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Antimalarial activity of cedronin

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Abstract

Cedronin was isolated from *Simaba cedron* Planchon (Simaroubaceae), a species popularly believed in South America to have antimalarial properties. It was examined for in vitro and in vivo antimalarial activities and for cytotoxicity against KB cells. Experimental results showed that cedronin was active against chloroquine-sensitive and resistant strain, with an IC_{50} of 0.25 μg/ml (0.65 μmol/ml). It was also found to be active in vivo against *Plasmodium vinkei* with an IC_{50} of 1.8 mg/kg (4.7 nM/kg) in the classic 4-day test. Cedronin belongs to the small group of quassinoids with a C_{19} basic skeleton and shows a rather low cytotoxicity against KB cells (IC_{50} = 4 μg/ml, 10.4 μM) as compared with C_{20} biologically active quassinoids; however its toxic/therapeutic ratio (10/1.8) remains lower than chloroquine (10/0.5).

Keywords: Cedronin; Quassinoids; Antimalarial activity; *Simaba cedron*; Simaroubaceae

1. Introduction

Quassinoids show a large spectrum of biological activities, which include antitumor, antiviral, antiamoebic and antimalarial (Polonsky, 1985; Wright and Phillipson, 1990). Sergeolide (**4** in Fig. 1) is highly active against chloroquine-resistant strains of *Plasmodium falciparum* and reduce the virulence of experimentally-induced *P. berghei* infections in mice (Fandeur et al., 1987). Highly active quassinoids like bruceantin (**5**), sergeolide (**4**), and simalikalactone D, are also extremely toxic. However, recent research has shown that their antimalarial activity does not always parallel their relative cytotoxic activity. (Wright and Phillipson, 1990). Quassinoids, with high cytotoxic activity, possess a C_{20} picrasan basic skeleton. In our attempt to find new antimalarial products, we report here on the in vitro and in vivo antimalarial activities of cedronin (**1**), a C_{19} basic skeleton quassinoid previously isolated by Polonsky (1960), and more recently by Jacobs et al. (1987) from *Simaba cedron* Planchon, which has been reported to have antimalarial properties in Central and South America (Grenand et al., 1987). Lecointe (1922) claimed it to be more effective than quinine.

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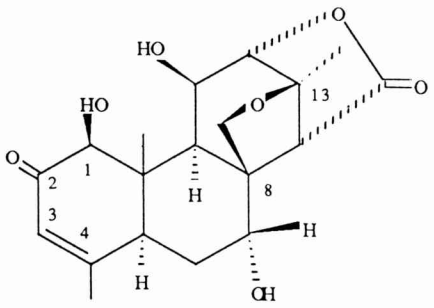
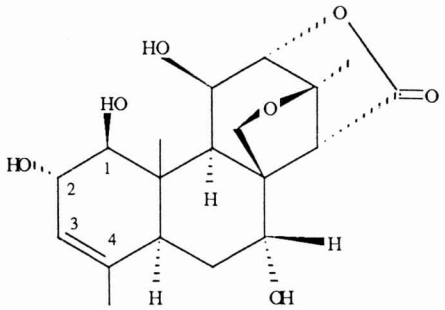
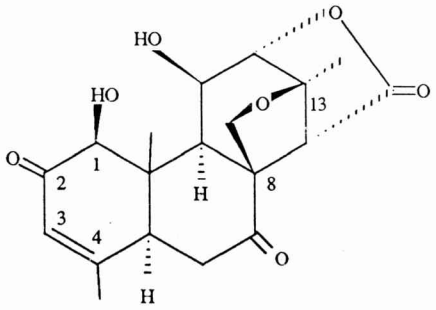


