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Absolute configuration determination of isoflavan-4-ol stereoisomers

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ABSTRACT

Elucidation of the correct stereochemistry of the metabolite is essential for the mechanistic study of bioactive compounds. Isoflavan-4-ol has the same chiropical chromophore as THD, the biosynthetic precursor of the potent phytoestrogen *S*-equol. Interested in the correct absolute configuration of isoflavan-4-ol stereoisomers and to compare the available practical approaches for the absolute configuration determination, complete absolute configuration analysis of isoflavan-4-ol stereoisomers has been carried out with by means of ECD and VCD spectroscopy as well as modified Mosher method. Theoretical TD-DFT computations resulted in a poor simulation of the observed experimental ECD spectra, and thus inconclusive absolute configuration assignments of isoflavan-4-ol stereoisomers were obtained. However, DFT-assisted VCD spectroscopic analyses successfully determined correct absolute configurations, and further confirmed by modified Mosher method.

Although various spectroscopic approaches are available, correct absolute configurational analysis of stereoisomers has never been an easy task for the chemists.[1] While single crystal X-ray crystallography is the only way to determine the absolute configuration unambiguously, enantiomer requires chemical derivatization with a chiral group to form the diastereomer. If X-ray crystallography is not accessible, the derivatized diastereomeric compound can be studied by NMR spectroscopy. Besides, NMR spectroscopy has been extensively used for the absolute configuration determination in the presence of chiral shift reagent or chiral solvent, even without chiral derivatization.[2] The chemical shift changes of the characteristic peaks assist determination of the absolute configuration, but sometimes ambiguously.

Optical rotation and CD (circular dichroism) are the accessible spectroscopic methods for the absolute configuration determination of the enantiomers. But these chiroptical methods are also limited in that it always requires reference compounds with known absolute configurations. Regardless, ECD (electronic circular dichroism) spectroscopy has been a useful tool to determine the absolute configuration, especially by adopting semi-empirical approaches for the ECD spectrum interpretation. For example, the Snatzke's modified octant rule has been successfully applied to aryl ketones,[3] and flavan-4-ol stereoisomers.[4] But, this empirical approach was not always being successful and couldn't be relied on, unless structural analogs exhibiting comparable chiroptical property were available. Therefore, different levels of quantum chemical calculations started to be used for the configurational analysis and ECD spectrum simulation. In principle, the methodology can simulate ECD spectra of any compounds if correct conformational analysis is achieved. The elucidation of the absolute configuration can be achieved even without reference compound. However, reliable ECD spectrum simulation requires high accuracy in the conformational analysis and precise comparison of stabilization energies among the conformers. Accordingly, tremendous amounts of computations are often necessary, which is not available for most chemists.[5] With the limited success of ECD spectroscopy, VCD (vibrational circular dichroism) spectroscopy has emerged as an alternative chiroptical spectroscopy. This relatively less recognized spectroscopic method utilizes the ground state energy transition by infrared energy. In practice, the outcome signals are less susceptible to the conformations of the molecule, and theoretical simulation of the chiroptical transitions is generally more reliable.[6]

Recently, we have reported the absolute configurations of four isoflavan-4-ol stereoisomers based on the semi-empirical CD spectrum interpretation by using the Snatzke's modified octant rule.[7] The isoflavan-4-ol stereoisomers have same chiroptical chromophore as THD (tetrahydrodaidzein or 7,4′-dihydroxyisoflavan-4-ol), the precursor of *S*-equol biosynthesis.[8] There are growing interests in *S*-equol, because it is the most effective metabolite from soybean isoflavonoids for preventing breast cancer[9] and stimulating estrogenic response.[10] Thus, correct absolute configuration determination of the isoflavan-4-ol stereoisomers is imperative for the related studies. Furthermore, it has been recently suggested that pterocarpan biosynthesis involve formation of isoflavan-4-ol intermediate, of which stereochemistry is important in the biological activities.[11] When we carried out quantum chemical calcula-

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tions to verify the ECD-based absolute configuration assignments,[7] we have found the semi-empirical ECD interpretation was not always consistent with the sophisticated quantum mechanical calculation. Here we report complete determination of the absolute configurations of isoflavan-4-ol stereoisomers by means of TD-DFT (time-dependent density functional theory) assisted ECD, DFT assisted-VCD, and the modified Mosher method.

