Anal. Calcd. for $C_7H_{12}O_2N(OCH_3)_3 \cdot HCl$: C, 44.19; H, 8.15; Cl, 13.05; OCH_3, 34.2. Found: C, 44.21; H, 7.93; Cl, 13.06; OCH_3, 34.5.

Preparation of 3,4,6-Trimethyl-N-methyl-L-glucosamine Hydrochloride (XI) from a Mixture of Pentaacetyl-N-methyl-α- and β-L-glucosamine.—A mixture of α- and β-pentaacetyl-N-methyl-L-glucosamine was prepared by acetolysis of crude methyl pentaacetyldihydrostreptobiosaminide according to the procedure of Stavely, *et al.*,[22] with the exception that the amount of acetolysis mixture was reduced to 25 ml. per gram of material. 2.32 g. of methyl dihydrostreptobiosaminide yielded 2.67 g. of crystalline mixed pentaacetates. This product (2.67 g.) was converted into a mixture of the anomeric methyl N-acetyl-N-methylglucosaminides by refluxing with 2% methyl alcoholic hydrogen chloride (65 ml.) for two hours. After removal of the hydrogen chloride by means of silver carbonate and of excess silver ion by hydrogen sulfide the methanol solution was evaporated to dryness. The crude residue (1.6 g.) was methylated in 3.2 ml. of water as described above, with a total of 14.4 ml. of carbon tetrachloride, 21.2 ml. of dimethyl sulfate and 26.7 ml. of 60% sodium hydroxide. The methylated product (1.33 g.) was removed by extraction with chloroform and was hydrolyzed with 3 *N* hydrochloric acid (19 ml.) for 2.5 hours. Evaporation of the hydrochloric acid after treatment with charcoal yielded a semi-crystalline residue, which upon recrystallization from alcohol–ethyl acetate yielded 3,4,6-trimethyl-N-methyl-L-glucosamine hydrochloride (XI) (519 mg.) in analytically pure form. This product as well as the α-diacetate of m.p. 118–119° prepared from it were identical with the products obtained from methylated N-acetyldihydrostreptomycin.

Degradation of 3,4,6-Trimethyl-N-methyl-L-glucosamine Hydrochloride (XI) to 2,3,5-Trimethyl-L-arabonamide (XII).—In a preliminary experiment 3,4,6-trimethyl-N-methyl-L-glucosamine hydrochloride (6.014 mg., 0.0221 millimole) was dissolved in potassium periodate solution and the utilization of periodate was followed iodometrically.[17] At the end of 18 hours 0.0216 millimole of periodate had been consumed, that is 0.98 mole of periodate per mole of substrate. In a subsequent preparative experiment 3,4,6-trimethyl-N-methyl-L-glucosamine hydrochloride (400 mg.) and finely powdered potassium periodate (339 mg.) were shaken with water (20 ml.) for 18 hours at room temperature. The undissolved potassium periodate (76 mg.) was filtered off and the aqueous filtrate was evaporated to dryness *in vacuo*. The residue was extracted with ethyl acetate and the filtered extract was evaporated to dryness *in vacuo*. A light yellow, low melting crystalline solid (150 mg.) remained, which was oxidized without further purification with bromine (0.3 ml.) in water (3 ml.) for 16 hours at room temperature. The excess bromine was removed by aeration under reduced pressure and the bromide ion was precipitated with silver carbonate. The resulting aqueous solution was carefully evaporated to dryness *in vacuo* so as not to lose the volatile 2,3,5-trimethylarabonolactone. The sirupy residue was transferred to a microdistillation apparatus and the product distilled at a bath temperature of 100–110° and a vacuum of 20 mm. The distillate was transformed into the amide by treating it for 15 hours at room temperature with methanol saturated with ammonia at −15°. Removal of the solvent *in vacuo* left behind a crystalline residue, which was recrystallized twice from acetone. The product (25.9 mg.) melted at 137° and had $[\alpha]^{25}D$ +17.4 (*c* 1.3 in water).

Anal. Calcd. for $C_8H_{17}O_5N$: C, 46.37; H, 8.35; N, 6.76; OCH_3, 44.9. Found: C, 46.56; H, 8.22; N, 6.91; OCH_3, 44.8.

2,3,5-Trimethyl-L-arabonamide has been reported[23] to melt at 132° and to have $[\alpha]D$ +15.8° (*c* 0.75 in water). An authentic sample of 2,3,5-trimethyl-L-arabonamide prepared from methyl L-arabofuranoside[24] by the procedure of Humphreys, Pryde and Waters[23] melted at 137.5–138° and had $[\alpha]^{23}D$ +16.9 (*c* 2.0 in water). A mixture of this material with that obtained by degradation of 3,4,6-trimethyl-N-methyl-L-glucosamine showed no depression in melting point. A mixture of the latter material with 2,3,5-trimethyl-D-arabonamide[13] began to sinter at 133–134° then resolidified and melted at 148.5–149.5°, the melting point characteristic for the D,L-form.[13]

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(22) H. E. Stavely, O. Wintersteiner, J. Fried, H. L. White and M. Moore, THIS JOURNAL, **69**, 2742 (1947).

