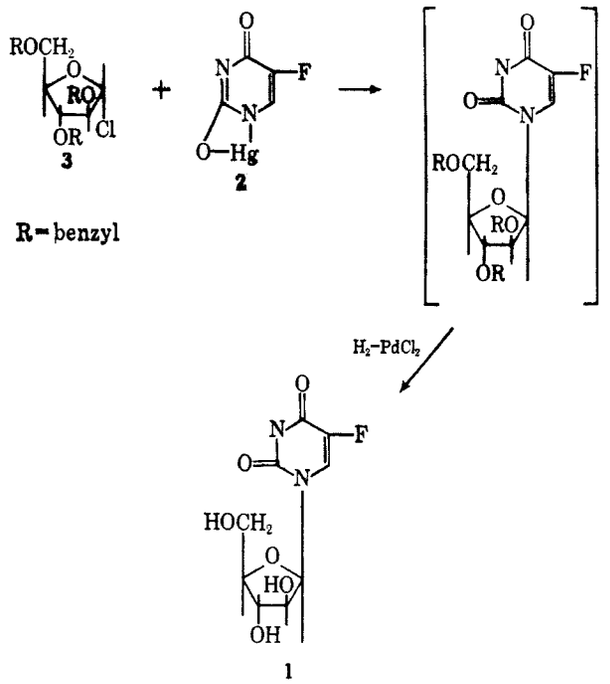
SCHEME I



(tlc, paper chromatography), and had constants in good agreement with those appearing in the literature.[3-5]

Experimental Section

1-(β-D-Arabinofuranosyl)-5-fluorouracil (1).—2,3,5-Tri-O-benzyl-1-(*p*-nitrobenzoyl)-D-arabinofuranose[12] (56.96 g, 0.1 mole), prepared in 90% yield from 2,3,5-tri-O-benzyl-β-D-arabinofuranose,[13] was added to dry methylene chloride (700 ml) which had been saturated with hydrogen chloride at 0°. The solution was maintained at 0° for 3 hr while protecting from moisture and maintaining a slow stream of hydrogen chloride through the reaction mixture. The *p*-nitrobenzoic acid which had separated in quantitative yield was removed by rapid filtration through a sintered glass funnel and the filtrate was concentrated to dryness *in vacuo* (bath 35°) and evacuated (0.1 mm) for 16 hr (25°). Monomercuri-5-fluorouracil[2,14] (16.43 g, 0.05 mole) was suspended in toluene (1 l.) and dried azeotropically by the distillation of a portion of the solvent (250 ml). A second portion of distillate (250 ml) was reserved for use in transferring the chloro sugar to the slightly warm, dried suspension of monomercuri-5-fluorouracil. Heating and stirring while protecting from moisture was recommended upon addition of the halo sugar. The mixture was refluxed for 15 min yielding a nearly clear solution which was cooled rapidly (ice bath) and filtered from a trace of insolubles. The filtrate was washed with 30% aqueous potassium iodide (two 250-ml portions) and water (250 ml), stirred with saturated sodium bicarbonate (500 ml) for 30 min, and washed with water (500 ml). The dried (magnesium sulfate) organic layer was concentrated to dryness *in vacuo* (30° bath) yielding a brown oil (50 g) which was debenzylated without further purification. The residual oil was dissolved in dry methanol (400 ml) and hydrogenated in two batches, each being added to palladium chloride (10.5 g) which had been suspended in dry methanol and prereduced just prior to the addition of the blocked nucleoside. The hydrogenation was carried out at an initial pressure of 3 atm, reduction being complete in approximately 40 min. The hydrogenation mixtures were filtered free of catalyst and the filtrates were brought to pH 5.5 by stirring with Dowex 2X8 (HCO_3^-) ion-exchange resin. The resin was removed by filtration and washed with methanol, and the combined filtrates and washings were concentrated to dryness *in vacuo* (35° bath) yielding a brown oil (21.6 g). The residue was dissolved in a 25:15 methanol–water mixture (400 ml) and stirred with freshly prepared Dowex 2X8 (OH^-) ion-exchange resin. After 15 min the supernatant appeared free of nucleoside by thin layer chromatography (silica gel; benzene–*n*-butylamine–water, 15:5:1). The resin was removed by filtration and washed with water until the washings were neutral (the filtrate and washings being discarded). The nucleoside was eluted from the resin by treatment with 5% acetic acid (six 200-ml portions) each portion being stirred for 10 min and the progress of the elution followed by thin layer chromatography. The combined acetic acid solutions were clarified by filtration through Celite and concentrated to dryness *in vacuo* (35° bath). Absolute ethanol (two 20-ml portions) was added to the residue and removed *in vacuo* to effect a final drying of the residue which was obtained as a foamed glass (10 g). This material on solution in ethanol (40 ml) and chilling afforded the product (I) as colorless crystals: 3.92 g, 30.2%, mp 183–185°, $[\alpha]^{26.0}D$ +129.4° (*c* 0.2, H_2O) [lit.[3,4] mp 187–188°, $[\alpha]^{24}D$ +128° (H_2O)]. On concentration of the mother liquors a second crop (0.282 g, 2.1%) was obtained: mp 176–179°. The material was homogeneous on paper chromatography (*n*-BuOH–H_2O, R_f 0.42) and showed the absence of 1-β-D-arabinofuranosyluracil (R_f 0.32). A portion of the first crop material was recrystallized from ethanol to yield the analytical sample: mp 184–186°, $[\alpha]^{26.0}D$ +125.0° (*c* 0.2, H_2O); $\lambda_{max}^{0.1\ N\ HCl}$ 270 mμ (ε 8900), $\lambda_{min}^{0.1\ N\ HCl}$ 234 mμ (ε 1310), $\lambda_{max}^{pH\ 7}$ 270 mμ (ε 8460), $\lambda_{min}^{pH\ 7}$ 237 mμ (ε 2990), $\lambda_{max}^{0.1\ N\ NaOH}$ 272 mμ (ε 7470), $\lambda_{min}^{0.1\ N\ NaOH}$ 247 mμ (ε 4030) [lit.[4] $\lambda_{max}^{pH\ 1}$ 270 mμ (ε 9080), $\lambda_{max}^{pH\ 7}$ 270 mμ (ε 8670), $\lambda_{max}^{pH\ 13}$ 272 mμ (ε 7590)].

