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# Structure Determination of Flavonoids Isolating from *Nymphaea tetragona* using NMR spectra and spin simulations

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**Abstract** *Nymphaea tetragona*, also known as waterlilies, is aquatic plant in the family of Nymphaeceae. Three flavonoids(**3**, **4**, and **5**) and one mixed flavonoids were separated from this plant. The mixed flavonoid consisted of two flavonoids; a well-known quercetin(**1**) and a new natural flavonoid(**2**). The latter also has two chiral centers and their configurations were established by ROESY experiment. Two glycoflavonoids were determined as isoquercetin and quercetin-3-*O*-β-xyropyranosyl-(1→2)-β-galactopyroside. The $^1$H NMR spectra for **4** and **5** dissoloved in DMSO-$d_6$ solvent showed resonance proximity and and severely overlap in the glycoside region, hindering the determination of the configurations of the stereogenic centers of the sugar moieties. This problem was solved through the spin simulation. Here, the exact NMR parameters for the sugar moieties of **4** and **5** were listed.

**Keywords** *Nymphaea tetragona*; quercetin; isoquercetin; 1D and 2D NMR; spin simulation

## Introduction

*Nymphaea tetragona* is aquatic plant in the family of Nymphaeaceae, commonly known as waterlilies. *N. tetragona* is widely distributed in Korea, especially abundant around Jeju Island. In traditional medicine, the flowers of *N. tetragona* has been used to treat fever, the roots for lung disease including asthma, and the aerial parts of this plant has been applied in the treatment of anxiety, diarrhea and ulcer.

*N. tetragona* has being paid increasing attention as natural active ingredients for anti-aging cosmetics. The extract of this plant showed the potent radical scavenging, elastase inhibition and MMP-1 expression inhibition activities in screening assay for native plants in Jeju Island.[1] The extract of *N. tetragona* roots could protect human epidermal keratinocytes against UVB-induced mitochondria-mediated apoptosis by regulating ROS-eliminating pathways.[2] Also, *N. tetragona* showed antimicrobial effect through modulating quorum sensing-mediated virulence factors of bacteria.[3,4]

Antioxidant property and hematopoietic repair capacity are important characteristics of radioprotective agents.[5] Naturally occurring antioxidants such as geraniin and 1,2,3,4,6-penta-*O*-galloyl-β-*D*-glucose (PGG), the major constituents of *N. tetragona*, are of interest in the search for effective and nontoxic compounds with radioprotective ability. Geraniin has been reported to possess protective effects against radiation-induced cell damage. Geraniin down regulated gamma radiation induced apoptosis by suppressing DNA damage.[6,7] Also, geraniin promoted recovery of hematopoietic cells after radiation exposure.[8] PGG protected splenocytes against radiation-induced apoptosis in murine splenocytes,[5] and attenuated $H_2O_2$-induced cell damage via antioxidant properties.[9]

In this paper, we report the isolation and structure

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determination of two mixed compounds and two flavonoid glycosides from the methanolic extract of *N. tetragona* (**Figure 1**). Among them, compound **2** was identified as a new natural product derived from **2**. The interpretation of 1D and 2D NMR spectra completely led to the NMR assignments for all isolated compounds.

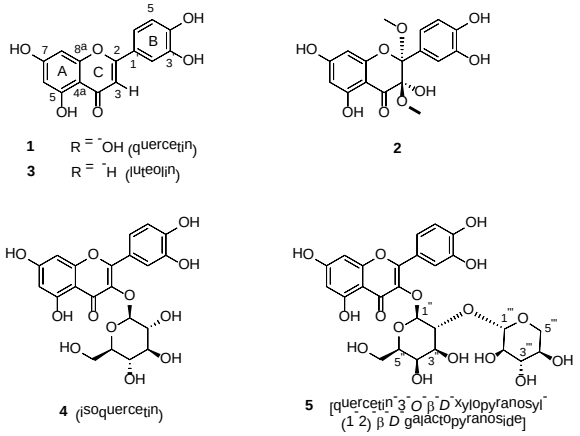


**Figure 1**. Four flavonoids isolated from N.tetragona

## Experimental Methods

***General Experimental*** - All NMR spectra were measured on a Varian VNMRS 500 spectrometer using DMSO-$d_6$ NMR solvent. The ESI mass spectra were acquired using an ABSCIEX QTRAP 3200 instrument. HPLC was performed using a Varian Prostar system with a 355 refractive index (RI) detector or Agilent 1200 Chemstation with DAD detector. The separation was performed using the YMC ODS-A column. All solvents were distilled prior to use.

***Plant material*** – *N. tetragona* were collected from the Medicinal Plant Garden, Seoul National University, Korea. Plant identification was authenticated by Dr. Min Hye Yang, Pusan National University, Korea. A voucher specimen (GNP-81) has been deposited in the Laboratory of Pharmacognosy, Gyeongnam National University of Science and Technology, Korea.

