Chapter 14
Macrocyclic Trichothecenes of *Baccharis*

Alessandra Caroline Montes Frade, Maria Beatriz de Oliveira Rabelo, Priscilla Rodrigues Valadares Campana, Rodrigo Maia de Pádua, and Fernão Castro Braga

Abstract Trichothecenes constitute a large group of sesquiterpene metabolites. They are classified into four different groups (types A, B, C, and D) according to their characteristic functional groups. These structural differences result in distinct biological activities. About 40 macrocyclic trichothecenes have been isolated so far from three species of *Baccharis* (*Baccharis megapotamica*, *Baccharis coridifolia*, and *Baccharis artemisioides*) and their chemical structures have been defined by spectroscopic analyses, especially by using nuclear magnetic resonance (NMR) and mass spectrometry (MS) data. It was initially supposed that trichothecenes found in *Baccharis* species originated from fungi present in the soil, such as *Myrothecium* species. Endophytic fungi synthesize exclusively simple trichothecenes, while some macrocyclic trichothecenes have been isolated from *B. coridifolia* and *B. megapotamica*, indicating that they are produced by the plants. The biosynthetic pathway for the production of macrocyclic trichothecenes is not fully elucidated and to date, only a few intermediates and final products have been isolated and characterized. Macrocyclic trichothecenes have been reported to possess antifungal, antimalarial, antitumor, and antiviral activities. The effects of trichothecenes on animal and plant cells include inhibition of DNA and RNA syntheses, inhibition of mitochondrial function, membrane destabilization, changes in cell division, and apoptosis. Additional phytochemical and biological studies with *Baccharis* species are demanded to better understand the chemical and medicinal properties of trichothecenes, aiming at future exploitation.

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1 General Aspects of Trichothecenes

Trichothecenes comprise an important group of mycotoxins, usually responsible for human and animal intoxications (for more information, see Chap. 11 "Livestock intoxication by *Baccharis*"), which also plays a significant role in the pathogenicity of some plants (Jarvis et al. 1991,1996).

The accumulation of trichothecenes in agricultural products, such as rice, oats, rye, barley, and wheat, leads to great economic losses due to the destruction of crops by plant intoxication (McCormick et al. 2011). Their ingestion by humans and animals causes clinical symptoms of acute intoxication, such as subcutaneous hemorrhaging, alimentary toxic aleukia (ALA), deterioration of throat tissue, exhaustion of bone marrow, abortion of fetuses, and even death (Kumari et al. 2016).

Trichothecin (6) was the first isolated trichothecene, resulting from the screening of antifungal metabolites in *Trichothecium roseum* culture. This compound is also produced by several species of fungi from the genera *Fusarium, Myrothecium, Trichoderma*, and *Cephalosporium*. Subsequently, other trichothecenes, designate verrucarins and roridins, were isolated from *Myrothecium verrucaria* and *Myrothecium roridum*, respectively (Ueno 1980).

These mycotoxins are divided into two groups: simple trichothecenes, such as T-2 toxin (1), diacetoxiscirpenol (2), and deoxynivalenol (3), and macrocyclic trichothecenes, like baccarinoids, roridins, and verrucarins. These compounds have several biological activities, including antimicrobial, cytotoxic, and insecticidal properties, besides being phytotoxic (Jarvis et al. 1991).

Before discovering the presence of macrocyclic trichothecenes in *Baccharis megapotamica* Spreng, these compounds had been isolated from soil fungus species. Thereafter, other trichothecenes isolated from *Baccharis coridifolia,* popularly known as "mio-mio" or "romerillo," have been reported (Jarvis et al. 1987c). Later, Rizzo and collaborators (Rizzo et al. 1997) found roridins and verrucarin in *Baccharis artemisioides.*

The genus *Baccharis* (Asteraceae) encompasses about 500 species, mainly distributed in Brazil, Argentina, Paraguay, and Uruguay (Verdi et al. 2005). In those countries, *B. coridifolia* is considered to be one of the principal poisonous plants to herbivorous mammals, causing severe losses among cattle (Habermehl et al. 1985).

Throughout this chapter, all macrocyclic trichothecenes isolated so far from *Baccharis* species (*B. megapotamica*, *B. coridifolia*, and *B. artemisioides*) will be reviewed. Also, the biosynthesis of these compounds will be discussed, as well as their already investigated biological activities.

2 Classification and Structure of Trichothecenes

Trichothecenes constitute a large group of sesquiterpene metabolites. They generally have a common nucleus, represented by a rigid tetracyclic ring, consisting of a cyclohexene ring (ring A with a C9-C10 double bond) attached to a tetrahydropyran ring (ring B) and a cyclopentyl ring (ring C), in addition to an epoxide at the C12-C13 position (Shank et al. 2011). The rigidity of the system results in a distinct stereochemistry, in which ring A adopts a half-chair conformation and ring B is found in a chair conformation (Jarvis et al. 1990). These chemical structures are represented in Fig. 14.1. The trichothecenes differ otherwise in the patterns of side-chain oxygenation and esterification at carbons 3,4,7,8,15 and in the presence of a keto group at C8 (represented by R in Fig. 14.1a) (Desjardins et al. 2007).

The trichothecenes are classified into different groups according to their characteristic functional groups. Type A compounds have the simplest structures, the C8 position is esterified, hydroxylated, or unsubstituted, while type B trichothecenes have a carbonyl at the C8 position. Type C derivatives have an additional epoxide goup at C7-C8 or C8-C9 positions, while type D trichothecenes have a macrocyclic ring between the C4 and C15 positions (Wu et al. 2013; Terciolo et al. 2018). These structural differences result in distinct biological activities. Examples of chemical structures representative of each group of trichothecenes can be seen in Fig. 14.2.

Typically, trichothecenes are resistant to degradation by environmental factors, like air and light, but may be affected by the presence of bacteria or fungi. They are nonvolatile compounds with a low molecular weight (between 250-550 Da), naturally occurring as colorless, crystalline, and optically active solids. They are highly soluble in acetone, ethyl acetate, chloroform, dimethyl sulfoxide (DMSO), ethanol, methanol, and propylene glycol. Trichothecenes are not inactivated by autoclaving but can be effectively deactivated under strong acid or alkaline conditions (Wu et al. 2013; Sudakin 2003).

Several methods can be used for the analysis and identification of these substances. When trichothecin (6) was first isolated in 1948, its structure could not be

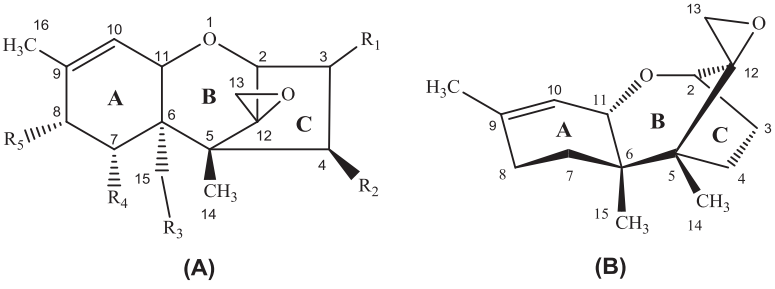


Fig. 14.1 (**a**) Chemical structure of the trichothecene core. (**b**) Three-dimensional stereochemistry of the trichothecene core. (Source: Shank, adapted (Shank et al. 2011))

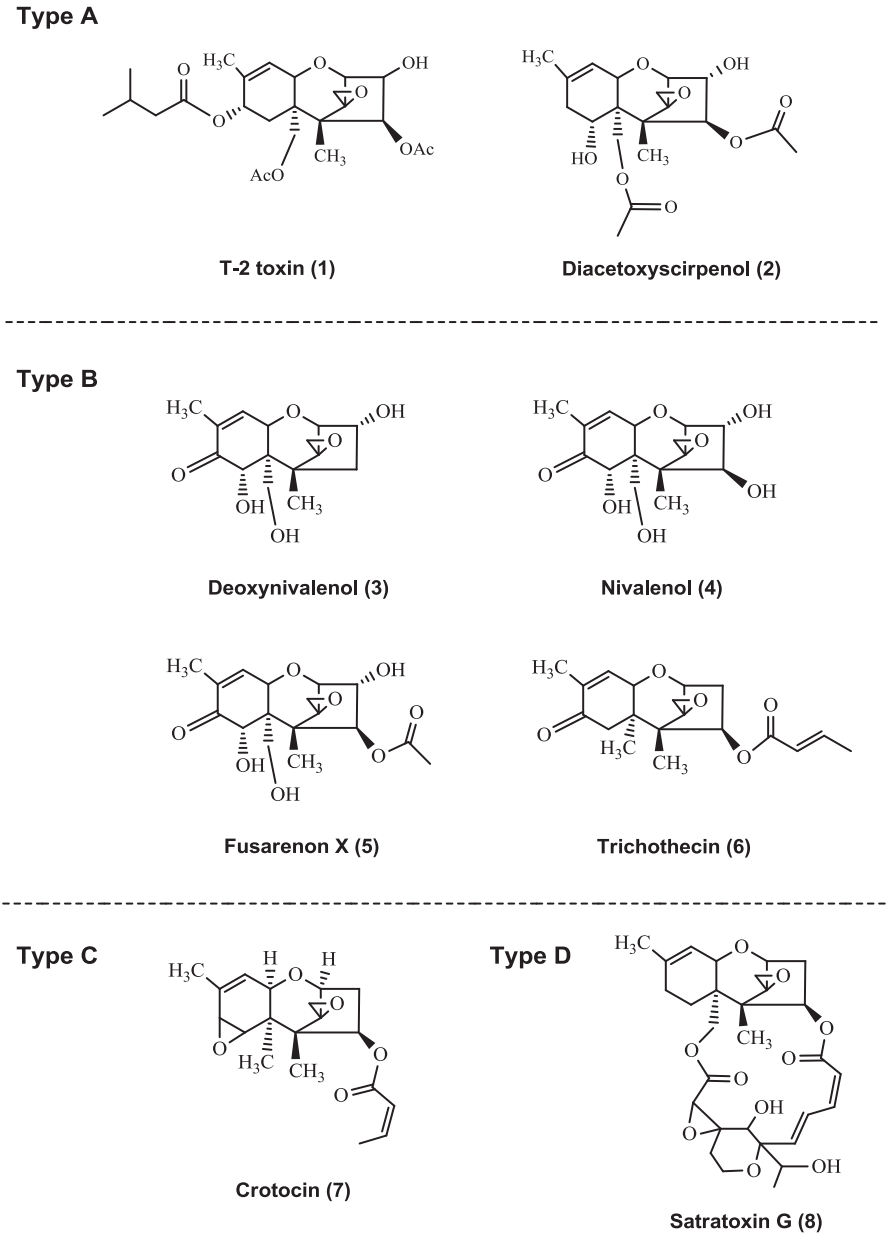


Fig. 14.2 Chemical structures of types A-D trichothecenes. (Source: Terciolo, adapted (Terciolo et al. 2018))

determined by the available methods (Shank et al. 2011). The chemical structure of trichothecin was reported only in 1959 by using chemical modifications, which allowed the determination of the toxin nucleus and substituent groups (Freeman et al. 1959).

The number of isolated trichothecenes with identified chemical structures started to increase after new structure elucidation techniques became available. A fundamental technique for structural elucidation and stereochemical assignment of trichothecenes was nuclear magnetic resonance (NMR) (Shank et al. 2011). Trichothecenes have some relevant structural features, easily identified by NMR data. For example, the ^{1}H methylene coupling of the epoxide ring is found between 2.7 to 3.4 ppm, with a scalar coupling constant of approximately 4 Hz. The methyl hydrogens at C-14 appear as a sharp singlet, while the methyl hydrogens at C-16 appear as a broad singlet, because of the long-range coupling with H-10. In addition, the rigidity of the ring system can explain the long-range coupling observed between H-7 and H-11 (Savard et al. 1987).

