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INDOLE ALKALOIDS FROM *Strychnos* SPECIES AND THEIR ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES*

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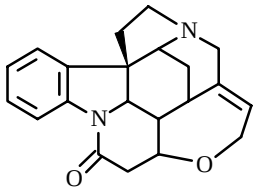
Indole alkaloids from the African Strychnos *species, their antiplasmodial and cytotoxic action, and structure - activity relationships are discussed in the review.*

Key words: *Strychnos*, indole alkaloids, antiplasmodial and cytotoxic activities.

Strychnos species (*Loganiaceae*) have been studied in the laboratory of Pharmacognosy of the University of Liege for more than fifty years. Although investigations into the *Strychnos* genus have been going on for a long time in the case of Asian tetanizing and American curarizing species, the first chemical and pharmacological screening on African species was undertaken in the early nineteen fifties by the late Prof. A. Denoel (University of Liege, Belgium), who examined twenty-five species of *Strychnos* collected in the Belgian Congo [1]. This research programme was also an offshoot of an inventory of medicinal and toxic plants in Rwanda, carried out during the years 1969–1970. The Belgian scientists were lucky to observe the tribe of Banyambo, living along the Akagera river on the border between Rwanda and Tanzania, where hunters prepared in front of them an arrow poison with *Strychnos usambarensis* roots and leaves as the main ingredients [2].



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Strychnos species have several ethnobotanical uses. A few species are well known for their incorporation into arrow and ordeal poisons, but play more a role in ethnomedicine against fever, rheumatism, worms, ulcers, leprosy, snake-bites, and so forth [3]. In fact, among about one hundred and ninety species, only six would contain strychnine (**1**) (*Strychnos nux-vomica* L. [4–7], *S. ignatii* P. Bergius [8, 9], *and S. wallichiana* Steud ex DC. [7, 10] from Asia, *S. lucida* R. Br. [7, 11] from Australia, *S. icaja* Baillon [1, 12] from Africa, and *S. panamensis* [13–15] from South America). *Strychnos* alkaloids are in fact an example of molecular and pharmacological biodiversity. More than 300 different *Strychnos* alkaloids have been isolated to date and they present various biological activities in several fields: parasitology (amoebiasis, paludism, etc.) [16–18], cancer [19–22], neurology (tetanizing or curarizing effects) [14, 23, 24–26], inflammation [27], and so on.

MALARIA

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths (principally young African children) out of 400 million cases each year (WHO world health report 2002) (Fig. 1).

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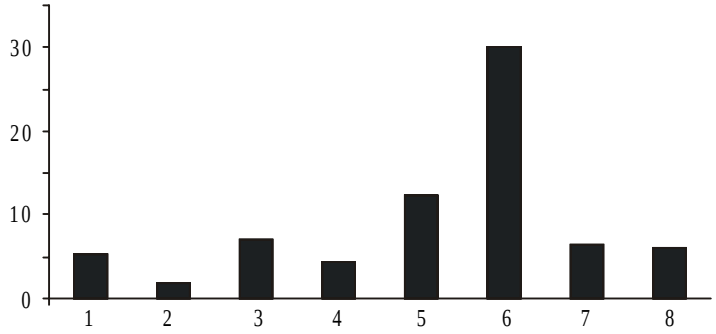
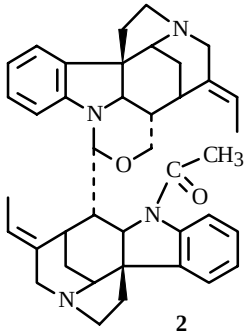


Fig 1. Causes of death the most distributed in the world. 1 – HIV/AIDS,
2 – Malaria (1080000), 3 – Respiratory infections, 4 – Perinatal conditions,
5 – Malignant neoplasms (6930000), 6 – Cardiovascular diseases,
7 – Respiratory diseases, 8 – Unintentional injuries.

More than half of the world's population live in areas where they remain at risk of malaria infection. In fact, more people could potentially be infected by malaria today than during the last century. During recent years, the situation has worsened in many ways, mainly due to malarial parasites becoming increasingly resistant to several antimalarial drugs [28]. This resistance concerns numerous drugs, but is thought to be most serious with chloroquine, the most widely used and cheapest drug to treat malaria. Urgent efforts are therefore necessary to identify new classes of antimalarial drugs. Furthermore, the control of malaria is becoming more complicated by the parallel spread of resistance of the mosquito vector to currently available insecticides. Malaria is caused by Protozoa of the genus *Plasmodium*. Four species of *Plasmodium* cause the disease in humans (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) but *P. falciparum* causes the most problems as a result of its prevalence, virulence, and drug resistance. *P. falciparum* may cause the conditions known as cerebral malaria, which is often fatal. The life cycle of the malaria parasite is complex and involves two stages: a sexual reproductive stage with multiplication (sporogony) wich occur in the midgut of the mosquito, and an asexual reproduction phase with multiplication (schizogony), which takes place in the human host. We could distinguish, in the human part of the cycle, two important phases: the exoerythrocytic (hepatic) phase and the erythrocytic phase.

