

Sara Hoet<sup>1,5</sup>  
Caroline Stévigny<sup>1,5</sup>  
Sébastien Block<sup>1</sup>  
Frederik Opperdoes<sup>2</sup>  
Pierre Colson<sup>3</sup>  
Brigitte Baldeyrou<sup>4</sup>  
Amélie Lansiaux<sup>4</sup>  
Christian Bailly<sup>4</sup>  
Joëlle Quetin-Leclercq<sup>1</sup>

## Alkaloids from *Cassytha filiformis* and Related Aporphines: Antitrypanosomal Activity, Cytotoxicity, and Interaction with DNA and Topoisomerases

### Abstract

*Cassytha filiformis* (Lauraceae), a widely distributed parasitic plant, contains several aporphine alkaloids and is often used in African folk medicine to treat cancer, African trypanosomiasis and other diseases. In a previous investigation, we showed that the alkaloid plant extract and the isolated aporphines possessed *in vitro* cytotoxic properties. In this paper, we evaluated the *in vitro* activity of the alkaloid extract ( $IC_{50} = 2.2 \mu\text{g/mL}$ ) and its three major aporphine alkaloids (actinodaphnine, cassythine, and dicentrine) on *Trypanosoma brucei brucei* as well as four related commercially available aporphines (bulbocapnine, glaucine, isocorydine, boldine). Only the three alkaloids from *Cassytha filiformis* were active on the trypanosomes *in vitro* ( $IC_{50} = 3-15 \mu\text{M}$ ). Additionally, we compared the cytotoxicity of these seven compounds on HeLa cells. Glaucine was the most

cytotoxic compound on HeLa cells ( $IC_{50} = 8.2 \mu\text{M}$ ) in the series. In order to elucidate their mechanism of action, the binding mode of these molecules to DNA was studied by UV absorption, circular and linear dichroism spectroscopy. The results of the optical measurements indicated that all seven aporphines effectively bind to DNA and behave as typical intercalating agents. Biochemical experiments showed that actinodaphnine, cassythine and dicentrine also interfere with the catalytic activity of topoisomerases in contrast to the four other aporphines. These interactions with DNA may explain, at least in part, the effects observed on cancer cells and on trypanosomes.

### Key words

Aporphine alkaloids · DNA interaction · *Cassytha filiformis* · Lauraceae · topoisomerase inhibition · antitrypanosomal activity · cytotoxicity

### Introduction

Over the past two decades, aporphine alkaloids have been extensively investigated as pharmacological tools and, in some cases,

as potential anticancer, antiviral, and antiparasitic agents [1], [2]. In this context, our previous investigation [3] has led to the isolation of aporphine alkaloids from *Cassytha filiformis* (Lauraceae), a sprawling parasitic herb widely used in African tradition-

### Affiliation

<sup>1</sup> Laboratoire de Pharmacognosie, Unité d'Analyse Chimique et Physico-Chimique des Médicaments, Université Catholique de Louvain, Bruxelles, Belgium

<sup>2</sup> Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Catholic University of Louvain, Brussels, Belgium

<sup>3</sup> Biospectroscopy and Physical Chemistry Unit, Department of Chemistry and Natural and Synthetic Drugs Research Centre, University of Liège, Liège, Belgium

<sup>4</sup> INSERM U-524 et Laboratoire de Pharmacologie Antitumorale du Centre Oscar Lambret, IRCL, Lille, France

<sup>5</sup> These authors contributed equally to this study

### Correspondence

Prof. J. Quetin-Leclercq · Laboratoire de Pharmacognosie · Unité CHAM · UCL 72.30 · Avenue E. Mounier 72 · 1200 Brussels · Belgium · Fax: +32-2-764-72-53 · E-mail: leclercq@cham.ucl.ac.be

### Funding

This research was supported by the Belgian National Fund for Scientific Research (fellowship to S.H.), by a grant from the "Fonds Spécial de Recherche" of the Catholic University of Louvain (fellowship to S.B.) and the Ligue Nationale Contre le Cancer (grant to C.B.). Support by the "Actions Intégrées Franco-Belges" Programme Tournesol is acknowledged (to C.B. and P.C.).

