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Stereochemical Structure Determination of Caffeine Complexes with Galloylated and Non-galloylated Catechins

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Stereochemical structures of a 1:1 complex of (−)-epicatechin (EC) and caffeine, and a 2:4 complex of (−)-epicatechin gallate (ECg) and caffeine were determined by X-ray crystallographic analysis, and noncovalent interactions forming between EC, ECg and caffeine moieties were also elucidate.

Catechins are a group of polyphenols that occur naturally in certain species of plants, including tea (*Camellia sinensis*, Camelliaceae), and are major ingredients in green tea infusions. The role of such catechins in the prevention of cancer and cardiovascular disease, antiaging, and dietietics has received a great deal of attention.[1–3] The catechins in green tea are mainly classified into two categories by the existence of a galloyl group on the oxygen atom at the C3 position; galloylated and non-galloylated catechins.[4] Generally, galloylated catechins show higher activities than non-galloylated catechins.[5–8] Caffeine is an alkaloid that has a central nervous system-stimulating effect and is also the other major component of tea. Interestingly, it is known that polyphenols form complexes with caffeine, especially in black tea and coffee.[9–11] Such complexes were thought to be a unique stereochemical structure and to form interesting noncovalent interactions between catechins and caffeine. Thus, many researchers have been investigating the structure of the complexes of catechins and caffeine.[12–14] These structural studies of the complexes of catechins and caffeine were performed in solution using NMR techniques, but their complete structures and the detailed interactions between catechins and caffeine have not been elucidated sufficiently.

We have investigated the stereochemical structures of the complexes of catechins and caffeine by X-ray crystallographic analysis.[15,16] In this study, we focused on (−)-epicatechin (EC) of the non-galloylated catechins and (−)-epicatechin gallate (ECg) of the galloylated catechins (Figure 1), which are included in Japanese green tea as the major catechins. The structural difference between EC and ECg is the presence of a galloyl group on the oxygen atom at the C3 position or absence, and all others are the same.

We prepared crystals of the complexes of EC, ECg and caffeine. X-ray crystallographic analysis of the complexes were performed to determine the crystal stereochemical structures and to elucidate the detailed noncovalent interaction between EC, ECg and caffeine moieties. Furthermore, we investigated the difference in the stereochemical structures having a galloyl group or not. A solution containing equimolecular amounts of EC and caffeine in water was lyophilized to give a colorless powder. The powder was recrystallized from methanol to afford colorless block crystals.

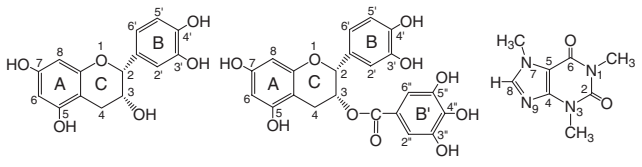

Figure 1.

A single crystal ($0.50 \times 0.40 \times 0.11\,mm^3$) was determined to be a 1:1 complex of EC and caffeine by X-ray crystallographic analysis and was monoclinic with space group $C2$ (No. 5).[17] One unit cell dimensions were $a = 27.2246(12)$ Å, $b = 6.6953(3)$ Å, and $c = 17.4322(8)$ Å, respectively. An ORTEP drawing of a unit of the 1:1 complex of EC and caffeine is shown in Figure 2a. One unit cell contained four units of the 1:1 complex of EC and caffeine and eight water molecules as crystal solvent (Figure 2b).

EC and ECg molecules have conformational flexibility, including orientation of the linkage between B, B′ and C rings owing to puckering of the pyran C ring. The caffeine molecule has a plain and rigid xanthine skeleton. The torsion angles of the EC moiety of the 1:1 complex (Table 1) indicated that the B ring and 3-OH group of EC were in equatrial and axial positions with respect to the C ring of the EC molecule, respectively.

