Reactions of Nitro Sugars. XXVI. Analysis and Separation of Stereoisomers by Nuclear Magnetic Resonance Spectroscopy and Column Chromatography of Benzylidene Derivatives[1]

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Stereoisomeric methyl 4,6-*O*-benzylidene-3-deoxy-3-nitrohexopyranosides proved to be distinguishable by the chemical shifts of their benzylidene methine proton signals, and ratios of such isomers in mixtures could be estimated by signal integration. The method was applied to a reinvestigation of the epimeric distributions in which methyl 3-deoxy-3-nitro-α-D-hexopyranosides arise, firstly, in the nitromethane cyclization of 2-*O*-[(*S*)-formyl(methoxy)methyl]-(*R*)-glyceraldehyde and, secondly, in their base-catalyzed configurational equilibration via nitronates. Previous results were essentially confirmed as far as the preferred configurations are concerned but require some revision as to the relative abundance of less favored configurations. By preparative chromatography of benzylidene acetals and subsequent debenzylidenation, some nitrohexopyranosides of the α-D series were isolated for the first time in crystalline condition. Separation of isomers in the β-D series was improved by use of chromatography and by a new method involving differential reactivities of compounds toward alkali.

Les composés méthyl 4,6-*O*-benzylidène-3-déoxy-3-nitrohexopyranosides stéréoisomériques peuvent être différenciés par les déplacements chimiques des signaux du proton méthine benzylidène; de même on peut estimer les proportions de tels isomères dans les mélanges en intégrant le signal. La méthode a été appliquée à une nouvelle étude des distributions épimériques dans lesquelles les composés méthyl 3-déoxy-3-nitro-α-D-hexopyranosides apparaissent, premièrement dans la cyclisation du nitrométhane du 2-*O*-[(*S*)-formyl(méthoxy)méthyl]-(*R*)-glycéraldéhyde, et deuxièmement dans leur équilibre configurationnel catalysé par les bases, via les nitronates. Les résultats précédents ont été essentiellement confirmés en autant que les configurations les plus stables sont concernées, mais il doivent être revisés en ce qui a trait à l'abondance relative des configurations moins favorisées. Par chromatographie préparative des acétals de benzylidène et par la débenzylidénation subséquente, on a isolé quelques nitrohexopyranosides de la série α-D pour la première fois à l'état cristallin. On a amélioré la séparation des isomères dans la série β-D en utilisant la chromatographie et au moyen d'une nouvelle méthode impliquant les réactivités différentielles des composés envers les alcalins.

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Introduction

In synthetic work with nitro sugars, as indeed with carbohydrates in general, benzylidene acetals play a paramount role (1, 2). The most recent, extensive use of *O*-benzylidenated nitro sugars was made in syntheses of epoxynitro (3) and halonitro (4) glycosides. Prior publications had recorded the preparation of 4,6-*O*-benzylidene acetals derived from several, but not all, of the known methyl 3-deoxy-3-nitrohexopyranosides. The acetals were obtained, by standard procedures, mostly from pure glycosides and occasionally from non-crystallizable mixtures of stereoisomers (2). In conjunction with various synthetic projects we have now elaborated further the techniques of separating and characterizing nitro glycosides by way of benzylidenation. With n.m.r. spectroscopy as an analytical aid and column chromatography as a preparative tool, some nitro sugar derivatives were isolated for the first time, and the preparation of others was facilitated. Special benefit, however, accrued from benzylidenation when it was applied in studies on the stereochemistry of the sugar dialdehyde – nitromethane cyclization and the epimerization of glycoside nitronates.

Results

A convenient means of differentiating stereoisomeric methyl 4,6-*O*-benzylidene-3-deoxy-3-nitrohexopyranosides was found in the sharp n.m.r. singlet given by the methine proton of the benzylidene group. The chemical shifts of these signals proved indicative of sets of isomers (Table 1). Although in chloroform-*d* solution the shifts differed little in compounds having the *gluco* and *galacto* configurations, they permitted differentiation between these and *manno* and

[1]For Part XXV see ref. 10.

TABLE 1. Benzylidene methine and methoxyl proton n.m.r. signals (τ-scale)