Received October 27, 2003 · Accepted February 7, 2004

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Planta Med 2004; 70: 407-413 · © Georg Thieme Verlag KG Stuttgart · New York  
DOI 10.1055/s-2004-818967  
ISSN 0032-0943

al medicine to fight, among others, parasites such as trypanosomes [4] and to treat cancers [5]. We showed that the alkaloid fraction and aporphines isolated therefrom possessed cytotoxic properties against cancer and non-cancer cell lines *in vitro* [3]. As *C. filiformis* is traditionally used to treat African trypanosomiasis, a disease for which appropriate and affordable treatment is scarce, we decided to analyze the *in vitro* effect of a crude alkaloid extract and isolated compounds on *Trypanosoma brucei brucei*, one of the causative agents of this disease.

Woo et al. [6] have suggested that dicentrine behaves as a DNA targeted "adaptative" intercalating agent. This consideration prompted us to investigate the interaction of several aporphine alkaloids with DNA and potential inhibition of the DNA-associated enzymes topoisomerases I and II, which both serve to resolve constraints in the genome. Topoisomerases have been proposed as potential targets for related aporphines [6], [7].

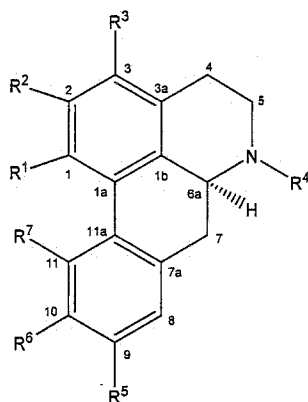
In this study, we compared the effects of three alkaloids isolated from *C. filiformis* (5–7) and four commercially available alkaloids (1–4) structurally related to the *Cassytha* aporphines (Fig. 1).

## Materials and Methods

### Drugs, chemicals and biochemicals

Three *S*-aporphine alkaloids (5–7) were isolated and purified from a crude alkaloid extract of *Cassytha filiformis* L. as previously described [3].

Four *S*-aporphine alkaloids (1–4) were purchased: bulbocapnine·HCl and isocorydine·HCl were obtained from ICN (Aurora, OH, USA), boldine from Federa (Brussels, Belgium) and glauci-



Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>
1 Bulbocapnine	-O-CH <sub>2</sub> -O-		H	CH <sub>3</sub>	H	O-CH <sub>3</sub>	OH
2 Isocorydine	O-CH <sub>3</sub>	O-CH <sub>3</sub>	H	CH <sub>3</sub>	H	O-CH <sub>3</sub>	OH
3 Boldine	O-CH <sub>3</sub>	OH	H	CH <sub>3</sub>	OH	O-CH <sub>3</sub>	H
4 Glaucine	O-CH <sub>3</sub>	O-CH <sub>3</sub>	H	CH <sub>3</sub>	O-CH <sub>3</sub>	O-CH <sub>3</sub>	H
5 Actinodaphnine	-O-CH <sub>2</sub> -O-		H	H	OH	O-CH <sub>3</sub>	H
6 Dicentrine	-O-CH <sub>2</sub> -O-		H	CH <sub>3</sub>	O-CH <sub>3</sub>	O-CH <sub>3</sub>	H
7 Cassythine	-O-CH <sub>2</sub> -O-	O-CH <sub>3</sub>		H	OH	O-CH <sub>3</sub>	H

Fig. 1 Structure of the studied compounds 1–7.

ne·HCl from Sequoia Research (Oxford, United Kingdom). A 5–10 mM stock solution was prepared in dimethyl sulfoxide (DMSO) for each compound. Stock solutions were kept at –20 °C and freshly diluted to the desired concentrations immediately prior to use with the suitable culture medium or buffer.

Calf thymus DNA (CT-DNA) and the double-stranded alternating polymer, poly(dA-dT)<sub>2</sub> were purchased from Sigma Chemical Co. (La Verpilliere, France). CT-DNA was deproteinized with sodium dodecyl sulfate (SDS) and dialysed against 1 mM sodium cacodylate buffered solution, pH 6.5. Concentrations were determined by applying a molar extinction coefficient of 6600 M<sup>-1</sup> cm<sup>-1</sup> for both polymers. All other chemicals were analytical grade.

