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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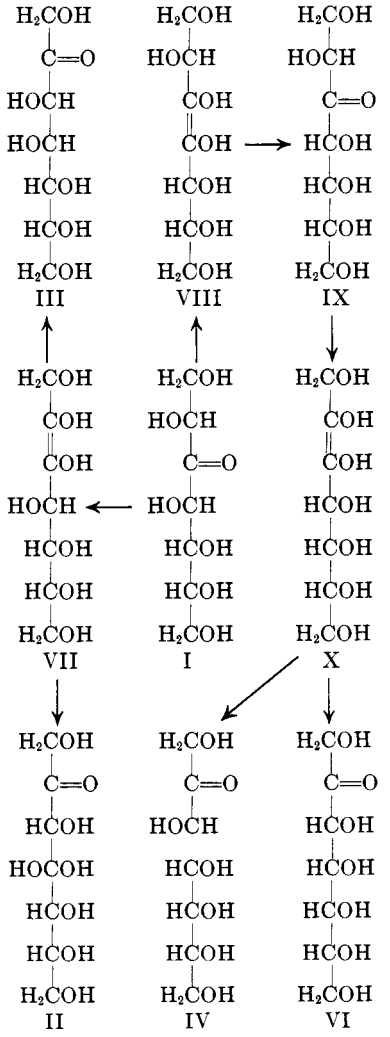
might correspond to D-*altro*-heptulose[10] (IV), or to D-*allo*-heptulose[11] (VI), or both. By the combined use of fractional crystallization and paper chromatography, compound II was isolated in a total yield of 40%, IV was obtained [as its crystalline 2,7-anhydride (V)] in 4% yield, and VI was crystallized as a hydrate[12] in 3% yield. Only a trace of III was detected, and it could not be crystallized from the mixture; no D-*manno*-3-heptulose was detected or recovered. Thus, the conditions of the Lobry de Bruyn–Alberda van Ekenstein reaction were found to affect the 3-heptulose profoundly. Assuming this behavior to be typical of such ketoses, it is understandable that the direct detection of 3-ketoses produced in alkaline isomerization reactions has not been realized.

The classical enolization pathway is accepted as the mechanism of alkaline interconversion of aldoses and 2-ketoses, and its occurrence is supported by the results of isotopic labeling studies.[13] The same mechanism can



account for the formation of the isomeric heptuloses that were isolated from the 3-ketose; this action, however, requires two pathways. The first of these, the route to compound II, involves enolization of I to D-*arabino*-heptitol-2-ene (VII), which is then isomerized to II. Compound III would be expected to form from this enediol intermediate, too. Because of the necessity of epimerizing the hydroxyl group at C-4 of compound I to form compounds IV and VI, the second pathway is more complex. Compound I is enolized to D-*arabino*-heptitol-3-ene (VIII), which, in turn, is isomerized to D-*altro*-3-heptulose (IX). This as-yet-unknown 3-heptulose is then transformed into the intermediate D-*ribo*-heptitol-2-ene (X), which finally is isomerized to IV and VI.

Interestingly, the composition and proportions of the ketoses isolated from the isomerization resemble those obtained by the aldol reaction of D-erythrose and 1,3-dihydroxy-2-propanone,[12] where the same heptuloses II, IV, and VI are formed, and where II constitutes the major ketose product, while its epimer III is present in a nonisolable amount. (The product compositions might have been even more alike had the aldol reaction mixture been exposed to the alkaline medium for a week, as in the isomerization, rather than for 22 minutes, the time required for the anionic addition.) The amounts of each heptulose isolated from the aldol synthesis and the isomerization reaction show that the major products in both instances have *threo* configurations at C-3 and C-4. These asymmetric centers are the newly created ones in the aldol reactions, where preference for products with *threo* configurations at the new asymmetric centers is experimentally well-established,[12] but more information regarding the isomerization of ketoses and aldoses in alkaline solution is required, in order to know whether there exists in these reactions, too, the general preference for products with this configuration.

