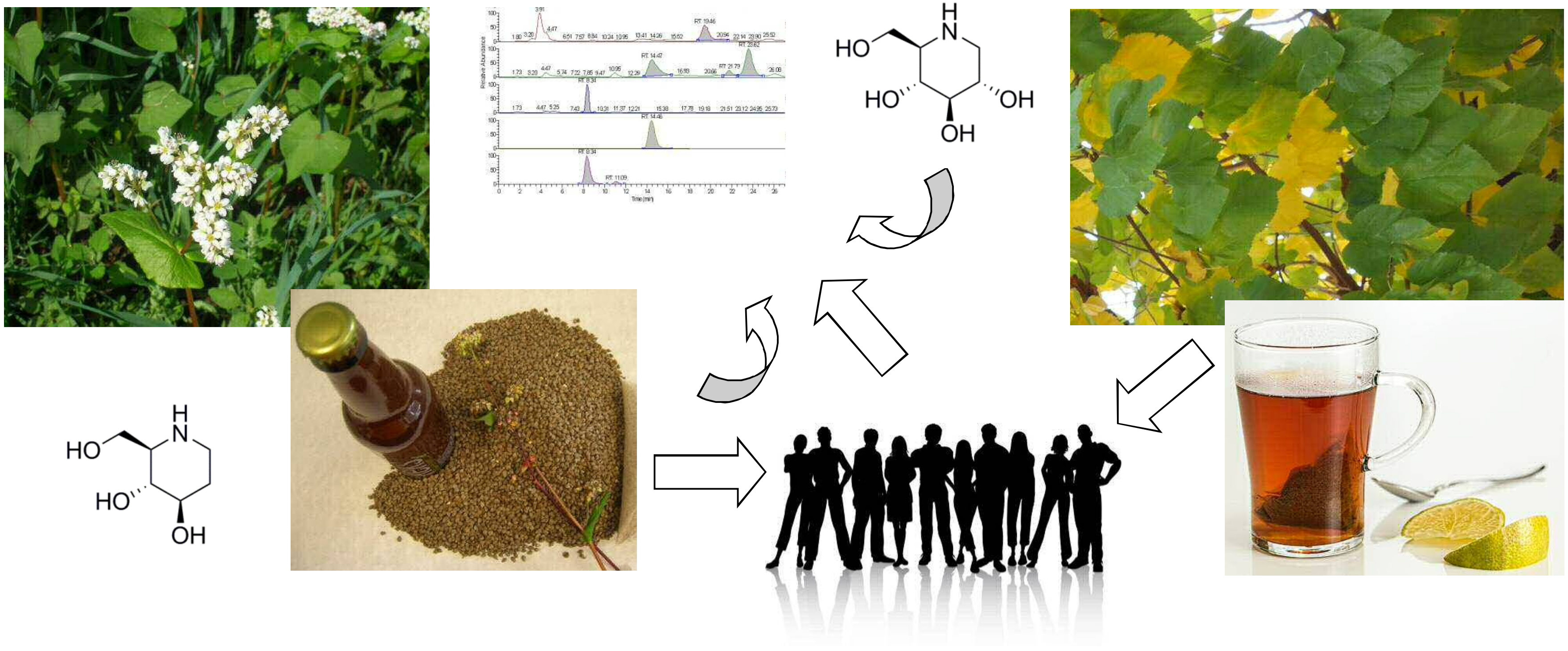

Relative Abundance
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Advances in the analysis of iminocyclitols: methods, sources and bioavailability

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Highlights

Iminocyclitols are sugar mimetics with multiple biological activities

Iminocyclitols are best analyzed using mass spectrometry

Iminocyclitol isomeric mixtures require chromatographic separations

Iminocyclitols are readily bioavailable and rapidly excreted in urine

Running title. Advances in iminocyclitol analysis

Abstract

Iminocyclitols are chemically and metabolically stable, naturally occurring sugar mimetics. Their biological activities make them interesting and extremely promising as both drug leads and functional food ingredients. The first iminocyclitols were discovered using preparative isolation and purification methods followed by chemical characterization using nuclear magnetic resonance spectroscopy. In addition to this classical approach, gas and liquid chromatography coupled to mass spectrometry are increasingly used; they are highly sensitive techniques capable of detecting minute amounts of analytes in a broad spectrum of sources after only minimal sample preparation. These techniques have been applied to identify new iminocyclitols in plants, microorganisms and synthetic mixtures. The separation of iminocyclitol mixtures by chromatography is particularly difficult however, as the most commonly used matrices have very low selectivity for these highly hydrophilic structurally similar molecules. This review critically summarizes recent advances in the analysis of iminocyclitols from plant sources and findings regarding their quantification in dietary supplements and foodstuffs, as well as in biological fluids and organs, from bioavailability studies.

Keywords. Iminocyclitols, iminosugars, fagomine, deoxynojirimycin, analysis, bioavailability

1. Introduction

1.1. Chemical structure and activity

Iminocyclitols, also referred to as iminosugars, azasugars or polyhydroxylated alkaloids, are small monocyclic or bicyclic polyhydroxylated piperidines, pyrrolidines, pyrrolizidines, indolizidines or nortropane (Figure 1). They are secondary metabolites that are present in many species of plants and bacteria [1] and are biosynthesized from simple sugars such as glucose in the case of piperidines [2] or from precursor amino acids in the case of indolizidines [3]. Factors such as geographical latitude, alkalinity and total nitrogen in the soil in which plants grow influence the biosynthesis of iminocyclitols [4]. As with other secondary metabolites, iminocyclitols can participate in plant defenses against certain microorganisms and predators, particularly insects [4, 5]. Since they are structurally related to sugars, iminocyclitols may interfere with physiologically relevant carbohydrate/protein interactions thereby influencing a variety of physiological processes and metabolic pathways. One such activity is the inhibition of intestinal glycosidases (e.g., brush border disaccharidases) [6]. By inhibiting glycosidases, plants prevent predators from digesting their food.

1.2. Natural sources and synthesis

The first natural iminocyclitol to be detected was the piperidine nojirimycin (NJ), which was isolated from a *Streptomyces nojiriensis* filtrate in 1966 [7]. D-Fagomine was the first iminocyclitol to be isolated from a plant, buckwheat (*Fagopyrum* e*sculentum* Moench, Polygonaceae), in 1974 [8]. Since then, many other iminocyclitols have been isolated, mainly from plant sources, for example: in 1976, 1-deoxynojirimycin (DNJ), also called moranoline, from the root bark of mulberry tree *Morus alba* [9] and (2R,3R,4R,5R)-2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) from the leaves of *Derris elliptica* (Fabaceae) [10]; in 1979, 1-deoxymannojirimycin (DMJ) from *Lonchocarpus sericeus* (Fabaceae) leaves [11]; in 1985, 1,4-dideoxy-1,4-imino-Darabinitol (DAB) from the fruit of *Angylocalyx boutiqueanus* (Fabaceae) [12]; and in 1988, α-homonojirimycin (α-HNJ) from *Omphalea diandra* (Euphorbiaceae) leaves [13]. Many other naturally occurring iminocyclitols have been isolated and are thoroughly reviewed elsewhere [1].

