Journal of Natural Products
Vol. 51, No. 4, pp. 736-744, Jul-Aug 1988

THE MYSTERY OF TRICHOTHECENE ANTIBIOTICS IN *BACCHARIS* SPECIES

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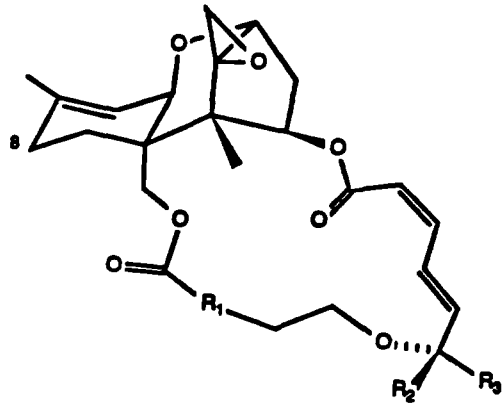
ABSTRACT.—The Brazilian higher plant *Baccharis coridifolia* has been shown to synthesize de novo a series of highly toxic macrocyclic trichothecene antibiotics heretofore found to be produced only by fungi. These compounds are produced only by female plants that have undergone pollination. Neither the male nor female plant is sensitive to the toxic effects of trichothecenes, whereas North American *Baccharis* species are. The macrocyclic trichothecenes found in *B. coridifolia* are the same as those produced by *Myrothecium* fungi, and it is suggested that the plant has acquired the toxin-producing genes from this fungus.

Several years ago, a Brazilian shrub, *Baccharis megapotamica* Spreng. (Asteraceae) was reported to contain a series of potent cytotoxic agents belonging to the trichothecene complex of antibiotics (1). This was surprising because the trichothecene mycotoxins heretofore had been found to be produced only by soil fungi, e.g., *Fusarium* and *Myrothecium* (2). Furthermore, the trichothecenes exhibit exceptionally high toxicity toward eucaryotic organisms, including high phytotoxicity (3,4). This is especially true of the macrocyclic trichothecenes (roridins, verrucarins, and baccharinoids; Figure 1), which have been shown to rank among the most potent phytotoxic agents (5). Subsequently, it was shown that, in addition to the baccharinoids (Figure 1) found in *B. megapotamica* (1), another Brazilian *Baccharis* species, *Baccharis coridifolia*, contained



1 Roridin A: R_1=CHOHCHCH$_3$, R_2=H, R_3=CHOHCH$_3$
2 Roridin D: R_1=CH−C(O)CH$_3$, R_2=H, R_3=CHOHCH$_3$
3 Roridin E: R_1=CH=CCH$_3$, R_2=H, R_3=CHOHCH$_3$
4 Baccharinoid B7: R_1=CHOHCHCH$_3$, R_2=H, R_3=CHOHCH$_3$, 8β-OH
5 Verrucarin A: R_1=CHOHCHCH$_3$, R_2,R_3=O
6 Verrucarin J: R_1=CH=CCH$_3$, R_2,R_3=O

FIGURE 1. Structures of the macrocyclic trichothecenes.

roridins A [**1**] and E [**3**] in appreciable quantity as well as smaller quantities of related toxins (6).

From the beginning of these initial findings, it was postulated that the true source of the plant-derived trichothecenes was a fungus. Evidence, albeit circumstantial, was obtained that supported this position: *B. megapotamica* fed roridin A [**1**] was shown to convert this fungal toxin into baccharinoid B7 [**4**], a compound found in this plant as it grows in Brazil (7). Furthermore, *B. megapotamica* grown in a local (University of Maryland) greenhouse over a period of several years did not produce trichothecenes, although none of these plants ever came into flower, a very important, though unrecognized point at that time (see below). Because these data were consistent with the hypothesis that fungus-produced roridins enter *B. megapotamica* and are transformed by this plant into baccharinoids (*B. coridifolia* presumably lacks the enzymatic system for the oxidative conversion of roridins to baccharinoids), we sought direct evidence of the participation of a fungus in this system. A chemical and microbiological survey of the *Baccharis* species was made in 1984, and the details of this investigation have been reported (8,9). No direct evidence of fungal participation was found, but we did show that in the case of *B. coridifolia*, the presence of roridins in this plant was widespread; i.e., plants collected from areas separated by 1000 km all contained very high concentrations of roridins that appeared to be concentrated in the flowering parts of the plants (9). The most interesting observation was that one sample, a single male *B. megapotamica* plant, contained no detectable quantity of macrocyclic trichothecenes, whereas all the other *B. megapotamica* and *B. coridifolia* samples (female or mixture of male and female plants) contained high quantities of these toxins (9).

