Development of High-Order Functions Using \((-\text{E})\)-Epigallocatechin-3-O-gallate in Water

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The high-order functions of molecular capture and chiral recognition of tea gallated catechins \((-\text{E})\)-epigallocatechin-3-O-gallate (EGCg) in water were investigated. A solution of equimolar amounts of a variety of heterocyclic compounds and EGCg in water afforded adhesive precipitates containing the heterocyclic compounds and EGCg at a molar ratio of 1:1, based on the integrated value of NMR proton signals. The molecular capture abilities of a variety of heterocyclic compounds using EGCg from the aqueous solutions were evaluated with the ratios of the heterocyclic compounds included in the precipitates of EGCg complex to the total heterocyclic compounds used. In the \(1^\text{H}-\text{NMR}\) spectrum of a solution containing cyclo-(L-Pro-Gly), cyclo-(D-Pro-Gly), and EGCg in a D\(_2\)O solution, a difference in the chemical shift of the \(1^\text{H}-\text{NMR}\) signal for some protons of the Pro residue was observed. Judging from the crystal structures of the 2:2 EGCg complexes of cyclo-(L-Pro-Gly), cyclo-(D-Pro-Gly), the difference in the chemical shift derived mainly from a magnetic anisotropic shielding effect by the ring current from the B ring of EGCg. In the \(1^\text{H}-\text{NMR}\) spectrum of a solution containing the pharmaceuticals racemic \((R,S)\)-propranolol, \((R,S)\)-diprophylline, \((R,S)\)-proxyphylline, and EGCg in D\(_2\)O, splitting of the \(1^\text{H}-\text{NMR}\) signals of the pharmaceuticals was observed. It was suggested that the pharmaceuticals formed diastereomers of EGCg complexes, as a result chirality of the pharmaceuticals was recognized by EGCg in the D\(_2\)O solution.

Key words \((-\text{E})\)-epigallocatechin-3-O-gallate; propranolol; diprophylline; proxyphylline; quantitative \(1^\text{H}-\text{NMR}\); X-ray crystallographic analysis

1. Introduction
Tea is drunk for its taste and aroma, and particularly in recent years, as a healthy beverage all over the world. Tea is commonly prepared by pouring hot or boiling water over leaves of the tea plant, \textit{Camellia sinensis}, Theaceae, which contain caffeine, tannins, vitamins, and theanine.\(^{1}\)

When a hot tea beverage cools, turbid and brown-white particles occur and then precipitate. This phenomenon is called a “creaming-down reaction.” Since the creaming-down reaction is a trigger that alters the original taste and appearance of tea, it is a serious problem in making a tea beverage.

Previously, Maruyama \textit{et al.} reported that the creaming-down reaction eventually occurs when an aqueous caffeine solution is poured into an aqueous solution of \((-\text{E})\)-epigallocatechin-3-O-gallate (EGCg), which is most abundant of the tea catechins\(^{1}\) (Fig. 1).

Therefore, we attempted crystallization of the precipitate of the creaming-down reaction made from an aqueous solution of caffeine and EGCg, and as a result obtained a crystal that was determined to be a 2:2 EGCg complex of caffeine by X-ray crystallographic analysis\(^{1–5}\) (Fig. 2a).

The caffeine moieties in the 2:2 complex were positioned in the space surrounding the top and bottom walls of the B’ rings of EGCg moieties and right and left walls of the A and B rings of EGCg moieties as shown in Fig. 2a. In the 2:2 EGCg complex of caffeine, intermolecular interactions between caffeine and EGCg moieties were mainly \(\pi-\pi\) interactions between a six-membered ring of caffeine and the B’ ring of EGCg (Fig. 3). Resultly, the caffeine molecules were captured by the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg in the 2:2 complex. Water molecules were not observed inside the space formed with the three aromatic A, B, and B’ rings of EGCg and existed outside the space, suggesting that the space had high hydrophobicity (Fig. 2b).

It was therefore considered that the precipitate of the creaming-down reaction occurred from the solution of caffeine and EGCg in water due to its high hydrophobicity.

\[\text{(-E)-Epigallocatechin-3-O-gallate (EGCg), Caffeine, Nicotinamide}\]

Fig. 1. \((-\text{E})\)-Epigallocatechin-3-O-gallate (EGCg), Caffeine, and Nicotinamide

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Nicotinamide, which is also one of the ingredients of tea, has an aromatic ring for the formation of \( \pi - \pi \) interactions with the B' ring of EGCg. The precipitate of the creaming-down reaction made from an aqueous solution of nicotinamide and EGCg was obtained, and then its crystal was prepared. The crystal was determined to be the 2:2 complex of nicotinamide and EGCg, similar to a 2:2 EGCg complex of caffeine, by X-ray crystallographic analysis\(^3\) (Fig. 4). Resultly, caffeine and nicotinamide molecules were captured by the hydrophobic space formed with the three aromatic A, B, and B' rings of EGCg in the 2:2 complex.

