Anal. Calcd. for $C_{26}H_{22}O_7$: C, 69.94; H, 4.97. Found: C, 69.76; H, 5.11.

Acknowledgments.—We wish to thank Dr. D. L. MacDonald of this Laboratory for performing the periodate oxidations. Analyses were carried out by the Institutes' Analytical Services Unit under the direction of Dr. W. C. Alford.

BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

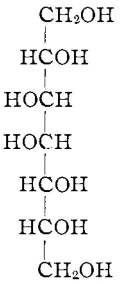
The Isolation of an Octulose and an Octitol from Natural Sources: D-*glycero*-D-*manno*-Octulose and D-*erythro*-D-*galacto*-Octitol from the Avocado and D-*glycero*-D-*manno*-Octulose from *Sedum* Species[1,2]

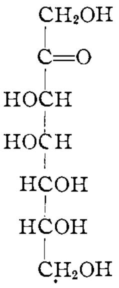
BY A. J. CHARLSON[3] AND NELSON K. RICHTMYER

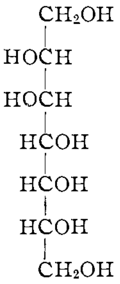
RECEIVED NOVEMBER 30, 1959

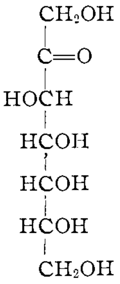
From the avocado, a long-known source of D-*manno*-heptulose and perseitol (D-*glycero*-D-*galacto*-heptitol), we have isolated small amounts of D-*glycero*-D-*manno*-octulose and D-*erythro*-D-*galacto*-octitol, and have obtained strong evidence for the presence also of D-*talo*-heptulose. From *Sedum* species, a well-known source of sedoheptulose (D-*altro*-heptulose), we have isolated small amounts of the same D-*glycero*-D-*manno*-octulose and also of β-sedoheptitol (D-*glycero*-D-*gluco*-heptitol). We have established the structures of the first known naturally occurring octulose and octitol by degradation studies and by the cyanohydrin synthesis from D-*glycero*-D-*manno*-heptose.

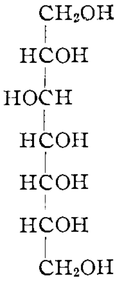
Nature has given us two heptitols and two heptuloses that have been known for a long time: perseitol(D-*glycero*-D-*galacto*-heptitol, I) from the avocado[4]; volemitol (D-*glycero*-D-*manno*-heptitol, III), discovered in the mushroom *Lactarius volemus* Fr.[5] and found later in the roots of several species of *Primula*,[6] in lichens,[7] and in brown and red algae[8]; D-*manno*-heptulose (II) from the avocado[9]; and sedoheptulose (D-*altro*-heptulose, IV), discovered in *Sedum spectabile* Bor.[10] and in other *Sedum* species, where it accumulates to the extent of about 1%, and found later to be widely distributed in nature (though usually in very small amounts) and to be a fundamental factor in plant and animal metabolism.[11] Thus, perseitol (I) and D-*manno*-heptulose (II) occur together in the avocado, and, in 1951, Nordal and Öiseth[12] isolated both volemitol (III) and sedoheptulose (IV), the latter being identified as the crystalline di-*O*-benzylidene derivative of sedoheptulosan, from the dried root stock of *Primula elatior* (L) Hill. We wish to announce that a third naturally occurring combination of heptitol and heptulose has now been established by our discovery that β-sedoheptitol (D-*glycero*-D-*gluco*-heptitol, V) accompanies sedo-

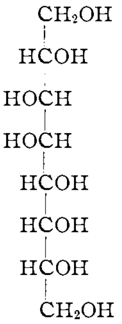

Perseitol (I)

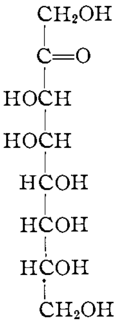

D-*manno*-Heptulose (II)

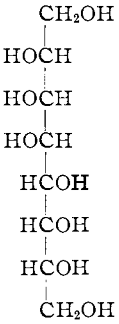

Volemitol (III)

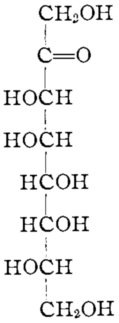

Sedoheptulose (IV)

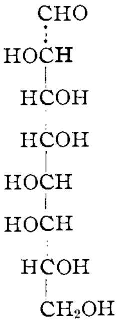

β-Sedoheptitol (V)

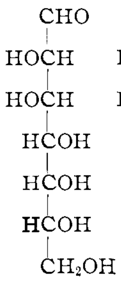

D-*erythro*-D-*galacto*-Octitol (VI)


D-*glycero*-D-*manno*-Octulose (VII)


D-*erythro*-D-*talo*-Octitol (VIII)


L-*glycero*-D-*manno*-Octulose (IX)


D-*threo*-L-*galacto*-Octose (X)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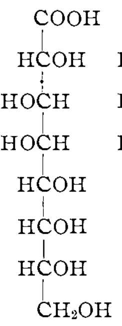
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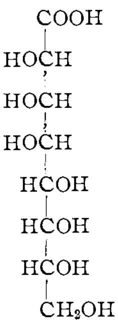
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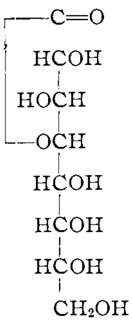
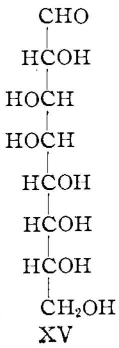

XIV


XV

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[1] Presented in part before the Division of Carbohydrate Chemistry at the Atlantic City Meeting of the American Chemical Society, September 15, 1959.

[2] A preliminary communication appeared in THIS JOURNAL, **81**, 1512 (1959).

[3] Visiting Scientist of the Public Health Service, April, 1958, to November, 1959.

[4] J. B. Avequin, *J. chim. méd., pharm. toxicol.*, [1] **7**, 467 (1831); see R. M. Hann and C. S. Hudson, THIS JOURNAL, **61**, 336 (1939).

[5] E. Bourquelot, *Bull. soc. mycologique France*, **5**, 132 (1889).

[6] J. Bougault and G. Allard, *Compt. rend.*, **135**, 796 (1902).

[7] Y. Asahina and M. Kagitani, *Ber.*, **67**, 804 (1934); B. Lindberg, A. Misiorny and C. A. Wachtmeister, *Acta Chem. Scand.*, **7**, 591 (1953). B. Lindberg, *ibid.*, **9**, 917 (1955).

[8] B. Lindberg and J. Paju, *Acta Chem. Scand.*, **8**, 817 (1954); B. Lindberg, *ibid.*, **9**, 1097 (1955).

