[CONTRIBUTION FROM THE MAX-PLANCK-INSTITUT FÜR MEDIZINISCHE FORSCHUNG, INSTITUT FÜR CHEMIE, HEIDELBERG, GERMANY, AND THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF.]

Cyclizations of Dialdehydes with Nitromethane. III.[1] Preparation of 3-Amino-3-deoxy-D-mannose

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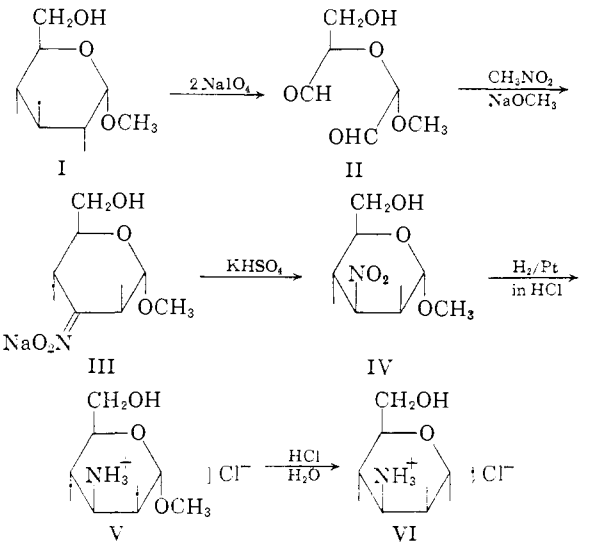
Cyclization, with nitromethane and sodium methoxide, of the dialdehyde produced by periodate cleavage of methyl α-D-glucopyranoside led to an *aci*-nitro condensation product (yield 80–85%) from which, upon acidification and hydrogenation, crystalline methyl 3-amino-3-deoxy-α-D-mannopyranoside hydrochloride could be prepared. This was hydrolyzed to give crystalline 3-amino-3-deoxy-α-D-mannose hydrochloride. The N-acetyl derivative of the latter was degraded to 2-acetamido-2-deoxy-D-arabinose.

Recently, a novel synthesis of 3-amino-3-deoxy sugars has been described.[1,3] The method has made possible the preparation of the D- and L-forms of 3-amino-3-deoxy-ribose hydrochloride in good yields with a reasonable outlay in time and materials. In addition, smaller amounts of 3-amino-3-deoxy-xylose derivatives are formed. The synthesis takes advantage of the well-known condensation reaction of nitroalkanes with aldehydes. As we have shown,[1,3] sugar "dialdehydes," formed by periodate cleavage of methyl glycosides, react with nitromethane and alkali in a twofold condensation involving *one* molecule of nitromethane with *both* aldehyde groups. The glycoside thus reconstituted then bears an *aci*-nitro group on carbon atom 3 and is obtained as the sodium salt. Acidification furnishes a mixture of epimeric methyl 3-nitro-glycosides, which can be hydrogenated to the corresponding methyl 3-amino-3-deoxy-glycosides. Conformational considerations have been employed for explaining the noteworthy fact that the stereospecificity of the condensation reaction is marked enough to permit the practical preparation of one favored product.

As already briefly mentioned,[3] this synthesis also proved to be applicable to the hexose series. The present paper reports the preparation of a new aminohexose, namely 3-amino-3-deoxy-α-D-mannose hydrochloride, using methyl α-D-glucopyranoside (I) as starting material. Periodate oxidation of I according to Jackson and Hudson gives D'-methoxy - D - hydroxymethyl - diglycolic aldehyde (II).[4] This sirupy dialdehyde, when treated with approximately equimolar amounts of nitromethane and sodium methoxide in methanolic solution at +4°, produced, in yields up to 85%, an amorphous powder which analyzed correctly for a methyl-*aci*-nitro-deoxy-hexoside sodium salt (III). The failure of this product to crystallize may be attributable to a lack in steric homogeneity. Support for this assumption came from a later stage of the synthesis. However, if the salt consisted of two or more stereoisomers, they were probably always formed in the same proportions since, in several parallel experiments and in successive fractions of an individual experiment, the products always exhibited, within narrow limits, the same rotational behavior. Immediately after dissolving III in water, the specific rotation was +52°. Thereafter a mutarotation was observed reaching a final value of about +100° after 5 days. The cause of this remarkable change is still unexplained.[5]

The methyl *aci*-nitro-hexopyranoside sodium salt (III) was acidified by means of solid potassium bisulfate. Ethyl acetate extraction of the dry salt mixture gave 90% of a methyl nitro-deoxy-hexopyranoside sirup ($[\alpha]_D$ +88°, in water). The results of the subsequent hydrogenation indicated that this sirup was a mixture. However, the methyl 3-nitro-3-deoxy-α-D-mannopyranoside (IV) appeared to represent a major part of it.



