FATE OF D-FAGOMINE AFTER ORAL ADMINISTRATION TO RATS

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ABSTRACT

D-Fagomine is an iminosugar found in buckwheat that is capable of inhibiting the adhesion of potentially pathogenic bacteria to epithelial mucosa and of reducing postprandial blood glucose concentration. This paper evaluates the excretion and metabolism of orally administered D-fagomine in rats and compares outcomes with the fate of 1-deoxynojirimycin. D-Fagomine and 1-deoxynojirimycin show similar absorption and excretion kinetics. D-Fagomine is partly absorbed (41-84%, dose 2 mg/kg body weight) and excreted in urine within 8 h while non-absorbed fraction is cleared in feces within 24 h. D-Fagomine is partially methylated (about 10% in urine and 3% in feces). The concentration of D-fagomine in urine from 1 to 6 h after administration is higher than 10 mg/L, the concentration that inhibits adhesion of *Escherichia coli*. Orally administered D-fagomine is partially absorbed and then rapidly excreted in urine were it reaches a concentration that may be protective against urinary tract infections.

Keywords. D-fagomine, 1-deoxynojirimycin, metabolism, Escherichia coli, urinary tract, mass spectrometry.

INTRODUCTION

D-Fagomine and 1-deoxynojirimycin (DNJ) are polyhydroxylated piperidines, also known as iminocyclitols, azasugars or iminosugars, that are synthesized by various plants and microorganisms as secondary metabolites [1,2]. D-fagomine and DNJ can be found in foodstuffs such as bread, pasta and biscuits made from buckwheat [3], and tea, snacks and biscuits made from mulberry [4]. Both of these iminocyclitols are glycosidase inhibitors with the capacity to reduce the postprandial glycemic response after oral administration of either sucrose or starch to rats and humans [5–8]. As D-fagomine and DNJ reduce the elevation of postprandial blood glucose they can be used as dietary supplements or functional food components to help maintain short-term homeostasis of blood glucose levels [9]. More recent observations have revealed that iminocyclitols may modify the composition of the gut microbiota by inhibiting bacterial adhesion to the intestinal mucosa [7]. It has been suggested that D-fagomine counteracts the short term metabolic alterations triggered by a high-energy-dense diet in rats [10], at least in part through the reduction of a diet induced excess of gut Enterobacteriales [11]. Thus, D-fagomine might also be administered to prolong maintenance of metabolic homeostasis.

Absorption, distribution, metabolism and excretion (ADME) studies in animals and humans have been published of naturally and non-naturally occurring bioactive iminocyclitols such as DNJ, *N*-methyl-DNJ, 1-deoxymannojirimycin (DMJ), 1,4-dideoxy-1,4-imino-d-arabinitol (DAB), *N*-hydroxyethyl-DNJ (miglitol or glyset), *N*-butyl-DNJ (miglustat or zavesca) [12–22]. In rats, DNJ is poorly absorbed in a dose-dependent manner, distributed in the intact form and rapidly excreted in urine [15,16,18,23]. The absorption and distribution of D-fagomine alone has never been reported. When administered as a minor component of an extract from mulberry twigs (Ramulus Mori, Chinese medicine) the D-fagomine absorption rate profile is similar to that of DNJ [21].

Bioanalytical methods for iminocyclitols have recently been reviewed [24]. Pharmacokinetics is effectively evaluated using radiolabeled derivatives. The technique is extremely sensitive but would fail to identify any putative transformation as a result of metabolism. More selective and safe hyphenated bioanalytical methods have also been developed. In particular, iminocyclitols are analyzed by hydrophilic interaction or cation exchange liquid chromatography coupled to different mass spectrometry detectors (single quadrupole (Q), triple quadrupole (QqQ), quadrupole-ion trap (QTrap), or time-of-flight (TOF)) [1,15–19,22].

The aim of this paper is to evaluate D-fagomine metabolism and excretion, and compare the results with those for DNJ: an iminocyclitol with putatively similar behavior.

MATERIALS AND METHODS

Reagents

A D-fagomine standard (assay > 98%) was provided by Bioglane (Barcelona, Spain). DNJ was from Carbosynth (Berkshire, UK). DMDP (2,5-hydroxymethyl-3,4-dihydroxypyrrolidine), the internal standard, was purchased from IRL (Lower Hutt, New Zealand). Lichrosolv grade methanol, together with analytical grade acetic acid and ammonium hydroxide were obtained from Merck (Darmstadt, Germany). HPLC-grade water (Millipore type I water from Merck) was used to prepare all of the aqueous solutions. Solid-phase extraction (SPE) cartridges for sample purification were Spe-ed, SCX (strong cation exchange) cartridges, 100 mg/mL from Applied Separations (Allentown, PA, USA). Nylon filters (0.45 μm) were obtained from Scharlab (Sentmenat, Spain). Microvette® CB 300 K2E Di-kalium-EDTA tubes were from Sarstedt (Nümbrecht, Germany), the gastric probe was from Harvard Apparatus (Holliston, MA, USA); and 25 G needles were from Novico Médica (Barcelona, Spain).

