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Advances in the research of curare and *Strychnos* alkaloids (**)

Riassunto. — Viene fatto il punto sullo stato attuale delle conoscenze sulla chimica degli alcaloidi dei Curari e quelli di *Strychnos*.

Nel caso dei Curari si può stabilire che gli alcaloidi siano quelli delle piante oppure da questi derivino per le reazioni che possono subire durante la preparazione.

Nel caso degli alcaloidi di *Strychnos* oggi in base alle strutture dei numerosi composti isolati si può considerare una comune origine biogenetica.

1. Introduction

The name of curare was attributed to a group of poisons elaborated in the Amazonas Orinoco basins by natives from plant extracts. These poisons have in common their peculiar physiological action blocking the neuro-muscular transmission.

Curare were and are used for hunting purposes, and there is no evidence that they were used in war, but their properties became soon of interest for the conquerors, and their renown soon spread in the form of legends into Europe.

Three hundred years of researches on curare from the Conquista to the end of the XIX century can be summarized in few facts and dates:

in 1516 Pietro Martire in Orbis Novus Decades reports the existence of peculiar arrow poisons;

in 1740 Father José de Gumilla, describes for the first time the property that curare is active only by injection;

in 1745 La Condamine gives the first account for the elaboration of curare by the Ticunas;

in 1782 Felice Fontana, in Florence, working on a sample of curare sent by Francisco Maldonado from Ecuador, demonstrate the particular physiological action, of curare extract;

in 1807 Alexander von Humboldt describes the elaboration of a curare at Esmeralda (Upper Orinoco) mainly from *Strychnos* root bark.

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(**) Questa Memoria è basata sulla relazione tenuta all'Universidad Mayor de San Marcos, Lima il 26 ottobre 1974.

1819 Roulin and Boussingault in Bogotà, separate the active principle of a curare made from Strychnos and do not find strychnine, but a water soluble salt!

1830–1880 Ethnopharmacological research in the acrea of Amazonas and Orinoco by the Castelnau, R. and E. Schomburgk, Jobert, Crevaux, Lacerda and Barbosa Rodriguez.

1856 The full physiological demonstration of the activity of curare by Claude Bernard in Paris.

Rudolf Boehm was the first, on the basis of all the material gathered and of the observation made during these years and of the personal chemical investigations, to propose a classification of the curares.

We must here underline that curare are elaborated in an area of the tropical rain forest of 5,000 km of lenght and 4,000 km width, that the plants used are therefore not always the same, and that the tribes which elaborate the poison have different cultural levels.

Many ethnographic observations are sometimes not sufficient to establish the origin of a curare, because this poison is a good which is transported and marketed by indian tribes through very large areas.

Anyhow Boehm established a particular classification, based mainly on ethnographical data, which corresponds rather well to a rough chemical classification.

The curares, according to Boehm (1888–1896) are:

tubo-curare, that is, curare contained in tubes, made from bamboo;

calabash curare that is curare contained in calabashes (dried fruit of squash);

pot-curare, curare contained in pots, made by the indians themselves.

For over fifty years all the researches on curare have been based on this classification.

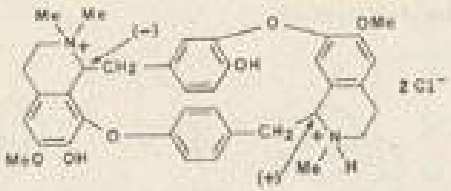
Tubo-curare according to Boehm, are formed by a mixture of tertiary and quaternary alkaloids belonging to the benzyl-isoquinoline group, calabash curare on the other hand are constituted by tertiary and quaternary alkaloids belonging to the indole group. Pot curare contains alkaloids from both of the above reported groups.

From the botanical point of view it is possible to establish on the ethnographical observations that tubo-curare main constituents are *Menispermaceae*; that calabash curare active principle take origin from *Strychnos* as Humboldt had established and that in pot curare both *Menispermaceae* and *Strychnos* are used.

35 years later King was able to establish in a tubo-curare the presence of a quaternary alkaloid, tubo-curarine, to which structure (I) was attributed.

Meanwhile botanical researches in the field by Krukoff and Moldenke and chemical studies by Wintersteiner and Dutcher could demonstrate that

the main constituents of tubo-curare were five species of *Chondodendron*, and that the active principle was the same (+) tubocurarine found by King in curare.



Few years later (1942) the work of Griffith and Johnson opened the new field of curarization in surgical anesthesia.

The structure of tubocurarine was taken as a model in 1946 by Bovet for elaborating the synthetical curare like drugs, which are now normally used as coadjuvant in anesthesia.

The search on calabash curares, which were also more potent than tubocurares, found more difficulties owing to the complexity of the alkaloids concerned.

2. The curare alkaloids

The first question to be solved was if the curare alkaloids were comparable or identical to those present in *Strychnos* plants.

