RESEARCH ARTICLE

The red clover isoflavone irilone is largely resistant to degradation by the human gut microbiota

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Received: May 20, 2009
Revised: June 29, 2009
Accepted: July 21, 2009

Intestinal bacteria may influence bioavailability and physiological activity of dietary isoflavones. We therefore investigated the ability of human intestinal microbiota to convert irilone and genistein *in vitro*. In contrast to genistein, irilone was largely resistant to transformation by fecal slurries of ten human subjects. The fecal microbiota converted genistein to dihydrogenistein, 6′-hydroxy-*O*-desmethylangolensin, and 2-(4-hydroxyphenyl)-propionic acid. However, considerable interindividual differences in the rate of genistein degradation and the pattern of metabolites formed from genistein were observed. Only one metabolite, namely dihydroirilone, was formed from irilone in minor amounts. In further experiments, *Eubacterium ramulus*, a prevalent flavonoid-degrading species of the human gut, was tested for transformation of irilone. In contrast to genistein, irilone was not converted by *E. ramulus*. Irilone only differs from genistein by a methylenedioxy group attached to the A-ring of the isoflavone skeleton. This substitution obviously restricts the degradability of irilone by human intestinal bacteria.

Keywords:
Genistein / Human intestinal microbiota / Irilone / Isoflavones / Red clover

1 Introduction

Owing to their purported beneficial effects on human health, isoflavone-containing dietary supplements are increasingly offered to consumers. In addition to soy-based dietary supplements, extracts of red clover (*Trifolium pratense* L.) are advertised as a natural and side-effect-free alternative to the conventional hormone replacement therapy for menopausal women. Red clover contains a wide variety of isoflavones, which structurally differ from those in soy [1, 2]. Besides formononetin (35–55%) and biochanin A (20–50%), irilone (4′,5-dihydroxy-6,7-methylenedioxy-isoflavone, structure in Fig. 7) (5–10%) represents one of the main isoflavones in red clover [1, 3]. Irilone and its glycosides were first isolated from rhizomes of *Iris germanica* L. and *I. florentina* L. and their structure elucidated [4, 5]. However, little is known about the bioactivity of irilone and its absorption and metabolic fate following ingestion. Based on its ability to inhibit cytochrome P450 1A1 and to induce NAD(P)H:quinone reductase, irilone was proposed to have a cancer chemopreventive potential [6].

Epithelial β-glucosidases, such as lactase phlorizin hydrolase, deglycosylate isoflavones in the small intestine [7]. Intestinal bacteria catalyze the deconjugation and further conversion of isoflavones. Only intestinal bacteria are able to cleave the basic isoflavone structure as has been unequivocally demonstrated by comparison of germ-free and human-microbiota associated rats [8]. The microbial metabolites

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Abbreviations: EI, electron impact; **DHG,** dihydrogenistein; **DHI,** dihydroirilone; **FS,** fecal slurry; **HPP,** 2-(4-hydroxyphenyl)-propionic acid; **OH-DMA,** 6′-hydroxy-*o*-desmethylangolensin; **rDA,** retro Diels-Alder; **TMS,** trimethylsilyl

*Both groups contributed equally to this work.

formed have biological activities that differ from those of the parent compounds [9, 10]. Thus, the gut microbiota plays a crucial role in determining the bioavailability, and thereby the biological activity, of isoflavones. Daidzein and genistein, the most studied isoflavones, are extensively metabolized in humans after ingestion of either pure compounds or soy [11–14]. The microbial degradation of both isoflavones has also been studied *in vitro* in human fecal suspensions [15–22]. Genistein (4′,5,7-trihydroxy-isoflavone, structure in Fig. 7) was shown to be transformed by human fecal microbiota to dihydrogenistein (DHG) and 2-(4-hydroxyphenyl)-propionic acid (HPP) [15, 17]. The intermediate 6′-hydroxy-*O*-desmethylangolensin (OH-DMA) was observed with rat but not with human intestinal microbiota [17, 23]. Three bacterial strains capable of converting genistein were isolated from human feces. Whereas *Clostridium* sp. HGH6 only catalyzes the reduction of the double bond in the C2/C3-position of genistein to form DHG [24], two strains of *Eubacterium ramulus*, DSM 16296 and Julong 601, cleave the C-ring of genistein yielding OH-DMA and finally HPP [25, 26]. Substantial interindividual differences in the formation of the microbial metabolite *S*-equol have been observed for daidzein [10]. These are most likely due to differences in gut microbiota composition. Similarly, *in vivo* and *in vitro* studies on the metabolism of genistein indicate an interindividual variability [13, 19–22].

So far, data on the fate of irilone following ingestion, in particular its conversion by intestinal bacteria, are not available. Irilone differs from genistein by the presence of a C6 to C7 methylenedioxy bridge instead of the single hydroxy group in the C7 position of the A-ring (structures in Fig. 7). The aim of this study was to test the human gut microbiota for its ability to transform irilone. As a positive control, the conversion of genistein was investigated under identical conditions.

