CXXVIII. THE PENTOSAN CONTENT OF *KLEINIA ARTICULATA*.

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THE results of this paper represent part of a preliminary survey of the metabolism of the succulent composite *Kleinia* (*Senecio*) *articulata*.

The formation of mucilaginous substances of a pentosan nature has been put forward as a direct cause of the succulent habit [McDougal *et al.*, 1918, 1919]. It is claimed that the peculiar metabolism of succulents involves a partial change from a hexose to a pentose metabolism, this change being facilitated by exposure to high temperatures and to a low water supply. This view has been discussed in a previous publication [Evans, 1932].

The sap of *K. articulata* after filtration did not appear to be at all mucilaginous, and some quantitative knowledge of the pentosan content seemed desirable.

Some investigators [Rosa, 1921; Doyle and Clinch, 1926] have distinguished between water-soluble pentosans; those insoluble in water but hydrolysed and brought into solution by 1 % HCl; and those insoluble in water but which require fairly concentrated (12 %) HCl to hydrolyse them and bring them into solution.

The exact nature of these three classes of pentosans and the part which they play in the metabolism or structure of the cell is unknown. Nevertheless it is fairly probable that the pentosans extractable by hot water alone are normally in solution (either true or colloidal) in the cell-sap, and that those which are only hydrolysed by 12 % HCl represent part of the wall structure.

It must be remembered however that the pentosans are not the only substances which contain pentose sugars in their molecule; in particular, hemicelluloses and pectic substances also generally contain pentoses, which are set free on hydrolysis. According to Onslow [1931], a certain class of compounds, of which glycuronic and galacturonic acids are members, produce furfuraldehyde under the same conditions as pentoses and pentosans. In most of the pentosan determinations such substances are also included, and the determinations thus approximate more closely to a measure of pentose metabolism than to a measure of any particular class of pentose-containing substances.

Those pentosans which are extracted by 1 % HCl, and especially by 12 % HCl, are probably part of the wall-structure, and possibly include pectic

substances and other complex polysaccharides brought into solution by the acid.

The pectic substances in particular have been shown to be of more or less universal occurrence in plants [Carré, 1929]. According to Onslow [1931] they are found to the greatest extent in fleshy and succulent tissues.

It is evidently those pentosans which are in solution in the cell-sap, and which presumably form the bulk of the pentosans extractable by water, which are of greatest interest in connection with the part played by pentosans in the causation of succulency.

Nevertheless the pentosan theory of succulency has been based upon the total pentosans (and other pentose-containing substances) extractable by 1 % HCl. No data are apparently available regarding the water-soluble pentosan content of succulents.

In the present work the three classes of pentosans, as separated by solubility in water, 1 % HCl and 12 % HCl respectively, have been determined wherever possible.

Method.

About 12 similar plants were taken for the determinations, their fresh weight being determined, and they were then cut into small portions about 0.5–1 cm. long. These small pieces were well mixed, and about 10 g. used for a dry weight determination.

The remainder were immediately killed by plunging into boiling alcohol containing a little strong ammonia, and boiled for about 30 minutes. They were then separated from the alcohol, placed in an oven at 95° and thoroughly dried. The alcohol belonging to each set was carefully kept.

The dried plants were finely ground in a mortar and transferred into a strong round-bottomed flask fitted with a reflux condenser, together with the alcohol that had been used for killing them.

The mixture was boiled gently for about 5 hours, by which time the alcohol-soluble sugars were entirely removed; the alcohol was filtered off, and the residue washed repeatedly with fresh portions of alcohol, which were added to the main bulk.

This procedure not only prevents any hydrolytic and enzymic changes in the tissue by almost instantaneous killing, but the preliminary drying and grinding of the tissues greatly shorten the time required for complete extraction of the sugars. The alcohol-soluble sugars were estimated in a few cases, but attention was paid in the main to the sugar-free residue.

This was dried and boiled with water for 5 hours. The residue was filtered off while hot and the watery extract kept for determinations of water-soluble pentosans. To the residue were added 150–200 cc. of 1 % HCl, and digestion was continued at the boiling-point for 3 hours. The mixture was filtered and the filtrate kept for the determination of the 1 % HCl-soluble fraction. The residue was retained for the determination of "residual" or 12 % HCl-soluble pentosans.

