Volume 26 March • 1963 Number 1

LLOYDIA

A Quarterly Journal of Pharmacognosy and Allied Biological Sciences

Published by THE LLOYD LIBRARY AND MUSEUM
and THE AMERICAN SOCIETY OF PHARMACOGNOSY

A Review of the Chemistry and Pharmacology of the Constituents of *Piper methysticum*[1]

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Captain James Cook in the account of his voyage to the South Seas in 1768 first described the native use of an intoxicating drink prepared from *Piper methysticum* Forst. (19). The properties ascribed to this beverage variously called kawa, kava, ava, awa, yangona, kawa-kawa, kava-kava, and wati, and the important role which it played in the social, economic, and religious life of the peoples inhabiting the regions where it was used have excited the curiosity of ethnologists, botanists, chemists, pharmacologists, and clinicians during a period of nearly two centuries. This interest of representatives of so many scientific disciplines has resulted in the accumulation of a great store of literature concerning *P. methysticum*, with the preponderance of this material dealing in the social sciences. A very comprehensive review of all aspects of the use of *P. methysticum* and current research on its constituents and their chemistry, pharmacology, and clinical effects was published in the form of a monograph by L. Lewin in 1886 (41). This admirable work is now, understandably, much out of date as regards chemistry and pharmacology. Two recent historical surveys (24, 55) covering the use of kawa have appeared, and a review (46) covering the chemistry of some of the compounds to be dealt with here, has just been published. No unified modern treatment of the chemistry and pharmacology of *P. methysticum* is currently available, however, and this paper has been prepared in an attempt to fulfill this need.

Piper methysticum Forst. has been assigned the following synonomy by Seemann (54).

Macropiper methysticum Miq.
Piper spurium Forst.
Piper inebrians Sol.
Piper decumanum Opiz.

The plant is an upright, large leaved shrub with a pithy, jointed stem, growing to an average height of approximately six feet (54), although in some forms (41) the plant is said to attain a height of ten to twelve feet. In Fiji, six varieties were recognized (54) by the natives, whereas in Tahiti no less than fourteen were known (41), and in the Marquesas twenty-one varieties were listed (24). The natives placed different values on these forms according to the strength of the drink which could be prepared from them; however, no systematic scientific survey appears to have been made as to the relative potency of extracts from the various forms of *P. methysticum*. The published studies generally have been carried out upon samples of plant material identified only as being the dried root of *P. methysticum* and since all of the growth forms would most likely not be thought worthy of

[1]This article was developed from a paper presented at the Third Annual Meeting of the American Society of Pharmacognosy (35).

recognition as separate taxa by plant taxonomists, this area remains one for possible future study and clarification.

The root and rootstock were generally used in making the kawa beverage which was prepared by first chewing the root, covering the masticated mass with water, straining out the fibrous residue, and serving the resulting fluid in ceremonies which have been well described by Seemann (54) and Lewin (41), as well as more recent writers (24, 55). Because of the ready availability of good accounts of the kawa ceremony in standard anthropology texts and available reviews (24, 55), no attempt will be made to describe the rituals here.

Kawa was principally renowned for its reputed intoxicating qualities wherein the drinker was said to attain a state of quiet relaxation differing from that produced by alcohol in that it induced a placid tranquility accompanied by incoherent dreams (54). Hocart reports (34) that the intoxication dulls the countenance and produces a lazy feeling without obscuring the reasoning. It is of interest to note that there is some disagreement among authors concerning the potency of the kawa beverage and the effects evoked by it. Steinmetz (55) claims that a wine glass full of kawa beverage induces a profound dreamless sleep, whereas Seemann (54) notes that the beverage causes incoherent dreams. Kawa was used medicinally by the natives as a diuretic, sudorific, analgesic, antipyretic, and for the treatment of venereal diseases, gout, rheumatism, diarrhea, asthma, and as a blood purifier (41, 52, 54). Over-use of kawa sometimes produced a curious affliction termed "kawaism," wherein the skin became flaky and the user's eyes became red and rheumy (34, 41, 54).

Physicians in Europe were quick to explore the use of kawa, employing it mainly for its sudorific and antivenereal activities. Seemann (54) quotes Dr. O'Rorke as stating, "The Kawa plant is the most powerful sudorific in existence. . . ." As noted by Schübel (52) it is peculiar that none of the early European medicaments containing kawa were directed toward its effect upon the central nervous system, but were instead intended as urinary antiseptic agents. Perhaps the most important of these preparations was "Gonosan," a kawa resin-sandalwood oil compound. It was not until the 1920's that a kawa preparation, "Neurocardin" (52) appeared on the European market which was claimed to be a clinically effective hypotensive and sedative agent. This preparation does not appear to have enjoyed wide usage. It is a strange commentary upon the kawa story that despite a wealth of investigation over a long period of time the history of the use of this material is full of contradictions as to its effectiveness.

A controversial point which has not been completely resolved to the present day (44, 56) regards the importance of the method of preparation in producing the activity ascribed to kawa. The fact that the original means of preparing the kawa beverage was by chewing the plant material has caused many people to believe that perhaps enzymatic breakdown was necessary to produce activity. Lewin claimed that if a chewed kawa preparation was carefully filtered none of the sedative action was observed and that the resulting filtrate was like a soft drink (41), whereas if particles of the finely divided resin were allowed to filter through a more coarse filter, activity would be obtained. He therefore believed that the physiologically active components resided in the resin and was convinced that the chewing did nothing more than to break up the root and emulsify the resin so that it could be assimilated into the liquid. van Veen in his later studies arrived at a similar conclusion (56).