Between 1930 and 1960, paludism has regressed, following the intensive use of quinine and chloroquine, two effective antimalarial agents. Unfortunately, from 1960, the reexpansion of paludism began with the emergence of resistances to the main antimalarial agents used. The parasite is nowadays more or less resistant to available treatments in much of the world. The only treatment possible in some regions is artemisinin and its derivatives.

To find new sources of natural antiplasmodial agents, more than 50 plant extracts have been screened against *Plasmodium falciparum*, and three plants were particularly active: *Strychnos usambarensis*, *S. icaja*, and *S. variabilis* [17]. *Strychnos variabilis* is a small tree endemic around Kinshasa in Congo. The golden fruit pulp is deliciously sweet but the root bark is said to be a violent poison from which twenty-one alkaloids have been isolated. Some of them belong to a new group of unsymmetrical bisindoline alkaloids: the strychnobilines (**2**) characterized by a carbinolamine ether group in a hexacyclic ring [29]. The others are monomers, like retuline (**3**) with sometimes an antiinflammatory activity similar to that of indomethacine but without adverse effects [30, 31].



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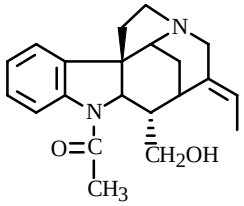

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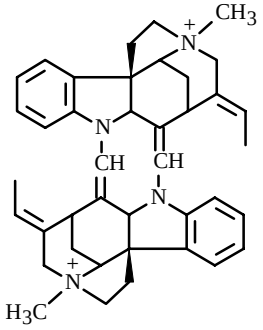
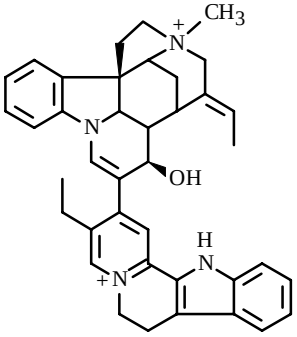
The two other active plants were *S. usambarensis* and *S. icaja*. *S. usambarensis* has a distribution in all tropical Africa. It is a climbing shrub in West Africa, while East and South African specimens are small trees. Roots and leaves of the plant are used to prepare a curarizing poison that is applied to long arrows prepared for hunting lions, buffalos, and antelopes [23]. Several years ago, the root barks were examined for tertiary and quaternary alkaloids. Up to now, twenty-two products have been isolated. The compounds could be divided according to their polarity and the characteristics of their amine functions. The symmetrical dimeric alkaloids like C-dihydrotoxiferine (**4**) were very active products. Three of them (dihydrotoxiferine, curarine, and calebassine) were previously isolated from South American curares and American *Strychnos* sp [32]. They were found and isolated for the first time in a *Strychnos* species growing outside of America. These bis-quaternary ammonium compounds offer potent neuromuscular blocking properties, and we may conclude that the lethal action of the arrow poison prepared by Nyambo hunters is mainly due to these products. The second binary compound was a new product and has been called afrocurarine (**5**). It is an unsymmetrical bisindole alkaloid a bit less active than the symmetrical dimeric compounds. We can add that some analogous derivatives have recently been found in a Brazilian sample of *Strychnos guianensis*. This Amazonian plant was the first botanically identified source of curare, but its chemistry remained unknown, probably due to the complexity of these unsymmetrical constituents [23, 26, 33, 34].



