OLIGOSACCHARIDES FROM SOME SPECIES OF THE GENUS CANAVALIA

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Gel Permeation Chromatography (G.P.C.) and High Performance Liquid Chromatography (H.P.L.C.) have been applied to determine the composition of low molecular weight carbohydrates in the green parts (leaves and branches) of *Canavalia gladiata*, *Canavalia gladiata* Var. *gladiata* and *Canavalia brasiliensis*. The analysis shows the presence of varying amounts of glucose, fructose and sucrose and a high D-3-O-Me-chiro inositol content. The occurence of the same prominent constituents on the three specimens is explained by their botanical classification proximity.

INTRODUCTION

The *Canavalia* genus is characterized by a high protective and recuperative ability for poor soils and by its high vegetative vigour. The number of its species amounts to forty[1], from which only eight were studied. The most throughly investigated has certainly been the jack bean, as a consequence of its nutritive value and the prestige it enjoys, conferred by the isolation of the first crystalline urease[2] from it. The others have been less systematically studied, usually in works concerned with the seeds. For instance, Kawamura[3,4] has included the *C. gladiata* in his study of oligosaccharides from seeds of five leguminosae species, using paper chromatography technique. Several other works deal with its starch, lectin, giberelin and canavanin content, as well as its nutritive properties[5]. In regard to *C. brasiliensis*, its lectin content has already been studied.

The high agronomical potential of the genus and the recent introduction of *C. gladiata* Var. *gladiata* in Brazil for nutritive purposes has directed our interest to it. The botanical material was cultivated by technicians of EPAMIG[7] and analysed by powerful techniques like GPC coupled with HPLC, GC, ^{13}C-NMR.

RESULTS AND DISCUSSION

Oligosaccharides contents of the three extracts have been determined by G.P.C. on Bio-Gel P2 – chromatograms are reported in *figure 1*. Three major fractions are present with PD (polymerization degree) none larger than 2 and a small content of higher PD. Each fraction has been collected and analyzed in composition by ion-exchange H.P.L.C. for sugar identification. In fraction A, glucose and fructose have been identified without ambiguity, another compound has been isolated and shown to be myo-inositol. Fraction B is constituted by our major products. Its mass spectrum $^{+}$(F.A.B.), exhibits a pseudomolecular ion (M + H^{+}) at 195; the ^{13}C-NMR chemical shifts, are identical with those reported by S.J. Angyal et al[10] for D-3-O-Me-chiro inositol. Sucrose is the constituent of peak C, peak D is a mixture of oligosaccharides with PD higher than 2. Actually a trisaccharide has been determined as inulotriose[11].

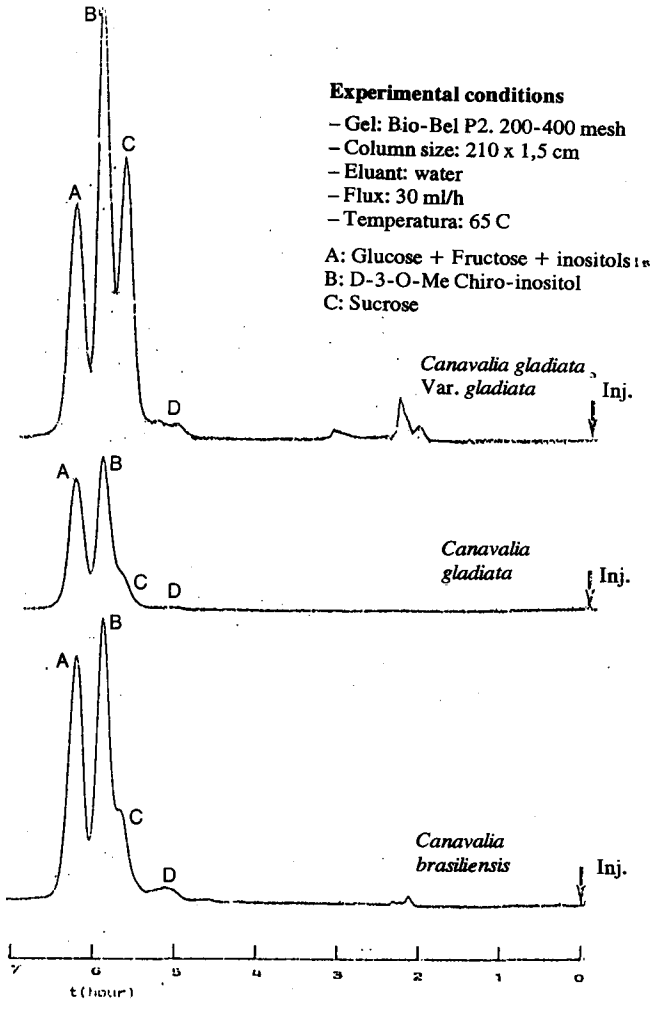

Figura 1. *G.P.C. Chromatograms obtained from ethanol/water (3:1) extracts of the 3* Canavalia *species.*