Herein we report the results of a study that leads us to conclude that our original hypothesis as to the involvement of fungi with *Baccharis* was incorrect; the true situation is, remarkably enough, that Brazilian *Baccharis* synthesize de novo these toxins, and, in addition, the biosynthesis occurs only in the female plants after pollination.

RESULTS AND DISCUSSION

There are more than 400 species of *Baccharis* found in the New World, with the vast majority of them located in South and Central America (10). The plants are dioecious, with male and female inflorescences appearing on separate plants. Although some dioecious plants undergo spontaneous change from one sex to the other, this has never been observed to occur with a member of the Asteraceae (11), in which the *Baccharis* are classified.

In March 1987, *B. coridifolia* was collected from four Brazilian sites west-northwest of Santa Maria and from three sites west of Curitiba, a location over 1000 km northeast of Santa Maria. Within each of these two areas, the collection sites were separated by 10–20 km. At each site, both male and female plants (root and aerial parts) growing close to one another were collected and stored separately. Analysis (hplc; see Experimental section for details) of the various plant parts showed that the toxins were restricted to the inflorescences of the female plants. Neither the male plants nor female plants not in flower contained macrocyclic trichothecenes, while all of the female plants in flower contained high quantities (from a few hundred to more than 4000 ppm) of macrocyclic trichothecenes.

The various plant parts were assayed for the trichothecenes, and they were found only in the inflorescences of the female plants, where they were concentrated in the seeds. The carpels and pappus contained only about 10% the concentration of toxins found in the seeds. The total concentrations of the major macrocyclic trichothecenes found in the seeds ranged from 1000 to more than 16,000 ppm (Table 1). Furthermore, the seeds were dissected into the seed coats (ca. 30% by wt, which also included the en-

TABLE 1. Concentration in μg/g (ppm) of Roridins A [1], D [2], and E [3], and Verrucarins A [5] and J [6] in Seeds of Female *Baccharis coridifolia*.

Specimen No.[a]	Compounds					
	1	2	3	5	6	Total
2	750	960	1450	1600	1650	6410
3	650	900	5650	850	2100	10150
5	500	550	2650	470	1140	5310
7	510	760	4000	510	1050	6830
9	850	5800	4280	1920	2000	14850
12	800	640	3750	1880	1600	8670
15	280	110	510	370	520	1790
18	450	450	2150	1000	960	5010
22	800	950	2500	350	750	6350
28	640	390	2320	240	850	4440
37	650	750	2600	1250	1800	7050
38	280	100	700	280	900	2260
41	310	90	280	140	240	1060
47	80	90	1050	160	480	1960
49	490	650	3650	450	1400	6640
55	520	350	11400	1250	2800	16320

[a]Samples 2–22 were collected west of Santa Maria, and samples 28–55 were collected west of Curitiba.

dosperm and fruit coats) and cotyledons (ca. 70% by wt), and analysis of the separated parts showed that the toxins were restricted to the seed coats. Thus, the seed coats contained 0 3–5% by dry wt of these highly toxic substances (LD_{50}'s in mice = ca. 0.5 mg/kg) (4). The seeds of *B. coridifolia* weigh 200–800 μg each, but our method of analysis (hplc) can easily quantitate the trichothecenes in a single seed.