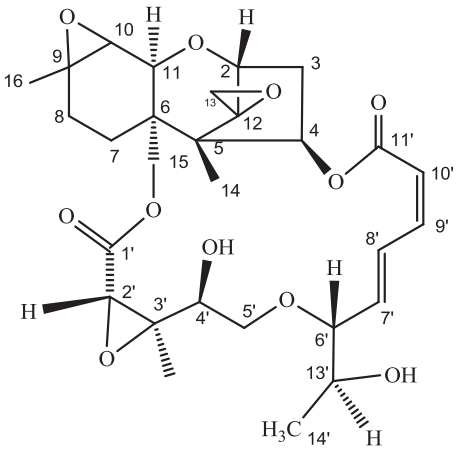
In the ^{1}H NMR spectra of macrocyclic trichothecenes, H-2′ resonates at 5.2 ppm in 2′-enes and at 3.4 ppm in 2′,3′-epoxides. In verrucarins, H-7′ and H-10′ appear as doublets between 5.8 and 6.2 ppm, H-9′ is found as a triplet between 6.5 and 6.6 ppm, and H-8′ is seen as a double doublet at 8.1 ppm. In roridins, the configuration at C-6′ determines the value of $J_{6',7'}$, which is 6 Hz in compounds where C-6′ has *S* configuration and 2 Hz if C-6′ has *R* configuration. Regarding ^{13}C NMR data, the chemical shift values reported for carbons of the trichothecene nucleus are very similar, whereas changes occur in the macrocyclic ring. For example, in (6′-*R*)-roridins, C-13′ resonates at higher frequencies (1-2 ppm) in 13′-*R* compounds in comparison to diastereoisomers with 13′-*S* configuration, as found in baccharinoids B13 (20) and B14 (21) (Grove 1993).

The ^{1}H and ^{13}C NMR spectral data reported for baccharin (9), a macrocyclic trichothecene, are depicted in Table 14.1.

Table 14.1 ^{1}H and ^{13}C NMR spectral data reported for baccharin (δ in ppm)[a]

Position	H	C	Position	H	C
2		78.1	1′		167.4
3	2.48	34.0	2′	3.37	56.0
4	5.80	73.8	3′		64.4
5		48.5	4′		75.3
6		42.3	5′		72.1
7		16.7	6′		86.5
8		25.7	7′	5.98	138.2
9		57.7	8′	7.48	125.1
10	3.11	56.9	9′	6.60	142.6
11		66.6	10′	5.82	117.4
12		65.2	11′		166.3
13	2.75 / 3.16	47.1	12′	1.65	11.6
14	0.75	6.5	13′		71.0
15	4.24 / 4.42	63.1	14′	1.20	17.7
16	1.83	21.6			

[a]Spectra measured in $CDCl_3$. Source: Kupchan (Kupchan et al. 1976; Kupchan et al. 1977)



Baccharin (9)

Rosen et al. (1986) converted some macrocyclic trichothecenes into their trimethylsilyl derivatives for their analysis by gas chromatography coupled to mass spectrometry (GC/MS), since the presence of polar, labile ester bridges in the molecules difficult their direct analysis by this technique. Other methods of detection and quantification were posteriorly introduced, with improved sensitivity, selectivity, and accuracy, by using direct chemical ionization tandem mass spectrometric techniques with minimal amounts of samples (Krishnamurthy and Sarver 1989).

Roridins and baccharinoids were identified in Brazilian *Baccharis* species by a direct chemical ionization tandem mass spectrometry (MS/MS) method. A mixture of these compounds was subjected to chemical ionization in the presence of ammonia followed by sequential collisionally activated dissociation of the specific adducts using argon. The identification occurs by detection of specific daughter ions and their associated parent ions (Krishnamurthy and Sarver 1989). Table 14.2 lists some chemical ionization MS data reported for macrocyclic trichothecenes.

Later, high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS), employing a reversed-phase column and thermospray ionization, allowed the unambiguous identification of isomeric baccharinoids (Krishnamurthy and Sarver 1989). The ions observed in the mass spectra revealed that structurally similar compounds undergo common fragmentation forms and neutral losses (Krishnamurthy and Sarver 1989). Thus, Krishnamurthyt and Sarver (1989) identified several daughter ions of macrocyclic trichothecenes. Table 14.3 lists the mass spectral data reported for the most significant daughter ions formed by thermospray ionization, whereas their chemical structures are depicted in Fig. 14.3.

The identification of daughter ions structures and neutral losses made it possible to propose fragmentation pathways for these compounds. Thus, it was observed that most of the fragments are formed by the bond break between the ester group and ether bonds in the exocyclic ring (Krishnamurthy and Sarver 1989).

Table 14.2 Chemical ionization mass spectra data (M^- ions) reported for some macrocyclic trichothecenes of *Baccharis*

Compound	MW	*m/z* (% relative abundance)
Baccharinol (11)	562	562 (3.8); 501 (10.1); 417 (100.0); 375 (2.4); 365 (29.9); 161 (1.4); 153 (19.9); 143 (12.8); 141 (1.2); 135 (12.9); 125 (12.2)
Roridin A (30)	532	402 (35.8); 401 (100.0); 153 (19.6); 145 (69.8)
Roridin D (31)	530	530 (1.7); 417 (1.3); 401 (100.0); 359 (2.3); 349 (1.0); 193 (2.5); 175 (1.1); 153 (7.1); 151 (1.2); 149 (2.5); 145 (3.1); 135 (13.1); 127 (1.2)
Roridin E (32)	514	514 (14.9); 484 (7.0); 470 (2.1); 403 (71.4); 386 (3.4); 385 (17.2); 359 (100.0); 154 (6.2); 153 (20.3); 136 (10.7); 134 (3.8); 111 (8.1)

Source: Krishnamurthy, adapted. (Krishnamurthy and Sarver 1989)

Table 14.3 Mass spectral data reported for the most significant daughter ions of some macrocyclic trichothecenes

Compound	MW	m/z
Baccharinol (11)	562	137, 247, 391
Roridin A (30)	532	249, 333, 403
Roridin D (31)	530	231, 249, 403
Roridin E (32)	514	137, 231, 361

Source: Krishnamurthy, adapted (Krishnamurthy and Sarver 1989)

Figure 14.4 shows the fragmentation pathway proposed for roridin A. Cleavage of C4-O and C6'-O bonds originates the neutral fragment of 154 Da, whereas cleavages between C3'-C4' and C6'-O result in neutral losses of 46 Da (ethanol). In its turn, cleavages at C1'-O and C6'-O bonds give neutral losses of 130 Da. The daughter ion at *m/z* 333 can be formed by neutral losses of 200 or 154 Da, followed by a loss of 46 Da. The other neutral losses observed are due to cleavages of bonds connected to C1'-O, C4-O, or C11'-O atoms (Krishnamurthy and Sarver 1989).

3 Biosynthesis of Trichothecenes

Trichothecenes are secondary metabolites mainly produced by filamentous fungi of the genera *Fusarium, Trichothecium, Stachybotrys, Mycothecium, Cephalosporium, Verticimonosporium*, and *Trichoderma* (Rocha et al. 2005; Arunachalam and Doohan 2013), being *Fusarium* spp. the principal producer (Kimura et al. 2007; Kumari et al. 2016).

Fusarium species are very diversified and have a wide geographic distribution (Smith 2007), commonly found as aerial parts colonizers of several vegetal species. They can be also found in association with roots of plants or in the soil, being part of the normal microflora or acting as pathogens (Nelson et al. 1994; Guarro 2013). Although fungi are the main producers of trichothecenes, some compounds

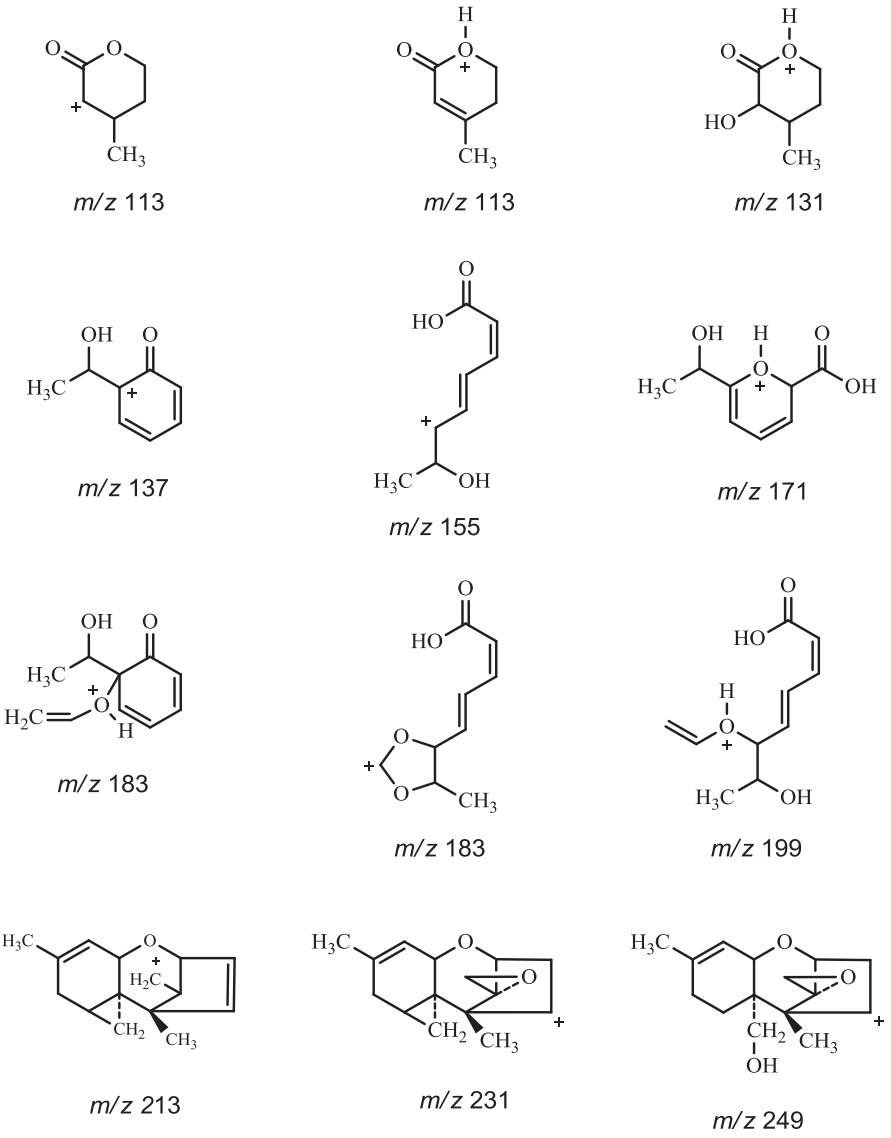


Fig. 14.3 Chemical structures of the most common daughter ions resultant from macrocyclic trichothecenes fragmentation by thermospray ionization. (Source: Krishnamurthy, adapted (Krishnamurthy and Sarver 1989))

of this class have already been isolated from plants of the genus *Baccharis*. It is important to mention that fungi exclusively produce simple trichothecenes, while some macrocyclic trichothecenes, such as baccharinoids, verrucarins A (36) and J (38), roridins A (30), D (31), and E (32), were isolated from *B. coridifolia* and *B. megapotamica* (Jarvis et al. 1991; Grove 1993).