Iminocyclitols may be obtained by extraction from plants and microorganisms, chemical synthesis and enzymochemical synthesis. Yields of extraction from plants are low both because iminosugar contents are low and due to the extracts usually being complex mixtures [14-16]. Some improvement can be obtained by mixed cultivation of bacteria with fungi (e.g., increased DNJ can be produced by microorganisms within the mulberry rhizosphere [16]) or bacterial fermentation of plant material (e.g., in the yield of DNJ from Okara (soy pulp) fermented in the presence of *Bacillus subtillis* B2 [17]). Chemical synthesis is usually preferred for the production of small organic molecules. However, in the case of iminosugars it is particularly complicated because of the polar nature and reactivity of the polyhydroxylated structures and their stereochemical complexity [18]. Recent advances in the synthesis of iminocyclitols are summarized elsewhere [19-22]. Biocatalysis appears to be particularly useful in the acquisition of iminocyclitols because it is compatible with minimal hydroxyl protection, polar solvents and provides high stereoselectivity [23, 24]. For instance, DNJ, DAB and D-fagomine can be stereoselectively formed in three steps using fructose-6-phosphate aldolase [25, 26].

1.3. Applications

Since they are sugar mimetics, the applications of iminocyclitols as health-promoting agents (drugs, nutraceuticals and functional food components) range from inhibiting intestinal brush border disaccharidases to modifying: i) the glycosylation of eukaryotic cells, ii) the metabolism of carbohydrates and glycoconjugates, iii) the carbohydrate-dependent properties of glycoproteins, such as folding and transport, and iv) the carbohydrate-mediated interaction of host cells with infective agents. Therefore, iminosugars could play a role in the prevention or cure of disorders such as obesity, diabetes, metabolic syndrome, Pompe's disease, immune response imbalances, cancer, viral pathologies, Leishmaniasis, leprosy, tuberculosis, influenza, autoimmune diseases, transplant rejection, malaria, lysosomal storage diseases (such as Gaucher's disease), HIV-related diseases, Niemann-Pick disease or Fabry disease [27-29].

DNJ derivatives such as Glyset (Miglitol) or Zavesca (Miglustat) are used in the treatment of T2DM and Gaucher's disease respectively [30, 31]. There is currently research into new iminosugar analogs with increased bioavailability and selectivity towards pharmacologically significant targets [32].

DNJ-rich mulberry extracts are commercialized as nutraceuticals due to their potential as a functional or medical food that modulates postprandial blood glucose [33]. The relatively high content of DNJ in mulberry and the capacity of the silkworm to accumulate it are reviving sericulture [34, 35]. Recently, D-fagomine, a dietary iminosugar naturally present in buckwheat, has been found to inhibit the adhesion of enterobacteria to the intestinal mucosa [28]. As the intestinal microbiota plays a role in host energy homeostasis and fat storage, this activity of D-fagomine may be relevant to weight management and the prevention of metabolic syndrome [36]. Three other properties make iminocyclitols particularly attractive as food components: they are water soluble, metabolically stable [18] and the absorbed fraction is rapidly excreted unchanged via urine [37].

Overall, iminocyclitols have far greater potential as drugs or functional food ingredients than the few examples mentioned may suggest. As they are so polar and UV transparent, they may well have been overlooked or discarded with aqueous phases in the extensive efforts made in the 1980s by the pharmaceutical industry to isolate new leads by extracting plant materials with organic solvents. Early in the current century, Watson and co-workers predicted that in the years to come, many water-soluble iminocyclitols would be reported from diverse sources, including plants and microorganisms from many taxa not previously considered to be alkaloid producers [1]. By that time, some specific analytical methods had already been developed and it was foreseen that more sensitive and selective techniques would be introduced [38]. New analytical procedures have been proposed to: i) identify and quantify new compounds in plants or microorganisms; ii) quantify selected iminosugars in enriched plant extracts and final products (supplements, functional foods, etc.); iii) assess the purity of synthetic iminosugars; and iv) monitor bioavailability. This review summarizes the technical advances in iminocyclitol analysis since the last published review [38], the structures newly identified and the recent studies of iminocyclitol determination in foodstuffs and biological fluids after their intake.

2. Recent advances in analysis

Table 1 summarizes the methods and improvements reported since 2002.

2.1. Extraction and purification

Because iminocyclitols can be found in many different matrices (i.e., plants, microorganisms, larvae, foodstuffs, drug formulations, and biological fluids and tissues) extraction methods have to be adapted to each case. The preferred methods generally include solvent extraction from the solid matrix and chromatographic purification. Conditions will depend on the amount of sample and the type of source. For identification and quantification from plants and microorganisms, the procedures generally involve relatively small samples (of the order of a few grams); while the solvents most commonly employed are water, acid water, aqueous ethanol, aqueous methanol and aqueous acetonitrile. Mixing is performed by vortexing or ultrasound. The extract is then separated from the solid matrix by centrifugation or filtration. To concentrate the extracts and to separate closely related molecules or other substances that may interfere, chromatographic steps are usually introduced. As iminocyclitols are ionizable amines, cation exchange resins (e.g., CM-Sepharose, Spe-ed SCX, Dowex 50 (H^+), Amberlite IR-120B (H^+)) are preferred [17, 39-50]. In a typical example, freeze-dried fungal mycelia (0.5 g) isolated from different plants that contain swainsonine are finely ground; the triturated sample is then extracted with methanol (100 mL) in a Soxhlet extractor for 24 h. After evaporation to dryness, the residue is dissolved in 2% acetic acid (10 mL), centrifuged and loaded into a column filled with BioRad AG 50 W cation exchange resin (10 mL). After washing with distilled water, the iminocyclitol is eluted with 1 mol L^{-1} aqueous ammonium hydroxide, the eluate is evaporated to dryness, dissolved in methanol (2 mL) and analyzed [50]. In the case of biological fluids and tissues, compounds that may interfere in the process, such as proteins, should be eliminated with pure acetonitrile or methanol prior to any further purification step [37, 51-54]. More recently, alternative extraction methods that mainly use water or water/ethanol mixtures in combination with ultrasound or microwaves have been reported. DNJ has successfully been extracted from mulberry by ultrasound treatment [4]. Those authors compared the effect of four extraction systems and concluded that immersion in aqueous HCl was more efficient than hot water, microwave-assisted hot water and 65% aqueous ethanol. Extraction combining temperature and pressure (supercritical carbon dioxide or subcritical water) are promising approaches that have successfully been used to extract other plant components [55] and that could be applied to iminocyclitols. Because iminosugars are metabolically stable, fermentation before extraction results in the concentration of the desired iminosugars from starchy sources. Recently, iminosugars, inositols and low-molecular-weight carbohydrates have been extracted from mulberry (*Morus alba*) by pressurized liquid extraction (PLE) with water and aqueous ethanol and methanol mixtures followed by elimination of the fermentable sugars by incubation with *Saccharomyces cerevisiae* [56].

2.2. Separation

After semi-preparative purification, the samples may require a further separation step using high-performance stationary phases before they can be characterized.

