Erythrina—Chemical and Pharmacological Evaluation II: Alkaloids of *Erythrina variegata* L.

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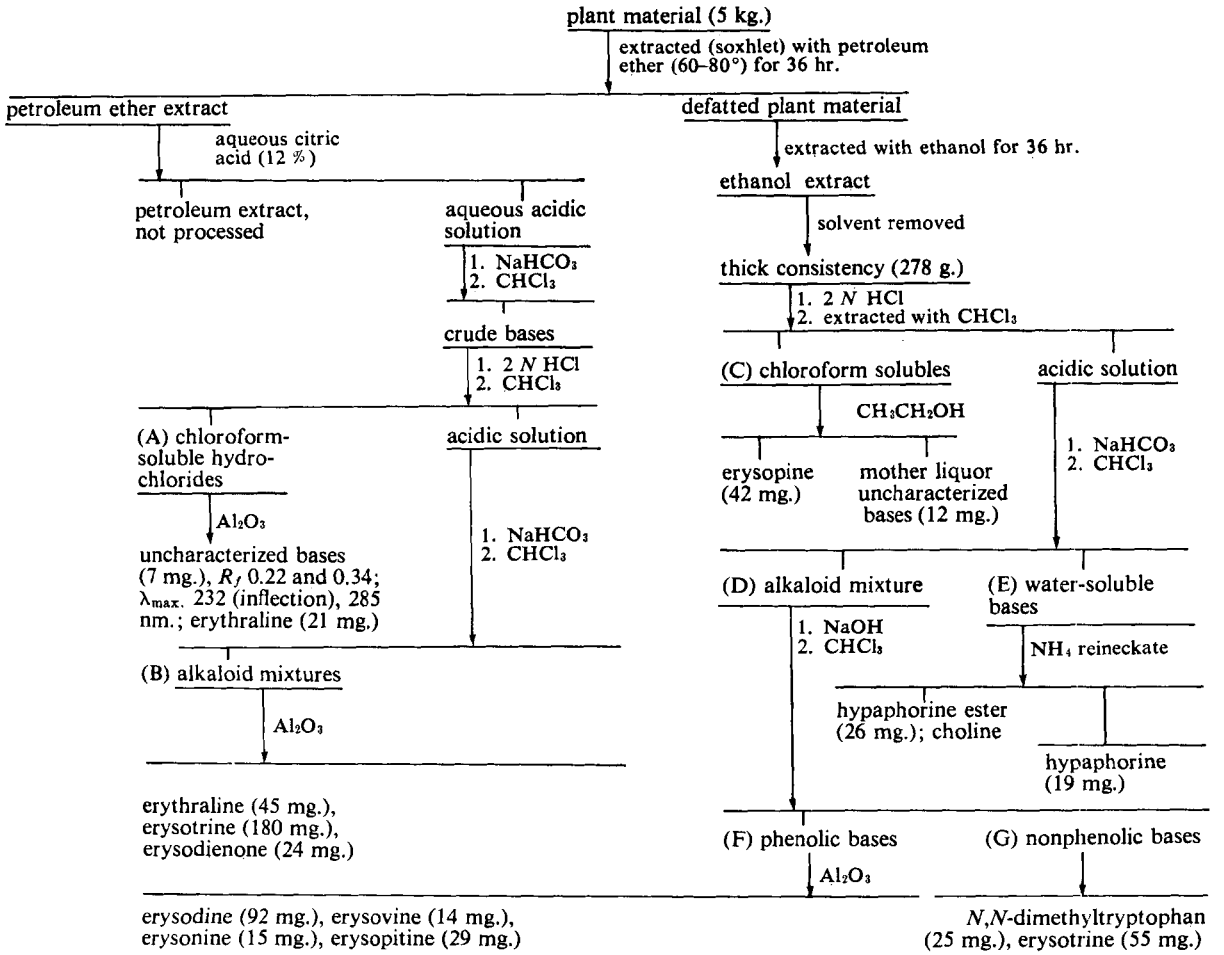
Abstract □ Eight spiroamine alkaloids (I–VIII) and three carboxylated indole-3-alkylamines (IX–XI) were isolated from the various parts of *Erythrina variegata*, and their identity was established by chemical and spectral (UV, IR, NMR, and mass) methods. Of the eight spiroamine bases isolated, three (I, VI, and VII) were previously unreported in nature, while methyl esters of hypaphorine (X) and *N,N*-dimethyltryptophan (XI) were isolated for the first time from this genus. Isolation of the two alkaloids (VI and VII), which lie in the natural biosynthetic pathway to the aromatic erythrina alkaloids, is of considerable biogenetic and chemotaxonomic significance. Selected pharmacological studies with the total alkaloid fraction from the bark showed several characteristic pharmacological effects: neuromuscular blocking, smooth muscle relaxant, CNS depressant, hydrocholeretic, and anticonvulsant effects, which are consistent with the reported uses of the plant extracts in the indigenous system of medicine.

