EFFECT OF A MACROCYCLIC DITERPENE FROM TOBACCO ON *TETRAHYMENA PYRIFORMIS* (GL)

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A macrocyclic diterpene (β-4,8,13-duratriene-1,3-diol) was extracted from burley tobacco and checked for physiological activity using *Tetrahymena pyriformis* (GL) as the test organism. The activity of the compound includes a continuum of effects, from decreased motility through physiological death. The effects are concentration-dependent and similar to those exerted by macrocyclic diterpenes from other sources.

As reported by Perkins and Ciereszko (1, 2), certain macrocyclic diterpenes exert a continuum of effects on *Tetrahymena pyriformis*, a ciliated protozoon, that extends from decreased motility to physiological death. To date, the macrocyclic diterpenes tested, primarily crassin acetate, have been extracted from gorgonian corals, but similar compounds exist in tobacco (3). Due to the known effects of tobacco products on ciliated cells and/or tissues (4), it seems reasonable to suppose that the macrocyclic diterpenes of tobacco also exert physiological activity. Perkins and Ciereszko (2) proposed that the macrocyclic diterpenes may possess chemotherapeutic activity. If this proposal is correct, acquisition of the compounds from a readily available source, such as tobacco, rather than an ecosystem, i.e., a coral grotto, is more feasible.

The following investigation was conducted to determine whether the macrocyclic diterpenes of tobacco are physiologically active and whether the activity closely agreed with the reported activity of other macrocyclic diterpenes.

MATERIAL AND METHODS

Crystalline β-4,8,13-duvatriene-1,3-diol (FW 306), herein referred to as βI, was extracted from burley tobacco (*Nicotiana tabacum*) and purified according to the procedure of Roberts and Rowland (5).

The biological model used in testing the activity of the above compound was *Tetrahymena pyriformis* (GL). Respective methods for data acquisition have been reported previously (2).

RESULTS

The effect of βI on motility

As shown in Figure 1, the only experimental concentration of β-4,8,13-duvatriene-1,3-diol (βI) which did not elicit a readily observable change in the motility of *T. pyriformis* was 0.027 mM. After exposure to 0.053 and 0.08 mM βI the cells could completely or partially recover from the initial effects of βI on motility. However, at concentration of 0.106 and 0.133 mM βI no recovery was observed.

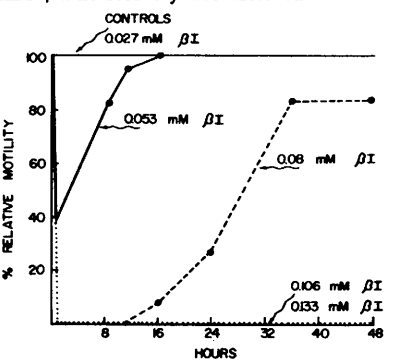

FIGURE 1. The effect of βI on the relative motility of *T. pyriformis* (GL).

The effect of βI on growth

Although a concentration of 0.027 mM βI did not cause any observable change in the motility of *Tetrahymena*, the cells grew at a lower rate and reached a final population density below that of the controls (Fig. 2 and Fig. 3). Increasing the concentration of βI twofold, i.e., to 0.053 mM,

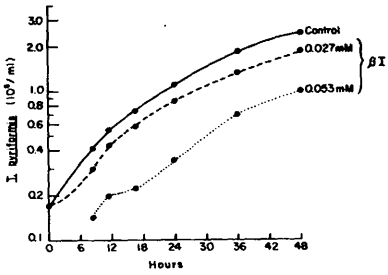

FIGURE 2. The effects of lower βI concentrations on the growth of *T. pyriformis* (GL).

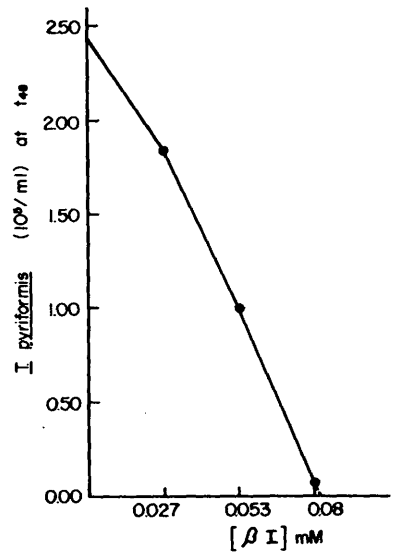

FIGURE 3. Population densities of *T. pyriformis* (GL) after 48 hr exposure to various concentrations of βI.

caused a further depression of growth rate and final population density. At a βI concentration of 0.08 mM, the cells did not reproduce (Fig. 3) even though the survivors did regain 80% normal motility (Fig. 1). The concentration of βI judged to be lethal for all cells is represented by the X- axis intercept of Figure 3 and the value is approximately 0.083 mM.

DISCUSSION

The above data establish the fact that βI elicits responses in *T. pyriformis* which are similar to, if not the same as, the responses brought about by crassin acetate, i.e., decreased motility, decreased population densities, and, therefore, decreased growth rate and/or death of the population.

However, crassin acetate (FW 376) produces a total kill of the population at approximately 0.162 mM, whereas βI brings about the same response at approximately 0.083 mM, i.e., βI is approximately twice as lethal as crassin acetate.

The exact mechanism whereby the above macrocyclic diterpenes exert their effect has, as yet, not been established, but their chemical characteristics suggest a membrane phenomenon. The compounds, being fat-soluble, should concentrate at the lipid matrix of the membrane with a concommitant alteration in passive and active transport phenomena.

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