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Ex Vivo Absorption of Thymol and Thymol-β-D-glucopyranoside in Piglet Everted Jejunal Segments

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Supporting Information

ABSTRACT: Food-producing animals are reservoirs of *Campylobacter*, a leading bacterial cause of human foodborne illness. The natural product thymol can reduce the survivability of *Campylobacter*, but its rapid absorption in the proximal gastrointestinal tract may preclude its use as a feed additive to reduce intestinal colonization of these pathogens. This work examined the *ex vivo* absorption of thymol and thymol-β-D-glucopyranoside in everted porcine jejunal segments, as the latter was hypothesized to be more resistant to absorption. A modified gas chromatography and extraction method was developed to determine 1.0−500 mg/L thymol. From 1 and 3 mM solutions, 0.293 ± 0.04 and 0.898 ± 0.212 mM thymol, respectively, p = 0.0347, were absorbed, and 0.125 ± 0.041 and 0.317 ± 0.143 mM thymol-β-D-glucopyranoside, respectively, p = 0.0892, were absorbed. Results indicate that thymol-β-D-glucopyranoside was absorbed 2.3 to 2.8 times less effectively than thymol, thus providing evidence that thymol-β-D-glucopyranoside may potentially be used as a feed additive to transport thymol to the piglet lower gut.

KEYWORDS: *gas chromatography, piglet everted small intestine, thymol, thymol-β-D-glucopyranoside*

INTRODUCTION

About 60 years ago the growth-promoting effect of subtherapeutic antibiotic use was discovered.[1,2] Growth-promoting agents used in intensive animal agriculture improve feed conversion and animal growth by 2−5% and 4−8%, respectively.[3,4] However, the mechanisms of growth promotion are still unknown.[5] There is concern, however, that growth-promoting use of antibiotics may lead to the development of resistant bacteria capable of rendering antibiotic treatment of human or animal infections ineffective.[6,7] The development of resistant bacteria was clearly demonstrated in Denmark in the case of the *Salmonella enterica* serovar Typhimurium DT104 outbreak in the 1990s.[8] Therefore, the use of antibiotics as growth promoters in the European Union (EU) countries has been curtailed by legislation.[9] Also, there may be a higher potential of transferring resistance to other strains of bacteria using antibiotic growth promoters.

Antibiotics provided in small amounts halt the growth of bacteria, prevent outbreaks of diseases, and enhance the rate of animal growth in intensively reared animals. It would be advantageous to find alternatives to antibiotic growth promoters. Candidates used to replace antibiotic growth promoters must be evaluated for their ability to kill bacteria, assessed for successful delivery to the animal gut, and evaluated for their ability to enhance feed efficiency. Aromatic plant extracts and plant-based products offer an opportunity in this regard, as many plants produce secondary metabolites that have antimicrobial or antiparasitic properties. These active components, generally recognized as being safe for human and animal consumption in the USA, have prompted scientists to examine their potential to improve production efficiency and health in livestock.[10]

Previous *in vitro* fermentations of the pig gut demonstrated that thymol (**1**) (Figure 1) at 1.717 mM, carvacrol at 1.698 mM, eugenol at 1.358 mM, or *trans*-cinnamaldehyde at 0.424 mM reduced the number of total anaerobic bacteria compared to the control with a probability of 99.7%.[11] Michiels et al.[12] found that 500 mM **1**, carvacrol, or *trans*-cinnamaldehyde in *in*

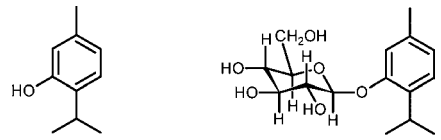

Figure 1. Chemical structures of thymol (**1**) and thymol-β-D-glucopyranoside (**2**).

