The second fraction, 2.05 g., m.p. 188–192°, eluted with 10% ethanol in ether, is recovered 15. The original pyridine-insol. residue was washed and dried: 5.49 g.; this plus the ether-insol. portion plus fraction two represent a recovery of 11.37 g. of 15.

11-Cholen-3α-ol (18) was prepared by $LiAlH_4$ reduction of 17, and crystallized from methanol as needles, m.p. 135.3–136.3°, $[\alpha]D$ +31.3° (1.77%); λ_{max}^{KBr} 3.05, 9.68, 13.81 μ.

Anal. Calcd. for $C_{24}H_{40}O$: C, 83.65; H, 11.70. Found: C, 83.71; H, 12.01.

The **acetate 19** crystallized from methanol as needles, m.p. 86.5–88.0°, $[\alpha]D$ +45.9°; λ_{max}^{KBr} 5.77, 8.01, 9.75, 13.83 μ.

Anal. Calcd. for $C_{28}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.99; H, 11.02.

The **tosylate 20** crystallized from acetone as plates, m.p. 141.5–143.5°, $[\alpha]D$ +31.7°; λ_{max}^{KBr} 8.53, 10.71, 10.83, 14.97 μ. Although repeated recrystallizations did not raise the m.p., analyses were erratic.

Anal. Calcd. for $C_{31}H_{46}O_3S$: C, 74.65; H, 9.30; S, 6.43. Found: C, 75.16, 74.80, 74.10; H, 9.10, 8.71, 9.08; S, 6.31, 6.72, 6.30.

Hydrogenation of 11-cholen-3α-ol in ethanol over Adams catalyst gave 3α-cholanol, m.p. 135.5–138.0°, mixture with authentic 3α-cholanol[5] m.p. 139.5–143.5°, mixture with 24-cholanol m.p. 110–130°, infrared spectrum (KBr) identical with that of known 3α-cholanol.

11-Cholene (21).—In a separate preparation of 11-cholen-3α-ol from crude tosylate 17, the total reduction product was chromatographed on alumina. The first fraction, eluted with ligroin, crystallized from methanol–benzene (4:1) as plates, m.p. 77.9–80.5°, $[\alpha]D$ +36.6°, λ_{max}^{KBr} 13.84 μ.

Anal. Calcd. for $C_{24}H_{40}$: C, 87.73; H, 12.27. Found: C, 87.79; H, 12.27.

3,11-Choladiene (22) was prepared by dehydrotosylation of 11-cholen-3α-yl tosylate (20) in refluxing 2,6-lutidine. The crude product crystallized when seeded with 3-cholene;[5] when recrystallized from methanol–benzene (2:1) it was obtained in the form of platelets, m.p. 53.0–56.0°, $[\alpha]D$ +37.2° (1.5%); λ_{max}^{KBr} 13.56, 13.82 μ.

Anal. Calcd. for $C_{24}H_{38}$: C, 88.27; H, 11.73. Found: C, 88.01; H, 11.81.

3β-Chloro-11-cholene (23) was prepared from the tosylate 20 and pyridinium chloride in pyridine at 78°. The crude product crystallized from methanol, m.p. 110–115°; this fraction was chromatographed on alumina and the portion eluted by ligroin crystallized from acetone in the form of platelets, m.p. 112.5–116.5°, $[\alpha]D$ +16.8° (1.66%); λ_{max}^{KBr} 7.82, 13.85, 14.06 μ.

Anal. Calcd. for $C_{24}H_{39}Cl$: C, 79.38; H, 10.82; Cl, 9.96. Found: C, 79.12; H, 11.04; Cl, 9.69.

24-Chloro-11-cholen-3α-ol (24), prepared similarly to 13 from the tosylate 17, crystallized from methanol–water (10:1), m.p. 103.5–104.8°, $[\alpha]D$ +32.3°; λ_{max}^{KBr} 3.01, 9.66, 13.79, 15.4 μ.

Anal. Calcd. for $C_{24}H_{39}OCl$: C, 76.05; H, 10.37; Cl, 9.36. Found: C, 75.98; H, 10.34; Cl, 9.53.

The **acetate 25** crystallized from acetone as transparent plates, m.p. 162.0–163.5°, $[\alpha]D$ +47.5°; λ_{max}^{KBr} 5.78, 5.80, 7.93, 8.02, 9.74, 13.83, 14.00 μ.

Anal. Calcd. for $C_{28}H_{41}O_2Cl$: C, 74.18; H, 9.82; Cl, 8.42. Found: C, 74.41; H, 9.81; Cl, 8.11.

