METHYL 3-NITRO- AND 3-AMINO-3-DEOXY-β-D-GALACTOPYRANOSIDE AND -MANNOPYRANOSIDE[1]

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ABSTRACT

The steric course of the nitromethane cyclization of L′-methoxy-D-hydroxymethyldiglycolic aldehyde was investigated. Methyl 3-nitro-3-deoxy-β-D-galactopyranoside was shown to arise as a second major reaction product in addition to the previously isolated principal stereoisomer, the *gluco* derivative. The corresponding *manno* stereoisomer is formed to a smaller extent. The configurations of the new methyl nitrodeoxyglycosides were established by conversion into the corresponding amino derivatives and hydrolysis of these latter to the known 3-amino-3-deoxy-D-galactose and -D-mannose hydrochlorides. All the products were obtained in a crystalline state. The reaction lends itself to a facile preparation of the nitrogenous galactose derivatives.

In a previous paper (1) the cyclization with nitromethane of the dialdehyde L′-methoxy-D-hydroxymethyldiglycolic aldehyde (I), which is readily available by periodate oxidation of methyl β-D-hexopyranosides, was reported to furnish a crystalline methyl 3-nitro-3-deoxy-β-D-hexopyranoside in a yield of 40% or better. The product was subsequently (2) established to possess the *gluco* configuration (II). There was evidence that the condensation reaction proceeded to virtual completion although no studies were made concerning the stereoisomeric composition of more than one-half of the mixture of products that was obtained. In view of the results recently elaborated in the anomeric α-hexoside series (3) as well as in anhydro sugars (4, 5) a more thorough investigation of the nitromethane condensation of I appeared to be of interest.

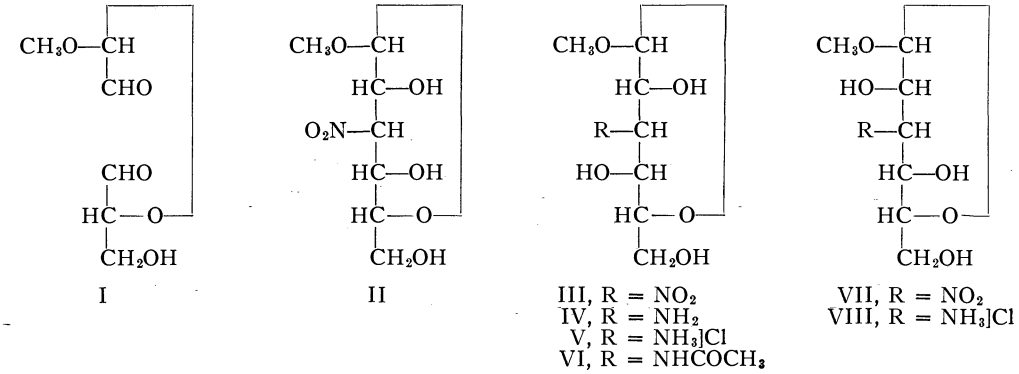
When the cyclization of I had been carried out in methanol in the presence of sodium methoxide, and had been followed by deionization of the solution and collection of the crystallizing nitroglycoside (40% of II and small fractions of undetermined composition[2,3]), there remained in the mother liquor a material which, at the time, failed to crystallize. Hydrogenation of this amorphous residue produced a crystalline aminoglycoside that had not been characterized further.[2] We have now found that the compound so obtained was methyl 3-amino-3-deoxy-β-D-galactopyranoside (IV). It is obvious, therefore, that the parent nitro compound, methyl 3-nitro-3-deoxy-β-D-galactopyranoside (III), was one of the products formed in the nitromethane cyclization in methanolic medium.

