Food Chemistry 116 (2009) 575-579

Contents lists available at ScienceDirect

**Food Chemistry** 

journal homepage: www.elsevier.com/locate/foodchem

# Analytical Methods Identification of natural epimeric flavanone glycosides by NMR spectroscopy

Federica Maltese<sup>a</sup>, Cornelis Erkelens<sup>b</sup>, Frank van der Kooy<sup>a</sup>, Young Hae Choi<sup>a,\*</sup>, Robert Verpoorte<sup>a</sup>

<sup>a</sup> Division of Pharmacognosy, Section Metabolomics, Institute of Biology, Leiden University, The Netherlands <sup>b</sup> Division of NMR, Leiden Institute of Chemistry, Leiden University, The Netherlands

### ARTICLE INFO

Article history: Received 17 September 2008 Received in revised form 17 January 2009 Accepted 1 March 2009

*Keywords:* Flavanone Citrus fruits Stereoisomers NMR

## ABSTRACT

Recently advanced analytical technology has provided evidence of the existence of stereoisomers of many natural products. Particularly, flavanones which might have two different configurations at C-2 exist in many food additives, e.g., citrus fruits. In this study, the possible stereoisomers of flavanone glycosides were identified by NMR spectroscopy. Based on NMR spectra of common flavanone glycosides such as naringin, hesperidin, and neohesperidin, the two existing diastereomeric forms of the molecules could clearly be distinguished. The <sup>1</sup>H NMR resonances of two diastereomers of each flavanone glycosides investigated in this study were fully assigned with the assistance of diverse 2D NMR spectroscopy methods.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Flavonoids represent one of the largest plant secondary metabolite groups with more than 6500 reported compounds up to the year of 2000. Flavonoids are largely present in our daily life, ingested via fruits, vegetables, legumes, and medicinal herbs. The importance of this huge class of plant phenolic constituents lies in their wide range of biological and physiological roles, mostly attributed to health benefits such as antioxidant, anti-inflammatory, oestrogenic, antiviral, and chemopreventive properties (Harborne & Williams, 2000).

Flavonoids are grouped in several subclasses, such as anthocyanins, flavans, isoflavonoids, flavones, flavanones, flavonols, and catechines, all of them chemically characterized by a 2-phenylbenzopyran-4-one structure. In particular the class of flavanones is so widely occurring in citrus fruits that several authors have chosen natural flavanones as chemotaxonomic markers and for the identification of adulterated processed juices (Albach & Redman, 1969; Kefford, 1959; Ooghe, Ooghe, Detavernier, & Huyghebaert, 1994; Wistuba, Trapp, Gel-Moreto, Galensa, & Schurig, 2006).

Among all the other flavonoids, the subclass of flavanones presents the unique characteristic of possessing a chiral center at the C-2 position, which potentially leads to the existence of two stereoisomeric forms (Fig. 1). Moreover, like other flavonoids, flavanones generally possess sugars attached to various –OH groups. The resulting changes of configuration at the stereogenic centers of the molecule determine that flavanone glycosides may exist as mixtures of diastereoisomers.

The determination of the isomeric composition of natural products in food products has received great attention for a long time. Positive bioactivities might be lost or transformed into undesirable toxicity or inactivity by the formation of stereoforms of natural compounds (Marder et al., 2003). It is believed that the involvement of a manifold of enzymes in the biosynthesis of phytochemicals results in the highly stereoselective production of the final natural products. However, this monolithic pressumption is now being challenged. In fact, recent advances in modern analytical technology used for the reassessment of formerly described natural products structures have allowed the discovery of natural stereoforms. In many cases, compounds which were believed to be present as one specific stereoform in nature were reviewed and found to occur as stereoisomers. NMR spectroscopy has been recognized so far as the most important tool for the structural elucidation of flavonoids (Fossen & Andersen, 2006) and it is also increasingly used as an analytical tool for the evaluation of quality traits in foods (Le Gall, Puaud, & Colquhoun, 2001; Vogels et al., 1996). Together with its undisputed potential in structural elucidation, NMR has other advantages such as a quick sample preparation and short analysis time, and its total sample recovery. Additionally, it also allows the analysis of stereoisomeric mixtures. Gaffield, Lundin, Gentili, and Horowitz (1975) used <sup>1</sup>H NMR as supporting material to circular dichroism measurements for an estimation of the isomeric composition of naringin in grapefruit at different ripening stages. The study allowed the authors to conclude that the (2S)-isoform of naringin in grapefruit, the main form present in premature fruits, undergo to racemization in maturing grapefruit. The isomeric composition of commercial enzymatically modified naringin used as food additive was also analysed by Akiyama, Yamada, Yamada, and Maitani (2000) by means of <sup>1</sup>H NMR and <sup>13</sup>C





<sup>\*</sup> Corresponding author. Tel.: +31 71 527 4471; fax: +31 71 527 4511. *E-mail address:* y.choi@chem.leidenuniv.nl (Y.H. Choi).