Analysis was carried out on 100 individual seeds of plant no. 12 (Table 1). All seeds contained appreciable quantities of macrocyclic trichothecenes with the total concentration ranging from 600 to 18,500 ppm. However, >90% of seeds had concentrations ranging between 2000 and 11,000 ppm. A statistical analysis showed a clear trend that the smaller seeds contained a higher concentration of toxins. Although this is consistent with the smaller seeds having higher surface areas relative to total weight, it is contrary to the expectation that larger seeds should accumulate higher concentrations of "defensive chemicals" such as trichothecenes (12). In general, the ratios of the toxins in the individual seeds were similar to the average values (Table 1) though a small percentage of the seeds did have extreme variations in their toxin ratios. The concentrations of the toxins were reasonably stable over a four-month period with the exception of roridin E [3], which dropped in concentration by 10–20% in seeds stored at room temperature.

In April of 1987, *B. coridifolia* plants that had been raised from seed in a local (University of Maryland) greenhouse began to flower. Three male and three female plants were placed together in an area, and a fourth female plant was placed in an isolated area. The inflorescences of these plants were monitored over a two-month period for the presence of macrocyclic trichothecenes. At no time were any of these toxins observed in the male plants or in the isolated female plant. However, as the three female plants that were mixed in with the male plants began to produce maturing seeds, the toxins were detected, and their concentrations in the seeds increased with time. Thus, it would appear that the biosynthesis of the macrocyclic trichothecenes in *B. coridifolia* is restricted only to pollinated female plants.

These data have now forced us to question seriously our original hypothesis that the initial source of the macrocyclic trichothecene toxins is a fungus. In part, this earlier no-

tion was very attractive because the major toxins observed in the seeds of *B. coridifolia* are exactly those same toxins typically produced by cultures of *Myrothecium verrucaria* and *Myrothecium roridum*: roridins A [**1**], D [**2**], and E [**3**], and verrucarins A [**5**] and J [**6**]. In fact, isolates of *M. verrucaria* and *M. roridum* have been found in the rhizosphere and on the leaves and flowers of these Brazilian *Baccharis* species (9); however, their number was small and represented no more than 1% of the fungal colonies isolated (9). In addition, careful examination for fungal endophytes (13) in root, leaf, and floral tissue of *B. coridifolia* by electron microscopy[1] was negative. Seeds of *B. coridifolia* that were surface sterilized, crushed, and cultured for fungi gave no positive indications for the presence of intra-seed fungi. *Baccharis* plants fed trichothecenes via the root system absorbed and translocated these compounds in an evenly distributed manner to the flowers as well as to the leaves; while in the Brazilian plants growing in the field, the toxins could not be detected in the leaves.

We believe that the conclusion is inescapable: *B. coridifolia* produces de novo macrocyclic trichothecenes. And, although we have yet to carry out detailed analyses of *B. megapotamica*, the current data give us every reason to believe the situation with this plant species is analogous to that of *B. coridifolia*.

Analyses of the seeds of *Baccharis halimafolia* native to and collected in Maryland and New Jersey in the fall of 1987 were negative for the presence of macrocyclic trichothecenes. In addition, this plant, as well as another North American species, *Baccharis sarothroides* (desert broom), is extremely sensitive to these toxins, whereas *B. megapotamica* (7) and *B. coridifolia* (both male and female, see below) show no apparent sensitivity to the trichothecene mycotoxins.

The question now is: How are these Brazilian *Baccharis* species able to produce exactly the same set of complex terpenoid antibiotics as is produced by *Myrothecium* fungi? If *Baccharis* species have acquired the ability to produce macrocyclic trichothecenes by convergent evolution, it is a remarkable feat considering the complex nature of these compounds, although there are some striking examples of this occurring in nature, e.g., prostanoids being produced by mammals and soft coral (14).