Based on the above findings, the high-order functions of molecular capture and chiral recognition of the tea gallated catechins EGCg in water were investigated.

Complexes of precipitates formed from aqueous solution of EGCg and a variety of heterocyclic compounds, that are not tea ingredients, were studied. The capture of heterocyclic compounds using precipitates of the EGCg complexes was investigated. And the molecular capture abilities of the heterocyclic compounds were estimated with the ratios of the heterocyclic compounds included in the precipitates to the total heterocyclic compounds used.

Furthermore, the C ring of EGCg has C\(_2\) and C\(_3\) of two chiral carbon atoms, and then the hydrophobic space formed with the three aromatic A, B, and B' rings of EGCg was a chiral space. Therefore, it was assumed that the space formed with the aromatic A, B, and B' rings of EGCg could recognize the chirality of compounds included in the space.

The diketopiperazines cyclo(Pro-Gly) was selected as a chiral compound because its molecular size was approximately the same as that of caffeine. Subsequently, the chiral recognition of diketopiperazines cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) by EGCg was investigated.

Many pharmaceuticals are used currently in racemic form, although it is desirable to be used a single enantiomer having greater potency judging from the viewpoint of adverse effects and pharmacokinetics. EGCg, which easily forms a complex with a variety of heterocyclic compounds in water and then precipitates from the solution in water, may be a potential new optical resolving agent for pharmaceuticals and natural products.

We applied chiral recognition to analyze pharmaceuticals currently used as racemic mixtures. The chiral recognition of the \( \beta \)-adrenergic receptor blocker propranolol, which is frequently used for the treatment of hypertension, and the bronchodilators proxyphylline and diprophylline, which have xanthine skeletons and are used to treat bronchial asthma, was investigated.

2. Results and Discussion

2.1. Crystal Structure of the 2:2 EGCg Complex of 2-Chloropyrimidine

2-Chloropyrimidine was used to investigate whether heterocyclic compounds that are not tea ingredient also form a 2:2 EGCg complex in the same form as the 2:2 complexes of caffeine, and nicotinamide with EGCg and then the complex precipitates. A solution of an equimolar amount of tea gallated catechin EGCg and 2-chloropyrimidine in water gave a precipitate of a colorless block crystal. The
crystal contained EGCg and 2-chloropyrimidine at a molar ratio of 1:1, based on the integrated value of corresponding proton signals in 1H-NMR spectra.

A single crystal was analyzed using X-ray crystallography. Based on an ORTEP drawing, the crystal had two crystallographically different EGCgs (EGCg A and EGCg B) and two 2-chloropyrimidines, which was a 2:2 complex facing the aromatic ring of the 2-chloropyrimidine and the B’ rings of each EGCg (Fig. 5a). One cell unit also contained four units of the 2:2 EGCg complex of 2-chloropyrimidine, and 24 H2O molecules of crystal solvent (Fig. 5b).

The torsion angles of the EGCg moieties (EGCgs A and B) of the 2:2 EGCg complex displayed that the B rings of EGCg A and B took both in equatorial positions and the B’ rings of EGCgs A and B took both in axial positions with respect to the C rings of EGCg (Table 1).

The layered structure of the 2:2 EGCg complex of 2-chloropyrimidine was formed with two layers (Fig. 6). In one layer 2-chloropyrimidine molecules were stacked between the B’ rings of EGCg A and in the other layer 2-chloropyrimidine molecules were stacked between the B’ rings of EGCg B, while 2-chloropyrimidine molecules positioned nearly the middle of the two B’ rings of EGCg A or B. These two layers existed parallel to the b axis.

Intermolecular interactions forming in the 2:2 EGCg complex of 2-chloropyrimidine, 2-chloropyrimidine and EGCg were determined (Fig. 7). π–π interactions occurred between a pyrimidine ring of 2-chloropyrimidine and the B’ ring of EGCg, B rings of EGCg, and A rings of EGCg, respectively. Furthermore CH–π interactions occurred between the C–H of 2-chloropyrimidine and B rings of EGCg A and B, respectively. Two OH…N intermolecular hydrogen bonds formed between 2-chloropyrimidine and EGCg.