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vitro incubations were very effective against coliform, lactobacilli, and streptococci bacteria. In *in vitro* cecal incubations, carvacrol, cinnamon oil, eugenol, and **1** showed a high efficacy against *Escherichia coli* K88 with minimum bactericidal concentrations (MBCs) of 100, 133, 300, and 100 μg/mL, respectively; against *E. coli* O157:H7 with MBCs of 283, 133, 466, and 166 μg/mL, respectively; and against Typhimurium DT104 with MBCs of 167, 100, 400, and 233 μg/mL, respectively, while showing little inhibition toward pig endogenous lactobacilli and bifidobacteria.[13]

It was demonstrated *in vitro* that 0.25 mM **1** in Mueller Hinton broth culture reduced the growth rate of *Campylobacter jejuni* and *Campylobacter coli* by 2-fold and 1.16-fold, respectively, and that 1.0 mM **1** reduced viable *Campylobacter* counts >5 $\log_{10}$ colony forming units when incubated in *in vitro* pig fecal suspensions.[14] Food-producing animals can be reservoirs for *Campylobacter*, a leading bacterial cause of human foodborne illness. *Campylobacter* differs from other gut bacteria in that they have a limited capacity to ferment carbohydrates but can utilize amino acids as a major energy source, a process that can be inhibited by **1**.[15] In order to evaluate palatability issues pertaining to feeding **1**, growing pigs were provided *ad libitum* access to standard growing diets supplemented with or without 0.0067% or 0.0201% **1** on a dry weight basis.[15] These doses were fed with the intent that if no adsorption or gastrointestinal degradation occurred, they would deliver sufficient **1** to achieve approximately 1× or 3× the 1.0 mM **1** considered to be an efficacious concentration in the lumen of the pig gut.[14] No effect of the treatment was observed on feed intake, on ileal or cecal *Campylobacter*, on cecal total culturable anaerobes, or on accumulation of the major fermentation end products within the collected gut contents. These findings suggested that appreciable quantities of **1** were absorbed or degraded in the proximal alimentary tract and that some type of encapsulation technology will be required to deliver effective concentrations of this compound to the lower gut to achieve *in vivo* efficacy against *Campylobacter*.[15]

Other than the work of Michiels et al.,[11] who studied degradation of **1** in piglet intestines, little data is available on absorption of **1** in the pig gut when **1** was administered orally. Information on the absorption and delivery technology of **1** is necessary for application and dosage of **1** in pig feeds and also with regard to potential resistance or tolerance development of gut bacteria to **1**.

This work focused on *ex vivo* absorption of **1** and its glucoside, thymol-β-D-glucopyranoside (**2**) (Figure 1), in the piglet small intestine. We hypothesized that **2** may be used to deliver **1** to the lower gut, where it would be released as a result of natural hydrolysis by β-glucosidases found in endogenous bacteria. Modified gas chromatography (GC) and extraction methods were developed for the determination of **1**.

MATERIALS AND METHODS

Chemicals and Materials. β-Glucosidase, isolated from sweet almonds, was obtained from MP Biomedicals Inc. (Santa Ana, CA, USA). Calcium chloride hexahydrate ($CaCl_2 \cdot 6H_2O$) was obtained from Fluka Analytical (Sigma-Aldrich, MO, USA). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) was obtained from Mallinckrodt (Paris, KY, USA). Sodium bicarbonate ($NaHCO_3$) was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). Sodium chloride (NaCl) was obtained from ACROS Organics (Fair Lawn, NJ, USA). Absolute ethanol, ethyl acetate, glucose, potassium chloride (KCl), sodium acetate, sodium dihydrogen phosphate (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), and **1** were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). T-61 was obtained from Intervet Canada (Whitby, ON, Canada). The glucoside **2** was obtained from Christof Senn Laboratories (Dielsdorf, Switzerland).

External Calibration Standard Preparation. The stock standard solution of **1** (1000 mg/L) was prepared by dissolving **1** in absolute ethanol and then storing at 8 °C. Working standard solutions were prepared daily by diluting the stock solution with ethanol. A concentration range of 1.0−500 mg/L **1** using nine calibration points was used for the GC external calibration curve. The constructed calibration curve had a correlation coefficient of 0.999 94.

