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Structural Features of the β-CD Complexes with Naringin and its Dihydrochalcone and Aglycon Derivatives by ^{1}H NMR *

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Abstract

The β-CD inclusion complexes of naringin **1**, naringin dihydrochalcone **2** and the aglycon of naringin dihydrochalcone **3** have been investigated by ^{1}H NMR spectroscopy. Continuous variation plots show the stoichiometry of all complexes to be 1 : 1. The structure of complexes was determined from 2D ROESY and 1D ROE experiments. For **1** and **2** the inclusion involves the aromatic rings leaving the disaccharide unit α-L-Rha-(1-2)-β-D-Glc outside the β-CD cavity. For naringin **1** the inclusion occurs preferentially from the wider rim of the β-CD truncated cone with the terminal phenolic ring deeply inserted into the β-CD cavity while for naringin dihydrochalcon **2** it occurs from the side of the β-CD narrower rim. The β-CD/aglycon **3** complex exists as an equilibrium of the two inclusion modes.

Introduction

Naringin **1** is the main flavonoid glycoside constituent of citrus fruits occurring principally in the peel. Its main characteristic is the bitterness which can be detected at very low concentrations (less than 10^{-4} M) [1]. For this reason high quantities of naringin are undesirable in citrus juices and various methods are used to lower the level of this flavonoid in their preparation. Among them direct addition of β-CD to the juices [2] or their treatment with insoluble β-CD polymers [3] are reported. Naringin exists as a mixture of two diastereoisomers because the chiral carbon C-2 of the dihydrobenzopyran ring is present in the R and S configuration. The interaction between β-CD and naringin was previously investigated in water by Colquhoun *et al.* [4] who determined in a detailed ^{1}H NMR study the association constants of the β-CD complexes of the two diastereoisomers in water. In this study on the basis of the ^{1}H chemical shift variations a reasonable structure of the complex was hypothesized having the terminal phenol ring included in the β-CD cavity from its wider entrance. The same complex was investigated also by S. Divakar [5] in dimethylsulfoxide solution using various techniques as ultraviolet spectroscopy, potentiometry and NMR spectroscopy. In this work information about the three dimensional structure of the complex was obtained through monodimensional stationary NOE measurements making use also of the NOE contacts produced by irradiation of the hydroxyl groups. Although the interpretation of the results achieved by this technique may be difficult because possible contributions to the magnetization transfer due to spin diffusion and/or chemical exchange phenomena cannot be excluded, the author concluded that the structure of the complex in dimethylsulfoxide is similar to that assumed in water. Here we present a rotating frame NOE study of the β-CD/naringin complex in water with the aim to provide in this solvent a detailed description of the spatial arrangement of the host and the guest molecules.

Furthermore in addition to naringin **1** we investigated also the β-CD complexes of naringin dihydrochalcone (DHC) **2** and of its aglycon derivative **3**. In contrast with the exceedingly bitter **1** the naringin DHC **2** is moderately sweet resembling in some way the behaviour of neohesperidin DHC **4** which is very sweet with respect to the parent neohesperidin. The structural difference between the naringin and neohesperidin glycosides with respect to their DHC derivatives resides in the central heterocycle ring which is present in the native glycosides as dihydrobenzopyran ring while it is opened in the reduction to the DHC derivatives. Such structural variation produces for both molecules a different behaviour with respect to the taste receptor, i.e. from bitterness to sweetness. As we will show below naringin and its DHC derivative behave differently also in their mode of complexation with β-CD thus suggesting that the central dihydrobenzopyran ring is crucial in determining the molecular recognition mechanism of molecules of this type. In a previous work [6] we have examined the complex of β-CD with neohesperidin DHC **4** showing that the inclusion occurs with the terminal isovanillin ring penetrating the β-CD cavity from the narrower rim of the truncated β-CD cone. Now we extend here the study to the complexes of naringin

* Dedicated to Prof. Paola Vita-Finzi on the occasion of her 70th birthday.