Another interesting possibility is that the genetic information necessary to synthesize these complex terpenoids was acquired from a fungus [*Myrothecium* is a likely candidate (15)]. Such transferences to higher plants of genetic material from viruses and bacteria are known to occur (16). However, to our knowledge, no verifiable case of such a transference and expression of fungal genes into higher plants is known (17). Although the number of genes involved in the synthesis of macrocyclic trichothecenes is unknown, it certainly must be substantial (18). If they were arranged together on a single plasmid, their transference into the plant chromosomes, in principle, might be accomplished. However, in the trichothecene-producing fungus *Fusarium*, the genes responsible for the biosynthesis of the trichothecenes are found not on plasmids but in the nuclear DNA.[2] Furthermore, the genes appear not to be arrayed contiguously but rather are dispersed at a variety of chromosomal sites.[2] It is difficult to see how such an arrangement of fungal genes could be "genetically engineered" into a plant host (19,20), though the evolutionary principles of such a transfer have been discussed (21).

If indeed *Myrothecium* and *Baccharis* share a common set of genes coding for macrocyclic trichothecene biosynthesis, the biosynthetic pathway leading from mevalonate to roridin E [**3**] should be the same (or at least very similar) for these two organisms.

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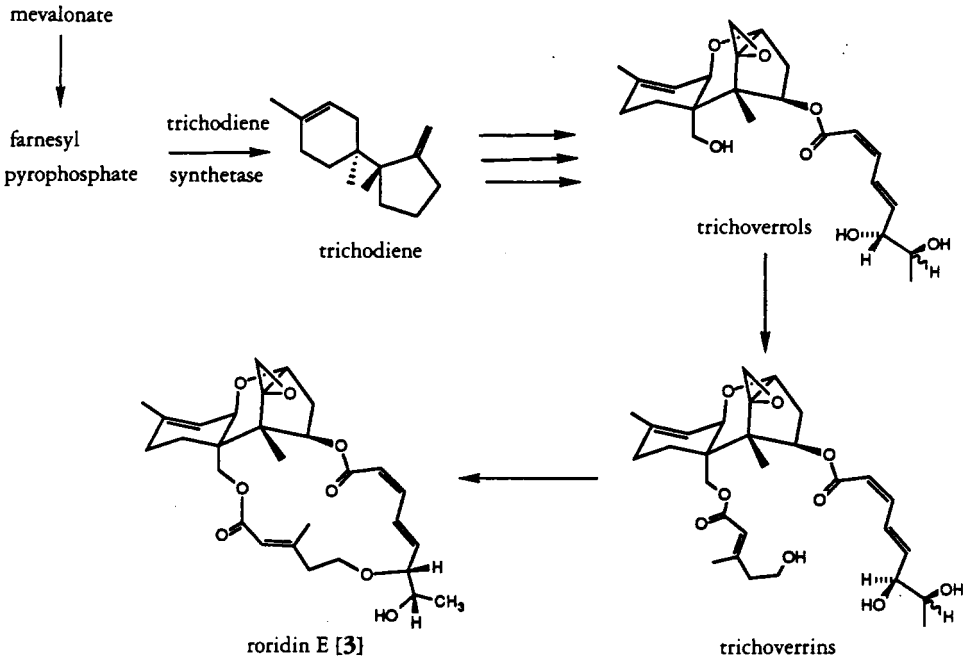
[1]J.D. Miller, Agriculture Canada, Ottawa, Canada, unpublished results.

[2]M. Beremand, A.E. Desjardins, and T.M. Hohn, NRRL, USDA, Peoria, IL, personal communication.

There is perhaps more known about the biosynthesis of trichothecenes in fungi (22,23) than any other sesquiterpene, and Scheme 1 outlines the general course of biosynthesis of the macrocyclic trichothecenes by *Myrothecium* (22,23).

