Biotransformation of (−)-Epicatechin 3-*O*-Gallate by Human Intestinal Bacteria

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The biotransformation of (−)-epicatechin 3-*O*-gallate (1) and related compounds was undertaken using a human fecal suspension. Of fifteen metabolites isolated, four compounds were new, namely, two epimers of 1-(3′-hydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)propan-2-ols (6, 19); 2″,3″-dihydroxyphenoxyl 3-(3′,4′-dihydroxyphenyl)propionate (14) and 1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)propan-2-ol (18).

(−)-Epicatechin (2), (−)-epigallocatechin (16) and their 3-*O*-gallates (1, 17) were extensively metabolized by a human fecal suspension after incubation for 24 h, whereas the gallates (1, 17) resisted any degradation by a rat fecal suspension, even after a prolonged incubation time (48 h), suggesting a difference in metabolic ability between two intestinal bacterial mixtures from different species.

Key words biotransformation; (−)-epicatechin 3-*O*-gallate; human intestinal bacteria; rat intestinal bacteria

Recently, increasing attention has been given to the role of free radicals and other oxidants in the pathophysiology of major chronic diseases such as heart disease, cancer, renal failure and inflammation.[1] This has unveiled an urgent need for potential antioxidants or free radical scavengers.

Flavan-3-ols and their derivatives make up one of the most important groups of antioxidants which are distributed in most medicinal plants (*e.g.* rhubarb, tea and hawthorn). These plants are rich sources of the flavan-3-ols, (−)-epicatechin (**2**), (−)-epigallocatechin (**16**) and their 3-*O*-gallates (**1**, **17**), as well as proanthocyanidins related to (−)-epicatechin. (−)-Epicatechin gallate (**1**), the main component of rhubarb tannins, was found to contribute mainly to the antiuremic action of aqueous rhubarb extract, which in turn protected the kidney against renal failure.[2] Compounds **1** and **17** were also found to be the most relevant antioxidant constituents of green tea, which protects us against lipid peroxidation,[3] inflammatory dermatoses and immunosuppression.[4] (−)-Epicatechin (**2**) was found to inhibit lung metastasis in mice.[5] Similarly, extracts of hawthorn (*Crataegus* sp.), which are rich in (−)-epicatechin (**2**) and related procyanidins,[6] find usage in Western medicine in the treatment of heart complaints. Likewise, herbal medicines still survive today in Europe and North America, whose beneficial action are believed to reside, at least in part, in their flavan-3-ol contents. The consumption of crude drugs rich in these compounds may protect us from major chronic diseases. However, studies dealing with the biotransformation of the flavan-3-ols, (−)-epicatechins by intestinal bacteria have been carried out only in a few instances. The metabolism of (−)-epicatechin (**2**) and its epimer (+)-catechin (**15**) in rats and guinea pig have long been studied.[7] The present report deals with the biotransformation of (−)-epicatechin 3-*O*-gallate (**1**) and related compounds *in vitro*, using human and rat fecal suspensions.

Results and Discussion

After anaerobic incubation with a mixture of human intestinal bacteria (HIB), the fermentation broth of **1**, **2** and **15** was separately extracted with a BuOH–Et_2O mixture (1 : 1) and then purified by Sephadex LH-20 and medium pressure liquid chromatography (MPLC) to afford fifteen metabolites (**2**—**14**, **18**, **19**) (Chart 1). Compounds **2**—**4** were obtained from **1** after incubation for 12 h (**4** was also obtained from **3**), **5**—**8** were mainly obtained from **1** and **2** after incubation for 24 h, and **9**—**14** could be mainly obtained from **1**, **2** and **15** after incubation for 48 h. Compounds **18** ($[\alpha]_D$ +20°) and **19** ($[\alpha]_D$ +12°) (two epimers of **5** and **6**) were obtained from **15** after incubation for 24 h. The structures of these metabolites were determined by chemical and spectroscopic methods as follows.

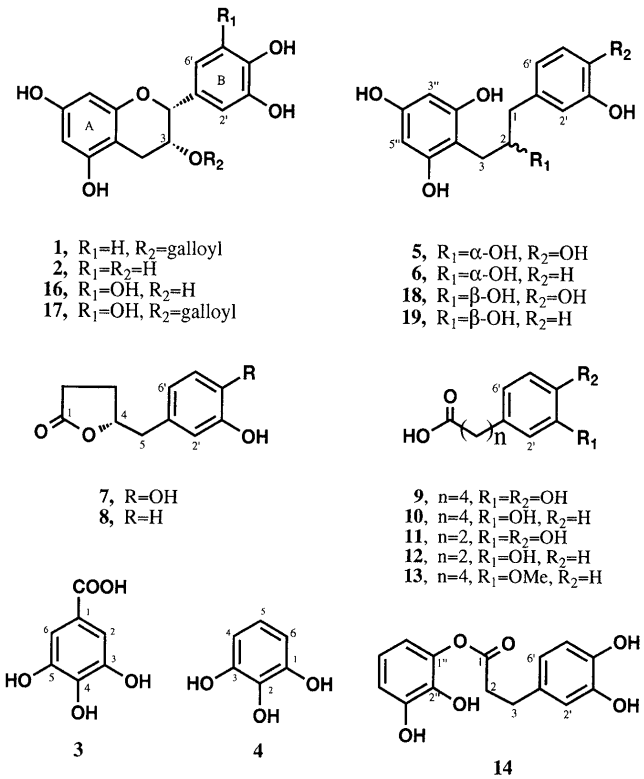

Chart 1. Structures of (−)-Epicatechin 3-*O*-gallate (**1**) and Its Metabolites

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Table 1. ^{1}H-NMR (500 MHz) Spectral Data of Compounds **5**, **6**, **18** and **19** (in CD_3OD)

