Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE ISOLATION OF D-*erythro*-L-*galacto*-NONULOSE FROM THE AVOCADO, TOGETHER WITH ITS SYNTHESIS AND PROOF OF STRUCTURE THROUGH REDUCTION TO D-*arabino*-D-*manno*-NONITOL AND D-*arabino*-D-*gluco*-NONITOL*

HUGO H. SEPHTON AND NELSON K. RICHTMYER
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014 (U.S.A.)

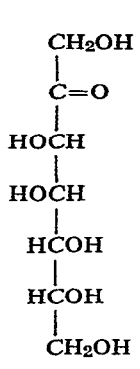
(Received February 17th, 1966; in revised form March 12th, 1966)

The application of chromatographic separation techniques has, in recent years, resulted in the addition of several higher-carbon sugars to those previously isolated from natural sources. D-*manno*-Heptulose (I)[1] and sedoheptulose (D-*altro*-heptulose, II) as the crystalline 2,7-anhydro-β-D-*altro*-heptulopyranose[2] had been isolated from plant sources by crystallization techniques before the era of chromatography. An investigation of the higher-carbon sugars of the avocado fruit (*Persea gratissima* Gaertn., family Lauraceae) has resulted in the isolation of D-*talo*-heptulose (III)[3], D-*glycero*-D-*galacto*-heptose (IV)[4], D-*glycero*-D-*manno*-octulose (V)[3–5], D-*glycero*-L-*galacto*-octulose (VI)[4], D-*erythro*-L-*gluco*-nonulose (VII)[6], and now D-*erythro*-L-*galacto*-nonulose (VIII). These octuloses and nonuloses have also been found in *Sedum* species (family Crassulaceae)[3,6], have recently been isolated from the roots of the primrose (*Primula officinalis* Jacq., family Primulaceae)[7], and have been indicated, by paper chromatography, to occur, with D-*manno*-heptulose and sedoheptulose, in at least five other genera of the Crassulaceae[8]. Sedoheptulose occurs widely in nature, and its role as an intermediate, both in carbohydrate metabolism and photosynthesis, is well understood. D-*manno*-Heptulose has been discovered in several plant families, but the mechanism of its biosynthesis is still obscure. The biosynthesis and possible role of the octuloses and nonuloses in carbohydrate metabolism are unknown, although several octuloses have been synthesized enzymically from aldoses[4,9–11].

It is interesting that the higher-carbon ketoses that have been shown to occur in plants can be divided into two well-defined groups with reference to their structures and possible modes of biosynthesis. The first group includes those that have a D-*threo* configuration at C-3 and C-4 (the "D-xylulose" configuration at C-1 to C-4); these should be capable of being synthesized by the enzymes transaldolase, transketolase, or aldolase. In this group belong sedoheptulose (II), D-*glycero*-L-*galacto*-octulose (VI)

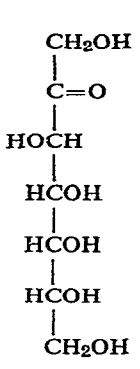
*Presented, in part, before the American Society of Plant Physiologists at the 45th Annual Meeting of the Pacific Division of the American Association for the Advancement of Science, Vancouver, B.C., Canada, June 1964, and, in part, before the Division of Carbohydrate Chemistry at the Winter Meeting of the American Chemical Society, Phoenix, Arizona, January 1966.

(which has been synthesized *in vitro* by an aldolase[4,9]), D-*erythro*-L-*gluco*-nonulose(VII), and D-*erythro*-L-*galacto*-nonulose (VIII). The configurations of the naturally occurring D-xylose, D-mannose, and D-glucose can be recognized in the lower portions of the octulose and two nonulose formulas, respectively, and this suggests that aldolase or



