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Absolute configurations of isoflavan-4-ol stereoisomers

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Abstract—Isoflavan-4-ol has been synthesized quantitatively from the reduction of isoflavone in the presence of Pd/C and ammonium formate under N_2 atmosphere. Isolation of *cis*- and *trans*-isomers was achieved by flash column chromatography and each enantiomer was separated by Sumi-Chiral column chromatography. Absolute configurations of four stereoisomers were determined by circular dichroism spectroscopy.

Isoflavonoids form a distinctive subclass of the flavonoids[1] and are mainly found in *Leguminosae* species. They are responsible for the protection against microbial infections in plants[2] and for the formation of nodules on *Leguminosae* species by nitrogen fixing bacteria.[3] Isoflavonoids also exhibit estrogenic, antiangiogenic, antioxidant, and anticancer activity to human, probably due to the structural similarity of the metabolites to human estrogens.[4–6] These metabolites are found in blood plasma, urine, and feces as a result of biotransformation by intestinal microorganisms.[7–9] For example, equol, produced from the sequential reduction reactions of a natural isoflavonoid daidzein (4′,7-dihydroxyisoflavanone), was reported as the most effective compound for preventing breast cancer[10] and stimulating estrogenic response[11] among the isoflavonoid metabolites. Interestingly, biological equol production was found stereoselective with formation of *S*-equol (3*S*-4′,7-dihydroxylisoflavan).[12] The metabolic pathway for *S*-equol production from daidzein has been suggested based on the isolated biotransformation products (Fig. 1).[13–17] While tetrahydrodaidzein formation from daidzein seems to be consistent, the next biotransformation of tetrahydrodaidzein to *S*-equol is still debatable. Direct two-electron reduction pathway and dehydration-reduction pathway of tetrahydrodaidzein seem to be equally plausible. Besides, the stereochemistry of dihydrodaidzein and tetrahydrodaidzein has never been elucidated to the best of our knowledge. For example, it is not clear yet which enantiomer of dihydrodaidzein or which stereoisomer of tetrahydrodaidzein is responsible for *S*-equol production. Here we report simple preparation of isoflavan-4-ols from isoflavone and elucidation of absolute configurations of isoflavan-4-ol stereoisomers, which can provide useful information on the mechanistic study of isoflavonoid biotransformation. Due to the structural similarity between isoflavan-4-ol and tetrahydrodaidzein, elucidation of stereochemistry of biotransformed tetrahydrodaidzein and biotransformation of isoflavan-4-ol stereoisomers as tetrahydrodaidzein analogs would be possible.

We have previously reported convenient synthetic method of 2′-hydroxydihydrochalcone from flavone, and isoflavan-4-one from isoflavone by catalytic hydrogen transfer reaction in the presence of Pd/C.[18,19] When we modified reaction conditions, isoflavan-4-ols were obtained from isoflavone in quantitative yield. The reduction of isoflavone was carried out in the presence of ammonium formate and Pd/C (Aldrich) in an inert atmosphere glove box. Isoflavone (200 mg, 0.90 mmol), Pd/C (200 mg), and NH_4HCO_3 (400 mg, 6.35 mmol)

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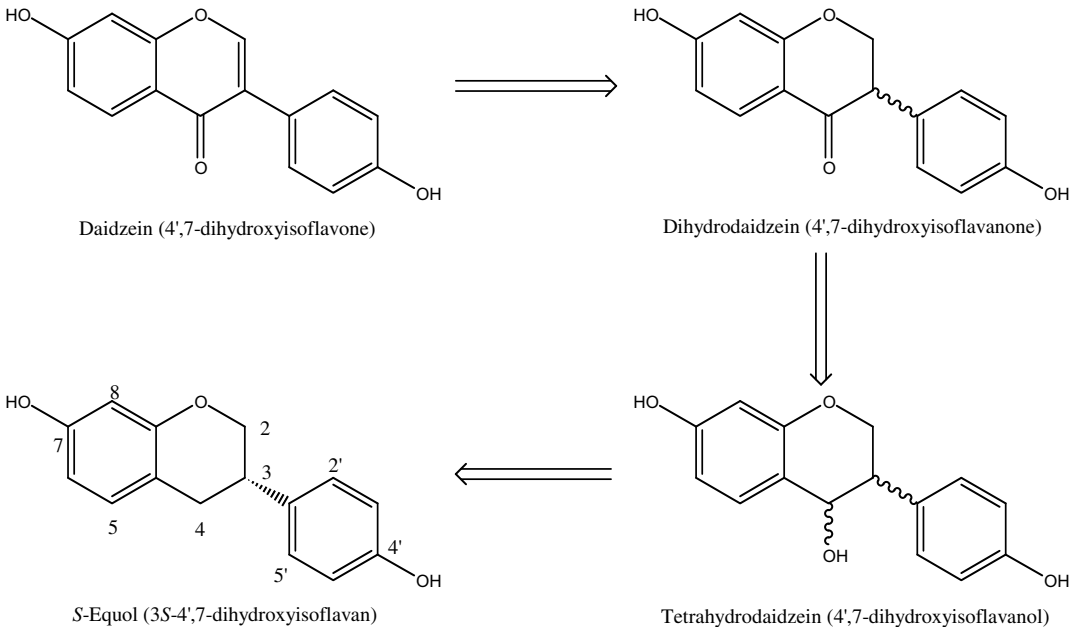

