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

Derivatives of N-Methyl-L-glucosaminic Acid; N-Methyl-L-mannosaminic Acid

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In a previous communication[1] there was described an improved synthesis of N-methyl-L-glucosaminic acid and preliminary work therein reported indicated that the epimeric acid would probably be isolable from the reaction mixture. N-Methyl-L-mannosaminic acid has now been isolated in the form of its acetylated nitrile, a readily purifiable substance, and this on hydrolysis of its acetyl and nitrile groups led to the synthesis of the pure acid. Suitable conditions for removal of the aforementioned groups were established with the previously reported[1] pentaacetyl-N-methyl-L-glucosaminic acid nitrile.

The two epimeric amino acids, being inner salts, have no melting points and in addition have low rotations ($-4.6°$ and $+6.7°$ in water at λ 5892.5 Å.). Their corresponding acetylated nitriles have true melting points and are more readily purified. Fischer and Leuchs[2] isolated only one acid from the cyanohydrin reaction on each of the enantiomorphous arabinosylamines and proved that the D-acid was related to the naturally occurring glucosamine which is now established[3,4] as 2-desoxy-2-amino-D-glucose. By an analogous procedure, Votoček and Lukeš[5] prepared a N-methylhexosaminic acid from D-arabinose and merely assumed that the acid they isolated was N-methyl-D-glucosaminic acid. A definitive proof of structure for this substance was offered by Folkers and co-workers[6] in a brief note without experimental detail. In view of the importance of this proof in relation to the structure of the antibiotic streptomycin, in which N-methyl-L-glucosamine is an integral part, and because we wished to establish beyond any doubt the structures of the two epimeric acids with which we are presently concerned, this work was repeated and the experiences of our Laboratory are herein recorded. The priority of Folkers and coworkers is fully acknowledged. Natural D-glucosamine was methylated to N-methyl-D-glucosamine, herein isolated as the α-D-pentaacetyl derivative, enantiomorphous with the sugar structure found in streptomycin. N-Methyl-L-glucosamine was oxidized to the acid and this was identical with the one most readily isolable in the cyanohydrin reaction on L-arabinosyl-N-methylamine. The acid of rotation $[\alpha]^{25}D - 4.6°$ (water) is therefore N-methyl-L-glucosaminic acid and that of $[\alpha]^{25}D + 6.7°$ (water) is N-methyl-L-mannosaminic acid.

We also report our experiences in reducing N-methyl-L-glucosaminic acid to the aldose sugar, a reaction likewise mentioned by Folkers and co-workers.[6] This was accomplished by reduction with sodium amalgam essentially according to the general procedure of Fischer and Leuchs.[2] This depends upon the reduction of the inner ester (lactone) and since lactonization appears to be hindered by the presence of the adjacent amino group, the reaction was not very satisfactory.

In the course of this work the hydrochlorides of N-methyl-L-glucosaminic acid and its nitrile were characterized. The latter substance is much more stable than the "free" N-methyl-L-glucosaminic acid nitrile. N-Methyl-L-glucosamine was prepared as the crystalline "free base" and this was found to have good stability. It was previously reported as a gum by Folkers and co-workers.[6] The second anomeric form of pentaacetyl-L-glucosamine is herein described. This is a β-L form and the previously recorded[6] anomer is therefore the α-L isomer. According to Hudson's[7] rules of isorotation, half the difference between the molecular rotations of the anomers of a sugar or a sugar derivative, is equal to the rotational effect of the lactol carbon atom. This value for the above pair is $-17{,}500$ while the corresponding one for the analogous derivatives of D-glucosamine[8] is $+17{,}960$. The racemic form of the α-isomer is reported. In the course of this work the chromatographic procedures[9,10] for separating sugar acetates found useful application.

Experimental

N-Methyl-L-glucosaminic Acid Nitrile Hydrochloride.—N-Methyl-L-glucosaminic acid nitrile[1] (5 g.) was dissolved without heating in 7 cc. of 12 *N* hydrochloric acid and diluted with 30 cc. of absolute ethanol. The product that crystallized immediately was removed by filtration and washed with ethanol and ether; yield 4.6 g., m. p. 138–140° (dec.), $[\alpha]^{22}D$ $-28.5°$ (initial) → $+54.2°$ (five days) → $+17.8°$ (final, eight days) (*c* 4.1, water). The substance is more stable than the previously described[1] free base. It has withstood storage for several months in a dry atmosphere.