Figure 2 shows the chromatograms obtained by ion-exchange chromatography of the three extracts and this system

appears to be a powerful tool in a fast quantitative determination of carbohydrates and cyclitols. Composition of 3:1 ethanol extracts are given in *Table I*. In view of the behaviour on different chromatografhic systems, the unknown compounds are probably inositols or inositol-containing oligosaccharides and have to be identified in a further study.

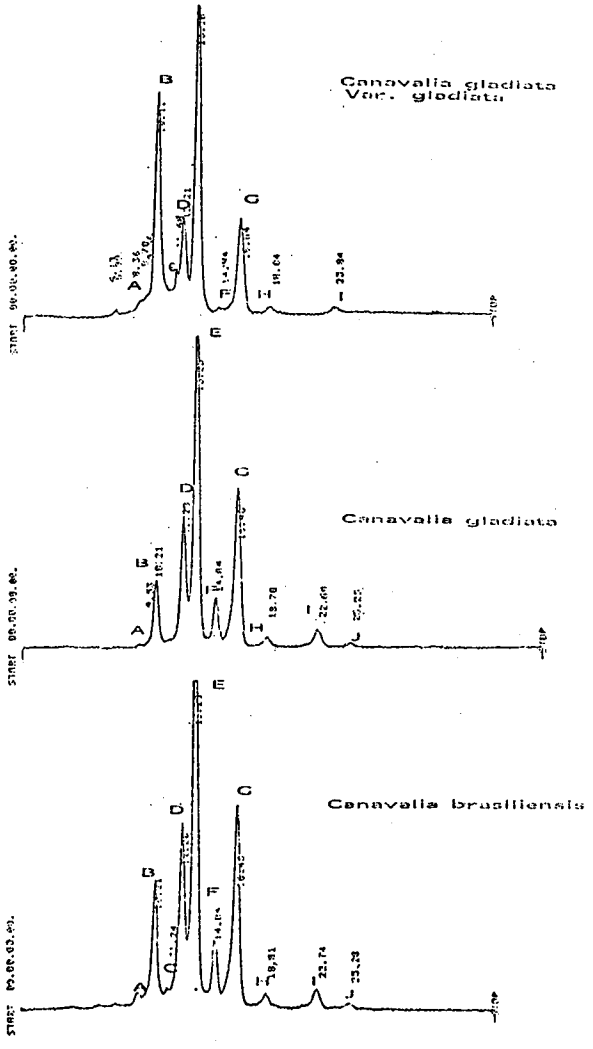

Figure 2. *HPLC chromatogram obtained from ethanol/water (3:1) extracts of three Canavalia species (Column: Aminex HPX-87P; eluant: H_2O; temp.: 85°C; flux: 0,6 ml/mn).*

CONCLUSION

The three species showed (see Fig. 2 and Table I) as major constituents saccharose (B), glucose (D), D-3-O-Me-chiro-inositol-(E), fructose (G), and myo – inositol (I); the constituents (A), (C), (F), (H), and (J) present in minor amounts were not identified.

From the data obtained, mainly the chromatogram profile on G.P.C. and HPLC, one concludes that the specimens of *Canavalia gladiata* and *Canavalia brasiliensis* we studied are identical. This has been confirmed by the botanists from EPAMIG after a botanical reinvestigation of the specimens, that showed that both specimens we worked out were from *Canavalia brasiliensis*.

EXPERIMENTAL

Preparation of the oligosaccharides mixtures

The whole dried, finely pulverized leaves and branches (100 g) of, respectively, *C. gladiata, C. gladiata* Var. *gladiata* and *C. brasiliensis,* were each extracted in a Soxhlet with CH_2Cl_2 until the completion of thirty cycles. The CH_2Cl_2 extracts were not examined in the present work. The insoluble residues were then each extracted with EtOH: H_2O (3:1) at room temperature for 24 hours with magnetic stirring. The EtOH: H_2O (3:1) extracts were each evaporated, affording, respectively, 4.0, 5.8 and 4.5 g of oligosaccharides after deionization with MB 3 ion exchange resin.

