	Moles of IO_4^- consumed per mole		$[\alpha]_D$ of dialdehyde produced
	2 hr.	12 hr.	
β-Anomer (I)	0.94	0.97	+16°
α-Anomer (II)	0.99	1.01	−25°

Chromatographic Studies.—In the ionophoretic system[26] requiring borate buffer, pH 6.2, 4 hr., 800 volts, the following migrations were obtained: for the α-anomer, +5.5 cm.; β-anomer, +5.6 cm.; and for 1-β-D-lyxofuranosylthymine, +7.8 cm. The chromatographic system[27] (ethyl acetate–acetic acid–water, 9:2:1, thin layer chromatography on Merck Silica Gel G.) without benzeneboronic acid present gave, for the α-anomer, R_f 0.60, and, for 5-methyluridine, R_f 0.59. The same system containing 0.5% benzeneboronic acid gave these R_f values: for the α-anomer, 0.86, and, for the β-anomer, 0.75.

Enzymatic Studies.—*Bacillus subtilis* 16, a pyrimidine-requiring organism,[28] was used as a source of pyrimidine nucleoside phosphorylase, since preliminary studies had indicated that extracts of this mutant contained substantial amounts of this enzyme. The organism was grown in the Biogen[29] in a chemically defined liquid medium[30] supplemented with 0.5 μmole per ml. of uracil. The cells were harvested at the end of the exponential phase of growth in a Sharples centrifuge and resuspended in 0.05 *M* phosphate–0.02 *M* cysteine hydrochloride buffer (pH 7.08); extracts were prepared by their disruption in a 10 KC Raytheon sonic oscillator. Purification of the enzyme was carried out according to the procedure of Tono and Cohen[31] to give a preparation purified 14-fold. This preparation was used for the experiments listed in Tables I–III.

A calibration curve relating the amount of thymine formed to the amount of 1-β-D-ribofuranosylthymine initially present in the incubation mixture was obtained. The results are shown in tabular form (Table II).

TABLE I

CLEAVAGE OF 1-D-RIBOFURANOSYLTHYMINES BY PURIFIED PYRIMIDINE NUCLEOSIDE PHOSPHORYLASE DERIVED FROM *B. subtilis* 16[a]

Time, min.	% thymine formed from β-Anomer	% thymine formed from α-Anomer
30	36	0
60	51	0
90	53	0
180	56	0

[a] Each incubation mixture contained 80 μmoles of phosphate buffer, pH 6.95, 12 μmoles of mercaptoethanol, 0.2 ml. of purified enzyme preparation, and 6.4 μmoles of the β-anomer or 19.2 μmoles of α-anomer in a total volume of 2.0 ml. Incubation was carried out at 36.2°; aliquots were removed at various time intervals and pipetted into 2.8 ml. of 0.1 *N* NaOH. The thymine present as a result of enzymic action was measured by following the change in optical density at 300 mμ, pH 13.

TABLE II

CLEAVAGE OF 1-β-D-RIBOFURANOSYLTHYMINE BY PURIFIED PYRIMIDINE NUCLEOSIDE PHOSPHORYLASE DERIVED FROM *B. subtilis* 16[a]

[A] 1-β-D-Ribofuranosylthymine, μmole/ml.	[B] Thymine formed, μmole/ml.	% cleavage, [B]/[A] × 100
1.0	0.42	42
2.0	0.80	40
3.0	1.14	38
4.0	1.47	37

[a] Each incubation mixture contained 80 μmoles of phosphate buffer, pH 6.95, 12 μmoles of mercaptoethanol, 0.2 ml. of purified enzyme preparation, and the amount of β-anomer as indicated in a total volume of 2.0 ml. Incubation was carried out at 36.2°; at the end of 180 min., the reaction was stopped and the amount of thymine present was determined by the change in optical density at 300 mμ, pH 13.

It is evident that a constant percentage of thymine is formed from the β-anomer irrespective of its initial concentration. Furthermore, the addition of various levels of the α-anomer to the reaction mixture did not affect the per cent cleavage of the β-anomer. These results are shown in Table III.

TABLE III

EFFECT OF 1-α-D-RIBOFURANOSYLTHYMINE UPON CLEAVAGE OF 1-β-D-RIBOFURANOSYLTHYMINE BY PURIFIED PYRIMIDINE NUCLEOSIDE PHOSPHORYLASE[a]

Anomeric mixture [A] β-anomer, μmole/ml	Anomeric mixture [B] α-anomer, μmole/ml.	Thymine formed, μmole/ml.	% thymine formed, [B]/[A] × 100
4.0	0	1.43	36
3.6	0.4	1.34	37
3.0	1.0	1.12	37

[a] Incubation conditions similar to those presented in Table II.

