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CICERITOL, A PINITOL DIGALACTOSIDE FROM SEEDS OF CHICKPEA, LENTIL AND WHITE LUPIN

BERNARD QUEMENER and JEAN-MARC BRILLOUET

Institut National de la Recherche Agronomique, Centre de Recherches Agro-Alimentaires, Laboratoire de Biochimie et Technologie des Glucides, rue de la Géraudière, 44072 Nantes Cédex, France

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Key Word Index—*Cicer arietinum*; chickpea; *Lens esculenta*; lentil; *Lupinus albus*; white lupin; Leguminosae; seeds; oligosaccharides; cyclitols; digalactosylpinitol; ciceritol.

Abstract—A new trisaccharide was isolated from an aqueous ethanolic extract of chick pea (*Cicer arietinum*) cotyledons by preparative PC. The trimethylsilyl derivative of this sugar had the same GC retention time as manninotriose previously wrongly reported in seeds of chickpea and lentil. HPLC analysis allowed a good separation between the new trisaccharide and manninotriose showing that the latter was absent in chickpea and in the other legume seeds under study. The composition and structure of the new sugar were determined by enzymatic hydrolysis assays, GC analysis of alditol acetate derivatives obtained after complete acid hydrolysis, GC analysis of by-products from dilute acid hydrolysis and GC/MS analysis of partially methylated or trideuteriomethylated alditol acetates. The new trisaccharide is an α-D-digalactoside of pinitol and is *O*-α-D-galactopyranosyl-(1–6)-*O*-α-D-galactopyranosyl-(1–2)-1D-4-*O*-methyl-*chiro*-inositol. As originating mainly from chickpea the name ciceritol is proposed. The seeds of seven commercially important legumes were analysed by HPLC and GC for cyclitols, cyclitol-derived oligosaccharides and sucrose α-D-galactosides. Ciceritol was detected in chickpea (2.80% per dehulled seed), lentil (1.60%), white lupin (0.65%), soya bean (0.08%) and bean (traces). The contribution of ciceritol and other α-D-galactosides to flatus is discussed on the basis of α-D-galactosidase sensitivity.

INTRODUCTION

Dry legume seeds are an economically important source of protein and carbohydrate. Several publications have reported the presence of oligosaccharides of the raffinose family (raffinose, stachyose, verbascose, ajugose and higher DP homologues) in legume seeds [1–11]. These oligosaccharides, especially stachyose and raffinose, could be responsible for flatulence [10, 12–14]. Moreover, some authors have noted the presence in pulses of cyclitols and of cyclitol-derived oligosaccharides. *Myo*-inositol, one of the nine stereoisomers of inositol, was first reported in seed of *Phaseolus vulgaris* [15] and was thereafter found in numerous legume seeds [5, 8, 16, 17]. Other cyclitols, especially D-*chiro*-inositol, sequoyitol (or 5-*O*-methyl-*myo*-inositol) and D-pinitol (or 1D-4-*O*-methyl-*chiro*-inositol) were also found in some legumes [1, 5, 8, 16–22]. The first galactoside of *myo*-inositol, 1L-1-*O*-(α-D-galactopyranosyl)-*myo*-inositol, or galactinol [23], was isolated from *Vicia sativa* [24]. It occurs in all plants producing raffinose and stachyose [25]. Small quantities of higher homologues (up to DP 4) were also detected in *Vicia sativa* [1]. Galactosides of D-pinitol, such as 1D-2-*O*-(α-galactopyranosyl)-4-*O*-methyl-*chiro*-inositol have been isolated from seeds of *Trifolium subterraneum* [16] and from soya bean [5]. Recently, other isomers, 1D-5-*O*-(α-D-galactopyranosyl)-4-*O*-methyl-*chiro*-inositol and 1D-2-*O*-(α-D-galactopyranosyl)-*chiro*-inositol, have also been isolated from soya bean [26].

