205. *Curare Alkaloids. Part IX. Examination of Some* Strychnos *Species from British Guiana: Characterisation of Diaboline, an Alkaloid from* Strychnos diaboli, *Sandwith.*

By Harold King.

The aim of this investigation was the survey of the *Strychnos* species of British Guiana, so far as they were accessible, in order to determine which contained alkaloids showing curare activity, in the hope that bases of use in medicine might be found. It has been established that several species contain alkaloids which exhibit curare activity, the most important being *S. toxifera*.

S. diaboli yields a base diaboline which has been characterised.

In 1931 Hartridge and West (*Brain*, **54**, 312) described a rigidity-removing (" lissive ") action of curare in experimental parathyroid tetany in dogs. This " lissive " effect was not observed with all samples of curare, and an explanation of the effect in terms of chemical constituents was not possible in view of the backward state of our knowledge of the alkaloids which contribute their effects to the various curares. During the past decade and a half considerable clarification has taken place; for instance, it is now known that the curares prepared by the natives of the northern part of South America come mainly from bush-ropes of the genus *Chondrodendron* or of the genus *Strychnos* or of both.

As a result of an approach made by Dr. Ranyard West to the Medical Research Council in 1931, a broad survey of the curare field was undertaken, and the present communication records the results which have been obtained on certain *Strychnos* species which grow in the Crown Colony of British Guiana. Specimens of the bark, sometimes of the stems, the leaves and in some cases of the flowers have been obtained through the kind co-operation of the Curator of Forests of British Guiana, and the identification of the species has been made finally by Mr. N. Y. Sandwith, M.A., of the Herbarium, Kew. The bark has been examined for alkaloidal content, and the alkaloidal extract tested for curare activity.

Sandwith, in the " Bulletin of Miscellaneous Information," No. 8, for **1933**, issued by the Royal Botanic Gardens, Kew, describes nine species of *Strychnos* which occur in British Guiana, for three of which there is very good evidence supplied by reliable explorers and botanists that they have been utilised in the preparation of curare. More recently Krukoff and Monachino (*Brittonia*, **1942**, **4**, 248) in a survey of American species of *Strychnos* mention eleven species as occurring in British Guiana, most of which have been recorded at some time or other as furnishing poisons of varying intensity.

The results of the examination of six of these species, and of some others about the identity of which there is some doubt, are recorded below.

Species.	Forest department no.	Alkaloid content.	Curare action.
Strychnos Erichsonii	2284	+++	None
,, *Melinoniana*	2279; 2286; 2303	+	,,
,, *Mitscherlichii*	2261	—	,,
,, ,,	2621	++	Weak
,, *diaboli*	2270	—	None
,, ,,	2295; 2468; 2473	+ to ++	,,
,, *toxifera*	2285; 2278	++	Strong
,, ,,	fruit	—	—
,, *guianensis*	4431	++	Weak
,, near *guianensis*	2467 (*a*)	—	None
,, ,, ,,	2620 (*b*)	++	Active
,, ,, *hirsuta*	2482	++	,,

(*a*, *b*) These are near *S. guianensis* but different from it and are not con-specific. (*b*) may be *S. glabra*, but in the absence of flowers exact identification was impossible.

Two of the species recorded by Richard Schomburgk (" Travels in British Guiana, 1840—1844 ") as entering into the curare of the Macusi Indians, namely *S. cogens* and *pedunculata*, have not been examined. They grow in a region infrequently visited.

All except one of the *Strychnos* species contain alkaloids, and four of them contain alkaloids with a curare-action. Of the four, *S. toxifera*, which Robert, brother of Richard, Schomburgk regarded as furnishing the main active ingredients of the curare of the Macusi Indians, gave the strongest curare preparations.

As there is considerable evidence that the Indians put the extracts of more than one plant into their curare preparations and even mix extracts from *Chondrodendron* species with those from *Strychnos* species, both of which furnish quaternary alkaloids with a curare-action, it is evident that the safest scientific approach to the problem of the origin and nature of the active principles of curare is by an examination of single, botanically identified *Strychnos* and *Chondrodendron* species.

Hitherto the only South American *Strychnos* species which has received more than a superficial chemical examination has been *S. toxifera* and that by Wieland and his pupils on material supplied by the Medical Research Council. The writer's own investigations were interrupted by the war.

