The Deamination of 1-Aminohexan-2-ol and 1-(Aminomethyl)pentyl β-D-Galactopyranoside; a Model Study of the Selective Cleavage of the Hydroxylysine-bound Glycosyl Residues of Collagen

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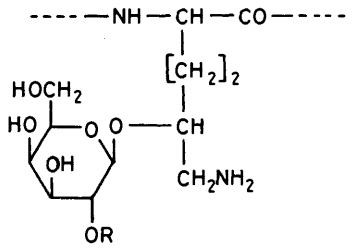
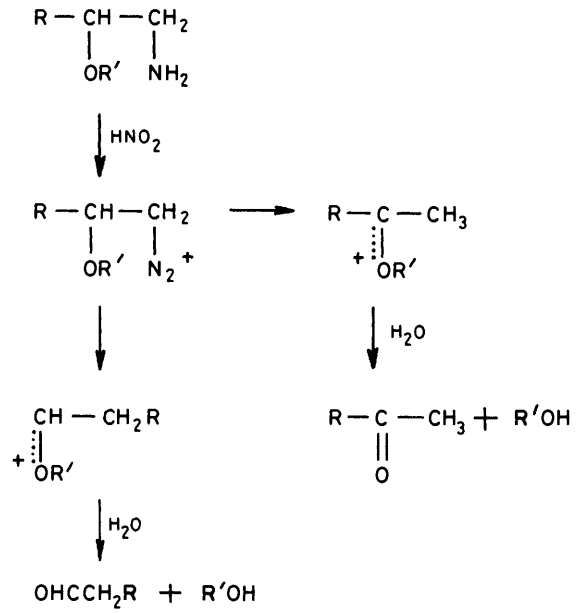
The reaction of 1-aminohexan-2-ol with nitrous acid gave a mixture of hexan-2-one, hexane-1,2-diol, 5-butyl-*N*-nitroso-2-pentyloxazolidine and 2,5-dibutyl-2-methyl-*N*-nitroso-oxazolidine, the proportions of which varied with the reaction conditions. The absence of 1,2-epoxyhexane from the product mixture is in marked contrast to results recently reported for a related aminoalcohol. 1-(Aminomethyl)pentyl β-D-galactopyranoside was synthesized as a model compound for the *O*-glycosylhydroxylysine residues of collagen, by the Lewis acid-catalysed reaction of β-D-galactopyranose penta-acetate with 1-nitrohexan-2-ol followed by reduction and deacetylation. The nitrosation of 1-(aminomethyl)pentyl β-D-galactopyranoside at pH 3.0—3.5 gave, as major products (>90%), D-galactose and hexan-2-one. This reaction constitutes a model for the selective cleavage, under very mild conditions, of the hydroxylysine-bound glycosyl residues of collagen.

The rearrangements that occur when primary aliphatic amines react with nitrous acid can result in the useful cleavage of carbon–carbon or carbon–oxygen bonds. Although such reactions are notorious for the large number of products that often result,[1] one pathway can predominate when the preferred conformation of the amine is favourable, and the reaction can then be used to effect the regiospecific cleavage, under very mild conditions, of a neighbouring bond. An example[2] of such an application is the cleavage of glycosides of 2-amino-2-deoxyaldopyranoses when the amino group is equatorially orientated in the preferred conformation.



(1) R = H or α-D-glucopyranosyl

In the deamination of α-hydroxy- and α-alkoxy-amines, 1,2-shifts of alkyl and hydride often predominate and it seemed possible that such a reaction might afford a useful method† for the selective release of the hydroxylysine-bound carbohydrate residues (1) of collagen.[3] The two pathways that would result in release of the carbohydrate (R'OH) are shown in Scheme 1. In the nitrosation of 1-amino-3-methylbutan-2-ol, Bunce *et al.*[4] found that the ketone (R = Me_2CH, Scheme 1) resulting from hydride shift predominated in the ether-soluble products. However, the extent of diol formation was not investigated.



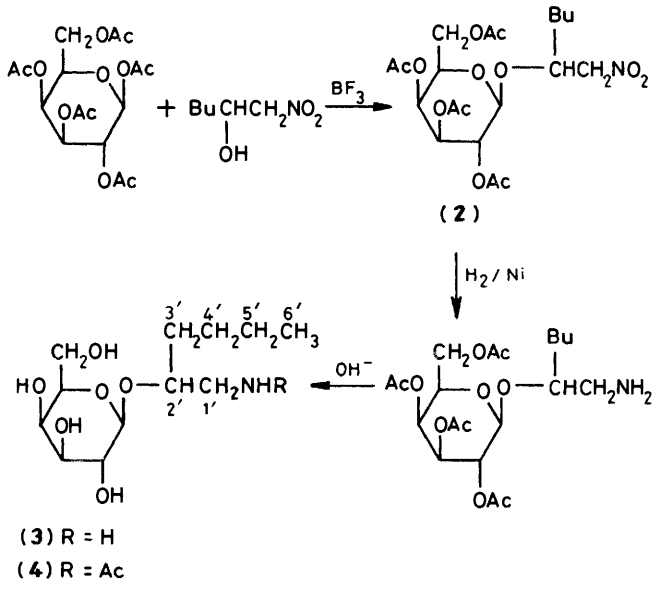
Scheme 1.