***Extraction and Isolation*** - The freeze-dried *N. tetragona* (1.2 kg) was powdered, and extracted three times with 80%-methanol for 3 h each in an ultrasonic apparatus. Removal of the solvent in *vacuo* yielded a methanolic extract (126.8 g). The methanolic extract was then suspended in distilled water and partitioned successively with *n*-hexane (18.2 g), chloroform (20.7 g), ethyl acetate (7.0 g), and *n*-butanol (21.3 g). The ethyl acetate and *n*-butanol soluble fractions that showed potent antioxidant activities in DPPH assay were subjected to column chromatography. The ethyl acetate fraction was subjected on a silica gel column using mixtures of ethyl acetate– methanol of increasing polarity as eluents to give 15 fractions (E1~15). E2 was further subjected to ODS gel column chromatography with a gradient elution of methanol– water (10→100% MeOH) to give 30 subfractions (E2-1~30). E2-24 was subjected to column chromatography on Sephadex LH-20 (MeOH) to give 10 subfractions (E2-1-24-1~10). E5-1-24-7 was further applied to C18 RP preparative HPLC to yield the mixture of compounds **1** and **2**. Compound **3** was obtained from E5-1-24-5. E4 subjected to ODS gel column chromatography with a gradient elution of methanol– water (30→50% methanol) to give 16 subfractions (E4-1~16). Compound **4** was obtained from E4-5 through recrystallization in methanol. The *n*-butanol fraction was subjected on a silica gel column using mixtures of chloroform– methanol– water of increasing polarity as eluents to give 40 fractions (C1~40). C27 was further subjected on a silica gel column using mixtures of chloroform– methanol– water of increasing polarity as eluents to give 8 subfractions (C27-1~8). C27-3 was subjected to column chromatography on Sephadex LH-20 (MeOH) to give 12 subfractions (C27-3-1~12). Compound **5** was isolated from C27-3-1~10.

## Results and Discussion

Five compounds, including two glycoflavonoids (**4** and **5**) were obtained from ethyl acetate and *n*-butanol soluble fractions of *N. tetragona* extract (**Figure 1**). Among these, compounds **1** and **2** were given as a mixture to a 3:2 ratio. The chemical structures of all compounds were determined by analyses of the 1D and 2D NMR spectra dissolved in

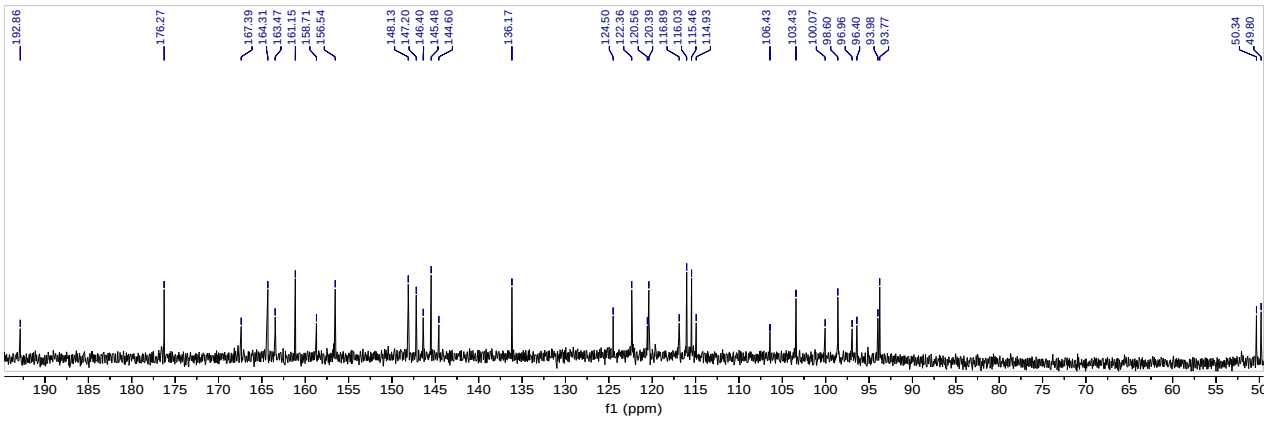


**Figure 2**. $^{13}$C NMR spectra of the mixture of **1** and **2**.

DMSO-$d_6$ solvent, along with their mass data. Furthermore, exact structure determination and the $^{1}$H assignments for **4** and **5** were performed through the spin analysis with Mnova NMR software (v. 12.0.3, Mestrelab Research S. L)