Of the other species recorded in the table, *S. Erichsonii* is very rich in alkaloids, but they seem to resinify with great ease and may need a special technique for their characterisation.

S. diaboli yields a crystalline water-soluble alkaloid *diaboline* with the formula $C_{21}H_{26}O_3N_2$. It is a non-phenolic mono-acidic base containing a methylenedioxy-group (Gaebel's test). It is doubtful whether an acylatable group is present; certainly no crystalline acyl derivative has been obtained, and nitrous acid gives a crystalline nitrite. The results obtained on methylation are of interest. When the base is boiled in methyl-alcoholic solution with methyl iodide it is converted partly into O-*methyldiaboline methiodide I* and if to the methyl-alcoholic mother liquor potassium hydroxide is added and the boiling continued with more methyl iodide, a second very soluble methiodide is formed which separates, when the solvent is changed to water, as an addition *compound* of potassium iodide and two molecules of *O*-methyldiaboline methiodide II. This additive product is much less soluble in *iso*propyl alcohol and crystallises therefrom in plates. If, however, it is repeatedly crystallised from water it may separate from concentrated solutions as O-*methyldiaboline methiodide II* free from potassium iodide and crystallising in large hemispherical masses.

It is tentatively suggested that the two methiodides, which differ considerably in optical rotation, may contain asymmetric nitrogen analogous to the metho-salts of canadine (Pyman, *J.*, 1913, **103**, 290), and that the third oxygen atom of diaboline is present as a non-phenolic carbinol group in propinquity to one of the nitrogen atoms.

Diaboline has little pharmacological activity. According to Dr. W. D. M. Paton of this Institute, *O*-methyldiaboline methiodide I has a curare-potency on the rabbit head-drop test one fifty-fourth that of *dextro*tubocurarine chloride, whilst that of the isomeric methiodide II is one seventy-ninth that of *dextro*tubocurarine chloride.

I am indebted to Dr. H. S. Forrest for determining the ultra-violet absorption spectrum of diaboline hydrochloride in water. It showed a maximum at 2490 A. in 0·0033% solution. It may be significant that this maximum is also shown by certain members of the calabash alkaloids described by Schmid and Karrer (*Helv. Chim. Acta*, 1947, **30**, 1167 and 2085). As these latter bases also orginate from South American *Strychnos* species, a common ultra-violet chromophore may be indicated. Apart from the striking colour reactions of the calabash alkaloids with the Otto–Wieland reagent there is no evidence of a close structural affinity between the alkaloids of *S. Nux Vomica* and the South American *Strychnos* species.

EXPERIMENTAL.

Preparation of Bark Extracts for Alkaloidal and Physiological Tests.—A known weight of finely powdered bark was slowly percolated with 1% aqueous tartaric acid until the extract no longer gave a reaction with Tanret's reagent or until the extract was very pale. The reaction of Tanret's reagent with a portion of the total percolate gave an indication of alkaloidal content. The test for curare activity was carried out on a neutralised portion of the solution, using the righting reflex in the frog as previously defined (King, *J.*, 1937, 1478), and I am indebted to Professor J. H. Gaddum, F. R. S., and Dr. G. L. Brown, F.R.S., for the physiological results.

When curare activity was found on the crude extract the solution was further purified by addition of basic lead acetate solution so long as a precipitate was formed. Lead ions were then removed from the filtrate and washings with hydrogen sulphide, and the solution prepared for the frog test by removing excess of hydrogen sulphide and final neutralisation. The cleaner solution usually gave a clearer physiological response.

Examination of S. Diaboli *Bark. Isolation of Diaboline.*—Finely powdered bark of *S.* ***diaboli*** (55·5 kg.) was percolated with 1% aqueous tartaric acid until the percolate gave only a faint turbidity with Tanret's reagent. The combined percolates were concentrated under reduced pressure at 50° to 22 l., and filtered from calcium and potassium tartrates. A portion (100 c.c.) was treated with aqueous 50% sodium hydroxide (25 c.c.), which kept a phenolic resin in solution, and then extracted 6 times with chloroform. On removal of this solvent the residual alkaloidal gum amounted to 1·55 g., corresponding to a crude alkaloidal content of 0·6% in the bark. Other batches of bark have given alkaloidal contents between 0·2 and 0·5%. The total crude base (199 g.) so obtained was neutralised to litmus with 3N-hydrochloric acid, and the solution extracted with chloroform to remove fat and some colouring matter. It was then concentrated to a syrup which, on keeping, slowly deposited *diaboline hydrochloride* as a very soluble salt which was then crystallised first from water and then from 7 parts of boiling absolute alcohol and then finally from water. By working up the mother liquors about 70 g. of pure salt were obtained.

