the medium, invoked no significant effect on the C^{14} distribution in glucose.

Although sufficient supporting evidence is lacking at this time, the distribution pattern data for the glucose resulting from the hydrolysis of the cellulose from culture 5 provides some indication for the hypothesis that cellulose formation even *via* the involved pathways inherent in bacteria is primarily a biosynthesis by direct polymerization.

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Isolation of Mannoheptulose and Identification of its Phosphate in Avocado Leaves[1]

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Mannoheptulose accumulates in avocado leaves and occasionally in the fruit. Isolation of this sugar and perseitol in good yield from avocado leaves is described. Brief photosynthesis in $C^{14}O_2$ produced labeled mannoheptulose monophosphate as well as sedoheptulose monophosphate and intermediates of carbohydrate synthesis. It was hydrolyzed to free mannoheptulose which was identified chromatographically. Paper chromatographic separation of borate complexes of these heptuloses and the hexoses has been accomplished.

The similarity of mannoheptulose to sedoheptulose suggested that it too might be involved in the regeneration of the carbon dioxide acceptor of photosynthesis and in the phytosynthesis of carbohydrate and aromatic compounds. The stereostructures of these heptuloses are related as are those of galactose and glucose. The nature of the interconversions of these isomeric pairs is not yet clearly understood.

Mannoheptulose had been previously isolated from one variety of avocado (*Persea gratissima*, var. Trapp) fruit[2,3] and we have found that it does not accumulate in several others. It has been identified in the dried root of *Primula elatior*[4] but may not occur widely in the plant kingdom. Avocado leaves appear to be a more reliable source of this sugar. They contain 0.5 to 1% wet weight of mannoheptulose. It was isolated from deionized leaf extract by crystallization from alcoholic solution after removal of hexoses by yeast fermentation.

As sedoheptulose is apparently derived from its 7-phosphate in sedum leaves it was suspected that mannoheptulose monophosphate should be found in avocado leaves. The labeled phosphorylated products of photosynthesis in $C^{14}O_2$ were fractionated by paper chromatography. The area containing sedoheptulose-7- and glucose-6-phosphates gave 12% radioactive mannoheptulose upon chromatography of the phosphatase hydrolysate.

Glucose and mannoheptulose cochromatographed in all the standard solvents used for sugar separations on paper. Glucose, mannoheptulose and sedoheptulose were therefore separated as borate complexes in a butanol–ethanol–borate buffered solvent, and the relative R_f values were 1.0, 0.74 and 0.54, respectively.

Experimental Part

Isolation of Mannoheptulose.—Fresh avocado (seedling tree) leaves (18 kg.) were macerated in Waring Blendors with three parts of cold water. The tissue was removed by filtration through cheesecloth and the yellow-brown aqueous solution was heated to 80–90° for one hour to precipitate proteins. After clarification by centrifuging, the solution was evaporated under reduced pressure to 10 l. Much color and the ionic constituents were removed by passing through columns of Dowex-50 and Duolite A3 ion-exchange resins (80–100 mesh). The pale yellow effluent solution was concentrated (< 40°) to a thin sirup which was mixed with two volumes of ethanol at 70°. The voluminous precipitate of slimy and pectic substances was filtered off and extracted twice with 1.5 l. of boiling 60% ethanol to extract any coprecipitated perseitol. The combined alcoholic filtrates were concentrated to a thick sirup (*ca.* 400 g.) at reduced pressure. This was taken up in 800 ml. of boiling methanol and stored at 10° for crystallization of perseitol[5] which continued several days.

The crude perseitol was dissolved in a minimum volume of hot 80% methanol, decolorized with charcoal, and allowed to crystallize. The yield after one further crystallization was 55 g., m.p. 187–188°.

Paper chromatographic examination of the mother liquor from the crude perseitol showed that the solution contained mannoheptulose and copious amounts of fructose, glucose and sucrose. To remove these, the solution was freed of methanol by evaporation, diluted with water to 2 l. and fermented with baker's yeast for 24 hours at 37°. The yeast was filtered off and the alcohol and some water removed by evaporation at reduced pressure. The residue was again diluted with water and the fermentation repeated. After three such fermentations, the solution contained very little hexose. The residue was concentrated *in vacuo* to a thick sirup and diluted with four volumes of hot methanol. Almost pure mannoheptulose separated on cooling and was recrystallized from 85% methanol. The yield of pure mannoheptulose was 160 g., m.p. 150–152°, with an appreciable amount of recoverable sugar remaining in the mother liquors.