CHEMISTRY

The chemical investigation of *Piper methysticum* began in 1860 with the publication of the work of Gobley and O'Rorke (25, 49) and the almost simultaneous study by Cuzent (21). These workers, aside from resinous products and inorganic salts, succeeded in isolating a crystalline neutral organic compound variously called methysticin (25, 49) and kawahin (21). The former name has taken

precedence. In 1874 Nölting and Kopp (45) succeeded in isolating a second neutral crystalline material, whose isolation was later repeated by Lewin, who named this compound yangonin (41). A resinous mixture called kawin by O'Rorke was separated into so-called α- and β-resin fractions by Lewin on the basis of their solubility in petroleum ether.

The period following the appearance of Lewin's monograph saw the first real progress being made in the chemistry of the constituents of *P. methysticum* with the publication of a series of fourteen papers by Borsche and his co-workers, between 1914 and 1933 (3–16). During this period these workers accomplished the isolation of two new constituents, kawain and dihydrokawain and succeeded in establishing structures for these as well as the three previously known components yangonin, methysticin, and dihydro-methysticin. In more recent times subsequent workers have isolated 5,6-dehydromethysticin (46), 11-methoxy-yangonin (46), desmethoxy-yangonin (36, 37), flavokawin "A" (29), flavokawin "B" (29), and an unidentified carbonyl compound (51) from kawa. The chemistry of these compounds will be dealt with separately below.

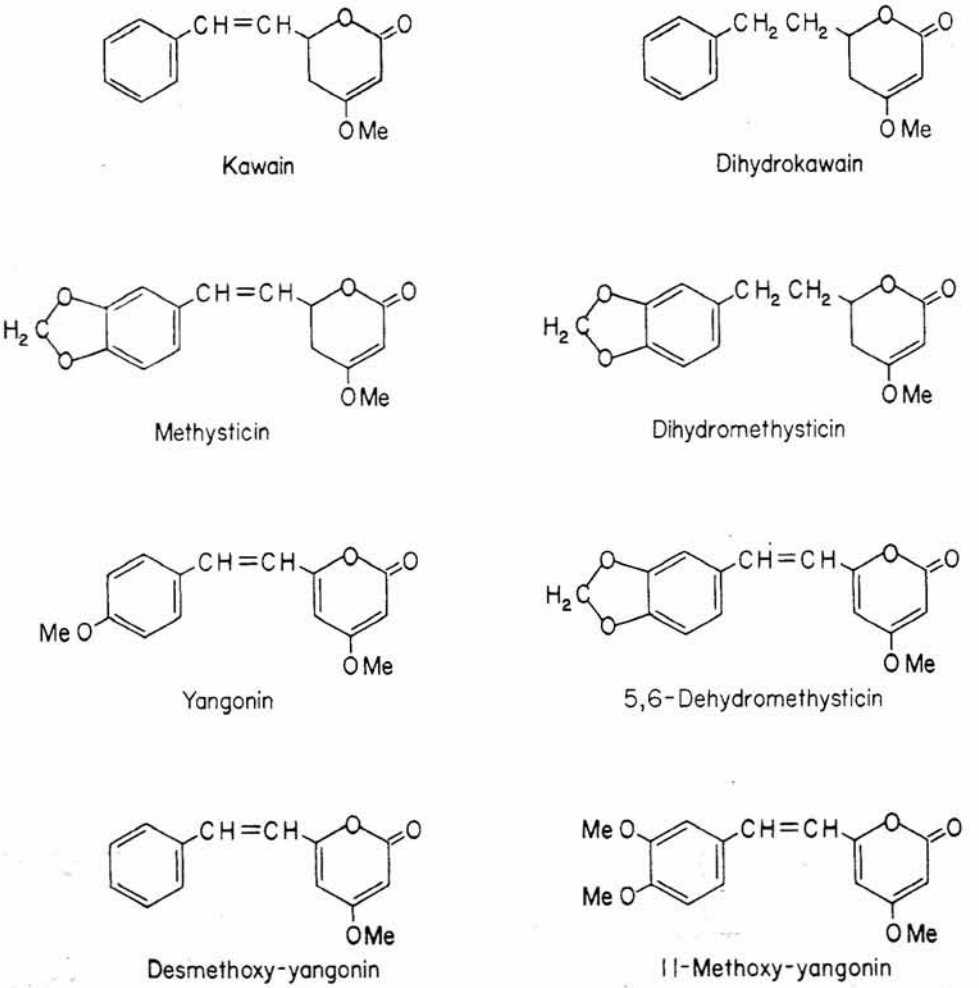
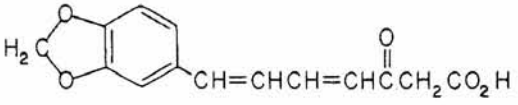
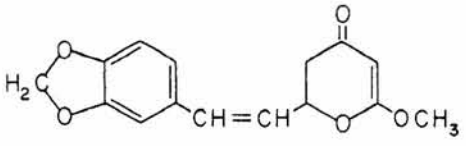


FIG. 1. *Compounds of established structure isolated from* ***P. methysticum.***

Methysticin.—This white, neutral compound was first obtained in a pure state by Pomeranz in 1889 (50), following its earlier isolation by Gobley (25), Cuzent (21), and Nölting and Kopp (48). Pomeranz succeeded in assigning an empirical formula, $C_{15}H_{14}O_5$, to this substance and also studied its hydrolysis and oxidation products. Upon treatment with hot alkali methysticin was found to yield an acid ($C_{14}H_{12}O_5$) called methysticic acid. This compound upon permanganate oxidation afforded 3,4–methylenedioxybenzoic acid. When methysticic acid was warmed with dilute mineral acid the substance lost carbon dioxide and gave a compound, $C_{13}H_{12}O_3$, which Pomeranz called methysticol. The ready decarboxylation of methysticic acid suggested the presence of a beta-keto acid grouping which, in conjunction with the other degradation data, led Pomeranz to propose the following structural formula

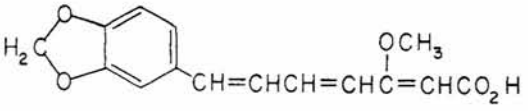


for methysticic acid and consider methysticin to be the corresponding methyl ester. The Japanese workers, Murayama and Shinozaki (47) noted that methysticin was optically active, however, and proposed a ring structure to accommodate this finding.



