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Gas - Liquid Chromatographic Determination of Major Constituents of *Piper methysticum*

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A procedure is described for the quantitative determination of seven known major constituents in sun-dried roots, rhizomes and commercially powdered samples of *Piper methysticum*. A 3.0–8.0-g amount of powdered sample is extracted with chloroform in a Soxhlet apparatus for 6 h. After evaporation of solvent the extract (about 0.35 g) is dried at 100 °C for 2 h and then dissolved in chloroform to about 0.7% *m/V* concentration. The resulting solution is analysed by gas - liquid chromatography using dual 1.5 m × 4 mm i.d. glass columns, containing 3% *m/m* of OV-1 on Chromosorb W HP, and dual differential flame-ionisation detectors with nitrogen as carrier gas, the column temperature being 210 °C. There is no interference from the eight other trace constituents, non-polar low-boiling compounds or polar "tarry" material.

Keywords: Piper methysticum *analysis*; *gas - liquid chromatography*

Chemical and pharmacological investigations of constituents of the sun-dried roots and rhizomes (known locally as "waka" and "lawena," respectively) of *Piper methysticum*, which began in 1860,[1–3] led to the publication of numerous papers and reviews.[4–6] To date at least seven major and eight trace constituents have been isolated from the roots of the shrub[4–7] (see Table I). A number of these constituents have been shown to possess interesting physiological properties such as sleep-producing,[8,9] antimycotic,[10] local anaesthetic,[11] anti-convulsive[12–14] and smooth muscle contraction[15] effects.

The beverage prepared by straining the powdered roots or rhizomes of *Piper methysticum* with water is a national drink, and is held in high esteem in all traditional Fijian ceremonies. It is also a common traditional drink in other Pacific islands such as Samoa, Tonga and Hawaii. During an investigation by the author of the quality of commercially powdered samples of *Piper methysticum* (known locally as "yaqona" or "kava"), it became necessary to determine the amounts of major constituents. Numerous reports[5–8,16] exist on the column and thin-layer chromatographic separation of the active constituents, but these methods are unsatisfactory and there does not appear to be a simple and rapid method for the quantitation of these constituents. This paper, which deals with the gas - liquid chromatographic analysis of the seven major constituents of *Piper methysticum*, aims to bridge this gap.

Experimental

Structures of the compounds concerned are shown in Fig. 1.

Apparatus

An electrothermal melting-point apparatus, a Hewlett Packard, Model 5730A, gas - liquid chromatograph, a Model 3380A integrator and a Model 7123A recorder were used.

All glassware was cleaned with chromic acid, washed with distilled water and oven dried at 100 °C.

Reagents

All reagents and solvents were of analytical-reagent grade unless otherwise stated. The solvents were chloroform, methanol and benzene. Aluminium oxide of Brockmann activity II (Merck) was used.

Standard Solutions

Standard solution A of major constituents

Prepare the following standard solution in chloroform (*m/V*): 0.12% 7,8-dihydrokawain (1), 0.12% kawain (2), 0.03% 5,6-dehydrokawain (6), 0.02% 5,6,7,8-tetrahydroyangonin (7), 0.075% 7,8-dihydromethysticin (5), 0.075% yangonin (4) and 0.12% methysticin (3).