In an attempt to improve the preparation of the *galacto* derivatives we investigated the possibility of epimerizing the sodium nitronate of the glucoside II. As was demonstrated recently (3), *aci*-nitroglycoside salts in aqueous solution undergo spontaneous epimerizations at carbon atoms adjacent to the nitro grouping. While the experiments pertaining to the behavior of the glucoside II in aqueous alkali have not yet been concluded, preliminary evidence indicates that II can in fact be converted partly into III in this way. We were thus prompted to transfer the mixture of *aci*-salts, produced in methanol, into an aqueous medium for a short time prior to deionization, or indeed to conduct the whole cyclization in water in the presence of one equivalent of sodium hydroxide. In either case

[1]*Part X in the series "Cyclizations of Dialdehydes with Nitromethane". For Parts IX and VIII see references 5 and 3, respectively.*
[2]*See p. 2869 of reference 1.*
[3]*These fractions have now been shown to contain some methyl 3-nitro-3-deoxy-β-D-mannopyranoside.*

the proportions of II and III appeared to be shifted in favor of the latter, for the yields of isolable II were decreased to 24–25%, and at the same time it became possible to isolate 34% of crystalline III. The results to be described were obtained from cyclization experiments carried out in aqueous solution.

When the mother liquor remaining after the collection of the crystalline nitroglucoside II was inspected by paper chromatography, a pattern of nitro compounds was observed consisting of two major spots (R_f 0.57 and R_f 0.68) and a number of faster-moving weak spots. Whereas a relatively strong spot (R_f 0.68) was due to residual II, an even stronger spot (R_f 0.57) represented two new nitroglycosides, III and VII, which were not separated on paper. Their isolation and configurational elucidation will be described here. The products migrating faster than II were present in small amounts only and have not been identified.[4]



The three nitroglycosides II, III, and VII were separated by fractional crystallization. Methyl 3-nitro-3-deoxy-β-D-galactopyranoside (III) was obtained in 34% yield and hence represented a major reaction product. It melted at 131–132° and had a specific rotation of $[\alpha]_D$ +32.6°. Catalytic hydrogenation furnished the corresponding amine, methyl 3-amino-3-deoxy-β-D-galactopyranoside (IV), with m.p. 175–176° (decomp.) and $[\alpha]_D$ −3.7°. A crystalline hydrochloride (V) and an N-acetyl derivative (VI) of the amino compound were also prepared. The amine IV proved to be identical with the aminohexoside that had been obtained previously but had not been characterized.[2]

Preliminary assignment to IV of the *galacto* configuration was suggested by the proximity of its specific rotation to that of a nitrogen-free analogue, methyl β-D-galactopyranoside ($[\alpha]_D$ −0.4°). It is a well-known rule that substitution of an amino group for a hydroxyl in glycosides has little influence upon the rotation.[5] Final proof was established by acid hydrolysis of IV (or V), which readily furnished known (6) 3-amino-3-deoxy-α-D-galactose hydrochloride. The identification of this amino sugar hydrochloride was corroborated by N-acetylation to 3-acetamido-3-deoxy-β-D-galactose and direct comparison of the latter with an authentic sample (6, 3).

Methyl 3-nitro-3-deoxy-β-D-mannopyranoside (VII) was present in the reaction mixture to a smaller extent than either of the glycosides II or III. It was rather difficult to obtain in pure condition, as multiple recrystallizations, causing loss of product, were

[4]*At least three compounds appeared to be present in the R_f range of 0.77 to 0.90. Accurate values are not available since the products do not separate well. Despite its subordinate amount, part of this material is of peculiar interest as it possesses a distinct growth-inhibiting activity against Bacillus subtilis. This merits further investigation.*

[5]*For literature see reference 4.*

required. Eventually the pure compound was isolated in a yield of about 2%, although in the mixture it amounted to at least 13%. The higher figure was based on the isolation of the corresponding amine following hydrogenation. The nitromannoside VII melted at 136–138° and showed $[\alpha]_D$ −81.7°. Configurational assignment was accomplished by experiments similar to those just described for the galactoside III. Catalytic hydrogenation afforded an aminoglycoside that was crystallized as hydrochloride VIII (m.p. 230–231°, $[\alpha]_D$ −68.5°). It was noted that the molecular rotation of VIII (M_D −15,600) agreed well with that of the nitrogen-free analogue, methyl β-D-mannopyranoside (M_D −13,400). Acid hydrolysis of VIII produced known (7) 3-amino-3-deoxy-α-D-mannose hydrochloride, thus proving the configuration of the glycosides VIII and VII.

