J. Appl. Biol. Chem. **51**(1), 17-19 (2008)

Article

Synthesis and the Absolute Configurations of Isoflavanone Enantiomers

Dongho Won, Bok-Kyu Shin, and Jaehong Han*

Metalloenzyme Research Group and Department of Biotechnology, Chung-Ang University, Anseong, 456-756, Korea

Received November 21, 2007; Accepted January 19, 2008

Isoflavanone has been synthesized from the reduction of isoflavone in nearly quantitative yield. Isoflavone with seven equivalents of ammonium formate in the presence of Pd/C in ethanol under N_2 atmosphere exclusively produced the two-electron reduced product in two hours. It was characterized by various spectroscopic methods, including UV-VIS, EI-MS, ^{1}H-NMR, ^{13}C-NMR and ^{1}H,^{1}H-COSY. The racemic mixture was separated by Sumi-Chiral column chromatography and the absolute configurations of the enantiomers were characterized by circular dichroism spectroscopy.

Key words : *absolute configuration, circular dichroism (CD), flavonoids, isoflavanone, palladium on activated carbon (Pd/C)*

Isoflavanone possesses a 3-phenylchroman skeleton and isoflavonoids form a distinctive subclass of the flavonoids [Marais *et al.*, 2006]. Isoflavanones, mostly found in *Leguminosae* species, are responsible for protection against microbial infection stresses in plants [Lozovaya *et al.*, 2004]. Some isoflavanones have chemical structures similar to those of human estrogens, and shows various physiological effects in human body [Jung *et al.*, 2003]. For example, it was reported that daidzein was converted to equol by equol-producing bacteria, and 4',7-dihydroxyisoflavanone (dihydrodaidzein) was known as one of the intermediates during the biotransformation [Wang *et al.*, 2005]. Biological equol production is stereoselective and only *S*-equol is produced. But stereochemistry of reaction intermediates has never been studied to the best of our knowledge. Hence, simple and reproducible preparation of isoflavanones from isoflavone can provide many useful bioactive compounds, and elucidation of absolute configurations of isoflavanone enantiomers can also provide useful information on the mechanistic study of isoflavonoids biotransformation.

We have reported convenient synthetic method of 2'-hydroxydihydrochalcone from flavone by catalytic hydrogen transfer reaction, in the previous work [Kim *et al.*, 2007]. On the line of our study, we have applied same reaction to isoflavone and prepared isoflavanone in nearly quantitative yield.

*Corresponding author
Phone: 82-31-670-4830; Fax: 82-31-670-4830
E-mail: jaehongh@cau.ac.kr

The reduction of isoflavone was carried out in the presence of ammonium formate and Pd/C (Aldrich) in an inert atmosphere glove box (Fig. 1). Isoflavone (200 mg, 0.90 mmol), Pd/C (200 mg), NH_4HCO_3 (400 mg, 6.35 mmol) 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) was disappeared in an hour and only isoflavanone (R_f=0.65) was observed on TLC (100% $CHCl_3$) in two hours. The reaction was stopped by filtering Pd/C, and the product was isolated by evaporating reaction solvent in a 94% yield (188.4 mg, 0.84 mmol).

UV-Vis spectrum of the isoflavanone in MeOH showed λ_{max} at 321 nm, 252 nm and 214 nm, which correspond to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$ transitions, respectively. It was identical to the reported value [Ibrahim *et al.*, 1990], and interestingly almost identical to 2'-hydroxydihydrochalcone because of same chromophores in the molecule [Kim *et al.*, 2007]. EI-MS measurement identified the molecular ion peak at m/*z* 224 and the base peak at m/*z* 120 ([M-Ph-CH=CH]$^+$). The two protons at C-2 on C-ring appeared at δ 4.69 (1H, *s*) and δ 4.67 (1H, *d*), and the proton at C-3 was shown at δ 4.01 (1H, *t*) on a 300 MHz ^{1}H-NMR spectrometer. Strong correlations among the protons at C-2 and C-3 were observed from ^{1}H,^{1}H-COSY. The carbonyl carbon C-4 was observed at δ 192.57, and the chemical shifts of C-2 and C-3 were observed at δ 71.67 and δ 52.46, respectively, on a 75 MHz ^{13}C-NMR spectrometer. The C=O stretching of isoflavone at 1639 cm^{-1} in FT-IR spectrum has moved to 1687 cm^{-1} due to the loss of double bond at C-ring of isoflavanone. All the spectroscopic characterizations have identified the product

