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A Chemical and Pharmacological Investigation of *Piper Methysticum* Forst.

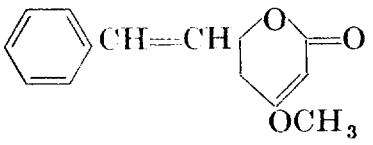
M. W. KLOHS, F. KELLER, R. E. WILLIAMS, M. I. TOEKES and G. E. CRONHEIM

Medicinal Chemistry and Biological Sciences Sections, Research Division, Riker Laboratories, Inc., Los Angeles, California

Introduction

Piper methysticum Forst. (family Piperaceae) is a shrub common to the islands of Polynesia, where it is known in various dialects as Kawa, Kava or Ava. The root of the kawa plant has been held in high esteem by the Polynesians from ancient times for its use in preparing an extract which, upon drinking, is reported to reduce fatigue and produce complete freedom from anxiety.[1]

The remarkable physiological action of *Piper methysticum* has prompted numerous chemical investigations, dating from that of GOBLEY and O'RORKE in 1850.[2] The most extensive work on the constituents of this plant was carried out by BORSCHE[3] and his co-workers, who, in a series of 13 papers, reported the isolation and structure of two new compounds, kawain (I)[4] and dihydrokawain (II),[4] and the structural elucidation of the other crystalline components, methysticin (III),[5] dihydromethysticin (IV)[6-a, b] and yangonin (V),[7] which had previously been isolated from this



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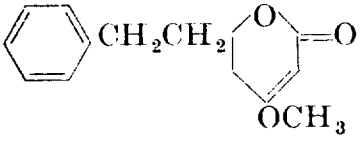

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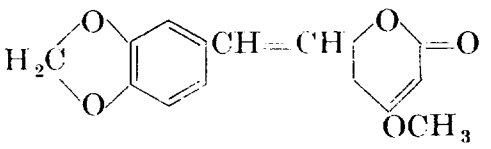

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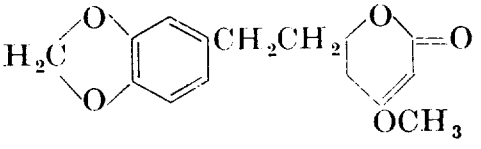

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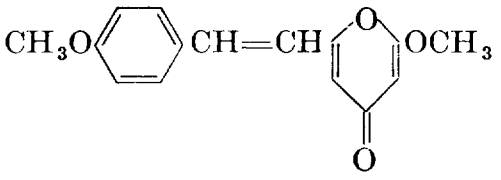

V

plant. At the conclusion of his work, Borsche stated that all of these compounds failed to exhibit the typical physiological action of the kawa root.[3] In a later study, utilizing chromatographic techniques, VAN VEEN isolated a substance 'marindinin'[1] which, when administered in a lecithin-water emulsion to pigeons and monkeys, exhibited the drug action of kawa; this compound was subsequently shown, however, to be identical with dihydrokawain.[8]

The intriguing physiological effects attributed to the use of *Piper methysticum* by the Polynesians and the lack of pharmacological evidence available to substantiate the activity of the drug, prompted us to investigate this plant species in a continuation of our search for compounds possessing desirable central nervous system activity. A chloroform extract of the kawa root was utilized for isolation studies, since pharmacological screening showed it to possess the action of the whole root as measured by its ability to antagonize strychnine convulsions in mice, to potentiate sodium pentobarbital-induced sleeping time and to cause increased incidence of fall-out in roller cage experiments. The extract obtained in this manner was subjected to chromatographic separation, and by this procedure all of the known constituents, kawain, dihydrokawain, methysticin, dihydromethysticin and yangonin, were readily obtained and their identity established by a comparison of their physical constants with those reported in the literature and by elementary analyses.

A small quantity of a new, optically inactive substance was also isolated from this extract, which we have designated 'compound A'. The elementary analysis and molecular weight determinations were in agreement with the empirical formula $C_{14}H_{12}O_3$; functional group analysis demonstrated the presence of one methoxyl group. Further work towards the structural elucidation of this substance is in progress and will be reported at a later date.

Pharmacology

The ground root of *Piper methysticum*, a chloroform extract obtained therefrom, and several of its crystalline constituents, were studied for effects on the central nervous system as determined by their ability to antagonize clonic strychnine convulsions and death in mice, cause fall-out in roller cage experiments and potentiate sodium pentobarbital-induced sleeping time. The results of these tests are tabulated in Table I.

