fructose, the second at R_f 0.92 (or, relative to D-fructose, R_f 3.18). On the same chromatogram, 1,3-dihydroxy-2-propanone gave a relative R_f 2.20.

Quantitative oxidation of the hydrolyzate of I. Total reducing sugars were determined according to Benedict[15]; 1 ml. of Benedict's reagent corresponded to 1 μM of D-fructose.

Calculations: 15 ml. of Benedict's reagent = 1.85 ml. of hydrolyzate soln. 1.48%. 10 ml. of Benedict's reagent = 1.22 ml. of hydrolyzate soln. 1.48%.

Hence, 1.48 mg. of hydrolyzate is equivalent in reducing power to 22.6 mg. of D-fructose (or 153% of a hexose). For aldose, an iodimetric estimation was used.[16]

Standardizations: 3.00 ml. of approx. $0.1N$ iodine soln. = 2.30 ml. of $0.1N$ sodium thiosulfate. 3.00 ml. of this iodine soln. + 1 ml. of a 1.37% D-fructose soln. = 2.27 ml. of $0.1N$ sodium thiosulfate.

Calculations: 3.00 ml. of this $0.1N$ iodine solution + 1 ml. of hydrolyzate 1.48% = 1.65 ml. of $0.1N$ sodium thiosulfate.

This equals 5.6 mg. aldohexose, or 2.8 mg. of aldotriose. Correspondingly 1 ml. of the hydrolyzate 1.48% contains 0.076 μM reducing sugar, including 0.031 μM aldose. The ratio aldose/total reducing sugar = 1:2.4.

Distillation of the hydrolyzate of I. Compound I (50 mg.) was dissolved in 5 ml. of $4N$ sulfuric acid and kept in a closed ampoule at 100° for 1 hr. The cooled hydrolyzate was transferred to a small distillation unit, and the distillate was collected in Dry Ice. It reduced Fehling's solution upon gentle warming. To 2 ml. of the distillate, 5 drops of phenylhydrazine, 0.1 g. of sodium acetate, and 10 drops of acetic acid were added, and the mixture was kept for 30 min. A crystalline mass settled out; it was dissolved in hot ethanol and concentrated in a stream of dry air. Crystallization set in (probably of sodium acetate) and the crystals were washed with little ethanol and removed by filtration. After several repetitions of this procedure, a small amount of yellow crystals appeared, m.p. 145° (pyruvaldehydeosazone, m.p. 148°). The low yield did not permit recrystallization.

This hydrolysis was repeated, using $0.2N$ sulfuric acid and reducing the time of hydrolysis to 30 min. The first drops of the distillate reduced Fehling's solution readily.

In a similar manner, 1,3-dihydroxy-2-propanone, D-fructose, and di-D-fructose dianhydride I were subjected to hydrolysis for 30 min. with $0.2N$ sulfuric acid and with $4N$ sulfuric acid. With $0.2N$ acid, none of these distillates exhibited any reducing action with Fehling's solution. With $4N$ acid, the reduction was negligible.[17] Using $6N$ sulfuric acid and the same conditions of hydrolysis, 1,3-dihydroxy-2-propanone yielded an appreciable amount of a volatile, reducing product.

JERUSALEM, ISRAEL

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(15) S. R. Benedict, *J. Am. Med. Assoc.*, **57**, 1193 (1911).

(16) F. A. Cajori, *J. Biol. Chem.*, **54**, 617 (1922).

(17) However, a paper chromatogram of these hydrolyzates revealed faint spots, identical with those for pyruvaldehyde.

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY, UNIVERSITY OF UTAH AND ARIZONA STATE UNIVERSITY]

Crystalline D,L-*xylo*-3-Hexulose, A New Hexose[1]

GEORGE U. YUEN[2] AND JAMES M. SUGIHARA

Received August 5, 1960

Monobenzoylation of 1,2:4,5-di-*O*-isopropylidene-D,L-galactitol followed by oxidation with chromium trioxide in pyridine yielded crystalline 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulose. Debenzoylation and deacetonation provided a crystalline hexose, presumed to be D,L,-*xylo*-3-hexulose. This series of reactions constitute the first synthesis of an unsubstituted 3-ketose.