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(27) E. J. Bourne, E. M. Lees, and H. Weigel, *J. Chromatog.*, **11**, 253 (1963).

(28) Obtained from Dr. R. Guthrie (Children's Hospital, Buffalo, N. Y.) who isolated and characterized this mutant with respect to its pyrimidine requirements.

(29) An instrument for the continuous cultivation of microorganisms (American Sterilizer Co.).

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Synthesis of Higher Ketoses by Aldol Reactions. II. Unsubstituted Heptuloses

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Unsubstituted D-erythrose undergoes a mixed aldol reaction with 1,3-dihydroxy-2-propanone to give D-*allo*-, D-*altro*-, and D-*gluco*-heptuloses in an over-all yield of 37%.

Work in this laboratory has shown that substituted higher ketoses[1] as well as branched-chain higher aldoses[2] are obtained by aldol reactions of carbohydrates that (a) are unable to form intramolecular hemiacetal linkages because of blocked γ- or δ-hydroxyl groups, and (b) are precluded from isomerizing to ketoses by substitution of the α-hydroxyl group. Recently, however, the aldol self-addition reaction of an *unsubstituted ring-forming* sugar (D-erythrose to D-*gluco*-L-*glycero*-3-octulose) was discovered,[3] and this indicated that a mixed aldol reaction of unsubstituted tetroses

(1) R. Schaffer and H. S. Isbell, *J. Org. Chem.*, **27**, 3268 (1962).

(2) R. Schaffer, *J. Am. Chem. Soc.*, **81**, 542 (1959).

(3) R. Schaffer and A. Cohen, *J. Org. Chem.*, **28**, 1929 (1963).

with, for example, 1,3-dihydroxy-2-propanone should take place also, to give higher heptuloses. There was a suggestion of this in the literature, for in 1951 Hough and Jones[4] wrote in a review article concerning the biosynthesis of monosaccharides: "Mr. K. B. Taylor, working with us, has observed that D-erythrose and dihydroxyacetone on condensation in alkaline solution give a product which on the paper chromatogram behaves as a heptulose and shows the same colour reaction. We are now attempting the isolation of this sugar with the view of its conclusive identification." Since then, however, no details have appeared, and the authors have ceased further investigation of this subject.[5] The value of a synthesis that would make unsubstituted heptuloses readily accessible impelled a more thorough investigation of the reaction of D-erythrose (I) and 1,3-dihydroxy-2-propanone (II) at this time.

Polarimetric observation of a limewater solution of I and II showed that reaction occurred readily, and paper chromatography employing solvent mixture A (see Experimental) and an orcinol spray[6] revealed that two blue spots, characteristic of heptuloses, were forming. These spots were preceded by one intensely gray spot and followed by one weakly gray-brown spot. The material responsible for the faster moving blue spot was found by chromatography to be composed of both D-*allo*-heptulose (III)[7] and D-*altro*-heptulose (IV).[8] To separate them, the mixture of the two heptuloses, eluted from a thick-paper chromatogram developed with solvent A, was rechromatographed with solvent mixture B. Compound III was then crystallized as a hydrate in 3.3% yield, and compound IV was separated, converted to its anhydride, and crystallized in 12% yield as 2,7-anhydro-β-D-*altro*-heptulopyranose hydrate (V).[8,9] From the material responsible for the slower moving blue spot, a 15.5% yield of D-*gluco*-heptulose (VI)[10] was isolated. The substance responsible for the intense gray color with the spray was the expected 4-(hydroxymethyl)-DL-*glycero*-pentulose ("dendroketose"),[1,11] the aldol self-addition product of II; the faintly colored, slowest moving ketose was D-*gluco*-L-*glycero*-3-octulose.[3] Column chromatography on cellulose of another sample of reaction mixture, using only solvent B as developer, led to the isolation of crystalline III in 3.3% yield, crystalline V in 11.7% yield, and crystalline VI in 21.7% yield. A small amount of the crystalline 3-octulose was also isolated. Thus, the preliminary observation of Hough, Jones, and Taylor that heptulose is obtained in the reaction of I and II has been verified, and the conclusive identification of three heptulose products has been made.

