

Conformational Study of 8-C-glucosyl-prunetin by Dynamic NMR Spectroscopy

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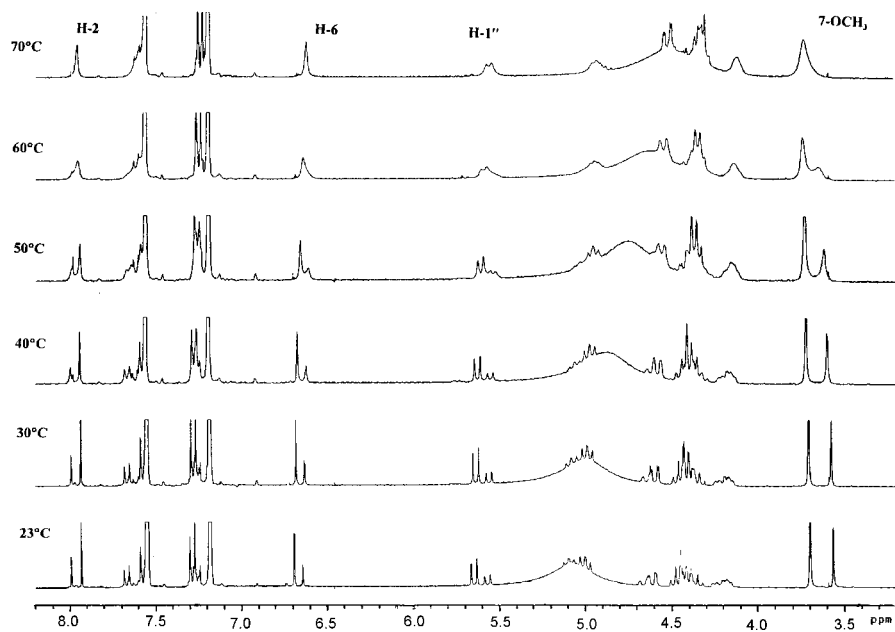
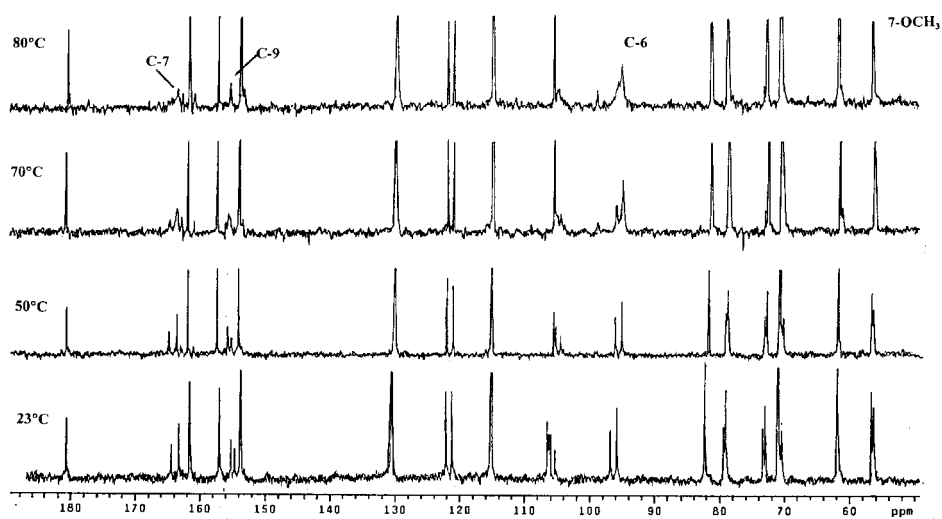
Abstract: By means of variable temperature NMR spectra, conformation of 8-C-glucosyl prunetin, isolated from the leaves of *Dalbergia hainanensis* (Leguminosae), was studied. The restricted rotation around the C (sp³)-C (sp²) bond in the C-glucosides isoflavonoid results in two main conformers (syn and anti). With the help of MM calculation, the preferred conformation A has H-1'' gauche to the 7-OCH₃. The barrier to rotation was 18.1 kcal/mol. This result agrees with the calculated value 16.2 kcal/mol of free energy of activation for the interconversion between the conformers.