It was found that 2-chloropyrimidine formed a 2:2 complex with EGCg in the same form as the 2:2 EGCg complex of caffeine, nicotinamide (Figs. 2a, 4). Resultly, 2-chloropyrimidine molecules were captured by the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg as shown in Fig. 8, and precipitated as the 2:2 complex from the solution of an equimolar amount of 2-chloropyrimidine and EGCg in water due to its high hydrophobicity.

2.2. Molecular Capture of a Variety of Heterocyclic Compounds Using EGCg

Aqueous solutions of equimolar amount of a variety of heterocyclic compounds (Table 2) other than 2-chloropyrimidine, which is not a tea ingredient, and EGCg also gave adhesive precipitates containing the heterocyclic compounds and EGCg at a molar ratio of 1:1, based on the integrated value of NMR proton signals. The precipitates were considered to be 2:2 EGCg complex of the heterocyclic compounds like the 2:2 EGCg complex of 2-chloropyrimidine, and the heterocyclic compounds were captured in the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg.

Therefore, we investigated the amount of heterocyclic compounds which could be captured in precipitate as the 2:2 complex from solution of the heterocyclic compounds and

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**Table 1. Torsion Angles of EGCg Moieties in the 2:2 EGCg Complex of 2-Chloropyrimidine**

| Torsion angle | A       | B       |
|--------------|---------|---------|
| C1-C2-C3-O   | 67.7(4) | 66.5(4) |
| H2-C2-C3-O   | 67.3    | 66.4    |

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**Fig. 5. Crystal Structure of the 2:2 EGCg Complex of 2-Chloropyrimidine**

The crystal solvent is omitted for clarity. (a) ORTEP drawing with a thermal ellipsoid at a 30% probability level; (b) One cell unit. (Color figure can be accessed in the online version.)

**Fig. 6. Layered Structure of the 2:2 EGCg Complex of 2-Chloropyrimidine**

Green and orange molecules are EGCg A and B, respectively. Hydrogen atoms and crystal solvent are omitted for clarity. (Color figure can be accessed in the online version.)
EGCg in water. The molecular capture abilities of a variety of heterocyclic compounds using EGCg were estimated based on the ratios of heterocyclic compounds included in the precipitates to the total heterocyclic compounds used (Table 2). The amounts were measured by an integrated value of the corresponding proton signals in the quantitative 1H-NMR spectrum in dimethyl sulfoxide (DMSO)-d$_6$. In Table 2, a heterocyclic compound having A (%) of 0% means that an aqueous solution of the heterocyclic compound and EGCg did not yield a precipitate.

It was considered that the molecular capture ability depended not only on the chemical structure of the heterocyclic compound but also on such physical factors as entropy, solubility, the degree of polymerization, etc. of the 2:2 EGCg complex. Particularly, the molecular capture ability of a heterocyclic compound with a hydroxyl group was low due to its high hydrophilicity.

The 2:2 complexes dissolved in various organic solvent, and readily decomposed into the constituent EGCg and the heterocyclic compounds, which can easily be isolated on column chromatography. Thus, the molecular capture ability is considered to be a useful indicator of the amount of the heterocyclic compound which can be isolated using EGCg from solution in water.

### 2.3. 2:2 EGCg Complexes of Cyclo(L-Pro-Gly) and Cyclo(D-Pro-Gly)

Interactions between EGCg and the diketopiperazines cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) (Fig. 9), which are mutual enantiomers, were investigated using X-ray crystallography and 1H-NMR spectral analysis. A solution of diketopiperazine cyclo(L-Pro-Gly) in H$_2$O was added to a solution of an equimolcular amount of EGCg in H$_2$O. The mixture afforded a colorless block crystal, which was determined to be a 2:2 EGCg complex of cyclo(L-Pro-Gly) by X-ray crystallographic analysis. Using the same method as for crystallization of the EGCg complex of cyclo(L-Pro-Gly), a single crystal of a EGCg complex of cyclo(D-Pro-Gly) was prepared and determined to be a 2:2 EGCg complex of cyclo(D-Pro-Gly) by X-ray crystallographic analysis. ORTEP drawings of the 2:2 EGCg complexes of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) are shown in Figs. 10a, b.

In the layered structures of the 2:2 EGCg complexes of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly), the two diketopiperazines were captured by the space formed with the aromatic A, B, and B' rings of EGCg and were located nearly in the middle of the two B' rings of EGCg A or B (Figs. 11a, b), the same as for caffeine and nicotinamide in the 2:2 EGCg complex (Figs. 2a, 4).

