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# Trypanocidal effect of boldine and related alkaloids upon several strains of *Trypanosoma cruzi*

A. Morello, I. Lipchenca, B. K. Cassels, H. Speisky, J. Aldunate and Y. Repetto

Department of Biochemistry, Faculty of Medicine; Department of Chemistry, Faculty of Sciences; and Institute of Nutrition and Food Technology, University of Chile,
P.O. Box 70086, Santiago 7, Chile

**The alkaloids boldine, glaucine, predicentrine, apomorphine, coclaurine, norarmepavine and codeine were tested against the epimastigotes of the Tulahuén and LQ strains and the DM 28c clone of *Trypanosoma cruzi*. The micromolar concentration to inhibit 50% of the culture growth (Tulahuén strain) for apomorphine, glaucine, predicentrine, boldine, norarmepavine, coclaurine and codeine were 29, 90, 85, 110, 310, 580 and >1000 respectively. Similar values were obtained with the LQ strain and the DM 28c clone. The most active compounds in inhibiting culture growth also inhibited cell respiration, suggesting that these drugs may act by blocking mitochondrial electron transport. The trypanocidal effects of these alkaloids appear to be correlated with their antioxidative activities.**

**Key words:** Alkaloids; Tulahuén strain; *Trypanosoma cruzi*; Trypanocidal effect.

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## Introduction

American trypanosomiasis (Chagas' disease) is caused by several strains of *Trypanosoma cruzi* and is a permanent threat to almost 20% of the population of Latin America. Approximately 24 million people suffer from the disease with around 70,000 deaths per year (Aldunate and Morello, 1993).

Nifurtimox and benznidazole are currently used to treat the disease; both drugs present toxic effects and are mutagenic (Castro and Díaz de Toranzo, 1988; Gorla *et al.*, 1989; Morello, 1988).

Blood transfusion plays an important role in the transmission of Chagas' disease, second only to the infection transmitted by triatomine vectors. Each year, about 25,000 cases of transfusion-induced Chagas' disease occur in Brazil (Rassi and De Rezende, 1975; De Rezende *et al.*, 1965). Crystal violet (Nussenzzweig *et al.*, 1953) is used to sterilize donor blood, but there is a report of potential mutagenicity (Thomas and MacPhee, 1984). This dye also imparts a purple color to the blood, with the consequence that it is not easily accepted by patients and physicians.

The proposal of this work is to look for trypanocidal compounds of plant origin. A few years ago, it was shown that several synthetic antioxidants inhibit the respiration and growth of *T. cruzi* in culture (Aldunate *et al.*, 1986; Ferreira *et al.*, 1988; Morello, 1988). As boldine and other aporphine alkaloids show potent antioxidative activity (Speisky *et al.*, 1991; Martínez *et al.*, 1992; Lissi *et al.*, 1993), their effects were studied on intact *Trypanosoma cruzi* epimastigotes.

Boldine is the major alkaloidal constituent of the boldo tree (*Peumus oldus* Mol., Monimiaceae), a medicinal plant of popular use which grows in abundance in Chile. Boldo is currently employed in infusions in the practice of traditional or natural medicine to treat digestive and hepatobiliary disorders (Reiche, 1901; Magistretti, 1980), and boldo preparations have

*Correspondence to*: A. Morello, Institute of Nutrition and Food Technology, University of Chile, P.O. Box 70086, Santiago 7, Chile.
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Table 1. Effect of several boldine-related drugs on the culture growth of the Tulahuén, LQ and clone DM 28c epimastigotes of *Trypanosoma cruzi*

| Compound | Tulahuén strain | | LQ strain | | DM 28c Clone | |
|---|---|---|---|---|---|---|
| | $I_{50}$ | $I_{95-100}$ | $I_{50}$ | $I_{95-100}$ | $I_{50}$ | $I_{95-100}$ |
| Apomorfine | 29 | 100 | 35 | 100 | 36 | 100 |
| Predicentrine | 85 | 500 | 104 | 500 | 105 | 500 |
| Glaucine | 90 | 500 | 83 | 500 | 100 | 500 |
| Boldine | 110 | 500 | 115 | 500 | 120 | 500 |
| Norarmepavine | 310 | > 500 | 300 | > 500 | 320 | > 500 |
| Coclaurine | 580 | > 1000 | 420 | > 1000 | 450 | > 1000 |
| Codeine | > 1000 | > 1000 | > 1000 | > 1000 | > 1000 | > 1000 |

