PHARMACOLOGIC ACTION OF ERYTHRINA ALKALOIDS
II. FREE, LIBERATED AND COMBINED ALKALOIDS

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In studies covering fifty-one out of the one hundred and five species of *Erythrina* known at present, it was found that the seeds of all species investigated possess curare-like action when tested on frogs (1). The curarizing potency of the seeds, varying in wide limits among the different species but surprisingly uniform in samples of any one given species gathered from widely separated regions, appears to be a characteristic feature of the genus, *Erythrina*. It has been shown by Folkers and his associates that the seeds of Erythrina contain a mixture of hitherto unknown alkaloids.

Subsequent to the isolation of erythroidine from *Erythrina americana* Mill. (2) other alkaloids have been isolated some of which, like β-erythroidine, are readily soluble in water as well as in chloroform. Among these alkaloids, erythramine, erythraline and erythratine have been obtained in an established pure form. Studies on the constitution of these substances (3) revealed that erythraline, $C_{18}H_{19}NO_3$, differs from erythramine, $C_{18}H_{21}NO_3$, by the presence of one additional ethylenic double bond; erythratine, $C_{18}H_{21}NO_4$, contains an additional non-phenolic hydroxyl group. All three alkaloids contain a tertiary nitrogen atom and react with methyl iodide to give the corresponding methiodides.

The pure alkaloids produce the same curarizing effect as that observed with crude seed extracts. However, the yield and potency of the alkaloids isolated from the chloroform fraction failed to account for the high curarizing activity of the seed extracts previously reported (1). For instance, seeds of *Erythrina glauca* Willd. (sample Haigh 9170) contain only 0.4% or 4 mgm. of these alkaloids per gram, an amount barely sufficient to paralyze 1 kgm. of frog, whereas the extract of one gram of seed was found to paralyze about 33.3 kgm. of frog. From this and other experiments it became apparent that the activity of the seed extracts did not always represent a measure of the amount of alkaloids which could be isolated as the free[1] fraction. This discrepancy between the potency of the seed extracts and the yield of free alkaloids suggested further chemical and pharmacological studies on the crude extracts. After removal of the free alkaloidal fraction, aqueous extracts of the seeds of *Erythrina glauca* Willd. and of other species were found to possess almost the same or only slightly less activity when retested on frogs. It was therefore concluded that the seeds

[1] This fraction has been designated as the free alkaloidal fraction to distinguish it from the fraction liberated by hydrolysis. The latter has been designated as the liberated alkaloidal fraction, and the combined alkaloidal fraction refers to this fraction before hydrolysis (5).

contain besides the free alkaloidal fraction other alkaloids of quite different solubility characteristics.

From the combined fraction Folkers and his associates have isolated by acid hydrolysis a new series of alkaloids of which erysopine, erysovine, erysodine and erysonine have been established in pure form (5, 6). The names of these liberated alkaloids were derived from the stem eryso- in order to distinguish them from the free alkaloids for which the stem erythr- was selected. Prior to hydrolysis, the liberated alkaloids exist in a combined form. Further chemical research showed the combined forms were alkaloidal esters of sulfoacetic acid (7). Thus, hydrolysis of two pure isolated combined alkaloids, erysothiopine and erysothiovine, gave erysopine and erysovine respectively and one molecule of sulfoacetic acid.

This paper is concerned with the pharmacologic action of various free, liberated and combined Erythrina alkaloids and some substances derived from these alkaloids. Their action has been compared to that of β-erythroidine (8).

The substances used in this study were obtained through the courtesy of Dr. Karl Folkers. The free alkaloids erythramine, erythraline and erythratine were available in the form of their hydrobromides and hydroiodides; dihydro-erythramine and dihydro-erythratine in the form of their hydrobromides. All substances are crystalline and readily soluble in water. The quaternary methosalts of erythramine and erythratine were obtained through the reaction of the tertiary bases with methyl iodide.

The liberated alkaloids erysopine, erysovine, erysodine and erysonine were available as free bases, and were used in aqueous solutions in the form of their hydrochlorides, erysodine also in the form of its sodium salt. Of the hydrogenated derivatives, tetrahydro-erysopine and tetrahydro-erysodine were obtained as hydrobromides. The combined alkaloids were represented by the sodium salts of erysothiopine and erysothiovine.