All possible conformers of (3*R*,4*R*)-isoflavan-4-ol for *cis*-isoflavan-4-ol and (3*S*,4*R*)-isoflavan-4-ol for *trans*-isoflavan-4-ol, respectively, were generated and optimized by AM1 semi-empirical calculation with Polak–Ribiere algorithm to 0.001 kcal/mol RMS gradient by HYPERCHEM (HYPERCHEM 7.5 for Windows, Hypercube, Gainesville, FL, USA).[12] Because ECD spectra of enantiomers are symmetric, only one enantiomers of *cis*- and *trans*-isoflavan-4-ols were studied for the ECD spectrum simulation. The conformers were defined by 3-Ph dihedral angle (π) of C2–C3–C1′–C2′ and 4-OH dihedral angle (ω) of C3–C4–O–H as shown in Figure 1. The previous work considered only the rotational helicity of C-ring of isoflavan-4-ol, because that is the only controlling factor in the semi-empirical ECD assignment.[4] The rotational helicity of (3*R*,4*R*)-isoflavan-4-ol was already determined as *P*, because it can form only equatorial 3-Ph conformation.[7] However, it can still produce a few stable conformers due to the freedom of rotation with ω and π.

From the AM1 semi-empirical geometry optimization of (3*R*,4*R*)-isoflavan-4-ol, three stable conformers were selected and labeled as RRa, RRb and RRc. While the dihedral angles of π for all stable conformers were found at the narrow range of 44° and 60°, the dihedral angle of ω were found at staggered positions of 4-OH proton. As suggested,[13] no 3-Ph axial conformers of *cis*-isoflavan-4-ol were found stable after geometry optimizations.

In case of *trans*-isoflavan-4-ol, it was reported the half chair conformation of C-ring formed two stable conformers of diaxial and diequatorial, with π dihedral angles at about −80° and −23° for diaxial conformers.[14] When we carried out geometry optimizations of all nine conformers (three for diequatorial and six for diaxial), seven conformers were merged to energy minima. Three diequatorial conformers were designated from SReqa to SReqc, and four diaxial conformers from SRaxa to SRaxd. Similar to *cis*-isoflavan-4-ol, all three merged to *trans*-diequatorial conformers showed very narrow range of π dihedral angles between −58° and −61°, and ω dihedral angles at three different staggered positions. For four diaxial conformers, two ranges of π dihedral angles were found stable, one at −49.46° and the other at the range of between −21° and −29°. For latter π dihedral angles, all three ω dihedral angles at the staggered position were merged. But for the conformers with π dihedral angle of −49.46°, only one ω dihedral angle of −67.31° found energy minima with a relatively high heat of formation. The other ω dihedral angles at this π dihedral angle of −49.46° didn't survive during the geometry optimizations, and merged to SRaxd conformer. Therefore, five *trans*-isoflavan-4-ol conformers, except high energy conformers of SRaxc and SRaxd, were subjected to further B3LYP 6-311g++(d,p) basis set DFT calculations.

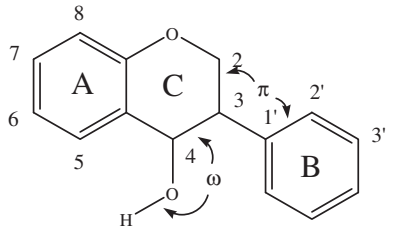

Figure 1. Structure of isoflavan-4-ol with numbering and dihedral angles.

