Selected Pharmacological Studies of an Alkaloidal Extract of *Banisteriopsis quitensis*

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Abstract □ The preparation and pharmacological testing of an alkaloidal extract of *Banisteriopsis quitensis* are presented. The extract was found to exert numerous effects on the CNS in mice, including sedation, transient paralysis, ataxia, prolongation of hexobarbital "sleeping" times, and prevention of reserpine-induced hypothermia. The preparation was without effect on the ECG, blood pressure, or respiration in unanesthetized rats. A testing method for analgesia is presented, utilizing a grid-shock apparatus. The extract produced an analgesic effect which was dependent upon intact supplies of serotonin but not norepinephrine.

Keyphrases □ *Banisteriopsis quitensis*, alkaloid extract—pharmacological screening, mice, rats □ CNS activity—*B. quitensis* extract, mice

In past years, studies involving various species of *Banisteriopsis* developed primarily in two directions: elucidation of the chemical composition of the crude drugs and observation of the psychological and somatic effects after administration of *Banisteriopsis* preparations to humans. Several investigators (1–3) reported that the majority of the alkaloids isolated from *B. caapi* and *B. inebriens* are harman derivatives. *B. rusbyana* was reported by Poisson (4) to contain primarily *N,N*-dimethyltryptamine, and this finding was confirmed by other investigators (5–7). *B. quitensis* contains both tryptamine derivatives and at least one carboline-type structure, and it is considered by some to be identical to *B. caapi* (8).

Considerable information exists concerning the effects of *Banisteriopsis* preparations on human subjects. Der Marderosian *et al.* (5) reported that *B. caapi*, *B. inebriens*, *B. longialata*, and other species have been used among South American natives for purposes of divination, prophesy, and increased bravery. The preparations are known as "caapi," "yaje," "ayahuasca," and "Natem," depending upon the area of South America where they are used. Among the effects reported are: hallucinations, in which a bluish "aureole" is present; prolonged, intense dreams of unusual clarity; immobility; and anesthesia.

Naranjo (9) studied the effects of harmala alkaloids contained in *Banisteriopsis* species used in the "montaña" region of South America. He found harmine ("telepathine," "yageine," and "banisterine") and harmaline to produce paresthesias, numbness, isolated physical symptoms, hallucinations, superposition of images, passivity of movement, and lethargy, and he noticed a distinct difference between the effects of these drugs and those of mescaline and lysergic acid diethylamide. He regarded the drugs as stimulants of the midbrain reticular formation. Udenfriend *et al.* (10) found the harmala alkaloids to have strong MAO-inhibitory effects. Harman, harmine, harmaline, and α-carboline produced 50% inhibition of serotonin degradation at a concentration of 10^{-6} *M* in rat brain tissue preparations. Reference was made to the use of the harmala alkaloids as far back as 1920 in the treatment of disorders now treated with MAO inhibitors.

In this paper, selected pharmacological studies of an alkaloidal extract of *B. quitensis* are presented, emphasizing the CNS effects of the drug.

EXPERIMENTAL

Preparation of Alkaloidal Extract—Powdered leaves and stems of *B. quitensis*[1] were placed in 70% ethanol (10 ml./g. powdered drug), stoppered, agitated for 4 hr., and then centrifuged at 3000 r.p.m. for 15 min.[2]. The supernatant was decanted, tested for the presence of alkaloids with Mayer's reagent, and evaporated to dryness in a vacuum oven at 45°[3]. The residue was dissolved in 70% ethanol (1 ml./5 g. original powdered drug) and diluted with distilled water to a final concentration representing 1 g. original powdered drug/5 ml. 7% ethanol[4]. A solution of 7% ethanol of equal volume to the extract was used for all "control" animals. To eliminate effects of ethanol at very high doses of extract, a suspension of the residue in distilled water was used for LD_{50} determinations.

Animals—Male Swiss-Webster albino mice, having an average weight of 25 g., were used for all studies except ECG, blood pressure, and respiration studies where male Wistar rats, having an average weight of 150 g., were employed.