Anal. Calcd. for $C_7H_{15}O_4N_2Cl$: C, 37.09; H, 6.66; N, 12.36; Cl, 15.64. Found: C, 37.13; H, 6.37; N, 12.13; Cl, 15.57.

Two grams of this material was acetylated by shaking with 12 cc. of acetic anhydride and 6 cc. of pyridine for thirty minutes. When the mixture was poured into ice and water, 3.5 g. of crystalline material separated; m. p.

(1) M. L. Wolfrom, A. Thompson and I. R. Hooper, *Science*, **104**, 276 (1946); THIS JOURNAL, **68**, 2343 (1946).

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

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

(4) E. G. Cox and G. A. Jeffrey, *Nature*, **143**, 894 (1939).

(5) E. Votoček and R. Lukeš, *Coll. Czechoslov. Chem. Commun.*, **7**, 424 (1935); *Chem. Listy*, **29**, 308 (1935).

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

(7) C. S. Hudson, *ibid.*, **31**, 66 (1909).

(8) C. S. Hudson and J. K. Dale, *ibid.*, **38**, 1431 (1916).

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

(10) L. W. Georges, R. S. Bower and M. L. Wolfrom, *ibid.*, **68**, 2169 (1946).

134°, $[\alpha]^{26}D$ −38.5° (*c* 4, chloroform). These constants identify this material as pentaacetyl-N-methyl-L-glucosaminic acid nitrile.[1]

N-Methyl-L-glucosaminic Acid Hydrochloride.—N-Methyl-L-glucosaminic acid (11 g.) was suspended in 40 cc. of ethanol and 5 cc. of 12 *N* hydrochloric acid was added. The material went into solution without being heated and then crystallized again as the hydrochloride; yield 6.0 g., m. p. 136–137°. Further recrystallization from methanol–ether did not alter the melting point; $[\alpha]^{22}D$ −4.7° (*c* 4.0, water).

Anal. Calcd. for $C_7H_{16}O_6NCl$: C, 34.21; H, 6.56; N, 5.69; Cl, 14.43; neutralization value (1 equiv.), 4.07 cc. of 0.1 *N* sodium hydroxide per 100 mg. Found: C, 34.29; H, 6.55; N, 5.67; Cl, 14.32; neutralization value, 3.90 cc. (methyl orange end-point).

Reduction of N-Methyl-L-glucosaminic Acid to N-Methyl-L-glucosamine Hydrochloride.—The reduction of N-methyl-L-glucosaminic acid to the aldose sugar has been mentioned by Folkers and co-workers[6] but no details have been published. The procedure followed in this Laboratory was a modification of that reported by Fischer and Leuchs[2] for the corresponding unmethylated structure. Ten grams of N-methyl-L-glucosaminic acid, or an equivalent amount of its hydrochloride, was dissolved in 20 cc. of water and 5 cc. of 12 *N* hydrochloric acid with heating. The solvent was removed under reduced pressure on a boiling water-bath. The sirup was dissolved in ethanol and the solvent was removed again by distillation. The sirup was transferred with a small amount of water to a container equipped with a mechanical stirrer and cooled to 0°. Sulfuric acid (20%, 20 cc.) was added, followed by 50 g. of 3% sodium amalgam[11] in small portions. Sulfuric acid and sodium amalgam were added, with the acid always in excess, in the above amounts until 250 g. of amalgam had been used. The time of addition was about four hours. The precipitate of sodium sulfate was removed by filtration. The solution was then treated with activated carbon, made just alkaline to phenolphthalein with sodium hydroxide and then slightly acid with hydrochloric acid. The solution was evaporated under reduced pressure to about 75 cc., 150 cc. of hot absolute ethanol added and the precipitated sodium sulfate removed immediately by filtration. Cooling the filtrate will cause the crystallization at this point of unchanged N-methyl-L-glucosaminic acid. Should such be present it is removed by filtration. Concentration and ethanol treatment may remove further quantities of sodium sulfate. The final sirup obtained on solvent removal under reduced pressure was dried by treatment with ethanol followed by distillation under reduced pressure. It was then dissolved in 15 cc. of methanol. Nucleation may cause the crystallization of N-methyl-L-glucosamine hydrochloride and more may be obtained by the addition of ethanol; crude yield 2.0 g., m. p. 166–168°, $[\alpha]^{18}D$ −108° (extrapolated initial value, *c* 1.9, water).

