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PRELIMINARY STUDIES OF THE BOTANICAL COMPONENTS OF TECUNA AND JAVA CURARE.*

BY KARL FOLKERS.

Curare, the arrow poison of the South American Indians, is composed of the combined extractives of many toxic plants. The curares of natives of different geographical locations vary in preparation, composition, physiological action, etc. The differences in action are no doubt primarily dependent upon what species of the genera of toxic plants are accessible and are used by the natives in each locality. There has been much scientific study, for many years, of the active principles of the curares. The two basic approaches to the study of these active principles are: *First*, studies starting with native curares, and *second*, studies starting with the botanical components. Certain recent studies involving both methods are those of H. King (1, 2, 3), Ranyard West (4, 5, 6), Wieland, *et al.* (7), Späth (8), Friese (9), Hauschild (10) and Santesson (11).

This paper is a contribution to the knowledge of curares as based on the second approach; that is, a study of the botanical components. B. A. Krukoff, on his Sixth Expedition to Brazilian Amazonia during 1935 and 1936, secured authentic crude material, backed by herbarium specimens, which was used by the Tecunas and Javas in the preparation of their arrow poison. Notes on the botanical components of these two curares were published by Krukoff and Smith (12). The preliminary chemical and pharmacological studies on this plant material, as herein described, were made from the point of view of determining which plants contained alkaloids of paralyzing action, and which ones did not. This selection, made upon authentic botanical material, would provide a basis for further studying the active principles of these curares as obtained from certain species of plants.

It seemed desirable to test first an extract of each plant material which would represent the total alkaloids. Since the "curarines" have been generally recognized to be quaternary ammonium bases, then this first extract should contain the qua-

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ternary and non-quaternary fractions in admixture. To this end, the general procedure was as follows:

Analytical Procedure.—The bark sample (25–40 Gm.) was ground to forty mesh, and extracted in a Soxhlet with ethanol for ten hours. The ethanol extract was distilled, and the last of the solvent removed *in vacuo* at 25–40°. There was added to the residue, 10 ml. water, 2 drops of concentrated hydrochloric acid (or more, if necessary) and 25 ml. of diethyl ether. The mixture was well shaken and stirred until all the residue had been both dissolved and suspended in solution. The entire mixture was then filtered with suction (no washing), and the combined aqueous and ethereal layers were transferred to a separatory funnel. As much as possible of the water layer was drawn off into a test-tube. The volume was noted, and the solution was boiled quickly to dispel the dissolved ether. Drops of water were added to bring the volume to the original level. Occasionally, most of the 10 ml. of water would be recovered, and again only 2 or 3 ml. of final extract would be obtained because of emulsions, etc. In all cases, the calculations on the paralysis potency and toxicity were made on the basis of the animal threshold dose and on the number of Gm. of bark represented by the volume of water added to the ethanol extraction residue. Such paralysis potency values are not strictly quantitative since several factors may influence the final numerical value. There is no necessary correlation between the paralysis potency and toxicity of a plant material since substances of paralyzing action, and others of non-paralyzing action, each of different toxicity, may be present together.

The motor nerve paralysis was determined with frogs by intralymphatic injection and application of an electrical stimulation to the sciatic nerve. The toxicity was determined in white mice by subcutaneous injection and the results calculated according to Behrens' method.

Discussion of the results will be made on the basis of the information supplied by Krukoff and Smith in their paper on the preparation of the two curares.

TABLE I.—PLANTS USED BY TECUNAS FOR CURARE.

Expt. No.	Botanical Name of Plant and Part.	Krukoff Botanical Number.	Alkaloids.	Curare Paralysis Gm./Kg. Frog.	Toxicity. White Mice Gm./Kg. L. D. 0.	L. D. 50.	L. D. 100.
110	*Strychnos Castelnæana* Wedd.	7533	..	.....	...	...	...
	Chondodendron limaciifolium	7534	+	Positive	...	...	...
	(Diels) Mold. (stem bark mixt.)	7535	..	.....	...	...	...
116	*Strychnos Castelnæana* Wedd.	7533	+	Positive	...	...	...
	(inner stem bark)	7534	..	.....	...	...	...
126	*Strychnos Castelnæana* Wedd.	7541	+	850.0	86.0	...	173.0
	(stem wood only)	7548	..	Negative	...	...	...
135	*Strychnos Castelnæana* Wedd. (stem bark)	7537	+	20.0 Positive	...	...	...
139	*Strychnos Castelnæana* Wedd. (root bark) (comparatively young plant)	7538	+	50.0 Positive	40.0	...	80.0
144	*Strychnos Castelnæana* Wedd. (stem bark) (comparatively young plant)	7538	+	200.0 Negative	4.0	...	40.0
129	*Strychnos toxifera* Rob. Schomb. ex. Benth. (stem bark)	7539	+	50.0 Positive	...	2.0	...
142	*Strychnos toxifera* Rob. Schomb. ex. Benth. (root bark)	7539	+	5.0 Positive	2.0	...	4.0
131	*Strychnos* cf. *Peckii* Rob. (var. ?) (stem bark)	7549	+	200.0 Negative	80.0	...	...
141	*Strychnos* cf. *Peckii* Rob. (var. ?) (root bark)	7549	+	20.0 Positive	4.0	...	40.0
149	*Chondodendron limaciifolium* (Diels) Mold. (stem bark) water percolation	7535	+	200.0 Negative	...	2.5	...

