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STUDY OF MUCILAGE AND PECTINS OF *PANCRATIUM SICKENBERGERI* ASCH Et. SFTH AND *ALLIUM SATIVUM* L.

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ABSTRACT

The mucilage of Pancratium sickenbergeri consists of rhamnose (2.02%), arabinose (6.02%), mannose (26.29%) and glucose (65.65%). The pectic substance of Allium sativum L. is composed of arabinose (2.41%), mannose (17.64%), glucose (78.63%) and galacturonic acid (38.4%). These investigations were achieved by TLC, GLC and GC/MS.

INTRODUCTION

Mucilagenous plants are used in food preparations, textile industries, in Pharmaceutical preparations and to treat constipation [1]. *Pancratium sickenbergeri* Asch Et. SFTH. (Amaryllidaceae) is a mucilagenous plant cultivated in Egypt for ornamental purposes [2].

Ahmed et al [3,4] stated that the mucilage of *Pancratium sickenbergeri* and *Pancratium maritimum* was found to be B-glucan type.

Balbaa et al [5] studied the mucilage of *Pancratium maritimum* using paper chromatography and reported the presence of L-rhamnose, L-arabinose, D-glucose, D-galactose and D-galacturonic acid.

Karawya et al [6] reinvestigated the mucilage of *Pancratium maritimun* L. and reported the presence of arabinose, galactose, glucose and galacturonic acid.

Allium sativum L. (Garlic) known in Arabic as "thome" is widely distributed edible bulb plant belonging to the family Alliaceae [7]. It is used as a spice, a popular remedy as antimicrobial, hypolipidermic agent and in lowering high blood pressure [8-10].

Rabie [7] studied the pectin of *Allium sativum* by paper chromatography and reported that the pectin hydrolysate of garlic most probably contains galacturonic acid, galactose, arabinose and rhmnose.

Recently polysaccharides became one of the most important natural constituents because of their chemotherapeutic antitumor action [1].

As little information is available regarding the mucilage and pectin content of these plants, it was deemed of interest to throw the light on the sugar composition of their polysaccharides.

EXPERIMENTAL

Plant Material:

Pancratium sickenbergeri Asch. Et. SFTH and *Allium sativum* L. were collected from the plants cultivated in the Experimental Station, Faculty of Pharmacy, Assiut University in April 1987. Identity of the plants was confirmed by Dr. A. Fayed, Associate Professor of Plant Taxonomy, Faculty of Science, Assiut University.

Preparation of the Polysaccharides [11,12]:

The fresh bulbs of the two plants (200 g each) were separately sliced, then dipped in boiling alcohol, left overnight and filtered. The dried marc in each case was reduced to moderately coarse powder and extracted as follows:

A-*Pancratium sickenbergeri* powder was extracted successively with water using cold extraction method [11] to give cold extraction mucilage (CEM) and hot extraction [12] to produce hot extracted mucilage (HEM).

The mucilage was vigorously stirred in chloroform and ether, filtered and dried in desiccator over anhydrous calcium chloride and weighed.

B-The dried marc of *Allium sativum* L. was extracted with 10 times its weight of 0.3% ammonium oxalate solution at 100°C [13,14] for 1 hour with continuous stirring and filtered while hot. The pectic substance was precipitated by adding alcohol acidified with 0.05 N HCl. The precipitate was collected by centrifugation, purified, washed with alcohol till free from acidity and dried in vacuum desiccator. The substance obtained gave positive tests for pectins [15].

Acid Hydrolysis [16]:

Each polysaccharide (50 mg) was separately hydrolysed by autoclaving in a sealed tube with 5 ml of 2 N trifluoroacetic acid (TFA) at 120°C/1 bar for one hour. Each hydrolysate was subjected to TLC analysis and GLC of the hydrolysate alditol acetates.

TLC of the Hydrolysates:

Each hydrolysate was chromatographed on silica gel G (E. Merck) using Acetonitrile-water (85:15) as developer and aniline diphenylamine phosphoric acid as spray reagent [17,18]. The results are listed in Table 1.

Preparation of Alditol Acetates:

The alditol acetates were prepared according to the method of Blakeney et al [19] as follows:

Reduction: After complete acid hydrolysis of each sample the acid was removed by co-distillation with distilled water three times. Each hydrolysate was

separately dissolved in 0.1 ml 1 N ammonia and 1 ml 2% $NaBH_4$ in DMSO (dimethylsulphoxide) was added and left for 90 minutes at 60°C. The excess $NaBH_4$ was decomposed by addition of 0.1 ml 18 M acetic acid.

Acetylation: To each reduced hydrolysate about 0.2 ml of 1-methyl imidazole and 2 ml acetic anhydride were added at room temperature for 10 minutes. The resulting alditol acetates were separately extracted with dichloromethane (3x2 ml). The combined organic phase in each case was washed with 0.1 N H_2SO_4 and finally dried under nitrogen atmosphere.