Compound	Configuration	PhCH		OCH_3	
		in $CDCl_3$	in DMSO-d_6	in $CDCl_3$	in DMSO-d_6
2	α-D-*manno*	4.34	4.24	6.61	6.67
3	α-D-*talo*	4.42	4.32	6.60	6.66
4	α-D-*gluco*	4.48	4.34	6.55	6.63
5	α-D-*galacto*	4.50	4.40	6.56	
17	β-D-*gluco*	4.48	4.33	6.46	6.57
18	β-D-*galacto*	4.48	4.38	6.40	6.57
19	β-D-*manno*	4.32	4.26	6.44	6.58

talo isomers. In dimethylsulfoxide-d_6 solution, on the other hand, the *gluco* and *talo* derivatives had similar shifts but could be distinguished from the *manno* and *galacto* derivatives. The variations observed were distinctly larger than those recorded (5) for a number of non-nitro analogs. In fact, the signal separation in certain mixtures of isomers readily allowed quantitative determination of the component ratio by integration (Table 2, mixtures A–D). Thus, in three-component mixtures containing **2**, **3**, and **4** in varying proportions, the percentage of each compound could be measured with an accuracy of $\pm 4\%$ or better. On the other hand, the chemical shift of the methine proton was scarcely influenced by the anomeric configuration. However, anomers may be distinguished by their methoxyl proton resonances (Table 1) which tend to occur at higher field for axial groups than for equatorial ones (6).

Prompted by the feasibility of spectral identification and quantitative assay of certain benzylidene acetals, we decided to reinvestigate the configurational distributions in which stereoisomeric methyl 3-deoxy-3-nitro-α-D-hexopyranosides are formed (7, 8) in the nitromethane cyclization of 2-*O*-[(*S*)-formyl(methoxy)methyl]-(*R*)-glyceraldehyde (**1**, "D-hydroxymethyl-D′-methoxydiglycolaldehyde") and also those in which they exist after thermodynamically controlled epimerization (8). In the earlier work, the nitro glycosides had not been obtained in crystalline form; approximate estimates of product ratios in the amorphous mixtures could be gleaned from the amounts of amino sugars that were isolated, upon catalytic hydrogenation, partly by direct crystallization and partly by chromatographic procedures. Although the configurational preferences in these reactions had thereby been reasonably well established, no great accuracy could be claimed in a quantitative sense. A renewed study using improved techniques therefore appeared worthwhile.

A mixture of nitro glycosides was prepared, essentially as described (8), by the reaction of dialdehyde **1** with nitromethane followed by

TABLE 2. Analysis of acetal mixtures by n.m.r. spectroscopy

Mixture*	Composition		PhCH signals	
	Compounds	Ratio	τ-Values†	Intensity ratios
A	**2**, **3**, **4**	16.7:49.8:33.5	4.34 4.41 4.48	19.0:48.2:32.8
B	**2**, **3**, **4**	33.3:14.5:52.2	4.34 4.41 4.49	29.6:15.1:55.3
C	**19**, **3**, **4**	28.9:42.6:28.5	4.32 4.41 4.48	27.3:41.8:30.9
D	**19**, **3**, **17**	39.7:25.1:35.2	4.33 4.41 4.48	35.3:27.5:37.2
E	**2**, **3**, **4**, **5**		4.34 4.42 4.49‡	37.1:11.8:51.1‡
			(4.24 4.33§ 4.40)	42.0:49.2§:8.8
F	**2**, **3**, **4**, **5**		4.34 4.415 4.49‡	15.5:52.6:31.9‡

*A–D, standards made by mixing weighed amounts of crystalline compounds; E, mixture from nitromethane cyclization of **1** (see text); F, mixture from nitromethane cyclization followed by epimeric equilibration (see text).
†In $CDCl_3$ except for the set in parentheses, which refers to DMSO-d_6.
‡**4** + **5**.
§**3** + **4**.

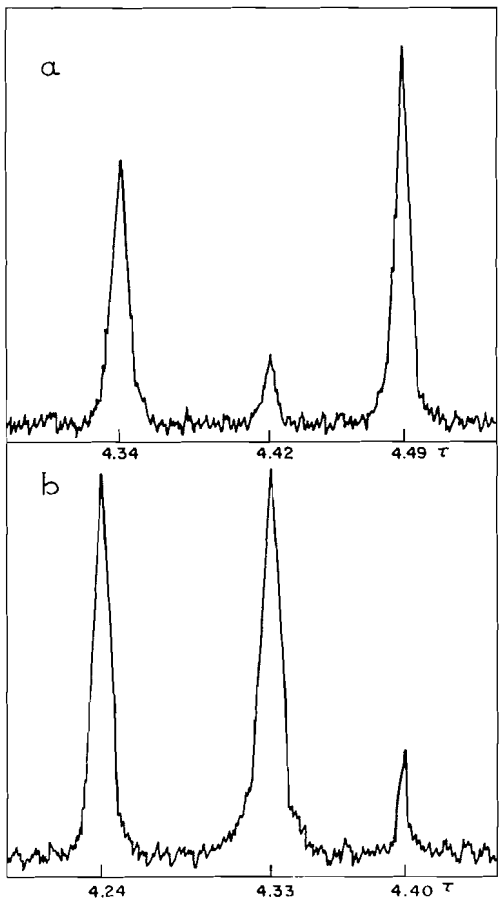


FIG. 1. Methine proton signals, in 100 MHz n.m.r. spectrum with 100 Hz sweep width, of nitromethane cyclization mixture from **1**, after benzylidenation; *a*, in $CDCl_3$; *b*, in DMSO-d_6.

