Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 10, pp. 1021-1027, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00069-7

The Specific Inhibition of Crystal Growth of Monohydrogen Potassium L-Tartrate by *d*-Catechin

Seiichi Imajo, Masaji Ishiguro,*[a] Kyoko Ishiguro,[c] Tokiko Murashima,[c] Koichiro Isoi,[c] Hirohumi Tanahashi[b] and Haruo Nishino[b]

[a]*Institute for Biomedical Research and* [b]*Research Institute for Enology, Suntory Ltd, Osaka 618 Japan,* [c]*School of Pharmacy, Mukogawa Women's University, Nishinomiya 663, Japan*

Abstract—Crystal growth of monohydrogen potassium L-tartrate in an ethanolic aqueous solution was specifically inhibited by *d*-catechin, but not by either its epimeric isomer at C3, *l*-epicatechin or by gallic acid and caffeic acid. 3D-Structure similarity search of *d*-catechin with two molecules of the tartrate and docking study of *d*-catechin with the crystal model of the tartrate suggested that *d*-catechin mimics a structure consisting of the two tartrate molecules in the inhibition. Differences in the conformation of the catechol moieties of *d*-catechin and *l*-epicatechin may explain the distinct inhibitory effects of the epimeric isomers.

Introduction

Polyphenolic compounds in Japanese or Chinese herbal medicine have been shown to have many interesting biological activities such as protective activity against bacterial infection.[1] Among these, *d*-catechin is one of the popular polyphenolic constituents in a variety of plants used as herbal remedies. Its mode of action, however, in biological events at molecular level remains uncertain. Hence, investigation of its physical or chemical characteristics will bring useful information for understanding its role in biological events.

d-Catechin is one of the major phenolic composites in green tea and red wine. It has been suggested that certain phenolic compound(s) may inhibit crystal formation of the tartrate.[2] This prompted us to investigate the inhibitory effect of phenolic compounds on crystal formation. We report herein a specific inhibitory effect of *d*-catechin on the crystal formation of tartrate in an ethanolic aqueous solution and computational evidence on a structural similarity between *d*-catechin and the tartrate which should be one of the major factors for the inhibition of the crystal formation of the tartrate through a specific molecular recognition.

There are reports on inhibitory proteins acting against crystal growth such as antifreeze protein[3] and calcium oxalate monohydrate crystal growth inhibitor in human urine.[4] Crystallization of organic and inorganic molecules in biological systems elicits severe diseases such as gout, which is caused by crystallization of uric acid at articular cartilage. Thus, prevention of crystal growth of organic and inorganic molecules is of importance in the medicinal field.

Results and Discussion

Inhibitory effects of polyphenolic compounds

Figure 1 shows an inhibitory effect on formation of the precipitate of the tartrate in the presence of selected phenolic compounds. The precipitation of crystal was completed within three days and prolonged time did not give any appreciable change of amount. Among those phenolic compounds listed in Figure 2, only *d*-catechin was found to prevent the precipitation in a concentration dependent manner, and a high inhibitory effect (> 90 %) was observed at more than 250 ppm, while other compounds did not show any significant inhibitory effect even at 1000 ppm. It is remarkable that *l*-epicatechin, the epimer of *d*-catechin at C3, did not inhibit the crystal growth at all. Fine crystals of the tartrate precipitated as shown in Figure 3 irrespective of the presence of *l*-epicatechin, caffeic acid or gallic acid, whereas the precipitated crystals in the presence of *d*-catechin have a characteristic anomaly at the surface (Figure 4). It is also noted that the tartrate precipitates as much larger crystals in the presence of *d*-catechin (see Figures 3 and 4). These results suggest a specific recognition of *d*-catechin in the growth of crystals of the tartrate.