$I_{50}$ data correspond to the micromolar concentration to inhibit 50% of the culture growth. $I_{95-100}$ correspond to the micromolar concentration to inhibit over 95% of culture growth. See Material and Methods for details.

been described in several pharmacopoeias. The toxicity of boldine to mammals is quite low, and this alkaloid shows no mutagenicity (Kreitmair, 1952; Speisky and Cassels, 1993). The fact that boldine and chemically related structures are potent antioxidants suggested that they might be useful as experimental drugs for the treatment of Chagas' disease.

## Material and Methods

### *Chemicals*

Tryptose, fetal calf serum yeast extract and tryptone were obtained from Difco. Apomorphine ([*R*]-5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo [de, g] quinoline-10,11-diol); boldine ([*S*]-5,6,6a,7-tetrahydro-1-10-dimethoxy-6-methyl-4H-dibenzo [de, g] quinoline-2,9-diol); predicentrine ([*S*]-5,6,6a,7-tetrahydro-6-methyl-1,9,10-trimethoxy-4H-dibenzo [de,g] quinolin-2-ol); glaucine ([*S*]-5,6,6a, 7-tetrahydro-6-methyl-1,2,9,10-tetramethoxy-4H-dibenzo [de, g] quinoline); (+)-coclaurine ([*R*, *S*]-1,2,3,4-tetrahydro-1-[4-hydroxyphenyl]methyl-6-methoxyisoquinolin-7-ol); (+)-norarmepavine ([*R*, *S*]-1,2,3,4-tetrahydro-1-[4-methoxyphenil]methyl-6-methoxyisoquinolin-7-ol); and codeine ([*R*]-7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-morphinan-6-ol) were obtained from Aldrich Chemical Co., isolated from boldo bark or synthesized in the laboratory. Hemin and all other chemicals were obtained from Sigma Chemical Co.

### *Parasites*

*T. cruzi* epimastigotes (Tulahuén and LQ strain and DM 28c clone) were grown at 28°C in Diamond's monophasic medium (Diamond, 1968; Aldunate *et al.*, 1986) with blood replaced by 4 $\mu$M hemin. Fetal calf serum was added to a 4% final concentration.

### *Inhibition of culture growth*

Drugs were added in dimethylsulphoxide at the concentrations indicated in tables and figures. *T. cruzi* epimastigote growth was followed by nephelometry using culture flasks with a side-arm tube (Aldunate *et al.*, 1986, 1992). The $I_{50}$ and $I_{95-100}$ data shown in Table 1 were calculated by interpolation of culture growth inhibition curves at different drug concentrations such as those in Fig. 1. Values were calculated on the seventh day of culture (exponential phase).

### *Oxygen uptake*

Respiration measurements were carried out polarographically with a Clark No. 5331 electrode (Yellow Spring Instrument) in a Gilson 5/6 oxygraph (Ferreira *et al.*, 1988). The volume of the chamber was 2.0 ml and the temperature 28°C. The number of parasites used for each essay was equivalent to 1.5 to 2 mg of protein (Lowry *et al.*, 1951), resuspended in 0.107 M sodium chloride 0.05 M potassium phosphate buffer, pH 7.4.

