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Formation of 1,5-Dideoxy-1,5-iminohexitols on Borohydride Reduction of 2-Amino-2-deoxyhexofuranurono-6,3-lactones

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1,5-Dideoxy-1,5-imino-D-mannitol and 1,5-dideoxy-1,5-imino-L-gulitol are formed on borohydride reduction of 2-amino-2-deoxy-D-mannofuranurono-6,3-lactone and the corresponding D-*gluco*-derivative, respectively. In the assumed mechanism 5-amino-5-deoxy-D-mannose and 5-amino-5-deoxy-L-gulose are first formed by partial reduction of the starting materials, and are then further reduced to the corresponding 1,5-dideoxy-1,5-iminohexitols.

The formation of 1,5-dideoxy-1,5-iminohexitols as artefacts in sugar analysis of complex carbohydrates containing 2-amino-2-deoxyhexuronic acid residues is discussed.

During sugar analysis of the capsular polysaccharide from *Streptococcus pneumoniae* type 12F,[1] involving acid hydrolysis, borohydride reduction and analysis of the resulting alditols by GLC of their acetates, we observed an artefact which, from its mass spectrum, was identified as a 1,5-dideoxy-1,5-iminohexitol. The polysaccharide contained 2-acetamido-2-deoxy-D-mannuronic acid as one of its sugar components and, as this sugar was the probable source of the artefact, the latter was assumed to have the D-*manno*-configuration. We now report further studies on the formation of this substance and of the corresponding L-*gulo*-derivative.

In the assumed reaction route the 2-acetamido-2-deoxy-D-mannuronic acid released during the acid hydrolysis is *N*-deacetylated and lactonized to 2-amino-2-deoxy-D-mannofuranurono-6,3-lactone (*1*). On treatment with aqueous sodium borohydride this lactone should be partially reduced, at C-1 and C-6, to 5-amino-5-deoxy-D-mannose (*2*) (that the amino group is now at C-5 is the result of nomenclature rules.). Analogous reductions of lactones to aldoses have been reported.[2] Compound *2*, in the pyranose form with nitrogen in the ring, is further reduced to 1,5-dideoxy-1,5-imino-D-mannitol (*3*). The corresponding reduction of 5-amino-5-deoxy-D-glucose, nojirimycin, to 1,5-dideoxy-1,5-imino-D-glucitol, has been reported.[3]

In order to test this hypothesis, the crystalline 2-acetamido-2-deoxy-D-mannofuranurono-6,3-lactone, prepared *via* the appropriate glycal by azidonitration,[4] was converted into *1* by acid hydrolysis. The best yields were obtained with strong acid and short reaction time (*e.g.* 4 M HCl, 100 °C, 7 min). Treatment of *1* with aqueous sodium borohydride followed by acidification with acetic acid yielded *3*, isolated as its amorphous hydroacetate (*3* × HOAc) (72 %), $[\alpha]_{578}$ −5°. The sodium salt of 5-amino-5-deoxy-D-mannonic acid (*4*) was also formed in about 16 % yield, demonstrating that part of the lactone was hydrolyzed. However, no 2-amino-2-deoxy-D-mannitol was found, indicating that the formation of *2* in cyclic form is faster than the reduction of the intermediary aldehyde to its alcohol. When *4* was converted into the lactone by treatment with acid and then treated with sodium borohydride, a further amount of *3* was formed. When the reduction of *1* was performed with borodeuteride, two atoms of deuterium were introduced at C-1 and one at C-6 in *3*, as demonstrated by mass spectrometry of the acetylated product. As expected, no *3* but only 2-acetamido-2-deoxy-D-mannitol was obtained when the *N*-acetylated uronolactone was treated with sodium borohydride.

Similar treatment of 2-amino-2-deoxy-D-gluco-furanurono-6,3-lactone (*5*), prepared from the 2-acetamido derivative, yielded 1,5-dideoxy-1,5-imino-L-gulitol (*7*), *via* the assumed 5-amino-5-deoxy-L-gulose (*6*). The yield of *7*, as its amorphous hydroacetate (*7* × HOAc), $[\alpha]_{578}$ +2°, was only

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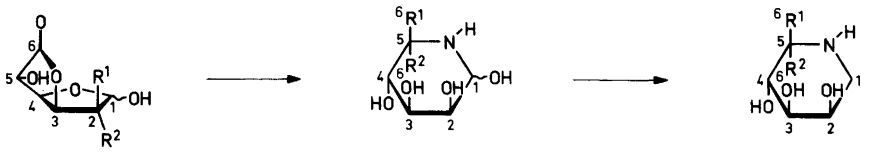
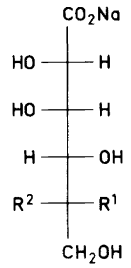