In an earlier communication from this laboratory, the preparation of crystalline 4-*O*-benzoyl-1,2:5,6-di-*O*-isopropylidene-D-*arabo*-3-hexulose was reported.[3] The compound was obtained by oxidation of 3-*O*-benzoyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol with chromium trioxide in anhydrous pyridine. Deacetonation and debenzoylation procedures applied to the substituted 3-hexulose failed to yield crystalline products and thus deterred an unequivocal synthesis of the parent 3-ketose. The failure of the debenzoylation reaction to proceed in a desired fashion was attributed to the formation of an enediol when the ester linkage was cleaved. This enediol would provide a mixture of four isomers.

An alternative path circumventing the formation of an enediol during the debenzoylation step was devised using 1,2:4,5-di-*O*-isopropylidene-D,L-galactitol[4] (I)(formula of D-isomer only given), α-diacetone dulcitol,[5] as the starting compound. Because this substance contains a primary and a secondary hydroxyl group, selective benzoylation of the former would be a distinct possibility.[6] Under controlled conditions, 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-galactitol (II) was obtained in a crystalline form. Oxidation of this compound in an anhydrous system with chromium trioxide in pyridine afforded crystalline 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulose (III).

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(1) This research has been supported by National Science Foundation Grant No. G7302.

(2) A member of the "Research Participation for College Teachers Program" at the University of Utah, Summer 1959, sponsored by the National Science Foundation.

(3) J. M. Sugihara and G. U. Yuen, *J. Am. Chem. Soc.*, **79**, 5780 (1957).

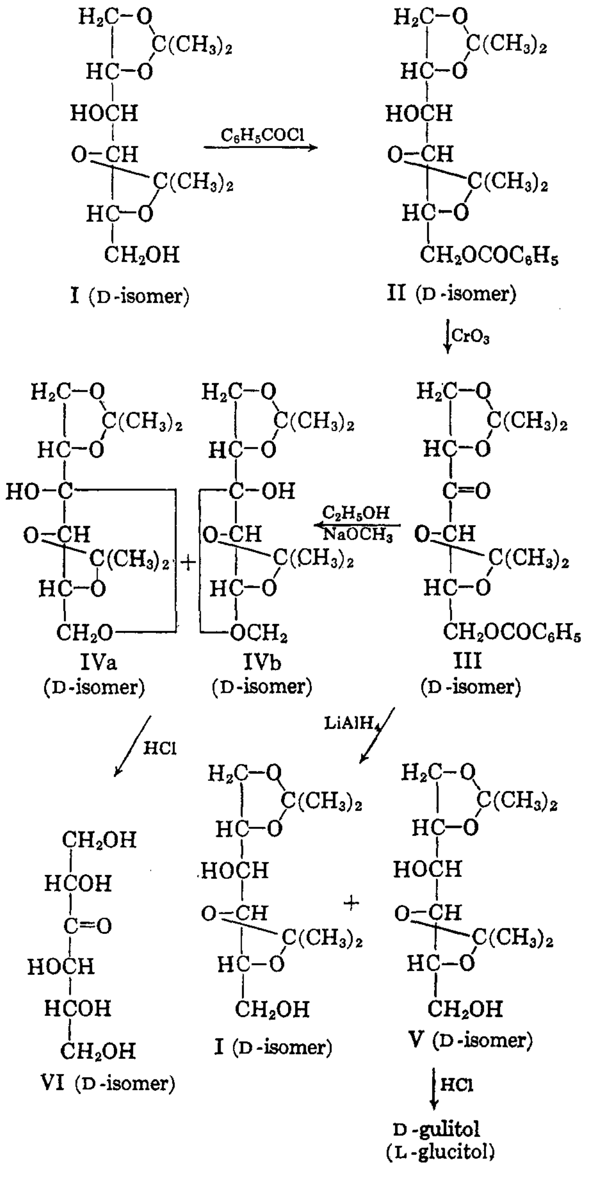
(4) This name is used in preference to 2,3:5,6-di-*O*-isopropylidene-D,L-galactitol suggested by Hann, Maclay, and Hudson (Ref. 5), as the name of the 3-hexulose would logically follow without inversion of the carbon chain.