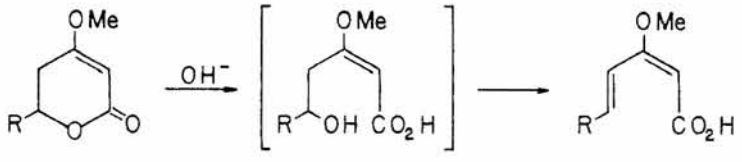
These workers also noted that they obtained a compound, isomethysticin, with the same empirical formula as methysticin when they treated methysticin with hot ten per cent sodium hydroxide.

Borsche, Meyer, and Peitzsch (10) reinvestigated these findings and determined that the isomethysticin obtained by the Japanese workers was in reality identical with the methysticic acid isolated by Pomeranz (50) and by Winzheimer (59). This compound was found to have retained the methoxyl group present in methysticin, contrary to earlier observations (50, 59), and methysticic acid was therefore formulated as being 3–methoxy–7–(3',4'–methylenedioxyphenyl)–2,4,6–heptatrienoic acid.



This structure also accounted for the formation of methysticol, since beta-alkoxy crotonic acids were known to be relatively stable to alkali but decomposed to an alcohol, a ketone (methysticol), and carbon dioxide, upon acid treatment. Methysticic acid was methylated with diazomethane and then treated with hydrochloric acid in methanol, whereby the enol ether was hydrolyzed yielding a substance identical with a synthetic sample of methyl–3–keto–7–(3',4'–methylenedioxyphenyl)–4,6–heptadienoate prepared earlier by Borsche, Rosenthal, and Meyer

(14). The change brought about by treating methysticin with base was therefore formulated (10) as follows:



R = 3,4-methylenedioxystyryl

This formula differs from the structure proposed by Murayama and Shinozaki (47) in that methysticin is represented as being an α-pyrone rather than a γ-pyrone derivative.

Klohs, Keller, and Williams (36) later succeeded in synthesizing *dl*-methysticin by the Reformatsky condensation of 3,4-methylenedioxycinnamaldehyde with γ-bromo-β-methoxy methyl crotonate in tetrahydrofuran. The product obtained exhibited identical infrared and ultraviolet spectra with those of the naturally occurring material. Further evidence for the structural identity was obtained by removing the center of asymmetry at C6 in the α-pyrone ring of *dl*-methysticin by basic hydrolysis, thereby forming methysticic acid which was identical to that obtained in the same manner from natural methysticin.

Dihydromethysticin (*Pseudomethysticin*).—An anonymous publication by the J. D. Riedel Company in 1908 (1) described the isolation of a new white neutral compound from kawa resin termed pseudomethysticin because of its similarity to the parent compound. Borsche and Peitzsch (11) investigated this material and showed it to be a mixture of methysticin and dihydromethysticin, the latter having been obtained by Goebel (26) in his studies upon the hydrogenation of methysticin. Borsche and Peitzsch (11) further showed that catalytic hydrogenation of pseudomethysticin gave pure dihydromethysticin (figure 1). *dl*-Dihydromethysticin has been obtained by the catalytic hydrogenation of synthetic *dl*-methysticin (36).

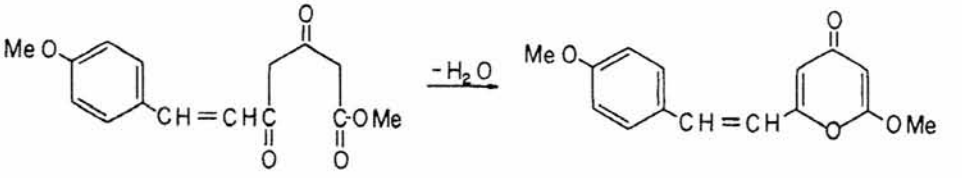
Yangonin.—This compound was first isolated from kawa by Nölting and Kopp (48) in 1874 and was later given its present name by Lewin (41). The substance was studied extensively by the J. D. Riedel group, which succeeded in establishing its empirical formula, $C_{15}H_{14}O_4$. They further demonstrated the presence of two methoxyl groups in the molecule and from a study of its behavior in alcoholic alkali they deduced the presence of a lactone ring. Yangonin on saponification with aqueous alkali formed yangonic acid, $C_{14}H_{14}O_5$, which lost carbon dioxide upon heating to form yangonol, $C_{13}H_{14}O_3$. The presence of an ethylene group in yangonin was shown by mild permanganate oxidation.

Borsche and Gerhardt (8) hydrogenated yangonin to the dihydro compound, $C_{15}H_{16}O_4$, and hydrolyzed this to the dihydro acid, $C_{14}H_{16}O_5$. When yangonic acid was heated with alkali under harsh conditions anisaldehyde and *p*-methoxycinnamic acid were obtained. The dihydro acid upon similar treatment yielded *p*-methoxyhydrocinnamic acid and anisylacetone.

