[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

Cyclizations of Dialdehydes with Nitromethane. VIII.[1] A Spontaneous Epimerization in *aci*-Nitro Glycosides and its Significance in the Preparation of Derivatives of 3-Amino-3-deoxy-D-mannose, -D-glucose, -D-talose and D-galactose

BY HANS HELMUT BAER[2]

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The stereochemistry of the previously reported cyclization reaction with nitromethane of D'-methoxy-D-hydroxymethyl-diglycolic aldehyde (I) has been further investigated. The mixture of stereoisomeric nitroglycosides (III) produced contains, in addition to 30–35% of the D-*manno* isomer, up to 60% of the D-*gluco* isomer. Certain modifications in the reaction conditions which do not alter noticeably this steric pattern offer a simplified preparatory route to 3-amino-3-deoxy-D-mannose. On the other hand, when the isolated sodium nitronates II of the nitroglycosides III are allowed to stand in aqueous solution, epimerization at C4 occurs resulting in the formation of D-*talo* and D-*galacto* derivatives. By virtue of this isomerization it has been possible to prepare and characterize the hitherto unknown 3-amino-3-deoxy-α-D-talose hydrochloride (X) and its methyl glycoside VI, the configuration of which has been proved by degradation to known 2-amino-2-deoxy-D-lyxose (XIII).

Previous communications of this series have demonstrated the usefulness of a cyclization reaction, with nitromethane, of dialdehydes for the preparation of nitro and amino derivatives of glycosides, sugars, anhydrosugars and inositols. In the course of those studies the hitherto unexplained observation was made that certain methyl 3-*aci*-nitro-3-deoxy-aldopyranoside sodium salts in aqueous solution undergo characteristic rotational changes.[3,4] In Fig. 1 some of these "mutarotations" are depicted. Since normal methyl glycosides are not known to mutarotate in neutral or weakly alkaline medium, this behavior of the *aci*-nitro derivatives prompted closer investigations. These have led to a widening of the scope of the nitromethane synthesis as will be shown here for the α-D-hexopyranoside series.

It was established in Part III[4] that D'-methoxy-D-hydroxymethyl-diglycolic aldehyde (I), when condensed in methanolic solution with nitromethane in the presence of sodium methoxide, gives rise to a mixture of stereoisomeric methyl 3-*aci*-nitro-3-deoxy-α-D-hexopyranoside sodium salts (II). From the isolated, solid salt mixture the methyl 3-nitro-3-deoxy-α-D-hexopyranosides (III) were liberated by reaction with potassium bisulfate in the "dry" state. Subsequent hydrogenation led to crystalline amino sugar derivatives in a way permitting the statement that the nitromethane synthesis proceeds with marked stereoselectivity. This, of course, was not surprising since it had to be expected that conformational features in the reactant I and in the theroretically possible forms of the nitro products (4 forms of II and 8 forms of III) would govern the final steric composition of the latter and hence of the amines obtained on hydrogenation.[5] As a matter of fact, under the conditions employed the D-*manno* configuration (as in IV) was distinguished by its favored formation to a minimal extent[6] of 32–36% which is strikingly above the 12.5% calculated for absence of selectivity. In the mother liquor which apparently contained still more of the *manno* derivative there was a second isomer also in large quantity, and a number of products in small or trace amounts. This second isomer, designated "by-product R_{gm} 1.32" had in part been isolated as its crystalline tetraacetate (m.p. 180–181°, $[\alpha]_D$ +111° in chloroform). A third isomer, designated "by-product R_{gm} 1.52" had been isolated as a crystalline hydrochloride in very small amount. Its R_f value appeared to coincide with that of the aminomannoside IV,[7] but the compounds differed in their rotations, X-ray diffraction patterns and infrared spectra. As already briefly announced,[8] we have now been able to identify these products. The crystalline acetate obtained from the amorphous hydrochloride with R_{gm} 1.32 was the 2,4,6-tri-O-acetyl-N-acetyl derivative Vb of methyl 3-amino-3-deoxy-α-D-glucopyranoside (Va).[9] The R_{gm} 1.52 compound was the hitherto unknown methyl 3-amino-3-deoxy-α-D-talopyranoside hydrochloride (VI). Proof thereof will be given below.

Having thus established that the D-*manno* and D-*gluco* configurations are the main steric species arising under the reaction conditions originally worked out, we proceeded to investigate the influences of modified conditions. The mode of acidification, with dry potassium bisulfate, of the *aci*-nitro salts which we had employed in the earlier work as a precautionary measure[3,4] appears to be of little significance to the over-all results. In subsequent work[10,11] the use of a cation-exchange

(1) Paper VII in this series: F. W. Lichtenthaler and H. O. L. Fischer, *J. Am. Chem. Soc.*, **83**, 2005 (1961).

(2) Department of Chemistry, University of Ottawa, Ottawa 2, Canada.

(3) H. H. Baer and H. O. L. Fischer, *J. Am. Chem. Soc.*, **81**, 5184 (1959).

(4) H. H. Baer and H. O. L. Fischer, *ibid.*, **82**, 3709 (1960).

(5) Steric preferences in nitromethane condensations are observed even in syntheses of straight-chain compounds, with less rigid spatial requirements; *cf.* J. C. Sowden, *Adv. in Carbohydrate Chem.*, **6**, 291 (1951).

(6) Actually isolated, as methyl 3-amino-3-deoxy-α-D-mannopyranoside hydrochloride (IV).

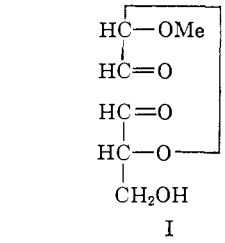
(7) We now have realized that a slightly faster migration of the by-product is noticeable on extended chromatography. Also, the shade of its ninhydrin coloration is different from that of IV; *cf.* Experimental.

(8) H. H. Baer, *Angew. Chem.*, **73**, 532 (1961).

(9) The lower specific rotation, $[\alpha]_D$ +101.9° (in $CHCl_3$), reported for Vb in the earlier literature [S. Peat and L. F. Wiggins, *J. Chem. Soc.*, 1810 (1938)] caused us to question this identity. In the meantime, values closer to ours have been recorded; *e.g.*, $[\alpha]_D$ +109° (in $CHCl_3$) by H. Ogawa, T. Ito, S. Kondo and S. Inoue, *Bull. Agr. Chem. Soc. (Japan)*, **23**, 289 (1959). Through the kindness of Dr. R. D. Guthrie, Leicester, England, an authentic specimen of Vb was obtained that had been prepared according to Peat and Wiggins. Identity with our product was established by mixed melting point, infrared spectra and X-ray patterns.