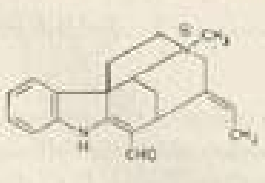
This question was first cleared by Berredo de Carneiro in 1938 who could demonstrate the similarity of *S. solimoesana* and Curare alkaloids, and later established by the fundamental work of Karrer and Schmidt and coworkers in Zürich, who have given the first structures for the quaternary alkaloids of *Strychnos*. These found to be identical or chemically related to the alkaloids isolated by Wieland from *S. toxifera* in 1941–47.

The main alkaloids obtained from curare responsible for its action and in part found also in *Strychnos* are:

Quaternary alkaloids.

(i) *Monomeric alkaloids.* A number of mono-quaternary alkaloids, which show only a very slight curarizing activity, have been found in various species.

They belong to a number of different structural types:

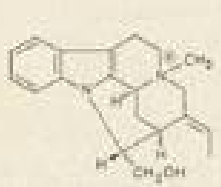


C-Fluorocurarine (Curarine III)

C-Hemitoxiferine

C-Fluorocurine

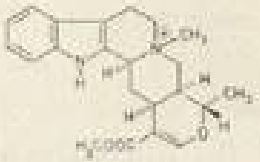
C-Fluorocurarine (C-curarine III) is a α-methylene-indoline base related to 18-deoxy-Wieland-Gumlich aldehyde. C-Hemitoxiferine is the methyl-quaternary salt of Wieland-Gumlich aldehyde. C-Fluorocurarine, on the other hand, is a pseudoindoxyl-derivative.



C-Mavacurine

Macusines

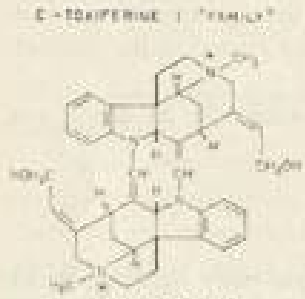
Macusine A, R = CH_2OH; R' = CO_2CH_3
Macusine B, R = H ; R' = CH_2OH
Macusine C, R = CO_2CH_3; R' = CH_2OH



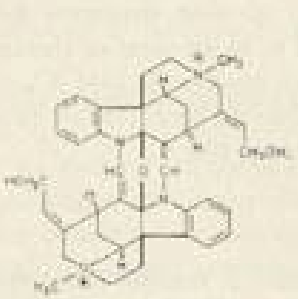
Melinonine A

C-Mavacurine, macusines A, B, and C, and melinonine A are all tetrahydro-β-carboline derivatives. The macusines are related to bases of the sarpagine and ajmaline types, while melinonine A is related to tetrahydroalstonine; the isomeric bases alstonine and serpentine have been found in the African *S. camptoneura* Gilg and Busse and also in several *Apocynaceae* plants.

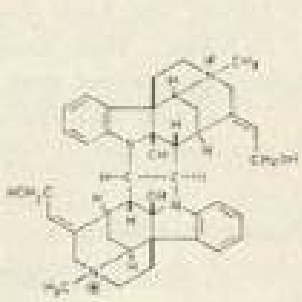
(ii) *Dimeric alkaloids.* The curarizing activity of Strychnos extracts as of curare, as shown by Bovet and later by Waser, is due mainly to the presence of dimeric alkaloids, containing two quaternary ammonium groups, which are formed by the union of two monomeric alkaloids.



C-Toxiferine I
(Toxiferine V, Toxiferine II)



C-Alkaloid E

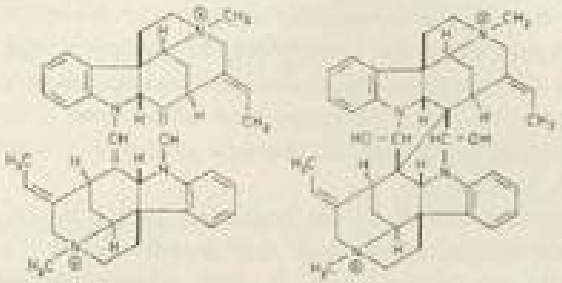


C-Alkaloid A

On the basis of current knowledge, these substances can be divided into three main groups or "families":

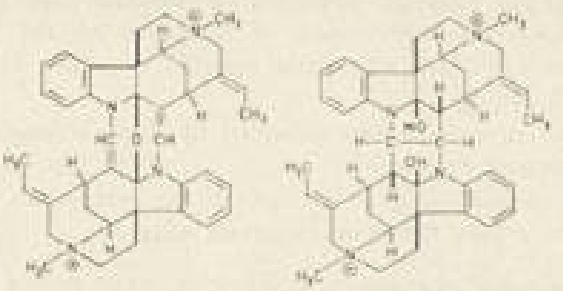
(*a*) The C-toxiferine I "family", formed by the union of two molecules of C-hemitoxiferine or by two molecules of Wieland-Gumlich aldehyde followed by quaternisation. The main alkaloids of this group are C-toxiferine I, C-alkaloids A and E, and toxiferine IX (= caracurine II methochloride).