The pentosan contents of the three fractions were determined by distillation with 12 % HCl under Kröber and Rimbach's conditions [1902], the pentosan being thus converted into furfuraldehyde which was precipitated as phloroglucide in the distillate. All the determinations were carried out under similar conditions so that the results are comparable.

Results.

In order to obtain some estimate of the degree of uniformity to be expected in the plants used, three samples were taken at the same time, each sample consisting of five similar plants.

The results of these determinations are given in Table I.

Table I. *Mature stems taken 12.30 p.m., October 30th.*

No of set	Dry matter %	% of dry weight			
		Water-sol.	1 % HCl-sol.	12 % HCl-sol.	Total
1	3.73	0.75	4.44	2.82	8.01
2	4.00	1.25	4.58	2.39	8.22
3	3.53	0.88	4.59	2.48	7.95

The total pentosan contents of these samples agreed fairly closely. Regarding the slight differences, it is interesting to note the inverse relation between total pentosan content and water content, though this is of doubtful significance.

The proportions of pentosans in the three fractions also differ slightly, but the main outcome of the experiment is to show that only about one-eighth of the total pentosan is in the water-soluble form.

The results of further pentosan determinations are given in Table II. Each sample of these experiments contained 12–15 similar plants. In general there is no great difference between the water-soluble pentosan content of leaves and young stems, and of more mature shoots, the slight difference that was shown in most of the experiments indicating a slightly higher amount in the former.

The total pentosan content of leaves however is much less than that of stems, the difference becoming more marked with increasing age of the stem. In the case of both stems and leaves the greater part of the pentosans is that fraction which is hydrolysable by 1 % HCl. In old joints however, as would be expected, the proportion of the residual or 12 % HCl-soluble pentosans is considerably higher.

In the case of stems the quantity of 1 % HCl-soluble pentosans increases considerably with age up to maturity, but decreases in very old stems. Most of the material denoted as "old" in the experiments of Table II had not passed the mature stage, but that of Exp. 3 was definitely older.

Table II. % of dry weight

Description of material	Part of plant	% of dry weight					Total "loose" i.e. 1 % HCl-sol. + water sol.
		Dry matter %	Water- sol.	1 % HCl- sol.	Residual or 12 % HCl- sol.	Total pento- san	
Exp. 1. <i>Kleinia articulata</i> . Outer house. Sept. 11th, 1930	Whole plants	—	—	—	—	—	—
Exp. 2. <i>K. articulata</i> . Similar material to that of Exp. 1, but slightly younger. Sept. 12th, 1930	„	6.46	—	—	1.94	7.34	5.40
Exp. 3. <i>K. articulata</i> . Old joints (1929 growth), young joints (1929-30 winter growth) bearing leaves. Inner house. Sept. 22nd, 1930	1. Old joints	5.72	0.664	2.34	3.64	6.64	3.00
	2. Young joints	4.27	0.757	5.44	2.60	8.80	6.20
	3. Leaves	4.30	0.740	—	—	—	—
Exp. 4. <i>K. articulata</i> . Long slender plants grown in shade from cuttings planted Nov. 20th, 1929, old joints 1929-30 growth, and young joints of 1930 summer growth bearing large leaves. Oct. 6th, 1930	1. Old joints	5.25	0.38	4.61	3.45	8.44	4.99
	2. Young joints	3.57	0.34	4.13	2.87	7.34	4.47
	3. Leaves	2.56	0.44	2.33	1.97	4.74	2.77
Exp. 5. <i>K. articulata</i> . Plants grown from cuttings made July 15th. From Sept. 18th onwards. I. Watered daily. II. Left without water. Analysed Oct. 18th, 1930	I. Young joints	3.54	—	—	2.02	6.17	4.15
	I. Old joints	5.64	—	—	4.07	9.77	5.70
	II. Young joints	5.68	—	—	1.96	6.76	4.80
	II. Old joints	8.00	—	—	3.61	8.41	4.80
Exp. 6. <i>K. articulata</i> . Plants from same box as material of Exp. 5, growing under normal conditions from July 15th to Oct. 30th. Oct. 30th, 1930	Young joints + leaves	3.85	—	—	2.12	5.67	3.55
	Old joints	5.23	—	—	3.56	9.94	6.38
Exp. 7. <i>Mesembryanthemum edule</i> . Leaves of one large plant used. Oct. 10th, 1930	Leaves	9.24	1.80	3.76	0.23	5.79	5.56
Exp. 8. <i>Fuchsia</i> leaves. Oct. 9th, 1930	Leaves	16.03	1.05	1.53	2.05	4.63	2.58

Plants which were grown in the shade (Exp. 4), which are thin and tender compared with normal plants and which have a relatively greater water content, have a very low pentosan content, this being especially so for the water-soluble fraction.

