The Ketoheptose Content of Some Tropical Fruits

James N. Ogata, Yoshihiko Kawano, Arthur Bevenue,* and Louis J. Casarett

Ten tropical fruits (17 varieties) were analyzed to determine their ketoheptose content. Paper chromatography and gas chromatography of the trimethylsilyl derivatives of the sugars were utilized to obtain the data. Avocado contained appreciable amounts of mannoheptulose (D-*manno*-heptulose), and trace amounts of this sugar were also found in mangos and passion fruit. Small quantities of sedoheptulose (D-*altro*-heptulose) were found in guava, mangos, papaya, and passion fruit. The analytical data coupled with the recent observation of an increased incidence of measurable quantities of sugar in the urine of local diabetics during the summer season when local fruits were consumed abundantly suggest a possible relationship between this phenomenon and the ingestion of increased amounts of fruits which contain mannoheptulose.

Mannoheptulose (D-*manno*-heptulose) was identified many years ago as a prominent constituent of avocado (LaForge, 1917). The observation that ingestion of the avocado may lead to the appearance of sugar in urine (Blatherwick *et al.*, 1940) suggested a biological significance for mannoheptulose in the diet. This sugar produces a hyperglycemia in man and animals when given by the oral route (Viktora *et al.*, 1969). The mechanism by which sugar levels are elevated involves an inhibition of secretion of insulin (Coore *et al.*, 1963), which is reversible and accompanied by a competitive inhibition of glucose phosphorylation (Malaisse *et al.*, 1968). In addition, mannoheptulose accelerates gluconeogenesis (Parry and Taylor, 1966). These and other facets of mannoheptulose action have been reviewed by Simon and Kraicer (1966).

In Hawaii, a variety of tropical fruits constitute an appreciable fraction of the diet during the summer season when the fruits are in abundant supply. It was recently reported locally (Ikezaki, 1969) that there was an increased incidence and quantity of measurable sugar in the urine of diabetics and an increased incidence of people who fit a classification of latent or asymptomatic ("chemical") diabetes (Malins, 1968).

This information prompted a survey of locally used tropical fruits for their mannoheptulose content. Analysis for sedoheptulose (D-*altro*-heptulose) was also included, although there has been a report of no diabetogenetic activity for this sugar on the basis of rat studies (Simon *et al.*, 1961).

EXPERIMENTAL

Gas Chromatograph. Varian Aerograph Series No. 1200, flame ionization detector; column temperature, 160° C; injection port temperature, 215° C; detector temperature, 220° C; range 1; attenuation 64. Gas flow rates: nitrogen 20 ml/min; hydrogen 20 ml/min; air 200 ml/min. Leeds and Northrup Speedomax H recorder, 1 mV full scale, chart speed 0.5 in./min. Gas chromatograph columns were 1/8 in o.d. by 6 ft borosilicate glass. Four columns were used for the chromatography of the per(trimethylsilyl) (TMS) sugar derivatives: (1) 3% SE-30 silicone on Chromosorb W, AW, DMCS, 60/80 mesh. (2) 3% SE-52 silicone on Chromosorb W, AW, DMCS, 80/100 mesh. (3) 4% SE-30 silicone and 6% QF-1 fluorosilicone on Chromosorb W, HP, 80/100 mesh. (4) 1.5% OV-17 and 1.95% QF-1 on Chromosorb W, HP, 100/120 mesh.

Numbers 3 and 4 packings were obtained from Supelco, Inc., Bellefonte, Pa.

Sugar Standards. D-*manno*-Heptulose was obtained from Nutritional Biochemicals, Cleveland, Ohio, and *myo*-inositol was obtained from Sigma Chemical Company, St. Louis, Mo. Sedoheptulose (D-*altro*-heptulose), isolated from *Sedum confusum*, was kindly furnished to us by Elizabeth McComb, University of California, Davis, Calif. A solution containing 10 μg/μl of each sugar was prepared for paper chromatography. Solutions containing 1 μg/μl of the sugars and 0.4 μg/μl *myo*-inositol, as the TMS derivatives, were prepared for gas chromatography.

TMS Reagents. Pyridine, hexamethyldisilazane (HMDS), and trimethylchlorosilane (TMCS) were purchased from the Pierce Chemical Co., Rockford, Ill.

