Absolute Configurations of (±)-Glabridin Enantiomers

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Concerned with ambiguous stereochemistry assignment of natural (+)-glabridin, absolute configurations of (±)-glabridin enantiomers were studied with synthetic glabridin. Synthetic glabridin enantiomers were separated by semi-preparative Sumi-chiral column chromatography, and characterized by UV-Vis and NMR spectroscopy. Three-dimensional molecular structure of glabridin was obtained as equatorial Ph-3 half chair chroman ring from semi-empirical PM3 calculation, and refined by coupling constants in ^{1}H NMR spectrum. Finally, absolute configurations of two enantiomers were determined by circular dichroism spectroscopy based on the empirical helicity rules. Absolute configuration of natural (+)-glabridin was confirmed as (*R*)-glabridin, as known.

Key Words: Absolute configuration, Circular dichroism (CD), Flavonoids, Glabridin, Isoflavan

Introduction

Recent growing interests in plant flavonoids are mainly due to their various biological activities without noticeable side-effects. Among these activities, not limited, estrogenic, antiangiogenic and anticancer activities to human appear owing to the structural similarity of the metabolites to human estrogens.[1,2,3] In nature, these flavonoids are often enantiomers and correct stereochemistry is necessary to study the biological activities at the molecular level.[4] For example, (3*R*)-equol and (3*S*)-equol are suggested to exhibit different biological activities.[5,6]

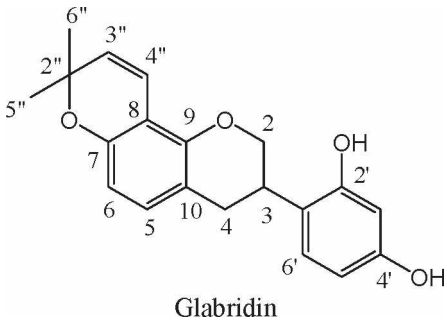
Spectroscopically, there are a few experimental approaches for the absolute configuration determination of enantiomers. Single crystal X-ray crystallography is the only way to determine the absolute configuration of the enantiomer unambiguously. But it requires derivatization of the enantiomer by a chiral group to form diastereomer. If crystallization failed, the derivatized compounds could be studied by ^{1}H-NMR spectroscopy. Even without chiral derivatization, NMR spectroscopy has been extensively used for the absolute configuration determination in the presence of chiral shift reagent.[7] The chemical shift changes of the characteristic peaks help determination of the absolute configuration, but sometimes ambiguously.

Without chemical derivatization or chiral shift reagents, only limited spectroscopic approaches are possible for the absolute configuration determination of the enantiomers. Optical rotation by polarimeter and optical dispersion by circular dichroism (CD) spectrometer are the accessible spectroscopic methods. But these methods are also limited in that it always requires standard compounds with known absolute configurations. Often oxidative degradation of the enantiomer to the simpler known standard compound is necessary.

On the other hand, optical rotation and circular dichroism spectra can be simulated by computational chemistry, even in the absence of standard compound. For example, simulation of CD spectra of dihydrodiols by *ab initio* calculations is successfully achieved.[8] Yet, theoretical method is not practically accessible to the most natural product compounds because of long computation times.

We have been studying the determination of the absolute structures of flavonoids by means of semi-empirical geometry-optimization and empirical helicity rules. In details, correct three-dimensional molecular structure of the flavonoid stereoisomer is the prerequisite for the next experiments, and that can be obtained from various sources, including single crystal X-ray crystallography, NMR spectroscopy, and computational chemistry. Reasonable structural information can be achieved by using at least two different experimental approaches, and that can be used for simulation of CD spectrum of the enantiomer. Empirical helicity rules are quick and an easy approach for the CD spectrum simulation, but which requires the proved database applicable to certain class of compounds.[9] In case of flavonoids, empirical rules for the helicity-induced CD spectrum changes are well established.[10] and empirical helicity rules can be applied to various flavonoids. At last and most importantly, CD spectrum of the enantiomer is obtained and compared with the simulation. In case of enantiomeric mixtures, separation of each enantiomer on chiral column chromatography precedes for CD spectra measurements.

For examples, we have isolated each stereoisomer and successfully determined absolute configurations of isoflavanone enantiomers and isoflavan-4-ol stereoisomers, by means of above methodology.[11,12] These compounds were perfect models for dihydrodaidzein (DHD) and tetrahydrodaidzein (THD), which were suggested as important intermediates of the (3*S*)-equol biosynthesis. Inspired by our successful absolute configuration determinations of the flavonoids and interesting structural similarity of (+)-glabridin to (3*S*)-equol,



Glabridin

Figure 1. Molecular structure of glabridin.

we have decided to study the absolute configuration of (+)-glabridin (Figure 1).

