl group was a small amount of methanol which had been used for transferring the amorphous aldehyde to the vessel in which the oxidation with sodium hypoiodite was carried out. Although only a few minutes elapsed before the solution was diluted with water, this must have sufficed to transform part of the aldehyde into the methyl hemiacetal which was then oxidized to the methyl ester.

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