2.2.1. Liquid chromatography

As predicted by Molyneux and co-authors [38], high-performance liquid chromatography coupled to mass spectrometry, both single (HPLC-MS) and tandem (HPLC-MS/MS), has become the most powerful and commonly used technique for the determination of iminocyclitols. The procedures include separation, ionization and detection by one or more mass analyzers. The chromatographic separation is crucial because MS detectors do not discriminate between isomers, except in those cases where information can be obtained from the fragmentation pattern, particularly by application of MS/MS techniques. This lack of selectivity would lead to overestimation of the amount of a given iminosugar if its isomers were not previously separated. The first reversed-phase liquid chromatography methods applied to iminocyclitols suffered from a lack of selectivity due to the structural similarity of these closely related polar molecules that are poorly retained in hydrophobic stationary phases [57]. Neither were iminocyclitols retained in an aminopropyl resin traditionally used for small polar molecules such as sugars and water soluble vitamins [58]. The same authors successfully performed chromatography on DNJ by employing hydrophilic interaction liquid chromatography (HILIC) using TSKgel-amide resin as the hydrophilic stationary phase eluted with acetonitrile/water pH 5.5 containing a high proportion of the solvent with lower polarity (acetonitrile) [58]. HILIC, developed as a tool to analyze highly polar compounds such as peptides, nucleic acids and complex carbohydrates [59, 60], has now been successfully applied to the separation of the piperidine iminosugars DNJ, 2-*O*-α-D-galactopyranosyl–DNJ (GAL-DNJ) and D-fagomine in a single run [34]. Recently, an in-depth comparison of three HILIC stationary phases (sulfoalkylbetaine zwitterionic, polyhydroxyethyl aspartamide and bridged-ethylene hybrid (BEH) particles with a trifunctionally bonded amide) showed that the BEH-amide phase gave the best results in terms of resolution, peak width and analysis time for the separation of iminosugars, simple sugars and inositols, from hyacinth, mulberry and buckwheat extracts [61]. As iminocyclitols include an ionizable basic function, cation exchange is also an option that may give better results in some instances. A weak cation exchange resin containing a carboxymethyl terminal group provided better selectivity than HILIC resins for the separation of D-fagomine from its diastereomers 3-*epi*-fagomine and 3,4-di-*epi*-fagomine [49]; those authors proved that the amount of D-fagomine in buckwheat may have been overestimated in previous studies that used less selective separation techniques.

2.2.2. Gas chromatography

Gas chromatography (GC) has also been applied to the separation of iminosugar mixtures. As iminocyclitols are not volatile, the prior formation of stable volatile derivatives is needed. DNJ has been analyzed after derivatization with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in the presence of trimethylchlorosilane (TMCS) [52]. More complex mixtures with more than one iminosugar or other related molecules require more sophisticated techniques. A combination of oximation and trimethylsilylation was effective for the separation of DNJ, D-fagomine, pipecolic acid derivatives and other polyols, such as both monosaccharides and disaccharides, *myo*-inositol and galactinol isomers, from mulberry extracts; while other derivatizations resulted in less resolution of the mixtures [62]. More recently, the same authors have reported an improved procedure that involves adjusting the temperature of the injection port to achieve better peak shape and separation, while minimizing degradation [63].

2.3. Detection

2.3.1. Mass spectrometry

Mass spectrometry (MS) is the most widely used detection method in iminocyclitol analysis. After separation, iminocyclitols are ionized by either thermospray (TSI), electrospray (ESI), atmospheric-pressure chemical ionization (APCI) or electron impact ionization (EI) [17, 34, 37, 51-54, 56, 61, 64-76]. Then, the ions may be analyzed using different MS detectors, depending on the information required: ion trap (IT) and time of flight (TOF) detectors are good for identification or semi-quantification, as shown for DNJ, α-HNJ and DMDP [33, 41]; while quantification is better accomplished by using single quadrupole (Q) and triple quadrupole (QqQ) detectors that provide the adequate selectivity, sensitivity, precision and accuracy [49, 51, 66, 74]. QqQ detectors provide a secondary fragmentation of selected primary fragments (MS/MS experiments), which facilitates the identification of isomers and complex structures. To analyze D-fagomine from buckwheat groats, ESI and a single quadrupole were used, which required careful assessment of the signal suppression caused by the matrix and its correction via the use of another iminocyclitol (DMDP) as an internal standard [49]. Other authors have used QqQ with multiple reaction monitoring (MRM) to obtain high selectivity and sensitivity by observing more than one pair of parent/daughter fragment ions [51, 66, 74]. Rodríguez-Sánchez and co-workers used a quadrupole TOF (QTOF) MS detector to analyze complex mixtures though MS/MS experiments; the compounds identified included DNJ, DMJ, HNJ, D-fagomine and some glycosylated derivatives never previously reported [61].

MS can be applied directly to plant tissue by ambient ionization (leaf spray); the method is fast and selective and has been applied to different native materials to detect phytochemicals that include a pyrrolidine-type alkaloid [77]. Xu and co-workers have proposed a direct-analysis in real time (DART) MS (QqQ) method to determine the DNJ content in mulberry leaves. The process includes a straightforward sample preparation step and fast MS analysis with no previous chromatography; it has proved to be as sensitive and accurate as conventional HPLC coupled to a fluorescence detector (FLD) [78].

2.3.2. Other detectors

Detectors other than MS have also been used to analyze iminocyclitols. Evaporative light scattering detection (ELSD) is a semi-universal detection method employed for sugar determination that may be applied to iminocyclitols. As it is sensitive to an array of different structures, ELSD requires careful sample pretreatment to avoid interference. The technique is very precise and accurate when coupled to HPLC, provided that the compounds in the mixtures are separated under isocratic conditions and the system temperatures are carefully controlled. ELSD does not require derivatization, which allows for simple and fast analysis of multiple samples. However, it is not as sensitive as MS, so it cannot be employed for low concentrations of analytes. ELSD has been used to analyze DNJ [17, 33], miglitol [79], swainsonine [50] and D-fagomine (Amézqueta, S, unpublished results). New detectors with higher sensitivity and reproducibility are being developed that might, in the near future, turn out to be a simple and more affordable alternative to MS.

More widely spread techniques such as HPLC coupled to ultraviolet or fluorescence detectors (HPLC-UV and HPLC-FLD, respectively) have also been applied to the analysis of iminocyclitols after derivatization, as the analytes lack chromophores in their structure [4, 35, 64, 80-90]. The stability of the derivatives is once again a crucial point. 9-Fluorenylmethyl (Fmoc) derivatization and separation in a reversed-phase C18 column has been used for the determination of DNJ from mulberry leaves [64]. The Fmoc derivative was stabilized by lowering the pH, which may not be compatible with the elution conditions used with the more selective HILIC mobile phases. Because retention on reversed-phase resins will be mostly due to the Fmoc part of the derivatives, some iminocyclitols may co-elute in C18 columns.

A pulsed amperometric detector (PAD) has also been used in combination with anion exchange liquid chromatography, which requires a post-column pH adjustment before the sample is introduced into the detector [91]. This detector is selective for compounds containing oxidizable functional groups, (e.g., the hydroxyl group) and does not require a previous derivatization step. However, this technique is less sensitive and reproducible than most of those described above.

Capillary electrophoresis (CE) has also been adapted for the analysis of iminosugars by using modifications aimed at improving the sensitivity. CE-DAD (CE coupled to a diode array detector) after in-situ complexation with borate ions was instrumental in the analysis of miglitol [92]. Calystegines A_3 and B_2 from potato have been analyzed by both CE coupled to UV and isotachophoresis coupled to a conductivity detector [92, 93].

2.4. Validation

The validation of analytical procedures, which is the proof of their suitability for the intended purpose, is essential to ensure that reliable data are obtained, to be able to compare results reliably, to spare unnecessary additional work and to aid in the correct decision-making process. Consequently, validation parameters have to be studied for every analytical method used and made publicly available. As a guide for method validation, EMEA (European Medicines Agency) has published free-access guidelines on the validation of bioanalytical methods [94].

