Central Activity of Aqueous Extracts of *Piper methysticum* (Kava)

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Aqueous extracts of *Piper methysticum* Forst. were tested for pharmacological activity. The roots of this plant have been used by the natives of many of the islands of the South Pacific in a beverage known as Kava to allay anxiety and reduce fatigue. The extracts were shown to have a depressant action on the central nervous system, evidenced by depression of spontaneous motor activity, marked reduction in irritability of rats having bilateral septal lesions, inhibition of the conditioned avoidance response in rats, and blockade of EEG arousal patterns in cats.

PIPER METHYSTICUM Forst. (*Piperaceae*) is a plant indigenous to many islands of the South Pacific. The roots of this plant have been used by natives of many of these islands in a beverage known as Kava, Kawa, or Awa to allay anxiety and reduce fatigue (1, 2). Consumption of large amounts of the drug are reported to induce intoxication with drowsiness and incoherent dreams (3). The pharmacologic actions of Kava have prompted a number of investigations and led to the isolation of several pure crystalline α-pyrones. Three of these—methysticin, dihydromethysticin, and dihydrokawain—possess sedative activity comparable to that of the whole root (4–10). The three α-pyrones are chloroform or ether soluble but water insoluble. Based on the reported methods used in the preparation and consumption of the crude drug, it seemed reasonable that a pharmacologically active water-soluble substance might be responsible for the peculiar central nervous system effects of this plant.

This report is concerned with the evaluation of the effects of different water-soluble extracts on the central nervous systems of several species of laboratory animals.

EXPERIMENTAL

Materials.—The plant material used was obtained from the S. B. Penick Co., New York, N. Y. It consisted of the finely pulverized roots of *P. methysticum* (*Piperaceae*) and was collected in Hawaii during the fall of 1959. The reference compounds—methysticin, dihydromethysticin, kawain, dihydrokawain, yangonin, and desmethoxyyangonin—were supplied through the courtesy of Riker Laboratories, Inc., Northridge, Calif. Chlordiazepoxide was kindly supplied through the courtesy of Hoffmann-La Roche, Inc., Nutley, N. J.

Phytochemical Screening.—A general phytochemical screening of the plant material was performed by using established methods to determine the presence or absence of alkaloids (11–14), tannins, flavonoids, saponins, unsaturated sterols (11), and cardiac glycosides (15).

Extraction Procedures.—A series of nine somewhat overlapping aqueous extraction procedures was used initially. Each extract was administered intraperitoneally to groups of mice (five mice per group), and the animals were observed. On the basis of these preliminary studies, three of the extracts were selected for further investigation. Only these preparations will be described.

Steam Extraction.—One-hundred grams of plant material was mixed with a suitable quantity of distilled water, so that a slurry having a volume of approximately 200 ml. was obtained. The slurry was steam distilled and the first 100 ml. of distillate collected, filtered, and lyophilized. The yield for each extraction was approximately 50 mg. of a yellow-white powder and was designated LE-I.

Waring Blendor Extraction.—One-hundred grams of plant material was mixed with 200 ml. of distilled water in a Waring Blendor (model 700A) for 15 minutes. The mixture was filtered and lyophilized. The yield for each batch was approximately 2.0 Gm. of a brown powder and was designated LE-II.

Chloroform Purification of Waring Blendor Extraction.—One-hundred grams of plant material was mixed with 200 ml. of distilled water in a Waring Blendor for 15 minutes. The mixture was filtered, then extracted successively with three 20-ml. portions of chloroform. The aqueous layer was separated and subjected to gentle heat to remove any chloroform present. The resulting amber-colored solution was filtered and lyophilized. The yield for each batch was approximately 1.90 Gm. of a dark brown powder and was designated LE-III.