H	5	6	18	19
2′	6.68 d (2.2)	6.62 br t (2.7)	6.66 d (1.9)	6.66 br t (2.1)
4′	—	6.68 dd (8.2, 2.7)	—	6.67 br dd (8.5, 2.1)
5′	6.66 d (8.2)	7.06 t (8.2)	6.65 d (8.1)	7.05 t (8.5)
6′	6.54 dd (8.2, 2.2)	6.59 dd (8.2, 2.7)	6.53 dd (8.1, 1.9)	6.58 dd (8.5, 2.1)
1	2.52 dd (13.7, 8.2)	2.60 dd (14.3, 8.8)	2.50 dd (14.1, 8.5)	2.57 dd (14.4, 8.5)
	2.68 dd (13.7, 3.8)	2.76 dd (14.3, 3.8)	2.69 dd (14.1, 4.4)	2.74 dd (14.4, 3.9)
2	3.90 dddd (8.2, 7.1, 4.4, 3.8)	4.03 dddd (8.8, 7.1, 4.4, 3.8)	3.94 ddd (8.5, 7.9, 4.4)	4.01 dddd (8.5, 8.1, 4.4, 3.9)
3	2.66 dd (13.7, 7.1)	2.72 dd (13.7, 7.1)	2.64 dd (13.9, 7.9)	2.69 dd (13.9, 8.1)
	2.86 dd (13.7, 4.4)	2.90 dd (13.7, 4.4)	2.87 dd (13.9, 4.4)	2.87 dd (13.9, 4.1)
6″, 8″	5.88 br d (2.2)	5.89 br s	5.87 br s	5.89 br s

δ values in ppm and coupling constants (in parentheses) are in Hz.

Table 2. ^{13}C-NMR (125 MHz) Spectral Data of Compounds **5**, **6**, **18** and **19**

C	5	6	18	19
1′	133.04 s	143.05 s	142.54 s	142.57 s
2′	118.11 d	114.55 d	117.59 d	113.77 d
3′	146.33 s	158.47 s	145.82 s	158.45 s
4′	144.59 s	117.95 d	144.35 s	117.31 d
5′	116.59 d	130.86 d	116.06 d	130.02 d
6′	122.31 d	122.67 d	121.80 d	121.83 d
1	44.29 t	44.92 t	43.78 t	44.34 t
2	75.86 d	75.72 d	75.34 d	75.01 d
3	32.04 t	32.33 t	31.55 t	31.75 t
1″	106.19 t	106.31 s	105.64 s	105.48 s
2″	158.15 s	158.17 s	157.64 s	157.69 s
3″	96.37 d	96.63 d	95.82 d	95.78 d
4″	158.70 s	158.77 s	158.19 s	158.22 s
5″	96.37 d	96.63 d	95.82 d	95.78 d
6″	158.15 s	158.17 s	157.64 s	157.69 s

Measured in MeOH-d_4. δ values in ppm and the multiplicities of carbons were determined by the aid of an HMQC experiment and indicated as singlet (s), doublet (d) and triplet (t).

Compounds **2** and **3** were identified as (−)-epicatechin and gallic acid by direct comparison of the spectral data with those of authentic samples.

Compound **4** was obtained in a relatively high yield (0.2%). Its proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed only AB_2-type aromatic protons at δ 6.50 (1H, t, J=8.5 Hz, H-5) and 6.40 (2H, d, J=8.5 Hz, H-4, H-6). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (see Experimental) showed signals characteristic of two sp^2 quaternary carbons at δ 146.92 and 134.09 (C-1 and C-2, respectively), and two aromatic methines at δ 120.08 and 108.24 (C-5 and C-4, respectively). Accordingly, **4** was identified as pyrogallol, whose ^{1}H- and ^{13}C-NMR data agreed with those of an authentic sample.

The ^{1}H- and ^{13}C-NMR spectra of **5** (Tables 1, 2) were similar to those of **2** with respect to the substitution patterns of two aromatic rings. However, the large upfield shift of C-1 in **5** (δ 44.29) relative to C-2 (δ 79.81) in **2**, was consistent with a 1,3-diarylpropan-2-ol skeleton. This was further supported by electron impact mass spectrometry [EI-MS (*m/z* 292 $[M]^+$)], ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and ^{1}H-detected heteronuclear multiple bond correlation (HMBC) experiments, and the structure of **5** was finally concluded to be 1-(3′,4′-dihydroxy-phenyl)-3-(2″,4″,6″-trihydroxyphenyl)propan-2-ol. Compound **5** was previously prepared by hydrogenolysis of (−)-epicatechin (**2**) at 180 °C and at 1000 psi of hydrogen (stereochemistry at C-2 is not given).[8] However, **5** obtained in this experiment was optically active ($[\alpha]_D$ −15°).

