

Trypanocidal Activity of Quinonemethide Triterpenoids from *Cheiloclinium cognatum* (Hippocrateaceae)

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Z. Naturforsch. **63c**, 207–210 (2008); received July 27/September 25, 2007

We report the trypanocidal activity of quinonemethide triterpenoids isolated from root extracts of *Cheiloclinium cognatum*, a plant of the Hippocrateaceae family, collected in the Cerrado Reserve at Universidade Federal de Goiás, Brazil. The trypanocidal activity assays showed an effect on the blood trypomastigote forms of the Y strain of *Trypanosoma cruzi* where tingenone and tingenol demonstrated activity on the parasite. Their structures were elucidated on the basis of spectral data, particularly COSY, HMQC and HMBC experiments, and chemical transformations.

Key words: Celastroids, *Trypanosoma cruzi*, Hippocrateaceae

Introduction

Chagas disease continues being one of the largest public health problems in Latin America. In spite of presenting a significant reduction of new cases the transmission of infection by blood transfusion has become the most important mechanism of transmission (Chiari *et al.*, 1991). Search for new, more effective and less toxic compounds than actually used is necessary due to the low effectiveness of the existent drugs for the therapeutics of the disease. For blood transfusion the only trypanocidal substance currently used to prevent infection is gentian violet, but its use is limited due to its toxic effects and the alarming colour communicated to the skin and urine of transfusion recipients (Sepulveda-Boza and Cassels, 1996).

Our interest for the chemical constituents of Brazilian Cerrado region plants, and particularly for those with biological activity, led us to isolate quinonemethide triterpenoids, known as celastroloids, from the Hippocrateaceae family (Lião, 1997; Jeller *et al.*, 2004). These compounds constitute a relatively small group of natural products encountered exclusively in plants of the Celastraceae and Hippocrateaceae families (Gunatilaka, 1996; Brüning and Wagner, 1978), and have been of interest due to their antitumour, antileukemic, antimicro-

bial (Brüning and Wagner, 1978), antimalarial (Pavanand *et al.*, 1989), and trypanocidal activities (Grijman *et al.*, 1984).

In this paper, we describe the trypanocidal activity of the quinonemethide triterpenoids tingenol (**1**) and tingenone (**2**), isolated from the root barks of *Cheiloclinium cognatum* (Miers) A. C. Sm., a tree used in folk medicine to treat fever and oedema. Tingenol was first isolated from *Peritassa campestris* (Lião, 1997; Rodrigues-Filho *et al.*, 2002), but its NMR data was not published. The known quinonemethide triterpenoids 22-hydroxytingenone, 20-hydroxy-20-*epi*-tingenone, celastrol, pristimerin (Gunatilaka, 1996), netzahualcoyondiol (González *et al.*, 1987) and netzahualcoyone (González *et al.*, 1983), were also isolated.

Results and Discussion

Tingenol (**1**) was isolated as an orange solid with an absorption in the UV-Vis region at 426.4 nm. Its mass spectrum showed the molecular ion peak at *m/z* 422, suggesting the molecular formula, $C_{28}H_{38}O_3$. The 1H NMR spectrum displayed pattern signals at δ 6.51 (*d*, $J = 1.2$ Hz, H-1), δ 6.99 (*dd*, $J = 1.2$ and 7.2 Hz, H-6), δ 6.34 (*d*, $J = 7.2$ Hz, H-7) and δ 6.96 (*brs*, OH-3), characteristic of the A and B rings of quinonemethide triterpenoids.

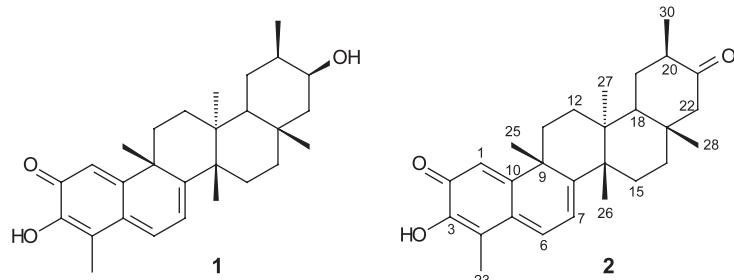


Fig. 1. Structure of tingenol (**1**) and tingenone (**2**).

