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COMMUNICATION

Selection of Kavalactones by Complexation of Kava Extract with Cyclodextrins

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ABSTRACT

Kavalactones, active ingredients extracted from Piper methysticum Forst. Piperaceae, *have many therapeutic properties including relaxing, anaesthetic, analgesic, and antifungic properties. Kavalactones are insoluble in aqueous vehicles. These active ingredients are inclused in cyclodextrins to improve their water solubility and to realize a galenic form.*

Gamma cyclodextrin (γ-CD) and beta cyclodextrin (β-CD) were used. The amounts of kavalactones inclused in cyclodextrins have been measured by HPLC with UV spectrophotometric detection.

The results showed that besides increasing the water solubility of kavalactones, the complexation leads to a selection of the most active compounds that are preferentially included.

INTRODUCTION

Piper methysticum Forst. Piperaceae is an Oceanian pepper plant. A drink prepared with the roots of this plant was used by the natives for its pharmacological properties including relaxing, local anaesthetic, analgesic, and antifungic properties (1,2).

The active ingredients responsible for these properties are mainly six kavalactones that have been extracted from *Piper methysticum Forst. Piperaceae* (3,4). These active ingredients are always used in the form of a total extract owing to the pharmacological synergy of their mixture (5).

Kavalactones have local anaesthetic effect superior to that of benzocaine (1) and equivalent to that of lidocaine (6) without any toxicity (7), two of these alpha-pyrones, kawaine and dihydrokawaine, are the more active components. Kavalactones are only soluble in organic solvents and oils.

Cyclodextrins (CD) are cyclic oligosaccharides made up of a variable number of glucose units (7 glucose units in β-CD and 8 glucose units in γ-CD) connected by α-

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(1.4) bonds. The ring formed by cyclodextrins externally is very hydrophilic and internally relatively apolar (8). These ring-shaped molecules can form inclusion compounds with many molecules both in liquid and solid medium and then can help to improve the dissolution of active ingredients (9), and in certain cases, to enhance their bioavailability (10), to diminish side effects (11,12), and perhaps to increase their stability (13).

The purpose of this work was to prepare a hydrophilic inclusion compound of kavalactones in order to realize a galenic form with local anaesthetic properties.

MATERIALS AND METHODS

Materials

β-CD was provided by Roquette Frères (Lestrem, France). γ-CD was provided by Wacker Chemie (Dusseldorf, Germany). Kava extract (containing more than 95% of kavalactones) was obtained by organic extraction from the roots of *Piper methysticum of chemotype H* from Vanuatu (Laboratoire de Pharmacognosie, Poitiers, France).

Hexan and dioxan were of HPLC grade from Rathburn (Walkerburn, UK). All of the other solvents were of analytical reagent grade from Labosi (Paris, France).

Methods

Inclusion Compounds

The inclusion of Kava extract has been realized in the β- then in the γ-cyclodextrin. Different proportions of host and guest molecules were used comparatively.

Three kavalactones, dihydrokawain (DHK), methysticin (M), and yangonine (Y), have then been inclused individually in the γ-cyclodextrin.

Finally, an equimolar mixture of these three kavalactones has been inclused in the same cyclodextrin.

Extraction

The aqueous solution of inclusion compound was submitted to three successive chloroformic extractions. The chloroformic phases were dried on sodium sulfate and evaporated under reduced pressure. The residue was weighed and diluted in chloroform (1/20) and the solution obtained was injected into the chromatograph.

High-Performance Liquid Chromatography

A Varian model 5000 chromatograph was used, equipped with a Rheodyne model 7125 injector and a Merck L 3000 photodiode array detector under computer control (Merck HPLC Manager). Analyses were conducted at 20°C.

Analytical HPLC was carried out on a normal phase Superspher Si 60 column (125 × 4 mm i.d., particle size 4 μm) (Merck) used with a Lichrospher Si 60 precolumn (4 × 4 mm i.d., particle size 5 μm). The mobile phase was hexan–dioxan (80:20 v/v) at a flow rate of 1 ml/mn. The injection volume was 10 ml and UV detection was at 240 nm (14).

A calibration curve was performed for each kavalactone. Working standard solutions containing 0.1–1 mg/ml kavalactones were prepared in chloroform. A 10-ml volume of each solution was injected into the chromatograph. The peak area was measured and the calibration curve was obtained for each kavalactone at 240 nm and for yangonin at 350 nm.

RESULTS AND DISCUSSION

Two cyclodextrins were selected to be used as host molecules according to the mean molecular weight of kavalactones (240) and the size of the cyclodextrins' internal cavities: 7.8 Å for β-CD and 9.5 Å for γ-CD.

The results showed that the inclusion is optimized by the use of the β-CD with a ratio of 1 mole of β-CD for

Table 1

Inclusion Yields of the Kava Extract in Cyclodextrins

	Inclusion Compound 1	Inclusion Compound 2	Inclusion Compound 3	Inclusion Compound 4
Cyclodextrin	β-CD	β-CD	γ-CD	γ-CD
β-CD:Kava ratio	1:1	1:2	1:2	1:3
Inclusion yield	37.5%	42.5%	33.3%	40%

2 moles of kavalactones (Table 1). The inclusion yield is 42.5% under these conditions.