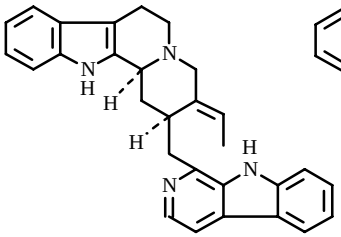
4 (Curarizing +++)



5 (Curarizing+)

Tertiary alkaloids **6–9** were found in the root bark of *S. usambarensis.* They were binary compounds devoid of any curarizing activity. According to some similarity with the structures of emetine-like alkaloids (isoquinoline alkaloids from ipecac) they were tested against protozoa. Usambarensine (**6**) possessed potent antiamoebic activities (3 times more active than emetine and thus as active as metronidazole), while dihydrousambarensine (**7**) showed an important activity against *Plasmodium falciparum*. The leaves were therefore studied. They contained fifteen new alkaloids slightly different from those present in the roots. They also possessed the usambarane skeleton but with a shift of the double bond located on the side chain. However, many are phenolic and, among them, strychnopentamine and isomers are curious alkaloids with five nitrogen atoms: a methylpyrrolidinic group is joined to the indole ring of the corynane part of the molecules **9**. Strychnopentamine and its isomer, isostrychnopentamine, are potent antiplasmodial alkaloids (IC_{50} = 0.1 mM), two times more active than quinine [16].

The third active species was *S. icaja*. *S. icaja* is a shrub which could become an important liana up to a hundred meters long and which is distributed in all central Africa. Roots have a characteristic red outer bark. The plant is mainly known for its use as a hunting or ordeal (trial) poison; nevertheless, the use of this *Strychnos* in traditional medicine, notably to treat chronic malaria, is also reported [3, 35]. *S. icaja* has been phytochemically fractionated, bioguiding all steps of fractionation by antiplasmodial activity testing. The roots have been extracted and fractionated by typical alkaloid methods. All extracts have been checked for antiplasmodial activity. The extracts showing the higher activity have been studied in priority. This has led to seven new alkaloids and could be separated in three groups: the sungucines (**10** bisindoline compounds), the strychnogucines (with one supplementary cycle as in the structure of strychnine **11–14**), and one trisindole alkaloid: strychnohexamine (**15**), which is the first example of such an indolomonoterpenic alkaloid isolated directly from a plant source. All structure elucidations have been conducted by means of spectroscopic analysis, using essentially advanced ^{1}H and ^{13}C 1D and 2D NMR methods, which was a very big part of the work [36–39]. In Fig. 2 are plotted the IC_{50} of these alkaloids compared to strychnine (scarcely active) and principally to chloroquine and quinine on several *Plasmodium falciparum* lines. Sungucine presented very little activity, but some compounds (strychnogucine B and 18-hydroxyisosungucine) displayed activities comparable to that of quinine. Generally, these alkaloids were more active against the chloroquine resistant strains.



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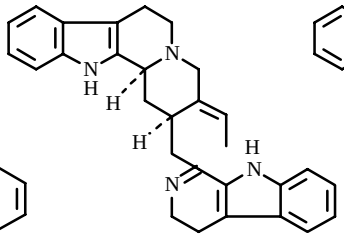

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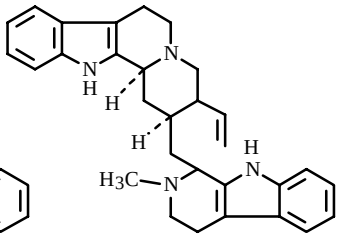

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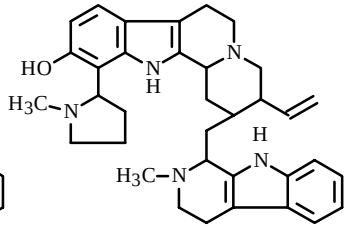

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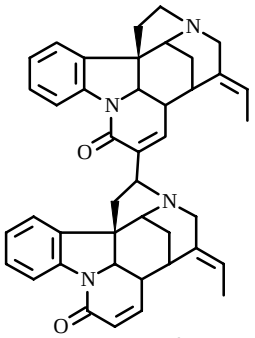

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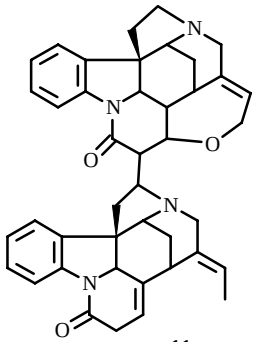

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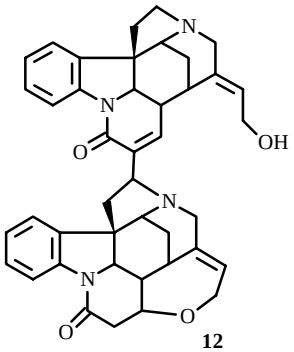

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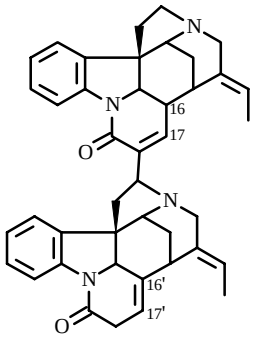