(*b*) The C-dihydrotoxiferine I "family", which derives from the union of two molecules of 18-deoxy Wieland-Gumlich aldehyde. C-Curarine, C-alkaloid D, and C-calebassine are the more important derivatives of this group.



C-Dihydrotoxiferine I (C-alkaloid K)

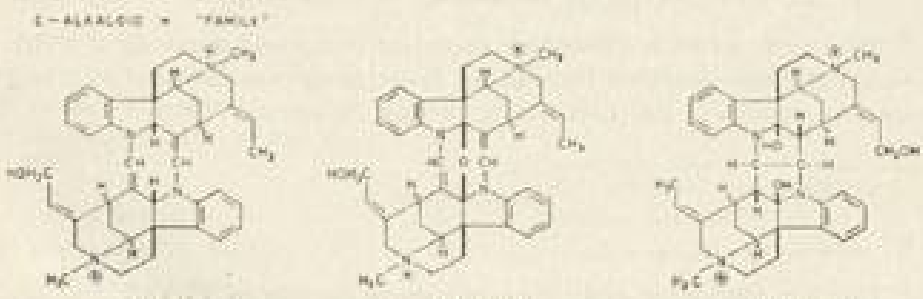
C-Alkaloid D



C-Curarine I

C-Calebassine

(*c*) The C-alkaloid H "family" is the third group and it is formed by the union of one molecule of Wieland-Gumlich aldehyde and one of 18-deoxy Wieland-Gumlich aldehyde. The main representatives are C-alkaloids H, G, and F.



C-Alkaloid H

C-Alkaloid G

C-Alkaloid F

3. New researches

In the international symposium on curare and curare-like drugs held in Rio de Janeiro in 1957, although the existence of bis-quaternary *Strychnos* alkaloids was not yet cleared but already near to a solution, still many questions were pending on the nature of the alkaloids present in curare and in *Strychnos*.

In effect the main source of information came from a number of studies on curares of different origin and the chemical studies of a very limited number of plants: i.e. *S. toxifera*.

The Rome group formed at the beginning by D. Bovet, Casinovi, Delle Monache, Galeffi, Iorio, Ciasca and the Author and by other colleagues joining temporarly our work, begun in 1953 a complete survey of a large number of *Strychnos*, and occasionally on curare samples.

In this work they were assisted, otherwise the organization could not be feasible, by prof. Adolfo Ducke a distinguished botanist from Fortaleza (Brazil) with the help of tte Conselho Nacional de Pesquisas de Brasil and later by dr. B. A. Krukoff former curator of Amazonas of the N. Y. Botanical Garden and their coworkers, who made possible to receive in Roma samples of plants, well determined.

Also samples of curares were provided by these colleagues as well by prof. Ettore Biocca, Rome.

Among the various questions left open at the Symposium on Curare from a chemical point of view the more important were:

1) What kind of changes of modification occur during the elaboration of curare in the structure of the alkaloids?

2) Are all the active principles present in the plants or are they formed during the preparation?

3) On a geo-botanical point of view there is any particular evidence to establish the difference between the alkaloid composition of american and other continents Strychnos?

4) Is curare composition identical to that prepared centuries ago?

5) Are they biogenetical relationships between alkaloids of Strychnos?

In order to establish some these important points and disposing of a rich botanical and ethnographical material the Rome group was dedicated to a long range investigation in this field during the last 20 years.

Methodology

a-1. *Analytical.*

The first difficulty to overcome is the scarce quantity of alkaloids generally obtainable from collected sample, and the number of constituents of the mixture.

TABLE 1.

The curarizing activity and alkaloids of Central and South American Strychnos species.