Preliminary Screening Tests—Observational studies, rotarod performance, acute LD_{50}, and effects on ECG, blood pressure, and respiration[5], were used initially to establish both a range of active dosages and a direction of pharmacological activity.

Hexobarbital "Sleeping" Time—Mice were pretreated with extract (2 ml./100 g. i.p.) 1 hr. prior to administration of hexobarbital (85 mg./kg. i.p.). The length of time between losing and regaining the righting reflex ("sleeping" time) was then determined for each animal, and the results were compared to controls (11).

Prevention of Reserpine Hypothermia—Mice were restrained in plastic holders, and their rectal temperatures were monitored using electronic thermometers equipped with temperature-sensing probes[6]. The rectal temperatures were recorded immediately after reserpine administration and at hourly intervals for 4 hr. following these zero-time readings. In two studies, reserpine was not used, and the zero time began 1 hr. after pretreatment. Treatments were as follows:

Group 1: reserpine (2.5 mg./kg. i.p.)
Group 2: 7% ethanol (2.4 ml./100 g. i.p.) 1 hr. prior to injection of reserpine (2.5 mg./kg. i.p.)
Group 3: extract (2.4 ml./100 g. i.p.) 1 hr. prior to injection of reserpine (2.5 mg./kg. i.p.)
Group 4: *p*-chlorophenylalanine (350 mg./kg. i.p.) 3 days prior to injection of 7% ethanol (2.4 ml./100 g. i.p.), followed in 1 hr. by injection of reserpine (2.5 mg./kg. i.p.)
Group 5: *p*-chlorophenylalanine (350 mg./kg. i.p.) 3 days prior to injection of extract (2.4 ml./100 g. i.p.), followed in 1 hr. by injection of reserpine (2.5 mg./kg. i.p.)
Group 6: 7% ethanol (2.4 ml./100 g. i.p.) 1 hr. prior to zero time
Group 7: extract (2.4 ml./100 g. i.p.) 1 hr. prior to zero time

Testing for Analgesic Effect—A 50.8 × 50.8-cm. (20 × 20-in.) wooden box was constructed and divided into two portions by a

[1] Identified by Dr. John D. Dwyer, Henry Shaw School of Botany, St. Louis, Mo.
[2] International model SBV, Rotor No. 823.
[3] Precision Scientific Co., No. 31468.
[4] Further characterization of active materials is being undertaken at this time.
[5] Physiograph Small Animal Study Unit, E&M Instrument Co.
[6] Tele-Thermometer model 43TA, with probes model 402, Yellow Springs Instrument Co.

Table I—Effects of Various Treatments[a] on Rectal Temperature in Mice

Treatment	Number of Mice	Difference at 4 hr.
Reserpine	9	−4.6 ± 0.6°
Ethanol, then reserpine	6	−4.0 ± 0.3°
Extract, then reserpine	9	−0.8 ± 0.9°
p-Chlorophenylalanine, then ethanol, then reserpine	9	−6.2 ± 0.8°
p-Chlorophenylalanine, then extract, then reserpine	10	−5.4 ± 0.6°
Ethanol	8	−1.4 ± 0.6°
Extract	8	−1.8 ± 0.4°

[a] For details of treatment, see text.

strip of plywood so that mice could be tested in both sides simultaneously. This box was prepared as follows. Strips of 0.96-cm. (0.38-in.) galvanized mesh wire were attached to the floor of the box in rows 0.32 cm. (0.125 in.) apart. Alternate rows were connected in series, so that a mouse placed into the box would touch two or more strips and complete an electrical circuit with his paws. Voltage was controlled by a rheostat[7], having a fixed amperage output within the range of voltages used, and was monitored by a voltmeter[8]. The grid floor of the box was lightly sanded, cleaned, and tested prior to each experiment.