2 Materials and methods

2.1 Chemicals

Irilone (purity 96.7%, HPLC) was obtained from Chromadex (Santa Ana, CA, USA). Genistein (purity 98.5%, HPLC) was purchased from Roth (Karlsruhe, Germany). HPP was from Acros Organics (Geel, Belgium), and 4-ethylphenol from Sigma (Deisenhofen, Germany). DHG and OH-DMA were prepared as previously described [27, 28]. The identity of the products was checked by ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) analysis (Bruker Avance 500). ST medium for the fermentation experiments were prepared as described previously [29].

2.2 Collection and processing of fecal samples

Fecal samples were collected from ten healthy human volunteers (seven females, three males; 20–51 years of age, mean age of 36.3 ± 13.6 years). The volunteers consumed a non-specified Western diet and had not undergone antibiotic therapy within 6 months prior to the study. The samples were collected in plastic boxes, kept under anoxic conditions using the AnaeroGen Compact System (Oxoid, Hampshire, UK) and stored at 4°C until further processing within 1 h. The fecal suspensions were prepared under anoxic conditions in a glove box (MACS Anaerobic Workstation, Don Whitley Scientific, Shipley, UK) containing a gas atmosphere of $N_2/CO_2/H_2$ (80/10/10, v/v/v). Aliquots (1 g) of human feces were suspended in 10 mL ST medium.

2.3 Fermentation experiments

Fermentation experiments were performed under strictly anoxic conditions in 16-mL Hungate tubes containing 2 or 10 mL ST medium and a gas phase of N_2/CO_2 (80:20, v/v). An irilone or genistein stock solution (20 mM in DMSO) was added with a syringe to a final concentration of approximately 190 μM. The final DMSO concentration was 1%. The media were inoculated with the above fecal suspensions (final concentration, 5%) or an exponentially growing culture of *E. ramulus* DSM 16296 (final concentration, 5%). Isoflavones in medium without an inoculum and bacteria in medium without isoflavones served as controls. The tubes were incubated at 37°C in a tube rotator for 96 h (4 d). At 0, 4, 8, 24, 48, and 96 h, aliquots of 200 μL were taken with a syringe and stored at −20°C until further processing and analysis.

2.4 Extraction of fermentation samples

Samples taken in the course of fermentation were thoroughly homogenized after thawing. An aliquot of 100 μL each was withdrawn, acidified with 10 μL 0.1 M HCl and extracted two times with 200 μL of ethyl acetate by vigorously vortexing. The phases were separated by centrifugation (12 000 × *g*, 2 min) and the combined upper layers were dried by vacuum centrifugation (Jouan RC 1022, Saint Herblain, France). Each residue was dissolved in 50 μL of 70% aqueous methanol v/v. After centrifugation (12 000 × *g*, 2 min), 10 μL of the supernatant were analyzed by HPLC. The recovery of the extraction procedure was determined by spiking medium with defined amounts of the individual compounds. The recoveries determined for irilone, genistein, DHG, OH-DMA, and HPP were 89, 88, 96, 84, and 99%, respectively. For quantification of these compounds in the course of fermentation, the obtained values were corrected according to the relative recoveries determined.

2.5 HPLC analysis

The HPLC system (Summit, Dionex, Idstein, Germany) was equipped with a high precision pump (P 680A LPG-4), an

autosampler (ASI-100T), a thermostated column compartment (TCC-100) with eluent preconditioner, a photodiode array detector (UVD 340U), and a LiChrospher 100 RP-18 column (250 × 4 mm id, 5 μm) (Merck, Darmstadt, Germany). The column temperature was maintained at 37°C. Aqueous 2% acetic acid (v/v, solvent A) and methanol (solvent B) served as the mobile phase in a gradient mode (B from 5 to 55% in 15 min, hold at 55% for 10 min, from 55 to 100% in 3 min) at a flow rate of 0.8 mL/min. Detection was at 280 nm. In addition, UV spectra were recorded in the range of 200–400 nm. For control of the HPLC system and data processing, the Chromeleon software version 6.40 (Dionex) was used.

2.6 MS analysis

After extraction, the samples of the fermentation experiments with irilone were analyzed for microbial metabolites by LC-ESI(+)-MS and GC-electron impact (EI)-MS. LC-MS analyses were performed on an LC system (Shimadzu, Langenfeld, Germany) consisting of two LC pumps (LC-10ADVP), a degasser (DGU-14A), an autosampler (SIL-HT-A), a column oven (CTO-10AVP), and an UV-detector (SPD-10AVVP) operating at 260 nm connected to an API 2000 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany) equipped with a turbo ionspray (pneumatically assisted ESI) source. For data collection and handling, the Analyst software version 1.4.1 (Applied Biosystems) was used. LC separation was carried out on a Supelco Discovery RP-18 column (150 × 2.1 mm id, 5 μm) (Sigma-Aldrich, Munich, Germany) kept at 40°C. Aqueous 0.1% formic acid (v/v, solvent A) and ACN (solvent B) served as the mobile phase in a gradient mode (B from 20 to 30% in 20 min, from 30 to 45% in 10 min, from 45 to 60% in 20 min, from 60 to 90% in 5 min) at a flow rate of 0.3 mL/min. The analytes were ionized in the interface with an ion spray voltage of 5000 V using 30-psi nebulizer gas and 40-psi drying gas with a temperature of 450°C. The analytes were detected in the positive ion mode using MS in MS/MS mode. The potentials were optimized under LC conditions for irilone, resulting in the following settings: declustering potential of 76 V, entrance potential of 11.5 V, focusing potential of 370 V, collision energy of 40 V, and collision exit potential of 6 V. MS/MS spectra were recorded between 100 u and the parent ion mass with a scan time of 2 s. For full-scan spectra (100–650 u), identical source parameters were used.

