SECTIONS

ERYTHRINA ALKALOIDS. V. COMPARATIVE CURARE-LIKE POTENCIES OF SPECIES OF THE GENUS ERYTHRINA.*

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In a recent paper (1), an historical review was made of the alkaloids of species of *Erythrina*, and data were presented showing in twenty-six species the presence of alkaloids which caused a curare-like action in the frog. This number of twenty-six species was reduced to twenty-three when the recent taxonomic revision of *Erythrina* by Mr. B. A. Krukoff (2, 3) was applied. The species' names used in this present paper are in full accord with his treatment of the genus.

This paper presents the data on the assay of twenty-eight additional species of *Erythrina*, and represents a total of fifty-one tested species out of the one hundred and five known species. For a great majority of the samples the identity of the *Erythrina* seeds used in these studies was established by accompanying herbarium material, and for the rest of the samples by other evidence which left no doubt regarding the identity.

The significant data on the twenty-eight additional species as well as new data on previously examined species are presented in Table I. Species from nearly every tropical and sub-tropical part of the world were examined. Most of the seeds were collected within the last three years and the same numbers were assigned to both the seed samples and to the botanical specimens taken from the same plants as the seeds. In most cases, a quantity of one to three Gm. of seeds

TABLE I.—DATA ON SPECIES OF ERYTHRINA.

Name of Plant.	Collectors' Names and Specimen Numbers.		Amount Gm. Seeds.	Fatty Fraction %.	Ethanol Extractives %.	Water Added Ml.	Threshold Dose Ml./Kg.	Paralysis Potency Value Gm. Frog/1 Gm. Seed.
E. fusca Lour.	(Buitenzorg)	9146	2.1067	7.2	14.8	1.0	0.05	9,500
E. fusca Lour.	(Buitenzorg)	9149	1.7112	8.9	14.1	1.0	0.05	11,700
E. glauca Willd.	Roig	9214	3.0018	6.6	15.2	20.0	0.2	33,300
E. glauca Willd.	Dugand	9203	3.0006	7.9	14.9	20.0	0.2	33,300
E. glauca Willd.	Haigh	9170	3.0014	10.4	14.0	20.0	0.2	33,300
E. glauca Willd.	Janz[1]	9343	3.0001	9.6	19.0	20.0	0.4	16,650
E. velutina Willd.	Walmsley	9211	3.0119	3.5	16.5	20.0	0.2	33,300
E. velutina Willd.	Gomez Parente	9169	3.0034	9.0	14.5	20.0	0.2	33,300
E. velutina Willd.	Krug[2]	9196	3.0001	7.6	15.6	20.0	1.5	4,400
E. velutina Willd.	Vasconcellos Sobrinho	9263	3.0000	8.6	17.0	20.0	0.4	16,650
E. velutina forma *aurantiaca* (Ridl.) Kruk.	Dias da Rocha	9272	3.0000	11.0	15.4	20.0	0.6	11,100
E. Grisebachii Urb.	Walsingham	9316	3.0000	10.3	16.3	20.0	0.8	8,300
E. crista-galli L.	Meyer	9212	3.0017	10.7	20.9	20.0	0.08	83,300
E. arborescens Roxb.	Sayeeduddin	9225	3.0001	14.2	19.4	20.0	0.1	66,600
E. speciosa Andr.	(undesignated)	9232	3.0000	9.2	13.1	20.0	1.0	6,600
E. edulis Triana	Robledo	9231	20.0	0.6	13.6	100.0	5.0	1,000
E. cubensis C. Wright	Acuna	9234	3.0008	13.3	16.9	20.0	0.2	33,300

* From the Research Laboratory of Merck & Co., Inc., and the Merck Institute of Therapeutic Research, Rahway, N. J.

TABLE I.—DATA ON SPECIES OF ERYTHRINA. (Continued from page 1019.)