GC Method. The gas chromatography methods of Michiels et al.[11] and Nozal et al.[16] were modified and carried out on a HP 6890 GC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary split/splitless inlet, total electronic pneumatic control of gas flow, autosampler, and flame ionization detector (FID). A 30 m × 0.25 mm × 0.25 μm film thickness Equity-1701 fused silica capillary column (Supelco, Bellefonte, PA, USA) was utilized for the GC separation of **1**. Hydrogen was used as the carrier gas at 1 mL/min. The mode used was split, with a split ratio of 5:1. Initial oven temperature was set at 130 °C and held for 1 min. A double temperature ramp program was used at the rate of 6 °C/min to 180 °C and then increased at 40 °C/min to 230 °C with a hold time of 4 min, resulting in a total run time of 14.58 min. Finally, the oven was brought back to 130 °C with a hold of 1 min. The injector and FID temperatures were 250 and 280 °C, respectively. Helium was used as makeup gas for the FID. The retention time of **1** was 5.8 min. Each extracted sample was analyzed four times by the GC method. All measured samples positive for **1** were within the limits of the external calibration curve. Chromatographic data were collected and analyzed using the Agilent ChemStation software (Rev. B.03.02 [341]).

GC−MS Method. Gas chromatography−mass spectrometry (GC−MS) was used to compare authentic **1** with the extracts of the internal contents of everted jejunal intestinal segments (EJSs) following absorption of **1** and **2**. GC−MS was carried out using a Finnigan Trace GC Ultra (Thermo Scientific, Waltham, MA, USA) interfaced to a Finnigan DSG Quadrapole mass spectrometer (Thermo Scientific) through a transfer line held at 200 °C. The separation of **1** was carried out on a 250 mm × 0.32 mm, 0.25 μm BP1 column (SGE Inc., Austin, TX, USA) with a constant flow of He gas at 1 mL/min. The GC oven temperature program was the following: 130 °C, held for 1 min, raised to 180 °C at a rate of 6 °C/min and then to 230 °C at a rate of 40 °C/min, and held for 1.0 min, giving a 11.58 min run time. The GC sample injection was in the split mode with a split ratio of 1:10 at a split flow rate of 10 mL/min and injector port temperature of 250 °C. The MS was conducted in the EI^+ mode at 70 eV with an ion source temperature of 190 °C and a mass range of m/z 40−175 at a scan rate of 500 Da/s.

Animals and Procedures. Two 7- to 8-week-old female piglets used in these trials were obtained from the Texas A&M University farm at an approximate weight of 30 kg. The piglets were fed a standard diet that satisfied their nutritional requirements.[17] All procedures were carried out in accordance with procedures approved by the Southern Plains Agricultural Research Center's Institutional Animal Care and Use Committee, Experimental Animal Protocol #2011004.

Everted Jejunal Intestinal Segment Preparation. Everted jejunal intestinal segments were prepared from piglet small intestines. The *ex vivo* procedure was carried out with piglet small intestines similarly to that described by Carstens and Kwang[18] using rat intestines. The jejunal intestinal segments were everted in order to expose the mucosal surface. The piglets were anaesthetized by intravenous injection of thiopental sodium (20 mg/kg body weight) and subsequently euthanized using 3 mL of T-61. The abdomen was immediately opened, and the proximal part of the jejunum 5 cm posterior of the duodenum was excised. The total length of the jejunum used was about 1 m. The EJSs were dissected into small segments each approximately 4 cm long and closed on one end by ligation with surgical silk No. 2/0, filled with approximately 5 mL of Beier's modified Tyrode's solution, pH 5.8, and then closed on the other end by ligation and used immediately. The pH was adjusted to

5.8 in order to simulate the physiological pH of the jejunum. The everted jejunal segments were immediately placed in cold Beier's modified Tyrode's solution. The composition of Beier's modified Tyrode's solution was the following: 137 mM NaCl, 2.7 mM KCl, 8.33 mM NaH_2PO_4, 1.8 mM $CaCl_2{\cdot}6H_2O$, 5.5 mM D-glucose, and 1 mM $MgCl_2{\cdot}6H_2O$, pH 6.5. Beier's modified Tyrode's solution was used because Tyrode's solution, of composition 137 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.2 mM Na_2HPO_4, 5.5 mM D-glucose, 1.8 mM $CaCl_2{\cdot}6H_2O$, and 1 mM $MgCl_2{\cdot}6H_2O$ at pH 6.5, required 6 to 7 days to adjust the pH to the desired pH of 5.8.