To obtain more accurate energy values of the stable conformers, second geometry optimizations of the selected structures were carried out by means of b3lyp/6-311g++(d,p) density functional theory calculation by the GAUSSIAN 03 program.[15] The energy differences among the conformers were calculated in Hartree (1 Hartree = 627.509 kcal/mol) and further converted to Gibbs free energy differences to estimate thermodynamic distribution of the conformers. In Table 1, the results of DFT calculations for (3*R*,4*R*)-isoflavan-4-ol and (3*S*,4*R*)-isoflavan-4-ol conformers are shown. Because RRb conformer was merged to RRc conformer during the DFT calculation, RRa and RRc conformers were only considered for the ECD simulations by mean of TD-DFT with B3LYP 6-311g++(d,p) basis set. The energy difference between RRa and RRc corresponded to 87.93 cal/mol, and which resulted in 54:46 distribution of RRa:RRc. The simulated ECD spectra of (3*R*,4*R*)-isoflavan-4-ol produced by mixing each ECD spectrum of RRa and RRc conformers according to the Boltzmann weighting are shown at Figure 2. The simulated ECD spectrum of (3*R*,4*R*)-isoflavan-4-ol was exactly matched to the experimental ECD spectrum of formerly assigned (3*S*,4*S*)-isoflavan-4-ol stereoisomer. The shifts of transition energies in both spectra could be due to the MeOH solvent used for ECD experiment or other factor, such as basis set of calculation. Regardless, the reported ECD spectra assignment for *cis*-isoflavan-4-ol enantiomers,[7] which was done by the semi-empirical approaches, did not match to the TD-DFT simulation. Therefore, stereoisomer C-1[16] formerly assigned as (3*S*,4*S*)-isoflavan-4-ol, was reassigned as (3*R*,4*R*)-isoflavan-4-ol with two characteristic positive Cotton effects at the regions of 226 nm and 275 nm. Accordingly, the other *cis*-isoflavan-4-ol enantiomer C-2 with negative Cotton effects at the same regions was assigned as (3*S*,4*S*)-isoflavan-4-ol.

The results of B3LYP 6-311g++(d,p) basis set DFT calculations of five (3*S*,4*R*)-isoflavan-4-ol conformers, three equatorial and two axial, are shown in Table 1. The SReqa was the most stable conformer from DFT calculations, and the SRaxa conformer was merged to SRaxb during the geometry optimization. The calculated energy of SReqc was much higher than the other conformers, which would result in practically no contribution to the ECD spectrum. Thus, the ECD spectrum of SReqc conformer was not simulated for Bolzmann weighting. The energy of the SReqb conformer was higher than the SReqa conformer by 557.4 cal/mol, and the SRaxb conformer was higher than the SReqa conformer by 594.0 cal/mol in energy. The contribution of the three conformers to the ECD spectrum was calculated to 57:22:21 for SReqa, SReqb, and SRaxb, respectively. Although the ellipticity of the simulated ECD spectrum was not exactly matched to that of the measured ECD spectrum of (3*S*,4*R*)-isoflavan-4-ol, the rotational strength distribution was relatively matched (Fig. 3). The simulated ECD spectrum of (3*S*,4*R*)-isoflavan-4-ol was similar to

Table 1
The dihedral angles and heat of formation in Hartree obtained from B3LYP 6-311g++(d,p) basis set DFT geometry optimization of (3*R*,4*R*)-isoflavan-4-ol and (3*S*,4*R*)-isoflavan-4-ol conformers

Structures	π	ω	Energy[b]
RRa	44.439	−66.655	−730.662686444
RRb[a]	58.055	157.690	−730.66254503
RRc	58.183	157.725	−730.66254632
SReqa	−59.759	−62.975	−730.66305941
SReqb	−63.456	69.725	−730.66217107
SReqc	−60.856	−174.333	−730.65972548
SRaxa[a]	−25.710	157.254	−730.66211274
SRaxb	−25.595	157.189	−730.66211278

[a] RRb and SRaxa conformers have merged to RRc and SRaxb, respectively, during the geometry optimizations.
[b] (RB+HF-LYP).

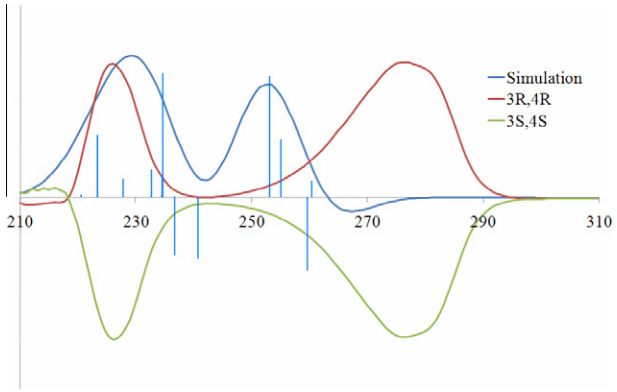

Figure 2. The CD spectra of the simulated (3*R*,4*R*)-isofavan-4-ol and experimentally measured *cis*-isofavan-4-ol. The C-1 corresponds to (3*R*,4*R*)-isofavan-4-ol based on TD-DFT ECD simulation. The blue bars show rotational strength of each transition.