Name of Plant.	Collectors' Names and Specimen Numbers.		Amount Gm. Seeds.	Fatty Fraction %.	Ethanol Extractives %.	Added Ml.	Threshold Dose Ml./Kg.	Paralysis Potency Value Gm. Frog/1 Gm. Seed.
E. Standleyana Kruk.	Acuna	9235	3.0006	14.9	13.7	20.0	0.6	11,100
E. mysorensis Gamble	Sutton	9270	10.0001	11.3	11.2	50.0	>40.0	<125
E. pallida Britton & Rose	Wortley	9257	3.0003	11.8	12.2	20.0	0.3	22,200
E. abyssinica Lam.	Louis	9259/5928	3.0000	14.8	16.4	20.0	0.6	11,100
E. sigmoidea Hua (?)	(Bur. Econ. Fr. Guin.)	9275	3.0003	9.8	13.8	20.0	0.15	44,400
E. orophila Ghesg.	Louis	9357	4.1444	16.2	14.1	27.6	0.8	8,300
E. falcata Benth.	Santini	9274	3.0005	9.3	18.0	20.0	0.6	11,100
E. suberosa Roxb.	Dorasami	9281	3.0001	6.3	19.0	20.0	0.08	83,300
E. Parcellii Bull.	Dorasami	9280	10.0000	9.9	12.4	50.0	10.0	500
E. stricta Roxb.	Ghose	9289	3.0001	12.2	20.4	20.0	0.3	22,200
E. stricta Roxb.	Biswas[2]	9290	3.0007	10.5	17.7	20.0	20.0	330
E. stricta Roxb.	Biswas[3]	9290	3.0002	10.4	18.0	20.0	10.0	660
E. stricta Roxb.	Broadfoot	9341	3.0012	8.0	19.9	20.0	0.2	33,300
E. stricta Roxb.	Bor	9335	3.0000	8.2	20.6	20.0	0.2	33,300
E. altissima A. Chev.	(Serv. For. Cote D'Ivoire)	9291	3.0003	8.9	20.0	20.0	1.0	6,600
E. glabrescens R. N. Parker	Bor	9334	3.0005	11.4	19.0	20.0	0.2	33,300
E. excelsa Baker	Thomas	9342	3.0005	9.0	24.0	20.0	0.2	33,300
E. americana Mill.	Mexia	9394/9022a	3.0001	9.1	17.0	20.0	1.0	6,600
E. breviflora forma *petraea* (Brand.) Kruk.	(undesignated)[4]	9397	2.8210	15.1	20.7	18.8	0.2	33,300
E. costaricensis Micheli	Niehaus	9200	3.6849	8.5	18.1	10.0	0.2	13,500
E. occidentalis Stand.	(undesignated)[4]	9398	1.2718	13.2	18.7	8.5	0.08	83,500
E. lanata Rose	(undesignated)	9399	1 2906	6.8	16.4	8.6	0.25	26,700
E. mexicana Kruk.	(undesignated)	9408	1.3458	11.7	29.3	9.0	0.08	83,500
E. Buchii Urb.	(undesignated)[4]	9414	0.8970	11.2	19.1	6.0	0.06	111,000
E. suberifera Welw. (?)	Louis	9416	3.0003	9.4	16.1	20.0	0.2	33,300
E. macrophylla DC.	Armstrong	9419/32	3.0000	9.4	16.1	20.0	0.06	111,000
E. Eggersii Kruk. & Mold.	(undesignated)[4]	9417	1.0370	17.2	17.3	6.9	0.04	166,000
E. chiapasana Kruk.	(undesignated)[4]	9429	0.9278	9.3	15.7	6.2	0.3	22,200
E. Standleyana Kruk.	(undesignated)[4]	9431	2.3395	17.8	13.0	15.6	2.5	2,700
E. Goldmanii Stand.	(undesignated)[4]	9428	1.5170	11.1	14.0	10.1	0.4	16,600
E. americana Mill.	(undesignated)[4]	9430	2.0449	14.0	17.6	13.6	0.6	11,100
E. coralloides DC.	(undesignated)[4]	9433	2.3613	19.6	14.8	15.8	0.05	134,000
E. americana Mill.	(undesignated)[4]	9432	2.4755	15.5	15.0	16.5	1.5	4,400
E. costaricensis Micheli	(undesignated)[4]	9435	1.4699	13.0	16.3	9.8	0.6	11,100
E. lanceolata Stand.	(undesignated)[4]	9436	1.9878	16.6	16.0	13.2	0.2	33,300
E. mexicana Kruk.	Giesemann	9240	3.0000	16.1	19.0	20.0	0.3	22,200
E. caffra Thunb.	Smithers	9279	3.0000	11.9	16.0	20.0	0.6	11,100