Significant amounts of an unknown oligosaccharide of presumed DP 3 have been detected several times in the seeds of chickpea [2, 10, 27] and lentil [6, 10]. Different authors have tentatively identified this sugar as manninotriose or *O*-α-D-galactopyranosyl-(1–6)*O*-α-D-galactopyranosyl-(1–6)-D-glucopyranoside and reported it in soya bean [5, 28], white lupin [11], mung bean [8, 11], chickpea [5, 8, 11] and lentil [5, 11]. More recently, it was noted that a discrepancy exists as to the chemical identity of this carbohydrate [10]. The purpose of the present study therefore, was to extract, purify, establish the structure of this presumed trisaccharide and to make a detailed investigation of the ethanol-soluble sugars of seven commercially important legume seeds.

RESULTS AND DISCUSSION

Preliminary characterization of ciceritol

Numerous peaks were observed in an HPLC chromatogram of an ethanolic extract of chickpea, most of them being characterized (Table 1). An unknown peak, 14, eluting between raffinose (peak 12) and stachyose (peak 18) represented a high proportion of total sugars (*ca* 25%). Its concentration (*ca* 3% in flour as raffinose) suggested that it could be manninotriose several times reported in chick pea [5, 8, 11]. Addition of manninotriose, however, to the chickpea extract produced an extra peak eluting between raffinose and stachyose at a RR_f very different from that of unknown peak 14 (Table 1). By GC of the sugar TMSi derivatives, however, added manninotriose was eluted close to the unknown peak 14 inducing a discrete shoulder. The RR_fs of manninotriose and unknown 14 were very close (1.10 and

Table 1. HPLC and GC RR_ts of legume seed low MW carbohydrates

No.	Peak	Legume seed	RR_t‡(HPLC*)	RR_t‡(GC†)
1	Unknown	—	0.34–0.35	—
2	Unknown	Faba bean	0.44	—
3	Pinitol	—	0.47–0.48	0.27
4	Unknown	Chickpea	0.51	—
5	Unknown	Bean	0.55	—
6	Sucrose	—	0.62	0.70
7	Galactopinitol A	—	0.67	0.75
8	Galactopinitol B	—	—	0.79
9	Galactosyl-*chiro*-inositol§	—	—	0.82
10	*Myo*-inositol	—	0.71–0.72	0.44
11	Galactinol	—	0.87–0.89	0.84
12	Raffinose	—	1.00	1.00
13	Unknown	Chickpea, lentil, pea, faba bean	1.05–1.07	1.05
14	Ciceritol	Chickpea, lentil, white lupin, soya bean	1.14–1.16	1.11
15	Unknown	Chickpea, lentil, pea	1.23	—
16	Manninotriose (standard)	—	1.41	1.10
17	Unknown	Lentil, pea, white lupin	1.43–1.45	—
18	Stachyose	—	1.64–1.67	1.44
19	Unknown	Chickpea, lentil, pea, white lupin	1.73–1.77	
20	Unknown	Chickpea, lentil, white lupin	1.85–1.90	1.63
21	Verbascose	—	2.74–2.80	—

*HPLC conditions: stationary phase: Lichrosorb NH_2 (5 μm); mobile phase: MeCN–H_2O (7:3); flow rate: 2 ml/min.

†GC conditions: TMSi derivatives; stationary phase: 3% OV-1 on Chromosorb Q (100–120 mesh); temp. programming: 140° to 320° at 6°/min; N_2 flow rate: 30 ml/min.

‡RR_t, Retention time relative to raffinose.

§Galactosyl-*chiro*-inositol corresponds to galactopinitol C described in ref. [5].

1.11, respectively) (Table 1), which could explain why many authors incorrectly identified manninotriose in chickpea by GC analysis [5, 8, 11].

Preliminary characterization of unknown peak 14, presumably a trisaccharide according to its elution volume, was carried out by various enzymatic assays on a whole chickpea extract and subsequent HPLC analysis of by-products. β-Galactosidase, β-fructofuranosidase and α- and β-glucosidases were ineffective in hydrolysing the sugar. On the other hand, α-galactosidase slowly hydrolysed the compound (*ca* 60% decrease in peak area in 48 hr). Liberated galactose was measurable using the β-D-galactose dehydrogenase–NAD system [29] indicating that galactose had the D-configuration. Therefore, unknown peak 14 was assumed to be an α-D-galactoside of presumed DP 3. It was named, thereafter, ciceritol as originating from chickpea, *Cicer arietinum*.