Anisylacetone and anisalacetone differed in their empirical formulas from yangonic acid and dihydroyangonic acid by $C_3H_2O_3$ and the beta-carbonyl formulation ($-C{:}O{\cdot}CH_2CO_2H$) was chosen for this fragment rather than the pyruvic acid structure ($-CH_2COCO_2H$) because of the ease with which yangonic acid lost carbon dioxide to form yangonol. Yangonic acid was therefore formulated as follows:

$$p\text{-}MeOC_6H_4CH{=}CH\text{-}CO\text{-}CH_2CO\text{-}CH_2CO_2H$$

and yangonin was thought to be the anhydride of the methyl ester of yangonic acid.



Confirmation for the γ–pyrone structure for yangonin was believed to be offered by the ready formation of salts and addition compounds, a property evidenced by known γ–pyrones.

Borsche and Walter (16) succeeded in synthesizing the diketone, yangonol, by reacting *p*–methoxycinnamoylchloride with sodioacetoacetic ester followed by decarboxylation, thus tending to confirm the structural assignment for yangonin.

The final proof for the structure of yangonin was considered to have been obtained when this compound was synthesized by Borsche and Bodenstein (7) as shown in figure 2. Lampé and Sandrowski (39) had independently investigated this method of synthesis but were unable to find the proper experimental conditions necessary for the elimination of the carboxyl group on the pyronone ring. An extension of this approach to the synthesis of methysticin likewise met with failure.

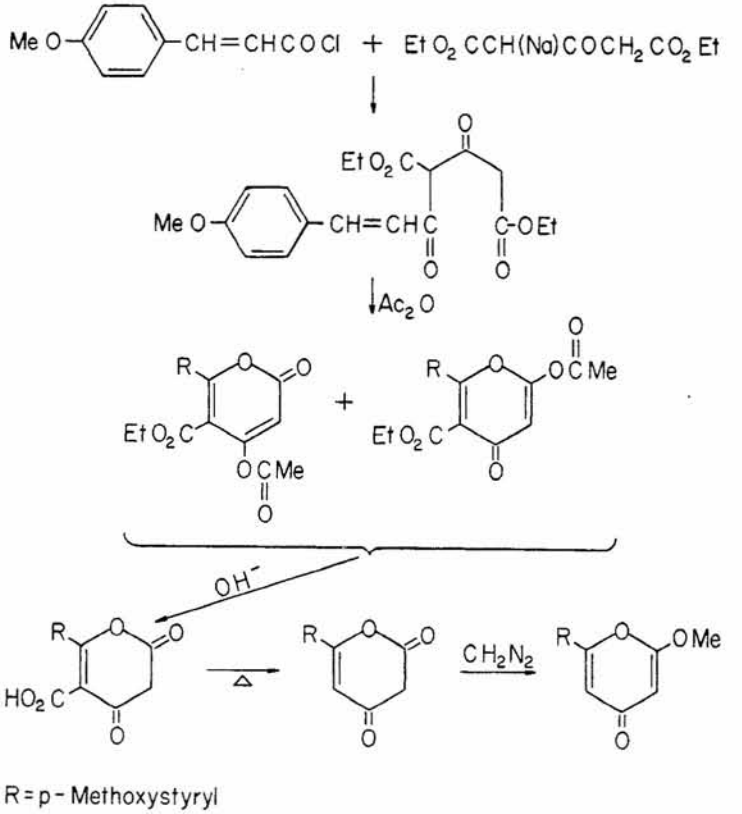


FIG. 2. *Synthesis of yangonin (Borsche and Bodenstein).*

Yangonin was thought to be the only naturally occurring 2-methoxy-4-pyrone until the structure of this compound was reinvestigated by a group of investigators at the University of Warsaw. Macierewicz (42, 43) showed that an analog of yangonolactone, styryl-6-dihydropyran-2,4-dione, gave the same 4-methoxy-alpha-pyrone derivative on methylation with diazomethane as was obtained in a cyclization experiment wherein the position of the substituents were unambiguously determined. This finding was contradictory to what presumably had happened in the methylation of yangonolactone and this reaction was therefore reinvestigated. It was found that methylation of yangonolactone by diazomethane gave a mixture of two compounds rather than the single compound, yangonin, as was reported by Borsche (7). The two compounds were separated on the basis of the differing

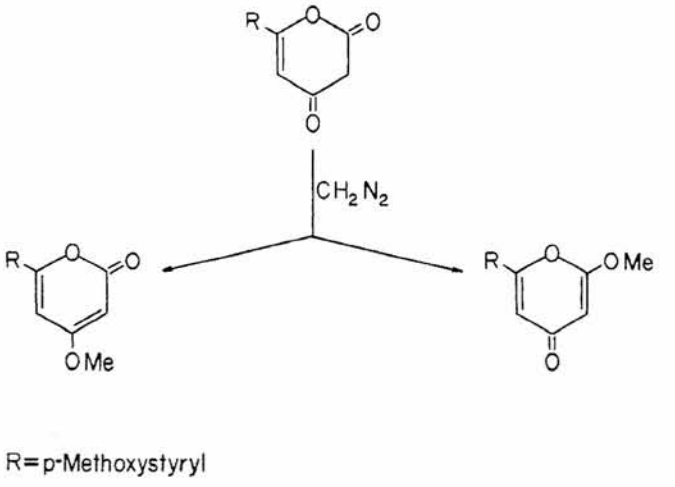


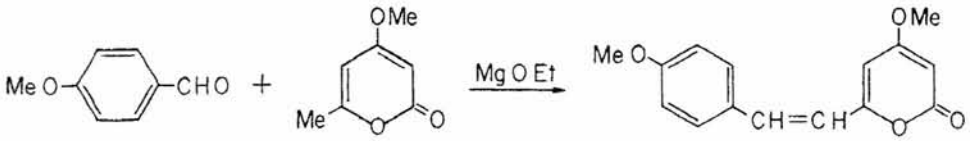
FIG. 3. *Methylation of yangonolactone.*

solubilities of their hydrochloride oxonium salts in ether. The substance which formed an ether insoluble hydrochloride was assigned the γ-pyrone structure and the ether soluble hydrochloride was formulated as an α-pyrone. The compound which behaved as an α-pyrone under these conditions was found to be identical to yangonin. The isomeric γ-pyrone with the structure which had previously been assigned to yangonin was designated pseudo-yangonin.