The mixture of compounds **1** and **2** showed two molecular ion $[M + Na]^+$ peaks at *m/z* 325 and 387 in the ESI-MS. The carbons in the mixture was observed to be 32 in the $^{13}$C NMR spectrum, composed of 30 in the range of 90 to 200 ppm and two in *ca* 50 ppm. As shown in **Figure 2**, the carbon resonances could be interestingly classified as high- and low-intensity signals. Compound **1** related with the high-intensity signals was identified to be quercetin, a well-known flavonoid, by analysis of 1D and 2D NMR spectra. The carbon chemical shifts were highly matched with those reported in the literature within ~ 1.2 ppm (**Table 1**).[10] On the other hands, the remaining 17 carbon signals also suggested to be a flavonoid (**2**) on the basis of the carbon chemical shifts, but the carbonyl carbon at 192.4 ppm and the two methoxy carbons at 49.4 and 49.9 ppm are not common signals in flavonoids. Rings A and B of **2** were determined to be equal to those of **1** from the correlations in the COSY and HMBC spectra and comparison with the corresponding carbon chemical shifts. Following this information, unassigned carbons to be pertained to ring C consisted of the two methoxy groups, the carbonyl carbon and two monporotonated carbons. Ring C of compound **2** was readily determined by the HMBC correlations with the protons ($\delta_C$ 106.0 / $\delta_H$ 6.84 and 7.01) in ring B and 3-OH ($\delta_C$ 192.4, 93.6, 106.0 / $\delta_H$ 7.04), as well as the two methoxy methyl protons ($\delta_C$ 93.6 / $\delta_H$ 2.87; $\delta_C$ 106.0 / $\delta_H$ 2.93. The determined compound **2** has been reported as one of the chemical reaction products of quercetin and DPPH radical in methanol, which was detected by mass spectrometer.[11] Here, we established the chemical structure of this compound by the NMR method for the first time. Moreover, this compound featured to possess the two chiral centers (C-2 and C-3) in ring C. The relative orientation of each methoxy group in 2- and 3-position can be favorably deduced to be an antiparallel direction due to steric hindrance from the close placement of the two groups. This was also supported by the ROE correlations from the two methoxy protons with the protons at $\delta_H$ 6.84 and 7.01 in ring B as shown in the expanded ROESY spectrum (**Figure 3**).

Compound **3** had a molecular formula $C_{15}H_{10}O_6$, and proton and carbon chemical shifts similar to those of **2**. The analysis of the 1D and 2D NMR spectra revealed to be the structure of luteolin, which substituted the hydroxyl group in 3-position with hydrogen.

Compound **4** had the molecular formula of $C_{21}H_{20}O_{12}$ on the basis of the pseudo molecular ion $[M + H]^+$ at *m/z* 487 in the ESIMS and the carbon resonances in the $^{13}$C NMR spectrum. The $^{1}$H and $^{13}$C NMR spectra, measured in DMSO-$d_6$ solvent,

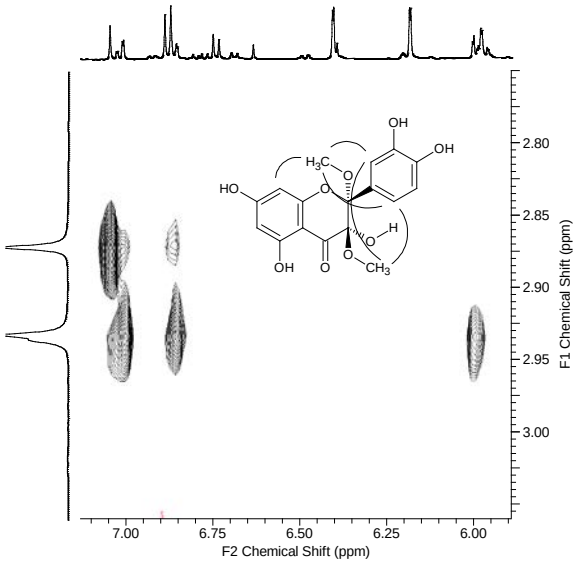


**Figure 3**. Key ROE correlations of **2**, indicating the relative orientation of the two methoxy groups.

showed a similiarity to those of compound **1**, quercetin, but contained additional signals corresponding to a sugar moiety (**Figure 4**). The hydroxyl protons in the $^1$H NMR signals of **4** are so broad that they were ignored in the determination of structure. Very similarly, the proton resonances of the sugar were also observed to be broad and severely overlapped. The assignment for the protons in the sugar was attempted by the COSY and TOCSY correlations, but resonance proximity confused to assign the exact proton signals. Moreover, broad lines hindered to measure the coupling constants between protons. The connection of the sugar moiety with quercetin was identified by the HMBC correlation from the anomeric proton at $\delta_H$ 5.47 (H-1″) to the monprotonated carbon $\delta_C$ 133.3 (C-3) in ring C. From this information, the planar structure of **4** was established.

Next step is to define the configuration of the protons in the pyranosyl unit for assigning the type of