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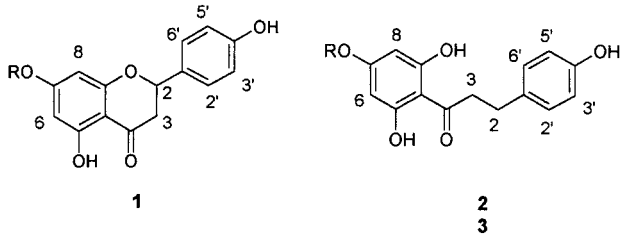
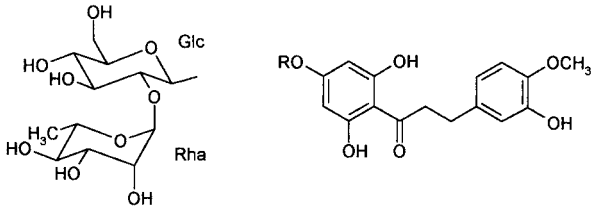


1 R=α-L-Rha-(1-2)-β-D-Glc (Naringin)
2 R=α-L-Rha-(1-2)-β-D-Glc (Naringin dihydrochalcone);
3 R=H (Aglycon of Naringin DHC)



R=α-L-Rha-(1-2)-β-D-Glc

4 R=α-L-Rha-(1-2)-β-D-Glc (neohesperidin DHC)

Chart 1. Structure and numbering of naringin **1**, naringin DHC **2**, naringin DHC aglycon **3**, and neohesperidin DHC **4**.

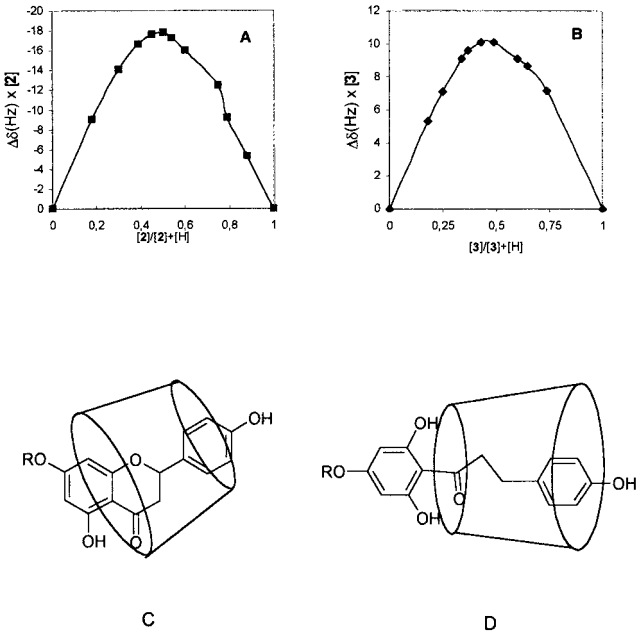


Figure 1. Top: Job's plots for the determination of the stoichiometry of β-CD complexes of **2** (A) and **3** (B). [H] = concentration of the host; $\Delta\delta$(Hz) frequency variation of the signal of the hydrogens $H_{3',5'}$. Bottom: qualitative picture of the preferential structure of the complexes **1**/β-CD (C) and **2**/β-CD (D).

DHC **2** and naringin DHC aglycon **3** with β-CD with the purpose of completing the description of the supramolecular structure of the structurally related molecules **1–4** and of evidencing possibly the contribution of the disaccharide unit to the complex formation.

Experimental

Naringin was a gift of Robertet, Grasse, France and was used with no further purification. Naringin DHC was obtained by catalytic reduction of naringin. The ^{1}H spectra were recorded on a Bruker AVANCE 500 spectrometer operating at 500 MHz. Spectral assignment of free and complexed molecules was made using 2D DQCOSY or TOCSY experiments and the NOE contacts were determined by 2D off resonance ROESY experiments using a mixing time of 200–400 ms. In many cases due to the complexity of the spectra the 1D equivalent of the corresponding 2D experiments (1D TOCSY and 1D ROE) were employed using a soft 90° or 270° pulse for the selective excitation of the signal of interest. The standard pulse programs of the Bruker library were used. The spectral analyses of free **1** and **2** were carried out in 2×10^{-3} M D_2O solutions while the spectrum of the almost insoluble 3 was obtained on saturated D_2O solution. The 1 : 1 β-CD complexes were carried out at concentrations 5 to 10×10^{-3} M. For NOE measurements many experiments were carried out in water containing only 10% of deuterated water for the instrument lock to avoid that the signals of the hydrogens H_6 and H_8 ortho to two hydroxyl groups disappear due to the exchange with the bulk water.