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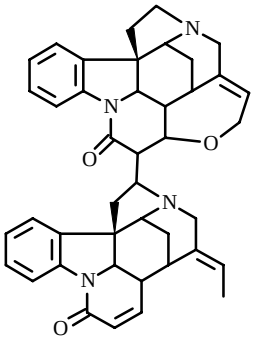

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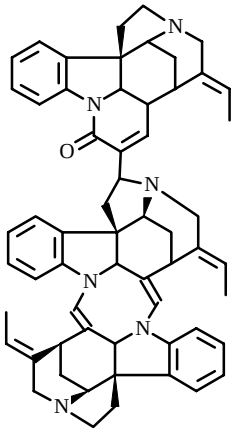

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In a continuation of the work, the antiplasmodial activity of indole alkaloids extracted from other *Strychnos* species has been studied (Fig. 3). Finally, the antiplasmodial activity of these compounds was compared with their cytotoxicity against human cell, and their selectivity index was determined. The higher this index, the more selective the compound against Plasmodium. We could remark that sungucine was not at all selective, while strychnogucine B presented relative selectivity. Dihydrousambarensine and 16-methoxy-isomatopensine also presented good selectivities [16].

Some structure/activity relationships could then be deduced: in the class of sungucine-like compounds, the presence of a double bond between C_{16} and C_{17} in the inferior moiety of the structure (isosungucine, **13**) as well as the cyclization of this inferior part (strychnogucine B, **12**) cause an increase in the activity. Some bisindole alkaloids displayed an activity similar to that of quinine, while all monomers present at the very most a weak activity. In fact, among 94 indolic alkaloids tested under the same conditions:

– fifty monoindoles were inactive and only five were slightly active (IC_{50} in the 10 to 30 micromolar range);

– seventeen bisindole were slightly active (2 to 20 micromolar);

– twenty-two bisindoles were active at a concentration inferior to 2 micromoles.

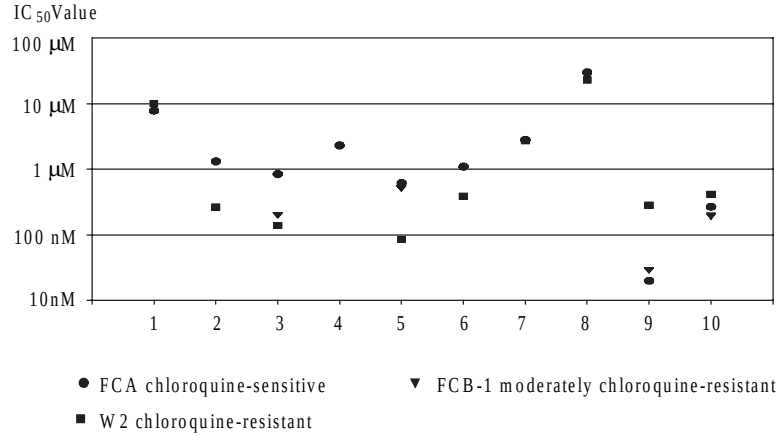

Fig. 2. IC_{50} of several alkaloids from *Strychnos icaja* against three *Plasmodium*. 1 – Sungucine (10), 2 – isosungucine (13), 3 – 18-hydroxy-isosungucine, 4 – strychnogucine A (11), 5 – strychnogucine B (12), 6 – strychnohexamine (15), 7 – bisnordihydrotoxiferine, 8 – strychnine (1), 9 – chloroquine, 10 – quinine.