(23) R. W. Humphreys, J. Pryde and E. T. Waters, *J. Chem. Soc.*, 1298 (1931).

(24) S. Baker and W. N. Haworth, *ibid.*, **127**, 365 (1925).

NEW BRUNSWICK, N. J.

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Streptomycin. XI.[1] Synthesis of 3,6-Dimethyl-N-methyl-D-glucosamine[2]

BY JOSEF FRIED AND DORIS E. WALZ

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The synthesis of 1,2,4-triacetyl-(VIII) and 2,4-diacetyl-3,6-dimethyl-N-methyl-D-glucosamine (IX) is described. 3,6-Dimethyl-D-glucose (II) was degraded by means of lead tetraacetate to produce in excellent yield 2,5-dimethyl-D-arabinose (III). The latter was converted by a cyanhydrin synthesis in the presence of methylamine into a mixture of 3,6-dimethyl-N-methyl-D-glucosaminic acid (V) and what appears to be the corresponding mannosaminic acid. The former after reduction with sodium amalgam followed by acetylation yielded the triacetate VIII. Chromatography of VIII on acetic acid-washed alumina produced the diacetate IX. 3,4,6-Trimethyl-N-methyl-D-glucosaminic acid (VI) prepared from 2,3,5-trimethyl-D-arabinose could not be reduced with sodium amalgam.

In the preceding paper we have described the degradation of methylated N-acetyldihydromannosidostreptomycin to the di- and triacetates of a O-dimethyl-N-methyl-L-glucosamine and the identification of the latter two substances as 2,4-diacetyl- and 1,2,4-triacetyl-3,6-dimethyl-N-methyl-L-glucosamine by comparison with the corresponding D-enantiomorphs prepared by synthesis. The present paper records this synthesis starting from 3,6-dimethyl-D-glucose.

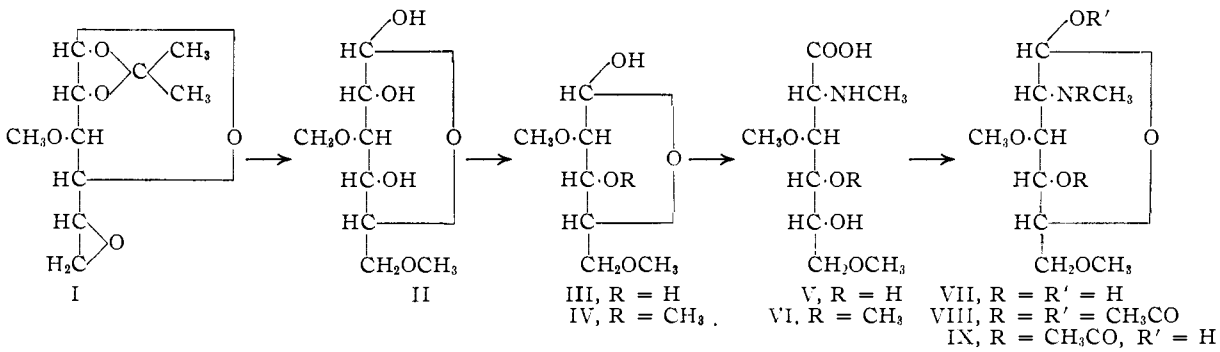
The preparation of 3,6-dimethylglucose has been described by Bell.[3] Starting with 1,2-monoacetone-3-methyl-D-glucose[4] this author effected introduction of the methyl group in position 6 by treat-

(1) Paper X of this series: J. Fried and H. E. Stavely, THIS JOURNAL, **74**, 5461 (1952).

(2) Presented in part before the Division of Biological Chemistry of the American Chemical Society in Chicago, Ill., April, 1948.

(3) D. J. Bell, *J. Chem. Soc.*, 1553 (1936), *cf.* also R. B. Duff and E. G. V. Percival, *ibid.*, 1675 (1947).

(4) K. Freudenberg, W. Dürr and H. v. Hochsteiter, *Ber.*, **61**, 1735 (1928).



ment of the 6-tosylate with sodium methoxide without isolating the intermediate 5,6-epoxide I. This latter reaction is patterned after the procedure of Levene and Raymond[5] for the synthesis of 1,2-monoacetone-6-methyl-D-glucose. In following this route we have found it advantageous to perform the reaction with sodium methoxide in two steps. The first step leading to the epoxide I was effected in chloroform solution at $-10°$, according to Vischer and Reichstein,[6] while the second step was allowed to proceed in methanol at room temperature as described in the Experimental part. In this manner it has been possible to increase by about 50% the yield of 1,2-monoacetone-3,6-dimethyl-D-glucose realizable by the original Bell procedure. The 3,6-dimethyl-D-glucose obtained by acid hydrolysis of its acetonyl derivative melted somewhat higher than reported by Bell but showed the equilibrium rotation value given by that author. The sugar was further characterized by its hitherto undescribed osazone.