24-Bromo-3-cholene (26).—To a solution of 3-cholene-24-yl tosylate[5] (1 g.) in 10 ml. of N,N-dimethylformamide was added 1.2 g. of pyridinium bromide (Metro Industries). The mixture was shaken and left at room temperature for 88 hours. The long needles which had formed and additional solid resulting from precipitation on addition of ice and water, after thorough washing with water, weighed 809 mg. (97%), m.p. 86.5–90°. Crystallization from acetone–water, then from acetone–methanol gave long rods, m.p. 88.5–91.0°, $[\alpha]D$ +17.5°; λ_{max}^{KBr} 14.70, 15.07, 15.58 μ.

Anal. Calcd. for $C_{24}H_{39}Br$: C, 70.72; H, 9.65; Br, 19.61. Found: C, 70.49; H, 9.85; Br, 19.30.

Further recrystallizations, although yielding products of sharper melting point, gave poorer analyses.

MEMPHIS, TENN.

[CONTRIBUTION FROM NATIONAL BUREAU OF STANDARDS, CHEMISTRY DIVISION]

Branched-chain Higher Sugars. II. A Diethylidene-octose[1]

BY ROBERT SCHAFFER

RECEIVED NOVEMBER 19, 1958

2,4-*O*-Ethylidene-D-erythrose (I) in calcium hydroxide solution undergoes aldol condensation to a branched-chain octose derivative, which is proved to be 1,3:5,7-di-*O*-ethylidene-3-*C*-formyl-D-*glycero*-D-*talo*-heptitol-3(1),6-pyranose. The 3-*C*-formyl-heptitol obtained on acid hydrolysis is converted by acid to a novel 3(1),1-anhydro-3(1),6-pyranose and converted by lead tetraacetate oxidation to D-*manno*-3-heptulose. Compound I is shown to crystallize as a dimer with the structure bis-(2,4-*O*-ethylidene-D-erythrose)-1,1′:1′,3-cyclic acetal.

Schaffer and Isbell[2] have shown that 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose[3] in calcium hydroxide solution undergoes aldol condensation to the branched-chain decose, 9-aldo-4-*C*-formyl-1,2:8,9-di-*O*-isopropylidene-L-*xylo*-L-*ido*-nono-1,4:9,6-difurano-4(1),7-α-pyranose. As aldol formation is not a characteristic reaction of tetroses or larger sugars, its occurrence with the pentose derivative was attributed to an inhibiting effect (of the substitution) on the enolizing and ring-forming properties of the pentose, with consequent enhancement of conditions for the condensation. In the present paper, a tetrose derivative, substituted (a) at carbon atom 2 to limit enolization to carbon atoms 1 and 2 only, and (b) at carbon atom 4 to preclude intramolecular hemiacetal formation, is shown to undergo aldol condensation in fulfillment of the prediction that suitably substituted sugars generally would undergo the reaction.[2] A proof of structure and configuration of the newly formed aldol product is also presented.

Discussion

The precursor to the aldol condensation used in this study, 2,4-*O*-ethylidene-D-erythrose (I), has been prepared in several laboratories,[4–6] but here-

(1) This work was conducted as part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Division of Research, Atomic Energy Commission, Dr. H. S. Isbell, project leader.

(2) (a) R. Schaffer and H. S. Isbell, THIS JOURNAL, **80**, 756 (1958); (b) R. Schaffer and H. S. Isbell, *ibid.*, **81**, 2178 (1959).

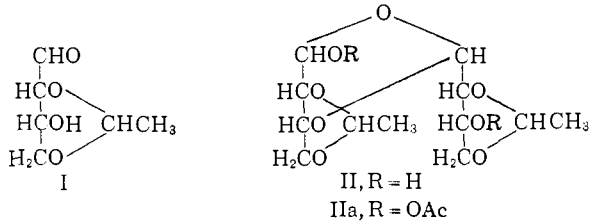
(3) K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941).

(4) E. J. Bourne, G. T. Bruce and L. F. Wiggins, *J. Chem. Soc.*, 2708 (1951).

(5) H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **51**, 307 (1953).