Purification and composition of ciceritol

Purification of ciceritol was achieved by 3MM Whatman descending PC. Purified ciceritol was analysed by HPLC and showed a single and symmetric peak eluting at the same R_t as unknown peak 14. Ciceritol was not reducing as shown by the Nelson procedure [30] and was not revealed by diphenylamine–aniline reagent [31]. GC analysis of alditol acetates obtained after TFA hydrolysis of ciceritol showed two peaks. Their RR_ts relative to *myo*-inositol were identical to those of authentic D-pinitol and D-galactose (0.61 and 0.74, respectively). Peak areas suggested that D-galactose and D-pinitol occurred in a *ca* 2:1 molar ratio.

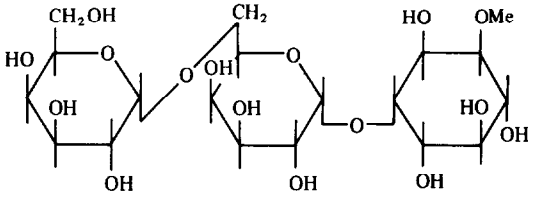
Mild acid hydrolysis of ciceritol yielded D-pinitol, galactose, galactopinitol A (or 1D-2-*O*-(α-D-galactopyranosyl)-4-*O*-methyl-*chiro*-inositol) [5, 26] and an unknown disaccharide peak, having an RR_t of 0.72 compared with raffinose (GC analysis), possibly a galactose dimer.

Methylation structural analysis of ciceritol

Dried ciceritol in DMSO was treated with dimsyl ion, then methylated by methyl iodide or deuterated methyl iodide [32, 33]. Three main GC peaks were detected of roughly equal areas on analysis of the partially methylated alditol acetates. GC/MS provided fragmentation patterns corresponding to pentamethyl-mono-*O*-acetylcyclitol (peak 1) [26], to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol (peak 2) and to 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol [33]. Such a distribution indicates that the ciceritol molecule must contain a terminal pyranoid galactose which is in good agreement with α-galactosidase sensitivity. Furthermore, a second galactopyranosyl molecule occurred at an intermediary 6-linked position. The occurrence of galactopinitol A in the trisaccharide as demonstrated by partial acid hydrolysis studies indicates that the intermediary galactose must be linked through its C-1 to C-2 of D-pinitol, thus confirming the non-reducing character of ciceritol.

Pertrideuteriomethylation of ciceritol, according to Schweizer and Horman [26], and subsequent hydrolysis followed by alditol acetate derivatization provided an identical GC chromatogram to that obtained above. GC/MS analysis allowed identification of peak 1 by comparison of its fragmentation pattern to that of a

previously described one [26]. Pertinent ions were found at *m/z* 91, 94, 150, 153 and 200 with relative proportions very similar to those given for 1D-1,3,5,6-tetra-*O*-trideuteriomethyl-2-*O*-acetyl-4-*O*-methyl-*chiro*-inositol [26]. From the above results, it can be deduced that the C-2 of D-pinitol is involved in a linkage with a galactose residue which fits well with the occurrence of galactopinitol A in ciceritol. Therefore, ciceritol would be the higher homologue of galactopinitol A, the methoxy group of pinitol being in the β-position relative to the glycosidic linkage at C-2. The chemical nomenclature must be *O*-α-D-galactopyranosyl-(1–6)-*O*-α-D-galactopyranosyl-(1–2)-1D-4-O-methyl-*chiro*-inositol.



It must be noted that ciceritol, the β-substituted isomer relative to the methoxy group of D-pinitol, represents as in the case of galactopinitol A [5, 16, 26] the essential isomeric form of the pinitol α-D-galactoside family.

Hydrolysis of α-D-galactosides by α-galactosidase

Enzymic hydrolysis curves of equal weights (20 μg) of sucrose α-D-galactosides (raffinose, stachyose and verbascose), pinitol α-D-galactosides (galactopinitol and ciceritol) and manninotriose by green coffee bean α-D-galactosidase are shown in Fig. 1. Rates of hydrolysis on an equimolecular basis are given in Table 2 as nmol galactose liberated in 15 min per 100 nmol of substrate.