### *Toxicity determinations*

Drugs at 1 mM concentration were added to parasite suspensions ($10^6$–$10^7$ cells/ml) in dimethylsulphoxide. After 1 and 20 hr at 28°C,

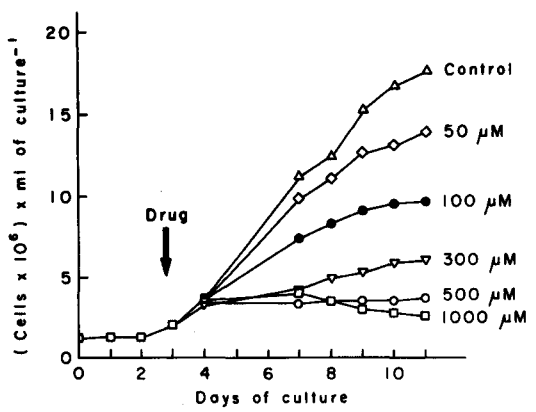


Fig. 1. Effect of several concentrations of boldine on *T. cruzi* (Tulahuén strain) culture growth. See Material and Methods for further details.

changes in motility and shape were observed (Letelier *et al.*, 1990).

All values shown in this communication represent the average of three or more independent experiments with no more than 15% variation among them.

## Results and Discussion

Figure 1 shows the effect of boldine on the growth of epimastigotes of the Tulahuén strain of *Trypanosoma cruzi*. At concentrations above 500 $\mu$M, growth is completely inhibited, all parasites are immotile, and their cells become rounded (microscopic observation) by the seventh day of culture. Similar results were obtained with the LQ strain and the DM 28c clone.

Table 1 shows the inhibition of the Tulahuén and LQ strain and the DM 28c clone expressed as the concentrations needed to inhibit 50% ($I_{50}$) or over 95% ($I_{95-100}$) of culture growth with several drugs. The alkaloids may be classified into four groups on the basis of their activity. The most active is apomorphine with an $I_{50}$ of 29–36 $\mu$M, followed by boldine and its *O*-methylated derivatives predicentrine and glaucine with an $I_{50}$ around 100 $\mu$M. The less active drugs were the benzyltetrahydroisoquinolines norarmepavine and coclaurine; codeine was inactive at 1 mM concentration.

The data in Table 1 also show no significant differences in susceptibility to the different drugs among the *T. cruzi* strains used in this experiment. The Tulahuén strain is much more strongly inhibited than the LQ strain when nifurtimox or benznidazole are used (Moncada *et al.*, 1989). This difference is mainly due to the differences in concentration of glutathione and trypanothione among the strains (Moncada *et al.*, 1989), as both drugs generate free

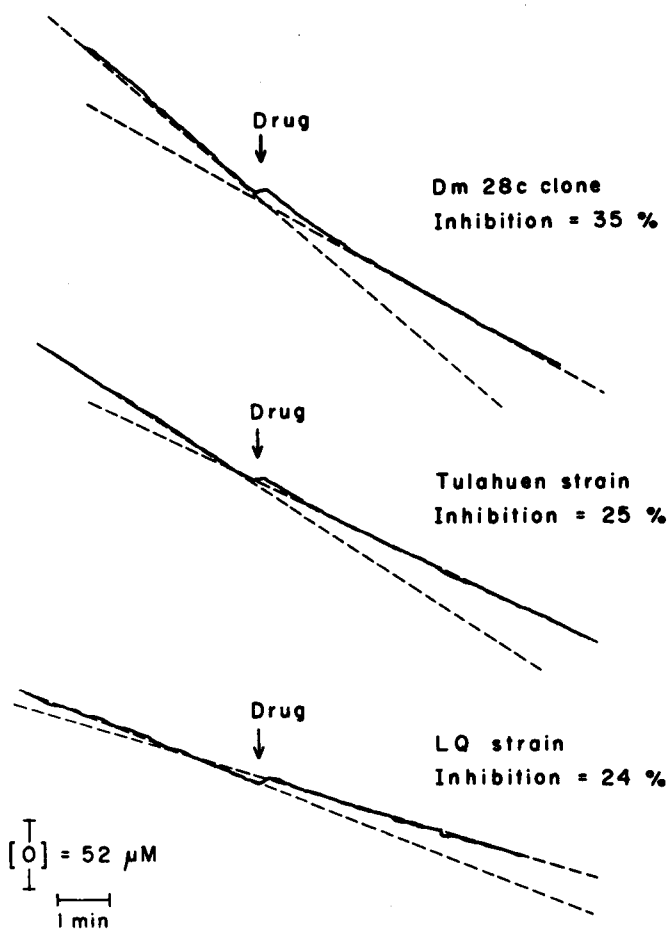


Fig. 2. Effect of boldine on oxygen uptake of *T. cruzi* epimastigotes of the Tulahuén and LQ strains and the clone DM 28c. Boldine was added at 1 mM final concentration in dimethylsulphoxide. Control respirations were 25.0 n at 0/min/mg of protein for the Tulahuén strain; 27.4 n at 0/min/mg of protein for the LQ strain; and 28.0 n at 0/min/mg of protein for the DM 28c clone. See Material and Methods for further details.