The pain threshold was determined by slowly increasing the voltage from zero to the point at which the mouse began rapidly lifting and lowering a rear foot. (Front-foot lifting, while occurring at slightly lower voltages, is not a dependable end-point, since it is commonly employed in preening or washing movements.) Mice were tested before and immediately after administration of the extract or control solution (zero time) and at 5-min. intervals for 60 min. following injection; the voltage required to elicit the pedal response was noted. The box was standardized prior to use by measuring the pain threshold in 200 untreated, male, Swiss-Webster mice; the mean threshold was found to be 11.4 ± 1.6 v. Standard analgesics such as morphine had been found previously to produce a dose-related elevation of analgesic threshold in mice when using this system. Treatments were as follows:

Group M1: extract (2.4 ml./100 g. i.p.)
Group M2: reserpine (2.5 mg./kg. i.p.) 16 hr. prior to administration of extract (2.4 ml./100 g. i.p.)
Group M3; *p*-chlorophenylalanine (350 mg./kg. i.p.) 3 days prior to administration of extract (2.4 ml./100 g. i.p.)
Group M4: *p*-chlorophenylalanine (350 mg./kg. i.p.) 3 days prior to administration of extract; reserpine (2.5 mg./kg. i.p.) 16 hr. prior to administration of extract (2.4 ml./100 g. i.p.)
Group M5: α-methyltyrosine (80 mg./kg. i.p.), three doses, at 24, 18, and 4 hr. prior to administration of extract (2.4 ml./100 g. i.p.)
Group M6: 7% ethanol (2.4 ml./100 g. i.p.)

Statistical Analysis—For comparing similar experimental and control groups, the difference between the means and the standard error of the difference between the means were determined and Student's *t* test was applied; *p* values of 0.05 or smaller were considered significant. For experiments involving multiple treatments or dosages, Duncan's New Multiple Range Test was employed, using an IBM 1130 computer, with a completely randomized block design.

The procedure involved was: (*a*) determine standard error, $s_{\bar{x}} = \sqrt{(\text{error mean square})/r}$, where r = replicates within each treatment; (*b*) obtain significant studentized ranges from appropriate tables and multiply these figures by $s_{\bar{x}}$ to give the least significant ranges; (*c*) rank the means; and (*d*) test the differences in the following order: largest minus smallest, largest minus second smallest, and so on until a difference is obtained from each mean utilizing every other mean. Each difference is declared statistically significant if it exceeds the corresponding least significant range; otherwise, it is declared not statistically significant.

[7] "Powerstat" type 116, Superior Electric Co.
[8] Triplett model 666-R.

Table II—Effects of Extract on Threshold Voltages in Mice[a]

Minutes Elapsed	Treatment[b] (Eight Mice/Group)			
0	M4 10.25	M1 10.38	M2 10.50	M3 11.00
5	M1 10.88	M4 12.88	M2 14.63	M3 18.38
10	M1 11.38	M4 14.63	M2 15.13	M3 20.13
15	M1 11.33	M2 14.25	M4 15.50	M3 22.63
20	M1 12.13	M2 14.13	M4 19.63	M3 20.25
25	M1 11.25	M2 16.00	M4 19.63	M3 23.25
30	M1 11.75	M2 15.88	M3 20.50	M4 21.50
35	M1 11.63	M2 16.13	M3 23.38	M4 24.13
40	M1 11.63	M2 16.88	M4 23.75	M3 24.75
45	M1 11.50	M2 16.75	M3 23.75	M4 24.25
50	M1 11.75	M2 14.38	M3 21.50	M4 23.25
55	M1 11.75	M2 13.50	M3 19.38	M4 22.00
60	M1 11.25	M2 13.00	M4 20.38	M3 20.50

[a] Mean threshold voltages are ranked beginning with the lowest value. Means for a given time interval not underlined by the same line are significantly different ($p = 0.05$). Standard error values are incorporated into the analysis (Duncan's New Multiple Range Test). [b] Treatments were as follows: M1, 0.3 ml./100 g. extract; M2, 0.6 ml./100 g. extract; M3, 1.2 ml./100 g. extract; and M4, 2.4 ml./100 g. extract. For details of treatment, see text.