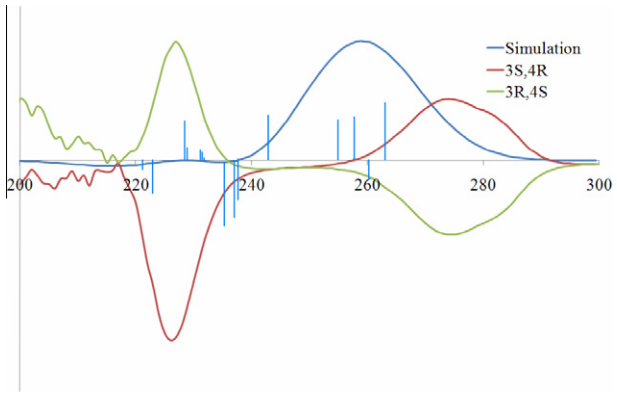

Figure 3. The CD spectra of the simulated (3*S*,4*R*)-isofavan-4-ol and experimentally measured *cis*-isofavan-4-ol. The T-1 corresponds to (3*R*,4*S*)-isofavan-4-ol based on TD-DFT ECD simulation. The blue bars show rotational strength of each transition.

the ECD spectrum taken from stereoisomer T-2. The results were consistent with the semi-empirical CD interpretation of *trans*-isoflavan-4-ol.

It was suggested that TD-DFT calculations for the ECD simulation was limited due to the difficulties of excited state energy calculation and conformational analyses. VCD has many advantages over ECD because it analyzes the chiroptically related ground state vibrational energy. Besides, the simulation of VCD spectrum is less susceptible to the molecular conformation due to the intrinsic property of IR transition. Hence, VCD spectra of T-1 and C-1 were measured on a modified Chiralir FT-VCD instrument[17] at 4 cm^{-1} resolution in $CDCl_3$.

The experimental procedure for the VCD spectrum simulation was basically same as the ones for the ECD spectrum simulation. Instead of TD-DFT method, frequency calculation (FREQ) by Stephens' theory[18] implemented in GAUSSIAN 03 was carried out with ground state b3lyp/6-311g++(d,p) DFT method after the conformational search. The VCD spectrum obtained from T-1 and the simulated spectrum of (3*S*,4*R*)- isofavan-4-ol are shown with IR spectra in Figure 4. The calculated IR peaks of (3*S*,4*R*)- isofavan-4-ol were found at the slightly higher energy region than those of observed ones, which is generally observed and probably due to the limitation of the DFT calculation.[5] The same tendency was observed for the VCD spectra comparison. But the rotational strength of each chiroptical transition in the observed and calculated spectra nicely matched as shown at Figure 4. Therefore, T-1 was assigned as (3*S*,4*R*)-*trans*-isofavan-4-ol. The VCD analysis of

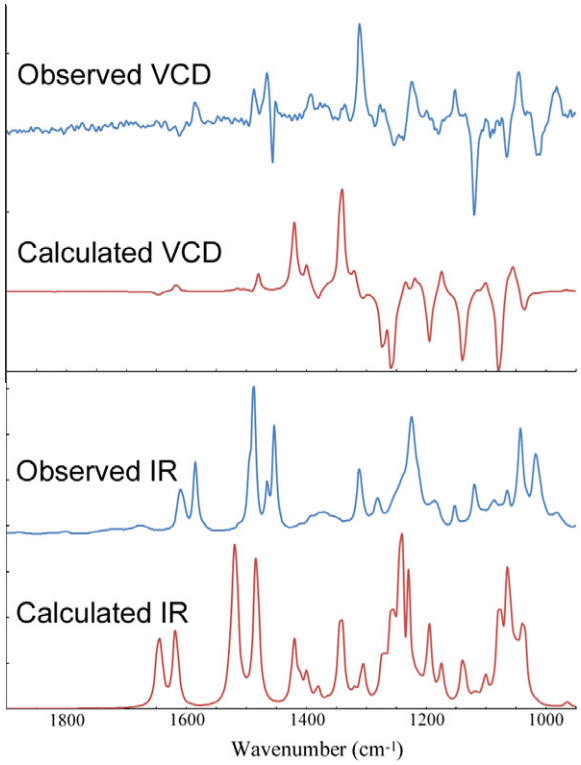

Figure 4. Comparison of IR and VCD spectra in the range of 1900–950 cm^{-1} for the observed (T-1) and calculated ((3*S*,4*R*)-isoflavan-4ol).

