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Article

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Curcuminoid Demethylation as an Alternative Metabolism by Human Intestinal Microbiota

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Abstract

Curcumin and other curcuminoids from *Curcuma longa* are important bioactive compounds exhibiting various pharmacological activities. In addition to the known reductive metabolism of curcuminoids, an alternative biotransformation of curcuminoids by human gut microbiota is reported herein. A curcuminoid mixture, composed of curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**) was metabolized by the human intestinal bacterium *Blautia* sp. MRG-PMF1. **1** and **2** were converted to new metabolites by the methyl aryl ether cleavage reaction. Two metabolites, demethylcurcumin (**4**) and bisdemethylcurcumin (**5**), were sequentially produced from **1**, and demethyldemethoxycurcumin (**6**) was produced from **2**. Until now, sequential reduction of the heptadienone backbone of curcuminoids was the only known metabolism to occur in the human intestine. In this report, a new intestinal metabolism of curcuminoids was discovered. Demethylation of curcuminoids produced three new colonic metabolites that were already known as promising synthetic curcumin analogues. The results could explain the observed beneficial effects of turmeric.

KEY WORDS: curcumin, curcuminoids, demethoxycurcumin, demethylation, human intestinal metabolism, turmeric

Introduction

Curcuminoids are major bioactive compounds derived from turmeric (*Curcuma longa* L.), which have pharmacological activities, such as anti-inflammatory, antibacterial, antifungal, antioxidant, antimutagenic, and anticancer effects. [1] Because of potential pharmaceutical applications, the metabolism of curcuminoids, including colonic metabolism, has been studied extensively. Similar to most dietary polyphenols, turmeric curcuminoids, composed of curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**) (Figure 1), are poorly absorbed and rapidly excreted from the body. [2] It is estimated that more than 75% of ingested curcuminoids are excreted from the body without absorption, and less than 1% is found as metabolites in circulating blood. [3,4] The major metabolites of curcumin in the plasma are curcumin glucuronide, curcumin sulfate, and hexahydrocurcumin. [5] A series of reduced curcumin metabolites are also produced by intestinal bacteria, such as *Escherichia coli* and *E. fergusonii*. [6,7] The enzyme responsible for the reduction was purified and characterized as a NADPH-dependent reductase. [8]

One the other hand, many curcumin derivatives have been synthesized to improve the bioavailability and pharmacological properties of curcuminoids. [1,9,10] Some of these exhibited even better pharmacological activities than the natural turmeric curcuminoids. For example, polyhydroxycurcuminoids have been described as promising Alzheimer's disease preventatives. [11] The epigenetic activity of bisdemethylcurcumin, a demethylated curcumin analogue, was particularly highlighted for its neuroprotective effects. [12,13]

The discrepancy in curcuminoid fluxes between the ratio of intestinal metabolites from gut microbiota and the distribution of phase II metabolites in the plasma attracted our attention. [3,4] In particular, less than 1% of reduced curcuminoids in plasma, resulting from microbial

metabolism, was too small to explain 25% of the orally administered curcuminoids that undergo intestinal metabolism. [14] Therefore, it was hypothesized that other types of curcuminoid metabolism should be operating in the intestine. To test this, curcuminoids were reacted with human fecal mixed cells. The result strongly indicated an alternative metabolism of curcuminoids different from the known reductive metabolism. Curcumin (**1**) and demethoxycurcumin (**2**) were biotransformed by the recently isolated *Blautia* sp. MRG-PMF1 that catalyzes the demethylation of the methyl aryl ether functional group. [15] This study describes an alternative metabolism of curcuminoids, and which may encourage the further study of curcuminoids as a valuable bioactive ingredient. [16]

Materials and Methods

The experimental protocol was evaluated and approved by the Institutional Review Board of Chung-Ang University (Approval Number: 1041078-201502-BR-029-01).

Chemicals and Bacterium

Curcuminoids mixture was purchased from Sigma Co. (St. Louis, USA). Curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**) were purchased from Phytobean AC. LTD (Yecheon-gun, Korea). HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Burdick & Jackson Laboratories, Inc. (Muskegon, MI, USA). Formic acid for mass spectrometry (98%) was purchased from Fluka (Buchs, Switzerland). Ethyl acetate (99.5%, EtOAc), acetic acid (99.5%), *N*,*N*-dimethylformamide (99.5%, DMF were purchased from Samchun Pure Chemicals (Gyeonggi-do. Korea). Gifu anaerobic medium (GAM) was from

Nissui Pharmaceutical Co. (Tokyo, Japan). The GAM broth was prepared following the manufacturer's instructions, and GAM plates were prepared by adding 1.5% (w/v) agar in GAM broth.

Human intestinal bacterium, *Blautia* sp. MRG-PMF1, isolated from our laboratory (GenBank accession number: KJ078647), was used in these experiments. Bacterial growth and substrate conversion experiments were performed under anaerobic conditions, according to published methods. [15,17, 18]

General HPLC Method

A Finnigan Surveyor Plus HPLC with a Thermo PDA Plus detector, equipped with a C18 Hypersil GOLD™ column (4.6×100 nm, 5 μm, Thermo Scientific, Waltham, MA, USA) was used for HPLC analysis. The injection volume was 10 μL and the flow rate was 1.0 mL/min. The mobile phase for the analysis of curcuminoid biotransformation was composed of 0.1% acetic acid in deionized water (solvent A) and 0.1% acetic acid in MeCN (solvent B). For the eluent gradient system, solution B was started at 20% and increased to 40% in 8 min, to 50% in 12 min, to 55% in 15 min, to 70% in 20 min, to 80% in 22 min, and finally to 20% in 30 min.