Curarizing effect in frogs. The potency of the alkaloids was tested in frogs kept at a constant temperature of 20°C. All alkaloids, with the exception of tetrahydro-erysodine, were found to possess curarizing effects. Electric stimulation of the sciatic nerve failed to elicit any response from the gastrocnemius muscle, whereas direct stimulation of the muscle was effective. The threshold for the direct stimulation remained unchanged during the paralytic stage. The minimum doses of the various substances causing complete block of the neuromuscular transmission are summarized in table 1.

Erythramine and erythraline possessed the same potency (10 mgm. per kgm.) whereas erythratine was much less effective. Dihydro-erythramine and dihydro-erythratine were markedly less effective than the parent substances, and the conversion of either one of the three free alkaloids into their respective methosalts decreased their curariform action. Among the liberated alkaloids erysovine was the most potent, closely followed by erysopine. Erysovine was equally effective in the form of the hydrochloride and as the sodium salt. Of the hydrogenated derivatives, tetrahydro-erysopine had only one-tenth of the activity of erysopine, and tetrahydro-erysodine failed to cause complete curarization when tested at 300 mgm. per kgm., thirty fold the effective dose of ery-

sodine. The combined alkaloids erysothiopine and erysothiovine were equally effective, surpassing in potency all other substances.

The average duration of the paralysis and its prolongation by increased doses of various alkaloids are summarized in table 2. The paralysis caused by the free alkaloids was short lasting, whereas the liberated and combined alkaloids had a prolonged effect. Erysopine had the most persistent action of any of the alkaloids. A threshold dose of this substance paralyzed the frogs for 24 hours;

TABLE 1

Potency of erythrina alkaloids in frogs

	MINIMUM CURARIZING DOSE BY INTRALYMPHATIC INJECTION
	mgm. per kgm.
Free alkaloids	
Erythramine HBr	10
Dihydro-erythramine HBr	300
Erythramine methiodide	40
Erythraline HBr	10
Erythraline methiodide	50
Erythratine HBr	75
Dihydro-erythratine HBr	100
Erythratine methiodide	300
Liberated alkaloids	
Erysopine HCl	4
Tetrahydro-erysopine HBr	40
Erysovine HCl	3
Sodium erysovinate	3
Erysodine HCl	10
Tetrahydro-erysodine HBr	>300
Erysonine HCl	100
Combined alkaloids	
Sodium erysothiopinate	1
Sodium erysothiovinate	1

ten-fold this amount produced paralysis lasting for about 5 days from which some frogs failed to recover. In the duration of its action, erysopine was distinctly different from erysovine. The combined alkaloids, erysothiopine and erysothiovine, however, had the same long lasting effect.

Toxicity in mice. The toxicity of some of the alkaloids was determined in mice following subcutaneous or oral administration, 10 animals being used for each dose level (table 3). All animals receiving lethal doses apparently died of respiratory failure due to paralysis of the diaphragm. Death usually occurred within 1 to 3 hours. The heart continued to beat for several minutes

after the respiration had ceased. Among the free alkaloids erythramine was less toxic than erythraline although both alkaloids were equally effective in frogs. Of the liberated alkaloids, erysopine was the most toxic. Erysothiopine, contrary to its effectiveness in frogs, was markedly less toxic than erysopine when tested on mice by subcutaneous injection. The oral toxicity was determined for erythraline, erysodine and erysopine. In all instances, the toxic doses were from 10% to 50% larger than those established by subcutaneous injection.