The complex stoichiometry of **2** and **3** was determined from Job's plots built up from the chemical shift variations of suitable signals. The overall concentration of the two components was maintained constant (range 1.0 or 2.0×10^{-3} M). The mixtures for the continuous variation plot were prepared mixing known volumes of the solutions of pure β-CD and pure compounds prepared by carefully weighing the two components and dissolving them in 100 ml of deionized water containing 10% of deuterated water. The proton spectra were run with the suppression of the strong peak of water using the presaturation technique. The chemical shifts (Table I) were referred to the internal standard tetramethyl ammonium bromide (3.17 ppm) which does not form complexes with CDs [7].

Results and discussion

The stoichiometry of the β-CD complexes of **2** and **3** was determined from the continuous variation plots obtained from the complexation induced chemical shifts shown by suitable signals of the guests or of the host as well. As an example in Figures 1A and 1B are reported the Job's plots of the hydrogens $H_{3',5'}$ of the terminal phenolic ring of **2** and **3** respectively, clearly showing that the stoichiometry of the complexes is 1 : 1. As reported in the reference [4] also the complex naringin/β-CD has a 1 : 1 stoichiometric ratio.

The above results demonstrate that a 1 : 1 complex exists in solution for molecules **1–3** and that the inclusion involves the aromatic rings. More detailed information on the molecular edifice of the complexes can be obtained from 2D ROESY spectra.

Naringin 1. The ROESY spectrum of the complex **1**/β-CD in water (10 mM) exhibits many NOE contacts. Among

Table 1. Chemical shifts (δ, ppm) of the β-CD complexes of compounds **1**, **2**, and **3** in water[a]

	1[b]		**2**[c]	**3**[c]
	Isomer I	Isomer II		
H-2	5.51	5.40	2.92(CH_2-2)	2.91(CH_2-2)
H-3	3.08	3.19	3.34–3.26(CH_2-3)	3.30(CH_2-3)
H-3′	2.97	2.80		
H-2′,6′	7.32	7.34	7.11	7.08
H-3′,5′	6.87	6.94	6.86	6.83
H-6	6.26	6.27	6.17	5.98
H-8	6.23	6.24	6.17	5.98
H-1 (Glc)	5.34	5.38	5.32	
H-2 (Glc)	3.64	3.64	3.64	
H-3 (Glc)	3.69	3.69	3.72	
H-4 (Glc)	3.50	3.51	3.51	
H-5 (Glc)	3.59	3.59	3.63	
H-6,6′(Glc)	3.89–3.72	3.89–3.73	3.92–3.74	
H-1 (Rha)	5.11	5.11	5.11	
H-2 (Rha)	4.05	4.05	4.06	
H-3 (Rha)	3.71	3.71	3.69	
H-4 (Rha)	3.42	3.42	3.43	
H-5 (Rha)	3.73	3.71	3.81	
CH_3 (Rha)	1.21	1.18	1.30	
H-1 (β-CD)	5.05		5.05	5.06
H-2 (β-CD)	3.64		3.64	3.65
H-3 (β-CD)	3.89		3.92	3.90
H-4 (β-CD)	3.57		3.57	3.60
H-5 (β-CD)	3.69		3.77	3.70
H-6,6′ (β-CD)	3.83		3.83 and 3.77	3.82 and 3.79

[a] Chemical shifts referred to the internal standard tetramethyl ammonium bromide taken at 3.17 ppm.
[b] Molar ratio 1 : 1, concentration 10.0 mM.
[c] Molar ratio 1 : 1, concentration 5.0 mM.

them the most interesting are the intermolecular cross peaks between the interior β-CD hydrogens H_3 and H_5 with the hydrogens of the terminal phenolic ring $H_{2',6'}$ and $H_{3',5'}$ resonating at 7.32/7.34 and 6.97/6.94 ppm respectively (there are two chemical shift values because naringin exists as mixture of two diastereisomers) and the hydrogens of the internal aromatic ring $H_{6,8}$ at 6.24/6.27 ppm. The spectrum shows the following trend: the cross peaks $H_{3',5'}/H_5(\beta\text{-CD})$ are much more intense than that $H_{3',5'}/H_3(\beta\text{-CD})$; the cross peaks $H_{2',6'}/H_5(\beta\text{-CD})$ and $H_{2',6'}/H_3(\beta\text{-CD})$ are of comparable intensity; finally the contacts $H_{6,8}/H_3(\beta\text{-CD})$ are strong while that $H_{6,8}/H_5(\beta\text{-CD})$ are practically absent. This behaviour suggests that the inclusion occurs preferentially from the wider rim of the β-CD truncated cone with the terminal phenolic ring deeply inserted into the β-CD cavity (Figure 1C). However the spectrum shows also week contacts between $H_{6,8}$ of **1** and $H_6(\beta\text{-CD})$ which are not consistent with the presence of the complex above. Such contacts may be explained taking in account that also the complex where the inclusion occurs from the primary rim of β-CD is present in solution as minor component.