63 **Animals, diets and *in vivo* tests**

64 Eighteen 8-week-old female Sprague–Dawley rats (Janvier, Le Genest-St-Isle, France) were housed
65 under controlled conditions of stable humidity (50% ± 10%), and temperature (22 ºC ± 2 ºC) with a
66 12 h light – 12 h dark cycle. To minimize circadian rhythm effects, all rat handling was carried out
67 in the morning. After overnight fasting, the rats were divided into six groups which were given
68 single doses of D-fagomine or DNJ, administered as aqueous solutions (5 mL/kg body weight)
69 using a gastric probe. Each group received 2.0, 10, or 100 mg/kg body weight of D-fagomine or
70 DNJ. The rats were then placed in metabolic cages to collect urine and feces 1, 2, 4, 6, 8 and 24 h
71 after administration. Samples were kept at –80 ºC until analysis. After that, the rats were placed in
72 standard cages and the remaining feces were collected by abdominal massage 48 h after
73 administration. The handling of the animals was in full accordance with the European Union
74 guidelines for the care and management of laboratory animals, and the pertinent permission was
75 obtained from the CSIC Subcommittee for Bioethical Issues (ref. AGL2009-12 374-C03-03,
76 CEEA-12-011, date of approval March 4th, 2013).

77 **Extraction and SPE clean-up**

78 After thawing, aliquots (60 µL) of urine were extracted with 70% aqueous methanol (5 mL) using
79 an orbital shaker (Intelli-mixer RM-2 device from Elmi; Riga, Latvia) for 20 min. Feces were cut
80 longitudinally and the analytes were extracted from half of the sample also with 70% aqueous
81 methanol (5 mL solvent/ 60 mg feces) using the orbital shaker for 20 min. After extraction the
82 suspensions were centrifuged in a 5810R centrifuge from Eppendorf (Hamburg, Germany) for 3
83 min at 8000 rpm and 20 ºC, filtered through a 0.45-µm nylon filter (Phenomenex; Torrance, CA,
84 USA) and the filtrates were diluted with water to a known volume in a volumetric flask (10 mL in
85 the case of urine; variable in the case of feces). Cation exchange SPE cartridges were conditioned
86 with HPLC-grade methanol (1 mL) and water (1 mL). Then aliquots from the previous step were

loaded onto the cartridges. The aliquot volumes were adjusted to equalize the response of the analysis (urine samples: 2500, 500 and 50 µL from the groups administered the 2.0, 10 and 100 mg/kg body weight doses respectively; feces samples: variable volumes depending on the dilution after the extraction step). SCX resin was then washed with water (4 mL) and vacuum-dried. Next, the analytes were eluted with 2 M aqueous NH_4OH (500 µL). The eluates were spiked with a DMDP solution in methanol (100 µL, 5 mg/L). The solution was evaporated to dryness under a stream of N_2 at 60°C and the residue was redissolved in water (500 µL) and filtered through a 0.45-µm nylon filter.

HPLC/ESI–QqQ-MS analysis

Chromatography was carried out on an Acquity H class system (Waters; Milford, MA, USA) equipped with a quaternary pump and fitted with a TSK-Gel CM2SW cation exchange column (25 cm×4.6 mm i.d., 5 µm particle size) (Tosoh Bioscience; Tokyo, Japan). The injection volume was 5 µL, the column temperature 25 °C and the total analysis time 30 min. The target compounds were separated with a binary system: 50 mM NH_4CH_3COO (pH 8.5) / methanol (4:1), under isocratic conditions at a flow rate of 0.8 mL/min. Mass spectrometric analysis of the column effluent was carried out on a triple tandem quadrupole Xevo-TQ-S spectrometer (Waters). The ESI-MS/MS parameters were: positive polarity, a capillary voltage of 3 kV, a desolvation temperature of 600 ºC, a desolvation gas flow of 1000 L/h, 7 bar of nebulizer gas, with a cone voltage of 20-45 V depending on the analyte, and a collision energy of 20-30 V depending on the analyte (Table 1). Analysis was carried out in the multiple reaction monitoring mode (MRM) using the following quantification transitions: 164 → 80 (DMDP and DNJ), 148 → 86 (D-fagomine), 162 → 100 (monomethyl-D-fagomine), 176 → 114 (dimethyl-D-fagomine), 190 → 128 (trimethyl-D-fagomine), 178 → 94 (monomethyl-DNJ), 192 → 108 (dimethyl-DNJ), 206 → 122 (trimethyl-DNJ). The retention times of the analytes were 4 min (DNJ), 5.5 min (DMDP), 7.5 min