Keywords: 8-C-Glucosyl prunetin, dynamic NMR, MM calculation, conformation.

Dalbergia hainanensis (Leguminosae), which is distributed in Hainan province of China, has been used as a Chinese folk medicine, for example an antiscabietic. In research for its biologically active compounds, two C-glucoside isoflavonoids, 8-C-glucosyl genistein (**1**) and 8-C-glucosyl prunetin (**2**)¹, were isolated from the leaves of the plant. C-glucoside isoflavonoids have many important bioactivities, for example, anti-complement activity and colony stimulating factor-inducing activity². It is interesting that two sets of signals are observed in the ¹H- and ¹³C-NMR measurements of **1** at the room temperature. Furthermore, it was confirmed that **1** was a pure compound, instead of a mixture of two compounds, by 2D NMR spectra (HMBC and HMQC). These suggested that two main conformational isomers unambiguously existed. Recently, conformations and barriers to conformational changes of organic compounds are of interest for organic chemists^{3,4}. Dynamic NMR spectroscopy, complemented with molecular mechanics calculations, have been useful in studying the stereodynamics of more complex amines⁵ and dimesityl sulfine⁶. In this paper, we report a dynamic NMR study of 8-C-glucosyl prunetin.

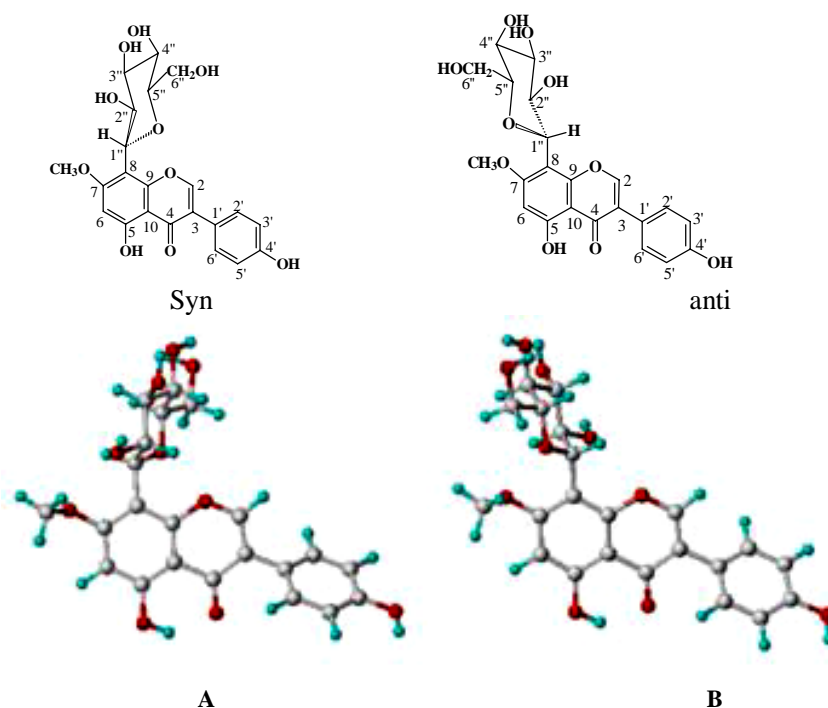
Results and Discussion

8-C-glucosyl prunetin (**1**) was isolated from the ethyl acetate fraction of ethanol extracts of the leaves of *Dalbergia hainanensis*. The ¹H-NMR spectrum of **1** displays at ambient