### *In vitro* test for antitrypanosomal activity

*Trypanosoma brucei brucei* (*T. b. brucei*) (strain 427) bloodstream forms were cultivated *in vitro* in a modified Iscove's medium containing 10% heat-inactivated fetal calf serum and bloodstream form supporting factors: 0.05 mM bathocuproine sulfonate, 1.5 mM L-cysteine, 1 mM hypoxanthine, 0.2 mM 2-mercaptoethanol, 1 mM sodium pyruvate, 0.16 mM thymidine known as HMI 9. Fifty microliters of HMI-9 were added to each well of a 96-well plate. Three-fold serial dilutions in duplicate were performed and 50 μL of an axenic culture of *T. b. brucei* containing 3000 bloodstream forms were added to each well. The plate was incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Control wells without compounds were included as well as control wells with DMSO. Background fluorescence of the compound-containing medium was determined for each concentration. The highest concentration of DMSO after serial dilutions with complete culture medium was 0.3%. After 68 hours of incubation, the fluorochrome Alamar Blue™ (Immunesource, Halle-Zoersel, Belgium) was added into each well and the fluorescence was quantified after a total incubation time of 72 h with a fluorescence plate reader (Millipore Cytofluor 2300) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm [8]. Fluorescence development was expressed as percentage of the control, considered as 100%, and IC<sub>50</sub> values (concentration of compound that reduced fluorescence intensity by 50%) were calculated by linear interpolation selecting values above and below the 50% mark according to Hills et al. [9]. The antitrypanosomal drugs, diminazene aceturate (Berenil®, Hoechst AG) and suramin (Sigma), were used as positive controls [8]. All experiments were performed at least in triplicate.

### Cytotoxicity assay

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum and penicillin (100 UI/mL). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. The effects of the alkaloids were evaluated using the tetrazolium salt MTT (Sigma) colorimetric method based on the reduction of the reagent by dehydrogenases in viable cells [10]. Briefly, 5000 cells/well were seeded in 100 μL of medium in 96-well plates. After 24 h, 100 μL of fresh medium containing various compound concentrations were added to each well while control wells received fresh medium containing analogous DMSO concentrations. Each concentration was tested in at least 7 wells. After 72 hours of incubation, the medium was replaced by 100 μL DMEM (without serum) containing 10 μL of MTT solution (3 mg/mL in PBS). After 45 min-

utes in the incubator, the medium containing MTT was removed and 100  $\mu\text{L}$  of DMSO were added to each well. Afterwards the plates were shaken and the absorbance was measured at 570 nm and 620 nm against a background control as blank (DMSO) on a microplate reader. The relative absorbance was expressed as a percentage of the control cells considered as 100%. Camptothecin (ICN) was used as a positive control [11]. The results are expressed by  $\text{IC}_{50}$  values (concentration of compound causing 50% inhibition of cell growth) calculated from graphs using at least five different concentrations of each alkaloid. All experiments were made at least in triplicate.

#### Absorption spectra and melting temperature studies

Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of  $T_m$  measurements, 6 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm path length) were heated by circulating water. The measurements were performed in BPE buffer pH 7.1 (6 mM  $\text{Na}_2\text{HPO}_4$ , 2 mM  $\text{NaH}_2\text{PO}_4$ , 1 mM EDTA) at an alkaloid concentration of 20  $\mu\text{M}$  together with CT-DNA or poly(dA-dT)<sub>2</sub> at 20  $\mu\text{M}$  [DNA-phosphate/drug ratio (P/D) = 1]. The temperature inside the cuvette was measured with a platinum probe; it was increased over the range of 35–100 °C for poly(dA-dT)<sub>2</sub> and over the range of 45–100 °C for CT-DNA, with a heating rate of 1 °C/min. The "melting" temperature  $T_m$  was taken as the mid-point of the hyperchromic transition.

The Uvikon 943 spectrophotometer was also used to record the absorption spectra. Absorption spectra measurements were performed in 1 mM sodium cacodylate buffer (pH 6.5) at a compound concentration of 20  $\mu\text{M}$  together with the CT-DNA at 200  $\mu\text{M}$  [DNA-phosphate/drug ratio (P/D) = 10]. A DNA blank at the same nucleotide concentration was performed concomitantly and used as a reference in the recording of absorption spectra.

#### Electric linear dichroism (ELD)

ELD measurements were performed with a computerized optical measurement system using the procedures previously outlined [12]. All experiments were conducted with a 10 mm path length Kerr cell having 1.5 mm electrode separation. The samples were oriented under an electric field strength varying from 1 to 14 kV/cm. Each alkaloid studied was present together with the DNA at a DNA-phosphate/drug ratio (P/D) = 20. This electro-optical method is a powerful optical tool to define the orientation of a ligand with respect to the DNA helix axis. In these experiments, the DNA is oriented by an electric field and the orientation of the molecules bound to DNA is probed using a linearly polarized light. ELD has the advantage that it senses only the orientation of the polymer-bound ligand: free ligand is isotropic and does not contribute to the signal [12].

