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Is the Alkaloid Pipermethystin Connected with the Claimed Liver Toxicity of Kava Products?

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Aim

Recently, a worldwide discussion on the potential liver toxicity of extracts obtained from Kava (Piperis methystici rhizoma) was initiated by a series of reports resulting in a ban by the German Federal Institute for Drugs and Medicinal Products (BfArM) that was followed by other countries [1]. However, most cases were evaluated as "doubtful" on causality assessment [2]. Several theories evolved as to why liver failure may have occurred [1]. Dragull et al [3] suggested the alkaloid pipermethystin (1) being responsible for hepatotoxicity.

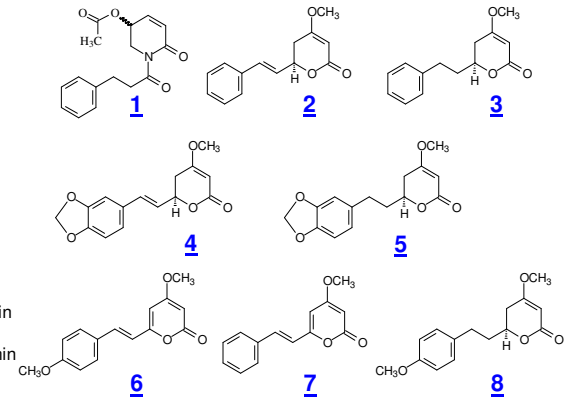
Material

We investigated various kava preparations including a series of retain samples of kava extract containing products from the German market, self-produced extracts from root and stem material obtained from two identified kava cultivars ("noble kava" Ava La'au from Samoa, "Tudei kava" Palisi from Vanuatu; extracted with ethanol 96% respectively acetone 75% or 100%), and an extract from the leaves of *Piper methysticum* (as a positive control).

No.	sample name (German)	origin	organ	company	type
1	MG1 Extrakt			Müller Göppingen	extract
2	MG2 Extrakt			Müller Göppingen	extract
3	MG3 Extrakt			Müller Göppingen	extract
4	Spissumextrakt Gehrlicher			Gehrlicher	extract (spissum)
5	Eukavan			Salus	capsule
6	W508	Vanuatu		Schwabe	crude drug
7	W504	Vanuatu		Schwabe	crude drug
8	Kavatonga	Tonga		Phytopharm	crude drug
9	Kavacur			Biocur	coated pill
10	Kavatino			Bionorica	capsule
11	Kavasedon			Harras Pharma	capsule
12	Kavosporal forte			Müller Göppingen	capsule
13	Kava von ct			ct-Arzneimittel	capsule
14	Limbao 120			Kanoldt	capsule
15	Laitan 100			Schwabe	capsule
16	Laitan (bras.)			Schwabe	capsule
17	Antares 120			Krewel	coated tablet
18	Aigin-Kava Hevert Dragees			Hevert Arzneimittel	coated pill
19	Aigin-Kava Hevert Tropfen			Hevert Arzneimittel	tincture
20	Kava ratiopharm			Ratiopharm	capsule
21	Kavacur 120 mg			Biocur	coated tablet
22	Maoni forte			Lichtwer	coated tablet
23 (I)	"noble kava" Ava La'au	Samoa	roots	HERBResearch	crude drug
23 (II)	"noble kava" Ava La'au	Samoa	peeling (stem)	HERBResearch	crude drug
24 (I)	"Tudei kava" Palisi	Vanuatu	rhizomes & roots	HERBResearch	crude drug
24 (II)	"Tudei kava" Palisi	Vanuatu	peelings (stem)	HERBResearch	crude drug
24 (III)	"Tudei kava" Palisi	Vanuatu	roots	HERBResearch	crude drug (chips)
25 Ac75	"noble kava" Ava La'au	Samoa	roots	Finzelberg	extract 75% acetone (spissum)
25 Eth	"noble kava" Ava La'au	Samoa	roots	Finzelberg	extract 96% ethanol (spissum)
26 Ac75	"Tudei kava" Palisi	Vanuatu	roots	Finzelberg	extract 75% acetone (spissum)
26 Eth	"Tudei kava" Palisi	Vanuatu	roots	Finzelberg	extract 96% ethanol (spissum)
27 Eth	Produktionsextrakt			Finzelberg	commercial extract 96% ethanol
28	Peeling Suva	Suva, Fiji	peelings	Nasigasiga Kava Dealer	crude drug
29	Kultivar "Matakaro"	Fiji	leaves	HERBResearch	crude drug

Structures

1 Pipermethystin
2 Kavain
3 Dihydrokavain
4 Methysticin
5 Dihydromethysticin
6 Yangonin
7 Desmethoxyyangonin
8 Tetrahydroxyyangonin



Analysis

GC-MS

Samples were analyzed for their content of pipermethystin (1) by GC-MS using total ion currency (TIC) and selective ion monitoring (SIM) detection. Limit of detection (LOD) was about 0.009% (SIM).

