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Aminocyclitols. XVI. A Synthesis and Configurational Study of Dibromo-dideoxyinosamine[1]

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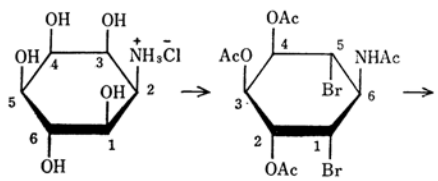
Bromination of *epi*-inosamine-2 in a mixture of acetyl bromide and acetic anhydride afforded 1, 5-dibromo-1, 5-dideoxy-*rac*-inosamine-6. From this compound, two hitherto unknown inosadiamines: *myo*-inosadiamine-2, 4 and *muco*-inosadiamine-2, 3, were obtained by displacement of the bromo groups by azide ions, followed by hydrogenation. The configurations of the new compounds obtained were established by the nuclear magnetic resonance spectra.

Bromination of inositols has been extensively studied by McCasland and his co-workers,[2] but that of inosamine or inosadiamine has never been well studied. Therefore, it seems of interest to study a bromination of aminocyclitols.

In the present paper, we wish to report the bromination of *epi*-inosamine-2 and the synthesis of two hitherto unknown inosadiamines from the dibromo-dideoxyinosamine obtained.

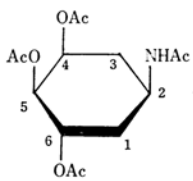
A catalytic hydrogenation of *epi*-inosose-2 phenylhydrazone[3] gave *epi*-inosamine-2 hydrochloride (I),[4,5] which was used as a starting material in the present study.

When I was heated in a mixture of acetyl bromide and acetic anhydride in a sealed tube at 145—150°C for 6 hr, and the product was acetylated with acetic anhydride in pyridine, tetraacetyl-dibromo-dideoxyinosamine (II) was obtained in a yield of 32.4%.

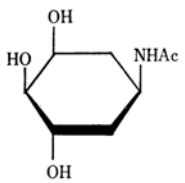


I

II



III



IV

To elucidate the structure of II, a catalytic debromination seemed to be feasible. Hydrogenolysis of II in the presence of Raney nickel and Amberlite IR-4B in aqueous ethanol afforded tetraacetyl-dideoxyinosamine (III) of mp 153—155°C. After acid hydrolysis, III showed a single spot of R_f 0.29 by ninhydrin spray in a paper chromatogram in an acetic acid-ethyl acetate-pyridine-water (1 : 5 : 5 : 3) system.[6] This provided a strong evidence that III was a single isomer.

A selective de-*O*-acetylation of III gave *N*-acetyl-dideoxy-inosamine (IV) of mp 160—161.5°C, which consumed 1.99 mol of periodate per each molecule. Accordingly, there might be two possible structures as follows: 1-acetamido-3, 4, 5-cyclohexanetriol (IVa) and 1-acetamido-2, 3, 4-cyclohexanetriol (IVb).

The nuclear magnetic resonance (NMR) spectrum of III revealed three sharp signals at τ 7.91 (6H), 7.97 (3H) and 8.03 (3H) which were attributed to two axial acetoxy, an equatorial acetoxy and an equatorial acetamido groups respectively. The doublet in the lowest field (τ 3.77) was a typical -NH- proton signal with a spin-spin coupling constant of 9.0 cps. Three ring protons on the carbon atoms which held acetoxy groups showed a signal at τ 4.90. A proton on the carbon atom which had an acetamido group appeared at τ 5.75 as a nine-line pattern, owing to the existence of two vicinal methylene groups (Fig. 1).