As the first part of a study of the potential of nitrosation as a method for the selective release of carbohydrate from collagen, 1-aminohexan-2-ol and its *O*-β-D-galactopyranosyl derivative (3) have been synthesized and their behaviour on treatment with nitrous acid has been studied.

Results

1-Nitrohexan-2-ol was isolated in 83% yield after the base-catalysed addition[5] of nitromethane to pentanal. Reduction of the nitroalcohol with lithium aluminium hydride in diethyl ether gave the hitherto unknown 1-aminohexan-2-ol, which was characterised as the crystalline hydrochloride salt. The crystalline acetate salt was obtained when reduction was effected catalytically over Raney nickel with acetic acid as solvent.

† The carbohydrate content of collagen is normally measured by a tedious and destructive procedure which involves alkaline hydrolysis.[3] An improved procedure would facilitate the study of the variation of carbohydrate content of collagens from different sources. The function of the carbohydrate is not known.

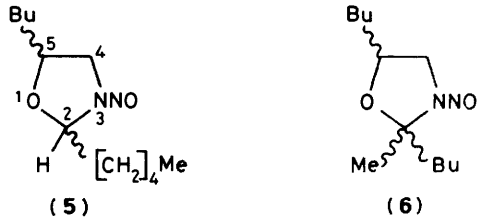


Scheme 2.

1-(Aminomethyl)pentyl β-D-galactopyranoside (**3**) was synthesized as shown in Scheme 2. Reaction[6] of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose with 1-nitrohexan-2-ol in the presence of BF_3–diethyl ether gave, after chromatography, the β-galactopyranoside (**2**) in 70% yield. Reduction of this nitroglycoside over platinum oxide gave poor yields (< 30%) of amine. Hydrogenation over Raney nickel in acetic acid at 66 bar was more successful but the syrupy product contained nickel ions. Passage of hydrogen sulphide gas into an aqueous solution of the product removed much of the nickel but a chelating resin was necessary to obtain a nickel-free product (75% yield). Deacetylation was effected using a strong base resin (Amberlite CG 400, OH^- form) to give the aminoglycoside (**3**) as an impure syrup containing some of the *N*-acetyl derivative (**4**). The latter was removed by absorption of the amine on a cation exchange resin, elution of the amide with water, and desorption of the amine with dilute ammonia. Analytically pure aminoglycoside (**3**) was obtained as the acetate salt by preparative paper electrophoresis. High-field n.m.r. measurements demonstrated the presence of two diastereoisomers; in the noise-decoupled ^{13}C n.m.r. spectrum at 75 MHz pairs of singlets were present for each carbon nucleus and the largest chemical shift difference between the carbon nuclei of the diastereoisomers was observed for C-2′* (1.34 p.p.m.). The configuration of the galactosidic linkage was shown to be β in both diastereoisomers by the ^1H n.m.r. spectrum ($J_{1,2}$ 7.5 Hz).

The nitrosation of 1-aminohexan-2-ol hydrochloride was first studied at pH 1 for 30 min at 0 °C followed by heating at 100 °C for 10 min according to the procedure of Bunce *et al.*[4] The product was almost exclusively hexan-2-one on the basis of ^1H n.m.r. analysis. Traces of yellow nitroso-oxazolidines were formed and the recovery of ketone was low (*ca.* 60%). These conditions seemed unnecessarily vigorous and in subsequent experiments no heating was used and the pH was kept between 3 and 6.5.

Hexan-2-one and hexane-1,2-diol were readily identified in the products by ^1H n.m.r. spectroscopy and g.l.c. These analytical methods and h.p.l.c. (high-pressure liquid chromatography) showed that hexanal and 1,2-epoxyhexane were absent. Yellow nitroso-oxazolidines ($\lambda_{max.}$ 358 nm) were present, and these were synthesized separately by reaction of 1-aminohexan-2-ol hydrochloride with hexan-2-one and with hexanal followed by nitrosation of the oxazolidines. In both cases a mixture of diastereoisomeric *N*-nitroso-oxazolidines resulted. 5-Butyl-*N*-nitroso-2-pentyloxazolidine (**5**) was isolated in 68% yield; the most useful ^1H n.m.r. signal for diagnostic purposes was the multiplet at δ 5.5—5.8 for 2-H. 2,5-Dibutyl-2-methyl-*N*-nitroso-oxazolidine (**6**) was isolated in 26% yield; the four singlets at δ 1.64, 1.74, 2.08, and 2.12 corresponded to the methyl protons of the four diastereoisomers.