[1] These seeds were obtained from a cultivated plant.

[2] These seeds were obtained from pods left over on a tree at the end of the fruiting season.

[3] This sample showed a very low paralysis potency value, whereas other samples of the species as well as those of the related *E. suberosa* and *E. glabrescens*, showed high values uniformly. This low potency might be simply the result of an abnormal plant.

[4] These seeds were old.

was used for an assay. This meant that micro quantities of active alkaloids were being determined. No attempt was made to discard the seed coat, all particles of the ground seeds being uniformly used. The manipulative details of the assay procedure were the same as those which were developed and described previously (1). Although this research is not directly concerned with the fatty oil content of the seeds, interesting data on the amounts of this fatty fraction in the seeds of various species were obtained since its initial removal facilitated the analytical procedure. There was one significant change in the assay procedure; the amount of water used to dissolve the ethanol extractives was always in the proportion of twenty ml. of water to three Gm. of powdered seeds. The ethanol extractives contained practically all of the active alkaloids together with extraneous material which was inert in the pharmacological test.

The extracts were tested by intralymphatic injection into a series of frogs, two animals being used for each dose level and the dose levels differing from each other by 10% to 25%. The validity of the results obtained by this procedure has been established and checked by an experiment with a large number of animals. Using twenty frogs per dose level it was found that the reaction of the frogs was consistently uniform. The minimum effective dose, as determined previously on two frogs per dose level, produced paralysis in seventeen out of twenty frogs; a dose 15% below the threshold dose paralyzed only three out of twenty frogs. The minimum dose which caused a complete curare-like paralysis was checked by electrical stimulation of both the nerve and muscle. From this minimum or threshold dose, the potency of the seed or the "paralysis potency value" was calculated. This value is defined as the number of Gm. of frog paralyzed by the extract of one Gm. of seed. The average error in the final paralysis potency values should be within 25%, a degree of accuracy which seemed sufficient for this type of investigation.

The paralysis potency values of different species were found to vary widely, the figures in the last column of Table I ranging from 125 to 166,000. These values correspond well with those previously reported (1), or 165 to 82,000 for twenty-three species of *Erythrina.*

Paralysis occurred within thirty to forty-five minutes after the injection, even by the smallest effective dose. The frogs injected with the threshold dose were paralyzed for approximately one hour and had completely recovered after three hours. Frogs injected with ten times the amount of the threshold dose recovered the next day. No deaths occurred even after the injection of a multiple of the threshold dose. Increase in dosage only prolonged the effect in frogs. The action of all extracts was rather transient. The short duration of the action of the *Erythrina* extracts contrasts markedly with the lasting paralysis caused by curare or by the extract of plants which are known as the essential ingredients of curare; *i. e.*, species of *Strychnos*.

During the testing of seeds of species of *Erythrina*, there was occasion to test seeds of species of related genera or of genera the seeds of which superficially resemble those of *Erythrina*. Seeds of these plants were tested by the same procedure, and the data are recorded in Table II. In no

TABLE II.—DATA ON OTHER PLANTS OF INTEREST.