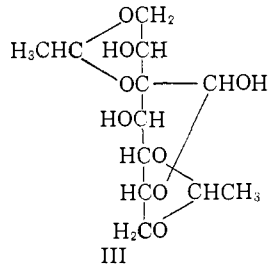
(6) A. C. Neish, *Can. J. Chem.*, **32**, 334 (1954).

tofore a crystalline modification has not been described in the literature.[7] A pure crystalline product was desired not only for the present study, but for the synthesis of carbon-14 labeled

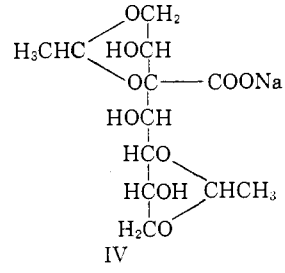


I

II, R = H
IIa, R = OAc



III



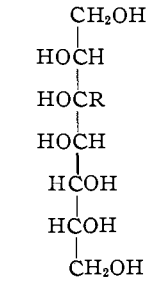
IV

sugars.[5,6,8,9] Crystallization of I is reported here. It was first isolated from solution in ethyl acetate, but a better product is obtained by recrystallization from ethanol and pentane.

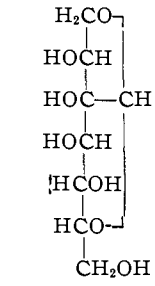
The infrared spectrum of the crystalline substance II shows no absorption in the carbonyl region, indicating that the aldehyde group shown free in I is present in a combined form in the crystalline state. A molecular weight determination, by freezing point depression, showed that the product is a dimer. Reaction with acetic anhydride in pyridine proceeds with two distinct rates; hence, the reactions indicate the presence of two kinds of hydroxyl group. The crystalline product IIa obtained from the acetylation reaction corresponds by analysis to a monoacetyl-monoethylidene-tetrose, but its molecular weight is also that of a dimer. It seems probable, therefore, that I crystallizes with a structure similar to that of crystalline, dimeric 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose,[10] which is a dimeric cyclic acetal-hemiacetal.[11] For the tetrose dimer, structure II is proposed, with the name bis-(2,4-*O*-ethylidene-D-erythrose)-1,1′:1′,3-cyclic acetal. Acid hydrolysis of II gives D-erythrose with a specific rotation of $-41°$. Values only as large as $-32.7°$ have been recorded previously.[12,13]

In the aldol condensation reaction, a solution of II in aqueous calcium hydroxide exhibits a change in optical rotation that is fairly rapid at first and then becomes slow and prolonged. A crystalline product (III) was isolated by working up the reaction mixture after completion of the faster phase of change in optical rotation.

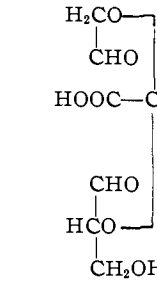
Compound III was found to be oxidized quantitatively by hypoiodite, as required for a diethylidene-aldo-octose. The crystalline sodium salt IV, corresponding in composition to a sodium diethylidene-octonate, was isolated. Thus, it was evident that an aldol condensation had taken place, giving III. In addition, IV, on acidification and hydrolysis, yielded a product conforming in analysis to a lactone of an unsubstituted octonic acid V.



V, R = COOH
VI, R = CHO



VII



VIII

Observation of the specific rotation of III, while heating it with dilute sulfuric acid, gave evidence for hydrolysis of the two ethylidene groups. Maintenance of the hydrolytic conditions beyond completion of hydrolysis to the unsubstituted 3-*C*-formyl-heptitol (VI) led to the formation of a non-reducing substance (VII). Analysis showed VII to correspond to a molecule of an eight-carbon sugar less one molecule of water. As sugars are commonly dehydrated by hot, dilute mineral acid to non-reducing, anhydro sugars,[14] the new substance (VII) was assumed to be an anhydro-octose. Information on the structure of VII was obtained by periodate oxidation. After 24 hr., the anhydro-octose reacted with only 3.1 moles of sodium metaperiodate per mole, yielding 2.09 moles of acid and no formaldehyde. Of the possible anhydro compounds that can be written, only VII conforms with these observations (yielding, presumably, equimolar amounts of formic acid and VIII).

Degradation of the eight-carbon sugar was necessary in order to relate its structure and configuration to known substances. Splitting off one carbon atom (that of the aldehyde group) would give a normal-chain ketose which should be capable of reduction to known heptitols. Degradation of the reducing carbon atom to give a 3-heptulose was performed using an equimolar proportion of lead tetraacetate (the method of Perlin and Brice).[15] Bromine oxidation was used to separate the ketose from any aldoses (by oxidation of the latter), and the ketose fraction was then reduced with sodium borohydride. From the reduction mixture, D-*glycero*-D-*ido*-heptitol[16](X) and D-*glycero*-D-*manno*-

(7) 2,4-*O*-Ethylidene-D-erythrose was also reported in papers presented orally by I. J. Goldstein, B. A. Lewis and F. Smith before the Division of Carbohydrate Chemistry at the 132nd Meeting of the American Chemical Society at New York, N. Y., September 11, 1957, and by C. E. Ballou before the Division of Carbohydrate Chemistry at the 134th Meeting of the American Chemical Society at Chicago, Ill., September 9, 1958.

