A New Trisaccharide in Tobacco Seeds

Sir :

In the course of our investigation on the sugars of tobacco seeds, a new trisaccharide, α -glucosido- β -fructosido- α -galactoside, has been found by the method of paper chromatography, using *n*-butanol-acetic acid-water¹⁾, phenol-water²⁾, and pyridine-*n*-butanol-water³⁾ as solvents. Filter paper *Toyo* No.2 was employed. Spots on the paper chromatograms were revealed by using the benzidine and resorcinol reagents.

Benzidine reagent : 0.5 g. benzidine, 20 cc. galacial acetic acid, 80 cc. ethanol.

Resorcinol reagent : 0.5 g. resorcinol, 20 cc. 50% trichloroacetic acid, 80 cc. ethanol.

The methanolic extract of tobacco seeds (*Nicotiana tabacum* L., "Bright Yellow") was concentrated *in vacuo* to the syrup which was dissolved in water. After treating the aqueous solution with lead acetate, the formed precipitate was removed by centrifugation. The supernatant was treated with hydrogen sulfide. After removing lead sulfide by filtration, the filtrate was concentrated to a small volume *in vacuo*. A small portion of it was separated on the paper chromatogram.

The chromatogram yielded no spot with the benzidine reagent, but two spots with the resorcinol reagent, the one ($R_f=0.11$ in *n*-butanol-acetic acid-water¹⁾) corresponding to an authentic sucrose and the other ($R_f=0.019$) not corresponding to raffinose ($R_f=0.025$) and stachyose ($R_f=0.003$).

The material corresponding to the latter spot was extracted with 80% ethyl alcohol from the chromatograms on number of spots have been run simultaneously. After concentrating the extract *in vacuo*, the residue was then hydrolyzed with 2% sulfuric acid, 5% oxalic acid or α -galactosidase and each hydrolysis product was rechromatographed by the same procedure.

Table I

Sugar	Phenol-water ²⁾	Rf value		Reaction to	
		<i>n</i> -Butanol-acetic acid-water ¹⁾	Pyridine- <i>n</i> -butanol-water ³⁾	Benzidine	Resorcinol.
Fructose	0.51	0.23	—	+	+
Glucose	0.39	0.17	0.37	+	—
Galactose	0.42	0.16	0.33	+	—
Sucrose	0.40	0.11	—	—	+
Lactose	—	0.06	—	+	—
Raffinose	0.29	0.025	—	—	+
Stachyose	0.15	0.003	—	—	+
"Disaccharide"	0.38	0.07	—	+	+
"Trisaccharide"	0.19	0.019	—	—	+

Table II

Hydrolysis Products of the Trisaccharide

With 2% H ₂ SO ₄	Glucose, Galactose and Fructose
" 5% Oxalic acid	Glucose and Disaccharide (Fructose-Galactose)
" α -Galactosidase	Sucrose and Galactose

Sulfuric acid hydrolysis yielded glucose, fructose and galactose. With oxalic acid small amounts of galactose and fructose were formed; but main products were glucose and an unknown sugar which was found to be a disaccharide consisting of fructose and galactose after the 2% sulfuric acid hydrolysis. The R_f value of the disaccharide ($R_f=0.07$) was between that of lactose ($R_f=0.06$) and sucrose ($R_f=0.11$) and the color reaction to the spraying reagents was different from those of the above two disaccharide. The trisaccharide treated with α -galactosidase from seeds of *Pinus Thubergii*, PARLTORÉ gave mainly sucrose and galactose.

From these evidences, the new trisaccharide seemed to be α -glucosido- β -fructosido- α -galactoside, glucose and fructose linking in the form of sucrose. This trisaccharide has not yet been reported to occur in the plant kingdom.

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Received March 30, 1951

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