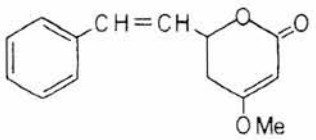
In order to obtain support for the new structural assignments, two additional pairs of isomers were prepared for a spectral study. The compounds were desmethoxy-yangonin (R = styryl) and dihydrodesmethoxy-yangonin (R = phenethyl) and their "pseudo" derivatives. In separating the "natural" and "pseudo" isomers of this series it was found that the hydrochloride salts were unsuitable; however, the isomers were easily separable through their perchlorates (18). The ultraviolet spectra of the dihydro "natural" series showed maxima at 280 mμ whereas the dihydro "pseudo" series showed maxima at 235–240 mμ. Using synthetic model compounds these workers had previously shown that the α-pyrones should have maxima at 280 mμ, and the γ-pyrones should have maxima at 240 mμ. The infra-red spectra in the two series also yielded diagnostic information, since the yangonin series (α-pyrone) showed a strong band at 5.80–5.85 μ which was shifted to 5.98–6.00 μ in the pseudo-yangonin (γ-pyrone) series.

Herbst, Mors, Gottlieb, and Djerassi (33) reëxamined the diazomethane methylation of 2,4 pyrononeS and their findings confirm the conclusions reached by the Polish group. These workers utilized chromatography as a means of separating the isomeric pairs of methylation products rather than salt formation.

Bu'Lock and Smith (17) have contributed another piece of evidence to confirm the α-pyrone structure for yangonin by an alternate synthesis wherein the use of the ambiguous diazomethane methylation as a final step is avoided as shown below.



Kawain and Dihydrokawain.—Borsche and his co-workers directed their attention to a study of the compounds obtained from the saponification of kawa resin after having isolated methysticin, dihydromethysticin, and yangonin by direct crystallization. One of the acidic products obtained from the saponification mixture, kawaic acid, behaved very much like the acid obtained previously by mild hydrolysis of methysticin. Kawaic acid methyl ester was partially demethylated by treatment with acidic methanol to give γ-cinnamalacetoacetic acid methyl ester. This substance proved to be identical with a synthetic sample prepared previously. Kawaic acid also behaved in the same manner as methysticic acid upon catalytic hydrogenation. It therefore appeared that kawaic acid differed from methysticic acid only in the absence of a methylenedioxy group. Borsche and Peitzsch (12, 13) taking into account the similar conditions used in forming kawaic acid from kawa resin and methysticic acid from methysticin, postulated that a pyrone with the structure corresponding to that of methysticin but lacking the methylenedioxy group must be present in the resin.



Upon reinvestigating kawa resin Borsche succeeded in isolating this postulated compound (kawain). Kawain was converted to kawaic acid by treatment with alkali and to dihydrokawain by catalytic hydrogenation. From the mother liquors of the kawain crystallization these workers also succeeded in isolating dihydrokawain with properties identical to those obtained by catalytic hydrogenation of kawain.

van Veen later isolated dihydrokawain from an extract of *P. methysticum* by chromatography on an acidic clay. He at first failed to recognize the identity of this compound and named it marindinin (56, 57); however, further investigation revealed the true nature of this material and the latter name has been withdrawn from general usage.

dl-Kawain has been synthesized by Kostermans (38) and in two different ways by Fowler and Henbest (23). Kostermans synthesized *dl*-kawain by the Reformatsky condensation of cinnamaldehyde with ethyl-4-bromo-3-methoxycrotonate, a procedure which was later modified to effect the synthesis of *d-l* methysticin in our laboratories.

Fowler and Henbest first synthesized *dl*-kawain by the sequence of reactions shown in figure 4.

An alternate synthesis involved treatment of 5,6-dihydro-4-hydroxy-6-styryl-2-pyrone with diazomethane. The *dl*-compound prepared by both groups differed in its melting point (142–4°, 145°) from that reported for the naturally occurring materials by Borsche (106°). However, the *dl*-compound was easily con-

verted to kawaic acid which agreed in all of its properties with those of the acid obtained from optically active kawain.

Viswanathan and Swaminathan (58) have synthesized *dl*-dihydrokawain by using hydrocinnamaldehyde in place of cinnamaldehyde in Kostermans' procedure for the synthesis of *dl*-kawain.

Beak and Abelson (2) in a study of the proton magnetic resonance spectra of kawain, yangonin, and methysticin confirmed that the styryl group in these compounds has a *trans* configuration, as had been suspected from the methods employed for their synthesis (7, 17, 23, 36, 38, 42).

Desmethoxy-yangonin.—In a chromatographic investigation of a chloroform extract of *P. methysticum* the Riker group (36, 37) was able to isolate a new pyrone derivative ($C_{14}H_{12}O_3$) in addition to those isolated previously. This compound was shown to be identical to desmethoxy-yangonin previously synthesized by Macierewicz (42) by a direct comparison of the naturally occurring material with a synthetic sample (28, 36).