RESULTS AND DISCUSSION

In simple observational screening tests, the alkaloidal extract was found to produce sedation, ataxia, lordosis, and transient paralysis of the rear limbs which were dose related (0.3–4.8 ml./100 g.). Rotarod performance was also impaired at dosage levels of 0.3 ml./100 g. and above. These effects were not unlike those reported following ingestion of *Banisteriopsis* preparations by South American natives, in which anesthesia, passivity of movement, and paresthesias were noted (5, 9). Control animals (receiving an identical quantity of 7% ethanol) were unaffected.

The preparation was without effect on blood pressure, ECG, or respiration in unanesthetized rats at dosage levels of 0.4–1.6 ml./100 g. i.v. Repeated injections were likewise ineffective.

The acute LD_{50} in mice was found to be 2.5 ml./100 g. i.p. of a suspension representing 800 mg. original powdered drug/ml. distilled water. This dosage is equivalent to 10.0 ml./100 g. of the final 7% ethanol preparation.

The hexobarbital "sleeping" time was prolonged significantly ($p = 0.05$) in animals pretreated with 2 ml./100 g. i.p. extract. The value for controls (10 mice) was 37.4 ± 7 min., and it was 59.1 ± 7 min. for extract-treated animals (10 mice). This effect is seen with many types of compounds, including certain sedatives, tranquilizers, and MAO inhibitors (12).

Reserpine-induced hypothermia was prevented to a significant degree ($p = 0.01$) in animals pretreated with the extract (2.4 ml./100 g. i.p.) as compared to those pretreated with control solution (Table I). Hypothermia produced in animals pretreated with control solution was not significantly different from that in animals with no pretreatment. Animals pretreated with *p*-chlorophenylalanine (350 mg./kg. i.p.) and control solution exhibited a significantly greater degree ($p = 0.05$) of hypothermia after reserpine treatment than was exhibited by animals receiving only control solution prior to reserpine treatment. This effect was not prevented to a significant degree

Table III—Effects of Various Treatments on Threshold Voltages in Mice[a]

Minutes Elapsed	Treatment[b] (Eight Mice/Group)					
0	M1 10.25	M3 10.25	M6 10.25	M4 12.00	M2 12.13	M5 12.25
5	M6 10.63	M3 11.25	M4 12.50	M1 12.88	M5 12.88	M2 13.38
10	M6 11.13	M3 11.88	M4 12.75	M5 14.13	M2 14.50	M1 14.63
15	M6 11.25	M3 12.00	M4 13.50	M2 15.00	M1 15.50	M5 16.63
20	M6 11.50	M3 12.75	M4 13.88	M2 15.13	M5 16.63	M1 19.63
25	M6 11.50	M3 13.13	M4 14.13	M2 16.00	M5 18.75	M1 19.63
30	M6 11.63	M3 12.75	M4 14.13	M2 15.75	M5 19.50	M1 21.50
35	M6 12.00	M3 12.75	M4 13.50	M2 15.00	M5 20.38	M1 24.13
40	M6 11.63	M3 12.63	M4 13.38	M2 15.00	M5 21.00	M1 23.75
45	M6 11.38	M3 12.38	M4 13.25	M2 14.88	M5 20.50	M1 24.25
50	M6 10.88	M4 12.13	M3 12.50	M2 15.00	M5 19.13	M1 23.25
55	M6 11.50	M3 11.75	M4 12.25	M2 14.00	M5 17.75	M1 22.00
60	M3 11.25	M6 11.50	M4 12.00	M2 13.63	M5 17.13	M1 20.38

[a] Mean threshold voltages are ranked beginning with the lowest value. Means for a given time interval not underlined by the same line are significantly different ($p = 0.05$). Standard error values are incorporated into the analysis (Duncan's New Multiple Range Test). [b] Treatments were as follows: M1, extract; M2, reserpine, then extract; M3, *p*-chlorophenylalanine, then extract; M4, *p*-chlorophenylalanine, then reserpine, then extract; M5, α-methyltyrosine, then extract; and M6, control solution. For details of treatment, see text.