The classical method[7] for the preparation of hexosamines involves the application of the cyanhydrin synthesis to the appropriate pentosimine followed by sodium amalgam reduction of the resulting hexosaminic acid. In order to utilize this procedure for the synthesis of the desired 3,6-dimethyl-N-methyl-D-glucosamine it was necessary to degrade 3,6-dimethyl-D-glucose to the corresponding pentose, 2,5-dimethyl-D-arabinose (III). The degradative procedure employed in this work is based on the incidental observation that 2,3,6-trimethyl-D-glucose is not attacked by aqueous periodate, presumably due to the absence in this medium of the open periodate oxidizable aldehydo-form. It was therefore not surprising to find that when 3,6-dimethyl-D-glucose was treated with excess aqueous periodate or with lead tetraacetate in glacial acetic acid only one mole of either reagent was consumed. In the case of the latter oxidizing agent the reaction proceeded with immeasurable speed. These findings were interpreted to indicate that cleavage had occurred between carbon atoms 1 and 2 with the formation of the desired 2,5-dimethyl-D-arabinose. That this was actually the case derives from the following facts: The vacuum-distilled, sirupy sugar ($[\alpha]$D $+23°$ (H_2O)[8]) from both the periodate and lead tetraacetate oxidations showed the correct analytical figures for a dimethylpentose and upon oxidation with bromine water yielded a dimethylpentonolactone, from which a crystalline amide could be prepared. The melting points and absolute specific rotation values of the latter two derivatives are in good agreement with those reported by Smith[9] for 2,5-dimethyl-L-arabonolactone and 2,5-dimethyl-L-arabonamide, respectively. For preparative purposes the lead tetraacetate procedure is preferred, since it results in a practically quantitative yield of III.

The conversion of III into 3,6-dimethyl-N-methyl-D-glucosamine has been accomplished under conditions analogous to those recently elaborated by Kuehl, Flynn, Holly, Mozingo and Folkers[10] and by Wolfrom, Thompson and Hooper[11,12] for the preparation of N-methyl-L-glucosamine from L-arabinose. The cyanhydrin reaction as in the above case[11,12] produced a mixture, which could be separated by fractional crystallization from alcohol into a dextrorotatory and a levorotatory component. Since N-methyl-L-glucosaminic acid rotates to the left, our dextrorotatory acid has been assigned the configuration of a D-glucosaminic acid (V), while the levorotatory component is believed to be the epimeric 3,6-dimethyl-D-mannosaminic acid. Accordingly, to complete the synthesis of 3,6-dimethyl-N-methyl-L-glucosamine the dextrorotatory acid was used, after conversion into the lactone, in the sodium amalgam reduction. The resulting aminosugar was isolated as the α-1,2,4-triacetate VIII. Partial deacetylation of VIII to the 2,4-diacetate IV was effected by chromatography on acetic acid-washed alumina. The fact demonstrated in the preceding paper[1] that VIII and IX are the enantiomorphs of the corresponding acetyl derivatives of 3,6-dimethyl-N-methyl-L-glucosamine obtained from mannosidostreptomycin fully confirms the assignment of the gluco-configuration to the dextrorotatory acid utilized in the sodium amalgam reduction.

It appeared of interest in this connection to pre-

(5) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **97**, 751 (1932).

(6) E. Fischer and T. Reichstein, *Helv. Chim. Acta*, **27**, 1332 (1944).

(7) E. Fischer and H. Leuchs, *Ber.*, **35**, 3787 (1902); **36**, 24 (1903).

(8) The specific rotation reported here is not in accord with that given by Smith ($[\alpha]$D $+46.6°$ (H_2O); *cf.* ref. 9) for his crude 2,5-dimethyl-L-arabinose obtained by hydrolysis of methyl hexamethyl-3-D-galactopyranosido-L-arabofuranoside. The high dextrorotation of that product is obviously due to incomplete separation from it of the highly dextrorotatory 2,3,4,6-tetramethyl-D-galactose ($[\alpha]$D $+118°$ (H_2O)), the presence of which is indicated by the isolation of some 2,3,4,6-tetramethyl-D-galactonolactone after oxidation of the crude sugar with bromine water.

(9) F. Smith, *J. Chem. Soc.*, 744 (1939).

(10) F. A. Kuehl, Jr., E. H. Flynn, F. W. Holly, R. Mozingo and K. Folkers, THIS JOURNAL, **69**, 3032 (1947).

(11) M. L. Wolfrom, A. Thompson and I. R. Hooper, *ibid.*, **68**, 2343 (1946).

