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Characterization of cyclitol glycosides by gas chromatography coupled to mass spectrometry

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ABSTRACT

Several cyclitol glycosides have been characterised as trimethylsilyl derivatives by their gas chromatographic (GC) retention data (linear retention indices) and electron impact mass spectrometric (MS) profiles. Both GC–MS results have been related to cyclitol glycosides structural features. Abundance ratios of characteristic *m/z* ions 133/129 and 260/265 have been proposed to distinguish glycosyl-inositols from glycosyl-methyl-inositols. These ratios in combination with the presence or absence of *m/z* 375 ion allowed the unequivocal characterization of cyclitol glycosides. These criteria have been applied to the characterization of new cyclitol glycosides in chickpea (*Cicer arietinum*) and adzuki bean (*Vigna angularis*) and in leaves of *Coriaria myrtifolia* and *Coriaria ruscifolia*.

1. Introduction

Cyclitol glycosides are considered pseudosaccharides, constituted by a chain of one to four galactosyl residues (linked in the C6 position) and an inositol or a methyl inositol. These compounds are naturally present in vegetal kingdom, mainly in seeds [1]. Its accumulation is associated with the acquisition of desiccation tolerance and longevity of seeds [2]. They are especially abundant in Leguminosae which are extensively consumed as food, feed and pasture. Other edible seeds such as buckwheat (*Fagopyrum esculentum*) [3] and pine nuts (*Pinus pinea*) [4] are also sources of these compounds.

Several bioactive properties, mainly associated with insulin resistance, have been attributed to cyclitols (e.g. *chiro*-inositol and pinitol) and their derivatives (e.g. fagopyritols). Thereby, these naturally occurring pseudosaccharides have been proposed for treating disorders such as diabetes mellitus, obesity and polycystic ovary syndrome [3,5].

Cyclitol glycosides are water soluble and either conventional solid liquid extraction [6] or advanced techniques such as pressurised liquid extraction [7] have been proposed for their effective extraction from natural sources. Different techniques such as nuclear magnetic resonance [8–10], liquid chromatography [11], gas chromatography (GC) and GC coupled to mass spectrometry (MS) [12–14], have been used for cyclitol glycoside instrumental analysis. Among these techniques, GC–MS can provide valuable structural information for the characterization of these compounds along with other soluble carbohydrates avoiding tedious fractionation processes.

Although GC relative retention times of several cyclitol glycosides can be found in the literature [6,15,16], linear retention index (I^T) data are still scarce [4,14]. Moreover, a systematic study of their mass spectra is also lacking.

The aim of this work is to provide I^T values and MS data of different cyclitol glycosides and correlate this information with their chemical structure in an attempt to establish criteria that can be used for identification of unknown compounds. The practical applicability of the proposed approach has been demonstrated by the characterization of several non-previously identified cyclitol glycosides in seeds such as *Cicer arietinum* (chickpea) and *Vigna angularis* (adzuki bean) and in leaves of *Coriaria myrtifolia* and *Coriaria ruscifolia*.

2. Materials and methods

2.1. Standards and samples

IUPAC recommendations for nomenclature of cyclitols have been followed through this paper [17].

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Sugars (sucrose, galactosyl-(1 → 6)-galactose, raffinose, stachyose and verbascose) and free inositols (*chiro*-inositol, *scyllo*-inositol, *myo*-inositol) were acquired from Sigma (St. Louis, MO). Free methyl-inositols, like sequoyitol, quebrachitol and pinitol were from Extrasynthèse (Genay, France), Carbosynth (Berkshire, UK) and Sigma, respectively, whereas bornesitol and ononitol were not available as commercial standards and were extracted from grass pea and black-eyed pea, respectively [14]. Galactinol was acquired from Sigma and other galactosyl-inositols, such as fagopyritol A1, fagopyritol B1, fagopyritol A2, fagopyritol B2 and fagopyritol A3 and di-galactosyl-*myo*-inositol (DGMI), were extracted from buckwheat (*Fagopyrum esculentum*) as previously described [14,18]. Regarding galactosyl-methyl-inositols, lathyritol was obtained from grass pea (*Lathyrus sativus*), whereas galactopinitol A1, galactopinitol B1 and ciceritol, were extracted from chickpea [14]; galactosyl-ononitol and digalactosyl-ononitol were identified in black-eyed pea (*Vigna unguiculata*) according to data reported elsewhere [19,20].

Samples (adzuki bean, black-eyed pea, buckwheat, chickpea and grass pea) were obtained from local markets in Madrid (Spain) and were ground using a domestic mill (Moulinex, Barcelona, Spain) and sieved through a 500 μm sieve before extraction.

Leaves from *C. myrtifolia* and *C. ruscifolia* were kindly supplied by Dr. R. Morales from Real Jardín Botánico de Madrid (CSIC).

2.2. Extraction of cyclitol glycosides

Exhaustive extraction of cyclitol glycosides and other low molecular weight carbohydrates from seeds and leaves was achieved by three consecutive extraction cycles of 1 g sample with 10 mL of hot Milli-Q water (60 °C) for 2 h under constant stirring as previously indicated [4,14]. The three extracts were combined, filtrated through a Whatman No. 4 paper and kept at −20 °C until analysis.

