

## Offset $\pi$ - $\pi$ Interaction in Crystal Structure of (–)-Gallocatechin-3-O-gallate

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The single crystal of (–)-gallocatechin-3-O-gallate (GCg) was prepared using a solution containing an equimolecular amount of GCg and (–)-epigallocatechin-3-O-gallate (EGCg) in water. The crystal structure of GCg determined by X-ray crystallographic analysis was monoclinic with the space group  $P2_1$  at 223 K. Offset  $\pi$ - $\pi$  interactions formed between the A and A rings, B and B rings, and gallate and gallate rings of GCg, and five intermolecular hydrogen bonds formed between GCgs, GCg and water. The B ring of GCg bonded to C2 was in the axial position and the gallate ring of GCg bonded to C3 was in the pseudoaxial position with respect to the C ring of GCg.

**Key words** (–)-gallocatechin-3-O-gallate; single crystal structure; offset  $\pi$ - $\pi$  interaction; intermolecular hydrogen bond; X-ray crystallographic analysis

Catechins belong to the family of flavan-3-ol flavonoids, and consist of two aromatic rings (A and B rings) connected by a pyran ring (C ring). It is known that these abound in certain species of plants, such as tea (*Camellia sinensis*, Camelliaceae), and have beneficial effects, such as anti-hypercholesterolemic,<sup>1,2)</sup> anti-bacterial,<sup>3,4)</sup> anti-oxidative,<sup>5,6)</sup> and anti-cancer.<sup>7,8)</sup> In particular, it is known that gallate-type catechins, such as (–)-epigallocatechin-3-O-gallate (EGCg) and (–)-gallocatechin-3-O-gallate (GCg) have high activities in tea catechins. Previously, most studies of catechins have focused on EGCg, which is thought to be most abundant with highest biological activity in catechins contained in tea. However, in a recent study, Ikeda *et al.* reported that GCg strongly inhibited cholesterol absorption<sup>9)</sup> and Lee *et al.* reported that GCg increased very low density lipoprotein receptor protein and activity to a greater extent than EGCg<sup>10)</sup>; therefore, GCg is attracting a great deal of attention presently. Also, various intermolecular interactions between catechins and biomolecules have been thought to regulate various bioactivities, and so Hayashi and Ujihara synthesized two water-soluble acyclic phase receptors, and investigated the binding of these receptors to eight catechin analogues with <sup>1</sup>H-NMR spectroscopy.<sup>11)</sup>

It is important to know the detailed structure of GCg to study of the relation between the structure and activity of GCg, but few structural studies of GCg have been reported to date. In particular, its crystal structure has not been reported because of the difficulty of crystallizing GCg. The crystallization of GCg was attempted and finally succeeded using a novel crystallization method. Originally, we attempted to synthesize a 1 : 1 complex of GCg and EGCg using a solu-

tion containing an equimolecular amount of GCg and EGCg in water<sup>12)</sup>; however, the solution afforded only a single crystal of GCg. The crystal structure of GCg was determined by X-ray crystallographic analysis. The interactions and conformation of the crystal structure of GCg were also investigated.

### Results and Discussion

GCg was crystallized using a different solubility between GCg and EGCg for water.

EGCg (10 mg,  $2.18 \times 10^{-2}$  mol) was dissolved in distilled water (120  $\mu$ l) at 90 °C, and then GCg (10 mg,  $2.18 \times 10^{-2}$  mol) was added to the solution and dissolved. The solution containing an equimolecular amount of GCg and EGCg was left at room temperature for a few days to afford a colorless block-shaped single crystal (0.40  $\times$  0.21  $\times$  0.15 mm) of only GCg, and EGCg and a part of GCg was still soluble in the