#### Circular dichroism (CD)

CD measurements were recorded on a Jobin-Yvon CD6 dichrograph. The same solutions as in the ELD experiments were used. Solutions of alkaloids and/or nucleic acids in 1 mM sodium cacodylate buffer pH 6.5 were scanned using a 1 cm path length cell. Three scans were accumulated and automatically averaged.

#### Topoisomerase-mediated DNA relaxation experiments

Topoisomerase I and II-mediated DNA relaxation assays were performed as described earlier [13]. Recombinant topoisomerase I protein was purified from baculovirus infected Sf9 cells according to a published procedure [14]. Some experiments were also performed with commercially available enzymes (topoisomerases I and II from TopoGen Inc.). For the topoisomerase I assays, supercoiled pLAZ3 DNA (130 ng) was incubated with 4 units topoisomerase I at 37 °C for 45 min in relaxation buffer (100 mM Tris-HCl, pH 7.9, 10 mM EDTA, 1.5 mM NaCl, 1% BSA, 1 mM spermidine, 50% glycerol) in the presence of varying concentrations of the compound under study. Camptothecin (Sigma) was used as a positive control.

For the topoisomerase II assays, supercoiled pLAZ3 DNA (130 ng) was incubated with 4 units human topoisomerase II at 30 °C for 30 min in relaxation buffer (50 mM Tris-HCl, pH 8.0, 120 mM KCl, 10 mM  $\text{MgCl}_2$ , 0.5 mM dithiothreitol, 0.5 mM ATP, 30  $\mu\text{g}/\text{mL}$  BSA) in the presence of varying concentrations of the compound under study. Etoposide (Sigma) was used as a positive control.

For both assays, reactions were terminated by adding SDS to 0.25% and proteinase K to 250  $\mu\text{g}/\text{mL}$ . DNA samples were then added to the electrophoresis dye mixture (5  $\mu\text{L}$ ) and electrophoresed at room temperature in 1% agarose gels. Gels were stained with ethidium bromide (1  $\mu\text{g}/\text{mL}$ ), washed, and photographed under UV light. Similar experiments were performed using ethidium-containing agarose gels.

## Results and Discussion

#### *In vitro* antitrypanosomal activity and cytotoxicity

Because of the use of *C. filiformis* in the traditional treatment of African trypanosomiasis, we analyzed the effects of a crude alkaloid extract on bloodstream forms of *T. b. brucei* *in vitro*. This extract revealed a significant antitrypanosomal activity ( $\text{IC}_{50}$  = 2.2  $\mu\text{g}/\text{mL}$ ) and a selectivity towards the parasite versus cancer cells ( $\text{IC}_{50}$  on HeLa cells = 35.2  $\mu\text{g}/\text{mL}$ ).

The major aporphines of this extract were then purified and tested for their *in vitro* antitrypanosomal activity as well as other aporphines commercially available (Table 1). The three alkaloids of *C. filiformis* (5–7) showed an  $\text{IC}_{50}$  on *T. b. brucei* of 3 to 15  $\mu\text{M}$ . Bulbocapnine (1), isocorydine (2), boldine (3) and glaucine (4) were inactive in our test. In a previous study, bulbocapnine showed no *in vivo* activity in mice infected with *T. b. brucei* [15].

As selectivity against trypanosomes was observed with the extract from *Cassytha filiformis*, we decided to compare under identical conditions the cytotoxicity of the 7 aporphines selected in this study. A cell growth inhibition assay using the human HeLa cancer cell line was employed. The cytotoxicity of all these molecules has already been reported in the literature but the cell line and the test procedure significantly varied making comparison difficult [1], [3], [6], [16]. Under our experimental conditions (3 days of continuous exposure), the three aporphines bulbocapnine (1), isocorydine (2), boldine (3) were found to be inactive (Table 1). The three alkaloids of *C. filiformis* (5–7) showed cyto-

Table 1 Antitrypanosomal activity and cytotoxicity of alkaloids 1–7

Compound	IC <sub>50</sub> (μM) <sup>a</sup>	
	<i>T. b. brucei</i> <sup>b</sup>	HeLa <sup>c</sup>
Bulbocapnine (1)	> 25	> 80
Isocorydine (2)	> 25	> 80
Boldine (3)	> 25	> 80
Glaucine (4)	> 25	8.2
Actinodaphnine (5)	3.2	15.0
Dicentrine (6)	14.6	35
Cassythine (7)	6.0	15.2
Camptothecin	nd <sup>d</sup>	0.4
Suramin	0.06	nd
Diminazene aceturate	0.02	nd

<sup>a</sup> Values indicate 50% inhibitory concentrations (IC<sub>50</sub>) in μM.

