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Fagopyritols: Occurrence, Biosynthesis, Analyses and Possible Role

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ABSTRACT

The discovery, isolation, purification, and molecular structure characterization of six fagopyritols found in common buckwheat (*Fagopyrum esculentum* Moench) seeds and milling fractions are described. The proposed roles of fagopyritols in seed maturation, seed desiccation tolerance, agronomic seed performance, and human health are outlined. The similarities in molecular structure of fagopyritols to a putative insulin mediator related to non-insulin dependent diabetes mellitus and polycystic ovary syndrome are described. The characterization of genes encoding enzymes capable of synthesizing buckwheat fagopyritols is highlighted.

Keywords: buckwheat, seed, fagopyritols, molecular structure, health-benefits

Abbreviations: GC, gas chromatography; **GC-MS**, gas chromatography-mass spectrometry; **GolS**, galactinol synthase; **DGMI**, digalactosyl *myo*-inositol; **FID**, flame-ionization-detector; **NIDDM**, non-insulin dependent diabetes mellitus; **PCOS**, polycystic ovary syndrome; **Rt-PCR**, reverse transcriptase polymerase chain reaction; **RACE-PCR**, rapid amplification of cDNA ends polymerase chain reaction; **STS**, stachyose synthase; **TGMI**, trigalactosyl *myo*-inositol; **TMS products**, trimethylsilyl products; **TMSI**, trimethylsilylimidazole; **UDP-gal**, uridine diphosphate galactose

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INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is an under-utilized crop (Marshall and Pomeranz 1982) known for its rich source of nutrients and health-related components in its edible seeds (Pomeranz 1983; Horbowicz and Obendorf 1992; Obendorf *et al*. 1993; Steadman *et al*. 2000, 2001a, 2001b; Li and Zhang 2001; Krkošková and Mrázová 2005; Christa and Soral-Smietana 2008). Unlike most seeds, buckwheat contains only small amounts of raffinose and stachyose, but instead accumulates mostly sucrose and galactosides of D-*chiro*-inositol, called fagopyritols after the species name *Fagopyrum*, as seed soluble carbohydrates (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998; Szczeciński *et al*. 1998; Obendorf *et al*. 2000; Steadman *et al*. 2001c; Horbowicz and Obendorf 2005). The chemical structures and biosynthesis of fagopyritols, as well as their health-related potential, are reviewed.

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Invited Review

CHEMISTRY

Chemical structures and nomenclature of *myo*-inositol and *chiro*-inositol

Two cyclic sugar alcohols called cyclitols, *myo*-inositol (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol) and D-*chiro*-inositol (*cis*-1,2,4-*trans*-3,5,6-cyclohexanehexol), are found in common buckwheat tissues. The six carbons of the *myo*-inositol ring are numbered in counter-clockwise direction (**Fig. 1**, top left) representing D-*myo*-inositol or in the clockwise direction (**Fig. 1**, top right) representing L-*myo*-inositol. In the absence of additional linkage groups to hydroxyl positions on the six-carbon *myo*-inositol ring, D-*myo*-inositol and L-*myo*-inositol represent the same compound called *myo*-inositol (**Fig. 1**, top center). However, when a hydroxyl position on a carbon in the *myo*-inositol ring has a linked group, such as a methyl ether (*O*-methyl) group as in D-ononitol (1D-4-*O*-methyl-*myo*-inositol) (**Fig. 1**, center), the car-bon with the attached linkage must be identified by the D- or L- numbering system. D-Ononitol is numbered in the D-direction to give the smallest number. D-*chiro*-Inositol (**Fig. 1**, bottom left) is numbered in counter-clockwise direction, whereas L-*chiro*-inositol (**Fig. 1**, bottom right) is numbered in the clockwise direction. *chiro*-Inositol is a symmetrical compound; therefore, linkage to the hydroxyl position on carbon-1 or on carbon-6 results in the same compound. Similarly, linkage to the hydroxyl position on carbon-2 or on carbon-5 results in the same compound, and linkage to the hydroxyl position on carbon-3 or on carbon-4 results in the same compound (reviewed by Horbowicz and Obendorf 1994).

In legumes, *myo*-inositol is converted to D-pinitol (1D-3-*O*-methyl-*chiro*-inositol) (**Fig. 1**, bottom center) through D-ononitol as an intermediate compound (Dittrich and Brandl 1987; reviewed by Horbowicz and Obendorf 1994).

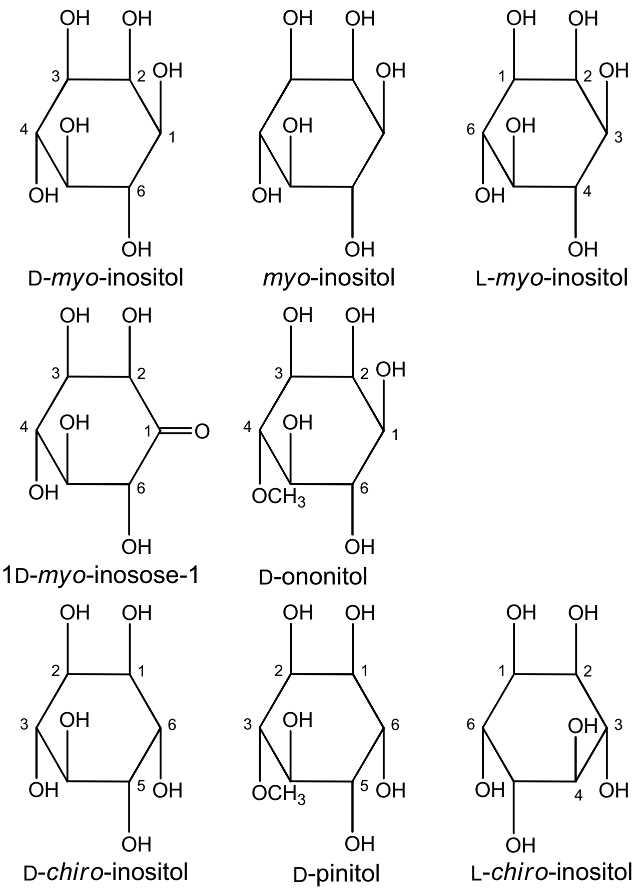

Fig. 1 Structures of *myo*-inositol and *chiro*-inositol, some related cyclitols, and the proposed biosynthetic intermediate product 1D-*myo*-inosose-1.

