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Potential α-glucosidase inhibitors from thermal transformation of (+)-catechin

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ABSTRACT

Thermal transformation of the (+)-catechin (**1**) with heating processing afforded a new oxidation product, gambiriin D (**2**), along with catechin [6′–8]-catechin (**3**), and (+)-epicatechin (**4**). The structure of a new catechin dimer with *C–C* linkage was determined on the basis of spectroscopic data interpretation. The catechin dimers **2** and **3** exhibited significantly improved inhibitory activities against α-glucosidase, with IC_{50} values of 0.16 ± 0.2 and 0.14 ± 0.2 μM, respectively, when compared to parent (+)-catechin. Kinetic analysis showed that the two effective compounds **2** and **3** have noncompetitive modes of action.

Diabetes mellitus is one of the most common chronic diseases in populations worldwide and belongs to the group of metabolic disorders which are characterized by high blood glucose levels. Diabetes mellitus is closely associated with cardiovascular disease, as the major cause of morbidity and mortality.[1] Also, the serious complications associated with diabetes mellitus, such as peripheral vascular disease, diabetic neuropathy, amputations, renal failure, stroke, and blindness result in increasing disability, reduced life expectancy, and enormous health costs.[2] It is an important and growing public health problem, due largely to the substantial financial loss incurred for appropriate disease control and management of chronic complications. The strategy of the most effective treatment for type 2 diabetes mellitus is to achieve optimal blood glucose levels after a meal. Recently, a more effective strategy for the treatment of type 2 diabetes mellitus has involved the disturbance of dietary monosaccharide absorption by inhibition of α-glucosidase.[3] Thus, α-glucosidase inhibitors are considered to be a valuable therapeutic reagent for treating type 2 diabetes mellitus in humans. Several α-glucosidase, including acarbose and voglibose from natural sources are representative anti-diabetic agents that affect blood glucose levels after food intake, are clinically used in the effective treatment of type 2 diabetes mellitus.[4] Only a few α-glucosidase inhibitors are clinically available and they are all sugar mimics with tedious multi-steps during preparation and problems of gastrointestinal side-effects. In recent years substantial efforts to discover effective non-sugar inhibitors of α-glucosidase from natural sources have been conducted and have received great attention because of the high levels of natural abundance and biological efficacies.[5,6]

Catechins are among the most well-known flavonoids and are potentially beneficial to human health. The naturally occurring compounds are widely distributed in various fruits, green tea, red wine, juices, and in chocolate.[7] Catechin derivatives have been shown to possess a wide spectrum of significant biological efficacies, such as antioxidant, anticancer, and anti-inflammatory properties. In addition, recent studies have demonstrated that naturally occurring catechin polymers, procyanidins, are also known to have various physiological functions, including antioxidant, anti-inflammation, antitumor, and vascular protective effects.[8–10] Recently, several researchers have attempted to create new useful procyanidin derivatives by structural modifications[11,12] and have verified that thermal processing of inactive compounds is a useful method for bringing about structural modifications and improving the bioactivity of natural products.[13,14] In addition, the presence of catechin derivatives by thermal processing in various primary foods is closely related to human health and it is necessary to evaluate their levels under thermal conditions because of possible biological effects and safety. As part of our continuing investigation into creating bioactive compounds of inactive ubiquitous natural

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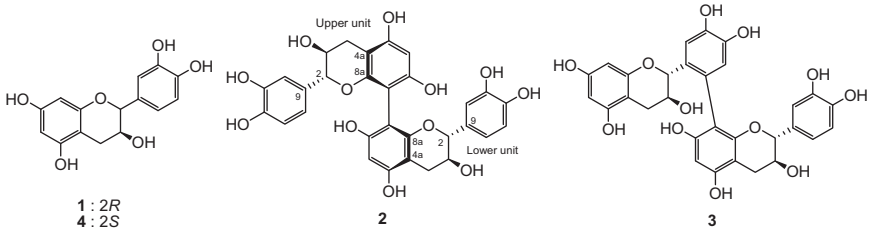

Figure 1. Structures of thermal transformation products **2–4** of (+)-catechin.