A comparison of the above results with those obtained earlier in the α-hexoside series leads to the following conclusion concerning the practical applicability of the nitromethane cyclization. The value of the method for the purpose of preparing nitrogenous hexose derivatives with *gluco*, *manno*, *galacto*, and *talo* configurations is complementary in the two anomeric series. In the α-series (3, 7), *manno* and *talo* derivatives are readily available by the method whereas *gluco* and *galacto* derivatives, although obtainable, are isolated only with some inconvenience. The β-series, on the other hand, lends itself to the facile preparation of *gluco* (1, 2) and, as was shown here, *galacto* derivatives.

EXPERIMENTAL

The melting points were determined in an aluminum block. The optical rotations were taken in 2-dm tubes and refer to solutions in carbon-dioxide-free water; *c*, approximately 1%. All evaporations were done *in vacuo* at 35–40° (bath temperature) unless otherwise indicated. Paper chromatography was performed by the descending technique on Whatman No. 1 paper. The nitro compounds were irrigated with 1-butanol – acetic acid – water (4:1:5, v/v, upper layer) and made visible by spraying with alkali (1 part *N* NaOH + 4 parts methanol + 5 parts butanol) and inspection under an ultraviolet lamp. The amino compounds were irrigated with pyridine – ethyl acetate – acetic acid – water (5:5:1:3, v/v, with pyridine – ethyl acetate – water, 11:40:6, v/v, in the bottom of the tank) and indicated with ninhydrin. R_{gm} = speed relative to glucosamine hydrochloride.

A. The Nitromethane Cyclization

(a) Condensation in Methanolic Solution Followed by Equilibration in Water

L′-Methoxy-D-hydroxymethyldiglycolic aldehyde (I) was prepared and condensed with nitromethane in the presence of sodium methoxide in methanolic solution as described previously (1). After the reaction period of 45 minutes, however, the solution was concentrated rapidly in an efficient evaporator at 20° (bath temperature) to the consistency of a thick sirup. The sirup was immediately dissolved in 10 parts of carbon-dioxide-free water and the solution was allowed to stand for 40 minutes at 23°. It was then cooled in ice water and deionized with Amberlite IR-120 (H^+). For deionization and subsequent crystallization and purification of the nitroglucoside II the earlier directions (1) were followed. There resulted a yield of 25% of purified II.

(b) Condensation in Aqueous Solution

A solution of dialdehyde I (0.08 mole, obtained from 15.52 g of methyl β-D-glucopyranoside) in 100 ml of water was carefully adjusted at 0° with sodium hydroxide to the point of a pink phenolphthalein coloration that persisted for at least 1 minute (3.6 ml of *N* NaOH was required). Upon addition of 4.44 ml of nitromethane (1 molar equiv.) another 80 ml of *N* NaOH was added over a period of 10 minutes at 0°. The solution was then brought to room temperature with running warm water and allowed to stand for 40 minutes. Thereupon the reaction mixture was deionized by being poured into an ice-cold aqueous suspension of 120 ml of Amberlite IR-120 (H^+); stirring was applied for 10 minutes. The solution was decanted from the resin, which was washed several times with water, and the combined liquid was passed over a column containing another 50 ml of fresh exchange resin. The column was finally rinsed with water. Evaporation of the effluent furnished a sirup which was dehydrated by two consecutive evaporations with ethanol and two with ethyl acetate. The partly crystalline residue was triturated with 30 ml of ethyl acetate and the crystals were collected after some standing in the refrigerator. The yield of crude nitroglucoside II was 4.3 g (24.1%).