(8) H. S. Isbell, H. L. Frush and R. Schaffer, *J. Research Natl. Bur. Standards*, **54**, 201 (1955).

(9) D. A. Rappaport and W. Z. Hassid, THIS JOURNAL, **73**, 5524 (1951).

(10) R. Schaffer and H. S. Isbell, *ibid.*, **79**, 3864 (1957), have characterized this crystalline dimer as bis-(5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose)-3,5′:5′,5-cyclic acetal.

(11) E. Spaeth and H. Schmid, *Ber.*, **74**, 859 (1941).

(12) W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 1363 (1949).

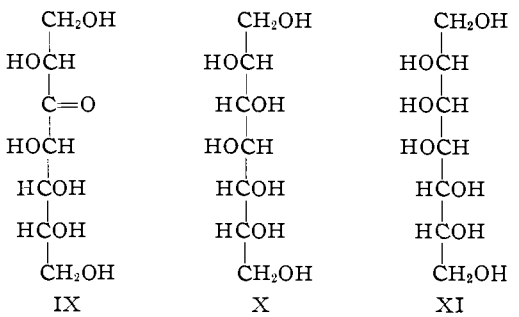
(13) A. S. Perlin and Carol Brice, *Can. J. Chem.*, **33**, 1216 (1955).

(14) J. W. Pratt and N. K. Richtmyer, THIS JOURNAL, **79**, 2597 (1957).

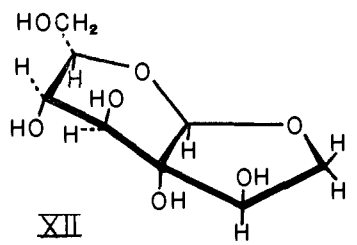
(15) A. S. Perlin and Carol Brice, *Can. J. Chem.*, **34**, 541 (1956).

(16) L. H. Philippe, *Ann. chim. phys.*, [8] **26**, 289 (1912).

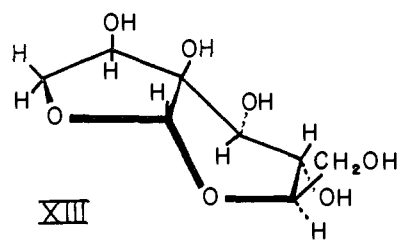
heptitol[17](XI) were crystallized. As these heptitols are epimeric at carbon atom 3, they must have resulted from reduction of D-*manno*-3-heptulose (IX). Although 3-hexuloses (or derivatives thereof) are known,[18–20] compound IX is the first 3-heptulose to be reported. The configuration of this as-yet-uncrystallized ketose establishes the configurations at all secondary carbon atoms of the eight-carbon precursors. Furthermore, that the degradation yielded a 3-heptulose shows the branching carbon atom to be carbon atom 3, in validation of the eight-carbon sugar as an aldol condensation product.



With an additional asymmetry at carbon atom 3, the precursor to the D-*manno*-3-heptulose has either the D-*glycero*-D-*talo* or the D-*glycero*-D-*ido* configuration. A choice between these alternatives was made by consideration of the structures of the anhydro sugar VII in these configurations. These anhydro compounds are shown by formula XII,



XII



XIII

which has the D-*glycero*-D-*talo* configuration, and by formula XIII, which has the D-*glycero*-D-*ido* configuration. The Fischer projection formula VII depicts the same compound as XII. Formula VII suggests that, in the D-*glycero*-D-*talo* configuration, the hydroxyl groups at carbon atoms 2, 3 and 4 should appear on the same side; however, the perspective formula XII shows them to be actually *trans*. Structure XIII, instead, has *cis*-hydroxyl groups at carbon atoms 2, 3 and 4. Of these *cis*-hydroxyl groups, the pair at carbon atoms 3 and 4 are at a projected angle of 60° because of their location on the boat-shaped pyranose ring; however, at carbon atoms 2 and 3, the hydroxyl groups are located on a furan ring and are therefore in a true *cis* arrangement. If the anhydro-octose has formula XIII, it would be expected to exhibit the behavior anticipated for a substance with a true *cis*-glycol grouping by complexing strongly with boric acid[21] and by rapidly reacting with lead tetraacetate.[22–24] On the other hand, if the substance has structure XII, boric acid should not complex with it, and lead tetraacetate should oxidize the glycol linkages only very slowly. On testing with these reagents, it was found that the substance (the anhydro-octose) did *not* increase the conductivity of a boric acid solution, and it reacted *very slowly* with lead tetraacetate, consuming only 0.6 mole per mole of anhydro-octose in 28 hr. It was, therefore, concluded that the anhydro-sugar has the D-*glycero*-D-*talo* configuration (XII).