GC-MS analyses were carried out on a Thermo Finnigan system (gas chromatograph model Focus hyphenated to a Polaris Q ion trap mass detector from Thermo Electron Corporation, Bremen, Germany). Standard compounds and irilone metabolites were derivatized with Silyl 991 for 30 min at 65°C. The trimethylsilyl (TMS)-derivatives were analyzed using a non-polar capillary column (Zebron ZB-5, 30 m × 0.25 mm id, 0.25 μM film thickness) (Phenomenex, Aschaffenburg, Germany) and a temperature gradient (150°C for 1 min, from 150 to 230°C at 25°C/min, hold for 3 min, from 230 to 258°C at 10°C/min, from 258 to 265°C at 0.3°C/min, hold for 5 min, from 265 to 320°C at 10°C/min, hold for 2 min). The injector port temperature was 250°C; injections were made in the splitless mode. Mass spectra were obtained by EI ionization at 70 eV and an ion source temperature of 200°C. Full scan spectra (mass range 50–650 u) were recorded.

3 Results

3.1 Fermentation experiments with irilone and human fecal slurries (FSs)

To test human intestinal bacteria for their ability to transform irilone, FSs from ten human subjects were spiked with irilone and incubated under anoxic conditions. Samples collected during fermentation for 96 h were extracted and analyzed by LC-DAD/MS for irilone and its metabolites. To identify compounds formed by intestinal bacteria, samples withdrawn from control incubations without irilone or fecal material were also analyzed. Under the conditions used, irilone was largely stable for 96 h. This applied to all FSs tested. Compared to the incubation of irilone in the absence of intestinal bacteria, no significant decrease in the irilone concentration was observed in their presence (data not shown). However, all collected samples were analyzed in more detail for metabolites formed in minor amounts. Following the incubation of irilone with one of the FSs (FS 1) for more than 4 h, a small peak (t_R = 23.4 min) in addition to irilone (t_R = 27.6 min) was observed (Fig. 1), which was not present in the corresponding control samples. The concentration of this metabolite reached its maximum after 8 h of incubation and remained largely stable until the end of incubation (96 h). This metabolite was identified as dihydroirilone (DHI) as described in

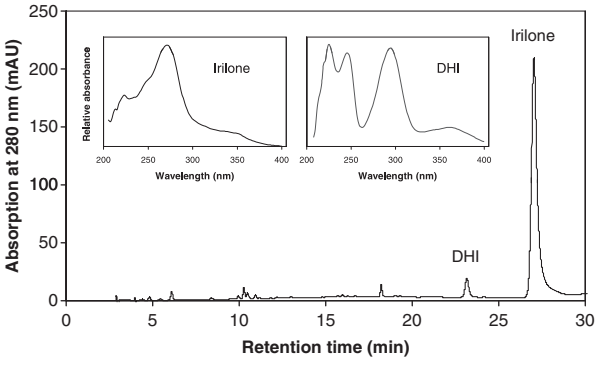

Figure 1. HPLC elution profile of a sample taken after 8 h of irilone incubation with a human fecal slurry (FS 1) exhibiting the peaks of irilone and dihydroirilone (DHI). The inserts show the UV spectra of irilone (λ_{max} 271 nm) and DHI (λ_{max} 225, 246, 294 nm).

Section 3.3. Triggered by the LC-MS data, samples withdrawn from irilone-spiked FSs other than FS 1 were analyzed by SIM. The molecular peak corresponding to DHI, m/z 301 $[M+H]^+$, was observed with two other FSs (FS 4, FS 6), after 4 to 8 h of incubation with irilone. Compared to FS 1, the DHI amounts formed by these FSs were even lower. Once formed, DHI concentrations hardly changed during further incubation. Since a standard substance was not available, DHI could not be quantified. However, the results demonstrate that DHI was formed at least in trace amounts by the fecal microbiota of three out of the ten human donors tested.