by pretreatment with extract. Treatment with extract alone (without reserpine) and with control solution alone (without reserpine) did not produce significant alterations in rectal temperature. These findings are interpreted to indicate that the alkaloidal extract acts by inhibiting MAO, since good correlation exists between prevention of reserpine-induced hypothermia and MAO inhibition (12). It also seems apparent that serotonin participates in the maintenance of body temperature, since the pretreatment that produced the greatest restriction on the availability of serotonin [*i.e.*, *p*-chlorophenylalanine pretreatment, which strongly inhibits serotonin synthesis but not norepinephrine synthesis to any significant extent (13)] produced the greatest degree of hypothermia after reserpine treatment. Similar effects have been reported for the harmala alkaloids by Udenfriend *et al.* (10).

When tested in the grid-shock apparatus, the extract was found to have analgesic effects which were dose related for most values (Table II). Equivalent volumes of control solution were without effect. Pretreatment with reserpine, *p*-chlorophenylalanine, or a combination of the two markedly inhibited the analgesic effect of the extract (Table III). Pretreatment with α-methyltyrosine, a known inhibitor of norepinephrine synthesis which has essentially no effect on serotonin synthesis (14), had considerably less effect on the analgesic threshold produced by the extract. Thus, it is suggested that serotonin, rather than norepinephrine, is the more important intermediary in pain response of this type.

SUMMARY

A study of selected pharmacological effects of an alkaloidal extract of *B. quitensis* was presented. The extract produced ataxia, lordosis, sedation, and transient paralysis; it impaired rotarod performance; and it significantly prolonged the hexobarbital "sleeping" time in mice. Reserpine-induced hypothermia was prevented to a significant degree by pretreatment with the preparation. An analgesic effect was observed with the preparation when a grid-shock apparatus was employed. This effect was prevented to a significant degree by pretreatment with reserpine, *p*-chlorophenylalanine, or both, but was modified considerably less by pretreatment with α-methyltyrosine. A relationship of serotonin to temperature maintenance and perception of pain stimuli is suggested.

REFERENCES

(1) V. Deulofeu, "Ethnopharmacologic Search for Psychoactive Drugs," No. 2, U. S. Public Health Service Publication 1645, 1967, p. 393.

(2) F. Elger, *Helv. Chim. Acta*, **11**, 162(1928).

(3) F. D. O'Connel and E. V. Lynn, *J. Amer. Pharm. Ass., Sci. Ed.*, **42**, 753(1953).

(4) J. Poisson, *Ann. Pharm. Franc.*, **23**, 241(1965).

(5) A. H. Der Marderosian, H. V. Pinkley, and M. F. Dobbins, IV, *Amer. J. Pharm.*, **140** (5), 137(1968).

(6) S. Szara, "Psychotropic Drugs," Elsevier, Amsterdam, The Netherlands, 1957, p. 460.

(7) S. Azurell, B. Holmstedt, and J. E. Lindgren, *Amer. J. Pharm.*, **140** (5), 148(1968).

(8) J. Cuatrecasas, *Webbia Raccolta Scr. Bot.*, **13**, 343(1957).

(9) C. Naranjo, "Ethnopharmacologic Search for Psychoactive Drugs," No. 2, U. S. Public Health Service Publication 1645, 1967, p. 385.

(10) S. Udenfriend, B. Witkop, B. C. Redfield, and H. Weissbach, *Biochem. Pharmacol.*, **1**, 160(1958).

(11) J. C. Cooper and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **114**, 409(1955).

(12) A. Pletscher, *Ann. N. Y. Acad. Sci.*, **80**, 1039(1959).

(13) B. K. Koe and A. Weissman, *J. Pharmacol. Exp. Ther.*, **154**, 499(1966).

(14) M. K. Menon, P. C. Dandiya, and J. S. Bapna, *Psychopharmacologia*, **10**, 437(1967).

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