The ^1H n.m.r. spectra of the products showed that little or no nitroso-oxazolidine (**5**) derived from hexanal was present; thus the nitroso-oxazolidine (**6**) could be assayed by u.v. measurements at 358 nm. The proportions of the products, ketone, diol, and nitroso-oxazolidine, varied with the conditions of nitrosation. A nitrite:amine molar ratio of 12:1 and perchloric acid at pH 3 gave the diol and nitroso-oxazolidine and no hexan-2-one. When acetic acid was used with a nitrite:amine ratio of 6:1 hexan-2-one was also formed (see entry 1 of Table). The absence of the epoxide, 1,2-epoxyhexane, was surprising in view of the epoxide formation reported for the nitrosation of 1-amino-3-methylbutan-2-ol. However, control experiments established that 1,2-epoxyhexane would not survive the conditions of nitrosation at pH values between 1 and 4.5. It did survive the pH 6.5 conditions, but it was not detected in the products of nitrosation at pH 6.5, even when the reaction mixture was stirred with diethyl ether to extract the ether-soluble products as they were formed. At pH 6.5 15% of unchanged 1-aminohexan-2-ol was recovered and much more diol (61% of product mixture) was formed. 1-Acetoxyhexan-2-ol was tentatively identified as a minor product (8%) from the singlet at δ 2.06 in the ^1H n.m.r. spectrum. The lowest yield of diol was obtained using Amberlite CG50 to generate the nitrous acid at pH 4.5 (see entry 3 of Table).

Nitrosation of 1-(aminomethyl)pentyl β-D-galactopyranoside was carried out using an aqueous solution of sodium nitrite and acetic acid at pH 3.0—3.5. The disappearance of the amine was followed by paper electrophoresis which showed that a nitrite : amine molar ratio of 24 : 1 and a reaction time of 50 h were necessary for complete reaction. H.p.l.c. and g.l.c. analysis showed that D-galactose and hexan-2-one were major products and that hexanal was absent. The yield of hexan-2-one was 93% (h.p.l.c.) and D-galactose was assayed by a colorimetric procedure (91%) (h.p.l.c.) and by g.l.c. analysis of the trimethylsilyl ethers (93%). G.l.c. analysis also revealed the presence of a minor product. This was isolated in 4% yield and shown to be the substitution product 1-(hydroxymethyl)pentyl β-D-galactopyranoside by high-field ^1H n.m.r. spectroscopy and by acid hydrolysis to D-galactose and hexane-1,2-diol.

Discussion

The absence of epoxide from the products of nitrosation of 1-aminohexan-2-ol is in marked contrast to the results of the nitrosation of 1-amino-3-methylbutan-2-ol.[4] Indeed, the fact that 1,2-epoxy-3-methylbutane survived a control experiment[4] which entailed a pH of 1 (our measurement) is surprising in

* Primed locants refer to the hexane aglycone chain numbering (see Scheme 2).

Table. Nitrosation of 1-aminohexan-2-ol

Entry	Solvent	Acid	Molar ratio $NaNO_2$/amine	pH	Reaction time (h)	Products % Ketone	Diol	Oxazolidine
1	Water	HOAc	6	3	4	19	22	56[a]
2	Water	HOAc	6	4.5	48	47	28	31[a]
3	Water–Et_2O	Amberlite CG50	6	4.5	48	69	12	31[a]
4[b]	Water	HOAc	2	6.5	48	17	61	14

[a] Calculated from u.v. absorption. [b] Product composition, calculated from n.m.r. spectrum, is given; diol monoacetate (provisionally identified) was 8%.

view of the lability of 1,2-epoxyhexane under similar and also less vigorous conditions. However, in the control experiment reported,[4] although the unchanged epoxide was identified qualitatively by g.l.c. it was not shown that there was no loss of 1,2-epoxy-3-methylbutane and the possibility of some conversion into the diol cannot be excluded. It is possible that some of the diol formed from 1-aminohexan-2-ol was derived from 1,2-epoxyhexane. However, the demonstration that 1,2-epoxyhexane was stable at pH 6.5, which gave the highest yield of diol, suggests that epoxide formation is not a favoured pathway at pH values of 6.5 and less.