TABLE 2

Duration of paralysis in frogs, expressed by the time interval between injection and complete recovery for the average of 10 frogs on each dose level

ERYTHRAMINE ERYTHRALINE		ERYSOVINE		ERYSOPINE		ERYSODINE		ERYSOTHIOPINE ERYSOTHIOVINE	
Mgm./ kgm.	Duration	Mgm./ kgm.	Duration	Mgm./ kgm.	Duration	Mgm./ kgm.	Duration	Mgm./ kgm.	Duration
10	3 hrs.	3	6 hrs.	4	24 hrs.	10	10 hrs.	1	24 hrs.
100	2 days	6	10 hrs.	8	1.5 days	20	24 hrs.	3	30 hrs.
200	3 days	10	15 hrs.	12	2.5 days	40	2 days	10	2 days
400	Died	20	24 hrs.	20	3.5 days	100	Died	50	Died
		30	2 days	40	5 days				
		100	Died		40% Died				
				100	Died				

TABLE 3

Toxicity of various erythrina alkaloids in mice

	LD 50 (MGM. PER KGM.)	
	Subcut.	Oral
Free alkaloids		
Erythramine HBr	104	
Erythraline HBr	72	80
Liberated alkaloids		
Erysopine HCl	14.8	18
Erysodine HCl	100	155
Combined alkaloids		
Sodium erysothiopinate	76	

Effect on circulation. The effect of intravenous injections of various alkaloids was studied in rabbits, cats and dogs anesthetized with sodium-pentobarbital. The free alkaloids, erythramine and erythraline showed no significant difference in their action upon the blood pressure and the heart rate; the intravenous injection of 3 to 5 mgm. per kgm. of either one of these alkaloids was fatal in cats. Both alkaloids caused a sharp fall in blood pressure returning to its original level within 3 to 15 minutes depending on the amount injected (0.25 to 1.5 mgm. per kgm.). Thus, the effect of these alkaloids on the blood pressure was of

the same short duration as observed with β-erythroidine (8) but the fall in blood pressure was more marked than that following β-erythroidine. Furthermore, in contrast to β-erythroidine, erythramine and erythraline significantly decreased the heart rate in cats, when doses smaller than the paralyzing dose

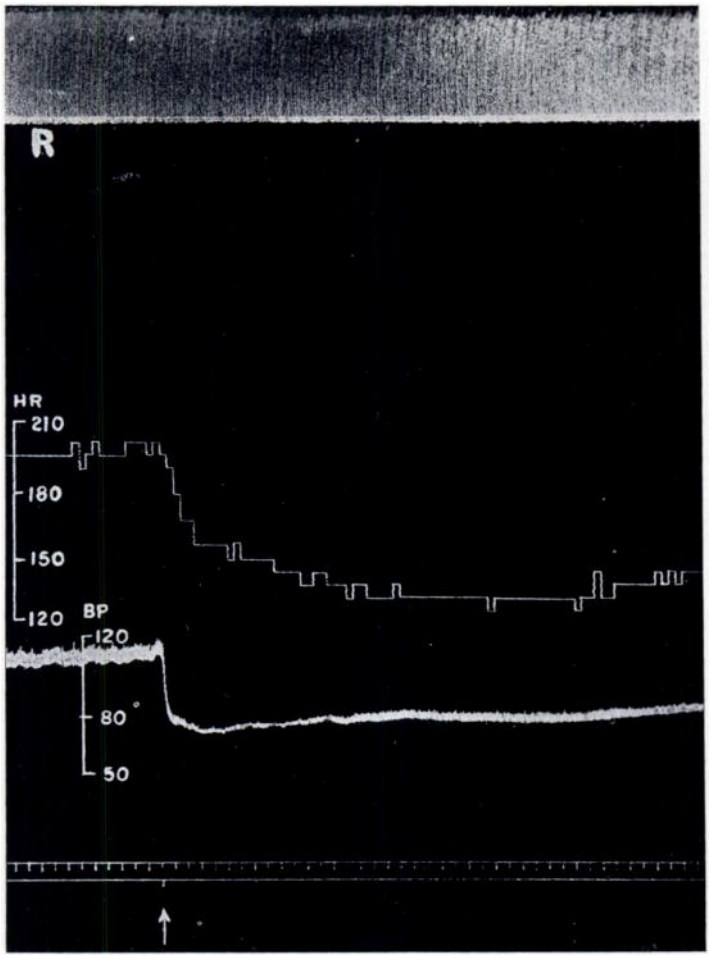

FIG. 1. EFFECT OF ERYSOPINE ON HEART RATE AND BLOOD PRESSURE

Cat, 2.6 kgm. anesthetized with 40 mgm. per kgm. sodium pentobarbital intraperitoneally. R: Rate of respiration. HR: Heart rate, beats per minute. BP: carotid blood pressure, mm. Hg. Time in 30 seconds. At the arrow Erysopine hydrochloride 1 mgm. per kgm.

were injected. The slowing of the heart rate was transient, its duration paralleling that of the depression of the blood pressure. Intravenous injections of atropine (2 mgm. per kgm.) failed to prevent either the decrease in heart rate or the fall in blood-pressure.