The proportions of mixed aldol products isolated are interesting. As in the ethylideneheptulose synthesis,[1] the heptuloses having a *threo* configuration at C-3 and C-4 (the positions where the new carbon-to-carbon bond was made) are the main products, and only one of the two potential products with an *erythro* configuration at those carbon atoms was isolated; however, in the new work it is D-*allo*-heptulose, whereas, in the older it is 4,6-*O*-ethylidene-D-*manno*-heptulose that was isolated. That D-*manno*-heptulose is absent from, or present to only a small extent in, the present study was evident from the lack of the greenish coloration that this heptulose characteristically develops with the orcinol spray; also, none could be induced to crystallize by seeding the mother liquors of the D-*gluco*-heptulose crystallizations, where the *manno* compound would have been expected from its chromatographic behavior. The predominance in both heptulose syntheses of products having the *threo* configuration shows that there is only a secondary influence exerted on the course of the reaction by the conformationally restricting acetal substituent. It is, however, significant that the absence of this substituent results in the production of D-*allo*-heptulose, an otherwise difficultly accessible ketose. Although its yield is low, the simplicity of synthesis by this method makes it now relatively easily available.

This clear evidence of aldol reactivity of unsubstituted tetroses raises the possibility that some higher ring-forming sugars may also be found that will participate in aldol reactions; although, at present, out of the many investigations of the reactions of sugars with alkali, the only recorded information that might be interpreted as evidence of this reactivity is the isolation of an incompletely characterized unbranched dodecitol, a by-product in the manufacture of hexitols by alkaline electroreduction of D-glucose.[12]

Experimental

Thick-paper chromatography was performed with Whatman seed-test paper by the descending method. Paper column chromatography was carried out using a Chro-Max Pressure Mantle, Type 3504.[13] The solvent systems employed for chromatography were: solvent A, 1-butanol–pyridine–water (6:4:3); and solvent B, 1-butanol–ethanol–water (4:1:1.2). Melting points were determined with a Hershberg apparatus. Infrared spectra were recorded with an Infracord spectrophotometer[14] with specimens in a Nujol mull.

The Aldol Reaction.—D-Erythrose (I) was prepared from 1.57 g. of dimeric 2,4-*O*-ethylidene-D-erythrose hemialcoholate as described previously.[15] The deionized sirupy product thus obtained and 1.44 g. of 1,3-dihydroxy-2-propanone (II) were dissolved in 50 ml. of limewater (prepared at 8°), and the solution was quickly warmed to 20°. After about 22 min. of reaction at this tempera-

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(5) Private communications from Dr. L. Hough and Professor J. K. N. Jones.

(6) A. Bevenue and K. T. Williams, *Arch. Biochem. Biophys.*, **34**, 225 (1951).

(7) J. W. Pratt and N. K. Richtmyer, *J. Am. Chem. Soc.*, **77**, 6326 (1955). The author is indebted to Dr. N. K. Richtmyer for supplying a solvated and a dried sample of this compound.

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(9) J. W. Pratt, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, **74**, 2200 (1952).

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(12) M. L. Wolfrom, W. W. Binkley, C. C. Spencer, and B. W. Lew, *J. Am. Chem. Soc.*, **73**, 3357 (1951).

(13) Product of the LKB Instruments, Inc., Bethesda, Md.

(14) Product of the Perkin-Elmer Corporation, Norwalk, Conn.

(15) R. Schaffer, *J. Am. Chem. Soc.*, **81**, 2838 (1959).

ture, the mixture was rapidly cooled to 0° and passed through a column containing 10 ml. of ice-cold Amberlite IR-120 (H^+). The effluent was concentrated at reduced pressure, transferred as a band at the origin to each of two sheets of thick chromatography paper, and developed with solvent A for 2.5 days. The ketose bands were made visible by pressing each wet chromatogram against a sheet of Whatman No. 1 paper, and then spraying the latter sheet. On elution of the slower heptulose bands from the two chromatograms, 0.325 g. of crystalline D-*gluco*-heptulose (VI)[10] was isolated. The combined material from the faster heptulose bands was rechromatographed on a single new sheet of thick paper using solvent B. The overlapping heptulose bands that resulted were located by the transfer technique, and the material corresponding to the more intense, slower band was cut away from that corresponding to the less colored, more mobile material. Crystalline D-*allo*-heptulose (III)[7] as a hydrate weighing 0.077 g. was separated from the faster moving material. From the slower portion, a sirupy product, D-*altro*-heptulose (IV), was separated. On heating a solution of the sirup in 0.2 *N* hydrochloric acid at 50° for 68 hr.,[16] there was obtained 0.251 g. of crystalline 2,7-anhydro-β-D-*altro*-heptulopyranose hydrate (V).[8,9]