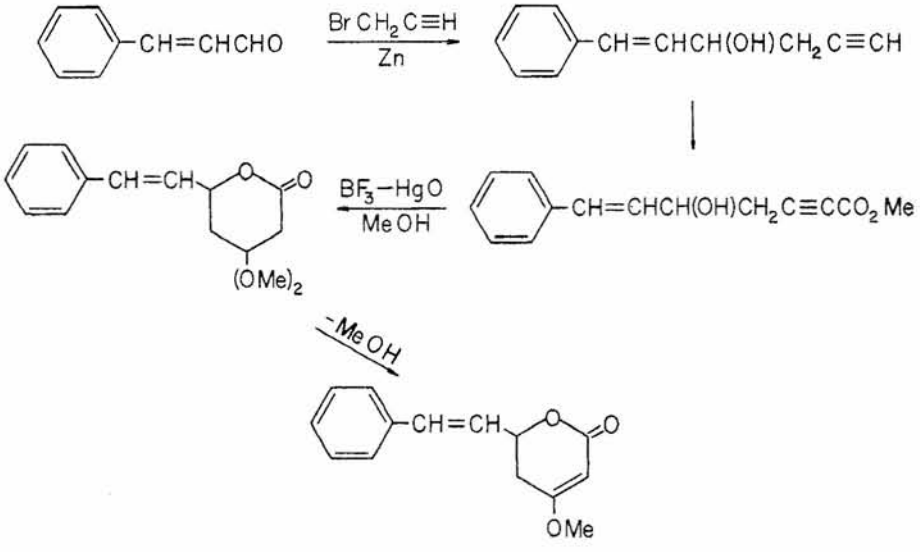


FIG. 4. *Synthesis of kawain (Flower and Henbest).*

This compound has also been isolated from *Aniba firmula* Mez. (*Lauraceae*) by Gottlieb and Mors (27) who refer to it as 5,6-dehydrokawain (28). This use of a second trivial name for a compound of known chemical structure serves little real purpose and it is therefore suggested that the compound be referred to by its systematic chemical name or by the name desmethoxy-yangonin, given to it originally by Macierewicz.

11-Methoxy-yangonin and 5,6-Dehydromethysticin.—In a preliminary announcement Mors, Magalhaes and Gottlieb (46) have reported the isolation of two new constituents of *P. methysticum*, 11-methoxy-yangonin and 5,6-dehydromethysticin. The identity of these compounds was confirmed by synthesis using the method of Bu'Lock and Smith (17).

Compounds of Undetermined Structure.—Recently Hänsel, Bähr, and Elich (29) described the isolation of two yellow compounds from kawa resin employing the technique of thin layer chromatography. These new compounds were designated flavokawin "A" and flavokawin "B."

The analysis of flavokawin "A" fit an empirical formula of $C_{18}H_{18}O_5$. Func-

tional group analyses indicated the presence of three methoxyl groups, a hydroxyl group, and an ethylenic center. Flavokawin "B" was obtained in amounts too small to allow more than a tentative empirical formula ($C_{17}H_{16}O_4$) to be assigned to this material. The ultraviolet absorption spectra of these two compounds indicated that they were chromones rather than pyrones.

Scheuer and Horigan (51) have reported the isolation of a small amount of a substance containing olefinic, carbonyl, and methylenedioxy groups by treating a fraction obtained in the purification of dihydromethysticin with Girard's reagent T. The new compound was purified by distillation after liberation from the Girard complex. A tentative empirical formula ($C_{11}H_{10}O_3$) was advanced on the basis of a 2,4-dinitrophenylhydrazone derivative. The empirical formula and functional group analyses would fit for a compound of the benzalacetone type; however, the data presented are too fragmentary to allow any definite assignment to be made.

Alkaloids.—The first mention of the presence of alkaloids in *P. methysticum* was made by Lavialle in 1889 (40). This finding was later confirmed by Winzheimer (59), who succeeded, some twenty years later, in extracting a liquid nitrogen-containing substance which gave a crystalline picrate. This claim of alkaloid material being present in kawa has quite recently been confirmed by Scheuer (51) with no experimental details being given. Other investigators have failed to encounter alkaloids in the lots of plant material with which they worked.

Farnsworth and Pilewski (22) have made the interesting observation that the naturally occurring α-pyrones isolated from kawa give positive tests when treated with Dragendorff's reagent and they advanced the opinion that this behavior may have accounted for some reports of alkaloids being present in *P. methysticum.*

TABLE 1. *Physical constants of compounds isolated from* **P. methysticum**

Name	Formula	Color	mp	$[\alpha]_D$
Kawain	$C_{14}H_{14}O_3$	White	106°(13) 106.5–108°(37)	+105°(EtOH) (13) +97°(EtOH) (37)
Dihydrokawain	$C_{14}H_{16}O_3$	White	56–58°(13) 55.2–56.2°(37)	+30°(EtOH) (13) +34°(EtOH) (37)
Methysticin	$C_{15}H_{14}O_5$	White	139–140.5°(37) 136–137°(11)	+95°(Acetone) (37) +94.30°(Acetone) (47)
Dihydromethysticin	$C_{15}H_{16}O_5$	White	117–118°(37)	+20.57°(MeOH) (11) +19°(MeOH) (37)
Yangonin	$C_{15}H_{14}O_4$	Yellow	155–156.5°(37) 153–154°(7)	———
Desmethoxy-yangonin	$C_{14}H_{12}O_3$	Yellow	138–140°(37)	———
5,6-Dehydromethysticin	$C_{15}H_{12}O_5$	Yellow	233–234°(46)	———
11-Methoxy-yangonin	$C_{16}H_{16}O_5$	Yellow	160–161°(46)	———
Flavokawin "A"	$C_{18}H_{18}O_5$	Yellow	114–116°(29)	———
Flavokawin "B"	$C_{17}H_{16}O_4$	Yellow	80–82°(29)	———