The formation of nitroso-oxazolidines from α-hydroxyamines has been reported previously.[7,8] The highest yields of nitroso-oxazolidines were obtained for the nitrosation of ethanolamine.[8] The relative importance of 1,2-hydride shift (to form a ketone) and 1,2-alkyl shift (to form an aldehyde) varies considerably with the structure of the aminoalcohol. Thus, little aldehyde was formed at a pH of *ca.* 7* from 1-amino-3-methylbutan-2-ol[4] whereas 1-aminopropan-2-ol, on nitrosation at pH 4.7, gave propanal (64%) and the nitroso-oxazolidine derived from propanal (23%).[9] Perhaps 1,2-alkyl shifts are favoured when the alkyl group is small (*e.g.* methyl).

In this work 1-(aminomethyl)pentyl β-D-galactopyranoside was used as a model for the *O*-glycosylhydroxylysine residues of collagen, (**1**). It was assumed that the neighbouring amide groups would not participate in the reaction to a significant extent. In the nitrosation of 1-aminohexan-2-ol, hydride shift to form the ketone and the oxazolidine (**6**) predominated at pH 3.0—4.5. Oxazolidine formation is impossible in the nitrosation of the glycoside (**3**), and the main products observed resulted from the hydride shift pathway. The high yield of D-galactose obtained suggests that the reaction could be used to release, under very mild conditions, the hydroxylysine-bound carbohydrate residues of collagen. This could therefore form the basis of a new method for the quantitative determination of the carbohydrate content of collagen and collagen-derived peptides. Such applications will be described separately.

Experimental

Methods.—M.p.s were measured on a Kofler hot-stage apparatus and are corrected. U.v. spectra were measured on a Perkin-Elmer 402 spectrophotometer and i.r. spectra on a Pye Unicam SP 1050. ^1H N.m.r. spectra were measured on Hitachi Perkin-Elmer R24B (60 MHz) and Varian HA 100 (100 MHz) spectrometers, and ^{13}C spectra on a Varian XL 100 spectrometer (30° flip angle, 1.6 s pulse-repetition time). $CDCl_3$ was the solvent and Me_4Si the internal reference unless otherwise stated; the internal reference for D_2O solutions was sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropanoate. A few high-field n.m.r. spectra were measured on a 300 MHz Bruker FT spectrometer. The *J* values quoted are the directly observed line spacings. T.l.c. was performed on Silicagel G and g.l.c. was carried out on a Pye 104 instrument with a 10% Carbowax 20M column (2 m) and a carrier gas flow rate of 60 ml min^{-1}. Paper electrophoresis was carried out using a pyridine–acetic acid buffer at pH 5.4, a voltage of 900 V, and a current of 30—35 mA. Light petroleum refers to the fraction boiling in the range 60—80 °C.

1-*Nitrohexan*-2-*ol.*—A solution of nitromethane (12.1 g, 0.2 mol) and pentanal (17.2 g, 0.2 mol) in an equal volume of ethanol was cooled to 10 °C. An aqueous solution (20 ml) of sodium hydroxide (8 g) was added slowly to the stirred solution so that the temperature did not exceed 10 °C. When the addition was complete the mixture was diluted with ice and glacial acetic acid until the precipitated salt dissolved and the pH was 6. Extraction with ethyl acetate (4 × 50 ml) followed by sequential washing of the organic layer with water, drying ($MgSO_4$), and evaporation under reduced pressure gave the crude product as a pale yellow oil. Distillation under reduced pressure gave the pure nitroalcohol (24.5 g, 83%), b.p. 69—70 °C at 0.2 mmHg (lit.,[10] b.p. 80 °C at 1 mm Hg); n_D^{16} 1.4485 (lit.,[11] n_D^{20} 1.4482); δ_H 0.90 (3 H, m, CH_3), 1.40 (6 H, m, 3 × CH_2), 3.12 (1 H, s, OH, removed by D_2O exchange), and 4.36 (3 H, m, CHO and CH_2NO_2); $\lambda_{max.}$(film) 1 560 (NO stretch) and 3 430 cm^{-1} (OH).