Column chromatography of a similar amount of the reaction products, using solvent B as developer, led to the separation of 0.5 g. of sirup containing "dendroketose"[1,11] from the effluent fractions in the volume from 1500 to 2475 ml.; 0.076 g. of III from effluent volumes 2550 to 2820 ml.; 0.227 g. of V from volumes 2820 to 3720 ml. (compound IV was eluted but was isolated as V after dehydration); and 0.455 g. of VI from volumes 3165 to 3720 ml. and 3720 to 3975 ml. About 0.005 g. of D-*gluco*-L-*glycero*-3-octulose[3] was crystallized from the 4500- to 5400-ml. fractions. The "dendroketose" and the 3-octulose were identified by the similarities to authentic materials of their chromatographic behavior and coloration with the orcinol spray. In addition, for the 3-octulose, identity was confirmed by its melting point (164–165°), undepressed mixture melting point, and its infrared spectrum. The infrared spectrum of compound III obtained from ethanol containing a little water agrees with that of an authentic specimen of the ketose,[7] and it shows an absorption at 1650 $cm.^{-1}$ which indicates that the solvation is due to water. Analysis showed irregular amounts of water that were in a nearly equimolecular ratio with the ketose. The desolvated product (heated under vacuum) melted at 128–131°, and this melting point was unchanged when a sample was mixed with some of the dried authentic product (lit.[7] m.p. 130–132°). The hydrated product showed $[\alpha]^{25}D$ −46° in water and the reported value[7] is $[\alpha]^{20}D$ −46.6°. Compound V showed $[\alpha]^{25}D$ −133.5°, m.p. 100–102°, and, when mixed with authentic material, the melting point was 100–102°. Its infrared spectrum corresponded with that of the known substance.[17] Compound VI showed $[\alpha]^{26}D$ +67°, m.p. 170–172°, and, when mixed with authentic material, m.p. 170–173°. Its infrared spectrum was identical with that of the known sugar.[17]

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The Isomerization of D-*manno*-3-Heptulose by Alkali

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The title compound isomerizes in alkaline solution by the enolization mechanism through two pathways: (a) *via* a 2,3-enediol to yield D-*gluco*-heptulose, and (b) *via* a 3,4-enediol, an isomeric 3-ketose, and another 2,3-enediol to yield D-*altro*- and D-*allo*-heptuloses.

Throughout the long history of the Lobry de Bruyn–Alberda van Ekenstein reaction (isomerization of sugars by alkali),[1] no direct evidence has been educed for the presence of 3-keto sugars among its products, although such compounds are theoretically capable of being formed from the 2,3-enediol intermediates which are believed to be responsible for the occurrence of ketoses epimeric at C-3, *e.g.*, D-tagatose (D-*lyxo*-hexulose) *and* D-sorbose (D-*xylo*-hexulose) from the isomerization of D-galactose.[2] Perhaps the clearest implication of the participation of a 3-ketose in such a system was found by Sowden and Thompson[3] who, using D-glucose-1-C^{14}, obtained data showing that isomerization by an enolization mechanism must have proceeded through all of the secondary carbon atoms of the chain to yield L-sorbose labeled predominantly at C-6. A direct investigation of the chemical behavior of a 3-ketose in such a system was undertaken when a suitable pure study material, namely D-*manno*-3-heptulose (I), became available; this sugar had been obtained as a sirup by the selective degradation of the reducing group of a related 3-*C*-formylheptitol,[4] but β-D-*manno*-3-heptulose hydrate[5] has now been crystallized.

Discussion

In the course of several days at room temperature, a solution of compound I in dilute limewater was found to undergo a change in optical rotation from levo- to dextrorotatory. Paper chromatography revealed that, during this time, the 3-ketose was being transformed to isomeric 2-ketoses: the gray-brown spot that forms on spraying the 3-heptulose with orcinol–trichloroacetic acid[6] gradually diminished, and, simultaneously, there appeared two faster moving blue spots (2-heptuloses)[7] which gradually increased in intensity. The slower blue spot was more intense than the faster one. The location of the slower spot on the chromatogram suggested that it might be due to D-*gluco*-heptulose[8] (II), or to D-*manno*-heptulose[9] (III), or both; however, its color showed none of the greenish blue that III gives with the spray. The location of the more mobile spot suggested that it

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