The reports published on iminocyclitol analysis (**Table 1**) do not always include a complete validation study. To be able to compare results obtained in different laboratories, at least the method accuracy and the limit of detection (LOD) or limit of quantification (LOQ) need to be given. **Table SM-1** includes the validation parameters reported for the analysis of iminocyclitols in natural sources, foodstuffs, pharmaceutical formulations, and biological fluids and tissues. Accuracy should preferably be evaluated using reference materials or by comparing the new method to a reference method. Unfortunately neither of these options is available for iminocyclitols; however, accuracy can be evaluated by calculating analyte recovery after spiking and analyzing replicate blank samples at different concentration levels. In those reports on iminocyclitols that include validation parameters, the recovery values vary between 35% and 104%. Methods with recoveries below 50% may also have low reproducibility and require proof of between-day accuracy that is not provided in any of the studies published so far. LOD and LOQ depend greatly on the detection technique employed. Values in the range 0.0001 – 30 mg kg^{-1} or mg L^{-1} have been reported. The best results have been obtained with LC or GC coupled to MS (TOF, Q or QqQ) [54, 56, 61, 74, 95]. When using MS after electrospray ionization, signal suppression or enhancement due to interfering components in the matrix should be checked for. This requires comparing the slope of the standard calibration curve with the corresponding matrix, e.g., as reported for plasma [51] and buckwheat [49].

In long-term studies, between-day precision and recovery studies should also be considered. Between-day recovery values are > 85% and between-day RSD (relative standard deviation) values are < 10% in all cases where data are available [49, 51, 54, 64, 66, 74, 78] which proves the long-term reliability of the methods employed.

3. Distribution in natural sources and derived products

The recent advances in analytical chemistry, mostly MS techniques, have revealed new sources of iminosugars, namely plants from different families: Combretaceae, Convolvulaceae, Erythroxylaceae, Euphorbiaceae, Fabaceae, Hyacinthaceae, Molyaceae, Moraceae, Polygonaceae and Solanaceae; worms fed mulberry leaves; bacteria belonging to the families: Bacillaceae, Nostocaceae and Streptomycetaceae; fungi belonging to the families: Clavicipitaceae and Pleosporaceae; and marine sponges belonging to the Dictyoceratida order (**Table 1**).

In plants, the most abundant iminocyclitols are: DAB (up to 16000 mg kg^{-1} in *Morus bombycis*), DNJ (up to 10160 mg kg^{-1} in mulberry), various calystegines (up to 10145 mg kg^{-1} in *Solanum tuberosum*), α-HNJ (up to 5500 mg kg^{-1} in *Hyacinthus orientalis*), DRB (up to 4800 mg kg^{-1} in *Morus bombycis*), swainsonine (up to 4500 mg kg^{-1} in *Oxytropis serioopetala*), 1-*epi*-australine (up to 2990 mg kg^{-1} in *Castanospermum australe*), australine (up to 1680 mg kg^{-1} in *Castanospermum australe*), (2*R*,3*S*)-3-hydroxy-2-hydroxymethylpyrrolidine (up to 1530 mg kg^{-1} in *Castanospermum australe*), D-fagomine (up to 660 mg kg^{-1} in *Morus alba*), 2-*O*-α-D-galactopyranosyl-DNJ (up to 530 mg kg^{-1} in *Morus bombycis*), homoDMDP (up to 441 mg kg^{-1} in *Scilla sociallis*), 6-*epi*-castanospermine (up to 390 mg kg^{-1} in *Castanospermum australe*), 3-*epi*-australine (up to 370 mg kg^{-1} in *Castanospermum australe*), DMDP (up to 344 mg kg^{-1} in *Stemona tuberosa*) and DMJ (up to 257 mg kg^{-1} in *Angylocalyx pynaertii*) (**Table 1** and **Table SM-2**).

Iminocyclitols are found in food supplements, teas and foodstuffs made using mulberry- or buckwheat-derived ingredients. To substantiate health claims associated with functional foods, accurate analysis of the individual functional components, such as iminosugars, in foodstuffs will become increasingly important. Moreover, determination in the finished product is important because the manufacturing processes may include high temperatures and oxidizing conditions that could degrade some of the components. DNJ is the major iminocyclitol in mulberry [4, 44, 62, 66, 71]; the shoots (3−4 g kg^{-1}) [4] and latex (3−5 g kg^{-1}) [44] are the parts with the highest DNJ content, followed by the leaves (0.1−1 g kg^{-1} DNJ, multiple entries in **Table 1**) which is the part of the tree mostly used as an ingredient in the production of food supplements and functional foods. The products derived from mulberry with the highest DNJ content (between 1 and 5 g kg^{-1}) are mulberry tea (loose leaves), mulberry powder and mulberry leaf tablets [35, 58, 91]. Certain mulberry snacks and cookies have been shown to contain 70 and 110 mg kg^{-1} DNJ respectively, as determined by anion exchange HPLC with a PAD [91]. Calystegines are found in fruit and vegetables pertaining to the Convolvulaceae and Solanaceae families. It is found in higher quantities in Solanaceae sprouts and peel than in the flesh [40, 45]. In *Erythroxylum novogranatense* var. *novogranatense*, the order of abundance is: young leaves > mature leaves > fruit ≈ old leaves ≈ flowers [42]. Buckwheat (groats and bran) contains D-fagomine as its major iminosugar (22−44 mg kg^{-1}), as determined by cation exchange (CEX) HPLC/ESI–Q-MS [49]. The use of this technique revealed that buckwheat also contains 3,4-di-*epi*-fagomine (1.0–43 mg kg^{-1}), an inactive isomer of D-fagomine that could not be separated by other methods. The foodstuffs with the highest amount of D-fagomine are buckwheat bread (24 mg kg^{-1}) and buckwheat cookies (15 mg kg^{-1}) [72]. D-Fagomine is highly stable under several preparation treatments that include boiling, baking, frying and fermentation [72]. The estimated daily intake of D-fagomine from a diet rich in buckwheat products is 3–17 mg per day (mean for both sexes; range from P5 to P95) [72].

4. Bioavailability

The first reports on the pharmacokinetics of iminocyclitols (DMJ and *N*-methyl-DNJ, miglitol) used classical sensitive measurements of radioactivity from radiolabelled ($[^3H]$– and $[^{14}C]$–tagged) compounds [96, 97]. The studies showed that natural iminocyclitols do not bind to plasmatic proteins, are rapidly cleared from plasma and are mainly excreted through the renal system. After oral ingestion, only a small portion was observed to be absorbed; whereas the *N*-alkylated derivatives, such as miglitol, were better absorbed than the intact natural species and then they were rapidly excreted. These pharmacokinetic studies, which are compulsory for drug candidates, require the use of expensive radiolabelled compounds. Moreover, since they monitor the radioactivity of single atoms, the procedures are blind to any metabolic changes in the molecules that may result in changes in the biological effects. Modern LC-MS techniques are sensitive enough to detect the intact molecules and their metabolites in most biological fluids and tissues.

DNJ (95% pure, extracted from mulberry leaves) was orally administered at a dose of 110 mg kg^{-1} to rats and determined in plasma by HILIC-MS using a TSK gel Amide-80 column and an ESI−TOF MS detector [37]. Only 1% of the ingested DNJ at doses of 1.1, 11, and 110 mg kg^{-1} body weight was detected in plasma. The concentration reached a maximum 30 min after administration and decreased rapidly thereafter. The incorporation of DNJ into plasma was dose dependent. The intact form of DNJ was detected intact in urine after 24 h (2% of the total ingested), as well as in the contents of the large intestine (7%) and small intestine (1%). DNJ concentrations in liver, kidney, pancreas, and spleen were at trace levels (< 1 mg (6 nmol) kg^{-1}). The results showed that only a small amount of orally administered DNJ is absorbed and then rapidly excreted in urine without accumulating in tissues. It may be speculated that bacterial degradation of DNJ in the gastrointestinal tract may occur before absorption or that DNJ is metabolized by drug-metabolizing enzymes such as cytochrome P450s in the liver. Since neither degradation products nor conjugated metabolites have ever been detected anywhere, this and other studies suggest that iminocyclitols are highly metabolically stable. If only a small portion of the ingested DNJ is degraded or metabolized, it may be too little to be detected with the HILIC–MS method under the conditions used.