Structural similarity of d-*catechin to the tartrate*

Although there was no obvious structural similarity between *d*-catechin and the tartrate, we supposed that *d*-catechin would correspond to a structure consisting of two molecules of the tartrate (hereafter dimeric tartrate) and would somehow mimic the structure at a growing crystal surface of the tartrate since the molecular weight of *d*-catechin (M_w = 290.28) is almost twice that of L-tartaric acid (M_w = 150.09). We first examined the three dimensional similarity of *d*-catechin to the dimeric tartrate, which was taken from the unit cell of the crystal structure of the tartrate, using the steric and electrostatic alignment molecular superposition program (SEA)[5] which estimates both steric and electrostatic similarity. Since we focused on sterical similarity for molecular recognition on the crystal surface, only the steric term was chosen for comparison. Figure 5 shows three out of eleven modes of superimposition of *d*-catechin on the dimeric tartrate

generated with SEA. This result indicates a good similarity (76–84 %) between *d*-catechin and the dimeric tartrate in their three dimensional shapes. There was not a large difference in the similarities of *d*-catechin and *l*-epicatechin due to only small structural differences, whereas both gallic acid (M_w = 170.12) and caffeic acid (M_w = 180.16) showed lower similarity (45–66 %) to the dimeric tartrate. These results with SEA are summarized in Table 1. Lower similarity (69–76 %) of gallic acid and caffeic acid to the tartrate monomer should be also noted.

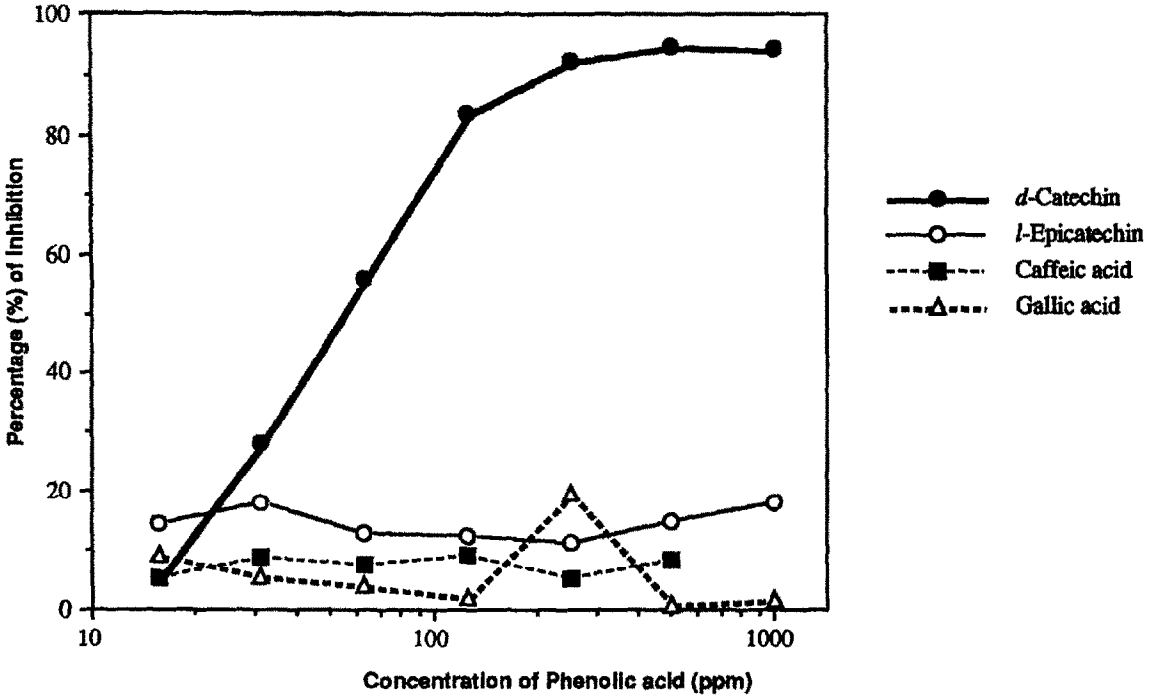
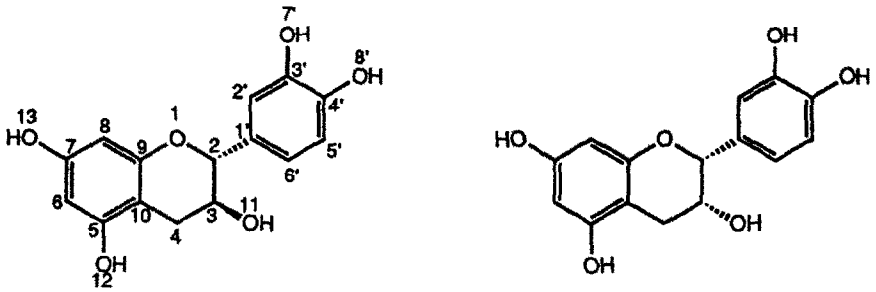


Figure 1. Inhibitory effect of four phenolic compounds on crystallization of the tartrate.