Figure 1 The observed temperature-dependent ^1H NMR spectra of **1** (in $\text{C}_5\text{D}_5\text{N}$)**Figure 2** The observed temperature-dependent ^{13}C NMR spectra of **1** (in $\text{DMSO}-d_6$)

temperature two unequally populated sets of signals, which broaden and coalesce into humps at higher temperature, and some narrower humps are seen to sharpen on further heating (**Figure 1**). The same situation is also observed in the ^{13}C NMR spectra (**Figure 2**). This model entails the existence of two possible conformers of different stability (sterelabile, diastereoisomers). In the ^1H NMR of **1** $J_{\text{H-1}''\text{2}''}$ (conformers A and B) were 9.6 Hz and H-2'' was triplet having $J_{\text{H-1}''\text{2}''} = J_{\text{H-3}''\text{2}''} = 9.6$ Hz. These data suggested that the glucosyl group is in chiral conformation in both conformers and both the conformers were not produced by the conformational changes of glycoside cycle. The crowding introduced by the substituents at the 9- and 7-positions of C-glucosides isoflavonoid results in restricted conformational motion about the C-8--C-1'' bond. While the sixfold barrier for an unhindered rotation about linking tetrahedral and trigonal planar centers is ordinarily very small, the conformations available to the C-glucosides isoflavonoid are restricted and the barrier for rotation about the C (sp^3)-C (sp^2) bond is substantial. We find evidence in variable temperature ^1H NMR studies for the existences of distinct conformations for the C-glucosides isoflavonoid of some different 7-O substituents (OCH_3 , $\text{OCH}_2\text{CH}_2\text{CH}_3$, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$), and have measured the barriers for interconversion between the conformations by full NMR analysis. Molecular mechanics (MM) studies provide a further basis for defining these conformations.

Figure 3 MM computed structures of the conformers A and B of 8-C-glucosyl prunetin (**1**)



For **1** just one signal is seen for each H nucleus at elevated temperature. Upon cooling, signals broaden and decoalesce eventually into two sets of signals for two

unequally populated conformers. The exchange process is most easily analyzed from the singlet change of signals of OCH₃ and H-2 that become two singlets and the H-1'' doublet signal that becomes two doublets at low temperature. At 313K, the ratio of conformer A to conformer B is about 2:1 and Δν is 40.5 Hz for 7-OCH₃ in the ¹H NMR (C₆D₅N). The free energy of activation for the interconversion between the conformers can be deduced using Eyring's Eq.1 and 2 as modified by Shanan-Atidi and Bar-Eli⁷.

Eyring's equation:

$$\Delta G_A^\ddagger = 4.57T_C \{10.62 + \log [X/2\pi (1-\Delta P)] + \log (T_C/\Delta\nu)\} \quad (1)$$

$$\Delta G_B^\ddagger = 4.57T_C \{10.62 + \log [X/2\pi (1+\Delta P)] + \log (T_C/\Delta\nu)\} \quad (2)$$

where $X = 2\pi\tau\Delta\nu$ and $\Delta P = P_A - P_B$

P_A , P_B representing the population of the species A and B ($P_A > P_B$, $P_A + P_B = 1$), respectively, and τ is the mean lifetime.

T_C and $\Delta\nu$ are the coalescence temperature and the chemical shift difference between A and B, respectively.

X is obtained by the use of Eq.3.

$$P_A - P_B = (X^2 - 2/3)^{3/2} / X \quad (3)$$

The calculated value of the activation energy ΔG^\ddagger for **1** was about 16.2 kcal/mol.

The conformational preferences for **1** were studied *via* molecular mechanics using the MM forcefield. Rotation through 360° for most of the bonds produces just two minima (A and B). The major and minor conformations for **1** were depicted in **Figure 3**. The MM calculation predicts approximately syn and anti alignments for H-1'' in the two conformations. The lowest energy conformation A is 0.46 kcal/mol lower in energy than conformation B and has H-1'' gauche to the 7-OCH₃. The barrier to rotation was 18.1 kcal/mol. This result agrees with the calculated value of the activation energy ΔG^\ddagger for **1** was about 16.2 kcal/mol.

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