(10) H. H. Baer, *Ber.*, **93**, 2865 (1960).

(11) A. C. Richardson and H. O. L. Fischer, *J. Am. Chem. Soc.*, **83**, 1132 (1961).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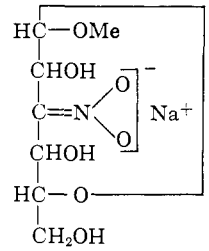
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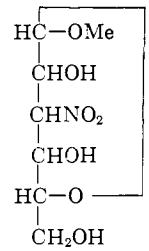
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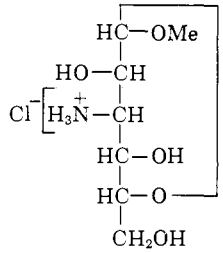
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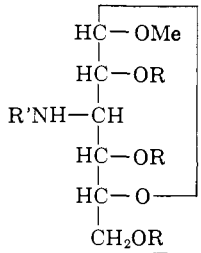
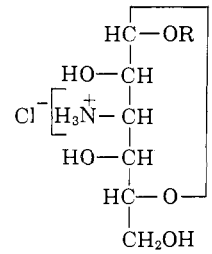
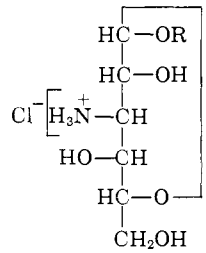
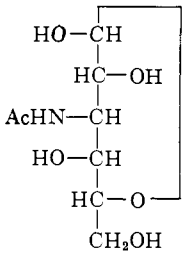
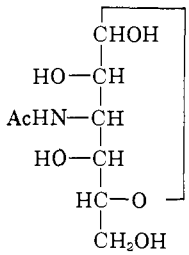

Va, R = H, R' = Cl⁻[H₂]⁺
b, R = R' = Ac


VI, R = Me
X, R = H


VII, R = Me
VIII, R = H


IX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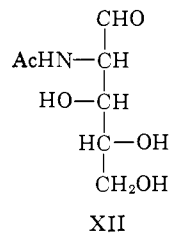
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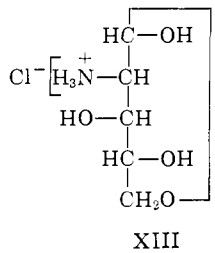

XIII

resin has proved to be just as feasible, and more convenient. When applied to the nitronate mixture II of the α-D-hexopyranoside series which need not be isolated, this modification offers a simplified preparatory route to III and its hydrogenation products, particularly the aminomannoside IV.

In the anomeric β-D-hexopyranoside series it had been found, moreover, that the nitromethane condensation furnished one and the same main product, the β-D-*gluco* derivative, regardless of whether the condensation was carried out in *methanolic* solution (with sodium methoxide) or in *aqueous* solution (with potassium hydrogen carbonate).[10] This holds also for the α-D-hexopyranoside series. After conducting a nitromethane condensation (I→II) in aqueous medium in the presence of 1 mole of $KHCO_3$ which required 3.5 days at 20°, deionizing the reaction solution (II→III) and hydrogenating III, 31% of crystalline aminomannoside hydrochloride IV was isolated, and an estimated 60% of aminoglucoside Va was formed of which about half was crystallized as its tetraacetate Vb.

Entirely different results were achieved, however, when the *isolated* nitronate mixture II was dissolved in water and allowed to stand at 20° until the mutarotation had reached a virtually constant value (4 days; Fig. 1). Deionization and hydrogenation followed by a preparative chromatographic fractionation revealed that the stereoisomeric composition of the *aci*-salt II had changed conspicuously during its standing in water. A much smaller quantity of the aminomannoside hydrochloride IV (about 10–12%) was now obtained; and the *gluco* derivative seemed to have largely disappeared, for from the corresponding fractions of the chromatographic column only very little of Va could be isolated as its readily crystallizing tetraacetate Vb. Instead, methyl 3-amino-3-deoxy-α-D-talopyranoside hydrochloride (VI) which had been encountered previously only as a minor by-product (see above), was now the predominant isomer. It could be isolated in a yield of about 40%; more of it obviously was contained in mixed fractions. Furthermore, a considerable amount of a new isomer occurred among the products. Although it failed to crystallize it was recognized as methyl 3-amino-3-deoxy-α-D-galactopyranoside hydrochloride (VII); the known sugars, 3-amino-3-deoxy-D-galactose hydrochloride (VIII) and 3-acetamido-3-deoxy-D-galactose (IX),[12] were obtained from it, and identified with authentic samples, by standard procedures. The aminogalactoside VII was present at an estimated 30%.

Methyl 3-amino-3-deoxy-α-D-talopyranoside hydrochloride (VI, m.p. 191–192° dec., $[\alpha]_D$ +90° in water) was hydrolyzed to furnish, in a yield of 84%, crystalline, reducing 3-amino-3-deoxy-α-D-talose

(12) R. Kuhn and G. Baschang, *Ann.*, **636**, 164 (1960).

hydrochloride (X). The amino sugar hydrochloride X was N-acetylated, and the resulting acetamido sugar XI was degraded by means of 1 mole of periodate to give a 2-acetamido-2-deoxypentose XII and, following acid hydrolysis, the parent pentosamine hydrochloride XIII in crystalline state. The degradation products XII and XIII were recognized by comparison with authentic samples to be N-acetyl-D-lyxosamine and D-lyxosamine hydrochloride, respectively.[13] Under the conditions employed D-lyxosamine can arise only from either 3-acetamido-3-deoxy-D-talose (XI) or 3-acetamido-3-deoxy-D-galactose (IX). Since our new amino sugar X and its N-acetate XI differ widely in their constants from known 3-amino- and 3-acetamido-3-deoxy-D-galactose,[12] the D-*talo* configuration of X and, of course, VI is established.

3-Amino-3-deoxy- (hydrochloride)	M.p., °C.	$[\alpha]_D$ in water
α-D-Galactose (VIII)	178–180	+115 → +89
α-D-Talose (X)	160–161	+ 29.5 → +23.7
3-Acetamido-3-deoxy-		
β-D-Galactose (IX)	170–172	+ 99 → +119
α,β-D-Talose (XI)	Amorphous	+3

Of the eight possible 3-amino-3-deoxy-hexoses of the D-series, seven had previously been synthesized[14] as such or in the form of derivatives. With the synthesis of 3-amino-3-deoxy-D-talose the last missing member of this group has now become known and available.