It is of interest to note that VII was obtained in 83% yield under conditions similar to those in which aldohexoses form 1,6-anhydropyranoses; however, VII has the D-*gluco* configuration in the pyran ring, a configuration leading with the normal hexoses to a minimal yield of the 1,6-anhydropyranose.[25] Although these facts seem at first contradictory, the presence on the pyranose ring of the (1,2-dihydroxyethyl) group in the octose in place of a hydrogen atom in the hexose should account for the difference in behavior. Whereas, the C1 conformation of the pyranose ring of D-glucose is the stable all-equatorial conformation,[26] it requires with the octose a higher-energy boat form of the pyranose ring to accommodate both the (1,2-dihydroxyethyl) group and the hydroxymethyl group in equatorial positions.

The anhydro sugar most probably has the B3 conformation shown by structure XII, because the alternative 3B form would have the furan ring and hydroxymethyl group at axial positions on the boat-ring and, furthermore, the hydroxyl group at carbon atom 2 would then have to be *endo* to the fused rings. In the B3 form the hydroxyl groups at carbon atoms 3 and 5 are in a true *cis* arrangement, and the pair at carbon atoms 2 and 4 are *cis*, but at a 60° projected angle. Slight distortion of the B3 conformation, with a small consequent increase of the bond distance between the true *cis*-hydroxyl groups, permits true *cis* alignment of the latter pair also. The molecule would then be capable of forming, on opposite sides of the fused rings, two hydrogen bonds, whose effect should be to stabilize the anhydro compound and which could account for its large yield. The infrared spectrum shows a splitting of the absorption

(17) F. B. LaForge, *J. Biol. Chem.*, **42**, 375 (1920).
(18) Laura E. Stewart, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2206 (1952).
(19) J. K. N. Jones, *ibid.*, **78**, 2855 (1956).
(20) J. M. Sugihara and G. U. Yuen, *ibid.*, **79**, 5780 (1957).
(21) J. Böeseken, *Adv. Carbohydrate Chem.*, **4**, 89 (1949).
(22) R. Criegee, L. Kraft and B. Rank, *Ann.*, **507**, 159 (1933).
(23) R. C. Hockett, M. H. Nickerson and W. H. Reeder, III, THIS JOURNAL, **66**, 472 (1944).
(24) R. E. Reeves, *Anal. Chem.*, **21**, 751 (1949).
(25) A. Thompson, Kimiko Anno, M. L. Wolfrom and M. Inatome, THIS JOURNAL, **76**, 1309 (1954).
(26) R. E. Reeves, *ibid.*, **72**, 1499 (1950).

band for the hydroxyl group, indicative of hydrogen bonding.

Following the rules of carbohydrate nomenclature,[27] III is 1,3:5,7-di-*O*-ethylidene-3-*C*-formyl-D-*glycero*-D-*talo*-heptitol-3(1),6-pyranose. Compound VII is 3(1),1-anhydro-3-*C*-formyl-D-*glycero*-D-*talo*-heptitol-3(1),6-pyranose.

Experimental

Preparation of Bis-(2,4-*O*-ethylidene-D-erythrose)-1,1′:1′,3-cyclic Acetal (Dimeric 2,4-*O*-Ethylidene-D-erythrose) (II).—A stirred solution of 23 g. of sodium metaperiodate in 175 ml. of water (externally cooled to 15–25°) was treated with 10 ml. of a solution of 10.3 g. of 4,6-*O*-ethylidene-D-glucose[28] in 50 ml. of water, and then with sufficient 2 *N* sodium hydroxide to bring the mixture to the methyl orange end-point. The remaining ethylidene-glucose solution was added in 10-ml. portions during the next hour, with readjustments of acidity made between additions. After 3 additional hours, the mixture was brought to *p*H 7.5 with dilute sodium hydroxide, and the mixture was freeze-dried. Three 75-ml. portions of ethyl acetate were used for extracting the dried residue. Concentration of the combined extracts at room temperature at reduced pressure (to about a 15-ml. volume) led to the separation of crystalline material. The product was obtained in 70% yield as needles by recrystallization from absolute ethanol and pentane; m.p. 149–150°, $[\alpha]^{22}D$ −40° (initial) → −43.5° in 2 hr. (*c* 1, water), $[\alpha]^{22}D$ −27.4° (*c* 0.5, ethanol).

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.9. Found: C, 49.2; H, 6.9.