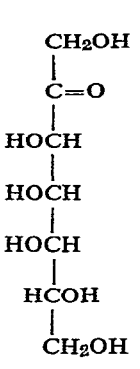
D-*manno*-Heptulose

I



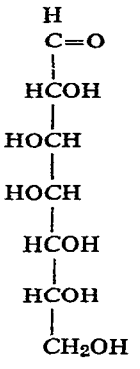
D-*altro*-Heptulose

II



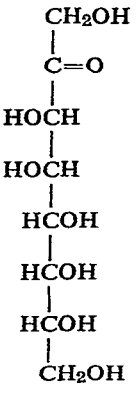
D-*talo*-Heptulose

III



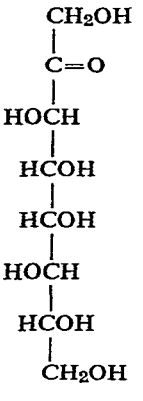
D-*glycero*-D-*galacto*-Heptose

IV



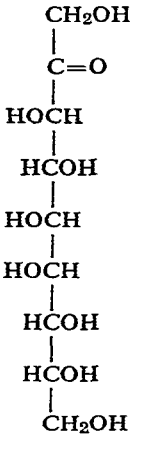
D-*glycero*-D-*manno*-Octulose

V



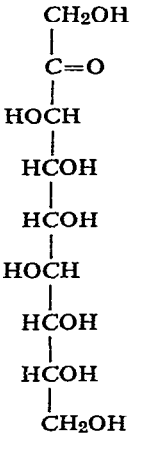
D-*glycero*-L-*galacto*-Octulose

VI



D-*erythro*-L-*gluco*-Nonulose

VII



D-*erythro*-L-*galacto*-Nonulose

VIII

transaldolase reactions are involved in their biosynthesis. The second group includes those higher-carbon ketoses that have an L-*erythro* configuration at C-3 and C-4, and whose mode of biosynthesis is at present obscure. D-*manno*-Heptulose (I), D-*talo*-heptulose (III), and D-*glycero*-D-*manno*-octulose (V) belong to this group.

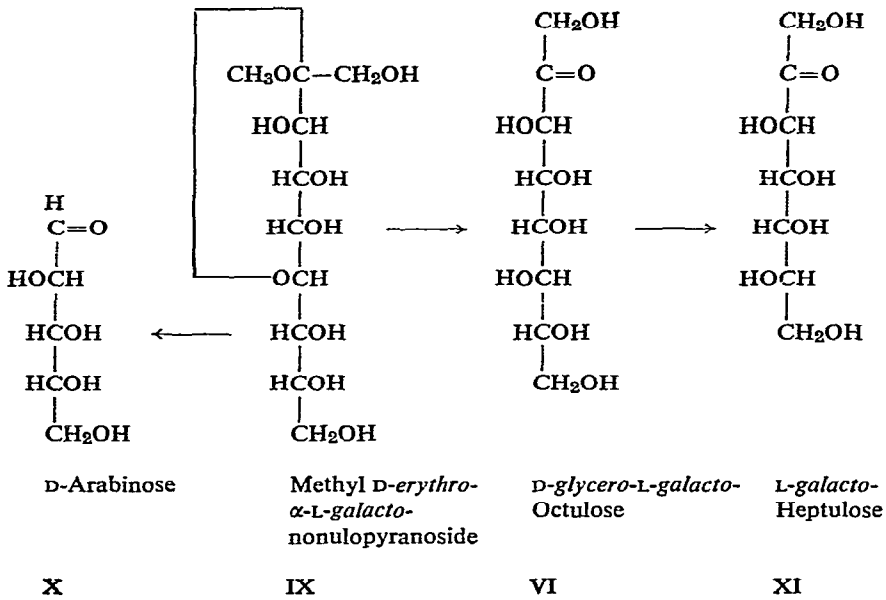
D-*erythro*-L-*galacto*-Nonulose (VIII), the primary subject of this article, was

isolated from the avocado fruit (Calavo, Hass variety) in very low yield, as a hygroscopic sirup, by repeated chromatography on cellulose columns. The structure of the nonulose was determined by two methods of degradation similar to those that had been used for D-*erythro*-L-*gluco*-nonulose[6], previously isolated from the avocado fruit. Oxidation from the reducing end with two molecular equivalents of lead tetraacetate, according to the procedure of Perlin and Brice[12], yielded as the main product a reducing sugar having the paper-chromatographic mobility of a glucose; it was shown to be D-glucose by its complete oxidation in the presence of D-glucose oxidase. The configurations of C-5 to C-8 of the nonulose were thus indicated to be the same as those of D-glucose. In agreement with this conclusion, minor components detected in the lead tetraacetate oxidation mixture corresponded to D-*glycero*-D-*gulo*-heptose and D-arabinose. The configurations of C-3 to C-6 were determined by application of the degradation procedure of Jones and Sephton[10] in which the methyl nonulopyranoside IX (or, probably, a mixture of the anomeric forms) was oxidized with two molecular equivalents of periodate at 0° and at 25°. At 0°, oxidation occurred mainly at the C-7, C-8 glycol outside the ring, producing a heptosuloside that, after reduction with potassium borohydride and hydrolysis with acid, gave a heptulose indistinguishable by paper chromatography from L-*galacto*-heptulose (perseulose, XI). An octulose, not separable from D-*glycero*-L-*galacto*-octulose (VI)[4], was also produced in small proportion by oxidation at C-8, C-9. When the borohydride reduction was omitted, the main aldose product (after acid hydrolysis) cochromatographed with D-arabinose (X); this indicated that a substantial portion of the nonuloside had been oxidized within the pyranose ring, with the lower portion of the nonulose being left intact. This observation indicated a relatively labile *cis*-glycol[13] at C-4, C-5, and is in agreement with the *galacto*-heptulose configuration indicated above for C-1 to C-6. When the periodate oxidation was carried out at 25°, followed by borohydride reduction and hydrolysis, the major product was a polyhydric alcohol cochromatographing with D-arabinitol; this result is consistent with the pyranoside structure ascribed to IX.

