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The Occurrence of Sedoheptulose in the Dried Root of *Primula elatior* (L) Hill

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For some time we have been engaged in investigating the distribution of heptoses in the plant kingdom [1–6] and have carried out screening tests on aqueous extracts of about 100 plants belonging to 60 different families. Initially we utilised the Bial reaction [7] but subsequently also made use of a new spraying reaction for ketoheptoses [5], and the results obtained encouraged us to continue the investigation.

For more detailed examination we chose several Primula species, as previous workers had demonstrated that the heptahydric alcohol volemitol occurs in several Primulas [8] and we felt it would be of interest to determine whether the corresponding sugars — sedoheptulose and mannoheptulose — are present in the same material. V. Ettel and L. Liebster [9], and others have shown that volemitol can be converted into these two sugars.

We have now examined the constitutents of aqueous extracts of dried root stock of *Primula elatior* (L) Hill and have identified sedoheptulose, fructose, glucose, saccharose and volemitol, and obtained evidence for the presence of mannoheptulose. All the carbohydrates were identified by paper chromatography, authentic samples being run simultaneously and the spots being detected by spraying with the reagent mentioned above which gives colours with fructose and saccharose as well as with ketoheptoses. Aldoses, on the other hand, do not react with this reagent, and were detected with *m*-phenylenediamine hydrochloride.

In addition, the sedoheptulose was isolated by partition chromatography on a column of cellulose, and identified by conversion into the dibenzylidene derivative of sedoheptulosan [10].

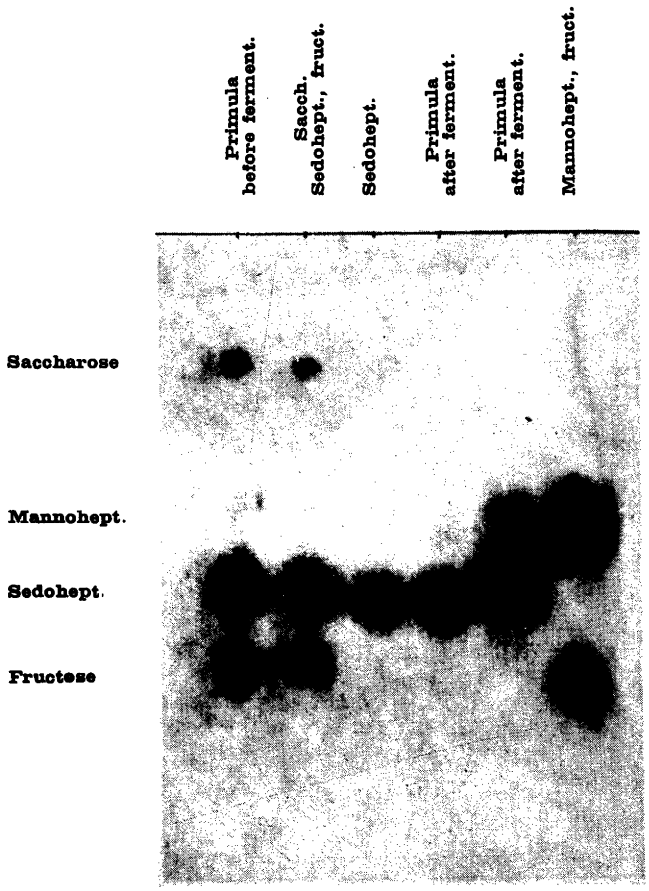

Fig. 1. The chromatogram has been run for 48 hours with ethyl acetate — acetic acid — water, then sprayed with orcinol — trichloroacetic acid reagent and heated at 100° for 10 min. The colours of the spots were:

Saccharose and fructose	*yellowish green*
Mannoheptulose	*green*
Sedoheptulose	*bluish green*

EXPERIMENTAL

The dried root (2.3 kg of commercial material shown by microscopical examination to consist mainly of *Primula elatior*) was extracted twice with a total of ten times its weight of water and the residue was pressed to remove as much of the liquid as possible. The extract was treated with a slight excess of basic lead acetate, the excess lead being removed with H_2S. The resulting solution was concentrated under reduced pressure (12—15 mm) to a weight of approximately 300 g, then set aside in the refrigerator. Most of the volemitol crystallised out and was collected. The mother liquor was precipitated

with alcohol, the insoluble material filtered off, and the alcoholic solution evaporated under reduced pressure. Paper chromatography of the resulting syrup indicated the presence of a considerable amount of sedoheptulose.

Attempts to prepare the dibenzylidene derivative of sedoheptulosan from this syrup were unsuccessful. The volemitol present reacted to form a tribenzylidene derivative [11], which inhibited the crystallisation of the derivative of the anhydro sugar. It was found necessary to remove the volemitol before successful preparation of the dibenzylidene sedoheptulosan.

Pure sedoheptulose was isolated from the syrup as follows: A part of the syrup (4 g) was dissolved in water (30 ml) and the resulting solution was freed from organic acids and salts by passage through "Zeokarb 215" and "Amberlite IR-4B" ion-exchangers. One drop of dilute sulphuric acid and a little phosphate were added to the resulting neutral solution, and the mixture was fermented with yeast until gas formation ceased (4 hours). The solution obtained was filtered, boiled, and evaporated on a water bath to yield a syrup (ca. 0.5 g) Chromatography of the syrup before and after fermentation, using ethyl acetate — acetic acid — water (3 : 1 : 3) as solvent, Whatman No. 1 paper, and orcinol — trichloroacetic acid for detection, gave the results shown in Fig. 1. Water saturated phenol as solvent gave similar results.

The volemitol present could be identified by paper chromatography using water-saturated *n*-butanol as solvent and ammoniacal silver nitrate (5 % $AgNO_3$ in ca. 90 % alcohol with excess ammonia) as developing agent.

The sedoheptulose and volemitol were separated by partition chromatography on a column of powdered cellulose (Whatman ashless tablets) using water-saturated *n*-butanol [12]. The fractions were tested for sedoheptulose, mannoheptulose, and volemitol with the reagents mentioned above. A trace of unfermented fructose passed through the column first, and was followed by the fractions containing the sedoheptulose.

The latter fractions were combined and evaporated under reduced pressure, and the residue (ca. 50 mg) was treated with conc. sulphuric acid (one drop) and benzaldehyde (one drop) with shaking for two hours, during which time crystallisation took place. The mixture was set aside in the refrigerator for 24 hours, and the solid was collected, washed with alcohol, water, and hot alcohol, and recrystallised from acetic anhydride: m.p. (micro) 242—245°.

This material consisted of small rods exhibiting parallel extinction, some prismatic with refractive indices 1 614, 1 624 and some acicular with a higher refractive index, 1 640 — in agreement with the data found previously for dibenzylidene sedoheptulosan [6].

SUMMARY

Partition chromatographic methods have demonstrated the presence of sedoheptulose, fructose, glucose, and saccharose, and the probable presence of mannoheptulose in dried root stock of *Primula elatior* (L) Hill. This is the first occasion on which sedoheptulose has been identified in a plant which is not a member of the family *Crassulaceae*.*

* Since this paper was submitted for publication we have noticed the appearance of a paper by A. A. Benson, J. A. Bassham, and M. Calvin (*J. Am. Chem. Soc.* **73** (1951) 2970). Studying the compounds formed during short periods of photosynthesis in $C^{14}O_2$– atmosphere, they isolated small amounts of labelled sedoheptulose monophosphate from several plants.

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