deionization.[2] Exhaustive benzylidenation with benzaldehyde and zinc chloride gave a mixture of 4,6-acetals that showed methine proton signals assignable to the α-D-*manno* (**2**), α-D-*talo* (**3**), α-D-*gluco* (**4**), and α-D-*galacto* (**5**) isomers. In $CDCl_3$, there occurred three signals having an intensity ratio of 37:12:51, corresponding to **2**, **3**, and **4** + **5**. In DMSO-d_6, there occurred three signals with an intensity ratio 42:49:9, corresponding to **2**, **3** + **4**, and **5** (Fig. 1 and Table 2, mixture E). It is concluded from these data that the *manno* and *gluco* compounds, **2** and **4**, were present as main products in similar amounts (about 40% each), whereas the *talo* and *galacto* compounds, **3** and **5**, were minor products present to the extent of about 10% each. The acetal mixture was then chromatographed on silica gel, and substantial amounts of each isomer could be isolated in crystalline form. Complete separation was not achieved, but the proportions of isomers contained in mixed fractions were estimated spectroscopically, and summation of the yields indicated a ratio of 42:8:41:9 for **2**:**3**:**4**:**5**, in good agreement with the ratio found in the mixture prior to chromatography.

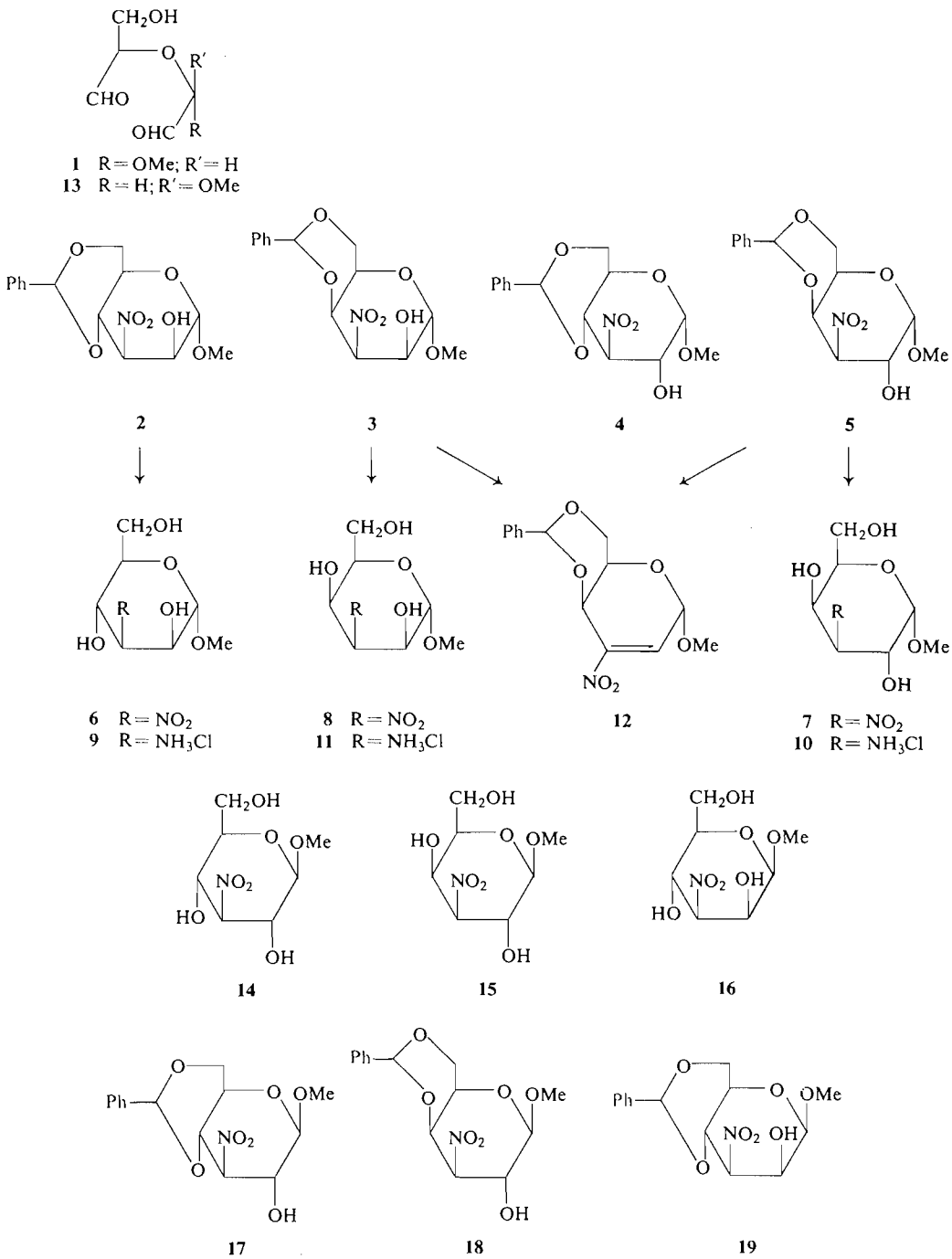
These results established in a more reliable, quantitative way what had been deduced earlier (8) from preparative separation of products on the amine stage, namely, that the *manno* and *gluco* configurations are favored in the nitromethane cyclization of **1** when kinetic control prevails. The results revealed, moreover, that the *talo* and *galacto* configurations are formed to a significant extent; the former had previously been observed to arise in an unspecified, small proportion and the latter had not been detected at all, under similar reaction conditions.[3]