3.2 Fermentation experiments with genistein and human FSs

In parallel to irilone, the structurally similar isoflavone genistein was incubated with aliquots of the same FSs of the ten human subjects that were used in the irilone fermentation experiments. Incubation, sampling, sample preparation, and HPLC-DAD analysis were done in the same way as described for irilone. Genistein metabolites were formed within 96 h of incubation by all but one of the FSs. However, the extent of genistein conversion revealed large differences (Table 1). The complete conversion of genistein was observed with three out of ten FSs within 4 to 48 h of incubation (FS 1, FS 2, FS 3). Not only the extent of conversion but also the pattern of metabolites formed from genistein varied considerably between the FSs (Table 1). Genistein was finally transformed to HPP by two of the FSs (FS 1, FS 2) within 24 h of incubation. The decarboxylation product of HPP, 4-ethylphenol, was not observed. DHG and OH-DMA were transiently formed at mean maximal concentrations of 9 and 17% of the initial molar genistein concentration, respectively. Figure 2 illustrates the time course of genistein degradation by the FS that exhibited the highest conversion rate (FS 1) in comparison with the incubation of irilone with FS 1. The conversion of genistein by FS 3 resulted in formation of DHG only. FS 4 to FS 10 metabolized genistein only incompletely. While FS 4 and FS 5 formed only DHG and FS 9 only OH-DMA, FS 6, FS 7, and FS 8 produced both metabolites from genistein. Although genistein decreased following its incubation with FS 10, no metabolites were detected. Based on the metabolites formed (Table 1), four different types of genistein conversion by human fecal microbiota may be distinguished (Fig. 3). Interestingly, the sole formation of DHG was only observed with FSs of male donors (FS 3, FS 4, FS 5) (Table 1). However, since the number of subjects included in the study was small, conclusions regarding sex-dependent differences are at best preliminary.

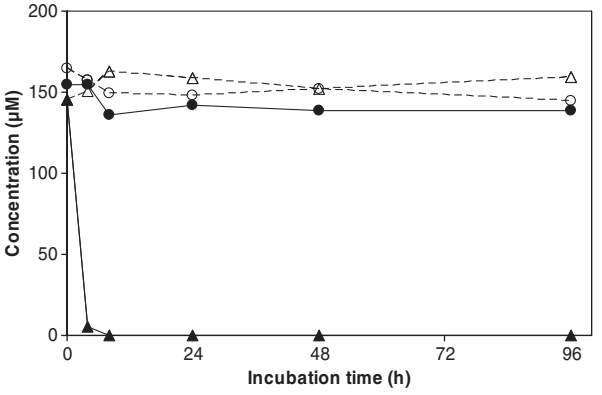

Figure 2. Time course of incubation of irilone (●) and genistein (▲), respectively, with a selected human fecal slurry (FS 1) compared with incubation of irilone (○) and genistein (△) without microbiota.

Table 1. Recovery of genistein after 24 h of incubation with human FSs and maximal amounts of metabolites formed from genistein within 96 h of incubation.

FS	Sex of donor	Genistein recovered after 24 h (%)	Maximal amount of metabolites (%) [at time (h)]		
			DHG	OH-DMA	HPP
1	f	0	12 [4]	23 [4]	69 [24]
2	f	0	7 [8]	12 [8]	65 [24]
3	m	16	119 [48]	ND[a)]	ND
4	m	85	4 [48]	ND	ND
5	m	100	3 [48]	ND	ND
6	f	76	16 [48]	4 [24]	ND
7	f	100	9 [24]	4 [24]	ND
8	f	97	3 [8]	1 [8]	ND
9	f	63	ND	30 [48]	ND
10	f	86	ND	ND	ND
Control[b)]	–	94	ND	ND	ND

a) Not detected.
b) Incubation of genistein without FS.

3.3 Identification of DHI

The metabolite formed from irilone by human fecal microbiota was further characterized using LC-MS and GC-MS techniques. For comparison, MS analyses of irilone were also carried out. The LC-MS scan of the metabolite revealed a base peak at m/z 301 $[M+H]^+$, indicating the formation of DHI from irilone ($[M+H]^+$, m/z 299). Comparing the ESI(+)-MS/MS spectra of DHI (Fig. 4A) and irilone (Fig. 4B), the difference of two mass units observed for their molecular peaks (m/z 301 *versus* 299) is also evident from several fragments formed thereof (m/z 271 *versus* 269, 243 *versus* 241, 149 *versus* 147, 121 *versus* 119). Signals at m/z 181, 123 and 107 are present in both spectra indicating that the fragmentation of irilone and DHI led to identical products. The main fragmentation pathways of DHI and irilone are shown in Fig. 5A and B, respectively. The fragment ions at m/z 181 and 121 for DHI and at m/z 181 and 119 for irilone are most likely formed by retro Diels-Alder (rDA) reactions, which have been described for isoflavones and for isoflavanones [30–32]. Since the A-ring in the irilone and DHI molecule has an identical structure, the formation of m/z 181 from both compounds is not unexpected. The fragment ions derived from the B- and C-rings, were characteristic of DHI (m/z 121) and irilone (m/z 119), respectively. The high abundance of m/z 107

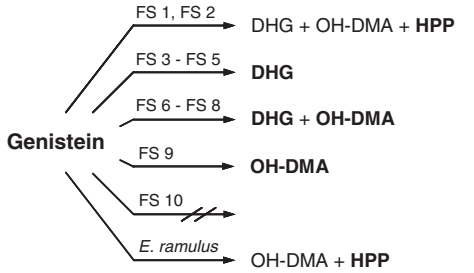

Figure 3. Pathways of genistein conversion by human fecal slurries and *Eubacterium ramulus*. The final products of conversion are highlighted in bold. The structures of compounds are given in Fig. 7.