[9] F. B. LaForge, *J. Biol. Chem.*, **28**, 511 (1917).

[10] F. B. LaForge and C. S. Hudson, *ibid.*, **30**, 61 (1917).

[11] For recent reviews see, for example, A. Nordal, *Tidsskr. Kjemi, Bergvesen Met.*, **5**, 77 (1956); A. Bonsignore, M. Orunesu, S. Pontremoli and P. Vegetti, *Giorn. biochim.*, **5**, 203 (1956).

[12] A. Nordal and D. Öiseth, *Acta Chem. Scand.*, **5**, 1289 (1951).

heptulose (IV) in the leaves and stems of *Sedum spectabile*.

The occurrence of more than one heptulose in the same plant material has been suggested by Nordal and Öiseth,[12] by Williams and Bevenue,[13] and by Williams, Potter and Bevenue.[14] Their evidence was based only on the number of spots on paper chromatograms that were revealed with orcinol–acid sprays, which we now know to be capable of detecting the non-reducing anhydroheptuloses as well. However, we have isolated from the avocado, through extensive fractionation, a small amount of a heptulose that is indistinguishable from D-*talo*-heptulose on paper chromatograms and that yielded, on degradation with oxygen in cold alkaline solution,[15] a lactone that was indistinguishable from D-talonic lactone on paper chromatograms. While we cannot consider the proof to be conclusive until we obtain more material and isolate the heptulose in crystalline form, we believe that we have at least strong evidence for the occurrence of D-*talo*-heptulose in the avocado.

Much more interesting is our discovery of an octulose and a closely related octitol in the avocado. The octulose was detected in the aqueous alcoholic extract of the Californian avocado (Calavo, Fuerte variety) when its paper chromatogram, sprayed with orcinol–hydrochloric acid and heated in an oven at 110°, revealed a crimson-colored spot that faded rapidly to gray; a similar spot was obtained with a synthetic octulose. Heptuloses, by contrast, give blue or bluish-green spots under these conditions. The avocado extract was freed from gums by precipitation with methanol, deionized, fermented, oxidized with hypobromite to remove aldoses, and the mixture of polyhydric alcohols and ketoses separated and purified by a combination of crystallization and cellulose column chromatography. The alditol fractions yielded *myo*-inositol and perseitol in addition to the octitol to be described later in this paper.

The octulose (1 g. from 27 kg. of avocado pulp) was a sirup, with $[\alpha]^{20}\text{D}$ +20° in methanol. As stated in our preliminary communication,[2] it was characterized by a crystalline 2,5-dichlorophenylhydrazone; on oxidation with two molar equivalents of lead tetraacetate according to the method of Perlin and Brice,[16] followed by acid hydrolysis, it yielded D-ribose; and on degradation with oxygen in cold alkaline solution[15] it yielded D-*glycero*-D-*manno*-heptonic lactone. On the basis of these degradations we concluded that the octulose must be D-*glycero*-D-*manno*-octulose (VII).

The octitol from the avocado was obtained as a monohydrate that melted at 169–170° and showed $[\alpha]^{20}\text{D}$ −11° in 5% aqueous ammonium molybdate and +66° in the acidified molybdate solution.[17] Its octaacetate melted at 99–100° and showed $[\alpha]^{20}\text{D}$ +2° in chloroform. Inasmuch as the reduction of D-*manno*-heptulose (II) furnishes the two alditols perseitol (I) and volemitol (III), both of which are found in nature, and sedoheptulose (IV) furnishes volemitol (III) and β-sedoheptitol (V), we reasoned that the avocado octitol should be related to the avocado octulose (VII) and therefore should be either D-*erythro*-D-*galacto*-octitol (VI) or D-*erythro*-D-*talo*-octitol (VIII). A test oxidation with *Acetobacter suboxydans* showed that the avocado octitol was oxidized to an octulose that migrated on a paper chromatogram more slowly than the avocado octulose (VII); from this we concluded that the avocado octitol could not be D-*erythro*-D-*talo*-octitol (VIII) for VIII could have been oxidized only to the avocado octulose (VII). On the other hand, rearrangement of the known D-*threo*-L-*galacto*-octose (X)[18] in boiling pyridine[19] afforded a solution that contained an octulose that migrated on paper chromatograms, run in four different solvent systems, at the same rate as the octulose produced by the action of *Acetobacter suboxydans* on the avocado octitol. Since the ketose expected from the pyridine rearrangement of the aldose X would be D-*glycero*-L-*manno*-octulose, and the ketose expected from the biochemical oxidation of D-*erythro*-D-*galacto*-octitol (VI) at C7 would be L-*glycero*-D-*manno*-octulose (IX), these two octuloses would be enantiomorphs, and that conclusion was supported by their identical R_f-values. Accordingly, the mixture of components from the pyridine rearrangement was reduced with sodium borohydride and the alditols were separated readily into two fractions, the larger one being the known D-*threo*-L-*galacto*-octitol[18] and the smaller one being the new D-*threo*-L-*talo*-octitol (= L-*erythro*-L-*galacto*-octitol). The latter was proved by its physical properties and those of its octaacetate to be the enantiomorph of the avocado octitol, which must therefore be D-*erythro*-D-*galacto*-octitol (VI).

Finally, the synthesis of the avocado octitol was undertaken, starting with some D-*glycero*-D-*manno*-heptose (XI) that was available from earlier studies in this Laboratory.[20] The addition of hydrogen cyanide to this heptose and subsequent hydrolysis of the nitrile mixture yielded a crystalline octonic acid; this was converted to a crystalline lactone, and this in turn to a crystalline phenylhydrazide whose dextrorotation indicated that the hydroxyl group at C2 was on the right and that the principal product in this synthesis was the D-*erythro*-D-*galacto*-octonic acid (XII) rather than the epimeric acid XIII. Reduction of a portion of the D-*erythro*-D-*galacto*-octonic γ-lactone (XIV) with excess sodium borohydride yielded D-*erythro*-D-*galacto*-octitol (VI) identical with the octitol isolated from the avocado.

Reduction of the remainder of the lactone with sodium amalgam afforded D-*erythro*-D-*galacto*-octose (XV); its mutarotation upward to $[\alpha]^{20}\text{D}$

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[13] K. T. Williams and A. Bevenue, *J. Assoc. Offic. Agr. Chemists*, **34**, 817 (1951).

[14] K. T. Williams, E. F. Potter and A. Bevenue, *ibid.*, **35**, 483 (1952).

[15] The method of O. Spengler and A. Pfannenstiel, *Z. Wirtschaftsgruppe Zuckerind.*, **85**, Tech. Tl. 547 (1935).

[16] A. S. Perlin and C. Brice, *Can. J. Chem.*, **34**, 541 (1956).

[17] N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **73**, 2249 (1951).