(c) Methyl 3-Nitro-3-deoxy-β-D-galactopyranoside (III)

The mother liquor that remained after removal of the crystalline nitroglucoside II (cf. preceding Section (*b*)) was shown by paper chromatography to contain major components of R_f 0.57 and 0.68 as well as minor

components in the range of R_f 0.77–0.90. The mother liquor was concentrated to a sirup that was inoculated with crystalline nitrogalactoside III.[6] Copious crystallization occurred at 0° within a few hours. The material was triturated with a little ice-cold ethyl acetate and the solid matter isolated and washed with cold ethyl acetate. The yield was 5.6 g (31.6%) of crude III (R_f 0.57) containing some of the *gluco* isomer II (R_f 0.68). Another 400 mg of III which was even purer than the main crop was obtained from the mother liquor, but, curiously enough, only after the nitromannoside VII had been isolated (cf. Section (*d*)). Thus the total yield of III was 34%.

The product was recrystallized by dissolution in the minimum amount of hot acetone and addition of chloroform to incipient turbidity. When the solution was cooled fine thin needles of III separated quickly; $[\alpha]_D^{23}$ +27.8°. Accompanying traces of II were rather difficult to remove. A sample that had been recrystallized five times was at last chromatographically uniform and showed $[\alpha]_D^{23}$ +32.6°. The air-dried product evidently contained solvent of crystallization. It melted upon rapid heating at 87–90°, but upon very slow heating or after drying at 80° at 3 mm the melting point was 131–132°. Slight changes in the infrared spectrum (in nujol) occurred when the air-dried substance was dried at 80° *in vacuo* for several hours, and the same changes resulted when it was kept in the molten state at 95° for a few minutes. The high-melting product when triturated with acetone–chloroform gave again the original spectrum. Anal. Calc. for $C_7H_{13}NO_7$ (223.2): C, 37.67; H, 5.87; N, 6.28. Found: C, 37.02; H, 6.20; N, 6.10.

(d) Methyl 3-Nitro-3-deoxy-β-D-mannopyranoside (VII)

The ethyl acetate filtrate from the main crop of crystalline III contained, besides remnant II and III, the nitromannoside VII, which was isolated in two portions. The first portion (A) crystallized together with II upon concentration of the filtrate and addition of a little ether. The second portion (B) was obtained from the remaining mother liquor by column chromatography.

The crystalline fraction A, after being washed with ice-cold, ether-containing ethyl acetate, weighed 490 mg, melted at 161–164°, and was shown by paper chromatography to contain a considerable amount of II. The material was recrystallized thrice from acetone–chloroform and subsequently treated with a small amount of boiling ethyl acetate. There remained 45 mg of crystals that did not dissolve in the ester. By means of their melting point (201–202°) and infrared spectrum they were identified as nearly pure nitroglucoside II. The acetone–chloroform mother liquors of the successive recrystallizations and the ethyl acetate extract were combined and the solvents removed, leaving a crystalline residue. From this a further 140 mg of somewhat less pure II was separated by one more recrystallization followed by extraction. The combined mother liquor and extract of this second sequence gave upon evaporation a residue which was recrystallized once again from acetone–chloroform. Thus 95 mg of a product with m.p. 133–134° and $[\alpha]_D^{23}$ −66.7° was obtained. It was nitromannoside VII (R_f 0.59) which was still accompanied by a small amount of nitroglucoside II (R_f 0.68).