For GLC analysis, each residue was separately dissolved in dichloromethane and 1-2 ul of the solution were injected.

Gas Chromatography:

1-GLC of alditol acetates:

Instrument: Varian 3500.
Column: WCOT glass capillary column (0.25 mm/25 m) 3% OV 225.
Carrier gas: Nitrogen (0.8 ml/min., split= 1:50).
Temperature Programme: 175°C-205°C (1°C/min), isotherm at 205°C for 15 minutes.
Detector: FID.

Preparation of Methyl Acetyl Sugars:

The methylation analysis was carried out according to the method of Harris et al [20] as follows:

a-Drying: The polysaccharide was dried over phosphorous pentoxide for at least 16 hours.

b-Preparation of the anion: 450 mg dried potassium hydride were ice cooled and mixed with 6.8 ml DMSO (dried over molecular sieve). The mixture was allowed to warm to room temperature and left for 40 minutes while stirring to give K-dimethylsulphonyl carbanion (anion).

c-Methylation: About 5-10 mg of each sample were dissolved in about 200 ml DMSO and 200 ul of the anion were added and the reaction mixture was left for 30 minutes at room temperature while stirring. After completion, the reaction mixture was ice cooled and 150 ul of methyl iodide were added and left while stirring at room temperature for 30 minutes.

All of the predescribed procedure must be carried out under argon atmosphere.

Each methylated sample was extracted by the addition of 3 ml of $CHCl_3$-MeOH (2:1) and 2 ml of distilled water. The upper phase was rejected by aspiration and the lower organic phase was washed three times with distilled water. Finally 2 ml of 2.2 dimethoxy propane and 20 ul of glacial acetic acid (18 M) were added and the solvents were concentrated by evaporation over a water bath at 90°C till a volume of 200 ul. The rest of the solvents were removed under nitrogen atmosphere.

d-Hydrolysis: Was acheived by autoclaving with 1 ml 2 N TFA at 120°C/1 bar for one hour. After completion the solution was freezed by liquid nitrogen after addition of 2 ml distilled water and then freeze dried.

e-Reduction: Was acheived by addition of freshly prepared solution of 0.5 M $NaBH_4$ in 2 M NH_4OH. The reaction mixture was left at 60°C for 1 hour and then 0.5 ml acetone was added and the solvents were removed at 40°C under nitrogen atmosphere.

f-Acetylation: Was carried out by addition of 0.2 ml of acetic acid, 1 ml ethyl acetate, 3 ml acetic anhydride and 1 ml perchloric acid (70%) and left for 10 minutes while stirring. The reaction mixture was cooled, 10 ml of distilled water were added and 0.2 ml 1-methyl imidazole were mixed and left for 10 minutes at room temperature and the partially acetylated methylated sugars were obtained by extractions with 1 ml dichloromethane and 1 ml 0.1 N H_2SO_4. The organic phase was separated by aspiration. The process was repeated three times and the combined organic phases were concentrated till dryness under nitrogen atmosphere, and subjected to GC/MS analysis.

2-GC/MS of Partially Acetylated Methylated Sugars:

Instrument: Hewlett-Packard GC 5890A (Capillary direct interface) with selective ion detector 5970B and HB work station 300.

Column: Durabond Fused-Silica capillary Column (0.25 mm/30 m). DB1701-30W, 0.25 mm. film thickness.

Carrier gas: Helium (0.8 ml/min., split= 1:50).

Temperature programme: 170-210°C (1°C/min). Isotherm at 210°C for 10 min.

Determination of methoxy group and galacturonic acid.

Following the method of the National Formulary 1975 [15]. The percentage of methoxy groups was found to be 6.2% and that of galacturonic acid was 38.4%.

RESULTS AND DISCUSSION

The sliced fresh bulbs of *Pancratium sickenbergeri* Asch and *Allium sativum* L. were treated with hot ethanol to destroy any enzymatic activity. The resulting crude mucilage of the first plant was found to be 10.4% (CEM) and 12.5% (HEM) while the crude pectic substance of the second plant was found to be 8.1%.

On hydrolysis with 2 N TFA and subsequent GLC examination of the hydrolysate the crude polysaccharide of the bulbs of the two plants exhibited the sugar composition as listed in Table 1. TLC of the hydrolysates revealed the presence of galacturonic acid only in the hydrolysate of *Allium sativum* (Table 2).

The polysaccharides of *Pancratium sickenbergeri* is composed of glucose, mannose and minor quantities of rhamnose and arabinose and the polysaccharide (pectic substance) of *Allium sativum* is composed mainly of glucose, mannose, minor amount of arabinose and galacturonic acid.