122	*Chondodendron limaciifolium* (Diels) Mold. (stem bark)	7535	+	Questionable, but symptoms at 25.0	...	2.0	...
128	*Chondodendron limaciifolium* (Diels) Mold. (stem wood)	7535	+	Questionable, but symptoms at 580.0	...	...	5.8
143	*Telitoxicum minutiflorum* (Diels) Mold. (stem bark)	7536	+	Questionable, but symptoms at 200.0	...	2.0	...
114	*Dieffenbachia seguina* (L.) Schott, var. *viridis* Engl. (succulent portion-stems)	7674	−	Negative	...	...	...
133	*Dieffenbachia seguina* (L.) Schott, var. *viridis* Engl. (succulent portion-stems)	7637	−	500.0 Negative	...	...	400.0
136	*Piper* sp. (roots)	7546	+	50.0 Negative	40.0	...	...
138	*Piper* sp. (Group of *P. obliquum* R. & P.) (roots)	7545	+	125.0 Negative	100.0	...	...
118	*Aristolochia* aff. *arcuata* Mast. (tuber)	7542	−	Negative	...	...	...
137	*Annona Ambotay* Aubl. sens. lat. (root bark)	7547	+	100.0 Negative	40.0	...	80.0
119	*Cyclanthera* sp. or *Melothria* sp. (tuber)	7544	+	Negative	...	...	...

Curare of the Tecunas.—Table I gives the results of certain tests made with the plants of the Tecunas. In Expt. 110, a mixture of shavings of the outer bark of *Strychnos Castelnæana* and bark of *Chondodendron limaciifolium* actually used by Indians in the making of curare was tested. The paralysis activity and toxicity showed that the long cold water extraction method of the Indians did not exhaust the active and toxic alkaloids from the barks. Thus, in correlating the alkaloids isolated from native curare and from plant materials, the question of selective extractions is not without interest. (See Expt. 149.) Likewise, it must be pointed out that the Indians extract green, fresh bark, whereas these laboratory tests were made with dried bark, and the alkaloids extracted by water in the two cases may be quite different.

Expt. 116 proved the presence of paralyzing alkaloids in the inner bark layer of stems of *Strychnos Castelnæana*, which the Indians believed to be free of "poison." Expt. 126 showed there were only traces of alkaloids in the stem wood, and the Indians knew of this lack of high toxicity. The presence of traces of alkaloid in the stem wood is not surprising since phloem tissues are scattered among the xylem in spp. of *Strychnos*. Expts. 135, 139 and 144 showed the potency of the stem and root barks of *Strychnos Castelnæana*. Expts. 139 and 144 were made on the material of a comparatively young plant of *Strychnos Castelnæana*.

The stem bark of *Chondodendron limaciifolium* (Expt. 149) did not give, on water percolation (the Indian method), an extract of curare paralyzing action, although the extract was highly active. The preliminary extracts of *Chondodendron limaciifolium* and *Telitoxicum minutiflorum*[1] and their tests were not sufficiently satisfactory to warrant a statement on their containing alkaloids of curare paralyzing action. Probably, the bark of these plants is so rich in complex and toxic alkaloids that a physiological test on an extract representing the total alkaloids was not satisfactory. It seems desirable to determine whether a quaternary alkaloid fraction is present in these plants and, if so, to study the quaternary and non-quaternary fractions separately. These studies will be described elsewhere.

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[1] B. A. Krukoff and H. N. Moldenke recently revised certain genera of Menispermaceous plants. Their paper is now in print. I am indebted to them for the corrected identifications of Menispermaceous plants that are used in this paper, as against those that were used by Krukoff and Smith (12) in 1937.

Strychnos cf. Peckii (var.?) showed negative action for small doses of extract of stem bark, but a strong curare action from the extract of root bark was obtained.

The bark of both stems and roots of *Strychnos toxifera* was very potent in paralyzing activity, as might be expected. King recently isolated 0.2% of amorphous curarine from *Strychnos toxifera* which was said to be identical with the amorphous curarine obtained by Boehm (3).

Expts. 114, 133, 136, 138, 118, 137, and 119 on other plants used by the Tecunas showed no paralyzing activity and low toxicities.