1 $R^1=NH_2$; $R^2=H$ *2* $R^1=CH_2OH$; $R^2=H$ *3* $R^1=CH_2OH$; $R^2=H$

5 $R^1=H$; $R^2=NH_2$ *6* $R^1=H$; $R^2=CH_2OH$ *7* $R^1=H$; $R^2=CH_2OH$



4 $R^1=NH_2$; $R^2=H$

8 $R^1=H$; $R^2=NH_2$

about 30 % but was increased to 61 % by treating the product, containing the sodium salt of 5-amino-5-deoxy-L-gulonic acid (*8*), with acid followed by a second borohydride reduction. The results suggest that the lactone with the D-*gluco*-configuration (*5*) is hydrolyzed more readily than the D-*manno*-isomer. Acidic aqueous solutions of *1* and *5* contain about 9:1 and 1:1, respectively, of the lactone *vs.* free acid at equilibrium, as demonstrated by NMR spectroscopy.

When 2-acetamido-2-deoxy-D-galacturonic acid[4] was treated with strong acid, followed by borohydride reduction, no 1,5-dideoxy-1,5-iminohexitol was formed. This was expected, as 2-amino-2-deoxy-D-galacturonic acid does not lactonize readily.

The mass spectra of the fully acetylated 1,5-dideoxy-1,5-iminohexitols with the D-*gluco*-, D-*manno*- and L-*gulo*-configurations are similar. The molecular ion, *m/z* 373, is observed in these spectra. The ions *m/z* 313 and *m/z* 300 are formed from the molecular ion by loss of acetic acid or the side chain, respectively. The elimination of an acetoxyl radical from the former ion to give *m/z* 254 is a less common reaction. A similar elimination was observed for the acetate of 1,5-dideoxy-1,5-imino-D-xylitol.[5] Other ions are formed by consecutive eliminations of acetic acid and ketene typical for this group of substances. Mass spectra of deuterated analogues of *7*, containing one deuterium atom on C-6 and/or two deuterium atoms on C-1, were consistent with the fragmentation routes indicated. Pertinent ions with some interpretations are given in the Experimental.

In the ^{13}C NMR spectrum of *3* × HOAc in D_2O, signals were observed at δ 24.5 (CH_3CO), 48.7 (C-1), 59.4 (C-6), 61.5 (C-5), 67.1, 67.3, 73.8 (C-2, C-3, C-4), and 182.8 ($C=O$). The corresponding values for *7* × HOAc were 24.5 (CH_3CO), 43.4 (C-1), 56.4 (C-5), 60.1 (C-6), and 63.8, 68.3, 69.6 (C-2, C-3, C-4), and 182.8 ($C=O$).

The ^1H NMR spectra of *3* and *7* as their hydroacetates are given in Experimental. The signals could readily be assigned by spin decoupling experiments, and the results support the assigned structures. It is obvious that *3* is present in the $^4C_1$ conformation while *7* is in the $^1C_4$ conformation. The spectrum of the D-*manno*-isomer (*3* × HOAc) is closely similar to the spectrum of the corresponding hydrochloride.[6]

In sugar analysis of polysaccharides involving acid hydrolysis, removal of the acid by distillation, borohydride reduction, acetylation and GLC of the alditol acetates, 2-amino-2-deoxyhexuronic acids which are readily lactonized may give 1,5-dideoxy-1,5-iminohexitols, as discussed above. Of the 2-amino-2-deoxyhexuronic acids found in Nature,[7] those with the D-*gluco*-, D-*manno*- and *gulo*-configurations lactonize but not those with the D- or L-*galacto* and L-*altro* configurations. On sugar analysis of three bacterial polysaccharides containing 2-acetamido-2-deoxy-D-mannuronic acid residues, from *Streptococcus pneumoniae* type 12F,[1] and *Haemophilus influenzae* types d[8] and e,[9] the yield

of 1,5-dideoxy-1,5-imino-D-mannitol was 60, 32 and 40 %, respectively, compared to those of the alditols from the non-acidic sugar components and estimated from the areas under the peaks on GLC. In the analysis of a fungal polysaccharide, from *Rhinocladiella mansonii*,[10] containing 2-acetamido-2-deoxy-D-glucuronic acid residues, the corresponding yield of 1,5-dideoxy-1,5-imino-L-gulitol was 20 %. The yield of 1,5-dideoxy-1,5-iminohexitols depends on several factors and was optimized only for the *S. pneumoniae* type 12F polysaccharide.

