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Phytochemical Studies of Egyptian *Plantago* Species (Glucides)

By Z. F. AHMED, A. M. RIZK, and F. M. HAMMOUDA

Mucilages are isolated from the seeds of eight Egyptian *Plantago* species by extraction with cold and hot water successively. The percentages, physical properties, as well as the qualitative and quantitative determinations of the sugar components of the different mucilage fractions are reported. The mono- and oligosaccharides identified in the seeds are planteose, planteobiose, sucrose, D-glucose, L-fructose, D-xylose, and L-rhamnose. The qualitative and quantitative determinations of the glucoside aucubin in the seeds are carried out.

THERE ARE 21 species of plantain in Egypt; some are very rare while others are common, particularly *Plantago albicans*, *P. ovata*, *P. major*, and *P. crypsoides*. *P. albicans* grows abundantly in the western Mediterranean regions. The majority of these *Plantago* species show xeromorphic characteristics, growing in deserts under severe conditions of drought.

The present work studies the glucides of the most common Egyptian *Plantago* species.

EXPERIMENTAL

Materials

P. notata.—Plants collected in April 1962 from Burg El Arab.

P. crypsoides.—Plants collected in April 1962 from Burg El Arab, along the road from Cairo to Alexandria opposite kilo 166 from Cairo, and from Sidi Barrani.

P. coronopus.—Plants collected in April 1962 from Burg El Arab.

P. crassifolia.—Plants collected in June 1962 from the marshy areas along the highway from Alexandria to Burg El Arab oppposite kilo 23–27 from Alexandria.

P. major.—Plants collected in June 1962 from gardens, fields, and along canal banks in Giza.

P. cylindrica.—Plants collected in April 1962 from the eastern and western deserts.

P. albicans.—Plants collected in April 1962 from Burg El Arab, Dabaa, Ras El Hikma, and Sidi Barrani in the western Mediterranean coastal region.

P. ovata.—Plants collected in April 1962 from sandy areas of the gravel desert along Cairo-Suez road and from the Lybian desert along Cairo-Alexandria road.

The systematic identification of the plants was carried out by Dr. K. H. Batanouny, Faculty of Science, Cairo University.

Spikes of the plants were collected and dried in an air oven at 50°, and the mature seeds were obtained from the spikes by hand, difficult with *P. crypsoides*, *P. coronopus*, and *P. crassifolia*, due to the compactness and hardness of their fruits.

Mucilages

The seeds of plantago were reported by some authors (1–7) to be an excellent source of acid polysaccharides, the mucilages of which appeared to be mixtures of at least two polysaccharides differing in their uronic acid content (5, 6).

Preparation and Fractionation of the Mucilages.—Ten grams of plantago seeds were mixed with 1 L. of distilled water slightly acidified with hydrochloric acid (pH 3.5) at a temperature of about 20° and stirred for 12 hr. The highly viscous mucilaginous solution was passed through folded muslin. The process was repeated three times, and the mucilage was precipitated from the combined extract by adding, dropwise while stirring, 4 vol. of 96% ethanol. The precipitated mucilage, obtained by centrifugation, was washed several times with 96% ethanol until free from chloride ions, then vigorously stirred with absolute acetone, filtered, and dried in a vacuum desiccator.

The seeds after exhaustive extraction with cold water were washed with cold water till free from chloride ions and then extracted completely with hot water (90–95°). The mucilage was separated and purified in the same manner as with the cold fraction. The corresponding percentages of the different mucilage fractions are shown in Table I.

The hot fractions, though darker in color, gave the same tests of purity as the cold fractions. Both were starch-free, had no odor or taste, did not reduce Fehling solution, and gave negative tests for nitrogen. A paper chromatogram of the mucilage proved the absence of any sugar contaminant. All the mucilage fractions left slight residue on ignition (Table I).

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TABLE I.—PERCENTAGES, SPECIFIC VISCOSITIES, ASH, INSOLUBLE RESIDUES (AFTER HYDROLYSIS), AND FINAL $[\alpha]_D$ OF SOLUBLE HYDROLYSATES OF MUCILAGES OF SEEDS OF EGYPTIAN *Plantago* SPECIES[a]

Species	Mucilage Fraction	%	η_{sp}	Ash	Insoluble Residue, %	α_D^{25}
P. notata	Cold	9.62	1.347	2.17	2.07	36.3
	Hot	4.05	0.319	2.38	2.70	39.5
P. crypsoides	Cold	14.73	5.375	3.12	2.31	45.1
	Hot	10.04	0.062	2.84	2.14	36.4
P. coronopus	Cold	16.52	24.870	2.42	2.06	53.2
	Hot	1.30	0.024	2.77	2.20	42.5
P. crassifolia	Cold	9.95	0.304	2.34	3.42	47.1
	Hot	13.66	0.182	1.95	2.83	34.7
P. major	Cold	13.25	17.496	3.24	2.36	51.4
	Hot	6.27	0.344	2.75	2.14	35.3
P. cylindrica	Cold	20.42	0.802	2.90	2.78	56.1
	Hot	2.55	0.134	2.64	2.38	41.6
P. albicans	Cold	26.82	1.015	2.61	2.82	52.3
	Hot	2.40	0.459	2.79	3.14	38.1
P. ovata	Cold	22.34	1.009	2.35	2.63	48.5
	Hot	3.24	0.141	2.44	2.92	37.4

[a] Mean values of three determinations.