Annona Ambotay was an interesting plant. The roots had a very pleasant odor. The sweet and penetrating odor of certain Tecuna curare is derived from this root. The ether layer in the extraction process on *Annona Ambotay* was found to contain this odoriferous principle. It was an oil, and amounted to 15% of the weight of the bark of the roots. It distilled quite constantly at 125–127° at 4 mm. The distilled liquid possessed the characteristic odor. It decolorized aqueous potassium permanganate solution and absorbed bromine from carbon tetrachloride; thus, it was unsaturated. The analyses were: Found, C, 74.02; H, 9.95; M. W. (Rast) 189. These results indicated the tentative empirical formula, $C_{12}H_{20}O_2$ (M. W. 196, C, 73.41; H, 10.27). Since this substance was not of interest to the immediate problem, it was not examined further.

TABLE II.—PLANTS USED BY JAVAS FOR CURARE.

Expt. No.	Botanical Name of Plant and Part.	Krukoff Botanical Number.	Alkaloids.	Curare Paralysis Gm./Kg. Frog.	Toxicity. White Mice Gm./Kg. L. D. 0.	L. D. 50.	L. D. 100.
154	*Piper* cf. *cinereonervosum* Tr. (roots)	7660	+	116.0 Negative	58.0	...	...
162	*Piper* cf. *tumidicondyli* Tr. (roots)	7661	+	160.0 Negative	...	...	...
156	*Roupala* aff. *adiantifolia* Kl. (stem bark)	7662	–	240.0 Negative	180.0	...	...
158	*Duguetia* cf. *Spixiana* Mart. (stem bark)	7659	+	310.0 Negative	...	80.0	...
155	*Duguetia* aff. *asterotricha* (Diels) R. E. Friese (stem bark)	7664	+	280.0 Negative	210.0	...	...
157	*Protium* sp. (stem and root bark)	7666	+	79.0 Negative	...	...	59.2
145	*Clavija* aff. *Poeppigii* Mez. (stem bark)	7665	+	220.0 Negative	176.0	...	...
164	*Weigeltia* sp. or *Stylogyne* sp. (stem bark)	7663	?	136.0 Negative	...	...	...
163	*Ipomoea* sp., probably *I. tiliacea* (Willd.) Choisy (stems)	7670	+	107.0 Negative	...	...	...
151	*Strychnos* sp. nov., related to *S. diaboli* Sandw. (stem bark) water percolation	7654	+	400.0 Negative	...	100.0	...
134	*Strychnos* sp. nov., related to *S. diaboli* Sandw. (stem bark)	7654	+	100.0 Positive	...	4.0	...
166	*Strychnos Jobertiana* Baill. (stem bark)	7657	+	4.4 Positive	...	...	...
161	*Capparis sola* Macbride (stem bark)	7667	+	30.0 Positive	30.0	...	...
160	*Capparis sola* Macbride (stem bark)	7658	+	25.0 Positive	...	...	...

Curare of the Javas.—In Table II are summarized the preliminary tests on the plants used by the Java Indians, who still use blow-guns and curare extensively. According to Krukoff and Smith, *Strychnos Castelnæana* was also used by the Javas. It is not listed in Table II since the preliminary tests on this plant are adequately presented in Table I. *Strychnos Jobertiana* (Expt. 166) possessed the paralyzing action, and so did the new species of *Strychnos* (Krukoff 7654) which is related to *Strychnos diaboli* Sandw. *Capparis sola*, which is considered as a very important component of curare by the Javas, on several tests showed a positive paralyzing action. Most of the other plants used by the Java Indians showed the presence of alkaloids, but they did not produce the paralyzing action, and none was very toxic.

CONCLUSIONS AND SUMMARY.

It has been found in regard to Tecuna and Java curare that five species of *Strychnos* possess alkaloids which cause a curare-like action in frogs. These species are *Strychnos* cf. *Peckii*, *Strychnos* sp. nov. (related to *Strychnos diaboli*), *Strychnos Jobertiana*, *Strychnos Castelnœana* and *Strychnos toxifera*. The extracts of two Menispermaceous plants, namely, *Chondodendron limaciifolium* and *Telitoxicum minutiflorum*, were found to be highly toxic, but the curare paralyzing action remains to be studied further. *Capparis sola*, a member of *Capparidaceæ*, has been found to contain alkaloids of curare-like action. It is an interesting plant and to our knowledge it has not been reported previously to contain alkaloids with such an action. Many other plants, as used by the Tecunas and Javas, have been eliminated as not being of interest to the problem of alkaloids of curare-like action. The interesting spp. of *Strychnos*, and *Capparis sola*, as well as plants of the *Menispermaceæ*, will be given further chemical and pharmacological study.

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ERYTHRINA ALKALOIDS. II. A REVIEW, AND NEW DATA ON THE ALKALOIDS OF SPECIES OF THE GENUS ERYTHRINA.*

BY KARL FOLKERS[1] AND KLAUS UNNA.[1]

Subsequent to the isolation of erythroidine from the seeds of *Erythrina americana* Mill. (1), and the developments which included the demonstration that this new alkaloid can cause a curare-like action, it was of considerable interest to make an examination of other species of the genus *Erythrina*. For the beginning of this

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