Figure 1. Suggested metabolic pathway for biological *S*-equol production.

were dissolved in anhydrous EtOH (25 ml). The reaction mixture was stirred at room temperature and the reaction was monitored by silica gel TLC. Isoflavone ($R_f = 0.53$) almost disappeared in an hour and isoflavan-4-one ($R_f = 0.65$) was observed as a major product on TLC (100% $CHCl_3$). In 6 h, *cis*-isoflavan-4-ol ($R_f = 0.37$) and *trans*-isoflavan-4-ol ($R_f = 0.29$) were observed on TLC, and the reaction was completed in 12 h. The reaction was stopped by filtering Pd/C, and the product was isolated by evaporating reaction solvent. The product was only isoflavan-4-ol stereoisomers and no other byproduct was observed. Although many hydrogenation reactions of isoflavanone were reported,[20,21] this is the first report that produced exclusively isoflavan-4-ol products, to our knowledge.

UV–vis spectra of the isoflavan-4-ol stereoisomers in MeOH were taken on Scinco S-3100 spectrophotometer, and showed λ_{max} at 283 nm ($\varepsilon = 1820\ M^{-1}\ cm^{-1}$), 275 nm ($\varepsilon = 2100\ M^{-1}\ cm^{-1}$) and 220 nm ($\varepsilon = 3950\ M^{-1}\ cm^{-1}$) (Fig. 2). ^1H NMR spectra of *cis*- and *trans*-isoflavan-4-ol in $CDCl_3$ were obtained on a 400 MHz NMR spectrometer.[†] In case of *trans*-isoflavan-4-ol, half-chair conformation of C-ring can form two conformers of diaxial and diequatorial in solution, due to the Ph-3 and OH-4 position (Fig. 3). Extensive ^1H NMR data assignment and conformation analysis of *trans*-isoflavan-4-ol have suggested equilibrium of diaxial and diequatorial conformers in MeOH solution, preferring thrice more diequatorial conformers.[22] Accordingly, it was found that the diequatorial conformation mainly exists from our experiment. The observed $J_{H3,4} = 8$ Hz has confirmed diequatorial and diaxial conformers of *trans*-isoflavan-4-ol co-exist[22] (Fig. 3). Because the detection time scale of NMR is relatively long, the observed $J_{H3,4}$ coupling constant reflects the statistic mean value of the coupling constants of both conformation ($J_{H3,4}$ of diequatorial conformer = 9.95 and $J_{H3,4}$ of diaxial conformer = 1.66).

The ^1H NMR spectrum of *cis*-isoflavan-4-ol is first reported in this work. It was characterized by broad singlet of H-4 at 4.852 ppm, and H-3, H-2α, and H-2β were observed at 3.374 ppm (dt), 4.377 ppm (ddd) and 4.652 ppm (dd), respectively. The conformation of *cis*-isoflavan-4-ol can be determined based on ^1H NMR measurement as above. The observed $J_{H3,4} = 3.4$ Hz corresponds to H–C3–C4–H dihedral angle of ca. 50°.[23] Because both conformers have similar dihedral angles close to 50° based on molecular mechanics calculations, actual conformation of *cis*-isoflavan-4-ol could not be elucidated based on the NMR data (Fig. 3). But diequatorial conformation is favored because of steric hindrance between phenyl and hydroxyl groups of diaxial conformers.[24]

Because hydrogenation of C-ring would generate four stereoisomers with C-3 and C-4 chiral centers, the diastereomeric mixture of isoflavan-4-ol was first separated by flash column chromatography and each enantiomer was purified further by a preparative Sumi-Chiral column. The enantiomers of *trans*-isoflavan-4-ol were separated isocratically with 35:65 ratio of MeCN:potassium phosphate (20 mM, pH 3.0) eluent system and the enantiomers of *cis*-isoflavan-4-ol were separated isocratically

