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Note

Reductive deamination of aminodeoxy groups in glycosides and polysaccharides

JAN ARNARP, PER J GAREGG, BENGT LENGSTAD, AND JORGEN LONNGREN
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

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The reductive deamination of aliphatic and aromatic amines and of amino acids with hydroxylamino-*O*-sulfonic acid (HOS) in aqueous sodium hydroxide has been briefly reported and the mechanism of the reaction has been discussed[1] This reaction is of potential interest in carbohydrate chemistry, *e g* , for the synthesis of deoxyglycosides from readily available aminodeoxyglycosides Transformation of aminoglycoside antibiotics and polysaccharides that contain aminodeoxy sugar residues into the corresponding deoxy compounds could provide useful modifications of biological activity Reductive deamination could provide a method for the specific degradation of polysaccharides that contain amino sugars, *e g* , the conversion of a 2-amino-2-deoxyhexosyl residue into a 2-deoxyhexosyl residue followed by acid hydrolysis under mild conditions, when the glycosidic linkage of the 2-deoxyhexosyl residues should be preferentially cleaved

We now describe an evaluation of this reaction for transformations in amino sugar chemistry

In order to obtain optimum conditions for the reductive deamination, buffered aqueous solutions of methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and methyl α-D-glucopyranoside (internal standard) in the ratio 1 1 were subjected to deamination with HOS The products were acetylated, and the ratios of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside to methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside were determined by g l c

Variations in the reaction conditions gave the following results The pH optimum for maximum yield of methyl 2-deoxy-β-D-*arabino*-hexopyranoside is 7–8 5, which is considerably lower than that previously reported for simple amines[1] In a typical experiment, the pH dropped from 7.5 to 7 3 at the end of the reaction. Above pH 10, up to 10% of D-glucal is formed The HOS, which decomposes in aqueous solution, must be freshly prepared and freshly recrystallized Additions of hydroxylammonium sulfate to the reductive-deamination reaction mixtures cause decreased yields Increases above a ten-fold molar excess of HOS did not significantly

alter the yields The highest yield (68% by g l c) was obtained in aqueous solution Other solvents gave the following yields *N,N*-dimethylformamide, 7%, formamide, 25%, and dimethyl sulfoxide, 3% A suitable reaction temperature is 4°, with a reaction time of 3 days

Results for the various deaminations, carried out on a preparative scale under the optimum conditions, are shown in Table I The yields given for the monosaccharides represent weighed, purified products, and those for the polysaccharides were determined by sugar analysis or by n m r spectroscopy The yield obtained in the deamination of the neuraminic acid derivative was low, but the others were in the range from 31 to 52% The *Streptococcus pneumoniae* type 14 capsular polysaccharide is constructed of tetrasaccharide repeating-units that contain branching 2-acetamido-2-deoxy-β-D-glucopyranosyl residues[2], and the *Vibrio cholera* O-antigen is a homo-

TABLE I

DEAMINATION OF AMINODEOXYGLYCOSIDES AND POLYSACCHARIDES CONTAINING AMINODEOXY SUGAR RESIDUES

Expt No	*Starting material*	*Product*	*Yield (%)*	*M p [f], $[\alpha]_D^{20}$ [h] (degrees)*	*Lit data m p, $[\alpha]_D^{20}$ (degrees)*
1	Methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride	Methyl 2-deoxy-β-D-*arabino*-hexopyranoside	52[a]	96–98[g] −24	96–98[6] −24
2	Methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride	Methyl 2-deoxy-α-D-*arabino*-hexopyranoside	31[a]	syrup[g] +122	syrup[7] +101
3	Methyl 3-amino-3-deoxy-β-D-allofuranoside hydrochloride[11]	Methyl 3-deoxy-β-D-*ribo*-hexofuranoside	44[a]	syrup[g] −30	— —
4	Methyl 6-amino-6-deoxy-α-D-galactopyranoside hydrochloride	Methyl 6-deoxy-α-D-galactopyranoside	48[a]	68–72[g] +141	64–65[8] +149
5	Methyl 5-amino-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranosidonic acid	Methyl 3,5-dideoxy-β-D-*gluco*-nonulopyranosidonic acid	19[b]	amorphous −42	— —
6	*Streptococcus pneumoniae* type 14 capsular polysaccharide[e]	Partly deaminated polysaccharide	55[c]	—	—
7	*Vibrio cholera* O-antigen[e]	Partly deaminated polysaccharide	60[d]	—	—

[a]Weighed yield of fully acetylated product [b]Weighed yield [c]Determined by sugar analysis comprising hydrolysis with 90% aqueous acetic acid at 100° for 2 h, sodium borohydride reduction, standard hydrolysis with acid, sodium borohydride reduction, acetylation, and g l c–m s analysis[9,10] [d]By ^{1}H-n m r analysis and integration (see Experimental) [e]*N*-Deacylated material [f]Melting points are corrected [g]For the fully acetylated product [h]Rotations for products of Expts 1–4 were recorded for solutions (*c* 1 0) in chloroform, and, for the product of Expt 5, for a solution (*c* 0 5) in water

polymer of 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues *N*-acylated with 3-deoxy-L-*glycero*-tetronic acid[3] These polysaccharides were *N*-deacylated and subjected to reductive deamination The yields obtained for the two polysaccharides (55 and 60%, respectively) suggest that this could be a useful method for the specific modification, under mild conditions, of polysaccharides that contain aminodeoxy sugars