We have carried out preliminary experiments with *Baccharis* to evaluate whether, similar to *Myrothecium*, these plants can convert trichoverroids (23) to the macrocyclic trichothecenes. A mixture of trichoverrins A and B (23) was absorbed by stems of both male and female *B. coridifolia* plants. Analysis of leaf and floral tissue after 1 day showed that both contained appreciable amounts of trichoverrins and roridin E [3]. After 7 days, the tissues contained high concentrations of both trichoverrins (>2000 ppm) and roridin E (500–1000 ppm). These results were observed both in stems without flowers and in stems bearing immature female flowers. Control plants that had not been treated



SCHEME 1. Biosynthesis of macrocyclic trichothecenes.

with the trichoverrins gave no evidence of containing either trichoverrins or roridin E [3]. When these experiments were repeated with *B. sarothroides*, surprisingly, this plant also absorbed the trichoverrins and converted them to roridin E [3] in a manner similar to that of *B. coridifolia*. However, the male and female *B. coridifolia* plants gave no sign of injury in these experiments, whereas *B. sarothroides* was severely damaged (leaves turned brittle and fell off after about 1 day). These experiments were repeated with trichoverrols A and B (Scheme 1), which are trichoverroids isolated from both *Myrothecium* (23) and *B. megapotamica* (24), and again *B. sarothoides* was rapidly killed by the trichothecenes whereas *B. coridifolia* plants showed no signs of injury. However, neither *Baccharis* species gave any indication that trichoverrols A and B had been transformed into either the trichoverrins or roridin E [3]. These experiments suggest that both *B. coridifolia* and *B. sarothroides* contain a cyclase that catalyzes the trichoverrin to roridin E conversion but lack the enzyme system necessary to transform earlier biosynthetic precursors to the macrocyclic trichothecenes. Presumably, these enzyme systems would be turned on in the female *B. coridifolia* plant following pollination, although this would have to be confirmed by further experimentation.

That Brazilian *Baccharis* species synthesize de novo the macrocyclic trichothecenes raises a number of intriguing questions. Although others have suggested that the common appearance of certain secondary metabolites in fungi and higher plants may be the result of interspecific gene transfer (25), proof of this through experimentation has been lacking. However, recent work with the biochemistry and genetics of trichothecene production in *Fusarium*[2] (26,27) suggests that the tools are now at hand, employing the techniques of molecular biology, to ascertain whether *Myrothecium* and *Baccharis* do indeed share a common (or near common) set of genes that code for the trichothecene synthetases.

Whether the origin of the trichothecene-coding genes in Brazilian *Baccharis* is the result of an interspecific genetic transfer or convergent evolution, it is a remarkable phytochemical tag. Thus, trichothecenes in *Baccharis* plants may serve as taxonomic markers: The appearance of these toxins can be employed to established the phylogenetic relationships (28,29) in *Baccharis* found through the New World.

EXPERIMENTAL

PLANT COLLECTIONS.—Four collections (sites I–IV) were made along a road west from Santa Maria to Santiago in Rio Grande do Sul state. Three collections (sites V–VII) were made along a road west from Curitiba to Palmeria in Parana. The plants, which were in flower at the time of the collection (late March 1987), were growing in pastures and clearly visible from the road. The male and female plants growing close to one another were collected separately along with root samples. The following (*B. coridifolia* unless otherwise stated) were collected from each site (specimen no.): site I, 2 female plants (nos. 2 and 3), 1 male plant (no. 1); site II, 2 female plants (nos. 5 and 7) 2 male plants (nos. 4 and 6); site III, 2 female plants (nos. 9 and 12), 2 male plants (nos. 8 and 13), soil from specimen 8 (no. 11), soil from specimen 9 (no. 10), 12 seedlings (not in flower) (no. 14); site IV, 3 female plants (nos. 15, 18, and 22), 3 male plants (nos. 16, 19, and 23), roots from specimens 15 (no. 17), 16 (no. 17a), 18 (no. 20), 19 (no. 21), 22 (no. 24), and 23 (no. 25); site V, 3 female plants (nos. 28, 37, and 38), 3 male plants (nos. 26, 28, and 36), roots from specimens 26 (no. 30), 29 (no. 31), 36 (no. 33), 28 (no. 32), and 37 (no. 34), an immature stem (no flowers) from specimen 28 (no. 35), and 1 male *B. trimera* plant; site VI, 2 female plants (nos. 41 and 47), an immature stem (no flowers) from specimen 41 (no. 40), 2 male plants (nos. 39 and 46), roots from specimens 40 (no. 42), 41 (no. 43), 46 (no. 44), and 47 (no. 45); site VII, 2 female plants (nos. 49 and 55), 3 male plants (nos. 51, 52, and 53), roots from specimens 49 (no. 48), 53 (no. 54), and 55 (no. 56), and 1 female *B. trimera* (no. 50). When possible, the samples were refrigerated, and, upon return to the United States, the samples used for chemical analysis were air-dried and stored at room temperature. All voucher specimens are stored at the Department of Botany, University of Maryland.