<sup>b</sup> *Trypanosoma brucei* bloodstream forms.

<sup>c</sup> Human cervix carcinoma cells.

<sup>d</sup> Not determined (nd).

toxic activity ranging from 15 to 35 μM and glaucine (4) was the most active compound in the series (IC<sub>50</sub> = 8.2 μM). The three *Cassytha* alkaloids (5–7) displayed a significant *in vitro* antitrypanosomal activity, especially actinodaphnine (5), but with a lower selectivity [selectivity index, IC<sub>50</sub> (HeLa)/IC<sub>50</sub> (*T. b. brucei*) < 5) than the alkaloid fraction (selectivity index = 16). It is also interesting to note that glaucine was cytotoxic to HeLa cells but was inactive on the trypanosomes.

#### DNA interaction and binding mode

Absorption measurements were performed to estimate the interaction of the compounds with DNA. The absorption spectrum of

each alkaloid at 20 μM was recorded in the absence and presence of 200 μM calf thymus DNA. Two typical absorption spectra obtained with actinodaphnine (5) and isocorydine (2) are shown in Fig. 2. Binding of actinodaphnine to DNA induced significant hypochromic effect and bathochromic shift in the absorption band of the aporphine chromophore centered at 305 nm, reflecting the interaction between the electronic states of the chromophore and the DNA bases. In contrast, the spectrum of isocorydine was only slightly affected in the presence of DNA. Fig. 3 shows the hypochromic effect (expressed in percentage) and bathochromic shift observed for each tested compound. This indicates that the three alkaloids isolated from *C. filiformis* (5–7) and bulbocapnine (1) have a stronger interaction with DNA than the other compounds, as can be concluded from both the higher hypochromic effect (30–40%) and bathochromic shift (> 4 nm) of the absorption band of the aporphine chromophore.

Melting temperature (*T*<sub>m</sub>) studies were performed with CT-DNA and the alternating polynucleotide poly(dA-dT)<sub>2</sub>. The difference in *T*<sub>m</sub> values between the drug-DNA complexes and free DNA or polynucleotide in solution provides a useful means to evaluate qualitatively the interaction of the molecules with double stranded DNA. The results of these *T*<sub>m</sub> measurements, carried out in BPE buffer at a DNA-phosphate/drug (P/D) ratio of 1 are presented in Fig. 4. Here again, the three alkaloids of *C. filiformis* (5–7) stabilized duplex DNA against heat denaturation. As expected, the stabilization due to these three compounds is significantly higher for the alternating poly(dA-dT)<sub>2</sub> polymer than for CT-DNA due to the lower intrinsic *T*<sub>m</sub> of the synthetic polymer compared to the naturally occurring DNA from CT which contains 42% GC base pairs and therefore melts at a higher temperature than poly(dA-dT)<sub>2</sub> [*T*<sub>m</sub> of 42 °C and 61 °C for poly(dA-dT)<sub>2</sub> and CT-DNA, respectively]. In this assay, bulbocapnine did not stabi-

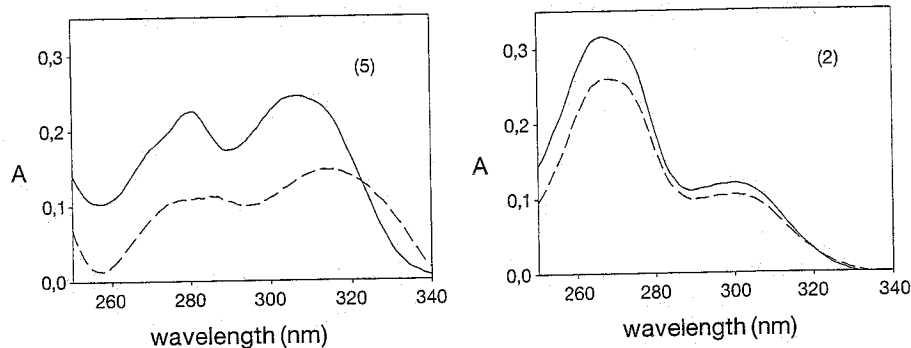


Fig. 2 Absorption spectra of actinodaphnine (5) and isocorydine (2) in the absence (solid line) and in the presence (dashed line) of calf thymus DNA at a DNA-phosphate/drug ratio (P/D) of 10. The absorption measurements were performed in 1 mM sodium cacodylate buffer at pH 6.5