Since degradation from the reducing end of the nonulose produced D-glucose, the *galacto*-heptulose obtained by degradation from the nonreducing end must belong to the L series (so as to overlap with D-glucose). The complete structure of the nonulose is, thus, indicated to be D-*erythro*-L-*galacto*-nonulose (VIII).

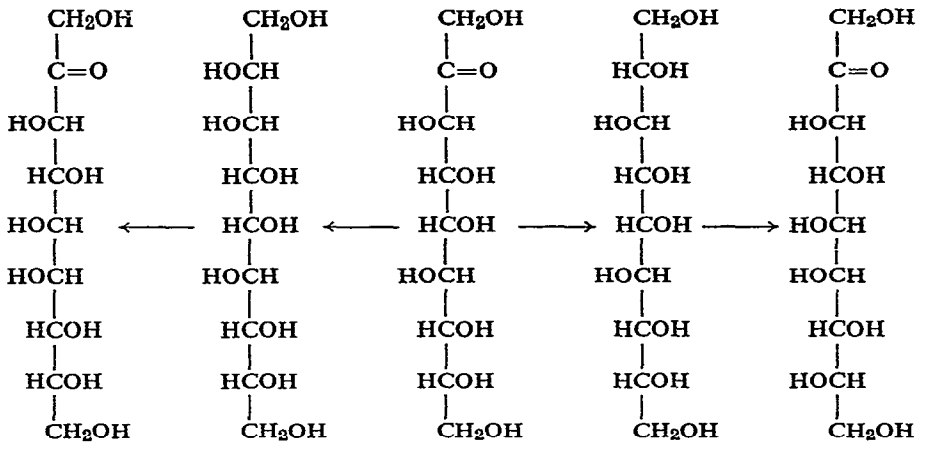
To confirm the structure thus determined by degradation, D-*erythro*-L-*galacto*-nonulose (VIII) was synthesized by (a) the nitroethanol method of Sowden[14] and (b) the diazomethane method of Wolfrom[15]. The addition of nitroethanol to D-*glycero*-D-*gulo*-heptose was carried out under a variety of conditions, all of which were found to give the desired nonulose, but in very low yield. The diazomethane synthesis from sodium D-*erythro*-L-*galacto*-octonate was somewhat better, giving VIII in 4.8% yield as a sirup having $[\alpha]_D^{20}$ −36.2° in water. A crystalline (2,5-dichlorophenyl)osazone of this sugar was prepared. The synthetic nonulose was found to have the same chromatographic mobility as the second avocado nonulose by paper chromatography and by gas–liquid chromatography. The identity of the synthetic

D-*erythro*-L-*galacto*-nonulose with the avocado nonulose was established by reduction of each with borohydride and isolation of corresponding, pairs of identical nonitols. Thus, each nonulose reduction mixture was chromatographed on a



column of Dowex 50W-X8 (Ba^{2+}) resin[16], and completely separated into fractions from which D-*arabino*-D-*manno*-nonitol (XII) and D-*arabino*-D-*gluco*-nonitol (XIII) were obtained in crystalline form. The identity of these two pairs of nonitols with each other was established by infrared and melting-point data. The assignment of configuration to the two nonitols was determined by oxidizing them to nonuloses with *Acetobacter suboxydans*; this organism oxidizes the penultimate hydroxyl group of a *cis* glycol when it is of the D configuration and next to a primary hydroxyl group. The main product thus obtained from the higher-melting nonitol (192–193°) was chromatographically inseparable from D-*erythro*-L-*gluco*-nonulose (VII), the first nonulose isolated from the avocado[6]; hence, this nonitol was D-*arabino*-D-*manno*-nonitol (XII). D-*erythro*-L-*galacto*-Nonulose (VIII), the second oxidation product to be expected, was, however, not detected. Oxidation of the second nonitol (m.p. 180–181°) gave a new nonulose (XIV) that was readily separable by chromatography from the two avocado nonuloses (VII and VIII); hence, the second nonitol was indicated to be D-*arabino*-D-*gluco*-nonitol (XIII). Emil Fischer[17] had obtained the higher-melting of these two nonitols by reduction of a synthetic nonose ("D-glucononose" or "D-α,α,α-glucononose"). C.S. Hudson[18] concluded that Fischer's nonose probably had the D-*arabino*-D-*manno* configuration because of its preponderance in the cyanohydrin synthesis, its weak dextrorotation, and the low solubility of its crystalline phenylhydrazone. The higher-melting of the above pair of nonitols was

shown to be identical with Fischer's nonitol by mixed melting point, by X-ray diffraction patterns, and by *Acetobacter suboxydans* oxidation. Hudson's assignment of the D-*arabino*-D-*manno* configuration was thus confirmed.