In higher plants, it has been proposed that D-*chiro*-inositol is synthesized by demethylation of D-pinitol (Scholda *et al.* 1964; reviewed by Hoffman-Ostenhoff and Pittner 1982 and Horbowicz and Obendorf 1994), but an enzyme for this reaction has not been identified. Neither D-ononitol, nor other *O*-methyl cyclitols have been detected in buckwheat (Horbowicz and Obendorf 1994; Horbowicz *et al.* 1998; Horbowicz and Obendorf 2005). In leaves of buckwheat (Ma *et al.* 2005) and soybean (*Glycine max* (L.) Merrill) (Gomes *et al.* 2005), D-*chiro*-inositol is synthesized from *myo*-inositol, most likely with 1D-*myo*-inosose-1 as an intermediate compound (**Fig. 1**, top left to middle left to bottom left). *Trifolium incarnatum* L. leaves also can synthesize D-*chiro*-inositol from 1D-*myo*-inosose-1 (Scholda *et al.* 1964) (**Fig. 1**, top center to center to bottom center). *Chlorella* can synthesize D-*chiro*-inositol from *myo*-inositol without the formation of methyl ether intermediates (Woeber and Hoffmann-Ostenhof 1969; Woeber *et al.* 1971). The conversion of *myo*-inositol to D-*chiro*-inositol also has been reported in microbial (L'Annunziata *et al.* 1977; Yoshida *et al.* 2006) and animal systems (Hipps *et al.* 1973; Pak *et al.* 1992, 1993). The conversion of *myo*-inositol to D-*chiro*-inositol is reduced in type 2 diabetic (NIDDM) rats compared to control rats (Sun *et al.* 2002). However, another study concludes that D-*chiro*-inositol is neither synthesized endogenously nor converted from *myo*-inositol in rodents (Lin *et al.* 2009b), although both D-pinitol (1D-3-*O*-methyl-*chiro*-inositol) and D-*chiro*-inositol are readily absorbed from dietary sources and appear to be solely derived from the diet (Lin *et al.* 2009b).

Chemical structures and nomenclature of fagopyritols

Fagopyritols are mono-, di-, or tri- α-galactosides of D-*chiro*-inositol. Six fagopyritols in two distinct series (**Figs. 2, 3**) are present in embryo tissues of common buckwheat seeds. In the fagopyritol B series (**Fig. 2**), the α-galactoside linkage is to the 2-carbon of D-*chiro*-inositol yielding fagopyritol B1 [α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol], fagopyritol B2 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol], and fagopyritol B3 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol] (Szczeciński *et al.* 1998; Obendorf *et al.* 2000; Steadman *et al.* 2001c). In the fagopyritol A series (**Fig. 3**), the α-galactoside linkage is to the 3-carbon of D-*chiro*-inositol yielding fagopyritol A1 [α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol], fagopyritol A2 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol], and fagopyritol A3 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol] (Obendorf *et al.* 2000; Steadman *et al.* 2001c). Horbowicz and Obendorf (1994) reviewed the nomenclature rules and some common mistakes in naming cyclitols and galactosyl cyclitols. All known fagopyritol structures, except fagopyritol B3, were confirmed by NMR spectral analysis (Szczeciński *et al.* 1998; Obendorf *et al.* 2000; Steadman *et al.* 2001c). The fagopyritol B3 structure was deduced from analysis of hydrolysis products (Steadman *et al.* 2001c) and recently has been confirmed by analysis of its NMR spectra (Gui W, Lemley BA, Keresztes I, Condo Jr. AM, Steadman KJ, Obendorf RL 2009 unpublished).

In addition to fagopyritols, buckwheat seeds also contain galactosides of *myo*-inositol, including galactinol [α-D-galactopyranosyl-(1→1)-L-*myo*-inositol, also known as α-D-galactopyranosyl-(1→3)-D-*myo*-inositol], digalactosyl *myo*-inositol [DGMI; α-D-galactopyranosyl-(1→6)-α-D-galacto-pyranosyl-(1→1)-l-*myo*-inositol], and trigalactosyl *myo*-inositol [TGMI; α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol] (**Fig. 4**). The structures of *myo*-inositol (Brown and Serro 1953) and galactinol (Brown and Serro 1953; Noguchi *et al.* 2000) have been confirmed. Recently, the structures of digalactosyl *myo*-inositol and trigalactosyl

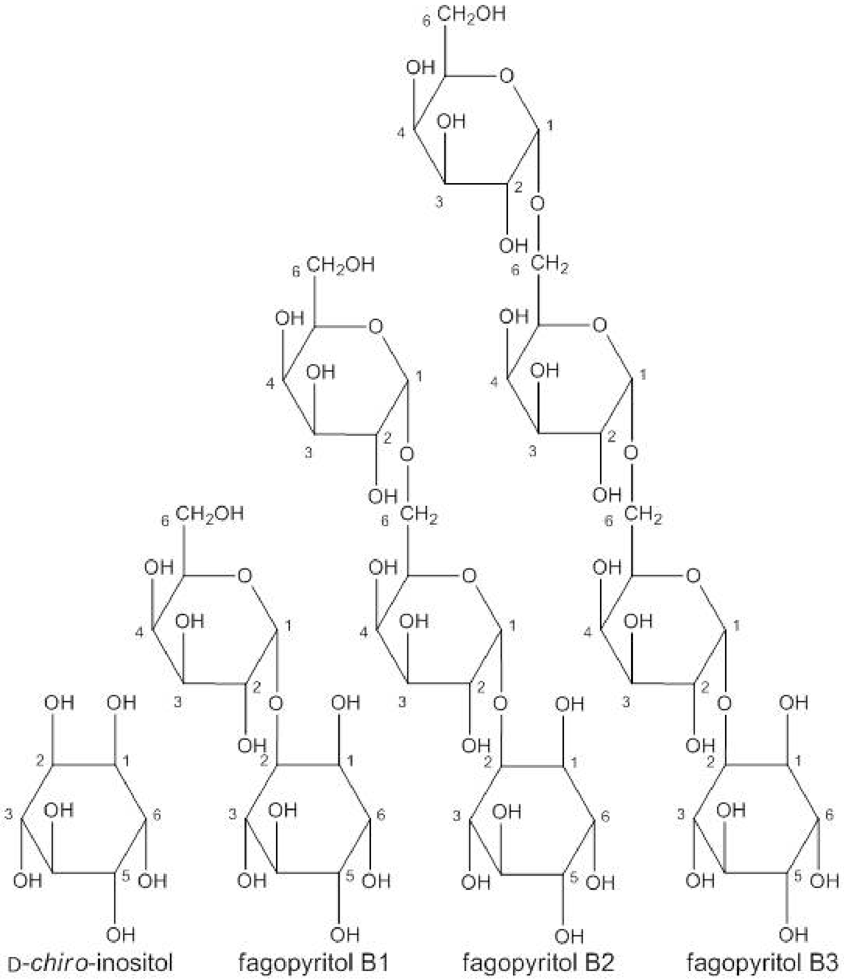

Fig. 2 Structures of galactosides of D-*chiro*-inositol of the fagopyritol B series including D-*chiro*-inositol, fagopyritol B1 (α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol), fagopyritol B2 (α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol), and fagopyritol B3 (α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol).