[18] W. D. Maclay, R. M. Hann and C. S. Hudson, *ibid.*, **60**, 1035 (1938).

[19] The method of S. Danilow, E. Venus-Danilowa and P. Schantarovitsch, *Ber.*, **63**, 2269 (1930).

[20] D. A. Rosenfeld, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **73**, 4907 (1951).

$+64°$ in water indicated the crystalline material to be the β-modification of the sugar. Rearrangement of this octose in pyridine resulted in the formation of an octulose that migrated on paper chromatograms in four solvent systems at the same rate as the avocado octulose (VII). Unfortunately there was not enough of the octose to rearrange and then attempt to isolate and identify the octulose with that from the avocado. Instead, the synthetic octose was converted to its phenylosazone and the latter proved to be identical with that derived from the avocado octulose through melting points, mixed melting points, infrared spectra and X-ray powder diffraction patterns.

Having discovered an octulose in the avocado, which is a good source of D-*manno*-heptulose, we reinvestigated the principal source of the other known naturally occurring heptulose and detected an octulose also in extracts of two *Sedum* species. We, therefore, attempted to isolate the *Sedum* octulose and, after extensive treatment and fractionation of the complex mixture of substances in the aqueous extracts of *Sedum*, succeeded in obtaining 0.16 g. of essentially pure octulose from 130 kg. of *Sedum*. The rotation of the sirupy octulose, $[\alpha]^{20}\text{D}$ $+20°$ in methanol, was the same as that of the avocado octulose. The two octuloses ran at the same rate on paper chromatograms in four different solvent systems, and their infrared spectra were identical. Degradation of the *Sedum* octulose with two molar equivalents of lead tetraacetate and with oxygen in cold alkaline solution yielded the same substances, as determined by paper chromatography, that we had isolated and characterized from the larger-scale degradations of the avocado octulose. Finally, the phenylosazone of the *Sedum* octulose was shown to be identical with those of the avocado octulose and the synthetic D-*erythro*-D-*galacto*-octose.

Thus we have shown that both the avocado[21] and *Sedum* contain D-*glycero*-D-*manno*-octulose (VII) and the avocado contains the closely related D-*erythro*-D-*galacto*-octitol (VI). We may predict that the same octitol will be found, though in very small amounts, also in *Sedum*. During the course of fractionation of our *Sedum* extracts we have isolated D-mannitol and *myo*-inositol, which have frequently been found in plant extracts. We have isolated, for the first time from a plant source, also β-sedoheptitol (V), as mentioned earlier. This substance is related to sedoheptulose (IV) in the same way that perseitol (I) is related to D-*manno*-heptulose (II) and the avocado octitol (VI) to the octulose (VII), that is, by the addition of hydrogen to the carbonyl group in such a way that the hydroxyl group introduced at C2 is *trans* to the one already present at C3.

In regard to the biosynthesis of these 7- and 8-carbon ketoses and alditols[22] we might note that in addition to the earlier-known heptulose syntheses through the action of aldolases, Racker and Schroeder[23] have shown recently that an octulose 8-phosphate is formed when D-ribose 5-phosphate and D-fructose 6-phosphate are incubated in the presence of a transaldolase, and that Sephton and Jones[24] have incubated D-fructose 1,6-diphosphate with each of the four pentoses D-ribose, D-lyxose, D-xylose and L-arabinose in the presence of purified rabbit muscle aldolase and isolated four octuloses, each with a D-*threo* configuration at C3 and C4 of the ketose formed.

We are continuing our investigations on the carbohydrate constituents of the avocado and *Sedum*, particularly in an attempt to isolate additional higher-carbon sugars and alditols.

Experimental

Paper chromatograms were run on Whatman No. 1 filter paper by the descending method using solvent A, 1-butanol–ethanol–water (40:11:19); solvent B, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); solvent C, 1-butanol–pyridine–water (6:4:3), or solvent D, 2-butanone–acetic acid–water saturated with boric acid (9:1:1). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid or orcinol–trichloroacetic acid for ketoses, *p*-anisidine hydrochloride for aldoses and ketoses, alkaline hydroxylamine–ferric chloride for lactones, and ammoniacal silver nitrate or sodium metaperiodate–potassium permanganate for sugars and alditols. The values for R_{sucr}, R_{pers}, and R_{lactone} refer to the rate of movement of the compounds on paper chromatograms relative to that of sucrose, perseitol and D-*glycero*-D-*manno*-heptonic lactone, respectively. All concentrations were carried out *in vacuo* at temperatures not over 50°.

Products from the Avocado. Isolation.—The crushed pulp from 94 ripe avocados (Calavo, Fuerte variety), weighing 27 kg., was extracted with 20% ethanol according to the general procedure of Montgomery and Hudson.[25] The extract was concentrated to 2 l. and the gums were precipitated by pouring the solution into 16 l. of methanol. The granular product was filtered, dissolved in 2 l. of water, and reprecipitated in the same manner. The combined methanol filtrates were concentrated and an aqueous solution of the residue was deionized by passage through columns of Amberlite IR-120 and Duolite A-4 ion-exchange resins. The solution was concentrated to 400 ml., 20 g. of D-glucose and 3 cakes of bakers' yeast were added, and fermentation was allowed to proceed for 48 hours at 37°. The solution was then filtered, deionized, and concentrated to a sirup that yielded readily, when dissolved in methanol, 1.1 g. of crystalline material. A paper chromatogram, run for 72 hours in solvent A and sprayed with ammoniacal silver nitrate, indicated that this material was a mixture of perseitol, the octitol (VI, R_{pers} 0.83), and *myo*-inositol (R_{pers} 0.45), whose separation and identification will be described below.

The filtrate from the 1.1 g. of polyols was concentrated to a sirup. Paper chromatograms, run in solvents A, B and C, and sprayed with aniline hydrogen phthalate, indicated the presence of arabinose and possibly xylose. Accordingly, all aldoses were removed by dissolving the sirup in 350 ml. of water and oxidizing with 4 ml. of bromine in the presence of 3 g. of barium benzoate for 20 hours in the dark. Excess bromine was removed by aeration, precipitated benzoic acid by filtration, and the solution deionized with Amberlite IR-120 and Duolite A-4 resins. Concentration of the solution left 16 g. of sirup that appeared to consist entirely of ketoses and alditols. Fractionation of this sirup was carried out on a cellulose column 325 mm. high and 50 mm. in diameter. Thus were obtained sirup A (6.4 g.) by elution with 1-butanol

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(21) Paper chromatograms of old mother liquors from the unfermented extracts of Florida avocados also showed the octulose spot, and so the octulose does not appear to be a constituent that is characteristic only of Calavo varieties. However, the relative amounts of octulose may change with the variety, as we know to be true of the D-*manno*-heptulose content; see reference 9; E. M. Montgomery and C. S. Hudson, THIS JOURNAL, **61**, 1654 (1939); and A. Nordal and A. A. Benson, *ibid.*, **76**, 5054 (1954).