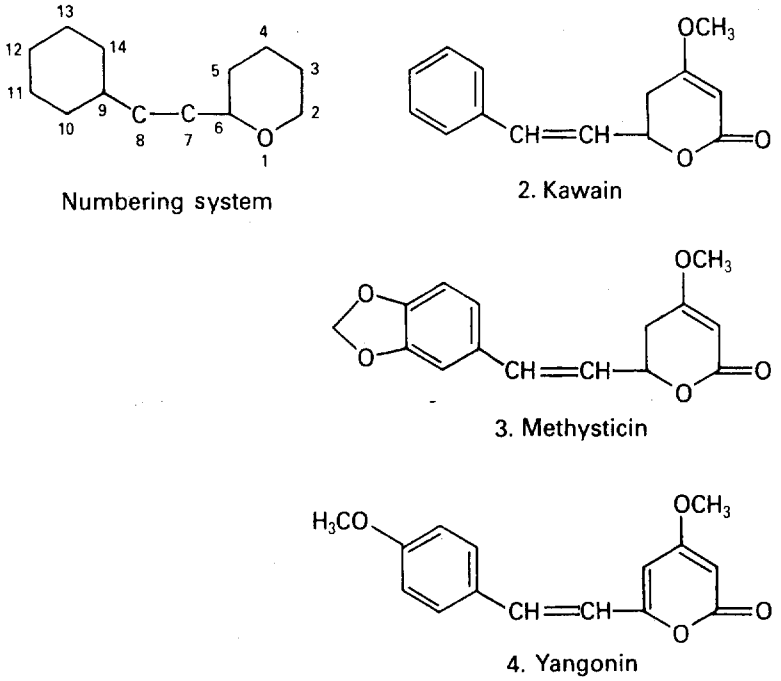

Fig. 1. Structures of the three parent compounds of *Piper methysticum*. The structural formulae for all other kava pyrones, 1 and 5–15, can be derived by using the numbering system above and diagrams 2, 3 and 4.

Standard solution B of trace constituents

The trace constituents were provided by Professor R. Hansel of Frieie Universität, Germany. Prepare a 0.003% *m/V* solution of each of *cis*-5-hydroxykawain (8), 7,8-dihydroyangonin (9), 5,6-dihydroyangonin (10), 5,6-dehydromethysticin (11), 11-methoxyyangonin (12), 11-hydroxyyangonin (13), 11-methoxy-12-hydroxy-5,6-dehydrokawain (14) and 10-methoxyyangonin (15).

Standard solution C of all active constituents

Prepare this solution by combining solutions A and B. It has concentrations of constituents as in the original solutions.

Standard solutions A, B and C have similar constituent ratios and concentrations to those in the samples and sample solutions. The standard and sample solutions are stable for at least 6 months if stored in a refrigerator.

Isolation of Major Constituents

Yangonin (4), methysticin (3), 7,8-dihydromethysticin (5), kawain (2), 5,6-dehydrokawain (6) and 7,8-dihydrokawain (1) were separated by column chromatography on aluminium oxide and purified by repetitive crystallisation as described elsewhere.[8] 5,6,7,8-Tetrahydroyangonin (7) was isolated by a combination of column chromatography[8] and the proposed gas - liquid chromatography and purified by crystallisation from methanol. 7,8-Dihydromethysticin (5), 7,8-dihydrokawain (1) and 5,6,7,8-tetrahydroyangonin (7) were obtained by catalytic hydrogenation of the parent compounds methysticin (3), kawain (2) and yangonin (4), respectively. Hydrogenation was carried out at room temperature on a 2-g sample using 0.1 g of platinum dioxide and 75 ml of ethyl acetate and was complete in 4 h. After filtration and distillation of the solvent *in vacuo*, the hydrogenated products were recrystallised.[8] The hydrogenated products had similar melting-points and mixed melting-points to the naturally occurring compounds.

Preparation of Samples

The dried roots and rhizomes were obtained from local markets, cut into thin slices and ground to pass a No. 25 sieve. Commercial powders were further ground to pass a No. 25 sieve.

Moisture Determination

Dry 5 g of prepared sample at 100 °C for 5 h.

Extraction of Constituents

Extract 3.0 g of prepared roots or 5.5 g of rhizomes or 8.0 g of commercial powders with chloroform in a Soxhlet apparatus for 6 h. Evaporate the solvent on a water-bath and dry the extract at 100 °C for 2 h. Dissolve the dried extract (about 0.35 g) in chloroform and make up to 50 ml with chloroform in a calibrated flask to give a concentration of about 0.7% *m/V* (sample solution D).