The molecular weight, determined in formamide by freezing-point depression, was 315. For the dimer, the theoretical value is 292. Hydrolysis of 0.2 g. of II in 10 ml. of 0.25 *N* sulfuric acid at the boiling temperature, with nitrogen bubbled through the solution, resulted in a quantitative distillation of acetaldehyde in less than 30 min. (The distillate was collected in hydroxylamine hydrochloride solution and titrated with alkali.) The colorless hydrolysis product, after deionization, showed a specific rotation for D-erythrose of −41°.

Preparation of 1,3′-Di-*O*-acetyl-(bis-[2,4-*O*-ethylidene-D-erythrose]-1,1′:1′,3-cyclic Acetal) (IIa).—A 0.1-g. amount of II in 8 ml. of pyridine was treated at 22° with 2 ml. of acetic anhydride. After 5 hr. the mixture was poured into ice-water and the solution was freeze-dried. A solution of the dried residue in ethanol gave IIa in 70% yield, m.p. 171.5–172°, $[\alpha]^{22}D$ −59° (*c* 0.6, ethanol).

Anal. Calcd. for $C_{16}H_{24}O_{10}$: C, 51.1; H, 6.4. Found: C, 51.3; H, 6.5.

From the changes in optical rotation during the acetylation reaction, two rates of acetylation were found: (1) a rapid levorotatory change with a half-time of about 4 min., and (2) a slower dextrorotatory change that was complete in 3 hr. The molecular weight of IIa, determined by the Rast camphor method, was 374. The value required for a dimer of ethylidene-erythrose with two acetyl groups is 376. The infrared spectrum of the product showed no absorption in the carbonyl region.

Preparation of 1,3:5,7-Di-*O*-ethylidene-3-*C*-formyl-D-*glycero*-D-*talo*-heptitol-3(1),6-pyranose (III).—A solution of 5.26 g. of II in 720 ml. of 0.05 *N* calcium hydroxide (prepared at 7°) was warmed to 22°. After 4 hr., it was cooled to 0° and deionized with an ice-cold mixture (70 ml. each) of cation-[29] and anion-exchange[30] resins. The effluent was concentrated under reduced pressure to near dryness. On further concentration, with ethanol added, III crystallized. The product was filtered, and washed with ethyl acetate. The yield was 15%, m.p. 228–229°, $[\alpha]^{22}D$ +49° (*c* 5, water).

Anal. Calcd. for $C_{12}H_{20}O_8$: C, 49.3; H, 6.9. Found: C, 49.6; H, 7.0.

At 60°, the specific rotation of a 0.1% solution of III in 0.25 *N* sulfuric acid decreases in 35 min. to about 6° (calculated for a monoethylidene-octose), and then increases over the next 5 or 6 hours to +17° (calculated for the octose VI). Continued heating at 75° resulted in a slow downward change of rotation (due to formation of VII) that was still incomplete after 10 hr.

Preparation of 3(1),1-Anhydro-3-*C*-formyl-D-*glycero*-D-*talo*-heptitol-3(1),6-pyranose (VII).—A solution of 1.0 g. of III in 10 ml. of 0.1 *N* sulfuric acid at the boiling temperature, with nitrogen bubbling through the solution, evolved acetaldehyde quantitatively in 15 min. Heating at reflux temperature was continued for 20 hr. more. The solution, brought to room temperature, was passed through a column containing 5 ml. of anion-exchange resin. The effluent was concentrated at reduced pressure; VII crystallized from a methanol plus 2-propanol solution of the concentrate; yield 0.68 g., m.p. 173.5–174°, $[\alpha]^{22}D$ −8.2° (*c* 3, water).

Anal. Calcd. for $C_8H_{12}O_7$: C, 43.2; H, 6.3. Found: C, 43.4; H, 6.4.

Compound VII does not react with Fehling solution. Treatment of VII with 5 mole-equivalents of sodium metaperiodate showed, after a 24-hr. reaction time, the consumption of 3.1 moles of periodate per mole of VII and the formation of 2.09 moles of acid per mole of VII. No formaldehyde was detected.

A solution of 0.1 *M* in VII and 0.25 *M* in boric acid had the same conductivity and *p*H as a solution 0.25 *M* in boric acid alone.

Aliquots of a solution 0.005 *M* in VII and 0.025 *M* in lead tetraacetate were tested periodically for lead tetraacetate consumption, using potassium iodide and sodium acetate for quenching the reaction and 0.02 *N* sodium thiosulfate for determination of the liberated iodine.[15] The number of moles of lead tetraacetate consumed per mole of VII was found to be 0.03 after 1 hr., 0.055 after 2 hr., 0.08 after 3 hr., 0.15 after 5.5 hr., and 0.62 after 28 hr.