Chromatography Procedures.—Furgiuele *et al.* (16) previously reported the chromogenic reaction of sulfuric acid with α-pyrones and the detection of these compounds on thin-layer chromatograms. Sulfuric acid produced color reactions with the six known α-pyrones in Kava. Kawain, dihydrokawain, yangonin, and desmethoxyyangonin produced yellow colors with this acid; the color was

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more predominant with yangonin and kawain. Methysticin and dihydromethysticin produced pink to deep violet with sulfuric acid; the intensity was roughly proportional to concentration. All colors were transient and persisted for only a few minutes. Preliminary studies had shown that the known α-pyrones in Kava, although primarily soluble in organic solvents, were also slightly soluble in water. Aqueous solutions of each of the extracts containing 1 mg./ml. were prepared, and 0.1 ml. of each was applied to Silica Gel G plates to determine the presence or absence of the known α-pyrones. In addition, a mixture consisting of 1 mg. of each α-pyrone was triturated for several minutes in a small agate mortar with 1 ml. of distilled water. The mixture was filtered and sufficient water added through the filter to make 1 ml. of a saturated aqueous solution of these components. A 0.1-ml. sample of this solution was applied to the same plate for comparison purposes. The spotted plates were developed with a Skelly B–ethyl acetate (7:8) solvent.

Spontaneous Motor Activity (SMA) and Rotarod Performance (RRP).—The effects of the lyophilized extracts on the SMA and RRP of male albino Swiss-Webster mice were evaluated by a method previously described by Furgiuele *et al.* (17, 18). One hour after receiving an intraperitoneal injection of either LE-I, LE-II, LE-III, or saline, groups of five mice were placed in photocell activity cages. A 15-minute count was begun 10 minutes later. Each extract was tested at four dose levels (six groups per dose), and the mean activities and standard errors of the means were determined. The significance of differences were assessed by the Student *t* test (19).

In the rotarod procedure, mice were trained to walk on a wooden rod rotating at 29 r.p.m. until they had learned to stay on for a minimum of 120 seconds. On treatment days, groups of 15 mice were injected intraperitoneally with either one of the extracts or saline. The time each animal remained on the rod was measured beginning 1 hour after injection. Maximum performance time was arbitrarily set at 120 seconds, and mean performance times and standard errors of the means were determined. The significances of differences were assessed as above. In both the activity cage and rotarod studies, all drugs were dissolved in water. Control groups received normal saline solution (0.9% NaCl), and the volume administered never exceeded 0.1 ml./10 Gm. body weight.

Septal Rats.—LE-I, LE-II, and LE-III were tested for antagonism to the exaggerated irritability and aggressiveness of rats having lesions in the septal area. The lesions were produced in a manner similar to that described by Randall *et al.* (20). Following a 3- or 4-day recovery period, the behavioral abnormalities of the lesioned rats were scored in a manner similar to that described by Schallek *et al.* (21). This involved scoring the responses obtained following (*a*) a puff of air on the back, (*b*) touching the whiskers with a probe, (*c*) prodding the animal's back gently with a probe, and (*d*) opening the cage door and approaching with a gloved hand.

Each test was rated on a scale ranging from 0 for no response to 6 for the most violent response. Different groups of five rats were tested 0.5, 1, and 2 hours after intraperitoneal injections of one of the extracts or saline. The average score of each drug-treated group obtained following each scoring session was compared to the average score of the saline-treated rats. The ED_{50}, or dose reducing the score to one-half the score of the control rats, was determined graphically by the method of Miller and Tainter (22). The sites of the lesions were confirmed histologically in all of the rats. Since chlordiazepoxide had been reported by Randall *et al.* (20) to be particularly effective in this preparation, it was included for comparison.

Conditioned Avoidance Response (CAR).—The pole climbing procedure of Cook and Weidley (23), as modified by Aceto *et al.* (24), was used. Responses to both tone and shock were recorded on paper by a four-pen polygraph (Lehigh Valley, No. 1321-4). During the training session, each rat was subjected to 10 cycles (15 seconds of tone, followed by a maximum of 30 seconds of shock at 2-minute intervals), and the number of times the animal responded to either tone or shock was recorded. After two or three sessions, the animals were able to avoid the shock consistently by responding to the tone signal. Trained groups of six male albino (Wistar strain) rats received intraperitoneal injections of one of the extracts, chlordiazepoxide, or saline, 1 hour prior to a 10-cycle session. The number of times the rat failed to respond to tone but did respond to the shock was a measure of the inhibition of the conditioned response. The ICR50, or that dose which produced inhibition of the conditioned response in 50% of the rats, was estimated graphically by the method of Miller and Tainter (22).