TABLE II.—PERCENTAGES OF DIFFERENT SUGAR COMPONENTS OF THE MUCILAGE FRACTIONS OF SEEDS OF EGYPTIAN *Plantago* SPECIES[a]

Species	Mucilage Fraction	GaU	Ga	Gl	Ar	Xy	Rh
P. notata	Cold	21.5				64.0	13.1
	Hot	6.8	4.0		12.6	75.1	
P. crypsoides	Cold	15.5	6.2		14.0	55.4	10.3
	Hot	4.0	3.4	2.1	12.5	63.2	13.6
P. coronopus	Cold	24.3	6.9		15.2	41.2	13.6
	Hot	7.6	4.5	3.1	13.8	70.1	
P. crassifolia	Cold	20.2			14.6	52.6	13.5
	Hot	6.4		4.3	12.5	62.1	16.0
P. major	Cold	24.0			13.2	61.0	
	Hot	6.2		3.0	11.4	78.0	
P. cylindrica	Cold	22.4	5.1		18.1	52.7	
	Hot	8.6			16.5	73.0	
P. albicans	Cold	27.2	6.5		9.3	44.8	10.4
	Hot	9.3	4.0		10.1	76.0	
P. ovata	Cold	20.4			18.0	51.4	11.3
	Hot	5.8			13.3	70.1	

[a] GaU, D-galacturonic acid; Ga, D-galactose; Gl, D-glucose; Ar, L-arabinose; Xy, D-xylose; Rh, L-rhamnose.

TABLE III.—MONO- AND OLIGOSACCHARIDES AS WELL AS PERCENTAGE OF AUCUBIN OF SEEDS OF EGYPTIAN *Plantago* SPECIES[a]

Species	Pl	Pb	Su	Gl	Fr	Xy	Rh	Un	Aucubin, %
P. notata	+		+	+	+		+		0.62
P. crypsoides	+	+	+	+	+		+		0.17
P. coronopus	+	+	+	+	+		+		0.10
P. crassifolia	+		+	+	+		+		0.11
P. major	+		+	+	+	+	+		0.37
P. cylindrica	+	+	+			+	+		0.14
P. albicans	+	+	+	+	+	+	+		0.56
P. ovata	+		+				+	+	0.21

[a] Pl, planteose; Pb, planteobiose; Su, sucrose; Un, unidentified; Gl, D-glucose; Fr, L-fructose; Xy, D-xylose; Rh, L-rhamnose.

Determination of Viscosity.—Viscosity determinations were carried out using an Ostwald viscosimeter, and the measurements were made at constant temperature of 30° using 5-ml. aliquots of 0.5% w/v of the different mucilage fractions. The specific viscosities η_{sp} (8) are shown in Table I.

Hydrolysis of the Mucilage and Identification of the Sugars in the Hydrolysates.—The hydrolysis of the different mucilages was effected by heating with 4% sulfuric acid for 20 hr. on a boiling water bath. Investigation of the hydrolysates of the different mucilages was carried out by paper chro-

matographic analysis (9–12). In addition, the identity of the sugars, separated on sheets of Whatman No. 3 MM, was confirmed by preparation of their osazones. It was possible to detect D-galactose, D-glucose, D-xylose, L-arabinose, and L-rhamnose. D-Galacturonic acid was proved by different color reagents—*viz.*, naphthoresorcinol reagent (13) and aniline diphenylamine reagent (13).

Quantitative Paper Chromatographic Analysis of the Sugars in the Mucilage Hydrolysates.—The phenol–sulfuric acid method of Dubois *et al.* (14) was applied in this work. The results obtained (mean of triplicate analyses) are shown in Table II.

Saccharides

The study of the free sugars of plantain seeds has received little attention. Greenway and Raymond (15) stated that the seed coat of *P. psyllium* contained sucrose, reducing sugars, and carbohydrates other than sugars. The trisaccharide, planteose, isomer to raffinose, was isolated by Wattiez and Hans (16) from the seeds of *P. major* L. and *P. ovata* Forsk. (*P. isphaghulata* Roxb.). The oligosaccharides of the roots of *P. rugellii* and *P. major* were studied by paper chromatography (17).

The separation and identification of the sugars present in the seeds of the different Egyptian *Plantago* species were carried out applying both paper and column chromatographic techniques.

Paper Chromatography.—The carbohydrates were extracted from the defatted powdered seeds of the eight species with the pyridine method (18). Investigation of the sugars was carried out by paper chromatography using the *n*-butanol–acetic acid–water system and applying the descending technique for 24 hr. Development of the sugar spots was carried out by *p*-anisidine–phosphoric acid reagent (19). The chromatogram obtained revealed significant differences (Table III).

The sugars detected in the different seeds were planteose, planteobiose, sucrose, D-glucose, L-fructose, D-xylose, and L-rhamnose in addition to an unidentified sugar in the case of *P. ovata*. The latter sugar had the same R_f as L-fucose, but gave different color with *p*-anisidene–phosphoric acid reagent. Hydrolysis of the oligosaccharides and paper chromatography of the hydrolysates were in agreement with the above findings.

Column Chromatography.—The findings of the paper chromatographic analysis were verified in the case of *P. albicans* by isolation of the various sugars using carbon (20) and cellulose column (20, 21) chromatography. The identification of the isolated compounds [sucrose, planteose (17, 20, 22, 23) and planteobiose (23–25)] from that species was established by the determination of the optical rotation, melting point, mixed melting point, and preparation of derivatives.

Aucubin

The glycoside, aucubin (26–28), previously isolated from members of *Plantaginaceae* (29, 30), was detected by paper chromatography in the species examined and the identity further established by isolation of that compound from *P. major* (31–36). Quantitative determination of aucubin in the different species of plantago was carried out spectrophotometrically (37), and the results obtained are shown in Table III.

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