The hydrogenation of the nitrohexoside mixture (IV plus stereoisomers) was carried out with a platinum catalyst in the presence of one molecular equivalent of dilute hydrochloric acid. Three molecular equivalents of hydrogen was taken up readily at room temperature. From the reaction mixture, colorless needles of melting point 205° dec. and $[\alpha]_D$ +60° (water) could be separated in a yield of 32–36%, based on the nitrohexoside mixture. The analytical data proved the product to be

(1) Communication II, H. H. Baer and H. O. L. Fischer, THIS JOURNAL, **81**, 5184 (1959).

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(3) H. H. Baer and H. O. L. Fischer, *Proc. Natl. Acad. Sci.*, **44**, 991 (1958).

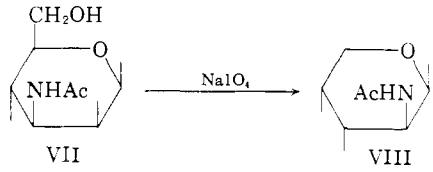
(4) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937). The open chain dialdehyde formula as pertinent to the following reactions is depicted, although it is acknowledged that dialdehydes of this type are apt to assume cyclic hemiacetal structures; *cf.* F. Smith, *et al.*, *ibid.*, **79**, 691 (1957); **80**, 4681 (1958).

(5) The crystalline methyl 3-*aci*-nitro-3-deoxy-β-D-ribopyranoside sodium salt and its enantiomorph undergo analogous mutarotations.[1] An investigation thereon is being undertaken.

a methyl amino-deoxy-hexoside hydrochloride (V). Acid hydrolysis afforded a crystalline, reducing amino-deoxy sugar hydrochloride VI showing decomposition at 165–167°, and exhibiting, in aqueous solution, a downward mutarotation[6]: $[\alpha]_D$ +17° (2 min.) → +6° (final).

As far as we are aware no crystalline 3-amino-3-deoxy-hexose hydrochloride, and only one corresponding methyl α-glycoside hydrochloride, has hitherto been described.[7] Because of this lack of compounds for comparison, an immediate configurational assignment of our products was not possible. Although some of the eight hexose configurations appeared more likely than others with regard to conformational considerations (see below), conclusive evidence as to how V and VI should be formulated had to be reached by the comparison of suitable derivatives. For this purpose, the amino sugar VI was N-acetylated; a crystalline acetamido hexose VII showing a melting point of 191–192° dec. and $[\alpha]^{28}_D$ −55° (initial) → −24° (water) was obtained. These data ruled out the possibility that VII was 3-acetamido-3-deoxy-D-glucose (N-acetyl-kanosamine).[8] The data, rather, suggested that our N-acetylated compound was identical with 3-acetamido-3-deoxy-β-D-mannose that was concurrently synthesized in an independent way by Kuhn and Baschang.[9] Full proof of the identity was established by comparison of the infrared spectra, X-ray powder photographs and R_f-values.

Moreover, we have degraded the 3-acetamido hexose VII by the action of one molecular equivalent of periodate and have thus obtained 2-acetamido-2-deoxy-β-D-arabinose (N-acetyl-β-D-arabinosamine, VIII). Again, the product was identified by comparison of its melting point (159–160°), rotation ($[\alpha]^{27}_D$ −154°, initial, → −97.3°, in water), R_f-value and infrared spectrum with a synthetic sample of R. Kuhn and G. Baschang.[10]



VII VIII

Since VIII can arise by degradation of either 3-acetamido-D-mannose or 3-acetamido-D-glucose only, and since the latter had been ruled out before, the periodate oxidation experiment constitutes further evidence that our acetamido sugar VII and, hence, also the compounds V and VI, are D-mannose derivatives as depicted in the formulas.

Paper chromatographic investigation of the mother liquor from the hydrogenation of the nitrohexoside sirup revealed at least seven ninhydrin-positive spots. Two of them, having R_{gm}-values of 1.52 and 1.32, could be distinguished as representing major constituents of the mother liquor, whereas the other spots were rather faint. Residual mannoside V (R_{gm} 1.52) was at first thought to be, and possibly was in part, responsible for the faster main spot. However, when a partial separation of the material was achieved on a cellulose column, a small fraction giving solely that spot yielded a crystalline aminoglycoside which was different from V, as indicated by melting point (194–196° dec.), rotation ($[\alpha]^{25}_D$ +83°, in water), infrared spectrum and X-ray powder photograph.

The slower-moving product with R_{gm} 1.32 was isolated in somewhat larger quantity. Since it did not crystallize, an attempt was made to characterize it by acetylation. The resulting crystalline tetraacetate had a melting point 181.5° and $[\alpha]^{29}_D$ +111° (chloroform).