In the 2:2 EGCg complexes of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly), intermolecular interactions occurring between the moieties were determined. In the 2:2 EGCg complex of cyclo(L-Pro-Gly), CH–π interactions formed between methine C–H$_5$ of cyclo(L-Pro-Gly) and the B rings of EGCg, and between methylene C–H$_7$ of cyclo(L-Pro-Gly) and the B rings of EGCg. In the same way as for the 2:2 EGCg complex of cyclo(L-Pro-Gly), two O–H···O intermolecular hydrogen bonds were formed between cyclo(L-Pro-Gly) and EGCg (Fig. 12a).

In the 2:2 EGCg complex of cyclo(D-Pro-Gly), CH–π interactions were observed between methine C–H$_1$ of cyclo(D-Pro-Gly) and the B' rings of EGCg. In addition, CH–π interactions were observed between methylene C–H$_3$ of cyclo(D-Pro-Gly) and the A rings of EGCg. In the same way as for the 2:2 EGCg complex of cyclo(L-Pro-Gly), two O–H···O intermolecular hydrogen bonds were formed between EGCg and cyclo(D-Pro-Gly), as shown in Fig. 12b.

In the 2:2 EGCg complexes of caffeine, nicotinamide, and 2-chloropyrimidine, π–π interactions formed between the B' ring of EGCg and a six-membered ring, while in the 2:2
EGCg complex of cyclo(Pro-Gly), CH–π interactions were observed between methine C–H of a six-membered ring of diketopiperazine skeleton and the B/ring of EGCg.

It was found that the hydrophobic space formed with the aromatic rings of A, B, and B/rings of EGCg took up cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) in water, and the precipitates of 2 : 2 EGCg complexes of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) formed from the aqueous solution due to its high hydrophobicity.

A solution of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) in D2O was added to a solution of an equimolar amount of EGCg in D2O. The 1H-NMR spectra of the mixture are shown in Figs. 13b, c. All proton signals derived from cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) appeared as broad proton signals, as compared with the corresponding proton signals of the 1H-NMR spectra of cyclo(L-Pro-Gly) alone in D2O (Fig. 10a). When cyclo(L-Pro-Gly) and, cyclo(D-Pro-Gly) formed complexes with EGCg, it was thought that the motion of their protons was restricted, leading to broadening of their proton signals.

Table 3 shows the chemical shift of 1H-NMR proton signals of a solution containing equimolar amounts of cyclo(L-Pro-Gly), cyclo(D-Pro-Gly), and EGCg in D2O, and indicates the shift values starting from the chemical shift of 1H-NMR proton signals of a solution containing cyclo(L-Pro-Gly) alone in D2O. A remarkable upfield shift in signal for the anomeric proton Hα of the Pro residue in cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) was observed, while a downfield shift of the signal for the α proton Hα of the Gly residue was observed. Upfield shifts in signals for H7α, H8α of the β and γ protons of the Pro residue of cyclo(o-Pro-Gly) were more remarkable than those of cyclo(i-Pro-Gly).

Judging from the crystal structures of 2 : 2 EGCg complexes of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly), the upfield shift in the signal of Hα was derived from magnetic anisotropic shielding by the ring current from the B/ring of the EGCg moieties (Fig. 12). Upon the formation of the 2 : 2 complex, the upfield shift values of the signals for H7α, H8α of the Pro residue of cyclo(L-Pro-Gly) were 0.087 and 0.065 ppm, respectively, and those of cyclo(D-Pro-Gly) were 0.118 and 0.113 ppm, respectively (Table 3). Such a difference between the upfield shift values of the proton signals of cyclo(L-Pro-Gly) and cyclo(D-Pro-Gly) were considered to be derived mainly from a difference of the magnitude of the magnetic anisotropic shielding by the ring current from the B/ring of EGCg.

Therefore, it was believed that a chirality of cyclo(Pro-Gly) was recognized by the magnetic anisotropic shielding effect of the ring current from the B/ring of EGCg.

As described above, EGCg recognized a chirality of the diketopiperazines cyclo(Pro-Gly) in aqueous solution. It was found that the hydrophobic space formed with the aromatic A, B, and B/rings of EGCg took up various heterocyclic compounds in water. Since the C ring of EGCg has C1 and C3 of two chiral carbon atoms, the hydrophobic space is also a chiral space, and the chirality of the induced compounds is recognized by EGCg.

It was an advantage of the chiral recognition that EGCg formed complexes with a variety of chiral compounds through weak non-covalent interactions such as hydrophobic and CH–π interactions, and hydrogen bond under mild conditions of neutrality and room temperature. This advantage allowed the chiral compounds to be easily isolated from the EGCg complexes after recognition.