The infrared absorption spectrum of VII shows on the absorption band characteristic of the hydroxyl group a shoulder at 3.05 μ and a splitting at 2.90 and 2.97 μ, which give evidence for hydrogen-bonding.

Preparation of Sodium 1,3:5,7-Di-*O*-ethylidene-D-*glycero*-D-*talo*-heptitol-3-*C*-carboxylate Dihydrate (IV).—An aqueous solution of 0.292 g. of III was treated alternately over a 10-min. interval with 2.5 ml. of 0.1 *N* potassium triiodide and dropwise with 3.75 ml. of 0.1 *N* potassium hydroxide until 25 ml. and 37.5 ml. of the respective reagents had been introduced. The mixture, cooled in ice, was then passed through a tube containing 30 ml. of ice-cold, cation-exchange resin into a stirred slurry of silver carbonate (0.025 equiv.) in water. After filtration and recooling to 0°, the filtrate was treated with an additional 10 ml. of the cation-exchange resin, and the effluent was neutralized with dilute sodium hydroxide. On concentration under reduced pressure, IV crystallized; yield 90%, $[\alpha]^{22}D$ −8.2° (*c* 7, water).

Anal. Calcd. for $C_{12}H_{19}O_9Na\cdot 2H_2O$: C, 39.3; H, 6.3; Na, 6.3. Found: C, 39.3; H, 6.4; Na, 6.3.

A solution of 0.671 g. of IV in 10 ml. of 0.433 *N* sulfuric acid showed an $[\alpha]^{22}D$ of −45.8° (calculated as diethylidene-octonic acid). At 60° the hydrolysis mixture required in excess of 30 hr. before the optical rotation appeared to be constant. Distillation of the liberated acetaldehyde at the reflux temperature led to a 7% increase in optical rotation. The final specific rotation observed was +26°. The hydrolysis mixture was freed of sulfuric acid, using barium hydroxide, and of excess alkali with carbon dioxide. After treatment with cation-exchange resin, concentrating the effluent, and taking up the concentrate in methanol, crystalline 3-*C*-carboxy-D-*glycero*-D-*talo*-heptitol-lactone was obtained; yield 0.44 g., m.p. 167–168°, $[\alpha]^{22}D$ +63.5° in 5 min. and +43° in 40 hr., reaction incomplete, (*c* 1.7, water).

Anal. Calcd. for $C_8H_{14}O_8$: C, 40.3; H, 5.9. Found: C, 40.0; H, 6.1.

The slow optical rotatory change accompanying self-hydrolysis of the lactone of V in aqueous solution suggests it to be a γ-lactone; however, from V two lactones are possible: the 3(1),1- and the 3(1),5-lactones, and the evidence is inadequate for a definitive assignment of its structure.

Degradation to D-*manno*-3-Heptulose (IX).—A solution of 1.0 g. of III in 10 ml. of 0.1 *N* sulfuric acid was kept for 15 min. at the boiling temperature, under nitrogen, for distillation of the acetaldehyde. The hydrolyzate was treated

(27) *Chem. Eng. News*, **31**, 1776 (1953).

(28) B. Helferich and H. Appel, *Ber.*, **64**, 1841 (1931).

(29) Amberlite IR-120 H, Rohm and Haas Co., Philadelphia, Pa.

(30) Duolite A-4, Chemical Process Co., Redwood City, Calif.

with 5 ml. of cation-exchange resin. The effluent was concentrated to a 2-ml. volume and then diluted with 200 ml. of glacial acetic acid. The solution was treated first with 1.475 g. of lead tetraacetate and, 15 min. later, with 0.4 g. of oxalic acid dissolved in glacial acetic acid.[15] The precipitate was filtered, the filtrate concentrated under reduced pressure, and the concentrate taken up in 10 ml. of 0.05 *N* hydrochloric acid and heated for 30 min. at 80° (for hydrolysis of formate ester groups). The solution was diluted with 50 ml. of water, and 1.5 g. of barium benzoate and 0.9 g. of bromine were introduced. After overnight reaction, the non-oxidized sugar was separated in the usual fashion (using cation- and anion-exchange resins for final purification). Paper chromatography using butanol–ethanol–water (4:1:5) showed the presence of a ketose with an R_f value only slightly less than that of glucose. Compound IX has not as yet been obtained in crystalline form. Compound IX, dissolved in water, was treated with a threefold excess of sodium borohydride. After 18 hr., cation-exchange resin was added to the stirred solution. When gaseous evolution had ceased, the mixture was filtered. Boric acid was removed from the filtrate by repeated reconcentrations with methanol. An aqueous ethanolic solution of the concentrate spontaneously gave small separate crops of two kinds of crystalline material. These were (a) D-*glycero*-D-*ido*-heptitol in the form of platelets with m.p. 125–127° and an undepressed mixed m.p. (using authentic heptitol[19] prepared by reduction of D-*glycero*-D-*ido*-heptose) and (b) D-*glycero*-D-*manno*-heptitol in the form of needles with m.p. 152–153° and an undepressed mixed m.p. (with authentic heptitol[20] prepared from D-*glycero*-D-*manno*-heptose).