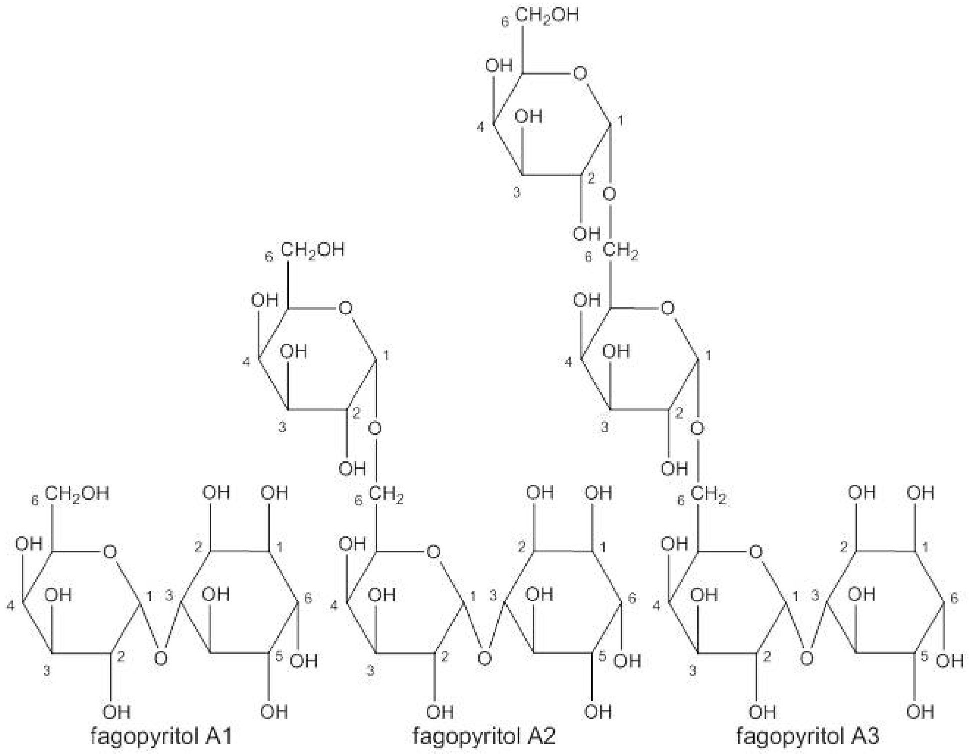

Fig. 3 Structures of galactosides of D-*chiro*-inositol of the fagopyritol A series including fagopyritol A1 (α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol), fagopyritol A2 (α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol), and fagopyritol A3 (α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol).

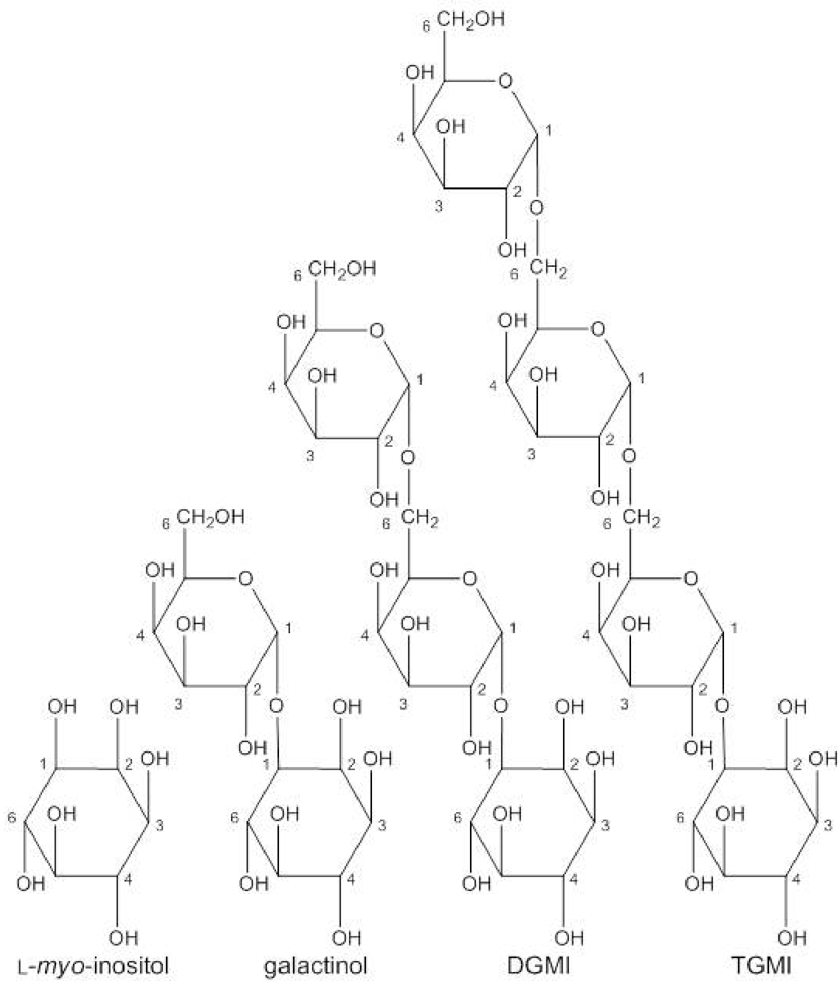

Fig. 4 Structures of the galactinol series compounds including L-*myo*-inositol, galactinol [α-D-galactopyranosyl-(1→1)-L-*myo*-inositol; also known as α-D-galactopyranosyl-(1→3)-D-*myo*-inositol], digalactosyl *myo*-inositol [DGMI; α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol], and trigalactosyl *myo*-inositol [TGMI; α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol].

myo-inositol have been confirmed by analysis of their NMR spectra (Gui W, Lemley BA, Keresztes I, Condo Jr. AM, Steadman KJ, Obendorf RL 2009 unpublished).