<sup>0308-8146/\$ -</sup> see front matter @ 2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2009.03.023



**Fig. 1.** Chemical structures of naringenin (1), naringin (2), hesperetin (3), hesperidin (4), and neohesperidin (5).

NMR spectroscopy. In an investigation by using <sup>1</sup>H NMR of "aged" samples of hesperidin extracted from Spanish oranges Nieto and Gutierrez (1986) detected two sets of proton signals, assigned to both C-2 isomers of the compound.

In order to evaluate <sup>1</sup>H NMR spectroscopy as a tool to rapidly detect the isomeric composition of natural flavanone glycosides present in food, we investigated grapefruit peels, followed by a study of reference compounds. Based on the <sup>1</sup>H NMR results with assistance of two dimensional NMR experiments, full <sup>1</sup>H resonance assignments of isoforms of flavanone glycosides were elucidated.

#### 2. Materials and methods

#### 2.1. Plant material

Peels from grapefruits purchased on the market were reduced to small pieces and extracted using chloroform and a solution made of methanol and deionized water 1:1. After ultrasonication for 20 min at room temperature and centrifugation, the two phases were filtered and separated. The MeOH–water fraction was dried under vacuum and redissolved in deuterated solvent.

#### 2.2. Chemicals and reagents

All solvents used in NMR analysis were purchased from Cortec (Paris, France). The reference compounds naringegin (1), naringin (2), hesperetin (3), hesperidin (4), and neohesperidin (5) were obtained from Sigma–Aldrich (Steinheim, Germany).

#### 2.3. NMR measurements

<sup>1</sup>H NMR, APT, J-resolved, <sup>1</sup>H-<sup>1</sup>H COSY, TOCSY, <sup>1</sup>H-<sup>13</sup>C HMBC, <sup>1</sup>H-<sup>13</sup>C HSQC, and NOESY experiments were recorded at 600 MHz on a Bruker DMX-600 spectrometer. NMR spectra were recorded in dimethylsulfoxide- $d_6$  or methanol- $d_4$ : water-d (1:1), and chemical shifts were referred to the corresponding dimethylsulfoxide ( $\delta_{\rm H} 2.54/\delta_{\rm C} 39.50$ ), or methanol ( $\delta_{\rm H} 3.30/\delta_{\rm C} 49.00$ ) signals.

### 3. Results and discussion

Our study started with the investigation of grapefruit (*Citrus paradisi* L.) peels by <sup>1</sup>H NMR spectroscopy. The high amount of naringin (**2**) in the peels is clearly visible in the <sup>1</sup>H NMR spectrum of the MeOH–H<sub>2</sub>O (1:1) extract. Fig. 2 shows the characteristic <sup>1</sup>H NMR resonances of naringin in CH<sub>3</sub>OH- $d_4$ : D<sub>2</sub>O (1:1). <sup>1</sup>H NMR resonances of the major flavanone glycoside, naringin, were assigned using two dimensional NMR spectra such as J-resolved, COSY, and HMBC. A close observation of the <sup>1</sup>H and the COSY spectra of the grapefruit peel extract showed the presence of another flavanone-



**Fig. 2.** <sup>1</sup>H NMR spectrum of grapefruit peel extract in the range  $\delta$  7.5– $\delta$  5.9 (a) and  $\delta$  5.6– $\delta$  4.2 (b) in CH<sub>3</sub>OH-*d*<sub>4</sub>:D<sub>2</sub>O (1:1). Resonances of naringin are assigned as it follows. (1) H-1<sup>*ii*</sup> (Rha), (2) H-1<sup>*i*</sup> (Glc), (3) H-2, (4) H-1 from sucrose, (5) H-6 and H-8, (6) H-3' and H-5', (7) H-2' and H-6'.