VII XII VIII XIII XIV

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 filter paper by the descending method at room temperature. The following solvent systems were used: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); B, butyl alcohol–ethyl alcohol–water (40:11:19); C, butyl alcohol–pyridine–water(6:4:3); and D, ethyl acetate–pyridine–water saturated with boric acid (12:5:4). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid for ketoses, and silver nitrate (ammoniacal, or in conjunction with sodium hydroxide in ethyl alcohol) for alditols, sugars, and other polyhydroxy compounds in general. With the orcinol–hydrochloric acid spray and heating at 100–110°, heptuloses give a pinkish orange color changing to blue (or greenish blue with *manno*-heptulose), octuloses give a pink to red color changing to a brownish gray, and nonuloses give a similar pink to red changing to a greenish gray. The octulose and nonulose spots fluoresce bluish white under ultraviolet light. All concentrations were carried out *in vacuo* at temperatures not over 50°; the final drying of sirups was completed in evacuated desiccators over granular calcium chloride. Melting points were determined on a Kofler micro hot-stage.

Isolation of D-erythro-L-galacto-*nonulose from the avocado*

A sirupy fraction (0.420 g) containing principally this nonulose was isolated

from the fruit pulp (95 kg) of 400 ripe avocados (Californian Calavo, Hass variety) by repeated chromatography on cellulose-powder columns as described in a previous paper[4]. The sirup was dissolved in a small volume of methanol, and polyhydric alcohols were removed from it by crystallization at low temperature. The mother liquor was concentrated to a sirup (0.282 g) that was dissolved in water (5 ml), the solution was filtered through a layer of decolorizing carbon (Darco X), and the filtrate was concentrated to a dry sirup (0.262 g). The presence of oligosaccharides in this sirup was indicated when acid hydrolysis of a small portion of it (5 mg) produced sugars that cochromatographed with D-glucose, D-xylose, D-*manno*-heptulose, and D-fructose, in addition to the nonulose. Oligosaccharides were removed from a portion (150 mg) of the nonulose fraction by chromatography on thick filter-paper (Whatman No. 3MM) with solvent D. A nonulose fraction (103 mg) having $[\alpha]_D^{20}$ −9.7° (*c* 1.24, water) was thus obtained. This product still contained a small amount of oligosaccharide contaminants.

Lead tetraacetate oxidation of the avocado D-erythro-L-galacto-*nonulose to* D-glycero-D-gulo-*heptose*, D-*glucose, and* D-*arabinose*

The nonulose (6.5 mg) was dissolved in glacial acetic acid (5 ml), and treated with a solution of two molecular equivalents of lead tetraacetate in glacial acetic acid (0.75 ml of a 0.116 N solution) at 22°. After 15 min, sufficient oxalic acid (10%) in glacial acetic was added to the solution to complete the precipitation of lead oxalate, which was removed by centrifugation. The supernatant liquor was concentrated to a sirup that was dissolved in aqueous 0.1 N sulfuric acid (5 ml), and the solution was heated on the steam bath for 5 h (to hydrolyze formyl and glycolyl esters), deacidified with Duolite A-4 ion-exchange resin, and concentrated to a sirup. The product was examined by paper chromatography with solvent systems A, B, and C, which indicated the following sugar components: a heptose not separable from D-*glycero*-D-*gulo*-heptose, a hexose (the major product) not separable from D-glucose, and a pentose not separable from D-arabinose. After incubation of a portion of the degradation products with D-glucose oxidase, the hexose component that had previously cochromatographed with D-glucose was completely absent, whereas the heptose and pentose were still detectable on paper chromatograms.

