net current flowing. Voltage clamp data obtained at these two potential levels, by applying step depolarizing and hyperpolarizing commands, are shown in Fig. 3B. At the holding potential (V_H) of −83 mV, increasing the level of the depolarizing voltage steps from −71 to −28 mV resulted in decrease in outward current. At the V_H of −21 mV, hyperpolarizing steps to −51 and −76 mV actually produced a small outward current. The current-voltage plots derived from the data obtained at the two different holding potentials are very similar, exhibiting a region of negative slope resistance in the voltage range −75 to −35 mV (Fig. 3C). It is evident from the plot that there is a small net inward current at voltages of −20 to −40 mV, which results in the *I-V* curve crossing the voltage axis in three places. In addition to the negative slope resistance (at −70 to −35 mV) and small net inward current, this cell exhibited an increase in outward currents for voltage steps more positive than +12 mV (evident in the voltage steps to +25 and +21 mV in Fig. 3B). This current is time-dependent, becoming activated approximately 30 msec after the depolarizing step to +25 mV from the holding potential of −83 mV (Fig. 3B) and slowly increasing, just saturating at the end of the 500-msec voltage step. The voltage clamp became unstable with larger depolarizing voltage steps, thereby preventing the outward current from being studied in greater detail.

This study demonstrates that voltage clamp techniques useful in the study of membrane properties of many types of cells can be used to study currents across the membranes of leukocytes. The region of negative slope resistance in the steady-state *I-V* curves, which is abolished by addition of barium to the bathing medium, and the outward currents that become activated at positive voltages, indicate that leukocytes exhibit at least two voltage-dependent conductances. Examining these conductances should provide information about the physiology of macrophages. Various nerve and muscle cells (*11*) as well as egg cells (*9*) exhibit N-shaped steady-state *I-V* curves. In excitable cells, N-shaped steady-state *I-V* curves have been implicated in pacemaker activity (*12*). The significance of this type of *I-V* curve in nonexcitable cells such as egg cells is not clear (*5*). Only 10 to 20 percent of the macrophages recorded from spleen cultures exhibit N-shaped *I-V* curves. Whether these cells are functionally different from other macrophages or simply reflect the absence of microelectrode-induced damage remains to be determined. Macrophages display a number of complex membrane-related functions such as motility, secretion, and endocytosis that may influence or be influenced by these conductances. The ability to directly study currents across the membranes of leukocytes and control membrane potential makes possible new experiments for the study of the role of ionic conductances in leukocyte function.

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13. I thank T. Pellmar, D. Livengood, and J. Freschi for critical review of the manuscript and T. Pellmar for helpful advice on the use of the single-electrode voltage clamp. Supported by Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Research Work Unit MJ 00020. The views presented in this report are those of the author. No endorsement by the Defense Nuclear Agency has been given or should be inferred.

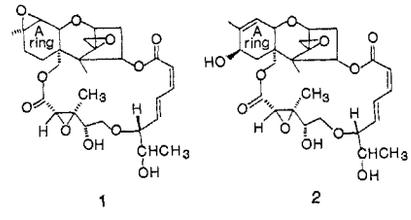
20 April 1981; revised 12 June 1981

Interaction Between the Antibiotic Trichothecenes and the Higher Plant *Baccharis megapotamica*

Abstract. *The Brazilian shrub* Baccharis megapotamica *contains significant amounts of antibiotic trichothecenes. When these plants are grown in the United States, they are devoid of the mycotoxins. Feeding experiments with fungus-produced trichothecenes show that* Baccharis megapotamica *absorbs, translocates, and chemically alters these compounds to ones with structures analogous to those found in the plant in its native habitat. The mycotoxins, which have no apparent ill effect in* Baccharis megapotamica, *kill tomatoes, peppers, and artichokes.*

We report that mycotoxins, presumably produced by a soil fungus, are absorbed, translocated, and stored in the higher plant *Baccharis megapotamica* Spreng (Asteraceae), a shrub found in Brazil. This situation, in which a significant quantity of a potent antibiotic is taken up, chemically modified, and stored by a higher plant with no apparent ill effect to that plant, appears to be unique (*1–4*).

Kupchan *et al.* (*5*) reported that an extract of *B. megapotamica* exhibited high activity in vivo against P388 mouse leukemia. From this extract was isolated a series of potent antileukemic sesquiterpenes belonging to the well-known class of trichothecene antibiotics, which previously had been isolated only from cultures of various saprophytic soil fungi (*6–8*). These particular compounds, the baccharinoids (**1** and **2**), are closely related to the macrocyclic trichothecenes known as roridins [for example, roridin A (**5a**)] and verrucarins [for example, verrucarin A (**6a**)]. The baccharinoids exhibit higher activity in vivo against P388 than do either the roridins or verrucarins. In addition, the presence of the trichothecene baccharinoids in *B. megapotamica* is curious because such compounds are normally highly phytotoxic (*6–8*), yet the baccharinoids are present



1 2

in *B. megapotamica* in such a high concentration (0.02 to 0.03 percent by dry weight) (*9*) that their presence seems unlikely to be the result of simple contamination by surface fungi.