MICROBIAL ANALYSIS.—Root tissue, soil surrounding roots, seeds, and leaf and floral tissues were cultured in a manner previously described (8,9) with the minor modification of employing a cellulose-Czapeks agar medium (30), which allows the more efficient isolation of *Myrothecium* species. Three species of *Myrothecium*, *Myrothecium cinctum* Corda, *M. roridum* Tode, and *M. verrucaria* Albertini and Schweinitz (31) were cultured from various sources (number of isolates/number of samples): roots (16/21), soil (15/21), and leaves (18/18). In spite of the relatively high frequency of isolation, *Myrothecium* spp. did not comprise more than 1% of the fungal colonies isolated. Of the *Myrothecium* isolates, 36 were tested for trichothecene production by bioassay (brine shrimp and tests in agar plates for growth inhibition of *Chlorella* sp. and *Ustilago* sp., the details of which are to be published elsewhere). A total of 21 isolates (19 *M. cinctum* and two *M. verrucaria*) were nontoxic; 3 isolates (two *M. cinctum* and one *M. verrucaria*) were moderately toxic, and 12 isolates (11 *M. verrucaria* and one *M. roridum*) were strongly toxic. Hplc and tlc analysis of extracts of rice cultures (9) of these isolates indicated that the strongly toxic isolates were producing roridins and verrucarins. From isolate MV-29 (strongly toxic), verrucarins A [**5**] and J [**6**] and roridin A [**1**] were isolated from the rice culture and their identities established by comparison of their spectral data with those of authentic samples.

CHEMICAL ANALYSIS.—Standard samples of roridins A [**1**], D [**2**], and E [**3**] and verrucarins A [**5**] and J [**6**] were obtained from cultures of *M. verrucaria* as described previously (15). Initially, plant samples were analyzed either as roots or upper parts (leaves and inflorescences combined). However, upon more careful examination, it became clear that the trichothecenes were concentrated in the inflorescences. Leaves and inflorescences from all of the female plants and male plants were separated by hand and analyzed separately as described below. The method of analysis (see below) was sufficient to detect and quantitate ca. 10 ppm in leaf samples and ca. 1 ppm in seed and root samples. This was determined by adding quantities

(0.5, 1, 5, 10, 25, 50 μg/g of plant tissue) of roridin E [3] to leaves of *B. coridifolia* and *B. halimafolia* and to seeds and roots of *B. halimafolia* followed by hplc analyses.