Periodate oxidation of the avocado methyl D-erythro-L-galacto-*nonuloside to* D-glycero-L-galacto-*octulose*, L-galacto-*heptulose*, D-*glucose, and* D-*arabinose*

The nonulose (6.0 mg) was refluxed with 2.5% methanolic hydrogen chloride (5 ml) for 5 h. The solution was cooled, neutralized to pH 5 by the gradual addition of a methanolic solution of potassium hydroxide, and concentrated *in vacuo* without heating. The sirupy residue was dissolved in water (5 ml), the solution was cooled to 0°, and a cold aqueous solution of 2 molecular equivalents of sodium metaperiodate (9.3 mg) was added to it. The solution was kept at 0° overnight, and divided into two equal volumes. One portion was treated with an excess of potassium borohydride for 4 h at 22°, decationized (Dowex 50W-X8), freed from boric acid by the usual

procedure, hydrolyzed in a 0.1 N aqueous sulfuric acid solution on the steam bath for 2 h, deionized with ion-exchange resins (Dowex 50W-X8 and Duolite A-4), and concentrated to a sirup. Upon examination by paper chromatography with solvent systems A, B, and C, it was found to contain some residual nonulose, an octulose not separable from D-*glycero*-L-*galacto*-octulose[4], and, as the major product, a heptulose not separable from L-*galacto*-heptulose. The second portion of the periodate oxidation mixture was hydrolyzed with aqueous 0.1 N sulfuric acid (5 ml) on the steam bath for 2 h, deionized with ion-exchange resins (Dowex 50W-X8 and Duolite A-4), concentrated to a sirup, and examined by paper chromatography with solvent systems A, B, and C. The principle aldose in this hydrolyzate was inseparable from D-arabinose. Minor components, inseparable from D-*glycero*-D-*gulo*-heptose and D-glucose, were also detected.

The methyl glycosides obtained from the avocado nonulose (5 mg) were also oxidized at 25° with two molecular equivalents of sodium metaperiodate, and the products were reduced with borohydride and hydrolyzed as described above. Paper chromatography revealed a main constituent that cochromatographed with D-arabinitol. Minor products (not separable from D-glucitol and erythritol), as well as some nonulose, were also detected.

Synthesis of D-erythro-L-galacto-*nonulose*

(*a*) *From* D-glycero-D-gulo-*heptose*

2-Nitroethanol (150 ml; Commercial Solvents Corporation) was purified by codistillation with phenyl ether (150 ml) under diminished pressure, with the precautions suggested by Noland[19]. The purified reagent was kept in the refrigerator.

In preliminary experiments, several methods of addition of 2-nitroethanol to D-*glycero*-D-*gulo*-heptose were tried, on a small scale, with different solvents and basic catalysts. These included sodium methoxide in methanol, aqueous sodium hydroxide, Amberlyst XN-1002 anion-exchange resin in pyridine, powdered potassium hydroxide in pyridine, sodium methoxide in methanol plus pyridine, sodium methoxide in methanol plus glycerol, and sodium hydroxide in methanol–water. The yields of nonuloses obtained were low in all cases.

D-*glycero*-D-*gulo*-Heptose[20] (57 g) was dissolved in distilled water (500 ml) by stirring at 60°. To the solution, cooled to 20°, 2-nitroethanol (30 g) was added, followed by the slow addition, with stirring, of sodium hydroxide (15 g) in water (300 ml). Methanol (400 ml) was added to provide a homogeneous solution. Stirring was continued at room temperature for 30 min, and the solution was then neutralized by the addition of Dowex 50W-X8 cation-exchange resin, filtered, and the filtrate concentrated to a small volume. Unreacted heptose (51.8 g) crystallized during the concentration, and was recovered by filtration and washing with methanol. The combined mother liquor and wash methanol, examined by paper chromatography, showed the presence of two nonuloses and a heptulose. It was combined with similar products obtained by re-treating the recovered heptose (51.8 g) with 2-nitroethanol as described above. The crystalline heptose (43 g) recovered from the second reaction

was treated a third time, to yield unchanged heptose (37 g) and a mother liquor that, upon further concentration, decomposed exothermally with evolution of gas and total loss of the product. The recovered heptose (37 g) was treated a fourth time with 2-nitroethanol in aqueous methanolic sodium hydroxide. The residual heptose (30 g) was recovered as described above, and the mother liquor containing the mixture of ketoses, the excess of 2-nitroethanol, and degradation products was combined, in aqueous solution (200 ml), with the mother liquor products previously obtained, and extracted with ethyl ether (5 times, 200 ml) to remove the excess of 2-nitroethanol and some of the degradation products. The residue was precipitated onto cellulose powder, in a slurry, by means of quarter-saturated aqueous butyl alcohol; the slurry was transferred to the top of a packed cellulose column (5 × 100 cm); and the column was eluted with quarter- to half-saturated aqueous butyl alcohol as described in a previous publication[4]. Fractions were collected on the basis of their ketose content as indicated by paper chromatography. D-*gluco*-Heptulose (6.7 g) and two nonulose fractions (1.295 g and 0.367 g) were obtained by concentrating appropriate fractions. The second of these two nonuloses was not separable from the avocado nonulose (VIII) by paper chromatography with solvent systems A, B, or C.