like compound. The presence of a side signal close to the signal of H-6 and H-8 at  $\delta$  6.17, together with the splitting pattern of H-2 at  $\delta$  5.33 (m), indicated an isomer of the compound present in the extract. Due to the signal overlapping in the grapefruit extract, especially in the range of  $\delta$  3.0– $\delta$  5.0 of the spectrum, for a complete assignment of the proton resonances, the <sup>1</sup>H NMR spectrum was compared with pure naringin obtained from citrus fruit (min. 95%, Sigma-Aldrich). It is known that commercial flavanones and glycoside derivatives are mixtures of enantiomers or epimers (Caccamese, Manna, & Scivoli, 2003; Uchiyama, Kim, Kawahara, & Goda, 2005). When measuring the NMR spectra of commercial naringin the presence of an additional set of signals belonging to second flavanone was detected. Both compounds were characterized by the presence of an AMX system which is typical of a flavanone structure with resonances at  $\delta$  5.56 (dd, J = 13.2, 3 Hz, H-2),  $\delta$  2.77 (dd, J = 17, 3.2 Hz, H-3<sub>ax</sub>),  $\delta$  3.36 (dd, J = 17, 14 Hz, H-3<sub>eq</sub>), and  $\delta$  5.52 dd, J = 13, 3 Hz, H-2),  $\delta$  3.43 (dd, J = 16, 13 Hz, H-3<sub>ax</sub>),  $\delta$  2.73 (dd, J = 16, 3.4 Hz, H-3<sub>ea</sub>), respectively. The assignment of these resonances was performed on the basis of 1D and 2D NMR experiments (<sup>1</sup>H NMR, APT, J-resolved, TOCSY, COSY, HSQC, HMBC, NOESY). One compound was identified as naringin, while the second compound was concluded to be one of the diastereomeric form of naringin. The complete assignment of the spectral signals of the two structures is given in Table 1.

The NOE correlation between H-3<sub>eq</sub> at  $\delta$  3.36 and the signal of H-2' and H-6' at  $\delta$  7.31 indicated an  $\alpha$ -orientation of the B-ring, thus identifying the compound bearing H-2 at  $\delta$  5.56 as the (2*S*)-isoform of naringin.

Biogenetically, chalcones are the immediate precursors of flavanones. In plants, flavanones are generally present as levorotatory (2S)-isomers because the enzymatic reaction which catalyzes the conversion of chalcones to flavanones is highly stereospecific (Grayer & Veitch, 2006). The enzyme chalcone isomerase (CHI)

Table 1	l
---------	---

NMR spectroscopic data (600 MHz, DMSO-d<sub>6</sub>) for naringenin (1), naringin (2), hesperetin (3), hesperidin (4), and neohesperidin (5). Coupling constants are (*J* in Hz) in parenthesis. <sup>i</sup>Coupling constant(s) not indicated because of overlapping with other protons.

Position	tion Naringenin (1) Naringin (2)		Hesperetin ( <b>3</b> )	Hesperidin (4)		Neohesperidin ( <b>5</b> )		
		(2S)	(2R)		(2S)	(2R)	(2S)	(2R)
2	5.47 dd (12.6, 2.7)	5.56 dd (13.2, 3.4)	5.52 dd (13.0, 3.2)	5.46 dd (12.3, 3.0)	5.54 dd (7.8, 3.0)	5.52 dd (7.2, 3.0)	5.55 dd (12.6, 3.2)	5.50 dd (12.6, 2.4)
3 <sub>ax</sub>	2.71 dd (17.0, 3)	2.73 dd (17.0, 3.4)	3.43 dd (16.4, 13.0)	2.74 dd (17.2, 3.0)	2.79 dd (17.4, 3.0)	3.42 dd (16.5, 8)	2.80 dd (16.3, 3.2)	3.35 dd (16.8, 13)
3 <sub>eq</sub>	3.30 dd (17.0, 12.9)	3.36 dd (17.0, 13.2)	2.77 dd (16.4, 3.2)	3.23 dd (17.2, 12.3)	3.31 dd (17.3, 8.1)	2.80 dd (16.8, 3)	3.31 dd (16.3, 12.6)	2.77 dd (17.0, 2.4)
6	5.91 s	6.11 d (2.4)	6.08 d (2.4)	5.91 d (1.9)	6.18 d (2.4)	6.17 d (2.4)	6.15 d (2.4)	6.15 <sup>i</sup>
8	5.91 s	6.10 s	6.07 d (2.4)	5.92 d (1.9)	6.16 d (3)	6.15 s	6.12 d (2.4)	6.13 d (2.1)
2′	7.35 d (8.4)	7.31 d (8.4)	7.32 d (8.5)	6.95 d (1.6)	6.97 d (1.6)	6.98 d (1.6)	6.97 d (2)	6.98 d (2)
3′	6.82 d (8.4)	6.80 d (8.4)	6.79 d (8.4)					
5′	6.82 d (8.4)	6.80 d (8.4)	6.79 d (8.4)	6.97 d (8.2)	6.83 d (8.4)	6.83 d (8.4)	6.94 d (8.0)	6.93 <sup>i</sup>
6′ 4′-OMe	7.35 d (8.4)	7.31 d (8.4)	7.32 d (8.4)	6.90 dd (8.2, 1.6) 3.81 s	6.99 dd (7.9, 1.6) 3.81 s	6.98 <sup>i</sup>	6.91 dd (8.0, 1.8) 3.81 s	6.91 <sup>i</sup>
Glc-1″		5.17 d (8.2)	l I		5.01 d (7.7)		5.17 d (7.4)	
Rha-1‴		5.13 d (2.2)			4.55 d (2.9)		5.14 d (2.9)	
5‴-Me		1.19 d (6.6)			1.12 d (6.6)		1.21 d (6.4)	