Considering the sucrose α-D-galactoside family, the trisaccharide, raffinose, was totally hydrolysed within 15 min whereas an increase of 1 DP unit (stachyose) produced a lower rate of hydrolysis, additional galactose resulting in a 50% decrease. However, on an equal molar

Table 2. Rate of hydrolysis of α-D-galactosides by green coffee bean α-D-galactosidase*

α-D-Galactoside	Hydrolysis rate (nmol galactose liberated/100 nmol substrate · 15 min)
Sucrose α-D-galactosides	
Raffinose	97.0
Stachyose	89.7
Verbascose	60.0
Pinitol α-D-galactosides	
Galactopinitol†	74.6
Ciceritol	37.6
Glucose α-D-galactoside	
Manninotriose	123.8

*Hydrolysis of 20 μg substrate by 0.25 units α-D-galactosidase (25 mM citrate buffer, pH 4.5, 20°).

†Galactopinitol is a mixture of galactopinitol isomers A and B [5, 26] in a 9:1 molar ratio.

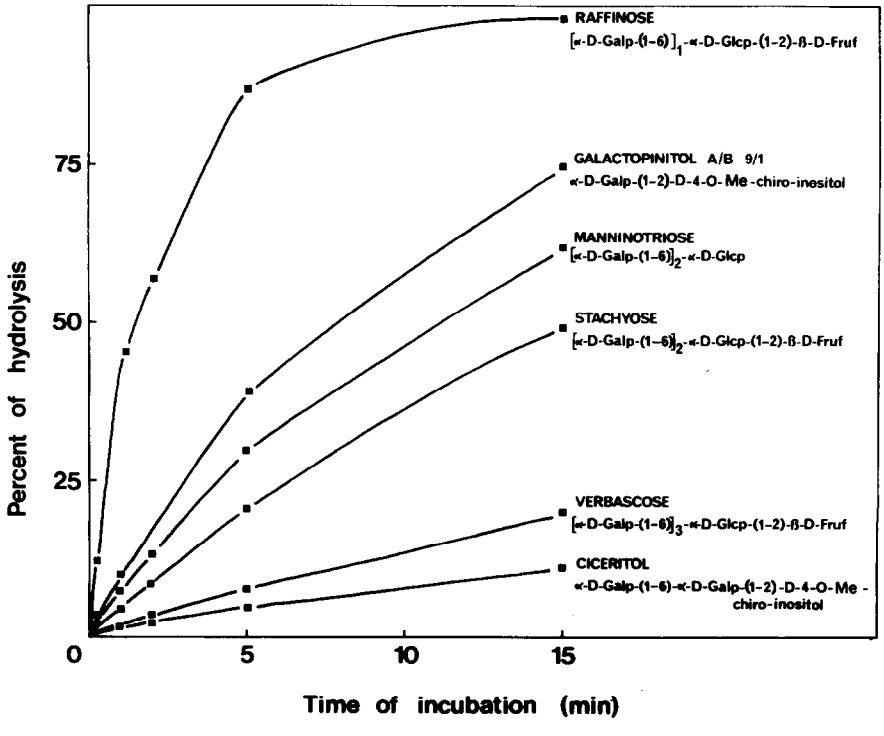


Fig. 1. Enzymatic hydrolysis of sucrose α-D-galactosides, pinitol α-D-galactosides and manninotriose by green coffee bean α-D-galactosidase (20 μg substrate/0.25 units enzyme, pH 4.5, 20°).

concentration basis, the rate of galactose release from stachyose was as high as 93% of that of raffinose. On the other hand, verbascose was hydrolysed at a lower rate (*ca* 62%) compared to raffinose. Therefore, the affinity of green coffee bean α-galactosidase is only slightly lower for stachyose than for raffinose, the decrease being more pronounced for verbascose. However, the activity of α-galactosidase on this pentasaccharide is, nevertheless, noticeable.