Biotransformation of Curcuminoids

All the experimental procedures for the biotransformation of curcuminoids were performed under anaerobic conditions (CO_2 5%, H_2 10%, N_2 85%) at 37 °C. Fresh fecal samples from three healthy volunteers were taken in sterilized vials and placed immediately in the anaerobic chamber. Using a cotton swab, the samples were diluted in saline solution, and the solution was filtered through sterile cheesecloth. For the preparation of mixed cell cultures, 1 mL of the

original filtrate was diluted to 10 mL with GAM broth and incubated. After 1 d, 100 μL of the mixed cell culture was transferred to an Eppendorf tube, and 1 μL of curcuminoids (a mixture of **1** – **3**, total 10 mM) was added to begin the biotransformation. After 6 h and 24 h, 1 mL of ethyl acetate was added to each tube and the reaction was stopped by vortexing. The solution was centrifuged (10770*g*) for 10 min, and the organic layer (800 μL) was dried under vacuum. The dried residue was dissolved in 10 μL of DMF and filtered through a 0.2-μm filter (Grace, USA) for HPLC analysis.

For the curcuminoid biotransformation with *Blautia* sp. MRG-PMF1, 60 μL of the solution containing the curcuminoids mixture, **1**, or **2** (10 mM in DMF) was added to GAM broth medium containing the MRG-PMF1 strain (6 mL, OD_{600} of 0.9). Aliquots (100 μL) of this reaction mixture were transferred to Eppendorf tubes after scheduled reaction times. The allocated media were extracted with 1 mL of ethyl acetate, and the supernatant (800 μL) collected after vortexing and centrifugation (10770*g* for 10 min) was dried under vacuum. The dried residue was dissolved in DMF (100 μL) and filtered through a 0.2-μm filter (Grace, USA) for HPLC analysis.

Structural Analysis of Curcuminoid Metabolites by HPLC-MS

The metabolites derived from curcumin (**1**) and demethoxycurcumin (**2**) were analyzed by a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA) equipped with a C18 reversed-phase column (Kinetex, 100 × 2.1 mm, 1.7 μm, Phenomenex, Torrance, CA) and a diode array detector (DAD). A Thermo Fisher Scientific LCQ fleet instrument (Thermo Scientific, Waltham, MA) was coupled to the HPLC system for electrospray ionization mass

spectrometry (ESI−MS) analysis. The mobile phase of the eluent gradient system was composed of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The initial composition of the mobile phase was 20% solvent B for 1 min. In a linear gradient, the composition was changed to 30% solvent B in 5 min, 40% solvent B in 15 min, 60% solvent B in 20 min, 80% solvent B in 22 min and then 20% solvent B remained for 2 min at a flow rate of 0.2 mL/min. ESI−MS analyses were performed in the positive-ion mode within the m/z range of 50−500 and processed with Xcalibur software (Thermo Scientific, Waltham, MA). ESI conditions: spray voltage, 5.4 kV; sheath gas, 15 arbitrary units; auxiliary gas, 5 arbitrary units; heated capillary temperature, 275 °C; capillary voltage, 27 V; and tube lens, 100 V.

Results

Curcuminoid Metabolism by Human Intestinal Bacteria

The curcuminoid mixture metabolized by three different mixed cell cultures exhibited different patterns of curcuminoid metabolism (see Figure S1). While two cultures produced putative reduced curcuminoid metabolites, the MS analysis identified demethylated metabolites from one of the mixed cell cultures. The culture with demethylation activity contained *Blautia* sp. MRG-PMF1, for which demethylation activity was reported previously, [15,18] thereby confirming that curcuminoids could be metabolized by the MRG-PMF1 strain. Most curcuminoids were metabolized within 2 d, and three metabolites were identified from HPLC chromatograms (Figure 2). Metabolite A disappeared in 24 h, but metabolite B remained even after 6 d of incubation. When each curcuminoid, curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**), was reacted with culture of the MRG-PMF1 strain, only

curcuminoids **1** and **2** were metabolized. Bisdemethoxycurcumin (**3**) was not metabolized (see Figure S2). The substrate curcumin (**1**) at the retention time of 14.42 min started to form the metabolite A at the retention time of 12.48 min after 3 h, and metabolite C appeared at the retention time of 10.80 min after 6 h. As metabolite A decreased over 30 h, the formation of metabolite C increased rapidly (Figure 3A). These results indicated that the metabolic pathway for curcumin (**1**) proceeded from **1** → A → C. For demethoxycurcumin (**2**), metabolite B found at the retention time of 12.25 min started to form after 3 h of incubation, and most metabolism was completed in 2 d (Figure 3B). This result indicated that the metabolic pathway for demethoxycurcumin (**2**) proceeded from **2** → B.