catalyzes the cyclization of chalcone with an apparent 100,000:1 preference for the synthesis of the (S)-isomer over the (R)-isomer (Jez, Bowman, Dixon, & Noel, 2000). As already mentioned, studies on citrus fruits revealed that flavanones and flavanones-7-0-glvcosides are present as enantiomeric and epimeric mixtures, respectively, in different ratios depending on the maturity grade of the fruit (Caccamese et al., 2003; Gaffield et al., 1975; Gel-Moreto, Streich, & Galensa, 2001). More precisely, it was reported by Gaffield et al. (1975) that the (2S)-isoform of naringin in grapefruit, the main form present in premature fruits, tends to convert to the (2R)-form, reaching an approximate epimeric ratio of about 3:2 at fruit maturity. According to the authors, a possible explanation of the diasteromerization of flavanones must be found in the ring-opening of flavanones under basic conditions. This conversion would lead to the formation of the corresponding chalcones, which are unstable and rapidly recyclize to flavanones in a non-stereospecific manner (Miles & Main, 1989). This chemical isomerization is also easily found in vitro, and it can also occur in vivo to the extent that moderate levels of anthocyanin can be formed (Heller & Forkmann, 1988). Moreover, flavanones are commonly synthesized via a Claisen-Schmidt condensation between 2-hydroxyacetophenone and a benzaldehyde and subsequent isomerisation of the 2'-hydroxychalcone intermediate (Grayer & Veitch, 2006). Condensation as well as isomerisation is catalyzed by acids or bases in homogeneous media, and also through electrochemical transformation, photochemical cyclization, and thermal isomerisation (Choudary, Vijeender, & Reddy, 2005). With the objective of detecting any isomerisation, the thermal conditions of the NMR probe were changed. If it actually occurred it would result in a change in the ratio of the two diastereoisomers. As shown in Fig. 3, increasing the temperature during the NMR measurement did not produce any change in the molar ratio of the two isoforms of commercial naringin. Furthermore, the addition of  $D_2O$  to the samples did not cause any modification in the epimeric ratio, the change in H-2 signal possibly due to shifting or overlapping rather than to a difference in the epimeric ratio of the isoforms.

In the case of the aglycon of naringin (naringenin, **1**) the separation of stereoisomers was not obtained in <sup>1</sup>H NMR spectrum because naringenin has only one chiral center at C-2, and the two possible enantiomers can not be differentiated by NMR. By the attachment of sugar, the enantiomeric protons become diastereomeric and can give resolved NMR spectra. As a further confirmation, the NMR spectra of another two flavanone glycosides, hesperidin (**4**) and neohesperidin (**5**) and their corresponding aglycone, hesperetin (**3**) as reference compounds were measured. Similarly to naringin, the <sup>1</sup>H NMR spectra of hesperidin (**4**) and neohesperidin (**5**) were also characterized by the presence of the two diastereoforms of the compound, while only the signals



**Fig. 3.** Effect of different temperatures on H-2 signals (1 and 2) and anomeric signals of glucose (3) and rhamnose (4) from naringin in the <sup>1</sup>H NMR spectrum (in DMSO-*d*<sub>6</sub>). (a) at 25 °C, (b) at 65 °C, (c) at 75 °C; (d) after addition of D<sub>2</sub>O at 25 °C, (e) after addition of D<sub>2</sub>O at 65 °C, (f) after addition of D<sub>2</sub>O at 75 °C.