An experiment was carried out to test the effect of dryness on the pentosan content.

From a batch of similar plants two samples were transplanted. One of these samples was watered daily for one month, the other was kept unwatered, and both were analysed at the same time (Exp. 5). Another sample which had been left under ordinary conditions was analysed a fortnight later (Exp. 6).

The differences are irregular and do not reveal any appreciable effect of dry conditions on pentosan content. The differences between samples I and II in terms of fresh weight are to be interpreted largely as the reflection of a reduction in the fresh weight of the unwatered plants which was used as the basis for the calculations.

For comparison with *Kleinia*, pentosan determinations were carried out for the leaves of *Mesembryanthemum edule* and *Fuchsia*; the results are given in Table II (Exps. 7 and 8).

It is obvious that, although the total pentosan content of *Kleinia* may be greater than that of *Mesembryanthemum* and *Fuchsia*, the proportion in the water-soluble form is much higher in the latter plants.

While the water-soluble pentosan (expressed as a percentage of the total) is about 5–15 % in *Kleinia*, in *Mesembryanthemum edule* it is 32 %, and in *Fuchsia* 44 %. The absolute amounts of water-soluble pentosans are also higher in these plants than in *Kleinia*. In all these experiments large samples were used and the results represent a mean value for these particular plants.

It may be objected that the preliminary extraction of the tissues with alcohol to remove soluble sugars may cause a physical change in the water-soluble pentosans so that subsequently they are not easily soluble in water. This would mean that the pentosans extractable by hot water did not correspond in quantity to those actually in solution in the sap of the intact plant.

In order to test this point, an experiment was carried out, in which the fresh juice was expressed and pentosan determinations made on the extracted sap, and on the residual tissues.

	Pentosan % fresh weight	
	Sap	Residue
Mature plants of 1931 summer growth. Taken 11 a.m. 5. ii. 32	0.028	0.462

The pentosan in solution in the cell-sap corresponds closely to the quantities previously determined in the watery extract. Calculating data from Table II as percentages of the fresh weight, the water-soluble pentosan of stems ranges from 0.012 to 0.038 and the corresponding total pentosan from 0.262 to 0.443.

For *Mesembryanthemum* the figures in terms of fresh weight are 0.167 water-soluble and 0.536 total; for *Fuchsia* 0.169 and 0.744.

SUMMARY AND CONCLUSIONS.

These determinations have shown that *Kleinia articulata* contains an amount of pentosan similar to that found in other succulents. It has also been shown however that only a small proportion of this is in solution in the cell-sap.

The major part of the pentosan comes within the fraction which is extracted by 1 % HCl, while there is also a considerable amount which is only extracted by 12 % HCl. It is probable that the greater part of this pentosan is derived from the breakdown of the cell-wall, and to some extent of the plant protoplasm.

As the water-soluble pentosans seem to be of the greatest interest in relation to the pentosan theory of succulence, the fact that *Kleinia* contains such a small amount does not lend much support to the theory, especially when it is considered that a plant like *Fuchsia*, which is not usually classed as succulent, contains greater quantities.

It is possible that the loosely bound pentosans extractable by 1 % HCl, in spite of their insolubility in water, may play a part in the water relations

of the cell, but it appears unlikely that a slightly greater quantity of these than that found in mesophytes is sufficient to transform the make-up of the plant, and to furnish it with the massive water-storage tissue characteristic of succulents.

I am indebted to Prof. D. Thoday for suggesting this work and for continued interest throughout.

REFERENCES.

- Carré (1929). *Food Investigation Special Report*, No. 33. London (H.M. Stationery Office).
Doyle and Clinch (1926). *Proc. Roy. Dub. Soc.* **28**, 219.
Evans (1932). *Biol. Rev.* **7**, 181.
Kröber and Rimbach (1902). *Z. angew. Chem.* **15**, 477.
McDougal and Spoehr (1918). *Plant World*, **21**, 245.
——— Richards and Spoehr (1919). *Bot. Gaz.* **67**, 405.
Onslow (1931). *The principles of plant biochemistry*. Part I. (Cambridge.)
Rosa (1921). *Mo. Agric. Exp. Sta. Res. Bull.* No. 48.