2.4. Chiral Recognition of Propranolol Using EGCg

Based on the findings of chiral recognition described above, we applied the chiral recognition using EGCg to the β-adrenergic receptor blocker propranolol, which is used for...
the treatment of hypertension and usually administered in a racemic form.\textsuperscript{10}

A solution of racemic (\textit{R})- and (\textit{S})-propranolols in a 1:1 ratio, in D\textsubscript{2}O was added to a solution of an equimolar amount of EGC\textit{g} in D\textsubscript{2}O. In the \textsuperscript{1}H-NMR spectrum of the mixture, the \textit{H}\textsubscript{2} proton signal of racemic propranolol was observed as two doublet-like signals (Fig. 14), suggesting that (\textit{R})- and (\textit{S})-propranolols each formed complexes with EGC\textit{g}, which are mutual diastereomers. The \textit{H}\textsubscript{2} proton signal of racemic propranolol alone was observed as a doublet-like signal.

By addition of EGC\textit{g}, the splitting of the \textit{H}\textsubscript{2} proton signal of (\textit{R})- and (\textit{S})-propranolols was observed, showing that EGC\textit{g} recognized the chirality of propranolol.

For assignment of the two doublet-like signals, (\textit{R})- and (\textit{S})-propranolols were added to a solution containing an equimolar amount of EGC\textit{g} and racemic propranolol in D\textsubscript{2}O (Fig. 15). Resultly, signals of 6.850 and 6.841 ppm were assigned to the \textit{H}\textsubscript{2} of (\textit{R})- and (\textit{S})-propranolols, respectively.
Kinetic analyses of the complex formations of \((R)\)- and \((S)\)-propranolols with EGCg were performed. The stability constants \(K_c\) for the complex formations of EGCg with \((R)\)-, and \((S)\)-propranolols at 35–70 °C were estimated, which assumed the order of the reaction \(n\). The reaction \(n\) values of the complexes of EGCg with \((R)\)-, and \((S)\)-propranolol at 35 °C were 0.86 and 0.87, respectively (Table 4), suggesting that \((R)\)- and \((S)\)-propranolol each formed a 1 : 1 EGCg complex.

From the dependence of \(K_c\) on temperature, the change in free energy \(\Delta G\), enthalpy \(\Delta H\) and entropy \(\Delta S\) of the complex formation were determined as shown in Table 4. The entropy (\(\Delta S\)) for the formation of the EGCg complex of \((R)\)-, and \((S)\)-propranolols had large negative values of \(-21.7\) and \(-6.7\) mol\(^{-1}\)K\(^{-1}\), respectively. It was suggested that \((R)\)-propranolol was fixed comparatively tightly in the EGCg complex of \((R)\)-propranolol, while \((S)\)-propranolol fitted comparatively loosely in the complex.

Nextly, changes in chemical shifts of each proton signal of \((R)\)- and \((S)\)-propranolols in \(^1\)H-NMR spectra by adding fixed amount of EGCg were measured. Upfield shifts in proton signals for \(H_9\) in the side chain and \(H_2, H_3, H_4, H_5, H_6, H_7,\) and \(H_8\) in the naphthalene ring of \((R)\)- and \((S)\)-propranolol were observed (Fig. 16). It was thought that the upfield shifts of proton signals were derived from the magnetic anisotropic shielding by the ring current from the B’ ring of EGCg when the naphthalene ring of propranolol was captured by the hydrophobic space formed with EGCg, as the caffeine and nicotinamide moieties of the 2 : 2 EGCg complex of caffeine and nicotinamide shown in Figs. 2a, and 4.

Upon formation the complex, intermolecular interactions...
between EGCg and propranolol moieties were investigated. The naphthalene ring of (R)- and (S)-propranolols was considered to be taken into the hydrophobic space formed with the aromatic of A, B, and B' rings of EGCg and undergo a π–π interaction with the B' ring of EGCg.

Judging from the entropy (ΔS) value (Table 4), the EGCg complexes of (R)-propranolol were thought to form not only the π–π interaction between the naphthalene ring of (R)-propranolol and the B' ring of EGCg, but also an intermolecular hydrogen bond between the hydroxyl group of the side chain of (R)-propranolol C10-OH and EGCg.

The intramolecular nuclear Overhauser effect (NOE) of (R)- and (S)-propranolols in the EGCg complexes in D2O were performed (Figs. 17b, c). For comparison, the intramolecular NOE of (S)-propranolol alone in D2O was also performed (Fig. 17a). Numerous NOEs were observed in (S)-propranolol in a free state, but not in (R)- and (S)-propranolols in the EGCg complex, suggesting that their conformers were fixed for the complex formation.