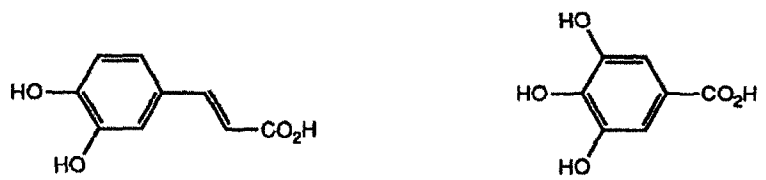


Figure 2. Typical phenolic compounds examined in this study.

Figure 3. Tartaric acid crystallized from aqueous ethanolic solution (× 100).

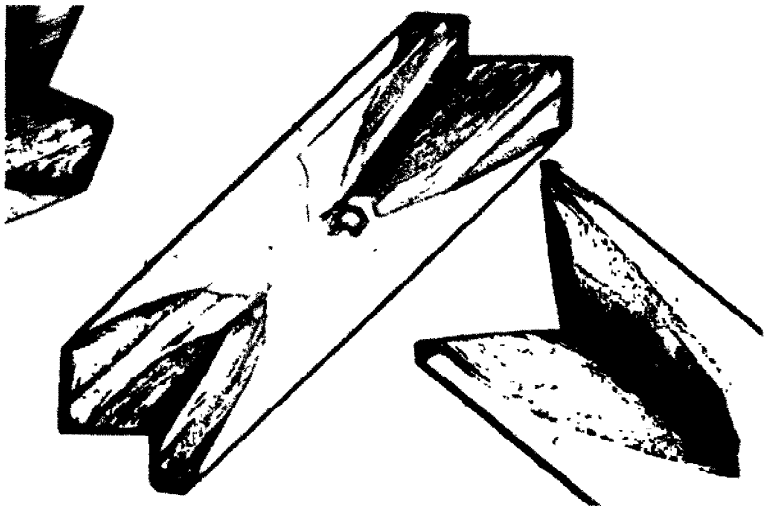


Figure 4. Crystal of the tartrate in the presence of 500 ppm of *d*-catechin (×100).

Table 1. Similarity index for the phenolic compounds to the dimeric tartrate obtained with SEA

Compounds	SEA Steric Energy	No. of superposition
Tartrate Dimer	-87.43*	1
d-Catechin	-73.35 ~ -66.63	11
l-Epicatechin	-73.63 ~ -69.59	9
Caffeic acid	-57.44 ~ -39.34	8
Gallic acid	-51.29 ~ -39.20	13

*Steric energy for the self superimposition. Percentage of similarity was estimated by dividing each energy value with this value (see text).

Docking study of d-*catechin with a model of the crystal nuclei of the tartrate*

Since the anomaly of the crystal surface shown in Figure 4 suggests an interaction of *d*-catechin at a specific face of crystal-growing sites, one of two stable conformers of *d*-catechin (see below) was docked on the surface of the model of the crystal nuclei consisting of four molecules of the tartrate with a docking program in COSMIC[6] which estimates energy for ligand interaction over the entire surface of host molecule(s). Figure 6 shows a complex structure model of *d*-catechin on a crystal surface at the lowest energy. A good surface complementarity of the molecules is demonstrated in Figure 7. *d*-Catechin, in the complex, forms extensive van der Waals contacts over the crystal surface at the same site where the dimeric tartrate can be found in the mature crystal. Figure 8 shows the superimposition of *d*-catechin with the dimeric tartrate found at the site. A similar mode of superimposition can be found in the mode No.6 (thin line in Figure 8) obtained with SEA. Hence, these results confirm the structural resemblance of *d*-catechin with the dimeric tartrate.