Name of Plant.	Specimen Numbers.[1]	Amount Gm. Seeds.	Fatty Fraction %.	Ethanol Extractives %.	Water Added Ml.	Threshold Dose Ml./Kg.	Paralysis Potency Value Gm. Frog/1 Gm. Seed.
Rhynchosia pyramidalis (Lam.) Urb.	9139	4.1286	0.7	4.7	1.8	10.0	43
Canavalia maritima (Aubl.) Thon.	9265	3.0002	1.0	8.6	20.0	(40.0)[2]	Negative
Clitoria arborescens Ait.	9299	1.3512	13.3	17.8	9.0	8.0	830
Clitoria mariana L.	9300	1.1231	18.1	18.2	7.5	(40.0)[2]	Negative
Mucuna urens (L.) DC.	9297	4.4732	4.3	4.1	30.0	(20.0)[2]	Negative
Mucuna deeringiana (Bart.) Small	9296	2.8100	4.5	7.8	18.5	(40.0)[2]	Negative
Mucuna pruriens (L.) Pers.	9298	3.0000	2.8	6.1	20.0	(40.0)[2]	Negative

[1] All the seeds tested were old.

[2] There was no curare-like paralysis of the frogs at this high dose. Tests with Mayer's, Valser's and Scheibler's reagents indicated the absence of alkaloids.

case was any marked degree of curare-like activity discovered. Seeds of five of the seven species listed in Table II were free of alkaloids and showed no action at all. Only the extracts of the seeds of *Rhynchosia pyramidalis* and *Clitoria arborescens* possess some curare-like action which, compared to the activity of the *Erythrina* seeds in Table I, was very weak. If the potency of the alkaloids in these two species responsible for the curare-like action is of the same order of magnitude as that of the *Erythrina* alkaloids, then *Rhynchosia pyramidalis* and *Clitoria arborescens* contained only traces of alkaloids. However, an examination of certain other species of these genera might reveal the presence of greater activity. Santesson (4) recently examined *Rhynchosia phaseoloides* and found positive alkaloidal and gucosidal reactions. However, he did not observe a curare-like action of the extract in the frog.

It is obvious that the paralysis potency values of the *Erythrina* seeds could be used to other advantages if the relationships between the species were better known. For example, knowledge of the identities, quantities and activities of the alkaloids of one species of *Erythrina* will enable one to suggest quite accurately the nature of the alkaloid content of related species on the basis of comparative paralysis potency values. After the recent taxonomic studies (2,3) on the genus *Erythrina*, it has been possible to arrange the species in the order of their probable relationship, to attach the corresponding paralysis potency values, and thus have in a compact form a series of values for interpretation and guidance. This arrangement has been shown in Table III, and only the significant data have been recorded. Where necessary, data were taken from the results already published (1). In preparing Table III it has been found desirable to list all species of *Erythrina* known to date even though an assay of all species has not yet been made. In this way, estimates can be made from existing data for those species which have not yet been available for actual testing.

TABLE III.—PARALYSIS POTENCY VALUES ON SPECIES OF THE GENUS ERYTHRINA.

	Species.	Names and Numbers.		Paralysis Potency Value.
Subgenus *Chirocalyx*.				
	Section *Merocraspedon* (African Species).			
1	*E. abyssinica* Lam.	Ross	9179	46,000[10]
	E. abyssinica Lam.	Louis	9259/5928	11,100
2	*E. comosa* Hua			
3	*E. Droogmansiana* De. Wild. & Th. Dur.			
4	*E. orophila* Ghesg.	Louis	9357	8,300
5	*E. Schliebenii* Harms			
6	*E. Addisoniæ* Hutch. & Dalz.			
7	*E. suberifera* Welw. (?)	Louis	9416	33,300
8	*E. mossambicensis* Sim.			
9	*E. Tholloniana* Hua			
10	*E. eriotricha* Harms			
11	*E. Sacleuxii* Hua			
12	*E. Dybowskii* Hua			
13	*E. Sigmoidea* Hua (?)	(Bur. Econ. Fr. Guin.)	9275	44,400
14	*E. decora* Harms			
15	*E. sudanica* E. G. Baker			
	Section *Dichilocraspedon* (African Species)			
16	*E. Mildbrædii* Harms			
17	*E. Klainei* Pierre			
18	*E. Buesgenii* Harms			
19	*E. altissima* A. Chev.	(Serv. For. Cote D'Ivoire)	9291	6,600
	Section *Dilobochilus* (African Species)			
20	*E. excelsa* Baker	Thomas	9342	33,300
21	*E. Burtii* E. G. Baker			