(monomethyl-D-fagomine), 9 min (D-fagomine), 10 min (dimethyl-D-fagomine), 10.5 min (trimethyl-D-fagomine) and 3.7 (monomethyl-DNJ). To confirm the identity of the metabolites, their exact masses were obtained using high resolution (HR) ESI–time of flight (TOF)-MS on an LCT Premier XE system (Waters) after HR separation using an HPLC Acquity system (Waters) fitted with a TSK-Gel CM2SW column.

Standard solutions

Stock standard solutions of 5 mg/L were prepared by dissolving D-fagomine, DNJ or DMDP (1.00 mg) in methanol (20 mL) and then diluting 1:9 in methanol. All solutions were stored at –20 °C. To prepare the working standard solutions, the corresponding aliquots of the D-fagomine or DNJ stock solutions were mixed with the DMDP stock solution (100 µL). The solvent was then evaporated to dryness under a stream of N_2 and the residue was dissolved in water (500 µL). These solutions were also stored at –20°C. The standard solutions were stable at this temperature for a period of at least 6 months.

Validation of the analytical method

As ESI techniques often suffer from matrix effects, the linear MS responses of D-fagomine/DMDP and DNJ/DMDP dissolved in water and matrix-matched solutions were evaluated and the slopes were compared. To prepare the matrix-matched solutions, urine and feces of rats that had still not been administered the iminosugars were used. These samples were subjected to the extraction and purification steps described in the 'Extraction and SPE clean-up' subsection. In the case of urine, 2500 µL of the extract (the aliquot that contains the highest amount of interfering compounds) were passed through the cartridge. The eluate obtained from the SCX resin was spiked with 100 µL of a DMDP solution in methanol (5 mg/L), and with different volumes of a D-fagomine + DNJ solution in methanol (5 mg/L of both compounds) to prepare a calibration curve in the range 4 – 83 mg D-fagomine/L urine or kg feces. Each solution was evaporated to dryness under a stream of N_2 at 60

°C and the residue was redissolved in water (500 µL) and then filtered through a 0.45-µm nylon filter. Calibration curves were constructed by plotting $A_{\text{D-fagomine or DNJ}}/A_{\text{DMDP}}$ against D-fagomine or DNJ concentration for each solvent. Then the SSE was calculated according to Eq. 1. An SSE value smaller than 100 means that the matrix causes signal suppression, and a higher value means that the matrix causes signal enhancement [25].

$$SSE(\%) = \frac{Slope_{matrix-matched\ calibration\ curve}}{Slope_{aqueous\ standards\ calibration\ curve}} \times 100 \qquad \text{[Eq. 1]}$$

In the assessment of linearity, calibration curves were plotted in the range 1.8 – 8300 mg/L and 8.3 – 8300 mg/L for D-fagomine and DNJ, respectively. Calibration curves with eleven calibration standards each were prepared across these ranges. All of the calibration standards were prepared using matrix matched solutions, and were spiked with the DMDP standard stock solution (100 µL, 5 mg/L). Linearity was evaluated for every analytical run batch to compensate for ESI variability. The concentration of the metabolites was expressed as DNJ of D-fagomine equivalents, due to the lack of commercial standards.

A precision and trueness study was carried out with the different matrices. The recovery study was performed by spiking the matrices with D-fagomine at three different concentrations (67, 330 and 3300 mg/L in urine; and 130, 670 and 6700 mg/kg in feces) in triplicate in three different days. Three standard solutions of D-fagomine were prepared in methanol (200, 1000 and 10000 mg/L), one for each of the three spiking levels. In the case of urine, the solvent of an aliquot (20 µL) of the corresponding standard was evaporated and the residue containing D-fagomine was suspended in the matrix under study (60 µL of urine). In the case of feces, a portion (60 mg) was spiked with an aliquot (40 µL) of the corresponding standard. The feces samples were processed 24 h after spiking, to ensure complete evaporation of the solvent. Next, thesamples were subjected to the purification step. After elution, the eluates were spiked with the DMDP standard stock solution

158 (100 µL, 5 mg/L) to correct for ESI variability. The solution was evaporated to dryness under a
159 stream of N_2 at 60 °C and the residue was redissolved in water (500 µL) and filtered through a
160 0.45-µm nylon filter. In parallel, the calibration curves were prepared using matrix-matched
161 standards. Finally, the samples and the calibration standards were analyzed by LC-MS/MS.
162 Recovery was determined by comparing the $A_{D\text{-fagomine}}/A_{DMDP}$ signal obtained from the spiked
163 samples with the $A_{D\text{-fagomine}}/A_{DMDP}$ signal from the calibration standards. Precision was evaluated by
164 calculating the RSDs obtained in within- and between-day recovery experiments. The limit of
165 quantification (LOQ) was established as the concentration at which a recovery value similar to that
166 of the trueness study was obtained with an RSD<20% when analyzing five spiked samples.