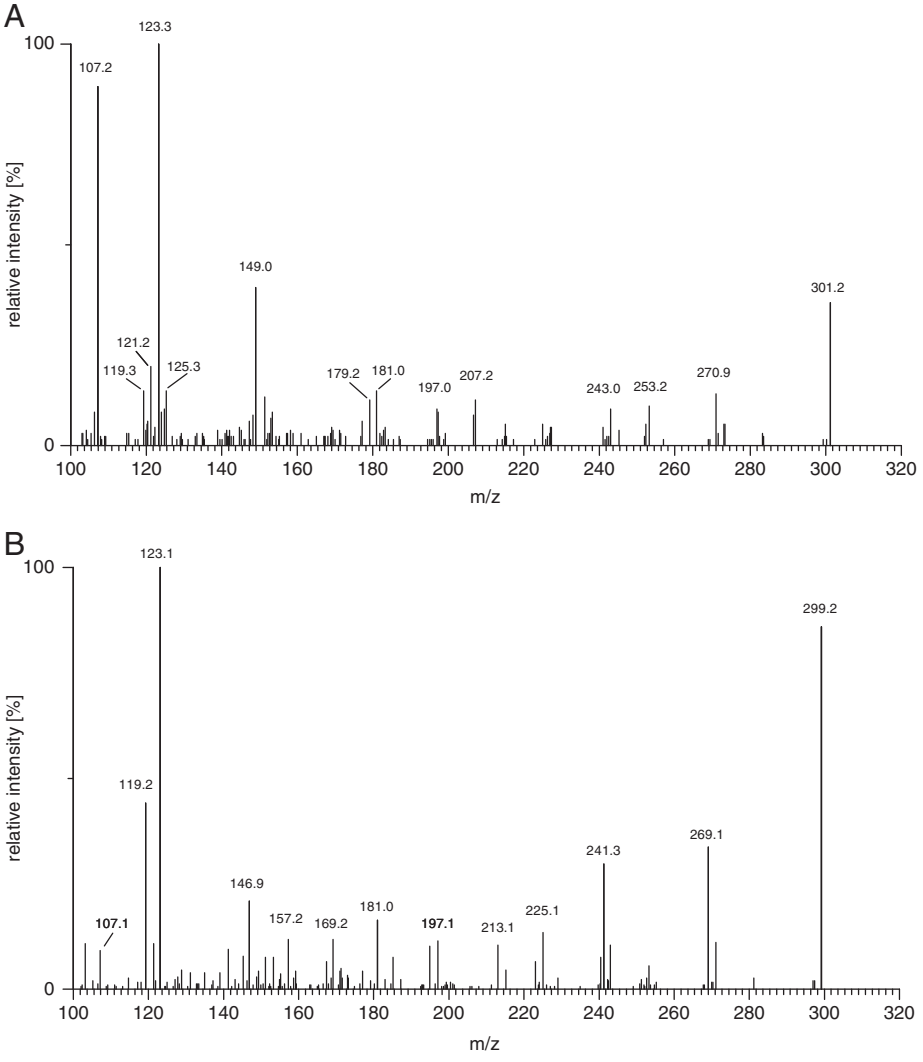

Figure 4. LC-ESI(+) mass spectra obtained in MS/MS mode on the molecular ion of dihydroirilone m/z 301 $[M+H]^+$ (A) and irilone m/z 299 $[M+H]^+$ (B).