Keyphrases □ *Erythrina variegata*—isolation, identification, pharmacological evaluation of eight spiroamine alkaloids and three carboxylated indole-3-alkylamines □ Spiroamine alkaloids—isolated, identified from *Erythrina variegata* □ Alkaloids, erythrina—isolation, identification, pharmacological evaluation of 11 constituents of *Erythrina variegata* □ Medicinal plants—isolation, identification, pharmacological evaluation of 11 constituents of *Erythrina variegata*

Erythrina variegata L. var. *orientalis* (L.) Merrill (syn. *E. indica* Lam.) (Family: Leguminosae) is a tall tree, reaching about 18 m. in height, and is widely distributed in India in deciduous forests (1). Extracts of different parts of the plant find use in the indigenous system of medicine for various purposes: the barks are used as an astringent and a febrifuge, in liver troubles and in epilepsy, as a nervine sedative and an anti-



Scheme I—Isolation of Alkaloids from Trunk Bark of E. variegata

asthmatic, and as a collyrium in ophthalmia; the leaves are used as a stomachic and diuretic and for relieving pain in joints.

In a recent communication (2) from this laboratory, the occurrence of six spiroamine alkaloids and hypaphorine in this species was reported. The initial phytochemical work has now been complemented by detailed identification of the minor basic constituents from the bark, localization of the alkaloidal entities in the various parts of the plant, and selected pharmacological studies with the total alkaloid from the bark, with a view to identifying the active principle(s) of this vegetable drug.

EXPERIMENTAL[1]

Chemistry—The principle of isolation and separation of the alkaloids involved utilization of differential solubility of the alkaloids in solvents of graded polarity (petroleum ether, chloroform, and ethanol) in the presence and absence of fat, difference in base strengths, and phenolic and nonphenolic characters. The alkaloid mixtures from the petroleum ether and ethanol extracts were broadly divided into two groups: chloroform-soluble hydrochlorides and chloroform-insoluble hydrochlorides. Subsequently, the alkaloids were separated into phenolic and nonphenolic entities in the usual way (3).

The individual alkaloids were obtained by repeated chromatography of the mixtures over neutral alumina (Brockmann grade III). Monitoring of the column chromatographic runs was accomplished at each stage of purification by TLC[2]. The water-soluble bases were isolated as their reineckates and precipitated at two pH levels (approximately 2 and 8). The bases were regenerated from their reineckate salts by passing their ethanolic solutions over columns of De-Acidite FF (pH ~ 8) (3). Petroleum ether (40–60°), benzene, chloroform, methanol, and different proportions of mixtures thereof were utilized as eluents in the column chromatographic runs. TLC was done with *n*-butanol–acetic acid–water (4:1:5) as the developer, and Dragendorff and Ehrlich reagents were used for staining purposes.

In a typical experiment, dried and ground trunk bark of *E. variegata*[3] was processed for alkaloids as shown in Scheme I.

The identity of the known alkaloids (Scheme I) was established by their co-TLC behavior with marker samples[4], by chemical transformations into suitable derivatives, and from correspondence of melting point, optical rotation, UV, IR, NMR, and mass spectra of the alkaloids and their derivatives with those reported in the literature (4–7). Characterization of the new alkaloids only is described here.

Erysodienone (VII)—The mixture of alkaloids from Fraction B (122 mg.) was chromatographed over alumina (18 × 1.2 cm.). Elution with chloroform gave erysodienone, which crystallized from ethanol as straw-colored needles, m.p. 222–225° [lit. (5, 6) m.p. 223 and 230°, respectively]; $\lambda_{max.}$: 240–242 (log ε, 4.32) and 285 nm. (log ε, 3.55); $\nu_{max.}$: 3533, 3286, 1672, 1655, and 1614 cm.$^{-1}$; δ 6.66 (s, 1H), 6.28 (t, 1H), 5.98 (s, 1H), 3.72 (s, 3H), 3.63 (s, 3H), and 2.2–3.7 (complex, 8H); *m/e* 313 (M^+, 74%), significant peaks at *m/e* 298 (35), 282 (base peak), 270 (18), and 254 (22). The spectral properties of the alkaloid are indistinguishable from those of erysodienone reported in the literature (6–8).