Most of the signals in the NMR spectra of **6** ($[\alpha]_D$ −14°) were similar to those of **5** (Tables 1, 2). However, the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum of **6** indicated that one of the spin systems in the aromatic region was integrated to four AB_2C-aromatic protons at δ 6.59 (dd, H-6′), 6.62 (br t, H-2′), 6.68 (dd, H-4′) and 7.06 (t, H-5′). Furthermore, the molecular ion peak (*m/z* 276 $[M]^+$) in the EI-MS was 16 mass units less than that of **5**. This observation suggested that *p*-dehydroxylation of **5** gives **6**. Further confirmation was substantiated by measuring the HMBC spectrum. Accordingly, **6** was determined to be 1-(3′-hydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)propan-2-ol.

The ^{1}H- and ^{13}C-NMR spectra of **7** (see Experimental) showed signals for three ABC-type aromatic protons, an oxygen-bearing methine at δ 84.05 (δ_H 4.72), and three methylenes at δ 28.63, 30.28 and 42.22 (δ_H 1.95—2.84). These findings suggested possible degradation of the phloroglucinol part (ring A)[9] in **5** to give **7** (after lactonization of C_2-OH with the residual carbonyl), as confirmed by EI-MS (*m/z* 208 $[M]^+$). Accordingly, **7** was identified as 5-(3′,4′-dihydroxyphenyl)γ-valerolactone. A similar compound was isolated by Watanabe[9] after incubation of **15** with rat intestinal bacteria (RIB), and two carbons of the lactone ring of the resulting phenyl-γ-valerolactone were shown to posses radioactivity when (+)[ring A-^{14}C]catechin (**15**) was used.[10]

The spectral data of **8** were close to those of **7**; however, AB_2-aromatic methines in **7** were changed to the AB_2C-type in **8**. This observation suggested the possible *p*-dehydroxylation of **7** to give **8**, which was further confirmed by EI-MS (*m/z* 192 $[M]^+$). Compound **8** was identified as 5-(3′-hydroxyphenyl)γ-valerolactone. A similar compound was previously isolated after incubation of **15** with RIB.[11]

Compound **9** was obtained as one of the major metabolites. Its EI-MS showed a molecular ion peak at *m/z* 210 $[M]^+$. The ^{1}H-NMR spectrum showed signals for ABC-aromatic protons at δ 6.48, 6.60 and 6.65, and four methylenes at δ 1.59, 2.28 and 2.46. Moreover, the ^{13}C-NMR spectrum showed a signal for a carbonyl

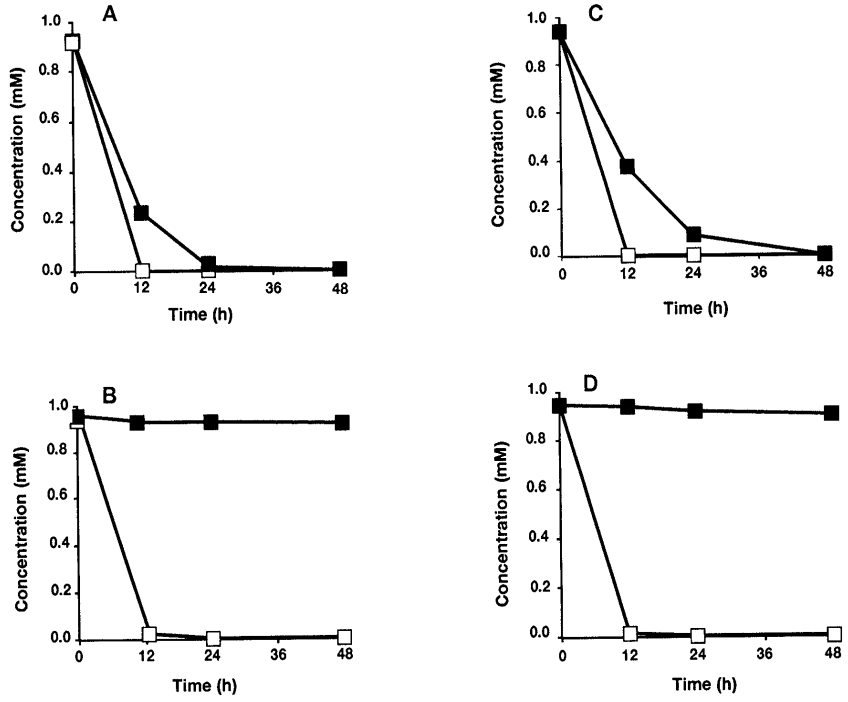

Fig. 1. Comparative Degradation Curves of Flavan-3-ols and Their Gallates by HIB (□) and RIB (■)
A, (−)-epicatechin (**2**); B, (−)-epicatechin gallate (**1**); C, (−)-epigallocatechin (**16**); and D, (−)-epigallocatechin gallate (**17**).

carbon at δ 175.10. From these data, **9** was identified as 5-(3′,4′-dihydroxyphenyl)valeric acid, and this was further confirmed by comparison of the spectral data with those of an authentic sample.

The structure of **10** was evident from EI-MS (*m/z* 194 $[M]^+$), which suggested *p*-dehydroxylation of **9** to give **10**. Further confirmation was obtained by inspection of ^{1}H- and ^{13}C-NMR spectra (see Experimental). Similarly, **10** was identified as 5-(3′-hydroxyphenyl)valeric acid.