Species	Curarizing activity	Alkaloids
S. chlorantha Prog.		Diaboline, acetyldiaboline, undetermined quaternay alkaloids
S. colombensis		No alkaloids in the bark
S. romeu-belemii Krukoff and Barneby		11-Methoxydiaboline
S. rondelletioides Spruce ex Benth.		Diaboline
S. macrophylla Barb. Rodr.	++	Macrophylline A and B, C-mavacurine, C-fluorocurine
S. brachiata		11-Methoxy-diaboline, Desacetyl-diaboline
S. trinervis (Vell.) Mart.	+++	C-Calebassine, C-curarine, C-alkaloids H, I, and K, C-fluorocurarine, C-fluorocurinine
S. panamensis Seem.		C-Alkaloids K, F, and G, C-fluorocurine, diaboline, strychnine, brucine
S. tabascana Sprague and Sandw.		Tabascanine, acetyltabascanine, strychnobrasiline, 10-methoxystrychnobrasiline, O-methyl-N-acetylstrychnosplendine
S. divaricans Ducke	+	C-Calebassine, C-curarine, C-mavacurine, C-fluorocurarine
S. medeola		11-methoxy-diaboline, nor-macusine B (deoxysarpagine)
S. toxifera Rob. Schomb.	+++	C-Toxiferine I-XII; C-toxiferine I, II, IIa, and IIb; C-toxiferine I, caracurine I-VIII, nor-dihydrotoxiferine; C-toxiferine I, macusine A, B, and C; C-mavacurine, fodamazine
S. tomentosa Benth	++	C-Alkaloid E, C-toxiferine I, C-fluorocurine C-curarine, C-fluorocurinine
S. diaboli Sandw	—	Diaboline; diaboline, deacetyldiaboline
S. javarieusis Krukoff	+	(=Wieland-Gumlich aldehyde)
S. javarieusis Krukoff	+	
S. windsorhiana Krukoff and Barneby	+	Desacetyldiaboline (= Wieland-Gumlich aldehyde), macrophylline A
S. jobertiana Baill	+	Diaboline, jobertine
S. pseudo-quina A. St. Hil.		Nor-dihydrotoxiferine
S. amazonica Krukoff	+	Alkaloids α, γ, δ, and ε, C-mavacurine, 11-methoxy diaboline, nor-dihydrofluorocurarine, macusine B, nor-dihydrotoxiferine

Continued: TABLE 1.

Species	Curarizing activity	Alkaloids
S. solimoesana Krukoff	+++	C-alkaloids C, D, E, F, and G, C-curarine, C-calebassine, C-calebassinine, C-fluorocurarine, C-fluorocurine, rubrocurarine I-III, solimoesine I-III, fluorosolimoesine I-IV, precurarine, premavacurine,curarine
S. froesii Ducke	++	C-Alkaloids E, I, J, and K, C-toxiferine I, C-fluorocurinine, C-mavacurine, nor-dihydrofluorocurarine, diaboline, desacetyldiaboline
S. pechii B. L. Robinson	+	
S. erichsonii Rich Schomb	—	
S. gardneri A. DC.		C-alkaloids H, I, and X, C-curarine
S. mitscherlichii Rich. Schomb		
var. *mitscherlichii*	—	
var. *amapensis* Krukoff and Barneby	++	C-Alkaloid D, C-calebassine, C-fluorocurine, C-mavacurine, C-curinine
S. solerederi Gilg.		Diaboline, desacetyldiaboline (=Wieland-Gumlich aldehyde)
S. guianensis (Aubl.) Mart.	+++	Guiacurarine I-VIII, guianine, guiacurine C-curarine, erythrocurarine I and II
S. glabra Sagot ex Prog.		Alkaloids with C.N.S. action
S. subcordata Spruce ex Benth	++	C-Mavacurine, desacetyldiaboline (Wieland-Gumlich aldehyde), C-fluorocurarine, C-fluorocurine, erythrocurarine III, guiacurarine III and X, fluorocordatine
S. hirsuta Spruce ex Benth	—	Undetermined tertiary alkaloids
S. cogens Benth	+	
S. melinoniana Baill		Melinonine A, B, E, F, G, I, K, L, and M, C-mavacurine, C-fluorocurarine
S. castelnaeana Wedd	+	Diaboline, C-alkaloid D
S. atlantica Krukoff and Barneby.	—	Undetermined tertiary alkaloids
S. parvifolia A. DC	++	C-Alkaloid I, C-calebassine, C-curarine, C-fluorocurarine, C-mavacurine
S. fulvotomentosa Gilg	++	
S. acuta Prog.	—	
S. brasiliensis (Spreng.) Mart.	+	Spermostrychnine, strychnobrasiline, strychnostilidine, strychnosiline, 12-hydroxy-11-methoxyspermostrychnine, 12-hydroxy-11-methoxystrychnobrasiline
S. pachycarpa Ducke	+	Undetermined tertiary alkaloids
S. tarapotensis		No alkaloids in the bark
Strychnos fendleri		Strychnofendlerine, diaboline, desacetyldiaboline

For this purpose paper and TLC chromatography and electrophoresis was largely applied and developed as analytical tool in order to establish the complexity of the alkaloid mixture.

Electrochromatography also showed good results.

After a long pioneering work on several samples a more or less complete picture was available of the analitycal composition of alkaloids of 20-25 different species.

a-2. Biological.

As a first test in order to establish the activity of the various extracts, a standard system was studied by Bovet and Marini-Bettòlo, based on the biological test of the toxicity of a standard weight of powdered plant material, extracted in the same conditions by a determined volume of an organic acid solution.

In these conditions all alkaloids are extracted and the results of toxicity may give a first information for further work on the sample. In Table 1 column 2 are reported the main results obtained by this method.

a-3. Separation.