Flexor Reflex.—The effects of intravenously administered LE-I and chlordiazepoxide on the flexor reflex of pentobarbitalized cats were evaluated by the method described by Berger (25) and modified by Furgiuele *et al.* (18).

Electroencephalographic Studies in Chronic Cats.—Cats with chronically implanted cortical and subcortical electrodes were prepared in a manner similar to that described by Horovitz and Chow (26). Bipolar electrodes were implanted into the amygdala (AP = 13, L = 9, H = −5), hippocampus (AP = 5, L = 12.5, H = −1.5), and pontine reticular formation (AP = 35 mm. anterior to F = 0, L = 0, H = 32 mm. at an angle of 25°) according to the atlas of Jasper and Ajmone-Marsan (27) and into the posterior hypothalamus (AP = 9, L = 0.5, H = −2.5) according to the atlas of Bleier (28). Monopolar electrodes were placed over appropriate cortical areas 5–10 mm. apart. Simultaneous recordings from three different leads were obtained on a Grass polygraph (model 5). A Grass square-wave stimulator was used to stimulate the posterior hypothalamus or brain stem reticular formation for a period of 15 seconds with pulses of 100 c.p.s. having a duration of 5 milliseconds and at 0.5 to 8.5 v. The animals were placed in a semisoundproof, constant environment chamber fitted with a one-way glass window and a small light. A total of four cats was used, and the method of conditioning each cat to the environment was standardized. All animals were treated according to the following procedure.

The animals were fed, placed in the chamber, and allowed a period of 1 hour to acclimatize. Control recordings were made and the thresholds for EEG arousal determined following electrical, visual

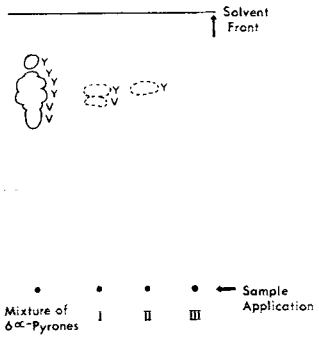

Fig. 1.—Thin-layer chromatograms of aqueous solutions of Kava extracts. Key: (after sulfuric acid spray): *Y* = yellow; *V* = violet. Broken line indicates trace concentrations.

(blinking light on and off), or auditory (clapping hands) stimulation. A desynchronization of the resting EEG for a period approximately twice the duration of the stimulus or longer was taken as the arousal response. Electrical stimuli were delivered to the posterior hypothalamus of two cats, and cortical activity was recorded bilaterally from the posterior sigmoid and anterior suprasylvian gyri; subcortical activity was recorded from the amygdala. The brain stem reticular formation of the two remaining cats was stimulated, and cortical activity was recorded bilaterally from the anterior sigmoid gyri, posterior sigmoid gyri, and anterior suprasylvian gyri; subcortical activity was recorded from the posterior hypothalamus. After control recordings, the animals were removed from the chamber, and an aqueous solution of LE-I or chlordiazepoxide was administered intraperitoneally. The effects of the drug on spontaneous EEG activity and upon arousal were observed 30, 60, 120, and 180 minutes following administration. An interval of at least 5 days was permitted between injections to insure recovery from the previous drug. At the termination of the study, the animals were killed by an overdose of pentobarbital sodium, the brains were perfused with 10% formalin, and the locations of the electrodes were verified.

RESULTS

Phytochemical Screening.—The standard alkaloid screening methods employed gave equivocal tests for alkaloids in Kava. After numerous failures to isolate alkaloid fractions, the possibility that the positive tests obtained were false was investigated. The results of this study clearly indicated that certain α-pyrone derivatives of Kava can induce false positive reactions (18). The plant material gave a positive test for tannins; however, flavonoids, saponins, unsaturated sterols, and cardiac glycosides were not detected.

Thin-Layer Chromatography.—After spraying the air-dried chromatograms with sulfuric acid, it was demonstrated clearly that on an equivalent basis known α-pyrones could not be detected in LE-III (Fig. 1). Neither methysticin nor dihydromethysticin was detected in LE-II, evidenced by an absence of the violet or red produced by these compounds. Trace amounts of either methysticin or dihydromethysticin appeared to be present in LE-I. LE-I and LE-II exhibited decreasing amounts, respectively, of one of the other α-pyrones known to produce yellow with sulfuric acid.

