Regular Article

Chiral Recognition of Pharmaceuticals Having a Xanthine Skeleton by (−)-Epigallocatechin-3-O-gallate in Water

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A mixture of pharmaceuticals having a xanthine skeleton, theophylline, proxphylline, diprophylline and (−)-epigallocatechin-3-O-gallate (EGCg) in water created a sticky precipitates, which were thought to be 2:2 complexes of the pharmaceuticals and EGCg. The molecular capture ability of the pharmaceuticals having a xanthine skeleton by EGCg was estimated by the amount of the pharmaceuticals included in the precipitates of the complexes, and measured by the integrated value of proton signals in the quantitative 1H-NMR spectra. Based on changes in chemical shifts of proton signals of the pharmaceuticals with a xanthine skeleton in 1H-NMR spectra by adding standard amounts of EGCg, the xanthine skeleton of the pharmaceuticals was considered to exist in the hydrophobic space formed by the three aromatic A, B, B’ rings of EGCg, and a part of the proxphylline and diprophylline side chains existed out of the hydrophobic space. In the 1H-NMR spectra of the mixture of (R)- and (S)-proxphylline, (R)- and (S)-diprophylline and an equimolecular amount of EGCg, the N3-CH3 signal of (R)- and (S)-proxphylline, and (R)- and (S)-diprophylline was clearly observed as two singlets. This suggested that EGCg recognized the chirality of proxphylline and diprophylline in water.

Key words chiral recognition; (−)-epigallocatechin-3-O-gallate; 1H-NMR; pharmaceutical having xanthine skeleton; proxphylline; diprophylline

Catechins are a group of polyphenols that occur naturally in certain species of plants, including tea (Camellia sinensis, Camelliaeaceae), and are major ingredients in green tea infusions. The physiological activity and function of catechins have been studied by many researchers. The role of such molecules in the prevention of cancer and cardiovascular disease has received a great deal of attention.1,2) Catechins may be scavengers of reactive oxygen species, which are thought to cause several diseases such as cancer, lifestyle diseases, aging, etc.3,4)

We previously studied the molecular recognition, molecular capture, and asymmetric recognition of tea catechins in water. The gallated catechin (−)-epigallocatechin-3-O-gallate (EGCg), which is most abundant in tea catechins, formed a hydrophobic space surrounding the top and lower walls of the B’ rings of EGCg, and right and left walls of the A and B rings of EGCg in water. Caffeine5) and nicotineamide,6) which are also components of tea, were captured by the hydrophobic space formed by the three aromatic A, B, B’ rings of EGCg, and 2:2 complexes of EGCg were formed. As a result, the 2:2 complex precipitated from the aqueous solution due to its hydrophobicity.

Furthermore, it was assumed that the space formed by the three aromatic A, B, B’ rings of EGCg recognized the chirality of compounds included in the space because 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.

On this assumption, we attempted the chiral recognition of diketopiperazines cyclo(-Pro-Gly) and cyclo(ω-Pro-Gly) by EGCg. Upon formation of 2:2 complexes of EGCg and cyclo(-Pro-Gly), cyclo(ω-Pro-Gly) in D2O, the chirality of cyclo(Pro-Gly) was recognized by a difference in the chemical shift of 1H-NMR signal for some methylene protons of the Pro residue.7) Based on the crystal structures of the 2:2 complexes of EGCg and cyclo(-Pro-Gly), cyclo(ω-Pro-Gly), such a difference in chemical shift may have been due to magnetic anisotropic shielding effects by the ring current from the B ring of EGCg (Fig. 1).

However, the splitting of the methylene protons of the Pro residue of cyclo(Pro-Gly) was difficult to observe in the chiral recognition due to small differences in their chemical shifts and overlap of signals.7) In this report we applied chiral recognition to pharmaceuticals, which are currently used in a racemic mixture. Thus, the chiral recognition of pharmaceuticals with a xanthine skeleton for bronchial asthma, proxphylline and diprophylline were investigated using EGCg in water (Fig. 2). As pharmaceuticals having a xanthine skeleton have two methyl groups, which were observed as a sharp, strong singlet, the split of the methyl signal was considered as easy to observe in 1H-NMR spectra.