(12) M. L. Wolfrom and A. Thompson, *ibid.*, **69**, 1847 (1947).

pare the fully methylated sugar 3,4,6-trimethyl-N-methyl-D-glucosamine by reduction of the correspondingly substituted glucosaminic acid lactone. The L-form of this sugar has been described as a hydrolysis product of methylated streptomycin.[1] For this purpose a mixture of α- and β-methyl 2,5-dimethyl-D-arabinosides was methylated with methyl iodide and silver oxide. Hydrolysis of the resulting mixture of glycosides yielded the sirupy 2,3,5-trimethyl-D-arabinose identified by conversion *via* 2,3,5-trimethyl-D-arabonolactone into the crystalline 2,3,5-trimethyl-D-arabonamide, the melting point and absolute specific rotation values of which are identical with those reported for the corresponding L-enantiomorph.[13] The application of the cyanhydrin reaction in the presence of methylamine to 2,3,5-trimethyl-D-arabinose afforded in contrast to the behavior experienced with the dimethyl sugar only a single crystallizable entity, to which the structure of a 3,4,6-trimethyl-N-methyl-D-glucosaminic acid (VI) is assigned on the basis of its dextrorotation ($[\alpha]^{24}D$ +8.2°, final in water). Unfortunately, two attempts to reduce this acid with sodium amalgam under the conditions found suitable for the reduction of V failed to yield the desired amino sugar, presumably due to the fact that the formation of the stable, amalgam-reducible γ-lactone is prevented by the presence of the methyl group in the 4-position.

Experimental

1,2-Monoacetone-3,6-dimethyl-D-glucose.—1,2-Monoacetone-3-methyl-5,6-anhydro-D-glucose (I) (37 g., $[\alpha]D$ −65° (acetone)) prepared from diacetone-D-glucose according to the procedure of Vischer and Reichstein[6] was added to a solution of sodium methoxide (from 8.44 g. of sodium) in methanol (370 ml.). After standing at room temperature for two days the mixture was carefully neutralized with concentrated hydrochloric acid (*ca.* 35 ml.) to *p*H 7.5 and, after the addition of ether (800 ml.) was filtered with suction and the precipitated sodium chloride washed with small portions of ether. The filtrate was concentrated *in vacuo* to remove ether, methanol and most of the water, taken up again in dry ether and dried over sodium sulfate. The ether solution upon concentration *in vacuo* left a sirup which was distilled in vacuum. The bulk of the product distilled at 97–101° (0.1 mm.) and amounted to 34.2 g. (80% yield), $[\alpha]D$ −33.5° (*c* 2.0 in acetone).

Anal. Calcd. for $C_9H_{14}O_4(OCH_3)_2$: OCH_3, 25.0. Found: OCH_3, 24.0.

3,6-Dimethyl-D-glucose (II).—Monoacetone-3,6-dimethyl-D-glucose (27.8 g.) was dissolved in a mixture of equal volumes of alcohol and 5% aqueous hydrochloric acid (695 ml.) and held at a temperature of 45° for 18 hours. During this time interval the specific rotation changed from an initial value of −39.5° to a value of +61°, which did not change on further warming. The solution was neutralized with silver carbonate (*ca.* 90 g.), filtered over a bed of charcoal and the excess silver ion precipitated with H_2S. After removal of the alcohol *in vacuo*, the aqueous concentrate was treated with charcoal, warmed on the steam-bath to coagulate the colloidal silver sulfide and filtered. The colorless filtrate was evaporated to dryness *in vacuo* and the last traces of water removed by distilling absolute alcohol off the sirupy residue. The latter crystallized readily when taken up in a small volume of ethyl acetate and allowed to stand at room temperature. First crop: 16.5 g., m.p. 124–126°; the mother liquors yielded a second crop (1.1 g.) which melted at 118–120° (75.5% yield), $[\alpha]^{23}D$ +101° (initial) → +64.7° (final, 5 hours) (*c* 1.2 in water); reported[3]: m.p. 115–116°, $[\alpha]D$ +61.5° (H_2O, final).

The osazone of 3,6-dimethyl-D-glucose was prepared by heating a solution of 3,6-dimethyl-D-glucose (200 mg.) in water (5 ml.) with phenylhydrazine (0.7 ml.) and glacial acetic acid (6 drops) on the steam-bath for 2 hours, while passing a stream of CO_2 through the solution. The resulting yellow oil was dissolved in benzene and the benzene solution after washing with 1% acetic acid and subsequent drying over sodium sulfate was chromatographed on acetic acid-washed alumina (10 g.). Elution with benzene and with benzene–ether 3:1 removed red and green impurities, while benzene containing 3% alcohol eluted a yellow band consisting of the osazone. Crystallization from ether–hexane and finally from ethyl acetate–hexane yielded the pure osazone as long needles melting at 114.5–115.5°, $[\alpha]^{25}D$ −139.4° (initial) → −50° (final, after 24 hours) (*c* 1.51 in 2 parts pyridine and 3 parts absolute alcohol).

Anal. Calcd. for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.43; OCH_3, 16.1. Found: C, 62.06; H, 6.69; N, 14.51; OCH_3, 15.8.