It has not yet been possible to establish the identity of these by-products of the synthesis. Since the nitromannoside IV was a main product of the acidification of the *aci*-nitro salt, and thus part of the latter must be represented by formula III, one should expect the formation of some methyl 3-nitro-3-deoxy-α-D-altroside (*i.e.*, the 3-epimer of IV) upon that acidification, and some of the corresponding 3-amino-altroside upon the subsequent hydrogenation.

Stereochemical Considerations.—The two asymmetric centers, derived from the carbon atoms 1 and 5 of methyl α-D-glucopyranoside, of the dialdehyde II were not involved in either the periodate scission I → II or the nitromethane condensation II → III. Hence, the resulting salt III is an α-hexopyranoside derivative of the D-series. Since III, as an *aci*-nitro-deoxy sugar, is symmetrical at carbon 3, there remain four configurations which the molecules may assume when they are being formed. These are the α-D-*manno*(*altro*), α-D-*gluco*(*allo*), α-D-*talo*(*ido*) and α-D-*galacto*(*gulo*) configurations. (Fig. 1.)

As previously pointed out[1] we believe that a selective principle as to what configuration will be favored is the tendency of the arising new hydroxyls (on C-2 and C-4) to enter into *trans* arrangements with respect to the adjacent substituents (on C-1 and C-5). The above formulas illustrate that a has two *trans* relationships, b and c have one each, and d has none at all.

The conformational situation existing during the formation of the *aci*-nitroglycoside salt may be evaluated by adopting Reeves' ideas of "instability factors,"[11] after making due allowance for the absence of asymmetry at carbon 3.[12] Accordingly, the α-D-*gluco*(*allo*) isomer existing in the C1 conformation and the α-D-*talo*(*ido*) isomer existing in the 1C conformation have to be assigned only one "instability unit" each, that is to say, they have to

(6) Since the sugar belongs to the D-series the crystals are the α-anomer.

(7) L. F. Wiggins, *J. Chem. Soc.*, 18 (1947), described methyl 3-amino-3-deoxy-α-D-altroside hydrochloride with m.p. 208-209° dec. and [α]D +88.2° (water). Earlier, S. Peat and L. F. Wiggins [*ibid.* 1810 (1938)] have prepared methyl 3-amino-3-deoxy-α-D-glucoside (free base) for which they reported [α]D +144.4° (water).

(8) N-Acetyl-kanosamine has m.p. 199–202° dec. and $[\alpha]^{25}_D$ +43° (equilibrium in water), according to R. U. Lemieux and associates, THIS JOURNAL, **80**, 2342 (1958).

(9) R. Kuhn and G. Baschang, *Ann.*, **628**, 206 (1959), have found m.p. 195–196° dec. and $[\alpha]^{25}_D$ −52° (3 min.) → −24° (water) for this compound, m.p. 202–203° dec. and $[\alpha]^{25}_D$ +17° (3 min.) → +45.6° (water) for 3-acetamido-3-deoxy-D-glucose.

(10) These investigators report m.p. 160–163° and $[\alpha]_D$ −149° (2 min.) → −97° (in water) *Ann.*, **628**, 193 (1959). We wish to express our gratitude to Prof. Kuhn and Dr. Baschang for providing samples for comparison of VII and VIII and for furnishing data prior to publication.

(11) R. E. Reeves, *Advances Carbohydrate Chem.*, **6**, 124 (1951).

(12) The *aci*-nitro substituent is aligned coplanar with the sugar ring.

be credited with about equal and highest possible stability. The respective 1C and C1 conformations, on the other hand, would involve a maximum of instability factors. The α-D-*galacto*(*gulo*) isomer, with two "instability units" in its C1 conformation, would likewise be unstable in the second chair form, 1C. The α-D-*manno*(*altro*) isomer, in contrast, has a nearly equal chance to assume *either* chair form. Although these are comparable in stability with the *galacto*(*gulo*) C1 form only and not with the more stable *gluco*(*allo*) C1 or *talo*(*ido*) 1C forms, this unique feature may play a favorable role in accommodating varying conformational shapes of the dialdehyde II.

Possibly both steric influences discussed contribute to selecting the *aci*-nitro salt configuration. For solving that problem, the identification of the by-products of the synthesis must be awaited. At any rate, one need not be surprised at the preferential formation of the *manno*(*altro*) salt III.

Acknowledgments.—The authors gratefully acknowledge the support of this work by the United States Public Health Service (Grant A-2425), the Nutrition Foundation Inc., New York, and the Max-Planck-Gesellschaft, Germany. Thanks are due to Professor Richard Kuhn, Heidelberg, for granting a leave of absence for America to H. H. B., and his interest in and permission for the work to be continued in his Institute. The skillful technical assistance of Mr. W. Dafeldecker is greatly appreciated.