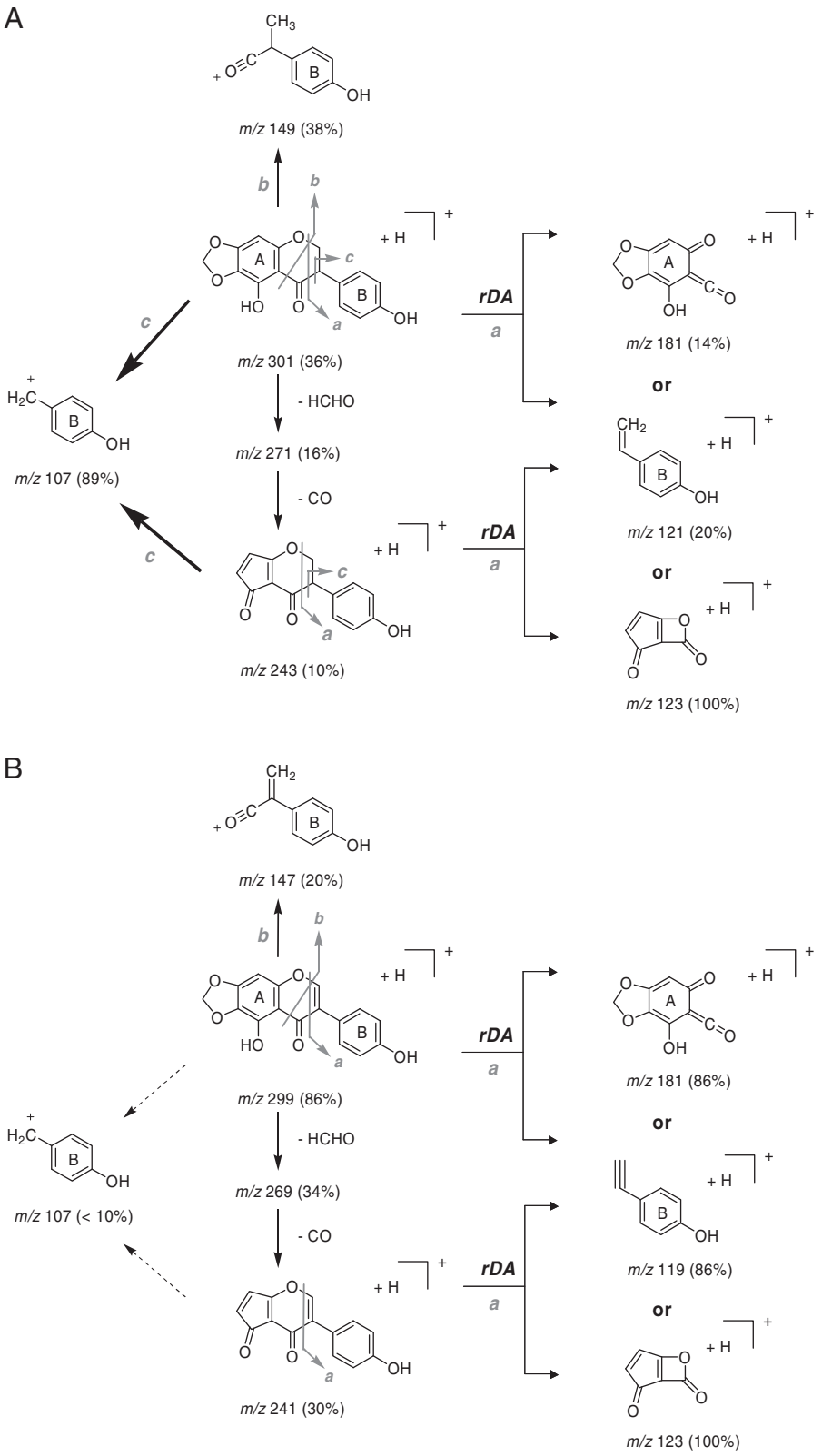

Figure 5. Main fragmentation pathways of dihydroirilone (A) and irilone (B) in LC-ESI(+)-MS.

observed for the microbial irilone metabolite (Fig. 4A) lends additional support to its identity as DHI (Fig. 5A, fragmentation pathways c). In the case of isoflavones, this fragmentation accounts for less than 10% of the relative intensity in the mass spectrum. However, after hydrogenation of the isoflavone molecule, this cleavage is facilitated and the 4-methylphenol cation constitutes a major fragment (relative intensity > 60%) [33, 34].

After TMS-derivatization of samples containing the assumed irilone metabolite DHI, the GC-EI-MS analysis revealed two metabolite peaks. The GC-MS spectrum of the higher peak showed a base peak at *m/z* 252 and an intense signal at *m/z* 429 (relative intensity, 93%) (Fig. 6A). The latter signal $[M\text{-}15]^+$ may be attributed to the elimination of a methyl group from the TMS-ether of DHI in position C-5. The base peak (*m/z* 252) corresponds to the rDA fragment of the A-ring and the signal at *m/z* 192 (relative intensity, 10%) to the respective rDA fragment resulting from the B-ring. The rDA fragmentation has been known to predominantly occur during GC-MS analysis of isoflavanones, as demonstrated for dihydrodaidzein and DHG [12]. The intact molecular ion of the TMS-ether of DHI was nearly absent. This was also true for the precursor irilone and has generally been observed for isoflavones with a hydroxy group in position C-5 [35, 36]. The second metabolite peak observed in the GC chromatogram could be assigned to the TMS-ether of the enolic tautomer of DHI. Its mass spectrum had a base peak at *m/z* 516 and an intense peak at *m/z* 325 (relative intensity, 96%) representing the rDA fragment of the A-ring (Fig. 6B). The corresponding fragment peak of the B-ring (*m/z* 191) was of low intensity. The other fragments probably originated from unspecific neutral losses, *e.g.* *m/z* 501 formed by elimination of a methyl group from the TMS-ether (Fig. 6B). The presence of two GC peaks after trimethylsilylation of isoflavanones due to keto-enol tautomerism has been described previously [11, 12]. The derivatization stabilizes both, the keto form as well as the enol form and the two derivatives may be chromatographically separated. Overall, the GC-EI-MS results confirmed the identity of the microbial irilone metabolite as DHI.