The metabolites A and C were identified as demethylcurcumin (**4**) and bisdemethylcurcumin (**5**), respectively, and metabolite B was identified as demethyldemethoxycurcumin (**6**), based on the UV and MS spectroscopic data (see Figure S3). The maximum UV absorptions of metabolites A and C were found at 428 nm, the same as curcumin (**1**), whereas the UV absorption of metabolite B was found at 423 nm, the same as demethoxycurcumin (**2**) (Figure 2). The ESI-MS analysis of metabolite A resulted in the $[M-H]^-$ peak of demethylcurcumin (**4**) at *m/z* = 352.95, smaller than the $[M-H]^-$ peak of curcumin by 14 Da due to the loss of a methyl group. The ESI-MS analysis of metabolite C showed a peak corresponding to the demethylated **4** at *m/z* = 338.94, and which was identified as bisdemethylcurcumin (**5**). The $[M-H]^-$ peak of metabolite B was found at *m/z* = 322.84, 14 Da less than demethoxycurcumin (**2**), and identified as demethyldemethoxycurcumin (**6**). The metabolites **4** – **6** were confirmed later by comparison of HPLC retention time and UV absorption with an authentic references which were obtained commercially.

Proposed Metabolic Pathway of Curcumin and Demethoxycurcumin

Curcumin (**1**) and demethoxycurcumin (**2**) were metabolized by the fecal samples and *Blautia* sp. MRG-PMF1. Based on the HPLC chromatographic changes (Figure 2 and 3), it was clear that the metabolism was performed mainly by *Blautia* sp. MRG-PMF1. Curcumin (**1**) was converted to bisdemethylcurcumin (**5**) via demethylcurcumin (**4**) by successive demethylation. Demethoxycurcumin (**2**) was directly metabolized to demethyldemethoxycurcumin (**6**) (Figure 4). Since new curcuminoid metabolism in the human intestine was identified in this work, curcuminoids **1** – **6**, as well as the reduced curcuminoinds metabolites, should be included for the study of the pharmacokinetics of curcuminoids.

Discussion

Study of the metabolic pathway of dietary polyphenols in the human intestine is important for human health. It can not only lead to new pharmaceutical compounds, but also provide insights into disease etiology and prevention. [19] Metabolic pathways of plant polyphenols by human gut microbiota have been extensively studied by us and others. Generally, intestinal metabolism of polyphenols includes hydrolysis of glycosides and esters, reduction of non-aromatic alkenes, and cleavage of the skeletons. [20,21] The biotransformation of turmeric curcuminoids, **1** – **3**, by human gut microbiota reported here is reminiscent of *S*-equol production from the soy isoflavone, daidzein (Figure 5). For example, repetitive reduction of curcuminoids **1** – **3** is known to produce a series of reduced curcumin metabolites. [7,8,22] Similarly, stereospecific reduction of daidzein produces dihydrodaidzein, tetrahydrodaidzein, and finally *S*-equol. [23-26]

When three different mixed cultures were reacted with curcuminoids, it was found that curcuminoids were metabolized differently depending on the fecal microflora. Similar to daidzein metabolism leading to *S*-equol formation, it appears that curcuminoid demethylation does not occur in everybody. Recently, Lou et al. also reported curcumin (1) demethylation by the fecal sample of a Chinese male. [27] However, it is still an open question whether dietary polyphenolics can alter human gut microbiota diversity or not.

Further study of one of the fecal samples which contained *Blautia* sp. MRG-PMF1 identified three new metabolites, **4** – **6**, arising from the different biotransformation of curcuminoids. From the biotransformation of each curcuminoid, the metabolic pathways for **1** and **2** were established as **1** → **4** → **5** and **2** → **6**, respectively. It is noteworthy that the potential pharmaceutical properties of the metabolites **4** – **6** had previously been studied as chemical derivatives of curcumin. For example, demethoxycurcumin (**4**) showed more potent cytotoxicity than curcumin (**1**) against human HCT116 colon cancer cells,[28] and the synthetic demethylated curcuminoids were reported to have better neuroprotective and anti-inflammatory properties. [12,13,29] Interestingly, the recently reported synthetic curcuminoids with preventative properties against Alzheimer's disease also included the metabolites **4** – **6**. The reported colonic metabolites **4** – **6**, were reported to activate neprilysin, an endogenous amyloid-β peptides degrading enzyme. Especially, metabolites **4** – **5** were reported to increase neprilysin mRNA expression in the brain. [11] In conclusion, our results support the thesis that the demethylated curcuminoids metabolites with strong pharmacological activities and better bioavailability than curcuminoids can be produced by human gut microbiota.

Supporting Information

198 Curcuminoid biotransformation by the three different mixed cell cultures (Figure S1),
199 bisdemethoxycurcumin (3) biotransformation by *Blautia* sp. MRG-PMF1 (Figure S2), and ESI-
200 MS spectra of curcuminoids and the metabolites (Figure S3). This Supporting Information is
201 available free of charge on the ACS Publications website at DOI:

202

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205 the Korean government (MSIP) (NRF-2015R1A1A3A04001198).

206

207 The authors declare no competing financial interest.

208

209 **References**

210 1. Prasad, S.; Gupta, S. C.; Tyagi, A. K.; Aggarwal, B. B. Curcumin, a component of golden
211 spice: from bedside to bench and back. *Biotechnol. Adv.* **2014***, 32,* 1053–1064.

212 2. Holder, G. M.; Plummer, J. L.; Ryan, A. J. The metabolism and excretion of curcumin (1,7-
213 bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in the rat. *Xenobiotica,* **1978**, *8,*
214 761–768.