Analytical methods

The methods for quantitative analysis of soluble carbohydrates have been reviewed (Kadlec *et al.* 2001; Obendorf *et al.* 2012). Sources of cyclitol galactosides, that are not available commercially, have been identified for use as reference compounds during analysis of soluble carbohydrates (Kadlec *et al.* 2001). High resolution gas chromatography (GC) is a favored method for analysis of soluble carbohydrates following their conversion to volatile trimethylsilyl (TMS) derivatives (Traitler *et al.* 1984; Horbowicz and Obendorf 1994; Obendorf *et al.* 1998; Kadlec *et al.* 2001; Gomes *et al.* 2005; Obendorf *et al.* 2009, 2012). In this method, 20 or more soluble carbohydrates that occur in plant tissues can be extracted, separated, and determined in a single assay (**Fig. 5**). Some capillary GC columns can separate with high resolution the TMS-carbohydrates from seed extracts within 15 min (Obendorf *et al.* 2012). Samples for GC analysis are extracted with ethanol-water (1:1, v/v), and the applied solvents are evaporated under a stream of nitrogen gas, followed by drying over P_2O_5 overnight to remove traces of water. If extracts are concentrated in a rotary speed evaporator, keep temperatures at 40°C or lower to minimize the potential for artifacts. Dry samples are derivatized with trimethylsilylsilimidazole (TMSI):pyridine (1:1, v/v) forming trimethylsilyl (TMS) derivatives at hydroxyl groups on the cyclitol and sugar. Drying is critical for complete derivatization, due to the rapid breakdown of the silylation reagent by traces of water. Separation of derivatized carbohydrates is commonly performed on a DB-1 (Supelco), an HP1-MS (Agilent Technologies) (15 m length, 0.25 mm inside diameter, 0.25 µm film thickness), or a Zebron ZB-1 (Phenomenex) glass capillary column (15 m length, 0.25 mm inside diameter, 0.10 µm film thickness, 100% dimethylpolysiloxane) and detected by a flame-ionization-detector (FID) or a mass spectra detector (GC-MS). GC analysis of soluble carbohydrates is sensitive and efficient. However, columns are commonly operated at near maximum temperatures for long periods of time (>40 min if using columns with 0.25 µm film thickness) especially for analysis of oligosaccharides with three to five rings (e.g., 3-5 mers or degrees of polymerization). For analysis of larger oligomers, liquid chromatography (HPLC) may be preferred, but sensitivity and resolution may be somewhat less with HPLC than with GC depending on the specific compounds being analyzed (Kadlec *et al.* 2001). The GC method outlined above for small molecular weight soluble carbohydrates does not efficiently separate nor detect charged compounds (zwitter ions, organic acids, amino acids, phosphorylated sugars or cyclitols) or their salts. Trimethylsilyl derivatives of glucose, fructose, galactose, and maltose capture the anomeric forms of the sugars as distinct TMS products (**Fig. 3**; Horbowicz and Obendorf 1994; Horbowicz *et al.* 1998). Acid (3 N trifluoroacetic acid) or enzyme (α-galactosidase) hydrolysis of fagopyritols, followed by GC analysis of the hydrolysis products, are useful for determination of the number and ratio of monomeric components and provides evidence of the α-linkages between monomeric components. Chiral capillary columns (Leavitt and Sherman 1982) have been used to distinguish the enantiomers D-*chiro*-inositol and L-*chiro*-inositol (Szczeciński *et al.* 1998; Obendorf *et al.* 2000; Steadman *et al.* 2001c) which typically co-chromatograph on most other columns.

Optical rotation values of selected free cyclitols and cyclitol galactosides

Optical rotation is sometimes used as one of several properties to identify a compound or the purity of a compound. Optical rotation values are expressed as: $[\alpha]_D^{20}$, where α is the value of optical rotation in degrees (°), subscript "D" is the concentration of chemical in grams per 100 milliliters of solvent, and superscript "20" is the temperature in °C. Information was assembled on optical rotation of fagopyritols and related cyclitols and galactosyl cyclitols (**Table 1**). In literature sources, only free cyclitols were crystalline (except pinpollitol). The galactosyl cyclitols generally form a white powder after freeze drying. Many were reported to be a colorless solid (probably means glass-like, not crystal). Therefore, melting points are not easily established and are reported infrequently and usually as broad ranges.

Some observed trends may be summarized as follows. D-*chiro*-Inositol and d-pinitol (with one *O*-methyl) are similar at +63° to +65° (Schweizer *et al.* 1978; Baumgartner *et al.* 1986) (**Table 1**). Adding a second *O*-methyl group (i.e., pinpollitol) is slightly lower at +50° (Angyal *et al.* 1976). *myo*-Inositol is optically inactive (no optical rotation). The D-ononitol value (+6.4°) (Binder and Haddon 1984; Richter *et al.* 1997) is low as expected from the *myo*-inositol ring. L-Bornesitol values (+32.05°, +34.8°) (Foster and Stacey 1953; Loewus FA 1994 unpublished) and D-bornesitol values (–26.4°, –28.4°, and –32.05°) (Bien and Ginsburg 1958; Ichimura *et al.* 1999; Obendorf *et al.* 2005) are higher than D-ononitol (note the reversal in direction of rotation for bornesitol). Our unpublished data for fagopyritol B1 (+166°) is similar to the value (+170°) obtained by Schweizer and Horman (1981). Galactinol and galactosyl ononitol (both *myo*-inositol containing) are +135.6° (Brown and Serro 1953) and +129.6° (Richter *et al.* 1997), whereas lathyritol, a galactosyl D-bornesitol, is +96.55° (Obendorf *et al.* 2005), consistent with D-bornesitol being levorotary. Digalactosyl ononitol is +162.5° (Peterbauer *et al.* 2003), a value higher than monogalactosyl ononitol, and similar to fagopyritol A2 (+165°) (Lewis BA and Obendorf RL 2000 unpublished). Galactopinitol A and trigalactopinitol A are similar at +181° and +179° (Schweizer and Horman 1981; Nicolas *et al.* 1984). Galactopinitol B is slightly lower at +159° (Schweizer and Horman 1981). Values for ciceritol (a digalactosyl pinitol A), trigalactosyl pinitol B, fagopyritol B3, or di- and tri-galactosyl *myo*-inositol have not been reported.

Methods of isolation and purification

Methods of isolation and purification of the fagopyritols have been reported (Horbowicz and Obendorf 1994; Horbowicz *et al.* 1998; Szczeciński *et al.* 1998; Obendorf *et al.* 2000; Steadman *et al.* 2001c; Horbowicz and Obendorf 2005). Mature dry seeds, seed parts, or seed milling fractions are ground to a fine powder. Small wet samples, or plant tissues containing oil, are frozen in liquid nitrogen and ground to a fine powder in a mortar pre-chilled with liquid nitrogen. Fagopyritols and other soluble carbohydrates are extracted from the pulverized sample with ethanol:water, 1:1 (v/v), and solvents are evaporated by freeze drying. The obtained residues are dissolved in a small amount of water forming a concentrated extract. The concentrated extract is placed on a carbon-Celite (1:1, v/v; Whistler and Durso 1950) column (100 mm × 180 mm bed volume). The column is eluted with water followed by increasing concentrations of ethanol in water (**Fig. 6**). D-*chiro*-Inositol and *myo*-inositol are eluted from the column with water, fructose and glucose are eluted with 2% ethanol, fagopyritol B1 is eluted with 4% ethanol, fagopyritol A1 is eluted with 5% ethanol, fagopyritol B2 is eluted with 10% ethanol, fagopyritol A2 is eluted with 14% ethanol, fagopyritol B3 is eluted with 20% ethanol, and fagopyritol A3 is eluted with 50% ethanol (**Fig. 6**). The obtained fractions (500 ml) are freeze-dried, concentrated in a small amount of water, filtered, dried, re-dissolved, and assayed for compositional analysis by GC (**Fig. 5**). Samples containing compounds of interest are pooled, re-chromatographed on a 25 mm x 900 mm bed of charcoal:Celite, and eluted with stepwise increases in ethanol concentration. Fractions containing a fagopyritol of interest are pooled and re-chromatographed as needed to provide an essentially pure (>95%) fagopyritol after freeze drying. The purified fagopyritols form a white powder, but not crystals, when dried.