Section (?) (African Species)

22 *E. rotundato — obovata* E. G. Baker

Group *Variegatæ* (Asiatic-Polynesian Species, Numbers 23–31; American Species, Numbers 32–33; Australian Species, Numbers 34–35)

23	*E. variegata* L.	(Buitenzorg)	9148	410[10]
23a	*E. variegata* L. var. *orientalis* (L.) Merr.	Haigh	9172	500[10]
	"	Otero	9131	240[10]
	"	(undesignated)	9129	450[10]
	"	Canicoza	9152	240[10]
24	*E. Parcellii* Bull.	Dorasami	9280	500
25	*E. mysorensis* Gamble	Sutton	9270	125
26	*E. rostrata* Ridl.			
27	*E. Merrilliana* Kruk.			
28	*E. euodiphylla* Hassk.			
29	*E. boninensis* Tuyama			
30	*E. tahitensis* Nadeaud			
31	*E. sandwicensis* Degener	L. W. Bryan	9136	40,000[10]
32	*E. velutina* Willd.	Walmsley	9211	33,300
	E. velutina Willd.	Gomez Parente	9169	33,300
	E. velutina Willd.	Krug	9196	4,400[7]
	E. velutina Willd.	Vasconcellos Sobrinho	9263	16,650
32a	*E. velutina* forma *aurantiaca* (Ridl.) Kruk.	Dias da Rocha	9272	11,100
33	*E. Grisebachii* Urb.	Walsingham	9316	8,300
34	*E. vespertilio* Benth.	Trist	9180	2,500[10]
35	*E. insularis* F. M. Bailey			

Subgenus *Euerythrina*

Group *Phlebocarpæ* (Australian Species)

36	*E. phlebocarpa* F. M. Bailey			

Group *Arborescentes* (Asiatic Species)

37	*E. arborescens* Roxb.	Sayeeduddin	9225	66,600

Group *Subumbrantes* (Asiatic Species)

38	*E. subumbrans* (Hassk.) Merr.	(Buitenzorg)	9151	35,400[10]

Group *Suberosæ* (Asiatic Species)

39	*E. suberosa* Roxb.	Dorasami	9281	83,300
40	*E. glabrescens* R. N. Parker	Bor	9334	33,300
41	*E. microcarpa* Koorders & Valeton			
42	*E. stipitata* Merr.			
43	*E. stricta* Roxb.	Biswas	9290	330[3]
	E. stricta Roxb.	Biswas	9290	660[3]
	E. stricta Roxb.	Ghose	9289	22,200
	E. stricta Roxb.	Broadfoot	9341	33,300
	E. stricta Roxb.	Bor	9335	33,300
44	*E. resupinata* Roxb.			

Group *Fuscæ* (Asiatic-Polynesian Species No. 45; American Species No. 46)

45	*E. fusca* Lour.	Canicoza	9153	9,200[10]
	E. fusca Lour.	(Buitenzorg)	9146	9,500
	E. fusca Lour.	(Buitenzorg)	9149	11,700
46	*E. glauca* Willd.	Campos Porto	9199	39,400[10]