Incubation Procedure. Piglet EJSs were placed into 100 mL flasks containing 80 mL of Beier's modified Tyrode's solution at pH 5.8, each flask contained one EJS, and there were three segments used for incubation at each chemical concentration carried out with 1.0 or 3.0 mM **1** or with 1.0 or 3.0 mM **2**. Aliquots of **1** and **2** were added from stock solutions (50 mg/mL) prepared in absolute ethanol to the 80 mL of Beier's modified Tyrode's incubation solutions resulting in 1.0 and 3.0 mM solutions. Amounts of ethanol added with the solution of **1** were less than 2% (v/v) of the suspension media, which would be expected to have minimal if any effect on absorption rates of **1** or **2**. Control incubations with three separate EJSs were carried out similarly to those with the noncontrols but without addition of either **1** or **2**. The flasks were incubated for 3 h at 37 °C in an incubator with a modified atmosphere (95% oxygen, 5% CO_2), while simultaneously being rotated on an oscillating plate at 40 rpm.

Sample Extraction and Preparation for GC Analyses. Immediately prior to incubations, samples of all suspension fluids were collected. Following incubation, samples of the suspension media and the EJS internal fluid (EJS fluid) were collected and prepared for determination of **1** and **2**. The extraction method used to extract **1** from samples was a modification of the method used by Michiels et al.[11] Briefly, extractions were carried out in 2.0 mL of polypropylene microcentrifuge tubes. Absolute ethanol (15 μL) and ethyl acetate (1 mL) were added to 0.25 mL of each sample. The mixtures were vortexed for 30 s and then left to equilibrate for 1 h at 8 °C. A recovery experiment was conducted to evaluate the time required for vortexing, 30 or 60 s, and if the samples could be left for 1 h at 8 °C or overnight at 8 °C for equilibration following the vortexing step. The extraction mixtures were then centrifuged at 8100*g* for 5 min at 25 °C. The supernatant was removed and placed in sealed vials for GC analyses.

Samples containing **2** were extracted for free **1** and also treated with β-glucosidase prior to extraction to determine the amount of released **1**. The difference in these two values resulted in the amount of conjugated **1**. The β-glucosidase enzyme was dissolved in 0.1 M sodium acetate, pH 5.0, resulting in a final concentration of 2.5 mg/mL β-glucosidase (2500 IU/mL), and 100 μL (250 IU) of this β-glucosidase solution was added to a 0.25 mL sample containing **2**. The mixture was vortexed for 30 s and incubated at 37 °C for 2 h. The incubated mixtures were cooled at 8 °C for 20 min, and then **1** was extracted using the same protocol as discussed above.

Calculations and Statistics. The values of **1** obtained from GC analysis were multiplied by 4 to adjust for the 0.25 mL sample size. Statistical analyses of intergroup differences of means were performed by a two-sample *t* test using Statistix9 analytical software (Tallahassee, FL, USA). Data were expressed as means ± standard deviation. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Our modified GC method worked well over the nine-point calibration curve from 1.0 to 500.0 mg/L **1**, resulting in a correlation coefficient of 0.999 94. All measured samples positive for **1** were within the limits of the external calibration curve.

Figure 2 shows recovery results from various amounts of **1** spiked into pH 5.8 Tyrode's solution and then using different extraction protocols. The extraction method of Michiels et al.[11] utilized ethyl ethanolate to extract **1** from 1 mL samples by vortexing for 30 s followed by a 30 min agitation on an orbital shaker, and then the samples were allowed to equilibrate for 8–12 h, followed by centrifugation. Our modified method uses ethyl acetate to extract **1** from 0.25 mL samples by vortexing for 30 or 60 s and then used a 1 h or overnight (18 h) equilibration period at 8 °C, followed by centrifugation. Figure 2 shows the results from these extraction trials. The percentage of recovery was satisfactory for all samples, suggesting that the samples could be extracted and then allowed to equilibrate at 8 °C overnight. However, the samples that were allowed to equilibrate at 8 °C for only 1 h resulted in better consistency, as can be seen by the standard deviations of the data points (Figure 2). We hypothesize that since **1** is a phenolic compound and Tyrode's solution consists of various salts, there may have been degradation of **1** in some of the samples held for 18 h in the Tyrode's solution. If this happened, then one would expect high standard deviations. The typical extraction protocol that was used for the rest of the study consisted of the following: 0.25 mL samples were vortexed for 30 s after the addition of 1 mL of ethyl acetate and 15 μL of ethanol, followed by a 1 h equilibration period at 8 °C, and then centrifuged at 8100*g* for 5 min at 25 °C.