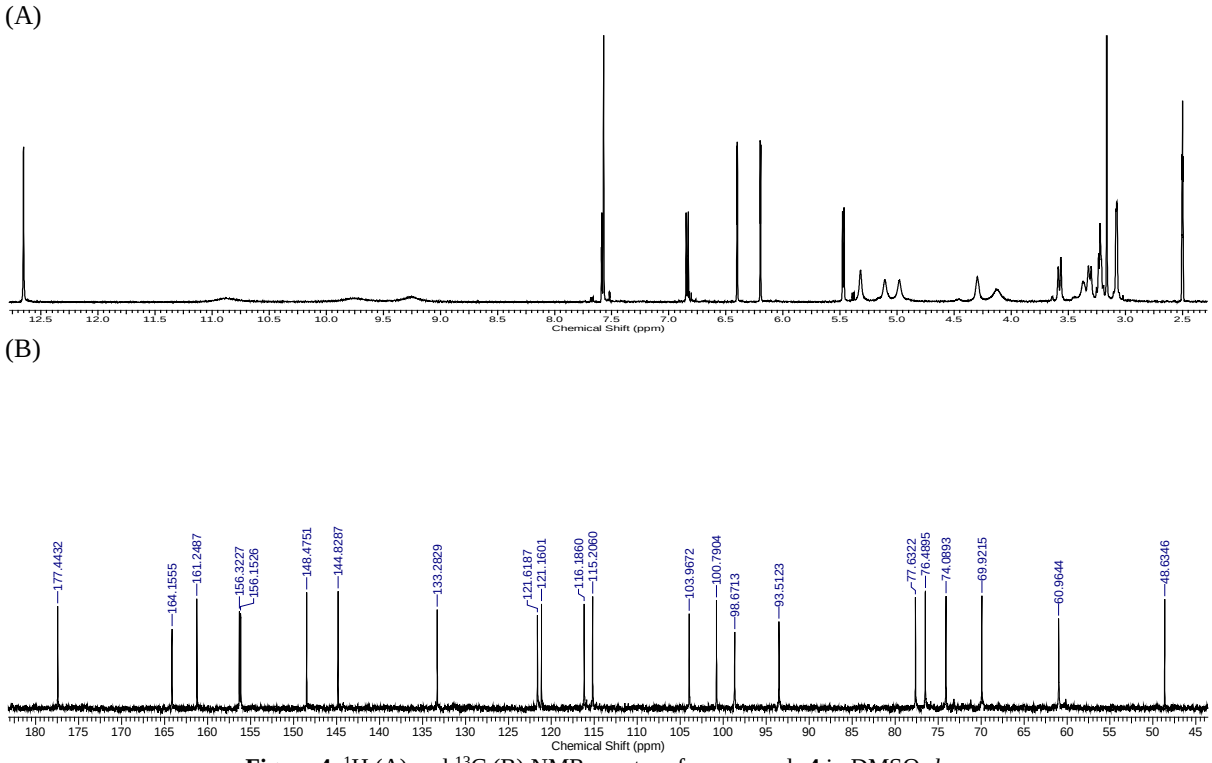


**Figure 4**. $^1$H (A) and $^{13}$C (B) NMR spectra of compounds **4** in DMSO-$d_6$.

sugar. In general, the relative configuration of sugar protons can be carried out from the coupling constants and/or NOE correlations between the protons in the pyranose ring. In this case, with the exception of the large coupling constant ($J_{HH}$ = 7.2 Hz) of the anomeric proton for the indication of β-form, any coupling constants for the configuration of the sugar could not be measured. Instead, the ROESY experiment was conducted to determine the relative orientation of the protons. From this spectrum, only two ROE correlations of H-1″/H-3″ and H-1″/H-5″ was obviously observed (**Figure 5**). On the basis of these information and the coupling constant of the anomeric proton, the configuration of the four protons (1″, 2″, 3″, and 5″) was established, but the configuration of H-4″ was still not clarified by the coupling constants or the ROE correlations.

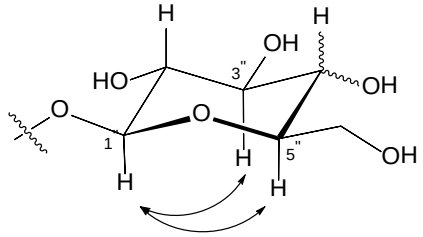


**Figure 5**. The ROE correlations observed in the pyranosyl moiety of **4**.

The configuration of H-4 was solved by spin simulation, which optimizes the NMR parameters to fit the observed $^1$H NMR spectrum. A wide variety of NMR processing softwares allows to present the simulated spectrum using the chemical shift ($\delta_H$) and coupling constant (*J*) values. Using Mnova software, the $^1$H spectra for the sugar moiety could be simulated depending on the input NMR parameter ($\delta_H$ and *J*) values. In the course of the simulation, the initial values of the chemical shifts for broad or overlapped signals were assessed from the COSY or HSQC spectra. Furthermore, the preliminary coupling constants were adopted as each trial value in the range of 2 ~ 5 Hz for *gauche* configurations and 7 ~ 10 Hz for *trans*, together with the observable coupling constants in the resolution-enhanced $^1$H spectrum. By comparing the calculated spectra with the observed one, the NMR parameters corresponding to *d* and *J* values were iteratively adjusted until giving optimum similarity between the two spectra. **Figure 6** shows the simulated spectrum (up) calculated by the optimized parameters and the observed $^1$H NMR spectrum (down) for the sugar moiety of **4**.