EXPERIMENTAL

General methods — Concentrations were performed under reduced pressure Merck silica gel was used for chromatography G l c was performed with a Perkin–Elmer 990 instrument fitted with a column of 3% OV-225 N m r. spectra were recorded with a JEOL FX-100 instrument, except for the ^{1}H-n m r spectra of methyl 3,5-dideoxy-β-D-*gluco*-nonulopyranosidonic acid and methyl 2,5,6-tri-*O*-acetyl-3-deoxy-β-D-*ribo*-hexofuranoside which were recorded with a Bruker WP-200 instrument Chemical shifts are recorded in p p m downfield from internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^{1}H-n m r. in D_2O), internal tetramethylsilane (^{1}H-n m r. in $CDCl_3$), or external tetramethylsilane (^{13}C-n m r in D_2O) ^{1}H-N m r data accorded with the structure proposed for each compound In some instances, spin-decoupling or addition of tris(dpm)Eu(III) was used to facilitate interpretation of the spectra All non-crystalline compounds gave single spots in t l c HOS was prepared and recrystallized as described earlier[4]

Deamination (*a*) *Pilot experiments* In a typical experiment, methyl α-D-glucopyranoside and methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (10 mg, molar ratio 1 1) were dissolved in water (2 mL), HOS (22 mg) and disodium hydrogenphosphate (45 mg) were added, and the pH was adjusted to 7.5 with M sodium hydroxide. The vessel was sealed and nitrogen was bubbled through the solution After 3 days at 4°, the mixture was concentrated to dryness and acetylated (acetic anhydride–pyridine, 2 mL) The mixture was concentrated to dryness, the residue was partitioned between chloroform and water, and the chloroform phase was concentrated to a small volume The residue was then analysed by g l c In separate experiments, the amount of HOS, the pH value, the solvent, the reaction time, and the temperature were varied

(*b*) *Expts 1–4* A solution of HOS (725 mg) and disodium hydrogenphosphate (1 50 g) in water (60 mL) at 0° was adjusted to pH 7 5 with M sodium hydroxide The aminodeoxyglycoside (100 mg) in water (1 mL) was added and, after nitrogen had been bubbled through the solution for 10 min, the vessel was sealed and kept at 4° for 3 days The solution was concentrated, the salts were crushed, and the mixture was treated with acetic anhydride (5 mL) and pyridine (5 mL) at 100° for 30–60 min. Concentration, partition between water and chloroform, drying (Na_2SO_4), filtration, and concentration yielded products that were purified by chromatography on a column of silica gel (toluene–ethyl acetate, 2 1) ^{1}H-N m r. data (200 MHz, $CDCl_3$) for methyl 2,5,6-tri-*O*-acetyl-3-deoxy-β-D-*ribo*-hexofuranoside δ 2 08 (dd,

1 H, H-3), 2 20 (m, 1 H, H-3'), 4 13 (dd, 1 H, H-6), 4 44 (m, 1 H, H-4), 4 49 (dd, 1 H, H-6'), 4 87 (s, 1 H, H-1), 5 08 (d, 1 H, H-2), 5 09 (m, 1 H, H-5), 3 35 (s, 3 H, OMe), and 2 07, 2 08, and 2 09 (3 s, 3 × 3 H, OAc), $J_{2,3}$ 5 6, $J_{3,3}$ 14 5, $J_{3,4}$ 6 3, $J_{3,4}$ 8 8, $J_{4,5}$ 6 3, $J_{5,6}$ 6 1, $J_{5,6}$ 3 1, and $J_{6,6}$ 12 5 Hz

(*c*) *Expt 5* A solution of methyl 5-amino-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranosidonic acid (100 mg) in water (60 mL) containing disodium hydrogenphosphate (1 12 g) was treated with HOS (560 mg), as described above The product was freeze-dried and the residue was extracted with hot ethanol (2 × 10 mL) The extract was filtered and concentrated, and the product was further purified by elution from a column (2 × 100 cm) of Biogel P2 (200–400 mesh) with water The amorphous product was recovered by freeze-drying ^{1}H-N m r (200 MHz, D_2O) data showed, *inter alia*, δ 1.40 (dd, 1 H, H-3ax), 1 61 (m, 1 H, H-5ax), 1 89 (m, 1 H, H-5eq), 2 25 (m, 1 H, H-3eq), and 3 20 (s, 3 H, OMe), $J_{3ax,3eq}$ 12 6, $J_{3ax,4}$ 11 1, $J_{5ax,5eq}$ 12 0, $J_{5ax,4}$ 12 0, $J_{5ax,6}$ 12 0, $J_{5eq,4}$ 4 2, $J_{5eq,6}$ 4 2, $J_{3eq,4}$ 4 8, and $J_{3eq,5eq}$ 1 8 Hz ^{13}C-N m r data (25 05 MHz, D_2O) 36 2 (C-5), 41 4 (C-3), 51 5 (OMe), 64 4 (C-9), 65 2 (C-4), 69 7 (C-7), 71 8 (C-8), 74 1 (C-6), 102 2 (C-2), and 177 2 (C-1)

(*d*) *Expts 6* and *7* The polysaccharides were *N*-deacylated as described in ref 5 (Expt 6) or by treatment with 0 5M trifluoroacetic acid at 100° for 16 h (Expt 7) A solution of the *N*-deacylated polysaccharide (10 mg) in water (2 mL) containing disodium hydrogenphosphate (45 mg) was treated with HOS (22 mg), as described above The mixture was freeze-dried, and the product was desalted on a column (2 × 60 cm) of Sephadex G-15 by elution with water The recoveries, after freeze-drying of the partly deaminated product, were 90 and 55%, respectively In the ^{1}H-n m r spectrum (D_2O, 85°) of the deaminated *V. cholera* material, H-6 appeared at δ 1 36 and 1 20 for the aminodeoxy and deoxy sugar residues, respectively Integration indicated a 60% yield of deoxy sugar residues

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