Naringin DHC ***2***. The ROESY spectrum of the complex **2**/β-CD in water (10 mM) shows the opposite trend with respect to naringin. In fact the observed NOEs are: $H_{3',5'}/H_5(\beta\text{-CD})$ small and $H_{3',5'}/H_3(\beta\text{-CD})$ strong; $H_{2',6'}/H_5(\beta\text{-CD})$ and $H_{2',6'}/H_3(\beta\text{-CD})$ of comparable intensity; $H_{6,8}/H_3(\beta\text{-CD})$ absent, $H_{6,8}/H_5(\beta\text{-CD})$ and $H_{6,8}/H_6(\beta\text{-CD})$ very strong. This trend clearly says that the inclusion occurs from the narrower side of β-CD with the primary methylene groups very near to the $H_{6,8}$ hydrogens (Figure 1D). This behaviour is identical to that observed for neohesperidin DHC [6].

Naringin DHC aglycon ***3***. In the case of the complex **3**/β-CD rather strong cross peaks are observed between $H_{3',5'}$ and $H_{2',6'}$ with both H_5/β-CD and H_3/β-CD. In addition the hydrogens $H_{6,8}$ shows contacts with H_3, H_5 and H_6 of β-CD thus suggesting that the two different inclusion complexes described above are in equilibrium in solution in comparable concentration. In Figure 2 are reported the 1D ROE spectra obtained by selective excitation of the $H_{6,8}$ hydrogens for the complexes of molecules **1–3** giving a simple and immediate picture of their different behaviour.

The data discussed above show that the aglycon **3** deprived of the disaccharide unit has a non preferential inclu-

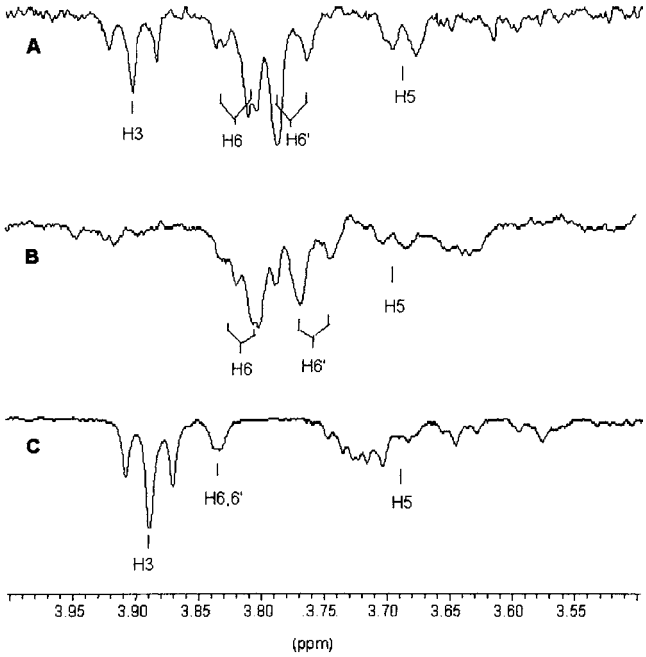


Figure 2. ROE 1D spectra obtained by selective excitation of the hydrogens $H_{6,8}$ of naringin DHC aglycon **3** (A), naringin DHC **2** (B) and naringin **1** (C).

sion mode with β-CD, while naringin **1** and naringin DHC **2** have opposite preferential inclusion modes. The factors contributing to the stabilization of the preferred structure cannot be clearly identified here. Going from naringin to naringin DHC the dihydrobenzopyran ring is opened leading to a change of the shape of the molecule and to the formation of an additional OH group in position 7. Possibly the presence of two OH groups for **2** may provide a further source of hydrogen bonding with respect to **1** thus enhancing the number of intermolecular interactions. In addition the disaccharide unit is probably important in establishing intermolecular contacts with the external surface of the host. Such interactions should be substantially different for **1** and **2** due to their different shape and flexibility. The existence of a interaction between the disaccharide unit and β-CD was proven in a previous FAB-MS study of the complex neohesperidin/β-CD [6].

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