[†] ^1H NMR of *trans*-isoflavan-4-ol (400 MHz, $CDCl_3$, ppm): 5.017 (dd, $J_{H\text{-}2\alpha} = 0.3$ Hz (unr), $J_{H\text{-}2\beta} = 5.2$ Hz, $J_{H\text{-}3} = 8$ Hz, H-4), 4.400 (dd, $J_{H\text{-}2\alpha} = 11.2$ Hz, $J_{H\text{-}3} = 3.6$ Hz, $J_{H\text{-}4} = 5.2$ Hz (unr), H-2β), 4.292 (dd, $J_{H\text{-}2\beta} = 11.2$ Hz, $J_{H\text{-}3} = 8.4$ Hz, $J_{H\text{-}4} = 0.3$ Hz (unr), H-2α), 3.211 (dt, $J_{H\text{-}2\alpha} = 8.4$ Hz, $J_{H\text{-}2\beta} = 3.6$ Hz, $J_{H\text{-}4} = 8$ Hz, H-3), ^1H NMR of *cis*-isoflavan-4-ol (400 MHz, $CDCl_3$, ppm): 4.852 (br s, $J_{H\text{-}2\alpha} = 12$ Hz (unr), $J_{H\text{-}2\beta} = 0.2$ Hz (unr), $J_{H\text{-}3} = 3.4$ Hz (unr), H-4), 4.652 (dd, $J_{H\text{-}2\alpha} = 12$ Hz, $J_{H\text{-}3} = 11.8$ Hz, $J_{H\text{-}4} = 0.2$ Hz (unr), H-2β), 4.377 (ddd, $J_{H\text{-}2\beta} = 12$ Hz, $J_{H\text{-}3} = 3.6$ Hz, $J_{H\text{-}4} = 1.2$ Hz, H-2α), 3.374 (dt, $J_{H\text{-}2\alpha} = 3.6$ Hz, $J_{H\text{-}2\beta} = 11.8$ Hz, $J_{H\text{-}4} = 3.4$ Hz, H-3).

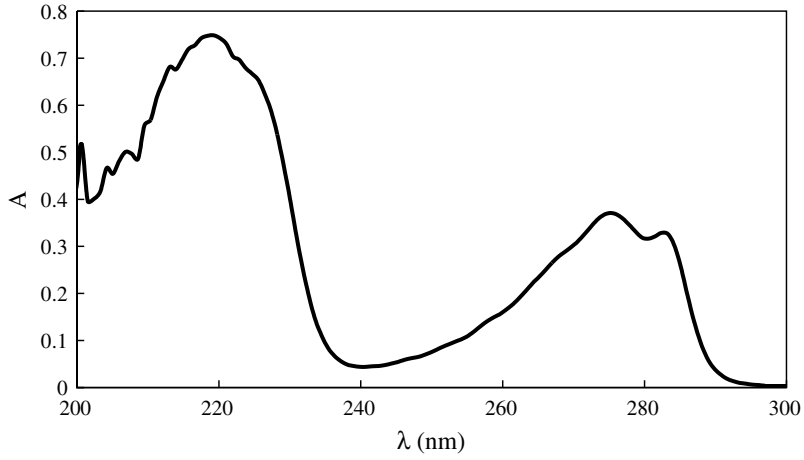

Figure 2. UV spectrum of isoflavan-4-ol.