215 3. Zeng, Y.; Qiu, F.; Liu, Y.; Qu, G.; Yao, X. Isolation and identification of phase 1 metabolites
216 of demethoxycurcumin in rats. *Drug Metab. Dispos.* **2007**, *35,* 1564–1573.

4. Hoehle, S. I.; Pfeiffer, E.; Sólyom, A. M.; Metzler, M. Metabolism of curcuminoids in tissue slices and subcellular fractions from rat liver. *J. Agric. Food Chem.* **2006**, *54,* 756–764.

5. Chiou, Y. S.; Wu, J. C.; Huang, Q.; Shahidi, F.; Wang, Y. J.; Ho, C. T.; Pan, M. H. Metabolic and colonic microbiota transformation may enhance the bioactivities of dietary polyphenols. *J. Funct. Foods.* **2014**, *7*, 3–25.

6. Tan, S.; Rupasinghe, T. W. T.; Tull, D. L.; Boughton, B.; Oliver, C.; McSweeny, C.; Gras, S. L.; Augustin, M. A. Degradation of curcuminoids by in vitro pure culture fermentation. *J. Agric. Food Chem.* **2014**, *62,* 11005–11015.

7. Tan, S.; Calani, L.; Bresciani, L.; Dall'asta, M.; Faccini, A.; Augustin, M. A.; Gras, S. L.; Rio, D. D. The degradation of curcuminoids in a human faecal fermentation model. *Int. J. Food Sci. Nutr.* **2015**, *66*, 790–796.

8. Hassaninasab, A.; Hashimoto, Y.; Tomita-Yokotani, K.; Kobayashi, M. Discovery of the curcumin metabolic pathway involving a unique enzyme in an intestinal microorganism. *Proc. Natl. Acad. Sci. USA.* **2011**, *108*, 6615–6620.

9. Appiah-Opong, R.; Commandeur, J. N. M.; Istyastono, E.; Bogaards, J. J.; Vermeulen, N. P. E. Inhibition of human glutathione S-transferases by curcumin and analogues. *Xenobiotica,* **2009**, *39*, 302–311.

10. Chen, W. F.; Deng, S. L.; Zhou, B.; Yang, L.; Liu, Z. L. Curcumin and its analogues as potent inhibitors of low density lipoprotein oxidation: H-atom abstraction from the phenolic groups and possible involvement of the 4-hydroxy-3-methoxyphenyl groups. *Free Rad. Biol. Med.* **2006**, *40*, 526–535.

11. Chen, P. T.; Chen, Z. T.; Hou, W. C.; Yu, L. C.; Chen, R. P. Y. Polyhydroxycurcuminoids but not curcumin upregulate neprilysin and can be applied to the prevention of Alzheimer's disease. *Scientific Rep.* **2016**, *6*, 29760.

12. Pinkaew, D.; Changtam, C.; Tocharus, C.; Govitrapong, P.; Jumnongprakhon, P.; Suksamrarn, A.; Tocharus, J. Association of neuroprotective effect of di-O-demethylcurcumin on Aβ25–35-induced neurotoxicity with suppression of NF-κB and activation of Nrf2. *Neurotox. Res.* **2016**, *29*, 80–91.

13. Pinkaew, D.; Changtam, C.; Tocharus, C.; Thummayot, S.; Suksamrarn, A.; Tocharus, J. Di-*O*-demethylcurcumin protects SK-N-SH cells against mitochondrial and endoplasmic reticulum-mediated apoptotic cell death induced by Aβ 25-35. *Neurochem. Int.* **2015**, *80*, 110–119.

14. Wahlstrom, B.; Blennow, G. A study on the fate of curcumin in the rat. *J. Acta Pharmacol. Toxicol.* **1978**, *43*, 86−92.

15. Kim, M.; Kim, N.; Han, J. Metabolism of *Kaempferia parviflora* polymethoxyflavones by human intestinal bacterium *Blautia* sp. MRG-PMF1. *J. Agric. Food Chem.* **2014**, *62,* 12377–12383.

16. Ho, J. N.; Jang, J. Y.; Yoon, H. G.; Kim, Y.; Kim, S.; Jun, W.; Lee, J. Anti-obesity effect of a standardised ethanol extract from *Curcuma longa* L. fermented with Aspergillus oryzae in ob/ob mice and primary mouse adipocytes. *J. Sci. Food Agric.* **2012**, *92*, 1833–1840.

17. Kim, M.; Kim, N.; Han, J. Deglycosylation of flavonoid *O*-glucosides by human intestinal bacteria *Enterococcus* sp. MRG-2 and *Lactococcus* sp. MRG-IF-4. *Appl. Biol. Chem.* **2016**, *59*, 443–449.

18. Burapan, S.; Kim, M.; Han, J. Demethylation of ppolymethoxyflavones by human gut bacterium, *Blautia* sp. MRG-PMF1. *J. Agric. Food Chem.* **2017**, *65*, 1620–1629.

19. Kumar, M.; Nagpal, R.; Verma, V.; Kumar, A.; Kaur, N.; Hemalatha, R.; Gautam, S. K.; Singh, B. Probiotic metabolites as epigenetic targets in the prevention of colon cancer. *Nutr. Rev.* 2012, *71*, 23–34.

20. Kim, M.; Lee, J.; Han, J. Deglycosylation of isoflavone *C*-glycosides by newly isolated human intestinal bacteria. *J. Sci. Food Agric.* **2015**, *95*, 1925–1931.

