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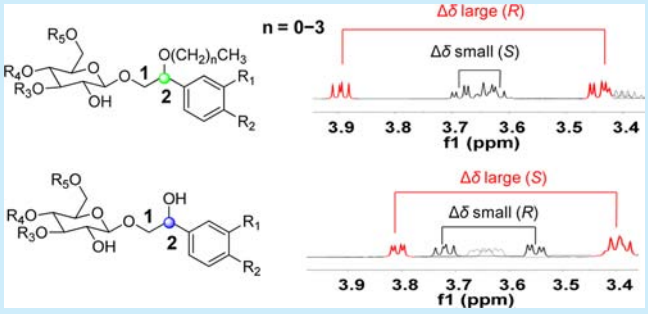
An Approach for Determining the Absolute Configuration of C-2 in 2-Oxygenated Phenylethanoid Glycosides by ^{1}H NMR Spectroscopy

Si-Yuan Shao, Fan Zhang, Ya-Nan Yang, Zi-Ming Feng, Jian-Shuang Jiang, and Pei-Cheng Zhang*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Supporting Information

ABSTRACT: The absolute configurations of 2-oxygenated phenylethanoid glycosides were conveniently determined by ^{1}H NMR spectroscopy. A comparison of the chemical shift differences ($\Delta\delta$) of the diastereotopic methylene protons (H-1) demonstrate that a large chemical shift difference corresponds to an *R* configuration and a small chemical shift difference indicates *S* for the 2-alkoxy form. However, the situation is contrary to that of the 2-hydroxy form. Furthermore, the mechanism underlying this result is discussed based on the visualized conformations of such compounds.



Determining the absolute configuration of a compound is vital for elucidating the structural characteristics and bioactivities of its chemical constituents. 2-Oxygenated phenylethanoid glycosides, a type of important secondary metabolite, consist of an aglycon with a chiral center (C-2), sugar moieties, and acyl groups. To date, there are approximately 60 such compounds that have been isolated from various natural plants.[1−21] The common method to determine the absolute configuration of C-2 is by hydrolyzing the compound and comparing the optical rotations of the aglycon with similar compounds (2-hydroxy-2-phenylethanol or 2-alkoxy-2-phenylethanol),[1] which often involves tedious multiple steps and introduces concerns about the stability of the compounds and the propensity for skeletal rearrangement, especially in 2-hydroxy phenylethanoid glycosides. The risk in this approach is apparent unless the aglycon can be isolated and identified from the hydrolysis products.[6] Moreover, other common approaches are not suitable because of the characteristics of 2-oxygenated phenylethanoid glycosides; the sugars make it difficult to grow crystals of the compounds. The acyl disturbs the comparison of the electronic circular dichroism (ECD) spectra; the existence of polyhydroxys limit the usage of Mosher's method.[22−25] Thus, a reliable and convenient aglycon stereochemistry determination method needs to be developed to help resolve this problem.

Recently, we investigated the chemical constituents of two traditional Chinese medicines (TCM), *Forsythia suspense* and *Leonurus artemisia*. Fourteen 2-oxygenated phenylethanoid glycosides (**1**−**14**) (Figure 1) were obtained using various column chromatography methods. When we tried to elucidate the stereochemistry of a pair of 2-butoxy phenylethanoid glycosides (**1** and **2**) using the hydrolysis method, too many hydrolysis products were found, with the result that the aglycon could not be isolated to determine the absolute configuration of C-2. However, during detailed assignments of the NMR data of compounds **1** and **2**, some interesting information was observed in the ^{1}H NMR spectra (in DMSO-d_6); two methylene protons of H-1 in **1** were clearly assigned at δ_H 3.78 (H-1a) and δ_H 3.43 (H-1b), while the corresponding protons in **2** were at δ_H 3.64 (H-1a) and δ_H 3.54 (H-1b) (Supporting Information, Table S2). Obviously, there was a disparity between the two chemical shift differences ($\Delta\delta$) of the methylene protons. Thus, one diastereomer (**1**) exhibited a large chemical shift difference ($\Delta\delta$ = 0.35), and the other diastereomer (**2**) showed a small chemical shift difference ($\Delta\delta$ = 0.10). Subsequently, the other five pairs of 2-oxygenated phenylethanoid glycosides (**3**−**6**, **9**−**14**) were studied. Indeed, all the compounds had a certain disparity. However, when we further compared their coupling constants in 2-alkoxy and 2-hydroxy phenylethanoid glycosides, an obvious difference was observed between them. For instance, compound **1** with a 2-butoxy group and **10** with a 2-hydroxy group had similar large chemical shift differences ($\Delta\delta$ = 0.35 and $\Delta\delta$ = 0.34), but the $J_{H\text{-}1a,2}$ in **1** and **10** were 8.0 and 3.0 Hz, respectively. This phenomenon implied that the dominant conformations of the 2-alkoxy and 2-hydroxy phenylethanoid glycosides were different. These findings inspired us to investigate this phenomenon thoroughly and reveal the relationship between the chemical shift differences ($\Delta\delta$) and the absolute configuration of C-2.