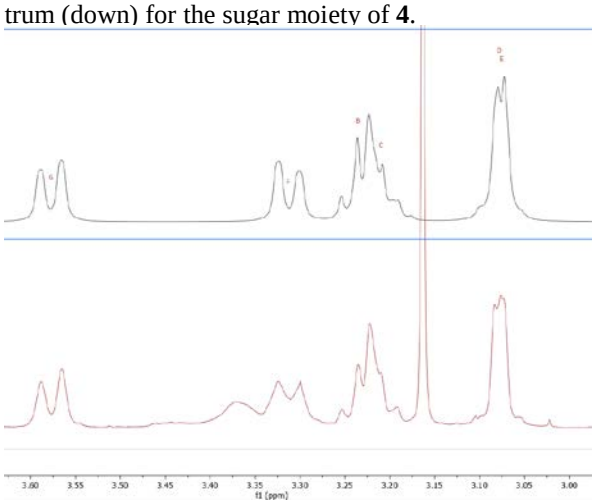

**Figure 6**. High similarity between simulated $^1$H NMR spectrum (up) and the observed spectrum (down) of **4**

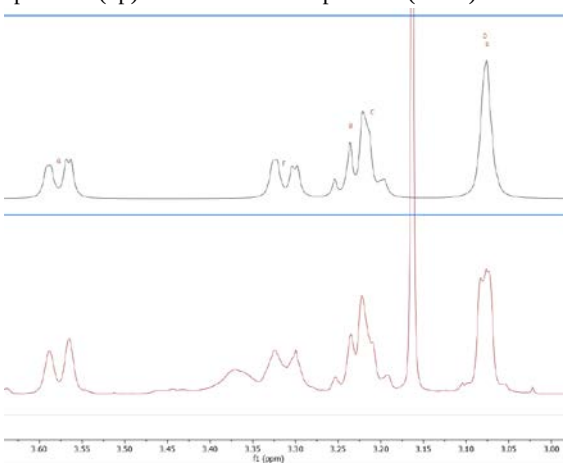

**Figure 7**. Difference between simulated $^1$H NMR spectrum (up) and the observed spectrum (down) of **4**

**Table 2** listed the 1H chemical shifts and the coupling constants obtained from this simulated process. Compared with the spectral data of 4 in Table 1, those in Table 2 were exact and informative for the (A) determination of the configuration of protons in the sugar. Specifically, the coupling constants of the critical H-4″ in the determination of sugar type was calculated as large value, indicating a glucopyranosyl moiety. Accordingly, compound 4 was determined to

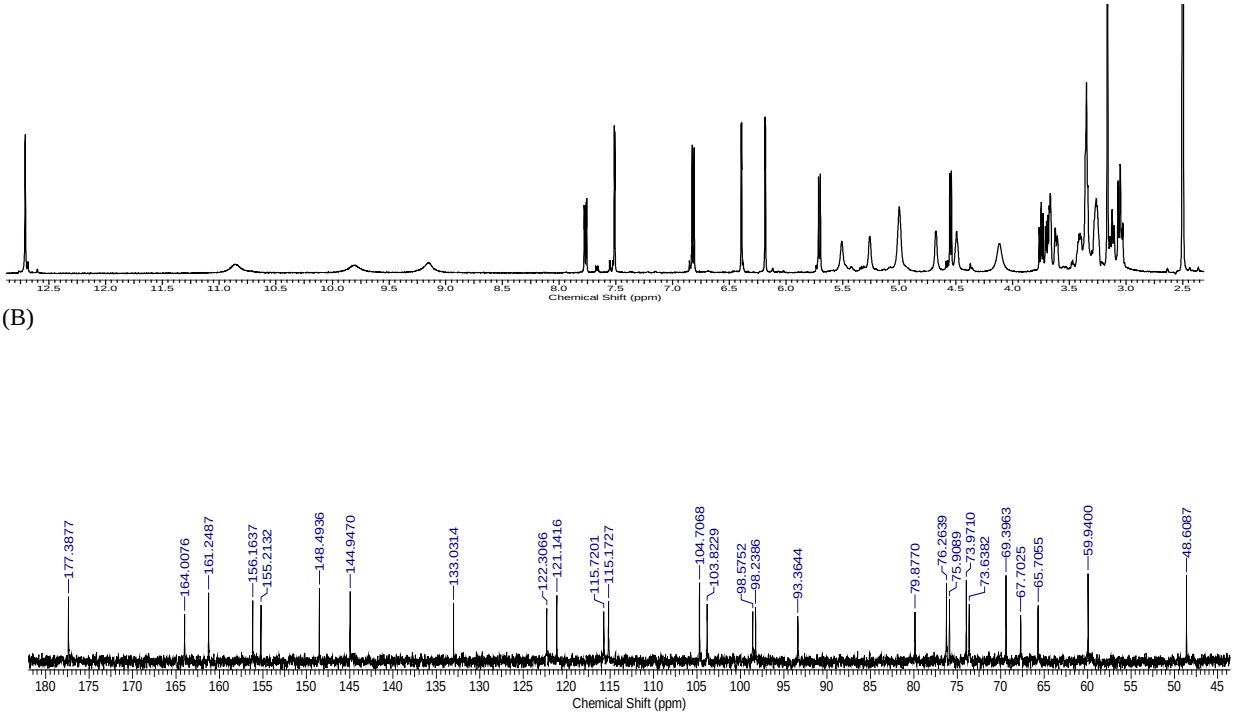


**Figure 8**. $^{1}$H (A) and $^{13}$C (B) NMR spectra of compounds **5** in DMSO-$d_6$.

be isoquercetin. If the coupling constants (*ca* 3 Hz) of H-4″, corresponding to galactopyranosyl moiety, are small, the predicted $^{1}$H spectrum will be predicted as presented in **Figure 7**, whose the lineshape in $\delta_H$ 3.05 ~ 3.10 region and splitting patterns are different from those in the observed spectrum.