3.4 Fermentation experiments with *E. ramulus*

The flavonoid-degrading human intestinal *E. ramulus* was tested for its ability to convert irilone. As a positive control, genistein was incubated with *E. ramulus* under identical conditions. Whereas genistein was rapidly degraded within 4 h of incubation, the irilone concentration remained unchanged during the incubation period of 96 h. Irilone-derived metabolites including DHI were not detected by HPLC-DAD analyses. Genistein was converted by *E. ramulus* to equimolar amounts of HPP (97% of the initial genistein concentration). The intermediate OH-DMA (7%) was transiently formed after 4 h of incubation. DHG was not detected in the course of genistein conversion by *E. ramulus*.

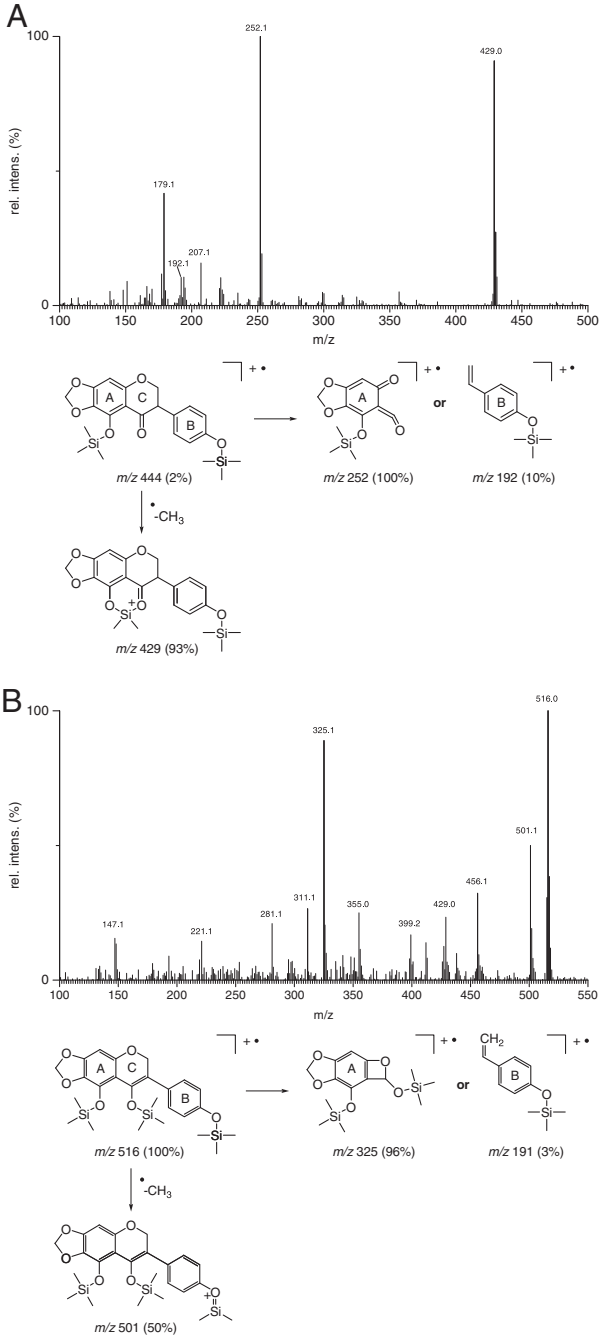

Figure 6. GC-EI mass spectra and interpretation of the main fragments of dihydroirilone in the keto form (A) and the enol form (B).

4 Discussion

Using human fecal suspensions, we investigated whether the human intestinal microbiota is capable of converting the isoflavone irilone. Irilone was hardly converted when incubated with FSs of ten human subjects of both sexes and varying age. Only one unknown metabolite was

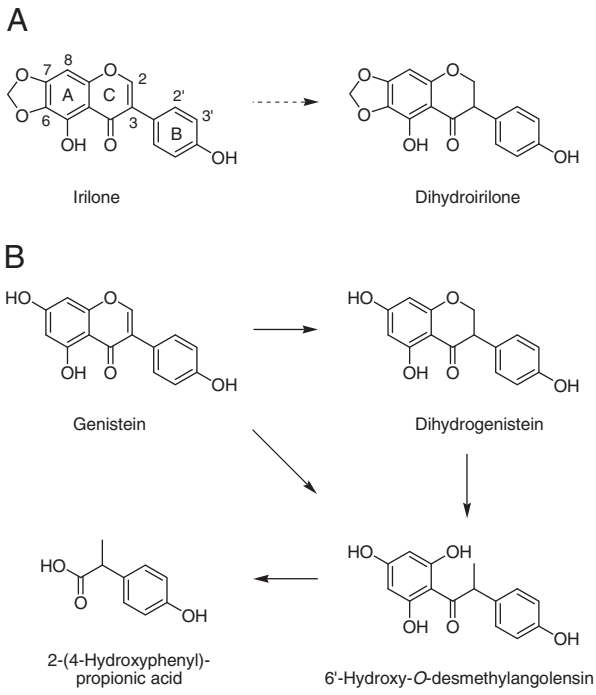

Figure 7. Conversion of irilone by the human intestinal microbiota (A) compared to microbial genistein transformation (B).