(*b*) *From sodium* D-erythro-L-galacto-*octonate*

The procedure of Barker[21] was adapted for the preparation of D-*erythro*-L-*galacto*-octonic acid heptaacetate. Freshly fused zinc chloride (7.5 g) was dissolved in acetic anhydride (100 ml) by magnetic stirring at 22° in a three-necked flask fitted with a calcium chloride tube, a thermometer, and a gas-dispenser tube. The solution was cooled to −10°, and powdered sodium D-*erythro*-L-*galacto*-octonate* (25 g) was added. Cooled in a freezing mixture at −20°, the solution was treated with dry hydrogen chloride at a low rate for 1.5 h with continuous stirring, care being taken to maintain the temperature of the solution below 0°. The solution thus saturated with hydrogen chloride at −10° was set aside to warm slowly overnight to 22°. The solution was then cooled to 0°, and chipped ice (500 g) was added slowly, with stirring, during 1.5 h. The acetylated octonic acid was extracted from the solution with dichloromethane (5 times, 200 ml), and the extract was dried (sodium sulfate). The dried solution was concentrated to a sirup that was dissolved in dry benzene (200 ml), and thionyl chloride (20 g) was added. The solution was heated under reflux, with protection against atmospheric moisture (calcium chloride tube), for 4 h, kept overnight at 22°, and the excess of reagent and the solvent were removed by concentration, followed by drying of the resulting sirup *in vacuo*. A solution of diazomethane in ethyl ether [prepared by swirling *N*-methyl-*N'*-nitrosoguanidine (20 g) in cold ethyl ether (400 ml) over a layer of cold 50% aqueous potassium hydroxide solution (10 ml), and drying over potassium hydroxide pellets] was decanted onto the dry, sirupy, acetylated octonyl chloride. After the sirup had dissolved, the solution was kept at

* The sodium octonate was made by earlier workers in this Laboratory from the lactone, which was prepared according to Hockett and Hudson (Ref. 22).

room temperature for 4 h, concentrated, and the sirupy residue dried *in vacuo*. A solution of the sirup in glacial acetic acid (250 ml), to which powdered copper (20 mg) and cupric acetate (trace) were added, was carefully heated to the point of vigorous gas evolution (114°), just below the boiling point. After the evolution of gas had subsided, the solution was heated at its boiling point for several min, cooled, and concentrated, and the sirup was dried *in vacuo* over potassium hydroxide (pellets). The sirup was dissolved in dry methanol (500 ml), the solution cooled to −5°, a catalytic amount of sodium methoxide in methanol (5 ml of 2.9 M) was added, and the solution was kept at −5° until deacetylation of the nonulose was complete, as indicated by paper chromatography (48 h). Sodium ions were removed from the solution by passing it through a column of Dowex 50W-X8 (H^+) resin, and the eluate was concentrated to a thin sirup. The sirup was precipitated onto cellulose powder slurried in quarter-saturated aqueous butyl alcohol, and fractionated by chromatography on a cellulose-powder column by elution with quarter- to half-saturated aqueous butyl alcohol as described in a previous publication[4]. The nonulose fraction obtained (1.15 g; 4.8% overall yield) was decolorized by treatment with activated carbon (Darco X), and filtered. It had $[\alpha]_D^{20}$ −36.2° (*c* 5.16, 90% aqueous methanol), and was chromatographically inseparable from the avocado nonulose (VIII) by solvent systems A, B, or C. The trimethylsilyl derivatives of this synthetic nonulose and the avocado nonulose were prepared by mixing a small quantity (about 1 mg) of each with pyridine (4 drops), hexamethyldisilazane (4 drops), and chlorotrimethylsilane (2 drops)[23]. The reaction mixtures were subjected to gas–liquid chromatography on a packed column (0.6 × 183 cm) of 3% SE 52 on Gas-Chrom A by injecting small volumes (1 μl) of each and heating the column from 75 to 280° at a rate of 11° per min, with a nitrogen flow-rate through the column of 100 ml per min. Each of the reaction mixtures produced a single, main chromatographic peak eluting at 275°, detected by hydrogen flame ionization; these peaks were not separated when the two preparations were cochromatographed. A crystalline (2,5-dichlorophenyl)-osazone was obtained from the synthetic nonulose (25 mg) by heating it under reflux for several days with (2,5-dichlorophenyl)hydrazine (50 mg) in absolute ethyl alcohol (5 ml) containing glacial acetic acid (0.5 ml). The osazone crystallized from the boiling reaction-mixture as nodular aggregates of fine needles; m.p. 247–249°. When mixed with the (2,5-dichlorophenyl)osazone of D-*erythro*-L-*gluco*-nonulose[6], the m.p. was 238–240°.

Anal. Calc. for $C_{21}H_{24}Cl_4N_4O_7$: C, 43.02; H, 4.13; Cl, 24.19; N, 9.56. Found: C, 43.33; H, 4.14; Cl, 23.88; N, 9.79.