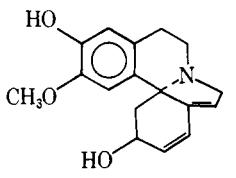
Erysodienol—Erysodienone (VII) (12 mg.) was dissolved in methanol (9 ml.), and sodium borohydride (48 mg.) was gradually added at ordinary temperature. The mixture was kept at room temperature for 2 hr. The solvent was removed, water (15 ml.) was added, and the suspension was extracted with chloroform (two 25-ml. portions). The organic layer was washed with water and dried (calcium chloride), and the solvent was removed when erysodienol was obtained as light-brown microneedles, m.p. 127–129° [lit. (6) m.p. 131°]; $\nu_{max.}$ (mineral oil): 3455 (OH) and 1645 (C=C) cm.$^{-1}$, and no carbonyl absorption was observed; *m/e* 315 (M^+, 100%), significant peaks at *m/e* 300 (24), 298 (52), 284 (78), and 241 (66).

Erysopitine (VI)—The mixture of phenolic bases from Fraction F was chromatographed over alumina (18 × 1.2 cm.). Elution was done with chloroform and chloroform–methanol. Chloroform–methanol (95:5) eluates gave erysopitine, $C_{17}H_{21}NO_4$, m.p. 168–171°; $[\alpha]_D^{25}$ +148° (c 0.52, ethanol); $\lambda_{max.}$: 285–287 nm. (log ε, 4.31); *m/e* 303 (M^+, 92%), significant peaks at *m/e* 288 (18), 271 (base peak), and 245 [(41), M—CH_2=$CHOCH_3$ (XII)].

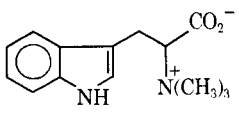
Transformation of VI into Erysotrine—Erysopitine (14 mg.) was dissolved in ether (20 ml.), and ethereal diazomethane was added in excess. The mixture was kept at ordinary temperature overnight. The gummy residue, obtained after removal of the solvent, was treated with methanesulfonyl chloride (0.5 ml.) and pyridine (0.5 ml.) at 0°. The mixture was kept at 0–5° for 3 hr. and then at ordinary temperature overnight. The product was triturated with water (10 ml.), basified (sodium bicarbonate), and extracted with chloroform (two 25-ml. portions). The chloroform layer was washed with water and dried (calcium chloride), and the solvent was removed. The thick brown liquid showed TLC behavior identi-

Table I—Relative Percent Yield and Abundance of *E. variegata* Alkaloids

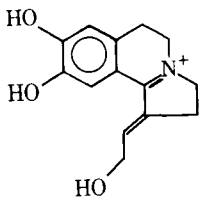
Component Alkaloids	Total Alkaloid: Root Bark (0.088%)	Trunk Bark (0.12%)	Leaves (0.04%)	Seeds (0.11%)
	Relative Abundance			
Erysotrine (I)	Traces	40	10	12
Erysodine (II)	34	15	14	Traces
Erysovine (III)	Nil	2	3	Nil
Erythraline (IV)	Traces	11	Nil	Traces
Erysopine (V)	9	7	3	10
Erysopitine[a] (VI)	Nil	5	Nil	Nil
Erysodienone (VII)	Nil	4	Nil	Traces
Erysonine (VIII)	3	2	Nil	Traces
Hypaphorine (IX)	42	3	45	51
Hypaphorine methyl ester (X)	Traces	4	6	6
N,N-Dimethyltryptophan methyl ester (XI)	4	2	2	3
Choline and uncharacterized bases	6	5	17	16



VIII: erysonine



IX: hypaphorine



XII

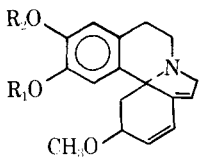
[1] Melting points were determined on a Toshniwal apparatus in open capillary and are uncorrected. Except where stated otherwise, IR spectra were measured in KBr pellets on a Perkin-Elmer 257 instrument and UV spectra were determined with aldehyde-free ethanol on a Cary 14 spectrophotometer. NMR spectra were run on a Varian A-60 D spectrometer in $CDCl_3$. Mass spectra were recorded with an A.E.I. MS-9 double-focusing spectrometer with an ionizing potential of 70 ev.; samples were directly inserted on a probe.