167

168 **RESULTS AND DISCUSSION**

169 **Set-up and validation of the analytical method**

170 The analytical method was adapted for biological fluids from a previously described procedure
171 developed for the analysis of plant sources (buckwheat and mulberry) [1]. Modifications were
172 introduced in the extraction step and, to improve selectivity and sensibility, triple quadrupole MS
173 was used instead of single quadrupole MS. First, the MS parameters such as desolvation
174 temperature, cone voltage or collision energy were optimized to improve better signal intensity for
175 the analytes under study. Then, selectivity, sensibility and linearity were checked by preparing the
176 D-fagomine and DNJ calibration curves using DMDP as an internal standard and two solvents:
177 water and the matrix resulting from control urine or feces subjected to the extraction and SPE
178 purification steps. The calibration curves generated with the two matrices showed significant signal
179 suppression/enhancement (Table 2), so the calibration standards used from that point on were
180 prepared using matrix-matched solutions. The assay response (area of the D-fagomine or DNJ peak
181 divided by the area of the DMDP peak) to D-fagomine or DNJ concentration was linear ($R^2 > 0.99$)

182 in the ranges under study (1.8 – 8300 mg/L and 8.3 – 8300 mg/L for D-fagomine and DNJ,
183 respectively). Within-day and between-day precision and trueness were studied using samples of
184 urine and feces spiked with D-fagomine. In the case of urine, the percentage recovery at the three
185 concentrations assayed (96%) was homogeneous (RSD: 12%), which demonstrated the precision
186 and trueness of the analytical procedure. The European Medicines Agency (EMEA) [26] recommends
187 a recovery value (trueness) in the range 85%–115% and a relative standard deviation value (RSD;
188 precision) of under 15% for bioanalytical methods. In the case of feces, within-day recovery was
189 47% (RSD: 10%): outside the EMEA suggested interval (85%-115%). Intra-day recovery was 51%
190 (RSD: 14%), which demonstrated the validity of the method despite the low recovery. The results
191 in this work are corrected for the recovery values. The LOQ values were 1.8 mg/L urine or kg
192 feces, for D-fagomine; and 8.3 mg/L urine or kg feces, for DNJ. DNJ shows a higher LOQ value
193 because its ionization in the MS detector was not as complete as that of D-fagomine.

194 The results of the validation study show that the new methods met all the requirements for a
195 bioanalytical process [26]. The methods were selective; the response was linear in the working range;
196 precision and trueness fell within the recommended range except for feces recovery; and the LOQ
197 was much lower than the D-fagomine and DNJ concentrations found after the maximum excretion
198 period. The methods were successfully applied to the determination of D-fagomine, DNJ and their
199 metabolites in the urine and feces of rats fed D-fagomine or DNJ. The identity of the compounds
200 was confirmed by HPLC/ESI–HR-TOF-MS with samples generated in a previous separate
201 experiment by administration of 250 mg of iminocyclitol per kg body weight (Table 3).

202 **Absorption and excretion**

203 The animals were administered 2.0, 10 or 100 mg/kg body weight of D-fagomine or DNJ (positive
204 control). The dose of 2.0 mg/kg body weight corresponded to the dose of D-fagomine that reduced
205 postprandial blood glucose concentration (20% reduction of the area under the curve between 0 and

120 min) after intake of glucose or starch [7]. At this active concentration part of the administered D-fagomine (0.2-0.4 mg) was absorbed and excreted in urine within 8 h, while the rest was recovered in feces within 24 h (Figure 1a and Table 4). Absorption appears to be limited due to saturation after a total amount of 3 mg of D-fagomine has been absorbed (Table 4). The highest excretion rate occurred in the period between 2 and 4 h in urine (Figure 2 and Table 5) and in the period between 8 and 24 h in feces (Table 6). D-Fagomine was not detected in feces collected by abdominal massage 48 h after intake (Table 6). Briefly the absorbed portion of D-fagomine is cleared in urine within 8 h and the non-absorbed portion in feces within 24 h after intake. The behavior of D-fagomine and DNJ (positive control) was similar (Figure 1 and Tables 4-6) and the absorption under our experimental conditions reached saturation at a total absorbed amount of 2 mg. As most (around 90% at the 3 doses) of both of the ingested iminocyclitols is excreted, whether in urine or feces, within the first 24 h after oral administration these compounds probably do not accumulate in tissues, as already suggested by Nakagawa *et al*. (2007) [15].