The pinitol α-D-galactosides were hydrolysed more slowly (Fig. 1, Table 2) than sucrose α-D-galactosides of corresponding DP. Furthermore, ciceritol was hydrolysed at half the rate of that of the lower homologue, galactopinitol, on a molecular basis. Finally, the best substrate for α-D-galactosidase was manninotriose which is quite understandable since the terminal reducing glucose resembles very much a galactosyl unit. The ease of hydrolysis of manninotriose by α-D-galactosidase would have been in favour of a strong flatus potential for chickpea and lentil in previous assumptions of manninotriose occurrence in these two seeds [5, 8, 11]. Ciceritol, having the same DP as manninotriose, was hydrolysed 3.3-fold slower (molecular basis). In conclusion, the occurrence of a pinitol moiety in an α-D-galactoside is a strong limit to α-galactosidase action. This is to be related with the decrease in the correlation coefficient between hydrogen production in rat and α-D-galactoside contents of several pulses, when ciceritol is added to the raffinose family [10]. As a consequence, we can assume that ciceritol may not be contributing in a significant extent to flatus.

Oligosaccharide composition of seven legume seeds

The oligosaccharide constitution of seven pulses was determined using ethanolic extracts either by HPLC or by GC analysis of TMSi derivatives (Tables 1 and 3). Up to 21 peaks were consistently observed by HPLC in pulses, some of them being found in only one seed. Most of these peaks were identified by both techniques using pure standards. Other unidentified components were thought to be saccharides or glycosides as in the case of vicine and convicine in faba bean [34]. Ciceritol was detected in chickpea (2.8% per dehulled seed), lentil (1.6%), white lupin (0.65%), soya bean (0.08%) and bean (traces) and was absent in other seeds. Our data are very similar to the manninotriose contents reported in chickpea and lentil by Schweizer *et al.* [5] (*ca* 2.4% and 1.4%) and Sosulski *et al.* [11] (2.3% and 1.4%) and in chickpea by Aman [8] (3.4%). Furthermore, our figures fit well with 'unknown I' reported by Fleming [10] in chickpea and lentil (2.8% and 1.7%) as well as with 'unknown II' described by Lineback and Ke [2] in chickpea (1.8%). Manninotriose was not detected in any seed. This degradation product of stachyose by β-fructofuranosidase is not, therefore, a constitutive oligosaccharide of these seven mature legume seeds from the Papilionoideae sub-family. Cyclitols, *myo*-inositol and pinitol or 1D-4-*O*-methyl-*chiro*-inositol were present in all the legume seeds examined in the present study. Pinitol concentration was always higher (0.08–0.45%) than that of *myo*-inositol (0.03–0.30%) except in pea and faba bean. Galactinol ranged from 0.05% in bean to 0.17% in white lupin and was absent in pea and soya bean. The highest galactopinitol content (0.56%) was found in chickpea, lentil and soya bean containing equivalent amounts (0.35%). Pea and faba bean were devoid of this pinitol α-galactoside. Similar data were obtained by Schweizer *et al.* [5]. Finally, the *myo*-inositol and derived oligosaccharide (galactinol) constituted 0.1–0.4% of seeds, whereas pinitol and corresponding galactosides (galactopinitol, ciceritol) represented 0.02–3.80% of cotyledon dry matter.

Oligosaccharides from the raffinose family were found in all legumes except verbascose which was not detected in chickpea. Raffinose contents ranged from 0.2% in faba bean to 0.7% for chickpea. Stachyose was more variable, from 0.85% in faba bean to 7.4% in white lupin. Verbascose percentage varied from 0.25% in bean to 3.1% in pea and faba bean. Taking into consideration the hydrolysis rates of α-D-galactosides by α-galactosidase (Table 2), it becomes obvious that, raffinose and stachyose being roughly equally sensitive and raffinose content being low, mainly stachyose has to be considered in the origin of flatulence. Fleming [10] obtained the best correlation coefficient (0.79) between hydrogen production in the rat and stachyose levels in pulses. The correlation coefficient for verbascose (−0.18) was unfavourable, whereas a significant galactosidase activity was observed in our case. However, as far as α-galactosidases are involved in the flatus phenomenon, differential affinities of bacterial and plant α-galactosidases for α-galactosides could be different from that of green coffee bean. Sucrose ranged from 1.9% in faba bean to 6.7% in soya bean. One must bear in mind that vicine, a glucoside of faba bean, was eluted at the same R_t as sucrose [34]. Our data are in good agreement with those previously reported [5, 11]. Finally, cyclitols and sugars represented from 6.1% of faba bean cotyledons to 13% of those of white lupin.