The liberated alkaloids, erysopine and erysovine caused decrease in the blood pressure and slowing of the heart rate which was more pronounced than

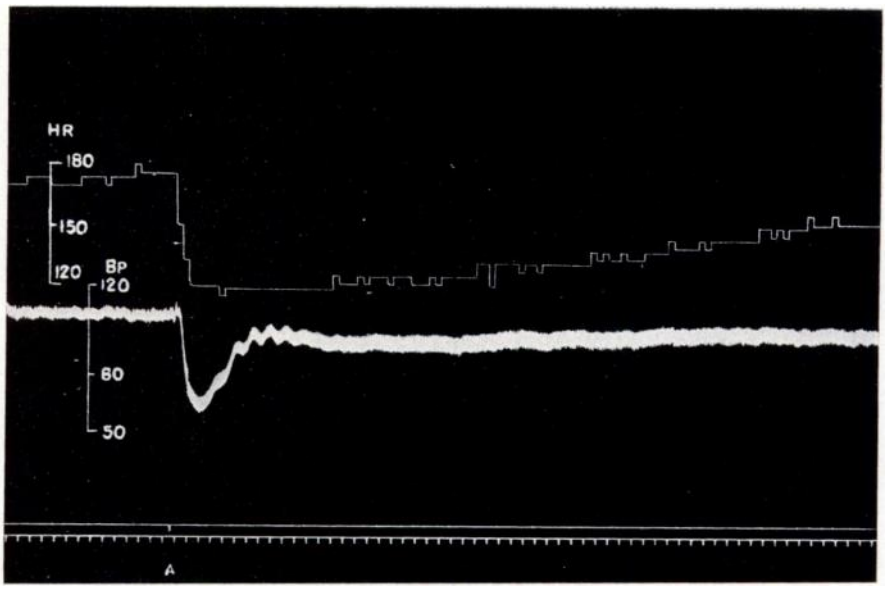

FIG. 2A. EFFECT OF ERYSOVINE ON HEART RATE AND BLOOD PRESSURE

Dog, 6 kgm., anesthetized with 36 mgm. per kgm. sodium pentobarbital intraperitoneally. HR: Heart rate, beats per minute. BP: carotid blood pressure, mm Hg. Time in 30 seconds. The animal was given a total of 4 mgm per kgm atropine sulfate intravenously. A, erysovine, 0.5 mgm per kgm.

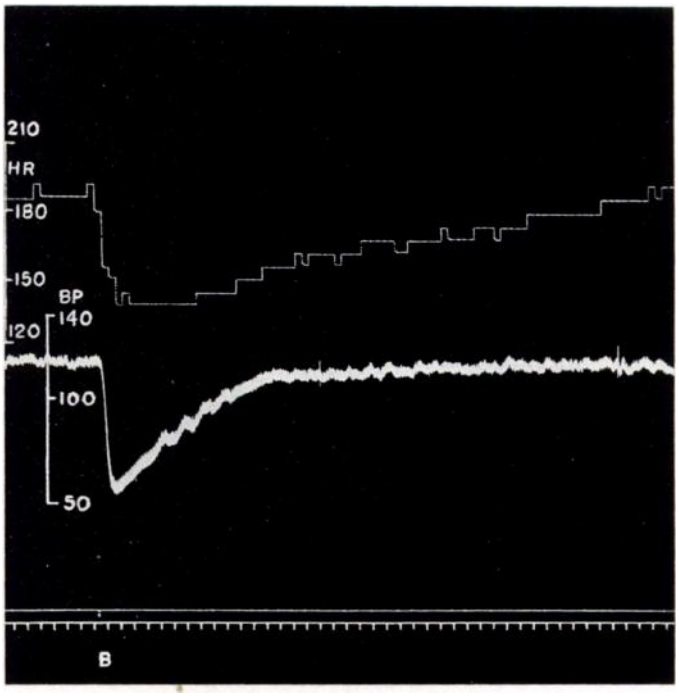

FIG. 2B. EFFECT OF ERYSOTHIOVINE ON HEART AND BLOOD PRESSURE

Same experiment as in Fig. 2A. B, erysothiovine, 0.25 mgm per kgm.