Anal. Calcd for $C_9H_{11}FN_2O_6$ (262.2): C, 41.22; H, 4.23; F, 7.25; N, 10.68. Found: C, 40.91; H, 4.30; F, 7.41; N, 10.45.

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(14) The 5-fluorouracil used in this work was generously provided by Hoffmann LaRoche, Inc., Nutley, N. J.

Anomeric Equilibria in Derivatives of Amino Sugars. 2-Amino-2-deoxy-D-mannose Hydrochloride[1,2]

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Crystalline 2-amino-2-deoxy-D-mannose hydrochloride[3] differs from other crystalline 2-amino-2-deoxy-D-hexose hydrochlorides[4] in that it has not been observed to exhibit mutarotation in water or dilute hydrochloric acid.[3-11] The observed specific rotation is about −3°. It has been proposed[12] that the molecule is stabilized as the β-D anomer in the *C1* chair conformation (2), by the formation of hydrogen bonds between hydrogens of the (axial) ammonium group at C-2 and the equatorial oxygen atoms at C-1 and C-4, with the result that conversion into the α-D anomer (1) is prevented. It has been pointed out[13] that a hydrogen bond from the am-

(1) Supported in part by Grants-in-Aid No. 19187 and 170200 from The Ohio State University Development Fund.
(2) Funds for purchase of the nmr spectrometer were provided by the National Science Foundation, Washington, D. C.
(3) P. A. Levene, *J. Biol. Chem.*, **39**, 69 (1919).
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monium group to O-4 is sterically impossible. The suggestion has been made[14] that the axial ammonium group reduces the rate of protonation of the ring oxygen atom to such an extent that mutarotation becomes too slow to be observed. An opposite viewpoint has been advanced[15] to explain why mutarotation in water is not observed with 2-amino-3,6-anhydro-2-deoxy-D-mannose hydrochloride; the ammonium ion is considered to act as a very effective proton donor to the ring oxygen atom, with the result that anomerization is fast, and an equilibrated mixture of anomers is already present when the first polarimetric measurement is made after dissolution of the sample. In the present work it is shown that equilibrium between tautomers is established very rapidly when crystalline 2-amino-2-deoxy-D-mannose hydrochloride is dissolved in water, to give a mixture of the α-D- and β-D-pyranose forms, with the latter preponderating. The α-D form is the more stable anomer in methyl sulfoxide solution.

Crystalline 2-amino-2-deoxy-D-mannose hydrochloride, prepared[8] by hydrolysis of crystalline, chromatographically homogeneous 2-acetamido-2-deoxy-D-mannose monohydrate,[8,16,17] had a melting point and specific rotation in good agreement with literature values. When dissolved in water, mutarotation was not observed. In methyl sulfoxide solution, the substance exhibited slow upward mutarotation, from −5 (12 min) to +0.6° (7 days). The crystalline substance showed absorption in the infrared at 12.06 μ, suggesting[18,19] that some of the α-D-pyranose form (1) was present.