For the isolation of the second portion of VII (B) the mother liquor from which fraction A had been separated was first reduced to a smaller volume and placed in the refrigerator. In the course of a few days a crystalline deposit was formed (400 mg) which by melting point and infrared spectrum was shown to be additional nitrogalactoside III (cf. preceding Section (*c*)). The mother liquor therefrom was then chromatographed on a cellulose column (3.7 cm×58 cm), the aforementioned solvent system for nitroglycosides being used. Although no satisfactory separation of the remaining nitroglycosides could be so achieved, the process served to remove fast-moving yellow impurities and unidentified by-products. The effluent containing the nitroglycosides afforded a material from which 100 mg of a crystalline mixture of II and VII, and 390 mg (2%) of pure VII (m.p. 136–138°; $[\alpha]_D^{23}$ −81.3°), could be obtained by fractional crystallization with ethyl acetate – ether. Anal. Calc. for $C_7H_{13}NO_7$ (223.2): C, 37.67; H, 5.87; N, 6.28. Found: C, 37.93; H, 5.88; N, 6.27.

B. Amino Derivatives

For hydrogenation of the nitroglycosides, by the use of a platinum catalyst in the presence of one equivalent of dilute hydrochloric acid, the directions given earlier (1, 3) for similar experiments were followed. Chloride ion was removed from the hydrogenated solutions with Dowex-1 (OH^-), grade X2.

(a) Methyl 3-Amino-3-deoxy-β-D-galactopyranoside (IV)

Nitrogalactoside III (223 mg) was hydrogenated to give, after recrystallization from 95% ethanol, 105 mg (54.5%) of colorless needles of IV showing m.p. 173–174° decomp., $[\alpha]_D^{23}$ −3.7° and R_{gm} 1.38. The infrared spectrum, mixed melting point, and R_{gm} value established the identity of the product with the methyl aminodeoxyhexoside that had been obtained previously as a by-product but had not been characterized in detail.[2]

The analytical sample was recrystallized once more from moist ethanol and then showed m.p. 175–176° decomp.; the specific rotation remained unchanged. Anal. Calc. for $C_7H_{15}NO_5$ (193.2): C, 43.51; H, 7.83; N, 7.25; OCH_3, 16.06. Found: C, 43.39; H, 7.54; N, 7.25; OCH_3, 16.20.

For the preparation of IV on a larger scale we found it convenient to hydrogenate directly, i.e., without prior isolation of crystalline III, the total residue from the mother liquor as it was obtained in the nitromethane condensations following removal of the main portion of II (cf. Sections A(*a*) and/or A(*b*)). The

[6]*Seed crystals were first obtained when a similarly prepared sirup was left in a refrigerator for several days.*

overall yields of pure IV were about 21% (based on the original starting material, methyl β-D-glucopyranoside).

Hydrochloride (V).—Evaporation with dilute hydrochloric acid of a sample of IV, followed by trituration of the residue with methanol, furnished clusters of fine needles that were recrystallized from ethanol (95%) – ethyl acetate. It was the hydrochloride V; $[\alpha]_D^{23}$ −3.1°. It melted with decomposition at about 227° on rapid heating; slow heating resulted in gradual darkening without distinct melting.

(b) Methyl 3-Acetamido-3-deoxy-β-D-galactopyranoside (VI)

N-Acetylation of the free base IV (200 mg) as applied earlier to the *gluco* isomer (1, 2) yielded 174 mg (71.3%) of white needles of VI; m.p. 239°, $[\alpha]_D^{23}$ +52°. Anal. Calc. for $C_9H_{17}NO_6$ (235.2): C, 45.95; H, 7.29; N, 5.96. Found: C. 46.13; H, 7.10; N, 5.88.

Hydrolysis of a sample in 4 *N* hydrochloric acid for 3 hours at 100° gave 3-amino-3-deoxy-D-galactose hydrochloride as the only sugar detectable by paper chromatography.

(c) 3-Amino-3-deoxy-α-D-galactose Hydrochloride

Methyl aminodeoxygalactoside IV (160 mg) was hydrolyzed with 32 ml of 4 *N* HCl for 3 hours at 100°. The hydrolyzate was evaporated with addition of several successive portions of water and finally of toluene. The residue upon trituration with ethanol started to crystallize at once and was collected after standing for 2 hours at 0°. The yellowish-white product was recrystallized by dissolution in the minimum amount of water required and addition of excess ethanol. The yields found in several parallel runs were 80–85%. The colorless crystals were chromatographically uniform (R_{gm} 0.90). $[\alpha]_D^{23}$ +109° → +82.0° (final, after 2 hours); final values of samples from parallel runs, +82.1°, +82.7°. Reported values (6), $[\alpha]_D$ +115° → +89° (2 hours). We were unable to confirm the melting point quoted (6), but observed slow decomposition above 200°.