Experimental

Periodate Oxidation of Methyl α-D-Glucopyranoside.—A solution of 8.56 g. of sodium metaperiodate in 100 ml. of water was cooled to +5°. Under swirling, methyl α-D-glucopyranoside (3.88 g.) was added portionwise in the course of 5 minutes. The solution was then allowed to stand in the dark at room temperature, the formic acid formed being gradually neutralized by the addition of portions of sodium bicarbonate solution. A total of 18 ml. of *M* sodium bicarbonate (90% of the equivalent amount) was employed; the *p*H of the reaction mixture was not allowed to become greater than 5–6 at any time. Completion of the oxidation usually took 2 to 3 hours, and was indicated by a negative starch–potassium iodide test of a withdrawn sample to which excess bicarbonate had been added.

A large part of the sodium iodate was precipitated from the solution by the addition of 200 ml. of absolute ethanol, then filtered off with suction, and washed with ethanol. The combined filtrate and washings were concentrated under reduced pressure at a bath temperature of 45°. After the addition to the concentrate, of more ethanol, the crystals that separated were removed. This procedure was repeated until no more alcohol-insoluble material could be filtered off. Finally the solution was evaporated (bath temperature 25°) to a sirup which still contained some salt. It was therefore taken up in a small quantity of absolute ethanol and evaporated again after filtration. A very small amount of salt crystals remaining in the final dialdehyde sirup need not be removed.

Condensation of D′-Methoxy-D-hydroxymethyl-diglycolic Aldehyde (II) with Nitromethane.—The sirupy dialdehyde II, obtained as described above, was dissolved in 25 ml. of absolute methanol. Nitromethane (1.1 ml., 1 molar equivalent) was added and the solution was chilled in an ice-bath. A chilled methanolic solution of 0.95 molar equivalent of sodium methoxide (14.5 ml. of a solution containing 3 g. of sodium per 100 ml.) was added dropwise, under swirling, at a moderately rapid rate. The reaction mixture was kept in the ice-bath for 15 minutes, during which time it slowly turned yellow. Then the solution was quickly concentrated under reduced pressure[13] at a bath temperature not exceeding 20°. When about one-third of the initial volume was reached, absolute ethanol was added to the point of a remaining turbidity. The evaporation was then continued until the precipitated reaction product formed a thick yellowish-white slurry. The product was transferred to a Büchner funnel by means of a little absolute ethanol, and washed, by trituration on the filter, with several portions of cold ethanol. This had to be done very quickly and with the smallest possible amount of moist air being allowed to contact the material.[14] The product, methyl 3-*aci*-nitro-3-deoxy-α-D-hexopyranoside sodium (III), was then dried in a desiccator over phosphorus pentoxide and potassium hydroxide.

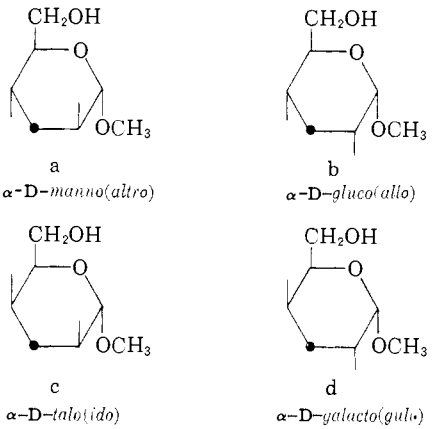


Fig. 1.—The four possible configurations of a methyl 3-*aci*-nitro-3-deoxy-α-D-hexopyranoside salt. •, Site of *aci*-nitro substituent.

Regardless of some precipitate which occurred on combining the washings with the mother liquor, the filtrate was concentrated *in vacuo*, thus affording a second crop of the nitroglycoside sodium salt. The weight ratio of the crops depended on how far the first solution was concentrated. In one typical experiment there were found 3.9 g. of first and 0.3 g. of second crop, in another experiment 2.7 and 1.2 g., respectively. The total yields, therefore, were 85 and 79%. The crops showed identical composition as revealed by their optical rotations.

The methyl 3-*aci*-nitro-3-deoxy-hexopyranoside sodium salt (III) was obtained as a faintly yellowish, amorphous, hygroscopic powder. It was easily soluble in water and in methanol, and insoluble in absolute ethanol.

The rotation of several crops was determined in carbon dioxide-free water, *c* 0.9–1.2, 2-dm. tube; the following is typical: $[\alpha]^{25}D$ +52.5° (2 min.) → +70.0° (1 hr.) → +87.6° (25 hr.) → +99.5° (120 hr.).

The ultraviolet absorption spectrum of III in CO_2-free water shows a strong maximum at 248 mμ and a low shoulder at 300 mμ.