(22) *Cf.* L. Hough and J. K. N. Jones, *Advances in Carbohydrate Chem.*, **11**, 185 (1956).

(23) E. Racker and E. Schroeder, *Arch. Biochem. Biophys.*, **66**, 241 (1957).

(24) H. H. Sephton and J. K. N. Jones, Abstracts of Papers, Atlantic City Meeting of the American Chemical Society, Sept. 13–18, 1959, p. 14E, and personal communications from those authors.

(25) E. M. Montgomery and C. S. Hudson, THIS JOURNAL, **61**, 1654 (1939).

half-saturated with water; sirup B (3.9 g.) by elution with 1-butanol three-quarters saturated with water; and finally sirup C (3.0 g.) by elution with 75% aqueous methanol.

Sirup A. Isolation of D-*talo*-Heptulose.—Examination of this sirup by paper chromatography indicated the presence of D-fructose, D-*manno*-heptulose and a second heptulose that ran somewhat faster than D-*manno*-heptulose. In order to purify the second heptulose, sirup A was fractionated on the cellulose column using 1-butanol–water (14:1) and 4.1 g. of sirup was removed; this sirup was refractionated using 1-butanol–water (28:1) and 2.2 g. of sirup obtained that still contained small amounts of D-fructose and D-*manno*-heptulose. Of this, 1.2 g. was fractionated on a cellulose column using benzene–methanol–water (300:100:4.) The 0.6 g. of sirupy heptulose thus obtained appeared to be free from D-*manno*-heptulose but still contained some D-fructose. Most of the latter was then removed by fermentation. Because the final sirup (0.48 g.) was believed to be principally sedoheptulose, it was dissolved in 25 ml. of 0.5 *N* sulfuric acid; however, its rotation did not change from $[\alpha]^{20}\mathrm{D}$ +2° on standing 48 hours at 20°. The solution was heated 2 hours on the steam-bath, deacidified with Duolite A-4, and concentrated to a sirup (0.44 g.). Paper chromatographic examination in all four solvent systems indicated that the major component was not sedoheptulosan but that it might be D-*talo*-heptulose (pure: $[\alpha]^{20}\mathrm{D}$ +12.9°[26]) containing small amounts of D-fructose and probably non-reducing anhydroheptuloses. Because the sirup could not be induced to crystallize when inoculated with authentic D-*talo*-heptulose, a 34-mg. sample of it was degraded by bubbling oxygen through its ice-cold solution in *N* potassium hydroxide for 20 hours. After removal of cations with Amberlite IR-120 and concentration, the residue was examined by paper chromatography in all four solvent systems; the major component was a lactone that ran at the same rate as D-talonic lactone.

Sirup B. Isolation of the Octitol VI and Characterization as D-*erythro*-D-*galacto*-Octitol Octaacetate.—A solution of sirup B in ethanol containing a small amount of water deposited 0.95 g. of needles melting at 166–168°. Paper chromatography in solvent A identified the material as the octitol (R_{pers} 0.83) contaminated with a very small amount of perseitol. The acetylation of 0.3 g. of this material by two successive treatments with acetic anhydride and pyridine yielded a sirup that crystallized. Recrystallization from ethanol afforded 0.35 g. of the octitol octaacetate as chunky prisms melting at 99–100° and showing $[\alpha]^{20}\mathrm{D}$ +2° in chloroform (*c* 3.4).

Anal. Calcd. for $C_{24}H_{34}O_{16}$: C, 49.83; H, 5.92; CH_3CO, 59.5; mol. wt., 578. Found: C, 49.74; H, 5.88; CH_3CO, 59.4; mol. wt., 570 (Signer method: isothermal distillation in acetone).

Sirup C. Isolation of *myo*-Inositol.—A solution of sirup C in ethanol deposited 0.4 g. of needles with R_{pers} 0.45 in solvent A (chromatogram sprayed with ammoniacal silver nitrate). The product was identified as *myo*-inositol through direct comparison on paper chromatograms, identical infrared spectra (Nujol mull), and melting point and mixed melting point of 228–229°. Confirmatory proof was obtained by acetylation to *myo*-inositol hexaacetate, whose melting point of 219–220° was not depressed when the substance was mixed with an authentic sample.

Purification of D-*erythro*-D-*galacto*-Octitol (VI).—The 1.1 g. of crystalline, mixed alditols that had separated before the bromine oxidation and subsequent fractionation on a cellulose column was combined with the mother liquor remaining after crystallization of the *myo*-inositol from sirup C, and the concentrated product was fractionated on the cellulose column. Elution with 1-butanol half-saturated with water followed by 1-butanol three-quarters saturated with water yielded, from the latter fractions, 1.2 g. of chromatographically pure octitol. The D-*erythro*-D-*galacto*-octitol monohydrate was recrystallized from aqueous ethanol, separating as elongated prisms melting at 169–170°. It showed $[\alpha]^{20}\mathrm{D}$ −11° in 5% aqueous ammonium molybdate (*c* 0.40) and +66° in the acidified molybdate solution (*c* 0.32).[17] Recrystallization from methanol containing only a small amount of water furnished the anhydrous octitol with the same m.p. of 169–170°.

Anal. Calcd. for $C_8H_{18}O_8 \cdot H_2O$: C, 36.92; H, 7.75. Found (hydrate): C, 37.01; H, 7.55. Calcd. for $C_8H_{18}O_8$: C, 39.67; H, 7.49. Found (anhydrous): C, 39.51; H, 7.80.

Isolation of D-*glycero*-D-*manno*-Octulose (VII) from Sirup B.—After removal of octitol from the ethanol solution of sirup B as described above, the mother liquor was concentrated and examined by paper chromatography. When the chromatogram was sprayed with orcinol–hydrochloric acid and heated at 110°, a crimson-colored spot developed (R_{sucr} 1.2 in solvent B and 2.6 in solvent D) and then faded to gray. This behavior is believed to be characteristic of an octulose, and the same color sequence was observed when a synthetic octulose was treated similarly. Fractionation of the remainder of sirup B was carried out on the cellulose column. Elution with benzene–methanol–water (200:100:6) yielded a fraction weighing 1.2 g., with $[\alpha]^{20}\mathrm{D}$ +20° in methanol (*c* 1.1). This sirup appeared to be essentially homogeneous and free from alditols when examined in solvent D, which is capable of separating the octulose from the octitol. For characterization a small portion of it was converted, by the procedure described by Mandl and Neuberg,[27] to **D-*glycero*-D-*manno*-octulose 2,5-dichlorophenylhydrazone.** The product was recrystallized from 40% aqueous ethanol as very pale yellow needles melting at 169–170°.