T-1 was completely opposite to the ECD results, and which shows limitation of ECD simulation as mentioned. Analogously, VCD spec-

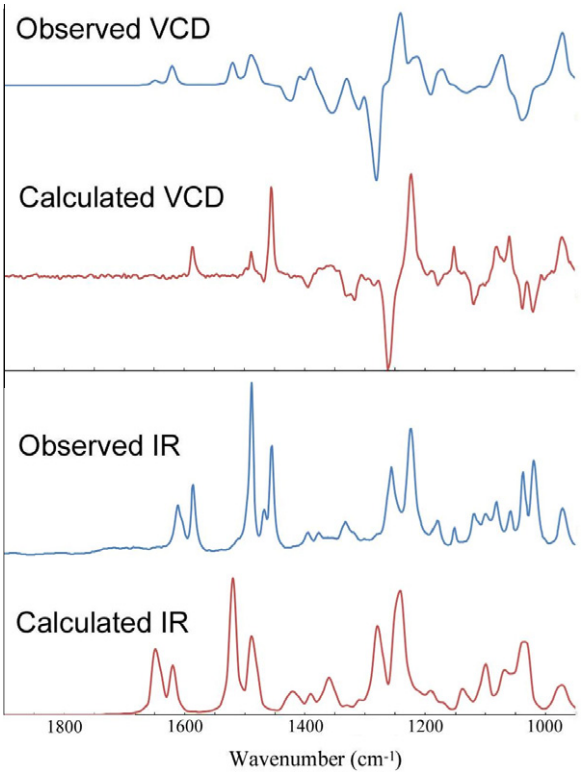

Figure 5. Comparison of IR and VCD spectra in the range of 1900–950 cm^{-1} for the observed (C-1) and calculated ((3*R*,4*R*)-isoflavan-4ol).

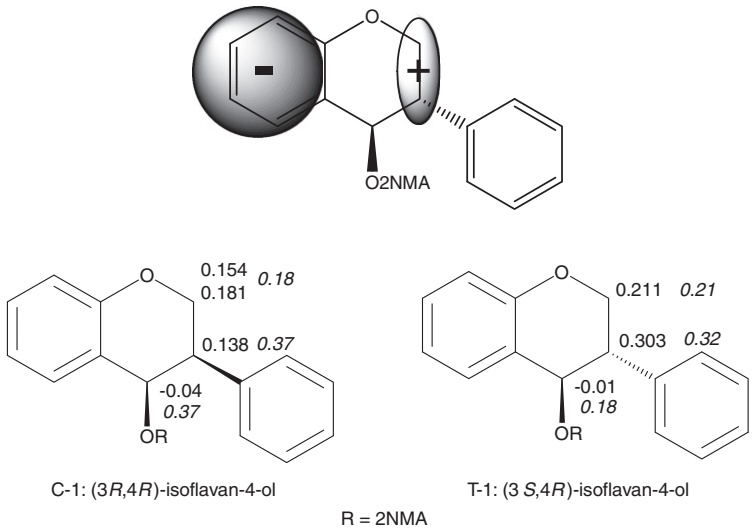

Figure 6. Expected $\Delta\delta$ changes for the 2NMA derivatives of (3*S*,4*R*)-isoflavan-4-ol and comparison of $\Delta\delta$ values of the 2NMA esters obtained for C-1 and T-1. The numbers in italic font represent ^{13}C NMR $\Delta\delta$ values.

Table 2
Experimental methods used for the absolute configuration determination of T-1 and C-1 and the results[a]

Methods	C-1	T-1
Semi-empirical ECD	(3*S*,4*S*)-isoflavan-4-ol	(3*R*,4*S*)-isoflavan-4-ol
TD-DFT ECD	**(3*R*,4*R*)-isoflavan-4-ol**	(3*R*,4*S*)-isoflavan-4-ol
VCD	**(3*R*,4*R*)-isoflavan-4-ol**	**(3*S*,4*R*)-isoflavan-4-ol**
Modified Mosher method	**(3*R*,4*R*)-isoflavan-4-ol**	**(3*S*,4*R*)-isoflavan-4-ol**

[a] Absolute configurations in bold are the correct assignments.

trum obtained from C-1 was interpreted as (3*R*,4*R*)-*cis*-isofavan-4-ol, and which was consistent with the ECD analysis of C-1 (Fig. 5).