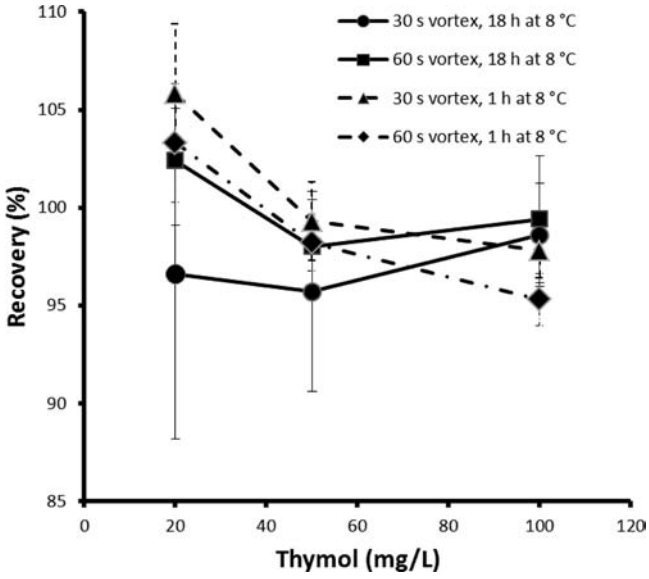

Figure 2. Thymol (**1**) recovery from samples of pH 5.8 Tyrode's solution using the described extraction method and modified GC method. The dashed lines show samples held for 1 h at 8 °C, and the solid lines show samples that were held for 18 h at 8 °C.

The absorption of **1** in the piglet EJSs relative to the concentration of **1** in the suspension fluid before incubation and after incubation of the EJSs is shown in Table 1. Also, **1** was not detected in the negative control suspension fluids or in the negative control EJS fluids containing no added **1** or **2**. This result was as expected, as the piglet had no prior exposure to **1**. Therefore, an effect of dietary treatment on the content of **1** was excluded.

For the EJSs incubated with 1.0 mM added **1** in the suspension fluid, the concentration of free **1** measured in the suspension fluid at the end of the incubation was significantly lower than at the beginning of incubation ($p = 0.0001$). The concentration of **1** in the EJS fluid was significantly lower than the concentration in the suspension fluid at both the beginning and the end of incubation ($p \leq 0.0002$), indicating that **1** was not freely diffusible across the jejunum intestinal wall. A similar pattern was observed when the incubation was conducted with

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Table 1. *Ex Vivo* Absorption of Thymol (1) in the Piglet Everted Jejunal Segment Fluids Following a 3 h Incubation

	suspension fluid (before incubation), mM[b]	EJS fluid[a] (after incubation), mM[b]	suspension fluid (after incubation), mM[b]
1.0 mM **1**[c]	1.035 ± 0.014[d]	0.293 ± 0.04[e]	0.673 ± 0.032
3.0 mM **1**[c]	3.069 ± 0.089[f]	0.898 ± 0.212[g]	2.01 ± 0.143

[a]EJS fluid = everted jejunal intestinal segment internal fluid. [b]Mean of 3 replicates ± standard deviation. [c]Evaluated in Beier's modified Tyrode's solution, pH 5.8 (n = 3). [d]This amount is 103.5 ± 1.4% of the expected amount of **1** present. [e]Average volume of EJS fluid for 1 mM **1** trials was 3.17 mL. [f]This amount is 102.3 ± 3.0% of the expected amount of **1** present. [g]Average volume of EJS fluid for 3 mM **1** trials was 4.1 mL.