Procedure. One-hundred-gram samples of ripe and unripe fruit were used for analysis. The peel and peeled fruits were analyzed separately. The procedure for the isolation of the seven-carbon sugars from the fruits utilized the extraction, deionization, and fermentation methods of Williams and Bevenue (1953). Initial sugar analyses were made by descending paper chromatography using a solvent system of ethyl acetate:pyridine:water (8:2:1 v/v). Orcinol reagent (0.5 g of orcinol, 15.0 g of trichloroacetic acid, 100 ml of isopropyl alcohol), which produces a specific blue color reaction with the ketoheptoses, was used for the detection of the spots on the paper chromatograms (Bevenue and Williams, 1960). The quantities of the sugars in the fruits were estimated by visual comparison with known amounts of mannoheptulose and sedoheptulose on the chromatograms. Confirmatory analyses were made by gas chromatography of the TMS derivatives of the sugars. Considerable work has been reported on the determination of sugars in biological materials whereby the TMS procedure was used (Davison and Young, 1969; McDonald and Newson, 1970; Kline *et al.*, 1970), and Johansson and Richtmyer (1970) used it to confirm the isolation of a *talo*-heptulose from the avocado.

Trimethylsilylation Reaction. An aliquot of the fermented fruit extract was concentrated to a syrup in a rotary vacuum evaporator and the method of Sweeley *et al.* (1963) was used to convert the sugars in the syrup to their derivatives. When large aliquots (equivalent to 25 g of the sample) of the extract

Departments of Agricultural Biochemistry and Pharmacology, University of Hawaii, Honolulu, Hawaii 96822.

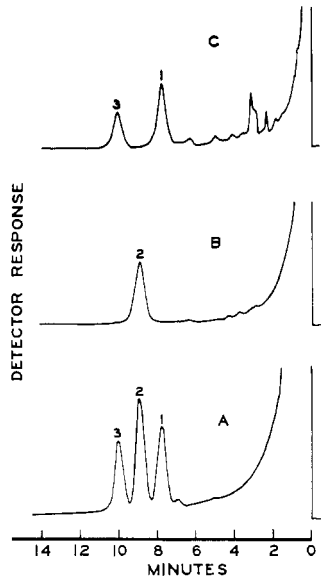

Figure 1. Gas chromatograph curves of (A) sedoheptulose 1, mannoheptulose 2, each 1 μg; and *myo*-inositol 3, 0.4 μg standards. (B) mannoheptulose in unripe avocado peel. (C) sedoheptulose 1 in ripe guava pulp. Gas chromatograph column 1.5% OV-17 and 1.95% QF-1 on Chromosorb W, HP, 100/120 mesh

Table I. Mannoheptulose Content of Avocados

Variety		P.C.[a] %	G.C.[a] %
Fuerte (Mexican–Guatemalan)	Unripe peel	0.68	0.53
	Unripe pulp	0.64	0.64
	Ripe peel[b]	0.06	0.04
	Ripe pulp	0.04	0.07
Frowe (West Indian)	Unripe peel	1.5	1.3
	Unripe pulp	2.5	2.5
	Ripe peel	0.50	0.55
	Ripe pulp	0.50	0.49
Itzamma (Guatemalan)	Unripe peel	2.0	2.0
	Unripe pulp	1.0	1.2
	Ripe peel	1.6	1.4
	Ripe pulp	0.20	0.16
Haas (Guatemalan)	Unripe peel	3.0	2.0
	Unripe pulp	1.0	0.86
	Ripe peel	0.40	0.60
	Ripe pulp	0.03	0.05

[a] P.C. = paper chromatography; G.C. = gas chromatography. Fruit ripened by storing at room temperature for 5–7 days.

were used, about 4 ml of pyridine was added, and up to a tenfold increase in the amounts of HMDS and TMCS was required. The mixture was shaken vigorously for about 1 min and allowed to stand overnight at room temperature. A 1-μl aliquot was injected into the gas chromatograph. A linear detector response was obtained within the examined range of 0.1–1 μg of mannoheptulose and sedoheptulose.