We then proceeded to examine in the same way the isomer ratio that exists after the aforementioned nitro glycosides have been allowed to epimerize in aqueous, alkaline solution to the thermodynamic equilibrium of their nitronates. Equilibration and subsequent deionization of the nitronate mixture was performed as previously described (8), and the product was then benzylidenated. The acetal mixture in $CDCl_3$ solution showed three methine-proton peaks (τ 4.34, 4.415, and 4.49), and their intensity ratios were 15.5:52.5:32 in a 3-day epimerization experiment[4] (Fig. 2 and Table 2, mixture F). The first two signals corresponded to the α-D-

[2]The reaction time allowed for the nitromethane cyclization was 45 min whereas in the previous work (8) it was 25 min.

[3]It may be expected that minor changes in such conditions as reaction time (see footnote 2), temperature, or alkalinity of the medium could produce measurable variations in product ratios, owing to the interplay of kinetic and thermodynamic factors. In the nitromethane cyclization leading to analogous 6-deoxy glycosides, for instance, the *gluco* to *manno* ratio was about 1:1 after a reaction time of 25 min, but about 2:1 after 40 min; *talo* and *galacto* derivatives were minor products in either event (9). See also the discussion on this subject in ref. 10.

[4]In an independent experiment we found almost no difference in spectra from benzylidenations performed after nitronate epimerizations running for 3 and 5 days. This indicated that epimerization was essentially complete within 3 days.



manno and α-D-*talo* isomers (**2** and **3**), respectively, and the third signal was due to the α-D-*gluco* (**4**) and α-D-*galacto* (**5**) isomers. Column chromatography confirmed this pattern, indicating the presence of **2**, **3**, **4**, and **5** in the ratio 15.5:51.5:20:13.

The direct evidence so obtained confirms the principal statement which was based on con-

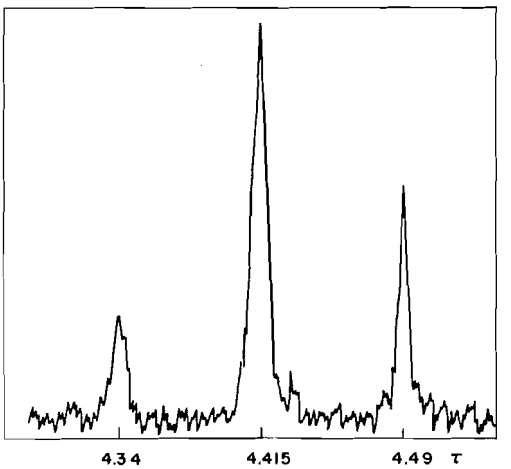


FIG. 2. Methine proton signals, in 100 MHz n.m.r. spectrum with 100 Hz sweep width, of nitromethane cyclization mixture from **1** after *epimerization* and subsequent benzylidenation; solvent, $CDCl_3$.

version into amine derivatives (8), namely that the nitro glycosides produced in the nitromethane cyclization of **1** epimerize, as nitronates, so as to generate chiefly the α-D-*talo* configuration at the expense of the α-D-*manno* and α-D-*gluco* configurations. Clearly, the nitronate of the α-D-*talo* compound is the thermodynamically most stable one. The experiments showed furthermore, that the amount of α-D-*galacto* isomer increases, if only slightly, during the epimerization. However, the relative stabilities of the nitronates of the α-D-*gluco* and α-D-*galacto* isomers have now been revealed to be in fact rather similar whereas, in the earlier work (8), the former had been underrated and the latter overestimated. The present results are for the most part in good harmony with those from a recent study (10) on analogous 6-deoxy glycosides in which, after nitronate equilibration, the *manno*:*talo*:*gluco*:*galacto* ratio was approximately 28:59:7:6.