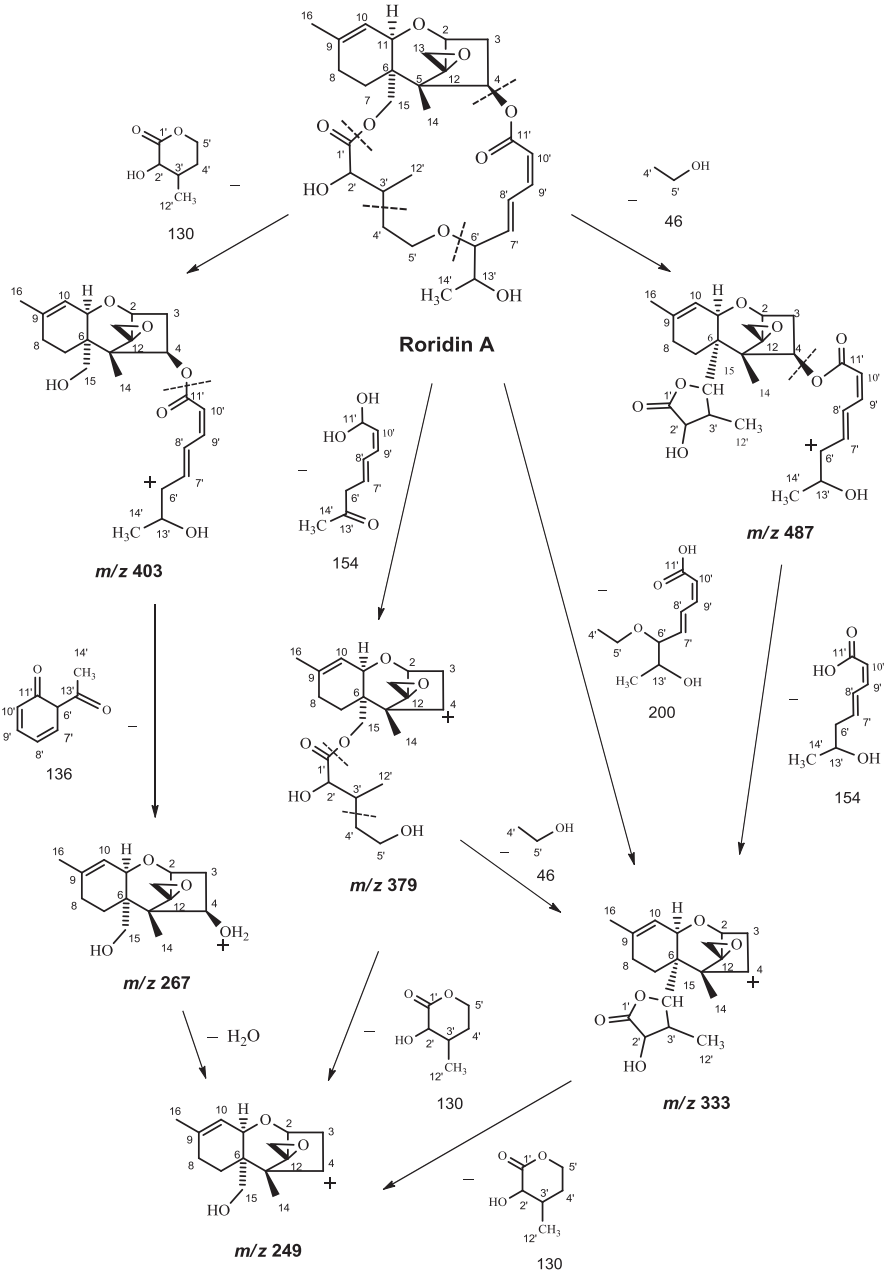


Fig. 14.4 Fragmentation pathway for roridin A. (Source: Krishnamurthy, adapted (Krishnamurthy and Sarver 1989))

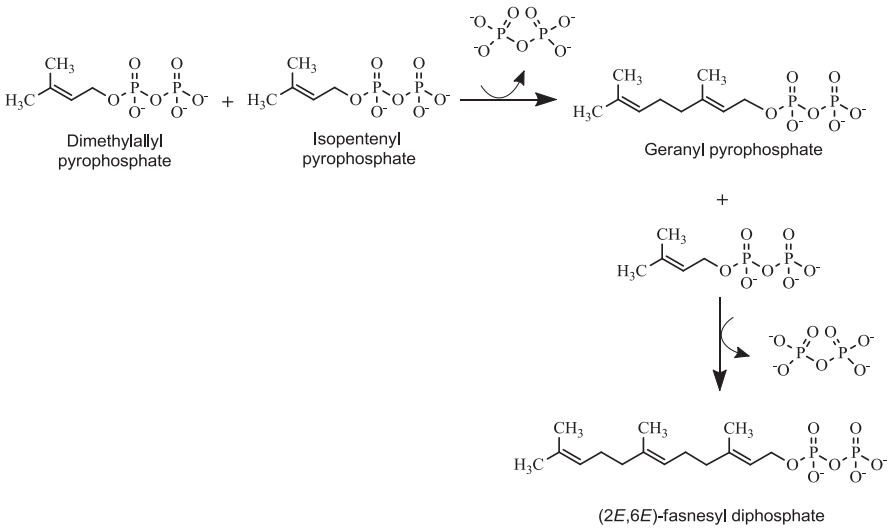


Fig. 14.5 Synthesis of farnesyl pyrophosphate. (Source: Dhar, adapted (Dhar et al. 2013))

The biosynthesis of trichothecenes in *Fusarium* and *Trichothecium* species was extensively studied. Trichothecenes have a carbon skeleton derived from farnesyl pyrophosphate (FPP), which is synthesized by the condensation reaction of dimethylallyl pyrophosphate with two units of isopentenyl pyrophosphate, having geranyl pyrophosphate as intermediate compounds (Kimura et al. 2007; Dhar et al. 2013), as shown in Fig. 14.5.

Figure 14.6 presents the biosynthetic pathway of trichothecenes proposed for *Fusarium* species. In the biosynthetic sequence, the cyclization reaction of an acyclic FPP molecule produces the bicyclic trichodiene (TDN), a precursor of trichothecenes (Kimura et al. 2007; Villafana et al. 2019). In its turn, TND is submitted to sequential and nonrandom oxidation reactions catalyzed by specific enzymes, at carbons 2, 12, and 13 (epoxidation reaction), along with C-11 and C-3, forming isotrichodiol. Subsequently, trichodiol formation occurs in a nonenzymatic reaction. The trichodiol molecule undergoes a second cyclization, giving rise to isotrichodermol, the first trichothecene of the biosynthetic pathway. Changes in the carbon skeleton, catalyzed by enzymes, lead to the formation of calonectrin, an important biosynthetic intermediate of different trichothecenes, such as deoxynivalenol (3), T-2 toxin (1), and nivalenol (4) (Villafana et al. 2019).

The biosynthetic pathway for the production of macrocyclic trichothecenes (Fig. 14.7) is not fully elucidated and to date, only a few intermediates and final products have been isolated and characterized from fungal cultures of *Myrothecium* species (Trapp et al. 1998). This pathway has isotrichodiol as a starting compound that undergoes nonenzymatic isomerization and cyclization to form 12,13-epoxytrichothec-9-ene. Next, hydroxylation occurs at C-15 and esterification takes place at C-4, with the introduction of a polyketide chain in this position. In the

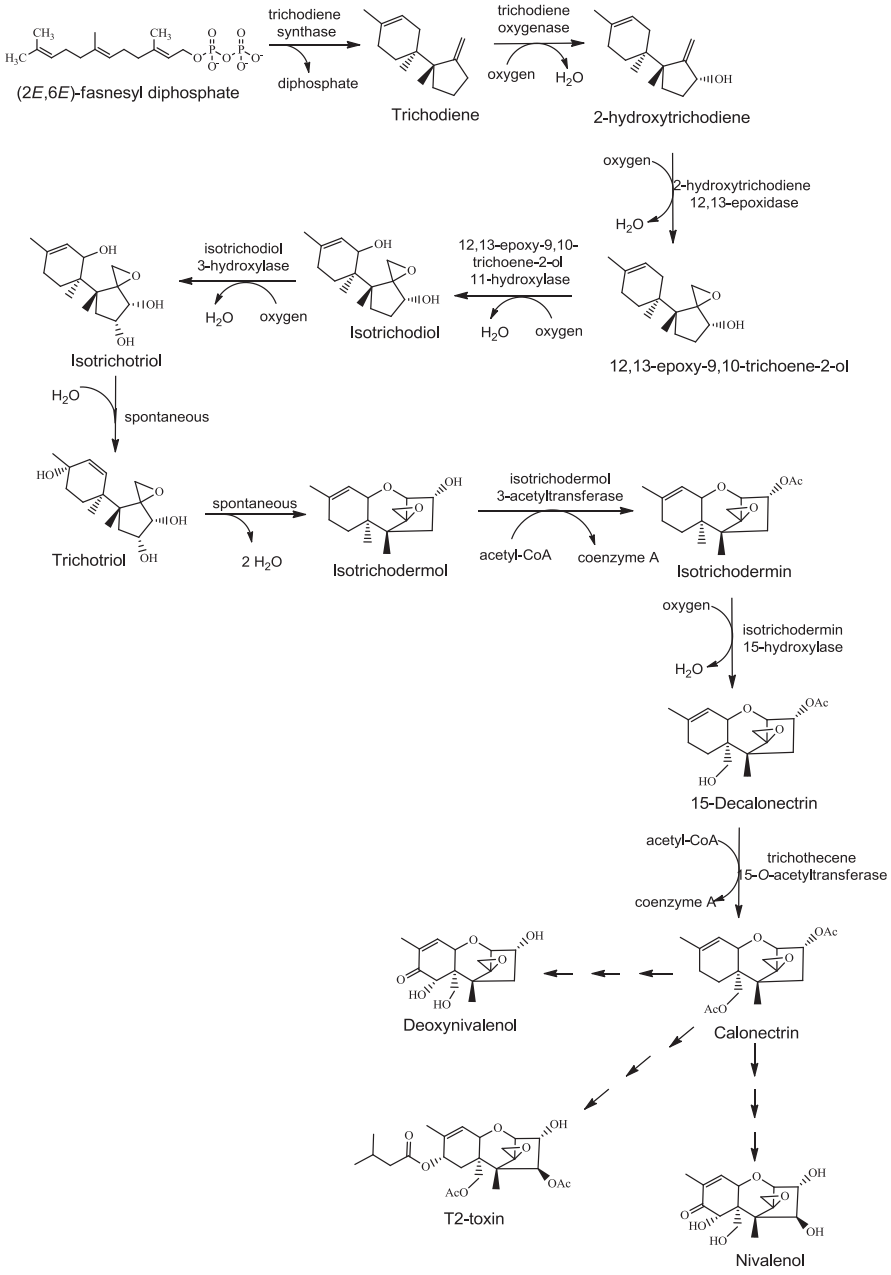


Fig. 14.6 Biosynthesis of trichothecenes in *Fusarium* species. (Source: Villafana, adapted (Villafana et al. 2019))