Table 1
^{1}H and ^{13}C NMR chemical shifts of compound **2**[a]

Position	Upper unit		Lower unit	
	δ_H[b] (*J* in Hz)	δ_C, mult.	δ_H[b] (*J* in Hz)	δ_C, mult.
2	4.61 (d, 8.4)	83.4	4.61 (d, 8.4)	83.4
3	4.02 (ddd, 8.4, 7.4, 5.4)	67.5	4.02 (ddd, 8.4, 7.4, 5.4)	67.5
4	2.90 (dd, 16.2, 5.4)	28.9	2.90 (dd, 16.2, 5.4)	28.9
4	2.51 (dd, 16.2, 8.4)		2.51 (dd, 16.2, 8.4)	
4a	—	100.9	—	100.9
5	—	155.2	—	155.2
6	6.00 (s)	96.8	6.00 (s)	96.8
7	—	155.0	—	155.0
8	—	105.1	—	105.1
8a	—	152.4	—	152.4
9	—	130.5	—	130.5
10	6.93 (d, 1.8)	115.4	6.93 (d, 1.8)	115.4
11	—	145.9	—	145.9
12	—	145.6	—	145.6
13	6.80 (d, 7.8)	115.8	6.80 (d, 7.8)	115.8
14	6.75 (dd, 7.8, 1.8)	120.0	6.75 (dd, 7.8, 1.8)	120.0

[a] ^{1}H NMR measured at 600 MHz, ^{13}C NMR measured at 150 MHz; obtained in acetone-d_6 + D_2O with TMS as internal standard. Assignments based on HSQC and HMBC NMR spectra.
[b] *J* values (Hz) are given in parentheses.

constituents using γ-irradiation and polyphenol oxidase,[15,16] we herein report the biotransformation of (+)-catechin using a heating processing, forming a new catechin dimer that exhibited significantly improved inhibitory effects against α-glucosidase than their parent (+)-catechin.

A sample solution containing (+)-catechin in H_2O was directly heated for 3 h[17] and the converted products were monitored by HPLC analysis. The dried resultant exhibited significant inhibitory activity, with an IC_{50} value of 11.3 ± 1.3 μg/mL in a α-glucosidase inhibition assay (Table 3). In the present investigation, column chromatographic purification of the 3 h reactant led to the isolation of the new catechin derivative, gambiriin D (**2**), along with catechin [6′–8]-catechin (**3**),[18] and (+)-epicatechin (**4**).[19] The known compounds were identified by comparison of their spectroscopic data with literature values (Fig. 1).

Compound **2** was obtained as a yellow amorphous powder, $[\alpha]_D^{25}$ − 134.3° (MeOH). Its molecular formula was determined to be $C_{30}H_{26}O_{12}$ using positive HRESIMS, which showed a pseudomolecular ion peak at *m*/*z* 601.1317 $[M+Na]^+$. The absorption maxima at 280 nm in the UV spectrum were attributed to a flavan-3-ol skeleton.[20] In the ^{1}H NMR spectrum of **2** (Table 1), the four protons in the aromatic region exhibited characteristic signals for one ABX-type aromatic signals at δ_H 6.93 (1H, d, *J* = 1.8 Hz, H-10), 6.80 (1H, d, *J* = 7.8 Hz, H-13), and 6.75 (1H, dd, *J* = 7.8, 1.8 Hz, H-14), one isolated aromatic proton at δ_H 6.00 (1H, s, H-6), indicating the presence of two aromatic ring systems with different substitution patterns. Also observed were two oxygenated methine protons at δ_H 4.61 (1H, d, *J* = 8.4 Hz, H-2) and 4.02 (1H, ddd, *J* = 8.4, 7.4, 5.4 Hz, H-3), which showed a characteristic large coupling constant associated with the 2,3-*trans* configuration,[21] and one methylene at δ_H 2.90 (1H, dd, *J* = 16.2, 5.4 Hz, H-4) and 2.51 (^{1}H, dd, *J* = 16.2, 8.4 Hz, H-4). Consistent with these ^{1}H NMR observations, the ^{13}C NMR and HSQC experiments of **2** further displayed the presence of signals for catechin skeleton at δ_C 83.4 (C-2), 67.5 (C-3), 28.9 (C-4).[22] These data closely resemble those for the parent compound, (+)-catechin (**1**),[18] except for the absence of an aromatic proton at the C-8 position in **1**. Peak at *m*/*z* 601 in the positive HRESIMS of the sodiated molecule was consistent with **2**, being a dimer of (+)-catechin (**1**). The absence of a H-8 proton signal in the ^{1}H NMR spectrum of **2** suggested that the intermolecular linkage was at C-8 in both halves of the dimer. HMBC experiments allowed for the complete assignment of the ^{1}H and ^{13}C NMR spectra of **2**, and a summary of key correlations observed for **2** is shown in Figure 2. The absolute configurations at C-2 and C-3 were determined by circular dichroism (CD) spectroscopy. The CD spectrum displayed a negative Cotton effect around 280 nm, indicating 2*R*,3*S*-configuration of the upper and lower units.[22,23] The *R*-configuration of the biphenyl moiety in **2** was supported by a negative Cotton effect at 212 nm and a positive Cotton effect at 241 nm by comparison of the CD spectrum of theasinensin C having an *R*