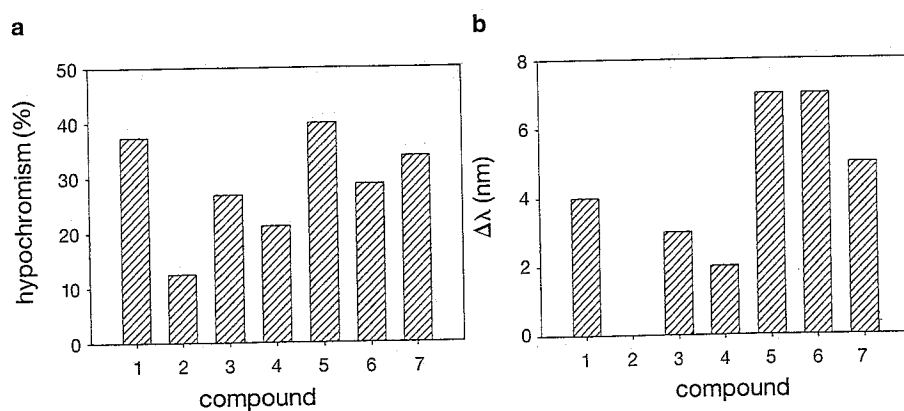


Fig. 3 Hypochromism percentage (a) and bathochromic effect ( $\Delta\lambda$ ) (b) of the maximum of the ligand absorption band upon addition of calf thymus DNA to a solution of each compound. Same experimental conditions as Fig. 2.

lize significantly the DNA helix even though its UV spectrum was significantly modified upon addition of CT-DNA. Isocorydine (**2**) had no stabilizing effect against thermal denaturation either for poly(dA-dT)<sub>2</sub> or CT-DNA. These observations clearly illustrate that absorption measurements (at room or increasing temperatures) give information about the presence of a ligand-DNA interaction but do not allow us to specify their binding mode.

Two spectroscopic methods with polarized light were applied to define the DNA binding process for the studied aporphines. The ELD experiments provided direct information on the DNA intercalating properties of the tested compounds. For all 7 aporphines, the reduced dichroism  $\Delta A/A$  was negative in the alkaloid absorption band (300–312 nm) reflecting the orientation of the chromophore perpendicular to the DNA helix axis (or electric field direction). Fig. 5 shows the  $\Delta A/A$  values measured for each compound at a DNA-phosphate/drug (P/D) ratio of 20. For most compounds, the reduced dichroism was close to that measured with DNA alone at 280 nm (dashed line in Fig. 5). For four drug-DNA complexes [the three alkaloids isolated from *C. filiformis* (**5–7**) and bulbocapnine (**1**)], the  $\Delta A/A$  values were lower than

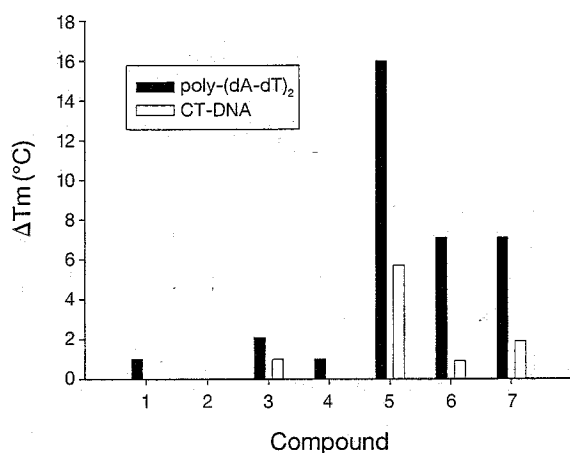


Fig. 4 Variation of the melting temperatures  $\Delta T_m$  ( $T_m^{\text{drug-DNA complex}} - T_m^{\text{DNA alone}}$ , in °C) of the complexes between DNA and the studied compounds.

