Tea leaves contain a wide variety of polyphenols that are beneficial to health and may have therapeutic applications. The polyphenolic compounds chafurosides A (II) and chafurosides B (IV) are flavone C-glycosides first isolated from oolong tea leaves (Fig. 1). A structural feature of chafurosides A and B is the unusual ether linkage between the C2-hydroxyl group of the mannose and the aglycone, which is generated by the fermentation process of tea leaves through the intramolecular ring-closing reaction of isovitexin-2'-sulfate (I) and vitexin-2'-sulfate (III) (prechafurosides A and B), respectively (Fig. 1). These flavonoids have a number of pharmacological activities. The oral administration of chafurosides A (1–10 µg/kg) and chafurosides B (100 µg/kg) reduced the skin reaction of sensitized mice after epicutaneous exposure to 2,4-dinitrofluorobenzene or 2,4,6-trinitro-1-chlorobenzene. This indicates that they have antiinflammatory effects.

Chafurosides have a number of beneficial pharmacological activities related to antiinflammation at various concentrations. However, no crystallographic study of chafurosides has yet been reported. In the present study, the crystal structures of chafurosides A and chafurosides B were investigated using single-crystal X-ray diffraction. The asymmetric unit of the chafurosides A crystal consists of one chafurosides A and two water molecules, and that of chafurosides B contains one chafurosides B and one water molecule. The flavone moiety of chafurosides A is curved, i.e., the angle between the best-fit planes of the chromene and phenyl rings is 18.9°, whereas that of chafurosides B flavone moiety is relatively flat. A comparison of the curvatures of the flavone moieties of various C-glycosides showed that the curvature of chafurosides A is significantly larger than those of the others. This structural feature might contribute to the differences between the strengths of the pharmacological activities of chafurosides A and B.

Key words flavone C-glycoside; oolong tea leaf; chafuroside; single-crystal X-ray diffraction

Introduction

Crystal Structures of Flavone C-Glycosides from Oolong Tea Leaves: Chafurosides A Dihydrate and Chafurosides B Monohydrate

Yasunori Iwao,* Hitoshi Ishida,† Shin-ichiro Kimura,‡ Toshiyuki Wakimoto,§ Hiromu Kondo,∥ Shigeru Itai,∥ and Shuji Noguchi∗

Graduate School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan; Faculty of Pharmaceutical Sciences, Hokkaido University; Kita-12, Nishi-6, Sapporo 060–0812, Japan; and Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.

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Chafurosides A and chafurosides B are flavone C-glycosides isolated from oolong tea leaves. They have a number of beneficial pharmacological activities related to antiinflammation at various concentrations. However, no crystallographic study of chafurosides has yet been reported. In the present study, the crystal structures of chafurosides A and chafurosides B were investigated using single-crystal X-ray diffraction. The asymmetric unit of the chafurosides A crystal consists of one chafurosides A and two water molecules, and that of chafurosides B contains one chafurosides B and one water molecule. The flavone moiety of chafurosides A is curved, i.e., the angle between the best-fit planes of the chromene and phenyl rings is 18.9°, whereas that of chafurosides B flavone moiety is relatively flat. A comparison of the curvatures of the flavone moieties of various C-glycosides showed that the curvature of chafurosides A is significantly larger than those of the others. This structural feature might contribute to the differences between the strengths of the pharmacological activities of chafurosides A and B.

Key words flavone C-glycoside; oolong tea leaf; chafuroside; single-crystal X-ray diffraction

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Experimental

Purification and Crystallization Chafurosides A and B were extracted from oolong tea leaves, purified with several column chromatographies, and crystallized from aqueous methanol solution using the method reported by Ishida et al.\textsuperscript{5}) Prior to extraction, the oolong tea leaves were heated at above 388 K to promote the conversion of prechafurosides A and B to chafurosides A and B, respectively.\textsuperscript{6}) Purified and recrystallized samples were stored in a desiccator over dry silica gel at room temperature until use.

Single-Crystal Structure Determination Details of the crystallographic data, data collection, and structure refinement are summarized in Table 1. The diffraction data for chafurosides A and B crystals were obtained at the SPring-8 BL02B1 and Aichi Synchrotron Center BL2S1, respectively. The initial structures were determined using SHELXT,\textsuperscript{13}) and the structures were crystallographically refined using SHELXL\textsuperscript{14}) and shelXle.\textsuperscript{15}) Hydrogen atoms were located in difference Fourier