Anal. Calcd. for $C_{14}H_{20}Cl_2N_2O_7$: Cl, 17.76. Found: Cl, 17.59.

Degradation of D-*glycero*-D-*manno*-Octulose (VII) to D-Ribose with Lead Tetraacetate.—To a solution containing 147 mg. of the sirupy octulose in 80 ml. of glacial acetic acid and 1.6 ml. of water was added 550 mg. (2.0 molar equivalents) of lead tetraacetate. After 15 minutes a 10% solution of oxalic acid in glacial acetic acid was added dropwise until no more lead oxalate precipitated. The solution was filtered and then concentrated to remove most of the acetic acid. The residue was dissolved in 30 ml. of water and the solution heated on the steam-bath for 9 hours to effect complete hydrolysis of formyl and glycolyl groups. The acids were removed by passage through a column of Duolite A-4 ion-exchange resin and the solution was evaporated to yield 72 mg. of sirup with $[\alpha]^{20}\mathrm{D}$ −10° in water (D-ribose shows $[\alpha]^{20}\mathrm{D}$ −20°). Paper chromatograms in all 4 solvent systems indicated that ribose was the major component of the sirup. It was identified as D-ribose by conversion to its crystalline *p*-tolylsulfonylhydrazone (30 mg.) with melting point 158–159° decomp. and $[\alpha]^{20}\mathrm{D}$ +17° in dry pyridine (*c* 0.3); the reported values[28] are 164° and +14°, respectively. A mixture with authentic material melted also at 158–159° dec.

Degradation of D-*glycero*-D-*manno*-Octulose (VII) to D-*glycero*-D-*manno*-Heptonic Lactone with Oxygen in Alkaline Solution.—A 210-mg. portion of the octulose sirup was dissolved in 15 ml. of *N* potassium hydroxide that had previously been cooled to 0° and saturated with oxygen. Oxygen was bubbled through the solution for 16 hours at 0° and then for 8 hours at 20–25°. The solution was freed of cations with Amberlite IR-120 and concentrated to a sirup. Paper chromatograms run in solvents A, B and C indicated that a lactone with R_{lactone} = 1.0 was the major component. The material was chromatographed on a cellulose column and the lactone eluted with 1-butanol half-saturated with water. The appropriate fractions were combined and concentrated to a sirup that yielded, with 95% ethanol, fine needles of D-*glycero*-D-*manno*-heptonic lactone. Upon recrystallization from 95% ethanol the 15 mg. of product melted at 164–165° to a thick sirup; this value was undepressed when the substance was mixed with freshly recrystallized authentic lactone.[20] The rotation, $[\alpha]^{20}\mathrm{D}$ +48° in water (*c* 0.2), was similar to the rotation +40.4° reported earlier. Infrared spectra of the two lactones in Nujol mull were identical.

Oxidation of D-*erythro*-D-*galacto*-Octitol (VI) by *Acetobacter suboxydans*.—A 100-mg. portion of the octitol from the avocado was dissolved in 10 ml. of distilled water containing 50 mg. of Difco yeast extract, 30 mg. of potassium dihydrogen phosphate and 5 mg. of D-glucose. The solution was sterilized in the autoclave and inoculated with 0.4 ml. of a culture of *A. suboxydans*[29] grown on a yeast extract and D-

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[26] J. W. Pratt and N. K. Richtmyer, This Journal, 77, 6326 (1955).

[27] I. Mandl and C. Neuberg, *Arch. Biochem. Biophys.*, 35, 326 (1952).

[28] D. G. Easterby, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 3416 (1951).

[29] American Type Culture Collection No. 621.

glucose medium. After 6 days at 30° the solution was diluted with 35 ml. of methanol, filtered, deionized with Amberlite IR-120 and Duolite A-4 ion-exchange resins, and concentrated. Paper chromatograms sprayed with the orcinol–hydrochloric acid reagent indicated the presence of an octulose through a typical crimson spot that faded rapidly to gray. In solvents B and D the octulose moved more slowly than the naturally occurring D-*glycero*-D-*manno*-octulose (VII). However, in solvents A, B, C and D the octulose produced by *A. suboxydans* moved at the same rate as the D-*glycero*-L-*manno*-octulose produced in the pyridine rearrangement of D-*threo*-L-*galacto*-octose (X), as described in the following paragraph. The octulose produced by biochemical oxidation thus appears to be the enantiomorphous L-*glycero*-D-*manno*-octulose (IX; see discussion in the first part of this paper) and a definitive proof of its structure will be sought when more of the octitol VI becomes available.

Syntheses. Pyridine Rearrangement of D-*threo*-L-*galacto*-Octose (X), Reduction of the Products, and Isolation of L-*erythro*-L-*galacto*-Octitol and Its Octaacetate.—A 5.8-g. sample of D-*threo*-L-*galacto*-octose (X) monohydrate[18] was heated at 100° *in vacuo* for 3 hours and the dried octose refluxed in 45 ml. of dry pyridine for 9 hours. The solution was concentrated and shown to contain octulose by paper chromatography (see preceding paragraph). The mixture of sugars was dissolved in 70 ml. of water and reduced with 1.5 g. of sodium borohydride. After removal of sodium ions and boric acid in the usual way, *i.e.*, by Amberlite IR-120 and evaporation with methanol, respectively, the mixture of solid alditols was shaken with 50 ml. of water. The insoluble portion (2.5 g.) was recrystallized from hot water and identified by melting point and mixed melting point of 231° as the known D-*threo*-L-*galacto*-octitol.[18] The aqueous extract, upon concentration, yielded an additional 1.1 g. of the same alditol. Finally, the mother liquor yielded 0.36 g. of the expected epimeric alditol melting at 150–160°. This was identified as D-*threo*-L-*talo*-octitol, which can also be named L-*erythro*-L-*galacto*-octitol and is then readily recognized as the enantiomorph of the naturally occurring octitol VI. Paper chromatograms run in solvent A for 48 hours and in solvent C for 18 hours showed identical rates of migration for the enantiomorphs. Upon acetylation the synthetic enantiomorph yielded 0.16 g. of large prisms that, after recrystallization from ethanol, melted at 97–98° and showed $[\alpha]^{20}\mathrm{D}$ −1° in chloroform (*c* 3) as compared with m.p. 99–100° and $[\alpha]^{20}\mathrm{D}$ +2° for the octaacetate of the naturally occurring octitol described earlier in this paper. The infrared spectra of the two octaacetates in chloroform solution were identical. Upon deacetylation of the synthetic product, the L-*erythro*-L-*galacto*-octitol monohydrate melted at 168–169° and showed $[\alpha]^{20}\mathrm{D}$ −70° in acidified molybdate solution (*c* 0.16) as compared with m.p. 169–170° and $[\alpha]^{20}\mathrm{D}$ +66° for the enantiomorph, D-*erythro*-D-*galacto*-octitol (**VI**).