The nmr spectrum of 2-amino-2-deoxy-D-mannose hydrochloride, measured shortly after dissolution of the crystalline material in deuterium oxide, was not noticeably different from spectra measured several hours later. Two narrow doublets, total integral one proton, were observed at lowest field: that at τ 4.60 was assigned to H-1 of **1** (equatorial in the *C1* conformation), and that at τ 4.78 was assigned to H-1 of **2** (axial in the *C1* conformation). Integration of the spectrum indicated that **1** and **2** were present in the relative proportions of 43 and 57%. The difference in chemical shift of the H-1 signal for the two forms is ~0.2 ppm, close to the difference in shift of 0.28 ppm observed[20] for the anomeric D-mannopyranoses. The signals assigned to H-1 of **1** and **2** appear about 0.2 ppm downfield from the H-1 signals of α-D- and β-D-mannopyranose,[20] presumably because of the extra inductive effect of the NH_3^+ group. Downfield shifts of this magnitude have been observed for the H-1 signals of the anomers of 2-amino-2-deoxy-D-glucose and -D-galactose hydrochlorides, in comparison[21] with the corresponding anomers of D-glucose and D-galactose. No signals in the region τ 4.3–4.4 were observed, indicating that no detectable proportion of furanose forms[15,20] was present. The signal for H-1 (axial) of **2** was a sharp doublet ($J_{1,2}$ = 1.5 cps); that for H-1 (equatorial) of the anomer (**1**) was also a narrow doublet, but it was less well resolved, probably because of virtual coupling.[22]

In methyl sulfoxide solution, the exchange of hydroxyl protons of simple sugars is slow, and well-defined signals for these protons can be observed in nmr spectra.[23,24] The spectrum of 2-amino-2-deoxy-D-mannose hydrochloride in methyl sulfoxide was complex and indicated that moderately rapid exchange was taking place between the hydroxyl and NH_3^+ hydrogens. Absorptions of the exchanging hydrogens in the τ 4–5.5 spectral region made it difficult to observe accurately the signals of the C-1 hydrogens of the anomeric forms. Deuteration of the sample simplified the spectrum, and the signals for the anomeric hydrogen of **1** and **2** were observed as narrow peaks at τ 4.78 and 5.00, in the intensity ratio 2:1. The latter ratio indicates that the α-D form (**1**) was the more stable anomer in methyl sulfoxide.

The H-1 signals of **1** and **2** in methyl sulfoxide are observed about 0.2 ppm upfield from their positions in the spectrum of the substance in deuterium oxide. These relative positions accord with the observations of Casu and co-workers[23] on simple sugars. Since discrete signals for the hydroxyl hydrogens were not observed in the spectra in methyl sulfoxide, it is evident that the NH_3^+ group facilitates exchange of hydroxyl protons in this aprotic solvent; a similar exchange has been noted[15] with 2-amino-3,6-anhydro-2-deoxy-D-mannofuranose hydrochloride.

In order to observe the equilibration between anomers in methyl sulfoxide, a sample of crystalline 2-amino-2-deoxy-D-mannose hydrochloride having all exchangeable hydrogens replaced by deuterium was prepared; it had an X-ray powder diffraction pattern identical with that of the nondeuterated material. The nmr spectrum in methyl sulfoxide, measured 2–4 min after dissolution, showed signals at τ 4.78 and 5.00, indicating that both anomers were present, with **2** preponderating over **1** by about 3 to 1. The relative proportions of the anomers showed little detectable change during 1 hr, but over a period of several days the proportion of **1** increased at the expense of **2**. At equilibrium, the α-D anomer (**1**) was preponderant. Addition of deuterium oxide causes a change in the composition, to give more of the β-D anomer (**2**).

The foregoing data establish that 2-amino-2-deoxy-D-mannose hydrochloride, as crystallized from water–ethanol–acetone, consists largely of the β-D-pyranose form **2**, apparently cocrystallized with some of the α-D-pyranose anomer **1**. A number of instances have been reported where cocrystallization of anomeric mixtures of sugars has taken place.[24] The data accord with the viewpoint[15] that the axial NH_3^+ group accelerates the process of tautomeric interconversion by permitting facile intramolecular protonation of the ring oxygen atom. Tautomeric interconversion in methyl sulfoxide is predictably slower than in water, because the former solvent cannot function as a good acceptor for the 1-OH proton during the ring-opening step.