A spin decoupling technique was a valuable aid in the analysis of a complex spectrum,[7] and was used in the present study. When N-C-H proton was irradiated, the doublet at τ 3.77 changed to a single peak. When the methylene protons were

1) Reported in part in the abstracts of papers, 20th Annual Meeting of the Chemical Society of Japan, Tokyo, March 31, 1967, p. 593.
2) G. E. McCasland, "Advances in Carbohydrate Chemistry," **20**, 12 (1965).
3) T. Posternak, *Helv. Chim. Acta*, **19**, 1333 (1936).
4) E. L. May and E. Mosettig, *J. Org. Chem.*, **14**, 1137 (1947).
5) H. E. Carter, R. K. Clark, Jr., B. Lytle and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1948).
6) F. G. Fischer and H. Dorfel, *Hoppe-Seyler's Z. physiol. Chem.*, **301**, 224 (1955).
7) G. E. McCasland, "Advances in Carbohydrate Chemistry," **20**, 54 (1965).

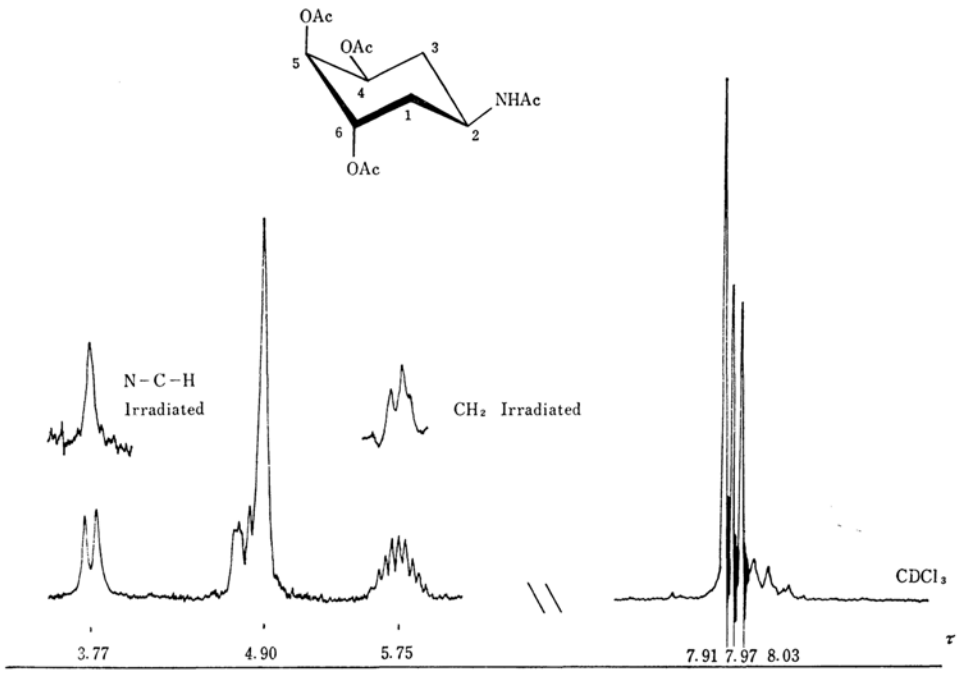
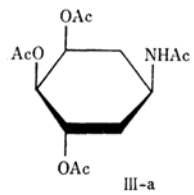


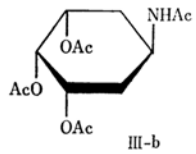
Fig. 1.

irradiated, the nine-line pattern collapsed to a doublet with a spin-spin coupling constant of 9.0 cps.

This fact suggested that the two methylene groups were located in the neighboring positions of the carbon atom which had the acetamido group. Thus the structure of IVb was excluded. Now the possible structures of III were limited in the following two configurations:



III-a



III-b

The compound, IIIa, had been synthesized in an optically active form by Fischer and Dangschat in 1934 from dihydroshikimic acid by Curtius degradation.[8] The infrared spectrum of the authentic sample[9] in chloroform was superimposable with that of III. Therefore, III was assigned to be DL-tetraacetyl-1, 3-dideoxy-*epi*-inosamine-2. Hence the configuration of II was also established except those of the two bromo groups.

A mechanism in bromination of inositols has been proposed by McCasland *et al.*,[10] and an acetoxonium ion is propounded as an intermediate in the conversion of *cis*-monoacetate to *trans*-2-acetoxycyclohexyl chloride by concentrated hydrochloric acid.[11] Yet, there were not enough data to predict the configuration of the bromo groups in the dibromo-dideoxyinosamine obtained.