β-Oxidation of **9** might give **11** (*m/z* 182 $[M]^+$ in the EI-MS), which was finally identified as (3′,4′-dihydroxyphenyl)propionic acid by ^{1}H- and ^{13}C-NMR spectral evidence.

Similarly, *p*-dehydroxylation of **11** gave **12**, as confirmed by EI-MS (*m/z* 166 $[M]^+$), ^{1}H- and ^{13}C-NMR (see Experimental), and **12** was identified as (3′-hydroxyphenyl)-propionic acid.

The ^{1}H-NMR spectrum of **13** showed an additional singlet at δ 3.64 integrated for three protons relative to that of **10**, and suggested the possible methylation of **10** to give **13**. This conclusion was evident from the EI-MS spectrum, which showed a molecular ion peak at *m/z* 208 $[M]^+$. Accordingly, the structure of **13** was identified as 5-(3′-methoxyphenyl)valeric acid.

The proposed structure for **14** relied on comparative NMR data with **4** and **11**, where the signal assignable for two equivalent protons (at δ 6.40) in **4** was changed to a spin system of the AB_2-type at δ 6.24, 6.25 and 6.94 in **14**, suggesting possible acylation of C_3-OH in **4**, as further confirmed by ^{13}C-NMR (see Experiment). Signals characteristic for ABC-aromatic protons and two methylene protons in the ^{1}H-NMR spectrum of **11** were also seen in those of **14** (see Experiment). However, the carbonyl carbon signal of **11** (δ 177.86) was shifted upfield (−12.9 ppm) in **14**, suggesting possible acylation of the phenyl propionate residue of **11** with a hydroxyl group of **4** to give **14**. This conclusion was further substantiated by HMQC. From the above mentioned data, **14** was determined to be 2″,3″-dihydroxyphenoxyl (3′,4′-dihydroxyphenyl)propionate.

Figure 1 shows the time course of degradation by intestinal bacterial mixtures for (−)-epicatechin (**2**), (−)-epigallocatechin (**16**) and their gallates (**1**, **17**). All of them were completely metabolized within 12 h by HIB when the reaction was monitored by thin-layer chromatography densitometry. On the other hand, 75—60% of **2** and **16** were metabolized by RIB for 12 h, and most of them within 24 h, indicating a slower conversion rate compared to that of HIB. In contrast to the case of HIB, the gallates **1** and **17** were hardly converted to any metabolites, even after prolonged incubation time up to 48 h. These findings revealed that the HIB mixture had appreciable activity of hydrolyzing 3-*O*-galloyl esters of flavan-3-ols, but the RIB mixture had little activity. However, both mixtures possessed metabolic ability of flavan-3-ols, even though their activities seem to be varied.

A number of metabolites obtained after incubation of **1** with HIB (Chart 1) indicates massive ring fission catalyzed by intestinal bacteria. Except for **2**, **5** and **6**, a phloroglucinol part of the original substrate (**1**) was missing in all metabolites. In this connection, reincubation of some intermediate metabolites provided evidence of their sequential relationships, which supports the pathway of **1** by HIB, as shown in Chart 2. Following the incubation of **2**, there was conclusive evidence that **5**—**9** were degradation products of **1**. When **7** was incubated with HIB, **9** and **12** were also obtained, together with **8**. Without any exception, **12** was found to be the main metabolite in all incubates when the incubation time was extended for 48 h.

The proposed metabolic sequence for the production of

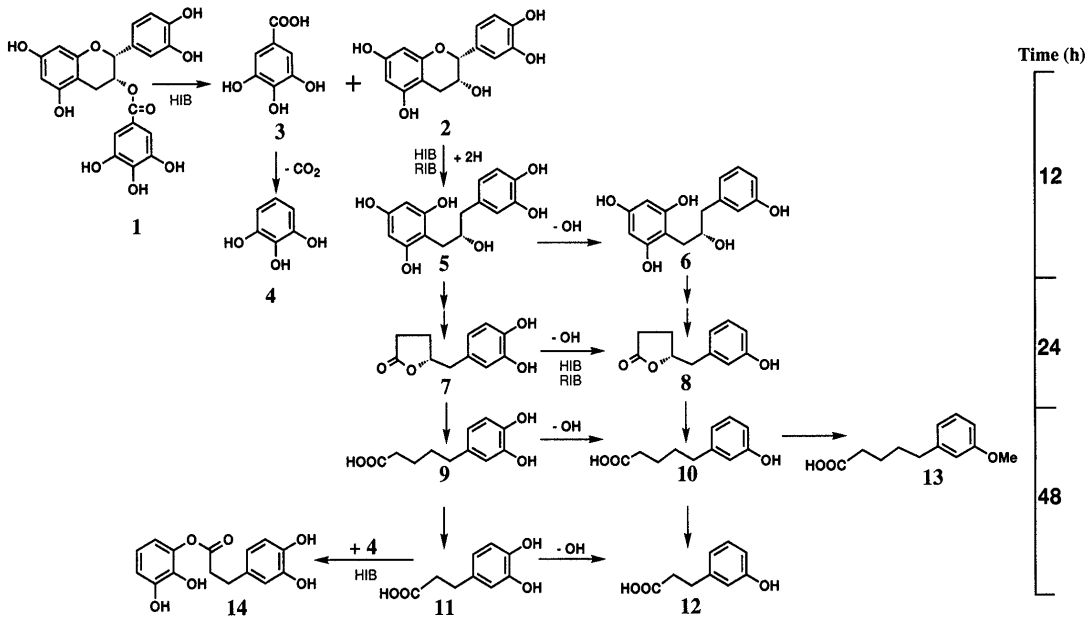

Chart 2. Possible Metabolic Pathways of (−)-Epicatechin 3-*O*-gallate (**1**) by HIB

