Studies on Amino-hexoses. XVII. Identification of the Deamination Products from D-Glucosaminitol

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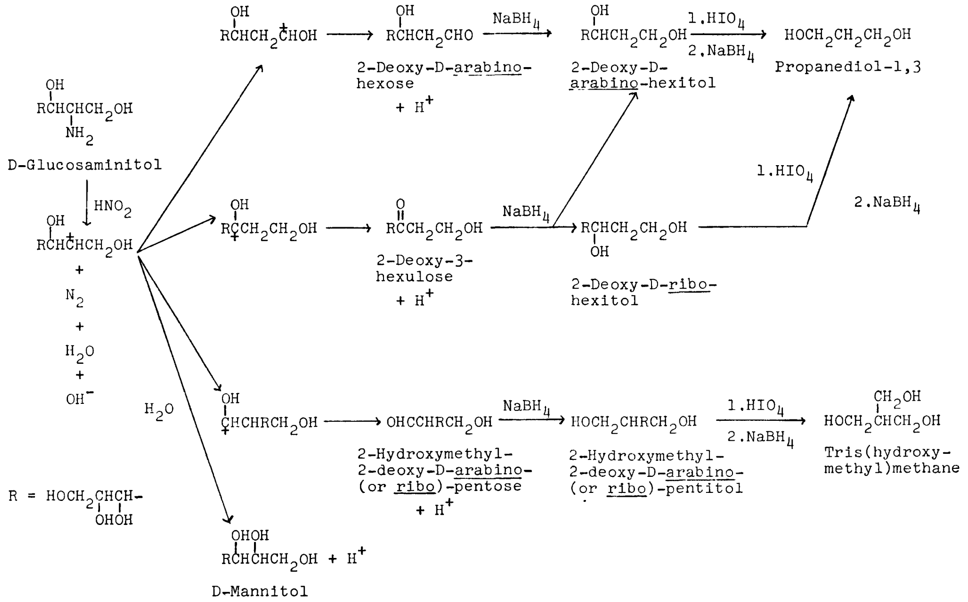
The nitrous deamination products of D-glucosaminitol were fractionated with a Dowex-1 free base column after the reaction mixture had been reduced with sodium borohydride. The chromatography gave discrete five peaks, four of which were identified as 2-deoxy-D-*arabino*-hexitol, 2-deoxy-D-*ribo*-hexitol, 2-hydroxymethyl-2-deoxy-D-*arabino*(or *ribo*)-pentitol and D-mannitol in a total yield over 90%. The nitrous deamination products fractionated with a Dowex-1 borate column were identified as 2-deoxy-D-*arabino*-hexose, 2-deoxy-D-*erythro*-3-hexulose, 2-hydroxymethyl-2-deoxy-D-*arabino*(or *ribo*)-pentose and D-mannitol. The formation of products other than D-mannitol was interpreted in terms of intramolecular rearrangement accompanying nitrous deamination.

The nitrous deamination reaction of amino-sugars was once of an unclarified nature in sugar chemistry.[1] However, owing to the progress in the theory of reactivity and molecular conformation,[2] it is now well understood that glucosamine and galactosamine give 2,5-anhydro-mannose and 2,5-anhydro-talose respectively by nitrous deamination, while mannosamine gives glucose, all of these amino-sugars having more or less rigid molecular conformation due to the ring structure.

One of the present authors demonstrated that nitrous deamination of glucosaminitol, which can take various conformations due to the chain structure, gave 2-deoxy-*arabino*-hexose(2-deoxy-glucose) in about 30% yield.[1] The remainder of the reaction products was not identified because of difficulty of analysis. In the present paper are described the results obtained using various chromatographic techniques in the analysis of the reaction products which, as expected, comprise various compounds. These compounds were identified in the following way: A mixture of nitrous deamination products which was highly reducing to Park-Johnson reagent[8] was reduced with sodium borohydride, the reducing groups of the products thus being converted into alcoholic groups. The mixture of the polyhydric alcohols obtained was then fractionated with a column of Dowex-1 free base[3-6] and each fraction was compared with the authentic samples by means of mixed melting point measurement, gas-liquid chromatography, or periodate oxidation.[7,8] On the other hand, the nitrous deamination mixture was directly fractionated with a Dowex-1 borate column,[9] each fraction separated being reduced with sodium borohydride, and the products were compared with the authentic samples by means of gas-liquid chromatography. A probable mechanism of the deamination reactions and the pro-



Scheme 1.

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1) Y. Matsushima, This Bulletin, **24**, 144 (1951).

2) S. Hase and Y. Matsushima, *J. Biochem.*, **66**, 57 (1969).