21. Lewandowska, U.; Szewczyk, K.; Hrabec, E.; Janecka, A.; Gorlach, S. Overview of metabolism and bioavailability enhancement of polyphenols. *J. Agric. Food Chem.* **2013**, *61*, 12183–12199.

22. Zeng, Y.; Qiu, F.; Liu, Y.; Qu, G.; Yao, X. Isolation and identification of phase 1 metabolites of demethoxycurcumin in rats. *Drug Metab. Dispos.* **2007**, *35*, 1564–1573.

23. Park, H. Y.; Kim, M.; Han, J. Stereospecific microbial production of isoflavanones from isoflavones and isoflavone glucosides. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 1173–1181.

24. Kim, M.; Won, D.; Han, J. Absolute configuration determination of isoflavan-4-ol stereoisomers. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4337–4341.

25. Kim, M.; Marsh, E. N. G.; Kim, S. U.; Han, J. Conversion of (3*S*,4*R*)-tetrahydrodaidzein to (3*S*)-equol by THD reductase: Proposed mechanism involving a radical intermediate. *Biochemistry,* **2010**, *49*, 5582–5587.

26. Kim, M.; Kim, S. I.; Han, J.; Wang, X. L.; Song, D. G.; Kim, S. U. Stereospecific biotransformation of dihydrodaidzein into (3*S*)-equol by the human intestinal bacterium *Eggerthella* strain Julong 732. *Appl. Environ. Microbiol.* **2009**, *75*, 3062–3068.

27. Lou, Y.; Zheng, J.; Hu, H.; Lee, J.; Zeng, S. Application of ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry to identify curcumin metabolites produced by human intestinal bacteria. *J. Chromatogr. B.* **2015**, *985*, 38–47.

28. Tamvakopoulos, C.; Dimas, K.; Sofianos, Z. D.; Hatziantoniou, S.; Han, Z.; Liu, Z. L.; Wyche, J. H.; Pantazis, P. Metabolism and anticancer activity of the curcumin analogue, demethoxycurcumin. *Clin. Cancer Res.* **2007**, *13*, 1269–1277.

29. Khanna, S.; Park, H. A.; Sen, C. K.; Golakoti, T.; Sengupta, K.; Venkateswarlu, S.; Roy, S. Neuroprotective and antiinflammatory properties of a novel demethylated curcuminoid. *Antioxid. Redox Signal.* **2009**, *11*, 449–468.

FIGURE CAPTIONS

Figure 1. Molecular structures of curcuminoids.

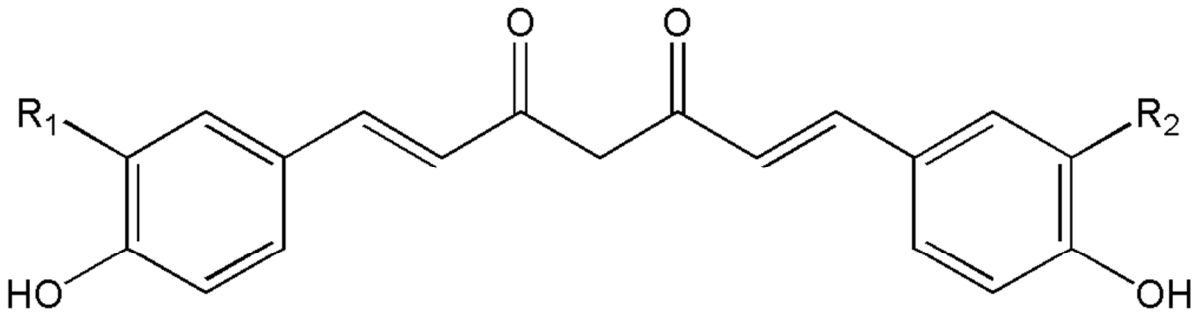
Figure 2. HPLC chromatogram changes (425 nm) of the curcuminoid mixture metabolism by *Blautia* sp. MRG-PMF1. The insets show UV spectra of the compounds.

Figure 3. HPLC chromatogram changes (425 nm) of curcumin (**1**) (a) and demethoxycurcumin (**2**) (b) metabolisms by *Blautia* sp. MRG-PMF1.

Figure 4. Proposed metabolic pathway of curcuminoids by human gut microbiota.

Figure 5. Established colonic metabolism of curcuminoids and daidzein. Sequential reduction of curcuminoids produces dihydro-, tetrahydro-, and hexahydro-curcuminoids. Dehydroxylation of the metabolite was also reported.[22] Analogously, stereospecific reduction of daidzein results in the formation of *S*-equol via dihydrodaidzein and tetrahydrodaidzein.