Glabridin is a major component of licorice extract, which has been used as a sweetener or a spice for a long time. It has also been reported to show various biological activities of antimicrobial,[13,14] antiinflammatory, anticancer,[15] antinephritis,[16] neuroprotective[17] and cardiovascular protective activities. Glabridin also inhibits lipid peroxidation of LDL,[18,19] some human cytochrome P450s,[20,21] NO production in mice,[22] osteoporosis and inflammatory bone diseases.[23,24] Regarding all these important biological activities, knowing the right absolute configuration of (+)-glabridin is essential for the molecular-level research. Nonetheless, we have found that absolute configuration of natural (+)-glabridin was ambiguously assigned. The chemical structure of (+)-glabridin was reported by Saitoh *et al.* for the first time.[25] The stereochemistry of C-3 was assigned as *R*-based on stereochemistry of the substitute isoflavans, of which 3*R*-stereoisomers showed positive Cotton effect in the region of 260 nm – 300 nm.[26] But such empirical stereochemistry assignment of isoflavan is not always valid as reported by the same authors, because some aromatically extended isoflavans do not follow the adopted empirical helicity rule.[27]

In this report, we have studied absolute configurations of the synthetic (±)-glabridin by means of chiral separation of the enantiomers, geometry-optimization by ^{1}H-NMR spectroscopy and semi-empirical calculation, and absolute configurational analyses by CD spectroscopy and empirical helicity rules. Based on the results, the absolute configuration of the natural (+)-glabridin was confirmed as (3*R*).

Experimental Section

The synthetic (±)-glabridin[28] was obtained from Prof. Nahm in Department of Chemistry at Yeungnam University, Gyeongsan, Republic of Korea. High performance liquid chromatography (HPLC)-grade solvents of acetonitrile and water were purchased from Fisher (Pittsburg, PA). Other commercial solvents were distilled under dinitrogen after pretreatment steps.[29]

^{1}H and ^{13}C NMR spectra of the respective compounds in $CDCl_3$ were respectively obtained by 400 MHz and 100 MHz, respectively, on a Bruker 400 NMR spectrometer at 293 K. The energy-minimized molecular structure of glabridin was obtained by Hyperchem v.7.0 (Hypercube, Inc. FL, USA). The geometry optimization of the built model was performed by semi-empirical PM3 method without configuration interaction, and Polak-Ribiere was chosen as the minimization algorithm. ^{1}H NMR spectrum simulations were carried out by ACDLABS HNMR viewer program. The glabridin enantiomers were separated by semi-preparative chiral column (Sumichiral OA-7000, 5 μm, 20×25 mm; Sumika Chemicals, Osaka, Japan). The mobile-phase was composed of 100% H_2O (A) and 100% acetonitrile (B). The elution started with A:B ratio at 50:50 (v/v) for 3 min, and then linearly changed to 30:70 (v/v) for 10 min.

Circular dichroism (CD) spectra of two glabridin enantiomers in ethanol were measured using a J-715 CD spectrometer (Jasco Corp., Tokyo, Japan) at NICEM in Seoul National University, Seoul, Republic of Korea. The spectra were recorded over 230 – 330 nm range using a cuvette with the optical path length of 0.5 cm.