To complete and verify the chiroptical ECD and VCD analyses, the modified Mosher method with 2NMA (methoxy-(2-naphthyl)acetic acid) has been adopted.[19] Even eight different reactions between four stereoisomers and two 2NMA enantiomers are possible for the chiral derivatization, practically four chiral derivatization reactions were enough for the complete assignment because four isoflavan-4-ol stereoisomers have the enantiomeric pairs. It is established that the 2NMA derivatives of secondary alcohol show unequal chemical shift changes of $\Delta\delta$ values ($\delta R_{ester} - \delta S_{ester}$, chemical shift differences between 2*R*-NMA and 2*S*-NMA derivatives) near the chiral center.[20] Based on the 600 MHz ^{1}H NMR chemical shift differences $\Delta\delta$ of T-1-2*R*-NMA and T-1-2*S*-NMA, 4-OH of T-1 has been assigned as 4*R* (Fig. 6).[21] Accordingly, the absolute configuration of T-1 was assigned as (3*S*,4*R*)-isoflavan-4-ol. From the ^{1}H NMR spectra of C-1-2*R*-NMA and C-1-2*S*-NMA, same tendency of $\Delta\delta$ value changes were observed. The 4-OH of C-1 was assigned as 4*R*, and which automatically assigned the absolute configuration of C-1 as (3*S*,4*R*). Along with ^{1}H NMR chemical shift changes, $\Delta\delta$ values of ^{13}C NMR can also be used for the absolute configuration determination. The results from the modified Mosher method were same to the absolute configurational assignments by VCD.

In this Letter, complete absolute configurational analysis of isoflavan-4-ol stereoisomers has been carried out by means of spectroscopic methods, including TD-DFT-assisted ECD simulation, DFT-assisted VCD simulation, and 2NMA-adopted Mosher method (Table 2). The semi-empirical[7] and TD-DFT ECD analyses were not reliable for isoflavan-4-ol stereoisomers, while the VCD experiments produced the results showing a good agreement with the modified Mosher method. Our results showed that Mosher method is more reliable than ECD spectroscopy for absolute configuration study. Besides, VCD can be a good spectroscopic approach for the absolute configuration determination when there is no functional group for the chiral derivatization.

Acknowledgments

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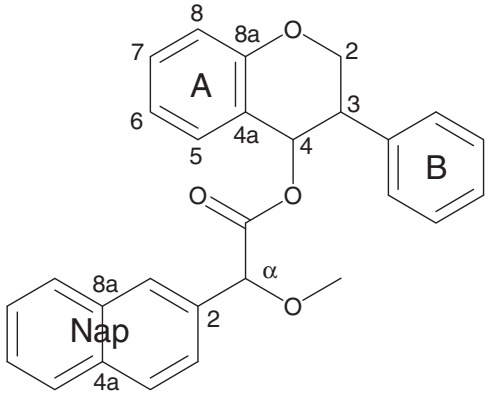
Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.074.