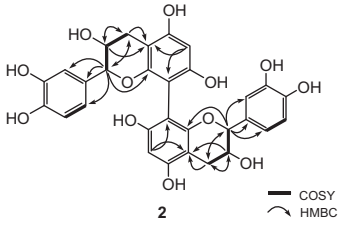

Figure 2. Selected COSY and HMBC correlations of **2**.

Table 2
Relative amounts of individual constituents in the reacted mixtures

Compound	Time (min)	1 h Content (%)	2 h Content (%)	3 h Content (%)	6 h Content (%)	12 h Content (%)
1	8.25	84.7	80.4	76.3	68.0	67.1
2	18.69	1.2	1.6	2.5	2.9	2.9
3	7.41	1.3	1.8	2.8	3.0	3.0
4	11.82	12.8	16.1	18.4	26.1	27.0

Table 3
Inhibitory effects of isolated compounds **2**–**5** on α-glucosidase

Reactant (h)	IC_{50} value[a] (μg/mL)	Compound	IC_{50} value[a] (μM)	Type of inhibition
1	52.1 ± 1.7	**1**	51.7 ± 1.4	NT[c]
2	27.7 ± 1.5	**2**	0.16 ± 0.2	Noncompetitive
3	11.3 ± 1.3	**3**	0.14 ± 0.2	Noncompetitive
6	18.2 ± 1.6	**4**	176.6 ± 2.2	NT[c]
12	25.1 ± 1.8	Acarbose[b]	300.1 ± 3.5	

[a] All compounds were examined in triplicate experiments.
[b] Used as positive control.
[c] NT is not tested.