(5) R. M. Hann, W. D. Maclay, and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 2432 (1939).

(6) J. M. Sugihara, *Advances in Carbohydrate Chem.*, **8**, 35 (1953).

The ketose formed a phenylhydrazone. Proof of structure of III, and in turn II, was accomplished by lithium aluminum hydride reduction of the hexulose and the isolation of 1,2:4,5-di-*O*-isopropylidene-D,L-galactitol (I) and 1,2:4,5-di-*O*-isopropylidene-D,L-gulitol (V) (synonym, 2,3:5,6-di-*O*-isopropylidene-D,L-glucitol), in turn characterized by hydrolysis to form D,L-gulitol (D,L-glucitol). The combined yield of the di-*O*-isopropylidenehexitols in crystalline form accounted for 80% of the ketose reduced. The galactitol derivative was obtained in an amount approximately four times as large as the gulitol compound. 1,2:4,5-Di-*O*-isopropylidene-D,L-gulitol has not previously been described.

Debenzoylation of the substituted ketose (III) was effected by transesterification with ethanol in the presence of sodium methoxide. A crystalline compound was obtained with a wide melting point range, which was not decreased by repeated recrystallizations. Adsorption chromatography using Magnesol did not provide a separation. Recrystallization from petroleum ether yielded crystals of two different types. Mechanical separation followed by recrystallization of each of the fractions gave samples with narrow melting point ranges, depressed upon mixing. Both compounds gave essentially the same ultimate analyses for carbon and hydrogen. On the basis of these observations and the common source of the two compounds, they have been assigned the structures IVa and IVb, namely, the α- and β-anomers of 1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-hexulofuranose.



The anomeric mixture of 1,2:4,5-di-*O*-isopropylidene-D,L-hexulofuranose was hydrolyzed with dilute hydrochloric acid at room temperature to yield a crystalline product analyzing as a hexose. The mild conditions employed to effect this reaction would hardly be expected to cause any isomerization of the 3-ketose formed. Moreover, the separation of the product in crystalline form suggests that such undesired reactions did not occur to any appreciable extent. Thus, it appears highly likely that the sequence of reactions described provides for a synthesis of D,L-*xylo*-3-hexulose (VI) and accordingly the first demonstration of preparation of an unsubstituted 3-ketose. The anomeric form of the crystalline hexulose is unknown.

A study of the properties of the 3-ketose and investigations related to those described in this communication are in progress.

EXPERIMENTAL[7]

*6-O-Benzoyl-1,2:4,5-di-O-isopropylidene-*D,L-*galactitol.* 1,2:4,5-Di-*O*-isopropylidene-D,L-galactitol was prepared by a modification of the procedure of Hann, Maclay, and Hudson.[5] The reaction mixture obtained after vigorous agitation for 24 hr. was placed in a refrigerator for at least 2 days. The amount of the crystalline product increased during this time. 1,2:4,5-Di-*O*-isopropylidene-D,L-galactitol, m.p. 142.5–144°, was obtained in a yield of 80%. Traces of hydrogen chloride adhering to the product did not cause hydrolysis provided that the crude product was stored in a dessicator. In general the substance was used without further purification.

In a 500-ml., three neck flask, affixed with condenser and mechanical stirrer, 90 g. (0.34 mole) of 1,2:4,5-di-*O*-isopropylidene-D,L-galactitol was added to 400 ml. of anhydrous pyridine (dried over calcium hydride). When all of the solid had dissolved, the solution was cooled and maintained at 0° while 44 ml. (0.38 mole) of benzoyl chloride was added dropwise from a dropping funnel. After standing at room temperature overnight, the reaction mixture was poured into 1000 ml. of ice and water. The water insoluble sirup was separated from the aqueous mixture by four ether extractions (500-ml. portions). The combined ethereal extraction was washed twice successively with dilute hydrochloric acid, aqueous sodium bicarbonate (sat.) and finally with water. After drying the ether solution overnight over anhy-

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(7) All melting points are corrected. Elemental analyses were performed by Drs. G. Weiler and F. F. Straus, Oxford, England, and by Dr. K. W. Zimmermann, University of Melbourne, Australia.

drous sodium sulfate, the solvent was removed under reduced pressure over a warm water bath to leave a sirup. The residue crystallized upon adding 100 ml. of absolute ethanol and cooling in an ice bath. The yield of crystalline 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-galactitol was 88 g. (70%); m.p. 73–76°. By recrystallizing four times from absolute ethanol, an analytical sample was obtained; m.p. 75–76°.