Reduction of 1-*Nitrohexan*-2-*ol.*—(a) *With lithium aluminium hydride.* A solution of 1-nitrohexan-2-ol (5.17 g, 35 mmol) in dry diethyl ether (50 ml) was added dropwise during 3 h to a stirred refluxing slurry of $LiAlH_4$ (6.4 g) in dry diethyl ether (100 ml). After the mixture had been refluxed for a further 2 h, excess of reducing agent was decomposed by dropwise addition of water (6.4 ml) with cooling, followed by 15% aqueous sodium hydroxide (6.4 ml) and then water (20 ml). The mixture was stirred for 0.5 h and the solid inorganic hydroxides were filtered off. The ether layer was separated and combined with the solution obtained by continuous overnight ethereal extraction of the filtered solid. The ethereal solution was dried ($MgSO_4$) and concentrated to give 1-aminohexan-2-ol (2.90 g, 70%). Distillation under reduced pressure gave the pure amine, b.p. 55 °C at 0.05 mmHg; δ_H 0.90 (3 H, m, CH_3), 1.38 (6 H, m), 2.60 (5 H, m), and 3.50 (1 H, m, CHO); $\nu_{max.}$(film) 1 470, 1 602 (NH bend), 3 300 and 3 375 cm^{-1} (OH and NH stretch). Addition of an equimolar amount of hydrochloric acid to an ethanolic solution of the amine gave 1-*aminohexan*-2-*ol hydrochloride*, m.p. 76 °C; δ_H (D_2O) 0.89 (3 H, m, CH_3), 1.39 (6 H, m), 2.85 (1 H, dd, CHN), 3.10 (1 H, dd, CHN), and 3.80 (1 H, m, CHO) (Found: C, 47.0; H, 10.8; N, 9.2. $C_6H_{16}ClNO$ requires C, 46.9; H, 10.5; N, 9.1%).

(b) *Catalytic hydrogenation.* A solution of 1-nitrohexan-2-ol (5.0 g) in glacial acetic acid (90 ml) containing Raney nickel (5 ml, settled volume in ethanol) was hydrogenated at 56 bar pressure. After 66 h t.l.c. (light petroleum—ethyl acetate, 3:2) showed that the reaction was incomplete, the starting material and the amine giving, respectively, yellow and violet colours with ninhydrin. The green solution was concentrated under

* Slightly less than 1 mol of HCl was added per mol of amine.

reduced pressure at 50 °C to a syrup which, on trituration with diethyl ether, gave a colourless crystalline solid. This was filtered off and washed with cold diethyl ether to give the amine acetate salt (3.68 g, 61%), m.p. 96 °C; δ_H 1.95 (3 H, s, OAc) and 6.9 (4 H, br s, NH_3^+ and OH). The acetate salt could be converted into the hydrochloride salt, identical with that formed above, in a mixture of ethanol and diethyl ether.

1-(*Nitromethyl*)*pentyl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*galactopyranoside* (**2**).—A mixture of β-D-galactopyranose penta-acetate (3.0 g), 1-nitrohexan-2-ol (4.2 g), and boron trifluoride–diethyl ether (4.5 ml) in dichloromethane (30 ml) was stirred at room temperature for 9 h with the exclusion of moisture. Aqueous sodium hydrogen carbonate (10%) was added to neutralize the boron trifluoride and the organic layer was washed with water, dried ($MgSO_4$), and evaporated to give an orange-brown syrup (4.85 g). Column chromatography on silica (Kieselgel-60, 130 g) with light petroleum–ethyl acetate (3:2) gave first a small amount of a compound that appeared to be the α-glycoside and then the pure β-*galactoside* (**2**) as a syrup (2.57 g, 70%), $[\alpha]_D^{20}$ +8.7° ($CHCl_3$); $\nu_{max.}$(film) 1 565 (N–O stretch) and 1 760 cm^{-1} (C=O of acetate); δ_H 0.92 (3 H, m, CH_3CH_2), 1.35 (6 H, m, 3 × CH_2), 1.96, 2.04, and 2.13 (12 H, 3 s, 4 × CH_3CO), 3.75—4.24 (3 H, m, 5-H and CH_2O), 4.24—4.80 (4 H, m, CH_2N, CHO, and 1-H), and 4.80—5.45 (3 H, m, 2-, 3-, and 4-H) (Found: C, 50.0; H, 6.7; N, 3.0. $C_{20}H_{31}NO_{12}$ requires C, 50.3; H, 6.5; N, 2.9%); δ_C 102.1 and 102.3 p.p.m. (C-1 of diastereoisomers).

1-(*Aminomethyl*)*pentyl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*galactopyranoside*.—A solution of the nitroglycoside (**2**) (5.685 g) from the preceding experiment in glacial acetic acid (100 ml) was hydrogenated over freshly prepared Raney nickel (W-4, 25 ml) at 66 bar pressure in a rocking autoclave at room temperature for 163 h. T.l.c. analysis (developer light petroleum–ethyl acetate, 3:2) showed only a trace of starting material (R_F 0.53). Removal of the solvent under reduced pressure gave a green syrup (7.08 g). An aqueous solution of a 2 g portion of the product was treated with hydrogen sulphide for 30 min. The resulting black precipitate was filtered off and the solution was adjusted to pH 2 and extracted with diethyl ether. The aqueous layer was then adjusted to pH 10 and extracted with diethyl ether (3 × 25 ml). The latter dried extracts on evaporation gave the syrupy amine (0.03 g, 15% yield). The other portion (5 g) of the crude product was dissolved in methanol (10 ml) and the solution was passed through a column of Amberlite IRC-718 (NH_4^+) chelating resin (250 ml). The resin was eluted with methanol until all the amine had been collected (as indicated by ninhydrin). Evaporation of the methanol gave the amine acetate salt as a pale brown syrup (3.75 g, 75%), δ_H 1.98—2.15 (15 H, m, 5 × CH_3CO) and 8.16 (3 H, br s, NH_3^+); t.l.c. (EtOH) R_F 0.2 (detected with ninhydrin).