In another reduction experiment, the above hydrochloride failed to crystallize and the sirup obtained on solvent removal under reduced pressure was acetylated overnight at room temperature with acetic anhydride (40 cc.) and pyridine (20 cc.). The mixture was poured into ice and water, extracted with chloroform and the extract was washed with a concentrated aqueous cadmium chloride solution, filtered from the pyridine–cadmium chloride complex, washed with water, dried and concentrated to a sirup under reduced pressure. The material was obtained crystalline from benzene–ether–petroleum ether; yield 2.0 g., m. p. 148–151°. Two recrystallizations from benzene produced pure material; m. p. 158.5–159.5° (cor.), $[\alpha]^{29}D$ −102° (*c* 4, chloroform). The constants previously reported[6] for this substance (pentaacetyl-N-methyl-α-L-glucosamine) are: m. p. 160.5–161.5° (micro-block), $[\alpha]^{25}D$ −100° (*c* 0.7, chloroform).

Further quantities of the anomeric pentaacetyl derivative were obtained in this experiment as described in the succeeding section.

One gram of pentaacetyl-N-methyl-α-L-glucosamine was suspended in 20 cc. of 4 *N* hydrochloric acid and heated in a boiling water-bath for forty-five minutes. The solution was filtered with decolorizing carbon and evaporated under reduced pressure at 50° to a sirup. A few drops of ethanol were added and the sirup slowly crystallized. The material (N-methyl-L-glucosamine hydrochloride) was filtered and washed with a small amount of cold ethanol; yield 0.5 g., m. p. 160–162°, $[\alpha]^{25}D$ −106° (initial, extrapolated) → −89° (final) (*c* 2, water). These constants are in agreement with those reported by Folkers and co-workers.[6]

Oxidation of N-methyl-L-glucosamine hydrochloride (0.5 g.) according to the procedure of Pringsheim and Ruschmann[12] for the analogous oxidation of D-glucosamine hydrochloride produced N-methyl-L-glucosaminic acid, in agreement with the finding of Folkers and co-workers[6]; yield, 0.3 g., m. p. 217–219° (dec., unreliable); $[\alpha]^{22}D$ −4.9° (*c* 3.0, water).

Pentaacetyl-N-methyl-β-L-glucosamine.—The material (0.75 g., m. p. 135–145°) obtained from the mother liquor from the above-described preparation of pentaacetyl-N-methyl-α-L-glucosamine was recrystallized from benzene–petroleum ether. After four recrystallizations no further changes were noted in the constants, which were: m. p. 153–153.5° (cor.), $[\alpha]^{25}D$ −16.5° (*c* 3, chloroform).

Anal. Calcd. for $C_{17}H_{25}O_{10}N$: C, 50.62; H, 6.25; N, 3.47. Found: C, 50.88; H, 6.15; N, 3.47.

N-Methyl-L-glucosamine.—N-Methyl-L-glucosamine hydrochloride (1.9 g.) was suspended in 20 cc. of methanol and 1 cc. of diethylamine was added. The material dissolved immediately and upon nucleation the free sugar crystallized; yield 1 g., m. p. 130–132° (dec.). The material was further purified by two recrystallizations from methanol–ethanol–ether; m. p. 130–132° (dec.), $[\alpha]^{24}D$ −64° (*c* 3.9, water, no detectable mutarotation). The substance has a sweet taste. The *p*H of a 4.0% aqueous solution was 10.5.

Anal. Calcd. for $C_7H_{15}O_5N$: C, 43.51; H, 7.82; N, 7.25. Found: C, 43.68; H, 7.85; N, 7.19.