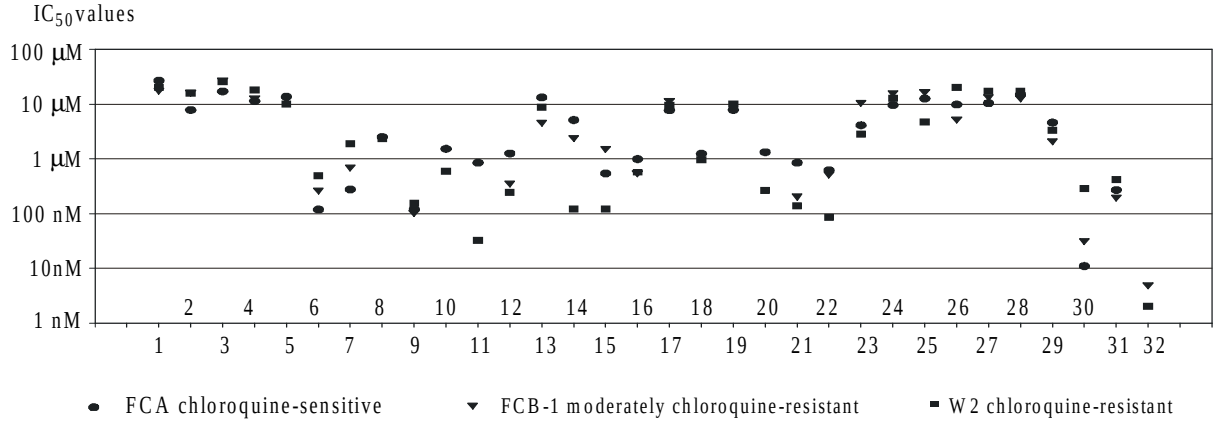

Fig. 3. IC_{50} of several indole alkaloids against three *Plasmodium falciparum* cell lines. 1 – Ngouniensine, 2 – epingouniensine, 3 – akagerine lactone, 4 – demethoxycarbonyl-3,14-dihydrogambirtannine, 5 – 9-methoxy-16(R)-E-isositsirikine, 6 – ochrolifuanine A, 7 – ochrolifuanine E, 8 – usambarine (8), 9 – isostrychnopentamine (9), 10 – usambarensine (6), 11 – dihydrousambarensine (7), 12 – matopensine, 13 – matopensine-N-oxide, 14 – 18-hydroxymatopensine, 15 – 16-methoxyisomatopensine, 16 – longicaudatine, 17 – longicaudatine F, 18 – tetradehydrolongicaudatine Y, 19 – sungucine (10), 20 – isosungucine (13), 21 – 18-hydroxyisosungucine, 22 – strychnogucine B (12), 23 – strychnofuranine, 24 – janussine A, 25 – janussine B, 26 – S-panganensine, 27 – panganensine Y, 28 – panganensine X, 29 – 16,17-dehydroisostrychnobiline, 30 – chloroquine, 31 – quinine, 32 –artemisinin.

A few of the products presented an activity under the micromolar range: isostrychnopentamine (from *S. usambarensis*, with an IC_{50} near 0.1 mM against all lines), ochrolifuanine A (from *S. potatorum*, with an IC_{50} between 0.1 and 0.5 mM), and strychnogucine B (from *S. icaja*, with IC_{50} between 0.1 and 0.6 mM)[16].

Concerning the structure-activity relationships, some converging points could be found in the class of indole alkaloids: some level of lipophilia and at least two basic amino centers as well as a certain steric cluttering (bis or tris-indolic compounds) are necessary for antiplasmodial activity.

CYTOTOXICITY

Six alkaloids have been submitted to the NCI (National Cancer Institute of the United States) screen. Two products have shown some selectivity against selected cancerous lines: isostrychnopentamine (ISP) and sungucine. The mode of action

of these compounds was investigated. Sungucine was able to induce apoptosis in HL-60 leukemia cells. This has been observed by several apoptosis tests: morphology (condensation of chromatin in the nucleus, etc.), induction of caspase 3, cleavage of PARP (Poly ADP-ribose-polymerase), and fragmentation of DNA [22, 36].

ISP is able to induce apoptosis in HCT-116 colon cancer cells. This has been shown by several classical tests [19]:

– firstly the morphology, where we can observe fragmented nuclei and blebbing in ISP-treated cells.

– secondly, the DNA laddering (indicating internucleosomal DNA fragmentation), the induction of caspase 3, and the exposition of phosphatidylserines (by FACS analysis).

But finally, the original point is that ISP could induce apoptosis and cell cycle arrest depending on the p-21 gene, and independently of the p-53 gene.

In this way, ISP is different from all DNA damaging agents, such as the topoisomerase inhibitors camptothecin or etoposide, or the tubulin poison taxol that induce, apoptosis controlled by this p-53 gene. This mechanism of induction independent of p-53 is really original and has been confirmed in the cell-line HCT-15, where the p-53 gene has been deleted [19].

CONCLUSIONS

Several types of indole alkaloids possessing interesting antiplasmodial properties or anticancer activities were discovered in our work, but pharmacological and toxicological studies must be continued. So the possible strychnine-like activity of sungucine derivatives are actually investigated because it is obvious that such properties could be disadvantageous for their potential exploitation as antimalarial drugs. Fortunately these bisindoline alkaloids are inactive on glycine receptors [40].

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