Owing the very scarce available quantities of alkaloid mixture extracted from plants we have adopted the separation on thick paper, by band chromatography.

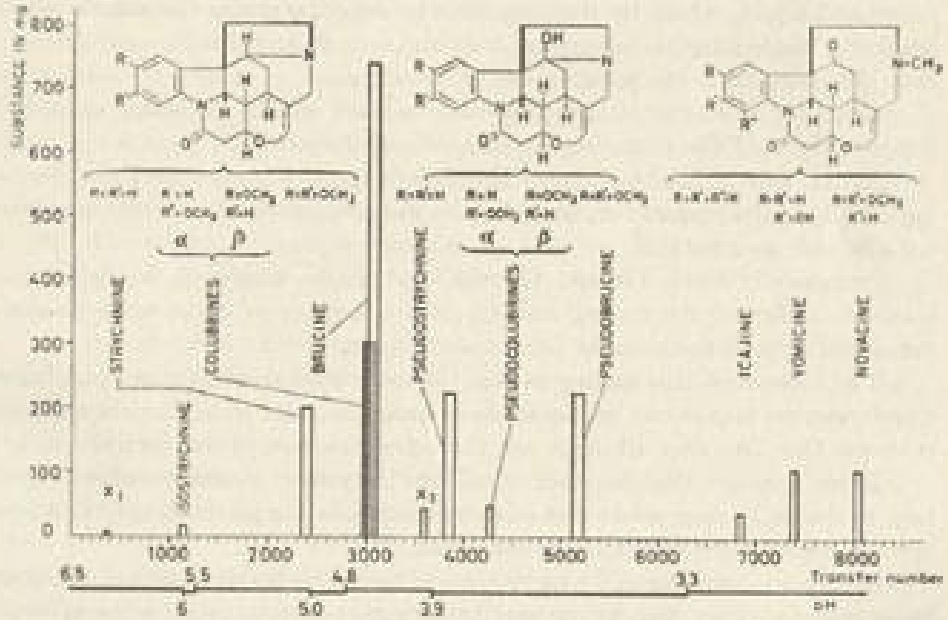


Fig. 1.

When possible column chromatography on cellulose was largely adopted using different solvents.

In the case of tertiary alkaloids a method proposed by Galeffi and others based on counter current distribution with progressive variation of the pH gave exceptional resolutions of mixture of very closely related alkaloids.

The method is based on the double distribution and dissociation equilibrium in the case of a weak base. Since the separation depends on the difference in the product of the dissociation constant k_b and the distribution coefficient k_r. To this purpose the use of a lower organic phase and an upper buffer phase whose pH is varied from neutrality to increasingly acidic values in such a way to extract alkaloids in order of decreasing $k_r k_b$.

We report in fig. 1 the separation of the mixture of alkaloids of *S. nux vomica* which yields, over the nine known alkaloids, three new substances.

b. Researches on Curares.

A new type of curare beyond the three of the Boehm's classification has been described in 1964.

Biocca in a scientific expedition in the areas between Rio Negro and Upper Orinoco found a new type of curare elaborated by the Yanoama indians (Guaicas).

This curare is prepared from the root bark of a *Strychnos* not specified, but most probably *S. toxifera*. The root bark is dried over the fire, powdered and extracted, on a funnel with made a leaf of a plant with hot water. The percolated liquid, which by the preparation method contains the soluble alkaloids of a single plant, is concentrated on the fire, when it has become siruppy, it is used to poison the point of the blow arrows.

Generally this type of curare is not disposed in any particular recipient, but directly used for preparing the arrows and darts.

Biocca, Bovet, Marini-Bettòlo and Galeffi could demonstrate the curarizing activity of the preparation, and establish the presence of caracteristic quaternary alkaloids as curarine.

Successively Padre Grossa, Caracas, and Padre Cocco, S. Maria de los Guaicas, confirmed the finding and supplied a number of darts, were curarizing quaternary alkaloids could be detected by us.

The interest of this finding is that, in these mild conditions no particular transformation may occur in the alkaloid structures, and thus there is a direct evidence that *Strychnos* alkaloids are the active fraction of the curare.

If we consider that in other conditions *Strychnos* alkaloids suffer a very long treatment, it is possible that in these conditions the alkaloids may undergo several oxidation or transposition reactions.

In effect it was shown by us that nor-dihydro toxiferine is rather common in several *Strychnos*, but before our investigation it was found only in very minute amounts both in plants and in curare because it undergoes rapidly to

transformation also in the presence of acids and heat, i.e. during extraction from plants.

It was known since the observations of Biocca and of Lazzarini Peckolt that some curares—in respect to their dry weight—were more active than the extracts of the corresponding plants.

The above mentioned Authors explained this property as due to the possible reactions which take place during the elaboration of the curare, i.e. permethylation reactions.