To explore this issue, 2-methoxy phenylethanoid glycosides with 2*R* and 2*S* (**A** and **B**) and 2-hydroxy phenylethanoid glycosides with 2*R* and 2*S* (**C** and **D**) were synthesized using

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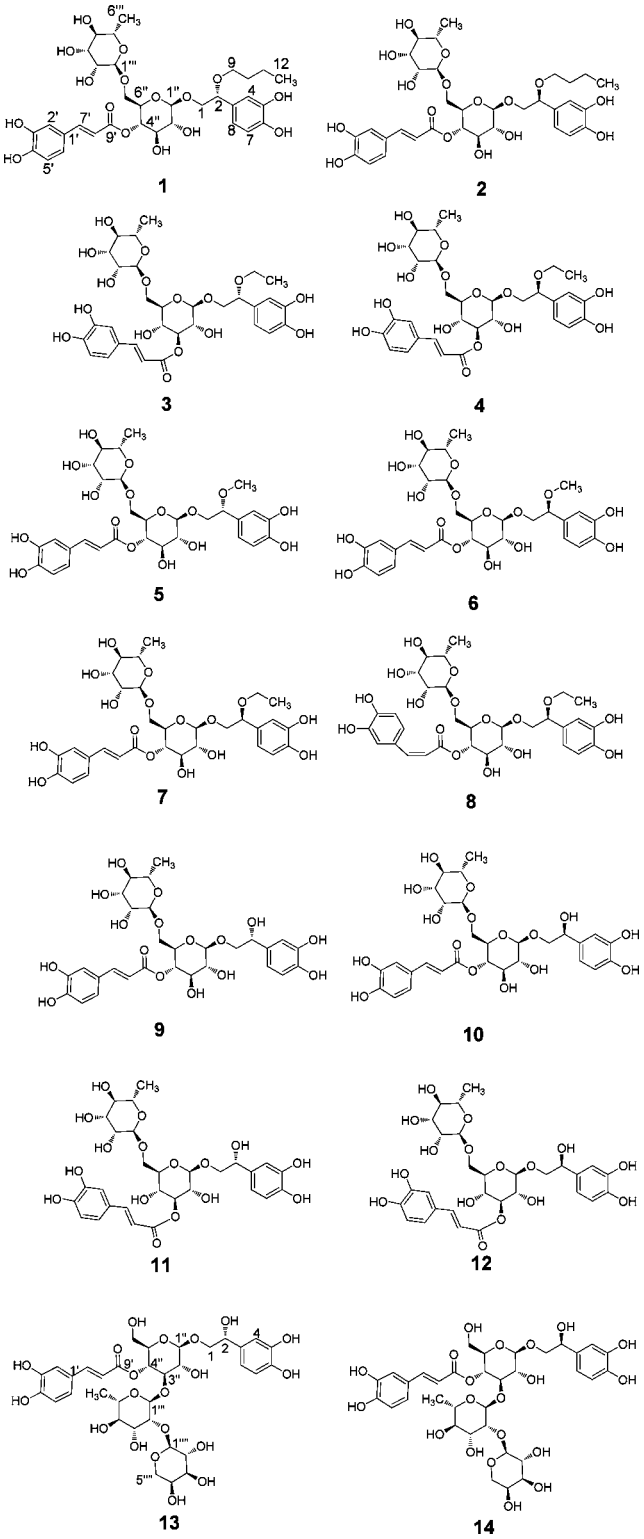

Figure 1. Compounds **1**−**14** purified from natural plants.

