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Phytochemical study and cytotoxic activity of alkaloids from *Uvaria chamae* P. Beauv.

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Phytochemical study of leaves of *Uvaria chamae* resulted in the isolation for the first time for the genus *Uvaria* of benzylisoquinoline alkaloids (+)-armepavine (**1**) and racem. O,O-dimethylcoclaurine (**2**). The aporphines nornantenine (**3**), nantenine (**4**) and corydine (**7**) are new for the species. The alkaloids were found to express cytotoxic activity against L 929 transformed cells. The highest activity was shown by **1**, **3**, and **5**. At a concentration corresponding to their IC_{50} against L929 cells, they were nontoxic against mouse thymocytes.

1. Introduction

The genus *Uvaria* L. (Annonaceae) is represented by more than 150 species, rich in isoquinoline alkaloids [1, 2]. It is widely distributed in tropical regions of Africa, Madagascar and Australia. *Uvaria chamae* P. Beauv. is a semi-shrub, distributed in dense humid forest of tropical Africa. There have been only limited chemical investigations of its alkaloid contents. Four aporphines and two proaporphines have been isolated from the species up to now [1].

Acetogenins with antibacterial and antihelmintic activities have been isolated *from U. hookeri* and *U. narum* [3], flavonoids with antimicrobial activity from *U. chamae* [4], and flavonoids with cytotoxic activity from *U. chamae* [5, 6]. In African traditional medicine *Uvaria* species are used as antimalarial remedies [7].

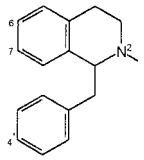
The present study describes the isolation and structural elucidation of seven isoquinoline alkaloids from leaves of *Uvaria chamae* grown in Guinea. These substances were tested for cytotoxic effect against mouse sarcoma cell line L 929 and mouse thymus-derived cells.

2. Investigations, results and discussion

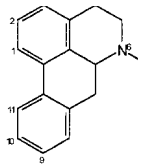
From an ethanol extract of the leaves of *Uvaria chamae*, non-alkaloid and alkaloid fractions were obtained. Seven known isoquinoline alkaloids were isolated from the alkaloid fraction by chromatographic procedures. The structures were elucidated by direct comparison of their UV, IR, ^{1}H NMR and mass spectral data with those of authentic samples. The main alkaloid is the benzylisoquinoline (+)-armepavine (**1**). The other alkaloid belonging to the same group is the racem. *O*,*O*-dimethylcoclaurine (**2**). The rest of the alkaloids – nornantenine (**3**), nantenine (**4**), asimilobine (**5**), thaliporphine (**6**) and corydine (**7**) are of the aporphine type.

To our knowledge, this is the first time benzylisoquinoline alkaloids have been isolated from the genus *Uvaria*. The alkaloids **3**, **4** and **7** are new for the species *Uvaria chamae*.

The alkaloids were tested for cytotoxic effect against mouse sarcoma cells L 929 and mouse thymocytes at a dose range from 25 to 500 μg/ml (approximately 1 to 20 mM). All the substances expressed lytic activity to L 929 cells (Table 1). The alkaloids **1**, **3** and **5** showed highest activity with IC_{50} values of 40, 28 and 30 μg/ml, respectively. The lowest activity was registered for **4**. The toxic effect of **1**, **3** and **5** was tested in cultures of mouse thymocytes. At a concentration above 100 μg/ml they dis-



	C-2	C-6	C-7	C-4′
1	CH_3	OCH_3	OCH_3	OH
2	H	OCH_3	OCH_3	OCH_3



	C-1	C-2	N-6	C-9	C-10	C-11
3	OCH_3	OCH_3	H	O-CH_2-O		
4	OCH_3	OCH_3	CH_3	O-CH_2-O		
5	OCH_3	OH	H			
6	OH	OCH_3	CH_3	OCH_3	OCH_3	
7	OH	OCH_3	CH_3		OCH_3	OCH_3

Table 1: Cytotoxic effect of *Uvaria* alkaloids on L 929 cells

Compd.	MW	IC_{50} μg/ml
1	313	40
2	313	150
3	325	28
4	339	300
5	267	30
6	341	70
7	341	80