	E. glauca Willd.	Dugand	9203	33,300
	E. glauca Willd.	Roig	9214	33,300
	E. glauca Willd.	Haigh	9170	33,300
	E. glauca Willd.	Janz	9343	16,650[1]
	Group *Cristæ-galli* (American Species)			
47	*E. crista-galli* L.	Diddell	9132	16,000[2,10]
	E. crista-galli L.	Meyer	9212	83,300
48	*E. falcata* Benth.	Thays	9188	41,000[10]
	E. falcata Benth.	Santini	9274	11,100
	Group *Vernæ* (American Species)			
49	*E. Pœppigiana* (Walp.), O. F. Cook	(Buitenzorg)	9150	51,400[10]
50	*E. Ulei* Harms			
51	*E. Dominguezii* Hassler	Schulz	9197/1569	49,000[10]
52	*E. verna* Vell.			
53	*E. flammea* Herzog			
	Group *Speciosæ* (American Species)			
54	*E. speciosa* Andr.	(undesignated)	9232	6,600
	Group *Edules* (American Species)			
55	*E. polychaeta* Harms			
56	*E. Schimpffii* Diels			
57	*E. edulis* Triana	Jaramillo	9182	165[4,10]
	E. edulis Triana	Robledo	9231	1,000
	Group *Leptorhizæ* (American Species)			
58	*E. breviflora* DC.			
58*a*	*E. breviflora* forma *petræa* (Brand.) Kruk.	(undesignated)	9397	33,300
58*b*	*E. breviflora* forma *oaxacana* Kruk.			
59	*E. leptorhiza* DC.			
60	*E. horrida* DC.			
61	*E. montana* Rose & Standley			
	Group *Corallodendra* (American Species)			
62	*E. Corallodendrum* L.			
62*a*	*E. Corallodendrum* var. *connata* Kruk.			
62*b*	*E. Corallodendrum* var. *bicolor* Kruk.			
63	*E. amazonica* Kruk.			
64	*E. similis* Kruk.			
65	*E. peruviana* Kruk.			
66	*E. Eggersii* Kruk. & Mold.	(undesignated)	9417	166,000[5]
67	*E. pallida* Britton & Rose	Wortley	9257	22,200
68	*E. mitis* Jacq.			
69	*E. leptopoda* Urb. & Ekm.			
70	*E. Buchii* Urb.	(undesignated)	9414	111,100[5]
	Group *Cubenses* (American Species)			
71	*E. cubensis* C. Wright	Acuna	9234	33,300
	Group *Herbaceæ* (American Species)			
	Subgroup *Lanatæ*			
72	*E. herbacea* L.	Brazol	9144	82,000[10]
	E. herbacea L.	(undesignated)	9125	13,700[5,10]

73	*E. coralloides* DC.	(undesignated)	9433	134,000[5]
74	*E. flabelliformis* Kearney	Marshall	9138	49,000[8,10]
	E. flabelliformis Kearney	Marshall	9138	50,300[9,10]
	E. flabelliformis Kearney	(undesignated)	9126	29,100[5]
75	*E. lanata* Rose	(undesignated)	9399	26,700[5]
76	*E. occidentalis* Stand.	(undesignated)	9398	83,500[5]
	Subgroup *Americanæ*			
77	*E. Berteroana* Urb.	Benitez (Guatemala)	9159	6,900[10]
	E. Berteroana Urb.	Armstrong (Guatemala)	9178	5,300[10]
	E. Berteroana Urb.	Otero (Puerto Rico)	9166	8,000[10]
	E. Berteroana Urb.	(undesignated)	9130	3,360[5,10]
	E. Berteroana Urb.	Montealegre (Costa Rica)	9193	11,400[10]
78	*E. americana* Mill.	Purpus	9145	1,870[10]
	E. americana Mill.	Conzatti	9135/5383	10,000[10]
	E. americana Mill.	Mexia	9394/9022a	6,600
	E. americana Mill.	(undesignated)	9430	11,100[5]
	E. americana Mill.	(undesignated)	9432	4,400[5]
79	*E. Standleyana* Kruk.	(undesignated)	9431	2,700[5]
80	*E. chiapasana* Kruk.	(undesignated)	9429	22,200[5]
81	*E. Goldmanii* Stand.	(undesignated)	9428	16,600[5]
	Subgroup *Rubrinerviæ*			
82	*E. rubrinervia* H. B. K.	Jaramillo	9181	29,300[10]
83	*E. mexicana* Kruk.	Giesemann	9240	22,200
	E. mexicana Kruk.	(undesignated)	9408	83,500[5]
84	*E. lanceolata* Stand.	(undesignated)	9436	33,300[5]
85	*E. hondurensis* Stand.	Chickering	9157/133	3,100[5,10]
86	*E. gibbosa* Cuf. od.			
87	*E. panamensis* Stand.			
88	*E. costaricensis* Micheli	(undesignated)	9435	11,100[5]
	E. costaricensis Micheli	Niehaus	9200	13,500
89	*E. Folkersii* Kruk. & Mold.	Kinloch	9167	4,200[10]
	Subgroup *Macrophyllæ*			
90	*E. macrophylla* DC.	Armstrong	9419/32	111,000
91	*E. cochleata* Stand.			
92	*E. chiriquensis* Kruk.			
93	*E. Smithiana* Kruk			
94	*E. colombiana* Kruk.			
	Group ? (African Species)			
95	*E. acanthocarpa* E. Mey.	Everitt	9198	66,600[10]
96	*E. Humeana* Spreng			
97	*E. Zeyheri* Harv.			
98	*E. caffra* Thunb	Robertshaw	9175	8,300[6,10]
	E. caffra Thunb.	Smithers	9279	11,100
99	*E. lysistemon* Hutch			
100	*E. senegalensis* DC.	(Bur. Affair. Econ. Fr. Guin.)	9202	20,000[10]
101	*E. Baumii* Harms			
102	*E. melanacantha* Taub.			