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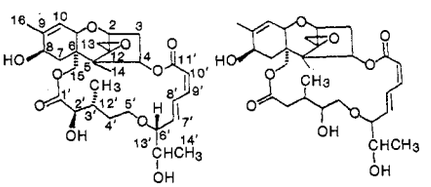
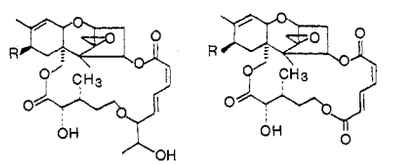
The baccharinoids are separable into two distinct classes: the 8β-hydroxyl derivatives (for example, **2**), which are present in high concentrations, and the 9β,10β-epoxides (for example, **1**) which are present in lower concentrations. Also isolated from *B. megapotamica* were baccharinoids **3** and **4** (*10–13*); the structure of **3a** was established by x-ray crystallography (*14*). Baccharinoids **3a** and **3b** differ in configuration from roridin A (**5a**) at C-2′. The configurations at C-6′ and C-13′ in **5a** are unknown (*8*). These particular patterns of A-ring substitution in the trichothecenes are found only in the baccharinoids (*15, 16*), and this suggests the possibility that the baccharinoids are the result of fungus-produced roridins being taken up and modified in vivo by *B. megapotamica*.



3a, C-13′=(R)
3b, C-13′=(S)

4



5a, R=H
5b, R=OH

6a, R = H
6b, R = OH

We have grown *B. megapotamica* from seed (*17*) in non-Brazilian soil and find that the plants do not contain baccharinoids (*18*). This strongly suggests that an agent necessary to trigger the production of baccharinoids is missing under these conditions. The most reasonable explanation is that plants grown in non-Brazilian soil lack the required trichothecene-producing soil fungus that serves as a source of roridins for uptake and further elaboration by the plant. When we fed *B. megapotamica* seedlings with roridin A (**5a**) and verrucarin A (**6a**) (*19, 20*), we found that not only are these mycotoxins efficiently taken up by the root system and rapidly translocated to the upper plant, but both are efficiently metabolized in high yield to their 8β-hydroxyl derivatives, **5b** and **6b**, respectively. The crude extracts of plants fed roridin A over a 3-day period at two different concentrations were analyzed by high-performance liquid chromatography (HPLC). Within 1 day, *B. megapotamica* had absorbed and converted a substantial amount of roridin A to its 8β-hydroxyl derivative **5b**. The total extracts (110 mg) of six plants used in a feeding experiment were combined (Fig. 1A) and subjected to flash chromatography (*21*) and then preparative HPLC to yield 2 mg of **5b**, which was identified by comparison [thin-layer chromatography (TLC), HPLC, and ^{1}H nuclear magnetic resonance (NMR) spectroscopy] with an authentic sample of 8β-hydroxyroridin A (*22*). Similar experiments with verrucarin A (**6a**) have shown that *B. megapotamica* also efficiently transforms **6a** to 8β-hydroxyverrucarin A (**6b**).