The results presented here for DNJ agree with two other studies that also used a pure compound [15,18]. Nakagawa *et al.* (2007) [15] found that about 0.6 mg of the DNJ (2%) was excreted in urine within 24 h after a 110 mg/kg body weight oral administration after fasting [15]. Kim *et al*. (2010) [18] reported that about 4 mg of the DNJ administered was excreted in urine within 24 h after a 30 mg/kg body weight oral administration; the non-absorbed fraction was excreted in feces within 48 h. When DNJ was administered as a component of a plant extract, the excretion profile was similar to that one obtained here. Xiao *et al*. (2014) reported that about 2.5 mg of DNJ in a mulberry root bark extract was excreted in urine within the first four hours [27]. Our results show that D-fagomine is absorbed as fast as DNJ is.

Metabolism

229 It has been reported that DNJ (positive control) is mainly excreted in the intact form [15,16,18,27].
230 Nakagawa *et al*. (2007) did not detect any degradation products (e.g., oxidized and alkylated
231 products) in plasma and reported that a small signal in the HPLC-MS single ion monitoring
232 chromatogram might correspond to an unidentified metabolite [15]. We found trace amounts of
233 monomethyl-DNJ in urine and several methyl derivatives of D-fagomine. A monomethylated
234 conjugate was present in urine (10%) and feces (3%), at the three doses, within the period of 0-8 h
235 (Figure 1a, Table 4). We also detected trace levels of dimethyl-D-fagomine and trimethyl-D-
236 fagomine in urine, at the three doses, within the period of 0-8 h. In a preliminary experiment with
237 samples obtained by administering a higher dose of D-fagomine (250 mg /kg body weight), signals
238 compatible with deoxygenated products were detected that were not found at the lower
239 concentrations used here. Metabolism of organic compounds consists mainly of their conversion
240 into more hydrophilic species that can be readily excreted [28]. Phase II conjugation into glucuronides
241 and sulfates are the most common transformations. Iminocyclitols such as D-fagomine and DNJ are
242 already highly water soluble and they are rapidly excreted without further modification.
243 Methylation is a less common phase II conjugation that reduces water solubility and plays the
244 physiological role of blocking biological activities by modifying chemically active functions, such
245 as those of amines and hydroxyls [29]. In the case of D-fagomine, as only a small percentage is
246 methylated, it is unlikely that methylation serves the purpose of deactivation. Its structure may
247 partially fit the requirements for the enzymes involved in the modification of other chemical
248 species, most likely sugars. The presence of methyl-D-fagomine in feces could be explained by
249 biliary excretion after modification in the liver. Alternatively, methyl-D-fagomine may be generated
250 by the gut microbiota as some species of the phylum Actinobacteria are capable of methylating the
251 terminal units of oligosaccharides [30].

252 The presence of intact D-fagomine in urine for several hours after oral ingestion may have
253 important implications for the maintenance of a healthy status in the urinary tract. The
254 concentration of D-fagomine in contact with the tissues along the urinary tract for the period of
255 from 1 to 6 h, in all the rats tested at any dose (Table 5, Figure 2) was higher than the concentration
256 (10 mg/L) that inhibits adhesion (95%) of *Escherichia coli* to the intestinal mucosa [7]. So, at the
257 dose that is active at lowering postprandial glucose concentration by 20% (2.0 mg/kg body weight:
258 the lowest dose tested) D-fagomine may protect against urinary tract infections. D-Fagomine may
259 also protect the intestinal tract against infections. We recently showed that D-fagomine, at a dose of
260 23 mg/kg body weight/day, reduced the increase in the population of Enterobacteriales induced by
261 a high-fat high-sucrose diet [11].

262 To sum up, orally administered D-fagomine is rapidly absorbed and excreted in urine within 8 h in
263 rats. The non-absorbed fraction is cleared in feces within 24 h. D-fagomine is partially methylated
264 (about 10% in urine and 3% in feces). The concentration of D-fagomine in urine 1 to 6 h after oral
265 administration of 2.0 mg/kg body weight is higher than the concentration that inhibits (95%) the
266 adhesion of E. coli to epithelial surfaces. Therefore, D-fagomine may protect the urinary tract
267 against infections caused by Enterobacteriales at a dose that is active at reducing postprandial blood
268 glucose concentration.