The group of Rome could demonstrate in a comparative study, both pharmacological and chemical, that in the case of Macu curare elaborated with *Chondodendron*, the curarizing activity of the alkaloids obtained from the tubocurare are ten fold more active than those extracted from the plant. Both alkaloids were submitted to methylation and again tested for their curarizing activity showed that in the case of alkaloids obtained from tubocurare no increase of potency can be observed whereas in the case of the alkaloids obtained from the plant the acitivity is increased.

In the case of calabash curare and *Strychnos* extracts, exhaustive methylation do not influence the curarizing activity of both samples.

These findings indicate that in the case of *Chondodendron* bis-benzylisoquinoline alkaloids, during the elaboration of the tubocurare some methylation reactions may occur both on the N= and OH groups, perhaps by some disproportion reactions. In the case of *Strychnos* indolic alkaloids no similar reactions take place.

We have examined for the first time the alkaloid composition of *Strychnos castelneana*, described by Castelnau as the main ingredient of Ticuna curare, a typical mixed curare, very well studied in the past centuries.

We have established that only a low percentage of quaternary alkaloids is present: in effect we have detected the presence of C-alkaloid D, and higher quantities of tertiary alkaloids like diaboline and jobertine. The overall curarizing activity is rather low as it is due only to alkaloid D. Therefore the activity of Ticuna curare is due not only to the latter but, we believe, mainly to the presence of bis-benzyl-isoquinoline derivative. A chromatographic assay on the Ticuna curare sample, brought in 1856 by de Castelnau and kindly supplied to us by the dr. Bauer of Volkerkunden Museum of Vienna, confirm these hypothesis.

In the chemical survey on curare deposited in various european Museum, dr. Bauer was able to demonstrate that the composition of curare of the same ethnographic origin is identical after more than one century, confirming thus the stability of these preparation.

Another important feature of the present evolution on curare chemistry is the finding by Angenot of an arrow poison from Rwanda, Central Africa, which contains C-curarine and, naturally, exerts curarizing activity and tertiary alkaloids like dihydro-toxiferine and a new bimolecular alkaloid usambarensine.

The arrow poison is elaborated from *Strychnos usambarensis*, a plant growing in Rwanda.

c. Recent researches on Strychnos alkaloids.

No complete account on Curare chemistry, especially in the group of calabash curare, can be given, without systematical chemical researches of a number of *Strychnos* species which are used in the elaboration of curare.

The present knowledge in this field is summarized in Table 1 and is due the result of mainly to the group of Rome, and to researches of the groups of Zürich, Bristol, Buenos Aires and Liège.

If we examine in detail the alkaloids of *Strychnos* we can divide them according to their chemical structures in monomeric tertiary, dimeric tertiary, monomeric quaternary and dimeric quaternary alkaloids. The latter as above reported were found in curare.

(i) *The monomeric tertiary alkaloids* found in American Strychnos, mainly by the group of Rome, can be grouped as follows:

Strychnine group: strychnine and brucine.

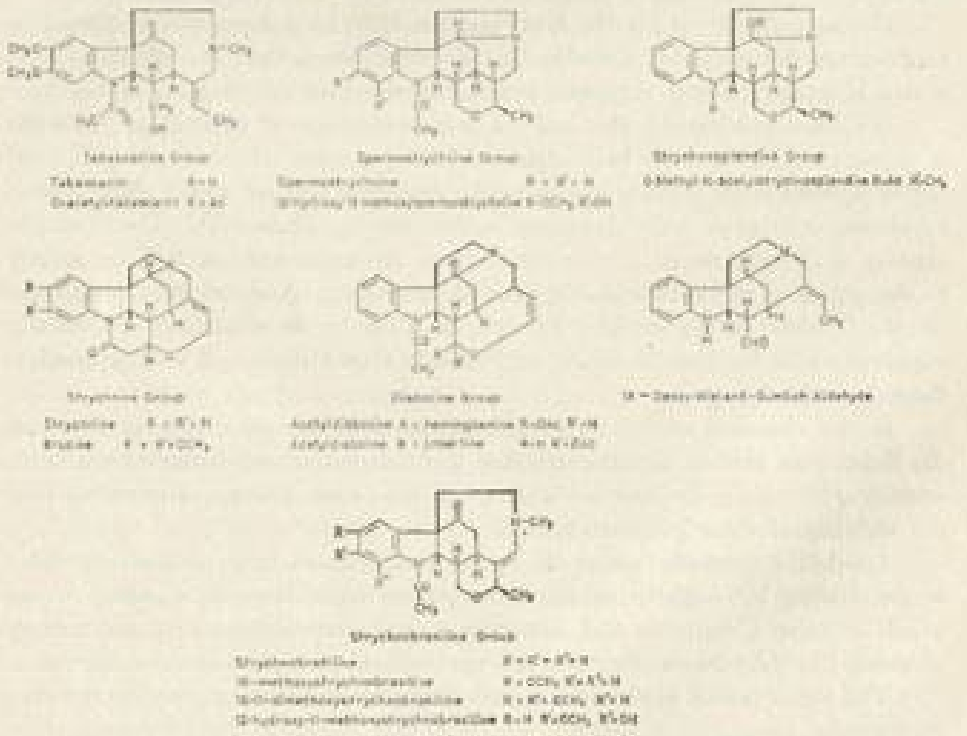
Diaboline group: diaboline, deacetyldiaboline (= Wieland-Gumlich aldehyde), acetyldiabolines A and B (henningsamine and jobertine), 11-methoxydiaboline.