The analytical sample was dried for 4 hours at 55° and 3 mm.

Anal. Calcd. for $C_7H_{12}O_7NNa$ (245.2): N, 5.71; Na, 9.38; OCH_3, 12.66. Found: N, 5.62, 5.81; Na, 9.56, 9.32; OCH_3, 12.98, 13.28.

Mixture of Methyl 3-Nitro-3-deoxy-α-hexopyranosides (IV).—The "dry" acidification with potassium bisulfate (17.5 g.) and anhydrous sodium sulfate (17.5 g.) of the above *aci*-nitroglycoside salt III (3–5 g.) was performed as described previously[1] for the liberation of the nitropentosides from their salts, except for the use of dry ethyl acetate instead of ether as an extractant. Two extractions of one hour each were sufficient. After evaporation of most of the solvent, a yellow sirup was obtained which was dried *in vacuo* over phosphorus pentoxide and potassium hydroxide at room temperature to constant weight. For purification, the product was dissolved in a small volume of water and

(13) In order to procure rapid evaporation, the condensate is cooled with an ice–salt mixture. If a capillary is used, it is connected with a soda and lime tube and a calcium chloride tube.

(14) Therefore, suction is employed only as long as necessary to remove most of the washings. In case parts of the salt have become sticky they are redissolved in a little methanol and combined with the mother liquor.

treated with activated charcoal. Upon evaporation, the filtrate left a honey-colored sirup of methyl 3-nitro-3-deoxy-α-D-hexopyranosides (IV), which, after drying in a desiccator, amounted to 84–89% of the theory (2.56, 2.99 and 3.84 g. of IV from 3.26, 3.70 and 5.0 g. of III, respectively); $[\alpha]^{24}D$ +88.2° (*c* 1, water), +87.2° (*c* 0.75, water).

Anal. Calcd. for $C_7H_{13}O_7N$ (223.2): C, 37.67; H, 5.87; OCH_3, 13.91. Found: C, 37.96; H, 5.15; OCH_3, 14.37.

In aqueous solution, the product exhibits a flat absorption curve in the ultraviolet region with a scarcely noticeable maximum at 270 mμ. Upon addition of sodium hydroxide solution, the strong maximum at 248 mμ of the *aci*-nitro salt appears instantly.

For a preparatory reconversion of the free nitrohexosides IV to the sodium salt III, a sample of 1.04 g. of IV was dissolved in 7 ml. of absolute methanol. Upon cooling of the solution to 0°, there was added 3.5 ml. of a sodium methoxide solution (3 g. of Na per 100 ml.). The reaction mixture, becoming dark yellow, was immediately worked up as described above for the isolation of III, yielding two salt crops of 607 and 79 mg., respectively: $[\alpha]^{22-25}D$ +52.6° (4 min.) → +69.0° (1 hr.) → +83.2° (26 hr.) → +92.7° (120 hr.) (*c* 0.46, CO_2-free water).

Methyl 3-Amino-3-deoxy-α-D-mannopyranoside Hydrochloride (V).—Platinum dioxide (5 g.) in 100 ml. of water plus 22.4 ml. of *N* hydrochloric acid was saturated with hydrogen at room temperature. A solution of 5.0 g. (22.4 m*M*) of nitroglycoside IV in 40 ml. of water was added and the hydrogen uptake measured. Consumption of 3 moles of hydrogen (calculated and found, 1.50 l.; corrected volume) required 4.3 hours.[15] The catalyst was filtered off and the small excess of free acid was removed with Amberlite IR-45 (OH^-) in an amount just sufficient to adjust the solution to *p*H 4. Evaporation at reduced pressure at 45° (bath temperature) afforded a colorless, partly crystalline residue, which was evaporated three times with absolute ethanol. Finally the residue was triturated with a little absolute ethanol and kept in the refrigerator for 3 hours. The crystals were collected, washed with a small portion of ice-cold ethanol and dried in a desiccator. The yield was 1.673 g. plus another 27 mg. from the mother liquor (33.2%). Similar experiments gave 32.8 and 36%. The white, rectangular prisms of the amino-mannoside hydrochloride V had $[\alpha]^{20}D$ +62.8° (*c* 1, water), and showed decomposition on heating at about 205°, after preliminary browning at 190°. On examination by paper chromatography[16] the product had R_{gm} 1.52, and was revealed to be accompanied by a slight impurity of R_{gm} 1.32 (ninhydrin spray). The impurity was removed by one recrystallization of the glycoside from a 20-fold amount of boiling ethanol containing 10% of water. The rotation of the purified material was $[\alpha]^{22}D$ +60.0° (*c* 2, water), the decomposition range was unchanged. The analytical sample was dried for 4 hours at 100°.