103 *E. Brucei* Schweinf.
104 *E. Livingstoniana* Baker
105 *E. Bagshawei* Bak. f.

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1 These seeds were from a cultivated plant.
2 These seeds were from a plant cultivated in Florida, U. S. A.
3 These seeds may be those of an abnormal plant.
4 These seeds had been poorly dried and molded in storage.
5 These seeds were old.
6 These seeds were of a horticultural variety; flowers orange.
7 These seeds were from pods left over on a tree at the end of the fruiting season.
8 This value represents the red seeds.
9 This value represents the buff-colored seeds.
10 Reference 1.

DISCUSSION.

These studies covering fifty-one out of the one hundred and five species of *Erythrina* known at present have shown that so far no species of this genus is devoid of alkaloids producing a curare-like paralysis in frogs, and therefore it seems justified to assume that the presence of alkaloids of this type constitutes a characteristic feature of the genus. As observed in Table III, this assumption has experimental justification because of the fact that the fifty-one tested species represent nearly all of the sections and groups of the genus and were collected from nearly all tropical and subtropical countries.

However, it is not surprising that there should be wide variations in the potency of the seeds of different species. Thus *E. variegata* and closely related species of the group *Variegatæ* showed a very low paralysis potency value. The large soft seeds of *E. edulis* (group, *Edules*) were quite low in activity, a result which is not so surprising since these seeds are consumed extensively, either cooked or fried, by people in Colombia. Undoubtedly, these low values represent only traces of the active alkaloids. The presence of only traces of active alkaloids in *E. variegata* probably accounts for the fact that they have not been discovered before in this well-known and widespread species. Higher values than these are found within the genus. The seeds of *E. Eggersii* were outstanding, their paralysis potency value being considerably higher than that of seeds of any other species tested so far.

It is interesting to observe that there exists a distinct uniformity in the potency of the seeds of the closely related species as their relationship is interpreted by taxonomists on the basis of botanical characters. This uniformity extends to certain taxonomic divisions of the genus, such as the groups *Suberosæ*, *Fuscæ* and *Vernæ*, as well as the subgroups *Lanatæ*, *Americanæ* and *Rubrinerviæ*. The complex and variable species, as a rule, showed considerable variations in their paralysis potency values, whereas those species which are uniform in their botanical characters and have a limited range showed more uniform paralysis potency values. It was found repeatedly that plants, represented by specimens which deviated somewhat from the more usual form of a given species, deviated also in their seed paralysis potency values. Certain species, such as *E. americana* and *E. coralloides*, which are difficult to distinguish without good herbarium material, or

E. Berteroana and *E. cubensis*, the seeds of which certainly cannot be distinguished in the absence of leaves, were found to be easily distinguished on the basis of their seed paralysis potency values. Furthermore, we have made numerous tests on seeds of certain species (such as *E. glauca*) from plants grown in widely separated regions and obviously on different soils and under different climatological conditions and found but slight variations. Thus, the soil and local climatological conditions are of minor importance for a given species. All these data lead to the conclusion that the curare-like potencies are primarily dependent upon the inherent characters of the plants.