2.3. GC–MS analysis

A two-step derivatization procedure (oximation + silylation) was carried out prior to GC–MS analysis [21]. This methodology gives rise to two peaks for reducing sugars and only one for non reducing sugars which results in a better GC resolution of cyclitol glycosides from other coextracted carbohydrates from plants. In brief, 0.5 mL of phenyl-β-D-glucoside (1 mg mL^{-1}) was added to 1 mL of standard solutions or plant extract and the solution was evaporated under vacuum. Then, samples were treated with 350 μL of 2.5% hydroxylamine chloride in pyridine (30 min at 75 °C). Trimethylsilyl (TMS) derivatives were obtained using 350 μL of hexamethyldisilazane (Sigma) plus 35 μL of trifluoroacetic acid (Sigma) at 45 °C for 30 min. After centrifugation, 1 μL of supernatant was taken for instrumental determination.

GC–MS analysis was carried out using a 7890A gas chromatograph coupled to a 5975C quadrupole mass detector (Agilent Technologies, Palo Alto, CA, USA) with an HT5 (5% phenyl (equiv.) polycarborane–siloxane) capillary column (25 m × 0.22 mm i.d. × 0.1 μm film thickness; SGE, Ringwood, Australia). Carrier gas was helium at 1 mL min^{-1}. Oven temperature was programmed from 180 °C (10 min) at 5 °C min^{-1} to 200 °C (15 min), then at 15 °C min^{-1} to 270 °C, at 1 °C min^{-1} to 290 °C, at 15 °C min^{-1} to 300 °C (15 min), and finally at 15 °C min^{-1} to 360 °C (15 min). Injections (1 μL) were carried out in split mode (1:20) at 320 °C. The mass detector was operated in electron impact (EI) mode at 70 eV, scanning the 50–650 *m*/*z* range. The transfer line and ionisation source were heated at 280 and 230 °C, respectively. HP ChemStation software (Agilent Technologies) was used for data acquisition and data treatment.

Table 1
Linear retention indices (I^T) on HT5 (5% phenyl (equiv.) polycarborane–siloxane) and mass spectrometric features (abundances of proposed *m*/*z* ratios) of glycosyl cyclitols. Some sugars have been included for comparison purposes.

Compound	Structure	t_R(min)	I^T	DP[a]	Ratios of *m*/*z* ions		Presence of *m*/*z* 375
					133/129	260/265	
Sucrose	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside	31.0	2516	2			
Galactopinitol A1	O-α-galactopyranosyl-(1 → 5)-3-O-methyl-D-*chiro*-inositol	33.0	2633	2	1.07	1.54	Yes
Galactopinitol B1	O-α-galactopyranosyl-(1 → 2)-3-O-methyl-D-*chiro*-inositol	34.1	2719	2	0.84	1.79	Yes
Fagopyritol A1	O-α-galactopyranosyl-(1 → 3)-D-*chiro*-inositol	34.3	2740	2	0.30	0.04	No
Lathyritol	O-α-galactopyranosyl-(1 → 3)-1-O-methyl-D-*myo*-inositol	34.6	2760	2	0.80	1.63	Yes
Fagopyritol B1	O-α-galactopyranosyl-(1 → 2)-D-*chiro*-inositol	35.0	2801	2	0.26	0.05	No
Galactosyl-ononitol	O-α-galactopyranosyl-(1 → 3)-4-O-D-methyl-*myo*-inositol	35.1	2811	2	0.92	1.56	Yes
Galactinol	O-α-D-galactopyranosyl-(1 → 3)-D-*myo*-inositol	35.6	2874	2	0.33	0.12	No
Raffinose	O-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside	40.1	3158	3			
Ciceritol	O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 5)-3-O-methyl-D-*chiro*-inositol	47.3	3489	3	0.77	1.34	Yes
Fagopyritol A2	O-α-galactopyranosyl-(1 → 6)-O-α-galactopyranosyl-(1 → 3)-D-*chiro*-inositol	49.1	3565	3	0.34	0.0	No
Digalactosyl-ononitol	O-α-galactopyranosyl-(1 → 6)-O-α-galactopyranosyl-(1 → 3)-4-O-D-methyl-*myo*-inositol	49.5	3580	3	0.64	2.08	Yes
Fagopyritol B2	O-α-galactopyranosyl-(1 → 6)-O-α-galactopyranosyl-(1 → 2)-D-*chiro*-inositol	51.1	3640	3	0.01	0.0	No
Digalactosyl-*myo*-inositol	O-α-galactopyranosyl-(1 → 6)-O-α-galactopyranosyl-(1 → 3)-D-*myo*-inositol	53.7	3734	3	0.21	0.0	No
Stachyose	O-[α-D-galactopyranosyl-(1 → 6)]$_2$-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside	62.1	3980	4			
Fagopyritol A3	O-[α-galactopyranosyl-(1 → 6)]$_2$-α-galactopyranosyl-(1 → 3)-D-*chiro*-inositol	72.4	4236	4	0.03	_[b]	No
Verbascose	O-[α-D-galactopyranosyl-(1 → 6)]$_3$-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside	76.1	4305	5			

[a] Degree of polymerization.
[b] *m*/*z* 260 and 265 ions not detected.