Diaboline hydrochloride crystallises as a felt of needles which shrink and turn brown near 260° and have no definite m. p. It usually crystallises as a monohydrate, sometimes as a hemihydrate (Found on air-dry solid : loss at 110°, 4·7. $C_{21}H_{26}O_3N_2,HCl,H_2O$ requires H_2O, 4·8%. On dried solid : C, 64·4; H, 6·9; N, 7·2; Cl, 8·9. $C_{21}H_{26}O_3N_2,HCl$ requires C, 64·5; H, 7·0; N, 7·2; Cl, 9·1%). When diaboline is submitted to a Herzig–Meyer process for the determination of *N*-methyl groups it shows the absence of a methoxyl group, but at 270—310° it may yield methyl iodide corresponding to as much as 0·2 of an *N*-methyl group which may arise from partial reduction of methylene di-iodide. The anhydrous salt showed $[\alpha]_{5461}^{20^\circ} + 184^\circ$ in water (*c*, 0·57), whence $[\alpha]_{5461}^{20^\circ} + 202{\cdot}7^\circ$ for the ion.

The *base* was extracted with chloroform from a strongly alkaline solution of the hydrochloride, and the gum left on removing the chloroform was boiled with ether. It gradually crystallised in silky needles, m. p. 187° (Found : C, 71·1; H, 6·8; N, 8·2. $C_{21}H_{26}O_3N_2$ requires C, 71·1; H, 7·4; N, 7·9%).

The *nitrate* was obtained as a white chalky crystalline powder by exact neutralization of the base with N-nitric acid. It was soluble in 8 parts of hot water and separated in clusters of prisms, m. p. 244° (decomp.) (Found : C, 60·3; H, 6·0; N, 9·6. $C_{21}H_{26}O_3N_2,HNO_3$ requires C, 60·4; 6·5; N, 10·1%);

$[\alpha]^{20^\circ}_{5461}$ + 170·7° in water (*c*, 0·44), whence $[\alpha]^{20^\circ}_{5461}$ + 200·5° for the ion, in close agreement with the value found using the hydrochloride.

The *hydrogen oxalate* crystallises from water in minute tablets, also in rods. When air-dried it melts partly near 100°, and when dried at 97° it has no definite m. p. but swells up between 170° and 180° (Found on air-dry solid : loss at 100° in a vacuum, 9·3. $C_{21}H_{26}O_3N_2,C_2H_2O_4,2{\cdot}5H_2O$ requires H_2O, 9·2%. On dried solid : C, 62·0 ; H, 6·3 ; N, 6·4. $C_{21}H_{26}O_3N_2,C_2H_2O_4$ requires C, 62·1 ; H, 6·4 ; N, 6·3%).

The *picrate* was obtained from the base (0·12 g.) and picric acid (0·08 g.) in boiling water (100 c.c.). It separated in irregular shaped plates or leaflets which required 440 parts of boiling water for re-solution ; m. p. 180—180° (efferv.). Great difficulty was experienced in the analysis of this substance. Although it suffers no loss in weight at 100°, most analyses by different micro-analysts agree with the presence of half a molecule of water (Found : C, 54·5 ; H, 5·1 ; N, 11·6. $C_{21}H_{26}O_3N_2,C_6H_3O_7N_3,0{\cdot}5H_2O$ requires C, 54·7 ; H, 5·1 ; N, 11·8%). On one occasion a picrate, small rectangular plates, m. p. 242° (decomp.), was obtained from a similar aqueous preparation, but this could never be repeated. It was found, however, that when the low-melting picrate was boiled with absolute alcohol it dissolved and then suddenly separated from the boiling solution in irregular plates, m. p. 244° (decomp.) (Found : C, 56·0 ; H, 4·7 ; N, 11·8, 12·4. $C_{21}H_{26}O_3N_2,C_6H_3O_7N_3$ requires C, 55·5 ; H, 5·0 ; N, 12·0%). This higher-melting picrate will not dissolve in boiling acetone or methyl ethyl ketone, but will do so if a trace of water is added. It is also very sparingly soluble in boiling ethyl or butyl alcohol.