A putative role of the five hydroxyl groups in d-*catechin for complex formation*

The phenol groups at C7 and C4' (Figure 8) show a good structural correlation with carboxylate oxygens in the tartrate, while no corresponding oxygen atom in the tartrate

was found for the phenols at C5 and C3'. Among those four phenol groups, the phenols at C7 and C3' in the complex model adopt distances for hydrogen-bonds (2.78 Å and 2.69 Å) with the carboxylate oxygens of the tartrate molecules (indicated by broken lines in Figure 6). Thus, the phenol at C3' should enhance the interaction of *d*-catechin with the tartrate crystal nucleus through an extra hydrogen-bond and should have particular contribution to the specific inhibitory effect. The other two phenol groups at C7 and C4' may have similar function of carboxylate oxygens of the tartrate. The phenol at C5 has neither specific interaction with the crystal nor significant structural correlation with the dimeric tartrate. The hydroxyl group at C3 has no specific interaction with any tartrate either. However, due to the equatorial configuration of this hydroxyl group the catechol moiety is rigidly fixed in two but very close conformers with torsion angles (ψ=137 ° and –45 °) of O1-C2-C1'-C2'. We used the most stable conformer (ψ=137 °) for the similarity search and docking study as described above. The other conformer (ψ = –45 °) turns its phenol group at C3' to the opposite direction to the tartrate surface. Thus, no hydrogen-bond with any tartrate can be expected for the phenol group. A conformational analysis revealed that *l*-epicatechin also has two stable conformers (ψ = 153 ° and –30 °). However, the most stable conformation of the catechol moiety of *l*-epicatechin has a larger torsion angle (ψ = 153 °) than that of *d*-catechin (ψ = 137 °) due to a sterical interaction with the axial hydroxyl group at C3, which results in a longer distance (3.03 Å) between the phenol group at C3' and the carboxylate oxygen. In addition, the axial hydroxyl group at C3 makes the catechol moiety more flexible than the equatorial hydroxyl group of *d*-catechin since the maximum rotational energy barrier at the C2–C1' bond is 5.5 kcal/mol for *d*-catechin, but 3.0 kcal/mol for *l*-epicatechin. These differences of the torsion angle (ψ) and the conformational flexibility may account for the stereospecific effect in the inhibition by *d*-catechin. Indeed, docking of *l*-epicatechin over the model of crystal nuclei of the tartrate with the docking program gave a totally different complex structure at the lowest energy, in which no significant structural similarity to the tartrate molecule(s) was found (data not shown).

In conclusion, we demonstrated the specific inhibitory effect of *d*-catechin on the crystal growth of the tartrate. Examination of the similarity of *d*-catechin and other polyphenolic compounds to the tartrate and docking study with the crystal model of the tartrate, showed a structural resemblance between *d*-catechin and the dimeric tartrate