Within the limits of detection, no trichothecenes could be detected in any of the root samples (specimen nos. 17, 17a, 20, 21, 24, 25, 30–34, 42–45, 48, 54, and 56), leaves of male plants (specimen nos. 1, 4, 6, 8, 11, 16, 19, 23, 26, 27, 29, 46, and 51–53), or immature plants lacking flowers (specimen nos. 14, 35, and 40). In only three of the specimens of female plants (nos. 7, 9, and 55) could trichothecenes be detected in the leaves and then only at trace levels (ca. 5–10 ppm), which could easily have resulted from contamination from the inflorescences. All of the male inflorescences also were devoid of measurable quantities of trichothecenes with the exception of specimen nos. 8 and 53, which showed traces of roridins A (<10 ppm) and E (<10 ppm). Again, these plants were growing in close proximity to female plants (specimen nos. 9 and 55, respectively) from which they may have been contaminated. Plant tissue (leaves and inflorescences combined, 1 g) was ground and extracted with MeOH (3 × 5 ml) under sonication, the MeOH removed under vacuum, and the residue taken up in 1.0 ml CH_2Cl_2, and 0.25 ml of this solution was passed through a small column (10 cm × 0.7 cm) of 40–60-μm Si gel. The column was washed with CH_2Cl_2 (8 ml, discarded) and 8 ml of 5% MeOH in CH_2Cl_2. The latter fraction was concentrated to dryness under N_2 and taken up to 1.0 ml of CH_2Cl_2. Aliquots (5 μl) of this solution were analyzed on a Gilson Model 302 hplc equipped with a Knauer variable wavelength monitor (at 260 nm) and a Shimadzu Chromatopac C-R3A integrator. Samples were injected onto a 5-μm silica Zorbex (250 × 4.6 mm) column and eluted with a mixture of 1% iPrOH in EtOAc (60%) and hexane (40%) at a flow rate of 1 ml/min. The following retention times were observed: verrucarin J, 5.5 min; verrucarin A, 6.6 min; roridin E, 8.4 min; roridin D, 9.3 min; roridin A, 11.0 min. Those peaks that matched in retention time the above standards were analyzed with an LKB 2140 Rapid Spectra Diode Array Detector interfaced with an IBM XT-PC microcomputer. This system recorded and plotted the uv spectra (220–300 nm) of the peaks on the chromatogram, and the uv spectra of the relevant peaks matched the uv spectra of the standards. In addition, the remaining extracts from the seeds of the female plants were combined, and samples of roridins A, D, and E and verrucarins A and J were isolated by chromatographic procedures. ^{1}H-nmr spectroscopy (200 MHz) confirmed the identity of the compounds.

The analysis of the seeds (ca. 100-mg samples) was carried out in essentially the same manner except that 5% MeOH in $CHCl_3$ was used as the extraction solvent because preliminary tests indicated that it was somewhat more efficient than 100% MeOH in extracting roridins and verrucarins. For quantitation, samples were run in triplicate, and standard curves for the roridins and verrucarins were generated by hplc analysis of solutions containing known amounts of the standards. The results reported (Table 1) are ± 10%.

Individual seeds (200–800 μg) from specimen no. 12 were weighed on a Cahn microbalance and extracted with 1 ml of 5% MeOH/$CHCl_3$ under sonication for 30 min. The solution was filtered through a plug of Si gel (2 cm in depth in a Pasteur pipette) that was washed with 2–3 ml of 5% MeOH/CH_2Cl_2. The eluate was concentrated to dryness under N_2 and taken up in 25 μl of $CHCl_3$, and 5 μl of solution was analyzed by hplc as above.

Seeds of *B. halimafolia* were collected from New Jersey (late October 1987) and Maryland (four collections from mid-October to mid-November 1987). Samples (ca. 500 mg each) of seeds were extracted as analyzed by hplc as described above, and no trace of the trichothecenes could be detected. The seeds appear to be relatively free of extractable material, since less than 2% by weight of these seeds was extracted by the 5% MeOH/$CHCl_3$ solvent. Seeds from each of these collections were shown to be viable by their ability to germinate, though under normal conditions (wet filter paper) they do so only after a two-week period of time.

ANALYSIS OF FLORAL PARTS.—The inflorescences (ca. 0.5 g each) of plants no. 12 and no. 55 were divided into three portions: calyx and petals, pappus (removed from the seed with a razor blade), and seeds. The tissues were extracted with 5% MeOH/$CHCl_3$ and subjected to hplc analyses as described above. The distribution of trichothecenes in each of the tissues was similar, but the total relative concentrations of the trichothecenes in the various tissues were ca. 10 (seeds): 1 (pappus): 1 (calyx + petals) in both plant specimens.