than that observed with any of the free alkaloids. Also, the duration of these effects on the circulation was prolonged. Erysovine appeared to be slightly more effective than erysopine. Erysopine, however, surpassed erysovine in the duration of its depressing effects upon both blood pressure and heart rate. Figure 1 illustrates the effect of erysopine on an anesthetized cat; the dose of 1 mgm. per kgm. did not influence the respiration, but slowed the heart rate from 200 to 130 beats per minute and lowered the blood pressure from 110 to 70 mm. Hg. Blood pressure and heart rate gradually regained their original levels within 45 minutes. In dogs, depression of the blood pressure and of the heart rate were observed with doses of 0.2 mgm. per kgm. of erysovine, representing less than 10% of the paralyzing dose. The effect of 0.5 mgm. per kgm. erysovine upon the heart rate and blood pressure of a dog is illustrated in figure 2A. The slowing of the heart rate (from 175 to 120 beats per min.) persisted much longer than the depression of the blood pressure. It subsided gradually and 45 minutes after the injection, the heart rate reached its previous frequency.

The effects on blood pressure and heart rate in these experiments were not influenced by atropine. In dogs anesthetized with sodium pentobarbital, atropine often failed to increase the heart rate significantly. In order to be assured that sufficient atropine had been given to paralyze the vagal endings, either the vagal nerves were stimulated or the effect of carbaminoyl choline was tested before and after atropine. Dogs anesthetized with sodium pentobarbital required comparatively large amounts of atropine for the abolishment of vagal responses. In atropinized dogs, erysovine (fig. 2A) and erysothiovine (fig. 2B) still produced a marked decrease in both heart rate and blood pressure. These effects were not significantly different from those obtained without atropinization.

Erysothiovine (fig. 2B) and erysothiopine caused a depression of both the heart rate and the blood pressure comparable to that obtained with erysovine and erysopine. The effective doses of the combined alkaloids, however, were smaller than those of the liberated alkaloids.

Discussion. All *Erythrina* alkaloids investigated possess true curare action, although the potency varies greatly among the different alkaloids. They also differ markedly in the duration of their action. Differences in potency and duration of effects between β-erythroidine and some substances derived from it, particularly dihydro-β-erythroidine, have already been reported (8). A comparison of the efficacy of the free, liberated and combined alkaloids and the duration of their action in frogs is presented in figure 3.

The free alkaloids, β-erythroidine, erythramine and erythraline share a characteristic feature in that their action is remarkably short. The smallest effective doses ranging from 3 to 100 mgm. per kgm. are similar in both the free and liberated alkaloids, but the action of the liberated alkaloids is more persistent. Erysovine, for instance, causes paralysis lasting more than twice as long as that following β-erythroidine, although the smallest effective doses of both substances are the same. On the other hand, the combined alkaloids, erysothiopine and erysothiovine, are effective in much smaller amounts than the

corresponding liberated alkaloids. The duration of their effect is either equal to, (erysothiopine) or more prolonged (erysothiovine) than, that of the liberated alkaloids.

The duration of the paralysis caused by these alkaloids can be prolonged by increasing the dose. Of the free alkaloids, erythramine, erythraline and β-erythroidine, frogs tolerate amounts from 20 to 80 times greater than the smallest effective dose, recovering completely after 3 to 5 days. The effect of the liberated alkaloids, erysodine and erysopine, however, becomes irreversible when 10 times the threshold dose is given. The difference in the spread from the minimum effective to the lethal dose between the free and liberated alkaloids apparently indicates that the short acting free alkaloids are metabolized at a faster rate than the liberated alkaloids.