1-(*Aminomethyl*)*pentyl* β-D-*Galactopyranoside* (**3**).—A mixture of the aminogalactoside tetra-acetate (1.15 g) from the preceding experiment and Amberlite CG 400 (OH^-) resin (30 ml) in methanol (100 ml) was stirred at 45 °C. T.l.c. (EtOH) after 1.5 h suggested that deacylation was complete. The resin was filtered off and washed with methanol. Evaporation of the combined filtrate and washings gave the crude aminogalactoside (**3**) as a pale yellow syrup (0.42 g, 74%). Paper electrophoresis at pH 5.5 showed an amine spot and a small amount of a neutral compound was detected at the origin by the silver nitrate–sodium hydroxide spray reagent. This impurity was removed by passing an aqueous solution (10 ml) of the product (1.08 g) through a column of Amberlite IR 120 (H^+) resin (45 ml). Water eluted the neutral impurity which was obtained as a pale yellow syrup (0.221 g), δ_H ([$^2H_4$]MeOH) 0.90 (3 H, m), 1.42 (6 H, m), 1.97 (3 H, s, $NCOCH_3$), 2.95—4.07 (10 H, m), and 4.30 (1 H, m, 1-H). Elution of the resin with ammonium hydroxide (20%, 400 ml) followed by evaporation gave the aminogalactoside (**3**) as a pale yellow foam (0.83 g, 77%), δ_C ([$^2H_4$]MeOH; 75.5 MHz) 14.33 and 14.37 (C-6′), 23.76 and 23.86 (C-5′), 28.54 and 28.62 (C-4′), 33.91 and 33.97 (C-3′), 45.94 and 46.04 (C-1′), 62.32 and 62.87 (C-6), 70.24 and 70.54 (C-4), 72.82 and 73.08 (C-2), 75.04 and 75.30 (C-3), 76.58 and 76.95 (C-5), 80.89 and 82.23 (C-2′), and 104.39 and 105.10 p.p.m. (C-1). Assignments were made by comparison with the spectra of lactose and octan-2-ol.

The glycoside amine was further purified by preparative electrophoresis on Whatman No. 3 paper at pH 5.4. Amine zones were located by spraying edge strips with ninhydrin. The amine was obtained as the syrupy *acetic salt* (80% recovery), δ_H ([$^2H_4$]MeOH; 300 MHz) 0.95 (3 H, m, 6′-H_3), 1.24—1.80 (6 H, m, 3 × CH_2), 1.92 (3 H, s, CH_3CO_2), 2.80—3.16 (2 H, m, 1′-H_2), 3.43—3.64 (3 H, m, 5-H and 6-H_2), 3.66—3.99 (4 H, m, 2′-, 2-, 3-, and 4-H), and 4.31 and 4.39 (1 H, 2 d, $J_{1,2}$ 7.5 Hz, 1-H) (Found: C, 49.1; H, 9.0; N, 4.3. $C_{14}H_{29}NO_8$ requires C, 49.5; H, 8.8; N, 4.1%).

Nitrosation of 1-*Aminohexan*-2-*ol Hydrochloride*.—To a solution of the amine hydrochloride (0.1 mmol) in water (10 ml) was added sodium nitrite (0.1—1.2 mmol, see Table) and the solution was cooled (< 10 °C) in an ice-bath. Acid (glacial acetic acid, 11M hydrochloric acid, Amberlite CG 50 H resin, or 60% perchloric acid) was added in three portions during 30 min and the reaction was followed by paper electrophoresis using ninhydrin to detect the amine. The products were extracted into diethyl ether (3 × 25 ml) and the ether layer was washed with aqueous sodium hydrogen carbonate (3 ×), dried ($MgSO_4$), and evaporated under reduced pressure at 0 °C, conditions that were shown by control experiments to involve no loss of any product. In some experiments the reaction mixture was stirred in the presence of diethyl ether (20 ml) so that products were extracted as soon as they were formed. Control experiments showed that all the products, but not starting material, partitioned into the ether layer under these conditions.

Each product was analysed by ^1H n.m.r. and u.v. spectroscopy and in some cases by g.l.c. N.m.r. spectroscopy and g.l.c. showed that the formation of hexanal and the derived oxazolidine (**5**) occurred only to a negligible extent. Thus the u.v. spectra gave the amount of ketone-derived oxazolidine (**6**).