Anal. Calcd. for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.98; H, 7.09.

*6-O-Benzoyl-1,2:4,5-di-O-isopropylidene-*D,L-*xylo-3-hexulose.* Chromium trioxide (18 g.) was added to 200 ml. of anhydrous pyridine while agitating mechanically. To this mixture, 15 g. of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-galactitol, dissolved in 30 ml. of anhydrous pyridine, was added quickly. After the attachment of a water cooled condenser to which a calcium chloride drying tube was affixed, the reaction mixture was heated on a water bath (bath temp. 60°) for 7 hr. with continuous stirring. The reaction mixture was then poured into 600 ml. of ice and water. The product, together with some unchanged starting material, was extracted from the aqueous mixture by shaking six times with ether (400-ml. portions). The combined ether solution was washed successively with dilute hydrochloric acid, aqueous sodium bicarbonate (sat.), and water. The ether solution was dried over anhydrous sodium sulfate and was concentrated under reduced pressure to a sirup (9 g.). Upon adding 15 ml. of ethanol and cooling, the sirup yielded 5.3 g. (34%) of a crystalline product; m.p. 64–67°. An analytical sample of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulose, m.p. 67.5–68.5°, was obtained after three successive recrystallizations from ethanol.

Anal. Calcd. for $C_{19}H_{24}O_7$: C, 62.62; H, 6.64. Found: C, 63.06; H, 6.73.

The *phenylhydrazone* of the substituted ketose was prepared by dissolving 1.0 g. of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulose and 0.32 g. of phenylhydrazine in 50 ml. of methanol and refluxing for 2 hr. Upon reducing the volume to 10 ml. and cooling, the solution yielded 0.4 g. of the phenylhydrazone; m.p. 102–107°. An analytical sample of the phenylhydrazone, m.p. 110.5–111.5°, was obtained by several recrystallizations from ethanol.

Anal. Calcd. for $C_{25}H_{30}O_6N_2$: C, 66.06; H, 6.65. Found: C, 66.25; H, 6.82.

*Reduction of 6-O-benzoyl-1,2:4,5-di-O-isopropylidene-*D,L-*xylo-3-hexulose.* A suspension of lithium aluminum hydride (1.6 g.) in 350 ml. of anhydrous ethyl ether was mechanically agitated for 1 hr. To this suspension, a solution of 10 g. of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulose in 200 ml. of anhydrous ether was added at a rate sufficient to maintain gentle reflux in the reaction flask. After the addition of the solution was completed, the resulting reaction mixture was mechanically agitated for an additional hour. Sufficient water was then added to destroy the excess lithium aluminum hydride and to precipitate the aluminum salts formed, without forming a distinct aqueous layer. The ether solution was decanted and the residue was extracted five times with 30-ml. portions of ether. The combined ether solution was concentrated under reduced pressure to a sirup, which partially crystallized upon cooling. Three grams of a crystalline solid (m.p. 141–145°) was collected and washed with acetone in a sintered glass funnel. Admixture of this crystalline solid with an authentic sample of 1,2:4,5-di-*O*-isopropylidene-D,L-galactitol did not depress this melting point. An additional 0.7 g. of the galactitol derivative was obtained by cooling the mother liquor.

After removal of the di-*O*-isopropylidene-D,L-galactitol, a second crystalline compound was obtained by allowing the acetone to evaporate slowly giving 0.9 g. of 1,2:4,5-di-*O*-isopropylidene-D,L-gulitol; m.p. 87–92°. By recrystallizing from acetone several times, an analytical sample (m.p. 90.5–92°) was obtained.

