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Cyclodextrins as carriers for kavalactones in aqueous media: Spectroscopic characterization of (*S*)-7,8-dihydrokavain and β-cyclodextrin inclusion complex

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ABSTRACT

Kavalactones represent the active constituents of kava–kava (*Piper methysticum* G. Forster), endowed with sedative and anaesthetic properties. Kavalactones are polar constituents, but poorly soluble in water with a low bioavailability. In this study, the formation of inclusion complexes of one of the most representative kavalactone isolated from kava–kava extract, (*S*)-7,8-dihydrokavain (DHK), with β-cyclodextrin (β-CyD) was investigated mainly by spectroscopic methods. NMR experiments were extensively used for the complete characterization of the complex and included ^{1}H NMR complexation shifts analysis, ^{1}H NMR diffusion measurements (DOSY), and ROESY experiments. In particular DOSY experiments demonstrated that in the presence of β-CyD the translational diffusion of kavalactone is sizably slowed down (2.5×10^{-10} m^2/s) with respect to the free drug (4.4×10^{-10} m^2/s) according to the inclusion of DHK in the cavity of (β-CyD). ROESY experiments confirmed the inclusion of DHK in the hydrophobic pocket of β-CyD through the primary hydroxyl rim, being the most relevant interactions between the H3′ of β-CyD and the *ortho* protons on the phenyl ring of the DHK, and between H5′ of β-CyD and the *meta*/*para* protons of DHK phenyl ring. The inclusion of the phenyl ring of DHK, leaving the lactone moiety outside of CyD was also confirmed by the induced CD effects. The binary solution DHK/β-CyD shows a 50% intensity increase of the negative band of the π–π* transitions of the phenyl ring with respect to the absorption observed with DHK alone. Molecular dynamics simulations results corroborated and further clarify observed spectroscopic data. It was found that the phenylethyl substituent at C6 has a preferential equatorial position in the free state, and an axial one in the complex, justifying the large downfield shift experienced by H6 of DHK upon binding. Finally the influence of β-CyD on water solubility of DHK was investigated by phase-solubility studies. In the range 2–4 mM of host, solubility of DHK was increased only two-fold, but being β-CyD also a penetration enhancer, *in vivo* studies will be performed to clarify a possible role of the complex on the bioavailability of DHK.

1. Introduction

Kava–kava (*Piper methysticum* G. Forster) is an herbal drug traditionally used in social and ceremonial life of Pacific islands inhabitants from ancient times for the soporific and narcotic effects. Kavalactones (kavapyrones) represent the active constituents of kava–kava, endowed with sedative and anaesthetic properties [1,2]. Kavalactones are polar constituents, but poorly soluble in water and for this reason the traditional kava–kava beverage was prepared with coconut milk [1]. In order to develop an innovative formulation whit increased bioavailability, for local anaesthesia, the enantiopure (*S*)-7,8-dihydrokavain (DHK see Fig. 1), one of the main characteristic kavalactones, was isolated from kava–kava extract by chromatographic separation, and investigated in its ability to form a supramolecular complex with β-cyclodextrin (β-CyD). CyDs are cyclic oligosaccharides that can interact with a wide variety of drugs and the formation of their supramolecular complexes can influence the dissolution rate, the aqueous solubility and permeation ability of the drugs improving their bioavailability profile [3]. In particular, in this study cyclodextrins were selected for their properties as carriers by keeping the hydrophobic drug molecules in the polar media and deliver them to the surface of the biological membrane, e.g. skin. In the literature, we found only one report about the interaction between CyDs and kava–kava constituents [4]. However, no data concerning the formation and characterization of inclusion complexes were reported in this preliminary evaluation of the interaction between a kava–kava extract or an artificial mixture of kavalactones and β- and γ-CyD. In the present investigation β-CyD was selected for evaluating the possible formation of inclusion complexes with kavalactones because

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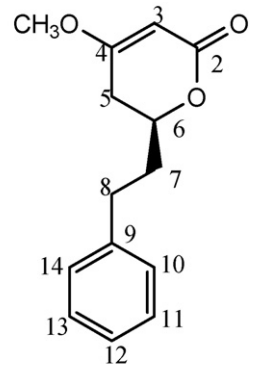

(*S*)-7,8-Dihydrokavain
(DHK)

Fig. 1. Chemical structure of (*S*)-7,8-dihydrokavain (DHK).

stationary phases based on β-cyclodextrin derivatives have been reported for chromatographic separations of kavalactones [5,6]. Moreover, β-CyD has been widely used in pharmaceutical applications because of its ready availability, low cost and cavity size suitable for the widest range of drugs [7]. Aim of this study was to provide a detailed structure and dynamic picture of the binding of the selected kavalactone DHK to β-CyD through circular dichroism (CD), NMR (^{1}H NMR complexation shifts analysis, ^{1}H NMR diffusion measurements, and ROESY) and by molecular dynamics simulations. The influence of β-CyD on the DHK solubility was also investigated by phase-solubility studies.