As to the stereochemistry of the nitromethane cyclization the experimental results suggest the following: In the condensation step which one can assume is kinetically controlled the molecules A and B are the most favored products. In our previous paper[4] we have already sought to explain, by invoking conformational rules, why A should be formed, and we have pointed out that B, too, might be considered a conformationally favorable molecule, even though at that time its actual formation had not yet been proved. However, A and B must not necessarily be the most stable forms thermodynamically. When they are allowed to stand in aqueous solution for a prolonged period of time, C and D are formed at their expense. As is seen from the formulas, this rearrangement involves an epimerization at C4, adjacent to the *aci*-nitro grouping. It is reminiscent of an explanation that has been proposed but not proved for the interconversion of certain nitrodeoxyinositols.[15] The mechanism of the rearrangement which would represent a type of epimerization novel to carbohydrate chemistry is under investigation. It is noteworthy that the nitromethane condensation of the dialdehyde I furnishes preponderantly A and B, *both* when the reaction takes place in *methanolic solution* (with $NaOCH_3$) *and* when it is carried out in *aqueous solution* (with $KHCO_3$). It is only when the *isolated* nitronate II is dissolved in water that the rearrangement prevails, possibly owing to a difference in *p*H. It is remark-

(13) R. Kuhn and G. Baschang, *Ann.*, **628**, 193 (1959).

(14) Only one, 3-amino-3-deoxy-D-glucose (kanosamine), has so far been discovered in nature.

(15) J. M. Grosheintz and H. O. L. Fischer, *J. Am. Chem. Soc.*, **70**, 1479 (1948).

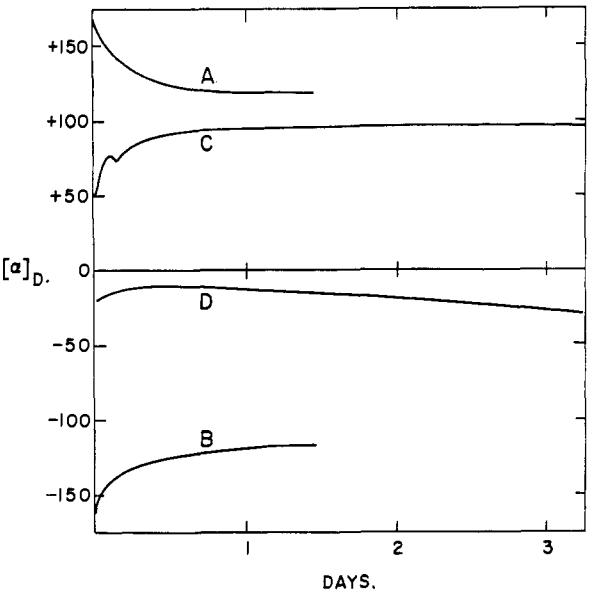
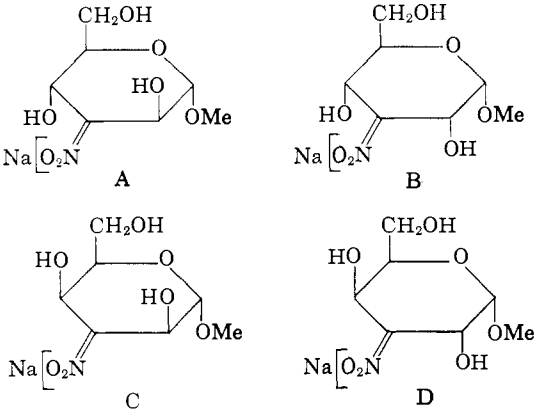


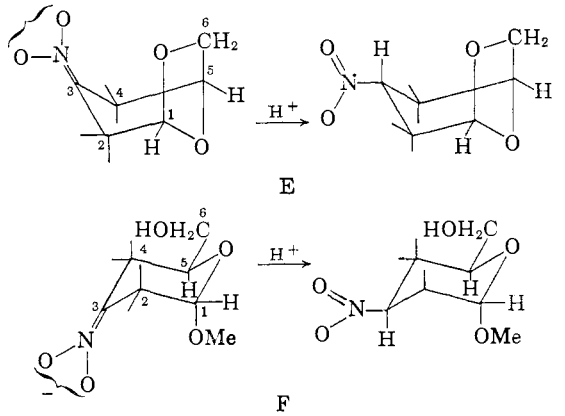
Fig. 1.—Changes of the specific rotations of methyl 3-*aci*-nitro-3-deoxy-glycoside sodium salts (*c* 1 in carbon dioxide-free water, 25°): A, crystalline β-L-ribopyranoside[3]; B, crystalline β-D-ribopyranoside[3]; C, α-D-hexopyranoside mixture[4]; D, β-D-hexopyranoside mixture (see footnote 7 in ref. 10).

able, furthermore, that in the conversion of the salts A, B, C and D into the corresponding free nitro glycosides the nitro group uniformly[16] acquires the same steric disposition, namely, the upward configuration in the Haworth formulas. Thus, the D-*manno*, D-*gluco*, D-*talo* and D-*galacto* derivatives arise, rather than the respective 3-



epimeric D-*altro*, D-*allo*, D-*ido* and D-*gulo* compounds. Conversely, the four latter configurations but not the former were obtained in a nitromethane cyclization of the dialdehyde from levoglucosan. In that case the products necessarily exist in a fixed 1C conformation (E), and it was reasoned that on acidification the bulky nitro group has a tendency to assume an equatorial position.[11] If in our case one presumes the same tendency, then one has to conclude that the molecules, at least while reacting with hydrogen ion, possess the C1 conformation (F).

(16) As far as can be told from the results of chromatography and preparative work-up.



E

F

Experimental

Melting points were taken with short stem thermometers. All evaporations were done *in vacuo* at 35–40°, unless otherwise stated. Paper chromatography was performed by the descending technique at 25° using Whatman No. 1 paper and the Fischer–Dörfel solvent system[17]; R_{gm} = speed relative to D-glucosamine hydrochloride.

