Table 2 Inhibitory activity (%) ± S on 5-LO.

Compound	Concentration (50 µM)
Cardanol fraction	41.4 ± 3.1
Cardanol 15:3	44.6 ± 8.9
Cardanol 15:2	46.9 ± 10.7
Cardanol 15:1	40.9 ± 11.6
Cardanol 15:0	44.1 ± 2.3
Grevillol	31.1 ± 5.5
Cardol fraction	90.0 ± 1.9
Cardol 15:3	90.5 ± 8.4
Cardol 15:2	66.2 ± 8.9

5-Lipoxygenase assay (8) was performed using intact porcine leukocytes (1.5×10^7 cells/ml), which were incubated with 15 µM Ionophor A 23187, 2 mM $CaCl_2$, 10 µM ETYA, and 0.1 µCi 1-[^{14}C]-arachidonic acid for 5 min at 37 °C. Arachidonic acid and its metabolites were separated and determined by reversed-phase-HPLC (8). Each experiment was performed with three different preparations of porcine leukocytes.

Table 3 Inhibitory activity (%) on CO.

Compound	Concentration (µM) 10.0	5.0	2.5	1.0
Cardanol fraction	39.6	—	—	—
Cardanol 15:3	63.4	24.9	—	—
Cardanol 15:2	50.8	25.4	—	—
Cardanol 15:1	30.9	17.9	—	—
Cardanol 15:0	19.3	—	—	—
Grevillol	90.4	54.2	30.3	—
Cardol fraction	90.2	—	—	—
Cardol 15:3	98.1	93.7	45.5	18.5
Cardol 15:2	95.3	91.6	40.6	25.9
Cardol 15:1	98.8	91.7	30.3	12.1
Cardol 15:0	9.3	—	—	—
4-Methyl-5-*n*-pentadecylresorcinol	84.7	49.9	—	—

Sheep seminal vesicles microsomes were isolated as described (9), and suspended in KH_2PO_4 buffer (pH 7.4) before storing at −70 °C in deviation from former experiments. After 5 min preincubation at 20 °C with the inhibitor, 5.3 µg of microsomal protein were incubated for 20 min at 37 °C with 1 µM 1-[^{14}C]-arachidonic acid, 1 mM 1-epinephrine bitartrate, 1 mM GSH, and 0.05 mM Na_2EDTA in 2 ml of Tris-HCl buffer (pH 8.0).

Acknowledgements

We thank Miss R. Mayer and S. Vollmer for excellent technical assistance.

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Cytotoxicity on Human Leukemic and Rat Hepatoma Cell Lines of Alkaloid Extracts of *Psychotria forsteriana*

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Received: January 23, 1989

Psychotria forsteriana A. Gray is a tropical plant endemic in the Vaté Islands. An alkaloidal extract of the leaves has been shown to be highly cytotoxic on cultured rat hepatoma cells (HTC). The isolation of its alkaloids have been reported. They are of the polyindolinic type and result from the condensation of several N_b-methyltryptamine units. These alkaloids were also cytotoxic on rat hepatoma HTC and mice leukemic L_{1210} cell lines (1, 2).

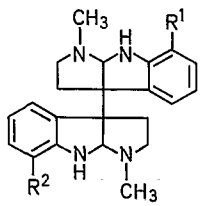
As a part of new developments on the pharmacognostical study of this genus, it seemed of interest to complete the study of the cytotoxic properties of this species by testing the biological activity of its different organs on other tumor systems. Alkaloid extracts of the different organs i.e. leaves (PFF), rootbark (PFER), stem bark (PFET) and fruits (PFFr) were tested on human leukemic ($Molt_4$) and rat hepatoma (FAZA) cell lines. Moreover, some of the isolated alkaloids from these organs were also tested.

Cell growth or survival was measured through the incorporation of ^3H-thymidine (^{3}HT). Thymidine being a precursor of the cells DNA, the amount of incorporated ^{3}HT is a function of the cell multiplication. These are the first evaluations on these types of compounds by this method.

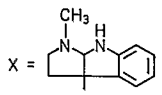
The percentage of incorporation of thymidine by the $Molt_4$ cells in the presence of increasing concentrations of the different alkaloid extracts clearly shows that they all exhibit cytotoxic properties comparable to the toxicity found with the leaves' extract (PFF). Doses above 10 µg/ml giving 100% cellular mortality; the extracts are still very active at doses of 2 µg/ml. PFER (root bark extract) seems to be the least active at 2 µg/ml with 30% of residual cells. These extracts seem unequally toxic to the cells. The difference in activity being probably related to differences in the amount, nature, and structure of their alkaloidal content.