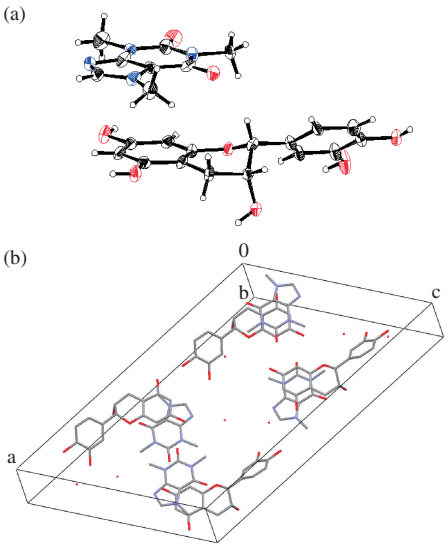

Figure 2. Crystal structure of the 1:1 complex of EC and caffeine. (a) ORTEP drawing with thermal ellipsoid at 30% probability level. Crystal solvent is omitted for clarity. (b) One unit cell. Hydrogen atoms are omitted for clarity.

Table 1. Torsion angle in EC alone, 1:1 complex of EC and caffeine, and 2:4 complex of ECg and caffeine

Torsion angle	EC alone[18]	EC in 1:1 complex	ECg in 2:4 complex	
			A	B
$\angle O_1$–C_2–C_3–H_3/°	179.60	179.56	175.15	174.05
$\angle H_2$–C_2–C_3–O_3/°	178.77	178.62	171.68	169.87
$\angle H_2$–C_2–C_3–H_3/°	60.68	60.23	68.46	71.06
$\angle H_3$–C_3–C_4–$H_{4\beta}$/°	43.42	31.52	40.69	45.18
$\angle O_3$–C_3–C_4–$H_{4\alpha}$/°	42.36	31.83	43.99	48.03

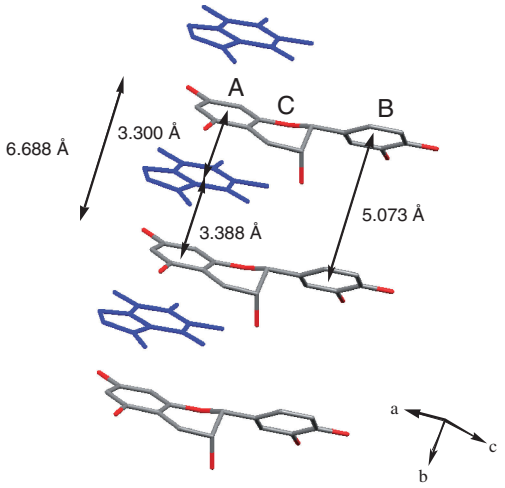

Figure 3. Layer structure of the 1:1 complex of EC and caffeine. Blue molecules are caffeine. Hydrogen atoms and crystal solvent are omitted for clarity.

In the layer structure shown in Figure 3, units of the 1:1 complex of EC and caffeine stacked in parallel in the same direction as the *b* axis. The A ring of EC and the six-membered ring of caffeine appear in turn along the *b* axis, and the six-membered rings of caffeines were located in almost the middle of the A rings of ECs. The A rings of both upper and lower ECs faced the six-membered ring of the caffeine (Figure 3).

A suspension containing equimolecular amounts of ECg and caffeine in water was heated at 90 °C, and left at room temperature to give a colorless powder. The powder was recrystallized from water to give colorless block crystals. A single crystal ($0.35 \times 0.25 \times 0.16\,mm^3$) was determined to be a 2:4 complex of ECg and caffeine by X-ray crystallographic analysis and was monoclinic with space group $P2_1$ (No. 4).[19] One unit cell dimensions were $a = 14.0190(7)$ Å, $b = 8.9403(4)$ Å, and $c = 31.4760(15)$ Å, respectively. An ORTEP drawing of a unit cell of the 2:4 complex of ECg and caffeine is shown in Figure 4a. In a unit of the 2:4 complex, the B and B′ rings of one ECg (ECg A) faced the caffeines and the A and B′ rings of the other ECg (ECg B) faced the caffeines. One unit cell contained two units of the 2:4 complex of ECg and caffeine and twelve water molecules as crystal solvent (Figure 4b).