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274

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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FIGURE CAPTIONS

Figure 1. Percentages of D-fagomine (a) and DNJ (b) including their methyl derivatives, excreted in feces and urine after oral administration at three different doses.

Figure 2. D-fagomine concentration in urine after oral administration of 2.0 mg/kg body weight.

382 **Table 1.** Cone Voltages and Collision Energies Applied in the ESI-MS/MS Analysis.

	Cone voltage (V)	Collision energy (V)
DMDP	45	20
DNJ	45	20
D-Fagomine	35	20
Methylfagomine	20	30
Dimethylfagomine	20	30
Trimethylfagomine	35	30

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385 **Table 2.** Signal Suppression and Enhancement Effect in Matrix-matched Solutions of Both Urine

386 and Feces.

		D-Fagomine/DMDP signal vs D-fagomine concentration	DNJ/DMDP signal vs DNJ concentration
Urine	Aqueous standards	y=0.58x-0.4	y=0.036x-0.06
	Matrix-matched standards	y=0.099x-0.3	y=0.0018x-0.005
	SSE (%)	17% (Signal suppression)	5% (Signal suppression)
Feces	Aqueous standards	y=0.103x+0.1	y=0.0025x-0.027
	Matrix-matched standards	y=0.47x+1	y=0.010x-0.08
	SSE (%)	458% (Signal enhancement)	390% (Signal enhancement)

387 y relative abundance, x concentration in mg/L or mg/kg, SSE signal suppression enhancement

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389 **Table 3.** Identification of D-Fagomine Metabolites by HPLC/ESI–HR-TOF-MS.

m/z	Retention time	Measured mass	Calculated mass	Formula	ppm	Compound
148	11.7	148.0970	148.0974	$C_6H_{14}NO_3$	-2.0	Fagomine
162	9.4	162.1129	162.1130	$C_7H_{16}NO_3$	-0.6	Methylfagomine
176	12.7	176.1282	176.1287	$C_8H_{18}NO_3$	-2.8	Dimethylfagomine
190	13.3	190.1441	190.1443	$C_9H_{20}NO_3$	-1.1	Trimethylfagomine

390 Measurements made from a sample of urine collected 1h after administration of D-fagomine (250
391 mg D-fagomine/kg body weight) during a preliminary pilot study with Sprague-Dawley rats.

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Table 4. Excretion of D-Fagomine and DNJ in Feces and Urine after Oral Administration.

Compound (dose)	Rat	mg Administered	mg excreted in urine (0-8 h), intact form	mg excreted in urine (0-8 h), methylated form	mg excreted in feces (6-24 h), intact form	mg excreted in feces (6-24 h), methylated form	% excreted in urine (0-8 h)	% excreted in feces (6-24 h)	Total (% feces + % urine)
D-Fagomine (2.0 mg/kg body weight)	1	0.53	0.41	0.04	0.10	-	84	19	103
	2	0.53	0.30	0.03	0.31	0.02	63	64	127
	3	0.57	0.21	0.02	0.39	0.02	41	71	112
DNJ (2.0 mg/kg body weight)	4	0.72	0.31	-	-	-	42	-	42
	5	0.80	0.55	-	0.09	-	69	11	80
	6	0.75	0.81	-	0.05	-	108	6	114
D-Fagomine (10 mg/kg body weight)	7	2.6	0.9	0.08	0.8	0.02	37	30	67
	8	2.7	1.6	0.25	1.6	0.03	70	60	131
	9	2.8	0.5	0.03	1.8	0.10	18	69	87
DNJ (10 mg/kg body weight)	10	2.6	2.2	-	0.6	-	83	23	105
	11	2.7	1.0	-	2.0	-	38	76	113
	12	2.7	1.6	-	1.1	-	59	42	101
D-Fagomine (100 mg/kg body weight)	13	26	2.3	0.07	17	0.52	9	66	75
	14	27	1.2	0.03	22	0.53	5	85	90
	15	27	5.4	0.02	18	0.95	20	69	89
DNJ (100 mg/kg body weight)	16	26	2.4	-	15	-	9	56	65
	17	27	1.4	-	26	-	5	95	101
	18	27	1.7	-	14	-	6	53	60

Table 5. D-Fagomine and DNJ Concentration (mg/L) in Urine during Different Periods after Oral Administration.