Synthesis of D-*erythro*-D-*galacto*-Octonic Acid (XII) from D-*glycero*-D-*manno*-Heptose (XI).—Twelve grams of D-*glycero*-α-D-*manno*-heptose hexaacetate[20] was deacetylated catalytically with sodium methoxide and the sirupy heptose treated with 1.8 g. of sodium cyanide by Hudson's modification[30] of the cyanohydrin synthesis. After saponification of the nitriles, removal of the sodium ions and concentration, the 5.9 g. of product was obtained as a solid residue. When this material was dissolved in 25 ml. of hot water it deposited 1.3 g. of elongated prisms of D-*erythro*-D-*galacto*-octonic acid monohydrate; once recrystallized from water it melted at 137° to a thick sirup and showed $[\alpha]^{20}\mathrm{D}$ −7° in water (*c* 1). When heated for 5 hours at 100° *in vacuo* the monohydrate lost about one molar equivalent of water (calcd. 6.57, found 5.91) and was converted to the anhydrous acid rather than to the lactone; a test for lactone was negative when a spot of the material on paper was sprayed with alkaline hydroxylamine followed by ferric chloride.

Anal. Calcd. for $C_8H_{16}O_9 \cdot H_2O$, C, 35.04; H, 6.62. Found: C, 35.24; H, 6.40. Calcd. for $C_8H_{16}O_9$: C, 37.50; H, 6.29. Found (dried at 100° *in vacuo*): C, 37.71; H, 6.51.

D-*erythro*-D-*galacto*-Octonic γ-Lactone (XIV) and D-*erythro*-D-*galacto*-Octonic Phenylhydrazide.—When 0.73 g. of the octonic acid monohydrate was dissolved in 40 ml. of boiling 2-ethoxyethanol, refluxed for 5 hours, and the solvent removed by distillation, the solid residue was found to consist of the lactone free from the acid as determined by paper chromatography. The lactone had R_{lactone} 0.83 in solvent B and 1.1 in solvent D. Recrystallization from ethanol containing a small amount of water afforded 0.47 g. of D-*erythro*-D-*galacto*-octonic γ-lactone. The product appeared to contain some solvent of crystallization and so it was dried for 8 hours *in vacuo* at 100°. It then melted at 152–153° and showed $[\alpha]^{20}\mathrm{D}$ −66° in water (*c* 1). Because the lactone was obtained only at a relatively high temperature it must be considered a γ-lactone.

Anal. Calcd. for $C_8H_{14}O_8$: C, 40.34; H, 5.92. Found: C, 40.70; H, 5.66.

The D-*erythro*-D-*galacto*-octonic phenylhydrazide was obtained by heating 0.1 g. of lactone and 0.1 ml. of phenylhydrazine in 1 ml. of water for a few minutes on the steam-bath. The mass solidified, and the product was purified by two recrystallizations from water as clusters of tiny needles. The phenylhydrazide melted at 193–195° and showed $[\alpha]^{20}\mathrm{D}$ +23° in water (*c* 0.2).

Anal. Calcd. for $C_{14}H_{22}N_2O_8$: C, 48.55; H, 6.40; N, 8.09. Found: C, 48.62; H, 6.41; N, 7.87.

Reduction of D-*erythro*-D-*galacto*-Octonic γ-Lactone (XIV) to D-*erythro*-D-*galacto*-Octitol (VI).—Reduction of 0.33 g. of the lactone with 0.5 g. of sodium borohydride according to the directions of Wolfrom and Wood[31] yielded *an* octitol and some octonic acid. The latter was removed on a column of Amberlite IRA-400 ion-exchange resin. The octitol obtained on concentration was recrystallized from ethanol containing a small amount of water. It weighed 0.15 g., melted at 170–171° both alone and in admixture with the naturally occurring D-*erythro*-D-*galacto*-octitol monohydrate, and showed $[\alpha]^{20}\mathrm{D}$ −12° in 5% ammonium molybdate (*c* 0.41) and +62° in acidified molybdate (*c* 0.33). The natural and synthetic octitols migrated at the same rates on paper chromatograms run in all four solvent systems. Acetylation of the synthetic octitol yielded an octaacetate that melted at 99–100°, both alone and when mixed with the octaacetate from the octitol from the avocado, and showed $[\alpha]^{20}\mathrm{D}$ +1° in chloroform (*c* 1.0). Infrared spectra of the two octaacetates in chloroform solution were identical.

D-*erythro*-D-*galacto*-Octose (XV).—The reduction of 4.4 g. of D-*erythro*-D-*galacto*-octonic γ-lactone (XIV) was carried out in ice-cold sodium hydrogen oxalate buffer solution by the general procedure of Isbell.[32] A total of 400 g. of 2% sodium amalgam was added in 50-g. portions at intervals of one hour while a *p*H of about 3 was maintained by the addition of oxalic acid dihydrate to the vigorously stirred solution. The next morning the mixture was filtered and an additional 250 g. of amalgam was added in the same manner to the filtrate. The mixture was again filtered, a considerable amount of the dissolved salts was precipitated by the addition of 3 liters of ethanol, and the remainder by deionization with Amberlite IR-120 and Duolite A-4 resins. Concentration of the solution left 3 g. of sirup that contained some unchanged lactone; the latter was removed by prolonged shaking of an aqueous solution of the sirup with Duolite A-4 resin. Concentration of the solution yielded 0.8 g. of a sirup that crystallized readily on addition of methanol. Upon recrystallization from methanol containing a small amount of water the octose separated as acicular prisms that melted at 174–175°. The rotation changed from $[\alpha]^{20}\mathrm{D}$ +45° (after a few minutes) to +64° (overnight, constant) in water (*c* 0.72) and the octose accordingly is to be designated D-*erythro*-β-D-*galacto*-octose. In relation to galactose, the octose showed R_{gal} 0.50 in solvent A and 0.46 in solvent B. On paper chromatograms it was detected as a dark green spot with the *p*-anisidine hydrochloride spray reagent.

Anal. Calcd. for $C_8H_{16}O_8$: C, 40.00; H, 6.71. Found: C, 40.26; H, 6.98.

Pyridine Rearrangement of D-*erythro*-D-*galacto*-Octose (XV).—A small sample of the octose was rearranged by boiling in dry pyridine for 8 hours. The solution then showed the presence of an octulose that moved at the same rate as the avocado octulose when paper chromatograms were run for extended periods of time in all four solvent systems.

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(30) C. S. Hudson, THIS JOURNAL, **73**, 4498 (1951); J. W. Pratt and N. K. Richtmyer, *ibid.*, **77**, 1906 (1955).