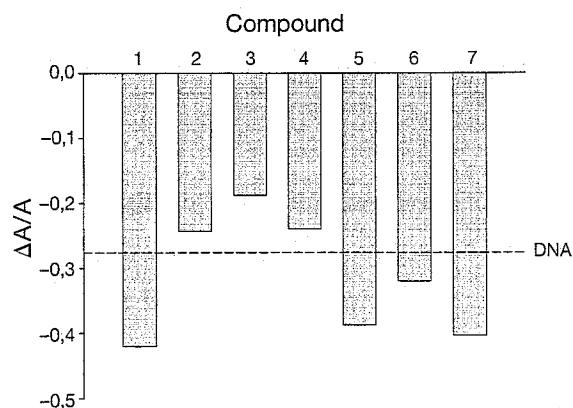


Fig. 5 Variation of the reduced electric linear dichroism ( $\Delta A/A$ ) of the complexes between calf thymus DNA and the tested alkaloids.  $\Delta A/A$  was measured in the alkaloid absorption band (300–312 nm) at 13.5 kV/cm and at a DNA-phosphate/drug ratio (P/D) of 20 in 1 mM sodium cacodylate buffer, pH 6.5. The dashed line marks the  $\Delta A/A$  value obtained for DNA alone at 280 nm (–0.276).

the value obtained with DNA alone. This likely reflects a drug-induced stiffening of the DNA target which increases the orientation of the polyanion molecules in the electric field.

All the CD spectra of the complexes between the aporphines and CT-DNA revealed weak amplitude variations compared to the spectra obtained with the molecule alone in the absorption band of this latter one (data not shown). This behavior is expected and often observed with intercalating molecules [17].

From the experiments using circularly and linearly polarized light, we concluded that the 7 aporphines are oriented parallel to the DNA base pairs, as expected for an intercalative binding mode. It was previously proposed that dicentrine could be an intercalator due to its capacity to unwind DNA [6]. However, aporphine alkaloids such as dicentrine lack the structural characteristics normally associated with conventional DNA intercalators such as the presence of two or three fused aromatic rings. They only have two aromatic rings separated by saturated rings making them non-planar molecules. In addition, these two aromatic rings are substituted with methylenedioxy and/or methoxy groups which may hinder access of the molecule to the intercalation sites. On the basis of molecular modeling studies, it was proposed that dicentrine was an “adaptive” intercalator, which undergoes a conformational change upon binding to DNA to adopt a strained planar conformation [6]. It is possible that the 7 aporphines included in this study use a similar DNA-controlled structural mechanism to intercalate between base pairs. However, even if all molecules intercalate into DNA, their affinity for the polymer and their ability to stabilize the DNA duplex against heat denaturation vary significantly from one aporphine to another.

#### Topoisomerase I and II inhibition

To analyze in greater detail the mode of action of these molecules, we evaluated their effects on the catalytic activity of topoisomerases I and II using a relaxation assay with supercoiled DNA.

Closed circular DNA was incubated with topoisomerase I in the absence and in the presence of the 7 aporphines (Fig. 6). The lanes marked TopoI show that supercoiled DNA was relaxed by the enzyme in the absence of the alkaloids. The gel shown in Fig. 6 indicates that the relaxation of DNA was already altered

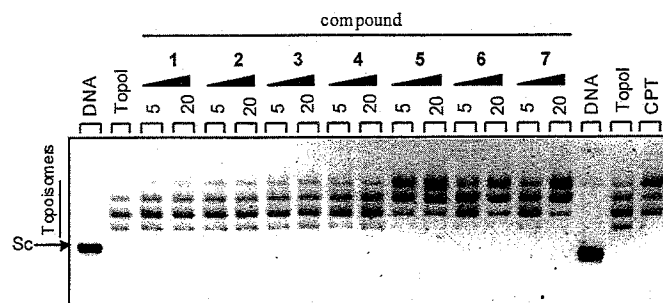


Fig. 6 Effect of the studied aporphines on the relaxation of plasmid DNA by topoisomerase I. Supercoiled pLAZ3 DNA (130 ng) (lane DNA) was incubated with 4 units topoisomerase I in the absence (lane TopoI) or presence of a given aporphine at the indicated concentration (5–20  $\mu\text{M}$ ). Camptothecin (CPT) was used at 20  $\mu\text{M}$ . Sc: supercoiled

