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Chiral recognition of diketopiperazines cyclo(Pro-Gly) using (−)-epigallocatechin-3-*O*-gallate

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ABSTRACT

Upon a formation of complexes of EGCg and cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) in D_2O, a chirality of cyclo(Pro-Gly) was recognized by difference of the chemical shift of ^1H NMR signal for $H_{7\alpha}$, $H_{7\beta,8\alpha}$ of the Pro residue. Judging from the crystal structures of the 2:2 complexes of EGCg and cyclo(L-Pro-Gly), cyclo(D-Pro-Gly), such a difference of the chemical shift might be due to magnetic anisotropic shielding effect by the ring current from the B ring of EGCg.

Tea is commonly prepared by pouring hot or boiling water over leaves of the tea plant, *Camellia sinensis*, Theaceae, which includes caffeine, tannins, vitamins, and theanine.[1] When a hot tea beverage cools down, it becomes turbid and brown-white particles settle out. This phenomenon is called a 'creaming' or 'creaming-down reaction'. Since creaming is a trigger deforming the original taste and flavor of tea, it is one of the most serious problems in making a tea beverage.

Previously, Ina and co-workers reported that creaming down eventually occurs when an aqueous caffeine solution is poured into an aqueous solution of gallated catechin (−)-epigallocatechin-3-*O*-gallate (EGCg), which is most contents in tea catechins (Fig. 1).[2] Then we had attempted a crystallization of the precipitate formed by creaming made from an aqueous solution of EGCg and caffeine, and as a result, got a crystal, which was determined to be a 2:2 complex of EGCg and caffeine by X-ray crystallographic analysis.[3]

The layer structure of the 2:2 complex of EGCg and caffeine was formed from two layers (Fig. 2a). Namely, caffeine A was stacked between the B′ rings of EGCg A and caffeine B was stacked between the B′ rings of EGCg B, and caffeine A or B was located almost in the middle of the two B′ rings of EGCg A or B. These were in parallel in the same direction as the *b*-axis.

As shown in Figure 2b, the caffeine moieties of the 2:2 complex were located in the space surrounding the top and lower walls of the B′ rings of EGCg moieties and right and left walls of the A and B rings of EGCg moieties. As a result, caffeine molecules were captured by the hydrophobic space formed by three aromatic A, B, B′ rings of the EGCg in the 2:2 complex. The water molecules existed outside the space formed by the three aromatic A, B, B′ rings of EGCg and were not observed in the space, suggesting that the space had high hydrophobicity. It was therefore thought that the sticky precipitate was formed by creaming precipitated from the aqueous solution of EGCg and caffeine due to its high hydrophobicity.

The hydrophobic space may be available for capture in various compounds in substitution for caffeine. Furthermore, the C ring of EGCg has two chiral carbon atoms, C2 and C3, and the hydrophobic space formed by the three aromatic A, B, B′ rings of EGCg was a chiral space. Therefore, it was assumed that the space formed by the three aromatic A, B, B′ rings of EGCg could recognize the chirality of compounds included in the space.

Thus, diketopiperazine cyclo(Pro-Gly) was selected as a chiral compound because the molecular size of cyclo(Pro-Gly) was about the same as that of caffeine. Subsequently, the chiral recognition of

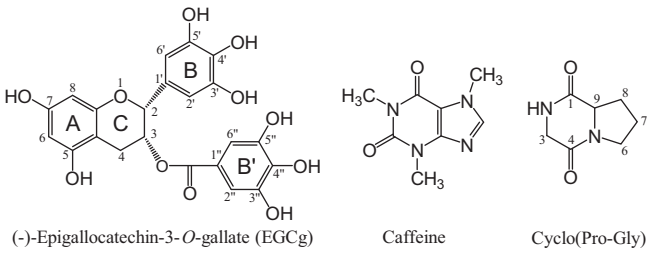

Figure 1. Epigallocatechin-3-*O*-gallate, caffeine and cyclo(Pro-Gly).

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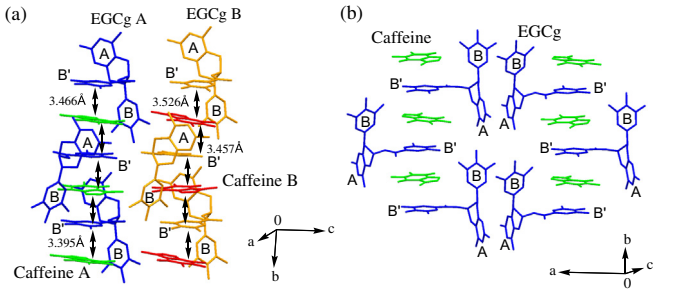

Figure 2. Layer structure of the 2:2 complex of EGCg and caffeine (a) two EGCg layers; (b) hydrophobic space formed by three aromatic A, B, B′ rings of EGCg.