Another study evaluated the absorption of highly purified DNJ and DNJ from a mulberry extract containing it, using GC–TOF–MS after derivatization with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in the presence of trimethylchlorosilane (TMCS) [52]. When highly purified DNJ was administered at different doses (3 or 6 mg kg^{-1} body weight), the concentrations of DNJ in plasma increased in a dose-dependent manner after 30 min. The intact iminocyclitol was recovered in urine and feces over a period of 48 h. In the case of the mulberry extract, the DNJ concentration in plasma increased to its highest level (12.01 µmol L^{-1}) 30 min after a single oral dose (1.7 g kg^{-1} body weight; 6 mg of DNJ) and then rapidly decreased. When administered at 0.98 mg of DNJ per rat, most of the DNJ was excreted in feces (1.27 ± 0.60 mg per rat) and only a small amount of DNJ was detected in urine (0.07 ± 0.07 mg per rat). More recently, a distribution study with a mulberry twig extract which contained DNJ, fagomine (diastereomeric purity not stated) and DAB, using HILIC–ESI–QqQ–MS/MS with separation in a TSKgel Amide-80 column, suggested that the clearance of fagomine and DAB from plasma is as fast as that of DNJ. MRM experiments of tandem MS provided high sensitivity with LOD and LOQ as low as 0.005 - 0.010 mg L^{-1} [74].

In humans, the results obtained in the 1990s with radiolabelled miglitol (fast absorption at doses lower than 50 mg) [97] have been confirmed by RP-HPLC–APCI–QqQ–MS/MS: the plasma concentration of miglitol, monitored by MRM transition experiments, increased up to 1.3 ± 0.2 mg L^{-1} 2.6 h after administration (50 mg) and then rapidly decreased [51]. DNJ administered orally as part of a mulberry extract (1.2 g; 6.3 mg of DNJ) was bioavailable in plasma in increasing concentrations up to 0.52 mg L^{-1} 1.5 h after administration and then rapidly decreased, as determined by a validated method using HILIC−QTRAP–MS/MS [73]. The concentration of DNJ in urine excreted during the first 24 h after administration was 7.0 mg L^{-1}, whereas the concentration over the next 24 h was at trace levels. As DNJ has a relatively short half-life in plasma, its tissue distribution and accumulation may not be particularly high [73].

So the usual pharmacokinetic behavior of natural iminocyclitols after oral ingestion is that only a small portion is absorbed and then it is rapidly excreted in the urine, mostly in the intact form. The alkylated derivative miglitol is better absorbed than its parent: natural DNJ. Iminosugars do not bind to plasmatic proteins and are not accumulated in tissues. The current most common detectors applied to bioavailability studies are time-of-flight, ion trap and triple quadrupole after electrospray ionization; whereas triple quadrupole and QTRAP detectors provide the highest sensitivity. The previous separation step is usually hydrophilic interaction chromatography.

Concluding remarks and future directions

Iminocyclitols are a versatile class of sugar mimetics with structural features that make them good candidates for drugs and nutraceuticals. They are highly stable, both chemically and metabolically. As they are carbohydrate mimetics, they have the potential to interfere with physiologically relevant recognition interactions, and to influence a variety of physiological processes and metabolic pathways. Moreover, as they are water-soluble, they can easily be formulated into foodstuffs and drinks.

When analyzing iminocyclitols, the two main drawbacks are that they are transparent to UV light and physicochemically similar to each other. Currently available analytical methods, mainly those involving high performance chromatography coupled to mass spectrometry detection, show adequate precision and recovery when determining the iminosugar content in plants and food products, as levels are usually higher than the limits of detection. The most commonly used modes of separation are GC and HILIC; the latter is simpler because it does not require derivatization, although it may not resolve diastereomeric mixtures, as in the case on D-fagomine, 3-*epi*-fagomine and 3,4-di-*epi*-fagomine; a mixture better separated by CEX. As the iminocyclitol content would be overestimated in the case of co-elution of structurally similar isomers even when MS/MS detectors were used, two independent separation runs with HILIC and CEX stationary phases would be a preferred option for initial analysis of new natural sources. MS detectors, particularly those (TOF, Q, Ion trap) that allow MS^n experiments, are the best option for: analysis of complex mixtures; searching of new entities; and bioavailability studies. ELSD is a more affordable alternative for quality control purposes once the purity of the mixtures has been established by other more selective and sensitive techniques. A new generation of ELSD detectors is expected to provide higher performance in the near future. As screening of potential new sources of bioactive iminocyclitols is likely to increase, automation is another improvement that may be expected soon. Automated multi-analyte extraction-purification-determination devices used in other areas, such as the determination of food contaminants, could easily be optimized and validated for iminocyclitols by adapting the methods described above. The application of these analytical techniques will not only allow the discovery of new iminosugars, but also the study of the taxonomic distribution in plant families and the study of factors that influence their biosynthesis (e.g., geographic locations, soil properties, meteorological factors, harvest time, etc.) in plants and microorganisms. Since iminocyclitols are being introduced as drugs and food additives, highly sensitive LC–MS^n techniques are progressively being applied to study their bioavailability, putative metabolization and excretion without using radioactive material. Analysis in biological tissues may be particularly challenging because biological fluids may cause significant signal suppression in MS, so that minute amounts of metabolites of highly stable iminocyclitols may be below the LOD. LC–MS should also be applied to the analysis of commercially available food-grade products that do not currently specify their content of functional components such as iminocyclitols.

Beyond DNJ, D-fagomine and alkylated derivatives such as miglitol, we may discover that nature and biotechnology offer more iminocyclitols and other cyclitols of use in functional nutrition and therapeutics, now that modern analytical techniques have made them more visible.

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Tables and Supplementary Material

Tables and Supplementary Material are included in a separated document.

Table 1. Reports of iminocyclitol analysis since 2002

Figure 1. Chemical structures of iminocyclitols

Table SM-1. Validation parameters pertaining to entries in Table 1

Table SM-2. Iminocyclitol composition and/or content pertaining to entries in Table 1