Table I.

	Strychnine ED50 w. 95% cl. in mg/kg	Roller cage		Sleeping time	
		dose, mg/kg	result	dose, mg/kg	sl. time in % of controls
Dihydrokawain	340 (270–430)	300	no effect	160	150
Yangonin	no protection at 1,000	300	no effect	160	150
Kawain	215 (160–290)	300	no effect	160	235
Compound A	no protection at 200	300	no effect	160	130
Methysticin	160 (110–232)	300	no effect	160	250
Dihydromethysticin	115 (97–152)	300	no effect	60	413
Chloroform extract	140 (121–162)	300	12/18 fall-out	160	340
Ground root	1,700 (1,400–2,100)	10,000	12/18 fall-out	10,000	400
Meprobamate	no protection at 700	ED50 = 165 (122–201)		160	250

The crude extract, methysticin and dihydromethysticin were particularly effective in affording protection against the lethal effects of strychnine, while yangonin and compound A were practically without effect. The time of peak action was determined for dihydromethysticin and was found to be around 60 min. (Table II).

Dihydromethysticin appeared to be the most potent agent in increasing pentobarbital-induced sleeping time. This was true not only for the condition of the experiments summarized in Table I, but also in additional experiments with varying doses of several compounds (Table III) or varying times after administering the drug (Table IV).

Using 'fall-out' from revolving (roller) cages as an index, none of the crystalline compounds had significant activity. This was

Table II. Effect of dihydromethysticin against strychnine convulsions and death

Time after drug, min.	30	60	240
ED50 in mg/kg (95% confidence limit)	115 (97–152)	64 (53–78)	130 (100–148)

in sharp contrast to the ground root and the crude extract. It thus appears likely that the activity of the root and of the extract

Table III. Pentobarbital sleeping time in % of controls ±standard deviation

Dose, mg/kg	Dihydromethysticin	Methysticin	Chloroform extract
10	152±30		
10	240±27		
40	457±43		
60	896	186	134
160	1800	257	250
250			440

in this test was due either to a synergistic action of the known constituents or to the presence of a compound or compounds not

Table IV. Pentobarbital sleeping time after dihydromethysticin (60 mg/kg)

Time after drug in h	½	1	4	24
Sleeping time in % of controls (±standard deviation)	410	630±160	370±80	54±8

as yet isolated. An indication of a synergistic action was found by testing a mixture of kawain (19·5 per cent), dihydrokawain

(33·4 per cent), methysticin (19·5 per cent), dihydromethysticin (5·5 per cent), yangonin (16·6 per cent) and compound A (5·5 per cent) against strychnine convulsions and death. These compounds were combined in the ratio in which they were isolated from the crude extract. The mixture showed an ED50 of 100 (79–127) mg/kg, indicating a potency at least as good as that of dihydromethysticin. Since the latter represented only 5·5 per cent of the mixture, and since the other constituents were less potent or inactive, a synergistic effect of the mixture appears most likely.

A most remarkable property of all these compounds was their quick absorption from the gastro-intestinal tract although they are insoluble in all applicable solvents.

Experimental*

Chemistry

Extraction of Piper methysticum *Forst.* The ground dried root of *Piper methysticum* Forst.† (5 kg) was extracted twice for 3 and 16 h, respectively, with chloroform (30-l. portions); the combined extracts were concentrated *in vacuo* on the steam bath to a yellow aromatic oil (321 g) which partially crystallized on standing.

Chromatography of Crude Extract from *Piper Methysticum* Forst.

The crude extract (38 g) was dissolved in 1 : 1 'Skelly B'-benzene (240 ml) and applied to a column of Merck acid washed alumina (750 g). Successive 100-ml fractions of eluant were collected and combined on the basis of their infrared spectra as indicated overleaf:

Isolation of Known Constituents

Dihydrokawain. Fractions 1–5 and 6–11 when crystallized from ether–'Skelly B' yielded dihydrokawain (1·3 g; 2·4 g,

* All melting points are uncorrected.

† This plant material was gathered in Hawaii and its identity confirmed by Dr. H. W. Younken, Massachusetts College of Pharmacy, Boston, Massachusetts.