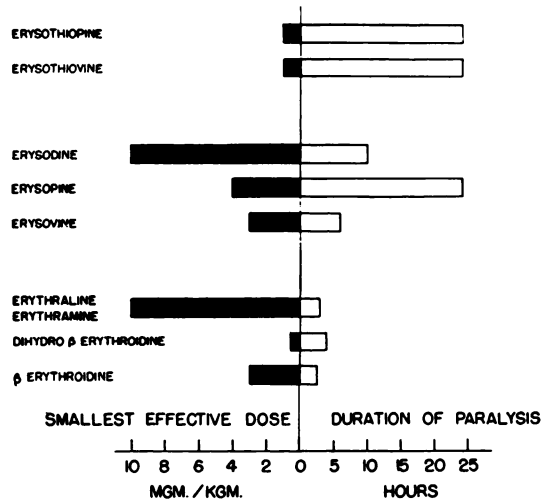

FIG. 3. COMPARISON BETWEEN THE EFFICACY AND THE DURATION OF THE ACTION OF ERYTHRINA ALKALOIDS IN FROGS

In general, the order of toxicity of the alkaloids in mice corresponds to that of their potency in frogs. A notable exception was found in erysothiopine which in the form of the sodium salt[2] was much less effective in mice than was anticipated from its high potency in frogs. On intravenous injection in cats, however, erysothiopine was more effective than erysopine; paralysis of the diaphragm and death were obtained with 0.8 mgm. per kgm. erysothiopine as compared to 2 mgm. per kgm. erysopine.

The circulatory effects of the various alkaloids reported in this study are more pronounced than those obtained with β-erythroidine (8). This is especially true for the liberated and combined alkaloids which lower the heart rate and depress the blood pressure in quantities which are only fractions of their paralyz-

[2] The sodium salts of the alkaloids were less stable on standing in aqueous solutions (pH 10–14) than solutions of the hydrochlorides which showed unaltered activity when retested after 3 months.

ing doses. Atropine given in doses sufficient to paralyze the vagal ending fails to prevent the decrease in heart rate in animals anesthetized with sodium pentobarbital. Hence, it is concluded that the bradycardia observed is due to a direct action of these alkaloids upon the heart.

Hydrogenated derivatives of free alkaloids, (dihydro-erythramine and dihydro-erythratine) and of liberated alkaloids, (tetrahydro-erysopine and tetrahydro-erysodine) were considerably less active on frogs than their parent alkaloids. This is in contrast to the findings with the hydrogenated derivatives of β-erythroidine; dihydro-β-erythroidine was about 6 times more active in frogs than β-erythroidine.

Conversion of erythramine, erythraline and erythratine into their methiodides decreased their curarizing action to one-fourth or one-fifth. An even greater decrease in activity had been obtained by the conversion of β-erythroidine into β-erythroidine methiodide. Thus, the quaternary metho-salts of all free alkaloids were found to be less active than the tertiary bases.

SUMMARY

1. The following 9 alkaloids isolated from seeds of species of the genus *Erythrina:* erythramine, erythraline and erythratine (free alkaloids), erysopine, erysovine, erysodine and erysonine (liberated alkaloids), and erysothiopine and erysothiovine (combined alkaloids) produce typical curare-like action.

2. The potency of these alkaloids and of some derivatives has been determined on frogs; the greatest activity was found with the combined alkaloids.

3. The combined and the liberated alkaloids have a more persistent action than the free alkaloids.

4. The order of toxicity of various alkaloids in mice following oral and subcutaneous administration followed, in general, that of their potency in frogs.

5. Intravenous injections especially of the liberated and combined alkaloids depressed the blood pressure and lowered the heart rate to a greater extent than did β-erythroidine.

6. Atropine failed to influence the bradycardia in cats and dogs anesthetized with sodium pentobarbital.

7. In contrast to the findings with β-erythroidine and dihydro-β-erythroidine, hydrogenated derivatives of erythramine, erythratine, erysopine and erysodine are less active than the parent alkaloids.

8. The quaternary metho-salts of erythramine, erythraline and erythratine are, like that of β-erythroidine, less active than the tertiary bases.

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