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SHORT COMMUNICATION

ANALYSIS FOR KAWA PYRONES IN EXTRACTS OF *PIPER METHYSTICUM**

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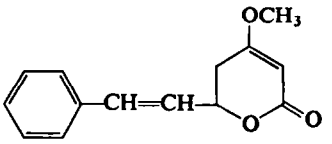
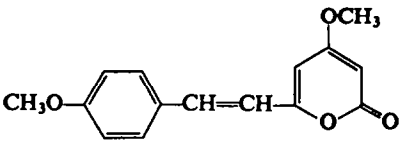
Abstract—The amounts of kawa pyrones (e.g. I and II) in ether extracts of *Piper methysticum* were determined using two-dimensional thin-layer chromatography on aluminium oxide. The concentrations of desmethoxyyangonin, yangonin, dihydromethysticin, methysticin, dihydrokawain, and kawain were determined by u.v. spectroscopy after elution of the spots with methanol. Recoveries of known compounds were 80–85 per cent for yangonin and 90–95 per cent for all others.

INTRODUCTION

DESPITE many years of extensive work on the extracts of *Piper methysticum* (Forst.)[1] a method for determining the concentration of known pyrones (e.g. I and II) in the extracts has not been published. An efficient quantitative analytical procedure would aid in the selection of varieties suitable for commercial cultivation and facilitate further isolation studies. Moreover, determination of the constitution of the extracts may help resolve the questions about



(I) Kawain



(II) Yangonin

varied physiological responses from test animals that have been reported by previous workers.[2, 3]

Determination of yangonin, desmethoxyyangonin, methysticin, dihydromethysticin, kawain, and dihydrokawain was the primary objective for an acceptable analytical procedure because of their dominant role in previous chemical work and because earlier researchers have ascribed to them primary pharmacological importance.[1, 5]

Quantitative thin-layer chromatography offers many advantages because of its simplicity, rapidity and ease of sample recovery for spectrophotometric analysis. One application of

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this technique for separation of kawa drugs has been reported.[4] Although that system, cyclohexane–ethyl acetate on silica gel, gave adequate resolution for qualitative work on mixtures arising in isolation work, we did not find it sufficient for quantitative determinations on the crude extracts.

RESULTS AND DISCUSSION

Thin-layer chromatographic resolution of the crude extracts was found to present an unexpectedly difficult problem, presumably because of the strong interactions between different pyrones (e.g. formation of mixed crystals).[6] The resulting perturbation of the adsorption isotherms accounts for the observation that mixtures of pyrones are less well separated than would be predicted from the chromatography of individual pyrones.

Trials of different development systems on silica gel and alumina layers led to selection of alumina (0·25 mm) as the best adsorbent for separation and recovery of the individual compounds in the amount of extract applied (150–180 μg). Two-dimensional development was used on this layer with *n*-hexane–ethyl acetate (7:3) in the first direction and carbon tetrachloride–*n*-butyl acetate (9:1) in the second. The plate was developed twice in the first dimension and two or three times in the second depending on the separations achieved. The progress of separation was observed under short-wave u.v. light. High concentrations of desmethoxyyangonin and yangonin showed as yellow fluorescent spots, while the other compounds appeared as dark, absorbing spots. Desmethoxyyangonin at low concentrations, as in extracts, also appeared as an absorbing spot. After extended drying, the absorbing spots were no longer visible, so the chromatograms were marked, the spots scraped off, and eluted soon after the solvent evaporated. Traces of solvent which may have remained did not interfere with the spectroscopic determination of pyrones since base line values for absorbance were observed when pyrone-free areas of the chromatograms were run against methanol.

Recovery of the sample from the TLC plate and subsequent quantitative determination was most easily accomplished by elution with pure methanol followed by u.v. spectroscopy. The eluent was passed through a 3–4 mm pad of acid-washed Celite 545 on a cotton filter to remove opalescence prior to examination. Other eluents such as sulfuric acid, dioxane, and chloroform offered no advantage.

The high values of the u.v. extinction coefficients for these pyrones provided a very convenient method for quantitative determination (Table 1). Occasionally kawain and yangonin were poorly separated; however, a correction could easily be made because yangonin absorbed at 355 mμ, whereas kawain did not absorb above 300 mμ. The amount of kawain in the yangonin spot could be determined by calculation from the enhanced absorbance at 244 mμ, the wavelength of the kawain maximum.

Recovery experiments with authentic compounds gave 80–85 per cent for yangonin and 90–95 per cent for all others.

Authentic compounds were isolated by previously described methods[6,7] (Table 1). The u.v. spectra, melting point, and colour reactions were the criteria for identity and purity. The samples isolated from the chromatograms were homogenous and identical to the authentic compounds in their u.v., when rechromatographed in another system[4] and when sprayed with $KMnO_4$–H_2SO_4 reagent at 50°.

[6] R. HÄNSEL and H.-V. BEIERSDORFF, *Arzneimittel-Forsch.* **9**, 581 (1959).
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TABLE 1. PHYSICAL CONSTANTS FOR KAWA PYRONES

Compound	Observed			Literature*	
	λ_{max} nm	Log ϵ	m.p.	Log ϵ	m.p.
Desmethoxyyangonin	342	4·37	138–140°	4·36	138–139°
Yangonin	355	4·48	155–157°	4·48	155–156·5°
	244	4·08			
Dihydrokawain	235	4·14	55·5–57°	4·08	55·2–56·2°
Kawain	244	4·43	106–108°	4·44	106·5–108°
Dihydromethysticin	286	3·64	117·5–119°	3·63	117–118°
Methysticin	305	3·96	141–143°	3·92	136–137°

* Reference 7.