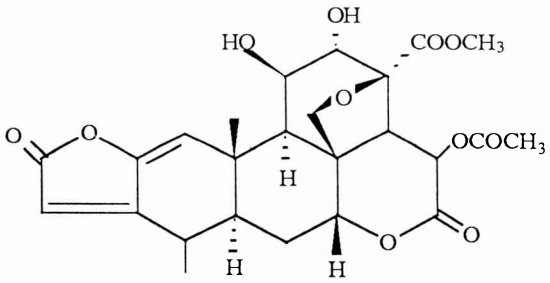
(1) Cedronin



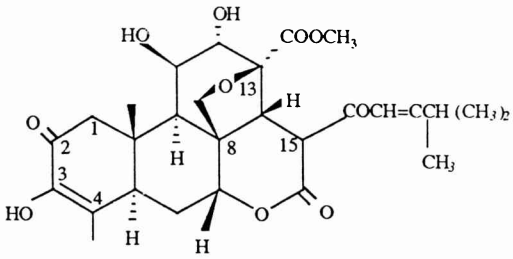
(2) Cedronolin



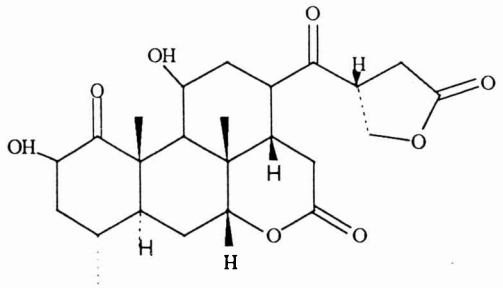
(3) Eurymolactone



(4) Sergeolide



(5) Bruceantin



(6) Simarolide

2. Material and methods

2.1. Extraction and isolation of quassinoids

Stem bark (8.4 kg) of *Simaba cedron* Planch. was collected in French Guyana. A voucher specimen (*Moretti 1020*) has been deposited in the ORSTOM herbarium of Cayenne. Quassinoids were isolated as pure compounds using the method already described (Fandeur et al., 1987). The major compound (1) was recrystallised in ethyl

acetate-methanol mixture, m.p. 240–241°, $[\alpha]_D$ +86 ($CHCl_3$; c0.5) (lit. +70°, Jacobs et al. (1987)). The UV spectrum of the more polar compound (**2**) did not reveal any significant absorption above 210 nm, which suggest a glycol substitution. Compounds (**1**) and (**2**) were identified as cedronin and cedronolin by their physical constants, and by comparing their spectral data (MS, ^1H and ^{13}C NMR) with literature data; their chemical structure were confirmed by complete interpretation of their NMR signals, using 2D NMR experiments.

2.2. In vitro antimalarial test

Two *Plasmodium falciparum* strains were used: FCC2 chloroquine sensitive and chloroquine resistant FZR8. Culture of parasites was carried out according to Trager and Jensen (1976), on glucose-enriched RPMI 1640 medium supplemented with HEPES and 10% human serum. The test procedure followed the method of Trager and Polonsky (1981). Giemsa-stained thin blood smears were examined under ×1000 magnification, and the percentage of parasited red blood (PRBC) cells was counted on at least 9000 red blood cells (RBC) observed for each concentration.

Percent growth inhibition of the parasite was calculated by the following formula:

$$\frac{\text{parasitemia in solvent} - \text{parasitemia with drugs}}{\text{parasitemia in solvent}} \times 100$$

2.3. In vivo antimalarial test

Quassinoids were directly dissolved in DMSO solution in order to obtain the final concentrations 1.2, 6.25, 12.5 mg/kg/day (3, 16, 33 nM/kg/day). Chloroquine was dissolved in saline. DMSO (Sigma Chemical Co) was used as control.

The in vivo antimalarial activity of the quassinoids was determined and compared with chloroquine by the classic 4-day suppressive test against *P. vinkei petteri*, 279 BY strain, in mice (Peters, 1980). The advantages of this *Plasmodium* species are: a biological cycle which is independent of the nycthemeral host cycle, synchroneous development in mice infected by frozen blood, and marked selectivity for mature erythrocytes (Carter and Walliker, 1975). Swiss male mice, mean body weight 20 ± 2 g, were infected with 10^8 parasitised cells in 0.9% saline on day 0. Groups of 10 mice were randomly treated i.p. from day 0 to day 3 with 100 ml containing increasing doses of cedronin (see above) or chloroquine (5 mg/kg/day). The suppressive effects of the different compounds were estimated on day 4 on Giemsa-stained thin blood films, by comparison with a group of 10 mice treated with DMSO solution.

Lethal dose (LD_{50}) of cedronin was determined using OF1 Swiss mice (Fandeur et al., 1987). Its IC_{50} value against KB human bucal carcinoma cells was determined by standard methods established at the National Cancer Insitute, currently used in ORSTOM laboratory (Rodriguez et al., 1992).