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Reference	Natural source (Family)	Compound	Amount (mg kg^{-1})^{a}	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Kato et al. [103]	*Castanospermum australe* (Fabaceae) seeds	australine, 1-*epi*-australine and other pyrrolizidine, indolizidine and piperidine alkaloids	up to 2990	850 g	50% ethanol	ion-exchange resins	nonenone	NMR
Haraguchi et al. [104]	*Ipomoea carnea* (Convolvulaceae) leaves, flowers and seeds	swainsonine 2-*epi*-lentiginosine, calystegines B_1, B_2, B_3, C_1	uup to 32 NA	NA	NA	NA	NA	NA
Gardner et al. [105]	*Astragalus lentiginosus* (locoweed) (Fabaceae)	swainsonine	NA	NA	NA	ion-exchange; aqueous methanol; liquid-liquid extraction with dichloromethane	NA	NA
Braun et al. [106]	locoweed with endophite infection (Fabaceae) locoweed without endophite infection	swainsonine	869 (mean, N = 79) 66 (mean, N = 84)	0.5 g	50 mL methanol; soxhlet	none	none	α-mannosidase inhibition assay
Friedman et al. [40]	potato flesh and peel	calystegines A_3 and B_2	up to 380	1 g	80% methanol; filtration	Dowex AG 50W X 8; 0.5% NH_4OH	GC: J & W SE-30 derivatizing agent: pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA)	MS (EI)
Schwarz et al. [65]	*Ipomoea carnea* Jacq. (Convolvulaceae)	swainsonine, calystegines B_1, B_2 and C_1	up to 1400	NA	ethanol	AG 50W-X8; 0.5% NH_4OH	LC: Keystone Scientific Betasil C18	Q (APCI)
Asano et al. [107]	*Scilla peruviana* (Hyacinthaceae) bulbs	DAB related compounds	up to 32	5 kg	6 L ethanol 50%; filtration	Amberlite IR-120B (H^+) resin; 0.5 M NH_4OH; Dowex 1-X2 (OH^-) resin; H_2O; Amberlite CG-50 (NH_4^+) resin; H_2O	none	NMR
Kimura et al. [58]	mulberry leaves, tea leaves, powder and tablets	DNJ	up to 4800	0.1 g	1 mL acetonitrile:water 1:1 (6.5mM ammonium acetate, pH 5.5); Sonication, centrifugation, filtration	none	LC: HILLIC (TSK Gel amide-80)	ELSD + TOF (ESI)
Asano et al. [108]	*Stemona tuberosa* (Combretaceae) roots	α-HNJ, DMDP and other piperidine and pyrrolidine alkaloids	up to 1010	1 kg	2 × methanol 50% (5 L)	Amberlite IR-120B (H^+) resin; 0.5 M NH_4OH; Dowex 1-X2 (OH^-) resin; H_2O; Amberlite CG-50 (NH_4^+) resin; H_2O	none	NMR
	Connarus ferrugineus (Combretaceae) leaves and twigs	1-deoxyaltronojirimycin and other piperidine and pyrrolidine alkaloids	up to 70	3 kg	2 × methanol 50%			
	Albizia myriophylla (Fabaceae) wood	DMDP and other pyrrolidine and piperidine alkaloids	up to 175	1 kg	2 × methanol 50%			

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})^{a}	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Kite et al.	[41]	*Suregada* Roxb. ex Rottl. (Euphorbiaceae) leaves	α-HNJ, DMDP	NA	200 mg	5 mL methanol 70%; centrifugation	Dowex 50 (H^+) resin; 2 M NH_4OH	GC: SGE BPX5 derivatizing agent: Sigma-Sil A	Ion Trap (APCI)
Ouyang et al.	[80]	*Morus alba* leaves	DNJ	NA	NA	50 mM chloridric acid	none	LC: HiQSiL C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
Brock et al.	[42]	*Erythroxylum* genus (Erythroxylaceae) strains	calystegine A_3, A_5, B_1 and B_2	up to 2600	50 – 100 mg	3 × 5 mL 50% methanol	cation exchange resins	GC: Hewlett Packard HP1 derivatizing agent: Pyridine with hexamethyldisilazane and trimethyl chlorosilane	FID
Schimming et al.	[43]	standards	swainsonine, castanospermine and several calystegines	NA	none	none	none	GC: DB1 derivatizing agent: pyridine and hexamethyldisilazane (HMDS)	MS (EI)
		Convolvulaceae and Solanaceae families	several calystegines		10 g	3 × 100 mL 50% methanol; centrifugation	Dowex 50WX8; 3.5% NH_4OH		
Konno et al.	[44]	*Morus bombycis* (Moraceae) latex	DNJ, DAB and DRB	up to 16000	2 mL	ethanol 70%	Amberlite IR-120B (H^+) resin; 0.5 M NH_4OH; Dowex 1-X2 (OH^-) resin; H_2O	none	NMR
		Morus alba latex	DNJ	up to 3200					
Kato et al.	[109]	*Scilla socialis* (Hyacinthaceae) bulbs	homoDMDP and other pyrrolidine and pyrrolizidine alkaloids	up to 441	2.3 kg	ethanol 50%	Amberlite IR-120B (H^+) resin; 0.5 M NH_4OH; Dowex 1-X2 (OH^-) resin; H_2O; Amberlite CG-50 (NH_4^+) resin; H_2O	none	NMR
Kimura et al.	[33]	*Morus alba* leaves	DNJ	up to 3800	0.1 g	1 mL acetonitrile:water 1:1 (6.5Mm ammonium acetate, pH 5.5); sonication, centrifugation, filtration	none	LC: HILIC (TSK Gel amide-80)	ELSD + TOF (ESI)
		Morus bombycis leaves	DNJ	up to 2000					
		Morus latifolia (Moraceae) leaves	DNJ	up to 1700					
Han et al.	[110]	*Morus multicaulis* (Moraceae) leaves	DNJ, D-fagomine, 2-*O*-α-D-galactopyranosyl-1-DNJ	NA	NA	NA	ion exchange resin, silica gel and Sephadex LH -20	NA	NA
Li et al.	[51]	Human plasma	miglitol	up to 1.3 mg L^{-1}	0.1 mL	0.5 mL acetonitrile, centrifugation, 0.2 mL water and 2 mL methylene chloride, Centrifugation	none	LC: Dalian Nucleosil C18	QqQ (APCI)

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})^{a}	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Nakagawa et al.	[37]	rat plasma, urine and tissues	DNJ	up to 15 mg L^{-1} in plasma, 2 mg L^{-1} in urine and 7 mg L^{-1} in large intestine	0.5 mL 0.5 g 1 mL	1 mL acetonitrile:water 72:28 (6.5mM ammonium formate, pH 5.5); Sonication, centrifugation, filtration	none	LC: HILIC (TSK Gel amide-80)	TOF (ESI)
Nuengchamnong et al.	[66]	mulberry shoots and leaves	DNJ	up to 3080	50 mg	2 × (10 mL 70% methanol; ultrasound, filtration)	none	LC: HILIC (TSK Gel amide-80)	QqQ (ESI)
Zhou et al.	[111]	Bombyx mori silkworm feces	DNJ, D-fagomine, 3-*epi*-fagomine	NA	NA	NA	Macroporous adsorbent resin, ion-exchange resin and sephadex LH 20	none	NMR
Zhang et al.	[81]	*Ramulus mori* (Moraceae)	DNJ	NA	NA	NA	NA	LC: Diamonsil C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
Kato et al.	[112]	*Baphia nitida* (Fabaceae) leaves	3-*O*-β-D-glucopyranosyl-DMDP and other pyrrolidine and piperidine alkaloids	up to 19	8 kg	Ethanol 50%	Ion exchange resin	HPTLC: Merck Silicagel $60F_{254}$	none
Yatsunami et al.	[113]	mulberry leaves	DNJ	up to 600	NA	Ethanol 75%	NA	LC	NA
Nakagawa et al.	[73]	human plasma and urine	DNJ	up to 0.52 mg L^{-1} in plasma and 7 mg L^{-1} in urine	0.1 mL	0.7 mL acetronitrile:water 1:6; Sonication, centrifugation, filtration	none	LC: HILIC (TSK Gel amide-80)	QTrap
Meng et al.	[82]	mulberry leaves	DNJ	NA	NA	NA	NA	LC: HiQSiL C18	FLD: Ex = 254 nm, Em = 322 nm
Xie et al.	[83]	*Morus alba* leaves	DNJ	1200	100 mg	2 × 10 mL 0.05 M chloridric acid	none	LC derivatizing agent: AQC	FLD
Xia et al.	[84]	mulberry twigs	several piperidine alkaloids	NA	100 mg	NA	NA	LC: Dikma C18 derivatizing agent: FMOC-Cl	UV: 264 nm
Li et al.	[114]	mulberry leaves	DNJ	NA	NA	NA	NA	LC: Zorbax NH_2	ELSD
Griffiths et al.	[45]	*Solanum tuberosum* group Phureja (Solanaceae) flesh, peel and sprouts	several Calystegines	up to 9158	1 g	80% methanol; filtration	Dowex AG 50W X 8; 0.5% NH_4OH	GC: J & W DB5-MS derivatizing agent: pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA)	MS (EI)
		Solanum tuberosum group Tuberosum (Solanaceae) flesh	calystegines A_3 and B_2	up to 101					