In the course of the chromatographic separations just mentioned, methyl 4,6-*O*-benzylidene-3-deoxy-3-nitro-α-D-mannopyranoside (**2**) and its α-D-*galacto* isomer (**5**) were isolated for the first time. These acetals as well as the previously described (11) α-D-*talo* isomer (**3**) were debenzylidenated to furnish methyl 3-deoxy-3-nitro-α-D-mannopyranoside (**6**), -α-D-galactopyranoside (**7**), and -α-D-talopyranoside (**8**) as new, crystalline compounds. The structures of **6** and **8** were ascertained by catalytic hydrogenation giving the known, crystalline amino glycoside hydrochlorides **9** and **11**, respectively. Similarly, **7** afforded the corresponding amine hydrochloride (**10**) in crystalline form. In this case, however, further structural proof was needed since the amine had been obtained earlier (8) as a syrup of questionable purity only, and data of comparison were lacking. The α-D-*galacto* configuration was established by dehydration of the acetal **5** with hot acetic anhydride and sodium acetate, which produced known methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-α-D-*threo*-hex-2-enopyranoside (**12**), *i.e.*, the same nitro olefin that was formed (11) by such dehydration from the α-D-*talo* isomer (**3**). The procedure for preparing **12** was improved in this connection.

Further preparative improvements to be recorded concern the separation of the three β-glycosides which are available (12, 13) by nitromethane cyclization of 2-*O*-[(*R*)-formyl-(methoxy)methyl]-(*R*)-glyceraldehyde (**13**, "D-hydroxymethyl-L′-methoxydiglycolaldehyde"), namely, methyl 3-deoxy-3-nitro-β-D-glucopyranoside (**14**), -β-D-galactopyranoside (**15**), and -β-D-mannopyranoside (**16**). Although preparation of **14** and **15** by direct crystallization is easy enough (14), it may be desirable to augment yields by working-up mother liquors that retain considerable quantities of material. Benzylidenation of such isomer mixtures from mother liquors has served (15) to separate components as their crystalline acetals (**17**–**19**), and in the Experimental we describe improved procedures involving fractional crystallization and column chromatography. Another method of separation was elaborated on the basis of the differential reactivities of **17**–**19** towards alkali; it represents a useful, preparative corollary to a recent communication on this subject (16). Thus, when a mixture of the three acetals (or a mixture of **18** and **19** alone) in chloroform solution was treated briefly with potassium hydroxide, the β-D-*manno* isomer **19** was quantitatively epimerized, and a mixture of **17** and **18** resulted. These two components then proved readily separable due to the fact that, under the conditions employed, the β-D-*galacto* isomer **18** forms a chloroform-insoluble, water-soluble nitronate whereas the β-D-*gluco* isomer **17** does not do so but remains soluble in chloroform and insoluble in water.

Experimental

The n.m.r. data were obtained from 100 MHz spectra taken on a Varian HA-100 instrument, using tetramethylsilane for the lock signal. For analysis of acetal mixtures, spectra were taken from approximately 10% solutions in chloroform-*d* or DMSO-d_6, at 100 Hz sweep width and 500 Hz sweep offset. The component ratios were determined by averaging three integrations of the methine proton signals and expressing each signal intensity in terms of percent of the sum of intensities. In standard mixtures, single integrations of a given signal deviated ± 1–2% from the average value, and the latter deviated ± 1–4% from the expected value.

The t.l.c. was performed on silica gel G (E. Merck AG) with carbon tetrachloride – ethyl acetate (7:3, v/v) as solvent unless specified otherwise. Ceric sulfate – sulfuric acid served as spray reagent. In column chromatography, silica gel refers to the 70-325 mesh ASTM product of E. Merck AG.

Separation of α-Glycosides

(a) Preparation of Nitro Glycoside and Acetal Mixtures

Preparation of the dialdehyde **1**, its cyclization with nitromethane in methanol solution containing sodium methoxide, and deionization of the product to give a mixture (E) of nitro glycosides was performed as previously described (8), with a minor modification.[2] The same applies to the preparation of the mixture (F) of epimerized nitro glycosides (8). Separation of crystalline methyl 3-deoxy-3-nitro-α-D-glucopyranoside by chromatography of mixture E on powdered cellulose has been recorded (15). Benzylidenation of mixtures E and F with benzaldehyde and zinc chloride had previously yielded the crystalline α-D-*gluco* (**4**) (15) and α-D-*talo* (**3**) (11) acetals, respectively. For the present studies, benzylidenated mixtures were prepared as previously described, but the syrups obtained were used for n.m.r. spectroscopy and column chromatography without prior removal of crystallizable components.