D'-Methoxy-D-hydroxymethyl-diglycolic aldehyde (I) was prepared as a sirup from pure methyl α-D-glucopyranoside according to the directions described earlier.[4]

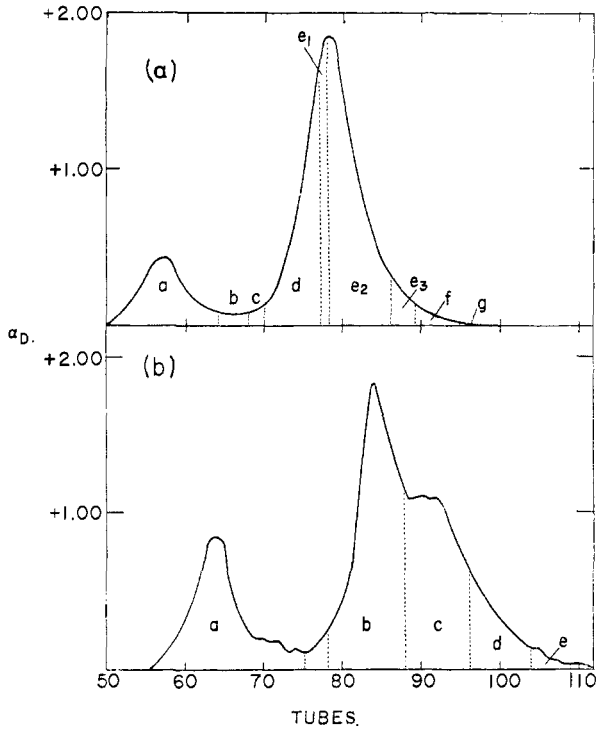


Fig. 2.—Chromatographic fractionations of methyl 3-amino-3-deoxy-α-D-hexopyranosides; column: length 75 cm., width 4.5 cm., content approximately one pound of powdered cellulose; eluant: pyridine–ethyl acetate–water–acetic acid, 5:5:3:1 (v./v.); flow rate: 80 ml. per hour; ordinate: α^{20}_{D} of effluent (2-dm tube); abscissa: numbers of the 20-ml. fractions collected.

Methyl 3-Amino-3-deoxy-α-D-mannopyranoside Hydrochloride (IV). A. Nitromethane Condensation in Methanol (Simplified Procedure).—The dialdehyde I obtained from 7.76 g. (0.04 mole) of methyl α-D-glucopyranoside was allowed to react, in 50 ml. of methanol, with 2.2 ml. (1 molar equivalent) of nitromethane and 29 ml. of a 3% (w./v.) solution of sodium in methanol (0.95 molar equivalent of $NaOCH_3$) as described previously.[4] After standing for 25 minutes at 0°, however, the reaction mixture was *not* worked up for the nitronates II which it contained. Rather, it was deionized by treatment with 100 ml. of methanol-washed Amberlite IR-120(H^+) in a manner described in detail in analogous work[10] in the β-series. The nitroglycoside mixture III thus produced ($[\alpha]^{20}D$ +100°, *c* 1.2 in water) was a colorless foam that failed to crystallize. Upon hydrogenation[4,10] with platinum catalyst[18] in the presence of 1 molar equivalent of hydrochloric acid the aminomannoside hydrochloride IV was obtained in over-all yields of 25–30%. Recrystallized from 95% ethanol, the long needles or thin prisms showed $[\alpha]^{20}D$ +60° (*c* 2 in water) and decomposed at 223°,[19] R_{gm} 1.50.

B. Nitromethane Condensation in Aqueous Solution.—Sirupy dialdehyde I (0.025 mole, obtained from 4.85 g. of methyl α-D-glucopyranoside) was freed from remnant alcohol by evaporation with water and then dissolved in 80 ml. of water. To the chilled (5°) solution nitromethane (1.4 ml.) and solid potassium hydrogen carbonate (2.5 g., 1 molar equivalent) were added under swirling. As the bicarbonate dissolved the nitromethane, too, went into solution. The reaction mixture was brought to 20° and made up to 100 ml. with water. The course of the reaction was followed polarimetrically with a 1-dm. tube: $\alpha^{20}D$ +5.30° (0.5 hr.) → +5.43° (2 hr.) → +6.05 (27 hr.) → +6.26° (65 hr.) → +6.28° (84 hr.). After 3.5 days the solution was ice-cooled, deionized with 50 ml. of Amberlite IR-120 (H^+) and finally adjusted to *p*H 5 with a little IR-45(OH^-). After being clarified with activated charcoal the pale yellow filtrate was brought to dryness and furnished upon repeated evaporation with ethanol and ethyl acetate an almost colorless foam weighing 4.62 g. (desiccator-dried). This nitroglycoside mixture III, according to its rotation of $[\alpha]^{20}D$ + 111° (*c* 1 in water), appeared to differ somewhat in the ratio of its components, from III as obtained under A; λ_{max} 250 mμ (in 0.01 *N* NaOH).

Anal. Calcd. for $C_7H_{13}O_7N$ (223.2): C, 37.67; H, 5.87. Found: C, 38.00; H, 5.94.

Hydrogenation with platinum of the product III, conducted as under A, resulted in the uptake of 3 moles of hydrogen (found 1308 ml., cor. vol.; calcd. 1350 ml.). Upon working-up[4] of the hydrogenation solution 1.08 g. (23.3%) of aminomannoside IV crystallized readily. It had a decomposition point of 221–223° and $[\alpha]^{20}D$ +61° (*c* 1.15 in water). Another 0.36 g. of IV was obtained from the chromatographic fractions c and d (see below) which increased the total yield to 31.2%.

Chromatographic Separation.—The remainder of the hydrogenated products was brought to dryness, dissolved in 5 ml. of pyridine and 3 ml. of water and placed onto a cellulose column followed by a mixture of 5 ml. of ethyl acetate and 1 ml. of acetic acid. The flask was rinsed with a little of the elution solvent. The performance of the fractionation is indicated in Fig. 2a. Fractions selected at adequate intervals were checked by paper chromatography as to their content of ninhydrin-positive aminoglycosides. This was performed by concentrating a sample in the air to about one-tenth of its volume and applying to the paper several droplets of this concentrate. The results permitted the pooling of the 20-ml. fractions as indicated in Fig. 2a. The large fractions thus obtained (a–g) were evaporated under several additions of water and then of toluene, if necessary, in order to remove the chromatographic solvent. The residues were taken up in water, the solutions clarified with activated charcoal and, with the exception of fraction a, carefully titrated with dilute hydrochloric acid so that the amino glycosides were present as their hydrochlorides. The fractions were then brought to dryness again and weighed.

(17) Pyridine–ethyl acetate–water–acetic acid (5:5:3:1, v./v.), with pyridine–ethyl acetate–water (11:40:8, v./v.) in the bottom of the tank; F. G. Fischer and H. Dörfel, *Z. physiol. Chem.*, **301**, 224 (1955).

(18) Similar hydrogenations have successfully been carried out with Raney nickel T4 catalyst; *cf.* ref. 1 and 11. Dr. A. C. Richardson, Bristol, informed the author that this catalyst may also be used for the preparation of IV.

(19) In the paper cited, ref. 4, a decomposition at about 205° with preliminary browning beginning at 190° was reported. A pure sample from that time was rechecked and was now found to decompose at 223°.