**Fig. 4.** H-2 signals in the <sup>1</sup>H NMR spectrum of neohesperidin for the (2*S*) isomer (a), and the (2*R*) isomer (b).

belonging to one single stereoisomer were present in the spectrum of hesperetin (data not shown). In the case of neohesperidin the <sup>1</sup>H NMR spectrum showed a bigger difference in the ratio of the two stereoisoforms of the molecule (Fig. 4), as compared to naringin with a much lower level of the (2*R*)-isoform. As previously reported the nonenzymatic chalcone formation is favoured in structures bearing a free 4'-OH group (Gaffield et al., 1975). Furthermore, Wistuba et al. (2006) detected the highest C-2 epimerization barrier for neohesperidin, which possesses a methoxy group in position 4', among several tested flavanones.

In addition to the results of the diastereomeric separation of flavanone glycosides, an interesting phenomenon was observed in the <sup>1</sup>H NMR spectrum of naringenin (**1**). At high temperatures a protonic exchange of H-6, and H-8 with the solvent takes place (Fig. 5). When measured at 90 °C, the signal of H-6, and H-8 at  $\delta$  5.91 of the spectrum of a DMSO- $d_6$  solution of naringenin kept overnight after addition of D<sub>2</sub>O, revealed the exchange with the deuterium of the solvent. The H/D exchange phenomenon has also been reported by Kolar (1971). The signals of H-6 and H-8 of some methylated and methoxylated flavonoid-like compounds dissolved in a solution of dioxane: $D_2O$  (3:1) exchanged with the deuterium of the solvent after heating at 95 °C prolonged for 16 h. According to the author the exchange reaction is a typical electrophilic aromatic substitution reaction being promoted by the Pyrex glass of the NMR tube. The kinetic and the reactivity differences between C-6 and C-8 toward H/D exchange was also studied by Kiehlmann, Lehto, and Cherniwchan (1988) on catechin derivatives in D2O at 90 °C. More recently, Jordheim, Fosse, Songstad, and Andersen (2007) studied the exchange H/ D reaction of anthocyanin molecules in acidified methanolic or aqueous solution. The reaction slowly takes place when the samples are kept for eight days in darkness at room temperature.

### 4. Conclusions

It is generally accepted that plants metabolites are produced in a stereospecific way because of the involvement of enzymes in many biosynthetic steps. However, diverse stereoisomers of the same compound may exist in nature, and thus in food products, as side-products of a certain enzyme or after chemical conversion. In this study, NMR spectroscopy allowed distinguishing stereoisomers and made it possible to identify the two epimeric forms of flavanone glycosides. The results obtained from this study show the importance of carefully checking the chemical structures of natural products in terms of their stereochemistry.



**Fig. 5.** <sup>1</sup>H NMR spectrum of naringenin in the range  $\delta$  7.5– $\delta$  5.0. Sample was dissolved in DMSO- $d_6$ , D<sub>2</sub>O was added and measured at 90 °C (a) after 3 h and (b) after 12 h. The signals are assigned as follows: (1) H-2, (2) H-6 and H-8, (3) H-3' and H-5', (4) H-2' and H-6'.

#### Acknowledgments

The authors would like to acknowledge Ms. E.G. Wilson for kindly revising the manuscript. The Post-doctoral Fellowship Program of Marie Curie Foundation (MEIF-CT-2005-023788) is gratefully acknowledged for the financial support to F.M.