Two 1,5-dideoxy-1,5-iminohexitols, the D-*gluco*-[11] and the D-*manno*-isomer,[6] are natural products and the former has been synthesized.[12] The present work describes the first synthesis of the D-*manno*-isomer. The D-*galacto*-isomer has also been synthesized recently.[13] Nothing is known about the biosynthesis of 1,5-dideoxy-1,5-iminohexitols. A route starting from the corresponding 2-amino-2-deoxyhexuronic acid, analogous to that described above, does not seem to be excluded.

EXPERIMENTAL

General methods. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded in the pulsed Fourier-transform mode using JEOL FX-100 (^{13}C NMR) or Bruker WH270 (^{1}H NMR) instruments. Chemical shifts are given relative to external TMS (^{13}C) and relative to the HDO peak at δ 4.78 (^{1}H). ^{1}H NMR spectra were interpreted on a first order basis. Mass spectra were recorded at 70 eV on a JEOL D-300 instrument connected with a Finnigan Nova-3 computer. For GLC, at 190 °C, a Perkin-Elmer 990 instrument fitted with a glass column (180 × 0.15 cm) containing 3 % OV-17 on Gas Chrom Q was used.

1,5-Dideoxy-1,5-imino-D-mannitol hydroacetate (3 × HOAc) and sodium 5-amino-5-deoxy-D-mannoate (4). 2-Acetamido-2-deoxy-D-mannofuranurono-6,3-lactone (31 mg) was dissolved in 4*M* hydrochloric acid (2 ml) and kept at 100 °C for 7 min. Hydrochloric acid was removed by distillation in a vacuum at 20 °C. Water was added in the beginning of the distillation in order to avoid high concentration of acid. The product was dissolved in water and freeze-dried. The crude product was dissolved in water (2 ml) and sodium borohydride (70 mg) was added. The solution was kept at room temperature overnight, acidified to pH 4 with 50 % acetic acid and boric acid was removed by co-distillation with methanol (3 × 2 ml). Purification of the crude product on a column of Sephadex G-15 (2.5 × 80 cm) irrigated with water gave amorphous *3* × HOAc (23 mg, 72 %) and *4* (5 mg, 16 %). Compound *3* × HOAc showed $[\alpha]_{578}$ −5° (*c* 1.1, water); ^{1}H NMR (270 MHz, D_2O): δ 1.89 (3 H, s, OAc), 2.94 (1 H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.1 Hz, $J_{5,6'}$ 3.3 Hz, H-5), 3.09 (1 H, dd, $J_{1a,1e}$ 13.6 Hz, $J_{1a,2}$ 1.0 Hz, H-1a), 3.28 (1 H, dd, $J_{1e,2}$ 2.9 Hz, H-1e), 3.64 (1 H, dd, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.2 Hz, H-3), 3.80 (1 H, dd, H-4), 3.81 (1 H, dd, $J_{6,6'}$ 12.2 Hz, H-6), 3.94 (1 H, dd, H-6'), and 4.16 (1 H, ddd, H-2). ^{13}C NMR (25.05 MHz, D_2O): δ 24.5 (OAc), 48.7 (C-1), 59.4 (C-6), 61.5 (C-5), 67.1, 67.3, 73.8 (C-2, C-3, C-4), and 182.8 (C=O). Compound *4* showed $[\alpha]_{578}$ −3° (*c* 0.3, water); ^{13}C NMR (25.05 MHz, D_2O): 56.7 (C-5), 59.5 (C-6), 69.0, 72.9 (C-3, C-4), 74.5 (C-2), and 179.6 (C-1).