The sugar linkages in each polysaccharide were determined by preparation of the methyl acetyl derivatives following Harris et al method. GC/MS analysis of methylated acetylated sugars (Table 3) showed that the mucilage of *Pancratium sickenbergeri* consists of 1,2-linked arabinose, 1-,1,4- and 1,6-linked glucose and

1,2-linked mannose while the pectic substance of *Allium sativum* L. consists of 1-2 linked arabinose; 1-,1,2-,1,6-,1,2,3- and 1,3,6-linked glucose and 1,2-,1,2,6-linked mannose. It is interesting that 1,2-arabinose (small amounts) is a common unit in the two polysaccharides.

The above mentioned data revealed that the polysaccharide of *P. sickenbergeri* is non-branched and the major and terminal sugar is glucose, while that of *Allium sativum* showing a significant difference in being branched one, the predominant localization of the external side chain found at glucose and mannose residue units.

The pectins of *Allium sativum* L. are characterized by having up to 6.2% methoxy groups and 38.4% galacturonic acid.

Table 1: GLC Analysis* of the Alditol acetates of *P. sickenbergeri* and *A. sativum*.

Sugar	Retention time	*P. sickenbergeri* 9%	*A. sativum* 9%
Rhamnose	7.48	2.023	-
Arabinose	9.20	6.020	2.416
Mannose	18.80	26.299	17.944
Glucose	22.32	65.657	78.639

* The relative percentages of the sugars was determined by calculating the peak area and following the method of internal normalization.

Table 2: TLC of the Polysaccharide Hydrolysate.

		Colour with the reagent		
Spot No.	HR_f	Pancratium sickenbergeri	Allium sativum	Reference sugars
1	64	yellowish	-	Rhamnose
2	46	yellowish blue	yellowish blue	Arabinose
3	40	blue	blue	Mannose
4	38	blue	blue	Glucose
5	26	-	bluish violet	Galacturonic acid *

* The percentage (38.4%) was determined by adopting the method of the National Formulary 1975.

Table 3: GC/MS data of Partially Methylated Alditol Acetates of *Pancratium sickenbergeri* Asch. Et. Sfth and *Allium sativum* L.

Components Identified	Retention Time (minutes)	*P. sickenbergeri* g%	*A. sativum* g%	Main Fragments m/z (rel. int. %)
1,2-arabinose	13.156	7.026	2.361	162(5), 161(62), 145(10), 129(100), 101(22), 89(5), 87(32), 71(13), 45(67).
1-glucose	15.431	4.269	3.960	162(3), 161(49), 145(44), 129(60), 117(52), 113(14), 102(12), 101(100).
1,2-mannose	20.673	21.749	7.665	190(2), 189(21), 161(30), 145(6), 130(8), 129(100), 113(4), 101(11), 99(11), 87(35), 71(9), 45(33).
1,2-glucose	21.135	--	36.605	189(18), 161(25), 129(100), 101(11), 99(11), 87(39), 71(10), 45(34).
1,4-mannose	21.609	4.549	--	234(3), 233(36), 161(11), 131(12), 129(14), 117(100), 113(37), 101(44), 99(31), 87(29), 71(12), 59(7), 58(6), 45(39).
1,4-glucose	22.604	3.135	--	234(5), 233(53), 131(14), 129(17), 117(100), 101(38), 99(42), 87(36), 71(18), 45(45).
1,6-glucose	23.352	59.245	34.090	234(3), 233(71), 189(17), 161(26), 129(57), 101(100), 99(61), 89(51), 87(45), 71(14), 117(73), 59(8), 45(24).
1,2,3-glucose	27.281	--	2.226	261(36), 201(17), 161(51), 129(100), 127(49), 101(32), 99(37), 87(38), 85(44), 71(19), 45(64).
1,3,6-glucose	30.661	--	2.795	233(10), 189(15), 159(13), 129(56), 117(100), 101(15), 87(32), 45(14).
1,2,6-mannose	31.090	--	0.334	189(28), 129(100), 99(21), 87(43), 71(6), 45(4).

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دراسة المواد الهلاميه والبكتينيه لنباتى

بنكريشيوم سيكنبرجيرى وآليوم ستيفوم "الثوم"

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وبعد دراسة تفصيليه استعمل فيها الباحثون طرق الفصل والتحليل المختلفة "كروماتوجرافيا الغاز وكذلك كروماتوجرافيا الغاز المتصله بجهاز قياس مطياف الكتله" امكن التعرف على تركيب المواد الهلامية فى كل نبات وكذلك النسب المئوية لها.

ففى نبات البنكريشيوم سيكنبرجيرى وجد أن المادة الهلامية تتكون من:- رامنوز "٢,٠٢%"، ارابينوز "٦,٠٢%" مانوز "٢٦,٢٩%" وجلوكوز "٦٥,٦٥%" اما فى نبات الثوم فوجد انها تتكون من: ارابينوز "٢,٤١%"، مانوز "١٧,٦٤%" وجلوكوز "٧٨,٦٣%" وحمض جلاكتويورونك بنسبة ٣٨,٤%.

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