Table 1. Crystal and Experimental Data of GCg Alone

GCg alone C <sub>22</sub> H <sub>20</sub> O <sub>12</sub>	
Formula weight	476.39
Crystal system	Monoclinic
Space group	$P2_1$
Crystal size	0.40 $\times$ 0.21 $\times$ 0.15 mm
Crystal characterization	Colorless block
Temperature	223 K
Wavelength $\lambda$ CuK $\alpha$	1.54187 Å
Unit cell dimensions	$a = 4.81345(11)$ Å $\alpha = 90^\circ$ $b = 16.8259(4)$ Å $\beta = 97.0571(15)^\circ$ $c = 12.4737(3)$ Å $\gamma = 90^\circ$
Volume, Z	1002.6(4) Å <sup>3</sup> , 2
Calculated density	1.578 g/cm <sup>3</sup>
Absorption coefficient	1.129 mm <sup>-1</sup>
$F(000)$	496
Index ranges	$h = -5 \rightarrow 5$ $k = -19 \rightarrow 20$ $l = -14 \rightarrow 14$
Collected reflections	9537
Unique reflections	3378
$R_1$ for $F_0 > 2$ sigma ( $F_0$ )	0.0343
$wR_2$ for all data	0.0979
Goodness of fit	1.002
Maximum difference peaks/e/Å <sup>3</sup>	0.49
Minimum difference peaks/e/Å <sup>3</sup>	-0.39

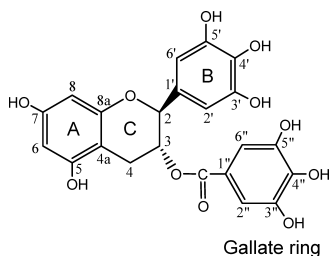


Fig. 1. (–)-Gallocatechin-3-O-gallate

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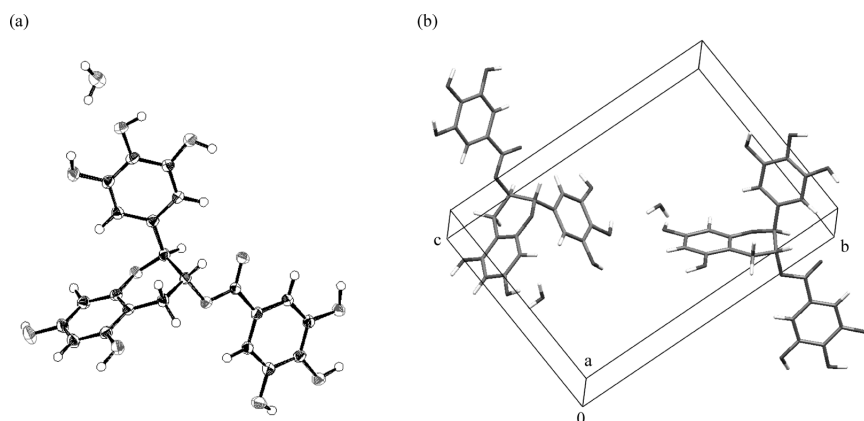


Fig. 2. (a) ORTEP Drawing Determined by X-Ray Crystallographic Analysis and (b) One Unit Cell of GCg Alone