A characteristic intramolecular NOE in the solution of (S)-propranolol and EGCg was observed between H2 proton signal in the naphthalene ring and H10 proton signal in the side chain of (S)-propranolol, but was not observed between them in the solution of (R)-propranolol. It is thought that the H2 proton signal of (R)-propranolol appeared in a lower field than that of (S)-propranolol under the influence of the oxygen atom of the hydroxyl group of (R)-propranolol in proximity. Therefore, it was concluded that a difference in the chemical shift of the H2 proton signal between (R)- and (S)-propranolols was derived from a difference in their conformations in the EGCg complexes, and then EGCg recognized a chirality of racemic propranolol in the D2O solution.

(S)-Propranolol is approximately 100-fold more efficacious clinically than (R)-propranolol, like because the C10-OH group of (S)-propranolol forms an intermolecular hydrogen bond at the site of interaction with the sympathetic β receptor, whereas that of (R)-propranolol does not.

This is interesting since the strength of the interaction between EGCg and (R)- and (S)-propranolols is determined by the presence or absence of the intermolecular hydrogen bond between EGCg and the C10-OH group of propranolol.

2.5. Interaction of Pharmaceuticals with a Xanthine Skeleton and EGCg

As described above, EGCg was shown to recognize the chirality of propranolol through splitting of the 1H-NMR proton signal. However, the splitting of 1H-NMR signal of racemic propranolol was difficult to observe due to overlap of proton signals and small differences in their chemical shifts. Therefore, the chiral recognition of pharmaceuticals with a xanthine skeleton for the treatment of bronchial asthma i.e., proxyphylline and diprophylline, were investigated using EGCg in water10 (Fig. 18). As pharmaceuticals with a xanthine skeleton have two methyl groups, which were observed as a strong, sharp singlet, the splitting of the methyl signals was expected to be easily observed in the 1H-NMR spectra.
2.5.1. Pharmaceuticals with a Xanthine Skeleton in the Complex with EGCg

Equimolar amounts of theophylline, proxyphylline, and diprophylline, which are pharmaceuticals with a xanthine skeleton, and EGCg in an aqueous solution divided into a supernatant liquid and a sticky precipitate, which contained the pharmaceuticals with a xanthine skeleton and EGCg at a molar ratio of 1:1 based on the integrated volume of ¹H-NMR proton signals. The precipitates were thought to be 2:2 EGCg complexes of the pharmaceuticals with a xanthine skeleton such as the 2:2 EGCg complex of caffeine and nicotinamide (Figs. 2a, and 4).

Before the investigation of proxyphylline and diprophylline, theophylline, which has a simple xanthine skeleton without a side chain and chiral carbon, was investigated.

Changes in chemical shifts of the proton signals of theophylline in the ¹H-NMR spectra observed after adding fixed amount of EGCg. Upfield shifts in proton signals for N₁–CH₃, N₃–CH₃, and H₈ were observed (Fig. 19).

It was thought that the upfield shifts of proton signals resulted from the magnetic anisotropic shielding by the ring current from the B’ ring of EGCg, and the whole theophylline molecule was captured by the hydrophobic space formed with EGCg, like as the caffeine and nicotinamide moieties in the 2:2 EGCg complex (Figs. 2a and 4).

Changes in chemical shifts of proton signals of theophylline in the ¹H-NMR spectra observed after adding fixed amount of EGCg. Upfield shifts in proton signals for N₁–CH₃, N₃–CH₃, and H₈ were observed (Fig. 19).

It was thought that the upfield shifts of proton signals resulted from the magnetic anisotropic shielding by the ring current from the B’ ring of EGCg, and the whole theophylline molecule was captured by the hydrophobic space formed with EGCg, like as the caffeine and nicotinamide moieties in the 2:2 EGCg complex (Figs. 2a and 4).

Then changes in chemical shifts of proton signals of (R)- and (S)-proxyphyllines in ¹H-NMR spectra after adding fixed amount of EGCg were shown in Figs. 20a and b. Upfield shifts in proton signals for N₁–CH₃, N₃–CH₃, and H₈ of the xanthine skeleton, and H₁₀ of its side chain were observed, as well as downfield shifts in proton signals for H₁₁ and H₁₂ of its side chain.

The part of (R)- and (S)-proxyphyllines having the xanthine skeleton and C₁₀ in its side chain were included in the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg in the 2:2 complex, and C₁₁ and C₁₂ of the side chain existed outside the hydrophobic space.

