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Studies on the hydrogen bond acidity, and other descriptors and properties for hydroxyflavones and hydroxyisoflavones

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ABSTRACT

An NMR method has been used to determine the hydrogen bond acidity, **A**, of a number of hydroxyisoflavones. Together with our previous studies on hydroxyflavones, these **A**-values enable the presence of intramolecular hydrogen bonding in hydroxyflavones and hydroxyisoflavones to be detected. A full set of Abraham descriptors has been assigned to all the monohydroxy and dihydroxy-flavones and isoflavones, and to some polyhydroxy-flavones and isoflavones as well. In these assignments, knowledge of the **A**-descriptor is crucial. These sets of descriptors can then be used to estimate values for a large number of physicochemical and biochemical processes.

1. Introduction

Flavones and isoflavones are classes of natural products that have a number of important biological properties including antioxidant, anticancer and neuro-protective activities [1–6]. Their hydroxy-derivatives are particularly important, and interaction with lipid structures depends on the number and position of the hydroxy-substituents [7–9], see Figs. 1 and 2. Because of the importance of hydroxy-derivatives, we studied the hydrogen-bond acidity, **A**, of a number of flavone derivatives by an NMR method and showed that the hydrogen-bond acidity depends crucially on the position of the hydroxy group [10].

We now report on further studies of the hydrogen bond acidity of hydroxyflavones and hydroxyisoflavones. These hydrogen-bond acidities are very important because they considerably aid the estimation of the overall Abraham descriptors for hydroxyflavones and hydroxyisoflavones, and hence the estimation of a large number of physicochemical and biochemical properties. For a few compounds there is available data on solubilities in organic solvents. We have previously described how solubility data for a given compound can be used to determine Abraham descriptors [11–15] and in the present work we use data on solubilities of the isoflavones daidzein and genistein to obtain descriptors. Pogodaeva et al. [16] have determined water–octanol partition coefficient for a number of flavones, and these log *P*oct values are of use in the determination of descriptors. The net result is the determination of Abraham descriptors for a large number of important hydroxyflavones and hydroxyisoflavones, followed by the estimation of numerous physicochemical and biochemical properties.

2. Materials and methods

2.1. Introduction

Initial investigations of genistein and daidzein demonstrated that both of these compounds were nearly insoluble in $CDCl_3$. Methylated derivatives of these compounds were therefore used to determine **A** for the hydroxyl groups at positions 5, 7 and 4′ of isoflavone.

2.2. Materials

All isoflavones were obtained at a purity of 98% or greater from Indofine Chemical Company, Inc. These included 7-hydroxyisoflavone (7HIF), 5,7-dihydroxy-4′-methoxy-isoflavone (Biochanin A), 4′-hydroxy-7-methoxyisoflavone (Formononetin), and 5,4′-dihydroxy-7-methoxyisoflavone (Prunetin). The deuterated solvents, $CDCl_3$ and DMSO-d_6, were obtained in sealed ampoules from Sigma Aldrich and each was 99.9 atom %D and contained 0.03% TMS.

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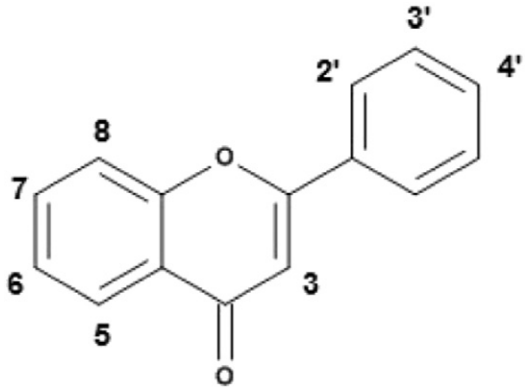

Fig. 1. Flavone.

3. Experimental

3.1. Instrument

A 300 MHz Bruker NMR Spectrometer equipped with field gradients and a jacketed probe regulated at 298 K (25 °C) was used for most measurements. Between 512 and 4096 transients were used to obtain high signal to noise ratios so that accurate values for chemical shift could be obtained. A Varian 400 MHz NMR spectrometer, also equipped with field gradients and a probe regulated at 298 K, was used for a titration assay of Biochanin A in the entire binary solvent system of $CDCl_3$/DMSO-d_6.

3.2. Methods

For each experiment, an aliquot of 1.0 mg of the isoflavone of interest was transferred to each of two 1.5 mL microcentrifuge tubes (snap-cap type). The two tubes were placed with caps open into a vacuum dessicator for an overnight period to remove adsorbed water. The dessicator was transferred to a glove box purged with dry nitrogen. The first sample was prepared by adding 1.0 mL of $CDCl_3$ (from a sealed ampoule) to the microcentrifuge tube which was then capped and agitated with a vortex mixer. The solution was transferred to an NMR sample tube with a screw-cap and Teflon seal to prevent contamination by water vapor. The second sample was prepared by adding 1.0 mL DMSO-d_6 (from a sealed ampoule) to the remaining microcentrifuge tube containing 1.0 mg of the isoflavone. The tube was capped and agitated using a vortex mixer and the solution was then transferred to an NMR sample tube with screw-cap and Teflon seal. The NMR sample tubes were removed from the glove box and then briefly heated at 35 °C to insure dissolution of the isoflavone. In the case of prunetin, a sonicating water bath was required to completely dissolve the solid sample in $CDCl_3$.