Fraction a: yellow sirup (392 mg.), ninhydrin-negative

Fraction b: colorless sirup (70 mg.); unidentified, fast migrating ($R_{gm} > 2$), ninhydrin-positive material

Fraction c: partly crystalline (42 mg.), gave upon recrystallization needles of IV with dec. p. 221–223° and $[\alpha]^{20}D$ +59° (*c* 0.75 in water)

Fraction d: colorless foam (1.20 g.) representing a mixture of the aminomannoside hydrochloride IV (R_{gm} 1.50) and the aminoglucoside hydrochloride Va (R_{gm} 1.32). On trituration with 3 ml. of ethanol containing 3 drops of water spontaneous crystallization of elongated prisms took place which was completed overnight at 4°. The crystals were collected, washed with ice-cold ethanol and ethanol–ethyl acetate (1:2), and air-dried; 324 mg. of dec. p. 216–217° and $[\alpha]^{20}D$ +68.5° (*c* 1 in water). One recrystallization from 95% ethanol afforded fine needles of IV with dec. p. 223° and $[\alpha]^{20}D$ +61° (*c* 1.1 in water). The mother liquor of fraction d which contained Va was worked up by acetylation as described below

Fraction e_1: colorless sirup (305 mg.) of $[\alpha]^{20}D$ +103° (*c* 5.9 in water); it was mainly Va together with some IV

Fraction e_2: colorless foam (1.37 g.) of $[\alpha]^{20}D$ +90° (*c* 1 in water); it contained Va with traces of IV

Fraction e_3: sirup containing hygroscopic crystals that were non-carbohydrate in nature and difficult to remove. There was about 130 mg. of sirup left which consisted mainly of Va contaminated with some slower-moving material of R_{gm} 1.0

Fraction f: similar to e_3 except that more of the unidentified R_{gm} 1.0-material was present; weight, approx. 100 mg.

Fraction g: sirup (approx. 50 mg.), R_{gm} 1.0

The total recovery from the column was 3.66 g. of material. When added to the crop of IV which had crystallized directly (1.08 g.) the products of the hydrogenation experiment are accounted for quantitatively (calcd., 4.64 g. of methyl aminodeoxy hexoside hydrochloride from 4.50 g. of methyl nitrodeoxy hexoside). Fractions e_1 and e_2 comprising the bulk of Va are dealt with in the following paragraph; the fractions e_3 – g were not investigated further.

Methyl 3-Acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-glucopyranoside (Vb).—After the removal of the crystallized IV the remainder of the above fraction d was brought to dryness and acetylated by refluxing for 5 minutes with 20 ml. of acetic anhydride and 5 g. of anhydrous sodium acetate. A part of the excess anhydride was distilled off *in vacuo* and the reaction mixture then poured onto ice and methanol. Following the decomposition of all the excess anhydride the solution was evaporated, the residue dissolved in 25 ml. of water, and the solution extracted five times with 40 ml. of chloroform. The combined extracts were washed twice with 10 ml. of a saturated solution of sodium bicarbonate, dried over anhydrous sodium sulfate and evaporated. From the near-colorless sirup white crystals separated readily upon trituration with a little ethanol. They were collected after standing overnight at 4°, and washed with ice-cold ethanol and ether; yield 536 mg. It was Vb showing m.p. 177–178° and $[\alpha]^{20}D$ +107.5° (*c* 1.14 in chloroform). Upon addition to the mother liquor of ether and cyclohexane crops of less pure crystals totalling 179 mg. were obtained.

Analogous acetylations of the fractions e_1 and e_2 furnished, respectively, 200 and 701 mg. of nearly pure Vb with m.p. 178–179° and slightly less pure second crops weighing 122 and 427 mg. Thus, this experiment yielded a total of 2.165 g. of isolated, crystalline Vb corresponding to 1.38 g. of the hydrochloride Va. This meant a preparative yield of 29.8% of the aminoglucoside. However, the sum of the weights of the fractions that contained Va suggested that about twice as much (58–60%) had actually been formed.

One recrystallization from 95% ethanol of the acetate Vb gave hexagonal prisms with m.p. 179–180° and $[\alpha]^{20}D$ +110° (*c* 2 in chloroform).

The product Vb was identical with the previously obtained[4] "by-product R_{gm} 1.32" and with an authentic specimen of methyl 3-acetamido-3-deoxy-2,4,6-tri-*O*-acetyl-α-D-glucopyranoside.[9]

Epimerization of the Methyl 3-*aci*-Nitro-3-deoxy-α-D-hexopyranoside Sodium Salts (II).—*aci*-Nitro salt II (0.05 mole) was prepared in methanolic solution, the dialdehyde I obtained from 9.70 g. of methyl α-D-glucopyranoside being used. Allowance being made for the larger amounts of reactants and solvent required here, the nitromethane condensation in its initial stage was performed precisely as before (see above under A and ref. 4). The condensation mixture was kept in an ice-bath for 15 minutes and then evaporated rapidly (bath, 20°)[20] to a sirupy consistency without the addition of ethanol. This process required 12 minutes. The sirup was then immersed in an ice-bath and quickly dissolved in about 200 ml. of chilled, carbon dioxide-free water. The yellow solution (217 ml.) was brought to 20° and stored in the dark at that temperature for 4 days. The rotational change was followed by the use of a 1-dm. tube; $\alpha^{20}D$ +4.33° (20 min. after the dissolving of the sirup)→4.40° (55 min.)→ +4.30° (140 min.) → +4.89° (19.3 hr.) → +5.95° (90 hr.). Assuming the presence of 12.26 g. of sodium nitronates (0.05 mole) the $[\alpha]D$ values conform to the mutarotation curve (Fig. 1, C) of previously isolated *aci*-nitro salt II.

Liberation of the Epimerized Mixture of Methyl 3-Nitro-3-deoxy-α-D-hexopyranosides (III_{ep}).—After 96 hours the above solution was chilled in an ice-bath and then slowly poured into an ice-cooled, magnetically stirred suspension in water of 100 ml. of Amberlite IR-120(H^+). The suspension which afterward reacted weakly acidic (*p*H 3) was stirred for 30 minutes and adjusted to *p*H 4.5 by the addition of some Amberlite IR-45(OH^-). The resins were filtered off and thoroughly washed by several decantations with water. The pale yellow combined filtrate and washings was treated with activated charcoal that removed some but not all of the color. The solvent was removed *in vacuo*, and further evaporation with additions of ethanol, ethyl acetate and finally benzene furnished a yellowish hygroscopic foam (III_{ep}) that was dried in a desiccator over phosphorus pentoxide and paraffin; yield 9.15 g. (82% based on the starting methyl glucopyranoside), $[\alpha]^{20}D$ +105° (*c* 1 in water).

Anal. Calcd. for $C_7H_{13}O_7N$ (223.2): N, 6.28. Found: N, 6.43.