The torsion angles of the ECg moieties (ECg A and B) of the 2:4 complex (Table 1) indicated that B rings of ECg A and ECg B were both equatorial positions and B′ rings of ECg A and ECg B were both axial positions with respect to the C rings of ECg molecules.

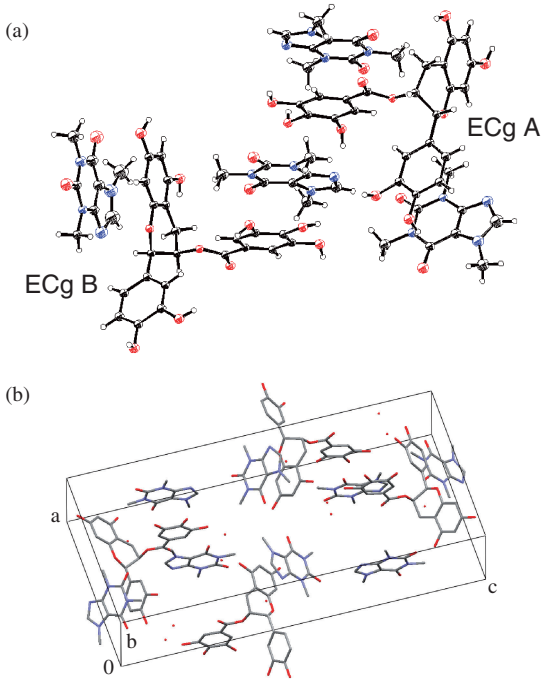

Figure 4. Crystal structure of the 2:4 complex of ECg and caffeine. (a) ORTEP drawing with thermal epllisoids at 30% probability level. (b) One unit cell. Crystal solvent and hydrogen atoms are omitted for clarity.

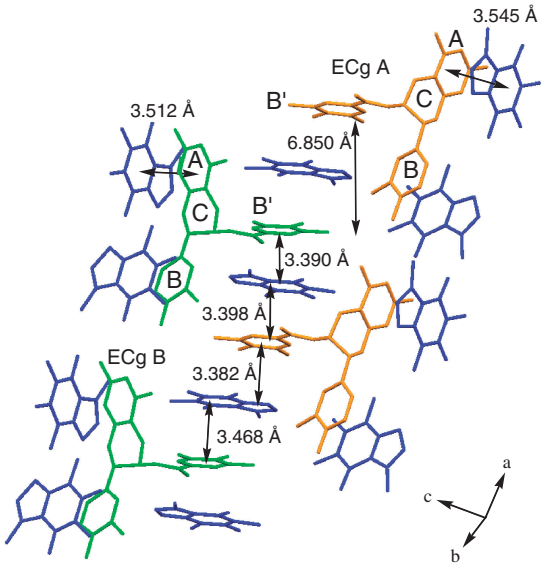

Figure 5. Layer structure of the 2:4 complex of ECg and caffeine. Orange and green molecules are ECg A and ECg B, respectively. Blue molecules are caffeine. Hydrogen atoms and crystal solvent are omitted for clarity.

In the layer structure shown in Figure 5, units of the 2:4 complex of ECg and caffeine stacked in parallel in the same direction as the *a* axis. The B′ ring of ECg and caffeine appear in turn along the *a* axis, and the caffeines were located in almost the middle of the two B′ rings of ECgs. Furthermore, the A and B rings of ECg faced the caffeines.