[2] Silica gel G, E. Merck.

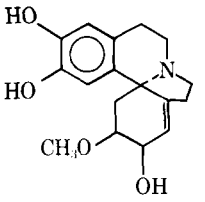
[3] The plant material used was supplied by United Chemical and Allied Products, Calcutta-1, India, and a voucher specimen is available with them.

[4] The authors are grateful to Professor V. Prelog for samples of erythrina alkaloids.

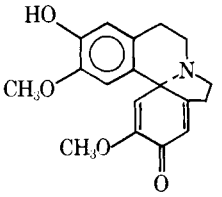
[a] Since the name erysotine has recently been given to a synthetic erythrina alkaloid (8), we propose to change the name of this naturally occurring base from erysotine (2) to erysopitine.



I: erysotrine ($R_1 = R_2 = CH_3$)
II: erysodine ($R_1 = CH_3$, $R_2 = H$)
III: erysovine ($R_1 = H$, $R_2 = CH_3$)
IV: erythraline ($R_1 + R_2 = -CH_2-$)
V: erysopine ($R_1 = R_2 = H$)



VI: erysopitine



VII: erysodienone

cal with an authentic sample of erysotrine; *m/e* 313 (M^+, 92%), significant peaks at *m/e* 298 (23) and 282 (base peak); $\lambda_{max.}$: 235 (log ϵ, 4.34) and 282–285 nm. (log ϵ, 3.51).

***N,N*-Dimethyltryptophan Methyl Ester (XI)**—Fraction G afforded a pale-brown gum, R_f 0.52, Ehrlich: blue; $\lambda_{max.}$: 222–224 (log ϵ, 4.42), 282 (log ϵ, 3.79), and 292–295 nm. (log ϵ, 3.76); $\nu_{max.}$: 3312 (NH), 2855 (NCH_3), and 1722 (CO_2CH_3) $cm.^{-1}$; $[\alpha]_D$ −59° (c 0.71, ethanol) [lit. (9) 65°, ethanol]; δ 2.29 [s, 6H, $N(CH_3)_2$], 3.25 (dd, 1H), 3.32 (s, 3H, OCH_3), 3.54 (dd, 1H), 4.45 (s, 1H), and 7.0–8.2 (complex, 5H); *m/e* 246 (M^+, 78%), significant peaks at *m/e* 183 ($M-CO_2CH_3$), 130 [base peak, $M-CH(CO_2CH_3)-N(CH_3)_2$], and 116 [$M-CH_2CH(CO_2CH_3)-N(CH_3)_2$]. The alkaloid methiodide crystallized from ethanol as prisms, m.p. and mixed m.p. 208–209°.

Hypaphorine Methyl Ester (X)—The reineckate salt from Fraction E, separated under basic condition, was dissolved in ethanol (30 ml.) and was passed over a column of De-Acidite FF (pH ~8). The regenerated based showed two spots, R_f 0.18 and 0.45, on TLC plates. The mixture was taken in water and treated with a drop of aqueous potassium iodide (10%). The solvent was removed on a steam bath, and the residue was crystallized from absolute alcohol. Hypaphorine methyl ester iodide separated as light-cream-colored needles, m.p. 207–209°.

Anal.—Calc. for $C_{15}H_{21}IN_2O_2$: C, 46.38; H, 5.41; I, 32.72; N, 7.21. Found: C, 46.26; H, 5.37; I, 32.04; N, 6.69.

The total percent yield of the alkaloids and the relative abundance of the individual bases in the various parts of *E. variegata* are shown in Table I. The mean of three determinations was taken.

PHARMACOLOGY

Details of the testing protocol of the positive findings are reported.

Animals—Albino mice (20–30 g., bred from CDRI strains), albino rats (150–200 g., bred from CDRI strains), rabbits (1–1.15 kg.), guinea pigs (0.5–0.75 kg.), frogs (75–100 g.), and mongrel dogs (10–15 kg.) were used.

Drug Preparation and Administration—A stock solution of the total alkaloids (50 mg./ml.) was prepared in dilute aqueous hydrochloric acid (final pH ~5). Subsequently, drug administration was by the intraperitoneal or intravenous route.