When to an aqueous solution of the product III_{ep} the equivalent amount of sodium hydroxide was added, the specific rotation (calcd. as sodium salt) reached *within 90 minutes* a constant value of +99°. This agrees with the equilibrium value attained after 5 days in the mutarotation of the isolated *aci*-nitro salt mixture II (Fig. 1, C); λ_{max} 249 mμ (in 0.01 *N* NaOH).

Hydrogenation of the Epimerized Nitroglycoside Mixture (III_{ep}) and Separation of the Products.—Nitroglycoside III_{ep} (3.75 g.) was dissolved in 10 parts of water, the solution clarified with a little charcoal, filtered, diluted to 100 ml. and added to pre-hydrogenated platinum catalyst (from 7 g. of PtO_2) in 70 ml. of water and 17 ml. of *N* hydrochloric acid. With an efficient shaking machine being used a rapid hydrogen uptake was observed which ceased after 3 hours (found 1320 ml. at 27° and 747 mm; 1165 ml., corrected for 0° and 760 mm; calcd. for 3 moles, 1130 ml.). After removal of the catalyst the colorless solution was adjusted to *p*H 4 – 5 by stirring with a little Amberlite IR-45(OH^-). Evaporation of the filtrate, finally with the addition of 3 consecutive portions of absolute ethanol gave a white foam that was dissolved in 30 ml. of warm 95% ethanol.[21] Upon inoculation and standing overnight in a refrigerator the solution deposited 237 mg. of fine needles of methyl 3-amino-3-deoxy-α-D-mannopyranoside hydrochloride (IV) that was isolated and washed with cold ethanol–ethyl acetate (2:1). It had $[\alpha]^{20}D$ +63° (*c* 0.94 in water) and decomposed at 221–222°. A second crop of IV (128 mg.) was obtained from the solution upon concentrating and addition of ethyl acetate to incipient cloudiness; total yield 9.9%. A second experiment afforded a yield of 10.2% of IV with $[\alpha]^{20}D$ + 60.4° (*c* 0.5 in water) and dec. pt. 222–223°.

Chromatographic Fractionation.—The mother liquor containing the bulk of the hydrogenated products was brought to dryness and the residue was applied to a cellulose column and chromatographed in the same manner as the hydrogenation products of III (see above). The rotational

(20) See ref. 4, footnote 13.

(21) At this point in one experiment a small amount of difficultly-soluble crystals formed which were removed after cooling of the solution and proved to be inorganic matter. In a second experiment this contamination did not separate beforehand but accompanied the first crop of crystallizing aminoglycoside, which was then purified by dissolving in 90% ethanol and centrifuging off the insoluble.

pattern of the separation is given in Fig. 2b. Paper chromatographic inspection of the small fractions permitted a pooling to large fractions (a – e) as indicated. Very similar results were achieved in a second experiment.

Fraction a: yellow sirup (510 mg.), ninhydrin-negative; λ_{max} 246 mμ (in 0.01 *N* NaOH)

Fraction b: nearly colorless foam (1.46 g.) mainly consisting of aminotaloside VI (R_{gm} 1.60) which probably was accompanied by aminomannoside IV (R_{gm} 1.52).[22] This fraction represented 38% of the theoretical of hydrogenation products, or 44% of that actually formed, allowance being made for the non-amino fraction a.

Fraction c: colorless glass (961 mg.) consisting of fast (R_{gm} 1.60 to 1.52) and more slowly (R_{gm} 1.32) migrating products. Judging from the intensities of the ninhydrin spots the latter preponderated three- to fourfold. An attempt at crystallization from ethanol and ethyl acetate furnished only 14 mg. of aminomannoside IV, with dec. pt. 223–224° after recrystallization. In view of the good crystallizability of IV one may presume that most of the fast moving part of fraction c was a different compound, most likely aminotaloside VI. Showing R_{gm} 1.32, the main component of fraction c traveled like the aminoglucoside Va. However, acetylation of the fraction produced only 35 mg. of Vb which is known to crystallize without difficulty; m.p. 178–179°, $[\alpha]^{20}D$ + 108° (*c* 1 in chloroform). The main product, therefore, was tentatively assumed to be aminogalactoside VII.

Fraction d: colorless sirup (670 mg.) containing aminogalactoside VII (R_{gm} 1.32). It also contained pyridine hydrochloride which crystallized but was not removed since it did not interfere with the subsequent acetylation. A comparison of the areas (Fig. 2b) of the fractions c and d as well as a comparison of the chromatographic spot intensities makes it reasonable to estimate roughly that c contained 0.7 g. and d 0.3 g. of VII, representing 30% of the amino glycosides formed.

Fraction e: yielded 35 mg. of inorganic crystals and 166 mg. of a brownish sirup. The sirup contained slow-moving, tailing, ninhydrin-positive material and was not investigated further.

The total recovery from the column was 3.40 g. of substance. When the 0.36 g. of IV is added that had crystallized directly, the products of the hydrogenation experiment are accounted for completely.

Methyl 3-Amino-3-deoxy-α-D-talopyranoside Hydrochloride (VI).—The amorphous material from the above fraction b was dissolved in 10 ml. of absolute ethanol. Upon careful addition, under agitating and scratching with a glass rod, of 5 ml. of ethyl acetate white crystals began to separate immediately. They were triturated under gentle warming of the flask while one more ml. of ethyl acetate was added. After standing for 2 hours at 25° and for 1 hour at 0° the crystals were collected and washed with ethanol–ethyl acetate (10:6). They weighed 564 mg. and melted at 192° dec. The mother liquor upon concentrating and enriching with warm ethyl acetate gave a second crop (140 mg.) with m.p. 190° dec. A third crop (127 mg.) of m.p. 189–190° dec. was obtained when the mother liquor was brought to dryness, the residue taken up in water, treated with charcoal, re-adjusted to *p*H 4 with a little dilute hydrochloric acid, and the above crystallization procedure repeated. Thus a total of 831 mg. of the crude fraction b could be obtained in crystalline state. The specific rotations of these and other crystallizates which came from parallel experiments were + 88 ± 2° (in water). The product was methyl 3-amino-3-deoxy-α-D-talopyranoside hydrochloride (VI) which was already fairly pure. Recrystallized twice from 95% ethanol with (i) or without (ii) addition of ethyl acetate the compound had $[\alpha]^{20}D$ +90° (*c* 2 in water). It separates quickly as small, short prisms of m.p. 191° dec. (i); or slowly as stout, hard prisms of m.p. 187–188° dec. (ii). These melting points of the pure substance are interconvertible. No loss in weight was observed on drying in a high vacuum at 56°.