RESULTS AND DISCUSSION

In the gas chromatograph portion of this study, the commonly used liquid phases SE-30 and SE-52 could not be used on the chromatograph column for the analysis of mannoheptulose, because of interference by *myo*-inositol, which is a common constituent of many plants. The TMS derivatives of the two compounds have similar retention times on these columns. Trials with other liquid phases showed that a mixture of 4% SE-30 silicone and 6% QF-1 fluorosilicone (Column No. 3) gave a partial separation, with mannoheptulose as a shoulder on the *myo*-inositol peak. A mixture of 1.5% OV-17 and 1.95% QF-1 fluorosilicone (Column No. 4) effectively separated the two compounds (Figure 1). The gas chromatographic technique is very effective in the study of the seven-carbon sugars in plant materials when the sugars are present in the low concentration of 0.01%. It may be difficult to extend the detectable limit below this amount for some fruits without additional cleanup procedures for the sample because of the increased background that appears on the recorder chart of the gas chromatograph.

Table II. Ketoheptose Content of Mangos

	Mannoheptulose				Sedoheptulose			
	Peel		Pulp		Peel		Pulp	
Variety	ripe	unripe	ripe	unripe	ripe	unripe	ripe	unripe[a]
Haden	T[b]	T	T	T	T	T	0.02–0.04	
Earlygold	T	T		T	T	T	0.01	T
Harries	T	T					T	
Zill	T	T	T	T	T	T		T

[a] Fruit ripened by storing at room temperature for 5–7 days. [b] T = trace (less than 0.01% in the fresh fruit).

Fruits analyzed for ketoheptoses included acerola (*Malpighia glabra L.*), avocado, banana (Bluefield and Williams varieties), dragon eye (*Euphoria longan* [Lour.] Steud), guava, lychee (*Litchi chinensis* Sonn), mangos, papaya, passion fruit, and pineapple. Of the ten fruits (17 varieties) analyzed, five contained ketoheptoses, of which three contained mannoheptulose. Only the avocado contained appreciable concentrations of mannoheptulose (Table I). The mannoheptulose content of the avocado was greater in the peel and pulp of the unripe fruit and decreased as the fruit ripened, an observation which agreed with the studies made by Davenport and Ellis (1959). The data for some of the avocado varieties are similar to those reported by LaForge (1917), who found about 1.4% in the pulp.

Trace amounts of mannoheptulose (less than 0.01% in the fresh fruit) were found in mango and passion fruit. The mango ranked second in ketoheptose content and in the consistency with which these sugars occurred in the fruit (Table II). Mannoheptulose was not present in the pulp of the Harries variety or the ripe pulp of the Earlygold; sedoheptulose was present in all ripe pulps of the mangos except the Zill variety.

Trace amounts of sedoheptulose were observed in ripe papaya peel and pulp and in the unripe peel and pulp of guava and passion fruit. About 0.04% of this sugar was found in ripe guava pulp. No mannoheptulose was present in papaya and guava.

Figs are also consumed locally, and a previous report (Bevenue *et al.*, 1961) showed that this fruit contains trace amounts of mannoheptulose and sedoheptulose.

Of the fruits analyzed, avocado is unique by virtue of its relatively high mannoheptulose content. The biological significance of the analytical findings reported herein remains to be assessed. Annual avocado production in Hawaii is about 1,000,000 lb, excluding home-grown fruit, and all of this is consumed locally. The dose of mannoheptulose obviously depends on the degree of ripeness of the fruit and the

amount consumed. It is not unusual for citizens to consume several avocados daily during the season.

Similarly, mangos produced in this State are not exported, trees and fruit are plentiful, and consumption is heavy during the season. They are eaten at all degrees of ripeness and are included in a variety of food forms, such as preserves, chutney, and pastries, which customarily require a low degree of ripeness. About 2,500,000 lb of passion fruit were processed in the form of canned juices, nectars, and sherberts in 1970, and much of this supply was consumed locally.

Compared to doses of mannoheptulose necessary to produce marked experimental hyperglycemia in animals and man, doses customarily ingested are likely to be low. It is reasonable, however, to expect that some ingestion patterns will result in doses of mannoheptulose similar to those found to be minimally effective experimentally. This possibility is augmented by the ingestion of several types of fruit during the season. Although a frank hyperglycemia may not be produced in a normal individual, a "chemical" or asymptomatic diabetic may require a smaller dose to produce hyperglycemia. It has been well demonstrated that small experimental doses which do not produce hyperglycemia still produce measurable changes in the secretion of insulin. These observations suggest that it would be profitable to explore the relation between diabetic symptomology and the oral ingestion of avocados and other tropical fruits.

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Received for review June 7, 1971. Accepted August 12, 1971. Journal Series No. 1333 of the Hawaii Agricultural Experiment Station.