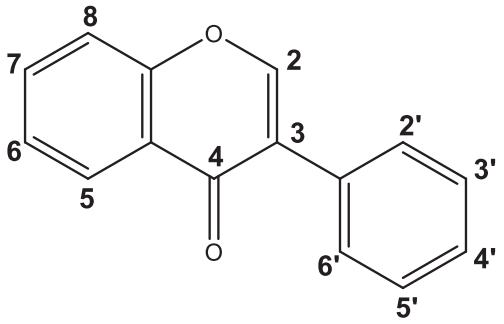

Fig. 2. Isoflavone.

The sample tubes were allowed to cool to room temperature (21 °C) before transferring them into the NMR spectrometer probe.

From previous work (10) it was found that sufficiently narrow signals for the isoflavone hydroxyl proton could be obtained by minimizing the H_2O contamination. The concentration of each isoflavone was approximately 4 mM in all samples and the integrated signal (singlet) for the hydrogen atom at position 2 was used as a quantitation standard. The samples with an isoflavone dissolved in $CDCl_3$ contained approximately 13 mM $CHCl_3$ (due to incomplete deuteration) and the integrated signal at 7.24 ppm was used as a quantitation standard. In spectra obtained using an appropriate acquisition delay (5 X T_1) the amount of H_2O present as a contaminant was between 5 mM and 10 mM. For samples prepared in $CDCl_3$, this was critical for obtaining signals sufficiently narrow to allow accurate chemical shift determination for the hydroxyl proton signal. In samples prepared in DMSO-d_6 the concentration of H_2O was approximately 10 mM. In a few samples prepared with DMSO-d_6, H_2O contamination was as high as 25 mM and this increased the linewidth of the isoflavone hydroxyl proton signal. In contrast, the chemical shift was not changed significantly by the presence of 25 mM H_2O.

Three separate experiments were performed for each compound and the three resulting values of **A** were averaged. The variation in chemical shift values accounted for an uncertainty of less than 0.01 in the value of A when the same NMR spectrometer was used. This was much lower than the reported uncertainty for using the published correlation used to calculate **A** ($\sigma = 0.05$). For each compound, chemical shifts were obtained in $CDCl_3$ and DMSO-d_6, and values of **A** were calculated from Eqs. (1) and (2) as described before [10]. The estimated error in **A** is about +/− 0.05.

$$\delta\Delta = \delta(\text{DMSO}) - \delta(CDCl_3) \quad (1)$$

$$\mathbf{A} = 0.0065 + 0.133\,\delta\Delta \quad (2)$$

When using different spectrometers to examine Biochanin A, differences in measured values of A were as great as 0.1 units. This was most likely due to slight differences in probe temperature calibration. The Bruker 300 MHz NMR had been recently installed with probe temperature calibrated by service personnel and therefore the values of A for each isoflavone were determined with this spectrometer.

With each isoflavone, a complete investigation of the binary system $CDCl_3$/DMSO-d_6 was made using a titration assay. Double reciprocal plots of $1/(\delta_o - \delta_f)$ versus $1/[D]_o$ were used to estimate the value of the association constant (K) for the binding of DMSO-d_6 to the isoflavone. The value of K was used to calculate the solute hydrogen bonding acidity parameter, α_2^H, so that it could be compared to A. This comparison was used as an assessment of whether self-association of the isoflavone was occurring. The details of this assay and theoretical arguments for using this approach to detect self-association were previously published [10]. For the Biochanin A position 7 hydroxyl group, a plot of chemical shift versus mole fraction of DMSO-d_6 has been provided in the Supporting Information. The graph exhibited a classic binding curve and similar results were obtained for the other isoflavones. A double reciprocal plot that was used to obtain the value of the association constant (K) between Biochanin A (7-OH) and DMSO-d_6 has also been included in the Supporting Information. Similar plots were made for the other isoflavones to obtain estimates of K and α_2^H.

When a hydroxyl group is engaged in an intramolecular hydrogen bond, it has the effect of reducing the overall polarity of the molecule and increasing the solubility in non-polar solvents. Monohydroxyflavones with hydroxyl groups at positions 6, 2′, 3′ and 4′ were insoluble in $CDCl_3$. Therefore, dihydroxyflavones with one hydroxyl group at position 3 or 5 and the other hydroxyl group at another position (6, 2′, 3′ or 4′) were used to determine A. As previously reported [10], these dihydroxyflavones each exhibited separate ^{1}H NMR signals for the two hydroxyl groups throughout the binary $CDCl_3$/DMSO-d_6 solvent