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, 36.76; H, 6.90; N, 6.15; Cl, 15.06, 15.11; OCH_3, 13.87.

3-Amino-3-deoxy-α-D-mannose Hydrochloride (VI).—Two grams of recrystallized aminoglycoside V was refluxed in 200 ml. of 0.5 *N* hydrochloric acid for 15 hours. Subsequently, the hydrolyzate was concentrated under reduced pressure at 50° (bath temperature) to a small volume. Water was added and the evaporation then continued. This procedure was repeated three times, most of the acid thus being removed. Finally the concentration was conducted further to give a colorless sirup, which was, while still moist, triturated with glacial acetic acid. Evaporation afforded a white solid that was dissolved in very little water. Upon addition of glacial acetic acid (8–10 volumes, to beginning turbidity) and inoculation[17] there crystallized 985 mg. (52.4%) of stout prisms of the 3-amino-mannose hydrochloride VI. The decomposition point was 165–167° (after preliminary browning at 160°); $[\alpha]^{26}D$ +17° (2 min.) → +14° (4 min.) → +12° (6 min.) → +8.5° (40 min.) → +7° (90 min.) → +6° (final, after 240 min.) (*c* 1, water, 2-dm. tube). The data were unchanged after one recrystallization from water–glacial acetic acid.

Anal. Calcd. for $C_6H_{14}O_5NCl$ (215.6): C, 33.42; H, 6.54; N, 6.50; Cl, 16.44. Found: C, 33.51; H, 6.47; N, 6.76; Cl, 16.62.

3-Acetamido-3-deoxy-β-D-mannose (VII).—Aminosugar hydrochloride VI (215 mg.), well-powdered silver acetate (167 mg.) and acetic anhydride (0.14 ml.) in 3 ml. of absolute methanol were stirred magnetically in the dark beginning at a temperature of 0°. During the first hour the reaction mixture was gradually allowed to come to 26°. After 5 hours, another two drops of acetic anhydride was added and the mixture heated on a steam-bath for 3 minutes. The solid was filtered off and washed well with hot water. The filtrate was acidified with a few drops of *N* hydrochloric acid, treated with activated charcoal, filtered again and evaporated *in vacuo*. After taking up the residue in 0.3 ml. of water, 2.7 ml. of absolute ethanol was added. Upon careful addition, to the alcoholic solution, of 4.5 ml. of ethyl acetate, and on scratching with a glass rod, crystallization of the acetamido sugar VII began. After the solution stood overnight in the refrigerator, the colorless crystals were isolated and washed with an ethanol–ethyl acetate mixture (3:4) and with pure ethyl acetate. The first crop weighed 50 mg. and had m.p. 191–192°. The mother liquor was worked up by slow evaporation in the air and by triturating the residue with methanol–ethyl acetate mixtures; additional crops amounting to 117 mg. were obtained, the total yield of VII thus being 79%. The product proved to be identical with 3-acetamido-3-deoxy-β-D-mannose prepared by Kuhn and Baschang[9] according to infrared spectrum, X-ray powder photograph, chromatography (R_{gm} 1.64, aniline phthalate spray)[16] and optical rotation, $[\alpha]^{28}D$ −55° (initial, extrapol.) → −53° (2 min.) → −24° (60 min., constant) (*c* 1, water).

Anal. Calcd. for $C_8H_{15}NO_6$ (221.2): C, 43.43; H, 6.84; N, 6.33. Found: C, 43.47; H, 6.89; N, 6.64.

2-Acetamido-2-deoxy-β-D-arabinose (VIII).—A mixture of 100.3 mg. of the acetamino mannose VII and of 97.3 mg. of sodium metaperiodate was dissolved in 5 ml. of water at 27°. The rotation of −1.32° observed in a 1-dm. tube 12 minutes after dissolving did not change within the next 50 minutes, indicating that the oxidation was complete within the first few minutes. A potassium iodide–starch test in the presence of excess bicarbonate, performed after one hour, showed all of the oxidant to be consumed. By the addition of 50 ml. of absolute ethanol and cooling to 0°, most of the sodium iodate formed was precipitated. The filtrate therefrom was evaporated, and the white residue treated with 10 ml. of warm absolute ethanol which was filtered after cooling to 0° and again evaporated. Now the residue was taken up in 10 ml. of water and 50 mg. of sodium bicarbonate was added. The solution was allowed to stand at 26° for 30 minutes and then at 55° for 15 minutes. Subsequently, the sodium ions were removed by stirring the solution with some Amberlite IR-120 (H^+). Remnant traces of iodic acid were removed by treatment with a little Amberlite IR-45 (OH^-) so that potassium iodide–starch paper no longer turned blue. The resins were filtered off and thoroughly washed by decantation with water. The filtrate and washings, after treatment with some activated charcoal, were evaporated under diminished pressure, eventually with the addition of two consecutive portions of absolute ethanol. The residue was dissolved in a little 95% ethanol. Upon addition of ethyl acetate, there crystallized colorless, diamond-shaped prisms of N-acetyl-β-D-arabinosamine (VIII), which were collected after standing overnight at 25° and washed with a mixture of ethyl acetate and 95% ethanol (1:1). The yield was 40 mg.; a second crop of 33 mg. was obtained from the mother liquor. After one recrystallization from ethanol–ethyl acetate, the m.p. was 159–160° dec., $[\alpha]^{27}D$ −154° (initial, extrapol.) → −138° (2 min.) → −97.3° (30 min., constant) (*c* 0.75, water).