Gas - Liquid Chromatography

Inject 3 μl each of standard solution A and sample solution D into the gas chromatograph using the following conditions: column, dual 1.5 m × 4 mm i.d. glass containing 3% *m/m* of OV-1 on Chromosorb W HP; detector, dual differential flame-ionisation; carrier gas, nitrogen at 300 kPa and 60 ml min^{-1}; fuel gases, hydrogen at 150 kPa and 60 ml min^{-1} and air at 180 kPa and 240 ml min^{-1}; column temperature, 210 °C; detector temperature, 300 °C; injection port temperature, 250 °C; and integrator, sensitivity 0.3–1.0 mV min^{-1}, attenuation 64, chart speed 5 mm min^{-1}.

Determination of Polar "Tarry" Material in Extract by Clean-up on an Aluminium Oxide Column

Wash 4–5 g of aluminium oxide contained in a glass column (12 mm diameter) with 20 ml of chloroform. Apply a suitable amount (generally about 0.35 g) of extract in 5 ml of chloroform to the washed column, elute with 50 ml of chloroform and collect the eluate in an already dried and weighed 100–150-ml conical flask. Evaporate the solvent on a water-bath and dry the extract at 100 °C for 2 h to give a cleaned-up extract. The difference in mass represents polar "tarry" material.

Thin-layer Chromatography

Thin-layer chromatography was carried out on 0.5-mm silica gel G plates with benzene - methanol (98.5 + 1.5) as developing solvents and the spots were revealed with iodine vapour.

Results and Discussion

The seven major and eight trace constituents of the roots and rhizomes of *Piper methysticum* are as presented in Table I and Fig. 1. The constituents are all derivatives of the three variant compounds, namely kawain (2), methysticin (3) and yangonin (4). The major constituents have traditionally been isolated by column chromatography[8,16] and the trace constituents by preparative thin-layer chromatography.[7] We have found that neither column nor thin-layer chromatography could be used for the quantitative determination of the constituents, as in the former the separation is incomplete and in the latter all of the major constituents have similar R_F values (Table I). The proposed gas - liquid chromatographic analysis of the major constituents overcomes this problem and offers a simple and rapid technique for their determination in *Piper methysticum*.

Extraction of Constituents

Chloroform,[8] diethyl ether[16] and ethanol[16] have been used for the extraction of constituents of *Piper methysticum*. Some of the constituents, such as methysticin (3) and yangonin (4), are insoluble in diethyl ether but all constituents are soluble in chloroform. We therefore used chloroform for the extraction work. It was found that 95% and 99% of the constituents are extracted in the first 2 h and 4 h, respectively, and we therefore used 6 h as the extraction time. The difference in the amounts of roots, rhizomes and commercial powders of *Piper methysticum* taken for extraction is to ensure that uniform amounts of extract (*ca.* 0.35 g) are obtained.

Quantification and Linear Range

A typical chromatogram is shown in Fig. 2, and the tested linear range limits and retention times of the various constituents are reported in Table I.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS AND CONCENTRATIONS OF CONSTITUENTS OF *Piper Methysticum*