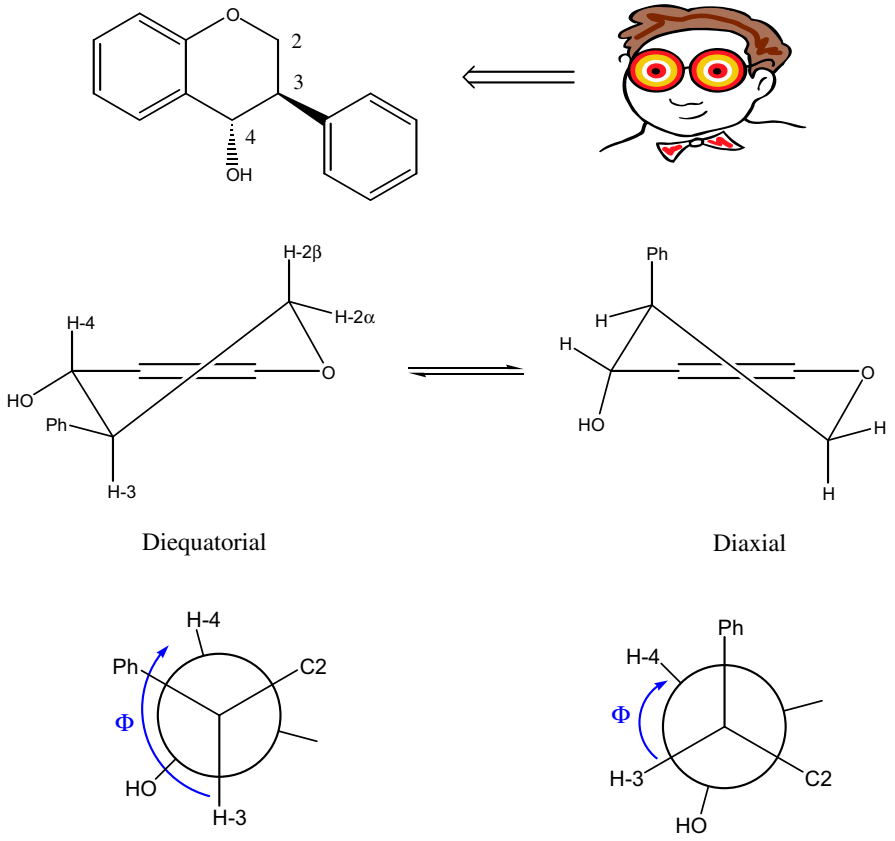

Figure 3. Half-chair conformations of *trans*-isoflavan-4-ol.

with 30:70 ratio of eluent system (Fig. 4). Each fraction was extracted with ethyl acetate and the solvent was removed under reduced pressure. CD spectra of four stereoisomers in MeOH were obtained on J-715 CD spectrometer and are shown at Figure 5. The *trans*-isoflavan-4-ol enantiomer isolated from the peak 1 showed negative Cotton effect between 250 and 300 nm, which corresponds to $^{1}L_{b}$ band $\pi \rightarrow \pi^{*}$ transition, and positive Cotton effect between 220 and 240 nm, which corresponds to $^{1}L_{a}$.[25] On the contrary, the other *trans*-isoflavan-4-ol enantiomer isolated from the peak 2 showed CD spectrum symmetric to peak 1. The conformation of isoflavan-4-ol can be visualized as half-chair conformation, due to the rigid chroman-4-ol structure (Fig. 6), and the peak 1 showing negative Cotton effect at the range between 250 and 300 nm was assigned as 3*R*, 4*S*-stereoisomer, and the peak 2 showing positive Cotton effect at the same region as 3*S*, 4*R*-stereoisomer, according to Snatzke's modified octant rule.[26–28] Likewise, the *cis*-isoflavan-4-ol enantiomer isolated from peak 1, showing positive Cotton effect between 245 and 300 nm, can be assigned as 3*S*, 4*S*-isoflavan-4-ol,