Experimental

NMR Experiments 1H-NMR spectra were recorded with a JEOL JMN-LA500 (Tokyo, Japan) operating at 500MHz. D2O (99.9 atom% D; Wako Pure Chemical Industries, Ltd.) was used as a solvent. Chemical shift values are expressed in ppm downfield using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. The nuclear Overhauser effect (NOE) difference experiments were typically conducted with 32K data points covering a spectral width of 10000Hz and with ca. 5s presaturation time.

Quantitative 1H-NMR (qNMR) Experiments qNMR was performed with the following optimized parameters: probe temperature, 25°C; spinning, off; number of scans, eight; spectral width, 20ppm; relaxation delay, 64s; pulse angle, 90°; internal standard, DSS-d6 (Wako Pure Chemical

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Dimethyl sulfoxide (DMSO)-$d_6$ (99.9 atom% D; Wako Pure Chemical Industries, Ltd.) was used as a solvent and DSS-$d_6$ (Wako Pure Chemical Industries, Ltd.) was used as an internal standard.

Preparation and qNMR of Sticky Precipitate Formed by EGCg and Pharmaceuticals Having a Xanthine Skeleton

EGCg (1.09×10^{-2} mmol) and pharmaceuticals having a xanthine skeleton (1.09×10^{-2} mmol) were dissolved in D$_2$O (70 µL) at 90°C, and left at room temperature for a day to obtain a supernatant liquid and sticky precipitate. After removing the supernatant liquid, the sticky precipitate was evaporated under reduced pressure to create a residue.

The residue was dissolved in DMSO-$d_6$ (520 µL) containing DSS-$d_6$ (1.44×10^{-3} mmol). The content of the resulting DMSO-$d_6$ solutions was measured by qNMR with the methyl group of DSS-$d_6$ as an internal standard.

Results and Discussion

Complex Formation of EGCg with the Pharmaceuticals Having a Xanthine Skeleton

Equimolecular amounts of pharmaceuticals having a xanthine skeleton, theophylline, proxyphylline, diprophylline 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 measurement of the integral volume of $^1$H-NMR signals. The precipitates were thought to be 2:2 complexes of the pharmaceuticals with a xanthine skeleton and EGCg such as the 2:2 complex of cyclo(Pro-Gly) and EGCg$^7$ (Fig. 1). The 2:2 complexes had a hydrophobic space formed by three aromatic A, B, B’ rings of EGCg, and the pharmaceuticals having xanthine were captured in the hydrophobic space from the aqueous solution.

The molecular capture ability of the pharmaceuticals with a xanthine skeleton by EGCg was estimated by the amount of pharmaceuticals included in the precipitates of the complexes, which was measured by the integrated value of proton signals in the $^1$H-NMR spectra (Table 1).

Changes in chemical shifts of proton signals of theophylline in $^1$H-NMR spectra by adding standard amounts of EGCg were observed. Upfield shifts in proton signals for all protons N$_1$-CH$_3$, N$_3$-CH$_3$ and H$_8$ were observed of EGCg, and the pharmaceuticals having xanthine were captured in the hydrophobic space from the aqueous solution.
(Fig. 3).

It was thought that the upfield shifts of proton signals resulted from the magnetic anisotropic shielding by the ring current from the B’ rings of EGCg, and the whole theophylline molecule was captured by the hydrophobic space formed by EGCg, such as the cyclo(Pro-Gly) moieties of the 2:2 complex of cyclo(Pro-Gly) and EGCg, as shown in Figs. 1 and 6a.

Next, changes in chemical shifts of proton signals of (R)- and (S)-proxyphyllines in ¹H-NMR spectra by adding standard amounts of EGCg were observed (Figs. 4a, b). Upfield shifts in proton signals for N₁-CH₃, N₃-CH₃, and H₈ of the xanthine skeleton, and H₁₀α,β of its side chain were observed, and downfield shifts in proton signals for H₁₁ and H₁₂ of its side chain were observed. It was thought that the downfield shifts in proton signals resulted from the deshielding by the ring current from the B’ rings of EGCg.

The part of (R)- and (S)-proxyphyllines containing the xanthine skeleton and C₁₀ in its side chain were included in the hydrophobic space formed by the three aromatic A, B, B’ rings of EGCg in the 2:2 complex, and C₁₁ and C₁₂ of the side chain existed out of the hydrophobic space, as shown in Fig. 6b.