#### References

- Akiyama, T., Yamada, M., Yamada, T., & Maitani, T. (2000). Naringin glycosides αglucosylated on ring B found in the natural food additive, enzymatically modified naringin. *Bioscience, Biotechnology and Biochemistry*, 64, 2246–2249.
- Albach, R. F., & Redman, G. H. (1969). Composition and inheritance of flavanones in citrus fruit. *Phytochemistry*, 8, 127–143.
- Caccamese, S., Manna, L., & Scivoli, G. (2003). Chiral HPLC separation and CD spectra of the C-2 diastereomers of naringin in grapefruit durino maturation. *Chirality*, 15, 661–667.
- Choudary, B. M., Vijeender, K., & Reddy, V. K. (2005). New synthesis of flavanones catalyzed by I-proline. *Tetrahedron Letters*, 46, 6991–6993.
- Fossen, T., & Andersen, Ø. M. (2006). Spectroscopic techniques applied to flavonoids. In Ø. M. Andersen & K. R. Markham (Eds.), *Flavonoids: Chemistry, biochemistry and applications* (pp. 37–142). Boca Raton, FL: CRC Press.
- Gaffield, W., Lundin, R. E., Gentili, B., & Horowitz, R. M. (1975). C-2 stereochemistry of naringin and its relation to taste and biosynthesis in maturing grapefruit. *Bioorganic Chemistry*, 4, 259–269.
- Gel-Moreto, N., Streich, R., & Galensa, R. (2001). Chiral separation of six diastereomeric flavanone-7-O-glycosides by capillary electrophoresis and analysis of lemon juice. *Journal of Chromatography A*, 925, 279–289.
- Grayer, R. J., & Veitch, N. C. (2006). Flavanones and dihydroflavonols. In Ø. M. Andersen & K. R. Markham (Eds.). Flavonoids: Chemistry, biochemistry and applications. Boca Raton, FL: CRC Press. pp. 917–1002.
- Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. Phytochemistry, 55, 481–504.
- Heller, W., & Forkmann, G. (1988). In J. B. Harborne (Ed.), The flavonoids: Advances in research since 1986. London: Chapman-Hall.

- Jez, J. M., Bowman, M. E., Dixon, R. A., & Noel, J. P. (2000). Structure and mechanism of the evolutionarily unique plant enzyme chalcone isomerase. *Nature Structural Biology*, 7, 786–791.
- Jordheim, M., Fosse, T., Songstad, J., & Andersen, Ø. M. (2007). Reactivity of anthocyanins and pyranoanthocyanins. Studies on aromatic hydrogendeuterium exchange reactions in methanol. *Journal of Agricultural and Food Chemistry*, 55, 8261–8268.
- Kefford, J. F. (1959). In C. O. Chicester, E. M. Mark, & G. F. Stewart (Eds.), Advances in Food Research. New York: Academic Press.
- Kiehlmann, E., Lehto, N., & Cherniwchan, D. (1988). Iodination and deuteration of catechin derivatives. Canadian Journal of Chemistry, 66, 2431–2439.
- Kolar, G. F. (1971). Nuclear deuterium exchange in methoxybenzenes and methylated flavonoids. *Journal of Labelled Compounds*, 7, 409–415.
- Le Gall, G., Puaud, M., & Colquhoun, I. J. (2001). Discrimination between orange juice and pulp wash by <sup>1</sup>H nuclear magnetic resonance spectroscopy: Identification of marker compounds. *Journal of Agricultural and Food Chemistry*, 49, 580–588.
- Marder, M., Viola, H., Wasowski, C., Fernandez, S., Medina, J. H., & Palladini, A. C. (2003). 6-Methylapigenin and hesperidin: New valeriana flavonoids with activity on the CNS. *Pharmacology, Biochemistry and Behavior*, 75, 537–545.
- Miles, C. O., & Main, L. (1989). Kinetics and mechanism of the cyclisation of 2,6dihydroxychalcone and derivatives. *Journal of the Chemical Society, Perkin Transaction*, 2, 1623–1632.
- Nieto, J. L., & Gutierrez, A. M. (1986). <sup>1</sup>H NMR spectra at 360 MHz of diosmin and hesperidin in DMSO solution. Spectroscopy Letters, 19, 427–434.
- Ooghe, W. C., Ooghe, S. J., Detavernier, C. M., & Huyghebaert, A. (1994). Characterization of orange juice (*Citrus sinensis*) by flavanone glycosides. *Journal of Agricultural and Food Chemistry*, 42, 2183–2190.
- Uchiyama, N., Kim, I. H., Kawahara, N., & Goda, Y. (2005). HPLC separation of hesperidin and the C-2 epimer in commercial hesperidin samples and herbal medicines. *Chirality*, 17, 373–377.
- Vogels, J. T. W. E., Terwel, L., Tas, A. C., van der Berg, F., Dukel, F., & van der Greef, J. (1996). Detection of adulteration in orange juices by a new screening method using proton NMR spectroscopy in combination with pattern recognition techniques. Journal of Agricultural and Food Chemistry, 44, 175–180.
- Wistuba, D., Trapp, O., Gel-Moreto, N., Galensa, R., & Schurig, V. (2006). Stereoisomeric separation of flavanones and flavanone-7-glycosides by capillary electrophoresis and determination of interconversion barriers. *Analytical Chemistry*, 78, 3424–3433.