The infrared spectra of the compounds considered in this paper will be presented in a forthcoming publication. Measurements in the 2 to 15 μ region were made with a Beckman IR-4 spectrometer using crystalline samples pressed into pellets of potassium chloride or iodide.

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Studies on the Sedimentation Behavior of Artificial Mixtures of Deoxyribonucleic Acid[1]

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The sedimentation behavior of two artificial mixtures of degraded and undegraded DNA were examined in the analytical ultracentrifuge equipped with ultraviolet optics. The components of the mixtures were found to sediment independently of each other with no pronounced interaction. The data confirm that very little experimental error is involved in the ultracentrifugal technique when applied to dilute solutions (*ca.* 0.003%) of DNA.

Johnston and Ogston observed[3] that an interaction occurred between proteins when they were subjected to ultracentrifugation in solution at a total concentration of solute between 3 and 8%. It was found that the material with lower sedimentation coefficient was enriched with material of higher sedimentation coefficient. This interaction, which has become known as the "Johnston–Ogston effect," was followed using an ultracentrifuge equipped with a schlieren optical system.

In a study of the ultracentrifugal heterogeneity of deoxyribonucleic acid (DNA), Peacocke and Schachman[4] prepared an artificial mixture composed of DNA of 20 *S* and a sonically degraded sample of sedimentation coefficient 8*S*. Again a schlieren optical system was used to follow the centrifugation and this necessitated concentrations of DNA of 0.12 to 0.17%. They stated that the interpretation of their study was made difficult due to the Johnston–Ogston effect observed at these relatively high concentrations.

The commercial availability of an ultraviolet optical attachment to the analytical ultracentrifuge[5–7] has made it possible to study the sedimentation behavior of DNA at much lower concentrations. Accordingly, the problem of the sedimentation of artificial mixtures was re-investigated to see whether or not, in dilute solutions, DNA preparations of different sedimentation coefficients would sediment separately from each other. This study was considered of special interest since it has been demonstrated, with many different preparations, that DNA at low concentrations (0.003%) exhibits a heterogeneity when examined in the ultracentrifuge equipped with ultraviolet optics.[5–8] Such a study would provide information on the interaction of the different molecular species as well as on the sensitivity of the ultracentrifugal technique.[9]

For this purpose in two separate experiments, a DNA preparation obtained by conventional methods was mixed with a DNA sample obtained therefrom by sonic degradation. It has been shown[10–12] that the sonic treatment of DNA leads to a diminution in length of the twin-helical chain[13] which is not accompanied by any denaturation, *i.e.*, separation of the twin strands.

These studies afforded an opportunity to determine the error in the quantitative assessment

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(2) Alfred P. Sloan Foundation Pre-doctoral fellow.

(3) J. P. Johnston and A. G. Ogston, *Trans. Faraday Soc.*, **42**, 789 (1946).

(4) A. R. Peacocke and H. K. Schachman, *Biochim. Biophys. Acta*, **15**, 198 (1954).

(5) K. V. Shooter and J. A. V. Butler, *Nature*, **175**, 500 (1955).

(6) K. V. Shooter and J. A. V. Butler, *Trans. Faraday Soc.*, **52**, 734 (1956).

(7) V. N. Schumaker and H. K. Schachman, *Biochim. Biophys. Acta*, **28**, 628 (1957).

(8) H. S. Rosenkranz and A. Bendich, *Federation Proc.*, **17**, 299 (1958); THIS JOURNAL, **81**, 902 (1959).

(9) A brief report of such a study has appeared, but no details were given; see discussion by S. S. Cohen in "Cellular Biology, Nucleic Acids and Viruses," Special Publication of the New York Academy of Sciences, V. 263 (1957).

(10) C. E. Hall and M. Litt, *J. Biophys. Biochem. Cyt.*, **4**, 1 (1958).

(11) P. Doty, B. B. McGill and S. A. Rice, *Proc. Nat. Acad. Sci., U. S.*, **44**, 432 (1958).

(12) M. Rosoff, A. Bendich and H. S. Rosenkranz, in preparation.

(13) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).