3. Results

Cedronin (**1**) showed in vitro antiplasmodial activity with an IC_{50} of 0.23 μg/ml (= 0.6 mM) against FCC2 chloroquine-sensitive strain, and with an IC_{50} of O.25 μg/ml against FZR8 resistant strains (Table 1). This quassinoid was also active in vivo with an ED_{50} value of 1.8 mg/kg/day, at a lower concentration than its lethal dose (LD_{50} = 10 mg/kg/day). It showed a cytotoxic activity against KB cells (IC_{50} = 4 μg/ml = 10.4 μM).

Cedronolin (**2**) did not demonstrate significant in vivo activity at the maximal tested dose (100 mg/kg/day), as expected for a quassinoid with a glycol substitution (Polonsky, 1985; Wright and Phillipson, 1990).

4. Discussion and conclusion

Cedronin (**1**) belongs to the few quassinoids with a C_{19} basic skeleton. Its in vitro antimalarial activity is similar to eurycomolactone (**3**) (IC_{50} = 0.21 μg/ml), another C_{19} quassinoid (Chan et al., 1986). Its IC_{50} values are similar for chloroquine-resistant and sensitive strains, suggesting that quassinoids may act upon the malaria parasite by means of a fundamentally different mechanism from chloroquine, as already presumed (Kirby et al., 1990). Quassinoids with other unusual structural skeletons were also investigated: the C_{25} quassinoid simarolide (**6**), previously iso-

Table 1
Antimalarial activity of cedronin (1) compared with chloroquine (CQ)

Concentration	Strains (in vitro)					
	FCC2			FZR8		
	Parasitemia	S.D.	Percent inhibition	Parasitemia	S.D.	Percent inhibition
10	0		100	0		100
5	0.1	0.05	98.2	0.2	0.03	95
2.5	0.3	0.1	94.4	0.4	0.05	90
1.25	0.75	0.11	86	0.88	0.09	78
0.62	1.42	0.24	73	1.09	0.9	73
0.31	1.82	0.23	66	1.57	0.11	61
0.15	3.94	0.26	26	2.93	0.27	27
0.07	5.17	0.07	2.5	4.1	0.21	
Control	5.3	0.26		4	0.22	
IC_{50} of (1)	0.23 μg/ml			0.25 μg/ml		
IC_{50} of CQ	0.04 μg/ml			0.38 μg/ml		
	Strain (in vivo)					
	Vinckei petteri					
12.5	0.94	0.61	96			
6.25	0.89	1.05	97			
3	9.57	2.62	63			
1.2	15.76	4.49	39			
Control	26.01	14.7				
IC_{50} of (1)	1.8 mg/kg					
IC_{50} of CQ	0.5 mg/kg					

S.D., standard deviation.

lated from *Simaba cf orinocensis* (Polonsky et al., 1981) was not active in vitro at the maximal tested dose (100 μg/ml). It is of interest to note that further examination of botanical material by taxonomic and chemical analysis of the quassinoid content led to the description of the new species *Simaba morettii* C. Feuillet (Feuillet, 1983).

Cedronin possesses some of the structural requirements for cytotoxic activities: an A-ring with unsaturated ketol at position 1 and 2, δ-lactone, and an oxide bridge between C-8 and C-15 (Polonsky, 1985); however it showed rather low cytotoxic activity compared with C_{20} biologically active quassinoids.

Nevertheless, we obtained a ratio of cytotoxicity to antimalarial activity (IC_{50}-KB/IC_{50}-Plasmodium) of 17 and 16 for the two strains, respectively, which was lower than the ratio obtained with chloroquine, which was equal to 182 for the resistant strain. The same trend occurred in vivo when we compared ED_{50} with LD_{50} obtained for cedronin and chloroquine (CQ: LD_{50} = 10 mg/kg/day; ED_{50} = 0.5 mg/kg/day). These results suggest that C_{19} quassinoid-like cedronin exhibits lower selective toxicity against plasmodium than against mammalian cells. It would be of interest to isolate and determine the activity of C_{19} quassinoid esters since it has been shown that the presence of an ester group in the molecule, which is lacking in cedronin, is important for biological activities.

Simaba cedron, named Quinquina de Cayenne in French creole (Grenand et al., 1987), is one of the

numerous drugs called 'falsa quina' in South and Central America, and used against malaria. The results presented here lend support to the traditional use of this plant. However, further studies are needed to secure its safety, given the relative toxicity of the major compound cedronin.

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