References and notes

1. *Stereochemistry of Organic Compounds*; Eliel, E. L., Wilen, S. H., Eds.; John Wiley and Sons: New York, 1994.
2. Frase, R. R. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1983. Chapter 9.
3. Snatzke, G. *Tetrahedron* **1965**, *21*, 413.
4. Snatzke, G.; Znatzke, F.; Tökés, A. L.; Rákosi, M.; Bognár, R. *Tetrahedron* **1973**, *29*, 909.
5. Gawronski, J. K.; Kwit, M.; Boyd, D. R.; Sharma, N. D.; Malone, J. F.; Drake, A. F. *J. Am. Chem. Soc.* **2005**, *127*, 4308.
6. (a) Nafie, L. A. *Nat. Prod. Commun.* **2008**, *3*, 451; (b) Freedman, T. B.; Cao, X.; Dukore, R. K.; Nafie, L. A. *Chirality* **2003**, *15*, 743.
7. Won, D.; Shin, B.-K.; Kang, S.; Hur, H.-G.; Kim, M.; Han, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1952.
8. Kim, M.; Kim, S.-I.; Han, J.; Wang, X.-L.; Song, D.-G.; Kim, S.-U. *Appl. Environ. Microbiol.* **2009**, *75*, 3062.
9. Sathyamoorthy, N.; Wang, T. T. Y. *Eur. J. Cancer* **1997**, *33*, 2384.
10. Setchell, K. D. R.; Clerici, C.; Lephart, E. D.; Cole, S. J.; Heenan, C.; Castellani, D.; Wolfe, B. E.; Nechemias-Zimmer, L.; Brown, N. M.; Lund, T. D.; Handa, R. J.; Heubi, J. E. *Am. J. Clinic. Nutr.* **2005**, *81*, 1072.
11. Kiss, L.; Kurtan, T.; Antus, S.; Benyei, A. *Chirality* **2003**, *15*, 558.
12. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
13. Wähälä, K.; Koskimies, J. K.; Mesilaakso, M.; Salakka, A.; Leino, T. K.; Adlercreutz, H. *J. Org. Chem.* **1997**, *62*, 7690.
14. Pihlaja, K.; Tähtinen, P.; Klika, K. D.; Jokela, T.; Salakka, A.; Wähälä, K. *J. Org. Chem.* **2003**, *68*, 6864.
15. (a) Seo, J.; Kang, S. I.; Ryu, J. Y.; Lee, Y. J.; Park, K. D.; Kim, M.; Won, D.; Park, H. Y.; Ahn, J. H.; Chong, Y.; Kanaly, R. A.; Han, J.; Hur, H. G. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 1451; (b) Seo, J.; Kang, S. I.; Kim, M.; Won, D.; Takahashi, H.; Ahn, J. H.; Chong, Y.; Lee, E.; Lim, Y.; Kanaly, R. A.; Han, J.; Hur, H. G. *Anal. Biochem.* **2010**, *397*, 29.

16. Each enantiomer was designated as T-1 and T-2 for *trans*-isoflavan-4-ols, and C-1 and C-2 for *cis*-isoflavan-4-ols, respectively, according to the elution order on the Sumi-chiral column chromatogram. See Ref. 8 for HPLC chromatogram of the stereoisomers.
17. Nafie, L. A.; Dukor, R. K.; Freedman, T. B.. In Chalmers, J. M., Griffiths, P. R., Eds.; John Wiley and Sons: New York, 2002; pp 731–744.
18. Cheeseman, J. R.; Frisch, M. J.; Devlin, F. J.; Stephens, P. J. *Chem. Phys. Lett.* **1996**, *252*, 211.
19. Kan, Y.; Satake, M.; Yasumoto, T. *Tetrahedron Lett.* **1996**, *37*, 7087.
20. Kusumi, T.; Takahashi, H.; Xu, P.; Fukushima, T.; Asakawa, Y.; Hashimoto, T.; Kan, Y.; Inouye, Y. *Tetrahedron Lett.* **1994**, *35*, 4397.
21. Each product was designated with the symbols of isoflavan-4-ol stereoisomers and 2NMA enantiomers. For example, C-1-2*R*-NMA was used for the esterification product of C-1 and 2*R*-NMA.