Compound **5** had the molecular formula $C_{26}H_{28}O_{16}$ on the basis of the pseudo molecular ion $[M + Na]^+$ at *m/z* 619 in the ESIMS and the carbons in the $^{13}$C NMR spectrum. As the previous compounds **1** and **4**, compound **5** also had the same quercetin skeleton based on the 1D and 2D NMR interpretation (**Figure 8**). The $^{1}$H NMR spectrum showed the signals for two anomeric protons ($\delta_H$ 5.70, d, $J$ = 7.7 Hz; $\delta_H$ 4.55, d, $J$ = 7.3 Hz). Careful examination of cross peaks in the COSY and TOCSY spectra led to assign the two sugar moieties. Similar to compound **4**, signal overlap and broadening in the sugar moiety frustrated the determination of all stereogenic centers. Two sugars were connected as (1→ 2) form by the HMBC correlation between $\delta_H$ 4.55 / $\delta_C$ 79.9. This substructure was revealed to link to 3-*O* in quercetin from the HMBC cross peak of $\delta_H$ 5.70 / $\delta_C$ 133.0. Based on the completed planar structure, the ROESY spectrum suggested the relative configuration of the sugar protons as shown in **Figure 9**.

Unlike the case of compound **4**, the sugars of compound **5** were designated as galactopyranose and xylopyranose, respectively, from the ROE correlation. Accordingly, compound **5** was named as quercetin-3-O-β-xylropyranosyl-(1→2)-b-galactopyranose). In the same way as compound **4**, the exact NMR chemical shifts and coupling constants of the sugar part of compound **5** could also be calculated by the spin simulation. **Figure 10** shows the good match between the simulated NMR spectrum (up) and the observed NMR spectrum (down) of **5**. **Table 2** summarized the exact chemical shift and coupling constants for the sugar part.

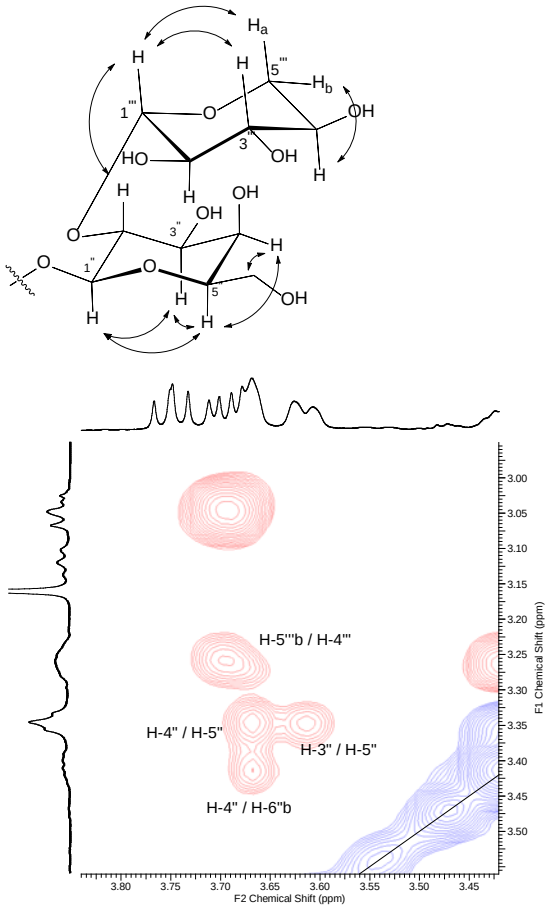


**Figure 9**. Key ROE correlations observed in the pyranosyl moiety of **5**.

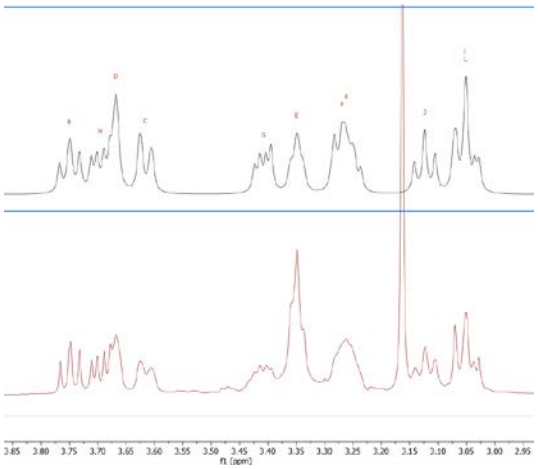

**Figure 10**. Similarity between simulated $^{1}$H NMR spectrum (up) and the observed spectrum (down) of **5.**

## Conclusion

In this study, the NMR data for five flavonoids were presented. Especially, the exact chemical shifts and coupling constants for the sugar part in isoquercetin and quercetin-3-O-xylopyranosyl-(1→20-galactopyranoside) was calculated through the spin simulation based on the assessment of the initial chemical shifts from the COSY or HSQC spectra. The use of DMSO-$d_6$ solvent ensures a high stability of the chemical shifts at different concentration of compounds, but causes large deviations in NMR spectrum at temperature, pH and also line broadening. Occasionally, sugar analysis by using NMR method might be blocked by the line broadening or resonance proximity. In this case, the spin simulation can be one of the solutions for determining the configuration by means of the coupling constants.

