

Studies of the Constituents of Crassulacean Plants

I. Paper Chromatographic Investigation of the Free Sugars of some *Sedum*, *Sempervivum*, *Echeveria*, and *Crassula* Species

A. NORDAL and R. KLEVSTRAND

Department of Pharmacognosy, Pharmaceutical Institute of the University, Oslo, Norway

The study of naturally-occurring sugars has been greatly facilitated by the introduction of paper partition chromatography¹, the substances being identified by their R_f -values with various solvent systems, and by the colours produced with various spraying reagents.

The plant family *Crassulaceae* is interesting from a biochemical point of view, among other reasons, because there has been found in certain species of this family a seven carbon ketose, *sedoheptulose* (D-althroheptulose, cf. *Advances in Carbohydrate Chemistry* I, p. 47)². Identification of this sugar has previously been effected by the isolation of derivatives such as the dibenzylidene derivative of the anhydro sugar or the osazone. Sedoheptulose gives, like the other heptoses, a colour reaction with orcinol and hydrochloric acid (Bial's reagent); in this case the colour is bluish-green. The reaction is, however, not specific, as pentoses and uronic acids give approximately the same colour. As described elsewhere³, we have found a spraying reagent which seems to give a specific colour reaction with ketoheptoses on paper chromatograms. As both the location of the spots on the chromatogram and the colour produced with this reagent are characteristic, we think that the method affords complete certainty for the detection of ketoheptoses. A few drops of fresh juice (the plants are succulents) are sufficient for the examination of the free sugars in the plants.

The objects of the investigations described here have been:

1. to study the distribution of sedoheptulose in the plant family *Crassulaceae* and possibly in closely related families,
and
2. to investigate the presence of other free sugars in these plants.

With respect to the last point there appears to be some disagreement in the relevant literature. According to Hudson (*Advances in Carbohydrate Chemistry* I, p. 14)² fermentable sugars are absent from the tissues in which heptuloses (ketoheptoses) occur, and our previous researches had supported this contention. This presupposes, however, a special form of carbohydrate metabolism, as the plants contain starch. In plantphysiological studies of Crassulacean metabolism investigators have made particular use of *Bryophyllum calycinum* Salisb.^{4, 5}, a Crassulacean plant which contains sedoheptulose. In this case they have shown that at least a part of the sugar mixture is fermentable, and have calculated this part as glucose in the usual way. It has also been found that there exists a genetic connection between starch and fermentable sugars on the one hand and organic acids on the other^{4, 5}.

Our investigations have shown that all the species examined contain sedoheptulose and in addition, fermentable sugars. The proportions of the individual sugars are, however, different. Sedoheptulose is the predominating sugar in most of the species we have examined. In the genus *Crassula* it seems, however, that the hexoses predominate. We have not carried out quantitative determinations of the sugars, but from the size of the spots and the intensity of the colour we have obtained some estimate of the relative proportions. Attention is drawn to the table in the experimental section.

It is our aim to investigate the free sugars of representatives of as many as possible of the genera belonging to the family *Crassulaceae*. Much remains to be done in this respect, however, as most of the genera have no representatives growing in the wild state in Norway. The species we have examined were practically all obtained from the Botanical Gardens of the University of Oslo, but even there only a minority of the genera are represented. Nevertheless it is our hope to be able to extend the investigations to cover more genera and species of this and closely related families.

EXPERIMENTAL

Juices pressed from fresh plants were used for the experiments, in some cases directly, in others after concentration on a water bath. About 0.01 ml of the juice was placed on the paper (Whatman No. 1). The solvent used was ethyl acetate-acetic acid-water (3 : 1 : 3)⁶ and the chromatogram was run for 24–48 hours, during which time the solvent ran off the lower edge of the paper. R_f -values were therefore unobtainable and

instead we noted the distance which the individual sugars had advanced in the course of 24 hours. The following sugars were used for comparison: sedoheptulose, fructose, glucose and sucrose, all in approximately 1 % solution, using 0.005–0.01 ml of the solutions each time.

As spraying reagents we used:

1. Orcinol and trichloroacetic acid in *n*-butanol, for the detection of ketoses³.
2. Aniline hydrogen phthalate in *n*-butanol⁷, and
3. *m*-Phenylenediamine in aqueous alcohol⁸, the latter two for the detection of both aldoses and ketoses.

The results are given in Table 1.

The reagent which has been used most extensively is orcinol and trichloroacetic acid, with which, however, glucose cannot be detected. General sugar reagents (nos. 2 and 3 above) were only used on chromatograms of 3 species, in order to demonstrate positively the presence of glucose. Although we have not demonstrated the presence of this in the other species, we consider it extremely probable that it occurs there also, together with fructose and sucrose.

Table 1. Free sugars of some Crassulacean plants, detected by means of paper partition chromatography. In cases where the presence of the same sugar has been demonstrated earlier, the reference is given. The sugars have not been determined quantitatively, but from the size of the spots and the intensity of the colours we have estimated the quantitative relationship.

The predominating sugar is denoted by ++; very weak spots are denoted by (+).

Species	Sedo-heptulose	Fructose	Glucose	Sucrose
<i>Sedum spectabile</i> Bor.	++ ⁹	+	+	
<i>S. acre</i> L.	++ ^{10, 11}	+		+
<i>S. rupestre</i> L.	++	+	+	+
<i>S. spurium</i> M. B.	++ ¹¹	+		+
<i>S. Telephium</i> L.	++ ¹¹	+	+	+
<i>Sempervivum Verloti</i> Lamotte	++	+		(+)
<i>Echeveria secunda</i> Booth var. <i>glauca</i> Hort.	++	+		+
<i>Crassula lactea</i> Soland	+	++		(+)
<i>C. portulacea</i> Lam.	+	++		(+)

Distance the substances had run in the course of 24 hours at ca. 18° C (in cm): 14.5–15.5 15–16 13–14 7–8

SUMMARY

The free sugars in the pressed out juices of some *Crassulacean* plants have been studied by means of paper partition chromatography. In this way sedoheptulose has been demonstrated to occur in some species in which it has not

been detected previously. Together with sedoheptulose, there was also found glucose, fructose and sucrose.

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