Then the NMR spectrum of II was measured in $CDCl_3$, which revealed four sharp signals with equal intensities at τ 7.78, 7.83, 7.91 and 7.93. The first two peaks were attributed to two axial acetoxy groups. The third signal was ascribed to an equatorial acetoxy group with deshielding by the neighboring bromo group. The last signal was assigned to an equatorial acetamido group which was shifted to a lower field with deshielding by the two vicinal bromo groups.[12] The signals of the ring protons were not well resolved to permit an analysis of the configuration.

Therefore, an attempt to establish the configuration by replacing bromo groups by nucleophilic reagents was made, and II was tentatively assigned to be DL-tetraacetyl-1, 5-dibromo-1, 5-dideoxy-*rac*-inosamine-6 by the reactions as described below.

When II was heated with sodium acetate in

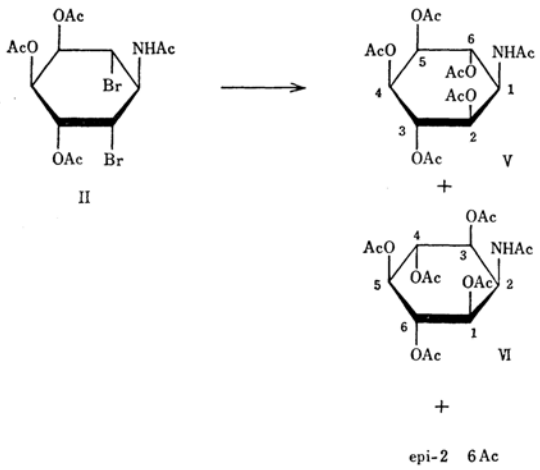
8) H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, **17**, 1200 (1934).

9) An authentic sample of tetraacetyl-1, 3-dideoxy-*epi*-inosamine-2 was synthesized from shikimic acid and presented by Doz. Dr. Frieder W. Lichtenthaler of Institut für Organische Chemie, Technische Hochschule, Darmstadt, Germany, to whom the authors' thanks are due.

10) G. E. McCasland and E. C. Horswill, *J. Am. Chem. Soc.*, **75**, 4020 (1953).

11) R. Boschan and S. Winstein, *ibid.*, **78**, 4921 (1956); E. S. Gould, "Mechanism and Structure in Organic Chemistry," Rinehart and Winston, New York (1956), p. 566.

12) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York (1962), p. 53.