Spontaneous Motor Activity (SMA) and Rotarod Performance (RRP).—The effects of the three lyophilized extracts on SMA and RRP of mice are summarized in Table I. Each of the three extracts caused a significant decrease ($p < 0.05$) in the SMA of mice, and this response was generally dose dependent. Loss of righting reflex was not observed, even at those doses which almost completely abolished SMA. In contrast to the decrease in SMA, RRP was relatively unaffected. None of the three extracts had an appreciable effect on RRP at doses which caused a marked decline in SMA.

Septal Rats.—The effects of the extracts on hyperirritability of septal rats are summarized in Table II. Since the average scores obtained following saline treatment were not statistically different from experiment to experiment (Student *t* test), the scores were combined and a single value used. Although each of the Kava extracts effectively reduced the hyperirritability of septal rats, it is apparent that LE-I was at least twice as potent as LE-II and LE-III. Slight ataxia was observed at the 720 and 960-mg./Kg. doses of extracts II and III, respectively. Ataxia was not observed in the septal rats

TABLE I.—EFFECTS OF THE LYOPHILIZED KAVA EXTRACTS ON MOTOR ACTIVITY OF MICE MEASURED BY THE PHOTOCELL ACTIVITY CAGE AND ROTAROD

			Photocell Activity Cage		Rotarod	
Drug	i.p. mg./Kg.	N[a]	Mean Activity Count/15-Min. Period ± S.E.	PN[b] Value	Group[c] Mean Performance Time, Sec. ± S.E.	PN[b] Value
Control	...	8	141 ± 8	100	113 ± 7	100
LE-I	42	6	46 ± 5[d]	33	113 ± 7	100
	84	6	33 ± 4[d]	23	91 ± 14	81
	120	6	16 ± 1[d]	11	108 ± 12	96
	240	6	12 ± 3[d]	9	107 ± 13	95
Control	...	8	175 ± 12	100	99 ± 9	100
LE-II	42	6	63 ± 13[d]	36	110 ± 7	109
	84	6	64 ± 11[d]	37	95 ± 11	96
	120	6	34 ± 8[d]	19	81 ± 11	82
	240	6	22 ± 5[d]	13	93 ± 9	94
Control	...	8	154 ± 6	100	109 ± 8	100
LE-III	42	6	64 ± 14[d]	42	88 ± 9	81
	84	6	22 ± 6[d]	14	104 ± 10	95
	120	6	6 ± 3[d]	4	79 ± 11[d]	73
	240	6	16 ± 5[d]	10	67 ± 12[d]	62

[a] *N*, number of groups of five mice tested. [b] *PN* = per cent normal. [c] Fifteen mice per group. [d] Significantly different from control ($p < 0.05$).

TABLE II.—EFFECTS OF THE LYOPHILIZED KAVA EXTRACTS AND CHLORDIAZEPOXIDE ON THE RAGE SCORE OF SEPTAL RATS

Drug	N[a]	i.p. mg./Kg.	Mean Score ±S.E.	ED50 ± % S.E. (mg./Kg.)
Control	20	...	62 ± 3	...
LE-I	5	50	47 ± 6[b]	
	5	100	37 ± 6[b]	170 ± 19
	5	200	26 ± 6[b]	
LE-II	5	240	43 ± 7[b]	
	5	480	32 ± 7[b]	380 ± 17
	5	720	5 ± 2[b]	
LE-III	5	240	47 ± 5[b]	
	5	480	35 ± 7[b]	570 ± 25
	5	960	19 ± 2[b]	
Chlordiazepoxide	5	10	48 ± 3[b]	
	5	20	26 ± 12[b]	17 ± 20
	5	40	14 ± 2[b]	

[a] N, number of rats tested. [b] Significantly different from controls ($p < 0.05$).

receiving LE-I. The rage response was also reduced by chlordiazepoxide, in confirmation of the data of Randall *et al.* (20). A moderate to marked ataxia occurred at the 20 and 40-mg./Kg. doses.