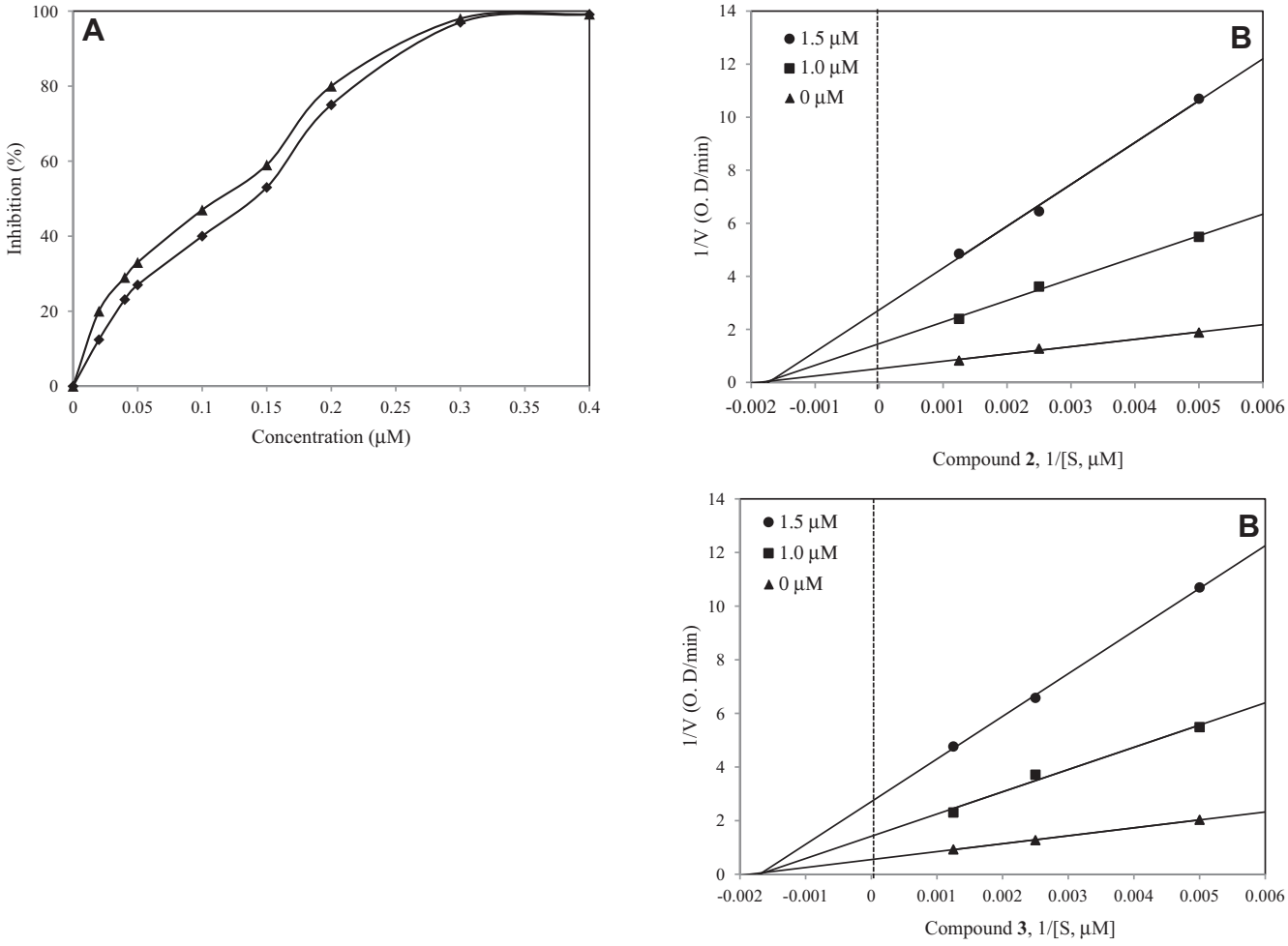

Figure 3. Effects of compounds **2** and **3** on the activity of α-glucosidase. (A) Dose dependent inhibition of α-glucosidase activity (compound **2**, ♦; compound **3**, ▲). (B) Lineweaver-Burk plot of PNP release by α-glucosidase in the presence of compounds **2** and **3** in phosphate buffer (pH 7.0) at 37 °C.

biphenyl bond.[24] Therefore, the structure of compound **2** was assigned as gambiriin D,[25] which is a new thermal transformation product of (+)-catechin.

All pure isolates obtained in the present investigation were evaluated for their anti-diabetic activity against the α-glucosidase[26] using acarbose as the positive control. The α-glucosidase

inhibitory activity of structurally modified catechin derivatives **2** and **3** were found to be significantly improved over that of their parent (+)-catechin (**1**) (Table 2). Catechin [6′–8]-catechin (**3**), with *C*–*C* linkage between C-6′ of B-ring for the upper unit and C-8 of B-ring for the lower unit, displaying the most potent α-glucosidase inhibitory activity, in a concentration-dependent manner (Fig. 3A), and with an IC_{50} value of 0.14 ± 0.2 μM. While, the isolated (+)-epicatechin (**4**) showed relatively weak inhibitory activity with an IC_{50} value of 176.6 ± 2.2 μM. As shown in Figure 3B, the inhibition kinetics of α-glucosidase of gambiriin D (**2**) and catechin [6′–8]-catechin (**3**) analyzed by Lineweaver–Burk plots showed compounds **2** (k_i, 0.25 μM) and **3** (k_i, 0.24 μM) were noncompetitive inhibitors because an increasing concentration of substrate resulted in a family of lines which declined with a common intercept on the *x*-axis.[27]

Thermal processing of pure (+)-catechin was carried out at 121 °C for five different times 1, 2, 3, 6, and 12 h. In order to estimate the relationship between activity and composition of the reactants, the relative contents of the active compounds was determined by HPLC (Table 2). While no significant production of **2** and **3**, as the most active compounds, were observed at the reacted mixtures, there was a maximal production of gambiriin D (**2**) and catechin [6′–8]-catechin (**3**) after 3 h thermal treatment. As the reaction time increased, the production of relatively inactive (+)-epicatechin slightly increased, and reached a maximum of 27% relative content in the 12 h reactant. On the other hand, the decreasing of (+)-catechin content was readily detected by thermal processing (Table 2).