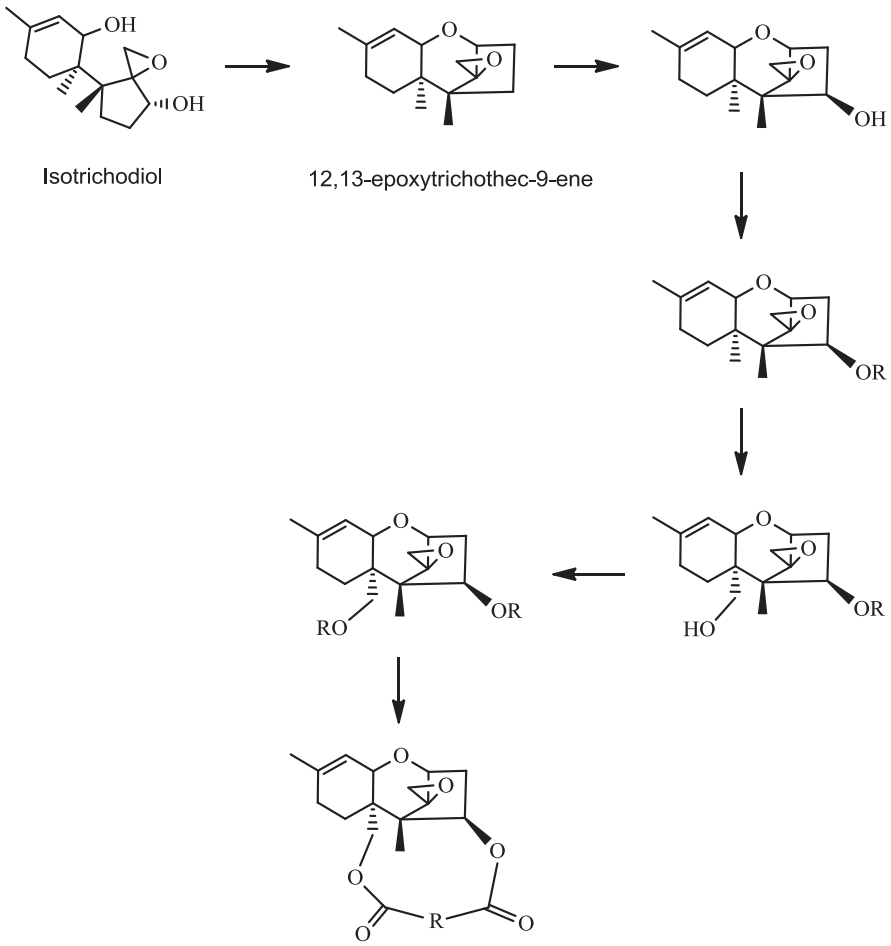


Fig. 14.7 Biosynthesis proposal for macrocyclic trichothecenes formation in *Myrothecium* species. (Source: McCormick, adapted (McCormick et al. 2011))

sequence, the hydroxyl group is esterified forming a polyketide ester at C-15. The condensation of the polyketide chains at C-4 and C-15 results in the formation of a macrocyclic trichothecene (McCormick et al. 2011).

It was initially supposed that trichothecenes found in *Baccharis* species originated from fungi present in the soil, such as *Myrothecium* species, which produce roridins. Thus, it was first accepted that in *B. megapotamica*, the roridins were absorbed, translocated, and metabolized in baccharinoids, whereas in *B. coridifolia*, they were only stored (Jarvis et al. 1981; Jarvis et al. 1987b, c).

However, in 1988, Jarvis et al. (1988b) refuted the original hypothesis of trichothecenes production by fungi in *Baccharis* species and claimed that these metabolites are synthesized by the plants themselves. The authors came to this conclusion

after analyzing species of *B. coridifolia*, for which neither *Myrothecium verrucaria* nor *Myrothecium roridum* could be detected. In addition, they could not isolate endophytic fungi from the species. The authors also found high concentrations of roridins A (30) and E (32) in *B. coridifolia* seeds.

In a later study of 1990, Kuti et al. (1990) sought to demonstrate the role of phytotoxins in seed maturation and germination of *Baccharis* species. The authors observed that *Baccharis* species from Brazil (*B. megapotamica* and *B. coridifolia*), which produce trichothecenes, had their germination completely inhibited when the seed husks were removed in comparison to American *Baccharis* (*B. halimifolia* and *B. glutinosa*), which presented better germination rates. Based on these results, the authors suggested that macrocyclic trichothecenes play a regulatory role in the reproduction and germination of *B. megapotamica* and *B. coridifolia*.

In relation to the distribution of these toxins between male and female individuals, it was observed that female *B. coridifolia* plants have a higher concentration of trichothecenes than the male ones, whereas for *B. megapotamica*, no difference was found. It is important to note that male individuals of *B. coridifolia* have miotoxin C (41) as a major component, whereas in female individuals, there is a higher concentration of roridins A (30) and E (32) (Jarvis et al. 1991). According to Jarvis et al. (1996), female plants of *B. coridifolia* are more toxic than male plants, since they contain five to ten times more trichothecenes.

4 Macrocyclic Trichothecenes in *Baccharis* Species

In 1976, Kupchan et al. (1976) isolated the first macrocyclic trichothecene from a plant species. In a screening of antitumor compounds from natural sources, the researchers observed the inhibitory activity of *B. megapotamica* ethanolic extract against KB cells (nasopharyngeal carcinoma) in vitro and in a model of P-388 murine leukemia in vivo. The phytochemical investigation resulted in the isolation of baccharin (9), isobaccharin (10), baccharinol (11), and isobaccharinol (12), considered responsible for the antileukemic activity (Kupchan et al. 1977). After that, other baccharinoids were isolated from the same species, including the baccharinoids B1 (13), B2 (14), B3 (15), B7 (16), B9 (17), B10 (18), B12 (19), B13 (20), B14 (21), B16 (22), B17 (23), B20 (24), B21 (25), B23 (27), B24 (28), B25(26), and B27 (29) (Jarvis et al. 1987a, b).

Due to the toxic effects caused by *Baccharis coridifolia*, associated with poisoning in animals, Habermehl et al. (1985) identified nine macrocyclic trichothecenes, considered responsible for the toxic effect: roridins A (30) and E (32), miotoxins A (39), B (40), C (41), D (47), and iso-D (48), along with miophytocens A (50) and B (51). It is noteworthy that roridins had already been isolated as secondary metabolites from fungal cultures, while miotoxins and miophytocens were unknown compounds. Later, Jarvis et al. (1996) isolated and characterized new trichothecene glycosides from the same species, including roridin A β-glucoside (33), roridin D β-glucoside (34), roridin E β-glucoside (35), verrucarin A β-glucoside (37),

miotoxin A β-glucoside (45), and miotoxin F β-glucoside (46), in addition to the new miotoxins E (42), F (43) and G (44). According to Rizzo et al. (1997), specimens of *B. coridifolia* collected in Argentina presented verrucarins A (36) and J (38), in addition to the roridins.

The first report on macrocyclic trichothecenes in *Baccharis artemisioides* occurred in 1977, when Rizzo et al. (1997) described the presence of roridins A (30), D (31) and E (32), along with verrucarins A (36) and J (38) in an Argentina specimen. Furthermore, the authors reported higher concentrations of toxins in *B. artemisioides* than in *B. coridifolia* (Rizzo et al. 1997).

The chemical structures of the macrocyclic trichothecenes here described are depicted in Fig. 14.8.

5 Biological Activities of Macrocyclic Trichothecenes

Antifungal, antimalarial, antitumor, and antiviral activities have been reported for macrocyclic trichothecenes. The toxic effects on eukaryotic cells are complex and varied; the toxicity and selectivity are dependent on the compound and cell type tested (Arunachalam and Doohan 2013).

The effect of macrocyclic trichothecenes on the growth of wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), and tobacco (*Nicotiana tabacum* L.) was investigated. The authors observed that roridin A (30), verrucarins A (36) and J (38) not only inhibited the growth but were also toxic to these species (Cutler and Jarvist 1985). In addition, roridins E (32) and H (49), along with verrucarins A (36) and J (38), presented phytotoxicity against cultures of duckweed (*Lemna pausicostata* L.) and kudzu (*Pueraria lobata* L.) (Abbas et al. 2002). In view of this phytotoxicity, the effects of roridins A (30) and E (32) on cell cultures of Brazilian *Baccharis* (*B. megapotamica* and *B. coridifolia*) and North American *Baccharis* (*B. halimifolia* and *B. glutinosa*) were compared, the last ones being more sensitive to the cytotoxic effects (Jarvis et al. 1988a).

Regarding the antifungal effect, roridins A (30) and D (31) were active against *Saccharomyces cerevisiae*, *Magnaporthe grisea*, and *Sclerotinia sclerotiorum* strains, with minimum inhibitory concentrations lower than or equal to fluconazole, used as positive control (Xie et al. 2008). Moreover, roridin A (30) and verrucarin A (36) inhibited *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum* growth, at lower or equal concentrations of the positive control ketoconazole (Liu et al. 2006).

Isaka et al. (1999) demonstrated the antimalarial activity of roridins A (30) and E (32), and verrucarins A (36) and J (38); they all showed lower EC_{50} than the reference drug artemisinin. In the same study, the authors reported high cytotoxic activity of these compounds against human oral epidermoid carcinoma KB cells, human breast cancer BC1 cells, and Vero cells. Zhang et al. (2002) described the inhibition

growth of *Plasmodium falciparum* strains induced by roridin E (32), with IC_{50} values lower than 1 ng/mL.

Roridins A (30) and E (32), along with verrucarins A (36) and J (38), were assayed against the Junin virus (JUNV), the etiologic agent of the Argentine hemorrhagic fever (AHF). All assayed trichothecenes inhibited virus replication at nontoxic concentrations, being verrucarin J (38) the most active compound, with a better selectivity index (García et al. 2002).

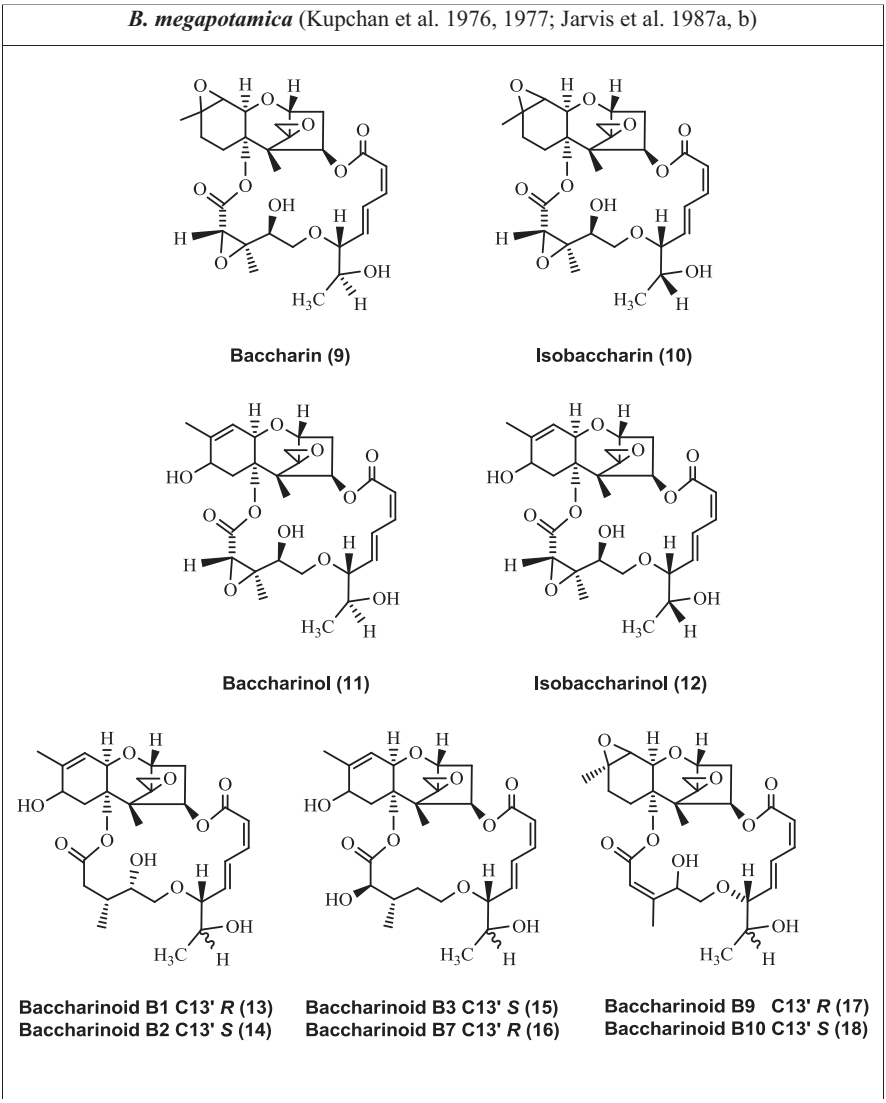
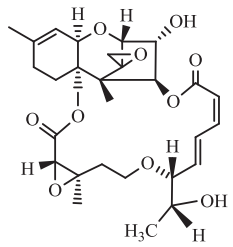
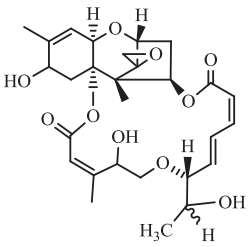