Table 2: Influence of armepavine, nornantenine and asimilobine on the viability of mouse thymocytes

Compd.	Concentration	% Living cells	Percentage of control
1	50	72	80.0
	100	65	72.2
	500	62	68.8
3	50	87	96.6
	100	80	88.8
	500	65	72.2
5	50	90	100.0
	100	80	88.8
	500	61	67.7
Control	–	90	100

played moderate toxicity of approximately 20–30%. At a concentration of 50 μg/ml the number of living cells was similar to that of the control. It might be concluded that the alkaloids expressed a selective lytic effect towards transformed rather than normal immune cells. Their investigation in assays with another transformed and normal cells warrants attention.

3. Experimental

3.1. Instruments

IR spectra were obtained on a Specord 75 IR spectrometer and Bruker Vector 22 spectrometer. EI- and CI-MS were recorded on a Varian MAT 311A spectrometer. ^{1}H NMR spectra were recorded on a Bruker DRX 250 MHz spectrometer in $CDCl_3$ with TMS as internal standard. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. Neutral alumina 90 (act. II–III, 70–230 mesh, Merck) was used for CC, silica gel 60 PF_{254} for PTLC and aluminium sheets silica gel 60 F_{254} for TLC. The mobile phase was petroleum ether/$CHCl_3$/MeOH/Me_2CO (4 : 4 : 1 : 1). Compounds were visualized by spraying with Dragendorff's reagent.

3.2. Plant material

The leaves of *Uvaria chamae* P. Beauv. were collected in East Guinea in April 1988. The plant was identified by Prof. M. Kandé and a voucher specimen was deposited at the Botanical Department of the University in Kankan (Guinea).

3.3. Extraction and isolation

After they had been dried at 40 °C and ground, leaves (900 g) were extracted exhaustively with 95% EtOH at room temperature. The combined EtOH extracts were evaporated *in vacuo* to give 321 g of ethanol concentrate. This was then acidified with 5% HCl (pH 1–2) and left at room temperature for 48 h. The insoluble material was removed by filtration, giving the non-alkaloid fraction (138.6 g). The purified acidic solution was made alkaline with 25% NH_4OH (pH 9–10) and extracted four times with $CHCl_3$. The combined organic extracts were dried over anh. Na_2SO_4 and evaporated *in vacuo* to give 1.7 g (0.19%) of crude alkaloid fraction. By consecutive use of CC on alumina eluted with a hexane/Me_2CO/MeOH gradient and PTLC from the alkaloid mixture the alkaloids **1** (11.9 mg), **2** (2.8 mg), **3** (8.8 mg), **4** (5.2 mg), **5** (5.9 mg), **6** (4.8 mg) and **7** (3.4 mg) were isolated.

3.4. Cytotoxic assay

Mouse sarcoma fibroblasts L 929 were placed on 96-well microplates (Falcon) at a concentration of 3×10^4 cells/well in RPMI 1640 medium (Gibco), supplemented with 10% FCS (Sigma), 2 mM glutamine and 100 U/ml penicillin-100 μg/ml streptomycin and incubated for 18 h at 37 °C in humidified air (5% CO_2). 0.4 μg/ml of actinomycin D (Sigma) was then added together with 100 μl of various dilutions of the alkaloids (final concentration from 25 to 500 μg/ml). After 18 h of incubation the supernatants were discarded, the cells fixed with 5% formaldehyde in PBS and stained with crystal violet. When the plate was dry, 100 μl of 33% glacial acetic acid was added to each well and the plate was read at 620 nm in ELISA Reader 530 (Organon Teknika). Dose-response curves were drawn and the concentration giving 50% lysis of cells (IC_{50}) was determined.

A single cell suspension of mouse thymocytes obtained from inbred ICR mice was prepared in RPMI 1640 medium. At a concentration of 1×10^6/well it was incubated for 24 h with the alkaloids at final concentrations of 50, 100 and 500 μg/ml. The live cells were then counted under the microscope in the presence of trypan blue.

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