In methyl sulfoxide solution, the α-D anomer is preponderant, as would be predicted from consideration of

(14) Reference 13, p 422.
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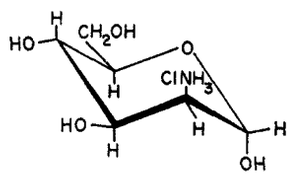
the anomeric effect.[25] In water, where considerable stabilization of an equatorial hydroxyl at C-1 can be achieved through solvation, the β-D anomer is the more stable.

It has been reported[12] that 2-amino-2-deoxy-D-mannose, prepared by treatment of an aqueous solution of the hydrochloride with sodium hydroxide, exhibits downward mutarotation. The present data clearly indicate that the free base present initially must have been an anomeric mixture. Since gross changes are possible under basic conditions, it is difficult to interpret the reported[12] mutarotation in terms of equilibria between tautomeric forms.



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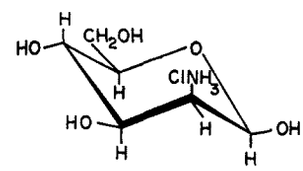

2

Experimental Section[26]

Nmr Measurements.—Spectra were measured with a Varian A-60 spectrometer; the temperature of the probe was approximately 40°. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (τ 10.00) was used as the internal standard for spectra measured in deuterium oxide, and tetramethylsilane (τ 10.00) was used as internal standard for spectra measured in methyl sulfoxide-d_6 or *N*,*N*-dimethylformamide. Chemical shifts were measured directly from spectra determined at a sweep width of 500 cps. The recorded J values are first-order coupling constants, as measured directly from spectra determined at a sweep width of 100 cps. Integrated peak intensities (±3%) are the mean of several integration curves, determined in both directions at a sweep width of 100 cps, and were also determined with the use of a planimeter. Deuteration was performed by adding 1 drop of deuterium oxide to the prepared sample. Each experiment was repeated a number of times, and concordant spectral data were recorded. Solutions were kept at room temperature except during spectral measurements.

2-Amino-2-deoxy-D-mannose Hydrochloride.—2-Acetamido-2-deoxy-D-mannose monohydrate[16,17] was hydrolyzed,[8] and the product was crystallized from water–ethanol–acetone as small, clear prisms: yield 88%; mp 178–180° dec; $[\alpha]^{22}D$ −3.7 ± 0.5° [3 min, unchanged after 24 hr (*c* 1.4, water)] [lit.[4,11] mp 178–180°, $[\alpha]^{25}D$ −3.2° (*c* 10, water)], $[\alpha]^{22}D$ −5.2 ± 0.6° (15 min) → −3.9 ± 0.6° (12 hr) → +0.6 ± 0.6° [7 days, final (*c* 3.6, methyl sulfoxide)]; λ_{max}^{Nujol} 12.06 μ (weak) (equatorial H-1 in pyranoid ring[18,19]); R_g[27] 1.13; X-ray powder diffraction data, 7.44 m, 6.71 s (4), 6.51 w, 5.61 m, 4.87 m, 4.62 vw, 4.33 vw, 4.21 s (3), 4.02 w, 3.95 vs (1,1), 3.69 vs (1,1), 3.41 s (2), 3.22 vw, 3.11 vw, 2.99 w, 2.87 vw, 2.79 w, 2.68 vw, 2.56 m, 2.51 vw, 2.44 m.

Anal. Calcd for $C_6H_{14}ClNO_5$: C, 33.26; H, 6.48; N, 6.50. Found: C, 33.04; H, 6.11; N, 6.66.

The X-ray powder diffraction pattern was identical with that recorded by Comb and Roseman.[7] The substance was recovered unchanged from the equilibrated solution in methyl sulfoxide, by evaporation and crystallization of the residue from water–ethanol–acetone.

2-Amino-2-deoxy-D-mannose hydrochloride, having all exchangeable hydrogens replaced by deuterium, was prepared by evaporating a solution of the sugar in deuterium oxide several times with small portions of deuterium oxide; the resultant syrup crystallized on standing. A portion of this product was dissolved in a small amount of deuterium oxide, ethyl deuterioxide was added, followed by acetone, and the substance was allowed to crystallize slowly. The clear prisms thus obtained had an X-ray powder diffraction pattern identical with that of the nondeuterated material.

Nmr Spectrum of 2-Amino-2-deoxy-D-mannose Hydrochloride.