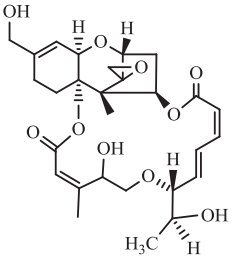
Fig. 14.8 Chemical structures of macrocyclic trichothecenes isolated from *Baccharis* species



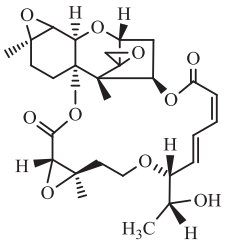
Baccharinoid B12 (19)



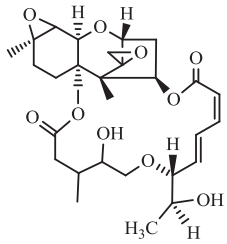
Baccharinoid B13 C13' *R* (20)
Baccharinoid B14 C13' *S* (21)



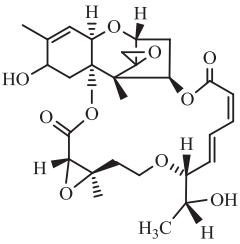
Baccharinoid B16 (22)



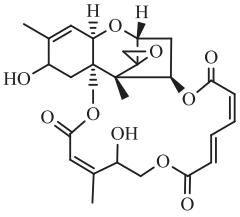
Baccharinoid B17 (23)



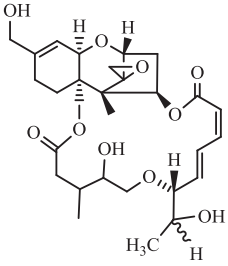
Baccharinoid B20 (24)



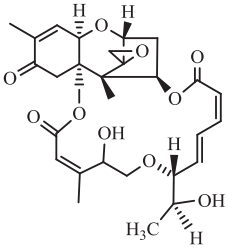
Baccharinoid B21 (25)



Baccharinoid B25 (26)



Baccharinoid B23 C13' *R* (27)
Baccharinoid B24 C13' *S* (28)



Baccharinoid B27 (29)

B. coridifolia (Habermehl et al. 1985; Jarvis et al. 1996; Rizzo et al. 1997)

Fig. 14.8 (continued)

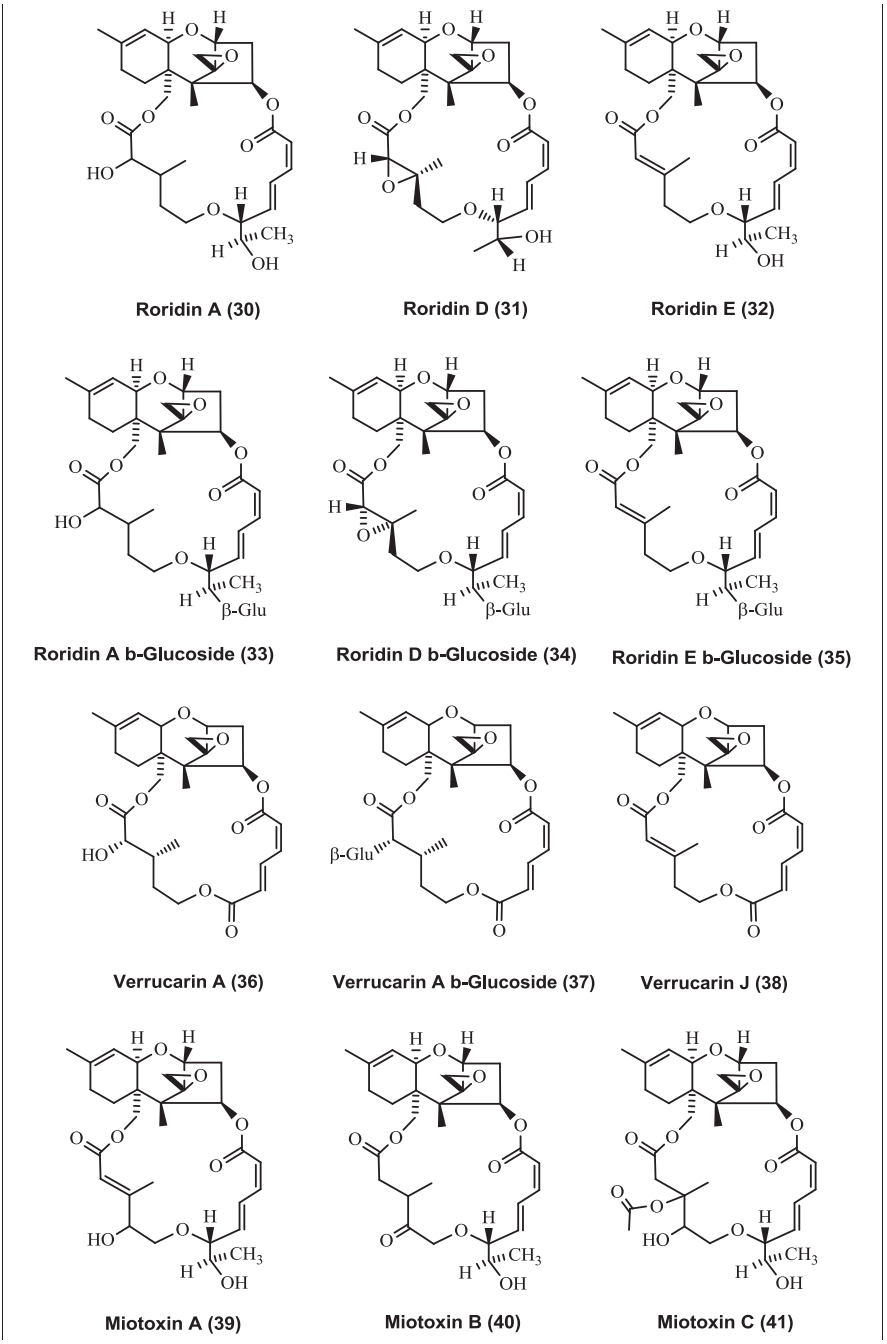


Fig. 14.8 (continued)

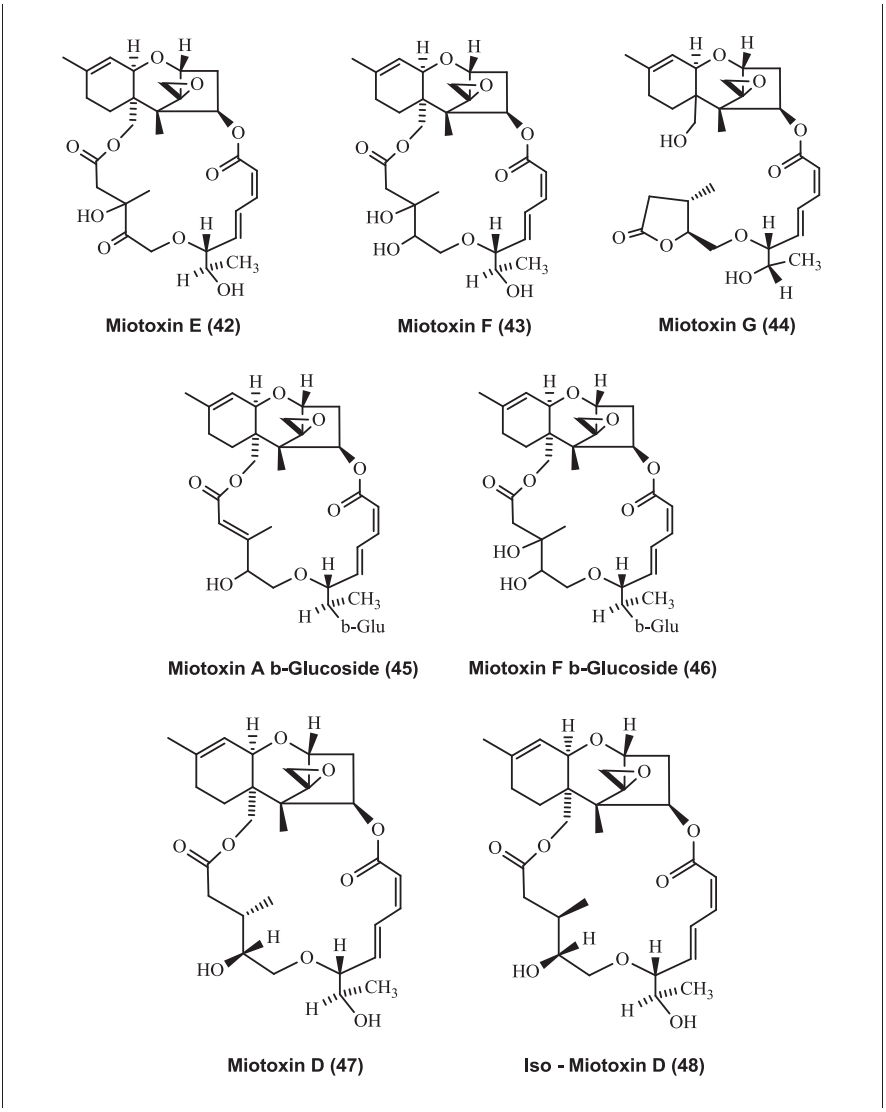


Fig. 14.8 (continued)

The cytotoxic activity of several mycotoxins against NIH/3 T3 cells (Swiss mouse embryonic fibroblast) and BE 12-6 cells (bovine embryonic cells) was evaluated and disclosed verrucarin A (36) and roridin A (30) as the most cytotoxic compounds (Terse et al. 1993). The cytotoxicity of roridins E (32) and H (49), along with verrucarins A (36) and J (38), has been demonstrated in NIH3T3 cells (Swiss mouse embryonic fibroblast), KA31T cells (oncogenically transformed 3 T3 mouse fibroblasts), H4TG cells (rat hepatoma), and MDCK cells (Madin-Darby canine