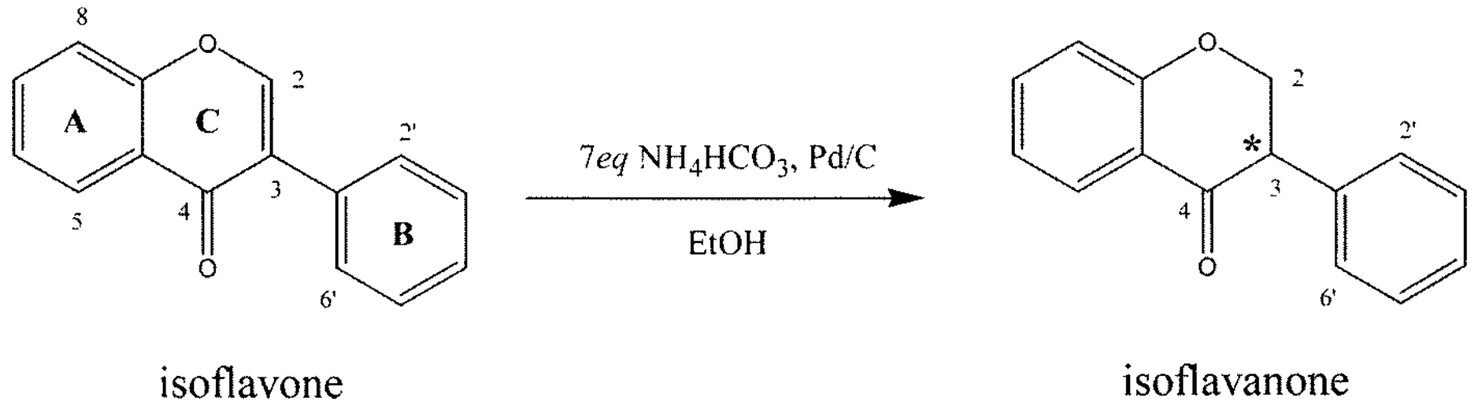

Fig. 1. Synthesis of isoflavanone.

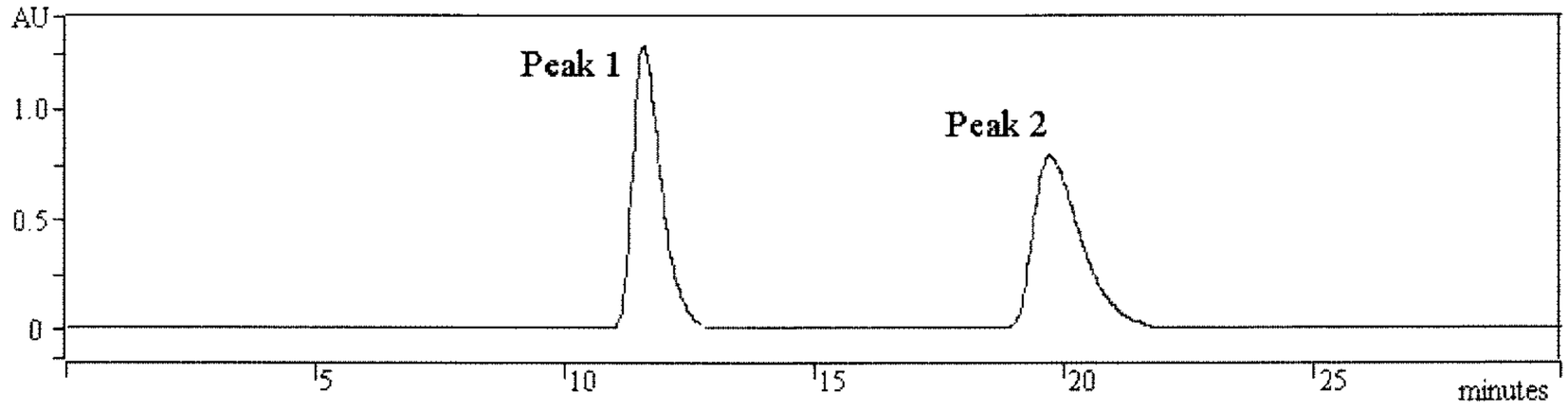

Fig. 2. Chromatogram of isoflavanone at 270 nm.