by the three alkaloids isolated from *C. filiformis* at a concentration of 5  $\mu$ M, as observed with the reference topoisomerase I poison, camptothecin (CPT lane). No effect was observed with the four other compounds. The inhibition could be either specific, resulting from direct interference of the aporphine with the enzyme-DNA complex (stabilization of topoisomerase-DNA cleavable complexes) or non-specific, resulting from the intercalation of the drug into DNA, which can also prevent the relaxation of the plasmid by the enzyme. The two types of inhibition can be differentiated using gels containing, or not, ethidium bromide during the electrophoresis. Ethidium-prestained gels (not shown) indicated that none of the aporphines promoted DNA cleavage by topoisomerase I. In other words, the alkaloids did not stabilize topoisomerase I-DNA complexes, as typically observed with topoisomerase I poisons such as camptothecin [18]. So we can conclude that actinodaphnine, dicentrine and cassythine (5–7) exhibit a non-specific topoisomerase I inhibition, most likely due to intercalation into DNA.

Similar experiments performed with human topoisomerase II have revealed that none of the aporphines are poisons of this enzyme. In this case, topoisomerase II-mediated double stranded DNA cleavage was detected in the presence of etoposide, the positive control, but not with the different alkaloids studied here (data not shown). Altogether, the results are consistent with the literature data indicating that dicentrine and bulbocapnine do not stabilize DNA-topoisomerase II complexes [6]. A weak inhibition of topoisomerase II has been reported with the planar oxoaporphine alkaloid liriodenine [7] but not with non-planar analogues like those tested here. Similarly, it was reported that dicentrinone, but not dicentrine, can inhibit specifically topoisomerase I [19].

The tested aporphines, which all behave as typical intercalating agents, can be divided into two groups depending on the presence or the absence of a methylenedioxy substituent. It is known that this substituent plays a role in modulating the biological activities of aporphinoids [1]. The first group includes isocorydine (2), boldine (3), and glaucine (4) which all three lack the methylenedioxy group. They exhibit a weak interaction with DNA as indicated by the absorption and melting temperature experiments. They have no effect on the catalytic activity of topoisomerase I and display no *in vitro* antitrypanosomal activity or cytotoxicity (except for glaucine). The second group includes bulbocapnine (1), actinodaphnine (5), dicentrine (6) and cassythine (7). These four molecules, bearing a methylenedioxy group, exhibit a higher affinity for DNA. The three alkaloids of *C. filiformis* (5–7) efficiently stabilize the DNA double helix against heat denaturation. They are cytotoxic *in vitro* and inhibit non-specifically the topoisomerase I activity through DNA intercalation. They also display a marked *in vitro* antitrypanosomal activity. Bulbocapnine (1), however, did not possess any of these properties. To explain this lack of effect, we may invoke the presence of a hydroxy group at position R<sup>7</sup> adjacent to a methylenedioxy group at R<sup>1-2</sup> which may prevent the molecule from adopting a flat conformation apparently necessary to stabilize DNA against thermal denaturation and inhibit the topoisomerase I, as suggested by Woo et al. [6].

In conclusion, our study sheds light on the mechanism of action of the studied aporphine alkaloids. Their cytotoxicity is probably independent of the interaction with DNA and topoisomerases; other mechanisms must be involved, at least for glaucine (4) which is the most cytotoxic compound in the present series.

In contrast, the antitrypanosomal activity of these aporphines seems to be related to their ability to stabilize the DNA double helix against heat denaturation and to inhibit the catalytic activity of topoisomerase I. Trypanosomes, as well as other trypanosomatids, are characterized by a prominent network of interconnected circular DNA molecules present in a specialized region of their single mitochondrion, called the kinetoplast. Treatment of trypanosomatids with DNA intercalating drugs, such as acriflavin and ethidium bromide has been shown to lead to the loss of the kinetoplast DNA networks and the subsequent formation of so-called dyskinetoplastic strains [20], [21]. Such strains are viable in the case of *Trypanosoma equiperdum* and *T. evansi*, but not in the case of the other African trypanosomes. Therefore, such compounds, especially ethidium bromide, have been used as successful drugs against cattle trypanosomiasis in large parts of Africa. This loss of kinetoplast DNA networks could perhaps explain the activity of the DNA intercalating aporphines isolated from *Cassytha filiformis* on *T. b. brucei*.

#### Acknowledgements

The authors wish to thank M-C Fayt for her skillful technical assistance and Professor C. Houssier for fruitful discussions.

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