(31) M. L. Wolfrom and H. B. Wood, Jr., *ibid.*, **73**, 2933 (1951).

(32) H. S. Isbell, U. S. Patent 2,632,005, March 17, 1953; see also H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952), and J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **75**, 4503, footnote 20 (1953).

Products from Sedum Species.—Isolation.—Preliminary experiments were carried out on an aqueous extract from 2600 g. of *Sedum Adolphi* Hamet. Gums were precipitated with methanol, and D-glucose, D-fructose and sucrose were removed by fermentation with bakers' yeast. Deproteinization, deionization, and concentration left 5.5 g. of a dry sirup. A paper chromatogram run in solvent C for 40 hours and sprayed with orcinol–trichloroacetic acid revealed a strong blue sedoheptulose spot; a very weak blue spot just above it corresponding to the second anhydride of sedoheptulose, namely, 2,7-anhydro-β-D-*altro*-heptulofuranose[33]; and a weak blue spot just below it corresponding to sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose). Below the sedoheptulosan spot was a weak gray spot, believed to be an octulose, and below that a weak, elongated, bluish spot (or probably, as the ammoniacal silver nitrate spray indicated, 3 separate spots that appeared as a single long spot with the orcinol reagent). A 2-year-old extract of *Sedum spectabile* that had been treated similarly showed the same 5 spots with orcinol–trichloroacetic acid.

Accordingly, a large-scale operation was begun in order to study further the presumed octulose from *Sedum* species as well as the substances responsible for the still slower moving spot or spots that appeared with the orcinol spray. For this purpose we had available the extracts, obtained over a 4-year period, from a total of 385 kg. of *Sedum* plants (principally *S. spectabile*). These had been precipitated with methanol to remove gums and were then treated with yeast to remove fermentable sugars. The resulting solutions were deproteinized, deionized, and heated in 0.2 *N* sulfuric acid at 40° to convert about 90% of the sedoheptulose to sedoheptulosan.[33] After deacidification with Duolite A-4 and concentration, about 2100 g. of sedoheptulosan hydrate was recovered by crystallization. The mother liquor was retreated with acid, and an additional 300 g. of sedoheptulosan hydrate obtained. On a paper chromatogram sprayed with aniline hydrogen phthalate the mother liquor appeared to contain about seven aldoses, and so it was oxidized with bromine water in the presence of a considerable excess of calcium carbonate. The solution was deionized and concentrated to a sirup that weighed 345 g.; calculations showed that about 100 g. of aldoses had been removed by the hypobromite oxidation. The sirup, chromatographed on paper in solvent C and sprayed with aniline hydrogen phthalate, showed negligible amounts of aldoses; with orcinol–trichloroacetic acid it showed the five spots representing ketoses and their non-reducing anhydrides; and with ammoniacal silver nitrate it showed about a dozen separate spots that included the ketoses, their anhydrides and polyhydric alcohols.

Fractionation on Cellulose.—The 345 g. of sirup obtained as described above was subjected to chromatography on a cellulose column 52 cm. high and 7.5 cm. in diameter. The sirup was divided into five portions varying from 55 to 88 g., and each portion was put on the column and eluted with mixtures of benzene and methanol containing 1% water, starting with 5:1 (+1% H_2O) and gradually decreasing the proportion of benzene until a 1:1 (+1% H_2O) ratio was used for the final elution. The many samples of eluate from the five runs were examined by paper chromatography and eventually distributed among eleven fractions, of which the first 4 have not yet been investigated. Fraction 5 contained principally sedoheptulosan, of which 50 g. has so far been obtained crystalline. Fraction 7 yielded D-mannitol and fraction 11 a considerable quantity of *myo*-inositol. Fractions 8–10 contained the octulose, and fractions 9–11 contained the material that appears as the slow-moving, elongated, blue spot on a paper chromatogram sprayed with orcinol and trichloroacetic acid. The sedoheptulose and its anhydrides, represented by blue spots that moved faster than the octulose, appeared in fractions 4–9. Because the separation of the large amount of complex mixture under the conditions described above was not clean cut, a 3.6-g. portion of fraction 9 was removed for the study reported in the following section, and the remainder of fractions 8–11, weighing 33.5 g., is currently being investigated by fractionation with other solvent systems.

Examination of Fraction 9. Isolation of D-*glycero*-D-*gluco*-Heptitol (β-Sedoheptitol, V).—From the first 115 g. of sirup that was fractionated on the large cellulose column (representing about 130 kg. of *Sedum* leaves and stems) was obtained 3.6 g. of sirup, called fraction 9, that seemed to contain the largest proportion of octulose. This sirup was refractionated on a smaller cellulose column (325 mm. high and 50 mm. in diameter) using 1-butanol half saturated with water until the eluate showed only a trace of octulose, and then changing to 1-butanol three-quarters saturated with water. Seven fractions were obtained, with fractions 9C and 9D showing only the gray, octulose spot and fractions 9E and 9F showing only a slower-moving blue spot on paper chromatograms sprayed with orcinol–hydrochloric acid. Fraction 9C (1.68 g.), when run on a paper chromatogram in solvent B for 2 days and sprayed with periodate–permanganate, showed the octulose spot (R_{sucr} 1.2) and another spot above it. The sirup was dissolved in a mixture of hot methanol and ethanol and the solvents allowed to evaporate slowly at room temperature. After a few days the crystals that had deposited were filtered and washed with methanol. The 0.4 g. of product melted at 131–132° and was identified as β-sedoheptitol by mixed melting point (no depression), identical R_f-values on paper chromatograms run in all 4 solvents, and identical infrared spectra on direct comparison with authentic material, and by its rotation of $[\alpha]^{20}\text{D}$ +46° in 5% ammonium molybdate as compared to the value of +49.6° reported in the literature.[34]

Isolation of D-*glycero*-D-*manno*-Octulose (VII).—The remainder of fraction 9C was combined with 9D (0.1 g.) and some additional β-sedoheptitol removed by crystallization. The residue (*ca.* 1 g.) was put on the small cellulose column and eluted with 1-butanol one-quarter saturated with water. Eight fractions were collected, with β-sedoheptitol being detectable by paper chromatography only in the first two fractions. The combined middle fractions 4–6 (0.16 g.) showed $[\alpha]^{20}\text{D}$ +20° in methanol (*c* 0.9), the same value that had been obtained for the sirupy octulose from the avocado. On paper chromatograms run in all four solvent systems the *Sedum* octulose had the same mobility as the avocado octulose. Infrared spectra of the thoroughly dried octulose sirups as films from methanol were identical.