Methylation of Diaboline.—The base, regenerated from diaboline hydrochloride (28·0 g., $[\alpha]_{5461}$ + 186°), was dissolved in methyl alcohol (150 c.c.) and methyl iodide (20 c.c.). After the solution had been boiled gently for 3 hours, one half of the solvent was distilled off, and on keeping a felt of prisms separated of crude methiodide I (13·8 g.), m. p. 282° (decomp.). The methyl-alcoholic filtrate was treated with methyl iodide (15 c.c.) and a solution of potassium hydroxide (7 g.) in methyl alcohol (40 c.c.) and boiled gently for an hour. The solution was then distilled to remove methyl alcohol, water being added in small portions to dissolve potassium iodide as it separated. Finally, crude methiodide II separated (16·1 g.), m. p. 232°. On careful fractionation of the mother liquors further crops of each methiodide were obtained.

A portion of crude methiodide I was crystallised from 4 volumes of hot water and then separated in prisms, m. p. 290° (decomp.) (Found : loss at 100°, 8·2. $C_{23}H_{31}O_3N_2I,2{\cdot}5H_2O$ requires H_2O, 8·1%. On substance dried at 100° : C, 53·8 ; H, 5·6 ; I, 24·9 ; OMe, 6·0 ; NMe, 5·9. $C_{23}H_{31}O_3N_2I$ requires C, 54·1 ; H, 6·1 ; I, 24·9 ; OMe, 6·1 ; NMe, 5·7%). Analysis shows that this substance is *O-methyldiaboline methiodide I*. The anhydrous salt showed $[\alpha]^{20^\circ}_{D}$ + 43·5° in water (*c*, 0·68). This gave a *picrate* crystallising from water in needles, m. p. 150—160°, which when recrystallised from methyl alcohol separated in thin leaflets, m. p. 213—214° (Found : C, 56·9 ; H, 5·2 ; N, 11·8. $C_{29}H_{33}O_{10}N_5$ requires C, 56·9 ; H, 5·4 ; N, 11·5%).

If crude methiodide II is crystallised from 20 volumes of *iso*propyl alcohol it separates in bold prisms, m. p. 235—238°, which consist of an *addition compound* of *O*-methyldiaboline methiodide II and half a molecule of potassium iodide [Found : loss at 100°, 4·0. $(C_{23}H_{31}O_3N_2I)_2,KI,1{\cdot}5H_2O$ requires H_2O, 4·3%. On anhydrous salt : C, 47·0 ; H, 5·2 ; N, 5·1 ; I, 31·6. $(C_{23}H_{31}O_3N_2I)_2,KI$ requires C, 46·5 ; H, 5·3 ; N, 4·7 ; I, 32·0%]. On ignition with sulphuric acid it left a residue of potassium sulphate.

If the crude methiodide II is crystallised from less than its own weight of water many times, it may lose the potassium iodide and then separates from water in large hemispherical masses built up compactly of prisms. *O-Methyldiaboline methiodide II* so obtained melts below 100° but when dried at 90° melts between 200° and 220° (Found : C, 48·9 ; H, 6·7 ; N, 5·3 ; I, 22·6 ; H_2O, 8·1, OMe, 5·9 ; NMe, 4·9. $C_{23}H_{31}O_3N_2I,2{\cdot}5H_2O$ requires C, 49·2 ; H, 6·5 ; N, 5·1 ; I, 22·9 ; H_2O, 8·1 ; OMe, 5·6 ; NMe, 5·2%). Unlike the addition compound with potassium iodide this salt is readily soluble in *iso*propyl alcohol. It is also soluble in acetone and in chloroform. The anhydrous salt showed $[\alpha]^{20^\circ}_{D}$ + 143·9° in water (c, 0·73). The picrate is amorphous, but a perchlorate is readily obtained, crystallising in diamond-shaped plates.

If *O*-methyldiaboline methiodide I is boiled for 3·5 hours with methyl-alcoholic potash (5% solution) and then for an additional 1·5 hours with excess of methyl iodide, the original methiodide I is recovered unchanged. If diaboline is boiled with methyl iodide and methyl-alcoholic potash, both methiodides I and II are formed.

The strongly alkaline aqueous solutions from which the chloroform-soluble bases, mainly diaboline, had been removed were neutralized and precipitated with mercuric chloride. The quaternary fraction freed from mercury ions was only obtained in the amorphous state and was devoid of noticeable pharmacological action.

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