	Rat	0-1 h	1-2 h	2-4 h	4-6 h	6-8 h	8-24 h
D-Fagomine (2.0 mg/kg body weight)	1	88	100	−	72	47	ND
	2	33	−	94	13	traces	ND
	3	68	−	47	ND	17	ND
DNJ (2.0 mg/kg body weight)	4	160	−	−	−	−	29
	5	430	−	−	−	280	20
	6	350	−	−	−	160	16
D-Fagomine (10 mg/kg body weight)	7	−	410	−	130	16	ND
	8	620	−	−	450	−	ND
	9	66	−	−	65	180	ND
DNJ (10 mg/kg body weight)	10	580	−	650	99	33	ND
	11	180	−	230	42	ND	ND
	12	350	640	−	440	100	ND
D-Fagomine (100 mg/kg body weight)	13	−	660	380	220	48	<40
	14	−	730	−	490	170	<40
	15	−	2000	360	460	64	<40
DNJ (100 mg/kg body weight)	16	−	−	1100	−	570	<40
	17	240	−	660	120	63	<40
	18	230	1200	1300	400	90	<40

ND: Not detected; − collected at the next time point as the animal did not excrete urine during all collecting periods.

Table 6. D-Fagomine and DNJ Concentration (mg/kg) in Feces Excreted in the 8-24 h Period after Oral Administration.

	Rat	6-8 h	8-24 h	48 h
D-Fagomine (2.0 mg/kg body weight)	1	−	32	ND
	2	ND	110	ND
	3	ND	170	ND
DNJ (2.0 mg/kg body weight)	4	ND	ND	ND
	5	ND	71	ND
	6	ND	34	ND
D-Fagomine (10 mg/kg body weight)	7	−	750	ND
	8	−	450	ND
	9	−	890	ND
DNJ (10 mg/kg body weight)	10	ND	150	ND
	11	ND	650	ND
	12	−	340	ND
D-Fagomine (100 mg/kg body weight)	13	5000	14000	ND
	14	4000	9200	ND
	15	−	8500	ND
DNJ (100 mg/kg body weight)	16	ND	11000	ND
	17	ND	8100	ND
	18	−	6300	ND

ND: Not detected; − collected at the next time point as the animal did not excrete feces during all collecting periods.

Figure 1.