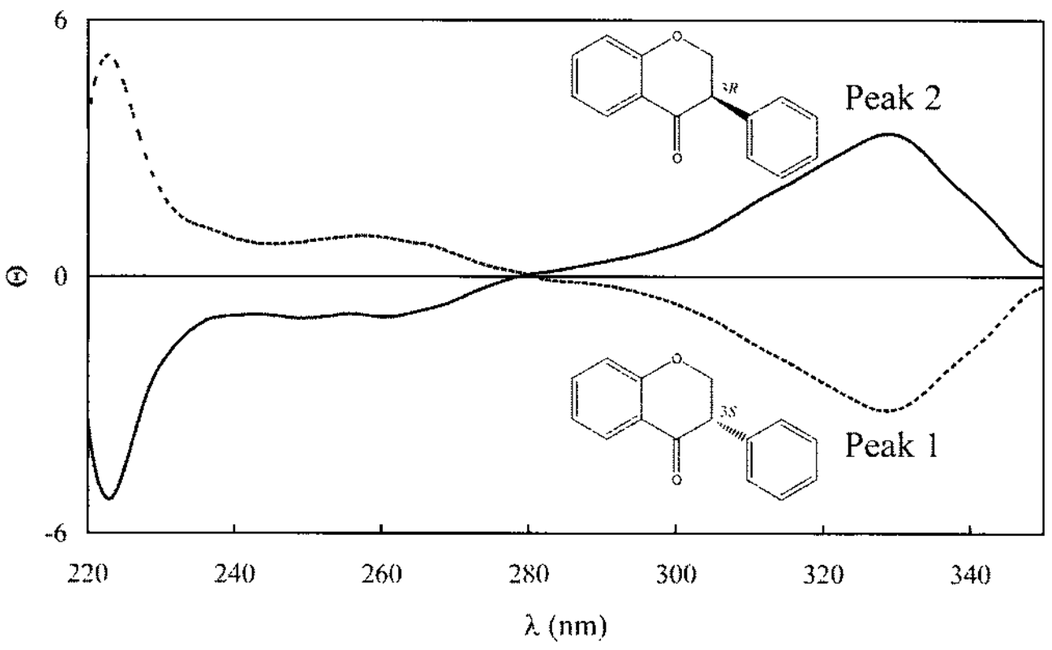

Fig. 3. CD spectra of isoflavanone enantiomers.

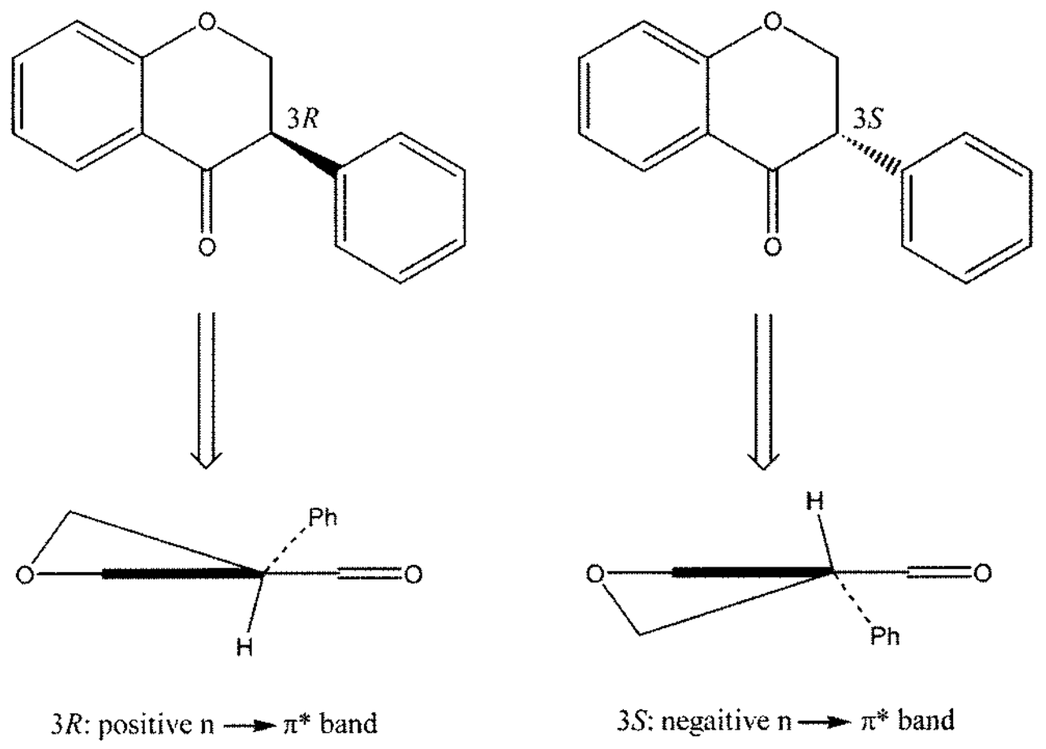

Fig. 4. Sofa conformations for Isoflavanones.

as isoflavanone undoubtedly.