The yields of the diol and ketone were determined by g.l.c. using cyclohexanol as an internal standard. The retention times of hexan-2-one and hexane-1,2-diol on g.l.c. analysis were 3.9 and 19.9 min, respectively, using a temperature program of 88 °C (isothermal) for 10 min, then increased to 180 °C at 20 °C min^{-1}. The nitroso-oxazolidine diastereoisomers eluted between 14.8 and 17.1 min. In one experiment (entry 4 of Table) the product *composition* was calculated from the n.m.r. spectrum. The figures are slightly in error because of the assumption that the nitroso-oxazolidine (a minor product) contains only nine (butyl) protons resonating at high field.

5-*Butyl*-N-*nitroso*-2-*pentyloxazolidine* (**5**).—A solution of 1-aminohexan-2-ol hydrochloride (153.5 mg, 1 mmol) in water (0.2 ml) and glacial acetic acid (0.2 ml) was stirred at 0 °C. A solution of hexanal (0.15 g, 1.5 mmol) in water (0.2 ml) was added dropwise. An aqueous solution (0.2 ml) of sodium nitrite (138 mg, 2 mmol) was then added during 30 min and the mixture was stirred overnight at 0 °C. The solution was neutralised with 10% aqueous potassium hydroxide and extracted with dichloromethane (3 × 25 ml). The combined extracts were washed with 5% hydrochloric acid, then with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and concentrated to give the nitroso-oxazolidine (**5**) as a yellow oil (164 mg, 72%) δ_H 0.9 (6 H, m, 2 × CH_3), 1.35 (12 H, m, 6 × CH_2), 2.1 (2 H, m), 2.8—4.3 (3 H, m, 5-H and 4-H_2), and 5.48 and 5.81

(1 H, m and t, 2-H). An analytical sample was obtained by flash chromatography using Kieselgel 60 and light petroleum–tetrahydrofuran (9:1) as eluant; $\lambda_{max.}$($CHCl_3$) 360 nm (ε 92) (Found: C, 62.7; H, 10.6; N, 11.8. $C_{12}H_{24}N_2O_2$ requires C, 63.2; H, 10.5; N, 12.3).

2,5-*Dibutyl*-2-*methyl*-N-*nitroso-oxazolidine* (**6**).—A similar reaction to the preceding one was carried out with hexan-2-one (150 mg, 1.5 mmol) instead of hexanal. The nitroso-oxazolidine (**6**) was obtained as an orange liquid (49.1 mg, 22%), δ_H 1.64, 1.74, 2.08, and 2.12 (4 s, 2-CH_3). An analytical sample was obtained by flash chromatography as in the preceding experiment; $\lambda_{max.}$ 358 nm (ε 67); δ_H: the δ 2.08 and 2.12 singlets were much less intense and the isomeric nitroso-oxazolidines (**5**) were present to the extent of 47.5% (m at δ 5.45—5.8). Hence ε for the purified diastereoisomers of (**6**) was calculated to be 46 (Found: C, 63.1; H, 10.7; N, 12.2. $H_{12}H_{24}N_2O_2$ requires C, 63.2; H, 10.5; N, 12.3).

1,2-*Epoxyhexane*.—1,2-Epoxyhexane prepared from hex-1-ene and 3-chloroperbenzoic acid was isolated as an oil (60% yield); $\nu_{max.}$(film) 750, 870, 890, and 1 030—1 080 cm^{-1} (C–O–C stretch); δ_H 0.92 (3 H, m, Me), 1.48 (6 H, m), and 2.37—3.00 (3 H, m, 1-H_2 and 2-H).

Hexane-1,2-*diol*.—The diol was prepared by oxidation of hex-1-ene with $KMnO_4$; yield after distillation was 50%; b.p. 86—87 °C at 0.3 mmHg; $\nu_{max.}$(film) 3 400 cm^{-1} (OH); δ_H 0.90 (3 H, m, Me), 1.37 (6 H, m), 3.24—3.62 (3 H, m, 1-H_2 and 2-H), and 4.42 (2 H, s, exchangeable with D_2O, OH).