2,5-Dimethyl-D-arabinose (III) by Degradation of 3,6-Dimethyl-D-glucose with Lead Tetraacetate. (a) Titration.—3,6-Dimethyl-D-glucose (23.9 mg.) was dissolved in 1.4% lead tetraacetate in glacial acetic acid (10 ml.) and 1 ml. aliquots were withdrawn for titration with 0.01 *N* $Na_2S_2O_3$ as described by Hockett and McClenahan.[14] A titration performed immediately after dissolving the 3,6-dimethylglucose indicated a consumption of 1.0 mole of lead tetraacetate per mole of substrate. The same value was obtained in subsequent titrations at one and two hour intervals.

(b) **Preparative.**—To a solution of 2,6-dimethyl-D-glucose (7.0 g.) in glacial acetic acid (70 ml.) was added with cooling (20°) a solution of lead tetraacetate (17.6 g. moist with acetic acid) in glacial acetic acid (105 ml.). The mixture was allowed to remain at room temperature for 10 minutes and the excess lead tetraacetate was determined titrimetrically. An amount of dimethylglycose (505 mg.) equivalent to the found excess was then added and the solution, which now no longer liberated iodine from a KI–sodium acetate solution, was lyophilized. The residue was extracted with ethyl acetate, the extract filtered and the filtrate evaporated to dryness *in vacuo*. The resulting sirupy 4-formyl-2,5-dimethyl-D-arabinose was deformylated by heating a solution of the latter in 0.5 *N* aqueous hydrochloric acid (100 ml.) on the steam-bath for 45 minutes. During this period of time the rotation of the solution changed from an initial value of 1.72° to a value of 1.28°, which did not change on further heating. The acid solution was neutralized with silver carbonate, filtered and freed from excess silver ion by means of hydrogen sulfide. A small amount of colloidal silver sulfide was removed by treatment with Darco G-60 and the solution lyophilized. The sirupy residue representing the practically pure 2,5-dimethyl-D-arabinose (5.78 g., $n^{20}D$ 1.4637) was used without further purification in the reactions described below.

For analysis and determination of its physical constants a portion of this material was distilled in high vacuum; b.p. 126° (0.03 mm.), $[\alpha]^{20}D$ +23° (H_2O), $n^{20}D$ 1.4648.

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.19; H, 7.92; OCH_3, 34.8. Found: C, 45.75; H, 7.78; OCH_3, 34.1.

2,5-Dimethyl-D-arabonolactone.—To a solution of 2,5-dimethyl-D-arabinose (III) (470 mg.) in water (8 ml.) was added bromine (1 ml.), and the resulting mixture allowed to stand in the dark for 24 hours. Nitrogen was then passed through the solution to remove excess bromine. The hydrobromic acid was neutralized with silver carbonate and excess silver ion precipitated with H_2S. The Darco-treated, H_2S-free solution was evaporated to dryness *in vacuo* and the residue distilled in a cold finger–sublimation apparatus. Three fractions totaling 290 mg. and melting at 50–57° were collected. Two recrystallizations from ether yielded the pure lactone as long needles, m.p. 59–60°, $[\alpha]^{24}D$ +62.2° (initial; *c* 1.09 in H_2O), no mutarotation. Reported[9] for the L-enantiomorph: m.p. 60°, $[\alpha]D$ −59.7° (initial, H_2O).

Anal. Calcd. for $C_7H_{12}O_5$: C, 47.73; H, 6.87; OCH_3, 35.2. Found: C, 47.57; H, 7.11; OCH_3, 35.09.

2,5-Dimethyl-D-arabonamide.—A solution of 2,5-dimethyl-D-arabonolactone (30 mg.) in methanol (1 ml.) was saturated with ammonia at 20°. After standing at room temperature overnight the solution was evaporated to dryness

(13) R. W. Humphreys, J. Pryde and E. T. Waters, *J. Chem. Soc.*, 1298 (1931).

(14) R. C. Hockett and W. S. McClenahan, THIS JOURNAL, **61**, 1667 (1939).

in vacuo and the residue recrystallized from alcohol–ether. The amide (23.2 mg.) crystallized in clusters of needles, which melted at 130.5–131°, $[\alpha]^{23}D$ −34° (*c* 0.82 in H_2O); reported[9] for the L-enantiomorph: m.p. 131°, $[\alpha]^{22}D$ +38° (H_2O).

Anal. Calcd. for $C_7H_{15}O_5N$: C, 43.53; H, 7.83; N, 7.25. Found: C, 43.31; H, 7.63; N, 7.06.

Titrimetric Oxidation of 3,6-Dimethyl-D-glucose with Periodate.—3,6-Dimethyl-D-glucose (10.6 mg.) was dissolved in 0.3% aqueous potassium periodate (10 ml.) and 1-ml. aliquots of this solution were withdrawn at 5-min. intervals to be titrated according to the procedure of Rappaport, Reifer and Weinmann.[15] Within the first five minutes 0.93 mole equivalent of periodate had been consumed and there was no further uptake within the ensuing 30 minutes. Under identical conditions 2,3,6-trimethyl-D-glucose (m.p. 111–112°) was stable toward periodate for at least 5 hours.