Gross General Observations—Groups of five albino mice were placed in cages 30 min. prior to administration of the test compound for general gross observations by the method of Irwin (10). Subsequently, these same groups were given the total alkaloids in doses of 10, 20, 40, or 80 mg./kg. i.p.; gross behavioral changes were recorded at 15, 30, 60, and 120 min. following injections.

Pentobarbital Sleeping Time—Forty rats, divided into four groups of 10 rats each, were used. One group received only the vehicle, while the three remaining groups received 2.5, 5, and 10 mg./kg. i.p. of the total alkaloids. Thirty minutes later, all groups were administered pentobarbital (30 mg./kg. i.p.). The time in minutes between injection of pentobarbital and the regain of the righting reflex was taken as the duration of sleeping time. The results are expressed in terms of percent increase in the sleeping time of the treated group over the control group.

Effect on Skeletal Muscle—Isolated frog's rectus abdominis muscle was used (11). The initial testing of the total alkaloids was done, using different concentrations (5–50 mcg./ml.), against acetylcholine- (5 mcg./ml.) and potassium chloride- (3 mg./ml.) induced spasms. The results were compared with those of tubocurarine. Spasmolytic ED_{50}'s of the test compound and of tubocurarine against acetylcholine were calculated by plotting log dose–percentage inhibition curves.

Rabbit Head Drop Method (12)—Ten rabbits, divided into two groups of five each, were used. Slow intravenous injection of the total alkaloids in doses of 5, 10, 15, 20, and 25 mg./kg. was given through the marginal ear vein of one group. The other group received tubocurarine in doses of 0.1, 0.25, 0.5, 0.75, and 1.0 mg./kg. The minimum dose required to produce the characteristic head drop and subsequent inability to lift it in spite of a sharp tap on the forehead was noted. The mean of five such experiments was taken.

Effect on Smooth Muscles—The effect of the total alkaloids was noted in isolated guinea pig ileum preparations (13) against spasms induced by acetylcholine (0.01 mcg./ml.), histamine (0.01 mcg./ml.), and barium chloride (0.2 mg./ml.). Likewise, the effect of pretreatment with the total alkaloids in isolated rat uterus preparations (14) against spasms induced by acetylcholine (0.1 mcg./ml.), serotonin (0.01 mcg./ml.), and oxytocin (0.01 U./ml.) was noted. The spasmolytic ED_{50} of the drug was calculated by plotting log dose–percentage inhibition curves against each spasmogen.

Effect on Perfused Frog Heart—The total alkaloids were administered to perfused frog heart in doses of 0.5–2.0 mg. The effect of atropine (10 mcg.) pretreatment on the drug response was also noted.

Effect on Biliary Flow (15)—In a pentobarbital- (35 mg./kg. i.p.) anesthetized dog, the bile duct was cannulated and the cystic duct was ligated. Bile secretions were collected at 30-min. intervals; when the bile flow stabilized, the drug was injected in a single dose of 1 mg./kg. i.v. Subsequently, bile samples were collected at 30-min. intervals for 180 min. The total solids per milliliter of each bile sample were also noted.

Anticonvulsant Effect—Sixteen rats, previously screened as "tonic extenders," were divided into four groups of four rats each. Three groups were given 2.5, 5, and 10 mg./kg. of the total alkaloids, while the last group received the vehicle. Thirty minutes after administration of the test compound, all rats were subjected to maximal electroshock, as described by Swinyard *et al.* (16). Anticonvulsant activity was considered when protection against the tonic extension of hind limbs was afforded to the animals. In another set of experiments, all rats, after administration of the total alkaloids, were tested against pentylenetetrazol-induced convulsions according to the method of Goodman *et al.* (17).

Determination of LD_{50}—Adult male rats in groups of four were given the total alkaloids in doses of 25, 50, 100, and 200 mg./kg. and were observed for 24 hr. The LD_{50} was calculated by the method of Litchfield and Wilcoxon (18).

RESULTS AND DISCUSSION

Eight aromatic erythrina alkaloids (I–VIII) and three carboxylated indole-3-alkylamines (IX–XI) were isolated from the trunk bark of *E. variegata*, and their identity was established by chemical reactions and spectral (UV, IR, NMR, and mass) evidence. The relative abundance of these alkaloids and a number of uncharacterized bases, in various parts of the plant, was also determined (Table I). Of the eight erythrina alkaloids isolated to date from this plant, three (I, VI, and VII) were not reported in nature until this investigation. Also, the occurrence of methyl esters of hypaphorine (X) and *N,N*-dimethyltryptophan (XI) was reported for the first time in this genus. Hypaphorine was earlier recorded in many erythrina species (4) and was recently found in two other genera—*viz.*, *Desmodium* (19) and *Abrus* (20), of the family Leguminosae; its occurrence outside this family is, however, not known so far.