18-deoxy Wieland-Gumlich aldehyde.

Tabascanin
Spermostrychnine } group

Strychnosplendine group

Strychnobrasiline group.



Surprisingly with more sofisticated techniques during the last years it was possible to isolate a number of new tertiary alkaloids from American *Strychnos* and to find in these species some alkaloids, before considered to belong only to the african, asiatic or australian species.

The main feature was the finding in *S. panamensis*, which contains quaternary dimeric alkaloids and the tertiary strychnine, brucine and diaboline. This demonstrates that there is not a substantial difference between american *Strychnos* and those of other continents.

A further confirmation of this fact was the finding both from the Rome group and by Comin and Iwataki in Buenos Aires, that in two species: one from the Central America and the other from Argentina, *S. brasiliensis* and *S. tabascana* respectively, of a group of new alkaloids (12-hydroxy-11-methoxyspermostrychnine, O-methyl-N-acetyl strychnosplendine) closely related to the spermostrychnine and strychnosplendine groups both found earlier in asiatic and australian species (*S. axillaris*, *S. pallosperma*).

Moreover two new structure type, chemically related to the latter were found in the Tabascanine group and the Strychnobrasiline group and recently in *S. fendleri*.

A certain interest is also shown by the finding in various samples of a number of tertiary alkaloids which can be considered intermediate for the building up of dimeric quaternary derivatives.

In effect as a result of our investigations, diaboline can be considered practically ubiquitous in all *Strychnos* species.

The first time we were able to detect a large amount of this alkaloid in *Strychnos ignatii* seeds from Malaya and anticipate that it could constitute the intermediate pathway for both the synthesis of strychnine and quaternary alkaloids, being also found in american *Strychnos* together with quaternary dimeric derivatives.

Other alkaloids of this type are desacetyl-diaboline known as Wieland-Gumlich aldehyde, and the two isomeric O-acetyl diabolines: jobertine and henningsamine.

Another substance of this type which discloses future possibilities is 11-*methoxy diaboline* and the corresponding desacetylderivative found by our group for the first time. It is now possible to argue that also dimeric 11-methoxy derivatives may be found, if desacetyl diaboline, as recently stated, could be considered the intermediate of the dimeric alkaloids biosynthesis.

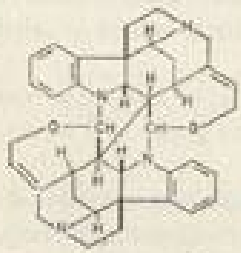
Deoxy-desacetyl diaboline, i.e. nor-dihydrofluorocurarine known also as a breakdown product of nor-dihydrotoxiferine and isolated by us in plants for the first time, was found to be a rather common constituents of several *Strychnos*. The product being supposed the precursor of the Curarine family its finding is of particular interest.

Among tertiary alkaloids the non-macusine-B was found recently by us in *S. medeola*. This alkaloids belongs to a particular structure, which is found in several plant families of the *Loganiacee* and *Apocynacee* groups.

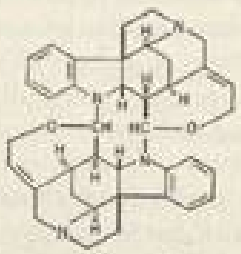
(ii) Dimeric tertiary alkaloids. The more important dimeric tertiary alkaloids isolated from *Strychnos* are nor-dihydrotoxiferine, caracurine II, and caracurine V. These bases may be considered as the dimerization products of 18-deoxy–Wieland-Gumlich aldehyde and of Wieland-Gumlich aldehyde, respectively.

Nor-dihydro-toxiferine (II) an alkaloid isolated before only in minute amounts, was found to be present in rather high percentage in a number of *Strychnos* species examined *S. pseudoquina* is particularly rich.

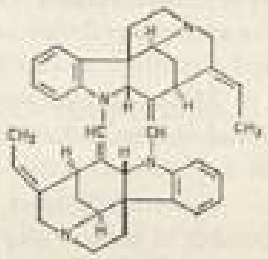
This alkaloid may constitute a precursor of C-dihydrotoxiferine. Its chemical reactivity is very high and thus the product very unstable. For this reason it is very probable that during extraction, in excess of mineral acids, the product may isomerize.