Unknown peaks were tentatively assigned considering their RR_ts. Peak 2, already reported in our previous work [9, 34], was found only in faba bean (0.2%) and could correspond to the "unknown I" reported in *Vicia faba* only (0.14%) by Naivikul and D'Appolonia [6]. Bean contains an unknown important sugar (1.2%) (peak 5) eluting just in front of sucrose. This material has not yet been reported in the literature. It did not appear on GC chromatograms, although perhaps elution with sucrose would explain the difference between GC and HPLC sucrose determinations in bean seed. Peak 17 (0.2% expressed as manninotriose) exhibited an RR_t slightly higher than that of manninotriose (Table 1). Peak 20 was detected only in chickpea, lentil and white lupin (Table 1) and was assumed to be a tetrasaccharide because of its elution after stachyose, possibly the higher homologue of ciceritol, a trigalactosylpinitol. Its content, expressed as ciceritol, was 0.2%, 0.15% and 0.10%, respectively, which could correspond to the unknown reported by Sosulski *et al.* [11] for the same pulses (0.18%, 0.12% and 0.06%, respectively) as well as the 'unknown II' of Fleming [10] (0.44% and 0.22% for chickpea and lentil).

EXPERIMENTAL

Plant materials. Legume seeds examined were chickpea (*Cicer arietinum* L., var. Aïn Temouchent), lentil (*Lens esculenta* M., var. Large blonde du Chili) and bean (*Phaseolus vulgaris* L., var. Michelet) from Algeria. Faba bean (*Vicia faba* L., var. Ascott), smooth pea (*Pisum sativum* L., var. Amino), lupin (*Lupinus albus* L., var. Kalina) and soya bean (*Glycine max* L., var. Yellow Tokyo) were from France.

Chemicals. Most reference sugars were obtained from Fluka (Switzerland). Stachyose ($4H_2O$) was from Serva (West Germany) and raffinose ($5H_2O$) from Merck (West Germany).

Table 3. Sugar and cyclitol distribution in legume seed cotyledons (% dry matter)

Legume seed	Pinitol*	*Myo*-inositol†	Sucrose*	Galactopinitol†		Galactosyl *chiro*-inositol†	Galactinol†	Raffinose*	Ciceritol*	Stachyose*	Verbascose‡	Total
				A	B							
Faba bean (*Vicia faba*)	0.02	0.03	1.90	—	—	—	0.06	0.20	—	0.85	3.05	6.10
Pea (*Pisum sativum*)	0.05	0.10	2.25	—	—	—	—	0.60	—	2.00	3.10	8.10
Lupin (*Lupinus albus*)	0.30	0.10	2.30	0.02	0.08	0.06	0.17	0.70	0.65	7.40	1.20	13.00
Soya bean (*Glycine max*)	0.20	0.03	6.75	0.30	0.05	—	—	0.50	0.08	4.25	0.30	12.50
Bean (*Phaseolus vulgaris*)	0.08	0.06	4.90	—	0.04	—	0.05	0.30	traces	3.80	0.25	9.50
Lentil (*Lens esculenta*)	0.11	0.07	2.00	0.25	0.11	0.03	0.12	0.30	1.60	3.10	1.40	9.10
Chickpea (*Cicer arietinum*)	0.45	0.30	3.50	0.50	0.06	0.08	0.08	0.70	2.80	2.40	—	10.90

*Average of HPLC and GC determinations.
†Determined by GC.
‡Determined by HPLC.

D-Pinitol and galactopinitol were gifts from Dr. Schweizer, Nestlé Products Technical Assitance (Switzerland). Manninotriose was prepared by incubation of 100 mg stachyose with 50 mg β-fructosidase in 50 ml H_2O. Enzymic hydrolysis was followed by measuring liberated fructose by HPLC as described below. α- and β-Galactosidases, β-galactose dehydrogenase and β-fructosidase were from Boehringer, Mannheim (West Germany). α- and β-Glucosidases, types III and II, respectively, were from Sigma (U.S.A.). CD_3I was Fluka.