Conditioned Avoidance Response (CAR).—The effects of the extracts on the rat CAR are summarized in Table III. The responses of saline-treated rats were not statistically different from experiment to experiment and were combined in a single value. Each of the three extracts caused a significant inhibition of the CAR. As in the test with septal rats, a greater potency was exhibited by LE-I than by either LE-II or LE-III. At all doses tested, LE-I caused no inhibition of the shock response. In contrast, a significant reduction in shock responses occurred after injecting 720, 1440, and 40 mg./Kg. of extracts II, III, and chlordiazepoxide, respectively.

Flexor Reflex.—In two cats, a single intravenous dose of 20 mg./Kg. of LE-I completely blocked the flexor reflex for approximately 3 hours without appreciably altering blood pressure or respiration. In two cats, chlordiazepoxide, at a dose of 3 mg./Kg. i.v., caused a 50–75% decline in the flexor reflex for approximately 2 hours.

Electroencephalographic Studies in Chronic Cats.—The effects of LE-I on the duration of the arousal response after threshold stimulus are summarized in Table IV. Low doses of LE-I (50 mg./Kg.) shortened the duration of the arousal response for approximately 1 hour. Although this effect was observed in recordings from both cortical and subcortical sites, no significant changes in EEG activity were evident. Larger doses (100 and 150 mg./Kg.) effectively blocked EEG arousal (Fig. 2) and caused a slowing of spontaneous cortical and subcortical activities. Mild to marked ataxia occurred after the 100 and 150-mg./Kg. doses, respectively. This was observed by removing the animals from the testing chamber and permitting them to walk about for 2 or 3 minutes. The duration of the arousal response after auditory stimulation was unaffected at the 50-mg./Kg. dose, was shortened after 100 mg./Kg., and was completely abolished after 150 mg./Kg. of LE-I. The EEG arousal caused by visual stimulation was unaffected after 50 mg./Kg., was shortened somewhat after 100 mg./Kg., and was blocked for more than 2 hours after 150 mg./Kg. of LE-I. After the recording sessions had been terminated, moderate ataxia was observed in those cats receiving 150 mg./Kg. This ataxia was still evident 12–14 hours later.

Thirty minutes after injection, chlordiazepoxide, at the 5-mg./Kg. dose, caused a 50% decrease in the duration of arousal in recordings from cortical and subcortical sites. The arousal response assumed predrug characteristics about 1 hour after administration of chlordiazepoxide (Table V). A dose of 10 mg./Kg. blocked cortical and subcortical arousal 10 minutes after injection into one cat; the response returned to threshold levels within 3 hours. Slight ataxia was observed even after the recording session had been terminated. In a second cat, duration of arousal was negligible if the stimulus was applied 30 minutes after administration of chlordiazepoxide. One hour after injection, the animal displayed slight ataxia accompanied by restless movements. These effects persisted for approximately 1 hour, at which time the experiment was terminated. Thirty minutes after an injection of 15 mg./Kg. of chlordiazepoxide to one cat, a marked decrease in duration of cortical arousal and a significant increase in the duration of hypothalamic arousal occurred. Thirty minutes after a dose of 15 mg./Kg. of chlordiazepoxide had been given to a second cat, the duration of cortical arousal was decreased, hypothalamic arousal was increased, and a state of uncoordinated excitation (resembling the symptoms of one cat receiving 10 mg./Kg.) was induced. The hyperexcitability subsided; blockade, or reduction of cortical and subcortical arousal, was evident within 30–60 minutes. Return to predrug values occurred in approximately 3 hours. The duration of the arousal response following auditory or visual stimulation was unaffected by chlordiazepoxide at the dosage levels used. A slight increase in slow wave activity appeared for 30–60 minutes in areas (cortical) of spontaneous fast activity. Hypothalamic activity was decreased markedly after administration of 15 mg./Kg. This

TABLE III.—EFFECTS OF THE LYOPHILIZED KAVA EXTRACTS AND CHLORDIAZEPOXIDE ON THE RAT CONDITIONED AVOIDANCE RESPONSE (CAR)

Drug	N[a]	i.p. mg./Kg.	% Inhibition of CAR	ICR50 ± % S.E.
Control	24	...	8	...
LE-I	6	50	48[b]	
	6	100	65[b]	82 ± 31
	6	200	70[b]	
	6	400	92[b]	
LE-II	6	240	22	
	6	480	35[b]	370 ± 17
	6	720	98[b]	
LE-III	6	240	33	
	6	480	48[b]	600 ± 15
	6	960	62[b]	
	6	1440	90[b]	
Chlordiazepoxide	6	10	26	
	6	20	37[b]	21 ± 14
	6	30	64[b]	
	6	40	100[b]	

[a] N, number of rats tested. [b] Significantly different from controls ($p < 0.05$).

