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ANTIMITOTIC ACTIVITY OF STRYCHNOPENTAMINE, A BISINDOLIC ALKALOID

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Summary

Strychnopentamine has been tested for its cytotoxic and antitumor activities and compared with two other bisindolic alkaloids that possess an usambarane skeleton. The presence of a *N*-methylpyrrolidine group increases the antimitotic activity of this type of alkaloids.

Introduction

The research program of the University of Liege on *Strychnos* alkaloids is an offshoot of an inventory of medicinal and toxic plants in the Eastern part of Rwanda, carried out during the years 1969--1970 (Angenot, 1970). Among the toxic plants, those species which are ingredients of arrow poisons, are really worth studying. An intriguing account of a hunter named Kahijama, gamekeeper in the National Park of Akagera became a special inspiration to initiation of our program. Kahijama belongs to the tribe of Banyambo, living along the Akagera river on the border between Rwanda and Tanzania. This tribe prepared an arrow poison in our presence (Angenot, 1971). The main ingredients of this previously unknown poison were *Strychnos usambarensis* leaves and roots which we had chemically and pharmacologically examined. A review of the phytochemistry of African *Strychnos* species, including *S. usambarensis*, has been recently presented in this journal (Ohiri et al., 1983).

One recent study describes an attempt to correlate results obtained from the NCI plant antitumor screening program with selected types of folkloric uses (Farnsworth et al., 1981). From these data, it would appear that one would increase by a factor of about five the number of plant species that could show experimental in vitro or in vivo cytotoxicity or antitumor activity if the plants were selected on the basis of alleged use as arrow poisons. Moreover, the chloroform extract of *S. usambarensis* leaves has been shown to be active against lymphatic leukemia P-388 in vivo in mice (pers. commun. of the late Professor Kupchan) (Rolfsen, 1980).

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Thus, we have carried out experiments on the cytotoxic and antitumor properties of pure alkaloids isolated from *S. usambarensis*, because this species is used as an arrow poison and has shown antitumor activity from a crude extract.

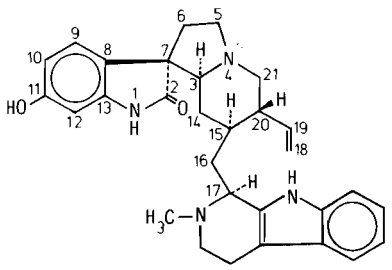
The cytotoxicity of two alkaloids (strychnofoline 1, dihydro-usambarine 2) isolated from *Strychnos usambarensis* leaves was demonstrated (Bassleer et al., 1982). These molecules showed a certain degree of antimitotic activity at relatively high doses (10 μg/ml and mainly 50 μg/ml) (Bassleer et al., 1982).

Strychnopentamine 3, another alkaloid from this African *Strychnos* has been tested for its cytotoxic and antitumor activities, either in vitro on cultured rat hepatoma, B16 melanoma and Ehrlich ascites tumor cells or in vivo on mice bearing an Ehrlich ascites tumor. The results of these experiments are described.

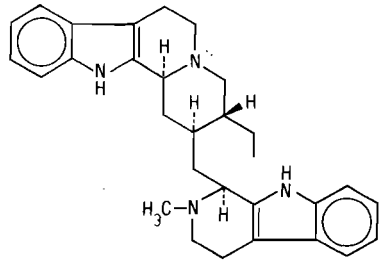
Materials and methods

Chemistry

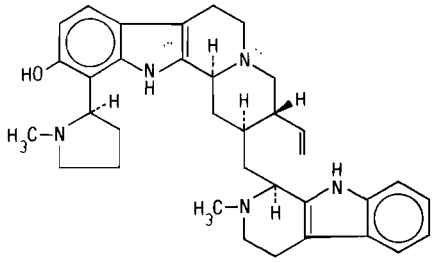
Strychnopentamine was extracted from *S. usambarensis* leaves. The plant material was collected in Tanzania and taxonomically identified by Leeuwen-



1 STRYCHNOFOLINE



2 18,19 - DIHYDRO USAMBARINE



3 STRYCHNOPENTAMINE

Fig. 1. Structures of *Strychnos* alkaloids strychnofoline (1), dihydro-usambarine (2) and strychnopentamine (3).

berg. A specimen is available at the Herbarium of the Agricultural University, Wageningen, The Netherlands. The dried leaves were extracted following our own method (Angenot et al., 1978). Strychnopentamine was identified by comparison with an authentic sample that was available from previous research (Dupont et al., 1977). Strychnopentamine possesses the "usambarane" skeleton but it is a curious indole alkaloid with five nitrogen atoms; a methylpyrrolidine group is joined to the benzene ring of the corynane part. The base was salified as acetate.

Cytotoxicity

Antimitotic activity tests were carried out as previously described (Bassleer et al., 1982) on: (a) cultured hepatoma cells derived from HW165 hepatoma of Wistar rats; (b) cultured B16 melanoma cells derived from C57BL mouse melanoma; (c) cultured Ehrlich ascites cells (line ELT) derived from a mouse mammary gland carcinoma and transplanted into C57BL mouse peritoneal cavity.

The cells were cultured in a liquid nutrient (MEM Gibco medium 90% complemented with 10% fetal calf serum) and 100 units/ml penicillin. The agent to be tested was added to the culture medium at various concentrations and for a maximum of 48 h.