3,6-Dimethyl-N-methyl-D-glucosaminic (V) and Mannosaminic Acids.—Into a boiling solution of 3,6-dimethyl-D-glucose (5.78 g.) in absolute alcohol (11.5 ml., dried over Mg) was passed for a period of 10 minutes a rapid stream of thoroughly dried methylamine. The solution was then allowed to cool to room temperature for a period of 30 minutes and freshly distilled hydrogen cyanide (4 ml.) was added in small portions so as not to exceed a temperature of 35–40°. When all the hydrogen cyanide had been added the solution was allowed to stand at room temperature for two hours. It was then cooled to −20° and slowly added to concentrated hydrochloric acid (25 ml.), which was likewise maintained at a temperature of −20° by immersion into an ice–salt-bath. The whole system was allowed to reach room temperature overnight and the solution, which now contained crystals of methylamine hydrochloride was evaporated to dryness in a good vacuum. The residue was taken up in water (25 ml.), barium hydroxide octahydrate (30 g.) was added and the mixture was refluxed for 3 hours. The barium ion was exactly precipitated with 2 *N* sulfuric acid, the solution was filtered and lead carbonate was added with stirring until there was no further evolution of carbon dioxide. The mixture was filtered with the aid of charcoal (G-60) and the remainder of the chloride ion removed by treatment with silver carbonate. Excess silver and lead were then precipitated with H_2S and the solution filtered again with the aid of a little charcoal. The colorless H_2S-free solution was lyophilized and the residue taken up in absolute alcohol (10 ml.). After centrifugation crystallization occurred spontaneously affording a mixture of 3,6-dimethyl-N-methyl-D-glucosaminic acid (V) and of the epimeric D-mannosaminic acid (1.76 g. total) which was separated by extensive fractional crystallization from absolute alcohol. The most dextrorotatory sample of 3,6-dimethyl-N-methyl-D-glucosaminic acid was obtained after six crystallizations of the crude mixture. It decomposed at 178° after browning at 171° and had $[\alpha]^{24}D$ +2.8° (*c* 2.8 in H_2O), no observed mutarotation.

Anal. Calcd. for $C_9H_{19}O_6N$: C, 45.57; H, 8.07; N, 5.90; OCH_3, 26.2. Found: C, 45.78; H, 8.06; N, 5.98; OCH_3, 26.2.

Continued fractionation of the mother liquors afforded the levorotatory 3,6-dimethyl-N-methyl-D-mannosaminic acid, m.p. 185–186°, $[\alpha]^{23}D$ −3.8° (initial) → −5.8° (final, after 20 hours) (*c* 2.13 in water).

Anal. Calcd. for $C_9H_{19}O_6N$: C, 45.57; H, 8.07; OCH_3, 26.2. Found: C, 45.57; H, 8.14; OCH_3, 24.4.

3,6-Dimethyl-N-methyl-D-glucosamine (VII).—A solution of 3,6-dimethyl-N-methyl-D-glucosaminic acid (V) ($[\alpha]D$ +2.5° (*c* 2.7 in H_2O), 400 mg.) in a mixture of water (8 ml.) and concentrated hydrochloric acid (2 ml.) was evaporated to dryness *in vacuo* to effect lactonization. The residual brown sirup was treated in the same manner for a second time and the resulting lactone hydrochloride freed from excess hydrochloric acid by vacuum distillation of its solution in absolute ethanol. To a solution of the resulting sirupy residue in water (4 ml.) maintained at 0° by immersion into an ice-bath was added with mechanical stirring 2.5% sodium amalgam (6 g.), immediately followed by cold sulfuric acid (20% by vol.), which was added from a buret at a rate adjusted to maintain the solution just acid toward congo paper. When the rate of consumption of the acid began to drop a second portion of sodium amalgam (6 g.) was added and the addition of acid continued until all the amalgam was spent. At this point a drop of the reaction mixture gave a strong positive test with Fehling solution. The slightly colored aqueous solution was diluted with water, charcoaled and adjusted to a *p*H of 6.0 with dilute sodium hydroxide. The solution was evaporated almost to dryness *in vacuo* and the residue extracted twice with hot methanol. The combined methanol extracts were evaporated to dryness *in vacuo*, the residue taken up in a small amount of absolute alcohol and evaporated again to dryness to remove the last traces of water.

The dried residue (410 mg.) was acetylated with pyridine (5 ml.)–acetic anhydride (5 ml.) at room temperature for 18 hours. The crude acetylation product when taken up in ethyl acetate–hexane crystallized spontaneously in well-formed needles, which melted at 137–138°. After one recrystallization the triacetate (VIII) (40 mg.) melted at 140–141°, $[\alpha]^{24}D$ +110° (*c* 0.98 in $CHCl_3$).[16] An additional amount (28 mg.) of material melting at 139–140° was obtained from the mother liquors.