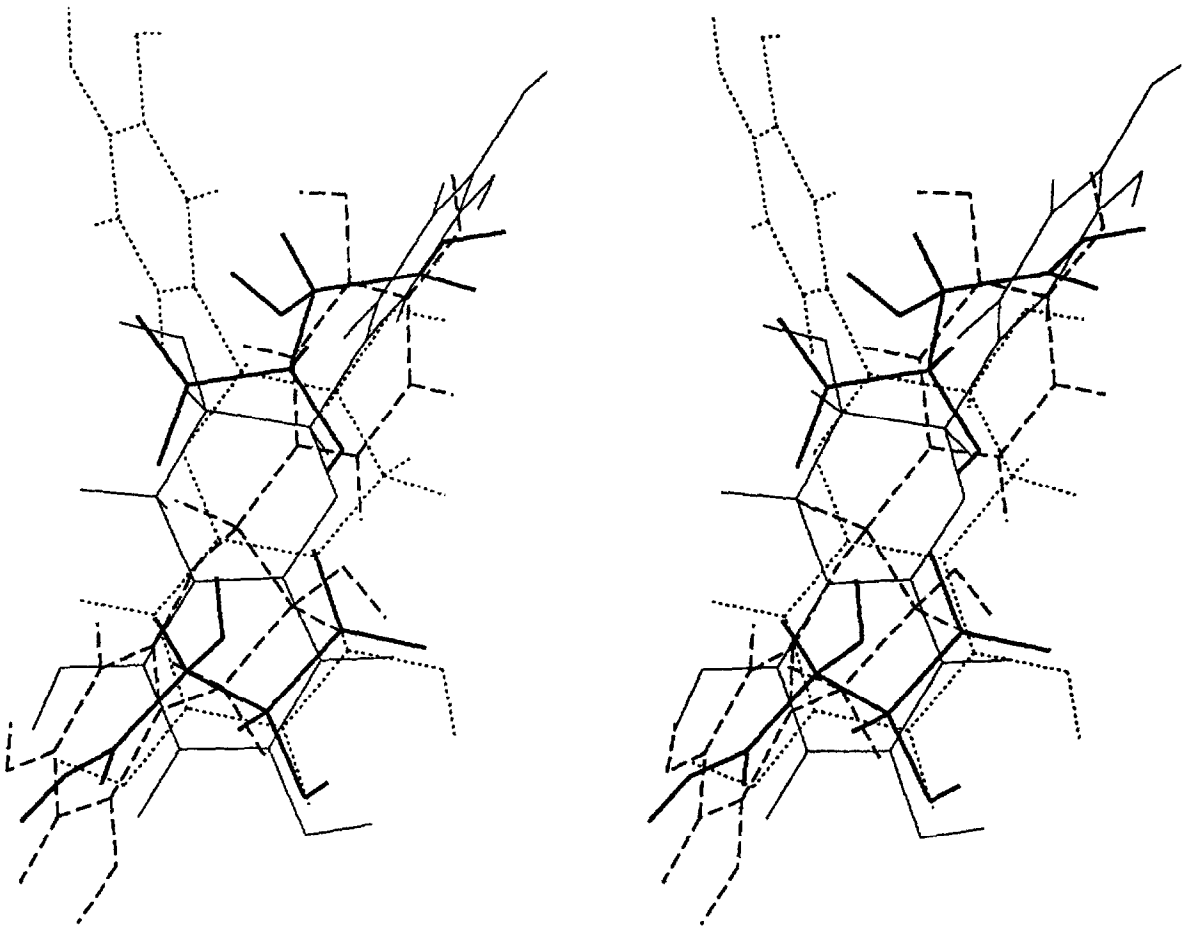


Figure 5. Superimposition of *d*-catechin over the dimeric tartrate (bold line). Three modes (Nos 1, 6 and 11) are shown in thin, broken, and dotted lines respectively.

and a good surface complementarity at the interaction sites. Hence, it can be proposed that *d*-catechin may efficiently inhibit the crystal growth of the tartrate through the specific molecular recognition at the crystal surface by mimicking the dimeric tartrate. The distinct role of the five hydroxyl groups for the recognition and the inhibition are noted. In particular, the equatorial hydroxyl group at C3 contributes to the rigid conformation of the catechol moiety of *d*-catechin. Thus, the phenol group at C3' may form a hydrogen-bond with the tartrate molecule. This hydrogen bond should have an important role for not only molecular recognition, but also an inhibitory effect for the crystallization as found in biochemical systems, where inhibitors or antagonists of enzymes and receptors mimic the structures of their natural ligands and also have extra interactions at different sites of the biomacromolecules. Thus, the mimicry of two-molecules structure with extra interaction as elucidated here may lead to the design of inhibitors of crystallization of molecules of particular interest in the biomedical field.