The results obtained led us to divide the kavalactones into three groups according to the saturation degree of the carbons 5-6 and 7-8 (Fig. 1). Group I corresponds to the insaturated kavalactones (Δ 5-6 and Δ 7-8), which are demethoxyyangonine (DMY) and yangonine (Y). Group II corresponds to the compounds with 1 insaturation between the carbons 7 and 8 (Δ 7-8), which are kawai (K) and methysticin (M). Group III corresponds to the saturated kavalactones on the carbons 5-6 and 7-8, which are dihydrokawain (DHK) and dihydromethysticin (DHM).

Regardless of the ratio, the kavalactones of the group I are less inclused in the β-CD than the kavalactones of the other groups.

The mean inclusion yields into β-CD are the following: DHK (group III): 45%; M (group II): 36%; DMY (group I): 20%.

The results obtained for the inclusions in γ-CD are the following: DHK (group III): 43%; M (group II): 31%; DMY (group II): 6.5%.

The differences observed in the percentage of inclusion between the three groups are more important with γ-CD. The γ-CD are apparently more selective than the β-CD.

One kavalactone of each group was then inclused in γ-CD individually: Y for group I, M for group II, and DHK for group III.

A ratio of two kavalactones for one γ-CD was chosen because increasing the quantity to three kavalactones (for one γ-CD) does not improve the inclusion yield significantly (Table 1).

The following results are obtained: DHK (group III): 53.3%; M (group II): 6.6%; Y (group I): 5%. The results showed that Y (group I) was less inclused than M (group II), which was less inclused than DHK (group III).

To verify the influence of the presence of other kavalactones on the complexation of these molecules, the complexation of a blend of equal proportions of these three kavalactones in γ-CD was also carried out. The same ratio of host and guest molecules was used. The inclusion yield of this mixture was 22.7%. The results for the inclusion of these blending kavalactones are the following: DHK (group III): 88.8%; M (group II): 9.2%; Y (group I): 2%.

These results show that the kavalactone belonging to group I (Y) presents a decrease of its inclusion yield ranging from 5% to 2%, when it is mixed with the other kavalactones.

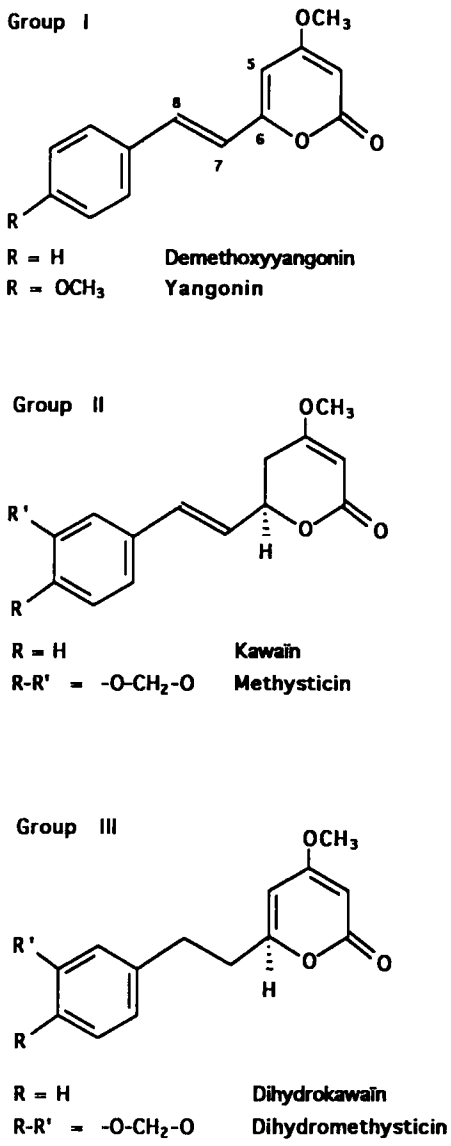

Figure 1. The formulas of three groups of kavalactones.

The inclusion yield of M (belonging to group II) is not significantly different (6.6% for M inclused individually and 9.2% when mixed with the other kavalactones); whereas for the kavalactone belonging to group III (DHK), an increase of its inclusion yield can be observed when mixed with the other kavalactones (from 53.3% to 88.8%).

Therefore, there is a competition between the kavalactones for their complexation in the γ-CD; furthermore, the inclusion yields of the mixture of the three kavalactones confirm the selectivity of the complexation according to the saturation degree of these molecules.

The less insaturated molecules (which are the most physiologically active) are the most inclused in cyclodextrins.

This might be explained by a greater flexibility of the insaturated molecules which could fit the cyclodextrins' cavity more easily.

CONCLUSION

The complexation of the Kava extract with cyclodextrins had permitted improved water solubility of the Kavalactones and led to selective inclusion of the most active molecules.

This will lead, in the absence of toxicity of the kavalactones, to the possibility of using these molecules as a substitute for lidocaine as a local anaesthetic.

These molecules could be used in the treatment of mucites related to immunodepression, especially for patients with AIDS syndrome or patients under chemotherapy.

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