Similarly, changes in chemical shifts of proton signals of (R)- and (S)-diprophyllines in ¹H-NMR spectra after adding...
fixed amounts of EGCg were shown in Figs. 21a, b. Upfield shifts in proton signals for N₁–CH₃, N₃–CH₃, and H₈ of the xanthine skeleton, and H₁₂α, β of its side chain were observed, and downfield shifts in proton signals for H₁₀α, β and H₁₁ of its side chain were observed. The part of (R)- and (S)-dipropyllines containing the xanthine skeleton and C₁₂ in its side chain were included in the hydrophobic space formed with the three aromatic A, B, and B’ rings of EGCg in the 2:2 complex, and C₁₀ and C₁₁ existed outside the hydrophobic space.

Figures 22a, b, and c show moieties of theophylline, proxyphylline, and dipropyphylline take into the hydrophobic space formed by the aromatic A, B, and B’/uni rings of EGCg, respectively.

Intramolecular NOEs of (R)- and (S)-dipropyphyllines, and (R)- and (S)-proxyphyllines in the EGCg complexes in D₂O were measured.

Intramolecular NOEs between H₈ and H₁₂ in (R)- and (S)-dipropyphyllines were observed in the EGCg complex of (R)- and (S)-dipropyphylline. While, intramolecular NOEs between H₈ and H₁₂ in (R)- and (S)-proxyphyllines were not observed in the EGCg complexes of (R)- and (S)-proxyphyllines. These findings suggest that the H₈ of (R)- and (S)-dipropyphyllines is in the vicinity of H₁₂, and that the H₈ of (R)- and (S)-proxyphyllines is not.

2.5.2. Chiral Recognition of Pharmaceuticals Having a Xanthine Skeleton by EGCg

In the ¹H-NMR spectra of an equimolar amount of racemic (R, S)-proxyphylline and EGCg (Fig. 23) in D₂O, the N₃–CH₃ signal of racemic (R, S)-proxyphylline was clearly observed as two singlets (Fig. 23). Based on the results shown in Figs. 20a and b, the singlets at 3.234 ppm and 3.223 ppm were assigned to the N₃–CH₃ proton signal of the racemic (R)- and (S)-proxyphylline, respectively.

While, split singlets for the N₁–CH₃ proton signal of the racemic (R, S)-proxyphylline or racemic (R, S)-dipropyphylline were not observed in the ¹H-NMR spectra (Figs. 23, 24).

Furthermore, although partly overlapping, splitting of the H₁₁ methine signal of (R)- and (S)-proxyphylline, which was bonded to the asymmetric carbon, and a splitting of the H₁₂ methylene signal of racemic (R, S)-dipropyphylline were observed in the ¹H-NMR spectra (Figs. 23, 24).

These findings suggested that (R)- and (S)-proxyphylline, and (R)- and (S)-dipropyphylline each formed complexes with EGCg, and are diastereomers of each other. Therefore, it was concluded that the chirality of proxyphylline and dipropyphylline was recognized by EGCg in water.

We then investigated the enantiomeric excess of dipropyphylline in water using the evidence of the clear splitting of the proton signal of the singlet derived from the N₁–CH₃ group of the racemic dipropyphylline.

An equimolar amount of EGCg was added to the aqueous solution of various ratios of (R)- and (S)-dipropyphylline. The enantiomeric excess ratio was calculated using the integrated values of the splitting singlets derived from the N₁–CH₃
groups of $R$- and $S$-forms of diprophylline in quantitative $^1$H-NMR spectra, and the correlation between the enantiomer -
ic excess ratio and those calculated from the ($R$) and ($S$) forms
used are shown in Table 5.

We plan to adapt this method to measure the enantiomeric
excess of various other water-soluble compounds.

3. Conclusion
We investigated molecular capture and chiral recognition, which are advanced functions of tea gallated catechins EGCg
in water.

It was found that the hydrophobic space formed with the aromatic A, B, and B’ rings of EGCg took various heterocy-
clic compounds to form 2:2 complex of the heterocyclic com-
pounds and EGCg, which precipitated from aqueous solution.
When the molecular capture abilities of various heterocyclic compounds using EGCg from the aqueous solutions were
evaluated with ratio of the heterocyclic compounds included
in the precipitates of EGCg complex to the heterocyclic com-
pounds used (Table 2).

It appeared that the molecular capture abilities of various heterocyclic compounds using EGCg depended not only on
the structure of the heterocyclic compound but also on such as physical factors of the degree of polymerization, entropy,
solubility, etc. of the 2:2 EGCg complex of heterocyclic com-
pound.

Interaction between EGCg and diketopiperazines cyclo(-
Pro-Gly), cyclo(-Pro-Gly), which are enantiomers each other,
was investigated using X-ray crystallography and $^1$H-NMR
spectral analysis.