Fractions	Eluant	Weight (g)
1–5	1:1 'Skelly B'-benzene	5·42
6–11	,, ,, ,,	6·26
12–17	,, ,, ,,	3·91
18–25	,, ,, ,,	2·87
26–42	,, ,, ,,	3·43
43–82	1:3 ,, ,,	8·16
83–102	benzene	3·52
103–142		2·5

respectively); on recrystallizing, the compound melted at 55·2–56·2°, $[\alpha]_D^{24}+34°$ (c, 1 in abs. ethanol); lit.[4] m.p. 56–58°, $[\alpha]_D^{19}+30°$ (c, 1 in abs. ethanol). The ultraviolet spectrum showed: $\lambda_{max.}^{alc.}$ (log ϵ), 205 mμ (4·11), 234 mμ (4·08), 343 mμ (2·92); $\lambda_{min.}^{alc.}$ (log ϵ), 219 mμ (3·91), 295 mμ (2·57). The infrared spectrum showed: $\lambda_{max.}^{Nujol}$ 5·87 μ (s), 6·14 μ (s).

For analysis the sample was dried to constant weight at room temperature (2 mm).

Anal. Calcd. for: $C_{14}H_{16}O_3$; C, 72·39; H, 6·92. Found: C, 72·24; H, 7·03.

Kawain. On dissolving fractions 12–17 and 17–25 in methanol-ethyl ether and allowing to stand, kawain (1·13 g; 0·4 g) was obtained; on recrystallization from ether, the compound melted at 106·5–108°. $[\alpha]_D^{25}+97°$ (c, 1·0 in ethanol); lit.[4] m.p. 106°; $[\alpha]_D^{20}+105°$ in ethanol. The ultraviolet spectrum showed: $\lambda_{max.}^{alc.}$ (log ϵ), 244 mμ (4·441); $\lambda_{min.}^{alc.}$ (log ϵ), 222 mμ (4·06). The infrared spectrum showed: $\lambda_{max.}^{Nujol}$ 5·87 μ (s), 6·14 μ (s).

For analysis the sample was dried to constant weight at room temperature (2 mm).

Anal. Calcd. for: $C_{14}H_{14}O_3$: C, 73·02; H, 6·13; OCH_3, 13·47; mol. wt., 230·25. Found: C, 72·65; H, 6·44; OCH_3, 13·99; mol. wt. (Rast), 245.

Yangonin. Fractions 43–82, when crystallized from methanol, yielded yangonin as yellow crystals (1·4 g); on recrystallization, the compound melted at 155–156·5°, $[\alpha]_D^{24}=0$ (c, 0·3 in ethanol); lit.[9] m.p. 153–154°; the ultraviolet spectrum showed: $\lambda_{max}^{alc.}$

(log ϵ), 217 mμ (4·43), 357 mμ (4·48), shoulder at 260 mμ (3·94); $\lambda_{min.}^{alc.}$ (log ϵ), 282 mμ (3·67). The infrared spectrum showed: $\lambda_{max.}^{Nujol}$ 5·87 μ (s), 6·04 μ (w), 6·15 μ (s), 6·22 μ (w), 6·60 μ (m), 6·67 μ (s).

For analysis the sample was dried to constant weight at 80° (2 mm).

Anal. Calcd. for: $C_{15}H_{14}O_4$: C, 69·75; H, 5·46. Found: C, 69·38; H, 5·49.

Dihydromethysticin. The material remaining in the mother liquors of fractions 43–82 after the crystallization of yangonin, yielded dihydromethysticin on further fractional crystallization from methanol. After recrystallization from methanol the material (0·8 g) had m.p. 117–118°, $[\alpha]_D^{24}+19°$ (c, 1·0 in MeOH). Lit.[6b] m.p. 117–118°, $[\alpha]_D+20·57°$ (c, 0·7 in MeOH). The ultraviolet spectrum showed: $\lambda_{max.}^{alc.}$ (log ϵ) 233 mμ (4·22), 287 mμ (3·63); $\lambda_{min.}^{alc.}$ (log ϵ), 217 mμ (4·00), 265 mμ (3·40). The infrared spectrum showed: $\lambda_{max.}^{Nujol}$ 5·86 μ (s), 6·17 μ (s), 6·67 μ (s), 6·72 μ (s).

For analysis the sample was dried to constant weight at room temperature (2 mm).

Anal. Calcd. for: $C_{15}H_{16}O_5$: C, 65·21; H, 5·84; OCH_3, 11·23. Found: C, 65·30; H, 5·99; OCH_3, 11·55.

