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Isolation and Purification of Kava Lactones by High Performance Centrifugal Partition Chromatography

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ABSTRACT

Kava kava (*Piper methysticum*) is currently used in a variety of herbal and homeopathic preparations to induce relaxation, treat anxiety, or induce sleep. The pharmacologically active compounds consist of a group of structurally related lactone derivatives that are concentrated in the roots, rhizomes, and root stems. The major constituents responsible for 95% of the total pharmacological activity are desmethoxyyangonin, dihydrokavain, yangonin, kavain, dihydromethysticin, and methysticin. The concern over the possible hepatoxicity of kava has led regulatory agencies in countries such as Germany, Switzerland, France, Canada, the United Kingdom, as well as the United States to respond by warning consumers about the potential risks of kava use, or by removing kava-containing

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products from the marketplace. The high demand for purified kava-lactones has emerged to facilitate necessary testing to investigate the rising concern over these cases of liver toxicity. A separation method using high performance centrifugal partition chromatography (HPCPC) has been developed for the isolation of each kava lactone from a CO_2 extract of *P. methysticum* in a single chromatographic run.

Key Words: Kava lactones; Centrifugal partition chromatography; HPCPC; *Piper methysticum*; Methysticin; Dihydromethysticin; Kawain; Dihydrokawain; Yangonin; Desmethoxyyangonin.

INTRODUCTION

Kava kava (*Piper methysticum*) has been used for centuries in social and religious settings throughout the Pacific islands of Micronesia. Fresh or dried root is crushed with water, filtered, and the liquid is then consumed as a beverage for its intoxicating, calming effects that promote sociability.[1] Due to the observed calming effects, dietary supplements containing kava kava were used in North America and Europe for anxiety disorders, stress disorders, nervous tension, and restlessness.[2,3] Today, several extracts are standardized for the biologically active kavalactones considered responsible for kava kava's calming effects. Although, clinical studies of kava kava in the last decade have shown it to be very effective, well tolerated, and non-addictive at therapeutic dosages, potential side effects can occur when very high doses (100–300 mg/daily) are taken for extended periods.[3,4] These can include an allergic skin reaction, a reversible gastrointestinal disturbance, as well as dopamine antagonism.[2] Recently, there has been several adverse event reports pertaining to kava kava in Europe and the United States due to severe cases of hepatotoxicity. According to the FDA, kava-containing products have been associated with liver-related injuries (including hepatitis, cirrhosis, and liver failure) in over 25 reports of adverse events in other countries.[5] A case of recurring necrotizing hepatitis was reported in Germany in 1998 and at the end of 2000 in Switzerland, a patient was subjected to a liver transplant because he developed stage IV encephalopathy associated with kava-kava consumption at 210–280 mg kavalactones/day.[6,7] This concern over the possible hepatoxicity of kava has led to regulatory agencies in countries such as Germany, Switzerland, France, Canada, the United Kingdom, as well as the United States to either warn consumers about the potential risk of kava use or remove kava-containing products from the marketplace.[8] However, the evidence is inadequate to implicate kava as the responsible agent since a direct causal relationship of the liver toxicity with kava has not been established.

Since kava has yet to be proven safe or not, further studies must be undertaken. This has led to the high demand for purified kavalactones (Fig. 1)

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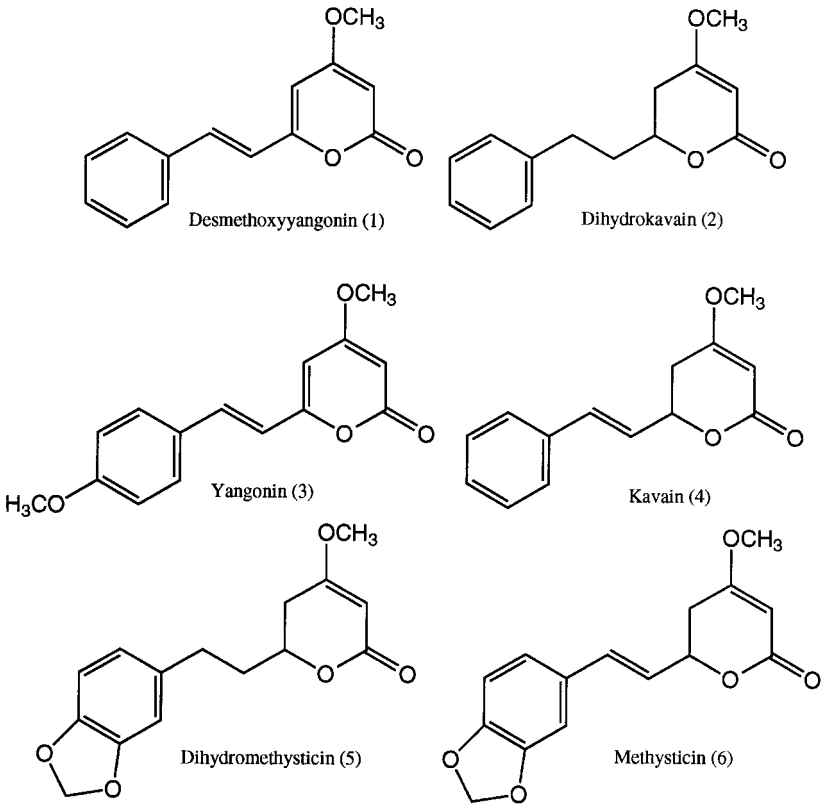

Figure 1. Structures of the kavalactones.

to facilitate necessary testing. A relatively simple and time efficient separation method for the isolation of the kavalactones[1–6] has been developed using high performance centrifugal partition chromatography (HPCPC).