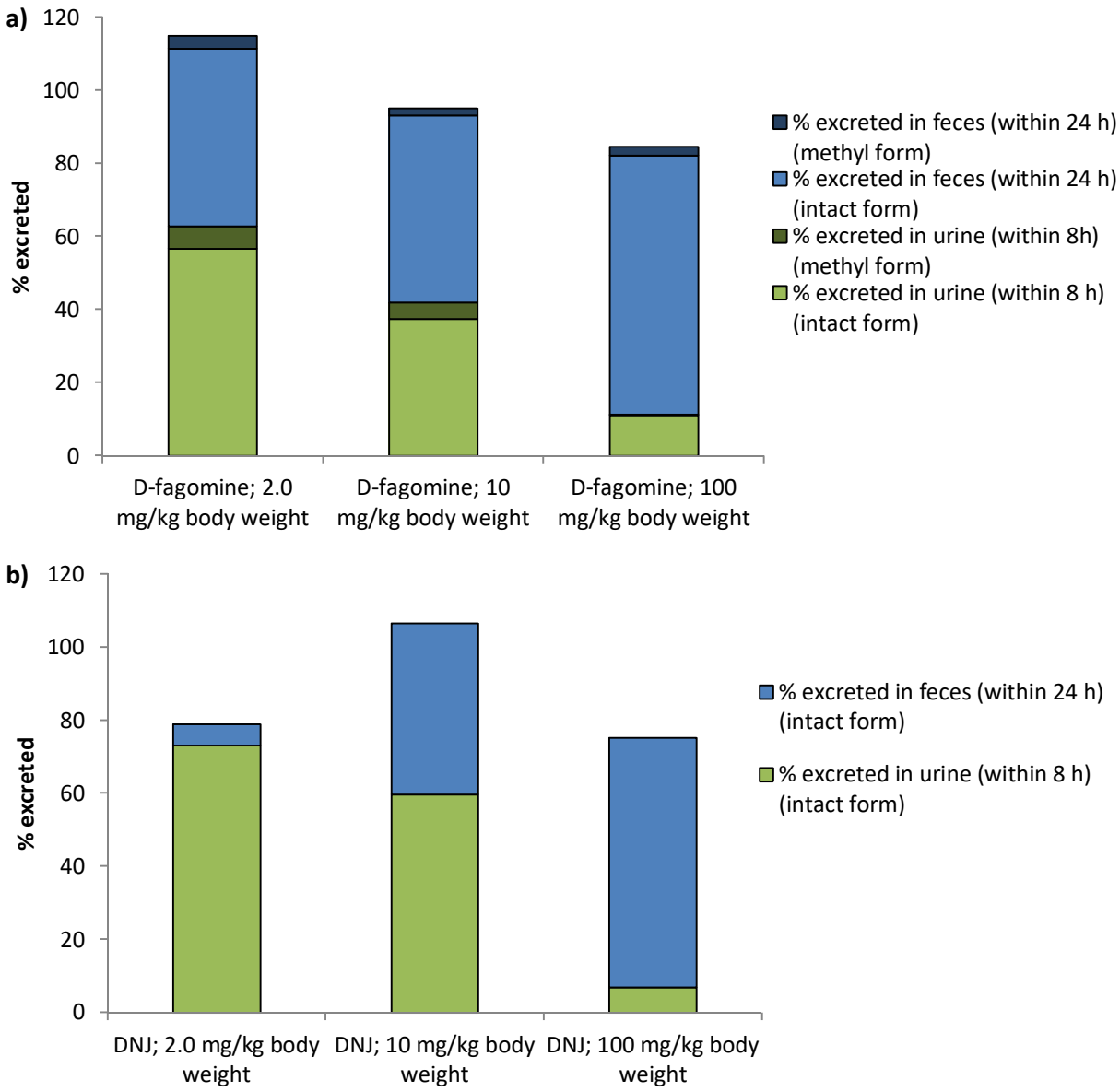
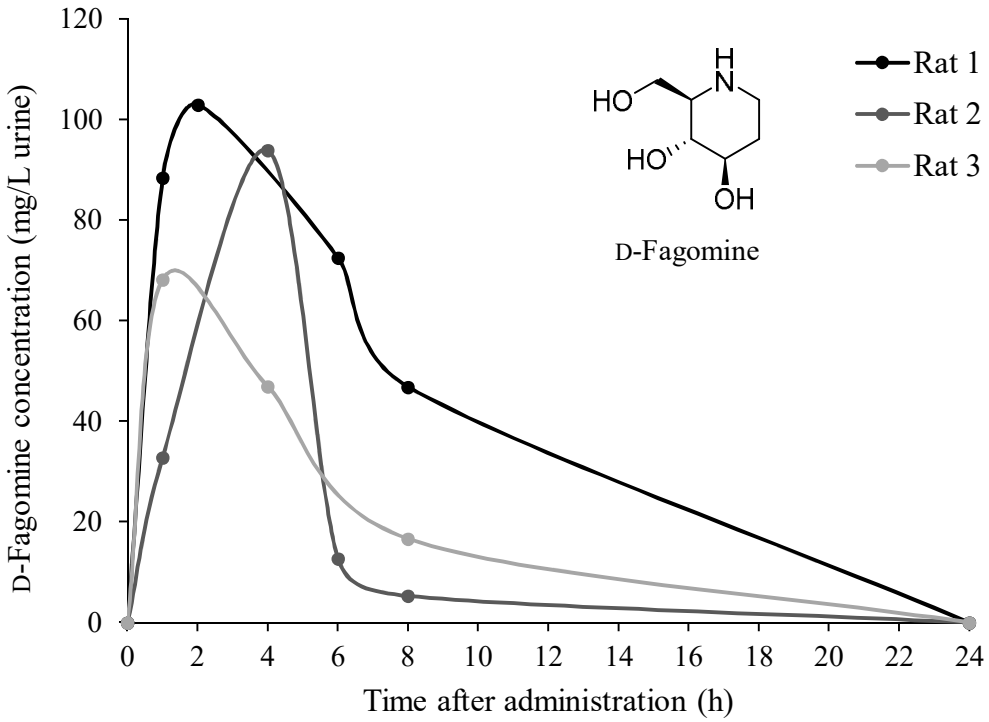

a)
120
100
80
60
40
20
0
% excreted
% excreted in feces (within 24 h) (methyl form)
% excreted in feces (within 24 h) (intact form)
% excreted in urine (within 8h) (methyl form)
% excreted in urine (within 8 h) (intact form)
D-fagomine; 2.0 mg/kg body weight
D-fagomine; 10 mg/kg body weight
D-fagomine; 100 mg/kg body weight
b)
120
100
80
60
40
20
0
% excreted
% excreted in feces (within 24 h) (intact form)
% excreted in urine (within 8 h) (intact form)
DNJ; 2.0 mg/kg body weight
DNJ; 10 mg/kg body weight
DNJ; 100 mg/kg body weight

Figure 2.


120
100
80
60
40
20
0
D-Fagomine concentration (mg/L urine)
0
2
4
6
8
10
12
14
16
18
20
22
24
Time after administration (h)
Rat 1
Rat 2
Rat 3
HO
H
N
HO
OH
D-Fagomine

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