3.0 mM added **1**; the concentration of **1** in the suspension fluid at the end of the trial was again significantly lower than at the beginning of the trial (p = 0.0004). It was previously reported that **1** biodegraded under aerobic conditions,[19] which may explain the observed difference in the content of **1** in both the 1.0 and 3.0 mM suspension fluids of **1** at the beginning and at the end of the incubations. However, Varel[20] found that **1** and carvacrol were both stable in swine waste under anaerobic conditions (nitrogen atmosphere) for 62 days, with 90–95% of **1** being recovered. Similarly, Broudiscou et al.[21] found no degradation of **1** over 24 h during *in vitro* caprine ruminal fermentation at a dose of 13.314 mM. Therefore, **1** released by enzymatic action in the pig gut would be expected to have a good lifetime without degradation.

In these studies, both of the investigated incubation concentrations of **1** (1.0 and 3.0 mM) demonstrated absorption of **1** in the piglet small intestine (Table 1, Figure 3). The 1.0 and 3.0 mM suspension fluids of **1** resulted in absorption of 0.293 ± 0.04 mM and 0.898 ± 0.212 mM **1**, respectively, in the EJSs fluid. Therefore, the EJSs absorbed 3.1 times as much **1**

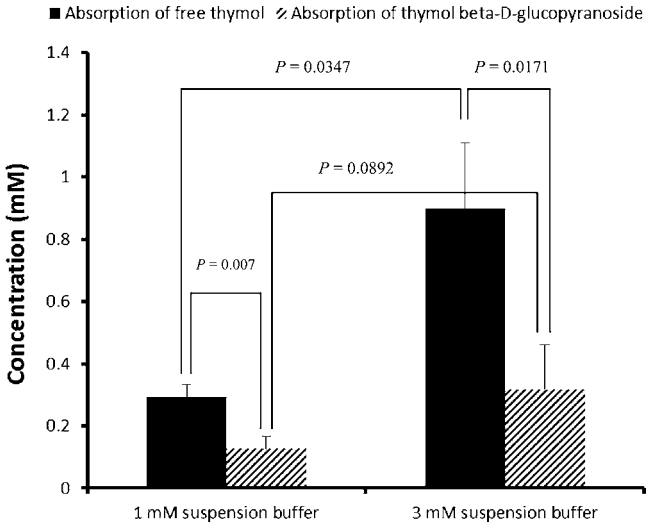

Figure 3. Absorption of free thymol (**1**) and thymol-β-D-glucopyranoside (**2**) in swine everted jejunal intestinal segments suspended in Beier's modified Tyrode's solution containing 1 and 3 mM **1** and **2**. Error bars indicate standard deviations (n = 3), and p values were generated via a two-sample t test.

when incubated in Beier's modified Tyrode's solution at pH 5.8 containing 3 mM **1** than when they were incubated in the solution containing 1 mM **1**. Michiels et al.[11] concluded that the absorption of all essential oils (including **1**) was fast and occurred primarily and nearly completely in the stomach and the proximal small intestine. Our findings showing that **1** was readily absorbed in the piglet intestine may explain the results obtained by Anderson et al.,[15] who observed no effect of **1** on *Campylobacter* in the cecum and lower gut of a pig fed diets supplemented with 0.0067% or 0.0201% **1** on a dry weight basis. In the study of Anderson et al.,[15] pigs in the respective treatment groups consumed on average 77 and 243 mg **1**/pig per d during the course of the 7 d feeding trial. On the basis of estimates of gut volume, these intakes would correspond approximately to 0.8 and 2.5 mM **1** within the gut lumen if no absorption or degradation had taken place. Thus, these concentrations are 0.8 and 2.5 times the 1 mM dose found to be effective in killing *Campylobacter* in pig fecal suspensions.[14] Therefore, the findings here have relevance with regard to the formulation of effective inclusion levels in feeds. The working concentrations of 1.0 and 3.0 mM **1** were chosen because the resulting EJSs absorbed **1** from the 1.0 and 3.0 mM solutions resulting in 0.293 and 0.898 mM **1**, respectively. The lower concentration of 0.293 mM is very close to the 0.25 mM **1** shown to be effective to reduce the growth rate of *C. jejuni* and *C. coli in vitro*.[14] With the results shown here that significant amounts of **1** are readily absorbed in the piglet intestine, other ways to deliver high concentrations of **1** to the small intestine should be investigated.