Sample preparation

a) Dried and ground plant material, extracts, total contents of capsules, aliquots of ground pills and coated tablets, aliquots of tinctures (amount: 0.1-1g)
b) Extraction with 10.0 mL methanol (Ultra Turrax® T8, 25000 rpm) / reduction to dryness / 1 ml MeOH-Water 10:90
c) RP18 SPE cartridge / washing step: 10 mL MeOH-water 10:90 / elution step: 10 mL MeOH-water 50:50 / reduction to dryness / 1.0 mL MeOH
d) GC analysis

Calibration

Pipermethystin (1) 1.76 - 75.0 µg/mL (in MeOH), Modus: SIM: m/z: 227, 131, 104.

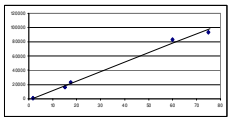


Fig. 1: Calibration curve of 1

GC/MS (EI)

Agilent Technologies: 5973 Mass Selective Detector, 6890N GC-System, 7683B Injector, Temp. 150°C→5°C/min→300°C (30 min isotherm.), Column: HP5MS 0.25mm×30m×0.25µm, Vol.: 1µL

Results

Chromatograms of raw extracts

Fig. 2 shows the chromatogram of a typical sample of Piperis methystici rhizoma (No.27Eth) without sample preparation simply dissolved in MeOH. Besides several signals of kavapyrones (2–8) no signal of 1 (expected R_t = 15.90 min) could be detected. Fig. 3 shows the same extract spiked with a certain amount of 1.

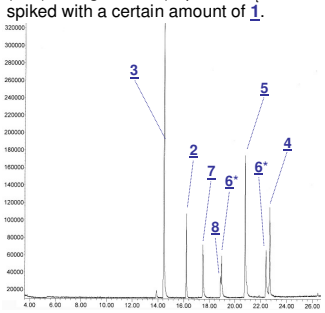


Fig. 2: TIC of extract No. 27, *cis/trans

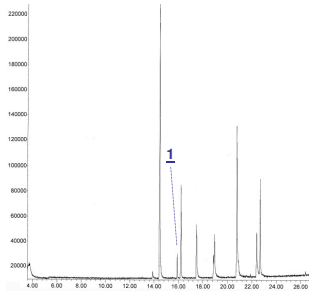


Fig. 3: TIC of extract (No. 27) spiked with 1

Chromatogram and mass spectrum of pipermethystin (1)

Fig. 4 shows the chromatogram of pure 1 (R_t=15.90min) and the corresponding EI-MS spectrum (Fig. 5). The recorded spectrum matched with the NIST database entry in very good quality. We decided to use fragments m/z= 227, 131 and 104 for quantification purposes (SIM).

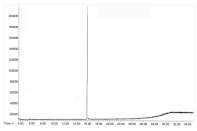


Fig. 4: TIC of pure 1

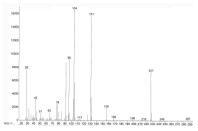


Fig. 5: EI-MS of 1

ID 110 393 in NIST database:
m/z = 287
5-(acetyloxy)-5,6-dihydro-1-(1-oxo-3-phenylpropyl-2(1H)-pyridinone
(= 1)

Chromatograms of leaves, "Noble Kava" and "Tudei kava"

The following GC's were registered after purification of the sample by using a RP-18 cartridge (step c, sample preparation) in order to prevent the column from contamination. 1 was quantitatively eluted from the cartridge by MeOH-H_2O 50:50.
Only the reference chromatogram of Kava leaves (No.29) showed a clear signal of 1 at a percentage of 0.2% (Fig.6). In all other samples no 1 above the LOD was detected. Only the SIM-chromatogram of No.2 showed a poor signal at 15.90 min corresponding to 0.02%; however an approval of its identity was not possible due to absence of this signal in "Scan-mode" (TIC). Fig. 7 and 8 show TIC-chromatograms of the "Noble Kava" (No.25Eth) and "Tudei Kava" (No.26Eth).

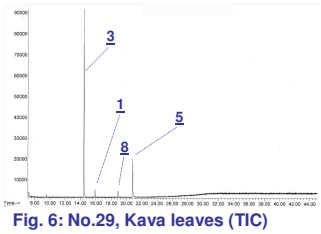


Fig. 6: No.29, Kava leaves (TIC)

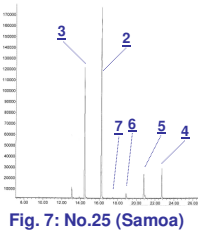


Fig. 7: No.25 (Samoa)

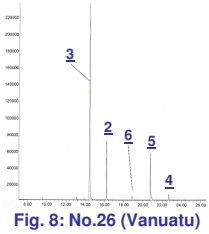


Fig. 8: No.26 (Vanuatu)

References

1. Anke, J. and Ramzan, I. (2004) Planta Med. 70: 193-6.
2. Schmidt, M. et al. (2002) Wien. Med. Wochenschr (WMW): 152, 382-8.
3. Dragull, K. et al. (2003) Phytochemistry 63 : 193-8.

Acknowledgement

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Conclusion

The data clearly show that pipermethystin (1) is absent from all samples above the limit of detection (0.009%). The positive control, leaves of the Kava plant No.29, as expected, shows an amount of 0.2% of 1. Thus, if there is hepatotoxicity, pipermethystin (1) should not be the responsible constituent.