A 16-mg. sample of the *Sedum* octulose was oxidized with 2 molar equivalents of lead tetraacetate as described for the avocado octulose. The resulting pentose in this case also had the same mobility as ribose when run on paper chromatograms in all four solvent systems. Another small sample was degraded with oxygen in cold alkaline solution; paper chromatograms run in solvents B and D indicated that the principal product here, as from the avocado octulose, was D-*glycero*-D-*manno*-heptonic lactone.

Preparation of D-*glycero*-D-*manno*-Octose Phenylosazone from the *Sedum* Octulose, the Avocado Octulose and D-*erythro*-D-*galacto*-Octose.—A 0.10-g. fraction of octulose sirup from *Sedum*, containing no detectable amount of β-sedoheptitol or other impurity, was dissolved in 3 ml. of water, together with 0.2 g. of phenylhydrazine hydrochloride and 0.3 g. of sodium acetate trihydrate, and the mixture heated for 30 minutes in a hot water-bath at 95°. A yellow osazone separated and, after cooling, it was centrifuged, and washed well with water and then ether. On recrystallization from 50% aqueous ethanol it separated as needles; yield, 10 mg. The phenylosazones were prepared in the same way from the sirupy avocado octulose and from the synthetic D-*erythro*-D-*galacto*-octose (XV). Each of the phenylosazone samples showed the same melting point, 188–189° with decomposition, with no significant depression of that value when mixed melting points were made. X-Ray powder diffraction patterns and infrared spectra in potassium bromide pellets were identical for all three phenylosazones.

Anal. Calcd. for $C_{20}H_{26}N_4O_6$: C, 57.40; H, 6.26; N, 13.39. Found (from *Sedum* octulose): C, 57.11; H, 6.30; N, 13.24. Found (from synthetic octose): C, 57.39; H, 6.10; N, 13.20.

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(33) N. K. Richtmyer and J. W. Pratt, THIS JOURNAL, **78**, 4717 (1956).

(34) A. T. Merrill, W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **69**, 70 (1947).

contributions of *Sedum spectabile;* Mr. John T. Sipes and Mr. Edward W. Tracy of this Laboratory for their help in processing the *Sedum;* Dr. John C. Keresztesy and his associates, Dr. Norman E. Sharpless, and Dr. Laura C. Stewart, of other laboratories of this Institute, for help in concentrating the *Sedum* extracts, obtaining the X-ray powder diffraction patterns and with the *Acetobacter suboxydans* study, respectively; and Mr. W. E. Clarke, III, of Lynchburg, Va., for his collaboration in the study of *Sedum Adolphi* while he was a participant in the High School Science Teacher Study–Work Institute during the summer of 1958.

BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

2-Deoxy-D-ribose. IV.[1] A Direct Synthesis of 2'-Deoxyadenosine and its Anomer through 2-Deoxy-D-ribose Derivatives[2]

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Mono-*p*-nitrobenzoylation of 2-deoxy-D-ribose diisobutyl dithioacetal affords a 5-*O*-*p*-nitrobenzoyl derivative. Demercaptalation of this, followed by further *p*-nitrobenzoylation, gives the two anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates. Conversion of these esters to amorphous 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride, followed by condensation with chloromercuri-6-benzamidopurine and removal of the protecting groups, has led to the isolation of 2'-deoxyadenosine, its anomer 9-(2-deoxy-α-D-ribofuranosyl)-adenine and a third nucleoside, probably 7-(2-deoxy-α-D-ribofuranosyl)-adenine. A direct preparation of 5-*O*-trityl-2-deoxy-α-D-ribose from 2-deoxy-D-ribose is described.

Chemical syntheses of nucleosides containing the 2-deoxy-D-ribofuranosyl moiety have heretofore relied on indirect pathways, preformed pentofuranosylpyrimidines[3] and purines[4] being reduced at carbon two of the sugar moiety through various ingenious transformations. In view of the relative accessibility of 2-deoxy-D-ribose,[5] the direct synthesis of such nucleosides through suitably substituted 2-deoxy-D-ribofuranosyl halides offers a number of attractive features. The present paper will describe such a direct synthesis.[6]

Zinner and co-workers[7] have shown that pentose dialkyl dithioacetals may be readily *p*-nitrobenzoylated at carbon five and that, through demercaptalation, 5-*O*-acylpentose dialkyl dithioacetals may be converted to 5-*O*-acylpentofuranoses. This method of obtaining pentofuranose derivatives was used in the course of the present research. 2-Deoxy-D-ribose was converted to its known diisobutyl dithioacetal[8] and mono-*p*-nitrobenzoylated to yield the crystalline 5-*O*-*p*-nitrobenzoyl derivative I. Demercaptalation with mercuric chloride and mercuric oxide then gave an amorphous 2-deoxy-5-*O*-*p*-nitrobenzoyl-D-ribose (II) which was further *p*-nitrobenzoylated, affording the two anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates (III and IV).

Either of these esters or a mixture of the two was dissolved in methylene chloride and treated with a very slight excess of hydrogen chloride.[9] *p*-Nitrobenzoic acid, which was precipitated in nearly quantitative yield, was removed, leaving a solution of 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride (V).[10] The amorphous, solvent-free halide was then condensed with chloromercuri-6-benzamidopurine in dimethyl sulfoxide solution. Protecting groups were stripped off with alkali and the product chromatographed on a cellulose column. 2'-Deoxyadenosine (VI, 9-(2-deoxy-β-D-ribofuranosyl)-adenine) was isolated in 10% yield. Its melting point, rotatory dispersion and ultraviolet absorption spectrum clearly showed it to be identical with the natural nucleoside.

A second crystalline nucleoside, isomeric with the natural product, was isolated through chromatography in 19% yield. In contrast with the first product, it was markedly dextrorotatory in the wave length range measured (589 to 340 mμ). In the ultraviolet it showed an absorption peak at 260 mμ characteristic of a 9-substituted adenine.[11] Hydrolysis with weak acid revealed only adenine, 2-deoxy-D-ribose and unchanged nucleoside. It appears highly probable that the substance is, therefore, the anomer of the natural nucleoside, namely, 9-(2-deoxy-α-D-ribofuranosyl)-adenine (VII).

Finally, the chromatography yielded a small amount (1%) of a third, isomeric nucleoside. From the dextrorotation of this substance and its absorption at 273 mμ (characteristic of 7-substituted adenines[11]) it may tentatively be assigned the structure 7-(2-deoxy-α-D-ribofuranosyl)-adenine.

Zinner, Nimz and Venner[12] have described an indirect method of preparing 2-deoxy-5-*O*-trityl-α-D-ribose; in the course of the present work the same substance was prepared rather more conveni-

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[1] 2-Deoxy-D-ribose. III, C. Pedersen, H. W. Diehl and H. G. Fletcher, Jr., THIS JOURNAL, **82**, 3425 (1960).

[2] For a preliminary communication describing this work see R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **81**, 4752 (1959).

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