3) Y. Matsushima, T. Miyazaki, and J. T. Park, *ibid.*, **54**, 109 (1963).

4) P.W. Austin, F.E. Hardy, J.G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, **1963**, 5350.

5) Y. Matsushima and T. Miyazaki, *J. Biochem.*, **55**, 464 (1964).

6) F. Yaku and Y. Matsushima, *Nippon Kagaku Zasshi*, **87**, 969 (1966).

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8) S. A. Barker and P. J. Somers, *Carbohyd. Res.*, **3**, 220 (1966).

9) J. X. Khym and L. P. Zill, *J. Amer. Chem. Soc.*, **73**, 2399 (1951).

10) J.T. Park and M.J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).

cedure of the analyses are summarized in Scheme 1.

Experimental

Deamination Reaction. To a solution of 2.0 g of D-glucosaminitol free base (mp 132—134°C)[1] in 40 ml of water was added 1.5 g sodium nitrite, and to this mixture was added dropwise 2.0 ml of acetic acid under mechanical stirring at room temperature. After being left to stand overnight, the reaction mixture showed strong reducing action to Park-Johnson reagent[10], ninhydrin reaction becoming negative.

Reduction of the Deamination Products. The pH of the deamination mixture was adjusted to 7—8 by adding potassium borate and to this solution was added 1.5 g of sodium borohydride. The whole mixture was allowed to stand overnight at 4°C. Excess borohydride was decomposed by treating with Dowex-50 free acid, and the filtrate from the resin was evaporated *in vacuo*. The residue was co-distilled three times with methanol to remove boric acid, and was used for the following column chromatography.

Separation of the Reduced Products. The reduced products corresponding to 2.0 g of glucosaminitol were dissolved in a small amount of water, and the solution was placed on a Dowex-1X4 (OH^- form, 100—200 mesh, 3×110 cm) column. Elution was carried out at the velocity of 1 ml per minute using water free from carbon dioxide as an eluent, each 10 ml fraction being collected. The chromatographic pattern as detected by a color reaction of formaldehyde[11] which had been produced by periodate oxidation of the substances is shown in Fig. 1.

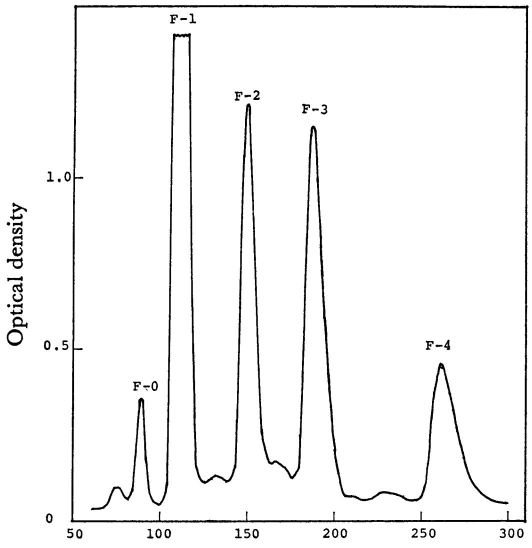

Fig. 1. Dowex-1X4 free base column chromatography of the reduced deamination products.

The results of gas-liquid chromatography of each fraction are shown in Table 1. We see that the fraction F-0 in the Dowex-1 chromatography seemed to be heterogenous and the yield was too low for further study. Crystallization occurred upon evaporation of the solvent *in vacuo* except for the cases of F-0 and F-3.

The yield of each fraction is given in Table 1. Physical constants, elemental analyses, and molecular formulae of each fraction are summarized in Table 2.

TABLE 1. RECOVERY, THEORETICAL YIELD, AND RETENTION RATIO (R_S) TO THE INTERNAL STANDARD (PENTAERYTHRITOL) IN GAS-LIQUID CHROMATOGRAPHY

The retention time of the internal standard is 5.6 min. The conditions of gas-liquid chromatography are identical with those described under Fig. 3.