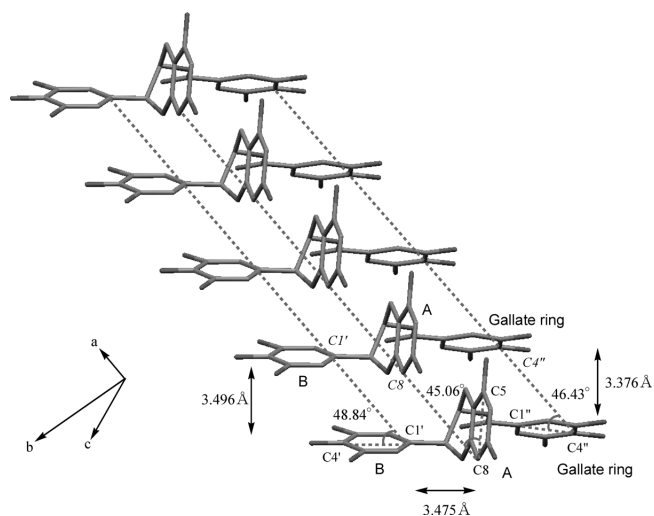


Fig. 3. Layer of GCg Alone

Hydrogen atoms are omitted for clarity. Arrows indicate the distance between planes. Dotted lines indicate angles. Italic  $C1'$ ,  $C8$  and  $C4''$  moved along the  $a$ -axis with  $C1'$ ,  $C8$  and  $C4''$ .

solution.

This single crystal of GCg was determined by X-ray crystallographic analysis at 223 K. The crystal and experimental data of GCg are shown in Table 1.

As a result, this single crystal was monoclinic with the space group  $P2_1$ . One unit cell dimensions were  $a = 4.81345(11)$  Å,  $b = 16.8259(4)$  Å and  $c = 12.4737(3)$  Å, respectively. The molecular conformation of GCg is shown in Fig. 2a. There are two GCg molecules and two water molecules in one unit cell (Fig. 2b). These GCg molecules are related by two-fold screw symmetry.

In the layer structure, GCg faced the same direction and accumulated parallel to the  $a$ -axis. The distances between the planes of A rings, B rings and gallate rings of GCgs in the layer structure were 3.475 Å, 3.496 Å and 3.376 Å, respectively. Also, the angles of  $C5-C8-C8$ ,  $C4'-C1'-C1'$ , and  $C1''-C4''-C4''$  in Fig. 3 were 45.06°, 48.84°, and 46.43°, respectively. Namely, A rings, B rings and gallate rings of GCg mutually shifted slightly.

Intermolecular interactions between GCg molecules to form the crystal structure were also investigated. As indicated in Fig. 3, offset  $\pi$ - $\pi$  interactions, which are not caused by the aromatic rings facing each other as in face-to-face

Table 2. Parameters of Selected Hydrogen Bonds of GCg Alone

D-H	A	D...A	D-H	H...A	$\angle D-H...A$
$C5-O-H$	$O=C$	2.728	1.014	1.750	160.77
$C4''-O-H$	$O-C5'$	2.734	0.953	1.810	162.29
$C5''-O-H$	$O-C7$	2.694	0.972	1.746	164.30
$C3'-O-H$	$O-C5$	2.801	0.919	1.933	156.53
$C7-O-H$	$O$ (water)	2.677	0.897	1.834	155.73

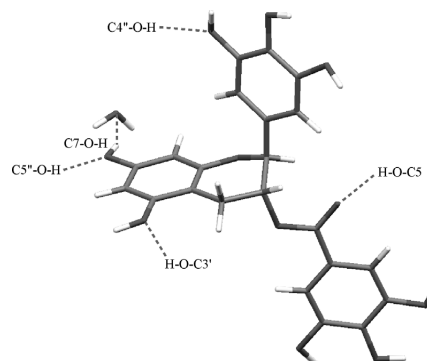


Fig. 4. Hydrogen Bonds of GCg Alone

Dotted lines indicate hydrogen bonds.

$\pi$ - $\pi$  interaction but the aromatic rings are mutually shifted a little, formed between A and A rings, B and B rings and gallate and gallate rings of GCgs; however, no face-to-face  $\pi$ - $\pi$  interaction was observed in the layer of GCg. Furthermore, five hydrogen bonds were observed between GCgs, GCg and water and, as a result, a network of hydrogen bonds formed in the crystal structure of GCg (Fig. 4, Table 2). Therefore, the driving forces behind forming the crystal structure of GCg were thought to be offset  $\pi$ - $\pi$  interactions and a network consisting of intermolecular hydrogen bonds.