Table 1. Crystallographic Data for Chafurosides A and B

| Crystal data | Chafuroside A dihydrate | Chafuroside B monohydrate |
|--------------|------------------------|---------------------------|
| Chemical formula | C\textsubscript{21}H\textsubscript{18}O\textsubscript{9}·2 (H\textsubscript{2}O) | C\textsubscript{21}H\textsubscript{18}O\textsubscript{9}·H\textsubscript{2}O |
| Space group | P\textsubscript{2}\textsubscript{1}2\textsubscript{1}2\textsubscript{1} | P\textsubscript{2}\textsubscript{1} |
| Crystal color | Yellow | Yellow |
| Cell parameters (Å) | | |
| a | 6.8007 (7) | 4.880 (4) |
| b | 10.7162 (11) | 19.650 (4) |
| c | 26.047 (3) | 9.720 (2) |
| Z | 4 | 2 |
| Data collection | | |
| Crystal size (mm) | 0.2 × 0.01 × 0.01 | 0.4 × 0.02 × 0.01 |
| Temperature (K) | 100 | 100 |
| Wavelength (Å) | 0.7006 | 0.75 |
| (sinθ/λ)\textsubscript{max} (Å\textsuperscript{−1}) | 0.649 | 0.666 |
| No. of reflections | | |
| Measured | 11792 | 30375 |
| Independent | 4288 | 4550 |
| Observed [I > 2σ] | 4157 | 4402 |
| R\textsubscript{int} | 0.0314 | 0.098 |
| Refinement | | |
| No. of reflections | 4288 | 4550 |
| R[F\textsuperscript{2} > 2σ (F\textsuperscript{2})] | 0.0306 | 0.0434 |
| wR(F\textsuperscript{2}) | 0.1002 | 0.124 |
| Δρ\textsubscript{max}, Δρ\textsubscript{min} (e Å\textsuperscript{−3}) | 0.33, −0.23 | 0.35, −0.43 |
maps and were treated as riding on their parent atom. The absolute structure of chafuroside A could not be reliably determined from the Flack parameter value (0.35(25)), and the absolute structure was set at that reported by Ishida et al. In the chafuroside B refinement, nine bond lengths and two angle distances of the disordered glucopyranoside ring were restrained to each of the average values calculated using Mogul in the CSD system. Although the hydrogen atoms of chafuroside B and a water molecule at the major site, with occupancies of 0.853(4), were located in difference Fourier maps, those at the minor site, with occupancies of 0.147(4), were not, and the hydrogen atoms were restrained so that reasonable hydrogen bonds were formed. Hydrogen atoms were treated as riding and the \( U_{\text{iso}}(H) \) values were treated as in the refinement of chafuroside A dihydrate. The Flack parameter of the chafuroside B monohydrate crystal, \(-0.11(15)\), suggested the correct assignment of the absolute structure, which is consistent with that reported by Ishida et al.

The atomic coordinates and diffraction data were deposited in the Cambridge Structural Database (CCDC numbers 1897680 for chafuroside A dihydrate and 1897681 for chafuroside B monohydrate).

Results and Discussion

Structure of Chafuroside A Dihydrate  
The asymmetric unit of the crystal consists of one chafuroside A and two hydration water molecules (Fig. 2a). The torsion angle between the phenyl and chromene rings, O1–C2–C11–C12, is \(-9.5(2)\)°. In the crystal, the phenyl and chromene rings of the flavone moieties are stacked alternately along the \( a \)-axis. The stacking distance, represented by C10 of the chromene ring at \((x, y, z)\) and C11 of the phenyl ring at \((x - 1/2, -y + 3/2, 1 - z)\) is 3.400(3) Å (Fig. 2c). One intramolecular hydrogen bond is formed at the chromene ring between O5 as the hydrogen donor and O4 as the hydrogen acceptor (Table 2). The phenolic hydroxyl oxygen atom O6 acts as a hydrogen donor and forms an intermolecular hydrogen bond with the hydroxyl ox-

Table 2. Hydrogen-Bond Geometry

| Hydrogen bond         | Donor (D) | Acceptor (A) | D…A (Å) | H…A (Å) | D–H…A (degree) |
|-----------------------|-----------|--------------|---------|---------|----------------|
| Chafuroside A dihydrate | O5        | O4           | 2.559   | 1.80    | 149            |
|                       | O7        | OW2          | 2.714   | 1.90    | 162            |
|                       | O8        | OW1          | 2.719   | 1.89    | 169            |
|                       | O6'       | O9'          | 2.752   | 1.98    | 153            |
|                       | O9        | O7(1)        | 2.842   | 2.04    | 159            |
|                       | OW1       | O6(2)        | 2.902   | 2.10    | 158            |
|                       | OW1       | OW2(10)      | 2.889   | 2.07    | 167            |
|                       | OW2       | O5(13)       | 2.846   | 2.01    | 168            |
|                       | OW2       | O4(10)       | 2.802   | 1.96    | 172            |
|                       | O5        | O4           | 2.598   | 1.85    | 148            |
|                       | OWA       | O7A          | 2.802   | 2.00    | 162            |
|                       | OWB       | O7B          | 2.92    | 2.17    | 149            |
|                       | O7A       | O4(10)       | 2.711   | 1.89    | 164            |
|                       | O7B       | O4(10)       | 2.96    | 2.15    | 162            |
|                       | O8B       | O5(13)       | 2.85    | 2.19    | 135            |
|                       | O9A       | O4(10)       | 2.809   | 1.97    | 173            |