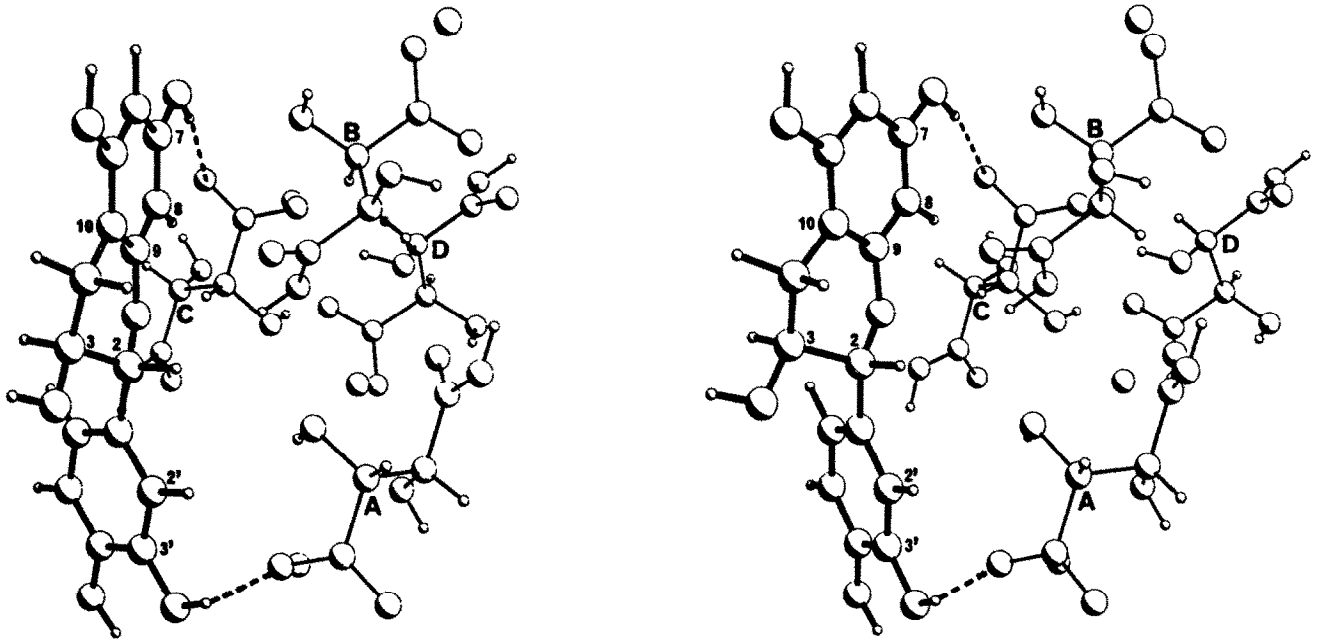


Figure 6. A complex structure model of *d*-catechin (bold line) on the tartrate crystal nucleus (four molecules: A, B, C and D; thin line) represented by a ball-and-stick model using PLUTO[11] (stereoview). Two possible hydrogen bonds are indicated by broken lines (O13 and carboxyl oxygen in molecule C and O7' and carboxyl oxygen in molecule A).

