

Bruceantin, a Potent Amoebicide from a Plant, *Brucea antidysenterica*

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Bruceantin, purified from an Ethiopian plant used to treat dysentery, killed *Entamoeba histolytica* in vitro (IC_{50} [the concentration of drug which decreased the number of colonies to half that of controls] = 0.018 $\mu\text{g/ml}$). Six related quassinoids of 17 tested were also amoebicidal. No relationship between quassinoid structure and amoebicidal activity was apparent.

Many antiparasitic drugs, such as quinine and emetine, have been derived from botanical sources (10). Bitter extracts of certain simarou-baceous plants have long been used to treat dysentery (7, 12, 13). Examples are extracts of *Brucea antidysenterica* in Ethiopia (16), of *B. sumatrana* (Ya-tan-tzu) in China, and of *Castela nicholsoni* or Chaparro amargoso (called Castamargina) in Mexico (7, 12).

The active agents in each case are quassinoid compounds (2, 13; Fig. 1 and 2). Two pure quassinoids, ailanthone and glaucarubin, were active against *Entamoeba histolytica* (the dysentery amoeba), both in vitro and in animals (3). Glaucarubin was effective in human amoebiasis (4, 15). Therefore, it was of interest to test other quassinoids against *E. histolytica* in vitro. The objectives of the study were: (i) to find related drugs equally or more active and (ii) to determine the relationship, if any, between chemical structure and antiamoebic activity.

(Some of these data were presented at the 9th Seminar on Amebiasis sponsored by the Instituto Mexicano del Seguro Social in Mexico City, 9-12 November 1981.)

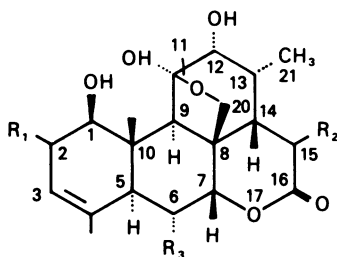
A convenient, precise, quantitative method for determining viability of *E. histolytica* is clonal growth of the parasites in semisolid agar media (8, 9). *Entamoeba histolytica* HM-1:IMSS (strain HM-1, ATCC 30459) was grown axenically in TYI-S-33 medium (6) containing 15% bovine serum. Cells were suspended by chilling and inverting the cultures and enumerated with a Coulter Counter. Stock drug solutions (2 mg/ml) were prepared in dimethyl sulfoxide. Before being used, the solutions were diluted 10-fold in phosphate-buffered saline (pH 7.2) and filter

sterilized. The final solutions contained 0.1% dimethyl sulfoxide or less, which did not affect viability. Diluted drug or dimethyl sulfoxide was added to standard tubes of media in duplicate for controls. Tubes of complete medium were warmed to 40 to 42°C, and concentrated melted agar (Difco Laboratories) was added (final concentration, 0.6% [8]). The tubes were removed from their warming bath, and duplicate diluted suspensions of organisms (0.1 ml of 5,000 organisms per ml) were added (total volume, 15 ml). The tubes were mixed to ensure a uniform suspension of the cells and chilled for 10 min in ice water to solidify the medium. After being incubated for 4 to 5 days at 35.5 \pm 0.5°C, the colonies which had grown from individual cells became visible to the unaided eye and were counted. The minimum lethal concentration (MLC) of a drug was the lowest concentration at which no colonies grew. The IC_{50} (calculated from semilogarithmic curves) was the concentration of drug which decreased the number of colonies to half that in controls (9).