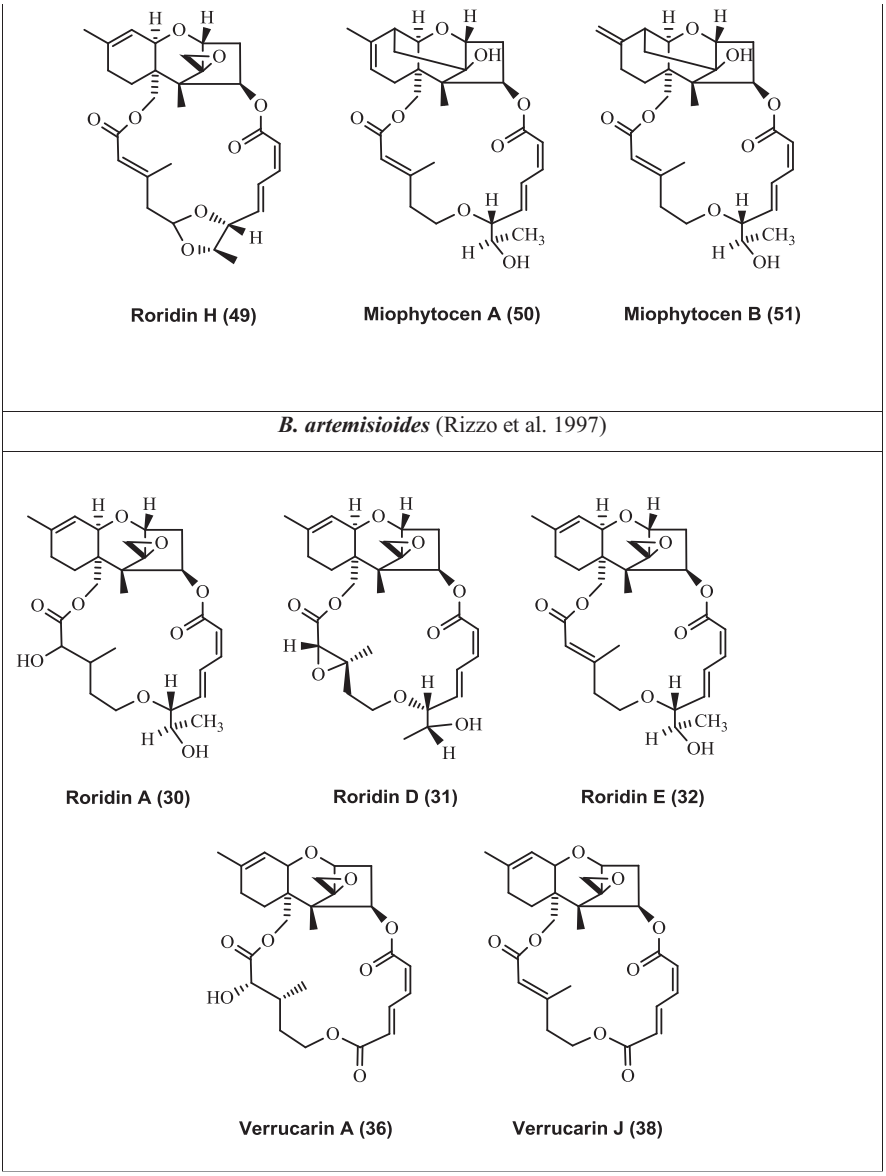


Fig. 14.8 (continued)

kidney) (Abbas et al. 2002). In addition, baccharin (9), baccharinol (11), roridins A (30) and H (49), and verrucarins A (36) and J (38) were shown to be active in vivo against P388 mouse leukemia (Jarvis et al. 1984).

Hughes et al. (1990) evaluated the effects of roridins A (30), D (31), and E (32); baccharinoid B12 (19); and verrucarins A (36) and J (38) on the viability and

mitogenesis of splenic lymphocytes of CD-1 mice. The trichothecenes showed cytotoxic effects at the concentration range from 10^{-6} to 10^{-4} M. Regarding the cell proliferation, roridin A (30) proved to be a good inhibitor, with concentrations lower than the maximum lethal effect.

Roridin E (32) was administered alone and in combination with linoleic acid to Sprague-Dawley rats. The combination produced more significant toxic effects, such as decreased blood glucose and glutathione levels, reduction of superoxide dismutase and glucose-6-phosphatase in the kidney, in addition to increased lipid peroxidation in the liver (El-Din et al. 1997).

6 Structure–Activity Relationship of Macrocyclic Trichothecenes

Most of the literature reporting structure-activity relationships is directed to simple trichothecenes, found in fungi species. As for macrocyclic trichothecenes, most studies were performed using two or more related compounds (de Carvalho et al. 2016). An example is the cytotoxic activity of roridin E (32) and its 12'-OH derivative against different leukemia lines (THP-1 cells – human monocytic leukemia and HL-60 cells – human promyelocytic leukemia) and against V79 cells (Chinese hamster lung fibroblast). Roridin E (32) showed higher cytotoxicity than the derivative, suggesting that a hydroxyl group at C-12′ position is required for the activity (Oda et al. 2010).

Amagata et al. (2003) studied the effect of some macrocyclic trichothecenes in tumor cell lines and in healthy cell lines, in order to analyze their cytotoxicity and selectivity. According to the authors, miophytocen C was the least potent compound and its lower potency would be related to the absence of the C12-C13 epoxide ring. On the other hand, trichothecenes like verrucarin A (36) and roridin A (30), which have an epoxide ring, were selectively active against the tumor cells. It can be therefore concluded that an epoxide ring at C12-C13 positions is required for selective cytotoxic effect. In addition, the authors concluded that the stereochemistry at C-6'and C-13'positions showed a small effect on cell selectivity.

Nineteen mycotoxins, all possessing the C12-C13 epoxide ring, were tested for their ability to inhibit protein synthesis in Vero cells and rat spleen lymphocytes. Trichothecenes with a C4-C15 hydrocarbon chain ring, namely, roridin A (30) and verrucarin A (36), showed higher potency than trichothecenes bearing open hydrocarbon chains at those positions, suggesting that the macrocyclic part is relevant for the cytotoxic effects (Thompson and Wannemacher 1986). Furthermore, 3D QSAR studies (Quantitative Structure–Activity Relationship) revealed that the toxicity is highly dependent on the conformation of the macrocyclic ring and that an additional epoxide ring at C9-C10 positions, as found in baccharin (9), increases the antileukemic activity (Steinmetz et al. 2009).

Verrucarins A (36) and J (38), along with roridin E (32), showed potent cytotoxic activity against *Artemia salina*. In this study, the verrucarins showed higher toxicity than roridin, suggesting that the presence of an ester at the C6'position is essential for the activity. In addition, since verrucarin A (36) was six times more active than verrucarin J (38), it seems that the C2'-OH group is capable of improving toxicity (Zhao et al. 2011).

The cytotoxicity of roridin H (49) and its 8α-OH and 8α-OAc derivatives was assayed against L929 cells (mouse fibroblast), MCF-7 cells (breast cancer), Hela KB3.1 cells, and A431 cells (skin cancer). The obtained results indicated that hydroxylation increased the cytotoxic activity against the tumor cells, whereas acetylation had the opposite effect. In addition, a hydroxyl group at C-8 position seems to be required for selectivity (de Carvalho et al. 2016).

7 Mode of Action of Macrocyclic Trichothecenes

Trichothecenes have several effects on animal and plant cells, including inhibition of DNA and RNA syntheses, inhibition of mitochondrial function, membrane destabilization, changes in cell division, and induction of apoptosis (Rocha et al. 2005). They also present effects on the inhibition of protein synthesis through interaction with ribosomes (Schindler et al. 1974; Cundliffe and Davies 1977).

In order to analyze the mode of action of some specific inhibitors of protein synthesis, trichothecenes were tested in H-HeLa cells. Roridin A (30) and verrucarin A (36) caused the accumulation of ribosomal structures, that were found to consist of 80S monosomes and 40S initiation complexes attached to the same mRNA strand, not normally present in control lysates. For this reason, it was suggested that these compounds can prevent the formation of 80S initiation complexes, in addition to inhibiting the function of ribosomes already attached to mRNA (Cundliffe and Davies 1977).

Loubresse et al. (2014) analyzed the interaction of verrucarin A (36) with the 80S ribosome of *Saccharomyces cerevisiae* using X-ray crystallography. According to the authors, the compound interacts with the A-site in the peptidyl transferase center. Also, the large macrocycle extends further toward the macrolide-binding site, thus confirming the relevance of stereochemistry on the biological effects of macrocyclic trichothecenes.

Trichothecenes are capable of inhibiting protein synthesis by binding to the ribosomal peptidyl transferase site. This inhibition results in cellular stress reactions, leading to the activation of mitogen-activated protein kinases (MAPKs), which are part of the apoptotic pathway. Gel electrophoresis revealed DNA fragmentation after the treatment of cells with some of these compounds, suggesting a role in apoptosis (Shifrin and Anderson 1999). This was confirmed by treatment of HL-60 cells (human promyelotic leukemia) with roridin A (30), which induced DNA fragmentation, with a minimal effective concentration of 0.001 μg/mL (Ueno et al. 1995).

Yang et al. (2000) investigated the relationship between the cytotoxic and apoptotic capacities of trichothecenes and the activation of MAPK. For this purpose, two myeloid models were employed: RAW 264.7 (murine macrophage) and U937 (human leukemic cells) cells. Upon evaluating the cytotoxic activity by the 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) assay, roridin A (30) and verrucarin A (36) have been shown to be highly toxic and, by using DNA fragmentation and fluorescence microscopy assays, the results suggest that cytotoxicity was mediated through an apoptotic process. In addition, the assessment of MAPK activation using Western blot analysis revealed that these trichothecenes activated some proteins of this group.

There are some studies evaluating the role of macrocyclic trichothecenes in immunological functions. An example is the inhibitory effect of roridin A (30) and verrucarin A (36) on human lymphocyte transformation, analyzed by the mitogen-induced blastogenesis assay. Both compounds were able to inhibit B and T cell subsets stimulation, after induction with leukoagglutinin, concanavalin A, and pokeweed mitogen (Pestka and Forsell 1988).

In order to study the capacity of the mycotoxins to alter immune functions, the effects of roridin A (30) and verrucarin A (36) on interleukin 2 (IL-2) production and viability were evaluated in a murine T-cell model. It was observed that IL-2 levels were significantly increased in cultures incubated with low concentrations of these compounds. However, in the presence of higher concentrations, IL-2 levels were depressed. Besides, cell viability determined by the MTT method was significantly decreased by 0.5 ng/mL of roridin A (30) and verrucarin A (36). The biphasic behavior of these compounds in the deregulation of cytokine production, where low concentrations superinduce IL-2 production and higher concentrations suppress, complicates the interpretation of different effects in in vivo experiments (Lee et al. 1999).

Kankkunen et al. (2009) demonstrated that roridin A (30) and verrucarin A (36) can activate the inflammasome-associated caspase-1, required for the formation of IL-1β and IL-18 in LPS-primed cells. Later, the same researchers showed that these compounds trigger activation of NLRP3 inflammasome through P2X7R and Src tyrosine kinase signaling–dependent pathway, using human primary macrophages (Kankkunen et al. 2014). Moreover, verrucarin A (36) inhibited IL-8 and NF-κB activation in HL-60 cells (human promyelocytic leukemia), at noncytotoxic concentrations (Oda et al. 2005).

Table 14.5 lists the biological activities reported for macrocyclic trichothecenes, together with the IC_{50}, EC_{50}, MIC, and 50%PSI values, in different models and cell lines.