EXPERIMENTAL

Materials

The supercritical CO_2 extract of *P. methysticum* was provided by Alden Botanica (Moreno Valley, CA). Solvents were Fisher Scientific chromatographic grade (Suwanee, Georgia).

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Methods

High Performance Centrifugal Partition Chromatography

CPC experiments were performed using a High Performance Centrifugal Partition Chromatograph, Series 1000 HPCPC Column Module (Sanki Engineering Limited, Nagaokakyo Kyoto, Japan). The solvents were pumped into the HPCPC rotating 1100 rpm with a Beckman 110B Solvent Delivery Module. Flow rate was set at 3 mL/min. Fractions were collected with a Bio-Rad Model 2110 fraction collector. A 1 g sample of kava extract was dissolved in 2.5 mL of a 50 : 50 mixture of both the organic phase and aqueous phase. A solvent system consisting of (3 : 1 : 2 : 1) (hexanes : ethyl acetate : methanol : water) separated five of the kavalactones collecting 300, 10 mL fractions. A solvent system of (4 : 1 : 3 : 1), (hexanes : acetone : methanol : water) separated all six kavalactones collecting 1145, 10 mL fractions. Both solvent systems were run in the ascending mode with the organic layer as the mobile phase and the aqueous layer as the stationary phase. Fractions were analyzed by TLC on silica gel, employing 30% ethyl acetate and 70% hexanes and developing the plate twice. The plates were sprayed with anisaldehyde spray reagent. Fractions were compared to pure compounds as standards. The fractions were also analyzed for purity and identity by HPLC.

Analytical High Performance Liquid Chromatography

HPLC was performed on a Waters Alliance 2695 Separations Module equipped with a Waters 996 photodiode array detector (Milford, MA). Separations were carried out on a Luna C8[2] reverse phase column (100 × 4.6 mm, 3 μm particle size) from Phenomenex (Torrance, CA). The mobile phase was mixed online to give a mixture of water/acetonitrile/reagent alcohol (65/20/15). The isocratic system had a flow rate of 0.75 mL/min with a 5 μL injection volume at 40°C. Run time was 20 min at a detection wavelength of 246 nm. The column was washed with methanol for 5 min and then re-equilibrated for 10 min. Samples (5 mg each) were dissolved in methanol (5 mL) and filtered through a Nylon 0.45 μm membrane filter into a HPLC vial for analysis.

Partition Coefficient Determination

About 10.0 mg of kava kava extract was partitioned between 2 mL upper phase and 2 mL lower phase of the solvent mixture; hexanes : acetone : methanol : water (4 : 1 : 3 : 1). The resulting layers were analyzed by HPLC for concentration of each kavalactone.

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Table 1. Partition coefficients for (1–6) in solvent mixture.

Compound	Partition coefficient
1	0.071
2	0.053
3	0.047
4	0.032
5	0.025
6	0.015

Hexanes : acetone : methanol : water (4 : 1 : 3 : 1).

RESULTS AND DISCUSSION

A relatively simple method for the isolation of all six kavalactones has been developed using HPCPC. This method purifies the kavalactones in a single chromatographic run using a hexanes : acetone : methanol : water, (4 : 1 : 3 : 1), solvent system. The partition coefficients of the kavalactones are summarized in Table 1. The six kavalactones are structurally very similar and chromatograph very closely on silica gel. The results obtained with the HPCPC method are substantially better than those from the conventional column chromatography in terms of peak resolution. Traditional chromatography using silica gel does not always afford fractions of purified kavalactones since the lactones frequently coelute. A solvent system, which employs (hexanes : ethyl acetate : methanol : water) (3 : 1 : 2 : 1) was able to purify five of the kavalactones and is relatively shorter to run as compared to the (hexanes : acetone : methanol : water) solvent system. The kavalactone yangonin could not be separated from kavain with this particular system, which would be preferable to use when isolation of yangonin was not a required purified product. The HPCPC was employed to separate a 1 g extract of kava kava. Using the hexanes : acetone : methanol : water solvent system afforded purified fractions of desmethoxyyangonin, 53.9 mg; dikydrokavain, 168.2 mg; yangonin, 29.5 mg; kavain, 138.0 mg; dihydromethysticin, 71.0 mg; methysticin, 50.7 mg. Upon recrystallization all had purities in excess of 98%.

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