Anal. Calcd. for $C_7H_{10}O_3N(COCH_3)_3(OCH_3)$: C, 51.86; H, 7.26; N, 4.03; OCH_3, 17.9; CH_3CO, 37.2. Found: C, 51.78; H, 7.44; N, 3.77; OCH_3, 17.5; CH_3CO, 36.9.

Chromatography of a solution of the combined mother liquors in benzene (5 ml.)–hexane (2.5 ml.) on acetic acid-washed alumina (6 g.) afforded after elution of amorphous material by benzene a crystalline fraction eluted by benzene–U.S.P. ether. This fraction (IX) after two recrystallizations from ethyl acetate melted at 162.5–163.5°, $[\alpha]^{24}D$ +71° (initial) → +31° (final, after one hour) (*c* 0.73 in water).[16]

2,3,5-Trimethyl-D-arabinose (IV).—A solution of 2,5-dimethyl-D-arabinose (III) (5 g.) in 1% methanolic hydrogen chloride (90 ml.) was allowed to stand at room temperature until a small aliquot no longer reduced Fehling solution, which required approximately 40 hours. The chloride ion was then removed with silver carbonate, the filtered solution treated with Darco G-60 and evaporated to dryness *in vacuo*. The resulting mixture of α- and β-methyl 2,5-dimethyl-D-arabofuranosides (5 g.) was methylated[17] as follows: To a well agitated solution of the sirup in methyl iodide (17.5 ml.) maintained at the reflux temperature was added silver oxide[18] (32.5 g.) in small portions. Fresh portions of methyl iodide were added so as to permit efficient stirring of the contents of the flask. When all the silver oxide had been added the mixture was heated for two more hours, allowed to stand at room temperature overnight and extracted with six 15-ml. portions of chloroform. The combined chloroform extracts were evaporated to dryness and the residue distilled in high vacuum, b.p. 66–68° (0.4 mm.), $n^{25}D$ 1.4321, yield 5.0 g. Efficient cooling of the receiver is necessary to prevent losses of material.

Anal. Calcd. for $C_9H_{18}O_5$: C, 52.43; H, 8.80; OCH_3, 60.1. Found: C, 51.82; H, 8.93; OCH_3, 59.1.

The above mixture of α- and β-methyl 2,3,5-trimethyl-D-arabinosides was hydrolyzed with 0.3 *N* hydrochloric acid (100 ml.) at 100° for three hours. During that period of time the specific rotation changed from +87° to a constant value of +38°. Reported[19] for 2,3,5-trimethyl-L-arabinose: −39.5° (water). The solution was freed from chloride ion by means of silver carbonate, excess silver ion removed with H_2S and the charcoaled solution evaporated to dryness *in vacuo;* yield of IV 3.7 g.

2,3,5-Trimethyl-D-arabonamide.—2,3,5-Trimethyl-D-arabinose (IV) (500 mg.) was oxidized with bromine water as described above. The distilled lactone solidified in an ice-bath and melted again at room temperature; reported[20] for 2,3,5-trimethyl-L-arabonolactone: m.p. 30°. The amide prepared from the above lactone melted at 137–139° and had $[\alpha]^{24}D$ −16.5° (*c* 1.15 in water).

Anal. Calcd. for $C_8H_{17}O_5N$: C, 46.37; H, 8.35; N, 6.76; OCH_3, 44.9. Found: C, 46.36; H, 8.30; N, 6.64; OCH_3, 44.4.

(15) F. Rappaport, J. Reifer and H. Weinmann, *Mikrochim. Acta*, 290 (1937).

(16) For the comparison of VIII and IX with 1,2,4-triacetyl- and 2,4-diacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine, respectively, see reference 1.

(17) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, 1028 (1903).

(18) B. Helferich and W. Klein, *Ann.*, **450**, 225 (1926).

(19) S. Baker and W. N. Haworth, *J. Chem. Soc.*, 365 (1925).

(20) J. Pryde and R. W. Humphreys, *ibid.*, 562 (1927).

A sample of 2,3,5-trimethyl-L-arabonamide prepared according to Humphreys, Pryde and Waters[13] melted at 137.5–138° and had $[\alpha]^{23}D$ +16.9° (*c* 2.0 in water). A mixture of equal amounts of the D- and L-forms melted at 149–150°.

3,4,6-Trimethyl-N-methyl-D-glucosaminic Acid (VI).—2,3,5-trimethyl-D-arabinose (IV) (3.7 g.) was subjected to a cyanhydrin synthesis as described above for 2,5-dimethyl-D-arabinose. The final product of the reaction, 3,4,6-trimethyl-N-methyl-D-glucosaminic acid (VI) (1.39 g.) crystallized readily from absolute alcohol, m.p. 206–206.5°, $[\alpha]^{25}D$ +9.5° (initial) → +8.2° (final after 20 hours), (*c* 2.1 in water). Three recrystallizations of the acid did not alter its melting point and specific rotation.