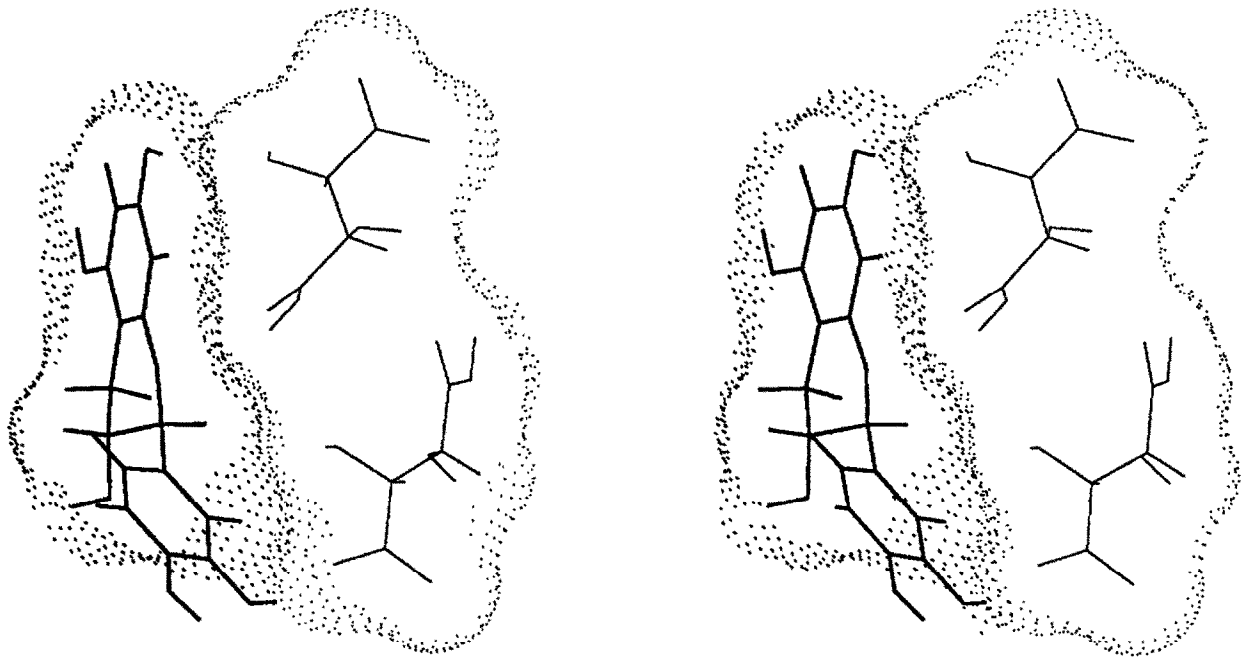


Figure 7. Complementary surfaces of *d*-catechin (bold lines and dots) and the tartrate crystal (thin lines and dots). For clarity, only dotted surfaces are clipped.

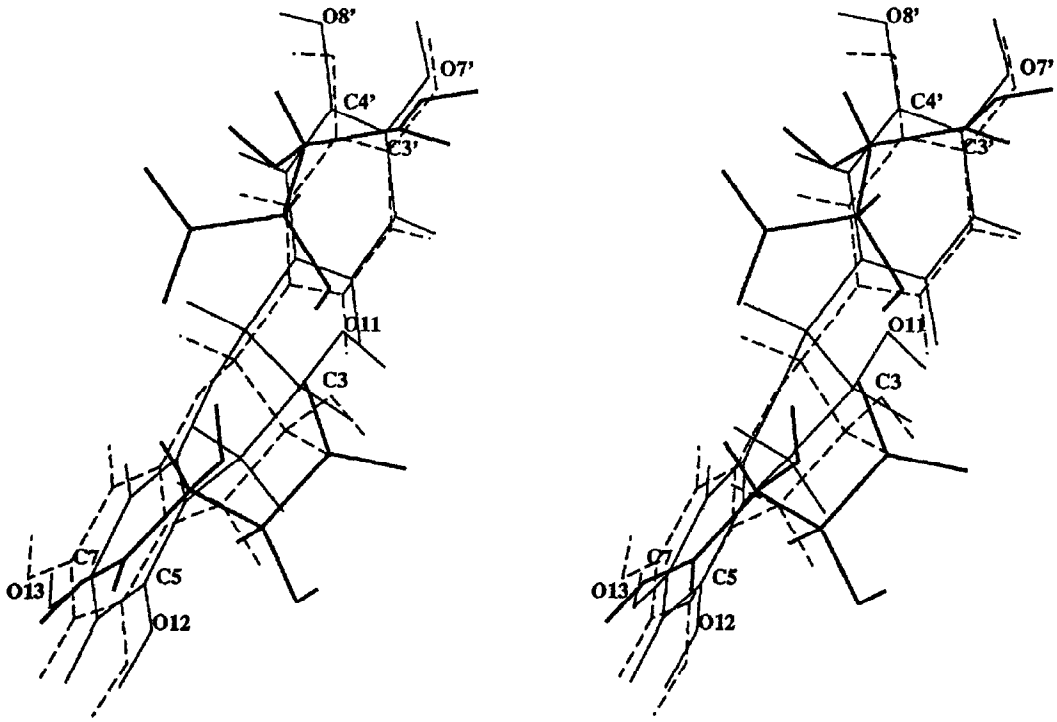


Figure 8. Superimposed line model of the dimeric tartrate (bold) and *d*-catechin (thin) obtained with the COSMIC program. The No. 6 of superimposition mode (see Figure 5) with SEA is shown in broken line model. The numberings of the hydroxyl groups are given on the thin line model.

Experimental

Chemicals

d-Catechin, *l*-epicatechin, caffeic acid and gallic acid were available from Tokyo Kasei Co., and the tartrate was bought from Merck Co., and those compounds were purified by recrystallization. Ethanol (99 %) was purchased from Nakarai Tesque Co.

Precipitation of the tartrate in the presence of phenolic compounds

Solutions of each phenolic compound in ethanolic (12 %(v/v)) aqueous solution of potassium hydrogen tartrate (2450 ppm) were prepared in concentrations of each phenolic compound of 1000, 500, 250, 125, 62.5, 31.25, and 15.63 ppm by two-fold dilution method. This solution was filtered through a membrane filter (0.45 μm, Millipore Co.) and left for 3 days at −4 ± 2 °C. Precipitated crystals were collected by filtration through membrane filters (0.45 μm), followed by drying in air and subsequent measurement of the weight of the resulting crystals. No appreciable impurity such as phenolic compound was found in the crystals as determined by NMR and elemental analysis. Percentage inhibition was estimated from the ratio of the unprecipitated tartrate to the control.