**Table 1**. $^{1}H$ and $^{13}C$ NMR spectral data for compounds **4** and **5** in DMSO-$d_6$ recorded at 500 MHz and 125 MHz

| | **1 (quercetin)** | | **2** | | **3 (luteolin)** | |
|---|---|---|---|---|---|---|
| no | $\delta_{H,}$ mult (Hz) | $\delta_C$ | $\delta_{H,}$ mult (Hz) | $\delta_C$ | $\delta_{H,}$ mult (Hz) | $\delta_C$ |
| 2 | | 146.8, C | | 106.0, C | | 163.9, C |
| 3 | | 135.8, C | | 93.6, C | 6.67, s | 102.8, CH |
| 4 | | 175.8, C | | 192.4, C | | 181.6, C |
| 4a | | 103.0, C | | 99.6, C | | 103.6, C |
| 5 | | 160.7, C | | 163.0, C | | 161.5, C |
| 6 | 6.18, d(2.0) | 98.2, CH | 5.97, d(1.8) | 96.5, CH | 6.18, d(1.5) | 98.9, CH |
| 7 | | 163.9, C | | 167.0, C | | 164.3, C |
| 8 | 6.40, d(2.0) | 93.4, CH | 5.99, d(1.8) | 96.0, CH | 6.44, d(1.5) | 93.9, CH |
| 8a | | 156.1, C | | 158.3, C | | 157.3, C |
| 1' | | 121.9, C | | 124.1, C | | 121.4, C |
| 2' | 7.67, d(2.2) | 115.0, CH | 7.01, d(2.1) | 116.5, CH | 7.39, d(1.8) | 113.3, CH |
| 3' | | 145.1, C | | 144.2, C | | 145.8, C |
| 4' | | 147.7, C | | 146.0, C | | 149.8, C |
| 5' | 6.87, d(8.4) | 115.6, CH | 6.74, d(8.3) | 114.5, CH | 6.88, d(8.4) | 116.0, CH |
| 6' | 7.53, dd(8.4, 2.2) | 120.0, CH | 6.84, dd(8.3, 2.1) | 120.1, CH | 7.41, dd(8.4, 1.8) | 119.0, CH |
| 2-$OCH_3$ | | | 2.93, s | 49.9, $CH_3$ | | |
| 3-$OCH_3$ | | | 2.87, s | 49.4, $CH_3$ | | |
| 3-OH | | - | 7.04, s | - | | |

**Table 1**. $^{1}H$ and $^{13}C$ NMR spectral data for compounds **4** and **5** in DMSO-$d_6$ recorded at 500 MHz and 125 MHz (Continued)

| | **4 (isoquercetin)** | | **5** | |
|---|---|---|---|---|
| no | $\delta_H$, mult (Hz) | $\delta_C$ | $\delta_H$, mult (Hz) | $\delta_C$ |
| 2 | | 156.2, C | | 155.2, C |
| 3 | | 133.3, C | | 133.0, C |
| 4 | | 177.4, C | | 177.4, C |
| 4a | | 104.0, C | | 103.9, C |
| 5 | | 161.3, C | | 161.3, C |
| 6 | 6.20, d (1.8) | 98.7, CH | 6.18, d (2.0) | 98.6, CH |
| 7 | | 164.2, C | | 164.0, C |
| 8 | 6.40, d (1.8) | 93.5, CH | 6.39, d (2.0) | 93.4, CH |
| 8a | | 156.3, C | | 156.2, C |
| 1' | | 121.2, C | | 121.1, C |
| 2' | | 148.5, C | 7.51, d (2.2) | 115.7, CH |
| 3' | 6.84, d (8.2) | 115.2, CH | | 145.0, C |
| 4' | 7.58, dd (8.2, 2.2) | 121.6, CH | | 148.5, C |
| 5' | | 144.8, C | 6.82, d (8.4) | 115.2, CH |
| 6' | 7.57, d (2.2) | 116.2, CH | 7.77, dd (8.4, 2.2) | 122.3, CH |
| 1" | 5.47, d (7.2) | 100.8, CH | 5.70, d (7.7) | 98.2, CH |
| 2" | 3.24, m | 74.1, CH | 3.75, dd (7.7, 9.3) | 79.9, CH |
| 3" | 3.21, m | 76.5, CH | 3.61, br d (9.3) | 73.6, CH |
| 4" | 3.08, m | 69.9, CH | 3.67, br s | 67.7, CH |
| 5" | 3.08, m | 77.6, CH | 3.35, br s | 75.9, CH |
| 6" | a 3.31, br d(11.4)<br>b 3.58, br d (11.4) | 61.0 $CH_2$ | a 3.265, m<br>b 3.41, dd (10.0, 4.7) | 59.9, $CH_2$ |
| 1"' | | | 4.55, d (7.3) | 104.7, CH |
| 2"' | | | 3.06, m | 74.0, CH |
| 3"' | | | 3.12, t (8.6) | 76.3, CH |
| 4"' | | | 3.258, m | 69.4, CH |
| 5"' | | | a 3.04, m<br>b 3.69, dd (11.3, 5.1) | 65.7, $CH_2$ |