Anal. Calcd. for $C_{12}H_{22}O_6$: C, 54.94; H, 8.46. Found: C, 55.06; H, 8.38.

*Hydrolysis of 1,2:4,5-di-O-isopropylidene-*D,L-*gulitol.* A solution of 0.3 g. of 1,2:4,5-di-*O*-isopropylidene-D,L-gulitol and 8 drops of 3*N* hydrochloric acid in 20 ml. of acetone was left overnight at room temperature. When the solvent and most of the hydrochloric acid were removed on a water bath under reduced pressure a sirup was obtained, which crystallized upon cooling. The melting point of the crude D,L-glucitol (0.2 g.) was 126–131°, reported[7] 135–137°.

The *hexaacetate* was formed by dissolving 0.2 g. of crude D,L-glucitol in a solution of acetic anhydride (2 ml.) and pyridine (15 ml.) and allowing the resulting solution to stand at room temperature overnight. Upon pouring into 50 ml. of ice and water, the crystalline hexaacetate precipitated from the aqueous solution. The crystals were collected and washed thoroughly with water, yielding 0.45 g. of D,L-glucitol hexaacetate; m.p. 116–118°, reported[7] 117–118°.

*Debenzoylation of 6-O-benzoyl-1,2:4,5-di-O-isopropylidene-*D,L-*xylo-3-hexulose.* To a solution consisting of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D,L-xylo-3-hexulose (14 g.) and absolute ethanol (150 ml.) sodium methoxide dissolved in methanol (0.1*N*, 20 ml.) was added. After refluxing for 3 hr., the solution was allowed to stand at room temperature for 24 hr. Solvent was then removed from the resulting mixture to yield a light amber colored sirup which crystallized in total upon cooling. The crystals were collected and washed with cold ethanol; yield 8 g. The melting point of the crystalline material, 56–69°, was not altered after several recrystallizations from ethanol to yield an analytical sample with the same melting point range.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.85; H, 7.74.

A sample of the crystalline solid was placed on a chromatographic column packed with acid-washed[8] Magnesol.[9] No separation was observed. The melting point of the crystalline material eluted from the absorbent was not significantly altered. The material recovered from the column was found in a region that was not oxidized by the alkaline potassium permanganate streak reagent,[10] which indicated that this material was resistant to mild alkaline oxidation conditions.

A small amount of the isolated solid from the debenzoylation reaction was dissolved in hot petroleum ether (b.p. 60–110°) and was left in the refrigerator for several days. During this period, two well defined crystalline solids had precipitated from solution; one crystalline solid was opaque and spherical in shape, and the other was long transparent prisms. These two solids were separated mechanically. The opaque spheres obtained melted at 63–71°. After three recrystallizations from petroleum ether (b.p. 60–110°) an analytical sample was obtained; m.p. 77–79°.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.41; H, 7.74.

The transparent prisms melted over several degrees, 69–77°. A pure sample (m.p. 79–81°) was obtained by several recrystallizations from petroleum ether.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.62; H, 7.81.

An admixture of pure samples of the opaque spheres and the prisms, assumed to be the α- and β-anomers of 1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulofuranose, depressed the melting point to 55–71°.

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(7) M. L. Wolfrom, B. W. Lew, R. A. Hales, and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 2343 (1946).

(8) J. M. Sugihara and M. L. Wolfrom, *J. Am. Chem. Soc.*, **71**, 3509 (1949).

(9) A product of Westvaco Chlorine Products Co., So. Charleston, W. Va.

(10) W. H. McNeely, W. W. Binkley, and M. L. Wolfrom, *J. Am. Chem. Soc.*, **67**, 527 (1945).