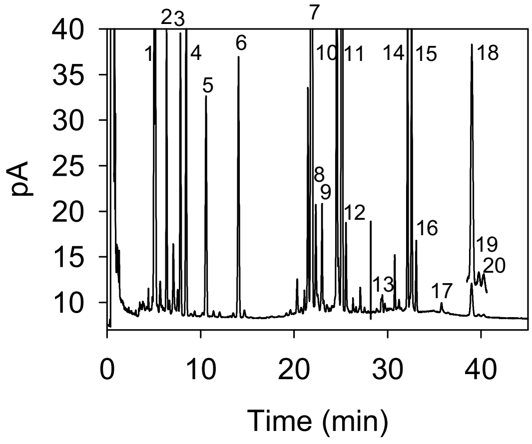

Fig. 5 Gas chromatogram of concentrated buckwheat seed (bran milling fraction) extract. Extract dry residues including the internal standard phenyl α-d-glucoside were derivatized with trimethylsilylimidazole (TMSI):pyridine and analyzed by gas chromatography (Horbowicz and Obendorf 1994) with minor changes (Gomes *et al.* 2005) using an HP-1MS capillary column and a flame ionization detector (FID). Identification of peaks: fructose (3 peaks) (**1**), glucose (2 peaks) (**2**, **4**), d-*chiro*-inositol (**3**), *myo*-inositol (**5**), phenyl α-d-glucoside (internal standard) (**6**), sucrose (**7**), maltose (two peaks) (**8**, **9**), fagopyritol A1 (**10**), fagopyritol B1 (**11**), galactinol (**12**), raffinose (**13**), fagopyritol A2 (**14**), fagopyritol B2 (**15**), digalactosyl *myo*-inositol (DGMI) (**16**), stachyose (**17**), fagopyritol A3 (**18**), fagopyritol B3 (**19**), trigalactosyl *myo*-inositol (TGMI) (**20**). Insert: peaks **18-20** are expanded. Trimethylsilyl derivatives of fructose, galactose (not shown), glucose, and maltose capture the anomeric forms of the sugars as distinct TMS products.

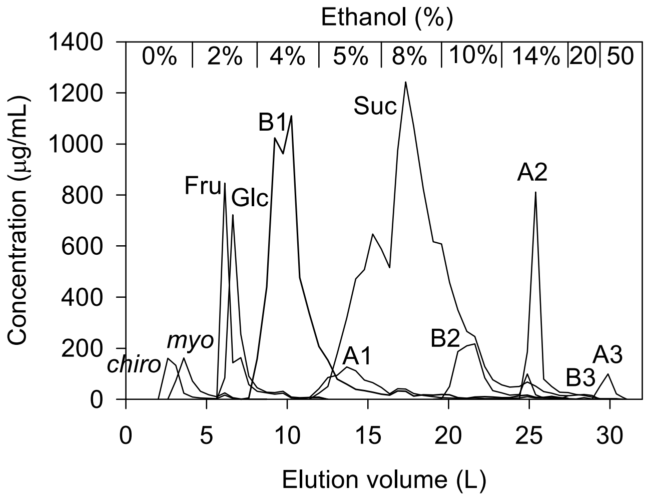

Fig. 6 Typical elution profile for fagopyritols from buckwheat bran extract concentrate on a primary Carbon-Celite column (100 mm × 180 mm). Collected elution fractions were 500 mL each. Fractions of interest were concentrated by freeze-drying.

Table 1 Optical rotation values reported for selected cyclitols and their galactosides.

Cyclitol, O-methyl cyclitol, or galactosyl cyclitol	Optical rotation values	References
D-*chiro*-Inositol	$[\alpha]_D^{20}$ +65° (c 0.1, water)	Schweizer *et al*. 1978
D-Pinitol	$[\alpha]_D^{20}$ +63° (c ??, water)	Schweizer *et al*. 1978
[1D-3-*O*-methyl-*chiro*-inositol]	$[\alpha]_D^{20}$ +64.5° (c 500, water)	Baumgartner *et al*. 1986
Pinpollitol [1D-1,4-di-*O*-methyl-*chiro*-inositol]	$[\alpha]_D^{22.5}$ +50°(c 0.79, methanol)	Angyal *et al*. 1976
myo-Inositol	Optically inactive	
D-Ononitol	$[\alpha]_D^{20}$ +6.55° (c ?, water)	Binder and Haddon 1984
[1D-4-*O*-methyl-*myo*-inositol]	$[\alpha]_D^{20}$ +6.4° (c 0.5, water)	Richter *et al*. 1997
D-Bornesitol	$[\alpha]_D^{18}$ –32.05° (c 3.5, water)	Bien and Ginsburg 1958
[1D-1-*O*-methyl-*myo*-inositol]	$[\alpha]_D^{30}$ –26.4° (c 0.5, water)	Ichimura *et al*. 1999
	$[\alpha]_D^{23}$ –28.4° (c 0.76, water)	Obendorf *et al*. 2005
L-Bornesitol	$[\alpha]_D$ +32.05° (c 0.8, water)	Foster and Stacey 1953
[1L-1-*O*-methyl-*myo*-inositol]	$[\alpha]_D^{28}$ +34.8° (c ?, solv ?)	Loewus FA 1994 unpub. data
Fagopyritol B1	$[\alpha]_D^{23}$ +170° (c 0.2, water)	Schweizer and Horman 1981
[α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol]	$[\alpha]_D^{20}$ +166° (c 0.6, water)	Lewis BA and Obendorf RL 2000 unpublished data
Fagopyritol B2 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol]	$[\alpha]_D^{20}$ +149° (c 0.3, water)	Lewis BA and Obendorf RL 2000 unpublished data
Fagopyritol A1 [α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol]	$[\alpha]_D^{22}$ +141° (c 0.2, water)	Lewis BA and Obendorf RL 2000 unpublished data
Fagopyritol A2 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol]	$[\alpha]_D^{22}$ +165° (c 0.2, water)	Lewis BA and Obendorf RL 2000 unpublished data
Fagopyritol A3 [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol]	$[\alpha]_D^{18}$ +144° (c 0.2, water)	Lewis BA and Obendorf RL 2000 unpublished data
Galactopinitol A [α-D-galactopyranosyl-(1→2)-1D-4-*O*-methyl-*chiro*-inositol]	$[\alpha]_D^{23}$ +181° (c 0.2, water)	Schweizer and Horman 1981
Trigalactopinitol A [α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-1D-4-*O*-methyl-*chiro*-inositol]	$[\alpha]_D^{23}$ +179° (c 0.5, water)	Nicolas *et al*. 1984
Galactopinitol B [α-D-galactopyranosyl-(1→2)-1D-3-*O*-methyl-*chiro*-inositol]	$[\alpha]_D^{23}$ +159° (c 0.2, water)	Schweizer and Horman 1981
Galactinol [α-D-galactopyranosyl-(1→1)-1L-*myo*-inositol]	$[\alpha]_D^{20}$ +135.6° (c ?, solv ?)	Brown and Serro 1953
Galactosyl ononitol [α-D-galactopyranosyl-(1→3)-1D-4-*O*-methyl-*myo*-inositol]	$[\alpha]_D^{20}$ +129.6° (c 0.5, water)	Richter *et al*. 1997
Digalactosyl ononitol [α-D-galactopyranosyl-(1→6)- α-D-galactopyranosyl-(1→3)-1D-4-*O*-methyl-*myo*-inositol]	$[\alpha]_D^{20}$ +162.5° (c 0.2, water)	Peterbauer *et al*. 2003
Lathyritol (galactosyl D-bornesitol) [α-D-galactopyranosyl-(1→3)-1D-1-*O*-methyl-*myo*-inositol]	$[\alpha]_D^{23}$ +96.55° (c 1.45, water)	Obendorf *et al.* 2005