The product VIII was shown to be identical with 2-acetamido-2-deoxy-β-D-arabinose prepared by Kuhn and Baschang,[10,18] according to melting point, rotation, infrared spectrum and R_{gm}-value (1.72).[16]

(15) Vigorous shaking of the hydrogenation vessel is essential. In some runs, the hydrogenation uptake became extremely slow after several hours. The process was then stopped, despite incomplete (*e.g.*, 90%) uptake, rather than being carried on longer than 6 hours.

(16) The authors are greatly indebted to Dr. Adeline Gauhe, Heidelberg, for running the chromatograms. Throughout this work the descending technique using Schleicher and Schüll 2043b paper and the solvent system ethyl acetate–pyridine–water–acetic acid, 5:5:3:1, according to F. G. Fischer and H. Dörfel, *Z. physiol. Chem.*, **301**, 224 (1955), was employed. $R_{gm} = R_{glucosamine\text{-}HCl}$.

(17) Seeding crystals were obtained when, in a preliminary experiment, some of the amino sugar sirup was exposed to the air for several days.

Investigation of the By-products of the Hydrogenation.—The mother liquor that remained after the crystallization of methyl 3-amino-3-deoxy-α-D-mannopyranoside hydrochloride (V) described above was evaporated *in vacuo*. Examination by paper chromatography of the resulting foamy or sirupy material led to the detection of ninhydrin-positive spots with the R_{gm}-values[16]: 2.23, 2.06, 1.92, (1.64), ***1.52***, ***1.32***, (1.0).

By use of a column (width, 5 cm.; length, 56 cm.) containing 330 g. of powdered cellulose[19] a partial separation of the mother liquor products could be achieved. Two grams of the amorphous product was developed with the Fischer–Dörfel solvent (*cf.* footnote[16]) at a flow rate of 1 drop per 5 seconds. The first 480 ml. of effluent did not contain optically active material and was discarded. Subsequently, fractions of 25 ml. were taken and examined by rotation and paper chromatography. Fractions 1–5 contained strongly dextrorotatory, ninhydrin-negative material (180 mg.).[20] Fractions 6–8 (38 mg.) and 9–14 (129 mg.), both weakly dextrorotatory, contained mixtures which gave spots having R_{gm} 2.23 + 2.06, 2.23 + 2.06 + 1.92, and 2.06 + 1.92. Fractions 15–16 (57 mg.) were mixtures of R_{gm} 2.06, 1.92, 1.64 and 1.52. So far the material was not investigated further.

From fractions 17–18 (126 mg., R_{gm} 1.52) there could be isolated 33 mg. of a crystalline aminoglycoside hydrochloride. Although it had the same R_{gm}-value as the aminomannoside IV, its rotation $[\alpha]^{25}D$ +83° (*c* 1, water), as well as its X-ray diffracton pattern and its infrared spectrum were different from those of IV.

Fractions 19–22 (469 mg., strongly dextrorotatory) were mixtures of the products of R_{gm} 1.52 and 1.32; they were discarded. In fractions 23–26 (645 mg., maximum of dextrorotation) mainly the substance of R_{gm} 1.32 was present, along with traces of that of R_{gm} 1.52. Fractions 27–36 (700 mg.) contained the R_{gm} 1.32 product only.