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})^{a}	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Kvasnicka et al.	[93]	Solanaceae family	calystegines A_3, A_3, B_2 and B_2	up to 132	50 g	200 mL 50% methanol; filtration	none	Capillary zone electrophoresis	DAD: 210 nm
Ralphs et al.	[46]	*Astragalus* genus	swainsonine	10 – 3700 (N = 131)	100 mg	chloroform and acetic acid	Cation exchange resin; NH_4OH	LC	MS
		Oxytropis genus	swainsonine	10 - 900 (N = 26)					
Russo et al.	[115]	Standards	calystegines A_5, B_4 and C_1	30	none	none	none	LC: Waters Acquity HILIC	ELSD
Dai et al.	[67]	mulberry leaves	DNJ	NA	NA	NA	NA	LC: Shimadzu HRC-NH_2	QqQ (APCI)
Guitton et al.	[68]	human plasma and cerebrospinal fluid	miglustat	NA	10 / 20 µL	1 mL acetonitrile:methanol 75:25; centrifugation	none	LC: Atlantis HILIC	QqQ (ESI)
Cook et al.	[47]	*Oxytropis sericea* roots, crown, scape, leaf, flower and pod	swainsonine	up to > 620	100 mg	Chloroform and acetic acid	Cation exchange resin; NH_4OH (d)	LC	MS
Nakagawa et al.	[34]	*Morus alba* leaves	DNJ and other piperidine alkaloids	up to 1600	50 mg	5 mL ethanol 50%; ultrasound, centrifugation, filtration	none	LC: HILIC (TSK Gel amide-80)	QqQ (ESI)
		Morus bombycis leaves	DNJ and other piperidine alkaloids	up to 1200					
Tan et al.	[116]	*Morus wittiorum* (Moraceae)	DNJ, D-fagomine, DAB and other piperidine alkaloids	NA	NA	NA	Ion exchange resins	NA	NMR
Wu et al.	[117]	*Morus atropurpurea* (Moraceae)	DNJ and other piperidine alkaloids	NA	NA	70% ethanol	NA	NA	NA
Kim et al.	[52]	rat plasma, urine and feces	DNJ	up to 4.2 mg L^{-1} in plasma, 4 mg in urine and 7 mg in feces	0.2 mL	methanol; sonication, centrifugation	NO	GC: DB-5MS derivatizing agent: BSTFA/TMCS	TOF (ESI)
Zhu et al.	[17]	fermented soy pulp (okara) with *Bacillus subtilis* B2 (Fabaceae)	DNJ	20	45 mL	80% ethanol; centrifugation, filtration, freeze-drying	Membrane dialysis, active charcoal, CM-Sepharose resin; TLC	LC: HILIC (TSK Gel amide-80)	ELSD + QqQ (ESI)
Yin et al.	[35]	mulberry leaf powder and silkworm larvae parts	DNJ	up to 1210	300 mg	2 × (0.05 M chloridric acid; ultrasound, centrifugation)	none	LC: Venus C18 derivatizing agent: FMOC-Cl	UV: 254 nm
Yoshihashi et al.	[91]	mulberry leaf tea, leaf tablet, snacks, cookies and drink powder	DNJ	up to 2360	100 mg	1 mL water; centrifugation, filtration	none	LC: Carbopac MA-1	PAD
Jones et al.	[118]	castanospermum australe leaves and steams	1-*epi*alexine	NA	3 kg	50% ethanol	Amberlite IR-120B (H^+) resin; 0.5 M NH_4OH; Amberlite CG-50 (NH_4^+) resin; H_2O; Amberlite CG-400; H_2O	GC: Varian VF-5ms derivatizing agent: Tri-Sil reagent	Q

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})^a	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Yu et al.	[48]	*Astragalus* genus	swainsonine	up to 520	NA	NA	NA	GC: AT.SE-54 derivatizing agent: Pyridine and BSTFA	MS-FID
		Oxytropis genus	swainsonine	up to 720					
		Endophytes	swainsonine	up to 570	0.5 – 1 g	50 mL methanol; soxhlet; evaporation; 2% acetic acid; centrifugation	BioRad AG 50 W; 1 M NH_4OH		
Gardner and Cook	[69]	Locoweed	swainsonine	up to 4300	100 mg	5 mL 2% acetic acid; centrifugation	none	LC: Thermo Fisher Betasil C18	QqQ (APCI)
Rodríguez-Sánchez et al.	[62]	*Morus alba* leaves, fruits and branches	DNJ and D-fagomine	up to 4750	1 g	10 mL chloridric acid 0.1%; Filtration	none	GC: Teknokroma TRB-1 derivatizing agent: Hexamethyldisylazane (HMDS) and trifluoroacetic acid (TFA)	QqQ (EI)
		Morus nigra leaves (Moraceae), fruits and branches	DNJ and D-fagomine	up to 2080					
Hu et al.	[85]	mulberry tree	DNJ	760 - 3580 (N = NA)	NA	0.05 M chloridric acid	none	LC: Agilent C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
		parasitic loranthusparasitized mulberry host trees	DNJ	400 - 1720 (N = NA)					
Li et al.	[86]	mulberry tree	DNJ	up to 10160	NA	0.05 M chloridric acid	none	LC: Agilent C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
		parasitic loranthusparasitized mulberry host trees	DNJ	up to 2720					
Wei et al.	[119]	*Streptomyces lavendulae* (Streptomycetaceae) strains	DNJ	up to 43000	NA	NA	NA	NA	NA
Jain et al.	[70]	human plasma	miglitol	NA	NA	Acetonitrile	NA	LC: Inertsil C18	QqQ (ESI)
Lou et al.	[4]	*Morus alba* shoots, roots and leaves	DNJ	up to 4400	1 g	2 × (80 mL 0.05 M chloridric acid; ultrasound, filtration); centrifugation	none	LC: Diamonsil C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
Cook et al.	[120]	*Astragalus mollissimus* (Fabaceae) roots, crowns, steams, leaves, flowers and pods	swainsonine	up to 2500	50 mg	1.5 mL 2% acetic acid; centrifugation; 0.95 mL 20 mM ammonium acetate	none	LC	MS
		Astragalus lentiginosus (Fabaceae) roots, crowns, stems, leaves, flowers and pods		up to 2500					
Vichasilp et al.	[71]	mulberry leaves and shoots	DNJ	up to 3500	50 mg	5 mL ethanol 50%; ultrasound, centrifugation, filtration	none	LC: HILIC (TSK Gel amide-80)	QqQ (ESI)