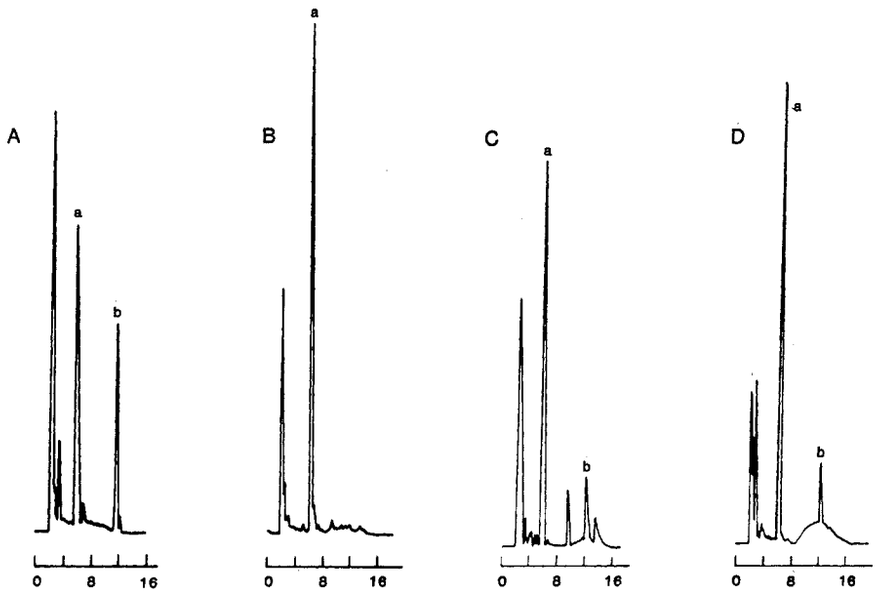


Fig. 1. Analyses by HPLC (gradient, 2 to 6 percent methanol in methylene chloride; flow rate, 1.5 ml/min; Supelcosil LC-Si column) of extracts of (A) *B. megapotamica*, (B) tomatoes, (C) artichokes, and (D) peppers fed roridin A for 3 days (*19*); *a*, roridin A (**5a**); *b*, 8β-hydroxyroridin A (**5b**).

Seedlings of tomatoes, peppers, and artichokes fed roridin A and verrucarin A showed severe damage and expired after the 3-day feeding period. All three of these plants absorb and translocate substantial amounts of roridin A; peppers and artichokes appear to convert **5a** into the 8β-hydroxy derivative **5b** in a manner analogous to that observed with *B. megapotamica* (Fig. 1) (*19*).

In its native habitat (Brazil), *B. megapotamica* grows in marshes in large areas measuring thousands of square feet; the only other higher plants that are present are native grasses (*23*). There appear to be few competitive plants in these areas, and the normally phytotoxic (*24*) and insecticidal (*25*) trichothecenes may be important in the ecology of this system. Heretofore, all reports of mycotoxicosis associated with trichothecene-producing strains of fungi have been the result of infestations of harvested crops (*6–8*). A serious health hazard would result if crops of agricultural importance were to interact with mycotoxins in a manner similar to that found with Brazilian *B. megapotamica* (*26*).

Note added in proof: The HPLC trace of the extract of *Baccharis* plants fed roridin A for a 1-week period shows a peak of slightly longer retention time than that of 8β-hydroxyroridin A (**5b**). As the feeding period is extended, this peak increases in size, and the peak due to **5b** diminishes. Two plants (~ 20 cm in height) fed roridin A for 6 weeks were harvested, dried, and extracted. From this extract were isolated by preparative TLC 0.4 mg of 8β-hydroxyroridin A (**5b**) and 2.0 mg of baccharinoid **3a**, whose identity was established by comparison (TLC, HPLC, and ^{1}H NMR spectroscopy) with an authentic sample. From a recently completed x-ray crystal structure (*27*), we now know that the configurations at C-6′ and C-13′ are (*R*) in roridin A. Thus, *B. megapotamica* not only hydroxylates C-8 but also epimerizes C-2′ from (*S*) to (*R*). These data strongly support the thesis that baccharinoids **3a** and **3b** result from the uptake and chemical modification of roridin A and isororidin A, respectively, by *B. megapotamica* in its native habitat.