During the last decade, structure modification of (+)-catechin to improve its bioactivity has been performed using microbial and enzymatic transformations.[11,28,29] In addition, previously studies have revealed that catechin oligomers from natural products are major inhibitors against α-glucosidase.[30,31] In this investigation, the structural modification of (+)-catechin using heating processing showed potentially enhanced anti-diabetic activity against α-glucosidase and this finding indicated that replacement of the A-ring at the C-8 of (+)-catechin is correlated with an increase in biological activity.

The results of the current study verify that natural (+)-catechin (**1**) is transformed into a new modified products, gambiriin D (**2**), along with catechin [6′–8]-catechin (**3**), (+)-epicatechin (**4**), and the structure of new compound was elucidated by interpretation of spectroscopic data. The catechin dimers **2** and **3** exhibited more potent anti-diabetic activity against α-glucosidase than the parent (+)-catechin. The biotransformation of (+)-catechin by heat processing may be a valuable and convenient strategy for structural modification and the enhancement of activity. More systematic structural modifications will be performed for the further development activity improvement of (+)-catechin.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.027.

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17. A sample solution of (+)-catechin (1.0 g) in H_2O (1.0 L) in chapped vials was autoclaved at 121 °C for 1, 2, 3, 6, and 12 h, respectively. The inhibitory activities against α-glucosidase are summarized in Table 3 and the 3 h heating reactant exhibited the most enhanced inhibitory activity when compared to parent (+)-catechin. The unreacted parent compound (**1**) (603 mg) after heating treatment for 3 h was removed by crystallization and the dried reactant was directly subjected to column chromatography over a YMC GEL ODS AQ 120-50S column (1.1 cm i.d. × 33 cm) with aqueous MeOH, to yield pure compounds **2** (4.3 mg, t_R 18.7 min), **3** (4.5 mg, t_R 7.3 min), and **4** (63.7 mg, t_R 11.7 min). HPLC analysis was carried out on a YMC-Pack ODS A-302 column (4.6 mm i.d. × 150 mm; YMC Co., Ltd) and the solvent system consisted of a linear gradient that started with 10% (v/v) MeCN in 0.1% HCOOH/H_2O (detection: UV 280 nm; flow rate: 1.0 ml/min; at 40 °C), increased to 80% MeCN over 23 min, and then increased to 100% MeCN over 5 min.
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25. Gambiriin D (**2**): Yellow amorphous powder, $[\alpha]_D^{25}$ − 134.3° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 280 (2.90), 230 (sh) (3.86), 206 (4.20) nm; CD (MeOH) Δε: 212 (−0.49), 241 (+0.11), 282 (−0.49) nm; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 601 [M+Na]$^+$, HRFABMS *m*/*z* 601.1317 [M+Na]$^+$ (calcd for $C_{30}H_{26}O_{12}Na$, 601.1322).
26. Assay of α-glucosidase inhibitory activity: a previously reported method with a minor modification (Kim, Y. M.; Wang, M. H.; Rhee, H. I. *Carbohydr. Res.* **2004**, *339*, 715.) was used for the evaluation of the ability of the compounds to inhibit α-glycosidase. Briefly, α-glucosidase (1 μL; EC 3.2.1.20) was incubated in 100 mmol L^{-1} potassium phosphate buffer (pH 6.8). Sample solution (1 μL) was premixed with 94 μL of 100 mmol L^{-1} potassium phosphate buffer (pH 6.8). After incubation at 37.5 °C for 20 min, substrate (3 mmol L^{-1} *p*-NPG) was added to initiate the reaction. The reaction mixture was incubated at 37.5 °C for 30 min, 100 μL of 0.1 mol L^{-1} Na_2CO_3 was then added to stop the reaction. The amount of released *p*-nitrophenol was measured at 410 nm using a UV microplate reader (Infinite F200; Tecan Austria GmBH, Grödig, Austria). The half-maximal inhibitory concentration (IC_{50}) value was calculated by linear regression analysis of activity under the assay conditions. Acarbose was used as a positive control and all assays were carried out in triplicate. Kinetic parameters were determined using the Lineweaver-Burk double-reciprocal plot at increasing concentrations of substrate and inhibitor.
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