Methysticin. Fractions 83–142 yielded methysticin (0·7 g) on crystallization from methanol, along with traces of yangonin and dihydromethysticin. On recrystallization from methanol, needles were obtained, m.p. 139–140·5°, $[\alpha]_D^{28}+95°$ (c, 1 in acetone), lit.[6b, 10], m.p. 136–137°, $[\alpha]_D^{20}+94·30°$ (c, 5 in acetone). The ultraviolet spectrum showed: $\lambda_{max.}^{alc.}$ (log ϵ), 226 mμ (4·39), 264 mμ (4·13), 305 mμ (3·92); $\lambda_{min.}^{alc.}$ (log ϵ), 253 mμ (4·08), 284 mμ (3·79). The infrared spectrum showed: $\lambda_{max.}^{Nujol}$ 5·87 μ (s), 6·04 μ (w), 6·5 μ (s), 6·22 μ (w), 6·60 μ (m), 6·67 μ (s).

For analysis the sample was dried to constant weight at room temperature (2 mm).

Anal. Calcd. for: $C_{15}H_{14}O_5$: C, 65·59; H, 5·15; OCH_3, 11·31; mol. wt. 274·26. Found: C, 65·30; H, 5·30; OCH_3, 10·94; mol. wt. (Rast), 285.

Isolation of compound A. Fractional crystallization from ethyl ether of the material remaining in the mother liquors of fractions 6–11, after the crystallization of kawain, yielded a hitherto

undescribed substance (0·45 g); on recrystallization from methanol, the compound melted at 138–139°, $[\alpha]_D^{24}$ 0° (c, 0·7 in ethanol). The ultraviolet showed: $\lambda_{max.}^{alc.}$ (log ϵ), 210 mμ (4·32), 225 mμ (4·20), 232 mμ (4·21), 225 mμ (4·13), 343 mμ (4·36); $\lambda_{min.}^{alc.}$ (log ϵ), 222 mμ (4·18), 228 mμ (4·18), 243 mμ (4·02), 273 mμ (3·73). The infrared spectrum showed: $\lambda_{max.}^{Nujol}$ 5·79 μ (s), 6·10 μ (m), 6·22 μ (m), 6·43 μ (m).

For analysis the substance was dried to constant weight at room temperature (2 mm).

Anal. Calcd. for: $C_{14}H_{12}O_3$: C, 73·67; H, 5·30; OCH_3, 13·6; mol. wt. 228. Found: C, 73·56; H, 5·56; OCH_3, 14·09; mol. wt. (Rast), 230.

Pharmacology

Methods

Male and female Carworth mice, in the weight range of 18–22 g, were used in all experiments. All materials to be tested were administered by the oral route, in 10 per cent Tween suspension, due to their insolubility in all applicable solvents.

(*a*) *Sleeping Time.* Pentobarbital sodium at a dose of 65 mg/kg by the intraperitoneal route was used for anaesthesia. Medication was administered 30 min or as indicated prior to the pentobarbital injection. Each compound was tested on at least one group of ten mice. The sleeping time of a group of treated animals was expressed as the percentage of that of a simultaneous control group. A value below 130 per cent was statistically insignificant ($p > 0·05$) and therefore considered a negative result.

(*b*) *Strychnine Convulsion.* A single intraperitoneal injection of 2·6 mg (LD90) of strychnine sulphate was given 15 min after medication. If the material protected the animals against strychnine-induced clonic convulsions and death, graded doses of the compound were tested and an ED50 (with 95 per cent confidence limits) was calculated according to LITCHFIELD and WILCOXON.[11] For the preliminary testing, ten mice were used. The ED50 was determined by using at least four groups of ten mice each, at four different dose levels.

(*c*) *Roller Cage.* The method has been described previously.[12]

A preliminary test was performed on 18 mice. The ED50 was determined by using four groups of 18 mice each, at four different dose levels.

Summary. The isolation of the known constituents of *Piper methysticum* Forst. by a chromatographic procedure is described, along with the isolation of a new substance designated as compound A ($C_{14}H_{12}O_3$). The results of pharmacological screening for central nervous system activity of these compounds, as well as the whole root and a crude extract, are presented. These substances are tested for their ability to antagonize strychnine convulsions, potentiate barbiturate sleeping time and cause 'fall-out' in roller cage experiments.

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