A difference between the chemical shift of some proton sig-
nals for the Pro residue in the $^1$H-NMR spectrum of cyclo(-
Pro-Gly) with EGCg and that of cyclo(-Pro-Gly) with EGCg
was observed. Judging from the crystal structures of the 2:2
EGCg complexes of cyclo(-Pro-Gly), and that of cyclo(-Pro-
Gly), the difference in the chemical shift was derived mainly
from a magnetic anisotropic shielding effect by the ring cur-
rent from the B ring of EGCg.

Since the C ring of EGCg has two C$_2$ and C$_1$ of two chiral
carbon atoms, the hydrophobic space formed with the aro-
matic A, B, and B’ rings of EGCg is a chiral space. It was in-
dicated that the chiral space of EGCg recognized the chirality
of compounds included in the space. The chiral recognition
of the pharmaceuticals, propranolol, proxyphylline, and dipro-
pylline which are usually administered in a racemic form in
medical field, was next investigated using EGCg.

In the $^1$H-NMR spectrum of a solution of racemic ($R$)- and
($S$)-propranolols in a ratio of 1:1, with EGCg in D$_2$O, the H$_2$
proton signal of racemic propranolol was observed as two

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Fig. 22. Pharmaceuticals with a Xanthine Skeleton in the Complexes with EGCg
(a) Theophylline; (b) Proxyphylline; (c) Diprophylline. Yellow zones show moieties of the pharmaceuticals with a xanthine skeleton taken into the hydrophobic space formed by the aromatic A, B, and B’ rings of EGCg. (Color figure can be accessed in the online version.)

Fig. 23. Splitting of the Racemic Proxyphylline H$_{11}$ and N$_3$–CH$_3$ Proton Signals
(Color figure can be accessed in the online version.)

Fig. 24. Splitting of the Racemic Diprophylline H$_{12}$ and N$_3$–CH$_3$ Proton Signals
(Color figure can be accessed in the online version.)
doublet-like signals, suggesting that (R)- and (S)-propranolols each formed complexes with EGCg, which are diastereomers of each other. It was found that the chirality of propranolol was recognized by EGCg. However, the splitting of the methylene protons of the Pro residue of cyclo(Pro-Gly) and H₂ of the naphthalene moiety of propranolol was difficult to observe in the chiral recognition due to overlap of signals and small differences in their chemical shifts.

Thus, the chiral recognition of pharmaceuticals having a xanthine skeleton, proxyphylline and diprophylline, which have two methyl groups, were investigated using EGCg in water. In the ¹H-NMR spectra of D₂O solution of racemic proxyphylline and diprophylline in the presence of EGCg, the N₁–CH₃ signal of racemic proxyphylline and diprophylline was clearly observed as two singlets (Figs. 23, 24), suggesting that the chirality of proxyphylline and diprophylline was recognized clearly by EGCg.

Then the enantiomeric excess of diprophylline in water were measured by using the evidence of the above clear splitting of the singlet derived from the N₁–CH₃ group of the racemic diprophylline in quantitative ¹H-NMR spectra (Table 5).

Table 5. Enantiomeric Excess of Diprophylline Calculated Using the Integrated Values in ¹H-NMR Spectra

| Ratio | Diprophylline (mg) | enantiomeric excess (e.e. %) | Integrated values | enantiomeric excess (e.e. %) |
|-------|-------------------|----------------------------|------------------|---------------------------|
|       | S-form | R-form | S-form | R-form |                        |                        |
| 1:1   | 2.712  | 2.714  | 0.04   | 100    | 101.31                 | 0.65                   |
| 1:3   | 1.348  | 4.043  | 48.92  | 100    | 303.16                 | 50.39                  |
| 3:1   | 4.052  | 1.358  | 49.80  | 100    | 37.23                  | 45.74                  |
| 1:6   | 1.348  | 8.089  | 71.43  | 100    | 605.25                 | 76.41                  |
| 6:1   | 8.085  | 1.354  | 71.31  | 100    | 17.54                  | 70.15                  |

a) The integrated values are those of the splitting singlets derived from N₁–CH₃ groups of R- and S-forms of diprophylline in ¹H-NMR spectra.

As described above, we have been developing the high-order functions of molecular capture and chiral recognition using EGCg. The findings obtained this time will be applied to the convenient new optical resolving. At present, many pharmaceuticals are used as racemic form in clinical practice, but it is desirable to use only the enantiomer having greater potency judging from the viewpoint of adverse effects and pharmacokinetics. Therefore, by using EGCg or its derivatives, we will be developing a method that the desired enantiomer can be easily isolating from an aqueous solution as a precipitate, or the undesired enantiomer can be removed from the solution as a precipitate and the desired enantiomer is still soluble.

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Conflict of Interest The author declares no conflict of interest.

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