Table 14.5 Biological activities of macrocyclic trichothecenes from *Baccharis* species

Macrocyclic trichothecenes	Biological activities	Model / cell line / microorganism	IC_{50} / EC_{50} / MIC / 50%PSI	Ref.
Baccharin	Cytotoxic	P-388 leukemia in mice	5.0 mg/kg	Jarvis et al. (1984)
Baccharinol	Cytotoxic	P-388 leukemia in mice	2.5 mg/kg	Jarvis et al. (1984)
Roridin A	Antiviral	Junin virus (JUNV)	3.1 ng/mL	García et al. (2002)
	Antifungal	*Saccharomyces cerevisiae, Magnaporthe grisea, Sclerotinia sclerotiorum*	31.25 μg/mL 125 μg/mL 31.25 μg/mL	Xie et al. (2008)
		Candida albicans, *Aspergillus niger, Trichophyton rubrum*	125 μg/mL 31.25 μg/mL 62.5 μg/mL	Liu et al. (2006)
	Antimalarial	*Plasmodium falciparum*	0.31 ng/mL	Isaka et al. (1999)
	Cytotoxic	KB cells BC1 cells Vero cells	1.0 ng/mL 5.0 ng/mL 1.7 ng/mL	Isaka et al. (1999)
		NIH/3 T3 BE 12-6	0.00028 μg/mL 0.00096 μg/mL	Terse et al. (1993)
		P-388 leukemia in mice	0.06 mg/kg	Jarvis et al. (1984)
		HL-60	0.0005 μg/mL	Ueno et al. (1995)
	Protein synthesis inhibition	Vero cells Rat spleen lymphocytes	12 nM 5 nM	Thompson and Wannemacher (1986)
Roridin E	Antiviral	Junin virus (JUNV)	3.1 ng/mL	Matsumoto et al. (1977)
	Antimalarial	*Plasmodim falciparum*	0.15 ng/mL	(Isaka et al. (1999)
		P. falciparum clone W2 *P. falciparum* clone D6	0.6 ng/mL 0.2 ng/mL	Zhang et al. (2002)
	Cytotoxic	THP-1 HL-60 V79	9.1 nM 7.9 nM 0.74 nM	Oda et al. (2010)
		Artemia salina	0.221 μg/mL	Zhao et al. (2011)
		KB cells BC1 cells Vero cells	0.5 ng/mL 0.7 ng/mL 0.4 ng/mL	Isaka et al. (1999)
		NIH/3 T3 KA31T H4TG MDCK	2.30 nM 7.68 nM 3.45 nM 1.74 nM	Abbas et al. (2002)

(continued)

Table 14.5 (continued)

Macrocyclic trichothecenes	Biological activities	Model / cell line / microorganism	IC_{50} / EC_{50} / MIC / 50%PSI	Ref.
Roridin D	Antifungal	*Saccharomyces cerevisiae, Magnaporthe grisea, Sclerotinia sclerotiorum*	62.5 μg/mL 250 μg/mL 31.25 μg/mL	Xie et al. (2008)
Roridin H	Cytotoxic	NIH/3 T3 KA31T H4TG MDCK	9.84 nM 8.64 nM 10.8 nM 3.98 nM	Abbas et al. (2002)
		P-388 leukemia in mice	12.5 mg/kg	Jarvis et al. (1984)
Verrucarin A	Antiviral	Junin virus (JUNV)	4.9 ng/mL	García et al. (2002)
	Antifungal	*Candida albicans, Aspergillus niger, Trichophyton rubrum*	31.25 μg/mL 250 μg/mL 125 μg/mL	Liu et al. (2006)
	Antimalarial	*Plasmodim falciparum*	0.90 ng/mL	Isaka et al. (1999)
	Cytotoxic	*Artemia salina*	0.001 μg/mL	Zhao et al. (2011)
		KB cells BC1 cells Vero cells	1.2 ng/mL 4.2 ng/mL 1.1 ng/mL	Isaka et al. (1999)
		NIH/3 T3 BE 12-6	0.00033 μg/mL 0.00092 μg/mL	Terse et al. (1993)
		NIH/3 T3 KA31T H4TG MDCK	3.03 nM 1.94 nM 2.50 nM 0.96 nM	Abbas et al. (2002)
		P-388 leukemia in mice	2 mg/kg	Jarvis et al. (1984)
	Protein synthesis inhibition	Vero cells Rat spleen lymphocytes	12 nM 3 nM	Thompson and Wannemacher (1986)

(continued)

Table 14.5 (continued)

Macrocyclic trichothecenes	Biological activities	Model / cell line / microorganism	IC_{50} / EC_{50} / MIC / 50%PSI	Ref.
Verrucarin J	Antiviral	Junin virus (JUNV)	1.2 ng/mL	García et al. (2002)
	Antimalarial	*Plasmodim falciparum*	0.20 ng/mL	Isaka et al. (1999)
	Cytotoxic	*Artemia salina*	0.006 µg/mL	Zhao et al. (2011)
		KB cells *BC1 cells* *Vero cells*	3.9 ng/mL 14 ng/mL 1.2 ng/mL	Isaka et al. (1999)
		NIH/3 T3 KA31T H4TG MDCK	4.55 nM 3.14 nM 4.56 nM 2.02 nM	Abbas et al. (2002)
		P-388 leukemia in mice	0.8 mg/kg	Jarvis et al. (1984)

8 Final Considerations

Phytochemical studies with *Baccharis* species were of great relevance to demonstrate that plants, in addition to endophytic fungi, can produce trichothecenes. Therefore, *Baccharis* spp. represent new sources of trichothecenes and suitable models for studying the biosynthesis of this class of metabolites. Additionally, they allow investigating evolutionary aspects and ecological relations between fungi and plants (Rizzo et al. 1997).

The toxicity of trichothecenes is a matter of concern, being the main cause of animal deaths in southern Brazil. The occurrence of intoxication with *B. coridifolia* was reported in cattle, sheep, and horses, while *B. megapotamica* intoxication was recorded in cattle and sheep (Rissi et al. 2005; Pedroso et al. 2010).

It is important to note that the number of studies performed with *Baccharis* trichothecenes is still limited. Additional phytochemical and biological studies are thus demanded to better understand their chemical and medicinal properties, aiming at future exploitation.

References

Abbas HK, Johnson BB, Shier WT, Tak H, Jarvis BB, Boyette CD (2002) Phytotoxicity and mammalian cytotoxicity of macrocyclic trichothecene mycotoxins from *Myrothecium verrucaria*. Phytochemistry 59:309–313. https://doi.org/10.1016/S0031-9422(01)00464-2

Amagata T, Rath C, Rigot JF, Tarlov N, Tenney K, Valeriote FA, Crews P (2003) Structures and cytotoxic properties of trichoverroids and their macrolide analogues produced by saltwater culture of *Myrothecium verrucaria*. J Med Chem 46:4342–4350. https://doi.org/10.1021/jm030090t

Arunachalam C, Doohan FM (2013) Trichothecene toxicity in eukaryotes: cellular and molecular mechanisms in plants and animals. Toxicol Lett 217:149–158. https://doi.org/10.1016/j.toxlet.2012.12.003

Cundliffe E, Davies JE (1977) Inhibition of initiation, elongation, and termination of eukaryotic protein synthesis by trichothecene fungal toxins. Antimicrob Agents Chemother 11:491–499. https://doi.org/10.1128/AAC.11.3.491

Cutler HG, Jarvist BB (1985) Preliminary observations on the effects of macrocyclic trichothecenes on plant growth. Environ Exp Bot 25:115–128

de Carvalho M, Weich H, Abraham WR (2016) Macrocyclic trichothecenes as antifungal and anticancer compounds. Curr Med Chem 23:23–35. https://doi.org/10.2174/0929867323666151117121521

Desjardins AE, McCormick SP, Appell M (2007) Structure-activity relationships of trichothecene toxins in an *Arabidopsis thaliana* leaf assay. J Agric Food Chem 55:6487–6492. https://doi.org/10.1021/jf0709193

Dhar MK, Koul A, Kaul S (2013) Farnesyl pyrophosphate synthase: a key enzyme in isoprenoid biosynthetic pathway and potential molecular target for drug development. New Biotechnol 30:114–123. https://doi.org/10.1016/j.nbt.2012.07.001

El-Din MO, El-Sawi NM, Meki ARMA (1997) Acute toxicity of the mycotoxin roridin E on liver and kidney of rats. J Appl Anim Res 12:145–152. https://doi.org/10.1080/09712119.1997.9706200

Freeman GG, Gill JE, Waring WS (1959) The structure of trichothecin and its hydrolysis products. J Chem Soc:1105–1132

García CC, Rosso ML, Bertoni MD, Maier MS, Damonte EB (2002) Evaluation of the antiviral activity against junin virus of macrocyclic trichothecenes produced by the hypocrealean epibiont of *Baccharis coridifolia*. Planta Med 68:209–212. https://doi.org/10.1055/s-2002-23134

Grove JF (1993) Macrocyclic Trichothecenes. Nat Prod Rep 10:429–448

Guarro J (2013) Fusariosis, a complex infection caused by a high diversity of fungal species refractory to treatment. Eur J Clin Microbiol Infect Dis 32:1491–1500. https://doi.org/10.1007/s10096-013-1924-7

Habermehl GG, Busam L, Heydel P, Mebs D, Tokarnia CH, Döbereiner J, Spraul M (1985) Macrocyclic trichothecenes: cause of livestock poisoning by the Brazilian plant *Baccharis coridifolia*. Toxicon 23:731–743. https://doi.org/10.1016/0041-0101(85)90003-0

Hughes BJ, Jarvis BB, Sharma RP (1990) Effects of macrocyclic trichothecene congeners on the viability and mitogenesis of murine splenic lymphocytes. Toxicol Lett 50:57–67. https://doi.org/10.1016/0378-4274(90)90252-H

Isaka M, Punya J, Lertwerawat Y, Tanticharoen M, Thebtaranonth Y (1999) Antimalarial activity of macrocyclic trichothecenes isolated from the fungus *Myrothecium verrucaria*. J Nat Prod 62:329–331. https://doi.org/10.1021/np980323x

Jarvis BB, Midiwo JO, Tuthill D (1981) Interaction between the antibiotic trichothecenes and the higher plant *Baccharis megapotamica*. Science 214:460–462

Jarvis BB, Midiwo J, Mazzolat EP (1984) Antileukemic compounds derived by chemical modification of macrocyclic. J Med Chem 27:239–244

Jarvis BB, Cómezoglu SN, Ammon HL, Breedlove CK, Miller RW, Woode MK, Streelman DR, Sneden AT, Dailey RG, Kupchan SM (1987a) New macrocyclic trichothecenes from *Baccharis megapotamica*. J Nat Prod 50:815–828. https://doi.org/10.1021/np50053a008

Jarvis BB, Comezoglu SN, Rao MM, Pena NB, Boettner FE, Williams M, Forsyth G, Epling B (1987b) Isolation of macrocyclic trichothecenes from a large-scale extract of *Baccharis megapotamica*. J Org Chem 52:45–56. https://doi.org/10.1021/jo00377a007

Jarvis BB, Wells KM, Lee YW, Bean GA, Kommedahl T, Barros CS, Barros SS (1987c) Macrocyclic trichothecene mycotoxins in Brazil species of *Baccharis*. Phytopathology 77:980–984

Jarvis BB, Kuti J, Bean GA, Park C (1988a) Phytotoxicity of macrocyclic trichothecenes toward *Baccharis* cell lines. JSM Mycotoxins:199–203

Jarvis BB, Midiwo JO, Bean GA, Aboul-Nasr MB, Barros CS (1988b) The mystery of trichothecene antibiotics in *Baccharis* species. J Nat Prod 51:736–744. https://doi.org/10.1021/np50058a012