The data obtained from ^1H NMR spectra are very closely related to those described for the known tingenone (**2**), except for the signals observed for α -carnonyl protons (H-22 α,β and H-20); they were shifted in comparison to those in tingenone. The shift observed for these protons was caused by the substitution of the carbonyl C-21 atom of **2** by a hydroxy group. The carbinolic proton (H-21) was observed at δ 3.85 (*brd*, $J = 2.4$ Hz). A similar situation was found in the ^{13}C NMR spectrum where the C=O group (δ 213.7) of **2** was replaced by a secondary carbinolic carbon atom in **1** (δ 71.2), confirmed by DEPT experiments. The coupling between the carbinolic proton with H-20, H-22 α and H-22 β confirmed the position of the hydroxy group at C-21. The assignment of the stereochemistry of OH-21 β was proposed from the coupling constants between H-21 and the vicinal protons ($J = 2.4$ Hz) requiring all protons to be equatorial according to the dihedral angle. Reduction of the carbonyl group of **2** with NaBH₄ afforded tingenol (**1**), supporting the structural proposal. All the evidence shown above led to the structure of an alcohol for this compound, namely tingenol (Fig. 1), very closely related to tingenone (**2**).

Tingenone was evaluated by Goijman and co-workers (1984), under epimastigote forms of parasite (Tulahuén strain), and presented an effective activity at a concentration of 30 μM ($\text{IC}_{50} = 10 \mu\text{M}$).

In the present study, we observed that tingenol and tingenone had a lower activity under trypomastigote forms, the infective form of the parasite. The results obtained showed significant activity of these substances up to 200 $\mu\text{g/ml}$. Table I summarizes the results obtained from the *in vitro* bioassay against trypomastigote forms of *T. cruzi*.

The IC_{50} values obtained for tingenol and tingenone are 306.9 μM and 486.6 μM , respectively, when evaluated under trypomastigote forms. This is simply explained by the variation on susceptibility, that occurs between different strains, and by different parasite forms evaluated in this work, the ones which present variation on the susceptibility to drugs. This fact can be explained by the difference between the composition of the plasmatic membrane of the epimastigote and trypomastigote forms. The first presents a larger percentage of sterols in its composition, mainly ergosterol, which plays an important role in the substances' transport through the plasmatic membrane of the parasite (Da Silveira *et al.*, 1979), turning the epimastigote form more susceptible to the action of exogenous substances.

These results suggest that tingenol and tingenone may be potential compounds for control and treatment of Chagas disease, where new derivatives should be obtained by chemical structure alteration in order to improve the activity on the parasite.

Compound	Inhibition (%)			
	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$
Tingenone (2)	18.6 \pm 2.6	53.3 \pm 3.1	80.2 \pm 4.9	97.1 \pm 1.9
Tingenol (1)	36.4 \pm 7.9	75.2 \pm 4.9	85.9 \pm 4.3	93.8 \pm 2.1

Table I. Results from tingenol and tingenone against trypomastigote form of *T. cruzi*.

Experimental

General experimental procedures

NMR: Bruker DRX 400, in CDCl_3 and TMS as internal standard. EIMS: low resolution on a VG Platform II (Fisons Instruments, Altrincham, England) instrument. IR: Bomem FTIR MB-102 spectrometer (ABB Bomem Inc., Quebec, Canada) in KBr pellets. $[\alpha]_D$: Perkin Elmer 241 instrument.

Plant material

The root barks of *Cheiloclinium cognatum* (Hippocrateaceae) were collected at Universidade Federal de Goiás, Goiânia, GO, Brazil, in June 2000 and identified by Dr. Júlio Antônio Lombardi (Departamento de Botânica do Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil). The voucher specimen (19797) is deposited at Herbarium of Instituto de Ciências Biológicas, UFG, Goiânia, GO, Brazil.

Extraction and isolation

The root barks of *C. cognatum* were dried in a circulating air stove and ground. The powder was extracted with CH_2Cl_2 by maceration for 3 weeks at room temperature. Solvent was removed by distillation under reduced pressure affording a dichloromethanic extract (3.0 g). The dichloromethanic extract was chromatographed on a silica gel column with hexane containing increasing amounts of acetone to yield quinonemethide triterpenoids in mixture and other terpenoids. Separation of the quinonemethide fractions by CC over silica gel, eluting with hexane/ $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (78:19:3 v/v) and hexane/diethyl ether (70:30 v/v), gave tingenol (**1**; 50 mg), tingenone (**2**; 500 mg), 22-hydroxytingenone (35 mg), 20-hydroxy-20-epi-tingenone (22 mg), celastrol (5 mg), pristimerin (10 mg), netzahualcoyondiol (65 mg) and netzahualcoyone (8 mg).