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1. There are a number of instances where toxins produced by pathogenic fungi (*2*), and to a far lesser extent by mycorrhizal fungi (*3*), are taken up and chemically modified by the host plant. However, these situations are quite different from the one reported here in that those toxins are clearly harmful to the plant, and, in fact, the outcome of the host-fungus battle strongly depends upon the chemical response of the host to the attacking microorganism (*4*).
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9. Three separate collections of *B. megapotamica* at varying times of the year have all yielded the same result with respect to the baccharinoids. These collections, which range from 3 to 1300 kg, were made from a marsh located near Curitiba, Brazil, approximately 1000 miles south of Rio de Janeiro. In addition to the four baccharinoids reported in the literature (*5*), *B. megapotamica* contains a large number of additional closely related macrocyclic trichothecenes, including roridins D and E (B. B. Jarvis, unpublished results).
10. Baccharinoids **1** to **4** were isolated as sets of diastereoisomers, epimeric at C-13′. We recently isolated isororidin A from a culture of *Myrothecium verrucaria* (ATCC 24571), which is epimeric with roridin A, also at C-13′ (*11*). This same culture also yields roridin E (*12*) and isororidin E (*13*), which we suspect also differ in configuration only at C-13′.
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15. A number of simple trichothecenes (*6*) and three recently isolated macrocyclic trichothecenes, verrucarin L and its acetate (*16*) and roridin K acetate (8α-acetoxyroridin E) (*11*), also possess a hydroxyl or ester group at C-8. However, all of these fungal metabolites are substituted at C-8 in the α configuration.
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17. These seeds, as well as 2-year-old plants grown from seed in a greenhouse, were provided by G. M. Christenson, Economic Botany Laboratory, U.S. Department of Agriculture, Beltsville, Md.
18. We also have carefully examined several other species of *Baccharis* (*B. halimifolia*, *B. pterimoides*, and *B. sarathroides*) and find no sign of baccharinoids. Furthermore, the National Cancer Institute has screened nearly 90 species of *Baccharis* and finds, because of lack of P388 activity in vivo, that none of the species of *Baccharis* is likely to contain baccharinoids (M. Suffness, personal communication).
19. Seedlings of *B. megapotamica* (about 15 cm tall) were suspended so that only the roots were in contact with an aqueous solution of the mycotoxin. The solutions were prepared by the addition of 25 mg of mycotoxin in 2.5 ml of ethanol or 50 mg of mycotoxin in 5 ml of ethanol to 50 ml of distilled water (10 μ*M* in calcium sulfate). After a suitable time, the plant was removed, and the roots were washed with water and separated from the upper portions of the plants. The plant material was freeze-dried and extracted (40°C overnight in absolute ethanol). The crude extracts were filtered through small portions of activated silica gel and the silica was washed with 20 percent methanol in methylene chloride. The resulting dark green gums were analyzed by HPLC (methanol in methylene chloride) on a silica gel column. The roots showed only a trace of trichothecenes. Controls with plants that had stood only in the aqueous solution with and without added ethanol showed no trace of mycotoxins.
20. Roridin A (**5a**) and verrucarin A (**6a**) were isolated from a large-scale (760 liters) fermentation with *Myrothecium verrucaria* carried out under the direction of R. Geoghegan, Frederick Cancer Research Center, Frederick, Md. A number of new trichothecenes were isolated from this fermentation [B. B. Jarvis, G. Pavanasasivam, C. E. Holmlund, T. DeSilva, G. P. Stahly, E. P. Mazzola, *J. Am. Chem. Soc.* **103**, 472 (1981)].
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28. Supported by National Cancer Institute grant CA 25967. We thank G. M. Christenson for assistance in obtaining seeds and plant samples of *B. megapotamica*.

22 May 1981; revised 14 July 1981

Direct Electrical Connections Between Transient Amacrine Cells in the Catfish Retina

Abstract. *Transient amacrine cells were identified by their intracellularly recorded response to flashes of light. These cells typically respond with a transient depolarization, often followed by a steady-state response during the stimulus. When two electrodes were placed in different transient amacrine cells, current of either polarity passed through one electrode produced a steady-state voltage change that was recorded by the electrode in the nearby cell. Following identification of the physiological type, transient amacrine cells were injected with horseradish peroxidase and the tissue was processed for light and electron microscopy. Both conventional chemical synaptic junctions and gap junctions were found to connect amacrine cells.*

Gap junctions have been found in both the outer and inner plexiform layers of the retina in several species (*1*). In the inner plexiform layer, amacrine cells form electrical junctions with bipolar cells (*2*). There are only two reports of gap junctions between amacrine cells: in the cat retina, between connected type A_2 (bistratified, narrow field) cells (*2*), and in the inner plexiform layer of the rat retina (*3*). However, physiological identification of these amacrine cells remains uncertain. Jensen and DeVoe (*4*) described a physiological type of amacrine cell which is probably similar to the transient amacrine cell discussed here. On the basis of dye coupling with other cells, they suggested that this type of cell might be electrically coupled to other amacrine cells. We report a class of amacrine cell which makes electrical connections with other physiologically identified amacrine cells.

Eyecup preparations of catfish retinas were continuously superfused with moist oxygen (*5*). Microelectrodes filled with 2*M* potassium citrate with or without horseradish peroxidase (HRP; 4 percent, weight to volume) were used to record from amacrine cells. After the electrophysiological experiment the retina was processed according to the procedure described by Christensen (*6*).

In some experiments two separate microelectrodes were used to record from individual transient amacrine cells locat-

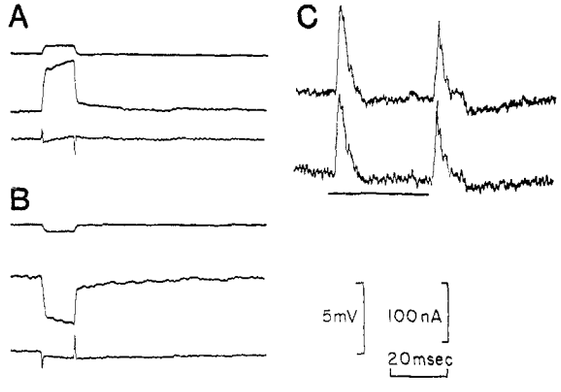

Fig. 1. (A and B) Voltage pulse applied to cell (top trace), voltage response from coupled cell (middle trace), and voltage response with electrode just outside the cell (bottom trace). (C) Intracellular response recorded with two separate electrodes, from transient amacrine cells during a flash of light. Length of bar indicates duration of light (0.5 second).

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