Extraction and purification of ciceritol. Chickpea oligosaccharides were extracted from whole flour (2.5 g) by boiling (EtOH–H_2O, 1:1) [35]. The extract was concd × 20 under vacuum (40°) to a yellow syrup (1 ml). *Ca* 250 mg of total sugars corresponding to 30 mg ciceritol was chromatographed on Whatman 3 MM paper with *n*-BuOH–pyridine–H_2O (6:4:3) for 48 hr. Standards of sucrose, raffinose and stachyose were run simultaneously. Narrow strips were cut off and sprayed with diphenylamine–aniline reagent [31]. Ciceritol was not revealed by the reagent but was located between raffinose and stachyose by HPLC analysis of H_2O eluted strips. Ciceritol aq. soln was concd under vacuum to 4 mg/ml as estimated by the orcinol method [36].

Characterization of ciceritol. Anomery of linkages occurring in ciceritol was determined by enzymic assays. Hydrolyses were performed by incubation of an EtOH chickpea extract containing 0.5–2 mg ciceritol with α-galactosidase (1 unit), β-galactosidase (1.5 units), α-glucosidase (20 units), β-glucosidase (10 units) and β-fructosidase (300 units). Hydrolysis products were analysed by HPLC.

Ciceritol was hydrolysed by 2 M TFA at 120° for 1.25 hr [37]. Liberated monosaccharides were alditol acetate derivatized [38] prior to GC analysis. Ciceritol and its constituent sugars obtained by partial acid hydrolysis [39] were trimethylsilyl (TMSi) derivatized [40] prior to GC analysis, using β-methyl-D-xyloside as int. standard.

Methylation of ciceritol. Ciceritol was permethylated or pertrideuteriomethylated in one step by MeI and CD_3I, respectively [32, 33]. After extraction by CH_2Cl_2, methylated or trideuteriomethylated ciceritol was sequentially hydrolysed by 90% HCO_2H (100°, 1 hr) then by 2 M TFA (100°, 3 hr) and the resulting partially methylated or trideuteriomethylated sugars were alditol acetate derivatized prior to GC/MS analysis [33].

Action of α-galactosidase on ciceritol and various α-galactosides. Performed according to the standard Boehringer method described for raffinose determination [29]. Enzymic hydrolyses were conducted on 20 μg substrate in the presence of 0.25 unit of α-D-galactosidase.

Oligosaccharide analysis of legume seeds. Legume seed oligosaccharides were extracted according to ref. [35], but using EtOH–H_2O (1:1) instead of (4:1) since for verbascose-rich pulses (3–3.5% dry wt), extraction of this pentasaccharide is only *ca* 90% in the latter mixture compared with the former. EtOH extracts were concd to 25 ml, purified by Carrez salts [9] and filtered through a Millipore filter (0.45 μm) before HPLC analysis. Oligosaccharides were also TMSi derivatized prior to GC analysis.

Chromatographic methods. HPLC analysis was carried out on a (250 × 0.46 cm i.d.) column packed with Lichrosorb NH_2 (particle size 5 μm) (Merck). Elution was performed with MeCN–H_2O (7:3) at a flow rate of 2 ml/min. Sugars were detected with a differential refractometer at an attenuation setting of 4 × [9].

Alditol acetates of monosaccharides from complete acid hydrolysis of ciceritol were analysed by GC on a (180 × 0.2 cm) column of 3% SP 2340 coated on Supelcoport (100–120 mesh) at 225° (N_2 flow rate: 20 ml/min).

TMSi derivatives of ciceritol, of its constituting sugars obtained by partial acid hydrolysis, and of legume seed oligosaccharides were analysed by GC on a (180 × 0.2 cm) column of 3% OV-1 coated on Gas Chromosorb Q (100–120 mesh) using temp. programming (140° to 320° at 6°/min) with N_2 flow rate of 30 ml/min [8].

Partially methylated or trideuteriomethylated alditol acetates were analysed by GC/MS [33] using a (280 × 0.2 cm) column of 3% OV-225 coated on Gas Chromosorb Q (100–120 mesh) at 170° (He flow rate: 20 ml/min). The column effluent was introduced into the mass spectrometer via a Brunnee separator. The spectrometer was operated at an inlet temp. of 250°, an ionization potential of 70 eV and an ion source temp. of 220°. Recorded spectra were analysed by comparison with those described for partially methylated alditol acetates [33] and for trideuteriomethylated acetylated cyclitols [26].

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