Tingenol [1(10),3,5,7-tetraen-3,21-dihydroxy-D:A-friedo-24,30-dinoroleanan-2-one-(20 β ,21 β)] (**1**): Intense orange crystals, $[\alpha]_D^{25} -43.0^\circ$ (c 0.56, CHCl_3). – UV (CHCl_3): λ_{max} ($\log \epsilon$) = 253.6 (4.00), 426.4 (4.12) nm. – IR (KBr): $\nu_{\text{max}} = 3380$, 1709, 1594, 1513 cm^{-1} . – ^1H NMR (CDCl_3 , 400 MHz): $\delta = 0.76$ (3H, *s*, CH_3 -27), 0.89 (3H, *d*, $J = 6.8$ Hz, CH_3 -30), 1.26 (3H, *s*, CH_3 -26), 1.27 (3H, *s*, CH_3 -28), 1.36 (1H, *brd*, $J = 14.0$ Hz, H-22 β), 1.45 (3H, *s*, CH_3 -25), 1.76 (1H, *m*, H-20), 1.90 (1H, *td*, $J = 12.0$ and 5.6 Hz, H-19 β), 2.00 (1H, *dd*, $J = 15.2$

and 3.6 Hz, H-22 α), 2.15 (1H, *m*, H-19 α), 2.19 (3H, *s*, CH_3 -23), 3.85 (1H, *brd*, $J = 2.4$ Hz, H-21), 6.34 (1H, *d*, $J = 7.2$ Hz, H-7), 6.51 (1H, *d*, $J = 1.2$ Hz, H-1), 6.96 (1H, *brs*, OH-3), 6.99 (1H, *dd*, $J = 7.2$ and 1.2 Hz, H-6). – ^{13}C NMR (CDCl_3 , 100 MHz): $\delta = 10.4$ (C-23), 18.6 (C-30), 21.4 (C-27), 21.8 (C-26), 25.0 (C-19), 28.9 (C-15), 30.2 (C-12), 30.6 (C-17), 31.4 (C-20), 34.0 (C-11), 35.4 (C-28), 37.4 (C-16), 38.9 (C-25), 40.7 (C-13), 43.2 (C-9), 44.3 (C-18), 44.4 (C-22), 45.2 (C-14), 71.2 (C-21), 117.4 (C-4), 118.3 (C-7), 119.8 (C-1), 127.8 (C-5), 134.1 (C-6), 146.3 (C-3), 165.1 (C-10), 170.3 (C-8), 178.6 (C-2). – EIMS: $m/z = 422$ (22) [$\text{M}]^+$, 241 (36), 202 (100), 201 (71), 95 (45), 84 (56) and 81 (28).

Biological assay

All assays were carried out using blood of mice experimentally infected with the Y strain (Nussenzweig *et al.*, 1953) of *T. cruzi*. This strain is deposited in the Parasitology Laboratory of Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil.

Trypomastigote forms were collected by cardiac puncture of Swiss albino mice in the parasitemic peak (7th day) after infection with the Y strain of *T. cruzi*. The blood was diluted with non-infected murine blood to give a concentration of ca. $2 \cdot 10^6$ trypomastigote forms/ml. Stock solutions of the compounds to be tested were prepared by dissolution in DMSO (dimethyl sulfoxide). The bioassays were performed in triplicate on microtitre plates (96 wells). For each sample, aliquots of the stock solutions were added to the diluted blood in such quantities as to give final concentrations of 100, 200, 500 and 1000 $\mu\text{g}/\text{ml}$ of mixture in the wells. The plates were incubated at 4 °C during 24 h, and the number of parasites was determined according to Brener (1962). As controls blood of infected mice without any addition (negative control), infected blood containing DMSO in equivalent amounts as the samples, and infected blood containing gentian violet (positive control) at 250 $\mu\text{g}/\text{ml}$ were used.

Acknowledgement

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), CAPES (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and Fundação de Apoio à Pesquisa da UFG (FUNAPE) for the financial support.

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