Results and Discussion

UV-Vis spectrum of glabridin, taken from PDA detector of HPLC, was shown at Figure 2 and identical to the reported values.[22] except the additional peak at 236 nm. ^{1}H and ^{13}C NMR spectra of glabridin in $CDCl_3$ were basically identical to the reported value.[30] Furthermore, we were able to identify all coupling interactions of the protons of the C-ring in glabridin, including H-3 (Figure 3). The assignment of the coupling constants of H-3 is first reported in this work. The peak of H-2$_{eq}$ was found at 4.37 ppm (*ddd*, $J_{H2eq,2ax}$ = 10.5 Hz, $J_{H2eq,3}$ = 3.7 Hz, $J_{H2eq,4eq}$ = 2.3 Hz), H-2$_{ax}$ at 4.02 ppm (*dd*, $J_{H2eq,2ax}$ = 10.5 Hz, $J_{H2ax,3}$ = 10.5 Hz), H-3 at 3.48 ppm (*dddd*, $J_{H2eq,3}$ = 3.7 Hz, $J_{H2ax,3}$ = 10.5 Hz, $J_{H3,4ax}$ = 10.5 Hz, $J_{H3,4eq}$ = 5.5 Hz), H-4$_{ax}$ at 2.98 ppm (*dd*, $J_{H3,4ax}$ = 10.5 Hz, $J_{H4ax,4eq}$ = 15.6 Hz), and H-4$_{eq}$ at 2.86 ppm (*ddd*, $J_{H2eq,4eq}$ = 2.3 Hz, $J_{H3,4eq}$ = 5.5 Hz, $J_{H4ax,4eq}$ = 15.6 Hz), respectively. Correct assignment of the coupling constants related to H-3 is critical for the conformational determination of glabridin, and the simulation of H-3 coupling interactions are shown at Figure 3. Molecular mechanics calculations for the geometry optimization have resulted in half chair conformation of C-ring with equatorial Ph-3, as energy minimized conformer (Figure 4). The calculated H_{2ax}-C2-C3-H_3 and H_3-C3-C4-H_{4ax} dihedral angles were 171° and 175°, respectively. In case of axial Ph-3 half chair conformer, these dihedral angles were calculated as 58° and 41°, respectively. The observed $J_{H2ax,3}$ = 10.5 Hz and $J_{H3,4ax}$ = 10.5 Hz clearly favored equatorial Ph-3 half chair

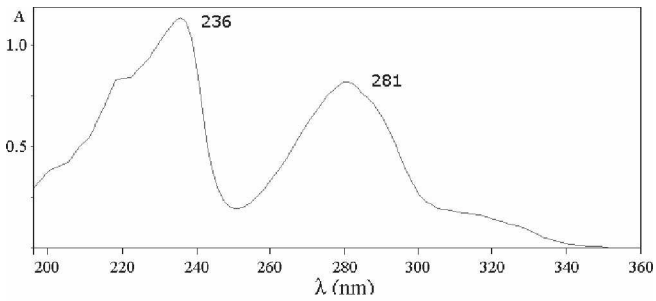


Figure 2. UV spectrum of glabridin.

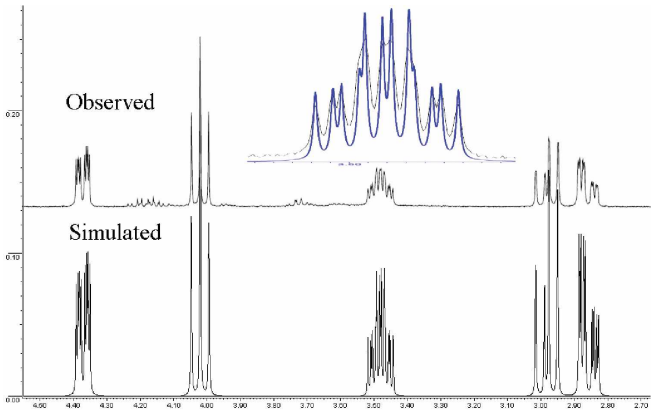


Figure 3. ^{1}H NMR spectrum and its simulation of grabridin C-ring region. The enlarged inset shows H-3 proton and the simulated peaks (blue).

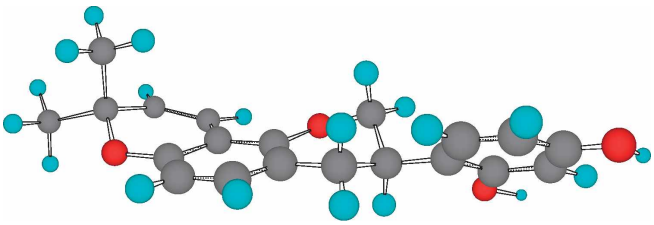


Figure 4. Three dimensional model of glabridin.

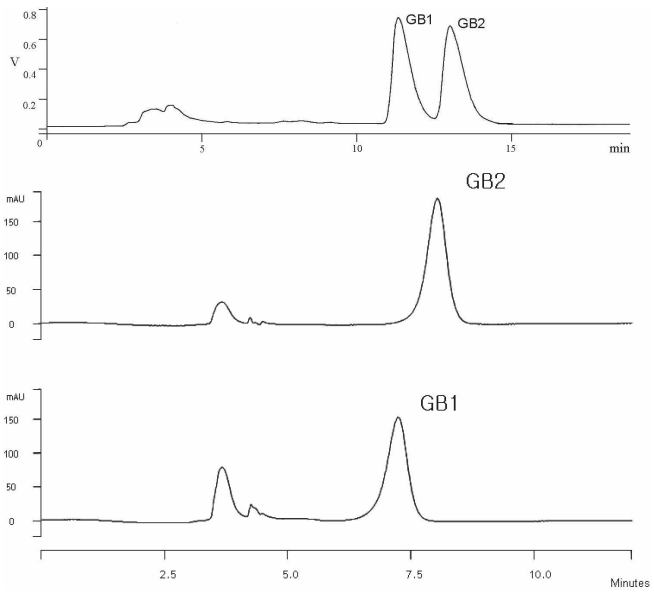


Figure 5. HPLC elution profiles for chiral separation of glabridin enantiomers.