(d) 3-Acetamido-3-deoxy-β-D-galactose

The above hydrochloride (1.25 g) was N-acetylated according to a known procedure (8, 2). There was obtained 996 mg (78%) of an acetamido sugar melting at 162–164°. Repeated recrystallization from ethanol – ethyl acetate afforded a product of m.p. 173° and $[\alpha]_D^{23}$ +92.5° → +118.0° (final, 2.5 hours). Reported (6), m.p. 170–172° and $[\alpha]_D$ +99° → +119° (2.5 hours). Our product and an authentic sample of 3-acetamido-3-deoxy-β-D-galactose kindly supplied by Professor R. Kuhn, Heidelberg, gave identical infrared spectra and a mixed melting point of 171–173°.

(e) Methyl 3-Amino-3-deoxy-β-D-mannopyranoside Hydrochloride (VIII)

(*i*) A sample of 62 mg of nitromannoside VII (not quite pure; m.p. 133–134°) was hydrogenated and afforded, upon evaporation without prior removal of chloride ion, a partly crystalline material. This was recrystallized from ethanol – ethyl acetate to give 45 mg (67%) of oblong, rectangular prisms of VIII; m.p. 230–231° decomp., R_{gm} 1.27, $[\alpha]_D^{23}$ −68.5°.

(*ii*) A nitromethane condensation with 0.04 mole of dialdehyde I had been performed in aqueous solution and had furnished about 25% of crystalline nitroglucoside II. The mother liquor had then been hydrogenated, giving 20.5% of crystalline aminogalactoside as free base IV. (See Sections A(*b*) and B(*a*).) The filtrate therefrom was now evaporated and the residue taken up in water and acidified with 20 ml of *N* hydrochloric acid. Evaporation with several consecutive portions of water followed by ethanol gave a quickly crystallizing residue of amine hydrochlorides. The material was allowed to stand overnight under some ethanol at 0°. The crystals were collected, washed with cold ethanol, and dried. The product (1.184 g, 12.9%) was VIII and showed m.p. 228–229° decomp., and $[\alpha]_D^{23}$ −68.3° after one recrystallization from aqueous ethanol. It was chromatographically uniform (R_{gm} 1.24) and was identified by infrared spectroscopy with the preparation obtained under (*i*). Anal. Calc. for $C_7H_{16}O_5NCl$ (229.7): C, 36.61; H, 7.02; N, 6.10. Found: C, 36.04; H, 6.89; N, 6.39.

The mother liquor after the crystallization of VIII deposited, on standing and careful addition of a little ethyl acetate, a small amount of crystals (36 mg), also melting with decomposition at about 228°. They were, however, identified as aminogalactoside hydrochloride (V) from the results of rotation ($[\alpha]_D^{23}$ −3.15°) and chromatography (R_{gm} 1.39). This was confirmed by acid hydrolysis to yield crystalline 3-amino-3-deoxy-D-galactose, which was identified by its infrared spectrum and chromatogram.

(f) 3-Amino-3-deoxy-α-D-mannose Hydrochloride

A 30-mg sample of pure VIII was hydrolyzed with 6 ml of 4 *N* hydrochloric acid for 3 hours at 100°. The reducing amino sugar hydrocholoride was obtained in crystalline condition as in the previously described (7) hydrolysis of the anomeric glycoside, and was found identical with a specimen from that work by comparison of the infrared spectra, R_{gm} values (1.12), and mutarotation data. $[\alpha]_D^{23}$ +16.5° → +6.1° (2 hours, final).

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