1,5-Dideoxy-1,5-imino-L-gulitol hydroacetate (7 × HOAc) and sodium 5-amino-5-deoxy-L-gulonate (8). 2-Acetamido-2-deoxy-D-glucofuranurono-6,3-lactone (32 mg) was *N*-deacetylated and treated with aqueous sodium borohydride as described for compound *3*. Purification of the product gave 7 × HOAc (10 mg, 30 %). In an analogous experiment the crude product was dissolved in 0.5 M hydrochloric acid (3 ml) and kept at 100 °C for 5 min. The solution was concentrated to dryness and dissolved in water. Sodium borohydride (60 mg) was added and the solution was kept at room temperature overnight, acidified to pH 4 with 50 % acetic acid and boric acid was removed by co-distillation with methanol. Purification of the crude product on a column of Sephadex G-15 (2.5 × 80 cm) irrigated with water gave amorphous 7 × HOAc (20 mg, 61 %) and *8* (9 mg, 28 %). Compound 7 × HOAc showed $[\alpha]_{578}$ +2° (*c* 1,0, water); ^{1}H NMR (270 MHz, D_2O): δ 1.89 (3 H, s, OAc), 3.05 (1 H, dd, $J_{1a,1e}$ 12.1 Hz, $J_{1a,2}$ 11.3 Hz, H-1a), 3.23 (1 H, dd, $J_{1e,2}$ 5.0 Hz, $J_{1e,3}$ 0.8 Hz, H-1e), 3.45 (1 H, ddd, $J_{5,6}$ 8.5 Hz, $J_{5,6'}$ 5.2 Hz, $J_{4,5}$ 1.8 Hz, H-5), 3.76 (1 H, dd, $J_{6,6'}$ 12.1 Hz, H-6), 3.84 (1 H, dd, H-6'), 4.01 (1 H, dd, $J_{3,4}$ 4.6 Hz, $J_{2,3}$ 2.8 Hz, H-3), 4.07 (1 H, dd, H-4), and 4.20 (1 H, ddd, H-2). ^{13}C NMR (25.05 MHz, D_2O): δ 24.5 (OAc), 43.4 (C-1), 56.4 (C-5), 60.1 (C-6), 63.8, 68.3, 69.6 (C-2, C-3, C-4), and 182.8 (*C*=0). Compound *8* showed $[\alpha]_{578}$ −9° (*c* 0.8, water); ^{13}C NMR (25.05 MHz, D_2O): 56.6 (C-5), 60.3 (C-6), 68.1, 73.7 (C-3, C-4), 74.0 (C-2), and 180.0 (C-1).

Sugar analyses. The polysaccharide (1 − 2 mg) was treated with 4 M hydrochloric acid (2 ml) at 100 °C for 2 h. The solution was worked up as described for *3* × HOAc. The product was dissolved in water (1 ml) and treated with sodium borohydride (~20 mg) at room temperature overnight. The solution was acidified with 50 % acetic acid, concentrated, and worked up as described above. The product was acetylated by treatment with acetic anhydride – pyridine (1:1, 1 ml) at 100 °C for 50 min. After work-up the alditol acetates were analyzed by GLC. The acetates of *3*, 7 and 1,5-dideoxy-1,5-imino-D-glucitol showed T_{Glc} = 1.41, 1.38 and 1.30,

Table 1. Mass spectra of fully acetylated 7. Relative intensities in and some plausible assignments in brackets. A. Non-deuterated. B. Dideuterated at C-1, monodeuterated at C-6. C. Dideuterated at C-1.

A	B	C
373(<0.1)[M^+]	376(<0.1)	375(<0.1)
330(0.3)[$M^+ - Ac$]	333(0.3)	332(0.4)
313(7)[$M^+ - HOAc$]	316(8)	315(8)
300(9)[$M^+ - CH_2OAc$]	302(9)	302(9)
254(24)[313 − OAc]	257(27)	256(25)
240(42)[313 − CH_2OAc]	242(48)	242(44)
212(4)[254 − $CH_2=C=O$]	215(3),214(4)	214(3),213(4)
198(17)[240 − $CH_2=C=O$]	200(19)	200(18)
180(13)[240 − HOAc]	181(9)	181(9)
152(9)	155(5),154(5)	154(7),153(4)
138(100)[198 − HOAc]	140(100)	140(95)
110(8)	113(4),112(4)	112(6),111(4)
96(95)	98(100)	98(100)

respectively (T_{Glc} = retention time relative to glucitol hexaacetate).

Mass spectrometry. Samples (0.5 − 1.0 mg) of *1* and *5* were converted into *3* and *7* as described above. For preparation of deuterated analogs of *3* and *7* sodium borodeuteride was used in the reduction step. In some experiments *8* was transformed into its lactone and then reduced with sodium borodeuteride to the analogue of *7* with two deuterium atoms at C-1. These compounds and 1,5-dideoxy-1,5-imino-D-glucitol were then acetylated and analyzed by GLC − MS. Some pertinent fragments in the mass spectra of fully acetylated *7* and its partially deuterated analogues are given i Table 1.

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