Experimental

Crystalline β-D-*manno*-3-Heptulose Hydrate.—Hydrolysis of 2,2′:4,6-di-*O*-ethylidene-2-(D-*glycero*-1,2-dihydroxyethyl)-D-glucopyranose by acid, degradation of the liberated branched-chain octose by lead tetraacetate, rehydrolysis, and removal of aldose contaminants have been described.[5] Duolite A-4 (amine form) was used to remove the sulfuric acid employed in the first hydrolysis step.[14] Thick paper chromatography of 0.9 g. of purified, sirupy, degradation product was used with 1-butanol–ethanol–water (4:1:1.2) as developer to separate the 3-heptulose band, which was visualized by pressing the wet, developed chromatogram between sheets of Whatman No. 1 paper and spraying the latter with orcinol, or silver nitrate and alkali. The material eluted from the 3-heptulose band weighed about 0.37 g., and, from a concentrated solution of it in 95% ethanol, 0.276 g. of crystalline β-D-*manno*-3-heptulose hydrate was obtained, m.p. 84–85.5°; $[\alpha]^{27}D$ −70°, 2.6 min. → −39°, final 1 hr. (*c* 1, water). Without chromatography, from 4.3 g. of sirupy degradation product in 95% ethanol, 0.62 g. of 3-heptulose crystallized, after seeding.

Anal. Calcd. for $C_7H_{14}O_7 \cdot H_2O$: C, 36.8; H, 7.1. Found: C, 36.6; H, 7.1.

D-*manno*-3-Heptulose Phenylhydrazone.—A solution of 0.035 g. of I in 2 drops of water and 10 drops of methanol was combined with 0.030 g. of crystalline phenylhydrazine–acetic acid (prepared by addition of pentane to a concentrated solution of phenylhydrazine and acetic acid in ether) and heated for 4 hr. at 50°. The solvent was then evaporated, and the product was crystallized from absolute ethanol, m.p. 147–148° dec., $[\alpha]^{25}D$ +80 ± 5° (*c* 0.5, methanol).

Anal. Calcd. for $C_{13}H_{20}N_2O_6$: C, 52.0; H, 6.7; N, 9.3. Found: C, 52.3; H, 6.9; N, 9.2.

Crystalline phenylosazones could not be isolated.

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(13) J. C. Sowden and R. Schaffer, *J. Am. Chem. Soc.*, **74**, 499, 505 (1952).

(14) The original publication had specified cation-exchange resin.

Isomerization of D-*manno*-3-Heptulose.—A solution of 0.114 g. of β-D-*manno*-3-heptulose monohydrate in 7.5 ml. of water was mixed with 2.5 ml. of limewater (prepared at 8°). After storage in the dark for about 1 week at room temperature, the slightly yellow solution was passed through a column containing 3 ml. of Amberlite IR-120 H^+ and 5 ml. of Duolite A-4, and then concentrated. The sirup was taken up in a little methanol and 95% ethanol, and seeded with compound II, whereupon nearly pure II crystallized, 0 0375 g., $[\alpha]^{26}D$ +67°, m.p. 169–173°. Its infrared spectrum corresponded to that of authentic material.[15] The mother liquor was chromatographed on Whatman No. 3 MM paper, using the solvents and techniques previously described for separating these isomeric heptuloses.[12] Three sirupy fractions, consisting principally of II, IV, and VI, respectively, were obtained. On seeding the fractions, 0.003 g. of additional II was obtained, and 0.003 g. of VI was isolated as a hydrate. The latter was identified by its chromatographic behavior and by comparison of its infrared spectrum with that of an authentic specimen.[11] The fraction containing IV was heated for 70 hr. at 50° in 0.2 *N* hydrochloric acid,[16] and, after deionization and concentration, was obtained as crystalline V, weight 0.004 g. Compound V was identified by comparison of its infrared spectrum[15] and chromatographic behavior with those of authentic material. The orcinol-sprayed chromatogram of the reaction mixture at the conclusion of the reaction showed neither the color nor the fluorescence of the starting material.