Compound No.	Constituents	Melting-point/°C	Retention time (GLC) at 210 °C/min	Tested limit of linear range/μg	Retention time at 240 °C/min	R_F (TLC)	Average content of constituents on dry mass basis, % *m/m*		
							Roots (6 samples)	Rhizomes (6 samples)	Commercial powder (12 samples)
	Major constituents—								
1	7,8-Dihydrokawain	55–57[17]	5.88	4.8	2.4	0.41	2.37	1.20	0.70
2	Kawain	107[17]	8.01	4.8	2.8	0.43	1.90	1.17	0.84
3	Methysticin	139–140.5[18]	27.0	—	8.0	0.39	2.12	1.00	0.69
4	Yangonin	155–157[19]	25.63	3.0	8.0	0.48	1.73	0.70	0.47
5	7,8-Dihydromethysticin	117–118[18]	18.33	3.0	6.4	0.39	1.12	0.69	0.61
6	5,6-Dehydrokawain	138–139[8]	10.27	1.2	3.6	0.52	0.81	0.32	0.18
7	5,6,7,8-Tetrahydroyangonin	99–100[7]	13.12	0.75	4.4	0.43	0.39	0.20	0.14
	Total of 1–7	—	—	—	—	—	10.44	5.28	3.63
7a	Tarry material	—	—	—	—	0.00	0.71	0.55	0.41
	Total of 1–7a	—	—	—	—	—	11.15	5.83	4.04
	Chloroform extract	—	—	—	—	—	12.21	6.61	4.69
	Other constituents—	—	—	—	—	—	1.06	0.78	0.65
	I Non-polar compounds		<5.0		<2.0	>0.65			
	II *Trace constituents*—								
8	*cis*-5-Hydroxykawain	120–122[7]	9.58	—	4.0	0.10			
9	7,8-Dihydroyangonin	104–106[7]	11.80	—	3.2	0.48			
10	5,6-Dihydroyangonin	122–124[7]	Decomposes	—	Decomposes	0.37			
11	5,6-Dehydromethysticin	230–231[7]	37.54	—	10.8	0.50			
12	11-Methoxyyangonin	155–157[7]	50.18	—	13.6	0.40			
13	11-Hydroxyyangonin	196–200[7]	50.18	..	13.6	0.18			
14	11-Methoxy-12-hydroxy-dehydrokawain	119–220[7]	50.18	—	13.6	0.19			
15	10-Methoxyyangonin	191–192[7]	56.12	—	14.8	0.42			

For 7,8-dihydrokawain (1), kawain (2), 5,6-dehydrokawain (6), 5,6,7,8-tetrahydroyangonin (7) and 7,8-dihydromethysticin (5), quantification was effected by comparisons of either standard and sample peak heights or integrated peak areas. However, the peak of yangonin (4) could not be completely separated from that of methysticin (3), which appears as a shoulder on the former. However, the peak height of yangonin (4) is linearly related to amount, irrespective of the amount of methysticin (3) present, and therefore can be determined. However, as the peak height of methysticin (3) is not linearly related to amount, the amount of methysticin (3) is determined by matching its peak heights in a standard and a sample. This is made simple by the fact that the ratio of yangonin (4) to methysticin (3) in roots, rhizomes and commercial powders of *Piper methysticum* is reasonably constant (1.7:2.1, 1.54:2.1 and 1.5:2.1, respectively).

Clean-up of Extract

The total amount of the major constituents 1–7 (see Table I) was found to be lower than that in the total chloroform extract. Thin-layer chromatography of standard C and a chloroform extract of *Piper methysticum* root sample showed that in addition to the major and trace constituents, the root extract contained some non-polar compounds and polar "tarry" material. The polar "tarry" material had an R_F value of 0. The amount of "tarry" material could be determined as described under Experimental. Between 10 and 60 ml of standard solution A, representing 0.056–0.336 g of major constituents, and between 10 and 40 ml of standard solution B, representing 0.0021–0.0084 g of trace constituents were subjected to the clean-up procedure, and subsequent analysis showed no significant adsorption of active constituents on the column at these concentrations. However, clean-up of 0.25–40 g of a chloroform extract of *Piper methysticum* roots on aluminium oxide showed an average adsorption of 0.7% *m/m* (based on the dry mass of the sample) of polar "tarry" material; thin-layer chromatography of the cleaned-up extract showed the absence of polar "tarry" material. However, the amount of the major constituents in the cleaned-up extract was the same as that in the unpurified extract. Hence, although the clean-up procedure provides a method for the determination of polar "tarry" material, it is not necessary for the determination of active constituents.

Interferences

Using the concentration ranges of standards and samples mentioned under Clean-up of Extract, it was established that heating for 6 h under reflux during extraction and drying for 2 h at 100 °C had no significant effects on the analysis. There was no interference by