Jarvis BB, Mazzocchi DB, Ammon HL, Mazzola EP, Flippen-Anderson JL, Gilardi RD, George CF (1990) Conformational effects in trichothecenes: structures of 15-hydroxy C4 and C8 ketones. J Org Chem 55:660–3662

Jarvis B, Mokhtari-Rejali N, Schenkel EP, Barros CS, Matzencachers NI (1991) Trichothecene mycotoxins from Brazilian *Baccharis* species. Phytochemistry 30:789–797

Jarvis BB, Wang S, Cox C, Rao MM, Philip V, Varaschin MS, Barros CS (1996) Brazilian *Baccharis* toxins: livestock poisoning and the isolation of macrocyclic trichothecene glucosides. Nat Toxins 4:58–71. https://doi.org/10.1002/19960402NT2

Kankkunen P, Rintahaka J, Aalto A, Leino M, Majuri M-L, Alenius H, Wolff H, Matikainen S (2009) Trichothecene mycotoxins activate inflammatory response in human macrophages. J Immunol 182:6418–6425. https://doi.org/10.4049/jimmunol.0803309

Kankkunen P, Välimäki E, Rintahaka J, Palomäki J, Nyman T, Alenius H, Wolff H, Matikainen S (2014) Trichothecene mycotoxins activate NLRP3 inflammasome through a P2X7 receptor and Src tyrosine kinase dependent pathway. Hum Immunol 75:134–140. https://doi.org/10.1016/j.humimm.2013.11.010

Kimura M, Tokai T, Takahashi-Ando N, Ohsato S, Fujimura M (2007) Molecular and genetic studies of fusarium trichothecene biosynthesis: pathways, genes, and evolution. Biosci Biotechnol Biochem 71:2105–2123. https://doi.org/10.1271/bbb.70183

Krishnamurthy T, Sarver EW (1989) Mass spectral investigations on trichothecene mycotoxins -VIII - Thermospray ionization and collisionally activated dissociation of macrocyclic trichothecenes. Biomed Environ Mass Spectrom 18:287–300. https://doi.org/10.1016/s0021-9673(01)97321-2

Kumari I, Ahmed M, Akhter Y (2016) Multifaceted impact of trichothecene metabolites on plant-microbe interactions and human health. Appl Microbiol Biotechnol 100:5759–5771. https://doi.org/10.1007/s00253-016-7599-0

Kupchan SM, Jarvis BB, Dailey RG, Bright W, Bryan RF, Shizuri Y (1976) Baccharin, a novel potent antileukemic trichothecene triepoxide from *Baccharis megapotamica*. J Am Chem Soc 98:7092–7093. https://doi.org/10.1021/ja00438a066

Kupchan SM, Streelman DR, Jarvis BB, Dailey RG, Sneden AT (1977) Isolation of potent new antileukemic trichothecenes from *Baccharis megapotamica*. J Org Chem 42:4221–4225

Kuti JO, Jarvis BB, Mokhtari-Rejali N, Bean GA (1990) Allelochemical regulation of reproduction and seed germination of two Brazilian *Baccharis* species by phytotoxic trichothecenes. J Chem Ecol 16:3441–3453. https://doi.org/10.1007/BF00982109

Lee MG, Li S, Jarvis BB, Pestka JJ (1999) Effects of satratoxins and other macrocyclic trichothecenes on il-2 production and viability of el-4 thymoma cells. J Toxicol Environ Health A 57:459–474. https://doi.org/10.1080/009841099157548

Liu JY, Huang LL, Ye YH, Zou WX, Guo ZJ, Tan RX (2006) Antifungal and new metabolites of *Myrothecium* sp. Z16, a fungus associated with white croaker *Argyrosomus argentatus*. J Appl Microbiol 100:195–202. https://doi.org/10.1111/j.1365-2672.2005.02760.x

Loubresse GN, Prokhorova I, Holtkamp W, Rodnina MV, Yusupova G, Yusupov M (2014) Structural basis for the inhibition of the eukaryotic ribosome. Nature 513:517–522. https://doi.org/10.1038/nature13737

Matsumoto M, Minato H, Uotani N, Matsumoto K, Kondo E (1977) New antibiotics from *Cylindrocarpon* sp. J Antibiot 30:681–682

McCormick SP, Stanley AM, Stover NA, Alexander NJ (2011) Trichothecenes: from simple to complex mycotoxins. Toxins 3:802–814. https://doi.org/10.3390/toxins3070802

Nelson P, Dignani M, Anaissie EJ (1994) Taxonomy, biology, and clinical aspects of *Fusarium* species. Clin Microbiol Rev 7:479–504. https://doi.org/10.1128/CMR.7.4.479

Oda T, Namikoshi M, Akano K, Kobayashi H, Honma Y, Kasahara T (2005) Verrucarin A inhibition of MAP kinase activation in a PMA-stimulated promyelocytic leukemia cell line. Mar Drugs 3:64–73

Oda T, Xu J, Ukai K, Nakazawa T, Namikoshi M (2010) 12′-Hydroxyl group remarkably reduces roridin E cytotoxicity. Mycoscience 51:317–320. https://doi.org/10.1007/s10267-010-0035-x

Pedroso PMO, Bandarra PM, Feltrin C, Gomes DC, Watanabe TTN, Ferreira HH, Driemeier D (2010) Intoxicação por *Baccharis megapotamica* var. *weirii* em ovinos. Pesqui Vet Bras 30:403–405. https://doi.org/10.1590/S0100-736X2010000500005

Pestka JJ, Forsell JH (1988) Inhibition of human lymphocyte transformation by the macrocyclic trichothecenes roridin A and verrucarin A. Toxicol Lett 41:215–222. https://doi.org/10.1016/0378-4274(88)90057-4

Rissi DR, Rech RR, Fighera RA, Cagnini DQ, Kommers GD (2005) Intoxicação espontânea por *Baccharis coridifolia* em bovinos. Pesq Vet Bras 25:111–114

Rizzo I, Varsavky E, Haidukowski M, Frade H (1997) Macrocyclic trichothecenes in *Baccharis coridifolia* plants and endophytes and *Baccharis artemisioides* plants. Toxicon 35:753–757. https://doi.org/10.1016/S0041-0101(96)00149-3

Rocha O, Ansari K, Doohan FM (2005) Effects of trichothecene mycotoxins on eukaryotic cells: a review. Food Addit Contam 22:369–378. https://doi.org/10.1080/02652030500058403

Rosen JD, Rosen RT, Hartman TG (1986) Capillary gas chromatography - mass spectrometry of several macrocyclic trichothecenes. J Chromatogr A 355:241–251

Savard ME, Blackwell BA, Greenhalgh R (1987) An ^{1}H nuclear magnetic resonance study of derivatives of 3-hydroxy-12 ,13-epoxytrichothec-9-enes. Can J Chem 65:2254–2262

Schindler D, Grant P, Davies J (1974) Trichodermin resistance: mutation affecting eukaryotic ribosomes. Nature 248:535–536. https://doi.org/10.1038/248535a0

Shank RA, Foroud NA, Hazendonk P, Eudes F, Blackwell BA (2011) Current and future experimental strategies for structural analysis of trichothecene mycotoxins-A prospectus. Toxins 3:1518–1553

Shifrin VI, Anderson P (1999) Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. J Biol Chem 274:13985–13992. https://doi.org/10.1074/jbc.274.20.13985

Smith SN (2007) An overview of ecological and habitat aspects in the genus *Fusarium* with special emphasis on the soil borne pathogenic forms. Plant Pathol Bull 16:97–120

Steinmetz WE, Rodarte CB, Lin A (2009) 3D QSAR study of the toxicity of trichothecene mycotoxins. Eur J Med Chem 44:4485–4489. https://doi.org/10.1016/j.ejmech.2009.06.012

Sudakin DL (2003) Trichothecenes in the environment: relevance to human health. Toxicol Lett 143:97–107. https://doi.org/10.1016/S0378-4274(03)00116-4

Terciolo C, Maresca M, Pinton P, Oswald IP (2018) Review article: Role of satiety hormones in anorexia induction by trichothecene mycotoxins. Food Chem Toxicol 121:701–714. https://doi.org/10.1016/j.fct.2018.09.034

Terse PS, Madhyastha MS, Zurovac O, Stringfellow D, Marquardt RR, Kemppainen BW (1993) Comparison of in vitro and in vivo biological activity of mycotoxins. Toxicon 31:913–919. https://doi.org/10.1016/0041-0101(93)90228-B

Thompson WL, Wannemacher RW (1986) Structure function relationship of trichothecenes in cell culture and in vivo. Toxicon 24:985–994

Trapp SC, Hohn TM, McCormick S, Jarvis BB (1998) Characterization of the gene cluster for biosynthesis of macrocyclic trichothecenes in *Myrothecium roridum*. Mol Gen Genet 257:421–432. https://doi.org/10.1007/s004380050666

Ueno Y (1980) Trichothecene mycotoxins mycology, chemistry, and toxicology. In: Draper HH (ed) Advances in nutritional research. Springer, Boston, pp 301–353. https://doi.org/10.1007/978-1-4757-4448-4_10

Ueno Y, Umemori K, Niimi E-C, Tanuma S-I, Nagata S, Sugamata M, Ihara T, Sekijima M, Kawai K-I, Ueno I, Tashiro F (1995) Induction of apoptosis by T-2 toxin and other natural toxins

in HL-60 human promyelotic leukemia cells. Nat Toxins 3:129–137. https://doi.org/10.1002/nt.2620030303

Verdi LG, Maria I, Brighente C, Pizzolatti G (2005) Gênero *Baccharis* (Asteraceae): aspectos químicos, econômicos e biológicos. Quim Nova 28:85–94

Villafana RT, Ramdass AC, Rampersad SN (2019) Selection of *Fusarium trichothecene* toxin genes for molecular detection depends on TRI gene cluster organization and gene function. Toxins 11:1–25. https://doi.org/10.3390/toxins11010036

Wu Q, Dohnal V, Ku K, Yuan Z (2013) Trichothecenes : structure-toxic activity relationships. Curr Drug Metab 14:641–660

Xie LW, Jiang SM, Zhu HH, Sun W, Ouyang YC, Dai SK, Li X (2008) Potential inhibitors against *Sclerotinia sclerotiorum*, produced by the fungus *Myrothecium* sp. associated with the marine sponge *Axinella* sp. Eur J Plant Pathol 122:571–578. https://doi.org/10.1007/s10658-008-9326-x

Yang GH, Jarvis BB, Chung YJ, Pestka JJ (2000) Apoptosis induction by the satratoxins and other trichothecene mycotoxins: relationship to ERK, p38 MAPK, and SAPK/JNK activation. Toxicol Appl Pharmacol 164:149–160. https://doi.org/10.1006/taap.1999.8888

Zhang HJ, Tamez PA, Aydogmus Z, Tan GT, Saikawa Y, Hashimoto K, Nakata M, Van Hung N, Xuan LT, Cuong NM, Soejarto DD, Pezzuto JM, Fong HHS (2002) Antimalarial agents from plants. III. Trichothecenes from *Ficus fistulosa* and *Rhaphidophora decursiva*. Planta Med 68:1088–1091. https://doi.org/10.1055/s-2002-36350

Zhao L, Liu L, Wang N, Wang S-J, Hu J-C, Gao J-M (2011) Potent toxic macrocyclic trichothecenes from the marine-derived fungus *Myrothecium verrucaria* Hmp-F73. Nat Prod Commun 6:1915–1916. https://doi.org/10.1177/1934578x1100601231