Anal. Calcd. for $C_{10}H_{21}O_6N$: C, 47.79; H, 8.42; N, 5.57; OCH_3, 37.01. Found: C, 47.87; H, 8.35; N, 5.48; OCH_3, 37.5.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Reductive Methylation of Steroid Ketones

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A dehydro derivative of methyl $\Delta^{9(11)}$-lithocholenate tentatively regarded by Fieser and Rajagopalan as a 3,9-oxide is actually the normal 3-ketone (I), and a product derived from it by hydrogenation in methanol–hydrobromic acid and characterized by the presence of an inert oxygen function is the 3β-methyl ether II. Comparable reductive methylations have been demonstrated with a saturated bile acid 3-ketone (VII), with coprostanone and with cholestanone.

On investigating the oxidation of methyl $\Delta^{9(11)}$-lithocholenate (V) and its oxide, Fieser and Rajagopalan[2] encountered two products that appeared to be of novel types. The product of oxidation of the 9,11-oxide was subsequently studied by Heymann and Fieser[3] and found to be a hemiketal with a 3,9-oxide bridge. That from the unsaturated hydroxy ester seemed abnormal because on hydrogenation in methanol containing hydrobromic acid it afforded a dihydro derivative that contained neither a hydroxyl nor a ketone function but appeared to contain an inert ether-oxygen atom. A 3,8-oxide formulation was tentatively considered,[2] although it failed to account for some of the observations.

At the time of the earlier work the Baird spectrophotometer available was incapable of distinguishing between a 3-ketone and an ester carbonyl group. The spectrum of Rajagopalan's original oxidation product, taken with improved apparatus at low speed and high resolution, has now revealed a distinct doublet in the carbonyl region with maxima at 5.79 and 5.83 μ (Chf). The inference that the substance is the normal oxidation product, methyl 3-keto-$\Delta^{9(11)}$-cholenate (I), was confirmed by formation of a semicarbazone and a 2,4-dinitrophenylhydrazone, and by formation of an identical product by Oppenauer oxidation of methyl $\Delta^{9(11)}$-lithocholenate, which excludes an allylic-type oxidation.

The product obtained by hydrogenation of I in methanol in the presence of hydrobromic acid, but not in absence of the acid, might still be a saturated, cyclic oxide. However, Dr. Rajagopalan's original samples as well as a fresh sample of the same properties all gave positive tests for unsaturation with tetranitromethane. The inference that the substance is in fact the unsaturated 3-methyl ether II was substantiated by the results of a methoxyl determination. The product obtained earlier[2] by further hydrogenation of II in acetic acid must then be the saturated 3-methyl ether III, and indeed an identical product was obtained by hydrogenation of methyl dehydrolithocholate (VII) in methanol–hydrobromic acid. The same ether also resulted from methanolysis of methyl lithocholate 3-tosylate (IV). Since in saturated systems tosylate displacement always proceeds with inversion, this result shows that the methoxyl group in II and III has the β-orientation.

The true structures of the alcohols[2] resulting from lithium aluminum hydride reduction of II and III follow from the revised formulations of these compounds, as summarized in the Experimental part. A further product, isolated by Rajagopalan as the acetate in less than 1% yield by acetolysis of the unsaturated methyl ether II, was characterized as a hydroxycholenic acid, m.p. 210°, αD + 44° Di; methyl ester acetate, m.p. 183°, αD + 40° Di. It now seems likely that this acetate arose not from II but from a 3-hydroxy compound present as an impurity. The fact that the hydroxycholenic acid differs but little in rotation from its acetate methyl ester provided a clue to its identity. In the series of 3α-hydroxy bile acids the increment $\Delta^{\text{acetate ester}}$ = [M_D (3-acetate methyl ester) − M_D (3-hydroxy acid)] has a large positive value, +79 for lithocholic acid, +95 for $\Delta^{9(11)}$-lithocholenic acid,[4] + 100 for Δ^{11}-lithocholenic acid.[4] In contrast, the M_D increment for conversion of the hydroxy acid in question into its acetate ester is only +7, which means that the acid must belong to some other series. That this is the β-hydroxy series was suggested by the fact that the acetate methyl ester (αD + 40° Di) differs in rotation from methyl 3α-acetoxy-$\Delta^{9(11)}$-cholenate (α_D + 62.9 An[4]) to an extent closely comparable to the difference between methyl 3β-acetoxycholanate (αD + 23° Chf) and the epimeric 3α-acetoxy compound (αD + 45° Di). The structure thus suggested, methyl 3β-acetoxy-$\Delta^{9(11)}$-cholenate (VIa) for the acetate methyl ester, was established by preparation of an identical substance by solvolysis of the tosylate of methyl $\Delta^{9(11)}$-lithocholenate (V) with

(1) Du Pont predoctoral fellow, 1951–1952.

(2) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **73**, 118 (1951).

(3) H. Heymann and L. F. Fieser, *ibid.*, **73**, 5252 (1951).

(4) E. Seebeck and T. Reichstein, *Helv. Chim. Acta*, **26**, 536 (1943).