TABLE IV.—EFFECTS OF LE-I ON DURATION OF AROUSAL FOLLOWING THRESHOLD STIMULATION IN CATS

Cat., No.	i.p. mg./Kg.	Area Stimulated	Area Recorded	Duration of Arousal, Sec. 0 Hr.[a]	0.5 Hr.	1 Hr.	2 Hr.	3 Hr.
4	50	Ret. Form.	P. Sigmoid	32	20	15	30	...
			A. Suprasyl.	32	20	15	30	...
			P. Hypothal.	32	20	15	30	...
3	50	P. Hypothal.	P. Sigmoid	25	13	9	22	...
			A. Suprasyl.	25	13	9	22	...
			Amygdala	25	13	9	22	...
2	50	P. Hypothal.	A. Sigmoid	40	30	21	35	...
			P. Sigmoid	40	30	24	35	...
			Amygdala	40	30	21	35	...
1	100	Ret. Form.	P. Sigmoid	29	0	0	16	22
			A. Suprasyl.	29	0	0	16	22
			P. Hypothal.	26	0	0	10	29
4	100	Ret. Form.	P. Sigmoid	42	10	0	0	36
			A. Suprasyl.	42	10	0	0	39
			P. Hypothal.	48	10	0	0	>48
4	150	Ret. Form.	P. Sigmoid	60	0	0	0	0[b]
			A. Suprasyl.	50	10	0	0	37[b]
			P. Hypothal.	50	2	0	0	0[b]
2	150	P. Hypothal.	A. Sigmoid	37	[c]	10	0	12
			P. Sigmoid	37	[c]	10	0	11
			Hippocampus	37	[c]	10	0	12

[a] Predrug. [b] Recovery in 4 hours. [c] Unable to record.

appeared within 30 minutes; the maximal effect was observed in 1 hour, and recovery occurred in approximately 3 hours. Moderate ataxia was still evident after the recording session had been terminated.

DISCUSSION

The lyophilized Kava extracts, LE-I, LE-II, and LE-III, were equipotent in reducing mouse spontaneous motor activity in doses having practically no effect on forced motor activity. However, LE-I exhibited a potency two to eight times that of LE-II and LE-III in reducing the rage of septal rats and altering conditioned avoidance response (CAR). Thus, extraction of an aqueous slurry of pulverized Kava root by steam and subsequent lyophilization yielded a water-soluble material, which was pharmacologically the most active extract. The results obtained on thin-layer chromatograms indicated that this activity was caused by substances other than the known α-pyrones

In reducing the behavioral abnormalities of septal rats, the Kava extracts acted in a manner similar to that of chlordiazepoxide. The rage response was reduced effectively by the extracts or by chlordiazepoxide in doses which exerted a specific blockade of the CAR. King and Meyer (29) have postulated that in the rat the septal area normally acts to dampen hypothalamic output associated with emotional states, whereas the amygdala may facilitate this hypothalamic activity. Destruction of the septal area should remove its restraining influence and result in a hyperirritable animal. Schallek *et al.* (21) related these findings to chlordiazepoxide and theorized that reduced activity in the amygdala is related to the psychodepressant effects of this drug.

One of the most consistent responses obtained after injecting aqueous Kava extracts in mice and rats was the apparent immobility and ataxia which suggested skeletal muscle relaxant activity. Blockade of the polysynaptic flexor reflex in cats substantiated this activity, and it appears that at least part of the altered behavioral effects observed in mice and rats were due to blockade of spinal interneurons. Chlordiazepoxide also suppressed interneuronal transmission in confirmation of the report of Randall (30).