Fraction	Recovery (mg)	Yield (%)	R_s of each fraction	R of authentic samples
F-0	25	1.67	0.535 1.83 2.0	—
F-1	827	55.14	3.22	3.23
F-2	213	14.21	3.34	3.36
F-3	249	16.60	2.74	—
F-4	73	4.44	5.11	5.12

TABLE 2. MELTING POINT, SPECIFIC ROTATION, ELEMENTAL ANALYSIS, AND MOLECULAR FORMULA ACCORDING TO ELEMENTAL ANALYSIS OF THE FRACTIONS F-1—4

	Mp (°C)	$[\alpha]_D^{20}$ in H_2O	Elemental analysis		Molecular formula
			C%	H%	
F-1	105—108	+16.2	43.09	8.45	$C_6H_{14}O_5$
F-2	88— 90	−13.1	42.79	8.41	$C_6H_{14}O_5$
F-3	sirup	−3.2	42.89	8.88	$C_6H_{14}O_5$
F-4	165—167.5	0	39.53	7.77	$C_6H_{14}O_6$

Calcd for $C_6H_{14}O_5$: C, 43.36%; H, 8.49%.
Calcd for $C_6H_{14}O_6$; C, 39.56%; H, 7.75%.

Periodate Oxidation of F-1—4. The oxidation products were reduced with sodium borohydride and analyzed by gas-liquid chromatography. The results are summarized in Table 3. As judged from the various evidence mentioned above, the fractions F-1—4 should be 2-deoxy-D-*arabino*-hexitol, 2-deoxy-D-*ribo*-hexitol, 2-hydroxymethyl-2-deoxy-D-*arabino* (or *ribo*)-pentitol, and D-mannitol respectively, the structural formulae of which are shown in Scheme 1.

TABLE 3. THE PERIODATE OXIDATION PRODUCTS OF THE FRACTIONS F-1—4 SUCCESSIVELY REDUCED WITH SODIUM BOROHYDRIDE

	HIO_4[7] (mol)	HCHO[11] (mol)	HCOOH[8] (mol)	Product
F-1	2.95	1.05	2.26	1,3-Propanediol
F-2	2.70	1.04	2.18	1,3-Propanediol
F-3	1.85	1.00	1.29	Tris(hydroxymethyl)methane
F-4	4.95	2.00	4.00	—

Direct Separation of the Deamination Products. The chromatographic pattern of the separation of the deamination products using a Dowex-1 borate column is shown in Fig. 2. Of the four peaks observed the peak F-4′ showed no reducing power. The patterns of gas-liquid chromatography of F-1′—4′ are shown in Fig. 3 (a—d). The yield of each fraction was calculated on the basis of the gas-liquid chromatogram of the deamination mixture shown in Fig. 4, and the data are summarized in Table 4. The borohydride-reduced fractions F-1′—3′ were also analyzed by gas-liquid chromatography, and the results are shown in Fig. 3(a—c) as broken lines. F-1′ gave 2-deoxy-D-*arabino*-hexitol, F-2′ 2-hydroxymethyl-2-deoxy-D-*arabino*(or *ribo*)-pentitol, and F-3′ 2-deoxy-D-*ribo*- and *arabino*-hexitol, F-4′ was a non-reducing substance which

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[11] T. Nash, *Biochem. J.*, **55**, 416 (1953).

was identified as D-mannitol. In conclusion, D-glucosaminitol gave the following four compounds by nitrous deamination, the structural formulae of which are also shown in Scheme 1: 2-deoxy-D-*arabino*-hexose, 2-hydroxymethyl-2-deoxy-D-*arabino*- (or *ribo*)-pentose, 2-deoxy-D-*erythro*-3-hexulose, and D-mannitol. A very small amount (0.6%) of a substance which showed retention time identical with 2,5-anhydro-D-mannitol[12] was observed in gas-liquid chromatography. However, the substance was not studied in detail due to the lack of material.