Pentaacetyl-N-methyl-α-D-glucosamine.—Folkers and co-workers[6] state that "methylation of D-glucosamine, followed by acetylation, yielded pentaacetyl-N-methyl-D-glucosamine; m. p. 160.5–161.5° (micro-block), $[\alpha]^{25}D$ +101°." This reaction was accomplished in our Laboratory by suspending D-glucosamine hydrochloride (10 g.) in 200 cc. of methanol containing 3.7 g. (2 moles) of sodium hydroxide. The mixture was shaken until the sugar was in solution whereupon 10 cc. of dimethyl sulfate was added and the mixture allowed to stand overnight. It was then filtered and evaporated to dryness under reduced pressure. The resultant sirup was dissolved in water, acidified with hydrochloric acid and boiled for thirty minutes. It was then evaporated under reduced pressure to a sirup and this was acetylated as described above. The sirup obtained on solvent removal was crystallized from benzene–ether; yield 1 g., m. p. 145–147°, $[\alpha]^{22}D$ +94° (*c* 3.8, chloroform). This material was dissolved in 35 cc. of benzene, placed on a column (45 mm. diam. × 160 mm.) containing 100 g. of Silene EF–Celite (5:1)[10] and developed with 1 liter of benzene–ethanol (100:1). Only one zone remained on the column. The effluent was evaporated under reduced pressure to a sirup and crystallized from benzene–ether; m. p. 158.5–159.5° (cor.), $[\alpha]^{17}D$ +102° (*c* 4.0, chloroform).

Pentaacetyl-N-methyl-α-D,L-glucosamine.—Equal quantities (0.16 g.) of the enantiomorphous isomers of pentaacetyl-N-methyl-α-glucosamine were dissolved in benzene and allowed to crystallize. After one recrystallization from benzene the constants were: m. p. 130–131° and $[\alpha]^{19}D$ 0° (*c* 2.3, chloroform).

Anal. Calcd. for $C_{17}H_{25}O_{10}N$: C, 50.62; H, 6.25; N, 3.47. Found: C, 50.57; H, 6.23; N, 3.48.

(11) W. B. Renfrow, Jr., and C. R. Hauser, "Org. Syntheses," Coll. Vol. **2**, 609 (1943).

(12) H. Pringsheim and G. Ruschmann, *Ber.*, **48**, 680 (1915).

Pentaacetyl-N-methyl-L-mannosaminic Acid Nitrile.—Dry hydrogen chloride was passed through the ethanolic mother liquor from the preparation of N-methyl-L-glucosaminic acid nitrile[1] (from 100 g. of L-arabinose), while maintaining the temperature near 25° by appropriate cooling, until a maximum precipitation of sirupy material occurred. The supernatant liquor was removed by decantation and the sirup was washed with ether by trituration and decantation. The sirup was placed under reduced pressure until the residual ether and ethanol were removed, whereupon it was acetylated (initial cooling) with acetic anhydride (300 cc.) and pyridine (150 cc.) as described above for the acetylation of N-methyl-L-glucosamine hydrochloride. The sirup obtained on solvent removal was dissolved in 30 cc. of benzene and pentaacetyl-N-methyl-L-glucosaminic acid nitrile (18 g.) separated on nucleation and was removed by filtration. The filtrate was concentrated under reduced pressure to a sirup which was crystallized from benzene–ether–petroleum ether; yield 25 g., m. p. 100–105°. Pure material was obtained on further crystallization from the same solvent mixture and from absolute ethanol; m. p. 111–112°, $[\alpha]^{23}D$ −28° (*c* 3.8, chloroform).

In another experiment, 3.5 g. of a sirup containing the crude product was dissolved in 50 cc. of benzene and placed on a column (80 mm. diam. × 180 mm.) containing 300 g. of Magnesol–Celite (5:1)[9] and developed with 2.5 liters of benzene–ethanol (100:1). The principal zone near the center of the chromatogram was sectioned and eluted with acetone; yield 0.9 g., m. p. 110–112°. After one recrystallization from ethanol the material exhibited the constants: m. p. 112–113.5°, $[\alpha]^{27}D$ −27.5° (*c* 4.1, chloroform).