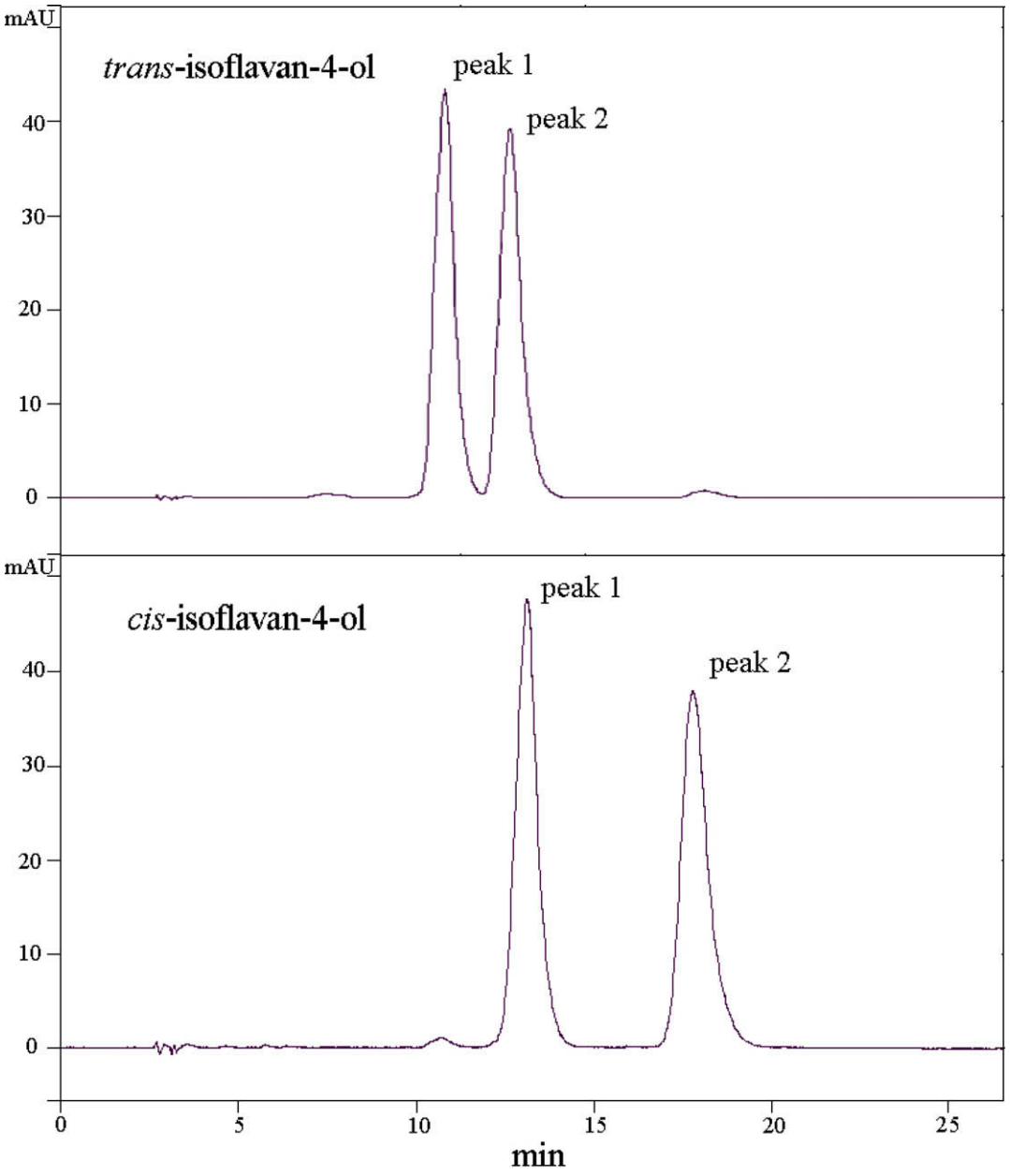

Figure 4. HPLC elution profiles for chiral separation of *cis*-isoflavan-4-ol and *trans*-isoflavan-4-ol.

and the other enantiomer from peak 2 with negative Cotton effect at the same region can be assigned as 3*R*, 4*R*-isoflavan-4-ol (Fig. 6). Interestingly, all 3*S*-enantiomers of (3*S*, 4*R*)-*trans*- and (3*S*, 4*S*)-*cis*-isoflavanol, as well as 3*S*-equol,[17] and 3*S*-isoflavanone,[19] showed short retention time on the Sumi-Chiral column, compared to the other relevant enantiomers.

We have observed co-existence of diequatorial and diaxial conformers of *trans*-isoflavan-4-ol from the NMR data. In case of (3*R*, 4*S*)-*trans*-isoflavan-4-ol, diequatorial conformer with *P*-helicity and diaxial conformer with *M*-helicity exist in about 3:1 ratio. Accordingly, the difference between absorbance of left circularly polarized and right circularly polarized light would be reduced, due to the circularly polarized lights mixing. In fact, it was observed that the molar ellipticity of *trans*-isoflavanol stereoisomers was twice smaller than that of *cis*-isoflavanol stereoisomers.

In summary, we have successfully prepared isoflavan-4-ol from catalytic hydrogenation of isoflavone without other byproducts. The four isoflavan-4-ol stereoisomers were efficiently isolated by Sumi-Chiral column chromatography and their absolute configurations were assigned by CD spectroscopy. This work could provide valuable information to the mechanistic study of the isoflavonoid biotransformation.