Conformational flexibility includes orientation of the linkage between B and gallate and C rings owing to puckering of the pyran C ring. It is expected that B and gallate rings of GCg take both equatorial positions rather than both axial positions because, generally speaking, the equatorial position of a large substitution group is more stable than the axial position. The torsion angles of  $H-C2-C3-H$  and  $C1'-C2-C3-O$  of GCg alone were 72.80° and 159.03°, respectively, indicating that B and gallate rings of GCg took axial and pseudoaxial positions with respect to the C ring, respectively (Fig. 5a, Table 3).

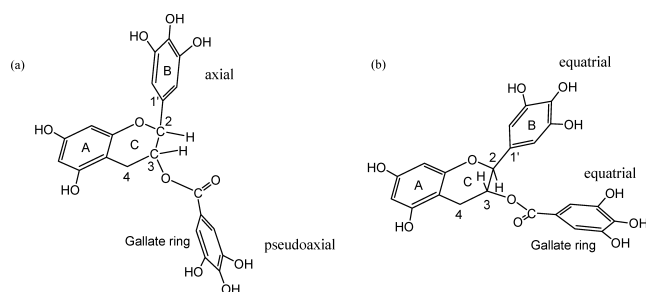


Fig. 5. Conformation of GCg Moiety in Crystalline State

(a) GCg alone, (b) 1 : 2 complex of GCg and caffeine.

Table 3. Torsion Angles in GCg Alone and 1 : 2 Complex of GCg and Caffeine

	GCg alone	1 : 2 complex of GCg and caffeine
Torsion angle (H–C2–C3–H)	72.80°	173.18°
Torsion angle (C1'–C2–C3–O)	159.03°	55.93°
Torsion angle (O–C2–C3–H)	171.33°	69.59°
Torsion angle (H–C2–C3–C4)	162.46°	54.81°

For comparison of conformation, the B and gallate rings of the GCg moiety in the 1 : 2 complex<sup>13)</sup> of GCg and caffeine, which was reported previously, were both in equatorial positions (Fig. 5b, Table 3).

The offset  $\pi$ – $\pi$  interactions form between A and A rings, B and B rings and gallate and gallate rings of GCgs. Furthermore, five hydrogen bonds form between GCgs and GCg and water in the crystal structure of GCg. These interactions have a significant influence on the conformation of GCg. As a result, it is thought that the cooperative effect of these interactions makes it possible to take axial and pseudoaxial positions in B and gallate rings.

## Experimental

**Material** GCg was supplied by Nagara Science Co., Ltd. and used without further purification.

**X-Ray Crystallographic Analysis** The single crystal of GCg alone was determined by X-ray crystallographic analysis. X-ray intensity data of 9537 reflections (of which 3378 were unique) were collected on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated CuK $\alpha$  radiation ( $\lambda$ =1.54187 Å). The data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods using SIR2004<sup>14)</sup> and expanded using Fourier techniques.<sup>15)</sup> Some non-hydrogen atoms were refined anisotropically, while the rest were refined isotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on  $F^2$  was based on 3378 observed reflections and 322

variable parameters and converged with unweighted and weighted agreement factors of:  $R = \sum ||F_o| - |F_c|| / \sum |F_o| = 0.0343$ ,  $R_w = [\sum (w(F_o^2 - F_c^2)^2) / \sum w(F_o^2)^2]^{1/2} = 0.0979$ .

The standard deviation of an observation of unit weight was 1.00. A Sheldrick weighting scheme was used. Plots of  $\sum w(|F_o| - |F_c|)^2$  versus  $|F_o|$ , reflection order in data collection,  $\sin \theta / \lambda$  and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.49 and  $-0.39$  e/Å<sup>3</sup>, respectively. All calculations were performed using the CrystalStructure crystallographic software package.<sup>16,17)</sup> These crystallographic data have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no.715904.

**Acknowledgements** The authors thank Dr. Kazunobu Harano for fruitful discussion about intermolecular interactions of the crystal structure of GCg.

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