1-*Acetoxyhexan*-2-*ol*.—A stirred suspension of neutral alumina (Merck 7—230 mesh size; dried at 200 °C; 17 g) in dry ethyl acetate (60 ml) containing hexane-1,2-diol (1.7 mmol) was refluxed at 80 °C for 24 h. H.p.l.c. analysis (Hypersil ODS 25 cm column; solvent 50% aqueous acetonitrile, flow rate 1 ml min^{-1}; R.I. detector) showed the presence of 1,2-diacetoxyhexane (R_t 5.7 min) as well as the expected [12,13] monoacetate (3.0 min) and starting material (2.1 min). The experiment was repeated using methyl acetate at 50 °C and monitoring by h.p.l.c.; the reaction was stopped before any diacetate had formed. ^1H N.m.r. spectroscopy showed the presence of 11% monoacetate (δ_H 2.08) which was presumed to be the 1-*O*-acetyl derivative.[12,13]

Nitrosation of 1-(*Aminomethyl*)*pentyl* β-D-*Galactopyranoside Acetate Salt*.—(a) *Assay of products*. A solution of the amine salt (16.95 mg. 0.05 mmol) and sodium nitrite (20.7 mg, 0.3 mmol) in water (1.019 ml) was cooled in an ice-bath and glacial acetic acid (3 × 50 μl) was added during 30 min to maintain the pH at 3. The reaction was followed by paper electrophoresis using ninhydrin and silver nitrate–potassium hydroxide to detect starting material and product respectively. Sodium nitrite (0.1 mmol) was added to the stirred mixture every 3 h, followed by glacial acetic acid (20 μl) to keep the pH between 3.0 and 3.5. After 1.2 mmol of sodium nitrite and 0.19 ml of acetic acid had been added the mixture was kept until the reaction was complete (50 h). Aliquots totalling 24 μl had been withdrawn for electrophoresis and the reaction solution (1.185 ml) was diluted to 5.0 ml with water. An aliquot (0.5 ml) of this solution was further diluted to 500 ml for the estimation of D-galactose by means of the ferricyanide colorimetric procedure. The yield of D-galactose was thus found to be 8.16 mg (91%). The remaining solution (4.5 ml) was extracted with diethyl ether (3 × 20 ml), and the combined extracts were washed with aqueous sodium hydrogen carbonate (10%; 3 ×) and dried ($MgSO_4$). The ether was removed under reduced pressure at 0 °C and the residue was dissolved in 50% aqueous acetonitrile (2.0 ml) for h.p.l.c. analysis on a Hypersil ODS column using the same solvent as eluant. Hexan-2-one was detected at 6.8 min (flow rate 0.5 ml min^{-1}; detection at 290 nm) and hexanal (at 9.2 min) was shown to be absent. The yield of hexan-2-one was 93% based on peak height measurements using a calibration obtained with standard solutions.

In a duplicate experiment an aliquot of the reaction mixture was treated with mannitol (1.19 mg) as internal standard. The solution was deionised on a column of Amberlite MB-3 resin (10 ml), the resin was washed with water, and the eluate was concentrated to dryness. D-Galactose was assayed by g.l.c. analysis of the trimethylsilyl ethers. The yield of D-galactose was 93%. In a separate experiment starting from 0.0267 mmol of amine acetate the g.l.c. analysis was repeated without adding mannitol. The peak area ratio of D-galactose to minor product was 96:4. The retention time of the latter relative to β-D-galactopyranose (as the silyl ethers) was 0.90 (column temperature 161 °C).

(b) *Isolation of minor product*. The amine salt (111.8 mg, 0.33 mmol) was nitrosated as above and the reaction mixture was deionised on a column of Amberlite MB-3 resin (100 ml), which was then washed with water until 300 ml of eluate had been collected. Concentration of this solution to dryness gave a syrup (65.7 mg); a solution of this in water (1 ml) was applied to a column of Amberlite IRA-400 (OH^-) (50 ml). The column was eluted with carbon dioxide-free water and fractions (13 ml) were collected. Fractions 7—30 contained carbohydrate (tested with H_2SO_4) and concentration of the pooled fractions gave 1-(hydroxymethyl)pentyl β-D-galactopyranoside as a syrup (4 mg, 4%), δ_H ([$^2H_4$]MeOH) 0.93 (3 H, m, Me), 1.45 (6 H, m), 3.44—3.90 (9 H, m, 1′-H_2, 2′-, 2-, 3-, 4-, 5-H, and 6-H_2), and 4.30 (1 H, m, 1-H).

The product was hydrolysed in aqueous solution containing Dowex AG 50W-X2 (200—400 mesh; 5 ml) at 100 °C for 1 h, the reaction being monitored by assay of the D-galactose content of aliquots by the ferricyanide colorimetric procedure. The cooled mixture was filtered, the resin was thoroughly washed with water, and one tenth of the solution was analysed for reducing sugar (D-galactose) by the ferricyanide method: yield 91%. The remaining solution was concentrated to dryness; the residue was dissolved in diethyl ether and shown to be hexane-1,2-diol by comparison with an authentic sample using g.l.c. (R_t 5.2 min at oven temperature of 184 °C and carrier gas flow rate of 65 ml min^{-1}) and ^1H n.m.r. spectroscopy (828 transients).

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