detected in small to minute concentrations in three of the ten FSs tested. Identification of the metabolite as DHI was accomplished by interpretation of mass spectra, since an authentic reference compound was not available. The hydrogenation of the C2-C3 double bond in the C-ring of irilone yielding DHI is analogous to the conversion of other (iso)flavonoids such as genistein whose conversion by human gut bacteria leads to the formation of DHG (Fig. 7) [15, 17, 24].

In accordance with previous studies [15, 17], human fecal suspensions converted genistein to DHG and HPP. In addition, we detected OH-DMA (Fig. 7). No decarboxylation of HPP to 4-ethylphenol was observed. After administration of genistein to humans, 4-ethylphenol has been detected in plasma and urine and proposed to be a microbial metabolite [37]. Most likely, the formation of 4-ethylphenol from HPP is only catalyzed by host enzymes.

In the study presented here, genistein degradation and metabolite formation were monitored over time. We observed considerable differences in the extent of genistein conversion and the pattern of metabolites between the microbiotas from different individuals. All but one of the fecal microbiotas tested formed at least one metabolite from genistein. Only two out of ten FSs completely transformed genistein *via* DHG and OH-DMA to HPP. Based on the pattern of metabolites formed, we observed four different types of genistein conversion as catalyzed by human intestinal microbiota (Fig. 3). Previous *in vitro* and *in vivo* studies already indicated an interindividual variability, which is most likely due to differences in microbiota composition. However, the studies including fecal microbiotas of several human subjects only monitored the degradation of genistein but did not analyze the products formed [19–22]. Only one study identified DHG as a product with the microbiota of one human donor [15], while the ensuing cleavage to HPP was observed for the microbiotas of two human donors in another study [17]. Interindividual differences in the quantities of microbial metabolites formed from genistein in blood and/or urine samples were also observed in *in vivo* studies [12, 38, 39]. Interindividual differences in genistein transformation indicate that the bacteria involved in this process are not common inhabitants of the human gut. Studies with pure cultures of human gut bacteria show that different species catalyze specific reaction steps. *E. ramulus*, which we included in this study (Fig. 3) has previously been shown to convert genistein to HPP *via* OH-DMA [25, 26]. Accordingly, DHG was not observed in the course of genistein conversion by *E. ramulus* in the present study. DHG is not used as a substrate by *E. ramulus* either [25]. DHG probably is the final product of genistein transformation by certain bacterial species such as *Clostridium* sp. HGH6 [24] and may be further transformed by other species of the complex microbiota as shown for dihydrodaidzein [40].

In contrast to genistein, irilone was hardly transformed by human intestinal microbiota. Similarly, *E. ramulus* rapidly converted genistein but not irilone. The 6,7-methylenedioxy bridge by which irilone differs from genistein did not undergo cleavage by gut bacteria and most likely prevents cleavage of the isoflavone basic structure by intestinal bacteria. So far, the dioxygenolytic cleavage has been described for aerobic bacteria only. For example, *Pseudomonas fluorescens* and *P. putida* are capable of converting 3,4-methylenedioxybenzoate [41, 42]. The latter species employs the 4-methoxybenzoate monooxygenase (*O*-demethylating, EC 1.14.99.15) for catalyzing this reaction [42]. In humans, the methylenedioxy group of a number of bioactive plant compounds or drugs such as safrol, myristicin, and stiripentol is cleaved [43–45]. Probably, this cleavage is catalyzed exclusively by human enzymes, which might also act on irilone, but not by the gut microbiota.

In summary, this is the first study on the metabolism of irilone by human intestinal microbiota. As a result, irilone was found to be largely resistant towards microbial degradation. The extent and pathway of genistein conversion depended on the human donor indicating that the metabolization of isoflavones such as genistein is largely influenced by the individual microbiota composition. Therefore, the potential health benefits ascribed to the parent compounds may differ between individuals. However, the bioavailability of irilone seems to be hardly affected by microbial activity. Thus, the intact irilone may be absorbed or display local effects in the human intestine.

We are indebted to Frank Lehmann and Michael Gütschow (Pharmaceutical Institute, University of Bonn, Germany) for synthesis of DHG and OH-DMA. We thank Sabine Zimmermann and Sabine Schmidt for technical assistance.

S. E. K. had the initial idea to investigate irilone; A. B. and M. B. designed the genistein experiments; A. B. performed the fermentation experiments and HPLC-DAD analysis; R. M. carried out the GC-MS analysis and contributed to the paper; R. M. and N. H. S. performed the LC-MS analysis; A. B., S. E. K. and M. B. wrote the paper; and all authors edited the paper.

The authors have declared no conflict of interest.

5 References

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