(b) Chromatography of Benzylidenated Mixture E

Crude, benzylidenated mixture E, obtained as a syrup (21.5 g) from **1** (0.068 mol, from 13.2 g of methyl α-D-glucopyranoside) was placed on a column (6.5 × 60 cm) containing 800 g of silica gel. Elution was performed with carbon tetrachloride – ethyl acetate mixtures of compositions (v/v) 7:3 (fractions 1–30), 1:1 (fractions 31–39), and 2:3 (fractions 40–80), the fractions being 75 ml in volume. All fractions were inspected by t.l.c., and the residues obtained after appropriate pooling and evaporation were examined by n.m.r. spectroscopy. Fractions 1–15 were free from carbohydrates but contained benzaldehyde (6.76 g). Fractions 16–21 yielded pure **2** (2.22 g). Fractions 22–30, 31–36, 37–39, 40–50, and 51 contained 4.49, 1.46, 0.43, 1.29, and 0.68 g of mixtures of **2**, **3**, and **4**. Fractions 52–63 and 75–80 were blank, and fractions 64–74 gave pure **5** (1.00 g). Thus the total, overall yield of nitro glycoside acetals was 11.57 g (54.7% of the theoretical based on starting methyl α-D-glucopyranoside). Individual yields, calculated by summation of pure fractions and spectroscopically-determined proportions of components in mixed fractions, were the following: **2**, 4.86 g (22.9%); **3**, 0.94 g (4.4%); **4**, 4.77 g (22.5%), and **5**, 1.00 g (4.7%).

When the column chromatography was performed with half the above glycoside load and with carbon tetrachloride – ethyl acetate mixture of constant composition (7:3), a somewhat better separation of the more mobile compounds, **2**, **4**, and **3** took place and parts of each were eluted as pure fractions, in that order, with mixed fractions occurring in between. However, the solvent was unsatisfactory for elution of slow-moving **5**.

(c) Chromatography of Benzylidenated Mixture E

A crude syrup (21.8 g) resulting from benzylidenation of a mixture (F) of epimerized nitro glycosides obtained from **1** was chromatographed. The technique detailed in section *b* was used except that the eluant composition was carbon tetrachloride–ethyl acetate in the ratios 4:3, 1:1, and 2:3 for fractions 1–24, 25–30, and 31–50, respectively. Fractions 1–10 contained benzaldehyde (5.6 g). Fractions 11–15 and 17–18 consisted of mixtures of **2** (1.20 g) and **4** (0.80 g), and of **4** (0.30 g) and **3** (0.55 g), respectively, the product ratios being determined from n.m.r. spectra. Fractions 16, 19–30, and 31–50 yielded virtually pure **4** (0.47 g), **3** (3.48 g), and **5** (1.03 g), respectively. Hence the total yield of nitro glycoside acetals was 7.83 g. Since the ratios of individual acetals were very similar before and after chromatography it appears that recovery was essentially complete. The weight deficit is attributed to benzaldehyde (5.6 g) and residual solvent in the starting syrup.

Characterization of α-D-Glycosides

Methyl 4,6-O-Benzylidene-3-deoxy-3-nitro-α-D-mannopyranoside (2)

Compound **2** as obtained from a chromatographic column was recrystallized for analysis from chloroform – petroleum ether; m.p. 138–139°, $[\alpha]_D$ +27.7° (*c*, 0.8 in $CHCl_3$).

Anal. Calcd. for $C_{14}H_{17}NO_7$ (311.3): C, 54.01; H, 5.51; N, 4.50. Found: C, 53.96; H, 5.56; N, 4.59.

Methyl 3-Deoxy-3-nitro-α-D-mannopyranoside (6)

The acetal **2** (300 mg) was debenzylidenated (17) by treatment with 3 ml of 90% trifluoroacetic acid at room temperature for 15 min. Completion of the reaction was ascertained by t.l.c. using ethyl acetate – petroleum ether (2:3). Water was added to the reaction mixture which was then evaporated. The residue was repeatedly evaporated with added water and ethanol, and the remaining syrup was dried *in vacuo* (206 mg, 96%; homogeneous on t.l.c.). Crystallization from tetrahydrofuran–ether furnished **6** (184 mg), m.p. 113–115°, $[\alpha]_D$ +44.5° (*c*, 0.9 in water).

Anal. Calcd. for $C_7H_{13}NO_7$ (223.2): C, 37.67; H, 5.87; N, 6.28. Found: C, 37.79; H, 5.93; N, 6.35.

A sample of **6** (100 mg) was dissolved in water (8 ml), 0.1 *N* HCl (5 ml), and platinum catalyst (from 50 mg of PtO_2) was added, and the mixture was hydrogenated overnight. Filtration and evaporation of the solution, followed by crystallization of the residue from 95% ethanol at 4° furnished 84 mg (81.5%) of methyl 3-amino-3-deoxy-α-D-mannopyranoside hydrochloride (**9**) in two batches, m.p. 234–236° (dec.) and 232–235° (dec.); $[\alpha]_D$ +57.5° (*c*, 0.3 in water). Reported: $[\alpha]_D$ +60° in water (7, 18), and decomposition at about 205° (7), 221–223° (8), 210–240° (18). Evidently the melting behavior is not very characteristic, it being probably influenced by traces of impurities and the mode of heating. A sample at hand from previous work in this laboratory melted with decomposition at 230–231° and caused no depression when admixed to new **9**. The i.r. spectra were identical.