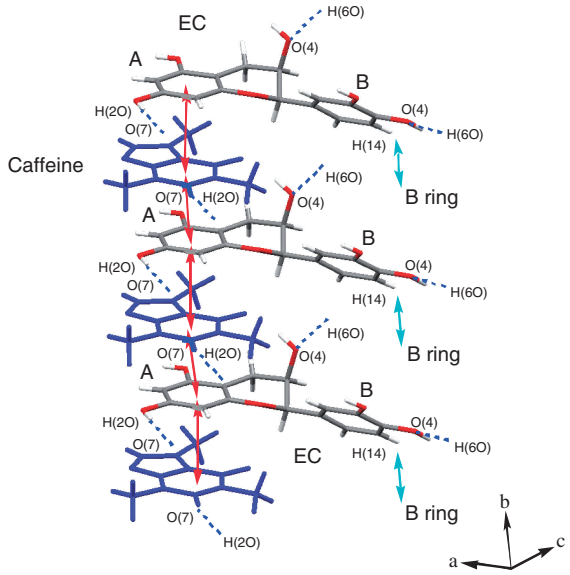

Figure 6. Molecular interactions between EC and caffeine for forming the crystal structure of the 1:1 complex. Red and aqua blue arrows indicate face-to-face π–π stacking interactions and CH–π interactions, respectively. Blue dotted lines indicate hydrogen bonds.

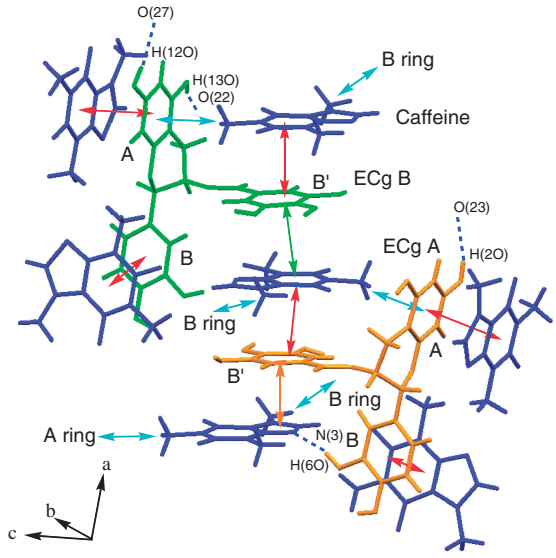

Figure 7. Molecular interactions between ECg and caffeine for forming the crystal structure of the 2:4 complex. Orange and green molecules are ECg A and ECg B, respectively. Red, green, and aqua blue arrows indicate face-to-face and offset π–π stacking interactions and CH–π interactions, respectively. Blue dotted lines indicate hydrogen bonds.

Noncovalent interactions are weaker forces than covalent bonding, but play a very important role in forming complexes, therefore noncovalent interactions among EC, ECg and caffeine moieties in 1:1 and 2:4 complexes were investigated.

In the 1:1 complex of EC and caffeine, face-to-face π–π stacking interaction formed between the A ring of EC and a six-membered ring of caffeine. Also, CH–π interaction formed between C(14)–H(14) of B ring and B ring (Figure 6). Two O–H···O intermolecular hydrogen bonds were also observed.

In the 2:4 complex of ECg and caffeine, face-to-face and offset π–π stacking interactions formed between the B′ ring of ECg and caffeine (Figure 7). Also, face-to-face π–π stacking interaction formed between the A ring of ECg and caffeine, and between the B ring of ECg and caffeine. CH–π interactions observed between the methyl group of N_1CH_3 of caffeine and the A ring of ECg, and methyl group of N_7CH_3 of caffeine and the B ring of ECg. Furthermore, four O–H···O and one O–H···N intermolecular hydrogen bonds were formed between ECgs and caffeine moieties.

In conclusion, 1:1 and 2:4 complexes were thought to be formed with the cooperative effect of three kinds (face-to-face π–π and CH–π interactions, and intermolecular hydrogen bonds) and four kinds (face-to-face and offset π–π stacking interactions, CH–π interaction, and intermolecular hydrogen bonds) of noncovalent interactions, respectively. Upon forming 1:1 and 2:4 complexes, π–π stacking interactions are thought to play an important role in binding EC and ECg with caffeine. The π–π complexation site of the non-galloylated catechin EC with caffeine was only the A ring, whereas that of the galloylated catechin ECg was aromatic A, B, and B′ rings.

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19 Crystal data for 2:4 complex of ECg and caffeine. 7999 independent reflections measured ($R_{int} = 0.032$), 7557 reflections of $I > 2\sigma(I)$, 1126 parameters used for refinements. R_1 and wR were 0.0527 (for $I > 2\sigma(I)$) and 0.1454 (for all data), respectively. GOF = 1.050. CCDC-765713.