D,L,-*xylo-3-Hexulose.* Ten milliliters of 0.5*N* aqueous hydrochloric acid was added to a solution containing 10 g. of the anomeric mixture of 1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulofuranose in 100 ml. of methanol. After 3 days at room temperature, the solution was neutralized with silver carbonate. The inorganic solids were removed by filtration and the filtrate was concentrated to a sirup under reduced pressure. When the sirup was dissolved in 30 ml. of ethanol and cooled, the crystalline sugar precipitated from solution. The crude product weighed 2.5 g. and melted at 115–119° (with slight decomposition). An analytical sample of D,L-*xylo*-3-hexulose (m.p. 123–124°, with decomposition) was obtained after two recrystallizations from ethanol.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.72. Found: C, 39.96; H, 6.89.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY,[3] UNIVERSITY OF WISCONSIN]

myo-Inositol-2-C^{14} and -4,5-C^{14}, and a Novel Degradation Reaction of *myo*-Inosose-2 Ethylene Disulfone

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The nitroinositol synthesis of Grosheintz and Fischer was used to prepare two varieties of *myo*-inositol, specifically labeled with C^{14} at position 2 and positions 4 and 5, respectively. The *myo*-inositol-2-C^{14} was extensively purified by chromatography. *myo*-Inosose-2 ethylene disulfone was synthesized. The alkaline dealdolization of this sulfone gives two products, one of which was partially characterized. Periodate degradation of the sulfone yields formic acid and 1,3-dithiolane 1,1,3,3-tetroxide, and is thus a convenient method for isolating C-2 from labeled *myo*-inositol.

When the senior author began to plan, some years ago, to undertake investigations of *myo*-inositol[4] metabolism, it was clear that some phases of this work would require inositol labeled with C^{14} at known positions in the ring. At the time (1955), the only known synthesis which was potentially capable of yielding *myo*-inositol so labeled was the nitroinositol synthesis of Grosheintz and Fischer.[5] We undertook the further development of this synthesis, and were able to prepare first *myo*-inositol-2-C^{14} then subsequently a *myo*-inositol-4,5-C^{14} (see formula VIIIa).

In connection with this work, certain degradation reactions were investigated which might serve for the isolation of individual carbon atoms from *myo*-inositol. Such reactions would be useful in tracer studies of inositol biosynthesis. The periodate oxidation of the ethylene disulfone (XI) of *myo*-inosose-2 (IX) was one of the reactions examined, and it was found to follow a novel course. Some information on the alkaline degradation of this sulfone was also obtained.

After our synthesis of *myo*-inositol-2-C^{14} was completed, Posternak and colleagues independently carried out a similar synthesis and described it fully.[6] We therefore propose to discuss only certain steps which were executed differently in the two laboratories, and in which our procedure appears to offer advantages. In its original form,[5] the synthesis involved the conversion of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (I) to 1,2-*O*-isopropylidene-D-xylofuranopentodialdose (III) and the alkaline condensation of the latter with excess nitromethane to give, after hydrolysis to remove the isopropylidene group, a mixture of 6-nitro-6-deoxy sugars (IV). Under appropriate conditions, the mixture IV cyclized to a nitrodeoxyinositol, which was reduced to an amine designated "aminodesoxyinositol III". Posternak[7] in 1950 identified the amine as *scyllo*-inosamine (VIa), and thus also established the configuration of the nitroinositol as Va. He found that free *scyllo*-inosamine could be deaminated with nitrous acid to a mixture of products including *myo*-inositol in 8–12% yield.

Two problems had to be solved to make the nitroinositol synthesis a feasible one for the preparation of carbon-labeled inositol: 1) When the label was to be introduced as nitromethane, the wastage of this compound at the condensation step had to be reduced; and 2) the yield in the deamination step had to be increased several fold.

In the Posternak synthesis[6] of *myo*-inositol-2-C^{14}, the molar ratio (7:1) of nitromethane and dialdose used in the condensation was that of the

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[4] The cyclitols discussed in this paper are named and numbered according to the system of H. G. Fletcher, Jr., L. Anderson, and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

[5] J. M. Grosheintz and H. O. L. Fischer, *J. Am. Chem. Soc.*, **70**, 1476, 1479 (1948).

[6] T. Posternak, W. H. Schopfer, and R. Huguenin, *Helv. Chim. Acta*, **40**, 1875 (1957).

[7] T. Posternak, *Helv. Chim. Acta*, **33**, 1597 (1950).