Methyl 4,6-O-Benzylidene-3-deoxy-3-nitro-α-D-talopyranoside (3)

Compound **3** collected from the column showed m.p. 177–178°, $[\alpha]_D$ +66.8° (*c*, 1.1 in $CHCl_3$), and its i.r. spectrum was identical with that of an authentic sample. Reported (11): m.p. 175°, $[\alpha]_D$ +68° (in $CHCl_3$).

Methyl 3-Deoxy-3-nitro-α-D-talopyranoside (8)

The acetal **3** (200 mg) was debenzylidenated with trifluoroacetic acid as described for **2**, above. The reaction was complete after 20 min as indicated by t.l.c. with chloroform–acetone (7:3). The syrupy product (117 mg, 82%) was crystallized from chloroform to give **8** (93 mg), m.p. 86–87°; $[\alpha]_D$ +87.5° (*c*, 0.9 in water).

Anal. Calcd. for $C_7H_{13}NO_7$ (223.2): C, 37.67; H, 5.87; N, 6.28. Found: C, 37.46; H, 6.08; N, 6.12.

A sample of **8** (40 mg) was catalytically hydrogenated for 3 h in water (3 ml) and 0.1 *N* HCl (2 ml), in the presence of platinum catalyst (70 mg of PtO_2). Upon recrystallization of the product from 99% ethanol by slow addition of ethyl acetate, methyl 3-amino-3-deoxy-α-D-talopyranoside hydrochloride (**11**) was obtained in 99% yield; m.p. 187–188° (dec.), $[\alpha]_D$ +88.3° (*c*, 0.3 in water). Reported (8): m.p. 187–188 (dec.), $[\alpha]_D$ +90° (in water). Identity with an authentic sample was established by a mixture melting point (186–188° with dec.), comparison of i.r. spectra, and paper chromatography (8).

Methyl 4,6-O-Benzylidene-3-deoxy-3-nitro-α-D-glucopyranoside (4)

Compound **4** collected from a column showed m.p. 166–167°, $[\alpha]_D$ +86.5° (*c*, 0.7 in ethanol), and its i.r. spectrum was identical with that of an authentic sample. Reported (15): m.p. 167°, $[\alpha]_D$ +87.2° (in ethanol).

Methyl 4,6-O-Benzylidene-3-deoxy-3-nitro-α-D-galactopyranoside (5)

Compound **5** collected by column chromatography was recrystallized for analysis from ethyl acetate–petroleum ether; m.p. 166–167°, $[\alpha]_D$ +205° (*c*, 0.5 in $CHCl_3$).

Anal. Calcd. for $C_{14}H_{17}NO_7$ (311.3): C, 54.01; H, 5.51; N, 4.50. Found: C, 53.83; H, 5.51; N, 4.41.

Methyl 3-Deoxy-3-nitro-α-D-galactopyranoside (7)

The acetal **5** (94 mg) was debenzylidenated with trifluoroacetic acid as described for **2**, above. The reaction was complete after 30 min as shown by t.l.c. Crystallization of the syrupy product (67 mg) from chloroform afforded **7** (32 mg), m.p. 145–146°, $[\alpha]_D$ +209° (*c*, 0.4 in water). The remainder of **7** (35 mg) was recovered as a syrup.

Anal. Calcd. for $C_7H_{13}NO_7$ (223.2): C, 37.67; H, 5.87; N, 6.28. Found: C, 37.92; H, 6.02; N, 6.16.

Methyl 3-Amino-3-deoxy-α-D-galactopyranoside Hydrochloride (10)

Syrupy **7** (35 mg) in water (5 ml) and 0.1 *N* HCl (2 ml) was hydrogenated for 3 h in the presence of prereduced PtO_2 (50 mg). Evaporation of the filtrate followed by two coevaporations with ethanol gave crystalline **10** (34 mg) which upon recrystallization from ethanol melted with decomposition at 219–221° and had $[\alpha]_D$ +146.5° (*c*, 0.2 in water).

Anal. Calcd. for $C_7H_{16}ClNO_5$ (229.7): C, 36.61; H, 7.02; Cl, 15.44. Found: C, 36.76; H, 6.79; Cl, 15.71.

In agreement with the earlier observation on amorphous **10**, the mobility of the compound in paper chromatography (8) was lower than that of its isomers **9** and **11**.