Similarly, changes in chemical shifts of proton signals of (R)- and (S)-diprophyllines in ¹H-NMR spectra by adding standard amounts of EGCg were observed (Figs. 5a, b). Upfield shifts in proton signals for N₁-CH₃, N₃-CH₃, and H₈ of the xanthine skeleton, and H₁₁ 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)-diprophyllines containing the xanthine skeleton and C₁₂ in its side chain were included in the hydrophobic space formed by the three aromatic A, B, B’ rings of EGCg in the 2:2 complex, and C₁₀ and C₁₁ existed out of the hydrophobic space, as shown in Fig. 6c.

![Fig. 4. The Chemical Shift Change of the (R)-Proxyphylline (a) and (S)-Proxyphylline (b) Protons by Adding EGCg](image)

Initial condition: solution of (R)- and (S)-proxyphylline (10 mM) in D₂O at 35°C.

![Fig. 5. The Chemical Shift Change of the (R)-Diprophylline (a) and (S)-Diprophylline (b) Protons by Adding EGCg](image)

Initial condition: solution of (R)- and (S)-diprophylline (10 mM) in D₂O at 35°C.

![Fig. 6. The Pharmaceuticals Having a Xanthine Skeleton in the Complex of EGCg; (a) Theophylline, (b) Proxyphylline, and (c) Diprophylline](image)

Each gray zone shows moieties of the pharmaceuticals with a xanthine skeleton that entered into the hydrophobic space formed by the three aromatic A, B, B’ rings of EGCg.
The intramolecular nuclear Overhauser effects (NOEs) of (R)- and (S)-diprophyllines, and (R)- and (S)-proxyphyllines in the complexes with EGCg in D_2O were measured. Intramolecular NOEs between H_8 and H_12 in (R)- and (S)-diprophyllines were observed in the complex of EGCg and (R)- and (S)-diprophylline. On the other hand, intramolecular NOEs between H_8 and H_12 in (R)- and (S)-proxyphyllines were not observed in the complex of EGCg and (R)- and (S)-proxyphyllines. This suggested that the H_8 of (R)- and (S)-diprophyllines was in the vicinity of H_12, and the H_8 of (R)- and (S)-proxyphyllines was not in the vicinity of H_12.

Chiral Recognition of Pharmaceuticals Having a Xanthine Skeleton by EGCg

In the 1H-NMR spectra of an equimolecular amount of racemic (±)-proxyphylline and EGCg (Fig. 7) in D_2O, the N_3-CH_3 signal of racemic (±)-proxyphylline was clearly observed as two singlets. Based on Figs. 4a and b, the singlets at 3.234 and 3.223 ppm were assigned to the N_3-CH_3 proton signal of (R)- and (S)-proxyphylline, respectively.

In the 1H-NMR spectra of an equimolecular amount of racemic (±)-diprophylline and EGCg (Fig. 8) in D_2O, the N_3-CH_3 signal of racemic (±)-diprophylline was clearly observed as two singlets. Based on Figs. 5a and b, the singlets at 3.225 and 3.238 ppm were assigned to the N_3-CH_3 proton signal of the racemic (R)- and (S)-diprophylline, respectively. On the other hand, split singlets for the N_1-CH_3 proton signal of the racemic (±)-proxyphylline or racemic (±)-diprophylline was not observed in the 1H-NMR spectra.

Furthermore, although partly overlapping, splitting of the H_11 methine signal of (R)- and (S)-proxyphylline, which was bonded to the asymmetric carbon, and a splitting of the H_12 methylene signal of racemic (±)-diprophylline were observed.

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

We will adapt this method to measure the enantiomeric excess of various racemic compounds in water.

Conflict of Interest The authors declare no conflict of interest.

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9) Pure enantiomers (R)- and (S)-proxyphylline were synthesized as follows: 1 equiv theophylline and 1.15 equiv of potassium hydroxide were dissolved at 70°C in water. 1.15 equiv of (R)- or (S)-1-chloro-2-propanol mixed with water was then added dropwise, and the reaction mixture was stirred over 24h at 70°C. The water was evaporated under vacuum, and the solid was dried. The solid was dissolved in EtOH, and heated for 3h and filtered. After evaporating EtOH, the crude product was purified by HPLC (YMC-Pack ODS-AL 250×20) with acetonitrile–H_2O (1:9).