A. In Deuterium Oxide.—A 28–35% solution of the sugar in deuterium oxide showed two doublets at lowest field, total integral of one proton, at τ 4.60 (broadened, $J_{1,2}$ = 1.1 cps, H-1 of α-D anomer, **1**) and 4.78 ($J_{1,2}$ = 1.5 cps, H-1 of β-D anomer, **2**), in relative proportion 43:57. The HOD signal was observed at τ 5.34, and the protons on C-2, -3, -4, -5, and -6 gave a six-proton multiplet, τ 5.85–6.60. There was little observable difference between a spectrum measured 30 sec after dissolution of the crystalline sample, and spectra measured 5 hr and 3 days later.

B. In Methyl Sulfoxide.—A 25% solution of the sugar in methyl sulfoxide-d_6 containing approximately 10% of deuterium oxide showed two signals, total integral 1 proton, below τ 5.5; a narrow, unresolved multiplet, 4.78, width at half-height of 3.2 cps (H-1 of α-D anomer, **1**) and a doublet at 5.00 [$J_{1,2}$ = 1.4 cps (H-1 of β-D anomer, **2**)]. Integration indicated that the α-D and β-D anomers were present in approximately 2 to 1 proportion. Signals of the protons on C-2, -3, -4, -5, and -6 gave a multiplet, τ 5.9–6.8. Addition of more deuterium oxide caused the signal at τ 5.00 to increase in intensity at the expense of the signal at 4.78.

The spectrum of the sugar, measured a few minutes after dissolution in dry methyl sulfoxide, showed a broad signal, τ 1.7–2.3 (NH_3^+) and a complex series of signals, 4.6–5.6. After 2 days the spectrum showed a broad signal, τ 1.90 (less than three protons, NH_3^+), a signal ~4.75 (H-1 of α-D anomer, **1**), and a broad multiplet, 4.95–5.3 (greater than four protons, OH, H-1 of β-D anomer, **2**). After deuteration, the solution gave a spectrum identical with that observed with a freshly prepared solution of the sugar in methyl sulfoxide–deuterium oxide.

Deuterium-exchanged 2-amino-2-deoxy-D-mannose hydrochloride (70 mg), which had been crystallized from a solvent mixture, was dissolved in methyl sulfoxide-d_6 (0.28 ml). Spectra measured 4, 8, 15, and 40 min after dissolution showed signals at τ 4.78 and 5.00 in approximate proportion 1:3; no difference in relative intensities of the two signals was observed during this period. After 2 days, the two signals were of approximately equal intensity, and after 4 days the intensity ratios were approximately 2:1, indicating a preponderance of the α-D anomer (**1**). Addition of a drop of deuterium oxide at this point caused little change in the intensity ratios of the two signals.

Deuterium-exchanged 2-amino-2-deoxy-D-mannose hydrochloride (crystallized syrup) gave signals at τ 4.78 and 5.00 in the approximate proportion 2:3 when first dissolved in methyl sulfoxide-d_6; after 4 days the relative proportions were 2:1.

C. In *N*,*N*-Dimethylformamide.—A freshly prepared solution of 2-amino-2-deoxy-D-mannose hydrochloride in *N*,*N*-dimethylformamide showed a complex series of signals in the range τ 3.0–5.5; deuteration of the sample gave, after 12 hr, two narrow doublets in this region, τ 4.54 ($J_{1,2}$ = 1.4 cps, H-1 of **1**) and 4.78 ($J_{1,2}$ = 1.6 cps, H-1 of **2**), in the approximate ratio of 11:9.

(25) Reference 13, pp 375–377.

(26) Melting points were determined with a Thomas–Hoover Unimelt apparatus (Arthur H. Thomas Co., Philadelphia, Pa.). Specific rotations were determined in a 2-dm polarimeter tube. Infrared spectra were measured with a Perkin-Elmer Infracord infrared spectrometer. Microanalytical determinations were made by W. N. Rond. X-Ray powder diffraction data give interplanar spacings (A) for Cu Kα radiation. Camera diameter was 114.59 mm. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

(27) Mobility relative to 2-amino-2-deoxy-D-glucose, by tlc on microcrystalline cellulose [M. L. Wolfrom, R. M. de Lederkremer, and D. L. Patin, *J. Chromatog.*, **17**, 488 (1965)] with 5:5:1:3 pyridine–ethyl acetate–acetic acid–water as the developer; indication with alkaline silver nitrate and with ninhydrin.

Vilsmeier Reaction of Methylpyrazine

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In view of our recent finding[2] that condensation of 2-amino-3-methylpyrazines with the Vilsmeier reagent

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