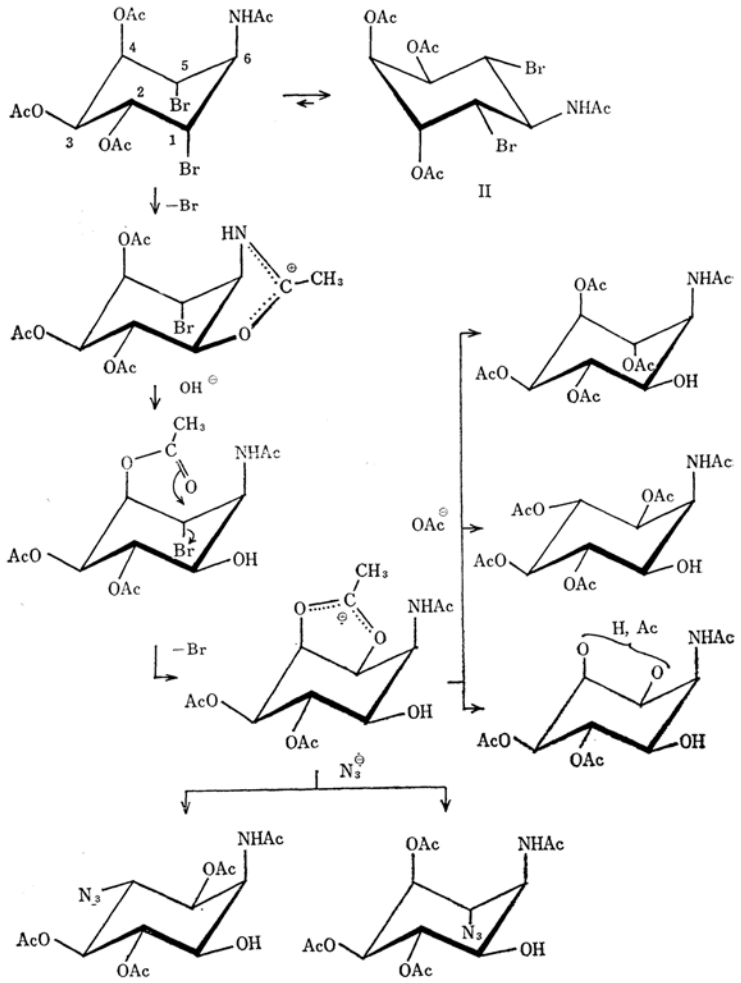


aqueous 2-methoxyethanol, three known inosamines: *muco*-1,[13] *myo*-2[14] and *epi*-inosamine-2,[4,5] were obtained.

From a mechanistic stand point, the following mechanism could be proposed.

Since the replacement of sulfonyloxy group by an acetate ion with a participation of vicinal acylamido group in cyclohexane ring gives *cis*-acylamido alcohol in an aqueous solvent,[15,16] an analogous mechanism could be proposed to the present reaction. That is, the bromo group on C-1 was displaced by a participation of the vicinal acetamido group through an intermediate oxazolinium ion to give *cis*-acetamido alcohol.

An acetoxonium ion was also pointed as an intermediate in the reaction of *trans*-2-acetoxycyclohexyl bromide with silver acetate. In wet

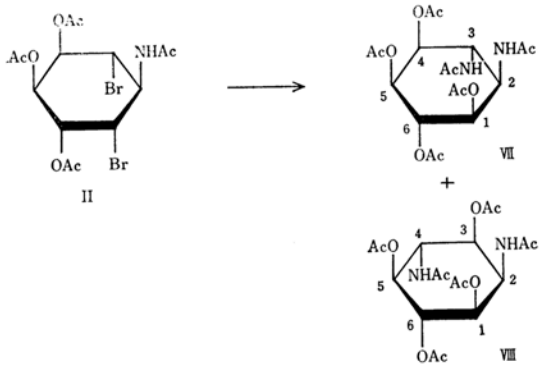


13) T. Suami and S. Ogawa, This Bulletin, **37**, 1238 (1964).
14) F. W. Lichtenthaler and H. O. L. Fischer, *J. Am. Chem. Soc.*, **83**, 2005 (1961).
15) S. Winstein and R. E. Buckles, *ibid.*, **64**, 2787 (1942).
16) G. E. McCasland, R. K. Clark, Jr., and H. E. Carter, *ibid.*, **71**, 637 (1949).

solvent, some of the ions were diverted to *cis*-1 2-cyclohexanediol monoacetate.[17] Therefore in the present reaction the displacement of another bromo group in II took place with a participation of the acetoxy group on C-4 to form an intermediary acetoxonium ion, which was attacked by an acetate ion or by water to give three isomers as shown in the following scheme.

This reaction was explained only with the above mentioned tentative configuration of II, but with the other configurations it was unable to elucidate it without contradictions.

When an analogous reaction was carried out with sodium azide and the azido-compounds obtained were catalytically hydrogenated, two hitherto unknown inosadiamines: hexaacetyl-*muco*-inosadiamine-2, 3 (VII) and hexaacetyl-*myo*-inosadiamine-2, 4 (VIII), were obtained.