Figure 1



$R_1 = OCH_3, R_2 = OCH_3$: Curcumin (**1**)
$R_1 = H, R_2 = OCH3$:Demethoxycurcumin (**2**)
$R_1 = H, R_2 = H$: Bisdemethoxycurcumin (**3**)

Figure 2

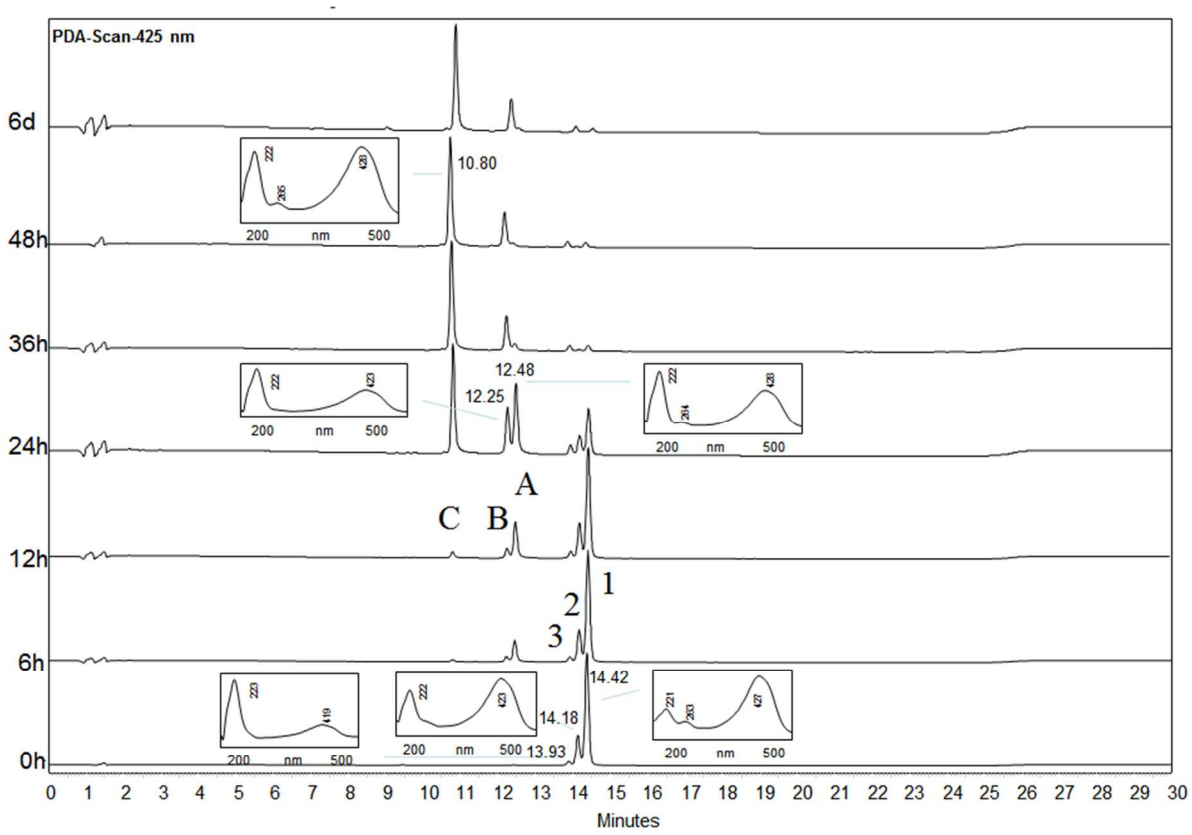
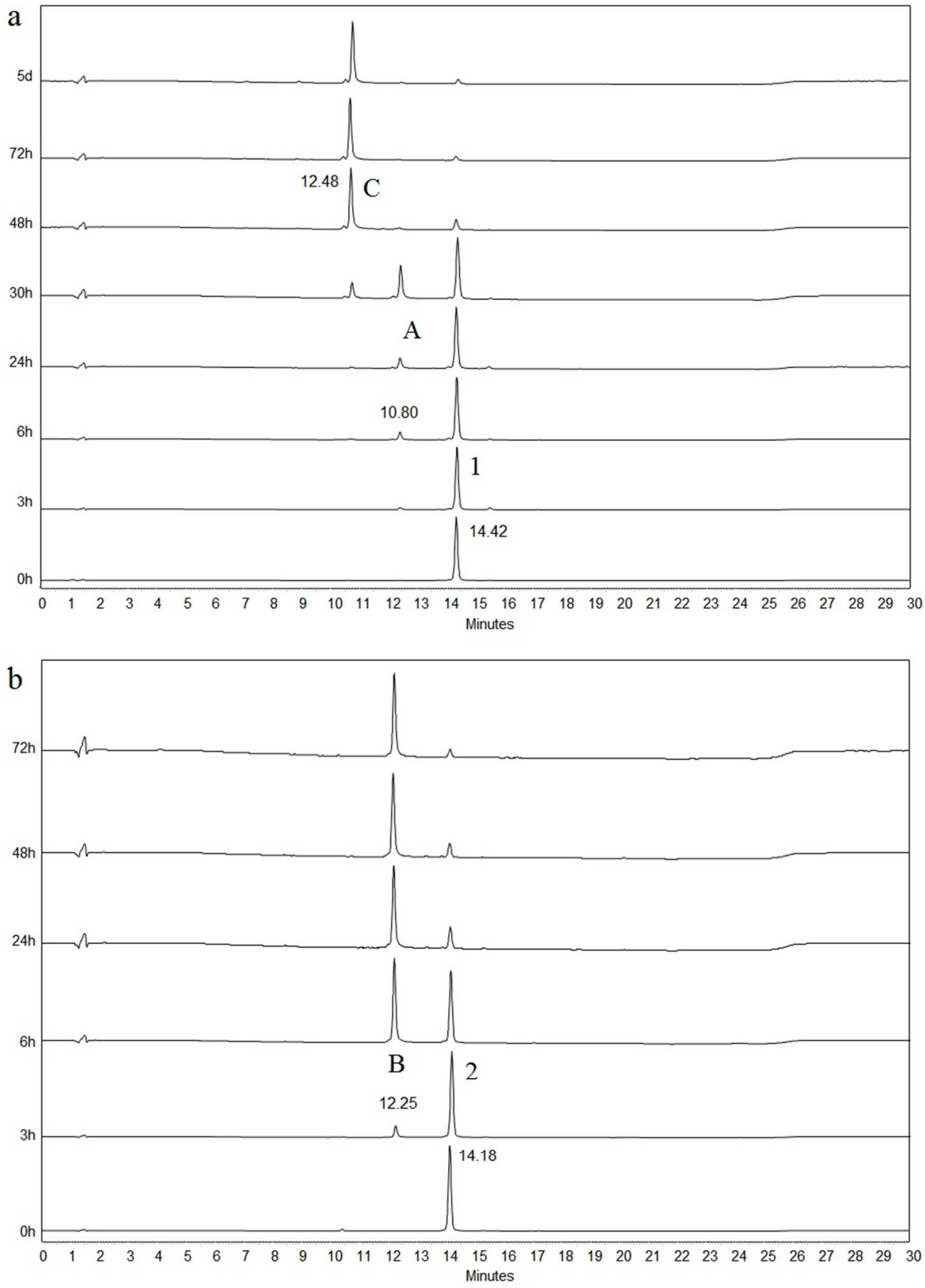