In the unanesthetized cat, LE-I caused a moderate slowing of cortical, hypothalamic, and hippocampal activity with accompanying ataxia and motor deficiency. After cortical activity had returned to predrug levels, subcortical (hypothalamus, hippocampus) activity was still reduced, evidenced by the absence of EEG arousal. Partial or complete arousal blockade was still evident even after both cortical and subcortical activity had returned. Although return of the arousal pattern generally marked the end of an experiment, ataxia and uncoordinated movements were still present, an

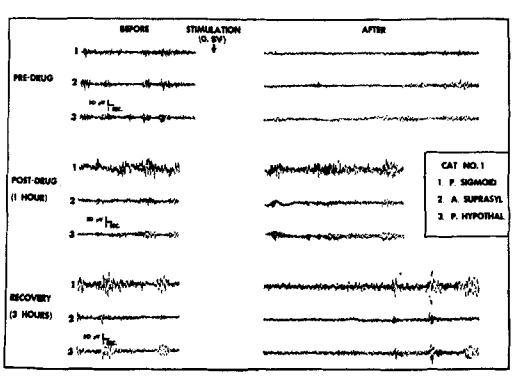

Fig. 2.—The effect of LE-I (100 mg./Kg. i.p.) on arousal after threshold stimulation of cat brain stem reticular formation.

TABLE V.—EFFECTS OF CHLORDIAZEPOXIDE ON DURATION OF AROUSAL FOLLOWING THRESHOLD STIMULATION IN CATS

Cat., No.	i.p. mg./Kg.	Area Stimulated	Area Recorded	Duration of Arousal, Sec. 0 Hr.[a]	0.5 Hr.	1 Hr.	2 Hr.	3 Hr.
2	5	P. Hypothal.	A. Sigmoid	40	23	32	45	...
			P. Sigmoid	40	23	30	45	...
			Amygdala	40	23	40	45	...
1	5	Ret. Form.	P. Sigmoid	38	5	18	41	...
			A. Suprasyl.	38	18	21	41	...
			P. Hypothal.	38	18	23	54	...
3	10	Ret. Form.	P. Sigmoid	32	2.5	>32	[b]	[b]
			A. Suprasyl.	32	2.5	>32	[b]	[b]
			P. Hypothal.	32	2.5	>32	[b]	[b]
1	10	Ret. Form.	P. Sigmoid	50	[c]	0	17	50
			A. Suprasyl.	60	[c]	0	17	50
			P. Hypothal.	50	[c]	5	8	50
2	15	P. Hypothal.	A. Sigmoid	30	[b]	20	0	32
			P. Sigmoid	30	[b]	15	0	32
			Amygdala	36	[b]	0	0	32
1	15	Ret. Form.	P. Sigmoid	38	13	0	12	32
			A. Suprasyl.	32	11	0	12	30
			P. Hypothal.	32	50	0	12	30

[a] Predrug. [b] Unable to record; animal excited. [c] Arousal blocked 10 minutes after injection.

indication that the drug was still exerting its muscle relaxant effect. It appeared, therefore, that LE-I exerted its predominant effects upon the spinal cord and progressively weaker depressant effects on the reticular formation, subcortex, and cortex, respectively.

Chlordiazepoxide effectively reduced cortical and subcortical arousal, and this effect was dose dependent. In two cats, chlordiazepoxide caused a period of excitation of sufficient intensity that recording was prevented. This stimulant action had been observed by others (21) and is thought to occur as a result of the drug's depressant action on the septum.

The results of these phytochemical and pharmacological investigations of *P. methysticum* indicate that aqueous extracts of this plant contain one or more substances (not α-pyrones) capable of exerting marked pharmacologic actions in laboratory animals.

SUMMARY

The effects of different aqueous extracts of *P. methysticum* Forst. (Kava) were investigated in mice and were found to depress spontaneous motor activity without appreciably altering rotarod performance. These extracts, though containing little or none of the α-pyrones known to be sedative constituents of Kava, also caused a marked reduction in irritability of rats having bilateral septal lesions and inhibited the rat conditioned avoidance response. A water-soluble lyophilized steam distillate of Kava root abolished the flexor reflex in cats and blocked the electroencephalographic arousal pattern produced in cats by electrical, visual, or auditory stimuli. The data suggest that the lyophilized distillate contains a compound or compounds which produce a depressant action and that the main site of action is in the spinal cord and subcortex of the brain.

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