The configurations of VII and VIII were established by the NMR spectra and chemical evidences. The spectrum of VII in deuteriodimethylsulfoxide revealed four sharp signals at τ 7.94 (6H), 7.98 (3H), 8.09 (3H) and 8.23 (6H), which were ascribed to two axial acetoxy, an axial acetoxy, an equatorial acetoxy and two equatorial acetamido groups respectively. The two –NH– protons showed two doublets at τ 2.26 and 2.48, suggesting that the two acetamido groups were conformationally nonequivalent. The di-*N*-acetyl derivative (IX) prepared from VII consumed 2.96 mol of periodate per each molecule. These facts were compatible with the configuration of *muco*-inosadiamine-2, 3.

The spectrum of VIII in deuterium oxide[18] revealed four signals at τ 7.90 (3H), 7.95 (6H), 7.99 (6H) and 8.07 (3H). The first three peaks were attributed to an axial acetamido and four equatorial acetoxy groups, and the last peak was ascribed to an equatorial acetamido group. The di-*N*-acetyl derivative (X) obtained from VIII consumed 1.86 mol of periodate per each molecule, where 0.89 mol of formic acid was determined.[19] These data were consisted only with the configuration of *myo*-inosadiamine-2, 4.

An analogous reaction mechanism as described above was proposed in the reaction with sodium azide. An intermediary acetoxonium ion was attacked by a highly nucleophilic azide ion, but not by water in this reaction. This reaction was also able to be interpreted only with the above mentioned configuration of II.

Therefore, the structure of II was assigned, but not conclusively, to be DL-tetraacetyl-1, 5-dibromo-1, 5-dideoxy-*rac*-inosamine-6.

Experimental

The melting points reported were determined in a liquid bath and uncorrected. The NMR spectra of the samples were recorded on Japan Electron Optics JNM-C-60 and JNM-4H-100 spectrometers at the frequency of 60 and 100 Mcps in deuteriochloroform, deuterium oxide or deuteriodimethylsulfoxide (d_6-DMSO) with tetramethylsilane, sodium trimethylsilylpropanesulfonate or tetramethylsilane respectively as an internal standard. The peak positions are given in τ-values.

DL-Hexaacetyl-*epi*-inosamine-2. DL-*epi*-Inosose-2 phenylhydrazone (10 g)[3] was hydrogenated in glacial acetic acid (100 m*l*) with Adams platinum oxide (300 mg) in a hydrogen stream at 30—35°C for 8 hr, and the reduction product was acetylated to give the crude product (14.1 g, 87.7% yield) of mp 157—160°C. Recrystallization from acetone-ethanol gave an analytically pure sample which melted at 158°C, resolidified and melted again at 189—190°C. (Lit.,[3] mp 192—194°C). (Found: C, 49.70; H, 6.16; N, 3.22%). NMR (d_6-DMSO): axial OAc, τ 7.80 (3H); equatorial OAc, τ 7.97 (3H), 8.02 (3H), 8.05 (6H); axial NHAc, τ 8.07 (3H).

DL-*epi*-Inosamine-2 Hydrochloride (I). Hydrolysis of DL-hexaacetyl-*epi*-inosamine-2 in 6 N hydrochloric acid yielded a crystalline product in 81.5% yield. Mp 227—230°C. (Lit. mp 223—226°C,[4] 234—236°C[5]).

DL-Tetraacetyl-1, 5-dibromo-1, 5-dideoxy-*rac*-inosamine-6 (II). A mixture of I (5 g), acetyl bromide (7 m*l*) and acetic anhydride (13 m*l*) was heated at 145—150°C for 6 hr in a sealed tube. Then the mixture was poured into 25 m*l* of ethanol and evaporated under reduced pressure. The residue was acetylated with acetic anhydride and pyridine overnight and then evaporated. The residue was extracted with chloroform (200 m*l*), and the solvent layer was washed with cold water (100 m*l*×3). After drying over anhydrous sodium sulfate, the chloroform solution was evaporated. The semicrystalline residue was dissolved in acetone (20 m*l*) and water was added to the solution until a slight turbidity appeared. After settled in a refrigerator overnight, the crystals (3.56 g, 32.4% yield) were collected by filtration and washed with cold aqueous acetone. Recrystallization from aqueous acetone gave needles of mp 227—230°C.

17) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Rinehart and Winston, New York (1965), p. 565.
18) F. W. Lichtenthaler, *Chem. Ber.*, **99**, 903 (1996).
19) J. M. Bobbitt, "Advances in Carbohydrate Chemistry," Vol. XI, Academic Press, New York (1956), pp. 1—41.

Found: C, 36.14; H, 3.99; N, 3.08; Br, 34.25%. Calcd for $C_{14}H_{19}NO_7Br_2$: C, 35.54; H, 4.05; N, 2.96; Br, 33.78%.

DL-Tetraacetyl-1, 3-dideoxy-*epi*-inosamine-2 (III). A mixture of II (1.0 g), 50% aqueous ethanol (40 m*l*) and Amberlite IR-4B (8 m*l*) was hydrogenated with Raney nickel at 30°C under 4.1 kg/cm² of hydrogen pressure for 20 hr. After the catalyst was removed by filtration, the filtrate was evaporated under reduced pressure. The residue was extracted with chloroform and the chloroform extract was purified by passing through a short aluminum oxide column. An excess solvent was evaporated and the residue was dissolved in ethanol. Petroleum ether was added to the ethanolic solution until a slight turbidity appeared to give needles (0.38 g, 57.0% yield). Recrystallization from ethanol-petroleum ether gave an analytical sample of mp 153—155°C.

Found: C, 53.51; H, 6.94; N, 4.83%. Calcd for $C_{14}H_{21}NO_7$: C, 53.32; H, 6.71; N, 4.44%.

The IR spectrum of the product in chloroform was superimposable with that of an authentic sample[8,9] in the same solvent.

DL-*N*-Acetyl-1, 3-dideoxy-*epi*-inosamine-2 (IV). A 200 mg portion of III was dissolved in 40 m*l* of methanol previously saturated with ammonia, and the solution was settled overnight at room temperature. After evaporation to dryness, the residue was crystallized from a mixture of ethanol and acetone to give 101 mg of the crude product (84.0% yield). Recrystallization from the same solvents afforded an analytical sample of mp 160—161.5°.

Found: C, 50.99; H, 8.17; N, 7.38%. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40%.

Periodate Oxidation. A 52.3 mg portion of the product was dissolved in 50 m*l* of a 0.0307 M sodium metaperiodate solution. Iodometric titrations with sodium arsenite[13] showed that 1.99 mol of periodate was consumed per each molecule of the sample in 26 hr.

Paper Chromatography. After hydrolysis in 6 N hydrochloric acid, III gave a single spot of R_f 0.29 in ascending paper chromatography in an acetic acid-ethyl acetate - pyridine - water(1 : 5 : 5 : 3) system[6] at 8—12°C. (R_f of D-glucosamine hydrochloride: 0.32).

DL-Hexaacetyl-*muco*-inosamine-1 (V) and Hexaacetyl-*myo*-inosamine-2 (VI). A mixture of II (1.0 g), sodium acetate (1.0 g) and 87% aqueous 2-methoxyethanol (40 m*l*) was heated under reflux for 45 hr. The solvent was removed under reduced pressure and the residue was acetylated with acetic anhydride in pyridine. After an insoluble material was removed by filtration, the filtrate was evaporated. The residue was crystallized in ethanol. The product was fractionally recrystallized from ethanol to give three hexaacetyl-inosamines. DL-Hexaacetyl-*muco*-inosamine-1 (0.25 g), mp 201—202°C, was obtained as prisms in 27.8% yield, which was identified with an authentic sample[13] by a mixed melting point determination and infrared spectrum. (Found: C, 50.27; H, 6.01; N, 3.15%). Hexaacetyl-*myo*-inosamine-2 (95 mg) was obtained as plates of mp 239—242°C in 10.5% yield, which was identified with the melting point and NMR spectrum[14] (Found: C, 50.36; H, 6.15; N, 3.19%). DL-Hexaacetyl-*epi*-inosamine-2 (106 mg) was recovered as needles in 11.8% yield, which was identified with an authentic sample by infrared spectrum and a mixed melting point determination.