PHARMACOLOGY

Lewin in his monographic study dealt with the pharmacological evaluation of the substances which had been isolated from kawa resin at that time, these being methysticin, yangonin, and the alpha and beta resins. This work was admirable in light of the period in which it was carried out; however, the lack of adequately characterized materials and changes in techniques have caused the data to have mainly historical significance. The pure compounds, methysticin and yangonin, were obtained in very limited quantity. Methysticin was stated to be inactive when injected intraperitoneally in doses of up to two grams in warm and cold-blooded animals. Yangonin was obtained in amounts so small that it

could be tested only in two frogs orally at doses of 0.05 g with no observable effects being noted.

The resin remaining after the crystallization of methysticin and yangonin was separated into alpha and beta fractions on the basis of their solubility in petroleum ether: the bulk of Lewin's experiments were performed with these materials. They were found to cause paralysis in frogs and to exhibit a local anaesthetic action. In experiments with a sparrow, a bat, and pigeons, these substances caused loss of use of the wings and the animals appeared deeply sedated. Guinea pigs were paralyzed after receiving doses of 1–2 g *per os* and didn't respond normally to stimulus. The animals did not recover. In cats (0.5–1 g *per os*) the materials caused salivation and vomiting, but when administered subcutaneously a state of deep sleep with local anaesthetic activity was evidenced.

Borsche in concluding his investigation of *P. methysticum* (6) stated that none of the compounds isolated from kawa resin, methysticin, dihydromethysticin, yangonin, dihydrokawain, or kawain, possessed the activity reputed to be present in the drug; likewise, the acidic fraction obtained from the hydrolyzed kawa resin was also found to be inactive. The possibility was then considered that the active principle might reside in the unsaponifiable fraction, the so-called neutral resin, but this hypothesis could not be substantiated because the solubility characteristics of the neutral resin did not lend themselves to biological testing. Borsche was thus unable to establish a relationship between the constituents of kawa root and the activity attributed to the beverage obtained from it, and therefore concluded that it was possible that the compounds which were isolated and had proved to be inactive were converted to active substances in the body. Borsche presented these conclusions without revealing any details of his methods of testing or data therefrom, and this phase of his work remains obscure.

During this period a pharmacological investigation of kawa was carried out by Schübel (52, 53), who found kawa resin to have a weak narcotic action, paralyze sensory nerves, and first stimulate, then paralyze smooth muscles. The hydrolysis products of the resin showed similar actions. The local anaesthetic action was attributed to compounds containing benzoic and cinnamic acid groups. Cinnamalacetoacetic acid, obtained from the resin, was found to produce an increased reflex excitability and lowered blood pressure (8–32 mg iv in rabbits). Vacuum distillation of the residue remaining after crystallization of the acid yielded a yellow nitrogen containing oil which had a pepper-like taste, a weak narcotic action, and which increased salivation. In the sense that this substance did not have profound physiological activity Schübel considered this nitrogeneous material to be nonalkaloidal. Schübel was unable to demonstrate any pharmacological activity when the pure substances yangonin and methysticin were administered to rabbits (1 g *per os*), to pigeons (0.1–0.5 g sc), or to frogs (0.5 g by direct injection into the lymph sacs). The solubility of these compounds complicated their administration and to circumvent this, a gum arabic suspension was employed as the vehicle for parenteral administration of these materials.

On the basis of experiments on the isolated frog heart, Schübel demonstrated that incubation of kawa root with saliva increased the potency of the kawa extract. This increase in activity was attributed to the enzymatic breakdown of the starch in the root, which led to a more efficient extraction of the active materials.

van Veen (56) published the results of a study shortly after the completion of Borsche's work in which this author employed animal assays to follow the active principles of kawa in his isolation procedure. As experimental subjects pigeons, monkeys, and rice birds were employed. In preliminary experiments it appeared that the rice birds were overly sensitive to the crude extract and monkeys too resistant. Pigeons were therefore used for routine assays. Approximately eight to fifteen minutes after administration of the extract, the pigeons were stated to become sleepy and atactic; a deep sleep would then set in lasting from

two to ten hours. The birds appeared to be fully recovered upon awakening. Monkeys required three to five times the dose used in pigeons. An effective dose caused preliminary loss of limb control, followed by sleep within a period of fifteen to thirty minutes, which often lasted for fifteen hours or longer. van Veen found that purified fractions had to be administered in an oil or lecithin water emulsion to obtain a maximal effect, and was of the opinion that chewing the root and admixing saliva only served to bring about emulsification and thus promote activity in this way.

van Veen isolated an active fraction by chromatography on an acid clay and from this fraction succeeded in separating a crystalline material which was designated "marindinin." The effective dose range of this compound for pigeons was found to be 50–100 mg. None of the other components previously isolated by Borsche and presumably present in the other fractions obtained by van Veen showed any activity by his test methods. He subsequently demonstrated (57) that marindinin was slightly impure dihydrokawain, a compound stated to be physiologically inactive by Borsche. van Veen also tested the purified dihydrokawain and showed that the physiological activity which he had been able to demonstrate was indeed exhibited by this refined material and was not due to the impurity.

Hänsel and Beirsdorf (30) investigated the constituents of kawa resin employing alumina chromatography as a means of isolation. These workers succeeded in re-isolating all of the compounds previously obtained by Borsche and his coworkers. Dihydrokawain and dihydromethysticin both appeared to be active in causing sleep in white mice and white rats when administered orally by stomach tube in the form of an oil solution or emulsion in a dose range of 50–200 mg/kg.