(22) Originally we had noticed no difference in the R_{gm} values of the mannoside IV and the "by-product R_{gm} 1.52" (see ref. 4) which has now been identified by infrared spectra and X-ray patterns with the taloside VI. They are, in fact, not readily separable; however, on prolonged chromatography a mixture of them gives a double ninhydrin spot, the front part of which is brownish-violet and the rear part violet.

Anal. Calcd. for $C_7H_{16}O_5NCl$ (229.7): C, 36.61; H, 7.02; N, 6.10; Cl, 15.44; OCH_3, 13.51. Found: C, 36.69; H, 7.15; N, 6.20; Cl, 15.51; OCH_3, 13.08.

The remainder of fraction b was brought to a sirup and hydrolyzed for 3 hours in a steam-bath with 50 ml. of 2 *N* hydrochloric acid. Paper chromatographic examination revealed the hydrolysate not to be uniform. However, the major product appeared to be 3-amino-3-deoxy-D-talose hydrochloride (X) which was accompanied by the same by-products that occur on hydrolysis of pure VI (see below). Some of aminomannose and possibly a trace of aminogalactose were also present. There was no evidence for aminoglucose.

3-Amino-3-deoxy-α-D-talose Hydrochloride (X). Preliminary Hydrolyses.—Samples of 25–80 mg. of VI were hydrolyzed in sealed tubes in concentrations of 1–1.6% under the following conditions; (a) in 1% hydrochloric acid at 103° for 28 hr.; (b) in 2 *N* hydrochloric acid at 98° for 2.5 hr.; (c) in concentrated hydrochloric acid at 23° for 15 and 23 hr. The final $[\alpha]^{20}D$ values of the hydrolysates were +24° in (a) and (b), +29° in (c). In each case crystalline, reducing X was isolated in good yield; dec. p. 156–158°. Since the pure amino sugar X has an equilibrium rotation of +23.7°, little room is left in the hydrolysates for the presence of by-products, particularly of an anhydro sugar which would possess a strong rotatory power. A sample of crystalline X was heated at 50° in half-concentrated hydrochloric acid for 30 min. whereby no change in the rotation was noticeable; unchanged starting material was thereafter recovered in good yield.

Preparative Hydrolysis.—The aminotaloside VI (200 mg.) was heated at 98° in 10 ml. of 2 *N* hydrochloric acid in a stoppered flask for a total of 2 hr. At intervals the hydrolysis was interrupted briefly for determination of the rotation: $\alpha^{20}D$ +0.85° → +0.58° → +0.49° → +0.45° (30, 60, 90, 120 min.; 1-dm. tube). Repeated evaporation with water furnished a colorless sirup which started to crystallize while still moist. The vessel was kept over potassium hydroxide in an unevacuated desiccator for 1–2 days. The almost completely solidified product was triturated twice with 1 ml. of 95% ethanol, then dried *in vacuo*. The yield was 160 mg. (84%) of fine prisms or needles decomposing at 158–160°. Chromatographically pure X decomposing at 160–161° was obtained upon one recrystallization from the minimum amount of water by the addition of excess glacial acetic acid; $[\alpha]^{20}D$ +29.5° (3 min.)→+23.7° (20 min., final, *c* 1 in water). The compound reduced Fehling solution strongly and gave a positive Elson–Morgan test. Its ninhydrin spot of R_{gm} 1.15 is initially yellowish-brown, whereas that of 3-amino-3-deoxy-D-mannose hydrochloride (R_{gm} 1.13) is brownish-violet. Both turn violet after several hours.

Anal. Calcd. for $C_6H_{14}O_5NCl$ (215.6): C, 33.42; H, 6.54; N, 6.50; Cl, 16.44. Found: C, 33.45; H, 6.46; N, 6.25; Cl, 16.33.

In the ethanolic washings of X there was detected chromatographically mainly additional X, a moderate amount of a compound with R_{gm} 1.70 (possibly anhydro sugar), and traces of three slow-moving reversion products.

3-Amino- and 3-Acetamido-3-deoxy-D-galactose (VIII and IX) from VII.—The sirupy material obtained from fraction d which contained an estimated 0.3 g. of an aminoglycoside with R_{gm} 1.32 was acetylated with acetic anhydride (4.5 ml.) in pyridine (3 ml.) at room temperature for 48 hr. The excess of anhydride was destroyed with 50 ml. of ice-cold methanol. Evaporation left a pyridine-containing sirup that was taken up in 50 ml. of chloroform. The pyridine was removed by shaking the solution briefly with 10 ml. of a saturated aqueous solution of cadmium chloride, filtering off with suction the precipitate and washing it exhaustively with chloroform. The filtrate upon drying with anhydrous calcium chloride and evaporating afforded a light brown sirup (304 mg., desiccator-dry).

Attempts at crystallization, from various combinations of solvents, of the peracetylated glycoside were unsuccessful. No crystal formation could be induced by seeding with easily crystallizing Vb. The product was then purified by a passage over a small column of aluminum oxide (Woelm, neutral, activity grade 1), the elution being performed with ether containing increasing concentrations of methanol. The column retained all the colored impurities; but the eluted, colorless acetate fractions totaling 204 mg. ($[\alpha]^{20}D$ +91 ± 3° in chloroform) stayed amorphous nonetheless.

The peracetylated glycoside was then converted by acetolysis into the peracetylated sugar. This was done by dissolving it at 0° in 5 ml. of acetic anhydride containing 0.1 ml. of concentrated sulfuric acid. After standing for 17 hr. at 24° the mixture was worked up according to standard procedure. The peracetyl amino sugar (188 mg.) obtained from the chloroform extracts did not crystallize. It was de-*O*-acetylated in 2 ml. of methanol containing 15 mg. of sodium methoxide (2 hr. at 0°). After dilution with methanol the solution was deionized with methanol-washed Amberlite IR-120(H^+), reconcentrated to a small volume, and the acetamido sugar IX was precipitated as an oil by the addition of excess ethyl acetate. It appeared chromatographically uniform[23] and was indistinguishable from authentic 3-acetamido-3-deoxy-D-galactose[12] (a), but well separated from 3-acetamido-3-deoxy-D-glucose[24] (b), 3-acetamido-3-deoxy-D-mannose[4] (c) and 3-acetamido-3-deoxy-D-gulose[11] (d). The ratios of migration were a:b:c:d = 0.96:1.00:1.10:1.13.

A small part of the oily acetamido sugar IX was crystallized from methanol–ethyl acetate and recrystallized from the same solvents; m.p. 168–169° dec., undepressed on admixture of authentic 3-acetamido-3-deoxy-D-galactose.