2. Materials and methods

2.1. Chemicals

Methanol was HPLC grade from Merck (Darmstadt, Germany). 85% Formic acid was purchased from Sigma–Aldrich (Fluka Chemicals, Sigma–Aldrich Division, Milano, Italy). Water was purified through a Milli-Q_{plus} system from Millipore (Milford, MA). Ethanol 96%, methanol and ethyl acetate were purchased from Riedel-de Haën Laborchemikalien GmbH & Co. KG. n-Hexane was purchased from Lab-Scan, Dublin. Dichloromethane and β-Cyclodextrin were purchased from Sigma–Aldrich. Spectroscopy-grade solvents (water and methanol) were purchased from Sigma–Aldrich. Deuterated solvents (D_2O, 99.8% and CD_3OD, 99.5%) were purchased from Merck.

2.2. Plant material

A commercial extract of kava–kava (lyophilized extract, lot no. 9A3847) was kindly offered by Aboca S.p.A. (Sansepolcro, Arezzo, Italy).

2.3. Isolation of (S)-7,8-dihydrokavain from the kava–kava extract

The lyophilized extract (3 g) was suspended in 4 mL of a solution of CH_2Cl_2/CH_3OH 1:1 and purified by column chromatography (Silica 9385, Kieselgel 60 Merck) at room temperature (elution mixture: hexane/ethyl acetate from 95:5 to 70:30, v/v). The fractions corresponding to DHK were collected, evaporated and crystallized from hexane, to obtain a white solid that was identified by HPLC/DAD/MS [8].

2.4. HPLC-DAD and HPLC-MS apparatus and methods

The HPLC system consisted of a HP 1100L instrument with a Diode Array Detector and managed by a HP 9000 workstation (Hewlett & Packard, Palo Alto, CA, USA). The reverse-phase column was a Prodigy ODS3 (5 μm, 150 × 2 mm, 100 Å, Phenomenex Torrance, CA, USA) maintained at 40 °C.

The eluents were: H_2O adjusted to pH 3.2 by HCOOH (A), and MeOH (B). The following solvent gradient was applied: 0–5 min A 70–50%; 5–10 min A 50–45%; 10–15 min A 45%; 15–30 min A 45–40%; 30–35 min A 40–30%; 35–40 min A 30–70%. The injected volume of sample was 10 μL of 1 mg/mL solution, the flow was 0.4 mL/min. UV–vis spectra were recorded in the range 200–590 nm, and chromatograms were acquired at 240, 254, 270, 350 and 590 nm.

The HPLC system was interfaced with a HP 1100 MSD API-electrospray (Hewlett & Packard, Palo Alto, CA, USA). The same column, mobile phase, time period and flow rate were used. Mass spectrometry operating conditions were optimised in order to achieve maximum sensitivity values: gas temperature 350 °C at a flow rate of 10 L/min, nebulizer pressure 30 p.s.i., quadrupole temperature 30 °C, and capillary voltage 3500 V. Full scan spectra from *m/z* 100 to 800 in the negative and positive ion mode were obtained (scan time 1 s).

2.5. Colyophilized products

Equimolar colyophilized products were prepared by freeze-drying (Lyovac GT2, Leybold-Heraeus) a solution obtained from a portion of the physical mixture of kavalactone/CyD dissolved in water and EtOH, followed by evaporation of the organic solvent. The 1:1 molar ratio of the dried powder was demonstrated through NMR.

2.6. Solubility studies

Phase-solubility studies were carried out in water, according to the method previously reported by Higuchi and Connors [9]. Briefly, excess amounts of DHK were added to water containing increasing concentrations of β-CyD (2–10 mM) and suspensions were shaken at constant temperature (25 ± 0.5 °C) for 1 day. After the equilibrium was reached, an aliquot was centrifuged and DHK concentration was determined by HPLC-DAD analysis. Each experiment was performed in triplicate (coefficient of variation (CV) <5%).

2.7. Spectroscopic measurements

NMR spectra were recorded at 298 K on a Varian INOVA 600 spectrometer operating at 14.1 T and referenced to the residual signal of HDO at 4.79 ppm. ROESY spectra were acquired using the standard pulse sequence with 0.6–0.8 s mixing times and continuous wave spin-lock (TROESY) with B_1 = 2800 Hz. DOSY experiments were performed with the DgcsteSL pulse sequence [10] using gradient pulses having 2 ms width and 1.17–70.5 G/cm strength. The samples consisted in the pure constituents and the colyophilized mixture kavalactone/β-CyD. The solids, in a total amount of about 3 mg, were dissolved in 0.5 mL 1:1 (v/v) CD_3OD/D_2O and sonicated for 10 min prior to perform measurements. The resulting solution was not completely clear even after filtration but this did not lead to apparent distortions of the NMR spectra.

CD spectra were recorded with a Jasco J-715 spectropolarimeter, using 0.02–0.2 cm cells, with the following conditions: bandwidth 1 nm, response 2 s, scanning speed 20 nm/min. The samples consisted in DHK 0.65 mM in CH_3OH/H_2O and a 1:1 colyophilized mixture of DHK/β-CyD 0.07 mM in H_2O.