The infrared spectrum of the osazone, recorded in a Nujol mull, showed distinctive absorption maxima at 745, 792, 845, 873, 920, 1013, 1047, 1090, 1170, 1205, 1248, 1258, 1268, 1455, 1473, 1587, 3275, and 3400 cm^{-1}.

Reduction of D-erythro-L-galacto-*nonulose, and isolation of the products:* D-arabino-D-manno-*nonitol and* D-arabino-D-gluco-*nonitol*

Synthetic D-*erythro*-L-*galacto*-nonulose (0.060 g), dissolved in water (25 ml),

was treated with an excess of potassium borohydride (0.10 g), and the solution was kept overnight at 22°. Cations were removed from the solution by stirring it with an excess of Dowex 50W-X8 (H^+) resin, and the acidic solution was filtered and concentrated to a sirup. Boric acid was removed from the product as (volatile) methyl borate. The residual sirup was dissolved in water (2 ml), placed on a column (1.2 × 140 cm) of Dowex 50W-X8 (200–400 mesh) in the Ba^{2+} form, and eluted with distilled water at a low rate (5 ml per h) according to the method described by Jones and Wall[16]. Small fractions (1 ml) were collected, and were examined by paper chromatography. The first crystalline nonitol (0.017 g) was recovered from fractions 151 to 220. After recrystallization from aqueous methanol, it had m.p. 192–193°.

Anal. Calc. for $C_9H_{20}O_9$: C, 39.70; H, 7.41. Found: C, 39.99; H, 7.06.

A second crystalline nonitol (0.022 g) was recovered from eluate fractions 285 to 380. After recrystallization from aqueous methanol, it had m.p. 180–181°.

Anal. Calc. for $C_9H_{20}O_9$: C, 39.70; H, 7.41. Found: C, 39.17; H, 7.46.

Duplicate infrared spectra obtained on each of these two nonitols (KBr discs) showed the following distinctive absorption maxima: first nonitol–860, 880, 1030, 1090, 1215, 1390, 1435, 1630, 2855, 2925, and 3410 (broad) cm^{-1}; second nonitol–625, 685, 855, 875, 920, 955, 1020, 1030, 1045, 1090, 1200, 1250, 1320, 1445, 1630, 2850, 2920, 2970, and 3380 (broad) cm^{-1}.

The avocado nonulose (VIII; 0.050 g) was also reduced with potassium borohydride (0.10 g), and a pair of crystalline nonitols (5 mg each) was obtained by chromatography on the Dowex 50W-X8 (Ba^{2+}) resin column as described above. After recrystallization from aqueous methanol, the first of these two nonitols had m.p. 193–194°. A mixture of this nonitol with the first nonitol obtained from synthetic D-*erythro*-L-*galacto*-nonulose melted at 192–193°. After recrystallization from aqueous methanol, the second nonitol obtained from the avocado nonulose had m.p. 180–181°. A mixture of this nonitol with the second nonitol from synthetic D-*erythro*-L-*galacto*-nonulose melted at 180–181°. The infrared spectrum of this second nonitol (KBr disc) clearly confirmed its identity with the second nonitol obtained from synthetic D-*erythro*-L-*galacto*-nonulose.

Oxidation of D-arabino-D-manno-*nonitol to* D-erythro-L-gluco-*nonulose, and of* D-arabino-D-gluco-*nonitol to* L-threo-L-gluco-*nonulose with* Acetobacter suboxydans

The first nonitol (0.8 mg) obtained from synthetic D-*erythro*-L-*galacto*-nonulose was oxidized with *Acetobacter suboxydans* (A.T.C.C. No. 621), grown on a mannitol–agar slant and suspended in nutrient broth (1.5 ml) containing 0.5% of yeast extract, at 25° for 4 days, with gentle agitation. The solution was filtered through decolorizing carbon (Darco X), evaporated to a sirup, and examined by paper chromatography. The main product gave the characteristic nonulose reaction with the orcinol reagent, and was not separable from synthetic D-*erythro*-L-*gluco*-nonulose[6] in solvent systems A, B, or C.

The second nonitol (2 mg) obtained from synthetic D-*erythro*-L-*galacto*-nonulose was oxidized with *A. suboxydans* in the same manner. The main product was shown

by paper chromatography to be a nonulose readily distinguishable from both D-*erythro*-L-*galacto*-nonulose and D-*erythro*-L-*gluco*-nonulose.