Although isoflavanone has been prepared from isoflavone by other metal hydride reagents, such as diisobutylaluminiumhydride or K/L-Selectride® [Salakka *et al.*, 2006], catalytic hydrogenation with Pd/C method is much convenient and simple [Szovó and Antal, 1973]. We have shown here that isoflavanone is quantitatively converted to isoflavanone in a relatively short time.

Hydrogenation of C2-C3 double bond on C-ring generates two enantiomers with C-3 chiral center. To elucidate absolute configuration, the enantiomeric mixture of isoflavanone was separated by preparative Sumi-Chiral column with 60 : 40 ratio of MeCN : potassium phosphate (20 mM, pH 3.0) eluent system (Fig. 2). Peak integration showed peak 1 and peak 2 were found in a ratio of 1 : 1.05 from the chromatogram, and the reaction of hydrogen transfer to isoflavone was not regioselective. Each fraction was extracted with ethyl acetate and the solvent was removed under reduced pressure.

Because absolute configurations for chiral molecules can only be obtained in the presence of chiral shift reagents in ^{1}H-NMR, circular dichroism (CD) spectroscopy was applied for the isoflavanone enantiomers. CD spectra of two enantiomers in MeOH were obtained on a J-715 CD spectrometer and the results are shown at Fig. 3. The enantiomer isolated from peak 1 showed negative Cotton effect between 280 nm and 350 nm, which corresponds to $n \rightarrow \pi^*$ transition, and positive Cotton effect between 220 nm and 280 nm, which corresponds to $\pi \rightarrow \pi^*$ transition. The conformation of isoflavanone can be visualized as sofa conformation, due to the plane chroman-4-one structure (Fig. 4). According to the octant rule [Moffitt *et al.*, 1961], the peak showing positive Cotton effect at the range between 280 nm and 350 nm was assigned as *R*-

stereoisomer, and the peak showing negative Cotton effect at the same region as *S*-stereoisomer [Slade *et al.*, 2005]. Therefore, peak 1 and peak 2 have been assigned as 3*S*-isoflavanone and 3*R*-isoflavanone, respectively.

In summary, we have successfully prepared isoflavaone from isoflavone and assigned absolute configurations of the isolated enantiomers.

Acknowledgments. This Research was supported by the Chung-Ang University Research Grants in 2008. The authors thank Dr Hor-Gil Hur (Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology) for graciously allowing us access to his HPLC facilities and thank Dr. Suil Kang for his help on the chromatographic separation of the isoflavanone enantiomers.

References

Ibrahim A-R and Abul-Hajj YJ (1990) Microbiological transformation of (±)-flavanone and (±)-isoflavanone. *J Nat Prod* **53**, 644-656.

Jung S-H, Cho S-H, Dang TH, Lee J-H, Ju J-H, Kim M-K, Lee S-H, Ryu J-C, and Kim Y (2003) Structural requirement of isoflavanones for the inhibitory activity of interleukin-5. *Eur J Med Chem* **38**, 537-545.

Kim M, Shin B-K, Won D, and Han J (2007) Synthesis of 2'-hydroxydihydrochalcone from flavone. *J Appl Biol Chem* **50**, 85-87.

Lozovaya VV, Lygin AV, Zernova OV, Li S, Hartman GL, and Widholm JM (2004) Isoflavonoid accumulation in soybean hairy roots upon treatment with *Fusarium solani*. *Plant Physiol Biochem* **42**, 671-679.

Marais JPJ, Deavours B, Dixon RA, and Efrreira D (2006) The stereochemistry of flavonoids. In *The Science of Flavonoids*, Grotewold E pp. 1-46, Springer, New York, USA.

Moffitt M, Woodward RB, Klyne MW, and Djerassi C (1961) Structure and the optical rotatory dispersion of saturated ketones. *J Am Chem Soc* **83**, 4013-4018.

Salakka AK, Jokela TH, and Wähälä K (2006) Mutiple hydride reduction pathways in isoflavonoids. *Beilstein J Org Chem* **2**, 16-24.

Slade D, Ferreira D, and Marais JPJ (2005) Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. *Phytochemistry* **66**, 2177-2215.

Szabó V and Antal E (1973) The selective reduction of isoflavone. *Tetrahed Lett* **14**, 1659-1662.

Wang X-L, Hur H-G, Lee JH, Kim KT, and Kim S-I (2005) Enantioselective synthesis of *S*-equol from dihydrodaidzein by an newly isolated anaerobic human intestinal bacterium. *Appl Environ Microbiol* **71**, 214-219.