We then determined the absorption characteristics of a carrier of **1**, the glucoside **2**. The glucoside conjugate, **2**, could in principle be used to deliver **1** to the lower gut, where the presence of naturally occurring bacterial β-glucosidase could cleave the molecule of **1**, making free **1** available in the lower gut. Recovery studies of **1** from **2** in Beier's modified Tyrode's solution at pH 5.8 for 1 mM and 3 mM solutions of **2** reacted with the β-glucosidase enzyme resulted in 82.6% and 82.0% recoveries of **1** with standard deviations of 0.258 and 0.921, respectively. Table 2 and Figure 3 show the results of *ex vivo* absorption of **2** in the piglet EJSs. In these incubations, a low but measurable amount of the glucoside **2** with absorption of 0.125 ± 0.041 mM was detected in the EJS fluid after 3 h of incubation with 1.0 mM **2**, and the concentrations of **2** did not

Table 2. *Ex Vivo* Absorption of Thymol-β-D-glucopyranoside (2) in Piglet Everted Jejunal Segment Fluids Following a 3 h Incubation

	suspension fluid (before incubation), mM[b]	EJS fluid[a] (after incubation), mM[b]	suspension fluid (after incubation), mM[b]
1.0 mM **2**[c]	0.764 ± 0.023[d]	0.125 ± 0.041[e]	0.737 ± 0.015
3.0 mM **2**[c]	1.874 ± 0.161[f]	0.317 ± 0.143[g]	1.799 ± 0.166

[a]EJS fluid = everted jejunal intestinal segment internal fluid. [b]Mean of 3 replicates ± standard deviation. [c]Evaluated in Beier's modified Tyrode's solution, pH 5.8 (n = 3). [d]This amount is 92.5 ± 2.8% of the expected amount of **2** synthetic product. [e]Average volume of EJS fluid for 1 mM **2** trials was 3.2 mL. [f]This amount is 76.2 ± 6.5% of the expected amount of **2** synthetic product. [g]Average volume of EJS fluid for 3 mM **2** trials was 3.45 mL.

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significantly differ (p = 0.1642) between the suspension fluid at the beginning and the end of incubation, indicating that there was little if any appreciable degradation of the glucoside conjugate at a 1.0 mM concentration in Beier's modified Tyrode's solution. In samples incubated with 3.0 mM **2**, absorption of 0.317 ± 0.143 mM glucoside was observed in the EJS fluid after the 3 h incubation, and this amount was significantly lower (p = 0.0003) than the concentration measured in the suspension fluid (1.799 ± 0.166 mM) at the end of incubation. No statistical difference (p = 0.6047) was observed in the concentration of **2** in the 3.0 mM suspension fluid of **2** at the beginning and at the end of incubation. A lower amount of the glucoside compared to the absorption of **1** was observed in the EJS fluid in samples incubated in 1.0 mM **2** (Figure 3), and this result was expected since the molecular weight of **2** (312.36) would not be expected to cross the intestinal epithelial barrier as well as the smaller molecular weight of **1** (150.22). Similar results were obtained from the incubation with 3.0 mM **2** (Figure 3). Table S1 (see the Supporting Information) shows the comparison of GC−MS data for authentic **1** with the analysis of the extracted internal contents of **1**- and **2**-treated piglet everted jejunal intestinal segments. The GC−MS data from the extracted internal contents of the EJSs were essentially identical to the GC−MS data for authentic **1**. The overall results of our study confirmed that **1** and, to a lesser extent, **2** were absorbed in the small intestine. Similarly, Michiels et al.[11] found that **1** was not significantly degraded in the jejunal gastrointestinal tract in *in vitro* fermentation simulations, but significant losses of up to 29% were found in cecal *in vitro* fermentation simulations. Further investigations are needed to determine the effective delivered concentration of **2** to the lower gut to achieve *in vivo* efficacy against *Campylobacter*. Therefore, delivery of **1** to the lower gut by the use of a glucoside carrier like that of **2** would allow **1** to be present in the gut region where **1** could be cleaved by normal gut bacteria containing β-glucosidase activity and be available to reduce the concentration of or remove *Campylobacter*.

■ ASSOCIATED CONTENT

Supporting Information

Table S1 with GC−MS data of authentic **1** is presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

EJS, everted jejunal intestinal segment; EJS fluid, everted jejunal intestinal segment internal fluid

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