Aminoglycoside R_{gm} 1.32.—The combined fractions containing the amino-glycoside R_{gm} 1.32 from an analogous column afforded, upon evaporation and drying *in vacuo* (14 days at 25°), a sirupy residue (924 mg.) which still contained acetic acid and pyridine; $[\alpha]^{28}D$ +56.2° (*c* 1, water). For purification the substance was stirred with Dowex-1 (OH^-) in aqueous solution, which rapidly became alkaline. Evaporation at reduced pressure, at last under repeated addition of absolute ethanol until the pyridine smell had disappeared, afforded a colorless sirup that was dissolved in water and carefully neutralized to *p*H 5 with dilute hydrochloric acid. The solution was then brought to dryness again, thus yielding 443 mg. of a colorless foam, $[\alpha]^{27}D$ +110° (*c* 1, in water). Analysis suggested the material to be a methyl aminodeoxy-hexoside hydrochloride: Calcd.: N, 6.10; OCH_3, 13.51. Found: N, 6.03; OCH_3, 14.54. For acetylation, 400 mg. of the above aminoglycoside was refluxed for 5 minutes with 2 g. of anhydrous sodium acetate and 10 ml. of acetic anhydride. The excess anhydride was distilled off *in vacuo*, and the residue was then dissolved in 50 ml. of water. Extraction with five 50-ml. portions of chloroform, washing the combined extracts with two 25-ml. portions of saturated sodium bicarbonate solution and with 25 ml. of water, and finally drying the chloroform with anhydrous sodium sulfate afforded an amorphous residue of $[\alpha]^{27}D$ +99° (in chloroform). Crystallization took place from 95% ethanol giving diamond-shaped prisms of m.p. 181.5° and $[\alpha]^{26}D$ +111.5° (*c* 0.75, chloroform). In another experiment crystallization was first achieved from acetone–ether–pentane. The product was recrystallized from carbon tetrachloride (m.p. 177–180° after preliminary softening) and subsequently from 95% ethanol (m.p. 180–181°); $[\alpha]^{26}D$ +111° (*c* 1.2, chloroform).

Anal. Calcd. for a tetraacetate, $C_{15}H_{23}O_9N$ (361.3): C, 49.85; H, 6.42; OCH_3, 8.59. Found: C, 49.92; H, 6.23; OCH_3, 8.69.

(18) M. L. Wolfrom and Z. Yosizawa, THIS JOURNAL, **81**, 3477 (1959), report m.p. 154–156° and $[\alpha]D$ +147.5° → +94° (in water) for the L-enantiomorph.

(19) Linterspulver, Schleicher and Schüll No. 124.

(20) Weights are approximate only; they were determined after drying the evaporation residues in a vacuum desiccator for several days. There were small amounts of solvent still present, however.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY AND THE DEPARTMENT OF CHEMISTRY OF THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA]

Plant Phospholipids. II. Isolation and Structure of Glycerophosphoryl Inositol[1,2]

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The location of the phosphate diester linkage in phosphatidyl inositol of plant origin has been examined. Glycerophosphoryl inositol was isolated from deacylated corn phosphatides by column chromatography on Dowex 2-acetate. Each of the other anionic derivatives of plant lipids was separated using gradient elution technique and labeled compounds for qualitative and quantitative analysis. Proton magnetic resonance spectrometry of glycerophosphoryl inositol heptaacetate revealed acetoxy hydrogen absorption corresponding to one axial acetoxy group. These results and hydrolysis product studies indicate that the plant phosphatide is 1-phosphatidyl-*myo*-inositol.

Phosphatidyl glycerol[3] and inositol are the most actively metabolized of the plant phospholipids.[4] The rapid transfer of phosphatidyl groups and the structural singularity of phosphatidyl inositol suggests function of enzyme systems for phosphatidylation of the *myo*-inositol at a specific hydroxyl group.

Of the six possible positions for the phosphate diester linkage, four are readily subjected to experimental approach. 2- and 5-glycerophosphoryl-*myo*-inositol may be expected to possess no optical rotatory contribution from the inositol moiety. Concurrent studies by Hawthorne and Hübscher[5] and by Brockerhoff and Hanahan[6] with phosphatidyl inositol of animal origin revealed asymmetry in the inositol ester. Ballou and Pizer[7] have established by synthesis the structure of the inositol monophosphate derived from hydrolysis of soy bean phosphoinositide as L-*myo*-inositol 1-phosphate. Studies by Brown, *et al.*,[8] of hydrolysis mechanisms of synthetic *cis* and *trans* analogs

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(2) Presented at the 136th meeting of the American Chemical Society, Atlantic City, N. J., September 13–18, 1959, Abstracts of Papers, p. 28C, and is excerpted from the thesis submitted by M. L. to the Graduate School of The Pennsylvania State University in partial fulfillment of the requirements for the Master of Science degree.

(3) A. A. Benson and B. Maruo, *Biochim. et Biophys. Acta*, **27**, 189 (1958).

(4) R. A. Ferrari and A. A. Benson, to be published.

(5) J. N. Hawthorne and G. Hübscher, *Biochem. J.*, **71**, 195 (1959).

(6) H. Brockerhoff and D. J. Hanahan, THIS JOURNAL, **81**, 2591 (1959).

(7) C. E. Ballou and L. I. Pizer, *ibid.*, **81**, 4745 (1959).

(8) D. M. Brown, G. E. Hall and H. M. Higson, *J. Chem. Soc.*, 360 (1958).