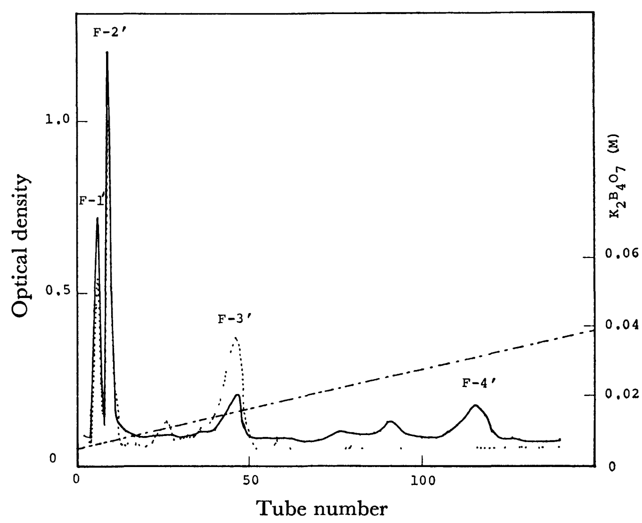
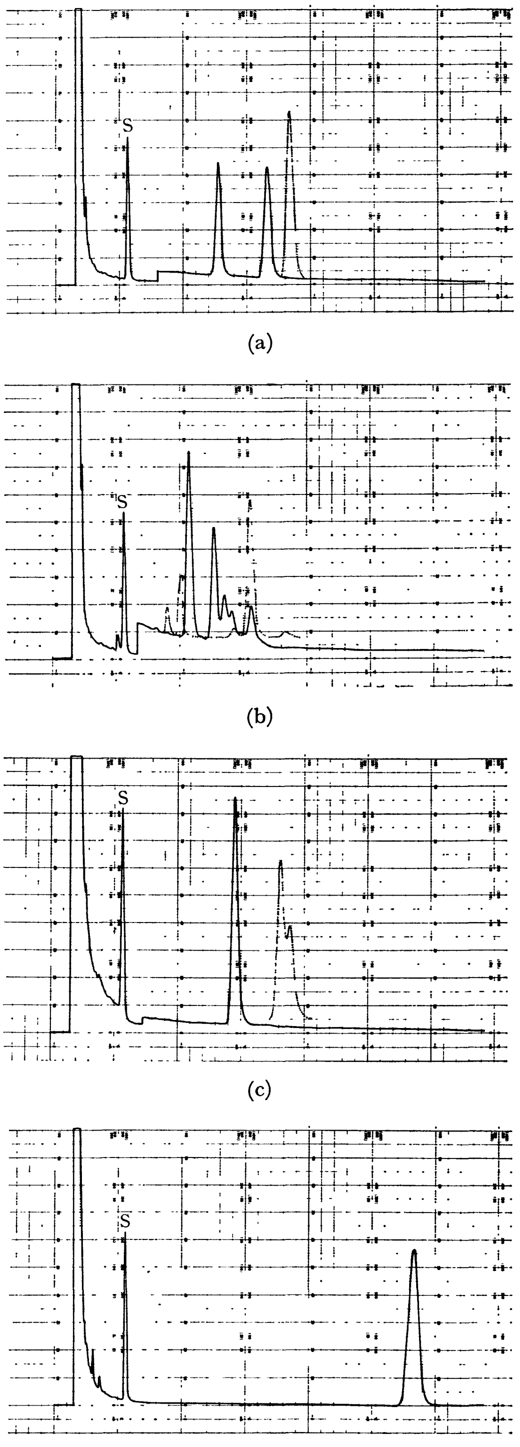


Fig. 2. Dowex-1X10 borate column (200—400 mesh, 0.9 × 17 cm) chromatography of the deamination mixture.
- - - - Concentration of potassium borate (eluent).
—— Formaldehyde determined by acetylacetone method[11] after the specimen had been oxidized with periodate.
······ Reducing power determined by Park-Johnson method.[10]

TABLE 4. YIELDS OF THE DEAMINATION PRODUCTS DETERMINED BY MEANS OF GAS-LIQUID CHROMATOGRAPHY

	Yield (%)
F-1'	45.8
F-2'	17.2
F-3'	22.8
F-4'	4.6

Preparation of the Reference Compounds. (a) 2-Deoxy-D-*arabino*-hexitol was prepared by reducing 250 mg of commercial 2-deoxy-D-*arabino*-hexose with sodium borohydride. Crystals appeared in ethanol solution giving 125 mg of the specimen which melted at 105—108.5°C, and had $[\alpha]^{20}_{D}$ +18.2 (*c*, 4.06 in H_2O). Found: C, 43.70; H, 8.50%. Calcd for $C_6H_{14}O_5$: C, 43.36; H, 8.49%. Bergmann *et al.*[13] gave mp 104—106°C, and $[\alpha]_D$ +17.5 (*c*, 10.0 in H_2O). (b) 2-Deoxy D-*ribo*-hexitol was prepared by reducing 2-deoxy-D-*ribo*-hexose which was synthesized by the known procedures.[14-17] Two hundred and ninety milligrams of the hexose was reduced



(a)

(b)

(c)

(d)

Fig. 3(a—d). Gas-liquid chromatography of the fractions F-1'—4'. A glass column (0.3 × 300 cm) was packed with 3% SE 52 on 90—100 mesh Chromosorb W which had been treated with dimethyldichlorosilane. The flow rate of the carrier nitrogen was 22 ml per minute, and the column temperature was 180°C. The internal standard(S) was pentaerythritol. Fig. 3(a) shows the anomers of 2-deoxy-D-*arabino*-hexose, the reduction product of which shows retention time identical with 2-deoxy-D-*arabino*-hexitol (broken line). Fig. 3(b) shows heterogeneity of the fraction F-2'. However, the main peak of the reduced F-2' had retention time identical with that of F-3. The fraction F-3'[Fig. 3(c)] gave upon reduction two hexitols, 2-deoxy-D-*arabino*- and *ribo*-hexitols. Fig. 3(d) shows D-mannitol.