The constituents of six different samples of *P. methysticum* were determined using this analytical method. The results in Table 2 show that this analytical method may be applied to mixtures arising from plant extracts and that other unknown compounds present in the extract will not interfere. They also show that an analysis may be achieved even when one component of the mixture is present in high concentration relative to the others. In addition the simplicity and precision of this method ($\pm$ 1·4 per cent or less) makes it readily applicable to pharmacological or plant-breeding problems.

TABLE 2. ANALYSES OF *Piper methysticum* SAMPLES

Sample	Oil (%)	DMY* (%)	Y (%)	M (%)	DHM (%)	K (%)	DHK (%)	Total percentage of oil
Pahoa 6009	15·7	3·2	9·8	12·5	14·2	24·0	12·6	72·6
		3·3	9·3	12·7	15·8	26·9	12·0	80·0
Pahoa 6000	16·0	2·3	6·3	12·9	14·2	17·8	13·0	66·5
		3·5	6·3	15·2	14·2	16·9	12·5	68·6
Pahoa 6006	17·1	7·1	8·3	12·0	11·8	16·4	7·7	63·3
		6·0	7·0	13·0	12·1	18·6	10·4	67·1
Pahoa 6011	14·6	3·0	9·1	10·3	9·9	9·6	5·8	47·7
		4·1	8·0	8·9	9·3	8·1	5·7	44·1
Wainiha (Green)	16·7	3·2	7·6	12·5	14·7	19·1	7·6	64·7
		4·1	7·1	11·1	14·6	21·9	7·5	66·3
Wainiha (Purple)	16·1	5·2	11·6	15·0	11·0	13·9	7·5	64·2

* Abbreviations—DMY, Desmethoxyyangonin; Y, yangonin; M, methysticin; DHM, dihydromethysticin; K, kawain; DHK, dihydrokawain.

These results also show that there is constancy in the concentration of most constituents between samples. Exceptions are DMY in Pahoa 6006, K in Pahoa 6009 and Wainiha Green, and DHK in Pahoa 6009 and Pahoa 6000. Morphological differences in kawa varieties are often small or obscure as illustrated by comparing the following selections: Pahoa 6009, 6000, and 6011. These selections do not differ morphologically, but the chemical data indicates that 6011 is quite different from 6009 and 6000. The taxonomic value of morphologic and chemical correlations in kawa must be shown by subsequent work in this area.

EXPERIMENTAL

The oil from 1–2 g of dried root (ground to pass a 20 mesh screen) was extracted (7·5 hr) with dry ether using a Goldfisch Fat Extractor. The ether was removed by evaporation and the oil dried at 100° and weighed. The yellow, semi-crystalline oil was stored at −30° until used, when it was diluted to a volume of 10 ml with freshly redistilled dioxane. Storage at 5° prevented decomposition for several months.

Glass plates coated with a 0·250 mm layer of aluminium oxide G were prepared in the usual manner. After air drying and equilibration overnight in a 66% r.h. chamber, the sample (150–180 μg) was applied to one corner of the plate, 15–20 mm from each edge. The plates were then developed twice in the first direction with *n*-hexane–ethyl acetate and up to three times in the second direction using carbon tetrachloride–*n*-butyl acetate (9:1).

After brief air drying the spots were marked under short wave u.v. light, scraped off the plate, and eluted with 2 ml of pure methanol through a filter of acid-washed Celite 545 on a surgical cotton support. The absorbance was determined on a Beckman DK2 recording spectrophotometer.

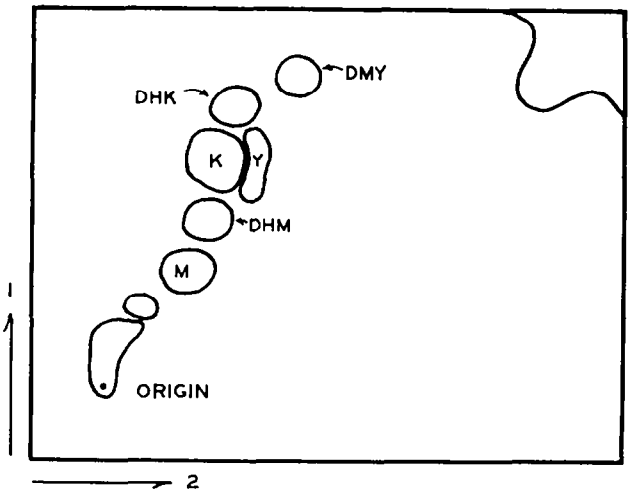

FIG. 1. TYPICAL CHROMATOGRAM OF KAWA ROOT EXTRACT.

1—First dimension, *n*-hexane–ethyl acetate (7:3); 2—second dimension, carbon tetrachloride–*n*-butyl acetate (9:1). See Table 2 for an explanation of the abbreviations.

Figure 1 shows a typical chromatogram obtained by this method. The molar extinction coefficients which were used in calculating the quantity of each compound present in the extract are shown in Table 1.

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