DL-Hexaacetyl-*muco*-inosadiamine-2, 3 (VII) and DL-*N*, *N'*-diacetyl-*myo*-inosadiamine-2, 4 (X). A mixture of II (2.0 g) and sodium azide (2.0 g) in 80% aqueous 2-methoxyethanol (80 m*l*) was heated under reflux for 50 hr. After evaporation in vacuo, the mixture was acetylated with acetic anhydride in pyridine. The mixture was evaporated again to give an oily residue. The residue was hydrogenated in 40 m*l* of ethanol in the presence of Adams platinum oxide for 8 hr under a hydrogen pressure of 3.4 kg/cm². After the catalyst was removed, the ethanolic solution was evaporated to give a residue, which was acetylated with acetic anhydride in pyridine. An excess acetylating reagent was removed by evaporation and the residue was crystallized in ethanol. The crystals were collected by filtration to give 0.50 g (27.6% yield) of the crude VII of mp 269—271°C. An analytical sample of mp 270—271°C was obtained by recrystallization from ethanol.

Found: C, 50.49; H, 6.02; N, 6.09%. Calcd for $C_{18}H_{26}N_2O_{10}$: C, 50.23; H, 6.09; N, 6.51%.

The ethanolic mother liquor was evaporated to give an oily residue, which was dissolved in 50 m*l* of methanolic ammonia. After settled overnight at room temperature, the solution was evaporated under reduced pressure to give 0.49 g (39.2% yield) of the crude X, mp 245—253°C. The product was recrystallized from ethanol to give 0.26 g (20.8% yield) of crystals, mp 260—261°C.

Found: C, 45.99; H, 7.16; N, 10.79%. Calcd for $C_{10}H_{18}N_2O_6$: C, 45.79; H, 6.92; N, 10.68%.

Paper Chromatography. After hydrolysis in 6 N hydrochloric acid, VII and X showed each single spot of R_f 0.25 and 0.12 respectively in an ascending paper chromatography in acetic acid - ethyl acetate - pyridine - water (1 : 5 : 5 : 3) system,[6] (R_f of D-glucosamine hydrochloride, 0.32—0.33).

Periodate Oxidation. A 51.7 mg portion of X was oxidized as described above to show 1.86 mol consumption of periodate and 0.89 mol of formic acid per each molecule of the sample was determined.[19]

DL-*N*, *N'*-Diacetyl-*muco*-inosadiamine-2, 3 (IX). A 400 mg portion of VII was dissolved in 50 m*l* of methanolic ammonia and settled overnight at room temperature. After evaporation under reduced pressure, the solution gave an oily residue, which was crystallized in a mixture of ethanol (5 m*l*) and ethyl acetate (2 m*l*). The crystals (196 mg) of mp 216—219°C were collected by filtration. Analytical sample (145 mg) of mp 221—222°C was obtained by recrystallization of the crude product from a mixture of ethanol, ethyl ether and water.

Found: C, 45.58; H, 7.09; N, 10.84%. Calcd for $C_{10}H_{18}N_2O_6$: C, 45.79; H, 6.92; N, 10.68%.

Periodate Oxidation. A 48.7 mg portion of IX was oxidized as descibed above to show 2.96 mol consumption of periodate per each molecule of the sample. 2.06 mol of formic acid was determined by titrating the oxidation mixture with 0.05 N sodium hydroxide solution.[19]

DL-Hexaacetyl-*myo*-inosadiamine-2, 4 (VIII). Acetylation of X (200 mg) in acetic anhydride and pyridine gave the crude product. The product was recrystallized from ethanol - petroleum ether to give 160 mg (48.7%) of crystals, mp 231—233°C.

Found: C, 50.50; H, 6.34; N, 6.42%. Calcd for $C_{18}H_{26}N_2O_{10}$: C, 50.23; H, 6.09; N, 6.51%.

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