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[12] L. F. Wiggins, *Nature*, **165**, 566 (1950).

[13] M. Bergmann, H. Shotte, and W. Leschinsky, *Ber.*, **56**, 1052 (1923).

[14] N. K. Richtmyer and C. S. Hudson, *J. Amer. Chem. Soc.*, **63**, 1727 (1941).

[15] D. A. Prins, *ibid.*, **70**, 3955 (1948).

[16] L. Vargha and J. Kuszmann, *Chem. Ber.*, **96**, 411 (1963).

[17] W. W. Zorbach and A. P. Ollapally, *J. Org. Chem.*, **29**, 1790 (1964).

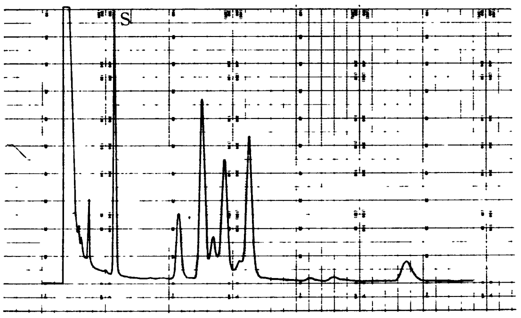

Fig. 4. Gas-liquid chromatography of the deamination mixture before fractionation. All the conditions of the chromatography were identical with those described under Fig. 3.

with sodium borohydride, giving 225 mg of the hexitol which crystallized from ethanol–ether mixture. mp 89—91°C, $[\alpha]_D^{20}$ $-9.8°$ (*c* 7.1, H_2O); Found: C, 43.15; H, 8.48%. Calcd for $C_6H_{14}O_5$: C, 43.36; H, 8.49%. (c) 2.5-Anhydro-D-mannitol was prepared by the known method. mp 100—102°C, $[\alpha]_D^{20}$ $+53$ (*c* 1.17, H_2O). Bera *et al.*[18] gave mp 100—101°C, $[\alpha]_D^{20}$ $+58.2$. Found: C, 44.00; H, 7.34%. Calcd for $C_6H_{12}O_5$: C, 43.90; H, 7.37%. (d) D-Mannitol was prepared by reducing commercial D-mannose with sodium borohydride. mp 167—168°C. Braham[19] gave mp 166—168°C. Found: C, 39.46; H, 7.73%. Calcd for $C_6H_{14}O_6$: C, 39.56; H, 7.75%. (e) Tris(hydroxymethyl)methane was prepared by lithium aluminum hydride reduction of the reaction mixture of ethyl malonate and formaldehyde[20]. To 16 ml of ethyl malonate was added 1.0 g of potassium bicarbonate, and to this mixture was added dropwise 7.0 ml of commercial formaldehyde solution under stirring. The mixture was extracted with ether after 2 hours, and the extract was washed with saturated ammonium sulfate and then with water. The ether solution was dried over anhydrous magnesium sulfate. Eight grams of lithium aluminum hydride was added to about 100 ml of the ether solution, and the solution was refluxed overnight. Excess lithium aluminum hydride was decomposed by treatment with ethyl acetate, and the reaction mixture was evaporated *in vacuo*. The residue was acetylated with acetic anhydride–sodium acetate, and the reaction mixture was extracted with chloroform. The chloroform extract was evaporated *in vacuo* and the residue was treated with sodium methoxide in methanol. The mixture of polyhydric alcohols thus obtained was analyzed first by gas-liquid chromatography using the standard trimethylsilylation method. The chromatogram showed three distinct peaks, two of which were identified as propanediol-1,3 and pentaerythritol. The remaining peak was expected to be tris(hydroxymethyl)methane. A preparative chromatography using Dowex-1 free base[4–6] was successful in fractionating three substances, one of which was identified as tris(hydroxymethyl)methane by elemental analyses of itself and its tri-3,5-dinitrobenzoyl derivative. mp 87—89°C. Found: C, 46.47; H, 9.70%. Calcd for $C_4H_{10}O_3$: C, 45.27; H, 9.50%. Tri-3,5-dinitrobenzoyl derivative gave mp 155.5—156°C. Found: C, 43.54; H, 2.60; N, 11.89%. Calcd for $C_{25}H_{16}O_{18}N_6$: C, 43.62; H, 2.34; N, 12.21%.

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[20] P. Block, Jr., "Organic Syntheses," Vol. 40, p. 27 (1960).