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B R I E F C O M M U N I C A T I O N S

CHEMICAL CONSTITUENTS OF THE WATER CALYX OF *Solanum cernuum*

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Solanum cernuum Vell is a shrub or a small tree of Solanaceae, native of Brazil. Aiming at further contributing to the understanding of the function of the water calyx in *S. cernuum* we describe in this work the major carbohydrates present in this mixture.

The inflorescences of *S. cernuum* were collected in January 2004. We performed initially a phytochemical screening using TLC (thin layer chromatography). With Dragendorf's reagent, no orange spots characteristic of alkaloids were observed. However, two gray spots with R_f 0.10 and 0.29 were observed when running a TLC using ethylacetate–methanol–water–acetic acid (13:3:3:4) as the mobile phase and vanillin/H_2SO_4 as the developing reagent. These spots were very similar in color and R_f to those observed for carbohydrate standards.

The mixture was then derivatized using trimethylsilylimidazole reagent and the reaction mixture analyzed by GC/MS under the conditions described in the experimental section. Standards of D-mannose, L-arabinose, D-xylose, D-glucose, D-maltose, saccharose and myo-inositol were subjected to the same procedure.

When TMS derivatives are made from free sugars, more than one product can be obtained of various anomeric and ring forms [1, 2]. In fact, analysis of the TIC (total ion chromatogram) of the carbohydrate standards displayed several peaks for the same sugar. Comparison with the TIC of the standards allowed us to identify D-glucose, myo-inositol, and saccharose in the mixture of carbohydrates. Analysis using AMDIS software and the equipment mass spectral database suggested that fructose in its two forms and sorbose are also present. Saccharose accounted for 67.90% of the total area of the chromatogram, followed by sorbose (17.56%). The results are summarized in Table 1.

The presence of water calyx in the buds of *S. cernuum* was described for the first time and its carbohydrate composition was determined by GC/MS. As there is no previous description of the presence of floral nectaries in this plant, ontogeny studies will be necessary to determine which structure in the plant is responsible for producing the water calyx in *S. cernuum*.

Calyx Water Collection. 71 buds were collected at the Federal University of Minas Gerais (UFMG) in Belo Horizonte City, Brazil. A voucher was deposited at the herbarium of UFMG under the code BHCB 16886. The liquid content of each bud was measured with a 500 μL Hamilton syringe and frozen in liquid nitrogen. The pooled frozen drops (4 mL) were transferred to an amber flask, lyophilized to afford 37 mg of dry material.

Thin-Layer Chromatographic Analysis. An aliquot of 1 mg of the lyophilized material, corresponding to the volume contained in 3 buds, was dissolved in Milli-Q water and chromatographed on two silica gel plates (Merck 60 F_{254}) eluted with a mixture of ethylacetate–methanol–water–acetic acid (13:3:3:4). The plates were inspected under UV light (λ_{254} nm and λ_{366} nm) and one of them was sprayed with Dragendorf's reagent and the other with vanillin/H_2SO_4 followed by heating at 100°C for 2 minutes.

Preparation of Trimethylsilyl (TMS) Derivatives. 500 μL of trimethylsilylimidazole in pyridine (Tri-Sil, Supelco) was added to 5 mg of lyophilized material suspended in 1 mL of pyridine and heated at 60°C during 40 min in a Teflon lined screw capped borosilicate glass vial. Separate reactions with D-mannose, L-arabinose, D-xylose, D-glucose, D-maltose, saccharose, and myo-inositol (1mg each plus 250 μL of Tri-Sil reagent) were run in parallel.

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TABLE 1. GC-MS Data Analysis of TMS-Ethers Carbohydrates Derivatives of Water Calyx of *S. cernuum*

Peak No.	Rt, min		Peak area, %	Relative intensities (%) of ion fragmentation at MS						MF	Carbohydrate
	Sample	St		191	204	217	305	318	361		
1	21.03	-	2.51	4.31	0.26	40.19	0.90	-	-	0.7	Fructose[a]
2	21.14	-	3.27	3.90	0.30	51.67	0.64	-	-	0.7	Fructose[a]
3	21.25	-	17.56	2.97	45.53	15.68	1.57	-	-	0.9	Sorbose
4	22.20	22.21	3.90	44.63	82.16	14.27	1.25	-	-	0.8	α-D-Glucose
5	23.34	23.36	2.00	50.31	79.48	16.27	1.80	-	-	0.8	β-D-Glucose
6	24.68	24.70	1.09	27.50	7.80	47.20	36.82	21.64	-	0.7	myo-Inositol
7	32.25	32.23	67.90	5.11	2.33	29.48	-		66.46	0.9	Saccharose

Rt: retention time; St: standard; MF: mach factor; intensity of *m/z* 73 is taken as 100%.

Gas Chromatography-Mass Spectrometry (GC/MS). The derivatized samples were analyzed in a Shimadzu QP5050 GC/MS spectrometer using a DB5 column (30 m × 0.25 mm) with helium as a carrier gas. The injector and interface temperature were set at 230°C and 240°C, respectively, split ratio 49, solvent cut 2 min, column pressure 68.7 kPa, temperature gradient 50°C to 250°C increasing 8°C/min, total flow rate 62.3 mL/min, and mass range 40 to 500 Da. The raw data obtained was analyzed using Shimadzu CLASS 5000 software and AMDIS (Automated Mass spectral Deconvolution & Identification System) of the National Institute of Standards and Technology.

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