conformer of glabridin.[31]

The enantiomeric mixture of (±)-glabridin was purified by a semi-preparative Sumi-Chiral column and rechromatography of the collected fractions achieved complete resolution of the enantiomers (Figure 5). Each fraction was dried under reduced pressure and redissolved in ethanol. CD spectra of two enantiomers in ethanol were obtained on J-715 CD spectrometer and are shown at Figure 6.

The glabridin enantiomer isolated from the peak 1 showed positive Cotton effect between 255 nm and 330 nm, which

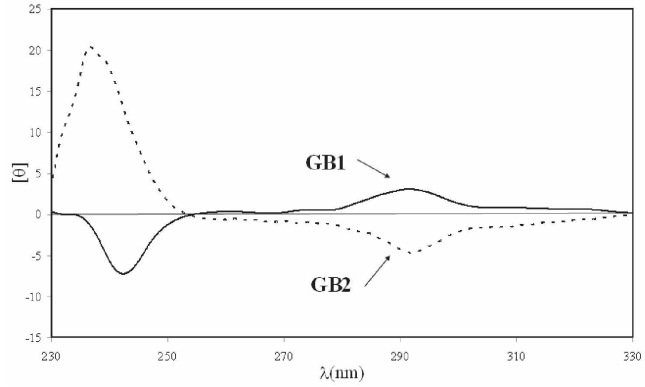


Figure 6. CD spectra of glabridin enantiomers.

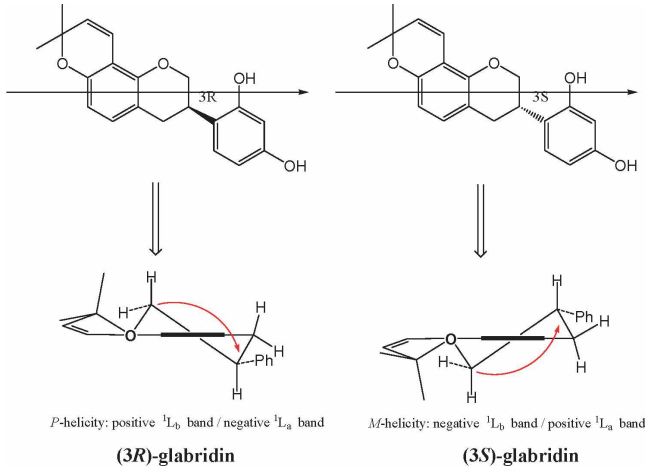


Figure 7. Half-chair conformations of glabridin enantiomers.

corresponds to ^1L_b band $\pi \rightarrow \pi^*$ transition, and negative Cotton effect between 230 nm and 255 nm, which corresponds to ^1L_a.[32] On the contrary, the other enantiomer isolated from the peak 2 showed CD spectrum symmetric to peak 1. Because the rigid chroman ring of glabridin exists only as half-chair conformation with equatorial Ph-3 conformer (Figure 4), (3*R*)-glabridin and (3*S*)-glabridin can show *P*- and *M*-helicity, respectively (Figure 7). In case of isoflavanes, numerous reports suggest inverse helicity rule is applied for Cotton effect.[7] Besides, absolute configuration of vestitol enantiomers, structural analog of (+)-glabridin, were clearly elucidated to follow the inverse helicity rule.[33] Accordingly, GB1 showing negative Cotton effect at the range between 230 nm and 255 nm was assigned as (3*R*)-glabridin, and GB2 showing positive Cotton effect at the same region as (3*S*)-stereoisomer. Because (+)-glabridin showed the same retention time as GB1 on the chiral column and the CD spectrum was identical to GB1, natural (+)-glabridin was assigned as (3*R*)-glabridin.

In summary, we have successfully separated glabridin enantiomers by Sumi-Chiral column chromatography and their absolute configurations were assigned by CD spectroscopy. The results have confirmed natural (+)-glabridin as (3*R*)-glabridin.

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