(*R*)- or (*S*)-2-methoxy-2-phenylethanol/(*R*) or (*S*)-2-hydroxy-2-phenylethanol and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (Supporting Information, S1). Their ^{1}H NMR and HSQC spectra were recorded in DMSO-d_6. A large chemical shift difference ($\Delta\delta$ = 0.46) was observed in the ^{1}H NMR spectrum of **A**, and a small chemical shift difference ($\Delta\delta$ = 0.07) was found in that of **B** (Figure 2). However, for the two 2-hydroxy phenylethanoid glycosides (**C** and **D**), a large

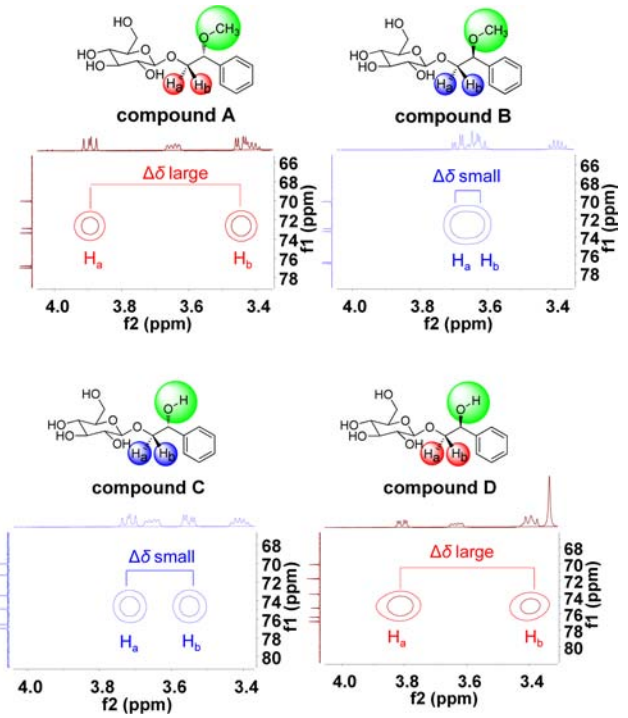

Figure 2. Large (red) and small (blue) chemical shift differences of compounds **A**−**D**.

chemical shift difference ($\Delta\delta$ = 0.41) was found in the ^{1}H NMR spectrum of **D** and a small chemical shift difference ($\Delta\delta$ = 0.16) was obtained in the ^{1}H NMR spectrum of **C**, which was contrary to the 2-alkoxy form (Figure 2). Then, the absolute configurations corresponding to the chemical shift differences were established.

The appearance of such chemical shift differences for these compounds could be explained by the anisotropic effects on the protons.[26,27] The influences of the pyran oxygen and phenyl groups toward the methylene protons can be readily recognized in their dominant conformations (Figure 3). In the compound **A**, the pyran oxygen can induce a deshielding effect on H_b, and the phenyl group has a shielding effect on H_a. However, for its

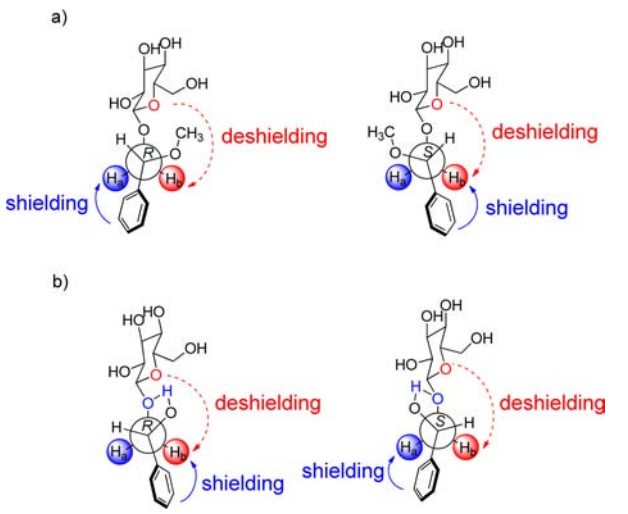

Figure 3. (a) Conformations of the (*R*)- and (*S*)-2-methoxy phenylethanoid glycosides. (b) Conformations of the (*R*)- and (*S*)-2-hydroxy phenylethanoid glycosides.