Chemical synthesis

Chemical synthesis of D-*chiro*-inositol and other inositols has been reviewed (Duchek *et al*. 2011). Fagopyritol B1 and fagopyriol A1 have been chemically synthesized (Kornienko *et al*. 1998; Cid *et al*. 2004). Reports of a partially characterized rat liver putative insulin mediator that contains galactosamine and D-*chiro*-inositol (Larner *et al*. 1988), partially characterized human liver putative insulin mediators (Caro *et al*. 1997), and a beef liver putative insulin mediator that contains a galactosamine D-pinitol manganese chelate (Larner *et al*. 2003) stimulated the synthesis of a number of related compounds (Berlin *et al*. 1990, 1991; Bonilla *et al*. 2002; Cid *et al*. 2002, 2003; Hart *et al*. 2001, 2004). The naturally occurring fagopyritol A1 with its unique α-(1→3)-linkage (Obendorf *et al*. 2000) is isosteric with 2-amino-2-deoxy-α-D-galactopyranosyl-(1→3)-D-*chiro*-inositol related to a putative insulin mediator (Berlin *et al*. 1990).

PHYSIOLOGY

Occurrence in plants

D-*chiro*-Inositol is present in leaves (Ma *et al*. 2005; Kosina *et al*. 2009) and seed embryos (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998; Gomes *et al*. 2005; Horbowicz and Obendorf 2005; Kosina *et al*. 2009, 2010) of common buckwheat and some legume plants and in citrus juice (mandarin, orange, grapefruit, lime, lemon) (Sanz *et al*. 2004). D-*chiro*-Inositol is synthesized in leaves of common buckwheat and soybean (Ma *et al*. 2005; Kosina *et al*. 2009) and perhaps other maternal tissues, is transported to seeds, is unloaded by seed coats (Ma *et al*. 2005; Kosina *et al*. 2009, 2010), and is absorbed by the embryos where it is stored primarily as fagopyritols, galactosides of D-*chiro*-inositol, in maturing seeds (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998; Obendorf *et al*. 1998; Szczeciński *et al*. 1998; Obendorf *et al*. 2000; Steadman *et al*. 2000, 2001c; Horbowicz and Obendorf 2005; Obendorf *et al*. 2009). L-*chiro*-Inositol, or galactosides of L-*chiro*-inositol, is rarely present in plants or seeds and is not detectable in buckwheat seeds (Szczeciński *et al*. 1998; Obendorf *et al*. 2000; Steadman *et al*. 2001c). Fagopyritol B1 is present in seeds of soybean, lupin, pigeon pea, cowpea, lentil, castor bean, and jojoba bean (Schweizer and Horman 1981; Horbowicz and Obendorf 1994; Górecki *et al*. 1997; Ogawa *et al*. 1997), and fagopyritol B2 is in sugar beet (*Beta vulgaris* L.), buckwheat, and other seeds (Shiomi *et al*. 1988; Horbowicz and Obendorf 1994; Górecki *et al*. 1997). Fagopyritol B3 is present in seeds of buckwheat (Steadman *et al*. 2000, 2001c) and certain genotypes of soybean (Obendorf *et al*. 2009) in small amounts. Fagopyritol A1, fagopyritol A2, and fagopyritol A3 are present only in buckwheat seeds (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998; Szczeciński *et al*. 1998; Obendorf *et al*. 2000; Steadman *et al*. 2000, 2001c; Horbowicz and Obendorf 2005).

Role in plants

In contrast to maturing embryos of many plant seeds that accumulate sucrose and the raffinose family of oligosaccharides (RFO), such as raffinose, stachyose and verbascose, as the predominant soluble sugars (Kuo *et al*. 1988; Horbowicz and Obendorf 1994), embryos of maturing common buckwheat seeds accumulate fagopyritols, galactosyl derivatives of D-*chiro*-inositol (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998; Szczeciński *et al*. 1998; Obendorf *et al*. 2000; Steadman *et al*. 2000, 2001c; Horbowicz and Obendorf 2005). Fagopyritols accumulate in embryo tissues during seed maturation (12 to 20 days after pollination of common buckwheat; embryo dry weight is maximum at 18 days after pollination) (Horbowicz *et al*. 1998), and fagopyritol accumulation is associated with the onset of seed desiccation tolerance in buckwheat (Horbowicz *et al*. 1998) and other seeds including soybean (Obendorf *et al*. 1998, 2009). Soybean seeds expressing the mutant *stc1* gene with low raffinose and stachyose (Hitz *et al*. 2002), accumulate fagopyritols to higher concentrations, especially fagopyritol B2 and fagopyritol B3 (Obendorf *et al*. 2009), tolerate imbibitional chilling injury (Obendorf *et al*. 2008), and have normal field emergence (Neus *et al*. 2005). Because fagopyritols accumulate during seed maturation, they are also seed reserve soluble carbohydrates. There is no evidence that fagopyritols accumulate in plant tissues other than seeds. Fagopyritols are proposed to substitute for the role of raffinose family oligosaccharides in seed desiccation tolerance (Horbowicz and Obendorf 1994; Obendorf *et al*. 2008). Fagopyritols (36.2 mg L^{-1}), D-*chiro*-inositol (2.6 mg L^{-1}), and *myo*-inositol (8.9 mg L^{-1}) have been reported to be present in concentrated extracts of *Momordica charantia* fruits (Xia and Wang 2007). However, the fagopyritols were not identified nor was galactinol reported. It is unclear if the fagopyritols were present in fruit tissues or in seeds contained within the fruits.