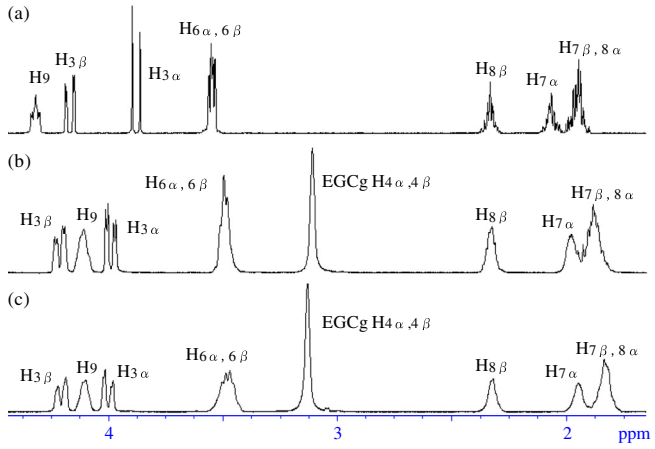

Figure 3. ^{1}H NMR spectra[6] of a solution containing (a) cyclo(L-Pro-Gly) or cyclo(D-Pro-Gly) (12.49 mg, 8.10×10^{-2} mmol) in D_2O (520 μL); (b) EGCg (37.14 mg, 8.10×10^{-2} mmol) and cyclo(L-Pro-Gly) (12.49 mg, 8.10×10^{-2} mmol) in D_2O (520 μL); (c) EGCg (37.14 mg, 8.10×10^{-2} mmol) and cyclo(D-Pro-Gly) (12.49 mg, 8.10×10^{-2} mmol) in D_2O (520 μL).

diketopiperazines cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) by EGCg was investigated.

A solution of the diketopiperazines cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) in D_2O was added to a solution of an equimolecular amount of EGCg in D_2O. ^{1}H NMR spectra of the mixture are shown in Figure 3b and c. All proton signals derived from cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) appeared as broad signals, as compared with the corresponding proton signals of the ^{1}H NMR spectra of cyclo(L-Pro-Gly), and cyclo(D-Pro-Gly) alone in D_2O (Fig. 3a). It was thought that cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) formed complexes with EGCg, and that the motion of their protons was restricted, leading to their signals being broadened.

Table 1 shows the chemical shift of ^{1}H NMR signals of a solution containing equimolecular amounts of EGCg and cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) in D_2O, and indicates the shift values started from the chemical shift of ^{1}H NMR signals of a solution containing cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) alone in D_2O. A marked upfield shift in the anomeric proton signal for H_9 of a proton in the α position of the Pro residue of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) was observed, while a downfield shift of the proton signal for $H_{3\alpha}$ in the α position of the Gly residue was observed. Upfield shifts in proton signals for $H_{7\alpha,\beta}$ and $H_{8\alpha}$ in the β and γ positions of the Pro residue of cyclo(D-Pro-Gly) were more marked than those of cyclo(L-Pro-Gly).

A solution of diketopiperazine cyclo(L-Pro-Gly) in H_2O was added to a solution of an equimolecular amount of EGCg in H_2O. The mixture afforded a colorless block crystal,[4] which was determined to be a 2:2 complex of EGCg and cyclo(L-Pro-Gly) by X-ray crystallographic analysis.[5] Using the same method as for crystallization of the complex of EGCg and cyclo(L-Pro-Gly), a single crystal of a complex of EGCg and cyclo(D-Pro-Gly) was prepared[4] and determined to be a 2:2 complex of EGCg and cyclo(D-Pro-Gly) by X-ray crystallographic analysis.[5] ORTEP drawings of the 2:2 complexes of EGCg and cyclo(L-Pro-Gly), EGCg and cyclo(D-Pro-Gly) are shown in Figure 4a and b. The 2:2 complexes were formed from two crystallographically different EGCgs (EGCg A and EGCg B) and two cyclo(L-Pro-Gly)s, two cyclo(D-Pro-Gly)s.

One unit cell contained one unit of the 2:2 complex of EGCg and cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) and seven water molecules as a crystal solvent (Fig. 5a and b). The torsion angles of the EGCg moieties (EGCg A and B) of the 2:2 complexes of EGCg and cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) indicated that the B rings of EGCgs A and B were both at equatorial positions, while the B′ rings of EGCgs A and B were both in axial positions with respect to the C rings of the EGCg molecules (Table 2).

In the layer structures of the 2:2 complexes of EGCg and cyclo(L-Pro-Gly), cyclo(D-Pro-Gly), cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) were captured by the space formed by the three aromatic A, B, B′ rings of EGCg and were located almost in the middle of the two B′ rings of EGCg A or B (Fig. 6a and b), the same as caffeine in the 2:2 complex of EGCg (Fig. 2b).

Table 1
^{1}H NMR chemical shift in proton signals of a solution containing cyclo(L-Pro-Gly), cyclo(D-Pro-Gly) (12.49 mg, 8.10×10^{-2} mmol) without and with EGCg (37.14 mg, 8.10×10^{-2} mmol) in D_2O (520 μL)

Proton	δ (ppm)	δ (ppm) in the presence of EGCg	Shift value (ppm)	
Chemical shift (ppm) of cyclo(L-Pro-Gly)				
9	4.319	4.108	−0.211	
3β	4.170	4.215	+0.045	
3α	3.881	3.989	+0.108	
6α,β	3.549	3.497	−0.052	
8β	2.332	2.325	−0.007	
7α	2.064	1.977	−0.087	
7β,8α	1.947	1.882	−0.065	
				cyclo(L-Pro-Gly)
Chemical shift (ppm) of cyclo(D-Pro-Gly)				
9	4.320	4.100	−0.220	
3β	4.170	4.207	+0.037	
3α	3.880	4.001	+0.121	
6α,β	3.548	3.480	−0.068	
8β	2.333	2.319	−0.014	
7α	2.065	1.947	−0.118	
7β,8α	1.947	1.834	−0.113	
				cyclo(D-Pro-Gly)