Caracurine II



Caracurine V



Nor-dihydrotoxiferine

This fact should be taken into account when discussing the transformation who may occurr in the elaboration of curares being this alkaloids rather common, according to our last studies in several species.

Two other alkaloids of this type Caracurine II and V were found in *S. toxifera* by Karrer and may be also considered as transformation products of nor-toxiferine.

Monomeric quaternary alkaloids have been found in several species, mainly macusine B, which is also common to other genera and families plants and probably constitutes an important intermediate compound.

Dimeric quaternary alkaloids. In *S. panamensis* dimeric quaternary alkaloids may be found with tertiary alkaloids.

Quaternary alkaloids are generally more abundant in root bark, but can be found also in stembark.

In addition to the three types of dimeric quaternary alkaloids above reported in *Strychnos* are also present a number of quaternary alkaloids of undertemined structure.

Owing to the difficulty in obtaining sufficient amounts of the alkaloids for the determination of their structures, many of the bases have so far only been characterized by their spectroscopic and chromatographic properties. Among these may be mentioned: guiananine, guiacurarines I-X, xanthocurine, macrophyllines A and B, erythrocurarines I-III, solimoesines I-III, urarine, fluoro-

cordatine, rubrocurarine, calebassinine, alkaloids α, β, and γ, and melinonines E, H, I, K, L, and M.

Some of them are of the anhydronium basis type.

The structures of these alkaloids has not yet been established owing to the limited amount of substance available and the difficulties to purchase the plants. Some comments may now illustrate the implications of these findings.

Conclusions

The most important results obtained during this systematic research programme are as follows:

(i) For the first time, strychnine, brucine, and diaboline, and dimeric quaternary alkaloids, have been found together in an American *Strychnos*: *S. panamensis*.

(ii) New alkaloids have been isolated from various species, e.g. 11-methoxydiaboline, jobertine (acetyldiaboline B), tabascanine and related compounds, and many alkaloids of undetermined structure (guiacurarines, solimoesines, etc.). These latter are an indication of the great versatility and multiplicity in the group of *Strychnos* alkaloids.

(iii) The occurrence in various species of a particular alkaloid which may be related to the special metabolism of the plants, e.g. *nor-dihydrotoxiferine, Wieland-Gumlich aldehyde, and diaboline*. These substances may be considered formally at least as precursor of more complicated alkaloids, although Schlatter and co-workers have not been able to find evidence for such a role in the case of the Wieland-Gumlich aldehyde, but recent researches demonstrate this possibility.

(iv) Confirmation that the alkaloids found in curare are identical with or closely related to the plant alkaloids or are formed from the latter through simple chemical reactions. In this connection may be mentioned the great sensitivity to heat and acids of nor-dihydrotoxiferine, which has been found to occur in considerable amounts in some *Strychnos* species. A very clear example of the relation between curare and *Strychnos* alkaloids is provided by Yanoáma curare which is obtained by simple percolation of dried and pulverized *Strychnos* bark and root.

(v) Dimeric curarizing alkaloids are present in reasonable amount only in the roots. The samples richest in these alkaloids come from the Amazon hylcia.

(vi) Examination of several samples of the same species and of the same part of the plant (bark of the lower, middle, and higher stem) shows in some cases very different alkaloid patterns. This variation in alkaloid pattern may reflect differences in alkaloid distribution at different ages of the plant.

(vii) The distribution of the alkaloids in the different parts of the plant shows that the branches and stem bark contain mainly tertiary alkaloids (these are also present in the fruit and seeds) and that in the lower stem bark and root bark mainly quaternary alkaloids occur.

About curare we may say:

(i) That some modification in alkaloids structure may take place during elaboration, both in tubocurare and in calabash curares.

(ii) In calabash curare we must take in account only the unstable alkaloids like nor-dihydrotoxiferine, and others which may undergo to oxidation processes during the preparation.

(iii) Although rare, out of the Amazonas hyleia, dimeric quaternary alkaloids were found in Central America and in Africa, where they are used for elaboration of " dart poisons ". On the other side strychnine and brucine were found in Central America with quaternary alkaloids species thus demonstrating a unique biogenetic pattern in *Strychnos* species.

(iv) Curare are very stable, once prepared, and differs very much in the composition of the alkaloids and of the plants used, but have always in common the presence of dimeric quaternary alkaloids although of different structure.

(v) We should here remember that curares are not a mere ethnographical curiosity, but even now, in the jet and moon exploration age, a daily tool, necessary to the survival of a number of indian tribes in the rain forest of South America.

We have learned from these populations the use of a very important group of new drugs, the curare and curare like drugs, which have now become indispensable for the progress of medicine.

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