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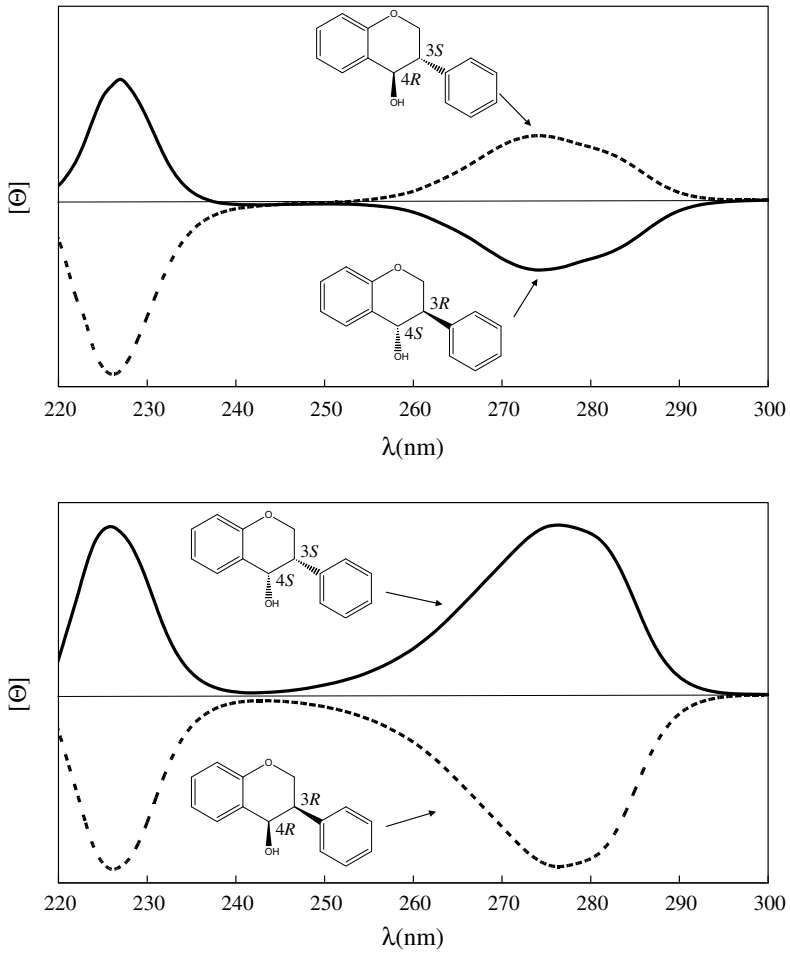

Figure 5. CD spectra of isoflavan-4-ol stereoisomers.

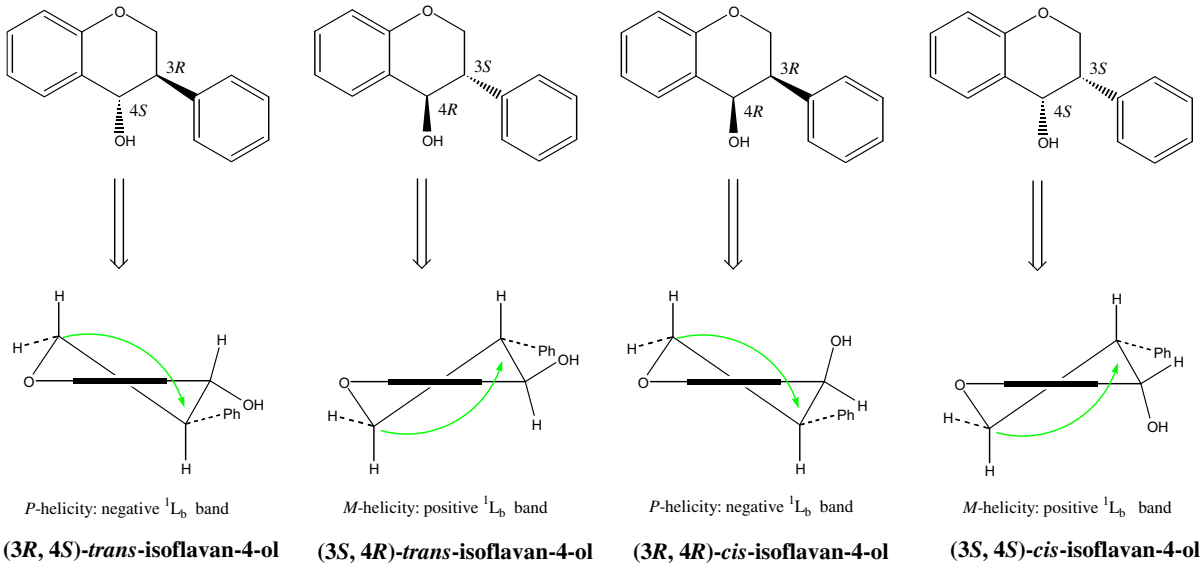

Figure 6. Half-chair conformations of isoflavan-4-ol stereoisomers.

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