C-1-2R-NMA: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ 3.112 (3H, s, OCH_3), 3.419 (1H, dt, J = 12 Hz, H-3), 4.416 (1H, dd, H-2a), 4.554 (1H, t, J = 12 Hz, H-2b), 4.648 (1H, s, Hα), 6.185 (1H, d, J = 2.76 Hz, H-4), 6.697 (1H, t, J = 6.4 Hz, B-Ph), 6.781 (1H, d, J = 8.0 Hz, B-Ph), 7.10–7.20 (7H, m, A and B-Ph), 7.25 (1H, d, Nap), 7.40 (2H, m, Nap), 7.60–7.66 (3H, m, Nap), 7.72 (1H, m, Nap). ^{13}C NMR spectrum (100 MHz, $CDCl_3$) δ 170.10 (CO_2), 154.61 (C-8a), 136.80 (C-4a), 133.37 (Nap-8a), 133.35 (Nap-4a), 133.13 (Nap-2), 130.99 (B-Ph), 130.55 (B-Ph), 128.60 (C-8), 128.60 (B-Ph), 128.44 (Nap), 128.34 (C-5), 128.11 (C-7), 128.11 (Nap), 127.69 (Nap), 127.43 (C-6), 126.35 (Nap), 126.35 (Nap), 126.22 (Nap), 124.31 (Nap), 120.74 (B-Ph), 120.22 (B-Ph), 116.94 (B-Ph), 82.74 (Cα), 69.30 (C-4), 64.83 (C-2), 57.53 (OCH_3), 42.29 (C-3).
C-1-2S-NMA: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ 3.210 (3H, s, OCH_3), 3.281 (1H, dt, J = 12.4 Hz, H-3), 4.235 (1H, dd, H-2a), 4.400 (1H, dd, J = 10.4 Hz, J = 12 Hz, H-2b), 4.681 (1H, s, Hα), 6.189 (1H, d, J = 1.36 Hz, H-4), 6.471 (2H, t, J = 8 Hz, B-Ph), 6.573 (2H, d, J = 6.8 Hz, B-Ph), 6.670 (1H, t, B-Ph), 6.848 (2H, d, A), 7.20–7.35 (2H, m, A), 7.38 (1H, d, Nap), 7.45 (2H, m, Nap), 7.56–7.70 (3H, m, Nap), 7.778 (1H, d, Nap). ^{13}C NMR spectrum (100 MHz, $CDCl_3$) δ 169.74 (CO_2), 154.58 (C-8a), 136.20 (C-4a), 133.42 (Nap-8a), 133.13 (Nap-4a), 133.09 (Nap-2), 132.27 (B-Ph), 130.79 (B-Ph), 128.58 (C-8), 128.28 (B-Ph), 128.13 (Nap), 127.74 (C-5), 127.74 (C-7), 127.38 (Nap), 127.10 (Nap), 127.10 (C-6), 126.88 (Nap), 126.54 (Nap), 126.53 (Nap), 125.10 (Nap), 120.80 (B-Ph), 120.80 (B-Ph), 117.0 (B-Ph), 82.60 (Cα), 69.34 (C-4), 64.65 (C-2), 57.21 (OCH_3), 41.92 (C-3).
T-1-2R-NMA: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ 3.311 (3H, s, OCH_3), 3.354 (1H, dt, H-3), 4.357 (2H, m, H-2), 4.886 (1H, s, Hα), 6.298 (1H, d, J = 7.32 Hz, H-4), 6.652 (1H, t, B-Ph), 6.66 (1H, d, B-Ph), 6.817 (1H, d, B-Ph), 7.12 (1H, t, B-Ph), 7.15–7.25 (5H, m, A and B-Ph), 7.49 (3H, m, Nap), 7.75–7.85 (4H, m, Nap). ^{13}C NMR spectrum (100 MHz, $CDCl_3$) δ 170.52 (CO_2), 154.67 (C-8a), 137.37 (C-4a), 133.43 (Nap-8a), 133.21 (Nap-4a), 133.08 (Nap-2), 129.75 (B-Ph), 128.77 (B-Ph), 128.69 (C-8), 128.46 (B-Ph), 128.15 (C-5), 128.14 (Nap), 127.81 (Nap), 127.80 (C-7), 127.67 (Nap), 127.47 (C-6), 126.58 (Nap), 126.39 (Nap), 126.26 (Nap), 124.20 (Nap), 120.74 (B-Ph), 120.0 (B-Ph), 116.64 (B-Ph), 82.70 (Cα), 71.01 (C-4), 67.67 (C-2), 57.33 (OCH_3), 43.90 (C-3).
T-1-2S-NMA: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ 3.051 (1H, dt, H-3), 3.392 (3H, s, OCH_3), 4.146 (2H, m, H-2), 4.910 (1H, s, Hα), 6.308 (1H, d, J = 6.72 Hz, H-4), 6.85–7.0 (7H, m, A and B-Ph), 7.12 (1H, d, A), 7.22 (1H, t, A), 7.38 (1H, d, Nap), 7.515 (2H, m, Nap), 7.72–7.86 (4H, m, Nap). ^{13}C NMR spectrum (100 MHz, $CDCl_3$) δ 170.37 (CO_2), 154.88 (C-8a), 136.99 (C-4a), 133.48 (Nap-8a), 133.24 (Nap-4a), 133.14 (Nap-2), 130.01 (B-Ph), 129.26 (B-Ph), 128.48 (C-8), 128.42 (Nap), 128.34 (B-Ph), 128.19 (C-5), 127.67 (C-7), 127.44 (Nap), 127.44 (Nap), 127.18 (C-6), 126.78 (Nap), 126.42 (Nap), 126.25 (Nap), 124.39 (Nap), 120.94 (B-Ph), 120.02 (B-Ph), 116.83 (B-Ph), 82.53 (Cα), 70.83 (C-4), 67.46 (C-2), 57.25 (OCH_3), 43.58 (C-3).