In a simultaneous study conducted in our laboratories (37) a chromatographic separation was also employed which yielded yangonin, kawain, dihydrokawain, methysticin, dihydromethysticin, and desmethoxy-yangonin. These compounds were administered orally to mice in ten per cent Tween suspensions and studied for their effects on the central nervous system as measured by their ability to antagonize strychnine induced convulsions in mice, potentiate barbiturate sleep time, and promote fall out from roller cages, and were compared with the crude extract, the ground root, and meprobamate. The results of these tests are summarized in "Table 2."

TABLE 2. *Biological evaluation of constituents of* ***P. methysticum*** (37).

	Strychnine	Roller cage		Sleeping time	
	ED_{50}, 95% conf. lim. mg/kg	dose mg/kg	result	dose mg/kg	sl. time in % of controls
Dihydrokawain	340(270–430)	300	no effect	160	150
Yangonin	no protect. at 1000	300	no effect	160	150
Kawain	215(160–290)	300	no effect	160	235
Desmethoxy-yangonin	no protect. 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	1700(1400–2100)	10,000	12/18 fall-out	10,000	400
Meprobamate	no protect. at 700	ED_{50}=	165(122–201)	160	250

Dihydromethysticin appeared to be the most active of the isolated constituents and, contrary to previous findings, dihydrokawain appeared to be relatively in-

active as judged by these tests. The activity shown by the chloroform extract and the crude root in the roller cage experiments was not evidenced by any of the pure isolated materials. It thus appeared that this activity was due to the presence of additional unisolated constituents or a possible synergistic action.

It has been suggested (44, 45) that the suspending agent influences the absorption of these compounds and that Tween is not as suitable as peanut oil for this purpose. It had been noted, however, that the absorption of these materials from the gastrointestinal tract when they were administered as Tween suspensions was remarkably rapid (37).

Meyer, Oberdorf, and Seifen (45) have examined the biological activity of dihydrokawain and dihydromethysticin and reported that these compounds had sedative activity when administered ip or *per os* to mice, rats, rabbits, and cats at a dose of 50 mg/kg. With higher doses (100–300 mg/kg.) there was a marked ataxic phase followed by loss of the righting reflex. The narcotic effect was observed to set in after ten minutes and appeared to reach a maximum one to two hours later with sleeptime lasting from two to ten hours depending upon the dose employed. The two compounds did not appear to affect blood pressure or heartbeat frequency in the rabbit or cat and respiration was only slowed slightly. The effect of dihydrokawain upon the rabbit EEG was characterized by increased spindle action and narcotic doses were stated to produce slow high waves. The threshold for arousal by electrical stimulation of the mesencephaloform reticular formation was found to be increased by the administration of dihydrokawain.

Meyer (44) has followed the above preliminary communication with a more detailed paper giving additional experimental data to further substantiate the earlier findings. When administered to mice as peanut oil solutions, both dihydrokawain and dihydromethysticin produced sedation, hypothermia, and a corresponding reduction in total oxygen consumption. In the unanaesthetized rabbit, blood pressure was only slightly influenced, even when doses near the LD_{50} were employed. Premedication with dihydrokawain or dihydromethysticin prolonged and deepened sodium hexobarbital anaesthesia and under these conditions metabolism was reduced to basal levels. This reduction appeared to be responsible for the observed hypothermia.

The strong sedative effects in mice obtained upon administration of dihydrokawain and dihydromethysticin by the modern German workers has not been observed in our laboratories. At doses of 50 and 200 mg/kg. in Tween suspensions dihydrokawain and dihydromethysticin only produced a slight decrease in motor activity when administered orally to mice. The reason for the disparity of results between laboratories is not immediately apparent. It may be noted that this sort of contradictory biological evidence has continually complicated the research on kawa since the earlier findings of Borsche and van Veen (*loc. cit.*).

A new approach to the pharmacological study of *P. methysticum* has recently been explored by Hart, Ray, Furgiuele, and Marrazzi (32). This group has evaluated the pharmacological properties of a water percolate (25°) of kawa. A dose of 0.1–0.5 ml/kg. (ip), of a 1 g root/ml extract, increased ease of handling mice and caused a 50 per cent reduction in activity cage counts without evidencing any overt signs of sedation. Intracarotid injection of 0.2 ml in the pentobarbital anaesthetized cat produced cortical synaptic inhibition in the transcallosal preparation, matching that of 50 μg of serotonin. A dose of 2–4 ml (ip) in thirsty rats, trained to obtain a water reward by pressing a lever at a tone signal, resulted in a prolongation of stimulus response latency to the twenty-second point where trials were terminated without reward. These investigators claimed that their cat and rat data was similar to that obtained with LSD–25 and pointed to a CNS inhibitory effect for the chemically uncharacterized principles present in the water extract. It was not stated how the previously isolated constituents behaved in these test procedures.

Clinical Findings.—A clinical pharmacological evaluation of *dl*-dihydromethysticin has been conducted (35). At doses of 500 mg/day, dihydromethysticin was found to be ineffective in the treatment of schizophrenia, as a skeletal muscle relaxant, and as an antiepileptic agent. It did appear to be active as a mild tranquilizer of the meprobamate type; however, on continued usage at the higher dose levels (300–800 mg/day) a high percentage of the patients developed an exfoliative dermatitis, characterized by a dry, scaly skin, and the trials were terminated. It is of interest to note that this same sort of skin affliction was observed in the Polynesians who were heavy drinkers of the kawa extract.

Received 1 February 1963.

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