Anal. Calcd. for $C_{17}H_{24}O_9N_2$: C, 50.99; H, 6.04; N, 7.00. Found: C, 50.85; H, 6.33; N, 7.30.

N-Methyl-L-mannosaminic Acid.—Pentaacetyl-N-methyl-L-mannosaminic acid nitrile (10 g.) was suspended in 20 cc. of 2 *N* hydrochloric acid and heated in a boiling water-bath for thirty minutes. The sirup obtained by solvent removal under reduced pressure was dissolved in water containing 19 g. of barium hydroxide octahydrate and boiled for thirty minutes. The barium ion was then removed as sulfate by the addition of the equivalent amount of sulfuric acid. The filtrate was treated with an excess of silver carbonate to remove the chloride ion and excess silver ion was removed as sulfide. Since decolorizing carbon had been added before each filtration the solution was colorless at this point. Most of the solvent was removed by evaporation under reduced pressure at 50°. Absolute ethanol was added and the material crystallized; yield 0.8 g., m. p. 195° (dec., unreliable). Pure material was obtained on further crystallization from water–ethanol, m. p. 195–197° (dec., unreliable) $[\alpha]^{21}D$ +6.7° (*c*, 2.3, water), $[\alpha]^{22}D$ 0° (initial) → −32° (twenty-four hours) → slowly diminishes (*c* 2.3, 2.5% hydrochloric acid).

Anal. Calcd. for $C_7H_{15}O_6N$: C, 40.18; H, 7.22; N, 6.71. Found: C, 40.04; H, 7.25; N, 6.81.

Hydrolysis of pentaacetyl-N-methyl-L-glucosaminic acid effected in the above-described manner, produced N-methyl-L-glucosaminic acid; m. p. 215–220° (dec., unreliable), $[\alpha]^{25}D$ −5.3° (*c* 3.7, water).

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Summary

1. The hydrochlorides of N-methyl-L-glucosaminic acid and its nitrile are described.

2. Laboratory details are recorded for the preparation of N-methyl-L-glucosamine hydrochloride from the corresponding acid and for the preparation of its enantiomorph (pentaacetyl derivative) from D-glucosamine—reactions previously cited by Folkers and co-workers.

3. Pentaacetyl-N-methyl-α-L-glucosamine and pentaacetyl-N-methyl-α-D,L-glucosamine have been synthesized.

4. N-Methyl-L-glucosamine is described.

5. N-Methyl-L-mannosaminic acid has been isolated, through its acetylated nitrile, from the cyanohydrin reaction on L-arabinosyl-N-methylamine.

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Antispasmodics. I. Cyclopentyl and Δ²-Cyclopentenyl Substituted Diethylaminoethyl Esters[1]

BY ROBERT BRUCE MOFFETT, CHARLOTTE ANNE HART AND WILLARD M. HOEHN

A search of the literature reveals that very few diethylaminoethyl esters of acids containing the cyclopentyl or Δ²-cyclopentenyl groups have been prepared. Miescher and Hoffmann[2] mention a few in two patents, but no pharmacological data are given. We have therefore prepared a series of such esters in which the cyclopentyl or Δ²-cyclopentenyl group is substituted in the alpha position.

These esters were prepared by refluxing the sodium salt of the corresponding acid with diethylaminoethyl chloride. In most cases the basic ester was distilled under reduced pressure and then converted to the hydrochloride. These diethylaminoethyl esters and their hydrochlorides are listed in Table II with their physical constants. The method of preparation is illustrated in the experimental part by diethylaminoethyl α-(Δ²-cyclopentenyl)-β-phenylpropionate, and its hydrochloride.

The necessary acids were prepared from the corresponding malonic esters. Considerable difficulty was encountered in the hydrolysis of some of the disubstituted malonic esters, but it was

(1) Presented before the 110th meeting of the Am. Chem. Soc. at Chicago, Ill., September 1946.

(2) Miescher and Hoffmann, U. S. Patents 2,265,184 and 2,265,185 (1941).