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})^{a}	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Amézqueta et al.	[49]	*Fagopyrum esculentum* groats, leaves, bran and flour	D-fagomine and 3,4-di-*epi*-fagomine	p to 44	100 mg	12 mL 70% methanol; centrifugation, filtration	Spe-ed SCX cartridge; 2 M NH4OH	LC: TSK Gel CM2SW	Single Q (ESI)
		Fagopyrum tataricum groats	D-fagomine and 3,4-di-*epi*-fagomine	up to 7					
		Morus alba leaves	D-fagomine and 3-*epi*-fagomine	up to 103					
		Castanospermum australe seeds	D-fagomine related compounds	NA					
Wang et al.	[121]	Streptomyces (Streptomycetaceae) strains	DNJ and miglitol	up to > 96	NA	NA	NA	LC	QqQ
Zhou et al.	[79]	Commercial standards (impurity test)	DNJ, miglitol	NA	NA	NA	NA	LC: Inertsil Amide column	ELSD
Li et al.	[122]	*Oxytropis serioopetala* (Fabaceae)	swainsonine	4510	NA	methanol; centrifugation	none	none	α-mannosidase inhibition assay
Singh and Kaur	[123]	*Metarhizium. Anisopliae* (Clavicipitaceae) strains	swainsonine	up to 1340 (N = 10)	none	broth supernatant; centrifugation; filtration	none	none	α-mannosidase inhibition assay
Yang et al.	[50]	*Undifilum oxytropis* (Pleosporaceae) mycelia	swainsonine	up to 5000	0.5 g	100 mL methanol; soxhlet; evaporation; 2% acetic acid; centrifugation	BioRad AG 50 W; 1M NH$_4$OH	LC: Waters XBridge HILIC	ELSD
Zhou et al.	[79]	synthetic miglitol and DNJ	miglitol and DNJ	NA	NA	NA	NA	LC: Inertsil Amide column	ELSD
Spieker et al.	[53]	mouse and human plasma	miglustat	up to 3.4 mg L^{-1}	25 µL	300 µL methanol, filtration, dilution	none	LC: Phenomenex Gemini C18	QqQ (APCI)
Amézqueta et al.	[72]	*Fagopyrum esculentum* foodstuffs	D-fagomine and 3,4-di-*epi*-fagomine	up to 24	100 - 800 mg	12 mL 70% methanol; centrifugation, filtration	Spe-ed SCX cartridge; 2 M NH$_4$OH	LC: TSK Gel CM2SW	Single Q (ESI)
Hu et al.	[89]	mulberry dry leaves	DNJ	up to 1472	90 mg	2 × 2.5 mL 0.05 M HCl, sonication centrifugation	none	LC: Waters XTerra RP-18 derivatizing agent: FMOC-Cl	DAD: 254 nm
Hunyadi et al.	[75]	*Morus alba* leaves	DNJ, 2-*O*-α-D-galactopyranosyl-DNJ, D-fagomine	NA	2.5 kg	30 L 70% methanol; evaporation; n-BuOH extraction	Merck Kieselgel 60; CH$_2$Cl$_2$/EtOH	LC: Agilent Zorbax Eclipse XDB-C8	QqQ (ESI)
Rodríguez-Sánchez et al.	[56]	*Morus alba* leaves	DNJ and D-fagomine	up to 1860	1 g	2 × 10 mL chloridric acid 0.1%; centrifugation, filtration	none	GC: Phenomenex ZB-1MS derivatizing agent: hexamethyldisylazane (HMDS) and trifluoroacetic acid (TFA)	QqQ (EI)
		Hyacinthus orientalis bulbs	α-HNJ and other piperidine alkaloids	up to 5500					

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})^{a}	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
		Fagopyrum esculentum grains	D-fagomine	up to 54					
Yan et al.	[124]	*Suregada glomerulata* leaves	pyrrolidine and piperidine alkaloids	10 (total)	14 kg	3 × 100 L water; evaporation; dilution with ethanol	H^{+} form resin; 0.5 N NH_4OH; OH^{-} form resin; HOAc	none	NMR
Rodríguez-Sánchez et al.	[76]	*Aglaonema treubii* (Araceae) root extract	several pyrrolidine and piperidine alkaloids	NA	0.08 g	0.8 mL of ultrapure water in a pressurized liquid extraction system	none	GC: HP-1 and BPX-50 derivatizing agent: hexamethyldisylazane (HMDS) and trifluoroacetic acid (TFA)	QqQ (EI)
Rodríguez-Sánchez et al.	[61]	*Morus alba* leaves	DNJ and other piperidine and pyrrolidine alkaloids	up to 1150	0.05 g	0.5 mL of ultrapure water in a pressurized liquid extraction system	none	LC: Waters BEH	Q (ESI)
		Hyacinthus orientalis bulbs	HNJ and other piperidine alkaloids	up to 1500					
		Fagopyrum esculentum seeds	D-fagomine	11					
Jiang et al.	[90]	mulberry leaves	DNJ	2000	0.5 g	0.05M HCl; centrifugation, filtration	none	LC: Diamonsil C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
Müller and Cooks	[77]	*Hibiscus moscheutos* and *syriacus* (Malvaceae) petals	Polyhydroxylated pyrrolidine	NA	Plant tissue	none	none	none	LTQ-Orbitrap (ESI)
Chen et al.	[88]	*Bombyx mori* silkworm larvae	DNJ	5000	NA	0.05M HCl; vortex, sonication	none	LC derivatizing agent: FMOC-Cl	FLD
Choi et al.	[95]	human plasma	miglitol	up to 2.5 mg L^{-1}	NA	NA	none	LC: Atlantis C18	QqQ
Wang et al.	[87]	mulberry leaves	DNJ	NA	solvent : sample, 1:12	55% ethanol, 80 ºC, 72 min, reflux; filtration	QiRui 732; NH_4OH 1 mM	LC: Kromasil C18 derivatizing agent: FMOC-Cl	FLD: Ex = 254 nm, Em = 322 nm
Gossan et al.	[125]	*Glyphaea brevis* (Malvaceae) roots	glyphaeasides A1–A4, B1–B5 and C	NA	NA	80% methanol; evaporation; successively partition with $CHCl_3$ and n-BuOH	Merck Kieselgel 60; $CHCl_3$–MeOH–H_2O; LiChroprep RP-18; MeOH–H_2O	none	NMR
Attimarad et al.	[54]	human plasma	miglitol	up to 1.2 mg L^{-1}	0.2 mL	acetonitrile; centrifugation; evaporate	none	LC: Zorbax Eclipse C18	Q (ESI)
Xu et al.	[78]	*Morus alba* leaves	DNJ	up to 2800	0.2 g	70% ethanol; ultrasound; centrifugation	none	none	direct analysis in real time (DART) mass spectrometry
Kato et al.	[126]	*Castanospermum australe* seeds and leaves	castanospermine and other pyrrolidine, pyrrolizidine, indolizidine and piperidine alkaloids	up to 2900	5.8 kg (seeds), 0.36 kg (leaves)	50% ethanol (seeds); 50% methanol (leaves)	Several resins (Amberlite IR-120B; Dowex 1-X2; Amberlite CG-50; and/or CM-Sephadex C-25)	none	NMR

Reference		Natural source (Family)	Compound	Amount (mg kg^{-1})[a]	Amount sample	Extraction	Purification Resin; eluent	Chromatographic analysis type: column	Detection Type (wavelength or ionization)
Yang et al.	[74]	rat plasma	DNJ, D-fagomine and DAB	up to 5.9 mg L^{-1}	0.1 mL	Tris, methanol and acetonitrile; centrifugation	none	LC: XBridge Amide column	QqQ (ESI)

[a] for liquid samples, mg L^{-1}, as indicated where appropriate; NA: Data not available

FIGURE 1

Chemical structure of iminocyclitols