Liquid chromatography

Analysis were performed by gel permeation chromatography (G.P.C.) and by high perfomance liquid chromatography (H.P.L.C.).

For gel filtration of oligosaccharides, a thermostated (65°C) column (210 x 1.5 cm I.D.) filled with polyacrylamide gel (Bio-Gel P2, 200-600 Mesh; Bio-Rad U.S.A) was used. The eluant (flow rate 30 ml/h) was distilled water.

Oligosaccharides were purified by H.P.L.C. on a preparative C column (PARTISIL M-20 < 10/50 ODS-2), purchased from Whatman, with pure water as eluant.

Monosacharides analysis were carried out with a HPX-87 P column from Bio-Rad.

The equipment used in H.P.L.C. included a Model 6000 A pump, an U6K injector and a model R-401 refractometric de-

Tabela I. Ethanol: water (3:1) extracts composition of three *Canavalia* species determined by HPLC.

COMPOSITION	SPECIES		
% W/W	*Canavalia gladiata*	*Canavalia gladiata* Var *gladiata*	*Canavalia brasiliensis*
A – DP > 2	0.2	2.1	1.0
B – Sucrose	7.7	27.9	11.6
C – n.i.	–	Tr**	1.3
D – Glucose	17.0	12.8	18.8
E – D-3-O-Me chiro-inositol	40.7	39.6	35.2
F – n.i.	6.5	Tr	6.4
G – Fructose	23.3	15.5	22.2
H – n.i.	1.5	1.1	1.1
I – myo-inositol	2.5	0.8	1.9
J – n.i.	0.5	–	0.4

* n.i.: not identified ** Tr: traces

tector, all from Waters Assoc. (Milford, MA, U.S.A). A Chromatopac C-Rib integrator (Shimadzu, Kyoto, Japan) was used to calculate peak areas.

Fast Atom bombardment Mass spectrometry (FAB-MS)

Spectra were recorded by using a quadripolar R 10.10C NERMAG mass spectrometer, an f.a.b., M SCAN-WALLIS atom-gun operating at 8 kV and a PDP 11/73 computer. The gas used was Argon. The samples (5 mg) were usually dissolved in water (0,5 ml), and the solution added to a drop of glycerol on a copper target.

^{13}C-NMR spectroscopy[9]

Samples were dissolved in D_2O. Experiments were performed with broad proton – decoupling at 25 MHz on a Bruker WP 100 spectrometer at 30°C.

Chemical shifts were expressed in p.p.m. downfield from DSS (sodium 4,4-dimethyl-4-silopentane-1-sulfonate) and compared with literature data.

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REFERENCES

1. Toms, G.C.; Western, A.; "Phytohaemagglutinins", in Harbone, J.B.; Boulter, D. and Turner, B.L., "Chemotaxonomy of the Leguminosae"; Academic Press, London and New York, (1971), Cap. 10, p. 367-462.
2. Summer, J.B.; *J. Biol. Chem.*(1926),**70**, 97.
3. Kawamura, S., *Chem. Abs.* (1958), **52**, 20430 b.
4. Narasaki, T; Kawamura, S.; *Chem Abs. (1958)*, **52**, 20430 d.
5. Penteado, M. de V. Camargo; Lajolo, F.M.; Rev. Farm. Bioquim. Univ. S. Paulo, (1983), **19**, 126.
6. Moreira, R.A.; Cavada B.Ss.; *Biol. Plant.* (1984), **26**, 113.
7. Empresa de Pesquisas Agropecuárias de Minas Gerais; Belo Horizonte, MG, Brasil.
8. Heyraud, A.; Rinaudo, M.; *Liquid. Chromatog.* (1981), **4**(Suppl.2), 175.
9. Bosso, C.; Defaye, J.; Heyraud, A.; Ulrich, J.; *Carbohydr. Res.* (1984), **125**, 309.
10. Angyal, S.J.; Odier, L.; *Carbohydr. Res.* (1983), **123**, 23.
11. Heyraud, A.; Rinaudo, M.; Taravel, F.R.; *Carbohydr. Res.* (1984), **128**, 311.