After the treatment, the cells were fixed and stained by Feulgen reaction for cytological analysis (light microscopy). Mitotic activity or degree of cell death was expressed as mitotic or pycnotic index (number of cells in mitosis of pycnosis for 1000 cells). In each case, 5—10,000 cells were analysed. The percentages of mitotic phases were calculated in view of detecting eventual mitotic disturbances. Mean index was compared with Student *t*-test.

Antitumor activity tests in vivo

C57BL/6J male mice were injected i.p. with 10^6 Ehrlich ascites cells. The tumor was allowed to grow for 4 days and at that time, the mice received strychnopentamine i.p. either in a single shot or in repeated injections at daily intervals for 3 days. Various dosages of the drug were used. Mice were killed by exsanguination. Peritoneal washings were performed to remove all tumor cells and the cell count was done with a hemocytometer or Coulter counter model B. Tests were controlled each time on 10 mice.

Results

Antimitotic activity in vitro

Strychnopentamine was tested on three tumors and the results are shown in Table 1.

After 24 h ED_{50} was about 3 μg/ml in hepatoma and ascites Ehrlich cells.

TABLE 1

CYTOTOXIC ACTIVITY OF STRYCHNOPENTAMINE

Treatment (μg/ml)	After 24 h				After 48 h			
	Mitotic index (‰)	*P*	Pycnotic cell index (‰)	*P*	Mitotic index (‰)	*P*	Pycnotic cell index (‰)	*P*
Hepatoma HW165								
Control	37.8	—	14.6	—	11.0	—	37.2	—
1	23.7	99.76	18.6	84.90	3.8	99.68	77.4	>99.9
3	17.6	>99.95	26.0	99.75	1.9	>99.95	154.0	>99.9
5	15.0	>99.95	63.0	>99.95	3.3	99.69	203.8	>99.9
10	0[a]	—	—	—	—[a]	—	—	—
Ascites Ehrlich cells								
Control	11.28	—	—[c]	—	4.3	—	—[c]	—
1	29.5[b]	>99.95	—	—	6.5	92.6	—	—
3	5.2	>99.95	—	—	—[a]	—	—	—
5	—[a]	—	—	—	—	—	—	—
B16 melanoma								
Control	8.2	—	41.6	—	—	—	—	—
1	4.9	99.1	247.7	>99.95	—	—	—	—
3	0	—	750.0	—	—	—	—	—
5	—[a]	—	—	—	—	—	—	—

[a]No cells left on the lamella.
[b]There were 63.4% of cells in metaphase in treated (1 μg/ml) for 31.25% in controls.
[c]Pycnotic index was not increased to any great extent.

TABLE 2

COMPARISON OF ANTIMITOTIC ACTIVITY

	ED_{50}		
	18,19-Dihydro-usambarine	Strychnofoline	Strychnopentamine
B16 melanoma			
After 24 h	—	—	1
After 72 h	>10	>10	—
	<50	<50	—
Ascites Ehrlich cells			
After 24 h	10	10	3
After 48 h	—	—	3[a]
HW165 hepatoma			
After 24 h	—	—	3
After 48 h	—	—	1
After 72 h	—	>10	—
		<50	—

[a]At 3 μg/ml no cells left on lamella.

A stronger activity was observed in B16 melanoma ($ED_{50} \simeq 1$ μg/ml). After 48 h, ED_{50} was less than 1 μg/ml in hepatoma and of course in the other tumors. We noted that the antimitotic activity generally depends on exposure time and the concentration used.

Antitumor activity in vivo

Considering the antimitotic activity as observed in cultured tumor cells, we have undertaken preliminary experiments to search for a possible antitumor activity of this molecule in the animal. Our experiments have been carried out on Ehrlich ascites tumor cells in male mice.

When mice received strychnopentamine at the dose of 400 μg in repeated injections at daily intervals for 3 days, there was no significant difference with control mice. When they received strychnopentamine in a single injection of 1 mg, there was a weak decrease in cell number (average in controls, 450×10^6 cells; average in treated, 290×10^6 cells).

At a single dose of 2 mg; a very strong decrease in tumor cell number was observed (controls, 407×10^6 cells, treated, 87×10^6). Unfortunately this dose is toxic, since four mice out of ten died very quickly after the injection.

Discussion

Strychnopentamine is about ten times more powerful than strychnofoline

and 18,19-dihydro-usambarine as an antimitotic agent in animal tumor cell cultures, since it inhibits 50% of the cells in mitosis at a concentration of 1.8 μM. The comparison of their molecular structures induces us to think that the presence of a *N*-methyl-pyrrolidine group increases the antimitotic activity of alkaloids having an usambarane skeleton (Table 2).

The in vivo experiments represent a first indication in favor of further studies of this type of alkaloid as antitumor agents. Indeed strychnopentamine exhibits in vivo activity against Ehrlich ascities tumor in mice. The very rapid regression of the ascites 24 h after treatment by a single i.p. dose of 2 mg is obvious, but mice, given strychnopentamine at that dose, rapidly show signs of intoxication.

In the future, similar tests will also be carried out with isomers of strychnopentamine and other derived alkaloids at various dosages in view of establishing a closer structure-activity relationship, and to look for less toxic compounds.

Acknowledgments

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