these metabolites involves the initial hydrolysis of the galloyl ester group in **1** to give **2** and **3**, where **2** underwent reductive cleavage to give **5**, while **3** was decarboxylated to **4**. It seems evident that the reductive cleavage of **2** is due to the presence of a free *p*-hydroxyl group ($C_{4'}$-OH) in **2**, since a 3′,4′,5,7-tetra-*O*-methyl ether of **2** was unchanged after incubation with HIB for 60 h (data not shown). Likewise, when acacetin, the 4′-methyl ether of apigenin, was orally given to rats, the extent of ring fission was appreciably reduced.[12]

Another pathway which has been prominent in most studies of flavan-3-ols to date involves the destruction of the phloroglucinol part (ring A) leading to CO_2, with the remainder of the molecule as phenolic lactone and sometimes phenolic acids.[10,11] In connection to this study, the possible lactonization of C_2-OH in **5** with a carbonyl carbon left after the degradation of ring A can be considered to give **7**, while *p*-dehydroxylation produced **6** which similarly gave **8**.

Massive ring fission of **7** and **8** produced phenolic acids (**9**—**13**) which were mainly detected in all incubates after prolonged incubation of **2** with either HIB or RIB (the same phenolic acids were obtained from **15**). Since aromatic compounds, coumarins and flavonoids are partly metabolized to phenolic acids by intestinal bacteria as well as by plants and aerobes,[13-15] it seems that ring fission in oxygen-containing heterocyclic compounds through reductive or oxidative cleavage is a common reaction of living organisms. Further evidence for the key role played by the intestinal bacteria in the catabolism of flavan-3-ols was obtained by Das and Griffiths,[16] who reported that the oral administration of aureomycin to guinea pigs suppresses the formation of ring fission products from ingested (+)-catechin (**15**).

Mono-methylation of an aromatic hydroxy group in **10** gave **13** (a similar reaction was also reported[17]). Condensation of **4** and **11** may also lead to the formation of **14**.

As regards the structural requirements for flavan-3-ols degradation, it is clear that the *p*-hydroxyl group ($C_{4'}$-OH) is a must. On the other hand, studies with 3-*O*-methyl-(+)-catechin indicated that this substrate was resistant to ring fission by RIB.[18,19] This experiment suggested a possible contribution of C_3-OH in the degradation of flavan-3-ols. Taking into account this conclusion and by comparing the degradation profiles for each pair, (**1**, **2**) and (**16**, **17**), after anaerobic incubation with HIB or RIB (Fig. 1), we can conclude that methylation or acylation of the hydroxyl group (C_3-OH) in **2** and **16** protects them from further degradation with RIB. This could be accounted for on the basis of species difference, which in turn reflects a difference in the types and numbers of potential bacteria in the particular species's intestinal microflora. Mitsuoka[20] showed that the population of *Bifidobacteria* in human intestine is almost 1000 times greater than that in the rat intestine. This fact was in accordance with results obtained in our experiment; when 32 defined bacterial strains were screened for their ability to metabolize **1**, the *Bifidobacterium* strains tested (seven strains) showed a high ability to metabolize **1** after incubation for 48 h (data not shown).

The present findings indicate that **1** undergoes several metabolic alterations when incubated with HIB, which was not demonstrated with RIB. This difference was also seen in the *in vivo* experiments, as a substantial amount of **1** could be detected in rat plasma following its oral administration,[21] but when fed to humans, **1** was completely absent in plasma or urine samples.[22] Therefore, it is not surprising that the 3-*O*-gallates (**1**, **17**) could be absorbed unchanged through the intestinal wall of rats, thus demonstrating their antioxidant activity. However, the human consumption of crude drugs rich in these compounds may lead to metabolites of diverse structures. Against this background, the question is unanswered whether the remaining substrate (*i.e.* **1**) or, more likely, its metabolites is/are mainly responsible for antioxidant

properties. This point will be a subject of particular interest to be discussed in the near future.

Experimental

General Experimental Procedure Optical rotations were measured in MeOH solutions using a JASCO DIP-360 automatic polarimeter at 25 °C. UV spectra were taken on a Shimadzu UV-2200 UV-VIS spectrophotometer (Kyoto, Japan) in MeOH solutions. ^{1}H- and ^{13}C-NMR spectra were taken on Varian Gemini 300 (^{1}H, 300 MHz; ^{13}C, 75.5 MHz) and Varian Unity Plus 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard. Mass spectra: 70 eV. TLC: Merck RP-18 $F_{254}S$. CC: Sephadex LH-20, Silica gel 60 silanized (RP-2) and RP-18.