In addition to the mentioned 11 alkaloids, two apparently new minor spiroamine alkaloids, which readily isomerized into substituted indoles, were isolated from this plant. Besides these bases, liberal amount of choline was isolated from all parts of *E. variegata*.

Two of the eight aromatic erythrina alkaloids reported in this paper are the recently established biosynthetic intermediates to

many erythrina alkaloids (8). Thus, incorporation of erysodienone (VII) into a number of aromatic erythrina alkaloids was recently shown by Barton *et al.* (5, 8). Also, erysotine, the C_{15}-methyl ether of erysopitine (VI), was shown to be a precursor of erythraline (IV) (8). In view of these observations, isolation of several spiroamine alkaloids and their precursors from *E. variegata* is of considerable biogenetic and chemotaxonomic significance.

The total alkaloids from the trunk bark of *E. variegata* showed several characteristic pharmacological effects: neuromuscular blocking effect of the antidepolarizing type, smooth muscle relaxant effect, and also hydrocholeretic, CNS depressant, and anticonvulsant effects.

The total alkaloids produced hind-limb paralysis in albino rats and mice in doses of 10–20 mg./kg. The ability of the animals to climb on an inclined plane and stay on a revolving drum was markedly reduced.

The total alkaloids in doses of 5 mg./kg. potentiated pentobarbital-induced hypnosis. The sleeping time in the drug-pretreated group was 85% more than in the control group. The mean sleeping time $\pm$ *SE* in the control group was 33.6 ± 4.1, whereas it was 62.4 ± 6.3 in the drug-pretreated group. The effect was statistically significant ($p < 0.005$).

The total alkaloids specifically blocked acetylcholine-induced spasms without altering the spasms induced by potassium chloride. The spasmolytic ED_{50} against acetylcholine was 10.2 mcg./ml. as against 1.05 mcg./ml. for tubocurarine. It also produced the characteristic head drop in rabbits in a dose of 10 mg./kg.; tubocurarine produced this effect in a dose of 0.75 mg./kg.

The total alkaloids showed a weak spasmolytic action against the spasmogens histamine, acetylcholine, and barium chloride on isolated guinea pig ileum. It produced a similar spasmolytic effect against the spasmogens acetylcholine, serotonin, and oxytocin on isolated rat uterus. The spasmolytic actions were nonspecific, and the ED_{50} of the drug against the spasmogens varied from 0.5 to 2.5 mg./ml.

The drug produced a moderate negative inotropic and chronotropic effect in doses of 0.5–2.0 mg. in perfused frog heart. The effect was a direct depressant one, since it was not blocked by atropine.

The total alkaloids produced a marked increase in bile secretion in a dose of 1 mg./kg. From the preinjection level of 2.4 ml., the bile volume increased to 6.1 ml. (2.5 times) 30 min. after drug administration and the effect passed off after 180 min. The effect was found to be only hydrocholeretic since the increase in bile volume was due to an increase of the fluid portion of the bile, the total solid decreasing with an increase in the bile volume.

The total alkaloids in a dose of 5 mg./kg. produced 80% protection ($p < 0.001$) to albino rats against maximal electroshock-induced convulsions and 56% protection ($p < 0.001$) to these animals against pentylenetetrazol-induced convulsions.

Acute toxicity of the drug following intraperitoneal administration, calculated as LD_{50} (mg./kg.) (with 95% fiducial limits, mg./kg.), was 127.8 (91.6–176.3).

The reported uses of the extracts of *E. variegata* in the indigenous system of medicine (1) as a remedy for biliousness, in liver troubles, in epilepsy, and as a nervine sedative could be correlated with the observed pharmacological effects of the total alkaloids from its bark. The other uses of this and related plants, such as laxative, diuretic, and antiasthmatic uses, would seem to be due to the intermediate alkaloids (dopamine, *N*-norprotosinomenine, and equivalents) to the spiroamines. A recent report of the occurrence of *N*-norprotosinomenine in *Erythrina lithosperma* Blume (21) is significant from this latter point of view. Further search for isoquinoline bases in *E. variegata* and in related taxa is underway.

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