ANALYSIS OF SEED COATS.—Ten seeds (4.5 mg dry wt) from specimen no. 55 were soaked for 2 h in H_2O and then dissected under a microscope into two parts: cotyledon (3.2 mg dry wt) and seed coat (which included the endosperm and fruit coat) (1.3 mg dry wt). Hplc analysis was carried out as described above for individual seeds. The cotyledon fraction appeared to contain traces of the trichothecenes, but the amounts (<1 ppm) were too low to quantitate or even to confirm their presence. However, the other seed part (1.3 mg, seed coat, endosperm, and fruit coat) contained the following trichothecenes (amount present): verrucarins A (5 μg) and J (10 μg), roridins A (2 μg), D (2 μg), and E (48 μg).

GREENHOUSE STUDIES.—Seeds of *B. cordifolia* collected in 1984 (9) were germinated on wet filter paper and transferred to potting soil. After a two-year period, the plants began to go into senescence in April 1987, and inflorescences began to appear. Three male and three female plants were placed next to one

another and a fourth female plant was transferred to another greenhouse. During the next two-month period, various parts of the plants were harvested (0.5–1 g each) and analyzed by hplc analysis. At no time during the course of this experiment were the trichothecenes detected in the leaves or inflorescences of male plants or from the leaves and inflorescences of the isolated female plant or from the leaves of the other female plants. Approximately 1 month after the first appearance of inflorescences in the female plants that were mixed in with the male plants, hplc analysis of the seeds revealed the presence of small amounts of trichothecenes (ca. 5–10 ppm). During the next six-week period, the maturing seeds were monitored by hplc analysis which indicated a steady increase in the concentration of verrucarins A [**5**] and J [**6**] and roridins A [**1**], D [**2**], and E [**3**] in all three plants. At the end of this period, the highest concentrations observed in the seeds were verrucarin A (95 ppm), verrucarin J (160 ppm), roridin A (70 ppm), roridin D (50 ppm), and roridin E (250 ppm).

ABSORPTION OF TRICHOTHECENES BY *BACCHARIS*.—Stem cuttings of *Baccharis* plants (ca. 30 cm) were set in a solution made by dissolving 0.05 g of trichoverrins or trichoverrols (23) in 0.5 ml of Me_2CO and adding this to a 100-ml solution of distilled H_2O containing 10 μM $CaSO_4$. Control seedlings were placed in a solution containing 0.5 ml of Me_2CO in 100 ml of 10 μM $CaSO_4$. After 1, 2, and 7 days, samples of plants (ca. 50 mg) were extracted and analyzed as described above.

Hplc analysis conditions were altered by adjusting the percent EtOAc (1% iPrOH)/hexane to 70% EtOAc (1% iPrOH)/30% hexane after 10 min run time. Under these conditions, the retention times of the trichoverrols and trichoverrins were 16 and 18 min, respectively. All three plants (male and female *B. coridifolia* and *B. sarothroides*) absorbed, translocated, and converted the trichoverrins on days 1 and 2 to essentially the same extent: day, concentration of trichoverrins, concentration of roridin E: day 1, 1000, 100; day 2, 1500, 250. However, the leaves of *B. sarothroides* began to fall off after a day or two, and by day 7 the plant was dead. For the *B. coridifolia* plants, the concentrations of trichoverrins and roridin E were 2000–3000 ppm and 500–600, respectively, on day 7 of the experiment. The structure assignment was based on hplc retention times and uv spectral analysis employing the diode array detector. Neither the male nor female *B. coridifolia* plants showed any obvious distress over this seven-day period. When this experiment was repeated employing trichoverrols, the test plants absorbed these trichothecenes to essentially the same degree as they had absorbed the trichoverrins, but at no time was any roridin E detected in any of the plants. Again, *B. sarothroides* was killed, whereas *B. coridifolia* appeared to be uninjured by the trichoverrols. The *B. corifidolia* stems used in these experiments either lacked flowers or, if female, had immature seeds.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (NSF-INT-86-01994) and the National Institutes of Health (NIH-CA-25967). We thank Dr. J.D. Miller for providing electron microscopy data and for helpful comments.

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Received 5 February 1988