Factors affecting accumulation of fagopyritols

Fagopyritols accumulate during rapid growth of buckwheat embryos during seed formation (Horbowicz *et al*. 1998; Horbowicz and Obendorf 2005). Fagopyritol A1 and fagopyritol B1 accumulations are favored by cool temperatures, and the higher oligomers, fagopyritol A2, fagopyritol B2, fagopryitol A3, and fagopyritol B3 are favored by higher temperatures (Horbowicz and Obendorf 2005) during seed maturation. Increasing the supply of D-*chiro*-inositol to seeds increases the accumulation of fagopyritols in mature seeds of buckwheat (Ma *et al*. 2005) and other plants (Obendorf *et al*. 2004; Gomes *et al*. 2005; Obendorf and Kosina 2011) including those that normally do not accumulate D-*chiro*-inositol (Lahuta *et al*. 2005a, 2005b, 2010; Lahuta and Goszczyńska 2010; Lahuta and Dzik 2011).

BIOSYNTHESIS

Biosynthetic pathways

Glucose-6-phosphate is a substrate for the biosynthesis of *myo*-inositol and other cyclitols. *myo*-Inositol phosphate synthase (EC 5.5.1.4) converts glucose-6-phosphate to *myo*-inositol-1-phosphate, which in turn is transformed to *myo*-inositol by *myo*-inositol-1-phosphate phosphatase (EC 3.1.3.25) (Górecki *et al*. 2001). *myo*-Inositol is a precursor to many other cyclitols, including D-*chiro*-inositol. In leaves of higher plants, the enzyme that converts *myo*-inositol to D-*chiro*-inositol is unknown, but it is proposed to be a two-step oxidoreductase reaction with 1D-*myo*-inosose-1 as an intermediate (**Fig. 1**, Horbowicz and Obendorf 1994; Obendorf 1997). D-*chiro*-Inositol is transported from leaves to seeds where it is stored as fagopyritols. D-*chiro*-Inositol is not synthesized in seeds, and fagopyritols are not synthesized in leaves.

Until the last decade, very little has been known about the enzymes, fagopyritol synthases, that catalyze the synthesis of fagopyritols in buckwheat. Obendorf *et al*. (2004) hypothesized two possible enzymatic reactions for fagopyritol synthase: (1) like galactinol synthase (GolS, UDP-galactose:*myo*-inositol galactosyltransferase; EC 2.4.1.123) it may use UDP-galactose (UDP-Gal) as the galactosyl donor and D-*chiro*-inositol as the galactosyl acceptor to synthesize fagopyritols (Frydman and Neufeld 1963), or (2) like stachyose synthase (STS), galactinol:raffinose galactosyltransferase; EC 2.4.1.67) it may use galactinol (α-D-galactopyranosyl-(1→1)-L-*myo*-inositol) as the galactosyl donor and D-*chiro*-inositol as the galactosyl acceptor to synthesize fagopyritols (Hoch *et al*. 1999). It has been reported that pea (*Pisum sativum* L.) seed GolS (Frydman and Neufeld 1963) and lentil (*Lens culinaris* L.) STS (Hoch *et al*. 1999) can form a product with D-*chiro*-inositol as substrate, although the product has not been confirmed to be a fagopyritol. The lack of activity of adzuki bean (*Vigna angularis* Ohwi et Ohashi) STS with D-*chiro*-inositol (Peterbauer and Richter 1998) and the very limited accumulation of stachyose in buckwheat seeds (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998) suggests that STS is not involved in the synthesis of fagopyritols in buckwheat. Ueda *et al.* (2005) favored the first reaction based on their observations from two preliminary *in vitro* enzyme assays using the crude enzyme extracts from immature embryos harvested at 20 days after pollination or from buckwheat bran prepared from mature dry seeds. First, no fagopyritol synthesis was detected in the assays when galactinol was used as the galactosyl donor. Second, when UDP-Gal was used as the galactosyl donor, fagopyritol B1 synthesis was detected, suggesting that fagopyritol synthase may have homology to GolS.

Based on the assumption that the multifunctional enzyme galactinol synthase (GolS, UDP-galactose:*myo*-inositol galactosyltransferase, EC 2.4.1.123) may have homology to the enzyme responsible for the synthesis of fagopyritols, a total of three different cDNA clones, two full-length and one partial, encoding GolS homologues have been obtained through reverse transcriptase polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE)-PCR assays using mRNA extracted from buckwheat seeds and degenerate oligonucleotide primers specific for galactinol synthase genes (Ueda *et al*. 2005). The two full-length cDNAs, designated as *FeGolS-1* (GenBank accession number AY126718) and *FeGolS-2* (GenBank accession number AY126716) are 1269 bp and 1326 bp in length and encode polypeptides of 38.3 kDa and 40.7 kDa, respectively. According to the deduced amino acid sequences, FeGolS-1 and FeGolS-2 share a high level of sequence similarity with GolSs in other plant species. However, FeGolS-2 and the partial cDNA clone FeGolS-3 (GenBank accession number AY126717) contain a unique insertion of 17 or 18 amino acid residues near the carboxyl terminus, respectively, which is absent in FeGolS-1 and other GolSs (Ueda *et al*. 2005).

Recombinant proteins have been prepared from *E. coli* by the bacterial expression of the two full-length *FeGolS-1* and *FeGolS-2* cDNAs. The *in vitro* enzyme assays performed with the recombinant proteins have confirmed that both FeGolS-1 and FeGolS-2 proteins exhibit GolS activities in the presence of UDP-galactose as galactosyl donor and *myo*-inositol as galactosyl acceptor. Furthermore, in the presence of UDP-galactose as galactosyl donor and D-*chiro*-inositol as galactosyl acceptor, FeGolS-1 catalyzes the synthesis of fagopyritol B1 whereas FeGolS-2 catalyzes the synthesis of both fagopyritol A1 and fagopyritol B1 in a 1:4 mole ratio which coincides with the observed mole ratio (1:4.4) for accumulated fagopyritol A series oligomers to accumulated fagopyritol B series oligomers in maturing embryos (Horbowicz and Obendorf 1994; Horbowicz *et al*. 1998). Thus, FeGolS-2 is capable of catalyzing the formation of the α-(1→3)-linkage unique to the fagopyritol A series as well as the α-(1→2)-linkage of the fagopyritol B series in buckwheat (Ueda *et al*. 2005). Under the corresponding conditions, the recombinant soybean GolS protein (GmGolS, AY126715) can synthesize fagopyritol B1 but not fagopyritol A1 (Obendorf *et al.* 2004). An enzyme(s) that adds additional galactosyl residues, forming fagopyritol B2, fagopyritol B3, fagopyritol A2, and fagopyritol A3, is not known but is assumed to be a multifunctional stachyose synthase or a similar enzyme.