Microscopic observation of the FAZA cells after GIEMSA coloration corroborates these results. The cytotoxic properties can qualitatively be appreciated and one can observe that the different extracts exhibit cytotoxic properties at doses of 10 µg/ml, with in most cases lysis of the cells.

The total alkaloid extracts were obtained as described in a previous communication (1). Comparison of the chromatographic profiles by TLC and HPLC showed some qualitative and quantitative differences in the alkaloidal content. Nevertheless, there seemed to be some common alkaloids between the extracts. Some major alkaloids from these extracts were isolated and identified on the basis of their spectral data in comparison with those found in the literature (1). These alkaloids, some of which are new compounds, are also of the polyindolinic type. They are polymers consisting of two to five N_b-methyltryptamine units (Fig. 1). Numerous possibilities of isomerism exist on account of the great number of asymmetric carbons in these structures. Their activities on cell viability was determined every 24 hours by counting the cells with a Neubauer microcytometer; the percentage of viability was measured as a function of culture time by the Trypan blue exclusion test as reported for HTC cells (2). It appears that these alkaloids produce variable effects as a function of their structure and dose. The most active in most cases are the ones with the higher relative molecular mass. Thus, some structural features such as the number of tryptamine units composing the molecules and consequently their relative molecular mass, the mode of polymerisation, structural and stereoisomerism, seem very important for the activities of these compounds. Moreover, the cytotoxic effects seem to interfere with DNA synthesis.



Alkaloid	R^1	R^2
chimonanthine	H	H
hodgkinsine	H	X
quadrigemine A	X	X
quadrigemine B	H	$(X)_2$
psychotridine	X	$(X)_2$
isopsychotridine	H	$(X)_3$



These preliminary results on the human cell line $Molt_4$ confirm the previously observed cytotoxic effects of polyindolinic alkaloids on rat and mice tumor cell lines.

$Molt_4$ cells seem to be much more sensitive than rat hepatoma HTC cells. Lower doses are needed to achieve 100% mortality. Moreover, $Molt_4$ cells are much more adapted to this technique in which cell growth is measured as a function of incorporation of ^3H-thymidine. This method is very convenient, rapid, more sensitive, and requires no particular skill. Both methods of evaluation i.e. classical microcytometer counting and ^3HT incorporation give similar responses. The activity on FAZA cells also confirms the cytotoxic properties of these substances. These alkaloids are cytotoxic *in vitro* in the micromolar range to both human leukemic and rat hepatoma tumor cell lines.

Moreover, their activities are a function of their structure and doses. More results on the stereochemistry of these compounds should allow us to establish some structure activity relationships. Further experiments in progress in our laboratory strongly support the evidence that these alkaloids can be considered as potent cytotoxic antitumoral agents *in vitro*.

Materials and Methods

$Molt_4$

Cytotoxicity tests were performed on human leukemic $Molt_4$ cells (established from the peripheral blood of a 19 year old male patient with acute lymphoblastic leukemia of T-cell origin) cultured in suspension in a RPMI 1640 medium supplemented with 10% V/V foetal calf serum (GIBCO). The assays were performed in multiwell plates: in each well were added successively the tested substance then the cells (2×10^5/ml). After 24 hours of incubation at 37 °C in CO_2 atmosphere, radioactive thymidine (^3HT) (0.5–1 μCi/well) AMERSHAM TRA 120, specific activity 5Ci/mM was added. After 40 minutes of incubation at 37 °C, the radioactivity was measured. The results are expressed as the percentage of incorporation of ^3H-thymidine in relation to the concentration of the test substance (μg/ml).

FAZA

The rat hepatoma cells (FAZA) are cultured in DMEM medium supplemented with 10% calf serum to form an adherent monocellular layer after 4 days of incubation at 37 °C. Test substances were added and the cells were incubated for 48 hours. The cells are observed after GIEMSA coloration under the microscope (× 300).

Extraction

Plant extracts were obtained through a classical acid base extraction procedure. The alkaloids were isolated by column chromatography and semi-preparative HPLC and were identified on the basis of their spectral data in comparison with those found in the literature (1).

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Planta Medica 55 (1989), 493–582, No. 6, December 1989

Planta Medica

Journal of Medicinal Plant Research

Reprint

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Rüdigerstraße 14 · D-7000 Stuttgart 30
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Thieme Medical Publishers Inc.
381 Park Avenue South, New York, NY 10016