Emil Fischer's "D-glucononitol"[17]* was found by us to have m.p. 192–194°, a value that was not depressed when this nonitol was mixed with the first nonitol obtained from synthetic D-*erythro*-L-*galacto*-nonulose. These two nonitol preparations gave closely similar X-ray diffraction spectra. When Fischer's nonitol (3.5 mg) was oxidized with *A. suboxydans* as described above, the main product obtained was chromatographically indistinguishable from D-*erythro*-L-*gluco*-nonulose.

ACKNOWLEDGMENT

The authors thank Dr. William C. Alford and his associates of the Section on Microanalytical Services and Instrumentation of this Laboratory of Chemistry for obtaining the elemental analyses and infrared spectra, and Dr. Norman E. Sharpless of the Laboratory of Physical Biology of this Institute for recording the X-ray diffraction patterns.

SUMMARY

D-*erythro*-L-*galacto*-Nonulose was isolated as a sirup from the avocado fruit, in very low yield, by chromatography. The structure of the sugar was determined by degradation from the reducing end with lead tetraacetate, and from the non-reducing end with sodium metaperiodate. The two sets of reaction products thus obtained were identified with known compounds whose structures could be "overlapped" to conform with the D-*erythro*-L-*galacto*-nonulose configuration. This sugar was synthesized, and its identity with that of the avocado nonulose was established by reduction, followed by isolation of crystalline D-*arabino*-D-*manno*-nonitol and D-*arabino*-D-*gluco*-nonitol from each nonulose. Assigment of configurations to the two pairs of nonitols was determined by oxidation with *Acetobacter suboxydans*.

REFERENCES

1 F.B. LaForge, *J. Biol. Chem.*, 28 (1917) 511.
2 F.B. LaForge and C.S. Hudson, *J. Biol. Chem.*, 30 (1917) 61.
3 A.J. Charlson and N.K. Richtmyer, *J. Am. Chem. Soc.*, 82 (1960) 3428.
4 H.H. Sephton and N.K. Richtmyer, *J. Org. Chem.*, 28 (1963) 1691.
5 A.J. Charlson and N.K. Richtmyer, *J. Am. Chem. Soc.*, 81 (1959) 1512.
6 H.H. Sephton and N.K. Richtmyer, *J. Org. Chem.*, 28 (1963) 2388.
7 R. Begbie and N.K. Richtmyer, *Carbohydrate Res.*, 2 (1966) 272.
8 H.H. Sephton, unpublished work done at the Laboratory for Comparative Biochemistry, San Diego, California.
9 E. Racker and E. Schroeder, *Arch. Biochem. Biophys.*, 66 (1957) 241.
10 J.K.N. Jones and H.H. Sephton, *Can J. Chem.*, 38 (1960) 753.
11 G.R. Bartlett and G. Bucolo, *Biochem. Biophys. Res. Commun.*, 3 (1960) 474.

*A sample of this nonitol from the Fischer collection was kindly supplied by Professor Clinton E. Ballou of the University of California (Berkeley).

12 A.S. PERLIN AND C. BRICE, *Can. J. Chem.*, 34 (1956) 541.
13 *Cf.* O. KJØLBERG, *Acta Chem. Scand.*, 14 (1960) 1118.
14 J.C. SOWDEN, *J. Am. Chem. Soc.*, 72 (1950) 3325; J.C. SOWDEN AND D.R. STROBACH, *J. Am. Chem. Soc.*, 80 (1958) 2532.
15 M.L. WOLFROM, S.W. WAISBROT, AND R.L. BROWN, *J. Am. Chem. Soc.*, 64 (1942) 2329, and other papers in the series, especially M.L. WOLFROM AND H.B. WOOD, JR., *J. Am. Chem. Soc.*, 77 (1955) 3096.
16 V.F. FELICETTA, M. LUNG, AND J.L. MCCARTHY, *Tappi*, 42 (1959) 496; J.K.N. JONES, R.A WALL, AND A.O. PITTET, *Chem. Ind.* (London), (1959) 1196; J.K.N. JONES, R.A. WALL, AND A.O. PITTET, *Can. J. Chem.*, 38 (1960) 2285; J.K.N. JONES AND R.A. WALL, *Can. J. Chem.*, 38 (1960) 2290.
17 E. FISCHER, *Ann.*, 270 (1892) 64.
18 C.S. HUDSON, *Advan. Carbohydrate Chem.*, 1 (1945) 1.
19 W.E. NOLAND, *Org. Syn.*, 41 (1961) 67.
20 N.K. RICHTMYER, *Methods Carbohydrate Chem.*, 1 (1962) 160.
21 R. BARKER, *J. Org. Chem.*, 25 (1960) 1670.
22 R.C. HOCKETT AND C.S. HUDSON, *J. Am. Chem. Soc.*, 60 (1938) 622.
23 C.C. SWEELEY, R. BENTLEY, M. MAKITA, AND W.W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497