Methyl 4,6-O-Benzylidene-2,3-dideoxy-3-nitro-α-D-threo-hex-2-enopyranoside (12)

A mixture of the acetal **5** (100 mg), anhydrous sodium acetate (200 mg), and acetic anhydride (2 ml) was heated on a steam bath for 30 min with continual swirling. The mixture was then poured into ice water (50 ml) which caused a white crystalline material to precipitate. The product, which was collected by filtration, washed well with cold water, and dried *in vacuo*, weighed 86 mg (91%) and melted at 175–177°. Recrystallization from ethyl acetate – petroleum ether raised the m.p. to 182–184°; reported (11), m.p. 185–186°. An i.r. spectrum was identical with that of **12** from the earlier work (11).

In like manner but with a reaction time of 15 min, **12** was obtained in 83% yield starting from the isomeric acetal **3**. Evidently, employment of steam-bath rather than reflux (11) temperature improved the yields in these dehydrations. Moreover, the procedure was more reliable than that formerly employed, which sometimes failed due to decomposition by overheating.

Separation of β-D-Glycoside Acetals

The preparation of dialdehyde **13** and its cyclization with nitromethane to give the nitro β-D-glycosides **14**, **15**, and **16** has been described (12–14*a*) as has the benzylidenation of these glycosides, individually as pure compounds and in mixtures recovered from mother liquors (14*b*, 15).

(a) Fractional Crystallization

Mother liquors that remained when upon nitromethane cyclization of **13** the bulk of crystallizable β-D-glucoside **14** had been collected (13) were evaporated, and the residue was benzylidenated. From the mixture of acetals so produced, the β-D-*galacto* isomer **18** can be obtained by fractional crystallization from ethanol in which it is the least soluble component. However, 6–8 recrystallizations are needed to achieve high purity. A more convenient procedure is to dissolve the mixture in a small amount of acetone and then to add chloroform, which causes precipitation of a material highly enriched in **18**. One subsequent recrystallization of the precipitate from ethanol usually is sufficient to give reasonably pure **18**, m.p. 229–231° (dec.). Reported (15): m.p. 230–231° (dec.). This procedure is probably the most convenient one for the preparation of larger quantities of **18**, as it renders unnecessary the use of isolated, crystalline **15** for benzylidenation.

The acetone–chloroform mother liquor from the above separation procedure is strongly enriched in the β-D-*manno* compound **19**, but purification of the latter by continued fractional crystallization could not be achieved. If pure **19** is desired without having to start with pure **16** and without resorting to chromatography, one may benzylidenate nitromethane cyclization mixtures from which the bulk of both **14** and **15** have been removed (13, 15). However, column chromatography proved advantageous for isolating **19**.

(b) Column Chromatography

The following example describes the separation of an acetal mixture containing mainly **18** and **19** (the latter preponderating) as well as a small proportion of **17**. The mixture (10 g) and a few grams of silica gel were intimately mixed by two coevaporations with acetone in a rotary evaporator, and the dry mass was then placed on a column (4.5 × 65 cm) containing 500 g of silica gel. Elution was performed with chloroform–acetone (7:3, v/v) at a rate of about 17 ml per

10 min. The eluate was inspected at intervals by t.l.c., and the fractions were suitably combined. The first 800 ml (approximately) of effluent was blank (it may be recycled). The following 300 ml contained β-D-*manno* derivative **19** which was obtained by evaporation and was recrystallized from ethanol to give a very pure product (5.23 g), m.p. 202°, $[\alpha]_D$ −112° (*c*, 1.2 in ethanol). Reported (15): m.p. 194–196° and 198–199°, $[\alpha]_D$ −107.5°. The next 60 ml of effluent yielded impure **19** (1.05 g, m.p. 189°) that contained some **17**. Continued elution with about 500 ml of solvent furnished the β-D-*galacto* derivative **18** (3.23 g) which emerged after a few blank fractions (or, in similar runs, with slight overlapping). Upon recrystallization from ethanol it showed m.p. 232°, $[\alpha]_D$ +25° (*c*, 1 in dimethylformamide). Reported (15): $[\alpha]_D$ +24.8°.

(c) Nitronate Formation

A mixture (1 g) containing mainly **18** and **19** along with a small proportion of **17** was dissolved in chloroform (40 ml), and an ethanolic, *N* potassium hydroxide solution (3.5 ml) was added. A voluminous precipitate occurred within 5 min, and t.l.c. of the reaction mixture, performed after 10 min with a neutralized sample, indicated absence of **19** and presence of both **17** and **18**. The reaction mixture was then shaken with 40 ml of water in which the precipitate dissolved. After phase separation the aqueous layer was extracted twice with chloroform which was combined with the organic phase. Excess acetone was then added to the aqueous phase and the solution was at once deionized with an acidic cation exchange resin. The filtrate was concentrated *in vacuo* to remove most of the acetone, whereby the β-D-*galacto* isomer **18** crystallized in chromatographically pure form (131 mg). The aforementioned chloroform phase was washed twice with water, dried over sodium sulfate, and evaporated to give chromatographically pure β-D-*gluco* isomer **17** (710 mg). The products were identified by their i.r. spectra and physical constants.

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