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Sorboses. IV. Syntheses of Thio Derivatives of 2,3-*O*-Isopropylidene-α-L-sorbofuranose[1]

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Derivatives of 1-thio- and 6-thio-2,3-*O*-isopropylidene-α-L-sorbofuranoses were prepared *via* the corresponding benzylthio-L-sorbofuranoses, which were readily obtainable by the reactions of *O*-*p*-toluenesulfonyl-L-sorbofuranoses with sodium benzylthiolate in liquid ammonia, not in alcohol.

Although thio sugars have received renewed attention in recent years not only from the standpoint of the chemistry involved but from biochemical and metabolic interest, only one paper has been published on the preparation of thioketoses,[2] because tosyloxy groups in ketoses undergo nucleophilic displacement except when they are hindered.[2-4] The present paper reports a new method of synthesis of thio derivatives of 2,3-*O*-isopropylidene-α-L-sorbofuranose.

The treatment of 1,6-di-*O*-*p*-toluenesulfonyl-2,3-*O*-isopropylidene-α-L-sorbofuranose (II)[5] with sodium benzylthiolate (I) in absolute methanol[6] gave colorless crystals (IV′), which had only one benzylthio group per sugar. This was also obtained from 6-*O*-*p*-toluenesulfonyl-1,4-anhydro-2,3-*O*-isopropylidene-α-L-sorbofuranose (III).[7] The acid-catalyzed acetonization of IV′ gave no acetonized compounds. Therefore, the structure of IV′ was considered to be 6-*S*-benzyl-2,3-*O*-isopropylidene-6-thio-α-L-sorbofuranose (IV). In order to prove the structure, the reaction of 6-*O*-*p*-toluenesulfonyl-2,3-*O*-isopropylidene-α-L-sorbofuranose (V) with I was carried out under the same conditions employed above. However, V was not converted into IV but into 2,3-*O*-isopropylidene-α-L-sorbofuranose (VI),[8] which was considered as the reaction product of V with a trace of water in the solvent. Dehydrating all the reagents or substituting I by other thio compounds such as thiol acetate brought no improvement. The treatment of V with I in liquid ammonia solution, which was prepared from metallic sodium and α-toluenethiol in liquid ammonia, at room temperature for 2 weeks gave IV. This was identical with IV′ by comparison of its rotation, infrared spectrum, and mixture melting point. Amino sugar was not isolated in this reaction. Furthermore, the treatment of II with I in liquid ammonia gave 1,6-di-*S*-benzyl-2,3-*O*-isopropylidene-1,6-dithio-α-L-sorbofuranose (VII) in good yield. (See Chart I.)

The tosylation of IV in pyridine gave 1-*O*-*p*-toluenesulfonyl-6-*S*-benzyl-2,3-*O*-isopropylidene-6-thio-α-L-sorbofuranose (VIII), which was converted into 1-anilino-6-*S*-benzyl-1-deoxy-2,3-*O*-isopropylidene-6-thio-α-L-sorbofuranose (IX) on heating with aniline. IX was also obtainable on treating I in liquid ammonia with 1-anilino-6-*O*-*p*-toluenesulfonyl-1-deoxy-2,3-*O*-isopropylidene-α-L-sorbofuranose (X), which was derived from the tosylation of 1-anilino-1-deoxy-2,3-*O*-isopropylidene-α-L-sorbofuranose (XI).

Derivatives of 1-*S*-benzyl-2,3-*O*-isopropylidene-1-thio-α-L-sorbofuranose (XV) were obtained in a similar manner. The treatment of 1-*O*-*p*-toluenesulfonyl-2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose (XII)[9] or its partially hydrolyzed product, 1-*O*-*p*-toluenesulfonyl-2,3-*O*-isopropylidene-α-L-sorbofuranose (XIII), with I yielded, in liquid ammonia, 1-*S*-benzyl-2,3:4,6-di-*O*-isopropylidene-1-thio-α-L-sorbofuranose (XIV) or XV, but in methanol solvent, 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose (XVI)[8] or VI, respectively. (See Chart II.)

The tosylation of XV, which was also prepared by partial hydrolysis of XIV, afforded 1-*S*-benzyl-6-*O*-*p*-toluenesulfonyl-2,3-*O*-isopropylidene-1-thio-α-L-sorbofuranose (XVII). This was converted into 1-*S*-benzyl-

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