Identification of Mannoheptulose Phosphate in Avocado Leaves.—The concentration of sedoheptulose-7-phosphate in many plants is near 10^{-4} *M* and it was suspected that mannoheptulose monophosphate concentration may be even lower. Therefore, labeled intermediates of $C^{14}O_2$ reduction were prepared in the manner generally used in this Laboratory.[6] After five minutes, photosynthesis in $C^{14}O_2$ was stopped suddenly by plunging the leaf into liquid nitrogen. The frozen leaf was ground and the powder dumped into boiling 80% ethanol for extraction. Two-dimensional (phenol–water, butanol–propionic acid–water) chromatograms were prepared from such extracts. Glucose-6-phosphate and sedoheptulose-7-phosphate are not generally separable in these solvents. Identification of the sugars in this monophosphate area is accomplished by dephosphoryl-

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ating with Polidase[7,8] and rechromatography of the free sugars. Authentic glucose and mannoheptulose failed to separate in any of the usual solvent systems used for sugars (phenol, butanol solvents, lutidine, collidine, etc.). It was therefore necessary to adapt the separation of the borate complexes[9,10] for the separation of this pair of sugars. Several *n*-butanol–ethanol–borate buffer (*p*H 6–8) solvents were tested. The more concentrated buffers gave good separation of glucose and mannoheptulose but had a tendency to give several sedoheptulose spots. The best solvent prepared was *n*-butanol 40 v.: ethanol 11 v.: borate buffer 19 v. (200 ml. H_2O, 1.25 g. of $Na_2B_4O_7 \cdot 10H_2O$, 0.25 g. of H_3BO_3). Tracer amounts of sugars or 40-μg. amounts separated on pre-equilibrated (6 hours) Whatman No. 1 paper after 40–48 hr. descending development. The lower edge was serrated to ensure linear solvent flow. Heptuloses were detected with orcinol–TCA–acetic acid reagent.[11-13] Presence of the buffer required more than one spraying to achieve maximum blue color. The mannoheptulose borate complex showed considerably more tendency to be adsorbed irreversibly on the cellulose than those of sedoheptulose or glucose. Forty hours development moved the mannoheptulose 24 cm. and the relative R_f values (compared to that of glucose) were: glucose, 1.0; mannoheptulose, 0.74; sedoheptulose, 0.54.

The ratio of glucose-6-phosphate concentration to mannoheptulose monophosphate in the products of five minutes photosynthesis (glucose phosphate was probably C^{14}-saturated while mannoheptulose phosphate may not have been saturated), was found to be 8:1. Of the free mannoheptulose in avocado leaves only a small fraction became labeled during the five-minute period compared to that previously observed for sugar phosphates.[14] No labeled mannoheptulose was detected among the otherwise normal products of ten-seconds photosynthesis in $C^{14}O_2$. A slow enzymatic exchange between free and phosphorylated mannoheptulose must therefore be assumed.

Fermentation of Glucose–Mannoheptulose Mixture Eluted from Chromatograms.—Chromatographic separation of heptuloses from relatively large amounts of glucose is best accomplished after removal of most of the glucose by yeast fermentation. Residual phenol and propionic acid from the eluted paper inhibited fermentation. To the eluate of five ether-washed "glucose" areas containing about 15,000 c.p.m. in 0.5 ml. was added 5 mg. of calcium carbonate and the mixture was boiled five minutes to remove volatile solvents. After cooling to 35°, 0.1 ml. of a fermenting mixture of baker's yeast in 2% glucose and an additional pea-sized lump of yeast was added and kept in suspension for 48 hours by frequent agitation. The solids were separated by centrifugation and washed several times with 0.5-ml. volumes of water. The extracts were concentrated and chromatographed. Without measurable diminution of heptulose content the glucose concentration had been reduced by about 90% which allowed improved separation of mannoheptulose from the remaining glucose.

Discussion

The occurrence of mannoheptulose phosphate in avocado leaves suggests that it is the direct precursor of free mannoheptulose in the leaves and fruit. The plant apparently transports the heptose as freely as it does the hexoses and sucrose which are formed in the leaf. Accumulation of free mannoheptulose in avocado fruits is probably due both to its translocation with the hexoses and sucrose during fruition and to the inability of the plant to utilize it readily as an energy and material source in its synthesis of new tissue.

Hudson[15] felt that the heptuloses played a prominent role in the metabolism of the tissues in which they occur. He had inferred that hexose metabolism in these tissues was negligible since fermentable sugars did not accumulate. In view of our observation of the normal sequence[3] of the early phosphorylated intermediates of photosynthesis in avocado leaves and the presence of considerable fermentable sugar, it seems only reasonable that plants of this type do not have an unusual metabolic pattern which Hudson supposed. Their uniqueness may lie rather in their particularly active mannoheptulose phosphatase and/or inactive mannoheptulokinase which leads to accumulation of heptulose. Heptulose metabolism in these tissues may be termed sluggish rather than particularly active. Other plants are known to have such heptulokinase activity[16] and it is almost certain that sugar synthesis and degradation proceeds only *via* phosphorylated intermediates. It is entirely possible that mannoheptulose phosphate may be a common metabolic intermediate whose identity has escaped notice.

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