PDA-Scan-425 nm
6d
48h
36h
24h
12h
6h
0h
10.80
12.48
12.25
A
C B
1
2
3
14.42
14.18
13.93
200 nm 500
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Minutes

Figure 3


a
5d
72h
12.48
C
48h
30h
A
24h
10.80
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1
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14.42
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0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Minutes
b
72h
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B
2
12.25
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Minutes

Figure 4

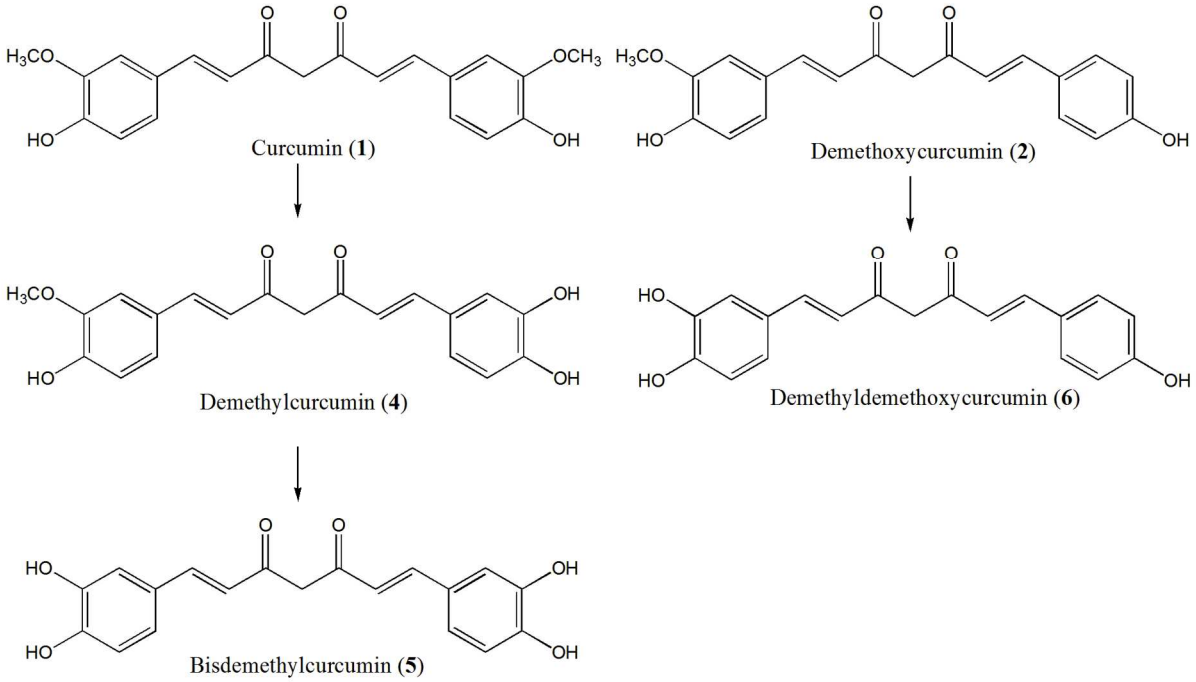
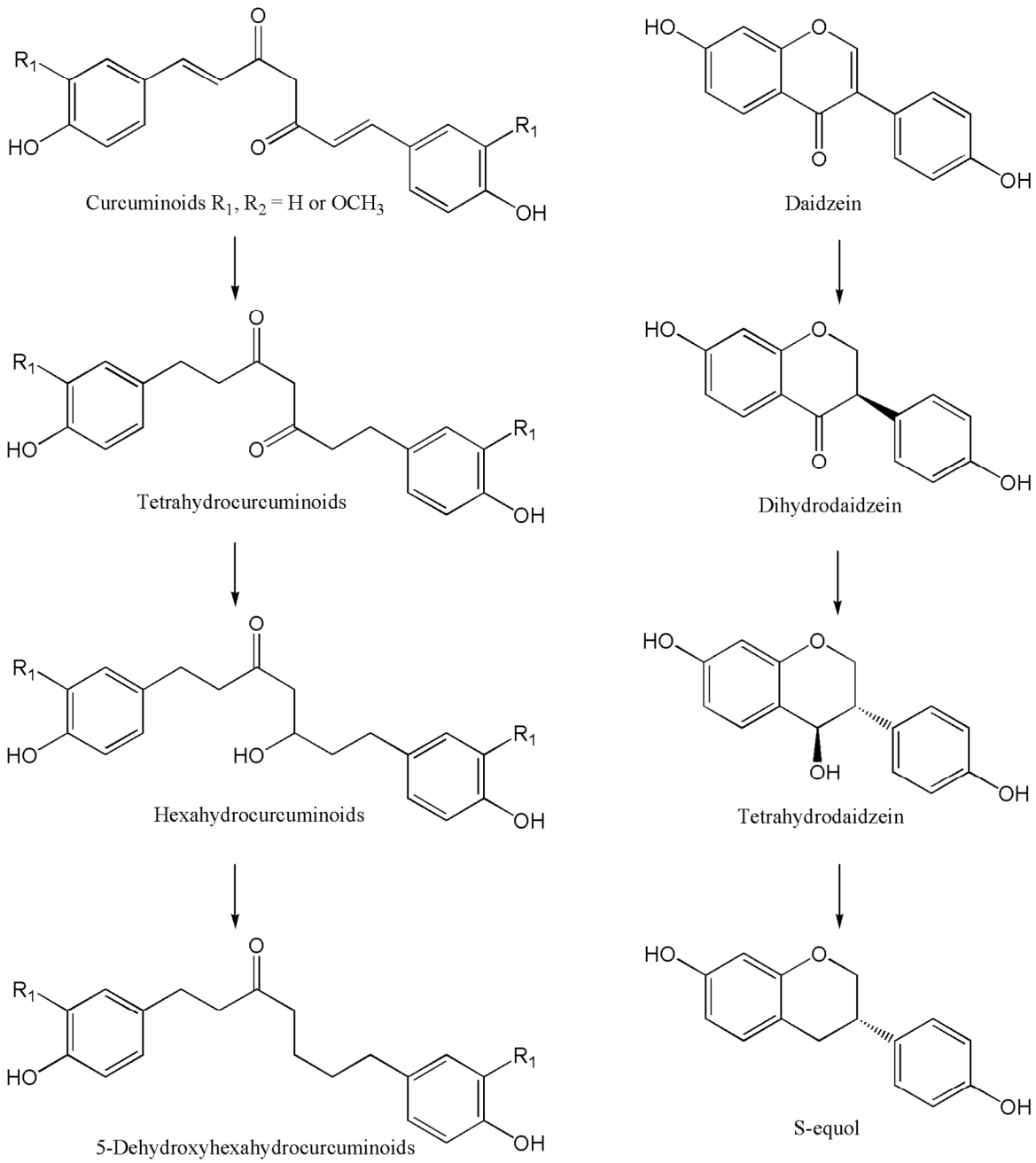
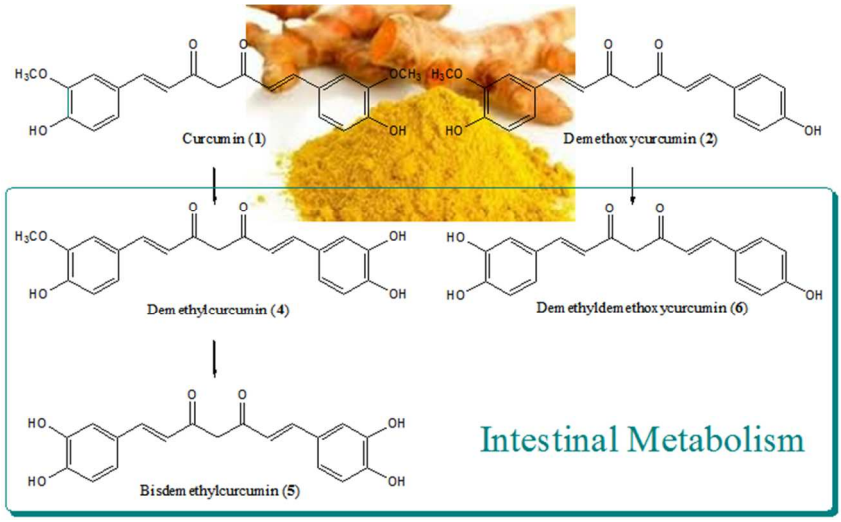

Curcumin (1)
Demethoxycurcumin (2)
Demethylcurcumin (4)
Demethyldemethoxycurcumin (6)
Bisdemethylcurcumin (5)

Figure 5


Curcuminoids R_1, R_2 = H or OCH_3
Tetrahydrocurcuminoids
Hexahydrocurcuminoids
5-Dehydroxyhexahydrocurcuminoids
Daidzein
Dihydrodaidzein
Tetrahydrodaidzein
S-equol

TOC Graphic


Curcumin (1)
Demethoxycurcumin (2)
Demethylcurcumin (4)
Demethyldemethoxycurcumin (6)
Bisdemethylcurcumin (5)
Intestinal Metabolism