Chemicals (−)-Epicatechin 3-*O*-gallate (**1**), and (−)-epigallocatechin 3-*O*-gallate (**17**) were purchased from Wako Pure Chemical Co. (Osaka, Japan), pyrogallol (**4**) from Nacalai Tesque, Inc. (Kyoto, Japan), and (−)-epigallocatechin (**16**) from Kurita Chemical Co. (Tokyo, Japan). (−)-Epicatechin (**2**) was a product of Sigma Chemical Co. (U.S.A.) and (+)-catechin (**15**) was isolated in our laboratory. Methylation of (−)-epicatechin (**2**) with dimethyl sulfate was carried out in our laboratory. The identity of these compounds was confirmed by $[\alpha]_D$, ^{1}H- and ^{13}C-NMR spectroscopy before use. General anaerobic medium (GAM) was purchased from Nissui Co. (Tokyo, Japan).

Preparation of HIB Mixture Fresh fecal sample (5 g), obtained from a healthy subject, was homogenized with K-phosphate buffer (50 ml, pH 7.25), and the sediments were removed by filtration through gauze. The suspension was made up to 100 ml with the same buffer, and was used as an HIB mixture in this experiment.

Preparation of RIB Mixture A fresh fecal sample (5 g), obtained from male Wistar strain rats (weighing approx. 250 g; Sankyo Labo. Service, Tokyo, Japan), was homogenized in K-phosphate buffer (50 ml). The suspension obtained after filtration was added to GAM broth (1 l) and incubated for 16 h at 37 °C under anaerobic conditions. The culture was centrifuged at 7800 × *g* for 10 min and the pellets were washed once with K-phosphate buffer. The pellets were suspended in the same buffer to give 5% and 10% suspension (as final concentrations), and used as the RIB mixture.

Incubation of (−)-Epicatechin 3-*O*-Gallate (1) with HIB (−)-Epicatechin 3-*O*-gallate (**1**, 500 mg in 5 ml MeOH) was added to an HIB suspension (500 ml) and incubated at 37 °C in an anaerobic incubator for 48 h. The reaction mixture was then extracted with a BuOH–Et_2O (1 : 1) mixture (4 × 500 ml). The organic layer was filtered through anhydrous $MgSO_4$ and evaporated under reduced pressure to give a residue (1.02 g). The residue was subjected to a column of Sephadex LH-20 (30 × 3.0 cm i.d.), the elution being started with EtOH and then EtOH–MeOH with a gradual increase in MeOH. Fractions were pooled to give fr. A—D. Repeated MPLC (RP-18, MeOH–H_2O; 3 : 7) of fr. A (32 mg) gave **2** (3 mg), **5** (3 mg) and **6** (2 mg). Fraction B (46 mg) afforded **7** (1 mg), **8** (2 mg) and **12** (3 mg). MPLC (RP-18, MeOH–H_2O; 4 : 6) of fr. C (59 mg) afforded **3** (2 mg), **4** (5 mg), **9** (14 mg), **10** (15 mg), **12** (9 mg), **13** (5 mg) and **14** (1 mg). CC/RP-2 (50% aq. MeOH) of fr. D (23 mg) gave an additional amount of **9** (2 mg) and **12** (3 mg).

Incubation of (−)-Epicatechin (2) with HIB An HIB suspension (1 l) was supplemented with 1 g of (−)-epicatechin (**1**, dissolved in 5 ml MeOH) and anaerobically incubated at 37 °C for 24 h. The reaction mixture was then treated as mentioned above to give **5** (30 mg), **6** (4 mg), **7** (10 mg), **8** (1 mg), **9** (33 mg) and **12** (21 mg).

Incubation of (+)-Catechin (15) with HIB Similarly, catechin (**15**, 1 g in 5 ml MeOH) was incubated with an HIB suspension (1 l) and incubated for 24 h. The reaction mixture was then treated as mentioned above to give **9** (14 mg), **12** (12 mg), **18** (11 mg) and **19** (6 mg).

Incubation of Gallic Acid (3) with HIB Gallic acid (**3**, 200 mg) was anaerobically incubated with an HIB suspension (100 ml) for 24 h. After incubation, the reaction mixture was extracted with Et_2O (2 × 100 ml) and the Et_2O-layer was evaporated under reduced pressure. CC/RP-2 (50% aq. MeOH) of the ether extract afforded **4** (115 mg).

Incubation of 7 and 8 with HIB The incubation conditions previously described were used for 24 h after **7** or **8** (4 mg each) was added. After extraction with Et_2O, two products (**8**, **9**) could be detected in the incubate of **7**, while **9** was mainly detected in the incubate of **8**, as determined by TLC (RP-18, 40% aq. MeOH).

Comparative Degradation of 1, 2, 16 and 17 by HIB and RIB Degradation was carried out in Eppendorff's tubes containing an HIB or RIB suspension (1 ml each) where (−)-epicatechin 3-*O*-gallate (**1**) and the related compounds (**2**, **16**, **17**) were separately added to the bacterial suspensions (to give a final concentration of 1 mM). After incubation in an anaerobic incubator at 37 °C for 48 h, the tubes were picked up at intervals (12, 24, 48 h) and extracted with a BuOH–Et_2O mixture (1 : 1, 1 ml). For determination of the degradation profiles, 20 μl of the upper layer of each incubate was applied to a TLC plate (RP-18), developed with 40% aq. MeOH and analyzed by TLC-densitometry. The spots were detected after spraying with anisaldehyde–H_2SO_4 reagent, followed by heating, and were analyzed by a chromatoscanner at a sample wavelength of 500 nm relative to a reference wavelength of 700 nm. The calibration lines for **1**, **2**, **16** and **17** were linear in a range of 0.4—50 nmol/spot.