**Table 2**. The NMR spectral data obtained by spin simulation process for sugar moieties for compounds **4** and **5**

| | **3 (calculated)** | | **4 (calculated)** | |
|---|---|---|---|---|
| no | $\delta_{H,}$ mult (Hz) | $\delta_C$ | $\delta_{H,}$ mult (Hz) | $\delta_C$ |
| 1" | 5.466, d (7.73) | 100.8, CH | 5.704, d (7.83) | 98.2, CH |
| 2" | 3.234, dd(7.73, 9.10) | 74.1, CH | 3.749, dd (7.83, 9.80) | 79.9, CH |
| 3" | 3.213, dd(9.10, 9.82) | 76.5, CH | 3.616, dd (9.80, 2.12) | 73.6, CH |
| 4" | 3.082, dd(9.82, 10.01) | 69.9, CH | 3.668, dd(2.12, 2.15) | 67.7, CH |
| 5" | 3.075, ddd(10.01,2.13,5.83) | 77.6, CH | 3.349, dd(2.12, 6.05) | 75.9, CH |
| 6" | a 3.313, dd(11.4, 5.83)<br>b 3.575, br d (11.4, 2.13) | 61.0 $CH_2$ | a 3.268, dd(10.03, 6.05)<br>b 3.408, dd (10.03, 4.65) | 59.9, $CH_2$ |
| 1"' | | | 4.546, d (7.45) | 104.7, CH |
| 2"' | | | 3.053, dd(7.45, 8.93) | 74.0, CH |
| 3"' | | | 3.123, dd(8.93, 9.70) | 76.3, CH |
| 4"' | | | 3.261, dd(9.70, 11.25) | 69.4, CH |
| 5"' | | | a 3.050, dd(11.25, 11.25)<br>b 3.695, dd (11.25, 5.14) | 65.7, $CH_2$ |

**Acknowledgements**

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**References**

1. Y. H. Kim, K. S. Kim, C. S. Han, H. C. Yang, S. H. Park, K. I. Ko, S. H. Lee, K. H. Kim, N. H. Lee, J. M. Kim, and K. H. Son, *J. Cosmet. Sci.* **58**, 19 (2007)
2. G. Park, Y. Sim, W. Lee, S H. Sung, and M. S. Oh, *Pharmacol.* **97**, 282 (2016)
3. M. A. Hossain, S. J. Lee, J. Y. Park, M. A. Reza, T. H. Kim, K. J. Lee, J. W. Suh, and S. C. Park, *J. Ethnopharmacol.* **174**, 482 (2015)
4. M. A. Hossain, J. Y. Park, J. Y. Kim, J. W. Suh, and S. C. Park, *Biomed. Res. Int.* **14**, 562 (2014)
5. S. J. Bing, M. J. Kim, E. Park, G. Ahn, D. S. Kim, R. K. Ko, N. H. Lee, T. Shin, J. W. Park, and Y. Jee, *Biol. Pharm. Bull.* **33**, 1122 (2010)
6. K. A. Kang, I. K. Lee, R. Zhang, M. J. Piao, K. C. Kim, S. Y. Kim, T. Shin, B. J. Kim, N. H. Lee, and J. W. Hyun, *Cell Biol. Toxicol.* **27**, 83 (2011)
7. S. J. Bing, D. Ha, M. J. Kim, E. Park, G. Ahn, D. S. Kim, R. K. Ko, J. W. Park, N. H. Lee, and Y. Jee, *Food Chem. Toxicol.* **57**, 147 (2013)
8. S. J. Bing, J. Cho, A. Kim, K. H. I. N. M. Herath, G. Ahn, N. H. Lee, J. W. Park, and Y. Jee, *Am. J Chin. Med.* **45**, 1003 (2017)
9. M. J. Piao, K. A. Kang, R. Zhang, D. O. Ko, Z. H. Wang, K. H. Lee, W. Y. Chang, S. Chae, Y. Jee, T. Shin, J. W. Park, N. H. Lee, and J. W. Hyun, *Food Chem.* **115**, 412 (2009)
10. E. Kyriakou, A. Primikyri, P. Charisadis, M. Katsoura, I. P. Gerothanassis, H. Stamatis, and A. G. Tzakos, *Org. Biomol. Chem.* **10**, 1739 (2012)
11. E. Hvattum, Y. Stenstorm, and D. Ekeberg, *J. Mass Spectrum*. **39**, 1581 (2004)