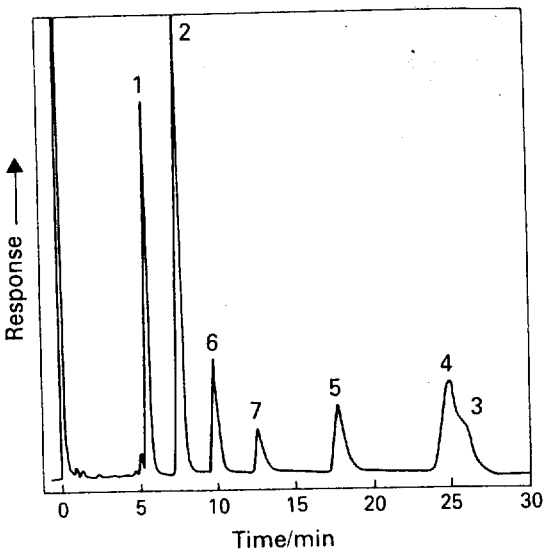

Fig. 2. Gas - liquid chromatogram obtained from a 3-μl injection of a 0.7% *m/V* solution of chloroform extract of *Piper methysticum*. Other conditions are as stated in text. Peaks: 1 = 7,8-dihydrokawain; 2 = kawain; 3 = methysticin; 4 = yangonin; 5 = 7,8-dihydromethysticin: 6 = 5,6-dehydrokawain; and 7 = 5,6,7,8-tetrahydroyangonin.

the trace constituents, as these have different retention times (see Table I). In addition, the comparatively low concentration of trace constituents in the samples did not give rise to any peaks under the given conditions of analysis. The analysis was complete within 0.5 h. A column temperature of 210 °C was used to distinguish the methysticin (3) peak from the yangonin (4) peak within a reasonable time. An increase in temperature resulted in the collapse of these two peaks, whereas a decrease in temperature only increased retention times without any improvement in the separation of the two peaks. It is clear from Table I that the proposed method is not satisfactory for the determination of trace constituents, owing mainly to the similar and relatively high retention times of some of the constituents. We are currently studying methods of analysis of trace constituents. There is also no interference by polar "tarry" material or non-polar low-boiling compounds, as the latter elute before any of the active constituents.

Precision

Satisfactory reproducibilities were obtained by the proposed method (Table II).

TABLE II

CONTENTS OF MAJOR CONSTITUENTS, CHLOROFORM EXTRACT AND POLAR "TARRY" MATERIAL FROM 10 RUNS ON A RHIZOME SAMPLE OF *Piper methysticum*

Constituent	Content, % *m/m*										Average	Standard deviation
7,8-Dihydrokawain	1.05	1.14	1.11	1.06	1.11	1.07	1.08	1.08	1.08	1.09	1.08	2.78
Kawain	1.22	1.22	1.25	1.25	1.28	1.31	1.21	1.27	1.29	1.28	1.26	2.38
Methysticin	1.15	1.10	1.12	1.21	1.17	1.17	1.13	1.17	1.19	1.17	1.16	2.59
Yangonin	0.82	0.82	0.87	0.84	0.87	0.79	0.81	0.83	0.84	0.86	4.84	3.57
7,8-Dihydromethysticin	0.68	0.66	0.66	0.65	0.71	0.62	0.66	0.68	0.68	0.69	0.67	2.99
5,6-Dehydrokawain	0.28	0.30	0.30	0.30	0.31	0.29	0.29	0.31	0.30	0.30	0.30	3.33
5,6,7,8-Tetrahydroyangonin	0.128	0.127	0.127	0.129	0.130	0.121	0.121	0.128	0.125	0.126	0.127	1.57
Polar "tarry" material	0.71	0.73	0.66	0.61	0.67	0.64	0.72	0.65	0.71	0.63	0.67	5.97
Chloroform extract	6.56	7.02	6.78	6.92	6.56	6.54	6.79	6.66	6.96	7.00	6.78	2.80

Conclusion

A simple and rapid gas - liquid chromatographic technique has been developed for the determination of the major constituents of *Piper methysticum*. This initial study has also shown considerable variations in the amounts of major constituents in roots, rhizomes and commercial powders. It is expected that the proposed method of analysis will find use in the column chromatographic separation and purification of the major constituents of *Piper methysticum*. The procedure described here is also being used for a study of variations in the amounts of constituents in roots, rhizomes and commercial powders of local *Piper methysticum* as part of a general survey of the quality of the local product.

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