Compound **2**: Amorphous powder, $[\alpha]_D$ −58° (c = 0.5, MeOH). EI-MS m/z: 280 $[M]^+$. The compound was identified as (−)-epicatechin by direct comparison of the ^{1}H- and ^{13}C-NMR spectral data with those of an authentic sample.

Compound **3**: ^{1}H-NMR (MeOH-d_4) δ: 7.05 (2H, s, H-2, H-6). The compound was identified as gallic acid.

Compound **4**: ^{1}H-NMR (MeOH-d_4) δ: 6.40 (2H, d, J = 8.5 Hz, H-4, H-6), 6.50 (1H, t, J = 8.5 Hz, H-5). ^{13}C-NMR (MeOH-d_4) δ: 108.24 (d, C-4, C-6), 120.08 (d, C-5), 134.09 (s, C-2), 146.92 (s, C-1, C-3). ^{1}H- and ^{13}C-NMR spectral data are comparable with those of authentic pyrogallol.

Compound **5**: Amorphous powder, $[\alpha]_D$ −15.0° (c = 0.5, MeOH). UV λ_{max} (log ε) (MeOH): 279 (2.05) nm. EI-MS m/z: 292 $[M]^+$, 274 $[M-H_2O]^+$, 169, 148, 124. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound **6**: Amorphous powder, $[\alpha]_D$ −14.0° (c = 0.3, MeOH). UV λ_{max} (log ε) (MeOH): 280 (1.80) nm. EI-MS m/z: 276 $[M]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound **7**: Amorphous powder, $[\alpha]_D$ −8.6° (c = 0.5, MeOH). UV λ_{max} (log ε) (MeOH): 282 (2.1) nm. EI-MS m/z: 208 $[M]^+$. ^{1}H-NMR (500 MHz, MeOH-d_4) δ: 1.94 (1H, dddd, J = 16.2, 8.9, 7.2, 5.9 Hz, H-3b), 2.22 (1H, dddd, J = 16.2, 11.7, 6.8, 5.9 Hz, H-3a), 2.32 (1H, ddd, J = 16.4, 11.7, 6.8 Hz, H-2b), 2.47 (1H, ddd, J = 16.4, 8.9, 4.7 Hz, H-2a), 2.70 (1H, dd, J = 14.1, 5.9 Hz, H-5b), 2.87 (1H, dd, J = 14.1, 5.9 Hz, H-5a), 4.71 (1H, q, J = 5.9 Hz, H-4), 6.56 (1H, dd, J = 8.1, 2.1 Hz, H-6'), 6.68 (1H, d, J = 2.1 Hz, H-2'), 6.69 (1H, d, J = 8.1 Hz, H-5'). ^{13}C-NMR (125 MHz, MeOH-d_4) δ: 27.83 (t, C-3), 29.49 (t, C-2), 41.42 (t, C-5), 83.27 (d, C-4), 116.32 (d, C-2'), 117.62 (d, C-5'), 121.87 (d, C-6'), 129.00 (s, C-1'), 145.17 (s, C-4'), 146.26 (s, C-3'), 180.34 (s, C-1).

Compound **8**: Amorphous powder, $[\alpha]_D$ −11.6° (c = 0.1, MeOH). UV λ_{max} (log ε) (MeOH): 282 (2.1) nm. EI-MS m/z: 192 $[M]^+$. ^{1}H-NMR (500 MHz, MeOH-d_4) δ: 1.95 (1H, dddd, J = 18.5, 9.4, 7.5, 5.1 Hz, H-3b), 2.24 (1H, dddd, J = 18.5, 9.4, 4.5, 2.9 Hz, H-3a), 2.36 (1H, ddd, J = 14.1, 9.4, 4.7 Hz, H-2b), 2.49 (1H, dd, J = 14.1, 8.9 Hz, H-2a), 2.86 (1H, dd, J = 13.8, 6.6 Hz, H-5b), 2.94 (1H, dd, J = 13.8, 6.6 Hz, H-5a), 4.75 (1H, q, J = 6.6 Hz, H-4), 6.66 (1H, d, J = 2.5 Hz, H-2'), 6.69 (1H, dd, J = 7.4, 2.5 Hz, H-6'), 6.70 (1H, dd, J = 7.4, 2.5 Hz, H-4'), 7.10 (1H, t, J = 7.4 Hz, H-5'). ^{13}C-NMR (125 MHz, MeOH-d_4) δ: 28.05 (t, C-3), 29.47 (t, C-2), 42.49 (t, C-5), 82.97 (d, C-4), 114.67 (d, C-2'), 117.36 (d, C-4'), 121.67 (d, C-6'), 130.50 (s, C-1'), 158.57 (s, C-3'), 180.14 (s, C-1).