Seventeen quassinoid compounds (Fig. 1 and 2) were screened for anti-amoebic activity at 2 $\mu\text{g/ml}$. This level was selected because it is in the concentration range in which metronidazole is active (1, 9; Table 1). The seven compounds with activity were studied more quantitatively. None of the 17 was active against the unrelated parasitic protozoa *Giardia lamblia* or *Trichomonas vaginalis* at 2 $\mu\text{g/ml}$ (data not shown). Bruceantin was more active than any of the other compounds by both the IC_{50} (0.018 $\mu\text{g/ml}$) and the MLC (0.076 $\mu\text{g/ml}$) measures (Table 1). Bruceantin was also more active than any of a number of unrelated drugs tested previously (1, 5, 9). The IC_{50} for metronidazole was 0.1 to 2.0 $\mu\text{g/ml}$, and the MLC was 0.6 to 4.0 $\mu\text{g/ml}$ in an earlier series of experiments (9). The potency of metronidazole was also compared directly with

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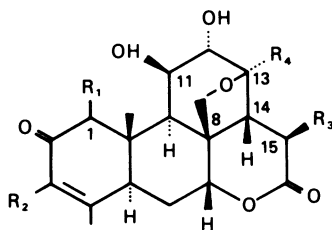
	R ₁	R ₂	R ₃
I. Active			
Ailanthinone	= O	O(CO)CH(Me)Et	H
Glaucarubolone	= O	OH	H
Glaucarubinone	= O	O(CO)C(Me)(OH)Et	H
Ailanthone 21: = CH ₂	= O	H	H
Glaucarubin	... OH	[S]O(CO)C(Me)(OH)Et	H
II. Inactive			
Chaparrin	... OH	H	H
Glaucarubol	... OH	OH	H
Holacanthone	= O	OAc	H
Undulatone	= O	OAc	O(CO)C(Me)=CHMe
6 α -Senecioloxy- Chaparrinone	= O	H	O(CO)CH=C(Me) ₂



Group A Quassinoids

FIG. 1. Structures of active and inactive (at 2 μ g/ml) group A quassinoid compounds.

	R ₁	R ₂	R ₃	R ₄
I. Active				
Bruceantin	H	OH	CO	$\begin{matrix} O \\ \\ \cdots C - OCH_3 \end{matrix}$
Simalikalactone D	OH	H	COCH(CH ₃)C ₂ H ₅	CH ₃
II. Inactive				
Brusatol	H	OH	COCH=C(CH ₃) ₂	$\begin{matrix} O \\ \\ \cdots C - OCH_3 \end{matrix}$
Bruceine B	H	OH	COCH ₃	"
Bruceantinol	H	OH	CO $\begin{matrix} OC - CH_3 \\ \\ O \end{matrix}$	"
Samaderine E	OH	H	H	CH ₃



Group B Quassinoids

FIG. 2. Structures of active and inactive (at 2 μ g/ml) group B quassinoid compounds.

TABLE 1. Quassinoid drugs effective against *E. histolytica*

Drug	IC ₅₀ (μg/ml)			MLC (μg/ml)		
	Mean	SD	Range (no. of expts)	Mean	SD	Range (no. of expts)
Bruceantin	0.018	0.006	0.012–0.03 (8)	0.076	0.02	0.06–0.1 (10)
Similikalactone D	0.047	0.027	0.015–0.08 (3)	0.24	0.13	0.1–0.5 (5)
Ailanthinone	0.068	0.06	0.01–0.15 (3)	0.25	0.17	0.06–0.5 (5)
Glaucarubolone	0.12	0.005	0.12–0.13 (2)	0.63	0.26	0.4–1.0 (3)
Glaucarubinone	0.14	0.025	0.12–0.17 (2)	0.54	0.26	0.2–1.0 (5)
Ailanthone	0.14	0.04	0.06–0.19 (5)	0.92	0.53	0.5–2.0 (6)
Glaucarubin	1.57	0.24	1.4–1.9 (3)	10.0		(4)
Metronidazole ^a	2.15	0.35	1.8–2.5 (2)	4.25	0.75	3.5–5.0 (2)

^a Metronidazole and bruceantin were tested in the same experiment.

that of bruceantin in the present studies, in which a mean IC₅₀ of 2.15 μg/ml and a mean MLC of 4.25 μg/ml were observed (Table 1). The greater potency of bruceantin (molecular weight 548) over metronidazole (molecular weight 171) is more evident if the compounds are compared on a molar rather than a weight basis.