It is, of course desirable to know next what these paralysis potency values represent. In such a pharmacological test as used for these assay experiments, the threshold dose observed and utilized for calculations is probably the effect of the most potent alkaloids present or the alkaloids present in predominating amounts. In other words, the threshold dose of these crude extracts may not necessarily represent the major alkaloid or alkaloids, but may express only the potency of a mixture of quite different alkaloids which may be present. Fully to explain and interpret these paralysis potency values, it would be necessary to know the amounts of the major alkaloids present and their individual pharmacological activities. This knowledge on the *Erythrina* alkaloids is being accumulated (5, 6, 7, 8) in connection with our studies on alkaloids of curare-like action, and it is expected that these chemical and pharmacological data will be used also at a future date for a full explanation of the paralysis potency values.

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SUMMARY.

Data have been presented which have shown the presence of alkaloids with curare-like action in twenty-eight additional species of *Erythrina*. Altogether, fifty-one species have been tested out of the one hundred and five species known to date. The pharmacological activity is expressed by "paralysis potency values." No species has been found so far which is devoid of alkaloids of paralyzing activity, although there is a wide variation in the potencies of the seeds of the different species. However, there is a considerable uniformity of the paralysis potency values of seeds of the closely related species. This uniformity often extends even to certain taxonomical divisions of the genus.

The paralysis potency values for seeds of *Erythrina* parallel closely the inherent botanical characters of the plants, and it is possible in some cases when proper herbarium material is absent to distinguish between certain seeds on the basis of this assay.

Species of the genera *Rhynchosia*, *Canavalia*, *Clitoria* and *Mucuna* were tested similarly for paralysis potency. All were found to be devoid of alkaloids with the

exception of the seeds of *Rhynchosia pyramidalis* and *Clitoria arborescens*. These were found to have some curare-like action which, compared to that of *Erythrina* seeds, was extremely weak and probably represented only traces of active alkaloids.

The curare-like action of the extracts of all species of *Erythrina* tested in frogs was of short duration when compared to the duration of paralysis of extracts of curare or curare plants.

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THE DETERMINATION OF IRON IN IRON SALTS OF ORGANIC ACIDS CONTAINING PHOSPHORUS.*

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The gravimetric estimation of iron is complicated by the presence of phosphorus. There are several methods mentioned in the literature which may be used to assay iron salts of organic acids containing phosphorus. One method is based upon the titration of the iron, previously reduced to the ferrous state, with an oxidizing agent as described by Kolthoff and Furman (1). Another, in which the iron is extracted as ferric chloride from a strong hydrochloric acid solution with ether, is described by Scott (2). Kolthoff and Furman (3) also mention a third one based on the reduction of ferric iron to ferrous iron by iodide and subsequent titration of the liberated iodine with thiosulfate. Also, modifications of these basic methods appear in the literature on this subject.

In the case of organic compounds interference due to organic matter must be considered. The methods mentioned above are adaptable only after destruction of the organic matter. We have used one which, in addition to being applicable after destroying organic matter, can apparently in some instances at least be employed without doing that. This method comprises the precipitation of the iron as sulfide (4, 5) in ammoniacal solution, separating it in this way from the phosphorus and other interfering constituents; the sulfide is then converted to the hydroxide, for weighing as ferric oxide. The method has been applied to samples of iron adenylate with satisfactory results.

Iron adenylate has the empirical formula $C_{10}H_{12}N_5O_7PFe$, containing 13.93% of iron. Values obtained after oxidation of the samples range from 13.36% to 14.42%. When the destruction of organic matter was omitted and the method was applied directly, the findings ranged from 13.72% to 14.05%.

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