The main part of IX was hydrolyzed in 10 ml. of *N* hydrochloric acid at 100° for 90 minutes. The hydrolysate was treated with acid-washed charcoal and then brought to a sirup under several additions of water to remove the hydrochloric acid. Paper chromatography revealed the product to be inhomogeneous. The main spot, however, was identical in its R_{gm} value of 0.86 and in the shade of its ninhydrin color (brownish-gray, turning gray-violet) with the spot given by authentic 3-amino-3-deoxy-D-galactose hydrochloride (VIII). It was clearly distinct from the spots of the hydrochlorides of 3-amino-3-deoxy-D-glucose, -D-mannose, -D-talose and -D-allose[25] which travel with R_{gm} values, respectively, of 1.05, 1.13, 1.16 and 1.13. In addition there were present faint spots in the area of R_{gm} 1.05–1.16 and traces of streaking slow-moving impurities.

Degradation of 3-Amino-3-deoxy-α-D-talose Hydrochloride (X) to D-Lyxosamine Hydrochloride (XIII). N-Acetylation.—Pure aminotalose hydrochloride X (165 mg.) was N-acetylated in the presence of Dowex-1 (CO_3^{--})[26] according to the direction given for the N-acetylation of 3-amino-3-deoxy-D-glucose.[24] A dry, colorless, foamy product (161 mg.) was obtained that failed to crystallize; $[\alpha]^{20}D$ +3° (*c* 1 in water). It was assigned formula XI.

(23) Aniline hydrogen phthalate spray; since acetamido sugars are not very perceptible to this indicator, small amounts of accompanying isomers may have escaped detection.

(24) H. H. Baer, *J. Am. Chem. Soc.*, **83**, 1882 (1961).

(25) Obtained by acid hydrolysis from 3-amino-3-deoxy-1,2;-5,6-diisopropylidene-α-D-allose (m.p. 92–94°) kindly supplied by Dr. D. H. Ball, Natick, Mass. *Cf.* R. U. Lemieux and P. Chu, *J. Am. Chem. Soc.*, **80**, 4745 (1958).

(26) S. Roseman and J. Ludowieg, *ibid.*, **76**, 301 (1954).

Periodate Degradation.—A solution in 10 ml. of water of 142 mg. of the above acetamido sugar XI and 138 mg. of sodium metaperiodate was kept in the dark at 20°. After 30 minutes the solution had become yellow owing to the formation of free iodine. The reaction solution was therefore briefly agitated with one big crystal of sodium thiosulfate which was removed after the color had disappeared. Excess ethanol was then added to precipitate sodium iodate, the chilled mixture was filtered and the filtrate evaporated. An aqueous solution of the evaporation residue was further desalted by stirring with mixed Amberlites IR-120 (H^+) and CG-45(OH^-). The filtrate from the resins was heated for 30 minutes at 50° with 80 mg. of sodium bicarbonate, in order to hydrolyze formyl ester which may have been still present. Following deionization with IR-120(H^+) the solution was evaporated with several additions of ethanol. A colorless foam of N-acetyl-D-lyxosamine (XII) resulted weighing 104 mg. (85% of the theoretical) and showing $[\alpha]^{20}D$ +19° (in water), in agreement with the literature.[13] Compound XII was chromatographically identical with authentic N-acetyl-D-lyxosamine[13] (a), but clearly distinct from specimens of N-acetyl-D-arabinosamine[4,13,24] (b) and N-acetyl-D-ribosamine[13] (c); ratios of migration, a:b:c = 1.33:1.18:1.43.

Like authentic N-acetyl-D-lyxosamine, and in contrast to the other N-acetyl-pentosamines, the product XII exhibited the Morgan–Elson color test *without heating* with sodium carbonate. This characteristic behavior is probably due to the presence in the equilibrium solution of sizable amounts of the furanose form.[13]

D-Lyxosamine Hydrochloride (XIII).—A solution of the N-acetate XII (100 mg.) in 10 ml. of 0.3 *N* hydrochloric acid was hydrolyzed for 2 hours on the steam-bath. The hydrolysate was neutralized with Amberlite CG-45 (OH^-), decolorized with charcoal, adjusted to *p*H 2 with dilute hydrochloric acid and evaporated to dryness. The solid obtained was not chromatographically pure; however, it gave a main spot identical with the one given by authentic D-lyxosamine hydrochloride.[13] The product was purified by precipitation with ethyl acetate from a solution in methanol–ethanol, the first flocculent parts of the precipitate being discarded. The solid was collected by centrifugation, then crystallized with some difficulty from methanol–ethanol when allowed to evaporate slowly on the air. The crystals (XIII) that were chromatographically pure, and an authentic specimen looked alike under the microscope, had the same R_f value, and gave almost superimposable infrared spectra. Especially characteristic for XIII was its complex mutarotation in half-saturated sodium borate solution. The curve given matched exactly the one determined by Kuhn and Baschang[13]; this distinguished XIII readily from D-ribosamine hydrochloride; $[\alpha]^{20}D$ +5° (*t* = 0, extrapol.) → −18.6° (10 min.)→ −93.5° (90 min.)→ −68.0° (5 hr., final) (*c* 0.75 in half-saturated $Na_2B_4O_7$ solution).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF IRWIN, NEISLER & CO., DECATUR, ILL.]

Stereospecific Syntheses of 2-Arylethyl-5-acyloxy-6-alkoxy-*cis*-octahydroisoindole Derivatives. The Stereochemical Course of the Epoxidation of *cis*-Δ⁴-Tetrahydrophthalic Anhydride

BY ALLAN P. GRAY, DONALD E. HEITMEIER AND HAROLD KRAUS

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Evidence has been adduced indicating that epoxidation of *cis*-Δ⁴-tetrahydrophthalic anhydride affords exclusively the β-epoxy derivative, and epoxidation of N-phenethyl-*cis*-Δ⁴-tetrahydrophthalimide gives predominantly the β-epoxide accompanied by a lesser amount of the α-isomer. These results have been interpreted as supporting the half-boat conformation (D) for both the anhydride and the imide. The epoxides have been converted, by stereospecific processes, to a series of 2-phenethyl-, 2-naphthylethyl- and 2-indolylethyl-5-acyloxy-6-alkoxy-*cis*-octahydroisoindole derivatives which have been examined for "reserpine-like" pharmacological activities.

A host of compounds more or less emulating structural features of the reserpine molecule[1] has been synthesized[2] in recent years. The present report is concerned with a stereospecific synthesis of a group of *cis*-octahydroisoindole derivatives,

(1) For the definitive discussion of the structure and stereochemistry of reserpine, see P. E. Aldrich, *et al.*, *J. Am. Chem. Soc.*, **81**, 2481 (1959).

(2) *E.g.*, see M. A. Karim, W. H. Linnell and L. K. Sharp, *J. Pharm. Pharmacol.*, **12**, 74 (1960), and the many references cited therein.