Compound **9**: Amorphous powder, EI-MS m/z: 210 $[M]^+$. ^{1}H-NMR (MeOH-d_4) δ: 1.59 (4H, m, H-2', H-3'), 2.28 (2H, br t, J = 7.0 Hz, H-4'), 2.46 (2H, br t, J = 7.0 Hz, H-1'), 6.48 (1H, dd, J = 8.0, 1.9 Hz, H-6), 6.60 (1H, d, J = 1.9 Hz, H-2), 6.65 (1H, d, J = 8.0 Hz, H-5).

Compound **10**: Amorphous powder, EI-MS m/z: 194 $[M]^+$. ^{1}H-NMR (MeOH-d_4) δ: 1.62 (4H, m, H-2', H-3'), 2.30 (2H, br t, J = 6.9 Hz, H-4'), 2.54 (2H, br t, J = 7.0 Hz, H-1'), 6.58 (1H, dd, J = 8.0, 1.9 Hz, H-6), 6.61 (1H, d, J = 1.9 Hz, H-2), 6.64 (1H, br d, H-4), 7.05 (1H, t, J = 8.0 Hz, H-5). ^{13}C-NMR (MeOH-d_4) δ: 24.19 (t, C-3'), 30.33 (t, C-2'), 33.63 (t, C-4'), 34.85 (t, C-1'), 112.59 (d, C-6), 115.10 (d, C-5), 118.90 (d, C-4), 129.10 (d, C-2), 143.39 (s, C-1), 157.23 (s, C-3), 174.53 (s, COOH).

Compound **11**: Amorphous powder, EI-MS m/z: 182 $[M]^+$. ^{1}H-NMR (MeOH-d_4) δ: 2.56 (2H, t, J = 7.1, H-2'), 2.84 (2H, t, J = 7.1 Hz, H-1'), 6.52 (1H, dd, J = 7.9, 2.0 Hz, H-6), 6.63 (1H, d, J = 2.0 Hz, H-2), 6.72 (1H, d, J = 7.9 Hz, H-5). ^{13}C-NMR (MeOH-d_4) δ: 33.01 (t, C-2'), 37.75 (t, C-1'), 115.14 (d, C-5), 117.16 (d, C-2), 121.55 (d, C-6), 131.49 (s, C-1), 159.49 (s, C-5), 177.86 (s, COOH).

Compound **12**: Amorphous powder, EI-MS m/z: 165 $[M-H]^+$. ^{1}H-NMR (MeOH-d_4) δ: 2.47 (2H, t, J = 6.6, H-2'), 2.75 (2H, t, J = 6.6 Hz, H-1'), 6.59 (1H, m, H-6), 6.64 (1H, m, H-2), 6.67 (1H, m, H-4), 7.06 (1H, t, J = 7.9 Hz, H-5). ^{13}C-NMR (MeOH-d_4) δ: 28.97 (t, C-2'), 37.48 (t, C-1'), 114.86 (d, C-5), 117.41 (d, C-2), 121.75 (d, C-6), 130.59 (s, C-1),

158.67 (s, C-5), 176.68 (s, COOH).

Compound **13**: Amorphous powder, EI-MS *m/z*: 208 $[M]^+$. ^{1}H-NMR (MeOH-d_4) δ: 1.61 (4H, m, H-2′, H-3′), 2.33 (2H, m, H-4′), 2.53 (2H, m, H-1′), 3.64 (3H, s, OCH_3), 6.58 (1H, m, H-6), 6.60 (1H, m, H-2), 6.63 (1H, m, H-4), 7.05 (1H, t, J=7.7 Hz, H-5).

Compound **14**: Amorphous powder, EI-MS *m/z*: 290 $[M]^+$. ^{1}H-NMR (500 MHz, MeOH-d_4) δ: 2.39 (2H, br t, H-2), 2.71 (2H, t, J=6.8 Hz, H-3), 6.24 (1H, dd, J=8.5, 2.1 Hz, H-2″), 6.25 (2H, dd, J=8.5, 2.1 Hz, H-4″), 6.53 (1H, dd, J=8.3, 1.9 Hz, H-6′), 6.64 (1H, d, J=1.9 Hz, H-2′), 6.90 (1H, d, J=8.3 Hz, H-5′), 6.94 (1H, t, J=8.5 Hz, H-3″). ^{13}C-NMR (125 MHz, MeOH-d_4) δ: 49.11 (t, C-2), 49.62 (t, C-3), 103.45 (d, C-2″), 107.01 (d, C-4″), 117.00 (d, C-2′), 117.20 (d, C-5′), 121.80 (d, C-6′), 131.00 (d, C-3″), 132.73 (s, C-1′), 138.21 (s, C-6″), 141.80 (s, C-4′), 144.60 (s, C-3′), 145.01 (s, C-5″), 164.99 (s, C-1).

Compound **18**: Amorphous powder, $[\alpha]_D$ +20.0° (c=0.5, MeOH). UV λ_{max} (log ε) (MeOH): 280 (2.50) nm. EI-MS *m/z*: 292 $[M]^+$, 274 $[M-H_2O]^+$, 169, 148, 124. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound **19**: Amorphous powder, $[\alpha]_D$ +12.0° (c=0.3, MeOH). UV λ_{max} (log ε) (MeOH): 273 (1.83) nm. EI-MS *m/z*: 276 $[M]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

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