Symmetry codes: (i) \( x - 1/2, -y + 3/2, -z + 1 \); (ii) \(-x + 1, y, -z + 1/2\); (iii) \(-x + 1/2, -y + 2, z - 1/2\); (iv) \(x + 1, y, z\); (v) \(-x, y + 1/2, -z + 1/2\); (vi) \(-x + 1/2, -y + 1, z - 1/2\); (vii) \(-x + 2, y + 1/2, -z + 2\); (viii) \(-x + 3, y + 1/2, -z + 2\); (ix) \(-x + 2, y + 1/2, -z + 1\).
oxygen of the glucopyranoside moiety, O9, at \((x - 1/2, -y + 3/2, -z + 1)\), and the O9 atom in turn is hydrogen bonded as a hydrogen donor to O7 at \((-x + 1/2, -y + 2, z + 1/2)\) (Fig. 2b). The oxygen atom OW1 of a hydration water molecule acts as the hydrogen bond donor in formation of a hydrogen bond with the other hydration water oxygen atom OW2 at \((x + 1, y, z)\) (Fig. 2c). This pair of hydration water molecules is surrounded by the hydroxyl and carbonyl oxygen atoms of chafuroside A to form a total of five hydrogen bonds.

**Structure of Chafuroside B Monohydrate** The asymmetric unit in the crystal contains one chafuroside B and one water molecule (Fig. 3a). The planes of the phenyl and chromene rings in the flavone moiety are twisted with a torsion angle \((O1–C2–C11–C12)\) of \(-8.9(3)°\). The glucopyranoside moiety of chafuroside B and a water molecule, which is hydrogen bonded to the hydroxyl oxygen O7 of the glucopyranoside moiety, are disordered and were modeled at two sets of sites (Table 2). The hydroxymethyl group \((-C22A–O9A)\) of the glucopyranoside moiety in the major conformer adopts a gauche–trans conformation and is hydrogen bonded as a hydrogen donor to the symmetry-related carbonyl oxygen atom O4 of the flavone moiety (Fig. 3b). The hydroxymethyl group in the minor conformer \((-C22B–O9B)\) adopts a gauche–gauche conformation and is hydrogen bonded as a hydrogen acceptor to the symmetry-related water molecule in the major conformation.

**Structural Relationship of Flavone C-Glycosides** Many hydrogen bonds are formed in the crystal structures of chafurosides and this might be involved in the stability of the structures, resulting in the poor water solubility of chafurosides. In addition, the flavone moiety of chafuroside A is curved, *i.e.*, the angle between the best-fit planes of the chromene and phenyl rings is 18.9°, whereas the chafuroside B flavone moiety is relatively flat. The curvatures of the flavone moieties of flavone C-glycosides glycosidated at chromene C6 or C8, as in chafurosides A and B, were compared on the basis of \(d_{\text{curve}}\), which is defined (Fig. 4a) as the distance between the best-fit plane of the chromene ring and the carbon atom of the phenyl group to which the hydroxyl group is bound (C14 in the cases of chafurosides A and B). Figure 4b shows a plot of the \(d_{\text{curve}}\)
values of the flavone C-glycosides for which crystal structures have been reported. The average \( d_{\text{curve}} \) value ± the standard deviation is 0.37 ± 0.28 Å. The \( d_{\text{curve}} \) values of the flavone C-glycosides, except for chafuroside A, are evenly distributed around 0.0 Å, which indicates that the curvature of the flavone moiety of chafuroside A (\( d_{\text{curve}} = 1.01 \) Å) is significantly large (Fig. 4c). As mentioned in Introduction, although each chafuroside shows various pharmacological activities at different concentrations, the strengths of their pharmacological activities depend on the interactions between each chafuroside and receptors that have not yet been identified. Since the product structure generally affects the interactions, we strongly suggest that differences among the curvatures of the flavone moieties in chafuroside A give high flexibility to the structure in solution, and this might be involved in the differences among the receptor affinities. Further studies of the structure–activity relationships are needed. However, this is the first evidence that curving of the flavone moiety may have important effects on the biological activities of the flavone C-glycosides chafurosides A and B.

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Conflict of Interest The authors declare no conflict of interest.

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