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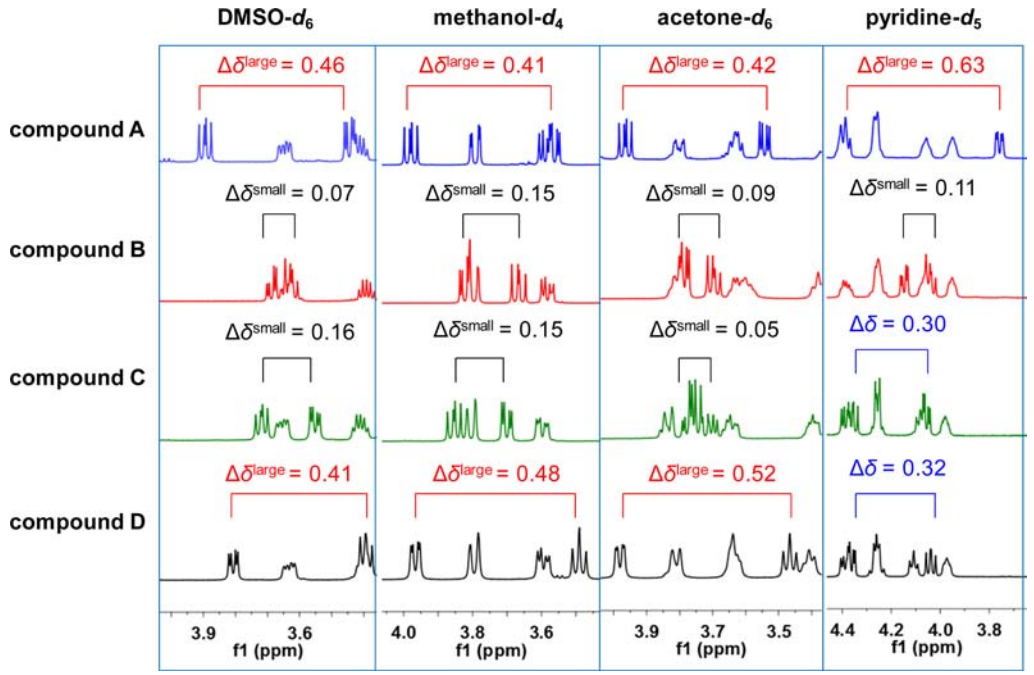

Figure 4. Influence of different deuterated solvents on the chemical shift differences of compounds **A**–**D**.

S configuration (**B**), H_b is deshielded by the pyran oxygen and shielded by the phenyl group. Thus, the chemical shifts of the methylene protons in these two structures are extremely different. For the *R* configuration, the chemical shift of H_b is located at low field and H_a is at high field. Thus, there is a large chemical shift difference. For the *S* configuration, H_b shifts downfield, and H_a stays in a normal field, which induces a small chemical shift difference. Following similar principles, 2-hydroxy phenylethanoid glycosides were analyzed. Because of the existence of the hydrogen bond, the phenyl group of compound **C** causes a shielding effect on H_b, and its pyran oxygen produces a deshielding effect. Both of them have no obvious effect on H_a; hence, the methylene protons ultimately generate a small chemical shift difference. In its *S* configuration (**D**), the shielding/deshielding effects of the phenyl group/pyran oxygen are similar to (*R*)-2-methoxy phenylethanoid glycoside, which has a large chemical shift difference.

Next, the effects of common solvents were investigated. The chemical shift differences of compounds **A**–**D** were recorded in DMSO-d_6, methanol-d_4, acetone-d_6, and pyridine-d_5 (Figure 4). Based on these experiments, all the small and large chemical shift differences of 2-methoxy phenylethanoid glycosides complied with the rule. Furthermore, a pair of natural products (**1** and **2**) were also recorded in different solvents and showed similar results (Supporting Information, Table S7). As for 2-hydroxy phenylethanoid glycosides (**C** and **D**), they also followed the rule with the only exception occurring in pyridine-d_5, in which their values were very close.

Based on the substituents of position C-2, the natural compounds (**1**–**14**) and synthesized compounds (**A**–**D**) were classified into two groups. As shown in Figure 5, when the substituent on C-2 is an alkoxy group, the large and small chemical shift differences are very obvious (Figure 5a); when the substituent is a hydroxyl group, although the gap is shortened, the absolute configuration can be readily recognized by the tendency (Figure 5b).