The colony method does not distinguish between inhibition of parasite growth and killing. Therefore, amoebae were exposed to bruceantin for various times in liquid medium and then survival was determined by the colony assay in the absence of drug. At concentrations of 0.2 μg/ml and above, all of the parasites were killed (Fig. 3). At 0.1 μg/ml, few parasites (approximately 1.5% of the control) survived at 48 h. In other experiments, all of the parasites were killed by exposure for 72 h to 0.1 μg of bruceantin per ml. The rates of amoeba killing by metronidazole (9) and bruceantin were similar at 15- to 30-fold-lower molar concentrations of the latter.

The quassinoid compounds of interest have the same backbone: four strained, six-membered rings. The position of an additional five-membered ring, containing an oxygen bridge, differs between group A and group B quassinoids. The former have a hemiketal ring from carbon-20 to carbon-11 (Fig. 1), whereas the latter have an ether ring from carbon-20 to carbon-13 (Fig. 2). Carbon-20 links the oxygen atom to the main skeleton at carbon-8 (2, 13). Some of these compounds have antitumor activity (2) which appears to be related to their ability to inhibit protein synthesis (11).

Attempts were made to correlate the structures of the various substituents of the group A (Fig. 1) and group B (Fig. 2) quassinoids with their amoebicidal activities. The same or similar substituents were found on both active and inactive compounds, although in different combinations. A comparison of the structures of bruceantin (active) versus bruceantinol and brusatol (both inactive) in group B showed only a minor difference in the ester side chain at car-

bon-15. Among active group A quassinoids, glaucarubolone has a free hydroxyl group at carbon-15, whereas glaucarubinone, which had similar activity, has a five-carbon ester at that position. The structure-activity relationships cited previously for antitumor activity (2), in which carbon-15 substituents were important, did not carry over to our test system. From the data available in the current study, there did not appear to be a simple relationship between structure and amoebicidal activity. The in vitro antimalarial activity of five quassinoids did correlate with their antitumor activity (14). Simili-

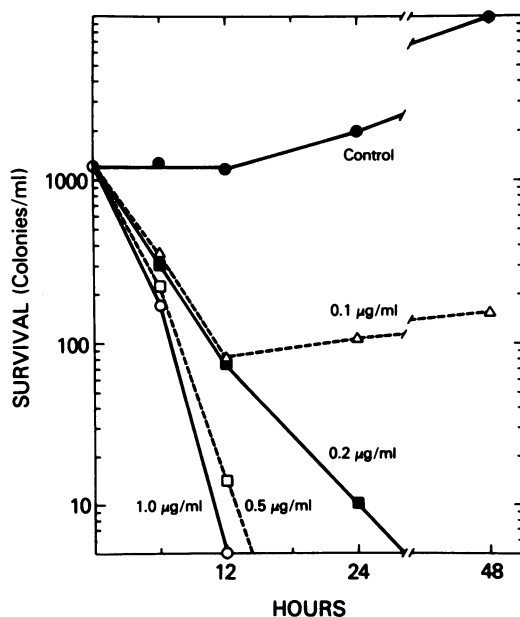


FIG. 3. Time course of killing of *Entamoeba histolytica* HM-1 by various concentrations of bruceantin. Amoebae were exposed to bruceantin (0.1 to 1.0 μg/ml) in liquid medium. At the times indicated in the figure, samples were diluted into agar medium without drug for assay of viability. The amount of drug transferred did not inhibit growth.

kalactone D and glaucarubinone (the only compounds tested in both studies) had both antimalarial and antiamoebic activity.

Since, in our studies, bruceantin was approximately 100 times more active against *E. histolytica* than glaucarubin was, it appears to merit testing in animals. Because of the toxicity of bruceantin (2), it must be established in animal studies whether the amoebicidal dose is safely below the toxic dose.

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