Table 2. Toxicity of boldine-related drugs toward *T. cruzi* epimastigotes

| | Tulahuen strain | | LQ strain | | DM 28c clone | |
|---|---|---|---|---|---|---|
| Compound | 1 hr | 20 hr | 1 hr | 20 hr | 1 hr | 20 hr |
| Control | 0 | 0 | 0 | 0 | 0 | 0 |
| Apomorphine | ++ | ++++ | ++ | ++++ | ++ | ++++ |
| Boldine | + | +++ | + | ++ | + | ++ |
| Glaucine | 0 | 0 | 0 | + | 0 | + |
| Norarmepavine | 0 | 0 | 0 | + | 0 | + |
| Codeine | 0 | 0 | 0 | 0 | 0 | 0 |

Toxicity grades expressed as 0, +, ++, +++, ++++, represent decrease in motility and change in shape evaluated microscopically (400 × magnification) after incubation at 28°C for 1 hr and 20 hr. See Material and Methods. Values represent the results of three or more independent experiments.

radicals, and glutathione and trypanothione are important factors in cellular defense mechanisms against such reactive species (Docampo and Moreno, 1984; Meister, 1983; Fairlamb and Cerami, 1985; Fairlamb *et al.*, 1985).

Figure 2 shows the effect of boldine upon the respiration of *T. cruzi* epimastigotes in the Tulahuén and LQ strains and the DM 28c clone. The inhibition is between 25 and 30%, and does not differ significantly among the strains used in this study. Similar results were obtained with apomorphine, predicentrine and glaucine. No significant inhibition of respiration was detected with the other drugs used at 1 mM concentration.

Table 2 shows the toxicity of the drugs used in these studies, as measured by microscopic observation. No important differences can be seen in the susceptibility to the chemicals among the *T. cruzi* strains. These results are in agreement with and are similar to the inhibition of culture growth (Table 1) and respiration (Fig. 1), confirming no significant differences among the *T. cruzi* strains used. Apomorphine was the most toxic alkaloid to *T. cruzi*, followed by boldine and glaucine.

It is intriguing that the antitrypanosomal activities of the alkaloids tested here appear to be correlated with their antioxidative activities. Thus, the catecholic apomorphine is by far the most potent isoquinoline alkaloid in these systems, followed by noncatecholic aporphines like boldine and glaucine and, at a considerable distance, the phenolic benzyltetrahydroisoquinolines coclaurine and norarmepavine. Considering that codeine is neither phenolic nor incorporates benzylic hydrogens alpha to amine nitrogen atoms, it would not be expected to show any striking antioxidative activity (Lissi *et al.*, 1993). Codeine showed no activity toward *T. cruzi*.

The low toxicity of boldine to humans is suggested by the prolonged traditional or pharmaceutical use of it. Also, doses of around 1 g/kg of body weight are required to induce lethality in several mammalian species (Kreitmair, 1952). Recently, boldine was demonstrated to be non-mutagenic (Moreno *et al.*, 1991) and non-genotoxic (Speisky *et al.*, 1993). The $LD_{50}$, i.p. in mice of apomorphine is 160 mg/kg (Cannon *et al.*, 1972).

The results presented in this communication suggest that boldine and related drugs may inhibit the growth of *T. cruzi* by a blockage of mitochondrial electron transport similar to the mechanism put forth to explain the trypanocidal action of BHT and BHA (Ferreira *et al.*, 1988; Aldunate *et al.*, 1986) and hydroquinones (Aldunate *et al.*, 1992).

Boldine and chemically related structures may be useful as experimental drugs for the treatment of Chagas' disease.

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