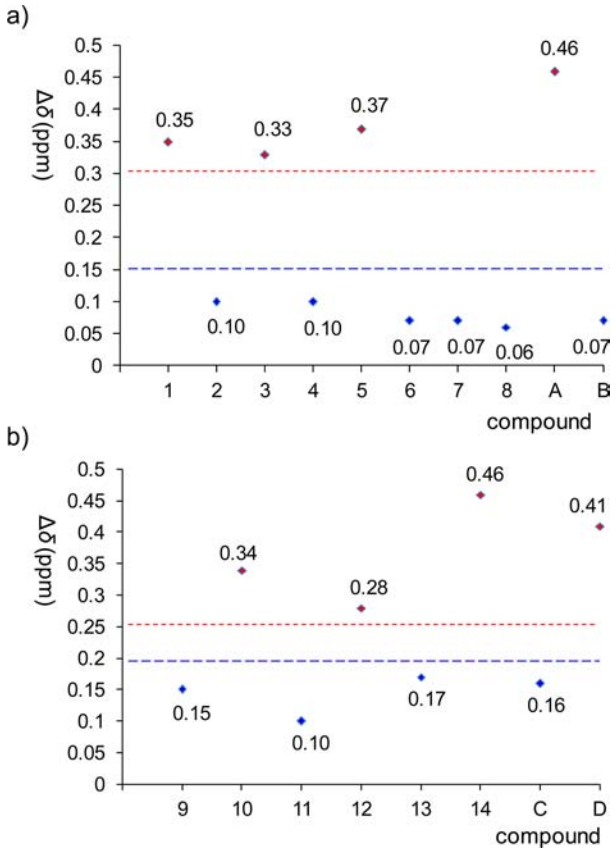

Figure 5. (a) The large and small chemical shift differences (Δδ) of compounds **1**–**8**, **A**, and **B**. (b) The large and small chemical shift differences (Δδ) of compounds **9**–**14**, **C**, and **D**.

In Nature, approximately 60 2-oxygenated phenylethanoid glycosides have been isolated to date.[1−21] Structurally, they involve most substituted types of such compounds. The aromatic rings have 3,4-disubstitution or 4-substitution. The

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substituent on position C-2 is hydroxy or alkoxy. The positions C-3, C-4, and C-6 of the glucose are substituted by caffeoyl, feruloyl, coumaroyl, or sugar moieties, including rha, rib, gal, and glc. On the basis of the reported information, most absolute configurations of these structures have not been determined. Among them, several studies of the structures mentioned discuss the stereochemistry, whereas some literature simply uses comparisons with previous vague literature.[1,6,8,14] However, their chemical shift differences in the ^{1}H NMR data have obvious differences; thus, the proposed rule can be used to settle these problems. As for compounds **1**−**14** that we isolated, they have been thoroughly studied via this method, because of the quantity yielded. This study indicated that these substituents hardly influenced the large and small chemical shift differences.

In conclusion, based on the ^{1}H NMR data of the aglycon methylene protons in 2-oxygenated phenylethanoid glycosides, the correlations between the absolute configuration of C-2 and the chemical shift difference of H-1 can be concluded as follows:

(a) 2-alkoxy phenylethanoid glycosides

R: = $\Delta\delta$ >0.30 ppm

S: = $\Delta\delta$ <0.15 ppm

(b) 2-hydroxy phenylethanoid glycosides

R: = $\Delta\delta$ <0.20 ppm

S: = $\Delta\delta$ >0.25 ppm

That is, when the chemical shift difference of H-1 in the aglycon of 2-alkoxy phenylethanoid glycosides is >0.30 or in 2-hydroxy phenylethanoid glycosides is <0.20, the absolute configuration of C-2 is determined to be *R*. The contrasting situation indicates an *S* configuration. Of course, it is noteworthy that the assignment of structures with substituents on the C-2 of glucose should be performed carefully, for its closer distance may affect the diastereotopic methylene protons.[4]

Thus, depending on the ability to correctly assign the ^{1}H NMR data, the absolute configurations at C-2 of the aglycon in 2-oxygenated phenylethanoid glycosides can be determined reliably and conveniently. These recent findings expand our knowledge of the methods used to determine the absolute configuration of compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01978.

A detailed experimental section; the general procedure for compounds **A**−**D**; physical data; and the IR, HRESIMS, 1D and 2D NMR spectra of compounds **1**−**14** and compounds **A**−**D** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pczhang@imm.ac.cn.

Notes

The authors declare no competing financial interest.

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