Coordinates for molecular modeling

The coordinates of *l*-epicatechin[7] are available from Cambridge Structure Database (CSD). Coordinates of *d*-catechin were generated by modification of the X-ray crystallographic data of 8-bromo-tetra-*O*-methyl-(*d*)-catechin[8] and optimized with MM2 in MACROMODEL.[9] A crystal structure of the tartrate[10] is available from CSD and the unit cell structure which consists of four tartrate molecules was used for our present study.

Conformational analysis and docking study of d-*catechin and l-epicatechin*

Conformational energy was calculated on each conformation generated by rotation of C2–C1' bond in 10 degree intervals with Multiconformation Input-Mode in MACROMODEL and minimized with FXTA constraint for the torsion angle ψ in MACROMODEL.[9]

Conditions used for SEA estimation[5] are as follows. Number of iterations: 500; translation scale factor:0.5; van der Waals scale factor: 0.8. Only the steric term was used for calculation. The smaller molecular weight molecule was scanned over the larger one. Hence, for comparison between the dimeric tartrate and the phenol compounds, the latter compounds were scanned over the dimeric tartrate while between the tartrate monomer and the phenol compounds the tartrate was scanned over the latter. Results obtained for the dimeric tartrate are shown in Table 1.

Interaction between *d*-catechin and the crystal structure of the tartrate which consists of four molecules in its unit cell was estimated with DOCK in COSMIC.[6] The electron density of each atom required for DOCK was calculated with CNDO/2. *d*-Catechin was moved on the four tartrate molecules which are fixed during calculation. The details of the other conditions for the calculation are described in Reference 6.

References

1. Sakanaka, S.; Kim, M.; Taniguchi, M.; Yamamoto, T. *Agric. Biol. Chem.* **1989**, *53*, 2307.

2. Pilone, B. F.; Berg, H. W. *Am. J. Enol. Vitic.* **1965**, *16*, 195; Tanahashi, H.; Nishino, H.; Nakai, T. *Proceedings of the 10th Symposium on Industrial Crystallization*, Bechyne, 1988, 631.

3. Sonnichsen, F. D.; Sykes, B. D.; Chao, H.; Davies, P. L. *Science* **1993**, *259*, 1154.

4. Nakagawa, Y.; Abram, V.; Kezdy, F. J.; Kaiser, E. T.; Coe, F. L. *J. Biol.Chem.* **1983**, *258*, 12594.

5. Smith, G. M. *SEA: Steric and Electrostatic Alignment Molecular Superposition Program*, QCPE program No. 567.

6. Vinter, J. G.; Davis, A; Saunders, M. R. *J. Computer-Aided Mol. Design* **1987**, *1*, 31.

7. Fronczek, F. R.; Gannuch, G.; Mattice, W. L.; Tobiason, F. L.; Broeker, J. L.; Hemingway, R. W. *J. Chem. Soc., Perkin 2* **1984**, 1611. CSD-refcode: COWHUR.

8. Engel, D. W.; Hattingh, M.; Hundt, H. K. L.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1987**, 695. CSD-refcode: BTMCAT.

9. Still, W. C.; Caufield, C.; Geller, G.; Glick, G. D.; Guida, W. C.; Lambiris, E.; Lipton, M.; Liskamp, R. M. J.; Mohadai, F.; Richards, N. G. J; Rothman, J.; Taylor, L. A.; and Walker, W. D. *MACROMODEL*, ver. 1.1, 1986; Department of Chemistry, Columbia University, NY 10027.

10. Bushman, J. B.; Luger, P. *Acta Cryst.*, C **1985**, *41*, 206. CSD-refcode: ZZZRZW01.

11. Allen, F. H.; Bellard, S. H.; Brice, M. D.; Cartwright, B. A.; Doubleway, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rogers, J. A.; Watson, D. G. *Acta Cryst.* **1979**, *B35*, 2331.

(*Received in U.S.A.* 10 *February* 1994; *accepted* 25 *May* 1994)