in field and laboratory tests. A start has been made in this direction by the sugar industry in Hawaii[1-3]).

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Phosphorylase Inhibitor in the Rind of Tapioca tuber

During an investigation on the phosphorylase activity of tapioca tuber *(Manihot utilissima)*, it was found that alcoholic extract of the rind and also the fleshy portion of the tuber to a much less extent, contain an inhibitor of phosphorylase activity. The presence of a similar inhibitor has been reported in potato tuber[1]). The present note deals with investigations on the inhibitor in tapioca rind.

The rind (consisting of the portion outside the Cambium layer) and the fleshy portion (mainly secondary xylem which is the storage tissue) of the fresh tuber collected from the Tuber Crops Research Station, Trivandrum, were extracted separately with absolute alcohol. The alcohol solutions were concentrated in vacuum and the solvent removed completely. The brown mass was taken up in water, the solution centrifuged and the clear supernatant was used for the study of inhibitor action. The juice of the fleshy portion of the tuber clarified by centrifugation was used as the source for phosphorylase. The phosphorylase activity was measured by the method of GREEN and STUMPF[2]). The results are given in the Table.

Table. *Effect of the alcoholic extract of the rind and the fleshy portion on phosphorylase activity*

The reaction system contains 1 cc. enzyme solution (clarified juice of fresh tapioca tuber) + 0·8 cc. inhibitor solution (0·8 cc. water in control) + 0·5 cc. of 0·5 M citrate buffer (Ph. 6·0) + 0·2 cc. of 5% starch solution + 1 cc. of 0·1 M. glucose-1-phosphate. — A Control; B enzyme + material from the alcoholic extract of the rind; C enzyme + material from alcoholic extract of the fleshy portion.

Experiment No.	Phosphorylase activity in units/cc.			% of inhibition	
	A	B	C	B	C
I	7·8	2·44	7·8	68·71	0·00
II	6·83	0·00	4·87	100·00	28·69
III*)	3·90	0·00	2·92	100·00	25·13
IV*)	1·46	0·00	0·97	100·00	33·56
V*)	2·19	0·487	1·95	77·76	10·95

*) Tuber collected during summer months.

The material obtained from the rind thus showed inhibition of phosphorylase activity ranging from 68·75% to 100%, while the fleshy portion showed only inhibitory activity ranging from 0 to 33%. Another observation made during these investigations was that for the same age group, the phosphorylase activity of the tuber was approximately 50% less in summer (May-June) than in other months.

Alcoholic solution of the material from the rind showing inhibitor activity, on paper chromatography using N-butanol — acetic acid — water (4 : 1 : 5) as the solvent and ferric chloride — ferricyanide solution as the spotting reagent showed the presence of nearly 13 polyphenolic compounds with *Rf* values ranging from 0·047 to 0·934. In this connection it may be mentioned that in potato, the inhibitor activity is supposed to be due to chlorogenic acid[1]). Detailed investigation on the isolation and nature of the inhibitor in tapioca rind is in progress.

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Large Accumulation of Oxaloacetic Acid in Leaves of Coleus Aromaticus (Hooker)

The recognition of keto acids as important intermediary plant metabolites has resulted in investigations showing their presence in a large variety of plant tissues[1]) and significant high values have been reported for α-ketoglutaric acid, pyruvic acid and glyoxylic acid. Presence of oxaloacetic acid has been shown in very few plants however and the highest value reported so far is of the order of 650 μg per 100 g fresh weight in strawberry leaves[2]); the other lower values being 106 μg for washed potato slices, 50 μg for *Oxycoccus quadripetalus* berries and 25 μg for *Vaccinium vitis-idaea* berries[3]). The low values of oxaloacetic acid are of course due to its key position in the operation of the citric acid cyle, being involved in the primary condensation reaction:

Oxaloacetic acid + Acetyl-CoA → Citric acid.

Analysis of keto acids, as their hydrazones, present in the leaves of *Coleus aromaticus*, a plant belonging to family Labiatae, has shown large accumulation of oxaloacetic acid which is of the order of 13,000 μg per 100 g fresh weight of the leaves. The keto acids were extracted as described by TOWERS et al.[4]) starting with 38 g fresh weight of the leaves. They were separated on special chromatographic grade of Whatman No. 1 filter paper by ascending chromatography in three solvent systems viz. n-butanol saturated with ammonia[5]), n-butanol-ethanol-water[6]) and two dimensional chromatography using combination of solvents 1 and 2. Keto acid hydrazones prepared from synthetic acids were run simultaneously for comparisons.

The keto acids were detected by their yellow spots and by viewing them under a chromatolite U.V. lamp. They were identified on the basis of their *Rf*, the characteristic colour reaction with alcoholic sodium hydroxide spray and their absorption spectra. The oxaloacetic acid hydrazone spot gave a characteristic dark-green-brown colour when sprayed with ethanolic sodium hydroxide. Microgram of oxaloacetic acid present was calculated in terms of absorption of α-ketoglutaric acid hydrazone since the synthetic oxaloacetic acid (NBCO) was contaminated with its breakdown products and was therefore unsuitable for being used as a standard. Moreover the E_{max} of OAA and α-KGA hydrazones have been shown to be similar, this being very close to 385 mμ[7]). A calibration curve was obtained with α-KGA hydrazone by measuring the optical density at 385 mμ using mercury line.

The striking similarity in the chromatograms showing the separation of keto acid hydrazones of *Coleus* leaf extract and of the synthetic oxaloacetic acid with its breakdown products, on a two-dimensional chromatogram and the large amount of oxaloacetic acid that is present in *Coleus* leaves may well suggest that oxaloacetic acid was probably the most dominant keto acid present in this tissue and that during extraction a part of it was decomposed to yield pyruvate and glyoxylate spots which therefore may or may not be natural constituents of Coleus leaves. The chocolate brown spot having *Rf* 0·28/0·54 does not correspond to any of the reference keto acid hydrazones and this unidentified keto acid is the other major component of the Coleus keto acid pool.

The metabolic events leading to the accumulation of OAA in *Coleus* leaves are under study.

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