The findings summarized above demonstrate that multifunctional GolS homologues in buckwheat seeds indeed confer fagopyritol synthase activities, and that the specificity for fagopyritol A1 synthesis may be mediated by a unique class of GolS homologues such as FeGolS-2 in buckwheat. Differences in amino acid sequence including the longer amino acid sequence (13–23 amino acids) near the carboxyl end may be related to the property of FeGolS-2 to form the unique α-(1→3)-linkage of the A series fagopyritols. Furthermore, these findings promise the future application of *FeGolS-2* gene for drug development for the treatment of insulin response disorders through biotechnology. A better understanding of the complete biosynthetic pathways leading to the fagopyritol production in buckwheat seeds would provide an insight into new strategies for the fagopyritol-based drug production. Future work should also address the formation of unique products related to insulin mediators and insulin mimetics useful in the study and treatment of non-insulin dependent diabetes mellitus (NIDDM) and polycystic ovary syndrome (PCOS).

MEDICINAL

Role in human health

D-*chiro*-Inositol is an insulin-sensitizing agent (Cheang *et al.* 2008; Galazis *et al.* 2011) and a component of D-*chiro*-inositol glycans active in insulin signaling and insulin resistance (Larner *et al.* 2010). Oral treatment with D-*chiro*-inositol reduces symptoms in at least some subjects with NIDDM (Larner *et al.* 2010) or PCOS (Nestler *et al.* 1999; Iuorno *et al.* 2002; Gerli *et al.* 2003; Cheang *et al.* 2008; Galazis *et al.* 2011).

Of the six fagopyritols found in common buckwheat, fagopyritol A1 is gaining considerable interest for medical applications. Fagopyritol A1 is isosteric with 2-amino-2-deoxy-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol (Berlin *et al.* 1990) which is related to a putative insulin mediator (Larner *et al.* 1988; Berlin *et al.* 1990). Because of their striking structural similarities, fagopyritols are of considerable value for the development of a novel plant-based drug aimed for the treatment of insulin response disorders such as NIDDM (Asplin *et al.* 1993; Ostlund *et al.* 1993; Fonteles *et al.* 1996; Cheang *et al.* 2008; Larner *et al.* 2010) and PCOS (Nestler *et al.* 1999; Iuorno *et al.* 2002; Baillargeon *et al.* 2008; Galazis *et al.* 2011).

When considering the strategies for the delivery of fagopyritols as insulin mediator supplements to the NIDDM or PCOS patients, several can be proposed. One way is to incorporate buckwheat bran, a commercial milling fraction (Steadman *et al.* 2000, 2001a) into a dietary schedule. Fagopyritols are concentrated in the axis and cotyledon tissues of embryos of buckwheat seeds (Horbowicz *et al.* 1998), and buckwheat bran is a rich source of fagopyritols (Steadman *et al.* 2000). It is not surprising to note that buckwheat has been used for the treatment of diabetes (Lu *et al.* 1992; Wang *et al.* 1992). Urinary D-*chiro*-inositol excretion is elevated in diabetic *db*/*db* mice and streptozotocin diabetic rats (Kawa *et al.* 2003a). A buckwheat extract concentrate containing fagopyritols (5.7%) and D-*chiro*-inositol (0.2%) reduces serum glucose in streptozotocin diabetic rats (Kawa *et al.* 2003b). Assuming the composition of the buckwheat extract to be comparable to that in **Fig. 5** (predominantly fagopyritol A1, fagopyritol B1, fagopyritol A2 and fagopyritol B2), it is likely that dietary fagopyritols are utilized by rodents. Dietary research indicates that rodents do not synthesize D-*chiro*-inositol, but they are solely dependent on dietary sources for their D-*chiro*-inositol and D-pinitol (Lin *et al.* 2009b). Both D-pinitol and D-*chiro*-inositol are readily absorbed by rodents from dietary sources. Absorption of labelled D-*chiro*-inositol administered orally was 98% (Lin *et al.* 2009b). Absorption of orally consumed fagopyritols is not known directly, but studies of rodent diets may provide indirect evidence. The sources of D-pinitol and D-*chiro*-inositol in rodent diets are mostly in alfalfa (leaves and young stems) and soybean meal (Lin *et al.* 2009b). D-Pinitol (1D-3-*O*-methyl-*chiro*-inositol) is 20-50% of the total soluble carbohydrates in alfalfa leaves (Horbowicz *et al.* 1995); galactopinitols are found only in seeds and are not present in alfalfa herbage (Beveridge *et al.* 1977; Horbowicz *et al.* 1995). By contrast, soybean meal contains very small amounts of free D-*chir*o-inositol and small amounts of free D-pinitol; most of the total D-*chir*o-inositol is present as its galactoside, fagopyritol B1, and about one-third of the total D-pinitol is present as its galactosides, galactopinitol A and galactopinitol B (Obendorf *et al.* 1998). The ratio of D-*chiro*-inositol to pinitol (1:3) in blood serum of rodents is higher than expected from the very low ratio in the diet, suggesting the metabolism of fagopyritols and/or the demethylation of pinitol followed by selective uptake of D-*chiro*-inositol by a stereospecific *myo*-inositol/D-*chiro*-inositol transporter (Ostlund *et al.* 1996; Lin *et al.* 2009a) in the presence of relatively large amounts of competing *myo*-inositol.

CONCLUSIONS

Mature, dry and edible seeds of common buckwheat contain sucrose and fagopyritols, galactosides of D-*chiro*-inositol, as the predominant soluble carbohydrates. Accumulation of fagopyritols is associated with the onset of desiccation tolerance in maturing seeds. Of the six fagopyritols identified in buckwheat seed extracts, fagopyritol A1 and its higher oligomers, fagopyritol A2 and fagopyritol A3, all have a unique α-(1→3)-linkage between galactose and the D-*chiro*-inositol ring. Fagopyritol A1 is isosteric with a putative insulin mediator that is insufficient in subjects with non-insulin dependent diabetes mellitus (NIDDM) and polycystic ovary syndrome (PCOS). NIDDM affects over 50% of people in certain populations, and PCOS affects about 10% of women of reproductive age. Oral administration of D-*chiro*-inositol reduces the symptoms in at least some of the subjects with NIDDM and PCOS. Feeding a concentrated buckwheat extract containing fagopyritols and D-*chiro*-inositol to diabetic rats reduced blood glucose. Fagopyritols have the potential to be a dietary treatment for reducing the symptoms of NIDDM and PCOS.

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